

Introduction to High Performance Liquid Chromatographic (HPLC) Modes

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

Introduction

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What does HPLC mean?

High pressure liquid chromatography

High priced liquid chromatography

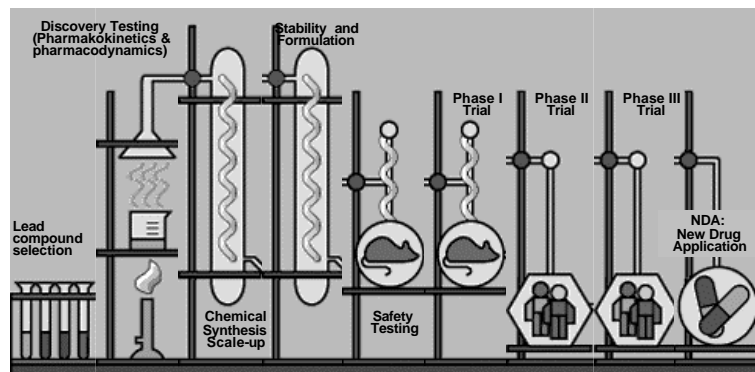
Hewlett-Packard liquid chromatography

High performance liquid chromatography

Hocus pocus liquid chromatography

High patience liquid chromatography

HPLC in Pharmaceuticals Technique No 1



APPLICATIONS OF HPLC

Veterinary



Environmental



Agriculture &
Food

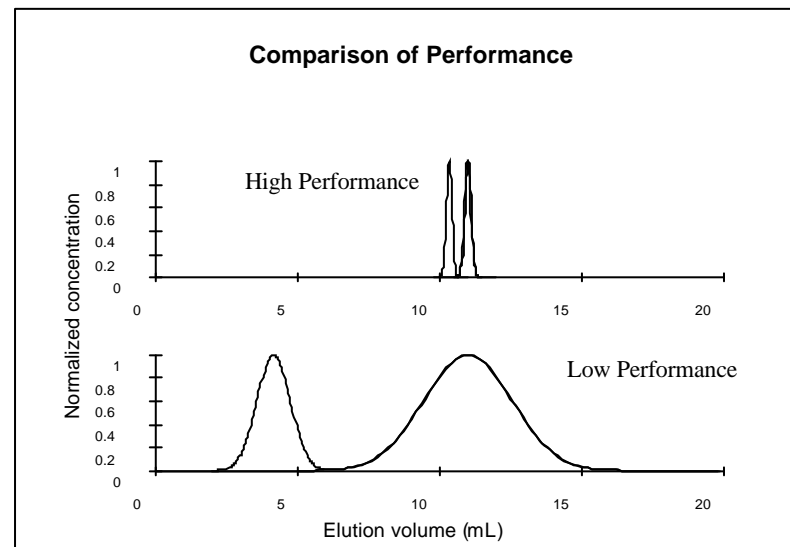
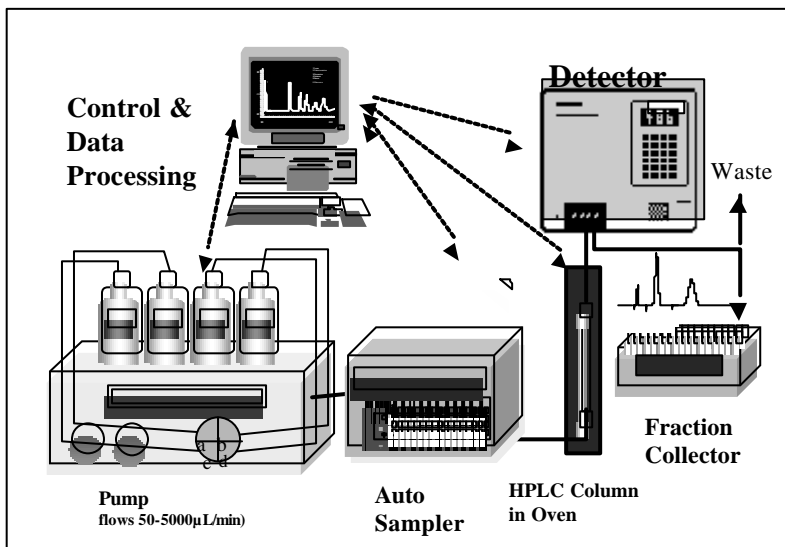
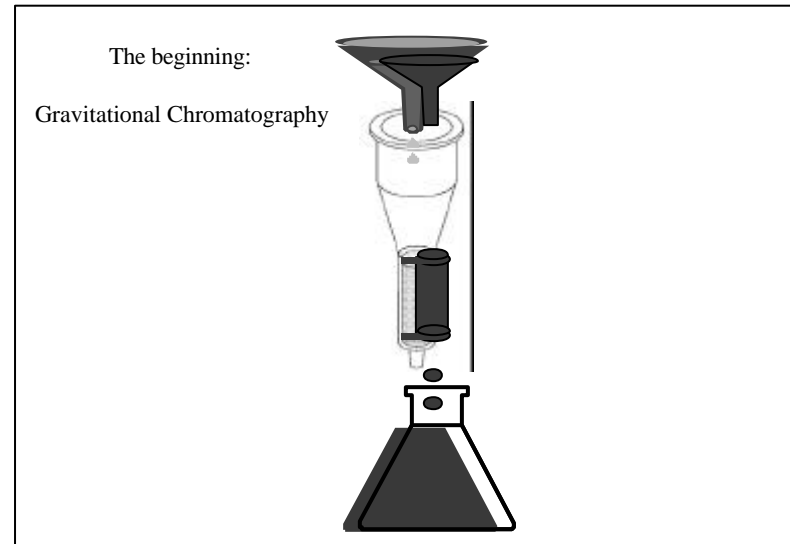
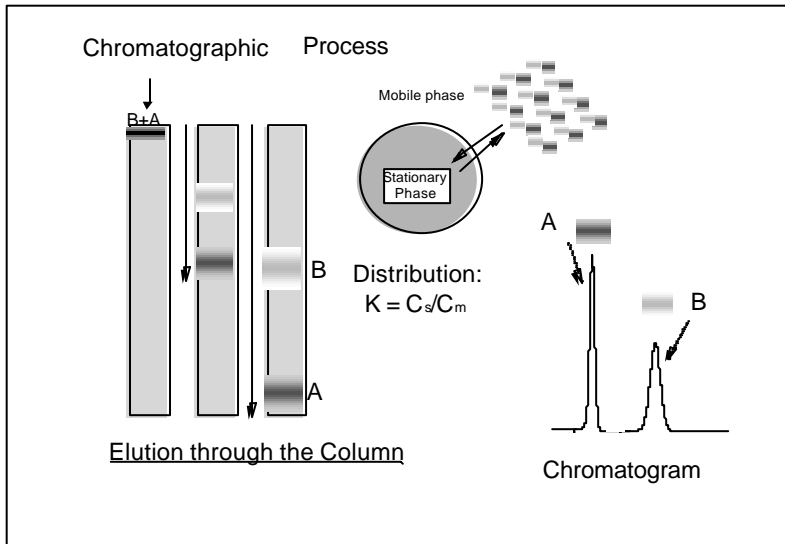


Biomedical and Clinical



Chemistry

Introduction

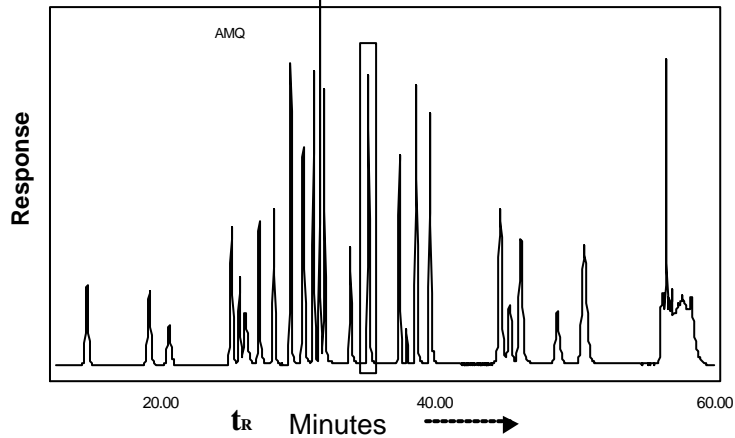


Introduction

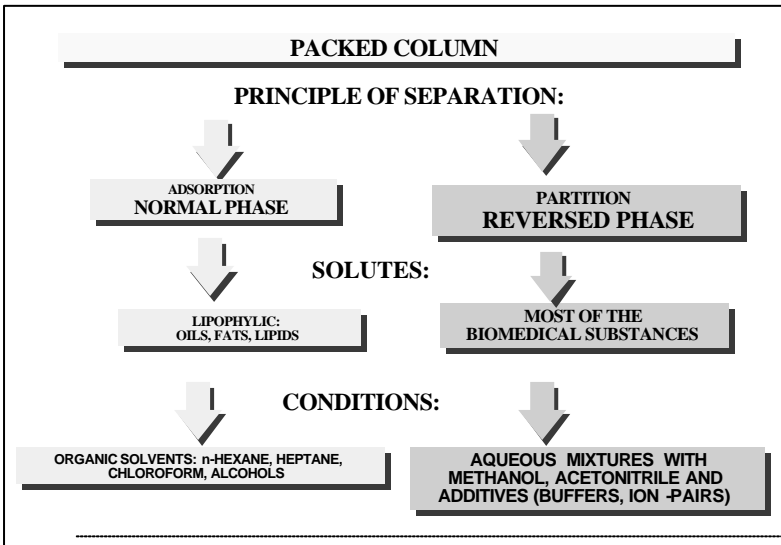
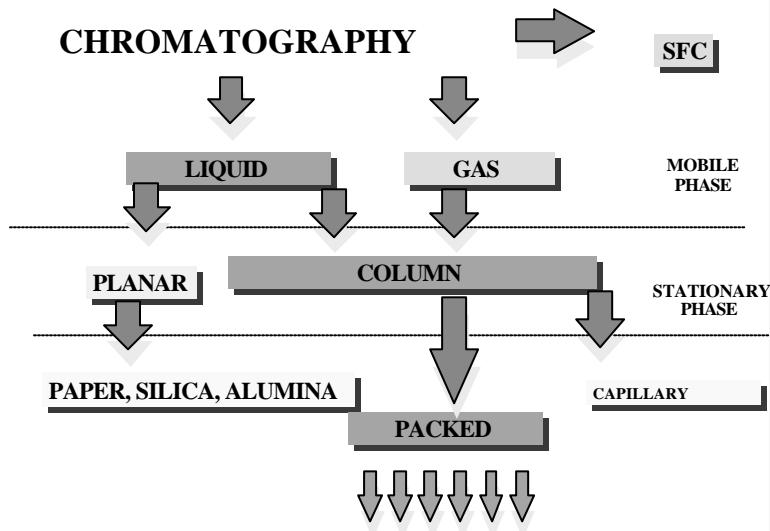
HPLC COURSE LAYOUT

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

Quantitative Determination and Identification



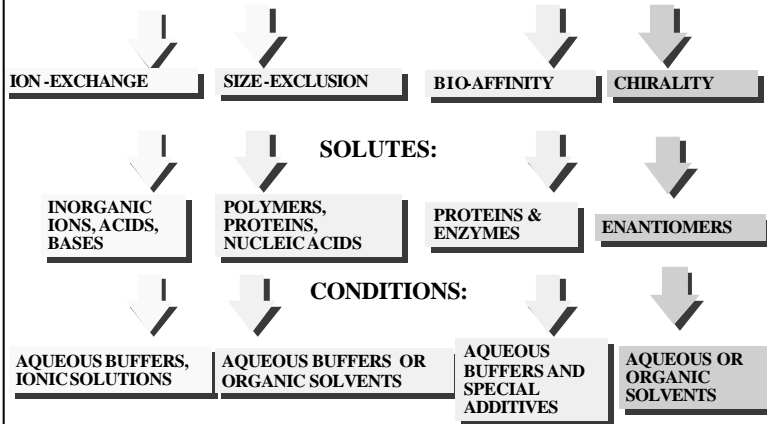
CHROMATOGRAPHY



Introduction

High Performance Liquid Chromatography

PRINCIPLE OF SEPARATION:



REVERSED PHASE

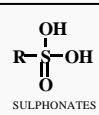
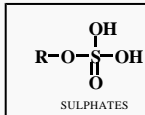
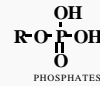
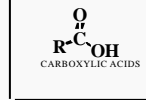
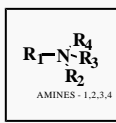
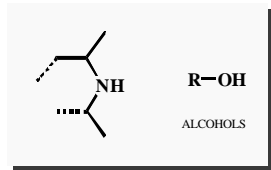
SOLUTES:

MOST OF THE BIOMEDICAL SUBSTANCES

CONDITIONS:

AQUEOUS MIXTURES WITH METHANOL, ACETONITRILE AND ADDITIVES (BUFFERS, ION -PAIRS)

IONIZABLE



MOBILE PHASE

SOLVENTS:
water, methanol, acetonitrile

ADDITIVES:
buffers, salts, ion-pairing reagents, complexants.



STATIONARY PHASE

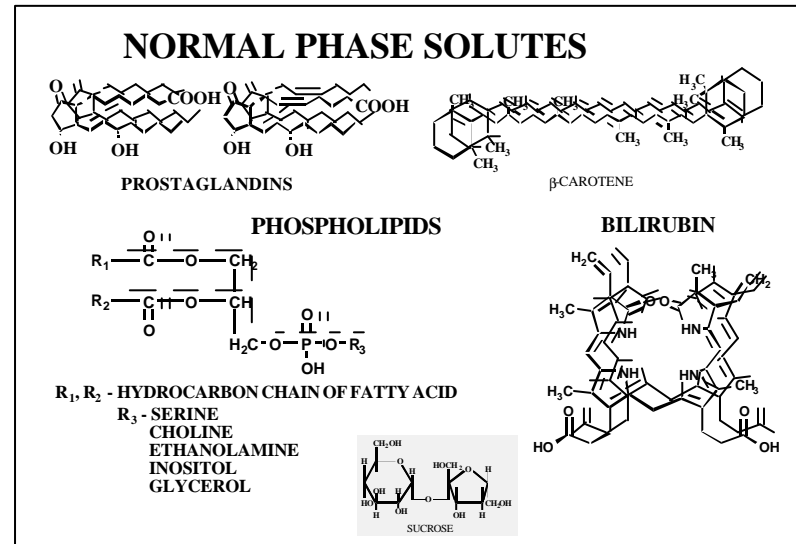
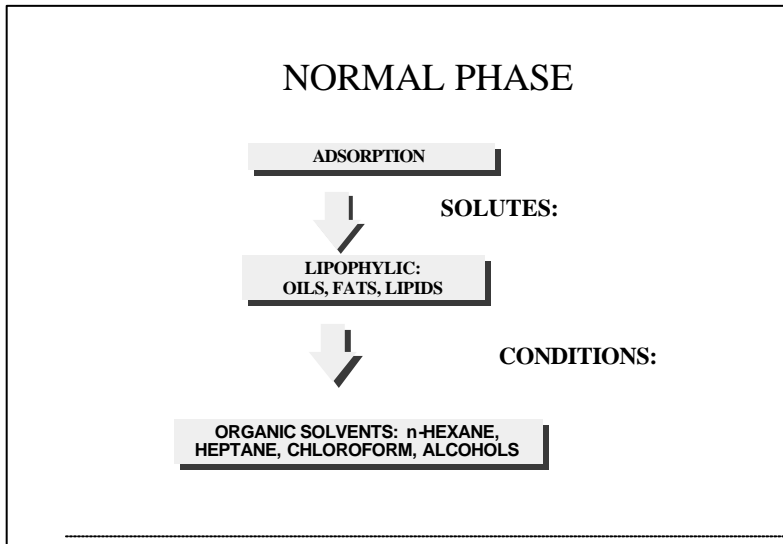
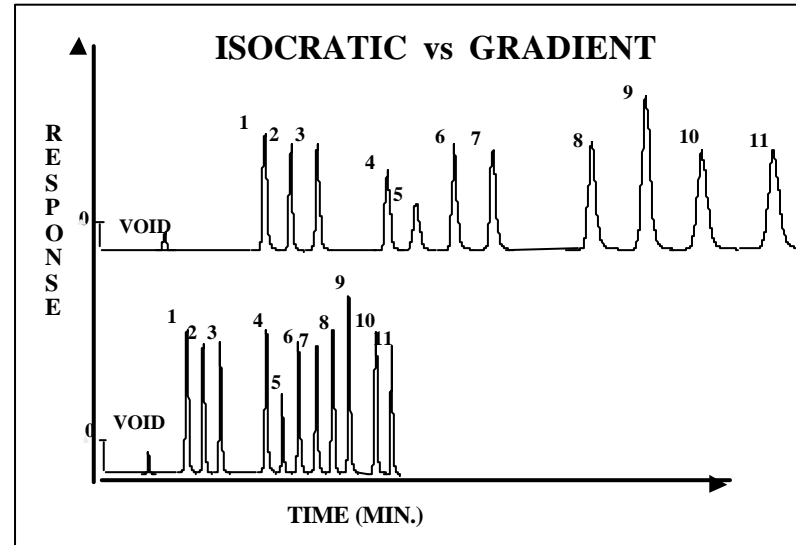
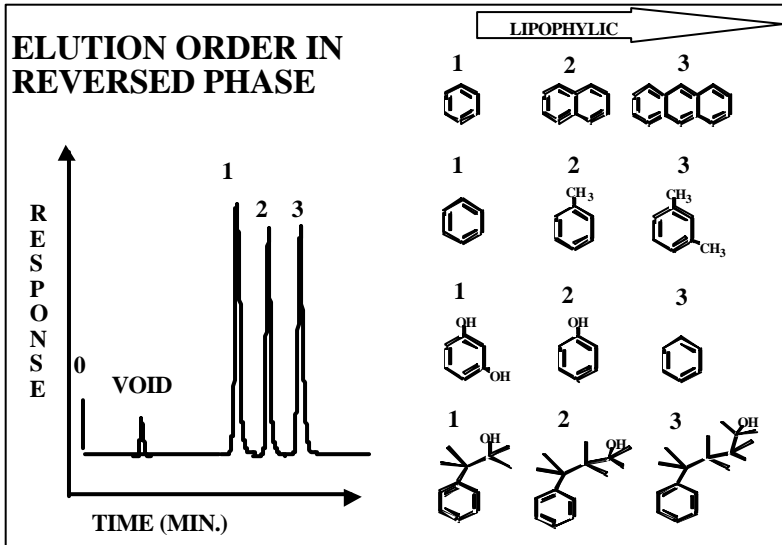
CHEMISTRY:

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

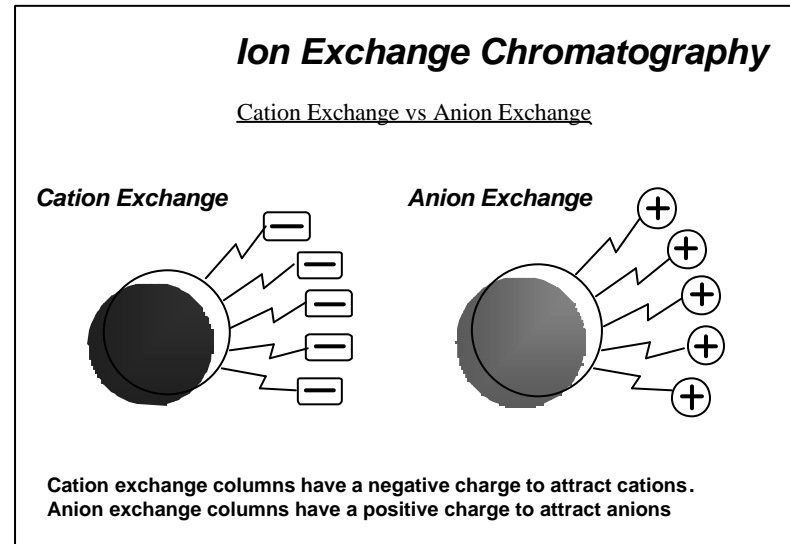
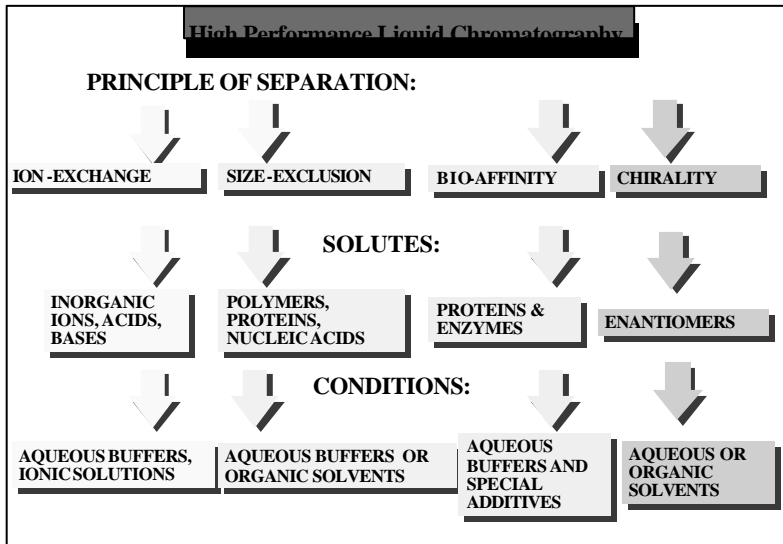
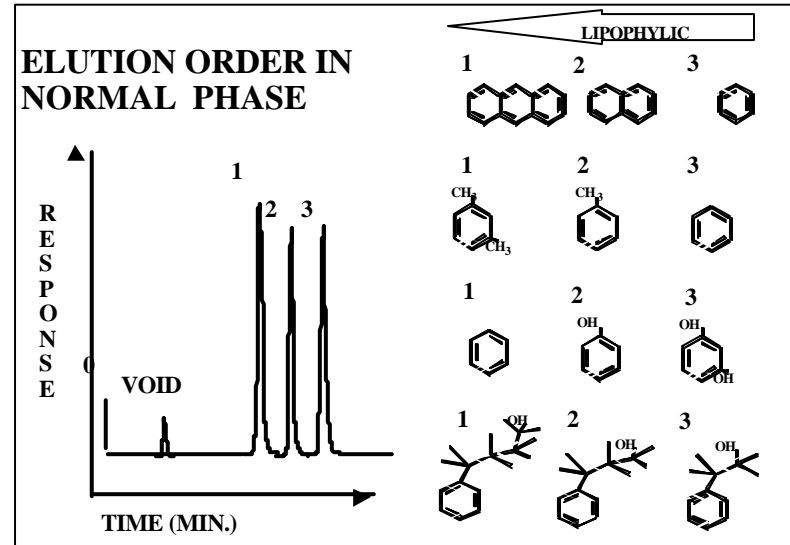
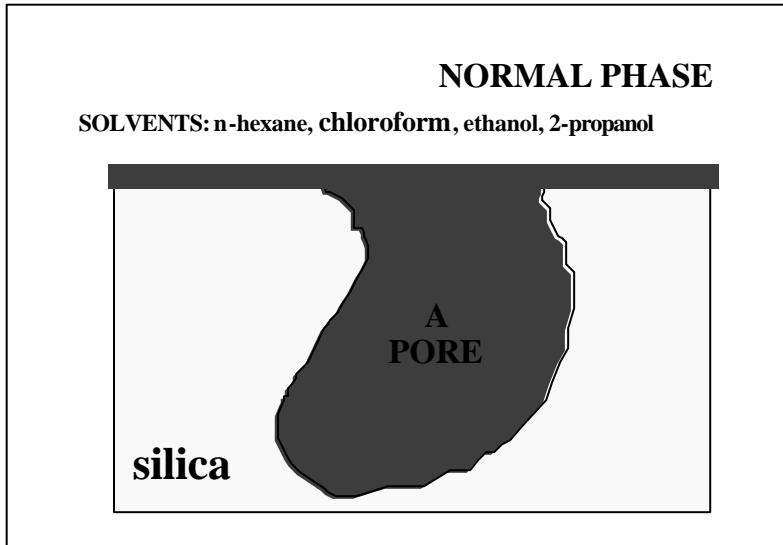
GEOMETRY

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

Introduction



Introduction



Introduction

Ion Exchange Chromatography

Strong vs. Weak Exchange Materials

Cation exchanger



STRONG

Anion exchanger



WEAK

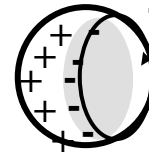


Strong Exchangers stay ionized as pH varies between 2 and 12.
Weak exchangers can lose ionization as a function of pH.

ION EXCHANGE

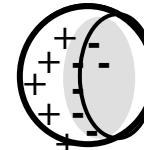
INSIDE A PORE IN THE STATIONARY PHASE

SAMPLE IONS IN



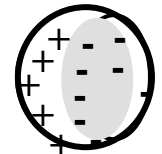
1. INJECTION

COUNTERIONS OUT



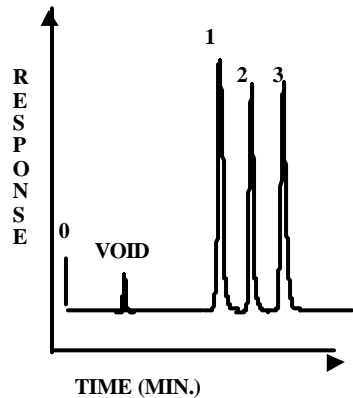
2. ADSORPTION:
DISPLACEMENT OF
COUNTER IONS

MOBILE PHASE
ADDITIVES



3. ELUTION

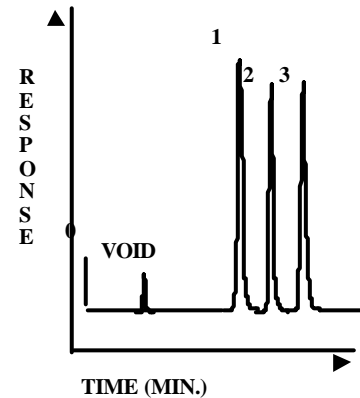
ELUTION ORDER IN ANION EXCHANGE



DENSITY OF CHARGE

1	2	3
OAc^-	OH^-	F^-
1	2	3
Cl^-	NO_3^-	NO_2^-
1	2	3
NO_3^-	SO_4^-	SO_3^-

ELUTION ORDER IN CATION EXCHANGE

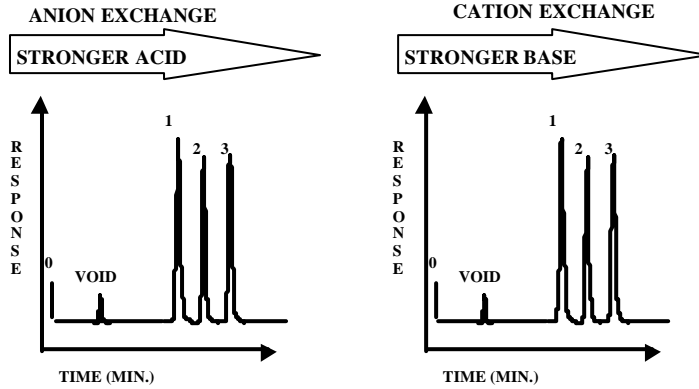


DENSITY OF CHARGE

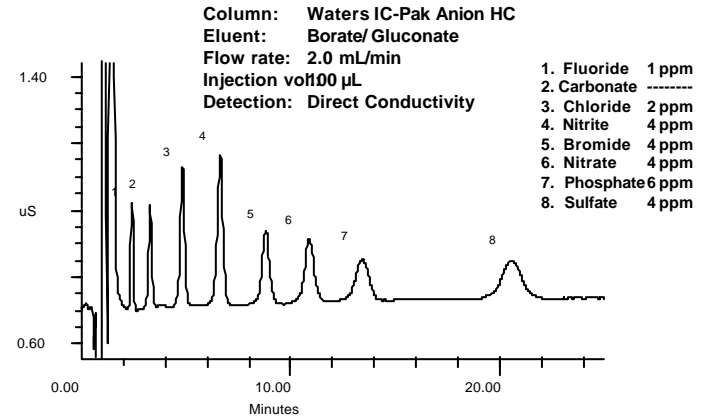
1	2	3
K^+	Na^+	Li^+
1	2	3
Ag^+	Zn^{++}	Al^{+++}

Introduction

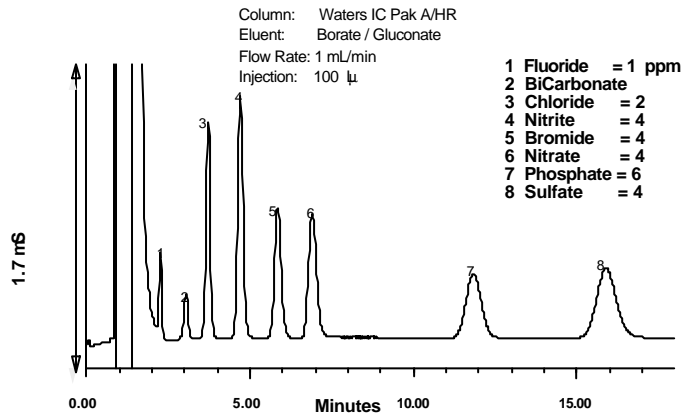
ELUTION ORDER IN ION EXCHANGE



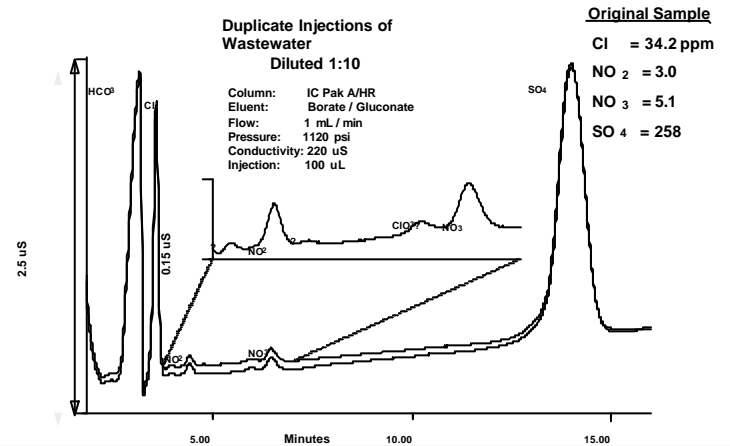
Analysis of Ions



Analysis of Anions

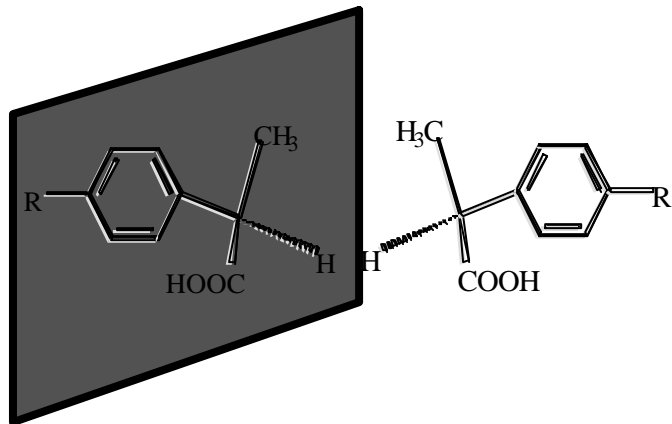


Analysis of Anions in Waste Water

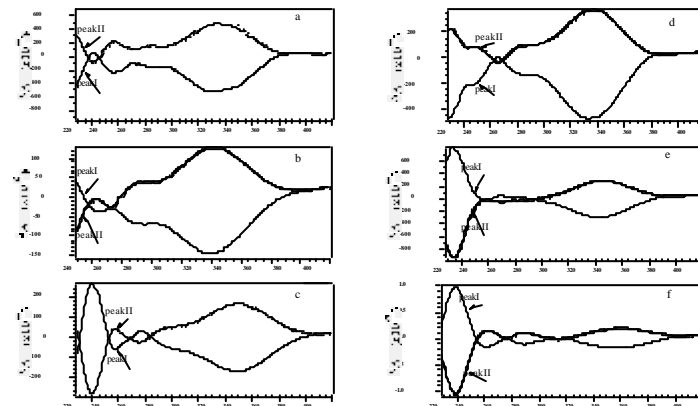


Introduction

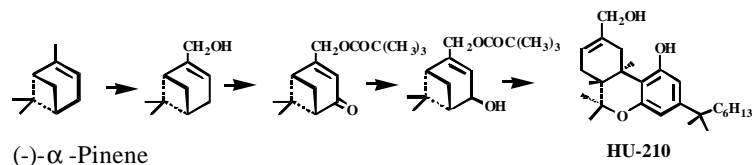
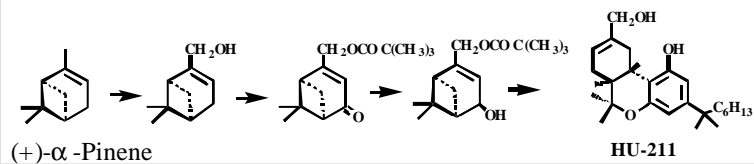
ENANTIOMERS: MIRROR IMAGES OF ONE ANOTHER



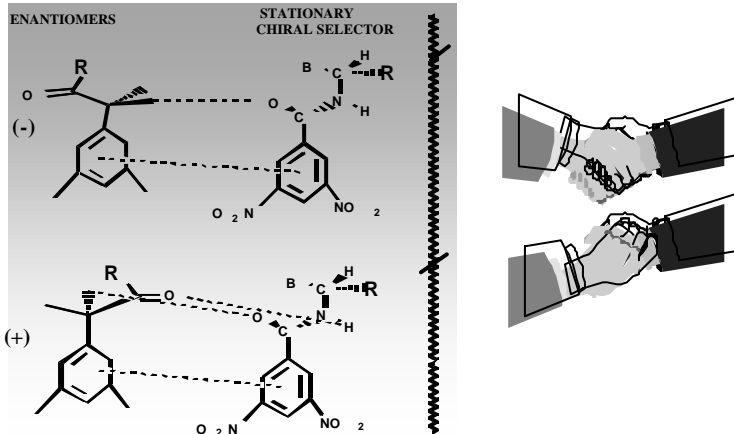
Circular Dichroism SPECTRA



Asymmetric Synthesis



BASIS FOR SEPARATION: CHIRAL RECOGNITION

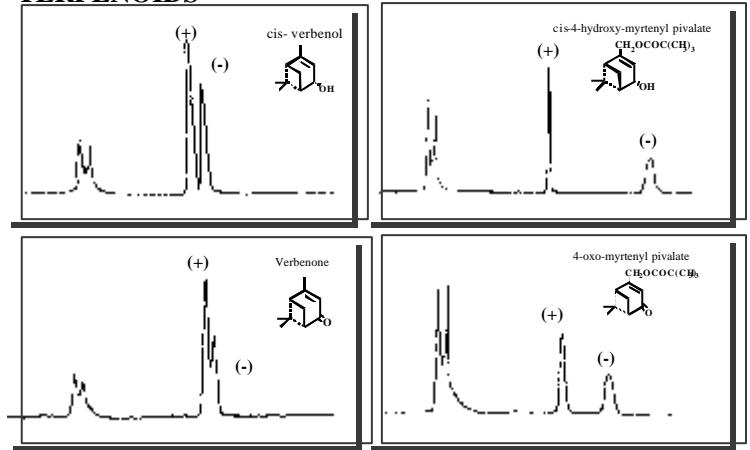


Introduction

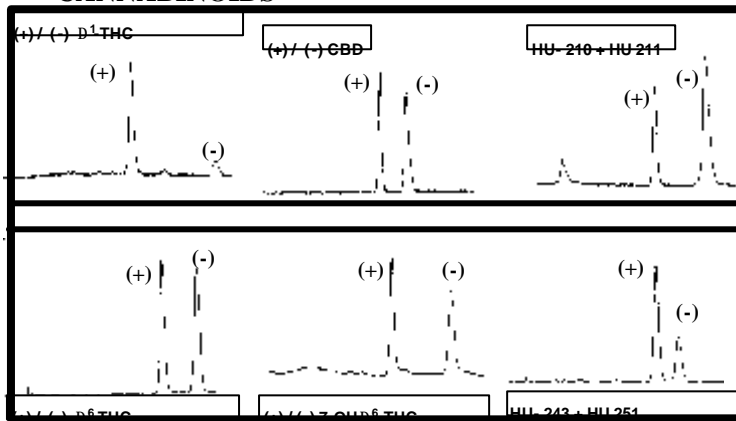
Chiral stationary phases:

- ❖ Ligand exchange
- ❖ p-Donor p-acceptor (Pirkle)
- ❖ Chiral Host-guest (cyclodextrin)
- ❖ Bonded macrocyclic antibiotics
- ❖ Immobilized/bonded proteins
- ❖ Immobilized/bonded polysaccharides

SEPARATION OF ENANTIOMERS OF TERPENOIDS



SEPARATION OF 6 ENANTIOMERIC PAIRS OF CANNABINOIDS

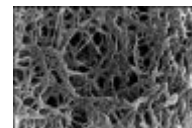


SIZE EXCLUSION CHROMATOGRAPHY

PRINCIPLE OF SEPARATION

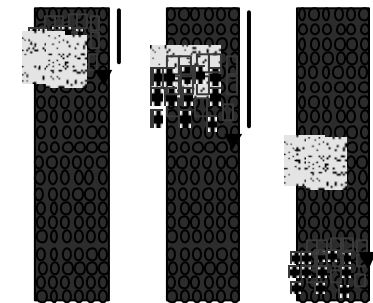


Gel Permeation mechanism



Scanning electron micrograph of an agarose gel. Magnification x 50,000. Ref. Anders S. Medin, PhD Thesis, Uppsala University 1995.

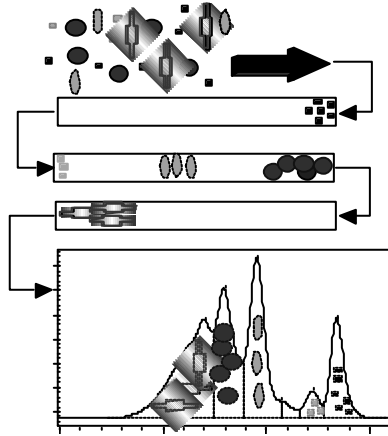
SEPARATION PROCESS:



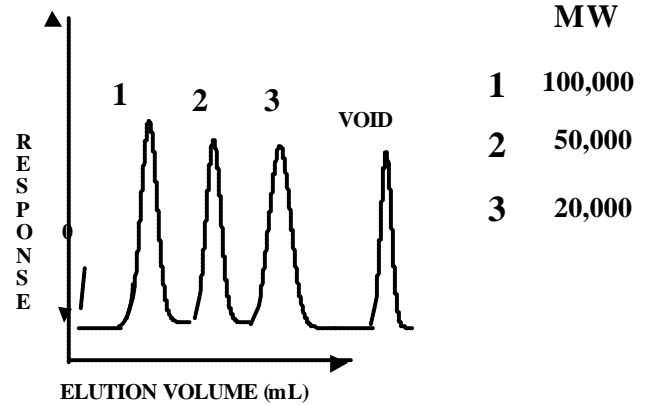
ELUTION ORDER:
LARGER ELUTE FIRST

Introduction

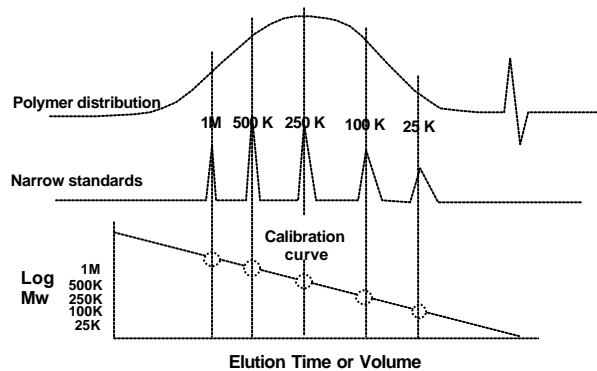
GPC Process



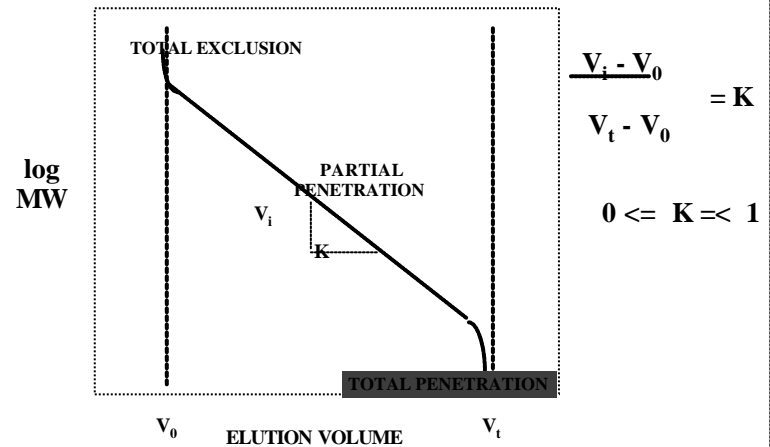
ELUTION ORDER IN SIZE EXCLUSION (GPC)



Gel Filtration/Size Exclusion/Gel Permeation

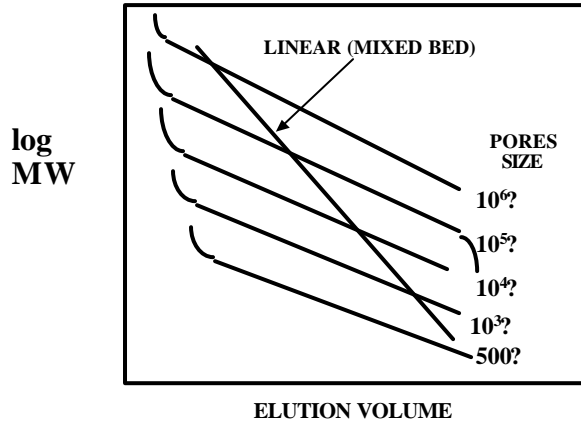


THEORETICAL CURVE OF THE STERIC EXCLUSION

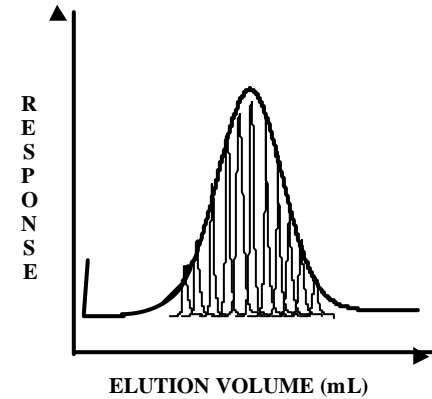


Introduction

ELUTION CURVES OF VARIOUS STATIONARY PHASES

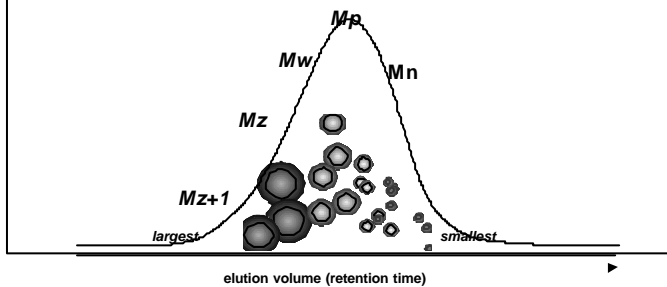


MOLECULAR WEIGHT DISTRIBUTION

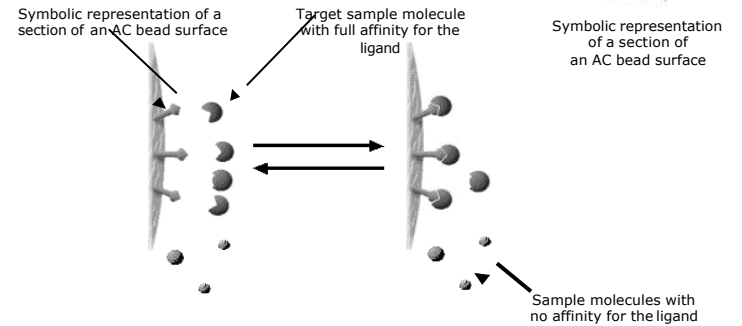


What is GPC?

► The elution profile represents the *molecular weight distribution* based upon the relative content of different molecular weights...



Affinity Chromatography



AC relies upon a reversible highly specific binding reaction.

Introduction

Affinity Chromatography

1. Equilibration

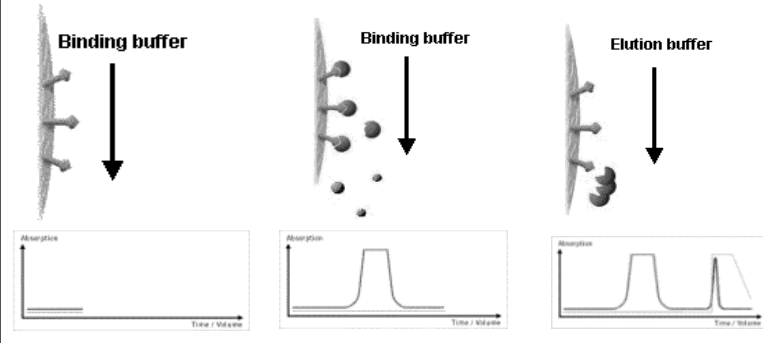
The column is conditioned to promote adsorption of the target molecule by equilibrating it with binding buffer.

2. Sample application and wash

The sample is applied under binding conditions. The target molecule binds specifically to the affinity ligands, while all other sample components are washed through.

3. Elution

The target molecule is desorbed and eluted by switching to elution buffer.



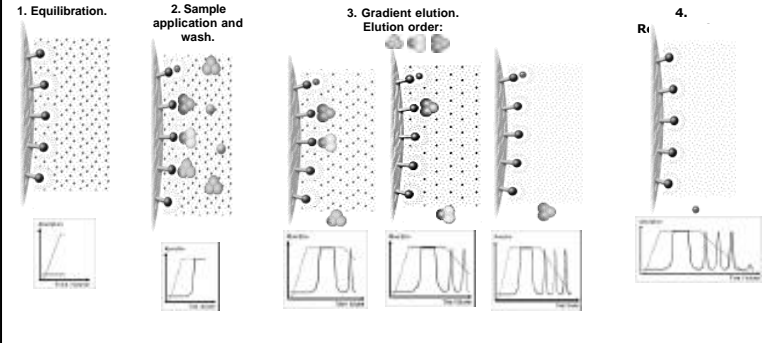
Hydrophobic Interaction Chromatography (HIC)

Slightly hydrophobic sample component.

Reasonable hydrophobic sample component.

Quite hydrophobic sample component.

Highly hydrophobic contaminant.



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

