A Review on Column Chromatographic Techniques as Separation Method

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Abstract-

A technique frequently used to extract compounds f rom complicated mixtures is column chromatograp hy. Column chromatography, as opposed to planar c hromatography, packs the stationary phase or poly mer (resins) into a column. The packed stationary p hase then is passed through the mobile phase toachi eve separation. This division may serve analytical or preparatory purposes.

Chromatography, which relies on the interaction of a stationary and a mobile phase, is a crucial method for purifying mixture components.

Indexed Terms- Column chromatography, Mobile phase, Stationary phase, Types of column chromatography, etc.

I. INTRODUCTION

Each of these distinctive components can be isolated using chromatographic techniques since proteins diff er in their size, structure, net charge, solid phase utilis ed, and binding ability. The most common applicatio n of these techniques is column chromatography.

Using this method, biomolecules can be made pure. The material to be separated is put to a column first (stationary phase), followed by wash buffer (mobile phase). Their flow via the inside column material, which is supported by fibreglass, is ensured.

The samples are gathered at the device's base in a time- and volume-dependent way.[1]

II. PRINCIPLE

The movement of the various parts of the mixture move at various speeds when the mobile phase and the mixture that needs to be separated are introduced from the top of the column. Compared to components with greater adsorption and affinity to the stationary phase, those with lower adsorption and affinity move more quickly. The elements that move quickly are eliminated first, while the elements that move slowly are eliminated last.

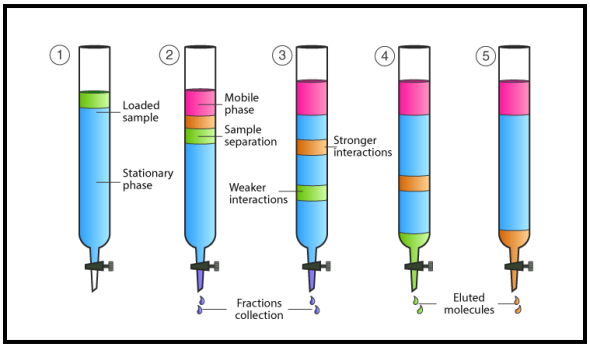

Figure 1: Column Chromatography

The reversible adsorption of solute molecules to the column takes place. The components' movement rate is stated as follows:[2]

The stationary phase and mobile phase in column chromatography are both solids. The silica and appropriate solvent are combined to prepare the column, which is then poured into the glass column. There are typically two ways to pack things: dry and damp. In the dry technique, dry powdered silica is initially added to the column. In the dry method, slurry of silica and solvent is first made and then put over the column using a funnels until the silica is settled into it. Then the mobile phase, an appropriate

solvent is pushed through it until all the silica are wet and settled.[3]

$$Rf = \frac{Distance\ travelled\ by\ the\ solute}{Distance\ travelled\ by\ the\ solvent}$$

Where,

R_f = The retardation factor.

III. COLUMN CHROMATOGRAPHY COMPONENTS

A typical chromatographic system with a gas or liquid mobile phase typically consists of the following elements:

- Stationary Phase: In general, it is a solid substance with strong adsorption abilities that should be suited for the separation of the analytes. It shouldn't obstruct the movement of the mobile phase in any way.
- Mobile Phase: Solvents that support the stationary phase make up this phase. In addition to serving as a solvent, the mobile phase also develops the sample by encouraging the separation of its constituent parts into bands and elutes the sample (to remove the components from the column that are separated during the experiment).
- Column:
- For liquid chromatography: stainless steel fabrication with internal diameter of 4mm and lengths of 2–50 cm
- For gas chromatography: 1-3m long and 2-4mm thick internally, made of glass or stainless steel.
- The stationary phase must be supported by the column's material and dimensions in order to facilitate successful separations.
- Injector System: Accountable for consistently sending test samples to the columns top.
- Detector or Chart recorder: As the analytes exit the column, this provides a continuous record of their presence in the eluate.The measurement of a physical parameter is necessary for detection (like visible or UV adsorption). Each separated analyte is represented by a peak on the chart recorder.The bottom of the column that is set up to collect the separated analytes has a collector at the bottom.

IV. COLUMN CHROMATOGRAPHY PROCESS

The following procedures are a part of column chromatography:

4.1. Setting up the column:

- The column is primarily made of a glass tube with the proper stationary phase.
- Glass wool/cotton wool or an asbestos pad is put into the bottom end of the column before the stationary phase is added.
- After packing the column, a paper disc is placed on top to prevent the stationary phase from being disturbed when the sample or mobile phase is introduced.

The uneven bands of separation are caused by the stationary phase's (the adsorbent layer) disturbance.

There are two distinct packing procedures for the column, namely:

a. Dry packing method: The required amount of absorbent is added to the column as a fine, dry powder, and the solvent is allowed to freely flow through the column until equilibrium is reached.
b. Wet packing method: The mobile phase and prepared adsorbent slurry are put into the column.

It is thought to be the best method for packaging.

Before usage, the column must be thoroughly cleaned and dried.

4.2. Introduction of the sample:

- The sample, which is a combination of components, is dissolved in the least amount of mobile phase possible.
- The sample is put into the column at once, where it is absorbed on the top of the column.
- The individual sample can be separated from this zone by the elution procedure.

4.3. The elution method:

This method totally separates the constituent components from the column.Two methods can be used to carry out the elution process:

a. Isocratic elution technique: A solvent with the same polarity or solvent composition is used throughout the entire process.

For example: Solely using chloroform

b. Gradient elution technique: Eluents with gradually increasing polarity or elution intensity are used throughout the separation process.

For example: Benzene → Chloroform → Ethyl acetate → Chloroform

4.4 Identifying Components:
Monitoring the separation process is easy if the mixture being separated in a column chromatography operation is one that involves coloured chemicals.Small fractions of the eluent are sequentially collected in tubes that are labelled if the substances being separated are colourless. The makeup of each fraction is identified via TLC.[4]

V. TYPES OF COLUMN CHROMATOGRAPHY

5.1. Adsorption column chromatography:
It is a sort of liquid chromatography that keeps the mixture's chemicals that have adsorbed on the stationary phase's surface.Van Der Waals forces and steric interactions are commonly used to attach the chemical to the solid support. Additionally, solid-liquid chromatography is another name for adsorption chromatography. Russian botanist Mikhail Tsvet developed this chromatography for the first time in 1901. Figure 1.2 shows an example of how molecules could adhere to the surface of a solid support.

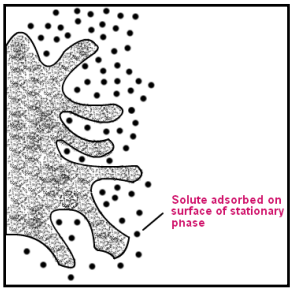

Figure 2: Principle of Adsorption Chromatography

5.2. Partition column chromatography:
In this method, both the stationary phase and the mobile phase are liquids.

5.3. Gel column chromatography:
With this type of chromatography, the separation is accomplished using a gel-filled column. A solvent is used to hold the stationary phase in the solvent's space.

5.4. Ion exchange column chromatography:
It is a type of chromatography in which ion exchange resin is always used as the stationary phase.

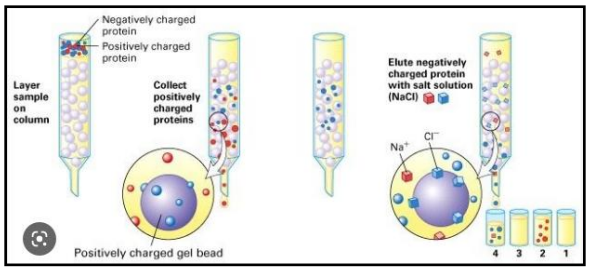

Figure 3: Ion Exchanging Chromatography

5.5. Affinity Chromatography:
In this kind of column chromatography, biomolecules separate from a mixture based on how they interact with the resin in particular ways. It uses the strong specificity and interaction of two biological molecules as the basis for its theory. When the molecule of interest (known as the ligand) interacts with its target bound to the surface of the stationary phase, the desired product is purified and separated from the mixture. Then, using a solvent that competes with it for binding sites on the stationary phase, it is eluted.

The removal of a protein from a mixture is the most typical application of affinity chromatography. It is typically processed using a resin or column that has antibodies bound to that protein. As a result, only the protein of interest attaches with its antibodies when the protein solution goes through the column, and the rest washes away. Affinity chromatography, however, also has certain drawbacks.

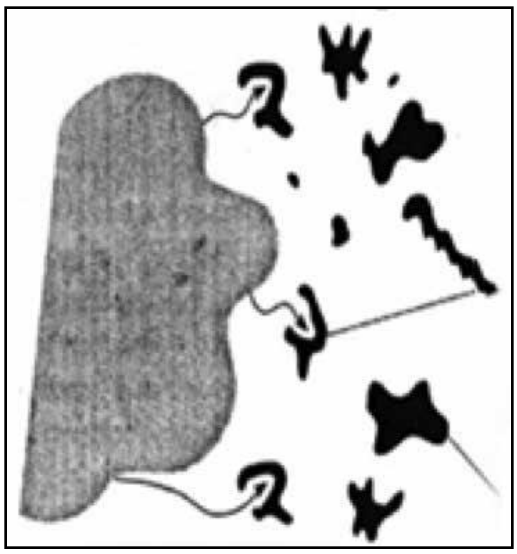

Figure 4: Affinity Chromatography

5.6. Gas Chromatography:

It is a different sort of chromatography that aids in the identification and examination of substances in vapour form. The examination sample is contained in an inert carrier gas that makes up the mobile phase. The stationary phase is a tiny layer of a liquid or polymer that is adhered to a column's interior. Experts refer to it as gas-liquid partition chromatography or vapor-phase chromatography. [5]

1.5 APPLICATION OF COLUMN CHROMATOGRAPHY:

One of the adaptable techniques for cleaning and separating both solids and liquids is column chromatography.

- To separate active ingredients
- To divide chemical mixtures
- To carry out a purifying process to remove contaminants
- To separate metabolites from bodily fluids
- To calculate medication concentrations in raw extracts or drug formulations.
- Further use of column chromatography is in purifying proteins based on different features including size, shape, and net charge. Therefore the technique uses the chemical, biological and physical properties of the protein for its purification. [6]
- Separation of mixture of compounds: Column chromatography can be used for the separation of several classes of drug and constituents like alkaloids glycosides amino acid etc.
- Removal of impurities or purification process: impurities present in a compound can be removed by using appropriate stationary and mobile phase.
- Isolation of active constituents: from plant extract, from formulation or other crude extracts, active constituents.
- Isolation of metabolite from biological fluid: eg.17-ketosteroids from urine cortisol ,other drug etc. from biological fluids like blood, plasma or serum , etc.
- Estimation of drugs in formulation or crude extracts
- Determination of %w/w of stychine in syrup of ferrous phosphate with quinine and strychnine.
- Determination of primary and secondary glycoside in digitalis leaf.
- Separation of diastereomers.
- Separation of inorganic ions like copper, cobalt, nickel ,etc.

CONCLUSION

Initially chromatographic techniques were used toseparate substances based on their color as was the case with herbal pigments. With time its application area was extended considerably. Nowadays, chromatography is accepted as an extremely sensitive and effective separation method. Column chromatography is one of the useful separation, and determination methods. Column chromatographyis a protein purification method realized especiallybased on one of the characteristic featuresof proteins. Besides, these methods are used tocontrol purity of a protein

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