(3) AB (P)

6) Explain the theory and principles of column chromatography.

In column chromatography, the stationary phase is a solid adsorbent, usually alumina (Al_2O_3) or (silica gel (SiO_2) and the mobile phase is liquid. The stationary phase is held in a tube called column, through which the mobile phase is forced either under pressure or gravity. The fundamental principle of column chromatography is the selective adsorption of various components of a mixture on the solid stationary phase called the adsorbent. Various components in the mixture are adsorbed in the various parts of the column in the order of their decresing adsorptive powers, and hence column chromatography is also known as adsorption chromatography. The initial separation of various components can be improved by passing a suitable solvent system called the 'eluent' through the column and the process is known 'elution', on elution, various components of the mixture are separated into different bands for zones of pure substance

each located at a different region in the column. The separation into different bands is known as the development of the chromatogram. When the process of elution is further continued with the eluent (solvent) each component travels down the column along with the solvent one by one. Thus, separation of the mixture into individual components can be achieved.

(7) Give the principle of chromatography?

Chromatography involves a mobile phase (liquid or gas) which passes over the surface of a fixed or stationary phase which may be a solid or a liquid immobilised by some method, as by adsorption on the surface of a solid. The components of the sample (which is inserted at or near the point where contact is first made between the two phases) are carried along at various rates depending on their relatives affinities for the two phases and are thus separated.

7) Explain the experimental procedure of column chromatography.

A glass column, with a narrow tube and a stop cock at the bottom, was packed with a finely divided adsorbent like alumina or silica gel. The adsorbent is wetted with the eluting solvent first and then a solution of sample mixture is introduced at the top of the column. The

column is then developed by elution i.e. by running the mobile phase until coloured bands of the components of the mixture appered at various positions along the length of the column. The components are then recovered following one of the methods given below.

(a) The adsorbent is carefully removed out of the column, each section is cut and separated, and finally the separation is treated with a suitable solvent to desorb the component into solution, which is separated by filtration (for the removal of desorbed and absorbent) followed by distillation (for the removal of the solvent). This is an old method, now seldom used. The method fails if the components will not form coloured bands (b) After the various components are separated into bands, elution is further continued with the mobile phase to get each component of the mixture one by one in the form of solution. The components is recovered from the solution by removing the solvent by distillation.

18) What is HPLC? Explain the theory and principle involved in High Pressure liquid chromatography (HPLC).

With the rapid advancement of the Efficient Gas-liquid chromatography (GLC) the classical column liquid chromatography went into back yard until High Performance liquid chromatography came into picture.

In classical column liquid chromatography, the mobile liquid phase flows slowly through the column under the influence of gravity. This method is generally characterised by low column efficencies and long separation times.

The availability of high pressure systems capable of operating at pressure upto 3000 psi ($2.0 \times 10^7 Nm^{-2}$), led to the development of High pressure liquid chromatography. In HPLC, small diameter columns (1-3 mm) with support particle sizes in the region of 30 μm are used and the eluent is pumped through the column at

a high flow rate. It has been found that separation by HPLC may be effected about 100 times faster than by the use of conventional liquid chromatography.

In comparison to classical column chromatography, where the column are gravity fed and a separation can take hours or even days, HPLC can provide analysis in 5-30 min, the times which are comparable with gas-liquid chromatography (GLC).

HPLC is particularly suitable to the analysis of those compounds which are not readily handled by GLC. For example, thermally labile compounds can be analysed at ambient temperatures by HPLC, highly polar compounds can be chromatographed without prior derivatisation and polymeric samples can also be analysed.

HPLC is defined as a method of separation or analysis of a mixture in which the stationary phase is contained in a column, one end of which is attached to a source of pressurised liquid eluent (mobile phase)

(19) Write about the important applications of High performance liquid chromatography

HPLC finds applications in a wide variety of fields some of which are considered here

(a) Inorganic chemistry: The utility of HPLC for the separation and analysis of mixtures of inorganic compounds is limited in comparison to organic compounds, only to those compounds which are soluble in solvents being compatible with HPLC columns. There are two classes of soluble inorganic compounds. (a) Ions (anions and cations) which are soluble in water giving aqueous solutions (b) Molecular compounds which are more readily soluble in organic solvents. Anions like F^-, Cl^-, Br^-, I^-, IO_3^-, SCN^-, ClO^-, ClO_4^-, BrO_3^-, $S_3O_3^{2-}$, SO_3^{2-}, SO_4^{2-}, S^{2-}, NO_2^-, NO_3^-, N_3^-, CN^-, HPO_4^{2-}, HPO_2^{2-}, CrO_4^{2-}, WO_4^{2-}, ReO_4^{2-}, TaO_4^{2-}, VO_4^{2-}, BO_3^{3-}, SeO_3^{2-}, SeO_4^{2-}, etc can be analysed by using suitable techniques.

(b) Forensic chemistry: In toxicology and criminology laboratories, very sensitive and rapid analysis of drugs of abuse are required by law enforcing agencies, because these analyses are important in determining the composition of the seized substance and the quantity of drug in a specified sample. HPLC has been found to be inherently suitable for dealing with such drug samples. Detection of cocaine, nor-cocaine and benzoylnor-cocaine in urine samples can be done with HPLC. The separation of 16 common street drugs, including phencyclidine, metho-done, cocaine, tetrahydrocannabinol, methylamphetamine, 2,5-dimethoxy-4-methyl amphetamine (STP), Methylenedioxy amphetamine (MDH), heroin, N,N-dimethyl triptamine (DMT), Lysergic acid diethylamide (LSD), diazepam (valium), mescaline, secobarbitone, amylobarbitone, phenobarbitone, and diphenyl hydantoin (Dilantin) has been achieved in 40 minutes using HPLC.

(c) HPLC is used in the analysis of natural and synthetic pharmaceutical drugs like antibiotics, sulphonamides.

(d) HPLC has been successfully used in the analysis of (i) alkaloids (ii) Amino acids and proteins (iii) carbonhydrates (iv) Vitamin (v) Nucleic Acids (vi) carcinogens like aflatoxins, metabolites of Aspergillus, fluvus etc. (vii). pesticides, insecti-cides, herbicides; fungicides, Rodenticides etc. (viii) Environ-mental-pollutants and (ix) Adultrant food additives.

(20) Write about the important applications of column chromatography.

Following are some of the areas in which the techique of column chromatography is used.

1. In establishing the identity (or) non-identity of two substances.
2. In determining the concentration of products in a given mixture.
3. In the separation of mixtures containing steroisomers.
4. Detection and estimation of contaminants in commercial products.
5. purification of commercial and technical products.

6. Identification of commercial products.
7. In testing the homogeneity of coloured compounds.
8. For the separation of 17-ketosteroids, plasma cortisel etc. in biochemistry.

(21) Why retention time is the basis for qualitative analysis in gas chromatography?

In gas chromatography the response of the detector is plotted as a function of time or carrier gas volume on a strip chart recorder. The components of the mixture are eluted from the column at different time intervals from injection. This time interval known as retention time is constant, if all the separation conditions remain the same on repeated injections. Consequently this is the basis for qualitative analysis because the retention time between a standard and an unknown are comparable.