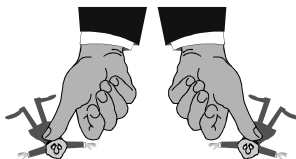


CHIRAL CHROMATOGRAPHY

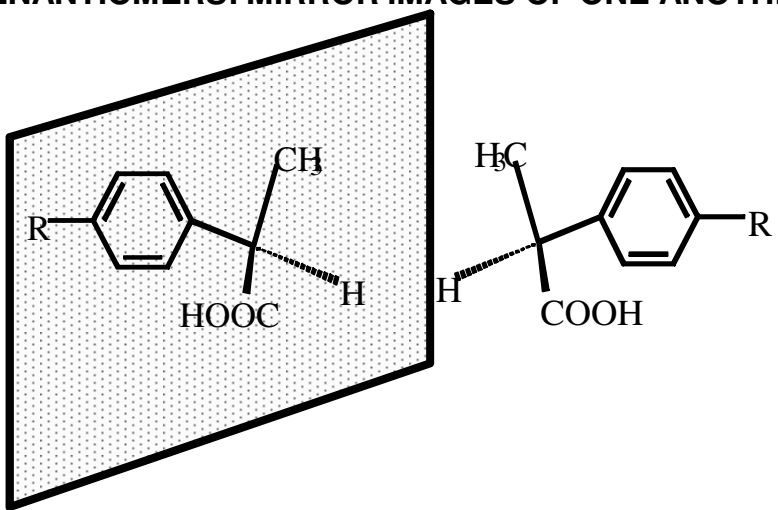
Separation of Optical Isomers (Enantiomers) by Chiral Chromatography



Dr. Shulamit Levin
Medtechnica, Isreal

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

CHIRAL CHROMATOGRAPHY

Stereospecificity in drug action

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

ENANTIOMERS vs RACEMATES

STEREOSELECTIVE PROPERTIES

Only one enantiomer is active:

The additional enantiomer is toxic:

Different pharmacokinetics:

Different rates and routes of metabolism:

One enantiomer is agonist, the other antagonist:

Different pharmacological action and tissue specificity:

POSSIBLE GAINS FROM USING ENANTIOMERS

Reduced dose and load on metabolism

Freedom in dose and broader use

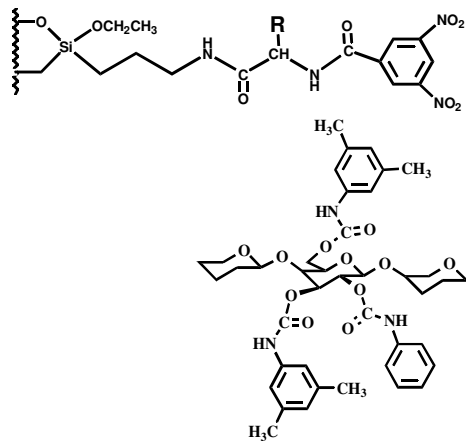
Better control of kinetics and dose

Freedom of dose, reduction of variability of patients' response

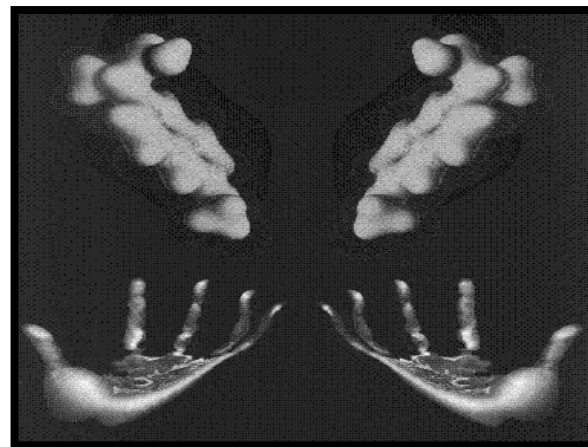
Enhanced activity and reduction of dose

Enhanced specificity and reduced side effects; use of the other enantiomer for different medication

Example of Chiral Features



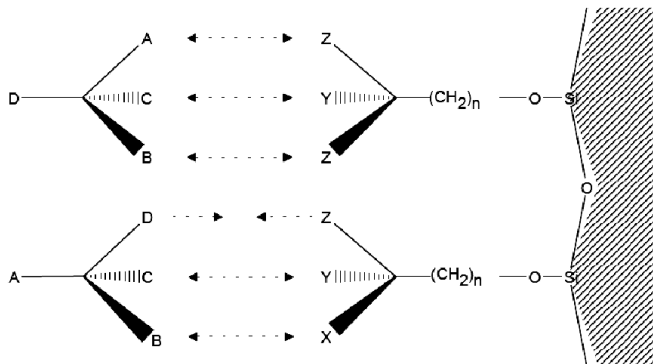
SEPARATION BY CHIRAL RECOGNITION



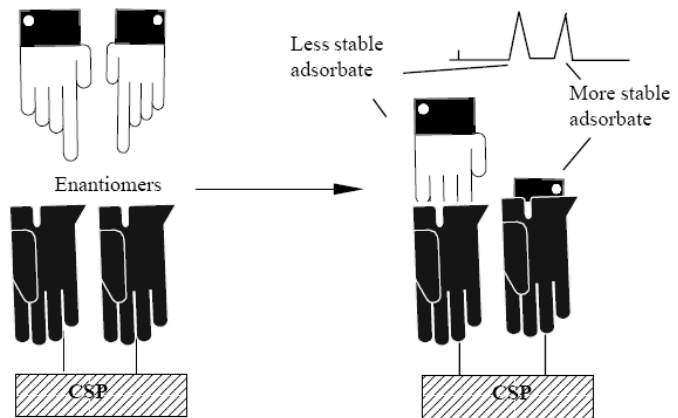
CHIRAL CHROMATOGRAPHY

Chiral Recognition

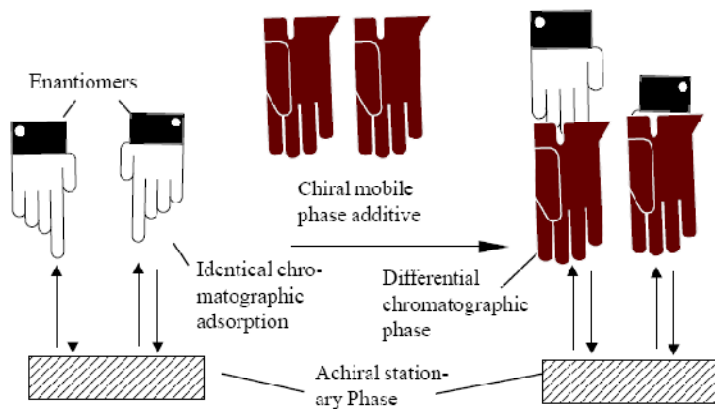
Chiral separations are generally accepted to involve a 3-point interaction with a system component.



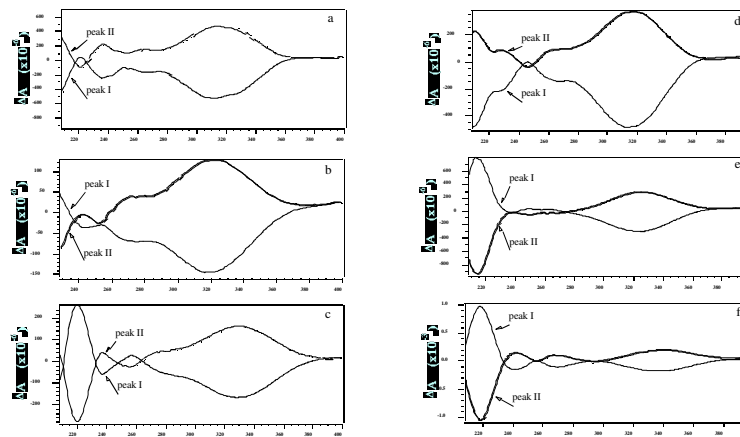
Chiral Recognition :Chiral Stationary Phases



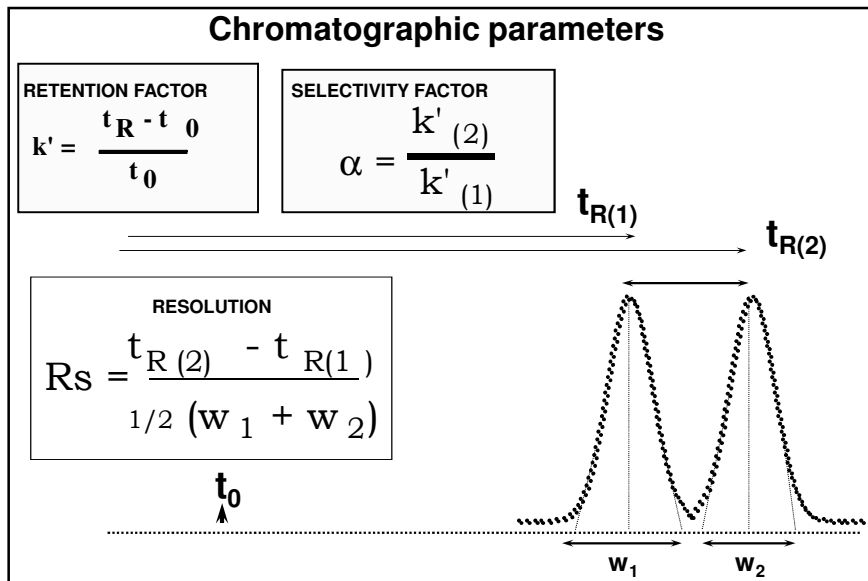
Chiral Recognition :Chiral Mobile Phases



Circular Dichroism: UV SPECTRA of POLARIZED LIGHT

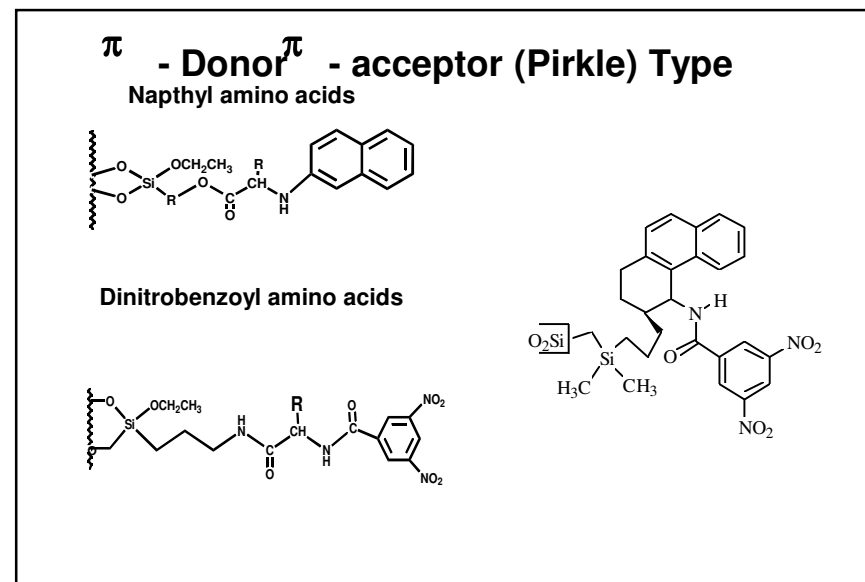


CHIRAL CHROMATOGRAPHY



- Types of Chiral stationary phases:**
- Ligand exchange
 - π -Donnor π -acceptor (Pirkle)
 - Chiral Host-guest (cyclodextrin)
 - Immobilized proteins
 - Immobilized polysaccharides
 - Macrocylic Antibiotics
 - Crown Ethers
-

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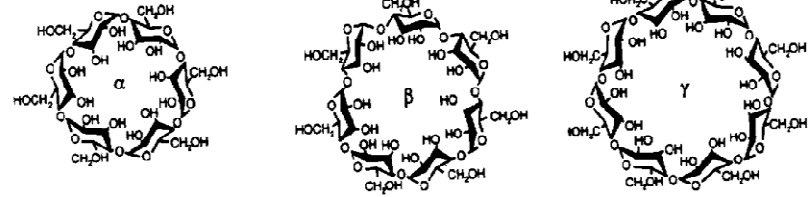
CHIRAL CHROMATOGRAPHY

Types of Chiral stationary phases:

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Chiral cavity by cyclodextrins



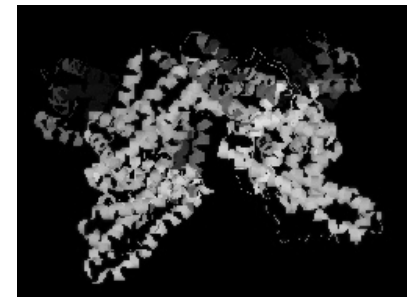
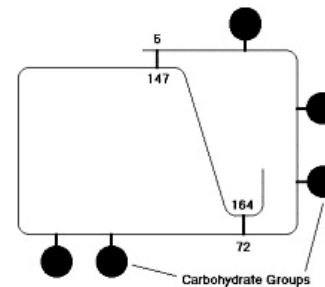
CD	No.Of units	size Å	Molecules included	Chiral centres
alpha	6	4.5-6.0	5-6 membered aromatic	30
beta	7	6.0-8.0	biphenyl or naphthalene	35
gamma	8	8.0-10.0	substituted pyrenes and Steroids	40

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Protein Columns



CHIRAL CHROMATOGRAPHY

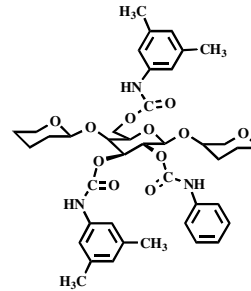
Types of Chiral stationary phases:

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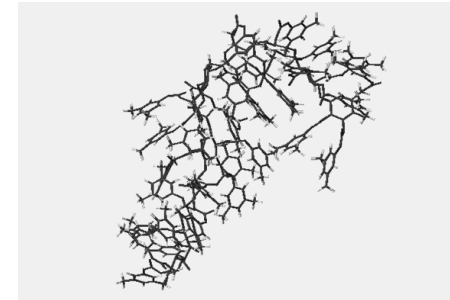


Immobilized polysaccharides:

Amylose
or
Cellulose



tribenzoate
tris phenylcarbamate
triacetate



Types of Chiral stationary phases:

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- Immobilized proteins
- Immobilized polysaccharides
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- Crown Ethers



macrocylic glycopeptide antibiotic eremomycin chemically bonded to silica

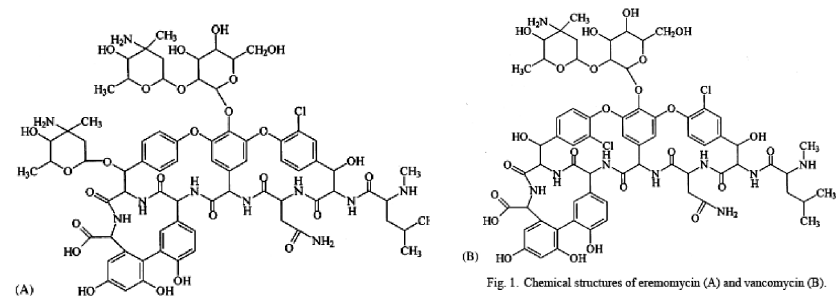
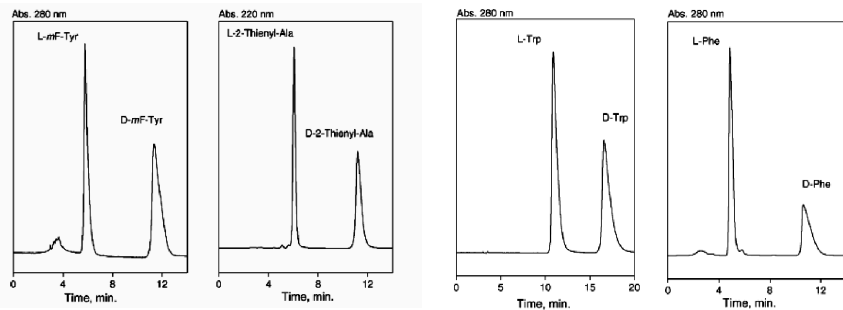


Fig. 1. Chemical structures of eremomycin (A) and vancomycin (B).

CHIRAL CHROMATOGRAPHY

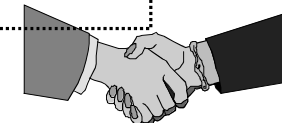
Separation of enantiomers of amino acids on eremomycin CSP. Column



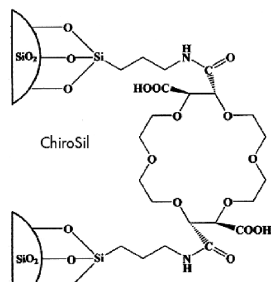
250mm4.0' mm. Eluent: methanol-0.1M NaH₂PO₄ (20:80, v/v), 0.7 ml/min.

Types of Chiral stationary phases:

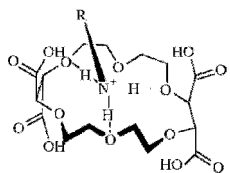
- Ligand exchange
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- Immobilized proteins
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Crown Ether Type of Chiral Stationary Phase

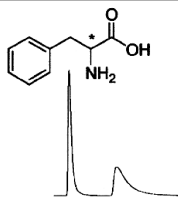


ChiroSil



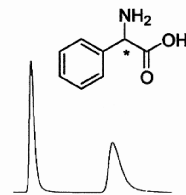
Phenylalanine

Phenylalanine
 Column: ChiroSil® RCA(+) or SCA(-) 15 cm x 4.6 mm
 Mobile Phase: (70/30) CH₃OH/H₂O
 +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

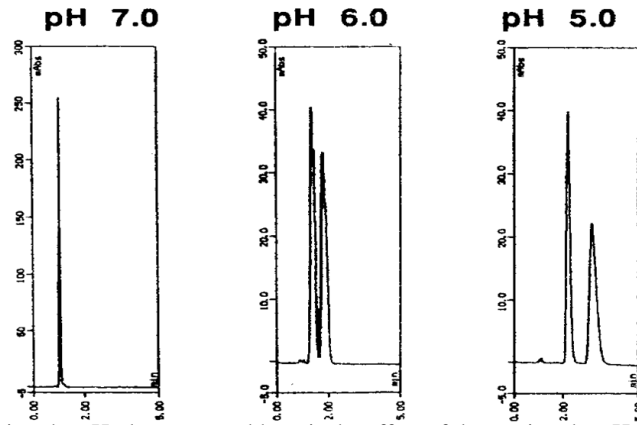
Phenylglycine
 Column: ChiroSil® RCA(+) or SCA(-) 15 cm x 4.6 mm
 Mobile Phase: (70/30) CH₃OH/H₂O
 +10 mM H₂SO₄ and 0.1% TEA
 Flow Rate: 1.0 mL/min
 Detection: UV 210 nm
 Run Time: 13.1 min
 k'₁: 3.14
 α : 2.60



Examples for Optimization in Chiral Separations: Aqueous and Non Aqueous

CHIRAL CHROMATOGRAPHY

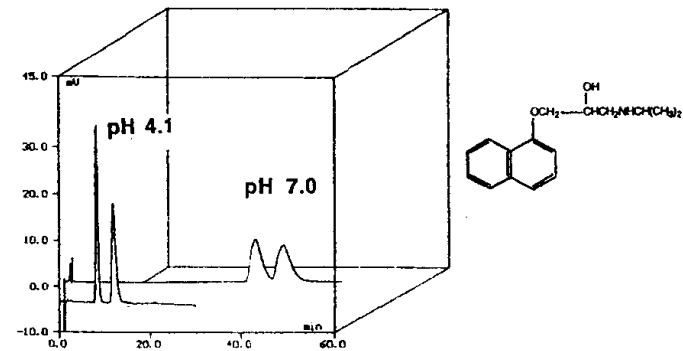
pH Affects Selectivity of Immobilized Protein Columns



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

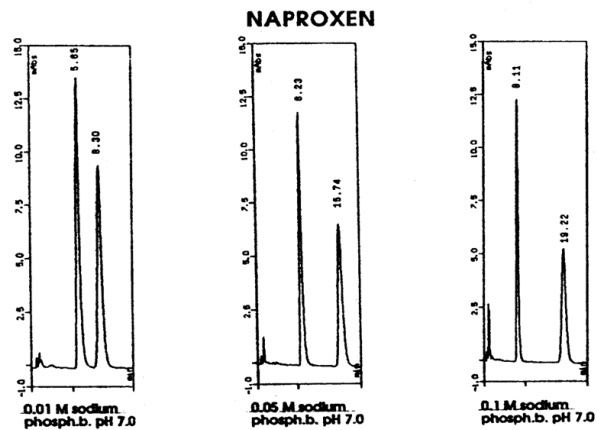
Amines

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

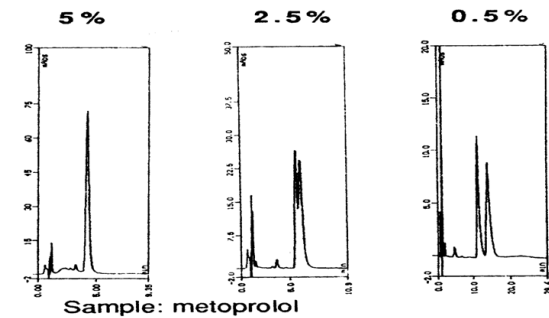
Changing the buffer concentration: By changing the buffer concentration in the mobile phase, it is possible to affect both the retention (k') and the enantioselectivity (α). These effects have been observed for acids and in special cases also for some amines. The chromatograms below demonstrate the effect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



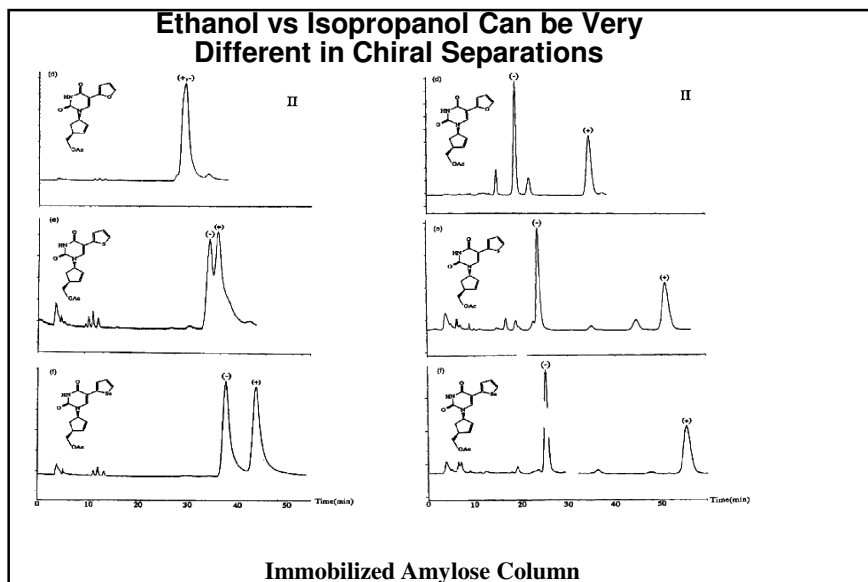
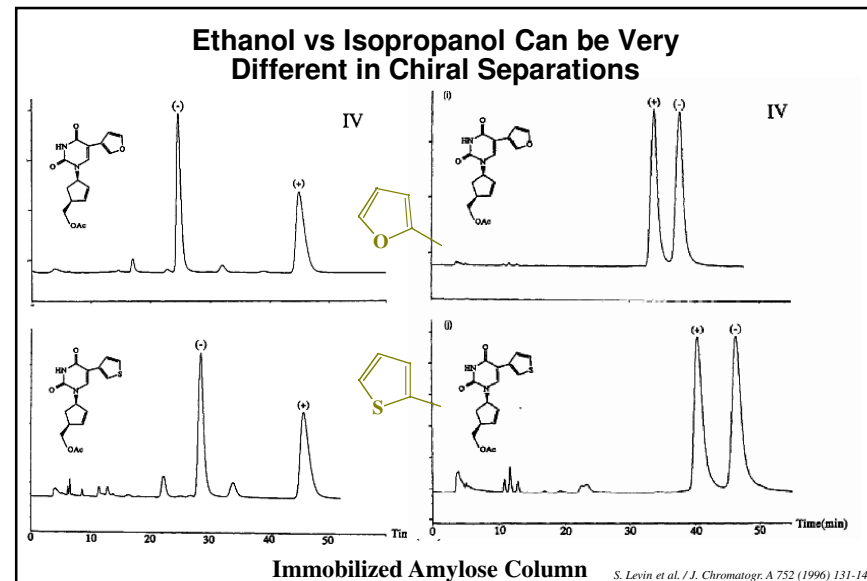
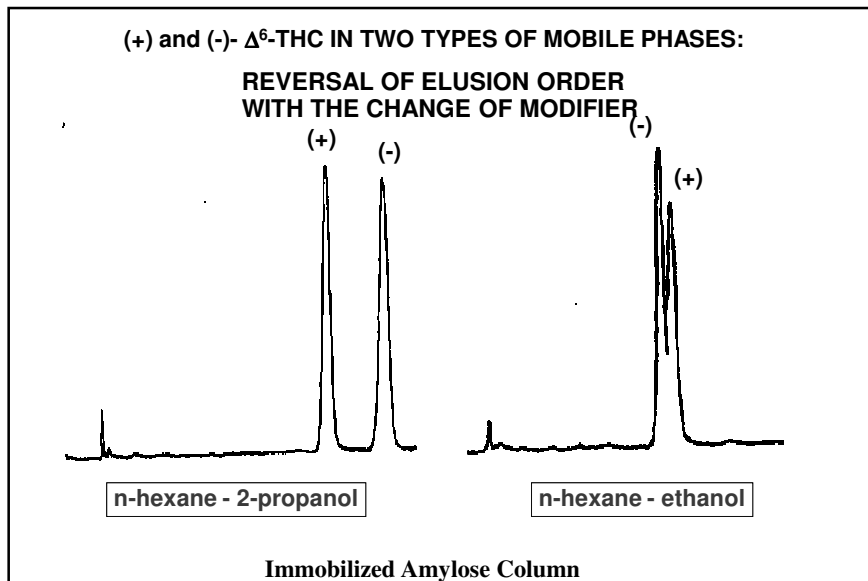
Effect of Modifier in Immobilized Protein Columns

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:

2-propanol concentration



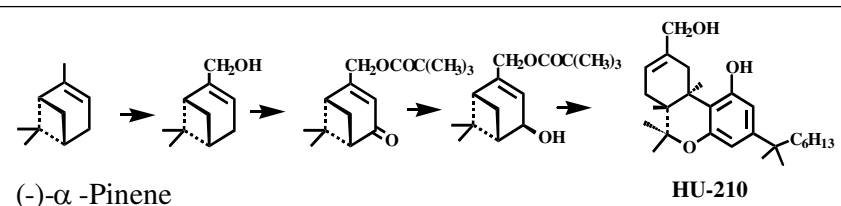
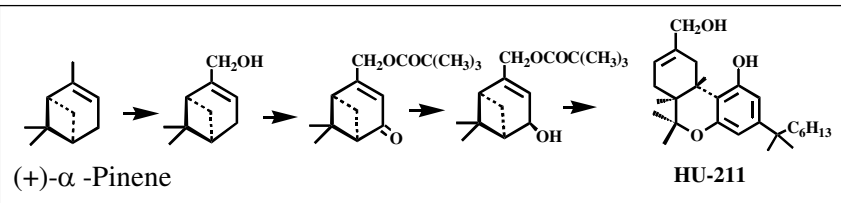
CHIRAL CHROMATOGRAPHY



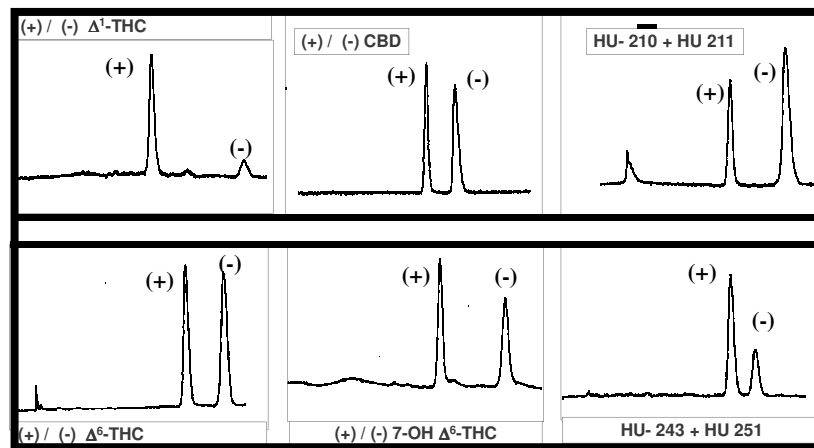
Structural Effects: What Causes Better Separation?

CHIRAL CHROMATOGRAPHY

FROM TERPENOIDS TO CANNABINOIDS: Chirality Preserving Synthesis

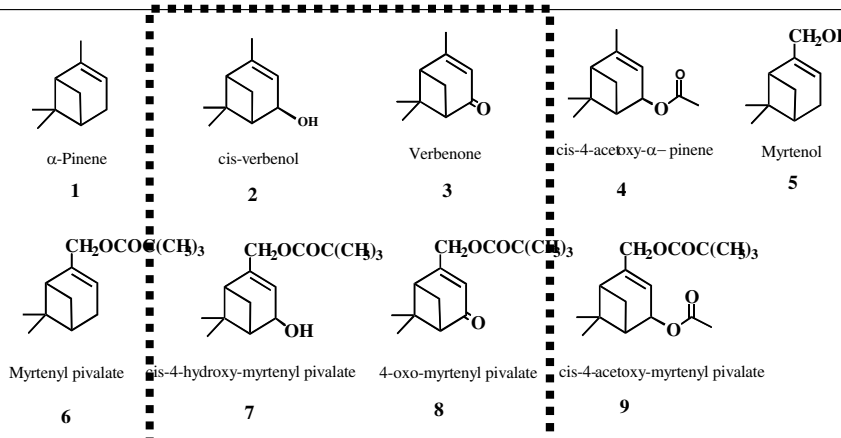


SEPARATION OF 6 ENANTIOMERIC PAIRS OF CANNABINOIDS

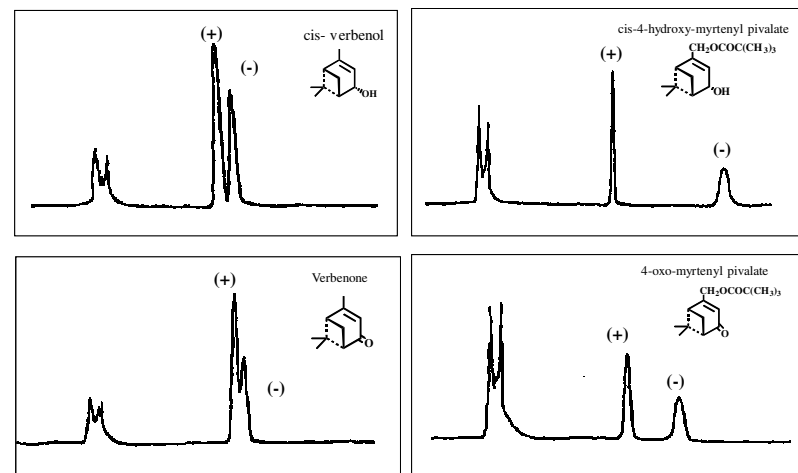


TERPENOIDS

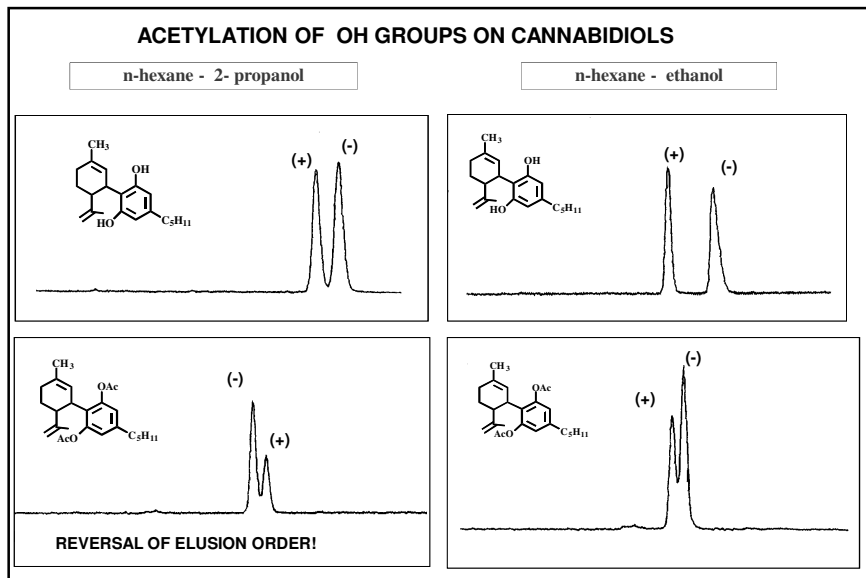
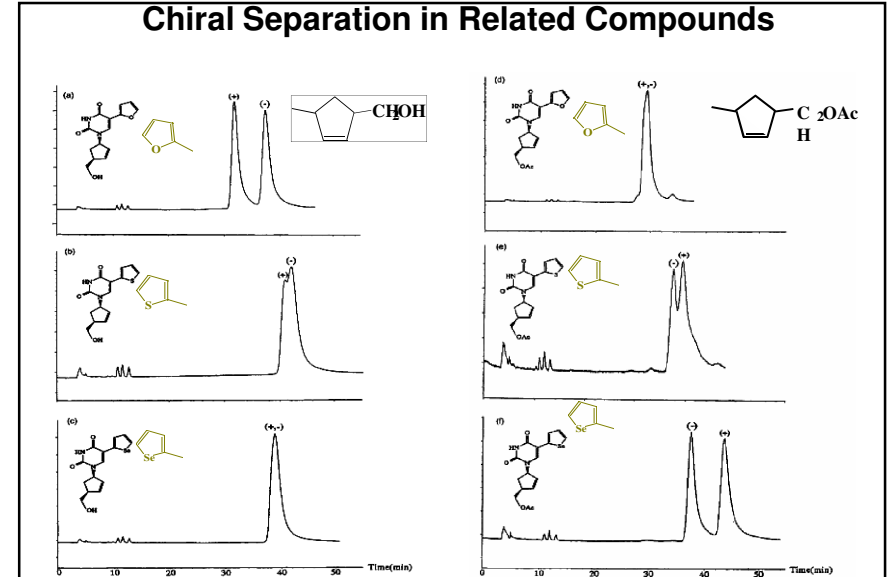
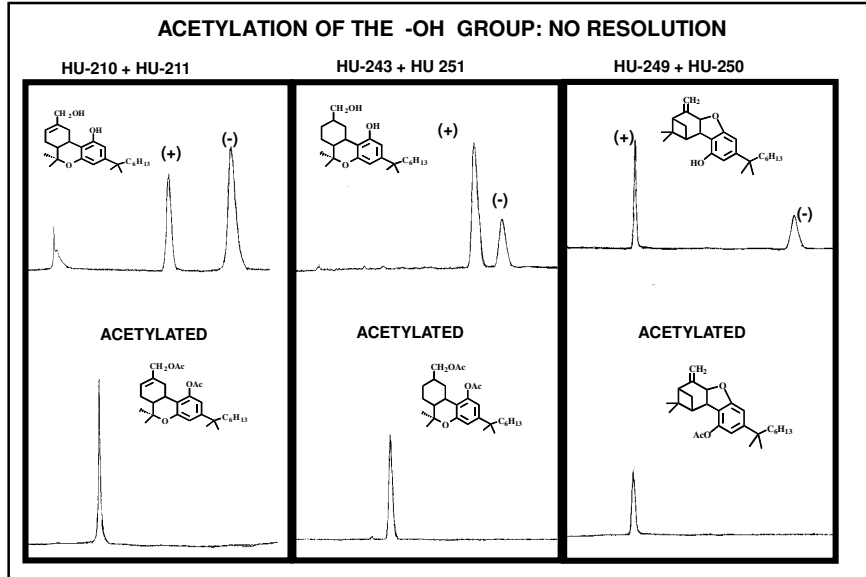
Separated Enantiomers: Only pairs 2, 3, 7, 8



SEPARATION OF ENANTIOMERS OF TERPENOIDS: Although very similar, some are separated better than others



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