

# High Performance Liquid Chromatography - HPLC

## Introduction

High Performance Liquid Chromatography (HPLC) for  
Pharmaceutical Analysis

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Homepage:

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

What does HPLC mean?

High Pressure Liquid Chromatography

High Priced Liquid Chromatography

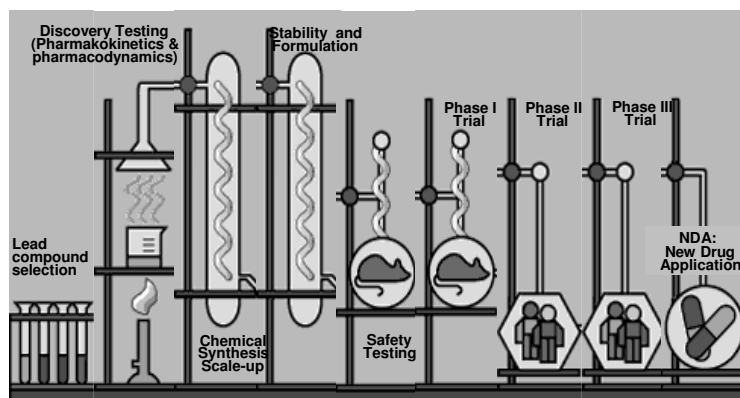
Hewlett-Packard Liquid Chromatography

High Performance Liquid Chromatography

Hocus Pocus Liquid Chromatography

High Patience Liquid Chromatography

HPLC in Pharmaceutics  
Quantitative Analytical Technique No 1



## APPLICATIONS OF HPLC

Veterinary



Environmental



Biomedical and Clinical

Agriculture &  
Food



Chemistry

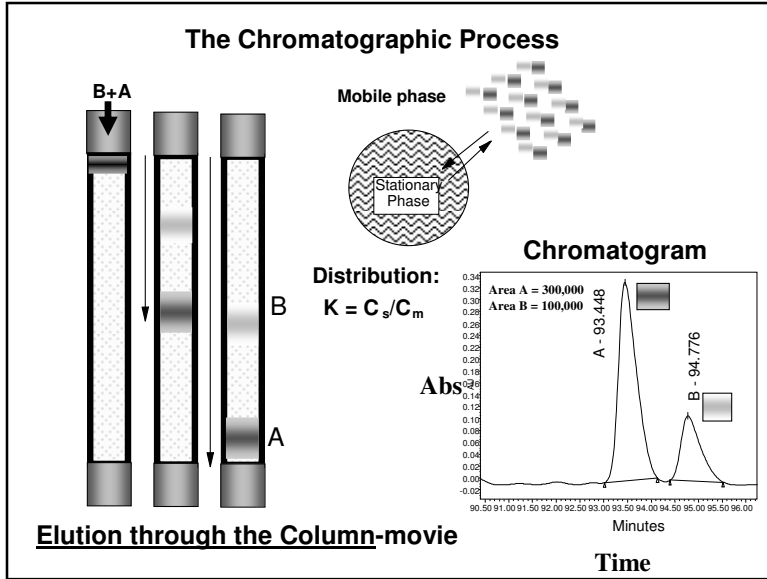


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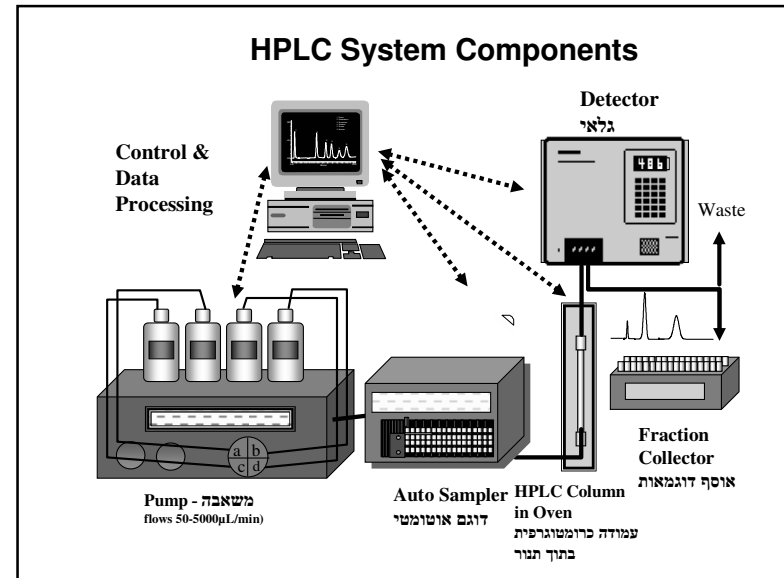
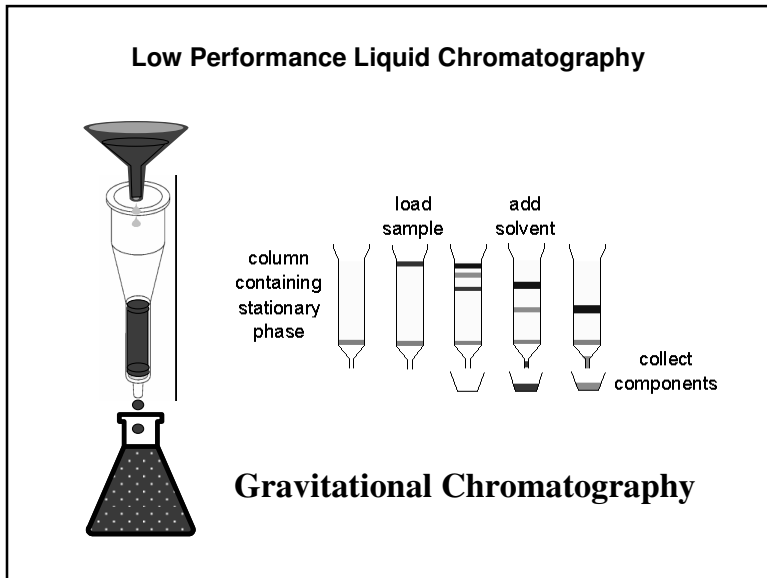


### Tswett's Experiment

- \* Tall Glass Open Column filled with Sand-Like Particles (1903)
- \* Ground-up Plant Extract in Solvent
- \* Poured into the Column and noticed Colored Bands develop as the Extract percolated down through the Column
- \* Different Compounds had Separated

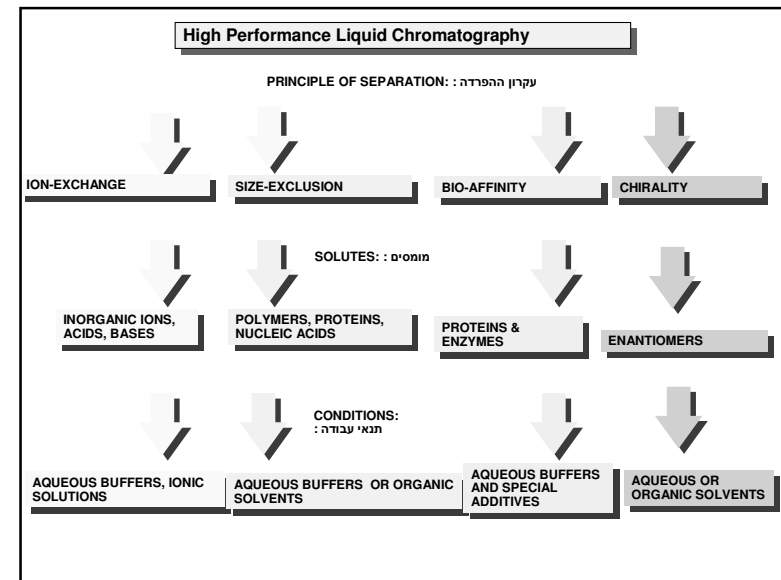
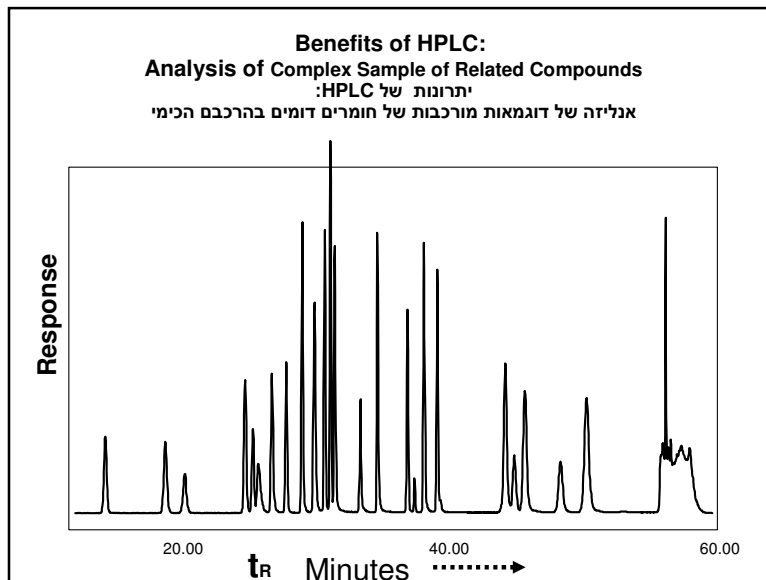
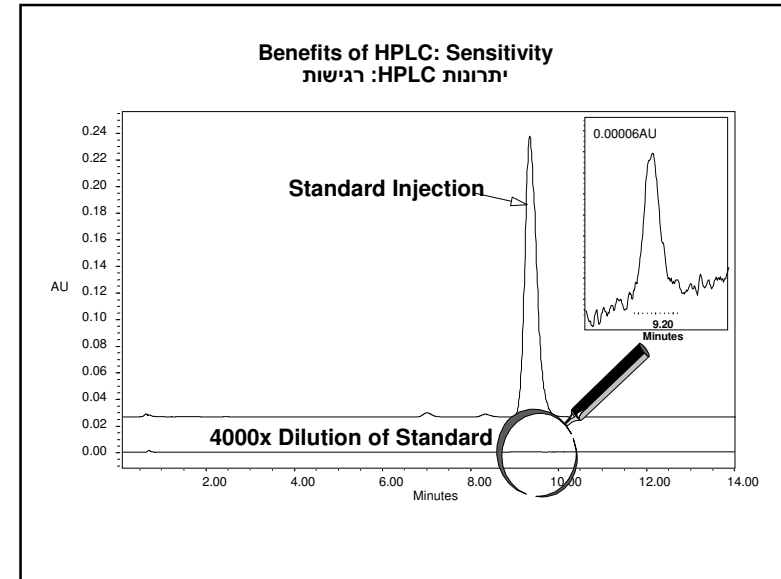
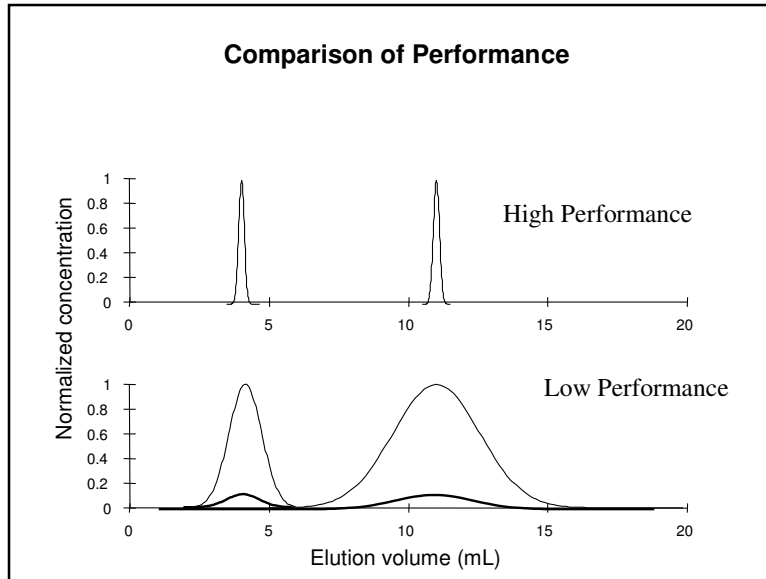
**Chromato = Color**  
**Graphy = Science/writing**

Note: Tswett means color in Russian



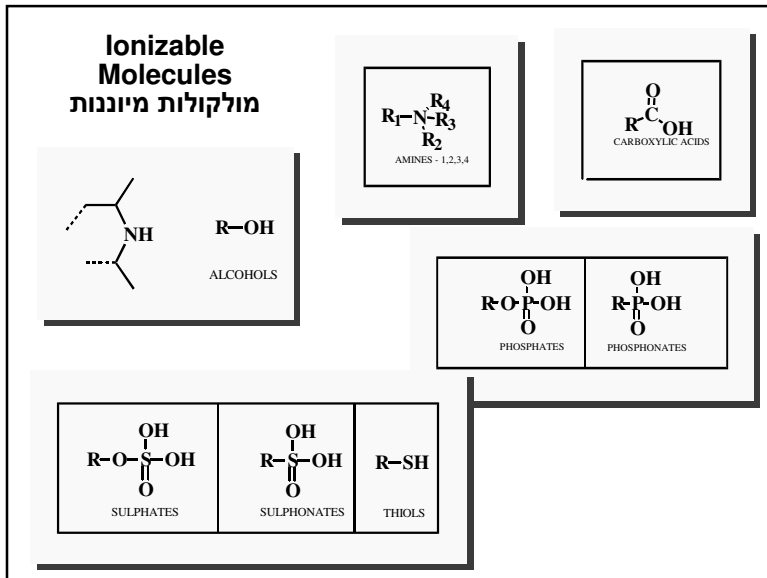
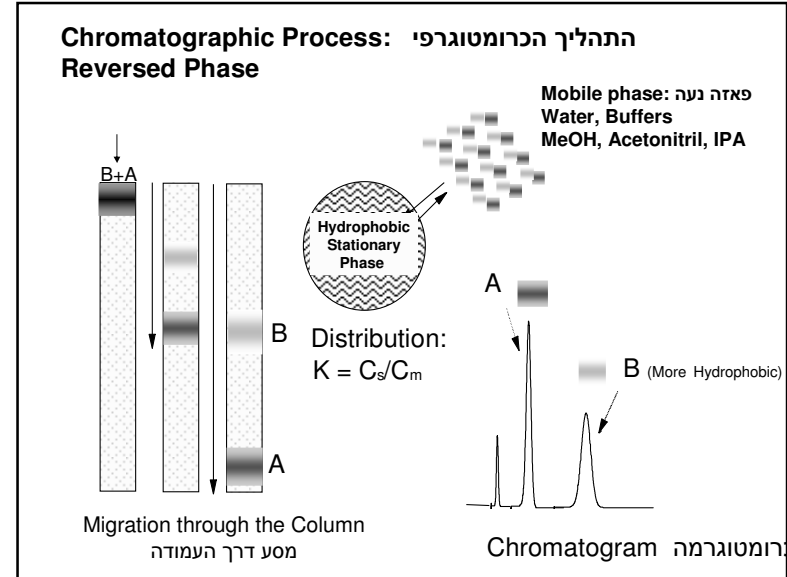
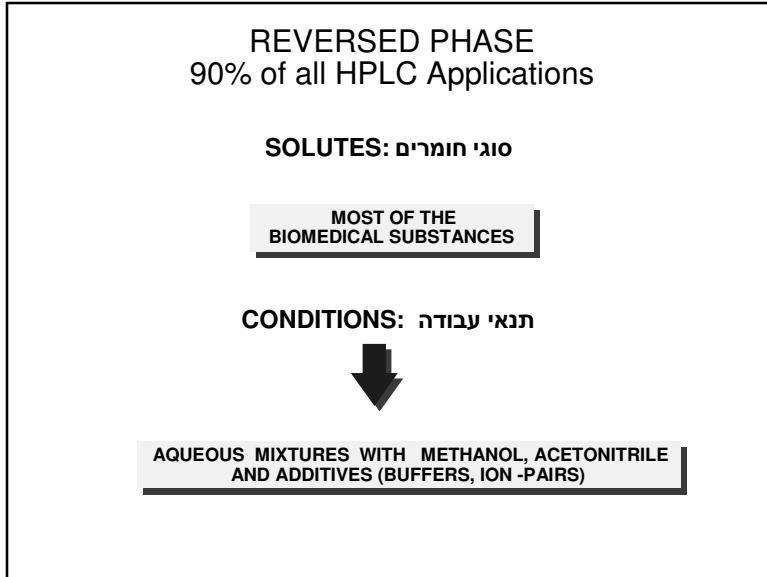
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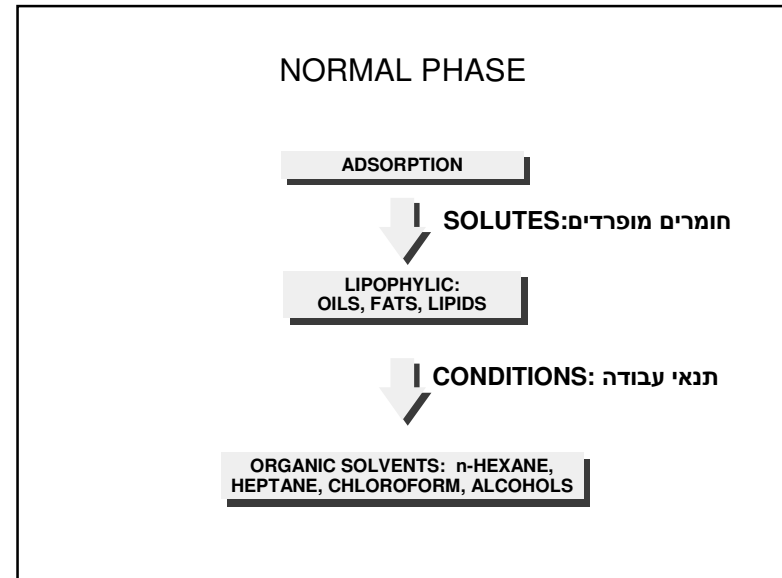
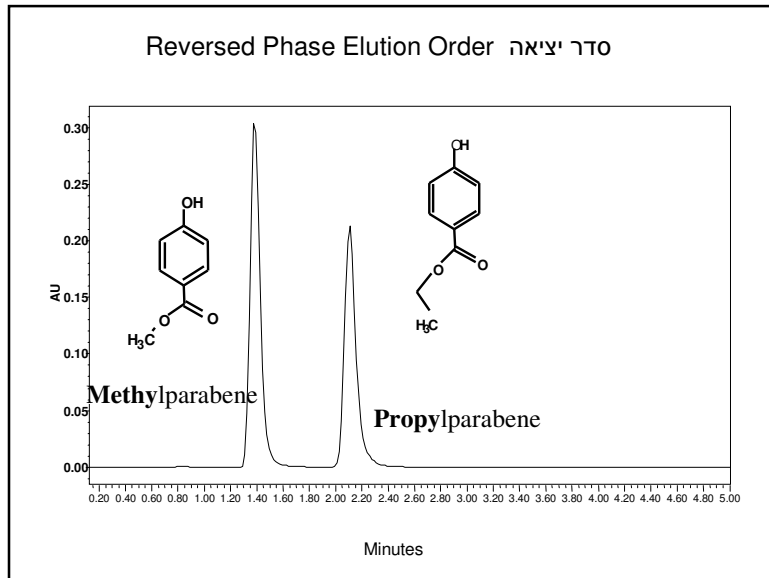
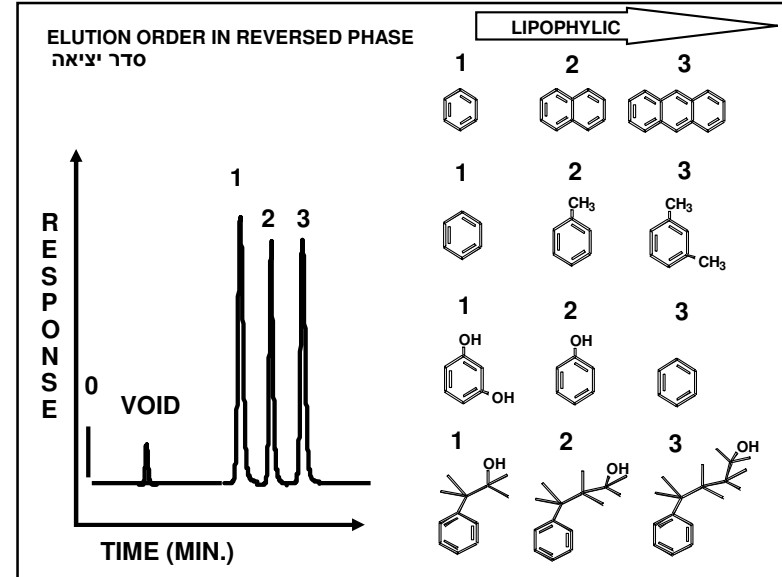
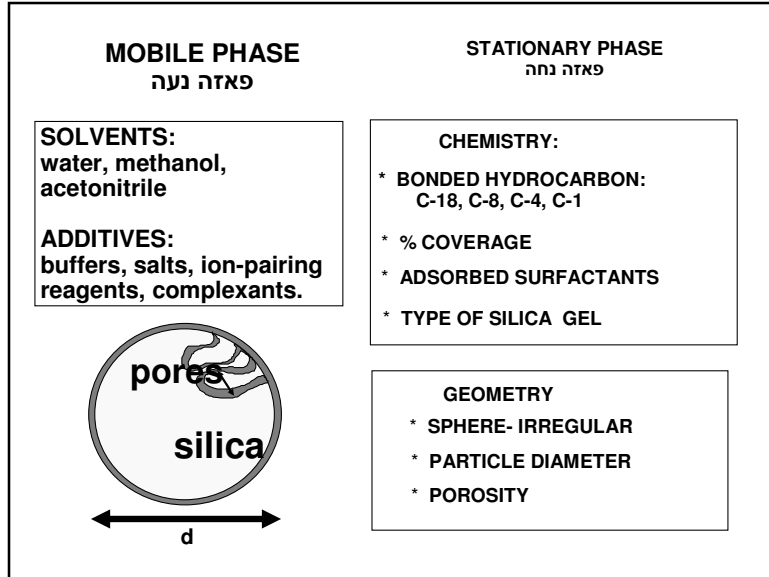


**Clinical Applications**  
יישומים רפואיים

- Chronic Alcohol-Abuse
- Monitoring Oxidative Stress
- Vitamin Profiling
- Risk Factor for Arterio-sclerosis
- Osteoporosis Diagnosis
- Biogenic Amines
- Therapeutic Drug Monitoring (TDM)
- Occupational Medicine
- Porphyrin Profiling
- Hemoglobin-Testing
- 6PLUS1 Multilevel Calibrator Sets
- HPLC-Instruments & Software Sets

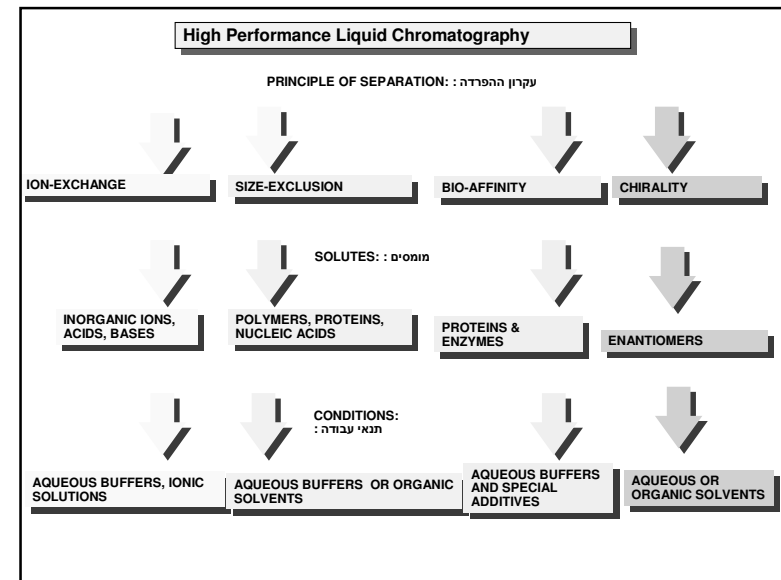
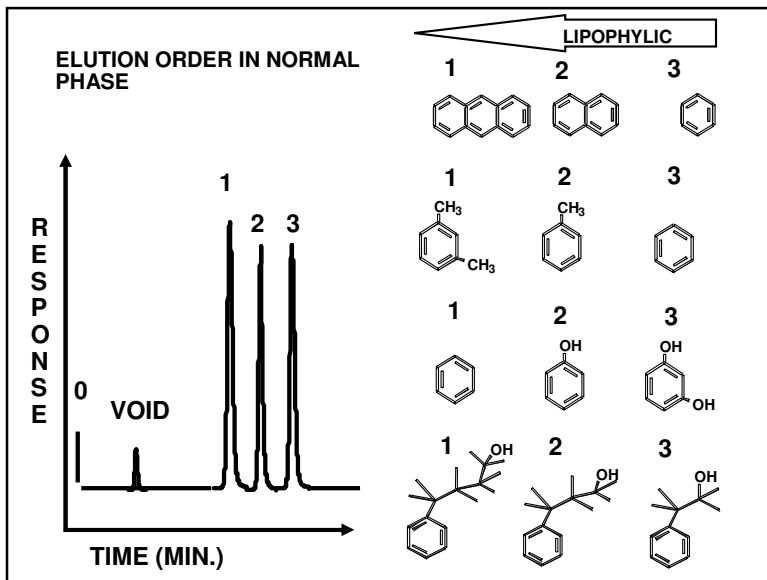
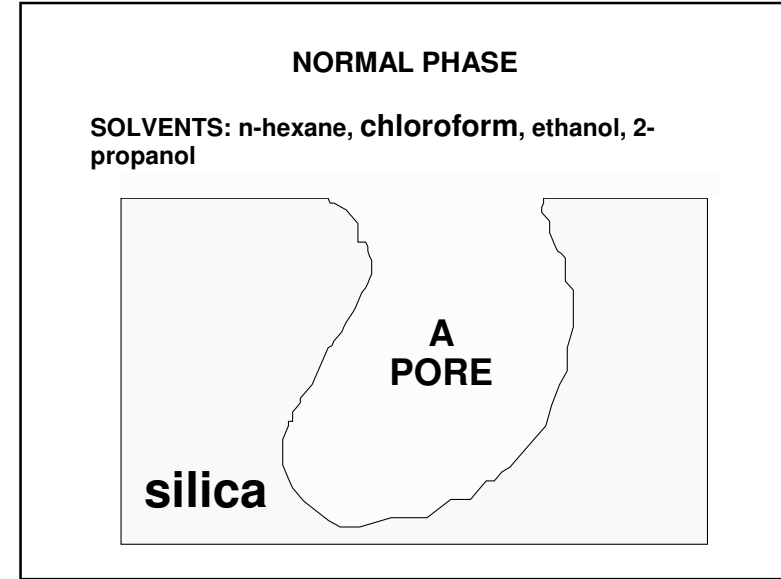
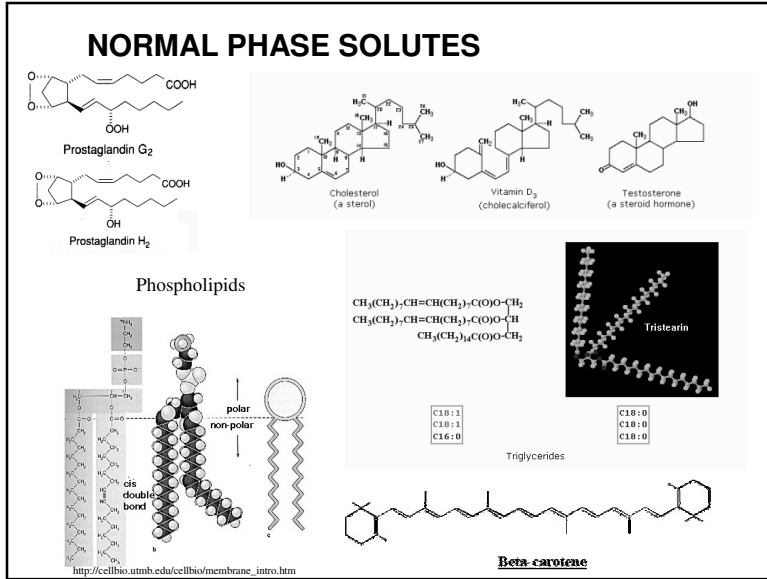
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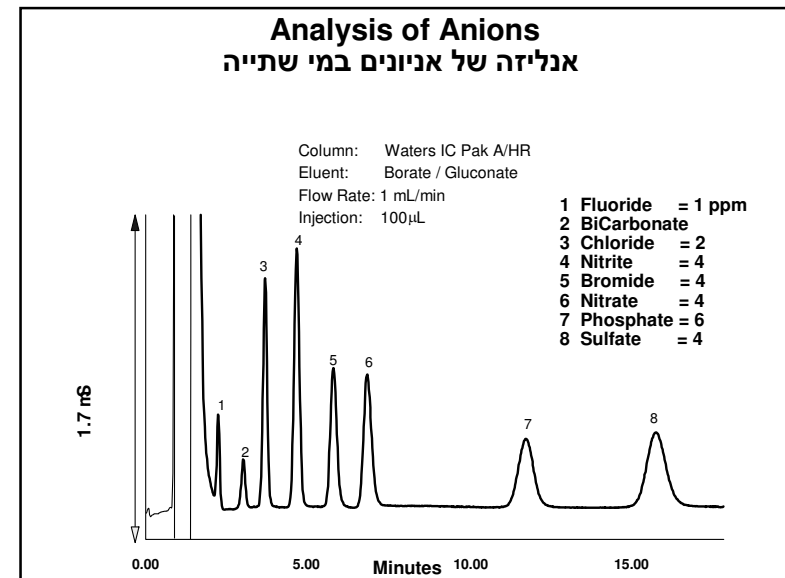
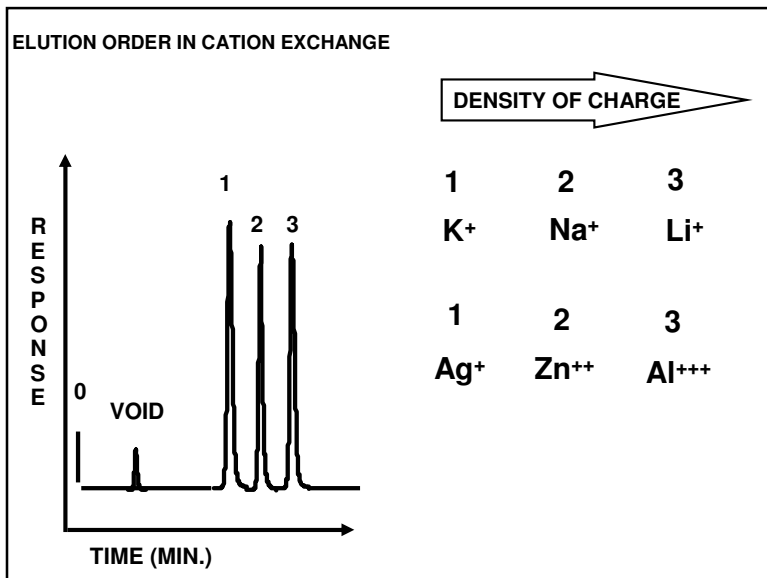
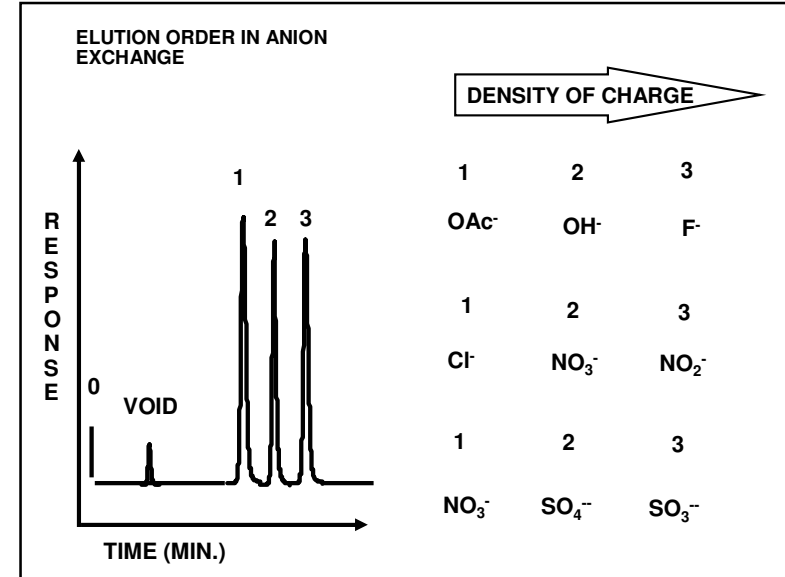
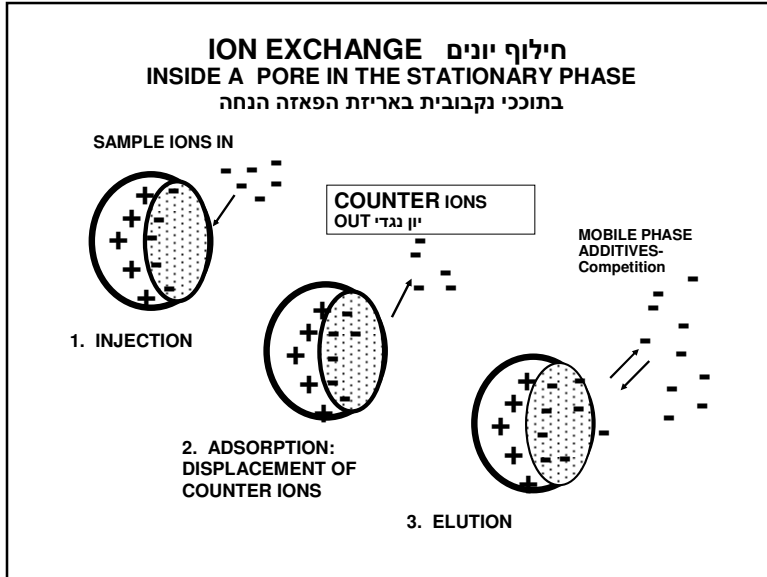
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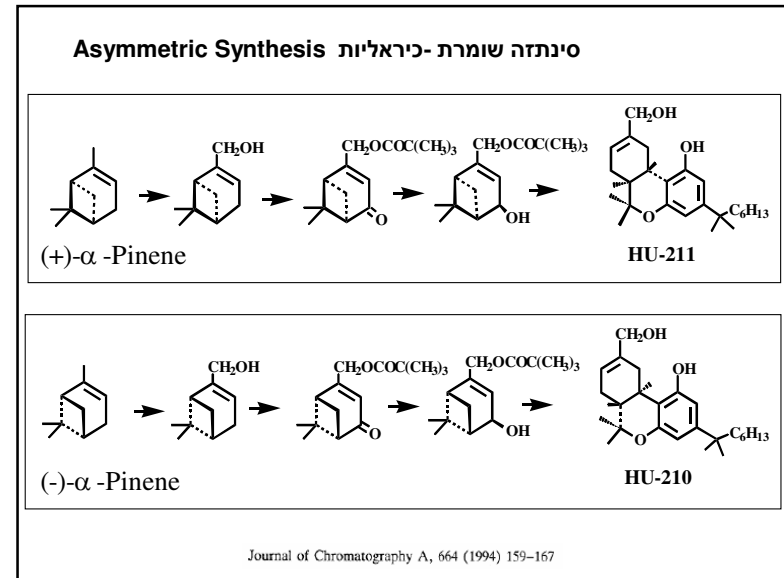
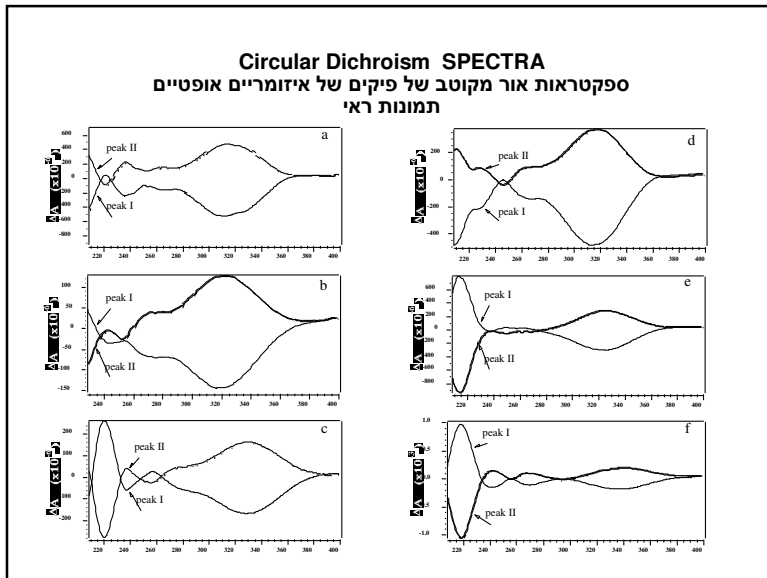
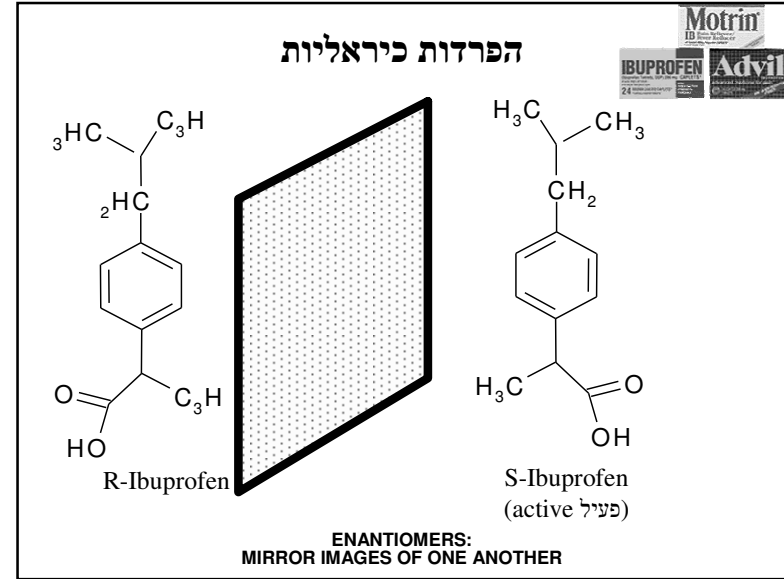
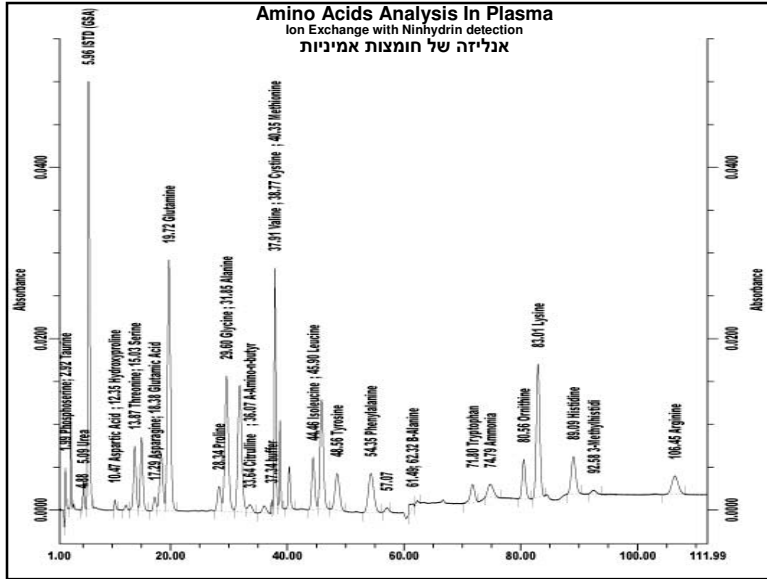
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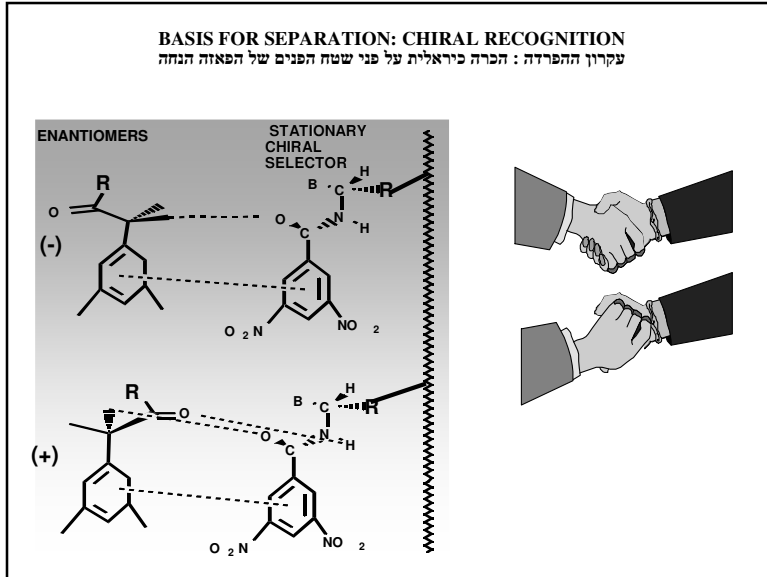
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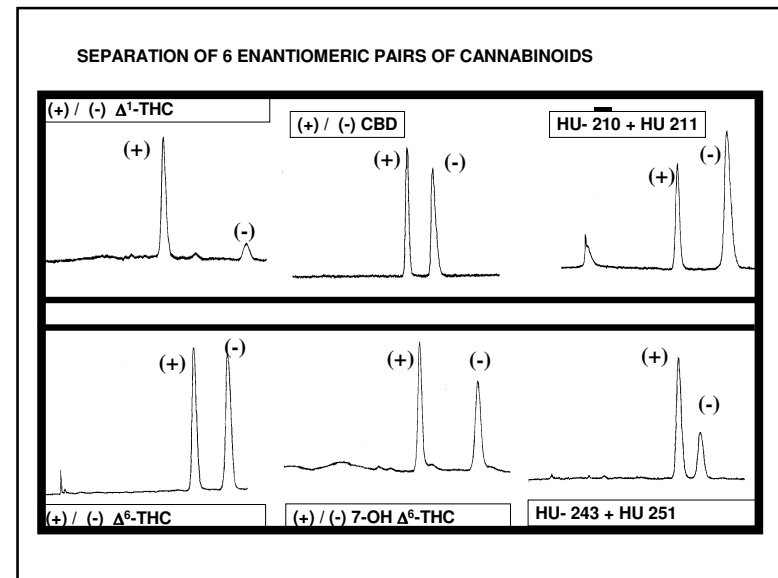
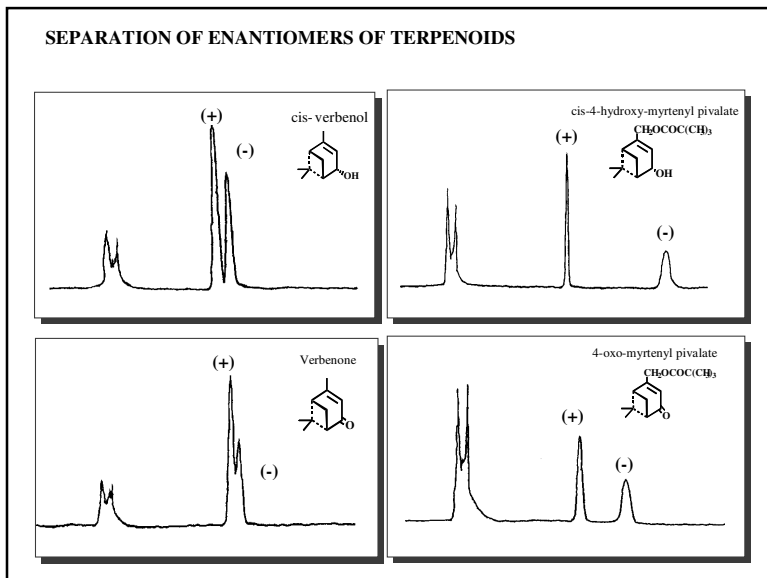
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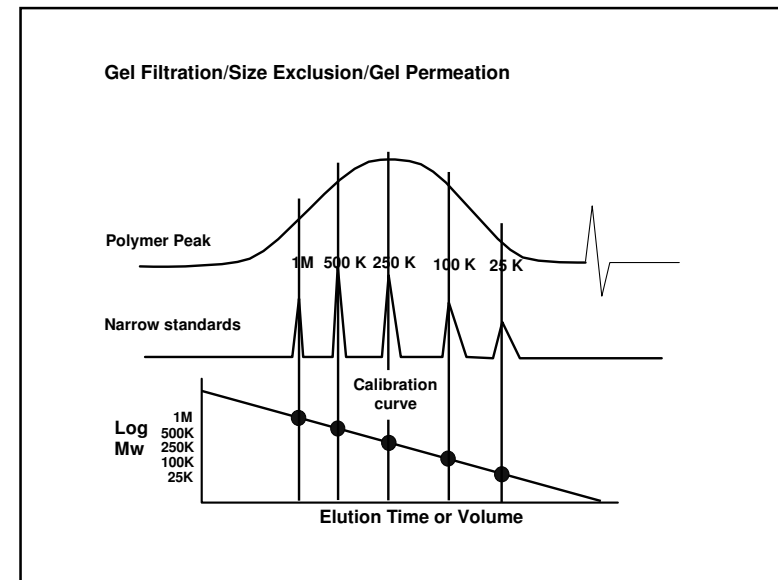
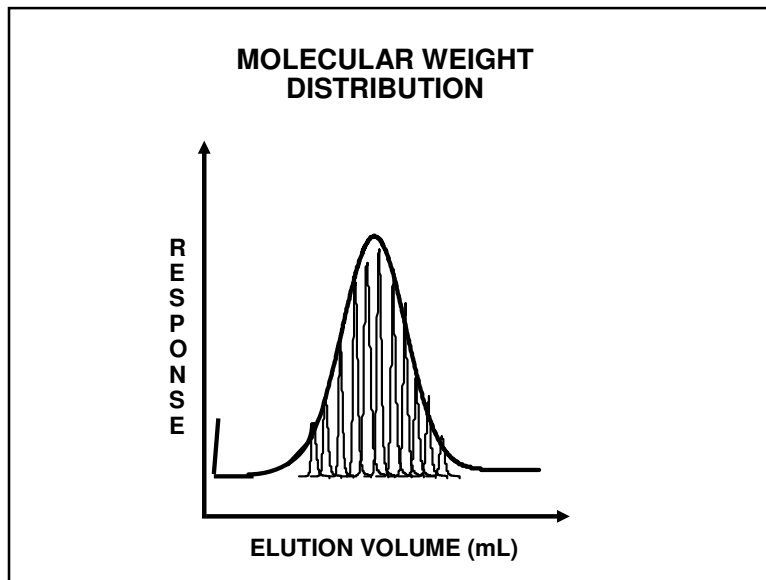
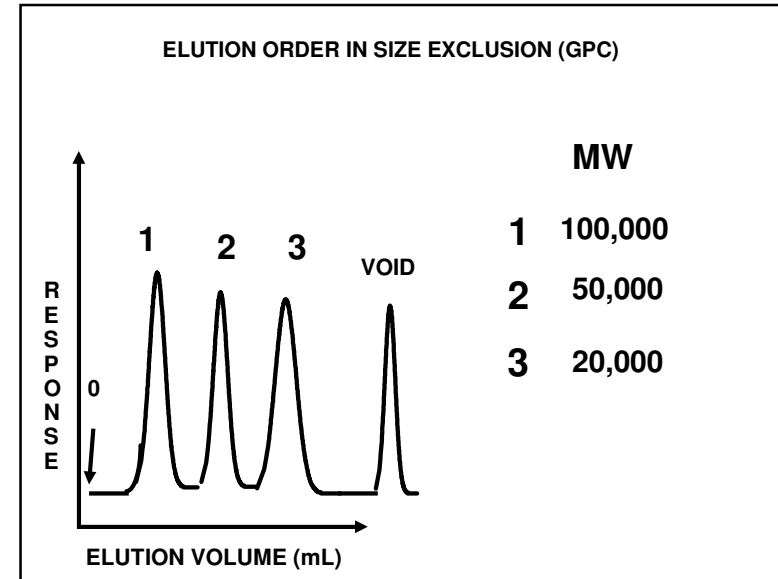
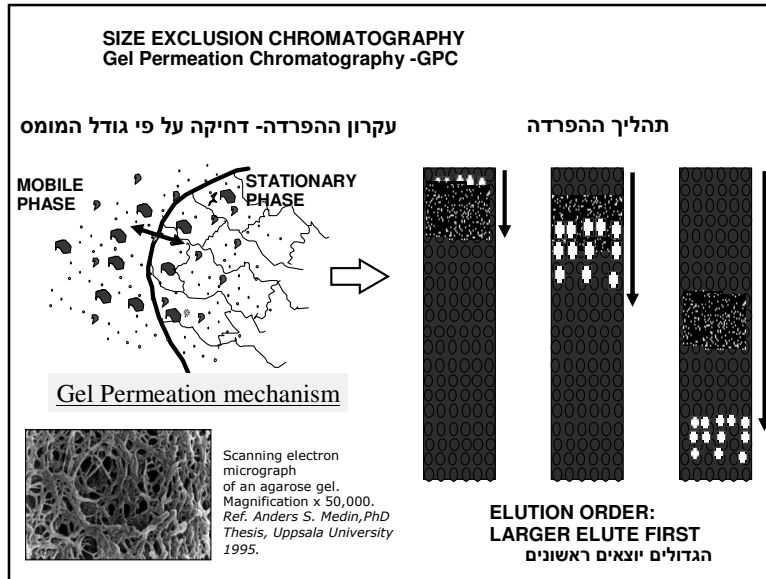
### Chiral stationary phases:

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



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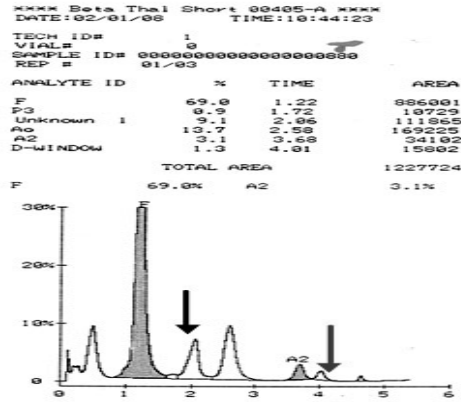


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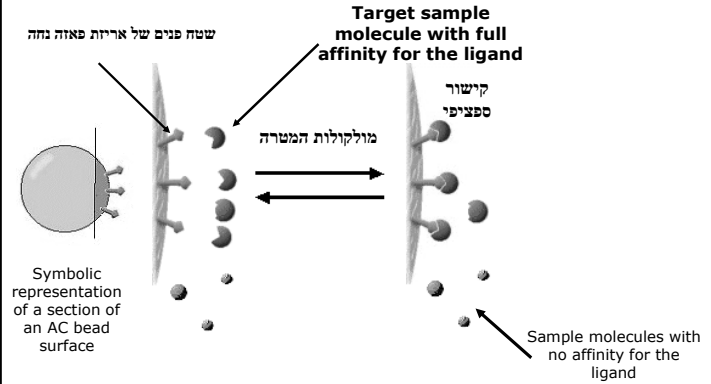
## Introduction

### Clinical Applications

Test of haemoglobin variant for Thalassemia  
 אנליזה של המוגלובין וריאנטים עבור תלסמיה



### זיקה Affinity Chromatography (AC) ביולוגית

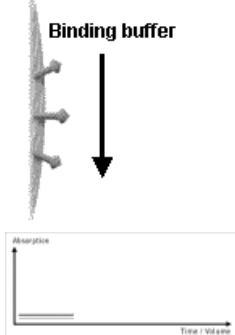


AC relies upon a reversible highly specific binding reaction.

### Affinity Chromatography

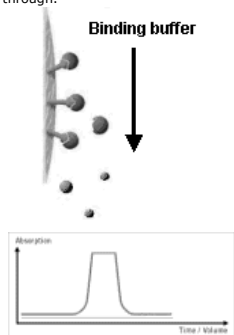
#### 1. Equilibration

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



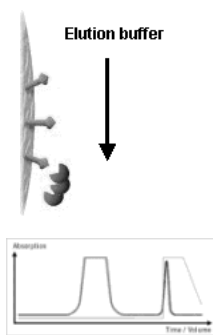
#### 2. Sample application and wash

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.

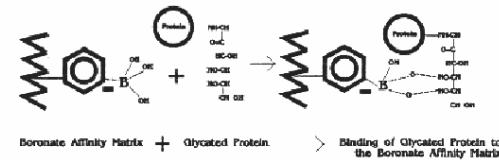


### Glycohemoglobin in Blood

Glycated proteins differ from non-glycated proteins by the attachment of a sugar moiety(s) at various binding sites by means of a ketoamine bond. Glycohemoglobin (GHb) thus contains 1, 2-cis-diol groups not found in non-glycated proteins.

These diol groups provide the basis for separation of glycated and nonglycated components by boronate-affinity chromatography (1-3). In this analytical technique, a boronate such as phenylboronic acid is bonded to the surface of the column support.

#### Affinity Binding of Glycated Protein



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