Chromatography

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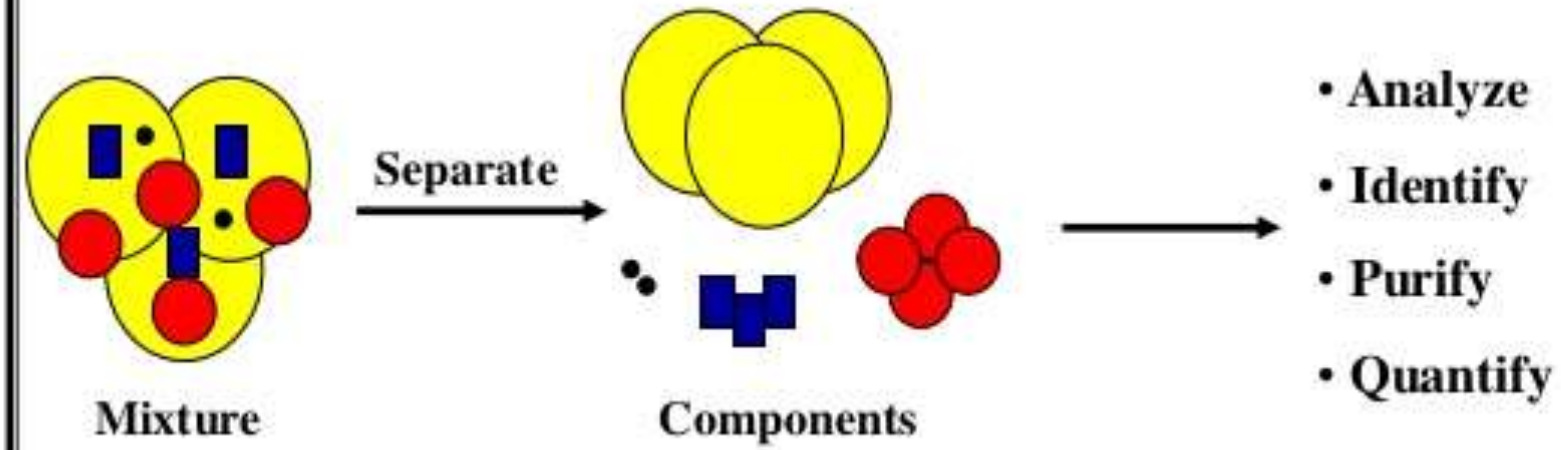
CHROMATOGRAPHIC SEPARATIONS

What is Chromatography?

- Chromatography is a technique for separating mixtures into their components in order to analyze, identify, purify, and/or quantify the mixture or components.



Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase.

Principle :

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.

It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases.

<u>The mobile phase:</u>

is the phase which moves in a definite direction.

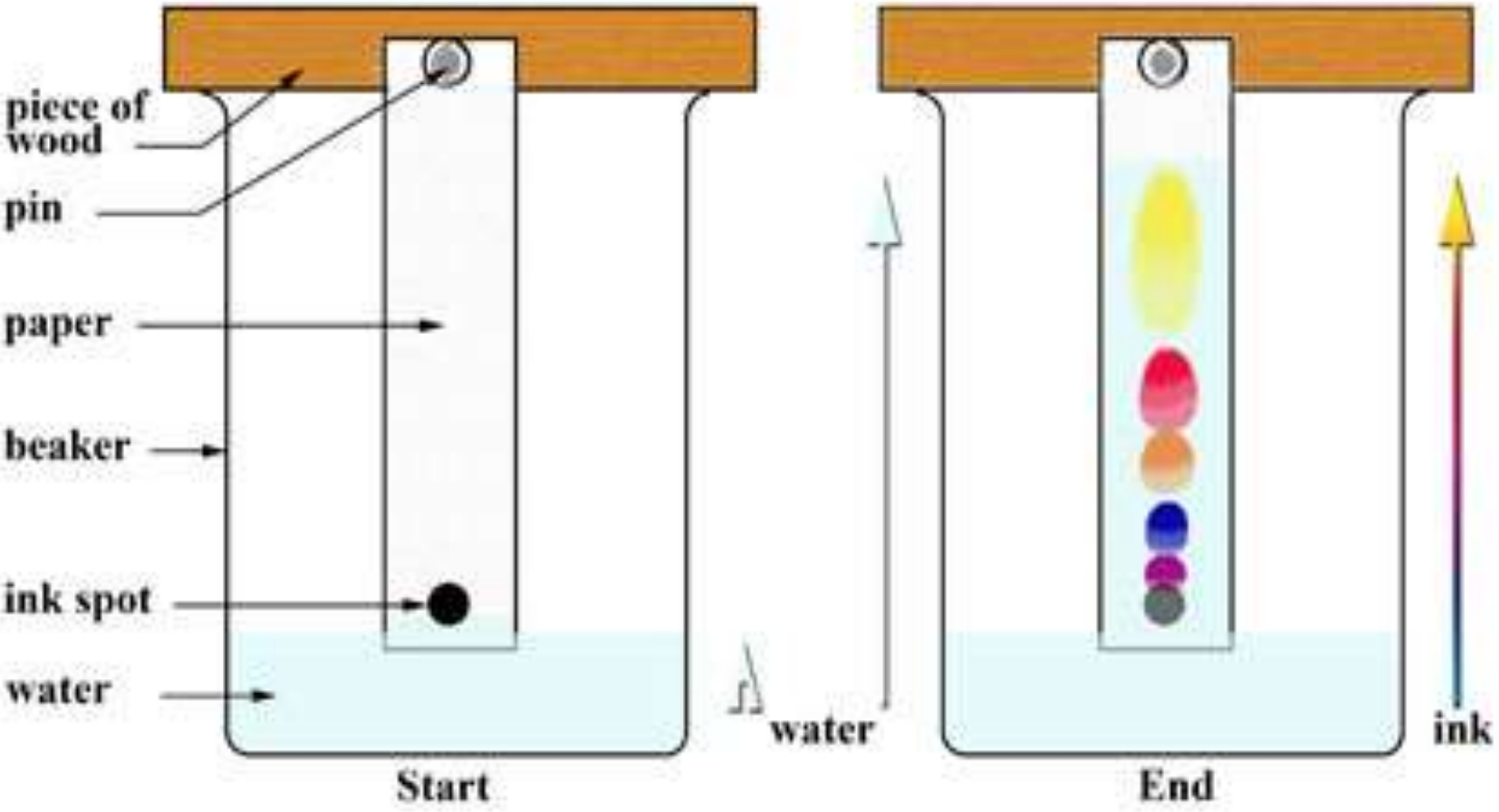
It may be a liquid or a gas.

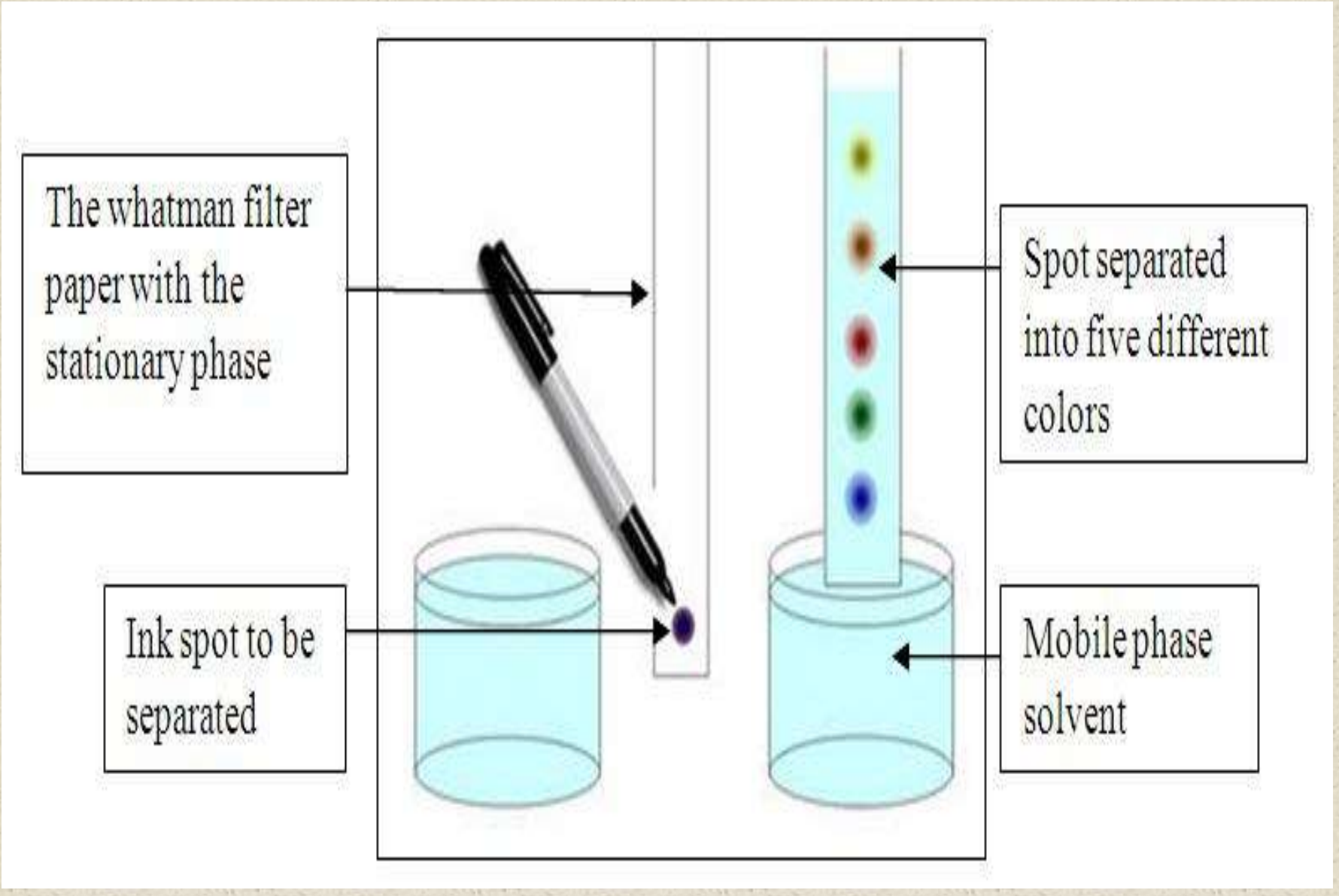
it consists of the sample being separated / analyzed and the solvent that moves the sample through the column.

<u>The stationary phase</u>

is the substance which is fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography.

Simple chromatography




The whatman filter paper with the stationary phase
Spot separated into five different colors
Ink spot to be separated
Mobile phase solvent

Function of Chromatography:

Chromatography may be preparative or analytical:

- The purpose of **preparative chromatography** is **to separate** the components of a mixture for further use (and is thus a form of **purification**).
- **Analytical chromatography** is done normally with smaller amounts of material **to measure** the relative proportions of analytes in a mixture.

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- **Separation of mixtures of:**
  - Mono- & polysaccharides.
  - Amino acids from peptides & proteins.
  - Proteins of different molecular weights.
  - Purification of enzymes.

Chromatography: classification

I. Classification of chromatography according to mobile phase:

1- Liquid chromatography (LC):

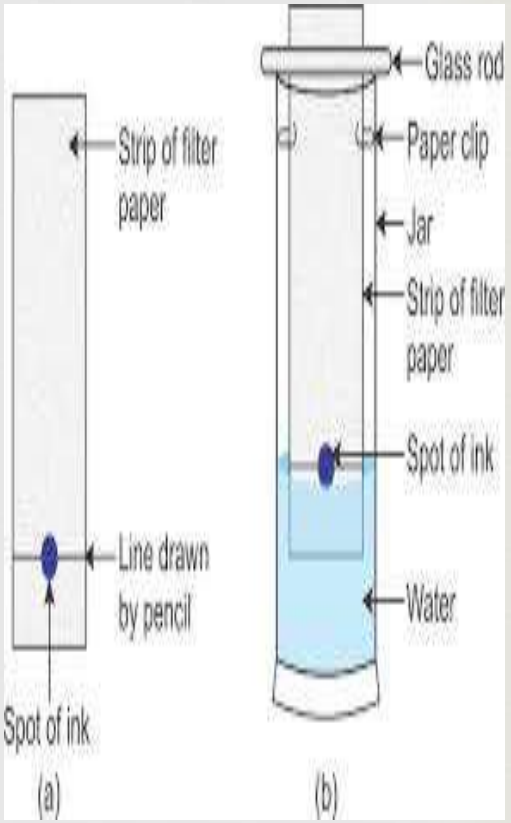
- **Mobile phase is a liquid**.
- It can be carried out either in a **column** or a **plane (paper & thin LC)**.
- LC that generally utilizes very ***small packing particles*** and a relatively ***high pressure*** is referred as high performance liquid chromatography **(HPLC).**

2- Gas chromatography (GC) :

- **Mobile phase is a gas.** It is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium).

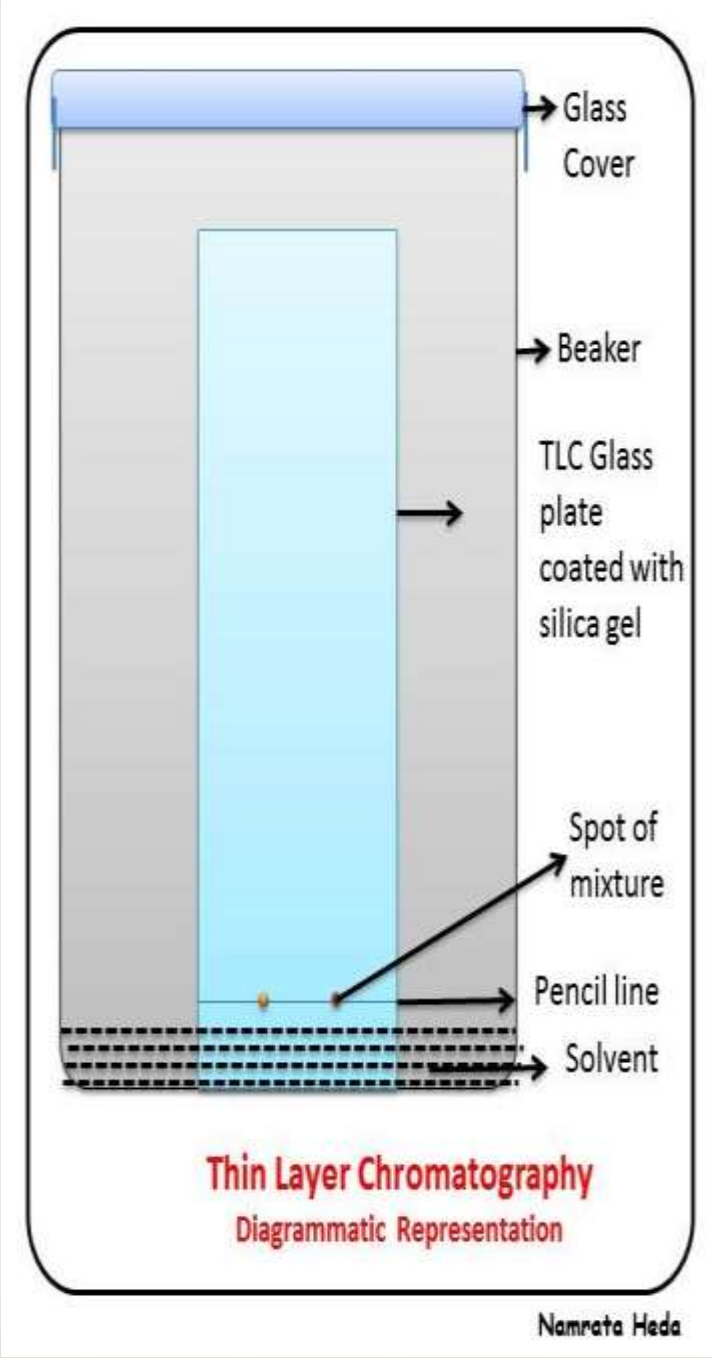
Paper Chromatography

- It is an analytical technique for separating and identifying mixtures that are or can be coloured, especially pigments.
- This is useful for separating complex mixtures of similar compounds, for example, amino acids.
- A small spot of the sample is applied to a strip of chromatography paper about 1 cm from the base, usually using a capillary tube for maximum precision.
- This sample is absorbed onto the paper and may form interactions with it.
- The paper is then dipped into a suitable solvent, such as ethanol or water, taking care that the spot is above the surface of the solvent, and placed in a sealed container.

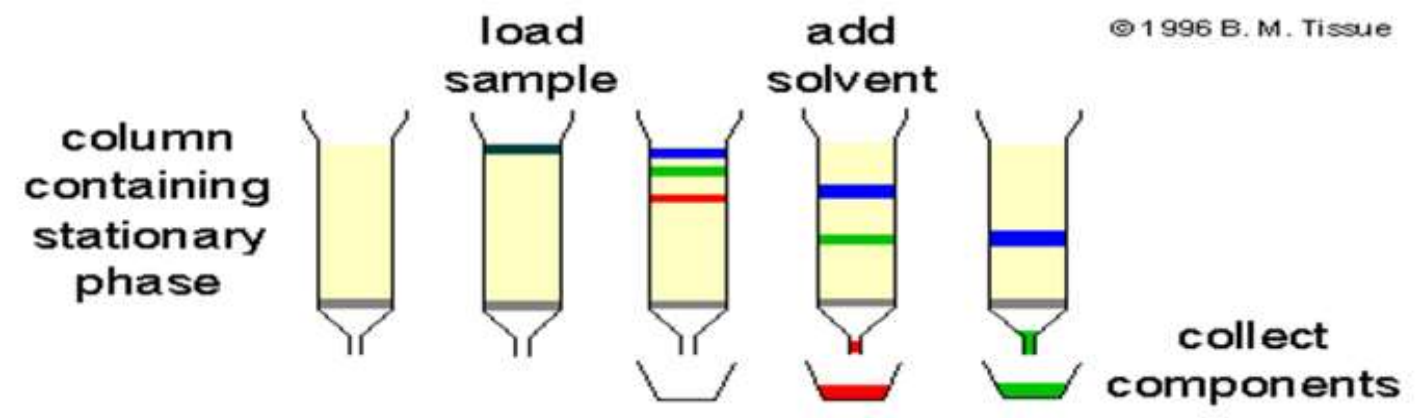


Thin Layer Chromatography (TLC)

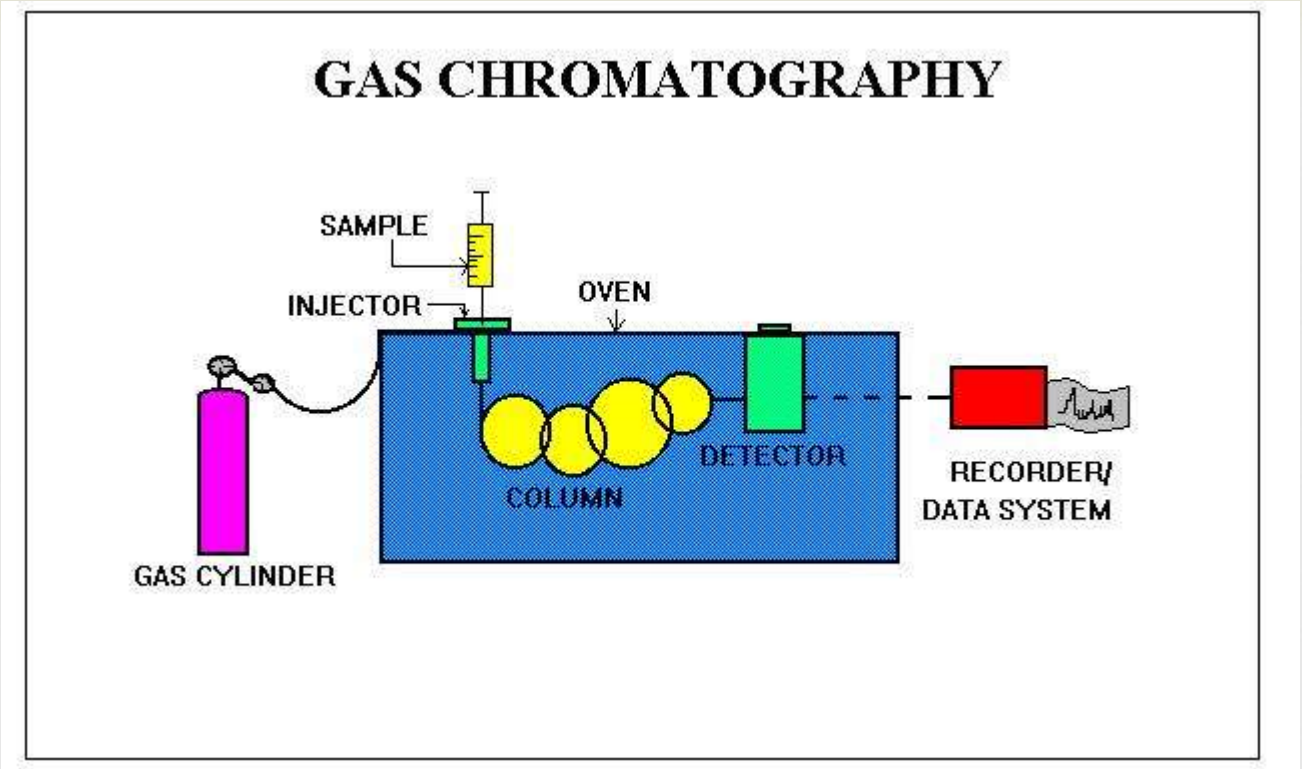
- TLC is a chromatographic technique that is useful for separating organic compounds. It involves a stationary phase consisting of a thin layer of silica gel.
- A liquid phase consisting of the solution to be separated dissolved in an appropriate solvent is drawn through the plate via capillary action, separating the experimental solution.
- Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.



liquid Chromatography



is a technique used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.



solution sample that contains organic compounds of interest is injected into the sample port where it will be vaporized. The vaporized samples that are injected are then carried by an inert gas (the mobile phase). The stationary phase is a microscopic layer of liquid inside a piece of glass or metal tubing called a column.

Application of Chromatography

- **Biochemical screening for genetic disorders**
- the levels of tyrosine in tyrosinemia type 1. (a specific marker).
- **Therapeutic drug monitoring and toxicology:**
- It can be used for the identification and/or quantification of :
  - drugs of abuse, therapeutic drugs, poisons, Organophosphorus pesticides.
- **test drinking water and to monitor air quality.**
- **prepare huge quantities of extremely pure materials, and also to analyze the purified compounds for trace contaminants**
- **Protein Separation like Insulin Purification, Plasma Fractionation and Enzyme Purification.**
- **separating, analyzing additives, vitamins, preservatives, proteins, and amino acids.**
- **Chromatography like HPLC is used in DNA fingerprinting and bioinformatics.**

Electrophoresis

Electrophoresis: definition

Definition:

- Electrophoresis refers to the migration of ***charged solutes*** or ***particles*** in a liquid medium under the influence of an **electrical field.**

Principle:

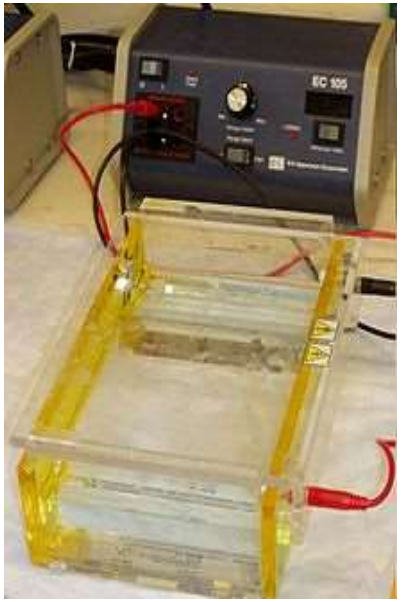
- Chemical species carrying an electrical charge move either to the **cathode** or to the **anode** in an electrophoresis system depending on the kind of charge on the molecule.

Importance of Electrophoresis:

  - Separation of plasma proteins, lipoproteins and hemoglobin.
  - Separation of iso-enzymes.
  - nucleic acids (DNA & RNA).

Electrophoresis

- The rate of migration is dependent on the following factors:
  - Net **electrical charge** of the molecule.
  - **Size and shape** of the molecule.
  - **Electrical field strength**.
  - Properties of the **supporting medium**.
  - **Temperature** of operation.
  - Composition, concentration and pH of the **buffer.**



Electrophoresis: Types

- **According to supporting media (matrix):**

➢ Celluloses acetate membrane.

➢ Gel (Agarose, polyacrylamide, …).

➢ Capillary.

Protein electrophoresis: Principle

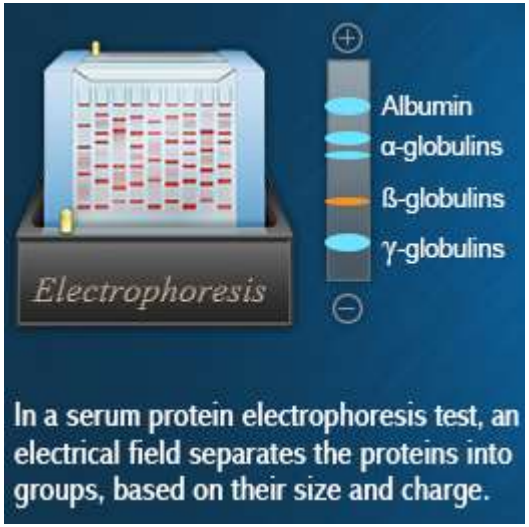
Protein is a molecule that can be either positively or negatively charged (Zwitterion).

In a solution more acidic than the iso-electric point of the solute, the ampholyte takes a positive charge and migrates toward the cathode (-Ve electrode).

In the reverse situation, the ampholyte is in the anionic form and migrates toward the anode (+ ve electrode).

- In a galva**n**ic (voltaic) cell, the a**n**ode is considered **n**egative and the **cathode** is considered **positive**. This seems reasonable as the anode is the source of electrons and **cathode** is where the electrons flow. However, in an electrolytic cell, the anode is taken to be **positive** while the **cathode** is now **negative**

- ***Proteins are separated by both electrical forces . The net charge on a protein is based on the sum charge of its amino acids, and the pH of the buffer. Proteins are applied to a solid matrix such as an agarose gel, or a cellulose acetate membrane in a liquid buffer, and electric current is applied. Proteins with a <u>negative</u> charge will migrate towards the positively charged anode. Albumin has the most negative charge, and will migrate furthest towards the anode***

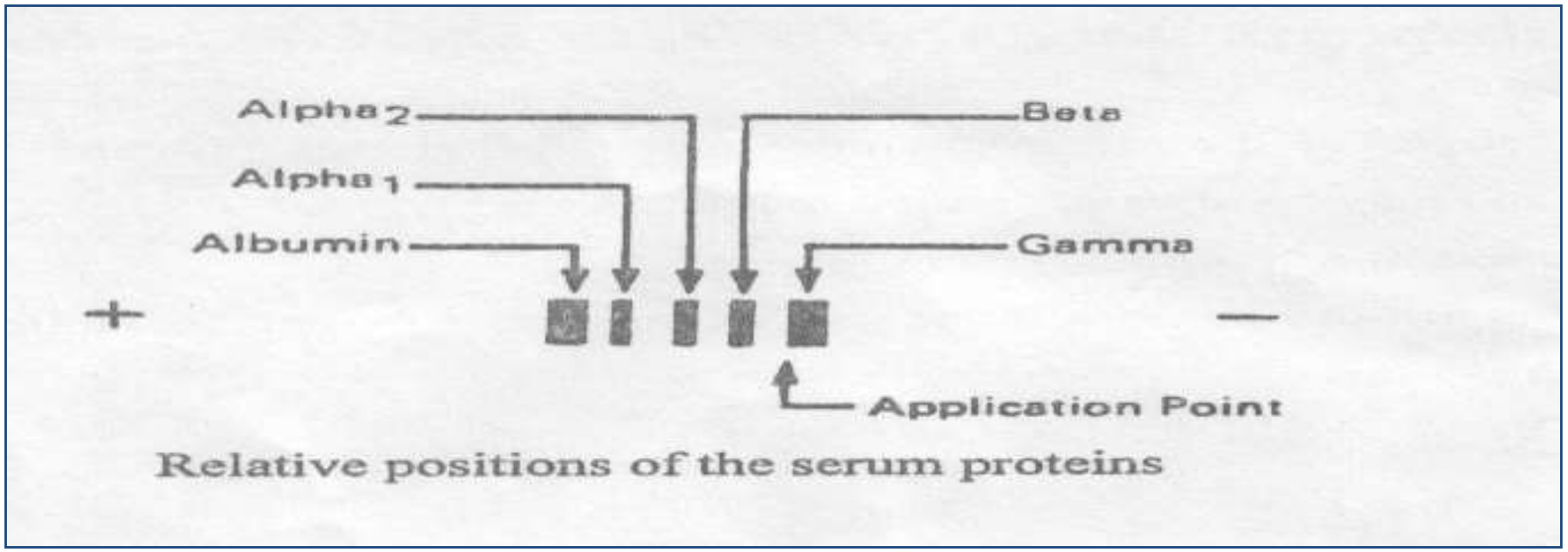


In a serum protein electrophoresis test, an electrical field separates the proteins into groups, based on their size and charge.

Electrophoresis: plasma proteins

By electrophoresis, plasma proteins will be fractionated into:

- Albumin 54-60%.
- α_1-globulins 3-4%.
- α_2-globulins 6-8%.
- β-globulins 6-9%.
- γ-globulins 16-20%
- Each of the albumin and globulin fractions consists of mixture of several proteins.



Relative positions of the serum proteins

Plasma protein electrophoresis: clinical application

- Changes in the electrophoretic pattern of plasma proteins occur in some diseases and may be value in their diagnosis e.g.:

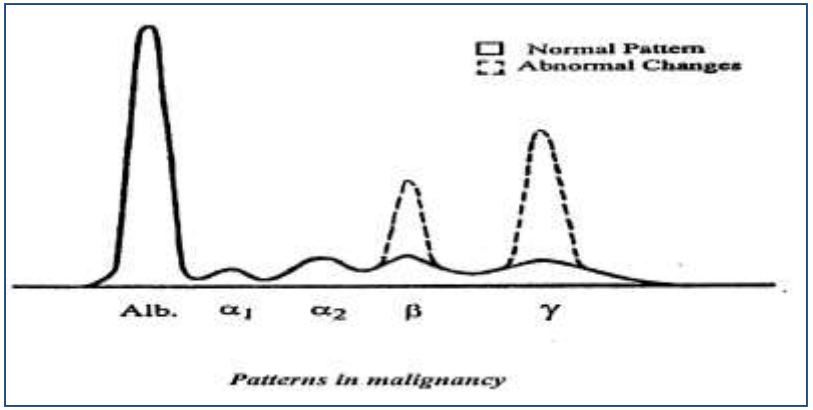
➢ **Myelomas and lymphoma (malignancy):**

  – The pattern shows diffuse increase in immunoglobulins, including the beta and gamma regions.

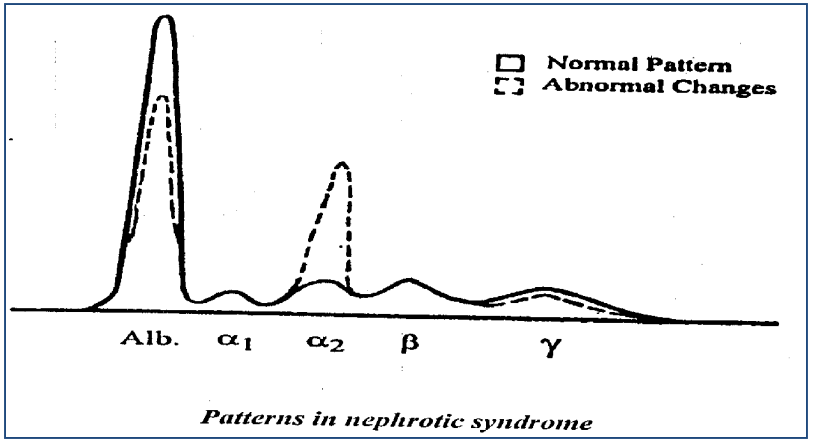
➢ **Nephrotic syndrome:**

  – There is decrease in albumin.

  – Increase in α2-globulins.

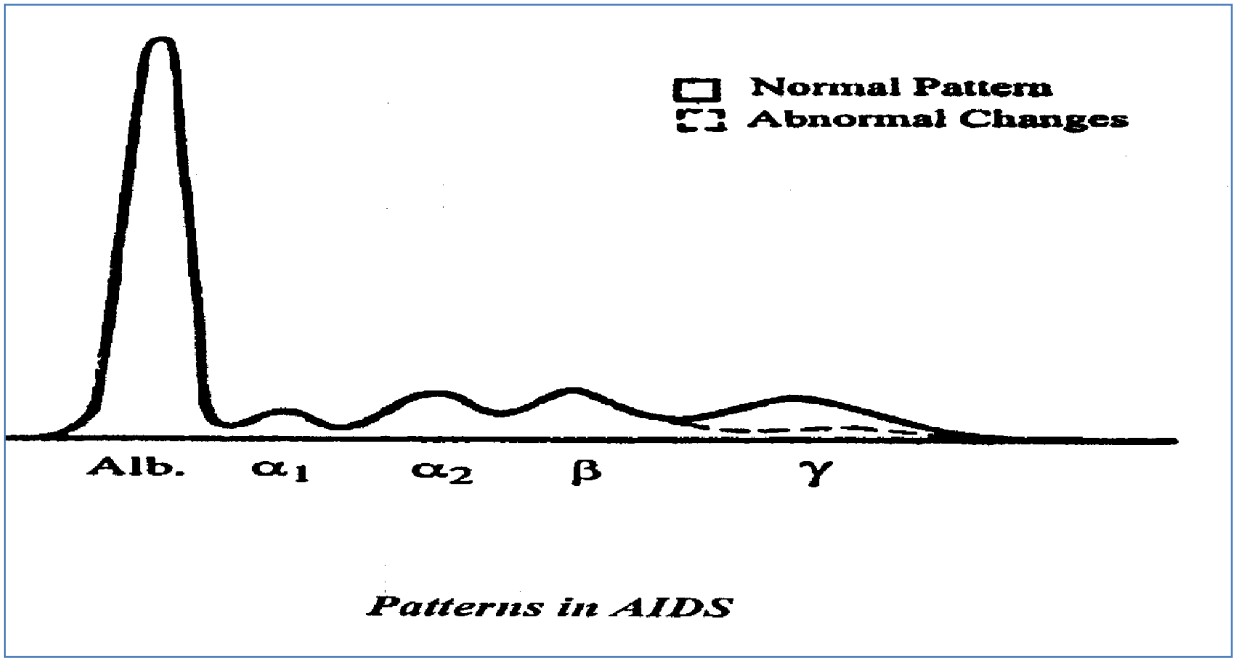


Patterns in malignancy



Patterns in nephrotic syndrome

- **AIDS:**
  - The pattern shows decrease or loss of γ-globulins.



Patterns in AIDS