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An Overview On The Chromatography

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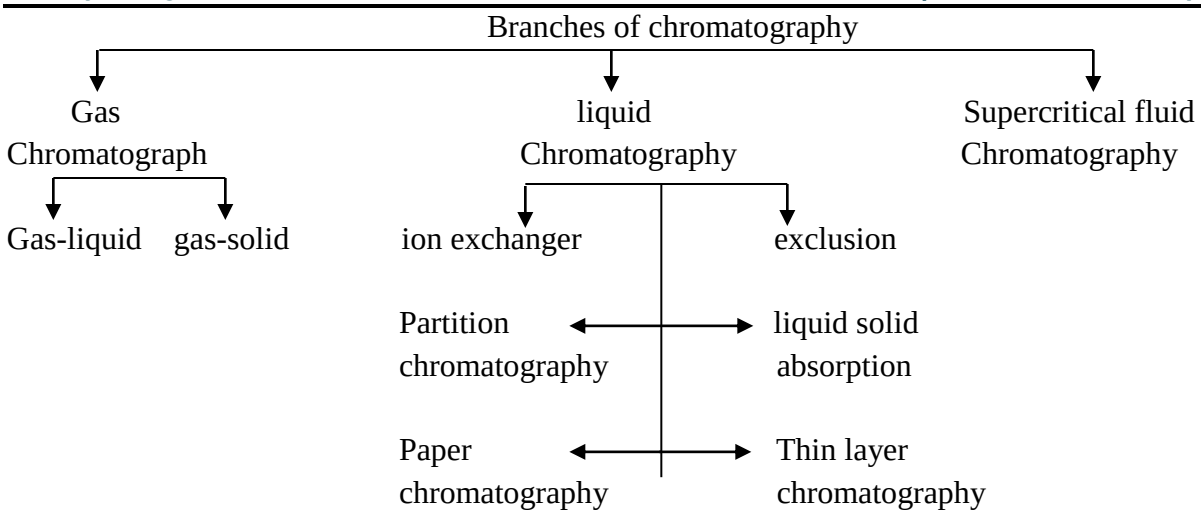
ABSTRACT:- In the present article attempt has been made to explain the basic ideas of different separation techniques (Chromatography). A sophisticated instrument, chromatography involve various type of chromatography divisions which allow to separate the crude drug or mixture of components. In this technique two phase are use mobile phase and stationary phase, in which one phase may be polar and another phase are non-polar according to the nature of sample. They focus on bio-analytical techniques which could help them in separating the most active elements from the crude mixtures. It also includes various different type of quantitative and qualitative techniques to estimation of biological and pharmaceutical samples. It is most versatile, safest, dependable and fastest techniques for qualitative control of drug components. While basic chromatography still prevails as the most important analytical tools in molecular chemistry. This article focuses on the various types of chromatography and it's principle, working and applications.

KEYWORD:- Gas chromatography, Liquid chromatography, Paper Chromatography, Thin layer chromatography, High Performance Liquid Chromatography, Supercritical Fluid Chromatography.

Introduction :- Chromatography is the analytical techniques use for separation, purification, identification of constituents from the mixture. This works on the principle of differential interaction of solute with two different phases (stationary phase and mobile phase). In this technique number of compound can be detected. They administer the mixture to be separated in a stationary phase (solid or liquid) and a pure solvent such as water or any mixed gas(mobile phase) is allowed to move slowly over the stationary phase, holding the components separately in the pure solvent as per their solubility.[1]

Type of chromatography:- Chromatography is divided into three types like chromatography of liquid, gas, and supercritical fluid.(fig.1) The gas chromatography is further divided into gas-liquid and gas-solid chromatography. And liquid chromatography is divided into ion exchange, exclusion, partition (paper chromatography) and liquid-solid interaction (thin layer chromatography).[2]



Various types of chromatography (fig.1)

GAS chromatography

G.C. was originally developed in the 1950s and also introduced in the late 1950s. As with all chromatographic techniques the theory depends on the partitioning of the analyte between a stationary and mobile phase. In the case of GC, the mobile phase is a gas which means that the analyte must be volatile to move through the column and majority analytes have little interaction with the mobile phase.[3]

It has two types-

1. **Gas-solid chromatography:-** the stationary phase is in solid at this technique. This functions on the absorbent principle.
2. **Gas- liquid chromatography:-** the stationary phase is in liquid at this technique. This functions on the principle of partition and absorption.

Components :-

Supply of carrier gas with attached pressure regulators and flow meters in a high pressure cylinder. Carrier gas: He, N_2, H_2 and argon methane (It is favored because of high thermal conductivity. N_2 is preferred when significant carrier g as consumption is used).Pressure regulators: Tank carrier gas moves through a toggle valve, a flow meter, (11000 mL / min), capillary restrictors, and a pressure gage (14 atm). The flow rate is balanced using a needle valve mounted at t he flow meter base and operated by capillary restrictors.[4]

Sample injection system:-

Liquid Sample is injected using microsyringe. To change in to vapour phase, the injector must be kept at a high temperature. The sample is directly injected into heated port which has a temperature higher than that of the oven. The volume to be injected for a packed column and capillary column are 1-20 µL respectively.

The separation column :-

The core of the gas chromatography is the column which is made of metals bent in coiled shape into an open spiral shape. Copper is useful up to 2500C. Depending on the requirements several sizes of columns are used.[4]

Column:- 5-50 m coiled stainless steel/glass/Teflon Types of columns depending on its use:

1. Analytical column 1-1.5 meters length, 4-6 mm diameter

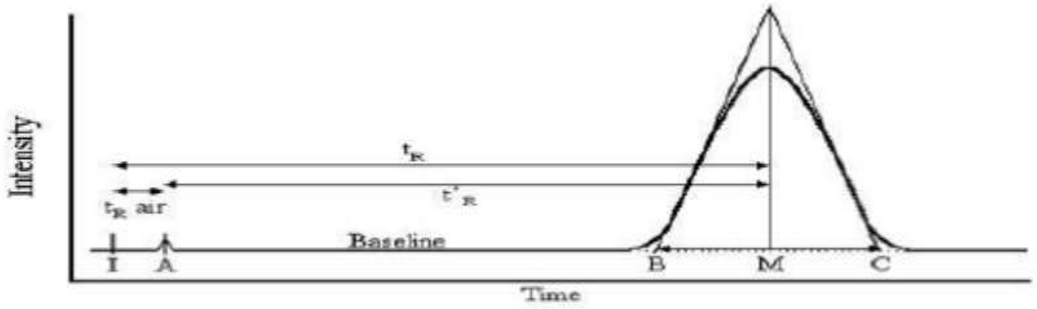
2. Preparative column 3-6 meters length, 5-9mm diameter

Column types depending on its nature:

- Packed column -partition and absorption
- Open tubular or Capillary column
- WCOT - partition
- SCOT - partition
- PCOT - adsorption

- Recorder
- Separate thermostat compartments for detectors and columns.

Procedure:- The mixture components are vaporized in the heat bar attach to the stationary phase in the column strongly or weakly. Mobile phase first takes the weakly bound component to column outlet. Thus, the components will be separated according to the boiling point, molecular weight, polarity, ability to hydrogen bonding with the stationary phase. The partial solute pressure in the gas and the detector's sensitivity to the sample dictates the magnitude of the output. The performance is reported as the amount of solvent that leaves the column as a functional of time, just a chromatogram.(fig.2)[5]



Chromatogram for gas chromatography (Fig.2)

Chromatographic Analysis:-

- The number of peaks obtained determines the number of components in a sample
- The sum of a given component in a sample is determined by the region under the peaks.
- The retention time aids in the identity of components

Detectors in Gas chromatography:-

In GC analysis the detector needs to be sensitive work at high temperature (0-400°C), stable and reproducible, linear response, wide dynamic range, quick response, easily (reliable), non-destructive, uniform response to all analytes for a fair GC study. The detectors used are thermal conductivity detector (TCD), atomic emission detector, thermionic detector, electron capture detector, flame photometric detector and flame ionization detector.[7]

Advantages:-

- Due to its very high resolution strength, complex mixtures can be solved in its components very high sensitivity with TCD.
- It is a micro method; even the small sample size is enough.
- Fast analysis is possible, gas as moving phase- rapid equilibrium.
- Relatively good accuracy & precision.
- Qualitative and quantitative analysis is possible.

Applications:-

- Quality management - Rt & RV are used for the identification & separation
- Purity control, pharmaceutical and Environmental analysis - chromatogram of the standard and sample are compared.
- Quantitative analysis of main and trace components - It is necessary to measure the peak height and peak area of each component.[8]

Liquid Chromatography

It is a simple column chromatography in which liquid acts as a mobile phase and as a stationary phase inert solid substances such as silica gel, alumina or cellulose supported in a column. While setting up a liquid chromatography column, it is vital that the stationary phase is saturated with solvent as any air present will interrupt the smooth flow and will result in inefficient or incomplete separation.[9]

Liquid chromatography is divided into different parts-

1. Ion exchange
2. Exclusion
3. Paper chromatography (partition)
4. Thin Layer Chromatography (liquid-solid interaction)

HPLC is the advance technology of liquid chromatography.

High performance liquid chromatography (HPLC):-

HPLC is a superior liquid chromatography method and one of the most commonly used analytical techniques. Due to the physical properties of HPLC column high pressure should be applied to have an eluent flow through the column. Because of this earlier the method was termed as high pressure chromatography.[8]

Principle:-

Separation is depended on the relative solubility between two liquid phases of the analyte. HPLC utilizes various types of stationary phase (typically, hydrophobic saturated carbon chains), a pump that pushes the mobile phase and analytes through the column, and a detector that provides a characteristic retention time for the analyte. The retention time of analyte varies depending on the column temperature, the ratio/composition of solvent used, and the mobile phase flow rate. For HPLC, a pump (rather than gravity) provides the higher pressure needed to propel the mobile phase through the densely packed column and analyte.[9]

Chromatographic analysis:-

The number of peaks recorded determines the number of components in a sample. The amount of a given component in a sample is determined by the region under the peaks. The retention time help within components identification. A stetionary phase of small particle size which increases the efficiency of separation forms the basis for HPLC. This is because the solute can balance between the two phases more quickly. Larger particle size stationary phase forms the basis of low-pressure liquid chromatography in which flow of the eluent through the column is either gravity-fed or pumped by a low pressure pump. It is cheaper to run but lacks the high resolution.[8] As the particles size of stationary phase decreases, surface area increases and indirectly number of plates are increases and hence resolution increased. Yet resistance to the mobile phase flow increases because increased capillary action with small particle sizes. Such resistance produce a backpressure that reduces the flow rate and make it more difficult to drain the column under gravity. To overcome this high pressure system has to be applied to maintain the flow of solvent.[10]

Mobile phases:-

Up to four different eluents can be handled by HPLC instruments. High purity eluents should be used. Single eluent or two or more eluents premixed in defined proportions may be made with a single pump whereas in gradient elution to produce two eluent in proportions predetermined by a gradient programmer. For reverse phase HPLC is eluent, which is strong for regular phase HPLC, is weak and the opposite is true too.

Buffer systems

Composition:-

The composition of elution buffer shouldn't impact resolution.

Column elution:-

Appropriate eluent stored in a buffer reservoir is passed through the column that a consistent uniform rate for sample separation containing the components. The sample is uniformly applied to the top of the stationary phase at the end.[11]

Filters and degassers:-

Membrane filter must eliminate dust particles. Degassing by heating, vigorously stirring with a magnetic stirrer, applying a vaccume, ultrasonic, and budding through the eluent reservoir of helium gas. As the size of the

particles of stationary phase decreases, surface area increases and indirectly number of plates increases and hence increased resolution.

Mobile phases:- HPLC instruments can accommodated up to four different eluent. It is appropriate to use high purity eluents. In isocratic elution, single eluent or two or more eluents premixed in defined proportions may be made with a single pump whereas in gradient elution separate pumps are used to deliver two eluents in proportions predetermined by a gradient programmer. Eluent, which is strong in normal phase and weak in reversed phase HPLC and the opposite is also true.

Pumping system:-

Special pumps are available for the transport of eluent and are one of the most important features of HPLC systems. The function of the pump is to force a mobile phase at a specific flow rate.

Sample introducer:-

The method of sample introduction is most commonly used by a loop injector. It consists of a small volume metal loop that can be filled with the sample [5 to 25microliters (µL)]. By changing the position of the valve, the eluent is channelled through the loop and the sample is flushed onto the column. HPLC injectors can be manual ones but in case of high number of samples an auto sampler is more convenient to apply.[12]

Columns:-

The column is often called the "heart of chromatography" in separation process, and the availability of stable, high performance stationary phases and columns is critical to the development of reproducible and robust methods. Silica is the most commonly used column packing material for its reliable strength and rigidity, relative inertness and ability to be modified chemically. [13]

Types of columns in HPLC depending on the use

- Analytical [internal diameter 1.0 – 4.5-mm; lengths 25 – 250 mm]
- Preparative [i.d. > 4.6 mm; lengths 50 – 250 mm]
- Capillary [i.d. 0.1 – 1.0 mm; various lengths] Tubing materials
- Stainless steel (the most popular; gives high pressure capabilities)
- Glass (mostly for biomolecules)

Reduced eluent consumption, ideal for interfacing with a mass spectrometer due to the slower flow rates and increased sensitivity due to the higher concentration of analytes that can be used are the advantages of open tubular columns over conventional. New columns are made of stainless steel with highly polished interior walls, plastic material, glass-lined inner surfaces and outer column surfaces made from the rigid polymer, soft polymeric material for the outer surface and by compressing the column radially.

HPLC is divided into normal phase (NP) HPLC and reversed phase (RP) HPLC methods based on the polarity of the stationary and the mobile phase. [14]

Applications:-

HPLC is suitable for the separation of the non-volatile and thermally unstable chemical and biological compounds.

- Pharmaceuticals like aspirin, ibuprofen, or acetaminophen.
- Potassium phosphate, sodium chloride and other salts.
- Proteins like blood protein, egg white.
- Organic chemicals like polymers (e.g., polystyrene, polyethylene).
- Motor oil and other hydrocarbons.
- Many natural products such as ginseng, herbal medicines, plant extracts.
- Thermally unstable compounds such as trinitrotoluene (TNT), enzymes.

HPLC instruments are everywhere in drug research and development, pharmaceutical manufacturing, quality assurance, diagnostics, toxicology, research and other laboratories.[15]

Ion Exchange Chromatography:-

IEC is part of ion chromatography which together with ion-partition/interaction and ion-exclusion chromatography is an important analytical technique for the separation and determination of ionic compounds. Ion chromatography separation is based on ionic interactions between ionic and polar analytes, ions present in the eluent and ionic functional groups fixed to the chromatographic support. Ion exchange due to competitive ionic attraction and ion exclusion due to repulsion between similarly charged analyte ions and the ions fixed on the chromatographic support, play a role in the separation in ion chromatography.[14]

Applications:-

- Separation to the mixtures of amino acids (amino acid analyzers).
- Analysis of charged compounds like anions, cations, peptides, amino acids, and proteins.[14]

Size-Exclusion chromatography:-

It is also known as the gel permeation chromatography (GPC). It separates the molecules according to their size by their ability to penetrate a sieve-like structure the stationary phase.With no retention time, molecular greater than the average pore size will exclude first.

Applications:-

- Preparative separations of biological origin (macromolecules)
- Purification of synthetic organic polymers
- Analysis of tertiary and quaternary structure of purified protein.

Paper Chromatography (Partition):-

In this method of separation the mixture of compounds by using specially designed chromatographic paper as stationary phase into individual compounds.

Components:-

- Stationary phase and papers – filter paper of different grades, paper impregnated with silica or alumina.
- Mobile phase – mixture of solvents, pure solvents (methanol: water - 4:1).
- Sample applicator.
- Chromatographic chamber.

Principle:-

In paper chromatography, partitioning and absorption occur both. However, the primary one is partition chromatography in which the compounds are divided in two liquid phases. The movement of mobile phase, due to the capillary action of pores in the paper, separates the mixture compounds.

Procedure:-

The sample mixture is placed on the piece of chromatography paper which is later place in a container solvent. Individual components travel to a varying degree of distances based on the various in their adsorbent and solvent affinity. Polar molecules are adsorbed onto the filter paper and transported to smaller distances while non-polar molecules migrate further. The extent of movement of components is measured by calculating the “Rf value”.[15]

Rf value is defined as the distance travelled by the component from application point divided by distance travelled by solvent from application point. Rx value is the ratio of distance travelled by the sample and the distance travelled by the standard. Rx value is always closer to one. Rf value is always less than one but Rx can be greater than one. The factors affecting the Rf value are the solvent system and its composition, temperature, pH of the solution, quality of paper and adsorbents and distance through which the solvent runs. [16]

Modes of paper chromatography:-

The following methods apply, based on the way of chromatogram is formed on the paper.

- **Ascending chromatography:** Like conventional type the solvent moves in upward direction on the paper. The solvent tank are at the bottom of the beaker.
- **Descending chromatography:** Here the solvent reservoir is at the top. Besides capillary action solvent movement is facilitated by gravity (solvent flows down the paper).

- **Ascending-descending mode:** A combination of two methods above, where the solvent first flow upward and than down the paper.
- **Circular/ Radial mode:** Here the solvent travels from the centre towards the periphery of circular chromatography paper. For the production of chromatogram the hole system is placed in sealed petridish. The solvent flows through a central packed wick on to the surface. The sample travels like concentric ring along with mobile phase to form spots of different compounds.
- **Two-dimensional development:** The samples are spotted on one end of rectangular paper and allow to be established. The second chromatogram is evolves opposite to the preceding one by eventually immerging it in the mobile phase. Hence, the chromatogram development takes place in two directions at right angles. [16]

Advantages:-

- Simple and easily available equipment.
- Better efficacy of separation.
- Closely related homologous, isomers, isotopes and very labile, reactive substances can be separated.

Applications:- Specially used for isolation of polar and non-polar compounds from mixtures. It also use for separation of amino acid, pigments, dyes and inks. To recognize organic and other biochemical compounds in urine, for hormones and medicine determination, evaluation of inorganic compounds like complexes and salts.

Thin Layer Chromatography (Liquid-Solid interaction):-

TLC exists mainly as a complementary technique to other column based liquid chromatographic methods to provide additional knowledge in separations (multi-modal separation techniques). TLC plays a crucial role in the early phase of drug development when there is insufficient information on impurities and degradation products in drug substance and drug product.[17]

Principle:-

TLC operates upon the absorption principle. Nonetheless there is normally adsorption and partition or a mixture of both. Elements with more affinity fly slower and vice versa.

Components:-

- TLC plates – stable and chemically inert plates used as a support for stationary phase (glass, plastic, or aluminium support)
- TLC chambers – used for the development of TLC plate, maintenance of uniform environment.
- Stationary phase (solid phase) – thin layer (0.25 mm thick) of adsorbent coated on a TLC plates.
- Filter paper – prevents the evaporation of solvents.
- Mobile phase – comprises of a solvent or solvent.

Procedure:-

The sample mixture spots are placed, near the bottom of the thin layer plate. Solvents are allowed to percolate up the plate by capillary action. The chamber is saturated with solvent vapor so as to prevent the solvent evaporating from the plate surface and also controlling the retention mechanism by surface deactivation. The plate is then placed in the chamber without allowing dipping of sample spot. A constituent that is strongly adsorbed will move slower. Results are represented by Rf value same as in paper chromatography.[18]

Factors determining the efficacy:-

The efficiency of chromatographic separation is determined by several factors such as selectivity of adsorbent's towards the separating substances and the adsorbent strength. Some of the commonly employed adsorbents in the decreasing order of their adsorptive power are as follows, alumina (Al_2O_3), charcoal (C), florisil [MgO/SiO_2 (anhydrous)] and silica gel (SiO_2).[18]

Advantages:-

- Quick, simple, inexpensive high sample throughput technique.
- Wide choice of the mobile phases.
- Sample preparation is minimum.

- ❖ Several samples can be run simultaneously using mobile phase in small quantity.
- ❖ Used in analytical laboratories for limited resources.

Applications:-

- ❖ It is used for separation of all types of natural products. E.g., acids, alcohols, amines, amino acids and proteins, etc.
- ❖ Mostly used for identification and purification.
- ❖ To check the performance of other separation processes.
- ❖ To measure the reaction process by assessment of intermediates, reaction course, etc.
- ❖ For separation of Inorganic Ions – Used for separating cationic & anionic substances.
- ❖ Separation of vitamins – Vitamin E, Vitamin D3, vitamin A.
- ❖ Quantitative analysis.

Supercritical Fluid Chromatography:-

Supercritical fluid chromatography (SFC) uses highly compressed gas over its critical temperature and pressure instead of an organic solvent as the solvent phase. The SFC detection systems are the ones that commonly used in GC. The main advantage of SFC is widely used in the detecting system in GC. i.e. FID, and the allowance for thermal unstable compounds in research.

Gases are commonly used such as carbon dioxide, nitrous oxide, and ammonia. Organic solvents such as methanol, isopropanol, methylene chloride, tetrahydrofuran, and acetonitrile are frequently employed in HPLC as modifiers to increase solvent strength.[19]

Principle:-

Supercritical fluid chromatography encompasses all chromatographic methods in which the mobile phase become supercritical under the analytical conditions, with the fluid's solvating properties having a measurable effect on the separation. SFC has several advantages over GC and HPLC: it expands the molecular-weight spectrum of GC, it can isolate thermally labile compounds at lower temperatures, compounds without chromophores can be sensitively detected, and the use of open-tubular and packed columns is feasible.[20]

Applications:-

SFC has been applied to a wide variety of materials, including Natural products, drug, foods, pesticides and herbicides, surfactants polymers, and polymer additives, fossil fuels, and explosives and Propellants.[20]

Conclusion :- Chromatography technique involve various type of techniques to separate the various types of component mixture, which include amino acid, bio-molecules, crude drug, medicines, oil, hydrocarbons, enzymes, organic polymer, biological resin, pesticides, herbicides, surfactant polymer, fossil fuels, acids, alcohols, amines, vitamins etc. chromatography can separate, purify, quantify, qualitatively measure the components. Selecting a right absorbing material can give a good result in separation due to different of the mixture which may be liquid, gas, volatile, insoluble etc. [20]

Abbreviation :- WCOT-wall coated open tubular, SCOT -sorbent coated open tubular, PCOT -porous layer open tubular, G.C.-gas chromatography, IEC- ion exchange chromatography, HPLC- high performance liquid chromatography, TLC- thin layer chromatography, HPTLC- high performance thin liquid chromatography, TCD-thermal conductivity detector, RT- retention time, Retardation factor, NP- normal phase, RP-reversed phase, RF-retardation factor, CC- column chromatography.

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