COLUMN CHROMATOGRAPHY

INTRODUCTION

Early chromatography columns wear glass tubes with diameters of of perhaps 40 to 50 mm that held 50 to 500 cm columns of solid particles of the stationary phase. To assure reasonable flow rate the particles of the solid were kept larger than 150 to 200 µm in diameter ordinally the head of of liquid above the packing suffered to force the mobile phase down the column flow rate were at best few tenths ml per minute separation tended to be time consuming. It was developed by the American petroleum chemist D.T .Day in 1900 M.S. Tswett the polish botanist in 1906 used adsorption columns in his investigation of plant pigments it was not until about 1930 that the method was used extensively by chemist column chromatography is also known as a adsorption chromatography

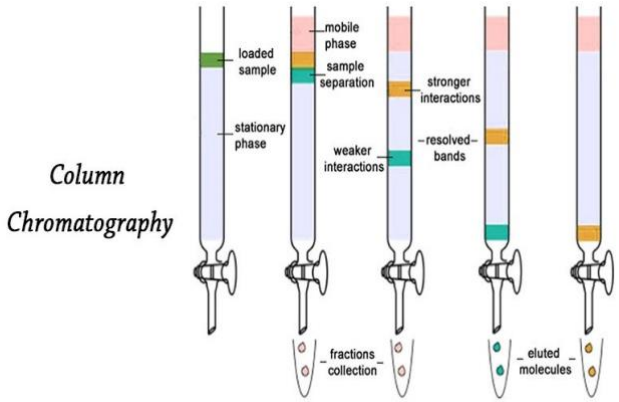

Fig no. 1 :- column chromatography.

❖ PRINCIPLE

1)It is known that the rate of adsorption varies with the given adsorbent for different material this principle of selective absorption is used in column chromatography - A solid stationary phase and a liquid mobile phase is used and the principle of separation is adsorption

2) The mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing the absorbent

3) The components which have greater absorbing power is in the upper part of column

4)The next component is absorb in lower portion of column which have less adsorbing power than the first component

The process is continued as a result the materials are partially separated and absorbed in the various part of the column .the type of interaction between the stationary phase and solute is reversible in nature

❖ INSTUMENTATION OF COLUMN CHROMATOGHRAPHY

A typical column chromatographic system using a gas or liquid mobile phase consists of the following components:

A stationary phase:

• Chosen to be appropriate for the analytes to be separated.

A column:

• In liquid chromatography these are generally 25- 50 cm long and 4mm internal diameter and made of stainless steel whereas in gas chromatography they are 1-3m long and 2- 4mm internal diameter and made of either glass or stainless steel.

They may be either of the conventional type filled with the stationary phase, or of the microbore type in which the stationary phase is coated directly on the inside wall of the column.
A mobile phase and delivery system:
• Chosen to complement the stationary phase and hence to discriminate between the sample analytes and to deliver a constant rate of flow into the column.

An injector system:
• To deliver test samples to the top of the column in a reproducible manner.

A detector and chart recorder:
• To give a continuous record of the presence of the analytes in the eluate as it emerges from the column.
• Detection is usually based on the measurement of a physical parameter such as visible or ultraviolet absorption or fluorescence.
• A peak on the chart recorder represents each separated analyte.

A fraction collector: For collecting the separated analytes for further biochemical studies .
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STEPS IN COLUNM CHROMATOGHRAPHY

A. Column preparation

• Column is prepared by packing solid absorbent into a cylindrical gas or plastic tube the size will depends on the amount of compound being isolated the base of the tube contains a filter either a cotton are glass Plug for glass frit to hold solid-phase in place a solvent reserve maybe attach attached at the top of the column

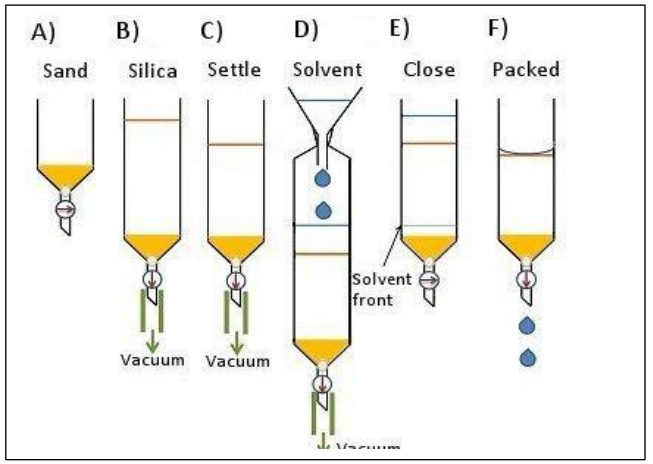


Fig no 2 :- Instrumentation

b. Introduction of sample

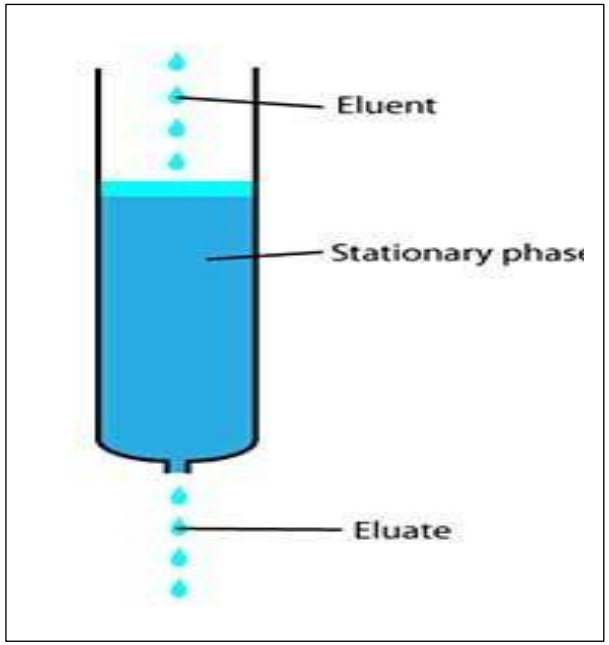
□□The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase.

□□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 ↑ polarity or ↑ 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.

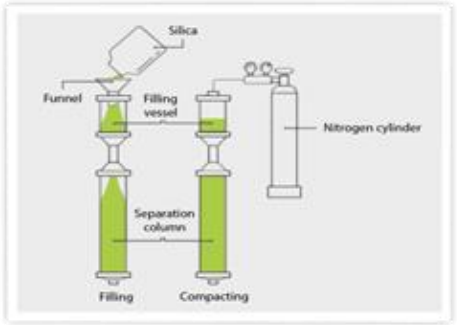


❖ **Two methods are generally used to prepare a column**

1. Dry method
2. Wet method

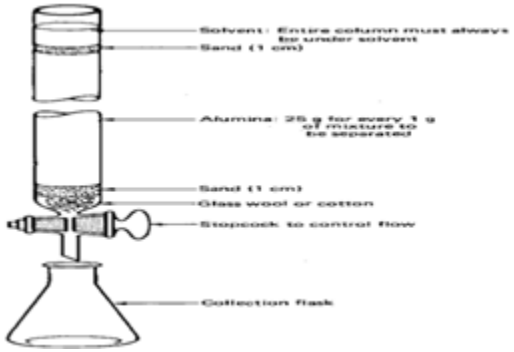
1).Dry method

For the dry method the column is first filled with dry stationary phase powder.
followed to the addition of mobile phase which is flushed through the column unit it is completely wet and from this point is never allowed to run dry



2) wet method

For a wet method of slurry is prepare of the eluent with the stationary face powder and then carefully poured into the column.
the top of the silica should be flat and top of the silica can be protected by a layer of sand
Eluent is slowly passed through the column To advance the organic material



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.

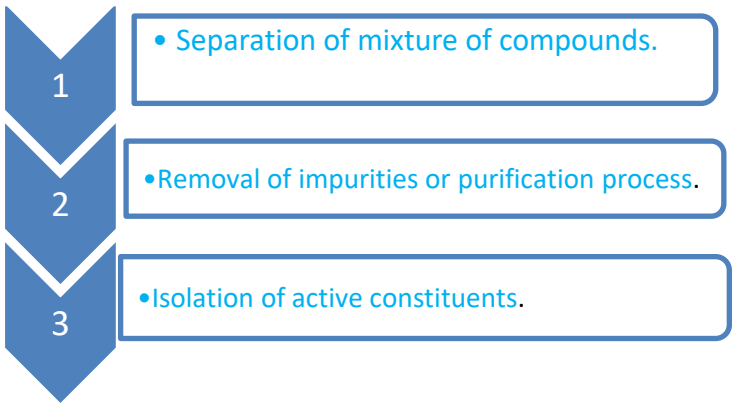
In preparative type, the sample can be separated and reused.

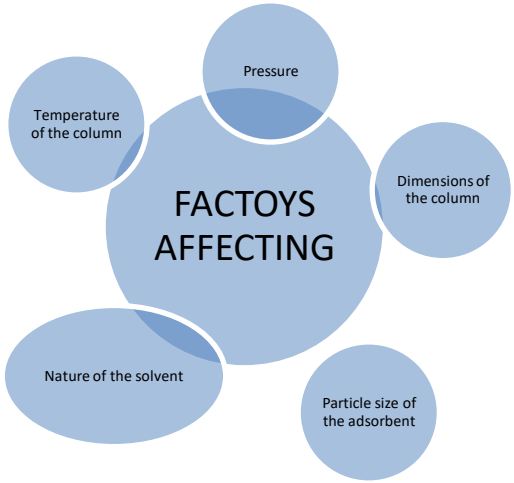
Automation is possible.

Disadvantages.

- Time consuming method.
- More amounts of solvents are required which may be expensive.
- Automation makes the technique more complicated and costly.

Application





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Prepared by :- Miss . Siddhi Kisan Shete

Mentor :- Mrs.Asmita V.Gaikwad

Department :- Pharmaceutical chemistry

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