CHIRALITY

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.”

The FDA Policy Statement for the Development of New Stereoisomeric Drugs, issued May 1, 1992. The policy noted that the term *stereoisomers* included enantiomers, geometric isomers (e.g., *cis/trans*), and diastereomers (isomers with more than one chiral center that are not mirror images of one another). Since the agency considered diastereomers and geometric isomers as chemically and pharmacologically distinct, and which can be separated without chiral techniques, these are generally treated as separate drugs, with some very specific exceptions (e.g., in vivo interconversion). There is no reason to develop a mixture of diastereomers or geometric isomers unless they fortuitously present a reasonable fixed-dose combination.

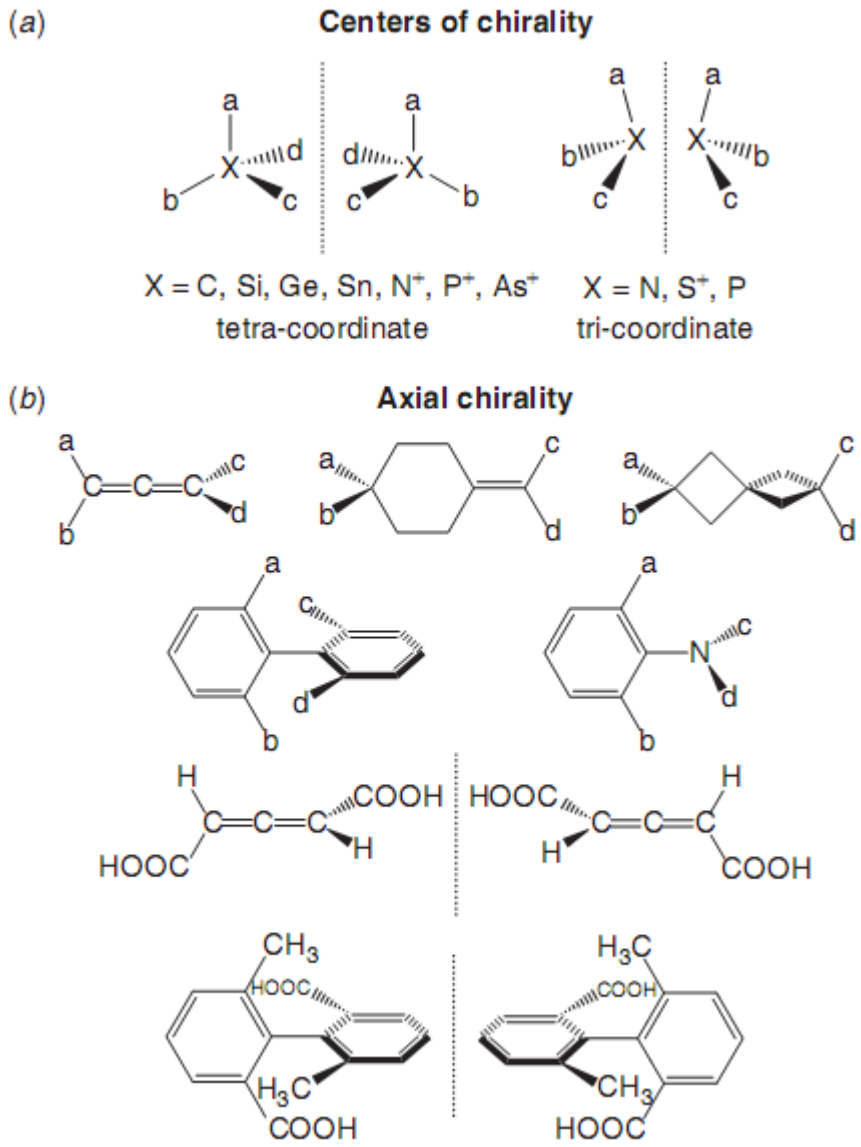
There are numerous examples of enantiomers with same qualities, such as:

- Dobutamine enantiomers are both positive ionotropes.
- Ibuprofen enantiomers are both anti-inflammatory agents.
- Warfarin and phenprocoumon enantiomers are both anticoagulants.
- Bupivicaine enantiomers both produce local anesthesia, but the incidence of severe arrhythmias in isolated rabbit heart is much less with the *S(−)*-enantiomer than the *R(+)*- isomer or the racemate.
- Quinolones and β-lactam antibiotic enantiomers are all antibacterial.

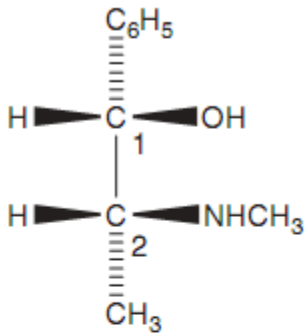
There are numerous examples of enantiomers with strikingly different qualities, such as:

- The *R(+)*-enantiomer of thalidomide has sedative action but the *S(−)*- enantiomer is a teratogen.
- The *l*-propranolol enantiomer is a β-adrenergic receptor antagonist (β-blocker), but *d*-propranolol is not.
- The *d*-sotalol enantiomer is a type 3 antiarrhythmic, whereas *l*-sotalol is a β-blocker.
- The *d*-levodopa isomer is associated with granulocytopenia.
- The *d*-levamisole isomer is associated with vomiting.
- The *d*-carnitine isomer is associated with myasthenia gravis symptoms.

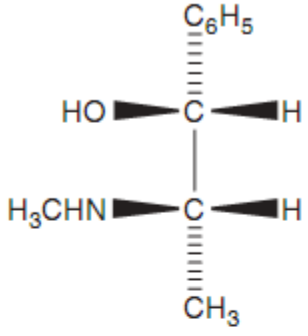
What is chirality? Chirality is a term used to describe an object or compound that is non-superimposable on its mirror image.



(*a*) erythro



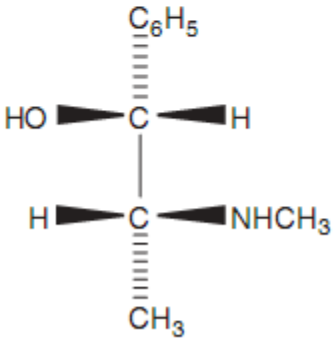
(1*S*, 2*R*)–(+)



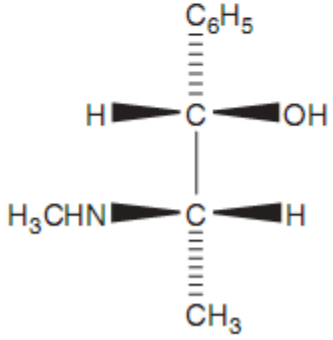
(1*R*, 2*S*)–(–)

ephedrine

(*b*) threo



(1*R*, 2*R*)–(–)



(1*S*, 2*S*)–(+)

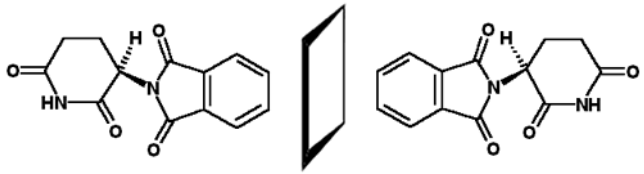
pseudo-ephedrine

Chiral Biological Macromolecules

- Proteins
  - Enzymes
  - Structural elements of membranes
  - Receptors
- Carbohydrates
- Nucleic acids
- Chiral “building blocks” of L-amino acids and D-carbohydrates.

Thalidomide was widely marketed in over 46 countries as a sleep aid. Its use during pregnancy was responsible for over 10,000 children born with profound birth defects. Thalidomide would have been approved in the United States were it not for the vigilance of a medical officer, Frances O. Kelsey.

The public health catastrophe prompted stronger regulation (Kefauver–Harris Amendment of 1962) for demonstrating the safety of a product prior to marketing. Although it was commonly thought the toxicity resided solely with the *S(−)* form of thalidomide, several in vitro studies showed that the drug could racemize quickly in various aqueous media. Metabolic inversion has been observed with other compounds, such as 2-arylpropionic acid (2-APA) and mandelic acid.



R-Thalidomide
(sleep-inducing)

S-Thalidomide
(teratogenic)

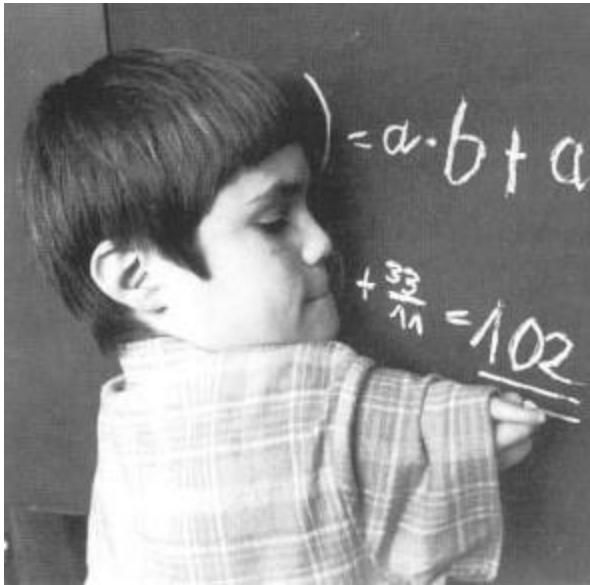


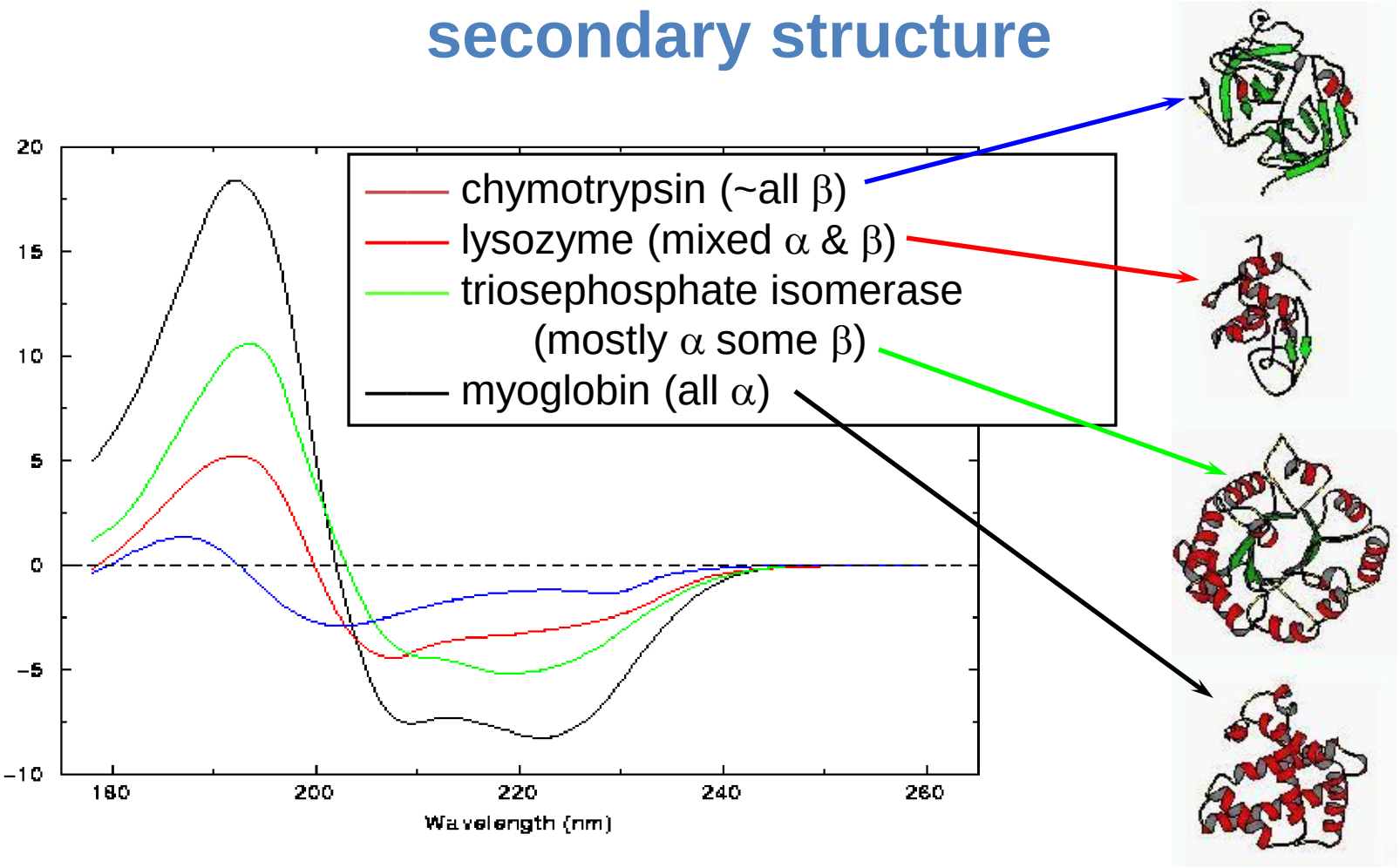
TABLE 1 Overview of Analytical Methods for Chiral Separation

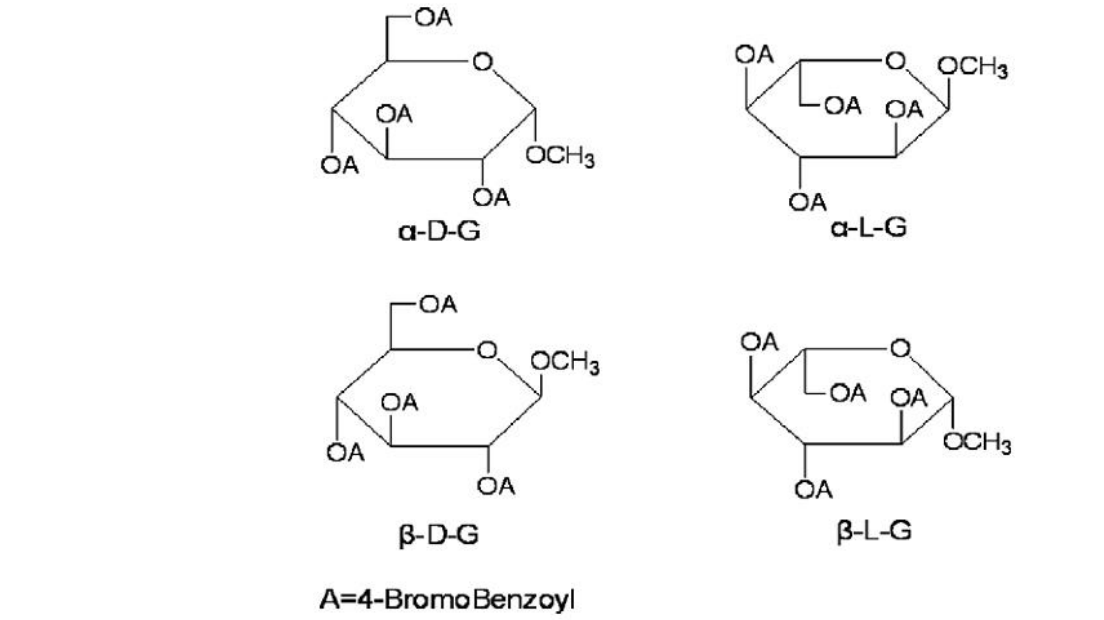
Method	Description of Principle and Application
Chiral HPLC	Chiral HPLC may be used to separate mixtures of enantiomers directly through the use of chiral stationary phases (CSPs) or chiral mobile-phase additives in conjunction with regular (achiral) columns. A key component of many CSPs is a polysaccharide-based material (e.g., derivatized amylose or cellulose polymers), although other materials have been used, such as proteins or vinyl polymers (e.g., Pirkle-type columns that use π-electron donor or π-electron acceptors).
Chiral GC	CSPs are also used and often modified with chiral agents for the separation of enantiomers. The first CSPs were derivatized amino acids, but they were not thermally stable. The CSPs used most commonly now are modified cyclodextrins.
Differential scanning calorimetry (DSC)	DSC is used routinely for polymorph assessment. The melting points may be used in distinguishing individual enantiomers from the racemate.
Optical rotation	This method can be used to distinguish between enantiomers because they rotate the plane of polarized light in opposite directions but in equal amounts. It is widely used but is not considered a very specific method for quantitative purposes.
Nuclear magnetic resonance (NMR)	NMR is a useful tool for the determination of enantiomeric purity or enantiomeric composition. This is accomplished by making the NMR signals for the protons of the enantiomers nonequivalent by the use of chiral lanthanide shift reagents, chiral solvating agents, or chiral derivatizing agents.
Optical rotary dispersion (ORD)	ORD measures the change of specific rotation of an optically active compound with the wavelength of the light used.
Circular dichroism (CD)	CD measures the differential absorption of left and right circularly polarized light by an optically active compound. These chiroptical methods can be used to identify and/or quantitate enantiomers.
Supercritical fluid chromatography (SFC)	A supercritical fluid is one that is above a critical temperature and pressure and exists in a supercritical state where its viscosity approaches that of a gas whereas the solvent strength is closer to that of a liquid. The lower-diffusion mobile phase can be pumped through the column at a higher rate, and diffusion is faster, both of which aid column efficiency.
Capillary electrophoresis (CE)	Although this method was developed in the 1980s and has been used for chiral separations. The advantage of CE is the minimal amount of sample preparation needed. However, the coupling of CE with mass spectroscopy has greatly enhanced the method utility.
Nonaqueous CE (NACE)	Nonaqueous CE (NACE) offers the advantage of analysis of compounds poorly soluble in water and avoids the solvents required for HPLC or GC.
Chiral CE	Chiral CE is based largely on separation by complex formation with cyclodextrins.
X-ray crystallography or x-ray powder diffraction (XRPD)	X-ray crystallography in the solid state could be used to determine the absolute configuration of molecules and to distinguish conglomerates from racemic compounds.

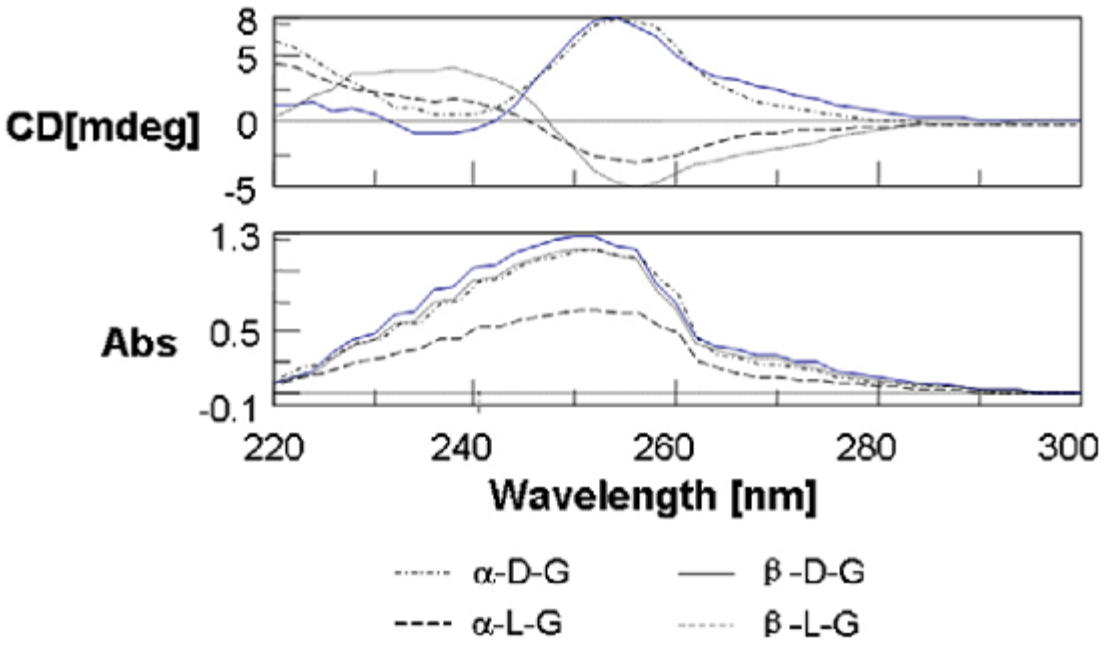
(*Continued overleaf*)

TABLE 1 (*Continued*)

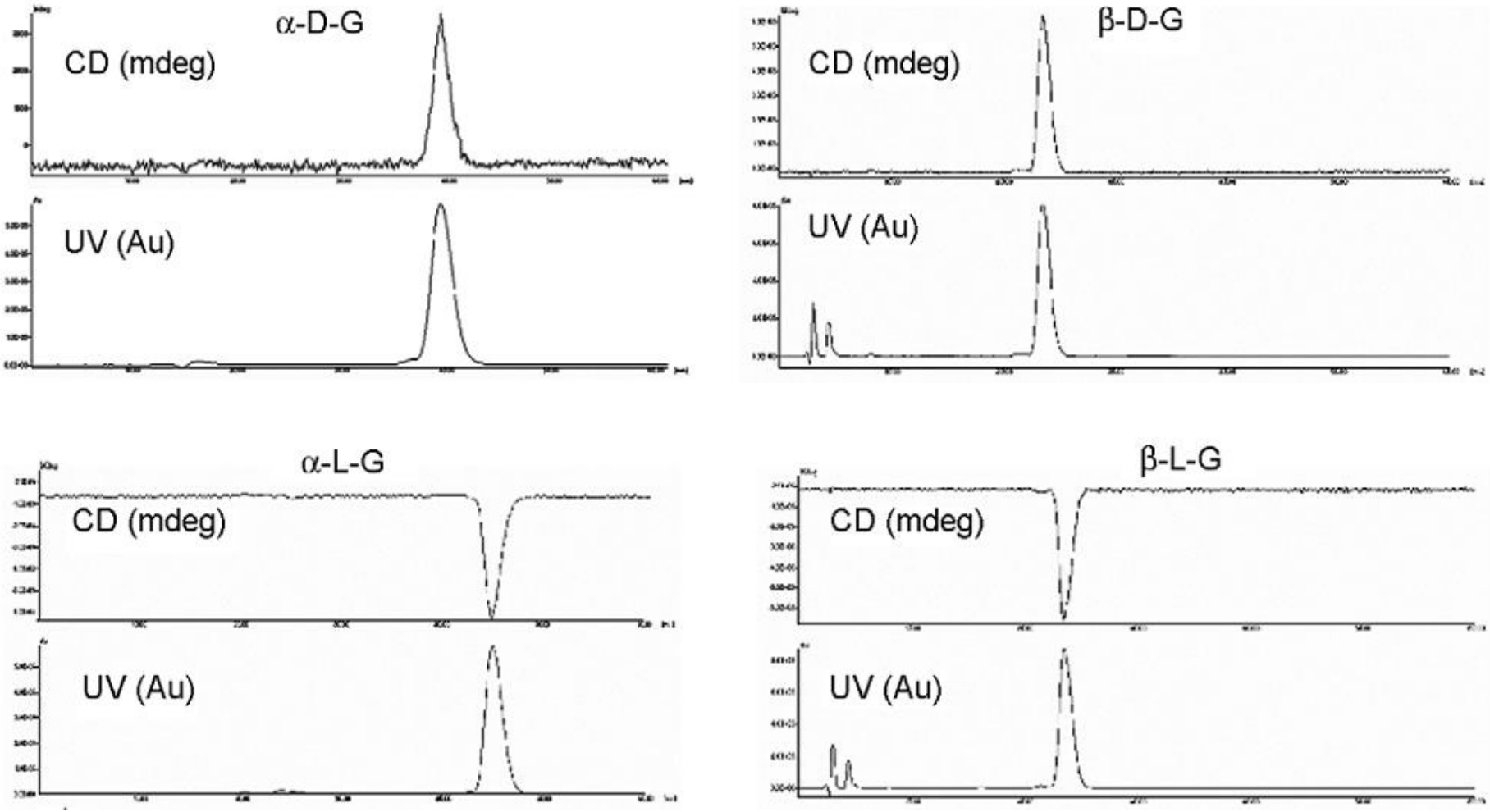
The CD signal for a protein depends on its secondary structure







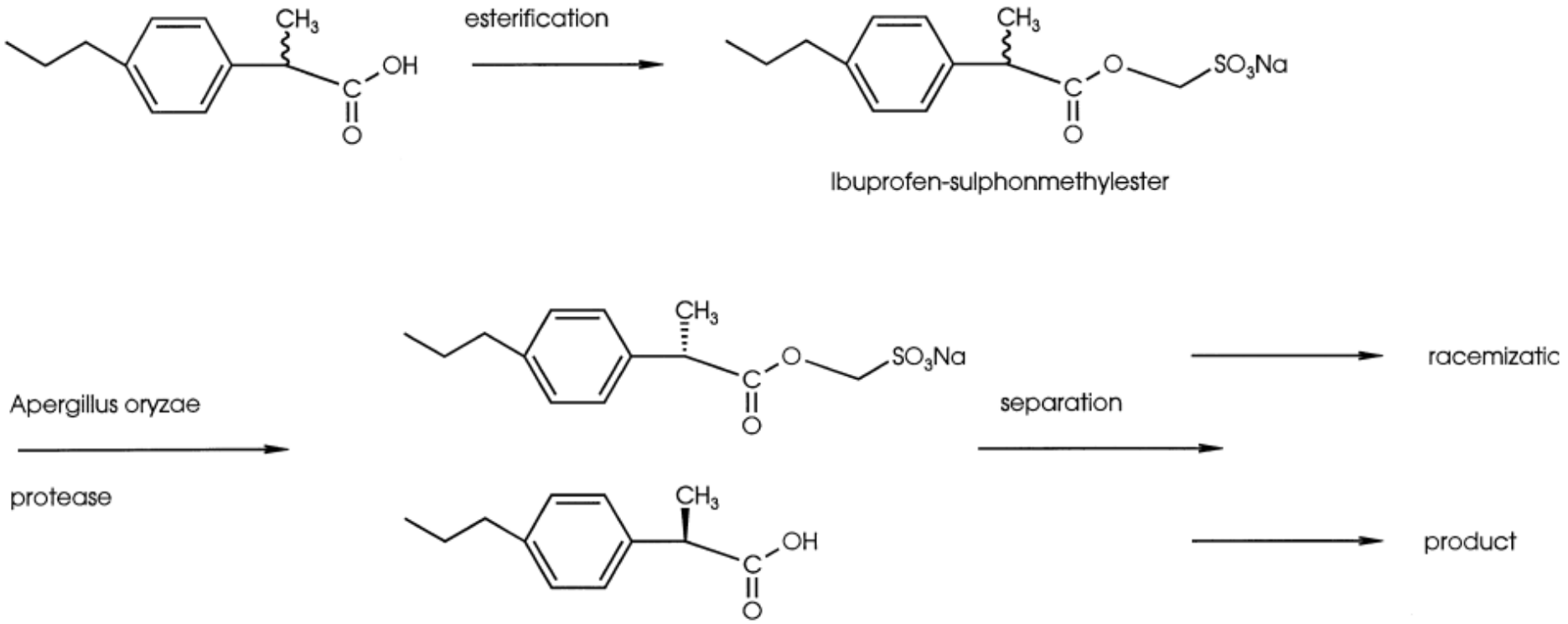
CD, and UV spectra of α-D/L-G and β-D/L-G.



Chromatograms of α-D-G, α-L-G, β-D-G and β-L-G.

S. Song et al. / J. Chromatogr. A 1179 (2008) 125–130

Enzymatic resolution and separation of ibuprofen sulphonmethyl ester.



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2009,** *57,* 2087–2095

REVIEW

Enantioselective Phytoeffects of Chiral Pesticides

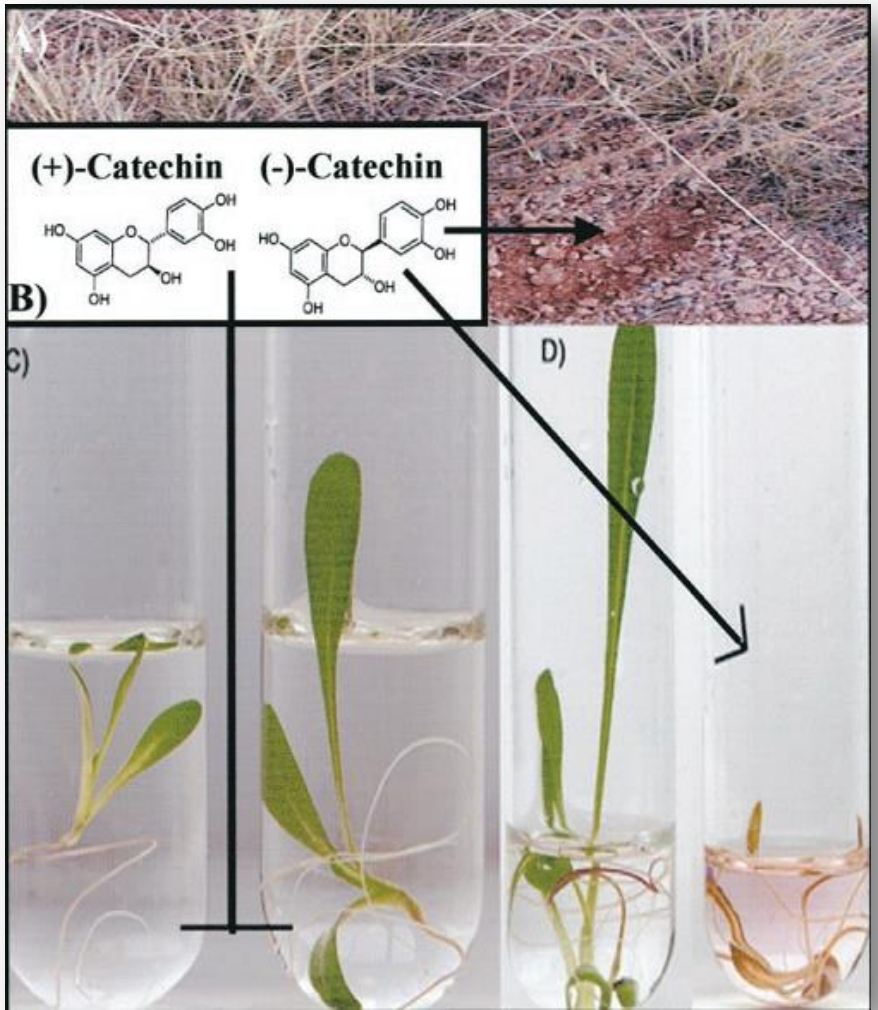
WEIPING LIU,*,† JING YE,‡ AND MEIQING JIN‡

Research Center of Green Chirality, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, People's Republic of China, and Institute of Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310027, People's Republic of China

J Chem Ecol (2009) 35:980–996
DOI 10.1007/s10886-009-9681-x

(±)-Catechin: Chemical Weapon, Antioxidant, or Stress Regulator?

Vladimir Chobot · Christoph Huber · Guenter Trettenhahn · Franz Hadacek



Food Chemistry 125 (2011) 1287–1290

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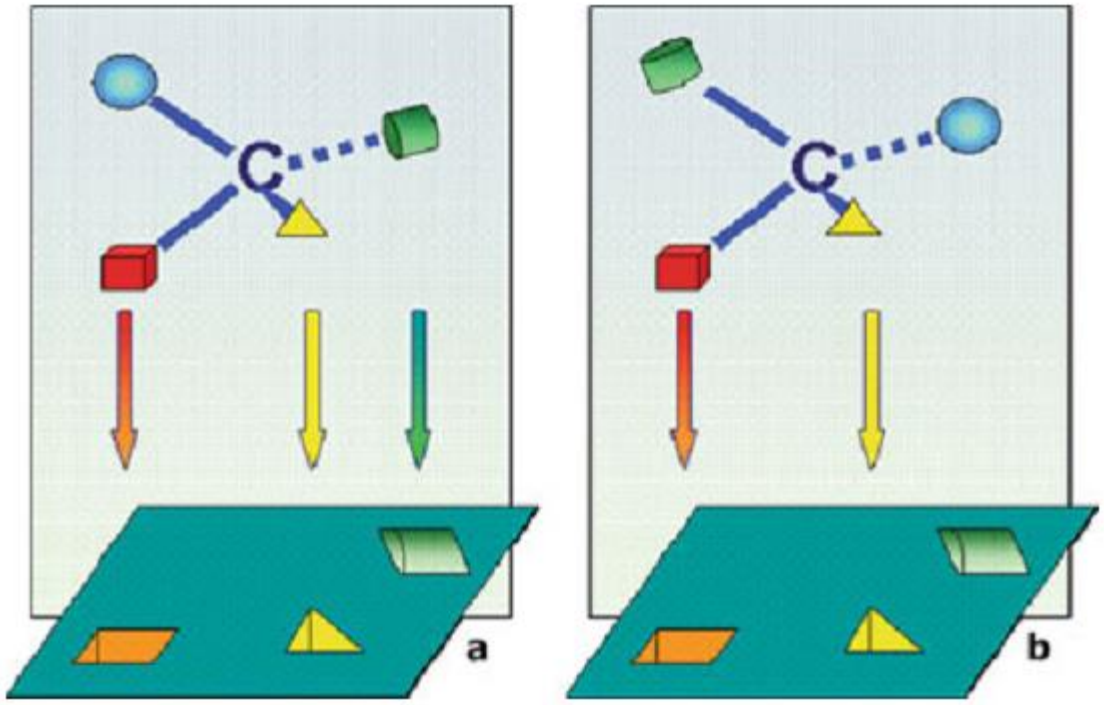
Short communication

Variation of Catechin, epicatechin and their enantiomers concentrations before and after wheat cultivar-*Puccinia triticina* infection

Alireza Ghassempour [a], Saeed Mollayi [a], Mohsen Farzaneh [b], Abbas Sharifi-Tehrani [b], Hassan Y. Aboul-Enein [c,*]

[a] *Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran*
[b] *Department of Plant Protection, University of Tehran, Karaj, Iran*
[c] *Department of Pharmaceutical and Medicinal Chemistry, National Research Centre, Cairo, Egypt*

The key step in enantiomer separation and chiral recognition is the formation of labile diastereoisomeric complexes between the enantiomers and the chiral selector.



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

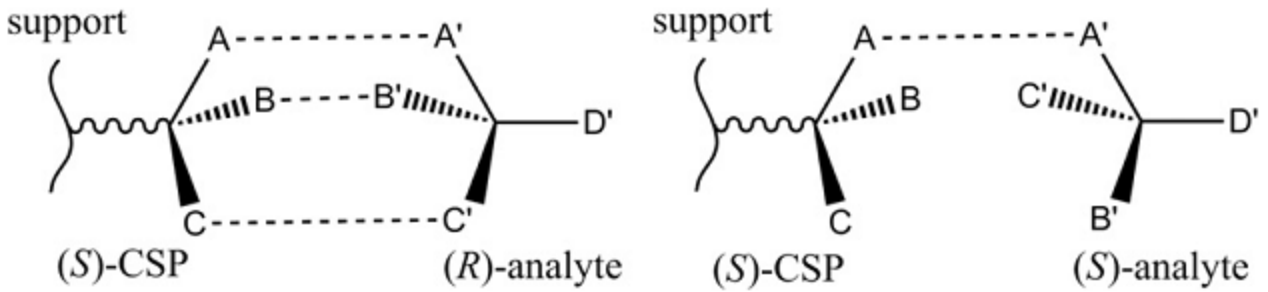
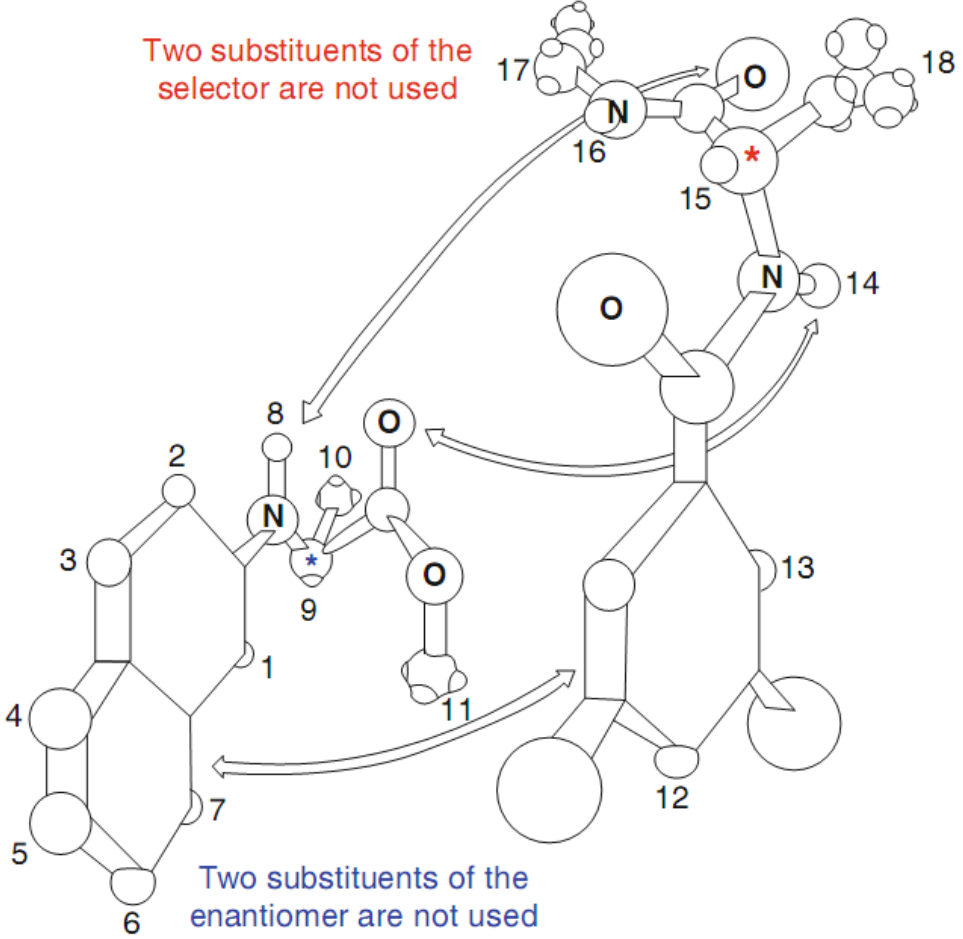


Figure 1. Three-point interaction model.

Trends in Analytical Chemistry, Vol. 39, 2012, 180

two of the three proposed interactions occur with the same substituents

This model cannot be right since two of the three proposed interactions occur with the same substituents



The three interactions must occur between three different substituents of both the chiral molecule and the chiral selector

Incorrect use of the three-point interaction model. 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

PirkleWH, Pochapsky TC (1987) J Am Chem Soc 109:5975–5982

In the original model, all interactions were attractive such as:

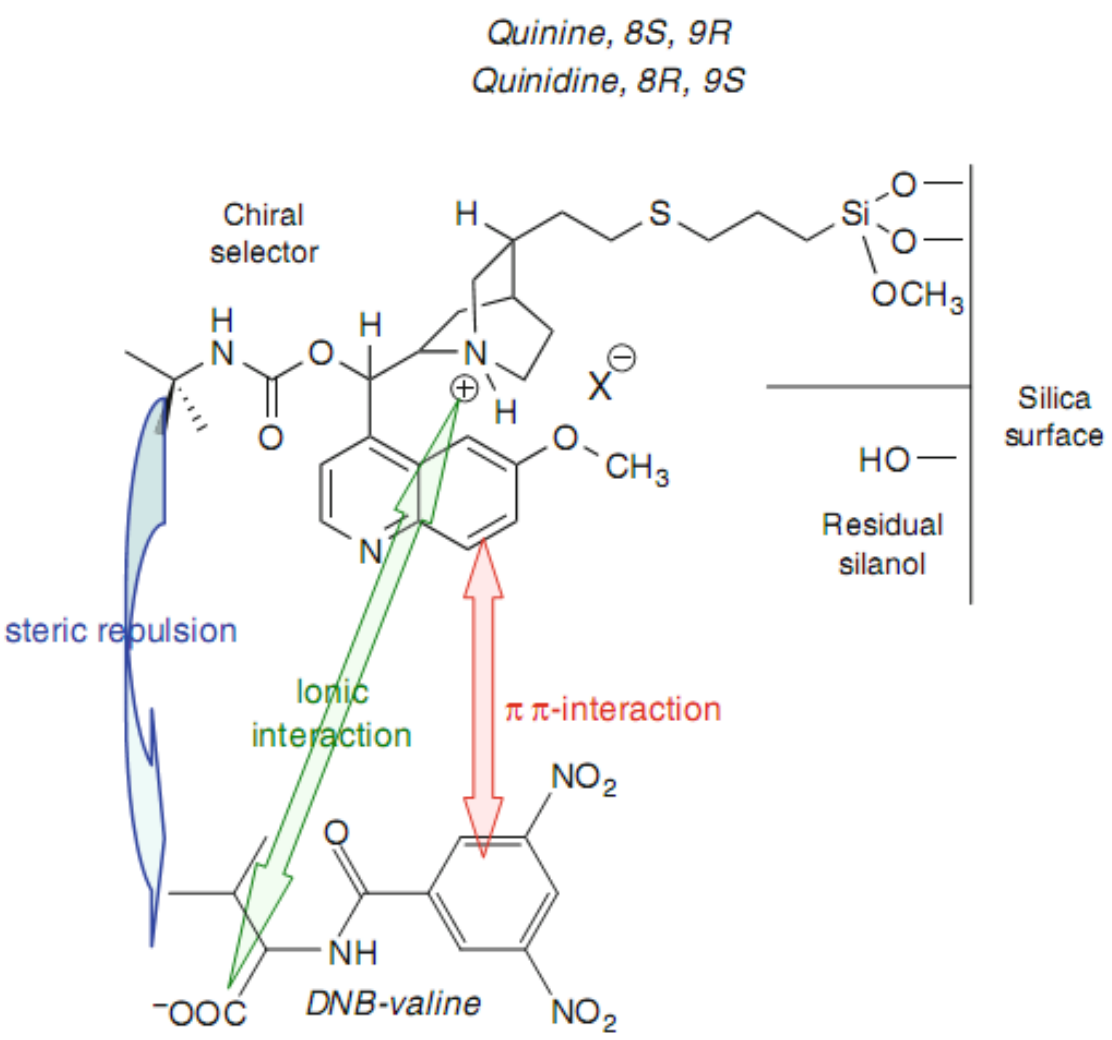
1. Steric fits in a cleft
2. Cavity
3. Repulsion
4. At least two geometrical points or a plane by at least three points

etc

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
$\pi-\pi$ 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 ($1/d^6$)

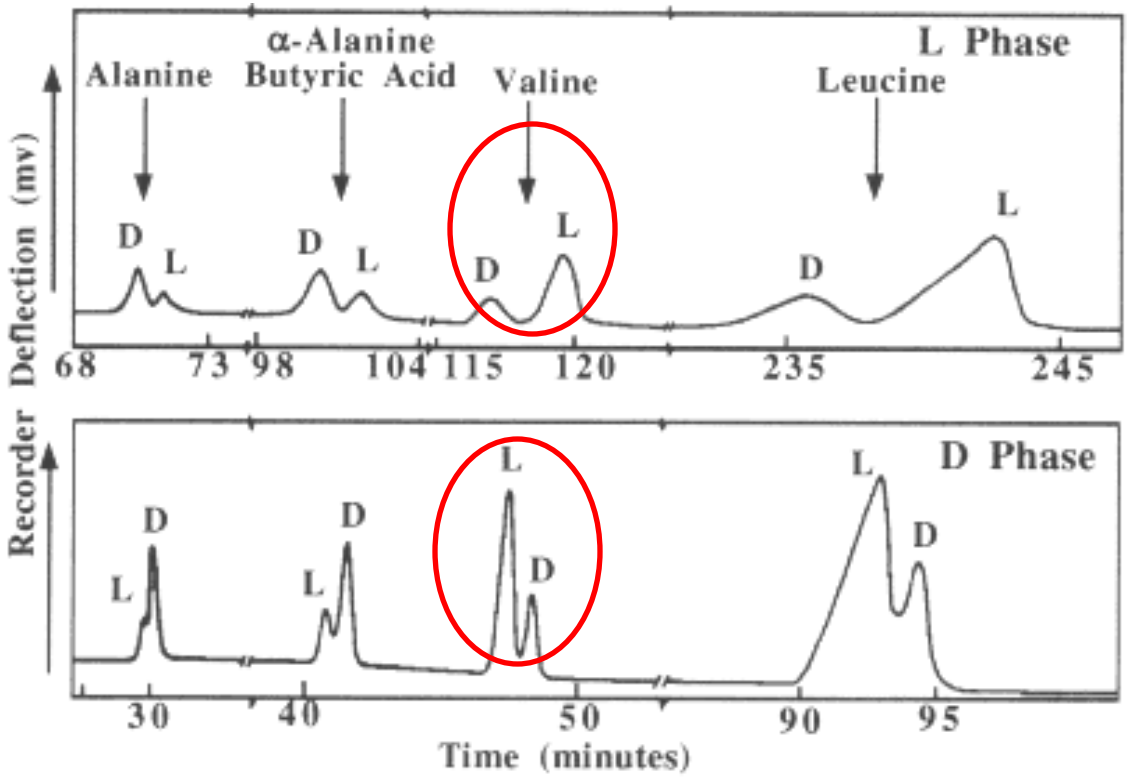
Selector	Mechanism	Primary interaction
Synthetic selectors		
Ligand exchange	Diastereoisomeric selector–metal-ion–analyte complex	Coulomb or ion–dipole
π-Complex	Transient three-point selector–analyte association	π–π
MIPs	Key-and-lock association	Selective shape interaction with the imprint
Chiral crown ethers	Inclusion complexation	Ion–dipole
Polymers	Diastereoisomeric selector–analyte complex	Hydrogen bond
Natural selectors		
Proteins	Multiple binding sites	Variable
Polysaccharides	Insertion into helical structures	Hydrogen bond, dipolar, or steric
CDs	Inclusion complexation	Hydrogen bond
Macrocyclic glycopeptides	Multiple binding sites	Variable
Cinchona alkaloids	Ion pairing	Coulomb



Top: the quinine and quinidine selectors. Bottom: Chiral recognition mechanism by a quinine-based chiral stationary phase (CSP). The strongest interaction is the ionic docking attraction between charges of opposite signs. DNB-D-valine is more retained by the quinine CSP than its L-enantiomer. DNB-L-valine is more retained by the quinidine CSP. All three interactions occur between three different substituents of both the quinine selector and amino acid selectand

Chiral GC

- Gil-Av *et al. used N-TFA-D-isoleucine* lauryl ester and *N-TFA-L-isoleucine lauryl ester as the stationary phase which were coated on the walls* of a capillary column 100 m long, 250 µm I.D.



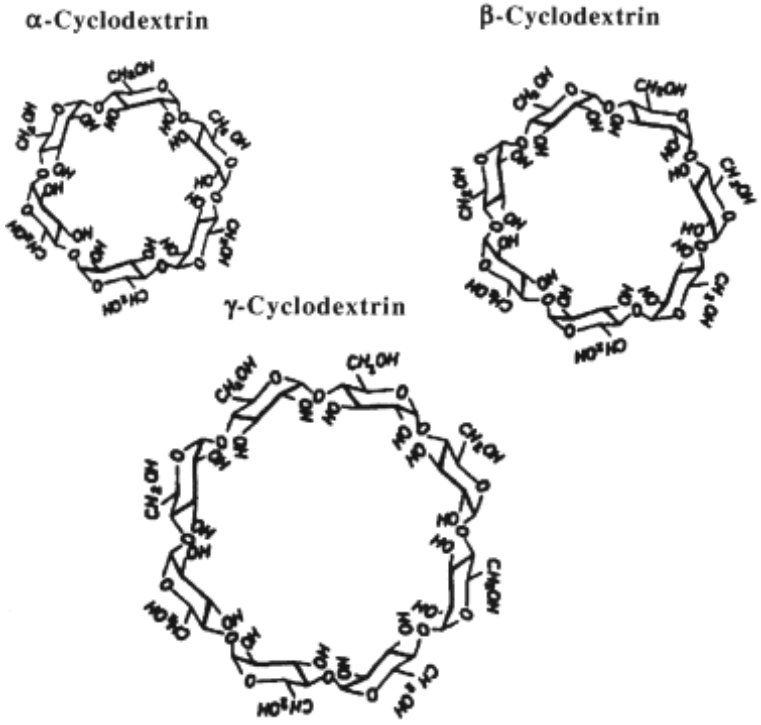
Chiral Stationary Phases for Gas Chromatography

- Amino acid derivatives
- Polysiloxane polymers
- Cyclodextrin derivatives
- Chiral Metal Chelating Stationary Phases
  - resolution of chiral unsaturated hydrocarbons, ethers, ketones, etc

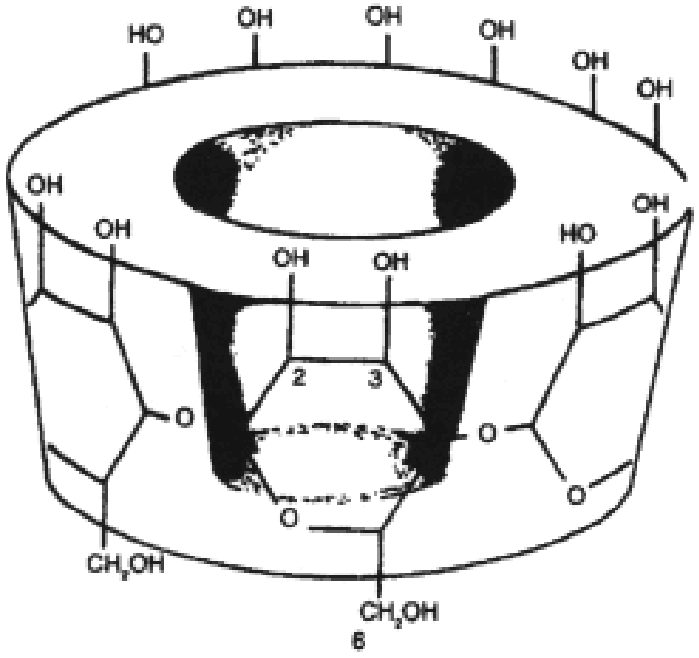
Cyclodextrin Chiral Stationary Phases

- The cyclodextrins are produced by the partial degradation of starch followed by the enzymatic coupling of the glucose units into crystalline, homogeneous toroidal structures of different molecular size.

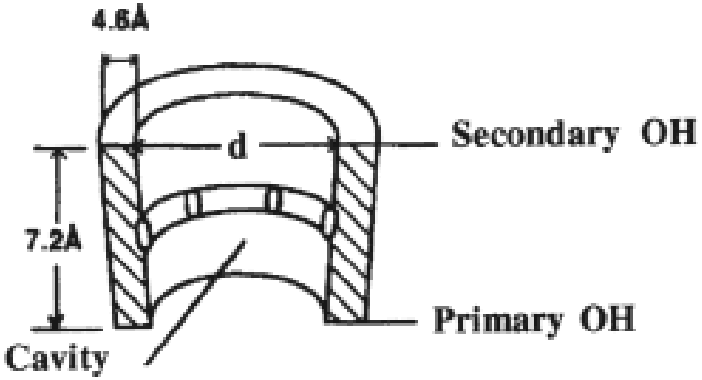
- *alpha-, beta- and gamma-cyclodextrins*
- *and have been* shown to contain
- 6 (cyclohexamylose), 7 (cycloheptamylose)
- and 8 (cyclooctamylose) glucose units,
- respectively.



The very effective chiral characteristics arise from the many chiral centers

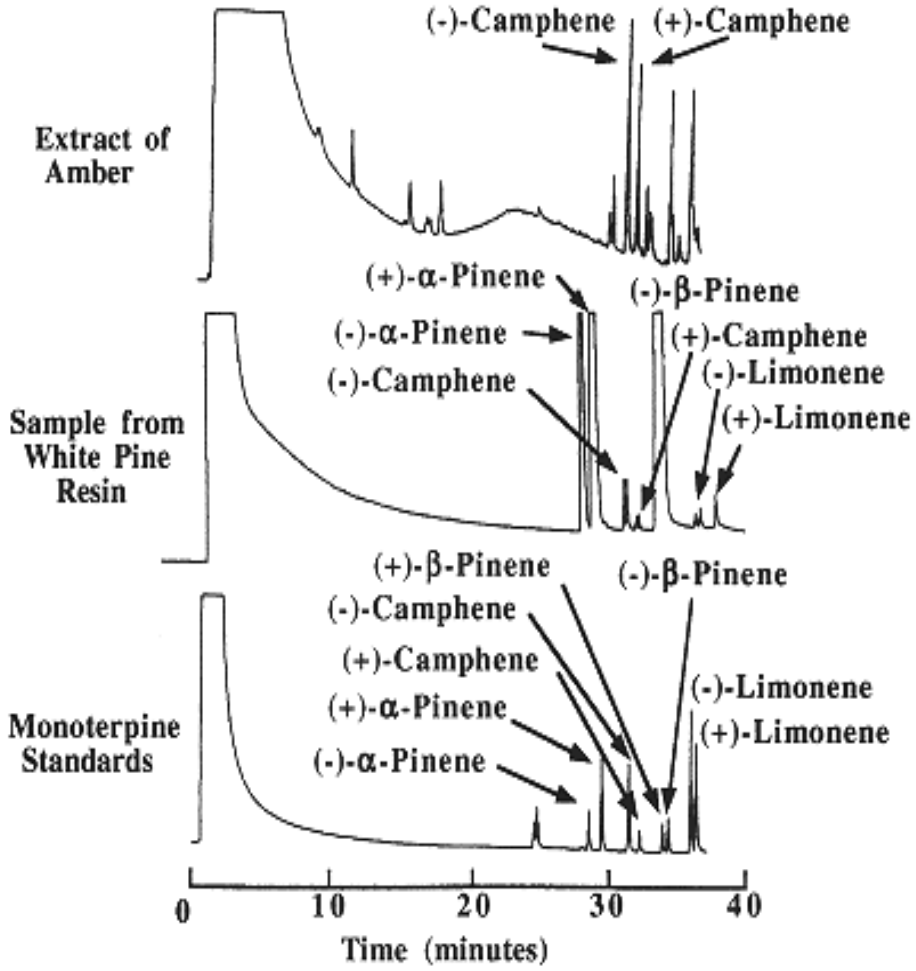


d
- α-Cyclodextrin: 4.7–5.2 A
- β-Cyclodextrin: 6.0–6.5 A
- γ-Cyclodextrin: 7.5–8.5 A

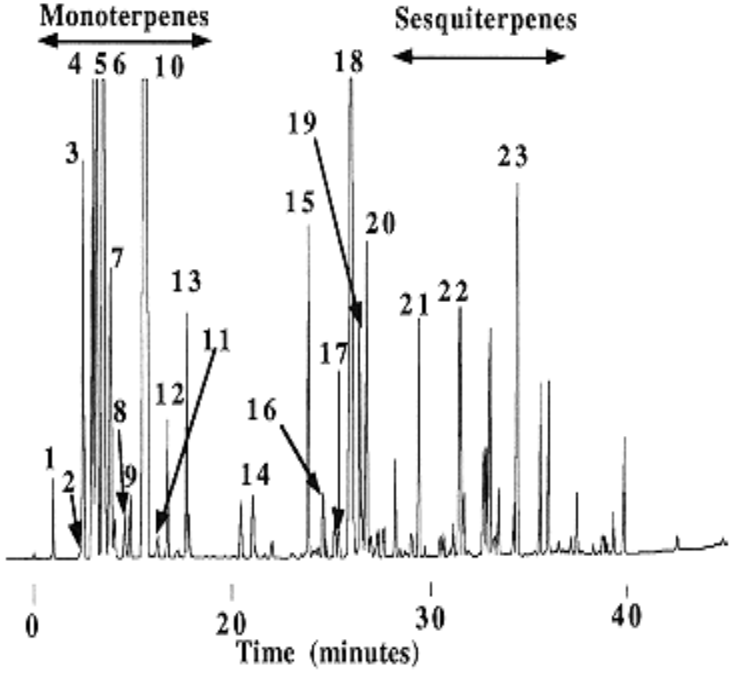


most effective stationary phases

Chiraldex B-PM is also a permethylated b-cyclodextrin



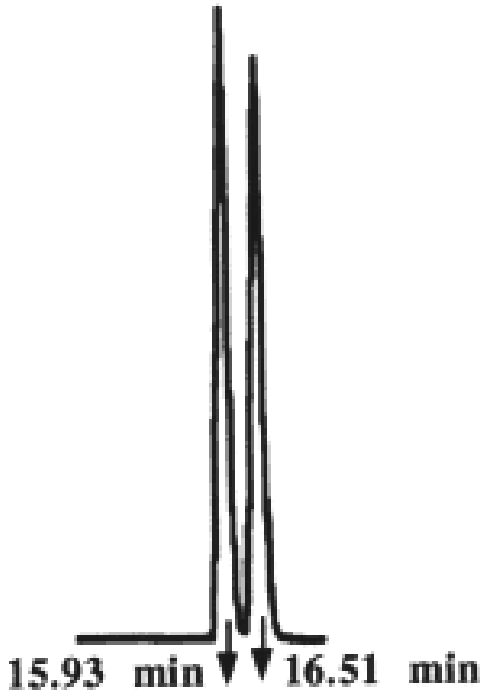
The column was 30 m long, 0.25 mm I.D., carrying a film of Chiraldex B-PM, 0.25 μm thick. The column was held at 40°C for 10 min and then programmed to 85°C at 2°C/min. Helium was used as the carrier gas at an inlet pressure of 10 p.s.i.



1 α-Thujene	7 (+)-Camphene	13 Terpinolene	19 Copanene
2 (-)-α-Pinene	8 (-)-Camphene	14 Linalool	20 (-)-β-Cadinene
3 (+)-α-Pinene	9 α-Terpinene	15 Camphor	21 Caryophylene
4 Tricyclene	10 (+)-Limonene	16 Terpinen-4-ol	22 γ-Cadiene
5 β-Myrcene	11 β-Phellandrene	17 Borneol	23 Gemacrene D
6 Sabinene	12 γ-Terpinene	18 Bornyl Acetate	

The columns were 30 m long, 0.25 mm I.D., carrying a film of stationary phase 0.25 μm thick of β-DEX™. The column was programmed from 40°C to 220°C at 4°C/min. The helium flow velocity was 35 cm/s.

The Separation of the
Enantiomers of **Ibuprofen**
on open tubular columns coated with derivatized b-cyclodextrin



Anal. Chem. **2004,** *76,* 6819−6822

Chiral Ionic Liquids as Stationary Phases in Gas Chromatography

Jie Ding,[†] Thomas Welton,[‡] and Daniel W. Armstrong*[,†]

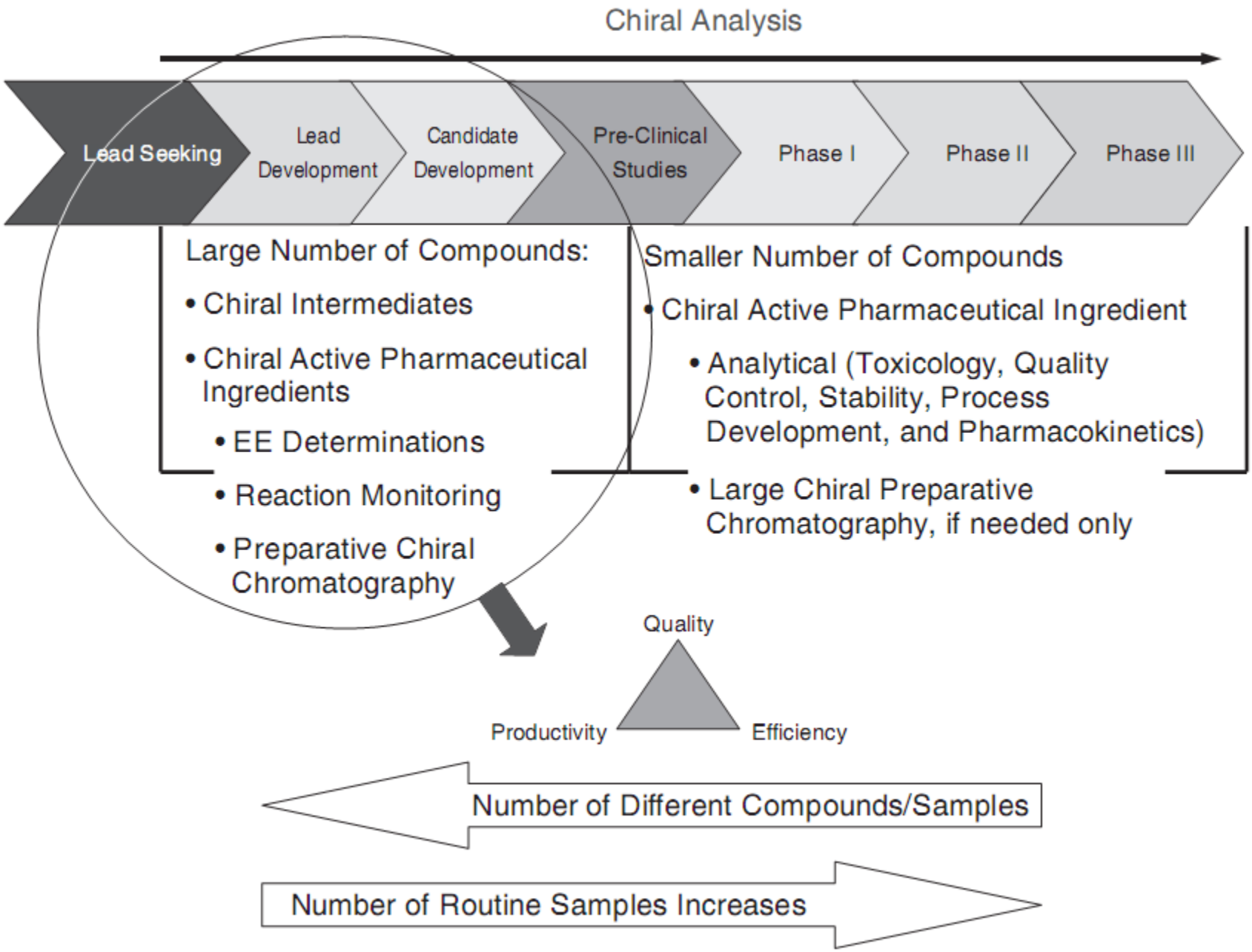
Department of Chemistry, Iowa State University, Ames, Iowa 50011

Recently, it has been found that room-temperature ionic liquids can be used as stable, unusual selectivity stationary phases. They show "dual nature" properties, in that they separate nonpolar compounds as if they are nonpolar stationary phases and separate polar compounds as if they are polar stationary phases. Extending ionic liquids to the realm of chiral separations can be done in two ways: (1) a chiral selector can be dissolved in an achiral ionic liquid, or (2) the ionic liquid itself can be chiral. There is a single precedent for the first approach, but nothing has been reported for the second approach. In this work, we present the first enantiomeric separations using chiral ionic liquid stationary phases in gas chromatography. Compounds that have been separated using these ionic liquid chiral selectors include alcohols, diols, sulfoxides, epoxides, and acetylated amines. Because of the synthetic nature of these chiral selectors, the configuration of the stereogenic center can be controlled and altered for mechanistic studies and reversing enantiomeric retention.

Room-temperature ionic liquids (RTILs) are low-melting (<100 °C) salts which represent a new class of nonmolecular, ionic solvents. These solvents possess a number of interesting properties in terms of their interaction/solvation parameters and abilities.[9] There also has been a great deal of interest in the application of the ionic liquids as novel biphasic catalysts,[10] extraction solvents,[11] highly selective transport membranes,[12] and stationary phases for gas chromatography.[13,14]

The first application of molten salts as gas chromatographic stationary phase was reported by Barber et al.[15] Since the early 1980s, Poole and co-workers have published a series of papers on using organic molten salts as GC stationary phases.[16−20] Although the initial alkylammonium- and alkylphosphonium-based molten salts had been used as GC stationary phases, they had limitations, such as relatively narrow liquid ranges, thermal instability, and poor wetability toward the surface of fused silica. Later-emerging ionic liquids containing alkylimidazolium or alkylpyridinium cations possessed improved properties (wider liquid range and better thermal stability) and were more suitable for GC stationary phases. Recently, we demonstrated that alkylimidazolium-based ILs can be used as stable, unusual selectivity stationary phases.[13,14] They show "dual nature" properties. They separate nonpolar compounds as if they are nonpolar stationary phases and separate polar compounds as if they are polar stationary phases. We also introduced the achiral ILs to the realm of chiral separations by dissolving the chiral selector (methylated

Chirality and HPLC

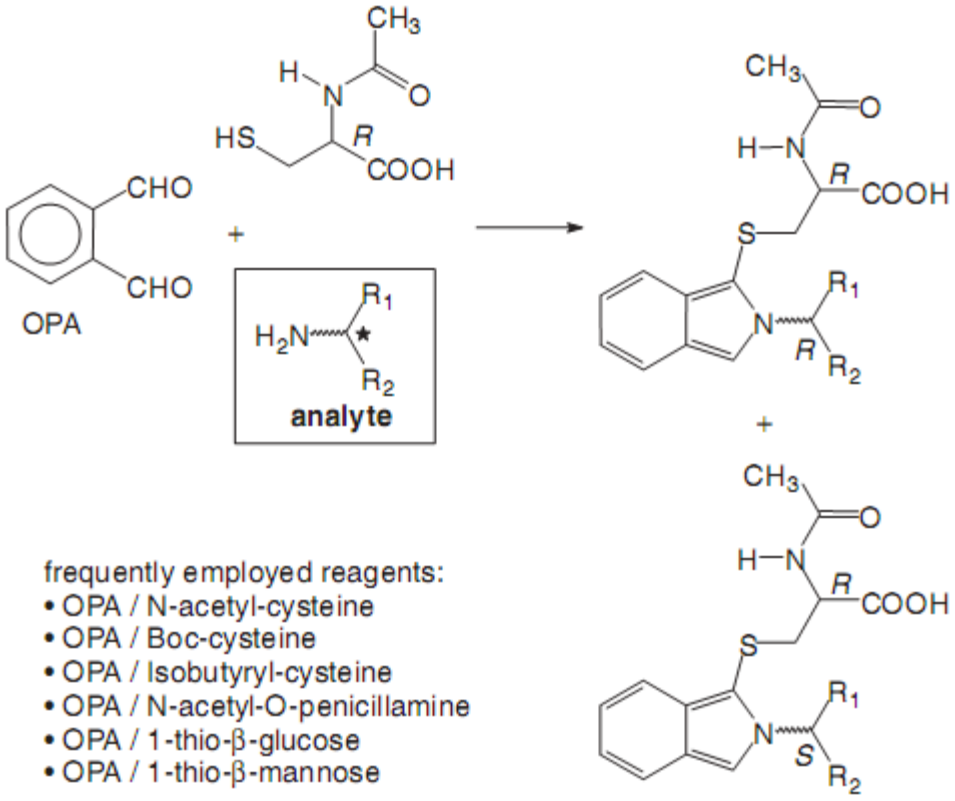


Typical pharmaceutical development stages.

Commonly Employed Chiral Derivatizing Reagents (CDR) and Their Application

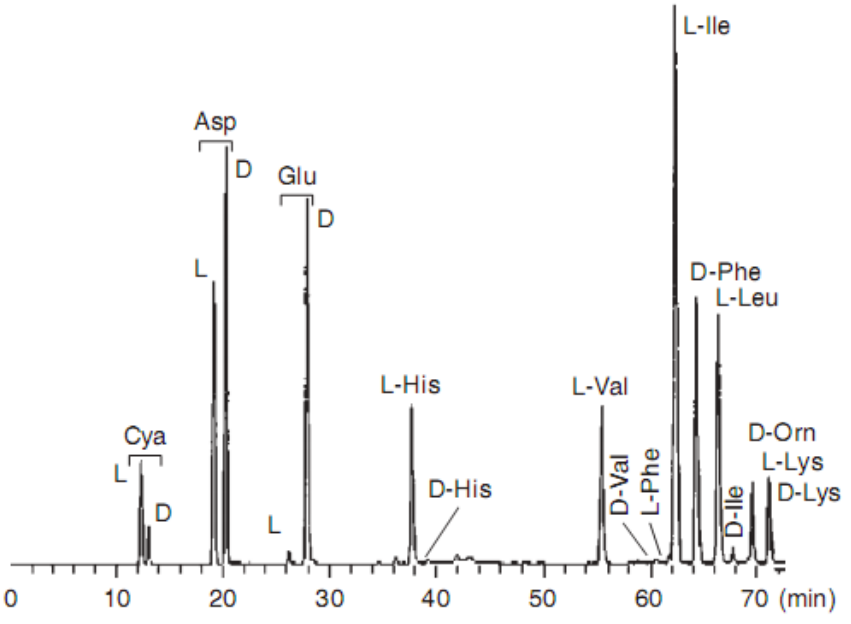
CDR	Analyte Class
1. (*R*) or (*S*)−α-methoxy-α-trifluoromethyl phenylacetic acid and corresponding acid chloride (Mosher's reagent)	Alcohols, amines
2. *O*,*O*′-dibenzoyl tartaric acid anhydride (DBTAAN)	Primary and secondary amines, alcohols, aminoalcohols
3. (*R*)- or (*S*)−1-(9-fluorenyl)ethyl chloroformate (FLEC)	Primary and secondary amines, amino acids
4. ortho-phthaldialdehyde (OPA) in combination with chiral thiols such as (*S*)-or (*R*)-enantiomers of *N*-acetyl-cysteine, *N*-t-Boc-cysteine, *N*-acetyl-penicillamine, 1-thio-β-glucose	Primary amines, primary amino acids
5. 1-fluoro-2,4-dinitrophenyl-5-(*S*)-alanine amide (FDAA) (Marfey's reagent)	Primary and secondary amines, amino acids, thiols
6. 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)	Primary and secondary amines, amino acids, thiols

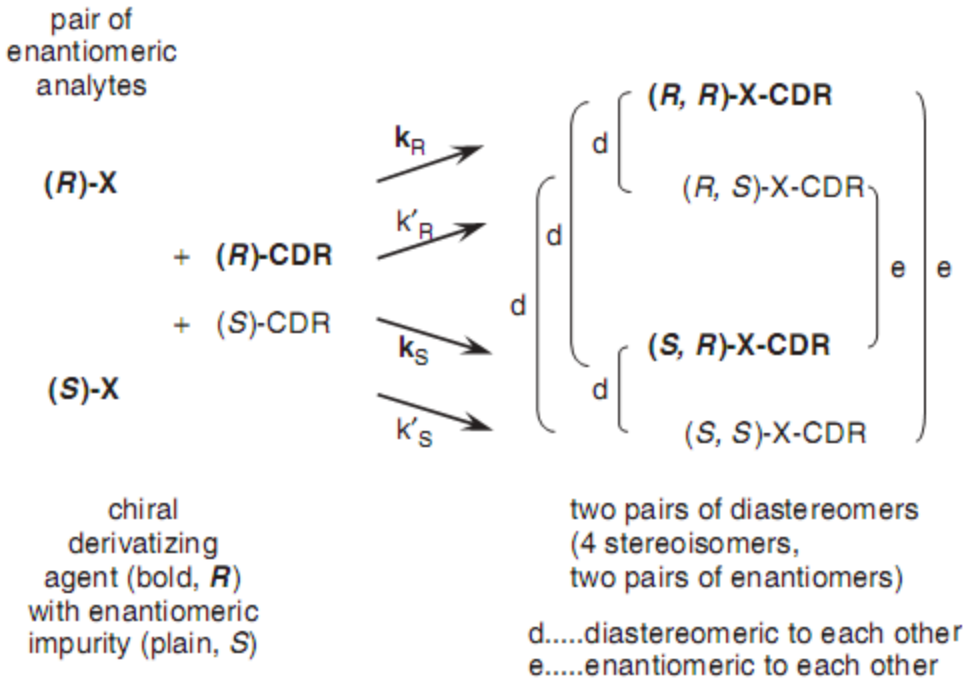
OPA-chiral thiol derivatization for the stereoselective analysis of primary amines and amino acids (indirect enantiomer separation).



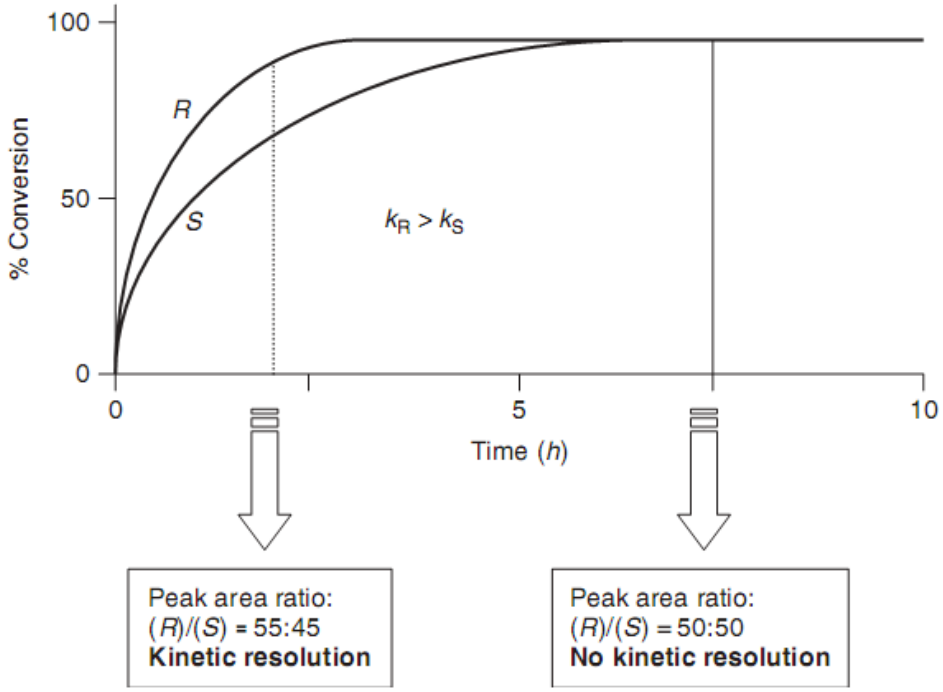
frequently employed reagents:
- OPA / N-acetyl-cysteine
- OPA / Boc-cysteine
- OPA / Isobutyryl-cysteine
- OPA / N-acetyl-O-penicillamine
- OPA / 1-thio-β-glucose
- OPA / 1-thio-β-mannose

Enantiomeric separation of amino acids obtained from the hydrolysis of baci-tracin A, followed by OPA-chiral thiol derivatization



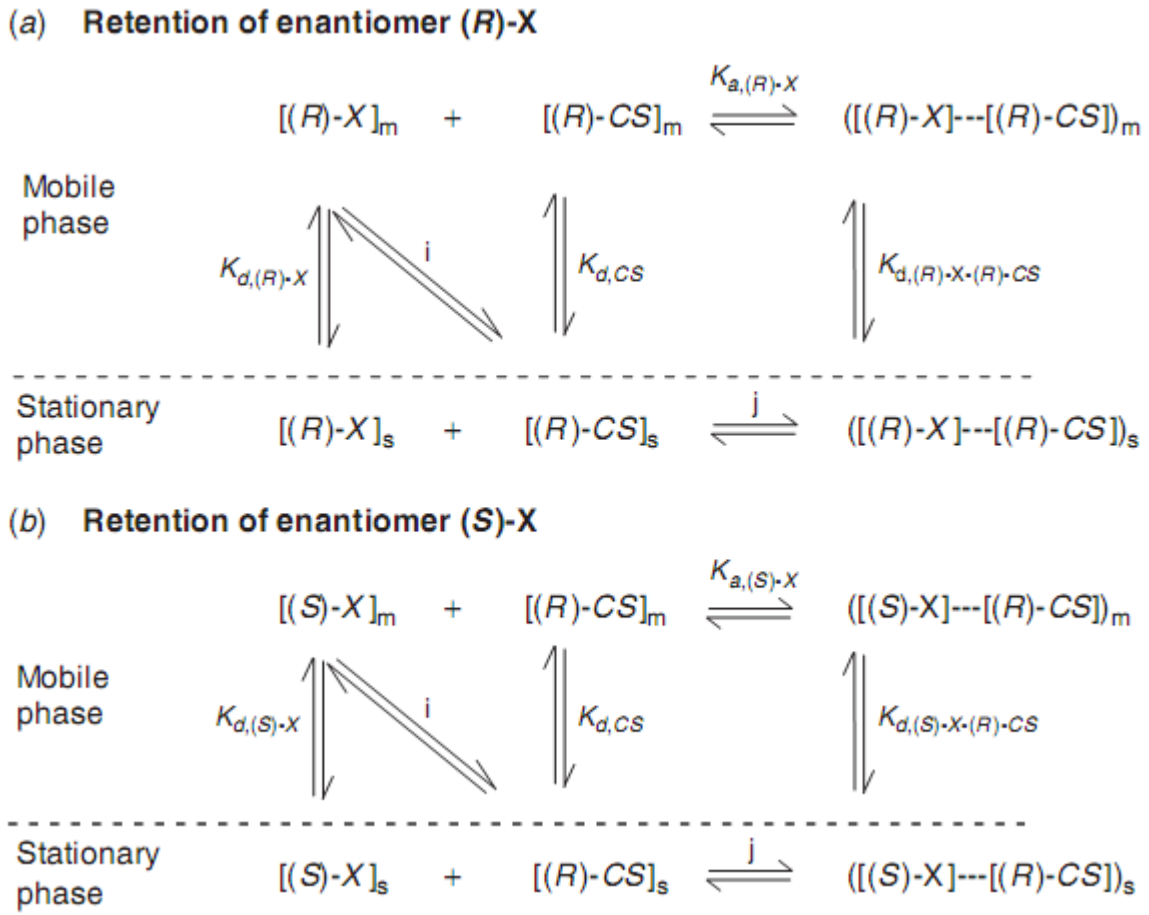


Reaction scheme for indirect HPLC enantiomer separation (in the presence of an S-CDR impurity in the chiral derivatization reagent R-CDR). All four stereoisomers areformed and two pairs of enantiomers, respectively (d, diastereomeric to each other; e, enantiomeric to each other).



Illustrative kinetic profiles for the reaction of a sample with a chiral derivatizing reagent. The two enantiomers are assumed to have different rate constants and an identical detector response for the resulting diastereomers.

Equilibria for the retention of R-and S- enantiomers (chiral–mobile-phase additive, CMPA) mode. Subscripts m and s refer to corresponding species in mobile and stationary phases; K_a and K_d represent association and distribution constants, respectively.



The addition of the **Chiral Selector** to the **mobile phase** is one of the simplest approaches for chi-ral separation. This approach is particularly simple and cheap in nano-LC but expensive when using HPLC.

5 type chiral stationary phase

- Type I : Form complexes based on pi donor and acceptor interactions
  - Ex: Brush Type (Pirkle) CSPs

- Type II : Seperation based on a combination of complex formation, usually due to H bonding, and the existence of cavities
  - Ex: Polysaccharide and Macrocyclic Antibody CSPs

- Type III : Separates primarily based on compounds ability to fit in a cavity
  - Ex: Cyclodextrin, Crown Ether and Polymer CSPs
- Type IV : Forms diastereomeric metal complexes
  - Ex: Ligand Exchange CSPs
- Type V: Based on hydrophobic and hydrophilic interactions
  - Ex: Protein Based CSPs
  - natural proteins bonded to a silica matrix

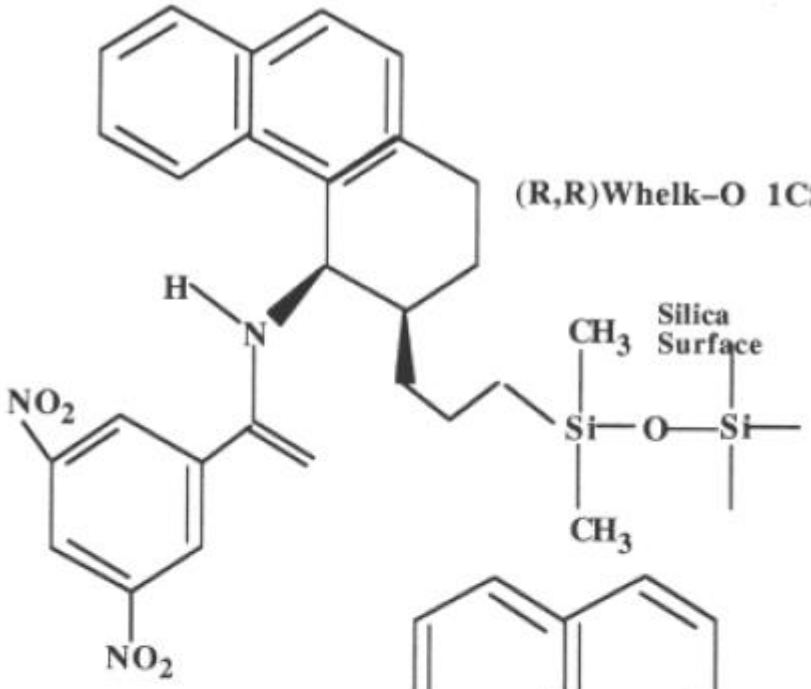
Brush Type

- Type I
- Developed by Pirkle
- First CSP
- Operates on pi donor and acceptor interactions

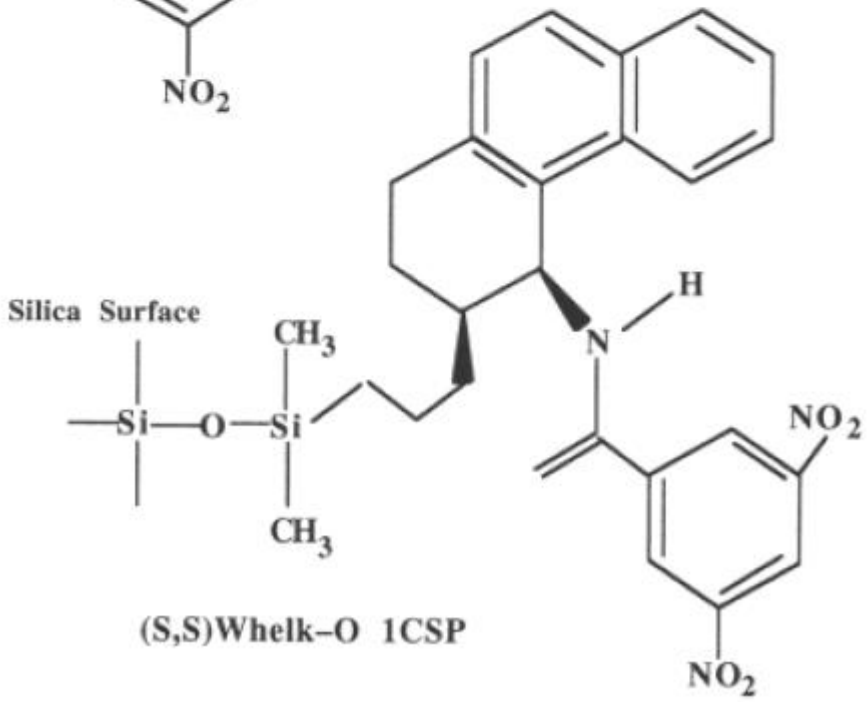
The small molecular weight chiral substances bonded to silica, commonly called the Pirkle phases, usually have a limited number of chiral centers but a large number of the groups bonded to the silica.

The system suffers from certain disadvantages which results from the spatial arrangement of the chiral center and other interacting moieties around it. The relative short bond between the chiral agent and the silica restricts the approach of some molecules, so that their chiral center can not interact with the chiral center of the stationary phase.

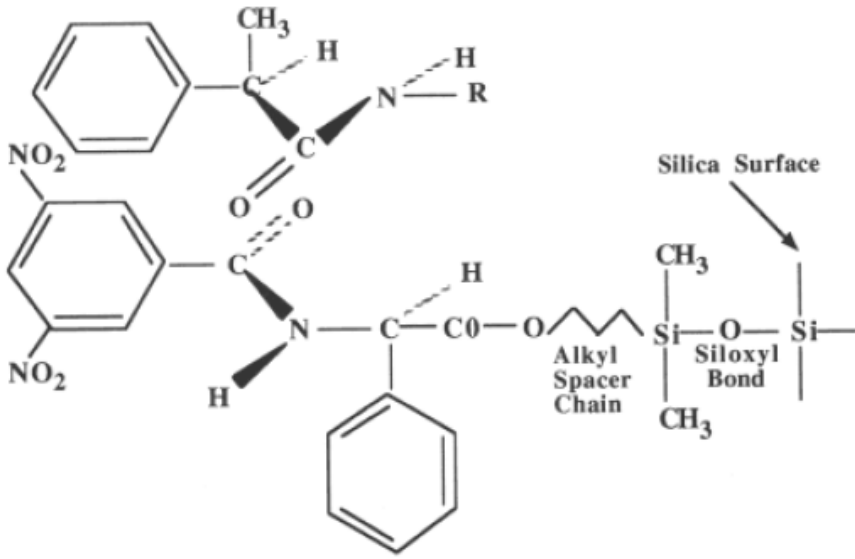
However, for certain molecules, the spatial arrangement can be ideal.



(R,R)Whelk-O 1CSP



(S,S)Whelk-O 1CSP



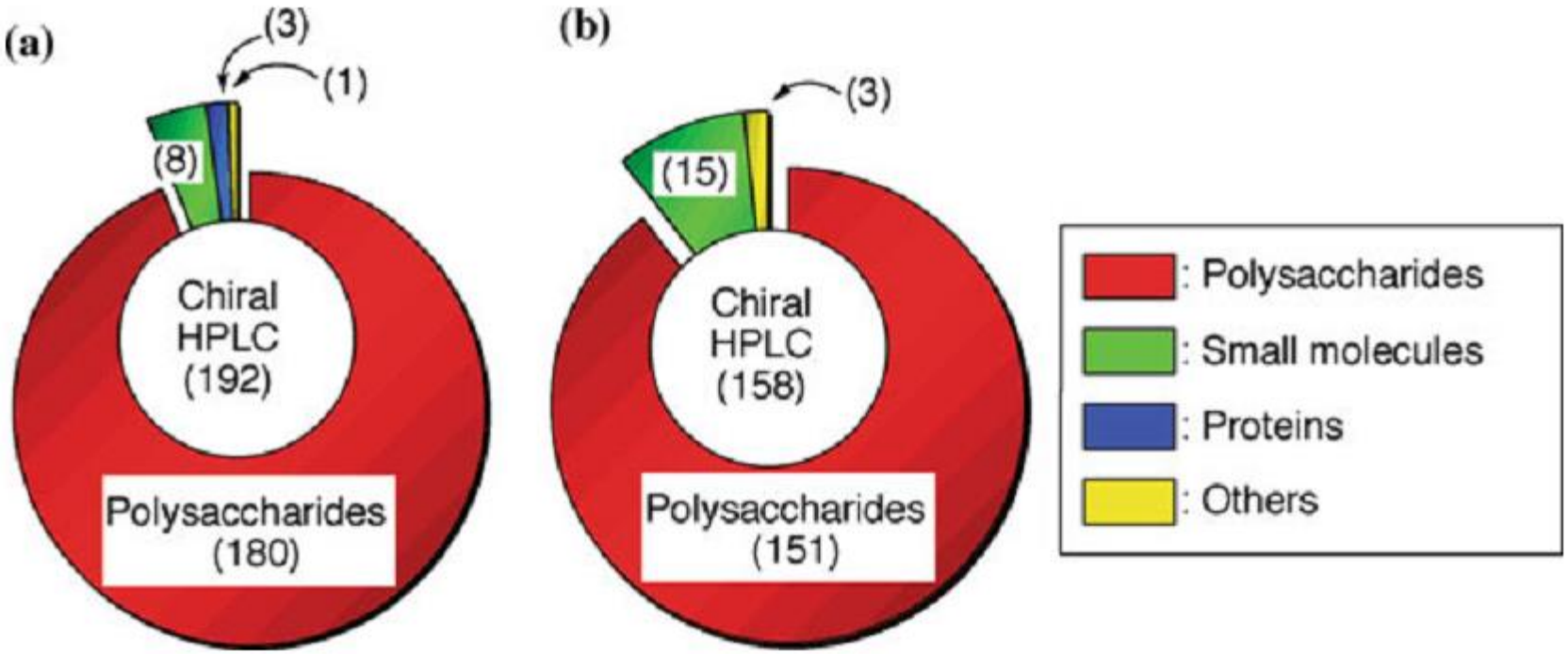
R= Naphthylmethylamide

Commercially Available Donor-Acceptor (Pirkle-Type) CSPs

Chiral Selector	Column Trade Name	Supplier
π-electron acceptor/π-electron donor phases		
3-[1-(3,5-dinitro benzamido)-1,2,3,4-tetrahydrophenanthrene-2-yl]-propyl-silica	Whelk-O 1	Regis
11-[2-(3,5-dinitroben-zamido)-1,2-diphenylethylamino]-11-oxoundecyl-silica	ULMO	Regis
3-[*N*-(3,5-dinitrobenzoyl)-(*R*) − 1-naphthyl-glycine-amido]propyl-silica	Chirex 3005 (Sumichiral 2500)	Phenomenex (Sumitomo)
π-electron acceptor phases		
3-[3-[*N*-[2-(3,5-dinitrobenzamido-1-cyclohexyl)]-3,5-dinitrobenzamido]-2-hydroxy-propoxy]-propyl-silica	DACH-DNB	Regis
3-[3-(3,5-dinitrobenzamido)-2-oxo-4-phenyl-azetidine-1-yl]-propyl-silica	PIRKLE 1-J	Regis
5-(3,5-dinitrobenzamido)-4,4-dimethyl-5-dimethyl phosphonyl-pentanyl-silica	α-Burke 2	Regis
11-[*N*-(3,5-dinitrobenzoyl)-3-amino-3-phenyl-2-(1,1-dimethylethyl) propanoyl]-undecyl-silica	β-Gem 1	Regis
3-[*N*-(3,5-dinitrobenzoyl) leucine-amido]propyl-silica	Leucine (DNBLeu)	Regis
3-[*N*-(3,5-dinitrobenzoyl) phenylglycine-amido] propyl-silica	Phenylglycine (DNBPG)	Regis (Merck)
π-electron donor phases		
3-[*N*-[(*R*)-(α-naphthyl) ethylcarbamoyl]-(*S*)-indoline-2-carboxamido] propyl-silica (urea linkage)	Chirex 3022 (Sumichiral OA 4900)	Phenomenex (Sumitomo)
3-[*N*-[(*R*)-1-(α-naphthyl) ethylcarbamoyl]-(*S*)-*tert*-leucine-amido] propyl-silica (urea linkage)	Chirex 3020 (Sumichiral OA 4700)	Phenomenex (Sumitomo)

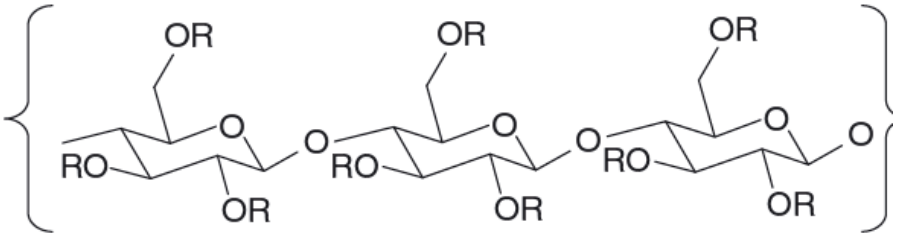
Polysaccharide

- Type II
- The first practical chromatographic use of polysaccharides came in 1973, when Hesse and Hagel prepared cellulose triacetate from microcrystalline cellulose and used it as a liquid chromatographic stationary phase.
- Most common CSP. These CSPs are well known to any practitioner of the art of HPLC-based enantiomer separation under the product names Chiralcel and Chiralpak. These phases were developed originally by Professor Yoshio Okamoto and co-workers at Osaka University and commercialized by Daicel Chemical Industries.
- They take two basic forms, those derived fromc ellulose polymers and those derived from amylose polymers; they are reported to have molecular weights of up to 40,000 Daltons. The basic difference between the two polymers is that the cellulose adopts a linear structure, whereas the amylose forms a helical structure. Both cellulose adn amylose unit contains 5 chiral centers.
- Cellulose or amylose bonded to silica Generally substituted with aromatic rings

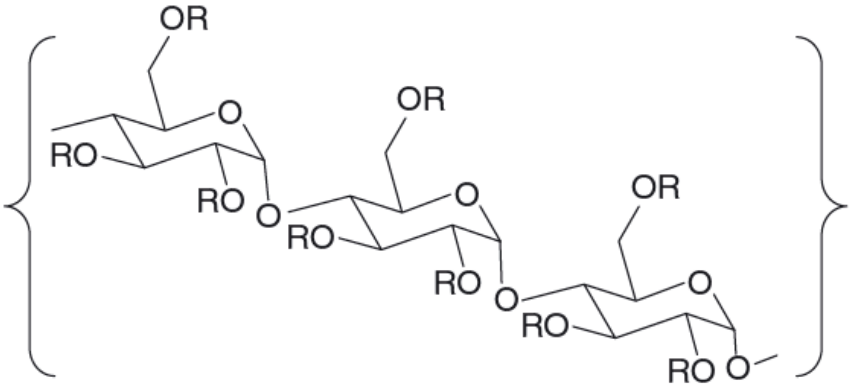


Distribution of CSPs for HPLC used for the determination of enantiomeric excess reported in Journal of the American Chemical Society in (a) 2005 and (b) 2007. The values in parentheses represent the number of the counted papers.

Due to the fact that the stationary phase is coated on the silica and not chemically bonded to it, certain limitations are placed on the type of solvents, the linear velocity of the mobile phase and the operating temperatures that can be employed. The solvents that are recommended for use as the mobile phase are heptane/alcohol mixtures which would indicate that the material is used largely in the polar phase mode (*i.e.* the dominant forces employed in the retention and selectivity are polar).

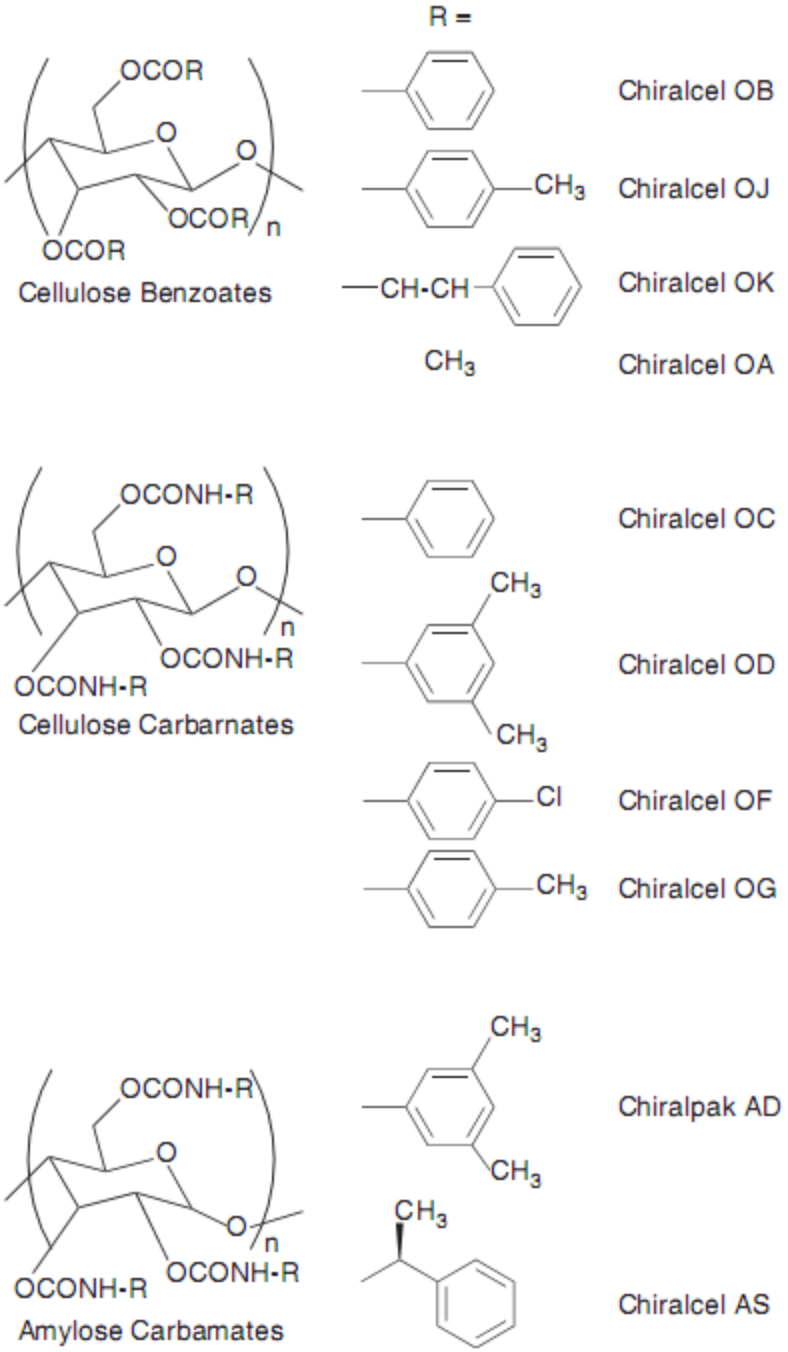


Cellulose



Amylose

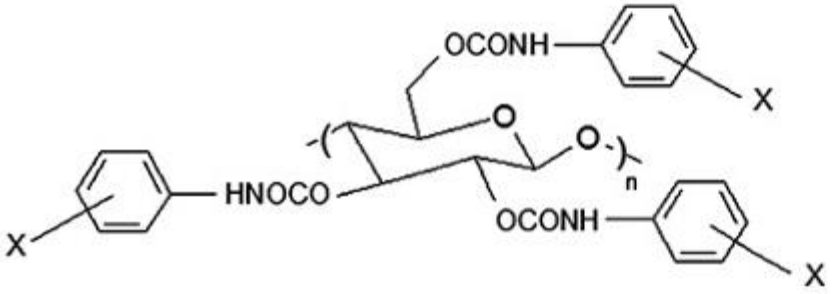
An incredible number of chiral separations have been and continue to be made with just four commercial chiral stationary phases: Chiralpak AD and AS and Chiralcel OD and OJ. Now these same problems can usually be solved with just three immobilized columns: Chiralpak IA, IB, and IC.



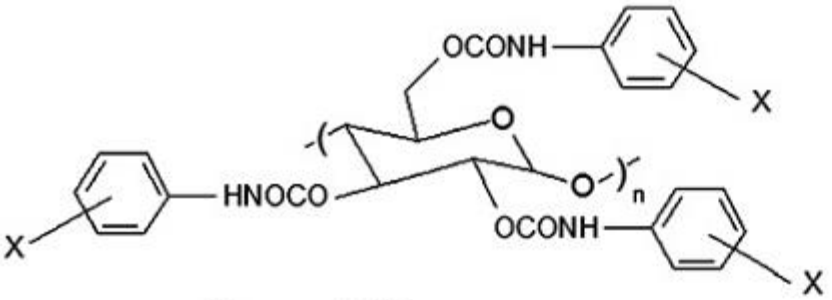
Commercially available polysaccharide-based CSP structures.

The 3,5-dimethylphenylcarbamate (CDMPC) and 3,5-dichlorophenylcarbamate appear to have the most general chiral selectivity. This high degree of selectivity is also exhibited by the 3,5- dimethylphenylcarbamate of amylose (ADMPC).

In this context it is interesting to note that in studies involving 510 racemic compounds, 315 showed either full or partial resolution on the CDMPC column. Similarly, of 384 racemates examined on an ADMPC column, 107 were resolved completely, and another 102 were resolved partially. In a combined screening experiment with 510 compounds, 129 were resolved only on CDMPC, 85 were resolved only on ADMPC, and 129 were resolved on both columns, with a combined success rate of about 78% (400 of 510 compounds).



Cellulose carbamates

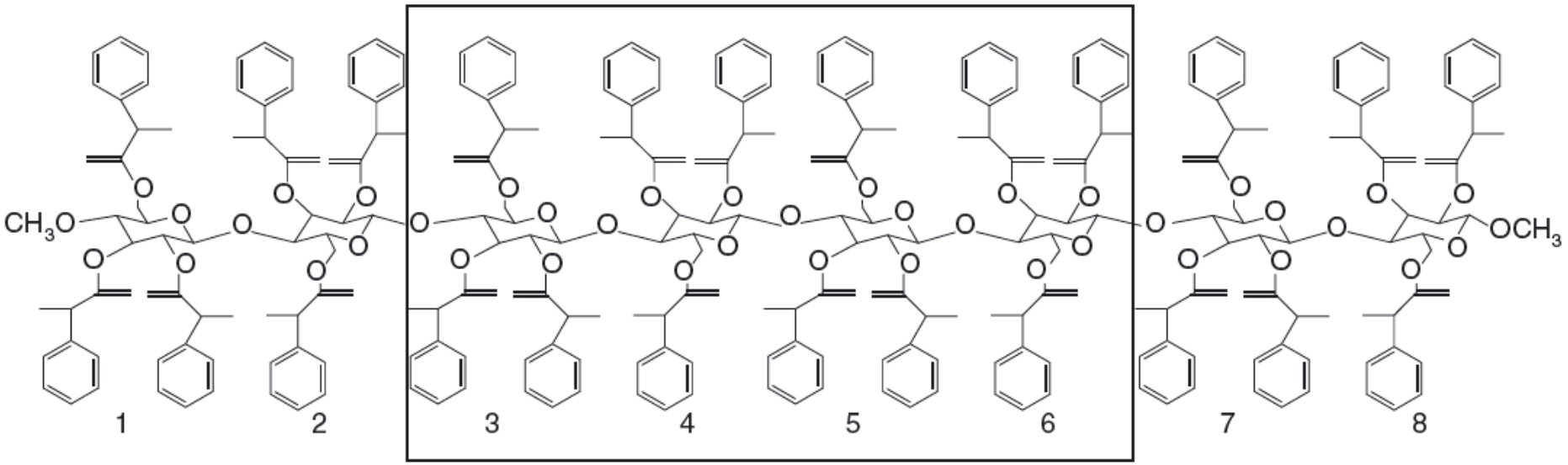


Amylose carbamates

X= a: 4-NO_2
b: 4-CF_3
c: 4-I
d: 4-Br
e: 4-Cl
f: 4-F
g: H
i: 4-Ph
j: 4-$Si(CH_3)_3$
k: 4-$C(CH_3)_3$
l: 4-$CH(CH_3)_2$
m: 4-C_2H_5
n: 4-CH_3
o: 4-OPh
p: 4-O-$CH(CH_3)_2$
q: 4-OC_2H_5
r: 4-OCH_3
s: 2-Cl
t: 2-CH_3
u: 2-OCH_3
v: 3-Cl
w: 3-CH_3
x: 3-OCH_3
y: 3-OC_2H_5
z: 3-$OCH(CH_3)_2$
aa: 3,5-$(OCH_3)_2$
ab: 3,5-$(CH_3)_2$
ac: 2,6-$(OCH_3)_2$
ad: 3,4-$(OCH_3)_2$
ae: 3,5-Cl_2
af: 3,4-Cl_2
ag: 2,6-Cl_2
ah: 3,5-F_2
ai: 3,5-$(CF_3)_2$

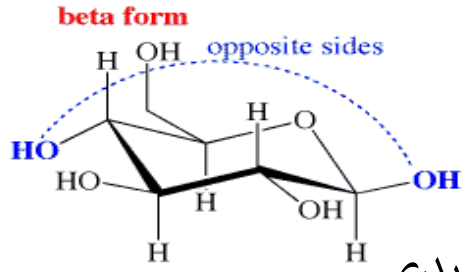
Structures of tris-phenylcarbamate derivatives of cellulose and amylose.

B. Chankvetadze / J. Chromatogr. A 1269 (2012) 26–51

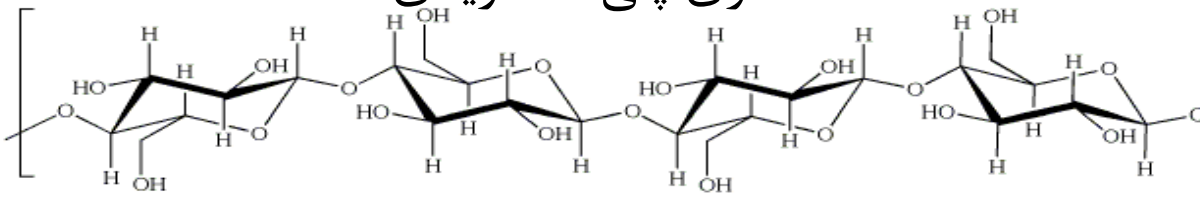


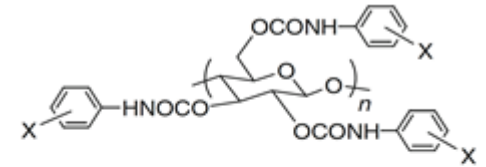
Octamer of CTPC reported by Yashima et al.

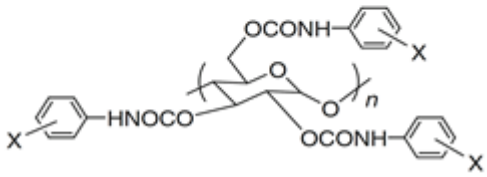
Kinds of Polysaccharides Phases

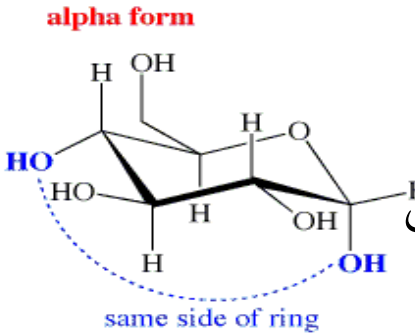


مشتقات استری پلی ساکاریدی

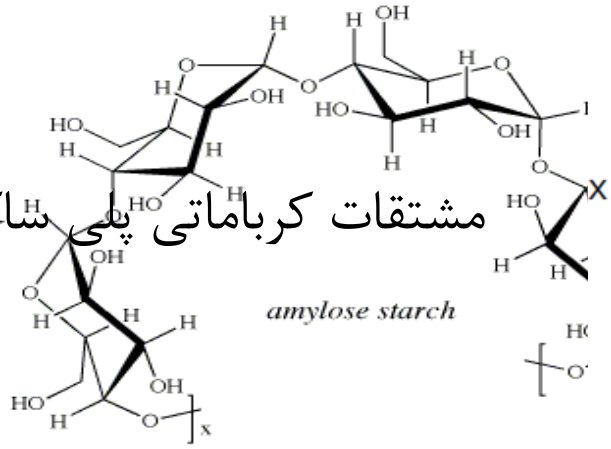


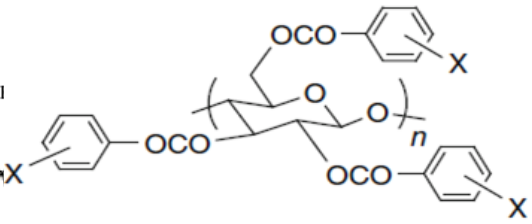


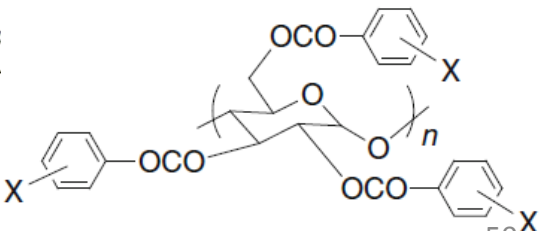




مشتقات کرباماتی پلی ساکاریدی

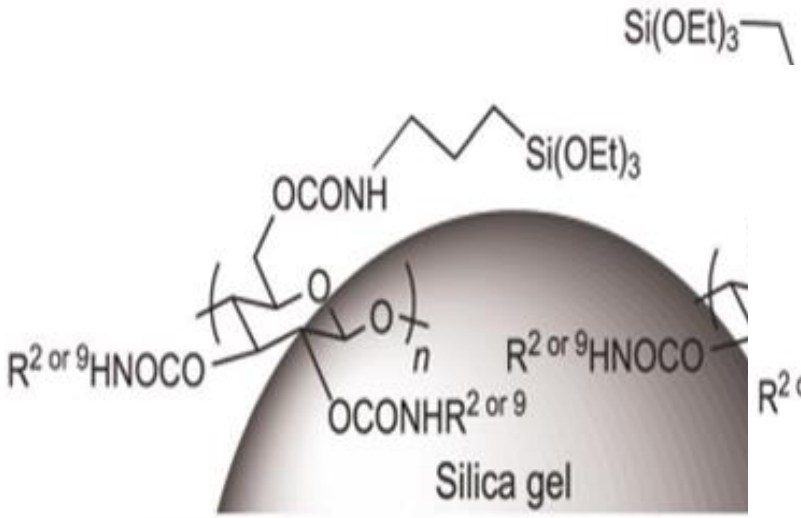




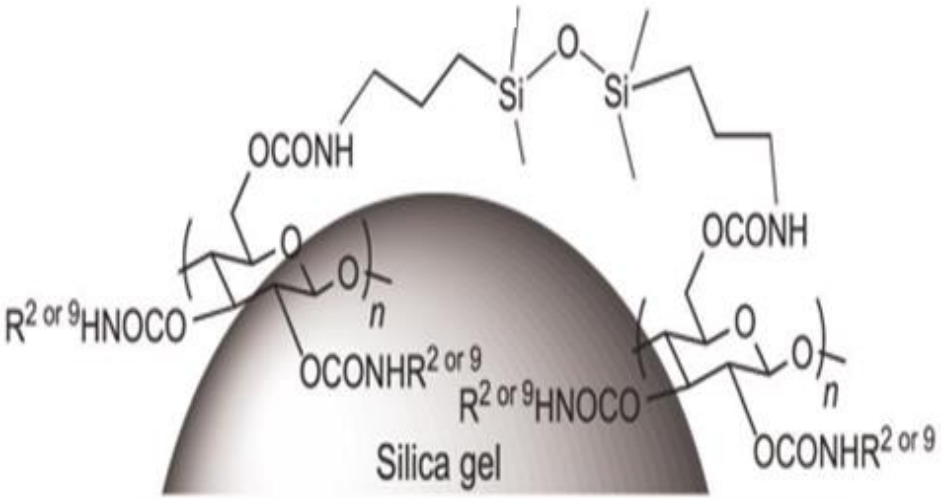


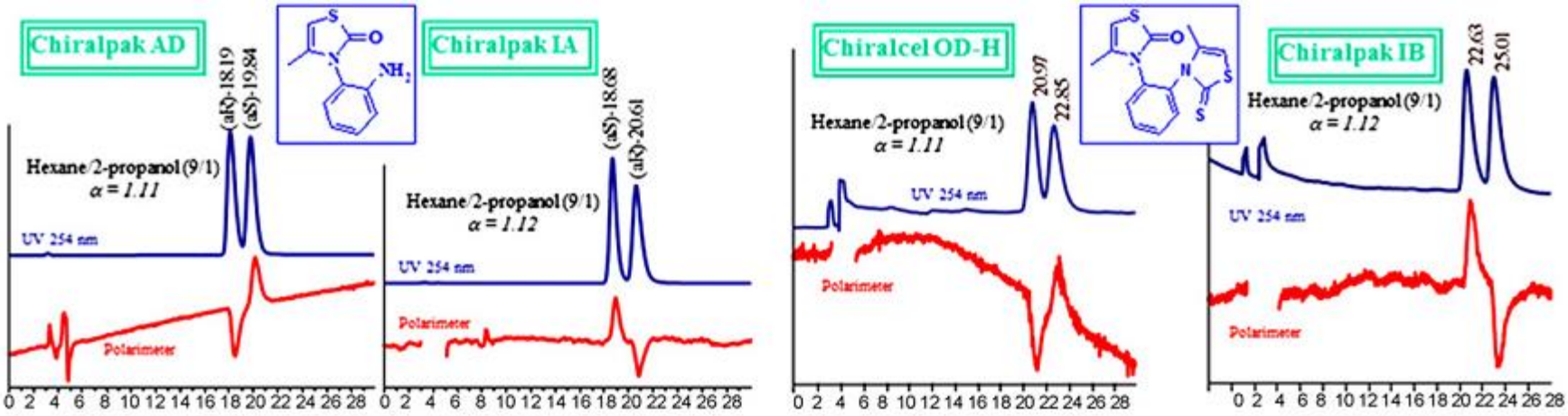
Polysaccharides Stationary Phases

Coated sp

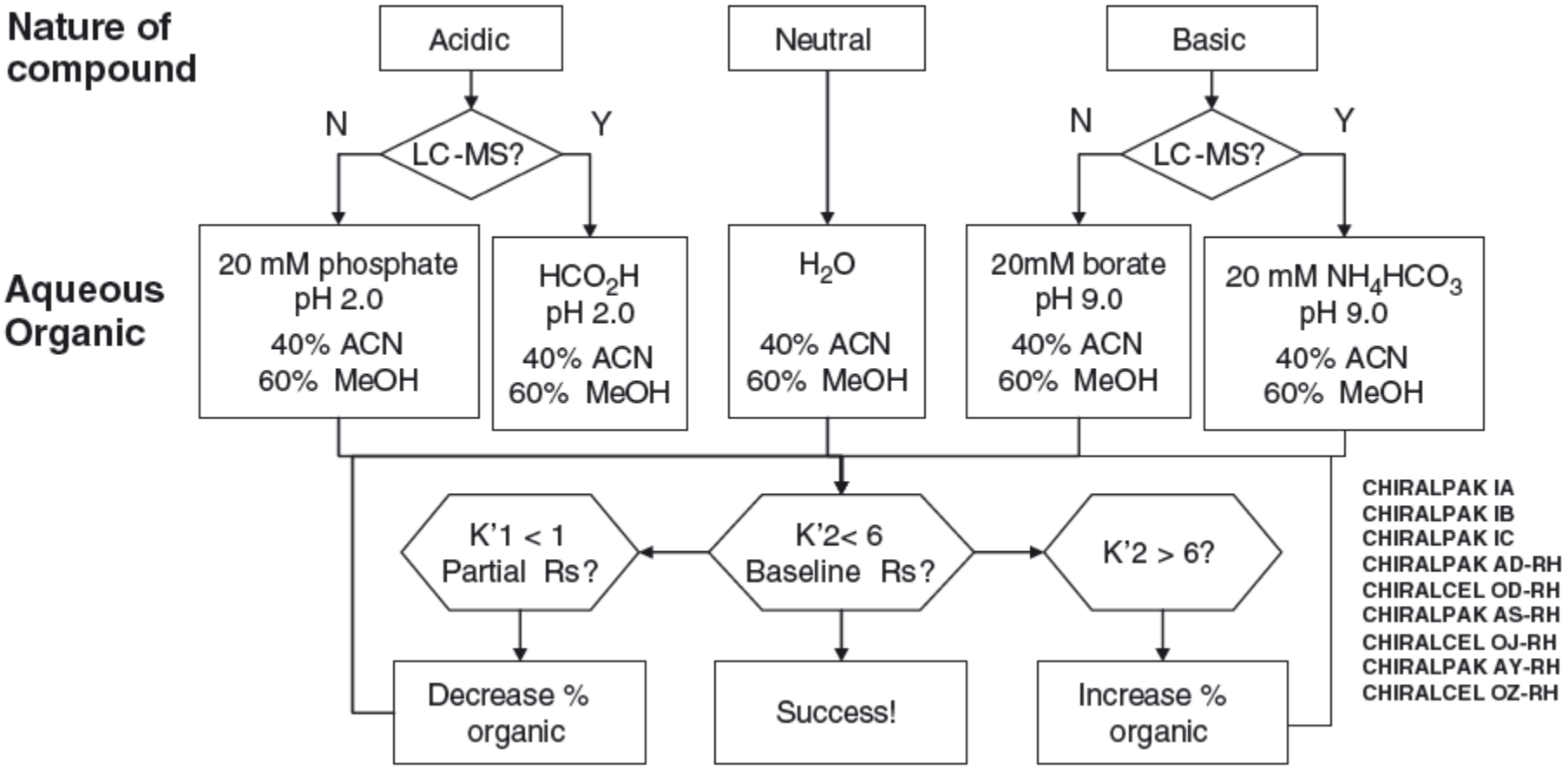


Imobbolized sp





Examples of opposite elution order of enantiomers on chiral columns containing coated and covalently immobilized versions of the "same" polysaccharide-based chiral selectors.

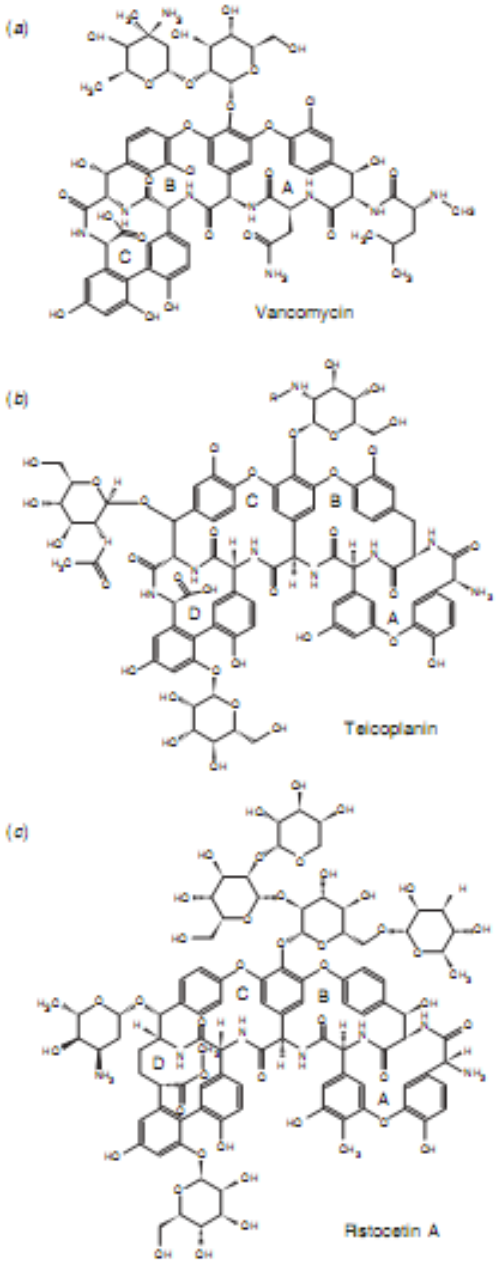

Nature of compound
Aqueous Organic
Acidic
Neutral
Basic
N
LC-MS?
Y
N
LC-MS?
Y
20 mM phosphate pH 2.0 40% ACN 60% MeOH
HCO2H pH 2.0 40% ACN 60% MeOH
H2O 40% ACN 60% MeOH
20mM borate pH 9.0 40% ACN 60% MeOH
20 mM NH4HCO3 pH 9.0 40% ACN 60% MeOH
K'1 < 1 Partial Rs?
K'2< 6 Baseline Rs?
K'2 > 6?
Decrease % organic
Success!
Increase % organic
CHIRALPAK IA
CHIRALPAK IB
CHIRALPAK IC
CHIRALPAK AD-RH
CHIRALCEL OD-RH
CHIRALPAK AS-RH
CHIRALCEL OJ-RH
CHIRALPAK AY-RH
CHIRALCEL OZ-RH

A number of significant advantages over coated columns:

1. Immobilized CSPs can be used with any organic solvent; consequently, the immobilized columns are very rugged and behave like any other HPLC column.
2. Any organic solvent can be used to dissolve a sample into solution. This feature can be especially useful for preparative separations, in which productivity is usually linked to sample solubility.
3. A broader range of solvent polarities, including a large group of medium polarity organic solvents, is now available to be used as mobile phases.
4. A greater variety of solvents is available for method development. Numerous separations have been developed on immobilized columns with extended-range solvents, for cases in which conventional solvents and coated solvents were unsuccessful.
5. More appropriate solvents can be used to flush undesired adsorbed material from the columns.
6. Solvent flushing can also be used to regenerate column selectivity.
7. Mobile phases can be chosen that are least likely to cause racemization of samples.
8. Immobilized columns can be used over a higher temperature range.

Macrocyclic Antibody

- Type II
- More universal than other CSPs
- Most recently developed CSP
- Up to 20 Chiral Centers on CSP



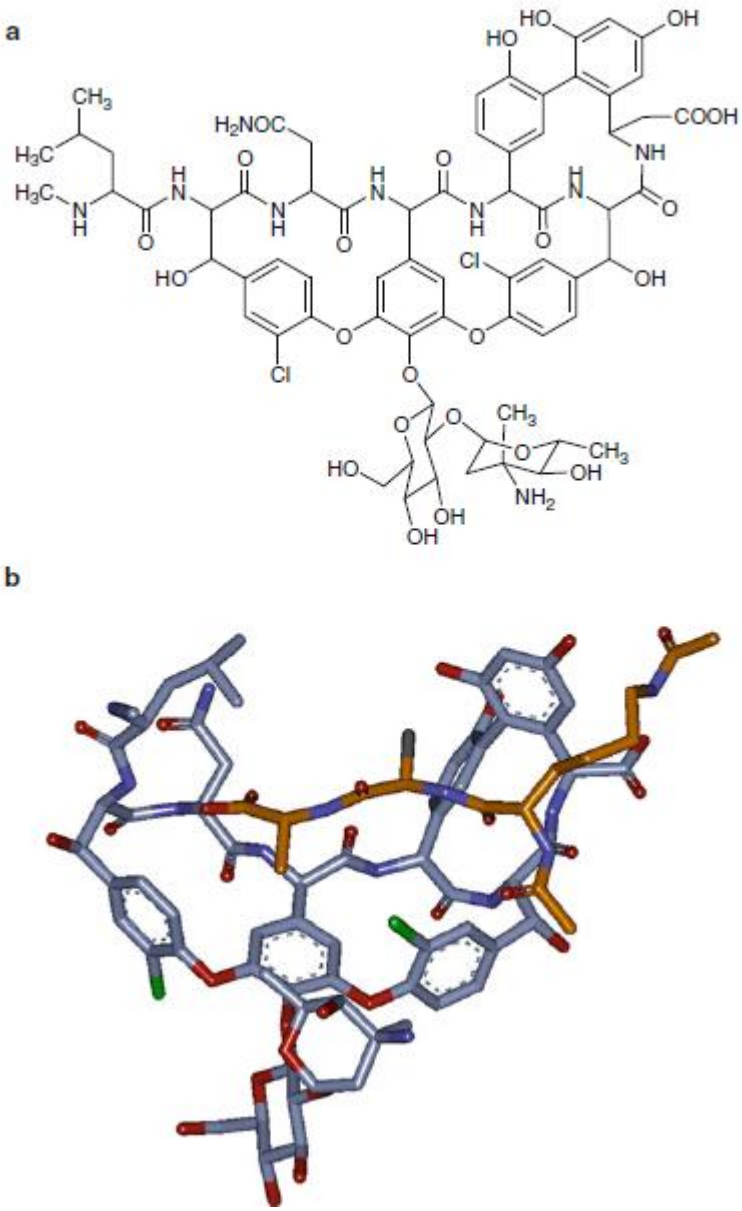
Chirobiotic Phases

- Macrocyclic glycopeptides linked to silica
- Contain a large number of chiral centers together with cavities for analytes to enter and interact
- Potential interactions:
  - π-π complexes, H-bonding, ionic interactions
  - Inclusion complexation, steric interactions
- Capable of running in RP-HPLC, normal phase, polar organic, and polar ionic modes

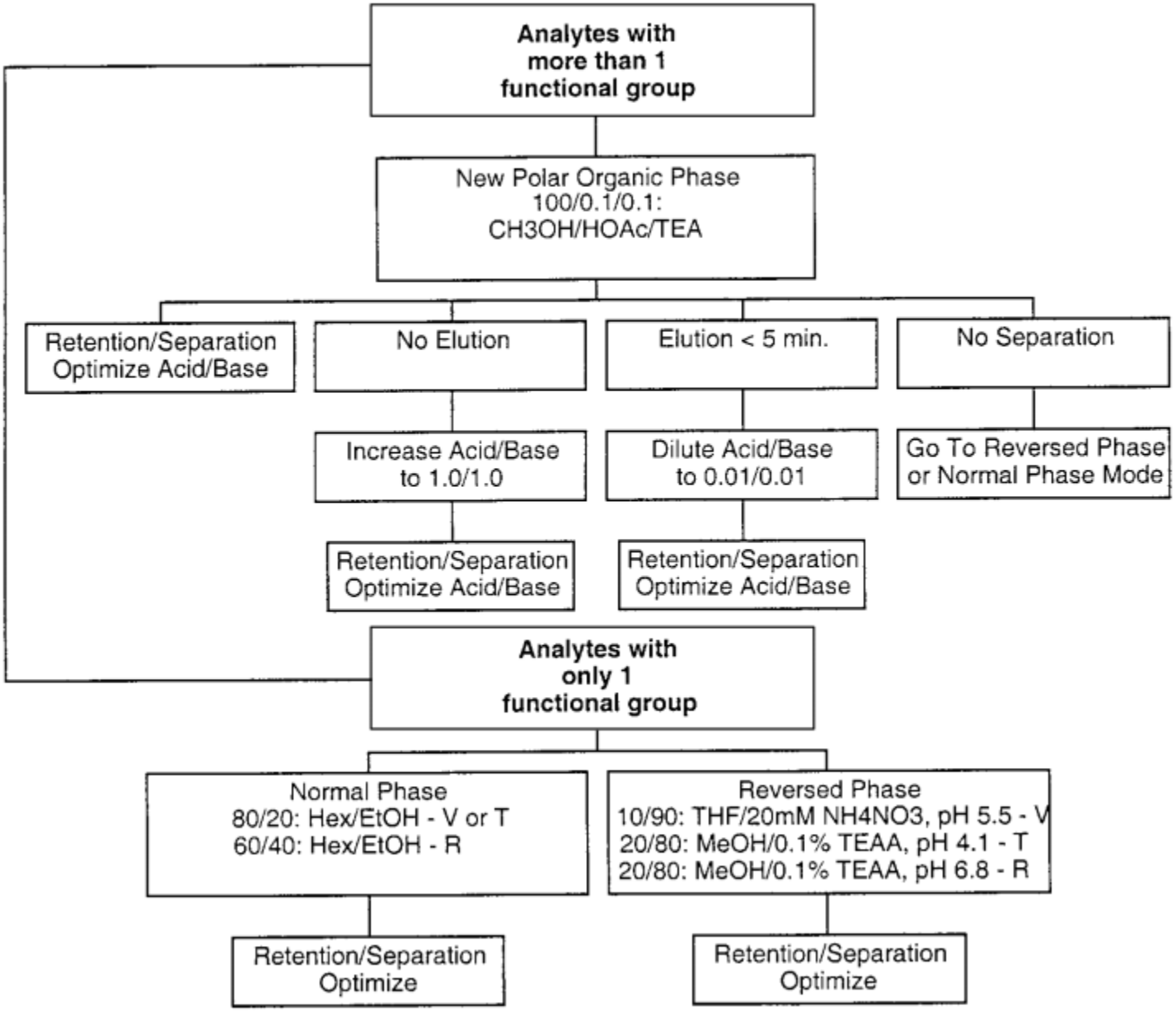
- Available columns:
  - Chirobiotic V and V2 (Vancomycin), Chirobiotic T and T2 (Teicoplanin), Chirobiotic R (Ristocetin A) from Astec

http://www.raell.demon.co.uk/chem/CHIbook/chiral.htm#Macrocyclic

(**a**) Structure of vancomycin and (**b**) X-ray crystal structure of the complex with *N* a , *N* w -diacetyl- L -Lys- D -Ala- D -Ala (in orange). The X-ray crystal structure image was generated with Accelrys Discovery Studio Visualizer 2.5 software from the coordinates from the Brookhaven Protein Data Bank (www.rcbs.org/pdb , fi le 1FVM).

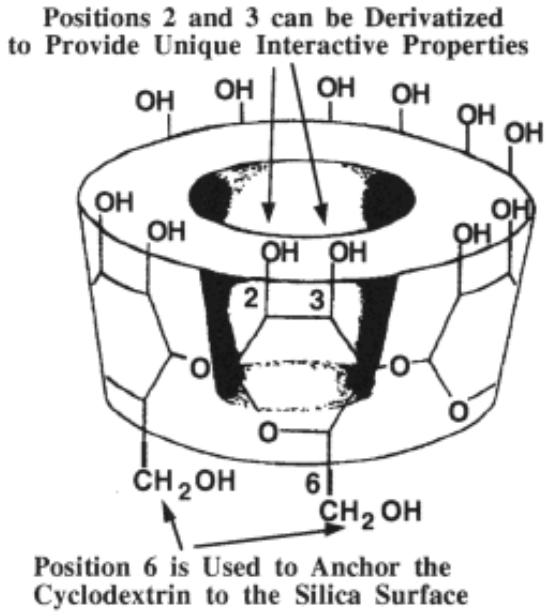


Method development protocols on 250 × 4.6 mm glycopeptide columns. V: vancomycin, T: teicoplanin, R: ristocetin A.



Cyclodextrin, Crown Ether, Polymer

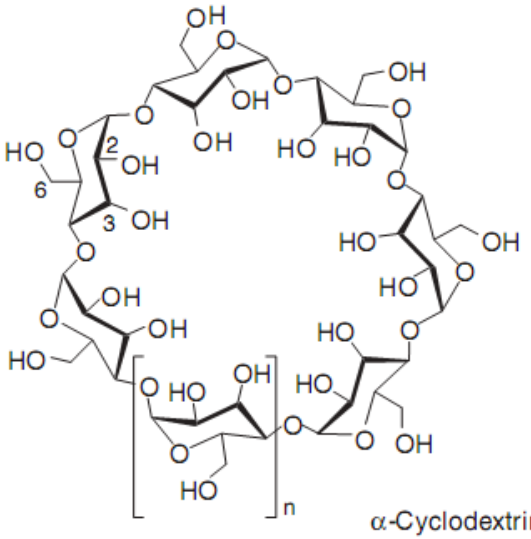
- Type III
- Compounds will selectively align in cavity
- Very selective to differently sized analytes

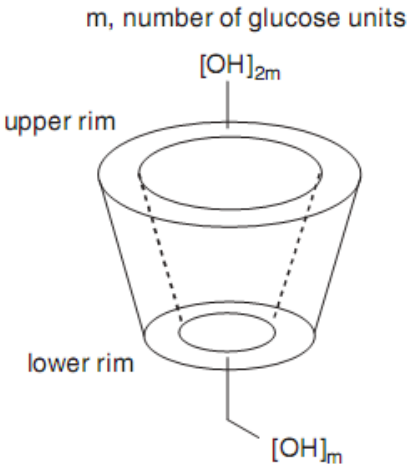


One or two of the primary hydroxyl groups (position 6) are used to link the cyclodextrin to the silica surface. The secondary hydroxyl groups (positions 2 and 3) can be derivatized selectively, usually first in position 2 and then subsequently in position 3. A number of different derivatives of the cyclodextrins have been synthesized to provide specific types of interaction to increase their chiral selectivity.

Structure of cyclodextrins and trade names of corresponding CSPs.

X-ray data has indicated that the b and g structures are quite rigid whereas the a structure appears to exhibit some flexibility. Thus solute molecules, if spatially suitable, can be included and interact by dispersive, polar of ionic forces with any neighboring groups to which they are approriately close.

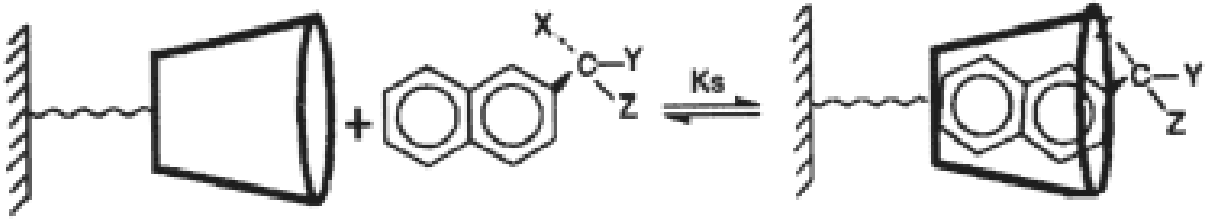




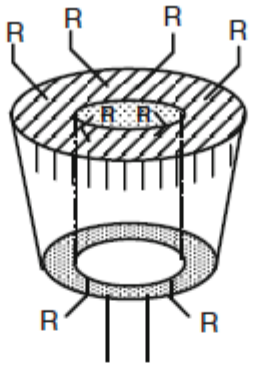
α-Cyclodextrin	n = 0, m = 6
β-Cyclodextrin	n = 1, m = 7
γ-Cyclodextrin	n = 2, m = 8

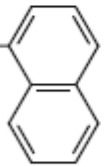
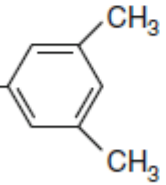
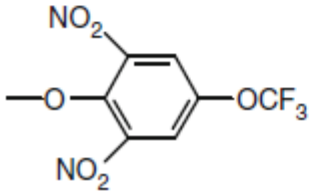
Commercial columns:

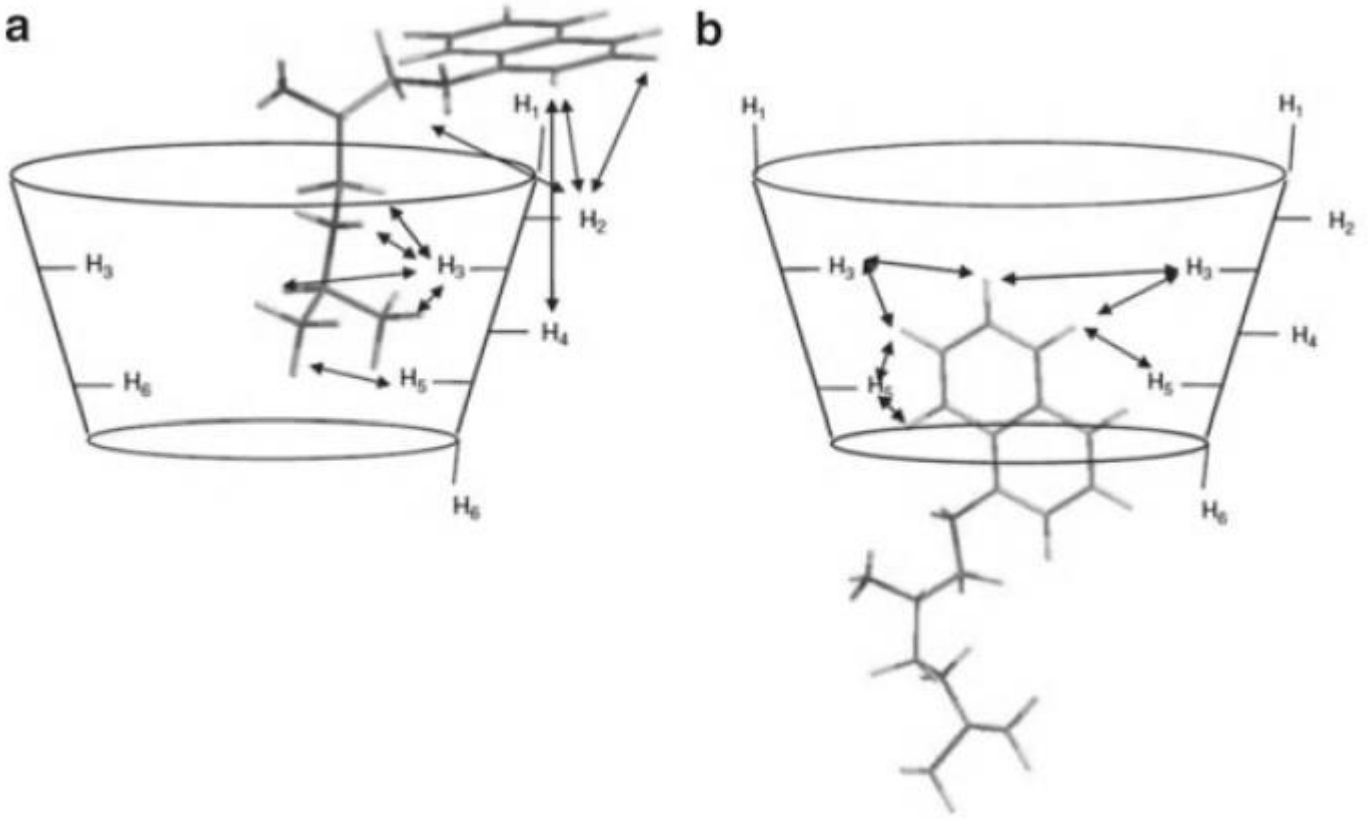
Cyclobond I (native β-CD), *II* (native γ-CD), and *III* (native α-CD) (from ASTEC)
Cyclobond I SP or *RSP* [(*S*)- or (*RS*)-2-hydroxypropylether-β-CD] (ASTEC)
Cyclobond I RN or *SN* [(*R*)-or (*S*)-1-(1-naphthyl)ethylcarbamate-β-CD] (ASTEC)
ChiraDex (native β-CD) and *ChiraDex Gamma* (native γ-CD) (from Merck)
Ultron ES-CD (native β-CD) and *Ultron ES-PhCD* (phenylcarbamoylated β-CD) (from Shinwa)



Summary of derivatives of Cyclobond



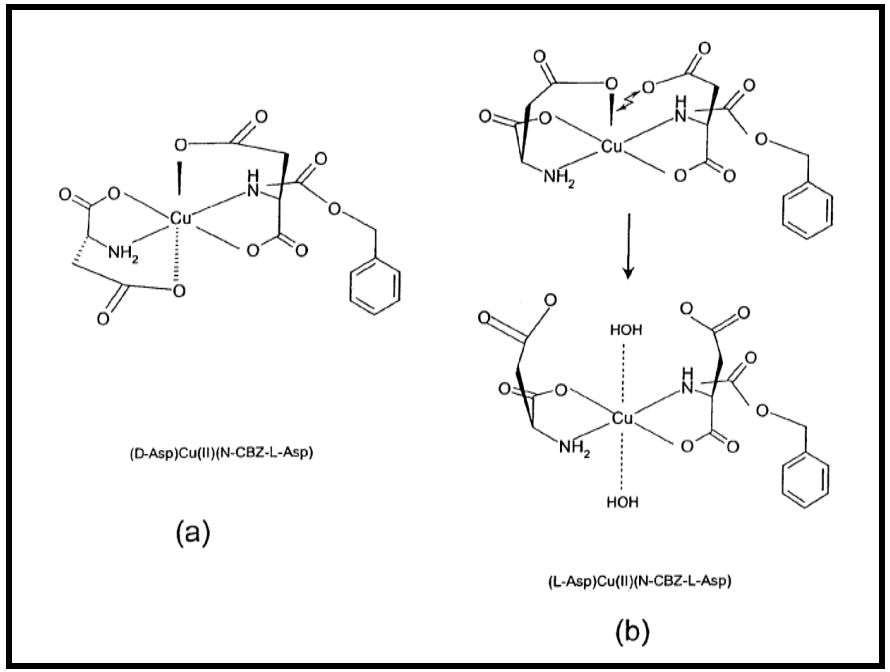
R =	CYCLOBOND I *2000* SUFFIX	CB-I
—CH_3	DM (2,3-di-O-methyl)	CB-DM
—$COCH_3$	AC (acetylated)	CB-AC
—CH_2*$CHCH_3$ (OH)	SP or RSP/HP-RSP (S or racemic RS hydroxypropyl ether)	CB-RSP CB-HP-RSP
—CONHCH*(CH_3) 	RN or SN (R or S naphthylethyl carbamate)	CB-RN CB-SN
—CONH  (CH_3, CH_3)	DMP (3,5-dimethylphenyl carbamate)	CB-DMP
	DNP 2,6-dinitro-4-trifluoro methylphenylether	CB-DNP



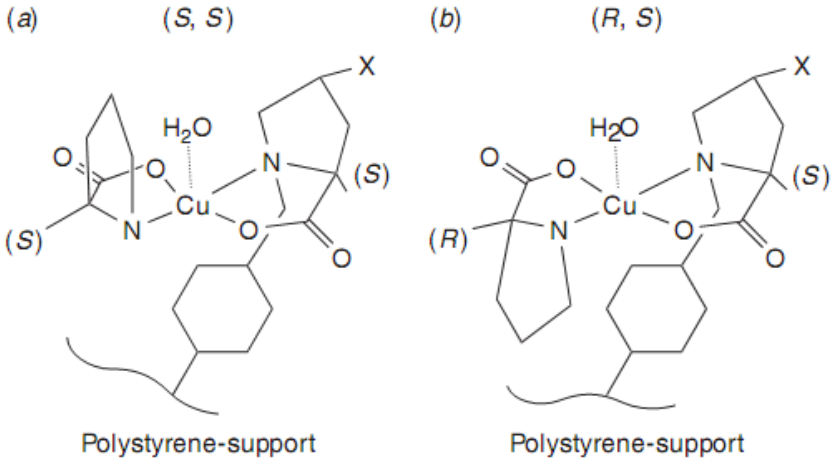
Structures of the complexes of (*R*)-propranolol with (**a**) heptakis (2,3-di-O-acetyl-6-O-sulfo)-b -CD in nonaqueous background electrolyte and (**b**) heptakis (2,3-di-O-methyl-6-O-sulfo)- b -CD in aqueous background electrolyte as derived from ROESY NMR experiments. The *arrows* indicate the observed intermolecular NOE upon irradiation of the respective protons

Ion and Ligand Exchange

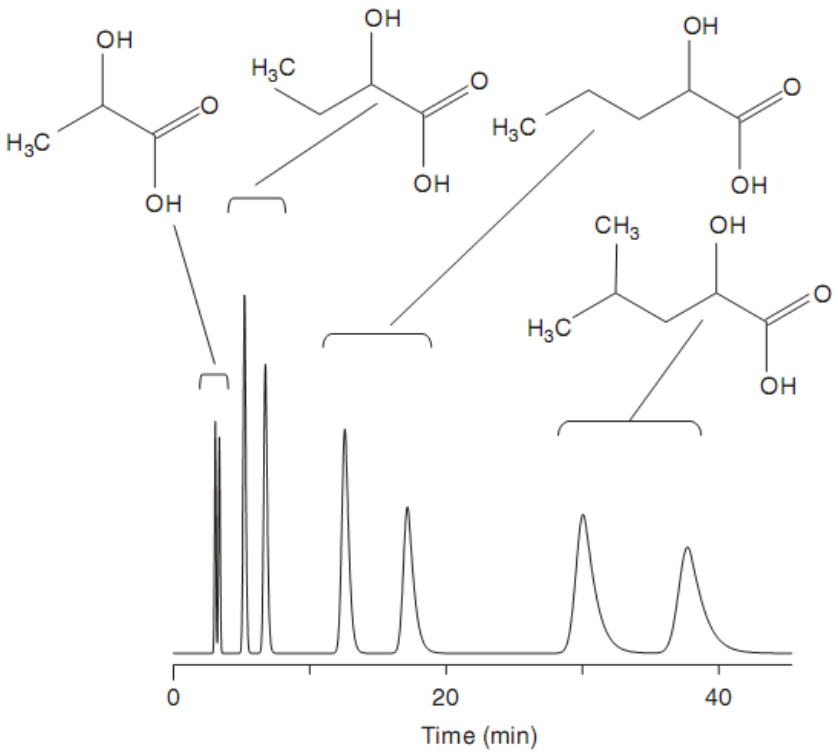
- Type IV
- Mainly used for amino acid separations
- Usually uses copper complexes
- Mobile phase must include metal



Basic principle of ligand exchange ***(3)*** is the involvement of a complexing metal ion into interaction between the analyte enantiomers to be resolved and the chiral selector, namely, through the formation of diastereomeric ternary complexes selector/metal ion/analyte. It is essential that the complexes be kinetically labile, i.e., they must form and dissociate at a high rate; otherwise the chromatographic column efficiency would be compromised. Complexes of Cu(II), Zn(II), Ni(II), and few other ions meet this condition while coordinating amino, carboxy, hydroxy, amido, thio, and few other electron donating functional groups. Here with, the lone electron pairs of the hetero atoms (N, O, S) of the functional groups, belonging to the analyte and selector, occupy definite positions in the coordination sphere of the central metal ion, to result in the formation of the ternary complex. During the chromatography process, the coordinated ligands are reversibly replaced by other ligands, such as molecules of water, ammonia, or other components of the eluent. Quick exchange of ligands in the metal ion coordination sphere dictates the name of the technique—ligand-exchange chromatography (LEC).



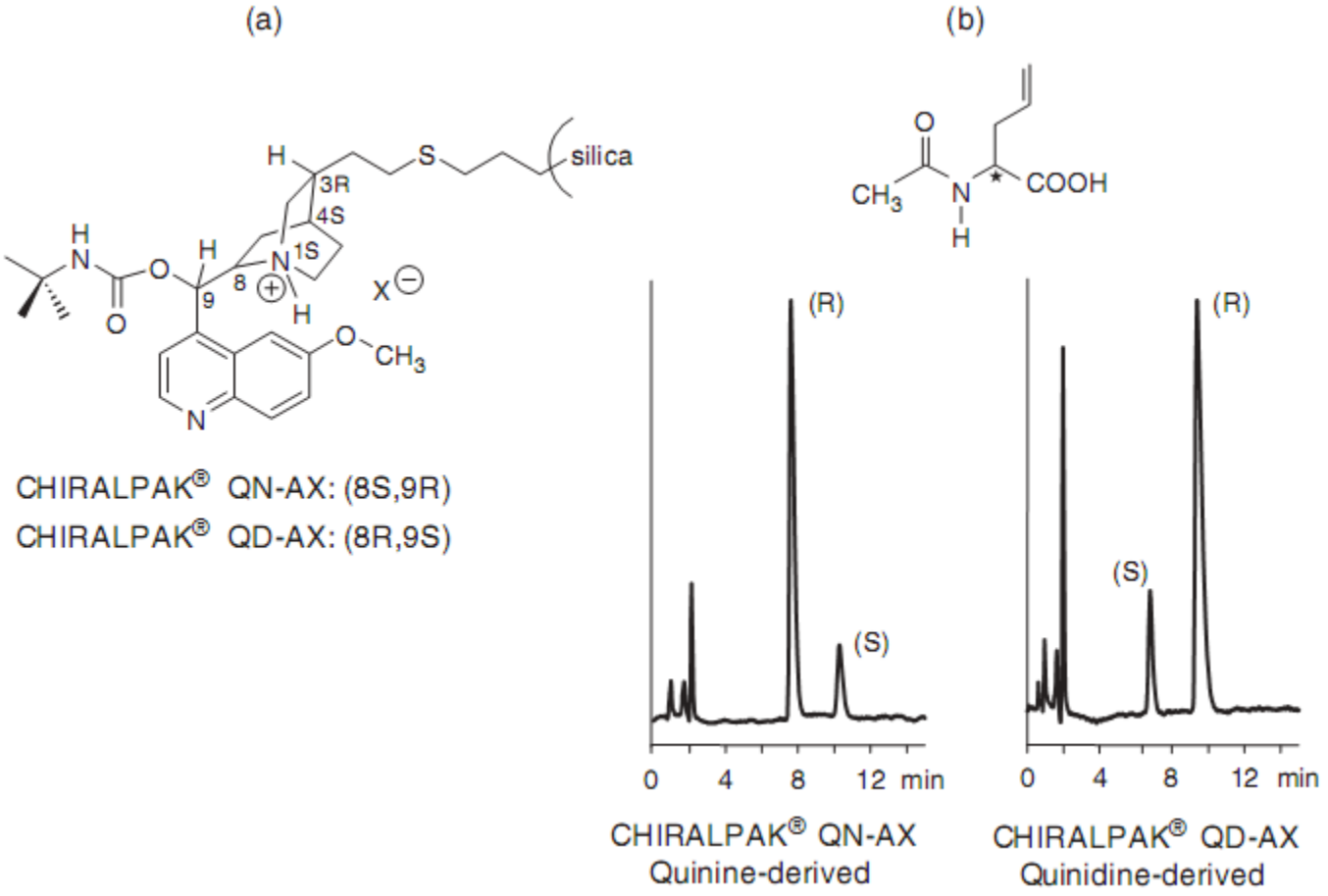
Principle of chiral ligand-exchange chromatography. Ternary diastereomeric Cu(II)-complexes of immobilized S-enantiomer of proline (X = H) (or hydroxyproline X = OH) ligand with S-and R-proline analytes, (a)and(b), respectively.



Enantiomer separation of hydroxy acids by chiral ligand-exchange chromatography (CLEC). Experimental conditions: column, CHIRALPAKMA; mobile phase, 10% ACN/H2Oplus2-mMCuSO4.

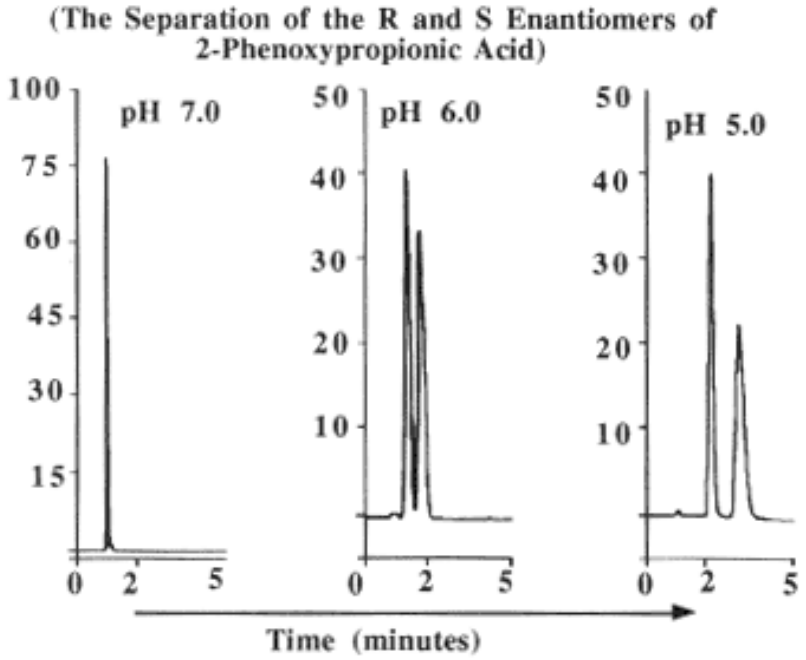
Ion exchange

Commercially available cinchona alkaloid-derived chiral anion-exchangers. (a) Structure; (b) illustration of a reversal of elution order by change from the quinine-derived CSP to the corresponding pseudoquinidine-derived CSP. Experimental conditions: Column dimension, 150 × 4-mm column; mobile phase, 1%acetic acid in methanol; temperature, 25◦C; flow rate, 1 mL/min; UV detection at 230 nm.



Protein Based

- The α1-acid glycoprotein, sold under the name Chiral-AGP
- Sensitive to variations in pH max 4-7
- Changing solvents changes retention non-linearly
- CHIRAL-AGP is commonly used for the separation of enantiomers carrying secondary and tertiary amines and for substances containing nitrogen in a ring.



Physical properties of proteins as chiral selectors.

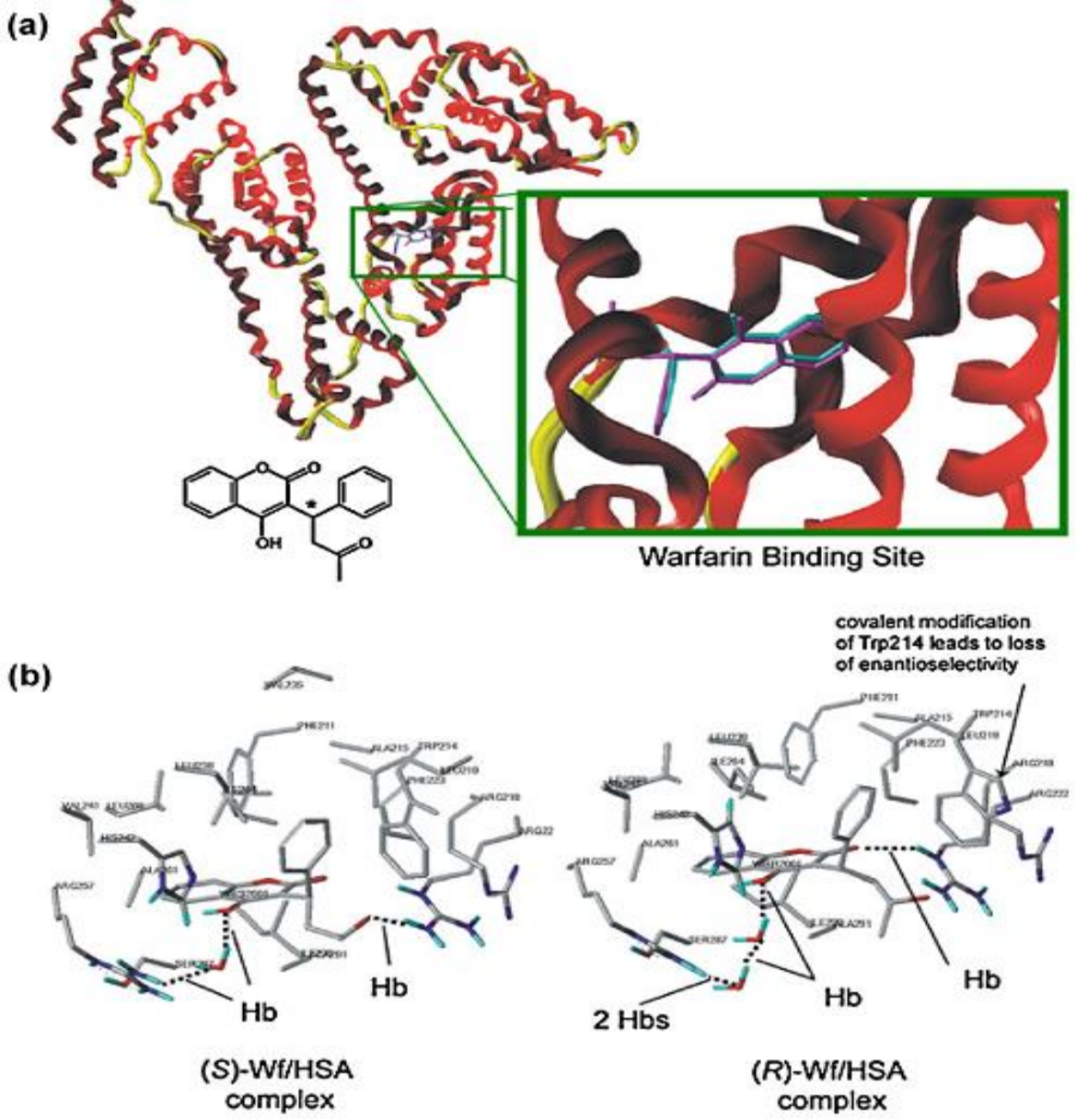
Protein[a]	Molecular mass (kDa)	Isoelectric point	Carbohydrate proportion (%)	Origin
Glycoproteins				
AGP	44	2.7	45	Human or bovine serum
OMCHI	28	4.1	30	Egg white
OGCHI	30	4.1	25	Egg white
AVI	77	10.0	7	Egg white
Streptavidin	66	7.0	–	Bacterium
RfBP	32–36	4.0	14	Egg white
Albumins				
BSA	66	4.7	–	Bovine serum
HSA	65	4.7	–	Human serum
Enzymes				
Fungal cellulase	60–70	3.6	6	Fungus
CBH I	64	3.9	6	Fungus
Lysozyme	14.3	10.5	–	Egg white
Pepsin	34.6	<1	–	Porcine stomach

[a] AGP: α-acid glycoprotein; OMCHI: ovomucoid from chicken egg whites; OGCHI: ovoglycoprotein from chicken egg whites; AVI: avidin; RfBP: riboflavin binding protein; BSA: bovine serum albumin; HSA: human serum albumin; CBH I: cellobiohydrolase I.

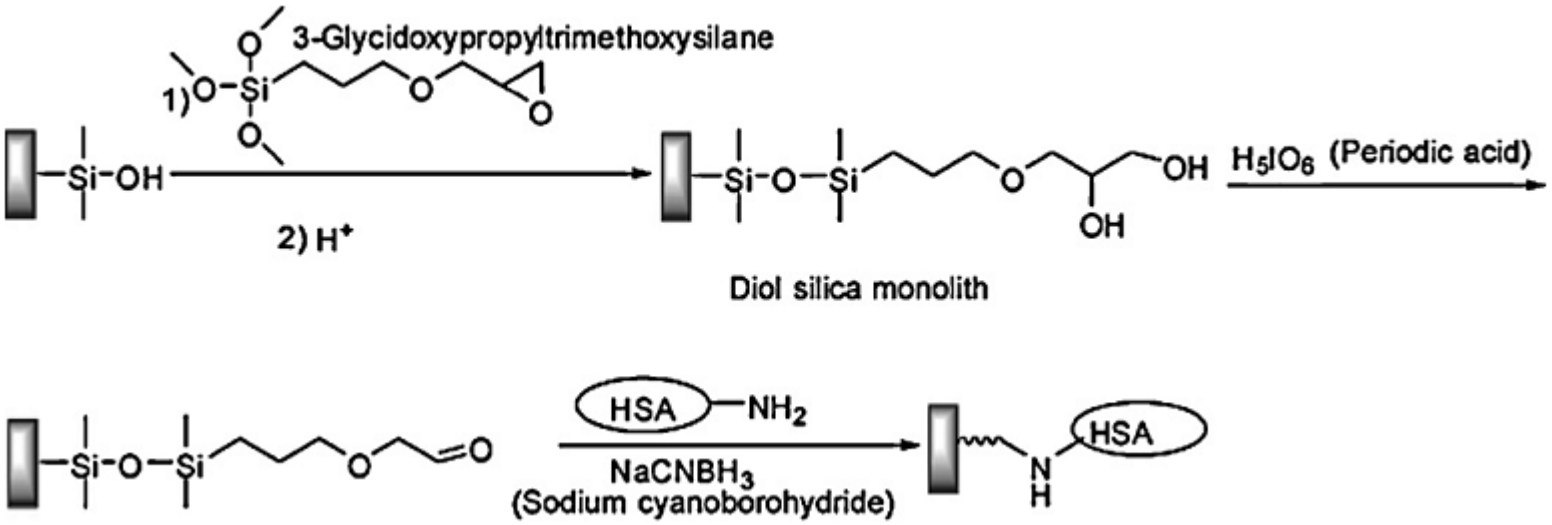
Summarization of monoliths with proteins as chiral selectors.

Monoliths backbone[a]	Types of protein[b]	Analytes	Ways of binding	Reference
Commercial silica monolith	AGP	Warfarin, propranolol	hydrazide immobilization method	[33,34]
GMA/EDMA	HSA	D,L-Amino acids, tryptophan	Epoxy means, Ethylene Diamine means	[86]
TMOS/MTMS	OVM, BSA	Tryptophan, benzoin, eperisone, chlorpheniramine	Encapsulation through sol–gel process	[89–92]
Commercial silica monolith	trypsin	Protein digestion and peptide analysis	Epoxy means	[93]
GMA/EDMA	HSA	Warfarin, tryptophan	Epoxy means	[94]
Commercial silica monolith, GMA/EDMA, silica particles	HSA	Warfarin, tryptophan	Epoxy, mild reduction	[95]
Commercial silica monolith	HSA	Warfarin	sulfhydryl immobilization method	[97]
Commercial silica monolith	trypsin	Protein digestion and peptide analysis	Epoxy means	[99]
Dimethylaminopropylmethacrylate, tetraethylene glycol dimethacrylate	Cytochrome c, lysozyme, myoglobin	–	Imprinting method	[115]
Imprinted polymers by the combined use of chitosan	bovine hemoglobin	–	Imprinting method	[120–122]
Metal surface and polymer	urease and BSA	–	Imprinting and coating	[124–126]
Microbeads	BSA	–	Coating imprinted thin film	[127]
Hybrid silica-based MIP monolith	BSA and lysozyme	–	Imprinting method	[134]
–	Canine serum albumin, BSA	Tryptophan derivatives	Partial filling	[138]
–	Penicillin G acylase	Ketoprofen	Partial filling	[139]
–	HSA	Bupivacaine	Partial filling	[140]
–	HSA	Promethazine, trimeprazine	Partial filling	[141]
–	HSA	Five antihistamines	Partial filling	[146]

[a] GMA: glycidyl methacrylate; EDMA: ethylene glycol dimethacrylate; TMOS: tetramethoxysilane; MTMS: methyltrimethoxysilane; MIP: molecularly imprinted polymer.
[b] AGP: α-acid glycoprotein; HSA: human serum albumin; OVM: ovomucoid; BSA: bovine serum albumin.



X-ray crystal structures of HSA–warfarin (Wf) complexes. (a) Superimposed complexes, (R)-Wf (magenta), (S)-Wf (cyan) and (b) active sites with binding modes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)



Reactions for the preparation of an HAS silica monolith

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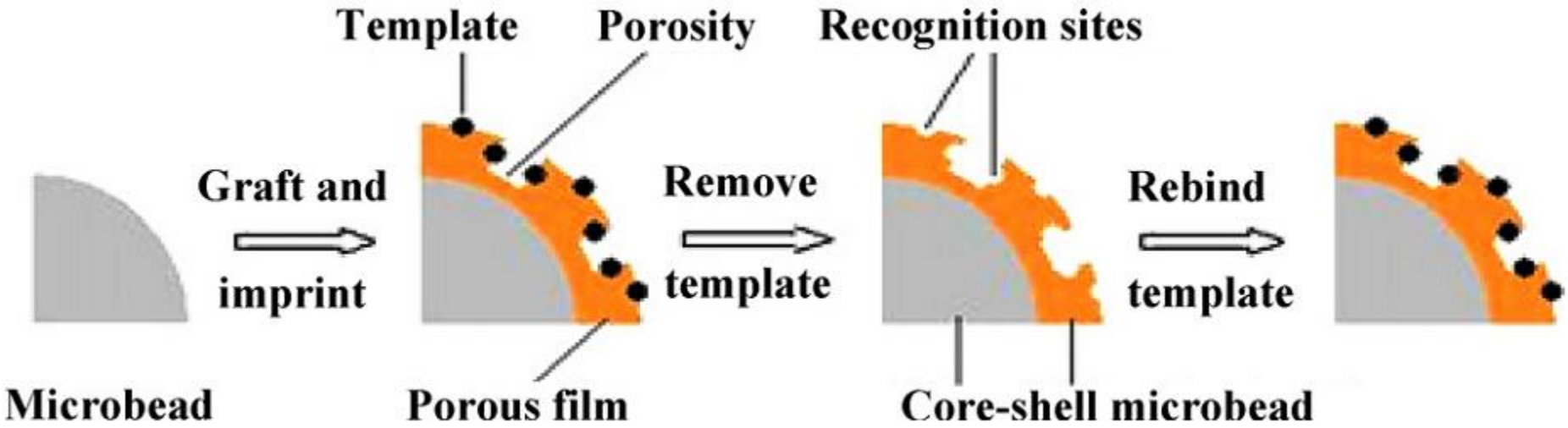

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Important Protein-Type CSPs and Column Trade Names, with Some Characteristics of the Protein Selectors

Protein	Molecular Weight (kDa)	Carbohydrate (%)	Isoelectric Point	Column Trade Name (Supplier)
Serum albumin				
Human (HSA)	67	0	4.7	Chiral HSA (ChromTech)
Bovine (BSA)	68	0	4.7	Resolvosil BSA (Macherey Nagel)
α_1-Acid glycoprotein (AGP)	44	45	2.7	Chiral AGP (ChromTech)
Ovomucoid (OVM)	28	17-34	4.5	Ultron ES-OVM (Shinwa Chemical)
Cellobiohydrolase I (CBH)	60–70	6	3.6	Chiral CBH (ChromTech)
Avidin	66	20.5	9.5–10	Bioptic AV-1 (GL Sciences)
Pepsin	70–78	-	6.1–6.6	Ultron ES-Pepsin (Shinwa Chemical)

Source: Adapted from [83].

Preparative chiral separations.

	Packing name	Chiral selector	Examples of (semi)preparative applications	Supplier or reference of the CSP
	CTA	Crystalline cellulose triacetate	[19,42,61–62]	Daicel, Merck
	TBC	Cellulose tribenzoate beads	[19,42]	[42]
	MMBC	Cellulose tris(3-methylbenzoate) beads	[19,42]	[43]
	PMBC	Cellulose tris(4-methylbenzoate) beads	[19,42]	[43]
Polysaccharides	Chiralcel OD	Cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel	[11,19,67,68]	Daicel, [67,68]
	Chiralcel OC	Cellulose tris(phenylcarbamate) coated on silica gel	[19]	Daicel
	Chiralcel OJ	Cellulose tris(4-methylbenzoate) coated on silica gel	[19]	Daicel
	Chiralcel OB	Cellulose tribenzoate coated on silica gel	[19]	Daicel
	Chiralpak AD	Amylose tris(3,5-dimethylphenylcarbamate) coated on silica gel	[19,69]	Daicel
	Chiralpak AS	Amylose tris[1-(*S*)-phenylethylcarbamate] coated on silica gel	[70]	Daicel
	–	Mixed cellulose 10-undecenoate/tris(3,5-dimethyl-phenylcarbamate) bonded on silica gel	[59,71]	[51]
	–	Mixed amylose 10-undecenoate/tris(3,5-dimethyl-phenylcarbamate) bonded on silica gel	[71]	[56]
Cyclodextrins	Cyclobond I	Cyclodextrin immobilized on silica gel	[19,22,72]	Astec
	Hyd-β-CD	Hydroxypropyl-β-cyclodextrin	[23]	Merck,[23]
Pirkle type	DNBPG-co	3,5-Dinitrobenzoylphenylglycine covalently bonded on silica gel	[19,21,73–78]	Regis
	ChyRoSine-A	3,5-Dinitrobenzoyltyrosine butylamide	[79]	Sedere
	Pirkle-1J	3,5-Dinitrobenzoyl-β-lactam derivative	[80]	Regis
	α-Burke 2	Dimethyl *N*-3,5-dinitrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl phosphonate bonded to silica	[80]	Regis
	ULMO	*N*-dinitrobenzoyl-*N*'-undecenoyl-diphenylethanediamine	[81]	Regis
	–	Cis-3-(1,1-dimethylethyl)-4-phenyl-2-azetidinone	[82]	[82]
		Quaternary ammonium derivative of 3,5-Dinitro-benzoyl-L-leucine on α-zirconium phosphate	[83]	[83]
	–	(*S*)-*N*-undecenoylproline 3,5-dimethoxyanilide bonded on silica gel	[80]	[80]
Polyacrylamides	Poly-PEA	Poly[(*S*)-*N*-acryloylphenylethylamine ethyl ester]	[21,84,85]	[84]
	PolyCHMA	Poly[(S)-*N*-methacryloyl-2-cyclohexylethylamine]	[84]	[84]
	D-ChiraSpher	Poly[(*S*)-*N*-acryloylphenylalanine ethyl ester]	[11,23,86]	Merck
LEC	Polystyrene-Prol	L or D-proline bonded to polystyrene	[87]	[87]
	Chirosolve-pro	L or D-proline bonded to polyacrylamide	[88]	UPS Chimie
	NucleosilChiral-1	L-hydroxyproline Cu^{2+} complexes bonded on silica gel	[33]	Macherey-Nagel

Chiral selector	Trade name	Manufacturer
Ligand-exchange CSPs		
(L)-Proline	Chiralpak WH	Daicel, Japan
	Chiralpak WH	J.T. Baker, USA
(L)-Hydroxyproline	Nucleosil Chiral-1	Macherey-Nagel, Germany
(L)-Hydroxy-proline	Chiral-Si 100 L-HyProCu	Serva, Germany
N,*S*-Dioctyl-(*D*)-penicillamine	Chirex 3126	Phenomenex, USA
	Sumichiral OA-5000	Sumika, Japan
	Chirex D-penicillamin	Phenomenex, USA
Binuclear Copper (II) of *N*-salicyliden-(*R*)-2-amino-1,1-bis(2-butoxy-5-tert-butylphenyl)-3-phenylpropanol	Sumichiral OA-5500	Sumika, Japan
(*R*,*R*)-tartaric acid-(*R*)-1-(α-naphthyl)ethylamide	Sumichiral OA-6000	Sumika, Japan
(*R*,*R*)-tartaric acid-(*S*)-valine-(*S*)-1-(α-naphthyl) ethylamide	Sumichiral OA-6100	Sumika, Japan
(D)-Pipecolinic	Chirosolve D-Pipec.	JPS Chimie, Switzerland
N,*N*-Dioctyl-(L)-alanine	MCI CRS10 W	Mitsubishi Kasei, Japan
N,*N*-Dioctyl-(D)-alanine	MCI CRS10WD	Mitsubishi Kasei, Japan
	Chiralpak MA(+)	Daicel, Japan
Cyclodextrin CSPs		
β-CD	Cyclobond I	Astec, USA
	Cyclobond I	Serva, Germany
	Cyclobond I	Technicol, UK
	Cyclobond I	Rainin, USA
	Cyclobond I	ICT, Germany
	Cyclobond I 2000	Astec, USA
	Cyclobond I 2000	Technicol, UK
	ChiraDex	Astec, USA
	Ultron ES-CD	Shinwakako, Japan
γ-CD	Cyclobond II	Astec, USA
α-CD	Cyclobond III	Astec, USA
(*R*)-naphthylethylisocyanate derivatized β-CD	Cyclobond I 2000 RN	Astec, USA
Acetylated β-CD	Cyclobond I Ac	Astec, USA
	Cyclobond I 2000 Ac	Astec, USA
	Cyclobond I 2000 Ac	Serva, Germany
β-CD (*S*) and (R,*S*)-hydroxypropylderivative	Cyclobond I RSP	Astec, USA
	Beta-RSP-2000	Astec, USA
	Cyclobond I RSP	Rainin, USA
β-CD-(*S*)-naphthylethyl carbamate derivative	Cyclobond I SN	Astec, USA
	Cyclobond I 2000 SN	Astec, USA
β-CD-(*S*)-hydroxypropylether derivative	Cyclobond I SP	Astec, USA
	Cyclobond I SP	Rainin, USA
Carboxymethylated β-CD	OR-Pak CDBS-453	Showa Denko, Japan
β-CD phenylcarbamate	Ultron ES-PhCD	Shinwakako, Japan
Perphenylcarbamate-heptakis(6-azido-6-deoxy) β-CD	CHIDEX-MKP	Chiral Sciences &Technologies Pte. Ltd, Singapore
Perphenylcarbamate-β-CD	CHIDEX-SKP	Chiral Sciences &Technologies Pte. Ltd, Singapore
Permethylated-β-CD	Nucleodex β-PM	Macherey Nagel, Germany
Permethylated-γ-CD	Nucleodexγ-PM	Macherey Nagel, Germany
Pirkle-type CSPs		
(*R*)-*N*-(3,5-Dinitrobenzoyl)phenylglycine	(R)-DNBPG covalent	Regis, USA
	Pirkle 1A covalent	Regis, USA
	Sumichiral OA 2000	Sumika, Japan
	Pirkle 1-A	Technicol, UK
	Chirex 3001	Phenomenex, USA
	Hi-Chrom Pirkle	HiChrom Ltd, UK

Chiral selector	Trade name	Manufacturer
(*S*)-*N*-(3,5-Dinitrobenzoyl)phenylglycine	DNBPG covalent	Regis, USA
	Chirasep DNBPG	Merck, Germany
(*R*)-*N*-(3,5-Dinitrobenzoyl)phenylglycine	Pirkle 1A ionic	Regis, USA
(*S*)-*N*-(3,5-Dinitroben zoyl)leucine	Pirkle-Leu covalent	Regis, USA
	Rexchrom	
	Pirkle covalent	Regis, USA
	L-leucine	J.T. Baker, USA
	S-DNBL covalent	HiChrom Ltd, UK
	Hi-Chrom Reversible-Leucine covalent	Interchim, France
	L-Leucine covalent	
(*R*)-3,5-Dinitrobenzoyl-leucine	Hi-Chrom Pirkle Covalent Leucine	HiChrom Ltd, UK
(*S*)-*N*-(3,5-Dinitroben-zoyl)leucine	S-DNBL ionic	Regis, USA
(*S*)-*N*-(3,5-DNB)-phenylalanine	Chirachrom A1	Interchim, France
(*S*)-dinitrobenzoyltyrosineamide	ChyRoSine-A	Sedere, France
(*R*)-DNB-Aminophosphonate derivative	(R)-alpha-Burke 1	Regis, USA
		Interchim, France
(*R*)-DNB-Aminophosphonate derivative	(R)-alpha-Burke 2	Regis, USA
(*S*)-DNB-Aminophosphonate derivative	(S)-alpha-Burke 2	Regis, USA
	(S)-alpha-Burke 2	Phenomenex, USA
Mono-3,5-dinitrobenzoyl-(R,R)-diphenylethanediamine	(R,R) ULMO	Regis, USA
Mono-3,5-dinitrobenzoyl-(*S,S*)-diphenylethanediamine	(S,S) ULMO	Regis, USA
3-(3',5'-Dinitrobenzoyl)amino-(*R*)-3-phenyl-(*R*)-2-tBu-propanoic acid undecyl ester	(R,R)-beta-GEM 1	Regis, USA
(3R,4*S*)-4-(3,5-Dinitrobenzamido)-3-[3-(dimethylsilyloxy)propyl]-1,2,3,4-tert-rahydrophenanthrene	(S,S) Whelk-O1	Regis, USA
(3R,4S)-4-(3,5-Dinitrobenzamido)-3-[3-(trioxysilyl)-propyl]-1,2,3,4-tetrahydro-phenanthrene	(R,R) Whelk-O2	Regis, USA
(*R*)-α-(1-Naphthyl)ethylamine urea	KK-Carnu	YMC, USA
(*S*)-Naphthylethylurea	SNU5 (S)- Naphthylurea	Supelco, USA
		Shandon, UK
(L)-Tartaric acid-(L)-pheny-lethylamine	Nucleosil chiral 2	Macherey-Nagel, Germany
cis-*N*-3-(3,5-Dinitrobenzoyl)-amino-4-phenyl-β-lactam	Pirkle 1-J	Regis, USA
(R,R)-*N,N'*-3,5-Dinitrobenzoyl-trans-1,2-diaminocyclohexane	(R,R)-DACH-DNB	Regis, USA
N-Dinitrobenzoyl-(*R*)-1-Naphthylglycine	Sumichiral OA-2500	Sumika, Japan
	Chirex 3005	Phenomenex, USA
	Sumichiral OA-2500R	Sumika, Japan
N-Dinitrobenzoyl-(*S*)-1-Naphthylglycine	Sumichiral OA-2500S	Sumika, Japan
(*R*)-*N*-(3,5-Dinitrobenzoyl)-1-naphthylglycine	Sumichiral OA-2500I	Sumika, Japan
3,5-Dinitrophenylaminocarbonyl-(*S*)-valine	Sumichiral OA-3100	Sumika, Japan
	Sumichiral OA-3100	Regis, USA
	Chirex 3010	Phenomenex, USA
	Sumichiral OA-3100	YMC, USA
N-(3,5-Dinitrophenylcarbamoyl)-*S*-tertiobutylglycine	Sumichiral OA-3200	Sumika, Japan
N-(3,5-Dinitrophenylcarbamoyl)-(D)-phenylglycine	Sumichiral OA-3300	Sumika, Japan
N-(*S*)-1-(1-naphthyl)ethy-laminocarbonyl-(L)-valine	Sumichiral OA-4000	Sumika, Japan
N-(*R*)-1-(α-Naphthy-l)ethylaminocarbonyl-(*S*)-valine	Sumichiral OA-4100	Sumika, Japan
	Sumichiral OA-4100	Interchim, France
	Chirex 3014	Phenomenex, USA
(*S*)-Benzoproline-(*S*)-1-naphthylethylurea derivative	Sumichiral OA-4800	Sumika, Japan
(*S*)-Benzoproline-(*R*)-1-naphthylethylurea derivative	Sumichiral OA-4900	Sumika, Japan
	Chirex 3022	Phenomenex,
	Sumichiral OA-4900	USA
		Rainin, USA
(*S*)-Proline-(*S*)-1-naphthylethylurea derivative	Sumichiral OA-4400	Sumika, Japan
	Chirex 3017	Phenomenex,
		USA
(*R*)-1-(1-Naphthyl)ethylaminocarbonyl-(L)-proline	Sumichiral OA-4500	Sumika, Japan
	Chirex 3018	Phenomenex,
		USA
tert-Butylcarbamoyl-quinine	Chiral AX QN-1	Bischoff, Germany

(*continued on next page*)

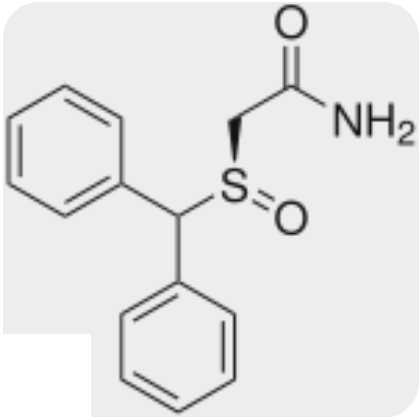
Chiral selector	Trade name	Manufacturer
Synthetic polymers		
(+)-Poly(triphenylmethylmethacrylate)	Chiralpak OT(+)	Daicel, Japan
		J.T. Baker, USA
Poly-[(*S*)-*N*-Acryloylphenylalanine-ethylester]	Chiraspher	Merck, Germany
		Bodman Chemical, USA
		Curtin-Matheson Scientific, USA
O,O'-di(3,5-dimethyl-ben-zoyl)-(2R,3R)-diallyl-tartardiamide	Kromasil CHI-I	EKA Chemical, Sweden
O,O'-di(4-tBu-phenyl)-(2R,3R)-diallyl-tartardiamide	Kromasil CHI-DMB	EKA Chemical, Sweden
	Kromasil CHI-II	EKA Chemical, Sweden
	Kromasil CHI-TBB	EKA Chemical, Sweden
N-(2-acryloylamine-(1R,2R)-cyclohexyl)-acrylamide radical-polymerized with 4,4'-azo-bis-(4-cyano)-valeric acid	(R,R)-P-CAP	Astec, USA
N-(2-acryloylamine-(1*S*,2*S*)-cyclohexyl)-acrylamide radical-polymerized with 4,4'-azo-bis-(4-cyano)-valeric acid	(S,S)-P-CAP	Astec, USA
Polysaccharide CSPs		
Microcrystalline cellulose triacetate	CTA-I	Merck, Germany
	MCTA	Merck, Germany
	CEL-AC-40-XF	Macherey-Nagel, Germany
	Chiralcel CA-1	Daicel, Japan
	CTA-I	Fluka, Switzerland
	Conbrio-TAC	Perstop Biochem, Sweden
Microcrystalline tribenzoylcellulose	Chiral Tribencel	Macherey-Nagel, Germany
Cellulose triacetate	Chiralcel OA	Daicel, Japan
	Optipak-TA	Waters, USA
	Chiralcel OA	J.T. Baker, USA
Cellulose tribenzoate	Chiralcel OB	Daicel, Japan
	Chiralcel OB-H	Daicel, Japan
	Chiralcel OB	J.T. Baker, USA
Cellulose triphenylcarbamate	Chiralcel OC	Daicel, Japan
Cellulose tris(3,5-dimethylphenylcarbamate)	Chiralcel OD	Daicel, Japan
	Chiralcel OD	J.T. Baker, USA
	Chiralcel OD-H	Daicel, Japan
	Chiralcel OD-R	Daicel, Japan
	Chiralcel OD-RH	Daicel, Japan
Cellulose tris(4-Chloro phenylcarbamate)	Chiralcel OF	Daicel, Japan
	Chiralcel OF	J.T. Baker, USA
Cellulose tris(4-Methyl phenylcarbamate)	Chiralcel OG	Daicel, Japan
Cellulose tris(4-methylbenzoate)	Chiralcel OJ	Daicel, Japan
	Chiralcel OJ-R	J.T. Baker, USA
	Chiralcel OJ-RH	Daicel, Japan
	Exp B101	Bio-Rad RSL, Belgium
Cellulose tricinnamate	Chiralcel OK	Daicel, Japan
	Chiralcel OK	J.T. Baker, USA
Amylose tris(3,5-dimethyl phenylcarbamate)	Chiralpak AD	Daicel, Japan
	Chiralpak AD-RH	J.T. Baker, USA
	Chiralpak AD-R	Daicel, Japan
Amylose tris[(*S*)-α-phenethyl]carbamate	Chiralpak AS	Daicel, Japan
	Chiralpak AS	J.T. Baker, USA
	Chiralpak AS-H	Daicel, Japan
Amylose tris(3,5-dimethylphenylcarbamate)	Chiralpak IA	Daicel, Japan
Cellulose tris(3,5-dimethylphenylcarbamate)	Chiralpak IB	Daicel, Japan
Macrocyclic antibiotic CSPs		
Ristocetin A	Chirobiotic R	Astec, USA

(*continued on next page*)

Chiral selector	Trade name	Manufacturer
Amphoteric Teicoplanin	Chirobiotic T	Astec, USA
Vancomycin	Chirobiotic V	Astec, USA
	Chirobiotic V	Technicol, UK
Teicoplanin aglycone	Chirobiotic TAG	Astec, USA
Methylated Teicoplanin aglycone	Chirobiotic MTAG	Astec, USA
Vancomycin aglycone	Chirobiotic VAG	Astec, USA
Crown ether-based CSPs		
(*R*)-18-Crown-6-ether	Crownpak CR(−)	Daicel, Japan
(*S*)-18-Crown-6-ether	Crownpak CR(+)	Daicel, Japan
		J.T. Baker, USA
	Crownpak CR(+)	
(+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid	Opticrown RCA(+)	US Mac Corporation
		USA
(*S*,*S*)-Pseudo-18-crown-6-ether	Sumichiral OA-8000	Sumika, Japan

Drug Name	Indication	Company	Phase	2008 World. Sales	Comments
Armodafinil. Nuvigil®	Narcolepsy, Jet lag disorder, obstructive sleep apnea and shift work sleep disorder	Cephalon	Marketed in 2009	-	Large-scale chiral chromatography has been used to significantly reduce the time to market and produce launch quantities More information
Escitalopram Lexapro®	Depression, generalized anxiety disorder	Lundbeck	Marketed in 2002	$1.24 billion	Currently produced by industrial chiral chromatography
Levitiracetam Keppra®	Epilepsy	UCB	Marketed in 1999	$1.75 billion	Currently produced by large-scale chiral chromatography More information
Radafaxine -	Restless leg syndrome, depression	GlaxoSmithKline	Discontinued	-	Thanks to the use of chiral chromatography for Radafaxine production, GSK was awarded twice for green chemistry More information
AMG221 -	Type 2 diabetes	Amgen	Phase I	-	The shorter reaction sequence and efficiency of the chromatographic separation make this route a more attractive option for long-term applications More information
Pagoclone -	Persistent developmental stuttering	Indevus Pharmaceuticals	Phase III	-	Chiral chromatography appeared to be the most time-efficient and cost-effective method in development More information

Armodafinil



CHIRALITY 20:896–899 (2008)

Use of Large-Scale Chromatography in the Preparation of Armodafinil

WILLY HAUCK,[1] PHILIPPE ADAM,[2] CHRISTELLE BOBIER,[2]* AND NELSON LANDMESSER[3]

[1]*Novasep Inc., Boothwyn, Pennsylvania*
[2]*Novasep SAS, Pompey, France*
[3]*Cephalon Inc., West Chester, Pennsylvania*

ABSTRACT Armodafinil, the (*R*)-enantiomer of modafinil, is a medication used to treat the excessive sleepiness associated with narcolepsy, obstructive sleep apnea/hypopnea syndrome, and shift work sleep disorder. We report here the chemical development of armodafinil and the investigations that led to a commercial route to prepare this pure enantiomer. Three synthetic approaches were used to provide the chiral sulfoxide. Resolution via preferential crystallization was used for phase I clinical trials and was subsequently replaced by chiral chromatography, enabling us to pursue a rapid filing and registration of the API. Finally, the commercial route was developed and employed asymmetric oxidation catalyzed by a titanium(IV) isopropoxide and diethyl tartrate system. The advantages of choosing a chromatographic development pathway to expedite registration while concurrently developing an economical chiral synthesis route is discussed in the context of armodafinil development. *Chirality 20:896–899, 2008.*

- To determine solubility of substrate in common eluent solvents,
- To screen a large number of chiral stationary phases (CSPs) and chiral intermediates
- To optimize the eluent system for use with the selected CSP/intermediate,
- To demonstrate the selected conditions on analytical and pilot scale, and
- To develop a process for large-scale separation.

❖ Based on the analyte solubility (greatest in MeOH 20 g/l at 208C), selectivity and productivity, Chiralpak AD 20 µm (Chiral Technologies) was selected as the CSP for the process. Conditioning of the CSP with isopropanol after column packing and prior to the VARICOL1 separation in methanol

- 0.5 kg (R)-modafinil/kg CSP/day, chromatographic yield was 93% and recovery yield of (R)-modafinil, including subsequent drying process, was 77.6%. The optical purity of the desired enantiomer
- was greater than 99.2% and the chemical purity was 99.7%

❖ CRYSTALLIZATION

theoretical yield for any crystallization process is only 50%.

❖

ASYMMETRIC SYNTHESIS

Solvent	% ee	LCAP Armod.	Yield
Toluene	93	>99	92 %
Ethyl acetate	99.5	>99	75 %
Methylene chloride	98	>98.5	61 %
Acetonitrile	99.3	>98.5	70 %
THF	99.7	>99	50 %
Acetone	99.6	>99	45 %

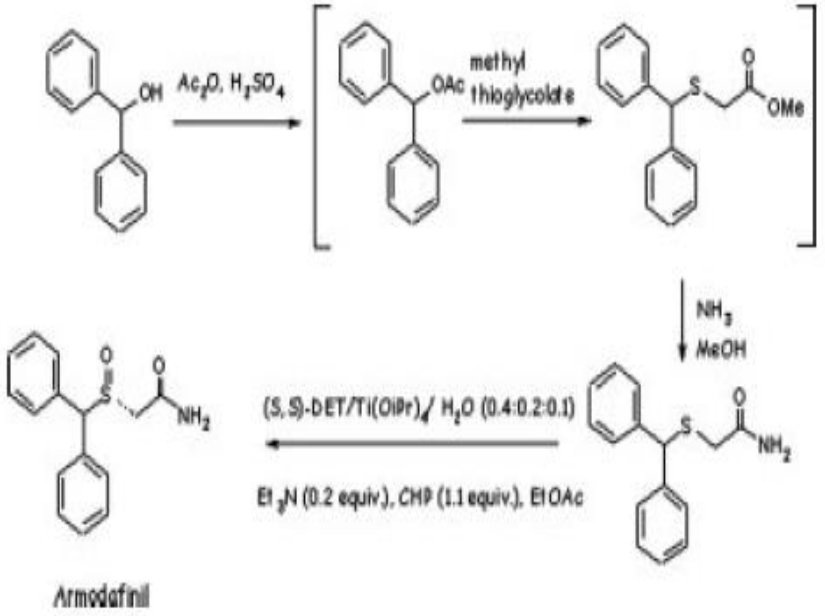
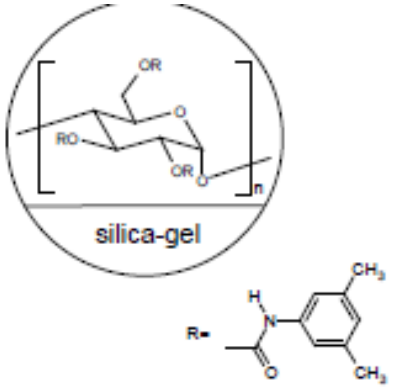


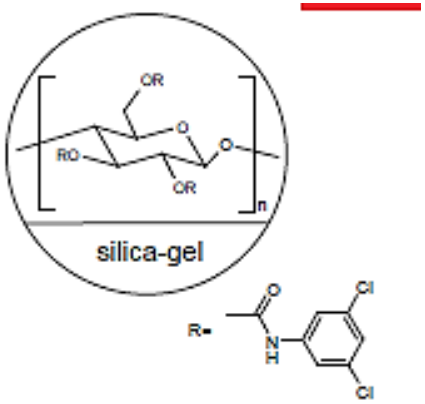
Fig. 4. Asymmetric synthesis of Armodafinil.

- **CHIRALPAK® IA**
- amylose tris (3,5-dimethylphenylcarbamate) immobilised on A 3 µm & 5 µm silica support

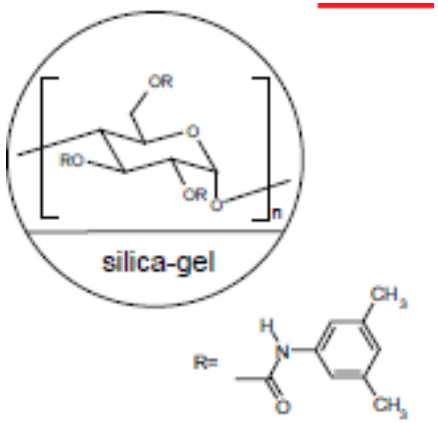


- **CHIRALPAK® IB**
- CELLULOSE tris (3,5-dimethylphenylcarbamate) immobilised on A 3 µm & 5 µm silica support

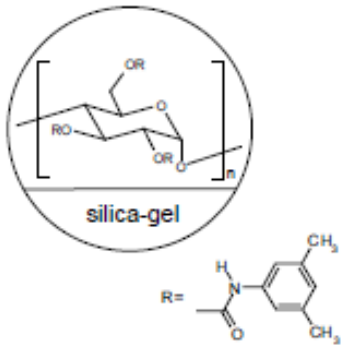
- CHIRALPAK AC
- CELLULOSE tris (3,5-DICHLOROPHENYLCARBAMATI 3 µm & 5 µm silica-gel



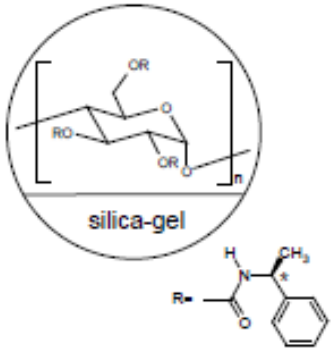
- CHIRALPAK AD & AD-H
- amylose tris (3,5-dimethylphenylcarbamate) on A 3 µm & 5 µm silica support



- COATED CHIRALPAK® AD
- amylose tris (3,5-dimethylphenylcarbamate) on a 10 µm silica support



- COATED CHIRALPAK® AS & AS-H
- amylose tris ((s) - α-methylbenzyLcarbamate) on a 3 silica support



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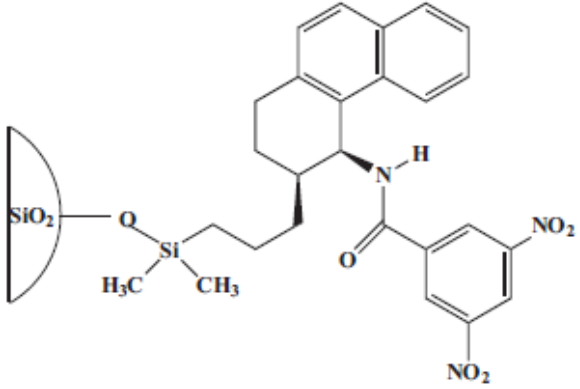


Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica			
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 4.6 mm i.d.	786101
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 10.0 mm i.d.	786102
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 30.0 mm i.d.	786105
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 4.6 mm i.d.	786201
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 10.0 mm i.d.	786202
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 30.0 mm i.d.	786205

Whelk-O® 1 *Analytical to Preparative Columns* The Whelk-O 1 is useful for the separation of underivatized enantiomers in a number of families including amides, epoxides, esters, ureas, carbamates, ethers, aziridines, phosphonates, aldehydes, ketones, carboxylic acids, alcohols and non-steroidal antiinflammatory drugs (NSAIDs). This -electron acceptor/-electron donor phase exhibits an extraordinary degree of generality. The broad versatility observed on the Whelk-O 1 column compares favorably with polysaccharidederived chiral stationary phases. In addition, because Whelk-O 1 is covalently bonded to the support, the phase is compatible with all commonly used mobile phases, including aqueous systems — a distinct advantage over polysaccharide derived chiral stationary phases. Other advantages include column durability, excellent efficiency, ability to invert elution order and excellent preparative capacity

KIRALYA

Preparative chromatography

High performance liquid chromatography

Polymer applications

Synthesis

Extraction and freeze-drying

High performance liquid chromatography purification (HPLC)

HPLC is one of the most efficient tools for separating, purifying and isolating the compounds coming from complex mixtures. After more than 20 years of acquired expertise in this technique, we are able to prepare from hundreds of mg to several dozens of kilos under GMP compliance.

The applications are numerous and varied; the following examples give an idea of what we have already accomplished for our clients:

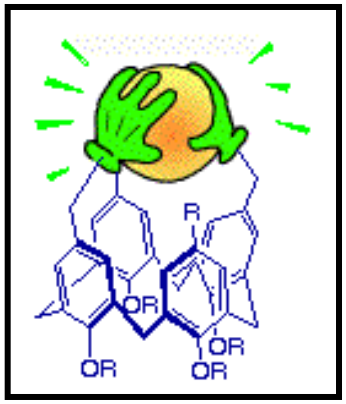
- Impuritiy isolation
- Plant extract purification
- Analytical standarc preparation
- Racemate resolution
- Intermediate and preclinical batch purification
- Clinical batch purif cation
- And so on...

Chiral Stationary Phases for HPLC

Applications

In biology

- Many biologically active molecules are chiral
- Enzymes, which are chiral, contain a glove-like cavity called the active site
- If the glove is “right-handed,” then one enantiomer will fit inside and be bound, the other will have a poor fit and is unlikely to bind



chiral ionic liquids (CILs)

- new chiral solvents should play a central role in enantioselective organic chemistry

- most of ILs possess a polymeric behavior and are highly ordered H-bonded liquids (three-dimensional networks of anions and cations linked together by hydrogen bonds).

- In addition, it was recently shown that hydrogen bonding is involved in controlling the *endo-selectivity of Diels*– Alder reactions. These findings suggest that CILs could be highly more efficient than classical chiral solvents for asymmetric induction.

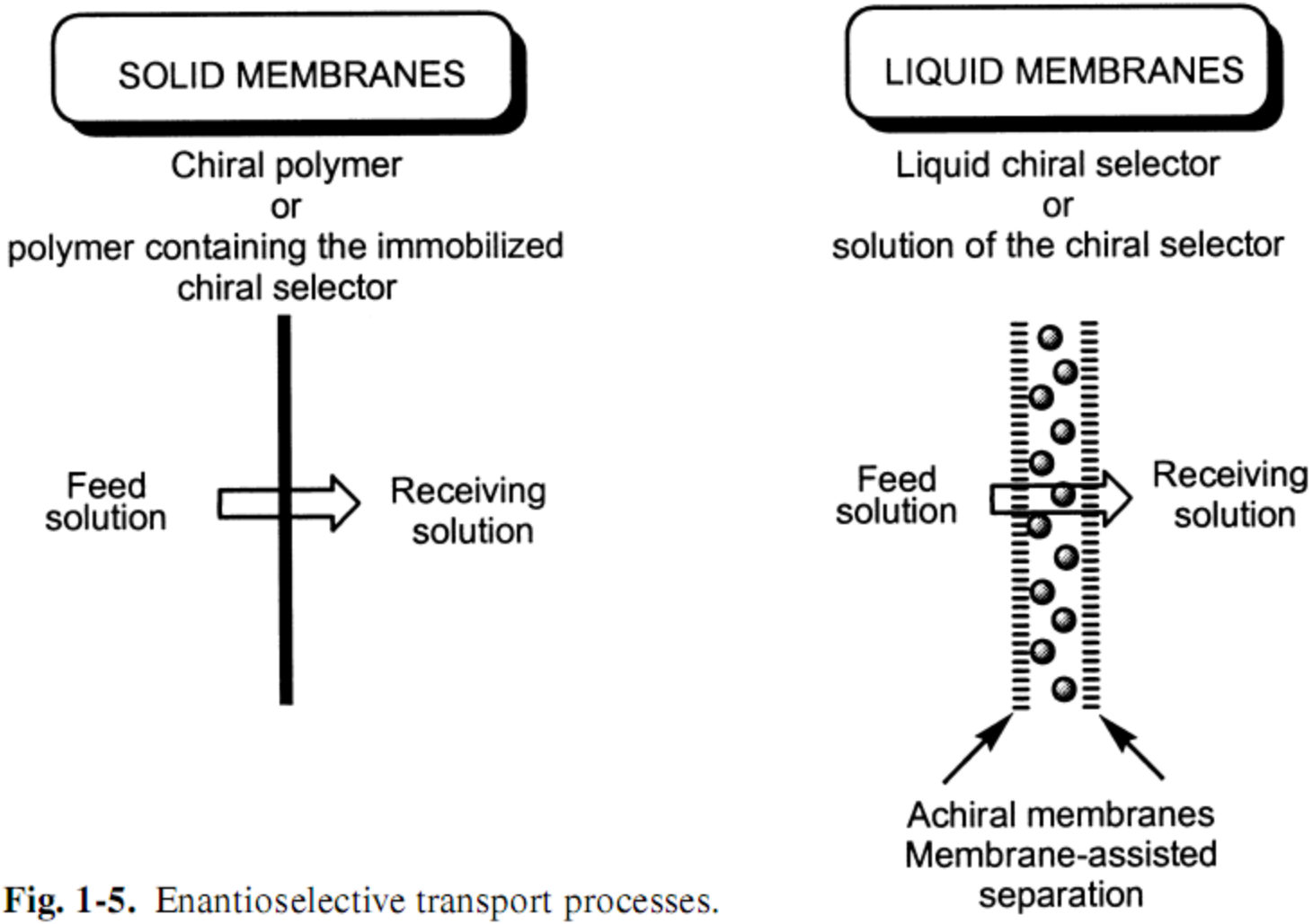


Fig. 1-5. Enantioselective transport processes.