

Column Chromatography

In chemistry, Column chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid. It separates substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated in fractions. This technique can be used on small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of [adsorption chromatography](#) technique.

Column Chromatography Principle

When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slow are eluted out last.

The adsorption of solute molecules to the column occurs in a reversible manner. The rate of the movement of the components is expressed as:

$R_f = \frac{\text{the distance travelled by solute}}{\text{the distance travelled by solvent}}$

R_f is the retardation factor.

Column Chromatography

Column Chromatography is another common and useful separation technique in organic chemistry. This separation method involves the same principles as TLC, but can be applied to separate larger quantities than TLC. Column chromatography can be used on both a large and small scale. The applications of this technique are wide reaching and cross many disciplines including biology, biochemistry, microbiology and medicine. Many common antibiotics are purified by column chromatography. To understand to uses of this separation technique, we can use the last experiment as an example. In the TLC experiment, we separated and analyzed the different components that makeup over-the-counter painkillers. The technique of TLC was useful in determining the type and number of ingredients in the mixture, but it was not helpful for collecting the separated components. We could only separate and visualize the spots. If we needed to collect the separated materials, column chromatography could be used. We could load 100 mg of a crushed Anacin tablet on a column made up of a silica stationary phase and separate the aspirin from the caffeine and collect each of these compounds in separate beakers. Column chromatography allows us to separate and collect the compounds individually. In this experiment, Column Chromatography (abbreviated CC) will be used to separate the starting material from the product in the oxidation of fluorene to flourenone and TLC will be used to monitor the effectiveness of this separation.

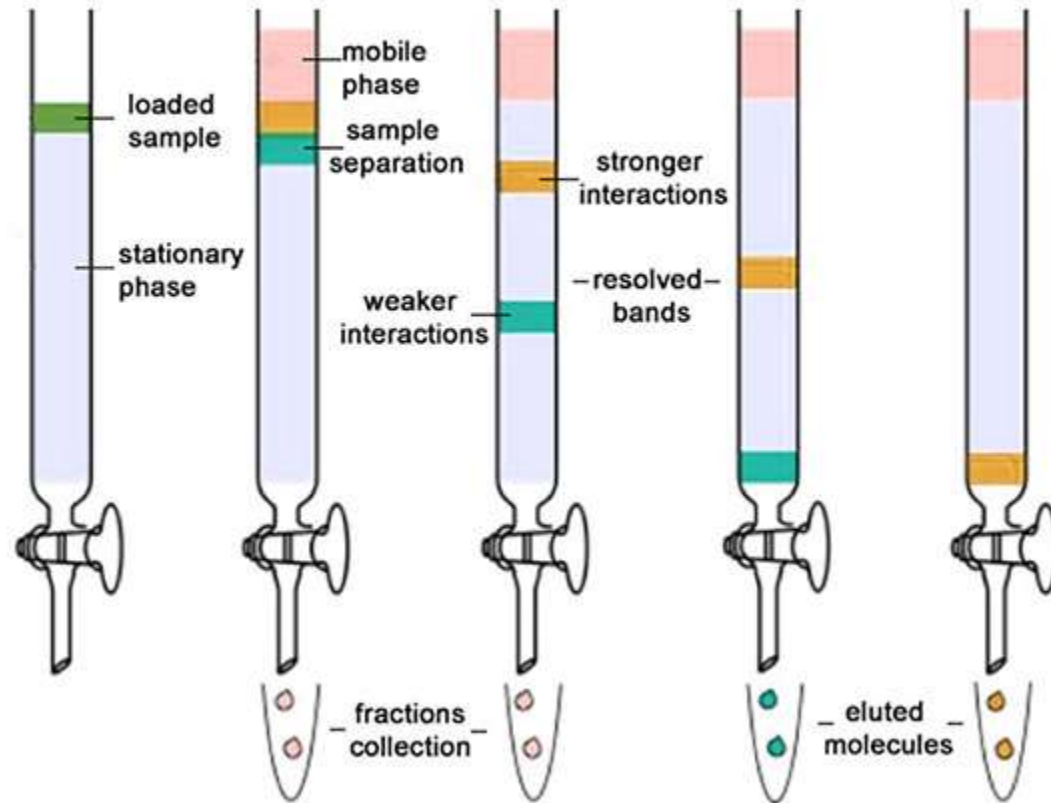
Choosing a Stationary Phase

As with TLC, alumina and silica are the two most popular stationary phases in column • chromatography. For these common phases, the partitioning works in an analogous manner. The more polar sample will be retained on the stationary phase longer. Thus the least polar compound will elute from the column first, followed by each compound in order of increasing polarity. PreLab Exercise Do not forget these additional sections in your PreLab. Alumina and silica 106 Although the interactions between the mobile and stationary phase are based on the same principles for CC and TLC, be careful when predicting the order of elution. Since the direction of the solvent flow in TLC moves up and in CC the solvent flows down, it appears that the order is “upside-down”. In TLC the more polar molecules will have lower R_f values, but in CC they will be retained longer on the column. Remember this when considering the polarities of the stationary phase as well as the polarity of the compounds being separated when predicting the order of elution. Stationary phases for CC can come in a variety of sizes, activities, acidic and basic variations for both alumina and silica. The types of stationary phase chosen are determined experimentally, or often based on results from a previous TLC experiment. The type of adsorbent, the size of the column, the polarity of the mobile phase as well as the rate of elution all affect the separation. These conditions can be manipulated to get the best separation for your mixture.

Choosing Solvents Solvent

systems for use as mobile phases in CC can be determined from previous •
TLC experiments, the literature, or experimentally. Normally, a separation will begin by using nonpolar or low polarity solvent, allowing the compounds to adsorb to the stationary phase, then SLOWLY switching the polarity of the solvent to desorb the compounds and allow them to travel with the mobile phase. The polarity of the solvents should be changed gradually. On a macroscale, the mixing of two solvents can create heat and crack the column leading to a poor separation. Some typical solvent combinations are ligroin-dichloromethane, hexane-ethyl acetate and hexane-toluene. Often an experimentally determined ratio of these solvents can sufficiently separate most compounds. Solvents such as methanol and water are normally not used because they can destroy the integrity of the stationary phase by dissolving some of the silica gel.

Column Chromatography



Type of column chromatography

There are two forms of column chromatography. •

Liquid chromatography (LC) •

Gas chromatography (GC) •

The most widely used forms of column chromatography are: •

Adsorption chromatography •

Partition chromatography •

Ion exchange chromatography •

Gel chromatography •

Types of Column Chromatography

1. Adsorption column chromatography – Adsorption chromatography is a technique of separation, in which the components of the mixture are adsorbed on the surface of the adsorbent. •
2. Partition column chromatography – The stationary phase, as well as mobile phase, are liquid in [partition chromatography](#). •
3. Gel column chromatography – In this method of chromatography, the separation takes place through a column packed with gel. The stationary phase is a solvent held in the gap of a solvent. •
4. Ion exchange column chromatography – A chromatography technique in which the stationary phase is always ion exchange resin. •

Preparation of the Column

The column mostly consists of a glass tube packed with a suitable stationary phase. •

A glass wool/cotton wool or an asbestos pad is placed at the bottom of the column before packing the stationary phase. •

After packing, a paper disc kept on the top, so that the stationary layer is not disturbed during the introduction of sample or mobile phase. •

Types of preparing the column

There are two types of preparing the column, they are: •

1. Dry packing / dry filling •

In this the required quantity of adsorbent is poured as fine dry powder in • the column and the solvent is allowed to flow through the column till equilibrium is reached.

2. Wet packing / wet filling •

In this, the slurry of adsorbent with the mobile phase is prepared and is • poured into the column. It is considered as the ideal technique for packing.

B. Introduction of the Sample •

The sample which is usually a mixture of components is dissolved in minimum • quantity of the mobile phase.

The entire sample is introduced into the column at once and get adsorbed on • the top portion of the column.

From this zone, individual sample can be separated by a process of elution. •

C. Elution •

By elution technique, the individual components are separated out from the • column.

It can be achieved by two techniques: •

Isocratic elution technique: Same solvent composition or solvent of same • polarity is used throughout the process of separation.

Eg. Use of chloroform alone. •

Gradient elution technique: Solvents of gradually \uparrow polarity or \uparrow elution strength are used during the process of separation. •

E.g. initially benzene, then chloroform, then ethyl acetate then chloroform •

D. Detection of Components •

If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. •

If the compounds to be isolated from column chromatography are colorless. •

In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analyzed by TLC. •

Applications

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids. Its major application includes: •

Column Chromatography is used to isolate active ingredients. •

It is very helpful in Separating compound mixtures. •

It is used to determine drug estimation from drug formulations •

It is used to remove impurities. •

Used to isolation metabolites from biological fluids. •

Advantages

- Any type of mixture can be separated by column chromatography.
- Any quantity of the mixture can also be separated.
- Wider choice of mobile phase.
- In preparative type, the sample can be separated and reused.
- Automation is possible.