UNIT I-4

CHROMATOGRAPHY-4

* Liquid - Liquid Partition chromatography :- (Partition chromatography) :-

* Principle :-

In Partition chromatography, the stationary phase is a liquid generally water held on a suitable inert porocss solid such as cellulose Powder of silica gel and a liquid mobile phase, such that the two phases are of limited miscibility. The components of the sample mixture participate in the partition between the stationary phase where they are held in fixed position and the mobile phase where they migrate those components which partitions more rapidly into the stationary phase are retained during their passage through the column wrto those which partition more into the mobile phase.

* Theoritical Basis For Liquid - Liquid chromatography (Partition chromatography) :-

The theory of partition chromatography is bassed on the concept of theoritical plates

* Theoritical plate :-

The theoritical plate is defined as imaginary layer of the column of such thickness that solution coming out from it has an equillibrium solute concentration, corresponding to average solute concentration of mobile phase with in the layer.

A chromatographic column may be regarded to contain a large no. of theoritical plates with the following properties.

① The thickness of each plate measured along the path of the mobile phase is identical because column packing is assumed to be uniform the parameters. It is called height equivalent of theoritical plate.

② The volume V_H of the mobile phase is const because the column is assomed to be uniform.

③ In each plate, partition occurs so that the fraction x of solute in the mobile phase is given by.

$$x = \frac{V_m}{V_m + K_d V_s}$$

where V_m, V_s are the volumes of mobile, station ary phase, respectively.

The effective plate volumes $V_H = V_m + K_d V_s$

$$= H (A_m + A_s).$$

where K_d is partition coefficient

A_m and A_s are the cross sectional area of the mobile and stationary phases. These area can be determined from the length of the colum Packing and the total volumes of two solvent contained in it.

* Advantages of partition chromatography and adsorption chromatography :-

① Partition is preferred for low concentration of mixture.

② Partition provides a large resolving power

than adsorption.

③ since Partition depends up on the solubility of two liquid small differences in molecular weight effect Partition. Thus Partitioning is good method for separation of homologous series.

④ Both Partition and adsorption are based on polarity differences as polarity increases the adsorption increases and hence it is difficult to elute polar molecules from the adsorbent. Thus adsorption is used for non polar molecule.

⑤ The important advantages of Partition over adsorption chromatography is that distribution isotherms in Partition systems are linear over a wide range of concentrations. The presence of over components does not effect the Partition Isotherms.

* Applications of Partition chromatography:-

Partition chromatography is powerful tool for the separation of closely related

③ substance based on differences in this solubility might be attributed to.

① No. of and Polarity of constituents.

② molecular size and shape.

③ chain length of aliphatic groups.

④ Number and location of C-C double bond, Thus based on the above criteria.

⑤ Resolution of numerous amino acids formed in the hydrolysis of proteins.

⑥ Analysis of closely related aliphatic alcohols.

⑦ Separation of carbonyllic acids and sugar, derivatives can be determined.

① separation of organic acids:-

In this experiment a variety of aliphatic acid will be separated as column packed with chloroform and butanol mobile phases and 0.5N H_2SO_4 on celite as stationary phase.

② separation of organic acids by Reverse Phase chromatography:-

In the reverse phase procedure a 1.1 X 60 cm column of so must Teflon-6 with

cyclo hexane as flat phase and water as mobile phase is emporyed to separate 1.5 mg of o-methoxy aniline from an equal no. of m-methoxy aniline use a flow rate of 1.3 ml/min.

* The supports :-

The essential requisites of a suppo rt are its ability to hold a certain amount of stationary liquid phase as well as insolubility in both the mobile and stationary liquids Preferably the support should be a porous granular solid of particle size using between 100 to 800 mesh. As supports kieselguhr, diatomaceous, earth with cellulose powder may be envisioned as constituting of connected. puddies of swollen fibrouss material. When treated with liquid by contrast or kieselguhr or diatomaccous earth holds the stationary liquid in droplet form between the spines and holes of the diatomic skeletones.

* The partitioning liquids :-

In normal partition column selection of solvents, involves of hydrophoblic solvents. serving as a mobile phase solvents may be classified on the basis of their ability to form hydrogen bonds. Then solvents form a sevies similar series in some respects to the elutropic sevies. The head of this sevies one solvents which are either donars or acceptors of e^- pairs and have the ability to form intermolecular hydrogen bridges The solvent pair selected should possess a low mutually solubility. Both the phases should be ingradients each phase must be saturated with the other at the operating temprature. In many applications water is bounded to the supports and a water immisible organic solvents forms the mobile phase. such a system is applicable to the separation of hydrophilic substances and substances of medium polarity. It is possible to add buffers to the stat water phase for adjus

The p^H value of the system are making agents to alter Partitioning hydrophylic organic solvents may be employed in place of water.

Ex:- The excellent Partitions of the Fatty acids have been carried out with methanol and liquid Partition mixtures. The method has also been extended to longer chain (C_{11} to C_{10}) Fatty acids by using a mixture of 2-Amino pyridine and furfuryl alcohol as the stationary liquid and n-hexane as the mobile phase.

* Eluents :-

It is difficult to Poldict the optimum combination of stationary and mobile liquid phase the Partitioning system may involve mixture solvents or buffers or complexing agents A further complication is that the 2 phases must be in equilibrium during the chromatographic separation. If 3rd or more components are Introduced into the equilibrium constant are effected.

Several Partition system for normal consume phase chromatography are gain in the tab.

* Reverse Phase chromatography :-

The column Partition chromatography can be extended by modifying the support. whe by the hydrophobic organic solvent becomes the stationary phase and the hydrophilic solven the mobile phase. This is known as reversed Pha chromatography. By exposing silica gel or cellulos powder in a closed container to the vapour of chloro silane. The surface of the support is rendered un wettabl by strongly polar solven and will retain the less polar phase of the num bous solvent systems. The solibility of the colu depends up on the interfecial tension of two phases. The reverse phase column with station ary phases of hydrocarbons are siliconiol require particular temperature control.

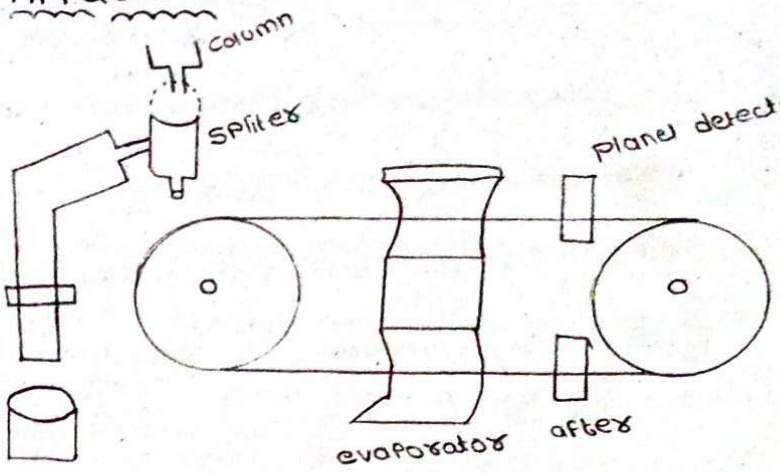
The variation in temparature will result in stripping of liquid on the solid support &

demining of the stat phase in the mobile phase. Typical reversed phase system include olive oil ethanol system and liquid paraffin eth and system on which fatty acids have been separated.

* Discuss about stationary and mobile phases :-

The partition chromatographic columns of finely divided solid as which a solvent is fixed tenacity it will not migrate. A second liquid phase immiscible with the stationary liquid substances flows over the latter Backed granular material solvening as a support provide contact over a very large interface. The components of sample mixture participate in a partition between the stat/ionary phase and the mobile phase. These components which partition is more rapidly into the stationary phase are retarded in their passage through the column with respective to those which partition more into the mobile phase.

* Apparatus :-



① Separation of organic acids.

② for eg separation of acetic formic and lactic acid separation.

column :- 45 cm long and 1 cm diameter.

S.P :- 0.5N H_2SO_4 on ceite.

M.P :- CHU_3 - Butanol, mixture

the S.P 9.6 ml of 0.5N H_2SO_4 is throughly which 12 gm of ceite the moist ceite is stirried with 10% butanol in chloroform and poume into the column excess m.p is attaced to drying the column.

The mixture of acids is introduced onto TOP of the column. the ~~dwebmint~~ development is continued with the organic liquid the flow rate is existed to 1 ml/min, 5ml portions of eluent is collected by acids

are determind by titrating with standard 0.1 N alkali Individual acids must be identfied comparision with known run on the same - column.

⑦ * High Performance Liquid chromatography :-

* Principle :-

In HPLC, eluent from the solvent reservoir is filtered, pressurised and pumped through the column. A mixture of solutes injected at the top of the column is separated into components. Individual solutes are monitored by the detector and recorded automatically.

* Theory :-

All forms of liquid chromatography (LC) are differential migration processes. Where sample components are selectively retained by a stationary phase. LC covers a variety of separation techniques such as liquid - solid, liquid - liquid, ion - exchange and exclusion chromatography, all involving a mobile liquid phase. Liquid - solid chromatography (LSC) is often termed as adsorption chromatography. Liquid - Liquid (partition) chromatography (LLC) is similar in principle to solvent extraction. LLC is divided into two categories, based on relative polarities of stationary and mobile phases.