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Column Chromatography

❖ Name Of 1ST Author : VIVEK TUKARAM SHINDE
❖ Name Of 2ND Author : R.S. GARAD
❖ Name Of 3RD Author : DR. SANTOSH JAIN
❖ Name Of 4TH Author : KAMBLE MANOJ
❖ Name Of 5TH Author : BANSODE BADAL

❖ **Designation of 1st Author : Student**
❖ **Designation of 2nd Author : Guide**
❖ **Designation of 3rd Author : Principle**
❖ **Designation of 4th Author : Student**
❖ **Designation of 5th Author : Student**

1. ABSTRACT

Column chromatography is used extensively in nucleic acid chemistry to purify or characterize products from chemical reactions. This appendix focuses on separations using silica gel or alumina. The procedures described include loading the column, assembling the apparatus, separating compounds and collecting fractions, and optimizing chromatography conditions.

Column chromatography is simple and the most popular separation and purification technique. Both solid and liquid samples can be separated and purified by column chromatography. Column chromatography consists of a stationary solid phase that adsorbs and separates the compounds passing through it with the help of a liquid mobile phase. On the basisof their chemical nature, compounds get adsorbed and elution is based on differential adsorption of a substance by the adsorbent. Various stationary phases, such as silica, alumina, calcium phosphate, calcium carbonate, starch, and magnesia, and different solvent compositions based on the nature of compounds to be separated and isolated, are used in column chromatography. Optimization of the method is an important task in the separation of different groups of compounds in extracts. In column chromatography, a cylindrical glass tube, which is plugged at the bottom by a piece of glass wool or porous disc, is filled with slurry (adsorbent) and a suitable solvent. Samples to be separated are mixed with silica and introduced at the top of the column and allowed to move with the solvent. With polarity differences, compounds are adsorbed at different regions and desorbed with suitable solvent polarity. The compound of higher adsorption ability will be adsorbed at the top and that withthe lower one will be at the bottom. By adding the solvent at the top, compounds get desorbed and pass through the column and this process is called elution.

2. INTRODUCTION OF CHROMATOGRAPHY

Chromatography is physical method of separation of the mixture into its individual Components. It is used as analytical technique to get information about what is present in the mixture, how much the individual compound is in mixture.

It is also used as a purification method to separate and collect the components of mixture.

Chromatography is Greek word where chromates mean colour and graphy means writing. So, basically chromatography is colour writing process.

The main advantage that differentiates chromatography from most other chemical and physical separation methods is that, two mutually immiscible phases brought into contact oneis stationary phase and other mobile.

Equilibrium of solute or components of mixture between stationary phase and mobile phaseis repeatedly achieved

Repeated interaction of species of sample in both phases may lead to gradual separation of sample into bands in the stationary phase.

The separation of components of mixture is achieved on the simple fact that different component of mixture having different affinity towards mobile phase and stationary phase The least affianced component emerges first; the most strongly affinated compound (retained)elutes last.

The concept of chromatography is first time put forwarded in 1906 by Great Russian botanist,Michael Tsweet, who has separated different plant pigments on solid support of calcium carbonate in a long tube. He observed that when methanolic extract of plant leaves are poured through the tube, various colour pigments are separated on calcium carbonate packed in tube. Each colour pigments occupy specific area in the column which is called as 'zone' and the coloured column is called as 'development of chromatogram.

DEFINITION OF CHROMATOGRAPHY

Chromatography is defined as it is a physical method of separation into its individual components when the mixture is distributed between two phases one is fixed phase called stationary phase and other is movable phase called mobile phase

OR

Chromatography is a method of separation of components of mixture in which a sample is introduced into a mobile phase which is carried along with a column and solid support called stationary phase.

Generally, the stationary phase is either solid or liquid and mobile phase is liquid or gas. Depending upon the stationary phase and mobile the chromatography is classified in different forms

DEFINITION OF STATIONARY PHASE:

The solid or liquid phase of a chromatography system on which the materials are to beseparated or selectively absorbed.

Stationary phase in chromatography is the one which does not move with the sample. The fluid (liquid or gas) that flows through a chromatography system, moving the materials to be separated at different rates over the stationary phase.

3. IMPORTANCE OF CHROMATOGRAPHY

A number of methods are available for the separation of organic and other compounds from the mixture and they are

(a.) Fractional distillation (b). Extraction (c)counter-current distribution (d) Crystallization

(e) Fractional distribution

Above all techniques are very useful in separation identification and purification of many compounds. But among all these available methods chromatography gains much more importance due to following advantages

1) It is very rapid accurate sensitive and gentle method.

2) very minute amount of sample is required for whole analysis.

3) Decomposition of compound can be avoided in this method this is useful especially for biological products.

4) It is non-destructive method of analysis means we can recover sample after the analysis

5) chromatography is useful in all fields such as biology, chemistry, dyes, medicine, forensics and preclinical studies etc.

4. PRINCIPLE OF CHROMATOGRAPHY

Different component of mixture having different affinity towards stationary phase and mobile phase is the basic principle of chromatography.

The components of the mixture are having different affinities or distribution coefficient kd towards the stationary md mobile phase. The basis of all type of chromatography is the partitionor distribution coefficient which describes the way in which compound distributes itself in two immiscible phases.

Suppose the compound is distributed in two phases, sat phase A and phase B, then the value of partition or distribution coefficient kd is given by:

$$\mathbf{Kd} = \frac{\text{Concentration of compound in phase A}}{\text{Concentration of compound in phase B}}$$

From the above equation the term effective distribution coefficient is defines as the total amount of substance present in one phase divided by total amount of substance present in another phase.

Suppose the mixture M is having three components A B and C is passed through the column packed with stationary phase and mobile phase is allowed to pass continuously through it as shown in figure the green component is having least affinity towards stationary phase, red having intermediate and blue having strong affinity. As the mobile phase passes continuously through column the 'green' component run faster because it is having least affinity towards stationary and mobile phase so interaction of green component between stationary phase and mobile phase is least. Red component run at intermediate speed in column because it is having intermediate affinity and Red occupy upper portion of column because it is having strong affinity towards stationary phase. In this manner all components get separated in chromatographic technique.

5. INTRODUCTION OF COLUMN CHROMATOGRAPHY

Chromatographic separation carried out in long tube filled with stationary material is led column chromatography. In this method, the separation is carried out in the glass tubes where the mixture is poured at the top of column packed with stationary phase and phase is allowedto pass through the column. The components of mixture get separated because of different component of mixture having different affinity towards stationary and mobile phase as a result of which components of mature travel with different rates in the column

The technique of column chromatography was invented by the American chemist D. T. Day in 1900 In 1906, the Polish botanist, M. S. Tsweet used adsorption columns in the oration of plant pigments; hence column chromatography is also known as adsorption Chromatography Glass tubes having diameter of 40 to 50 mm are used as chromatographic columns in columnchromatography. The glass tubes are filled with stationary phase having particle diameter 150 to 200 um. The column dimension is not critical. It ranges from millimetres to few centimetres and lengths from centimetres to meters. For a given set of column, greater efficiency will be obtained with long narrow column than with a short thick one but if the column is too long the flow rate will be very low.

6. PRINCIPLE OF COLUMN CHROMATOGRAPHY

Components of the mixture are having different affinity Le different distribution coefficient or partition coefficient towards stationary and mobile phase, on the basis of which separation of mature can be achieved. This is the basic principle of column chromatography In column chromatography, the column is packed with stationary phase and the mixture to be separated is dissolved in a suitable solvent and allowed to pass through the adsorbent column. The rateof adsorption varies with type of adsorbent (stationary phase) filled in the column and the type of mixture to be separated. The component from the mixture which has greater adsorbing power, is adsorbed at the upper part of the column while the component having less adsorbing power than the first component is adsorbed at the lower portion of the column. Thus, column chromatography works on the principle of selective adsorption. The adsorbed sample components form a distinct band in the column and travel down along the mobile phase and get eluted. The portion of column which is occupied by a particular substance is called as its zone. Different zones appear in the adsorbent column which is termed as chromatogram and the

chromatographic operations are called as development of chromatogram.

- Two common procedures may be adopted to estimate various constituents of the samplemixture.

Those are:

a) After development, the column of adsorbent may be pushed out of the tube, variouszones are cut with knife at boundaries and the substances present in zones are extracted

With suitable solvent. This process of recovery of constituents from the chromatogram isknown as elution.

b) After development, the column may be washed with more solvent now termed the eluent and each component is collected separately as it reaches the end of the column. The process of separation of mixture of two substances A and B has been represented in the

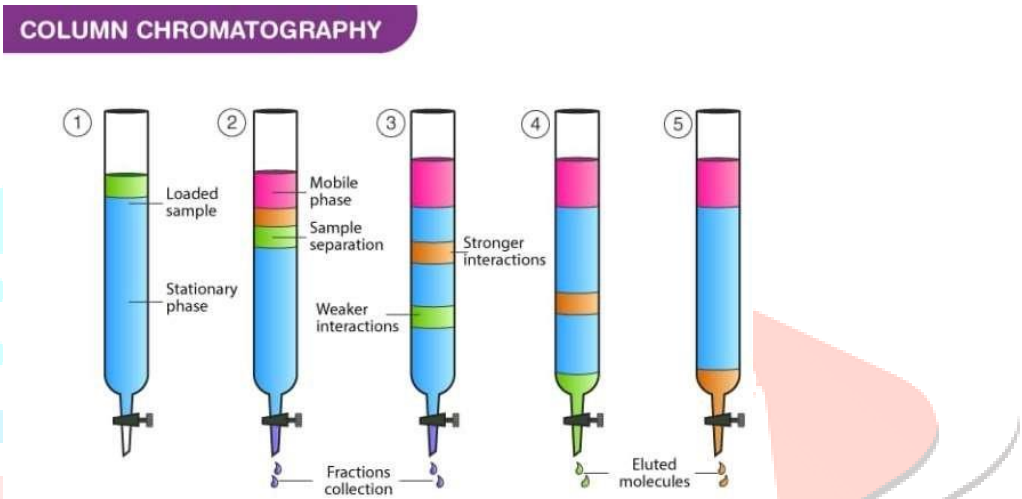


Fig. 1

7. TYPES OF COLUMN CHROMATOGRAPHY:

On the basis of type of stationary phase used in the separation, column chromatography isclassified into two types:

1)Adsorption column chromatography2)Partition column chromatography 3)Gel column chromatography

1. Adsorption Column Chromatography: In adsorption column chromatography, the separation is achieved by allowing the mixture to pass through the stationary and mobile phase in which stationary phase is solid and mobile phase is liquid solvents. In this method, the mixtures are made to adsorb on the surface of adsorbent and mobile phase allowed to flow through it.

2. Partition Column chromatography: In this technique, the mixture is to be separated is distributed between liquid stationary phase and liquid mobile phase.

Partitioning of mixture between two liquid phases takes place in this method. Depending upon the affinity of compounds towards liquid phase, compounds get separated in the column. The liquid is coated on a solid support to render it stationary. Experimental detailed both types of column chromatography are almost same, but they differ only in the use of stationary material for the separation.

3. Gel Chromatography: Gel chromatography, also called Gel Filtration, in analytical chemistry, technique for separating chemical substances by exploiting the differences in the rates at which they pass through a bed of

a porous, semisolid substance. The method is especially useful for separating enzymes, proteins, peptides, and amino acids from each other and from substances of low molecular weight. The separation of the components of a mixtureby gel chromatography is based on the differences in the molecular sizes of the components. Small molecules tend to diffuse into the interior of the porous particles so that their flow is restricted, while large molecules are unable to enter the pores and tend to flow unhindered. Thus, the components of highest molecular weight leave the bed first, followed by successively smaller molecules. The bed materials most extensively used are polyacrylamide and a polymer prepared from dextran and epichlorohydrin. The dry polymers are usually suspended in suitable agents to form a homogeneous, semisolid mixture.

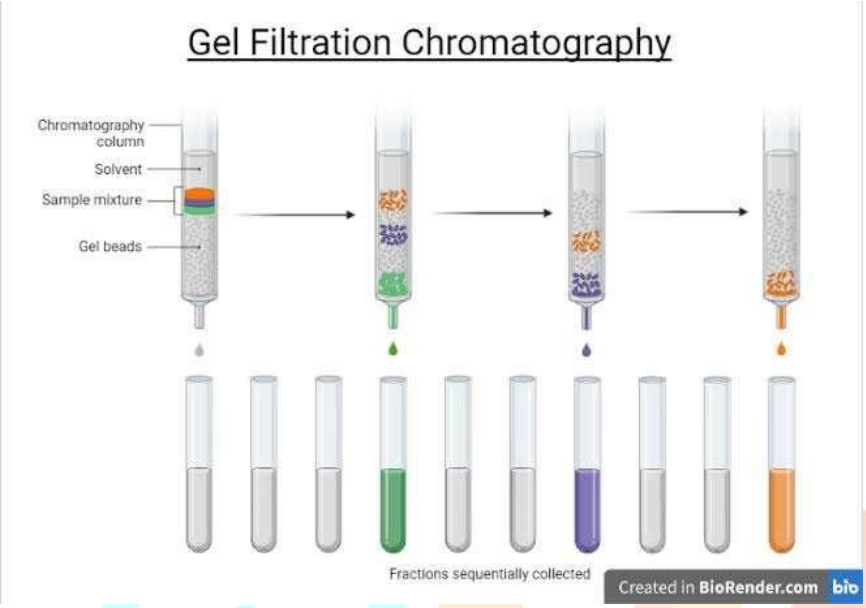


Fig.2

8.EXPERIMENTAL DETAILS OF COLUMN CHROMATOGRAPHY:

A. Chromatographic Column (Apparatus):

A simple straight glass tube tapered at the bottom and fitted with a top is commonly used as a column. The length to diameter ratio should be greater than 20: 1. The ratio of 40 I can be takenas standard. The glass tube is about 20-30 cm long and 2-3 cm in diameter. The glass tube can hold 50 to 100 grams of adsorbent (stationary phase). Long and narrow tubes are generallyused for difficult separations. Column fitted with cooling jackets are used when constant temperature conditions are required.

B. Stationary Phase (Adsorbents):

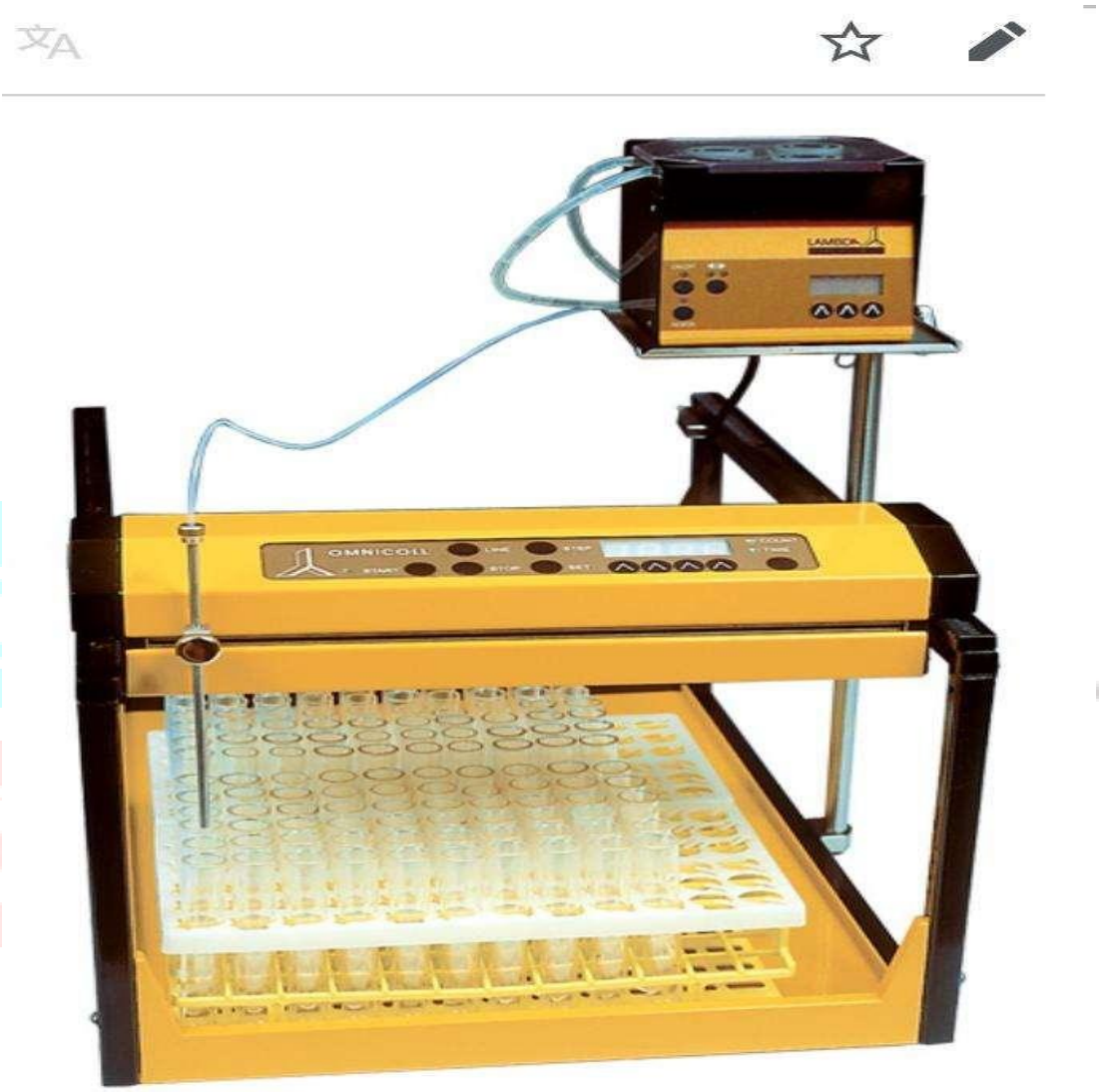
Coating materials used in TLC are employed as adsorbents in column chromatography. The usual adsorbents used are; silica, alumina, calcium carbonate, magnesia, starch, sucrose, activated magnesium silicate, cellulose, etc. Adsorbents should have following features

1. The adsorbent should not react chemically either with mobile phase or with samplecomponents

2. The adsorbent particles should have spherical shape and uniform size.3.The adsorbent should contain small number of soluble components.

4. The adsorbent should be inert by nature.

5. The adsorbent should be mechanically stable to prevent formation of fine dust which mightbe deposited in the channels of the packing.



Automated fraction collector and sampler for chromatography technique

Fig.3

The Stationary phase or adsorbent in column chromatography is a Solid. The most common stationary phase for column chromatography is Silica gel, the next most common being alumina. Cellulose powder has often been used in the past. A wide range of Stationary phases are available in order to perform ion exchange chromatography, reversed-phase chromatography (RP), affinity chromatography or expanded bed adsorption (EBA). The stationary phases are usually finely ground powders or gels and/or are microporous for an increased surface, though in EBA a fluidized bed is used. There is an important ratio between the stationary phase weight and the dry weight of the analyte mixture that can be applied onto the column. For silica column chromatography, this ratio lies within 20:1 to 100:1, depending on how close to each other the analyte components are being eluted.

C. Preparation of column

A column is prepared by packing a solid adsorbent into a cylindrical glass or plastic tube. Thesize will depend on the amount of compound being isolated. The base of the tube contains a filter, either a cotton or glass wool plug, or glass frit to hold the solid phase in place. A solvent reservoir may be attached at the top of the column.

Two methods are generally used to prepare a column: the dry method and the wet method. For the dry method, the column is first filled with dry stationary phase powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet,and from this point is never allowed to run dry. For the wet method, a slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. The top of the silica should be flat, and the top of the silica can be protected by a layer of sand. Eluent is slowly passed through the column to advance the organic material.

The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column, they elute one at a time. During the entire chromatography process theeluent is collected in a series of fractions. Fractions can be collected automatically by means of fraction collectors. The productivity of chromatography can be increased by running several columns at a time. In this case multi stream collectors are used. The composition of the eluent flow can be monitored and each fraction is analysed for dissolved compounds, e.g. by analytical chromatography, UV absorption spectra, or fluorescence. Coloured compounds (or fluorescent compounds with the aid of a UV lamp) can be seen through the glass wall as moving bands.

D. Solvent Used (Mobile phase):

sample mixture should not get dissolved in the chosen solvent. The solvents used t column chromatography should possess boiling point between 40°C to 85°C Most used solvents are light petroleum. Other solvents used are cyclohexane, carbon disulphide benzene, chloroform, carbon tetrachloride, methylene chloride, ethyl acetate, acetone, elle pyridine, formamide, etc.

The solvents used in chromatography have to perform three functions: They serve to introduce the mixture to the column. They affect the process of development by which the zones of chromatograms separated to their fullest extent. When used for this purpose the solvents are termer as developers

They are also used to remove the required content of each zone from the mechanically separated parts of the column, or from the column as a whole after its partly developed. The solvent used for this purpose are called eluents The mobile phase or eluent is a solvent or a mixture of solvents used to move the compounds through the column. It is chosen so that the retention factor value of the compound of interest is roughly around 0.2 - 0.3 in order to minimize the time and the amount of eluent to run the chromatography. The eluent has also been chosen so that the different compounds can be separated effectively. The eluent is optimized in small scale pretests, often using thin layer chromatography (TLC) with the same stationary phase. There is an optimum flow rate for each particular separation. A faster flow rate of the eluent minimizes the time required to run a column and thereby minimizes diffusion,resulting in a better separation. However, the maximum flow rate is limited because a finitetime is required for the analyte to equilibrate between the stationary phase and mobile phase, see Van Deemter's equation. A simple laboratory column runs by gravity flow. The flow rateof such a column can be increased by extending the fresh eluent filled column above the topof the stationary phase or decreased by the tap controls. Faster flow rates can be achieved by using a pump or by using compressed gas (e.g., air, nitrogen, or argon) to push the solvent through the column (flash column chromatography). Photographic sequence of a column chromatography the particle size of the stationary phase is generally finer in flash column chromatography than in gravity column chromatography. For example, one of the most widely used silica gel grades in the former technique is mesh 230 – 400 (40 – 63 µm), while the latter technique typically requires mesh 70 – 230 (63 – 200 µm) silica gel. A spreadsheet that assists in the successful development of flash columns has been developed. The spreadsheet estimates the retention volume and band volume of analytes, the fraction numbers expected to contain each analyte, and the resolution between adjacent peaks. This

information allows users to selectoptimal parameters for preparative-scale separations before the flash column itself is attempted.

E. Development of Column:

The development of chromatographic column is done by two methods:

1. Successive Elution: In successive elution method, the development of chromatographiccolumn is done by using series of solvents. Here instead of mixture of solvents, the development is done by using single solvent and after completion of development; the fractionsare collected in beaker. For example, particular solvent may dissolve particular group ofcompounds such as tannins, leaving rest of the compound undissolved at the upper part of column. After the group of compounds that is soluble in the first solvent has been eluted from the column, a new solvent a used to dissolve and separate another class of compounds says aromat compounds. In this way, series of solvents are used to separate the different kinds of compounds. This is called successive elution.

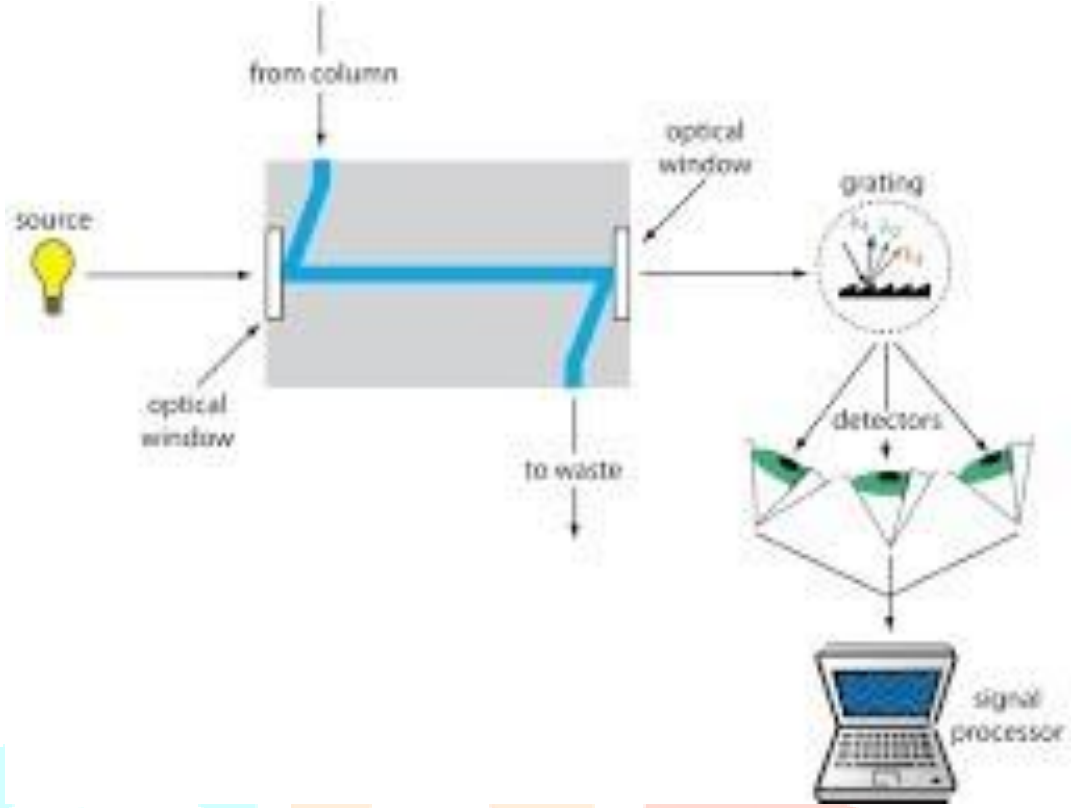
2. Gradient Elution: The continuous change in solvents described above sometimes leads to problems, particularly in collecting fractions near the interface between the two succeeding mobile phases. This technique has been improved upon by using process of gradient elution. In this system, two solvents may be used that are completely miscible in each other, but with different dielectric constants. After the sample is loaded on the top of the column, the first solvent, which has a lowe solvent strength, is used as the mobile phase. After a suitable period of time, a small amount of the second solvent is introduced. With time, its concentration in the first savent progressively increases until the mobile phase consists entirely of the second

spvent Throughout the operation, there is a gradient in the concentrations of the two solvents. This leads to a progressive change in solvent strength and in the solubility of some of the components of the sample. Any two solvents may be used, provided that they are miscible Frequently, solvents that are somewhat different in their properties are selected in order to separate a greater range of components Popular pairs of solvents include water-methanol water-acetonitrile, and dioxane-tetrahydrofuran

F. Detectors:

The auent emerging from the column is exposed to the detector for quantitative determination of the sample. Different types of detectors can be employed for this purpose.

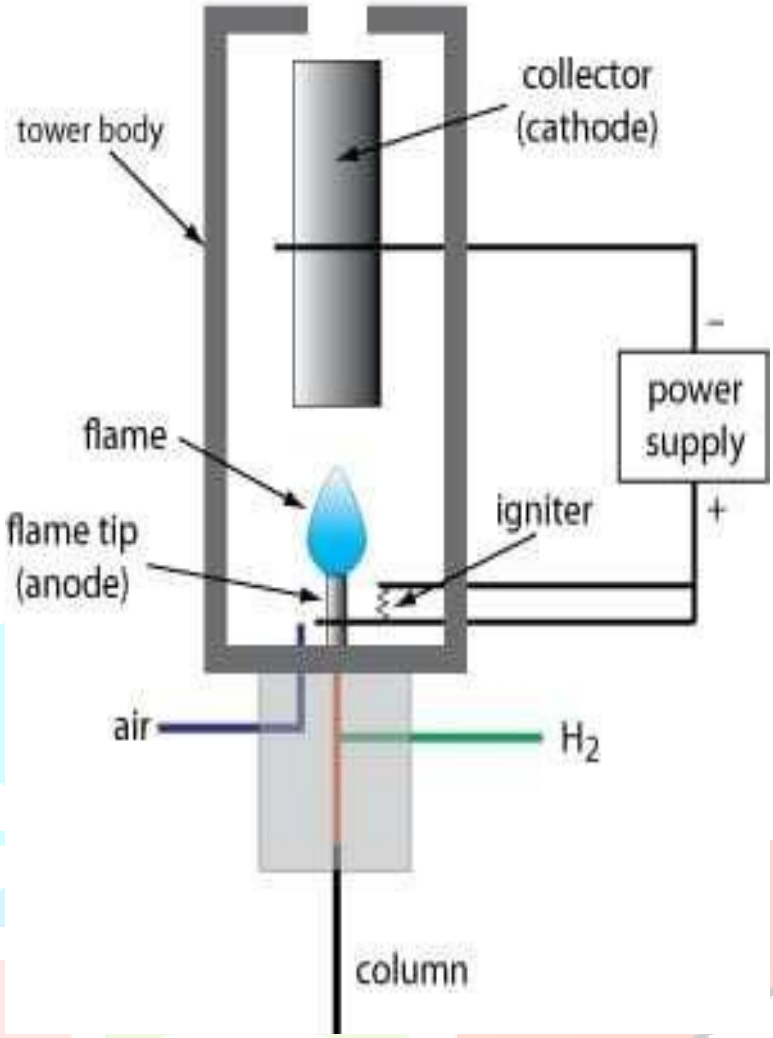
I). Optical detectors :carry a small cell which is made from glass or quartz UV or visible light radiations are used for continuous photometric analysis of the sample. If the samples do not exhibit appreciable absorption in the cited regions reagents can be added to produce colour reactions which can be measured photometrical.



OPTICAL DETECTOR:-

Fig.4

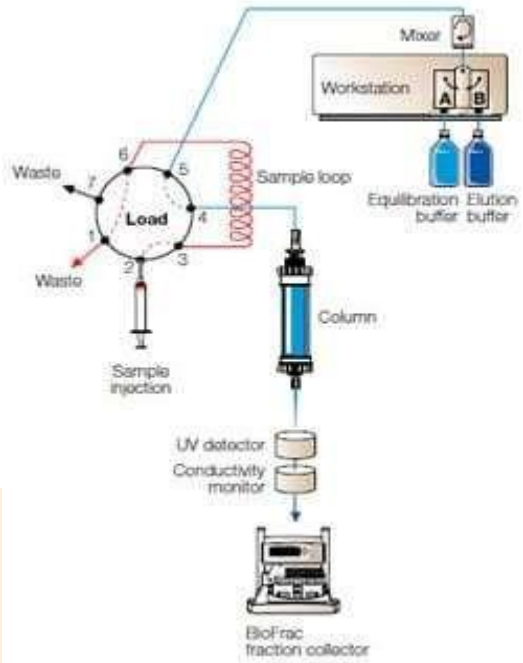
II). Flame lionization Detectors: In flame ionization detector, there is an endless metal wire which is passed by the column exit. The decomposition products of the substances which are transported by the wires are led to flame ionization or argon detectors.



FLAME IONIZATION DETECTOR

Fig.5

III). Conductivity Detectors: These are suitable for ionized substances in aqueous solution. The effluent is passed through a measuring cell of the detector contains two or three platinum electrodes within a Wheatstone meter bridge excult and is operated by alternating concurred.



CONDUCTIVITY DETECTOR

Fig.6

IV). Detectors Based on Heat of Adsorption: These detectors are known as micro adsorption detectors. In these detectors the liquid emerging from a separating column is passed through two cells, one located on top of the other while lower cell is filled with an adsorbent. In the centre of the packing of each of the cell the, glass- covered measuring point of a small thermistor is located. The total deflection (+ve and-ve peak) is proportional to the concentration.

G. Method of introducing the sample:- The sample mixture is taken initially; the topof the column is covered by a plug of cotton wool. Some solvent is put over it through a funnel.The rate of flow of solvent through the column is controlled by a suction pump. The solvent should populate at the rate of 4 to 10 cm per minute in a 5 cm tube. Now the solution is added through a funnel for a tap funnel. The top of the column must remain covered with the liquid throughout the procedure. If this precaution is not taken, the column may dry and shrink. Too much suction must also be avoided to prevent evaporation. After the entire portion of the solution has passed through the developing liquid is led into the column slowly. After all the liquid has passed through and various zone become well-defined, the system is allowed to drivein air or oxygen or nitrogen.

H. Analysis:-For the analysis of the separated zones, that sorbent may be pushed out completely with wooden pestle or plunger and the zones may be separated as they leave the tube. Each zone is then dropped immediately into the element and the suspension is filtered on the sintered glass funnel to get rid of the adsorbent. In some cases, the column of adsorbent is not removed from the glass tube. The developed chromatogram is treated either with a single eluent or with a succession of solvents having increasingly powerful eluent actions. The various portions of the column are this washed out one by one and collected in different receivers. Further estimation is achieved using different analytical techniques. Efficiency

9.FACTORS AFFECTING COLUMN EFFICIENCY:

1. **Nature of Solvents:** Solvents of low viscosity are generally used for high efficiency separations. The reason for this is that rate of flow is inversely proportional to viscosity and hence, it becomes necessary to select a solvent of lowest viscosity and proper elution strength.

2. **Dimensions of Columns:** It is possible to improve the column efficiency by increasing the length/width ratio of the column. For the common preparative separations, sample/column packing ratios have found to range from 20:1 to 1000:1

3. **Particle Size of Column Packing:** it is possible to increase the column efficiency by decreasing the particle size of the adsorbent. The usual particle size ranges from 100 to 200 meshes.

4. **Pore Diameter of Column Packing:** Pore diameter of the column affects the efficacy of chromatographic separation in column chromatography. According to the study, a decrease in average pore diameter from 170-20 Å does not affect efficiency. If the pore diameter of stationary phase is above the average range, this will directly affect the separation process of chromatography.

10. AUTOMATED COLUMN CHROMATOGRAPHY

Column chromatography is an extremely time-consuming stage in any lab and can quickly become the bottleneck for any process lab. Many manufacturers like Biotage, Buchi, Interchim and Teledyne Isco have developed automated flash chromatography systems (typically referred to as LPLC, low pressure liquid chromatography, around 350–525 kPa or 50.8–76.1 psi) that minimize human involvement in the purification process. Automated systems will include components normally found on more expensive high performance liquidchromatography (HPLC) systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent. Typically, these automated systems can separate samples from a few milligrams up to an industrial many kilograms scale and offer a much cheaper and quicker solution to doing multiple injections on prep-HPLC systems.

The resolution (or the ability to separate a mixture) on an LPLC system will always be lower compared to HPLC, as the packing material in an HPLC column can be much smaller, typically only 5 micrometres thus increasing stationary phase surface area, increasing surfaceinteractions and giving better separation. However, the use of this small packing media causes the high back pressure and is why it is termed high pressure liquid chromatography. The LPLC columns are typically packed with silica of around 50 micrometres, thus reducingback pressure and resolution, but it also removes the need for expensive high-pressure pumps. Manufacturers are now starting to move into higher pressure flash chromatography systems and have termed these as medium pressure liquid chromatography (MPLC) systems which operate above 1 MPa (150 psi).

An automated ion chromatography system

Fig.7

11. COLUMN CHROMATOGRAM CALCULATION

Typically, column chromatography is set up with peristaltic pumps, flowing buffers and thesolution sample through the top of the column. The solutions and buffers pass through the column where a fraction collector at the end of the column setup collect the eluted samples.Prior to the fraction collection, the samples that are eluted from the column pass through a detector such as a spectrophotometer or mass spectrometer so that the concentration of the separated samples in the sample solution mixture can be determined.

For example, if you were to separate two different proteins with different binding capacities to the column from a solution sample, a good type of detector would be a spectrophotometerusing a wavelength of 280 nm. The higher the concentration of protein that passes through the eluted solution through the column, the higher the absorbance of that wavelength.

Because the column chromatography has a constant flow of eluted solution passing throughthe detector at varying concentrations, the detector must plot the concentration of the elutedsample over a course of time. This plot of sample concentration versus time is called a chromatogram.

The ultimate goal of chromatography is to separate different components from a solution mixture. The resolution expresses the extent of separation between the components from the mixture. The higher the resolution of the chromatogram, the better the extent of separation of the samples the column gives. This data is a good way of determining the column's separationproperties of that particular sample. The resolution can be calculated from the chromatogram.

The separate curves in the diagram represent different sample elution concentration profilesover time based on their affinity to the column resin. To calculate resolution, the retention time and curve width are required.

Retention time is the time from the start of signal detection by the detector to the peak heightof the elution concentration profile of each different sample.

Curve width is the width of the concentration profile curve of the different samples in thechromatogram in units of time.

A simplified method of calculating chromatogram resolution is to use the plate model. The plate model assumes that the column can be divided into a certain number of sections, or plates and the mass balance can be calculated for each individual plate. This approach approximates a typical chromatogram curve as a Gaussian distribution curve. By doing this, the curve width is estimated as 4 times the standard deviation of the curve, 4σ. The retentiontime is the time from the start of signal detection to the time of the peak height of the Gaussian curve.

From the variables in the figure above, the resolution, plate number, and plate height of thecolumn plate model can be calculated using the equations:

Resolution (R_s)

$R_s = 2(t_{RB} - t_{RA})/(w_B + w_A)$
Where:

t_{RB} = retention time of solute Bt_{RA} = retention time of solute A
w_B = Gaussian curve width of solute B w_A = Gaussian curve width of solute A Plate Number (N):
$N = (t_R)^2/(w/4)^2$
Plate Height (H):
$H = L/N$
Where L is the length of the column.

12. ADVANTAGES OF COLUMN CHROMATOGRAPHY:

1.Using column chromatography all kinds of complex mixtures can be separated.2.Any amount of mixture can be separated by column chromatography,

3.A broad range of mobile phases.

4.Analytes can be separated and reused, in preparative type chromatography5.It can be possible to run automation.

6.This is a robust method.

13.DISADVANTAGES OF COLUMN CHROMATOGRAPHY:

1. It takes more time to separate the compounds.
2. Column chromatography has of low separation power relative to advance.
3. Higher quantities of solvents are essential, which is more expensive.
4. Automation makes more complex and costly.

14.APPLICATION

1. **Analytical Uses**:
Capillaries made up of glass or copper; of 0.05-2mm Internal diameter and 1-20 m length are used for analytical purposes. The internal surface of the narrow tubing serves as adsorbent or support for the liquid phase. Glass capillaries whose internal surfaces treated with concentrated ammonia at 300°C can separate amino acids using butanone/Pyridine/dilute acetic acid (5:5:1) or xylose/glucose/maltose using butanone/acetic acid/water (3:1:6). Scientist Vestergaard and Sayegh separated 7 urinary steroids within 5 hours which. 36 hours on a normal column. They have used narrow Teflon tubing packed with Aluminium oxide or silica gel. An optical detectoris used for the analysis of steroids by gradient elution using acetone in chloroform.
2. **Separation of Diastereomers**: Separation of diastereomeric 7-chloro-azibicyclo (4:1:0)- heptane is done on silica gel using pentane/ diethyl ether as solvent.

3. **Separation of Tautomeric Mixtures**: Separation of tautomeric mixture is possible at High temperature gas chromatography cannot be employed. However, the separation Of these could be done by column chromatography. The keto and enol forms of p-hydroxy-phenyl pyruvicacid and indolyl pyruvic Acid could be separated in a liquid phase. The separation is done in weakly acidic Medium. The enols form appears in elute before the keto form.

4. **Separation of Geometrical Isomers**: Separation of cis and trans isomers is based on the steric factor. Isomers whose functional group can approach the surface of the adsorbent more easily are more strongly adsorbed. The first chromatographic separation of cis and trans isomers of bixin and crocetin dimethyl ether was reported by scientist Winterstein. Later, Zechmeister separated cis and trans isomers carotenoids on calcium carbonate, Aluminium oxide and other adsorbents. Cis and Trans isomers of carboxylic acids have been separated on charcoal and silica gel.
5. **Separation of Racemates**: The first successful separations of racemates using organic solvents were achieved on lactose.

15. CONCLUSION:

Column chromatography is one of the most useful methods for the separation and purificationof liquids and solids. In this experiment, it was used to successfully separate and purify a mixture of two organometallic compounds. The experiment was successful and there were no human errors that interfered with the results. The ferrocene and acetylferrocene mixture were separated, dried, and the melting point was obtained that determined the two were pure compounds.

Column chromatography is a conventional tool for separation of phytochemicals, removal ofimpurities and purification of drugs.

Effective separation of constituents from different sources in preparative scale (milligram togram) can be achieved by column chromatography.

Availability of wide range of stationary phases makes the technique to be used for differentkinds of mechanisms.

Understanding the basic principles of column chromatography enables us to find solutions forcurrent research problems.

16. REFERENCE

1. Shusterman, AJ; McDougal, PG; Glassfuls, A (1997). "Dry-Column Flash Chromatography". J Cham Educ. 74 (10): 1222. Bibcode:1997JChEd.74.1222S. doi:10.1021/ed074p1222. ISSN 0021-9584.

2. "How to set-up a flash chromatography silica column and actually succeed at separation". Reachdevices.com. REACH Devices, LLC. Retrieved 3 Jan 2019.

3. Still, WC; Kahn, M; Mitra, A (1978). "Rapid chromatographic technique for preparative separations with moderate resolution". J Org Chem. ACS. 43 (14): 2923– 2925. Doi:10.1021/jo00408a041.

4. Harwood LM, Moody CJ (13 Jun 1989). Experimental organic chemistry: Principlesand Practice (Illustrated ed.). London: Blackwell. Pp. 180–185. ISBN 978-0-632- 02017-1. OCLC 1079261960.

5. "Material Harvest Silica Gel for Normal Phase Column Chromatography". Material Harvest. 2008. Retrieved 3 Jan 2019.

6. Fair, JD; Kormos, CM (2008). "Flash column chromatograms estimated from thin- layer chromatography data". J Chromatograph A. 1211 (1–2): 49–54. Doi:10.1016/j.chroma.2008.09.085. ISSN 0021-9673. PMID 18849041.

7. Harrison RG, Todd PW, Rudge SR, Petrides DP (2003). Bioseparations science and engineering (2nd ed.). New York, NY: Oxford University Press. ISBN 9780190213732. OCLC 899240244.

8. Jeffry G.H., J. Mendham et al. Vogel's Text book of Quantitative Chemical Analysis,5th Edition, 1989, Longman Scientific & Technical, Bath Press, Great Britain.

9. G.R. Chatwal, SK Anand. Instrumental methods of chemical analysis (Analytical Chemistry), ed. 1995, Himalaya Publishing House, Bombay.

10. A.H. Beckett & J.B. Stenlake. Practical Pharmaceutical Chemistry, Part Two, ed. 4th, 1997, (reprint 2003) CBS Publishers & Distributors, New Delhi.