ELECTION OF THE CHIRAL SELECTOR FOR ENANTIOSEPARATIONS BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

Capillary electrophoresis is an effective tool for the resolution of enantiomers, which is accomplished by supplying the background electrolyte with a chiral selector capable of discriminating between the enantiomers concerned. A large number of chiral selectors are currently available, especially prominent among which are cyclodextrins, chiral crown ethers, chiral surfactants, ligand-exchange complexes and linear polysaccharides. The most suitable chiral selector for each specific purpose is usually elected by trial and error, which is expensive and time-consuming. This paper reviews the separation capabilities of chiral selectors and provides criteria for their election in terms of molecular size, charge, and the presence of specific functional groups or substructures in the analytes with a view to minimizing the number of trials needed. Such criteria are summarized in tabular form and their application illustrated with selected examples.

1. INTRODUCTION

That stereochemistry plays a crucial role in the interaction of some compounds with biological targets is widely recognized. Thus, enantiomerism is extremely significant in the pharmaceutical, environmental and nutritional fields. A large number of active principles are chiral and exhibit substantial pharmacological, pharmacokinetic or toxicological differences between their enantiomers [1]. This has led competent regulatory bodies to issue guidelines on the development of drugs with stereogenic centres [2].Following such guidelines, single enantiomers of new chiral compounds should be brought onto the market wherever possible. This is also the case with some cosmetics, pesticides, aromas and sweeteners. There is thus a growing demand for methods enabling the separation of chiral compounds.

The enantiomers of a compound are identical as regards physico chemical properties, so they can only be distinguished in a chiral environment. There are two types of methods for resolving enantiomers. One is indirect chiral separation, by which enantiomers are derivatized into diastereomers using an optically pure reagent; because diastereomers differ in their chemical properties, they can be separated in an achiral environment. On the other hand, direct chiral separation relies on the formation of labile diastereomers with chiral selectors present in the mobile phase and/or the stationary phase. The application of chromatographic techniques in chiral separations was recently reviewed and the extensive use of capillary electrophoresis (CE) for this purpose noted.

CE is an effective choice for resolving enantiomers, as reflected in a number of literature reviews[3-4] and monographs [5]on the topic. Worth special note among the reviews are that by Verleysen [6], which includes more than 350 references, and that by Gübitz[7], which reports the conditions for the enantioresolution of more than 250 analytes. These separations are usually conducted in the capillary zone (CZE) or micellar (MEKC) mode, using a chiral selector in the background electrolyte (BGE) to discriminate between the two enantiomers (*i.e.* the direct method).

Capillary electrophoresis provides a number of advantages in chiral separations, namely:

(*a*) A high separation efficiency that enables resolution even in those chases where the enantiomers of the selector and selectand interact virtually identically. Such a subtle difference could probably never be detected by any other technique.

(*b*) The ability to readily alter the nature and concentration of the chiral selector.

(*c*) That of using several selectors in combination to improve chiral resolution.

(*d*) Low sample and reagent consumption, which allows the use of expensive selectors.

These advantages make CE a firm candidate for a variety of separations (particularly those involving compound families). A number of selectors have been reported, a variety of racemic drugs enantioresolved and the theoretical foundation of the technique examined ever since the earliest use of Chiral CE was developed. This has led some authors to claim that Chiral CE has passed its state of maturation and is currently being intensively implemented in practical fields. However, the key to successful CE-based enantioresolution (*viz.* the selection of a suitable chiral selector) continues to rely on trial and error.

The purpose of this work was to critically review available experience in Chiral CE with a view to developing criteria for helping the user elect the most suitable selector for the enantioseparation of compounds not previously reported. By examining the specific selectands resolved by each selector, we identified the (sub)structures most likely to be enantioresolved by a given selector. While not absolute in nature, these criteria can help sort the wide range of chiral selectors currently available and reduce the number of preliminary tests required, thereby saving on time and money. The discussion is illustrated with selected examples of the separation modes most frequently used for this purpose, namely: capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). This paper does not include the latest innovations in chiral separations, which are reviewed periodically, or alternative, less commonplace separation modes such as non-aqueous CE or capillary electrochromatography (CEC).

2. General considerations

To be effective, a chiral selector must meet several requirements, namely:

(*a*) It should be stereoselective and form a transient diasteromeric complex with each enantiomer;

(*b*) it should be soluble and chemically stable in the BGE;

(*c*) it should not interfere with the detection; and

(*d*) it should exhibit a fast complexation kinetics.

Some substances such as cyclodextrins (CDs), chiral crown ethers, macrocyclic antibiotics, chiral surfactants, ligand-exchange complexes and linear polysaccharides fulfill many of the previous requirements. Figure 1 shows the relative use of these types of selectors over the period 1997 2002.

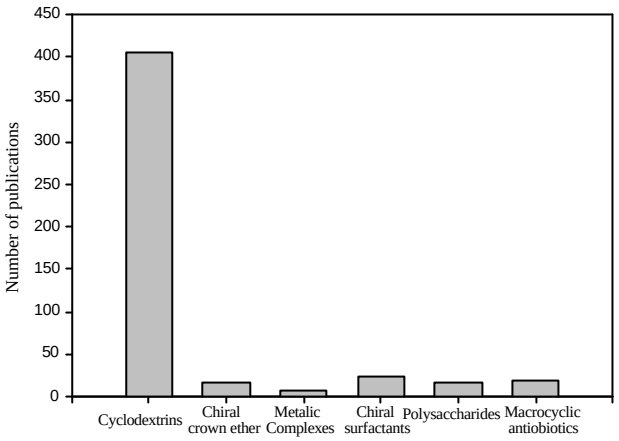


Figure 1. Frequency of use of various types of chiral selectors from 1997 to 2002. Source: CAPLUS Database.

As can be seen, CDs accounted for roughly 80% of all applications. This increase from 67% in 1997 reveals a growing trend in the use of this type of selector, promoted by the commercial availability of new cyclodextrins (particularly charged CDs). Notwithstanding their high stereoselectivity, other, classical chiral selectors initially employed in HPLC and subsequently adopted in CE such as proteins, ligand-exchange complexes and macrocyclic antibiotics are scarcely used at present, mainly as a result of their low chemical stability, high absorptivity which interferes with UV detection of analytes or tendency to be adsorbed on capillary walls. Chiral surfactants have grown in use by effect of the increasing interest in synthetic chiral surfactants. These synthetic selectors have opened up new avenues for the design of selectand-tailored chiral selectors as they allow the *R* and *S* forms of the surfactant to be synthesized and hence the enantiomer migration sequence to be elected. In summary, the current picture is rather complex; however, a relatively small number of selectors allows the effective enantioresolution of a large number of compounds by capillary electrophoresis. Below is discussed the enantioresolution potential of the most common types of chiral selectors. Based on this discussion, criteria for electing the most suitable chiral selector for each purpose summarized in Table 3 have been compiled.

3. Cyclodextrins

Naturally occurring á-, â- and ã-cyclodextrins are cyclic oligosaccharides consisting of six, seven and eight glucopyranose units, respectively. They have a shape of a truncated cone with a relatively hydrophobic open cavity and a hydrophilic outside by effect of the presence of hydroxyl groups (positions 2, 3 and 6 on the glucopyranose ring). The most widely accepted mechanism for enantioresolution with a CD involves the inclusion of the chiral analyte in its cavity (to form a host guest or inclusion complex) and the establishment of secondary interactions with the hydroxyl groups on the CD rim. These interactions differ between enantiomers and are responsible for the differences in the inclusion constants (K_1 and K_2). Resolution between two enantiomers can be expressed mathematically as:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{(K_1 - K_2)[CD]}{(1+K_1[CD])(1+K_2[CD])}\right)\left(\frac{m^f - m^c}{m_{avg} + m_{eof}}\right)$$

where N is the number of theoretical plates; μ^f and μ^c are the mobilities of the enantiomers in free and fully complexed form, respectively; μ_{avg} is the average mobility of the two enantiomers; and μ_{eof} is the mobility of the electroosmotic flow (EOF). The analyte will only be included if it fits into the CD cavity; inclusion reflects in a marked increase in the migration time for the analyte with respect to the same BGE containing no CD. Although it does not ensure enantioresolution, it is a necessary condition, so the size and shape of the selector and selectand should be carefully controlled. Natural cyclodextrins can include a variety of compounds (particularly if they contain some aromatic ring).

Analytes with one aromatic ring bearing a single or no substituent can form inclusion complexes with á-CD that with the smallest cavity. This group of analytes includes structures with several, unfused rings at least one of which must be aromatic. Aminoglutethimide [8], hexobarbital [9] and several other substances [10] possess a free, scarcely functionalized ring and have been successfully enantioresolved with á-CD. â-CD is the natural cyclodextrin with the highest enantioresolution power; in fact, analytes with a doubly substituted aromatic ring, two fused aromatic rings or other six-membered rings seemingly fit into its cavity. Consequently, they are the best candidates for enantioresolution with this cyclodextrin. Typical examples of compounds with one or two doubly substituted rings that have been resolved with â-CD include various arylpropionic acids [11] and catecholaminesv [12]. Analytes with six-membered heterocycles such as hexobarbital [13] have also been

successfully enantioresolved with â-CD. Structures with more than two fused rings or containing 5-, 7- or 8-membered heterocycles can also be enantioresolved in this way provided they possess some aromatic ring.

ã-Cyclodextrin is seemingly the most suitable CD for resolving analytes with three (*e.g.* promethazine, oxomemazine, isothipendyl) or four fused rings (*e.g.* isolysergic acid), or even a single, extensively substituted ring (*e.g.* secobarbital) [7]. Substituted aromatic rings exhibit steric hindrance that restricts inclusion into the â-CD cavity and can be better enantioresolved with ã-CD.

However, the extent of chiral discrimination exhibited by naturally occurring CDs is quite modest and only 15% of the compounds listed in the review by Gübitz[9] can be resolved with these CDs. According to Easton [14], such a low enantioselectivity can be ascribed to the inherent symmetry of natural CDs. Increased chiral discrimination can thus be expected from the use of modified CDs with an increased degree of asymmetry. The hydroxyl groups on the rim of natural cyclodextrins can be modified to obtain CDs in variable degrees of substitution. Modifications can be of two types. Thus, hydroxyl substituents can be replaced at random to obtain a complex mixture of isomers where the overall effect is that of a symmetric distribution. Therefore, this type of substitution does not alter the symmetry or enantioselectivity of the cyclodextrin. The other type of modification has arisen from a new trend to producing selectively substituted derivatives. This may induce changes in the asymmetry of the CD and increase enantioselectivity as a result. This modification of neutral CDs can also lead to neutral or charged CDs.

The neutral modified CD derivatives are obtained by replacing hydroxyl groups with alkyl groups, which increase the solubility and flexibility of the cyclodextrins. These two factors help accommodate the guest and increase the stability of the resulting complex [14]. Overall, neutral modified CDs exhibit improved inclusion and enantiodiscrimination capabilities. This has reflected in extensive use, particularly of methyl derivatives such as *heptakis*-(2,3,6-tri-*O*-methyl)-â-CD (TM-â-CD) and *heptakis*-(2,6-di-*O*-methyl)-â-CD (DM-â-CD). These are two single-isomer derivatives which can enantioresolve 26% of the compounds listed in the review by Gübitz [7]. The reason for such extensive use is not only their ready availability from chemical distribution companies but also their frequently complementary enantioselectivity with respect to â-CD. For example, DM-â-CD is the most effective resolving agent for some sympathomimetic drugs such as doxylamine and isoproterenol, which are poorly if at all resolved by â-CD [15]. Similarly, â-CD is ineffective in the enantioseparation of chiral arylpropionic acids, where TM-â-CD exhibits excellent chiral recognition capabilities [16] (see Fig. 2).

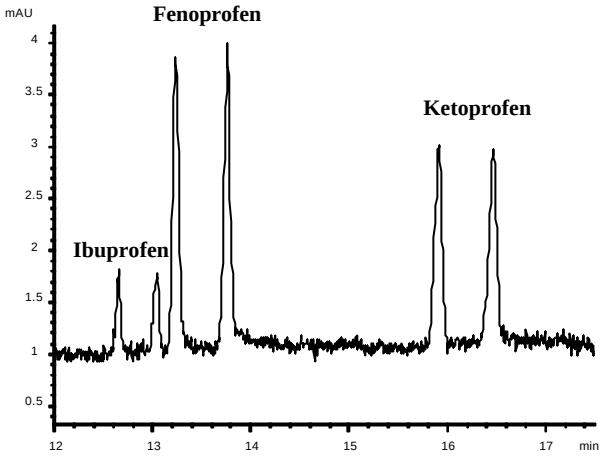
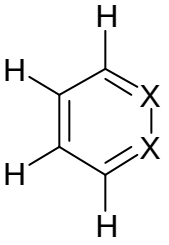


Figure 2. Simultaneous enantioresolution of various profen drugs with 25 mM TM-â-CD, using a 20 mM TEA/phosphoric acid buffer of pH 5, 20 kV and 35 ºC. Taken from reference 16.

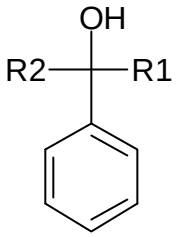
Koppenhoefer [17], studied the influence of the structure of the analyte on its enantioresolution with â-CD, and TM-â-CD and defined a *migration retardation factor* (R_m) as the ratio of the migration time for the analyte in the presence of CD in the BGE to that in its absence. This factor is a measure of analyte inclusion into the CD; on the other hand, the enantiodiscrimination capacity is measured by the separation factor, á. TM-â-CD was found to enantioresolve 55% of the analytes tested, as compared to only 22% with â-CD despite its increased inclusion capacity.

Analytes bearing substructure 4H (*viz.* structure I with *X* denoting any atom or substituent) have a higher affinity for CD and a high rate of success of chiral separation. The influence of cavity size in the cyclodextrin was examined by comparing TM-á-CD, TM-â-CD and TM-ã-CD. The results revealed the superiority of TM-â-CD, even though TM-á-CD provided the largest inclusion constants which shows that R_m and á are mutually independent. The increased enantioresolving power of TM-â-CD is assumed to be related to the presence of an odd number of glucose rings, which decreases local symmetry in the cyclodextrin.



(1)

One other widely used single-isomer derivative is 2-hydroxypropyl-â-cyclodextrin (HP-â-CD). This cyclodextrin can enantioresolve analytes possessing some aromatic ring in addition to a hydroxyl group on the asymmetric carbon. In fact, 60% of the analytes listed in the review by Gübitz [7]and enantioresolved with this CD possess the following substructure:



(2)

Various hypertensives such as nadolol and propanolol, and bronchodilators such as epinephrine possess this substructure and have been successfully enantioresolved with HP-â-CD [7]. The enantioresolution of analytes with such a disparate structure as that of the hormone homatropine [18], which still contains substructure 2, confirms these results.

HP-â-CD was recently found to successfully enantioresolve lactic acid [19], an á-hydroxyacid having no aromatic ring. This suggests that non-bulky structures containing no hydrophobic moiety can also be enantioresolved with CDs, via a mechanism that involves not inclusion but the attachment of the analyte to the outside of the cavity. Holzgrabe [20] have confirmed this mechanism from experimental NMR spectroscopic measurements.

HP-â-CD can also enantioresolve other analytes not possessing the substructure 2 such as dimethindene [21] and various other compounds [22]. These compounds have been enantioseparated with no other neutral CD, so HP-â-CD is to some extent complementary to all other neutral CDs in its ability to resolve enantiomers. The fact that HP-â-CD can also enantioresolve analytes with several fused rings [23] such as nefopam or mianserin suggests that the presence of the hydroxypropyl chain makes this CD flexible enough to include bulky analytes.

Figure 3 illustrates some of the previous conclusions on CDs. The structure of isoproterenol is amenable to the resolution of its enantiomers with â-CD (a substituted aromatic ring) and its methyl analogues. The results testify to the enantioresolving power of modified CDs and the ability of HP-â-CD to resolve analytes possessing the substructure 2.

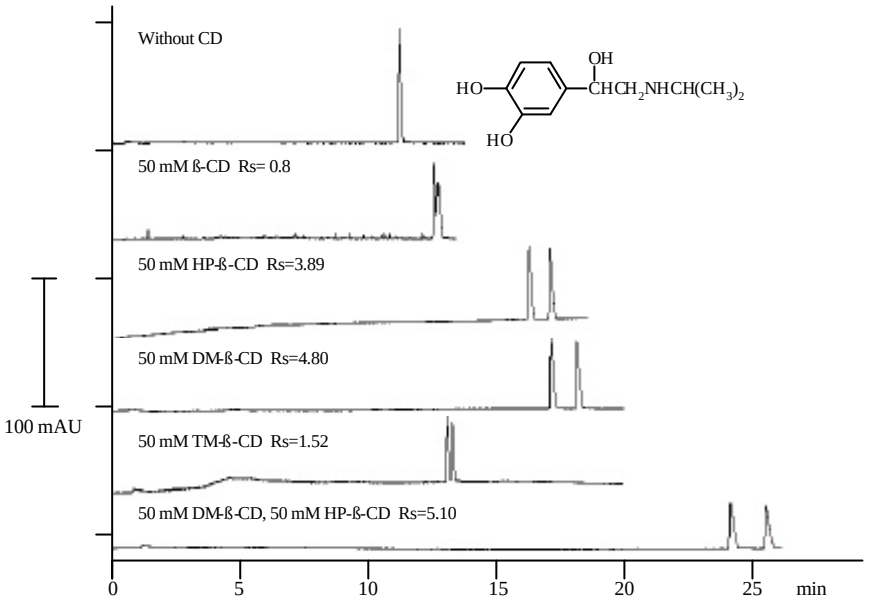


Figure 3. Enantioresolution of isoproterenol with â-CD and its methyl analogues using a 50 mM phosphoric acid/TEA buffer of pH 3 and 30 kV.

Despite their widespread use, neutral CDs can only enantioresolve charged analytes. The possibility of resolving neutral and charged analytes fostered the synthesis of charged CDs that provided high chiral resolutions. This superiority of charged CDs accounts for the slogan of the Beckman company in promoting their recently developed highly sulphated cyclodextrins as the cure for chiral headaches ; in fact, these modified CDs successfully resolved 131 out of 135 compounds tested [24].

Notwithstanding such brilliant results, charged CDs are not the definitive solution as they are subject to several shortcomings, namely:

(*a*) Not all charged CDs are commercially available.

(*b*) Their presence substantially increases the passage of current through the system.

(*c*) The CD analyte complex can migrate to the cathode or the anode, which entails performing experiments under direct and inverse polarity conditions.

(*d*) The high intrinsic mobility of the CD can result in excessive electrodispersion and hence in broad, distorted peaks.

(*e*) As a result, the robustness of the separation (in terms of migration times and resolution) is diminished.

In spite of these shortcomings, charged CDs are effective routine chiral testing agents on account of their ability to discriminate enantiomers of opposite sign. Especially prominent in terms of use are carboxymethyl-â-CD (CM-â-CD), sulphated-â-CD (S-â-CD) and sulphobutylether-â-CD (SBE-â-CD), all anionic, and 2-hydroxypropyl-trimethylammonium-â-CD (QA-â-CD), a quaternary ammonium-substituted cationic CD. The uses of these and other, less common CDs (carboxyethyl, phosphate and succinyl CDs), were recently reviewed [25].

CM-â-CD is a commercial anionic CD the carboxyl groups in which are protonated (and result in an electrically neutral CD) below pH 4 but dissociated (to form a negatively charged CD that can be used as a moving stationary phase to resolve neutral analytes) above such a pH value. This cyclodextrin has been used for the enantioseparation of a variety of drugs (all cationic). It can also resolve some acidic compounds provided the pH is below that where its carboxyl groups are dissociated [26]. The interaction of this CD with the analyte changes markedly with pH, so much so that a cationic analyte can behave formally as an anion and yet migrate to the cathode if it interacts strongly enough with the cyclodextrin. This is accompanied by a change in the enantiomer migration sequence. Figure 4 illustrates the enantioseparation of a secondary amine (fluoxetine) with CM-â-CD at a variable pH. As can be seen, fluoxetine behaves as an anion even though it is protonated and at pH 4.5 it must be detected at the anode.

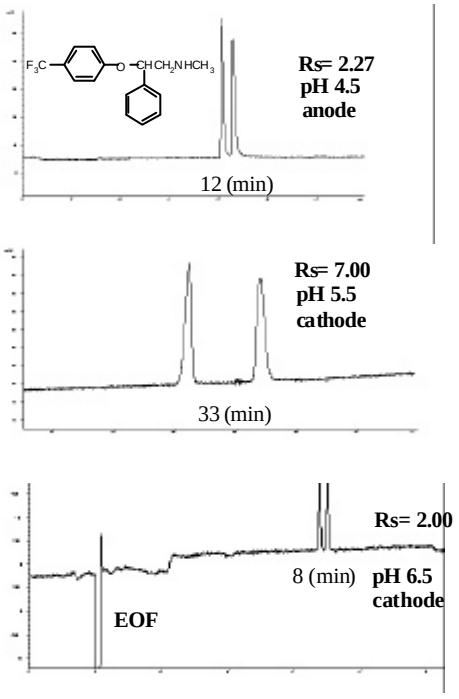


Figure 4. Enantioresolution of fluoxetine using 10 mM CM-â-CD in a 20 mM citric acid buffer and 30 kV.

In contrast to CM-â-CD, sulphated-â-CD (S-â-CD) is negatively charged throughout the pH range used in CE. Stalcup [27] used a mixture of these CDs in different degrees of substitution to enantioresolve 56 neutral and cationic compounds in an acidic BGE. Under these conditions, all analytes migrated to the anode following complexation with the CD and 40 of the 56 tested were successfully enantioresolved. The enantioresolution of the neutral analytes was thought to be governed by inclusion; thus, the analytes with no hydrophobic moiety or some such moiety but no additional functional group causing the analyte to be included in the CD cavity exhibited very low or no resolution.

Sulphobutylether-â-CD (SBE-â-CD) is an anionic CD throughout the pH range that was introduced for the enantioresolution of ephedrine and related compounds [28]. Since then, it has proved highly effective for resolving a variety of compounds [29] including illicit drugs, stimulants and drugs of forensic interest.

Cationic CDs have been used to a lesser extent than anionic CDs. Their primary application is the separation of carboxylic acids and aminoacids. 2-Hydroxypropyl-3-trimethylammonium-â-CD (QA-â-CD), which is commercially available, is the most widely used cationic cyclodextrin. Its earliest application was reported in 1996 and involved the separation of cyclodrine and cyclopentolate [30]. Since then, it has been used to resolve various types of substances including anticoagulants, organic acids and peptides [31].

Single-isomer charged and amphoteric cyclodextrins have recently been developed. Such is the case with *heptakis*-(2,3-diacetyl-6-sulphate)-â-CD (HDAS-â-CD) and *heptakis*-(2,3,-dimethyl-6-sulphate)-â-CD (DMS-â-CD), which have provided excellent results in the enantioseparation of Dopa and related compounds [32]. This CDs are expected to outperform their randomly substituted analogues in resolution and robustness. Also, various synthetic amphoteric CDs have been prepared to resolve both cationic and anionic analytes by altering the pH of the BGE with a view to obtaining as universal as possible a chiral selector [33]. Such interesting approaches are currently under evaluation, so any conclusions regarding performance are still premature.

4. Chiral crown ethers

Crown ethers are macrocyclic polyethers with a relatively polar cavity that form inclusion complexes with akali, alkaline-earth and primary ammonium cations. To date, only (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C6H_4$) has proved effective as a chiral selector for CE. Its selective ability to discriminate analytes bearing a primary amine make it highly suitable for resolving drugs possessing this function [34]. Primary amines are held inside the cavity via three hydrogen bonds in a tripod-like arrangement (see Fig. 5).

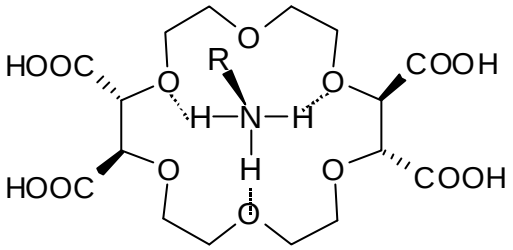


Figure 5. Host guest complex between a primary amine and 18C6H4.

However, additional chiral interactions between substituents of the crown ether and the ligand are still needed [35]. Tris and triethylamine/citric acid buffers supplemented on 18C6H4 have been successfully used to resolve primary amines. These BGEs contain no alkali or alkaline-earth ions, which exhibit a high affinity for the ether and would diminish enantioresolution. Although Tris is a primary amine, it does not compete with the analyte as it possesses a comparatively small inclusion constant [36]. The chemical structure of the enantiomer strongly influences the ability of the crown ether to enantioresolve it. Thus:

(*a*) A primary amine functionality of the analyte is essential (second and tertiary amines cannot provide the type of complexation required for chiral recognition).

(*b*) Bulky substituents on the asymmetric carbon provide better enantioresolution (*e.g.* naphthylethylamine is very well resolved whereas phenylethylamine is not resolved at all) [37].

(*c*) The distance between the amine functional group and the stereogenic centre exerts a strong influence on the separation. As a rule, the closer to the amine function the asymmetric carbon is, the higher is the resolution.

(*d*) Structures where the asymmetric carbon is thoroughly substituted are less likely to be enantioresolved because complexation by the ether is sterically hindered.

18C6H4 has been successfully used to resolve aminotetralins and aminodecalins [38], as well as sympathomimetic drugs [39]. However, aminoacids and its derivatives constitute the most frequent targets for this chiral selector. Figure 6 illustrates the simultaneous enantioseparation of two aminoacid derivatives, Dopa (3,4-hydroxyphenyl-L-alanine) and benserazide {DL-serine-2-[(2,3,4- trihydroxyphenyl)methyl]hydrazine}, with 18C6H4.

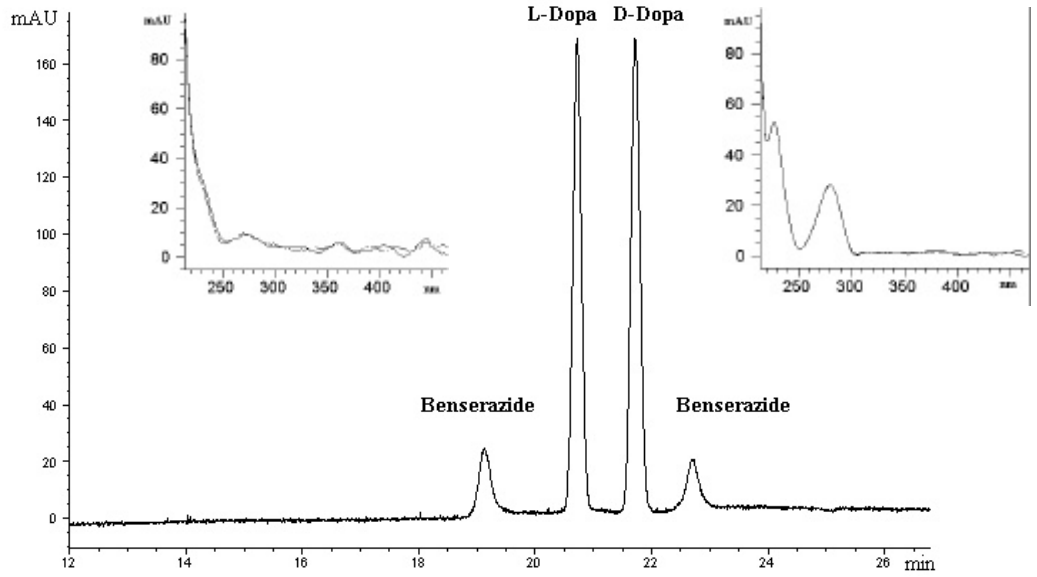
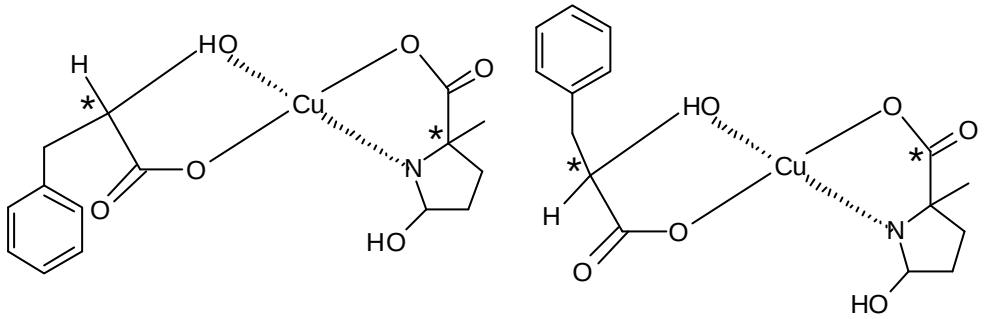


Figure 6. Chiral separation of benserazide and Dopa using 18C6H4. UV Vis spectra for benserazide (left) and Dopa (right) enantiomers. Conditions: 10 mM Tris/Citric acid at pH 2.5, 12 mM 18C6H4, 15% MeOH, 15 ºC, 30 kV.

5. Ligand-exchange complexes

Enantioresolution by ligand-exchange complexation relies on the formation of a multi-component chelate complex consisting of a central cation [basically Cu(II), but also Ni(II) or Zn(II)] and at least two chiral bifunctional ligands (usually L-aminoacids) added as selectors to the BGE (Fig. 9). The analyte replaces one ligand in the selector complex to form a ternary mixed complex.

The enantioseparation is based on the difference in stability between the complexes of the *R* and *S* analytes. These selectors can enantioresolve analytes with functional groups capable of forming the complexes (*viz.* chelate complex-forming compounds such as aminoacids, aminoalcohols and hydroxyacids).



L-3-phenyl-lactic acid L-hidroxiproline D-3-phenyl-lactic acid L-hidroxiproline

Figure 7. Possible structures for the ternary complexes formed between the enantiomers of 3-phenyl-lactic acid and L-hydroxyproline. [Reproduced from reference 54].

This principle was first used to enantioresolve some Dansyl-Aminoacids with Cu(II) histidine complex added to the BGE. In recent years, some selectors based on *N*-alkyl-aminoacids have proved better than their unmodified analogues for the enantioseparation of aminoacids, dipeptides, á-hydroxyacids and aminoalcohols [40-41]. Some short-chain organic acids (*viz.* malic, tartaric) in natural products have also been successfully resolved with Cu(II) L-tartrate and Cu(II) D-quinic acid as chiral selectors [42]. For a comprehensive review of the selectands involved and selectors used, interested readers are referred to a review by Schmid [43].

The most severe constraints on the use of these selectors are the detection problems (*e.g.* low sensitivity, rough baselines) arising from the high absorption of the BGE and the tendency of some analytes to be poorly resolved owing to their rather slow ligand-exchange kinetics. On the other hand, their greatest asset is their ready availability.

6. Chiral surfactants

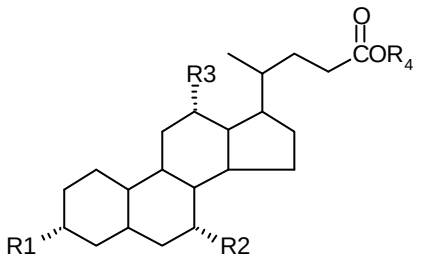
Chiral surfactants have usually been employed in MEKC for the enantiodiscrimination of cationic and neutral analytes. They can be of two different types, namely: naturally occurring detergents and synthetic chiral surfactants derived from natural sugars and aminoacids. The natural chiral surfactants used in Chiral CE can be classified into three families, *viz.* bile salts, digitonins and saponins. Bile salts are by far the most common.

Bile salts are optically active surfactants that consist of four saturated fused rings with side chains containing hydroxyl groups and a carboxyl moiety bonded to either taurine or glycine. Deoxycholic derivatives (particularly taurodeoxycholic acid, STDC) have proved more effective for enantiodiscrimination purposes; this increased efficiency as a chiral selector must thus be related to the absence of a hydroxyl group at position 2 [44]. Structures such as those of dansyl-aminoacids, binaphthyl derivatives, diltiazem and trimetoquinol have been successfully resolved with STDC [45]. All these analytes have a common rigid structure of fused rings. As a

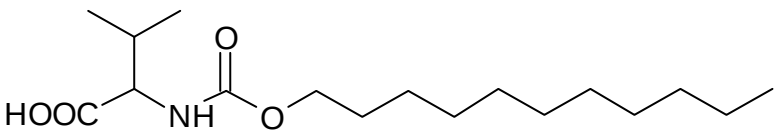
result, enantiodiscrimination with bile salts appears to be especially favourable in analytes with restricted rotation. In this respect, bile salts are complementary of CDs as this type of polycyclic structure is unlikely to be resolved with CDs owing to their decreased inclusion capacity. Digitonin and saponins such as glycyrrhizic acid and â-escin have been used in the enantioseparation of dansyl-aminoacids using mixed micelles with SDS [46].

Table 2. Structure of bile salts

Bile salt	Abbreviation	R_1	R_2	R_3	R_4
Sodium cholate	SC	OH	OH	OH	ONa
Sodium taurocholate	STC	OH	OH	OH	$NHCH_2CH_2SO_3Na$
Sodium deoxycholate	SDC	OH	H	OH	ONa
Sodium taurodeoxycholate	STDC	OH	H	OH	$NHCH_2CH_2SO_3Na$



Recently, a large variety of synthetic chiral surfactants have been reported that can be classified into the following families: *N*-alkanoyl-L-aminoacids, *N*-dodecoxycarbonyl aminoacids, alkylglucoside chiral surfactants, tartaric acid-based surfactants and steroidal glucoside surfactants. Some authors have examined the effect of systematic alterations of the surfactant structure with a view to optimizing the enantioresolution of various analytes [47]. This promising approach is still at an incipient stage and exclusively accessible to a few research groups with synthesis expertise. The *N*-alkanoyl-L-aminoacid (*R*)-*N*-dodecoxycarbonylvaline (structure 3) is one of the most effective chiral surfactants; it is available in its two forms (*R* and *S*), which allows the enantiomer migration sequence to be elected [48].



(3)

Worth special mention here is the ability of tartaric acid-base surfactants to enantioresolve polycyclic structures in a manner similar to that of bile salts [49].

Table 3. Some clues for the correct election of chiral selectors in CE

Type of selector	**Type of analyte or structure**
Naturally ocurring cyclodextrin	
α-CD	Analytes with a scarcely functionalized aromatic ring
β-CD	Analytes with a double functionalized aromatic ring (structure 4H)
γ-CD	Analytes with a three or four fused rings Analytes with a single extensively substituted ring
Neutral modified cyclodextrins	
DM-β-CD	Like β-CD but more universal in scope
TM-β-CD	Like β-CD but more universal in scope
HP-β-CD	Analytes with some aromatic ring and a hydroxyl group on chiral carbon.
Charged modified cyclodextrinss	
Anionic CDs (CM--β-CD, S-β-CD, SBE-β-CD)	Neutral or cationic analytes (particularly those having some aromatic ring)
Cationic (QA-β-CD)	Neutral or anionic analytes
Chiral crown ethers ($18C6H_4$)	Primary amines
Chiral surfactants	
Bile salts	Analytes with a rigid structure of fused rings
Ligand-Exchange complexes	Aminoacids, aminoalcohols and hidroxyacids
Macrocyclic Antibiótics	
Ansamycins	Neutral or cationic analytes (particularly those having some amino group)
Glycopeptides	Neutral and anionic analytes with an anionic moiety and an aromatic ring or a carbonyl or amide group close to stereogenic carbon
Polysaccharides	
Heparin	Analytes with a heterocyclic nitrogen ring and another ionizable nitrogen in the molecule.
Chiral derivatizing agents (indirect method)	Derivatizable analytes with no groups capable of establishing stereoselective interactions.

7. Macrocyclic antibiotics

Macrocyclic antibiotics can be classified into ansa compounds (ansamycins) and glycopeptides. Ansa compounds are selectors consisting of a chromophore bonded to a hydrocarbon chain bearing various substituents. Rifamycin A and rifamycin SV are the two most salient compounds of this type. On the other hand, glycopeptides consist of three or four fused macrocyclic rings composed of linked aminoacids and substituted phenols. Some fused rings bear various sugar or saccharide moieties.

Ansamycins and glycopeptides share some structural features including the presence of

(*a*) Multiple stereogenic centres;

(*b*) Aromatic rings, hydrophobic cavities and groups forming hydrogen bonds;

(*c*) Groups at an appropriate distance for the tripod-like interaction required for chiral recognition to be established; and

(*d*) Ionizable groups influencing chiral recognition and electrophoretic behaviour.

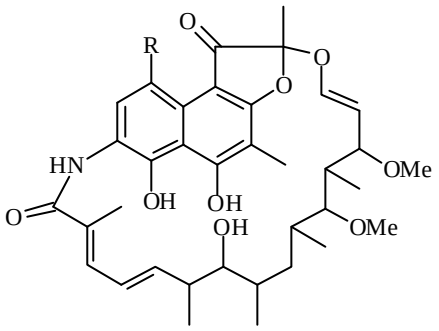


Figure 8. Basic structure of the two most common ansa compounds, *viz.* rifamycin B (R = OCH_2CO_2H) and rifamycin SV (R = OH).

Although the precise mechanism by which these chiral selectors provide enantiorecognition has not yet been unequivocally established, there is evidence that it involves inclusion into hydrophobic cavities, dipole dipole interactions, hydrogen bonding and electrostatic or ð ð interactions. These variety of potential interactions allows these selectors to enantioresolve analytes with widely different structures.

Ansa compounds possess a lower enantiodiscrimination power than glycopeptides. Rifamycin B is an acid that occurs in anionic form under standard operating CE conditions, whereas rifamycin SV is a neutral molecule.

The former has been used to enantioseparate cationic compounds and is specially adept at enantioresolving hydrophilic amine-containing compounds [50]. Some electrostatic interaction between the anionic rifamycin B and the cationic analyte is seemingly required. Both selectors absorb strongly in the UV Vis region, which facilitates direct and indirect detection provided a high enough wavelength (> 350 nm) is used to avoid absorption by most analytes. Short-chain alcohols improve their enantioresolution as they disrupt any micellar aggregates formed by these species in solution [51].

Glycopeptide antibiotics are highly flexible selectors capable of resolving about 500 different selectands. In fact, some of the highest enantioresolution values obtained in Chiral CE (R_s > 20) have been achieved with selectors of this type such as vancomycin [52]. Other glycopeptides used in this context include teicoplanin, avoparcin and ristocetin A. The last is the most universal of the four glycopeptides and avoparcin the least. These compounds exhibit interesting complementary properties, so if one of them allows only partial enantio-resolution of a given analyte, another is bound to resolve it to the baseline. Although these selectors are amphoteric, they have proved especially efficient for the separation of neutral and anionic compounds, so they are complementary to ansamycins in this respect

Chiral enantioresolution with these four glycopeptides is favoured by the presence of the following structural features in the selectand:

(*a*) Some anionic moiety (*e.g.* carboxylate, phosphate or phosphonate groups). The initial contact of the analyte may involve such a moiety and the amino groups in the glycopeptide. The situation is especially favourable if the anionic moiety is attached to the asymmetric carbon or some adjacent atom.

(*b*) A carbonyl or amide group, or an aromatic ring, near the asymmetric carbon.

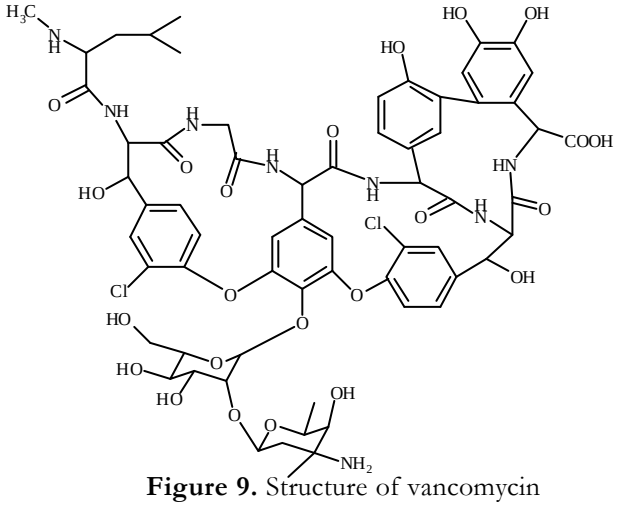


Figure 9. Structure of vancomycin

Macrocyclic antibiotics have been widely used in the past but are scarcely employed at present owing to their strong absorption in the UV Vis region, low chemical stability, ready adsorption on capillary walls and high cost. On the other hand, the aqueous solutions of glycopeptide antibiotics degrade at temperatures above 35 ºC and in both acid and basic media (4 > pH > 8). Ristocetin A is the glycopeptide antibiotic least strongly affected by these problems; however, it is extremely expensive.

8. Linear polysaccharides

Cyclodextrins are the polysaccharides most widely used as chiral selectors; there are, however, other, non-cyclic saccharides capable of enantioresolving analytes by CE. Such linear polysaccharides can be classified into the following families: chondroitin sulphates, dextrans, dextrins, aminoglycosides and heparin. Figure 10 shows the parent structure of a chondroitin, a dextran and a dextrin, as well as the structural formulae of kanamycin (an aminoglycoside) and heparin. As with macrocyclic antibiotics, the enantioseparation mechanisms of these selectors involve a variety of interactions and hence enable the enantioresolution of a widely different structures. Chondroitin sulphates are ionic polysaccharides available in three forms: A, B and C. Chondroitin sulphate A (R_1 = SO_3H and R_2 = H in Fig. 10) and C (R_1 = H and R_2 = SO_3H in Fig. 10) have proved effective in the enantioresolution of basic drugs. Chondroitin sulphate C is more effective than the other two, which has been ascribed to the presence of the sulphate group at position 6 in B and to steric hindrance in A.

Dextrans have been less often used as chiral selectors, possibly because they exhibit poorer enantioresolution capabilities than Chondroitin C or heparin, for example. Also, the fact that they are electrically neutral restricts their use to charge analytes.

Dextrans are intermediate products in the hydrolysis of starch in different degrees of polymerization (DP) and can be classified according to their dextrose equivalent (DE). Dissolved dextrin forms a helix that can include organic molecules. Maltodextrins, classified as á(1-4) linked D-glucose units with a DE < 20, are the most successful chiral selectors in this family. Several maltodextrins have been tested for the enantioseparation of drugs such as 2-arylpropionic acids and anticoagulants with excellent results [52]. The nature and chirality of the BGE were found to affect the enantioselectivity achieved with these selectors. A maltodextrin with DE = 10 was used for the resolution of sugar enantiomers. This can only be accomplished with a borate-containing BGE, which suggests that complexation of the sugars by borate ion must play some role in the chiral recognition. Soini evaluated the use of various maltodextrins with DE values of 10 20 for enantioresolving some acidic and basic

drugs [53]. They found that, for the analytes studied, positioning of the stereogenic centre next to the aromatic ring was favourable in order to achieve chiral recognition.

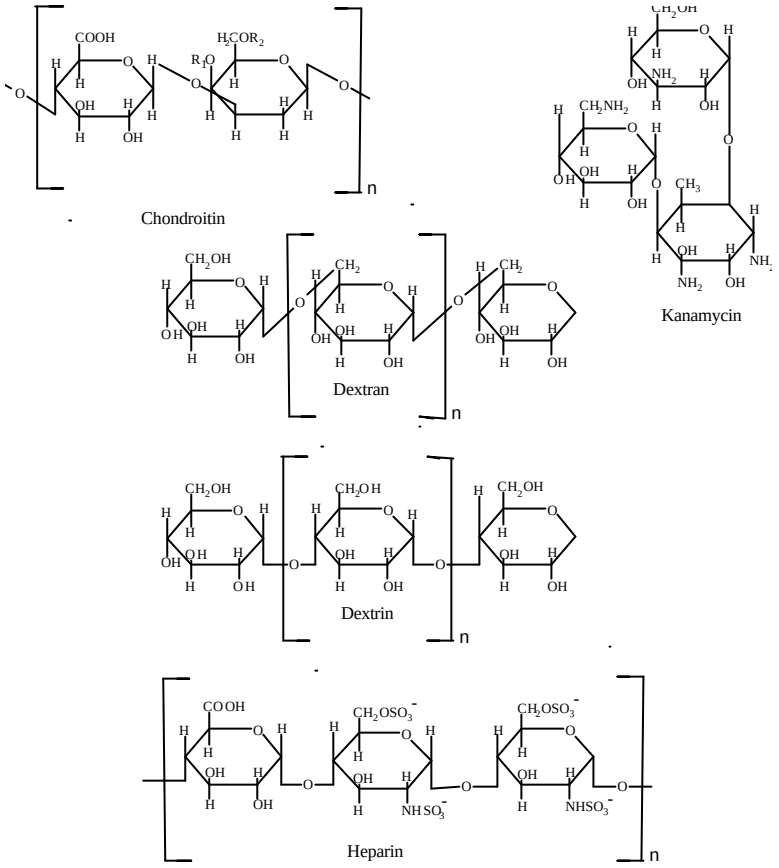


Figure 10. Structures of some polysaccharides used as chiral selectors in CE

Three aminoglycosides (*viz.* kanamycin, streptomycin sulphate and fradiomycin) have been used as chiral selectors in CE [54]. However, their reduced enantiodiscriminating power and their ready adsorption on the capillary walls have restricted their use. On the other hand, heparin has proved highly effective for the enantioseparation of widely variable analytes. This glycosaminoglycan (Fig. 10) is a heterogeneous mixture of sulphate polysaccharide chains and has successfully enantioresolved various antihistamines (doxylamine, dimethindene) and antimalarial drugs (primaquine, mefloquine) [55]. All these structures possess a heterocyclic nitrogen-containing ring and another ionizable nitrogen atom, so they are the best candidates for enantioseparation with this selector.

9. Miscellaneous approaches

This section describes alternative approaches to achieving chiral resolution. The indirect method is advisable with readily derivatized compounds containing no groups capable of establishing stereospecific interactions (*e.g.* aminoacids, short-chain organic acids and sugars). Some aminoacids have been enantioresolved using classical chiral derivatizing agents such as L-fluoro-2,4-dinitro-5-L-alanine (Marfey's reagent) [56]. Derivatization with a fluorescent agent such as ( )-[1-(9-fluorenyl)-ethyl]chloroformate [( )-FLEC]] can be used to improve detectability in the analyte [57]. The indirect method is rarely used in CE as it its more complicated and requires using a derivatizing agent of a high optical purity; also, the subsequent separation of diastereoisomers is not always easy.One other approach involves the combined use of several chiral selectors in the BGE. The combinations, which usually include a CD and some other chiral selector, have provided satisfactory results [58]. The most frequent choice is the use of two CDs. The variation of enantioresolution can be predicted from two terms in eq. (1), *viz.* the affinity term (K_1 K_2) and the mobility term (î$_i$ ì$_c$). If the two chiral selectors alter the mobility term in the same direction (*i.e.* if both accelerate or delay migration of the analyte relative to the absence of the CDs), then the same chiral recognition pattern can be used to increase enantioresolution. On the other hand, when the mobility term is of the opposite sign for the two selectors, then the opposite recognition pattern will be required [59]. Figure 6 illustrates this principle: DM-â-CD and HP-â-CD have the same effect (delay) on the analyte mobility and exhibit the same migration sequence for the enantiomers [( )-isoproterenol is the first to migrate]. The combined selector effectively improves the enantioresolution of the analyte.

Additional alternative approaches include the use of recently developed or unconventional selectors such as proteins [60], chiral calixarenes [61].The range of choices is completed with CE operational modes based on alternative separation principles (*e.g.* capillary electrochromatography, CEC) [62.

10. Conclusions

The number of chiral selectors available for CE is quite large and continue to increase. As a result, choosing the best selector for a specific purpose is a difficult task. Based on our own experience and on the literature on the topic, in this review we discuss the resolving potential of the most commonplace chiral selectors and the analytes most likely to be effectively resolved with them. The ensuing selection criteria are intended to minimize the number of preliminary experiments required to resolve enantiomers. The authors recommend using the

proposed criteria and testing the more usual selectors before resorting to a more uncommon one. Elucidating the enantiorecognition mechanism for each chiral selector would no doubt help refine the criteria.

Acknowledgements

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