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Modified Cyclodextrins for Chiral Separation

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Preface

Chiral separation has attracted considerable attention in both academia and industry, particularly in biological, pharmaceutical, and agrochemical fields. Such interests can be attributed largely to a heightened awareness that many compounds of biological and pharmaceutical interest are chiral and that their enantiomers often exhibit divergent biological properties in terms of, for example, pharmacodynamics, pharmacokinetics and toxicity. The demand for single enantiomers in developing chiral drugs has led to burgeoning development of new strategies toward asymmetric synthesis on the one hand and new methods of chiral resolution on the other. As such, various chromatographic and electromigration techniques have been developed to meet the need for characterizing chiral compounds to greater extents as well as with greater accuracy and precision.

Successful chiral separation in various analytical techniques is largely dependent on the chiral selectors used in either stationary phases or mobile phases. Among all chiral selectors explored in literature, cyclodextrins (CDs) and their derivatives are the most widely used ones. CDs are torus-shapedcyclic D-GLUCO-OLIGOSACCHARIDES, comprising 6, 7, and 8 glucopyranose units connected through glycosidic α-(1,4) linkages. CDs are chiral in nature and feature a hydrophobic interior cavity and hydrophilic edges due to the presence of hydroxyl groups. The hydrophobic cavity endows CDs with their unique inclusion complexation capability with organic guest molecules, which serves as the foundation of their applications in enantioseparation of racemic compounds. In tailoring for chiral separation in various analytical techniques, natural CDs have been extensively modified to increase their stereoselectivity for guest enantiomers, CDs' solubility in desired solvents, and chemical bonding to supporting materials like silica gel.

Modified Cyclodextrins for Chiral Separation focuses on the strategies in cyclodextrin modifications for chiral separation in various chromatographic and electroseparation techniques on an analytical scale. It is not the aim of this book to give a comprehensive overview of the principles for all chiral separation and methods development in various analytical techniques, since there are already many reviews and series of specialized books on this topic. However, hitherto there is no

specific book on CD's modification targeting their applications as chiral selectors in either stationary phases or mobile phases for the various analytical techniques.

Modified Cyclodextrins for Chiral Separation begins with an introduction to cyclodextrin and the general strategies for CDs' modification (Chap. 1). Chapters 2, 3, and 4 summarize functionalized cyclodextrins based chiral stationary phases for the chiral separations using gas chromatography, high-performance liquid chromatography, and supercritical fluid chromatography. Chapters 5, 6, and 7 review the neutral and charged CDs including both anionic and cationic ones for the chiral separation using capillary electrophoresis. These chapters differ from conventional articles because primary emphasis is set on giving overview of different modified CDs for specific analytical techniques.

Ten experienced researchers from both Tang's and Ng's research laboratories have contributed to *Modified Cyclodextrins for Chiral Separation*. We want to express our thanks to all of our authors for making their expertise and knowledge available to those who are not already versed in this area.

This book should be helpful to analytical chemists, pharmaceutical chemists, organic chemists, and pharmacologists, both in research institutions and in industry.

Nanjing, People's Republic of China — Weihua Tang
Nanyang Technological University, Singapore — Siu-Choon Ng
Nanjing, People's Republic of China — Dongping Sun

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Contents

Chapter 1
Modification of Cyclodextrin

Jian Tang and Weihua Tang

Abstract The general information of cyclodextrins is firstly provided in this chapter. The efficient strategies for selective modification of cyclodextrins are then summarized for the development of new chiral selectors for different chromatography and electromigration analytical techniques. The modification strategies here are specially tailored for the interest of researchers in the field of applying cyclodextrin chemistry to chiral separation.

Abbreviations

CD	Cyclodextrin
Mess-N_3-CD	6^A-Azido-6^C-mesitylenesulfonyl-β-cyclodextrin
per-6-NH2-β-CD	Heptakis(6-amino-6-deoxy)-β-cyclodextrin
β-CD-EA	Heptakis(6-hydroxyethylamino-6-deoxy)-β-cyclodextrin
β-CD-OMe (VII)	Heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin
TBDMS	*tert*-Butyldimethylsilyl

1.1 Introduction

Cyclodextrins (CDs) are also known as Schardinger dextrins, cycloamyloses, and cycloglucoamyloses, which comprise a family of cyclic oligosaccharides obtained from starch by enzymatic degradation [1]. They were discovered in 1891 by Villiers

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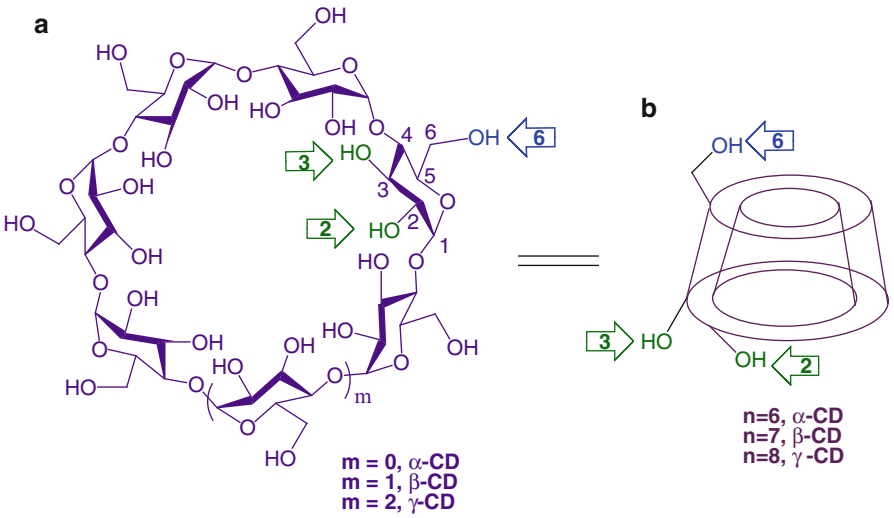


Fig. 1.1 Schematic illustration of α-, β-, and γ-CD (**a**) and their equivalent truncated cone structures; (**b**) 6-hydroxyl groups at the primary rim and the 2- and 3-hydroxyl groups at the secondary rim of CD construct a hydrophilic exterior surface

[2], but the first detailed description of the preparation process and isolation was made in 1903 by Schardinger [3]. In the preparation process, the starch is treated with a group of amylases called cyclodextrinases. The starch helix is hydrolyzed off, and its ends are joined together through α-1,4 linkages [4, 5]. Since these enzymes are not very specific as to the site of hydrolysis, the product contains α-, β-, and γ-cyclodextrins together with small amounts of higher analogues consisting of up to 13 glucose units [6–8].

Naturally occurring α-, β-, and γ-cyclodextrins are cyclic oligosaccharides consisting six, seven, and eight α-1,4-D-glucopyranose units, respectively. Built of glucopyranoside units in the $^4C_1$ conformation, CDs are pictured as a shallow truncated cone with a cavity lined with H3 and H5 protons and lone pairs of glycosidic oxygen atoms lying in a plane thus endowing the cavity with hydrophobic character, while the bases formed by the primary and secondary OH groups bestow a hydrophilic character (Fig. 1.1) [9]. Due to the hydrophilic cavity exteriors, native CDs are water-soluble, while the hydrophobic cavity interiors endow CDs with the capability to encapsulate hydrophobic moieties of a wide range of guest molecules either completely or partially inside the cavity to form "host-guest" inclusion complexes. As the host molecules, cyclodextrins are "all-purpose molecular containers for organic, inorganic, organometallic, and metallo-organic compounds that may be neutral, cationic, anionic, or even radical" [11, 12]. The principal factors involved in the inclusion complexation are van der Waals forces and hydrophobic interactions, while hydrogen bonding and steric effect may play certain roles [12, 13]. The inclusion complexation ability is of great significance to CDs in research and applications including pharmaceutical [14–16], food [17–19], the chemical industry, and, to the greatest extent, separation science [1, 17–20].

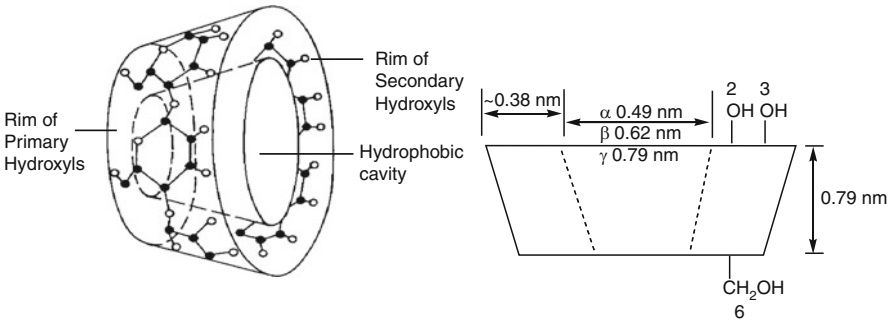


Fig. 1.2 Schematic representation of the cyclodextrin torus

Table 1.1 Some physical and chemical properties of native cyclodextrins

	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Number of glucose units	6	7	8
Formula (anhydrous)	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$
Molecular weight	972.85	1134.99	1297.12
Approximate cavity volume (nm^3)	1.74	2.62	4.27
α_D (deg.)	+150.5	+162.0	+177.4
p*K*a (25°)	12.33	12.20	12.08
Solubility (g/100 ml water, 25 °C)	14.5	1.85	23.2

In general, a cyclodextrin molecule can be briefly described as a torus but is somewhat more realistically pictured as a shallow truncated cone possessing multiple stereogenic centers with a partially blocked base, a hydrophobic interior cavity, and hydrophilic edges due to the presence of hydroxyl groups. All the glucose units in this toroidal structure are in their chair conformation. The interior of the CD cavity is relatively hydrophobic, while the outside rim is more hydrophilic [21, 22]. The rim on the wider side of the CD cavity contains the chiral secondary hydroxyl groups, while the opposite smaller opening is occupied by achiral primary hydroxyl groups. The dimensions are schematically shown in Fig. 1.2.

The sizes of the hydrophobic cavities are different for different types of cyclodextrin; the α-CD can accommodate a single phenyl ring, while β-CD and γ-CD can accommodate substituted single and multiple ring systems. This inclusion alone is not enough for chiral recognition: interactions between substituents on the asymmetric center of the analyte and the hydroxyl groups on the CD rim are responsible for chiral recognition. Some physical properties of these three CDs are quite different (as shown in Table 1.1) [23–27].

CDs are able to be regarded as "hosts" for "guest" molecules capable of entering (in whole or in part) the cavity and forming noncovalent host-guest inclusion complexes. Almost all applications of CDs involve complexation. The mechanism of inclusion complexation between CD-host and molecule-guest is schematically shown in Fig. 1.3. A combination of weak forces such as hydrophobic interaction, electrostatic interaction, van der Waals interaction, hydrogen bonding, and dipole-dipole

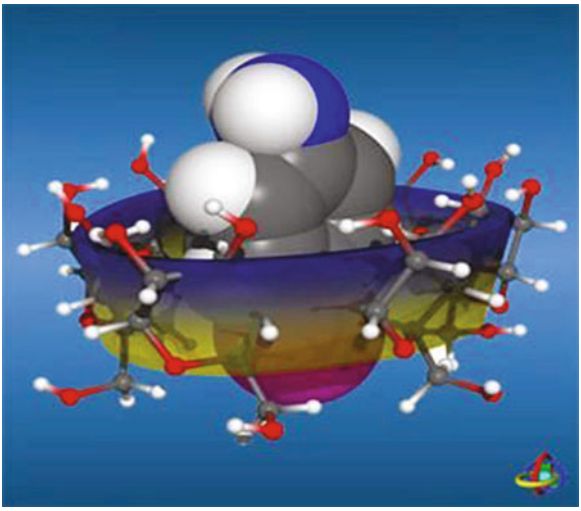


Fig. 1.3 Inclusion complexation between CD-host and molecule-guest

interaction cooperatively governs the inclusion complexation behavior of the cyclodextrin hosts. These weak interactions between CDs and guest molecules result in a selectivity-structure correlation, and this correlation forms the basis for chiral separation and other applications using CDs. An important feature of this technique is that CDs introduce a shape-selective effect that is beneficial for the separation of structural, geometrical, and optical isomers. The inclusion complex between the CDs and solutes depends on the size and shape of molecules, as well as the interior size of the CDs. The attractive properties of CDs have led many researchers to develop novel CD derivatives. The hydroxyl groups present on the rim of the CDs can be easily modified by chemical reactions. Modified CDs may have very different properties compared with native CDs. These include increased solubility, possibility for different secondary bonds, different hydrophobicity of the cavity, and potentiality for the analysis of highly hydrophobic and uncharged compounds.

When CDs selectively form inclusion complexes with enantiomeric guest, chiral recognition occurs. CDs and their derivatives are the most widely used chiral selectors for enantiomeric separation of racemic guest molecules, especially those with biological, pharmaceutical, and agrochemical interest [28, 29]. There are literally thousands of cyclodextrin derivatives that have variable ring size and random or site-specific chemical functionalization [14]. The modified CDs harvest better solubility in certain solvents and improved chiral selectivities towards specific guest molecules in various practical applications.

Despite the numerous CD derivatives were investigated in the past decades for the purpose of chiral separation, a great need still exists for further effort in synthetic chemistry aiming at the development of novel CDs with improved chiral recognition ability towards a large pool of racemic analytes with shorter separation time and greater analysis accuracy.

Strategies for selective CD modification have been well reviewed in other literature for wide applications such as analytical, catalytic, biological, and pharmaceutical [30, 31]. This chapter provides a specific overview of the modification strategies of various CD derivatives as chiral selectors for analytical chromatographic techniques and capillary electrophoresis.

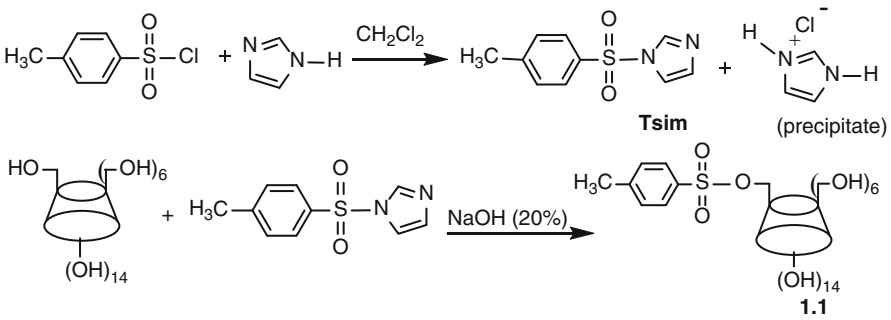
1.2 Cyclodextrin Modification

There are two primary factors needed to be considered in the chemistry of cyclodextrins for their modification: the nucleophilicity of the hydroxyl groups and the ability of cyclodextrins to form complexes with the reagents used [31]. All modifications of cyclodextrins take place at the hydroxyl groups. Since hydroxyl groups are nucleophilic in nature, the initial reaction, which directs the regioselectivity and the extent of modification (mono, di, tri, per, etc.) of all subsequent reactions, is an electrophilic attack on these positions.

The best method to provide cyclodextrins of any size, shape, and most importantly containing any functional groups is to selectively convert the hydroxyl groups to other desired functionalities. The modification of cyclodextrins offers chemists both enormous opportunities and challenges. Hydroxyl groups present at the 2-, 3-, and 6-positions compete for the reagent used, which makes selective modification extremely difficult. Of the three types of hydroxyl groups present in CD rims, the most basic (and often most nucleophilic) are those at the 6-position, the most acidic are those at the 2-position, and the most inaccessible are those at the 3-position [32, 33]. Thus, under normal circumstances, the 6-position is easily attacked by an electrophilic reagent (Fig. 1.1). The less reactive reagents will attack the hydroxyl groups more selectively. Thus, more reactive reagents will not only react with hydroxyl groups at the 6-positions but also with those on the secondary side; whereas, less reactive reagents will react more selectively with the 6-position hydroxyl groups. For instance, the less reactive reagent *tert*-butyldimethylsilyl chloride (TBDMSCl) will react selectively with hydroxyl groups at the 6-positions [34], while the more reactive reagent trimethylsilyl chloride (TMSCl) will indiscriminately react with all hydroxyl groups in CD rims [35].

1.2.1 Monosubstitution at the 6-Position of Cyclodextrins

As discussed above, the primary hydroxyl groups of the CD are the most nucleophilic; this may be selectively modified by reaction with electrophilic species. For example, cyclodextrins react with *p*-toluenesulfonyl chloride in pyridine or DMF containing a base, to give a mixture of products arising from sulfonation of these groups [36, 37]. The corresponding C(6) CD monotosylates may then be obtained by separation from the mixtures via chromatography in the case of α-CD, and

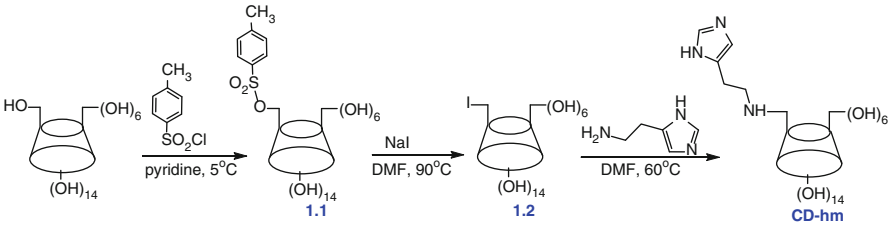


Scheme 1.1 Synthesis of mono-6-tosyl-β-cyclodextrin

through recrystallization from water for the β-CD derivative. As a nonuser-friendly solvent of choice for this reaction, pyridine forms a pyridinium complex with the cavity and complicates the work-up process. However, the major advantage of this solvent is its ability to direct the reaction to the 6-position as compared to DMF where sulfonation occurs on both faces of cyclodextrin. The method of choice [38] for the synthesis of monotosylcyclodextrin is to react cyclodextrin with tosyl chloride in 1:1 equivalent ratio in aqueous alkaline medium for a short time to give the mono-6-tosylate in fairly good yield. The product is obtained in a reasonable purity either by repeated crystallization from water or by chromatography on a charcoal column [38].

The synthesis of mono-6-tosyl-β-cyclodextrin was recently improved by taking advantage of CD's complexation property to introduce one tosyl group specifically to 6-position or 2-position. Facile synthesis of mono-6-tosyl-β-CD is achieved by preparing the key intermediate compound, 1-(*p*-toluenesulfonyl)imidazole (Tsim), for better yield and easier handling. As shown in Scheme 1.1, a two-step protocol was used to eliminate the drawbacks of the previously reported approach using pyridine [39, 40]. With tosyl imidazole as key intermediate compound, mono-6-tosyl-β-cyclodextrin **1.1** can be synthesized in aqueous base solution in an overall yield of 36 %. This approach features easy work-up and selective modification with good yield.

With the readily obtained mono-6-tosyl-cyclodextrins at hand, a further nucleophilic attack of mono-6-sulfonylcyclodextrin with a reagent containing the appropriate group has thus been developed into the most popular method for monomodifications at the 6-position of cyclodextrins. Mono-6-tosyl-cyclodextrins are important precursors for a variety of modified cyclodextrins because a nucleophile can attack the electrophilic carbon atom at the 6-position to produce a corresponding functionality. A nucleophilic displacement of the tosyl group may be realized by using suitable nucleophiles such as iodide, azide, thioacetate, hydroxylamine, alkyl, or poly- (alkylamines) to afford monoiodo- [31], ^{0}azido- [41], thio- [42, 43], (hydroxylamino)- [44], or (alkylamino)cyclodextrins [45].



Scheme 1.2 Synthesis of CD-hm (Reprinted with permission from Ref. [46]. Copyright 1991 American Chemical Society)

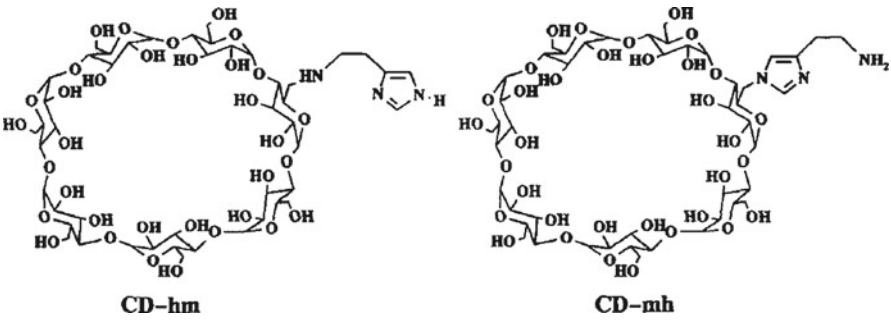
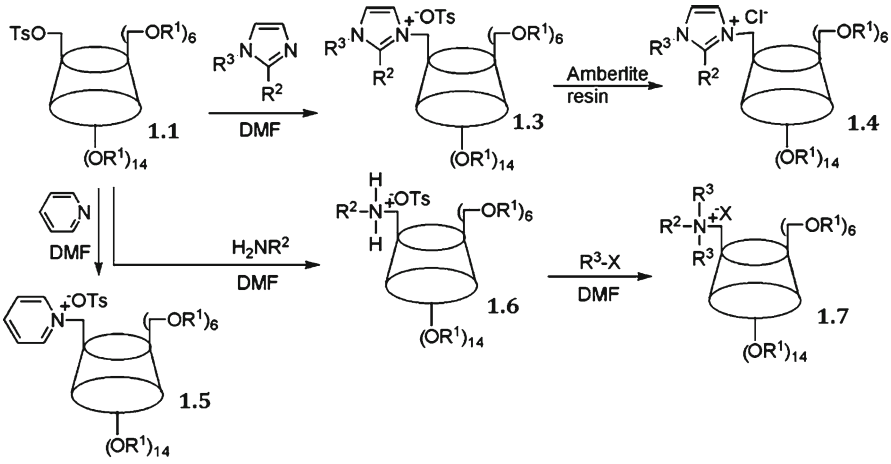


Fig. 1.4 The structure of CD-hm and CD-mh (Reproduced from Ref. [47] by permission of John Wiley & Son Ltd.)

An example of the using of nucleophilic displacement of mono-6-tosyl-cyclodextrin with NaI to prepare monoiodocyclodextrins and further histamine-substituted β-CD [6-Deoxy-6-(N-histamino)-β-cyclodextrin, CD-hm] is shown in Scheme 1.2 [46–48].

By linking the histamine group onto the upper rim of CD linked by the imidazole nitrogen, Marchelli and his coworkers also developed the analogue of CD-hm, 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]-β-cyclodextrin (CD-mh) (structure in Fig. 1.4) [47]. Good enantiomeric separation for dansylated amino acids was obtained by using low concentrations of the selectors (1–3 mM). In order to modulate the number and the position of the positive charges, the electrolyte pH was increased from 5 to 7.5, where the chiral discrimination decreased along with the deprotonation of the imidazolyl moiety when pH increased. The results showed that CD-mh presented better chiral resolution ability than CD-hm under the same analytical conditions, indicating the type and position of cation groups are crucial for the chiral separation using electrostatic interactions as the driving forces besides inclusion complexation.

A library of cationic β-CDs substituted at the 6-position with imidazolium (**1.3**, **1.4**), pyridinium (**1.5**), and quaternary ammonium (**1.6**, **1.7**) moieties (Scheme 1.3) were firstly reported in Ng's group [40, 49]. Starting from mono-6-tosyl-β-CD, a nucleophilic substitution with alkylimidazole, pyridine, or alkylamine and a further

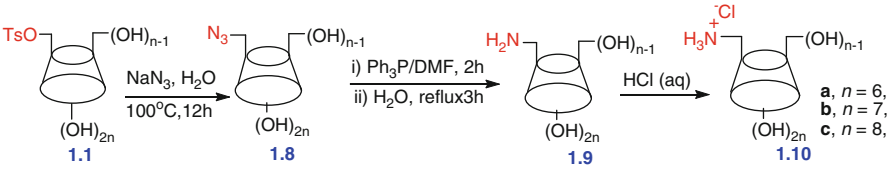


Scheme 1.3 Synthesis of mono-6-alkylimidazolium-, pyridinium-, and quaternary ammonium-substituted β-CDs (Reprinted from Ref. [49], Copyright 2005, with permission from Elsevier)

Table 1.2 Yields and melting point data for cationic β-CDs [49]

CDs	R^1	R^2	R^3	X^-	Yields (%)	Melting point (°C)
1.3a	H	H	CH_3	OTs	99	257–259 (dec)
1.3b	H	H	*n*-C_4H_9	OTs	97	254–256 (dec)
1.3c	H	H	*n*-C_3H_{17}	OTs	98	256–258 (dec)
1.3d	H	CH_3	CH_3	OTs	62	257–258 (dec)
1.4a	H	H	CH_3	Cl	93	233–235 (dec)
1.4b	H	H	*n*-C_4H_9	Cl	93	249–250 (dec)
1.4c	H	CH_3	CH_3	Cl	82	199–200 (dec)
1.3e	Ac	H	CH_3	OTs	64	129–130
1.3f	Ac	H	*n*-C_4H_9	OTs	76	115–116
1.3g	Ac	CH_3	CH_3	OTs	62	125–126
1.5	H	H	–	OTs	85	239–241 (dec)
1.6a	H	C_3H_5	–	OTs	92	249–250 (dec)
1.6b	H	*n*-C_3H_7	–	OTs	98	260–261 (dec)
1.6c	H	*n*-C_4H_9	–	OTs	97	263–264 (dec)
1.6d	H	*n*-C_5H_{11}	–	OTs	96	266–267 (dec)
1.7	H	*n*-C_3H_7	CH_3	I^-	89	241–243 (dec)

anion exchange afford the monosubstituted alkylimidazolium β-CDs, pyridinium, and alkylammonium β-CDs with varied alkyl chain length. For CDs **1.3** and **1.4**, a general methodology involves the heating of mono-6-tosyl-β-CD with alkylimidazoles in DMF at 90 °C for 2 days. Furthermore, the tosylate anion was converted into chloride by ion exchange through Amberlite 900 (Cl) resin (Scheme 1.3). The CDs **1.5** was prepared by refluxing mono-6-tosyl-β-CD with pyridine at 90 °C for 2 days. For CDs **1.6** and **1.7**, similar methodology is adopted by refluxing alkylamine together with mono-6-tosyl-β-CD **1.1** in DMF for 5 h. While the melting points of these compounds are too high (115–267 °C) (Table 1.2) for ionic liquid



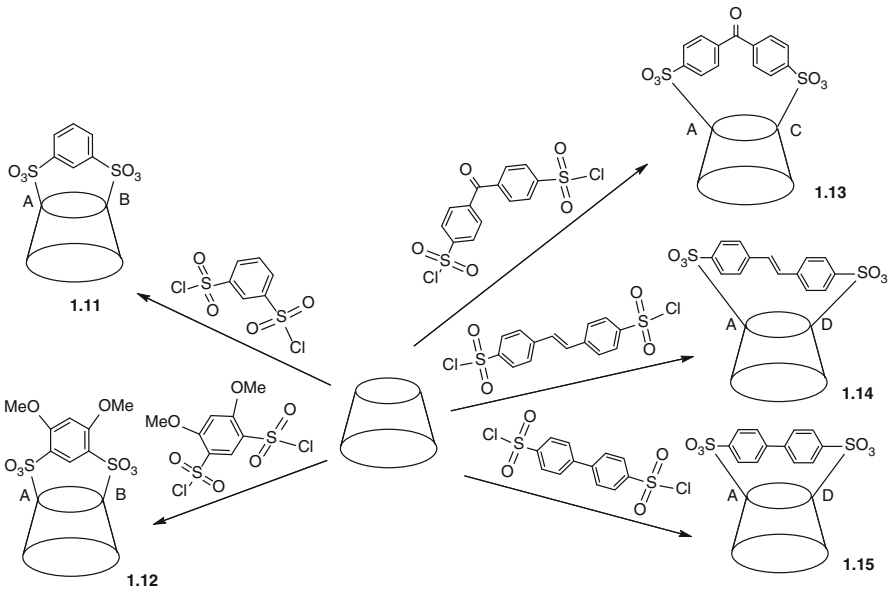
Scheme 1.4 Synthetic approach to mono-6-ammonium CDs

applications, these cationic cyclodextrins are proved highly successful stationary phases for the enantioseparation of aromatic carboxylic acids at low concentrations (3–10 mM). This is presumably due to the strong electrostatic interaction between cationic CD and the anionic analytes [50–52].

Monoamino derivatives of cyclodextrin are conveniently obtained by nucleophilic displacement of tosyl group with ammonia under pressure (10^6 N m^{-2}) for 18 h at room temperature [53] or azide substitution of CD tosylate and followed by hydrogenation with Pd/C for 12 h [54]. Both procedures are low- yield and include incomplete reactions, thus undesirable for large-scale production. An innovative methodology was developed by Tang et al. (Scheme 1.4) [55, 56], which involved the reduction of 6-azido CD (**1.8**) by using of triphenylphosphine and a further hydrolysis to afford monoamino CD (**1.9**). A subsequent treatment of monoamino CDs with dilute hydrochloric acid yields the targeted highly water-soluble cationic CD (**1.10**). These steps are almost quantitative with yield higher than 90 %. This approach has been greatly improved with mild reaction conditions and higher yields than previously reported methods.

1.2.2 Disubstitution at the 6-Position of Cyclodextrins

A particularly efficient method to obtain disubstituted sulfonates of cyclodextrins is by reaction of arenedisulfonyl chlorides with cyclodextrins to give AB, AC, and AD isomers [57–59]. Although these disulfonyl chlorides give a mixture of regioisomers, they show distinct regiospecificity based on their structures. An elegant method to control the regiospecificity to produce AB, AC, or AD isomers by using the geometry of the reagents has been described [60]. For example, as shown in Scheme 1.5, *trans*-stilbene and biphenyl-based capping reagents preferentially give AD isomers **1.14** and **1.15** in yields of 20 and 18 % [60, 61]. Benzophenone-based reagents give AC isomers **13** in 40 % yield [58, 60]. 1,3-Benzenedisulfonyl chlorides [62] (especially the electron-rich 4,6-dimethoxybenzene-1,3-disulfonyl chloride [63]) gives the AB isomers **1.11** and **1.12** in 40 and 12 % yields. Anthraquinone-2,6-disulfonyl chloride gives AC and AD isomers in low yield after purification by HPLC [64]. Bis(9,10-dicyanoanthracenesulfonyl) chloride produces two isomers (AD and AC) in a ratio of 3:1, showing some degree of selectivity [65]. Among the three kinds of regioisomers, the 6^A,6^B-capped CDs are the most stable and less susceptible to hydrolysis [30].



Scheme 1.5 Using the geometry of reagents to direct the regiospecificity in disubstitution of cyclodextrins

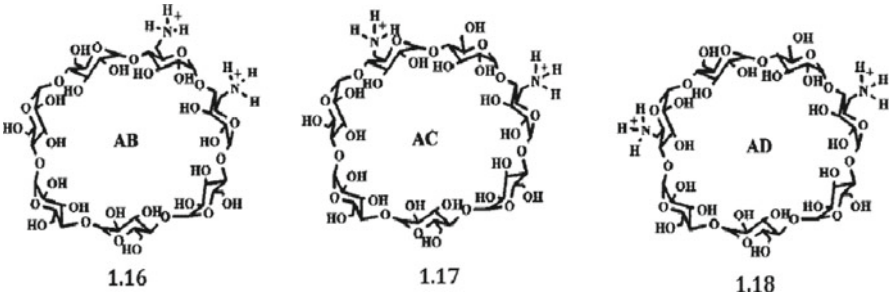
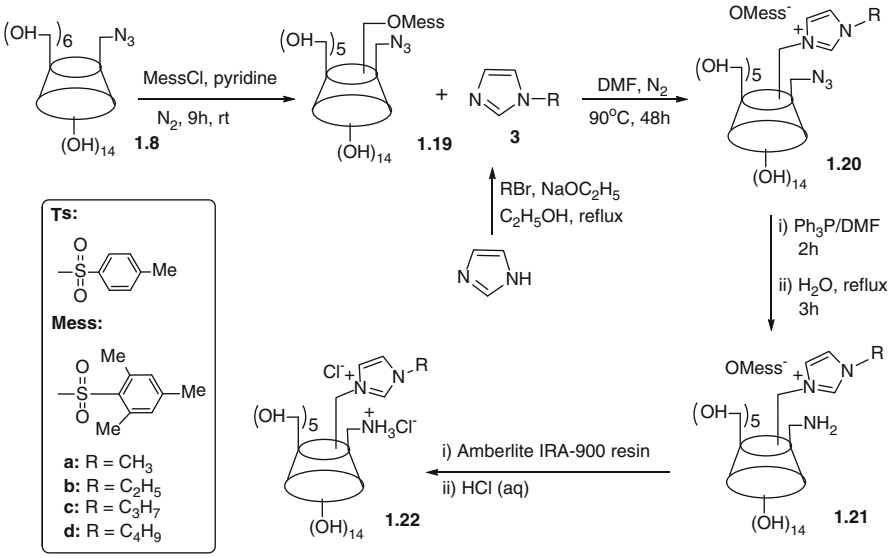


Fig. 1.5 Structure of the three 6,6′-dideoxy-6,6′-diamino-β-cyclodextrins AB (**1.16**), AC (**1.17**), and AD (**1.18**) (Reproduced from Ref. [66] by permission of John Wiley & Sons Ltd.)

The disubstituted sulfonates of cyclodextrins can be used as important precursor for many disubstituted CDs with the same functionalities at 6-positions. For example, selectively modified 6,6′-dideoxy-6,6′-diamino-β-cyclodextrins (AB, AC, AD) was successfully developed by Marchelli and his coworkers [66]. Disulfonated AB, AC, and AD isomers of β-CD were synthesized with high selectivity by using appropriate capping reagents, i.e., 4,6-dimethoxybenzene-1,3-disulfonylchloride, benzophenone-3,3′-disulfonylchloride, and biphenyl-4,4′-disulfonylchloride, respectively [60, 62, 63]. The disulfonated CDs were further nucleophilic substituted with sodium azide [67], followed by reduction with the Staudinger reagents [68] to afford the desired diamino CD derivatives (**1.16, 1.17, 1.18** in Fig. 1.5).



Scheme 1.6 Synthetic route to 6^A-ammonium-6^C-alkylimidazolium-β-cyclodextrin chlorides (Reproduced from Ref. [69] by permission of The Royal Society of Chemistry)

For the case of disubstituted CDs with different functionalities, a facile approach was recently developed in Tang's group [69, 70]. As depicted in Scheme 1.6, the synthetic route for dicationic CDs was established as following: an azide group was first introduced onto C(6A) position of CD [39]; mono-azido-β-CD **1.8** was further nucleophilic substituted with 2-mesitylenesulfonyl chloride to give a mixture of three regioisomers, 6^A-azido-6^X-mesitylenesulfonyl-β-cyclodextrin (X = B, C or D). The mixture was then subjected to a reversed-phase column liquid chromatography [71] to afford the desired AC regioisomer, 6^A-azido-6^C-mesitylenesulfonyl-β-cyclodextrin (Mess-N_3-CD) **1.19**, with a yield of 42 %.

The AC regiochemistry was supported by comparison with the known AC [58], AB [60], and AD [63] regioisomers synthesized with capping of CD. The AC regioisomer structure **1.19** is confirmed by its most characteristic absorptions [101.68 (C1), 81.53 (C4)] in ^{13}C NMR, where the strong peaks centered at 71.82 are assigned to C2, C3, and C5 while peak at 59.67 assigned to C6 adjacent to hydroxyl group on primary rim of CD (Fig. 1.6). The as-prepared Mess-N_3-CD **1.19** was then reacted with 3-alkylimidazoles via nucleophilic addition to introduce imidazolium cation onto C(6C) position of CD. Staudinger reaction was further employed to achieve 6^A-amino-6^C-alkylimidazolium-β-cyclodextrin mesitylene sulfonate **1.21**. The final dicationic CDs were synthesized via protonation with dilute hydrochloric acid and anionic exchange with Amberlite (Cl) resin. An example of the confirmation of the final products is shown in Fig. 1.6b for 6^A-ammonium-6^C-butylimidazolium-β-CD chlorides (**1.22d**) [70].

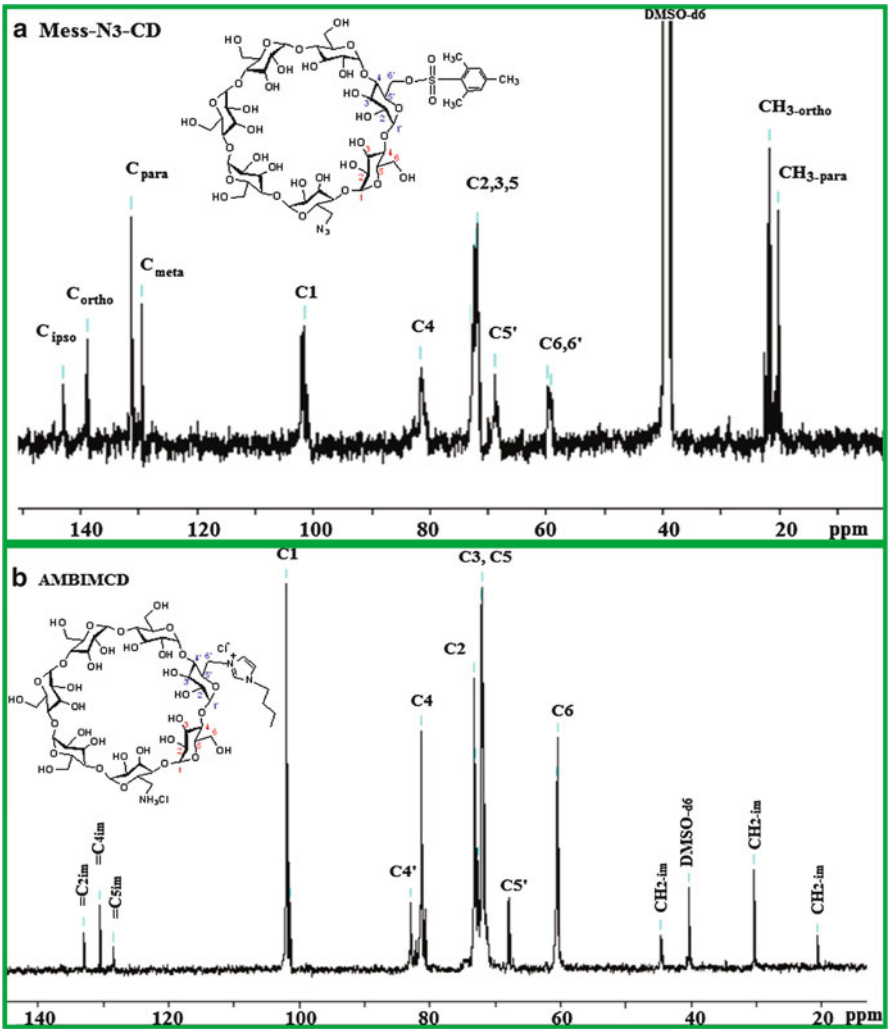
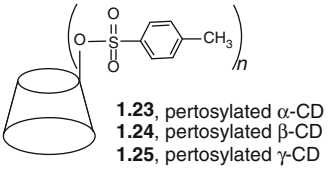


Fig. 1.6 (**a**) 500 MHz ^{13}C NMR spectrum of Mess-N_3-CD **1.19** in DMSO-$_{d6}$, where C_{ipso}, C_{ortho}, C_{para}, C_{meta} refers to the respective carbon atoms in benzyl ring of mesitylenesulfonyl moiety while $CH_{3\text{-}ortho}$ and $CH_{3\text{-}para}$ are assigned to the respective carbon atoms of methyl groups. (**b**) 500 MHz ^{13}C NMR spectrum of **1.22d** in DMSO-$_{d6}$

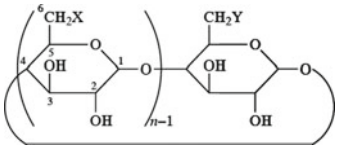
1.2.3 Persubstitution at the 6-Position of Cyclodextrins

According to statistical calculations, permodification of the primary face should give 57 % yield (assuming 91 % yield per reaction; $0.91^6 = 0.57$) in the case of α-cyclodextrin. However, the actual yield of hexa-6-substituted cyclodextrin is often found to be much lower. Steric crowding, the geometry of the molecule, the

Table 1.3 Structure of 6-persubstituted CDs



1.23, pertosylated α-CD
1.24, pertosylated β-CD
1.25, pertosylated γ-CD



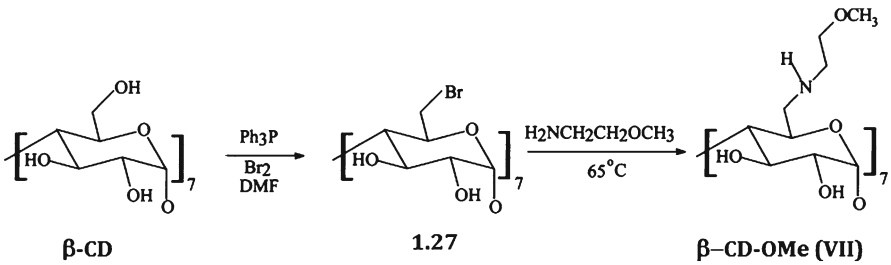
1.26-1.38

	X = *Y*	*n*	Abbreviation
1.26	Br	6	Ref. [75]
1.27	Br	7	Refs. [75, 76]
1.28	Br	8	Ref. [75]
1.29	I	6	Ref. [77]
1.30	I	7	Ref. [78]
1.31	Cl	7	Ref. [79]
1.32	NH_2	7	per-6-NH2-β-CD [78, 80]
1.33	$NHCH_3$	7	heptamethylamino-β-CD [81, 82]
1.34	$NHCH_2CH_3$	7	heptaethylamino-β-CD [81]
1.35	NH_3^+	6	Per-NH_3^+-α-CD [83]
1.36	NH_3^+	7	Per-NH_3^+-β-CD [83]
1.37	$NHCH_2CH_2OH$	7	β-CD-EA [77, 78]
1.38	$NHCH_2CH_2OCH_3$	7	β-CD-OMe (VII) [79]

type of inclusion complex formed, and positional isomerism decrease the yield of the product as the degree of substitution increases [72].

Persulfonates are generally prepared directly from cyclodextrins and a large amount of sulfonyl chloride in pyridine. The reaction between CD persulfonates with sodium halides was used to prepare CD polyhalides [73, 74]. More directly, CD polybromides **1.26–1.28** (Table 1.3) were prepared by treatment of native α-, β-, or γ-CD with methanesulfonyl bromide in DMF, followed with sodium methoxide [75]. The treatment of β-CD with triphenylphosphine and iodine afforded polybromides 1.27 in 93 % yield [76]. The α- and β-CD polyiodes **1.29–1.30** were directly prepared with α- or β-CD with triphenylphosphine and iodine in 80 and 88 % yields, respectively [77, 78]. Reaction of β-CD with methanesulfonyl chloride and imidazole in DMF afforded the chloride **1.31** in 90 % yield [79].

With CD persulfonates and CD polyhalides at hand, further nucleophilic substitution can be employed to prepare a large library of 6-persubstituted CD derivatives. For example, a reaction of polyiodine **1.30** with sodium azide, followed by Staudinger reduction, afforded the polyamine **1.32** [78, 80]. Direct substitution of halides such as **1.26–1.30** with alkylamine gives the corresponding per-alkylamino-substituted CDs [84, 85]. Alternatively, the per-methylamino-substituted CD **1.35** and per-ethylamino-substituted CD **1.36** were directly prepared by treated β-CD with methylamine or ethylamine, respectively. The treatment of per-amino-substituted CDs with



Scheme 1.7 Synthesis route for heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)] (Reprinted from Ref. [87], Copyright 1998, with permission from Elsevier)

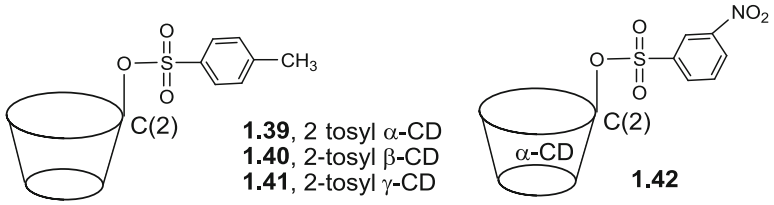
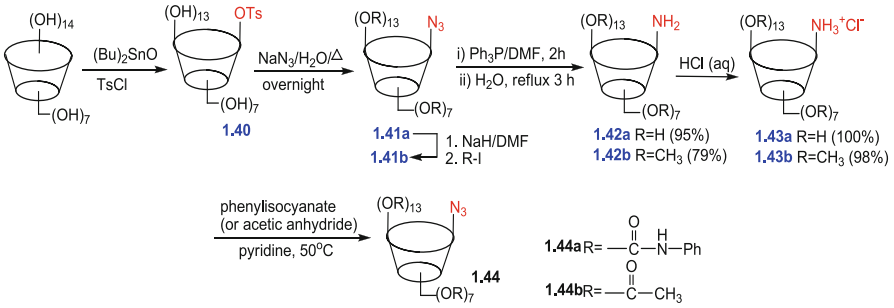


Fig. 1.7 Structure of mono-2-sulfonate CDs

dilute HCl solutions can afford salt form of CDs **1.35** and **1.36** [83]. The heptakis (6-hydroxyethylamine-6-deoxy)-β-cyclodextrin (β-CD-EA) [50, 86] and heptakis (6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)] [87] were prepared in 60–65 % yield by direct nucleophilic substitution of polybromide **1.27** with hydroxyethylamine or methoxyethylamine, respectively. The synthetic route for approach β-CD-OMe (VII) is shown as Scheme 1.7.

The secondary hydroxy groups of the CDs are the most acidic, with pKa values near 12.2 [88]. And due to the presence of twice the number of hydroxy groups at the secondary rim of CD than the primary rim, the steric hindrance for substitution at secondary side of CDs is larger than that at the primary side. Moreover, the hydrogen bonding between hydroxy groups at the 2- and 3-positions makes them rigid and less flexible as compared to C-6 hydroxy groups. All these factors make the secondary side less reactive and harder to be selectively functionalized than the primary face. During the course of a reaction, as the degree of substitution increases, the secondary side becomes even more crowded. The increased steric hindrance with the incoming nucleophile forces the attacking group towards the other face, which decreases the selectivity [31].

The C(2) secondary hydroxy groups are the most nucleophilic above pH 10–11, whereas under neutral and acidic condition, it is the primary hydroxy groups [30]. The selective modification of the C(2) secondary hydroxys of CD is thus generally realized through treatment with electrophilic reagents. The preparation of mono-2-tosyl β-CD **1.40** (Fig. 1.7) was achieved by treatment of



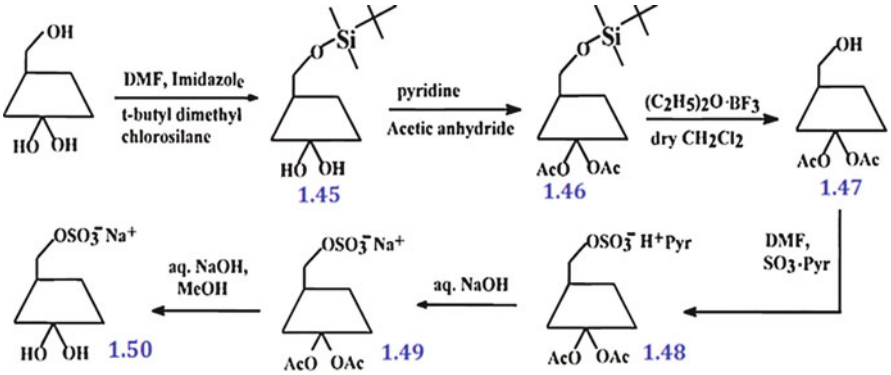
Scheme 1.8 Synthesis route for mono-2-functionalized CDs

β-CD with *p-toluenesulfonyl* chloride and sodium hydride in DMF [89], whereas the corresponding mono-2-tosyl α-CD **1.39** was prepared using *p*-toluenesulfonyl chloride and α-CD in aqueous alkaline solution [90]. The reaction of α-CD with *m*-nitrobenzenesulfonyl chloride at pH 12 afforded C(2) sulfonate **1.42** [91]. The sulfonate **1.40** was also accessible with the reaction of β-CD with *m*-nitrophenyl tosylate in pH 10 aqueous solution [92].

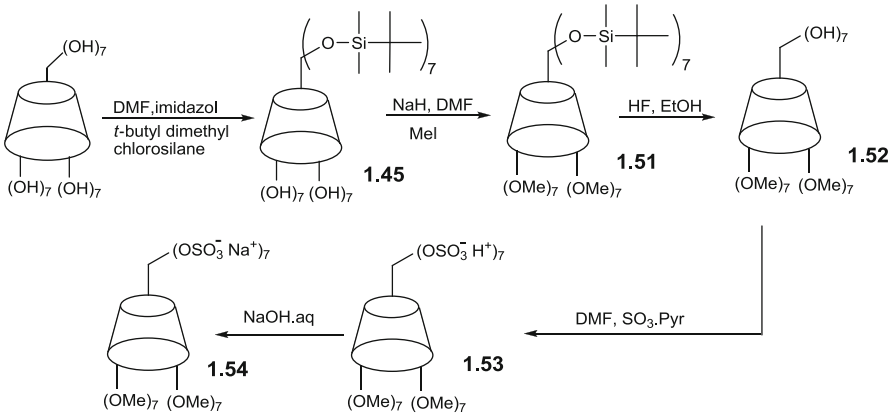
Alternatively, the mono-2-tosylate **1.39–1.41** can be synthesized by using dibutyltin oxide. The C(2) hydroxy group was activated by the reaction of dibutyltin oxide with 1,2-diols at the secondary rim of a CD glucopyranose unit. The mono-2-tosylate **1.39–1.41** can be important precursors for large library of 2-functionalized CDs. As shown in Scheme 1.8, the reaction of mono-2-tosyl β-CD **1.40** with sodium azide afforded mono-2-azide β-CD **1.41** [40]. Followed reduction with triphenylphosphine generated mon-2-amino β-CD **1.42**, which was further transferred into salt form in almost quantitative yields. The rest of hydroxy groups at C(6), C(3), and C(2) positions can be fully substituted with methyl, phenylcarbamate, or acetyl functionalities with nonselective nucleophilic substitution [93].

Besides pH, the formation of an inclusion complex by the cavity of cyclodextrins and the relative orientations of reactive groups in the complexes also play a very prominent role in the regioselectivity of reaction of CDs. An included reagent may react with the hydroxy groups at the 2-, 3-, or 6-positions depending on the nature of the complex [92]. Some of these problems can be overcome by protection of the primary side before modification of the secondary side [31]. For example, per-6-silyl-mono-2-tosylcyclodextrin is synthesized by the reaction of 6-silylated cyclodextrin with tosyl chloride in THF with NaH as a base in 32 % yield after purification [94]. An advantage of this strategy is that the reaction as well as the purification steps can be carried out in organic solvents and the desilylation can be carried out easily to yield the desired tosylate.

The protection strategy was widely used to prepare C(2) and C(3) persubstituted CD derivatives. As shown in Scheme 1.9 [95], heptakis-(2,3-diacetyl-6-sulfato)-*β-cyclodextrin* **1.49** is produced by first reacting β-cyclodextrin with dimethyl-*tert*-butylchlorosilane [96], purifying the intermediate **1.45** with gradient elution preparative column chromatography [97] on silica gel using *n*-hexane/ethyl acetate/ethanol as



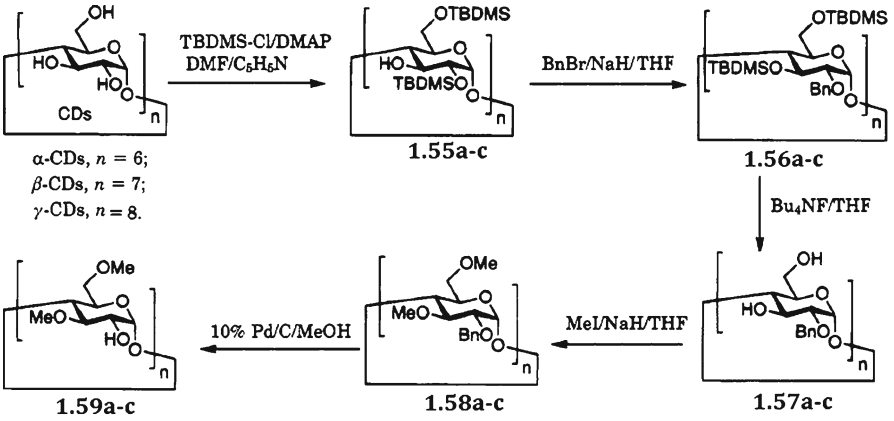
Scheme 1.9 Synthesis scheme for heptakis(2,3-diacetyl-6-sulfato) -β-cyclodextrin (Reprinted with the permission from Ref. [95]. Copyright 1997 American Chemical Society)



Scheme 1.10 Synthesis scheme for heptakis(2,3-dimethyl-6-sulfato)-*β*-cyclodextrin (Reprinted with the permission from Ref. [100]. Copyright 1998 American Chemical Society)

eluent. The purified intermediate was then peracetylated with acetic anhydride. The purified heptakis(2,3-diacetyl-6-dimethyl-*tert*-butylsilyl)-β-cyclodextrin **1.46** was then reacted with boron trifluoride etherate to remove the dimethyl-*tert*butylsilyl-protecting group [96]. Next, the pure heptakis-(2,3-diacetyl)-*β*-cyclodextrin **1.47** was reacted with SO3•pyridine in DMF to completely sulfate the primary hydroxyl groups of the cyclodextrin [98]. By neutralizing the aqueous solution of **1.48** with NaOH, the desired heptakis-(2,3-diacetyl-6-sulfato)-*β*-cyclodextrin **1.49** was obtained. The deacetylation of **1.49** by reacting with 25 % methanol in pH 12 aqueous solution for 12 h afforded sodium salt of hepta-6-sulfato-*β*-cyclodextrin **1.50** [99].

By using similar protection strategy, heptakis(2,3-dimethyl-6-sulfato)-*β*-*cyclodextrin* was prepared from per-6-6-dimethyl-*tert*-butylsilylated *β*-CD. After complete methylation at secondary side of CD, the intermediate **1.51** (Scheme 1.10)



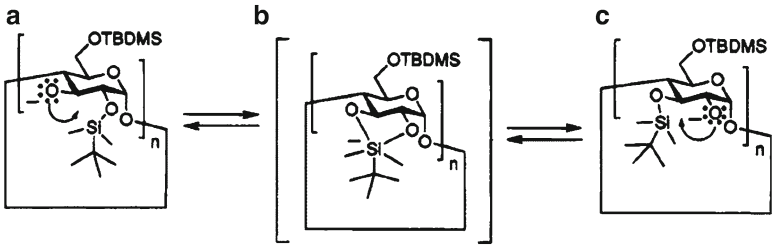
Scheme 1.11 Synthesis of the per(3,6-di-O-methyl)-cyclodextrins (Reprinted with the permission from Ref. [101]. Copyright 1995 American Chemical Society)

was then reacted with HF in ethanol to remove the *tert-butyldimethylsilyl-protecting* group to afford **1.52**. The primary hydroxy groups were further sulfated with pyridinesulfonate in DMF, followed by treatment with aqueous NaOH solution to generate the desired product [100].

The persubstitution of the C(2) hydroxy groups is generally achieved by first protecting the primary and C(3) hydroxy groups, to avoid the competing processes. As illustrated in Scheme 1.11, a convenient synthesis of per-O-benzyl CDs **1.57a-c** is achieved with a first silyl protection step for both C(6) and C(2) positions of CDs and a second migration step of silyl protection groups between C(2) and C(3) hydroxy groups [101]. The migration of silyl protection groups was thought to be an intramolecular process, which exposed the C(2) alkoxides for functionalization. Hence, the treatment of intermediate compound **1.55a-c** with benzyl bromide, followed by desilylation of the product of **1.56a-c** with tetrabutylammonium fluoride, affords the corresponding per-O-benzyl CDs **1.57a-c**. Similarly, the reaction of **1.55a-c** with methyl iodine, followed by desilylation of the resultant product, gives heptakis(2-O-methyl)-β CD.

The mechanism for the migration of silyl group was explained as in Scheme 1.12, where a five-membered ring intermediate **B** containing a pentacovalent silicon atom was most likely involved. The alkylation occurs under kinetic control, i.e., the species **A** is more stable than the isomeric species **C** but **C** is more reactive as a result of the proximity of the 2-OH to the anomeric center [101]. The yields of these reactions are 78 % for β-CD, 76 % for γ-CD, and 49 % for α-CD (49 %). The lower yield of the benzylation of the α-CD derivative is probably a consequence of the higher steric hindrance of the smaller α-CD ring and the bulky substituents, i.e., TBDMS and benzyl groups.

Due to the high inaccessibility of those hydroxy groups at the 3-position of CD, the selective modification of C(3) hydroxy groups is realized by using special reagents to



Scheme 1.12 The plausible mechanism for TBDMS migration from the O-2 to the O-3 positions of the CDs (Reprinted with the permission from Ref. [101]. Copyright 1995 American Chemical Society)

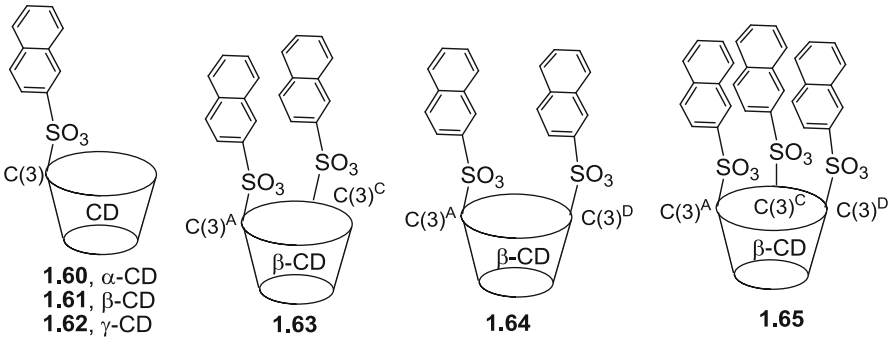
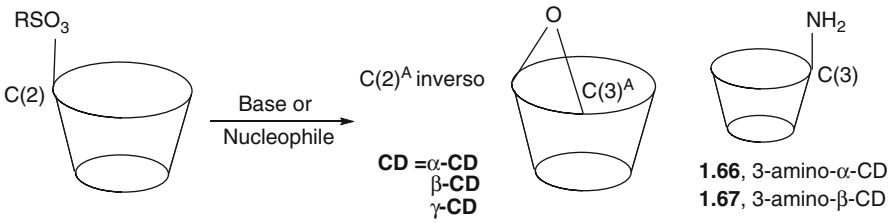


Fig. 1.8 Structures of mono-3-sulfonate CDs, $3^A,3^C$- and $3^A,3^D$-disulfonate β-CDs, and $3^A,3^C,3^E$-trisulfonate β-CD

avoid the competition of more reactive C(2) and C(6) hydroxys. The mono-3-tosylated α- and β-CDs are generally prepared with β-naphthalenesulfonyl chloride to give the corresponding monosulfonate **1.60** (Fig. 1.8) [91] and **1.61**, respectively [102]. Using the same approach, pure monosulfonate **1.62** can be obtained through chromatography separation from a mixture of C(2) and C(3) derivatives [103]. The 3-disusulfonate β-CDs **1.63** and **1.64** can be obtained by chromatography separation from their mixture with **1.61**. The reaction mixture was achieved by the treatment of β-CD with β-naphthalenesulfonyl chloride in 30 % aqueous CH_3CN at 40 °C, the initial pH of β-CD solution was adjusted to 12, and the pH was allowed to decrease during the reaction. The reversed-phase chromatography afforded pure sulfonates **1.61** with a yield of 18.0 %, **1.63** with 4.5 %, and **1.64** with 4.4 % [102]. The trisulfonate β-CD, however, was obtained much easier in comparison to disulfonate ones. The only one isomer ($3^A,3^C,3^E$-trisulfonate β-CD, **1.65**) out of five 3,3,3-tri-O-sulfonyl-β-CDs was obtained with a yield of 17.8 % by treatment of β-CD with β-naphthylsulfonyl chloride in alkaline aqueous acetonitrile (pH 12) [104].

There are still some 3-substituted CDs prepared using CD C(2) sulfonate by taking advantage of the displacement reactions of CD C(2) sulfonate. As illustrated in



Scheme 1.13 Displacement reactions of CD C(2) sulfonates afford *manno*-2,3-epoxy CDs

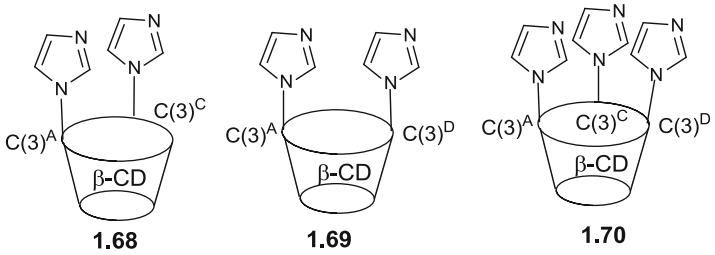


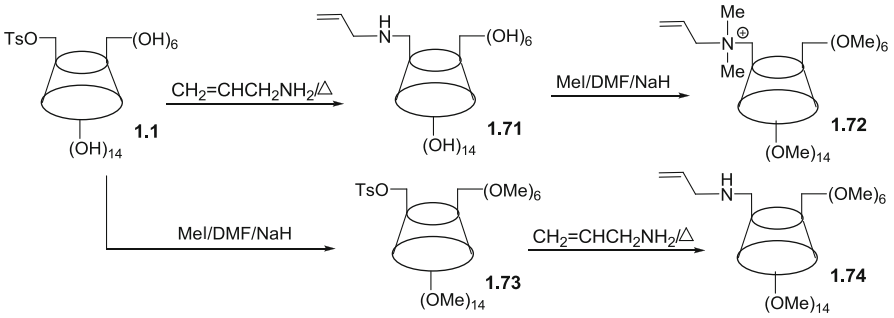
Fig. 1.9 Structures of $3^A,3^C$- and $3^A,3^D$-imidazole β-CDs and $3^A,3^C,3^E$- and $3^A,3^C,3^E$-imidazole β-CD

Scheme 1.13, the C(2) sulfonate of CDs occurs the displacement reaction under base or nucleophile attack to generate so-called *manno*-2,3-epoxyCDs [90, 91, 105, 106]. The nucleophilic ring-opening reactions of the *manno*-2,3-epoxyCDs afford C(3) substituted CD derivatives, with an overall inversion of stereochemistry at both C(2) and C(3) positions of the modified glucopyranose residue [30]. For instance, a treatment of *manno*-2,3-epoxyCDs with ammonia affords the amines **1.66** and **1.67** [107]. Besides, the TBDMS migration from the O-2 to the O-3 positions of the CDs provides an alternative method to generate per-3-subsituted CDs. As shown in Scheme 1.12, by benzyl removal of **1.58a-c** affords the 3-permethylated CDs **1.59a-c** [101].

The regiospecific sulfonate can also be employed for the preparation of regioisomer CDs. For example, the imidazole regiospecifically modified CDs **1.68–1.70** (Fig. 1.9) are prepared by a reaction of imidazole with the di- or tri-*allo*-epoxides of **1.63–1.65** [104].

1.2.4 Permodification at All Three Positions of Cyclodextrins

All of the hydroxyl groups can be unselectively converted to ester functionalities under appropriate conditions by using suitable reagents. Organic acid chlorides attack all three positions indiscriminately in pyridine or any other solvent containing



Scheme 1.14 Synthesis of mono(6^{A}-allylamino-6^{A}-deoxy)permethylated β-CD

a tertiary amine as a base to produce the esters of cyclodextrins. The size of the alkyl or aromatic group has little effect on the substitution pattern, and homogeneous per-substituted products are produced. Acetylation [35, 108, 109], benzoylation [35, 72, 110], and methylation [111–113] are achieved with acetic anhydride, benzoyl chloride, and methyl iodide, respectively, in pyridine when reactions are allowed to run for a long time.

Another strategy to produce a persubstituted cyclodextrin is to react a partially modified cyclodextrin with an appropriate reagent to attach desired groups on the remaining free hydroxyl groups. For example, 6-monocationic per-phenylcarbamated CDs can be accomplished by a later phenylcarbamation on all the rest of hydroxy groups [114]. Care should be taken when the reagent used for the late persubstitution reacts with the existing functionality. Adjustment of reaction procedure may be a good choice. For example, in the synthesis of mono(6^{A}-allylamino-6A-deoxy) permethylated β-CD **1.74** (Scheme 1.14) [115], the amino group in compound **1.71** was fully methylated and a charged CD derivative **1.72** was isolated. With a view to avoid methylation of the amino group, an alternative synthesis involves the direct permethylation of mono-6-tosylated β-CD **1.1** and a second nucleophilic substitution of sulfonate **1.73** with allylamine to afford the desired compound **1.74** under mild conditions with 74 % yields.

1.3 Conclusion

The strategies for selective modification of cyclodextrins outlined above mainly concentrate on the substitution of the C(6), C(2), and/or C(3) hydroxy groups on the CD rims. With the aim to develop selectively modified cyclodextrins as chiral mobile phase or chiral stationary phases for different analytical applications, the modification strategies here are specially tailored for the interest of researchers in the field of applying cyclodextrin chemistry to chiral separation. For a wider view of selective modification of CD for other purposes, please refer to the excellent

reviews published previously [30, 31, 116]. We hope that the researchers who require specific structures and functional groups in cyclodextrin molecules can decipher the underlying chemistry of the synthetic methods with the guidance of these general strategies and develop their own synthetic methodologies.

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Chapter 2
Cyclodextrin-Based Chiral Stationary Phases for Gas Chromatography

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Abstract In this chapter, cyclodextrin-based chiral stationary phases (CSPs) developed for the enantiomeric separation using gas chromatography are reviewed. A correlation of the CD structure and the enantioselectivities of the resultant CSPs are discussed.

Abbreviations

GC	Gas chromatography
GC-MS	Gas chromatography
HPLC	High-performance liquid chromatography
SFC	Supercritical fluid chromatography
FID	Flame ionization detection
CSP	Chiral stationary phase
CD	Cyclodextrin
CMP	Chiral mobile phase additives
Chiraldex PH-A	Permethylated S-hydroxypropyl-α-CD
Chiraldex PH-B	Permethylated S-hydroxypropyl-β-CD
Chiraldex PH-G	Permethylated S-hydroxypropyl-γ-CD
Chiraldex DA-A	2,6-*O*-Dipentylated 3-*O*-acetylated α-CD

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Chiraldex DA-B	2,6-*O*-Dipentylated 3-*O*-acetylated β-CD
Chiraldex DA-G	2,6-*O*-Dipentylated 3-*O*-acetylated γ-CD
Chiraldex TA-A	2,6-O-Dipentylated 3-O-trifluoroacetylated α-CD
Chiraldex TA-B	2,6-O-Dipentylated 3-O-trifluoroacetylated β-CD
Chiraldex TA-G	2,6-O-Dipentylated 3-O-trifluoroacetylated γ-CD
Chiraldex PN-G	2,6-O-Dipentylated 3-O-propionyl-γ-CD
Chiraldex BP-G	2,6-O-Dipentylated 3-O-butyryl-γ-CD
Chiraldex DM-B	Di-O-dimethyl-β-CD
Hydrodex B	Permethylated β-CD + polysiloxane
Chiraldex B	Permethylated β-CD + poly(dimethylsi1oxane)
Lipodex A	2,3,6-Tri-O-pentyl-α-CD
Lipodex B	2,3,6-Tri-O-pentyl-3-O-acetyl-α-CD
Lipodex C	2,3,6-Tri-O-pentyl-β-CD
Lipodex D	2,3,6-Tri-O-pentyl-3-O-acetyl-β-CD
Lipodex E	2,3,6-Tri-O-pentyl-3-O-acetyl-γ-CD
α-Dex 120	Permethylated α-CD (20 %)-SPB 35
β-Dex 110	Permethylated β-CD (20 %)-SPB 35
β-Dex 120	Permethylated γ-CD (20 %)-SPB 35
perMe-β-CD	2,3,6-Permethyl-β-cyclodextrin
perMe-γ-CD	2,3,6-Permethyl-γ-cyclodextrin
DMP-B	2,6-Di-O-methyl-3-O-pentyl-β-cyclodextrin
DMP-G	2,6-Di-O-methyl-3-O-pentyl-γ-cyclodextrin
CP-Chirasil-Dex CB	Heptakis(2,3,6-tri-O-metil)-β-cyclodextrin
OPP	Organophosphorus pesticide
PMHP-α-CD	Permethyl-*O*-(S)-2-hydroxypropyl-α-cyclodextrin
PMHP-β-CD	Permethyl-*O*-(S)-2-hydroxypropyl-β-cyclodextrin
PMHP-γ-CD	Permethyl-*O*-(S)-2-hydroxypropyl-γ-cyclodextrin
DBTBCD	Heptakis(2,6-di-O-butyl- 3-O-trifluoroacetyl)-β-CD
DNTBCD	Heptakis(2,6-di-O-nonyl-3-O-trifluoroacetyl)-β-CD
DDTBCD	Heptakis(2,6-di-O-dodecyl-3-O-trifluoroacetyl)-β-CD
DBBBCD	Heptakis(2,6-di-O-butyl-3-O-butyryl)-β-CD
DPABCD	Heptakis(2,6-di-O-pentyl-3-O-acetyl)-β-CD
20Me/P6COOMe	6^{I}-O-Methoxy-carbonyl-6^{I}-deoxy-$2^{I–VII}$,$3^{I–VII}$,$6^{II–VII}$eicosa-*O*-methylcyclodextrin
20Me/P6OCH_2CO OMe	6^{I}-*O*-Methoxycarbonylmethyl-$2^{I–VII}$,$3^{I–VII}$,$6^{II–VII}$-eicosa-*O*-methyl-cyclodextrin
20Me/P2OCH_2COOMe	2^{I}-*O*-Methoxycarbonylmethyl-$2^{II–VII}$,$3^{I–VII}$,$6^{I–VII}$-eicosa-*O*-methyl-cyclodextrin

2.1 Introduction

As one of the leading techniques in analytical separation field, gas chromatography (GC) has numerous advantages such as high efficiency at high speed, sensitive, straightforward detection formats, temperature-programming tools, multicolumn

operations, and simple operation [1–3]. Eiceman summarized all these merits and introduced the gas chromatography techniques in detail, such as columns principles and technology, models for separations, GC detection methods, and instrumental hyphenation like gas chromatography-mass spectrometry (GC-MS) [4–6]. Gas chromatography (GC) has been used in enantiomer separation field for more than 45 years [7–9], as a part of continuing efforts to improve the safety and efficacy of drugs, food, and spice.

Compared with the two most popular analysis techniques, i.e., high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC), GC possesses better column efficiencies and sharper peaks because its higher diffusivity leads to lower mass transfer resistance. Because of its enormous separation power, enantioselective GC makes it possible for simultaneous analysis of enantiomeric mixtures and other potential impurities or contaminants in one run, assuming molecules of interest can be volatilized [10, 11]. In addition, flame ionization detection (FID), which is commonly used in GC systems, provides a straightforward and universal detection format, making it a practical alternative for compounds lacking strong chromophores. Carboxylic acid and alkane racemates, which cannot be separated by HPLC or SFC due to lack of strong UV chromophores in functional groups, can be applied successfully in GC.

Chiral separations using GC can be performed indirectly or directly, and all direct enantiomeric separations are done with chiral stationary phases (CSPs). Generally three classes of GC-CSPs have been developed. They are (1) non-racemic chiral amino acid and derivatives-based CSPs via hydrogen bonding, such as Chirasil-Val [12–15]; (2) non-racemic chiral metal coordination compounds-based CSPs via complexation, such as Chirasil-Nickel [16, 17]; and (3) cyclodextrin and (CD) derivatives-based CSPs via inclusion, such as Chirasil-β-Dex [18–21]. Since 1983, native cyclodextrin (CD) was first used in enantioselective gas–liquid chromatography as chiral stationary phase [22]. After that various CD derivatives have been extensively studied [23, 24]. The chemical modifications of the free hydroxyl groups of CD allow a change of the polarity of CD derivatives and then influence their chiral recognition. CD-based CSPs generally present a fuctionalization of the three different hydroxyl groups, with each glucose unit remaining identical.

During the past decades, Armstrong and coworkers developed numerous CD-CSPs used in GC and investigated the enantiomeric recognition mechanism [19, 25–32]. They found that the CD-CSPs gave unusual selectivities, which could be accounted for only by inclusion complex formation. A variety of CD derivatives were prepared, like octakis (2,6-di-O-pentyl)-β-cyclodextrin [29], permethyl-*O*-(2-hydroxypropyl)-α- and β-cyclodextrins, as well as trifluoroacetylated octakis(2,6-O-dipentyl)-γ-CD [25]. All these CD-CSPs proved to be sufficiently effective. Meanwhile, Schurig and his coworkers [33–38] also concentrated on the development of multiple CD-CSPs; they synthesized different CD derivatives and used them as chiral selectors, such as Chirasil-Dex [33], Chirasil-γ-Dex [1], and C11-ChirasiI-Dex [36]. In addition, they also have an excellent performance in GC chiral separation. Apart from the two famous scientists, a lot of chemists developed abundant CD-CSPs applied in GC to separate various enantiomers. In this chapter, we commit to illustrate the current situation about GC chiral separation based on

CD-CSPs, highlight the different kinds of CD-CSPs and the application in a series of enantioseparation experiments, and depict generally the mechanism of the separation process.

2.2 GC Chiral Columns Based on CD-CSPs

The most important part for GC chiral separation is the chiral column; herein we focus on CD-CSPs chiral columns and further discuss about their applications in GC enantioseparation. Cyclodextrins (CDs) are cyclic oligomers of 1,4-linked, α-D-glucose monomers. They are employed for molecular recognition because of the guest–host complexation of the hydrophobic cavity. Cyclodextrin derivatives belong to the most frequently used CSPs and have been used for a long time for enantiomer separation in HPLC both as chiral mobile phase additives (CMPs) and as chiral CSPs. For CD-CMPs, native cyclodextrins or their derivatives are added into the mobile phase as chiral selectors to achieve enantioseparation, which is a little consumptive. For CD-CSPs, the common method is to make a packing column using both native and modified cyclodextrins based on silica gel or polymers, which can also be applied to SFC. However, GC CD-CSPs are somewhat different from former two situations.

At first, scientists attempted to use native CDs and simple derivatives in GC for years but just with little success. CD-CSPs should have superior selectivities due to inclusion complex formation, but the result is not as good as expected. It is assumed that the selectivity of these stationary phases are affected by the solvent used in the coating process. The fact is that native CDs and most of their simple derivatives (e.g., dimethyl, permethyl, peracetyl) are crystalline solids with very high melting or decomposition points, which makes them difficult to use as GC-CSPs [29]. Attempts to dissolve the CDs in various high-boiling-point solvents only obtained poor efficiencies, and high temperatures also need to be avoided [39–41]. Schurig and coworkers found that results would be better when permethylated β-CD was dissolved in a typical GC liquid stationary phase, such as OV-1701, and coated onto glass capillary columns [42].

By now, different approaches have been developed to prepare the cyclodextrins used in GC enantioseparations. The first approach utilizes undiluted permethylated β-cyclodextrin coating on a glass capillary column as a CSP in a supercooled state [43]. The second approach was developed by Schurig and Nowotny [42] and will be described in the latter section. A logical extension of this approach is to link the modified CDs, e.g., permethylated β-cyclodextrin, via a polymethylene linker to polydimethylsiloxane to yield a chiral polysiloxane-containing cyclodextrin (Chirasil-Dex) [44, 45].

Recently, there have been several reports of new types of GC CD-CSPs focusing on versatility, stability, and utility of the newest generation of CD derivatives. Since Juvancz first applied permethylated β-cyclodextrin as a stationary phase to capillary gas chromatography [22], CD derivatives have been widely used for the separation

of chiral volatiles by GC. A huge number of articles about GC chiral separation based on CD-CSPs have been published; both commercial columns and self-made columns will be emphatically described in the following sections.

2.3 CD-CSPs Columns and Their Applications in GC

For GC CD-CSPs columns, there are two main types, i.e., "wall-coated" columns and "wall-immobilized" columns (so-called "bonded" type). For the first type of column, physical coating method is used. For the second type of column, the cyclodextrin moieties are attached through a single linkage to form a "necklace"-type polymer in which the chiral moieties are spaced along the siloxane polymer like "pendants." Armstrong [46] produced a branched or "star" oligomer of cyclodextrin and a short-chain, hydrogen-terminated methyloligosiloxane. When attached to the wall of fused-silica capillary and cross-linked, it produces a stable immobilized CSP, which is usually more robust and stable than coated analogues. This provides obvious benefits in regard to column lifetime, temperature limit in GC. Besides, there are several kinds of commercial GC columns supplied by different companies. Table 2.1 summarizes some typical commercial GC CD-CSPs columns including both "wall-coated" and "bonded" type [30].

2.3.1 Commercial GC CD-CSPs Columns and Their Applications

Commercial columns provide convenience for GC enantioseparations and possess good chiral enantioseparation abilities. Figure 2.1 gives the structures of four common commercial GC columns, and their characteristics are summarized in Table 2.1.

Table 2.2 lists the separation results of 25 racemic analytes on these four columns. For compounds 1–7, the isothermal retention data obtained on β- and γ-cyclodextrin stationary phases varied greatly. An increase in selectivity was observed on the three γ-cyclodextrin CSPs when the substituent was changed from methyl to vinyl. In this case, it appears that the additional double bond adjacent to the sulfur stereogenic center decreases the enantioselectivity of the γ-cyclodextrin CSPs. A combination of size and polarity/electronegativity of the para substituent on methyl-phenyl-sulfoxides **3–7** appears to have an effect on enantioselectivity. Compounds ortho-substituted sulfoxides **11**, **12**, **13** produced the best separations on **DM-B** column. Selectivity appears to be influenced by the size and polarity of the ortho substituent. Compounds **14–20**, the liquid sulfinate esters, could be separated at a much lower temperature. **TA-G** and **DM-G** columns performed best in separating these seven compounds. Compounds **21–23** are three sulfoxides; these sulfoxides presented an interesting case with the γ-cyclodextrin CSPs. Compounds **24** and **25** are a chiral sulfinate ester and a chiral sulfoxide, respectively. Sulfoxide **25** was

Table 2.1 Characteristics of the capillary chiral GC columns [19, 30, 31, 47]

Trade name	Chiral phase description	Company	Length (m)	Film thickness (m)	Code
Chiraldex PH-A	Permethylated S-hydroxypropyl-α-CD	Astec	10	0.125	PH-A
PH-B	Permethylated S-hydroxypropyl-β-CD		10	0.125	PH-B
PH-G	Permethylated S-hydroxypropyl-γ-CD		10	0.125	PH-G
Chiraldex DA-A	2,6-*O*-Dipentylated 3-*O*-acetylated α-CD	Astec	10	0.125	DA-A
DA-B	2,6-*O*-Dipentylated 3-*O*-acetylated β-CD		10	0.125	DA-B
DA-G	2,6-*O*-Dipentylated 3-*O*-acetylated γ-CD		10	0.125	DA-G
Chiraldex TA-A	2,6-*O*-Dipentylated 3-*O*-trifluoroacetylated α-CD	Astec	10	0.125	TA-A
TA-B	2,6-*O*-Dipentylated 3-*O*-trifluoroacetylated β-CD		10	0.125	TA-B
TA-G	2,6-*O*-Dipentylated 3-*O*-trifluoroacetylated γ-CD		5.30	0.125–0.25	TA-G
Chiraldex PN-G	2,6-*O*-Dipentylated 3-*O*-propionyl-γ-CD	Astec	20	–	PN-G
BP-G	2,6-*O*-Dipentylated 3-*O*-butyryl-γ-CD		20	–	BP-G
Chiraldex DM-B	Di-O-dimethyl-β-CD	Astec	20	–	DM-B
Hydrodex B	Permethylated β-CD + polysiloxane	M. N.	10	0.25	Hydrodex
Chiraldex B	Permethylated β-CD + poly(dimethylsi1oxane)	Astec	10	0.125	PM
Cyclodex B	Permethylated β-CD + DB1701	J&W	25	0.25	Cyclodex
Chirasil-Val	L-valine tert-butylamide coated	MN	25		CT. C. Sil-Val
Permabond L-Chirasil-Val	L-valine tert-butylamide bonded	MN	25	bonded	PB.C.Si1-Val
Lipodex A	2,3,6-Tri-O-pentyl-α-CD	MN	10		Lipodex A
Lipodex B	2,3,6-Tri-O-pentyl-3-O-acetyl-α-CD	MN	10		Lipodex B
Lipodex C	2,3,6-Tri-O-pentyl-β-CD	MN	10		Lipodex C
Lipodex D	2,3,6-Tri-O-pentyl-3-O-acetyl-β-CD	MN	10		Lipodex D
Lipodex E	2,3,6-Tri-O-pentyl-3-O-acetyl-γ-CD	MN	10		Lipodex E
α-Dex 120	Permethylated α-CD (20 %)-SPB 35 (80 %)	Supelco	30		α-Dex 120
β-Dex 110	Permethylated β-CD (20 %)-SPB 35 (80 %)	Supelco	30		β-Dex 110
β-Dex 120	Permethylated γ-CD (20 %)-SPB 35 (80 %)	Supelco	30		β-Dex 120
perMe-β-CD	2,3,6-Permethyl-β-cyclodextrin	Middelbu	25	0.25	PM-B
perMe-γ-CD	2,3,6- Permethyl-γ-cyclodextrin	rg	25	0.25	PM-G
DMP-B	2,6-Di-O-methyl-3-O-pentyl-β-cyclodextrin	MEGA	25	0.25	DMP-B
DMP-G	2,6-Di-O-methyl-3-O-pentyl-γ-cyclodextrin		25	0.25	DMP-G
CP-Chirasil-Dex CB	Heptakis(2,3,6-tri-O-metil)-β-cyclodextrin	Varian	25	0.25	CP-Chirasil-Dex CB

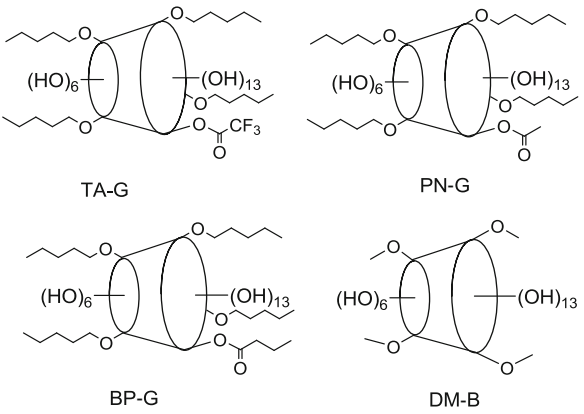


Fig. 2.1 Simplified schematics of the four cyclodextrin CSPs: TA-G, PN-G, BP-G, and DM-B

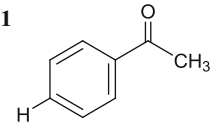
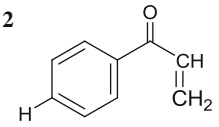
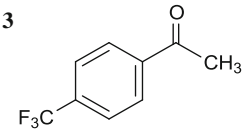
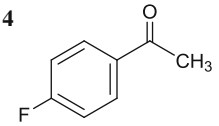
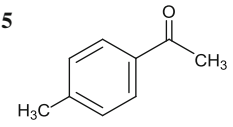
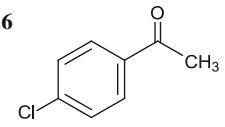
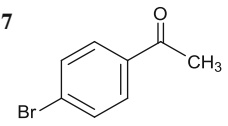
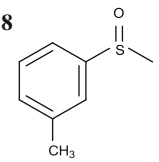
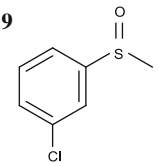
easily separated by all CSPs; DM-B was capable of resolving the enantiomers of compound **24**.

Table 2.3 gives the enantiomeric separation data for another 19 compounds using **TA-G**, **TA-B**, **PN-G,** and **DM-B**. Excellent results were achieved; all 19 compounds were baseline separated, most with high-resolution factors [32]. The retention factor (k'), selectivity (α), resolution (R_s), and separation temperature (T) for each compound are summarized in Table 2.3. The 19 β-lactams investigated in this work can be divided into four categories: bicyclic (compounds **1–7**), aromatic tricyclic (compounds **8–10**), aliphatic tricyclic (compounds **11–12**), and aryl-substituted (compounds **13–19**).

The **TA-G** was found to be the most powerful CSP with the broadest enantioselectivity, which produced 18 baseline and 1 partial separations for the 19 compounds examined. Compounds **1** to **8**, with the only exception of compound **7**, showed resolutions over 4.0. While showing comparable resolving power to **TA-G**, the smaller **TA-B** displayed complementary separations to its γ-cyclodextrin analog. All racemates except compound **12** and compound **17** were nicely separated on this column. Comparing **PN-G** with **TA-G**, the resolution of compound **11** increased from 2.6 to 4.6 when the **PN-G** column was used. An increase in resolution from 1.5 to 2.0 was also observed for compound **13** with the **PN-G**. The Chiraldex B-DM column separated 11 analytes with baseline resolution. Compounds **10** and **15**–**17** were partially separated on this column under the same conditions used with other columns. All these four CD-CSPs show excellent chiral separation abilities, and examples of the best and worst separation results obtained are shown in Fig. 2.2.

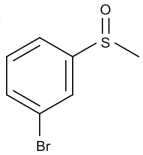
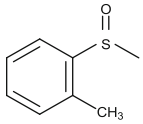
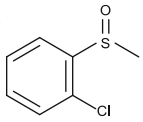
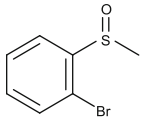
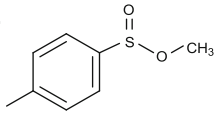
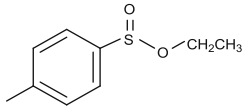
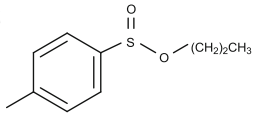
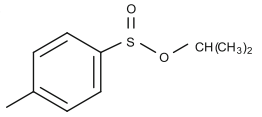
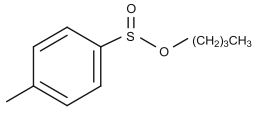
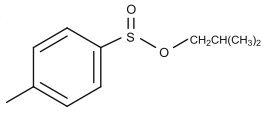
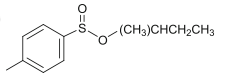
Alfredo et al. [47] also used the commercial column CP-Chirasil-Dex CB (summarized in Table 2.1) to separate 13 organophosphorus pesticides (OPPs) which are widely employed for crop protection. Numerous OPPs are enantiomeric compounds with phosphorus or carbon atoms as chiral centers. Thus, it would be desirable to use single enantiomers as pesticides to reduce the toxic effects on untargeted organisms. A lot of commercial GC CD-CSP columns were introduced above; in the following section self-made CD-CSP columns will be described in detail. In

Table 2.2 Structure and retention data for 18 chiral sulfoxides and 7 sulfinate esters separated by four different CSPs

Structure	T (°C)	k_1'	k_2'	α	R_s	n_1	n_2	Column
1 	150	4.29	5.79	1.35	6.3	515	253	TA-G
	150	5.82	7.74	1.33	6.6	708	485	PN-G
	150	4.76	5.90	1.24	2.7	185	166	BP-G
	110	18.1	18.6	1.03	0.8	1,111	642	DM-B
2 	150	4.38	4.74	1.08	2.8	1,174	823	TA-G
	150	6.30	6.74	1.07	2.3	1,533	1,203	PN-G
	150	5.03	5.33	1.06	1.3	763	517	BP-G
	120	12.1	12.4	1.02	0.4	726	219	DM-B
3 	140	5.30	5.41	1.02	1.1	2,620	2,124	TA-G
	130	10.8	11.1	1.03	1.2	1,814	1,452	PN-G
	120	15.0	15.6	1.04	0.7	398	278	BP-G
	120	12.9	14.1	1.09	2.9	1,309	983	DM-B
4 	150	3.39	3.8	1.12	3.7	1,128	797	TA-G
	150	4.97	5.52	1.11	3.2	1,173	940	PN-G
	150	4.03	4.35	1.08	1.2	330	249	BP-G
	120	11.8	12.5	1.06	1.7	389	299	DM-B
5 	150	6.65	7.10	1.07	2.0	809	535	TA-G
	150	8.19	8.68	1.06	1.9	1,021	839	PN-G
	150	7.25	7.61	1.05	0.5	121	121	BP-G
	120	20.2	21.2	1.05	0.9	416	218	DM-B
6 	150	8.27	8.62	1.04	1.4	1,067	570	TA-G
	150	12.5	13.0	1.04	1.8	1,915	1,649	PN-G
	150	10.7	11.1	1.04	0.6	286	258	BP-G
	120	36.6	39.5	1.08	1.2	276	178	DM-B
7 	150	13.5	13.9	1.03	1.3	1,598	771	TA-G
	150	21.3	21.9	1.03	1.3	1,703	1,483	PN-G
	150	17.2	17.7	1.03	0.5	306	157	BP-G
	120	66.3	71.6	1.08	1.2	196	193	DM-B
8 	150	6.13	8.79	1.44	6.2	280	166	TA-G
	150	7.66	11.0	1.44	10	1,164	605	PN-G
	150	6.56	8.66	1.32	3.4	159	150	BP-G
	120	16.9	18.1	1.07	1.4	442	342	DM-B
9 	150	7.57	12.7	1.68	8.5	357	129	TA-G
	150	11.2	19.4	1.74	20	2,045	893	PN-G
	150	9.39	13.8	1.47	6.3	482	188	BP-G
	120	24.5	25.7	1.05	0.9	335	202	DM-B

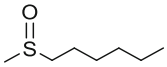
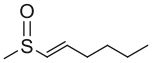
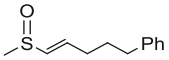
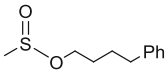
(continued)

Table 2.2 (continued)

Structure	T (°C)	k_1'	k_2'	α	R_s	n_1	n_2	Column
10 	150	12.2	19.2	1.57	13.1	1,795	304	TA-G
	150	18.5	29.3	1.58	15	1,942	668	PN-G
	150	15.7	21.7	1.38	5.4	370	200	BP-G
	120	41.8	43.9	1.05	1.2	530	396	DM-B
11 	150	7.24	8.82	1.22	4.8	741	263	TA-G
	150	9.12	10.3	1.13	3.9	1,123	924	PN-G
	150	7.86	8.49	1.08	1.1	203	172	BP-G
	120	18.7	24.5	1.31	7.8	818	705	DM-B
12 	150	6.63	7.84	1.18	3.7	637	214	TA-G
	150	8.98	10.2	1.14	5.1	1,690	1,453	PN-G
	150	7.41	8.15	1.10	1.8	399	331	BP-G
	120	17.6	20.6	1.17	3.2	454	313	DM-B
13 	150	11.0	12.9	1.17	3.4	396	234	TA-G
	150	15.4	17.0	1.11	3.9	1,381	1,178	PN-G
	150	12.6	13.7	1.09	1.7	409	334	BP-G
	120	31.6	37.9	12.0	3.3	347	236	DM-B
14 	110	22.4	23.3	1.04	1.5	712	690	TA-G
	100	60.4	61.6	1.02	0.4	625	174	PN-G
	100	43.4	44.3	1.02	0.7	2,696	747	BP-G
	120	11.8	12.9	1.09	3.5	1,529	1,275	DM-B
15 	110	28.5	31.9	1.12	4.1	940	673	TA-G
	110	38.4	43.4	1.13	4.8	1,502	1,171	PN-G
	110	29.8	32.5	1.09	2.9	1,069	785	BP-G
	120	13.2	14.5	1.10	4.8	2,909	1,738	DM-B
16 	110	42.2	45.6	1.08	2.5	641	560	TA-G
	110	56.5	58.8	1.04	1.5	1,648	1,227	PN-G
	110	44.6	45.9	1.03	1.0	1,440	724	BP-G
	120	20.5	21.9	1.07	3.2	2,457	1,813	DM-B
17 	110	27.0	30.2	1.12	4.3	1,146	587	TA-G
	110	35.8	37.9	1.06	2.6	2,289	1,501	PN-G
	110	29.5	31.3	1.06	2.4	1,857	1,034	BP-G
	90	67.8	69.9	1.03	1.3	2,785	1,590	DM-B
18 	110	50.8	53.3	1.05	3.0	2,456	1,574	TA-G
	110	92.0	93.9	1.02	0.8	1,780	615	PN-G
	100	43.3	44.2	1.02	0.7	1,448	665	BP-G
	120	35.2	36.6	1.04	1.2	906	467	DM-B
19 	110	46.3	47.2	1.02	1.0	2,600	1,940	TA-G
	100	111	112	1.01	0.5	2,957	1,341	PN-G
	100	54.2	58	1.07	0.5	195	22	BP-G
	120	24.0	24.7	1.03	1.6	3,623	2,618	DM-B
20 	120	24.8	25.3	1.09	0.9	1,794	1,677	TA-G
	120	34.5	35.0	1.04	0.9	1,957	6,560	PN-G
	120	28.1	28.4	1.04	0.8	2,994	3,455	BP-G
	120	22.8	23.3	–	–	–	–	DM-B

(continued)

Table 2.2 (continued)

Structure	T (°C)	k_1'	k_2'	α	R_s	n_1	n_2	Column
21 	140	5.53	5.75	1.04	1.0	585	362	TA-G
	130	8.51	9.02	1.06	0.9	294	225	PN-G
	110	23	25.5	1.11	0.9	66	52	BP-G
	90	47.5	50.3	1.06	0.9	227	187	DM-B
22  **trans**	150	38.8	–	–	–	165	–	TA-G
	150	49.2	–	–	–	421	–	PN-G
	150	45.5	–	–	–	1,780	–	BP-G
	155	21.4	22.0	1.03	0.8	1,471	526	DM-B
23  **trans**	150	40.1	–	–	–	293	–	TA-G
	150	49.7	–	–	–	283	–	PN-G
	150	46.4	–	–	–	1,218	–	BP-G
	150	27.1	27.9	1.03	1.0	1,152	722	DM-B
24 	140	12.3	12.4	1.01	0.7	2,430	1,455	TA-G
	130	31.3	31.6	1.01	0.8	2,810	1,918	PN-G
	120	44.3	45.2	1.02	0.9	1,925	1,390	BP-G
	150	8.26	8.51	1.03	1.4	3,203	2,368	DM-B
25 	180	11.2	12.5	1.12	6.3	1,993	1,946	TA-G
	180	15.9	17.1	1.08	3.7	1,876	1,989	PN-G
	180	14.3	15.3	1.07	1.2	291	250	BP-G
	180	8.81	9.78	1.11	4.8	2,533	1,938	DM-B

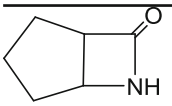
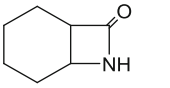
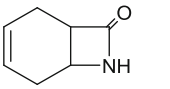
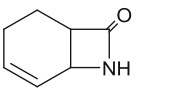
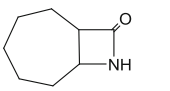
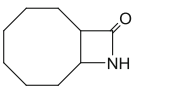
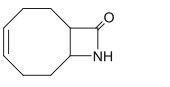
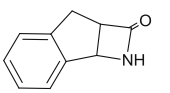
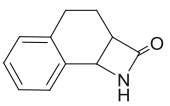
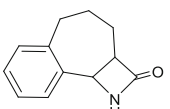
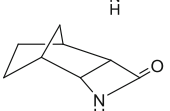
α: enantioselectivity; k_1'& k_2': retention factors for two enantiomers; R_s: resolution factors; n: efficiency

order to make a clear illustration of the self-made CD-CSP columns, it is divided into two parts: the "wall-coated" and "bonded" columns.

2.3.2 Self-Made "Wall-Coated" GC CD-CSP Columns

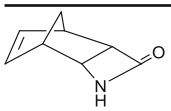
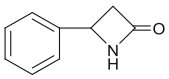
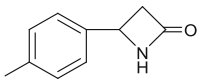
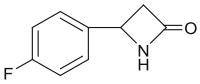
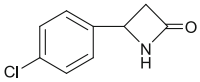
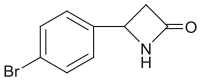
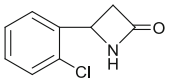
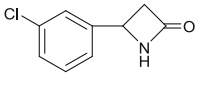
Since the first use of native CD as chiral stationary phase in enantioselective gas–liquid chromatography in1983 [22], various CD derivatives have been extensively studied. For any chromatography chiral separation situation, the most important element is the CSP. CSPs based on CD present generally the functionalization of the three different hydroxyl groups, each glucose unit remaining identical [48]. The chemical modifications of the free hydroxyl groups of CD allow a change of the polarity of CD derivatives and then influence the chiral recognition. In order to improve the gas chromatographic performance, cyclodextrins have been modified by derivatization of the free hydroxyl groups of the glucose units. A wide array of alkylation and acylation strategies has been applied [24]. Blocking the 6 hydroxyl position of the glucose unit with a bulky silyl group and subsequent modification of the 2, 3-hydroxy groups resulted in useful CSPs [49, 50]. In the following paragraph, different CD-CSPs and their applications in gas chromatographic enantioseparation will be illustrated in detail.

Table 2.3 Enantiomeric separation of 19 β-lactams with GC-CSPs

Structure	CSP	T (°C)	k_1'	α	R_s
	TA-G	150	3.82	1.53	10.5
	TA-B	150	4.26	1.46	6.2
	PN-G	150	4.02	1.20	5.6
	DM-B	150	2.41	1.08	1.8
	TA-G	150	8.88	1.30	11.1
	TA-B	150	7.19	1.39	12.4
	PN-G	150	8.30	1.20	9.8
	DM-B	150	5.24	1.10	3.3
	TA-G	150	10.22	1.25	7.1
	TA-B	150	7.89	1.34	12.7
	PN-G	150	9.92	1.14	5.7
	DM-B	150	5.67	1.14	3.4
	TA-G	150	6.70	1.15	6.4
	TA-B	150	5.45	1.20	9.3
	PN-G	150	6.46	1.09	4.5
	DM-B	150	4.57	1.14	4.5
	TA-G	150	13.68	1.20	8.2
	TA-B	150	10.82	1.15	7.0
	PN-G	150	13.37	1.15	6.7
	DM-B	150	8.46	1.10	3.4
	TA-G	170	8.22	1.08	4.6
	TA-B	170	6.63	1.13	6.0
	PN-G	170	8.41	1.05	2.5
	DM-B	N.A.	N.A.	N.A.	N.A.
	TA-G	160	14.23	1.02	1.4
	TA-B	160	11.80	1.06	2.1
	PN-G	140	45.49	1.02	0.5
	DM-B	N.A.	N.A.	N.A.	N.A.
	TA-G	180	7.62	1.08	4.1
	TA-B	180	6.91	1.06	2.2
	PN-G	190	5.95	1.05	2.4
	DM-B	190	4.00	1.05	1.5
	TA-G	180	11.11	1.05	2.6
	TA-B	180	10.15	1.13	6.7
	PN-G	190	8.58	1.03	1.5
	DM-B	200	4.22	1.14	5.3
	TA-G	180	20.71	1.04	2.4
	TA-B	180	16.35	1.08	4.4
	PN-G	180	27.00	1.05	2.9
	DM-B	180	16.43	1.02	0.9
	TA-G	150	14.43	1.05	2.6
	TA-B	150	10.83	1.05	2.7
	PN-G	150	14.00	1.10	4.6
	DM-B	150	9.01	1.04	1.7

(continued)

Table 2.3 (continued)

Structure	CSP	T (°C)	k_1'	α	R_s
	TA-G	170	3.82	1.03	1.5
	TA-B	N.A.	N.A.	N.A.	N.A.
	PN-G	150	9.95	1.05	2.5
	DM-B	N.A.	N.A.	N.A.	N.A.
	TA-G	160	13.96	1.02	1.5
	TA-B	160	13.01	1.06	2.4
	PN-G	160	13.62	1.05	2.0
	DM-B	160	9.65	1.13	4.2
	TA-G	160	21.99	1.03	1.6
	TA-B	160	22.50	1.05	3.1
	PN-G	160	20.73	1.02	0.7
	DM-B	160	N.A.	N.A.	N.A.
	TA-G	160	17.06	1.02	1.5
	TA-B	160	19.93	1.05	2.4
	PN-G	160	20.06	1.03	1.5
	DM-B	160	19.78	1.02	0.5
	TA-G	160	47.92	1.03	1.8
	TA-B	160	60.49	1.05	2.0
	PN-G	160	58.73	1.02	0.7
	DM-B	160	31.48	1.03	0.8
	TA-G	160	80.89	1.03	2.0
	TA-B	160	N.A.	N.A.	N.A.
	PN-G	160	99.15	1.02	0.4
	DM-B	160	51.08	1.04	0.9
	TA-G	160	31.15	1.05	1.5
	TA-B	160	26.95	1.05	1.5
	PN-G	160	35.41	1.04	2.4
	DM-B	160	20.34	1.13	3.6
	TA-G	160	44.04	1.04	2.4
	TA-B	160	41.91	1.07	2.0
	PN-G	160	52.43	1.03	1.6
	DM-B	160	26.31	1.12	1.9

In the 1990s a wide variety of cyclodextrins chiral stationary phases were developed for the gas chromatographic separation of enantiomers. Much of the early work on the use of native cyclodextrins as GC stationary phases was done by Smolkova-Keulemansova and coworkers [51–54] and Sybilska and associates [22]. Recently, Konig and coworkers produced lipophilic alkyl and alkyl–acyl derivatives of cyclodextrins that were liquids [55]. Also, Schurig has dissolved native and permethylated cyclodextrins in various GC stationary-phase liquids, thereby obtaining viable CSPs, as well as Armstrong and coworkers, who developed pentyl-substitute cyclodextrin derivatives and permethyl-O-((S)-2-hydroxypropyl)-cyclodextrins and gained good separation results of dozens of enantiomers in GC.

In 1990, Armstrong et al. [29] synthesized three kinds of di-O-pentylcyclodextrins, namely, 2,6-di-*O*-pentyl-α-, β-, and γ-cyclodextrin (as shown in Fig. 2.3), and

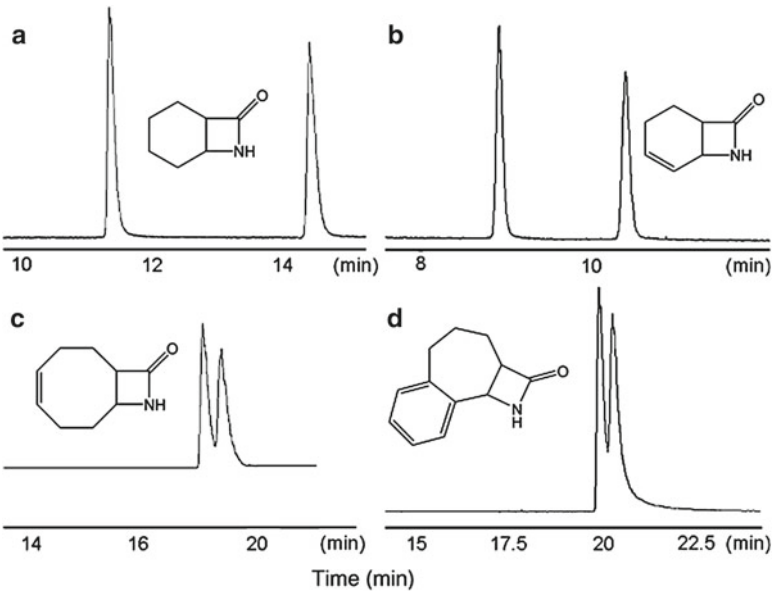


Fig. 2.2 Examples of the best (**a**, **b**) and worst (**c**, **d**) enantiomeric separations for β-lactams obtained by GC-CSPs. (Analytes in chromatogram C and D are baseline separated using other CSPs; see Table 2.3) (**a**) Chiraldex TA-G column, 150 °C, helium carrier gas, FID. (**b**) Chiraldex TA-B column, 150 °C, helium carrier gas, FID. (**c**) Chiraldex G-TA column, 150 °C, helium carrier gas, FID. (**d**) Chiraldex B-DM column, 180 °C, helium carrier gas, FID (Reprinted from Ref. [32], with kind permission from Springer Science + Business Media)

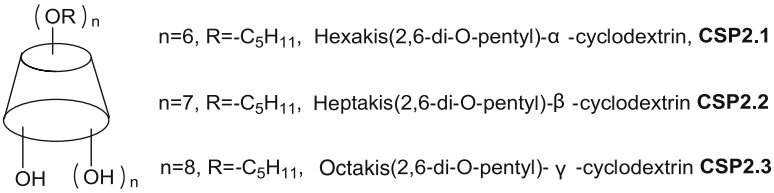


Fig. 2.3 Structures of these derivatized cyclodextrins

examined their physical properties and performance as GC choral stationary phases respectively. These three CD-CSPs are Hexakis(2,6-di-*O*-pentyl)-α-cyclodextrin (**CSP 2.1**), heptakis(2,6-di-*O*-pentyl)-β-cyclodextrin (**CSP 2.2**), and Octakis(2,6-di-*O*-pentyl)-γ-cyclodextrin (**CSP 2.3**), respectively. A series of 65 racemic compounds were individually injected into capillary columns coated with these three CD-CSPs. Very different enantioselectivities were observed. Only the data for racemates that were effectively resolved are given in each table. It is apparent that a variety of enantiomeric compounds can be resolved by capillary GC, including alcohols, amines, ammo alcohols, amino acids, epoxides, carboxylic acids, esters, lactones, ethers, sugars, and haloalkanes. The largest number of compounds was

separated on the derivatized β-CD column, followed by the α-CD, and then the γ-CD column. Table 2.4 summarizes the compounds and pertinent separation data generated with the **CSP 2.2** capillary column, and Fig. 2.4 shows the enantiomeric separation of trifluoroacetylated 1-aminoindan, 1,2,3,4-tetrahydro-1-naphthylamme, and 1-(1-naphthyl)ethylamine on a 30-m fused-silica capillary coated with **CSP 2 at** the column temperature of 160 °C.

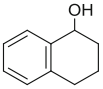
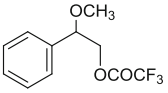
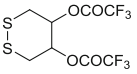
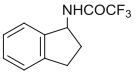
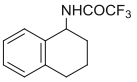
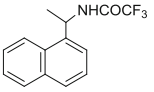
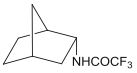
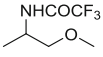
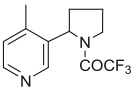
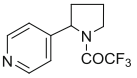
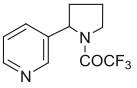
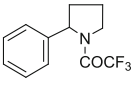
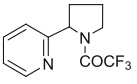
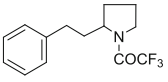
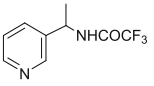
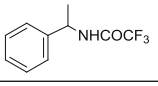
From Table 2.4, we can find that **CSP2.2** effectively resolved a number of compounds such as esters and amino alcohols. Herein, the chiral separation results of hexakis(2,6-di-*O*-pentyl)-α-cyclodextrin (**CSP2.1**) and octakis(2,6-di-*O*-pentyl)-γ-cyclodextrin (**CSP2.3**) are omitted. From the data in the table, it is easy to obtain that these three kinds of CD-CSPs are effective and all of the recently reported derivatized "liquid" CD-CSPs used in GC are hydrophobic. Currently, several liquid CD derivatives having hydrophilic groups are under development.

Armstrong and coworkers developed other "polar-liquid" CD-CSPs for GC also in 1990, including *O*-(S)-2-hydroxypropyl-derivatized α-, β-, and γ-cyclodextrins, named permethyl-*O*-(S)-2-hydroxypropyl-α-cyclodextrin (PMHP-α-CD, **CSP2.4**), permethyl-*O*-(S)-2-hydroxypropyl-β-cyclodextrin (PMHP-β-CD, **CSP2.5**), and permethyl-*O*-(S)-2-hydroxypropyl-γ-cyclodextrin (PMHP-γ-CD, **CSP2.6**), respectively [25]. The comparisons of **CSP2.4**, **CSP2.5**, and **CSP2.6** for the resolution of racemic compounds are made in Table 2.5, and the injection port temperature was 200 °C, and N_2 was used as the carrier gas. These PMHP-CDs CSPs have similar enantioselectivity with the former three di-*O*-pentylcyclodextrins CSPs, and their enantioseparation results were shown in Tables 2.6, 2.7, and 2.8. In contrast to the alkyl-substituted cyclodextrins, permethyl-*O*-(S)-2-hydroxypropyl-CDs are relatively hydrophilic (they dissolve in water) and seem to be relatively polar (they coat better on undeactivated, polar surfaces).

As shown in Table 2.5, the PMHP-derivatized cyclodextrins seem to be very highly enantioselective for alkylamines, epoxide, lactones, and amino alcohols. In addition we obtain that PMHP-β-CD, **CSP2.5** has better chiral separation selectivity of the analytes except 1,2-dihydro-2-ethoxypyran and *trans*-1,2-dithiane-4,5-diol. In reversed-phase HPLC on cyclodextrin-bonded phases, inclusion complexation plays a major role in the enantioseparation process. There is a question as to whether or not it plays a major role in GC. The formation of an inclusion complex is significantly affected by the size and shape of the solute being complexed. Consequently, binding differences due to analyte size are seen between α-, β-, and γ-cyclodextrins which have different internal diameters. Although it is somewhat less pronounced, there also appears to be size selectivity between α-, β-, and γ-versions of the lipophilic cyclodextrin GC phases. One of the unusual features of the current hydrophilic PMHP-CD phases is that there does not seem to be significant selectivity differences based on the size of the racemate. Separation factors of the largest PMHP-γ-CD are relatively small, and easier separation on α- and β-analogues can be obtained.

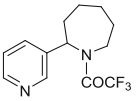
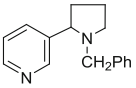
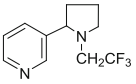
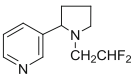
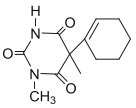
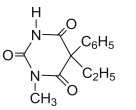
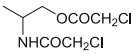
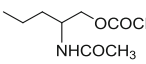
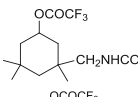
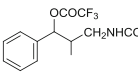
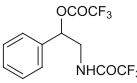
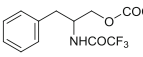
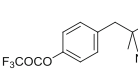
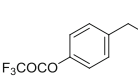
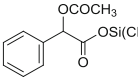
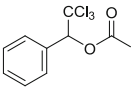
Tables 2.5 and 2.6 give the enantioseparation comparison of PMHP-α-CD and dipentyl-α-CD and PMHP-β-CD and dipentyl-β-CD, respectively. It is easy to find that selectivities of almost analytes in dipentyl-α-CD are better than PMHP-α-CD, but the difference between dipentyl-β-CD and PMHP-β-CD is not obvious. Besides,

Table 2.4 Enantiomeric separation of racemic compounds by capillary GC with **CSP2.2** [29]

Compound	Structure	Column temperature (°C)	Column length (m)	α
Alcohols				
1,2,3,4-Tetrahydro-1-naphthol		100	10	1.05
2-Methoxy-2-phenylethanol		100	10	1.02
trans-1,2-Dithiane-4,5-diol		80	10	1.07
Amines				
1-Aminoindan		140	20	1.05
1,2,3,4-Tetrahydro-l-naphthylamine		130	20	1.01
1-(1-Naphthyl) ethylamine		150	10	1.06
endo-2-Aminonorbornane		100	10	1.03
2-Amino-1-methoxypropane		115	30	1.02
4-Methylnornicotine		170	10	1.05
2-(4-Pyridyl)pyrrolidine		170	10	1.03
Anabasine		180	10	1.03
2-Phenylpyrrolodine		140	10	1.03
2-(2-Pyridyl)pyrrolidine		140	10	1.02
2-Phenethylpyrrolidine		140	20	1.01
1-(3-Pyridyl)ethylamine		140	20	1.02
1-Phenylethylamine		120	20	1.03

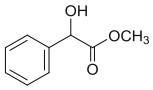
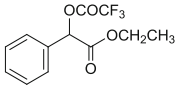
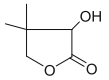
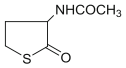
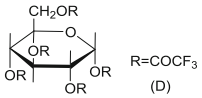
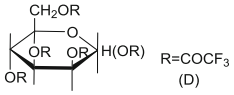
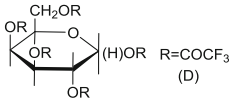
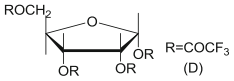
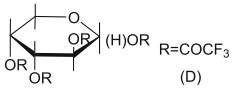
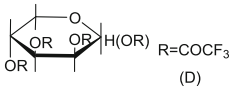
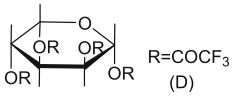
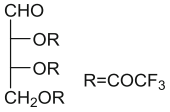
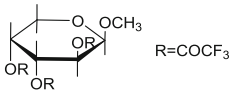
(continued)

Table 2.4 (continued)

Compound	Structure	Column temperature (°C)	Column length (m)	α
2-(3-Pyridyl)-1-azacycloheptane		140–195[a]	20	1.01
N′-Benzylnornicotine		160–200[a]	20	1.01
N′-(2,2,2-Trifluoroethyl) nornicotine		140	20	1.01
N′-(2,2-Difluoroethyl) nornicotine		160	30	1.02
Hexobarbital		180	10	1.02
Mephobarbital		180	10	1.02
Amino alcohols				
2-Aminopropan-2-ol		140–180[a]	30	1.01
2-Aminopentan-1-ol		125	30	1.02
3-Aminomethyl-3,5,5-trimethylayclohexanol		150	30	1.06, 1.01[b]
ψ-(±)Ephedrine		120	20	1.04
Octopamine		140–200[a]	30	1.01
2-Amino-3-phenylpropanol		130	20	1.02
Amino acids				
α-Methyl-*p*-tyrosine methyl ester		170–200[a]	30	1.02
Tyrosine methyl ester		170–200[a]	30	1.02
Carboxylic acids esters				
O-Acetylmandelic acid		130	30	1.03
α-(Trichloromethyl) benzyl acetate		150	30	1.04

(continued)

Table 2.4 (continued)

Compound	Structure	Column temperature (°C)	Column length (m)	α
Mandelic acid methyl ester		100	30	1.02
Mandelic acid ethyl ester		100	30	1.02
Lactones				
Pantolactone		110	30	1.05
N-Acetyl homocysteine thiolactone		145	30	1.02
Sugars				
DL-Glucose		100	10	1.21
DL-Mannose		100	10	1.23
DL-Galactose		90	10	1.04
DL-Ribose		80	10	1.07
DL-Arabinose		70	10	1.21
DL-Xylose		70	10	1.04
DL-Lyxose		80	10	1.05
DL-Erythrose		80	10	1.07
DL-Sorbose		100	10	1.04
1-O-Methyl-β-DL-arabinopyranoside		90	10	1.11

[a]This separation used a temperature gradient of 1 °C min^{-1}

[b]Two pairs of enantiomers (a total of four stereoisomers) exist for this material. Both dried nitrogen and hydrogen were used as carrier gases

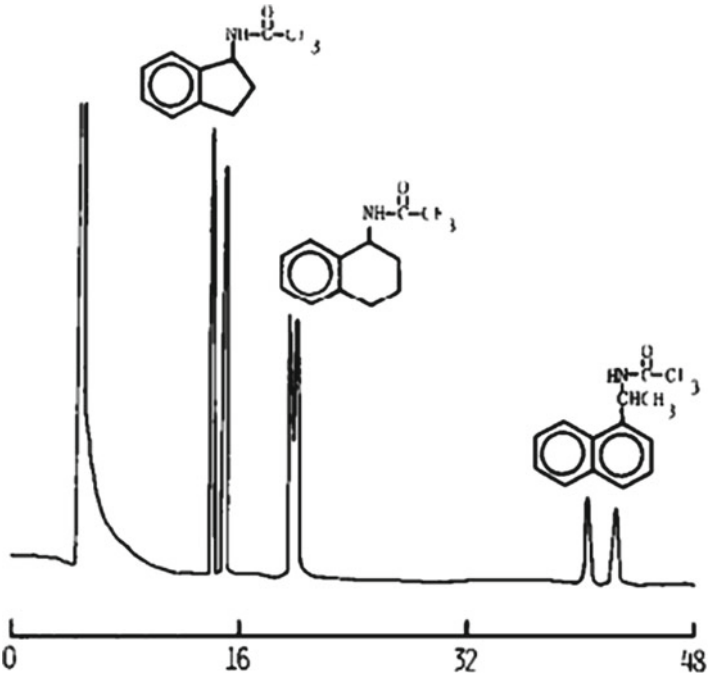


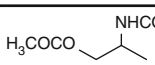
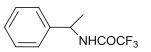
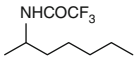
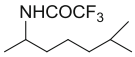
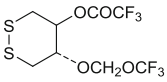
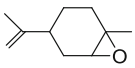
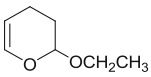
Fig. 2.4 Isothermal enantiomeric separation of trifluoroacetylated 1-aminoindan, 1,2,3,4-tetrahydro-1-naphthylamme, and 1-(1-naphthyl)ethylamine on a 30-m fused-silica capillary coated with **CSP2.2** (column temperature 160 °C) (Reprinted from Ref. [29], Copyright 1990, with permission from Elsevier)

both the two kinds of the β-CDs derivatives have excellent enantioselectivity of sugars. The substitutions of dipentyl-CDs and PMHP-CDs are different, but they all well changed the structure of the cyclodextrins and prepared excellent cyclodextrins derivatives chiral separation selector used in GC.

Armstrong and his coworkers also compared **CSP2.4** and **CSP2.5** with a new "liquefied" CD-CSP, 2,6-di-*O*-pentyl-3-*O*-(trifluoroacetyl)-γ-cyclodextrin (G-TA, **CSP2.7**) [26]. Split injections of 0.1–0.2 μL of samples were done with a split ratio 1/100. The injection port and detector temperature were set at 250 °C. N_2 was used as the carrier gas with a linear velocity of ~10 cm/s for the 20 m columns and 9 cm/s for 10 m columns. The structures and chiral separation data of compounds including tetralins, indans, octahydrophenanthrenes, and isoprenoids by using **CSP2.4**, **CSP2.5,** and C**SP2.7** are shown in Table 2.8. Separation factors ranged from 1.01 to 1.1. In some cases, i.e., 1,4-dimethyltetralin and the methyl-substituted octahydrophenanthrene, **CSP2.5** showed the greatest enantioselectivity. For the remaining substituted racemic tetralins, the **CSP2.5** and the **CSP2.7** (G-TA) columns gave similar enantioselectivities.

Armstrong et al. [27] also synthesized 2,6-di-*O*-penty1-3-*O*-(trifluoroacetyl)-β-cyclodextrin (DP-TFA, **CSP2.8**) and investigated its chiral separation property

Table 2.5 Comparisons of enantiomeric separation data for compounds resolved on **CSP2.4**, **CSP2.5,** and **CSP2.6**

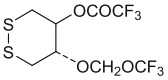
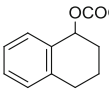
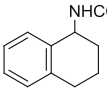
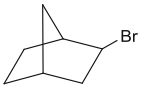
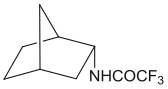
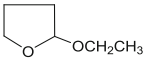
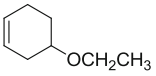
Compound	Structure	T (°C)	Column length (m)	α	CSP
2-Amino-1-propanol		120	9	1.02	**CSP2.4**
		120	20	1.04	**CSP2.5**
		120	9	1.02	**CSP2.6**
1-Phenylethylamine		90	9	1.02	**CSP2.4**
		100	9	1.04	**CSP2.5**
		100	9	1.04	**CSP2.6**
2-Aminoheptane		90	9	1.05	**CSP2.4**
		80	9	1.12	**CSP2.5**
		90	9	1.02	**CSP2.6**
2-Amino-6-methyl-heptane		90	9	1.04	**CSP2.4**
		80	9	1.15	**CSP2.5**
		90	9	1.03	**CSP2.6**
trans-1,2-Dithiane-4,5-diol		100	9	1.12	**CSP2.4**
		100	10	1.06	**CSP2.5**
		100	9	1.03	**CSP2.6**
Limonene oxide		70	9	1.03	**CSP2.4**
		100	20	1.03	**CSP2.5**
		70	9	1.03	**CSP2.6**
1,2-Dihydro-2-ethoxypyran		60	9	1.16	**CSP2.4**
		50	9	1.05	**CSP2.5**
		50	9	1.04	**CSP2.6**

comparing with **CSP2.7**. Table 2.9 summarizes the retention data for three homologous series of compounds: amines, diols, both TFA derivatized, and alkyl esters of 2-bromobutyric acid. For both CSPs and all three homologous series studied, nearly identical values were obtained within a series regardless of the chain length or branching of the "tail." In the amine-containing homologous series, the smallest member had a slightly smaller a value than the higher molecular weight analytes. In the other two series, the smallest members had slightly larger values than the rest.

In order to get more insight in the influence of long-chain alkyl substituents of CD on chiral selectivity, Nie et al. [3, 56] synthesized a series of CD-CSPs: heptakis(2,6-di-O-butyl-3-O-trifluoroacetyl)-β-CD (DBTBCD, **CSP2.9**), heptakis (2,6-di-O-nonyl-3-O-trifluoroacetyl)-β-CD (DNTBCD, **CSP2.10**), and heptakis (2,6-di-O-dodecyl-3-O-trifluoroacetyl)-β-CD (DDTBCD, **CSP2.11**). Then, these three **CSP2.10**, **CSP2.11,** and **CSP2.8** were dissolved in dichloromethane and then coated statically to the same deactivated 20 m × 0.25 mm ID fused-silica capillary columns (0.3 % w/v) to prepare capillary GC columns.

Nie [57] also synthesized heptakis(2,6-di-O-butyl-3-O-butyryl)-β-CD (DBBBCD, **CSP2.12**). Some complex chiral compounds, such as some drug intermediates and insecticides, can be satisfactorily resolved on the mixed CD derivative chiral stationary phases. So Nie and coworkers prepared the columns containing two

Table 2.6 Enantiomeric separation data for compounds resolved on permethyl-O-(S)-2-hydroxypropyl-derivatized α-cyclodextrin (**CSP2.4**) [25] and dipentyl-α-CD stationary phase (**CSP 2.**) [29]

Compound	Structure	*T* (°C)	Column length (m)	*α*	CSP
trans-1,2-Dithiane-4,5-diol		100	9	1.12	**CSP2.4**[a]
		70	10	1.30	**CSP2.1**
1,2,3,4-Tetrahydro-1-naphthol		100	9	1.03	**CSP2.4**
		160	20	1.07	**CSP2.1**
1,2,3,4-Tetrahydro-l-naphthylamine		130	9	1.03	**CSP2.4**
		170	20	1.05	**CSP2.1**
exo-2-Bromonorbornane		90	9	1.01	**CSP2.4**
		30	10	1.05	**CSP2.1**
endo-2-Aminonorbornane		90	9	1.02	**CSP2.4**
		30	10	1.08	**CSP2.1**
2-Ethoxytetrahydrofuran		60	9	1.22	**CSP2.4**
		30	10	1.10	**CSP2.1**
3,4-Dihydro-2-ethoxy-2*H*-pyran		60	9	1.16	**CSP2.4**
		30	10	1.20	**CSP2.1**

[a]Injection port temperature was 200 °C and N_2 was used as the carrier gas

mixture CD-CSPs: **CSP2.10** + **CSP2.13** (permethylated β-CD, PMBCD) and **CSP2.12** + **CSP2.13**. The stationary-phase film thickness of all the columns prepared was ca. 0.20 μm. Both the injector and detector temperatures were maintained at 250 °C with hydrogen as the carrier gas; all the separations were performed isothermally, with a split ratio of 1:100. In addition, Nie [56] developed another CD-CSP: heptakis(2,6-di-O-pentyl-3-O-acetyl)-β-CD (DPABCD, **CSP2.14**).

Table 2.10 summarizes the capacity factors (k') and separation factor (α) for different racemates on the different **CSP2.10**, **CSP2.11,** and **CSP2.8** to investigate the influence of long-chain alkyl substituent on the chiral selectivity. The racemates separated on the three cyclodextrin CSPs included amines, alcohols, diols, carboxylic acids, amino acids, halohydrocarbons, epoxides, and ketones. It is observed that **CSP2.10** (DNTBCD) exhibits the best chiral selectivity among the three stationary phases for most racemates investigated. As shown in Table 2.10, almost all the chiral compounds separated on **CSP2.8** can be separated on **CSP2.10**, but the racemates separated on **CSP2.11** is significantly less than on **CSP2.8**, which mean the chiral recognition ability of **CSP2.11** is inferior to that of **CSP2.10** or **CSP2.8**. As illustrated in the table, for almost all the racemates tested, the chromatographic separation results are given at the same temperature. It is observed that for the majority of the racemates tested, separation factors on **CSP2.10** are significantly higher than

Table 2.7 Enantiomeric separation data for compounds resolved on permethyl-O-(S)-2-hydroxypropyl-derivatized β-cyclodextrin (**CSP2.5**) [25] and dipentyl-β-CD stationary phase (**CSP2.2**) [29]

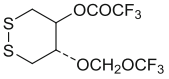
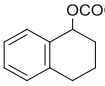
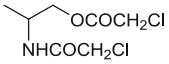
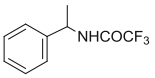
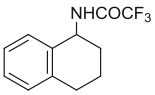
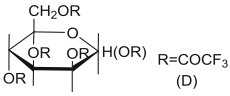
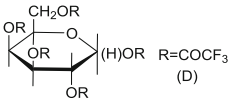
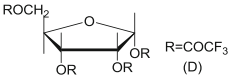
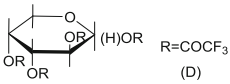
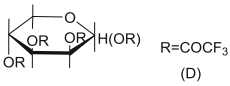
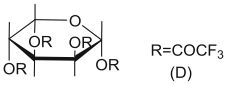
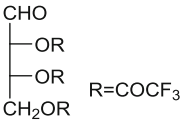
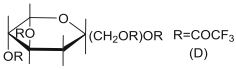
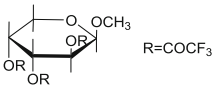
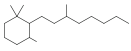
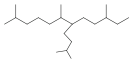
Compound	Structure	*T* (°C)	Column length (m)	*α*	CSP
trans-1,2-Dithiane-4,5-diol		100	10	1.06	**CSP 2.5**
		80	10	1.07	**CSP 2.2**
1,2,3,4-Tetrahydro-1-naphthol		120	20	1.07	**CSP 2.5**
		–	–	–	**CSP 2.2**
2-Amino-1-propanol		120	20	1.04	**CSP 2.5**
		140	30	1.01	**CSP 2.2**
1-Phenylethylamine		100	9	1.04	**CSP 2.5**
		120	20	1.03	**CSP 2.2**
1,2,3,4-Tetrahydro-l-naphthylamine		150	20	1.04	**CSP 2.5**
		130	20	1.01	**CSP 2.2**
DL-Mannose		90	9	1.02	**CSP 2.5**
		100	10	1.21	**CSP 2.2**
DL-Galactose		100	9	1.05	**CSP 2.5**
		90	10	1.04	**CSP 2.2**
DL-Ribose		100	9	1.08	**CSP 2.5**
		80	10	1.07	**CSP 2.2**
DL-Arabinose		90	9	1.04	**CSP 2.5**
		70	10	1.21	**CSP 2.2**
DL-Xylose		80	9	1.03	**CSP 2.5**
		70	10	1.04	**CSP 2.2**
DL-Lyxose		80	9	1.04	**CSP 2.5**
		80	10	1.05	**CSP 2.2**
DL-Erythrose		80	20	1.03	**CSP 2.5**
		80	10	1.07	**CSP 2.2**
DL-Sorbose		90	9	1.12	**CSP 2.5**
		100	10	1.04	**CSP 2.2**
1-*O*-Methyl-β-DL-arabinopyranoside		90	20	1.09	**CSP 2.5**
		90	10	1.11	**CSP 2.2**

Table 2.8 Relevant structure information and separation data for the gas chromatographic separation [26]

Compound	Structure	k'	α	T (°C)	Column length (m)	CSP
Tertralin						
1,8-Dimethyl-		6.9	1.03	110	10	**CSP2.5**
2,7-Dimethyl-		38.4	1.02	70	10	**CSP2.5**
		21.1	1.05	120	10	**CSP2.4**
1,5,8-Trimethyl-		10.3	1.05	120	10	**CSP2.5**
2,6-Dimethyl-		35.4	1.03	70	10	**CSP2.5**
		12.42	1.04	100	10	**CSP2.4**
1,4-Dimethyl-		21.5	1.06[a]	80	10	**CSP2.5**
		58.4	1.01[a]	70	10	**CSP2.7**
2-Ethyl-		40.5	1.01	70	10	**CSP2.5**
		12.9	1.02	100	10	**CSP2.4**
Indan						
1-Isopropyl-		19.4	1.02	90	10	**CSP2.4**
1-Propyl-		19.4	1.02	90	10	**CSP2.4**
1-Ethyl-		15.0	1.05	90	10	**CSP2.4**
4a-Methyl-1,2,3,4,4a,9,10,10a-octa-hydro-phenanthrene		34.4[b]	1.01[b]	105	10	**CSP2.6**
		48.9	1.03			
		20.12[b]	1.01[b]	120	10	**CSP2.4**
		29.22	1.02			
1,1,3-Trimethyl-2-(3-methyloctyl)-cyclohexane		80.2[c]	–	70	20	**CSP2.5**
2,6,10-Trimethyl-7-(3-methylbutyl)dodecane		70.4	1.01[d]	100	10	**CSP2.5**

k': value is for the first eluted enantiomer. α: the separation factor

[a]This compound exists as a pair of enantiomers and a meso compound. The α value is for the enantiomeric pair only

[b]This compound exists as two pair of enantiomers; hence, two values are given

[c]There are three stereogenic centers (four pairs of enantiomers) for this sample. The k' value is for the first eluted enantiomer of the first pair

[d]This compound also contains three stereogenic centers. However, all eight stereoisomers could not be resolved

those determined on **CSP2.8** at the same temperature. Therefore, **CSP2.10** possesses the best long-chain nonyl substituent among these three CSPs.

Table 2.11 lists satisfactory enantioseparation data of some synthetic pyrethroids achieved on different CD-CSPs. *cis*-Chrysanthemic methyl ester (P1) was baseline resolved on **CSP2.13**, but *trans*-chrysanthemic methyl ester was not resolved at all on column **CSP2.10**. On **CSP2.12**, *trans*-chrysanthemic methyl ester can be

Table 2.9 Retention and selectivity of three homologous series on 2,6-dipentyl-3-(trifluoroacetyl)-cyclodextrin stationary phases, **CSP2.7** and **CSP2.8** [27]

Compound[a]	T (°C)	k'^{b}	α	Stationary phase
2-Aminobutane	90	1.55	1.14	**CSP2.8**
2-Amino-3,3-dimethylbutane	90	2.10	1.22	**CSP2.8**
2-Aminopentane	90	2.40	1.22	**CSP2.8**
1,3-Dimethylbutylamine	90	2.80	1.22	**CSP2.8**
2-Aminoheptane	90	8.15	1.22	**CSP2.8**
1,5-Dimethylhexylamine	90	12.15	1.22	**CSP2.8**
1,2-Propanediol	70	1.61	1.49	**CSP2.7**
1,2-Pentanediol	70	3.50	1.23	**CSP2.7**
1,2-Hexanediol	70	6.64	1.23	**CSP2.7**
1,2-Octanediol	70	29.3	1.23	**CSP2.7**
Methyl 2-bromobutanoate	80	5.07	1.56	**CSP2.8**
		6.71	1.57	**CSP2.7**
Ethyl 2-bromobutanoate	80	5.25	1.29	**CSP2.8**
		9.93	1.16	**CSP2.7**
Isopropyl 2-bromobutanoate	80	6.57	1.08	**CSP2.7**
N-Butyl 2-bromobutanoate	80	15.5	1.16	**CSP2.8**
		21.5	1.09	**CSP2.7**
N-Pentyl 2-bromobutanoate	80	32.3	1.16	**CSP2.8**
		39.5	1.08	**CSP2.7**
N-Hexyl 2-bromobutanoate	80	66.4	1.16	**CSP2.8**
		80.0	1.09	**CSP2.7**

[a]All amines and alcohols were resolved after trifluoroacetylation

[b]For the first eluted enantiomer

resolved, but *cis*-chrysanthemic methyl ester cannot be resolved at all. It is expected that the mixed chiral stationary phase consisting of **CSP2.13** and **CSP2.12** can combine their chiral recognition property to separate chrysanthemic methyl ester. As expected, diastereomer and enantiomer of chrysanthemic methyl ester can be resolved on the mixed chiral stationary phase (**CSP2.10 + 2.13**). Better resolution of chrysanthemic methyl ester can be achieved at lower temperatures.

As for chrysanthemic *L*-menthyl ester (P2), the diastereomers and enantiomers of chrysanthemic *L*-menthyl ester can be satisfactorily resolved on either of the mixed chiral stationary phases (**CSP2.10 + 2.13**, **CSP2.12 + 2.13**). On **CSP2.13** or **CSP2.10**, only *trans*-isomer can be satisfactorily resolved. As seen in Table 2.11, for 3-(2,2,2-trichloroethyl)-2,2-dimethylcyclopropanecarboxylic methyl and ethyl esters (P4 and P5), the values of the separation factor (α) measured on the two mixed chiral stationary phases are greater than those obtained on **CSP2.13**, **CSP2.12,** and **CSP2.10** containing single cyclodextrin derivative phases. It is obvious that for P4 and P5, positive synergistic effects exist in the mixed cyclodextrin derivative chiral stationary phases. This indicates that the mixing of **CSP2.13** with **CSP2.12** or **CSP2.10** can improve the enantiomeric separation of 3-(2,2,2-trichloroethyl)-2,2-dimethyl-cyclopropanecarboxylate esters. Likewise, a positive synergistic effect was observed on **CSP2.10 + 2.13** for *trans*-permethrinic methyl ester. It is obvious that the mixing CD-CSPs GC columns have better enantioseparation ability.

Table 2.10 Enantioseparation results for different racemates on different CD-CSPs [3]

		DNTBCD, **CSP2.10**			DDTBCD, **CSP2.11**			DP-TFA, **CSP2.8**		
Compounds	Derivative group	α	k_1'	T/°C	α	k_1'	T/°C	α	k_1'	T/°C
2-Butylamine	TFA[a]	1.140	1.72	100	1.103	0.87	100	1.113	2.47	100
2-Heptylamine	TFA	1.075	1.89	130	1.082	1.35	130	1.105	3.10	130
2-Octylamine	TFA	1.148	3.19	130	1.090	2.36	130	1.206	5.43	130
o-CH_3O-α-PEA	TFA	1.039	2.74	160	1.051	3.62	160	1.039	2.58	160
m-CH_3O-α-PEA	TFA	1.021	4.48	160	NS[b]			1.022	4.35	160
p-CH_3O-α-PEA	TFA	1.032	5.18	160	1.019	6.18	160	1.021	5.80	160
o-CH_3-α-PEA	TFA	1.068	3.89	140	NS			1.034	3.81	140
m-CH_3-α-PEA	TFA	1.042	4.49	140	NS			1.022	4.15	140
p-CH_3-α-PEA	TFA	1.050	5.27	140	NS			1.020	5.26	140
2-Butanol	TFA	1.171	0.43	60	1.093	0.70	48	1.128	0.77	60
2-Pentanol	TFA	1.156	0.59	65	1.100	0.83	50	1.142	1.43	60
2-Octanol	TFA	1.030	3.06	80	1.049	2.23	80	1.091	5.53	80
2-Nonanol	TFA	1.056	3.05	100	1.030	2.29	100	1.047	4.46	100
2-Nonanol	acetyl	1.121	5.67	110	1.057	7.23	100	1.091	4.88	110
3-Nonanol	acetyl	1.050	4.71	110	NS			1.041	2.94	110
4-Nonanol	acetyl	NS			NS			1.010	2.62	110
2,3-Dibromo propanol	TFA	1.088	1.93	100	NS			1.027	1.00	100
3-Methyl-2-butanol	TFA	1.031	1.74	70	NS			1.052	1.14	70
2-Methyl-1-pentanol	TFA	1.037	1.64	70	NS			1.018	3.36	70

1,2-Propanediol	TFA	1.140	0.65	100	1.118	0.46	90	1.149	0.61	100
1,3-Butanediol	TFA	1.135	1.63	100	1.072	0.95	100	1.062	1.48	110
2,3-Butanediol	TFA	NS			1.037	2.44	100	NS		
2,3-Butanediol	acetyl	1.051	1.80	110	1.084	2.53	100	1.049	1.23	110
2-Bromopropionate	IP[c]	1.050	1.75	100	1.049	1.70	90	1.036	1.53	100
Alanine	TFA-IP	1.088	1.74	120	1.036	1.21	120	1.094	1.99	120
Valine	TFA-IP	1.053	3.29	120	1.047	1.57	120	1.022	2.65	120
Threonine	TFA-IP	1.093	2.26	130	1.079	4.12	120	1.124	1.61	130
Isoleucine	TFA-IP	1.071	3.42	120	1.065	2.50	120	1.034	3.94	120
Leucine	TFA-IP	1.064	3.47	120	1.062	2.61	120	1.026	3.71	120
Proline	TFA-IP	1.110	5.88	130	1.090	4.40	130	1.041	6.85	130
1,2-Dibromopropane		1.040	3.00	80	NS			1.040	1.99	80
Epichlorohydrin		1.170	2.05	80	1.071	2.02	70	1.260	2.04	80
Epibromohydrin		1.100	1.45	100	1.035	2.07	90	1.130	1.37	100
2-Methylcyclohexanone		1.096	2.80	100	1.052	2.74	100	1.058	2.08	100
3-Methylcyclohexanone		1.099	4.47	100	1.053	3.55	100	1.043	2.71	100

[a]Trifluoroacetate
[b]No separation observed
[c]Isopropyl

Table 2.11 Separation results of pyrethroids on five different CD-CSPs columns [56]

		T/°C	**CSP2.13**		**CSP2.12**		**CSP2.10**		**CSP2.10+2.13**		**CSP 12+13**	
P1	*cis*	105	16.65	1.041	14.31	1.000			17.71	1.013		
	trans		21.03	1.000	17.45	1.009			22.13	1.010		
P2	*cis*	150	31.05	1.022	27.15	1.000	25.26	1.000	28.80	1.011	28.46	1.008
	trans		32.67	1.105	28.18	1.080	26.66	1.077	30.21	1.094	30.11	1.086
P3	*cis*	90	39.93	1.051	33.78	1.000	23.97	1.000	37.89	1.020	28.89	1.025
	trans		51.45	1.000	44.28	1.018	29.89	1.032	53.489	1.024	34.60	1.030
P4	*cis*	110	20.18	1.022	22.98	1.000	9.81	1.026	27.24	1.046	19.75	1.039
	trans		27.10	1.022	32.70	1.000	11.01	1.032	38.41	1.000	25.10	1.034
P5	*cis*	110	20.48	1.032	17.13	1.000	10.00	1.025	28.70	1.038	19.69	1.040
	trans		29.54	1.028	21.19	1.000	11.23	1.032	41.81	1.000	25.03	1.035

P1: chrysanthemic methyl ester, 3-(2, 2-dimethylethenyl)-2, 2-dimethylcyclopropanecarboxylic methyl ester
P2: chrysanthemic *L*-menthyl ester, 3-(2, 2-dimethylethenyl)-2, 2-dimethylcyclopropanecarboxylic (1R, 2S, 5R)-2- isopropyl-5-methylcyclohexanyl ester
P3: permethrinic methyl ester, 3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropanecarboxylic methyl ester
P4: 3-(2, 2, 2-trichloroethyl)-2, 2-dimethylcyclopropanecarboxylic methyl ester
P5: 3-(2, 2, 2-trichloroethyl)-2, 2-dimethylcyclopropanecarboxylic ethyl ester

Schurig et al. [58] also studied the mixed CD-CSPs with effort to simultaneously enantioseparate racemic unfunctionalized alkanes and racemic α-amino acid derivatives. They developed a new kind of mixed **CSP2.15** column by dissolving the commercial Lipodex G (listed in Table 2.1) and synthesized heptakis(2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin in a polysiloxane, which was then coated onto a 50 m × 0.25 mm i.d. fused-silica capillary column. With mixed binary chiral selector (structures of each CSP are shown in Fig. 2.5) system present in one CSP, the simultaneous gas chromatographic enantioseparation of racemic alkanes and of racemic derivatized α-amino acids are achieved in a single run. Combining β- and γ-cyclodextrin derivatives, the enantioseparation scope of this **CSP2.15** could be extended as compared with the conventional use of the single CD selectors in GC.

It was dissolved in a semipolar polysiloxane (PS 255) and then coated to the capillary column, and the structure of this CSP is given under Fig. 2.6. It was applied as a chiral stationary phase to GC enantioseparation, and its chiral separation result was compared with the commercial Lipodex E (listed in Table 2.1).

Engel et al. [48] also developed a γ-CD-CSP; they employed methoxymethyl chloride as acetalization reagent to introduce the methoxymethyl (MOM) moiety at the 2, 3-hydroxyl rim of γ-cyclodextrin to synthesize the novel cyclodextrin derivative: octakis(2,3-di-*O*-methoxymethyl-6-*O*-tert-butyldimethylsilyl)-γ-cyclodextrin (2,3-MOM-6-TBDMS-γ-CD, **CSP2.17**), as shown in Fig. 2.7. It was demonstrated to be a CSP suitable for enantiodifferentiation of a broad spectrum of chiral volatiles from various chemical classes including alcohols, aldehydes, ketones, acids, esters, and acetal.

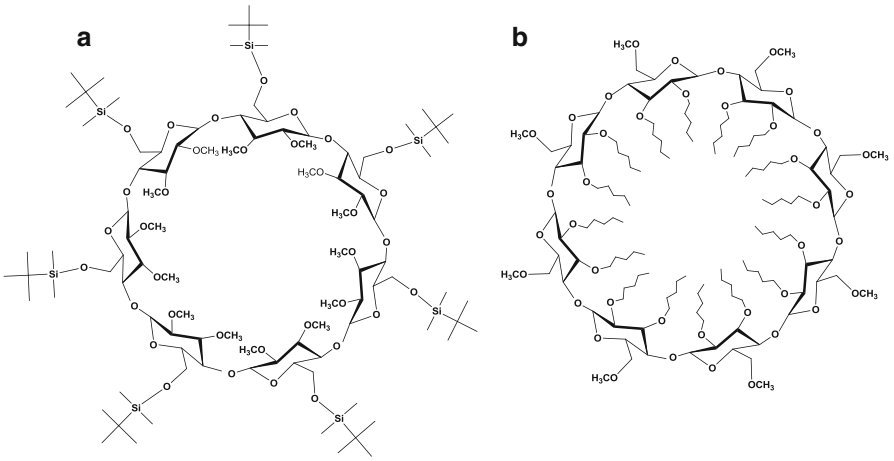


Fig. 2.5 Structure of the heptakis(2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin (**a**) and Lipodex G (**b**) in the mixed binary chiral selector system (diluted in polysiloxane PS 086) for enantioselective gas chromatography (Reprinted from Ref. [58], Copyright 1990, with permission from Elsevier)

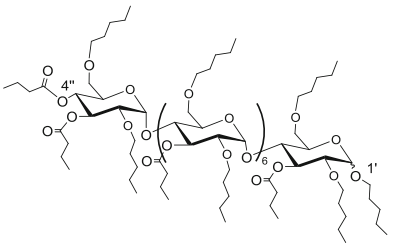


Fig. 2.6 Structure of octakis[(3-*O*,-4″*O*)-butanoyl-(1″-*O*,2,6-di-*O*)-n-pentyl] maltooctaose, CSP2.16

Bouillon and coworkers [59] exploited another γ-CD-CSP named 6^{I}-O-Benzyl-$2^{I–VIII}$,$3^{I–VIII}$,$6^{II–VIII}$-tricosa-O-methyl-γ-cyclodextrin (**CSP2.18**). Two synthesis methods were employed, the indirect strategy and the direct method. Figure 2.8 gives the direct synthesis process of this γ-cyclodextrin derivative. This CD derivative was diluted at 15 % (w/w) in OV 1701 and coated in a fused-silica column (10 m × 0.25 mm) according to the static method. The **CSP2.18** presents generally low-resolution values for the solutes tested and only linalool and α-terpineol enantiomers. This result might be the consequence of additional π-stacking interactions between these solutes and the benzyl group of the CD derivative.

Combret et al. [60] made three new GC capillary columns based on 6^{I}-O-methoxycarbonyl-6^{I}-deoxy-$2^{I–VII}$,$3^{I–VII}$,$6^{II–VII}$-eicosa-*O*-methylcyclodextrin (20Me/P6COOMe, **CSP2.19**), 6^{I}-*O*-methoxycarbonylmethyl-$2^{I–VII}$,$3^{I–VII}$,$6^{II–VII}$-eicosa-*O-methyl*-cyclodextrin (20Me/P6OCH$_2$COOMe, **CSP2.20**), and 2^{I}-*O*-methoxycarbonylmethyl-$2^{II–VII}$,$3^{I–VII}$,$6^{I–VII}$-eicosa-*O*-methyl-cyclodextrin (20Me/P2OCH$_2$COOMe, **CSP2.21**).

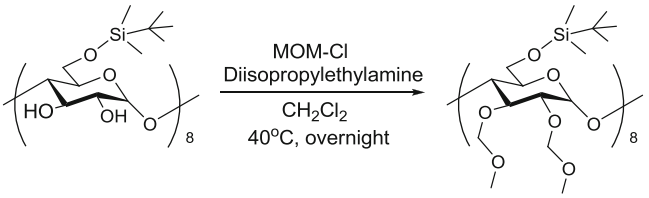


Fig. 2.7 Synthesis of 2,3-MOM-6-TBDMS-γ-CD, **CSP 17**

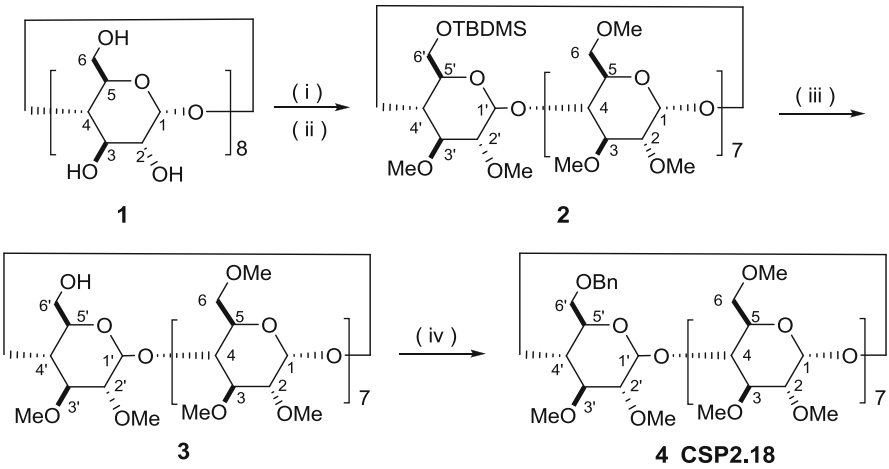


Fig. 2.8 Reagents and conditions: (i) TBDMSCl, imidazole, DMF, rt (24 h); (ii) NaH, DMF, 0 °C (30 min) to rt (2 h) then MeI, 0 °C (1 h) to rt (24 h) (two steps, **2**: 30 %); (iii) Bu_4NF, THF, rt (12 h) (**3**: 54 %); (iv) NaH, THF, △ (2 h) then BnCl, 0 °C (1 h) to reflux (12 h) (**4**: 76 %) (Reprinted from Ref. [59], Copyright 2008, with permission from Elsevier)

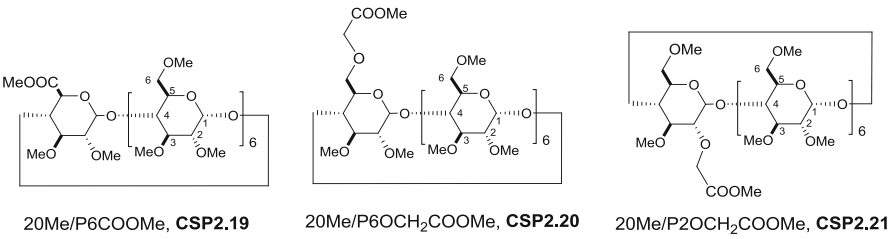
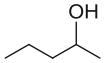
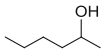
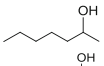
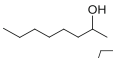
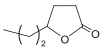
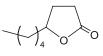
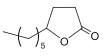
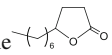


Fig. 2.9 Structures of the three CSPs

More than 60 solutes have been used for studying their enantioselectivity, and enantioseparation results of second alcohols and γ-lactones using these three CSPs (their structures are shown in Fig. 2.9) are listed in Table 2.12.

As shown in the Table 2.12, the separation results obtained on **CSP2.20** (20Me/P6OCH2COOMe) and **CSP2.21** (20Me/P2OCH2COOMe) indicate that the position of the ester group has no influence on the selectivity. The resolution values for

Table 2.12 Enantioseparation results of the enantiomers of secondary alcohols and γ-lactones

Compounds	T (°C)	CSP2.18			CSP2.19			CSP2.20		
		k	α	R_s	k	α	R_s	k	α	R_s
2-Pentanol 	60									
2-Hexanol 	60	2.38	1.03	1.46	2.24	1.00	–	2.87	1.03	2.02
2-Heptanol 	60	6.12	1.02	1.92	6.03	1.03	0.91	7.62	1.03	1.69
2-Octanol 	60	14.28	1.00	–	15.39	1.00	–	16.84	1.00	–
γ-Heptalactone 	110	9.35	1.02	1.90	11.90	1.03	2.49	10.57	1.04	3.74
γ-Nonalactone 	110	32.46	1.02	1.89	41.93	1.03	2.91	36.85	1.03	3.57
γ-Decalactone 	110	71.24	1.00	–	88.91	1.00	–	77.07	1.00	–
γ-Undecalactone 	110	106.8	1.00	–	146.2	1.03	1.94	124.4	1.03	2.97

CSP2.21 are better than **CSP2.20**, indicating that the substituted group on **CSP2.19** is a little longer which will influence the formation of complexation if it is twisted and included in the cavity. As for **CSP2.19**, a decrease of enantioselectivity was observed which might because the ester group is directly connected to the rim.

The above section focuses on the "wall-coated" CD-CSP column, which is one type of the most widely applied GC columns and includes multiple categories. "Wall-coated" CD-CSP columns play an important role in GC enantioseparation field, while another main type of CD-CSP columns is "wall-bonded" column. In the next section, this immobilized type of CD-CSP columns applied in GC will be introduced.

2.3.3 Self-Made "Wall-Bonded" GC CD-CSPs Columns

Schurig et al. [35, 61] made a great contribution to develop the applications of CD-CSP columns in GC enantioseparation field. He synthesized a polysiloxane-anchored permethylated β-CD-bonded capillary column: Chirasil-Dex [61]. Now this kind of column can be purchased as listed in Table 2.1, and the CD-CSP used here is mentioned before (**CSP2.13**). The CD was anchored to a polysiloxane to make this column, which was applied in both GC and SFC for enantiomeric separation. Table 2.13 lists the enantiomeric separation of *rac*-proline in various derivatized forms on this kind of "bonded" columns. In this table, a decrease in selectivity in the order free acid > methyl ester > isopropyl ester is observed for the acid group. Further improvements in selectivity can be gained by using cyclic derivatives such as methylthiohydantoins (MTH). However, these MTH derivatives required the

Table 2.13 Enantioseparation of rac-proline in various derivatized forms on Chirasil-Dex CSP[a] [58]

Derivatives of groups[b]				
Carboxy	Amino	Mode	Temperature (°C)	Selectivity (α)
H	TFA	SFC	60	1.096
H	Ac	SFC	60	1.107
iPr	TFA	GC	110	1.008
Me	TFA	GC	110	1.028
Me	Ac	GC	110	1.035
MTH		GC	150	1.046
MTH		SFC	60	1.132

[a]GC: 10 m × 0.1 mm i.d., column $d_f = 0.15$ μm; SFC: 8 m × 0.1 mm i.d., column $d_f = 0.2$ μm, density = 0.5 g cm^{-3}

[b]Abbreviation of derivatives: *H* free, *TFA* trifluoroacetyl, *Ac* acetyl, *Me* methyl, *iPr* isopropyl, *MTH* methylthiohydantoin

analytical temperature should be higher than 150 °C in the GC mode, at which temperature of these compounds thermally degrades to some extent. Therefore, SFC is an appropriate analysis method for the MTH amino acids, because the low analysis temperature does not cause any degradation.

In Chirasil-type stationary phases, the chiral selector is anchored to a polysiloxane backbone; permethylated β-cyclodextrin was chemically linked to an apolar polydimethylsiloxane backbone via a trimethylene, pentamethylene, or octamethylene spacer, known as Chirasil-Dex (a chiral polysiloxane-containing cyclodextrin). In Chirasil-Dex, the CD selector is embedded in the apolar environment; if Chirasil-Dex can be thermally immobilized on the inner surface of fused-silica capillaries, it can be used in all contemporary modes of enantioselective open-tubular chromatography (GC, SFC, and LC) including a unified approach employing the same single column. In order to further separate the chiral recognition unit (CD) and the supporting polymeric matrix (polysiloxane), a tether of extended length, i.e., the undecamethylene spacer, has now been introduced into Chirasil-Dex by Schurig et al. [35]. A new CSP C11-Chirasil-Dex (**CSP2.22**) was prepared as Fig. 2.10. This CSP shows excellent separation properties for the enantiomers of polar secondary alcohols (Table 2.14). The structures of these second alcohols are shown in Fig. 2.11.

The C11-Chirasil-Dex column was demonstrated to have a excellent enantioseparation ability of alcohols from the data in the Table 2.14. For the compound **11**, the resolution value is as high as 10.66, and R_S values for at least six compounds are more than 4.0. In the case of the enantioselective transesterification of alcohols **7** and **14**, the reaction was very fast. It is thus reasonable that the prolongation of reaction time causes the faster reacting enantiomer to reach its state of equilibrium more rapidly.

Ghanem [62] demonstrated the enantiomeric separation ability of **CSP2.22** by simultaneously separating a set of cyclopropane derivatives. All these cyclopropane derivatives were prepared according to literature [63, 64]. The GC parameters including oven temperature (T), retention time (t_R, t_S), resolution (R_s), and the separation

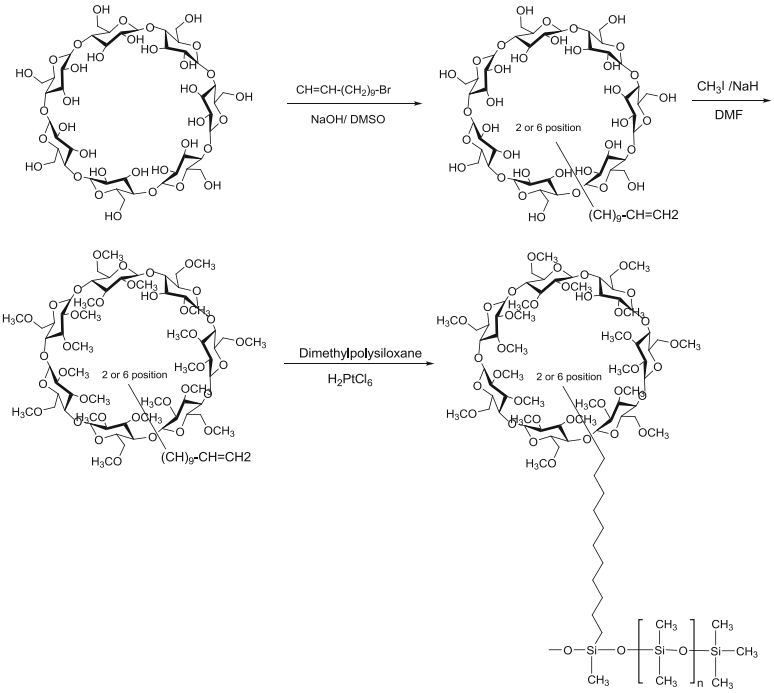


Fig. 2.10 Synthesis of permethylated β-cyclodextrin with a new 11 spacer (Chirasil-β-Dex with 11 spacer) bonded to a polysiloxane backbone (Reprinted from Ref. [35], with kind permission from Springer Science + Business Media)

Table 2.14 Baseline chiral separation data of racemic secondary alcohols

Compounds	Oven temperature (°C)	t_R	t_S	R_S	α
1	95	10.1	11.2	5.83	1.12
2	100	14.2	15.2	4.20	1.08
6	80	27.1	26.1	2.02	1.04
7	85	4.6	4.8	1.88	1.05
8	50	5.4	5.8	1.78	1.07
9	110	20.5	22.1	4.76	1.08
10	105	10.6	11.5	4.68	1.09
11	90 °C for 13 min, then increase to 120 °C at the rate of 10 °C/cm	18.2	20.0	10.66	1.10
12	105	9.3	10.5	6.63	1.14
13	100	22.1	21.4	1.79	1.03
14	120	16.7	15.9	2.68	1.05
15	100	10.6	10.3	1.64	1.04
16	90 °C for 4 min, then increase to 120 °C at the rate of 10 °C/cm	10.9	11.3	3.47	1.04

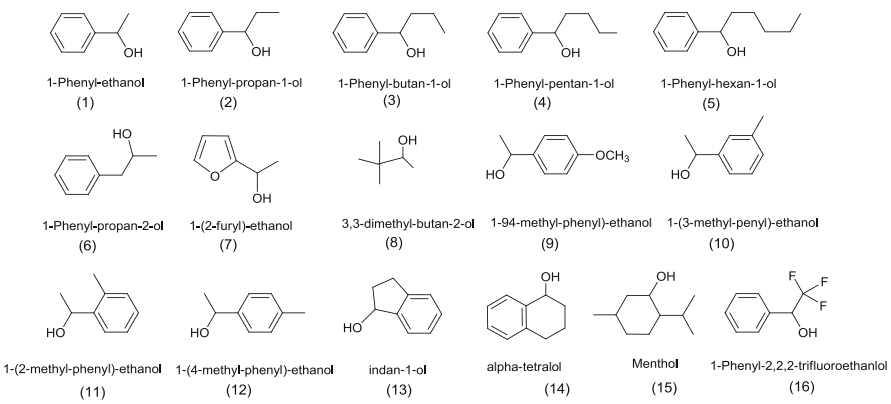


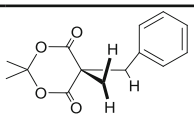
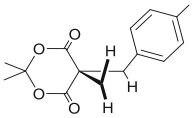
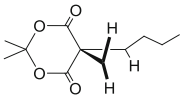
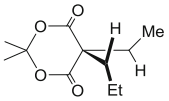
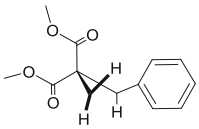
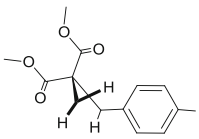
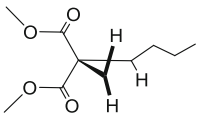
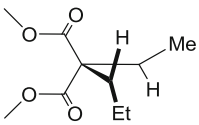
Fig. 2.11 Structure of the secondary alcohols

factor (α) are recorded in Table 2.15. From the data, we can see that **CSP2.22** is absolutely a good selector as all the resolution values for these eight compounds are higher than 1.5. It means all the enantiomers are baseline separated, and the selectivities for all the analytes are also excellent. All these eight compounds are hydrophobic which can form inclusion with the cavity of the cyclodextrin. Complex plays a significant role in the enantioseparation process of the cyclodextrins.

Schurig et al. [36] developed another three new CD-CSPs: $2^{\text{I–VII}},3^{\text{I–VII}},6^{\text{I–VI-eicosa-O}}$-methyl-$6^{\text{I}}$-*O*-(oct-7-enyl) cyclomaltoheptaose (**CSP2.23**), $2^{\text{I–VI}},3^{\text{I–VII}}$, $6^{\text{I–VII-eicosa}}$-*O*-methyl-2^{I}-*O*-(oct-7-enyl) cyclomaltoheptaose (**CSP2.24**), and $2^{\text{I–VII}},3^{\text{I–VI}},6^{\text{I–VII}}$-eicosa-*O*-methyl-$3^{\text{I}}$-*O*-(oct-7-enyl) cyclomaltoheptaose (**CSP2.25**). Each of these three CDs was anchored by hydrosilylation to a hydridomethyldimethylsiloxane copolymer to yield unambiguously *O*-2-, *O*-3-, and *O*-6-immobilized CD-CSP columns, which were evaluated in enantioselective GC. The synthetic routes for these CD-CSPs are shown in Fig. 2.12. Schurig also studied their enantioseparation ability and got that **CSP2.23** displayed slightly inferior enantioselectivity relative to either **CSP2.24** or **CSP2.25**, which will not be discussed here.

Armstrong [28] synthesized two β-CD derivatives named allyl-permethylated and 5-pent-1-enyl-permethylated β-CD. By changing the proportion of derivatized β-CDs and hydrogen-terminated polydimethylsiloxane, they made three different GC columns classified as (A) 1:6 (w/w) ratio of allyl-permethylated β-CD bonded to PS537 (APβCD I, **CSP2.26**), (B) "wall-bonded" CD-CSP 1:4 (w/w) ratio of allyl-permethylated β-CD bonded to PS537 (APβCD II, **CSP2.27**), and (C) 1:6 (w/w) ratio of 5-pent-1-enyl-permethylated β-CD bonded to PS537 (PPβCD I, **CSP2.28**). In the GC enantioseparation process, the injection port and detector temperatures were set at 250 °C. Nitrogen was used as the carrier gas with a linear velocity of 10 cm/s. Several chiral compounds were resolved by capillary GC on three different CD "wall-immobilized" capillary columns; Table 2.16 summarizes the enantiomeric separation results. From the data, it is hard to tell the difference between these CD-CSPs, and they are all demonstrated to be excellent chiral

Table 2.15 Oven temperature (T), retention time (t_R, t_S), resolution (R_s), and the separation factor (α) of the simultaneous baseline separation of racemic cyclopropane derivatives [48]

Entry	Structures	Oven temperature (°C)	t_R	t_S	R_S	α
1		100	40.4	42.8	4.96	1.06
2		140	20.7	22.0	3.25	1.06
3		100	20.9	22.1	2.96	1.05
4		110	21.0	22.3	3.45	1.04
5		130	20.8	22.20	3.36	1.06
6		120	20.8	22.1	3.25	1.06
7		100	15.7	16.2	1.50	1.02
8		100	32.7	33.7	1.71	1.05

selectors. The resolution values of 1-aminoindan and oxyphene are both over 1.10 on CSP **2.26** and **CSP 2.28,** respectively. For 1,3-dimethylbutylamine, the resolution values of both **CSP 2.26** and **CSP2.27** are higher than 1.10 which means the racemic is well separated.

Schurig [37] also synthesized a really interesting mixture Chirasil-Calixval-Dex (**CSP2.29**) by bonding Chirasil-Dex (**CSP2.13**) and Chirasil-Calixval on the same polymeric backbone as Fig. 2.13 shows. In this situation, a resorcinarene with pendant *L*-valine diamide groups (used as hydrogen-bonding selector) and a **CSP2.13** (used as inclusion-type selector) were chemically bonded to poly(hydromethyl) dimethylsiloxane in a one-pot reaction via Pt-catalyzed alkene hydrosilylation.

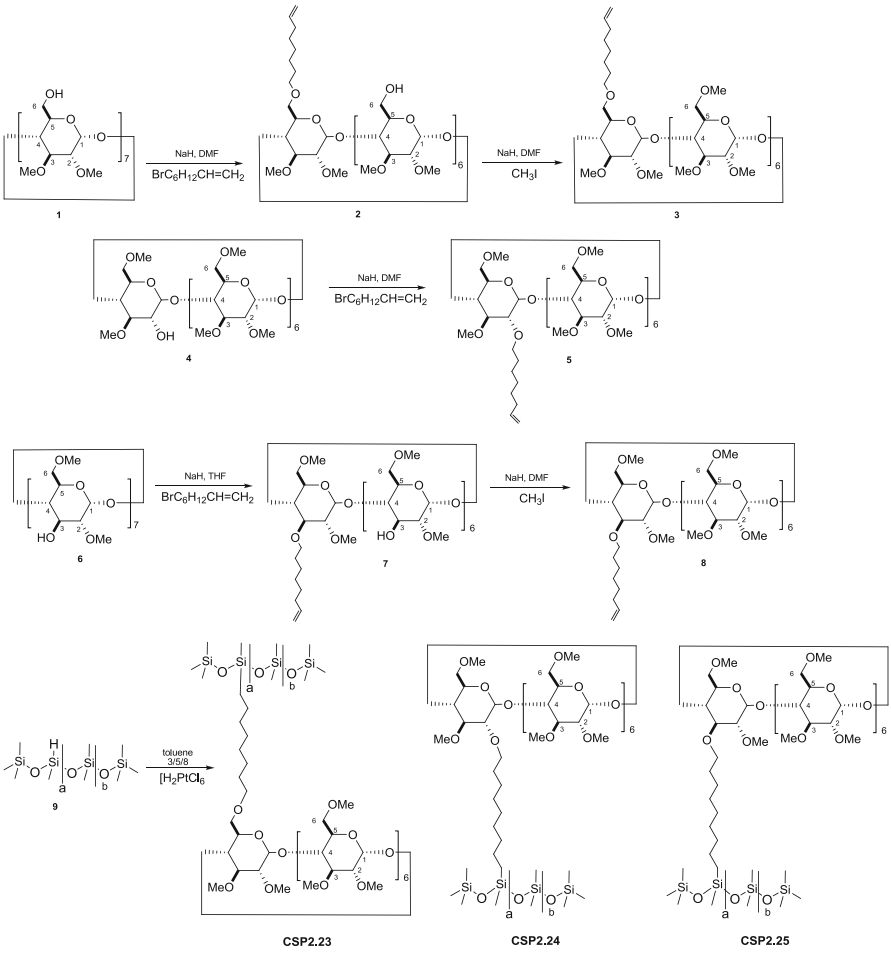
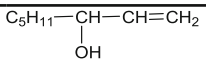
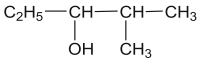
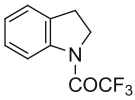
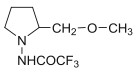
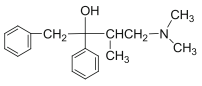
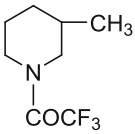
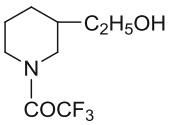
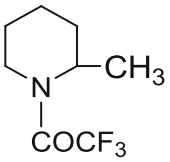
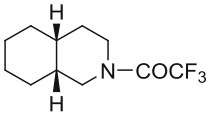
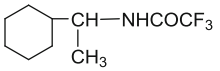
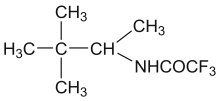
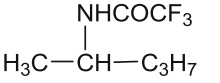
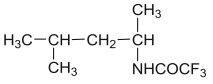
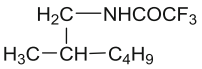


Fig. 2.12 Synthesis of the selectively bonded **CSP2.23** [from $2^{I\text{-}VII},3^{I\text{-}VII},6^{I\text{-}VI}$-eicosa-O-methyl-$6^{I}$-O-(oct-7-enyl) cyclomaltoheptaose (**3**)], **CSP2.24** $2^{I\text{-}VI},3^{I\text{-}VII},6^{I\text{-}VII}$-eicosa-O-methyl-$2^{I}$-O-(oct-7-enyl) [from cyclomaltoheptaose (**5**)], and **CSP2.25** [from $2^{I\text{-}VII},3^{I\text{-}VI},6^{I\text{-}VII}$-eicosa-O-methyl-$3^{I}$-O-(oct-7-enyl)cyclomaltoheptaose (**8**)] by hydrosilylation with hydridomethyldimethylsiloxane copolymer **10** (Reproduced from Ref. [36] by permission of John Wiley & Sons Ltd)

The novel mixed **CSP2.29** was used successfully in enantioselective gas chromatography for the chiral separation of apolar hydrocarbons as well as polar amino acid derivatives. Representative separation spectra are shown in Fig. 2.14. **CSP2.29** retains the individual enantioselectivities of the single component. Thus, the enantiomers of a polar hydrocarbons as well as polar amino acid derivatives can be separated with the mixed **CSP2.29**.

This section describes in detail the "immobilized" CD-CSP columns used in GC; dozens of racemic compounds were well separated on these CD-CSPs. Cyclodextrins

Table 2.16 Enantioseparation of several racemic compounds on **CSP2.26**, **CSP2.27,** and **CSP2.28**

Compound	Structure	k'	α	T (°C)	CSP
1-Octen-3-ol		17.7	1.02	65	**CSP2.26**
		19.6	1.03	65	**CSP2.27**
		12.5	1.02	65	**CSP2.28**
2-Methyl-3-pentanol		11.7	1.03	40	**CSP2.26**
		16.3	1.02	40	**CSP2.27**
		7.16	1.04	40	**CSP2.28**
Aminoindan		13.6	1.11	120	**CSP2.26**
		11.8	1.07	120	**CSP2.27**
		9.80	1.06	120	**CSP2.28**
1-Amino-2-(methoxymethyl)-pyrrolidine		14.2	1.02	80	**CSP2.26**
		11.7	1.06	100	**CSP2.27**
		12.5	1.05	80	**CSP2.28**
Oxyphene		34.6	1.08	170	**CSP2.26**
		22.9	1.07	190	**CSP2.27**
		32.8	1.10	170	**CSP2.28**
3-Methylpiperidine		8.66	1.04	85	**CSP2.26**
		9.60	1.02	80	**CSP2.27**
		6.07	1.04	85	**CSP2.28**
2-Piperidineethanol		6.32	1.02	135	**CSP2.26**
		14.3	1.02	110	**CSP2.27**
		12.9	1.03	110	**CSP2.28**
2- Methylpiperidine		9.21	1.06	85	**CSP2.26**
		10.3	1.10	80	**CSP2.27**
		6.53	1.05	85	**CSP2.28**
trans-Perhydroisoquinoline		6.00	1.03	150	**CSP2.26**
		16.1	1.02	110	**CSP2.27**
		15.1	1.03	130	**CSP2.28**
1-Cyclohexyl-ethylamine		10.2	1.06	110	**CSP2.26**
		14.8	1.07	100	**CSP2.27**
		14.9	1.04	95	**CSP2.28**
2-Amino-3,3-dimethylbutane		9.76	1.07	55	**CSP2.26**
		3.90	1.04	70	**CSP2.27**
		7.26	1.06	55	**CSP2.28**
1-Methyl-butylamine		5.60	1.05	65	**CSP2.26**
		7.90	1.04	60	**CSP2.27**
		4.75	1.04	60	**CSP2.28**
1,3-Dimethylbutylamine		5.45	1.12	75	**CSP2.26**
		5.70	1.10	75	**CSP2.27**
		4.09	1.08	75	**CSP2.28**
2-Ethylexylamine		13.8	1.03	100	**CSP2.26**
		34.7	1.01	40	**CSP2.27**
		15.1	1.04	90	**CSP2.28**

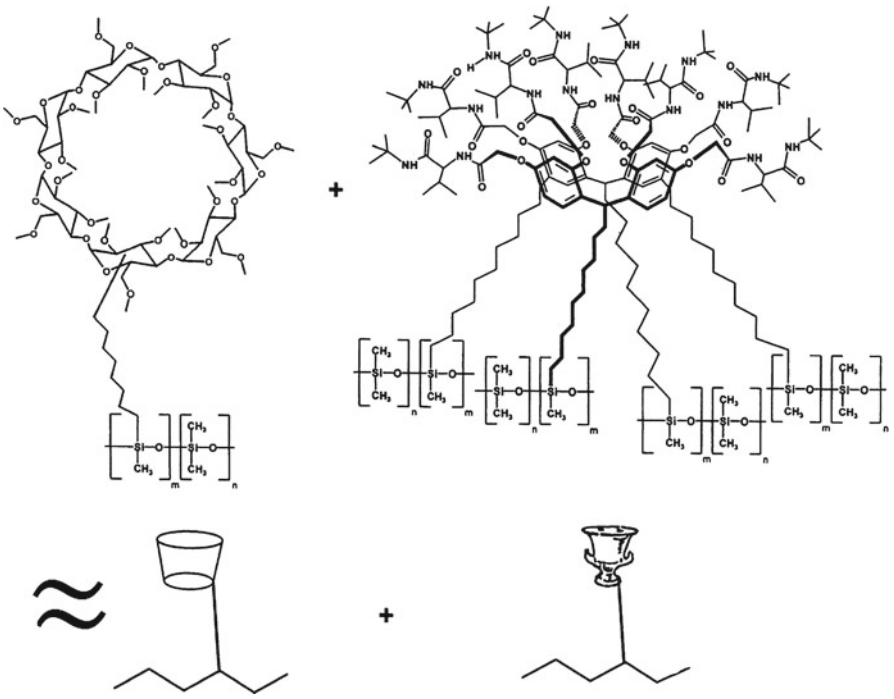


Fig. 2.13 Structures of the two CSPs Chirasil-Dex (*top*) and Chirasil-Calixval (*bottom*), **CSP 2.29** was synthesized by bonding these two CSPs on the same polymeric backbone at molar ratio of 1:1. Depending on the reaction conditions, the octamethylene linker arises from the O6- and/or O2-position of cyclodextrin (Reprinted from Ref. [37], Copyright 2003, with permission from Elsevier)

and their derivatives are the most commonly used selectors in different analytical separation chromatography. Moreover, cyclodextrin derivative, featuring inclusion complexation as the chiral recognition driving force, is unambiguous a type of excellent chiral selector. As we know, inclusion plays a definite role in the chiral recognition, which is the advantage of CD-CSPs in chiral separation process.

2.4 Summary

Chiral separations using GC proved to be a useful chromatographic chiral separation approach. In this chapter, different GC columns coated with modified cyclodextrins or bonded with cyclodextrins derivatives are introduced and applied for enantiomeric analysis. In total, 30 kinds of commercial CD-CSP columns and 29 types of self-made CD-CSP columns were mentioned above. These columns possess prominent chiral separation ability towards different kinds of racemic compounds like

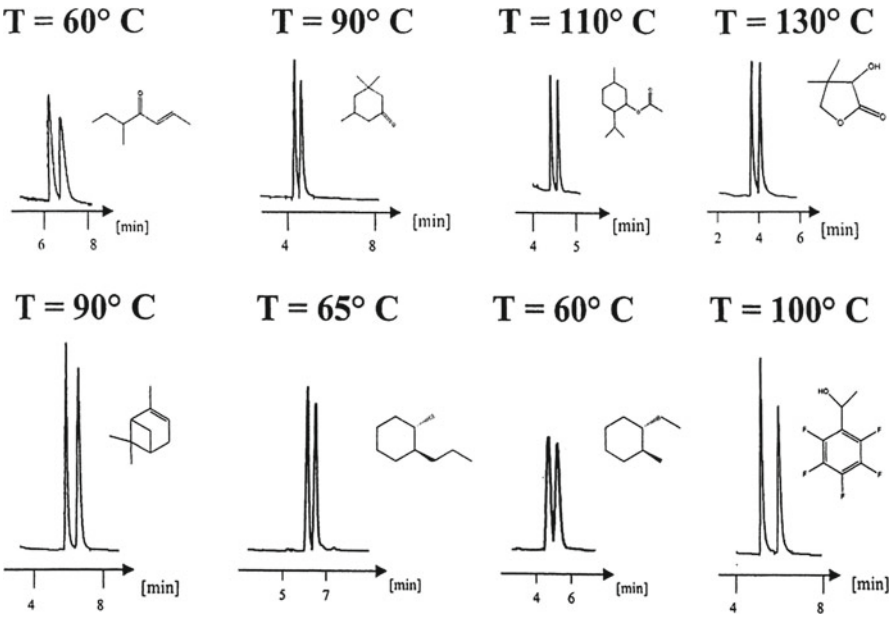


Fig. 2.14 Gas chromatographic enantiomeric separation of different classes of compounds on **CSP2.28**. 20 m×0.25 mm fused-silica capillary; film thickness: 250 nm; carrier gas: 0.5 bar hydrogen; split: 1:100 (Reprinted from Ref. [29], Copyright1990, with permission from Elsevier)

alcohols, ketones, acids, esters, and acetal. The resolution values for some special analytes are even more than 10.0, which demonstrate that cyclodextrins and their derivatives are absolutely excellent chiral selectors.

GC, as one of the most commonly used analytical separation chromatography, is increasingly becoming the focus of the research. In addition, the most important element in the GC enantioseparation process is the chiral selector. Cyclodextrin derivatives, as the most efficient chiral selectors, will be further studied.

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Chapter 3
Cyclodextrin-Based Chiral Stationary Phases for High-Performance Liquid Chromatography

Yun Dai, Shuye Wang, Weihua Tang, and Siu-Choon Ng

Abstract In this chapter, the recent progress in the development of structurally well-defined cyclodextrin (CD)-based chiral stationary phases (CSPs) via covalent linkages for the enantioseparation of racemic compounds using high-performance liquid chromatography is summarized. An overview of amide-bonded, amine-bonded, ether-bonded, urea-bonded, and triazole-bonded CD CSPs is presented herewith. The correlations between CD structures and the enantioselectivities of the resultant CSPs are also discussed.

3.1 Introduction

The development of efficient enantioseparation techniques has become increasingly demanding, especially in the pharmaceutical industry, as optical isomers often produce different biological activities, some of which are detrimental to further drug development. The analysis and preparation of a pure enantiomer often involve the resolution from its antipode. Amongst all the enantioseparation techniques, chiral high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) has proven to be one of the most versatile, convenient, and robust platform for resolving racemic compounds for both analytical and

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preparative purposes. CSP development has plateaued, with several CSPs now dominating selective screening protocols.

Chromatographic enantioseparation via HPLC dates back to 1960 when Klem first reported the use of a silica gel support for chiral chromatographic separation [1]. From then on, the application of liquid chromatography (LC), in enantioseparations, has burgeoned [2]. Development of stable and solvent-compatible reversed-phase CSPs has played an important role in the early studies of chiral drugs and metabolites. Several prior reviews have afforded an overview of numerous publications on this subject based on different approaches [1–6]. More recently, Armstrong delivered a complete review on commercially available cyclodextrin (CD)-derived CSPs including the Cyclobond, ChiraDex, and Orpark series and their applicable HPLC modes such as reversed phase, polar organic phase, and normal phase in enantioseparations, encompassing 137 references published in the period from 1983 to 2002 [7].

In this chapter, we will update on the research endeavours on the development of structurally well-defined CD-based CSPs. Depending on the type of covalent linkers between CD chiral selectors and silica support, the CSPs are herein classified into amino, ether, urethane, urea, and triazolyl-bonded CSPs. Also discussed are the synthetic approaches and enantioseparation results for these CSPs. It is envisaged that a vivid correlation of the CSP structure enantioseparation performance relationship would afford apt guidelines into the rational design of CD CSP.

3.1.1 History and Development

In the early days, CDs were polymerized and simply coated onto suitable support materials [8–10]. The first preparation of insoluble CD polymer-epichlorohydrin resins was reported in 1965 together with their selectivities in binding to various substances [11]. Thereafter, several other CD-containing resins, e.g., CD-polyurea and CD-poly(vinyl alcohol), were developed and successfully applied in chromatographic separation of natural amino acids [12], alkaloids [9], and other optical isomers [13, 14]. The polymerized CD CSPs, however, are very sensitive to the mobile phase used and demonstrated poor mechanical strength, long equilibrium time, poor anti-pressure ability, and low efficiency. Consequently, the application of these CSPs to modern HPLC would be impractical.

The full utility of CDs in HPLC would not be realized until a stable and efficient packing could be developed. In 1983, Fujimura and Shono first reported their preparation of silica gel with chemically bonded CDs and the retention behaviours of some aromatic compounds [15, 16]. These initial attempts brought forth a new era in development of bonded-phase CD-based CSPs for HPLC, even though these CSPs encountered some problems in their subsequent applications such as hydrolytic instability, low selector loading, unexpected influences of the amino moieties present, and tedious syntheses. Thereafter, the application of CSPs based on chemically anchored CDs and the understandings of their properties have been broaden tremendously.

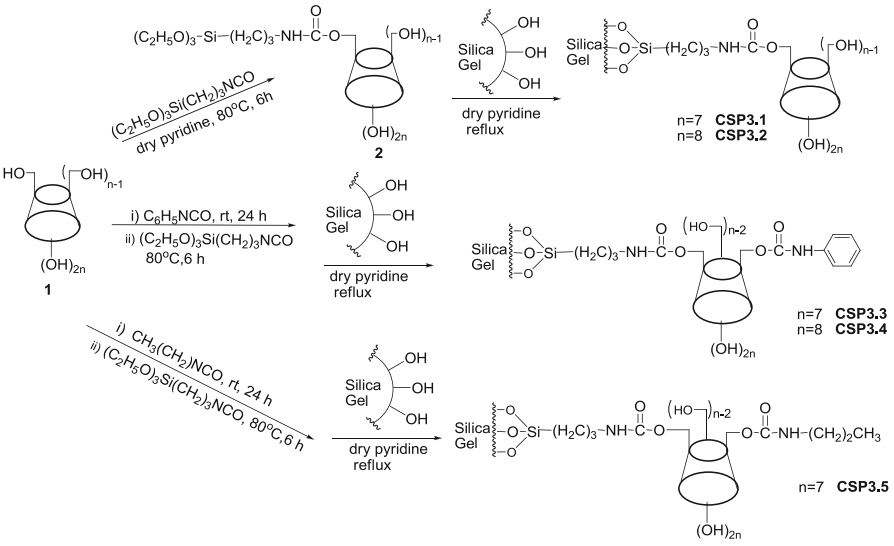
In 1984, Armstrong reported new LC stationary phases based on chemically bonded CDs [17]. These phases demonstrated improved enantioseparation ability towards a variety of difficult-to-separate isomers. Subsequently, this group released their first commercial CSP with CD bonded to silica via an 8–10 atom spacer [18], which is hydrolytically stable under standard reversed-phase LC conditions. The spacer also restricts the motion of the CD and provides additional sites for interaction with the complexed guest molecules. However, Armstrong's approach and other similar procedures, which used ether or carbamoyl covalent linkages to afford solvent tolerant CD CSPs [19, 20], involve post-immobilization modification of CDs [21]. Since the functionalization procedures involve heterogeneous solid–liquid phase reaction, a partial and random substitution of the CD hydroxyl groups is often the result. Consequently, the post-immobilization modification approach would not easily afford structurally well-defined CSP, and the control of batch-to-batch reproducibility may be challenging.

Alternative immobilization approaches which aim to solve this problem have been attempted by many researchers. Thus, Konig successfully synthesized a series of structurally well-defined CD derivatives which was then immobilized onto the surface of silica to be evaluated as CSPs [22]. Felix prepared a series of β-CD CSPs with different spacer lengths and investigated the influence of the spacer length on the enantioseparation process [23, 24]. Ciucanu immobilized a variety of peralkylated β-CDs on silica gel via hydrosilylation [25, 26]. One of our research groups reported the preparation of three π-acid-type chiral stationary phases based on chlorophenylcarbamates of β-CD via hydrosilylation [27]. We have also reported a novel methodology for the preparation of a series of structurally well-defined CD CSPs by covalently linking monoazido perfunctionalized CD with aminized silica gel via stable urea linkage(s) using the Staudinger reaction [28–30]. Using selectively modified CDs, a large spectrum of CD CSPs with amino, ether, urethane, urea, and triazolyl linkages have been developed in recent years. Herein, their syntheses and performance in enantioseparations are summarized in this chapter.

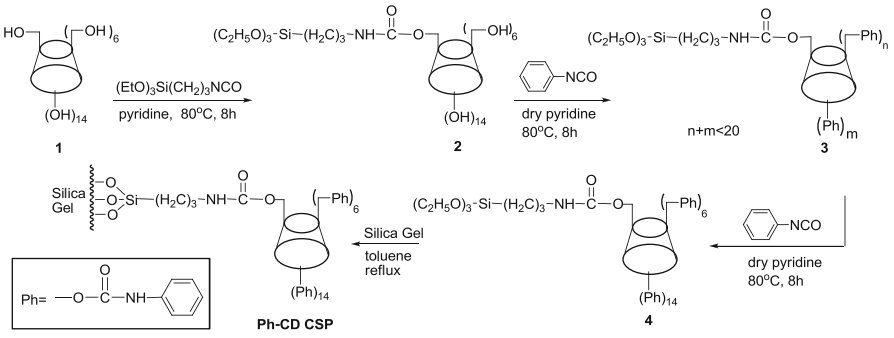
3.2 Chemically Bonded CD CSPs Based on Urethane Linkage

Fujimura prepared five series of CSPs through bonding native and carbamoylated β-CD or γ-CD onto silica support via the urethane covalent linkage (Scheme 3.1) [31].

Their enantioseparation performance was evaluated using racemic dansylated (Dns) α-amino acids as analytes. The native γ-CD bonded phase was found very effective for the separation of not only said enantiomers but also the homologues of Dns amino acids. A chiral recognition mechanism of Dns amino acid based upon a competitive inclusion model consisting of the formation of an inclusion complex of CD with dansyl group as well as a side chain of the analyte was proposed in their study.



Scheme 3.1 Preparation of modified or unmodified CD CSPs with urethane linkage



Scheme 3.2 Preparation of PhCD-bonded CSP

Under reversed-phase condition, native and derivatized β-CD-bonded stationary phases have been successfully used in HPLC for enantioseparation of a wide variety of positional, structural, and optical isomers [32–34]. However, racemic compounds are not always well resolved on these CD columns, and accordingly, further improvement is needed. Several different approaches have been used for the preparation of CD-bonded phases for use in HPLC [35, 36].

Wada's group proposed a facile procedure to modify CD stationary phases with linkers affording potent hydrophilic or hydrophobic interactions to enhance the chiral recognition by CD [37]. As shown in Scheme 3.2, a bifunctional β-CD-based CSP (PhCD CSP) was prepared by immobilizing perphenylcarbamoylated β-CD onto silica with a single urethane covalent bond. One of the C6-hydroxyl groups of β-CD

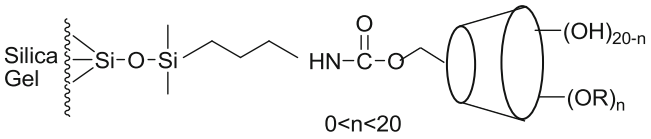


Fig. 3.1 Structures of phenylcarbamoylated CD-bonded CSPs

was used to construct the urethane linkage between CD and silica support, while the remaining 6 primary and 14 secondary hydroxyl groups on the larger opening of the CD cone were all derivatized to phenylcarbamoyl groups via reaction with phenylisocyanate. The presence of phenyl moieties was afforded a hydrophobic cluster above the larger opening of the CD cone. By forming this cluster, the derived stationary phase will be bifunctional in chiral recognition: one is chiral inclusion by the CD cavity and the other hydrophobic interaction by the phenyl cluster.

Comparison of the chromatographic characteristics of PhCD CSP with those of underivatized native CD CSPs would suggest that the additional hydrophobic interaction between phenyl clusters and hydrophobic parts of the analytes could improve the resolution of phenylalkyl alcohols and amines, which had a hydrophobic aromatic ring and a chiral carbon of several Angströms distant from the ring. For instance, PhCD CSP demonstrated good separation for β-blocker drugs such as propranolol and its ester derivatives in aqueous mobile phases.

Meklati recently reported a improved synthesis procedure for **CSP3.1** via a one-pot synthesis of **CSP3.1** involving firstly reaction of β-CD with triethoxy(3-isocyanatopropyl)silane in pyridine at 80 °C for 2 h and then further reaction with silica gel for 24 h under reflux [38]. In comparison with the previously reported CSPs [31, 39], this CSP demonstrated higher resolutions towards analytes including two aryloxyphenoxy propionate herbicides, eight pharmaceutical compounds (benzodiazepines, anticoagulant agents, and nonsteroidal anti-inflammatory drugs), and some other optically active solutes.

The first successful CD-based normal-phase enantioseparation was accomplished by Armstrong with different series of partially derivatized β-CD CSPs [39]. The derivatization of native CD immobilized on silica was affected by reaction with acetic anhydride, 2,6-dimethylphenyl isocyanate, p-toluoyl chloride, and (*R*)- or (*S*)-(1-naphthyl)ethyl isocyanate. The CDs modified with the larger, bulkier substituents consistently had a lower degree of substitution on the secondary hydroxyl, presumably due to steric reasons. These derivatized CD CSPs demonstrated good enantioseparation ability towards 20 selected analytes.

In order to expand the application of derivatized β-CD phases in enantioseparation, Iida et al. prepared one native and four phenylcarbamoylated β-CD bonded-phase HPLC columns and investigated their enantioseparation ability towards phenylthiocarbamoyl amino acids (PTC-AAs) [40]. Figure 3.1 shows the structures of the β-CD stationary phases. Apart from the CSP with native β-CD, the substitution of phenylcarbamoylation on β-CD moiety was controlled by varying the quantity of the derivatization reagent, phenylisocyanate, in a way similar to that in a prior report [41].

Table 3.1 Enantioseparation of PTC-AAs on the five phenylcarbamoylated CD CSPs

	Glu		Ser		Ala		His		Phe	
Chiral phase	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
Native CD	0.91	1.19	1.38	1.00	1.56	1.00	2.02	1.07	5.84	1.26
0.2Ph/CD	0.53	1.15	0.86	1.00	0.93	1.00	1.38	1.07	4.06	1.29
3.3Ph/CD	1.04	1.18	1.51	1.05	1.65	1.07	2.31	1.11	7.20	1.34
7.5Ph/CD	0.35	1.23	0.79	1.00	0.90	1.12	1.87	1.16	3.44	1.39
16.9Ph/CD	0.37	1.00	1.03	1.00	1.59	1.00	2.86	1.00	8.81	1.05

Condition: 100 mM ammonium acetate (pH 6.5)/methanol (80/20, v/v) as the mobile phase, 1.0 mL min^{-1} flow rate

Table 3.2 Capacity factor (k') and separation factor (α) obtained for PTC-AAs on 3.3Ph/CD column [40]

	k'				k'				k'		
PTC-AAs	D-form	L-form	α	PTC-AAS	D-form	L-form	α	PTC-AAs	D-form	L-form	α
Ala	2.40	2.54	1.06	His	3.19	3.56	1.12	Pro	2.23	3.44	1.54
Arg	3.74	4.25	1.14	Ile	4.04[a]	5.13	1.27	Ser	2.28	2.40	1.05
Asn	2.20	2.31	1.05	Leu	4.44	5.75	1.30	Thr	1.99	2.23	1.12
Gln	2.18	2.49	1.14	Lys	11.26	12.59	1.12	Trp	13.72	18.92	1.38
Glu	1.57	1.88	1.20	Met	3.85	4.45	1.16	Tyr	6.34	6.70	1.06
Gly	2.25			Phe	13.61	18.46	1.36	Val	3.12	3.74	1.20

Condition: 100 mM ammonium acetate (pH 6.5)/methanol (90/10, v/v) as the mobile phase, 1.0 mL min^{-1} flow rate

[a]This is D-allo-Ile, which is a stereoisomer of D-Ile at the β-carbon position

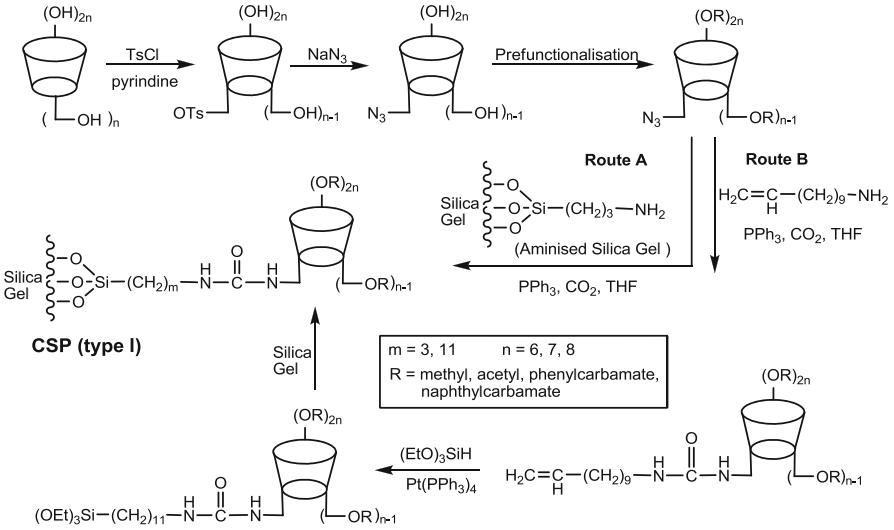
Accordingly, the resulting four CSPs of phenylcarbamoylation were prepared from 1 mol of phenylcarbamoylated β-CD with Ph/CD ratios of 0.2, 3.3, 7.5, and 16.9.

In the evaluation of enantioseparation, PTC-Glu, PTC-Ala, PTC-His, PTC-Ser, and PTC-Phe, which represent acidic, neutral, basic, hydroxyl, and aromatic amino acids, were selected as the model analytes. Among the five different β-CD CSPs, the 3.3 Ph/CD demonstrated the broadest range of enantioseparation towards the selected amino acids (Tables 3.1 and 3.2). As shown in Table 3.1, PTC-Ser enantiomers were only separated on the 3.3Ph/CD (separation factor $\alpha = 1.05$), and the other PTC-AAs enantiomers were also successfully separated with $\alpha = 1.07–1.34$.

The enantioseparation of 18 pairs of PTC-AAs and PTC-Gly with the 3.3Ph/CD column (Table 3.2) further demonstrated that a certain degree phenyl replacement of hydroxyl groups on β-CD face is beneficial for enantioseparation of PTC-AAs arising from widening of the secondary rim of the CD cone with consequent accentuated penetration into or interaction with the β-CD cavity.

3.3 Chemically Bonded CD CSPs Based on Urea Linkage

In the course of the early developmental stages on the application of CDs in separation methods, β-CD was dissolved in aqueous urea solutions and applied as mobile phases for TLC separations [41, 42]. However, the coupling of CDs onto stationary



Scheme 3.3 Synthetic approach for CD CSPs bearing a single C6-urea bond (Type I)

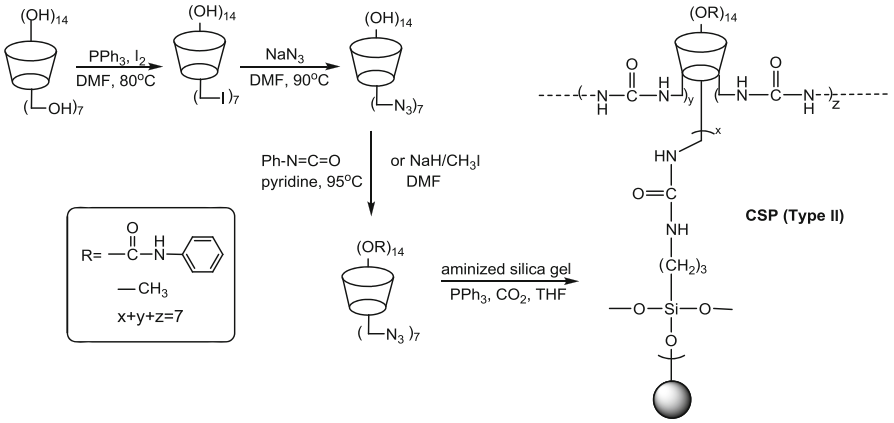
phases for TLC separations, similar to those used in HPLC, has been limited due to the lack of a commercial source for such plates.

Efforts to couple CDs to silica gel were initially attempted by Fujimura et al. through amino covalent linkages [15]. The same group further explored the preparation of bonded-phase CD CSPs via a two-step protocol, comprising firstly a reaction of CD derivatives with (3-isocyanatopropyl)triethoxysilane and a further grafting onto silica surface to afford the urethane bond [31]. The problem subsequently encountered by these CSPs is their instability to hydrolysis, which limits their utility in reversed-phase mode.

One of our research groups has been actively engaged in developing new synthetic methodologies for immobilization of perfunctionalized CDs onto silica gel via urea-covalent linkages with varying chain lengths of spacer arms [28–30, 43, 44]. We have explored the use of perfunctionalized CD derivatives with the azido moiety as key intermediates for preparation of chemically bonded CD CSPs on silica with a single urea bond at the C6-position (designated herein as Type I), CD CSPs having multiple urea bonds at the C6-positions (Type II), and CD CSPs having a single urea bond at the C2-position (Type III).

3.3.1 CD CSPs Bearing a Single C6-Urea Linkage (Type I)

For Type I CSPs, a generic methodology involved the use of mono-(6-azido-6-deoxy) perfunctionalized CDs for immobilization onto the surface of silica gel using Staudinger reaction [45, 46] under mild reaction conditions (Scheme 3.3).



Scheme 3.4 Synthetic pathway of CD CSPs having multiple urea bonds (Type II) (Reproduced from Ref. [30] by permission of John Wiley & Sons Ltd.)

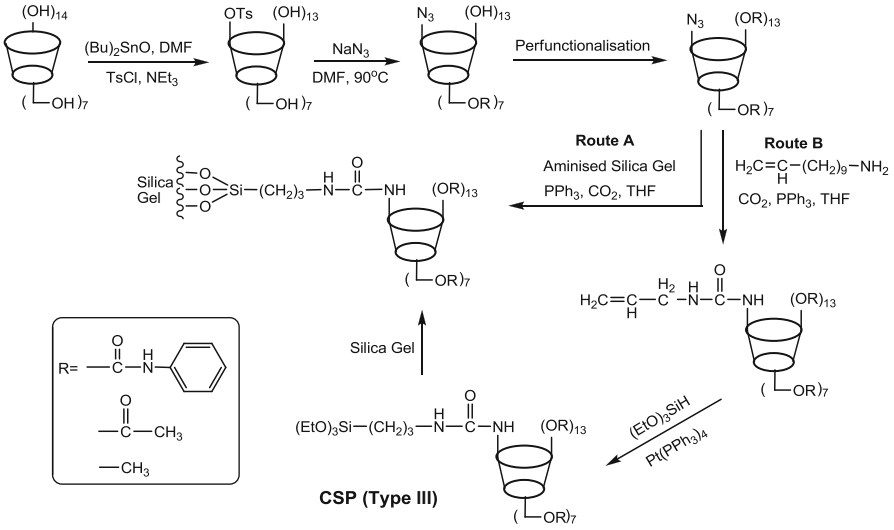
Two approaches were investigated for the immobilization of perfunctionalized CDs onto silica. In the first approach (route A), mono-(6-azido-6-deoxy) perfunctionalized CDs were immobilized by means of the Staudinger reaction onto aminized silica gel [35], which can be easily obtained by treating silica with aminopropyltriethoxysilane. Likewise, the facile preparation of analogous perfunctionalized α-CD and γ-CD CSPs can also be effected by using this synthetic methodology [47]. The CSPs obtained by this approach would however contain residual amine functional groups on the silica surface, which can result in extensive tailing in chromatographic applications particularly when in conjunction with acidic/weakly acidic analytes. In order to avoid such undesirable effects, an alternative synthetic route (route B) was developed.

In route B, mono-(6-azido-6-deoxy) perfunctionalized CDs were first coupled with an ω-alkenylalkylamine via Staudinger reaction to afford the ω-alkenyl-substituted β-CDs. Thereafter, the ω-alkenyl-substituted CDs were reacted with triethoxysilane in the presence of catalytic amounts of tetrakis(triphenylphosphine) platinum(0) to afford reactive ω-triethoxysilanyl CD intermediates which were then reacted with silica gel to afford the required CD CSPs. This method would afford CSPs without amine residues on silica surfaces.

3.3.2 CD CSPs Bearing Multiple C6-Urea Linkages (Type II)

On the basis of the previous work, we extended our research work to afford durable CSPs bearing multiple covalent urea linkages to the silica support (Type II) [48, 49] using the synthetic approach depicted in Scheme 3.4.

Herein the synthetic route entailed the preparation of heptakis (6-azido-6-deoxy)-β-CD, which was then reacted with phenylisocyanate to afford heptakis



Scheme 3.5 Synthetic pathway of CD CSP (Type III) bearing a single C2-urea bond (Reproduced from Ref. [30] by permission of John Wiley & Sons Ltd.)

(6-azido-6-deoxy)-2,3-di-O-phenylcarbamoyl-β-CD [48] or reacted with methyl iodide in the presence of sodium hydride to afford heptakis(6-azido-6-deoxy)-2,3-di-O-methyl-β-CD [49]. The key intermediate compounds obtained were then immobilized onto aminized silica gel via Staudinger reaction to afford the final CSPs. These CSPs possess high hydrophobicity together with multiple urea linkages, which would enhance their stability particularly in acidic media. The perphenylcarbamoylated β-CD CSP derived has the phenylcarbamoyl moieties at the C2- and C3-positions on the wider mouth of the β-CD cone, which has the impact of further widening the cavity in addition to enhancing its hydrophobicity as well as the prospect of affording additional π–π interactions with analytes, all of which may play important roles in the enantioseparation process.

3.3.3 CD CSPs Bearing a Single C2-Urea Linkage (Type III)

The secondary rim of CDs is reported to be catalytically very important and modifications on this face are believed to produce valuable derivatives for catalysis, enzyme mimics, etc. [50, 51]. However, the secondary hydroxyl groups (in C-2 and C-3 positions) on the secondary rim of CD are relatively less reactive in comparison to the primary hydroxyl groups (in the C-6 position). One of our research group has successfully immobilized mono(2-azido) perfunctionalized CD onto silica gel using the synthetic methodology outlined in Scheme 3.5 to afford CSPs bonded at the C2-position on the wider CD rim (Type III) [52, 53]. Using this approach, the

regioselective immobilization of CD at the C2-position was achieved via a single urea-covalent linkage to the silica support to afford perphenylcarbamoylated, peracetylated, and permethylated CD CSPs.

As shown in Scheme 3.5, mono-2-(p-toluenesulphonyl)-β-CD was prepared selectively from β-CD followed by an azidation reaction [54]. The resulting secondary monoazido-β-CD precursor was then perfunctionalized with phenyl isocyanate, acetic anhydride, or methyl iodide to afford mono (2-azido) perphenylcarbamoyl-, peracetyl-, or permethyl-β-CDs, respectively. The immobilization of the perfunctionalized CDs was again carried out using two approaches. In the first approach (Scheme 3.5, route A), the perphenylcarbamoylated and peracetylated mono(2-azido)-CDs were directly coupled to the aminized silica support using the Staudinger reaction to afford chemically well-defined perfunctionalized CD CSPs. In the second method, the perfunctionalized mono(2-azido)-CDs were first reacted with allylamine using the Staudinger reaction followed by hydrosilylation and then immobilized onto silica surface to afford the perfunctionalized CD CSPs (Scheme 3.5, route B). The latter would afford CSPs without amine residues on the silica surfaces.

The enantioseparation abilities of the CD CSPs (Types I–III) have been evaluated using analytes[49] comprising racemic aromatic substituted alcohols, flavonoids, acids, weak acids, antihistamines, amines, amides, amino alcohols, β-blockers, alkaloids, and neutral compounds [57]. These analytes were found to be resolvable using CD CSPs of Type I, II, or III under normal- and/or reverse-phase conditions. Moreover, the preparative enantioseparations of pharmaceutical compounds such as atropine, bendroflumethiazide, propranolol, O-acetyl-propranolol, isoproterenol, and alprenolol were also achieved using these CSPs.

3.3.4 Effect of CD Type on Enantioseparation Abilities of C6-Urea-Bonded CSPs (Type I)

The influence of the cavity size of CD on CSPs (Type I) was investigated using perphenylcarbamoyl-α-CD (**CSP3.6**), perphenylcarbamoyl-β-CD (**CSP3.7**), and perphenylcarbamoyl-γ-CD (**CSP3.8**) as model CSPs. It is evident that the surface coverage for perphenylcarbamoyl-γ-CD is much lower than that for perphenylcarbamoyl-α-CD and perphenylcarbamoyl-β-CD. This may be attributable to the perphenylcarbamoylated γ-CD moiety having a larger molecular volume than the perphenylcarbamoylated α- or β-CD, which consequently afforded stronger steric hindrance towards immobilization. Three CSPs (**CSP3.6-3.8**) gave column efficiencies of 33,300, 36,000 and 34,500 plates/metre, respectively, using biphenyl as test sample under normal phase (hexane and isopropanol in 90/10 v/v ratio, 1 mL min^{-1}).

The enantioseparation abilities of these CD CSPs for several representative racemic alcohols were studied under normal-phase conditions [47, 54]. For p-bromophenylethanol, the enantioseparation ability of **CSP3.7** appeared better

than with **CSP3.6** and **CSP3.8**. For 2-naphthylethanol, no resolution was obtained for **CSP3.6**, whereas **CSP3.7** and **CSP3.8** afforded partial separation of enantiomers. When bulky analytes such as chloroquine and pindolol were tested (refer to Fig. 3.3 for all analyte structures), **CSP3.8** was found to resolve them both, while these same compounds were not resolvable on **CSP3.6** and **CSP3.7**. Accordingly, a larger cavity size, for example, as afforded by β-CD, is necessary for enantioseparation of sterically encumbered analytes. The enantioselectivities of **CSP3.6–3.8** under reversed-phase condition were studied using compounds having a stereogenic centre at their aliphatic pendant chain, not immediately adjacent to the aromatic moiety, such as chloroquine, metoprolol, pindolol, oxprenolol, and alprenolol. Fujimura et al. had proposed a chiral recognition mechanism based on the competitive inclusion model, involving the formation of an inclusion complex of CD with an aromatic group as well as with a side chain [31]. On the basis of Fujimura's competitive inclusion model, a bulky cyclic moiety of the analyte molecules cannot be included in the cavity of the α-CD, but the aliphatic pendant chain containing the chiral centre could on the other hand be readily included.

3.3.5 Enantioseparation Abilities of Multiple C6-Urea-Bonded CSPs (Type II)

The enantioseparation abilities of multiple urea-bonded perphenylcarbamoyl-α-CD (**CSP3.9**), perphenylcarbamoyl-β-CD (**CSP3.10**), and perphenylcarbamoyl-γ-CD (**CSP11**) were evaluated using a wide range of racemic compounds comprising flavonoids, β-blockers, amines, and substituted phenyl- alcohols under both normal- and reverse-phase conditions [48, 55]. The enantioseparation data of flavonoids with **CSP3.9–3.11** under reverse-phase conditions are summarized in Table 3.3.

As shown in Table 3.3, γ-CD-derived **CSP3.11** demonstrated outstanding enantioseparation towards all ten flavonoids, β-CD-based **CSP3.10** resolved 90 % analytes, while α-CD-based **CSP3.9** only afforded partial resolution to two analytes [56]. This phenomenon could be explained by Armstrong's inclusion (host–guest) mechanism for chiral recognition in CD-based CSPs. The observed stereoselectivity is due to the differences in fit or inclusion of the enantiomers into the CD cavities of the CSP [17].

The presence of phenylcarbamoyl groups at the 2- and 3-positions of the CD rims would enlarge the cavity, thus allowing for the inclusion of larger molecules. However, most of the flavonoids are too bulky to fit into the hydrophobic cavity of **CSP3.9** but are easily included in **CSP3.10** and **CSP3.11**. Consequently, **CSP3.10** & **3.11** were found superior to **CSP3.9** in resolving chiral molecules with larger steric configuration. To further strengthen this viewpoint, the enantioseparation of hesperetin (Table 3.3; entry 9), which is the most bulky molecule among the ten flavanones, can only be separated by using **CSP3.11**, which has the largest hydrophobic cavity amongst the three CSPs.

Table 3.3 Separation results of flavonoids in reverse-phase mode using CD CSP (Type II)

			HPLC data			
Entry	Flavonoids	CSP Type II	k'_1	k'_2	α	R_s
1	Flavanone	**CSP3.9**	–	–	–	0.0
		CSP3.10	11.14	14.4	1.30	3.33
		CSP3.11	10.76	14.1	1.31	3.45
2	2-Hydroxyflavanone	**CSP3.9**	8.08	8.37	1.04	0.47
		CSP3.10	6.91	8.17	1.18	2.45
		CSP3.11	8.54	8.73	1.02	1.42
3	4′-Hydroxyflavanone	**CSP3.9**	–	–	–	0.00
		CSP3.10	10.02	13.7	1.37	3.03
		CSP3.11	8.51	12.7	1.49	3.01
4	6-Hydroxyflavanone	**CSP3.9**	–	–	–	0.00
		CSP3.10	9.71	11.9	1.23	2.67
		CSP3.11	8.54	10.7	1.25	2.80
5	4′-Methoxyflavanone	**CSP3.9**	–	–	–	0.00
		CSP3.10	10.96	11.8	1.08	1.17
		CSP3.11	13.67	16.6	1.21	2.52
6	5-Methoxyflavanone	**CSP3.9**	–	–	–	0.00
		CSP3.10	8.72	9.75	1.12	1.56
		CSP3.11	9.47	11.1	1.18	2.43
7	6-Methoxyflavanone	**CSP3.9**	–	–	–	0.00
		CSP3.10	19.6	25.3	1.29	2.29
		CSP3.11	16.6	25.0	1.51	4.04
8	7-Methoxyflavanone	**CSP3.9**	–	–	–	0.00
		CSP3.10	18.97	26.3	1.39	2.79
		CSP3.11	16.35	20.6	1.26	2.46
9	Hesperetin	**CSP3.9**	–	–	–	0.00
		CSP3.10	–	–	–	0.00
		CSP3.11	12.15	13.7	1.13	1.43
10	Naringenin	**CSP3.9**	11.62	11.9	1.03	0.35
		CSP3.10	14.71	15.9	1.08	0.71
		CSP3.11	10.29	13.8	1.29	2.63

Conditions: 1 % TEAA buffer (pH4.65)/MeOH (50/50 v/v) mobile phase, 0.5 mL min^{-1} flow rate. Structures refered to in Fig. 3.2

3.3.6 Effect of Functionality on Enantioseparation Abilities of Single C2-Urea-Bonded CSPs (Type III)

In order to investigate the influence of functionalities on the secondary face of CD on the enantioseparation abilities, perphenylcarbamoyl-, peracetyl-, and permethyl-β-CD selectors were immobilized onto silica via a single urea-covalent linker to afford **CSP3.12–3.14**, respectively [52, 53]. Racemic aromatic alcohols, β-adrenergic blockers, flavonoids, and antihistamines were used as analyte.

The separation data are summarized in Table 3.4 (refer to Fig. 3.2 for all analyte structures). Good enantioseparation results were obtained for bendroflumethiazide,

Table 3.4 Separation results of analytes in reverse-phase mode using CD CSPs (Type III)

S/N	Analytes	CSP Type	Conditions	HPLC data				Ref.
				k'_1	k'_2	α	R_s	
1	Flavanone	**CSP3.12**	I	1.60	2.68	1.68	3.89	[52]
		CSP3.13	I	3.12	3.44	1.10	0.83	[52]
		CSP3.14	II	5.29	6.06	1.15	1.16	[57]
2	4′-Hydroxyflavanone	**CSP3.12**	I	1.24	1.95	1.58	2.72	[52]
		CSP3.13	III	3.33	3.33	1.00	0.00	[52]
		CSP3.14	IV	6.06	6.34	1.05	0.74	[57]
3	4′-Methoxyflavanone	**CSP3.12**	I	1.42	2.08	1.46	1.74	[52]
		CSP3.13	III	3.69	3.92	1.06	0.29	[52]
		CSP3.14	V	5.07	5.55	1.09	0.95	[57]
4	5-Methoxyflavanone	**CSP3.12**	I	1.27	1.60	1.26	1.97	[52]
		CSP3.13	I	3.63	4.06	1.12	0.88	[52]
		CSP3.14	I	1.29	1.36	1.05	0.85	[57]
5	6-Methoxyflavanone	**CSP3.12**	I	3.54	5.30	1.50	3.67	[52]
		CSP3.13	I	1.74	2.13	1.22	1.14	[52]
		CSP3.14	I	1.47	1.53	1.04	0.51	[57]
6	7-Methoxyflavanone	**CSP3.12**	I	3.01	5.13	1.70	3.71	[52]
		CSP3.13	I	3.26	3.61	1.11	0.91	[52]
		CSP3.14	II	8.11	9.04	1.10	1.43	[57]
7	Bendroflumethiazide	**CSP3.12**	VI	5.22	12.5	2.41	6.26	[52]
8	Tolperisone	**CSP3.12**	VI	2.13	3.32	1.56	2.77	[52]
9	Indapamide	**CSP3.12**	VI	3.01	3.50	1.16	0.94	[52]
10	Ancymidol	**CSP3.12**	VI	1.44	1.63	1.13	0.63	[52]
11	Chlorpheniramine	**CSP3.12**	VI	0.26	0.33	1.27	0.36	[52]
12	Brompheniramine	**CSP3.12**	VI	0.38	0.49	1.29	0.64	[52]
13	Propranolol	**CSP3.12**	VI	0.58	1.16	2.01	2.09	[52]
14	Alprenolol	**CSP3.12**	VI	0.23	0.46	2.02	1.25	[52]
15	Acebutolol	**CSP3.12**	VI	0.03	0.14	1.10	0.82	[52]
16	Pindolol	**CSP3.12**	VI	0.04	0.10	1.05	0.49	[52]
17	Metoprolol	**CSP3.12**	VI	0.20	0.29	1.45	0.42	[52]
18	Etilefrine	**CSP3.12**	VI	0.35	0.35	0.35	0.00	[52]
19	Atropine	**CSP3.12**	VI	0.33	1.50	4.55	2.94	[52]

Conditions: I. MeOH/water (50/50) mobile phase, 1.0 mL min^{-1} flow rate; II. 1 % TEAA buffer (pH 5.0)/MeOH (65/35), 0.5 mL min^{-1}; III. MeOH/water (50/50), 0.8 mL min^{-1}; IV. 1 % TEAA buffer(pH 5.5)/MeOH (65/35), 0.5 mL min^{-1}; V. ACN/water (5/95), 0.5 mL min^{-1}; VI. 1 % TEAA buffer (pH 5.11)/MeOH (70/30), 1.0 mL min^{-1}

tolperisone, propranolol, alprenolol, and atropine (as shown in Table 3.4; entries 7, 8, 13, 14, and 19) using **CSP3.12** under reverse-phase conditions. **CSP3.12** also afforded better enantioseparation than Cyclobond I; for example, propranolol, which could not be baseline resolved by Cyclobond I ($R_s = 1.25$) [58], can be well separated by CSP12 ($R_s = 2.09$). Under reverse-phase conditions, assuming that inclusion phenomenon takes place at the secondary face of the CD [59, 60], then the orientation of the CD in the CSP bonded at the C-2 position would be less favourable towards inclusion, as it may limit the number of analyte molecules entering the wider opening of the CD

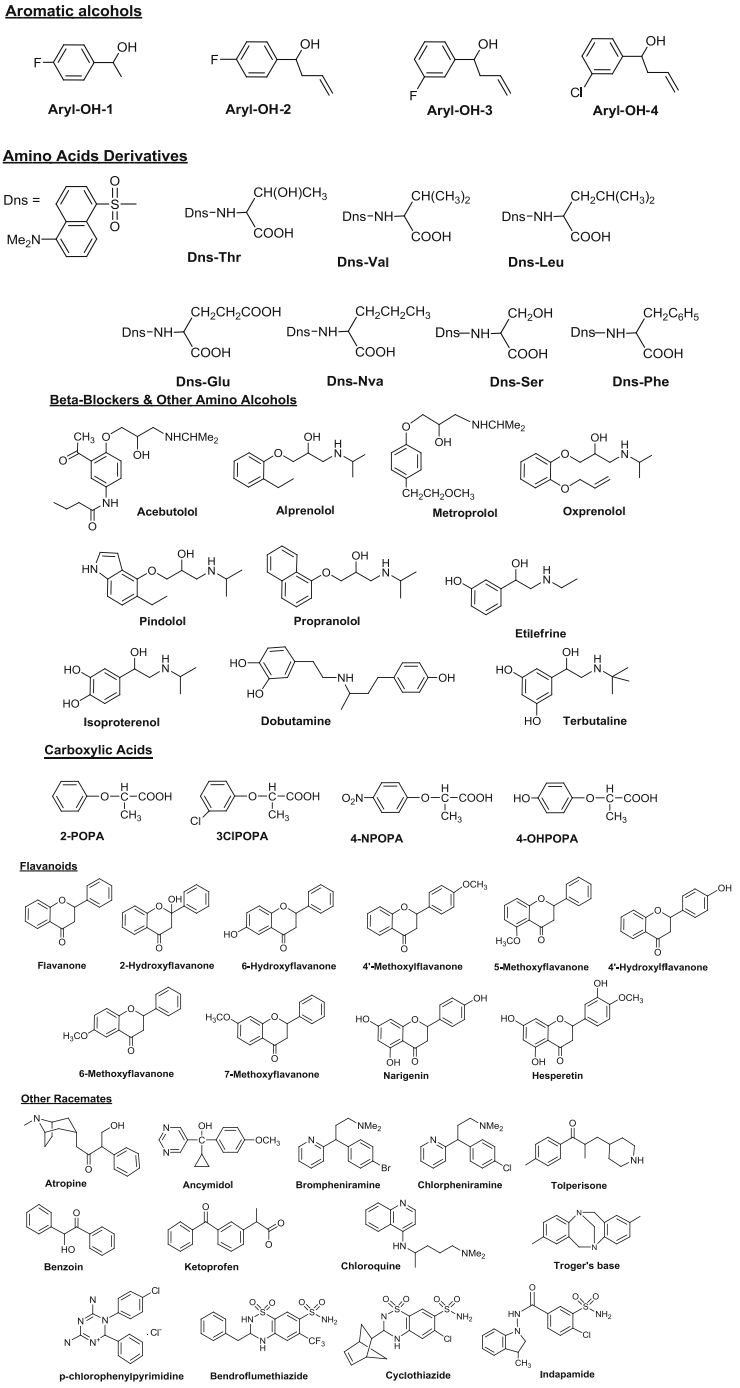


Fig. 3.2 Chemical structures of chiral compounds discussed in this chapter

cavity which is now facing inwards towards the silica support. The smaller retention factors show that there was early elution of all the racemates and the selectivity factors (α) were found to be lower for most racemic analytes compared to analogous CSP derived from immobilization via the primary rim of CD [52]. In contrast, high chiral resolution is obtainable for bendroflumethiazide using **CSP3.12**, presumably ascribable to a combination of inclusion complexation between the CD cavity with the analyte as well as π–π interactions between the phenylcarbamoyl groups and the analyte. In addition, hydrogen bond formation may also occur between the urea linkage on the CSP spacer arm and the –NH and $–NHSO_2$ moieties of bendroflumethiazide.

Under reverse-phase conditions, the perphenylcarbamoylated CD (**CSP3.12**) afforded obviously better enantioseparation compared to the peracetylated CD (**CSP3.13**) and permethylated CD (**CSP3.14**) CSPs. This can be ascribed to inclusion complexation and π–π interaction between the aromatic rings in the phenylcarbamoyl groups of **CSP3.12** and flavanone.

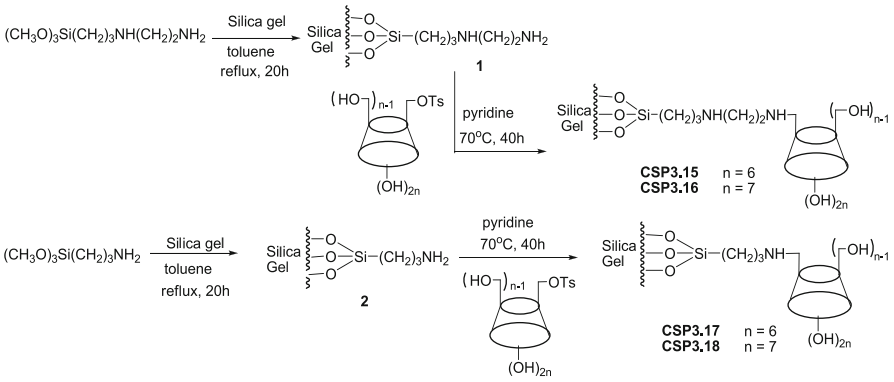
3.4 Chemically Bonded CD CSPs Based on Amino Linkages

CD derivatives with one of the glucosidic 6-OH replaced by amino, alkylamino, or histamine moiety have previously been synthesized [61–64] and used for enantioseparation of anionic analytes in chiral CE [65, 66]. Since the CD molecules are chiral, they can form a diastereomeric pair of inclusion complexes with each enantiomer of racemates. CD derivatives mentioned above can also form stable inclusion complexes with racemates through host–guest hydrophobic interactions such as van der Waals forces, hydrogen bonding, and especially electrostatic interaction between the chiral selector and analyte molecules. These CDs have been used extensively as CSPs in LC on account of their ability in recognizing enantiomeric molecules through the formation of inclusion complexes under reversed-phase mode [67–69].

The first bonded-phase CD CSPs having amino covalent linkers was reported by Fujimura et al. and used in reverse-phase LC [15]. Four amino-bonded CD CSPs were synthesized using a two-step protocol, involving synthesis of aminized silica gel by firstly reacting silica with N^1-3-(trimethoxysilyl)propylethane-1,2-diamine or 3-trimethoxysilylpropan-1-amine followed by nucleophilic attack of tosylated α- or β-CD with the as-synthesized aminized silica (Scheme 3.6).

The achiral separation capabilities of these CD CSPs were evaluated under reversed-phase conditions with 1- and 2-naphthylamines as well as *m*- and *o-nitroanilines* as model analytes. It is noteworthy that very high separation factors were observed for **CSP3.16** in comparison to **CSP3.15**, **CSP3.17**, and **CSP3.18**. This would suggest that the influence of such factors as the chain length of the spacer, steric hindrance, and the size of the CD cavity would be important considerations for the formation of inclusion complexes.

Shono's group also investigated amino-bonded CSPs by immobilizing unmodified α- or β-CD stationary phase by reaction with succinamidopropyl silica (Su-Silica) [16, 50, 70]. The resultant CSP however contained both amido and secondary amino



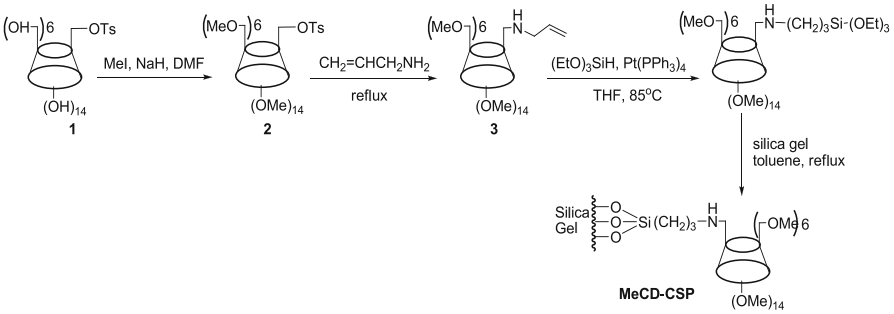
Scheme 3.6 Synthesis of amino-bonded CD CSPs (**CSP15-18**) (Reprinted with the permission from Ref. [15]. Copyright 1983 American Chemical Society)

(–NH-) groups, and it is probable that the –NH moieties would affect the solute retention in some cases. Though these CSPs were found effective for enantioseparation of certain analytes, their applications were limited by several disadvantages including (1) hydrolytic instability, (2) low CD loading, (3) separation selectivity affected by amine linkage, and (4) a tedious synthesis [7].

One of our research groups has recently synthesized a novel methylated β-CD CSP, which demonstrated good enantioseparation ability towards a selection of flavour and fragrance compounds using HPLC under reverse-phase conditions [67]. However, the use of aminized silica in this procedure would invariably result in remnant unreacted amine moieties on the silica gel surface. The presence of free amine groups on the surface may be undesirable under some conditions because they may interact with analytes through H-bonding. In order to overcome this problem, we subsequently developed a convenient synthesis for the CSP (designated as **PICD** herein) using mono(6^{A}-*N*-allylamino-6^{A}-deoxy)perphenylcarbamoylated β-CD as the chiral selector immobilized subsequently via a hydrosilylation approach [71, 72]. This CSP exhibited accentuated enantioseparation abilities towards a range of racemic compounds. Given the impetus stated above, we further reported a facile synthesis of mono(6^{A}-*N*-allylamino-6^{A}-deoxy)permethylated β-CD to afford the target **MeCD-CSP** (Scheme 3.7) [73], which is anticipated to show complementary enantioseparation ability in comparison to the perphenylcarbamoyl analogue [74].

Efficient chiral separations for a wide range of analytes on **MeCD-CSP** were demonstrated and the separation conditions optimized under both normal-phase and reverse-phase mode.

Generally, the **MeCD-CSP** demonstrated enantioseparation abilities towards all the six flavanones under all the four chromatographic conditions (see Table 3.5). It should be noted that all flavanones with the exception of 4′-hydroxyflavanone

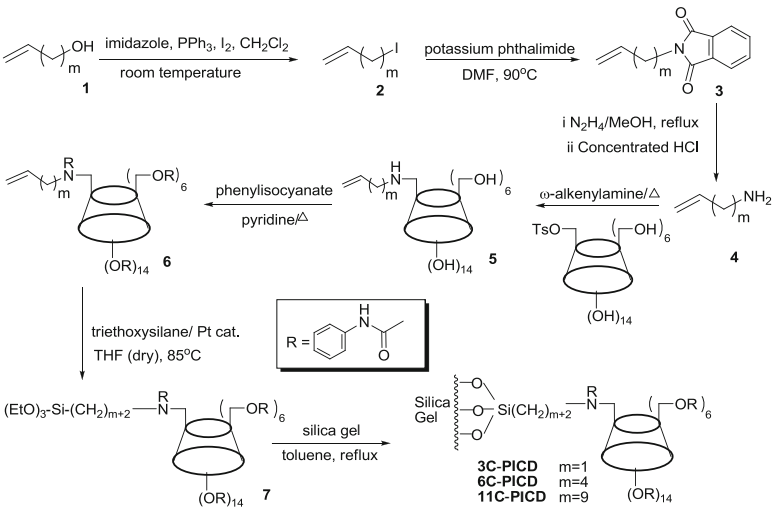


Scheme 3.7 Synthesis of permethylated **MeCD-CSP** (Reprinted from Ref. [73], Copyright 2004, with permission from Elsevier)

Table 3.5 Enantioseparation of flavanones on MeCD-CSP under reversed-phase conditions

	Chromatographic result						
Compound	t_1 (min)	t_2 (min)	k_1	k_2	α	R_S	Cond.
Hesperetin	112.5	134.0	16.31	19.62	1.20	1.88	I
	67.0	78.0	9.98	11.79	1.18	1.63	II
	24.8	28.0	3.20	3.75	1.17	1.33	III
	128.5	141.2	16.13	17.83	1.11	0.50	IV
6-Methoxyflavanone	43.5	49.2	5.69	6.57	1.15	1.31	I
	29.5	32.5	3.84	4.33	1.13	1.15	II
	13.4	14.4	1.27	1.44	1.13	0.59	III
	46.5	54.1	5.20	6.21	1.19	1.29	IV
7-Methoxyflavanone	50.3	62.8	6.74	8.66	1.28	2.28	I
	33.4	40.6	4.48	5.66	1.26	2.06	II
	14.7	16.7	1.49	1.83	1.23	1.61	III
	54.2	68.2	6.23	8.09	1.30	1.67	IV
4′-hydroxyflavanone	46.0	49.5	6.08	6.62	1.09	0.81	I
	38.1	41.0	5.25	5.72	1.09	0.93	II
	14.4	15.2	1.44	1.58	1.10	0.43	III
	53.5	57.8	6.13	6.71	1.09	0.59	IV
6-Hydroxyflavanone	45.2	50.8	5.95	6.82	1.15	1.45	I
	36.4	39.5	4.97	5.48	1.10	1.20	II
	14.1	14.7	1.39	1.49	1.07	0.42	III
	47.2	54.1	5.29	6.21	1.17	1.27	IV
Flavanone	37.5	41.4	4.77	5.37	1.13	1.31	I
	31.8	34.5	4.21	4.66	1.11	0.95	II
	13.5	14.1	1.29	1.39	1.08	0.32	III
	36.0	40.9	3.80	4.45	1.17	1.07	IV

Conditions: flow rate 0.5 mL min^{-1}, aqueous buffer (1 % aqueous TEAA, pH 5.5); I. methanol/buffer (25/75); II. methanol/buffer (35/65); III. methanol/buffer (50/50); IV. methanol/H_2O (15/85)



Scheme 3.8 Synthetic route to perfunctionalized CD immobilized onto silica gel via amine linkage (Reprinted from Ref. [75], Copyright 2011, with permission from Elsevier)

achieved the highest R_s value under condition I (methanol/buffer, 25/75) and three of the six racemates achieved the highest α value under condition IV (methanol/water, 15/85). This indicates that conditions I and IV might be the universal optimum conditions on MeCD-CSP for separation of flavanones under reverse-phase conditions.

As an extension of our study on mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated β-CD-based CSPs, we investigated the effect of spacer length on the enantioseparation abilities of **PICD** CSPs [75]. CSPs with a spacer length of 3, 6, and 11 carbons were prepared and designated as **3C-PICD**, **6C-PICD**, and **11C-PICD**, respectively (Scheme 3.8). Their chromatographic performance under normal phases was evaluated with ten racemic analytes including aromatic alcohols, flavanone compounds, amines, and non-protolytic compounds.

In many cases, CSP **6C-PICD** with the highest surface loading displayed the best enantioseparation ability (Fig. 3.3) and strongest retention to all the racemic samples. The data would suggest that there may be an optimum spacer length (approximately 6 carbons) for optimal enantioseparation of this series of CSPs. The existence of an optimum spacer length might be explained by the fact that a longer spacer arm would allow for the CD selectors in moving further away from the silica surface, which would in turn have the consequence of reducing achiral hydrogen bonding interactions between the analytes and the hydroxyl groups on silica surface. A diminished participation of achiral molecular interactions would be expected to accord with improved enantioselectivities. On the other hand, if the spacer arm is too long, the chiral selectors would be able to contact each other freely with consequent increase of mutual interactions between the CD chiral selectors (CD) in the stationary phase. Accordingly, this could lead to a decrease

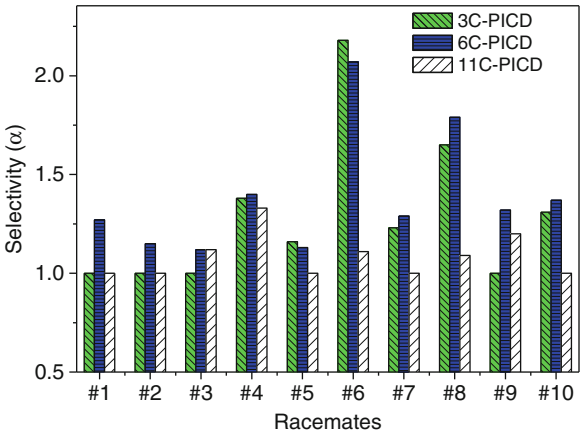


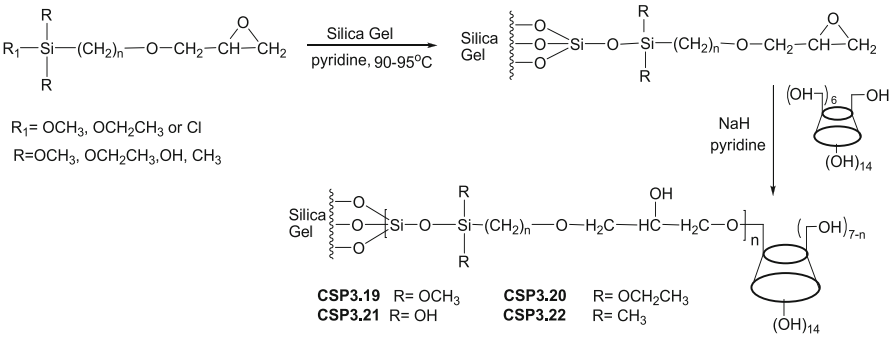
Fig. 3.3 Selectivity factor (α) versus spacer arm length for selected racemic samples. Analytes: #1. indapamide, #2. ancymidol, #3. 4-chromanol, #4. flavanone, #5. 6-methoxyflavanone, #6. 1-4-bromophenyl-3-buten-1-ol, #7. 1-3-chlorophenyl-3-buten-1-ol, #8. 1-(4-bromophenyl) ethanol, #9. 1-(4-chlorophenyl)ethanol, and #10. 1-(3-hydroxylphenyl)ethanol (Reprinted from Ref. [75], Copyright 2011, with permission from Elsevier)

in enantioselective interactions between the chiral selectors and analytes [76]. The balance of these opposing influences would accordingly afford an optimal spacer length for HPLC.

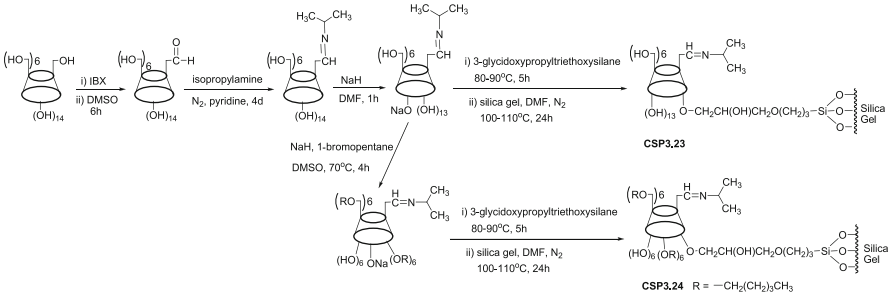
3.5 Chemically Bonded CD CSPs Based on Ether Linkages

Bonded phase CD CSPs with amido, urethane, and amino covalent linkers have extensively been used in HPLC, GC, or supercritical fluid chromatography (SFC) where there were numerous literature reports [2, 30, 77–79]. The key problem subsequently encountered with these CSPs would be their instability towards hydrolysis, which places severe limitations on their applications in aqueous mobile phases. An alternative approach for immobilizing native β-CD using hydrolytically more stable ether linkages was developed by Armstrong [18]. As outlined in Scheme 3.9, activated silica with a reactive epoxy end group was first obtained by reacting an anhydrous slurry of silica gel in toluene with appropriate silylating reagents. Subsequent reaction of the reactive epoxy moieties on silica with native CD in the presence of NaH in DMF or pyridine would afford the desired CSPs.

Recently, Zhou and his coworkers developed a series of CD CSPs with ether linkages for enantioseparation applications in HPLC. They initially prepared two new chiral stationary phases (CSPs), 6-deoxyisopropylimino-β-CD bonded on the silica gel (**CSP3.23**) and heptakis[2,6-o-diamyl-6-deoxyisopropylimino]-β-CD bonded on the silica gel (**CSP3.24**) by introducing the rigid imino group to β-CD (Scheme 3.10) [80].



Scheme 3.9 Immobilization of native β-CD on silica gel via ether bond to achieve **CSP3.19–3.22**.



Scheme 3.10 Synthetic approach for **CSP3.23** and **CSP3.24** (Reprinted from Ref. [80], Copyright 2005, with permission from Elsevier)

The presence of Schiff base moiety in these two CD CSPs afforded better selectivity in the series of amino acid analytes used. This could be due to enhanced π–π, hydrogen bonding, and polar–polar interactions. The column efficiency of **CSP3.23** and **CSP3.24** was determined to be 24,045 and 30,845 plates/metre, respectively, under normal phase [isopropanol/hexane (10/90, v/v)] with resorcinol as test compound. **CSP3.24** demonstrated enhanced retention of amino acids, presumably due to larger steric hindrance of **CSP3.24** compared to **CSP3.23**. It was proposed that the amylation modification above the secondary CD rim would partially occlude the mouth of the CD cavity, sterically affecting the formation of inclusion complexes, thus leading to a change in solute retention.

Following the development of **CSP3.23–3.24**, Zhou's group explored the synthesis of other imino-functionalized β-CD CSPs by taking advantage of the feature of Schiff base in enhancing π–π, hydrogen bonding, and polar–polar interactions between CSP and analytes [81, 82]. As shown in Fig. 3.4, the imino-functionalized β-CD CSP derivatives explored included mono(6-deoxy-*N*-1-phenylethylimino)-β-CD (**CSP3.26**),

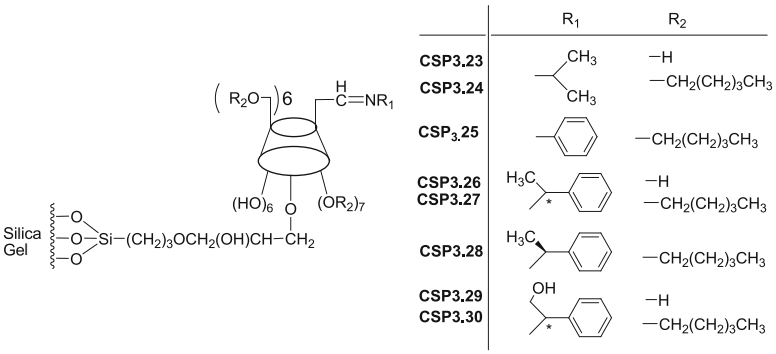


Fig. 3.4 The structure of imino-substituted β-CD **CSP3.23–3.30** based on ether linkage

mono[6-deoxy-*R*-(−)-*N*-1-(2-hydroxyl)phenylethylimino]-β-CD(**CSP3.29**),heptakis(2,6-o-diamyl-6-deoxy-phenylimino)-β-CD (**CSP3.27**), heptakis[2,6-o-diamyl-6-deoxy-*R*-(−)-N-1-phenylethylimino)]-β-CD (**CSP3.28**) [81], and heptakis[6-deoxy-R-(−)-*N*-1-(2-hydroxyl)-phenylethylimino)]-β-CD (**CSP3.30**) [82].

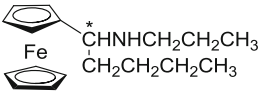
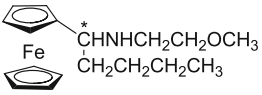
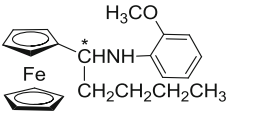
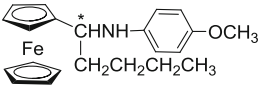
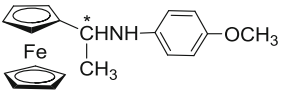
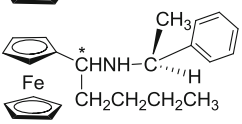
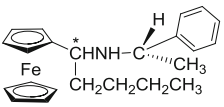
Their chromatographic properties were evaluated using a wide range of structurally diverse racemic compounds such as disubstituted benzenes, amino acids, aromatic alcohol analytes, and ferrocene analytes, with the latter being effectively baseline resolved. These investigations on the effect of functionalities on β-CDs would indicate that separation ability of the CSPs could be optimized by tuning the hydrophobic interactions, π–π, hydrogen bonding, dipolar–dipolar interactions, and conformational inductive effects.

The separation data for racemic ferrocene derivatives on **CSP3.26–3.28** are summarized in Table 3.6. All CSPs demonstrated outstanding enantioseparation towards ferrocene analytes, with R_s ranging from 1.07 to 8.16. As observed, **CSP3.27** is more suitable for enantioseparation of alkyl-substituted ferrocene amine. Comparing the separation results of model ferrocene on the **CSP3.26** and **CS3.28**, the multi-amyl groups substituted on CD were observed to have little effect on the enantioseparation of these compounds, which may suggest inclusion complexation cannot always play a major role in the chiral recognition.

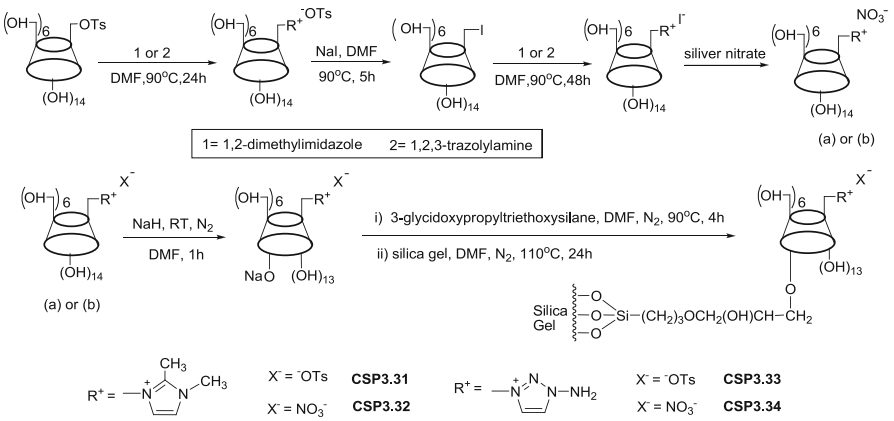
Recently, following our work in development of ionic CD derivatives as chiral additives for enantioseparation [83–86], Zhou et al. explored the chemical immobilization of ionic β-CDs onto silica gel via ether linkages [87]. Four novel ionic β-CDs were derived incorporating the imidazolium and 1,2,3-triazolium cation and nitrate or the tosylate counteranions. The synthetic approach for these ionic β-CDs CSPs is shown in Scheme 3.11.

The enantioseparation performance of these ionic CD-based CSPs was investigated using racemic aromatic alcohols as analytes, with the enantioseparation data summarized in Table 3.7. As seen, **CSP3.31–3.34** demonstrated better retention capacities for all analytes in comparison to **CSP3.29**–**3.30**, due to secondary steric interaction between the cation and anion moieties on the selectors and the analytes

Table 3.6 The separation data for ferrocene derivatives on **CSP3.26**, **CSP3.27** and **CSP3.28** [87]

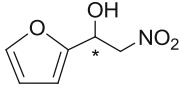
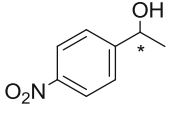
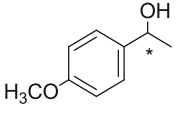
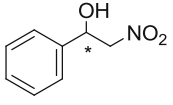
Entry	Compound	CSPs	Cond.	HPLC data k'_1	k'_2	α
1		**CSP3.26**	I	1.39	1.77	1.27
		CSP3.27	II	0.13	0.68	5.26
		CSP3.28	II	0.13	0.39	3.00
2		**CSP3.26**	II	0.12	0.37	3.09
		CSP3.27	II	0.15	0.70	4.69
		CSP3.28	II	0.18	0.53	2.96
3		**CSP3.26**	II	1.80	2.72	1.51
		CSP3.27	II	5.47	7.06	1.29
		CSP3.28	II	4.64	6.22	1.34
4		**CSP3.26**	II	1.13	1.77	1.57
		CSP3.27	II	3.34	4.68	1.40
		CSP3.28	II	2.66	3.62	1.36
5		**CSP3.26**	II	0.88	1.25	1.42
		CSP3.27	II	3.67	4.44	1.21
		CSP3.28	II	1.21	1.70	1.41
6		**CSP3.26**	II	0.12	0.38	4.12
		CSP3.27	II	0.12	0.98	8.16
		CSP3.28	II	0.19	0.61	3.21
7		**CSP3.26**	II	0.16	0.39	2.43
		CSP3.27	II	0.50	0.98	1.96
		CSP3.28	II	0.16	0.61	3.81

Separation conditions: 0.1 % TEAA (pH 6.0); I. ACN/TEAA (10/90), 0.3 mL min^{-1} flow rate; II. ACN/TEAA (30/70), 0.6 mL min^{-1} flow rate



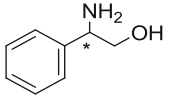
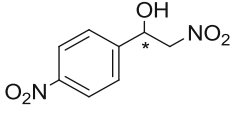
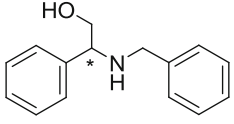
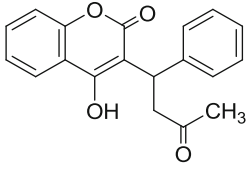
Scheme 3.11 Synthetic pathway of ionic CD **CSP3.31–3.34** having a single C2-ether linkage (Reprinted from Ref. [87], Copyright 2010, with permission from Elsevier)

Table 3.7 Chiral separation data for aromatic alcohols **CSP3.29–3.34**

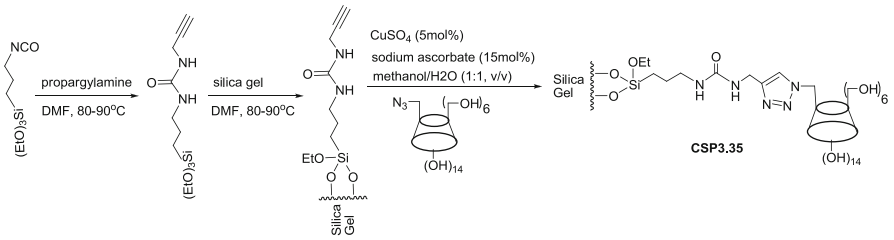
Entry	Structure	CSPs	Cond.	Separation data				Ref.
				k'_1	k'_2	α	R_S	
1		**CSP3.30**	I	1.52	1.73	1.14	1.71	[87]
		CSP3.29	I	1.39	1.65	1.19	2.12	[87]
		CSP3.31	III	0.63	0.76	1.21	1.17	[93]
		CSP3.32	III	0.55	0.72	1.32	1.6	[93]
		CSP3.33	III	0.59	0.81	1.37	1.47	[93]
		CSP3.34	III	0.52	0.77	1.49	2.44	[93]
2		**CSP3.30**	I	1.84	1.84	1.00	–	[87]
		CSP3.29	I	1.53	1.53	1.00	–	[87]
		CSP3.31	III	0.55	1.00	1.83	1.94	[93]
		CSP3.33	III	1.22	1.85	1.52	1.74	[93]
		CSP3.34	III	0.66	0.78	1.19	1.30	[93]
3		**CSP3.30**	I	1.50	1.80	1.20	1.84	[87]
		CSP3.29	I	1.25	1.38	1.38	3.53	[87]
		CSP3.31	IV	0.58	0.84	1.47	2.80	[93]
		CSP3.32	IV	0.48	0.84	1.74	3.90	[93]
		CSP3.33	IV	0.52	0.83	1.60	2.99	[93]
		CSP3.34	IV	0.43	0.78	1.80	3.96	[93]
4		**CSP3.30**	I	1.50	1.73	1.15	1.70	[87]
		CSP3.29	I	1.38	1.66	1.20	2.00	[87]
		CSP3.31	III	0.63	0.71	1.12	0.73	[93]
		CSP3.32	III	0.58	0.76	1.31	1.21	[93]
		CSP3.33	III	0.58	0.81	1.39	1.30	[93]
		CSP3.34	III	0.52	0.77	1.50	2.17	[93]

(continued)

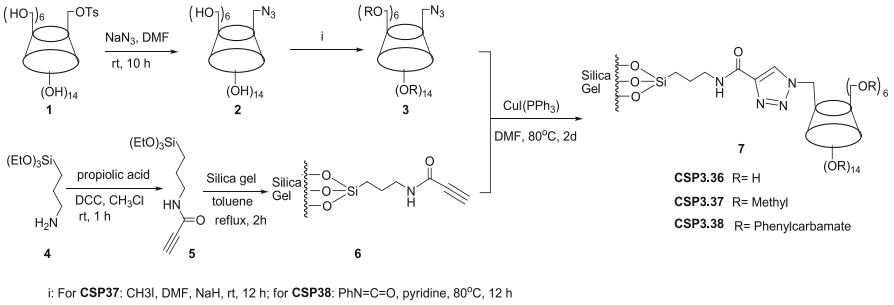
Table 3.7 (continued)

Entry	Structure	CSPs	Cond.	Separation data				Ref.
				k'_1	k'_2	α	R_S	
5		**CSP3.30**	I	1.66	1.84	1.11	1.04	[87]
		CSP3.29	I	1.83	2.12	1.16	1.96	[87]
		CSP3.31	I	0.26	0.89	3.42	6.87	[93]
		CSP3.32	III	0.29	0.89	3.09	6.40	[93]
		CSP3.33	III	0.66	0.85	1.29	1.29	[93]
		CSP3.34	IV	0.82	0.82	1.00	0	[93]
6		**CSP3.30**	I	1.54	1.86	1.21	1.98	[87]
		CSP3.29	I	1.42	1.83	1.29	2.50	[87]
		CSP3.31	IV	0.64	0.82	1.28	1.68	[93]
		CSP3.32	I	0.57	0.79	1.39	2.04	[93]
		CSP3.33	I	0.59	0.81	1.37	1.95	[93]
		CSP3.34	IV	0.53	0.84	1.59	2.13	[93]
7		**CSP3.30**	II	2.84	6.42	2.26	6.19	[87]
		CSP3.29	II	3.10	8.53	2.75	6.89	[87]
		CSP3.31	IV	0.71	1.29	1.82	4.57	[93]
		CSP3.32	III	0.67	1.42	2.21	6.08	[93]
		CSP3.33	III	0.70	1.42	2.04	5.97	[93]
		CSP3.34	I	0.53	1.35	2.53	6.27	[93]
8		**CSP3.30**	I	1.51	1.74	1.15	1.02	[87]
		CSP3.29	I	1.35	1.63	1.21	1.61	[87]
		CSP3.31	IV	0.72	2.63	3.65	6.54	[93]
		CSP3.32	I	0.62	1.80	2.91	3.67	[93]
		CSP3.33	I	0.72	2.30	3.21	5.02	[93]
		CSP3.34	c	0.48	0.81	1.69	2.29	[93]

Separation conditions: I. ACN/MeOH/AcOH/TEA (80/20/1/1); II. MeOH/H_2O (50/50); III. ACN/MeOH/AcOH/TEA (80/20/1/2); IV. ACN/MeOH/AcOH/TEA (480/20/2/1)



Scheme 3.12 The preparation process of **CSP3.35** (Reprinted from Ref. [92], Copyright 2008, with permission from Elsevier)



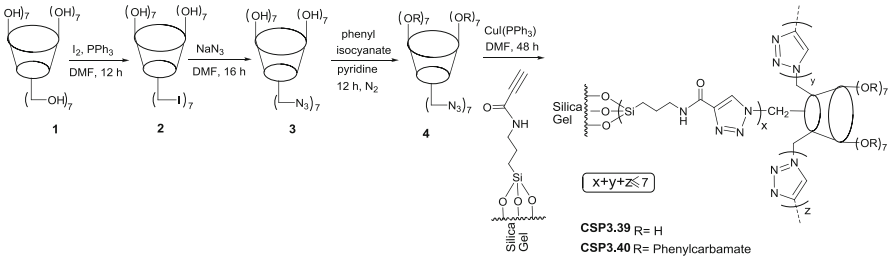
Scheme 3.13 Synthetic routes to **CSP3.36–3.38** via click chemistry

beside inclusion complexation. **CSP3.31–3.34** exhibited good resolution for all analytes investigated, whereas **CSP3.34** affords higher resolution factors for most of the analytes tested. Entries 7 and 8 (Table 3.7) achieved highest R_s of up to 6.54 with **CSP3.34** and baseline separation with **CSP3.31–3.33**.

3.6 Chemically Bonded CD CSPs Based on Triazolyl Linkages

Click chemistry was coined by Sharpless and has become a versatile approach in organic synthesis [88]. Kacprzak reported the preparation of a CSP by immobilizing Cinchona alkaloid derivatives onto silica via click chemistry [89]. Liang and coworkers have explored the application of click chemistry for the preparation of silica-based CSPs for HPLC [90, 91]. Recently, this research group also prepared a novel triazolyl-bonded β-CD CSP (**CSP35**) [92] via click chemistry of an azido-β-CD [64, 93] with alkyne-modified silica (Scheme 3.12) [90].

Meanwhile, our research group reported the use of click chemistry in affording β-CD derivative-based CSPs with the triazolyl linkage using an organic soluble copper (I) catalyst [94]. Three novel CSPs (**CSP3.36–3.38**) were prepared which exhibited good stability and excellent enantioselectivity. The synthetic route (Scheme 3.13) involved first the synthesis of ω-alkynyl functionalized



Scheme 3.14 Preparation of the CSPs **3.39** and **3.40** (Reprinted from Ref. [98], Copyright 2010, with permission from Elsevier)

silica **6**, followed by click chemistry with monoazido-β-CD derivatives, in the presence of catalytic quantity of $CuI(PPh_3)$, under relatively mild reaction conditions.

Enantioseparation results of these three CD CSPs were elaborated in detail [95–97].

Considering the potentially unstable single triazolyl linkage particularly after long exposure to buffer, we investigated the preparation of multiple triazolyl covalently bonded CD **CSP3.39–3.40** by application of "multiple click" reactions between heptakis (6-deoxy-6-azido)-β-CD and heptakis(6-deoxy-6-azido- perphenylcarbamoylated)-β-CD with ω-alkynyl functionalized silica (Scheme 3.14) [98].

For **CSP3.39–3.40**, the smaller opening of the CD cone is almost blocked off by the presence of multiple triazolyl linkages. Accordingly, these materials demonstrated quite different enantioseparation properties to the previous series of "click"-derived **CSP3.36–3.38**. The enantioselectivities of CD **CSP3.36–3.40** were evaluated under reverse-phase conditions in HPLC with a wide spectrum of racemates including aromatic substituted alcohols, β-blockers, carboxylic acids, dansyl-amino acids, and flavonoids (refer to Fig. 3.2 for all the analyte structures).

Perusal of Schemes 3.13 and 3.14, **CSP3.38** and **CSP3.40** bear the same O-functionality on the CD chiral selector but different number of triazolyl linkage, with the latter possessing multiple triazolyl linkages. Their comparative enantioseparation capabilities towards aromatic substituted alcohols and β-blockers were summarized in Table 3.8. **CSP3.38** afforded better separation of aromatic substituted alcohols in comparison to **CSP3.40**. Amongst the aromatic alcohols, entries 2, 3, and 4 displayed higher selectivity values (α) and the retention factors in comparison to entry 1 on both **CSP3.38** and **CSP3.40**, indicating that inclusion is the predominant factor for the chiral separation of these analytes. The better separation of latter three analytes compared with entry 1 would suggest enhanced chiral discrimination by virtue of π–π interactions between the allyl group of the analyte guest molecule and phenyl groups of the host. Steric factors

Table 3.8 Enantioseparation of alcohols and β-blockers with **CSP3.38** and **CSP3.40**

				HPLC data				
Entry	Racemic compounds	CSPs	Cond.	k'_1	k'_2	α	R_S	Ref.
1	Aryl-OH-1	**CSP3.38**	a	0.86	0.95	1.11	0.91	[97]
		CSP3.40	f	0.99	1.03	1.04	0.42	[98]
2	Aryl-OH-2	**CSP3.38**	a	2.25	2.93	1.30	3.56	[97]
		CSP3.40	f	1.93	2.18	1.13	1.96	[98]
3	Aryl-OH-3	**CSP3.38**	b	3.35	4.09	1.22	3.12	[97]
		CSP3.40	f	1.94	2.18	1.12	1.84	[98]
4	Aryl-OH-4	**CSP3.38**	b	4.96	5.52	1.11	1.50	[97]
		CSP3.40	f	2.76	2.93	1.06	1.24	[98]
5	Alprenolol	**CSP3.38**	c	3.64	4.38	1.20	1.40	[97]
		CSP3.40	g	1.66	2.02	1.20	2.02	[98]
6	Etilefrine	**CSP3.38**	d	0.75	1.11	1.48	0.60	[97]
		CSP3.40	h	3.03	3.28	1.08	0.77	[98]
7	Terbutaline	**CSP3.38**	e	–	–	–	0.62	[97]
		CSP3.40	i	0.61	1.13	1.84	2.26	[98]
8	Propranolol	**CSP3.38**	c	8.19	10.13	1.24	1.36	[97]
		CSP3.40	g	3.24	4.10	1.27	2.20	[98]
9	Pindolol	**CSP3.38**	c	2.66	2.94	1.10	<0.4	[97]
		CSP3.40	g	1.04	1.12	1.07	0.88	[98]
11	Dobutamine	**CSP3.38**	d	1.11	1.31	1.18	0.96	[97]
		CSP3.40	j	1.09	1.80	1.84	1.86	[98]

Separation conditions: flow rate 0.7 mL min^{-1}; (a) MeOH/H_2O (50/50, v/v); (b) ACN/H_2O (20/80); (c) MeOH/1 %TEAA (pH 6.5) (30/70); (d) MeOH/1 %TEAA (pH 6.5) (50/50); (e) MeOH/1 %TEAA (pH 6.5) (20/80); (f) ACN/H_2O (35/65); (g) MeOH/0.1 %TEAA (pH 4.2) (35/65); (h) MeOH/0.1 %TEAA (pH 4.2) (35/65); (i) MeOH/TEAA (pH 4.2) (5/95); (j) MeOH/0.1 %TEAA (pH 4.2) (50/50)

would afford better performance of **CSP3.38** than **CSP3.40**, since **CSP3.40** would be more sterically encumbered with consequent greater steric hindrance between CD and the analytes.

Similarly, **CSP3.36** and **CSP3.39** have the same functionalities on the CD (free –OH) but different number of triazolyl linkages. The separation data for carboxylic acids and dansyl-amino acids on **CSP3.36** and **CSP3.39** are summarized in Table 3.9. Generally, **CSP3.39** afforded better enantioseparation towards carboxylic acids than **CSP3.36**, presumably due to enhanced interaction between CSP and analytes. **CSP3.39** possesses multiple triazolyl linkages on the smaller opening of the CD, allowing the single benzene ring of carboxylic acids to enter the CD cavity to form inclusion complex. In addition, the multiple triazolyl moieties of **CSP3.39** may afford more hydrogen bonding and π–π interaction sites which are favourable for chiral discrimination. However, for dansyl-amino acids, the presence of the bulky naphthalene ring makes it more difficult for these molecules to penetrate into the CD cavity of multiple triazolyl-bonded **CSP3.39** than **CSP3.36**.

Table 3.9 Separation results of carboxylic acids and dansyl-amino acids on **CSP3.36** and **CSP3.39**

				HPLC data				
Entry	Analytes	CSPs	Cond.	k'_1	k'_2	α	R_S	Ref.
12	2-POPA	**CSP3.36**	a	2.93	3.2	1.09	0.97	[95]
		CSP3.39	d	2.02	2.44	1.21	2.00	[98]
13	3ClPOPA	**CSP3.36**	b	5.26	5.65	1.07	0.67	[95]
		CSP3.39	d	3.52	3.82	1.09	1.06	[98]
14	4-NPOPA	**CSP3.36**	a	6.09	6.54	1.07	0.90	[95]
		CSP3.39	d	5.50	5.83	1.06	1.17	[98]
15	4-OHPOPA	**CSP3.36**	b	3.13	3.35	1.07	0.67	[95]
		CSP3.39	d	1.37	1.58	1.15	1.25	[98]
16	Dns-Thr	**CSP3.36**	c	1.75	2.48	1.41	2.32	[95]
		CSP3.39	e	2.92	3.46	1.18	0.91	[98]
17	Dns-Val	**CSP3.36**	c	1.68	2.37	1.41	2.36	[95]
		CSP3.39	e	2.67	3.29	1.23	1.45	[98]
18	Dns-Leu	**CSP3.36**	c	3.68	7.35	1.99	5.08	[95]
		CSP3.39	e	3.05	3.33	1.09	0.61	[98]
19	Dns-Glu	**CSP3.36**	c	2.21	3.71	1.22	1.25	[95]
		CSP3.39	e	3.81	4.51	1.19	1.35	[98]
20	Dns-Nva	**CSP3.36**	c	1.23	1.60	1.30	1.42	[95]
		CSP3.39	e	2.39	2.68	1.11	0.51	[98]
21	Dns-Ser	**CSP3.36**	c	1.53	1.92	1.25	1.36	[95]
		CSP3.39	e	2.46	2.81	1.14	0.53	[98]
22	Dns-Phe	**CSP3.36**	c	2.50	3.76	1.50	2.43	[95]
		CSP3.39	e	3.80	14.5	1.05	0.40	[98]

Separation conditions: (a) 1 % TEAA buffer (pH 4.11)/MeOH (70/30), flow rate 0.3 mL min^{-1}; (b) 1 % TEAA buffer (pH 4.11)/MeOH (70/30), flow rate 0.4 mL min^{-1}; (c) 1 % TEAA buffer (pH 5.11)/MeOH (50/50), flow rate 0.7 mL min^{-1}; (d) MeOH/0.1 %TEAA (pH 5.2) (50/50), flow rate 0.7 mL min^{-1}; (e) ACN/TEAA (pH 5.2) (35/65), flow rate 0.7 mL min^{-1}

Consequently, the formation of inclusion complex between dansyl-amino acids and **CSP3.39** would be more difficult than **CSP3.36**. As a result, **CSP3.36** would be more appropriate for the enantioseparation of dansyl-amino acids.

CSP3.38–3.40 demonstrated good resolution towards flavonoids, with **CSP3.40** presenting the best enantioseparation results except entry 26 (Table 3.10).

The enantioseparation results of other racemates tested are summarized in Table 3.11. It is apparent that the enantiomers of atropine and bendroflumethiazide were well separated on **CSP3.38** and **CSP3.40**, while other racemates were separated to a less significant extent.

In this section, the preparation and enantioseparation capabilities of six bonded-phase CD CSPs with triazolyl linkers and synthesized via "click" chemistry were described. The "click"-immobilized perphenylcarbamoylated and permethylated CSPs demonstrated good enantioseparation ability for a wide range of racemic analytes including aryl alcohols, carboxylic acids, dansyl-amino acids, and flavonoids.

Table 3.10 Separation result of flavonoids on **CSP3.38–3.40** under reversed-phase mode2

S/N	Racemic compounds	CSPs	Cond.	HPLC data				Ref.
				k'_1	k'_2	α	R_S	
23	Flavanone	**CSP3.38**	a	7.9	8.27	1.05	0.75	[97]
		CSP3.39	b	22.4	23.1	1.03	0.41	[98]
		CSP3.40	c	4.79	5.53	1.15	2.54	[98]
24	4′-Hydroxylflavanone	**CSP3.38**	a	11.2	12.1	1.04	<0.4	[97]
		CSP3.39	b	24.1	25.4	1.05	0.64	[98]
		CSP3.40	c	3.61	3.98	1.10	1.51	[98]
25	6-Hydroxylflavanone	**CSP3.38**	a	11.7	12.1	1.05	0.45	[97]
		CSP3.39	b	23.3	24.5	1.05	0.62	[98]
		CSP3.40	c	3.81	4.07	1.07	1.34	[98]
26	5-Methoxylflavanone	**CSP3.38**	a	6.07	6.27	1.03	<0.4	[97]
		CSP3.39	b	15.3	17.5	1.14	1.65	[98]
		CSP3.40	c	4.03	4.10	1.02	1.06	[98]
27	6-Methoxylflavanone	**CSP3.38**	a	10.31	10.8	1.06	0.87	[97]
		CSP3.39	b	19.2	20.8	1.08	1.09	[98]
		CSP3.40	c	7.43	8.37	1.13	2.12	[98]
28	7-Methoxylflavanone	**CSP3.38**	a	10.8	11.7	1.09	1.52	[97]
		CSP3.39	b	18.7	20.4	1.09	1.15	[98]
		CSP3.40	c	7.55	9.27	1.22	2.91	[98]

Separation conditions: (a) MeOH/H_2O (30/70), flow rate 0.5 mL min^{-1}; (b) MeOH/H_2O (35/65), flow rate 0.7 mL min^{-1}; (c) MeOH/H_2O (50/50), flow rate 0.7 mL min^{-1}

Different derivatization on the CD selector afforded quite different enantiorecognition properties due to the difference in π–π, dipole–dipole, and hydrophobic interactions with analyte molecules.

3.7 Summary

Cyclodextrin-based CSPs have been successfully employed in the resolution of over 1,000 racemic compounds as well as numerous diastereomers and structural isomers. Over 400 papers have been published on the use of CD-based CSPs in LC since 1983. The development of robust CSPs with facile synthetic methodologies and reproducible application calls for structurally well-defined cyclodextrin bonded onto supports with robust and solvent-stable covalent linkers. We have afforded in this chapter an update on recent efforts in the development of bonded-phase CD-based CSPs incorporating ether, urea, and triazolyl covalent linkers and outlined their corresponding enantioseparation performance. It is anticipated that the correlation of the structure enantioseparation performance relationship would provide guidelines to the rational design of efficacious novel CD CSP.

Table 3.11 Enantioseparation results of the analytes on different CD CSPs

Entry	Racemic compounds	CSPs	Cond.	HPLC data				Ref.
				k'_1	k'_2	α	R_S	
29	Benzoin	**CSP3.35**	a	24.82	–	1.11	1.87	[92]
		CSP3.38	b	12.45	12.96	1.04	0.41	[97]
30	Ketoprofen	**CSP3.35**	c	10.3	–	1.09	0.89	[92]
		CSP3.39	d	8.37	8.87	1.06	0.65	[98]
31	Atropine	**CSP38**	e	0.74	1.21	1.63	2.89	[97]
		CSP3.40	f	0.81	1.44	1.78	3.57	[98]
32	Brompheniramine	**CSP3.36**	g	9.04	9.78	1.09	0.81	[95]
		CSP3.39	h	2.38	2.56	1.07	0.42	[98]
		CSP3.40	i	3.10	3.21	1.03	<0.3	[98]
33	*p-Chlorophenyl-pyrimidine*	**CSP3.38**	j	1.11	1.32	1.18	0.67	[97]
		CSP3.39	k	2.12	2.40	1.13	0.96	[98]
34	Bendroflumethiazide	**CSP3.36**	l	8.71	9.47	1.11	0.75	[95]
		CSP3.38	m	1.86	2.38	1.28	2.82	[97]
		CSP3.40	n	5.80	6.31	1.09	1.48	[98]
35	Cyclothiazide	**CSP3.39**	o	2.12	2.78	1.31	1.05	[98]
		CSP3.40	p	5.31	5.83	1.10	1.08	[98]
36	Tröger's base	**CSP3.35**	q	18.6	–	1.09	1.05	[92]
		CSP3.39	r	16.6	19.7	1.18	1.64	[98]

Separation conditions: (a) methanol/1 %TEAA (pH 4.94) (10/90), flow rate 1.00 mL/min; (b) MeOH/H_2O (20/80) flow rate 0.7 mL min^{-1}; (c) ACN/H_2O (0.5 % acetic acid) (15/85); (d) ACN/1 %TEAA (pH 5.2) (25/75), flow rate 0.7 mL min^{-1}; (e) MeOH/1 %TEAA (pH 6.5) (50/50), flow rate 0.7 mL min^{-1}; (f) MeOH/1 %TEAA (pH 4.2) (35/65), flow rate 0.7 mL min^{-1}; (g) MeOH/1 %TEAA (pH 4.94) (40/60), flow rate 0.5 mL min^{-1}; (h) ACN/1 %TEAA (pH 5.2) (25/75), flow rate 0.7 mL min^{-1}; (i) MeOH/1 %TEAA (pH 4.2) (35/65), flow rate 0.7 mL min^{-1}; (j) MeOH/1 %TEAA (pH 6.5) (50/50), flow rate 0.7 mL min^{-1}; (k) MeOH/1 %TEAA (pH 4.2) (35/65), flow rate 0.7 mL min^{-1}; (l) MeOH/1 %TEAA (pH 4.11) (30/70), flow rate 0.5 mL min^{-1}; (m) ACN/H_2O (40/60), flow rate 0.7 mL min^{-1}; (n) ACN/H_2O (40/60), flow rate 0.7 mL min^{-1}; (o) ACN/1 %TEAA (pH 5.2) (35/65), flow rate 0.7 mL min^{-1}; (p) ACN/H_2O (40/60) flow rate 0.7 mL min^{-1}; (q) MeOH/1 %TEAA (pH 4.94) (25/75), flow rate 1.00 mL min^{-1}; (r) MeOH/H_2O (35/65), flow rate 0.7 mL min^{-1}

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Chapter 4
Cyclodextrin-Based Chiral Stationary Phases for Supercritical Fluid Chromatography

Yun Dai, Weihua Tang, and Siu-Choon Ng

Abstract In this chapter, a general introduction of supercritical fluid and supercritical fluid chromatography (SFC) is firstly provided. An overview of commercially available or newly developed packed columns for enantiomeric separation based on cyclodextrin chiral stationary phases (CSPs) in SFC is then presented. The correlations between cyclodextrin structures and their enantioselectivities are also discussed. Furthermore, the analytical conditions for optimization of enantiomeric separation with these cyclodextrin-based CSPs are discussed in detail.

4.1 Supercritical Fluids

Supercriticality is a state of a certain fluid which is reached at a temperature higher than its critical temperature and at a pressure higher than its critical pressure [1]. In the region above the critical temperature and pressure, a substance cannot be classified as either a gas or a liquid since it has properties of both. In other words, supercritical fluids can be thought of as gases that have been compressed to densities at which they can exhibit liquid-like interactions. Vapor–liquid phase diagram is often used to describe the supercritical state of pure compound; Fig. 4.1 shows the phase diagram of carbon dioxide [2]. There is an equilibrium between vapor and the liquid; it can be illustrated by a curve as $P=f(T)$. This equilibrium curve exists only

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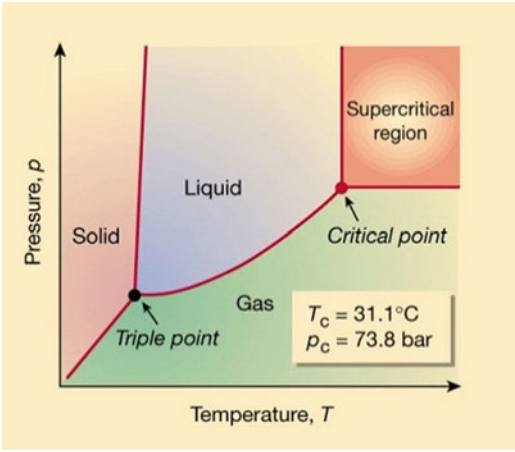


Fig. 4.1 PT phase diagram of carbon dioxide; the critical point of CO_2 is at $T = 31.1$ °C, $P = 73.8$ bar

between the triple point (T) and the critical point (C). At the triple point, the solid, the liquid, and the gas coexist in equilibrium. At temperatures below the triple point, the gas phase is in equilibrium with the solid phase and a thermodynamically stable liquid cannot exist. When the temperature of a gas/liquid system is in equilibrium above the triple point, the density of the liquid phase decreases, which can be seen clearly from the phase diagram of carbon dioxide.

The supercritical fluid corresponds to the condition that the pressure exceeds the critical pressure and the temperature exceeds the critical temperature. Table 4.1 lists the molecular weight, the critical temperature, the critical pressure, and the critical density of a few solvents [3]. Figure 4.2 shows the pressure–density isotherms of carbon dioxide. Above the critical temperature (31.1 °C), large changes are seen in the density of carbon dioxide as the pressure is varied [4]. A supercritical fluid has properties that are intermediate between liquids and ordinary gases. The density affects the solvation power of the solvent: the higher the density, the greater the solvent strength (Fig. 4.2) [5].

At high densities, supercritical fluids have solvent strengths similar to those of liquids. Both liquids and supercritical fluids have high solvent strengths and can dissolve many different types of solutes. By changing the density of the fluid through temperature and pressure variation, the solvation strength of a supercritical fluid can be altered. Depending upon the density, the viscosities of supercritical fluids can be similar to gas or intermediate between gas and liquid [6]. Finally, solute diffusion coefficients of supercritical fluids are intermediate between those in gases and in liquids. In view of such powerful properties of supercritical fluid, advanced chromatography technique was developed using supercritical fluid as mobile phase. As shown in Table 4.1, among the various considered supercritical fluids, carbon dioxide exhibits easily accessible critical conditions ($T_c = 304.12$ K, $P_c = 73.74$ atm) and offers several advantages, like nontoxic, cheap, and environmental friendliness [7]. Hence, supercritical carbon dioxide becomes the most widely used fluid in various fields including the chromatographic mobile phase.

Table 4.1 Critical properties of some common fluids [5]

Solvent	MW	T_c (K)	P_c (atm)	D_c (g/ml)
Methane (CH_4)	16.04	190.6	46.4	0.162
Ammonia (NH_3)	17	405.4	113.5	0.235
Water (H_2O)	18.015	647.1	220.6	0.322
Ethylene (C_2H_4)	28.05	282.3	50.4	0.215
Ethane (C_2H_6)	30.07	304.3	48.7	0.203
Methanol (CH_3OH)	32.04	512.6	80.9	0.272
Propylene (C_3H_6)	42.08	364.9	46.0	0.232
Carbon dioxide (CO_2)	44.1	304.12	73.74	0.469
Nitrous oxide (N_2O)	44	309.6	72.5	0.45
Propane (C_3H_8)	44.09	369.8	42.5	0.217
Ethanol (C_2H_5OH)	46.07	513.9	61.5	0.276
Acetone (C_3H_6O)	58.08	508.1	47.0	0.278
Trifluoromethane (CHF_3)	70.01	298.97	48.4	0.526

MW, molecular weight; T_c, critical temperature (K); P_c, critical pressure (atm); D_c, critical density (g/ml)

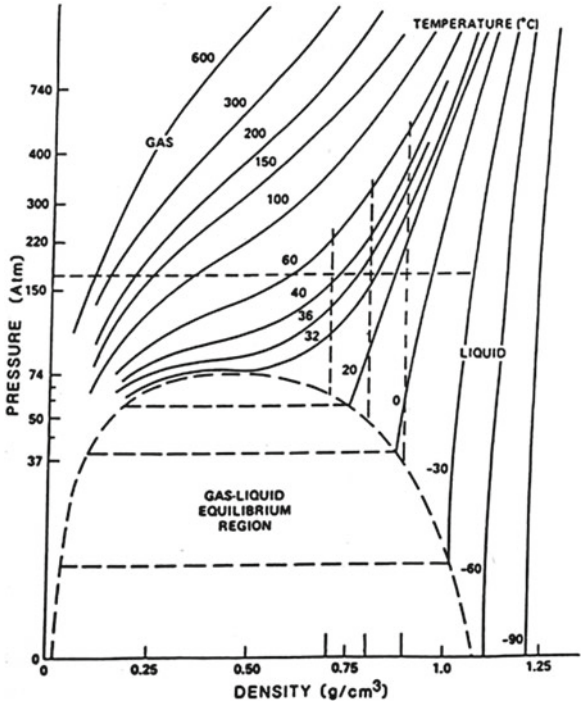


Fig. 4.2 Pressure–density behavior of carbon dioxide at various temperatures (Reprinted with the permission from Ref. [4]. Copyright 1988 American Chemical Society)

4.2 Application of Supercritical Fluid in Chromatography (SFC)

The use of supercritical fluids as eluents in chromatography was first reported by Klesper and his co-workers in 1962, and this technology was called supercritical fluid chromatography (SFC) [8]. They separated porphyrin mixtures by using supercritical chlorofluoromethanes as mobile phase. Above the critical state, fluids have solvating strength approaching to liquids, yet they still retain the low viscosity and high diffusivity as gases [9]. During the past few decades, several scientists such as Novotny, Gere, Greibrokk, and Schoenmakers have contributed to the development of SFC. Carbon dioxide remains the most widely used supercritical fluid because of its low cost, low toxicity, and modest critical parameters. Therefore, if not particularly pointed out, the eluents of supercritical fluid chromatography all refer to supercritical carbon dioxide.

Owing to the nonpolar nature of CO_2, polar modifiers are often added to increase its solvent strength, especially when polar solutes are to be separated, or to reduce the retention volume of the analyte in the column [10]. The elution of strongly polar compounds, such as acid and basic analytes, can be achieved through the use of additives into the mobile phase [11]. Table 4.2 summarizes several useful modifiers that have been utilized in SFC [6, 13]. Modifiers can provide control over both retention and selectivity. Because modifiers compete with the analytes for sites on the stationary phase, retention decreases as the modifier concentration increases. A useful rule of thumb is that retention doubles each time the modifier concentration is cut in half [14].

Table 4.2 Frequently used modifiers in supercritical fluid chromatography [12]

Modifier	T_c (°C)	P_c (atm)	Molecular mass	Dielectric constant (20 °C)	Polarity index
Methanol	239.4	79.9	32.04	32.70	5.1
Ethanol	243.0	63.0	46.07	24.3	4.3
1-Propanol	263.5	51.0	60.10	20.33	4.0
2-Propanol	235.1	47.0	60.10	20.33	4.0
1-Hexanol	336.8	40.0	102.18	13.3	3.5
2-Methoxy ethanol	302	52.2	76.10	16.93	5.5
Tetrahydrofuran	267.0	51.2	72.11	7.58	4.0
1,4-Dioxane	314	51.4	88.11	2.25	4.8
Acetonitrile	275	47.7	41.05	37.5	5.8
Dichloromethane	237	60.0	84.93	8.93	–
Chloroform	263.2	54.2	119.38	4.81	4.1
Propylene carbonate	352.0	–	102.09	69.0	6.1
N,N-dimethylacetamide	384	–	87.12	37.78	6.5
Dimethyl sulfoxide	465.0	–	78.13	46.68	7.2
Formic acid	307	–	46.02	58.5	–
Water	374.1	217.6	18.01	80.1	10.2
Carbon disulfide	279	78.0	76.13	2.64	–

Table 4.3 Supercritical fluids have densities, viscosities, and other properties intermediate between gas and liquid [17]

Property	Gas	Supercritical fluid	Liquid
Density (g/cm^3)	$(0.6–2)\times10^{-3}$	0.2–0.5	0.6–2
Diffusion coefficient (cm^2/s)	$(1–4)\times10^{-1}$	10^{-3}–10^{-4}	$(0.2–2)\times10^{-5}$
Viscosity (g/cm s)	$(1–4)\times10^{-4}$	$(1–3)\times10^{-4}$	$(0.2–3)\times10^{-2}$

At very low modifier concentrations, however, peak shapes tend to degrade. Recent studies provided an insight into the rational choice of modifier based on whether the inter-molecular interaction needs to be enhanced or reduced [15].

The addition of these solvents into a supercritical fluid phase will modify the polarity of the eluent due to the high dielectric constants or polarity indexes associated with the organic modifiers [12]. As summarized in Table 4.2, modifiers have quite different critical temperatures and pressures. This suggests that when using modifiers, caution must be taken to assure the components are miscible over the range of temperatures and pressures that are used eluent.

Although modifier has many benefits in chromatography separation, it is added to the supercritical fluid eluent to eliminate adsorptive effects exhibited by solutes only in packed-column SFC. When it turns to capillary column SFC, pure CO_2 is the most widely used.

4.3 Advantages and Drawbacks of SFC in Comparison to GC and HPLC

4.3.1 Mobile Phase and Gradient

The definition of supercritical fluid chromatography comes from its mobile phase, supercritical fluid. So the advantages of SFC compared with GC and HPLC focus on the superiority of supercritical fluid over both liquid and gas. Advantages of supercritical fluids over liquid phases rest with improved mass transfer processes due to lower fluid viscosities and higher analyte diffusivities, while advantages over gas phases rest with increased molecular interactions due to higher densities [16]. Figure 4.2 shows that the density of supercritical fluid is the function of both temperature and pressure, and density is proportional to solvent strength. This aspect gives rise to some unique features that can not found in HPLC and GC. This peculiar property of supercritical fluids provides an opportunity to enhance the performance by the implementation of gradients that results in increased chromatographic separation ability. Moreover, the diffusivity of supercritical CO_2 is approximately two orders of magnitude greater than those exhibited by liquid solvents. Similarly, the viscosity of supercritical CO_2 is at least 20 times larger than the viscosities associated with liquid media [17], and Table 4.3 summarizes the densities,

viscosities, and other properties of general gas, supercritical fluid, and liquid [18]. As noted by Giddingset al. [19], these properties provide an opportunity to analyze nonvolatile or thermally labile samples that are not suitable for GC and to achieve higher efficiencies than HPLC.

Eventually, SFC's merits can be summarized in three points. Firstly, it cuts the consumption of organic solvents to the bone. Secondly, the physiochemical properties of supercritical fluid, e.g., viscosity and diffusivity, are favorable compared to liquids, thus leading to lower pressure drop and higher column efficiency. Last, the retention behavior of solutes in supercritical fluids shows a strong dependence on the mobile phase density and hence could be used to impose a gradient on the separation process, thereby increasing the productivity.

Supercritical fluid chromatography does have some drawbacks in comparison to liquid chromatography and gas chromatography that supercritical fluid chromatography must be carried out at high pressure, which makes the process more complicated and potentially more dangerous.

4.3.2 Instruments

For general SFC systems, an HPLC injection equipment, an HPLC syringe pump, a capillary column or a packed column, a GC oven, and either a HPLC UV–vis or GC-FI detector make up the major components. A syringe pump is used because it provides pulseless constant flow even at low flow rates and because it is easy to control for pressure programming. In these major components, column and detector are worthy of careful discussion.

Capillary column and packed column are all successfully applied to SFC and have obtained good separations of many substances especially drugs and enantiomers [20–23]. Klesper et al. [8] first reported the SFC with packed column. Then, Novotny proposed the capillary column SFC in 1981, opening a new page of SFC [24]. In the following years, capillary column SFC techniques were developed to a relatively mature level especially in Novotny and Lee's groups. Recently, the techniques for packed-column SFC have also been improved and studied more than capillary column. It can be said that capillary SFC is more similar to GC, and packed-column SFC is more similar to HPLC. Both packed and capillary columns have their advantages when used in SFC. A packed column is basically a tube filled with porous material. The total surface area of the packing is much greater than the surface area of a capillary tube, giving it a larger sample capacity. The primary advantage of capillary columns in SFC is that they offer a greater number of theoretical plates than packed columns [4].

SFC has the advantage over HPLC that both HPLC and GC type detectors can be used for SFC [25]. The type of detector used depends upon the chromatographic system. FI detection is most commonly coupled to capillary systems, while the UV–vis detector is common in packed-column systems.

4.3.3 Developments of Separation Method

Development of chiral analytical methods is quite similar in SFC and LC, but SFC offers more flexibility in parameter selection. For SFC separation method development, modifier and experiment temperature and pressure are the key considerations.

Modifiers can provide control over both retention and selectivity. Because modifiers compete with the analytes for sites on the stationary phase, retention decreases as the modifier concentration increases [26]. A useful rule of thumb is that retention doubles each time the modifier concentration is cut in half [27]. At very low modifier concentrations, however, peak shapes tend to degrade. Recent studies provide insight into the rational choice of modifier based on whether the inter-molecular interaction needs to be enhanced or reduced [15].

Temperature and pressure are rarely optimized in HPLC separations, but both parameters can alter retention, selectivity, and resolution in SFC. As the temperature increases, selectivity decreases to a point where the enantiomers coelute in the column. Above this point, the elution order reverses, and additional increases in temperature increase the selectivity [28].

Higher flow rates can be used in SFC to take advantage of the high diffusivity of supercritical fluids without compromising efficiency. Flow rates have significant effects on the performance of SFC, especially the separation efficiency. Packed-column SFC is now rapidly replacing many normal-phase HPLC methods for chiral separations because SFC can meet the analytical requirements associated with stereoisomeric drug development [29].

4.3.4 Applications

According to published literatures, a wide variety of substances, including natural products, drugs, foods, pesticides, herbicides, surfactants, and so on, have been separated successfully by SFC [30–34]. Among all these applications of SFC, chiral separation is considered to be the most important one. Chiral separation by SFC was first documented in 1985 by Mourier [35]. Due to the high efficiency, fast flow rate, low temperature analysis, and applicability to wide variety of detectors, SFC has now become an attractive alternative for chiral drug separation [36]. In addition, enantioseparation of chiral compounds has been attracting great interests these years [37–39]. This chapter focuses on the chiral separation performance of SFC, and the following pages will detail this subject.

4.4 Chiral Separation in SFC

Molecular chirality is necessary to be understood in both drug discovery and pharmaceuticals manufacturing, and it is a fundamental consideration in clinical use. As more drugs are developed in single-enantiomer form, the determination of

enantiomeric purity of starting materials, intermediates, and bulk drugs has become a necessity. Enantioselective chromatography has played an increasing role not only as an analytical tool for chiral analyses but also as a preparative technique to obtain pure enantiomers from racemates quickly from a wide diversity of chemical structures [40]. Although a large number of approaches have been used to isolate single enantiomers [28, 29, 41], enantioselective chromatography using SFC has become the most widely utilized technique in the context of obtaining limited quantities (from mg to multi-grams) of pure enantiomers quickly, particularly in drug discovery [42].

In chiral SFC, the majority of separations are performed by the direct approach, which uses a chiral selector to enable the enantioseparation. In this approach, two variants are applied: one selector is bonded onto a chromatographic support, and the other is present in the mobile phase. In the first variant, a column or a capillary, filled with chiral stationary phase (CSP), is used under SFC conditions. This is also the most frequently used variant in the literature. In the second variant, achiral column, 非手性 is used as stationary phase (SP), and the selector is added to the chiral mobile phase (CMP). The mobile phase in SFC consists of carbon dioxide combined with an organic modifier to change the polarity of the mobile phase and thus the elution behavior of the compounds in SFC. The chiral selector can be polysaccharide, macrocyclic antibiotic, polymeric, chiral crown ether, proteins, or other things, but cyclodextrins (CDs) are the most commonly used chiral selector in all kinds of enantioseparation methods.

Cyclodextrins (CDs) and their derivatives are the most extensively used chiral selectors in capillary electrophoresis (CE) [43, 44], HPLC [45, 46], as well as SFC [23, 47]. CDs are cyclic oligosaccharides consisting of at least six *D*-glucopyranose units bonded through α-1,4 linkages [48]. Each glucose unit that is part of the macrocyclic ring of native cyclodextrins has two secondary hydroxyl groups on C-2 and C-3 position and one primary hydroxyl group on C-6 position, so the exterior of cyclodextrins has a very good hydrophilic ability. Moreover, cyclodextrins have a special cavity structure, which can form inclusion complexes with analytes. The chiral recognition mechanisms of cyclodextrins as chiral selectors are believed to involve a combination of π–π, inclusion, hydrogen bonding, dipole stacking, and steric interactions. The hydroxyl groups can readily be derivatized by a wide variety of substituents [49]. Therefore, CD and its derivatives have been used for enantiomer separation frequently, both as a mobile phase additive and as a chiral stationary phase (CSP).

4.5 CD-CSPs for Chiral Separation in SFC

The application of packed-column SFC to enantiomeric separations was first reported by Mourier in 1985 [35]. Since the first commercialized CD-bonded CSP was introduced in the reversed-phase mode HPLC by Armstrong [50], a wide variety of derivatized CDs have been developed as multi-model CSPs (Table 4.4) [51, 52].

Table 4.4 Commercially available CD-CSPs [28, 40]

Chiral selector	Commercial name
α-Cyclodextrin	Cyclobond I [28]
β-Cyclodextrin	Cyclobond II [28]
	Chiraldex [28]
	Chiral β-dex [28]
γ-Cyclodextrin	Cyclobond II [28]
Acetylated α-cyclodextrin	Cyclobond III Ac [28]
Acetylated β-cyclodextrin	Cyclobond I Ac [28]
β-Cyclodextrin-derived (S)-2-hydroxy-propyl	Cyclobond I SP [28]
β-Cyclodextrin-derived 2-hydroxy-propyl (racemic)	Cyclobond I RSP [28]
β-Cyclodextrin-derived (S)-[1-(1-naphthyl)ethyl]carbamate	Cyclobond I SN [28]
β-Cyclodextrin-derived (R)-[1-(1-naphthyl)ethyl]carbamate	Cyclobond I RN [28]
β-Cyclodextrin-derived [1-(1-naphthyl)ethyl]carbamate (rac)	Cyclobond I RSN [28]
β-Cyclodextrin-derived 3,5-dimethylphenylcarbamate	Cyclobond I DMP [28]
β-Cyclodextrin-derived 4-methylphenylcarbamate	Cyclobond I PT [28]
Mono-6-O-pentenyl-β-CD, thioether linkage	β-Cyclose-6-OH-T [40]
Mono-6-O-pentenyl-β-CD, sulfone linkage	β-Cyclose-6-OH [40]
Mono-2-O-pentenyl-β-CD, thioether linkage	β-Cyclose-2-OH-T [40]
Mono-2-O-pentenyl-β-CD, sulfone linkage	β-Cyclose-2-OH [40]
Heptakis(2,3,6-*tri-O*-methyl)-β-cyclodextrin	Sumichiral OA-7500 [23]

Capillary column just like capillary GC column which has CDs and derivatives coated on the capillary wall. Successful applications in SFC using this selector coated on a CSP have also been reported [53, 54].

4.5.1 Commercial Packed Columns

By now, a lot of cyclodextrin-based commodity columns have been developed; Table 4.4 lists the most commonly used chiral columns [28, 40]. Enantiomeric separation of a variety of drugs and related compounds (ancymidol, coumachlor, ibuprofen, mephenytoin, tropicamide, verapamil, etc.) on Cyclobond I 2000 SN and Cyclobond I 2000 RN using SFC has been accomplished [21, 53, 55, 56]. Williams et al. [21, 53, 55] studied a lot of enantiomer compounds and their chiral separation on both Cyclobond I 2000 SN and Cyclobond I 2000 RN in 1990s, and he also compared the SFC chiral separation result with LC.

In 1996, Williams [53] first reported the use of the Cyclobond I 2000 SN in SFC enantioseparation. Table 4.5 lists the analytes (as shown in Fig. 4.3) and their chromatographic separation results that the enantiomers of both acidic and basic compounds were readily resolved. The influence of modifier, temperature, and pressure on the chromatographic process was also studied, as summarized in Tables 4.6, 4.7 and 4.8, respectively [53].

Table 4.5 Chromatographic results of compounds in Cyclobond I 2000 SN[a]

Compound	k'	α	R_s	Modifier (%)[b]
Ancymidol	6.32	1.08	1.3	10
Bendroflumethiazide	9.95	1.11	1.9	30
Benzoin	2.29	1.06	0.8	5
Coumachlor	17.54	1.05	0.7	15
Cromakalim	10.25	1.08	1.5	4
5-(4-Hydroxyphenyl)-5-phenyl hydantoin	29.75	1.06	0.8	15
Ibuprofen	6.14	1.06	1.0	5
Mephenytoin	3.15	1.25	3.0	5
Phensuximide	1.93	1.07	1.0	2
4-Pheny-2-oxazolidinone	7.07	1.08	1.2	5
Piperoxan	3.88	1.08	0.7	10
Proglumide	16.20	1.04	0.6	8
Suprofen[c]	20.55	1.06	0.7	20
Tropicamide[c]	12.48	1.15	2.1	10
Verapamil	10.28	1.05	1.0	10

[a]Capacity factor (k') for the first eluting enantiomer, $(t_1-t_0)/t_0$; selectivity (α), k'_2/k'_1; resolution (R_s), $2(t_2-t_1)/w_1+w_2$

[b]Percentage of modifier in carbon dioxide

[c]Ethanol used as the modifier

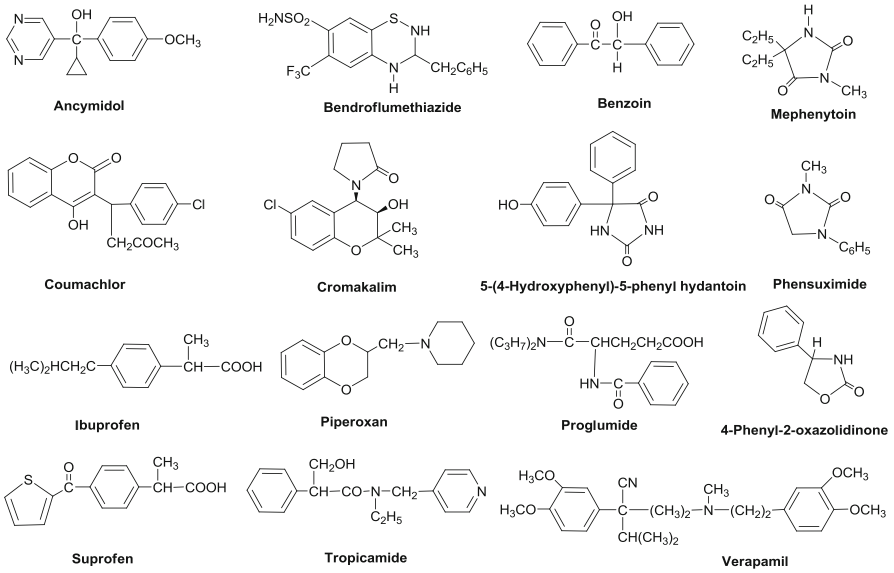


Fig. 4.3 Chemical structures of all analytes studied

Table 4.6 Influence of modifier on selectivity and resolution[a]

	Mephenytoin		Ancymidol		4-Phenyl-2-oxazolidinone	
% Modifier(MeOH)	α	R_s	α	R_s	α	R_s
2.5	1.32	3.9	–	–	1.11	1.4
5.0	1.21	2.9	1.10	1.4	1.07	1.1
7.5	1.16	2.3	1.09	1.2	1.06	0.9
10.0	1.15	1.9	1.07	1.1	1.05	0.7
12.5	1.13	1.7	1.06	1.0	1.04	0.6
15.0	1.12	1.5	1.06	0.9	1.04	0.6
17.5	–	–	1.06	0.8	–	–

[a]Conditions: mobile phase, CO_2/methanol; flow rate, 2.0 ml/min; temperature, 30 °C; pressure, 150 MPa

Table 4.7 Influence of temperature on retention, selectivity, and resolution[a]

	Mephenytoin			Ancymidol			Bendroflumethiazide		
Temperature	k'	α	R_s	k'	α	R_s	k'	α	R_s
25	2.44	1.20	2.8	5.75	1.08	1.2	8.94	1.14	2.0
30	2.56	1.21	2.8	5.79	1.07	1.1	8.43	1.13	2.0
35	2.82	1.20	2.9	6.10	1.07	1.1	8.19	1.12	1.9
40	3.23	1.20	3.0	6.26	1.06	1.0	7.74	1.11	1.8
45	3.45	1.20	2.9	6.63	1.05	0.9	7.89	1.10	1.7
50	3.81	1.19	2.9	7.14	1.05	0.8	8.05	1.09	1.6

[a]Conditions: mobile phase, CO_2/methanol; flow rate, 2.0 ml/min; pressure, 150 MPa

Table 4.8 Effect of pressure on retention, selectivity, and resolution[a]

Pressure	k'	α	R_s
100	3.18	1.20	2.8
125	2.94	1.20	2.9
150	2.65	1.21	2.9
175	2.51	1.21	2.9
200	2.29	1.21	2.8
225	2.19	1.21	2.8
250	2.13	1.22	2.8
275	2.03	1.21	2.7

[a]Conditions: CO_2/methanol (95:5); flow rate, 2.0 ml/min; temperature, 30 °C

It was demonstrated that several of the analytes in Table 4.5, such as 5-(4-hydroxyphenyl)-5-phenylhydantoin and suprofen, exhibited very strong retention in SFC. Because the polarity of carbon dioxide is comparable to hexane [57], the eluents used in SFC were substantially less polar than the mobile phases used in LC. Therefore, the large capacity factors observed for some analytes are not surprising. However, chiral resolution on the Cyclobond I 2000 SN can be performed with

a very simple eluent in SFC. Therefore, it is highly advantageous when exploring the applicability of the CSP for separation of a particular analyte.

Due to elevated modifier concentrations, the viscosity of the eluent was increased [53], so the selectivity factor (α) for mephenytoin decreases from 1.32 at 2.5 % methanol to 1.12 at 15 % methanol. Moreover the decrease in retention was accompanied by a slight decrease in selectivity at higher modifier concentrations. For mephenytoin and bendroflumethiazide, when the modifier proportion is 2.5 %, they obtained the best result. With the increasing of the modifier concentration, the chromatographic separation efficiency decreased.

Temperature is obviously a very important parameter for SFC separation process, and selectivity typically decreases as the temperature increases [17]. Increases in retention at higher temperatures are probably caused by the reduced density of the supercritical fluid. Although the highest selectivity was observed at low temperature, little variation in enantioselectivity was actually observed within the temperature range investigated. As shown in Table 4.7, resolutions of ancymidol and bendroflumethiazide decreased when the temperature decreased, but mephenytoin was not influenced by the temperature, and its resolution value increased contrarily.

Pressure is another key factor in SFC. Table 4.8 shows the effect of pressure on the chromatographic behavior of mephenytoin. Although increases in pressure reduced retention, little change in selectivity or resolution was observed within the range examined while retention is obviously decreased. Retention is linked with the increased density of the mobile phase, when at higher pressures the eluent strength increased, which caused the decrease of the retention [58].

Williams et al. [22] completed a good comparison study of SFC and LC for the enantioseparation using Cyclobond I 2000 SN CSP and Cyclobond I 2000 RN CSP. The chromatographic separation difference of Cyclobond I 2000 SN CSP and Cyclobond I 2000 RN CSP on SFC was studied, and their comparison with LC was also discussed. The advantages of SFC for chiral separations were strengthened on these CSPs including simple eluents, rapid optimization of selectivity, and improved resolution compared to LC results for the same columns. For SFC, the concentration of methanol modifier was 10 %. Table 4.9 provides a summary of the results comparing LC and SFC on the Cyclobond I 2000 SN CSP column. α values and R_s for all the compounds are obtained in both SFC and LC, but the results of SFC are obviously better than LC. The article also reported that the SFC process is timesaving and the mobile phase in SFC is CO_2 with little MeOH added, which is environment friendly.

Williams et al. [55] also found that in LC the elution order is often in reverse to that in the normal-phase mode when switching from the Cyclobond I 2000 RN CSP column to the Cyclobond I 2000 SN CSP column, so a comparison of elution order for the two columns was conducted in SFC. The chromatographic results are tabulated in Table 4.10. A comparison of LC and SFC results for underivatized agricultural and pharmaceutical compounds on the Cyclobond I 2000 SN CSP and Cyclobond I 2000 RN CSP is shown in Table 4.11. Selectivities of the Cyclobond I 2000 RN CSP column and the Cyclobond I 2000 SN CSP column in SFC are summarized in Table 4.12. The chromatographic results about the polar organic

Table 4.9 Comparison of chromatographic results for *N*-(3,5-dinitrobenzoyl) derivatized analytes in LC and SFC on the Cyclobond I 2000 SN CSP [22]

Compound	Method	k'^{a}	α	R_s	Mobile phase
Alanine methyl ester	LC	4.12(D)	1.49	3.7	70:30[b]
	SFC	4.23(D)	1.31	4.7	90:10[c]
Alanine ethyl ester	LC	2.70(D)	1.60	4.2	70:30
	SFC	3.78(D)	1.31	4.6	90:10
Norleucine methyl ester	LC	2.25	1.65	4.4	70:30
	SFC	3.55	1.31	4.6	90:10
Valine methyl ester	LC	2.58(D)	1.79	4.9	70:30
	SFC	2.80(D)	1.43	5.8	90:10
Phenylalanine methyl ester	LC	4.10(D)	1.29	2.3	60:40
	SFC	5.11(D)	1.25	4.0	85:15
4-Chlorophenylalanine ethyl ester	LC	3.13	1.25	2.0	60:40
	SFC	5.08	1.14	2.3	85:15
2-Aminoheptane	LC	6.55	1.17	1.2	90:10
	SFC	9.27	1.14	2.6	95:5
1-Cyclohexylethylamine	LC	3.60(R)	1.23	1.7	80:20
	SFC	6.73(R)	1.45	5.9	90:10
α-Methylbenzylamine	LC	3.29(R)	2.10	6.8	70:30
	SFC	3.55(R)	1.56	7.8	80:20
1,2,3,4-Tetrahydro-1-naphthylamine	LC	2.15	1.92	5.0	70:30
	SFC	3.51	1.46	6.4	80:20

[a]Configuration of the first eluting enantiomer shown in parentheses
[b]Volume ratios of hexane: 2-propanol for LC mobile phases
[c]Volume ratios of CO_2: methanol for SFC mobile phases

Table 4.10 Comparison of chromatographic results in SFC for *N*-(3,5-dinitrobenzoyl)-derivatized compounds on the Cyclobond I 2000 RN CSP (RN) and Cyclobond I 2000 SN CSP (SN)

Compound	CSP	k'^{a}	α	R_s	% Modifier[b]
Alanine methyl ester	RN	6.029 (L)	1.21	5.2	10
	SN	4.23 (D)	1.31	4.7	10
Alanine ethyl ester	RN	5.22(L)	1.18	4.6	10
	SN	3.78(D)	1.31	4.6	10
Leucine methyl ester	RN	4.51(L)	1.27	5.8	10
	SN	2.79 (D)	1.29	4.1	10
Valine methyl ester	RN	3.73(L)	1.37	8.3	10
	SN	2.80 (D)	1.43	5.8	10
Phenylalanine methyl ester	RN	7.22(L)	1.31	6.3	15
	SN	5.11 (D)	1.25	4.0	15
1-Cyclohexylethylamine	RN	9.44(L)	1.64	11.7	10
	SN	6.73 (D)	1.45	5.9	10
α-Methylbenzylamine	RN	5.41(L)	1.28	5.6	20
	SN	3.55 (D)	1.56	7.8	20

[a]Configuration of the first eluting enantiomer shown in parentheses
[b]Volume percentage of methanol in mobile phase; flow rate, 2.0 ml/min; temperature, 30 °C; pressure, 15 MPa

Table 4.11 Comparison of chromatographic results for SFC and reversed-phase LC on the Cyclobond I 2000 RN CSP (R) and Cyclobond I 2000 SN CSP (S) [22]

Compound	CSP		k'	α	R_s	Mobile phase
Ancymidol	SN	LC	4.72	1.14	1.3	80:20(7.0)[a]
	SN	SFC	6.32	1.08	1.3	90:10[b]
Bendroflumethiazide	SN	LC	6.36	1.22	1.9	70:30(4.5)[a]
	SN	SFC	9.95	1.11	1.9	70:30[b]
Cromakalim	SN	LC	2.19	1.00	0.0	80:20(4.5)[a]
	SN	SFC	10.25	1.08	1.5	96:4[b]
5-(4-Hydroxyphenyl)-5-phenylhydantoin	RN	LC	8.51	1.10	0.7	80:20(4.5)[a]
	RN	SFC	36.24	1.15	1.5	85:15[b]
Ibuprofen	SN	LC	3.26	1.14	0.6	70:30(4.5)[a]
	SN	SFC	6.14	1.06	1.0	95:5[b]
Mephenytoin	SN	LC	1.29	1.22	1.3	70:30(4.1)[a]
	SN	SFC	3.15	1.25	3.0	95:5[b]
Piperoxan	SN	LC	1.20	1.15	0.6	80:20(4.5)[a]
	SN	SFC	3.88	1.08	0.7	90:10[b]
Tolperisone	SN	LC	1.63	1.11	0.9	80:20(4.5)[a]
	SN	SFC	6.77	1.00	0.0	90:10[b]
Tropicamide	SN	LC	1.56	1.22	1.1	70:30(4.5)[a]
	SN	SFC	12.48	1.15	2.1	90:10[b]

[a]Volume ratios of triethylammonium acetate buffer-acetonitrile for LC mobile phases; pH given in parentheses

[b]Volume ratios of CO_2/MeOH for SFC mobile phases

composition are listed in Table 4.13. The mobile phase compositions and chromatographic conditions in Table 4.13 for LC and SFC yielded optimum selectivity and resolution for the compounds examined.

In Table 4.10, the elution order of all the compounds on the Cyclobond I 2000 RN CSP was the opposite of that observed on the Cyclobond I 2000 SN CSP, which corresponded with elution order patterns for these compounds have been observed in LC [55]. The chromatographic separation results in Table 4.10 show that all compounds were well separated, while Cyclobond I 2000 RN CSP gained better separation data [56]. Examination of the data in Table 4.11 reveals that retention, measured by the capacity factor (k'), was higher in SFC than in LC for the compounds studied [57]; the structures of these compounds are shown in Fig. 4.3. An extreme case of this difference of retention is illustrated by the chromatographic data for 5-(4-hydroxyphenyl)-5- phenylhydantoin. Retention in SFC ($k' = 36.24$) was much longer than in LC ($k' = 8.51$). Carbon dioxide is nonpolar, so the eluents in SFC were substantially less powerful than the mobile phases used in LC. In Table 4.11, the resolution value and α value are also higher in SFC than LC, showing a better chiral separation of SFC. The composition of the mobile phase used in SFC is simpler than that used in LC.

In Table 4.12, Cyclobond I 2000 RN CSP column exhibited higher enantioselectivity than the Cyclobond I 2000 RN CSP column. In fact, three of the compounds in Table 4.12 were only resolved on the Cyclobond I 2000 RN CSP column. The

Table 4.12 SFC separation of underivatized compounds on the Cyclobond I 2000 RN CSP and Cyclobond I 2000 SN CSP [22]

Compound	CSP	k'	α	R_s	% Modifier[a]
Ancymidol	RN	6.90	1.09	1.3	10
	SN	6.32	1.08	1.3	10
Bendroflumethiazide	RN	9.11	1.00	0.0	30
	SN	9.95	1.11	1.9	30
Benzoin	RN	2.70	1.04	0.9	5
	SN	2.29	1.06	0.8	5
Cromakalim	RN	13.29	1.03	0.7	4
	SN	10.25	1.08	1.5	4
Ibuprofen	RN	5.81	1.04	0.5	5
	SN	6.14	1.06	1.0	5
Mephenytoin	RN	4.02	1.09	1.2	5
	SN	3.15	1.25	3.0	5
4-Phenyl-2-oxazolidinone	RN	9.16	1.05	0.6	5
	SN	7.07	1.08	1.2	5
Piperoxan	RN	4.78	1.00	0.0	10
	SN	3.88	1.08	0.7	10
Tropicamide[b]	RN	14.66	1.10	1.3	10
	SN	12.48	1.15	2.1	10
Verapamil	RN	19.29	1.00	0.0	10
	SN	10.28	1.05	1.0	10

[a]Volume percentage of MeOH in mobile phase; flow rate, 2.0 ml/min; temperature, 30 °C; pressure, 15 MPa
[b]Ethanol used as the modifier

Table 4.13 Comparison of SFC and polar organic LC on the Cyclobond I 2000 RN CSP [22]

Compound		k'	α	R_s	Mobile phase
2-(4-Chlorophenoxy)-propionic acid	LC	0.87	1.18	2.1	95:5:0.6:0.4[a]
	SFC	30.90	1.14	2.0	80:20[b]
Coumachlor	LC	0.33	1.27	1.5	98:2:9.8:0.6[a]
	SFC	19.99	1.06	1.1	85:15[b]
Proglumide	LC	1.07	1.19	1.8	95:5:0.8:0.6[a]
	SFC	15.44	1.10	1.9	92:8[b]
Suprofen[c]	LC	3.23	1.10	1.0	95:5:0.2:0.2[a]
	SFC	21.50	1.05	0.6	80:20[b]

[a]Volume ratios of acetonitrile-methanol-acetic acid-triethylamine for LC mobile phases
[b]Volume ratios of CO_2/MeOH in SFC
[c]Ethanol used as the modifier for SFC

nonequivalent selectivities of the Cyclobond I 2000 RN CSP and Cyclobond I 2000 RN CSP in SFC suggest that analogous chiral recognition processes are available in SFC. The comparison of chromatographic separation results in polar organic LC and SFC on the Cyclobond I 2000 RN CSP is listed in Table 4.13 [22]. The analytes were well separated except suprofen. Comparisons between LC and SFC demonstrated

Table 4.14 Enantioseparation chromatographic results for *N*-(3,5-dinitrobenzoyl) derivatized analytes in LC and SFC on the Cyclobond I 2000 SN CSP [22]

Compound		k'	α	R_s	Mobile phase
Alanine methyl ester	LC	4.12	1.49	3.7	70:30[a]
	SFC	4.23	1.31	4.7	90:10[b]
Norleucine methyl ester	LC	2.25	1.65	4.4	70:30
	SFC	3.55	1.31	4.6	90:10
Valine methyl ester	LC	2.58	1.79	4.9	70:30
	SFC	2.80	1.43	5.8	90:10
Phenylalanine methyl ester	LC	4.10	1.29	2.3	60:40
	SFC	5.11	1.25	4.0	85:15
2-Aminoheptane	LC	6.55	1.17	1.2	90:10
	SFC	9.27	1.14	2.6	95:5
1-Cyclohexylethylamine	LC	3.60	1.23	1.7	80:20
	SFC	6.73	1.45	5.9	90:10
α-Methylbenzylamine	LC	3.29	2.10	6.8	70:30
	SFC	3.55	1.56	7.8	80:20
1,2,3,4-Tetrahydro-1-naphthylamine	LC	2.15	1.92	5.0	70:30
	SFC	3.51	1.46	6.4	80:20
Ancymidol	LC	4.72	1.14	1.3	80:20
	SFC	6.32	1.08	1.3	90:10[b]
Cromakalim	LC	2.19	1.00	0.0	80:20
	SFC	10.25	1.08	1.5	96:4
Ibuprofen	LC	3.26	1.14	0.6	70:30
	SFC	6.14	1.06	1.0	95:5
Mephenytoin	LC	1.29	1.22	1.3	70:30
	SFC	3.15	1.25	3.0	95:5
Piperoxan	LC	1.20	1.15	0.6	80:20
	SFC	3.88	1.08	0.7	90:10
Tolperisone	LC	1.63	1.11	0.9	80:20
	SFC	6.77	1.00	0.0	90:10
Tropicamide[c]	LC	1.56	1.22	1.1	70:30
	SFC	12.48	1.15	2.1	90:10

[a]Volume ratios of hexane:2–propanol for LC mobile phases
[b]Volume ratios of CO_2/MeOH for SFC mobile phases
[c]Ethanol used as the modifier for SFC

that Cyclobond I 2000 RN CSP and Cyclobond I 2000 SN CSP are both excellent chiral separation substances. Although selectivity was sometimes lower in SFC than in LC, the improved efficiency in SFC obtained a higher resolution in SFC than in LC. Most of the compounds investigated were resolved using a CO_2/MeOH eluent. The alcohol modifier played an important role in enantioselectivity in SFC, and the nature of this role was not the same for all analytes.

In 1991, Armstrong et al. [54] reported the comparison of Cyclobond I 2000 SN CSP and Cyclobond I 2000 RN CSP in the chiral separation. Furthermore, chromatographic results in LC were also studied, and they proved the advantage of SFC that the column equilibration and parameter optimization were generally accomplished more rapidly in SFC than in LC. Table 4.14 lists comparisons of

chromatographic results for SFC and reversed phase LC on the Cyclobond I 2000 SN CSP [22]. Resolution of cromakalim was not obtained on the (S)-naphthylethylcarbamoylated-β-cyclodextrin CSP using LC but was readily accomplished using SFC. The separation of the enantiomers of *N*-(3,5-dinitrobenzoyl)valine methyl ester, ancymidol, and proglumide was also obtained in a single run using carbon dioxide–methanol eluent, whereas the same separations in LC required three different mobile phases.

As shown in Table 4.14, compounds resolved on the Cyclobond I 2000 RN and SN CSPs under normal-phase conditions in LC were also resolved in SFC. It provides a summary of chromatographic data of LC and SFC. With the exception of the results for 1-cyclohexylethylamine, selectivities in SFC were lower than those observed by LC for the compounds. However, the decrease in selectivity was offset by an increase in resolution relative to LC. Separation of the enantiomers of *N*-(3,5-dinitrobenzoyl)-DL-valine methyl ester on the Cyclobond I SN CSP is illustrated in Table 4.14; selectivity was lower in SFC ($\alpha = 1.43$) than in LC ($\alpha = 1.79$). The discrepancies in selectivity observed for cromakalim and tolperisone (Table 4.14) indicate that LC and SFC cannot be considered interchangeable for the compounds examined. However, SFC provides a rapid and convenient method of evaluating CSPs for a desired separation. Elimination of aqueous buffers in the eluent system is also likely to extend column life.

The alcohol modifier played an important role in enantioselectivity in SFC, and the nature of this role was not the same for all analytes. Investigation of different alcohol modifiers can provide an insight into the similarities between the chiral recognition mechanisms in SFC and the reversed-phase mode in LC. The effect of three different alcohol modifiers on the enantioresolution of piperoxan in SFC is shown in Fig. 4.4, where the best separation was obtained with 10 % addition of MeOH. Enantioselectivity was reduced significantly when ethanol was used as a modifier and no separation was achieved with 2-propanol. The more hydrophobic alcohols interact more strongly with the nonpolar CD cavity and may block inclusion of the analyte. Therefore, it appears that inclusion complexation, which is known to be important in the reversed-phase mode in LC, may also contribute to enantioseparation in SFC of the compounds.

The enantioseparation chromatographic results for SFC on the Cyclobond I 2000 RN CSP are shown in Table 4.15. Substantial differences in retention for the two techniques were observed, retention rates of 2-(4-chlorophenoxy)-propionic acid and Coumachlor were extremely high, and resolution values were also good.

The tremendous versatility of the NEC-CD-CSPs in SFC is highlighted by the chromatogram in Fig. 4.5 [22]. The separation of the enantiomers of N-(3,5-dinitrobenzoyl)-valine methyl ester, ancymidol, and proglumide was achieved in a single run using a carbon dioxide–methanol eluent with 25 mins. The same separations in LC would require three different mobile phases.

Kasai et al. [59] studied heptakis(2,3,6-*tri*-*O*-methyl)-β-cyclodextrin (Sumichiral OA-7500) to separate three different racemates:neutral *rac*-*α*-tetralol, acidic *rac*-*2*-*phenylpropionic* acid, and basic *rac*-1-phenylethylamine in SFC. The influence of experimental conditions on the chiral separation like the type of alcohol modifier, column oven temperature, mobile phase composition, flow rate, and pressure was

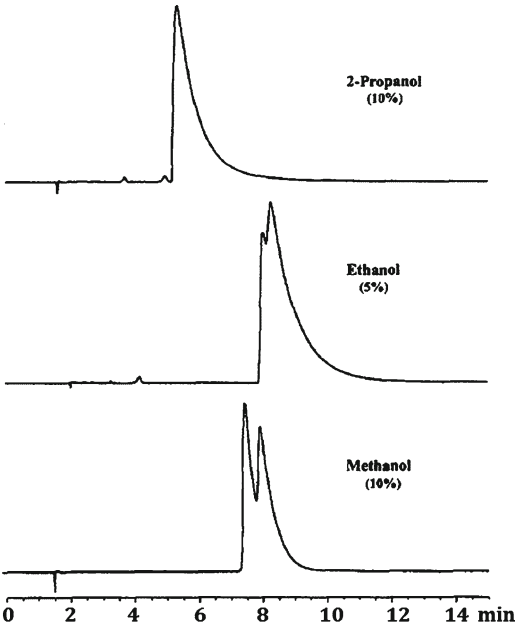


Fig. 4.4 Effect of modifier on the separation of piperoxan on the S-NEC-CD-CSP. Chromatographic conditions, carbon dioxide alcohol, 2.0 ml/min, 30 °C, 15 MPa (Reprinted from Ref. [22], Copyright 1996, with permission from Elsevier)

Table 4.15 Enantioseparation chromatographic results for SFC on the Cyclobond I 2000 RN CSP [22]

Compound	k'	α	R_s	Mobile phase
2-(4-Chlorophenoxy)-propionic acid	30.90	1.14	2.0	80:20[a]
Coumachlor	19.99	1.06	1.1	85:15
Proglumide	15.44	1.10	1.9	92:8
Suprofen[b]	21.50	1.05	0.6	80:20

[a]Volume ratios of CO_2/MeOH for SFC mobile phases
[b]Ethanol used as the modifier for SFC

fully examined. It was reported that lower alcohol content improved the separation of *rac-α*-tetralol, and decrease outlet pressure improved the separation of *rac-2-phenylpropionic* acid. Moreover, he compared the chiral separation ability of Sumichiral OA-7500 with the common used tris(3,5-dimethylphenylcarbamate) of amylose column (Chiralpak AD-H) and obtained optimal conditions and separation parameters of the chiral separation of each racemic compound. The chemical structures of racemates are shown in Fig. 4.6 as well as two CSPs, and Table 4.16 summarizes the chiral separation result of the three analytes on the Sumichiral OA-7500 and Chiralpak AD-H.

In Table 4.16, we can find that *rac-α*-tetralol was achieved on both the Sumichiral OA-7500 and Chiralpak AD-H columns, and 2-PrOH-modified CO_2 was appropriate to be used as the eluent. On the Sumichiral OA-7500 column, the α and R_s values obtained with sub-SFC were higher than those obtained with SFC, while SFC was

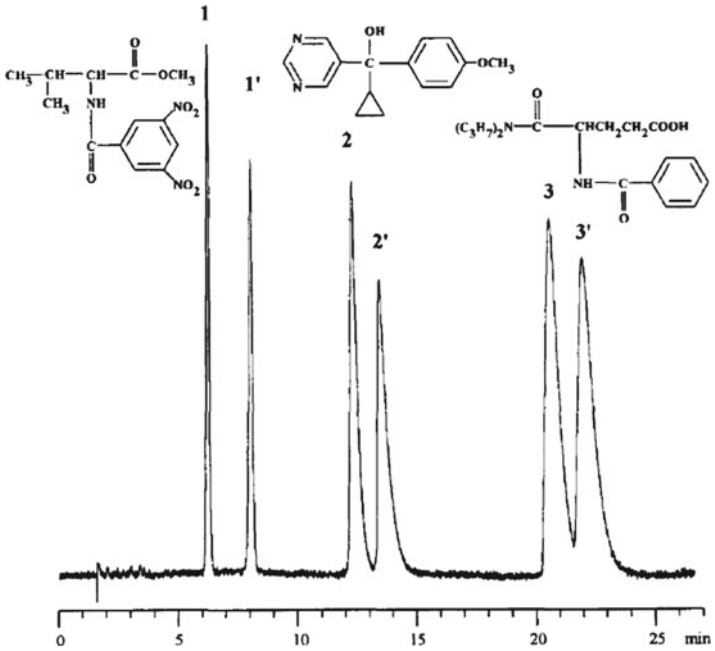


Fig. 4.5 Separation of N-(3,5-dinitrobenzoyl)-DL-valine methyl ester (**1** and **1′**), ancymidol (**2** and **2′**), and proglumide (**3** and **3′**) on the Cyclobond I 2000 RN CSP. Chromatographic conditions, carbon dioxide methanol (90:10), 2.0 ml/min, 30 °C, 15 MPa (Reprinted from Ref. [22], Copyright 1996, with permission from Elsevier)

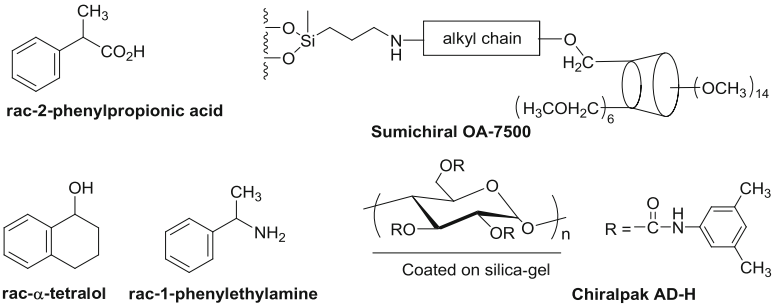


Fig. 4.6 Structure of the three racemates and the two chiral stationary phases

more appropriate for the Chiralpak AD-H column. The α values obtained on the Chiralpak AD-H column were higher than those on the Sumichiral OA-7500 column. Enantiomeric separation of rac-2-phenylpropionic acid was achieved with MeOH-modified CO_2 eluent on the Chiralpak AD-H column with SFC. 2-PrOH containing 0.1 % DEA-modified CO_2 eluent on the Chiralpak AD-H column resulted in improved peak separation of rac-1-phenylethylamine with sub-FC. The Sumichiral OA-7500 column did not separate enantiomers of either rac-2-phenylpropionic acid or

Table 4.16 The optimal conditions and enantioseparation parameters of the racemates using SFC

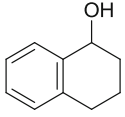
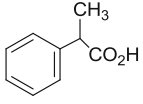
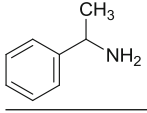
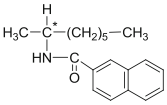
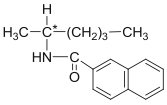
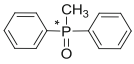
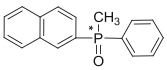
Compound	Column	Alcohol% (v/v) in CO_2	Column temp. (°C)	Flow rate (ml/min)	Pressure (MPa)	t_1 (min)	t_1 (min)	k_1	k_2	α	R_s
	Sumichiral OA-7500	2 % 2-PrOH	25	5	9.8	3.8	4.2	2.8	3.2	1.1	2.2
	Chiralpak AD-H	6 % 2-PrOH	40	4	13.7	5.9	7.4	4.9	6.4	1.3	7.5
	Chiralpak AD-H	4 % MeOH	40	5	7.9	3	3.1	1.7	1.9	1.1	1
	Chiralpak AD-H	4 % PrOH with 0.1 % DEA	25	5	11.8	11	20	13.6	25.5	1.4	2.4

Table 4.17 Comparison of chiral separation results for enantiomeric amides and phosphine oxides in SFC [58]

Solute	α	R_s	$t_{analysis}$	CSP
	1.056	1.20	5.30	β-CD-bonded phase (CO_2/methanol = 94:6)
	1.086	1.60	6.71	Pirkle phase (CO_2/2-propanol = 95:5)
	1.064	1.30	4.83	β-CD-bonded phase (CO_2/methanol = 94:6)
	1.071	1.29	6.60	Pirkle phase (CO_2/2-propanol = 95:5)
	1.11	1.75	12.55	β-CD-bonded phase (CO_2/methanol = 94:6)
	1.00	–	13.20	Pirkle phase (CO_2/2-propanol = 95:5)
	1.09	1.30	20.58	β-CD-bonded phase (CO_2/methanol = 94:6)
	1.00	–	20.51	Pirkle phase (CO_2/2-propanol = 95:5)

For β-CD-bonded phase: flow rate, 4.5 ml/min; average column pressure, 150 bar
For pirkle phase: flow rate, 4.0 ml/min; average column pressure, 230 bar
Other conditions: temperature, 25 °C; detection, 234 nm

rac-1-phenylethylamine under the conditions used in this study. The chromatographic results obtained in this study provide additional insight into the usefulness of SFC for chiral separation.

All the commercial columns are packed columns, and they show excellent chiral separation ability and have wide applications [53]. Actually, these kinds of columns were ever used in HPLC, but they are also applicable in SFC; it is the technology development trend of SFC columns. Therefore, a lot of packed columns and their use in chiral separation of racemate drugs by SFC have been reported [17]. Cyclodextrin chiral stationary phase-based packed columns no doubt become the focus of research.

4.5.2 Self-Made Packed Columns

The SFC chiral separation studies we discussed above are all based on commercial columns, and self-made native cyclodextrin-based chiral stationary phases are also generally used in SFC; Macaudiere et al. [58] reported the enantioseparation of racemic amides and phosphine oxides on a β-CD-CSP. This is the first example of cyclodextrins stationary phase using for the resolution of racemics. The chiral separation in SFC using a β-CD-bonded phase was reported and compared those separation results with the other pirkle phase column. As expected, β-CD-bonded phase showed a superior result than pirkle phase, like its higher stereoselectivity, less resolution time, and less analysis time. Table 4.17 gives the comparison of SFC separation

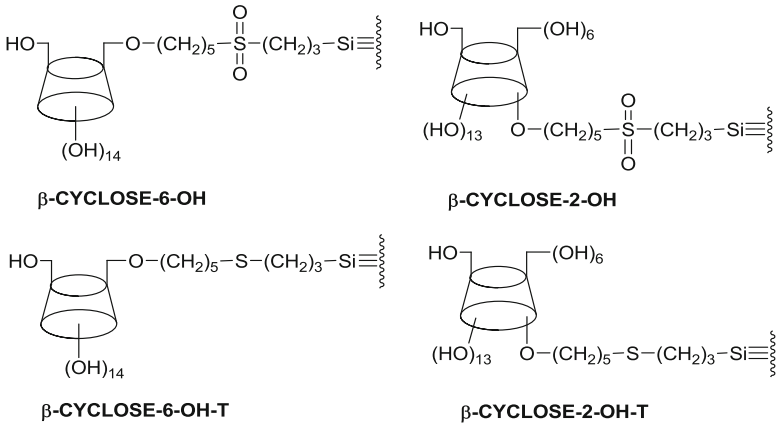


Fig. 4.7 The structures of the four mono-2 and mono-6 substituted β-CDs-based CSPs

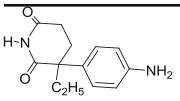
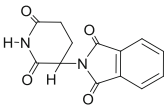
results in the above two chiral stationary phases, and (*R*)-*N*-(3,5-dinitrobenzoyl) phenylglycine was used as the pirkle phase.

A comparison of the data presented in Table 4.17, β-CD-bonded phase was demonstrated as an excellent chiral selector. The separation process in β-CD-bonded phase column is timesaving and effective. The attempt leads to the research of this kind of CD, and its derivatives based chiral stationary phase and their application in chiral separation process in SFC. The values of the analytes are larger in β-CD-bonded phase column than in pirkle phase column except the first one. In addition, the separation processes in β-CD-bonded phase are much faster than those in pirkle phase column. All these four racemates have at least one benzene ring which can form complex with CD's hydrophobic cavity. Due to the impressed inclusion activity, chiral separation result was improved. Besides, size of β-CD is more suitable for compounds containing benzyl ring, as indicated by the best chromatographic separation results obtained for the third solute.

Duval et al. [60] exploited four kinds of mono-2 and mono-6-pentenyl-β-cyclodextrin-based chiral stationary phases and studied the chiral separation of aminoglutethimide and thalidomide by HPLC and SFC. These CD-CSPs were synthesized by linking four cyclodextrins through covalent bond to mercaptopropyl silica gel (thiol-Si) based on thioether or sulfone linkage. The structures of these four kinds of CD-CSPs are shown in Fig. 4.7, and their chiral separation results in SFC and HPLC are shown in Table 4.18.

Table 4.18 shows that mono-2 and mono-6-CD-CSPs possess different enantioselectivities; β-Cyclose-2-OH-T and β-Cyclose-2-OH only show very small α values a little higher than 1.0 for aminoglutethimide, but they are efficiently resolved on both β-Cyclose-6-OH-T and β-Cyclose-6-OH with the α value of 1.44 and 1.25, respectively. The result shows that the thioether functional group in the spacer arm is more powerful in enantiorecognition than the sulfone. It is also surprising to find that

Table 4.18 Comparison of SFC and HPLC separation results for aminoglutethimide and thalidomide [60]

Solute		β-Cyclose - 6-OH	β-Cyclose- 2-OH	β-Cyclose- 6-OH-T	β-Cyclose- 2-OH-T
 Aminoglutethimide	SFC[a]	$\alpha = 1.002$	$\alpha = 1.44$	$\alpha = 1.002$	$\alpha = 1.28$
	HPLC[b]	$\alpha = 1.00$	$\alpha = 1.00$	–	$\alpha = 1.00$
 Thalidomide	SFC[a]	$\alpha = 1.00$	$\alpha = 1.00$	$\alpha = 1.00$	$\alpha = 1.00$
	HPLC[b]	$\alpha = 1.00$	$\alpha = 1.04$	$\alpha = 1.12$	$\alpha = 1.08$

[a]CO_2/methanol (0.2 % DEA/isocratic 70/30) = 70:30, v/v; flow rate, 3 ml/min; △P, 15 bar; UV detection at 254 nm; 30 °C

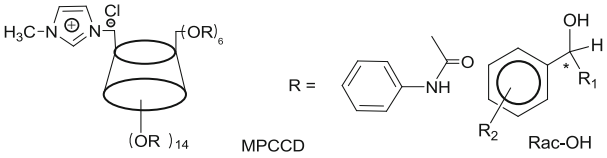
[b]TEAA(0.1 %, v/v, pH 5.0)/MeOH = 30:70,v/v; flow rate, 1 ml/min; △P, 115 bar; UV detection at 254 nm; AUFS, temperature, 25 °C

thalidomide cannot be separated in theses CD-CSPs. HPLC separation results show that aminoglutethimide is not separated; maybe it is due to the strong ionic interactions between the sulfates groups present on the CSP and the protonated amino group, in aqueous acidic buffers, of the aminoglutethimide. However, thalidomide shows a good α value of 1.12 in HPLC but 1.0 in SFC for all the four CD-CSPs. So in this study, HPLC shows a better separation for thalidomide than SFC.

Recently, Ng and co-workers [46, 61–66] have reported the syntheses of cationic cyclodextrins containing imidazolium, pyridinium, or ammonium substituted-β-CDs derivatives and their applications as chiral selectors or chiral additives. These cationic chiral additives have demonstrated efficient enantioseparations for phenyl hydroxyl acids, phenyl carboxylic acids, and dansyl amino acids. They also developed a series of cationic β-cyclodextrin derivatives, which were physically coated onto or chemically bonded to porous spherical silica gel to obtain novel chiral stationary phases (CSPs) [41, 67, 68].

A cationic CD derivative, mono-6-(3-methylimidazolium)-6-deoxy-erphenylcarbamoyl-β-cyclodextrin chloride (MPCCD), was synthesized and physically coated onto silica gel at varying weight percentages (%, w/w) by Ong et al. [41]. The chiral columns consisting 15, 20, and 35 % (w/w) of MPCCD are named as MPCCD-C15, MPCCD-C20, and MPCCD-C35, respectively. These three CD-CSPs were used as chiral selectors to pack the chiral columns, and the enantioseparation abilities of these columns were tested using ten racemates of aromatic alcohols as chiral analytes by HPLC and SFC. These three columns were studied in order to investigate the effect of loading of MPCCD on enantioseparation. Further studies on the column's chiral separation ability on SFC using an MPCCD-C20 column were also conducted. The structure of MPCCD and the ten enantiomers are shown in Table 4.19.

Table 4.19 Structure of MPCCD and racemic aryl alcohols



Analyte	R_1	R_2
Rac-OH-1	CH_3	*p*-F
Rac-OH-2	CH_3	*p*-Cl
Rac-OH-3	CH_3	*p*-Br
Rac-OH-4	CH_3	*p*-I
Rac-OH-5	CH_3	H
Rac-OH-6	CH_2-CH_2=CH_2	*p*-F
Rac-OH-7	CH_2-CH_2=CH_2	*p*-C_6H_5
Rac-OH-8	CH_2-CH_2=CH_2	*p*-CF_3
Rac-OH-9	CH_2-CH_2=CH_2	*p*-Br
Rac-OH-10	CH_2-CH_2=CH_2	*m*-F

MPCCD-C15 column and MPCCD-C35 column were used for enantioseparation studies of racemic aryl alcohols using HPLC, and only MPCCD-C20 column was used for both SFC and HPLC. Table 4.20 lists the chromatographic separation results in SFC and HPLC. It can be observed from the data in the table that MPCCD-C20 column has better separation ability under the HPLC condition. In addition, R_s values of these 10 racemate are all more than 1.0, and the R_s values of Rac-OH-1and Rac-OH-2 are even larger than 5.0. For SFC, the separation results are not that excellent, only half of these ten racemics are well separated, and Rac-OH-4 shows the biggest R_s value of 3.28. Further studies were conducted for the MPCCD-based CD-CSPs, and better enantioseparation was bound to achieve. Among the three CSPs being studied, MPCCD-C20 gave the best resolution ability. This indicated that the best loading concentration to attain the best resolution ability should be approximately between 20 and 35 % (w/w).

On the basis of previous study of Ong, Wang et al. [41] synthesized another three kinds of CDs: mono-6-(3-methylimidazolium)-6-deoxyper(3,5-dimethylphenyl carbamoyl)-β-cyclodextrin chloride (MDPCCD), mono-6-(3-octylimidazolium)-6-deoxy-perphenylcarbamoyl-β-cyclodextrin chloride (OPCCD), and mono-6-(3-octylimidazolium)-6-deoxy-per(3,5-dimethylphenylcarbamoyl)-β-cyclodextrin chloride (ODPCCD). Their structures are similar to former mentioned MPCCD; the synthesis process is also the same. The three new cyclodextrins were also coated to silica gels to get the CD-CSPs. The synthesis route for the novel cationic functionalized β-CDs is shown in Fig. 4.8, and for structures refer to Table 4.21.

The performances of these CSPs were studied on HPLC and SFC using 18 racemic aryl alcohols as test analytes, and Rac-OH-1 to Rac-OH-10 are as the same in literature [46]. Chromatographic separation results in Table 4.22 are not very good; α and R_s are all higher in LC than in SFC. Rac-OH-4 obtained the best separation result with α=2.26, 2.15 in HPLC and SFC, while 4.02, 4.13, respectively. Herein, analytes having electron

Table 4.20 Separation results obtained for racemic aromatic alcohols using MPCCD-C20 under HPLC and SFC

	HPLC[a]				SFC[b]			
Analytes	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Rac-OH-1	2.85	4.18	1.46	5.65	3.42	4.24	1.24	0.85
Rac-OH-2	3.21	5.18	1.61	5.16	6.22	8.42	1.35	1.06
Rac-OH-3	3.78	6.89	1.82	3.83	8.14	13.64	1.68	1.83
Rac-OH-4	3.86	8.10	2.09	4.63	12.28	24.98	2.03	3.28
Rac-OH-5	3.34	5.87	1.76	2.61	3.15	–	–	–
Rac-OH-6	1.59	2.79	1.75	5.11	2.15	2.74	1.28	0.55
Rac-OH-7	1.84	2.09	1.13	1.25	9.36	11.50	1.23	0.80
Rac-OH-8	1.69	7.57	4.49	3.59	2.16	4.99	2.31	1.21
Rac-OH-9	3.91	7.66	1.96	2.81	6.28	12.65	2.02	1.84
Rac-OH-10	1.47	2.87	1.94	3.48	1.99	2.71	1.36	0.65

[a]Mobile phases: *n*-hexane/2-propanol (97/3, v/v); flow rate of 1 ml/min; temperature at 25 °C
[b]Mobile phases; CO_2/2-propanol (97/3, v/v); flow rate of 3 ml/min; back pressure at 17.0 MPa; temperature at 40 °C

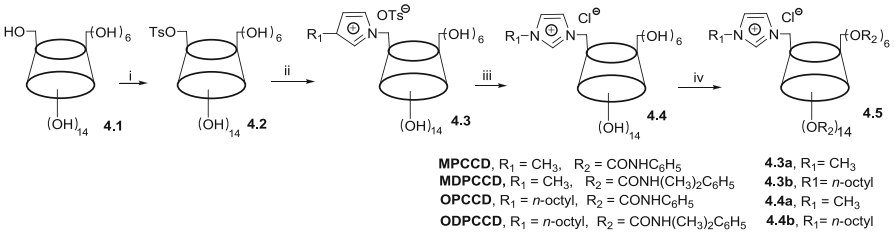


Fig. 4.8 Synthetic route for the novel cationic functionalized β-CDs. Conditions: (i) tosylimidazole, rt for 3 h; (ii) methylimidazole or *n*-octylimidazole, reacted at 90 °C for 48 h; (iii) amberlite resin; (iv) phenylisocyanate or 3,5-dimethylphenylisocyanate reacted at 85 °C for 18 h (Reprinted from Ref. [41], Copyright 2008, with permission from Elsevier)

Table 4.21 Chemical structures of the four cationic functionalized β-cyclodextrins

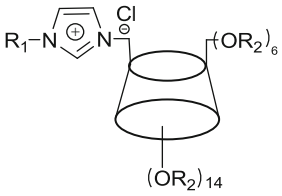
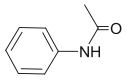
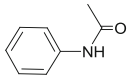
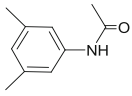
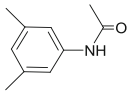
Chemical structure	Chiral selectors	R_1	R_2
	**MPCCD**	$-CH_3$	
	OPCCD	$-C_8H_{17}$	
	MDPCCD	$-CH_3$	
	ODPCCD	$-C_8H_{17}$	

Table 4.22 Chiral separation results of different analytes on column OPCCD-C20

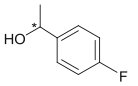
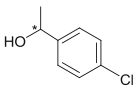
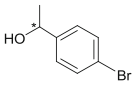
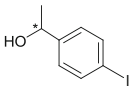
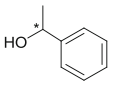
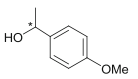
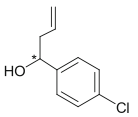
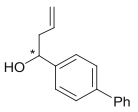
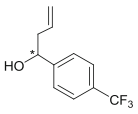
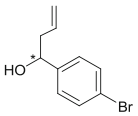
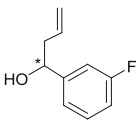
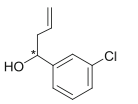
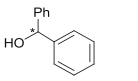
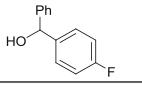
Analytes		Chemical structure	α	R_s	
Rac-OH-1	1-(*p*-Fluorophenyl)ethanol		1.66	4.02	HPLC
			1.33	1.35	SFC
Rac-OH-2	1-(*p*-Chlorophenyl)ethanol		1.88	3.19	HPLC
			1.49	2.14	SFC
Rac-OH-3	1-(*p*-Bromophenyl)ethanol		2.27	3.99	HPLC
			1.72	1.96	SFC
Rac-OH-4	1-(*p*-Iodophenyl)ethanol		2.62	4.02	HPLC
			2.15	4.13	SFC
Rac-OH-5	1-Phenylethanol		1.17	0.87	HPLC
			–	–	SFC
Rac-OH-12	1-(*p*-Methoxyphenyl)ethanol		1.09	0.79	HPLC
			1.09	0.57	SFC
Rac-OH-6	1-(*p*-Chlorophenyl)-3-butene-1-ol		2.55	3.59	HPLC
			1.90	2.62	SFC
Rac-OH-7	1-(*p*-Phenylphenol)-3-butene-1-ol		1.12	0.83	HPLC
			1.12	0.51	SFC
Rac-OH-8	1-(*p*-Trifluoromethylphenyl)-3-butene-1-ol		5.52	3.27	HPLC
			2.30	1.76	SFC
Rac-OH-9	1-(*p*-Bromophenyl)-3-butene-1-ol		3.02	4.00	HPLC
			2.24	3.10	SFC
Rac-OH-10	1-(*m*-Fluorophenyl)-3-butene-1-ol		2.03	2.94	HPLC
			1.43	0.91	SFC
Rac-OH-11	1-(*m*-Chlorophenyl)-3-butene-1-ol		1.25	1.10	HPLC
			1.25	0.79	SFC
Rac-OH-13	Diphenylmethanol		1.07	0.40	HPLC
			–	–	SFC
Rac-OH-14	1-*p*-Fluorophenyl-1-phenyl-methanol		1.58	1.84	HPLC
			1.17	0.82	SFC

Table 4.23 Chiral selectivities on MPCCD-C20, OPCCD-C20, and ODPCCD-C20

Analytes	$\alpha_{MPCCD-C20}$	$\alpha_{OPCCD-C20}$	$\alpha_{ODPCCD-C20}$
Rac-OH-12	1.00	1.09	1.00
Rac-OH-6	1.72	1.90	1.17
Rac-OH-9	2.02	2.24	1.41
Rac-OH-8	2.31	2.30	1.17
Rac-OH-7	1.23	1.12	1.00
Rac-OH-10	1.36	1.43	1.00
Rac-OH-11	1.21	1.25	1.00
Rac-OH-1	1.24	1.33	1.00
Rac-OH-2	1.35	1.49	1.12
Rac-OH-3	1.68	1.72	1.19
Rac-OH-4	2.03	2.15	1.46
Rac-OH-5	1.00	1.00	1.00
Rac-OH-14	1.00	1.00	1.00

withdrawing substitution groups on the aryl ring achieve good separations on OPCCD-C20 (as shown in Table 4.22). Comparison between three *p*-substituted 1-phenyl-3-butene-1-ols, such as Rac-OH-6, Rac-OH-9, and Rac-OH-8 shows that when the volume of substitution group at the p-position of the aryl ring increases, the selectivity is also increased. Comparison between four p-substituted 1-phenylethanols, such as Rac-OH-1, Rac-OH-2, Rac-OH-3, and Rac-OH-4, also illustrates that a greater electron withdrawing substitution group at *p*-position of the aryl ring affords greater selectivity.

OPCCD-C20 and MPCCD-C20 are similar in structure, wherein the methyl group on the imidazolium moiety of MPCCD-C20 is changed to *n*-octyl group in imidazolium moiety of OPCCD-C20. The interactions between racemates and alkyl substitute may influence chiral resolution, and almost all the samples give better selectivity on OPCCD-C20 (shown in Table 4.23). The longer alkyl chain can also prevent two cyclodextrin rings from being too close to each other so that they can work individually and result in better selectivity [67].

Among these four CSPs, OPCCD shows the best separation results for all analytes on both HPLC and SFC analyses. Chromatographic studies reveal that the CSPs consisting of an *n*-octyl group on the imidazolium moiety and phenylcarbamoyl groups on the cyclodextrin ring provide enhancement of analyte–chiral substrate interactions over CSPs bearing the methyl group on the imidazolium moiety and 3,5-dimethylphenylcarbamoyl groups on the cyclodextrin ring. The derivatizing groups in OPCCD-C20 are phenylcarbamates, while in ODPCCD-C20 they are 3,5-dimethylphenylcarbamates. It is thought that the additional two methyl groups are sticking points when the phenyl groups in analytes try to form π–π conjugation with the phenylcarbamates' aryl ring and make the interaction weaker than those without methyl groups, which may be the reason why most samples can achieve better enantioseparations on OPCCD-C20 than on ODPCCD-C20.

Wang et al. [68] further synthesized two cationic β-CD perphenylcarbamoylated derivatives, and the two CDs were chemically bonded onto vinylized silica using a radical copolymerization reaction. Their enantioseparation abilities were successfully demonstrated through the separation of 14 racemates (as shown in Fig. 4.9).

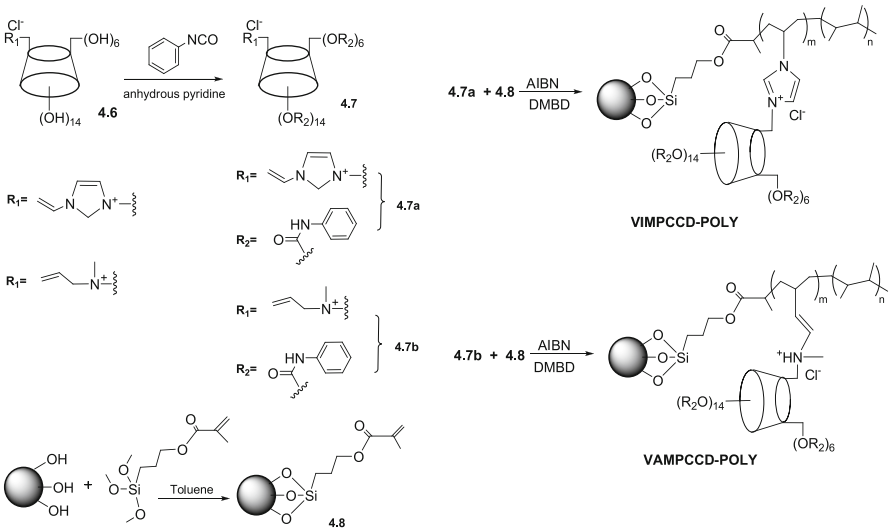


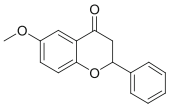
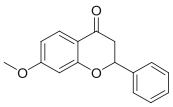
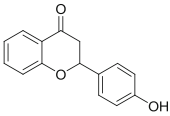
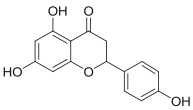
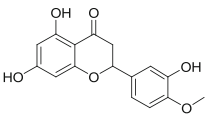
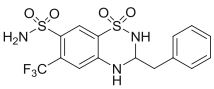
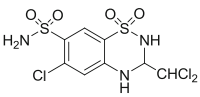
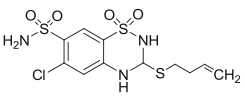
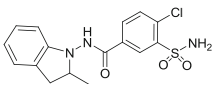
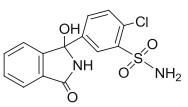
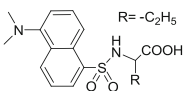
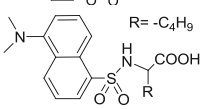
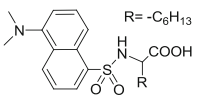
Fig. 4.9 Synthesis process of cationic β-CD derivative and functionalized silica gel and preparation of chemically bonded cationic β-CD on silica surface (Reprinted from Ref. [68], Copyright 2012, with permission from Elsevier)

Table 4.24 shows chromatographic results of the flavanones, thiazides, and amino acid derivatives on VIMPCCD-POLY [68]. The enantioseparations of five flavanone derivatives were progressed on VIMPCCD-POLY; 4′-hydroxyflavanone has the best selectivity and resolution. The interactions between phenolic groups and chiral selector may not always result in higher stereoselectivity, whereas the position of phenolic group on flavanone is important for enantioseparation: cationic CSPs show enhanced enantioselectivity if the phenolic group is on 4′-position; their enantioselectivities towards flavanone derivatives with multiple phenolic groups were reduced especially for flavanone derivatives with phenolic groups both 4′- and 6′-position.

Comparing the selectivities and resolutions among the dansyl amino acids, it was found that when the chain length of the alkyl group was lengthened from ethyl to n-hexyl substituent, VIMPCCD-POLY CSP demonstrated increased chiral selectivity from1.09 to 1.18. It can therefore be inferred that hydrophobic inclusion between the CD cavity and the alkyl group was involved in the enantioseparation processes for dansyl amino acids. The alkyl group with higher hydrophobicity afforded dansyl-DL-phenylalanine a better enantioselectivity of 1.29. The same CSP demonstrated a lower chiral selectivity (1.18) towards dansyl-β-aminocaprylic acid which had an *n*-hexyl group. The phenyl group's interaction with CD cavity in VIMPCCD-POLY was apparently more favorable for enantioseparation than an alkyl chain (n-hexyl group), and the separation result of dansyl-DL-phenylalanine is the best during dansyl amino acids with $\alpha = 1.31$ and $R_s = 1.74$.

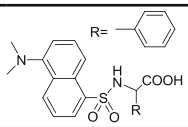
Enantioseparations of bendroflumethiazide might require π–π stacking, while the substituents in their bonded CSP could only form hydrogen bonding with the analytes. Enantioseparation results of thiazides are better than the other two kinds of racemates according to Table 4.24. In addition, trichlormethiazide has the highest

Table 4.24 Chromatography results on VIMPCCD-POLY

Analytes	Structure	Conditions	α	R_s
6-Methoxyflavanone		a	1.10	1.22
7-Methoxyflavanone		a	1.20	0.50
4′-Hydroxyflavanone		b	1.38	1.94
Naringenin		c	1.16	1.38
Hesperetin		c	1.22	1.67
Bendroflumethiazide		d	1.84	3.51
Trichlormethiazide		d	1.71	3.94
Althiazide		d	1.50	2.72
Indapamide		d	1.19	1.87
Chlorthalidone		d	1.29	2.44
Dansyl-DL-α-amino-*n*-butyric acid		e	1.09	0.72
Dansyl-DL-norleucine		e	1.15	1.08
Dansyl-DL-α-aminocaprylic acid		e	1.18	1.29

(continued)

Table 4.24 (continued)

Analytes	Structure	Conditions	α	R_s
Dansyl-DL-phenylalanine		e	1.31	1.74

Conditions: flow rate, 1.0 ml/min; oven temperature, 40 °C; back pressure,15 MPa; modifier content in CO_2 (a, 1 vol% 2-propanol; b, 3 vol% 2-propanol; c, 10 vol% 2-propanol; d, 10 vol% MeOH; e, 40 vol% 2-propanol)

α and R_s values ($\alpha = 1.71$, $R_s = 3.94$) among 14 racemates. As mentioned above, enantioseparations of bendroflumethiazide might require π–π interaction and hydrogen bonding between analyte and the selector. Sulfur-oxygen double bond can form hydrogen bonding with the nitrogen atom in the substituted group of VIMPCCD-POLY, and aromatic rings of the analytes can easily form inclusion complexes with cyclodextrin cavity. Therefore, thiazide derivatives afforded a higher chiral separation efficiency.

4.6 Chiral Separations in SFC Using CD-CSPs and Capillary Column

Chiral separations by SFC using column is also an important branch in analytical application of SFC technique. The capillary columns used for chiral separations in SFC generally have an inside diameter (I.D.) between 50 and 100 μm and a length ranging from 2.5 to 20 m [20]. The first use of capillary column in SFC was reported by Novotny et al. in 1981 [24]. Generally, two different approaches have been used. The first approach involved extensive derivatization of the cyclodextrin with one or more hydrophobic and/or moderately polar substituents in order to obtain a gelatinous or amorphous material [69]. The second approach employed simple methoxy-functionalized cyclodextrins and dissolved them in an appropriate GC liquid stationary phase (e.g., polysiloxane, polyethylene glycol) [70].

Five novel β- (or α-) cyclodextrin-hexasiloxane copolymers have been prepared in Lee's group [71, 72]. The copolymeric phases provided excellent enantiomeric separation of a variety of chiral solutes in open tubular column SFC. The CDs (**4.25**–**4.29**) were prepared as in Fig. 4.10 [69]; the key step was the reaction of partially alkylated β- (or α-) cyclodextrin with *p*,*p*′-methylenebis (benzenesulfony1 chloride) to form a bissulfonate ester on the smaller rim of cyclodextrin.

Copolymers (**4.33**–**4.36**), shown in Fig. 4.11, were synthesized by the hydrosilylation of **4.25**–**28** and **4.32** with dodecamethylhexasiloxane. Copolymers were

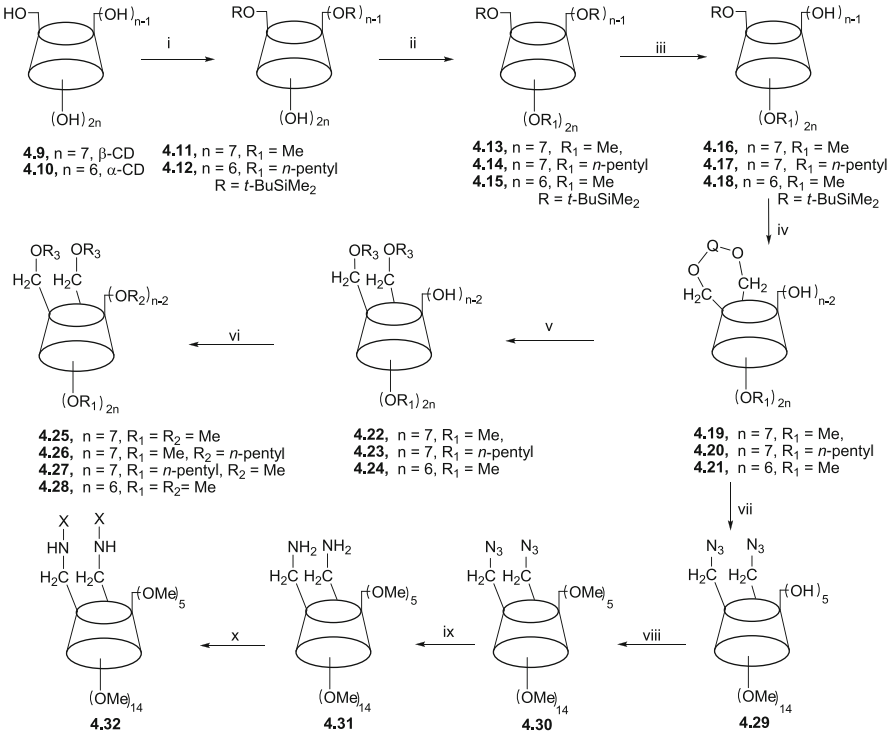
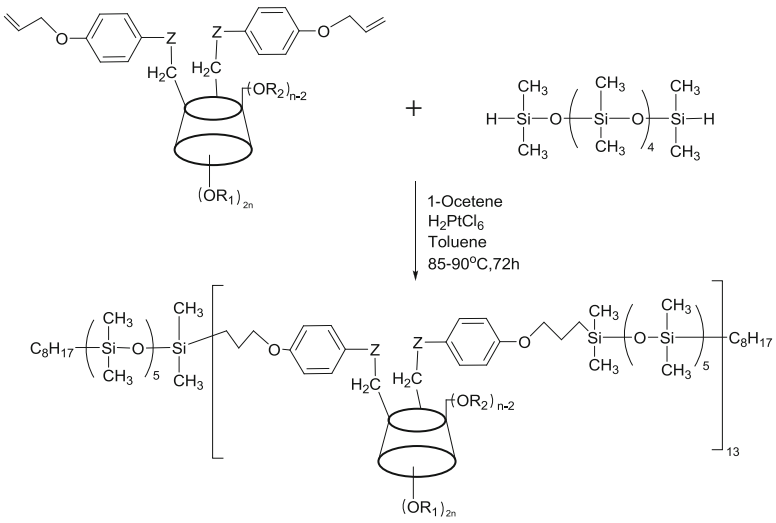


Fig. 4.10 Preparation of bisallyl-substituted cyclodextrins. Conditions: (i), *t*-BuSiMezCl, imidazole, DMF; (ii) NaH, MeI, DMF; (iii) NH_4F, MeOH for **4.16** and **4.18**, $(n\text{-Bu})_4NF$,THF for **4.17**; (iv) *p*,*p*′-methylenebis(benzenesulfony1 chloride), pyridine;(v) sodium *p*-(allyloxy)phenoxide, DMF; (vi) NaH, MeI, DMF;(vii) NaN_3, DMF; (viii) NaH, MeI, DMF; (ix) H_2,PtO_2; (x) *p*-(allyloxy)benzoyl chloride, NEt_3, toluene (Reprinted with the permission from Ref. [69]. Copyright 1993 American Chemical Society)

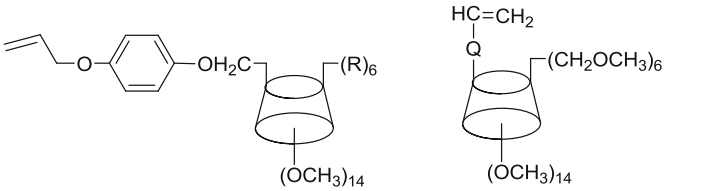
coated to capillary column, for example, copolymer **4.33** was coated on a 5 M×50 μm I.D. fused silica column with a film thickness of about 0.25 μm as previously reported [72]. Capillary column based on copolymer **25** was used for the enantioseparation of diethyl tartrate, 2-phenylcyclohexanol, l-phenylethanol, ibuprofen, pantolactone, and 1,2-di-phenyl-1,2-ethanediol. All of the five compounds were baseline separated with high selectivity and resolution value.

Yi et al. [73] also developed a kind of permethyl-substituted β-cyclodextrin polysiloxane which was used as chiral stationary phases in capillary chromatography. These permethyl-substituted β-CDs (as shown in Fig. 4.12) synthesized with polyhydromethylsiloxane **4.26** in a manner similar to that previously reported [69]. The preparation process is depicted in Fig. 4.13. Separation of (±)-trans-1,2-cyclohexanediol enantiomers on β-cyclodextrin-bound polysiloxane phase **4.29** was



Copolymer	*n*	R_1	R_2	Z
4.33	7	Me	Me	O
4.34	7	Pentyl	Me	O
4.35	7	Me	Pentyl	O
4.36	6	Me	Me	O
4.37	7	Me	Me	-CONH-

Fig. 4.11 Preparation of cyclodextrin-hexasiloxane copolymers



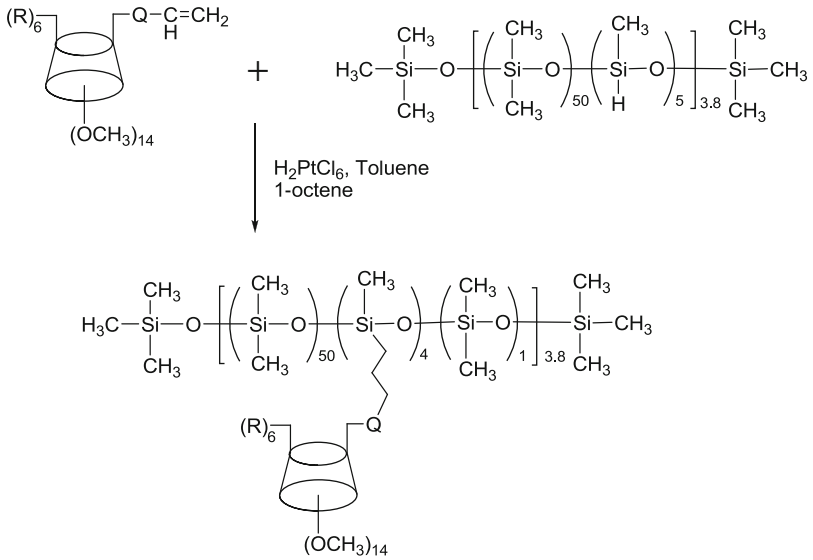
4.38, R = CH_2OCH_3
4.39, R = $CH_2OC(O)CH_3$
4.40, R = CH_3

4.41, Q = CH_2CH_2
4.42, Q = CH_2OCH_2
4.43, Q = $CH_2O(CH_2)_8CH_2$
4.44, Q = None

Fig. 4.12 Structures of persubstituted monoalkenyl-β-cyclodextrins

tested in both GC and SFC, and SFC showed a better separation result with high resolution and less time consumption.

Peterssona et al. [74] developed two series of β-cyclodextrin-based chiral stationary phases, i.e., copolymeric series and side-arm substituted series, to improve the applicability of cyclodextrin-based CSPs in capillary column SFC. Altogether 21 kinds of CD-CSPs were prepared, with their structures listed in Fig. 4.14. Table 4.25 lists the compounds used for the comparisons of enantioseparation



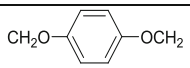
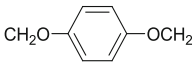
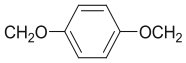
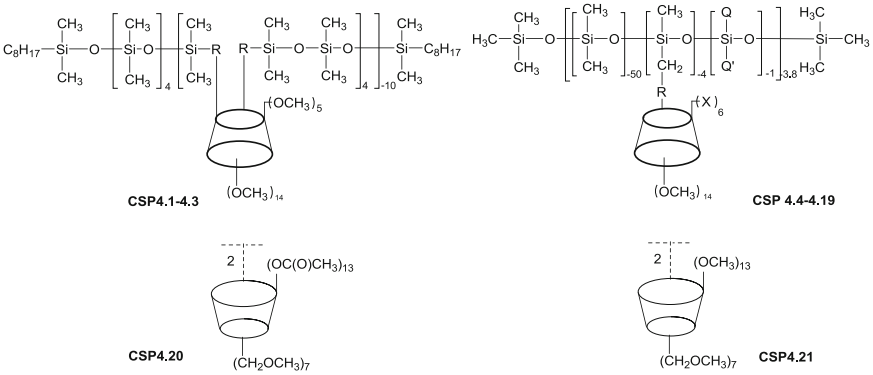
Polymer	Q	R
4.45		CH_2OCH_3
4.46		$CH_2OC(O)CH_3$
4.47		CH_3
4.48	CH_2CH_2	CH_2OCH_3
4.49	CH_2OCH_2	CH_2OCH_3

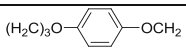
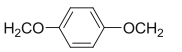
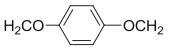
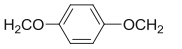
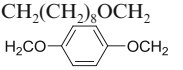
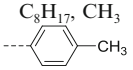
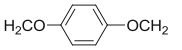
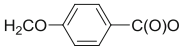
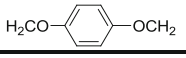
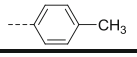
Fig. 4.13 Preparation of persubstituted β-cyclodextrin-bound polymethylsiloxanes

abilities of these CSPs. Table 4.26 shows the SFC separation results at optimal conditions using the same 100 μm I.D. column coated with **CSP4.18**. **CSP4.18** is an excellent chiral selector shown by the resolute values in the table (almost higher than 1.0, baseline separation). Moreover, temperature differences seem to have little effect on the chiral separation of **CSP4.18**. Obviously, capillary column SFC is very important in analytical chemistry, but their small separation capacities and poor separation results caused their replacement by packed columns. Recently, there is lack of studies about capillary columns.

4.7 Chiral Separation in SFC Using CD-CMPs

Another important application of cyclodextrin derivatives in SFC is used as chiral mobile phase. Chiral mobile phase (CMP) additive used for enantiomeric separation is proved to be a convenient way [75–77]. An overview of the molecules



CSP	R[a]	Q,Q'	X	% n/n[b]	% w/w[c]
CSP4.1					56
CSP4.2	$(CH_2)_3OCH_2$				59
CSP4.3	$(CH_2)_{10}OCH_2$				57
CSP4.4-CSP4.11[d]		C_8H_{17}, CH_3	CH_2OCH_3	0.4-9.5	12-72
CSP4.12		C_8H_{17}, CH_3	$CH_2OC(O)CH_3$	3.6	59
CSP4.13		C_8H_{17}, CH_3	CH_3	3.6	50
CSP4.14	None	C_8H_{17}, CH_3	CH_2OCH_3	3.6	56
CSP4.15	CH_2CH_2	C_8H_{17}, CH_3	CH_2OCH_3	3.6	56
CSP4.16	CH_2OCH_2	C_8H_{17}, CH_3	CH_2OCH_3	3.6	55
CSP4.17	$CH_2(CH_2)_8OCH_2$	C_8H_{17}, CH_3	CH_2OCH_3	3.6	53
CSP4.18			CH_2OCH_3	4.3	56
CSP4.19[e]		C_8H_{17}, CH_3	CH_2OCH_3	4.0	53
CSP4.20		C_8H_{17}, CH_3		3.6	59
CSP4.21				4.3	56

Note [a]the left side of the spacer is attached to the siloxane [b]amount of CD expressed in % by substituent [c]amount of CD expressed in % by weight. The spacer is assigned to the achiral part of the CSP [d]synthesized in eight versions with different amounts of cyclodextrin (0.4, 0.8, 1.5, 2.1, 2.7, 3.6, 5.4 and 9.5 % by substituent, or 12, 21, 34, 42, 46, 53, 63 and 72 % w/w) [e]contains 4.0 % CD, 1.0 % octyl and 5 % cyanopropyl by substituent

Fig. 4.14 The structure of as-prepared 21 CSPs

separated by means of analytical SFC using a chiral SP and a cyclodextrins chiral selector in the mobile phase is given in Table 4.27 [40]. Salvador and co-workers investigated enantioseparation of several racemates using dimethyl-β-CD (DM-β-CD) as CMP in SFC and studied the influence of modifier, temperature, pressure, and some other parameters on the chiral separation results [78, 79].

Table 4.25 Compounds used to compare chromatographic performance on **CSP4.1–4.21** in SFC

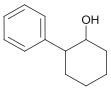
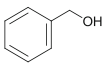
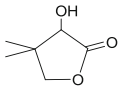
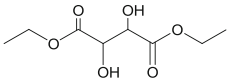
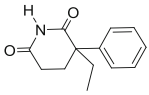
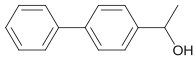
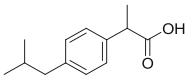
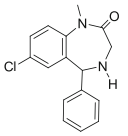
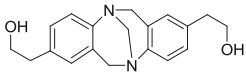
 (±)-Trans-2-phenylcyclohexanol	 (±)-1-Phenyl-1-ethanol	 (±)-Pantolactone
 (±)-Diethyl tartrate	 (±)-Glutethimide	 (±)-1-(4-Phenyl)phenylethanol
 (±)-Ibuprofen	 (±)-Dihydrodiazepam	 (±)-2,8-Di(2-hydroxyethyl)-6H, 12H-5,11-methanodibenzo-[b,f]-[1,5]-diazocine

Table 4.26 Comparison of SFC and GC at optimal conditions using the same 100 µm I.D. column coated with **CSP4.18**

	SFC 5 m × 50 µm I.D.[a,b]				SFC 10 m × 50 µm I.D.[a,c]			
Compound	T (°C)	ρ (g/ml)	t_{R2} (min)	R_s	T (°C)	ρ (g/ml)	t_{R2} (min)	R_s
(±)-trans-2-Phenylcyclohexanol	60	0.35	14.1	1.6	40	0.20	56.1	1.6
(±)-1-Phenyl-1-ethanol					48	0.17	31.8	1.6
(±)-Pantolactone					50	0.16	56.8	1.3
(±)-Diethyl tartrate					50	0.18	55.6	1.4
(±)-Glutethimide					58	0.27	63.0	1.1
(±)-1-(4-Phenyl)phenylethanol					51	0.29	59.9	1.6
(±)-Ibuprofen					60	0.30	62.3	1.0
(±)-Dihydrodiazepam					58	0.52	14.2	1.6
(±)-2,8-Di(2-hydroxyethyl)-6H,12H-5,11-methanodibenzo-[b,f]-[1,5]-diazocine					53	0.61	64.9	1.5

[a]For both the 50 and 100 µm I.D. column, the film thickness was ca. 0.25 µm.
[b]$CO_{2\ at}$ an average linear velocity of 1.9 cm/s at 60 °C and 0.30 g/ml
[c]$CO_{2\ at}$ an average linear velocity of 2.5 cm/s at 60 °C and 0.30 g/ml

Table 4.28 summarizes influence of polar modifier on retention factor (k_1, first eluted enantiomer) and enantioselectivities of some racemates (Fig. 4.15). In most cases, acetonitrile or methanol provides highest retentions. Clearly, retention decreased as a function of the chain length of the alcohols. For most solutes, higher enantioselectivities are obtained using acetonitrile or methanol as polar modifier [79]. The addition of DM-β-CD in SFC mobile phase allows efficient chiral separations, and good enantioselectivities were obtained with 80:20:2 or 95:5:0.5 (v/v/mM) CO_2-polar modifier-MeCD, with moderate column temperature (41 °C) and outlet pressure (110 bars). Acetonitrile or methanol can be used as polar modifier, which is dependent on the solute.

Table 4.27 Substances separated by SFC using a cyclodextrin chiral selector in the mobile phase

Substance	CSP or SP/chiral selector	Mobile phase composition (ratio and percentage, v/v)	Refs.
Benzoxazine derivative	Hypercarb100×4.6 mm/ dimethyl-β-CD (DM-β-CD)	CO_2/MeOH/CD (95:5:0.75 v/v/mM)	[78]
Tofizopam, warfarin, lorazepam, flurbiprofen, chlorthalidone, benzoxazine derivative, temazepam, methyl phenyl hydantoin	Hypercarb 100×4.6 mm/ DM-β-CD	80–95 % CO_2, 5–20 % ACN, MeOH, or EtOH 0.5–2 mM CD	[78, 79]

Table 4.28 Influence of polar modifier on retention factor (k, first eluted enantiomer) and enantioselectivity

Mobile phase	Solute		Polar modifier			
			Methanol	Ethanol	*n*-Propanol	Acetonitrile
80:20:2 (v:v:mM) CO_2-polar modifier-MeCD	Tofizopam	k_1	11.6	9.0	7.1	9.9
		α	1.06	1.04	1.00	1.40
	Warfarin	k_1	12.3	8.9	7.7	13.8
		α	1.22	1.30	1.23	1.42
	Benzoxazine derivative	k_1	3.6	2.2	1.5	2.6
		α	1.21	1.20	1.20	1.24
	Lorazepam	k_1	11.1	9.6	6.6	17.9
		α	1.18	1.22	1.19	1.38
	Flurbiprofen	k_1	7.9	4.6	2.2	18.6
		α	1.07	1.03	1.00	1.06
	Temazepam	k_1	3.5	2.9	–	3.4
		α	1.11	1.12	–	1.00
	Chlorthalidone	k_1	21.4	20.0	14.4	>66.0
		α	1.07	1.10	1.08	–
95:5:0.5 (v:v:mM) CO_2-polar modifier-MeCD	Benzoxazine derivative	k_1	6.9	5.6	4.6	9.0
		α	1.34	1.38	1.20	1.43
	Temazepam	k_1	15.1	16.45	13.9	12.7
		α	1.12	1.11	1.00	1.07
	Me-phenyl hydantoin	k_1	6.3	7.0	1.2	39.9
		α	1.21	1.26	1.00	1.34

Column: Hypercarb (100×4.6 mm I.D.); flow rate, 3 ml/min; temperature, 418 °C; outlet pressure, 110 bars

Herbreteau and his co-workers also examined the influences of methanol percentage in the mobile phase, pressure, and column temperature on the chiral separation using DMCD as CMP [78, 79]. The results showed that the enantioselectivities

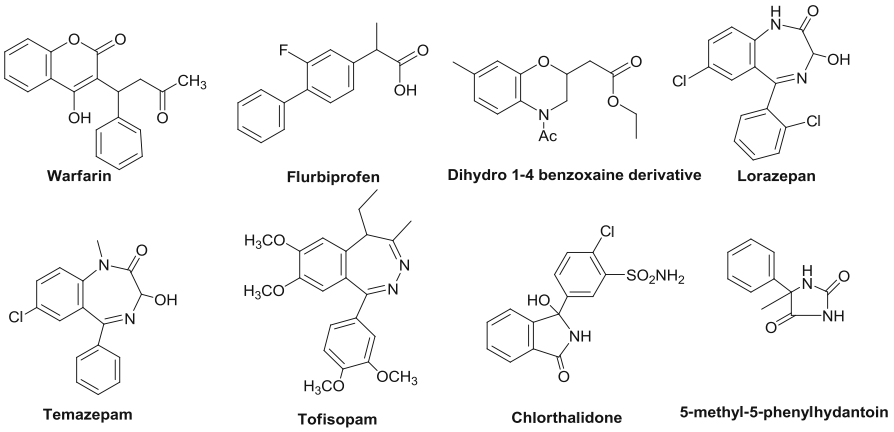


Fig. 4.15 Structure of solutes

increased gradually with the increase of DMCD concentration. In terms of pressure, decreased results were obtained as the pressure increased from 50 to 250 bar. However, enantioselectivities were proportional to the increasing temperature. In Table 4.28, all analytes showed a favorable separation results because the selectivities in the two kinds of mobile phases were very good. Ethanol seemed to be a better modifier in CD-CMPs SFC chiral separation process compared with methanol.

4.8 Conclusion

Today, SFC is a separation technique similar to high-performance liquid chromatography (HPLC) which uses mostly the same hardware and software as HPLC. The mobile phase is a binary or ternary mixture with CO_2 as the main component, which is environment friendly and has high diffusion coefficient. As a result, the analyte separation process is fast and highly efficient. As a kind of the most commonly used natural macromolecule chiral selectors, CD and derivatives have been universally employed in SFC.

In this chapter, we firstly gave a brief introduction to SFC, which features numerous practical advantages relative to reversed-phase HPLC such as higher efficiency, higher throughput, more rapid equilibration, and shorter cycle time. SFC has become an increasingly fast-growing, effective, and environment-friendly technique worldwide in the field of analytical chemistry including chiral analysis. For chiral separation in SFC, the development of cyclodextrin-based chiral selectors was summarized, and their applications as chiral stationary phases or chiral mobile phase additives were discussed in details.

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Chapter 5
Native β-Cyclodextrins and Their Neutral Derivatives for Capillary Electrophoresis

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Abstract In this chapter, a general introduction of capillary electrophoresis is firstly provided. An overview of native and neutral cyclodextrins for enantiomeric separation using capillary electrophoresis is then elaborated, where a correlation of cyclodextrin structure and their enantioselectivities is discussed. In addition, the analytical parameters for optimization of enantiomeric separation with these cyclodextrins are also discussed in detail.

Abbreviations

ACE	Affinity capillary electrophoresis
2-APA	2-Arylpropionic acids
6-AMCD	6-Monoamino-6-monodeoxy-β-cyclodextrin
BGE	Background electrolyte
BHPA	1, 1′-Binaphthyl-2, 2′-diylhydrogenphosphate
CD	Cyclodextrin
β-CD-NH_2	Mono(6-amino-6-deoxy)-β-cyclodextrin
CEC	Capillary electrochromatography
CIEF	Capillary isoelectric focusing
CMC	Critical micelle concentration

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CMCD	Carboxymethyl-β-cyclodextrin
CZE	Capillary zone electrophoresis
DMCD	Heptakis-(2, 6-di-O-methyl)-β-cyclodextrin
2,3-DMCD	Heptakis-(2,3-di-O-methyl)-β-CD
3,6-DMCD	Heptakis-(3,6-di-O-methyl)-β-CD
DMSO	Dimethyl sulfoxide
EOF	Electroosmotic flow
GC	Gas chromatography
HBCD	2-O-(2-Hydroxybutyl)-β-CD
HECD	Hydroxyethyl-β-cyclodextrin
HPCD	(2-Hydroxypropyl)-β-cyclodextrin
HSCD	Heptakis-6-sulfato-β-cyclodextrin
2-HPCD	2-O-(2-Hydroxypropyl)-β-cyclodextrin
HPLC	High-performance liquid chromatography
IPR	Ion-pairing reagent
LOD	Limits of detection
MCD	Heptakis-O-methyl-β-cyclodextrin
MEKC	Micellar electrokinetic chromatography
MI-S-β-CD	Randomly sulfate-substituted β-cyclodextrin
NACE	Nonaqueous capillary electrophoresis
PM-CD	Permethylated-β-cyclodextrin
PS	Polymeric surfactant
SDS	Sodium dodecyl sulfate
SP-CD	Sulfopropyl-β-cyclodextrin
STDC	Sodium taurodeoxycholate
TBDMS	Tert-butyldimethylsilyl
TEA	Triethylamine
TMA-CD	2-Hydroxypropyltrimethylammonium salt of β-cyclodextrin
TM-6-AMCD	Permethylated 6-monoamino-6-monodeoxy-β-cyclodextrin
TMCD	Heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin

5.1 Introduction

Capillary electrophoresis (CE) has been regarded as a major analytical tool for enantiomeric separation analyses [1–3]. CE has various unique advantages encompassing high peak efficiency, short analysis time, good sample compatibility, and low selector consumption in comparison with other established techniques. Moreover, CE shows remarkable versatility due to the applicability of a wide variety of chiral selectors and quick equilibration between analytes and the chiral selector. Many available pharmacological compounds are racemates which require separation into enantiomers for pharmaceutical research. Accordingly, the CE technique has been in rapid development as a viable tool for accessing enantiomeric purities of drug molecules.

Meanwhile, cyclodextrins (CDs) and their derivatives are most widely used as chiral selectors in CE due to good water solubility, excellent chiral selectivity, and their relatively low cost [4–6]. CDs are essentially cyclic oligosaccharide molecules comprising various glucose units, e.g., β-CD which possesses seven glucose moieties. The bowl-shaped CD has a narrow rim with primary 6-hydroxyl groups and secondary 2-, 3-hydroxyl groups on the wider rim. Moreover, a racemic analyte can form temporary diastereomeric associations with the chiral CDs by molecular interactions before or during the electrophoretic separation in CE. It has been established that the chiral selectivity and resolution can be influenced by various factors such as the structure and concentration of CDs, the pH and composition of the background electrolyte (BGE), the organic modifier or additives, as well as the temperature of operation [7, 8]. In this chapter, the application of native β-CD and its neutral derivatives for the enantioseparation by means of CE will be reviewed and discussed.

5.2 Fundamental Aspects of CE

5.2.1 Development History of CE

The development of the CE technique can be traced back to the 1930s. It was widely acknowledged that Tiselius first used electrophoresis as a separation technique in 1937 [9]. Moreover, he established the method of moving boundary electrophoresis wherein five proteins were successfully separated using this approach. Thereafter, Hjerten first afforded the capillary zone electrophoresis (CZE) methodology which is widely regarded as the origin of CE mid-1960s [10]. In 1981, Jorgenson and Lukacs [11] applied fused-silica capillary with 75 μm i.d. in CZE, obtaining an extremely high efficiency of 4×10^5 plates per meter. The demonstrated potential and efficiency of this drives its rapid development from then on. Subsequently, various separation methods were developed such as micellar electrokinetic chromatography (MEKC) [12] and capillary electrochromatography (CEC) [13]. The first commercially available CE instrument entered the market in 1989, and thereafter application aspects and literatures burgeoned from 1990 [14–20]. Hitherto, the CE technique has been devoted largely to research pertaining to chiral analysis.

5.2.2 Characteristics of CE

CE is an effective analysis technique for enantiomers, the method exhibiting unparalleled advantages compared with other practical methodologies for chiral analyses [21]. The process of CE separation based on the electrokinetic chromatography is developed using fused-silica capillary with an inner-diameter range between 25 and 100 μm, a voltage range of 10–30 kV, and an electrical field between 10 and 30 kV/ cm.

The buffers used in CE usually possess very high electrical resistance which can limit the phenomenon of Joule heating which will directly affect the shape of plug flow. Moreover, buffers can be varied among aqueous, organic, and polar organic types which can afford very different interactions. The flat profile of plug flow offers high efficiency ($N > 10^5$) [22] and reduces mass transfer resistance. CE requires small solvent consumption for successful separation with little sample injection (1–50 μL), and the analysis time may be less than 1 s [23]. The flexibility in the choice of chiral selector used is particularly notable. Almost all the selectors which have been applied in high-performance liquid chromatography (HPLC) or gas chromatography (GC) are also applicable in CE [3, 6, 24]. The chiral selector can be an additive into buffer mobile phase or as chiral stationary phase. In the former, the low consumption of selector allows for the use of valuable selectors. In addition, it is noteworthy that the CE technique can provide flexibility in choosing separation modes, such as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), nonaqueous capillary electrophoresis (NACE), capillary electrochromatography (CEC), affinity capillary electrophoresis (ACE), and capillary isoelectric focusing (CIEF). These advantages show strong potential as an apt methodology for enantiomeric separations.

5.2.3 Enantioseparations Mechanism

Enantioseparation in CE is based on the mobility and distribution difference between the components of enantiomers under the high-voltage and high electric field in the separation channel. There are two widely recognized approaches in achieving the enantioseparation: direct enantioseparations method (via temporary diastereomeric pairs during the separation process) and indirect method (via stable diastereomeric associations before the electrophoretic process). However, enantioseparation in CE differs from the conventional electrophoretic separation. Electrophoretic separation is a result of different migration velocities due to different charge-to-mass (size) ratio of the analytes. Enantioseparation in CE is not only based on the electrophoretic principle because the enantiomers do not differ from each other in terms of the charge densities in achiral phenomenon. However, chiral additives are added to create a chiral environment in CE. Accordingly, the electroosmotic flow (EOF), the mobility of the analytes, and their combination would differentiate the enantiomers during the enantioseparation process.

Ionic enantiomers would afford effective electrophoretic mobility (i.e., self-mobility, μ_{eff}) in the electric field, where the μ_{eff} is zero for neutral analytes. However, EOF often generated on the inner surface of capillary is present in the separation process. Moreover, the EOF is superimposed on the electrophoretic mobility. Consequently, an apparent mobility would result from a combination of electrophoretic mobility and the EOF, namely, $\mu_{\text{app}} = \mu_{\text{eff}} + \mu_{\text{EOF}}$. Figure 5.1 depicts schematically the generation of EOF. Fused silica is the most widely used capillary material in CE; the inner surface of capillary has numerous silanol groups which will afford

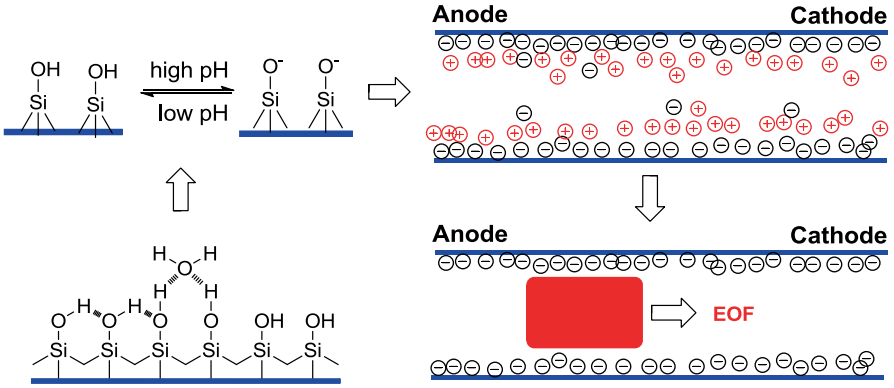


Fig. 5.1 Generation of EOF in CE

oxygen anions at high pH value of buffer, resulting in an accumulation of cations to afford a nonuniform distribution of charges. As a result, a layer of strongly adsorbed cations and solvent molecules will cover the wall which will be mobile in an electrical field to afford the EOF.

The EOF can carry ionic or neutral molecules in the same direction, allowing them to be detected online with a single detector. Essentially, EOF plays a similar role like the HPLC pump, except that the EOF would generate plug flow with high velocity, allowing for greater efficiency and resolution than pressure-driven methods which are characterized by a parabolic flow profile. The magnitude of the electroosmotic flow can directly affect the observed mobility and chiral resolution in the enantioseparation process [11].

A successful enantioseparation in CE means that each enantiomer is detected in a different period of time after simultaneous injection at the capillary inlet, i.e., the enantiomers must migrate with different velocities along the same length in the capillary. In the process of separation, the selectors and enantiomers can form transient diastereomeric associations. The mobility difference of the enantiomers ($\Delta\mu$) can be expressed by the following equation [25, 26]:

$$\Delta\mu = \mu_R - \mu_S = \frac{\mu_f + \mu_{cR} K_R [C]}{1 + K_R [C]} - \frac{\mu_f + \mu_{cS} K_S [C]}{1 + K_S [C]} \tag{5.1}$$

where μ_R and μ_S are the apparent mobilities of the *R*- and *S*- enantiomers, μ_f is the mobility of the analytes in free form, and μ_{cR} and μ_{cS} are the mobilities of analytes in complex form (temporary diastereomeric complexes). K_R and K_S are the stability constants of the *R*- and *S*-enantiomers with the selector, respectively, and [*C*] is the concentration of the selector. As mentioned above, the mobilities of the enantiomers are equal in free form in achiral environment (μ_f) as well as in the associated form ($\mu_{cR}=\mu_{cS}=\mu_c$), while the interaction parameters of the *R*- and *S*-enantiomers with the chiral selector may be different ($K_R \neq K_S$).

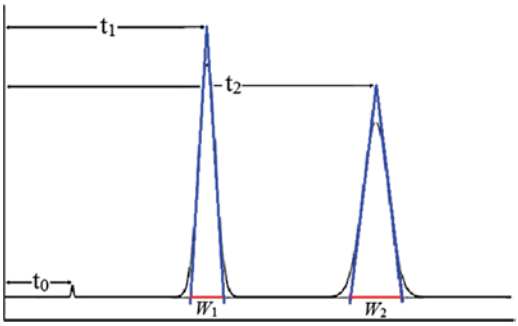


Fig. 5.2 Chromatogram of the enantioseparation in CE

Accordingly, the Eq. (5.1) can be simplified to the following:

$$\Delta\mu = \frac{(\mu_f - \mu_c)(K_R - K_S)[C]}{1 + (K_R + K_S)[C] + K_R K_S [C]^2} \tag{5.2}$$

This simplified Eq. (5.2) has been extensively used to optimize the enantioseparation process in CE. The optimal concentration value can be obtained through calculation from Eq. (5.2), resulting in attainment of maximal mobility difference between the enantiomers. In addition, when the analytes and selector possess opposite charge, they will migrate in opposite direction under the electrical field, so the absolute value of ($\mu_f - \mu_c$) will significantly increase to produce higher mobility difference.

As shown in Eq. (5.2), efficient enantioseparations are based on the mobility difference between the free and complex analytes ($\mu_f - \mu_c \neq 0$) and the difference between the association constants with selectors ($K_R - K_S \neq 0$), with the former requirement being essential for enantioseparation. Consequently, neutral enantiomers cannot be resolved with uncharged selectors due to the absence of mobility difference, because the migration mobilities of enantiomeric pairs are equal to the EOF in both free and associated forms. On such occasion, charged CDs or charged achiral micellar compounds must be added into the BGE to generate a mobility difference. The main separation mechanism of enantiomeric pairs in CE is the interaction between the selectors and analytes. Accordingly, the chiral selectivity and resolution can be influenced by the mobilities of the temporary diastereomeric pairs, the binding constants, or both of them simultaneously.

5.2.4 Calculation of Parameters for Enantioseparation

There are two main measurement parameters of enantioseparation in CE: enantioselectivity (α) and chiral resolution (R_s). The parameters are shown in Fig. 5.2.

The following part outlines the calculation of key parameters used in accessing enantioseparation.

The capacity is calculated using retention time as follows:

$$k = \frac{t_R - t_0}{t_0} \tag{5.3}$$

The USP calculation methods are as follows:
Theoretical plates:

$$n = 16\left[\frac{t_R}{W}\right]^2 \tag{5.4}$$

Enantioselectivity:

$$\alpha = \frac{k_2}{k_1} \tag{5.5}$$

Chiral resolution:

$$R_s = \frac{2\left(t_2 - t_1\right)}{\left(W_1 + W_2\right)} \tag{5.6}$$

where n is theoretical plates, t_0 is the dead time (the elution time of the retained solvent or marker), t_1 and t_2 are the retention times for the enantiomeric pairs, and W is the width of the base of the component peak using tangent method.

5.3 Suitability of CDs for Applications in Chiral CE

5.3.1 High Efficiency

CDs are commonly used in high-efficiency analysis techniques. However, the chiral selectivity values of CDs in enantioseparation rarely exceed two. Nonetheless, limited selectivity can be compensated by the high efficiency of capillary columns. In some cases, the CDs afford baseline separation with high efficiency of 10^5–10^6 plates per meter despite low α-values in the range of 1.01–1.03 [6].

We discuss herein considerations for achieving successful enantioseparations. The limited solubility of analytes and selectors is an important factor which is responsible for the reduction of chiral selectivity. Thus, CDs are oftentimes not just the chiral selectors but serve the apt role of solubilizing the analytes through inclusion phenomenon. Hence, the modification of CDs, proper concentrations of CDs, and the addition of organic modifiers (e.g., methanol, acetonitrile) can play important roles in improving the solubility of analytes and selectors [27–29]. The compatibility of CDs and the BGE is another important

consideration which would diminish efficiency. For instance, multi-charged CDs may not match with the BGE resulting in dramatic decrease of efficiency in some cases. Accordingly, using the correct type and an appropriate concentration of BGE must be used to preserve the high efficiency of the system by affording a balanced conductivity [5].

5.3.2 Broad Application and Chiral Selectivity Spectra

CDs and their derivatives have been found to be apt selectors for chiral CE. An important reason is that CDs have exhibited exceedingly broad chiral selectivity spectra despite their limited selectivity values in CE. The phenomenon can be attributable to their natural chiral features where every glucose unit possesses five chiral centers. Moreover, the functional group on each chiral center has different orientations even in the same unit. Funtionalization with other substituent groups can also afford additional chiral centers on the CD rims, thus expanding the chiral recognition capabilities. CD derivatives can also change their shapes to form stable associations through molecular interactions with one of the enantiomeric pairs on account of their flexible structures. These complexes may afford accentuated chiral recognition in enantioseparation.

The broad applicability of CDs as chiral selectors for the enantioseparations of analytes is notable. Thus, CDs can be used in aqueous media and polar or apolar organic buffers where they can afford different chiral selectivity. For example, proteins, antibiotics, and chiral acrylates can only separate aliphatic amino acids, whereas CDs can deal with most amino acids [30]. Moreover, enantiomers with heteroatomic chiral centers (S, N, P, and Si) [26, 31] and regioisomers can also be separated using CDs as selectors. Pharmaceutical molecules and their metabolites can also be analyzed using only one process [32].

5.3.3 Reversal of Migration Order

Under normal conditions, one compound peak should not overlap with another one if the enantiomers are in equal molar amount. However, tailing of the major peak which elutes first may overlap with the minor one when enantiomeric excess is high. In such circumstances, reversal of the elution order such that the minor component elutes first can afford a solution to this problem. There are various approaches which can afford a reversal of migration order, such as applying different selectors with opposite charge [33], eliminating or reversing of EOF [33], and addition of achiral additives [34].

CDs afford strong capability for the reversal of migration order for enantiomers in comparison with other selectors. Various CD derivatives can afford the reversal under different conditions, making for facile analysis of pharmaceutical drugs [35–37]. For

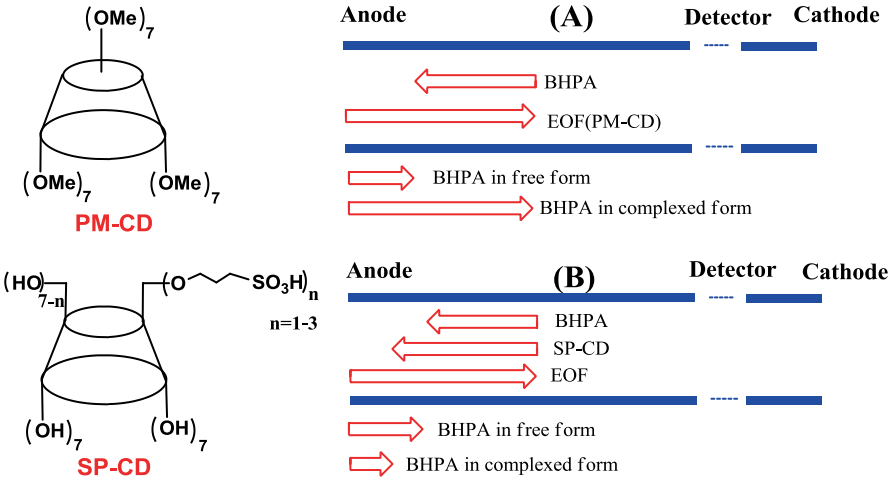


Fig. 5.3 Migration for BHPA with (**a**) PM-CD and (**b**) SP-CD

instance, Mayer et al. [38] applied permethylated-β-CD (PM-CD) and sulfopropyl-β-CD (SP-CD) to resolve the 1,1′-binaphthyl-2,2′-diylhydrogenphosphate enantiomers (BHPA). This reversal of migration is described in Fig. 5.3. Herein, the EOF is the driven flow with the highest mobility in both systems. Neutral PM-CD has the same migration mobility and direction with EOF. Accordingly, the enantiomer, which can form steady diastereomeric association, will be detected first (Fig. 5.3a). On the other hand, the mobility of SP-CD is higher than BHPA and opposite to EOF. Accordingly, the more tightly bound enantiomer could be decelerated and migrates as second peak (Fig. 5.3b). Thus, a reversal of migration order was obtained by using different CDs as selectors.

5.3.4 Dual Systems

The burgeoning application of CDs in CE is attributable to their unique features and facile conversion to various new derivatives. However, in those cases where using a single CD derivative cannot achieve enantioseparations of neutral analytes, the dual systems can be used successfully. A dual system refers to a buffer which contains two different selectors, e.g., two CDs or one CD and other chiral selector of other type [39, 40]. This method can combine the advantages or compensate for the disadvantages of both selectors. Consequently, significant increases in resolution can be obtained in most cases.

Moreover, the dual system can also afford the simultaneous resolution of numerous enantiomers in a single chromatographic run. Thus, the simultaneous separation of six herbicide enantiomeric pairs was attained through the combination of two CDs [41]. The two CDs, 6-monoamino-6-monodeoxy-β-CD (6-AMCD) and permethylated

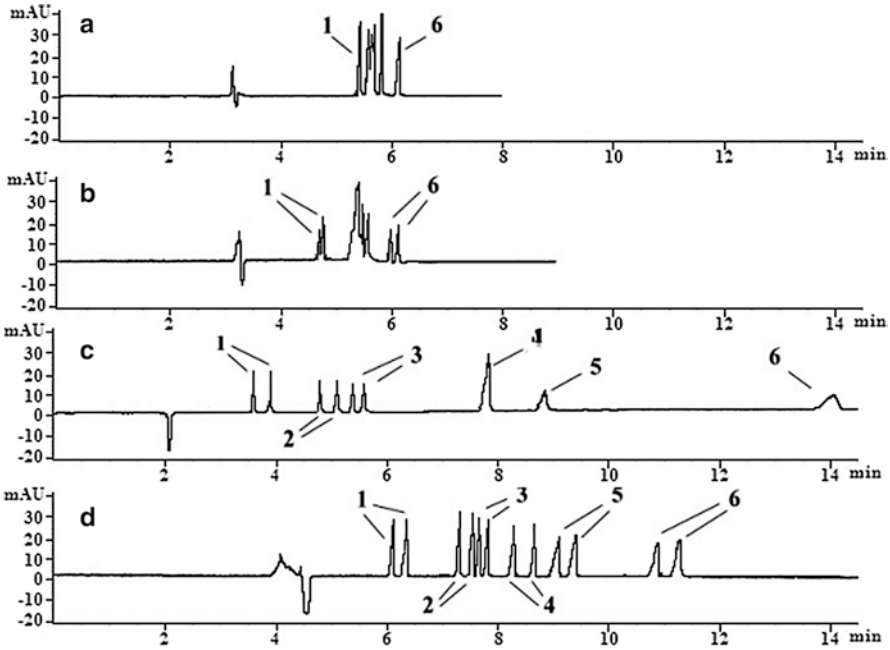


Fig. 5.4 Simultaneous separation of six herbicides in CE. (**a**) no CD, (**b**); 1 mM 6-AMCD, (**c**) 10 mM TM-6-AMCD, (**d**) 1 mM 1 mM 6-AMCD and 10 mM TM-6-AMCD. Compounds 1–6 represent, respectively, (+)- and (−)-2,4,5,-trichloro-phenoxypropionic acids; (+)- and (−)-2-methyl-4-chloro-phenoxypropionic acids; (+)- and (−)-2,4-dichloro-phe-noxypropionic acids; (+)- and (−)-3-chloro-phenoxypropionic acids; (+)- and (−)-4-methyl-phenoxypropionic acids; (+)- and (−)-phenoxypropionic acids (Reproduced from Ref. [41] by permission of John Wiley & Sons Ltd.)

6-monoamino-6-monodeoxy-β-CD (TM-6-AMCD), have different enantiomeric resolution abilities with the chosen analytes as can be seen in Fig. 5.4. Electrophoregram B shows that only compounds (1) and (6) can be separated by 6-AMCD, while compounds 1, 2, and 3 were successfully separated using TM-6-AMCD, as can be seen in electrophoregram C. However, combination of the two CDs afforded excellent chiral resolution with successful enantioseparation of all the six herbicides as shown in electrophoregram D

5.4 Influence Factors of Enantioseparations in CE

The separation of enantiomeric pairs can be affected by various factors, such as the structure and concentration of chiral selectors, the pH and the composition of buffer, additives used, voltage applied, and temperature.

5.4.1 The Structure and Concentration of CDs

CD is a cyclic oligosaccharide which is hydrophilic outside the cavity but hydrophobic on the inside. Invariably in CE enantioseparations, native CDs and CD derivatives which have different functional groups in different positions and with various degrees of substitutions are used. Native CDs can be transformed into neutral, anionic, or cationic derivatives, which are suitable for separating different analytes. Numerous randomly and selectively functionalized CD derivatives have been used as chiral selectors in CE over the past decade [1, 41]. However, randomly functionalization often affords mixtures of CD derivatives with different degrees of substitutions, which would lead to poor reproducibility in synthesis and poor repeatability in analysis applications from batch to batch. Accordingly, single-isomer CD derivatives (monosubstituted or persubstituted) are strongly recommended for CE enantioseparations [42–44]. These single-isomer derivatives have additional chiral centers affording unique chiral recognition.

However, it is extremely difficult to predict a priori successful enantioseparation with given racemates and selectors. Essentially, there are triple interactions in the enantioseparation, including hydrogen bonding, inclusion complexation, and electrostatic interaction. These interactions are closely related to the structure of selectors. However, the presence of these interactions cannot guarantee a successful enantioseparation. Of course, enhancing these interactions can usually increase the chiral selectivity and resolution. Sometimes, the hydrogen bonding may serve as a key interaction especially if it occurs in position adjacent to the asymmetric center [33]. In addition, steric hindrance must be taken into consideration in such situation. Thus, it is acceptable that the presence of more hydrogen bonds may afford more stable transient diastereomeric associations which may vastly affect the enantioseparations.

At the onset of the development of CE technique, inclusion complexes were regarded as the necessary prerequisite for chiral recognition with CDs. However, in the case of (2-O-methyl-3, 6-di-O-sulfo)-β-CD [45], the CD can hardly form inclusion complex due to the bulky substituent on the entrance to the CD. Surprisingly, a chiral drug aminoglutethimide (AGT) was separated with good resolution in CE. Meanwhile, the H-NMR spectrum of the diastereomeric pair afforded measurable differences in chemical shifts, clearly corroborating the existence of chiral interactions. However, studies of Overhauser effect indicated absence of inclusion phenomenon between the selector and analytes. Accordingly, inclusion complexation between CDs and the guest molecules does not appear to be a prerequisite for chiral recognition because CD selectors may also form stable external complexes with analytes [46]. Of course, inclusion complexation appears to afford somewhat superior chiral recognition in enantioseparation, mostly in aqueous buffer [47, 48]. With the development of ionic CD derivatives, electrostatic interaction would play a more important role in CE. Electrostatic interactions between the selectors and oppositely charged analytes are vastly stronger in water-based buffer, affecting chiral recognition simultaneously.

As mentioned above, the optimal concentration of selectors can be obtained from Eq. (5.2) so as to achieve the maximum mobility difference. Consequently,

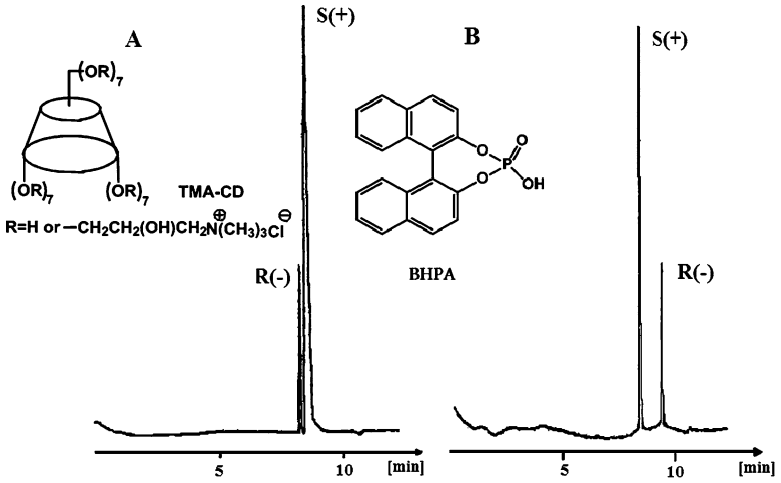


Fig. 5.5 Electropherograms of racemic BHPA with (**a**) 0.05 mg/mL and (**b**) 10 mg/mL of TMA-CD (Reprinted from Ref. [49], Copyright 1997, with permission from Elsevier)

the change of concentration will directly affect the elution time and chiral resolution. In general operation, a concentration range is usually applied within the appropriate concentration value. Moreover, change of selector concentration may lead to a reversal of migration order via decreasing or increasing the migration mobility, especially when the chiral selectors were used as carriers in the mobile phase. For example, this phenomenon was clearly observed in the enantioseparation of the anionic chiral compound BHPA [49] using 2-hydroxypropyltrimethylammonium salt of β-CD (TMA-CD) as shown in Fig. 5.5.

In practical CE enantioseparations, the structure and concentration of CD selectors will definitely impact upon the elution time and chiral resolution. Different selectors may exhibit different chiral recognition mechanisms during the separation. Although the success of enantioseparation cannot be predicted with given CDs, some general conclusions can be drawn on the basis of theory and experience.

5.4.2 The Composition and pH of Buffer

The buffer usually comprises background electrolytes with certain concentration of chiral selectors. The buffer may be aqueous, organic, or apolar organic media. Common guidelines for determination of suitable buffer composition are based on the following considerations:

1. The buffers must exhibit excellent buffering ability in the applied pH range.
2. The selected analytes can be detected in a relatively narrow ultraviolet range.
3. The buffer should normally afford low mobility, i.e., high volume with low charge density to afford relative low current.

Suitable buffer for CE would vary with different pH values. Thus, sodium phosphate would be applicable over relatively broad pH range, with sodium borate buffers used in a high pH range, while Tris–HCl buffer applicable in the low pH range. Meanwhile, acetic acid/ ammonium acetate buffer would be the common choice in CE-MS.

In general, the first consideration would be to vary the buffer concentration before changing the parameters when $R_s < 1.5$ or >3. Since high ionic strength would decrease the EOF, the elution time would accordingly be lengthened. The buffer concentration is usually increased only when $R_s < 1.5$. On the other hand, use of lower buffer concentration would commonly be considered when $R_s > 3$.

The applied pH values of buffer usually vary with the pK_a of analytes [50]. For instance, a pH of 2.5 is used widely in the enantioseparation of basic drugs. An appropriate pH value would be helpful to maximize intermolecular interaction, especially the hydrogen bonding between CDs and the analytes. This would result in good enantioseparation while enhancing the electrophoretic migration in applied electric field due to the protonated amino groups of basic drugs. However, low pH buffer is not appropriate for the acidic analytes. The dissociation of the acidic moieties may be limited by the low pH, and it will decrease the EOF and lengthen elution time. Accordingly, the pH value of buffer would be pivotal for successful enantioseparation.

5.4.3 Organic Modifiers in BGE

The presence of organic modifier in the BGE is expected to afford a fine-tuning of enantioseparations [51]. Organic modifier can affect both the elution time and chiral resolution of enantiomers, especially when inclusion complexation is involved in enantioseparations. Moreover, organic modifiers can improve the resolution but afford a negative effect on the binding constants of each enantiomer, and the addition of organic modifiers can improve the solubility of selectors and/or analytes. Commonly used modifiers would include protic organic solvents, methanol, triethylamine, and acetonitrile. A theoretical model developed by Wren [52] on the effect of organic solvent in BGE suggested that organic modifiers diminish the resolution when the concentration of chiral selectors is at or below the optimum value. This effect could be ascribable to the change in formation constants of inclusion complexes, which modifies the optimum concentration of chiral selectors.

5.4.4 The Influence of Temperature

Temperature also plays an important role in CE enantioseparation. An increase in applied temperature usually leads to a decrease in the viscosity of buffer and an increase of EOF, which will directly result in diminishing both the migration time and resolution value. A change of temperature generally originates from the Joule heating in the capillary. Heat is produced by the electrical current in the capillary. In

accordance with a practical experience, the viscosity of BGE solution will change by 2–3 % for every °C change in temperature. An effective approach to reduce Joule heating is to apply buffers with high electrical resistance.

All of the factors mentioned above must be taken into consideration in the enantioseparation of any given analytes. Fillet et al. [40] has developed a useful strategy for general enantioseparations of basic, acidic, or neutral drugs based on numerous experiment data (see flowchart in Fig. 5.6). Over 96 % of the selected compounds attain baseline enantioseparation easily and rapidly using the chart, with particularly high-resolution values ranging from 3 to 30. By this strategy, most chiral drugs can be resolved with significantly reduced cost and time.

5.5 Application of Native β-CDs and Their Neutral Derivatives

Neutral β-CDs including native CDs show great potential in enantioseparations due to their relatively low cost, good solubility, and high stereoselectivity. Moreover, there are few reproducibility problems associated with the enantioseparation when CD derivatives are used.

The β-CDs usually are physically or chemically bonded onto the silica when used as chiral stationary phases in HPLC, but they are mostly used as chiral mobile phases in the buffer to afford enantioseparations in CE. A commonly used derivatization approach for β-CD is to selectively or randomly convert the hydroxyl groups to desired functional groups. However, the hydroxyl groups located at the 2-, 3-, and 6-glucosidic positions in CD would compete for the substitution, which makes selective modification extremely difficult. The primary hydroxyl groups at 6-positions are most reactive towards derivatization reactions in comparison with the other secondary hydroxyl groups at 2- and 3- glucosidic positions.

A great variety of neutral derivatives of β-CDs have been synthesized and applied in the enantioseparations of various analytes. The most common β-CD derivatives used are alkylated β-CDs such as heptakis-O-methyl-β-CD (MCD) [53], heptakis-(2,6-di-O-methyl)-β-CD (DMCD) [54, 55], heptakis-(2,3-di-O-methyl)-β-CD [56] (2,3-DMCD), heptakis-(3,6-di-O-methyl)-β-CD [56] (3,6-DMCD), heptakis-(2,3,6-tri-O-methyl)-β-CD (TMCD) [54, 55], hydroxyethyl-β-CD (HECD) [57], and (2-hydroxypropyl)-β-CD (HPCD) [58–60], etc. Some new derivatives have also been reported recently including 2-O-(2-hydroxybutyl)-β-CD (HBCD) [61, 62] and mono-3-O-phenylcarbamoyl-β-CD [63] (see Fig. 5.7). Moreover, further classes of CDs are being explored for the application in chiral analyses. Since the majority of β-CD derivatives represent mixtures of products, their purification would be an imperative. Accordingly, a popular trend would be to synthesize selectively modified CDs derivatives. The application of neutral β-CDs or dual CD system is used extensively in approximately two-thirds of chiral CE publications between 2000 and 2006 [4]. In the ensuing section, the application of neutral β-CDs will be discussed.

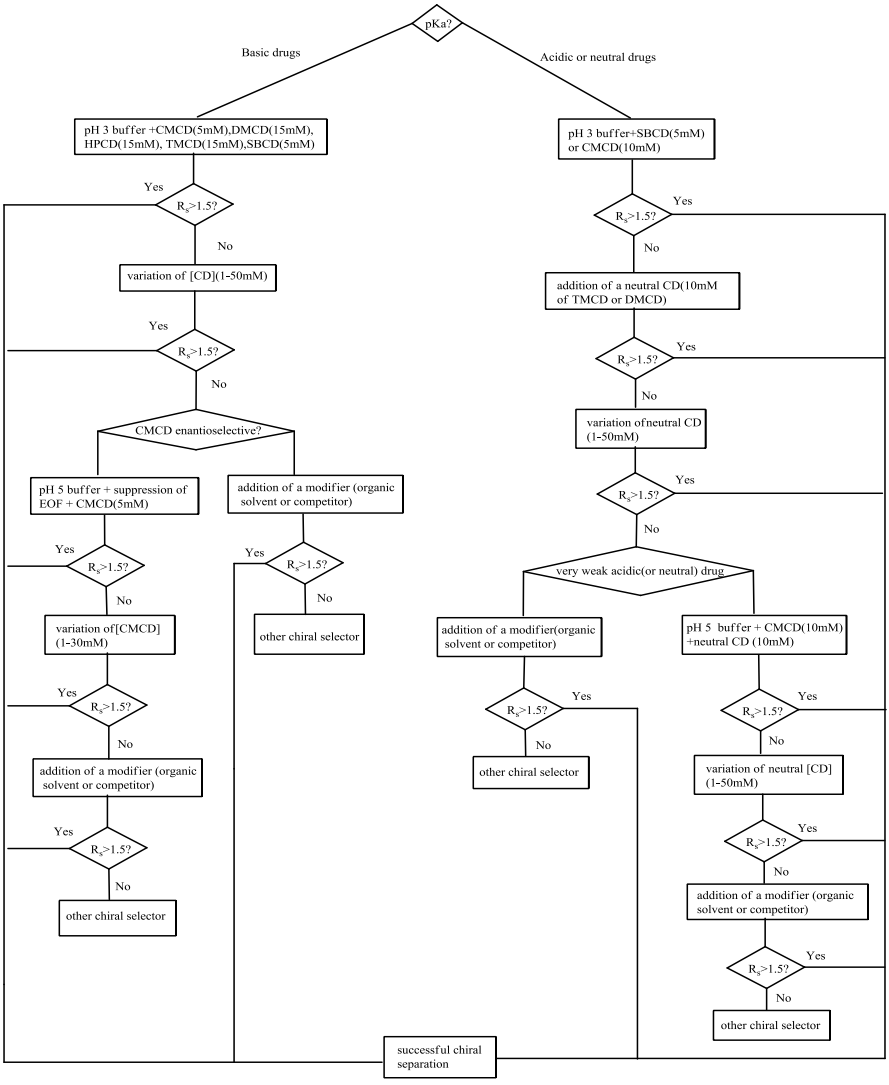


Fig. 5.6 Flowchart for enantioseparation considerations in CE (Reproduced from Ref. [40] by permission of John Wiley & Sons Ltd.)

5.5.1 Application of Native β-CD

There are many successful applications of native β-CD in enantioseparations. For example, Chu et al. [63] studied the degradation behaviors of the imazalil enantiomers in soil using CD-modified CZE (Fig. 5.8).

As shown in Fig. 5.8, the enantioseparation was achieved successfully. The separation resolution was as high as 4 with analysis time within 10 min.

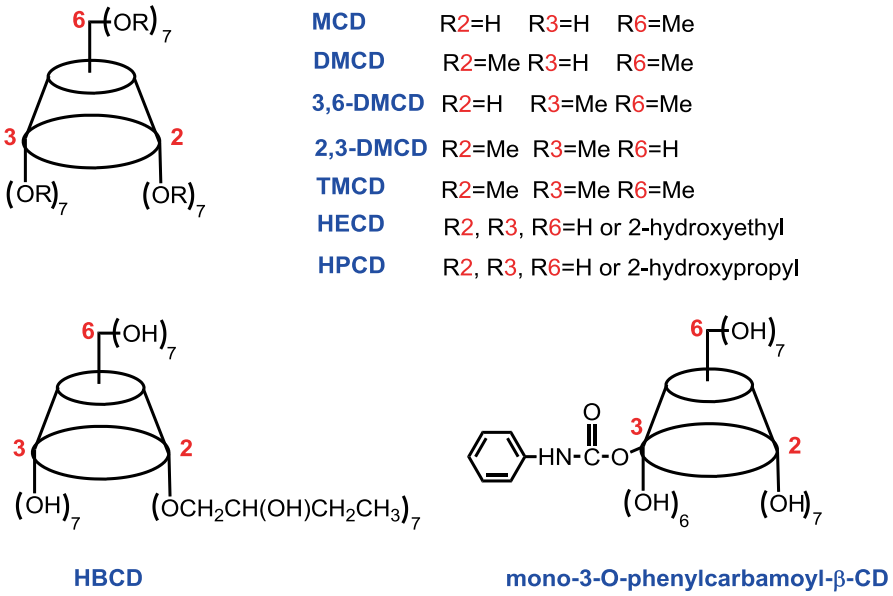


Fig. 5.7 Structures of various neutral β-CD derivatives

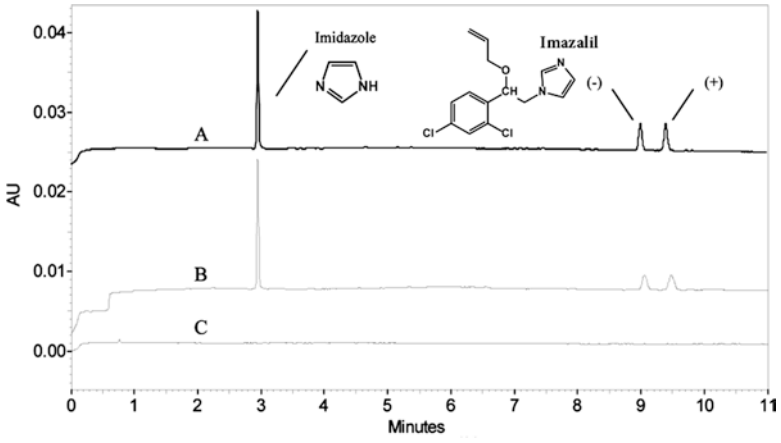


Fig. 5.8 Electropherograms of (**a**) 10 μg/mL imazalil standard, (**b**) concentrated sample from soil extracts (group B, day 5), and (**c**) extracted soil sample without imazalil addition (blank). CE conditions: separation buffer, 50 mM of NaH_2PO_4 containing 5 mM of $NH_4H_2PO_4$ and 5 mM of β-CD (pH = 3.0); running sample, 100 μL sample mixed with 10 μL of 500 μg/mL imidazole (internal standard) and 90 μL of separation buffer; capillary, 75 μm id, total length 50 cm, effective length 40 cm; Injection, 0.5 psi 5 s; separation voltage, +25 kV; UV detection wavelength, 214 nm; temperature, 20 ± 1 °C (Reproduced from Ref. [63] by permission of John Wiley & Sons Ltd.)

The electropherograms of both imazalil enantiomers show two peaks of imazalil enantiomers at 9.0 and 9.4 min, which are attributed respectively to (−)-imazalil and (+)-imazalil. Moreover, it was found that in the process of enantioseparation, β-CD was associated with imazalil enantiomers wherein the phenyl group was inserted into the cavity, while the imidazole ring and the allyl group were excluded from the cavity. However, the imidazole ring and the alkyl group interacted strongly with the larger rim of β-CD. It would be reasonable to attribute poor separation to the use of carboxymethyl-β-cyclodextrin (CMCD) in comparison with native β-CD because the substituents on the rim of the CD cavity may obstruct inclusion due to steric hindrance.

Denola et al. [50] studied the effects of different parameters on the enantioseparation of ten basic drugs using native β-CD as chiral selector. The optimal conditions for the enantioseparation of ten basic drugs are summarized in Table 5.1. Reproducibility of the elution time and resolution were obtainable under optimum conditions for the ten drugs. In addition, the capillary with 20 μm i.d was found to have relatively low sensitivity but higher limits of detection (LOD) of 0.036 mg/mL terbutaline in comparison with 50 μm i.d affording LOD of 0.016 mg/mL terbutaline. The 20 μm i.d capillary is invariably used for CE analysis of the basic drugs with concentration of 0.1 mg/mL, given relatively high LOD.

5.5.2 Enantioseparation with Neutral CD Derivatives

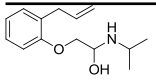
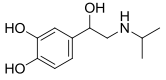
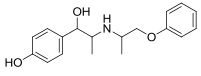
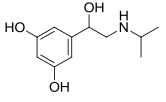
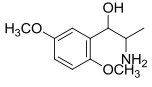
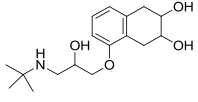
Recently, the application of native β-CD alone has become less popular because of their limited solubility and chiral selectivity, especially in aqueous media. However, application of β-CD derivatives could overcome these problems. Among them, methylated and hydroxyalkylated β-CDs are the most extensively used chiral selectors.

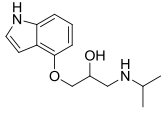
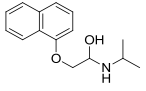
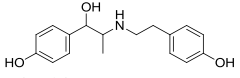
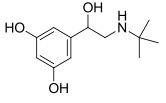
5.5.2.1 Methylated β-CDs

Methylation of Native β-CD

Methylated β-CDs can be obtained by converting the hydroxyl groups of native β-CD to methyl groups at different positions and with varying extent of derivatization. Methylated derivatives are generally divided into those randomly functionalized and those selectively functionalized. There are three main methodologies for methylation: Kuhn-Trischmann methylation [64, 65], the Wacker's industrial method [66], and the Hakomori methylation [67, 68]. The Wacker's industrial method often produces randomly methylated CD mixture using methylchloride under pressure. The methylation of CD can dramatically increase the water solubility in comparison with native CD, wherein the solubility increases until the number of methyl groups reaches 13–14 and then decreases till it reaches the maximum of 21 per CD molecule. The

Table 5.1 Enantioseparations of ten basic drugs using 20 i.d. capillary at optimal conditions

Basic drug	t_1/min	t_2/min	R_s	C_b/mM	C_s mM	T °C	Modifier
 Alprenolol	70.75 ± 2.20	72.19 ± 2.20	1.03 ± 2.26	200	20	15	15 % MeOH + 1 %TEA
 Isoproterenol	20.12 ± 2.71	20.47 ± 2.80	1.69 ± 2.94	100	50	20	1 %TEA
 Isoxsuprine	14.48 ± 2.29	14.79 ± 2.33	1.68 ± 2.29	100	15	30	0
 Metaproterenol	9.53 ± 0.11	9.76 ± 0.12	2.25 ± 0.00	75	20	30	0
 Methoxamine	8.02 ± 1.10	8.33 ± 1.16	1.82 ± 1.17	75	20	30	0
 Nadolol	21.03 ± 0.82	21.51 ± 0.70	2.01 ± 3.00	100	50	15	0

 Pindolol	37.78 ± 0.43	38.44 ± 0.43	1.55 ± 2.56	200	50	15	10 % MeOH + 0.6 %TEA
 Propranolol	19.01 ± 0.79	19.30 ± 0.79	1.63 ± 1.00	75	20	15	20 %MeOH
 Ritodrine	16.29 ± 0.44	16.50 ± 0.44	1.54 ± 2.69	150	10	15	2 %TEA
 Terbutaline	10.02 ± 0.34	10.41 ± 0.35	2.97 ± 1.04	75	20	30	0

Conditions: Injection, 4 kV, 2 s; applied voltage, 30 kV. Capillary: 20 × 375 μm; total length, 48.5 cm, effective length, 40 cm. Detection wavelength: 210 nm. In addition, C_b and C_s represent, respectively, concentration of buffer and β-CD; H_3PO_4-TEA buffer pH 2.5 + urea (urea concentration = 2 M when [β-CD] < 20 mM; urea concentration = 4 M when [β-CD] > 20 mM)

Table 5.2 The composition of methylated β-CD

Methylated β-CD	d.s.[a]	Number of methyl groups per β-CD (% of content)											
		8	9	10	11	12	13	14	15	16	17	18	19
DMCD (Kuhn-Trischmann method)	14.2				4	7	11	39	22	14	3		
DMCD (Aldrich Chemical Co., Inc.)	14.4	1	2	3	4	5	7	22	30	16	7	3	
DMCD (Toshin Chemical Co., Ltd.)	14.5						8	41	44	7			
DMCD (Chinoin Chem. Pharm. Works)	14.3		2	3	4	5	8	30	31	10	3	2	2
Partially ethylated β-CD (2-O-64 %,3-O-43 %, 6-O-99 %; Sanraku Inc.)	14.3			3	3	6	6	37	26	12	5	2	

Purification through recrystallization twice in DI water

[a]d.s. represents the average degree of substitution

commercially available methylated β-CDs have an average degree of substitution (d.s.) of 1.4–1.8 per glucose unit. Accordingly, the synthesis of methylated β-CD must be systematically studied to achieve good water solubility, especially when dealing with insoluble or poorly soluble drugs. Among these derivatives, DMCD and TMCD are the most commonly used methylated CDs for CE enantioseparation.

The Kuhn-Trischmann procedure often affords random methylation of β-CD using $Ba(OH)_2$ as a base and dimethyl sulfoxide (DMSO) and N,N-dimethylformamide (DMF) as solvents, yielding products in which the C2 and C6 hydroxyl groups are primarily methylated. Moreover, this approach could afford an isomeric purity of DMCD > 50 %, although only partial methylation of CDs is achieved.

The technical importance of methylated β-CDs and the difference between this method and others were re-investigated by Pitha's team [69]. In their research, it was shown that a 65 % yield of DMCD was obtained through the Kuhn-Trischmann methylation. The composition of the mixed products in comparison with some commercial methylated CDs is shown in Table 5.2. Herein, it is apparent that the number of methyl groups in the product is clustered at 13–15, with the average substitution close to that of the commercial methylated CDs of 14.3–14.5.

In contrast to the first two approaches, the Hakomori's method would afford selectively methylated CDs. In this procedure, methylation was achieved with methyl iodide as methylation reagent, DMF or DMSO as solvent, and sodium hydride as base. The base initially converts CD into its alkoxide form before further reactions. During Hakomori's methylation, tert-butyldimethylsilyl (TBDMS) is commonly used as a protective group with good selectivity [70]. Chen et al. [71] described a convenient synthesis of mono-6-hydroxy-permethylated β-CD, which is outlined in Fig. 5.9. In their strategy, TBDMS is used to selectively protect one of the glucosidic 6-OH groups. The TBDMS groups were stable under the reaction conditions but can be easily removed using ammonium fluoride, avoiding the use of acid which can decompose the parent β-CD. Cousin

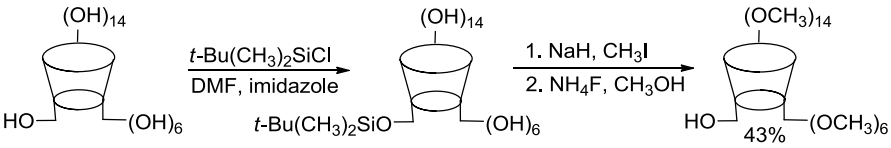


Fig. 5.9 Synthetic strategy for mono-6-hydroxy-permethylated β-CD

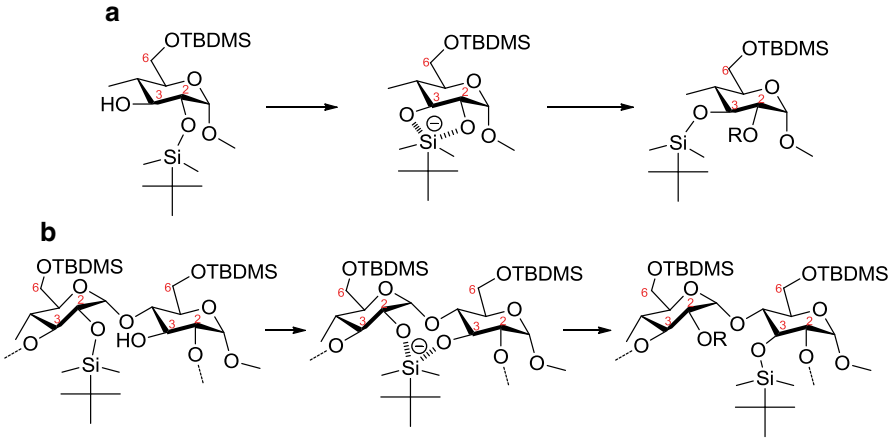


Fig. 5.10 Silyl migration from C2 to C3 position (**a**) intra- and (**b**) inter-glucosidically

et al. [72] developed an analogous strategy for the syntheses of three isomeric mono 2-, 3-, or 6-hydroxy-permethylated β-CDs.

It is noteworthy that the migration of secondary 2-O-tert-butyldimethylsilyl group from O-2 to O-3 would occur when 2,6-di-O-tert-butyldimethylsilylated CD are treated with methyl iodide and sodium hydride in dry tetrahydrofuran, yielding 2-O-alk(en)yl-3,6-di-O-tert-butyldimethylsilyl-CDs. Icheln et al. [73] have investigated the synthetic potential of 2, 6-TBDMS-β-CD for the selective modification at the C3 position, depicting an unexpected migration from the C2 to C3 position. As described in Fig. 5.10, silyl migration might occur intra- or inter-glucosidically, which may be driven thermodynamically. Accordingly, the observed migration could be utilized for the selective modification at C2 position of CD.

Applications of Methylated β-CD

Fanali et al. [54] utilized methylated β-CDs, DMCD, and TMCD for the enantioseparations of several racemic 2-arylpropionic acids (2-APA) in CE. These compounds are shown in Fig. 5.11. In their work, the optimizations of enantioseparation as well as the practical factors that would influence chiral resolution such as the type and concentration of CD and the BGE composition were also described.

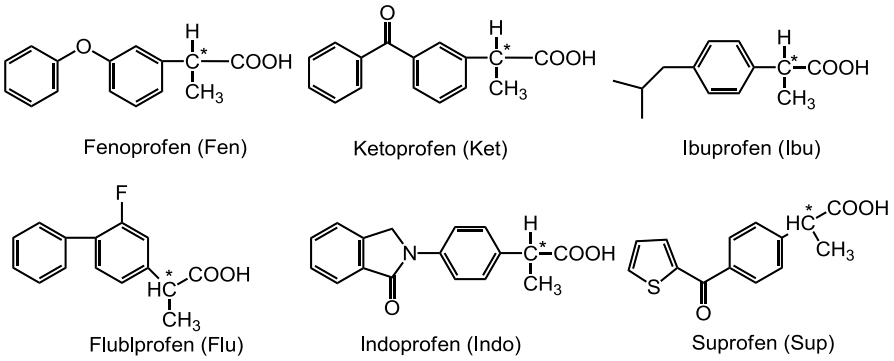


Fig. 5.11 Structures of the studied 2-APAs

These results revealed that TMCD is a better selector as it can achieve the resolution of all selected racemic analytes, whereas DMCD is only able to resolve some of them. Meanwhile, an increase in elution time of these 2-APAs was observed with the increase of the TMCD concentration, which was attributable to the probable complexation order Ibu > Indo ≈ Flu > Fen > Sup > Ket. The effect of the organic modifier on the enantioseparation was also discussed for four enantiomers (Fen, Ibu, Ket, and Flu) using 5 mM TMCD. It was found that increased concentration of methanol resulted in longer elution time. Moreover, the results revealed that the enantioseparation of fenoprofen and flubiprofen was achieved with a resolution of 0.7 and <0.5, respectively, without organic modifier, although baseline separation was not achieved. However, addition of methanol significantly improved the resolution of flubiprofen as shown in Fig. 5.12.

Enantioseparation of gemfibrozil racemic analogues was achieved using TMCD as chiral selector by Ammazzalorso et al. [74]. The optimum separation conditions were also obtained through varying the CD type, pH, and concentrations of running buffer and chiral selector. The migration order of analytes was further determined under optimum conditions by adding a pure optical isomer to the racemic mixture. It was found that chiral selector concentration did not change the migration order but changed the elution time. Under all conditions, (−)-isomers showed higher migration mobility than (+)-isomers (see Fig. 5.13) because the (−)-isomers is able to form more stable inclusion complexes with TMCD.

5.5.2.2 Hydroxyalkylated β-CDs

Hydroxyalkylation of β-CDs

Hydroxyalkylated β-CD derivatives can be readily synthesized through etherification by using alkylene oxide such as ethylene oxide, propylene oxide, or glycidol, leading to the introduction of 2-hydroxyethyl, 2-hydroxypropyl, or 2,3-dihydroxpropyl groups

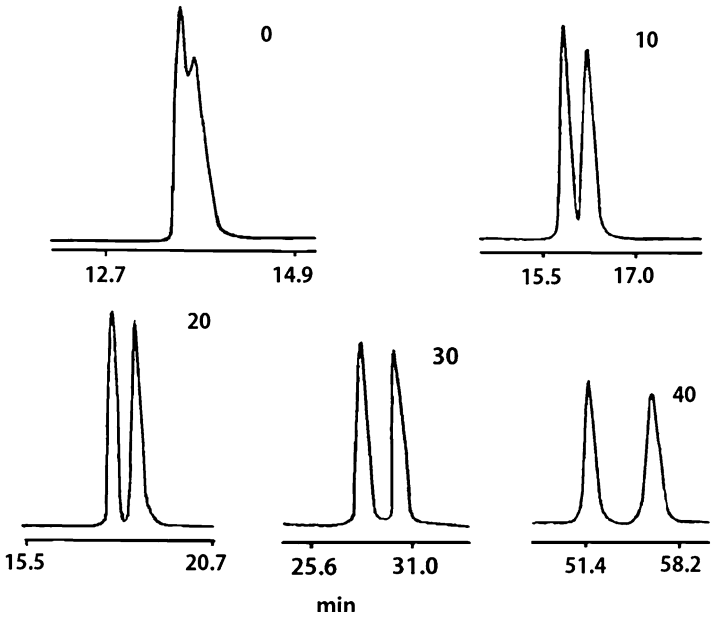


Fig. 5.12 Electrophoretograms of the enantioseparation of flubiprofen with increase methanol concentration (0–40 %). Separation conditions: BGE, 100 mM of MES (pH = 5); Applied voltage, 20 kV, 6.6–3.5 μA (Reprinted from Ref. [54], Copyright 1995, with permission from Elsevier)

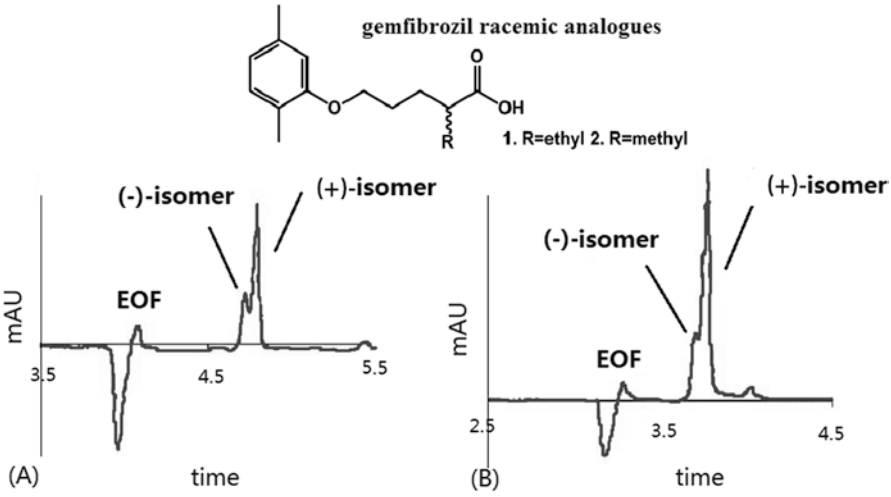


Fig. 5.13 Migration order of pure optical (S)-(+)-isomer enriched mixture, using 50 mM Tris phosphate, pH 6.0: (**a**) 15 mM TMCD and (**b**) 25 mM TMCD (Reprinted from Ref. [54], Copyright 1995, with permission from Elsevier)

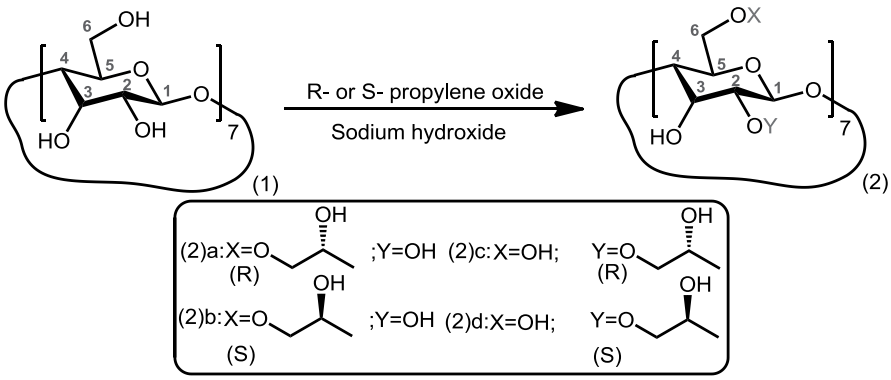


Fig. 5.14 Synthesis of 2- or 6-(2-hydroxypropyl)-β-CDs

to the hydroxyl rims, respectively. The extent of modification is measured by the degree of substitution, namely, the number of alkyls per molecule. In general, these hydroxyalkyl-β-CD derivatives are mixtures of many products. The preparation of hydroxyalkyl-β-CDs is already a mature technology, but the key challenge would be the purification of the products after reaction. At present, the most widely used hydroxyalkyl-β-CD derivative is the commercially available HPCD from several suppliers, like Wacker, Janssen, and Cerestar. Furthermore, the substituent groups possess polar and apolar features affording good solubility and accentuated chiral selectivity. Moreover, additional chiral centers are introduced to the native β-CD which results in broader chiral selectivity spectra.

Pitha and his coworker [75] developed a facile methodology for the preparation of pure 2- and 6-hydroxypropyl-β-CDs. This approach depended on the controlling the alkalinity of the medium (synthesis scheme is shown in Fig. 5.14). In this procedure, an excess of β-CD was reacted with either (R) – or (S)-propylene oxide in 10.7 M aqueous sodium hydroxide, affording the desired 6-O-(2-hydroxylpropyl)-β-CD (HPCD) derivatives 2a or 2b, respectively. Meanwhile, 2-O-(2-hydroxypropyl)-β-CD (2-HPCD) derivatives 2c or 2d were also obtained in dilute aqueous sodium hydroxide (0.37 M), albeit in rather low yields. This different regioselectivity may be attributable to the distribution of the hydroxyalkyl groups corresponding to alkali concentration used in the alkylation. Under strong alkaline conditions, the more accessible primary C6 hydroxyls is more reactive, while the weaker basicity favors the less acidic secondary C2 and C3 hydroxyls.

Hao et al. [76] improved the Pitha's method by modifying the reaction conditions as shown in Fig. 5.15. Herein, the yield increased from 5 to 28 %. The yield is temperature sensitive. Higher temperatures may lead to the decomposition of the propylene oxide, while lower temperatures may limit the alkylation at the C2 position, reducing the product yield.

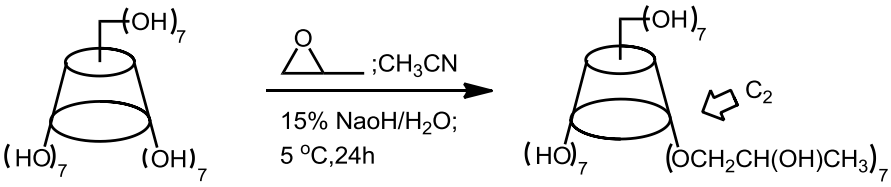


Fig. 5.15 Improved synthesis route of 2-HPCD

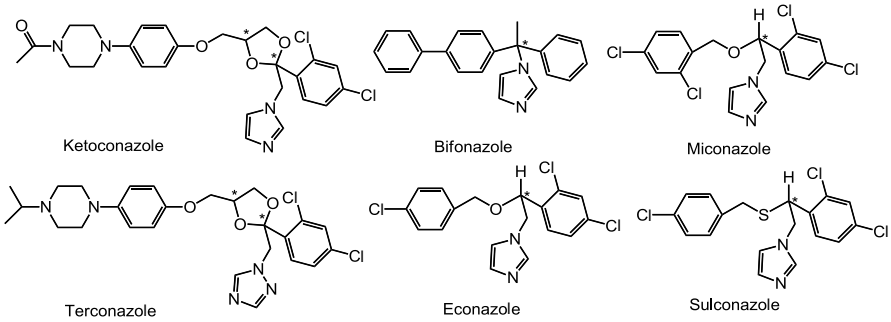


Fig. 5.16 The structures of the six selected compounds

Application of Hydroxyalkyl-β-CDs

Among the hydroxyalkylated β-CD derivatives, the HPCD is most frequently used in chiral analyses. Zhao et al. [77] used CZE for optical purity determination of four aromatic 1,2-diol enantiomers using HPCD as chiral selectors; $R_s > 2.2$ was attainable. Furthermore, this approach's precision was expressed as relative standard deviation (RSD), and the RSD values of elution time and peak area were obtained within 1 and 4 %, respectively. This method allowed for the determination of ee (%) values of targeted isomers as high as 99.6 %. The resolution of imazalil enantiomers in orange was obtained in CE using HPCD as a chiral selector by Kodama et al. [78]. The chiral resolution of imazalil could attain a high value of 6.03, which was higher than that of the case when native β-CD, DMCD, and TMCD were used.

In Castro-Puyana's research [79], the enantioseparations of six weakly basic azole compounds (see Fig. 5.16) were achieved using three neutral β-CDs as chiral selectors: native β-CD, HPCD, and TMCD. The separation results and apparent binding constants calculated for the six compounds at different concentrations of CD selectors are summarized in Table 5.3.

Apparently, native β-CD could not afford high chiral selectivity in comparison with the other two neutral selectors. It is noteworthy that better resolutions of miconazole, econazole, and sulconazole were observed with HPCD, whereas for ketoconazole, terconazole, and bifonazole, TMCD was found to be a better choice. The interactions between the six compounds studied and the native β-CD were

Table 5.3 The enantioseparation results using native CD, HPCD, and TMCD

Compound	Concentration range (mM)	$K_1(M^{-1})$	$K_2(M^{-1})$	α	C_{opt} mM	C_R mM	R_s
β-CD							
Ketoconazole	1–15 ($r=0.998$; $n=5$)	566 ± 105	566 ± 105	1.00	–	–	0.0
Terconazole	1–15 ($r=0.998$; $n=5$)	610 ± 110	610 ± 110	1.00	–	–	0.0
Bifonazole	1–15 ($r=0.998$; $n=5$)	2,767 ± 552	2,767 ± 552	1.00	–	–	0.0
Miconazole	0.5–15 ($r=0.999$; $n=6$)	749 ± 91	932 ± 120	1.24	1.2	1	1.8
Econazole	0.5–15 ($r=0.999$; $n=6$)	622 ± 97	737 ± 121	1.18	1.5	2	1.3
Sulconazole	0.5–15 ($r=0.999$; $n=6$)	2,357 ± 611	2,394 ± 640	1.02	0.4	0.5	0.5
HPCD							
Ketoconazole	0.1–2 ($r=0.998$; $n=4$)	2,477 ± 421	2,495 ± 347	1.01	0.4	1	0.9
	10–120 ($r=0.999$; $n=6$)	142 ± 23	146 ± 22	1.03	7.0	25	1.9
Terconazole	0.1–2 ($r=0.999$; $n=4$)	1,488 ± 84	1,504 ± 38	1.01	0.7	1	0.5
	0–120 ($r=0.999$; $n=5$)	47.4 ± 3.2	46.8 ± 3.2	1.01	21.2	60	0.9
Bifonazole	0.1–20 ($r=0.999$; $n=8$)	2,487 ± 557	2,487 ± 557	1.00	–	–	0.0
Miconazole	0.1–20 ($r=0.999$; $n=8$)	855 ± 62	1,012 ± 78	1.18	1.1	1	2.2
Econazole	0.1–20 ($r=0.999$; $n=8$)	584 ± 32	719 ± 42	1.23	1.5	2	2.0
Sulconazole	0.1–20 ($r=0.999$; $n=8$)	2,161 ± 306	2,406 ± 389	1.11	0.4	0.5	1.2
TMCD							
Ketoconazole	5–30 ($r=0.996$; $n=6$)	53.8 ± 3.7	62.9 ± 3.9	1.17	17.2	20	5.2
Terconazole	2–30 ($r=0.992$; $n=7$)	89 ± 10	104 ± 11	1.16	10.4	10	1.7
Bifonazole	0.1–10 ($r=0.999$; $n=6$)	1,590 ± 239	1,885 ± 252	1.19	0.6	2	1.4
Miconazole	2–30 ($r=0.997$; $n=7$)	113 ± 10	122 ± 10	1.09	8.5	10	1.1
Econazole	20–50 ($r=0.999$; $n=6$)	91.6 ± 2.9	90.4 ± 3.3	1.01	11.0	50	0.5
Sulconazole	2–30 ($r=0.998$; $n=7$)	198 ± 19	215 ± 21	1.09	4.8	5	0.9

K_1 and K_2 represent apparent binding constants for the first- and second-migrating enantiomer, respectively; C_{opt} and C_{Rs} represent optimal CD concentrations experimental CD concentration for maximum enantiomeric resolution, respectively; and r and n represent correlation coefficient of linear fitting and the number of experimental, respectively

stronger than TMCD. Two different HPCD concentration ranges were employed to study the interaction and resolution. Stronger interactions were observed between enantiomers and HPCD at the low concentration range than at the high concentration range. Moreover, bifonazole, econazole, and sulconazole with β-CD were found to interact more strongly with HPCD, while for miconazole, similar interactions were established with β-CD and HPCD. However, the stronger selector-analyte interactions do not necessarily afford better chiral recognition or higher enantioselectivity [55]. Thus, although higher resolutions of econazole, miconazole, and sulconazole were obtained with HPCD, only miconazole presented a higher binding constant in comparison with β-CD.

Reversal of elution order for ketoconazole enantiomers was also observed using different concentrations of HPCD as shown in Fig. 5.17 [79]. The concentrations were varied from 0.1 to 25 mM. The mobility difference decreased with the increase of concentration until it became zero at 5 mM HPCD concentration. Thereafter, the mobility difference would increase again with HPCD concentrations exceeding

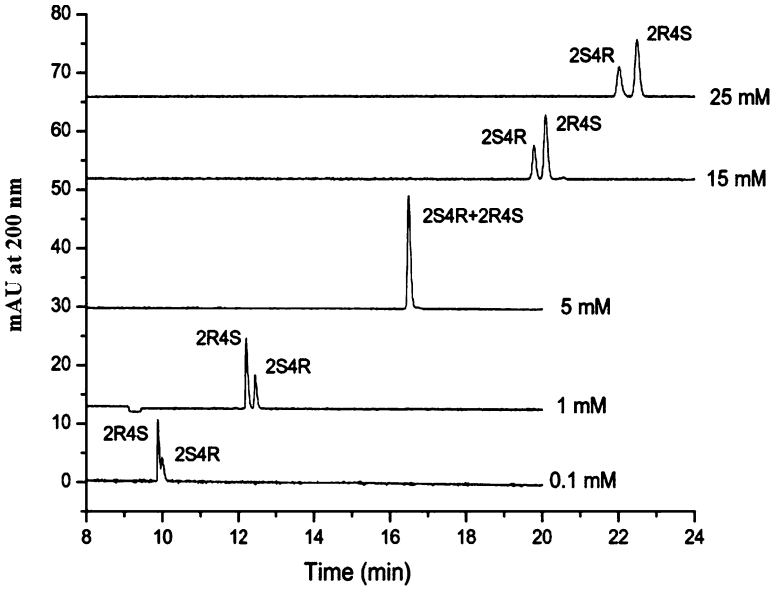


Fig. 5.17 Electropherograms of enantioseparation of ketoconazole enantiomers in 0.1M phosphate buffer at pH 3.0 with different concentrations of HPCD (Reproduced from Ref. [79] by permission of John Wiley & Sons Ltd.)

5 mM. This interesting phenomenon could lead to a reversal in the elution order for ketoconazole enantiomers. The pure form of 2R, 4S-ketoconazole was added to identify the enantiomers by spiking the feature peak. The first migrating peak was corresponding to the 2R, 4S-enantiomer when CD concentration was lower than 5 mM. On the contrary, 2S, 4R-form migrated when the CD concentrations exceeded 5 mM.

5.5.2.3 Enantioseparation in Dual Systems

Native β-CD and its neutral derivatives have been used in CE enantioseparations as chiral selectors alone, given its commercial availability, low cost, and reasonable solubility. Meanwhile, chemical modification of native CDs leads to significant changes in their physical and chemical properties as well as their chiral recognition ability. However, the application of neutral CDs can be limited by factors such as their neutrality, relatively low solubility (particularly when compared with charged CDs). Charged CDs derivatives or other chiral selectors have their own electrophoretic mobility, allowing them to function as carriers in CE for separation of both neutral and ionic analytes. A key disadvantage of neutral selectors is that they cannot separate neutral analytes when used alone. Therefore, attempts have been made to overcome this issue in analyses of neutral enantiomers. Systematic approaches

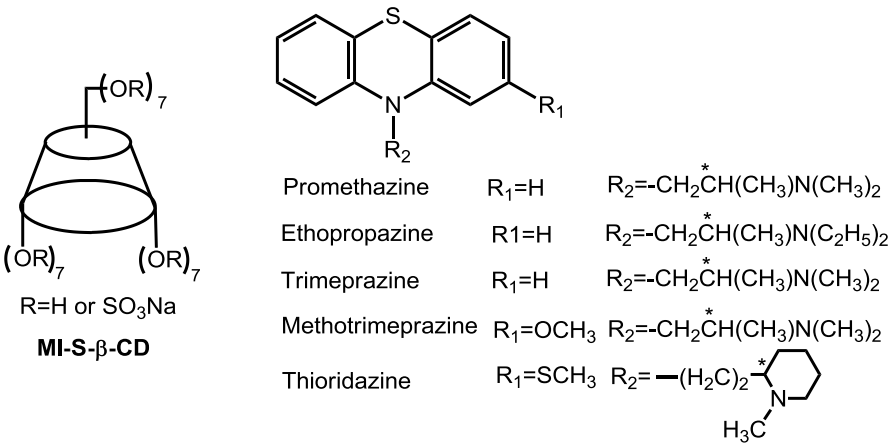


Fig. 5.18 Structures of MI-S-β-CD and five phenothiazines studied

have been developed for CE using dual systems. This approach aimed at achieving higher enantiomeric resolution with lower consumption of chiral selectors. Furthermore, the application of dual systems is rapidly becoming the definitive approach nowadays with the development of various effective selectors [80, 81].

Dual Cyclodextrins System

Neutral β-CDs are more frequently used as one component of dual systems in combination with charged β-CD derivatives. It has been found that the combination of neutral and charged CDs often improves or even enables enantioseparation. For example, Lin et al. [82] demonstrated the enantioseparations of five phenothiazines, including promethazine, ethopropazine, trimeprazine, methotrimeprazine, and thioridazine in CZE using a dual CD system comprising HPCD and randomly sulfate-substituted β-CD (MI-S-β-CD) (Fig. 5.18). It was found that MI-S-β-CD is an excellent chiral selector for ethopropazine and promethazine in concentrations range of 0.5–1.0 % w/v while HPCD interacts strongly with thioridazine and trimeprazine. Consequently, the enantioselectivity of these two phenothiazines was remarkably and synergistically enhanced using the dual system. Moreover, a reversal of elution order for promethazine was observed by varying the concentration of β-CD in the presence of MI-S-β-CD (0.75 % w/v), which may be attributable to the opposite effects of charged and neutral CDs on the mobility of enantiomers.

Lelievre et al. [80] showed the intrinsic selectivity of arylpropionic acid enantiomers using dual systems comprising DMCD or TMCD and cationic mono(6-amino-6-deoxy)-β-cyclodextrin (β-CD-NH_2). It was found that the intrinsic selectivity of both dual systems was equal to the neutral CDs when DMCD or TMCD was used alone, indicating that the β-CD-NH_2 was not stereoselective in such situation but

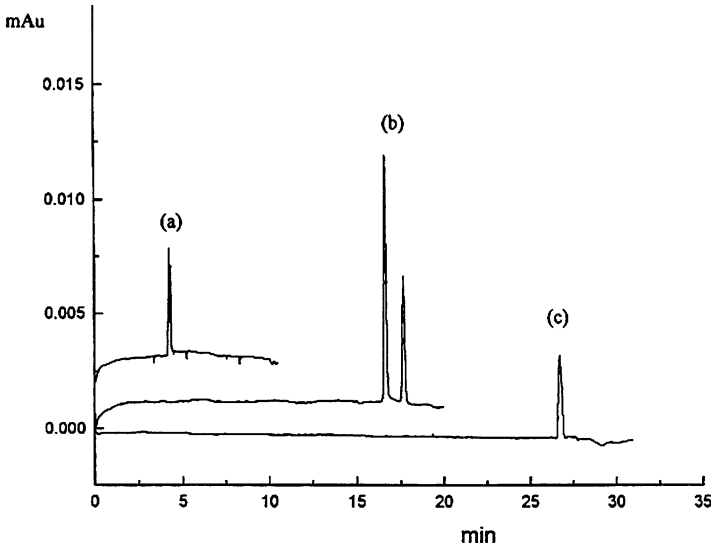
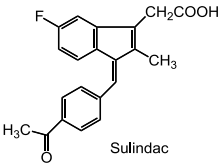
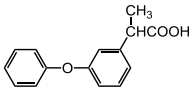
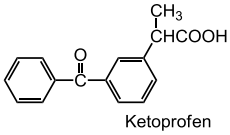
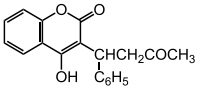
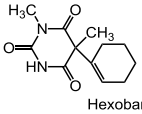


Fig. 5.19 Enantioseparation of ketoprofen. Buffer, 100 mM phosphate solution (pH = 3.0) containing (**a**) 2.5 mM HSCD, (**b**) 2.5 mM HSCD CD and 30 mM TMCD, and (**c**) 30 mM TMCD. Capillary: total length, 44 cm; effective length, 37 cm; 50 μm i.d. Temperature, 25 C.; voltage, −25 kV; detection wavelength, 210 nm; injection time, 6 s (Reprinted from Ref. [2], Copyright 2000, with permission from Elsevier)

that the neutral CDs offered the chiral recognition. They also revealed the potential of the neutral chiral agents for neutral analytes and thereby developed some new strategies. For example, they synthesized new charged CDs, which retain the main advantages of the stereoselectivity of neutral CD. Fillet et al. [2] used a dual system comprising TMCD and anionic heptakis-6-sulfato-β-CD (HSCD) to separate ketoprofen enantiomers. As shown in Fig. 5.19, racemic ketoprofen which was in uncharged form at pH 3 could be only partially resolved with HSCD, while no enantioseparation was observed with TMCD. However, high chiral resolution was attainable using a dual system containing the two CDs.

Comparative chiral resolution studies among the commonly used neutral CDs with an anionic sulfobutyl ether β-CD (SBE) were also performed by Fillet et al. [83]. The results suggested a significant increase in enantiomeric resolution for various acidic drugs using such dual systems comprising neutral and charged β-CDs (see Table 5.4). At pH 3.0, the analytes are hardly ionized and exist mainly in neutral form and consequently cannot be separated using neutral CDs alone. In this study, the anionic afforded enantioselectivity which can be vastly improved upon by addition of the neutral β-CDs, including native β-CDs, MCD, DMCD, TMCD, and HPCD. Addition of DMCD afforded the best enantioselectivity among these neutral β-CDs, attaining a resolution as high as 9.3 for warfarin which is rare in CE enantioseparation. However, HPCD afforded the poorest resolution among the neutral CDs towards these analytes.

Table 5.4 Enantiomeric resolutions by addition of different neutral CDs in the presence of SBE

Analytes	Enantiomeric resolution (R_s)					
	No CD	CD	MCD	DMCD	TMCD	HPCD
 Sulindac	1.4	2.3	3.5	3.8	1.6	<0.7
	<0.7	1.0	1.5	2.8	2.8	<0.7
 Ketoprofen	1.1	1.5	2.1	4.6	2.1	1.3
 Warfarin	2.2	5.1	9.2	9.3	4.3	2.7
 Hexobarbital	1.7	0.8	1.2	3.0	1.9	<0.7

Buffer: 5 mM SBE in 100 mM phosphoric acid at pH 3.0 with triethanolamine containing no CD or CD, MCD, DMCD, TMCD, and HPCD (10 mM)

Cyclodextrins and Crown Ethers

Crown ethers [84] are macrocyclic polyethers which can afford stereoselective inclusion complexes with primary amines. The formation of hydrogen bonds between the amine hydrogens and the lone pairs of the macrocyclic ether oxygen would contribute to the chiral recognition. 18-Crown-6 and 18-crown-6-tetracarboxylic acid have been widely used as additives in CD systems for the separation of amino acids, amino acid derivatives, and various drugs containing primary amino groups. Several novel chiral crown ethers, such as (S,S)-1,7-bis(4-benzyl-5-hydroxy-2-oxo-3-azapenzyl)-1,7-diaza-12-crown-4 [85], (S,S)-1,7-bis(4-benzyloxazolin-2-yl-methyl)-1,7-diaza-12-crown-4 [86], and (S,S)-1,7-bis(4-phenyl-5-hydroxy-2-oxo-3-azapentyl)-1,7-diaza-12-crown-4 [87] were also reported. Crown ether phases have been commercially available from Daicel (Crownpack CR) and USmac Corporation (Opticrown) (see Fig. 5.20). It clearly shows that the substituent groups of Opticrown are perpendicular to the plane of the macrocyclic ring, which divides the space available for the analytes into two domains leading to the formation of two different diastereomeric inclusion complexes.

Combination of β-CDs with crown ethers has been employed in CE enantioseparation applications by Armstrong and his coworkers [88]. The addition of

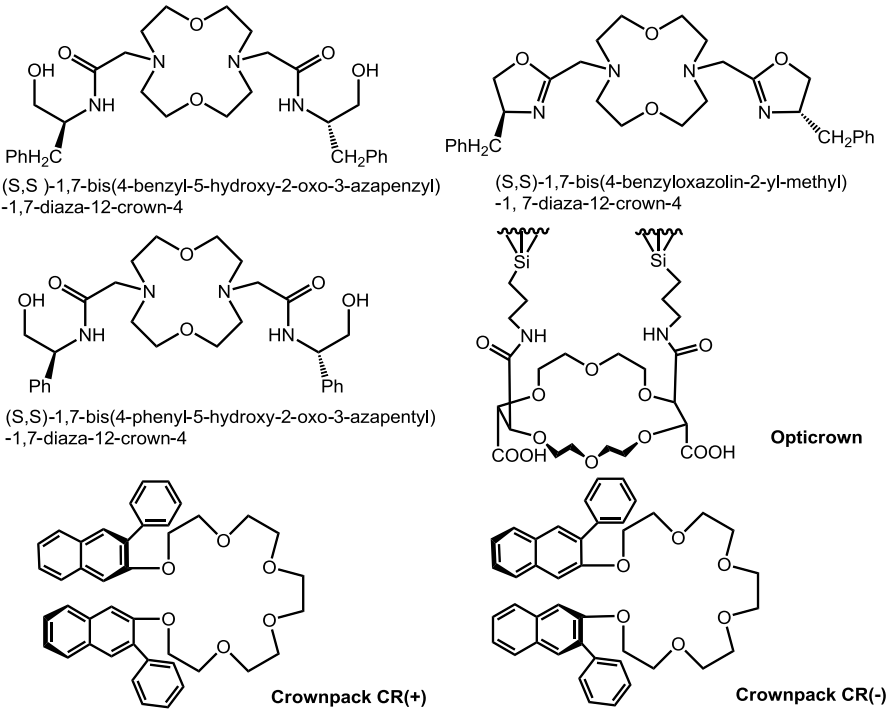


Fig. 5.20 Structures of crown ethers

achiral crown ether (18-crown-6) was shown to significantly improve the chiral resolution of organic enantiomers containing a primary amine moiety. All the analytes studied are depicted in Fig. 5.21. The chiral resolutions of various analytes were induced or enhanced electrophoretically using a buffer comprising 50 mM sodium dihydrogen phosphate; 30 mM of 18-crown-6 with 20 mM of native β-CD or 30 mM of DMCD, TMCD, or HPCD at pH 2.2; and an applied voltage of 15 kV. The effects of concentration of the additives as well as the influence of pH were examined. However, there appears an obvious way of predicting the optimal concentration of 18-crown-6 or the best type of CD for the enantioseparation of a given analyte. Nonetheless, the study revealed that approximately 10 mM CD and approximately 10–15 mM 18-crown-6 would be the minimal concentration required for attaining a baseline enantioseparation in most cases.

As postulated, chiral recognition mechanism was ascribed to the formation of hydrogen bonding between the amine nitrogen and the lone pairs of the macrocyclic ether oxygen atoms. It would be noteworthy that the complexes were not in static states but in dynamic equilibrium whereby chiral resolution and the elution time was a reflection of equilibration among the two-body complexes [(a) and (b) of Fig. 5.22] and the three-body complex (c).

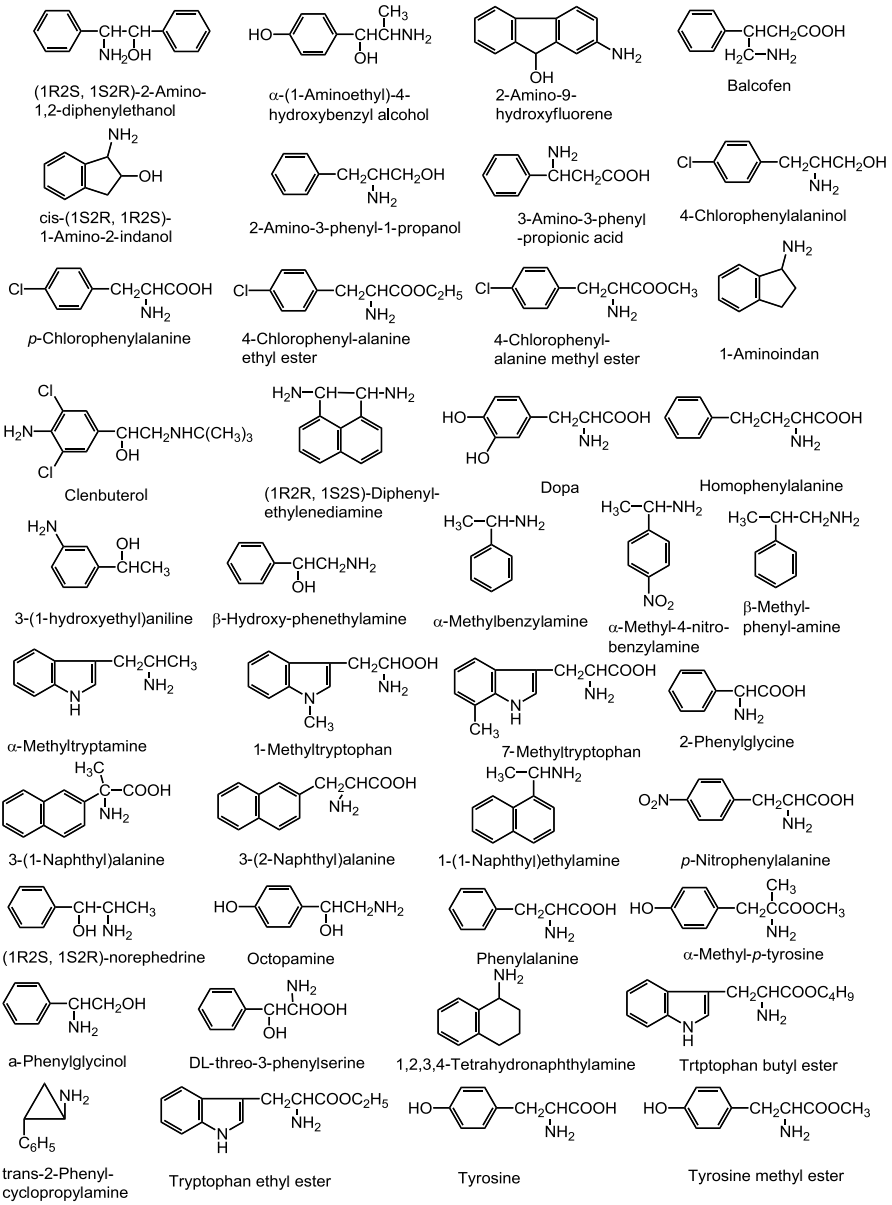


Fig. 5.21 Structures of the applied analytes (all contain primary amino group)

Cyclodextrins and Ion-Pairing Reagents

Chiral ion-pairing reagents (IPRs) can separate racemates by forming diastereomeric ion pairs. These ion pairs differ from each other in mobile phase. Recently, anionic,

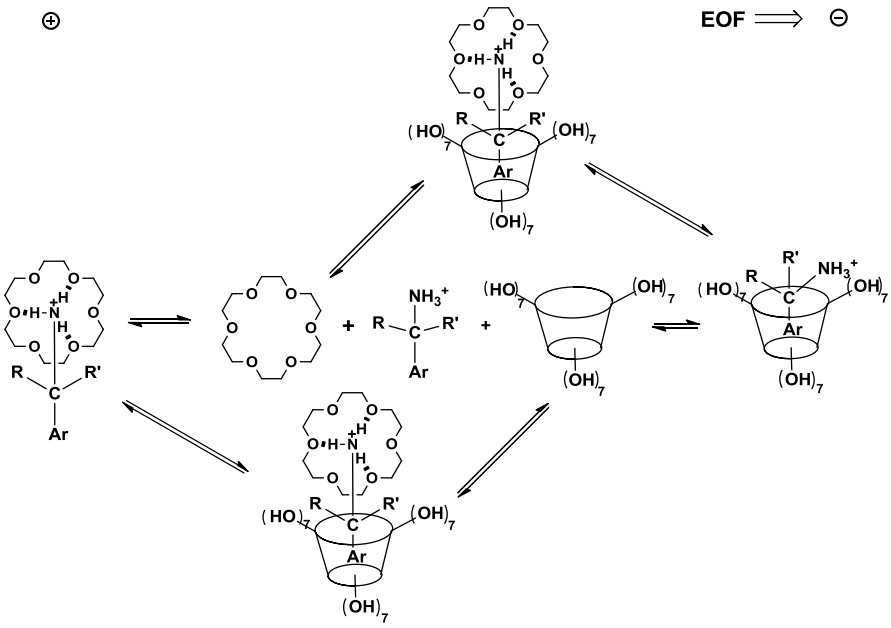


Fig. 5.22 Main equilibrium steps in the formation of the "two-body" and "three-body" complex (Reprinted from Ref. [88], Copyright 1998, with permission from Elsevier)

cationic chiral, and achiral IPRs have been used in enantioseparations in dual systems containing neutral β-CDs. For example, Jira et al. [89] investigated the applicability of ionic chiral as well as achiral IPRs with various β-CD derivatives in enantioseparation by CE. The effects of various IPRs on separation of basic and acidic analytes in the presence of β-CD derivatives are summarized in Table 5.5. The mechanism of separation may be ascribable to diastereomeric ion pairs, which would be responsible for an increase in chiral resolution, while a decrease in elution time and increase in chiral selectivity would be observed simultaneously. The IPRs probably altered the position of the analytes in CD cavity arising from their own inclusions, so that the chiral center of analytes could be a better position for chiral recognition.

Meanwhile, ionic liquids (ILs) have attracted intense interests in recent years in the field of analytical chemistry. In particular, chiral ILs are applicable to the development of new enantioseparation methodologies. Francois et al. [90] focused on the applications of two chiral ILs (ethylcholine and phenylcholine bis (trifluoromethylsulfonyl)imide) (EtCholNTf$_2$, PhCholNTf$_2$) admixed with β-CDs in chiral CE for the enantioseparations of a series of 2-arylpropionic acids as model compounds (see Fig. 5.23).

No direct enantioselectivity for the analytes was observed using these two chiral ILs alone. However, an increase in chiral resolution was observed upon adding these ILs with DMCD or TMCD than when the CDs were used alone. Furthermore, simultaneous increase in enantioselectivity and resolution for

Table 5.5 Influence of various IPRs on enantioseparation of given analytes in the presence of CD

Analytes	Cyclodextrins	IPRs	
Basic analytes	100 mM phosphate buffer + 1.8 % CD + 40 mM IPR(pH 2)		
Cyclodrine	β-CD	CSA, PrSA, PeSA, HxSA, HpSA, OcSA, SCy	+
	MeCD	CSA	+
Cyclopentolate	β-CD	CSA, PrSA, PeSA, HxSA, HpSA, OcSA, SCy	+
	MeCD	CSA	+
Butetamate	β-CD	CSA, PrSA, PeSA, HxSA, SCy	+
Prilocaine	MeCD	CSA	–
Bupivacaine	MeCD	CSA, HxSA	–
Disopyramine	MeCD	CSA	–
Brompheniramine	β-CD	HxSA	+
Doxylamine	β-CD	CSA, PrSA, PeSA, HxSA, HpSA, SCy	–
Basic analytes	100 mM phosphate buffer + 1.8 % CD + 40 mM IPR(pH 7.2)		
Biperidene	β-CD	HxA, HpA, OcA	–
Brompheniramine	β-CD	HxA, HpA, OcA	–
	HPCD	OcA	+
Bupivacaine	MeCD	HxA, HpA, OcA	–
Cyclodrine	β-CD	HxA, HpA, OcA	+
	HPCD	HxA, HpA, OcA	+
Cyclopentolate	β-CD	HxA, HpA, OcA	+
	HPCD	HxA, HpA, OcA	+
	MeCD	OcA	+
Dipiproverine	HPCD	HxA, HpA, OcA	–
	MeCD	HxA, HpA, OcA	+
Homatropine	β-CD	HxA, HpA,	–
	HPCD	HxA, HpA, OcA	–
	MeCD	HxA, HpA, OcA	–
Pheniramine	β-CD	HpA, OcA	–
Terbutaline	β-CD	HxA, HpA, OcA	–
	HP-β-CD	OcA	–
	Me-β-CD	OcA	–
Acidic analytes	100 mM phosphate buffer + 1.8 % CD + 40 mM IPR(pH 6.9)		
α-(1-Hydroxycyclopentyl)-phenylacetic acid	β-CD	CSA, HxSA	+
	MeCD	CSA	–
Phenprocoumon	HPCD	CSA	+
	MeCD	CSA	+

CSA camphersulfonic acid, *PrSA* propanesulfonic acid, *PeSA* pentanesulfonic acid, *HxSA* hexanesulfonic acid, *HpSA* heptanesulfonic acid, *OcSA* octanesulfonic acid, *SCy* sodium cyclamate, *HxA* hexanoic acid, *HpA* heptanoic acid, *OcA* octanoic acid

Separation effect: "+" represents increased and "–" represents decreased

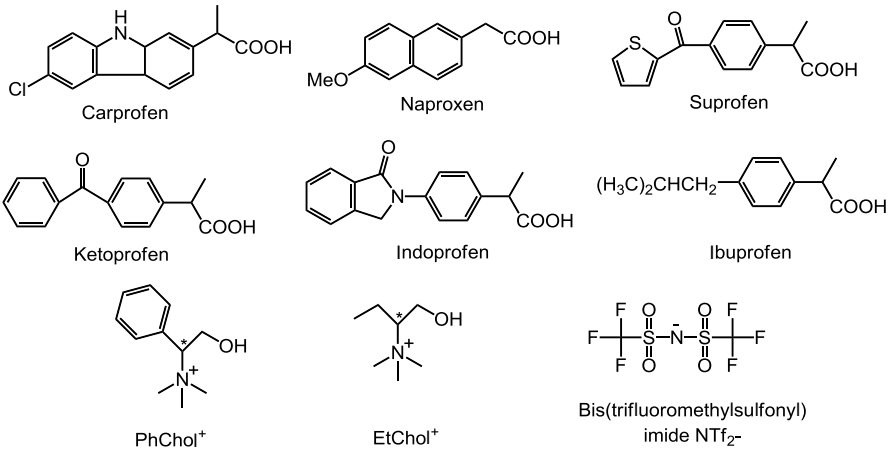


Fig. 5.23 Structures of 2-arylpropionic acids and EtCholNTf2, PhCholNTf2

Table 5.6 Cases of simultaneous increase in enantioselectivity and chiral resolution

Selectors	C_{salt} (mM)	H_2O/MeOH (V/V)	α_{eff}	R_s
TMCD	0	90/10	1.00	0.00
TMCD + EtChol	60	90/10	1.15	2.90
TMCD + EtChol	5	90/10	1.09	0.81
TMCD + PhChol	5	90/10	1.11	1.06
TMCD	0	75/25	1.00	0.00
TMCD + EtChol	5	75/25	1.05	1.09
TMCD + PhChol	5	75/25	1.05	0.41
DMCD	0	100/0	1.00	0.00
DMCD + PhChol	5	100/0	1.12	0.98
DMCD	0	90/10	1.00	0.00
DMCD + EtChol	5	90/10	1.05	0.99
DMCD + PhChol	5	90/10	1.05	0.78
DMCD	0	75/25	1.00	0.00
DMCD + EtChol	5	75/25	1.05	1.06

C_{salt} represents the salt concentration of the buffer. Conditions: capillaries: 50 μm i.d. × 35 cm (effective length, 26.5 cm); applied voltage: 25 kV; temperature: 25 °C. UV wavelength: 230 nm

carprofen was obtained in some cases as summarized in Table 5.6, indicating a synergistic effect between chiral IL and CD derivatives. The synergistic effect could even enable the enantioseparation with α_{eff} increasing to 1.15 from 0 by adding 60 mM EtChol to TMCD buffer. However, the presence of the phenyl moiety in IL cation appeared less important in promoting these synergistic effects when using methanol or a low salt concentration in the BGE. It was also

found that analyte and IL cation competed to form complexes with CDs during the enantioseparation process.

Herein, the application of anionic/cationic ion-pairing reagents with CDs appears to be a promising analytic methodology for separating acidic and basic enantiomers in CE. This analysis approach would depend on the type of CDs and IPRs and their concentrations.

Cyclodextrins and Surfactants

Surfactants are amphiphilic molecules comprising a hydrophilic head and a hydrophobic tail. Micelles are formed when the surfactant concentration exceeds the critical micelle concentration (CMC). MEKC is one of the most popular techniques in CE, where neutral and charged analytes can be analyzed [91]. Various ionic chiral or achiral micelles can be used for enantioseparation in CD-modified MEKC (CD-MEKC), e.g., the conventional micelles of sodium dodecyl sulfate (SDS) and bile salts.

Bile salts are natural chiral surfactants, which have been used with CDs for enantioseparations. As an example, Okafo et al. [92] reported the enantioseparations of some Dns-DL-amino acids using a mixture of taurodeoxycholic acid (50 mM) and β-cyclodextrin (20 mM) which afforded excellent resolutions. Sodium taurodeoxycholate [93] (STDC) was also used in the presence of native β-CD for enantioseparation of amino acid enantiomers in CE; 19 enantiomeric pairs could be well resolved in this dual system. The resolution was found to be far superior to that achieved by using β-CD alone (see Table. 5.7). The chiral resolutions for valine, isoleucine, and phenylalanine could exceed 5.0 with STDC concentration >30 mM. Moreover, it would be interesting to note that the mobility difference ($\Delta\mu = \mu_R - \mu_L$) between enantiomeric pairs did not significantly increase in this dual system. However, the summed mobility ($\sum\mu = \mu_R + \mu_L$) of the enantiomeric pairs would be decreased as a result of the decrease in EOF. The change in EOF was probably responsible for the improved chiral resolution. Consequently, the change of EOF should be taken into account as an important factor in dealing with the chiral resolution. This observation would probably help explain the resolution improvement in some dual-selector systems, where the mechanism is still unclear at present.

In addition, polymeric surfactants (PSs) are also applicable for enantioseparations. As an example, Akbay et al. [94] have reported the enantioseparations of 12 mono-methylbenz[*a*]anthracene (MBA) isomers using poly(sodium 10-undecenyl sulfate) (poly-SUS) in combination with β-CD, DMCD, TMCD, and HPCD. It was found that these chiral CDs exhibited different enantioselectivity and chiral resolutions towards the analytes in such dual system. Valle et al. [95] realized the enantioseparations of binaphthyl derivatives using β-CD in combination with various polymeric surfactants. The elution order of enantiomers was

Table 5.7 Enantioseparations with various concentrations of STDC

	C_{STDC} (0 mM)		C_{STDC} (15 mM)		C_{STDC} (30 mM)		C_{STDC} (60 mM)	
Analytes	R_S	$N/10^5$	R_S	$N/10^5$	R_S	$N/10^5$	R_S	$N/10^5$
Aspartic acid	1.76	2.3	1.87	2.4	2.32	2.8	2.53	2.0
Glutamic acid	3.22	2.3	3.68	2.4	4.89	3.4	5.25	2.0
Lysine	0.67	3.3	2.08	3.6	2.60	4.3	1.20	2.4
Cysteine	1.31	3.1	2.76	3.5	2.15	3.1	1.29	2.0
Tyrosine	1.00	2.6	2.70	2.5	2.07	3.8	1.11	2.4
Valine	3.16	3.7	4.28	3.8	6.78	4.6	7.51	3.2
Isoleucine	2.71	3.6	4.20	4.2	6.01	4.6	5.88	2.8
Phenylalanine	2.52	4.3	3.72	3.9	6.10	4.2	7.66	3.0
Proline	2.05	3.7	2.07	3.5	2.78	3.0	2.95	2.3
Leucine	1.99	4.0	2.94	4.4	3.90	4.2	4.06	3.0
Tryptophan	1.68	3.5	2.87	4.2	4.54	4.3	3.79	2.2
Methionine	1.62	3.7	2.15	3.9	3.14	4.3	3.25	2.9
Histidine	1.23	3.3	1.99	3.4	3.47	4.2	4.02	3.2
Threonine	1.25	3.8	1.69	3.9	2.22	3.9	2.43	2.5
Asparagine	1.27	3.9	1.58	3.9	2.02	3.3	2.52	3.3
Serine	1.05	3.0	1.34	3.1	2.07	3.8	2.04	2.3
Glutamine	0.97	3.5	1.29	3.6	1.69	3.4	2.00	2.8
Arginine	–	–	–	–	1.36	4.7	1.52	3.1
Alanine	0.60	3.4	0.93	3.7	1.18	3.2	1.25	2.6

N is the geometrical plate numbers of the enantiomers. C_{STDC} is the concentration of STDC. Separation conditions: 25 kV and 25 °C; capillary, 60 cm (50 cm effective), 75 mm i.d; buffer, 18 % v/v of isopropanol and 150 mM borate at pH 8.0 with 30 mM β-CD

further investigated in the presence of β-CD and poly(sodium N-undecanoyl D-alaninate) [poly(D-SUA)], poly(sodium N-undecanoyl D-leucinate) [poly(D-SUL)], poly(sodium N-undecanoyl D-valinate) [poly(D-SUV)], poly(sodium N-undecanoyl L-alaninate) [poly(L-SUA)], poly (sodium N-undecanoyl L-leuci-nate) [poly(L-SUL)], and poly(sodium N-undecanoyl L-vali-nate) [poly(L-SUV)]. It would be noteworthy that a reversal of migration order for (±)-1,1′-binaphthyl-2,2′-diamine (BNA) was observed with three D-PSs. Take poly(D-SUA) as example (see Fig. 5.24); R-BNA was eluted first using 6 mM poly(D-SUA) alone with a resolution of 1.35. The enantiomers were simultaneously eluted upon the addition of 3 mM β-CD within a shorter time. The reversal of migration order was observed when a β-CD concentration was increased to 5 mM, which afforded an increased resolution of 1.42, and then further to 1.43 with shorter retention time when 8 mM CD was used. However, no such phenomenon was observed for three L-PSs. Meanwhile, there was an increase in both enantioselectivity and resolution with increasing β-CD concentration, indicating that the S-enantiomer of BNA has a greater affinity for the β-CD. This clearly demonstrated an enhancement in chiral recognition of BNA enantiomers when a dual chiral selector system is used.

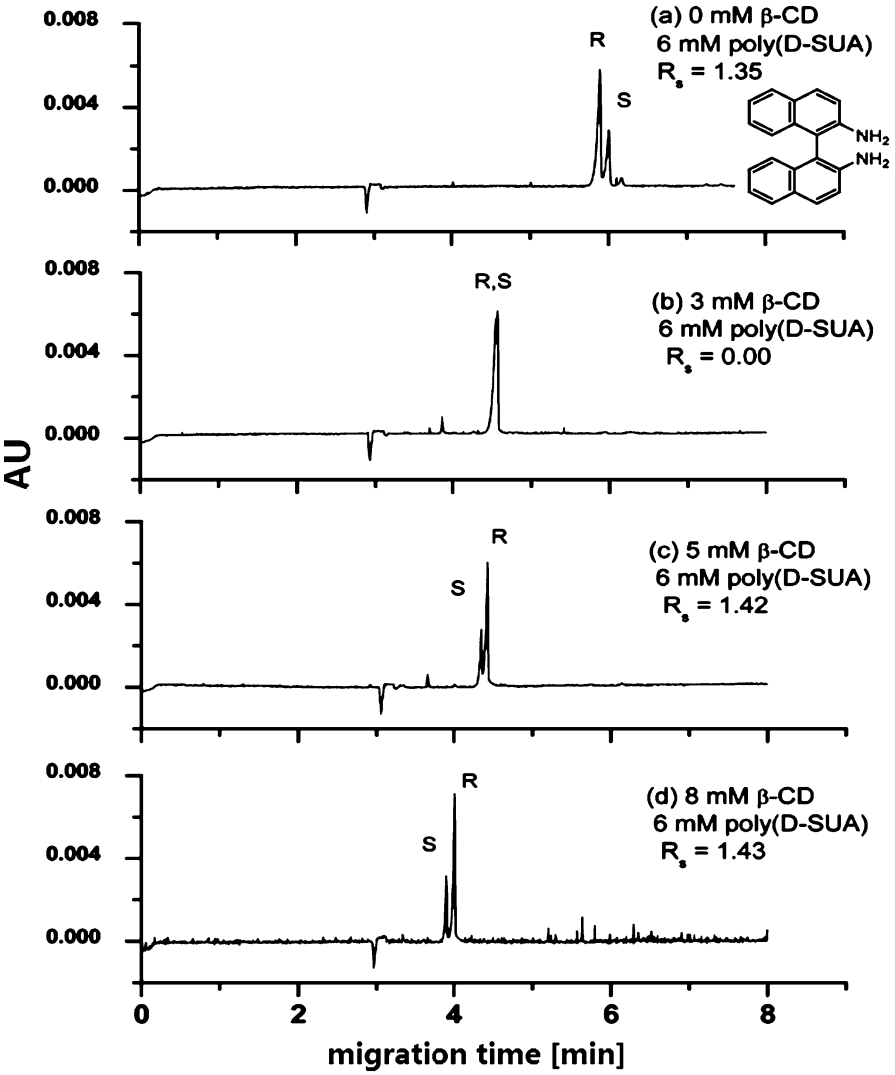


Fig. 5.24 Chromatograms of enantioseparations for BNA enantiomers using 6 mM poly(D-SUA) with (**a**) 0 mM β-CD, (**b**) 3 mM β-CD, (**c**) 5 mM β-CD, and (**d**) 8 mM β-CD. Conditions, 100:10 mM Tris/borate buffer; pH 10; 30 kV; UV detection at 254 nm (Reproduced from Ref. [95] by permission of John Wiley & Sons Ltd.)

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Chapter 6
Anionic Cyclodextrins for Capillary Electrophoresis

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Abstract An overview of negatively charged (anionic) cyclodextrins for enantiomeric separation using capillary electrophoresis (CE) is presented. Starting from commercially available multisubstituted anionic cyclodextrins, this chapter gives an updated summary of single-isomer anionic CDs for chiral CE. The correlation of cyclodextrins structure and their enantioselectivities, as well as the optimization of analytical parameters for enantiomeric separation with these cyclodextrins, are discussed.

Abbreviations

CE	Capillary electrophoresis
CD	Cyclodextrin
CEKC	Capillary electrokinetic chromatography
EOF	Electroosmotic flow
SBE-β-CD	Sulfobutyl ether β-CD
CM-β-CD	Carboxymethyl-β-CD
SEE-β-CD	Sulfoethyl ether β-CD
S-β-CD	Sulfated β-cyclodextrin
CZE	Capillary zone electrophoresis
HP-β-CD	2-Hydroxypropyl-β-cyclodextrin
LPME	Liquid-phase microextraction
DCIT	Desmethylcitalopram
PPF	Propafenone
5OH-PPF	5-Hydroxy-propafenone

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NOR-PPF	N-despropyl-propafenone
CE-β-CD	Carboxyethylated β-CD
DHPs	1,4-Dihydropyridines
CM-γ-CD	Carboxymethyl-γ-CD
Su-β-CD	Succinylated β-CD
THP	Trihexyphenidyl
M-β-CD	Methylated β-cyclodextrin
DM-β-CD	Heptakis(2,6-di-O-methyl)-β-cyclodextrin
SBE-γ-CD	Sulfobutyl ether γ-CD
DOPA	Dihydroxyphenylalanine
MDOPA	Methyldihydroxyphenylalanine
CDOPA	Hydrazinomethyldihydroxyphenylalanine
HDAS β-CD	Heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin
HP-γ-CD	Hydroxypropyl-γ-CD
HxS-α-CD	Hexakis(6-O-sulfo)-α-cyclodextrin
HS-β-CD	Hepta-6-sulfato-β-cyclodextrin
OS-γ-CD	Octa(6-O-sulfo)-γ-cyclodextrin
HDMS-β-CD	Heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin
HxDAS-α-CD	Hexakis(2,3-di-O-acetyl-6-O-sulfo)-α-CD
ODAS-γ-CD	Octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin

6.1 Introduction

The field of enantioseparation in pharmaceutical analysis and bioanalysis for the monitoring of drugs, drug impurities, synthetic precursors, side products, or metabolites has been extensively explored over the last two decades [1–4]. Indirect chiral separations have been performed with several chromatographic techniques such as GC, HPLC, and SFC [1]. Capillary electrophoresis (CE), as a new, efficient, and fast separation technique, has become a routine method for chiral separation in drug development, due to its features of high efficiency, fast analysis, easy method development and minimum consumption of chiral selectors and analytes [1–4].

The first separation of enantiomers by CE dated back to 30 years ago by Zare and coworkers [5]. The field of chiral CE remained in relative obscurity until the appearance of two key papers in 1989. Fanali [6] demonstrated the potential of natural macrocycles, cyclodextrins (CD), as chiral selectors in background electrolyte (BGE) for free-solution CE, and Terabe [7] showed the first application of a charged CD as a potential chiral selector in capillary electrokinetic chromatography (CEKC). Native and neutral derivatized CDs have been employed very extensively as chiral selectors ever since. Besides the often-used neutral CDs, charged CDs are gaining increased attentions because of their ability to perform fast chiral separations at low concentrations and the possible chiral separations of neutral analytes. Moreover, charged CDs are expected to give the best resolving power towards oppositely charged analytes, because the interactions between the CDs and analytes are now not only based on

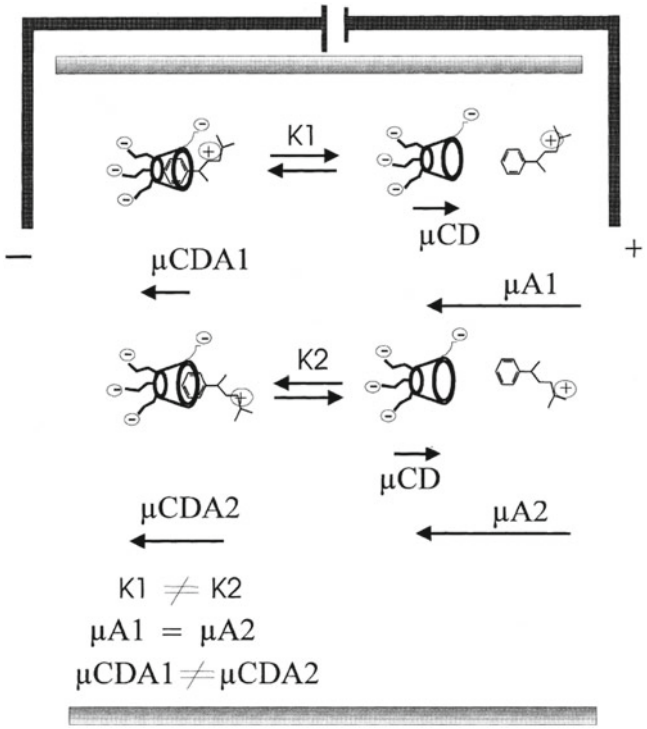


Fig. 6.1 Illustration of the separation of a cationic analyte with an anionic CD, under the conditions that the EOF is eliminated and that both analyte and CD are fully charged (Reproduced from Ref. [1] by permission of John Wiley & Sons Ltd.)

inclusion complexation but also on strong electrostatic interactions [1]. Take the cationic analyte with an aninonic CD as example; the mobilities of the anionic CD, the cationic analyte, and their inclusion complexation products (CDA1 and CDA2) under the ideal conditions can be depicted in Fig. 6.1, where the CD and analyte are fully charged and electroosmotic flow (EOF) is eliminated. As shown in Fig. 6.1, the mobilities of two enantiomers are equal (μA1 = μA1), whereas the mobility of the CDA1 complex is smaller than the mobility of the CDA2 complex. The reason is explained by the stronger interactions including both inclusion complexation and electrostatic interaction for CDA1 than the single inclusion complexation for CDA1. Therefore, the equilibrium constants (binding constants, K_1 and K_2) are different for inclusion complexation between the CD and two enantiomers. In a word, both the binding constants and mobilities of the complexes CDA1 and CDA2 are different, which offer the driving forces for enantiomer discrimination.

In practical CE analysis where the EOF is present, the EOF direction can be altered by changing the polarity of the applied voltage in CE. In the normal mode of CE as shown in Fig. 6.1, the EOF is directed towards cathode, while the mobility

of CD is towards the anode. This "countercurrent" flow of the negatively charged additive with respect to the EOF is advantageous to obtain the maximum resolution for the analytes. This behavior was observed by Stalcup et al., who reported the first application of randomly substituted polyanionic β-CD, sulfobutyl ether β-CD (SBE-β-CD), as chiral selector in CE [8]. The SBE-β-CD achieved superior enantioseparation to those possible with neutral selectors such as β-CD or heptakis(2,6-dimethyl)-β-CD. Their research demonstrated that the maximum opportunity for chiral separation might exist when the electrophoretic mobility of the chiral selector is opposite to that of the analyte. The use of sulfonated cyclodextrins has been further highlighted by Lurie [9] and Terabe [10] and Blaschke [11] after they noted the advantage of the "countercurrent" flow of the negatively charged additive with respect to the EOF. Charged CDs are, thus, valuable for the chiral separation in CE due to the fact that a large library of pharmaceutically interesting compounds can be easily protonated. By taking advantage of electrostatic interactions and countercurrent flow of the chiral additive, charged CDs have been widely employed in chiral CE. In some cases where only inclusion complexation or electrostatic interactions occurs, the capability of enantioseparation is reduced because of the lower chiral recognition for one of the enantiomers. Due to the difference of interaction between CD and analytes, the migration order of analytes may thus be altered when using different types of charged CDs, as depicted in Fig. 6.2 [12].

Charged CDs are developed through chemical modification of hydroxy groups on the rims of CD. In particular, the β-CD derivatives have been found to exhibit an excellent resolving ability for chiral molecules containing a (substituted) aromatic ring and, for instance, a chargeable group like amino or a carboxyl group. For anionic CDs, the hydroxy groups are substituted with either strongly charged functional groups (e.g., sulfate or sulfoalkyl ether groups) or weakly charged functional groups (e.g., carboxymethyl or carboxyethyl groups). Sulfated CDs and the often-used SBE-β-CD can be used over a wide range without affecting their net charge. Due to the extensive application of anionic CDs as chiral selectors in CE, several anionic CDs including carboxymethyl-β-CD (CM-β-CD), SBE-β-CD, and sulfoethyl ether β-CD (SEE-β-CD) have been developed as commercially available [13]. And the research efforts devoting to the use of anionic CDs in chiral CE have been summarized in several excellent reviews [1–4, 13].

The objective of this chapter is to update the use of the anionic CDs as chiral selectors for enantiomer separation in pharmaceutical analysis, starting with sulfated β-cyclodextrin.

6.2 Sulfated β-Cyclodextrin (S-β-CD)

The utilization of sulfated β-cyclodextrin (S-β-CD) as a chiral additive for capillary zone electrophoresis (CZE) has been reported in many publications since Stalcup's report in 1995 [14]. The same group [8] reported the chiral separations of 56 compounds of pharmaceutical interest by the use of S-β-CD whose average degree of

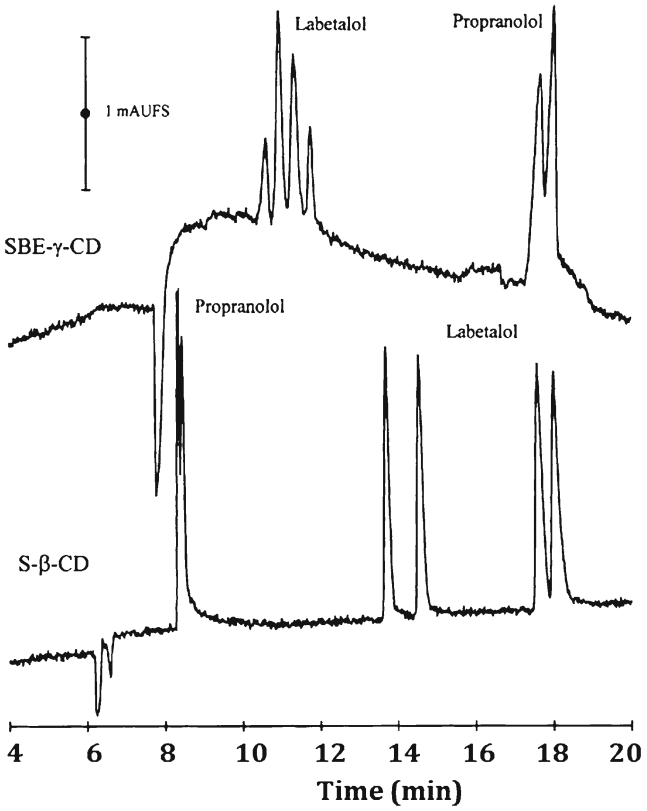
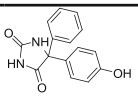
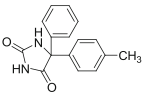
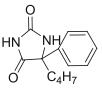
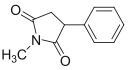
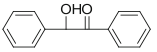
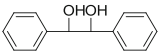
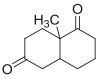
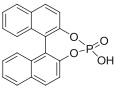
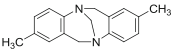
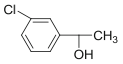
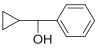
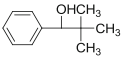
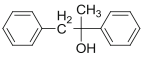
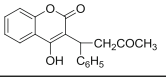


Fig. 6.2 Electropherogram showing the reversal of the migration order of racemic labetalol and propranolol using 30 mM phosphate buffer containing either SBE-γ-CD (20 g/L) or S-β-CD (7.7 g/L) (Reprinted from Ref. [12], Copyright 1998, with permission from Elsevier)

substitution is 7–11. A variety of neutral racemic compounds that are difficult to separate with neutral CDs were successfully resolved by using S-β-CD in background electrolytes (BGEs) at different pH values. Afterwards, sulfated CDs have been applied for the separation of a number of neutral and charged enantiomers such as anesthetics, antihistamines, antiarrhythmics, and β-blockers [9–12, 14, 15].

The chiral resolution data of neutral compounds using S-β-CD in either low- or high-pH BGEs are listed in Table 6.1 [14]. As shown in the table, most of the solutes having a chiral center located on a ring or locked ring structures were resolved. The results demonstrated that S-β-CD has sufficient solubility in an electrolyte to act as a good enantioselective agent for the resolution of studied neutral enantiomers. Comparing the data obtained from different CE conditions, one may find that better resolutions were achieved with the reduction or complete lack of EOF in low pH BGEs. It should be noted that phensuximide had the longest migration time (t, 51.12/60.65 min), implying the weakest interactions with the S-β-CD of all the

Table 6.1 Electrophoretic data for neutral analytes resolved using S-β-CD as chiral agent in different CZE condition

Analyte	Structure	Migration time	R_s	CE conditions
5-(4-Hydroxyphenyl)-5-phenylhydantoin		15.61/16.72	2.94	Buffer A, 15 kV
		14.47/15.62	5.6	Buffer B, 15 kV
5-(4-Methylphenyl)-5-phenylhydantoin		15.20/16.06	2.96	Buffer C, 15 kV
		12.88/14.04	5.4	Buffer B, 15 kV
5-Cyclobutyl-5-phenylhydantoin		11.53/12.36	3.95	Buffer C, 15 kV
		21.30/32.33	26.0	Buffer B, 15 kV
Phensuximide		19.32/20.1	1.94	Buffer D, 8 kV
		51.12/60.65	8.3	Buffer B, 15 kV
Benzoin		26.79/28.20	1.77	Buffer D, 8 kV
		19.55/21.16	2.8	Buffer B, 15 kV
Hydrobenzoin		8.63/10.12	4.4	Buffer A, 15 kV
		30.31/57.95	26.0	Buffer B, 15 kV
9-Methyl-$\Delta^{5(10)}$-1,6-dione		17.88/18.46	2.94	Buffer E, 8 kV
		42.72/48.79	6.5	Buffer B, 15 kV
1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate		24.31/25.19	2.43	Buffer A, 8 kV
		16.33/16.92	2.4	Buffer B, 15 kV
1,1′-Bi-2-naphthol		9.35/10.17	2.72	Buffer A, 15 kV
		35.41/50.24	11.1	Buffer B, 15 kV
Troger's base		16.81/17.78	2.44	Buffer F, 15 kV
		10.00/13.06	16.1	Buffer B, 15 kV
1,2,3,4-Tetrahydro-1-naphthol		16.66/17.22	2.2	Buffer B, 15 kV
1-(3-Chlorophenyl) ethanol		29.49/31.91	4.9	Buffer B, 15 kV
α-Cyclopropylbenzyl alcohol		34.24/47.01	15.7	Buffer B, 15 kV
2,2-Dimethyl-1-phenyl-1propanol		12.50/13.32	2.6	Buffer B, 15 kV
1,2-Diphenyl-2-propanol		18.42/19.18	2.3	Buffer B, 15 kV
2-Phenylcyclohexanone		24.15/25.34	2.7	Buffer B, 15 kV
Warfarin		25.20/30.89	8.6	Buffer B, 15 kV

Buffer A, 2 %CD in10 mM pH 8.0 Na_2HPO_4; Buffer B, 2 %CD in10 mM pH 3.8 Na_2HPO_4; Buffer C, 10 %MeOH+2 % CD in 10 mM pH 8.0 Na_2HPO_4; Buffer D, 3 %CD in 10 mM pH 7.0 Na_2HPO_4; Buffer E, 2 %CD in 10 mM pH 6.0 Na_2HPO_4, pH 6.0; Buffer F, 30 % MeOH+2 % CD in 10 mM pH 8.0 Na_2HPO_4

analytes in Table 6.1. Comparing the migration times and enantioresolution obtained for phensuximide with those of other studied enantiomers, the single phenyl ring of phensuximide, with no para-hydrogen bonding (e.g., 5-(4-hydroxyphenyl)-5-phenylhydantoin (t, 14.47/15.63 min)) or hydrophobic moiety (e.g., 5-(4-methylphenyl)-5-phenylhydantoin (t, 12.88/14.04 min)) to assist in immobilizing the analyte within the CD cavity, may partially account for its relatively weak binding. In the case of 5-cyclobutyl-5-phenylhydantoin, the decreased binding to CD, as indicated by longer migration times, resulted in dramatically enhanced resolution ($R_s = 26.0$) relative to 5-(4-hydroxyphenyl)-5-phenylhydantoin ($R_s = 5.6$) or 5-(4-methylphenyl)-5-phenylhydantoin ($R_s = 5.4$).

The chiral resolution data for the cationic compounds using S-β-CD are tabulated in Table 6.2. The combination of ion-pairing and inclusion complexation leads to stronger binding between the cationic compounds and the sulfated CD than that with the neutral analytes. This is proved by the data that the migration times for the basic compounds used ($t_{av} = 13 \pm 7$ min) were shorter than the neutral analytes ($t_{av} = 25 \pm 11$ min). As described in the above tables, a large number of structurally diverse analytes, neutral as well as cationic, were successfully enantioresolved.

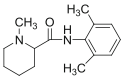
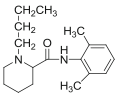
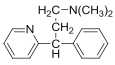
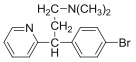
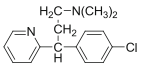
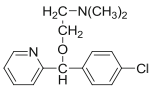
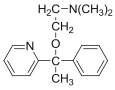
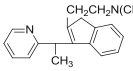
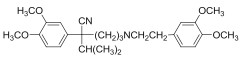
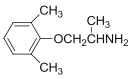
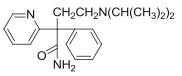
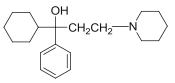
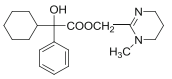
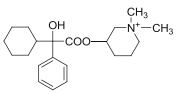
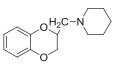
The sulfated β-cyclodextrin and carboxymethyl β-cyclodextrin were also employed as the chiral selectors for the enantioseparation of 37 chiral sulfoxides and sulfinate esters [16]. Table 6.3 shows the structures, migration times, mobility difference ($\Delta\mu$, $cm^2/kV \times min$), resolutions obtained for the chiral sulfoxides, and sulfinate esters separated using S-β-CD or CM-β-CD as a run buffer additive.

Most sulfoxides and sulfinate esters could be separated by using S-β-CD, and many of them gave baseline or better resolutions. The CM-β-CD separated far fewer compounds. Compounds that could be separated with both chiral selectors usually had better resolution with the S-β-CD. Only in one case (for compound # 7) could a separation be achieved with CM-β-CD but not SBC. Though much less CM-β-CD was used than S-β-CD, these were the optimized conditions for each selector. Moreover, compound 10 had the greatest enantioseparation with the lowest concentration of chiral selector in the running buffer. Only 20 mg/mL of S-β-CD was required to obtain a resolution of 6.0 and a mobility difference of 3.4 $cm^2/kV \times min$. This compound has a diphenyl substituent, providing more bulk next to the stereogenic sulfur than any other compound. Clearly large, bulky groups in this position have a strong enhancing effect on enantioseparation.

The structure of *para*-substituent attached to the phenyl ring (compounds 1–6) and the size of substituent next to the stereogenic sulfur exert significant effect on the chiral separation in CE with S-β-CD (Fig. 6.3). Overall, for compounds 1–6, larger groups with more electronegative para-substituents tended to give worse separations. For compounds 1, 9–11, 22, and 24, the observed effect of substituent was different from what was seen for compounds 1–6. The much bigger t-butyl group, benzyl, or diphenyl moieties directly linked to the stereogenic sulfur gave a better separation than either of the smaller methyl (compound 1) or olefinic groups (compound 24).

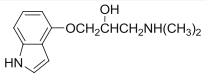
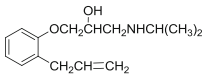
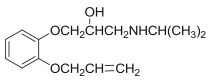
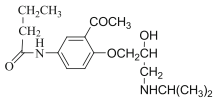
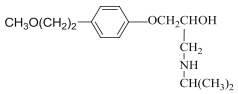
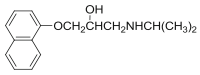
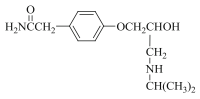
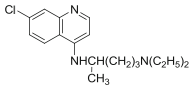
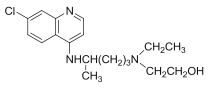
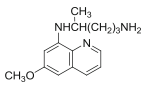
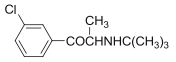
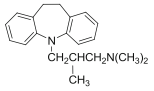
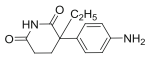
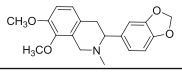
All of the compounds in this group have, at most, two possible sites for inclusion complexation (i.e., the two organic substituents attached to the stereogenic sulfur) (Table 6.3). In some cases (e.g., the methyl phenyl sulfoxides, compounds

Table 6.2 Electrophoretic data for cationic analytes using 2 % S-β-CD as the chiral selector in 10 mM pH 3.8 phosphate buffer at 15 kV applied voltage

Compound	Structure	t_1, min	t_2, min	R_s
Ketamine		13.08	13.62	3.0
Mepivacaine		19.79	21.43	4.0
Bupivacaine		24.43	25.22	2.6
Pheniramine		8.60	8.68	1.0
Brompheniramine		8.26	8.44	1.9
Chlorpheniramine		8.55	8.80	2.3
Carbinoxamine		8.63	8.96	3.2
Doxylamine		8.88	8.97	0.6
Dimethindene		8.87	9.32	4.1
Verapamil		10.45	11.02	5.0
Mexiletine		12.40	13.08	3.6
Disopyramide		20.83	22.64	7.4
Trihexyphenidyl		8.25	8.37	1.3
Oxyphencyclimine		8.19	8.26	0.8
Mepenzolate		9.74	9.98	1.7
Piperoxan		8.15	8.61	6.1

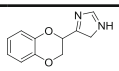
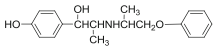
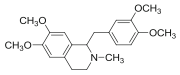
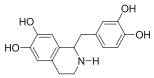
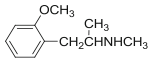
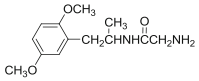
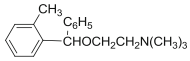
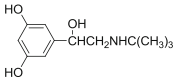
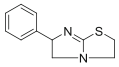
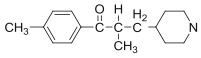
(continued)

Table 6.2 (continued)

Compound	Structure	t_1, min	t_2, min	R_s
Pindolol		10.87	11.12	2.3
Alprenolol		11.62	12.12	4.0
Oxprenolol		26.92	30.98	8.4
Acebutolol		30.47	31.23	1.9
Metoprolol		9.69	9.69	0
Propranolol		10.43	10.43	0
Atenolol		13.46	13.46	0
Chloroquine		10.27	10.58	2.6
Hydroxychloroquine		10.38	10.75	3.8
Primaquine		8.60	8.60	0
Bupropiontranyl cypromine		8.34	8.56	1.6
Trimipramine		8.55	8.69	1.1
Tranylcypromine		10.27	10.64	2.4
Nefopam		10.75	10.91	1.1
Aminoglutethimide		8.75	9.48	5.8
Canadine		8.32	8.71	3.4

(continued)

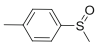
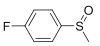
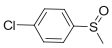
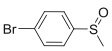
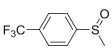
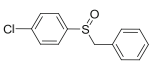
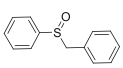
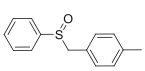
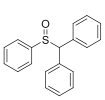
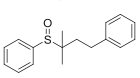
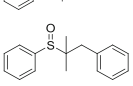
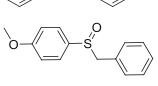
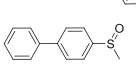
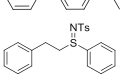
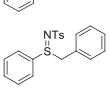
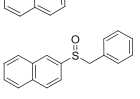
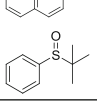
Table 6.2 (continued)

Compound	Structure	t_1, min	t_2, min	R_s
Idazoxan		8.83	9.15	2.2
Isoxsuprine		10.24	10.37	1.0
Laudanosine		33.97	37.87	8.1
Tetrahydropapaveroline		10.58	12.56	5.6
Methoxyphenamine		17.16	22.81	12.6
Midodrine		14.00	16.53	4.6
Orphenadrine		8.01	8.20	1.5
Terbutaline		8.37	8.75	2.8
Tetramisole		11.35	11.98	3.8
Tolperisone		9.26	9.82	3.4

1–6, 34–36, and 14), it can be argued that the single aromatic substituent is the only possible (or at least a much more favored) site for inclusion complexation. There are two other noninclusion interaction sites that are identical for all of the chiral sulfoxides and sulfinate esters in this study, i.e., the oxygen moiety and the lone pair of electrons on the stereogenic sulfur (see structures in Table 6.3). These are available for hydrogen bonding interactions, for example. Compounds that have two aromatic substituents, or one aromatic substituent plus a comparable (in size or hydrophobicity to an aromatic ring) alkyl substituent, have two hydrophobic binding or complexation sites.

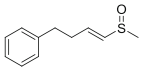
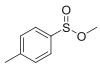
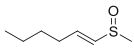
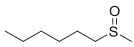
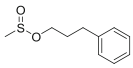
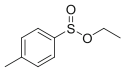
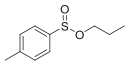
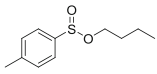
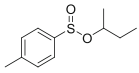
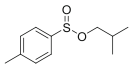
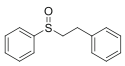
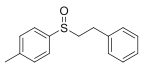
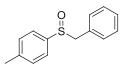
By using the methods described by Rundlett and Armstrong [17], the binding constants of some analytes to both chiral selectors were measured in order to examine and help explain the observed migration behavior and enantioselectivity trends (Table 6.3) [16]. A comparison of the binding constants with two anionic CDs is shown in Table 6.4; one may find that migration time of the studied compounds with CM-β-CD as the chiral selector was longer than the other one with S-β-CD. This was true even though all of the analytes showed much stronger binding to carboxymethyl β-cyclodextrin than to sulfated β-cyclodextrin. Overall, sulfated β-cyclodextrin separated a greater number of compounds and had better separating capabilities than did carboxymethyl β-cyclodextrin for these analytes.

Table 6.3 Separations of chiral sulfoxides and sulfinate esters with S-β-CD[a] or CM-β-CD[b]

Compound	Structure	t_1, min	t_2, min	$\Delta\mu$	[CD], mM	R_s
1		11.81	12.61	0.76	150	2.2
2		10.22	10.71	0.57	150	1.4
3		8.90	9.19	0.58	150	1.3
4		14.69	15.39	0.58	150	0.8
5		15.19	15.88	0.41	150	1.7
6		9.50	9.63	0.22	150	0.7
7		22.85	23.45	0.040	20[b]	1.2
8		17.68	20.52	1.10	150	5.4
		17.61	17.94	0.038	20[b]	0.8
9		14.56	16.79	1.28	150	6.1
		20.98	21.43	0.036	20[b]	1.2
10		5.16	6.75	3.40	20	6.0
		29.90	31.33	0.056	20[b]	1.6
11		14.50	16.28	1.06	150	5.0
12		6.18	6.63	0.66	30	2.1
		32.05	32.55	0.017	20[b]	0.5
13		12.83	15.03	1.60	150	7.0
		20.37	20.84	0.040	20[b]	1.4
14		18.55	22.01	0.98	75	4.2
15		13.49	13.93	0.43	125	1.7
16		9.33	10.33	1.46	150	4.5
17		13.34	13.71	0.37	125	1.4
18		8.36	9.05	1.28	150	2.1
19		8.89	11.32	3.40	150	8.3
		17.17	17.95	0.091	20[b]	2.6
20		18.20	18.79	0.29	125	1.6
21		11.62	14.52	1.97	100	6.7
		27.23	27.66	0.021	20[b]	0.6

(continued)

Table 6.3 (continued)

Compound	Structure	t_1, min	t_2, min	$\Delta\mu$	[CD], mM	R_s
22		No separation				
23		10.32	10.82	0.63	103	1.9
24		11.87	12.29	0.52	44	1.5
25		10.98	11.60	0.37	150	1.2
26		8.57	8.90	0.33	130	1.0
27		No separation				
28		8.82	9.85	1.50	80	3.3
29		9.45	10.48	1.46	100	4.0
30		10.66	11.57	1.03	100	2.3
31		10.45	11.15[a]		100[c]	–
		10.32	10.82[b]		20[c]	–
32		10.63	11.69	1.19	100	3.9
33		10.00	10.65	0.46	150	1.4
34		7.35	7.71	0.48	100	2.0
		22.83	23.22	0.026	20[b]	0.8
35		12.62	13.07	0.20	150	0.7
36		8.63	9.66	0.74	100	2.9
37		8.20	9.10	0.72	100	2.5
38		15.50	18.70	1.55	100	5.4
		19.08	19.62	0.052	20[b]	1.3

[a]All samples were run at a pH of 8.3 in 10 mM phosphate buffer and +9.00 kV, with the exceptions of 12, 15, and 22, which were run at 13, 11, and 11 kV, respectively. SBC concentration is in mg/mL. EOFs were typically in the range of 16 $cm^2/kV \times min$. Separation results of compounds in Table 6.3 were operated in this condition without explanation

[b]All samples run at a pH of 5.2, +15 kV, and 10 mM phosphate run buffer. Typical EOFs under these conditions were about 13.5 $cm^2/kV \times min$

[c]This compound had two chiral centers and peaks could not be identified. The two main peak times are listed

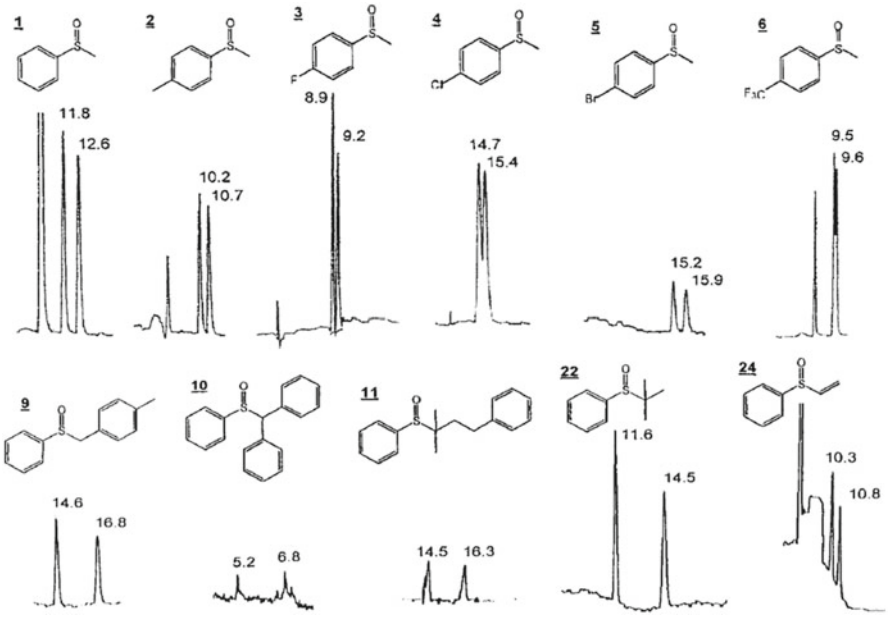
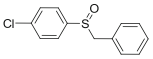
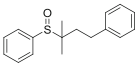
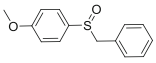
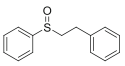
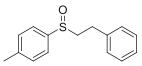


Fig. 6.3 Electropherograms of compounds 1–6, 9–11, 22 and 24. Experimental conditions for all compounds except compound 4: 150 mg/mL S-β-CD in 10 mM phosphate buffer, pH 8.3; +9 kV. Compound 4 was run at +7 kV (Reproduced from Ref. [16] by permission of John Wiley & Sons Ltd.)

Table 6.4 Binding constants for selected compounds

Compound structure		Method	S-β-CD			CM-β-CD		
			K_1	K_2	α	K_1	K_2	α
7		y-reciprocal	20	–	–	640	738	1.15
		Double-reciprocal	20	–	–	560	658	1.18
11		y-reciprocal	27	31	1.15			
		Double-reciprocal	29	33	1.14			
13		y-reciprocal	19	22	1.16			
		Double-reciprocal	18	21	1.17			
17		y-reciprocal	39	40	1.03			
		Double-reciprocal	33	34	1.03			
20		y-reciprocal	15	20	1.33	201	226	1.13
		Double-reciprocal	22	28	1.27	175	205	1.17
37		y-reciprocal	51	61	1.20			
		Double-reciprocal	34	40	1.18			
38		y-reciprocal	20	23	1.15			
		Double-reciprocal	19	22	1.16			

Reproduced from Ref. [16] by permission of John Wiley & Sons Ltd.

By varying BGE pH, selector concentration, and organic modifier concentration, the separation with S-β-CD may be further optimized. However, the addition of methanol was found to result in decreased resolution and mobility difference for both S-β-CD and CM-β-CD. The most notable effect was the addition of large bulky groups next to the stereogenic sulfur, which greatly enhanced the enantiomeric peak to peak separation.

Inspired by these study, the polyanionic S-β-CD has been widely explored in the analysis of chiral compounds in real samples of interest in pharmaceutical, medical, biological, and environmental fields [18–23]. A simple, fast, accurate, precise, rugged, and sensitive chiral CE method with desired selectivity was achieved by using randomly S-β-CD through a systematic approach to method development based on theoretical predictions [18–24]. The enantiomeric purity of the studied compounds was thus quantitatively determined.

Chiral separation of triazole fungicides was explored in a variety of CE running systems. The S-β-CD-mediated system turned out to be the only system that enantioseparated all of the compounds [19]. The 14 fungicides investigated were bitertanol, cyproconazole, difenoconazole, diniconazole, flutriafol, hexaconazole, myclobutanil, paclobutrazol, penconazole, propiconazole, tebuconazole, tetraconazole, triadimefon, and triadimenol. Under the optimal conditions, excellent enantioseparation was achieved for all the 14 fungicides, including those fungicides containing two chiral centers. The optimal chiral separation was obtained by using a phosphate buffer containing 2 % S-β-CD (pH 3.0), together with a reversed high voltage. For the most hydrophobic triazole fungicides, chiral separation was enhanced by the addition of urea.

Chiral dihydrofurocoumarin compounds are currently the focus of industrial and pharmacological research. These derivatives have been shown to possess many physiological properties that could be medically beneficial. Several different cyclodextrin chiral selectors were examined to evaluate their effectiveness in the enantioseparation of dihydrofurocoumarins [20]. When comparing the techniques, the optimum enantioseparation was obtained in the reversed-polarity mode with S-β-CD as the chiral selector. S-β-CD gave better separations than CM-β-CD due to its greater enantioselectivity for these analytes. S-β-CD was also more successful than either SBE-β-CD or in combination with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), and it produced shorter run times when compared to the micelle-based enantioseparation method. These studied dihydrofurocoumarin compounds can be well separated in 5 min by using S-β-CD as the chiral selector. The result also showed that optimization of BGE pH and CD concentration had substantial effects on the enantioseparation of dihydrofurocoumarins.

Samples of citalopram and its pharmacologically active metabolite desmethylcitalopram (DCIT) were prepared by liquid-phase microextraction (LPME) based on a rodlike porous polypropylene hollow fiber. LPME-CE was developed and investigated as a combination for enantiomeric determination of drugs and metabolites in biological matrices [21]. Excellent chiral separation was obtained by using 1 % S-β-CD as chiral selector in combination with 12 % acetonitrile in 25 mM pH 2.5 phosphate. This study established LPME-CE as a suitable tool for enantiomeric determination of chiral drugs and metabolites in biological matrices.

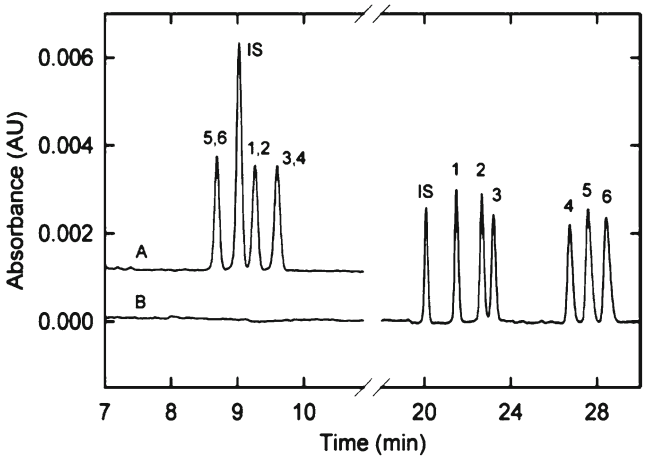


Fig. 6.4 Electropherograms obtained with standards for (*A*) analysis of 1 mg/mL amounts in absence of a chiral selector and (*B*) analysis of about 5 mg/mL of each enantiomer in presence of 0.6 % sulfated β-CD. Data of graph A are plotted with a y-axis offset of 0.0015 AU. Experimental conditions as described in Ref. [22]. *Note*: *1* S-PPF, *2* R-PPF, *3* S-5OH-PPF, *4* R-5OH-PPF, *5* S-NOR-PPF, *6* R-NOR-PPF, *IS* atenolol (Reproduced from Ref. [22] by permission of John Wiley & Sons Ltd.)

Thormann et al. [22] firstly described a robust, inexpensive, sensitive, and selective CE method for the simultaneous determination of the enantiomers of propafenone (PPF), 5-hydroxy-propafenone (5OH-PPF), and N-despropyl-propafenone (NOR-PPF) in serum and in in vitro media. It is based upon liquid–liquid extraction at alkaline pH followed by analysis of the reconstituted extract by CE in presence of a pH 2.0 running buffer composed of 100 mM sodium phosphate, 19 % methanol, and 0.6 % highly sulfated β-CD. For each compound, the S-enantiomers are shown to migrate ahead of their antipodes, and the overall run time is about 30 min, which can be obviously observed in Fig. 6.4.

A CE method for the chiral separation of phenylglycidate enantiomers using S-β-CD has been developed [23]. Under optimal conditions, phenylglycidates and a variety of its substituted derivatives can be baseline separated. A capillary electrophoresis (CE) method for the enantioseparation of phenylglycidates has been developed. When the migration time was set at the threshold value, it was found that the best enantioseparation was obtained at 10 kV with 3 % (w/v) sulfated β-cyclodextrin at pH 6.5. A range of substituted phenylglycidates was successfully separated by using this method and the results shown to be superior to those obtained by using GC.

The S-β-CD-mediated CE method was described by Chou et al. for the enantioseparation of cetirizine using achiral cefazolin as an internal standard [24]. In the preliminary study on enantioseparation of cetirizine, several kinds of neutral cyclodextrins including α-CD, β-CD, γ-CD, and HP-β-CD were investigated in 5 mM

borate buffer (pH 8.7). For the two enantiomers of cetirizine, no chiral recognition was observed with the neutral CDs alone in borate buffer. The enantioseparation of the drug was achieved with 1 % S-β-CD in the same BGE. Under optimized conditions, a baseline separation of two enantiomers was achieved in less than 7 min. Using cefazolin as an internal standard (IS), the linear range of the method for the determination of levocetirizine was over 1.0–50.0 μg/mL; the detection limit (signal-to-noise ratio = 3) of levocetirizine can reach 0.5 μg/mL. This analytical method was also applied to enantioseparation of cetirizine in plasma samples after liquid–liquid extraction as sample pretreatment.

6.3 Carboxymethyl-β-CD (CM-β-CD)

Another series of ionizable β-CD derivatives and in particular those containing carboxy groups, such as carboxymethyl-, carboxyethyl-, or succinyl-β-CD, have also been successfully developed as the chiral selector for the enantioseparations of neutral and basic analytes by CE since the last 1990s [13, 25–32]. An anionic CD derivative, mono-2-O-carboxymethyl-β-CD, was firstly used by Terabe et al. in CE as the moving pseudostationary phase [25], where no chiral recognition was reported. In 1993, Smith described the enantioseparation of some neutral analytes using CM-β-CD as chiral selector at pH 12.4 [26]. Engelhardt et al. achieved the chiral separation of 7 neutral and basic analytes using CM-β-CD, carboxyethylated β-CD (CE-β-CD), and succinylated β-CD in both charged and the uncharged modes by adjusting BGE pHs [27]. The reversal of the migration order of the stereoisomers was achieved for analytes when using a coated capillary with suppressed EOF and employing chargeable cyclodextrins in the "uncharged and charged mode." The mobilities of the analytes are presented schematically in Fig. 6.5.

CM-β-CD offered better resolution for doxylamine, ephedrine, dimetinden, and propranolol in both modes; separation results obtained in optimal condition are summarized in Table 6.5. Hexobarbital, binaphthol, and oxazolidinone were selected as the model to investigate the influence of a methylene spacer on resolution and selectivity. Studies found that the two CDs' chiral recognition ability for hexobarbital and 1,1′-binaphthyl alcohol is different, which ascribed this effect to the spacer length. Chankvetadze et al. [29] studied the anionic cyclodextrin derivatives such as CM-β-CD, SEE-β-CD and SBE-β-CD for the separation of racemic 1,1′-bi-2-naphthol, 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, and 1,1′-binaphthyl-2,2′-diamine. The results indicated all the anionic CDs exhibit a chiral recognition ability for not only neutral and cationic, but also anionic racemates. The enantiomers of the antidepressant drug, mianserine, and its 11 structural analogues were well resolved ($R_s > 1.4$) with CE using CM-β-CD at rather lower concentrations (5 mM) [30]. Crommen et al. [31] further explored the potential of carboxymethyl-, dimethyl-, and hydroxypropyl-β-cyclodextrin as chiral selectors for the enantioseparation of seven basic drugs in free-solution CE [31]. The best results with respect to chiral resolution were obtained with CM-β-CD, which resolved the enantiomers of all compounds.

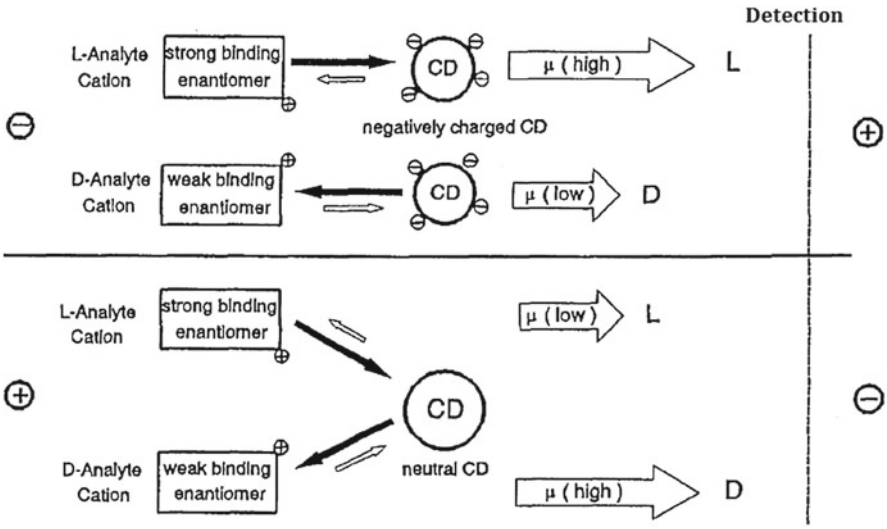
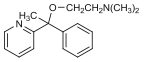
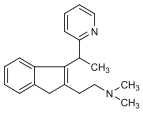
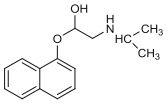
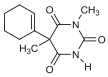
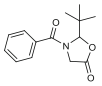
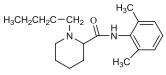
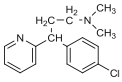


Fig. 6.5 Inversion of migration order with pH using chargeable CDs. In "charged mode" CD functions as carrier and analyte with stronger affinity to CD has highest mobility. In "uncharged mode" analyte with stronger affinity has lowest mobility because of longer incorporation time in CD (Reprinted from Ref. [27], with kind permission from Springer Science + Business Media)

The enantioseparation of 1,4-dihydropyridines (DHPs) with CE has been described using neutral and negatively charged CDs since 1990s [23, 34]. The SBE-β-CD and CM-β-CD appeared to be the most effective chiral additives. Marina et al. [36] realized the individual enantiomeric separation of 12 DHP derivatives using CM-β-CD, although not all were baseline resolved. In comparison, carboxymethyl-γ-CD (CM-γ-CD) and succinylated β-CD (Su-β-CD) cannot perform as well as CM-β-CD. Zhang et al. [37] developed the methods for the enantioseparation of *m*-nisoldipine, a new 1,4-dihydropyridine calcium ion antagonist [37]. The elaborated enantiomers separation methods of *m*-nisoldipine were successfully performed using an anionic CD, SBE-β-CD or CM-β-CD, as chiral selector. Although the results indicated that SBE-β-CD was a better chiral selector for enantioseparation of the neutral *m*-nisoldipine, the CM-β-CD (15 mM) still achieved a R_s of 1.3 using 20 mM phosphate buffer (pH 5.0), an applied voltage of 25 kV, and a temperature of 20 °C.

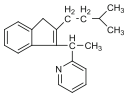
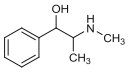
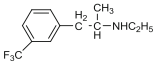
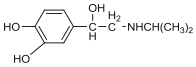
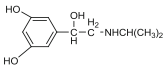
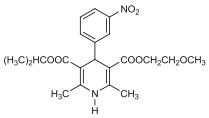
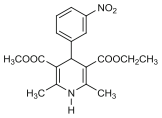
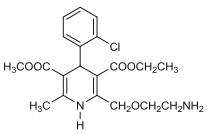
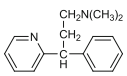
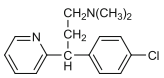
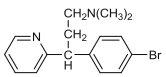
A rapid and simple method for the analysis of salbutamol was developed by Marina et al. [38] using CM-β-CD as the chiral selector, where the enantiomers of salbutamol could be separated in about 2 min. Three different pharmaceutical preparations (two syrups, one oral solution, and two kind of tablets) containing a racemate of salbutamol were injected directly into the CE system, following dilution in dimethyl sulfoxide. A simple, sensitive and low-cost method using capillary electrophoresis coupled with field-amplified sample stacking (FASS) technique has been developed for enantioselective separation and quantification of

Table 6.5 Separation of neutral and basic drugs using CM-β-CD or CE-β-CD at different pH BGEs

Compounds	Structure	[CM-β-CD]	R_s	CE conditions	Ref.
Doxylamine		2 % (w/v)	2.3	20 mM citric acid at pH 2.7	[27]
Dimetinden		2 % (w/v)	9.6	20 mM pH 2.7 citric acid	[27]
Propranolol		1.5 % (w/v)	3.6	20 mM pH 5.8 phosphate-Tris	[27]
		10 mM	4.4	100 mM pH 3 phosphate-TEA	[28]
		15 mM CE-β-CD	1.6	100 mM pH 3 phosphate-TEA	[28]
Hexobarbital		0.66 % (w/v) CE-β-CD	0.8	20 mM pH 5.5 phosphate-Tris	[31]
Binaphthol		0.66 % (w/v) CE-β-CD	3.6	20 mM pH 5.5 phosphate-Tris	[27]
Oxazolidinone		1.5 % (w/v) CM-β-CD	3.6	20 mM pH 5.8 phosphate-Tris	[27]
1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate		NA	$\alpha = 1.06$	50 mM pH 6.0 phosphate buffer	[29]
1,1′-Binaphthyl-2,2′-diamine		NA	$\alpha = 1.14$	50 mM pH 3.0 phosphate buffer	[29]
Mianserine and analogues		5 mM	>1.4	50 mM pH 3.0 phosphate buffer	[30]
Bupivacaine		15 mM	2.2	100 mM pH 3.0 phosphate-TEA	[31]
Chlorpheniramine		15 mM	23.7	The same as above	[31]

(continued)

Table 6.5 (continued)

Compounds	Structure	[CM-β-CD]	R_s	CE conditions	Ref.
Dimethindene		10 mM	18.2	The same as above	[31]
Ephedrine		15 mM	6.3	The same as above	[31]
		1.5 % (w/v)	3.5	20 mM pH 5.8 phosphate-Tris	[27]
Fenfluramine		15 mM	2.1	The same as above	[31]
Isoprenaline		15 mM	4.8	The same as above	[31]
Terbutaline		5 mM	10.4	The same as above	[31]
Nimodipine		10 mM	3.70	20 mM pH 4.6 phosphate borate buffer +1 % urea	[33]
Nitrendipine		10 mM	5.7	20 mM pH 4.6 phosphate borate buffer +1 % urea	[33]
Amlodipine		10 mM	8.0	20 mM pH 4.6 phosphate borate buffer +1 % urea	[33]
		2.5 mM	>1	50 mM pH 3.0 phosphate buffer	[34]
Pheniramine		14 mM CE-β-CD	<1	20 mM pH 5.8 phosphate buffer	[35]
Chlorpheniramine		14 mM (2 %, w/v) CE-β-CD	<1	20 mM pH 5.8 phosphate buffer	[35]
Brompheniramine		14 mM (2 %, w/v) CE-β-CD	<1	20 mM pH 5.8 phosphate buffer	[35]

TEA Triethanolamine, *Tris* Tris(hydroxymethyl)-aminomethane

trihexyphenidyl (THP) enantiomers in human serum [39]. In this work, three kinds of modified β-CD were tested as chiral selectors in CE. Among the CDs studied, THP enantiomers could only be separated by CM-β-CD. A systematic study of the parameters (CD concentration and pH value in CE buffer, separation voltage and temperature, composition of sample solvent, injection voltage, and time) affecting chiral separation and on-line concentration of THP enantiomers were investigated and optimized. Baseline separation can be achieved for E-6006, a thienylpyrazolylethanamine derivative, with 10 mM CM-β-CD in pH 3.0 BGE [40].

Fan et al. [41] developed a CE method for the simultaneous separation of two pairs of anisodamine enantiomers in plasma. Good resolution was achieved with 25 mM CM-β-CD in pH 2.5 BGE. Interestingly, a water soluble carboxymethylcyclodextrin polymer (CM-β-CD polymer) was synthesized and used as CE chiral selector for the successful separation of verapamil and thiopentorusodium [42].

Scriba et al. [43] reported the enantiomeric separation of LL- and DD-enantiomers of several dipeptides and tripeptides by CE with anionic CD as chiral selector. Except for Phe-Ala the peptide enantiomers could be separated with at least one of the two CD derivatives at one of the two buffer pH values studied. The CM-β-CD was more universal for enantioseparations than Su-β-CD; most of the studied peptides can be resolved by CM-β-CD. Reversal of the enantiomer migration order upon increasing the buffer pH from 2.5 to 3.5 was observed in some cases (Table 6.6). Complexation constants and complex mobilities vary with pH as both the charge of the peptide and the charge of CD depend on pH.

The separation of dipeptides was found to be strongly dependent upon the concentration of CM-β-CD (Fig. 6.6). While the resolution of the enantiomers of Ala-Phe increased with increasing concentrations of CM-β-CD, a decrease of the resolution was observed for Phe-Phe. For both peptides the complex between the chiral selector and the weaker bound enantiomer possessed the higher mobility compared to the complex with the stronger bound stereoisomer. Thus, as the concentration of the CD is increased, the complex mobility becomes a more dominant factor. In the case of Ala-Phe, no resolution of the enantiomers is observed at low concentrations of CM-β-CD, as the higher complexation constant between the CD and the DD isomer and the higher mobility of the complex CD-LL enantiomer "neutralize" each other. At high concentrations of the CD the complex mobility becomes dominant resulting in an enantioseparation. In contrast, the enantioresolution of Phe-Phe due to a difference in the complexation constants observed at low concentrations of CM-β-CD is lost at high concentrations when the influence of the mobilities of the diastereomeric complexes increased [43].

Synthetic antimalarial drugs including chloroquine, erythro-mefloquine, primaquine, quinacrine, and tafenoquine are all administered as racemates. Their structures consist of two or three condensed aromatic rings and aliphatic or alicyclic side chains containing center(s) of chirality and amine group(s). Numerous results were reported on the resolution of antimalarial drugs by CE using various selectors like CDs [8, 35, 44] or other polysaccharide derivatives having poor reproducibility due to their heterogeneity. Németha et al. [45] developed the CE methods for chiral resolution of five antimalarial drugs (primaquine, tafenoquine, mefloquine, chloroquine,

Table 6.6 Migration order of the peptide enantiomers at pH 2.5 and 3.5 using 15 mg/mL CM-β-CD or 20 mg/mL Su-β-CD

	CM-β-CD		Su-β-CD	
	pH 2.5	pH 3.5	pH 2.5	pH 3.5
Gly-Phe	D > L	D > L	ns	ns
Ala-Phe	**LL > DD**	**DD > LL**	**LL > DD**	**DD > LL**[e]
Phe-Ala	ns	ns	ns	ns
Ala-Tyr	DD > LL[b]	DD > LL[c]	**LL > DD**	**DD > LL**
Phe-Phe	LL > DD	LL > DD	**LL > DD**	**DD > LL**
Gly-Ala-Phe	DD > LL	DD > LL	ns	ns
Ala-Gly-Phe	DD > LL	DD > LL	LL > DD	ns
Ala-Trp	LL > DD	LL > DD	LL > DD	ns
Ala-Leu	**LL > DD**[d]	**DD > LL**[c]	LL > DD	LL > DD
Ala-cHAla[a]	DD > LL	DD > LL	ns	ns
Ala-PheOMe	LL > DD	LL > DD[c]	LL > DD	LL > DD
Ala-$PheNH_2$	ns	DD > LL	ns	ns
Glu-$PheNH_2$	LL > DD	LL > DD	LL > DD	ns
Asp-PheOMe	LL > DD	LL > DD	LL > DD	ns
Asp-$PheNH_2$	DD > LL	DD > LL	ns	ns
Gly-Asp-$PheNH_2$	DD > LL	DD > LL	DD > LL	DD > LL
PheOMe	L > D	L > D	ns	L > D
$PheNH_2$	D > L	D > L	D > L	D > L

Reproduced from Ref. [43] by permission of John Wiley & Sons Ltd.
ns no separation
[a]Alanyl-cyclohexylalanine
[b]25 mg/mL CM-β-CD
[c]5 mg/mL CM-β-CD
[d]50 mM pH 2.4 phosphate buffer
[e]30 mg/mL Su-β-CD. The faster migrating enantiomer is listed first. The pH-dependent reversal of the migration order is indicated in bold letters

and quinacrine) by using a wide selection of neutral and anionic CD derivatives. The use of SBE-β-CD and CM-β-CD resulted in good resolution of quinacrine and tafenoquine, respectively. These two anionic CDs also provided improved resolution for primaquine. The CM-β-CD provided not only the best separation of primaquine from quinocide but also the simultaneous complete resolution of both compounds.

6.4 Sulfobutyl Ether-β-CD (SBE-β-CD)

Sulfobutyl ether-β-CD (SBE-β-CD) has been extensively used as the chiral selector for the separation of acidic, neutral, and basic enantiomers [10, 11, 29, 30, 46–49]. The synthesis and characterization of the sulfoalkyl ether β-cyclodextrin derivatives was described by Rajewski et al. [50, 51], as depicted in Fig. 6.7. The former SBE-β-CD usually had a degree of substitution (DS) of 4.0, followed by DS of 7.0. That's

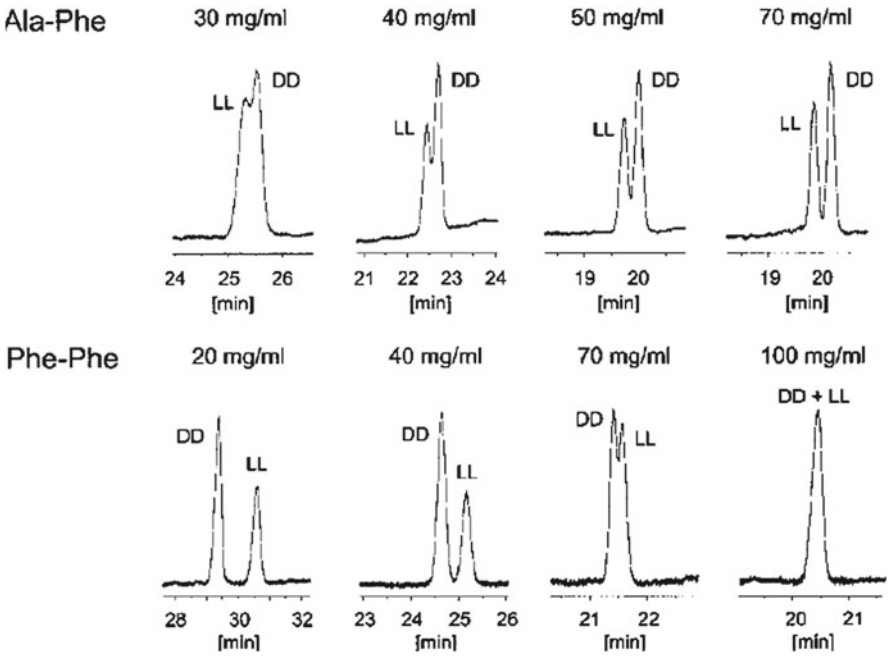


Fig. 6.6 Dependence of the chiral separation of Ala-Phe at pH 3.8 (*top*) and Phe-Phe at pH 3.5 (*bottom*) on the concentration of CM-**β**-CD. Conditions: polyacrylamide-coated capillary, 50 mM phosphate buffer, –25 kV (Reproduced from Ref. [43] by permission of John Wiley & Sons Ltd.)

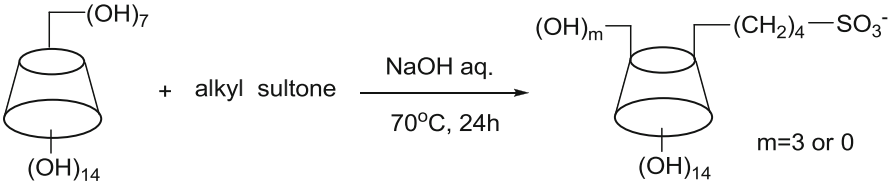


Fig. 6.7 The synthesis of sulfobutyl ether-β-CD

to say, SBE-β-CD has four butyl chains, each with a sulfo group at one end, are connected by ether bridges to four of the seven OH groups at the primary hydroxyl rim.

Dette et al. [10] developed two methods for the enantiomeric separation of chiral ephedrine alkaloids with SBE-β-CD, where 20 mM phosphate buffer (pH 2.5) or 20 mM borate buffers (pH 9.25, 9.5, 9.75, or 10.0) was used. Good separations were obtained for ephedrine, methylephedrine, and methylpseudoephedrine in borate buffer, while no baseline separation was achieved for norephedrine in all buffers. As can be seen from Table 6.6, norephedrine was only partially separated, while the

other three analystes all achieved an R_s value over 1.1. For the studied enantiomers, SBE-β-CD afforded a much broader pH range to obtain a sufficient enantiomeric resolution than the neutral CDs. The successful separation of ephedrine derivatives were also discussed by Stobaugh utilizing the same CD [46].

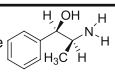
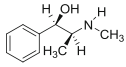
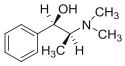
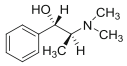
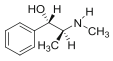
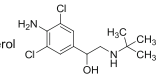
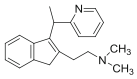
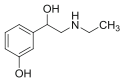
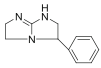
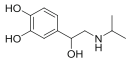
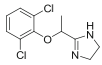
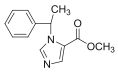
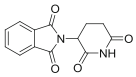
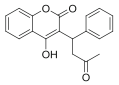
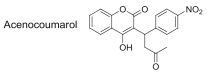
Chankvetadze [11] reported the separation of nine basic racemic drugs using SBE-β-CD at concentration as low as 40–100 μM (Table 6.7). The high efficiency of SBE-β-CD is ascribed to its countercurrent mobility to the racemic solute. Examples of the chiral separation in countercurrent flows of discrete zones of the chiral selector and the racemic compound, as well as separation of the neutral racemic compound thalidomide, were demonstrated with SBE-β-CD in a micellar electrokinetic chromatography-like mode. Detailed CE and ^{1}H NMR studies of the chiral recognition of racemic metomidate with SBE-β-CD indicate that ^{1}H NMR spectrometry is a useful technique for the investigation of the chiral recognition mechanism in CE [47].

Stalcup et al. [8] also investigated enantioseparation of racemic 1,1′-bi-2-naphthol, 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, and 1,1′-binaphthyl-2,2′-diamine using SBE-β-CD and SEE-β-CD. Almost at the same time, SBE-β-CD and SEE-β-CD were used to separate 12 antidepressant drugs at lower concentration (0.1 or 0.5 mM) in comparison to CM-β-CD. Based on the ^{13}C NMR studies, SBE-β-CD is more likely stereoselective binding to the chiral select and which is an important reason for its high chiral resolving power [30].

A study of the enantiomeric separation of a variety of underivatized anionic and cationic compounds of pharmaceutical interest and uncharged phenyl alcohols and dansyl-amino acids by using SBE-β-CD as the selector were investigated [48]. At low pH 2.5, among the studied basic drugs, only terbutaline was resolved by utilizing the SBE-β-CD as the chiral selector. By increasing the pH to 6.0 containing different amounts of SBE-β-CD, the enantiomeric separation of several compounds was successful. Table 6.7 gives the compounds and their resolution in the BGE contained optimal concentration of chiral selector. Enantiomeric resolution with good peak shapes was achieved for warfarin, acenocumarol, promethazine, and terbutaline when the lower concentration of chiral selector was used (1 or 3 mg/mL). However, poor enantiomeric resolution was obtained for metoprolol, oxprenolol, atenolol, pindolol, and propranolol ($R_s < 0.5$). What's more, for phenyl alcohols and dansyl-amino acids, different pH value and concentration of chiral selector were investigated to study these factors to the enantiomeric resolution. Some satisfactory results were given in Table 6.7. SBE-β-CD was effective for the enantiomeric separation of most of the amino acid derivatives studied, especially amino acids with nonpolar chains.

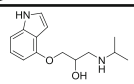
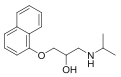
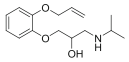
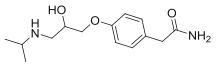
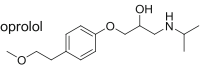
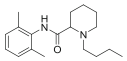
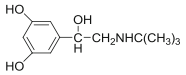
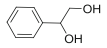
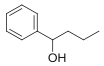
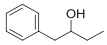
SBE-β-CD combined with neutral cyclodextrins was developed as a theoretical model for separation of enantiomers of neutral species [49]. Resolution and selectivity can be calculated based on model parameters. The validity of the model was demonstrated by resolving the stereoisomers of LY213829 and its sulfoxide metabolites with a BGE containing 10 mM SBE-β-CD and 7 mM β-CD. The model is very useful in predicting the migration times and resolution of test substances, and baseline separation was achieved for all species.

Table 6.7 Electrophoretic data for basic, acid, and neutral analytes resolved using SBE-β-CD as chiral agent in optimal CZE condition

Compounds	[SBE-β-CD]	R_s	CE conditions	Ref.
Norephedrine 	40 mM	0.71	20 mM pH 10.0 borate buffer	[10]
Ephedrine 	40 mM	1.13	20 mM pH 10.0 borate buffer	[10]
Methylephedrine 	40 mM	1.27	20 mM pH 10.0 borate buffer	[10]
Methylpseudoephedrine 	40 mM	3.08	20 mM pH 10.0 borate buffer	[10]
Pseudoephedrine 	40 mM	0	20 mM pH 10.0 borate buffer	[10]
Clenbuterol 	1.00 mM	0.68	50 mM pH 3.1 phosphate buffer	[11]
Dimethindene 	0.08 mM	1.18	50 mM pH 3.1 phosphate buffer	[11]
Etilefrine 	1.00 mM	1.43	50 mM pH 3.1 phosphate buffer	[11]
Imafen 	1.00 mM	4.53	50 mM pH 3.1 phosphate buffer	[11]
Isoprenaline 	1.00 mM	1.04	50 mM pH 3.1 phosphate buffer	[11]
Lofexidine 	1.00 mM	3.15	50 mM pH 3.1 phosphate buffer	[11]
Mefloquine 	0.08 mM	1.51	50 mM pH 3.1 phosphate buffer	[11]
Metomidate 	1.00 mM	2.21	50 mM pH 3.1 phosphate buffer	[11]
Mianserine 	0.04 mM	1.1	50 mM pH 3.1 phosphate buffer	[11]
Thalidomide 	2 mM	1.0	50 mM pH 4.35 phosphate buffer	[11]
Warfarin 	3 mg/mL; 20 mg/mL	1.09; 2.11	50 mM pH 6 phosphate buffer	[48]
Acenocoumarol 	3 mg/mL; 20 mg/mL	1.09; 2.13	50 mM pH 6 phosphate buffer	[48]

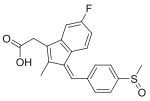
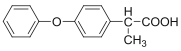
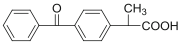
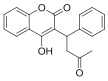
(continued)

Table 6.7 (continued)

Compounds	[SBE-β-CD]	R_s	CE conditions	Ref.
Pindolol 	1–20 mg/mL	<0.5	50 mM pH 6 phosphate buffer	[48]
Propranolol 	1–20 mg/mL	<0.5	50 mM pH 6 phosphate buffer	[48]
Oxprenolol 	1–20 mg/mL	<0.5	50 mM pH 6 phosphate buffer	[48]
Atenolol 	1–20 mg/mL	<0.5	50 mM pH 6 phosphate buffer	[48]
Metoprolol 	1–20 mg/mL	<0.5	50 mM pH 6 phosphate buffer	[48]
Promethazine 	3 mg/mL; 20 mg/mL	0.78; 2.0	50 mM pH 6 phosphate buffer	[48]
Bupivacaine 	3 mg/mL; 20 mg/mL	<0.5; 1.36	50 mM pH 6 phosphate buffer	[48]
Terbutaline 	1 mg/mL; 20 mg/mL	1.56 5.10	50 mM pH 6 phosphate buffer	[48]
1-Phenyl-1,2-ethanediol 	20 mg/mL	1.35	50 mM pH 9.0 borate buffer	[48]
2-Phenyl-2-butanol 	20 mg/mL	2.12	50 mM pH 9.0 borate buffer	[48]
1-Phenyl-1-propanol 	10 mg/mL	1.09	50 mM pH 6.0 phosphate buffer	[48]
1-Phenyl-1-butanol 	20 mg/mL	1.47	50 mM pH 6.0 phosphate buffer	[48]
α-Ethyl-phenethyl alcohol 	20 mg/mL	0.76	50 mM pH 6.0 phosphate buffer	[48]
Dns-Phe	20 mg/mL	4.00	50 mM pH 6.0 phosphate buffer	[48]
Dns-Trp	20 mg/mL	1.89	50 mM pH 6.0 phosphate buffer	[48]
Dns-Met	20 mg/mL	2.96	50 mM pH 6.0 phosphate buffer	[48]
Dns-Val	20 mg/mL	0.95	50 mM pH 6.0 phosphate buffer	[48]
Dns-Norval	20 mg/mL	2.71	50 mM pH 6.0 phosphate buffer	[48]
Dns-Leu	20 mg/mL	4.37	50 mM pH 6.0 phosphate buffer	[48]

(continued)

Table 6.7 (continued)

Compounds	[SBE-β-CD]	R_s	CE conditions	Ref.
Dns-Norleu	20 mg/mL	4.28	50 mM pH 8.0 phosphate buffer	[48]
Sulindac 	5 mM	1.4	100 mM pH 3.0 phosphoric-triethanolamine buffer	[52]
Fenoprofen 	5 mM	<0.7	100 mM pH 3.0 phosphoric-triethanolamine buffer	[52]
Ketoprofen 	5 mM	1.1	100 mM pH 3.0 phosphoric-triethanolamine buffer	[52]
Warfarin 	5 mM	2.2	100 mM pH 3.0 phosphoric-triethanolamine buffer	[52]
Hexobarbital 	5 mM	1.7	100 mM pH 3.0 phosphoric-triethanolamine buffer	[52]

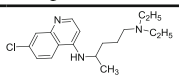
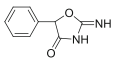
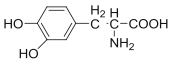
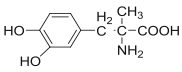
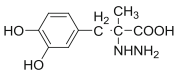
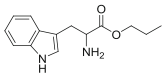
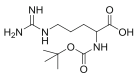
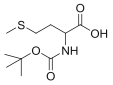
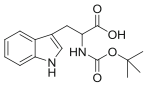
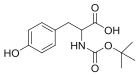
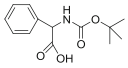
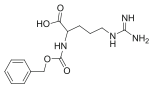
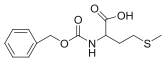
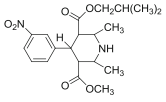
SBE-β-CD with uncharged β-CD was used to separate the enantiomers of acidic drugs, namely, sulindac, fenoprofen, ketoprofen, warfarin, and hexobarbital, in a buffer of pH 3 [52]. The single SBE-β-CD can achieve satisfactory results for the studied compounds, though combined CDs were better chiral selectors. It's worthwhile to mention that the enantioresolution of warfarin highly depends on the selected neutral CDs. A particularly high resolution values (R_s>9.0) were obtained with methylated β-cyclodextrin (M-β-CD) and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) in the buffer which might be related to the fact that optimum concentrations of M-β-CD and DM-β-CD for the enantioresolution of warfarin were found to be very close to the cyclodextrin concentration used in these experiments (10 mM).

Several β-cyclodextrin derivatives have been investigated for chiral separations and among them SBE-β-CD proved to be effective for the stereoselective resolutions of the investigated herbicides. Analysis of a commercial formulation of flamprop isopropyl herbicide (pure R-enantiomer) demonstrated the actual applicability of CE to the stereoselective analysis of real samples of environmental interest [53].

In addition, the preparation, characterization, and evaluation of sulfobutyl ether γ-CD (SBE-γ-CD) were also described by Francotte [54]. The new CD combined the good enantioselectivity of γ-cyclodextrin with the advantages of a negatively sulfobutyl ether group, giving a good chiral ability for the enantiomers. The order of enantiomer separation of dansyl-*tert*-leucine by CE was opposite using a 30 mM phosphate buffer (pH 7) containing SBE-γ-CD or SBE-β-CD (Table 6.8).

SBE-β-CD showed great capability among the nine chosen native CD and modified CD in the separation of chloroquine and pemoline [54]. An R_s of 2.1 for chloroquine and 1.4 for pemoline was achieved with 2.5 mM SBE-β-CD and 1.0 mM

Table 6.8 Enantioseparation of compounds with different SBE-β-CD concentration under optimal CE conditions

Compounds	Compounds	SBE-β-CD, mM	R_s	CE condition	Ref.
Chloroquine		2.5	2.1	50 mM pH 3.0 Na_2HPO_4 buffer	[54]
Pemoline		1.0	1.4	50 mM pH 3.0 Na_2HPO_4 buffer	[54]
2-Amino-3-(3,4-dihydroxyphenyl) propionic acid (DOPA)		5.1	3.8	40 mM pH 2.5 phosphate buffer	[55]
2-Amino-2-methyl-3-(3,4-dihydroxyphenyl) propionic acid (MDOPA)		3.0	4.2	40 mM pH 2.5 phosphate buffer	[55]
2-Hydrazino-2-methyl-3-(3,4-dihydroxyphenyl) propionic acid (CDOPA)		1.7	2.9	40 mM pH 3.0 phosphate buffer	[55]
		2.0	13.94	20 mM pH 2.5 NaH_2PO_4	[56]
Tryptophan butyl ester		15	10.90	pH5.5 phosphate buffer + 30 vol% MeOH	[57]
N-*tert*-Boc-arginine		15	2.37	pH 4.0 phosphate buffer	[57]
N-*tert*-Boc-Methionine		15	1.94	pH 2.5 phosphate buffer + 15 vol% MeOH Buffer + 15 vol% MeOH	[57]
N-*tert*-Boc-Tryptophan		15	3.73	pH 2.5 phosphate buffer	[57]
N-*tert*-Boc-Tyrosine		15	2.36	pH 2.5 phosphate buffer + 15 vol% MeOH	[57]
N-*tert*-Boc-Phenylglycine		15	0.95	pH 4.0 phosphate buffer	[57]
N-CBZ-Arginine		15	0.88	pH 2.5 phosphate buffer	[57]
N-CBZ-Methionine		15	0.68	pH 2.5 phosphate buffer	[57]
m-Nisoldipine		20	1.4	30 mM pH 7.9 phosphate buffer	[37]

SBE-β-CD, respectively, in 50 mM pH 2.5 sodium phosphate buffer. SBE-β-CD was also used for the separation of structurally related compounds, namely, dihydroxyphenylalanine (DOPA), methyldihydroxyphenylalanine (MDOPA), and hydrazinomethyldihydroxyphenylalanine (CDOPA) [55]. For DOPA and MDOPA, good enantioresolution was achieved by using the buffer at pH 2.5 containing a low concentration of SBE-β-CD and applying 24 and 30 kV, respectively, in the normal polarity mode. However, due to the strong complexation between CDOPA and SBE-β-CD, the optimum SBE-β-CD concentration was 1.7 mM, giving an enantioresolution of 2.9 in the reversed-polarity mode.

Blaschke et al. [56] studied the enantiomeric separations of DOPA and CDOPA using several native, neutral, and anionic cyclodextrins as chiral additives. A high resolution value of 15.63 was obtained for DOPA enantiomers with a buffer containing 20 mM single isomer, heptakis(2,3-diacetyl-6-sulfato)-β-cyclodextrin. The enantiomers of CDOPA were separated using 2 mM SBE-β-CD to achieve a resolution of 13.94.

The enantioseparation of estrogens and stereoisomers were performed using anionic SBE-β-CD in the run buffer successfully [58, 59]. The amount of SBE-β-CD required is markedly less than the neutral CD used in previous reports. A study is to perform PM3 (Parametric Method 3) semiempirical molecular orbital calculations on these six inclusion complexes (12 different orientations) for each SBE-β-CD to correlate the migration order with their relative stability.

The use of experimental design in method development was studied for the chiral separation of several amino acid derivatives in CE [57]. The aim of the study was to define rapidly experimental conditions under which the enantiomers can be sufficiently separated for quantification and to derive a methodology for the separation of new compounds. The three CDs, HP-β-CD, CM-β-CD, and SBE-β-CD, have shown to be very effective and should lead to the separation of a wide range of analytes. Experimental designs appear to be very efficient to determine in a few experimental conditions, where baseline separation of the compounds can be achieved. The separation of the N-*tert*-Boc and N-CBZ amino acids with the highest resolution values obtained in the screening using SBE-β-CD under the given fractional factorial design. The strategy defined will be used in a future work as a base for the development of a more general strategy for the chiral separations of different families of compounds.

The enantioseparations of the chiral antimalarial drugs (erythro-mefloquine and its analogues) were studied by CE using 23 cyclodextrins (CDs) as chiral selectors [60]. A satisfactory results can be obtained by employing a lower SBE-β-CD concentration as small as 0.2 mM, which is more than tenfold smaller than other studied CDs.

The DHP derivatives as calcium channel blockers are largely applied in cardiovascular diseases, angina pectoris, and hypertension. The chiral separation of DHPs with CE has been reported mainly utilizing SBE-β-CD and CM-β-CD as the chiral selectors [34, 36, 37, 61]. Five neutral CDs and SBE-β-CD and CM-β-CD have been examined for the resolution of amlodipine enantiomers in CE [34]. Neutral CDs yielded no enantioselectivity for amlodipine, CM-β-CD showed

poorer enantioselectivity; however, SBE-β-CD gives a robust separation. Through neutral and negatively charged CDs, the racemates of 29 acidic, neutral, and basic DHPs were separated by Holzgrabe [61]. SBE-β-CD showed better capability for the separation of neutral DHPs than the sulfated β-CD and heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS β-CD). All studied neutral DHPs could be baseline-separated with 1–7 mM SBE-β-CD employing both the normal and reversed-polarity mode. Better resolution was obtained when running the reversed-polarity mode in comparison with the normal polarity mode. Most of the basic DHPs can also be enantioseparated by SBE-β-CD at a lower concentration (<1 mM). However, CM-β-CD also enabled well enantiomeric separation of DHP derivatives by Marina [36].

The methods for the enantioseparation of m-nisoldipine using SBE-β-CD and CM-β-CD as chiral selectors were developed and validated with CE by Lantong Zhang et al. [37]. The obtained results suggested that SBE-β-CD was a better chiral selector for enantioseparation of m-nisoldipine than CM-β-CD. For method validation, the SBE-β-CD with a higher degree of substitution (DS) of 7.0 was chosen which induced better enantioresolution than the one with low DS (4.0). The possible chiral recognition mechanisms of dihydropyridines were also discussed.

An enantioselective capillary electrophoresis method for the determination of the enantiomeric impurity of armodafinil was established by Wang [62]. Among several investigated native and derivatized CDs, namely, β-CD, γ-CD, HP-β-CD, M-β-CD, and hydroxypropyl-γ-CD (HP-γ-CD), only SBE-β-CD was able to separate the modafinil enantiomers as revealed by initial experiments. From the electropherograms of armodafinil and (S)-modafinil, the enantiomeric impurity (S)-modafinil migrates faster to the detector than armodafinil, which makes the method useful for the enantiomeric purity control of armodafinil. In order to get a satisfactory enantioresolution, the optimization of the CE condition was carried out. The finally adopted condition was 20 mmol/L phosphate buffer at pH 7.5, containing 20 mmol/L SBE-β-CD and 20 % methanol, at temperature of 25 °C.

The established method was proven to display good selectivity, repeatability, linearity, and accuracy. The proposed method was applied to determine the enantiomeric impurity of armodafinil in bulk samples. (S)-modafinil was not detected in a bulk sample of armodafinil. Thus, a sensitive and effective method of determining enantiomeric impurity at a concentration of 0.1 % can be estimated so that the impurity control of armodafinil can be regarded as reliable.

An efficient and sensitive CE method for the chiral analysis of a novel antidiabetic drug, sitagliptin, was developed [63]. The acid–base profiling of the analyte was carried out using both CE and NMR pH titrations. Altogether 30 CD derivatives in acidic conditions were used to study the apparent complex stability and chiral separation properties. The effects of dual CD systems on separation were also extensively studied. Complete separation of racemic sitagliptin with good resolution ($R_s = 2.24$) was achieved within a short time (15 min) with optimized parameters (40 mM pH 4.4 phosphate buffer, 10 °C) of a SBE-β-CD (averaged degree of substitution ~4) and native β-CD dual system. On the basis of ROESY studies, a

geometric model of the inclusion complex was constructed with the trifluorobenzene moiety of sitagliptin and an inclusion through the wider CD rim.

The formation of the inclusion complexes of neutral red (NR) with CDs including β-CD, HP-β-CD, and SBE-β-CD was studied by fluorescence and UV–vis absorption spectroscopy, while the binding constants of the inclusion complexes were obtained by steady-state fluorescence measurements [64]. The results suggested that NR exists in two molecular forms in aqueous solution (the acidic form and the neutral form). CDs were most suitable for inclusion of the neutral form of NR, which contributed to the effect of hydrophobicity and could cause enhanced fluorescence. Among the CDs examined, SBE-β-CD was the most suitable for the inclusion of NR, due to the important role of electrostatic effect between negative SBE-β-CD and positive NR played in the inclusion process.

As we all know, CD usually forms a 1:1 inclusion complex with a drug molecule. The solubility of the resulting complex is typically higher than that of the drug molecule, and the apparent drug solubility increases linearly with the CD concentration. And it is also known that the complexation constant can help us to predict the solubilizing effect offered by CDs. Based on the studies of prior researchers, Merzlikine et al. [65] described a new set of 142 experimentally determined complexation constants between SBE-β-CD and diverse organic guest molecules, and 78 observations reported in literature, were used for the development of the QSPR models by the two machine learning regression methods-Cubist and Random Forest. Similar models were built for β-CD using the 233-compound dataset available in the literature. These results demonstrate that the machine learning regression methods can successfully describe the complex formation between organic molecules and β-CD or SBE-β-CD. The developed QSPR models can be used to predict the solubilizing effect of CDs and to help prioritize experimental work in drug discovery.

6.5 Single-Isomer Sulfated Cyclodextrins

A family of single-isomer sulfated cyclodextrins, the sodium salt of hexakis(6-O-sulfo)-α-cyclodextrin (HxS-α-CD), hepta-6-sulfato-β-cyclodextrin (HS-β-CD), and octa(6-O-sulfo)-γ-cyclodextrin (OS-γ-CD), has been synthesized, analytically characterized, and used as the resolving agent for the capillary electrophoretic separation of the enantiomers of nonionic, weak acid, and weak base analytes mainly by Vigh's group [15, 66–68]. The structures of representative single-isomer sulfated cyclodextrins are listed in Fig. 6.8.

Hepta-6-sulfato-β-cyclodextrin (HS-β-CD) has been synthesized (depicted in Fig. 6.9) and used for the separation of the enantiomers of numerous noncharged, acidic, basic, and zwitterionic analytes in CE (Table 6.9) [66]. HS-β-CD proved to be a much stronger complexing agent for all the analytes tested, in both low-pH and high-pH background electrolytes, than the previously synthesized, moderately hydrophobic heptakis-(2,3-diacetyl-6-sulfato)-β-cyclodextrin (HDAS-β-CD) [67]. Though heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) forms

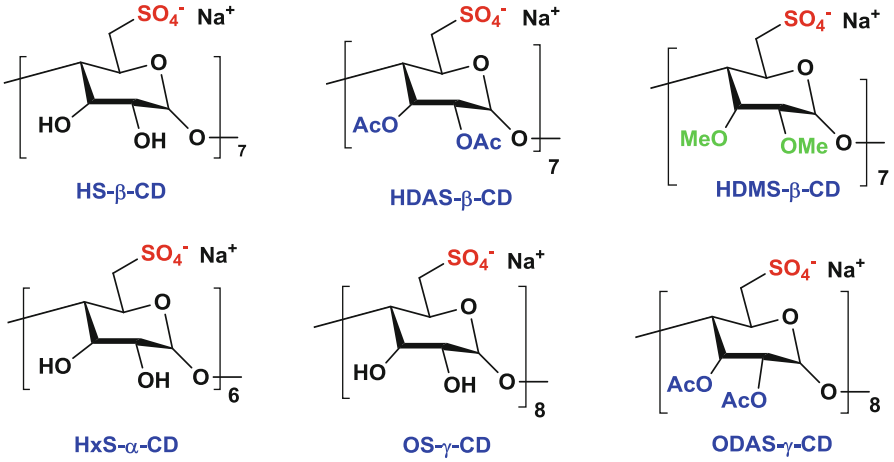


Fig. 6.8 Structures of single-isomer sulfated CDs

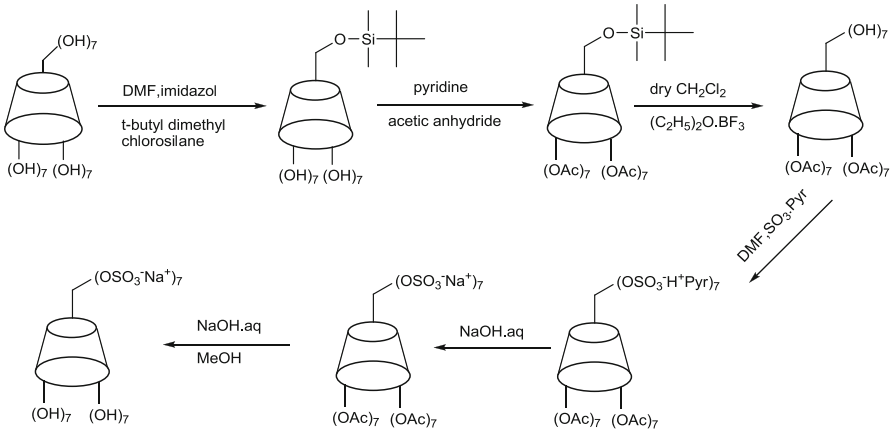


Fig. 6.9 Synthesis scheme for hepta-6-sulfato-β-cyclodextrin

complexes much less strongly with any of the analytes tested here than HDAS-β-CD and HS-β-CD, it offered excellent enantioselectivities, complementary to those of the other two single-isomer, differently functionalized charged cyclodextrins [68].

Scriba et al. [69, 70, 86] firstly described the application of single-isomer sulfated β-CD derivatives for the peptide enantiomer separations. The enantiomer migration order depended to a greater extent on the CD than on the amino acid sequence of the peptide although small structural differences such as formation of a peptide amide or ester affected the chiral recognition by the randomly substituted CD derivatives.

Table 6.9 The enantioseparation of various enantiomers using single-isomer sulfated CDs

Compounds	[CD]	Condition	Ref.
Neutral, strong base, weak base, weak acid, and zwitterionic enantiomers	10–50 mM	pH 2.5 or pH 9.5 HS-β-CD BGEs	[66]
Noncharged, weak acid, strong base, weak base, and zwitterionic racemates	10–50 mM	pH 2.5 or pH 9.5 HDAS-β-CD BGEs	[67]
Neutral, acidic, basic, and zwitterionic enantiomers	10–50 mM	pH 2.5 or pH 9.5 HDMS-β-CD BGEs	[68]
Dipeptide and tripeptide enantiomers	2 mg/mL	pH 2.5–3.5 HS-β-CD, HDAS-β-CD, HDMS-β-CD and SBE-β-CD, S-β-CD	[69]
	10–40 mg/mL	HS-β-CD, HDAS-β-CD pH 2.5 or 5.3	[70]
Compounds with multiple stereogenic centers, eucatropine, fenoterol, nadolol, nafronyl, nylidrin, and pentapiperide	5–30 mM	pH 5.5–10.5 TRIS-phosphate buffer, HS-β-CD, HDAS-β-CD, HDMS-β-CD	[71]
Chiral aryl alkyl and aryl benzyl sulphoxides	20 mM	pH 2.0 HS-β-CD, HDAS-β-CD	[72]
Antiarrhythmic drugs, propafenone and diprafenone, and their metabolites and analogs	0.2–10 mg/mL	Triethanolamine phosphate buffer, HS-β-CD, HDAS-β-CD, HDMS-β-CD	[73]
Antihistamines, antimalarials, β-agonists, β-blockers	50 mM	50 mM pH 3.0 HS-β-CD BGE	[15]
Catecholamines and structurally related compounds	0–1.3 % w/v	200 mM pH 3.0 phosphate buffer, H S-β-CD	[74]
Phenothiazines	0.5–2.0 % w/v	100 mM pH 3.0 citrate BGE, HS-β-CD	[75]
Naphthalene compounds	2–10 mM	pH 7.5 HDMS-β-CD	[76]
Basic pharmaceuticals (β-blockers, local anesthetics, sympathomimetics)	5–30 mM	25 mM potassium camphor SO_3^- BGE consisting of a methanolic solution of 0.75 M formic acid, HDMS-β-CD	[77]
β-Blockers (atenolol, celiprolol, and propranolol) and local anesthetics (bupivacaine, mepivacaine, and prilocaine)	10–30 mM	HDMS-β-CD in methanol acidified with 0.75 M formic acid and containing an electrolyte salt	[78]
Diastereoisomers of 6-oxycodol and nor-6-oxycodol and their analysis in biological samples	2.05 % w/v	HDAS-β-CD in 100 mM pH 2.0 phosphate buffer	[79]
Atropine, scopolamine, ipratropium, and homatropine	5 mM	10 mM pH 9.2 sodium tetraborate buffer with 0.8 % octane, 6.6 % 1-butanol, 2.0 % SDS and 90.6 % (w/w) CD: HS-β-CD, HDAS-β-CD or HDMS-β-CD	[80]

(continued)

Table 6.9 (continued)

Compounds	[CD]	Condition	Ref.
Enantiomers of nonionic, weak acid, and weak base analytes	0–50 mM	pH 2.5 or pH 9.5 HxS-α-CD BGEs	[81]
Enantiomers of nonionic, weak acid, weak base, and ampholytic analytes	0–75 mM	pH 2.5 HxS-α-CD BGEs	[82]
Nonelectrolyte, weak acid, weak base, and ampholytic enantiomers	0–25 mM	pH 2.5 OS-γ-CD BGEs	[83]
Neutral, acidic, basic, and amphoteric enantiomers	0–25 mM	pH 2.5 ODAS-γ-CD BGEs	[84]
Nonionic, acidic, basic, and ampholytic enantiomers	0–50 mM	pH 9.4 OS-γ-CD BGEs	[85]

A series of chiral aryl alkyl and aryl benzyl sulfoxides was well separated by using sulphated-β-CDs as chiral selectors in reversed-polarity capillary electrophoresis [72]. The single-isomer sulfated β-CDs, HDAS-β-CD and HS-β-CD gave better capability for the separation of the majority of enantiomeric pairs. In addition, the electrophoretic migration order was R- before S-sulphoxide, indicating generally greater affinities between the R-enantiomers and the sulphated β-CDs.

Three singer-isomer sulfated β-cyclodextrins were used to separate several compounds with multiple stereogenic centers [71]. Resolution of four isomers was achieved for several of the test compounds under counter-EOF conditions in less than 10 min. The migration order of isomers can be manipulated by changing either the CD concentration or buffer pH. Different types of sulfated cyclodextrins were employed as chiral selectors for the enantioseparations of basic pharmaceutical compounds [87]. The results elucidated that random sulfated β-CD gave better resolution than the applied singer-isomer sulfated β-CDs.

The single-isomer (HS-β-CD) material and the mixed isomer (S-β-CD) additive exhibited different ability for the same enantiomers [15, 73]. The amount or the position of the sulfate groups differs for the two additives, which influence the effectiveness of the enantioseparation. The randomly substituted sulfated β-CD were better suitable for the enantioseparation of antiarrhythmic drugs than the single-isomer sulfated β-CDs [73]. However, HS-β-CD, HDAS-β-CD, and HDMS-β-CD were excellent chiral selectors for the separation of basic drugs, naphthalene compounds and atropine, scopolamine, ipratropium, and homatropine [74–80].

A family of single-isomer, sulfated α-CDs, the sodium salt of hexakis(2,3-di-O-acetyl-6-O-sulfo)-α-CD (HxDAS-α-CD) and HxS-α-CD, has been synthesized and used for the initial capillary electrophoretic separation of the enantiomers of nonionic, weak acid, weak base, and ampholytic analytes [81, 82]. In comparison to the larger-ring analogs, HS-β-CD and OS-γ-CD, sulfated α-CDs interacted less strongly with many of the analytes tested. For some of the analytes, the separation

selectivities obtained with HxS-α-CD were complementary to those observed with HS-β-CD and OS-γ-CD.

The member of the single-isomer sulfated γ-cyclodextrins, OS-γ-CD and the sodium salt of octakis(2,3-diacetyl-6-sulfato)-γ-cyclodextrin (ODAS-γ-CD) have also been synthesized and used to separate the enantiomers of nonelectrolyte, acidic, basic, and ampholytic analytes by CE in acidic aqueous BGEs [83, 84]. Both the effective mobilities and the separation selectivites were found to be in agreement with the predictions of the CHARM model of CE enantiomer separations. The sulfated γ-cyclodextrins proved to be a broadly applicable chiral resolving agent.

OS-γ-CD was also used to separate the enantiomers of nonionic, acidic, basic, and ampholytic analytes by CE in high-pH aqueous BGEs [85]. The effective mobilities and separation selectivities were found to follow trends similar to those observed earlier in acidic aqueous BGEs. OS-γ-CD has been proved to be a broadly applicable chiral resolving agent and afforded adequate peak resolution values with short separation times for a number of nonionic, weak acid, weak base, and ampholytic analytes.

6.6 Conclusions

The enantiomeric separation of drugs by using a series of randomly carboxyl CDs, sulphated CDs and single-isomer sulphated CDs are summarized. The randomly sulphated CDs are widely used for the separation of neutral analytes, cationic analytes, chiral sulfoxides and sulfinate esters et al. The single-isomer sulphated CDs in various forms are the complements to separation of the chiral drugs. Studies found that carboxylated CDs have special abilities for the enantioseparation of synthetic antimalarial drugs, trihexyphenidyl enantiomers and 1,4-dihydropyridines. This chapter will give a reference for the enantioseparation of new chiral drugs, which have structures similar to those in the literature.

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Chapter 7
Cationic Cyclodextrins for Capillary Electrophoresis

Shuye Wang, Linyi Bian, and Weihua Tang

Abstract The development of different types of modified cationic cyclodextrins including C(6) persubstituted, randomly multi-substituted, selectively disubstituted, and monosubstituted derivatives as well as C(2) monosubstituted derivatives for enantiomeric separation in capillary electrophoresis is summarized in this chapter. The analytical parameters for optimization of enantiomeric separation with these cyclodextrins are elaborated. The NMR study to reveal the recognition mechanism of cyclodextrin derivatives towards selectands is also discussed.

7.1 Background

Currently, chiral separation is mainly achieved via direct separation methods in which the enantiomers are put in a chiral environment with the addition of appropriately selected chiral selectors. Chiral separation is realized by the formation of diastereoisomers between enantiomers and chiral selectors. Different chromatographic and electrophoretic separation methods have been developed in the last decades to meet the demand of characterizing a broader range of chiral compounds with greater accuracy and precision. Chromatographic and electrophoretic separation methods mainly focus on chiral HPLC, GC, SFC, and CE [1–7]. The chiral selectors are incorporated into the chiral stationary phases (CSPs) via either chemical bonding or physical coating in chromatographic techniques or added to the mobile phase in electrophoretic ones. For CSP approach, enantiomers differ in their interactions with the same CSP and thus display different retention time in the CSP. With chiral mobile phase approach, diastereoisomeric complexes between enantiomers and

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chiral selectors differ in their stability. As such, enantiomers are separated due to the differences in either retention time or the stability of the two complexes [3].

The most widely used chromatographic technique for chiral separation is HPLC due to its variable separation modes (reverse phase, normal phase, ion pair etc.) and applicability to compounds with a wide range of polarity [1–3]. GC and SFC are limited in chiral analysis of pharmaceuticals due to the consideration of volatility (GC) and polarity (SFC) for the analytes [3, 8]. Chromatographic methods using packed columns have limited separation efficiency, so they require high-selectivity chiral selectors [2]. CE, however, has become quite popular over the recent years, mainly due to its high efficiency, fast analysis, small quantities of chiral selector and analytes needed, and flexibility in the course of method development [9].

For direct separation methods, native CDs and their derivatives are the most frequently used chiral selectors. Native CDs, however, only exhibit modest chiral discrimination to very limited racemate pool due to their inherent symmetry [10, 11]. Increased chiral discrimination can thus be expected from the use of modified CDs with an increased asymmetry. CD modification is often performed by varied substitution of the hydroxyl groups on both CD rims. However, random replacement of hydroxyl moieties does not alter too much the symmetry or enantioselectivity of parent CDs. More often, selective substitution of hydroxyl moieties with functional groups is adopted to introduce larger asymmetry and improved enantioselectivity to the CD derivatives. The modification of CD can lead to neutral or charged CD derivatives [12]. The use of CDs as chiral selectors is the subject of several selective reviews [1, 2, 10, 11, 13].

7.2 Fully, Randomly, or Disubstituted Cationic CD Derivatives in the C6 Position

Compared to negatively charged (or anionic) CD derivatives, positively charged (or cationic) CD derivatives have been developed slowly in the research fields. Their limited application might be due to their absorption to the column wall and the more complicated synthesis procedure [14]. Despite that, they demonstrate an advantage over anionic CDs, since their use in CE lowers the migration time of the solutes. They can be either strong electrolytes, when they are functionalized with quaternary ammonium groups, or weak electrolytes.

7.2.1 Fully Substituted Cationic CD Derivatives

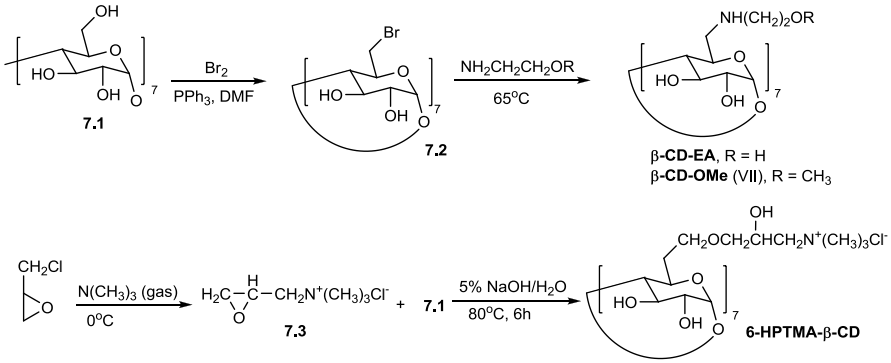
The polycationic CD derivatives, which are completely aminated in the C6 position of the glucopyranisinic unit, were first investigated because they demonstrated good batch-to-batch reproducibility and good enantioseparation abilities [15–19].

Fanali et al. [20] first reported the use of mono- and dimethylamino-β-CDs for the enantioseparation of racemic mandelic acid and its derivatives. 6^A-Methylamino-β-CD and hepta-methylamino-β-CD have been used as chiral selectors for the enantiomeric separation of a number of acidic and basic compounds by capillary electrophoresis [20]. The effect of the CD type, CD concentration, and the pH of the background electrolyte on the mobility and chiral resolution of the analytes was studied. The use of monomethylamino-β-CD in a coated capillary allowed the enantiomeric resolution of phenyl lactic acid, warfarin, and acenocoumarol but was not successful for tiaprofen and its 3-isomer. The hepta-methylamino derivative, under the same experimental conditions, was a better chiral selector than the monosubstituted CD towards the arylpropionic acids and phenyl lactic acid, while the anticoagulant drugs showed poor or no chiral resolution. They also studied the enantiomeric separation of several arylpropionic acids, namely, carprofen, cicloprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, naproxen, and suprofen, by using capillary zone electrophoresis with different chiral selectors including hepta-methylamino-β-CD [21].

O'Keeffe et al. [16] presented heptakis(6-hydroxyethylamino-6-deoxy-β-cyclodextrin) (β-CD-EA), as a chiral host-guest additive for the enantioseparation of various classes of chiral anionic analytes. Most CE separations of anionic drugs and herbicides were accomplished in the pH range of 4.0–7.0 with a reverse polarity configuration. At pH 5.0, enantioseparation of a mixture of three structurally related anti-inflammatory agents (fenoprofen, flurbiprofen, and ibuprofen) was possible in about 30 min. However, other chiral acids, such as a series of phenoxypropionic acid, herbicides, and dansylated amino acids (glutamic acid and aspartic acids), were best separated at pH 6.0 or 7.0. Enantiomeric separations of six anionic and two neutral racemates were achieved using β-CD-EA as the chiral selector by Shamsi and his coworkers [17].

Heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)] was successfully used as a chiral selector for enantiomeric separation of nonsteroidal anti-inflammatory drugs (NSAIDs) and phenoxypropionic acid herbicides (PPAHs) by the same group [15]. The synthetic route for the above CD was depicted in Scheme 7.1. Separation parameters such as pH and concentration were found to have major influences on enantiomeric resolution of the NSAIDs and PPAHs. Results indicate that β-CD-OMe (VII) performs exceptionally well for the enantiomeric resolution of NSAIDs: indoprofen and fenoprofen ($R_s = 11$ and 14, respectively). In addition, baseline enantiomeric separation of a mixture of six pairs of PPAHs was achieved in 30 min (Fig. 7.1).

Chiral recognition of α-amino acid derivatives by charged β-cyclodextrins has been studied by means of ^{1}H NMR spectroscopy [22]. Protonated heptakis(6-amino-6-deoxy)-β-cyclodextrin (per-NH_3^+-β-CD) forms complexes with the (S)-enantiomers of N-acetylated Trp, Phe, Leu, and Val in their anionic forms more preferentially than the (R)-enantiomers, though the difference in the binding constants (K) between the enantiomers is small. The K values for the per-NH_3^+-β-CD–guest anion complexes are much larger than those for the mono-NH_3^+-β-CD complexes. Heptakis(6-amino-6-deoxy)-β-cyclodextrin (per-6-NH_2-β-CD) was successfully used as a chiral



Scheme 7.1 Synthetic route for the polycationic CD derivatives (Reprinted with the permission from Ref. [16]. Copyright 1997 American Chemical Society)

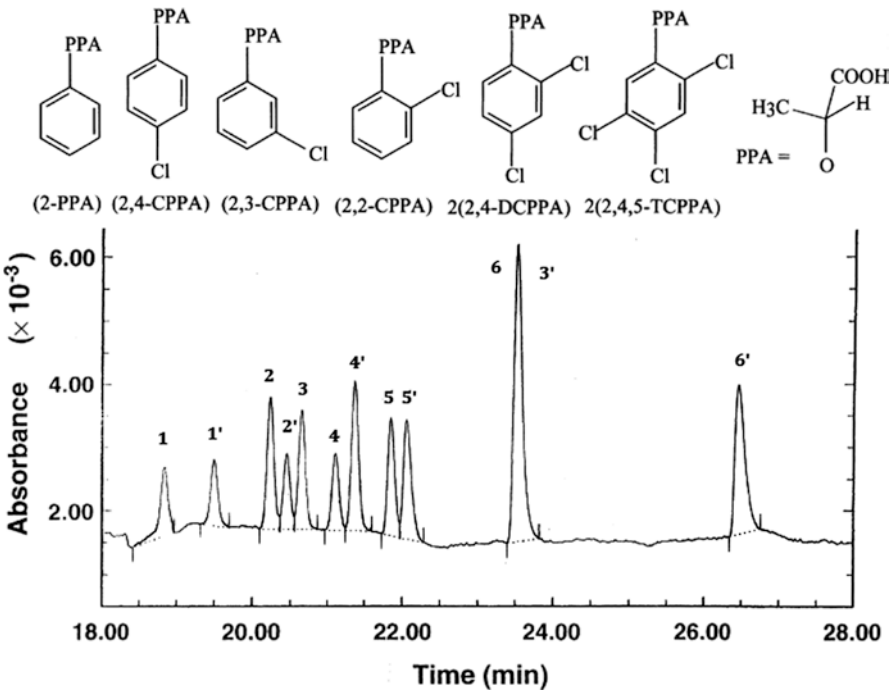


Fig. 7.1 Enantiomeric separation of a standard mixture of 12 (±) PPAH enantiomers. The BGE contains 50 mM NaH_2PO_4 adjusted to pH 6; 3 mM β-CD-OMe (VII); applied voltage was −15 kV, −25 μA; pressure injection 85 kPa.s; sample concentration 0.1 mg/ml in methanol/water (1:1, v/v). 1,1′ 2-PPA, 2,2′ 2,4-CPPA, 3,3, 2(2,4-DCPPA), 4,4, 2,2-CPPA, 5,5, 2,3-CPPA, 6,6, 2(2,4,5-TCPPA) (Reprinted from Ref. [15], Copyright 1998, with permission from Elsevier)

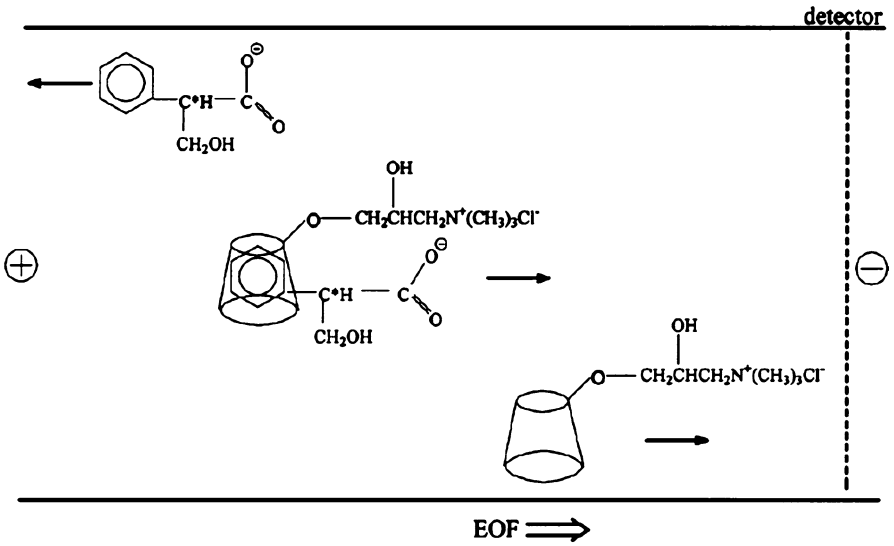


Fig. 7.2 Move direction of the species on the uncoated capillary (Reproduced from Ref. [23] by permission of John Wiley & Sons Ltd.)

selector for the enantioseparation of different anionic analytes (mandelic acid and carboxylic acids, arylpropionic acids, and amino acid derivatives) [18]. The running buffer pH and chiral selector concentration were the crucial parameters in achieving the maximum possible enantioresolution. Enantiomeric separation of a mixture of seven carboxy-benzyl-amino acids was achieved in 24 min.

Another charged highly water-soluble CD derivative, 6-O-(2-hydroxy-3-trimethylammonio-propyl)-β-CD (6-HPTMA-β-CD), was synthesized and successfully used as a chiral selector for enantiomeric separation of some acidic compounds by using CZE in an uncoated capillary [23]. Substitution with 2-hydroxy-3-trimethylammoniopropyl groups at the primary hydroxyl group of the CD was aimed at influencing the magnitude and selectivity of analyte–CD interactions. The behavior of 6-HPTMA-β-CD was compared with that of the commercially available randomly multi-substituted quaternary ammonium-β-CD (QA-β-CD) under the same separation conditions.

Since HPTMA-β-CD and other persubstituted cationic CD are all amines as QA-β-CD, they will adsorb onto the walls of the capillary under general CE experimental conditions used for enantioseparation as expected [15]. Such adsorption arises from electrostatic interactions that operate between the positively charged selector and the negatively charged walls of the uncoated capillary. As a result of adsorption, EOF is reduced in the case of running buffers containing cationic CDs. Reversal of EOF was observed in the case of buffers containing QA-β-CD or other persubstituted cationic CDs. Under the applied CZE conditions, cationic HPTMA-β-CD moves towards the cathode due to the action of the electric field and EOF (Fig. 7.2)

7.2.2 Randomly Multi-Position-Substituted Cationic CD Derivatives

A randomly multi-position-substituted cationic cyclodextrin, quaternary ammonium β-cyclodextrin ([empirical formula: $(C_6H_{10-n}O_5)_7$ $(C_6H_{15}ONCl)_n$, $n = 3.8$]), was developed and employed for the chiral separation of nonsteroidal anti-inflammatory drugs (profens), 1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate, N-[1-(1-naphthyl) ethyl]phthalamic acid, and derivatized amino acids by using capillary electrophoresis (CE) in both aqueous and nonaqueous media [24]. Several profens and amino acids could only be separated by QA-β-CD in pure formamide system. No chiral separation of the profens was achieved in the following solvents: N-methylformamide, methanol, dimethyl sulfoxide, and water; however, chiral separations of most of the amino acids were obtained in all of these solvents. The effects of other experimental parameters such as the CD concentration and apparent pH were also investigated. The first application of nonaqueous CE chiral separation of ketoprofen in a commercially available sample, Actron, was also examined.

Quaternary ammonium β-CD (DS = 3.5) and a commercial amphoteric β-CD (AM-β-CD) were used for the enantiomer separations of various acidic racemates [25]. Eleven acidic racemates were successfully separated using QA-β-CD by changing the CD concentration and the buffer pH. These enantiomer separations were compared with the results using five neutral CD derivatives. When QA-β-CD was employed, the enantiomer separations were successful at low concentrations below 5 mM. Enantiomers of five acidic racemates and ten dansylated amino acids (Dns-amino acids) were separated using AM-β-CD. Although the baseline separation of racemic 4-chloromandelic acid was not achieved with either QA-β-CD or five neutral CDs, AM-β-CD showed complete resolution. Furthermore, the simultaneous enantiomer separation of eight Dns-amino acids was also achieved with AM-β-CD. Both QA-β-CD and AM-β-CD were analyzed by CE and mass spectrometry (MS) in order to identify their compositions due to their nature of a mixture having different degrees of substitution. QA-β-CD consisted of six components having from one to six quaternary ammonium groups. In case of randomly multi-position-substituted derivatives, besides limited reproducibility of their synthesis, their resolution ability may vary from batch to batch, the dissimilarity of isomeric structures can lead to uncertainty in their practical applications.

7.2.3 Disubstituted Cationic CD Derivatives

The selective functionalization of two different C6-hydroxy groups on β-CD rim can be realized by using appropriate capping reagents, i.e., rigid systems bearing two reactive sulfonyl chloride groups at the right distance tailor to the desired disulfonated AB (4,6-dimethoxybenzene-1,3-disulfonylchloride, benzophenone-3,3′-disulfonylchloride), AC (benzophenone-3,3′-disulfonylchloride), and AD

Table 7.1 Enantiomeric separation of α-hydroxy acids and carboxylic acids with β-CD-$(NH_3)_2^{2+}$ (AB), (AC) and (AD)

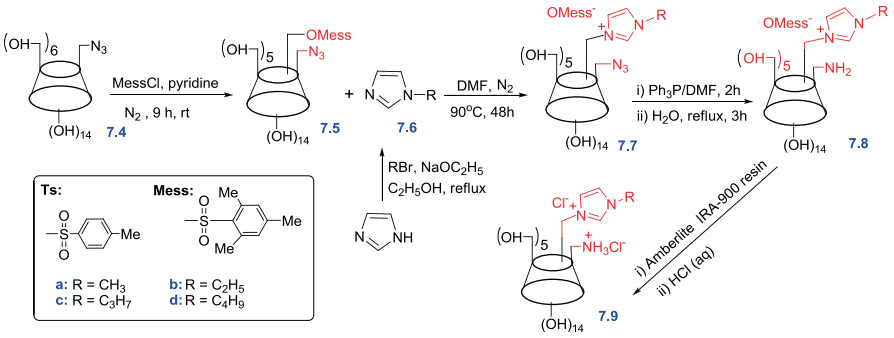
	β-CD-$(NH_3)_2^{2+}$ (AB)			β-CD-$(NH_3)_2^{2+}$ (AC)			β-CD-$(NH_3)_2^{2+}$ (AD)		
Racemates	t_R	α	R_s	t_R	α	R_s	t_R	α	R_s
α-Hydroxy acids									
Mandelic	7.73	1.03	0.7	8.57	1.05	1.6	8.04	1.04	1.2
p-Hydroxymandelic	9.51	1.03	1.3	8.73	1.06	2.3	9.40	1.06	2.0
3-Hydroxy-4-methoxymandelic	9.31	1.03	1.3	9.56	1.04	1.7	9.06	1.03	1.0
2-Phenyllactic	10.26	1.04	0.7	9.40	1.09	2.0	9.92	1.08	1.5
3-Phenyllactic	12.51	1.18	3.2	12.22	1.16	3.3	10.36	1.11	2.4
3-(4-Phenyl)lactic	15.35	1.26	4.1	12.02	1.18	3.8	12.79	1.15	3.7
Carboxylic acids									
2-Phenylpropanoic	14.47	1.10	2.4	12.71	1.00	0	10.84	1.00	0
2-Phenylbutanoic	32.80	1.40	3.6	19.65	1.00	0	15.51	1.00	0
3-Phenylbutanoic	28.80	1.09	1.0	23.34	1.06	0.5	24.75	1.00	0

Conditions: 1 mM β-CD-$(NH_3)_2^{2+}$, 100 mM acetate buffer, pH 5.0; temperature, 25 °C; applied voltage, 5 kV; coated capillary, 27 cm × 57 μm (20 cm to the detector); UV detector ($\lambda = 200$ nm); $\alpha = t_R/t_S$; $R_s = 2(t_R - t_S)/(w_R + w_S)$

(biphenyl-4,4′-disulfonylchloride) regioisomers with high selectivities [26]. Fujita et al. [27] established the convenient preparation method of 6^A6^B, 6^A6^C, and 6^A6^D-disulfonates of β-cyclodextrin and their isolation through single column chromatography. The separation method using the reversed phase column is also quite suitable for the elimination of the unreacted cyclodextrin and salts such as pyridinium tosylate.

By adopting the above-mentioned strategy for selective modification of CD, the 6,6′-dideoxy-6,6′-diamino-β-cyclodextrins (AB, AC, AD) (structure is shown in Fig. 1.4, Chap. 1) were successfully developed by Marchelli and his coworker [28]. These selectively disubstituted CDs were successfully used for the enantioseparation of hydroxy acids and carboxylic acids (particularly, phenoxyalkanoic acid herbicides) in CE. Chiral separations of hydroxy acids were obtained at low CD concentration (1 mM) with good α and R_s (Table 7.1). The different position of the cationic groups on the upper rim of CD greatly influenced the separation, which is due to the electrostatic interactions between the protonated amino groups of the CDs and the carboxylate of the selectands. As shown in Table 7.1, the best enantiomeric separation of α-hydroxy acids was obtained with AC regioisomer of CD, whereas the carboxylic acids were well resolved only by the AB regioisomer. Based on the proposed recognition model, the orientation of the guest in the inclusion complex is determined by the electrostatic interactions between the selectand and the upper rim of CD.

In review of the successful applications of diamino-β-cyclodextrins (AB, AC) in the enantioseparation of acids, it is intriguing for us to think whether we can develop efficient regioisomers with both ammonium and imidazolium functionalities as chiral selectors for the enantioseparation of acids. Recently, Tang's group prepared a seriesofdicationicACregioisomercyclodextrins:mono-6^A-ammonium-6^C-alkylimidazolium-



Scheme 7.2 Synthetic approach to 6^A-ammonium-6^C-alkylimidazolium-β-cyclodextrin chlorides (Reproduced from Ref. [29] by permission of The Royal Society of Chemistry)

β-cyclodextrin chlorides and used as chiral selectors for enantioseparation [29]. As depicted in Scheme 7.2, the synthetic route for dicationic CDs was established. A further nucleophilic substitution of mono-azido-β-CD **7.4** with 2-mesitylenesulfonyl chloride afforded a mixture of three regioisomers, 6^A-azido-6^X-mesitylene-sulfonyl-β-cyclodextrin (X = B, C, or D). After a liquid chromatography purification [26], the desired AC regioisomer 6^A-azido-6^C-mesitylene-sulfonyl-β-cyclodextrin (Mess-N3-CD) **7.5** was obtained. The AC regiochemistry was supported by the comparison of the known AC, AB, and AD regioisomers synthesized by capping of the CD [26, 27]. The as-prepared Mess-N3-CD **7.5** was then reacted with 3-alkyl-imidazoles **7.6** to introduce an imidazolium cation onto the C(6C) position of the CD. A Staudinger reaction, a protonation reaction, and a further anionic exchange were employed to achieve the desired 6^A-ammonium-6^C-alkylimidazolium-β-cyclodextrin chlorides **7.9**.

One of the desired cyclodextrins, 6^A-ammonium-6^C-butylimidazolium-β-cyclodextrin chlorides (**7.9d**, R=C_4H_9, AMBIMCD), demonstrated outstanding chiral recognition ability towards both acidic and even neutral racemates at a low concentration of 0.5 mM, which was even better than those of its mono-imidazolium or ammonium-substituted counterpart CDs at ninefold higher concentrations [30]. As depicted in Table 7.2, the effective mobilities of all studied analytes were found to decrease with the concentration of AMBIMCD.

From Table 7.2, R_s values of 2.4 and 3.0 were obtained for Dns-Aca with 1 and 1.5 mM AMBIMCD, respectively, while a maximum R_s of 2.0 was obtained with 10 mM mono-6^A-ammonium-β-cyclodextrin chloride ($CDNH_3Cl$). A high R_s of 15.4 was even achieved for 4-H MA with only 1.5 mM AMBIMCD. For enantioseparations in CE, few cationic chiral selectors can demonstrate such outstanding enantioselectivities towards both ampholytic and acidic racemate at CD concentration as low as 0.5 mM [31, 32]. Take the resolution of 3-PLA as example; an R_s of 3.1 could be obtained with 1.0 mM AMBIMCD, while 3.0 mM CD-hm only contributed a 2.24 R_s under the similar CE conditions with 27 cm × 75 μm ID capillary [33]. Inclusion complexation in combination with electrostatic interactions seemed to account for the enhanced chiral discrimination process.

Table 7.2 Effective mobility (μ_2^{eff}, in 10^{-5} cm^2V^{-1} s^{-1} units), separation selectivity (α), and peak resolution (R_s) of selected racemates in 1 mM AMBIMCD BGEs with different pH values

		pH 5.5			pH 6.0			pH 6.5			pH 7.0		
Analyte	CD	μ_2^{eff}	α	R_s	μ_2^{eff}	α	R_s	μ_2^{eff}	α	R_s	μ_2^{eff}	α	R_s
Dns-Aca	a	−12.67	1.19	1.8	−11.84	1.21	2.4	−12.03	1.17	1.9	−11.22	1.12	1.6
	b	−11.84	1.02	0.7	−10.32	1.08	0.9	−10.87	1.07	0.8	−9.14	1.07	0.8
	c	−10.28	1.12	1.7	−9.82	1.27	1.9	−9.95	1.19	1.8	−8.26	1.17	1.5
Dns-Nle	a	−16.65	1.03	1.6	−16.08	1.03	1.9	−18.97	1.03	1.7	−17.81	1.03	1.7
	b	−15.83	1.03	0.5	−14.26	1.04	0.8	−11.53	1.03	0.8	−10.48	1.03	0.6
Dns-Phe	a	−14.69	1.04	1.6	−13.76	1.03	1.7	−14.81	1.04	1.6	−13.49	1.01	1.6
	b	−12.32	1.15	1.9	−11.92	1.09	1.6	−12.32	1.07	1.5	−10.23	1.09	1.3
	c	−11.99	1.04	1.6	−10.04	1.28	2.5	−9.53	1.20	2.3	−8.03	1.16	1.7
Dns-Ser	a	−16.42	1.01	1.5	−15.02	1.02	1.9	−14.56	1.01	1.6	−13.84	1.01	1.4
	b	−13.77	1.04	0.9	−11.80	1.05	0.8	−13.36	1.03	0.9	−12.22	1.03	0.8
3-PLA	a	−19.64	1.05	2.9	−18.85	1.12	3.2	−18.81	1.09	2.7	−17.92	1.08	3.4
	c	−19.34	1.26	4.1	−18.09	1.23	4.9	−16.54	1.19	3.1	−12.92	1.07	3.1
HPLA	a	−22.42	1.14	2.2	−21.50	1.17	3.5	−20.47	1.10	3.3	−18.51	1.08	2.8
MoPLA	a	−26.52	1.16	3.6	−21.36	1.17	5.6	−27.28	1.06	3.9	−26.52	1.03	1.7
PCPA	a	−15.79	1.11	1.6	−14.65	1.11	1.8	−14.95	1.12	1.9	−13.75	1.11	1.7
2-PPA	a	−18.78	1.01	1.4	−17.62	1.02	1.6	−20.61	1.03	1.9	−19.62	1.06	1.6
2-TPA	a	−22.97	1.03	1.9	−21.59	1.03	2.4	−23.05	1.02	2.3	−21.03	1.02	2.1
2POPA	a	−20.35	1.01	1.3	−19.09	1.04	1.6	−22.16	1.05	2.5	−20.76	1.03	2.0
3-Cl POPA	a	−24.82	1.09	1.9	−24.20	1.09	1.8	−23.82	1.09	2.1	−23.38	1.06	1.9

[a]1 mM AMBIMCD
[b]5 mM BIMCDCl
[c]5 mM $CDNH_3Cl$

The chiral recognition ability of dually cationic CDs was further demonstrated with the outstanding resolution of eight model analytes including dansyl amino acids and acidic racemates with AMBIMCD (Fig. 7.3).

7.3 Synthetic Approaches to Monosubstituted Cationic Cyclodextrins

Ng and Tang's research groups have been actively engaged in developing selectively modified CDs as chiral selectors for chiral chromatographic and electromigration techniques [34–43]. For chiral HPLC and SFC, a large spectrum of perfunctionalized CDs have been prepared by immobilizing CD onto silica gel via urea linkage with spacer arms of various chain length. The synthetic methodologies have been well reviewed by Ng and his coworkers [34, 35]. For chiral CE, a wide range of monosubstituted cationic CDs have been explored by introducing imidazolium, ammonium, and pyridinium moieties with varied alkyl chain length and CD types [36–43].

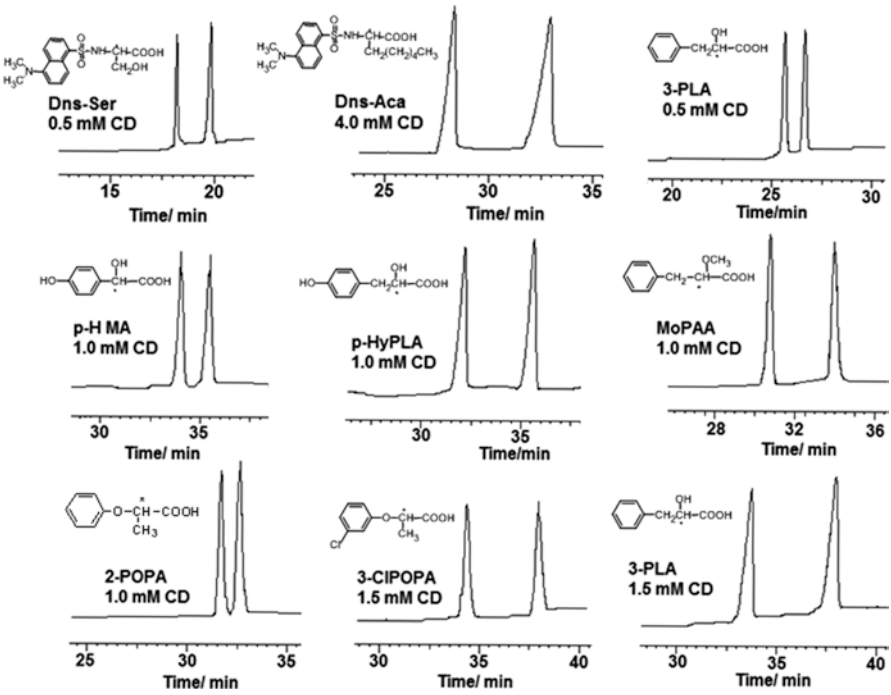
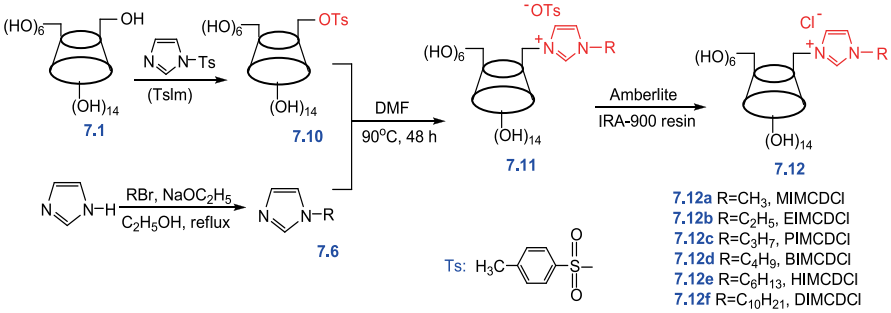
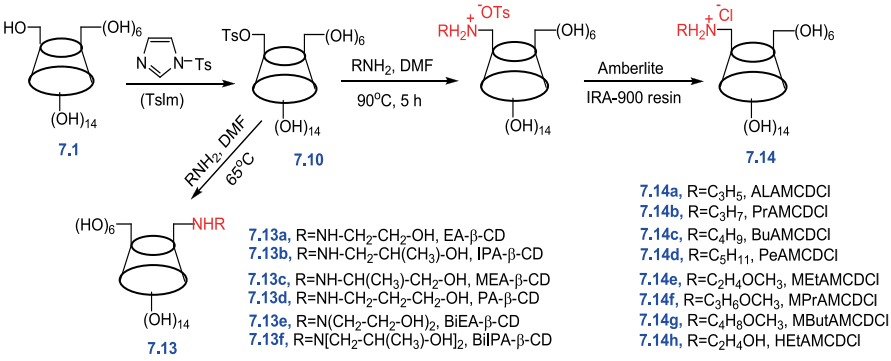


Fig. 7.3 Capillary electrochromatography of racemates using 1.0 mM BAMIMCD in 50 mM pH 6.0 phosphate buffer (Reproduced from Ref. [29] by permission of The Royal Society of Chemistry)

Tang et al. [38] have exploited the "clever" method in CD modification by using the shortest synthetic routes to obtain the desired primary products. Considering the existence of three types of hydroxyl groups on the CD rims, we take advantage of CD's complexation property to introduce one tosyl group specifically to 6-position or 2-position. Facile synthesis of mono-6-tosyl-β-CD is achieved by preparing the key intermediate compound, 1-(*p*-tolunesulfonyl)imidazole (Tsim) for better yield and easier handling. Starting from mono-6-tosyl-β-CD, a nucleophilic substitution with alkylimidazole or alkylamine and a further anion exchange afford the monosubstituted alkylimidazolium β-CDs (designated herein as Series **I**) and alkylammonium or (hydroxy)alkylammonium β-CDs (Series **II**) with varied alkyl chain length. For Series **I** CDs, a general methodology involves the heating of mono-6-tosyl-β-CD with alkylimidazoles in DMF at 90 °C for 2 days. Furthermore, the tosylate anion was converted into chloride by ion-exchange through amberlite 900 (Cl) resin (Scheme 7.3). For Series **II** CDs, similar methodology is adopted by refluxing alkylamine or (hydroxy)alkylamine together with mono-6-tosyl-β-CD in DMF (Scheme 7.4). A detailed step-to-step synthetic protocol for the synthesis of Series **I** and **II** CDs was exemplified using BIMCDCl (**7.12d**) [38].



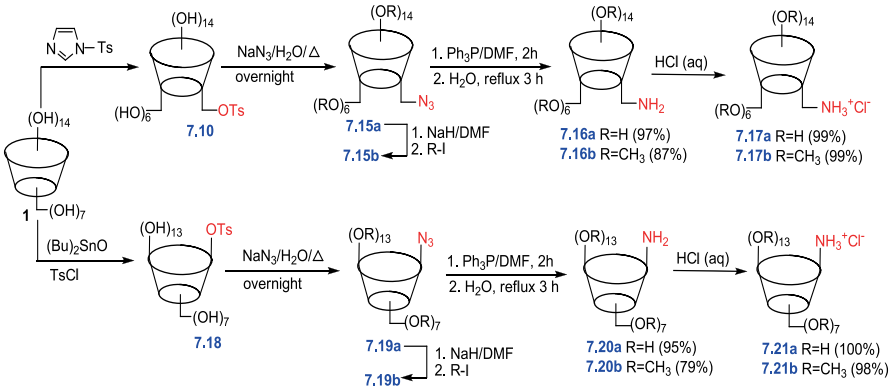
Scheme 7.3 Synthetic approach to mono-6-alkylimidazolium β-CDs (Series **I**) (Reproduced from Ref. [36] by permission of John Wiley & Sons Ltd.)



Scheme 7.4 Synthetic approach to mono-6-alkylammonium β-CDs (Series **II**) (Reproduced from Ref. [36] by permission of John Wiley & Sons Ltd.)

For the synthesis of the structurally simplest cationic β-CD (mono-6 or 2-amino-β-cyclodextrin hydrochlorides, Series **III**) (Scheme 7.5), we developed an innovative methodology involving the reduction of 6-azido β-CD (**7.15a**) by the use of triphenylphosphine and a further hydrolysis to afford mono-amino β-CD (**7.16a**). A subsequent treatment of mono-amino β-CDs with dilute hydrochloric acid yields the targeted highly water-soluble cationic CD (**7.17a**). These steps are almost quantitative with yield higher than 90 %. The approach has been greatly improved with mild reaction conditions and higher yields than previously reported methods, i.e., one is nucleophilic displacement of tosyl group with ammonia under pressure (10^6 N m^{-2}) for 18 h at room temperature [44]; the other is azide substitution of CD tosylate and a following hydrogenation with Pd/C for 12 h [45]. Both procedures are low yielding and incomplete reactions, thus undesirable for large-scale production.

We also extended the above methodology to the synthesis of mono-2-amino-β-cyclodextrin hydrochlorides (**7.21a**) by starting from mono-2-tosyl-β-CD (**7.18**). Reduction of 2-azido-β-CD (**7.19a**), further hydrolysis, and final treatment with



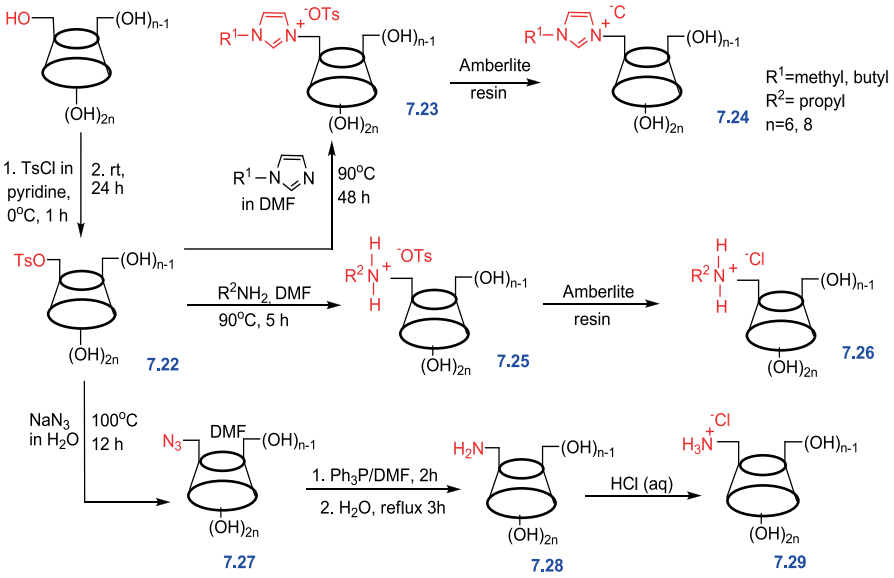
Scheme 7.5 Synthetic approach to mono-6 and 2-amino-β-CD hydrochlorides (Series **III**) (Reproduced from Ref. [36] by permission of John Wiley & Sons Ltd.)

HCl lead to the synthesis of mono-2-amino-β-cyclodextrin hydrochloride with satisfactory yield. For the concern of solubility and enantioselectivity, we can further substitute all rest hydroxyl groups at CD rims with functional groups. Herein, we have explored the synthesis of permethylated mono-6 or 2-amino-β-cyclodextrin hydrochlorides (**7.17b**, **7.21b**) by adopting the perfunctionalization on 6 or 2-azido-β-CD (**7.15b**, **7.19b**). The approach works well and good yields are achieved (Scheme 7.5).

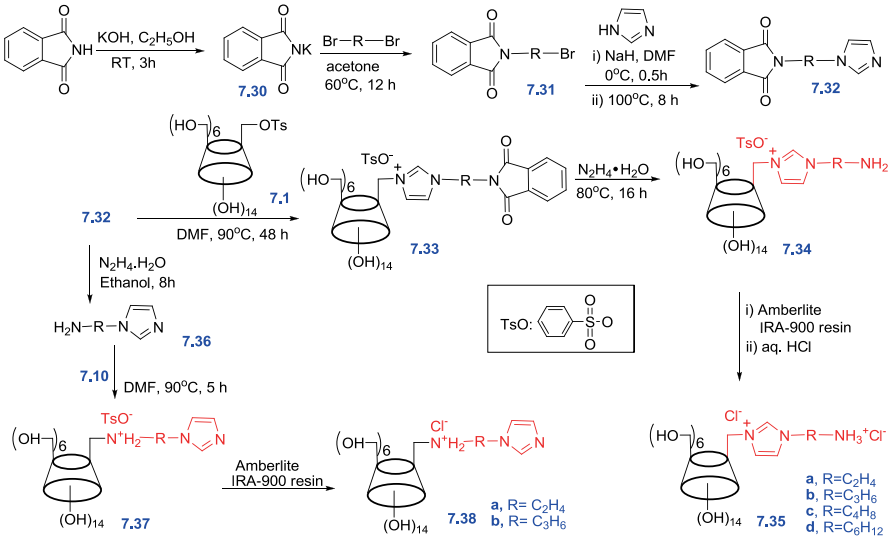
With the view of the effect of cavity dimension on the enantioselectivity of monosubstituted cationic CDs, Tang's group has extended their research efforts to α- and γ-cyclodextrin systems (Series **IV**). The synthesis of monosubstituted cationic α- and γ-CDs with alkylimidazolium, alkylammonium, and amino moieties has been explored (Scheme 7.6) by following the above-mentioned methodologies. The highly water-soluble CDs were characterized in terms of structure and their potential in chiral separation, corroborating the success of the synthetic approach [43]. A detailed step-to-step synthetic protocol for the synthesis of mono-6-amino-6-deoxy-α-, β-, γ-cyclodextrin hydrochlorides (**7.17a**, **7.29**) is available [37].

Marchelli investigated if and how the protonation of CD-hm and CD-mh, placing one or two positive charges at different distances from the cavity, would affect the enantiomeric separation in CE. Their study revealed that imidazole group directly anchored to CD derivative (CD-mh) demonstrated good enantioselectivities only for amino acids [46], while amino-linked counterpart CD (CD-hm) displayed good enantioselectivities for acidic racemates [33]. It is challenging to design cationic CDs to achieve versatile enantioselectivities for a wide range of both ampholytic and acidic racemates.

Moreover, Tang's group developed monosubstituted dually cationic cyclodextrins (Series V) recently, as depicted in Scheme 7.7. These cationic CDs exhibit satisfactory enantioselectivities for amino acids and acidic racemates in aqueous capillary electrophoresis [47]. Using the general Gabriel synthesis [48], (bromoalkyl)phalimides **7.31** was reacted with imidazole in the



Scheme 7.6 Synthetic approach to mono-6-substituted cationic α- and γ-CDs (Series **IV**) (Reproduced from Ref. [36] by permission of John Wiley & Sons Ltd.)



Scheme 7.7 Synthetic route for dually cationic CDs (Series **V**) (Reproduced from Ref. [47] by permission of The Royal Society of Chemistry)

presence of NaH to give N-[(1*H*-imidazolyl)alkyl]phthalimide **7.32**. A key step of nucleophilic attack of *p*-tolylsulfonyl β-cyclodextrin **7.1** by derivative **7.32** [38, 41] afforded the key monosubstituted CD intermediate **7.33**. The phthalimide removal with hydrazine monohydrate gave mono-6^{A}-[3-(aminoalkyl)-imidazol-1-ium]-6^{A}-deoxy-β-CD tosylate **7.34** with a yield of 60–81 %. Further anion exchange of **7.34** with Amberlie (Cl) resin and treatment with dilute hydrochloric acid gave the final product, mono-6A-[3-(ammonioalkyl)-imidazol-1-ium]-6^{A}-deoxy-β-CD chloride **7.35** as a white solid.

By deprotection of N-[(1*H*-imidazolyl)alkyl]phthalimide **7.32** with hydrazine [34], (1H-imidazol-1-yl)alkylamine **7.36** was first obtained, which can undergo nucleophilic attack of *p*-tolylsulfonyl β-cyclodextrin **7.10** to afford ammonium-substituted cationic CD **7.37**. Similar anionic exchange offered the second series of dually cationic CDs **7.38** in good yields.

7.4 Application of Cationic CDs in Chiral Separation Using CE

The enantioseparation abilities of the as-synthesized cationic CDs (Series **I–V**) in chiral CE have been evaluated using derivatized amino acids (Fig. 7.4), hydroxy acids, and carboxylic acids (Fig. 7.5).

In order to optimize the chiral separation, we have broadly investigated the effect of buffer pH, selector structure, CD concentration, separation temperature, organic solvents, and spectroscopic measurements on the enantioseparation of selected analytes [39–43, 49–56]. Among these cationic CDs, the CD derivatives with hydroxyl or methoxyl group in the sidearm showed good enantioseparation for neutral and acid racemates [54–56].

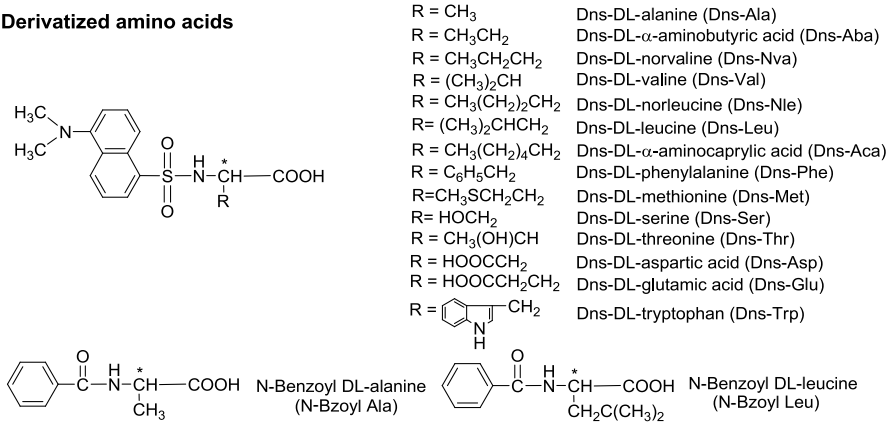


Fig. 7.4 The structure of derivatized amino acids

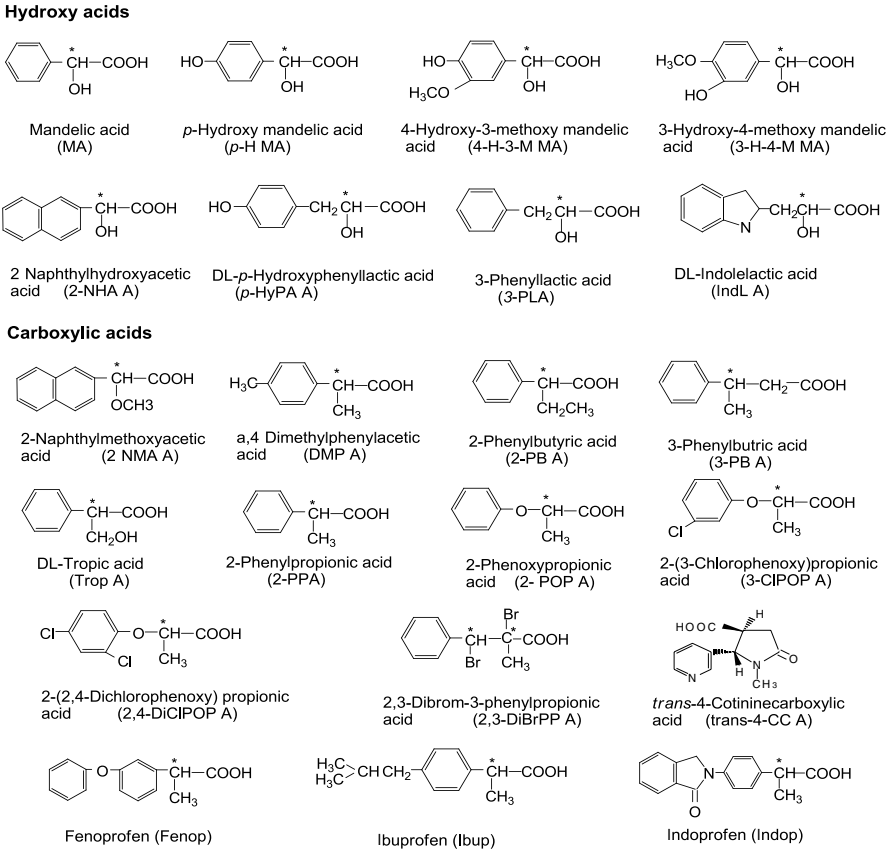


Fig. 7.5 The structure of hydroxy acids and carboxylic acids

7.4.1 Choice of Buffer in Chiral CE

Buffer pH is an important concern for enantioseparation using CE as it determines the charges on both the analytes and CDs. Altering the charge state of the analyte and CDs can affect both the degree and the enantioselectivity of the inclusion. Considering the pK_a values (3.5–4) of hydroxyl and carboxylic acids [49] and p*I* values (5.5–6.5) of native amino acids [50, 51], all chiral separations were carried out in buffers with pH ranging from 4 to 9.6 to ensure all acids were sufficiently dissociated. Phosphate buffers (50 mM) were used for wide pH range from slightly acidic (6.0) to basic. Acetate buffers (50 mM) were used for acidic pH range beyond pH 6.0. Under such conditions, these cationic CDs are completely protonated (pK_a ~8.2), and the strong electrostatic interactions between CD and enantiomers play a role in the chiral separation of enantiomers besides inclusion complexation. The optimum buffer pH for chiral separation varied for different

Table 7.3 The migration time of first enantiomer (t_1, min), chiral selectivity (α), and resolutions (R_s) of racemates with 5 mM MPrAMCD in various pH buffers

	pH 6.0			pH 6.5			pH 7.0		
Analytes	t_1	α	R_s	t_1	α	R_s	t_1	α	R_s
Dns-Nva	18.70	1.04	3.2	17.68	1.01	1.4	11.77	1.01	0.7
Dns-Nle	12.73	1.02	2.1	11.60	1.01	0.6	11.21	1.01	0.5
Dns-Phe	18.22	1.02	1.1	12.63	1.02	0.9	–	–	–
Dns-Ser	22.74	1.06	2.5	20.70	1.02	0.9	14.45	1.01	0.6
Dns-Thr	20.61	1.02	1.6	17.44	1.01	0.6	14.32	1.01	0.5
3-PLA	21.82	1.21	23.7	15.73	1.13	7.2	13.13	1.11	4.6
HPLA	19.14	1.14	6.7	13.54	1.11	6.2	12.53	1.10	3.3
IndL A	18.73	1.14	12.4	15.15	1.10	4.1	11.21	1.02	1.1
MA	23.81	1.16	14.8	20.42	1.12	4.1	19.43	1.10	4.5
p-H MA	21.31	1.15	13.4	19.45	1.10	3.6	17.70	1.02	1.0
4-H-3-M MA	27.73	1.14	7.1	25.14	1.06	3.2	22.49	1.04	1.4
2-Cl MA	22.46	1.23	21.1	22.61	1.13	5.8	18.65	1.12	5.3
p-HyPA A	14.34	1.11	9.2	13.10	1.10	7.2	12.18	1.09	4.4

series of CDs. For Series **I** CDs, MIMCDCl (**7.12a**) was found to present best resolution abilities towards dansyl amino (Dns-amino) acids at pH ~8.0 [49], while towards the same analytes, the other Series **I** CDs enjoyed better enantioseparation abilities at pH 6.0 [50].

For Series **II** CDs, it is worth to note that ALAMCDCl (**7.14a**) is very powerful in the enantioseparation of Dns-amino acids [53]. Most analytes were satisfactorily separated at an optimum pH 7.0 except Dns-Thr. Surprisingly, the CDs which contain methoxy group in the sidearm present better resolution abilities towards hydroxy and carboxylic acids [54, 55]. Taking mono-6^A-(3-methoxypropan-1-ammonium)-6^A-β-cyclodextrin chloride (MPrAMCD) [54] as an example, excellent separations were achieved for most hydroxyl and carboxylic acids at pH in the range of 6.0–7.0. From Table 7.3, under optimum pH 6.0, chiral resolutions over 10 can be readily achieved for acidic racemates with CD concentration below 10 mM. This cationic CD exhibits great potential for versatile chiral separation in CE probably due to the enhanced hydrogen bonding. A comparison study of a family of single-isomer amino-β-CDs containing an amino or (hydroxy)alkylamino group conducted by Iványi et al. [56] also revealed that the (hydroxy)alkylamino groups have higher enantioselective power than the alkyl ones. They attributed the higher enantioselectivity of hydroxyalkyl amino containing CDs to their higher polarity or hydrogen-bond-forming ability.

7.4.2 Concerns of CD in Chiral CE

For the chiral recognition with CD-based chiral selectors, CDs' cavity structure plays a very important role in determining their enantioseparation ability [50, 51], since the observed stereoselectivity was due to differences in fit or inclusion of the

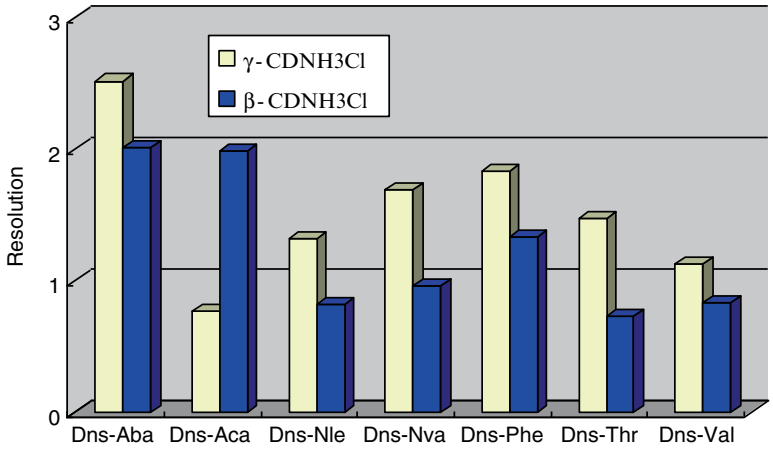


Fig. 7.6 Effect of CD type on enantioseparation of Dns-amino acids. Conditions: 10 mM CD, 50 mM pH 6.0 phosphate buffer (Reproduced from Ref. [36] by permission of John Wiley & Sons Ltd.)

enantiomers in CD cavities. For selected Dns-amino acids, mono-6-amino-γ-CD hydrochloride (γ-CD-NH_3Cl) provided better resolution than β-CD analogue (β-CD-NH_3Cl) as shown in Fig. 7.5. It indicates that the inclusion of dansyl group into the cavity of γ-CD would be more favorable than that into the cavity of β-CD to form the tight-fit complexes. One exception is Dns-Aca, probably due to the competitive inclusion of the dansyl group and its longer hexyl group directly connected to the chiral carbon. Due to cavity dimension, mono-6-amino-α-CD hydrochloride (α-CD-NH_3Cl) can provide poor resolution or even no recognition capability to the targeted Dns-amino acids [43] (Fig. 7.6).

β-CD-NH_3Cl (**7.17a**) is versatile and powerful in chiral resolution of anionic acids. As shown in Fig. 7.7, a standard mixture of eight Dns-amino and hydroxyl acids was baseline separated within 35 min. This separation was achieved using pH 6.0 buffer with 20 mM β-CD-NH_3Cl, and the migration order of analyte enantiomers in the standard mixtures was verified by injecting each racemate individually [41].

Besides CD cavity, the cation structure on the CD rims plays a role in the enantioselectivity of cationic CDs. Interestingly, when we made a comparison on the resolution ability of the as-developed mono-6-amino-β-CD hydrochlorides [41], mono-6-alkylamino-β-CDs [40, 51–53], and mono-6-alkylimidazolium-β-CDs [39, 49, 51] towards Dns-amino acids, it was found that Series **I** CDs with shorter alkyl chain ($R = C_nH_{2n+1}$, $n \leq 4$) and ALAMCDCl provided the outstanding resolutions to Dns-amino acids. Similar results can also be found in the imidazoyl directly linked 6-deoxy-6-[4-(2-aminoethyl)imidazoyl]-β-CD (CD-mh), which showed more powerful resolution abilities towards Dns-amino acids than amino directly linked 6-deoxy-6-N-histamino-β-CD (CD-hm) [46]. This may be related to the chiral recognition mechanism where host–guest interactions and ion pair interactions occur.

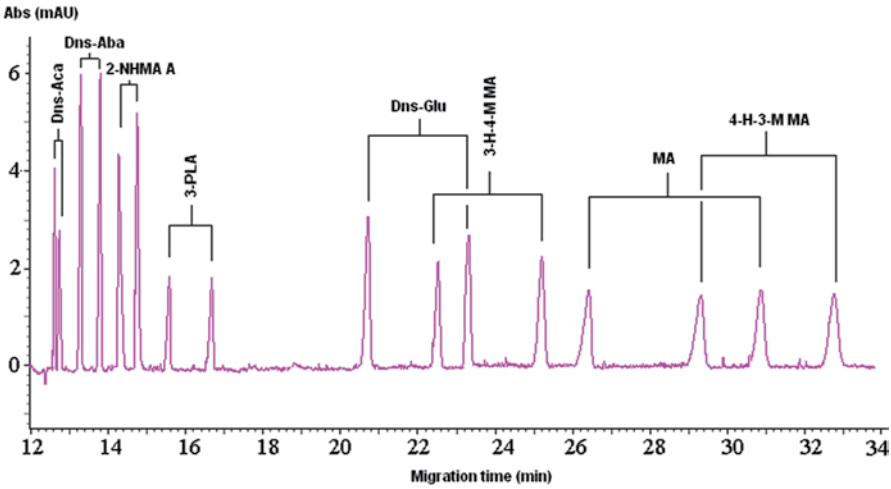


Fig. 7.7 Baseline separation of an eight-acid mixture using β-CD-NH_3Cl at 254 nm detection wavelength (Reprinted from Ref. [41], Copyright 2005, with permission from Elsevier)

In addition to this, the hydrogen bonding can also enhance the CD's recognition ability. Table 7.4 showed the enantioseparation of different type racemates with various MPrAMCD concentrations in pH 6.0 buffers.

The chiral resolution of α-hydroxy acids (from 3-PLA to *p*-HyPA A) with MPrAMCD is impressive, with $R_s > 10$ readily achieved even at CD concentration ([CD]) as low as 2.5 mM. The outstanding enantioselectivities increase dramatically with the increment of [CD] before reaching their maxima at 10 mM [CD]. Taking 2-ClMA as an example, its R_s increases from 11.8 to 19.1, 20.6, and 21 with [CD] gradually increasing from 2.5 to 10 mM. Further increasing [CD] to 15 mM leads to a decreased R_s of 4.0 for 2-ClMA. Similar behavior is also observed for carboxylic acids, where R_s of analyte increases with increasing [CD] before reaching their local maxima at [CD] of 10.0 mM. Though R_s values for all carboxylic acids are relatively lower than those for hydroxy acids due to lack of hydrogen bonding to enhance MPrAMCD's enantioselectivity, R_s over 2 can be easily achieved with only 2.5 mM CD.

The effect of alkyl chain length on the CDs chiral recognition ability was systematically investigated for Series **I** CDs. Results show that CDs with shorter alkyl chain ($R = C_nH_{2n+1}$, $n \leq 4$) in the imidazolium demonstrate better chiral discrimination towards Dns-amino acids than HIMCDCl. Such difference was revealed by the complex stability between CDs and analytes [50]. The resolution ability of Series **II** is also not strongly dependent upon the alkyl chain length of alkylammonium cation, probably because the alkyl chains are too short to compete in the inclusion complication between CDs and analytes (Table 7.5).

The optimum selector concentration is a second concern in screening efficient chiral selectors. For hydroxyl acids, PrAMCDCl (**7.14b**), BuAMCDCl (**7.14c**), and

Table 7.4 Effective mobilities of the first enantiomer (μ_{eff1}, in 10^{-5} cm^2/V s units), chiral selectivity (α), and chiral resolution (R_s) of racemates with various MPrAMCD concentrations in pH 6.0 buffers

	2.5 mM			5.0 mM			7.5 mM			10.0 mM			15.0 mM		
Analytes	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s
Dns-Aba	−9.73	1.08	2.7	−8.78	11.03	3.3	−8.74	1.03	3.3	−8.02	1.04	4.0	−6.31	1.05	7.4
Dns-Nva	−15.38	1.03	1.1	−14.34	1.04	3.2	−11.61	1.08	2.0	−11.51	1.01	1.1	−10.98	1.01	3.4
Dns-Nle	−11.36	1	0	−10.60	1.02	2.1	−10.11	1.02	2.0	−9.92	1.02	2.7	−9.63	1.03	2.7
Dns-Met	−12.15	1	0	−12.03	1.01	0.9	−11.76	1.1	1.5	−11.95	1.01	3.0	−11.10	1.02	4.5
Dns- Ser	−14.92	1	0	−12.13	1.06	2.5	−11.13	1.02	2.5	−12.37	1.03	2.6	−11.71	1.04	4.0
Dns-Thr	14.70	1	0	−11.94	1.02	1.6	−11.51	1.01	1.4	−12.11	1.03	2.7	−9.18	1.01	1.5
3-PLA	−12.57	1.11	11.0	−10.90	1.21	23.7	−10.81	1.15	11.6	−10.69	1.10	23.7	−6.31	1.05	11.4
HPLA	−10.36	1.03	3.4	−9.26	1.14	6.7	−10.01	1.13	12.4	−9.88	1.08	16.1	−5.21	1.10	8.4
IndL A	−10.31	1.08	9.7	−13.50	1.14	12.4	−10.06	1.20	12.4	−9.71	1.13	16.2	−9.45	1.03	2.7
MA	−19.12	1.11	12.4	−19.74	1.16	14.8	−19.02	1.15	14.1	−18.56	1.25	20.7	−10.17	1.10	7.7
p-H MA	−17.17	1.08	9.4	−17.30	1.15	13.4	−17.03	1.13	15.8	−15.32	1.17	18.1	−9.14	1.01	2.1
4-H-3-M MA	−13.97	1.02	3.0	−19.26	1.14	7.1	−19.44	1.07	8.4	−19.50	1.12	10.3	−10.25	1.02	3.0
2-ClMA	−16.21	1.11	11.8	−17.40	1.23	21.1	−17.12	1.24	20.6	−17.13	1.22	21.0	−9.40	1.04	4.0
p-HyPA A	−12.35	1.10	7.5	−12.07	1.11	9.2	−10.88	1.09	9.5	−10.73	1.08	12.9	−7.26	1.01	1.5
2-PBA	−9.84	1.02	3.0	−9.73	1.02	2.2	−9.13	1.01	2.6	−7.84	1.01	4.4	−6.31	1.05	4.0
2-PPA	−13.17	1.07	1.9	−11.04	1.01	3.4	−11.45	1.04	2.2	−10.98	1.04	4.2	−6.01	1.01	3.4
Trop A	−15.97	1.02	2.5	−15.70	1.03	2.7	−15.73	1.04	4.7	−15.05	1.09	10.7	−13.54	1.03	1.4
2,3-DiBrPPA	−8.34	1.01	2.3	−6.70	1.01	2.0	−6.25	1.01	2.1	−8.24	1.01	2.4	−6.43	1	0
2-POPA	−16.13	1.06	6.2	−15.83	1.10	8.2	−15.73	1.10	14.2	−15.49	1.12	15.9	−13.23	1.13	10.7
3-ClPOPA	−12.60	1.08	3.1	−11.95	1.20	6.3	−11.24	1.07	10.1	−11.33	1.08	10.9	−10.70	1.02	3.0
2,4-DCPOPA	−14.28	1.02	2.6	−11.73	1.05	3.3	−11.12	1.03	4.0	−10.19	1.03	6.1	−10.13	1.04	2.6
2-TolylPA	−10.00	1.02	2.1	−9.71	1.02	2.3	−9.51	1.01	2.3	−8.27	1.01	2.7	−6.14	1.01	1.5

Table 7.5 Effect of CD concentration on chiral separation of hydroxy acids

		2.5 mM			5 mM			7.5 mM			10 mM			20 mM		
Analytes	**CD**	t_1	α	R_s	t_1	α	R_s	t_1	α	R_s	t_1	α	R_s	t_1	α	R_s
MA	**7.14b**	15.79	1.03	1.7	16.25	1.05	2.5	16.35	1.06	3.2	17.95	1.08	3.7	20.08	1.11	6.5
	7.14c	15.89	1.06	2.0	16.37	1.07	2.5	17.73	1.08	2.8	18.63	1.09	3.5	20.74	1.13	4.5
	7.14d	18.06	1.04	1.7	18.50	1.07	2.9	19.58	1.09	3.3	20.13	1.11	4.1	20.29	1.13	5.2
p-H MA	**7.14b**	13.54	1.04	1.4	14.14	1.06	3.0	15.74	1.07	2.9	15.05	1.01	4.0	15.2	1.09	3.2
	7.14c	13.35	1.07	3.5	14.05	1.08	3.4	14.38	1.08	3.1	14.66	1.09	3.5	16. 35	1.11	3.9
	7.14d	15.69	1.05	1.8	16.22	1.08	3.5	16.96	1.09	3.6	14.37	1.09	3.2	17.33	1.12	4.9
4-H-3-M MA	**7.14b**	14.22	1.01	0.7	15.21	1.02	1.1	15.3	1.02	1.4	17.45	1.03	1.6	21.23	1.05	3.1
	7.14c	19.26	1.02	0.9	16.64	1.03	1.4	17.64	1.03	1.6	19.49	1.04	2.0	24.64	1.08	3.2
	7.14d	16.07	1.01	0.7	17.36	1.02	1.0	20.52	1.04	1.5	21.55	1.05	2.2	24.35	1.09	3.6
3-H-4-M MA	**7.14b**	13.63	1.02	1.6	14.32	1.04	2.3	13.75	1.05	2.1	16.13	1.06	2.9	16.26	1.07	4.1
	7.14c	18.7	1.04	1.7	14.87	1.05	2.7	16.26	1.06	1.8	17.12	1.08	3.3	19.43	1.12	3.9
	7.14d	15.34	1.03	1.3	16.03	1.05	2.1	17.94	1.08	3.2	18.13	1.09	3.5	18.99	1.12	5.4
2-NHA A	**7.14b**	9.83	1.05	2.2	9.89	1.05	1.2	10.43	1.04	2.0	10.09	1.03	1.9	10.70	1.02	1.8
	7.14c	12.22	1.07	2.5	9.88	1.04	1.3	9.96	1.04	1.4	10.46	1.03	1.6	11.57	1.01	1.1
	7.14d	10.82	1.05	2.4	10.88	1.05	1.5	10.79	1.04	1.7	11.1	1.04	1.6	12.17	1.02	1.7
p-HyPA A	**7.14b**	12.01	1.07	2.4	11.21	1.07	4.2	11.76	1.08	3.8	14.25	1.09	3.9	17.58	1.09	3.4
	7.14c	10.67	1.15	6.1	11.18	1.08	4.6	10.81	1.08	3.7	10.12	1.06	2.9	12.08	1.05	2.0
	7.14d	17.28	1.12	3.1	11.68	1.08	3.0	13.35	1.09	3.1	13.42	1.12	3.6	14.36	1.14	4.1
IndL A	**7.14b**	15.02	1.06	1.3	14.35	1.06	2.6	14.8	1.08	3.0	19.42	1.11	3.6	20.42	1.12	3.8
	7.14c	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	7.14d	18.43	1.04	1.3	14.28	1.04	1.7	15.98	1.05	1.6	16.11	1.08	2.1	17.54	1.08	2.3

Abbreviations: t_1 migration times (min), α chiral selectivity, R_s resolution

Conditions: 50 mM phosphate buffer; pH 6.0, containing different CD concentration; applied potential, 15 kV; 25 °C. /: not available

PeAMCDCl (**7.14d**) displayed excellent enantioseparation abilities over a wide concentration range of CD (Table 7.5). For most hydroxy acids, the chiral R_s increased with increasing CD concentration over the whole range from 2.5 to 20 mM. The higher resolution of hydroxy acids may be attributed to their ability to form secondary interactions (e.g., hydrogen bonding) with the CDs.

According to the theoretic model proposed by Wren for inclusion complexation [57], the optimum selector concentration can be predicted by the equilibrium constants (K) between free enantiomer and complexed enantiomer, when the apparent electrophoretic mobility difference reaches its maximum,

$$\Delta\mu = \frac{[CD](\mu_1 - \mu_2)(K_2 - K_1)}{1 + [CD](K_1 + K_2) + K_1 K_2 [CD]^2} \tag{7.1}$$

$$[CD]_{opt} = \frac{1}{\sqrt{K_1 K_2}} \tag{7.2}$$

where [CD] is the concentration of selector, μ_1, μ_2 are the apparent electrophoretic mobilities for two enantiomers, and K_1, K_2 are the equilibrium constants. The dependence of apparent mobility difference upon the Series **II** CD's concentration is depicted in Fig. 7.8 [40]. The apparent mobility difference of hydroxyl and Dns-amino acids increased with BuAMCDCl concentration and slowed down after 10 mM, indicating the maximum resolutions of these acids had not reached, which is confirmed by the resolution data in Table 7.4. The peak resolution values increased with the increment of CD's concentration. The optimum CD concentration for obtaining best separations should therefore be higher than 20 mM. For carboxylic acids, however, a local maximum of apparent mobility difference reached at BuAMCDCl concentration ranging from 2 to 5 mM. Correspondingly, the best resolution of these acids was obtained in this range of CD concentration. According to Eq. (7.2), the equilibrium constants (K_1, K_2) for α-hydroxy acids should be smaller than those for carboxylic acid. This may be explained by the inclusion complexation. Structurally, enantiomers of α-hydroxy acids bind more strongly with the cationic CD than the carboxylic acids. Therefore, the exchange rate between free and complexed enantiomers was lower for α-hydroxy acids, while the complexes between carboxylic acids and CD may form much faster, so does the enantiomer leave the CD cavity.

The theoretical determination of equilibrium constants between various acids and β-CD-NH_2 were calculated according to the x-reciprocal method [56], where equilibrium constant (K) is given by the slope of the fitting curve, when plotting $\left(\mu_{eff} - \mu_{free}\right)/[CD]$ against $\left(\mu_{eff} - \mu_{free}\right)$.

$$\frac{\left(\mu_{eff} - \mu_{free}\right)}{[CD]} = -K\left(\mu_{eff} - \mu_{free}\right) + K\left(\mu_{cplx} - \mu_{free}\right) \tag{7.3}$$

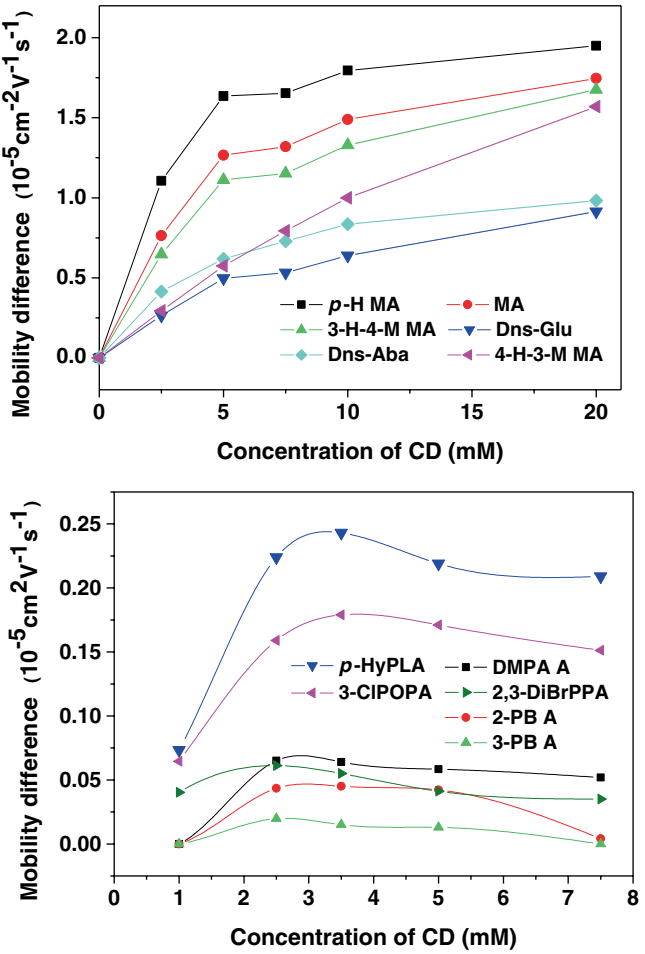


Fig. 7.8 Apparent mobility difference for hydroxyl and amino acids (*top panel*) and carboxylic acids (*bottom panel*) as a function of BuAMCDCl concentration (Reproduced from Ref. [40] by permission of John Wiley & Sons Ltd.)

The K values between β-CD-NH_2 and hydroxyl/Dns-amino acids fall in the range of 86–174 M^{-1} by linear regression as described from five different CD concentrations between 2.5 and 20 mM [58]. The K values between Series **I** CDs and nine Dns-amino acids were also calculated by increasing CD concentrations from 1 to 20 mM in pH 5.0 buffers (Table 7.6) [52]. The K values range from 130 to 210 M^{-1}. In general, the K values between HIMCDCl and most Dns-amino acids are lower than its analogue CDs with shorter alkyl chains in the imidazolium ring. It is noted that the side chains of the Dns-amino acids have considerable effect on the stability of the inclusion complex formed between Dns-amino acids and CDs. Dns-amino

Table 7.6 Apparent complex stability constants (K_1 and K_2) of mono-alkylimidazolium β-CDs with Dns-amino acids [52]

K_1 and K_2 (M^{-1})	MIMCDCl	EIMCDCl	PIMCDCl	BIMCDCl	HIMCDCl
Dns-Aba	181	183	187	181	176
	199	195	198	193	182
Dns-Val	173	169	165	166	164
	192	185	175	179	176
Dns-Nva	153	155	149	138	135
	161	165	163	151	140
Dns-Nle	137	137	137	138	136
	145	144	144	145	142
Dns-Aca	128	128	128	127	126
	136	135	135	134	131
Dns-Phe	182	181	183	182	181
	206	206	198	194	193
Dns-Ser	143	138	149	142	137
	158	147	162	154	146
Dns-Thr	151	149	154	151	145
	163	164	169	169	159
Dns-Glu	186	187	186	184	181
	197	198	197	193	189

acids with increasing alkyl chain (from Dns-Aba to Val, Nval, Nle, Aca) possessed lower *K* values with CDs, mainly due to the steric hindrance, similar to the effect of alkyl chain from the alkylimidazolium β-CDs on the complexes formation. Moreover, the polarity of the side chain of the Dns-amino acids also affects the complex formation. Since repulsion will occur between the hydroxyl groups from the amino acids and CDs, the stability of complex formed decreased. However, this repulsion effect reduced when increasing the alkyl chain and thus resulted in higher stability (higher *K* values from Dns-Thr than Dns-Ser) [52].

A close examination of *K* values, it is noted that the side chains of the Dns-amino acids have considerable effect on the stability of the inclusion complex formed between dansyl substitute of Dns-amino acids and CDs. Interestingly, Dns-amino acids with increasing alkyl chain (from Dns-Aba to Val, Nval, Nle, Aca) possessed lower *K* values with CDs, mainly due to the steric hindrance, similar to the effect of alkyl chain from the alkylimidazolium β-CDs on the complexes formation. Moreover, the polarity of the side chain of the Dns-amino acids also affects the complex formation. Since repulsion will occur between the hydroxyl groups from the amino acids and CDs, the stability of complex formed decreased, which can be easily proved when comparing *K* values between Aba and Ser as well as those between Val and Thr. On further comparison on the *K* values between Ser and Thr, it is noted that such repulsion effect reduced when increasing the alkyl chain and thus resulted in higher stability (higher *K* values from Dns-Thr). Dns-Glu presented higher *K* values due to the existence of a carboxylic group on the side chain, which is a ready site for hydrogen bonding with CD's hydroxyl group. Among the analytes

Table 7.7 Maximum resolution (R_s^{max}) and calculated optimum selector concentration (c_{opt}) of mono-alkylimidazolium β-CDs with Dns-amino acids [52]

	MIMCDCl	EIMCDCl	PIMCDCl	BIMCDCl	HIMCDCl
R_s^{max}					
Dns-Aba	2.39(20 mM)	2.31(15 mM)	2.07(15 mM)	1.75(20 mM)	1.89(20 mM)
Dns-Val	2.33(20 mM)	1.43(5 mM)	1.46(15 mM)	1.76(20 mM)	1.75(20 mM)
Dns-Nva	1.09(15 mM)	0.9(5 mM)	0.86(5 mM)	1.83(20 mM)	0.79(15 mM)
Dns-Nle	1.56(10 mM)	0.9(5 mM)	0.9(5 mM)	2.12(20 mM)	1.17(20 mM)
Dns-Aca	2.93(10 mM)	2.31(15 mM)	1.49 (20 mM)	2.89 (20 mM)	1.06 (15 mM)
Dns-Phe	5.06(15 mM)	5.19(15 mM)	4.6(15 mM)	3.89(15 mM)	3.39(15 mM)
Dns-Ser	0.98(20 mM)	1.17(10 mM)	0.87(10 mM)	1.32(20 mM)	1.12(15 mM)
Dns-Thr	2.46(20 mM)	1.77(10 mM)	1.73(5 mM)	2.62(20 mM)	1.62(20 mM)
Dns-Glu	2.69 (10 mM)	1.66 (10 mM)	1.55 (10 mM)	2.23(7.5 mM)	1.26 (10 mM)
C_{opt}					
Dns-Aba	5.3 mM	5.3 mM	5.2 mM	5.3 mM	5.6 mM
Dns-Val	5.5 mM	5.6 mM	5.9 mM	5.8 mM	5.9 mM
Dns-Nva	6.4 mM	6.2 mM	6.4 mM	6.9 mM	7.3 mM
Dns-Nle	7.1 mM	7.1 mM	7.1 mM	7.1 mM	7.2 mM
Dns-Aca	7.6 mM	7.6 mM	7.6 mM	7.7 mM	7.8 mM
Dns-Phe	5.2 mM	5.2 mM	5.3 mM	5.3 mM	5.3 mM
Dns-Ser	6.6 mM	7.0 mM	6.4 mM	6.8 mM	7.1 mM
Dns-Thr	6.4 mM	6.4 mM	6.2 mM	6.3 mM	6.6 mM
Dns-Glu	5.2 mM	5.2 mM	5.2 mM	5.3 mM	5.4 mM

studied, Dns-Phe possesses the highest apparent complex stability constants with different alkylimidazolium CDs, which is attributed to its high hydrophobic benzene group. Hence, theoretical optimum CD concentration for achieving maximum resolution of Dns-Phe is lower than other analytes (as shown in Table 7.7) [52].

It is worthwhile to note that the maximum resolutions were obtained at higher CD concentrations than the theoretically calculated values [57]. This can be seen particularly in the cases of Dns-Aba and Dns-Phe. This phenomenon might be related to the degree of peak dispersion depending on the apparent complex stability constant, though the intrinsic mechanism of this phenomenon has not been cleared up yet.

7.4.3 Consideration of Temperature in Chiral CE

The influence of buffer temperature on CE separation was investigated for the chiral separation of Dns-Thr with 20 mM BIMCDCl in pH 6.0 buffer by gradually increasing capillary temperature from 15 to 45 °C [39]. As expected, the migration time of Dns-Thr decreased by increasing the capillary temperature; while chiral resolution and selectivity decreased when increasing separation temperature [59]. According

to Lindner [60], a linear relationship between ln α and 1/T in the Van't Hoff plot exists due to the equation

$$\ln \alpha = -\Delta G = -\Delta H / \mathrm{RT} + \Delta S / \mathrm{R} \tag{7.4}$$

where the $\Delta\Delta G$, $\Delta\Delta H$, and $\Delta\Delta S$ are the Gibbs energy difference, enthalpy difference, and the entropy difference of the interactions between chiral selector and analyte. A Van't Hoff plot of ln α and 1/T shows an approximately linear relationship in the temperatures range from 20 to 45 °C. The linear relationship between ln α and 1/T shifts away beyond 20 °C, indicating a significant change in the migration behavior and consequently separation of the diastereomeric complexes as a result of additional, predominantly entropy-controlled effects [59].

7.4.4 Addition of Organic Solvent in Chiral CE

Both the degree of inclusion of analyte into CD cavity and the degree of enantioselectivity can be altered by the nature of surrounding solvent system [44]. CDs are often thought to act via the inclusion of a nonpolar portion of the guest molecule inside their hydrophobic cavity. On the basis of this simple approximation, it can be expected that any decrease in the hydrophobicity of the surrounding solvent will lead to the decrease in the degree of inclusion. The organic solvents added to the aqueous buffer can affect both migration time and chiral resolution when the inclusion-complexation mechanism is involved in the chiral recognition process. Herein, two typical additives, i.e., protic organic solvent methanol and aprotic organic solvent acetonitrile, were selected in the study of chiral separation of Dns-amino acids with BIMCDCl [39] and mono-6^A-(2-methoxyethyl-1-ammonium)-6^A-β-cyclodextrin chloride (**7.14e**, MEtAMCD) [55]. The effect of methanol on the enantioseparation was examined in the range of 0–20 % (v/v) with 5 mM BIMCDCl. The separation results of Dns-amino acids are summarized in Table 7.8. A general increase of both chiral resolution and selectivity was observed upon the addition of methanol into the buffer, whereas the selectivity displayed a maximum at 10 % (v/v) addition of methanol. However, a general increase in migration times (ca. 20–40 % with 10 % methanol) of analytes was observed. The increase might be attributed to the decreased electroosmotic flow (EOF), probably via the interaction of the modifier with the capillary wall by reducing the adsorption of cationic CD and thus changing the ζ potential, the driving force of EOF.

The influence of acetonitrile is quite different from that of methanol. Figure 7.9 compares the electropherograms of the enantiomeric separations of 2-chloromandelic acid using 2.5 mM MEtAMCD buffer containing different content of methanol or acetonitrile. Note that the peaks of 2-chloromandelic acid shifted to much longer migration times by increasing the concentration of organic solvents. Moreover, both the chiral resolution and selectivity increased with the content of methanol in buffer, while the trend was totally different with acetonitrile [55].

Table 7.8 Influence of methanol on chiral separation of Dns-amino acids with BIMCDCl

	0			5 % (v/v)			10 % (v/v)			20 %(v/v)		
Analytes	t_1	α	R_s	t_1	α	R_s	t_1	α	R_s	t_1	α	R_s
Dns-Aba	19.58	1.03	1.08	26.63	1.04	1.23	29.06	1.06	1.62	33.88	1.04	1.41
Dns-Leu	15.48	1.01	1.04	20.98	1.03	1.56	24.79	1.06	1.80	26.41	1.04	1.91
Dns-Nle	18.47	1.02	0.76	28.46	1.02	0.89	31.59	1.05	1.21	36.49	1.04	1.26
Dns-Ser	20.31	1.02	0.82	24.79	1.03	1.04	26.81	1.04	0.81	28.69	1.03	1.10
Dns-Thr	22.17	1.03	1.51	27.48	1.04	1.71	30.15	1.05	1.50	32.58	1.04	1.74
Dns-Val	20.24	1.03	1.61	24.21	1.04	0.92	26.25	1.09	3.65	30.23	1.05	1.42

Conditions: 5 mM BIMCDCl, 50 mM acetate buffer (pH 6.0), 25 °C

The behavior of methanol in this cationic CD separation systems is similar to that found in the chiral separation of terbutaline with sulfated β-CD in phosphate buffer [61]. With the addition of 5 and 10 % methanol, enantioselectivity increased but decreased in equilibrium constants. In this case, the migration time of analyte was largely elongated. The increase in enantioselectivity is caused by the equilibrium constants for the more weakly binding enantiomer decreasing at a much faster rate than the more strongly binding one [44].

7.4.5 Spectroscopic Measurements (^{1}HNMR Measurements (TOCSY) and 2-D NMR ROESY Experiments)

The inclusion complexation behavior was also investigated by mono-dimensional (1-D) ^{1}HNMR measurements (TOCSY) and *2-D NMR* ROESY experiments. By taking mono-6^A-[3-(6-ammoniohexyl)-imidazol-1-ium]-6^A-deoxy-β-cyclodextrin chloride (Series **V, 7.35d**, AMHIMCD) as CD host and single enantiomers of 3-phenyllactic acid (3-PLA) (i.e., *R*-3-PLA and *S*-3-PLA) as mode guests, the inclusion complexation mode of AMHIMCD/*R*-3-PLA and AMHIMCD/*S*-3-PLA complexes (10 mM) was revealed with ^{1}H NMR measurement in D_2O (pD 6.0) [47]. As shown in Fig. 7.10, when *R*-3-PLA was mixed with CD-X in aqueous solution, the split of the homologs of benzene was changed. The overlapped Hp and H_m proton signals for pure *R*-3-PLA were well separated to present.

A close look at the chemical shift for the protons of guest molecules in their complexes with AMHIMCD, the proof for molecular recognition can be found. High-field shifted chemical shifts obviously observed for -CH-α and CH_2-β of *R-3-PLA* are mainly attributed to the electrostatic interactions formed between *R*-3-PLA and CD. Down-field shifted chemical shifts for aromatic moiety of 3-PLA are also visible, mainly due to the shield effect of CD cavity. Among three kinds of aromatic protons, Ho exhibited the largest chemical shift increment. All these demonstrated that the benzene ring and CD formed the inclusion complexes. For MPrAMCD, its chiral recognition ability to acidic enantiomers was also conducted through the 1-D TOSEY ^{1}H NMR study [54]. The molecular recognition mode of MPrAMCD/*R-3-PLA* and PrAMCD/*R*-3-PLA complexes was further examined by two-dimensional nuclear magnetic resonance (2-D NMR) ROESY experiments (Fig. 7.11).

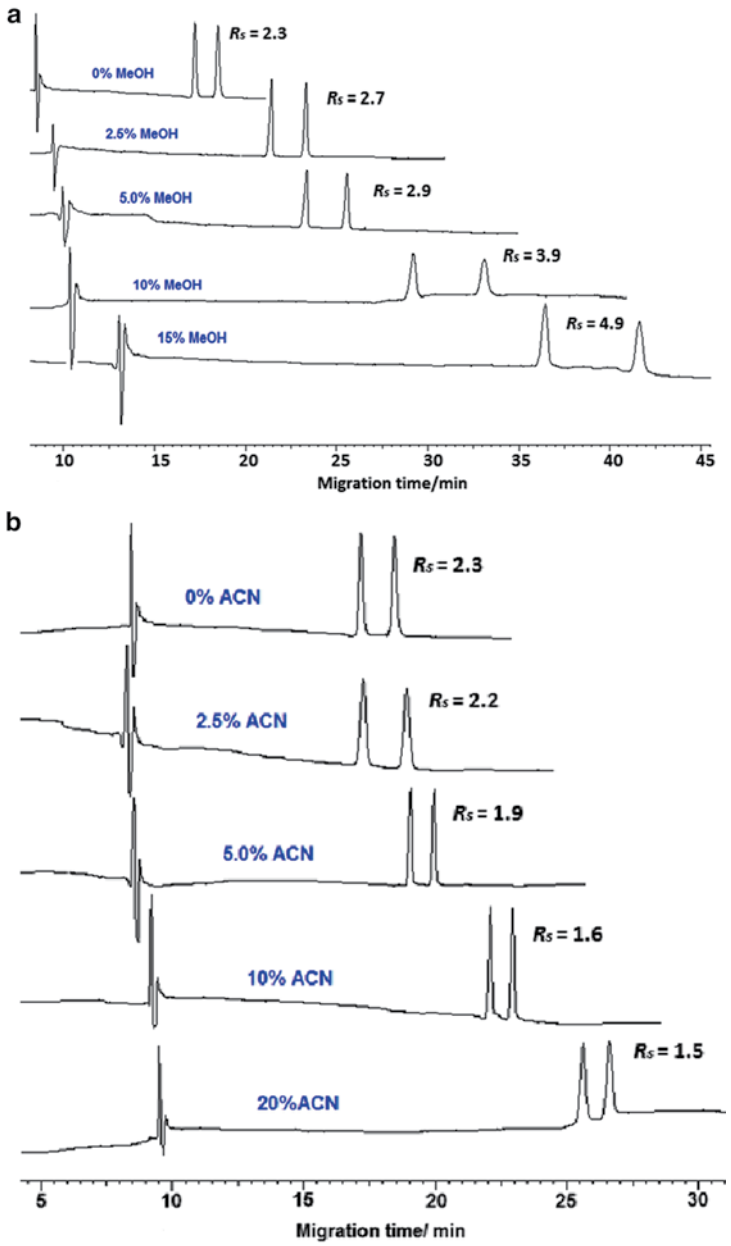


Fig. 7.9 Influence of methanol (**a**) and acetonitrile (**b**) on the enantiomeric separation of 2-Cl MA with 2.5 mM MEtAMCD in 50 mM pH 6.5 BGEs (Reprinted from Ref. [55], Copyright 2013, with permission from Elsevier)

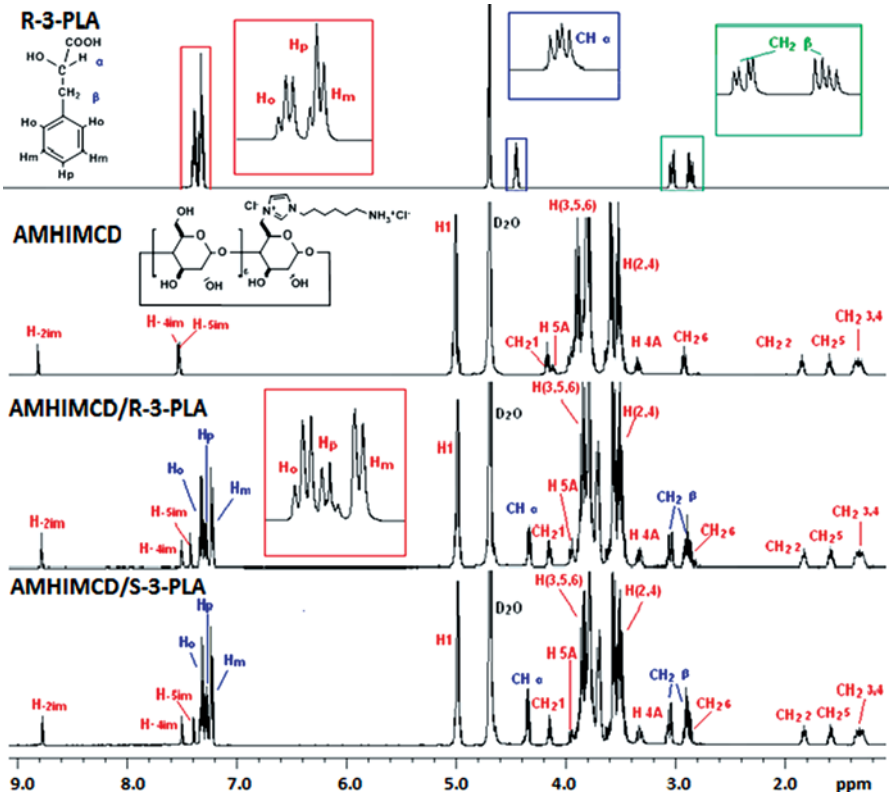


Fig. 7.10 ^{1}H NMR spectra of AMHIMCD, *R*-3-PLA and the mixture of AMHIMCD/*R*-3-PLA and AMHIMCD/*S*-3-PLA (1:1, 10 mM, pD 6.0) (Reproduced from Ref. [47] by permission of The Royal Society of Chemistry)

Intermolecular cross peaks are observed between –OH of *R*-3-PLA (5.61 ppm) and H 5A and –OCH_3 of CD (3.51–3.54 and 3.23 ppm) for complex MPrAMCD/*R-3-PLA* (Fig. 7.11a), while no cross peaks are observed in this region for complex PrAMCD/*R*-3-PLA (Fig. 7.11b). More importantly, the cross peaks between Hα (4.02 ppm) and -CH_2 **c** (2.91 ppm) in the former complex are also stronger than those between Hα (4.05 ppm) and -CH_2 **a** (2.93 ppm) in the latter. Besides, cross peaks between Hβ of *R*-3-PLA (2.72–2.78 ppm) and -CH_2 **c** (2.91 ppm) of MPrAMCD sidearm are much stronger than those between Hβ (2.76–2.81 ppm) and -CH_2 **a** (2.93 ppm) of PrAMCD. These results are consistent with the recognition model in which external hydrogen bonding is formed between –OH of *R*-3-PLA and the methoxy group of MPrAMCD, which contributes in the chiral recognition events.

By taking advantage of hydrogen bonding as extra driving force, as revealed in the NMR study, MPrAMCD demonstrated stronger molecular recognition for R-3-PLA

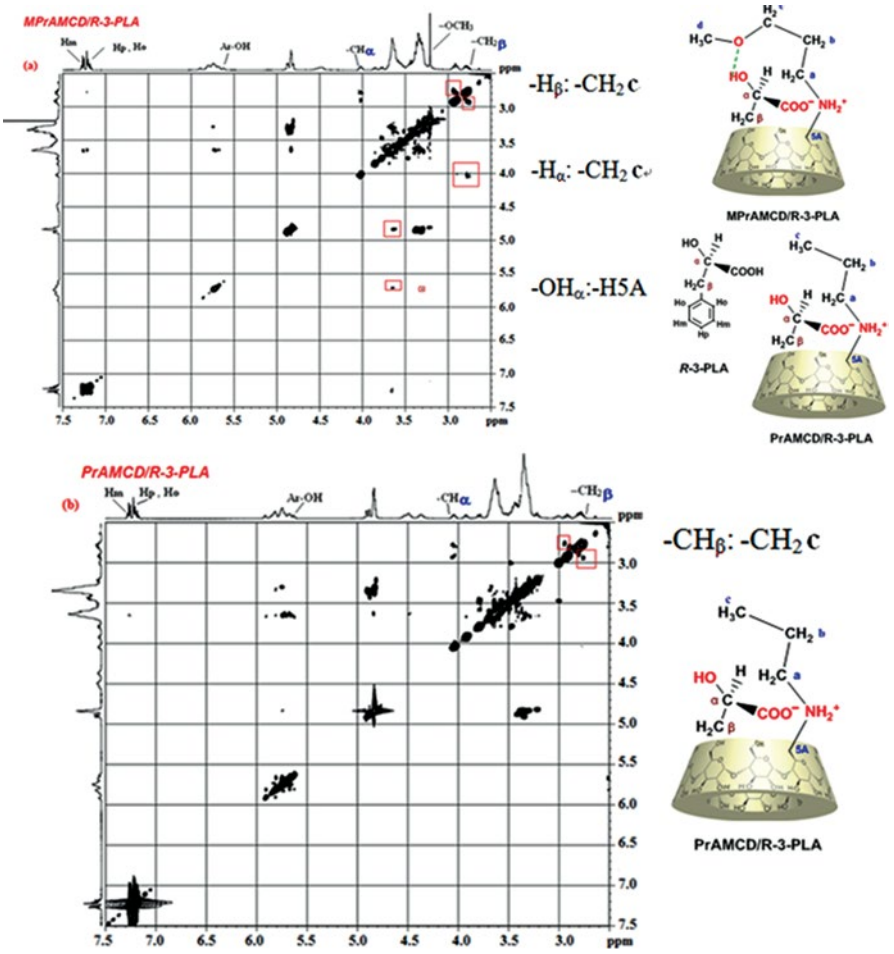


Fig. 7.11 Portion of 2-D ROESY NMR spectra of MPrAMCD/*R*-3-PLA (**a**) and PrAMCD/*R-3-PLA* complexes (1:1, 10 mM) in DMSO-$_{d6}$ (**b**) (Reprinted from Ref. [54], Copyright 2012, with permission from Elsevier)

than PrAMCD and stronger chiral recognition for R-3-PLA than S-3-PLA. It is reasonable to expect that MPrAMCD will demonstrate better chiral resolution capability than the reference chiral selector, PrAMCD, which has already demonstrated excellent enantioselectivities for acidic racemates [52]. A comparison study of enantioselectivities of MPrAMCD and PrAMCD (10 mM CD, pH 6.0 buffer) was conducted with ten model racemates including both ampholytic and acidic analytes. As shown in Fig. 7.12, MPrAMCD demonstrates nearly threefold higher chiral resolutions towards selected analytes than PrAMCD, with most Rs over 10. To our best knowledge, these high R_s values are rare among all enantioseparations achieved with chiral selectors [14, 16–21, 33–56].

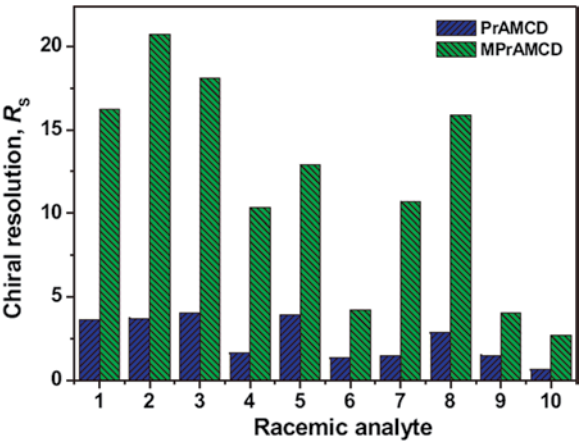


Fig. 7.12 Chiral resolutions (R_s) of ten typical racemates with MPrAMCD and PrAMCD using 10 mM CD and pH 6.0 buffer. Note *1* IndL A, *2* MA, *3* p-H MA, *4* 4-H-3-M MA, *5* p-HyPA A, *6* 2-PPA, *7* Trop A, *8* 2-POPA, *9* Dns-Aba, *10* Dns-Nle (Reprinted from Ref. [54], Copyright 2012, with permission from Elsevier)

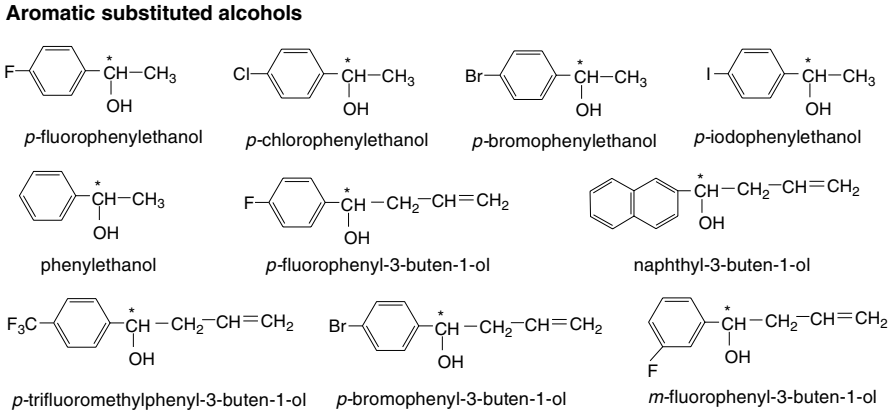


Fig. 7.13 The structure of aromatic substituted alcohols

7.4.6 Application of Cationic CD-Based CSPs in HPLC and SFC

In view of the successful application of the cationic CDs in the chiral separation of amino acids and anionic analytes, their potential as chiral selectors in HPLC and SFC has been explored in Ng's lab. CSPs were prepared by coating mono-6-(3-methylimidazolium)-6-deoxyperphenylcarbamoyl-β-cyclodextrin chloride (MPCCD) on silica gel, and their chiral resolution ability towards neutral analytes (Fig. 7.13) was evaluated [36]. MPCCD was prepared by perfunctionalization of MIMCDCl (Scheme 7.3) with phenyl isocyanate at 85 °C in pyridine for 15 h and purified by flash column chromatography. The chiral resolution ability of these CSPs is strongly influenced by the CD loading content; 20 % (w/w) loading of MPCCD provided the best

enantioseparations. Under normal-phase HPLC (mobile phase: *n*-hexane/2-propranol 97/3, v/v at 1 ml/min flow rate), all selected alcohols can be nicely separated with chiral resolution in the range of 1.25–5.65 with 20 % CD loading CSP. Except phenylethanol, other nine alcohols were resolved by the same CSP in SFC separation.

7.5 Conclusions and Future Prospects

The use of selectively substituted cyclodextrins is strongly recommended in performing mechanistic studies and developing validated chiral separation assays. For efficient chiral CE separation, charged CDs are advantageous for the chiral recognition of oppositely charged enantiomers at low concentration and neutral analytes with their electrophoretic mobilities. Compared with the large spectrum of anionic CDs developed so far, more research efforts in cationic CDs are demanding. The results recounted above describe the research efforts in developing selectively substituted charged CDs for the chiral separation by the use of electrophoretic and chromatographic methods, starting from the design of synthetic methodologies for these structurally well-defined cationic CDs and leading to the thorough investigation in optimizing the enantioseparation towards targeted racemates by considering all separation parameters and CD structures. This chapter provides researchers with great reference in their future exploration in novel CD-based selectors for chiral separation techniques.

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