(4) Explain the general procedure of thin layer chromatography (TLC).

Chromatography using thin layers of adsorbents on glass plates is commonly known as thin layer chromatography. It is a simple, versatile, inexpensive chromatographic technique of great importance.

A glass plate is coated with a slurry of an adsorbent. The slurry will adhere to the surface of the glass plate as a thin layer after drying.

The unknown substance and reference materials are dissolved in suitable solvents in separate test tubes and then applied in a raw of spots, about 2cm from the edge of the plate with the help of capillary tubes.

After getting the spots dried, the chromatoplate is placed in a jar containing the solvent for development, care should be taken to see that the row of spots are just above the level of eluent solvent. As the solvent ascends through the layer by capillary action, sample mixture is resolved into various fractions at different heights from the point of application.

The plate is carefully withdrawn after the solvent front has travelled about 80% of the height of the plate. The plate is then dried and sprayed with a spray reagent to locate the components as ~~red~~ black spots, or alternatively, the plate is exposed to iodine vapours by keeping it in an iodine chamber, to locate the various components of the mixture under investigation as brown spots.

(5) Write about the applications of thin layer chromatography.

(1) TLC has been used as an rapid method for isolation. identification and purification of organic compounds.

(2) A mixture of 34 amino acids, proteins and peptides have been successfully separated and isolated from urine using silicagel plates.

(3) TLC has been applied for the separations of alcohols and glycols using loose layers of alumina.

(4) A large number of vitamins, antibiotics and food products have been separated using TLC. The important food stuffs that are separated and analysed by TLC are (a) fruits for aminoacids (b) milk for amino acids (c) wine for glucose and sorbitol d) meat for carboxylic acids (e) beer for aromatic alcohols (f) orange juice for amino acids.

(5) In organic chemistry, TLC is used for checking the purity of samples, for studying various reactions and their progress, in the characterisation of a number of compounds such as alcohols, acids, amides, alkalodis, amino acids, antibiotics, vitamins etc.

Q. 1. *What is solvent extraction? Explain briefly.*

When two mutually immiscible or partially miscible liquids are brought in contact together, the substance or substances to be extracted will distribute between the two liquids in a definite way concentrating predominantly in one of them. The process is known as solvent extraction.

In otherwords, removal of a solute present in a solvent using another liquid which is immiscible with the original solvent is known as solvent extraction.

Extraction arises from differential solubility of a solute in two mutually immiscible liquids. In these extraction processes one of the liquids is usually water and the other is an organic liquid. In such processes advantage is taken of the fact that the distribution ratio of most of the inorganic substances is in favour of water, while most of the organic substances is in favour of organic solvents. This is due to the fact that organic compounds (non - polar or less polar covalent compounds) are generally more soluble in non - polar organic solvents like benzene, ether, carbon tetrachloride, chloroform etc than in water and most of these organic solvents are immiscible with water. Hence organic compounds are easily extracted into non - aqueous organic layer when an aqueous solution containing both inorganic and organic compounds are brought in contact with an organic solvent, Finally, the non - aqueous layer is separated from the aqueous layer, and the solvent is removed by distillation to get the organic substance.

(2) (9) What is paper chromatography?

Paper chromatography is defined as the technique in which the analysis of an unknown substance is carried out mainly by the flow of solvent on specially designed filter paper. It is the passage of a mobile phase (solvent) through the porous structure of the paper which contains a stationary liquid phase. Development is terminated before the mobile phase reaches the edge of the paper so that the zones are distributed across the paper. The stationary phase and the mobile phase should be mutually immiscible or partially miscible with each other. The separation is effected by differential migration of the substance in the mixture. This takes place as a result of difference in partition coefficients

(2)

(10) Explain the experimental procedure of paper chromatography.

A drop of the mixture to be analysed is applied near the edge of a ribbon of chromatographic paper, dried and then

Immersed into a cylinder with a suitable solvent whose level is below the applied drop. The solvent raises by capillary action of the paper and various components of the mixture are carried with it at different rates. In fact, each component of the mixtures is distributed between the mobile and stationary phases in accordance with their values of partition coefficient. The lower the values of partition coefficient, the more of the component will pass over into the mobile phase and move at a higher speed together with the solvent. Hence, as the solvent front moves along, separation of the components takes place and this separation depends on the ratio of the partition coefficients of the components in the two phases of the chromatographic system. As a result, each of the components of the initial mixture is concentrated at different distances from the place of initial application. Thus, various components of the mixture are distributed as zones and a chromatogram is obtained. If the zones are colourless, the

chromatogram has to be developed by applying reagents, called visualising agents.

(11) short
~~Explain~~ the experimental procedure of paper chromatography.

Mention some of the applications of paper chromatography.

Applications of paper chromatography are numerous. paper chromatography has been widely used for quantitative as well as qualitative analysis of mixture containing inorganic and organic compounds of biochemical, pharmaceutical, medicinal and industrial interest.

1. Paper chromatography can be used for the identification and separation of complex mixture of drugs, dyes, metal ions etc.

2. paper chromatography has been widely used for the analysis of mixture of aminoacids using the spray reagent ninhydrin.

3. Paper chromatography is extensively used in the analysis of mixture of carbohydrates, using the spray reagent aniline hydrogen phthalate.

4. Paper chromatography is ideally suited for rapid anlysis of reaction mixtures.

(12) S.F.D

Explain two dimensional paper chromatography.

In two dimensional paper chromatography a drop of the mixture to be separated is applied at one corner of a square sheet of a chromatographic paper. After drying, the paper is successively developed twice with two different solvent systems. The paper to which sample is applied is developed first with one solvent system and the solvent is dried by evaporation. After evaporation of the solvent, the paper is rotated 90° in a required proper way and is again developed with the second solvent system. After evaporating the solvent, the chromatogram is developed by applying visualising reagents and the components are identified. Two dimensional chromatography is specially suitable for those substances which cannot be separated effectively by one dimensional paper chromatography. This happens when the R_f values of the constituent components of the

mixture are very close and nearly the same. such mixture are obtained with bio chemical compounds like amino acids, carbohydrates, porphyrins etc.

Q.7. *Explain the following* Imp
(a) Ascending Paper chromatography (b) Descending Paper chromatography (c) Radial paper chromatography.

(a) Ascending Paper chromatography : In this technique the chromatographic paper, after application of the sample mixture, is suspended vertically using a horizontal glass rod as support, in a glass tank containing the eluting solvent (mobile phase) The paper is dipped in the solvent in such a way that the sample spot is slightly above the level of the eluting solvent. The glass tank is closed with a cover. The solvent rises up by the capillary action of the paper. Hence this technique is known as Ascending paper chromatography. After elution the paper is removed from the tank, the solvent is allowed to evoporate and then developed using visualising reagents. The R_f values of each component are calculated.

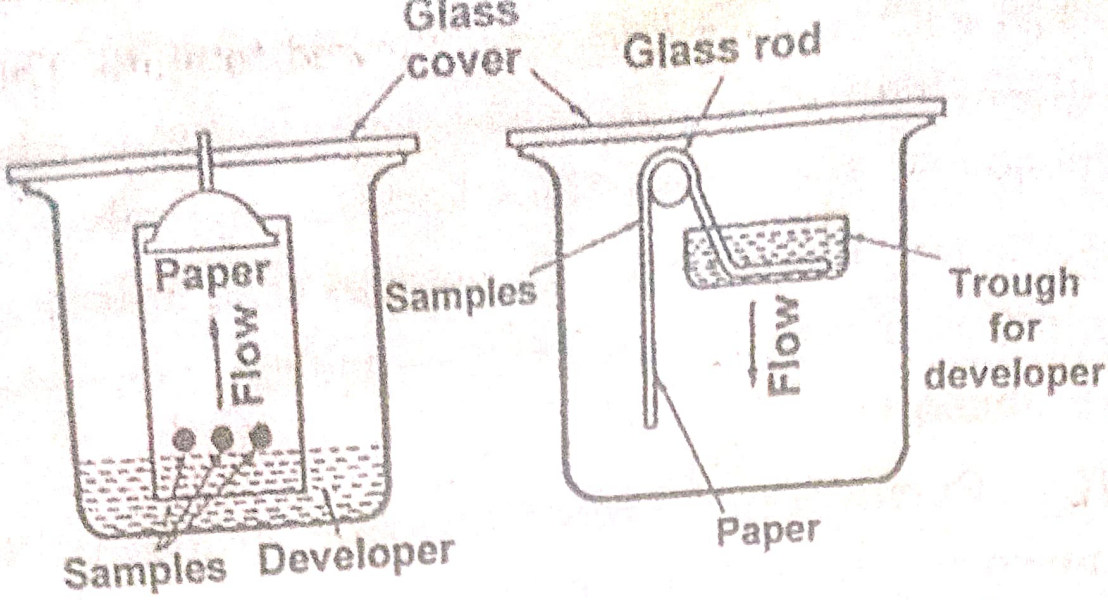

Fig. Apparatus for paper chromatography
(a) Acending flow (b) Descending flow

(b) Descending paper chromatography : In this technique, the eluting solvent (mobile phase) is kept in a trough mounted near the top of the glass tank. The upper end of chromatographic paper, which contains the applied sample is kept dipped in the eluting solvent. The eluting solvent moves downwards the paper and hence this technique is known as descending paper chromatography. The advantage of this technique is that development can be continued indefenitely till the solvent runs off in the trough.

After the elution, the paper is removed from the tank, allowed the solvent to evaporate and then developed by using visualising agents. Finally, the analysis is completed by calculating the R_f values of the different components of the mixture.

(c) Radial paper chromatography : In this method, a circular piece of chromatographic paper having a wick, cut parallel to the radius from the edge to the centre is used. The sample mixture is applied as a spot at the centre of the paper at the upper end of the wick. After drying the spot, the paper is horizontally fixed over the trough containing the eluting solvent in such a way that the wick of the paper partially dips in the solvent. The trough along with the paper is now kept in a glass

tank, covered with a plate and then allowed to elute. The solvent ascends through the wick and then flows radially through the paper. Hence this method is known as Radial chromatography. When the solvent front has moved through the required distance, various components in the sample mixture are separated in the form of concentric circular bands. Hence this method is also known as circular paper chromato-graphy. After elution the paper is removed from the tank, the solvent is allowed to evaporate, and the circular bands are identified using visualising agents. Analysis will be done by calculating the R_f values of various components of the sample.

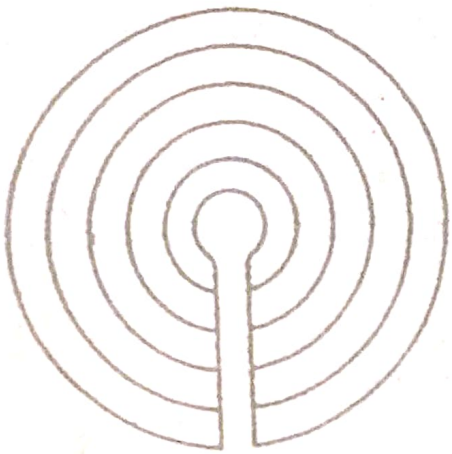

Fig. Radial or Disc development