B.Sc. IVth Semester

CHEMISTRY

UNIT – VIII – Separation Techniques

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Chromatography –

- Chromatography may be defined as a method of separating mixture of components into individual components through the equilibrium distribution between two phases. Essentially, the technique of chromatography is based on the different rate at which component of mixture move through porous medium called stationary phase under the influence of some solvent or gas called as mobile phase.
- The chromatography method of separation in general involves following steps –
  i) Adsorption or Retention of substances or substance on the stationary phase.
  ii) Separation of adsorbed substance by the mobile phase.
  iii) Recovery of separated substances by continuous flow of mobile phase, the method being called as elution.
  iv) Qualitative and quantitative analysis of eluted substance.
- Chromatography is a non-destructive procedure for dissolving the multi-component mixture of trace, minor or major constituents into individual fraction.
- Chromatography is relatively new technique which was first invented by **M. S. Tswett**, a botanist in 1906 in Warsaw, Poland. In that year he was successful in doing the separation of chlorophyll & xanthophyll and several other colored substances by calculating vegetable extract through a column of $CaCO_3$. The $CaCO_3$ column act as adsorbent and the different substances get adsorb to different extent & this give rise to colored bands at different position of the column. Tswett termed this system of color band as **chromatogram** and the method is chromatography after the Greek word 'Chroma' and 'graphes' meaning 'color' and 'writing' respectively. However, in the majority of chromatography procedure no color produces or form which is termed as **misnomer**.
- In 1930, chromatography in the form of Thin-layer chromatography and Ion-exchange chromatography was introduced as a separation technique.
- In 1941, **Martin** and **Synge** introduce Partition chromatography and paper chromatography.
- In 1952, **Martin** and **Synge** introduce Gas chromatography.

Types of Chromatography –

1. Column Chromatography (Adsorption).
2. Partition Chromatography.
3. Paper Chromatography.
4. Thin-layer Chromatography.
5. Gas-liquid Chromatography.
6. Gas-solid Chromatography.
7. Ion-exchange Chromatography.

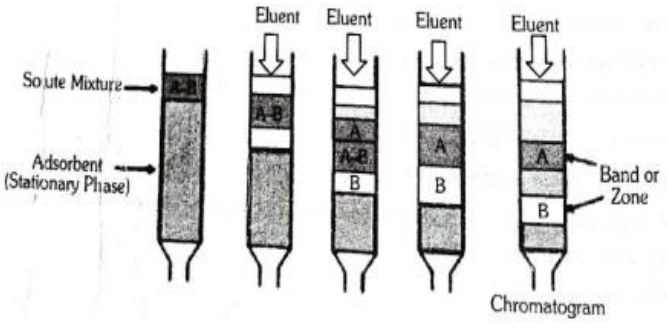

Figure : Adsorption Chromatography

Theoretical Principle of Chromatography technique –

- The basis of all form of chromatography is the partition or distribution coefficient which describes the way in which a compound distributes itself into two immiscible phases.
- For a compound distributing itself between equal volume between two immiscible solvents i.e., solvent A & solvent B, the value of coefficient at the given temperature is given by the expression –

$$k_d = \frac{\textbf{Concentration of ion in Solvent A}}{\textbf{Concentration of ion in Solvent B}}$$

The distribution of compound can however be described not only in term between two solvent but also by distributing between any two phases such as solid/liquid or gas/liquid phase. Thus, a distribution coefficient of substance between Salicylic acid and benzene is 0.5 which means the concentration of substance in benzene is twice that in Salicylic acid.

- Basically, all the chromatography systems consist of two phase-
  1) Stationary phase – which may be solid, gel, liquid or solid-liquid mixture.
  2) Mobile phase – which may be liquid or gaseous and flow over through stationary phase.
- The choice of stationary & mobile phase is made so that the compound to be separated have different distribution coefficient that may be achieved by setting up –
  a) Adsorption equilibrium between stationary solid and liquid phase. (Adsorption chromatography)
  b) A partition equilibrium between stationary liquid and mobile phase. (Counter current chromatography or Partition chromatography)
  c) A partition equilibrium between stationary liquid and mobile gas phase. (Gas-liquid chromatography)
  d) An ion-exchange chromatography obtained by setting up equilibrium between ion-exchange resin stationary phase and mobile electrolyte phase. (Ion-exchange chromatography)

Theory of Chromatography –

Two theories have been forwarded regarding the rate of migration of solute and the development of peaks in the chromatograms. They are (i) Plate theory and (ii) Rate theory or Kinetic theory.

(i) **Plate theory** –

- According to Plate theory developed by Martin and Synge, a chromatography column consist of series of discrete a continuous horizontal layers which are formed at the theoretical plate and equilibration of solute between the stationary & mobile phase takes place. At each these plates migration of solute is then assume to occur by a series of step-wise form between one plates to other immediately below. The efficiency of separation in a chromatography column get increase as the number of theoretical plates increases. This is because of number of equilibration will also corresponding increase. The number of theoretical plate (N) refers to measure of column efficiency. If the length of column (L) and height (H) is increase then,

$$N = \frac{L}{N}$$

(ii) **Rate theory or Kinetic theory** –

- The rate theory is able to explain the affect variable such as mobile phase velocity and adsorbilities which determine the width of an elution band. It also relates with the effect of these variables on the time taken by solute to make its appearance at end of the column. Migration of solute particle in a

column occurs in the state of confusion, each solute molecule progressing in a stop & go sequence independent of any other molecule.

- If the molecule is attached to stationary phase its migration down the column is temporarily stops but the zone passes on i.e., to say one molecule may caught immobilize temporarily on the column while other molecule migrate. But this manner molecule alternate rapidly between adsorbed-disorbed stages. The time of molecule spending in either phase is highly regular. Some solute molecules may migrate rapidly whereas other may remain behind the rate, all this random process is a symmetric or average separation of molecule.

Column chromatography –

The separation by adsorption column chromatography involves following steps –

i. Preparation of Column –
The glass column of smaller diameter are generally prepared for the reasons of smooth & uniform, one end of the column is tapered and fitted with stop cock. The column is clamped on stand vertically and a sintered glass disk or small plug of glass wool is inserted at the bottom of column to support the packing.

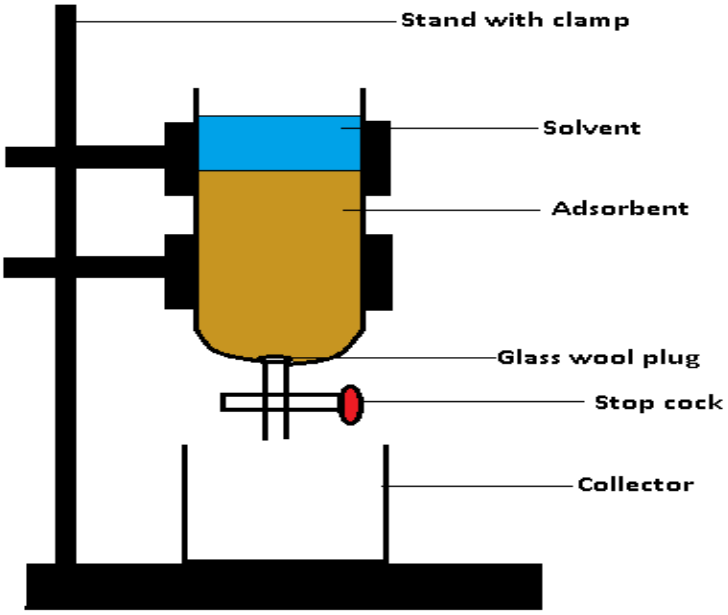

Figure : Adsorption Column Chromatography

Dry packing –
The dry powdered adsorbent such as $CaCO_3$, $CaSO_4$, Silica, Alumina powder is introduce in a small installment through the open end of the column then it is tapped gently for its setting. The process is repeated until the filled column of desired height is obtained.

Wet packing –
More commonly thin slurry of adsorbent in suitable solvent, for example CCl_4 (Carbon tetrachloride), Benzene, Acetone etc., is made and pour in the open end of column and allow to settle down and the stop cock is open to allow the level of liquid to run down taking care of level of liquid must never be

allow to go down below the top of packing otherwise, air bubbles will get trapped causing channeling in the column.

ii. Selection of an adsorbent –
The choice of adsorbent depend upon a number of factors, these are as follows –
a) It should be insoluble in solvent use for working in the column.
b) It should be not react with substance to be separated.
c) It should be colorless and
d) It should have a uniform composition.

iii. Selection of Solvent or Mobile phase –
A relatively non-polar solvent is used to place the separating mixture on the column then a somewhat polar solvent is used to elute the adsorbed component. The rate at which component are eluted is control by the activity of adsorbent and the polarity of solvent.
In general, more polar is solvent the more rapidly will move that component. Some of the common solvents in there decreasing order of polarity are given below –
Organic acid > Pyridine > Water > Methanol > Ethanol > Chloroform ($CHCl_3$) > Ethyl-acetate > Benzene > Ether > Carbon disulphide (CS_2) > Cyclo-hexane > CCl_4 > Petroleum ether

iv. Application of sample –
The mixture to be separated is poured onto the column in the form of solution with the help of pipette the care is taken to avoid sticking of the sample to inner walls of the column elsewhere except on the top of adsorbent liquid mixture may be applied directly to the column.

v. Development of Column –
The components of mixture are eluted by continuous downward flow of the solvent because each component to passed down the tube. In certain cases where packing material consists of very fine particle for example – Charcoal and Celite, the pressure is applied down the top of column to maintain reasonable flow rate of eluting solvent. Sometime quantity of eluting solvent is kept just sufficient to separate the component into colored zone.

Factors affecting Column chromatography efficiency –

i. Nature of Solvent.
ii. Dimensions of Column.
iii. Pore-diameter of column.
iv. Particle size of adsorbent.
v. Polar adsorbent should possess a pore diameter of 20 Å.
vi. Particle size of adsorbent using smaller size 100-200 mesh.

Applications or Uses of Column Chromatography –

i. Separation of mixture into pure individual components.
ii. Removal of immunities and in the purification of compound.
iii. Identification of unknown compound.
iv. Column chromatography has been used in separation of geometrical isomer, diastereomers, racemates and tautomers.

v. Create application of column chromatography has been separation & identification of inorganic anions (-) and cations (+).

Partition Chromatography or Liquid-liquid Chromatography –

- Partition chromatography is a technique in which mixture of substances are separated by means of partition between moving solvent and stationary liquid which is held on suitable solid support.
- When the solvent (moving phase) is a liquid it is known as Liquid-liquid chromatography.
- When the moving phase (solvent) is gas then the technique is known as Gas-liquid chromatography.
- The liquid-liquid separation are carried out on cellulose or moist silica gel which may be in the form of thin sheet (paper chromatography), thin layers (thin-layer chromatography) are packed in column (partition chromatography). The medium each which case acts as support of water
- Eluotropic series of common solvent –
  When the solvent arrange in order of increasing eluting power in the following order are –
  Light petroleum < Cyclo-hexane < CCl_4 < Toluene < Benzene (C_6H_5) < $CHCl_3$ < Ether < Acetone < n-propanol < Ethanol < Methanol < Water < Pyridine < Organic acid < Inorganic bases

Procedure of Partition chromatography –

In partition chromatography, a column is prepared by a glass tube filled with porous plate and filled with powered Silica gel. Usually, the stationary liquid phase is introduced to the support to packing of column. Consider the separation of mixed organic acid carried out in 1952 by **Hays**, the Silica gel is first saturated with methyl-alcohol and poured in the column, either dried or slurry form with petroleum ether. The powder is allow to be settle down, a closely filled disc or filter paper is place in contact with packing to facilitate movement of liquid. The acid solution under analysis is prepared in petroleum ether and added in column, the solvent moving downward contain within those acid have the smallest distribution ratio. Acid has high ratio are held by column thus facilitates separation.

Theory of Partition Chromatography –

- Theoretical plate theory –
  According to **Martin** and **Synge**, the theoretical late is an imaginary layer of column of such thickness that solution coming out from it an equilibrium solute concentration corresponding to average solute concentration of immobile phase within the layer. The column can be divided into number of plates where perfect equilibrium is assumed in each plate for mobile & stationary phase.
- The plate number and height is useful index of column efficiency to describe the extent to which peaks and zones are brought through action of physical transport phenomenon. The general theory of partition chromatography is similar to fractional distillation.

Liquid-liquid Partition chromatography –

- Liquid-liquid partition chromatography consists of column of finely divided solid support on which is fixed and made immobile and second phase is mobile phase which is immiscible with stationary liquid substance flow over it.
- The components of sample mixture participate in partition between the stationary & mobile phase. When they migrate down the column, those components which partition more readily into the

stationary phase are retarded in the passage through the column. Those into mobile phase the support consist of solid porous material with a mesh size of 100-300 mesh. Holding about 50% weight of stationary liquid.

- As regard partitioning liquid, the selection of solvent is confined to hydrophilic solvent on stationary support and hydrophobic solvent serving as mobile phase.
- Solvent can be classified on the basis of hydrogen bonding. Solvent which are either donor or acceptor of electron pairs have the ability to form inter-molecular hydrogen bonding (H-bonding).
- The solvent pair selected should have low mutual solubility substance with medium porosity can then be separated. Buffer can be added to the water phase for adjusting the pH of complexation.

Reverse phase partition chromatography or Extraction chromatography –

- As the name implies that the phases used in liquid-liquid partition chromatography are reversed, this means that instead of hydrophilic stationary phase hydrophobic solvent be used as the stationary phase on a suitable stationary support.
- Similarly, instead of using the hydrophobic phase as the mobile phase hydrophilic phase is used as the mobile phase.
- There is a specific technique employed to coat the stationary phase with hydrophobic water repellant usually stationary phase support consist of Silica gel, Alumina, Teflon etc. They are hydrophobic by exposure to vapour of di-ethyl chloro silane.
- Several solubility solvent like tri-butyl phosphate (TBP), tri-butyl phosphate oxide (TBPO), tri-octyl phosphine oxide (TOPO) can be used for hydrophobic phase. Similarly, there solvent which are used in several extraction processes.

Advantages of Reverse phase extraction chromatography –

The principle advantage of this is the use of previous knowledge of an element from a point of extraction for development of new separation with the knowledge of batch extraction is fully known in what acidity and what concentration and extraction of particular metal is extracted.

Application of Reverse phase extraction chromatography –

1. It is used extensively in metal separation at microgram concentration (10^{-6}). This technique combines advantage of solvent extraction and column chromatography to effect selective separation. For example – With TBP as the stationary phase excellent separation have been made for Fe(III), Cr(VI), MO(IV)&(V), Au(III), Hg(II) from metal component mixture.
2. It has also been possible to use Amberlite as stationary phase for separation of Sc & In by extracting their anionic malonate complex. It is possible to separate Bismuth from alloy such as Woods, metals, solder, white metal and various borenge.