ANALYTICAL CHEMISTRY CODE : 18K5CHELCH1

UNIT –II

2.1 Separation and Purification techniques:

❖Purification of organic compung is important because they made in the laboratory contains impurities.

❖The common method for purification are crystallisation, sublimation, distillation, chromatography etc.,

Principle of separation by precipitation

❖**Precipitation** is a technique used to separate a mixture based on the solubility of its components. The solubility of a compound depends on the ionic strength of the solution, its pH, and temperature. Manipulation of these factors can cause a compound to become an insoluble solid, and fall out of solution.

❖**Solubility product principle:**

Solubility product of a sparingly soluble electrolyte is defined as the product of its ions in its saturated condition at 25 ºC. The value of solubility product of a sparingly soluble salt is always constant at given temperature. For example the solubility product of AgCl at 25ºC is 1.6 × 10-10ºC. It represents the level at which a solute dissolves in solution.

Let us consider a sparingly soluble electrolyte AB that dissolves very slightly in water. It dissociates into cations and anions.

$AB \rightleftharpoons A^{+} + B^{-}$ $AB \rightleftharpoons A^{+} + B^{-}$

At first, AB dissociates to give A+ and B–. As the concentration of A+ and B– is increased, the backward reaction initiates and the reaction comes under equilibrium. When the solution is saturated at 25ºC, the concentration of AB, A+ and B– remains constant. Now,we can apply the law of mass action to this equilibrium system.

$K = [A^{+}][B^{-}][AB]$ $K = [A^{+}][B^{-}][AB]$

or, $K \times [AB] = [A^{+}][B^{-}]$ or, $K \times [AB] = [A^{+}][B^{-}]$

At saturated condition in 25ºC,

$K \times [AB] = Ksp$ $K \times [AB] = Ksp$

$\therefore Ksp = [A^{+}][B^{-}]$ $\therefore Ksp = [A^{+}][B^{-}]$

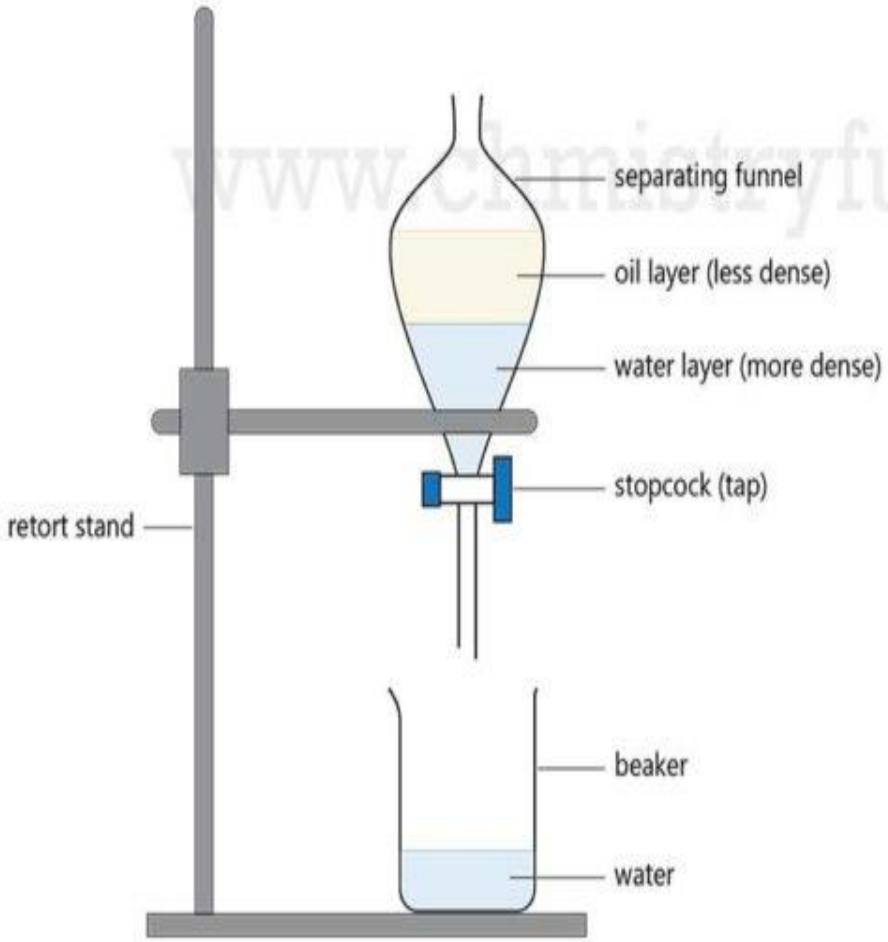
The above expression defines the solubility product principle.

Principle of solvent extraction:

When the solute (liquid or solid) is add to a heterogeneous system of two immiscible liquids (in both of which the solute is soluble), the solute distributes between the two liquids. This distribution governed by Nernst distribution law.

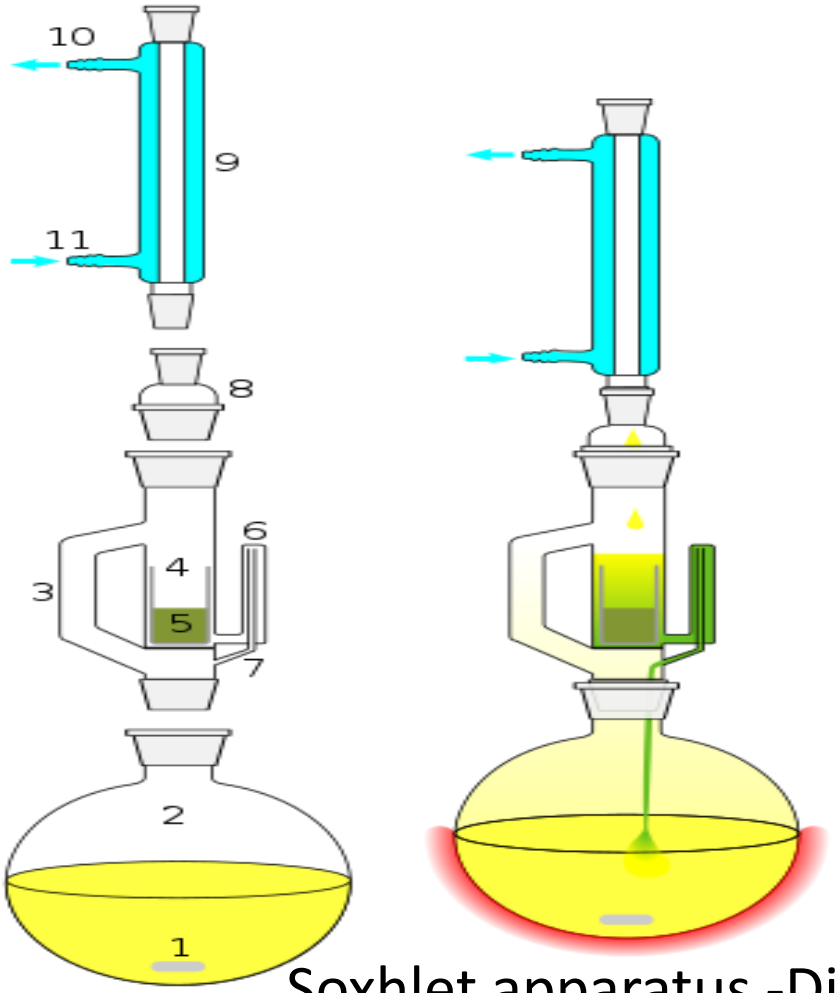
Importance of the process:

- The Solvent Extraction(**S.E.**) process was first developed as a tool of analytical chemistry. Every metallic element of the periodic table could be virtually separated by this process.
- Back in 1940's , **S.E.** was primarily used to separate nuclear and rare earth elements.
- However, availability of inexpensive and effective reagents led to the establishment of large scale **S.E.** processes for extraction of non-ferrous metals from hydrometallurgical leach liquors.



Seperatory funnels

A **Soxhlet extractor** is a piece of laboratory apparatus invented in 1879 by Franz von soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, Soxhlet extraction is used when the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.



1: Stirrer bar/anti-bumping granules
2: Still pot (extraction pot) - still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber.
3: Distillation path
4: Soxhlet Thimble
5: Extraction solid (residue solid)
6: Syphon arm inlet
7: Syphon arm outlet
8: Reduction adapter
9: Condenser
10: Cooling water out
11: Cooling water in

Soxhlet apparatus -Diagram

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 system on which is fixed a material called the stationary phase.

❖The Russian botanist Mikhail Tswett coined the term chromatography in 1906.

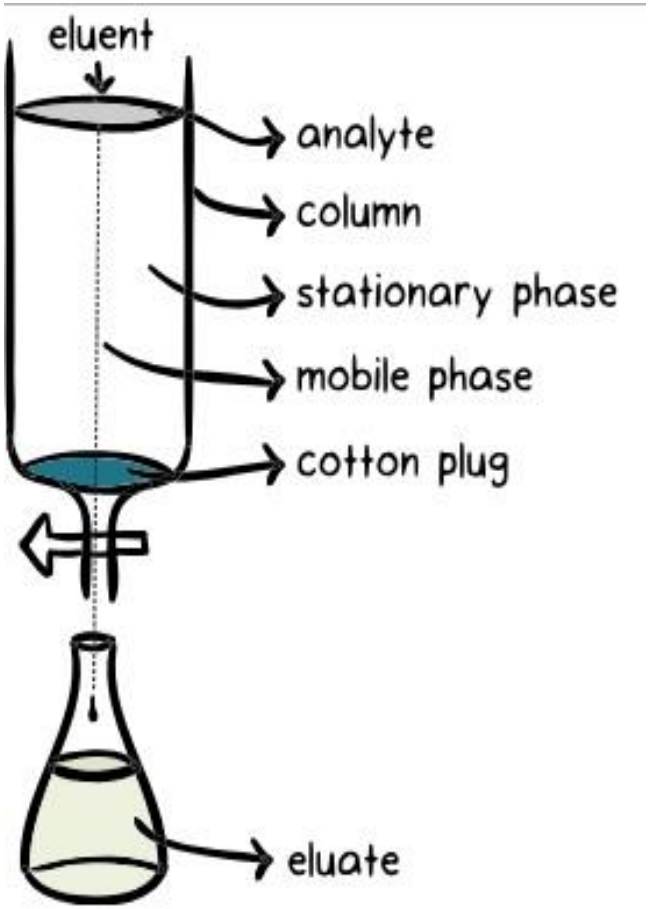
❖The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of **fatty acid** mixtures.

❖A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials.

❖It is a powerful separation tool that is used in all branches of science and is often the only means of separating components from complex mixtures.

Principle of Chromatography

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.



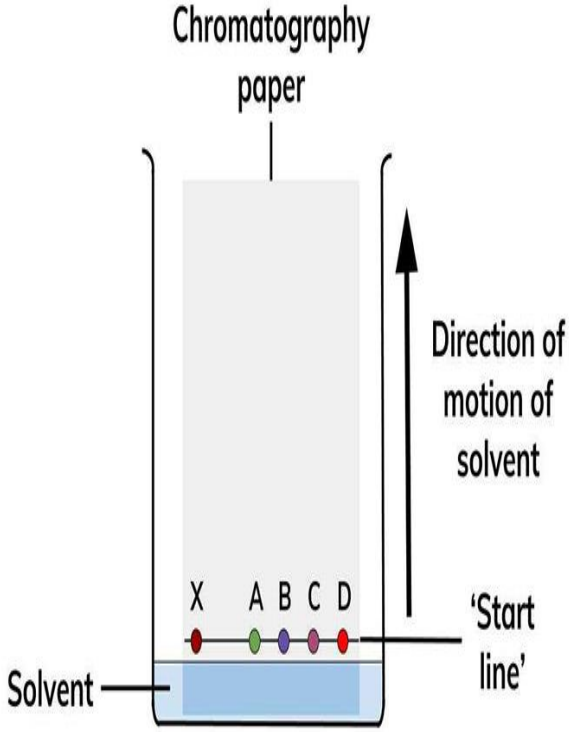
Term	Definition
Mobile phase or carrier	solvent moving through the column
Stationary phase or adsorbent	substance that stays fixed inside the column
Eluent	fluid entering the column
Eluate	fluid exiting the column (that is collected in flasks)
Elution	the process of washing out a compound through a column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

Paper Chromatography

❖Paper chromatography (PC) is a type of a planar **chromatography** whereby chromatography procedures are run on a specialized paper.

❖PC is considered to be the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification and quantitative determination of organic and inorganic compounds.

❖It was first introduced by German scientist Christian Friedrich Schonbein (1865).



Types of Paper chromatography

Paper Adsorption Chromatography

Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase.

Paper Partition Chromatography

Moisture / Water present in the pores of cellulose fibers present in filter paper acts as stationary phase & another mobile phase is used as solvent In general paper chromatography mostly refers to paper partition chromatography.

Instrumentation of Paper chromatography

- Stationary phase & papers used
- Mobile phase
- Developing Chamber
- Detecting or Visualizing agents

Steps in Paper Chromatography

In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. The basic steps include:

- **Selection of Solid Support**
- **Selection of Mobile Phase**
- **Saturation of Tank**
- **Sample Preparation and Loading**
- **Development of the Chromatogram**

Different types of development techniques can be used:

- **ASCENDING DEVELOPMENT**
- **DESCENDING TYPE**
- **ASCENDING – DESCENDING DEVELOPMENT**
- **CIRCULAR / RADIAL DEVELOPMENT**

Drying of Chromatogram

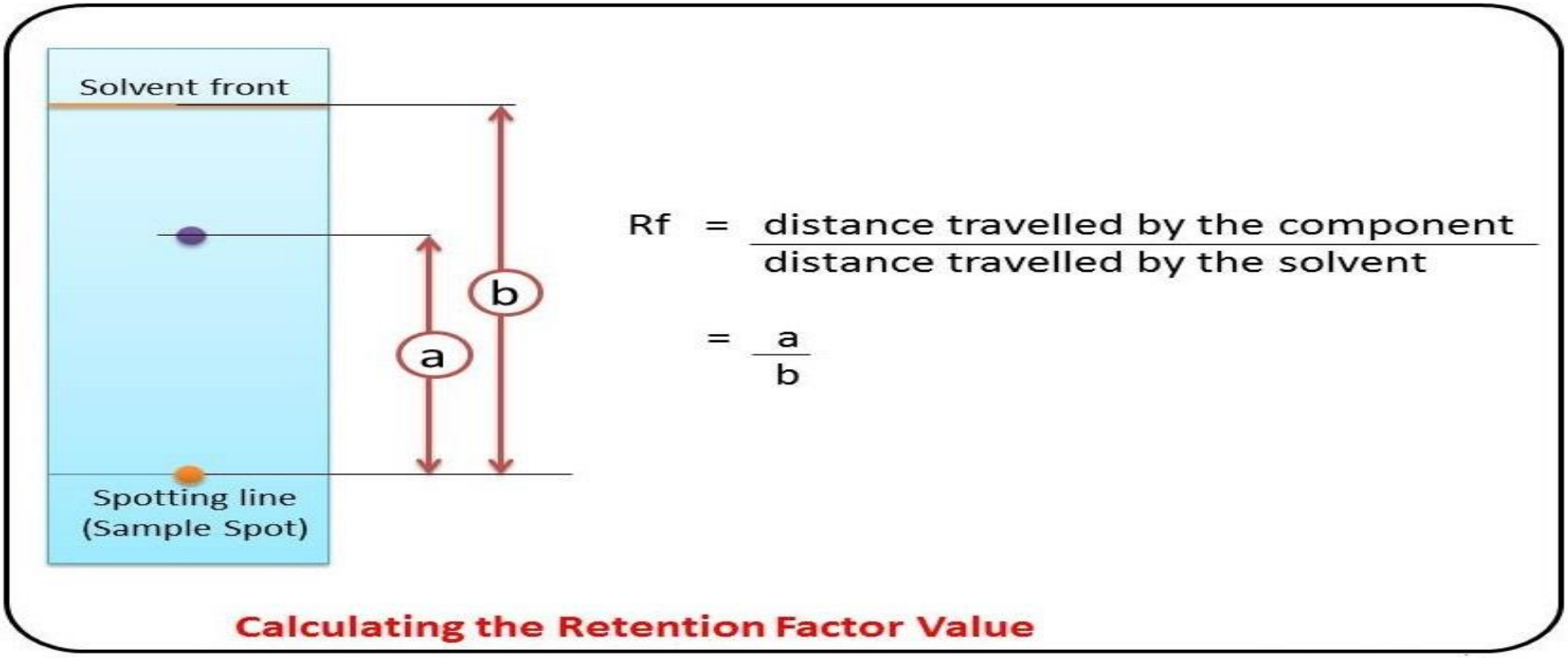
After the development, the solvent front is marked and the left to dry in a dry cabinet or oven.

Detection

- Colourless analytes detected by staining with reagents such as iodine vapour, ninhydrin etc.
- Radiolabeled and fluorescently labeled analytes detected by measuring radioactivity and florescence respectively.

R_f values

Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the base line. The distance travelled relative to the solvent is a constant for a particular compound as long as other parameters such as the type of paper and the exact composition of the solvent are constant. The distance travelled relative to the solvent is called the Rf value.



Applications of Paper Chromatography

- To check the control of purity of pharmaceuticals,
- For detection of adulterants,
- Detect the contaminants in foods and drinks,
- In the study of ripening and fermentation,
- For the detection of drugs and dopes in animals & humans
- In analysis of cosmetics
- Analysis of the reaction mixtures in biochemical labs.

Advantages of Paper Chromatography

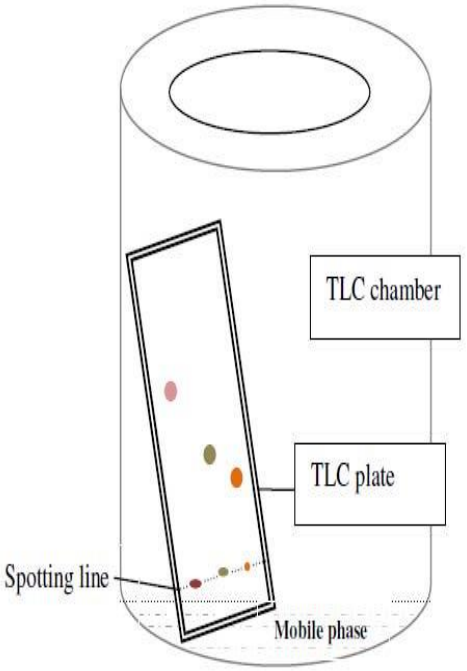
- Simple
- Rapid
- Paper Chromatography requires very less quantitative material.
- Paper Chromatography is cheaper compared to other chromatography methods.
- Both unknown inorganic as well as organic compounds can be identified by paper chromatography method.
- Paper chromatography does not occupy much space compared to other analytical methods or equipments.
- Excellent resolving power

Limitations of Paper Chromatography

- Large quantity of sample cannot be applied on paper chromatography.
- In quantitative analysis paper chromatography is not effective.
- Complex mixture cannot be separated by paper chromatography.
- Less Accurate compared to HPLC or HPTLC

Thin Layer Chromatography

Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a plate and liquid as a mobile phase.

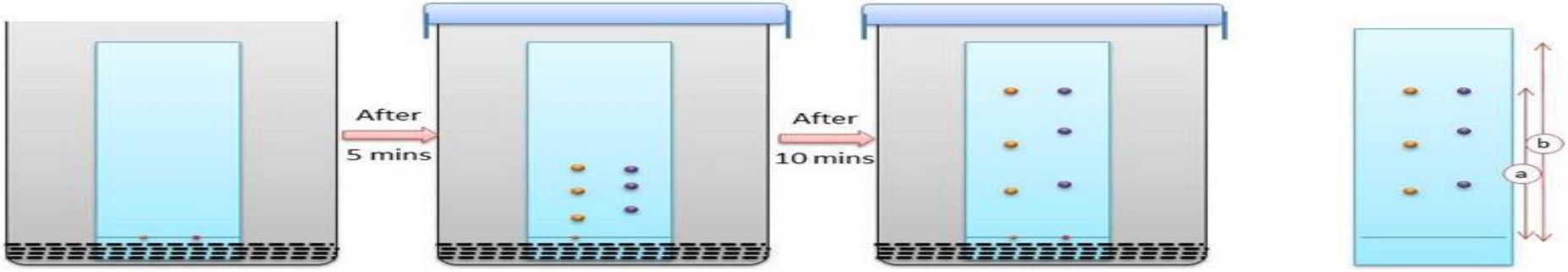


Principle of Thin Layer Chromatography (TLC)

Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase.

Components of Thin Layer Chromatography (TLC)

- ❑**TLC plates**
- ❑**TLC chamber**
- ❑**Mobile phase**
- ❑**A filter paper**



Steps of Thin Layer Chromatography

1.With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots.
2Then, samples solutions are applied on the spots marked on the line in equal distances.
3The mobile phase is poured into the TLC chamber to a leveled few centimeters above the chamber bottom.
4A moistened filter paper in mobile phase is placed on the inner wall of the chamber to maintain equal humidity.
5Now, the plate prepared with sample spotting is placed in TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid.
6The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent) for development.
7.Sufficient time is given for the development of spots.
8.The plates are then removed and allowed to dry.
9.The sample spots are then seen in a suitable UV light chamber, or any other methods as recommended for the given sample.

Applications of Thin Layer Chromatography (TLC)

- In monitoring the progress of reactions
- Identify compounds present in a given mixture
- Determine the purity of a substance.
- Analyzing ceramides and fatty acids
- Detection of pesticides or insecticides in food and water
- Analyzing the dye composition of fibers in forensics
- Assaying the radiochemical purity of radiopharmaceuticals
- Identification of medicinal plants and their constituents

Advantages of Thin Layer Chromatography (TLC)

- It is a simple process with a short development time.
- It helps with the visualization of separated compound spots easily.
- It helps in isolating of most of the compounds.
- The separation process is faster and the selectivity for compounds is higher (even small differences in chemistry is enough for clear separation).
- The purity standards of the given sample can be assessed easily.
- It is a cheaper chromatographic technique.

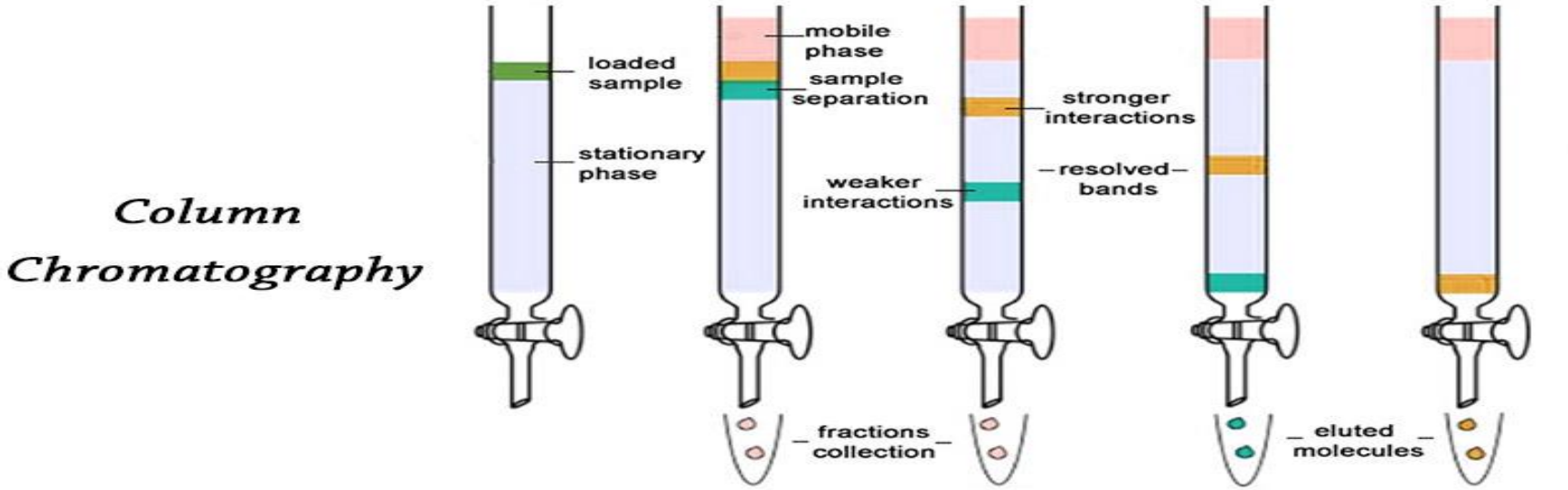
Limitations of Thin Layer Chromatography (TLC)

- It cannot tell the difference between enantiomers and some isomers.
- In order to identify specific compounds, the Rf values for the compounds of interest must be known beforehand.
- TLC plates do not have long stationary phases. Therefore, the length of separation is limited compared to other chromatographic techniques.

Column Chromatography

❖ Column chromatography is a technique in which the substances to be separated are introduced onto the top of a column packed with an adsorbent, passed through the column at different rates that depend on the affinity of each substance for the adsorbent and for the solvent or solvent mixture, and are usually collected in solution as they pass from the column at different times.

❖ It was developed by the American chemist D.T Day in 1900 while M.S. Tswett, the Polish botanist, in 1906 used adsorption columns in his investigations of plant pigments.



Forms of Column Chromatography

- There are two forms of column chromatography.
- Liquid chromatography (LC)
- Gas chromatography (GC)

The most widely used forms of column chromatography are:

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Gel chromatography

Principle of Column Chromatography

- In column chromatography the stationary phase is packed into a glass or metal column.
- The mixture of analytes is then applied and the mobile phase, commonly referred to as the eluent, is passed through the column either by use of a pumping system or applied gas pressure.

Instrumentation of Column Chromatography

A typical column chromatographic system using a gas or liquid mobile phase consists of the following components:

- A stationary phase
- A column
- A mobile phase and delivery system
- An injector system
- A detector and chart recorder
- A fraction collector

Factors Affecting Column Efficiency

- Dimensions of the column
- Particle size of the adsorbent
- Nature of the solvent
- Temperature of the column
- Pressure

Steps in Column Chromatography

A. Preparation of the Column

1. Dry packing / dry filling

2. Wet packing / wet filling

B. Introduction of the Sample

C.Elution

1.Isocratic elution technique

2.Gradient elution technique:

D. Detection of Components

Applications

- Separation of mixture of compounds.
- Removal of impurities or purification process.
- Isolation of active constituents.
- Isolation of metabolites from biological fluids.
- Estimation of drugs in formulation or crude extracts.

Advantages

- Any type of mixture can be separated by column chromatography.
- Any quantity of the mixture can also be separated.
- Wider choice of mobile phase.
- In preparative type, the sample can be separated and reused.
- Automation is possible.

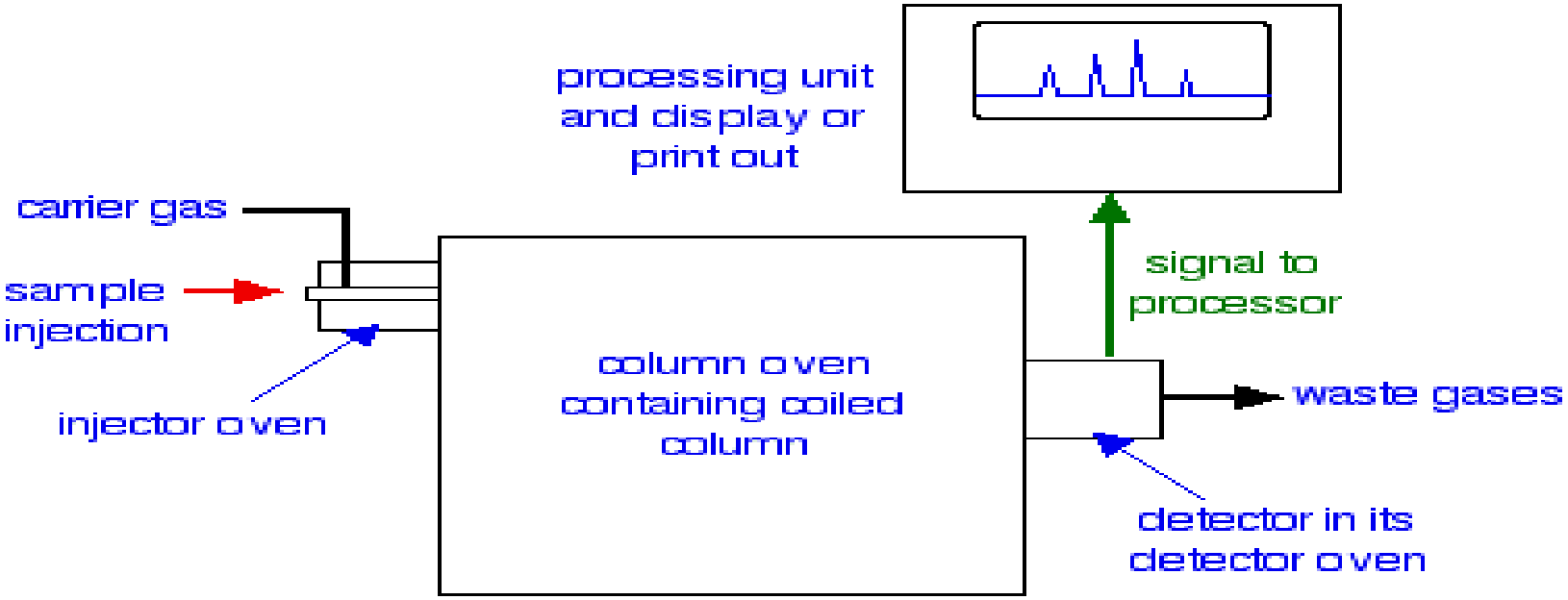
Limitations

- Time consuming method.
- More amounts of solvents are required which may be expensive.
- Automation makes the technique more complicated and costly.

GAS-LIQUID CHROMATOGRAPHY

Gas-liquid chromatography (often just called gas chromatography) is a powerful tool in analysis. It has all sorts of variations in the way it is done - if you want full details, a Google search on gas chromatography will give you scary amounts of information if you need it! This page just looks in a simple introductory way at how it can be carried out.

A flow scheme for gas-liquid chromatography



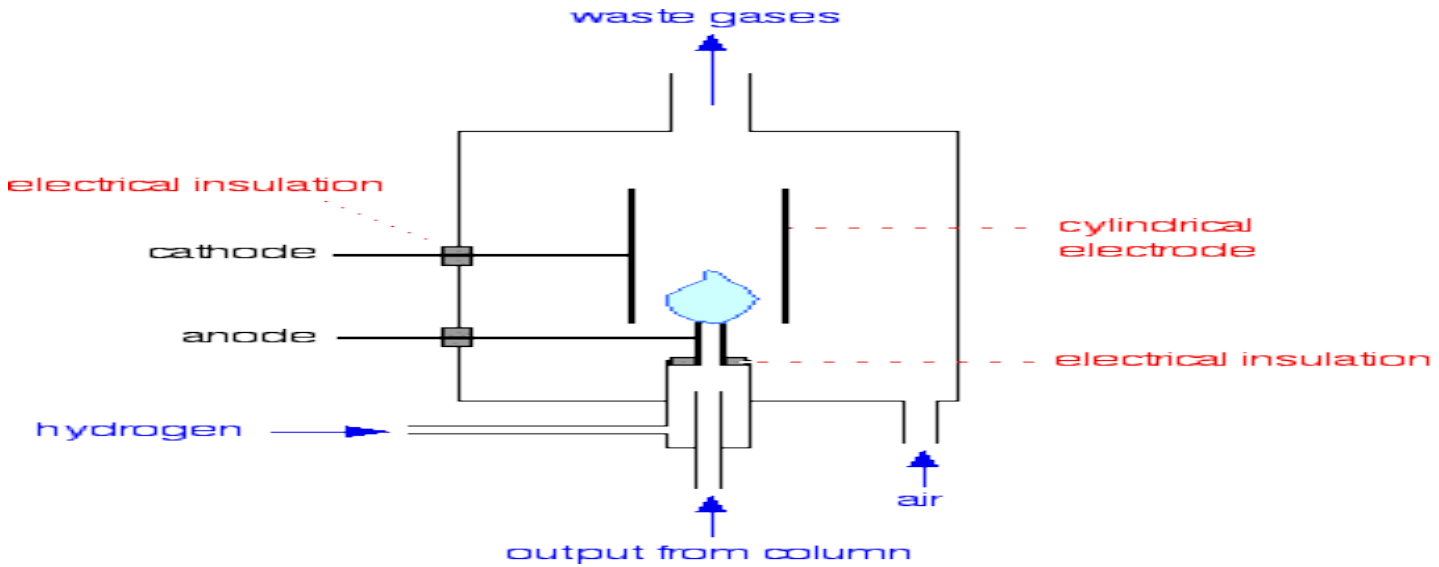
Works on the column

One of three things might happen to a particular molecule in the mixture injected into the column:

- It may condense on the stationary phase.
- It may dissolve in the liquid on the surface of the stationary phase.
- It may remain in the gas phase.

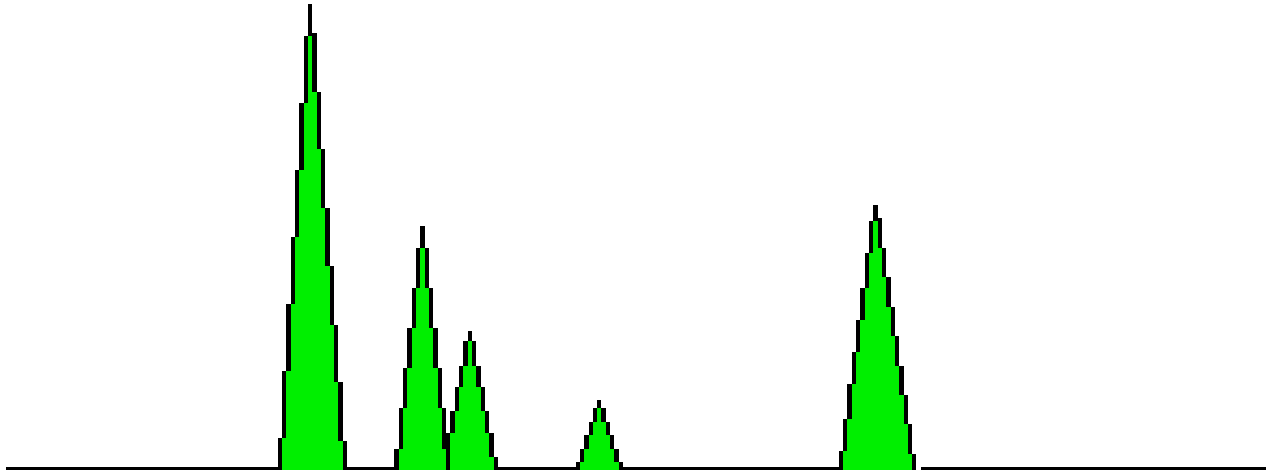
A flame ionisation detector

The whole detector is enclosed in its own oven which is hotter than the column temperature. That stops anything condensing in the detector.



Interpreting the output from the detector

The areas under the peaks are proportional to the amount of each compound which has passed the detector, and these areas can be calculated automatically by the computer linked to the display. The areas it would measure are shown in green in the (very simplified) diagram.

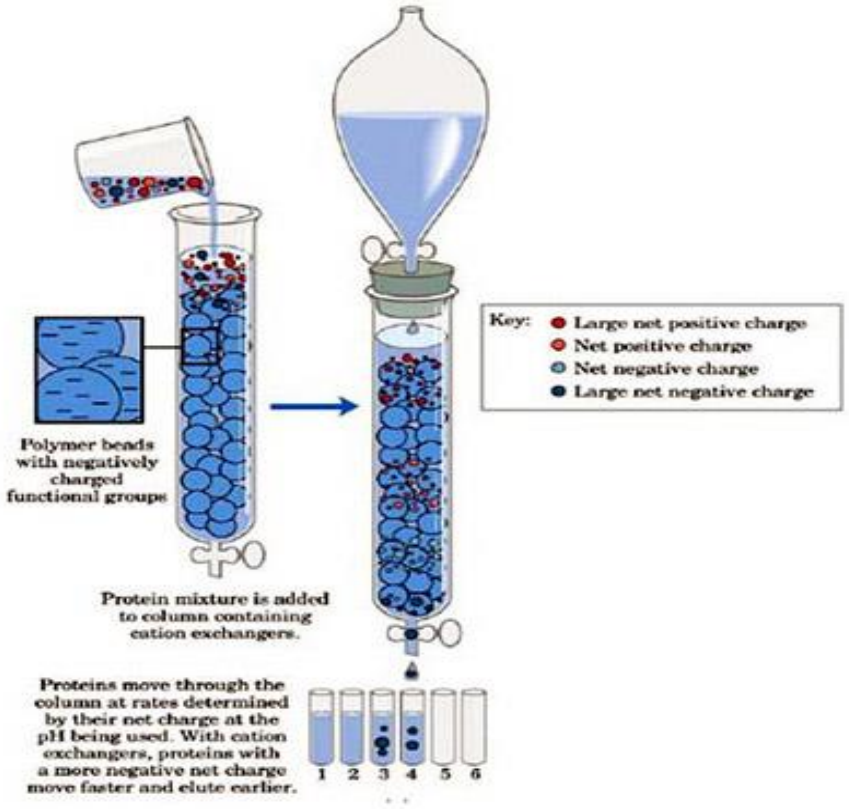


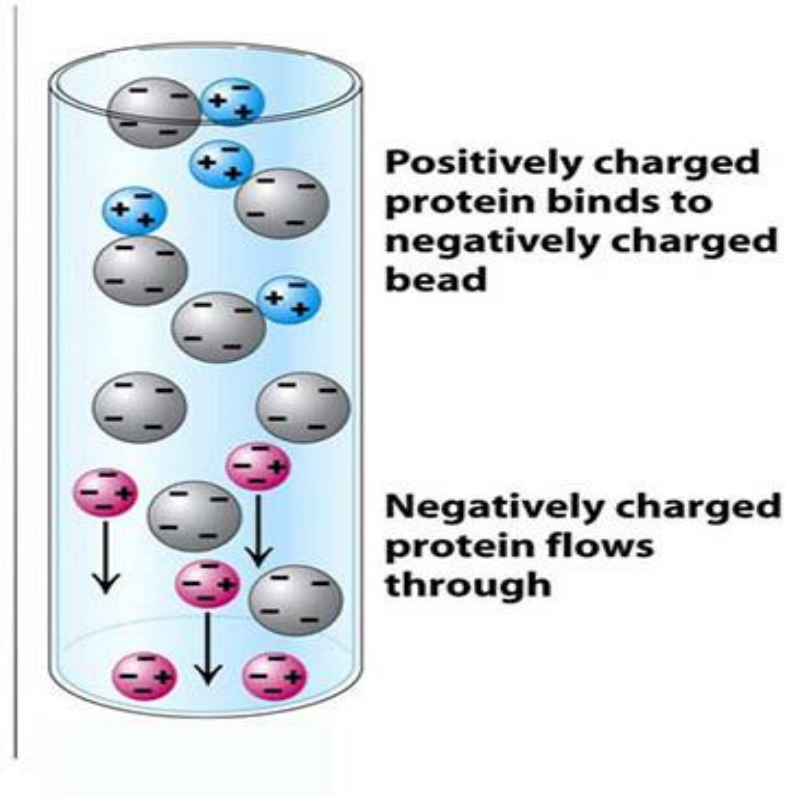
Ion Exchange Chromatography

Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers.

Cationic exchangers "Acidic ion exchange"

Anionic exchangers **"**Basic ion exchange"





Working Principle of ion exchange chromatography

- The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.
- The charged groups of the matrix can be positively or negatively charged.
- When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.
- In this “ion cloud”, ions can be reversibly exchanged without changing the nature and the properties of the matrix.

Instrumentation of ion exchange chromatography

- Pump
- Injector
- Column
- Suppressor
- Detector
- Recorder or data system

Applications of ion exchange chromatography

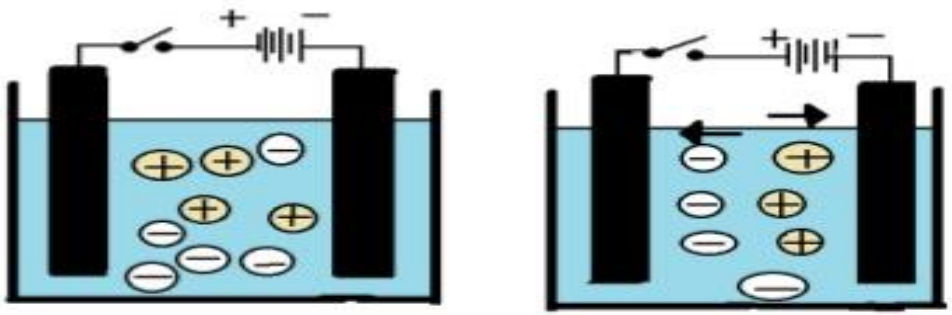
- An important use of ion-exchange chromatography is in the routine analysis of **amino acid** mixtures.
- The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated and used in clinical diagnosis.
- This is most effective method for water purification. Complete deionization of water (or) a non-electrolyte solution is performed by exchanging solute cations for hydrogen ions and solute anions for hydroxyl ions. This is usually achieved by method is used for softening of drinking water.
- In the analysis of products of hydrolysis of nucleic acids. In this way, information is gained about the structure of these molecules and how it relates to their biological function as carriers of hereditary information.
- Chelating resins are used to collect trace metals from seawater.
- To analyze lunar rocks and rare trace elements on Earth.

Electrophoresis

- **Definition**="migration of charged particles or molecules under the influnce of electric current."
- **Literally**= greek word means transport by electricity.

▪Principle:

Any charged ion or molecule migrates when placed in an electric field the rate of migration depend upon its net charge size,shape and the applied electric current.



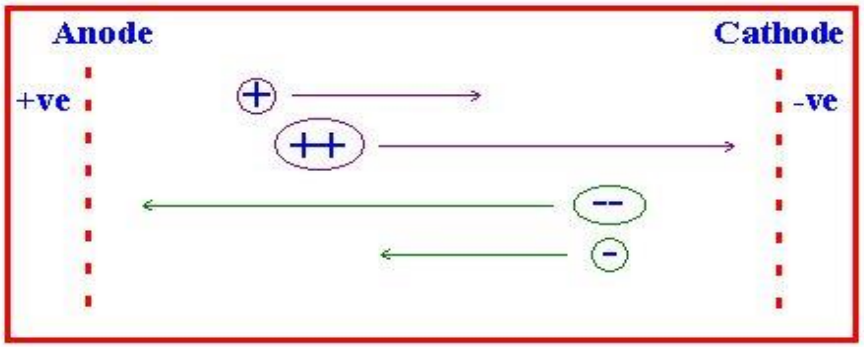
(a) no electric field

(b) with electric field

Electrophoresis

Separation, analytical or preparative, of charged molecules by migration through a matrix due to application of an electric field, with net movement towards electrode of opposite charge

General Principle



The rate of movement depends on the field strength and the number of charges. Biomolecules such as proteins possess surface charge due to the presence of acidic and basic amino acids

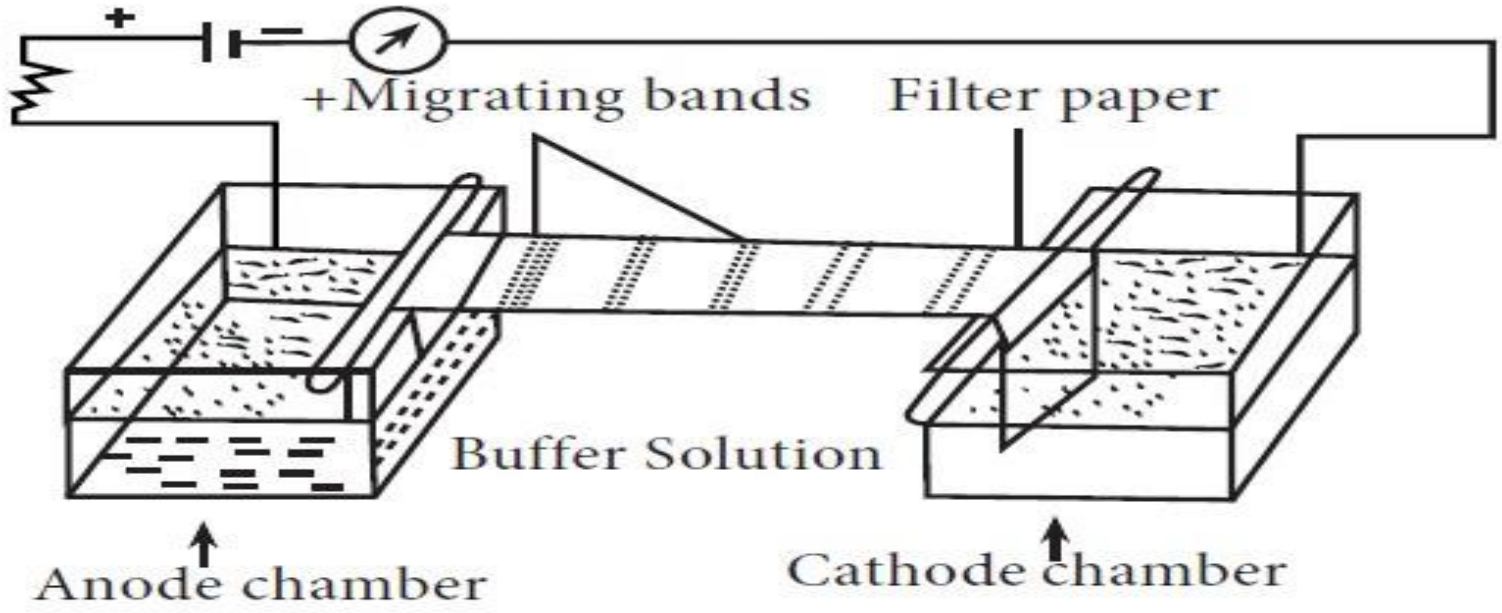


Figure 10.9 Paper electrophoresis

Applications of Electrophoresis

How the Process Works. **Organic molecules** often have a positive or a negative charge, which causes them to respond to an electric current. ...

DNA Analysis. One leading use of electrophoresis is in the identification and study of DNA and DNA fragments. ...

Protein and Antibody Interactions. ...

Testing Antibiotics. ...

Testing Vaccines.

UNIT –V
5.1 Colorimetric Analysis

A **colorimeter** is a device that is used in Colorimetry. It refers to a device which helps specific solutions to absorb a particular wavelength of light. The colorimeter is usually used to measure the concentration of a known solute in a given solution with the help of the Beer-Lambert law. The colorimeter was invented in the year 1870 by Louis J Duboscq.

Principle of Colorimeter

It is a photometric technique which states that when a beam of incident light of intensity I_o passes through a solution, the following occur: A part of it is reflected which is denoted as I. A part of it is absorbed which is denoted as I. Rest of the light is transmitted and is denoted as I.

Beer's law:

According to this law the amount of light absorbed is proportional to the solute concentration present in solution.

$\text{Log10 } I_o/I_t = a_s c$

where,

a_s is absorbency index

c is the concentration of solution

Lambert's law:

According to this law the amount of light absorbed is proportional to the length as well as thickness of the solution taken for analysis.

$A = \text{log10 } I_o/I_t = a_s b$

Where,

A is the test absorbance of test

a_s is the standard absorbance

b is the length / thickness of the solution

The Beer-Lambert law states that:

for a given material sample path length and concentration of the sample are directly proportional to the absorbance of the light.

he Beer-Lambert law is expressed as:

$A = \varepsilon Lc$

where,

A is the amount of light absorbed for a particular wavelength by the sample

ε is the molar extinction coefficient

L is the distance covered by the light through the solution

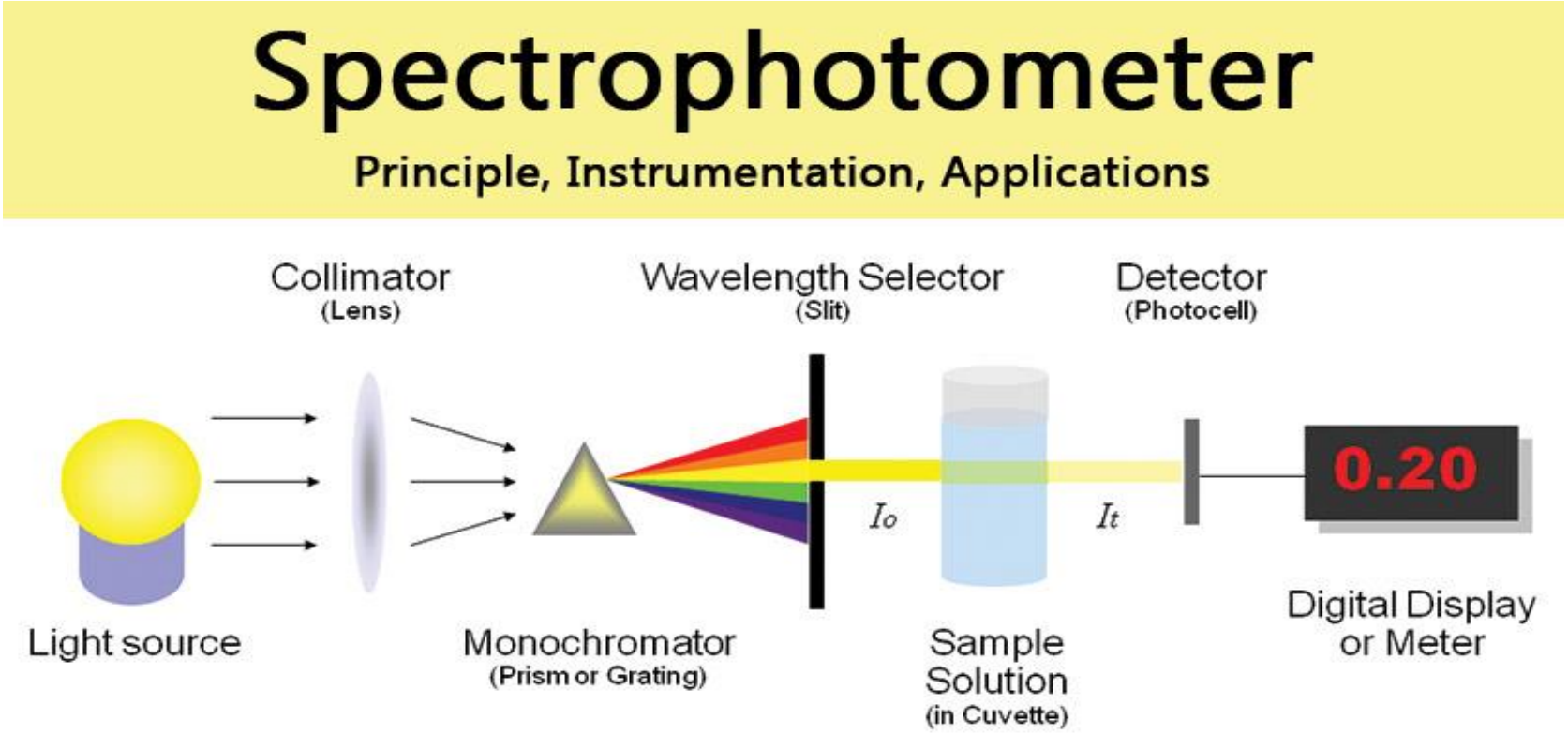
c is the concentration of the absorbing species

Following is an equation to solve for molar extinction coefficient:

$\epsilon = ALc$

But Beer-Lambert law is a combination of two different laws: Beer's law and Lambert law.

Nessler cylinders are used for **colorimetric** analysis, such as APHA color. The color of the substance contained in a **Nessler** cylinder is visually compared with the model. The **tubes** are often used to carry out a series of calibration solutions of increasing concentrations, which functions as a comparative scale.



Stark –Einstein Law of photochemical equivalence law, fundamental principle relating to chemical reactions induced by light which states that for every quantum of radiation that is absorbed, one molecule of the substance reacts. A quantum is a unit of electromagnetic radiation with energy equal to the product of a constant (Planck's constant, *h*) and the frequency of the radiation, symbolized by the Greek letter nu (ν). In chemistry, the quantitative measure of substances is expressed in terms of gram moles, one gram mole comprising $6.022140857 \times 10^{23}$ (Avogadro's number) molecules. Thus, the photochemical equivalence law is restated as: for every mole of a substance that reacts $6.022140857 \times 10^{23}$ quanta of light are absorbed.

The photochemical equivalence law is also sometimes called the Stark–Einstein law after the German-born physicists Johannes Stark and Albert Einstein, who independently formulated the law between 1908 and 1913.

Use of colorimetry

Determination of Nickel:

Chemicals required:

1. Standard Nickel Ammonium sulphate solution
2. 1% DMG (Di methyl Glyoxime)
3. Saturated bromine water
4. Ammonium hydroxide solution
5. Rosy red color solution measured in 445 nm.

Determination of Copper:

Chemicals required:

1. Standard copper sulphate solution
2. 1% potassium Ferro cyanide
3. Choclate brown color solution measured in 540 nm

Determination of Iron:

Chemicals required:

1. Standard Ferric Ammonium sulphate solution
2. Potassium thio cyanate
3. Con HCl
4. Blood red color solution measured in 540 nm

Principle and applications of atomic absorption

A photoelectric **flame photometer** is a device used in inorganic chemical analysis to determine the concentration of certain metal ions, among them sodium, potassium, lithium, and calcium. Group 1 and Group 2 metals are quite sensitive to **Flame Photometry** due to their low excitation energies.

n principle, it is a controlled flame test with the intensity of the flame color quantified by photoelectric circuitry. The intensity of the colour will depend on the energy that had been absorbed by the atoms that was sufficient to vaporise them. The sample is introduced to the flame at a constant rate. Filters select which colours the photometer detects and exclude the influence of other ions. Before use, the device requires calibration with a series of standard solutions of the ion to be tested.

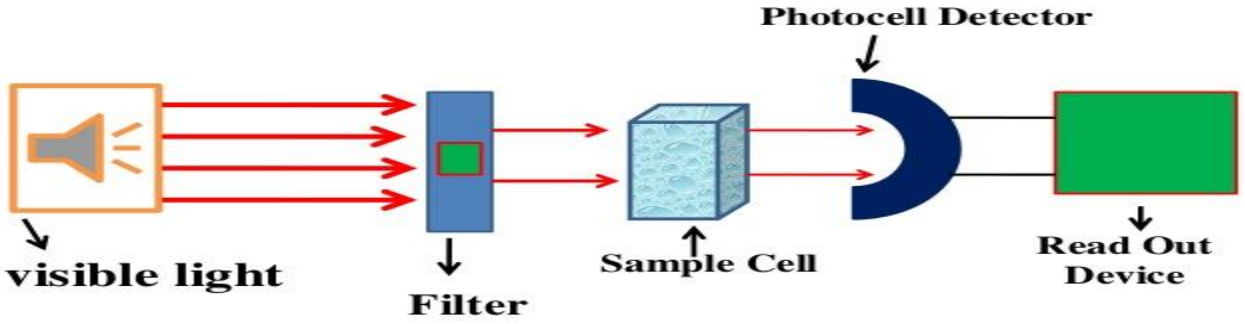
Flame photometry is crude but cheap compared to flame emission spectroscopy where the emitted light is analysed with a monochromator

NEPHELOMETRY AND TURBIDIMETRY

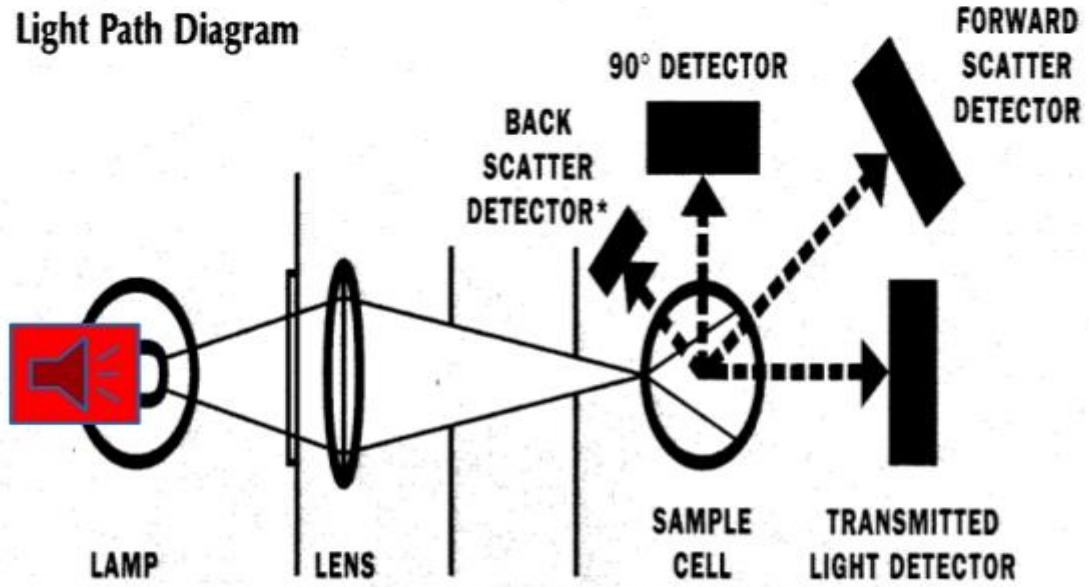
INTRODUCTION:

When electromagnetic radiation (light) strikes on a particle in solution, some of the light will be absorbed by the particle, some will be transmitted through the solution and some of the light will be scattered or reflected. The amount of light scattered is proportional to the concentration of insoluble particle.

Turbidimeter



NEPHELOMETER



- Light Scattering Phenomenon:
- The **blue color** of the sky and the **red color** of the sun at sunset result from scattering of light of small dust particles, H_2O molecules and other gases in the atmosphere.
- The efficiency with which light is scattered depends on its wavelength, λ.
- ***The sky is blue because violet and blue light are scattered to a greater extent than other longer wavelengths***.

Turbidometric measurements are used in the determination of suspended material in natural waters and in processing streams. The technique is also used for determination of sulfur in coal, oil, and other organic materials; the sulfur is precipitated as barium sulfate.

Nephelometry is a technique used in immunoloy to determine the levels of several blood plasma proteins. For example the total levels of antibodies isotopes or classes: Immunoglobulin M, Immunoglobulin G, and Immunoglobulin A, It is important in quantification of free light chains in diseases such as multiple myeloma. Quantification is important for disease classification and for disease monitoring once a patient has been treated (increased skewing of the ratio between kappa and lambda light chains after a patient has been treated is an indication of disease recurrence).