

High Performance Liquid Chromatography - HPLC Introduction

High Performance Liquid Chromatography (HPLC) for Clinical and Biomedical Applications

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Homepage: <http://www.forumsci.co.il/HPLC>

HPLC COURSE LAYOUT

- Introduction & Applicability
- Modes of Chromatography
- Quantitative work and System Qualification.

What does HPLC mean?

High pressure liquid chromatography

High priced liquid chromatography

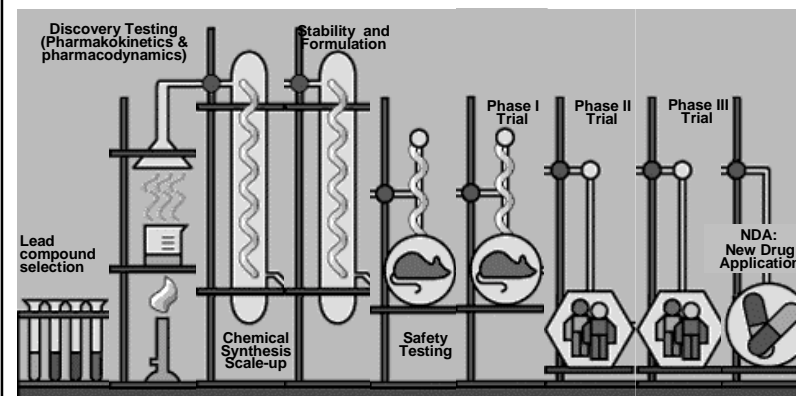
Hewlett-Packard liquid chromatography

High performance liquid chromatography

Hocus pocus liquid chromatography

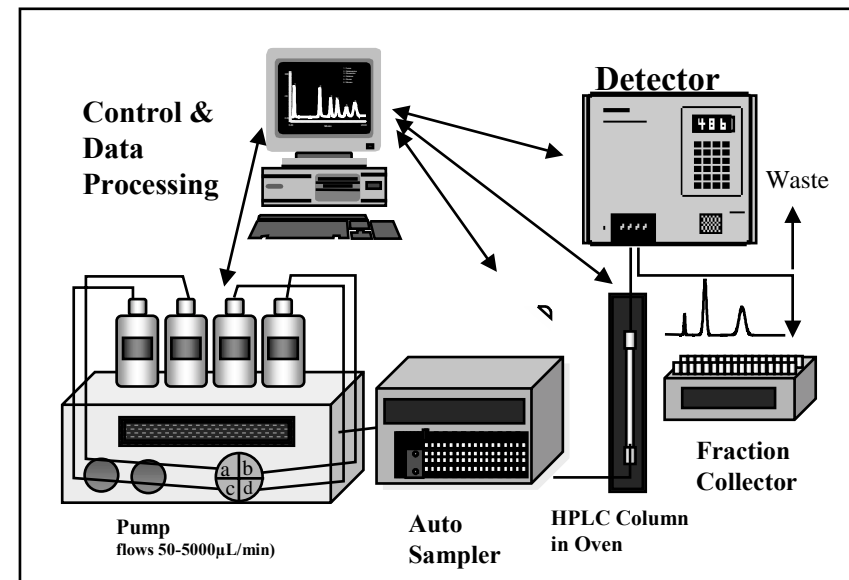
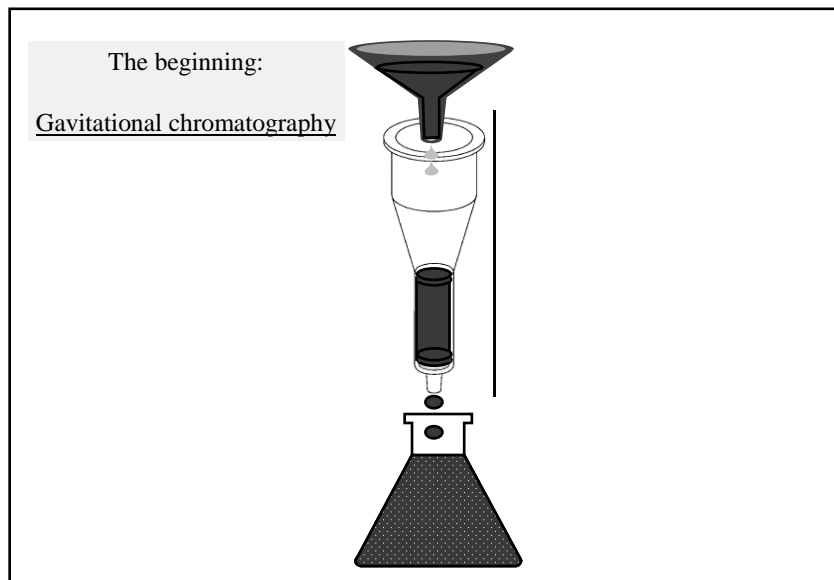
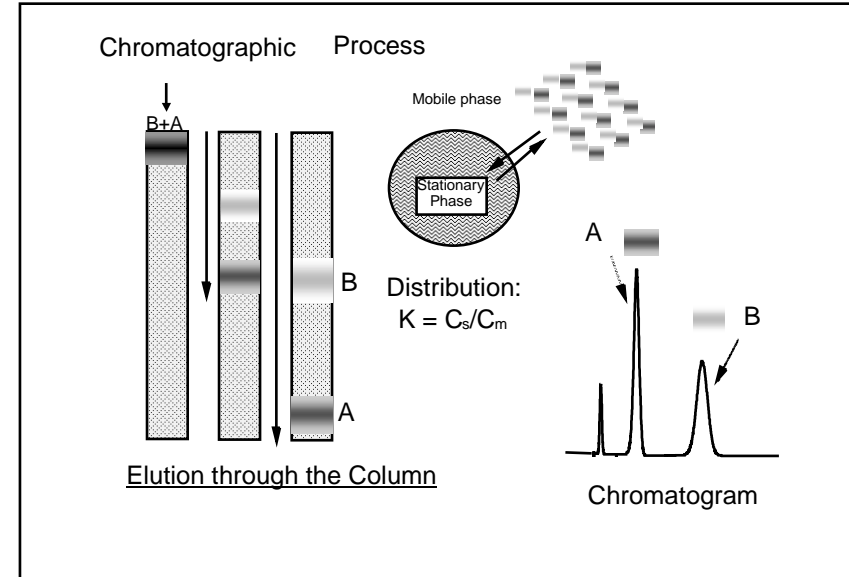
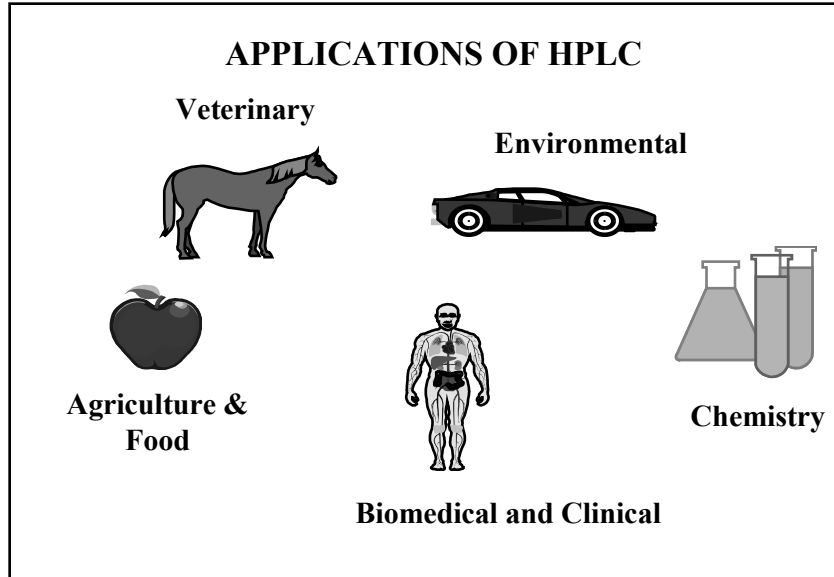
High patience liquid chromatography

HPLC in Pharmaceutics Technique No 1



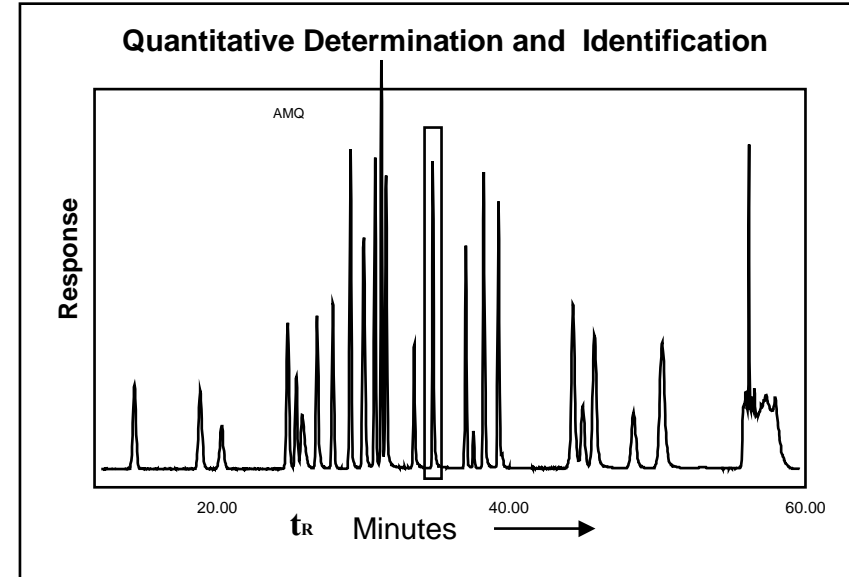
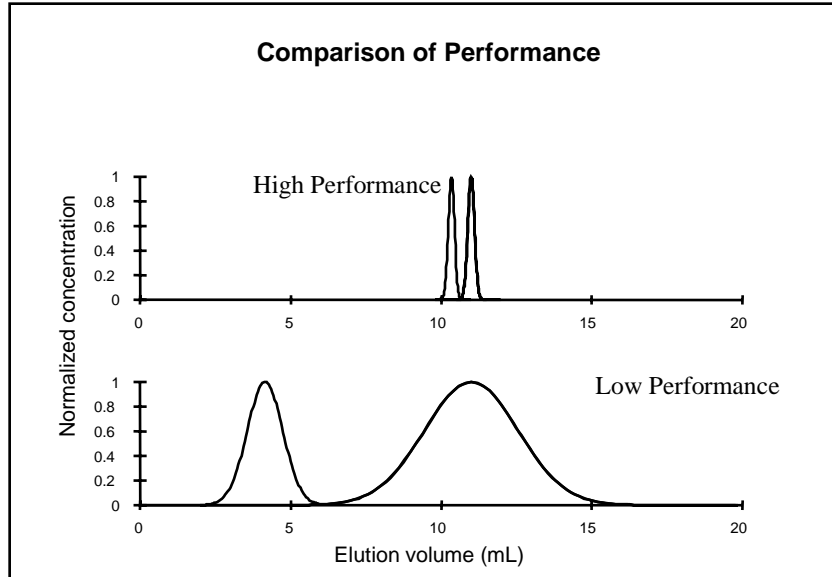
High Performance Liquid Chromatography - HPLC

Introduction



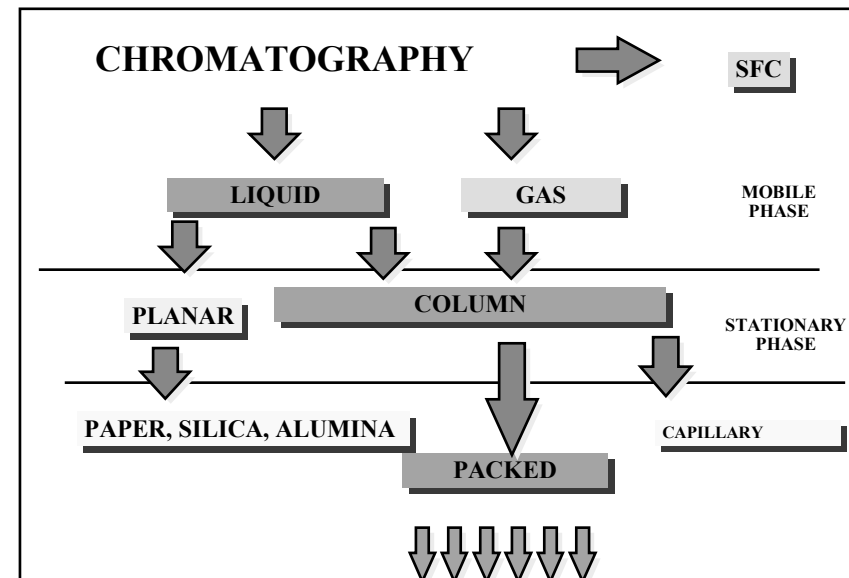
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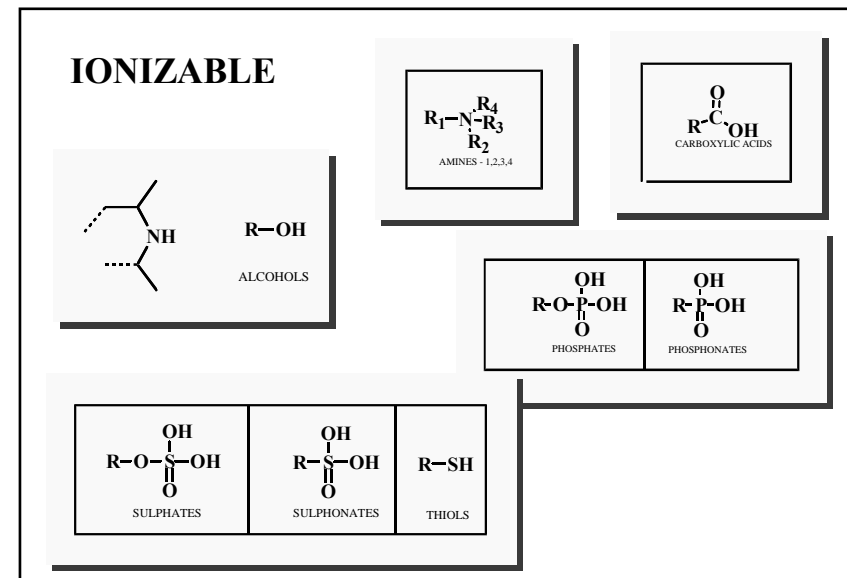
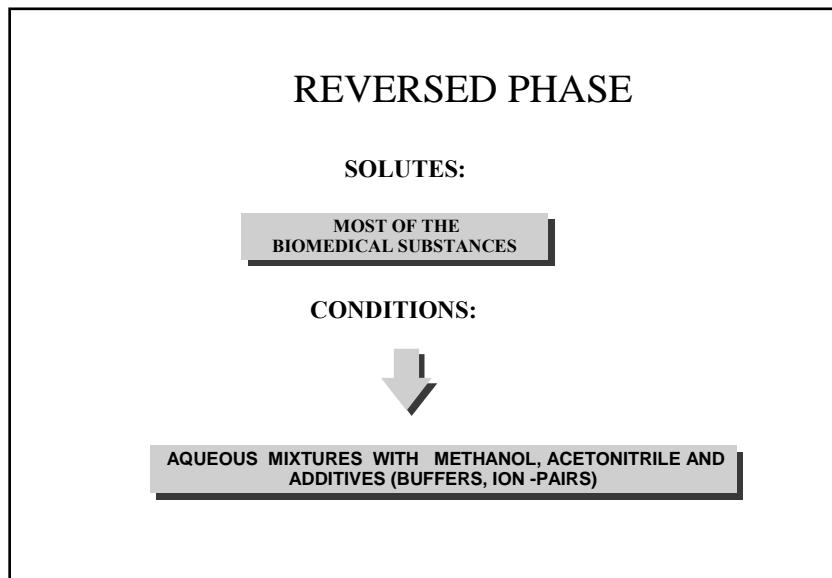
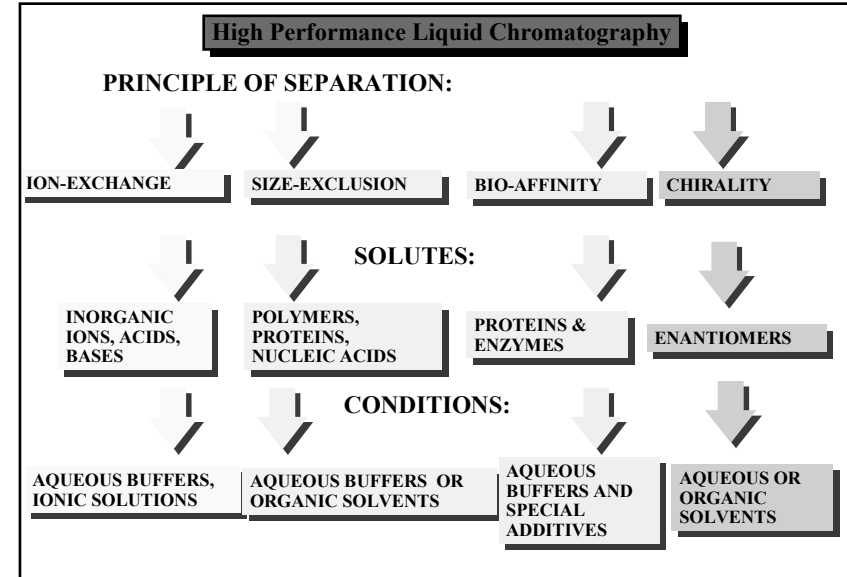
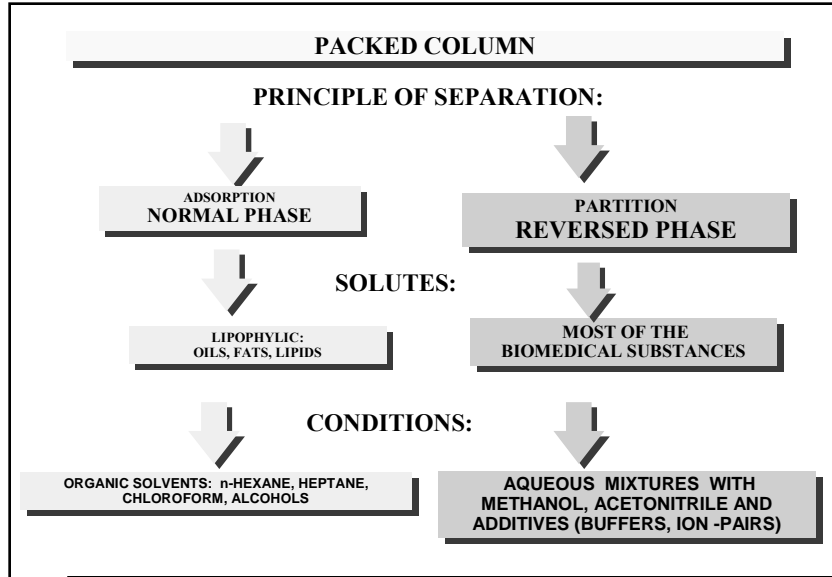
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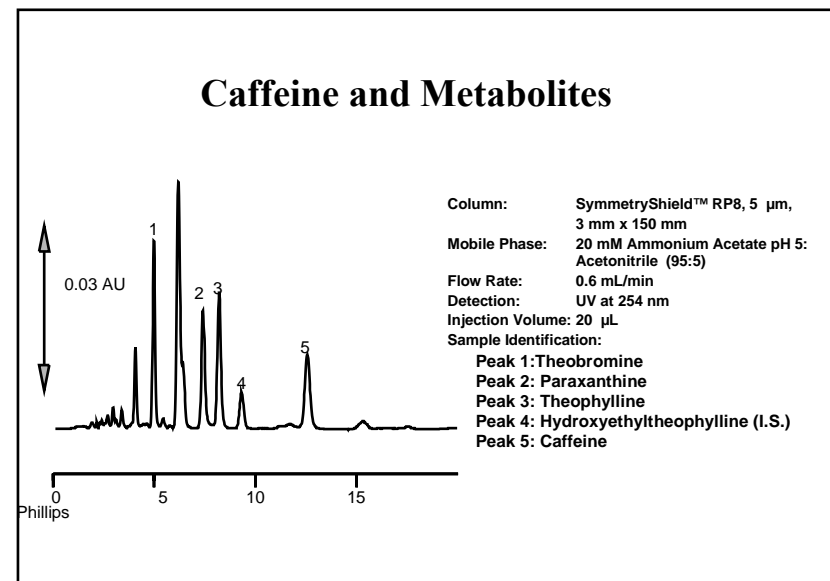
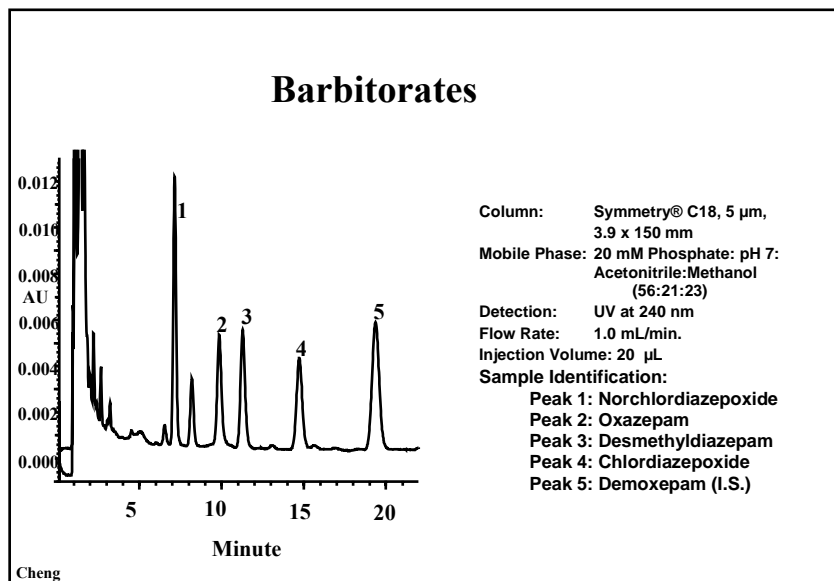
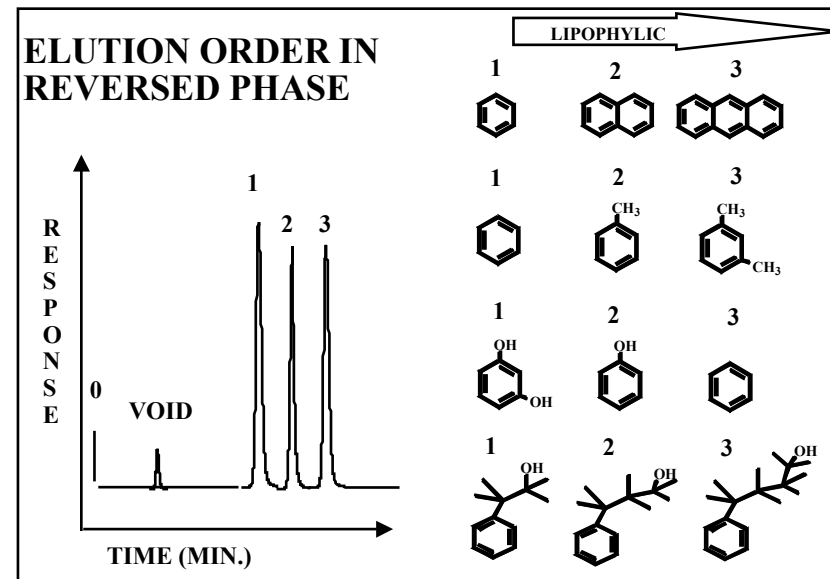
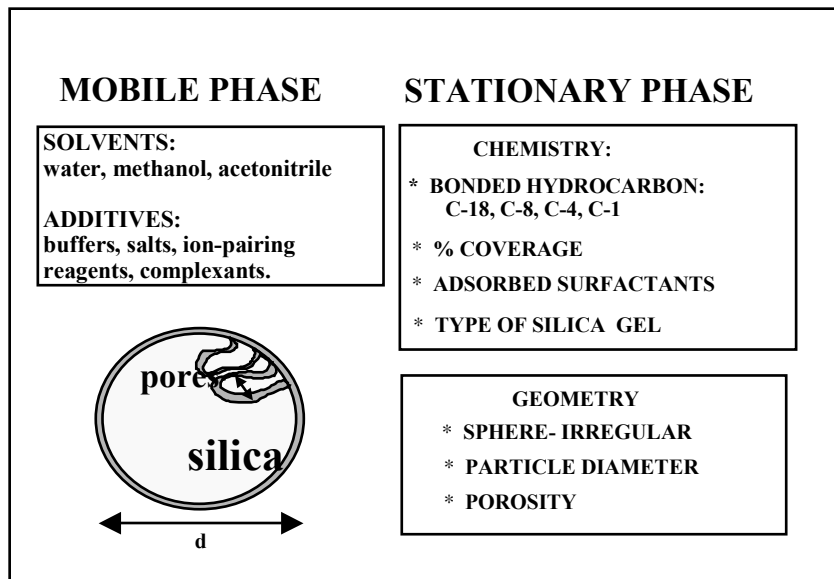
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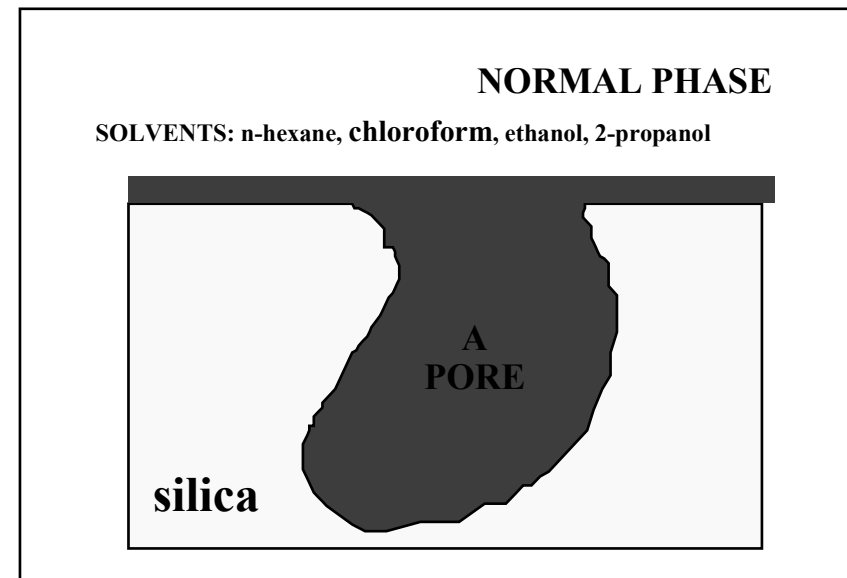
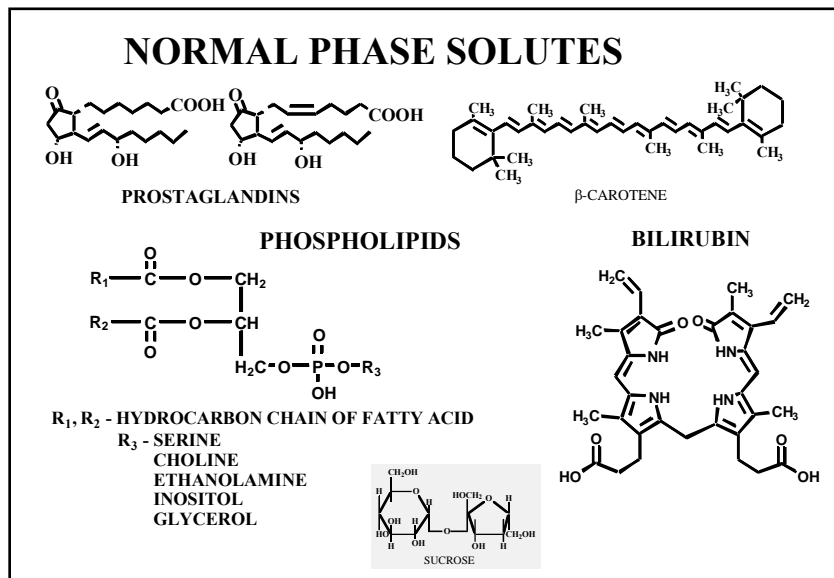
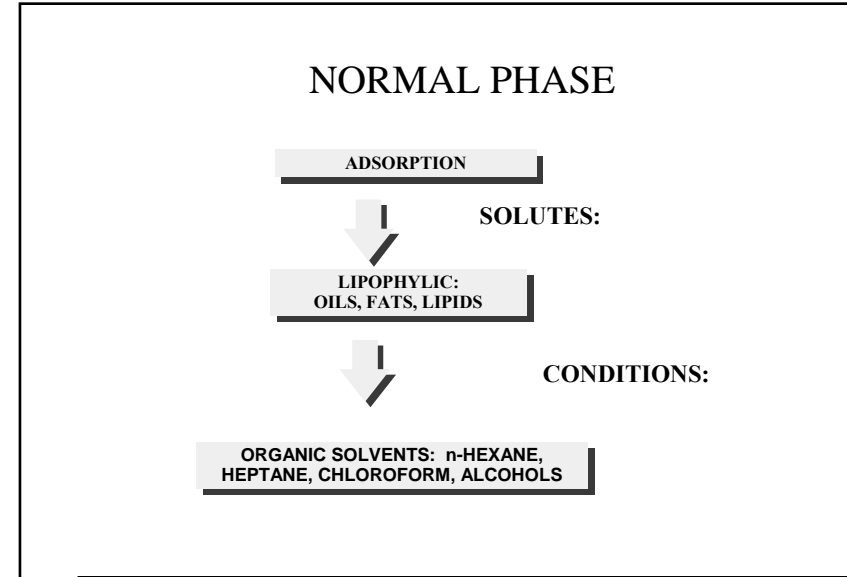
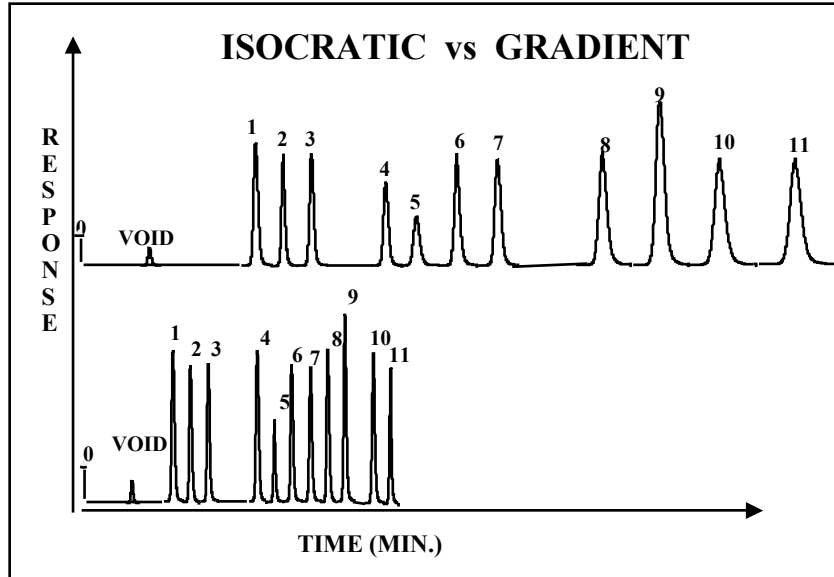
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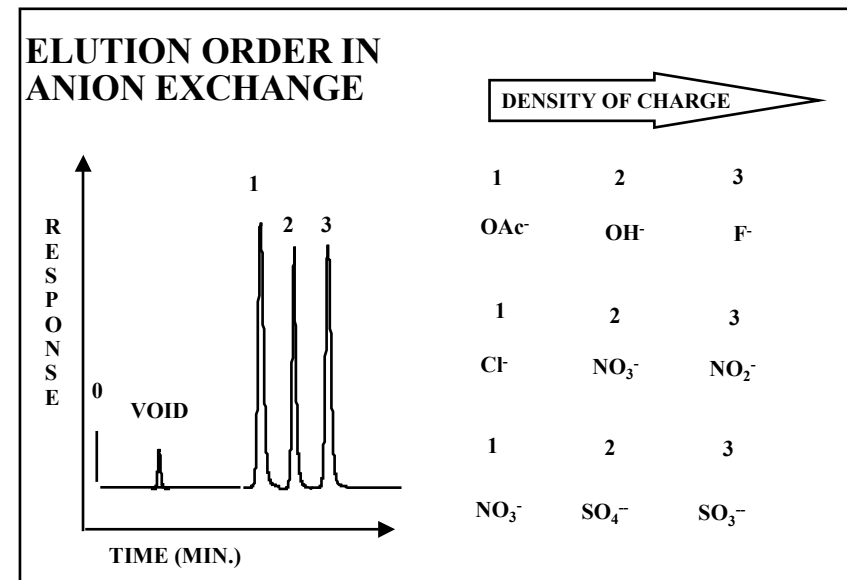
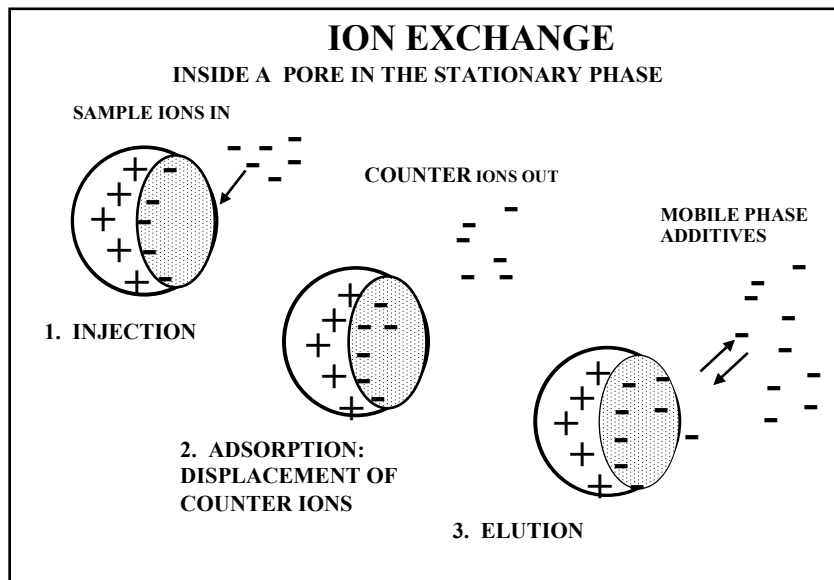
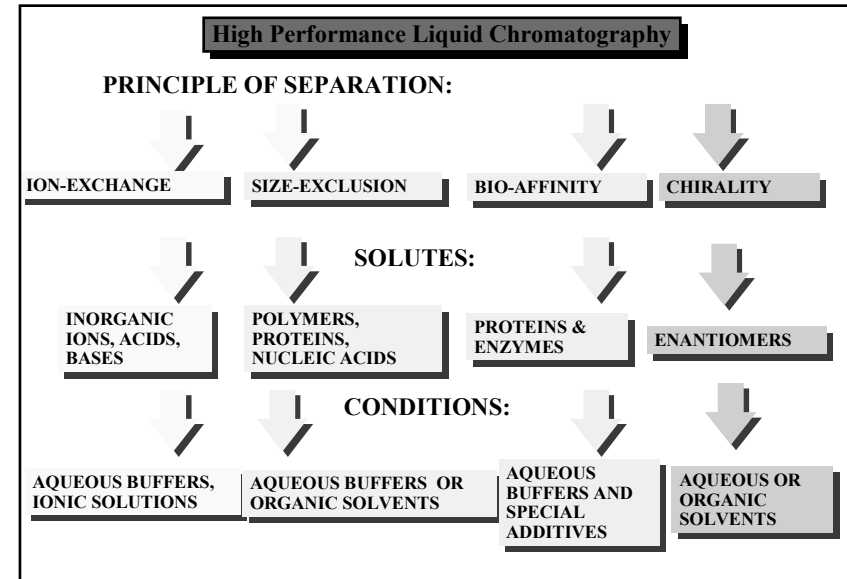
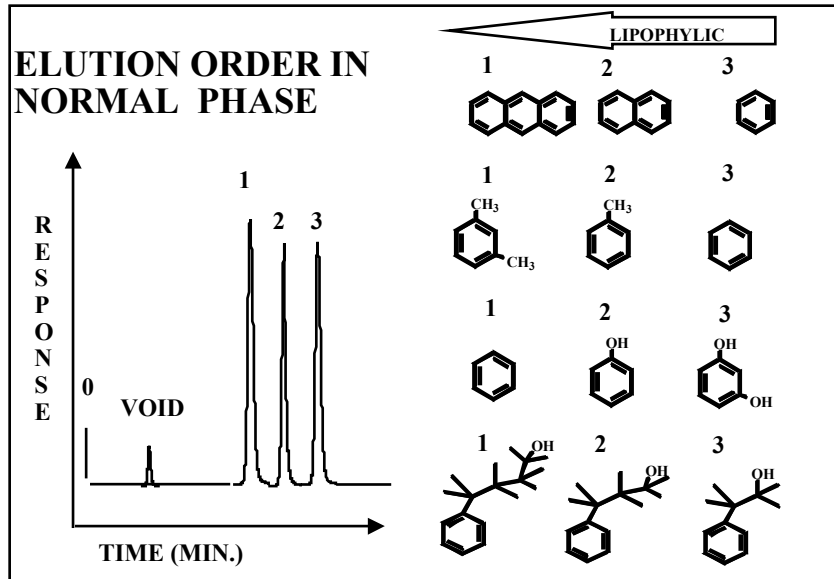
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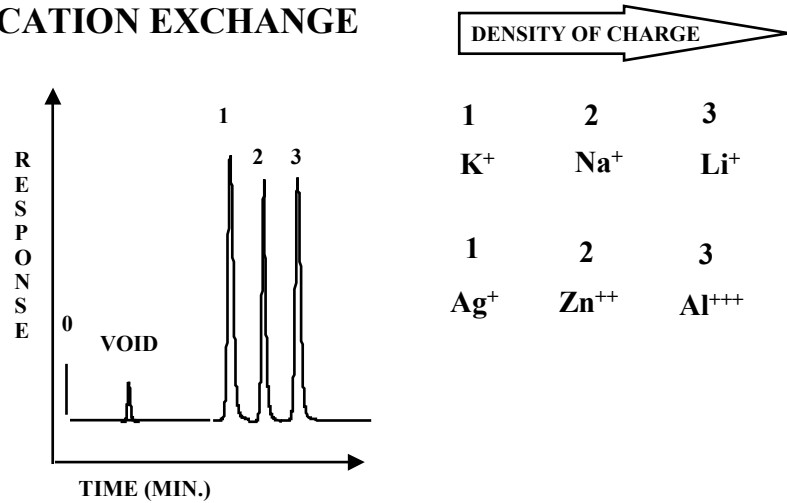
Introduction



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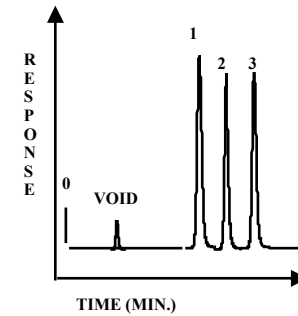
ELUTION ORDER IN CATION EXCHANGE



ELUTION ORDER IN ION EXCHANGE

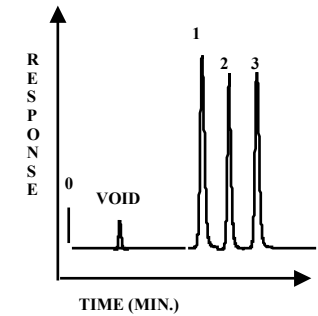
ANION EXCHANGE

STRONGER ACID →

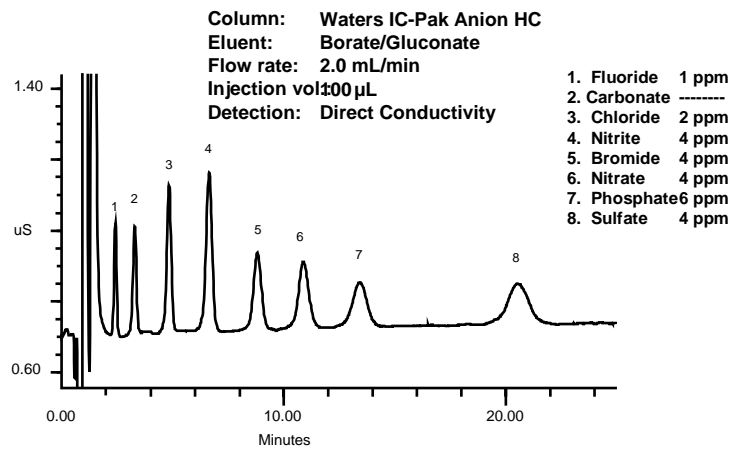


CATION EXCHANGE

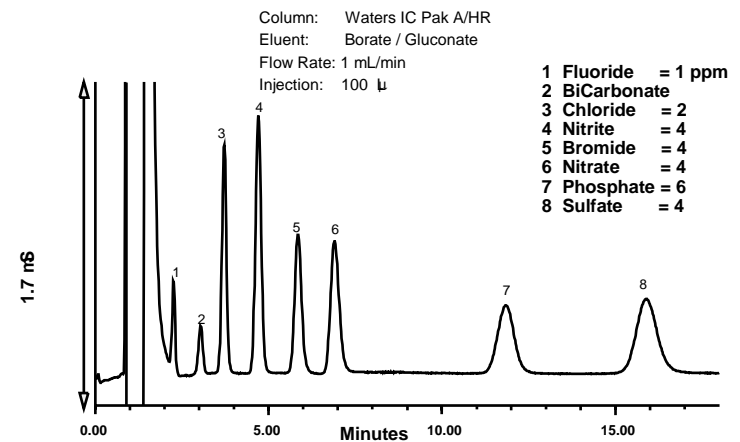
STRONGER BASE →



Analysis of Ions

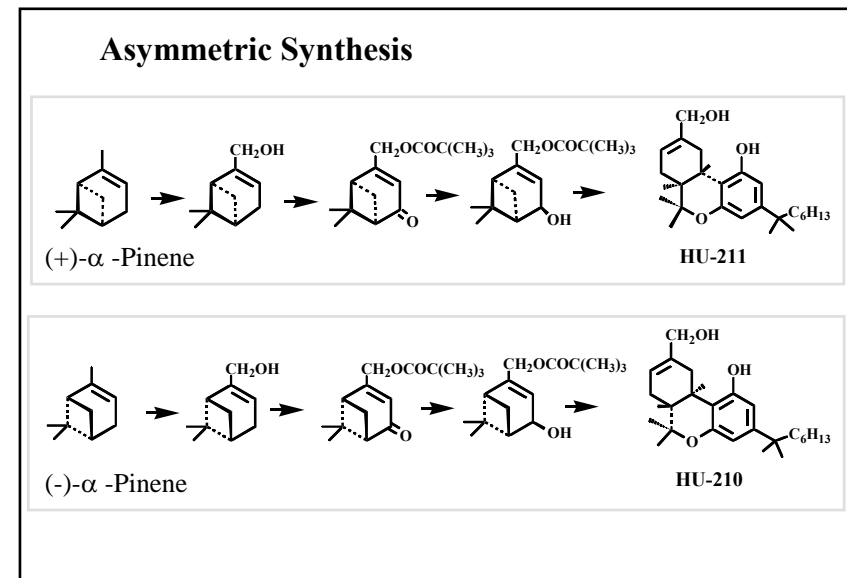
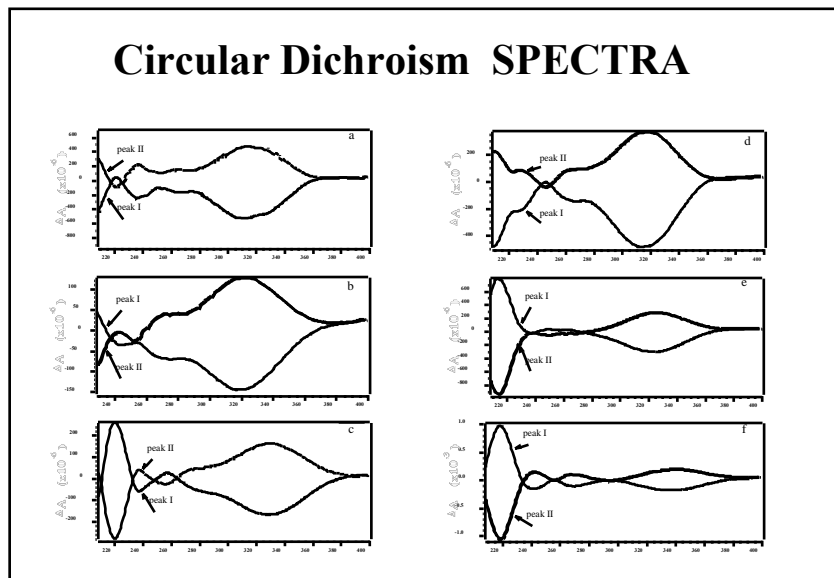
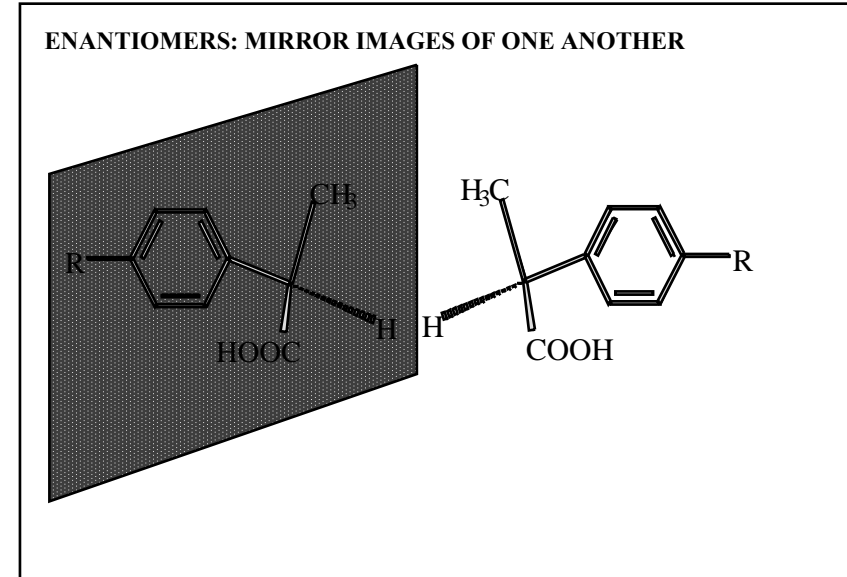
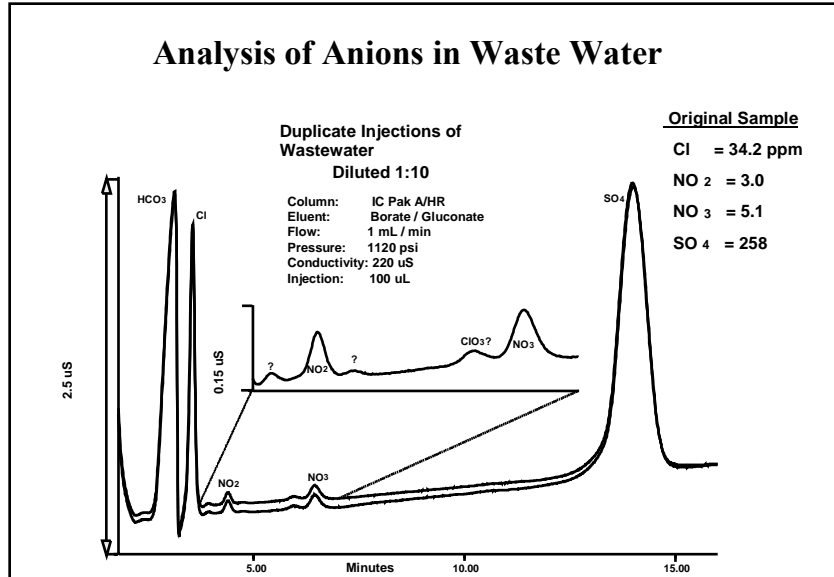


Analysis of Anions



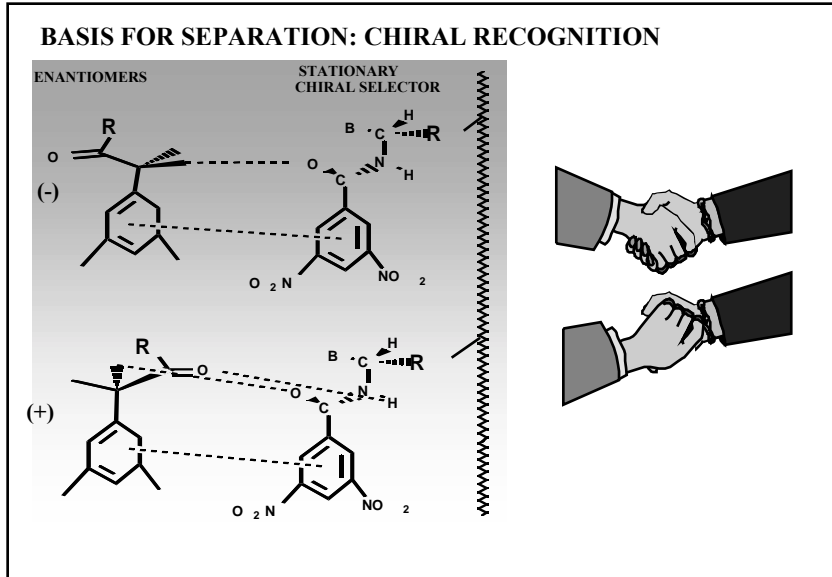
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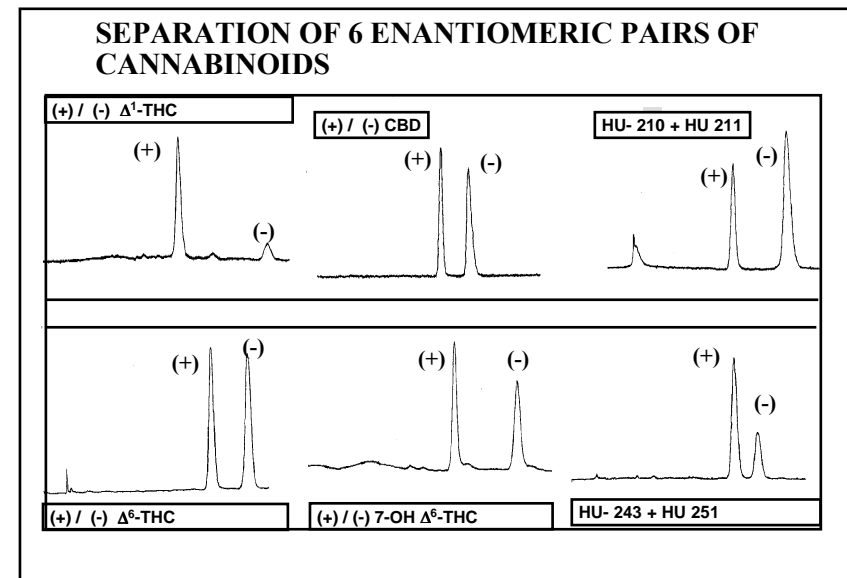
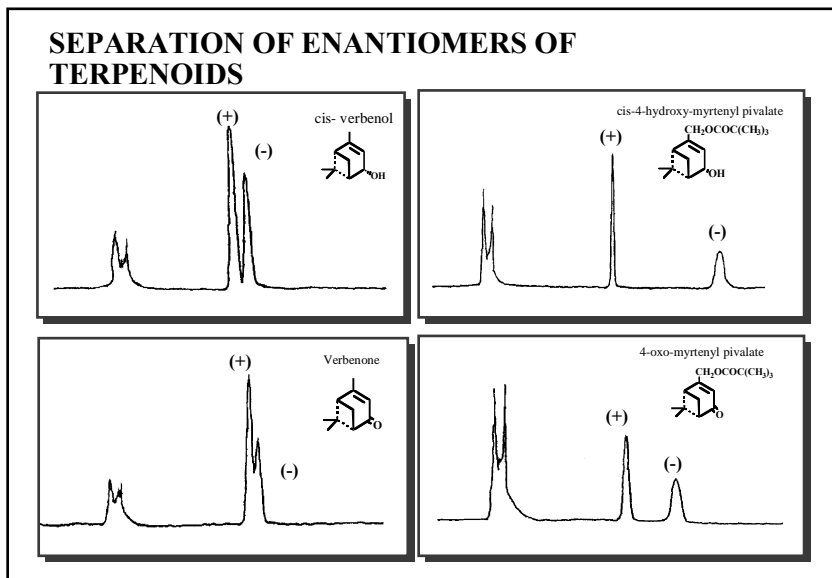
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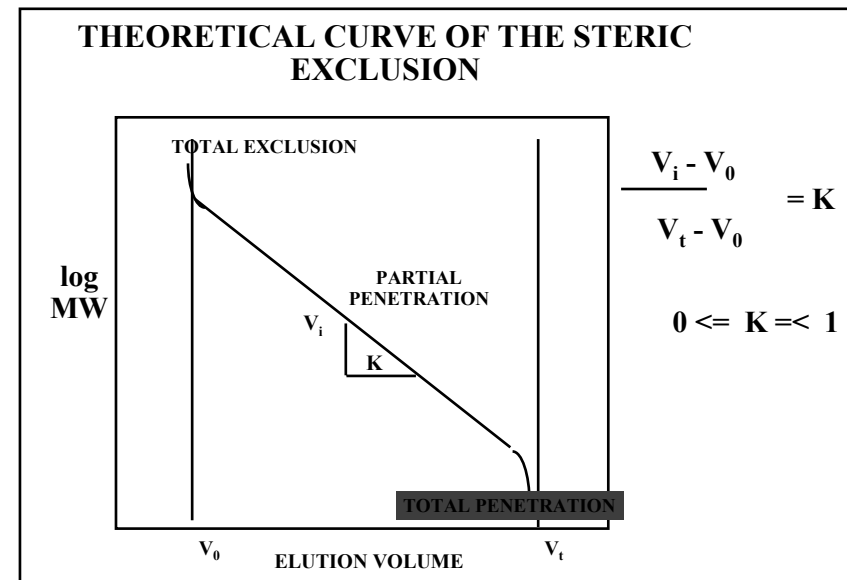
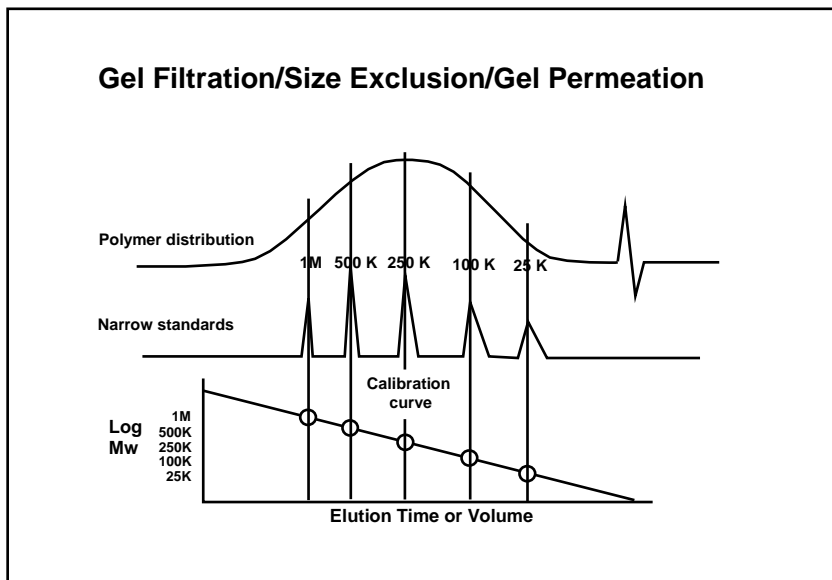
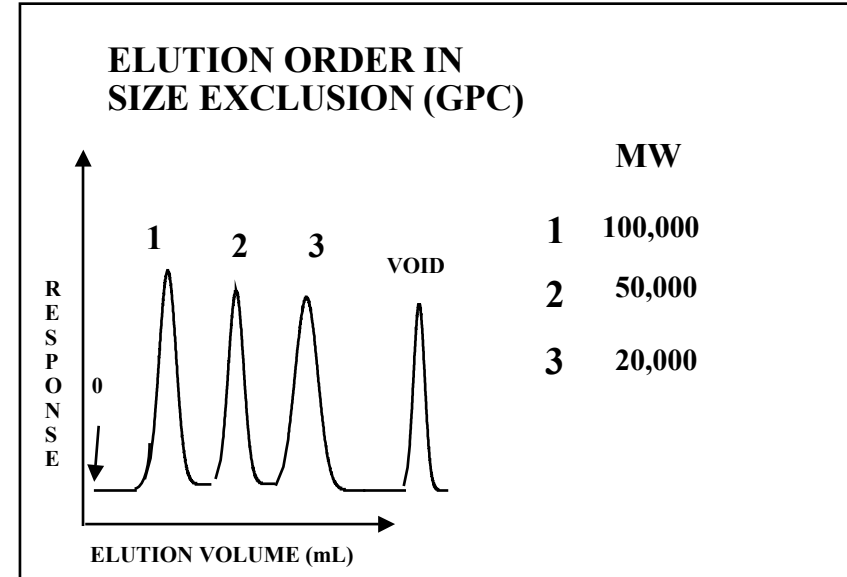
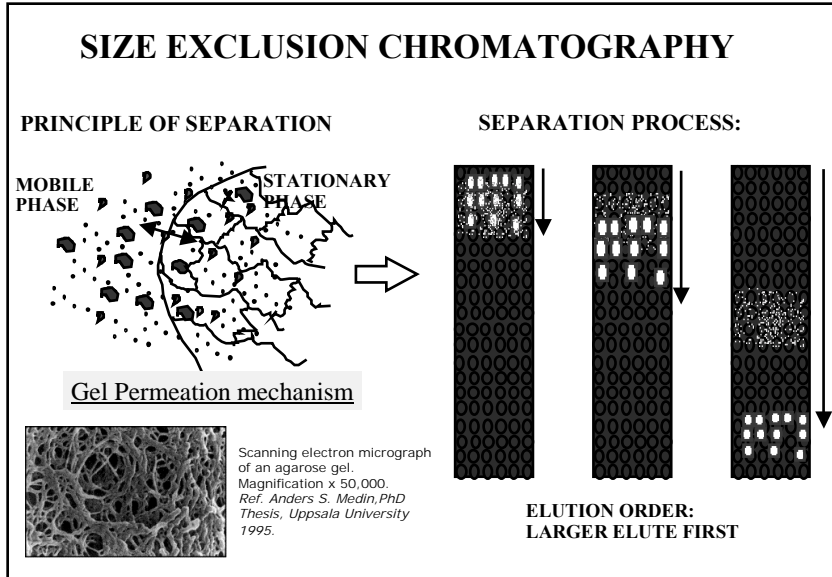
Chiral stationary phases:

- * Ligand exchange
- * π -Donor π -acceptor (Pirkle)
- * Chiral Host-guest (cyclodextrin)
- * Immobilized proteins
- * Immobilized polysaccharides



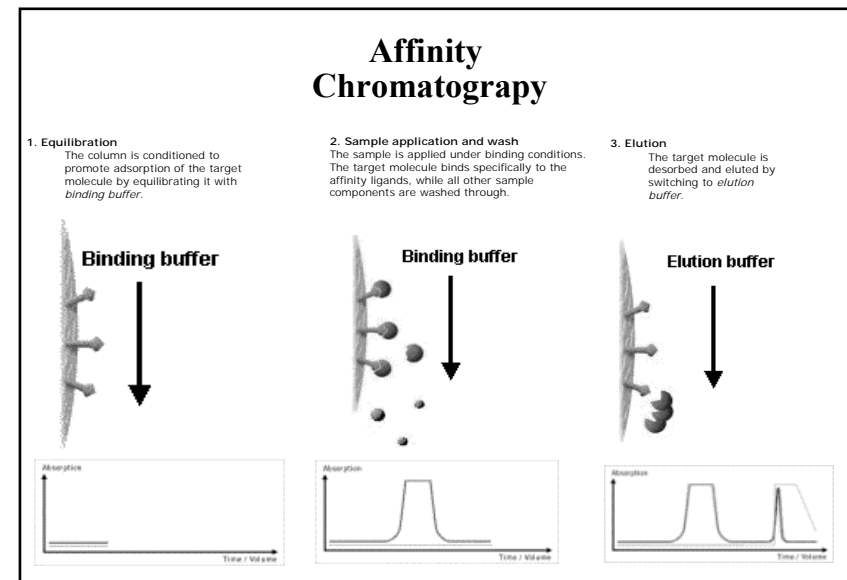
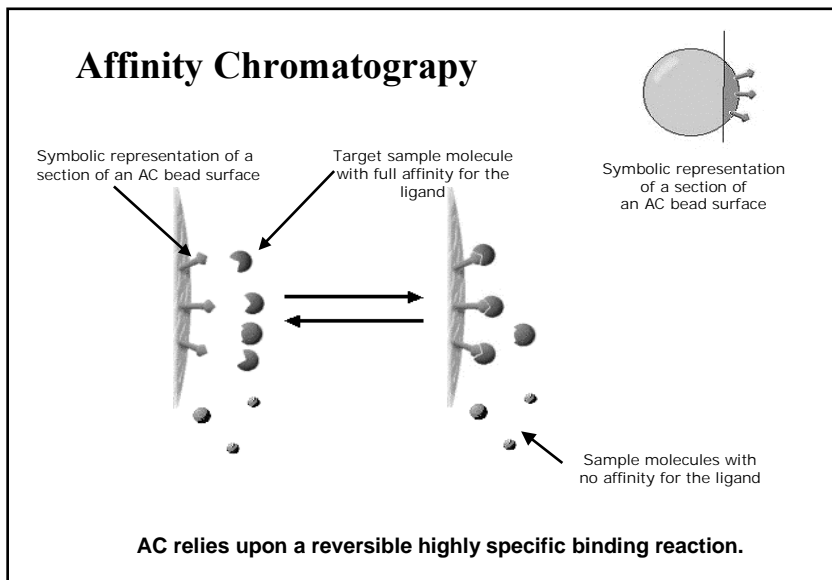
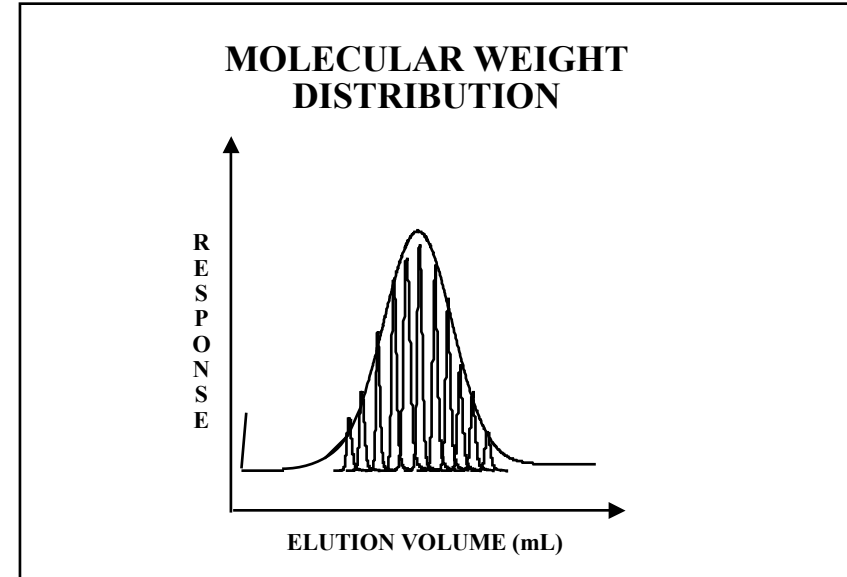
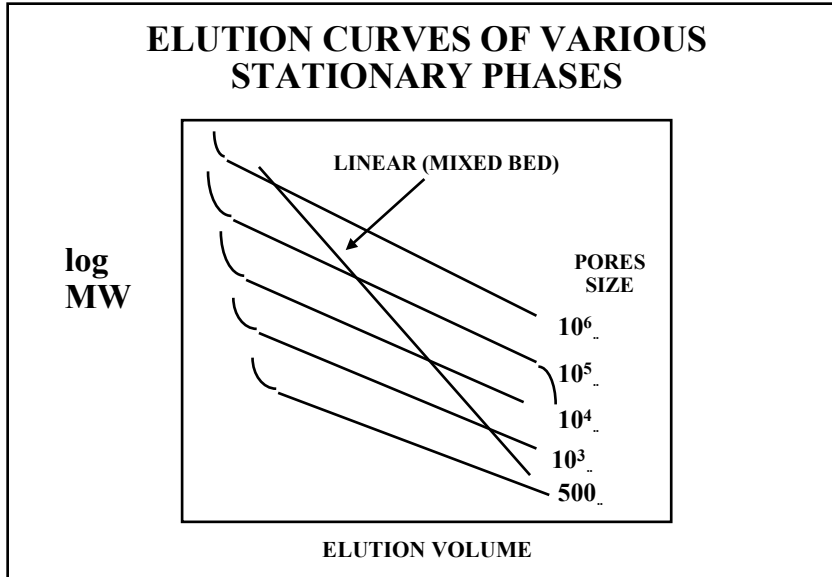
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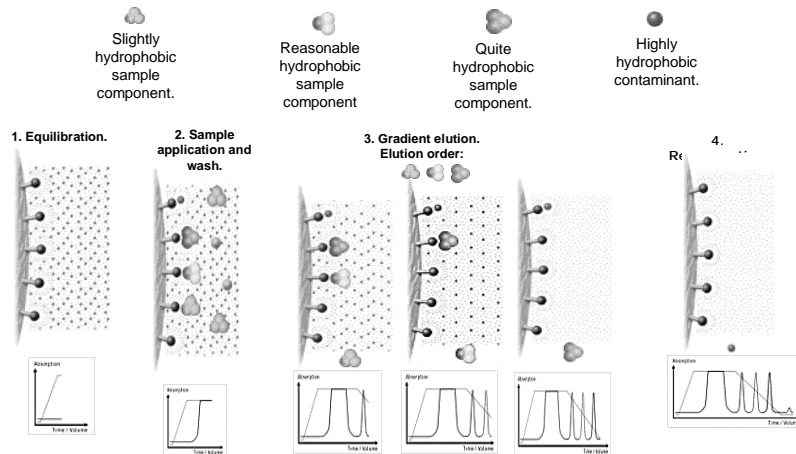
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High Performance Liquid Chromatography - HPLC

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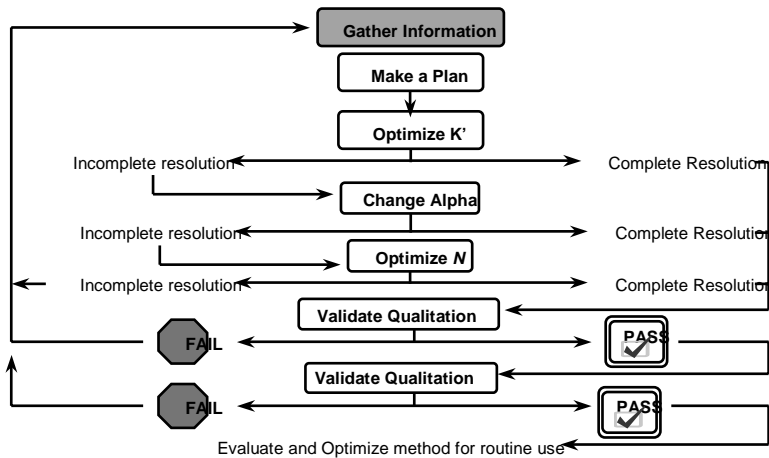
Hydrophobic Interaction Chromatography (HIC)



Seven Basic Considerations in Choosing HPLC Operating Parameters

- 1) Solubility - Hexane, Chloroform, Methanol, Water (buffer pH), other?
- 2) Molecular Weight - Would GPC be useful in either the analysis or sample prep?
- 3) Functional Groups - Any ionizable groups? Acidic, Basic, or Neutral?
- 4) Sample Matrix - What amounts are expected in matrix for either analytical or preparative isolation?
- 5) Levels in Matrix - What amounts are expected in matrix for either analytical or preparative isolation?
- 6) Detectability - Any chromophores or fluorophores? Consider Redox or derivatization. Together with point #5, an appropriate detector is chosen.
- 7) How Do Species Differ - An important clue to manipulate selectivity the separation, especially if compounds are similar in their structure.

Methods Development Strategy



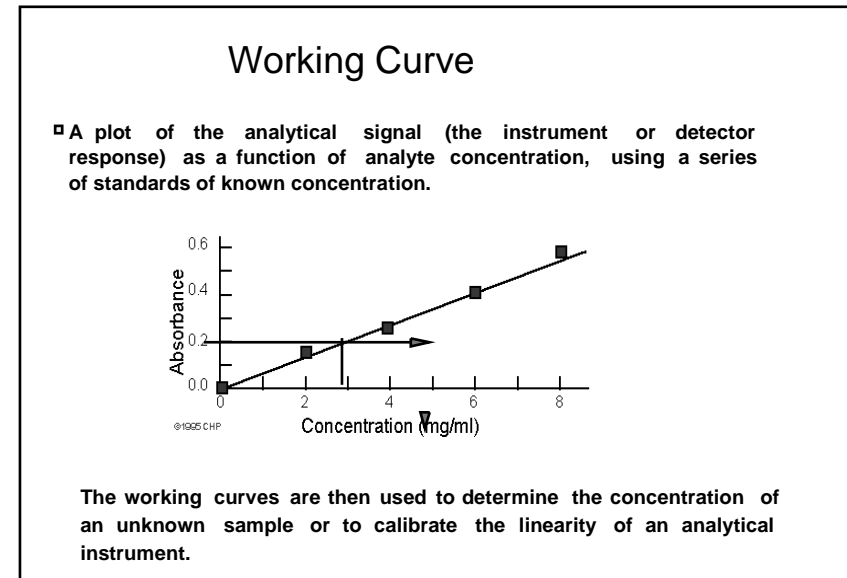
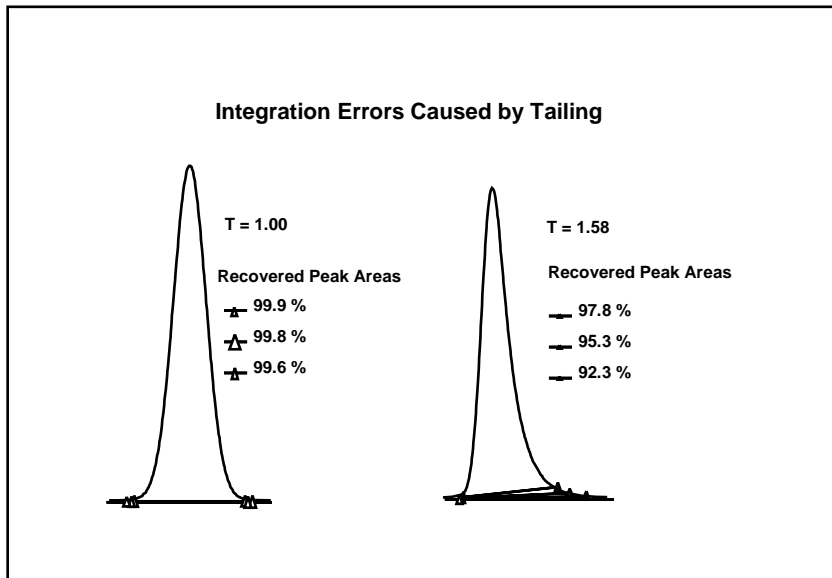
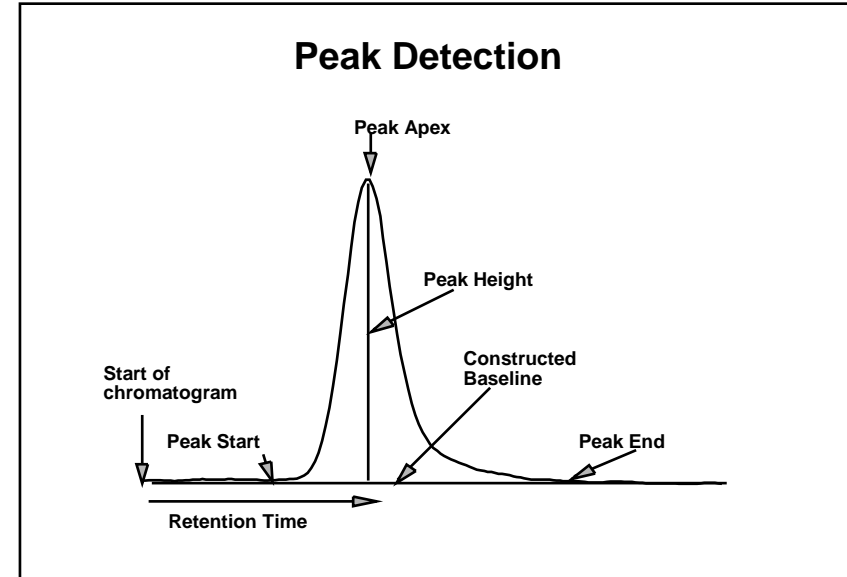
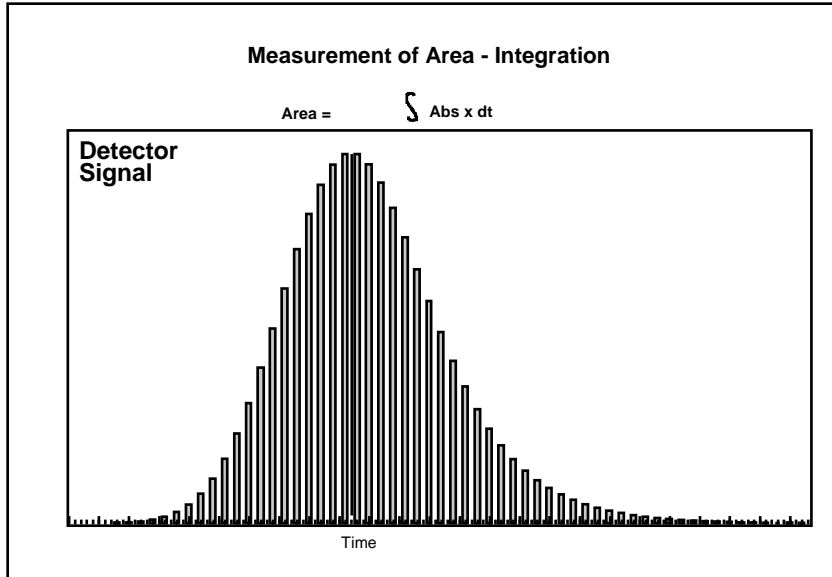
- Step by step method development strategy -

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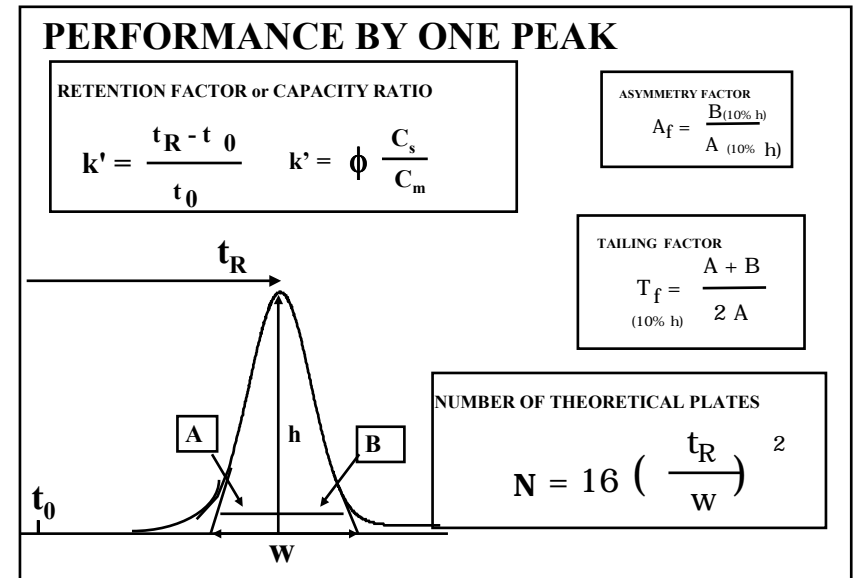
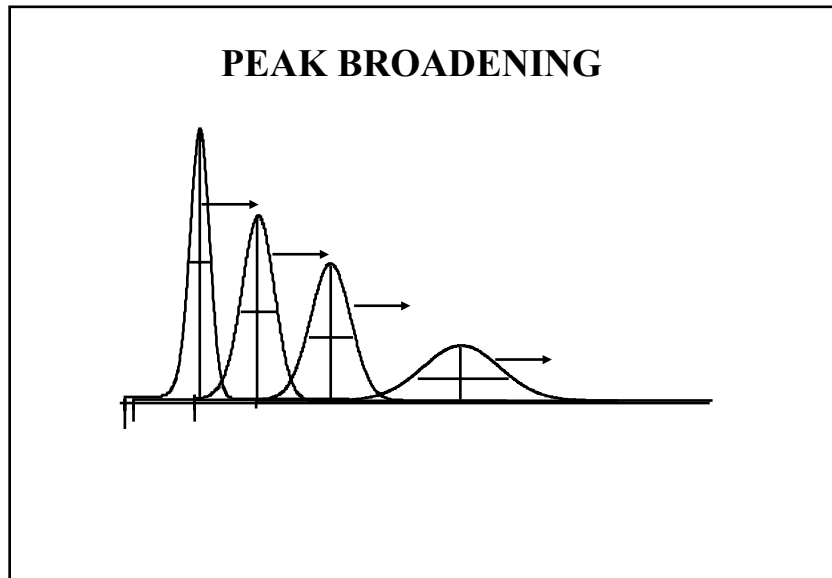
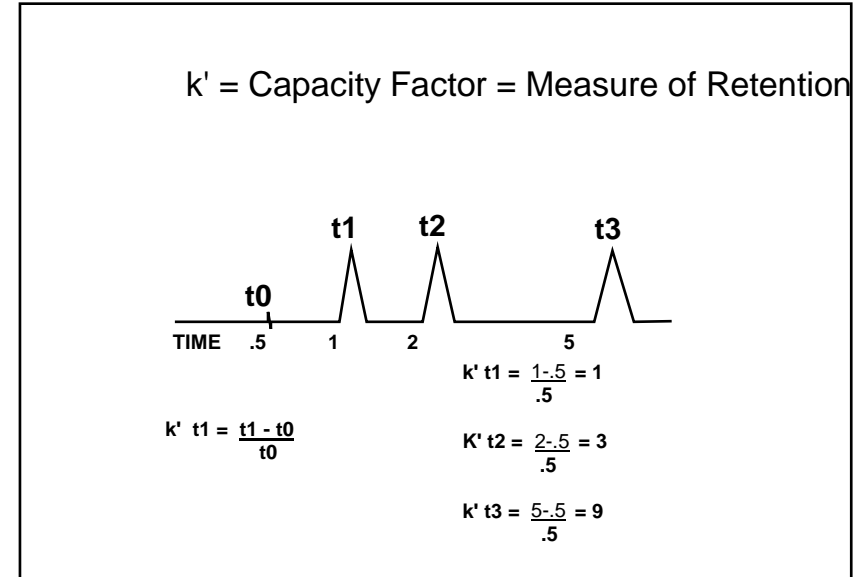
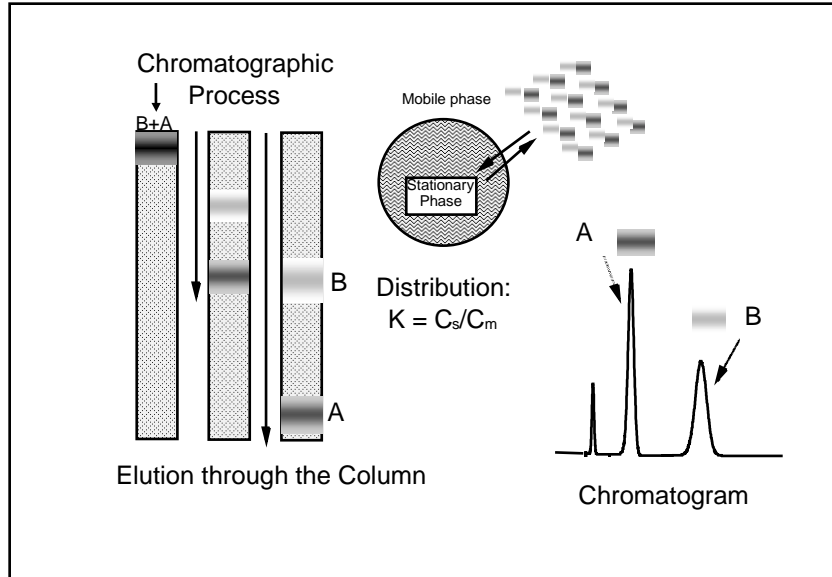
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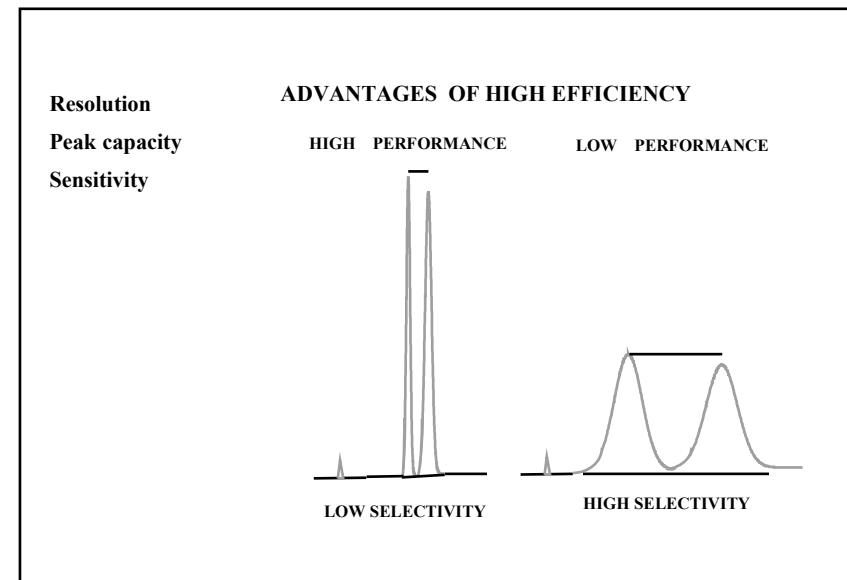
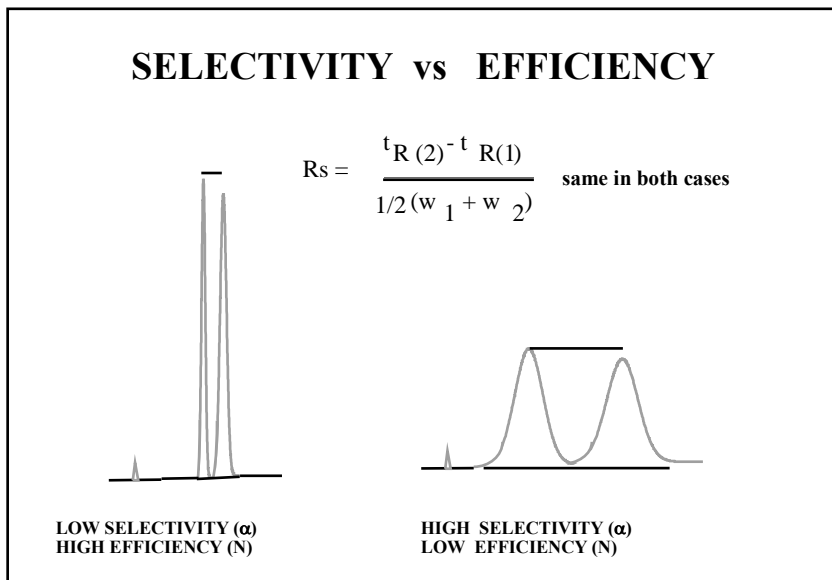
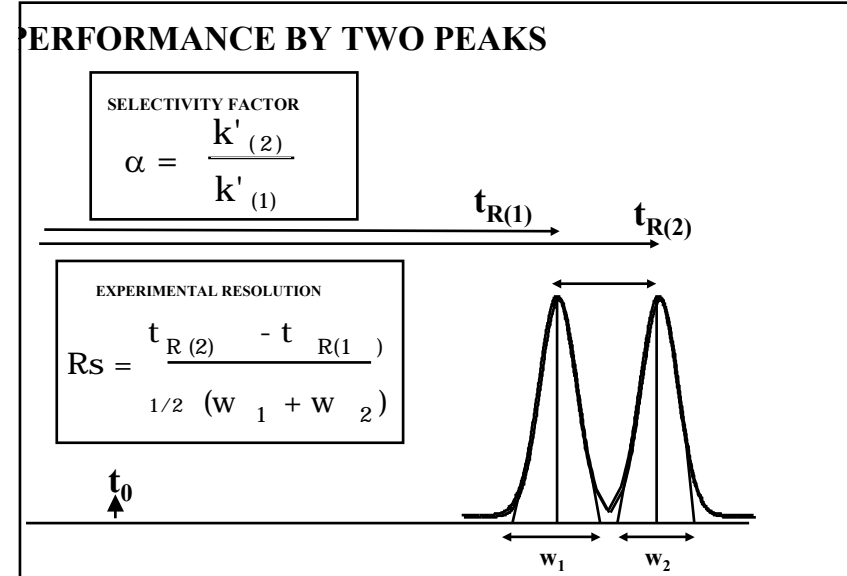
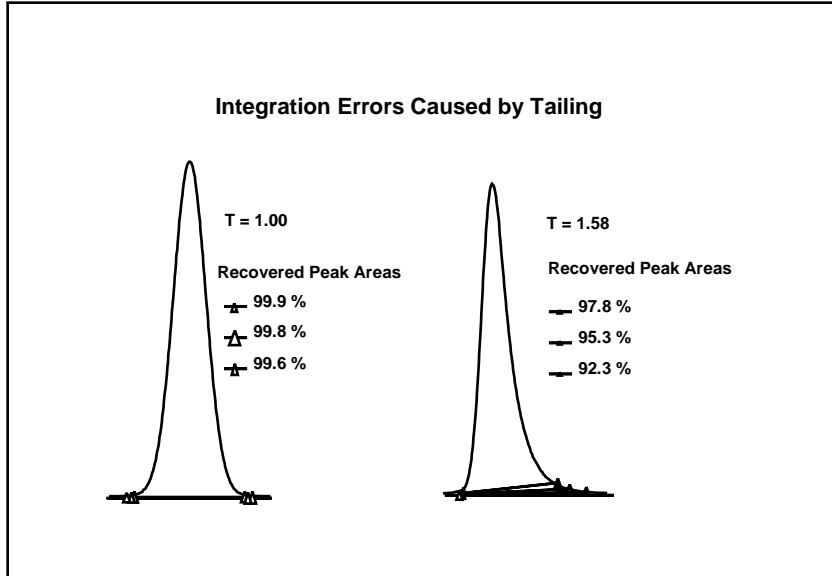
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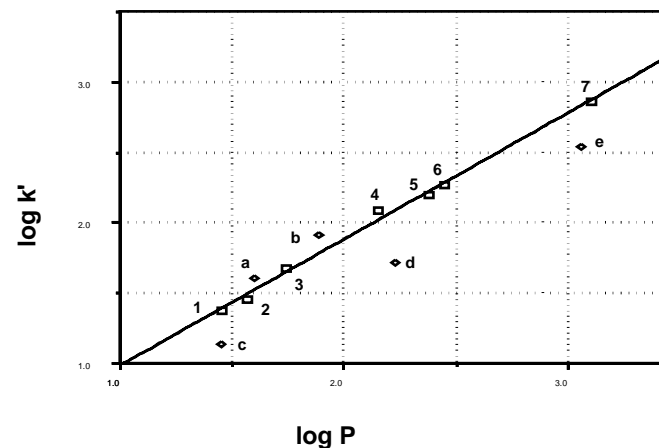
Introduction

Reversed Phase HPLC

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Medtechnica

http://www.Geocities.com/CapeCanaveral/8775/HPLC_guide_h.html

Hydrophobicity

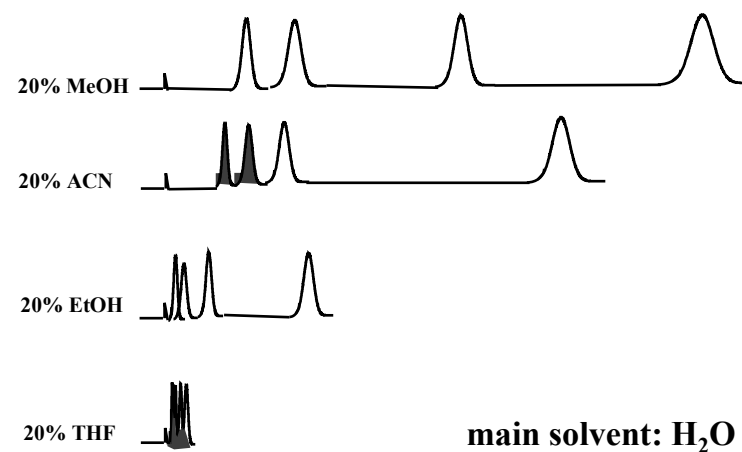


MOBILE PHASE

- * TYPE OF MODIFIER (MeOH, ACN)
- * SOLVENT STRENGTH (% modifier)
- * pH
- * TYPE OF BUFFER (phosphate, acetate)
- * IONIC STRENGTH (Salts, buffer concentration)
- * ION-PAIRING REAGENTS (alkyl-amines, -sulfonates)

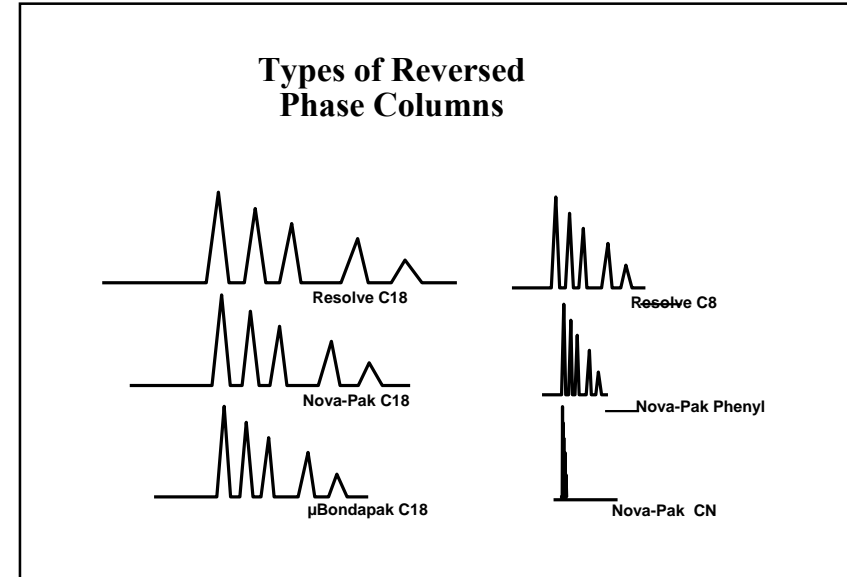
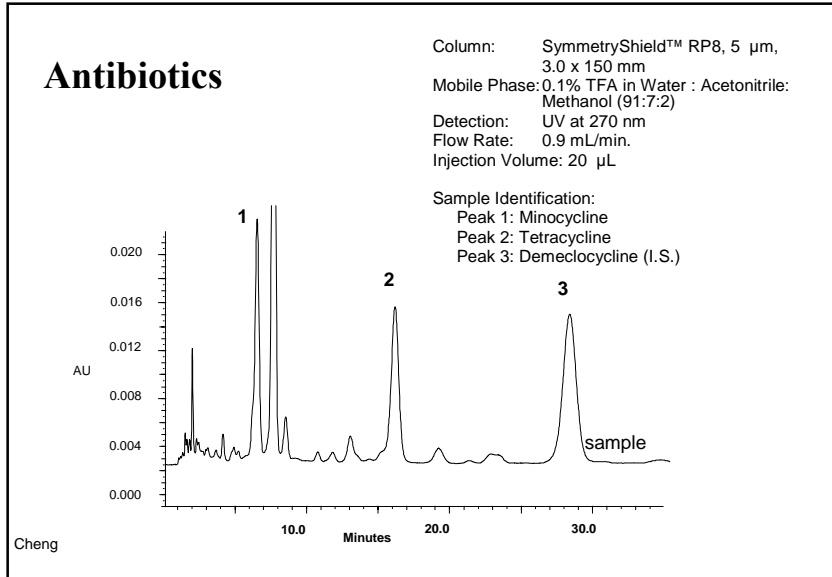
OPTIMIZATION: CHOICE OF SOLVENTS

REVERSED PHASE



High Performance Liquid Chromatography - HPLC

Introduction



Stationary Phase Properties

CHEMISTRY:

- * BONDED HYDROCARBON: C-18, C-8, C-4, C-1, CN, phenyl
- * % COVERAGE
- * TYPE OF SILICA GEL

pores

silica

d

GEOMETRY

- * SPHERE- IRREGULAR
- * PARTICLE DIAMETER
- * POROSITY

Stationary Phase Supports

Stationary phase	Functionality
C ₁₈	-Si(CH ₃) ₂ C ₁₈ H ₃₇
C ₈	-Si(CH ₃) ₂ C ₈ H ₁₇
tC ₂	-SiC ₂ H ₅
Aminopropyl	-Si(CH ₃) ₂ NH ₂
Cyanopropyl	-Si(CH ₃) ₂ (CH ₂) ₃ CN
Diol	-Si(CH ₃) ₂ O CH ₂ CH(OH)CH ₂ OH

Retention time

Chain length CN Phenyl NH₂ C₄ C₈ C₁₈

High Performance Liquid Chromatography - HPLC

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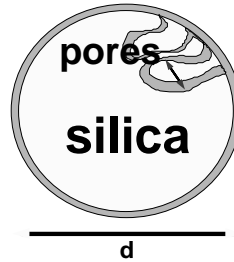
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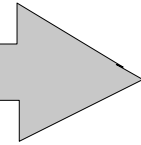


CARBON LOAD

Increasing carbon load on a similar geometrical shaped particles increases retention.

Retention time

Carbon load 5% 7% 9% 12% 15% 17%



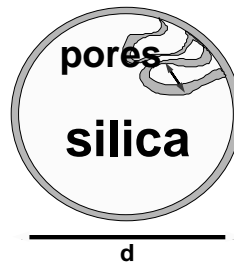
Stationary Phase Properties

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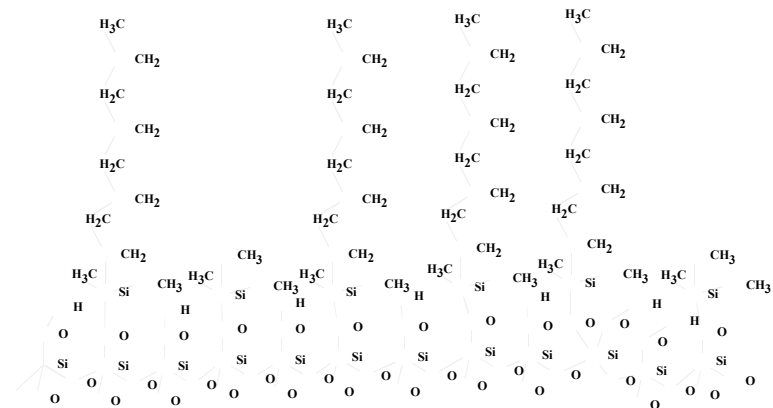
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Surface of a Reversed- Phase Packing

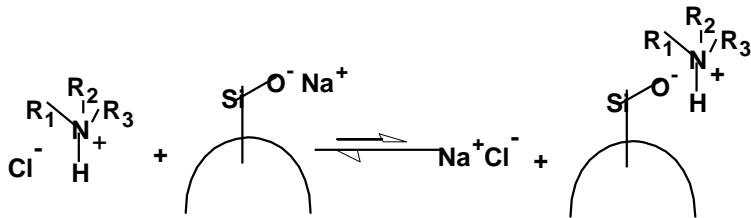


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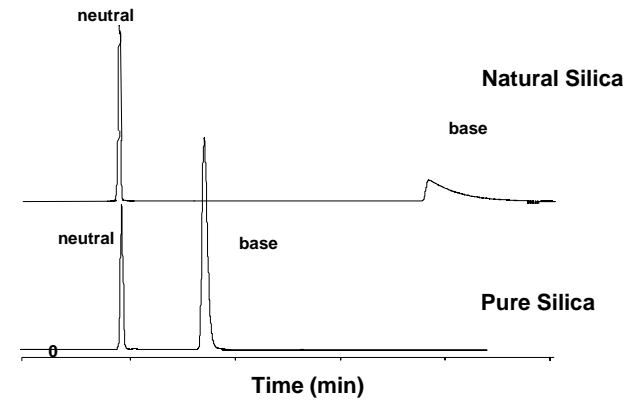
Introduction

What Causes Tailing?

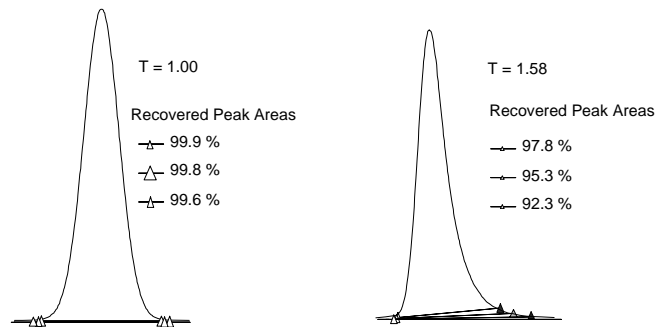
- Mixed-mode retention:
 - hydrophobic - interaction with bonded phase
 - ion exchange - interaction with charged sites



Quality of Columns Performance



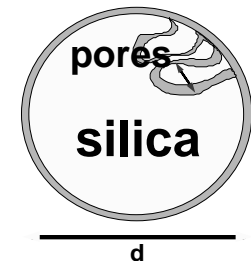
Integration Errors Caused by Tailing



Stationary Phase Properties

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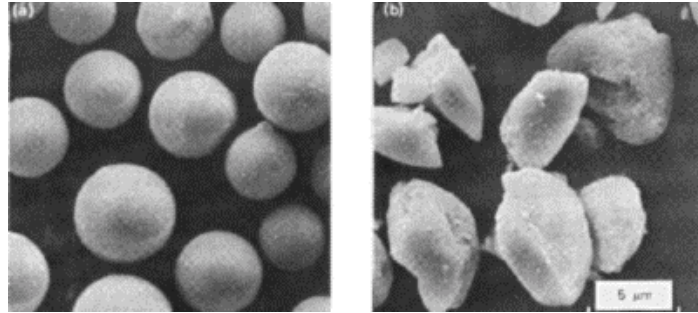
- GEOMETRY**
- * SPHERE- IRREGULAR
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 - * POROSITY



High Performance Liquid Chromatography - HPLC

Introduction

Spherical and Irregular particles

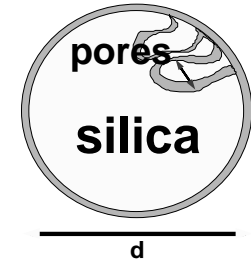


Electron microphotograph of spherical and irregular silica particles. [W.R.Melander, C.Horvath, Reversed-Phase Chromatography, in HPLC Advances and Perspectives, V2, Academic Press, 1980]

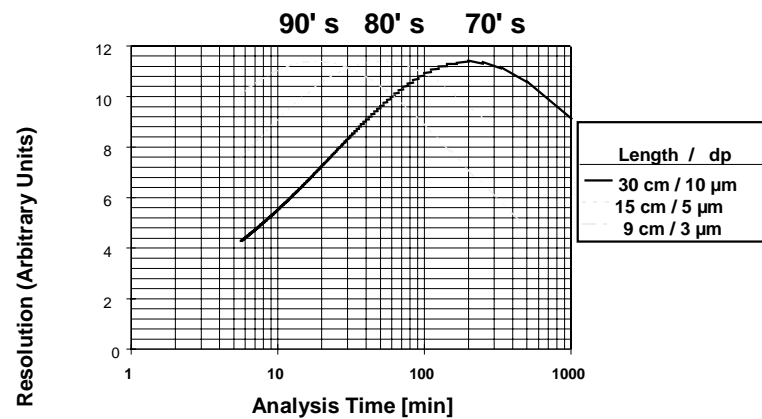
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 * POROSITY



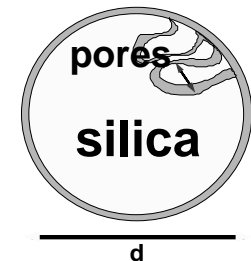
Resolution - Time Diagram



Stationary Phase Properties

CHEMISTRY:
 * BONDED HYDROCARBON:
 C-18, C-8, C-4, C-1, CN, phenyl
 * % COVERAGE
 * TYPE OF SILICA GEL

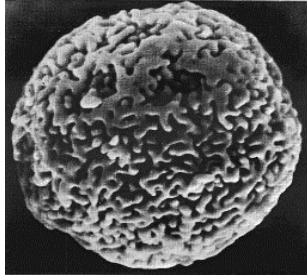
GEOMETRY
 * SPHERE- IRREGULAR
 * **PARTICLE DIAMETER**
 * POROSITY



High Performance Liquid Chromatography - HPLC

Introduction

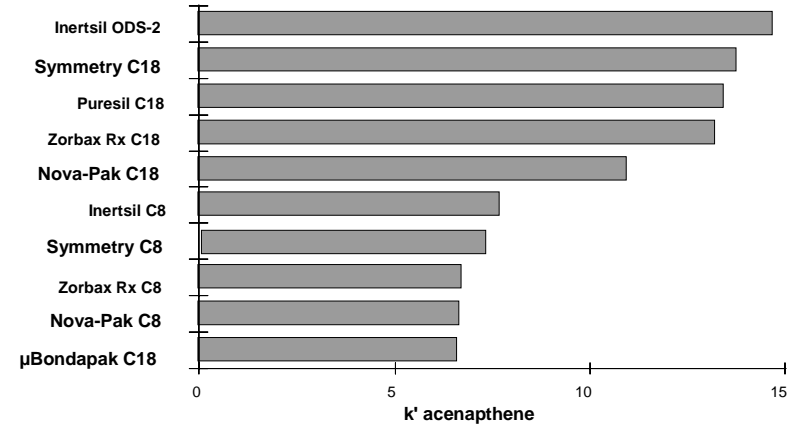
Pore size, shape and distribution



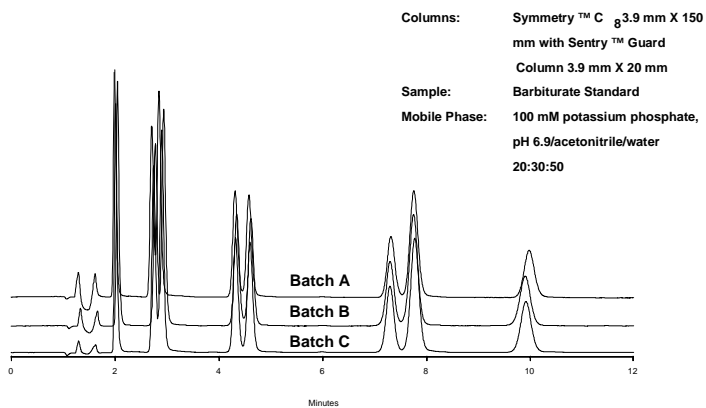
▣ Macroporous spherical silica particle. [K.K.Unger, Porous silica, Elsevier, 1979]

Pore size defines an ability of the analyte molecules to penetrate inside the particle and interact with its inner surface. This is especially important because the ratio of the outer particle surface to its inner one is about 1:1000. The surface molecular interaction mainly occurs on the inner particle surface.

Relative Hydrophobicities of General Purpose Analytical Packings



Batch-to-Batch Reproducibility of Columns



Chromatogram of lifetime test

