CHROMATOGRAPHY

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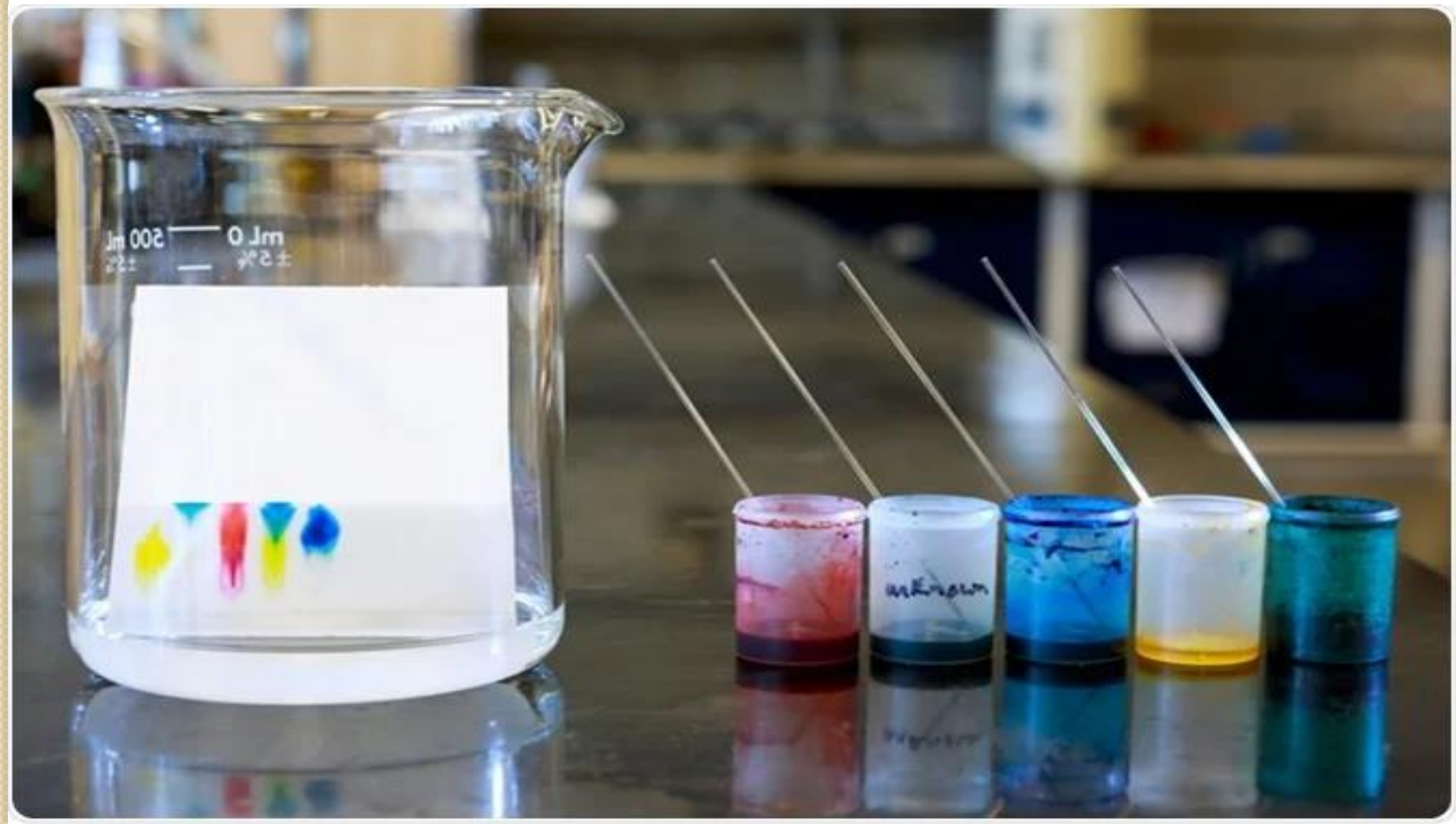


CONTENTS

Definition

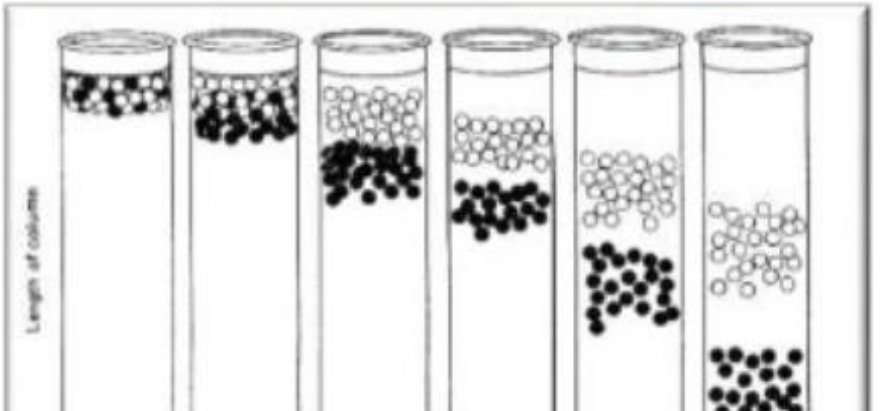
Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction

Stationary phase

Solid
Liquid (supported on a solid or gel)

Mobile phase

Liquid
Gas



Distribution equilibrium:

-Substances are eluted from the chromatographic column in inverse order of their distribution coefficients with respect to the stationary phase.

-The distribution of solutes between two phases is governed by an equilibrium constant known as distribution coefficient, K (or partition coefficient in certain types of chromatography).

$K = C_{stationary} / C_{mobile}$

$C_{stationary}$ = concentration of solute in stationary phase

C_{mobile} = concentration of solute in mobile phase

Larger K value

More time is spent in the stationary phase

Smaller K value

The solute will be eluted very fast with the mobile phase

Rate of travel

Factors limiting rate of travel:

1-Velocity of the mobile phase

2-The ratio of volume of the stationary phase to the volume of the mobile phase

3-The value of the distribution coefficient (it is characteristic for each component)

Determination of the rate of travel:

1- By measuring the distance travelled by each solute after a fixed time (planar chromatography)

2- By measuring the time interval at which each component appears after a fixed distance (column chromatography)

Development

It is the process by which chromatographic separation takes place by allowing the mobile phase to pass through the stationary phase

Elution

Removal of adsorbed solutes from stationary phase using Eluent

Eluate

Fraction of the mobile phase that comes out the column containing the solutes

Clip slide

Effluent

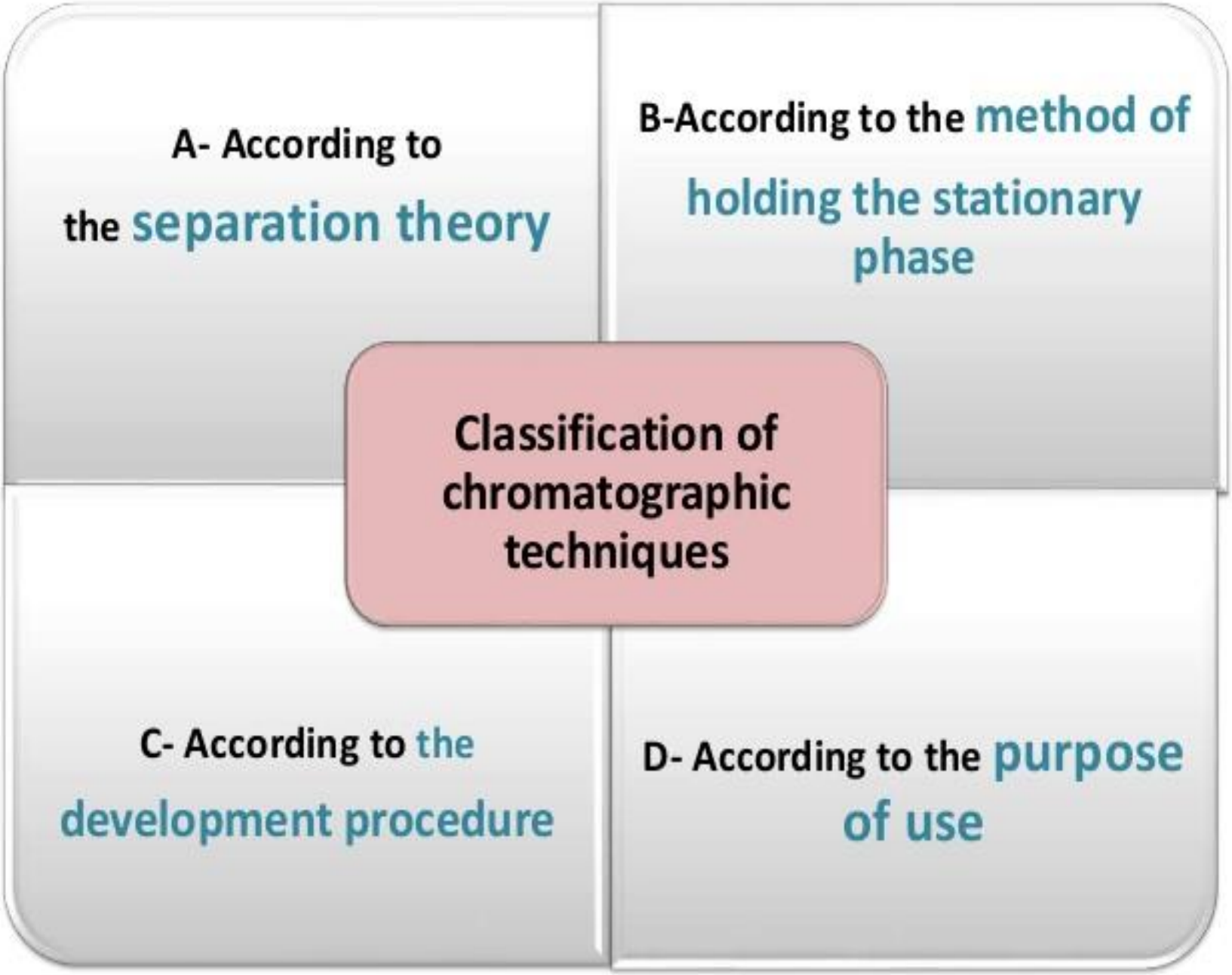
Any solvent that passes out the column

Retention volume

It is the volume of the mobile phase that comes out the column before elution of any solutes

Retention time (R_t)

Denotes the time needed before any solute comes out of the column


A- According to the separation theory
B-According to the method of holding the stationary phase
Classification of chromatographic techniques
C- According to the development procedure
D- According to the purpose of use

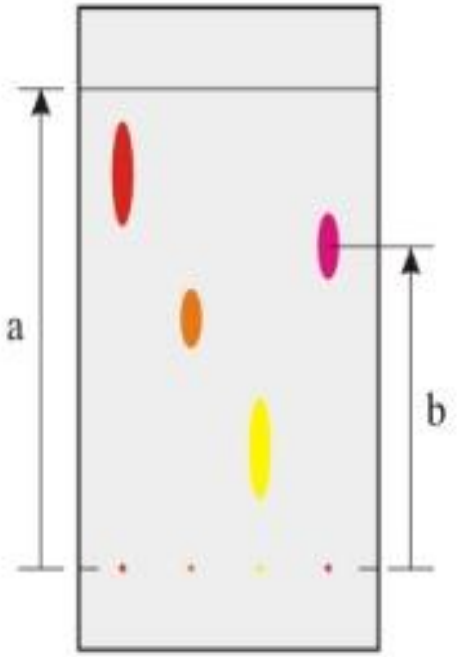
A- According to the mechanism of separation (separation theory)

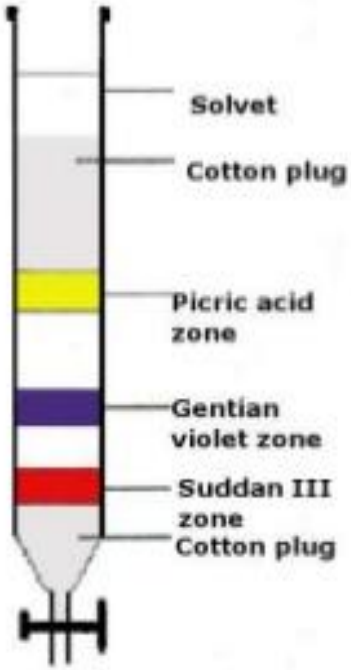
- Adsorption
- Partition
- Ion exchange
- Molecular exclusion
  (Gel permeation , Gel filtration)
- Affinity chromatography

B-ACCORDING TO THE METHOD OF HOLDING THE STATIONARY PHASE

i- **Planar** chromatography

ii- **Columnar** chromatography





C-According to the development procedure

i- Ascending development

ii- Descending development

iii- Radial development

iv- Horizontal development

D- According to the Purpose of use

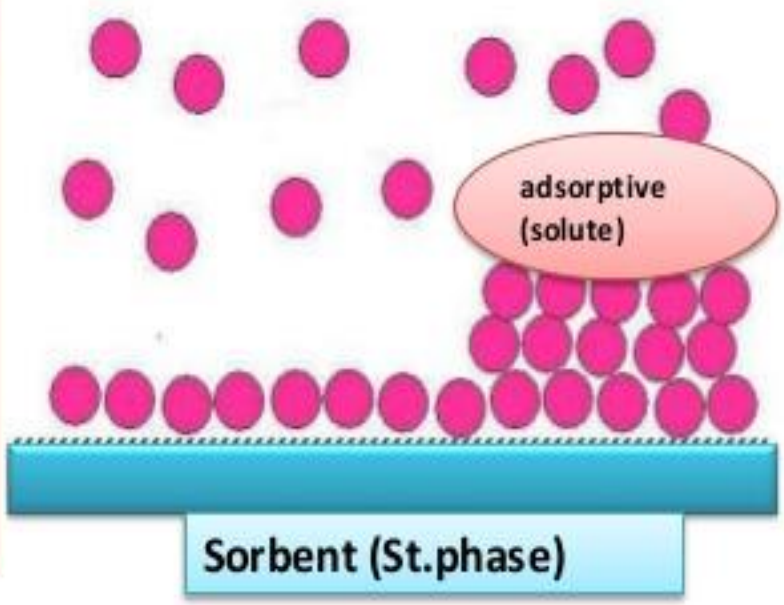
Analytical chromatography (qualitative and quantitative chromatography)

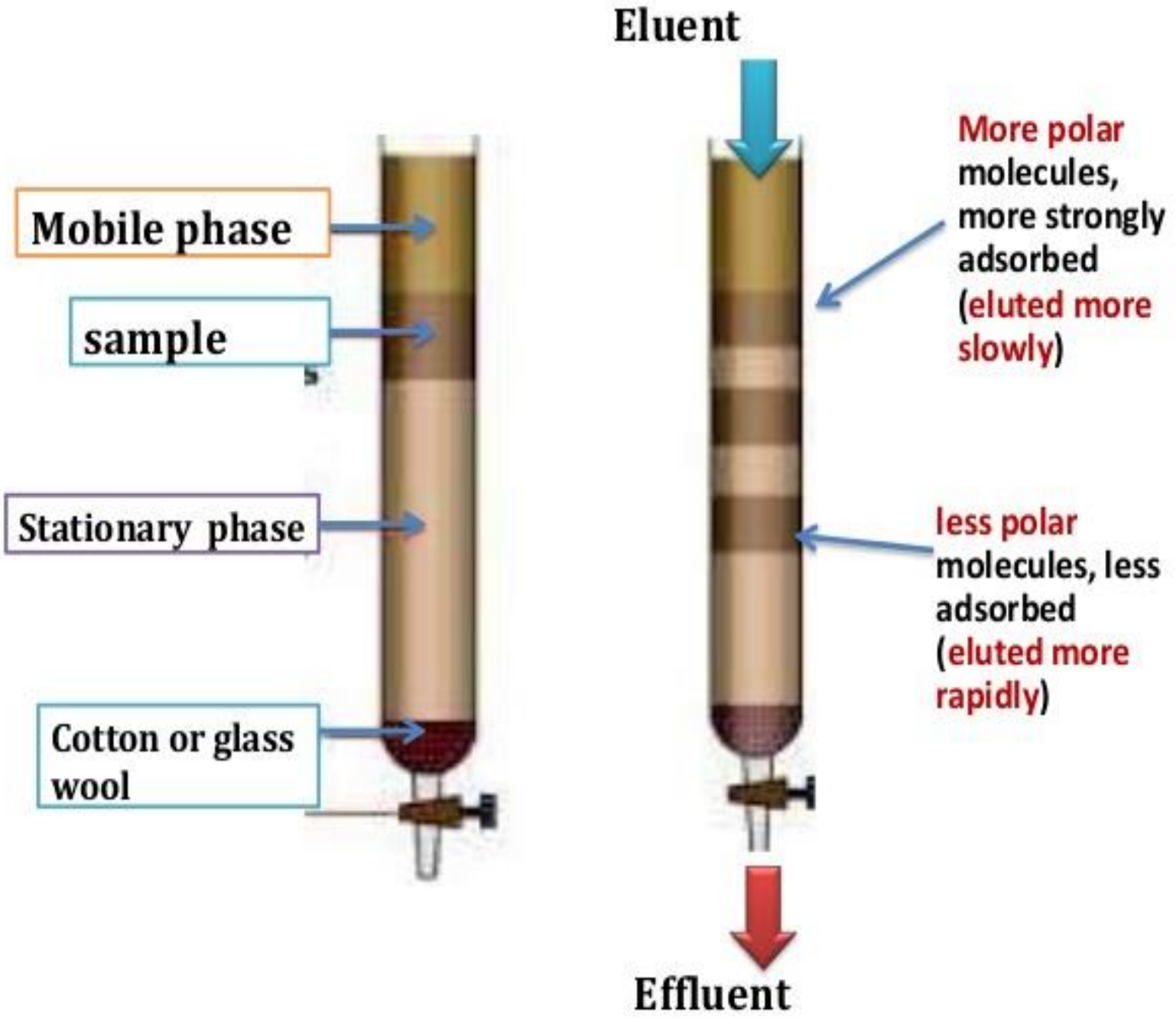
Preparative chromatography (isolation of sample components)

Adsorption chromatography

Adsorption is a surface phenomenon

- In adsorption, the concentration at the interface solid stationary phase/ mobile phase is higher than in the surrounding medium
- More polar molecules, will be more strongly adsorbed (eluted more slowly)




Eluent
Mobile phase
sample
Stationary phase
Cotton or glass wool
More polar molecules, more strongly adsorbed (eluted more slowly)
less polar molecules, less adsorbed (eluted more rapidly)
Effluent

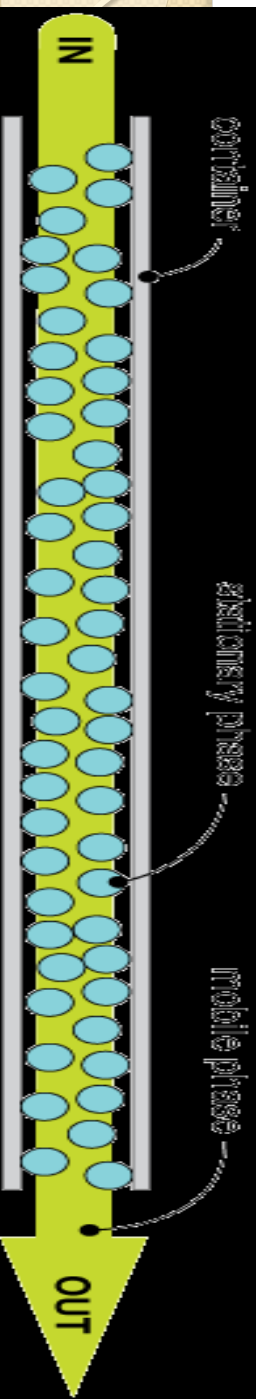
stationary phase is Solid

- Liquid Solid chromatography LSC
- TLC
- HPLC

- Gas solid chromatography GSC

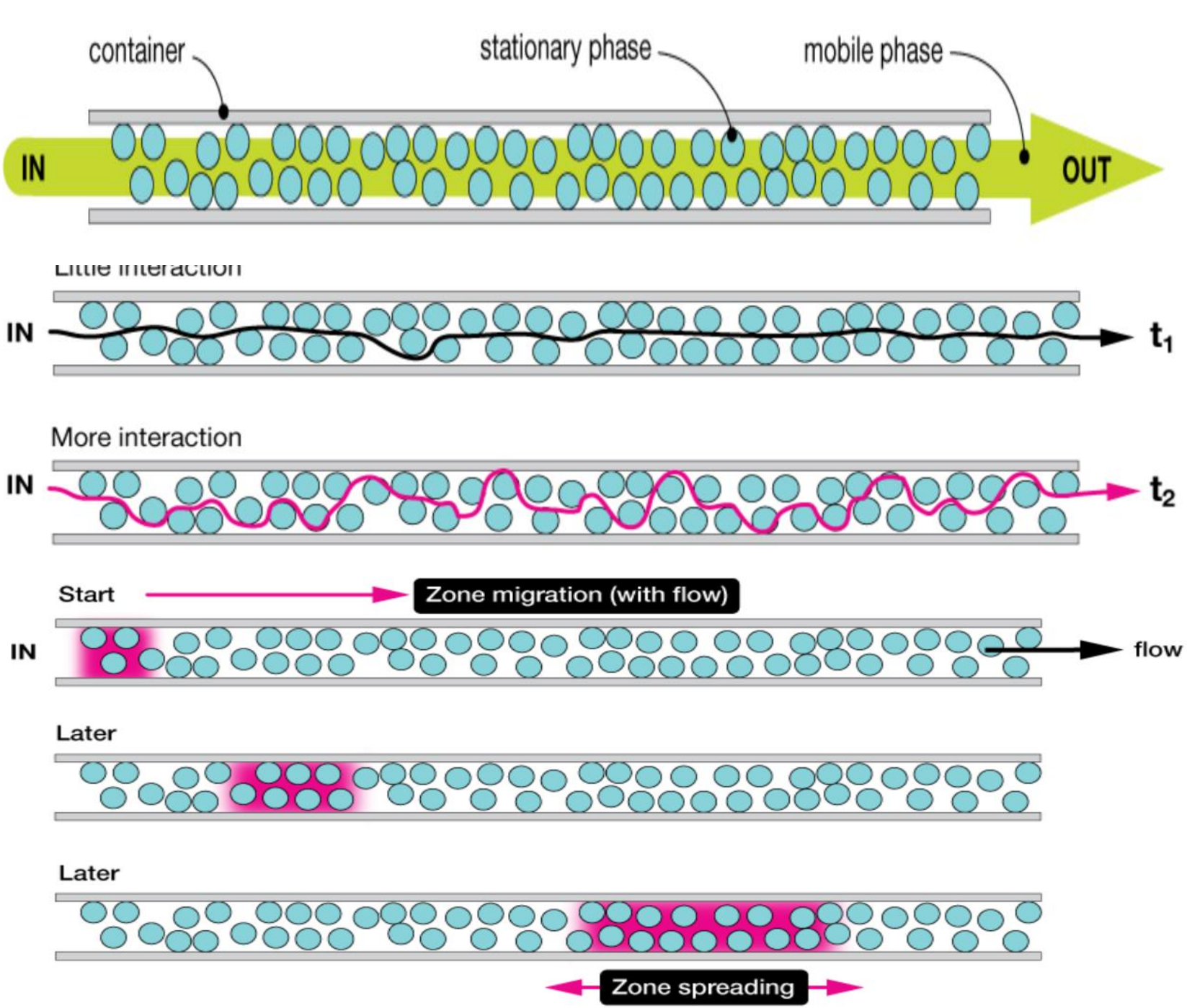
ii-Common adsorbents (Stationary phase)

- ✓ Alumina
- ✓ silica
- ✓ magnesium oxide
- ✓ Charcoal
- ✓ calcium carbonate



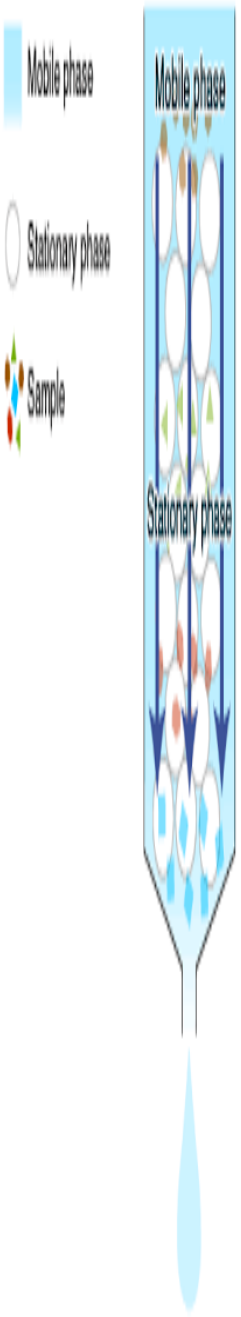
Chromatography

- 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.


container
stationary phase
mobile phase
IN
OUT
Little interaction
IN
t_1
More interaction
IN
t_2
Start
Zone migration (with flow)
IN
flow
Later
Later
Zone spreading

History

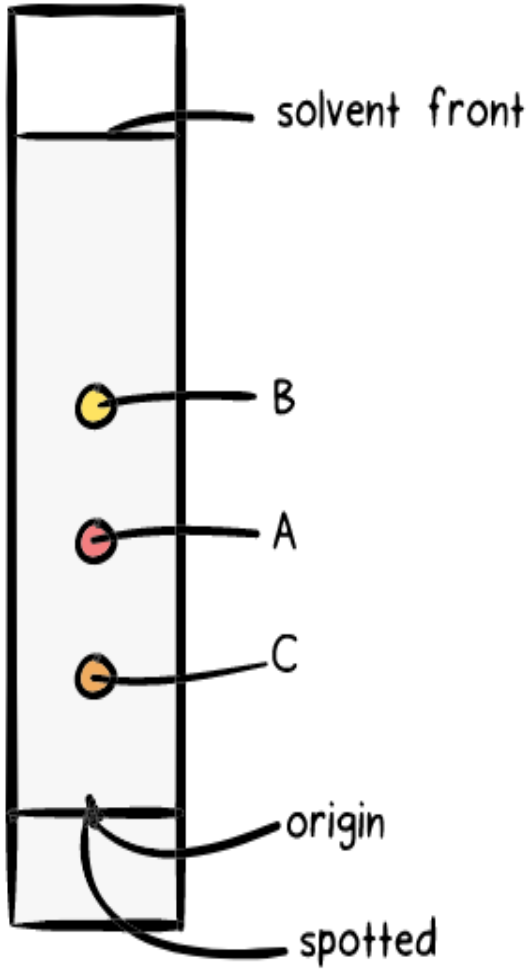
- Chromatography, literally "color writing", was first employed by Russian scientist Mikhail Tsvet in 1900.
- He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls.
- Since these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



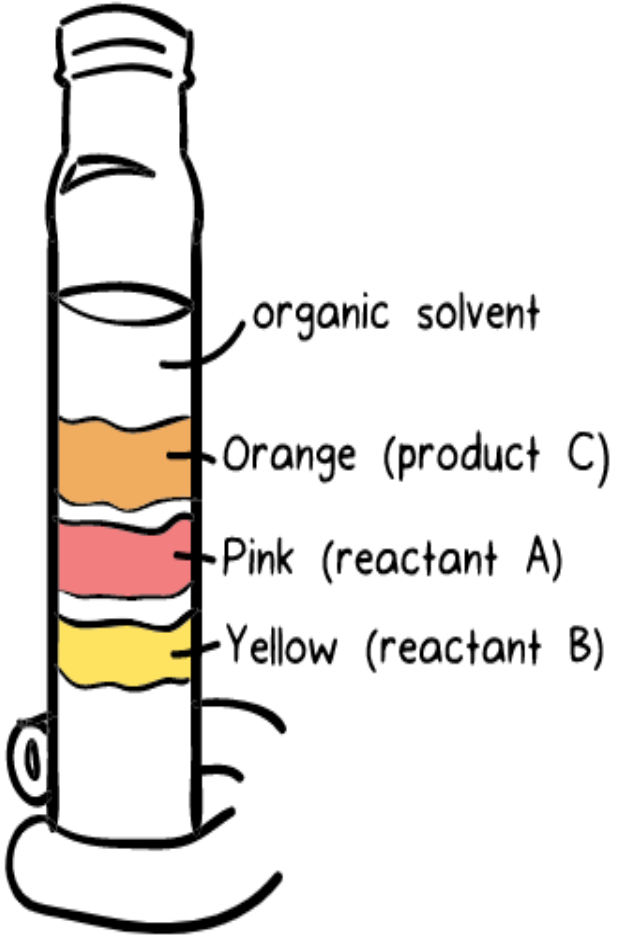
Principles

- 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.

Thin Layer Chromatography (TLC)



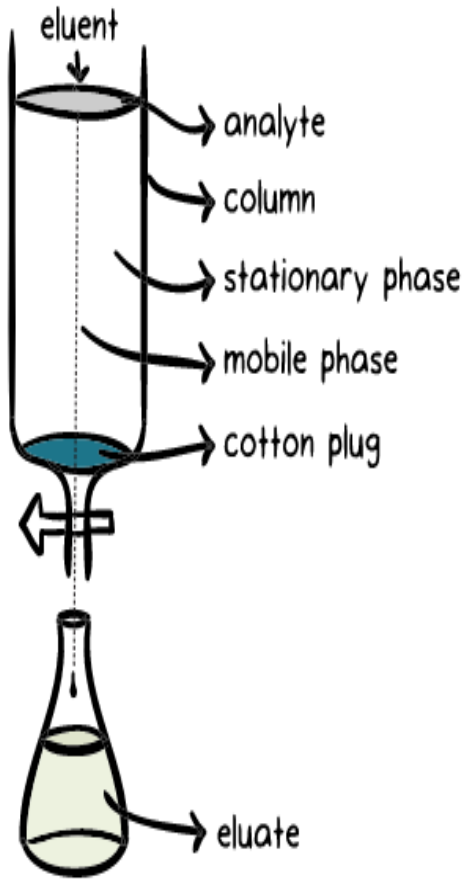
Column Chromatography



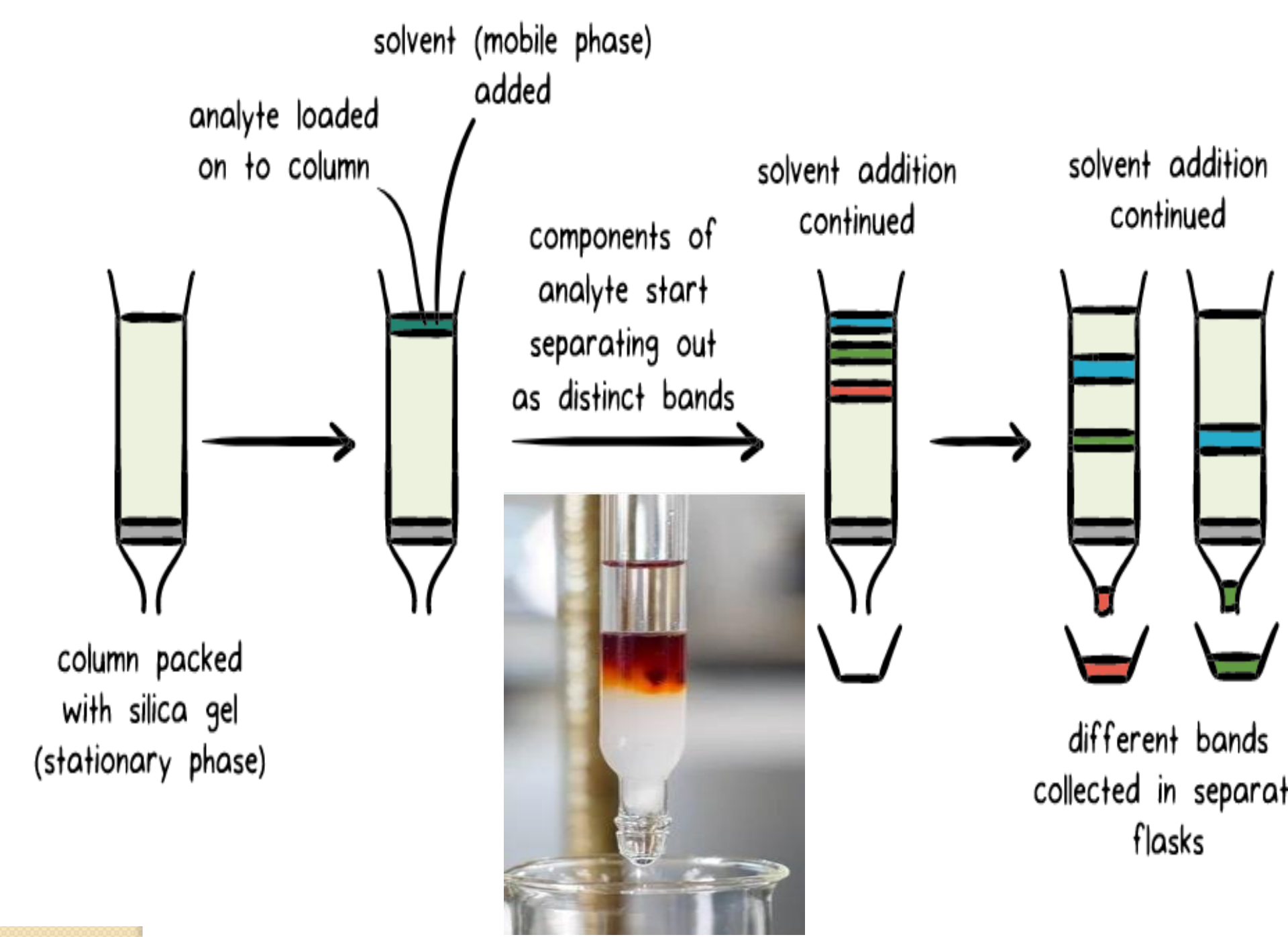
Glass column packed with silica in an organic solvent, reaction mixture loaded on the silica bed with help of a glass pipette

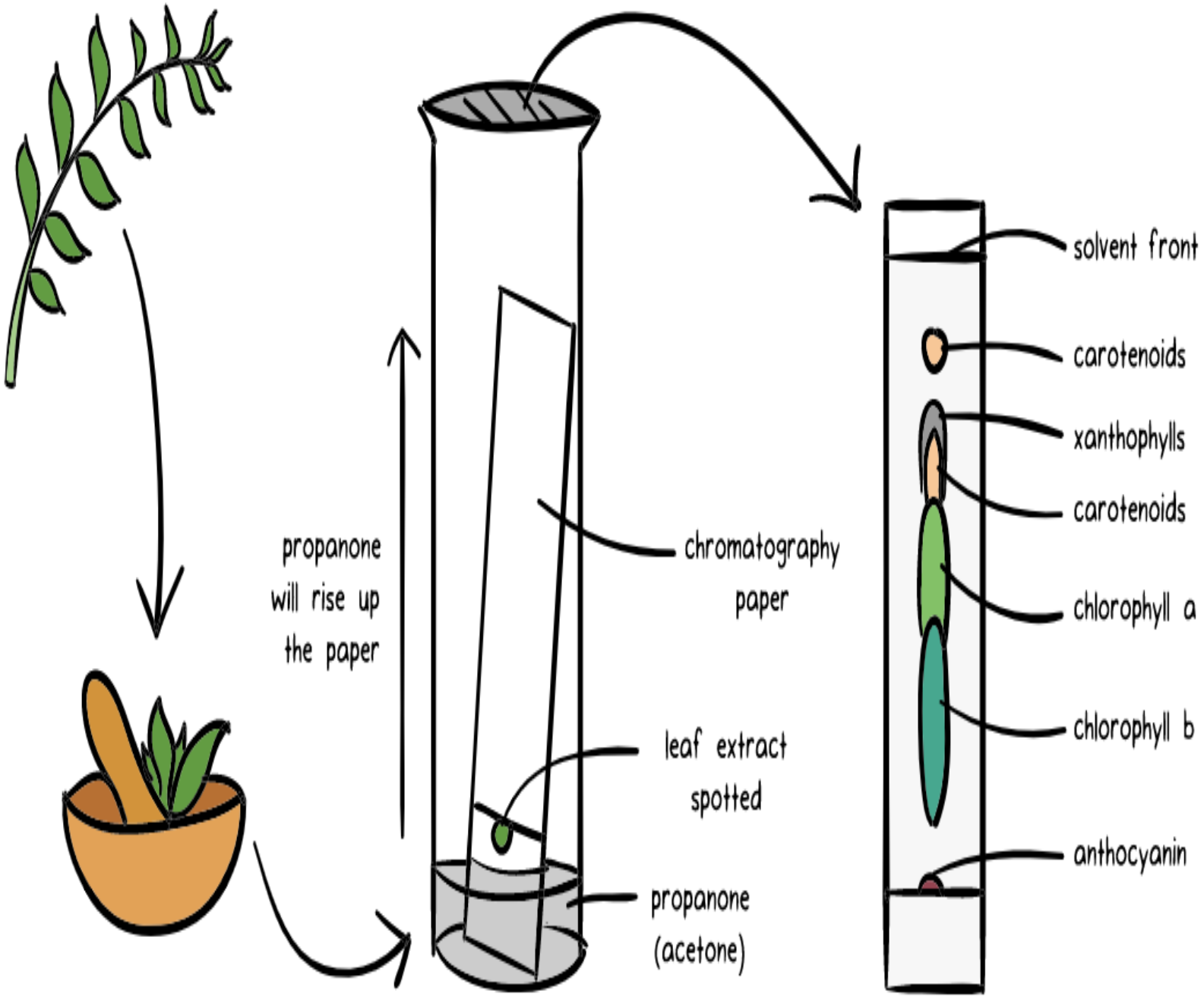
Principles of chromatography

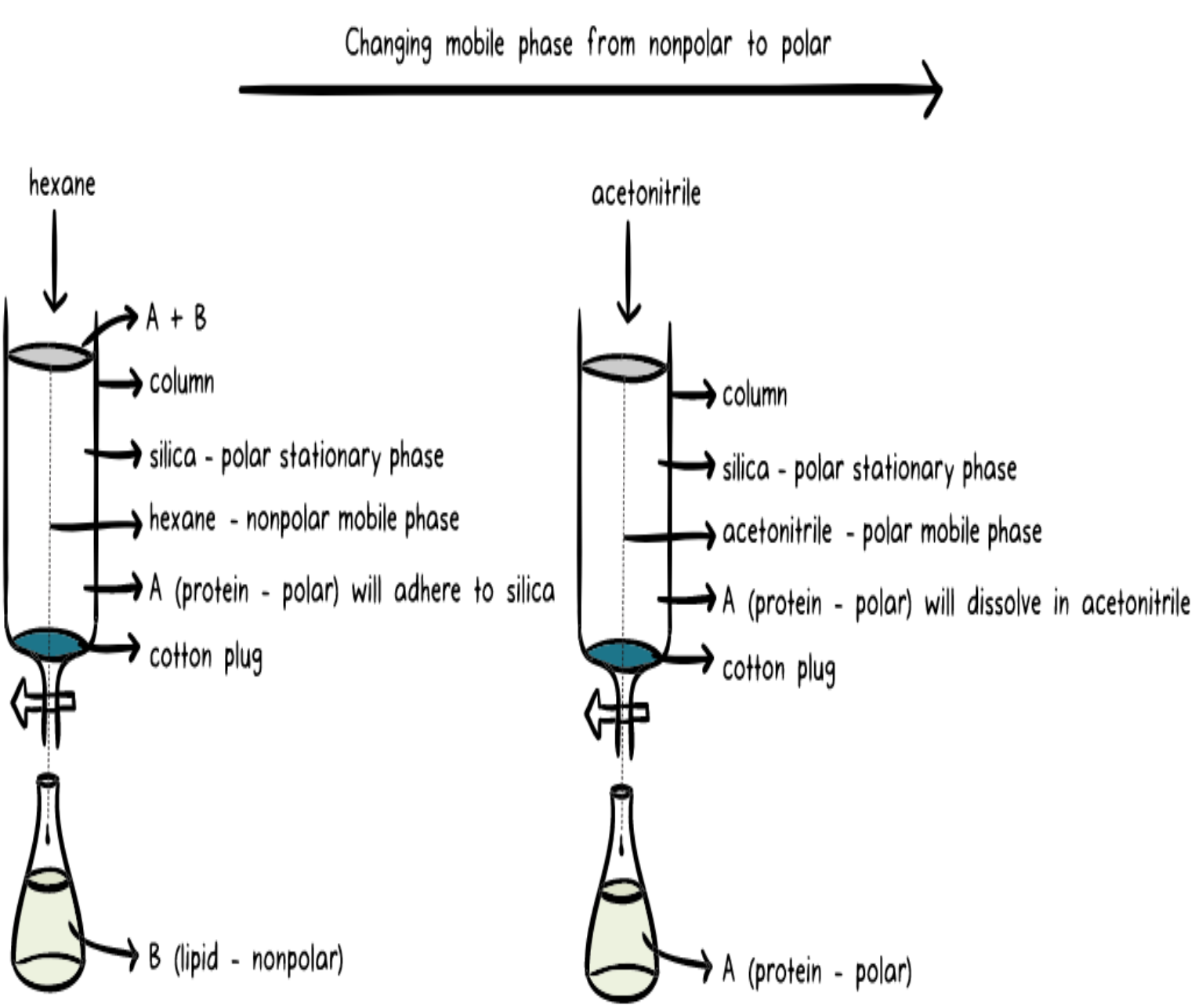
Let's first familiarize ourselves with some terms that are commonly used in the context of chromatography:



Term	Definition
Mobile phase or carrier	solvent moving through the column
Stationary phase or adsorbent	substance that stays fixed inside the column
Eluent	fluid entering the column
Eluate	fluid exiting the column (that is collected in flasks)
Elution	the process of washing out a compound through a column using a suitable solvent
Analyte	mixture whose individual components have to be separated and analyzed


solvent (mobile phase) added
analyte loaded on to column
components of analyte start separating out as distinct bands
solvent addition continued
solvent addition continued
column packed with silica gel (stationary phase)
different bands collected in separat flasks


propanone
will rise up
the paper
chromatography
paper
leaf extract
spotted
propanone
(acetone)
solvent front
carotenoids
xanthophylls
carotenoids
chlorophyll a
chlorophyll b
anthocyanin


Changing mobile phase from nonpolar to polar
hexane
A + B
column
silica - polar stationary phase
hexane - nonpolar mobile phase
A (protein - polar) will adhere to silica
cotton plug
B (lipid - nonpolar)
acetonitrile
column
silica - polar stationary phase
acetonitrile - polar mobile phase
A (protein - polar) will dissolve in acetonitrile
cotton plug
A (protein - polar)

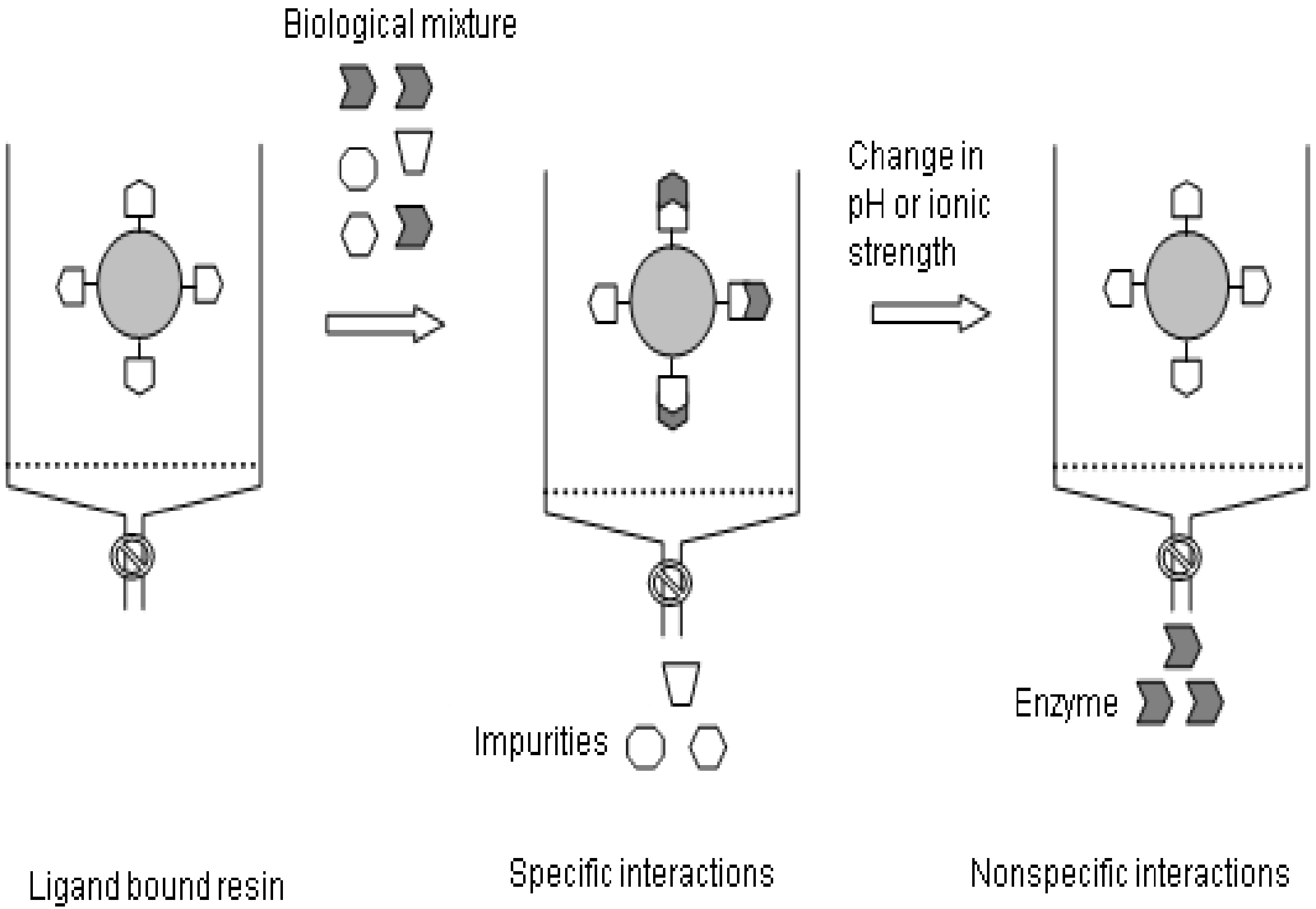


Fig. 1.24 Schematic representation of affinity-adsorption chromatography

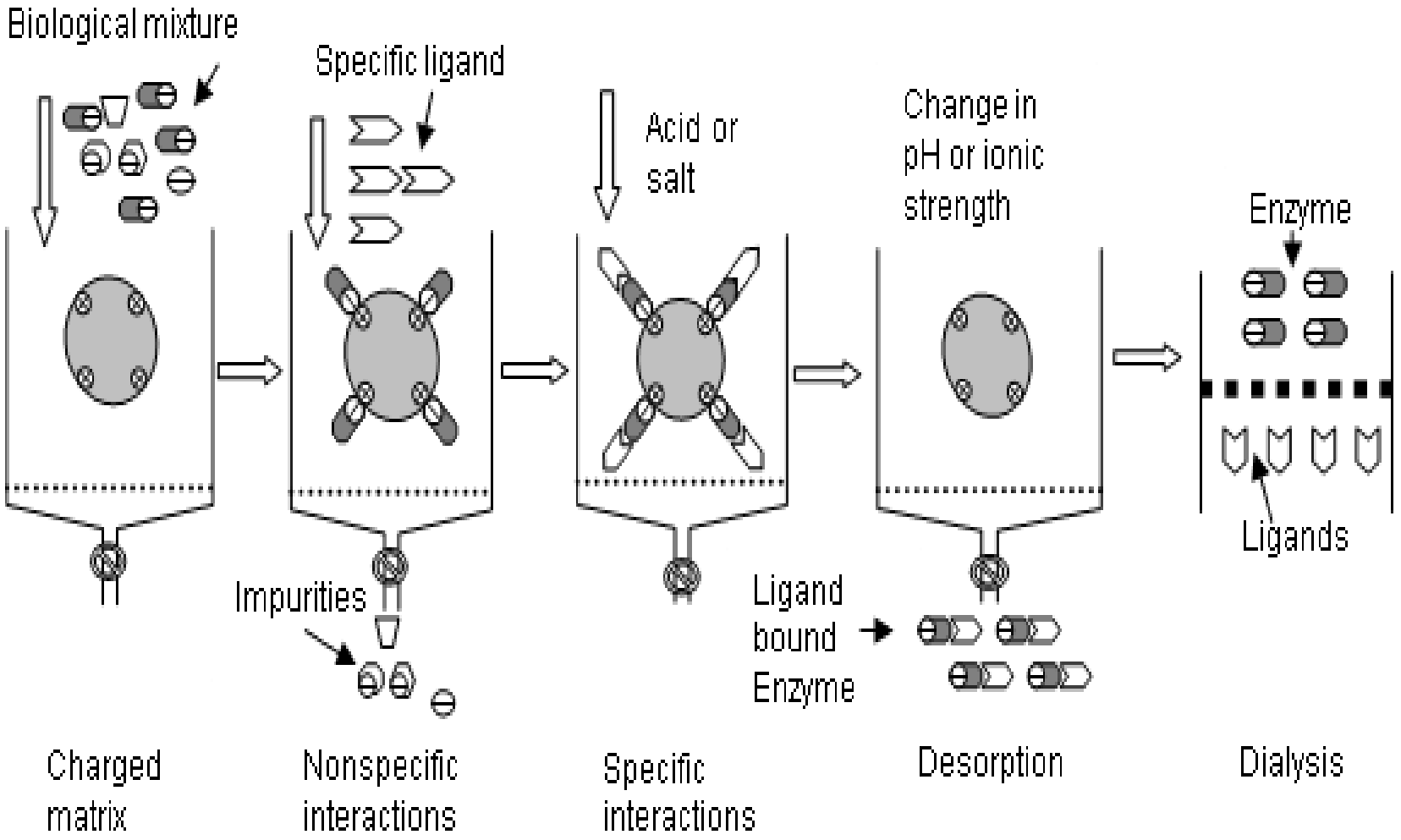


Fig. 1.25 Schematic representation of affinity-elution chromatography

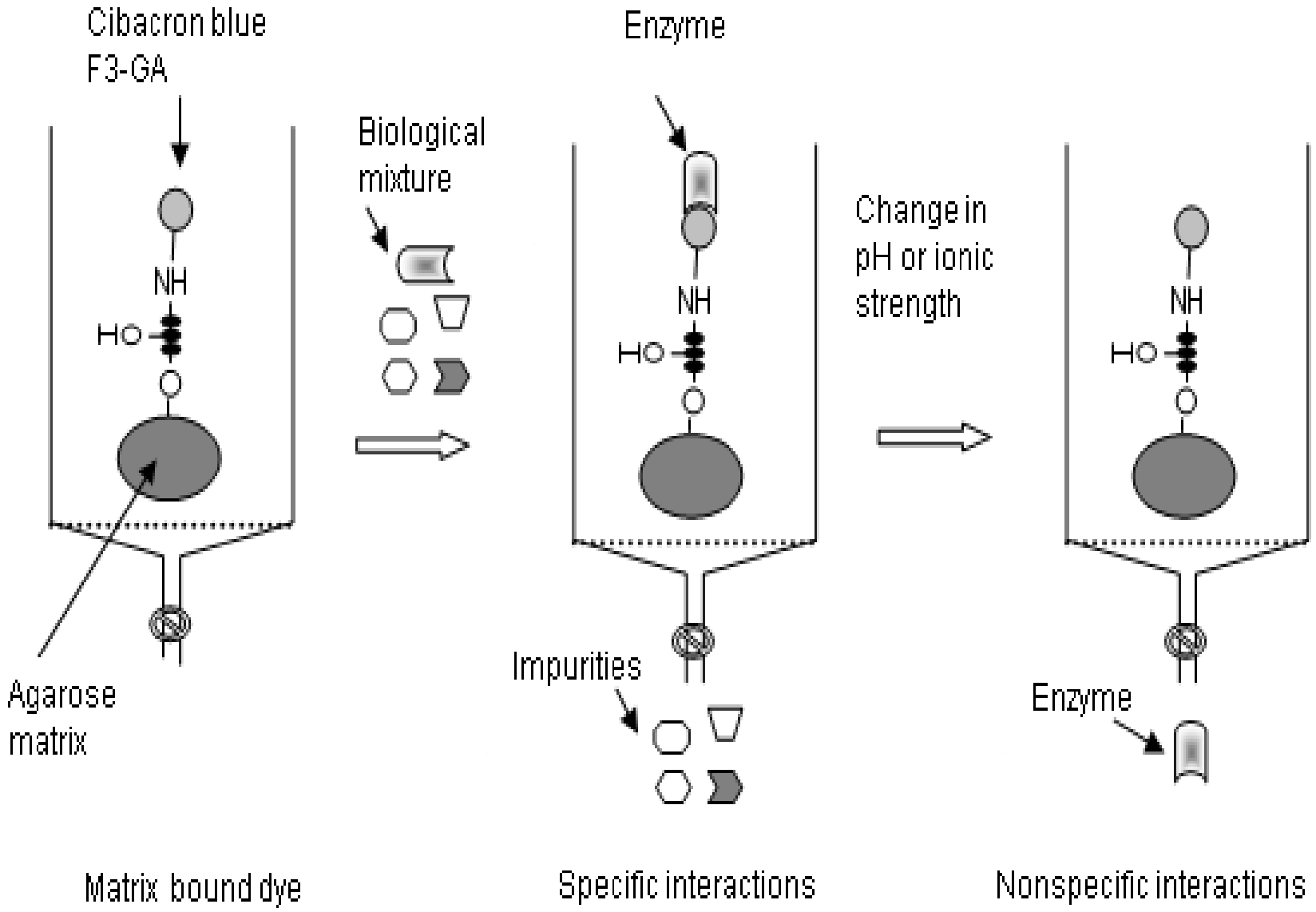


Fig. 1.26 Schematic representation of dye-ligand chromatography

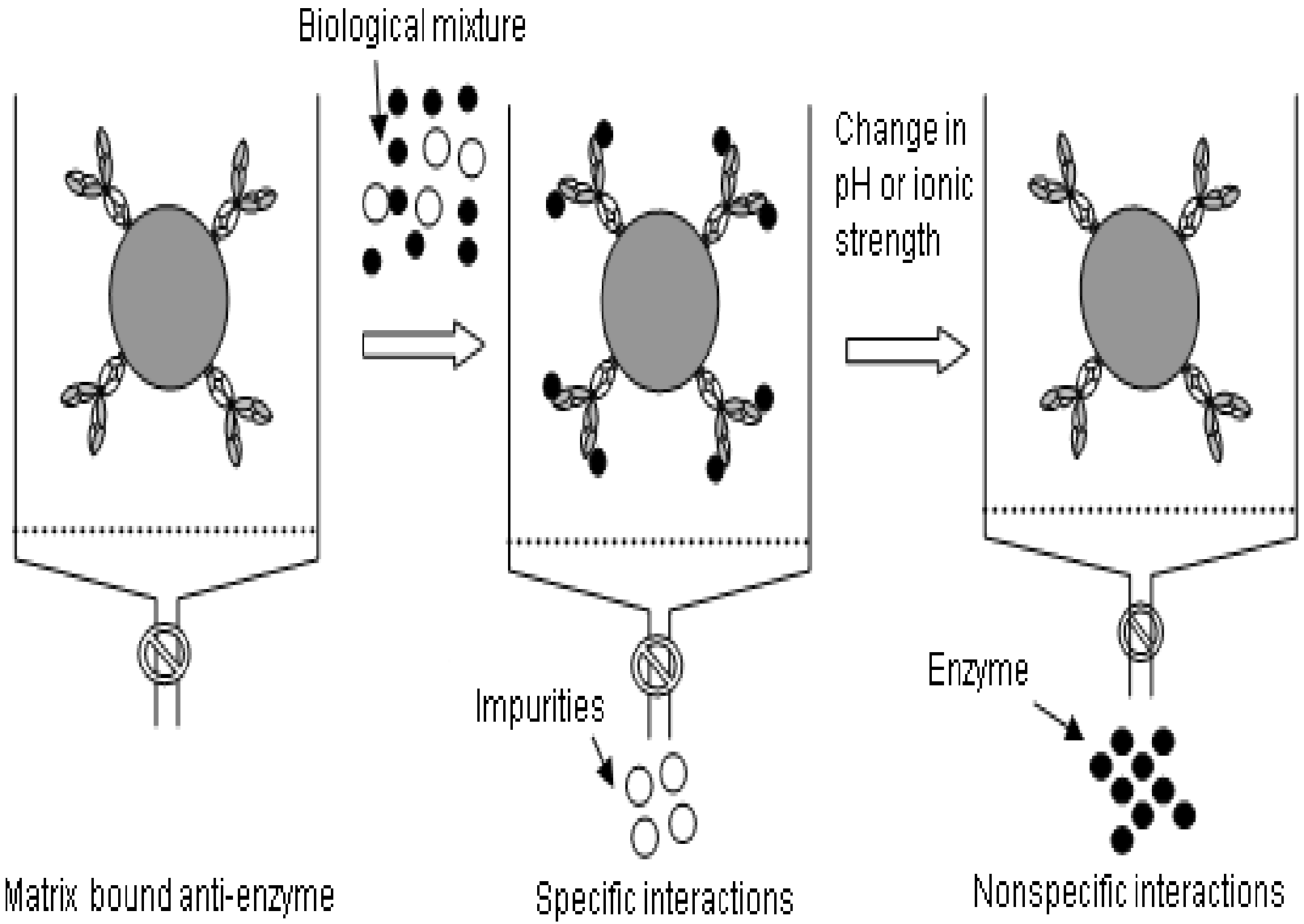


Fig. 1.27 Schematic representation of immuno-adsorption chromatography

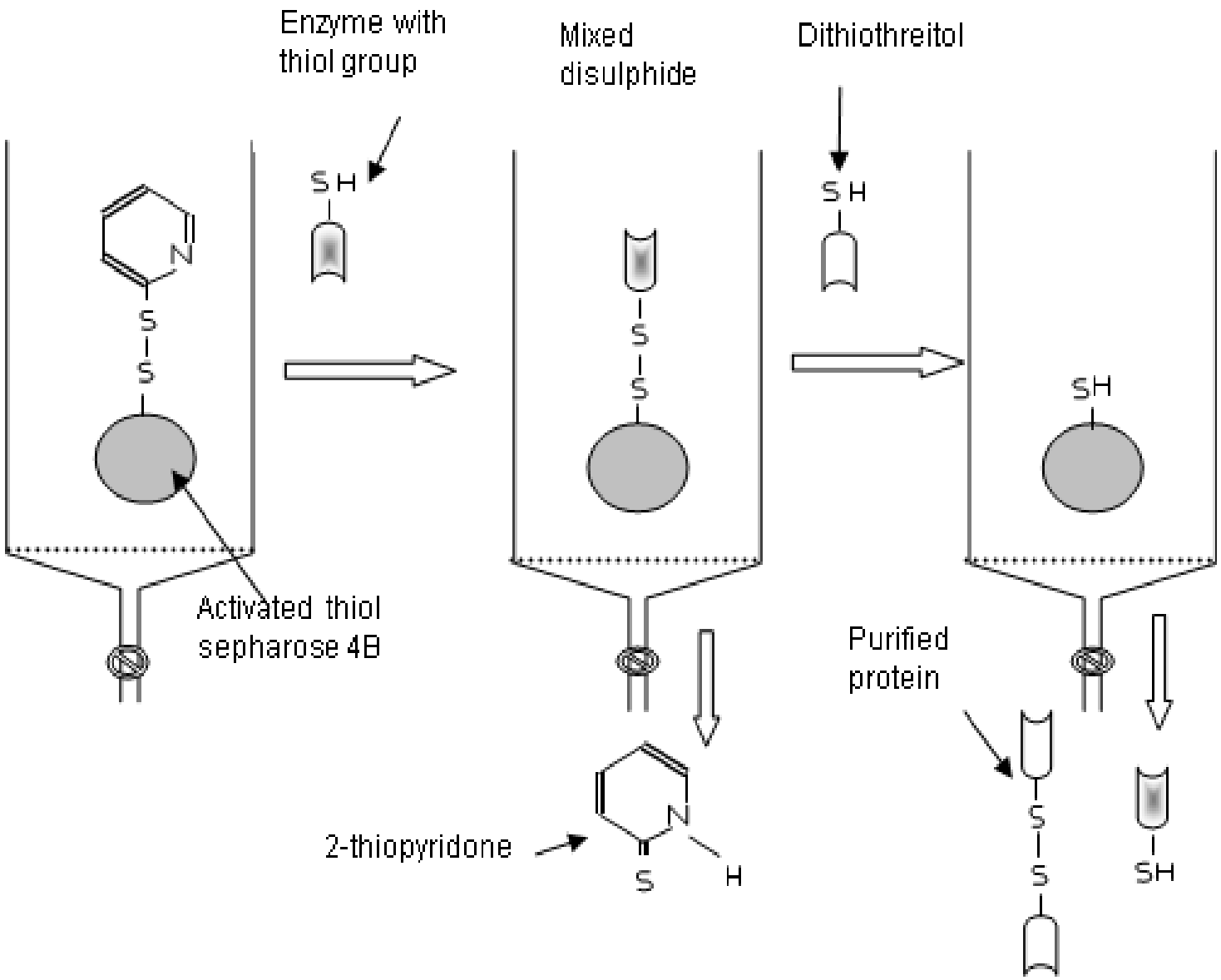


Fig. 1.28 Schematic representation of covalent chromatography

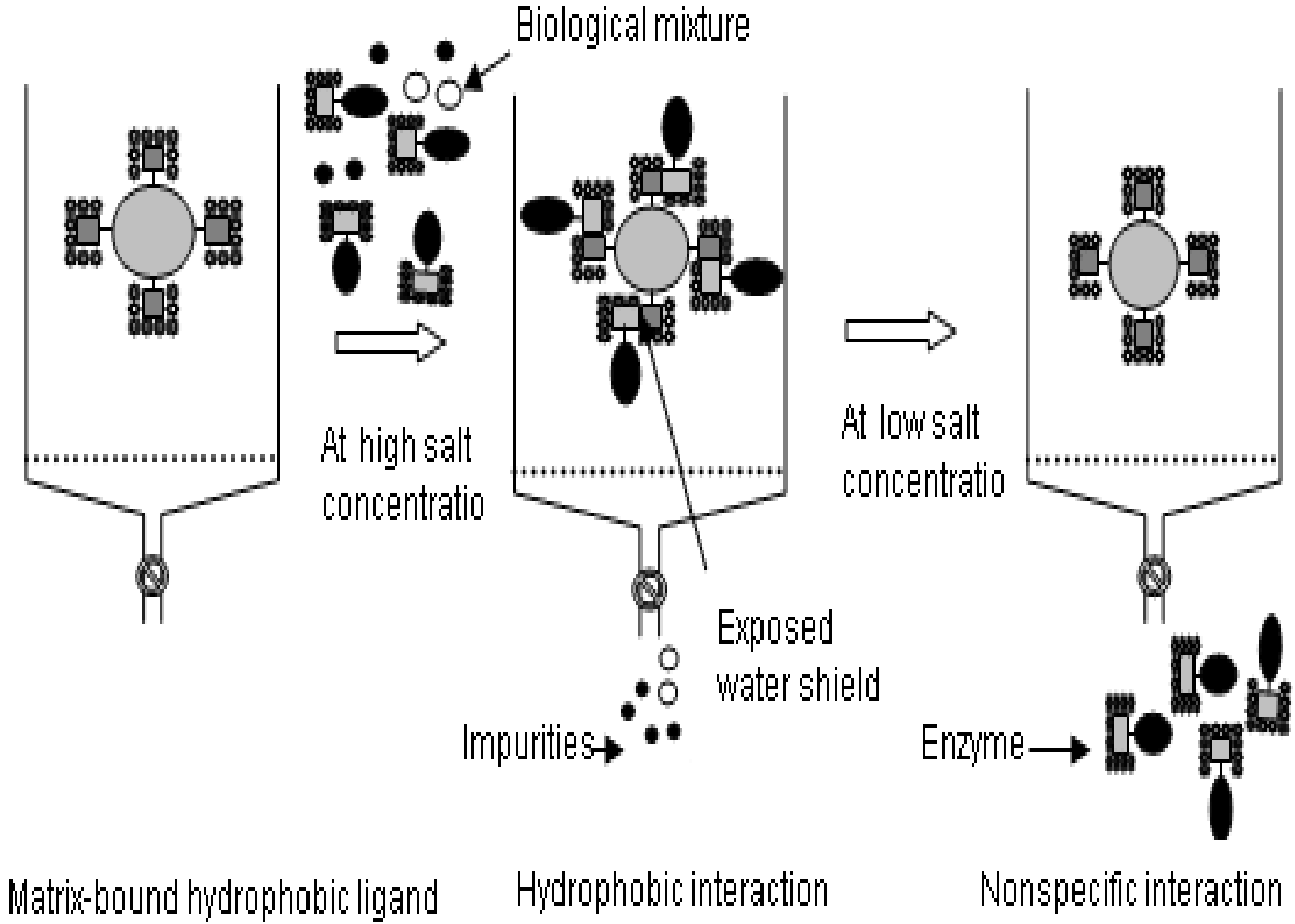


Fig. 1.32 Schematic representation of hydrophobic interaction chromatography.

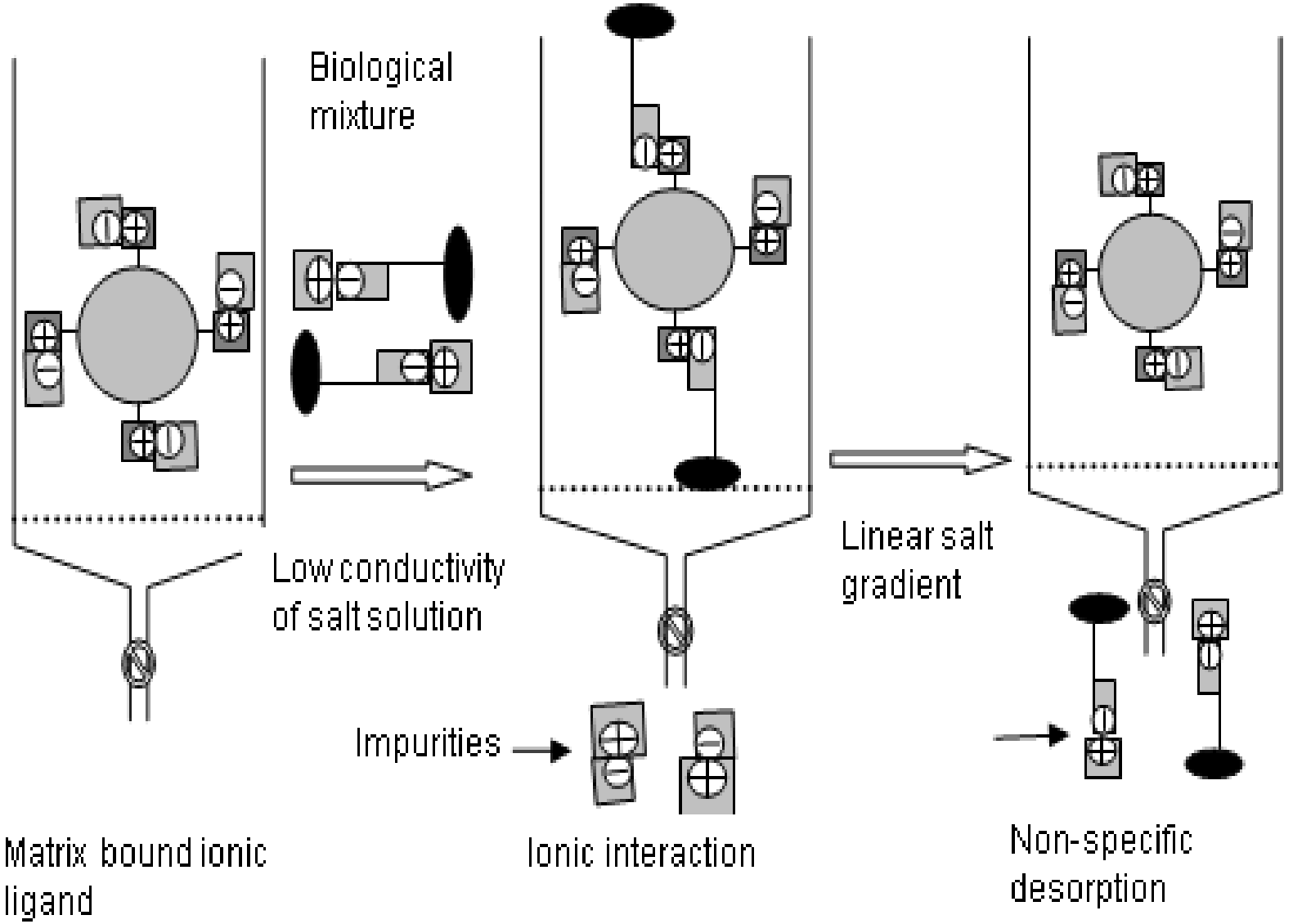


Fig. 1.33 Schematic representation of ion exchange chromatography

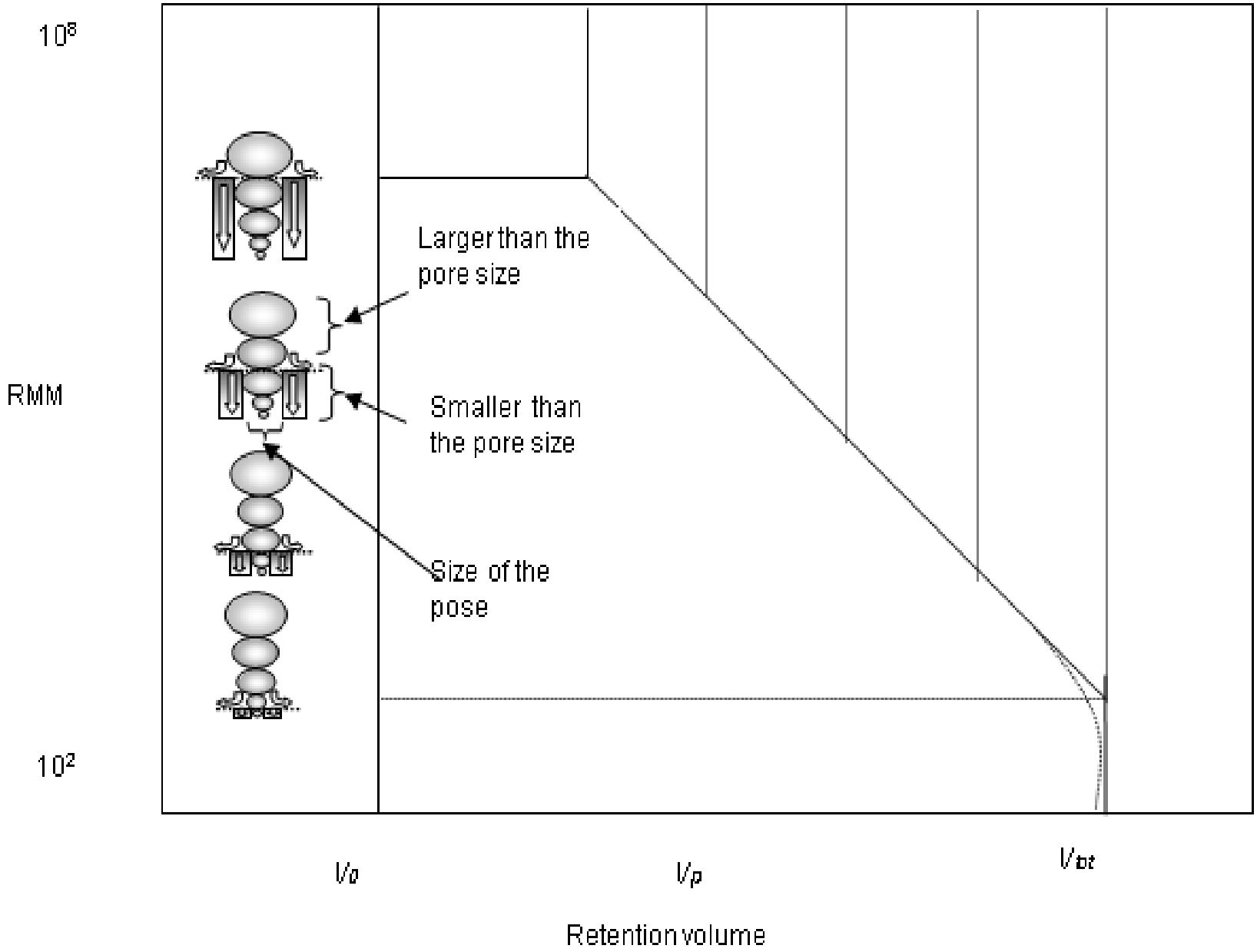


Fig. 1.36 Schematic representation of molecular sieving chromatography with graph

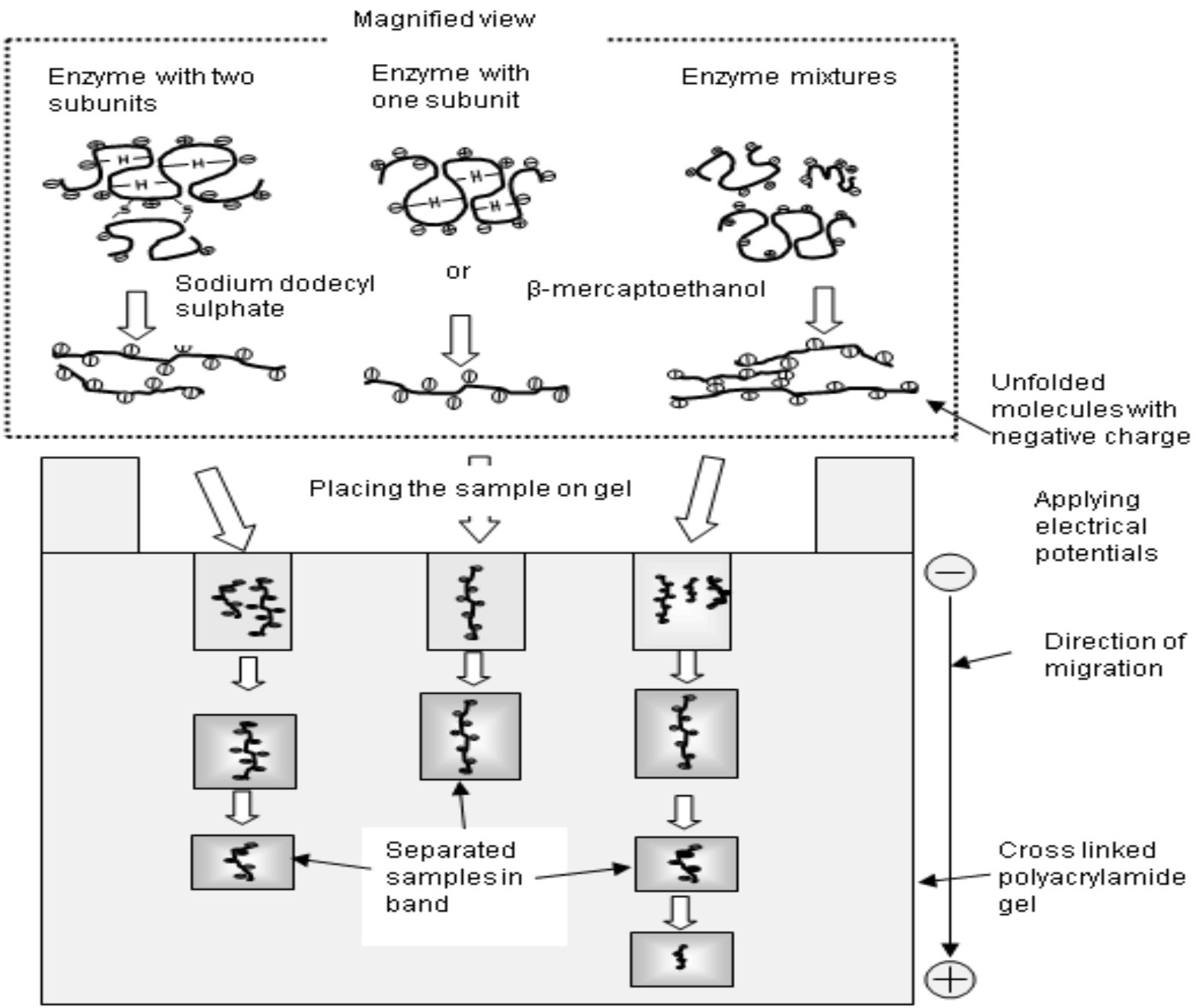


Fig. 1.35 Schematic representation of electrophoretic separation of enzymes based on size

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

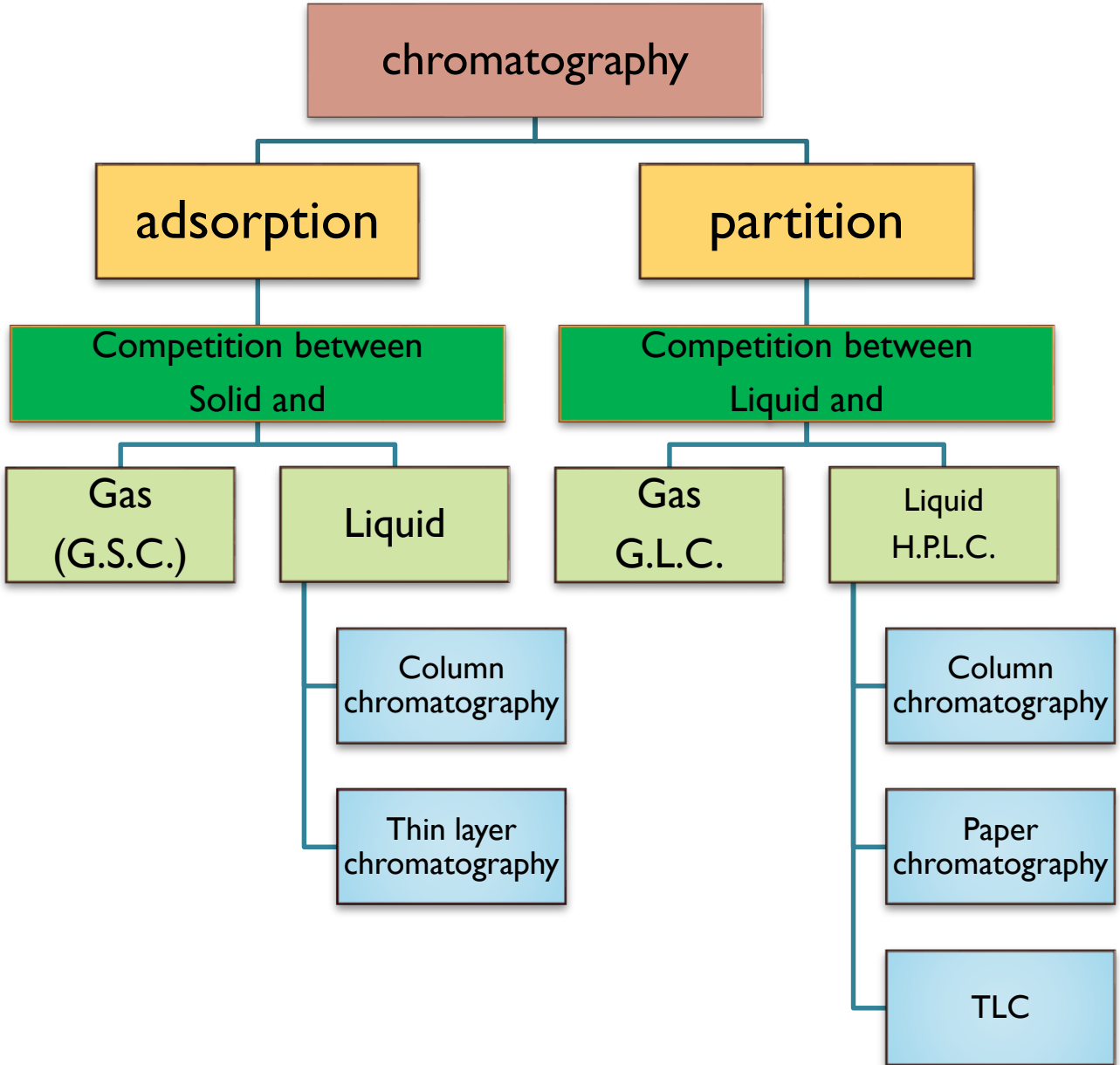
Chromatographic terms

- 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.

Classification of chromatography

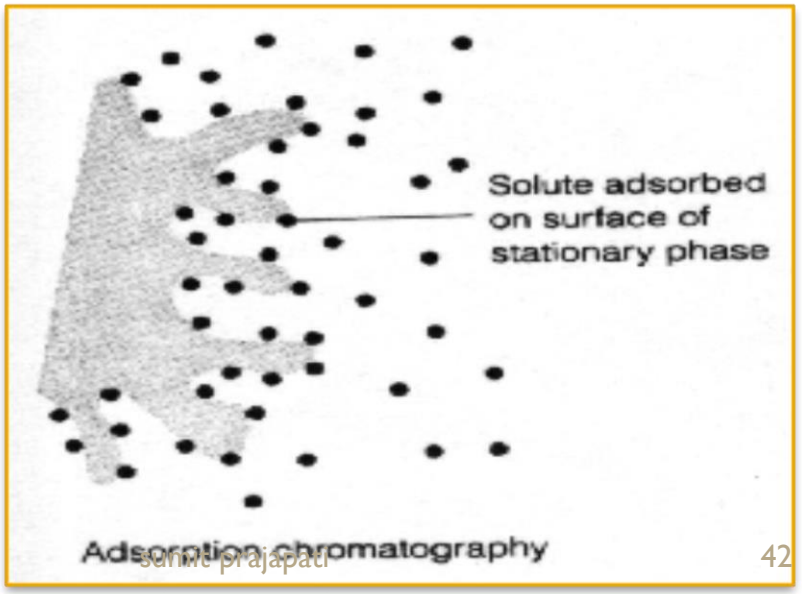
1. **Based on mechanism of separation**
   - I. adsorption chromatography
   - II. Partition chromatography
2. **Based on phases**
   - I. Solid phase chromatography
     - i. Solid-liquid chromatography
     - ii. Solid-gas chromatography
   - II. Liquid phase chromatography
     - i. Liquid-liquid 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 chromatograohy

- 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

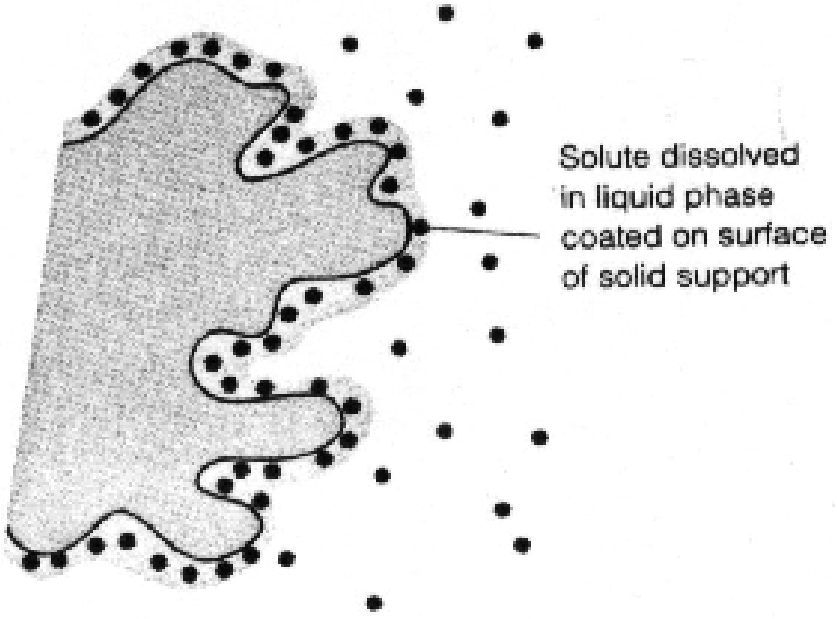


Partition chromatography

- 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

Gas-Solid chromatography(G.S.C.)

- Gas chromatography employs an inert gas as the mobile phase
- The mobile phase is a gas, often nitrogen, but sometimes helium, hydrogen or occasionally another gas. It is called the "carrier gas".
- Common solids are charcoal, a synthetic zeolite called "molecular sieve", or a combination of the two.
- Separation depends on the relative partial pressures of the sample components above the stationary phase.
- Gas-solid chromatography is relatively rare, but it is used to separate atmospheric gases

Solid-Liquid chromatography

- Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid.
- The preferred mobile phase is a nonpolar or slightly polar...
- Liquid chromatography can be carried out either in a column or a plane
- In **liquid-solid chromatography** the porous adsorbent is polar and separation is based on the properties of classes of compounds—e.g., amines (alkaline) from alcohols (neutral) and esters (neutral) from acids
- Popular adsorbents are Silica and Alumina.

Liquid-Gas Chromatography

- The mobile phase is an unreactive gas, such as nitrogen (the carrier gas)

- The stationary phase comprises of a small amount of liquid held on a finely-divided inert solid support.

- Gas-liquid chromatography is very sensitive and can be used to detect small quantities of substances
  it is often used in forensic tests

❖ Stationary phase used in (LGC)

- **Dimethyl Polysiloxane (350°C)**
  Hydrocarbons, Polynuclear aromatics
- **Poly(phenyl methyl) siloxane (250°C)**
  Steroids, Pesticides, Glycols

Liquid-Liquid Chromatography

- The first liquid-liquid system was reported by A. J. P. Martin who used water supported on silica gel as the stationary phase and n-heptane as the mobile phase

- Liquid-liquid chromatography is a chromatography separation technique in which the mobile phase is a liquid (usually a solvent or a simple binary solvent mixture) and the stationary phase is also a liquid (which must be immiscible and insoluble in the liquid mobile phase).

- The system is inherently unstable, as the stationary phase will always have some solubility in mobile phase

Planner chromatography

- **Planar chromatography** is a separation technique in which the stationary phase is present on a plane.

- The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (paper chromatography) or a layer of solid particles spread on a support such as a glass plate (Thin layer chromatography).

- Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase.

- The specific Retention factor (R_f) of each chemical can be used to aid in the identification of an unknown substance.

Column Chromatography

- Column chromatography is a separation technique in which the stationary bed is **within a tube**.

- The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column).

- Differences in rates of movement through the medium are calculated to different retention times of the sample

Important properties of liquid stationary phase

- Liquid phase should have low volatility and high stability at elevated temperatures
- Liquid phase should not permeate too deeply into the fine pores of the support structure as slow diffusion in and out of pores affects column efficiency
- Support should be deactivated before use as undesirable surface impurities can cause decomposition of the sample or stationary liquid
- Small particles of support give higher efficiency as HETP is proportional to particle diameter but particle size reduction increases back pressure

Conclusion

- In overall ranking Chromatography techniques , it can be judge SFC falls somewhere between HPLC or GC.
- In field of pharmaceutical chemistry and bioanalytical application gained its applications

References

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- www.wikipedia.com
- www.studymafia.org
- www.pptplanet.com


The End