Column Chromatography

13

Abstract

Column chromatography is a common chromatographic technique. It is a type of adsorption chromatography that is widely used for the separation of individual components of interest present in mixture. This technique can be used on small as well as on large scale for the isolation and purification of components of interest. This chapter briefly describes the basic principle involves in it, its, working, factors that may affect on its working. Moreover, advantages, disadvantages, and applications of column chromatography have also been discussed in this chapter.

Keywords

Principle of column chromatography · Working of column chromatography · Applications of column chromatography

13.1 Introduction

An American chemist D.T Day was the first scientist who introduced this technique in chemical analysis in 1900 while, in 1906, Polish botanist M.S. Tswett investigated some plant pigments using adsorption columns. A schematic representation of this technique has been illustrated in Fig. 13.1. This chromatographic technique is widely used for both the separation and purification of solid and liquid components of interest present in mixture. Column chromatography is actually a solid–liquid technique, where solid is the stationary phase and liquid is the mobile phase.

13.2 Principle

A mixture or compound that needs to be separated is dissolved first in mobile phase which is then introduced from the top of the column. Components present in the mixture then move at different rates depending upon their relative affinities towards

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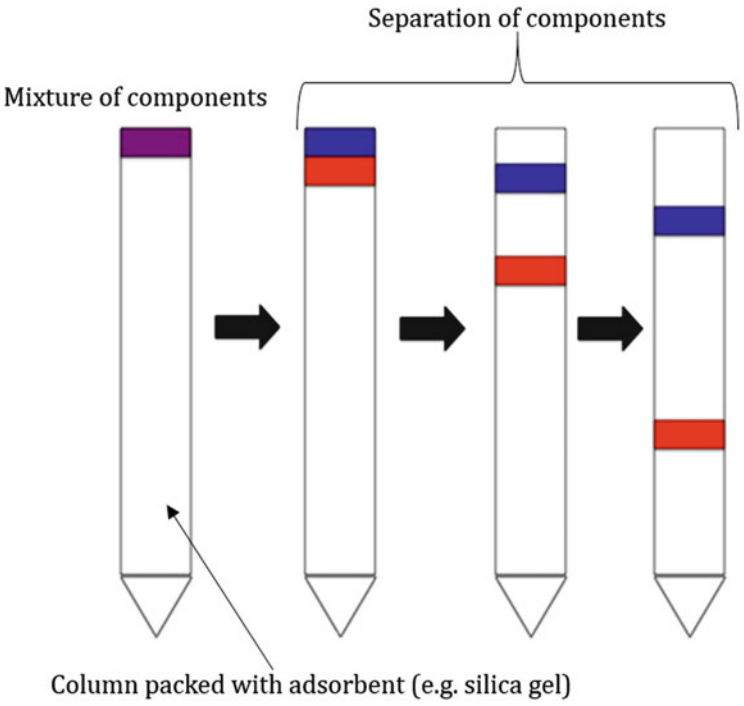

Fig. 13.1 Schematic representation of column chromatography

the stationary phase. The components having lower adsorption rate and less affinity with stationary phase will move faster as compared to those components having more adsorption and more affinity with stationary phase. The components moving faster are eluted out first, whereas those moving slowly are removed last. From the distance travelled by solute, a retardation factor is calculated:

$$R_f = \text{Distance travelled by solute}/\text{total distance travelled by the solvent.}$$

13.3 Types of Column Chromatography

Following are the most common types of column chromatography. These techniques have been briefly described in Chap. 11.

1. Absorption chromatography
2. Partition chromatography
3. Gel chromatography
4. Ion exchange chromatography

13.4 Components of Column Chromatography

Following are the main components of column chromatography:

13.4.1 Stationary Phase

Stationary phase is solid in column chromatography which should have good adsorption property. Stationary phase should be selected properly to achieve the success of column chromatography. It depends on the following conditions:

1. Removal of impurities present in the compound.
2. Number of components to be separated.
3. Affinity differences between the components.
4. Length of the column used.
5. Quantity of adsorbent used.

A stationary phase should have the following properties:

1. Should have uniform shape and size of particles
2. Mechanically stable
3. Chemically inert
4. Allow free flow of the solvent
5. Should be colorless
6. Inexpensive
7. Free availability

13.4.1.1 Adsorbents

Silica, calcium phosphate, calcium carbonate, starch, and magnesia are the most commonly used adsorbents in column chromatography. For less polar compounds' alumina is preferred. Silica gel also gives good results for compounds having polar functional groups. Adsorbents should have the following properties:

1. Particles should be uniform in size and have spherical shapes.
2. High mechanical stability.
3. Chemically inert.
4. Useful for the separation of many compounds.
5. Inexpensive and freely available.

13.4.1.2 Mobile Phase

Mobile phase is liquid in case of column chromatography which dissolves the mixture and transfer it to column. It acts as a,

1. Solvent: to introduce the sample mixture into the column.
2. Developing agent: to separate the components of interest present in mixture in the form of bands.
3. Eluting agent to remove the separated components out of the column.

The solvent is chosen on bases of the solubility properties of the mixture. Low boiling point and polarity of the solvents are the important factors in the selection of a solvent in column chromatography. The mostly used solvents are carbon tetrachloride, petroleum ether, ether, esters, cyclohexane, acetone, toluene, benzene, and water.

13.4.2 Column

It is used to hold the adsorbent or stationary phase. It is made up of neutral glass which should be of good quality so that it cannot affect the solvent. Usually, a burette is used as a column having length and diameter ratio of 10:1, 30:1, or 100:1. Selection of the column's dimensions depends upon the number of components in the sample, type of stationary phase, sample quantity under analysis, and components affinity towards the stationary phase. A narrow column is preferred over the short and thick column to achieve better separation.

13.4.2.1 Preparation of Column

Column is first washed properly with purified water and then with acetone. Then, it is dried properly to remove the impurities. Then column is hanged along a stand with the help of clamp in such a way that its outlet facing should be downward. Column should be packed from the bottom with a cotton wool, glass wool, filter paper, or asbestos pad so that adsorbent will not fall out. After packing, a paper disc is placed on the top of the column so that the adsorbent layer is not disturbed when the sample or mobile phase is introduced.

13.4.2.2 Packing of Column

Two techniques are used for the packing of column. One is dry packing or dry filling and second one is wet packing or wet filling.

Dry Packing of Column

Column is firstly filled with the adsorbent in dry form in this method and then the solvent is flushed through the column until equilibrium is reached. Air bubbles may be entrapped between the mobile phase and stationary phase. Cracks or void space may appear in the adsorbent layer. To address these problems tapping is done while packing of the column. A schematic representation of dry packing of column has been represented in Fig. 13.2.

Fig. 13.2 Schematic representation of dry packing of column

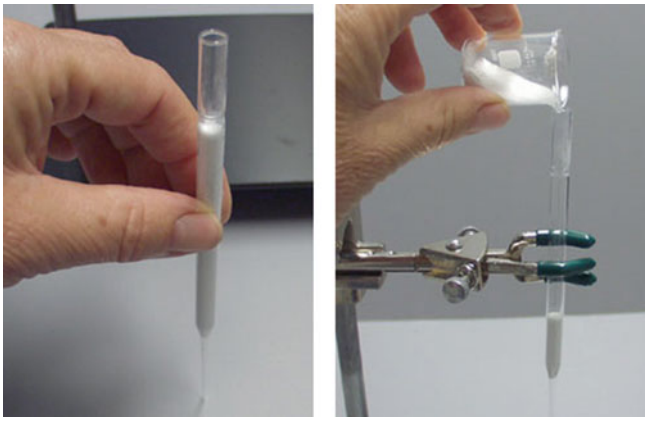

Fig. 13.3 Schematic representation of wet packing of column

Wet Packing of Column

Formations of air bubbles and cracks are the drawbacks in dry packing. Therefore, wet packing is preferred in which a slurry made up of adsorbent and solvent is generally added to the column in portions. Stationary phase (adsorbent) settles uniformly and no cracks are formed in the column. The solid settle down while the solvent remains upward. The solvent is then removed and again a cotton plug is placed in the bottom of column. A schematic representation of dry packing of column has been represented in Fig. 13.3.

13.5 Working of Column Chromatography

Column is firstly packed either by dry packing or wet packing. Then the sample is dissolved in minimum quantity of mobile phase and is introduced into the column at once. Then mobile phase is flushed through the column until 1/3 length of column is filled with solvent. By the process of elution, the components of interest are separated out from the column. A schematic representation of working of column

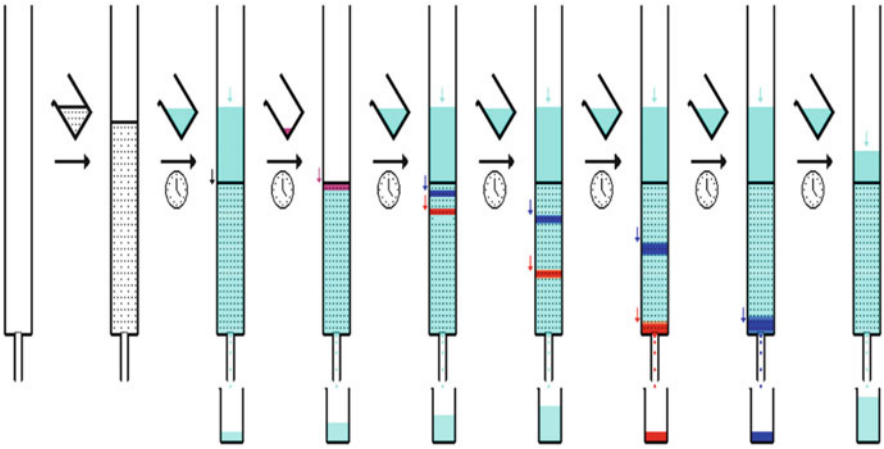

Fig. 13.4 Schematic representation of working of column chromatography

chromatography has been described in Fig. 13.4. There are two techniques which are involved in the separation process.

13.5.1 Isocratic Elution Technique

Solvents having the same composition and same polarity are used in this technique throughout the process of separation. One of the most commonly used solvents for isocratic elution technique is chloroform.

13.5.2 Gradient Elution Technique

Solvents having gradually high polarity or high strength of elution are used in this technique during the process of separation. For example, initially benzene, then chloroform, then ethyl acetate then chloroform.

13.6 Detection of Components

Detection of components of interest can be done visually if colored bands are appeared as separated components. But if the components of interest to be separated appear as colorless bands then small fractions of the eluent are collected in labeled tubes and then TLC is performed on each section separately to detect the composition of each fraction.

13.7 Factors Affecting on Column Chromatography

1. Column's dimension, length of the column should be more than its width. Normally 10:1, 30:1, or 100:1 ratios of length and diameter are used.
2. Particle size of the adsorbent should be small.
3. Proper activation of the adsorbent is needed.
4. Column's temperature should be managed properly as high temperature can enhance the process of elusion.
5. Column should be properly packed with the adsorbent and bottom should be also filled with cotton wool or anything else used for this purpose.
6. Less viscous solvents give better results.

13.8 Advantages

1. It is simple and easy technique.
2. Mixture of any type or quantity can be easily separated using this technique.
3. Many types of solvent/mobile phase can be used in this technique.
4. Automation is possible when using this technique.
5. It is an inexpensive technique.
6. This technique can be used both on small and on large scale.

13.9 Disadvantages

1. It is a time consuming process, especially when components show colorless bands.
2. In this technique, a large amount of the solvent is required for the proper elusion.
3. It is a simple technique but if automated then it becomes more complex and hence more expensive.

13.10 Applications

1. It is used to separate a mixture of compounds into its components of interest.
2. It is used for purification process.
3. The active constituents of many drugs can be isolated by using this technique.
4. It is used to determine the drug estimation in drug formulations.
5. It is also used to isolate many metabolites from the biological fluids like blood or serum.
6. Primary and secondary glycosides in digitalis leaf can be isolated by using this technique.

7. This technique is also used for the separation of diastereomers.
8. This technique is best employed for the separation of active principles of plant materials like alkaloids, glycosides, resins, tannins, and flavonoids.
9. Multistage column chromatography can be used to study the nucleotide sequences in RNA.

Further Reading

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