CHROMATOGRAPHY

CONTENTS

Chromatography

* Chromatography (from Greek chroma "color and graphein "to write") is the collective term for a set of laboratory techniques for the separation of mixtures.
* The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase.
* The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.

History

* Chromatography, literally "color writing", was first employed by Russian scientist Mikhail Tsvet in 1900.
* He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls.
* Since these components have different colors (green, orange, and yellow, respectively) they gave the technique its name.

Principles

* Chromatography usually consists of mobile phase and stationary phase. The mobile phase refers to the mixture of substances to be separated dissolved in a liquid or a gas.
* The stationary phase is a porous solid matrix through which the sample contained in the mobile phase percolates.
* The interaction between the mobile phase and the stationary phase results in the separation of the compound from the mixture.

Applications of chromatography

* The chromatographic technique is used for the separation of amino acids,proteins & carbohydrates.
* It is also used for the analysis of drugs,hormones,vitamins.
* Helpful for the qualitative & quantitative analysis of complex mixtures.
* The technique is also useful for the determination of molecular weight of proteins.

The chromatographic method of separation, in general, involves following steps

* Adsorption or retention of substances on the stationary phase
* Separation of the adsorption of substances by the mobile phase
* Recovery of the separated substances by a continuous flow of the mobile phase; the method being called elution
* Qualitative and Qantitative analysis of the eluted substances

Retention Time

* Time taken by any component from point of injection till it reaches the detector.
* Represented by t_r

Time of Unretained Phase

* Time taken by mobile phase in coming out of column is called time of mobile phase or time of unretained phase.
* Represented by t_m

Adjusted Retention Time

* Adjusted Retention time = $t_r - t_m$
* Represented by t_r'

Chromatographic terms

- The **analyte** is the substance to be separated during chromatography.
- A **chromatogram** is the visual output of the chromatograph.
- The **eluate** is the mobile phase leaving the column.
- The **eluent** is the solvent that carries the analyte
- The **detector** refers to the instrument used for qualitative and quantitative detection of analytes after separation.

Relative Retention

* Relative retention is also calculated by formula,
* RRT = t_2 / t_1
* For each peak in the chromatogram, the capacity factor, , is defined as
* Capacity factor (k') = tr-tm/tm

Linear Flow Rate

* Linear flow rate is distance per unit time traveled by solvent.

Its units are m/S

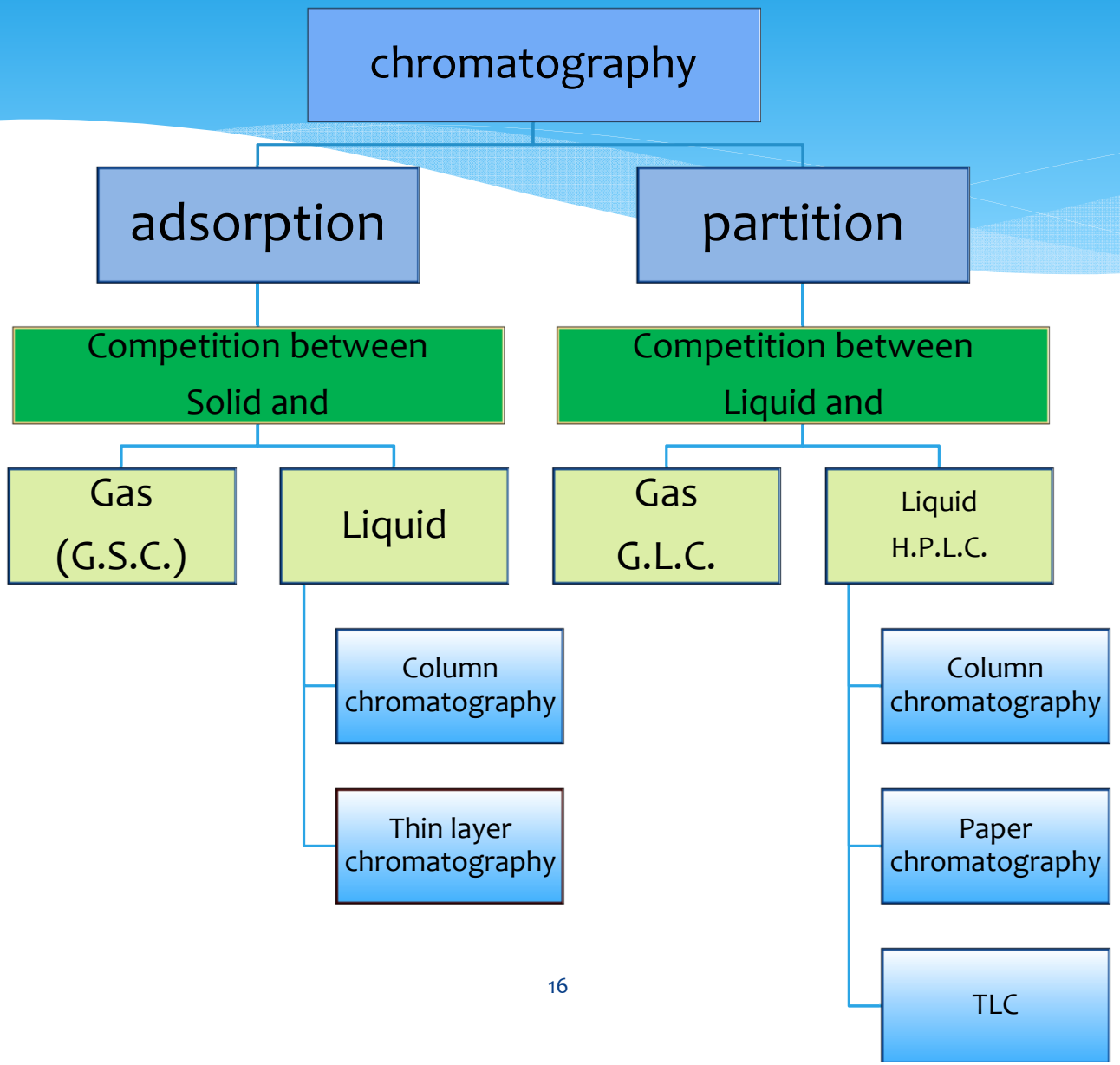
Volume Flow Rate

* Volume per unit time travelled by solvent.
* Its units are dm3/s

Classification of chromatography

1. **Based on mechanism of separation**
   - I. adsorption chromatography
   - II. Partition chromatography
2. **Based on phases**
   - I. Solid phase chromatography
     - i. Solid-liquid chromatography
     - ii. Solid-gas chromatography
   - II. Liquid phase chromatography
     - i. Liquid-liquid chromatography
     - ii. Liquid –gas chromatography
3. **Based on shape of chromatographic bed**
   - I. Planner chromatography
     - i. Paper chromatography
     - ii. Thin layer chromatography
   - II. Column chromatography
     - i. Packed column chromatography
     - ii. Open tubular column chromatography

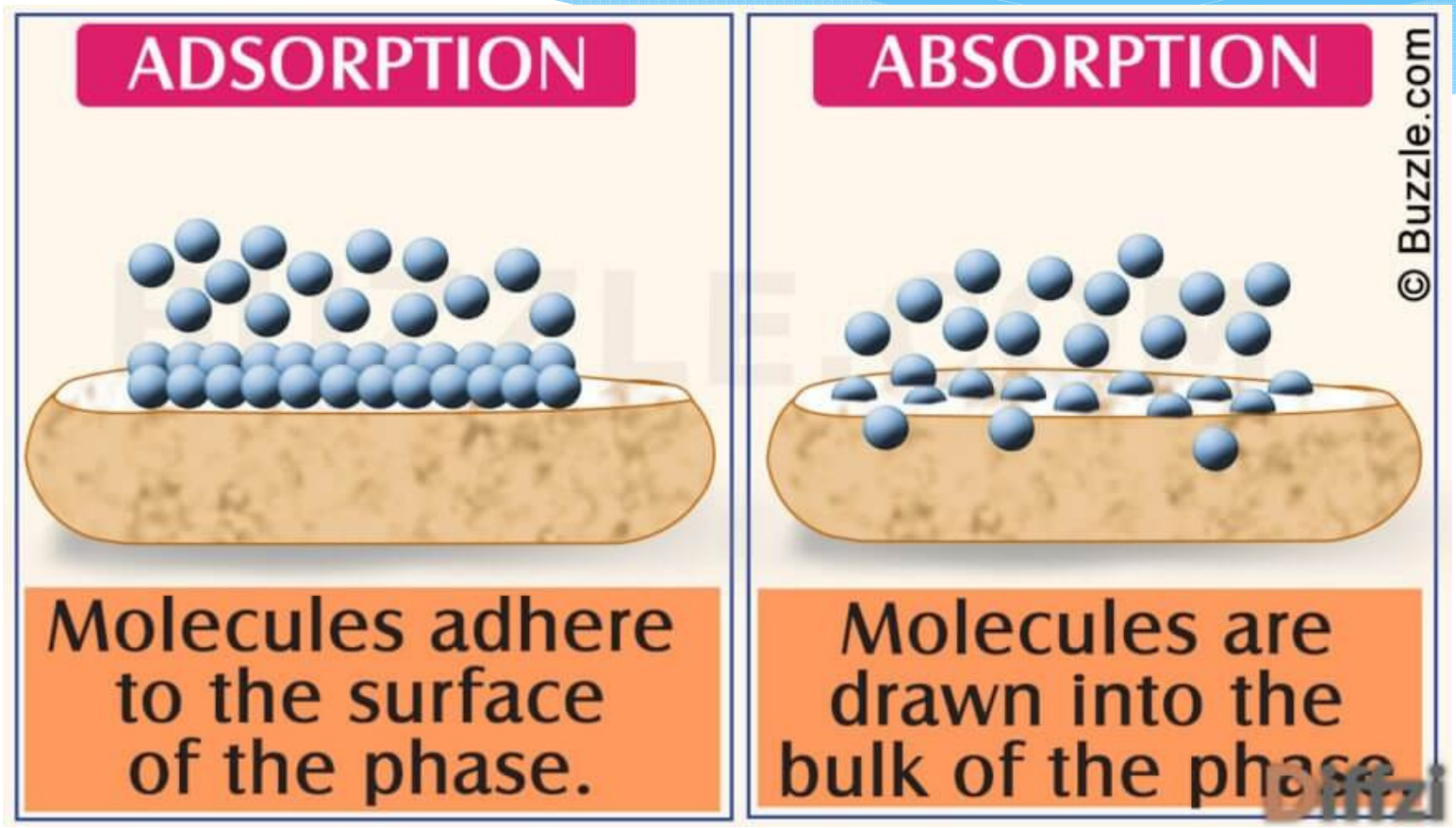
Flow chart diagram of chromatography



Adsorption chromatography

* Stationary Phase must be a solid material.
* Mobile phase may be a liquid or gas.
* Adsorption is process of surface attachment.
* Sample attach on the surface of stationary phase.
* It is different from absorption.

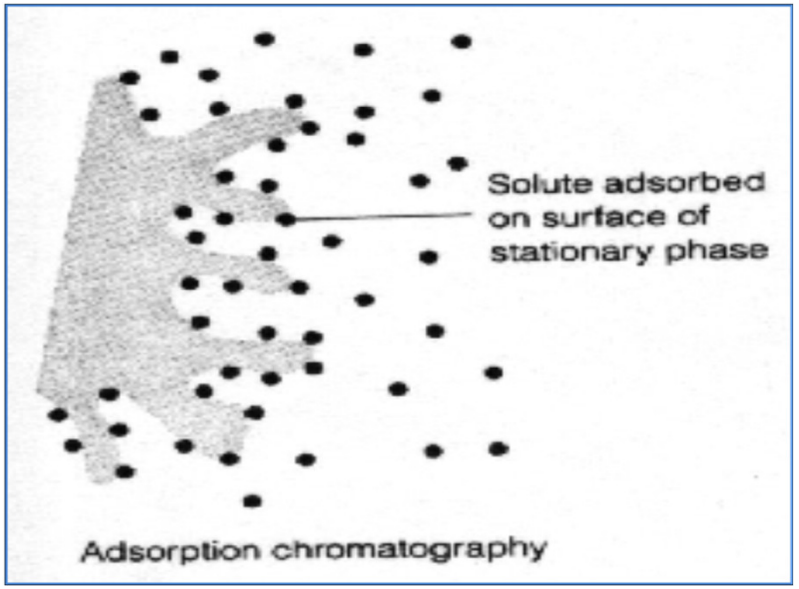
Difference between Adsorption and Absorption



Difference between Adsorption and Absorption

Adsorption chromatography

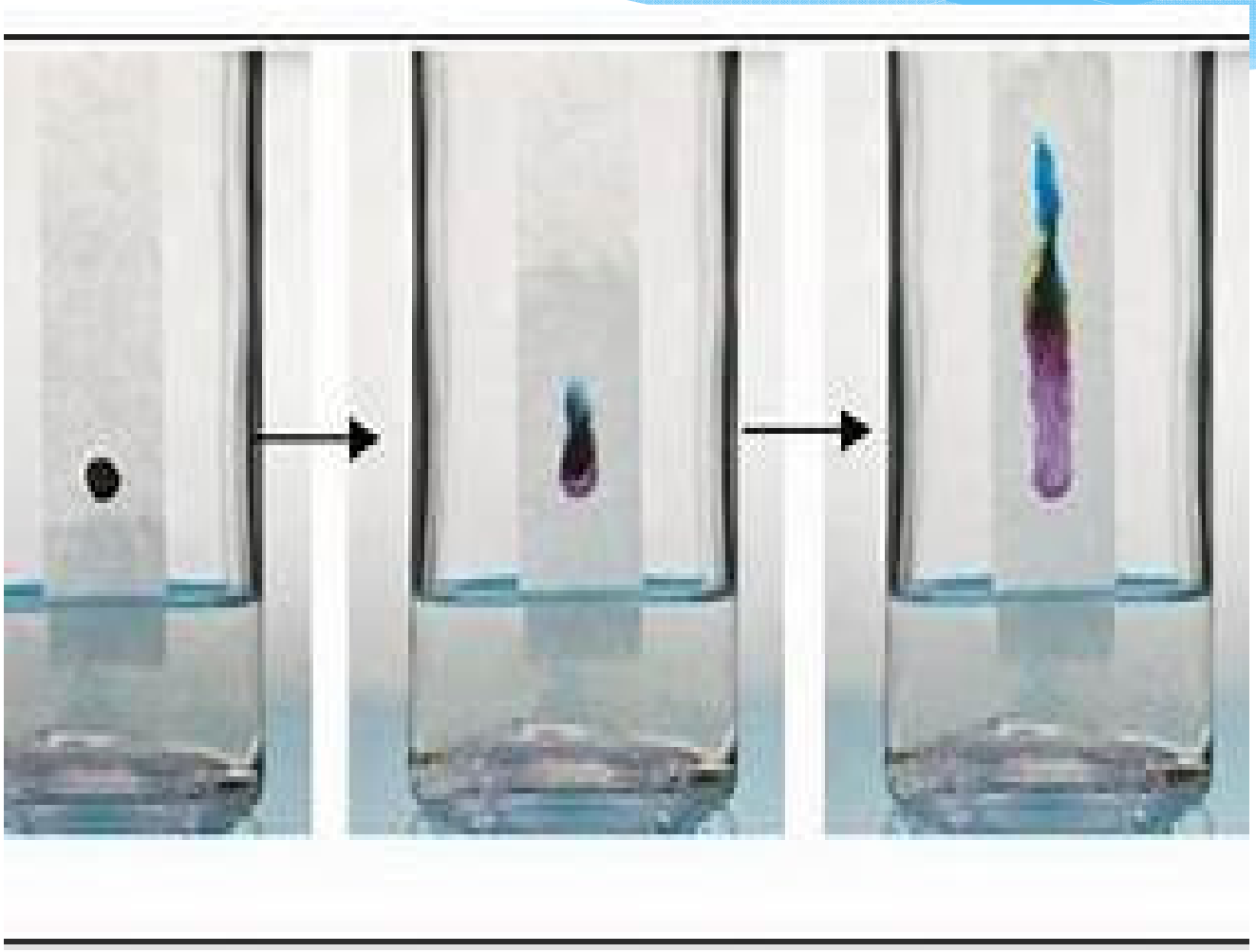
- Adsorption chromatography is process of separation of components in a mixture introduced into chromatography system based on the relative difference in adsorption of components to stationary phase present in chromatography column
- Adsorption chromatography is one of the oldest types of chromatography.
- The equilibriation between the mobile and stationary phase accounts for the separation of different solutes.
- It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase



Adsorption chromatography

Partition chromatography

* Stationary phase must be a liquid.
* Mobile phase may be a liquid or gas.
* Stationary phase is coated on some support.
* e.g. Paper chromatography.
* Stationary phase is water molecule present in structure of cellulose of paper.
* Cellulose is just support.

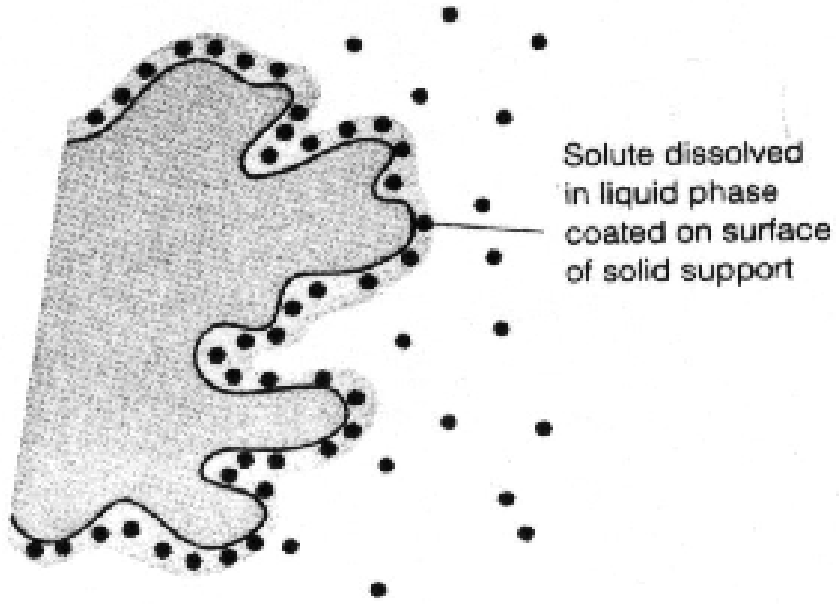


Partition chromatography

- Chromatography in which separation is based mainly on differences between the solubility of the sample components in the stationary phase or on differences between the solubility of the components in the mobile and stationary phases

- This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase

- Solute equilibrates between the mobile phase and the stationary liquid.



Partition chromatography

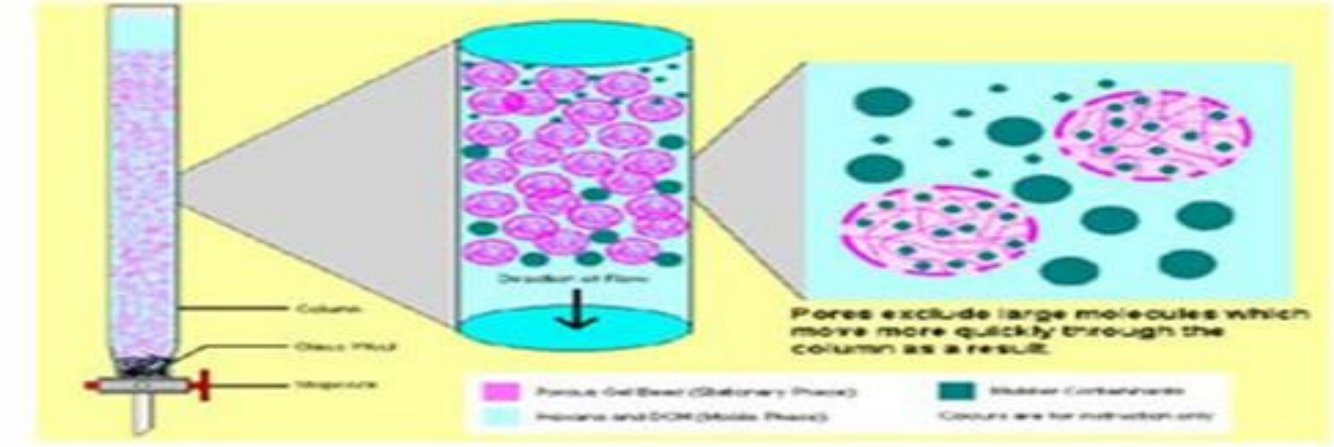
Classification Based on Interaction of Sample with Stationary Phase

* 1- Adsorption chromatography.
* 2- Partition chromatography.
* 3- Ion exchange chromatography.
* 4- Gel filtration chromatography.
* 5- Affinity chromatography.

Gel filtration chromatography/ Size Exclusion Chromatography

* **Size-exclusion chromatography** (**SEC**), also known as **molecular sieve chromatography**, is a chromatography method in which molecules in solution are separated by their size, and in some cases weight of molecule. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.

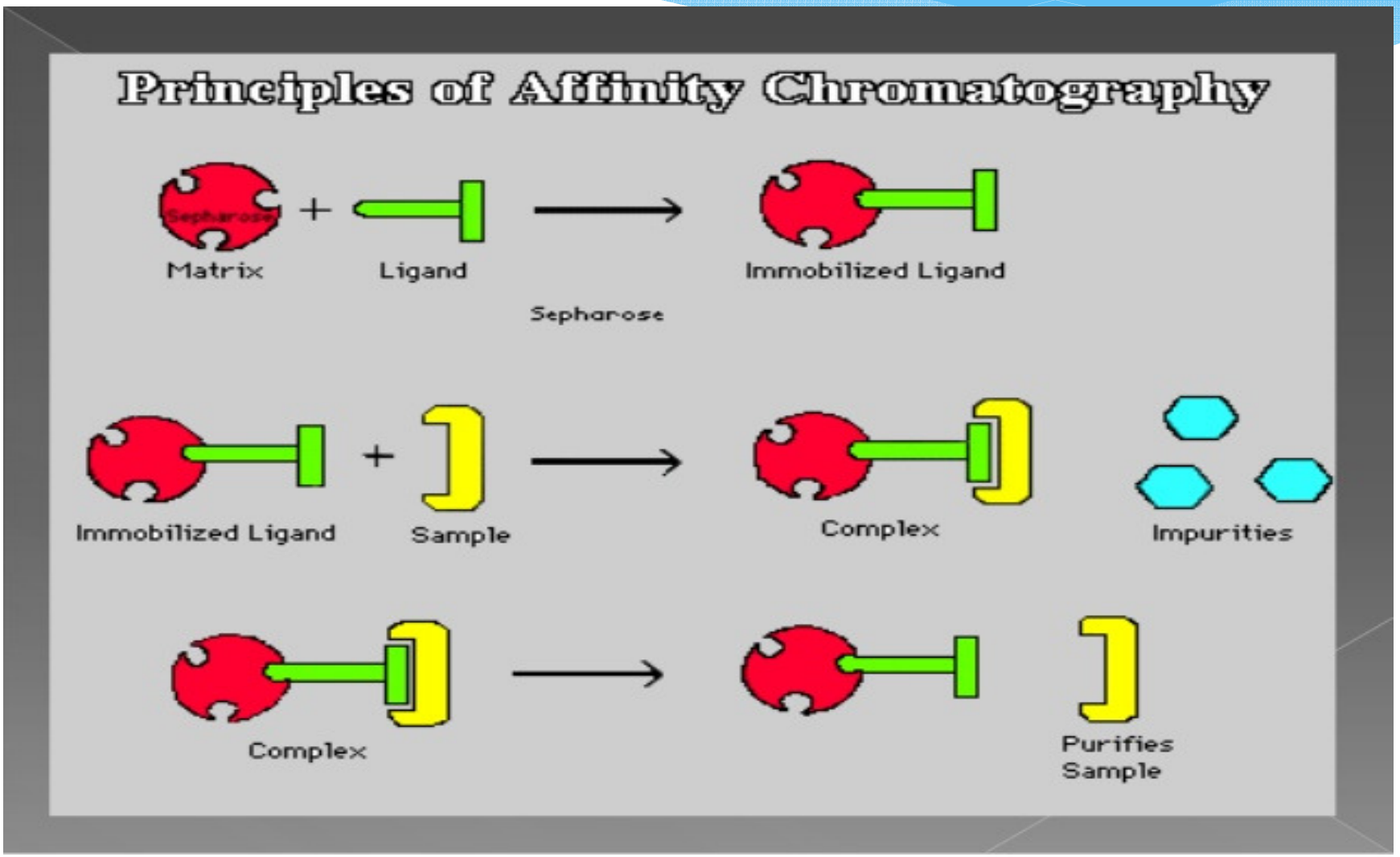
Gel filtration chromatography



known as molecular exclusion chromatography, or Molecular Sieve chromatography or permeation chromatography

Affinity Chromatography

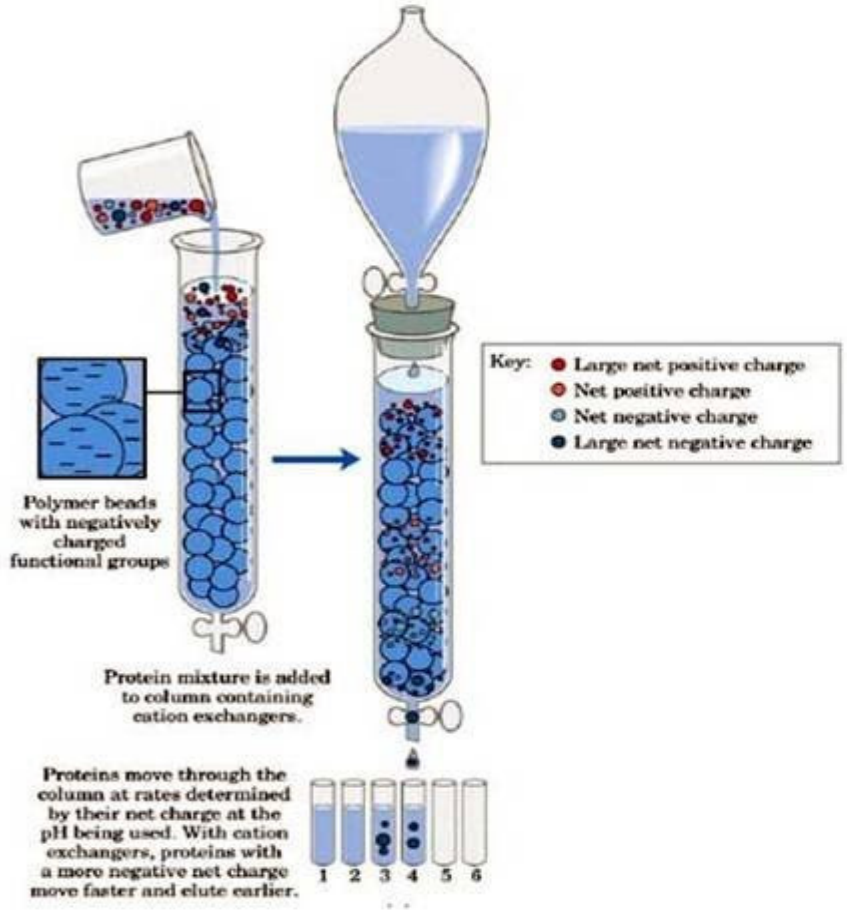
* **affinity chromatography** that based on a highly specif ic biologic interaction such as that between antigen a nd antibody, enzyme and substrate, or receptor and li gand. Any of these substances, covalently linked to an insoluble support or immobilized in a gel, may serve a s the sorbent allowing the interacting substance to be isolated from relatively impure samples; often a 1000-fold purification can be achieved in one step.

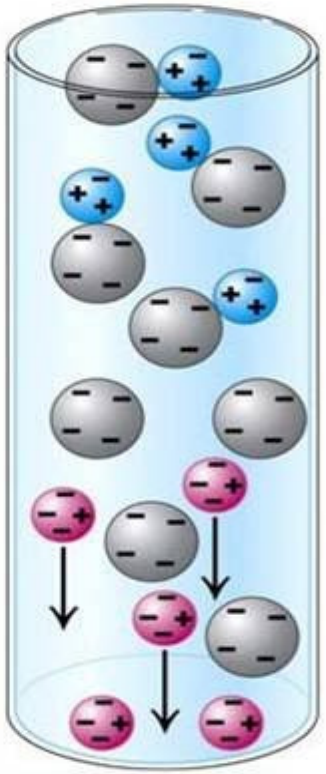

Principles of Affinity Chromatography
Sepharose
Matrix
Ligand
Sepharose
Immobilized Ligand
Immobilized Ligand
Sample
Complex
Impurities
Complex
Purifies
Sample

Ion Exchange Chromatography

* **ion exchange chromatography** that utilizing **ion exchange RESINS**, to which are coupled either cations or anions that will exchange with other cations or ani ons in the material passed through their meshwork.

Ion Exchange Chromatography Principle





Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

Classification according to the packing of the stationary phase:

1- Thin layer chromatography (TLC): the stationary phase is a thin layer supported on glass, plastic or aluminium plates.

2- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support.

3- Column chromatography (CC): stationary phase is packed in a glass column.

How Does Chromatography Work?

* In all chromatographic separations, the sample is transported in a **mobile Phase**
* The mobile phase is then forced through a **stationary phase** (SP) held in a column or on a solid surface.
* Therefore, separation of sample in to their components is based on the d/ce in the migration rates.
* Samples that interact greatly with SP, then appear to move more slowly.
* Samples that interact weakly, then appear to move more quickly. Consequence: separate bands, or zones are obtained (use full for qualitative and quantitative purpose)

Theoretical Plates

* A. J. P. Martin (1910-2002) and R. L. M. Synge (1914-1996) formulated a theory of chromatography using the theoretical plate concept (2,3). These researchers went on to win the Nobel Prize in Chemistry in 1952 for the invention of partition chromatography.
* The number of theoretical plates is proportional to column length, and depends upon column design.

Theoretical Plates

* According to this concept a column can be divided into large number of theoretical zones called theoretical plates.
* To compare distillation efficiencies among columns of different lengths, column length, *L*, is divided by plates, *N*, to give the height equivalent of a theoretical plate.
* H = L/N

Theoretical Plates

* . Separation is uniform throughout a chromatographic column.
* 2. A column can be divided into equal lengths, stages, or segments.
* 3. Within each stage, there is sufficient time for equilibrium to be reached, as solutes partition between mobile and stationary phases.
* 4. Solutes are sufficiently dilute so that their retention characteristics, i.e., thermodynamic properties, are independent of one another.

Theoretical Plates

* 6. The number of theoretical plates generated by a solute can be calculated by representing each peak as a Gaussian distribution.
* 7. Each theoretical plate is considered to be a discrete site (a nano-size separatory funnel, if you wish), in which solutes distribute between two phases.
* 8. After equilibrium, solute is carried by the mobile phase to the next theoretical plate and the process repeated until components emerge from the column with characteristic retention times and peak widths, as described by a Gaussian distribution.