Chiral Recognition in Separation Methods

Alain Berthod
Editor

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Mechanisms and Applications

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Preface

What drives a scientist to edit a book on a specific scientific subject such as chiral mechanisms in separation methods? Until December 2005, the journal *Analytical Chemistry* of the American Chemical Society (Washington, DC) had an A-page section that was dedicated to simple and clear presentations of the most recent techniques or the state of the art in a particular field or topic. The "A-page" section was prepared for a broad audience of chemists including industrial professionals, students as well as academics looking for information outside their field of expertise. Daniel W. Armstrong,[1] one of the editors of this journal and a twenty-year+ long friend, invited me to present my view on chiral recognition mechanisms in a simple and clear way in an "A-page" article. In 2006, the "A-page" section was maintained as the first articles at the beginning of each first bi-monthly issue but the pagination was no longer page distinguished from the regular research articles published by the journal. During the time between the invitation and the submission, the A-page section was integrated into the rest of the journal and the article appeared as (2006) Anal Chem (78):2093–2099.

The article was well received. John Dorsey,[2] another very long time friend and colleague, invited me to present it as a lecture in his Dal-Nogare Award session of the 2008 Pittsburg Conference in New Orleans. I presented a talk focusing on the only part of chiral mechanisms that I really know and worked on: chiral recognition mechanism with the macrocyclic glycopeptide chiral selectors. Steffen Pauly, Senior Editor Chemistry for the publisher Springer, heard the talk and asked me to edit a book on the subject. It was so well paid (sigh!) that I could not refuse the offer. . . and now, you have the book in hand.

Author invitations, article redaction time, reviewing and revising process, and text editing took almost 2 years. The book opens with my own general view of chiral mechanisms in separation methods. I was very fortunate in recruiting some of the most distinguished researchers in the field. In many cases, the originators of some of these powerful separation methods agreed to contribute and provide

[1] University of Texas at Arlington
[2] Florida State University, Tallahassee

their unique insight. For instance, Yoshio Okamoto, the discoverer of the powerful carbohydrate-based chiral stationary phases (CSP), and his co-workers prepared a chapter on mechanisms and applications of these CSPs. Cyclodextrins are another class of very useful CSPs. Thomas Beesley, CEO of Astec Inc, recently incorporated in the Supelco-Sigma-Aldrich group, gives his views on cyclodextrin CSPs. Daniel Armstrong introduces the macrocyclic glycopeptide CSPs. In addition, he presents here, with his group, a new class of potentially very powerful CSPs: the cyclofructan CSPs. In capillary electrophoresis (CE) the chiral selector must be added to the mobile phase since there is no real chromatographic stationary phase. Bezhan Chankvetadze of the Tbilisi State University details all possible mechanisms of chiral separations in CE. The sixth chapter written by Brian He of Bristol-Myers Squibb provides the point of view of an expert in chiral separations from the pharmaceutical industry. Next, the macrocyclic glycopeptide CSP properties and interaction mechanisms are presented by Dan Armstrong, people of his group and myself. Tim Ward of Millsaps College, Mississippi, reminds us that vancomycin, one of the macrocyclic glycopeptide selectors, has strong antibiotic properties and proposes, using vancomycin as an example, and that the antibiotic and enantioselective interactions are related. The ninth chapter, presented by Cristina Minguillon of University of Barcelona, deals with countercurrent chromatography and chiral interactions in liquid phases. Eric Peyrin of Grenoble University explains aptamers capabilities in chiral separation and the book ends with a chapter by the Isiah Warner group (Louisiana State University) on another new class of chiral selectors: the chiral ionic liquids.

In drawing this preface to a close, while all authors presented their unique point of view on chiral mechanisms in enantiomeric separations, they would like to impress upon the readers that we are still a very long way from full understanding of the enantiomer–chiral selector interactions leading to chiral separation. For instance, solvents are used. Solvent effects are very important and yet very difficult to predict accurately. The different author approaches should give an idea to the reader on the complexity of the chiral separation problem.

I want to acknowledge and to thank all the authors for the hard work and amount of effort and information that they put in their chapters. We all sincerely wish that this book will be useful to beginners and students as well as to confirmed practitioners in this unique separation field.

Villeurbanne, France Alain Berthod
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List of Abbreviations

AA	Amino acid
ACN	Acetonitrile
ADP	Adenosine diphosphate
AGP	Acid glycoprotein
AGT	Aminoglutethimide
AMP	Adenosine monophosphate
APCI	Atmospheric pressure chemical ionization
AQC	Amino quinilyl carbamate
ATP	Adenosine triphosphate
ATPS	Aqueous two phase systems
ATR-IR	Attenuated total reflection – infrared
AVI	Avidin
BGE	Background electrolyte
Bis–Tris	Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane acetate buffer
BNDA	Binaphthyl diamine
BSA	Bovine Serum Albumin
C12-Pro	*N*-Dodecyl-L-proline
CB-AC	Acetylated-beta-cyclodextrin
CB-DM	Dimethyl-beta-cyclodextrin
CBH	Cellobiohydrolase
CB-RN	*R*-naphthylethyl carbamate-beta-cyclodextrin
CB-RSP	Hydroxypropylated-beta-cyclodextrin
CBZ	Carboxybenzoxy
CCC	Countercurrent chromatography
CCD	Central composite design – or – charged coupled device
CCS	Charged chiral selector
CD	Cyclodextrin
CE	Capillary Electrophoresis
CEC	Capillary electrochromatography
CF	Cyclofructan
CGE	Capillary gel electrophoresis

CHARM	Charged resolving agent model
CICS	Complexation-induced chemical shift
CIEF	Capillary isoelectric focusing
CIL	Chiral ionic liquid
CIP	Cahn–Ingold–Prelog rule
CMPA	Chiral mobile phase additive
CPC	Centrifugal Partition Chromatography
CS	Chiral selector
CSP	Chiral Stationary Phase
CZE	Capillary zone electrophoresis
DEA	Diethylamine
DFT	Density functional theory
(DHQD)2PHAL	Bis-1,4-(dihydroquinidinyl)phtalazine
DIM	Dimethindene
DM	Dual-mode
DMA	Dimethylacetamide
DMO	Desmethyl meloxifene
DNA	Deoxyribonucleic acid
DNB-(±)-Leu	*N*-(3,5-dinitrobenzoyl)-(±)-leucine
DNB-(±)-Leu-*t*Bu	*N*-(3,5-dinitrobenzoyl)-(±)-leucine-*t*-butylamide
DNS	Dansyl (5-sulfonyl chloride)
DNS-(±)-Nle	Dansyl-(±)-norleucine
DNS-D-Nle	Dansyl-D-norleucine
DNS-L-Nle	Dansyl-L-norleucine
DNZ-(±)-NPG	*N*-(3,5-dinitrobenzyloxycarbonyl)-(±)-neopentylglycine
EDDP	Ethylidene dimethyl diphenyl pyrrolidine
EDTA	Ethylene diamine tetraacetic acid
ee	Enantiomeric excess
EFGF	Electric field gradient focussing
EKC	Electrokinetic chromatography
ELISA	Enzyme-linked immunosorbent assay
EMO	Enantiomer migration order
ENFB	Ethoxynonafluorobutane
EOF	Electroosmotic flow
ESI	Electrospray ionization
FAB	Fast atom bombardment
FCCE	Flow counterbalanced capillary electrophoresis
FDA	Food and drug administration
FLEC	Fluorenyl ethyl chloroformate
GC	Gas chromatography
HILIC	Hydrophilic interaction chromatography
HP-CD	Hydroxypropylated cyclodextrin

HPLC	High Performance Liquid Chromatography
HSA	Human serum albumin
IL	Ionic liquid
IPA	Isopropyl alcohol
IUPAC	International union of pure and applied chemistry
$\boldsymbol{Ka_{R/S}}$	Association constant CS/enantiomer
$\boldsymbol{K_D}$	Distribution ratio
LC	Liquid chromatography
LLE	Liquid–liquid extraction
LSER	Linear solvation energy relationship
MALDI	Matrice-assisted laser desorption ionization
MD	Molecular dynamics
MDM	Multidual mode
MED	Micromachinated electrophoretic device
MEKC	Micellar electrokinetic chromatography
MIBK	Methyl isobutyl ketone
MIP	Molecular imprinted polymer
MLR	Multilinear regression
MM	Molecular mechanic or molecular modeling
MS	Mass spectrometry
MTBE	Methyl *tert*-butyl ether
NARP	Nonaqueous reversed phase
NEC	Naphthyl ethyl carbamoyl
NIR	Near infra-red
NMF	*N*-methyl formamide
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NP	Normal phase
NPLC	Normal-phase liquid chromatography
$\mathbf{NTf_2}$	Bis-trifluoromethyl sulfonylamide anion $[(CF_3SO)_2N]^-$
OVM	Ovomucoid
PBD	Plackett–Burmann design
PIM	Polar ionic mode
POM	Polar organic mode
PEG	Polyethylene glycol
PTC	Phenyl thiocarbamate
PGA	Penicillin G acylase
PCA	Principal component analysis
PLS	Partial least square
QD	Quinidine
QN	Quinine
QSAR	Quantitative structure activity relationship

Rs	Resolution factor
RPLC	Reversed-phase liquid chromatography
R	Ristocetin
RP	Reversed phase
ROESY	Rotating Overhauser exchange spectroscopy
RNA	Ribonucleic acid
RTIL	Room temperature ionic liquid
SCCE	Synchronous cyclic capillary electrophoresis
SDS	Sodium docecyl sulfate
SELEX	Systematic evolution of ligands by exponential enrichment
S_f	Stationary-phase fraction retained in a CCC column
SFC	Supercritical fluid chromatography
SMB	Simulated Moving Bed
SPE	Solid phase extraction
SR	Stereocenter recognition
SULL	Sodium undecanoyl-L-leucine leucinate
SULV	Sodium undecanoyl-L-leucine valinate
***S*-β-CD**	Sulfated β-cyclodextrin
T	Teicoplanin or Absolute temperature in K
TAG	Teicoplanin aglycon
TEAA	Triethylammonium acetate
Tf^-	Triflate anion (CF_3SO^-)
TFA	Trifluoroacetic acid
TFAE	Trifluoroanthryl ethanol
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TOF	Time of flight
TPI	Three-point interaction
UHPLC	Ultra high pressure liquid chromatography
UV-vis	Ultraviolet-visible light
V	Vancomycin
VCD	Vibrational circular dichroism
VP	Verapamil
±-WSA	Racemic mixture of the Whelk-O® selector analogue
XRD	X-ray diffraction
α_{CCC}	Enantioselectivity factor in CCC
α_{HPLC}	Enantioselectivity factor in HPLC
ΔG	Gibbs free energy
ΔH	Enthalpy variation
ΔS	Entropy variation
$18C6H_4$	(+)-(18-crown-6)-tetracarboxylic acid

Chiral Recognition Mechanisms in Enantiomers Separations: A General View

Alain Berthod

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Abstract In 1858, Louis Pasteur, the first to accomplish the separation of two enantiomers wrote: "Most natural organic products, the essential products of life, are asymmetric and possess such asymmetry that they are not superimposable on their image. This establishes perhaps the only well-marked line of demarcation that can at present be drawn between the chemistry of dead matter and the chemistry of living matter." Enantiomers have exactly the same properties in isotropic conditions.

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They behave differently only in anisotropic conditions. Chiral–chiral interactions are needed for enantiomeric separations. The fundamental mechanisms for chiral separations are listed along with the commercially available chiral selectors. Two chemometric examples are commented: one on quantitative structure enantioselectivity relationship and the second one on linear solvation energy relationships. It is shown that the solvents used in the mobile phase may play the most critical role in the chiral mechanism.

1 Introduction

After the thalidomide tragedy (1957–1961), a strict control of the purity of enantiomers used in medicine was inducted. Worldwide, governmental agencies control all active drugs produced by the pharmaceutical industry with a special attention on the enantiomeric purity in case of chiral drugs. With time, less and less new drugs are introduced as racemates. Figure 1 shows the evolution of the numbers of news drugs introduced worldwide as pure enantiomers, achiral molecules, and racemates over the last 20 years. The steady increase of pure enantiomers is associated with the sharp decrease of racemate introduction with only seven racemates introduced over the 2003–2006 4-year period. This figure should be compared to the 246 pure enantiomers and 131 nonchiral drugs introduced over the same period of time [1–3]. It is interesting to note that 99% of the pure enantiomers had a natural or semi-synthetic origin when most of the nonchiral molecules were synthetic drug substances [3]. Such concern on the interaction of enantiomers with the living world is now going beyond the pharmaceutical industry expanding to the food and agriculture industries and wherever animal and vegetable organisms are involved.

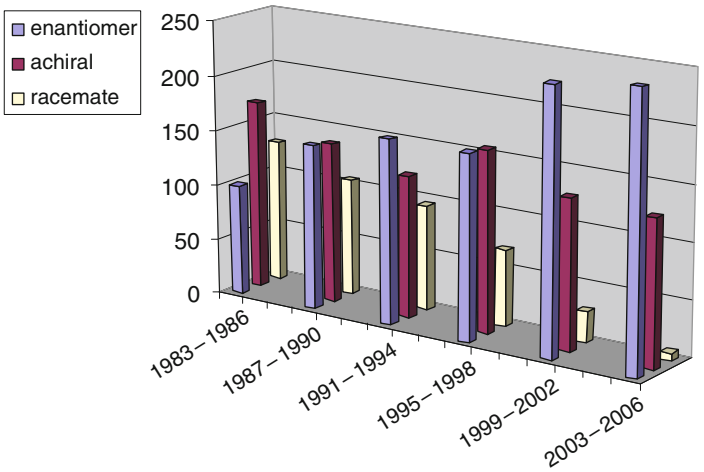


Fig. 1 Time distribution of the number of worldwide newly approved drugs according to chirality character (data from [1–3])

Enantiomer separation and chiral mechanisms go together. The separation of enantiomers is a very difficult task that cannot be achieved without a different pure enantiomer called the selector. This is the first most important point to understand: two enantiomers have exactly the same properties in anistropic, asymmetric, or achiral environments. Some differences in enantiomer behavior can occur only in isotropic or chiral environments. For reasons beyond the scope of this chapter, nature uses single enantiomers of, e.g., amino acids and carbohydrates to build asymmetric living organisms which produce very different interactions with chiral molecules. The metabolic pathway of the (*R*)-thalidomide enantiomer produced the desired sedative effect when that of (*S*)-thalidomide displayed dramatic teratogenic effects in pregnant women. It is because living organisms are asymmetric that chiral separation and pure enantiomers gained such a high significance.

This first chapter will present the world of chiral separation by listing and defining the terms used in the field, giving a brief historical view followed by a general description of enantiomeric interactions and mechanisms involved in enantioseparations. Chromatographic techniques are greatly emphasized due to the background of the author.

2 Nomenclature

2.1 Term Definitions

Absolute configuration	The fully identified spatial arrangement of all stereogenic centers in a chiral molecule.
Achiral molecule	A molecule that does not contain any asymmetric center. Its mirror images are superimposable upon each other.
Asymmetric center	The carbon atom bearing four substituents in a chiral molecule. The tetrahedral sp^3 hybridization of carbon with four different substituents is responsible for more than 95% of the chirality in the living world. Figure 2 shows chiral molecules that do not contain a defined asymmetric center.
Chiral molecule	A molecule with at least one asymmetric center. Its mirror images are not superimposable. The use of the adjective "chiral" is extended to describing involvement with enantiomers, e.g., chiral chromatography, chiral separations.
Diastereoisomers:	Isomers differing by the spatial arrangement of their functional groups not being mirror image of each other. They may contain multiple asymmetric centers. Diastereoisomers may or may not be optically active.

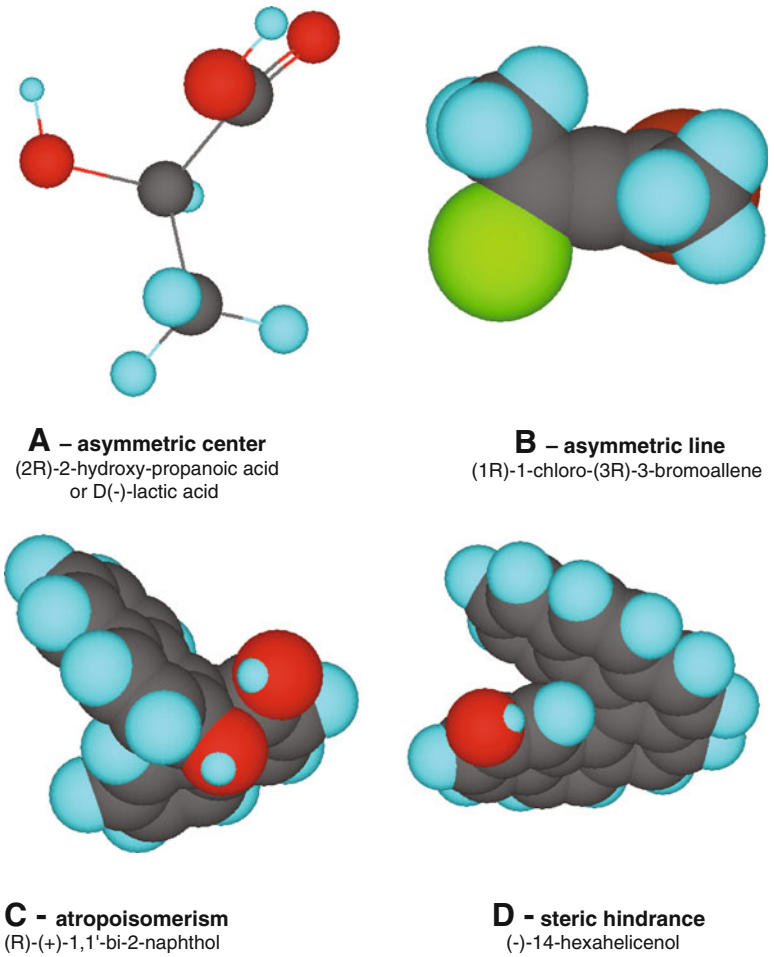


Fig. 2 Examples of chiral molecules with and without asymmetric center. **a** The sp^3 hybridized carbon bearing four different substituents is by far the most common asymmetric center. **b** The C=C=C allene arrangement forms a chiral axis. The 1-chloro-3-bromoallene is chiral. **c** Atropoisomerism occurs when the free rotation around a σ bond is hindered. **d** Steric hindrances create a chiral plane in helicenes

Enantiomer:	One member of a pair of molecules that are mirror images of each other and not superimposable. Enantiomers are optically active.
Enantiomeric excess	The percent excess of an enantiomeric form over the racemate in a mixture of a pure enantiomer and its racemate. Symbol *ee*, it is also termed "optical purity," the specific optical rotation of an enantiomer mixture over the specific rotation of the pure enantiomer. For example, if the *ee* or optical purity of a mixture of

	two enantiomers is 40%, it contains 70% of one enantiomer and 30% of the other. These percentages are seen as 60% of the racemate nonoptically active and 40% "excess" of an optically active enantiomer.
Enantiopure	Quality of a compound that is made of a single isomer not containing its enantiomer according to available analytical methods.
Epimers	Diastereoisomers differing in configuration at one of the two or more asymmetric centers, e.g., sugars. Epimers are optically active.
Meso compound	A diastereoisomer with two or more asymmetric centers and a plane of symmetry within the molecule reducing the number of possible enantiomers. A meso compound is not optically active.
Optical purity	Measure of the enantiomeric excess determined by optical rotation measurement, see "enantiomeric excess".
Racemate	Synonymous of racemic mixture or racemic compound containing exactly the same amount of both enantiomers.
Racemic mixture	A mixture composed of equal amount of enantiomers. This mixture is not optically active.
Specific rotation	The angular rotation $[\alpha]$ observed if a 1 dm length unit tube is used with a compound present at a 1 g/mL unit concentration. $[\alpha]$ is usually expressed in degree $cm^2\ g^{-1}$.
Stereoisomers	Isomers that differ from each other only in the way atoms are oriented in space. There are two types of stereoisomers: enantiomers and diastereoisomers.

2.2 Molecule Nomenclature

The internationally accepted nomenclature for chiral molecule uses the Cahn–Ingold–Prelog (CIP) rules for sp^3 hybridized carbons [4]. The four substituents are sorted by increasing mass of the first atom attached to the asymmetric center. If two atoms are identical (carbons in the case of 2-butanol, Fig. 3), the next heaviest atom one bond further away is considered and so on. Next, the molecule is held by the lightest substituents (-H for 2-butanol in Fig. 3) and the way the three other substituents are arranged in decreasing mass order define the R-enantiomer (Fig. 3 for 2-butanol with the order OH → ethyl → methyl rotating clockwise), R is for the latin word "rectus" right. The mirror image of (*R*)-2-butanol is the S-enantiomer (S is for the latin word "sinister" or left). These rules allow for the absolute configuration of any chiral compounds.

Historically, the first chiral separation of the enantiomers of sodium ammonium tartrate by Louis Pasteur in 1858 was done separating the crystals by hand

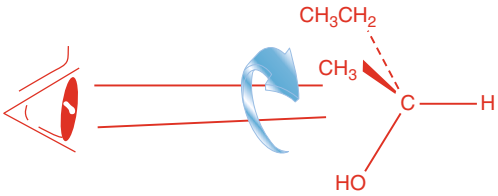


Fig. 3 The Cahn–Ingold–Prelog rules applied to 2-butanol. The decreasing substituents mass order is -OH > -CH_3-CH_2>-CH_3>-H. Seeing the molecule held by the lightest – H atom, the substituent masses decrease rotating clockwise: the R-enantiomer is pictured

using tweezers and a magnifier [5]. In the nineteenth century, the optical activity of the solutions was the only mean to recognize chiral molecules that were sorted in *d*- and *l*-isomers for dextrorotatory or levorotatory the right and left, respectively, optical rotation of the vertically polarized orange sodium light (589 nm). The *d*- and *l*-nomenclature is no more in use today supplanted by the (+) or (–) signs associated with the (R) and (S) CIP notation. Indeed, there is no known relation between the absolute molecular configuration of a compound and its optical rotation. In 1891, Emil Fisher devised a method of representing a three-dimensional molecule on a page. By a lucky guess, he correctly defined the structures of D- and L-glyceraldehyde and consequently of D- and L-tartaric acid [6]. His method was used to name sugars and amino acids for more than 50 years. It is still accepted today for these natural compounds only. Setting glycine apart since it is nonchiral, it must be noted that all amino acids found in proteins are L-amino acids and also have the S-configuration at the exception of cysteine whose –CH_2-SH substituent precedes the carboxylate-COOH in mass making L-cysteine the R-enantiomer.

3 Interaction Between Molecules

3.1 The Bases: The Three-Point Interaction Model

As already said, two enantiomers have exactly the same properties in anisotropic environment. To separate enantiomers, interactions with an isotropic selector are needed. The key step in enantiomer separation and chiral recognition is the formation of labile diastereoisomeric complexes between the enantiomers and the chiral selector. The selector will be able to discriminate between the two enantiomers if there are at least three point of interaction between the chiral selector and one or both of the enantiomers as illustrated by Fig. 4. The left image shows that a chiral molecule can match exactly three sites of the selector. Its mirror image on the right, after all possible rotations, can present a maximum of two groups able to interact with only two sites of the selector. The experimental binding constant of enantiomer (a) will be higher than that of its mirror image (Fig. 4). This difference can be used to separate the two enantiomers. Easson and Stedman were the first to propose in 1933 a minimum of three points of attachment to explain the different physiological

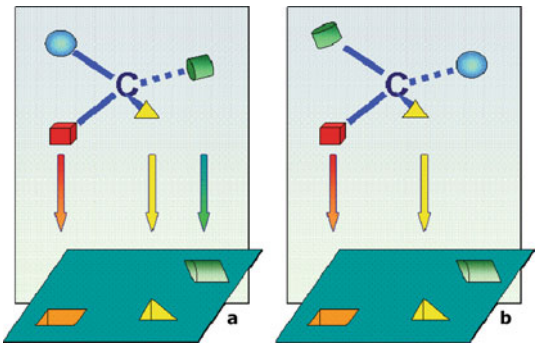


Fig. 4 The three-point interaction model. Enantiomer (**a**) presents three groups that match exactly three sites of the selector when its mirror image, Enantiomer (**b**) can interact with a maximum of two sites of the selector

activities of dissymmetric drugs [7]. Dalgliesh later adapted the model to explain the separation chiral aromatic amino acids that he obtained on paper chromatography, the first use of a cellulose chiral stationary phase! [8].

The "three-point interaction model" was useful in the design of some of the earlier chiral stationary phases (CSP). It is still used to rationalize mechanisms for chiral discrimination. It is very important to use it correctly. Figure 5 shows a fancy

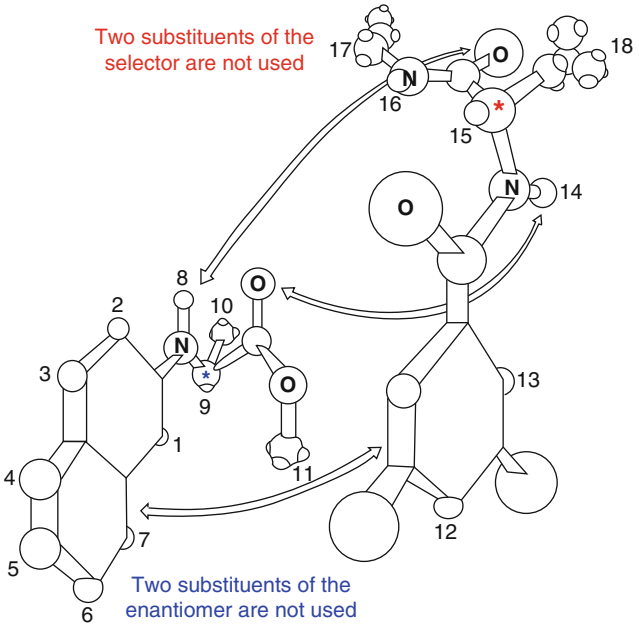


Fig. 5 Incorrect use of the three-point interaction model seen in [9]. Interaction of methyl-*N*-(2-naphthyl)alaninate with the chiral selector *N*-(3,5-dinitrobenzoyl)-(*S*)-leucine *n*-propylamide. Switching Hydrogen 15 and Group 18 on the selector asymmetric center (*) would produce the other enantiomeric form. Switching hydrogen 9 and methyl 10 of the leucine asymmetric center (*) would make the (*R*)-leucine enantiomer. In both cases, the three interactions mentioned would be similarly possible not allowing for any chiral discrimination

molecular modeling that was published by prominent experts in chiral separation in one of the best scientific journal [9]. Sorry to say, this model cannot be right since two of the three proposed interactions occur with the same substituents of the derivatized (L)-leucine chiral molecule. Switching the hydrogen atom 9 for the methyl group 10 (Fig. 5) would produce the (*R*)-leucine enantiomer that could interact with the selector through exactly the same proposed three interactions. This erroneous figure was unfortunately used over and over as an illustration of the three-point interaction model [10–12]. The three interactions must occur between three different substituents of both the chiral molecule and the chiral selector (Fig. 4).

The model is not readily applicable to all cases. The simplification of considering a point of interaction is not appropriate for all enantiomer–selector binding. Steric fits in a cleft or cavity can correspond to more than one interaction. In the original model, all interactions were attractive. From a stereochemical point of view, repulsion is considered as productive an interaction as attraction. For example, two of the interactions can be repulsive if the third interaction is strong enough to promote the formation of at least one of the two possible diastereoisomeric selector–ligand complexes [12]. Also the three-point interaction model can be considered as a geometric model. When the formation of the intermediate diastereoisomer complex involves interaction with a line or a plane or other rigid structures, this interaction can be counted for two or even three. So that this can agree with the idea of the three point of interaction considering that a line is defined by at least two geometrical points or a plane by at least three points [13].

3.2 Intermolecular Forces

All chiral separation methods involve an intermediate diastereoisomeric complex formed between the enantiomers to be separated and a chiral selector. All molecular interactions can play a role in the enantiomer–chiral selector-binding process. Table 1 lists these forces along with their strength, direction, and range.

The strongest interaction is obtained with the *Coulomb force*. The attraction between two electric charges of opposite signs is responsible for the high cohesion of salts. The Coulomb interaction can be attractive as well as repulsive if the two charges have the same sign. The *hydrogen bond* (H-bond) interaction occurs between the positively polarized hydrogen atom of a hydroxyl (or amine) group and the negatively polarized oxygen (or nitrogen) atom of another hydroxyl (or amine) group. H-bonds can be very strong because the negative site can come very close to the hydrogen atom depleted of any remaining repulsive electrons. *Steric hindrances* are due to the intrinsic room needed for an atom or group of atoms. This volume cannot be occupied by another atom or group of atoms. Steric hindrances are repulsive, very strong on very short range.

π–π *interactions* are observed when π-electron molecular assemblies, mainly aromatic rings, interact with each other. Aromatic structures are said to be π-acceptor or π-acid where the ring has electron-rich substituents, mainly $-NO_2$

Table 1 Strength, direction, and working distances of molecular interactions

Type of interaction	Strength	Direction	Working distance
Coulomb or electric	Very strong	Attractive (+/–) or repulsive (same charges)	Medium range ($1/d^2$)
Hydrogen bond	Very strong	Attractive	Long range
Steric hindrance	From weak to very strong	Repulsive	Short range
π–π interaction	Strong	Attractive (donor/acceptor)	Medium range
Ion–dipole	Strong	Attractive	Short range
Dipole–dipole	Intermediate	Attractive	Short range ($1/d^3$)
Dipole-induced dipole	Weak	Attractive	Very short range ($1/d^6$)
London dispersion or van der Waals forces	Very weak	Attractive	Very short range ($1/d^6$)

groups. They are said to be π-donator or π-basic when the π-electron can delocalize such as in a naphthyl group or when electron donating substituents, such as methyl groups, are attached to the aromatic ring. π–π interactions involved in chiral recognition mechanisms are most often attractive with a π-acceptor or π-acid group of the enantiomer interacting with a π-donator or π-basic group of the selector or vice versa. *Ion–dipole*, *dipole–dipole*, and *dipole-induced dipole* interactions act with molecule having a dipole moment. The strongest ion–dipole interaction combines the Coulomb force between the ion and the partial charge of the dipolar molecule. It is always attractive since, by constitution, a permanent dipole structure combines a partial positive charge with an equal partial negative charge. For the same reason, the dipole–dipole interaction is also attractive although weaker than the ion–dipole interaction.

The weakest interaction is that occurring between a permanent dipolar molecule and a dipole induced by the electric field. The *London forces*, part of the van der Waals interactions, are the weakest intermolecular forces. Being the weakest forces does not mean that they have no importance and/or no significant role to play in molecular behavior: these forces are, for example, responsible for the hydrophobic effect that is responsible for a great part of reversed-phase liquid chromatography (RPLC) compound separations and for entropy-driven forces causing oil to separate from water.

4 Assessing Mechanisms

4.1 Rationale of Chiral Recognition Mechanisms

Molecular interactions are responsible for slightly different binding constants between the transient diastereoisomeric complexes formed with the chiral selector and the enantiomers. A full knowledge of the chiral recognition mechanism would

allow predicting which selector will be best to separate the enantiomers of any chiral compounds. The full rationale of chiral recognition is far from being in sight yet although progress is continuous. Chiral recognition mechanisms can be studied most effectively when the exact structure of the chiral selector is known. This is mainly true for the smaller selectors. Most derivatized macromolecules and polymers have little-known structures. However, even with small selectors, too often in liquid chromatography (LC), beautiful molecular modeling studies of chiral molecule—selector association explain a posteriori a particular enantioseparation and have no predictive ability because they do not account for critical solvent effects.

4.2 Methods to Study Mechanisms

Information on chiral recognition mechanisms is mostly obtained by studying differences between binding energies of enantiomers and a chiral selector. Table 2 lists the different methods.

Table 2 Methods for investigating chiral recognition mechanisms

Spectroscopic methods
Circular dichroism and optical rotatory dispersion
NMR
X-ray crystallography
Fluorescence anisotropy
Separation methods
Liquid chromatography
Gas and supercritical fluid chromatography
Capillary electrophoresis
Computer methods
Molecular modeling
Structure properties relationships and handling data

4.2.1 Spectroscopic Methods

Spectroscopic methods can work with the chiral selector associated with the ligand either in solid state or in solution. The chiroptical spectroscopies, circular dichroism, and optical rotatory dispersion, represent an important means for evaluating structural properties of selector–ligand adducts [14]. NMR can specifically investigate ^{1}H proton or ^{13}C carbon atom positions and differentiate one from the other. X-ray crystallography is a powerful technique to investigate the absolute configuration of diastereoisomeric complexes but in the solid state only. Fluorescence anisotropy is a polarization-based technique that is a measure, in solution, of the rotational motion of a fluorescent molecule or a molecule + selector complex [15].

4.2.2 Separation Methods

Separation methods use chiral selectors to separate the enantiomers. Multiple selector–ligand association–dissociation steps occur between the mobile and stationary phase. In chromatography, the selector is most often attached to the stationary phase producing a chiral stationary phase (CSP). The enantiomers are introduced in the mobile phase that is a liquid chromatography (LC), a gas chromatography (GC), or a supercritical fluid chromatography (SFC). They move at slightly different average velocities according to their binding constants with the chiral selector. In capillary electrophoresis (CE) there is not actually a stationary phase: the chiral selector bears a charge, is added to the electrolyte, and moves in the electric field according to its electrophoretic mobility, differentially binding to the two enantiomers. The dissolved chiral selector can be treated as a pseudophase. Alternatively, the chiral analyte may be charged and the selector can be neutral. The migration times of the enantiomers give access to their binding constants. This book focuses on separation methods to obtain insights into chiral recognition mechanisms.

4.2.3 Thermodynamics

Working at different temperatures allows one to perform thermodynamic studies which, in some cases, can provide information on the chiral mechanism. Chromatographic methods give the enantiomer retention factors, k. It is relatively easy to measure the k factors at different temperatures. The slope and intercept of the Van't Hoff plots (Ln k versus $1/T$) contain, respectively, the enthalpy, ΔH, and entropy, ΔS, variations of each enantiomer–selector global (chiral + achiral) interaction.

$$\ln k = -\Delta H/RT + \Delta S/R + \ln \varphi \tag{1}$$

In Eq. (1), R is a perfect gas constant, T is the absolute temperature (°C + 273 in Kelvin) and ϕ is the column phase ratio (ratio of the stationary phase volume over the mobile phase volume).

Comparing the selectivity values α (ratio of the two retention factors k_1 over k_2) for the two enantiomers gives information on the enantioselective part of the interaction [16].

$$\Delta(\Delta G) = -RT \ln \alpha = \Delta(\Delta H) - T\Delta\ (\Delta S) \tag{2}$$

In Eq. (2) $\Delta(\Delta G)$, $\Delta(\Delta H)$, and $\Delta(\Delta S)$ are, respectively, the chiral part of the Gibbs free energy change of the enantiomer–selector phase transfer, the chiral part of the enthalpy and entropy changes occurring with the transfer [16].

The thermodynamic parameters obtained, binding constant , enthalpy, or entropy changes, correspond to the global ligand–chiral selector association. Information concerning the enantioselective separation mechanism can sometimes be inferred

by changing the experimental conditions in a controlled/sequential manner. These changes include the composition of the mobile phase, the pH, the polarity or ionic strength, and substituting and/or derivatizing a chemical group of the analyte and/or the selector.

A statistical thermodynamic study of CSP–enantiomer interaction demonstrated that the possible enantioselectivity factor α was not significantly different when an interaction dominated the two others or when the three interactions were of comparable strength. However, in the former case, ln α should be a linear function of $1/T$, with T, the absolute temperature and a departure from this Van't Hoff behavior would suggest that multiple retention modes compete [17].

Bi-Langmuir adsorption isotherms of enantiomeric pairs and CSPs were determined to gain information on chiral mechanisms. In the few cases fully studied, it was found that the two isomers interacted with type I nonselective sites as well as with type II enantioselective sites [18]. The bi-Langmuir equation is expressed as:

$$q_{R,S} = \frac{q_{\mathrm{I}} b_{\mathrm{I}} C_{R,S}}{1 + b_{\mathrm{I}}} + \frac{q_{\mathrm{II},R,S} b_{\mathrm{II},R,S} C_{R,S}}{1 + b_{\mathrm{II},R,S} C_{R,S}} \tag{3}$$

in which q is the amount of compound at equilibrium per unit of volume of CSP. The subscripts *R, S,* I, and II refer to the R- or S-enantiomers and the type -I or type II adsorption sites. The constants b subscript I and b subscript II with R and S references depend on the site adsorption energies. C is the enantiomer concentration in the mobile phase. The q_{I} contributions and type I b_{I} constants are identical for the two enantiomers making two unknown parameters. There are a total of six unknown parameters in the two q_R and q_{S} in Eq. (3): q_{I} and b_{I} for the nonselective type I sites and $q_{\mathrm{II},R}$ and $q_{\mathrm{II},S}$ and $b_{\mathrm{II},R}$ and $b_{\mathrm{II},S}$ for each R- and S-enantiomers. The six parameters were fully determined for several enantiomeric pairs allowing to obtain the true enantioselectivity factor α as the ratio of the q_{II} b_{II} products for the two enantiomers [19]. In all cases, it was found that the less retained enantiomer interacts with the enantioselective type II sites [18]. For six enantiomeric pairs well separated with enantioselectivity factors over 1.9, the relative chiral contribution to the retention factors of the less retained enantiomers was between 25 and 77% and between 40 and 89% for the most retained enantiomers [18]. The adsorption studies demonstrated also that heterogeneous mass transfer kinetics was the essential explanation for the poor efficiency of protein CSPs. The adsorption results confirmed that the kinetics of adsorption/desorption is much slower on the chiral selective sites than on the nonselective ones [19].

4.2.4 Molecular Modeling and Statistical Analyses

Computer methods use chemical theory to establish chiral recognition mechanisms. Software computes the atom coordinates and calculates the best molecular conformation that minimizes energy between the chiral selector and the ligand.

Beautiful models of chiral molecule–selector association are particularly useful in crystallography and GC. In LC, they may well explain a particular enantioseparation but often have no predictive ability because, so far, models ignored critical solvent effects in a particular interaction.

Another computer approach is to compile a large amount of results and performs quantitative structure retention relationships. This approach classifies experimental results associating conditions, selectors, and enantiomeric pairs successfully separated, not giving great information on the chiral recognition mechanism [20]. However, using the database with probability rule and a statistical approach was proved to have a very good predictive ability [21]. Section 6 of this chapter will detail parts of the author's personal work on associating chemometry and chiral separations.

5 Chiral Selectors in Separation Methods

5.1 Chiral Separations

Enantiomers need an isotropic medium to show different properties. In separation methods, there are three ways to make enantiomers and chiral selectors interact: (1) a chiral derivatization agent can be used to react with the enantiomeric pair turning it into a diastereoisomeric pair that can be separated by classical means; (2) a chiral selector can be added to the mobile phase so that labile diastereoisomers can be formed with the enantiomeric pair during the separation process. Again a classical column will be able to separate the formed diastereoisomers; (3) a chiral selector can be attached to the stationary phase. Labile diastereoisomers can be formed with the chiral stationary phase (CSP) producing different progression of the two enantiomers within the chiral column.

All three methods are used. However, the third method has a significant advantage over the two other methods: a lower than 100% enantiomeric purity of the CSP will not produce erroneous results in chiral analyses. Indeed, a drawback of method 1 is that the derivatization agent used to prepare the diastereoisomers of an enantiomeric pair may be less that 100% pure. If it is only 99% pure, all optical purity analyses done on the diastereoisomeric pair obtained will be systematically biased by 1%. Also, the chemical reaction involved to prepare the diastereoisomers may change the initial optical purity of the enantiomeric pair. Chiral additives to the mobile phases must also have the highest optical purity in order to give accurate results. When a CSP is used to separate enantiomers, e.g., 99% optical purity can be tolerated: the two peaks corresponding to the two enantiomers will be separated by only 99% of the maximum possible resolution factor. However the peak areas will be correct producing accurate optical purity results. The use of CSPs is by far the preferred method in gas and liquid chromatography chiral separations. Chiral mobile phase additives are used in capillary electrophoresis chiral separations.

5.2 Different Classes of Chiral Selectors

The quest for chiral selectors can be arbitrarily separated in two paths: the synthetic route and the natural route. The synthetic route studies the chiral molecule evaluating possible interactions (Table 1) and designs a selector that will interact differently with an enantiomeric form than with its mirror image. The natural route follows Pasteur and uses the fact that the living world is made of countless chiral selectors and produces pure enantiomers. Once a natural chiral selector has been selected, it is tested with its natural chiral target(s) and with many other enantiomers. The observation of the results allows estimating a posteriori possible chiral mechanisms.

Actually, neither of these two classes of selectors is 100% pure: the semi-synthetic class would almost be the actual class since many synthetic selectors are based on a natural molecule and many natural selectors are chemically modified to enhance their initial properties. Table 3 lists most of the selectors used for the separation of enantiomers sorted according to their main origin: synthetic or natural.

Table 3 Chiral selectors and their primary interaction

Appellation	Mechanism	Primary interaction
Synthetic selectors[a]		
Ligand exchange	Diastereoisomeric selector/metal ion/analyte complex	Coulomb or ion-dipole (lone electron pair coordination)
π-complex selectors	Transient 3-point selector/analyte association	π–π interaction
Molecular imprinted polymers	Key and lock association	Selective shape interaction with the imprint
Chiral crown ethers	Inclusion complexation	Ion (primary amino group)-dipole
Polymers	Diastereoisomeric selector/analyte complex	H-bond
Natural selectors[a]		
Proteins	Multiple-binding sites	Variable
Polysaccharides	Insertion in helical structures	H-bond or dipolar or steric
Cyclodextrins	Inclusion complexation	H-bond
Cyclofructoses	Inclusion of NH_2 + multiple-binding sites	Variable
Macrocyclic glycopeptides	Multiple-binding sites	Variable
Cinchona alkaloids	Ion pairing	Coulomb

[a]Most ligand-exchange and π-complex selectors have a natural amino acid core and most natural selectors are artificially derivatized to enhance their performance.

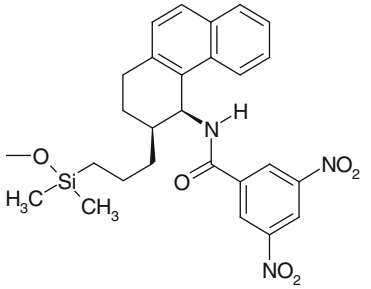
5.2.1 Ligand Exchange Mechanism

The chiral ligand-exchange principle was established in the late 1960s [22]. The basic mechanism involves a metal ion, most often Cu^{2+}, that will be at the core of a complex with the enantiomers and the chiral selectors. To insure an acceptable

chromatographic efficiency, the complex must be kinetically labile forming and dissociating at a high rate. The central metal ion has definite positions in its coordination sphere (six for Cu^{2+}) that can each be occupied by a lone electron pair of organic groups (or water molecules). The chemical functional groups meeting these requirements, a lone electron pair and lability, are the amino, carboxy, hydroxy, amido, and thio derivatives, all bearing at least one lone electron pair on the heteroatom. The chiral selector is an amino acid derivative and other analogous chiral bidentate ligands. Through its amino and carboxylic groups, it occupies two positions of the copper ion coordination sphere. Two positions are occupied by small water molecules leaving two positions for the ligand. The enantiomer analytes must be able to form bidentate chelates. They are α- or β-amino acids, amino alcohols, hydroxyl acids, diamines, amino amides, and dicarboxylic acids. The two interactions described are necessary but not sufficient; the third interaction, required for chiral recognition, is provided by steric- or dipole-type interaction with the selector. Bulky and/or rigid groups in the analyte situated close to the stereogenic center will greatly enhance the chiral recognition as indicated by the good chromatographic enantioselectivity of the separation [22].

5.2.2 Molecular Adjustment for Three-Point Interaction

The π-donator or π-acceptor chiral selectors were introduced in the late 1970s [23]. Later, the (*R*)-*N*-(3,5-dinitrobenzoyl) phenyl glycine selector was specifically designed to have π-bonding capabilities [24]. The π-donator character of the dinitrobenzoyl group of the selector can interact with an added π-acceptor substituent of the enantiomer. Dipole stacking, H-bond, and steric repulsion will provide the two other necessary interactions. The interest of the concept was demonstrated when it was shown that, making the (S) version of the phenyl glycine selector, it was possible to observe the reversal of the elution order of the π-donator substituted enantiomers [25]. Some rigidity in the molecule enhances chiral recognition. At the moment, the most successful π-complex selector, the Whelk-O-1, has two stereogenic centers that are part of a ring and two bonds with two bulky π-electron-rich (acid and basic) substituents.



The synthetic (3R,4S) Whelk-O-1 selector

5.2.3 Key and Lock Recognition with MIPs

Molecular imprinted polymers (MIP) are prepared in solvent solution with the imprint pure enantiomer, a functional monomer (e.g., methacrylic acid), a cross-linker (e.g., ethylene glycol dimethacrylate), and an initiator (e.g., 2,2-azobis-(2-methylpropionitrile)). The mixture is reacted for several hours at elevated temperature. The resultant bulk rigid polymer should be ground in a sieved powder and the template enantiomer will be washed off. Knowing the way the MIP was prepared makes it easy to understand that it will have a strong affinity for the enantiomer that served as template. The interactions are mainly steric and shape recognition associated with other interaction solute depending [26]. The drawback is that MIPs are too specific. They essentially play no role in practical/commercial enantiomeric separations. They are limited by their poor capacity and the lability of the imprint to varying solvent conditions.

5.2.4 Host Crown Ether and Chiral Guest

Chiral crown ether selectors are derivatized forms of polyoxyethylene crown-6 [27]. This crown ether has a cavity that exactly match the size of an ionized primary amine group, $-NH_3^+$. The host–guest ammonium-crown ether interaction, one point of attachment, is the driving force of the enantiomer with this class of chiral selector. The two other necessary interactions are a steric and a hydrophobic one. They will occur between the crown ether substituents and the host substituent. Chiral crown ether can only discriminate chiral molecules with a primary amine group at low pH (where the amine is protonated).

Crown ether type-cyclic oligosaccharides could soon become another class of very efficient chiral selectors. The crown ether cavity could be used as well as the fructose sugar on the ring. Derivatized forms of cycloinulooligosaccharides showed excellent chiral recognition ability for primary amines and a variety of chiral compounds (Armstrong, 2009, personal communication).

5.2.5 Synthetic Polymers

The helical polytriphenylmethyl methacrylate was the first synthetic chiral polymer able to separate a very limited number of enantiomers [28]. Recently a fully synthetic chiral stationary phase based on polymerized diacryloyl derivative of *trans*-1,2-diaminocyclohexane [either (R, R) or (S, S)] bonded to silica gel in the form of a very thin layer was proposed as a new LC CSP [29]. This CSP could not resolve many enantiomeric pairs. However, when it could resolve a racemate, it was shown that the amount that could be loaded was much larger than that on most other CSPs. It means that the number of active sites is large. Hydrogen bonds were found to be pivotal in the chiral recognition mechanism of this CSP. The enantioselectivity was adjusted by the methanol content in the organic mobile phase. Polysodium *N*-undecanoyl-L-leucyl-leucinate (poly-SULL) and −L-leucyl-valinate

(poly-SULV) were dipeptide polymers forming micelles that were found very useful in micellar electrokinetic chromatography with a broad range of applications [30].

5.2.6 Proteins

Proteins were very early introduced as natural chiral selector [31]. This was a highly logical choice since such bio-macromolecules are responsible for the chiral discrimination of drugs and nutrients in the living body. Proteins can discriminate a wide spectrum of charged and neutral molecules. However, they may be difficult to use since small changes in the experimental conditions, pH, ionic strength, added organic solvent, may cancel the enantiorecognition. It is not possible to give a simple mechanism since a single protein may contain several sites acting as chiral selectors. All listed interactions may be involved.

5.2.7 Polysaccharide Selectors

Cellulose, amylase, and chitin are the most abundant optically active natural polymers. They can be readily modified to carbamates or esters through reactions with isocyanates and acid chlorides, respectively [32]. These selectors are very successful and have broad selectivity. They associate individual chiral carbohydrate monomers in a long-range helical secondary structure, also chiral. This association was found to be highly effective for HPLC enantiomer separations. Since the most popular selectors (Chiralcel® OD and Chiralpack® AD in coated forms or Chiralpak® IA and IB in bonded forms) are cellulose and amylose derivatized with 3,5-dimethylphenyl carbamate, a π-donator or π-basic group, it is likely that π–π interactions will be part of the mechanism. However, these chiral polymers offer so many possible interacting sites that many enantiomers are discriminated finding three different points of interaction without possibility to know exactly the mechanism.

5.2.8 Inclusion Complexation

Cyclodextrins (CD) are small cyclic polysaccharides forming a cone-shaped cavity with 6, 7, or 8 glucopyranose units for the α-, β-, or γ-CD, respectively. The interior of the cavity is rather nonpolar with ether groups; the larger and smaller rims of the cavity are lined with polar primary and secondary hydroxyl groups, respectively. Inclusion complexation is the driving interaction in chiral recognition by CDs. Native CDs were proposed in the early 1980s as chiral selectors [33]. Polar secondary interactions with the hydroxyl groups were predominant. Derivation of these hydroxyl groups produced a wide variety of CDs with adjusted polarities and functionalities. Derivatized CDs were able to separate a broad spectrum of enantiomers [34]. For example, naphthyl-ethyl carbamate-substituted CDs associated $\pi-\pi$ interactions, H-bond, and inclusion complexation widening the applicability