FREQUENTLY ASKED QUESTION:

Q.1: What is a chromatogram?

Ans. A chromatogram is a graph showing the detector response as a function of elution time.

Q.2: How do planar and column chromatography differ?

Ans. In Column chromatography, the stationary phase is placed in a column through which the mobile phase moves under the influence of gravity or pressure. The stationary phase is either a solid or a thin, liquid film coating on a solid particulate packing material or the column's walls. While as in planar chromatography, the stationary phase coats a flat glass, metal, or plastic plate and is placed in a reservoir containing the mobile phase which moves by capillary action carrying with it the sample components.

Q.3: How does TLC differ from paper chromatography?

Ans. Thin layer chromatography is similar to paper chromatography, but the stationery phase is a thin layer of solid such as alumina or silica supported on an inert base such a glass, aluminum foil or insoluble plastic. The mixture is spotted at the bottom of the TLC plate and allowed to dry. The plate is placed in a closed vessel containing solvent (mobile phase) so that the liquid the liquid is below the spot. TLC has advantages over paper chromatography in that its results are reproducible, and that separations are very efficient because of the much smaller particle size of the stationary phase. The solvent ascends the plate by capillary action, the liquid filling the spaces between the solid particles.

Q.4: Describe the fundamental difference between adsorption and partition chromatography? **Ans.** Adsorption chromatography includes the interactions of solute on surface of stationary phase for polar non-ionic compounds while as partition includes the interface based on the relative solubility of analyte in mobile and stationary

phases.

Q.5: Describe the fundamental difference between ion-exchange and size-exclusion chromatography?

Ans. Ion exchange describes the interaction between the analyte and stationary phase via attraction of ions of opposite charges e.g., for ionic compounds anions or cations while as size exclusion (gel filtration, gel permeation) separates molecules by size, sieving –not real interaction, small molecules travel longer**.**

Q.6: Describe the difference between gel-filtration and gel permeation chromatography?

Ans. Gel permeation chromatography separates based on the size or hydrodynamic volume (radius of gyration) of the analyte. This differs from other separation techniques which depend upon chemical or physical interactions to separate analyte. Separation occurs via the use of porous beads packed in a column while as in gel filtration chromatography, the stationary phase consists of porous beads with a well defined range of pore size. The stationary phase for gel filtration is said to have a fractionation range, meaning that the molecules within that molecular weight range can be separated.

Q.7: What is purpose of chromatography?

Ans. The purpose of chromatography is both analytical and preparative. In analytical purposes, chromatographic separations determine the chemical composition of the sample while as in preparative phase chromatography serves to purify and collect one or more components of a sample.

Q.8: What is retention factor?

Ans. The retention is the measure of the speed at which a substance moves in a chromatographic system. In continuous development system like HPLC or GC, where the compounds are eluted with the eluent, the retention is usually measured as the retention time Rt or tR , the time between injection and detection. In interrupted development system like TLC the retention is measured as the retention factor Rf.

Q.9: What is physical principle of chromatography?

Ans. The principle of chromatography is based on the partition of analyte between liquid, mobile phase and immobilized liquid stationary phase via partition, adsorption, ion-exchange or size exclusion mechanisms.

Q.10: How the measurement is made and what is data obtained?

Ans. Sample is introduced as small liquid aliquot into column and elution measured at end of column. The data is procured as peak areas proportional to concentration and characteristic retention times.

Q.11: Name two different types of column chromatography techniques?

Ans. There are two types of chromatography, affinity chromatography and ion exchange chromatography. Affinity uses an antibody to bind to a protein of interest so that we know proteins which are the ones that we want. Ion exchange binds with oppositely charged molecules so if we put negatively charged beads in than positively charged molecules will separate.

Q.12: How does molecular exclusion differ from other types of chromatographic methods?

Ans. Molecular exclusion differs from other types of chromatography in that no equilibrium state is established between the solute and the stationary phase. Instead, the mixture passes as gas or a liquid through a porous gel. Thus separation is according to particle size.

Q.13: How is HPLC differentiated from Normal Liquid Phase Chromatography?

Ans. HPLC is distinguished from traditional (low pressure) liquid chromatography because operational pressures are significantly higher (50-30 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile through the column. Due to the small sample amount separated in analytical HPLC typical column dimensions are 2.1-4.6 mm diameter, 30-250mm length. Also HPLC

column are made with smaller sorbent particles (2-50 micrometers in average particle size). This gives HPLC superior resolving power when separating mixtures, which is why it is popular chromatographic technique.

Q.14: What is difference between isocratic and gradient elution?

Ans. The separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning constant composition). The mobile phase composition does not have to be remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution.

Q.15: what is distribution coefficient? What are its applications?

Ans. A partition or distribution coefficient is the ratio of concentrations of a compounds in a mixture of two immiscible phases at equilibrium. These coefficients are a measure of the difference in solubility of the compound in these two phases. In medical practice, partition coefficients are useful for example in estimating distribution of drugs within body. Hydrophobic drugs with high octanol/water partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs (low octanol/water partition coefficient) preferentially are found in hydrophilic compartments such as blood serum.