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2nd stage

introduction to Chromatography

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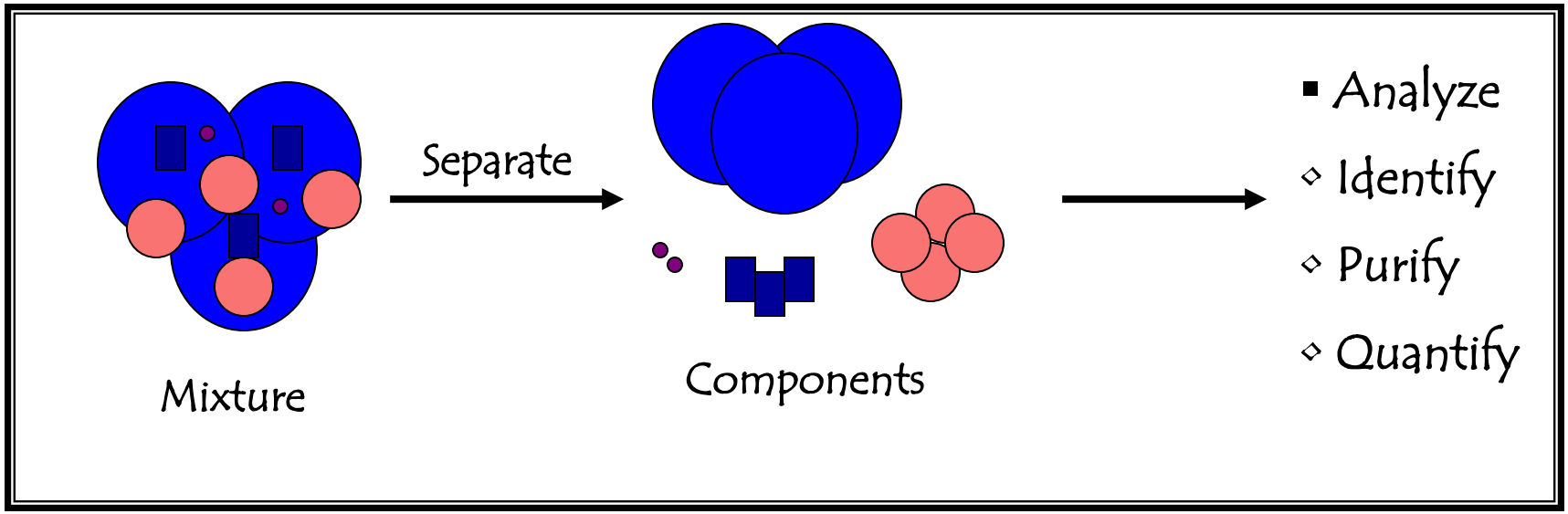
DEFINITION

It is a physical separation method which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.

CHROMATOGRAPHY



Glossary...

- ✓ **Chromatograph:** Instrument employed for a chromatography.
- ✓ **Eluent:** Fluid entering a column.
- ✓ **Eluate:** Fluid exiting the column.
- ✓ **Elution:** The process of passing the mobile phase through the column.
- ✓ **Flow rate:** How much mobile phase passed / minute (ml/min).
- ✓ **Linear velocity:** Distance passed by mobile phase per 1 min in the column (cm/min).

Components of Chromatography

Mobile Phase – gas or liquid that carries the mixture of components through the stationary phase.

Stationary Phase – the part of the apparatus that holds the components as they move through it, separating them.

Uses for Chromatography

Chromatography is used by scientists to:

- Analyze – examine a mixture, its components, and their relations to one another
- Identify – determine the identity of a mixture or components based on known components
- Purify – separate components in order to isolate one of interest for further study
- Quantify – determine the amount of the a mixture and/or the components present in the sample

Uses for Chromatography

Real-life examples of uses for chromatography:

- ✓Pharmaceutical Company
- ✓Hospital
- ✓Law Enforcement
- ✓Environmental Agency
- ✓Manufacturing Plant

CHROMATOGRAPHY TERMS

Chromatogram:

It is the visual output of the chromatograph.

Chromatograph:

It is equipment that enables a sophisticated Separation.

Stationary phase (bounded phase):

It is a phase that is covalently bonded to the support particles or to the inside wall of the column tubing.

CHROMATOGRAPHY TERMS

Mobile phase:

It is the phase which moves in a definite direction.

Analyte (Sample):

It is the substance to be separated during chromatography.

Eluate:

It is the mobile phase leaving the column.

CHROMATOGRAPHY TERMS

Retention time:

It is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.

Eluent:

It is the solvent that will carry the analyte.

TYPES OF CHROMATOGRAPHY

- **Liquid Chromatography** – separates liquid samples with a liquid solvent (mobile phase) and a column composed of solid beads (stationary phase)

- **Gas Chromatography** – separates vaporized samples with a carrier gas (mobile phase) and a column composed of a liquid or of solid beads (stationary phase)

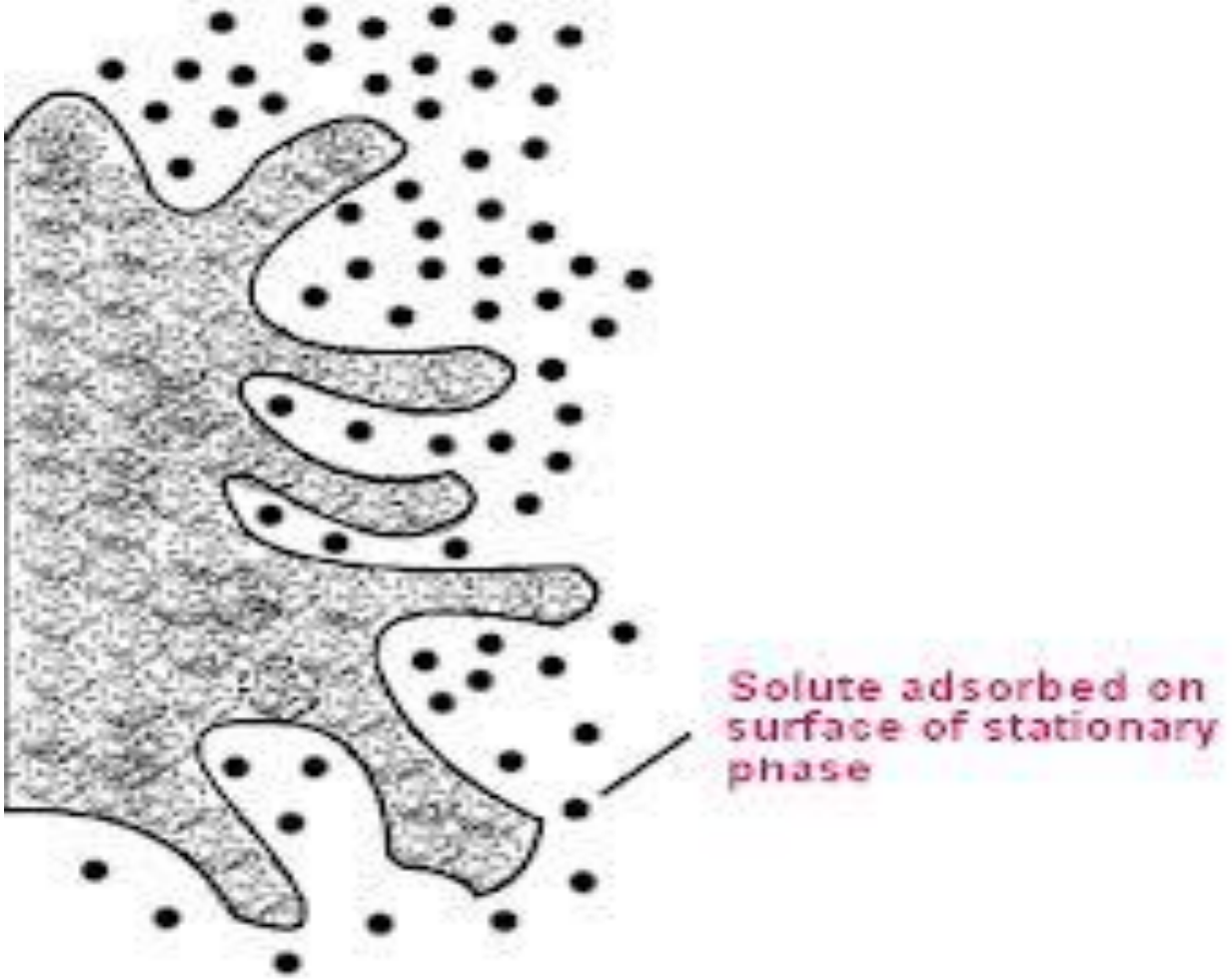
- **Paper Chromatography** – separates dried liquid samples with a liquid solvent (mobile phase) and a paper strip (stationary phase)

- **Thin-Layer Chromatography** – separates dried liquid samples with a liquid solvent (mobile phase) and a glass plate covered with a thin layer of alumina or silica gel (stationary phase)

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid chromatography (LC) (mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas chromatography (GC) (mobile phase: gas)	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical-fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

classification according to the mechanism of separation (according to the mode of separation). It is perhaps more useful to divide chromatographic forms according to the mode of separation on which each is based . These basic forms of molecular interaction , determine chromatographic behavior. There are several basic mechanisms of chromatography by which separation can occur , & more than one mechanism may be responsible during a given separation:

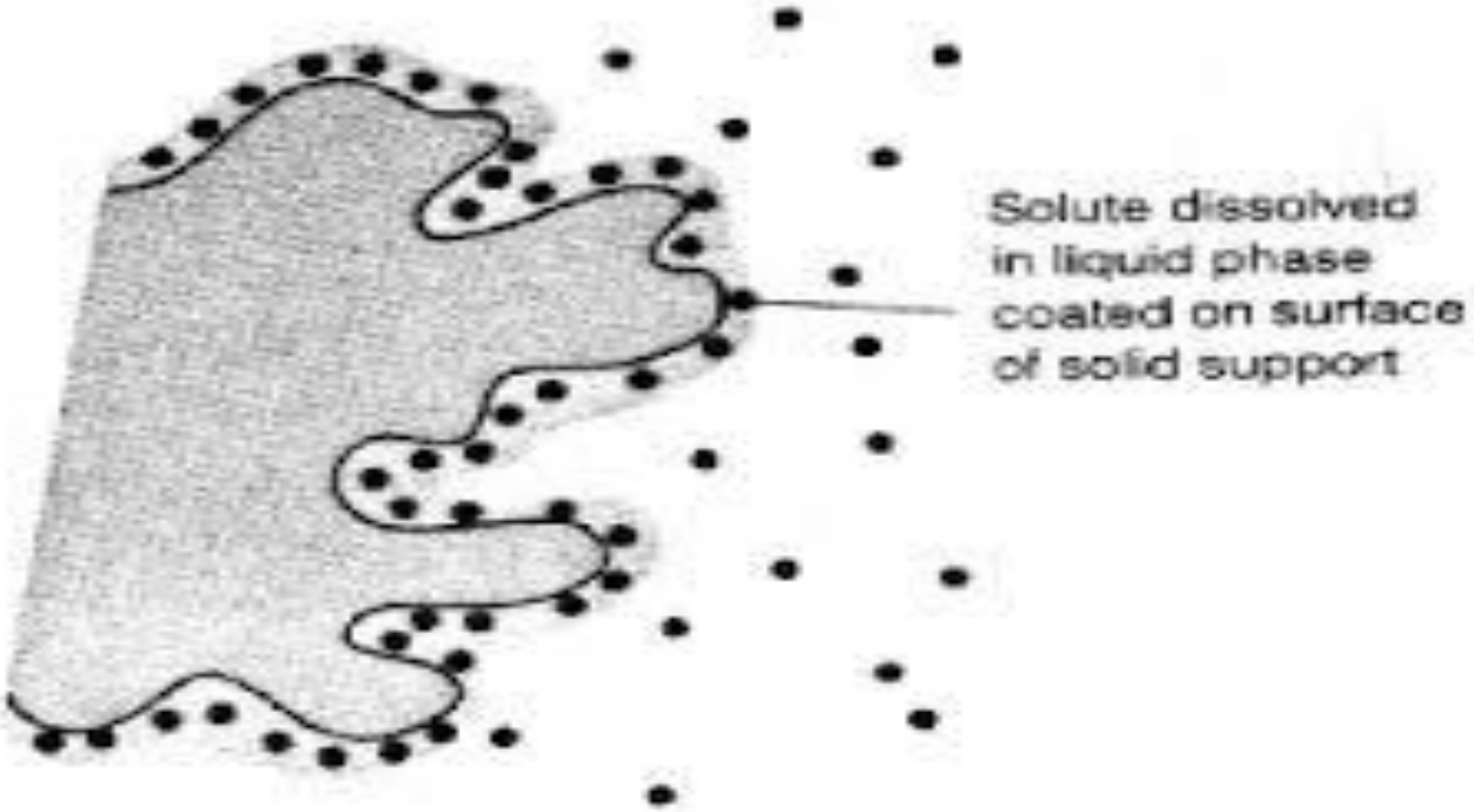
Adsorption chromatography: This involves mobilization of molecule between the surface of a solid stationary phase & liquid mobile phase. The dynamic equilibrium of solutes as they switched between the stationary & mobile phases (processes of sorption & desorption , respectively) is specific for each molecule & is affected by competition that exists between solutes & solvent for sites on the stationary phase.


Solute adsorbed on surface of stationary phase

- This is a purely physical process involving the formation of no chemical bonds , but only the relatively weak forces of hydrogen bonds , Van der Waal forces , & dipole-dipole interactions. For this reason almost any inert material can in theory be used as an adsorbent , Common example of adsorbent include silica (as a column or as a TLC stationary phase) , cellulose , styrene, alumina etc.

Partition chromatography: This type of chromatography employs the separation principle of liquid-liquid extraction. The mechanism involves the relative solubility of the compound between the sorbent (stationary phase) & the solvent (mobile phase).

Compounds that are more soluble in the mobile phase will migrate up the plate to a greater extent than components that are more soluble in the stationary phase.



Partition chromatography

- When one of the liquids is coated onto a solid support, such as a column of cellulose coated with water , or a silica TLC plates coated with adsorbed water , a stationary phase is created on which separation can be carried out with an immiscible/organic mobile phase , employing the principles of liquid-liquid extraction with the advantages of chromatography.

- However, this method suffers from the dis advantages that the liquid stationary phase tends to be stripped (leached) from the column as a result of shear forces acting on it from the movement of the mobile phase & by the solubility of the liquid stationary phase in the mobile phase.

- Normal phase/reverse phase : If the stationary phase is more polar than the mobile phase , this is normal phase chromatography. An example of this is a column of silica with its polar silanol groups & a mobile phase of an organic solvent. When the stationary phase is less polar than the mobile phase , this is reverse phase chromatography, exemplified by the hydrocarbons bound to the silica support & a water/acetonitrile mobile phase. Reverse phase chromatography is very widely used as a form of HPLC, & most natural products have a region of hydrophobicity that leads to their retention to some extent on a reverse phase column.

Comparison between adsorption & partition chromatography:

- Separation in chromatography depends on the type of the distribution of the solute (the substance to be separated) between the mobile phase & the stationary phase. In adsorption chromatography the distribution of the solute is between a solid phase which is called adsorbent & a liquid or gas phase which is called the mobile phase. In partition chromatography the distribution of the solute is between a liquid stationary phase & a mobile phase which could be a liquid or a gas.

GEL FILTRATION

- ✓ Gel filtration separates molecules according to the differences in size as they pass through the filtration medium packed in the column.
- ✓ It is well suited for biomolecules that are sensitive to pH ,concentration and harsh environment.
- ✓ Parameters that affects gel filtration are, particle size, flow rate, packaging density, porosity of the particle and viscosity of the mobile phase.

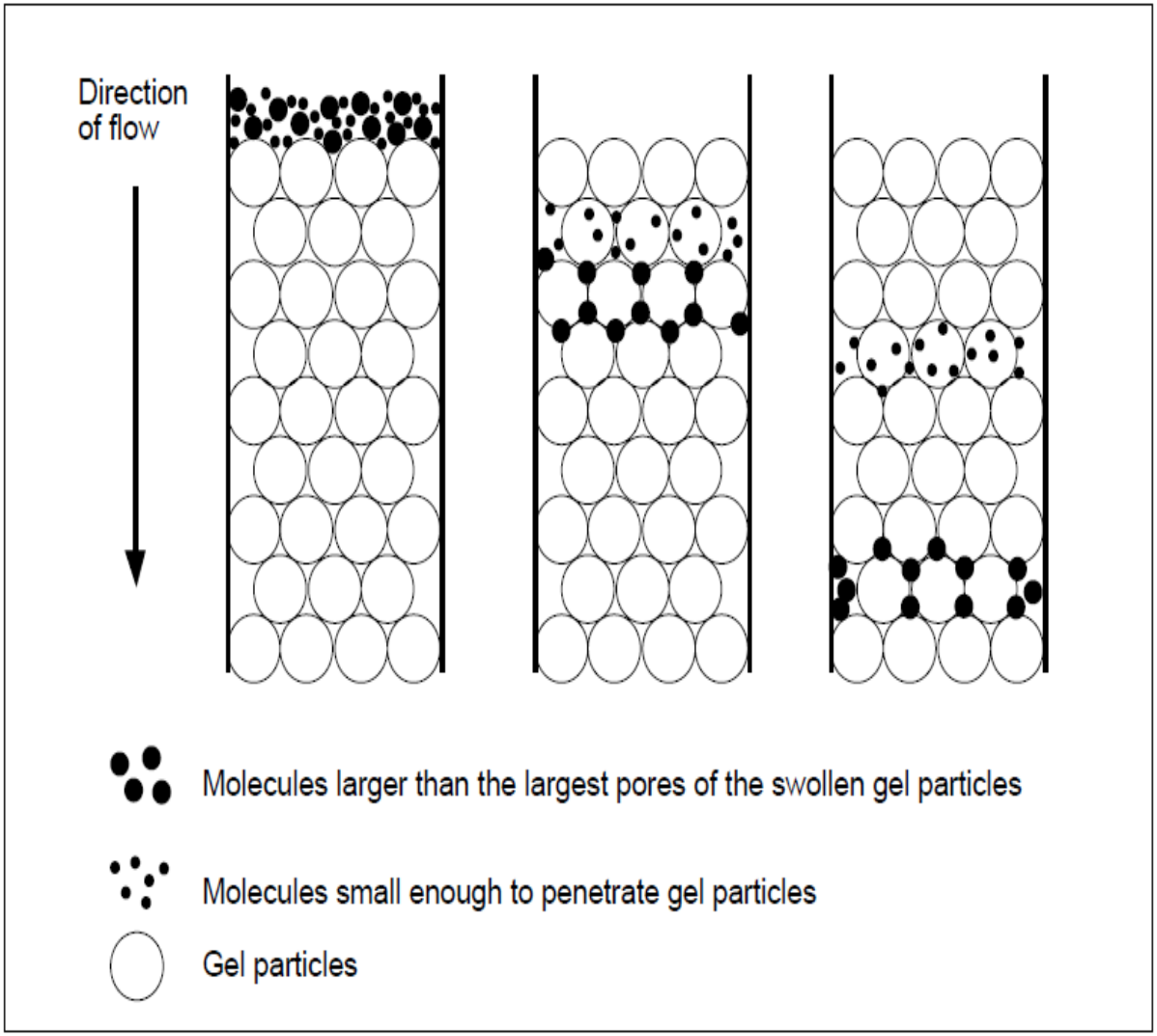


Figure 4 Gel permeation chromatography

MATERIALS REQUIRED

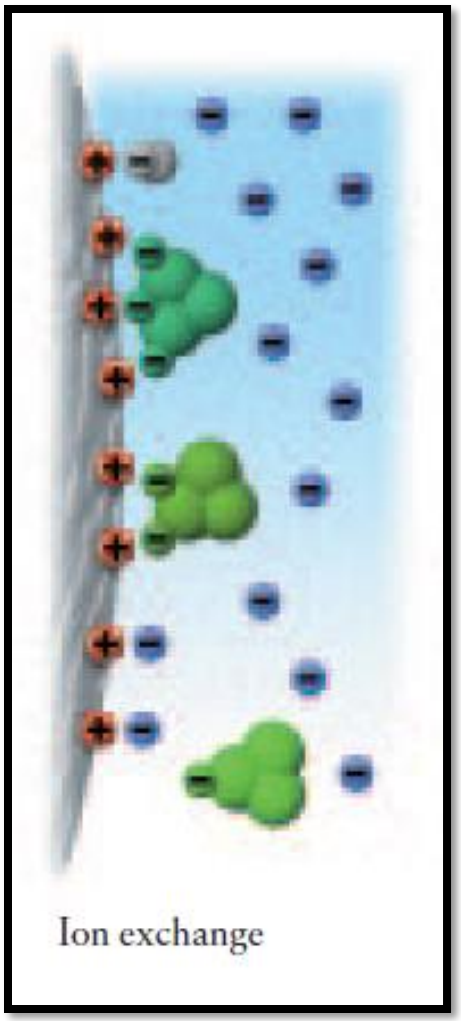
- ✓ Cross linked dextrans (sephadex)
- ✓ Agarose (sepharose)
- ✓ Polyacrylamide
- ✓ Porous glass gel.

APPLICATIONS

- ✓ Fractionation (purification of the desired protein using suitable gel)
- ✓ Molecular weight determination

ION EXCHANGE

- ✓ *Ion exchange chromatography is used to remove ions of one type from a mixture and replace them by ions of another type.*
- ✓ *The basic principle is reversible competitive binding*



Ion exchangers

- *Cation exchangers (negative ions – stationary)*
- *Anion exchangers (positive ions - stationary)*

Four types of polymers are commonly used. They are,

- *Synthetic hydrophobic polymer resins crosslinked with divinylbenzene.*
- *Naturally occuring as well as synthetic polymers(cellulose)*
- *Synthetic hydrophilic polymers*
- *Silica gel*

AFFINITY CHROMATOGRAPHY

- Affinity chromatography includes bioaffinity, dye-ligand affinity and immobilized metal ion afffinity techniques.
- It is based on the formation of the specific and reversible complexes between a pair of biomolecules.

Affinity

Affinity chromatography: This type of chromatography depends on specific interactions of biological molecules such as an antibody-antigen interaction , enzyme-inhibitor interaction , DNA-DNA binding , DNA-protein interaction , or a receptor-agonist/antagonist interaction . The ligand (receptor) is covalently bound to the packing material.

6- Electrophoresis.

HPLC

- HPLC is a physical separation technique in which a sample dissolved in a liquid is injected into a column packed with small particles and it is separated into its constituent components
- HPLC is probably the most important and widely used analytical technique for quantitative analysis of organics and biomolecules
- HPLC is applicable to many kind of samples:
- Most useful for pharmaceuticals, biomolecules, and labile organics

“Education is not the learning Of Facts, But the training Of The Mind To Think”

Albert Einstein