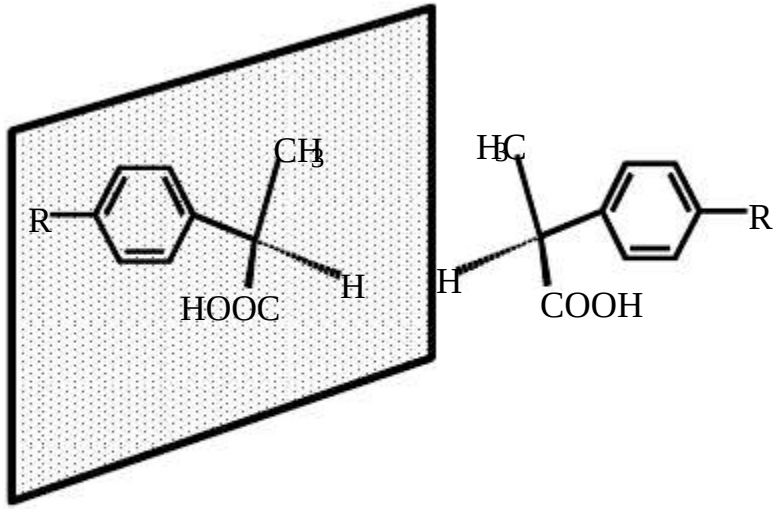
Separation of Optical Isomers (Enantiomers) by Chiral Chromatography

Dr. Shulamit Levin

Introduction: Chirality of Drugs

ENANTIOMERS: MIRROR IMAGES OF ONE ANOTHER



60 seconds on stereochemistry

Asymmetric	**Lacking an alternative axis of symmetry, existing as enantiomers**
Chiral, "handed"	**having the potential to exist as two nonsuperimposable mirror images**
Enantiomers (stereoisomers)	**Two nonsuperimposable compounds, mirror images of one another**
Diastereomers	**Optical isomers that are not mirror images on one another**
Enantioselectivity	**Selective preference of one enantiomer over the other**
Optical activity [(+) or (-)]	**Experimentally observed rotation of the plane of monochromatic plane-polarized light**
R or S	**Absolute configuration about a dissymmetric center**
Racemate	**50:50 mixture of two enantiomers**

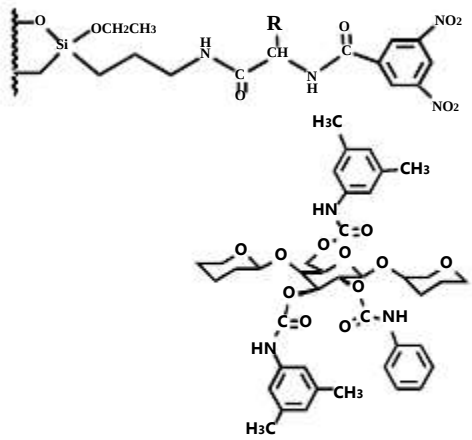
Stereospecificity in drug action

* binding to proteins
* transport through membranes
* receptor recognition
* metabolism
* clearance

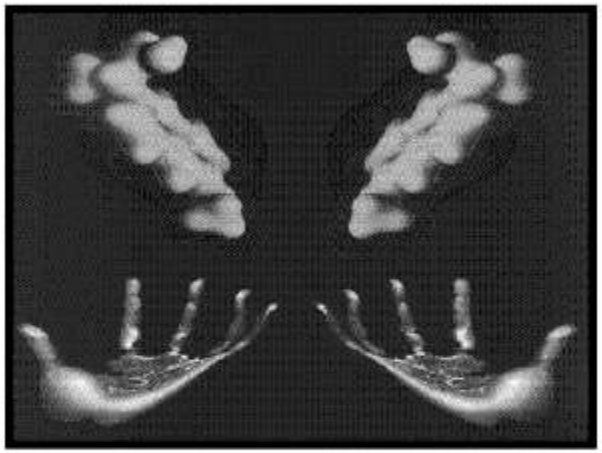
ENANTIOMERS vs RACEMATES

STEREOSELECTIVE PROPERTIES	POSSIBLE GAINS FROM USING ENANTIOMERS
Only one enantiomer is active:	Reduced dose and load on metabolism
The additional enantiomer is toxic:	Freedom in dose and broader use
Different pharmacokinetics:	Better control of kinetics and dose
Different rates and routes of metabolism:	Freedom of dose, reduction of variability of patients' response
One enantiomer is agonist, the other antagonist:	Enhanced activity and reduction of dose
Different pharmacological action and tissue specificity:	Enhanced specificity and reduced side effects; use of the other enantiomer for different medication

Example of Chiral Features



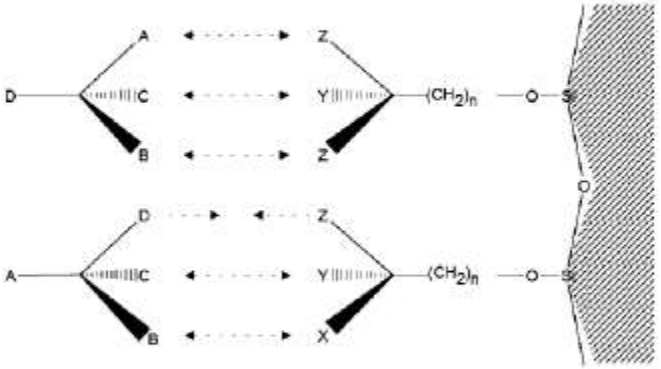
SEPARATION BY CHIRAL RECOGNITION



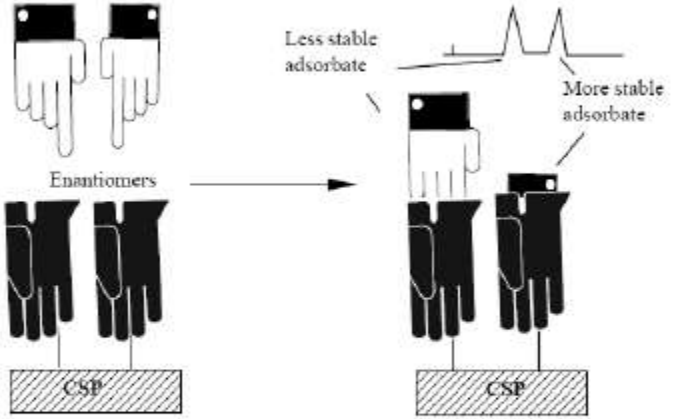
CHIRAL CHROMATOGRAPHY

Chiral Recognition

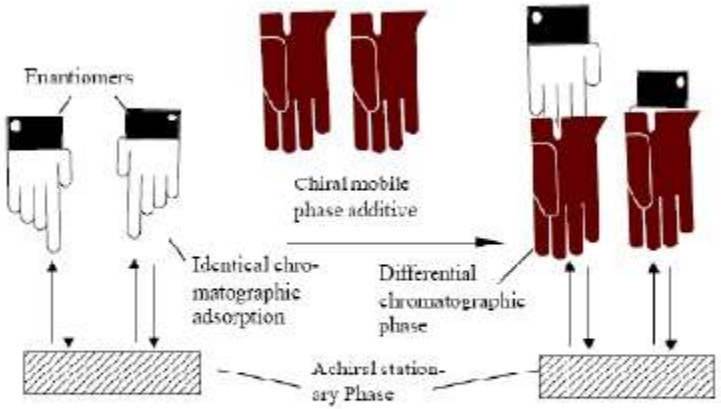
Chiral separations are generally accepted to involve a 3-point interaction with a system component



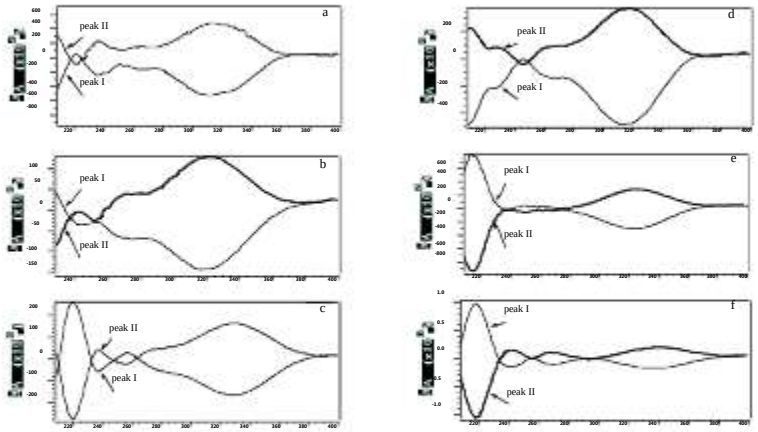
Chiral Recognition :Chiral Stationary Phases



Chiral Recognition :Chiral Mobile Phases



Circular Dichroism: UV SPECTRA of POLARIZED LIGHT



Shulamit Levin,

Chromatographic parameters

RETENTION FACTOR

$$k' = \frac{tR - t_0}{t_0}$$

SELECTIVITY FACTOR

$$\alpha = \frac{k'(2)}{k'(1)}$$

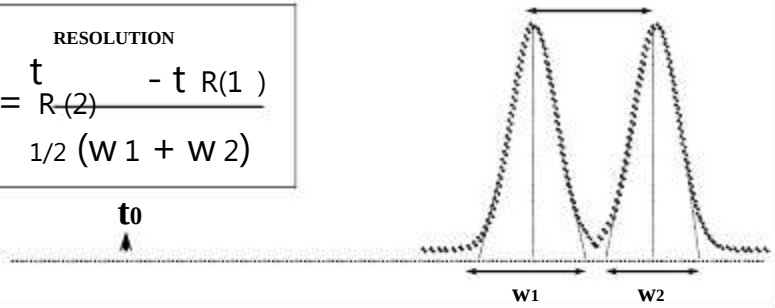
$t_{R(1)}$

$t_{R(2)}$

RESOLUTION

$$Rs = \frac{t_{R(2)} - t_{R(1)}}{1/2\,(W1 + W2)}$$

t_0



Types of Chiral stationary phases:

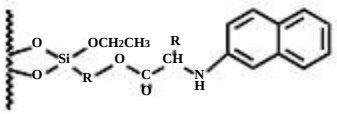
- Ligand exchange
- □-Donnor □-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides
- MacrocyclicAntiobiotics
- Crown Ethers

Types of Chiral stationary phases:

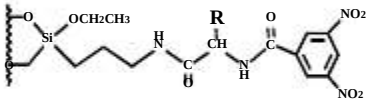
- Ligand exchange
- □-Donnor □-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides
- MacrocyclicAntiobiotics
- Crown Ethers

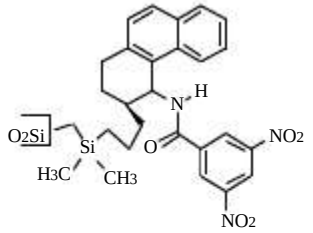
□ - Donor □ - acceptor (Pirkle) Type

Napthyl amino acids



Dinitrobenzoyl amino acids

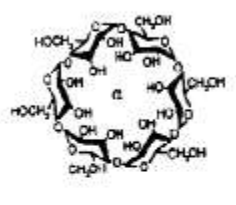


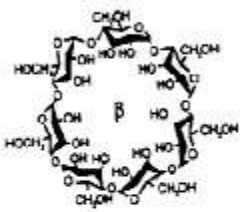


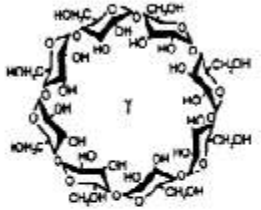
Types of Chiral stationary phases:

- Ligand exchange
  - □-Donnor □-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides
- MacrocyclicAntiobiotics
- Crown Ethers

Chiral cavity by cyclodextrins





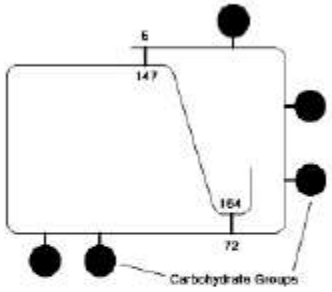


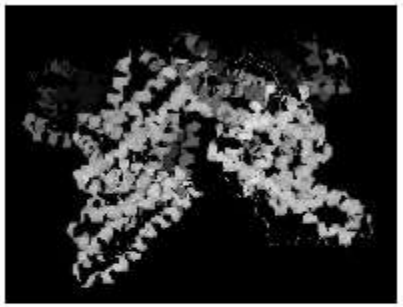
CD	No.Of units	size A	Molecules included	Chiral centres
alpha	6	4.5-6.0	5-6 membered aromatic	30
beta	7	6.0-8.0	biphenyl or naphthalene	35
gamma	8	8.0-10.0	substituted pyrenes and Steroids	40

Types of Chiral stationary phases:

- Ligand exchange
  - □-Donnor □-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides
- MacrocyclicAntiobiotics
- Crown Ethers

Protein Columns

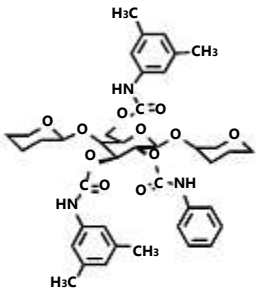




Types of Chiral stationary phases:

- Ligand exchange
  - □-Donnor □-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides
- MacrocyclicAntiobiotics
- Crown Ethers

Immobilized polysaccharides:



tribenzoate
tris phenylcarbamate
triacetate

Amylose
or
Cellulose

Types of Chiral stationary phases:

- Ligand exchange
  - □-Donnor □-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides
- MacrocyclicAntiobiotics
- Crown Ethers

macrocyclic glycopeptide
antibiotic eremomycin chemically bonded to silica

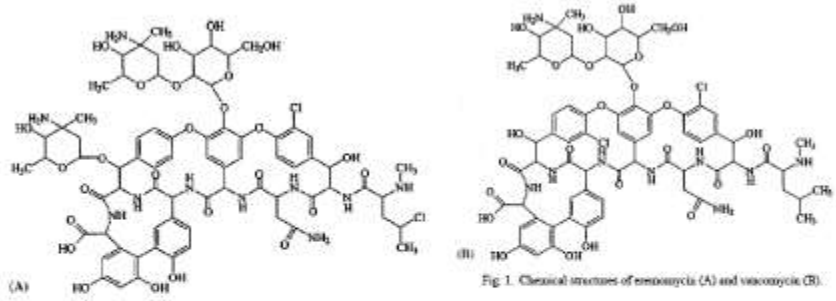
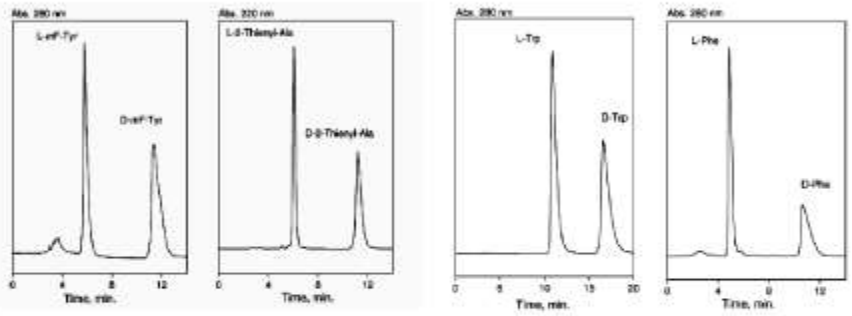


Fig. 1. Chemical structures of eremomycin (A) and vancomycin (B).

Journal of Chromatography A, 1108 (2006) 263–267

Separation of enantiomers of amino acids on eremomycin CSP. Column

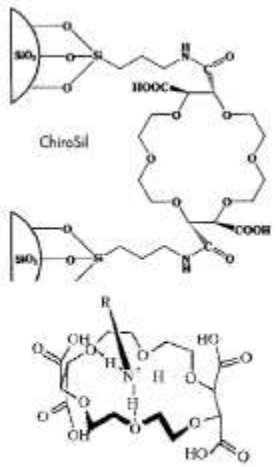


250mm4.0 mm. Eluent: methanol–0.1M NaH2PO4 (20:80, v/v), 0.7 ml/min.

Types of Chiral stationary phases:

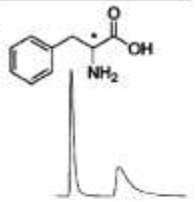
- **Ligand exchange**
- **□-Donnor □-acceptor (Pirkle)**
- **Chiral Host-guest (cyclodextrin)**
- **Immobilized proteins**
- **Immobilized polysaccharides**
- **MacrocyclicAntiobiotics**
- **Crown Ethers**

Crown Ether Type of Chiral Stationary Phase



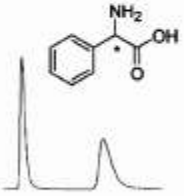
Phenylalanine

Phenylalanine
Column: ChiroSil® RCA(+) or SCA(-) 15 cm x 4.6 mm
Mobile Phase: (70/30) CH_3OH/H_2O +10 mM Acetic acid
Flow Rate: 1.5 mL/min
Detection: UV 210 nm
Run Time: 8.9 min
k'_1: 2.66
α: 2.57



Phenylglycine

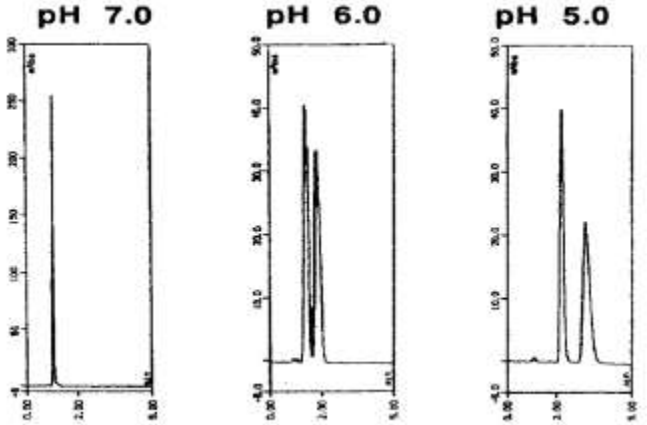
Phenylglycine
Column: ChiroSil® RCA(+) or SCA(-)15 cm x 4.6 mm
Mobile Phase: (70/30) CH_3OH/H_2O +10 mM H_2SO_4 and 0.1% TEA
Flow Rate: 1.0 mL/min
Detection: UV 210 nm
Run Time: 13.1 min
k'_1: 3.14
α: 2.60



Examples for Optimization in Chiral Separations: Aqueous and Non Aqueous

CHIRAL CHROMATOGRAPHY

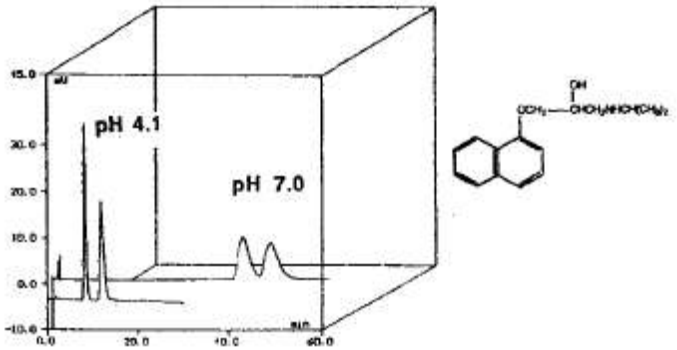
pH Affects Selectivity of Immobilized Protein Columns



* Changing the pH: demonstrated here is the effect of decreasing the pH when chromatographing an acid, 2-phenoxypropionic acid:

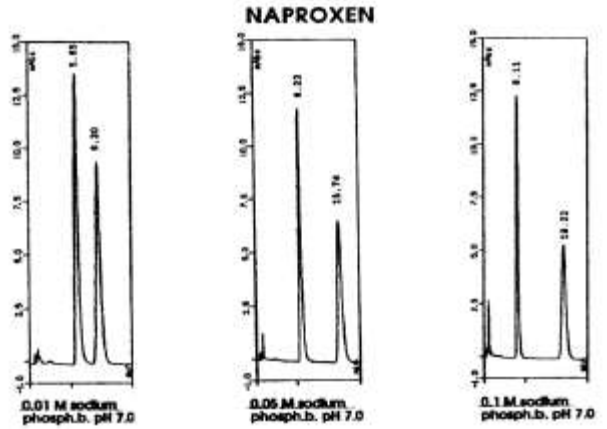
Amines

When chromatographing hydrophobic amines a pH of 4-5 is preferred. In this pH range the protein has a lower negative charge compared to pH 7, which means that the affinity of the amines is decreased, i.e., lower retention. For some compounds even a decrease to pH 6 may give large improvements compared to pH 7.



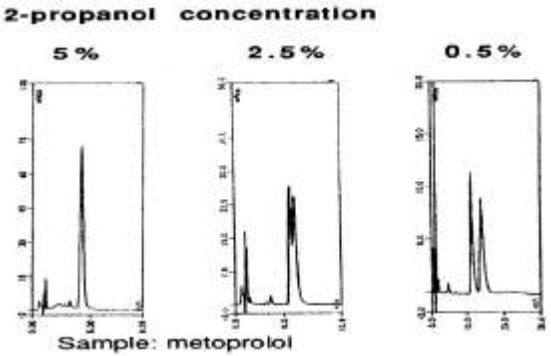
Effect of ionic strength in Immobilized Protein Columns

Changing the buffer concentration: By changing the buffer concentration in the mobile phase, it is possible to affect both the retention (k') and the enantioselectivity (a). These effects have been observed for acids and in special cases also for some amines. The chromatograms below demonstrate the effect of changing the buffer:



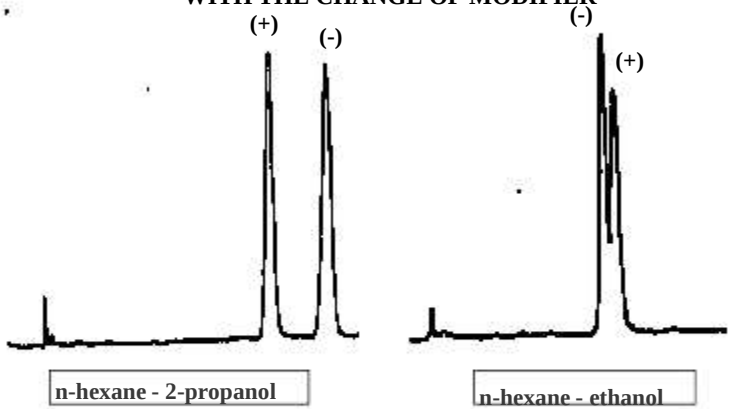
Effect of Modifier in Immobilized Protein Columns

Changing the modifier concentration: On the Chiral-AGP column, the most frequently used organic modifiers are: 2-propanol, acetonitrile, methanol, ethanol, 1-propanol and tetrahydrofuran. Normally increasing modifier concentration will give decreasing retention and decreasing enantioselectivity for all types of compounds. This is illustrated in the chromatogram below:



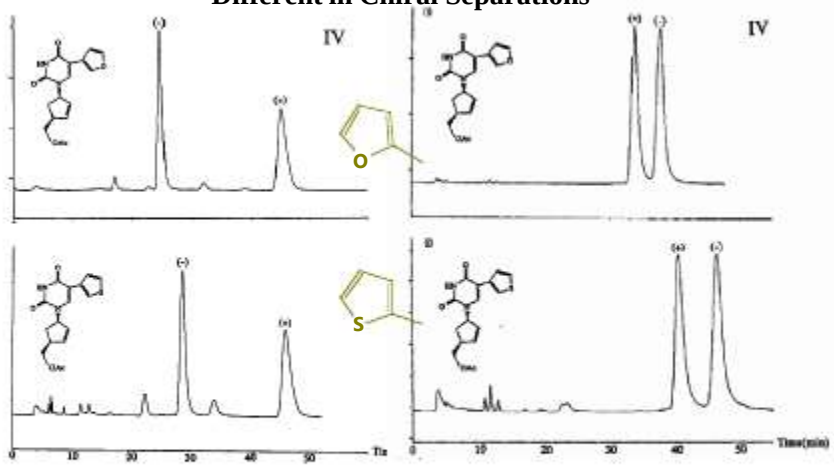
CHIRAL CHROMATOGRAPHY

(+) and (-)- Δ6-THC IN TWO TYPES OF MOBILE PHASES:
REVERSAL OF ELUSION ORDER
WITH THE CHANGE OF MODIFIER



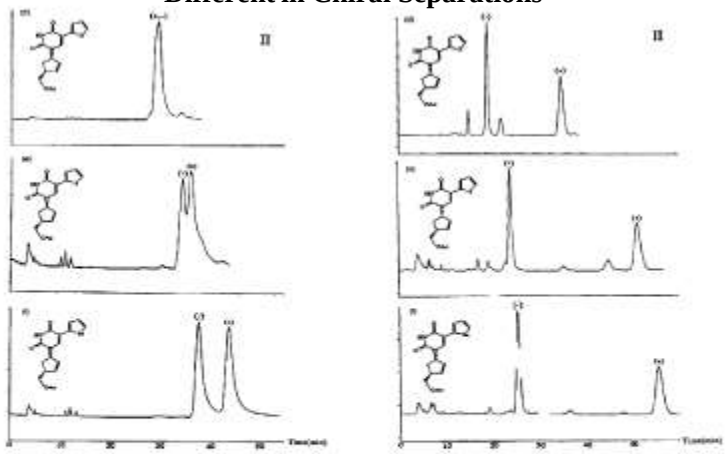
Immobilized Amylose Column

Ethanol vs Isopropanol Can be Very Different in Chiral Separations



Immobilized Amylose Column *S. Levin et al. / J. Chromatogr. A 752 (1996) 131-146*

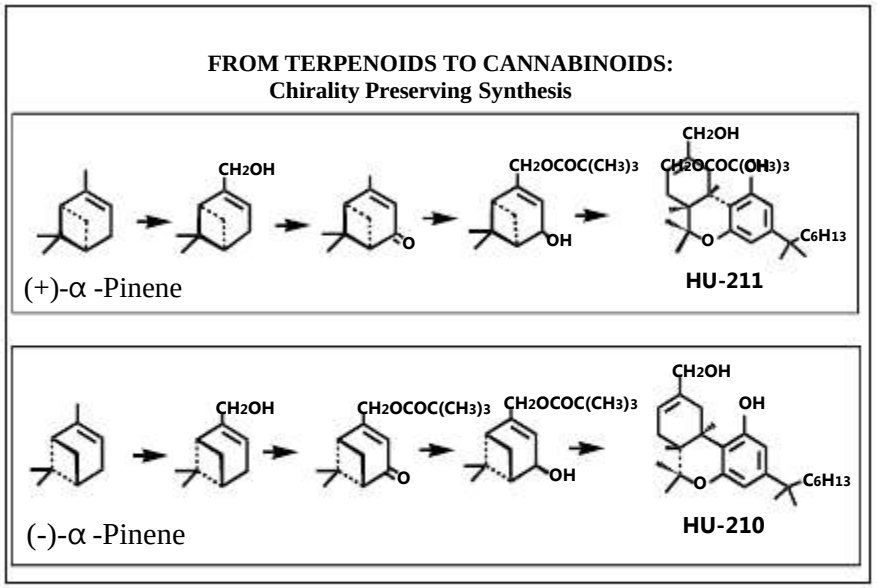
Ethanol vs Isopropanol Can be Very Different in Chiral Separations

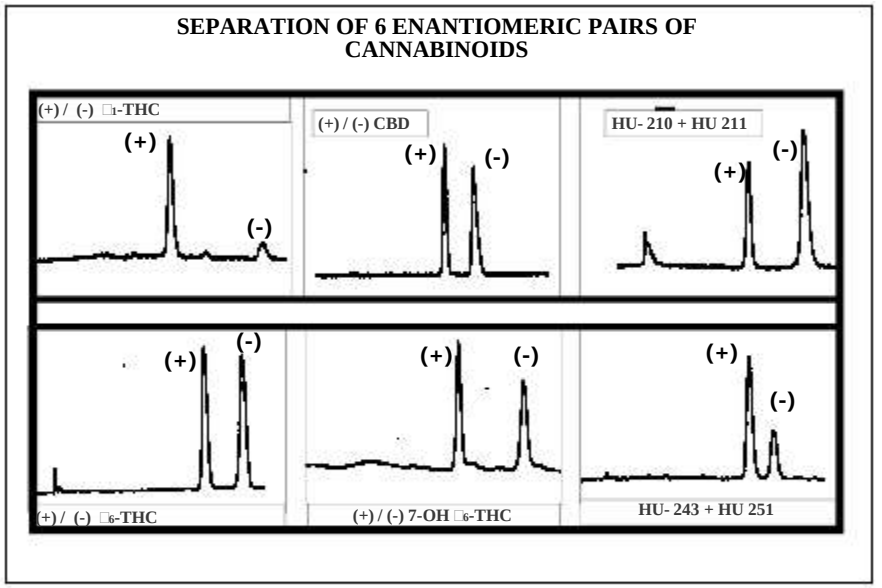


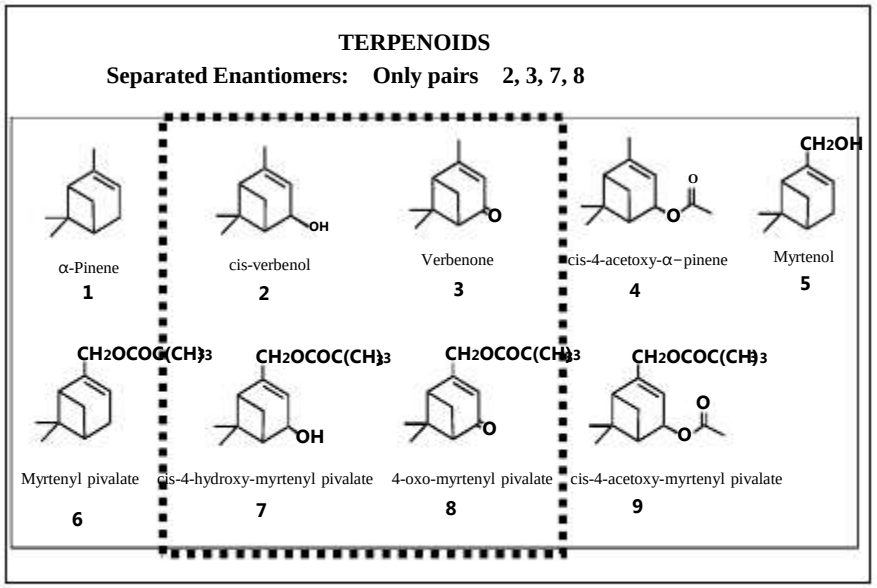
Immobilized Amylose Column

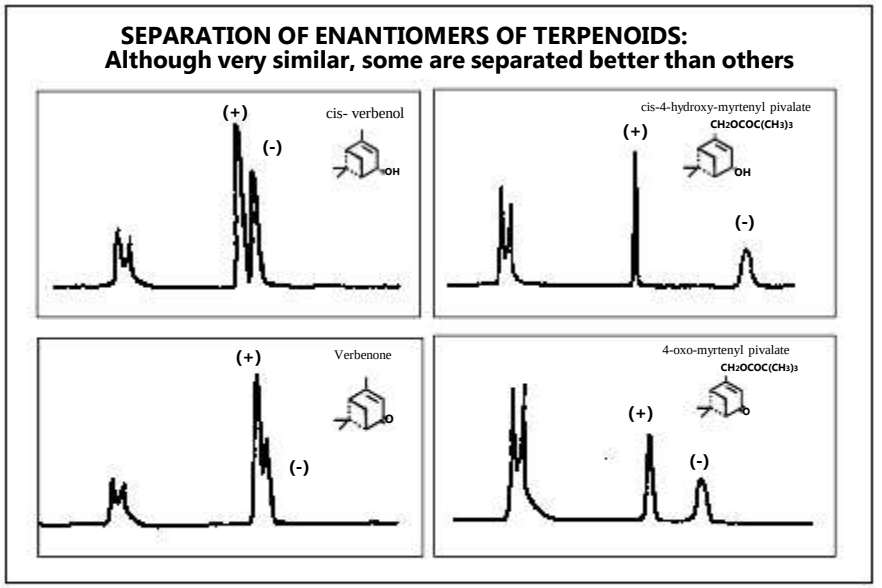
Structural Effects: What Causes Better Separation?

CHIRAL CHROMATOGRAPHY

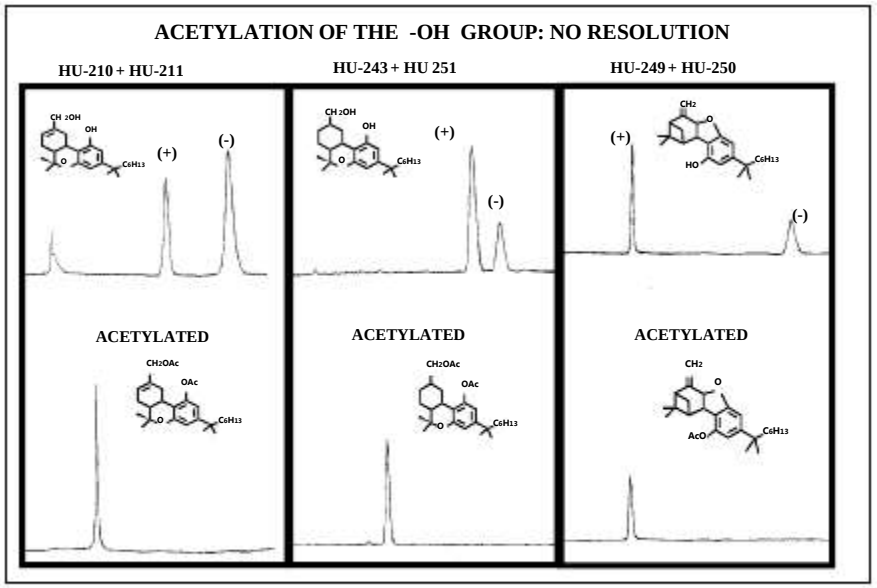


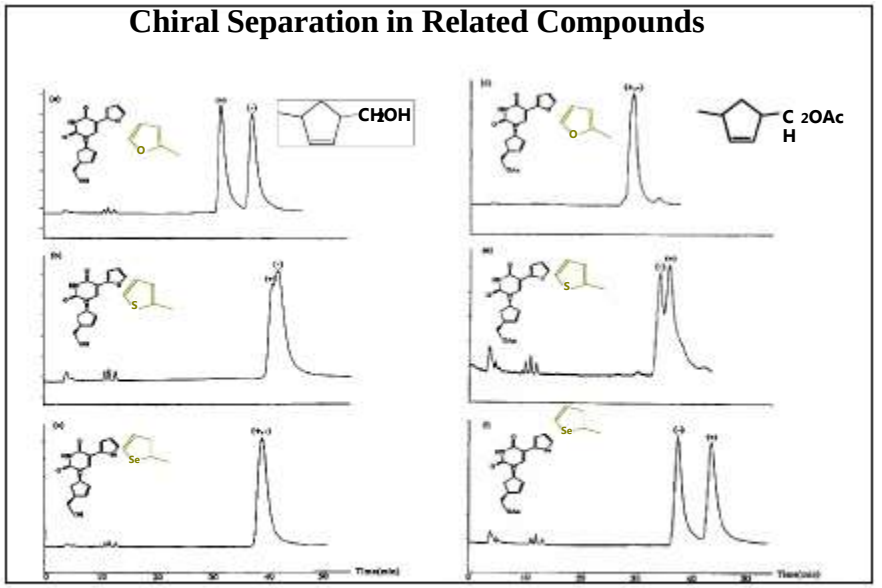


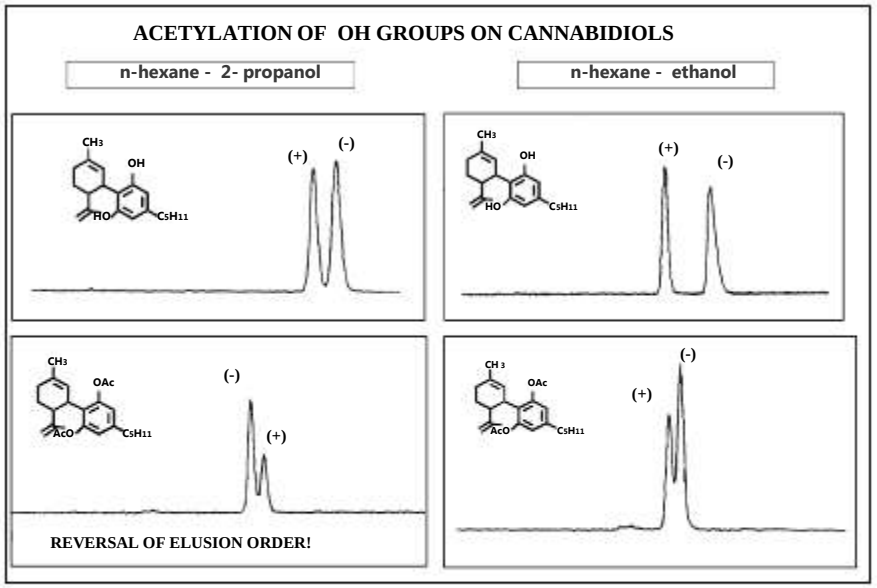




CHIRAL CHROMATOGRAPHY







Conclusions

- Solvents affect differently than Normal Phase and Reversed Phase: Elution order might be changed
- There is no way to predict in advance whether a pair will be separated or not, even if a related compound was separated
- pH and ionic strength are effective in the Protein immobilized type of columns