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Use of cyclodextrins for separation of enantiomers

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Abstract. The review surveys data on the use of cyclodextrins as chiral selectors in high-performance liquid chromatography and capillary electrophoresis. The mechanisms of enantiomer separation and the factors influencing the selectivity and efficiency of separation in various methods of chromatography and capillary electrophoresis are considered. Numerous examples of the practical application of cyclodextrins in separation of optical isomers are given. The bibliography includes 325 references.

I. Introduction

The necessity of separating stereoisomers (in particular, enantiomers) is, to a large extent, associated with their various biological activities.[1–4] This problem arises in different fields of chemistry, biochemistry, pharmacology and agrochemical industry. The enantiomeric compositions of chiral compounds are generally determined by chromatographic methods and capillary electrophoresis (CE).

Enantiomers can be separated only with the use of systems containing a chiral selector, which should recognise the three-dimensional configurations of two isomers identical in chemical and physical properties.[5] Two modes of separation are possible. In one mode, a chiral stationary phase (SP) and an achiral mobile phase (MP) are used. By contrast, another mode employs achiral stationary and chiral mobile phases. Chromatography more often employs the former mode of separation, whereas the latter mode is more commonly used in capillary electrophoresis.

Enantioselectivity of a chiral selector is based on the difference in its interactions with a pair of enantiomers. Two types of enantioselectivity, *viz*., kinetic and thermodynamic, are considered. Kinetic enantioselectivity reflects the difference in the rate of interaction of a chiral selector with two enantiomers, whereas thermodynamic enantioselectivity reflects the difference in stability of two possible diastereomeric adducts formed in the course of interaction. The mechanisms of separation of optical isomers are explained using models of chiral recognition, which are often based on a model of a 'three-point interaction' proposed by Dalgliesh.[6,7] Interactions between a chiral selector and enantiomers are determined by a combination of various attractive and repulsive forces. Repulsive interactions occur generally due to steric hindrance and, more rarely, to dipole – dipole repulsions, whereas attractive interactions may be of diversified nature and include hydrogen bonds, Coulombic or dipole – dipole attractions, the formation of charge-transfer complexes (CTC), hydrophobic interactions (in aqueous systems), *etc*.

The formation of charge-transfer and inclusion complexes is of most importance in enantioselective sorption. The presence of π-electron systems in interacting compounds is necessary for the formation of CTC. Aromatic systems acting as both donating and withdrawing components often form stable CTC. Such π – π interactions together with Coulombic interactions and hydrogen bonds provide high activity of chiral selectors. Inclusion complexes are formed by compounds with specific structural features (the presence of a cavity), due to which their molecules can trap and hold suitable guest molecules. Rigid requirements providing the possibility of the formation of host – guest complexes assume stereoselectivity of this process, *i.e*., enantiomeric guest molecules can be completely separated with the use of chiral host molecules. Cyclodextrins and crown ethers belong to a class of chiral selectors whose action is based on the inclusion effect.

Finally, in some cases chiral recognition can be performed based only on steric compatibility. The idea of 'molecular fingerprints' consists of creating a rigid chiral cavity in a polymer network such that it is suitable only for one of two enantiomers.[2]

Of a large number of known chiral selectors, polysaccharides [8] and cyclodextrins (CD) [9–12] find extensive application in chromatography and capillary electrophoresis. Cyclodextrins are particularly versatile because their modifications allow one to prepare selectors possessing new properties. Cyclodextrins modified with polar groups have not been adequately explored as selectors in HPLC. Aminated β-CD remains virtually unstudied. The use of this chiral selector in HPLC was first described by us.[13,14]

The wide application of CD in liquid chromatography is favoured by the preparation of CD-based stable chiral sorbents by chemical modifications of silica gels. A sorbent of this type was first prepared by Fujimura *et al*.[15] by the reaction of tosylated CD with aminopropylated silica gel, but this sorbent was readily subjected to hydrolysis. Armstrong and Mond [16] used spacers containing from 2 to 20 carbon atoms and prepared cyclodextrin-modified silica gels possessing high hydrolytic stability by remov-

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ing non-selective polar adsorption centres. This gave impetus to the rapid development of chiral liquid chromatography. Recently, Crini *et al.*[17] synthesised chiral phases by treating silica gel with cyclodextrin-containing polymers. However, sorbents of this type are insufficiently selective and their further improvement is required.

Cyclodextrins find application as chiral selectors in gas [18–20] and liquid chromatography and capillary electrophoresis. The use of CD in enantiomer separation was examined in a large number of studies and surveyed in a number of reviews [10, 12, 21–35] devoted to the discussion of both the principal theoretical problems and practical application of chiral separations on CD. However, Russian publications on chiral separations are scarce (many of these studies were carried out by Davankov and co-workers and were devoted to chiral ligand-exchange chromatography [21, 36–38]). Reviews of cyclodextrin chiral selectors are lacking.

The aim of the present review is to close this gap. We consider the mechanisms of enantiomer separation and factors responsible for enantioselectivity of cyclodextrins with different structures. A large number of examples of the practical application of CD in separation of optical isomers are given. Taking into account a huge number of studies in this field, we focus our attention on the results of studies by high-performance liquid chromatography, capillary electrophoresis and electrocapillary chromatography published in 2000–2001. In the review, chiral separations on CD by gas and liquid chromatography and capillary electrophoresis are compared.

II. Cyclodextrins and their derivatives

Cyclodextrins are cyclic oligosaccharides similar in shape to crown ethers. Cyclodextrins are produced under the action of *Bacillus macerans* amylase on starch and consist of 6–12 D-glucose residues linked to each other by α-(1,4) bonds. Cyclodextrins containing 6–8 glucose residues are called α-, β- and γ-CD, respectively (Fig. 1).

The physical and chemical characteristics of CD are given in Table 1. As can be seen from this table, solubility of β-CD in water is very low compared to that of other oligosaccharides. However, its solubility increases substantially in aqueous organic solutions (methanol or ethanol, < 30%), at high pH, with the use of a supporting electrolyte and also on going to various β-CD derivatives.

The hydroxy groups of CD can be rather readily modified by chemical reactions giving rise to CD derivatives with various degrees of substitution. The chemical compositions of the products are determined by a number of factors, such as reaction conditions, properties of the reagents used, their ratio, *etc.*

Table 1. Selected chemical and physical characteristics of cyclodextrins.[10, 39]

Parameter	Cyclodextrin		
	α	β	γ
Number of glucose residues	6	7	8
Molecular mass	972	1135	1297
Inner diameter /nm	0.57	0.78	0.95
Depth of the cavity /nm	0.78	0.78	0.78
Specific rotation (25 °C) /deg	150.5	162.5	177.4
Solubility (25 °C) /g/100 ml of H_2O	14.5	1.85	23.2
pK_a	12.33	12.20	12.08

Depending on the substituents introduced into CD, either uncharged or charged modified CD are produced. Methylated, acylated, hydroxyethylated and hydroxypropylated cyclodextrins belong to the former group, whereas aminated, methylaminated, carboxymethylated, sulfated, phosphated, *etc.* cyclodextrins belong to the latter group.[10, 13, 14, 23, 39]

It should be noted that the preparation of cyclodextrin derivatives is a rather complicated process in the course of which either all hydroxy groups or only some of them are replaced. In some cases, the structures of the selectors thus formed were not studied in detail. In original publications, the names of selectors were often not detailed and only the types of substituents were reported. As a result, a large number of trivial names exist, which hinders a comparison of the results of investigations and their interpretation. In the present review, we give names of selectors which were used by authors of the cited studies.

The properties of modified CD differ from those of non-modified CD. The selectivity of enantiomer separation can be improved by increasing solubility, using the ability of CD derivatives to form secondary bonds or controlling the degree of hydrophobicity of the CD cavity.[10] For example, dimethyl-β-CD (DM-β-CD) has a higher solubility and possesses a larger interaction surface compared to β-CD. The hydroxy groups of a substituent in β-CD, which are either charged or can be charged, may be very useful for optimisation of separation by HPLC and CE.

β-Cyclodextrin bearing hydroxypropyl substituents is of interest for separation of enantiomers containing one aromatic or heterocyclic ring and a two–four-membered carbon chain with substituents, which can form hydrogen bonds or serve as proton acceptors.[23] The presence of hydroxy groups in substituents modifying CD provides additional centres for hydrogen bonding. It should be noted that acylated β-CD and β-CD containing hydroxypropyl groups act both in reversed-phase and 'polar

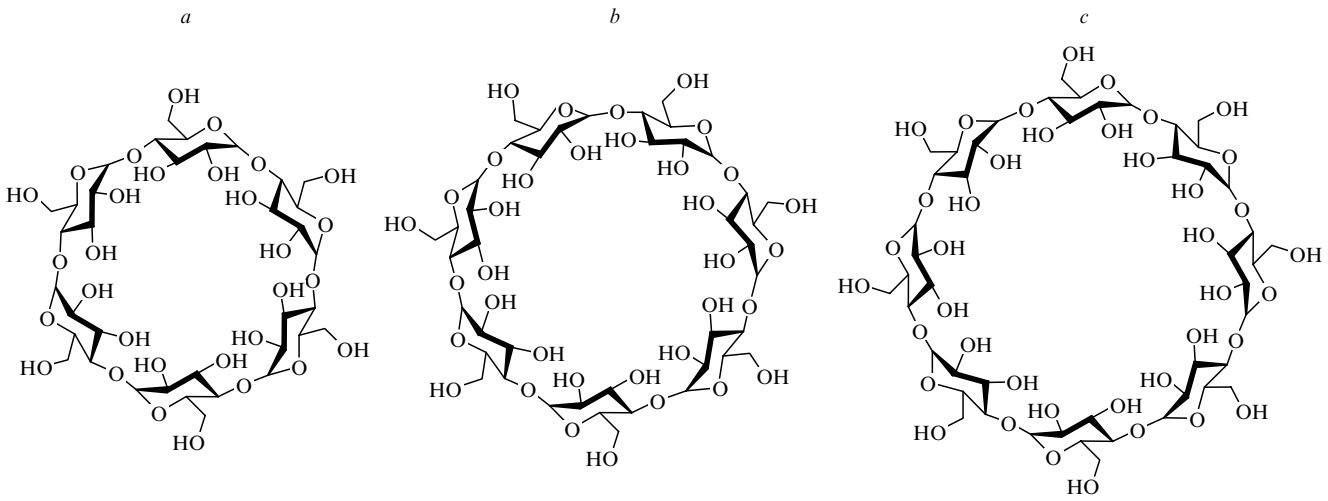


Figure 1. Molecular structures of cyclodextrins: α-cyclodextrin (*a*); β-cyclodextrin (*b*); γ-cyclodextrin (*c*).

organic' HPLC as well as CE, whereas methylated β-CD is used only in reversed-phase HPLC.

β-Cyclodextrins with bulky substituents, for example, (*S*)- and (*R*)-naphthylethylcarbamates and 3,5-dimethylphenylcarbamates, are commonly used for separation of enantiomers by HPLC.[10]

III. Cyclodextrins as chiral selectors in high-performance liquid chromatography

Enantiomer separation is most often carried out by high-performance liquid chromatography. In the last decade, this method has been extensively developed. Considerable progress in this area was made due to thorough investigations of the processes of chiral recognition of enantiomers and factors influencing this process. The use of cyclodextrins in HPLC provided a deeper insight into the characteristic features of enantiomeric recognition and extended the field of its application.

1. Mechanisms of separation on cyclodextrin chiral phases

In aqueous media, cyclodextrins adopt a toroidal conformation with a hydrophobic inner surface, because all hydroxy groups are located outside the cavity of the molecule. For this reason, hydrocarbon fragments of many organic compounds readily occupy the inner cavity to form inclusion compounds with CD. Cyclodextrins can be involved in both hydrophobic and polar interactions as well as in hydrogen bonding with sorbed molecules due to which this chiral selector has a broad spectrum of action. The main prerequisite for enantioselectivity of separation on CD is a particular size of the sorbate molecule, because the formation of an inclusion complex of the sorbate with CD plays a decisive role.

Stability of an inclusion complex depends substantially on the degree of hydrophobicity and steric compatibility between the guest and host (CD) molecules. Evidently, an inclusion complex cannot be formed if the diameter of the molecule is larger than the size of the CD cavity. If the diameter of the molecule is smaller than the size of the CD cavity, the complex can be formed, but the constant of its formation is much smaller than that for a molecule, which can be rigidly fixed in the cavity. It was experimentally found that compounds bearing one aromatic ring (for example, benzene) have a size suitable for α-CD, compounds with two aromatic rings (for example, naphthalene) are suited to the separation on β-CD, and compounds such as substituted pyrenes are suitable for the separation on γ-CD. The presence of one or several polar groups in the sorbate molecule is favourable for hydrogen bonding with the hydroxy groups of CD.

The selectivity of complex formation is determined by the energy difference between inclusion complexes of CD with enantiomers. In most cases, enantiomers contain an aromatic ring (often more than one ring). It was found[40,41] that separation depends on the position of the chiral centre and its environment. The enantiomeric resolution is higher if a chiral centre is involved in the ring and is bound to two or three sp^2-hybridised carbon atoms. Complexes with a chiral centre enclosed in a sandwich consisting of two π systems, one of which is an aromatic ring and the other one is either a carbonyl group or the second aromatic ring, are best suited to the enantiomer separation. Successful separation is associated with the high rigidity of the molecule, the chiral centre serving as a point about which fragments of the molecule can rotate.

Most of the published experimental data demonstrate that the size of the β-CD cavity is optimum for the separation of a broad spectrum of optically active compounds, including those used in the pharmaceutical industry.[23] It should be emphasised that the sorbate molecule does not need to be completely located inside the CD cavity. Numerous examples of separation on β-CD of enantiomers containing three or four aromatic rings have been given.[10] It appeared that it is quite sufficient if the sorbate molecule is only partially located in the cavity and forms an inclusion complex.

The overwhelming majority of enantiomer separations were carried out with the use of β-CD and its various derivatives.[10] To reveal the reasons for this advantage in separation of various compounds, let us consider the structure of the CD molecule (Fig. 2). Hydrophobic molecules analogous to benzene can enter into and leave the CD cavity and are reversibly sorbed on this surface. However, in the absence of hydrophobic sorbates, any other particles, including ions, can penetrate into the cavity. In addition, sorbate molecules can form hydrogen bonds with the hydroxy groups of the CD surface and/or interact with substituents located outside the cavity (in modified CD) and the residual silanol groups of silica gel.

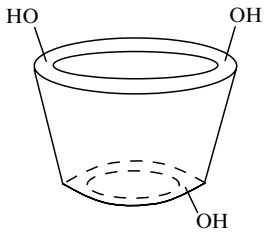


Figure 2. Shape of the β-cyclodextrin molecule in aqueous solutions.[23]

To extend the field of application and enhance the selectivity of chiral stationary CD phases, substituted CD with various functional groups are used.[10,23] The presence of such groups provides additional interactions between the sorbate and the surface, which allows one to extend substantially the spectrum of racemates separated. Extensive investigations in this area are being continued. We proposed a new chiral selector, *viz*., aminated β-CD. Silica gel modified with aminated β-CD serves as an efficient sorbent for separation of amino acid derivatives and its application made it possible to extend substantially the spectrum of amino acid derivatives which can be separated. For example, separations of *N*-*tert*-butoxycarbonyl derivatives of amino acids were carried out. Earlier, the latter derivatives had been separated only with the use of hydroxypropyl derivatives of β-cyclodextrin.[13,14]

In the case of modified β-CD with hydrophobic substituents, for example, with (*S*)-naphthylethylcarbamate or 3,5-dimethylphenylcarbamate, interactions on the surface occur through another mechanism. Depending on the type of the mobile phase, the following interactions between the sorbate and the surface prevail on these chiral stationary phases: the formation of π complexes in a normal phase, hydrogen bonding in a polar organic phase, and the formation of inclusion complexes in a reversed phase.[10]

The above-described chiral selectors are multimodal, because they are successfully used for separation of a large amount of various enantiomers and interact with the latter according to different mechanisms. The choice of a mobile phase is governed by the structure of the compound analysed, its stability and solubility in the mobile phase. The normal-phase method is most preferable, the 'polar organic' method ranks next, and the reversed-phase method occupies the last place because of its low resolution, relatively short lifetime of such chromatographic columns and small capacity factors.

Cyclodextrins find wide application as chiral additives to a mobile phase in enantiomer separations. In this case, recognition of enantiomers occurs due to the distribution of the sorbate between the stationary phase and the inclusion complex in the mobile phase. The resolution and selectivity in these systems depend on the type and concentration of the CD, the nature of the mobile and stationary phases and temperature. In some cases,

CD can be sorbed on the surface of a mobile phase and form a pseudostationary phase.[42]

2. Separation of enantiomers in reversed-phase high-performance liquid chromatography

Most separations on CD-based chiral stationary phases are carried out by reversed-phase liquid chromatography, because inclusion complexes can be formed only in aqueous or aqueous – organic solutions. The stability constants of complexes are generally larger in water and decrease as the fraction of an organic solvent increases. It is known that the addition of various organic solvents to an eluent can change the selectivity of enantiomer separation. This effect is observed also for CD-based stationary phases.[10]

a. Choice of a mobile phase

Methanol and acetonitrile are used as organic additives to mobile phases. Ethanol, propanol, dioxane, dimethylformamide and tetrahydrofuran are used much more rarely. Water has the smallest eluting ability. For alcohols, the polarity increases in the series methanol < ethanol < propanol. Tetrahydrofuran and acetonitrile are similar in polarity to propanol. Solvents which are less polar than water and whose molecular sizes correspond to the sizes of the CD cavities are prone to be retained on stationary CD phases. Therefore, the capacity factors of sorbates depend on how rapidly the solvent molecules displace the compound to be determined from the CD cavity. For example, it is necessary to use a larger percentage of methanol than that of propanol to obtain comparable retention times in both cases.

Different ways of optimising the concentration of an organic solvent in a mobile phase are available. There are three possible types of chromatographic behaviour of compounds on the surface of the stationary CD phase. The corresponding dependences of the retention on the concentration of an organic solvent in a mobile phase are shown in Fig. 3.[43] The most general dependence is represented by curve *1*, which shows that the retention decreases as the concentration of an organic component in the mobile phase increases. The curve reaches a minimum, *i.e.*, the 'dead time', and the further addition of the organic solvent has no effect on retention. This chromatographic behaviour is typical of compounds soluble in organic solvents (phenols, anilines and other aromatic and heterocyclic compounds).

Curve *2* illustrates the unique properties of stationary CD phases, which enable one to extend substantially the range of the compounds separated due to the application of 'polar organic' liquid chromatography. A decrease in the buffer/organic solvent ratio in the mobile phase leads to a decrease in retention (and resolution). The retention minimum is achieved in the range of 50% – 80% of an organic solvent in the mobile phase.[43] A further increase in the percentage of an organic solvent increases retention. Therefore, it can be assumed that the mechanism of recognition on stationary CD phases in elution of sorbates with mobile phases with a dominant concentration of an organic solvent differs substantially from that taking place in reversed-phase chromatography.

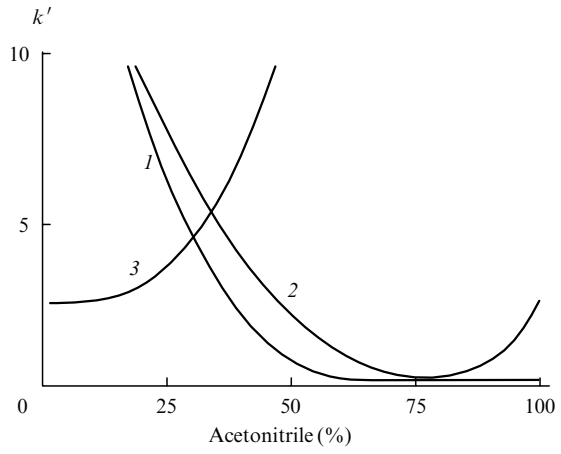


Figure 3. Influence of the composition of a mobile phase on the retention of various compounds.[43]
The description of curves (*1* – *3*) is given in the text.

The third type of chromatographic behaviour is described by curve *3*, which can also have a minimum. However, in this case the curve shape is not associated with the unique properties of stationary CD phases; instead, this is a consequence of insolubility or low solubility of sorbates in organic solvents. This behaviour is shown by many amino acids. Analogous dependences for amino acids were obtained also on traditional reversed-phase columns.[43]

The curve is shifted to the right or left by changing from one organic solvent to another, the character of the dependence remaining unchanged.

b. Salt effect and influence of pH of a mobile phase

In some cases, the nature of the organic solvent is of less importance than pH and the ionic strength of the mobile phase. The effect of the concentration of the salt additive on the retention and enantioselectivity was studied for 5-dimethylaminonaphthalene-1-sulfonyl derivatives of amino acids (dansyl amino acids). As can be seen from Table 2, the addition of small amounts of a salt to the mobile phase leads to substantial changes in retention (k') and peak resolution (R_s) of enantiomers, whereas it has virtually no effect on the selectivity coefficient (α). The resolution is improved due to a decrease in the chromatographic band broadening. In some cases, the column performance is increased by a factor of 4.[10]

A comparison of the experimental data obtained with the use of additives of various salts to a mobile phase shows that the presence of the salt always leads to a decrease in the capacity factor of the column and an increase in resolution (Table 3). The effect of a decrease in retention of sorbates upon the addition of a salt to the mobile phase can be compensated by varying pH and concentration of the salt.

From the published data, it is known that triethylamine acetate is recommended as an additive to a mobile phase, because this reagent allows one to vary pH using buffer solutions. The effect of pH on the retention of dansyl-L-valine is shown in Fig. 4. Curve *2* characterises the dependence of the retention on the concentration of an organic solvent in the mobile phase (a mixture of methanol and water) in the absence of the salt additive. It should be noted that curve *2* has a minimum, which is absent in curves *1* and *3*. It should also be emphasised that the behaviour of sorbates in acidic media differs from that in alkaline media (curves

Table 2. Effect of the ionic strength of a mobile phase on the separation of enantiomers of dansyl amino acids.[43]

Compound	$[Na_2HPO_4]$ /mol litre^{-1}	k'_1 (see [a])	α	R_s
Dansyl-DL-valine	0	3.9	1.16	0.4
	0.005	0.6	1.20	0.5
	0.01	0.7	1.20	0.6
	0.02	1.4	1.17	1.1
	0.03	1.2	1.18	1.1
Dansyl-DL-phenylalanine	0	10.5	1.15	0.5
	0.005	1.7	1.18	0.7
	0.01	2.0	1.19	0.9
	0.02	3.4	1.16	1.1
	0.03	3.2	1.17	1.2

Note. Methanol – water (25 : 75) as the mobile phase, the flow rate was 1 ml min^{-1}. [a] The capacity factor of the first enantiomer.

Table 3. Influence of various salt additives on the separation of enantiomers of dansyl amino acids.[43]

Additive [a]	Dansyl-DL-valine			Dansyl-DL-phenylalanine		
	k'_1 (see [b])	α	R_s	k'_1 (see [b])	α	R_s
$LiClO_4$	1.7	1.16	1.3	3.9	1.17	1.4
$NaClO_4$	1.8	1.17	1.4	3.9	1.18	1.5
$KClO_4$	2.2	1.16	1.3	4.7	1.16	1.3
CsBr	2.4	1.16	1.5	4.9	1.17	1.6
NH_4Cl	2.4	1.16	1.5	5.3	1.19	1.5
$Ca(NO_3)_2$	1.7	1.15	1.2	3.5	1.15	1.3
$Cd(NO_3)_2$	2.3	1.14	1.4	4.6	1.15	1.3

Note. Methanol – water (40 : 60) as the mobile phase, the flow rate was 1 ml min^{-1}. [a] The concentration of the salt was 0.02 mol $litre^{-1}$; [b] the capacity factor of the first enantiomer.

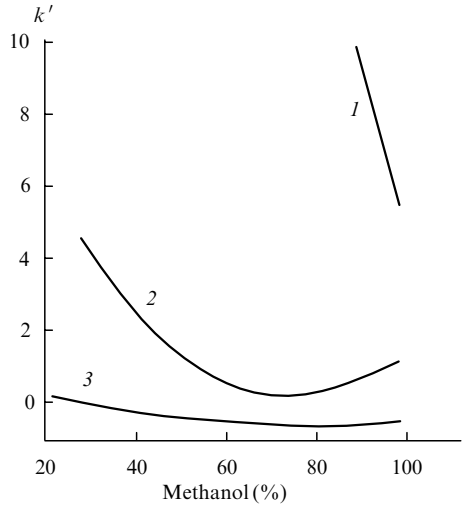


Figure 4. Influence of pH and concentration of an organic solvent on the retention of dansyl-DL-valine.
A 0.1% buffer solution of triethylamine acetate [pH 4.1, curve (*1*); pH 8.0, curve (*3*)] and pure water [curve (*2*)] were used as an aqueous component of the mobile phase.[43]

1 and *3*, respectively). High enantioselectivity of stationary CD phases in separation of compounds of an acidic or neutral nature is most often observed at pH ~ 4.0. This is associated with the ionic state of sorbates and depends on their pK_a. For instance, pH ~ 4 is optimum for most amino acid derivatives separated on β-CD, because compounds exist predominantly in the molecular form under these conditions.

c. Temperature effect

It is known that the constants of interactions between sorbates and the cyclodextrin surface depend on the temperature.[10] As can be seen from Table 4, the selectivity coefficient and resolution increase as the retention temperature decreases. However, an increase in the resolution ability is not always achieved, because at low temperatures the mass transfer rate on the surface decreases and the chromatographic band is broadened. Therefore, an increase in selectivity has no positive effect because of an increase in the peak width at low temperatures. The use of low temperatures becomes favourable if there is a possibility of narrowing the chromatographic band by changing from one organic solvent to another or introducing various additives into the mobile phase.

In the temperature range of 60 – 90 °C, most inclusion complexes decompose on stationary CD phases. This fact is exploited in elution of sorbates when their retention on the surface is very strong. It should be noted that the influence of the temperature on the retention of sorbates on stationary CD phases is more pronounced than that on traditional reversed-phase sorbents.

Table 4. Effect of the temperature on the separation of enantiomers of dansyl amino acids.[43]

Compound	T /°C	k'_1 (see [a])	α	R_s
Dansyl-DL-valine	23	2.4	1.16	1.5
	4	3.7	1.23	1.7
Dansyl-DL-phenylalanine	23	5.3	1.19	1.5
	4	8.8	1.3	1.8

Note. Methanol – 0.02 M NH_4Cl (40 : 60) as the mobile phase, the flow rate was 0.5 ml min^{-1}. [a] The capacity factor of the first enantiomer.

d. Effect of the rate of a mobile phase

The standard flow rate which is used in commercial 250 × 4.6-mm CD columns packed with a sorbent with a grain size of 5 – 10 μm varies from 0.5 to 2 ml min^{-1}. For some compounds, the chromatographic band is substantially broadened at a flow rate of 1 – 2 ml min^{-1} in the absence of a buffer or salt additives to a mobile phase. This broadening occurs due to the characteristic features of the kinetics of formation of an inclusion complex, for which the mass transfer rate on the surface of a stationary phase is low. A decrease in the rate of the mobile phase from 1 – 2 ml min^{-1} to 0.4 – 0.5 ml min^{-1} can improve the column performance by a factor of 1.5 – 3.[10]

3. Enantiomer separation at high concentrations of an organic solvent in a mobile phase ('polar organic' method)

The characteristic features of separation by chromatography with mobile phases containing from 90% to 100% of acetonitrile or methanol differ substantially from those of separation by reversed-phase chromatography. If an organic solvent is dominant in a mobile phase, no inclusion complexes are formed because the CD cavities are occupied by solvent molecules (Fig. 5). Armstrong and co-workers [22, 44] hypothesised that this is a new modification of chromatography, which was called a 'polar organic mode of liquid chromatography'.

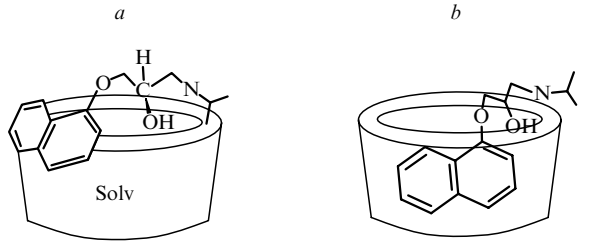


Figure 5. Scheme of the enantioselective mechanism of retention on β-cyclodextrins in the 'polar organic' mode (*a*) and normal-phase (*b*) chromatography.[10]

a. Structural characteristics of chiral recognition

In elution with mobile phases in which an organic solvent prevails, the retention and enantioselectivity of separation on CD are determined by hydrogen bonding and dipole – dipole interactions between the sorbate molecules and hydroxy groups of CD rather than by hydrophobic interactions (formation of inclusion complexes).[10, 22] In addition, steric factors can be of considerable importance in chiral recognition, which is typical of stationary phases based on CD derivatives.

Compounds separated in the 'polar organic' mode should be able to form hydrogen bonds. For instance, the replacement of

hydrogen at the secondary nitrogen atom adjacent to the chiral centre by the methyl group leads to a decrease or loss of enantioselectivity. This example suggests that the presence of the acidic hydrogen atom, *i.e.*, the proton donor, is of importance for recognition of enantiomers.[44]

To separate enantiomers on nonmodified CD, it is necessary that the sorbate molecules contain at least two groups capable of hydrogen bonding.[10] The retention and separation on cyclodextrin derivatives occur due to the formation of $\pi-\pi$ complexes or hydrogen bonding. Groups grafted to the CD surface also contribute to chiral recognition as a result of the appearance of steric interactions.

Mobile phases with a high content of an organic solvent offer advantages over the reversed-phase and normal-phase modes of liquid chromatography and allow one to extend substantially the range of racemates which can be separated. For example, separation of compounds such as β-blockers (propranolol, atenolol, timolol, *etc.*) can be carried out on β-CD only in the 'polar organic' mode.[10]

b. Influence of the mobile-phase composition

The retention and enantioselectivity of sorbates in the 'polar organic' mode of chromatography depend on the nature and concentration of an organic solvent. Acetonitrile is most commonly used in enantiomer separations.

Since molecules of acetonitrile serving as the major component of a mobile phase, unlike sorbate molecules, do not form hydrogen bonds with cyclodextrin, the retention of the compounds analysed may be very high. The presence of small additives of methanol, which can form hydrogen bonds with cyclodextrin and sorbate molecules, in a mobile phase leads to a decrease in retention to a reasonable time. However, a high excess of methanol can cause a loss of enantioselectivity. To improve recognition and efficiency of separation, small amounts of triethylamine and acetic acid ($\leqslant 1\%$) are recommended to be added to a mobile phase.[10,44] The ratio between these additives in a mobile phase is determined primarily by selectivity of separation and depends on the nature of the sorbates.

One of the advantages of the 'polar organic' mode of chromatography is that it allows one to separate a series of water-insoluble organic salts. This problem cannot be solved by normal-phase chromatography in which sorbates should be electroneutral.[23]

4. Enantiomer separation by normal-phase high-performance liquid chromatography

Cyclodextrin-based stationary phases are used for separation of some compounds by normal-phase chromatography.[10,23] Hexane–isopropanol mixtures in different ratios are used as mobile phases. In this case, components of a nonpolar mobile phase tend to occupy cyclodextrin cavities and, hence, the retention and separation occur through hydrogen bonding between the sorbate and hydroxy groups of cyclodextrin and also due to $\pi-\pi$ interactions. Satisfactory results were obtained in separations of various structural isomers (substituted benzoic acids, phenols, anilines, *etc.*) by normal-phase chromatography.[23]

Studies of enantiomer separations on CD-based stationary phases by normal-phase liquid chromatography are few in number. All these studies were devoted to β-CD derivatives. In most cases, their enantioselectivity is higher than that of traditional chiral polysaccharide phases for normal-phase chromatography.

5. Practical application of enantiomer separation

Molecules of most compounds which are of interest from the viewpoint of pharmacology and biochemistry contain one or several asymmetric centres. Since optical isomers differ generally in pharmacological and biological activities, the enantiomer separation has attracted growing interest. High-performance liquid chromatography has gained wide acceptance, because it provides high efficiency and selectivity of enantiomer separation, analyses being relatively inexpensive and time-saving.

The differences in the physiological activity of enantiomers of β-adrenoblockers (so-called β-blockers) gave impetus to investigations of chromatographic separation of enantiomers of such compounds. It is known that (*S*) enantiomers of such compounds are often 50–100 times more efficient than (*R*) enantiomers. The latter can even be toxic. These compounds are too polar to be separated by gas chromatography without preliminary derivatisation. Therefore, HPLC is the method of choice. Data on the use of β-CD and its derivatives for the determination and separation of enantiomers of β-blockers have been published in the literature. The recognition ability of the following cyclodextrins was examined: β-CD,[9,45] perphenylcarbamate (PPC) β-CD,[46] carboxymethyl-β-CD (CM-β-CD),[47] heptakis(2,3-di-*O*-methyl)-β-CD,[9] phenylcarbamate (PC) β-CD[48] and mono-6-azido-6-deoxy-PPC-β-CD.[49] In addition, the determination of β-blockers is of great interest in studies of the composition of human blood and urine. For example, propranolol enantiomers were quantified in human urine and plasma; the sensitivity of analysis was 1.5 ng ml^{-1} for each enantiomer. It was experimentally found that the concentration of the (*S*) enantiomer is always higher than that of the (*R*) enantiomer.[50]

Enantiomer separations of hydantoin and its derivatives exhibiting anticonvulsive activity were carried out with the use of chiral additives of β-CD;[51,52] β-CD, charged β-CD and CM-β-CD;[53] α-CD, β-CD and their permethylated (PM) derivatives;[54] and chiral stationary phases based on β-CD and trimethyl-β-CD (TM-β-CD).[55] Charged β-CD was also used as a stationary phase for separation of antiinflammatory drugs.[56]

The chromatographic behaviour of the following compounds was investigated: antidepressants and their metabolites on acylated β-CD;[57] drugs used in cancer treatment[58] and anticonvulsive drugs and their metabolites on β-CD;[59] antiepileptics and their metabolites,[60] drugs used for treatment of allergic rhinitis[61] and imidazole derivatives[62,63] with the use of an additive of β-CD to a mobile phase and imidazole derivatives on β-CD;[64] the local anaesthetic procalin[65] and the antiinflammatory drug naproxen[66,67] using various β-CD derivatives both as chiral additives to a mobile phase and as a stationary phase; profens on β-CD.[68] A procedure was developed for the detection of the antianxiety agent lorazepam in human plasma on a β-CD derivative.[69]

Pentostatin was detected and separated with the use of a mobile phase in the presence of an additive of sulfated β-CD;[70] methylsalsolinol and its derivatives were detected and separated with the use of an additive of β-CD to a mobile phase. The biological and toxic properties of these compounds were studied.[71] The behaviour of substituted decalins on various chiral stationary CD phases was investigated.[72] The detection of the following pharmaceuticals and biologically active compounds was examined in a series of studies: methadone and its metabolites in main human biological liquids on β-CD and its derivatives,[73] 7-desmethylormeloxifene (metabolite of levormeloxifene)[74] with an additive of sulfated β-CD to a mobile phase, pirlindole on β-CD,[75] tropicamide on 2-hydroxypropyl-β-CD (HP-β-CD),[76] doxazosin on CM-β-CD,[77] trimipramine with the use of an additive of β-CD to a mobile phase;[78] carbisocaine on β-CD,[79] simendan and levosimendan on β-CD,[80] salsolinol and methylsalsolinol,[81,82] terfenadine,[83] thalomide[84] using an additive of β-CD to a mobile phase; zopiclone and its metabolites on β-CD,[85] *etc.*[9,47]

Cyclodextrins as chiral additives to a mobile phase (α-CD,[86] β-CD[87] and α-CD together with β-CD[88]) are used also for separation of terpenes [(±)-α-pinene, (±)-β-pinene, (±)-camphene and (±)-limonene]. The enantiomeric purities of these hydrocarbons were, generally, determined by polarimetry, because separation of these nonpolar compounds devoid of functional groups which can undergo derivatisation or interact with a selector presents difficulties. Consequently, a chromatographic method for the enantioselective separation of racemates of satu-

rated hydrocarbons should be based on the formation of inclusion complexes, because in this case steric effects play a decisive role. That is the reason why enantiomer separations are carried out with the use of cyclodextrins.

The separation of amino acid derivatives on chiral cyclodextrin stationary phases was extensively studied in the early 1990s. Amino acid derivatives containing the following modifying substituents were investigated in most detail: 4-dimethylaminoazobenzene-4′-sulfonyl (dabsyl), dansyl, 9-fluorenylmethoxycarbonyl (FMOC), 2,4-dinitrophenyl (DNP), 3,5-dinitropyridyl (DNPY), phthalyl, benzyloxycarbonyl (Z), benzoyl, *tert*-butoxycarbonyl (BOC) and derivatives of *o*-phthalic anhydride (OPA).[10,23] The mechanism of retention and separation of enantiomers was studied for the following compounds: dansyl amino acids on PM-β-CD,[89] dansyl- and 3,5-dinitrobenzoylamino acids on methyl-β-CD (M-β-CD),[90] DNP-amino acids on 3-*O*-methyl-β-CD,[91] dansyl and dabsyl amino acids on β-CD,[92] FMOC amino acids and selected peptides on β-CD and γ-CD,[93] FMOC and dansyl derivatives of DL-serine on γ-CD,[94] dansyl amino acids on β-CD, heptakis(2,3-di-*O*-methyl)-β-CD[9] and γ-CD, which was modified with azobenzene derivatives,[95] 3,5-dinitrobenzoyl and dansyl amino acids on naphthylethylcarbamate (NEC) β-CD,[96] 1-fluoro-2,4-dinitrophenyl-5-L-alanineamido derivatives of amino acids on β-CD,[97] Z, benzoyl, dansyl, DNP, DNPY, OPA, BOC, FMOC and phthalyl derivatives of amino acids on heptakis(6-amino-6-deoxy)-β-CD.[13] The chiral ability of this aminated β-CD was compared with that of nonmodified β-CD [14] for various derivatives of amino acids.

Interesting experimental chromatographic data were obtained in the separation of dansyl derivatives of amino acids on a chiral stationary phase based on β-CD modified with L-tyrosine.[98] Separations of nonmodified amino acids, *viz*., enantiomers of DL-selenomethionine [99] and various amino acids from tobacco,[100] were carried out on β-CD.

As can be seen from the above-considered data, most separations were carried out on CD as chiral stationary phases. However, in some cases chiral additives of CD to a mobile phase were used. As an example we refer to the separation of naphthalenesulfo derivatives of amino acids.[101]

Enantiomer separations of the following compounds were carried out: bicyclic 1,3-amino alcohols on NEC-β-CD,[102] analogues of crown ethers on β-CD and heptakis(2,3-di-*O*-methyl)-β-CD,[9] polychlorobiphenyls and certain pesticides on heptakis(tri-*O*-methyl)-β-CD (HTM-β-CD),[103] enantiomers of warfarin on β-CD,[104] phenoxypropionates on permethylated β-CD,[105] a number of carboranes on β-CD and acylated β-CD,[106] compounds analogous to isoflavone on β-CD,[107] fluoxetine and norfluoxetine with the use of CD both as chiral additives to a mobile phase and as a stationary phase.[108,109]

A number of studies were devoted to the quantification and separation of biologically active compounds, in particular, of amino alcohols belonging to one of the most important classes of chiral compounds. These compounds play an important role in metabolic processes in organisms and find extensive application in medicine and pharmacology. The optical purity of adrenaline was determined using 2,6-DM-β-CD as the stationary phase.[110] Adrenaline and its analogues were quantified in biological liquids using additives of TM-β-CD to a mobile phase.[111] Quantification of adrenaline and noradrenaline was carried out on β-CD.[112] Ephedrine and methylamphetamine were quantified on β-CD.[113] Methylenedioxyamphetamines were detected on β-CD and HP-β-CD,[114] and methamphetamine was quantified on phenyl-β-CD.[115]

IV. Enantiomer separation by capillary electrophoresis

Capillary electrophoresis is a modern method which allows one to perform rapid and highly efficient separations of compounds. Since enantiomers possess identical physicochemical properties, it is necessary to change selectively their effective, *i.e*., intrinsic, electrophoretic mobilities with the aim of carrying out their successful separation by CE. This is achieved upon the formation of stereoselective complexes through hydrophobic, π–π, dipole–dipole and van der Waals interactions and hydrogen bonds. To separate enantiomers by capillary electrophoresis, a chiral selector is either added to a supporting electrolyte (SE) or chemically grafted to a capillary wall or a stationary phase. In separations with the use of cyclodextrins as chiral selectors, the compounds are either completely or partially included into the CD cavity to form inclusion complexes stabilised by secondary bonds between polar groups of the chiral selector and the compounds that are separated.

1. Selected aspects of the mechanism of chiral separations by capillary electrophoresis

The model of separation of an enantiomer pair by CE was first proposed in 1992.[116] It is assumed that chiral separation takes place if enantiomers have different affinities for a chiral selector, *i.e*., different binding constants K_1 and K_2, and if the electrophoretic mobility of the free enantiomer differs from that of the enantiomer involved in a complex. The difference in the electrophoretic mobilities is expressed by the following equation:[116]

$$\Delta\mu = \frac{[\mathrm{C}](\mu_1 - \mu_2)(K_2 - K_1)}{1 + [\mathrm{C}](K_1 + K_2) + K_1K_2[\mathrm{C}]^2}, \quad (1)$$

where μ_1 and μ_2 are the electrophoretic mobilities of the free enantiomer and the enantiomer involved in a complex, respectively (it is assumed that these parameters are identical for both enantiomers) and [C] is the concentration of the chiral selector. The optimum concentration of the chiral selector can be determined by solving the equation $d\Delta\mu/d[\mathrm{C}] = 0$. In this model, the concentration depends only on the binding constants K_1 and K_2:

$$[\mathrm{C}]_{\mathrm{opt}} = \frac{1}{\sqrt{K_1K_2}}. \quad (2)$$

From Eqn (1), it follows that the difference in electrophoretic mobilities is larger if the enantiomer–chiral selector complex migrates in a direction opposite to the direction of migration of the free enantiomer. This is confirmed by experimental results obtained with the use of charged CD, when the compounds that are separated and the chiral selector migrate in different directions.

The influence of the following two factors providing enantioselective separation of dansyl amino acids by CE was examined:[117] the difference in the binding constants of enantiomers with the chiral selector (HP-β-CD) and the enantioselective change in pK_a upon complex formation in the pH range $pK_a \pm 2$ resulting in different charges of enantiomer–chiral selector complexes and, consequently, in different effective mobilities of the complexes. Intra- and intermolecular interactions often have a strong effect on acid–base equilibria and lead to changes in pK_a of acidic and basic groups in the molecule. Enantiomers are included into the CD cavity in different fashions to form hydrogen bonds which differ in strength and direction. Enantiomers also differ in the involvement in dipole–dipole interactions, which leads to different changes in pK_a of the amino and carboxy groups for two enantiomers. An enantioselective change in pK_a upon complex formation can lead to a difference in pK_a for two enantiomers up to 0.4 (for the carboxy group of dansyl tryptophan). In the pH range $pK_a \pm 2$, a change in the concentration of a chiral selector leads to the reversal of the enantiomer migration order. This phenomenon is observed if a more stable enantiomer–chiral selector complex possesses a higher effective mobility and the mobility of the complexes is lower than that of the free enantiomers.

The pH-dependent reversal of the migration order of di- and tripeptide enantiomers with the use of β-CD and its derivatives

was investigated.[118] The possibility of this reversal depends on the structure of cyclodextrin, the amino acid composition and amino acid sequence in peptides. Phenylalanine containing the carboxy group at one end of the amino-acid chain is favourable for the reversal of the migration order. It was noted that the pH-dependent reversal of the migration order in the case of di- and tripeptides is observed at pH $\approx$ pK_a for carboxy groups.

2. Factors influencing enantiomer separation in capillary electrophoresis

The efficiency of enantiomer separation by CE is influenced by the following factors: the type and concentration of a chiral selector, pH and the composition of SE, organic and inorganic agents modifying SE and the capillary temperature. The effects of all the above-mentioned factors are briefly discussed below.

a. Type and concentration of a chiral selector

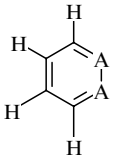
α-, β- and γ-Cyclodextrins contain different numbers of glucose residues and, consequently, their cavities differ in the inner diameter. Since the mechanism of enantiomer separation is based on the possibility of inclusion of a substrate into the CD cavity, both the sizes and shapes of the guest and host molecules are of great importance in chiral separation. These effects were examined in the studies.[119–129] The importance of the choice of the type of CD as a chiral agent was demonstrated in different CE methods (capillary gel electrophoresis, isotachophoresis, capillary zone electrophoresis).[119–121]

Various methylated β-CD as applied to the enantiomer separation of dansyl derivatives of amino acids were examined.[130] Methylation of CD at position 6 has no effect on the stereoselectivity of separation compared to the starting (native) CD, whereas the modification of position 2 renders chiral separation impossible. These data are indicative of the key role of the hydroxy groups of CD in the chiral recognition of dansyl amino acids. The importance of the type of CD in the chiral separation of several herbicides using selectively modified α-, β- and γ-CD was demonstrated in the study.[131]

The influence of the concentration of new positively charged CD, *viz*., heptakis(6-deoxy-6-methoxyethylamino)-β-CD, on separation of enantiomers of profens and herbicides (chlorophenoxypropionic acids) was studied.[132] The order of the decrease in the degree of complex formation of profen enantiomers with a chiral selector was revealed (from the difference in the migration times in the presence and absence of the selector). It was noted that the peak resolution decreases in the reverse order independently of the concentration of the CD derivative. The best resolution of enantiomer peaks was achieved for indoprofen and fenoprofen (R_s are 11 and 14, respectively). For chlorophenoxypropionic acids, the formation of more stable complexes with the chiral selector improves the peak resolution. A mixture of enantiomers of six different herbicides was successfully separated.

Various cyclodextrins were extensively investigated.[133–136] For example, racemates of 42 of 86 drugs under study were separated with the use of HP-β-CD.

The review[137] surveyed data on the study of enantiomer separation using TM-β-CD. Of 86 pharmaceuticals, 47 compounds were successfully separated. The influence of the structures of the compounds analysed and chiral selectors (TM-α-CD, TM-β-CD, TM-γ-CD) on enantiomer separation was examined. Based on abundant data, it was found that the so-called 'substructure 4H',



where A = C, N, *etc*., influences substantially the electrophoretic separation. Compounds containing this substructure not only have high affinity for cyclodextrins but also can be separated with a high probability into enantiomers using the above-mentioned chiral selectors.

Interesting results were obtained with the use of various CD[123] (α-, β-, γ-, DM-α-, DM-β-, TM-α-, TM-β-CD) for separation of enantiomers of dansyl amino acids.

The advantage of charged CD in the chiral separation was demonstrated in the study[122] devoted to separation of enantiomers of dansyl amino acids on charged mono-(6-amino-6-deoxy)-β-CD.

Sulfobutyl ether of β-CD (SBE-β-CD) is an important chiral selector, which finds wide application in CE. The advantages of this CD derivative for separation of a large number of racemates of chiral compounds were demonstrated in many investigations.[138–146] It should be noted that peaks observed in capillary electrophoresis with the use of charged CD are often smeared, particularly at low pH in analysis of basic compounds.[147] A mixture of sulfated cyclodextrins, which differ in the degree of substitution, was used[148] for separation of enantiomers of 56 drugs.

The separation of dansyl amino acids with the use of new CD derivatives, *viz*., 6-deoxy-6-*N*-histamino-β-CD and 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]-β-CD, was investigated.[149] The former chiral selector is a monosubstituted positively charged β-CD in which the histamine moiety is bound to the C(6) atom of the monosaccharide fragment of the CD ring through the amino group. In the latter chiral selector, the histamine moiety is bound to the C(6) atom through the imidazolyl group. The number of positive charges in the molecules of these chiral selectors can be varied depending on pH, because the amino and imidazolyl groups have different pK_a. The best separation of dansyl amino acids was achieved with the use of the latter chiral selector. The same CD derivatives were used in the separation of enantiomers of α-hydroxy and carboxylic acids.[150] Separation with the use of the former chiral selector was attained at a concentration of 1 mmol litre^{-1}, whereas separation with the use of the latter chiral selector did not occur, which indicates that a short distance between the positive charge and the CD cavity is of importance for chiral separation.

New CD derivatives were proposed[151–153] for separation of enantiomers of neutral and charged compounds. Heptakis(2,3-dimethyl-6-sulfo)-β-CD forms less stable complexes with the compounds analysed compared to two other derivatives, *viz*., heptakis(2,3-di-*O*-acetyl-6-sulfo)-β-CD (HDAS-β-CD) and heptakis(6-sulfo)-β-CD, and exhibits the highest stereoselectivity of these three derivatives. An increase in the concentration of the chiral selector leads to a change in the elution order of enantiomers of neutral compounds. Enantiomers of weak bases and acids were separated at pH lower and higher than 7, respectively.[151]

Cyanoethylated β-CD was proposed for separation of basic and acidic racemic compounds.[154] The advantage of this chiral selector is that its solubility in water and organic solvents is relatively high.

Mono(6-glutamylamino-6-deoxy)-β-CD is a zwitterionic CD suited to the separation of uncharged compounds.[155] The charge of the chiral selector can be varied by changing the pH of SE: the chiral selector is positively charged at pH 2.3 and negatively charged at pH 10.3–11.2.

Enantiomers of herbicides, dansyl amino acids and profens were separated on heptakis(6-hydroxyethylamino-6-deoxy)-β-CD.[156] The stereoselectivity of enantiomer separation depends on the pH of SE. Chiral separations were obtained in the pH range 4–7.

Soluble polymers, which were prepared by the reactions of β-CD or carboxymethyl-β-CD with epichlorohydrin, were successfully used in chiral CE.[157–161]

The concentration of CD in the supporting electrolyte is of primary importance in CE. Chiral separations very often do not take place, although the addition of CD causes a decrease in effective mobilities of enantiomers. One of the reasons is an

improper choice of the CD concentration. The existence of the optimum CD concentration (Eqn 2), at which the maximum enantiomer separation would be expected, was theoretically described in the study.[116] In some cases, the maximum peak resolution is not attained (R_s increases as the concentration of CD is increased), because the optimum concentration of cyclodextrin cannot be achieved due to its low solubility in water.

Based on the data published in the literature, it can be concluded that the β-CD cavity (unlike the α- and γ-CD cavities) is most suitable for the chiral separation of a large number of compounds, particularly those involved in modern drugs. Most commercially available cyclodextrin derivatives were synthesised based on β-CD. As mentioned above, modified cyclodextrins differ substantially in their properties from native β-CD (they are readily soluble, can form secondary bonds and their cavities have different hydrophobicities), which can be used for improving the selectivity of enantiomer separation. The experimental difficulties in using charged cyclodextrins are associated with the fact that most commercially available selectors are complex mixtures containing a large number of isomers. A change in the concentration of charged cyclodextrins in SE leads to substantial changes in the ionic strength. In the presence of cyclodextrins, it is difficult to determine the precise rate of electroosmotic flow (EOF).

Charged β-CD derivatives find application in separations of enantiomers of neutral and charged compounds. In the case of the latter, the use of charged chiral selectors leads to larger R_s compared to neutral selectors. As mentioned above, the migration of a chiral selector in a direction opposite to the direction of migration of the compounds analysed facilitates enantiomer separation, because it increases the difference in the electrophoretic mobilities of the free enantiomer and the enantiomer involved in a complex.[116]

b. Concentration and pH of a supporting electrolyte

The choice of an appropriate supporting electrolyte (the type and concentration of a buffer solution, ionic strength, pH) is of great importance for achieving the optimum experimental conditions for enantiomer separation. The electroosmotic flow can be controlled and the ionic state of both the compounds separated and a chiral selector can be changed by varying the pH. The influence of the pH of a supporting electrolyte on the enantioselectivity was examined in virtually all the above-mentioned studies. The ionic strength influences both EOF and migration times of the compounds separated. Electromigration dispersion can be decreased by increasing the ionic strength of the buffer, which is, however, accompanied by an increase in the current in the system. The effect of the ionic strength on separation of enantiomers of warfarin was studied;[162] *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonate containing CD as a chiral additive was used as the buffer; the ionic strength was increased by adding sodium chloride. An increase in the ionic strength of the buffer led to a decrease in the rate of EOF, an increase in the migration time and an improvement of peak resolution of warfarin enantiomers. In the study,[163] the optimum separation of isoproterenol enantiomers was achieved at relatively high SE concentrations (250 mM sodium acetate) with maximum efficiency, selectivity and peak resolution.

c. Capillary temperature

The capillary temperature is a very important parameter, which is necessary to control in chiral analyses by electromigration methods. The temperature influences the mobility of the compounds analysed, the kinetics and thermodynamics of complex formation with cyclodextrins, *etc*. The influence of the capillary temperature on the chiral separation was examined in the studies.[164–171] It was found that the migration time and the peak resolution decrease as the temperature increases. In some cases, a reverse dependence was observed.[172, 173]

d. Organic and inorganic modifying agents of supporting electrolytes

The addition of achiral compounds to SE changes the electroosmotic flow, viscosity, solubility of the compounds analysed and/or the chiral selector with a resulting change in electrophoretic mobilities giving rise to enantiomer separation. Enantiomers of *threo*-chloramphenicol and ketotifen were separated using heptakis(2,6-di-*O*-methyl)-β-CD with the addition of hydrophilic cellulose derivatives to SE.[174] In the cited study, an improvement of separation was attributed to a decrease in both EOF and adsorption of the compounds analysed on capillary walls. An analogous effect was observed in the study of the influence of polyvinyl alcohol on the chiral separation of several tocainides.[175] The introduction of short-chain alkyl ammonium cations into SE has a strong effect on the chiral separation of some pharmaceuticals. Separations of enantiomers of 22 drugs using β-CD, DM-β-CD, TM-β-CD, HP-β-CD with additives of tetramethyl- or tetrabutylammonium bromide were investigated.[176] The use of ammonium salts causes the reversal of EOF, an increase in the migration time and improvement of resolution of enantiomer peaks. The separation of enantiomers of several anaesthetics was also improved with the use of the tetrapropyl- and tetrabutylammonium cations in DM-β-CD-containing SE.[177] The influence of small organic cations on the chiral separation of compounds possessing basic properties was examined in the study.[178] Optical isomers of alprenolol, amphetamine, anisodamine, isoprenaline, pinacidil, synephrine and verapamil were separated with the use of HP-β-CD in the presence of tris(hydroxymethyl)methylamine (Tris), diethanolamine, triethanolamine, diethylamine or triethylamine. Of the additives used, triethanolamine proved to be most efficient for improving the selectivity.

Enantiomers of nonpolar primary amines are difficult to separate by CE with the use of only CD. To perform or improve the chiral separation of amines, DM-β-CD, hydroxyethyl-β-CD (HE-β-CD) and γ-CD in combination with 18-crown-6 were used.[179] The compounds analysed form inclusion complexes both with the chiral selector, which recognises amine enantiomers, and 18-crown-6. The mechanism of enantiomer separation with the use of CD and 18-crown-6 was elucidated in the study.[180] Compounds containing the amino group were separated on β-CD or its derivatives in the presence of an achiral crown ether (30 mmol litre^{-1}). A combination of a chiral selector (β-CD or succinyl-β-CD) and an achiral complex-forming agent (a borate buffer) made it possible to separate enantiomers of phenylethane-1,2-diol, 3-benzyloxypropane-1,2-diol, hydroxybenzoin and 3-(*N*-benzyl-*N*-methylamino)propane-1,2-diol.[181] The borate anion serves as a complex-forming agent for vicinal diols. The cationic ion-pair reagent quinine has a strong effect on separation of enantiomers of acidic and basic compounds on cyclodextrins.[182]

The introduction of organic modifying agents into SE can change parameters, such as the binding constants of the chiral selector and enantiomers, the rate of EOF, conductivity of SE, solubility of the chiral selector and/or compounds analysed with a resulting change in the time required for analysis. The introduction of organic modifying agents into SE can either improve or impair the enantiomer separation. Chiral separations of several amino acids were performed in a capillary filled with polyacrylamide gel with the addition of 10% of methanol to CD-containing SE.[119] Propranolol was separated into enantiomers only in the presence of methanol (10%–30%) in SE (phosphate buffer–urea–β-CD).[183] An improvement of chiral separation upon the addition of methanol was observed for mandelic acid,[170] warfarin [162] and imidazole derivatives.[184] The influence of the organic modifying agents on the chiral separation was theoretically examined in the study.[185] In some cases, the addition of this agent impairs the chiral separation because the binding constants and the difference between the effective mobilities of enantiomers decrease when the optimum CD concentration is attained. Examples are separations of clenbuterol,[186] denopamine and trimetoquinol,[187] deprenyl,[188] dioxypromethazine,[189] verapamil and

propafenone,[190] dansyl derivatives of phenylalanine, leucine and propranolol with the use of β-CD and in the presence of additives of alcohols containing from one to six carbon atoms in SE.[191]

3. Methods for optimisation of separation using cyclodextrins

The use of a large number of commercially available cyclodextrins in CE makes it difficult to formulate general rules for choosing conditions of enantiomer separation. The present knowledge of the processes of enantiomer separation does not allow one to choose *a priori* the best chiral selector for a particular racemate. The charged resolving agent migration (CHARM) model proposed by Williams and Vigh [192] can be used for a rational choice of conditions for separation. This model enables one to estimate rapidly the ability of various charged cyclodextrins to perform chiral separation of any racemate.

Successful enantiomer separation depends on many parameters. Instrumental factors (separation potential, temperature) are rather well and predictably controlled in most modern instruments. The selectivity of separation in CE is determined by the ratio between the effective mobilities of the compounds separated and can be optimised based on the dependence of the effective mobility on the composition of the supporting electrolyte. However, the effect is predictable only for some of the compositional variables of SE (pH, the concentration and charge of the chiral selector used, the effective charge of the enantiomer, the rate of EOF). Such factors as the structure of a chiral selector and the addition of organic or inorganic modifying agents can cause essential but unpredictable changes. The CHARM model is based on the consideration of equilibria between protonation and complex formation of enantiomers with a charged chiral selector (the assumed enantiomer : selector ratio is 1 : 1) and the influence of these equilibria on the effective charges of enantiomers, their effective mobilities and selectivity of separation. Analytical expressions for calculations of the above-mentioned parameters of compounds of differing nature (non-electrolytes, strong and weak electrolytes) were reported in the study.[192] It was demonstrated that the calculated values depend on the ionic mobilities of enantiomers involved in a complex and the free protonated and non-protonated forms of enantiomers, the protonation constants, the formation constants of enantiomer – selector complexes for protonated and non-protonated forms, pH and the concentration of the selector in SE. The theoretical resolution can be calculated if the binding constants, ionic charges and ion mobilities of different forms of enantiomers are known.

The CHARM model helps one in making a rational choice of the composition of the supporting electrolyte. For selectors which are strong multicharged electrolytes, the separation of neutral and charged analytes is independent of pH in the absence of EOF. For weak electrolytes, R_s is high at low pH for separation of weak acids in a non-dissociated form and for separation of weak bases as ions. At high pH, R_s is high for separation of weak acids as ions and for separation of weak bases in a non-dissociated form. Consequently, in the absence of EOF (*i.e.*, in a capillary with a neutral coating), it is necessary to perform only two series of measurements to find the best conditions for separation: at low pH of the supporting electrolyte (for example, at pH 2.2) and at high pH (for example, pH 9.5). At each pH, the type and concentration of CD are varied to find the maximum R_s. If the optimum chiral selector is known, the rate of EOF can be varied by changing the properties of capillary walls (anionic or cationic coating, uncoated walls) to improve the peak resolution, accelerate separation or change the enantiomer migration order. Finally, to carry out or improve separation, one can try to use organic modifying agents, surfactants or systems consisting of two chiral selectors. However, this does not necessarily improve the results.

Electromigration dispersion caused by the difference in mobilities of the ions of the sample and buffer often leads to broadening of the peaks (frontal peaks or peaks with 'tails'). This phenomenon can be reduced by increasing the ionic strength of SE or with the use of a buffer whose conductivity is equal to that of the band of the sample.

4. Micellar electrokinetic chromatography

Micellar electrokinetic chromatography (MEKC) allows one to separate uncharged components of a sample due to the difference in the probability of their presence in an aqueous mobile and pseudostationary phases. Sodium dodecylsulfate (SDDS) is the surfactant most generally used in capillary electrophoresis for separation of enantiomers in the presence of cyclodextrins (CD-MEKC method). Micelles of SDDS bear a negative charge and, consequently, possess electrophoretic mobility toward the anode. A scheme of electrophoretic separation of uncharged compounds by CD-MEKC with the use of SDDS is shown in Fig. 6; the electroosmotic flow is directed toward the cathode. If the contribution of electrophoretic mobility of micelles is smaller than the contribution of electroosmotic mobility, micelles move toward the cathode, *i.e.*, the detector. Uncharged enantiomers form inclusion complexes with CD in an aqueous phase or are included into micelles and move at the rate of the latter. The separation of neutral enantiomers is based on the difference in their distribution between a CD-containing buffer solution and the interior of micelles. Since molecules of a sample interact with a pseudostationary phase, the metrological characteristics of CD-MEKC correspond to the characteristics of chromatography. It should be emphasised that MEKC on CD allows one to separate both uncharged and charged enantiomers if they are included into micelles.

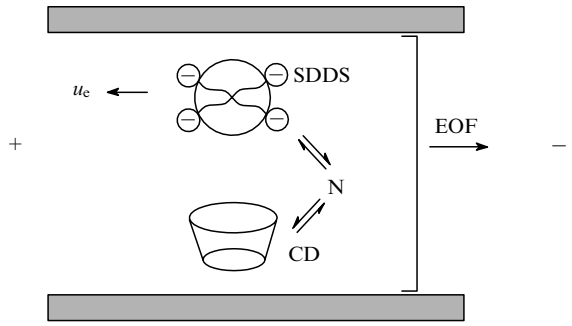


Figure 6. Scheme of electrophoretic separation of an uncharged compound by CD-MEKC;[39]
u_e is the rate of electrophoretic migration, N is an enantiomer.

In the first study devoted to separation of enantiomers by CD-MEKC,[193] the influence of the nature of a chiral selector on the chiral separation of thiopental, pentobarbital, phenobarbital and barbital was examined using α-, β-, γ-, DM-β- and TM-β-CD in a buffer containing 50 mM SDDS and 4 M urea (pH 9.0) as examples. γ-Cyclodextrin containing a cavity with the largest diameter proved to be the most efficient chiral agent. The addition of other chiral compounds together with CD (for example, with D-camphor-10-sulfonate) improved the chiral separation of the compounds under study.

The MEKC method was employed in chiral separations of various compounds: amino acid derivatives,[194–197] aromatase and barbiturate inhibitors,[165] β-blockers,[198] triazole derivatives,[199,200] dihydropyridine derivatives,[201] cicletanine,[202] mephenytoin, phenytoin and their 4-hydroxy derivatives,[203] peptide derivatives,[204,205] pesticides,[206] *etc*. In CD-MEKC, bile acids belonging to chiral surfactants and their salts are also used for separating enantiomers of dansyl amino acids,[207–209] baclofen and its analogues, mephenytoin and fenoldopam and their derivatives,[207] amino acid derivatives, such as *N*-substituted 1-cyanobenzo[*f*]isoindoles (CBI amino acids),[208] OPA amino acids,[210]

phenylethylamines (diclofensine, ephedrine, methamphetamine, β-blockers)[211] and binaphthyl derivatives.[212] Mixed micellar mixtures of SDDS and taurodeoxycholic acid with β-CD are used to separate CBI-selenomethionine and selenoethionine enantiomers.[213] Enantiomers of dansyl amino acids were separated[214] with the use of an achiral polymeric surfactant (sodium salt of butyl acrylate – butyl methacrylate – methacrylic acid copolymer) in combination with β-CD; the peak resolution obtained in the cited study was better than that attained with the use of the β-CD – SDDS system.

5. Non-aqueous chiral capillary electrophoresis

The review[31] surveyed data on enantiomer separations by non-aqueous CE. The effects of the concentration and the nature of the chiral selector used, apparent pH, the ionic strength, temperature and the nature of organic solvents on the mobility of enantiomers, selectivity and efficiency of enantiomer separation were discussed.

One of the most important advantages of non-aqueous CE is that it enables one to study compounds insoluble or poorly soluble in water. Solubility of cyclodextrins in many solvents is much higher than that in water. For example, the solubility of β-CD in *N*-methylformamide is higher than 700 mmol litre^{-1}, whereas its solubility in water is 18 mmol litre^{-1}.[215] Therefore, the chiral separation can be improved at higher cyclodextrin concentrations in organic media.[215–223] The chiral separation of basic tricyclic drugs was investigated[216] with the use of β-CD, M-β-CD, HP-β-CD and γ-CD in formamide, *N*-methylformamide and *N,N*-dimethylformamide. In this case, the character of interactions between enantiomers and the chiral selector changes. It is assumed that hydrophobic interactions between enantiomers and the chiral selector are virtually absent and the formation of inclusion complexes proceeds to a very low extent. Apparently, the planes of the compounds separated lie on the cyclodextrin 'cone', and the compounds interact with the hydroxy groups of CD. This assumption is consistent with the binding constants of trimipramine, mianserin and thioridazine, which decrease from $\sim 10^4$ in water to $\sim 10^{-2}$ in *N,N*-dimethylformamide. The formation of inclusion complexes proceeds more readily in a polar solvent, for example, in formamide, than in less polar *N*-methylformamide because the difference in polarity of the solvent and the CD cavity is larger in the former case. The best results of chiral separations of tricyclic compounds were obtained with the use of β-CD and its derivatives, but not with γ-CD, although the diameter of the cavity in the latter is larger and is closer to the size of the molecules separated.

The drawback of the above-mentioned solvents is their intense absorption at 214 nm and at lower field. Ultraviolet-transparent supporting electrolytes can be prepared from methanol or mixtures based on this solvent. Enantiomers of 40 basic compounds in the H_3PO_4 – NaOH – CH_3OH supporting electrolyte were separated with the use of heptakis(2,3-dimethyl-6-sulfo)-β-CD.[224] The dependences of the effective mobilities, selectivity coefficients and peak resolution on the concentration of a chiral selector were studied in the range of 0 – 40 mmol litre^{-1} and it was found that these dependences obey the theoretical predictions of the CHARM model with the use of a charged chiral selector.[192]

6. Electrochromatography

Capillary electrochromatography (CEC) is a modern method which combines the advantages of HPLC and CE. Chiral CEC employs the principles of molecular recognition of enantiomers by a chiral stationary phase (CSP) and the electric field as the driving force for the transfer of enantiomers. The main advantage of CEC over HPLC is a higher column performance, which is a result of a planar plug-like profile of the mobile phase flow.

The main principles of chiral CEC were discussed in the review.[225] The transfer of enantiomers through a capillary column occurs primarily due to EOF caused by the electric double layer in the vicinity of the charged surface of a stationary phase. The linear rate of osmotic flow u_{eo} is proportional to the ζ potential, which characterises the electric double layer and depends also on the properties of a mobile phase (dielectric permeability ε, viscosity η, ionic strength) and the electric field strength E. In addition to the transfer caused by EOF, charged compounds have inherent electrophoretic migration in a direction determined by the ion charge. The linear migration rate of compounds can be increased by applying pressure to a column. The apparent linear migration rate of a compound u_{app} is the sum of the linear rate of EOF u_{eo}, the rate of electrophoretic migration u_{ep} and an increase in the rate due to the applied pressure u_{press}:

$$u_{app} = u_{eo} + u_{ep} + u_{press}. \quad (3)$$

The chiral separation is always based on enantioselective molecular interactions between the compound and CSP or pseudo-SP (chiral additives), whereas the electrophoretic migration makes a contribution only to the total transfer of compounds, because enantiomers possess identical electrophoretic mobilities. The elution time t_e depends on the column length L, the chromatographic retention factor k and the linear migration rate of the compound u_{app}:[226]

$$t_e = \frac{L(1+k)}{u_{app}}. \quad (4)$$

Two varying approaches are used for the direct separation of enantiomers in CEC: a chiral selector can be added to a mobile phase or immobilised on a solid support or a capillary wall (CSP).

Commercial reversed-phase achiral columns are generally used for the enantiomer separation in the presence of chiral additives in a mobile phase. In this version of CEC, the mechanism of separation is rather complex because it includes electrophoretic (for charged enantiomers) and chromatographic components. The enantioselectivity of separation is achieved due to the difference in the formation constants of enantiomer – chiral selector complexes, the difference in effective mobilities of the free enantiomer and the enantiomer involved in a complex and separation of diastereomeric complexes on SP.[226] If EOF and electrophoretic migration of charged compounds occur in different directions, the elution order of enantiomers can be reversed by changing the mobile phase (this effect is well known in CE).[117] It should be noted that α-, β-, γ-CD and their derivatives are most commonly used as additives to a mobile phase. Chlorthalidone enantiomers were separated on a column packed with octadecyl silica gel (ODS) (3 μm) with HP-β-CD as a chiral selector.[227] Enantiomers of phenylephrine and synephrine were separated on a silica gel column using HP-β-CD as a chiral selector.[228] In the study,[226] salsolinol enantiomers were separated by CEC on a column based on ODS in the presence of β-CD as a chiral additive and sodium heptanesulfonate as an achiral ion-pair reagent. The mechanism of separation in CEC in the presence of an additive of a chiral selector in MP was discussed in detail, and a theoretical model of enantioselectivity in such systems applicable to both neutral and charged compounds was proposed. Capillary electrochromatography with the use of a chiral selector in a mobile phase has no advantages over enantioselective CE.

A chiral stationary phase holds the most promise for separating enantiomers by CEC. There are three different approaches to CSP-based capillary columns.[229]

1. Open tubular columns, whose walls are chemically or physically modified with a chiral selector. Tubular columns with an inner diameter of ~ 50 μm, which is optimum simultaneously for high column performance and UV detection (standard detection in most apparatus for CE) are required for enantiomer separation. The electroosmotic flow is created by residual silanol groups on a silica gel surface (for neutral chiral selectors). The only drawback of this approach is a small surface area accessible for the modification with a chiral selector. As a consequence, columns can be easily overloaded, which is manifested in peak

asymmetry and deterioration of the efficiency. The efficiency of separation is influenced by the thickness of the SP layer. Larger theoretical plate (t.p.) numbers are obtained on thinner films.

Enantiomer separation on an open tubular column using a 0.2-μm thick film of polydimethylsiloxane with permethylated β-CD (16%) (Chirasil-Dex) covalently grafted through the octamethylene spacer was first carried out for 1-phenylethanol and other compounds.[230] In another study,[231] a capillary modified with permethylated γ-CD (Chirasil-γ-Dex) was used for the chiral separation of ibuprofen, flurbiprofen, cicloprofen and 1-phenylethanol. The effects of the pH of the buffer, the applied stress and the thickness of the SP layer were studied. An increase in the film thickness from 0.2 to 0.4 and 0.8 μm led to a substantial loss of efficiency: from 30 500 (300 000 t.p. m^{-1}) to 24 000 and 9500 t.p. per column, the selectivity of separation remaining virtually unchanged (1.17, 1.19 and 1.12, respectively). Enantiomer separations with the use of Chirasil-Dex films in open tubular columns were discussed in the review.[232]

2. Packed columns with CSP using standard chromatographic supports (silica gel or polymers) chemically modified with a chiral selector. For such chiral stationary phases, quartz capillaries with an inner diameter of 75 or 100 μm are used. Columns of this type are analogous to standard chromatographic columns but allow one to obtain a larger theoretical plate number. For CSP, smaller particles and/or longer columns can be used, because there are no such strong limitations on the pressure in CEC compared to HPLC. It should be noted that external pressure applied to a capillary can decrease the time of analysis but simultaneously leads to distortion of the planar profile of the flow typical of electromigration systems to give a parabolic profile of the flow resulting in a decrease in the column performance. As mentioned above, enantiomer separation can be performed using normal-phase, reversed-phase and 'polar organic' modes of HPLC. The latter two modes are predominantly used for CEC; the conditions of separations by HPLC can often be directly extended to CEC without loss of enantioselectivity. The mechanism of separation of enantiomers by CEC is identical to that by HPLC and was discussed above. The strength of interactions between an enantiomer and a chiral selector can be controlled by varying the composition of a mobile phase. This is more difficult to perform in CEC than in HPLC, because control over the retention and/or selectivity of separation in CEC always influences the rate of EOF and, in the case of charged compounds, the electrophoretic migration. In most cases, mobile phases include acetonitrile-based mixtures, which produce large EOF, and buffers with a low ionic strength. Acetonitrile as an organic modifying agent is best suited to attain a high rate of EOF and is, generally, preferred over methanol. A high rate and stability of EOF are important conditions in CEC. The ζ potential of the operating conditions as well as the rate and direction of electroosmotic flow are determined by the properties of mobile and chiral stationary phases. The choice of the corresponding support for the preparation of CSP is of more importance in CEC than in HPLC. Silica gels can differ substantially in the number of silanol groups resulting in differences in the rate of EOF for CSP prepared based on these silica gels. Examples of the use of this type of column are given below. Enantiomers of benzoin, hexobarbital, dansyl and 3,5-DNP amino acids were separated on capillary columns packed with CSP containing β-CD (5 μm, Cyclobond I).[233] The use of a solution of triethylammonium acetate instead of a phosphate buffer led to the reversal of EOF and improved the separation of acidic compounds. This reversal of EOF observed for mobile phases containing small amounts of methanol (no more than 15%) cannot be attributed to adsorption of the triethylammonium ions on the SP surface and the formation of a dynamic anion exchanger. Chlorthalidone and mianserin enantiomers on CSP based on HP-β-CD (5-μm commercial column Cyclobond I 2000 RSP) were separated in the study.[227] In the investigation,[234] CSP based on permethylated β-CD (pore size was 300 Å, 5 μm) was used for the enantiomer separation of several barbiturates, benzoin, glutethimide, methylthiohydantoin proline and methyl mandelate (the efficiency varied from 20 000 to 40 000 t.p. m^{-1}). A comparison of the separation of mephobarbital enantiomers by micro-HPLC and CEC on this capillary column demonstrated that the use of CEC led to a threefold increase in the efficiency of separation and improved the peak resolution from 2.08 to 3.39 without loss of enantioselectivity. Separations of enantiomers of the same compounds and esters of aryloxycarboxylic acids performed on permethylated β-CD covalently bound through a spacer (octamethylene) to polydimethylsiloxane on silica gel (pore size was 300 Å, Chirasil-Dex) led to a further improvement of the column performance to 70 000 t.p. m^{-1}.[235] The hydrophobic microenvironment and protection of polar silanol groups with a polysiloxane film improve the enantioselectivity. For example, 1-(2-naphthyl)ethanol was separated only on Chirasil-Dex rather than on covalently grafted permethylated β-CD as a chiral stationary phase.

3. Specially designed monolithic CSP prepared by *in situ* polymerisation in a capillary; such CSP have chiral selectors or chiral cavities introduced into a continuous polymeric matrix. Positively and negatively charged polyacrylamide gels (which give cathodic and anodic EOF, respectively) physically or chemically modified with β-cyclodextrin were prepared in capillaries, which already contained methacrylate groups.[236–238] The advantage of these monolithic CSP is that there is no need to introduce an organic modifying agent, which can decrease enantioselectivity, into a mobile phase with the aim of performing enantiomer separation. Separations of terbutaline, propranolol and benzoin enantiomers were carried out using negatively charged gel.[236, 237] Dansyl amino acids and some other acidic compounds were separated on positively charged polyacrylamide gel with covalently grafted allylcarbamoyl-β-CD.[238] The column performance was as high as 150 000 t.p. m^{-1}.

7. Practical application of enantiomer separation

Data on enantiomer separations by CE with the use of cyclodextrins as chiral selectors were generalised in the reviews.[29, 39, 239–244] Examples of chiral separations carried out in 2000–2002 (which were grouped according to the type of CD) are given in Table 5.

In spite of abundant data on the application of cyclodextrins in chiral separations by CE, studies devoted to quantification of enantiomers in real samples are few in number. Enantiomers in real samples are quantified in pharmaceutics, medicine, biology and investigations of environmental objects. It should be emphasised that when performing stereoselective analyses of drugs, it is necessary to quantify an undesirable enantiomer at a concentration lower than 1%. The precise determination of the enantiomer ratio of 99.9 : 0.1 is often a more complicated problem than the 'simple' separation of racemates. Examples of quantification of enantiomers in real samples were given in the reviews.[29, 39] The results obtained in 2000–2002 are presented in Table 6.

The principles and most important aspects of chiral analysis of pharmaceuticals were discussed and the data on quantification of enantiomers of drugs and their metabolites in biological liquids were surveyed in the reviews.[245, 246] These reviews also gave examples of the practical application of the determination of enantiomers for an explanation of the possible mechanisms of pharmacokinetics and stereoselectivity of drug metabolism.

The review[247] was devoted to chiral separations of amino acids and peptides. A large number of examples which illustrate the state-of-the-art separation of enantiomers and diastereoisomers of amino acids and peptides were offered. Ways of improving the selectivity and methods for optimisation of the parameters of chiral separation using the mathematical design of experiments were discussed.

Table 5. Examples of enantioselective separations by CE using various types of cyclodextrins.

Compound	CD	Supporting electrolyte, method of detection [a]	Ref.
Aromatic derivatives of aminophosphorous and aminohypophosphorous acids	α-CD	100 mM phosphate buffer, pH 7.5, and 40 mM CS [b]	248
5,11-Dihydro-11-[2-[2-[(*N,N*-dipropylaminomethyl)-piperidin-1-yl]ethylamino]carbonyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one	sulfo-α-CD	25 mM triethylammonium phosphate, pH 2.5 and 5% CS	249
Lobeline, benzhexol	β-CD	50 mM Tris – phosphoric acid, pH 2.5 and 14% of a mixture: β-CD, P_2O_5, L-glutamic acid	250
Brompheniramine	β-CD	100 mM phosphoric acid – triethanolamine, pH 3.0 and 18 mg ml^{-1} CS	251
OPA amino acids	β-CD	40 mM borate buffer, pH 9.0, 60 mM sodium taurocholate, 15% propan-2-ol and 30 mM β-CD	210
Promethazine	β-CD	30 mM Na_2HPO_4, 15 mM citric acid, pH 2.5 and 1 mM CS; amperometric detection	252
Ibuprofen	β-CD	200 mM MES,[c] pH 4.4, 0.3% hydroxyethylcellulose and 15 mM CS	253
Thioureas (products of the reaction of fluorescein-5-isothiocyanate with amino acids)	β-CD	80 mM borate buffer, pH 9.3, 30 mM sodium taurocholate and 20 mM β-CD; FD [d]	254
Dextromoramide	HP-β-CD	100 mM phosphoric acid – triethanolamine, pH 3.0 and 15 mM CS	255
2-(4,5-Dihydro-1*H*-imidazol-2-yl)-5-fluoro-2-methoxybenzo[1,4]dioxane	HP-β-CD	50 mM ammonium formate, pH 4 and 10 mM CS; CE-MS [e]	256
Cizolirtine and its dehydration products	HP-β-CD	50 mM borate buffer, pH 9.2, 150 mM SDDS, 5% n-butanol and 60 mM CS	257
3,4-Methylenedioxymethamphetamine and two of its metabolites	HP-β-CD	50 mM phosphoric acid, pH 2.5 and 50 mM CS	258
Bis(phenethyl)amines, their cyclic analogues	HDAS-β-CD	50, 100 mM phosphate buffer, pH 3.0, 6.0, 7.5 and 0.5 – 9 mM CS	259
Synthetic analogue of wasp poison	HDM-β-CD [f]	50 mM 6-aminocaproic acid, pH 4.0 and 30 mM CS	260
1-(1-Naphthyl)ethylamine, 1-aminoindan, 1,2,3,4-tetra-hydro-1-naphthylamine	HDM-β-CD [f]	200 mM Tris – 300 mM borate buffer, pH 7.0, 10 mM 18-crown-6 and 10 mM CS	261
Mianserin, cyclopentolate, fluoxetine, nisoxetine, verapamil, aminoglutethimide, dimethindene, doxapram, hydroxyzine, meclozine, ketoprofen	HDMC-β-CD [g]	50 mM phosphate buffer, pH 7.0 and 18 – 72 mM CS	262
[5-(4-Hydroxybutyl)-5′-methyl-2,2′-bipyridyl]-bis(2,2′-bipyridyl)Ru(II) bis(hexafluoroantimonate)	CM-β-CD	40 mM borate buffer, pH 9.5 and 7.5 mg ml^{-1} CS	263
4-(3-Isobutylamino-2-hydroxypropoxy)-3-alkoxymethylacetophenone derivatives	CM-β-CD	80 mM Tris-acetate buffer, pH 4.7 and 10 mM CS	264
Chlorpheniramine, methadone	CM-β-CD	100 mM phosphoric acid – triethanolamine, pH 4.0; 3.5 and 5 mM CS	265
Adrenaline, noradrenaline (1), ephedrine, pseudoephedrine (2)	CM-β-CD	(1) 20 mM phosphate buffer, pH 2.5, 12 mM 18-crown-6 and 5.2 mg ml^{-1} CS; (2) 100 mM NaOH, pH 12.9 and 16; 3.9 mg ml^{-1} CS; amperometric detection	266
Selenomethionine, selenocysteine, selenoethionine	sulfo-β-CD	35 mM citrate buffer, pH 7 and 1% CS	267
Fungicides	sulfo-β-CD	phosphate buffer, pH 3.0 or phosphate buffer, 2 M urea, pH 3.0 and 2% CS	268
Ormeloxifene and its derivatives	sulfo-β-CD	10 mM NaH_2PO_4, pH 3.0 and 2% CS	269
Spiro(2*H*-benzofuro[2,3-*a*]quinolizine-2,4′-(1′*H*)-pyrimidine)-2′(3′*H*)-one derivative, *cis*-aminoindanol	sulfo-β-CD	25 mM Na_3PO_4, pH 2.5 and 1.5% CS, PVA-coated capillary; 25 mM Na_3PO_4, pH 6.5 and 0.3% CS	270
Clenbuterol, salbutamol, tulobuterol	sulfo-β-CD	50 mM phosphate buffer, pH 2.24 and 25% CS	271
Nicardipine	sulfo-β-CD	20 mM triethanolammonium phosphate, pH 3 and 5% CS	272

Table 5 (continued).

Compound	CD	Supporting electrolyte, method of detection [a]	Ref.
Voriconazole	SBE-β-CD	50 mM NaH_2PO_4, pH 7.0 and 1 mM CS	273
Flobufen	HTM-β-CD	87.5 mM acetate buffer, pH 5.5, 10% methanol and 30 mM CS	274
Dimethindene	HTM-β-CD	100 mM phosphoric acid – triethanolamine, pH = 3.0 and 150 mg ml^{-1} CS	275
Naproxen	HTM-β-CD	25 mM H_3PO_4 – 25 mM CH_3COOH – 25 mM H_3BO_3 – NaOH, pH 5.0 and 3 mM CS, polyacrylamide-coated capillary	276
Propranolol, mexiletine, lobeline, propafenone, isoprenaline, bupivacaine, verapamil, ketamine	MPC-β-CD [h]	40 mM borate buffer, pH 9.18 and 2.5 mM CS	190
Methyl 4-(3,4-difluorophenyl)-6-methoxymethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate, hydrolysis products	QA-β-CD [i]	25 mM Tris-acetate buffer, pH 8.3 and 2.5% CS	277
2-(2,4-Dichlorophenoxy)-, 2-(4-chloro-2-methylphenoxy)propionic acids	EC-β-CD [j]	45 mM NaH_2PO_4, pH 5 and 12 mM CS	278
Di- and tripeptides	β-CD, PM-β-CD, HDM-β-CD, HTM-β-CD, HP-β-CD	50 mM phosphate buffer, pH 2.5 – 3.5 and 20 mg ml^{-1} CS	118
3-(3,4-Dihydroxyphenyl)alanine, 3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid	HDAS-β-CD, CM-β-CD	20 mM phosphate buffer, pH 2.5 and 20 mM CS	279
1,3,4-Thio-, 1,3,4-selenodiazine derivatives	HE-β-CD, HP-β-CD, M-β-CD	25 mM CH_3COONH_4 – 1 M CH_3COOH or 25 mM citric acid – 12 mM Tris in methanol, formamide or a formamide : acetonitrile mixture (1 : 2) and 200 mM CS	280
Alkyl esters of amino acids, BOC and Z amino acids	HP-β-CD, CM-β-CD, SBE-β-CD	100 mM phosphoric acid – triethanolamine, pH 2.5; 4.0; 5.5; 0% – 30% methanol and 5 – 30 mM CS	281
Propranolol, hydrobenzoin, atenolol, nefopam, chlorcyclizine, 1,1′-binaphthyl-2,2′-diyl phosphate, trimipramine, primaxin, canadine, piperoxan	sulfo-β-CD, phosphated β-CD	50 mM phosphate buffer, pH 5.0 and 2% CS	282
Synthetic arginine-containing peptide and its derivatives	SBE-β-CD + β-CD	50 mM citrate buffer, pH 2.5 and 20 mM CS_1 + 5 mM CS_2, PVA-coated capillary	283
Sertraline	sulfo-β-CD + + HP-β-CD	20 mM borate buffer, pH 9.0, 50 mM sodium cholate, 15 mM CS_1 + 5 mM CS_2	284
Fenoprofen, flurbiprofen, ibuprofen, ketoprofen	sulfo-β-CD + + HTM-β-CD	100 mM phosphoric acid – triethanolamine, pH 2.5 and 2 – 3 mM CS_1 + 10 – 20 mM CS_2	285
Aminoglutethimide	α-CD + HDM-β-CD; α-CD + HTM-β-CD	100 mM phosphate buffer, pH 2.5 and 10% – 95% CS_1 + 90% – 5% CS_2, total concentration of CD was 15 mM	286
DNP derivatives of aspartic acid, alanine, glutamine, homoserine, proline, norvaline and glutamic acid	copolymer of allylamine and '2-hydroxy-3-methacryloyl-β-CD'	50 mM acetate buffer, pH 4.0 and 4 – 20 mM CS	287
2-Phenoxy-, 2-(4-chlorophenoxy)-, 2-(3-chlorophenoxy)propionic acids	trehalose cysteamine-HSD [k]	22 mM Na_3PO_4, pH 6.0 and 2.5 mM CS	288
	trehalose cysteamine-HSD, trehalose ammonium-HSD, trehalose β-alanine-amide-HSD	22 mM phosphate buffer, pH 6.0 and 1.50 – 5.00 mM CS; 22 mM borate buffer, pH 9.5 and 5.00 mM CS	289
Reaction product of homocysteine with 4-aminosulfonyl-7-fluoro-2,1,3-benzooxadiazole	γ-CD	50 mM Na_3PO_4, pH 2.25 and 50 mM CS	290
1-Methyl-3-phenylpropylamine, 1,2,3,4-tetrahydro-1-naphthylamine	γ-CD	50 mM phosphate buffer, pH 2.0, 10 mM 18-crown-6 and 40 mM CS	291

Table 5 (continued).

Compound	CD	Supporting electrolyte, method of detection [a]	Ref.
Thioureas (reaction products of isothiocyanates with amino acids)	γ-CD	100 mM borate buffer, pH 9.4, 30 mM SDDS and 10 mM CS; FD	292
1-Aminoindan, β-blockers, bupivacaine, bupropion, chlophedianol, 4-chloroamphetamine, chlorpheniramine, epinephrine, isoproterenol, ketamine, metaproterenol, methoxyphenamine, 1-(1-naphthyl)ethylamine, oxyphencyclimine, phenylpropanolamine, piperoxan, propafenone, terbutaline, tetrahydropapaveroline, tetrahydrozoline, tolperisone, trihexyphenidyl	ODAS-γ-CD [l]	25 mM H_3PO_4, 12.5 mM NaOH, CH_3OH and 2.5 – 30 mM CS	293
Alkyl aryl phosphates	ODAS-γ-CD [l]	25 mM MES, 12.5 mM NaOH, 0% – 10% methanol and 5 – 20 mM CS	294
Uniconazole, diniconazole	CM-γ-CD	50 mM phosphate buffer, pH 6.5 and 5 mM CS	295
Ephedrine and amphetamine derivatised by 4-fluoro-7-nitrobenzofurazan	sulfo-γ-CD	50 mM phosphate buffer, pH 7.35, 1.5 mM SDDS and 10 mM CS; FD	296
Promethazine, ethopropazine, trimeprazine, thioridazine	β-CD, HP-β-CD, γ-CD	40 mM phosphate buffer, pH 3.0 and 0.02 – 3.0 mM CS_1 or 0.4 – 6.0 mM CS_2 or 2.5 – 6.0 mM CS_3	297
Acebutolol, bepridil, cloperastine, diperodon, disopyramide, doxylamine, homochlorcyclizine, hydroxyzine, ketamine, oxyphenonium bromide, pheniramine, propiomazine	CM-β-CD, CM-γ-CD	100 mM phosphate or borate buffer, pH 6, 7, 8 and 2.5% CS	298
Polychlorobiphenyls	CM-γ-CD, β-CD, PM-β-CD	50 mM MES, pH 6.5, 2 M urea and 20 mM CS_1 + 10 mM CS_2 or 20 mM CS_1 + 20 mM CS_3	299
26 Drugs and their synthetic intermediates	SBE-β-CD + γ-CD + HDM-β-CD	50 mM NaH_2PO_4, pH 2.5 and 5% CS_1 + 25 mM CS_2 + 25 mM CS_3	300

[a] The method is not given if a UV detector was used; [b] CS is a chiral selector; [c] MES is 2-morpholinoethanesulfonic acid; [d] FD is fluorescence detection; [e] MS is mass spectrometry; [f] HDM is heptakis(2,6-di-*O*-methyl); [g] HDMC is heptakis(2-*N*,*N*-dimethylcarbamoyl); [h] MPC is mono-3-*O*-phenylcarbamoyl; [i] QA is quaternary ammonium salt; [j] EC is ethyl carbonate; [k] HSD is hemispherodextrin; [l] ODAS is octakis(2,3-diacetyl-6-sulfo).

Table 6. Enantioselective separations using cyclodextrins in analysis of real samples by CE.

Compound	Sample	CD	Supporting electrolyte, method of detection [a]	Ref.
Epimers of amygdalin	Chinese herbal remedies	α-CD	20 mM Tris – HCl, pH 9.0, 80 mM sodium deoxycholate and 25 mM α-CD	301
CBI baclofen	plasma	α-CD	50 mM borate buffer, pH 9.5 and 7 mM CS; FD	302
Rogletimide	serum	α-CD	10 mM NaH_2PO_4 – 5 mM $Na_2B_4O_7$, pH 2.5, 0.03 mM HTAB [b] and 60 mM CS	303
CBI aspartic acid	rat brain	β-CD	50 mM borate buffer, pH 9.0, 50 mM SDDS, 15% methanol and 20 mM CS; FD	304
CBI derivatives of seleno-methionine and selenoethionine	commercial L-SeMet	β-CD	30 mM NaH_2PO_4 – 10 mM H_3BO_3, pH 7, 50 mM SDDS, 50 mM taurodeoxycholic acid and 20 mM β-CD	213
Methadone, cocaine, amphetamine and its 3,4-methylenedioxy derivatives	heroin	β-CD	100 mM K_3PO_4, pH 3.23 and 20 mM CS	305
Lactic acid	food products	HP-β-CD	90 mM phosphate buffer, pH 6.0 and 240 mM CS	306
	biological liquids	HP-β-CD	200 mM phosphate buffer, pH 6.0 and 413 mM CS, polyacrylamide-coated capillary	307
Terbutaline	the same	HP-β-CD	50 mM phosphate buffer, pH 2.5 and 10 mM CS	308
Methylphenidate	commercial samples	HDM-β-CD	100 mM H_3PO_4 – triethanolamine, pH 3.0 and 15 mM CS	255

Table 6 (continued).

Compound	Sample	CD	Supporting electrolyte, method of detection [a]	Ref.
Ephedrine, propranolol, adrenaline, phenylalanine	spinal puncture, pharmaceuticals Gripex and Nurofen	CM-β-CD	20 mM $Na_2B_4O_7$, pH 7.5 and 20 mM CS	309
Amphetamine, methamphetamine	biological liquids	CM-β-CD	100 mM phosphate buffer, pH 2.5 and 10 mM CS	310
Metaprolol, its metabolite, *O*-desmethyl metaprolol	the same	CM-β-CD	100 mM acetate buffer, pH 4.0, 5% propan-2-ol and 10 mM CS	311
Albendazole sulfoxide	spinal puncture	sulfo-β-CD	20 mM Tris, pH 7.0 and 3% CS	312
Atropine	ophthalmological solution	sulfo-β-CD	55 mM phosphate buffer, pH 7 and 2.9 mM CS	313
Ofloxacin	biological liquids	sulfo-β-CD	50 mM phosphate buffer, pH 2.5 and 0.35 mM CS	314
Praziquantel, *trans*-4-hydroxy-praziquantel	plasma	sulfo-β-CD	20 mM borate buffer, pH 10, 20 mM sodium deoxycholate and 2% CS	315
Disopyramide, mono-*N*-des-alkyl disopyramide	"	sulfo-β-CD	20 mM acetate buffer, pH 5.0 and 0.2% CS	316
Eticlopride, sulpiride	serum	sulfo-β-CD	20 mM citrate buffer, pH 2.90 and 2% CS	317
Ofloxacin, desmethyl ofloxacin, ofloxacin *N*-oxide	biological liquids	SBE-β-CD	100 mM phosphate buffer – triethylamine, pH 2.0 and 0.65 mg ml^{-1} CS; FD	318
Tramadol, its metabolites	plasma	SBE-β-CD	40 mM ammonium acetate, pH 4.0 and 2.5 mg ml^{-1} CS; PVA-coated capillary; CE-MS	319
Venlafaxine, its metabolite	clinical samples	phosphated γ-CD	50 mM phosphate buffer, pH 2.5 and 20 mg ml^{-1} CS	320
Tramadol	biological liquids	CM-β-CD + M-β-CD	50 mM borate buffer, pH 10.0 and 40 mg ml^{-1} CS_1 + 22.5 mg ml^{-1} CS_2; FD	321
Carvedilol	plasma	succinyl-β-CD + M-α-CD	150 mM phosphate buffer, pH 3.0 and 7.5 mg ml^{-1} CS_1 + 15 mg ml^{-1} CS_2; FD	322
Amphetamine, methamphetamine, ephedrine, norephedrine, their derivatives (simultaneous detection)	biological liquids	β-CD + HDM-β-CD	75 mM Tris – H_3PO_4, pH 2.5 and 3 mM CS_1 + 10 mM CS_2	323
Diastereomers of flavanone-7-*O*-glucosides	citron juice	γ-CD	200 mM borate buffer, pH 10.0 and 5 mM CS	324
Vestitone, medicarpin (simultaneous detection)	extracts from transgenic plants	HP-β-CD + HP-γ-CD	25 mM borate buffer, pH 10.0, 10% methanol and 2 mM CS_1 + 20 mM CS_2	325

[a] The method is not given if a UV detector was used; [b] HTAB is hexadecyltrimethylammonium bromide.

V. Conclusion

A huge amount of the published data on the application of cyclodextrins in enantiomer separations demonstrates the important role of these chiral selectors in various methods of chromatography and capillary electrophoresis. Each method has advantages and limitations. Hence, it is useful to compare the possibilities of chiral separations by capillary gas chromatography, high-performance liquid chromatography, capillary electrochromatography and capillary electrophoresis and to discuss the prospects of the use of CD-based chiral phases.

High-performance liquid chromatography finds the most extensive application. Enantiomers are separated with the use of various CD and their derivatives (both polar and hydrophobic). The advantages of HPLC are associated with the availability of commercial columns, their high selectivity and rather high performance, diversity and high sensitivity of detectors, good reproducibility of the results and a large amount of published data on separations of enantiomers of differing nature.

Capillary gas chromatography with cyclodextrin phases is employed more rarely because of technological difficulties in the column manufacture. However, the number of CD used in capillary gas chromatography is rather large (like in liquid chromatography). This method enables one to separate enantiomers with a boiling point of no higher than 260 °C. Unlike HPLC, gas chromatography allows one to separate compounds devoid of an aromatic ring. The development of capillary gas chromatography with CD phases provided a deeper insight into processes responsible for separation of optical isomers. Depending on the type of CD, either the formation of inclusion complexes (β-CD and its polar derivatives) or surface interactions, *viz*., hydrogen bonding and π – π interactions with substituents in the ring of CD (γ-CD and hydrophobic derivatives of β-CD), can play a decisive role. A predominance of hydrogen bonding and π – π interactions with substituents in the ring of CD provides an increase in the efficiency of chiral separations, because the rates of these processes are substantially higher than the rate of formation of inclusion complexes. Such selectors make it possible

to separate enantiomers possessing similar thermodynamic characteristics.

The advantages of capillary electrophoresis are a higher efficiency of separation and the ease of variation in the nature of a selector by adding various CD to a supporting electrolyte. The choice of chiral selectors in CE is limited by their solubility in aqueous solutions serving as SE. In some cases, aqueous–organic mixtures (with acetonitrile, methanol or dimethyl sulfoxide) can be used. This is the main limitation on the application of CE to separation of optical isomers. This difficulty is partially compensated by the ease of variation in the composition of a supporting electrolyte. Thus, a selector, its concentration and the pH of an electrolyte can be varied, mixed chiral selectors can be used and specific achiral additives can be introduced.

An essential problem in capillary electrophoresis is associated with the reproducibility of the results. To solve this problem, each subsequent separation must be performed using a fresh solution of the supporting electrolyte and the capillary must be washed with this solution between separations. This leads to a high consumption of a chiral selector and an increase in the time of analysis.

Yet another problem is the detection of enantiomers. A UV detector is most commonly used. Sorbates are detected as diastereomeric complexes with a chiral selector. Due to adsorption of the selector involved in SE, the sensitivity of determination of sorbates decreases. The sensitivity can be substantially different for various enantiomers that form diastereomeric complexes with different stability.

The experimental technique used in capillary electrochromatography approaches that employed in HPLC. Therefore, the nature of a selector can be varied over a wider range, which increases the sensitivity of determination and improves the reproducibility of the results. The retention of sorbates increases substantially (the capacity of a stationary phase increases), which improves the selectivity of separation, the efficiency of separation remaining comparable with that in CE.

All the aforesaid provides evidence that CEC holds considerable promise. In the future, CEC may displace both CE and HPLC as applied to chiral separations. However, the necessary condition is the development of technology for the production of columns for CEC, an increase in the stability of their characteristics and availability of various types of commercial columns.

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