Column Chromatography

Lab 8

- Column chromatography is described as the useful technique in which the substances to be isolated are presented onto the highest point of a column loaded with an adsorbent (stationary phase), go through the column at various rates that rely upon the affinity of every substance for the adsorbent and the solvent or solvent mixture, and are typically gathered in solution as they pass from the column at various time.

- The two most common examples of stationary phases for column chromatography are silica gel and alumina while organic solvents are regarded as the most common mobile phases.



- The main principle involved in column chromatography is the adsorption of the solutes of the solution with the help of a stationary phase and afterward separates the mixture into independent components.

- At the point when the mobile phase together with the mixture that requires to be isolated is brought in from the top of the column, the movement of the individual components of the mixture is at various rates.

- The components with lower adsorption and affinity to the stationary phase head out quicker when contrasted with the greater adsorption and affinity with the stationary phase. The components that move rapidly are taken out first through the components that move slowly are eluted out last.

- The adsorption of solute molecules to the column happens reversibly.

- A chromatography column is a glass or plastic tube that you set vertically and fill with a stationary phase. The bottom end of the column is packed with a glass wool/cotton wool or an asbestos pad ,after which the stationary phase is packed.

- The steps included in the column chromatography are:

1. Preparation of the column:

Column packing in chromatography is a critical step in your separation procedure. A poorly packed column can lead to uneven flow and band broadening, both of which give rise to poor separation.

- There are two common ways you can fill a chromatography column:

a. Dry Packing

The amount of absorbent needed is added as a fine dry powder in the column and the solvent flows freely through the column until equilibrium is achieved.

b. Wet Packing

Wet packing technique – The slurry of adsorbent is prepared along with the mobile phase and is poured into the column.

It is regarded as the ideal technique for packaging because it’s usually faster and seems to use up less liquid.

2. Introduction of the sample

The sample (a mixture of components) is dissolved in the minimum amount of the mobile phase.

At one instant, the sample is introduced into the column and on the top portion of the column, it is absorbed.

Through the elution process, the individual sample can be isolated from this zone.

3. Elution technique

Through this technique, the individual components are separated completely from the column.

- The process of elution can be carried out by employing two techniques:

a. Isocratic elution technique – Throughout the procedure, a solvent of the same polarity or same solvent composition is utilized.
Example: Use of chloroform alone

b. Gradient elution technique – Throughout the separation procedure, solvents of gradually increased polarity or increased elution strength are utilized.
Example: Benzene → Chloroform → Ethyl acetate → Chloroform

➢ Detection of Components

In case the mixture separated in a column chromatography procedure are colored compounds, then monitoring the separation progress is simple.

In case the compounds undergoing separation are colorless, then small fractions of the eluent are sequentially collected in tubes that are labeled. Through TLC, the composition of each fraction is determined.

➢ Column chromatography uses

Column chromatography is one of the versatile methods for purifying and separating both solids and liquids. Major applications:

To isolate active constituents

To separate compound mixtures

To remove impurities or carry purification process

To isolate metabolites from biological fluids

To estimate drugs in drug formulations or crude extracts

Types of Column chromatography

- Adsorption column chromatography – Technique of separation in which compounds to be separated (solute) is retained or adsorbed on the surface of the adsorbent (stationary phase).
- Partition column chromatography – It is based on the variance in partition coefficient of the individual components of the mixture, where the stationary phase and the mobile phase both are in the liquid state.
- Gel column chromatography – Here, the separation is carried out through a column packed with gel and possesses a porous stationary phase. It is also referred to as size exclusion chromatography.
- Ion exchange column chromatography – The basis relies on the charge of the molecules. The separation is done when molecules get attracted to the oppositely charged stationary phase.

- Affinity chromatography is a method for purification of a specific molecule or a group of molecules from complex mixtures , based on highly specific biological interactions between two molecules such as interactions between enzyme and substrate, receptor and ligand, or antibody and antigen.

- Gas Chromatography (GC).

- High-Performance Liquid Chromatography (HPLC).