Chromatography

Chromatography is a physical method of separation, identification and purification of components of a mixture.

This technique of chromatography was introduced by Tswett and developed by Martin and co-workers in 1941.

Chromatography involve two steps

1) Stationary phase: Finely divided solid or liquid coated on inner wall of solid support act as stationary phase
2) Mobile phase: It may be liquid or gas

Chromatography is a physical process where the components (solutes) of a sample mixture are separated as a result of their differential distribution between stationary and mobile phases.

Chromatography

Principle:

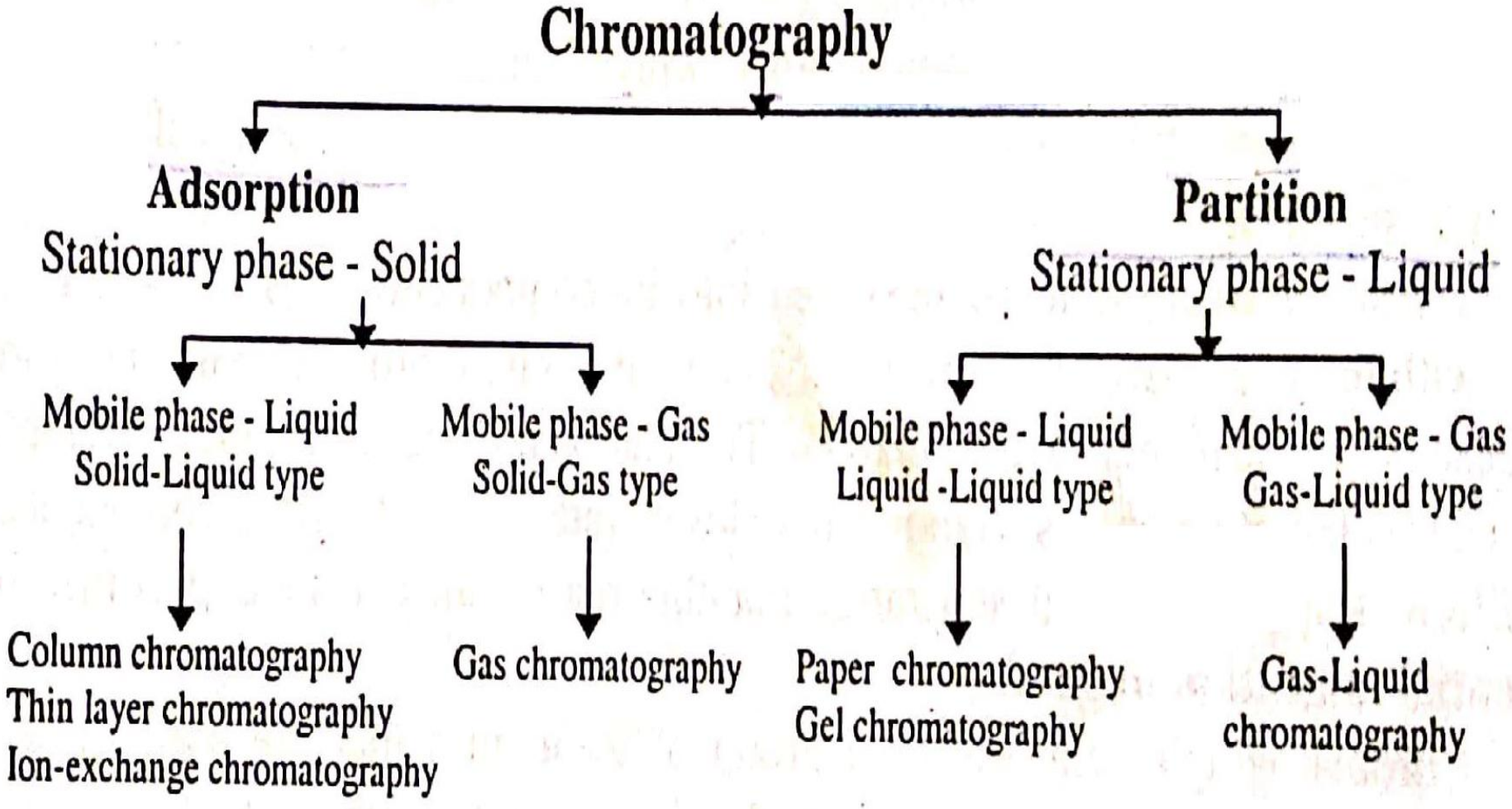
Mobile phase act as a driving force while stationary phase act as retarding force. Retardation is due to the adsorption of components on solid surface or partition of components between two phases. Constituents of mixture differ in their adsorption and desorption behavior, hence get separated from each other.

Match box model

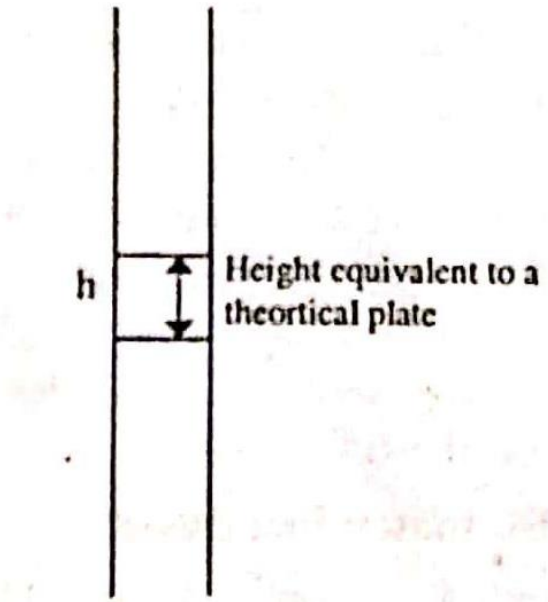
This model explain separation mechanism of two solutes on the basis of their different solubility in two solvents (i.e., stationary and mobile phase)

It explain the mechanism involved in liquid -liquid chromatography.

Types of Chromatography/ Classification



Theoretical plates and efficiency of chromatographic column



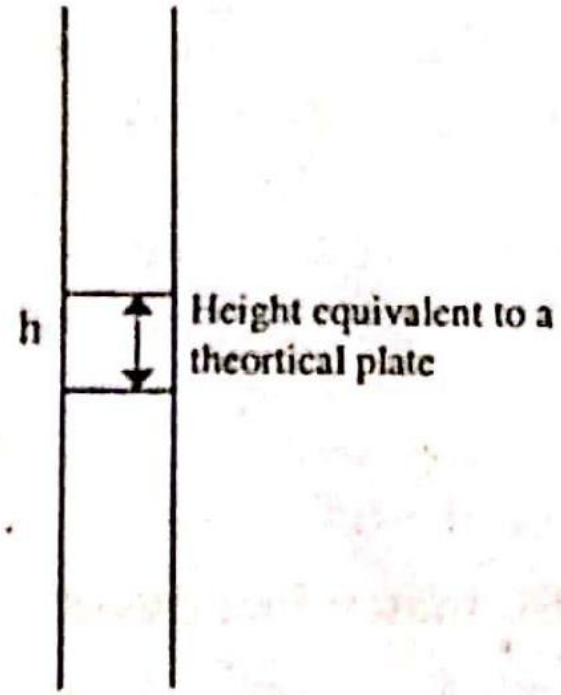
A column and HETP

The efficiency of the chromatographic column is described in terms of theoretical plates.

During chromatographic separation there is distribution and redistribution of solute between stationary phase and mobile phase.

Equilibrium between solute and solvent is due to the theoretical plates present on the chromatographic column.

Theoretical plates and efficiency of chromatographic column



A column and HETP

In one chromatographic column there are several thousands of such plates.

As number of theoretical plates increases efficiency of column also increases

For a given length (l) of chromatographic column , number of theoretical plate (n) are more if height (h) of plate(HETP) is small

Thus,

$$n = l/h$$

Column Chromatography

Adsorption Chromatography

Stationary phase is solid

Stationary phase adsorbed the components of a mixture while mobile phase desorb the components of a mixture.

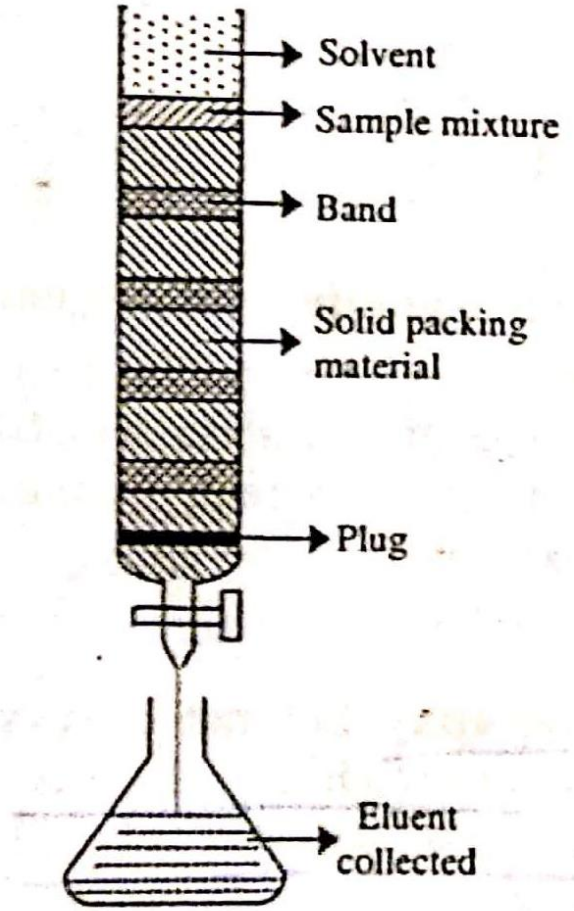
Different components of a mixture adsorb to different extent on stationary phase hence causes separation

Partition Chromatography

Stationary phase is liquid

Separation take place due to differential partition(distribution)of components of the mixture

Column Chromatography



Column chromatography

Preparation of column

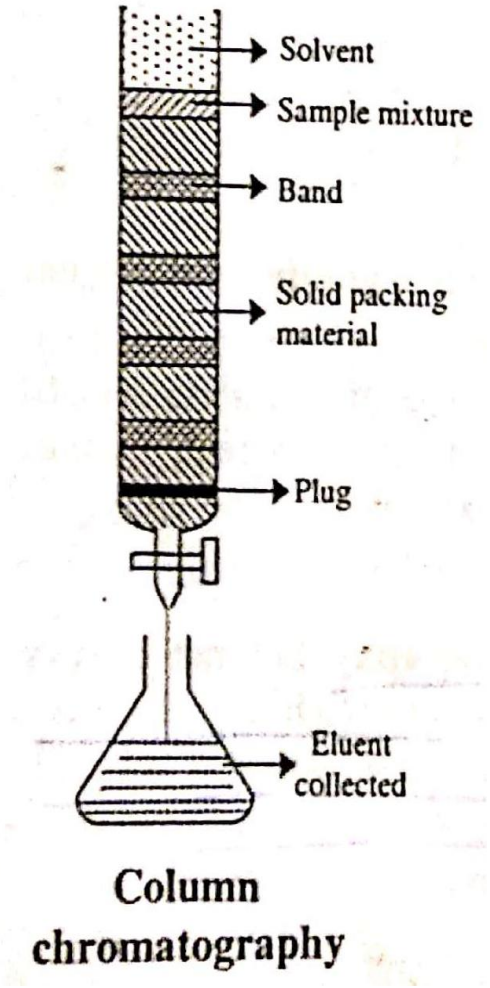
Column is prepared by carefully packing solid material. (silica gel, alumina etc.)

Height of column is generally 10 to 90 cm and inner diameter is 0.5 to 2 cm.

While preparing a column care is taken that no air bubble should remain in the column.

At least half of the column is filled with stationary phase.

Column Chromatography



Column chromatography

Method of separation

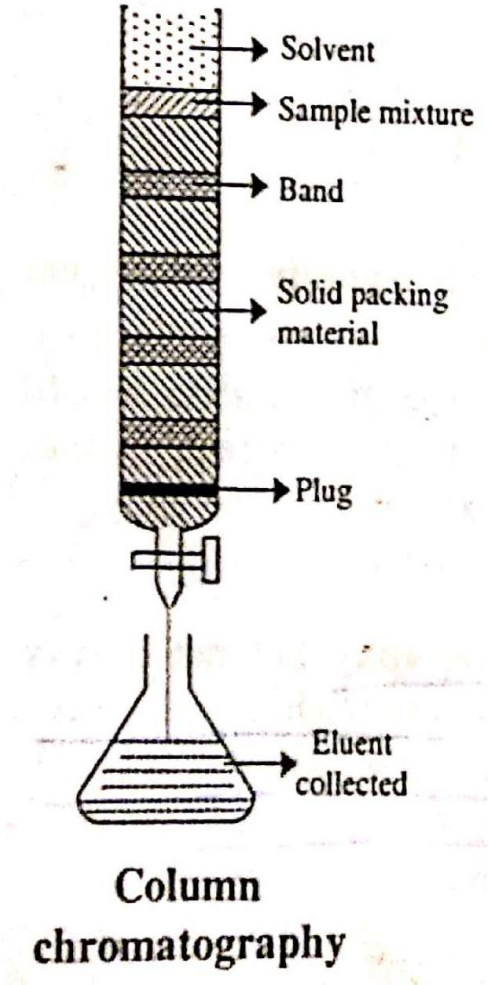
A small amount of mixture to be separated is placed at the top of the column.

The mobile phase called as eluting solvent is poured in to the column with constant rate.

As solvent passes over sample band desorption or partition of the sample components take place.

A component which is weakly held (less adsorbed) is move first while component which strongly held is move afterward. Thus, different zones of sample components are formed in chromatographic column.

Column Chromatography



Column chromatography

Separation take place on the basis of extents of adsorption of different components of mixture on solid surface.

Elution

The solution coming out of the column is called eluate.

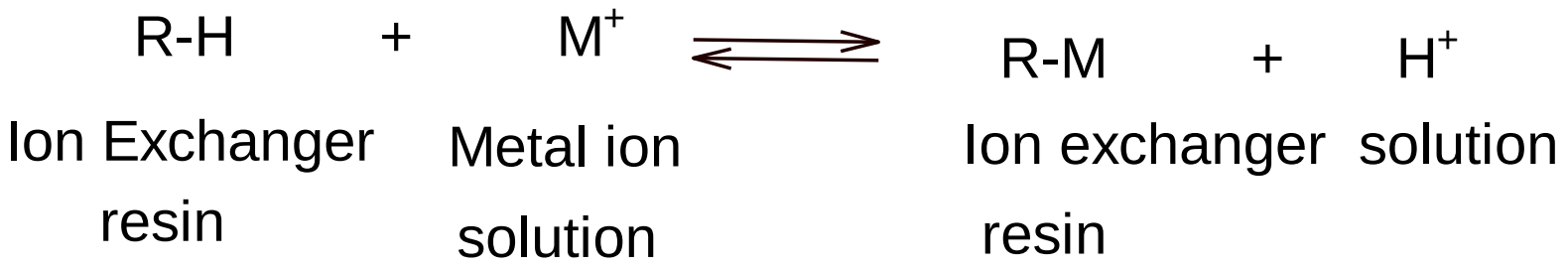
Different zones of the components are eluted by mobile phase and collected in separate container.

Ion Exchange Chromatography

Ion exchange chromatography is the process in which a mixture of similar charged ions can be separated by using an ion-exchange resin.

Ion exchange resins are high molecular weight polymers having sufficient cross linking and exchangeable ions.

Ion exchange resin exchange ions according to their relative affinities.



Ion Exchange Chromatography

Properties of ion exchangers

They are insoluble in water and organic solvents such as benzene, carbon tetrachloride, ether etc.

They are chemically inert and denser than water.

They are complex in nature (polymeric in nature).

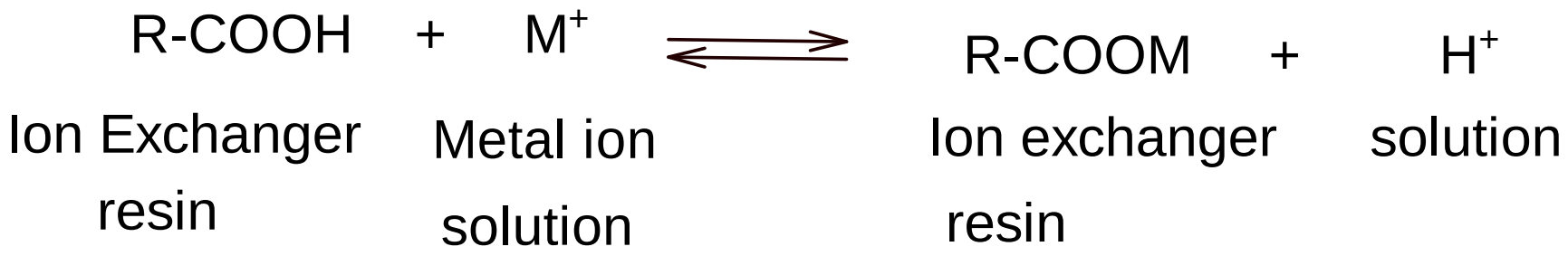
They have active ions that can exchange with other ions present in a surrounding solution.

Ion Exchange Chromatography

Cation Exchangers

These are the resins having sulphonic acid (-SO_3H) or carboxylic acid (-COOH) group

The cation exchange can be represented by the following equation



Ion Exchange Chromatography

Types of Cation Exchangers

a) Strong acid resins (Polystyrene sulphonic acid resin)

They contain -SO_3H group. They are effective from pH 1 to 14.

These are used for the separation of amino acids, vitamins, peptide, rare earth elements etc.

Examples Dowex-50, Doulite C-20, Amberlite IR-120

b) Weak acid resins (polymethylacrylic acid resin)

They contain -COOH group. They are effective from pH 5 to 14.

These are used for the separation of Transition metals, acids, antibiotics organic bases etc.

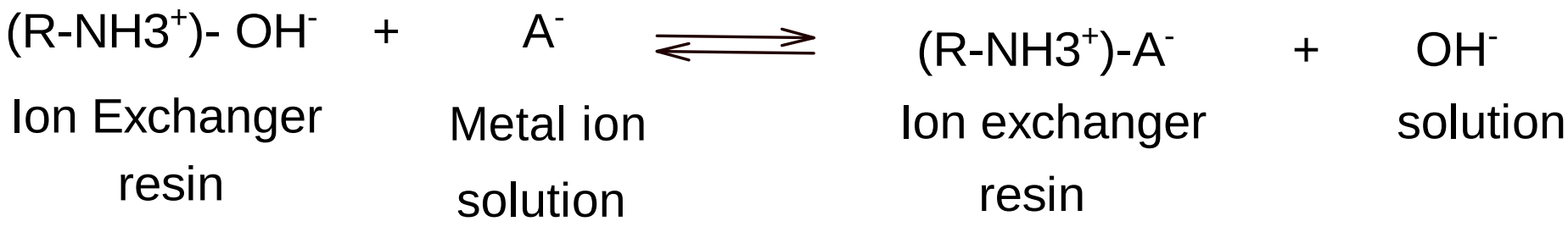
Example: Amberlite IRC-50

Ion Exchange Chromatography

Anion Exchangers

These are the polymers of having amine or quaternary ammonium group.

The anion exchange can be represented by the following equation



Ion Exchange Chromatography

Types of Anion Exchangers

a) Strong base resins (Polystyrene quaternary ammonium resin

b) They contain $(R\text{-}NH_3^+)\text{-}OH^-$ or $(R\text{-}NH_3^+)\text{-}C^{l-}$ group

They are effective from pH 0 to 12.

These are used for the separation of alkaloids, fatty acids, vitamin b complex etc.

Examples Amberlite IRA-400

b) Weak base resins (polystyrene tertiary amine resin)

They contain primary, secondary or tertiary amine groups. $(-NH_3^+Cl^-)$

They are effective from pH 0 to 9.

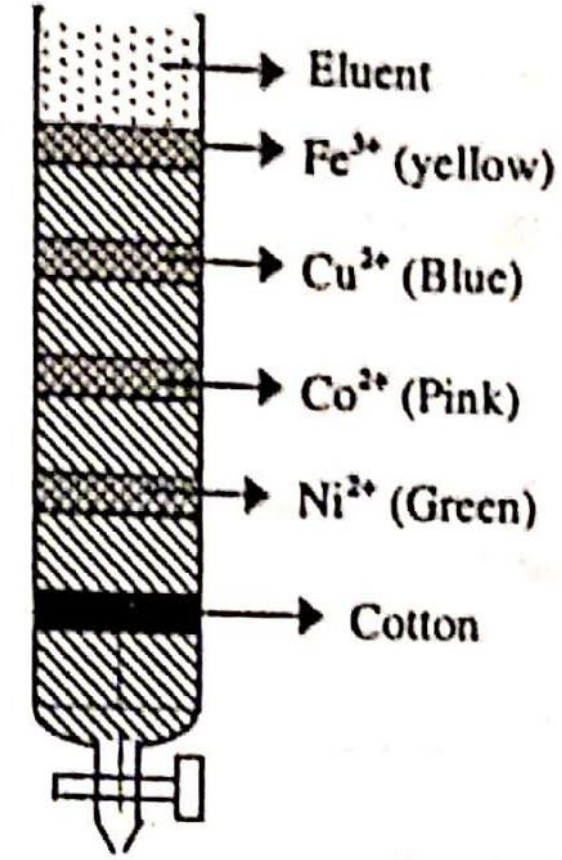
These are used for the separation of anions and amino acids.

Example: Amberlite IR-45

Ion Exchange Chromatography

Type of Exchanger	Functional Exchanger Group	Trade Name
Cation		
Strong acid	Sulphonic acid $-SO_3H$	Dowex – 50, Amberlite IR 120, Rexyn 101, Permutit Q
Weak acid	Carboxylic acid $-COOH$	Amberlite IRC 50, Rexyn 102, Permutit H – 70
Anion		
Strong base	Quaternary ammonium ion $-N(CH_3)_3^+ Cl^-$	Dowex–1, Amberlite IRA 400, Rexyn 201, Permutit S–1
Weak base	Amine group $-\overset{+}{N}H_3Cl^-$	Dowex 3, Amberlite IR 45 Rexyn 203, Permutit W

Ion Exchange Chromatography



Ion Exchange

Preparation of column

Procedure of preparation of column chromatography is used for preparation of column of ion exchange chromatography. In this column ion exchange resin is used as stationary phase.

Method of separation

$$R - H + M^+ \rightleftharpoons R - M + H^+$$

In solution In solution

or $-NH_3)^+ - OH^- + A^- \rightleftharpoons (R - NH_3)^+ A^- + OH^-$

In solution In solution

Elution

The process of removing exchanged ions from resin is called elution. Solvent used for elution is called eluent and solution coming out of the column is called eluate.

Ion Exchange Chromatography

Applications

It can used for laboratory separation, purification of water, extraction of metal etc.

1) Separation of metal ions on anion exchangers

Metal ions are first converted to anionic complexes by using complexing agents.(Cl^-, F^-, Br^- etc)

For example iron can be converted to $[FeCl_4]^-$

Concentrated HCl can form complex with many metal ions, except alkali metals. Thus these metals can easily separated by using conc. HCl.

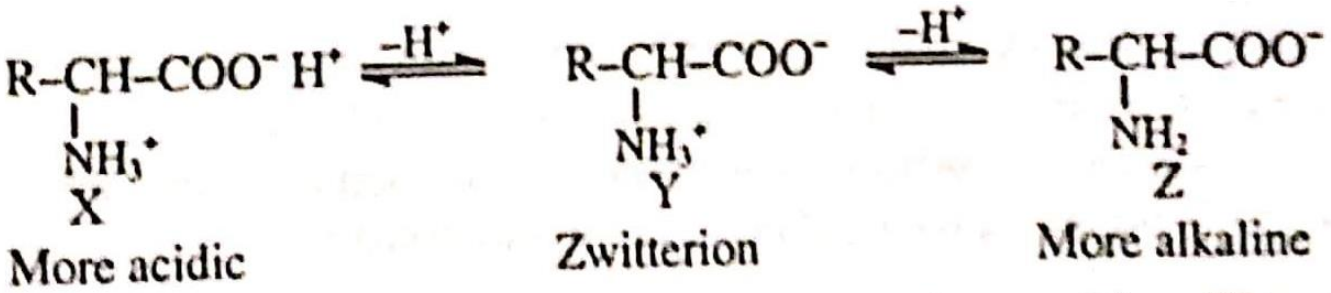
When there are many metal ions, they can be separated by decreasing HCl concentration to a level at which KD of other metal ion is low .

Ion Exchange Chromatography

Applications

2) Separation of amino acid

Adjustment of suitable pH is very important in separation of amino acids. Amino acids are exists in three forms



Thus positively charged amino acids(X form) are separated by using cation exchanger, while negative form (Z form) is separated by using anion exchanger. Amino acids exist as Zwitter ion form(Y form) at isoelectric point. This form pass through both cation and anion exchanger and get separated.

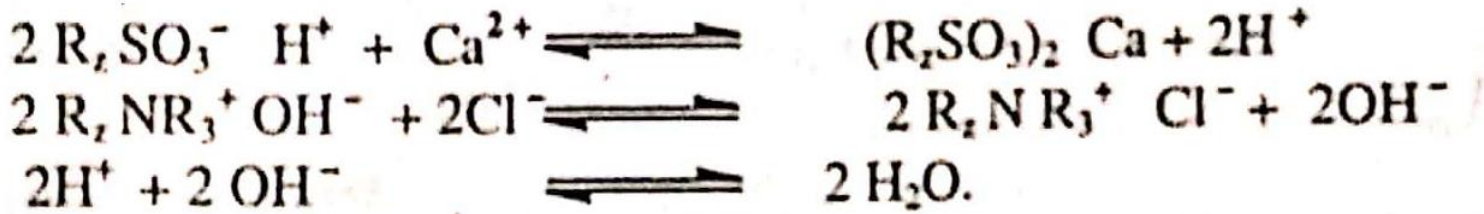
Ion Exchange Chromatography

Applications

3) Purification of water

Deionised or demineralised water is the water free from any cationic or anionic impurities.

Water is allowed to passed through mixed ion exchange resin. Cations and anions present in water exchanged with cations and anions present on resin.



Uses of Deionised water: 1) Used in conductivity experiments. 2) used in biological studies 3) used in high pressure boiler 4) used in lead storage battery 5) used in preparation of $AgNO_3$ solution

Paper Chromatography

Analysis of unknown substance is carried out mainly by flow of solvent on specially prepared filter paper is called paper chromatography.

Paper chromatography is a partition chromatography.

In this chromatography water act as stationary phase (paper is solid support on which stationary phase is coated) and organic solvent is mobile phase.

Mobile phase move over stationary phase by capillary action

The solutes which are more soluble in water move slowly while solutes more soluble in mobile phase move faster, thus separation of components take place.

Paper Chromatography

Steps involved in Paper chromatography

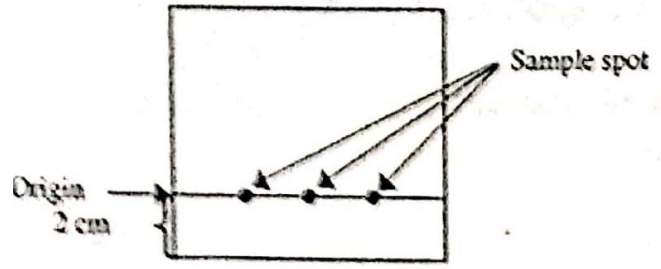
a) Application of sample on the paper

A whatman filter paper of suitable size (15 to 30 cm in length and 10 to 15 cm in width) is used

A thine pencil line is drawn at the distance 2 cm from the bottom.

A small quantity of mixture is dissolved in minimum quantity of volatile solvent

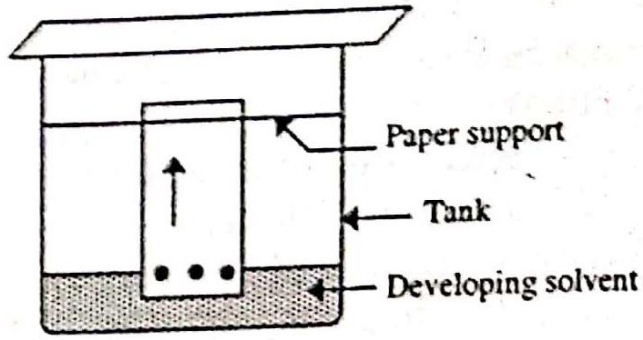
The sample solution is spotted on base line with the help of capillary tube.



Spotting of the sample solution

b) Saturation of the tank

The atmosphere of the tank must be saturated with mobile phase before starting the development. For this solvent is added and tank is closed.



Paper chromatographic set up

Paper Chromatography

Steps involved in Paper chromatography

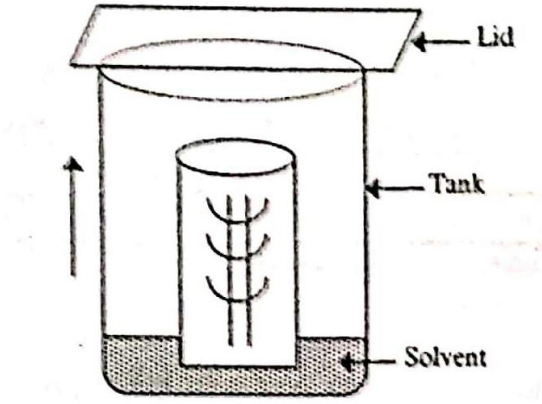
c) Development of the chromatogram

The paper is placed in the chamber with one end dipping in the developing solvent.

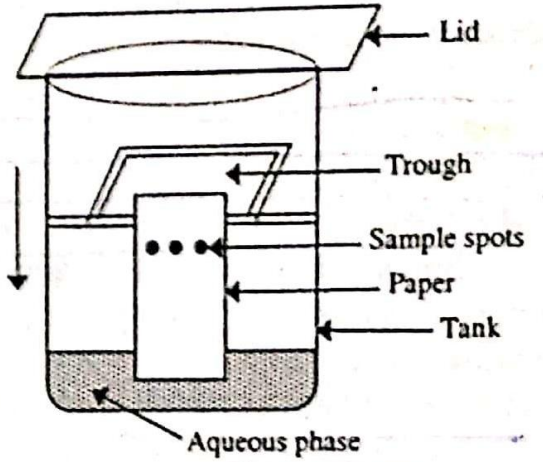
Ascending development: developing solvent move in upward direction by capillary action.

Descending development: Developing solvent move in downward direction by capillary action.

Development: The process of separation of components of the mixture in the form of bands or spots of pure substances at different places on chromatogram is known as development.



Ascending development



Descending development

Paper Chromatography

Steps involved in Paper chromatography

d) Location of the spot:

i) Physical method: The paper is observed under U.V. light to locate the colour components.

ii) Chemical method: Colourless components can be converted to coloured by reaction with chemical reagents.

Following chemicals are generally used for this purpose

K_2CrO_4 crystals

Iodine crystals (Vapours of iodine in iodine chamber)

Methyl alcohol, ethyl alcohol, t-butyl alcohol

Ninhydrin spray (for amino acids)

Paper Chromatography

Steps involved in Paper chromatography

e) Identification of components:

Identification id done by calculating R_f (retardation factor)values

R_f is the ratio of the distance travelled by the given substance from the origin to the distance travelled by the solvent from the origin.

$$R_f = \frac{Distance\ travelled\ by\ the\ substance}{Distance\ travelled\ by\ the\ solvent}$$

Under constant experimental conditions Rf vales are reproducible.

Paper Chromatography

Applications

1) Separation of very small amount of substance (in Biochemistry)
2) Separation of amino acids
3) Separation of complex mixture (Proteins)
4) Separation of organic as well as inorganic substances
5) Analysis of mixture of sugars

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is an important technique used for identification and separation of mixture into its individual components.

TLC is solid liquid chromatography consists of

Solid stationary phase (Thin layer of silica is coated on glass plate)

Liquid mobile phase (This liquid flow over stationary phase)

Thin Layer Chromatography (TLC)

Principle:

It involves the distribution of components of a mixture to be separated between two phases.

Different components of a mixture have different solubility and adsorption in two phases

In TLC separation of the individual substances is based on their relative affinities towards stationary and mobile phases.

Components with more affinity towards stationary phase travels slower.

Components with less affinity towards stationary phase travels faster.

Thin Layer Chromatography (TLC)

Commonly used stationary Phases

Silica Gel, Alumina, Cellulose powder

Sometimes binding agents like calcium sulphate and plaster of paris are added

Commonly used mobile Phases

Pure solvent or mixture of two or more solvent is used as mobile phase

Ethyl acetate, Petroleum ether, Benzene, Carbon tetrachloride etc

Thin Layer Chromatography (TLC)

Preparation of surrey

A small amount of stationary phase material is dissolved in suitable solvent. Dry TLC plate is dipped in to the slurry for development of uniform thin layer.

Plate is dried to evaporate solvent and activate at 110^{o}C.

Chromatoplate: Plate having uniform layer of finely devided solid on it is called chromatoplate

Saturation of the Tank

Pure solvent or freshly prepared mixture of solvent is poured in the tank and the tank is covered with lid for saturation

A mixture to be analyzed is dissolve in small amount of solvent and applied on TLC plate as a spot using capillary tube.

Plate is kept in the tank for sometime and removed after development.

Thin Layer Chromatography (TLC)

Types of Thin Layer Chromatography

1) Adsorption Thin Layer Chromatography

This is the most common form of TLC. Generally silica gel or alumina is used as a stationary phase.

2) Liquid-liquid partition Chromatography

Int his case adsorbed or residual water is act as a stationary phase.In this case chromatoplate is not activated by heating.

3) Ion exchange TLC

Ion exchange resin powder is used for preparation of chromatoplate. Strong acid cation exchangers and strong base anion exchangers are selected on the basis of mixture to be separated.

Thin Layer Chromatography (TLC)

Types of Thin Layer Chromatography

4) Size Exclusion TLC

Superfine sephadex is used for preparation of chromatoplate. Gel is soaked in water for four days and then spread on the plate. Development time is 8 to 10 hours.

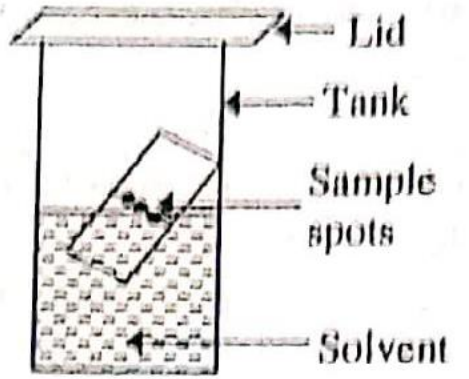
Mobile phase used in TLC

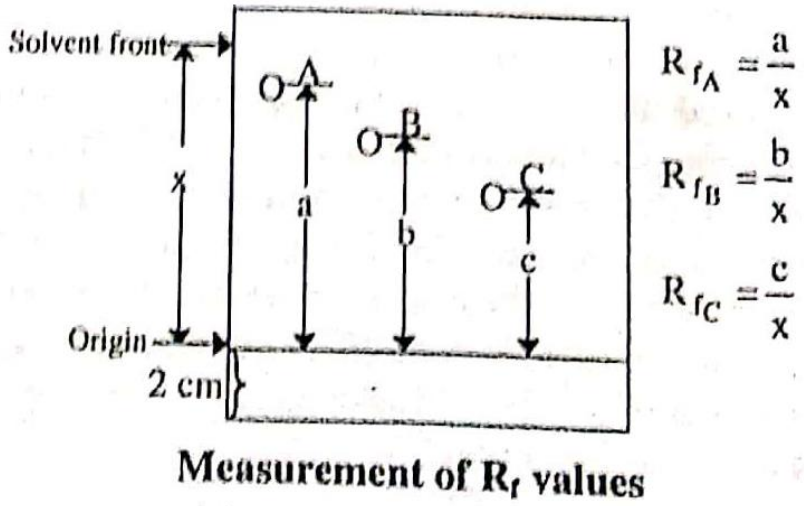
Pure solvent or mixture of two or more solvent is used as mobile phase

Ethyl acetate, Petroleum ether, Benzene, Carbon tetrachloride etc

Generally mixed solvents gives better separation

Thin Layer Chromatography (TLC)





Measurement of R_f values

Detection of the spots

1) **Use of Iodine chamber:** developed plate is exposed to iodine vapours, colourless spots become coloured.
2) **Use of Sulphuric acid:** 4 ml H_2SO_4 in 100 ml methanol is spread on the plate for the detection of organic compounds.
3) **Ninhydrine spray** is used for detection of amino acids
4) Plate is observed under **U.V. light**

Thin Layer Chromatography (TLC)

Advantages of TLC over Paper chromatography

1) It gives very sharp separation.
2) It can be used in organic, inorganic, biochemistry and pharmaceutical chemistry.
3) It is faster than paper chromatography
4) It gives reproducible Rf values.
5) It can be used for adsorption, partition, ion exchange etc.

Chromatography

Advantages and Limitations of Chromatographic technique

Advantages:

1) The components which shows great similarities in physical and chemical properties are easily separated by this technique
2) Technique is very sensitive. Even 0.1 microgram of compound can be detected
3) Apparatus/equipments requires are very simple and easily available

Limitations

1) Incomplete recovery of sample
2) Adsorption depends on the physical state of adsorbent. It is difficult to get adsorbent of same quality every time.

Applications of Chromatography

1) It is used for purification and identification.
2) In synthetic chemistry it is used to find out the progress of the reaction and quality of product.
3) Detection of mixture of terpenes oils, vitamins, chlorophyl, steroids etc.
4) Identification of sugar in urine.
5) In forensic science it is used for detection of poison and metal ions.

Applications of Chromatography

Solved Problems

1. In an experiment of paper chromatographic separation of silver, lead and mercury, the solvent front was 27 cm. while front due to these metals were 24 (Ag), 18 (Pb) and 9 (Hg) cm. What are the R_f values of these metals ?

Solution : In paper chromatography, we use the formula

$$R_f = \frac{\text{distance travelled by solute front}}{\text{distance travelled by solvent front}}$$

For Ag $\rightarrow R_f = \frac{24}{27} = 0.88$ For Hg $\rightarrow R_f = \frac{9}{27} = 0.33$ For Pb $\rightarrow R_f = \frac{18}{27} = 0.66$

Applications of Chromatography

3. Separation of cations was done using ion exchange chromatography. Calculate the number of plates n and height of theoretical plate h, if column exit time d is 4.67 minutes after sample adsorption with the width of elution curve $W_e = 0.215$ minutes and height of the column *l* is 27.45 cm.

Solution : We use the formula $n = 16\left(\frac{d}{W_e}\right)^2$

where n = number of plates
d = exit time = 4.67 minutes
W_e = width of elution curve = 0.215 minutes

$$n = 16\left(\frac{4.67}{0.215}\right)^2 = 7.55 \times 10^3$$

Now we use the formula $h = l/m$

where h = height of the plate in cm.
l = height or length of the column in cm

n = number of plates

$$h = \frac{27.45}{7.55 \times 10^3} = 3.64 \times 10^{-3} \text{ cm}$$

THANKS