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Review Paper

An Overview on The Chromatography and Chromatography Technique

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ABSTRACT

Chromatography is a widely used technique that separates and identifies the components of complex mixtures. It works on the principle of differential movement of compounds through a stationary phase when influenced by a mobile phase. Depending on the interaction between the mixture's components and the phases, the compounds move at different rates, leading to their separation. There are several types of chromatography, including liquid chromatography (LC), gas chromatography (GC), thin-layer chromatography (TLC), ion-exchange chromatography, and size-exclusion chromatography. Liquid chromatography (LC) is commonly used to analyze liquid mixtures in fields like pharmaceuticals and environmental studies. Gas chromatography (GC) is ideal for analyzing volatile compounds, such as in environmental monitoring or forensic analysis. Thin-layer chromatography (TLC) is a simpler and more affordable method for qualitative analysis, like identifying compounds or tracking reaction progress. Ion-exchange chromatography separates charged particles, and size-exclusion chromatography is used for separating molecules based on size, often applied in protein and polymer analysis. Chromatography instruments typically include columns, detectors (such as UV/Vis or mass spectrometers), pumps, and injectors. The choice of method and equipment depends on the specific analysis needs. This technique plays a crucial role in areas like drug development, food safety, and biotechnology, where precise separation and identification of substances are critical.

INTRODUCTION

Chromatography is an analytical method used to identify, separate, and purify the components of a mixture. This operates on the basis of the solute's differential interaction with two distinct phases (the stationary phase and the mobile phase). Many compounds can be found using this method. They manage. Instead of keeping the components separately in the pure solvent according to their solubility, the combination to be separated in a stationary phase (solid or liquid) and a pure solvent, such as water or any mixed gas (mobile phase), is permitted to flow slowly.(1)

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Chromatography can be analytical or preparatory. Preparatory chromatography is a type of purification since its goal is to separate a mixture's constituent parts for use at a later time. Typically, analytical chromatography is carried out using lower quantities of material and is used to determine whether analytes are present in a mixture or to measure their relative amounts. There is no mutual exclusion between the two.(2).

2)History of Chromatography:

1. The first real chromatography technique is usually credited to Mikhail Tswet, a Russian-Italian botanist.
2. In the 1890s, Tswet developed column separation methods to isolate different petroleum compounds. His approach was inspired by earlier experiments using filter paper.(3)
3. He later used a liquid adsorption column filled with calcium carbonate to separate plant pigments like xanthophylls (yellow), carotenes (orange), and chlorophylls (green).
4. Over time, chromatography has become a very important tool in both biological and physical sciences, helping scientists study and separate different substances.
5. In fact, between 1937 and 1972, twelve Nobel Prizes were awarded for research where chromatography played a key role, showing how valuable this technique is in scientific discoveries.(4)

3) Principle Of Chromatography:

Chromatography is a technique used to separate the different components of a mixture. It works on the idea that when a mixture moves through a material (called the stationary phase) with the help of another substance that flows (called the mobile phase), the individual parts of the mixture separate from each other. The separation happens because different molecules interact differently with the stationary and mobile phases. Some stick more to the stationary phase and move slowly, while others move faster with the mobile phase. This difference in movement helps separate the components.(5)

There are three main parts in any chromatographic method:

1. **Stationary Phase** – Usually a solid or a liquid coated onto a solid.
2. **Mobile Phase** – A liquid or a gas that carries the mixture.
3. **The Sample** – The mixture that needs to be separated.

4) Types Of Chromatography:

4.1) Adsorption Chromatography
4.2) Partition Chromatography
4.3) Thin Layer Chromatography
4.4) Paper Chromatography
4.5) Gas Chromatography
4.6)High Performance Liquid Chromatography
4.7)High Pressure Thin Layer Chromatography(6)

4.1) Adsorption Chromatography:

Adsorption chromatography is one of the oldest methods used for separating mixtures. It works by passing a liquid or gas (called the mobile phase) through a solid material (called the stationary phase). The different components in the mixture stick to the solid surface to different extents. The ones that stick more strongly move slowly, while those that stick less move faster. This difference in movement helps separate the components. Common types of adsorption chromatography include column chromatography, gas-solid chromatography, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC).(7)

Applications of Adsorption Chromatography:

1) Amino Acid Separation:
- Its helps in separating individual amino acids from a mixture for analysis or research.
2) Antibiotic Purification:
- Used to isolate specific antibiotics from complex mixture during drug development.
3) Fat and Fatty Acid Analysis:
- Helps in distinguishing and identifying various fats and fatty acids, often used in nutrition and food testing.
4) Protein and Peptide Studies:
- Used to isolate and study proteins and peptides, especially in biochemical and pharmaceutical research.

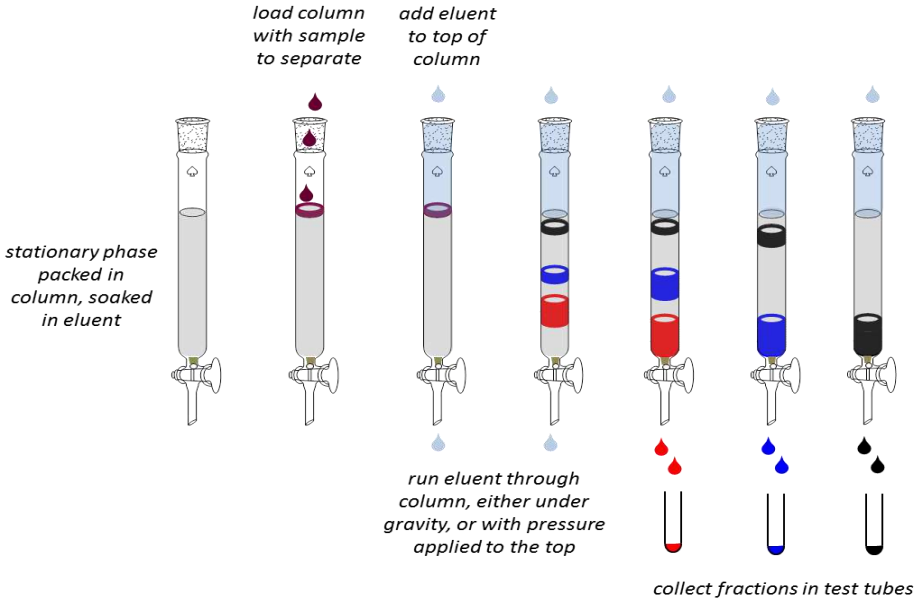

Fig no.01

4.2) Partition Chromatography:

Partition chromatography works by using a thin layer of liquid that coats the surface of a solid support. In this method, the separation happens based on how the solute distributes itself between this stationary liquid layer and the moving (mobile) phase. The solutes that dissolve more in the stationary liquid move slowly, while those that prefer the mobile phase move faster. Common types of partition chromatography include paper chromatography, thin-layer chromatography (TLC), gas-liquid chromatography (GLC), high-performance liquid chromatography (HPLC), and partition column chromatography.(2,4)

Applications of Partition Chromatography:

1) Cleaning protein solution:
- It is used to remove unwanted detergents from solutions containing proteins.
2) Separation of specific compound:
- Helps in separating substances like mycotoxins, bile acids, and steroids from complex mixtures.
3) Removing harmful chemical:
- Useful for getting rid of insecticides, pesticides, and phenols from samples during analysis.
4) Detecting trace metal:
- Can be used to measure very small amounts of metal ions in water and other liquid samples.

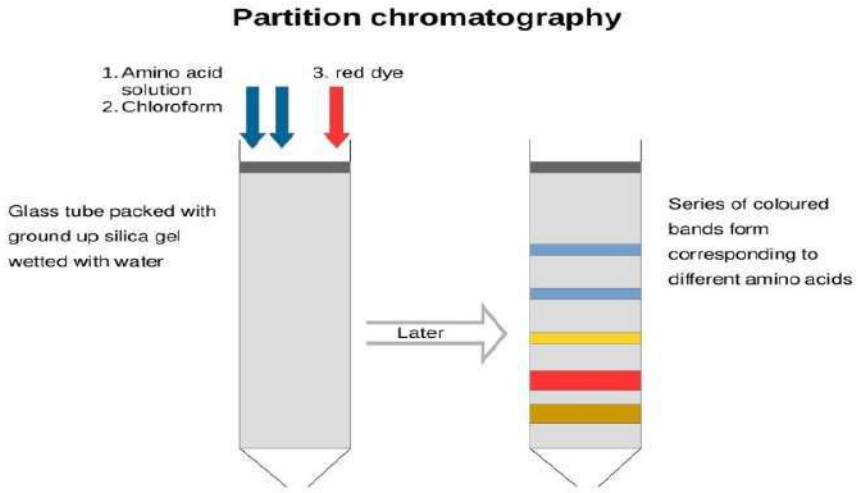

Fig no.02

4.3) Thin layer Chromatography:

Thin Layer Chromatography, or TLC, is a quick and simple method used to separate and analyze the components of a mixture. It helps identify how many compounds are present, check the identity of substances, and determine the purity of a sample. TLC is also useful for tracking the progress of a chemical reaction by showing how products form or reactants disappear. It's a very sensitive technique, able to detect even very small amounts (as little as a microgram), and the entire process usually takes only 5 to 10 minutes.(8) After drying a TLC (Thin Layer Chromatography) plate, we can see the separated spots or bands either by their absorbance or fluorescence. TLC is a common method used in labs to identify the compounds in a mixture and to track the progress of chemical reactions. The separation in TLC happens because the components of the mixture interact differently with the stationary phase (a thin solid layer) and the mobile phase (a liquid solvent).There are various chromatography methods like column chromatography and paper chromatography. Among these, TLC is one of the most frequently used. It's quite similar to paper chromatography, where paper serves as the stationary phase. But in TLC, instead of paper, a thin layer of adsorbent material (like silica gel) is used, which allows the process to be faster and gives more precise separation.(9)

Principle of Thin Layer Chromatography (TLC):

In Thin Layer Chromatography (TLC), a solid surface—usually a glass or plastic plate—is coated with a thin layer of materials like silica gel or aluminium oxide. This coating is called the stationary phase. The mobile phase is a liquid solvent chosen based on the chemical properties of the substances being tested.To perform TLC, a small amount of the substance or mixture is placed as a dot near the bottom of the plate. This spot acts as the starting point. The plate is then placed upright in a closed container (called a developing chamber) that has a shallow layer of the solvent at the bottom.As the solvent moves up the plate by capillary action, it carries the components of the mixture with it. Depending on how well each substance dissolves in the solvent, they will either move along with the solvent or stay attached to the stationary phase.(10) The movement of each compound depends on its molecular structure and functional groups. The rule **"like dissolves like"** helps explain which compounds will travel farther—substances that are more similar to the

solvent will dissolve better and move up the plate. Meanwhile, substances that interact more strongly with the stationary phase will stay behind or move less. In short, TLC separates substances based on how strongly they stick to the plate versus how easily they dissolve in the solvent.(10)

Procedure of TLC:

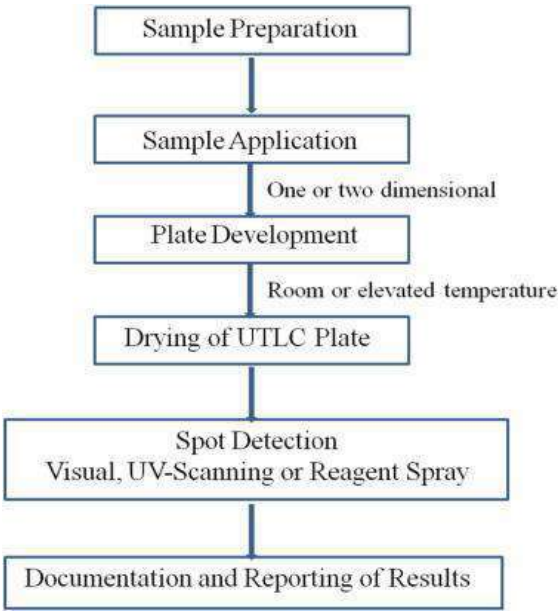

Fig no.03

Rf value: In Thin Layer Chromatography (TLC), how a compound moves is measured using a value called **R*f*** (Retention factor). It's written as a decimal number. To find the R*f* value, you divide the distance the compound moved from the starting point by the distance the solvent moved (which is called the solvent front). This helps us understand how far a particular substance travels on the TLC plate.(1)

$$R_f = \frac{\text{Distance of centre of spot from starting point}}{\text{Distance of solvent front from starting point}}$$

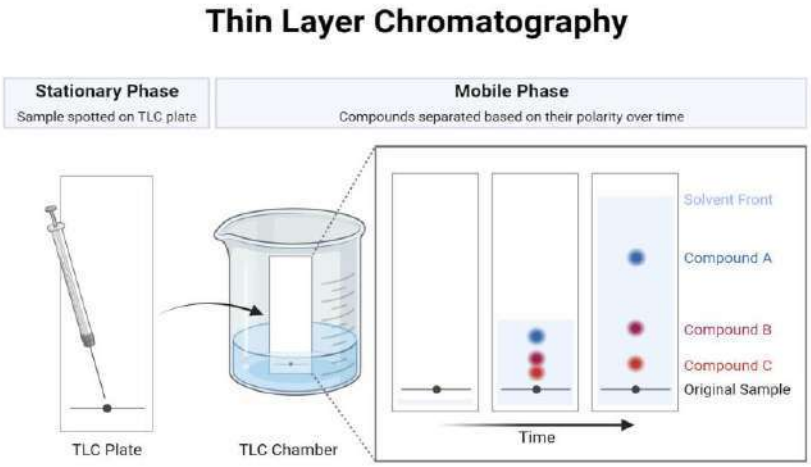

Fig No.04

Applications of Thin Layer Chromatography (TLC):

 It helps in separating different types of natural substances like acids, alcohols, amines, and large molecules such as amino acids and proteins.

 It is often used to identify and purify different compounds.
 It can be used to check how effective other separation techniques are.
 It is useful for separating vitamins like Vitamin E and Vitamin D3.
 It allows for the accurate measurement of Vitamin A content.(11)

Advantages of TLC:

 It's a straightforward method and doesn't require expensive equipment.
 It's a fast process and much quicker than column chromatography.
 Even small amounts of substances, like in milligrams, can be separated effectively.
 It can be used to analyzed almost any kind of compound.
 Very fine particles can be used, which improves how well substances are separated. Also, flow rate control isn't a big concern in this method**.**(12)

Disadvantages of TLC:

 Since the TLC plate is short, the separation can only happen over a limited distance.
 It can't be used to separate substances that easily evaporate, which limits its usefulness.
 TLC works only if the components in the mixture can dissolve in the chosen solvent.
 It can be hard to get the exact same results each time you repeat the experiment.
 TLC mainly helps in identifying what's in a sample, but it's not suitable for measuring exact amounts.(11)

4.4) Paper Chromatography:

Chromatography is a powerful method used to separate and study the different parts of a mixture. It works by using two phases: one that stays still (called the stationary phase) and another that moves (called the mobile phase). As the mobile phase flows through the stationary phase, the different substances in the mixture move at different speeds based on how they interact with each phase. This allows scientists to identify, purify, and measure the components. It's widely used in labs and industries for analyzing chemical mixtures.(13)

Principle of paper chromatography:

Paper chromatography works on the principle of partitioning, where the components of a mixture are divided between two liquid phases. In this method, the filter paper acts as the stationary phase by holding water inside its tiny pores, while the mobile phase is a solvent that travels across the paper. As the solvent moves, the substances in the mixture separate based on how much they are attracted to the water in the paper and the moving solvent. This helps to sort out the different chemicals in the mixture.(14) The separation in paper chromatography mainly happens because of capillary action—this is when the liquid moves through the tiny pores in the paper, carrying the mixture's components along with it. Each substance moves at a different speed based on how well it dissolves in the solvent and how it interacts with both phases. In some cases, separation can also happen through adsorption, where the substances stick to the paper's surface, which acts as the stationary phase. While partitioning is the main method at work, adsorption can also influence the separation in certain situations.(15) In partition chromatography, both the stationary and mobile phases are liquids. The stationary phase is usually water that's absorbed into the paper, and it's polar in nature. A small amount of the sample is placed near one edge of the paper and left to dry. Then, the edge of the paper is placed in a solvent. Due to capillary action, the solvent starts to travel across the paper—either upwards (ascending) or downwards (descending). As the

solvent moves, it carries the different parts of the sample with it, and they separate into visible spots or zones along the paper.(13)

Modes Of Paper Chromatography:

4.4.1) Ascending Chromatography: Ascending chromatography is a technique where the solvent moves upward through a strip of paper, carrying the components of a sample along with it. First introduced by Condens, Gordon, and Martin—and later improved by Williams and Kirby—this method involves placing a spot of the sample on a piece of paper and then dipping the bottom edge of the paper into a solvent in a container. As the solvent travels upward by capillary action, it separates the different parts of the sample based on how they interact with the solvent and paper. It's important to make sure the sample spot is above the solvent level and to use a paper size that fits well in the container to avoid it getting bent or crumpled.(15)

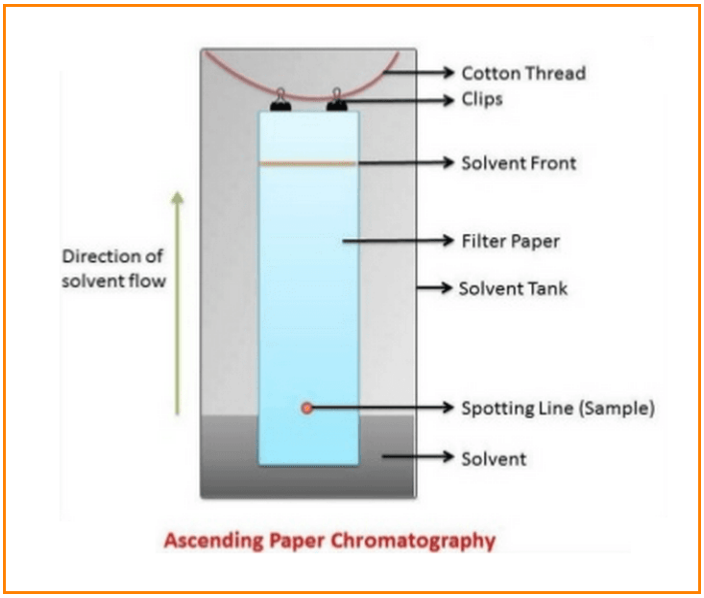

Fig no.05

4.4.2) Descending Chromatography:

Descending chromatography is a method where the solvent flows downward along the paper, helped by gravity. In this setup, the solvent is placed at the top, allowing it to move down over the sample. This technique works well for separating substances that don't travel far with the solvent (i.e., those with low Rf values) and usually takes less time than ascending chromatography. It also offers a steady flow of solvent. However, it often requires bigger or more specialized equipment compared to the ascending method.(16)

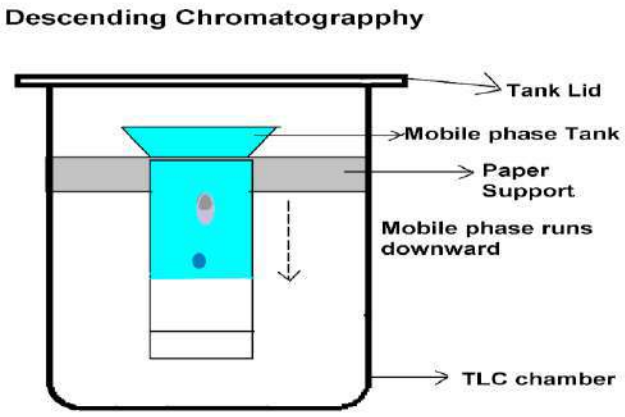

Fig no.06

4.4.3) Ascending- Descending Chromatography: This method allows the solvent to travel both upward and downward on the same piece of paper. It takes advantage of both ascending and descending chromatography, offering benefits like faster separation, better separation of components with higher Rf values, and a longer path for the solvent, which improves the clarity of the results. However, even with these improvements, the Rf values usually remain similar to those from regular ascending or descending methods.(17)

4.4.4) Two-Dimensional Chromatography: Two-dimensional chromatography, also called circular chromatography, is a great method to separate different organic and inorganic substances. In this method, we use a circular paper and put a small drop of the sample near one edge or corner. First, we let the solvent move in one direction and spread the sample. Once that's done, we rotate the paper at a right angle (90 degrees) and dip it again in the same solvent. This time, the solvent moves in a different direction. This two-way process helps to separate the components more clearly and effectively.(18)

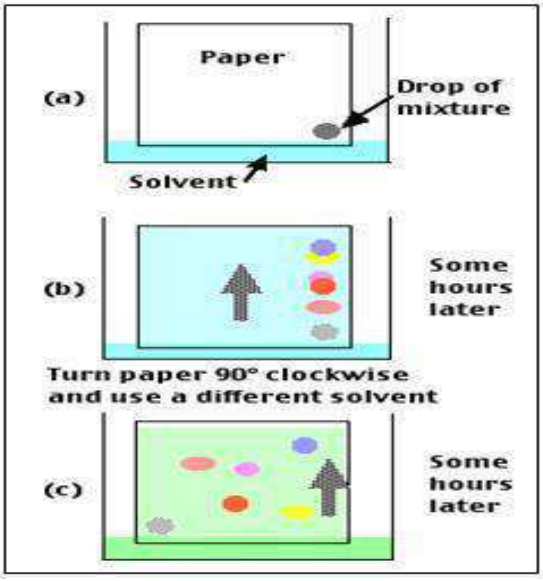

Fig no.07

Advantages of Paper Chromatography:

1. **Needs Only a Small Sample**: Paper chromatography works well even with tiny amounts of material, which is useful when the sample is limited.

2. **Low Cost**: It's a budget-friendly method since it doesn't require expensive tools or materials.

3. **Helps Identify Unknowns**: This technique is good for figuring out what substances are present in a sample, especially for unknown chemicals or inorganic compounds.

4. **Takes Up Little Space**: It doesn't need much room or large equipment, so it's ideal for small or crowded labs.(19)

Disadvantage of paper Chromatography:

1. **Small Sample Handling**: Paper chromatography can't manage large amounts of sample, so it's not ideal for experiments that need to process bigger quantities.

2. **Not Suited for Accurate Measurements**: This method isn't the best for precise or detailed measurements, especially when compared to more advanced techniques like HPTLC or HPLC.

3. **Difficulty with Complex Samples**: It may not separate complicated mixtures effectively, which can limit how useful it is for analyzing more detailed or mixed samples.(20)

4.5) Gas Chromatography:

Gas chromatography (GC) is a popular method used to separate and study gases or substances that easily turn into gas. It was invented in 1952 by James and Martin. Even though it was first used to separate amino acids, today it's used in many different fields because it's fast and very accurate. GC can be used to identify substances (qualitative analysis) and to measure how much of a substance is present (quantitative analysis), even if it's just a tiny amount. In this method, the sample is dissolved in a liquid, turned into gas, and then separated. GC works by using two phases — the stationary phase (which stays still) and the mobile phase (which moves). The mobile phase is usually an unreactive gas like helium or nitrogen. One special thing about GC is that the moving gas doesn't react with the substances being tested.(21)

Principle Of Gas Chromatography:

In gas-solid chromatography, a solid material is used to separate components based on how they stick to it. In gas-liquid chromatography, a liquid-coated solid helps in the separation. The sample is first turned into gas and mixed with a carrier gas like helium or nitrogen. This mixture passes through a column, where different parts of the sample move at different speeds and get separated.A detector measures how long each part takes to come out and how much of it is present. To find out the concentration of a substance, we compare the results to a known standard sample.(22)

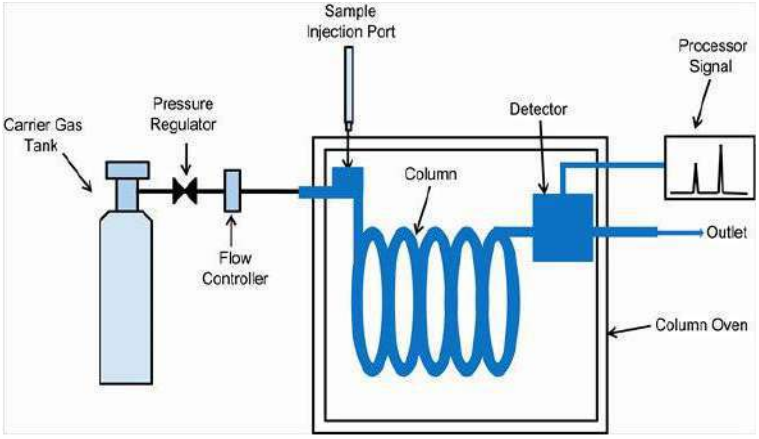

Fig no.08

Instrumentation Of Gas Chromatography:

1)Pressure Regulator: A cylinder regulator reduces the high pressure from a gas cylinder to a safe level for the Gas Chromatograph (GC). There are two types: **single-stage** and **double-stage**. Single-stage regulators are less stable and need frequent adjustments, while double-stage regulators provide more consistent pressure, making them better for GC use.(23)

2)Sample Injector: The GC column, the sample should be small and injected quickly, like a single shot or "slug." If the sample is too big or injected slowly, it can spread out and reduce accuracy. Usually, a microsyringe is used to inject the sample through a rubber septum into the injector port at the top of the column. The injector is heated to about 50 °C higher than the boiling point of the least volatile part of the sample. For packed columns, sample size is between a fraction of a microliter to about 20 microliters. But for capillary columns, much smaller samples are used—around 0.001 mL. In capillary GC, a split/splitless injector is used depending on how much sample you need to enter the column.(23)

3)Columns: There are two main types of GC columns: packed columns and capillary (open tube) columns.

Packed columns are filled with a fine solid material (like diatomaceous earth) that's coated with a liquid stationary phase. They are usually 1.5 to 10 meters long and 2 to 4 mm in inner diameter. Capillary columns are much thinner, with an inner diameter of only a few tenths of a millimeter. There are two types:

- WCOT (Wall Coated Open Tube): the liquid stationary phase is coated directly on the inner wall.
- SCOT (Support Coated Open Tube): the inner wall has a thin layer of solid support (like diatomaceous earth), which holds the liquid phase.(23)

4) Detector: The most common detectors in GC are FID (Flame Ionization Detector) and TCD (Thermal Conductivity Detector).

TCD is non-destructive, which is a benefit, but it's not very sensitive for most substances, so it's not used as often.

FID is much more sensitive—especially for hydrocarbons—and is better at detecting organic compounds. However, FID doesn't detect water or carbon dioxide. Overall, FID is 2–3 times more sensitive than TCD and is great for analyzing

organic substances in environmental samples.(23,24)

Advantages of Gas Chromatography:

- High sensitivity and accuracy
- Fast analysis
- Good separation of complex mixtures
- Requires small sample size
- Can be automated

Disadvantage of Gas Chromatography:

- Only works for volatile and thermally stable compounds
- Expensive equipment
- Not suitable for large or non-volatile molecules
- Requires skilled operators

4.6) High Performance Liquid Chromatography:

High-Performance Liquid Chromatography (HPLC) is a technique used to separate, identify, and measure components in a mixture. It works by pushing a liquid sample through a column under high pressure. Different compounds in the sample interact differently with the column material, causing them to come out at different times (retention times). HPLC uses a pump, column, and detector, and the separation depends on the chemical properties of the sample, the solvents used, and the type of column.(25)

Principle Of HPLC:

The basic principle of HPLC is that a liquid sample is injected into a column filled with a solid, porous material (called the stationary phase). A liquid (the mobile phase) is pumped through this column at high pressure. The separation happens because different parts of the sample stick to the stationary phase with different strengths, depending on their chemical properties.(26)

HPLC is a type of column chromatography, but it works much faster because it uses high pressure to move the liquid through the column. This pressure allows the use of very small particles in the column, which improves how well the system separates the components and reduces the time needed for analysis compared to traditional methods.(27)

Advantages Of HPLC:

 HPLC gives sharp and clear separation of compounds quickly.
 It provides a large surface area for interaction between the sample and the stationary phase.
 It operates under high pressure, which helps push the solvent through the column efficiently.
 A variety of stationary phases are available, making it suitable for analyzing many types of compounds.
 The flow rate of the mobile phase is accurately controlled for consistent and reliable results.(28)

Disadvantages Of HPLC:

 HPLC systems are quite expensive to buy and set up.
 It needs top-quality parts and accessories to work properly.
 The chemicals and columns used can be costly.
 It requires frequent upkeep and calibration, which increases overall expenses.
 Advanced software is needed to analyze the data accurately.
 The cost of developing new methods or doing research with HPLC is also high.(28)

4.7) High Pressure Thin layer Chromatography:

High performance thin-layer chromatography, or HPTLC, is a more sophisticated and automated form of thin-layer chromatography (TLC) with better and enhanced separation efficiency and detection limits. It is also known as high-pressure thin layer chromatography, planar chromatography, or flat-bed chromatography. It is a useful analytical method that can be applied to both quantitative and qualitative issues. Depending on the kind of adsorbents used on the plates and the solvent system used in the development, separation may result from partitioning, adsorption, or both. Applications have included finger print analysis, biomedical analysis, phytochemical analysis, herbal drug quantification, analytical analysis, and the possibility of hyphenation (HPTLC-MS, HPTLCFTIR, and HPTLC-Scanning Diode Laser). Principle, theory, and comprehension are just a few of the facets of HPTLC fundamentals that have been discussed.(29)

Principal Of HPTLC:

This is a powerful analytical method that is effective for both quantitative and qualitative assessments. The nature of the adsorbents used on the plates and the solvent system chosen for the development can lead to separation through either partitioning, adsorption, or a combination of both processes. The solvent in the mobile phase moves through the medium due to capillary action. The substances migrate toward the adsorbent based on their affinities. Components with a stronger attraction to the stationary phase exhibit slower movement. Conversely, components with a weaker attraction to the stationary phase travel more rapidly. As a result, the components are effectively separated on a chromatographic plate.(29,30)

Advantages Of HPTLC:

1)shorter time frame for analysis.

2)Reduce how much solvent is required.

3)Handling multiple samples.

4)High levels of adaptability

5)Nanograms of the sample can be detected.

6)enhanced sensitivity and accuracy.

Disadvantages Of HPTLC:

1)require a large space.

2)sophisticated machinery

3)It is necessary to have someone with technical expertise.(29,31)

CONCLUSION:

chromatography is an essential analytical technique that enables the separation and identification of complex mixtures. With various types like liquid chromatography, gas chromatography, and thin-layer chromatography, it serves a wide range of applications across industries, including pharmaceuticals, environmental monitoring, food safety, and biotechnology. By leveraging different stationary and mobile phases, chromatography provides precise results for both qualitative and quantitative analysis. The choice of method and instruments, such as detectors and chromatographic columns, ensures optimal separation based on the sample's nature. Ultimately, chromatography is vital for advancing research and ensuring quality control in numerous fields

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