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An Overview On Analytical Methods

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ABSTRACT

Separation is crucial in chemistry because it helps analyze substances more accurately. It allows scientists to split components in a sample into separate groups or areas, either in space or time. This separation is important because it isolates different parts of the sample, making it easier to study them without interference from other substances mixed in. It's also useful for purifying specific substances from mixtures or natural sources so scientists can study them more closely. Separation helps make tests more sensitive by gathering the substances we want to study in higher amounts. Modern techniques for separation are well-established and widely used in scientific research. However, as analytical tasks become more complex, there's a growing need for even more advanced separation methods. This has motivated us to put together this collection, which explores trends in this practical, diverse, and always evolving field.

KEYWORD

chromatography, analytical techniques, hplc , uplc

INTRODUCTION

Pharmaceutical Analysis is a branch of analytical chemistry focused on studying drugs, both in their pure forms and in their formulated products. Its main goals are to identify substances, purify them, separate mixtures, and determine the structures of chemical compounds.

Types of Pharmaceutical Analysis:

1. Qualitative Analysis: This method identifies the chemical compounds present in a sample.
2. Quantitative Analysis: This measures the amounts of individual components in a mixture.
3. Semi-Quantitative Analysis: This estimates the presence of specific impurities in a sample.

Analytical Techniques:

Various techniques are used in pharmaceutical analysis, including:

Chromatography

- Electrochemical methods
- Titration
- spectroscopy
- Electrophoresis

These methods help in analyzing both drug substances and drug products in the pharmaceutical industry.

CHROMATOGRAPHY

Chromatography is a method used to separate a mixture into its individual parts. The term comes from Greek words meaning "colour" and "write." In chromatography, a mixture is dissolved in a fluid called the mobile phase, which moves through a stationary phase.

There are two main types of chromatography:

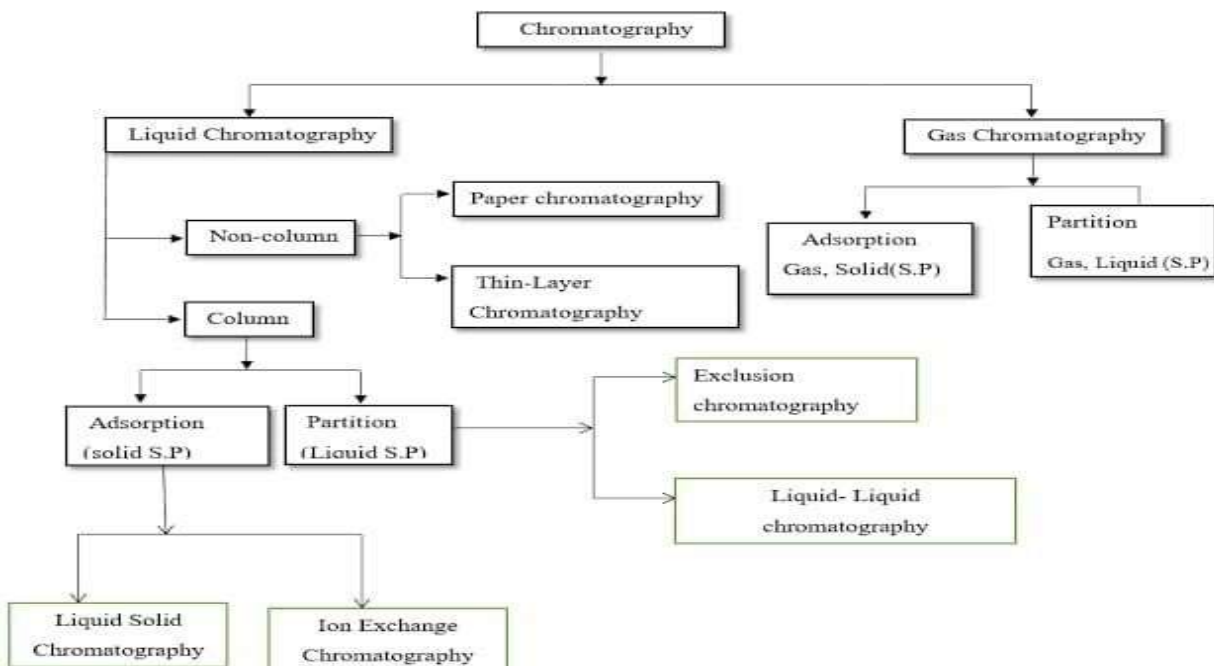
1. **Preparative Chromatography** : This is used to separate and purify the different components of a mixture.
2. **Analytical Chromatography** : This involves smaller amounts of material and is used to identify or measure the amounts of different substances in a mixture.

HISTORY

Chromatography was first created in Russia by Mikhail Tsvet, an Italian-born scientist, in 1900. He used a column filled with calcium carbonate (chalk) to separate plant pigments in a colourful way. In the 1930s, new types of chromatography were developed, making the technique useful for various separation processes.

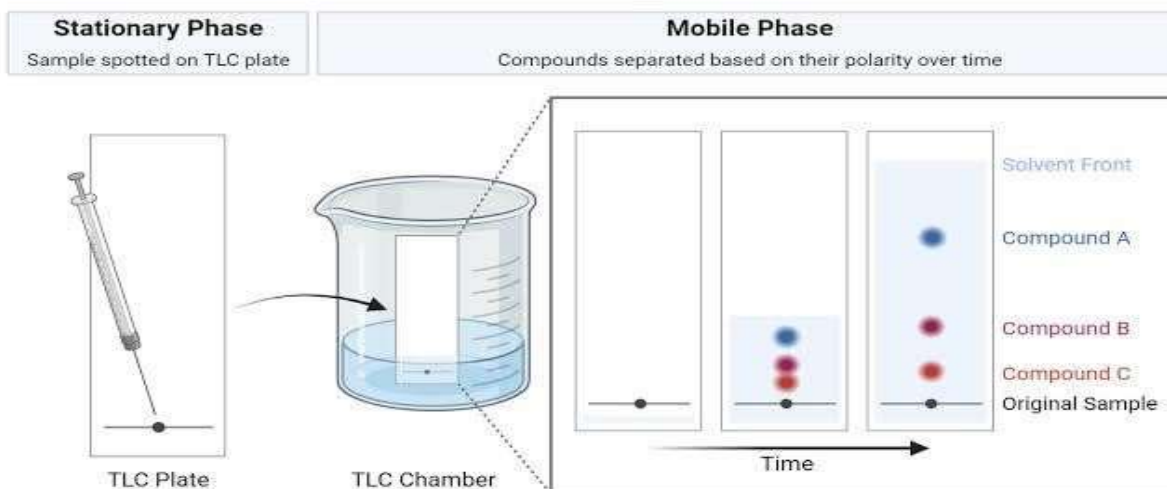
In 1952, Archer John Porter Martin and Richard Laurence Millington won the Nobel Prize in Chemistry for their work on partition chromatography. Their research helped advance chromatography methods, leading to techniques like paper chromatography, gas chromatography, and high-performance liquid chromatography (HPLC). Since then, the technology has improved rapidly.

Principle of Chromatography: Chromatography is a physical separation method where the components of a mixture are separated using two phases. One phase is the stationary phase, which is a solid or a liquid that doesn't move, and the other is the mobile phase, which is a fluid that flows over the stationary phase.



THIN LAYER CHROMATOGRAPHY (TLC)

Thin Layer Chromatography



Thin-Layer Chromatography (TLC) :

What It Is: Thin-layer chromatography (TLC) is a technique used to separate non-volatile mixtures. It is done on a flat surface, like glass, plastic, or aluminum foil, that is coated with a thin layer of adsorbent material, typically silica gel, alumina, or cellulose. This coating is called the stationary phase, while the liquid used for separation is the mobile phase.

How It Works:

The separation happens through *adsorption chromatography*. Small amounts of the mixture are applied to the adsorbent layer. A solvent (the mobile phase) moves up the plate through capillary action. The different components of the mixture travel at different speeds based on how much they stick to the adsorbent. Those that stick more move slowly, while those that stick less move faster.

Advantages:

- Simple and Cost-Effective: Easy to set up and requires inexpensive equipment.
- Fast: Quick process for separating mixtures.
- Easy Detection: Components can be easily spotted and analyzed.
- Less Resource-Intensive: Requires less solvent and time compared to other methods like column chromatography.

Disadvantages:

- Time-Consuming: Can take longer for complex separations.
- Higher Solvent Costs: Requires more solvent for larger samples, making it more expensive.
- Complex Automation: Automating the process can be complicated and costly.
- Low Separation Power: May not separate very similar compounds effectively.

Applications:

- Separation of Mixtures: Useful for separating drugs, plant extracts, and other substances.
- Analysis of Nutrients: Separates carbohydrates, vitamins, antibiotics, proteins, alkaloids, and glycosides.
- Identification: Helps identify related compounds in pharmaceuticals.
- Contaminant Detection: Can detect unwanted substances in drugs.

COLUMN CHOMATOGRAPHY

Column chromatography was invented by American chemist D.T. Day in 1900. In 1906, Polish botanist M.S. Tsweet used this method to separate plant pigments, which is why it's also called adsorption chromatography.

How It Works:

- Equipment: It uses glass tubes with a diameter of 40 to 50 mm, filled with a stationary phase (the material that helps separate the components) that has particle sizes between 150 to 200 micrometers.
- Column Size: The columns can vary in size from a few millimeters to several centimeters in diameter and can be several centimeters to meters long.

Efficiency:

- Longer, narrower columns usually provide better separation than shorter, wider ones.
However, if a column is too long, the flow rate can be very slow, making the process inefficient.

PRINCIPLE

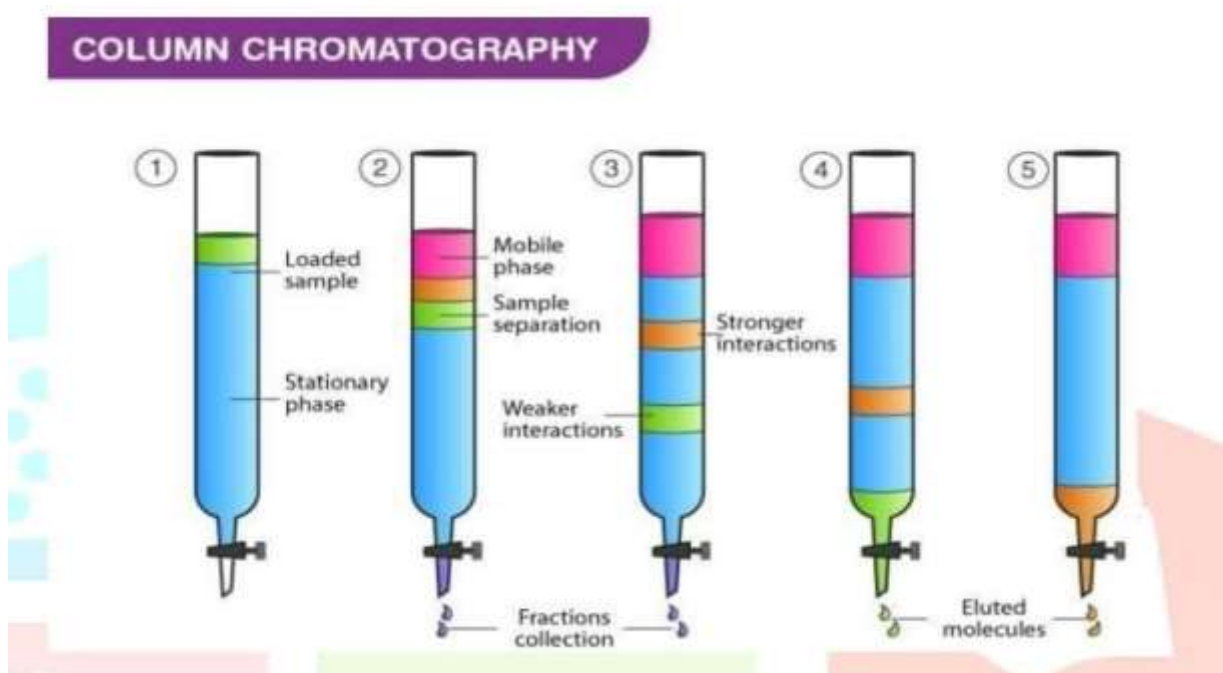
The principle of column chromatography is based on the different affinities that components of a mixture have for the stationary phase (the material in the column) and the mobile phase (the solvent).

In this technique, the column is filled with a stationary phase, and the mixture to be separated is dissolved in a suitable solvent. As the mixture moves through the column, different components interact with the stationary phase differently. Components that are more strongly attracted to the stationary phase move more slowly and are retained higher in the column, while those that are less attracted move down more quickly.

As a result, distinct layers or "zones" of different components form in the column, creating a chromatogram. There are two common ways to collect the separated components after the chromatography process:

1. ***Elution by Cutting***: After the separation is complete, the column can be removed from the tube, and the different zones can be carefully cut out. The substances in each zone are then extracted using a suitable solvent.
2. ***Sequential Collection***: Alternatively, the column can be washed with additional solvent (called the eluent). Each component is collected separately as it exits the column.

This process effectively separates the components of the mixture based on their differing affinities for the stationary and mobile phases.



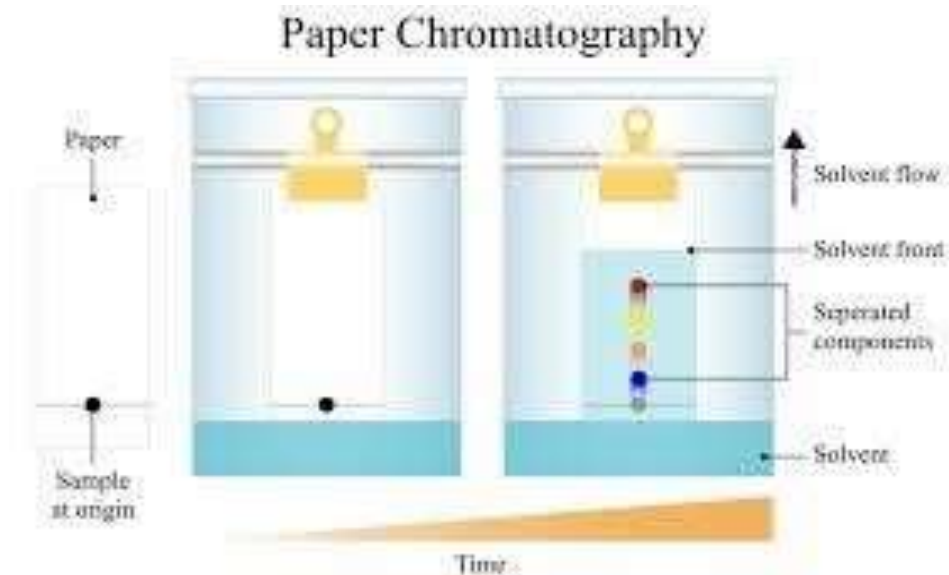
Advantages of Column Chromatography:

1. Versatile: It can separate any type of mixture.
2. Flexible Quantity: You can separate small to large amounts (from micrograms to milligrams).
3. Variety of Solvents: There are many options for the mobile phase.
4. Reusable Samples: In preparative chromatography, separated samples can be collected and reused.
5. Automation: The process can be automated for efficiency.

Disadvantages of Column Chromatography:

1. Time-Consuming: It can take a long time to complete the separation.
2. High Solvent Use: It requires a large amount of solvent, which can be costly.
3. Complex Automation: While automation helps, it can also complicate the technique and increase costs.

PAPER CHROMATOGRAPHY



Definition:

Paper chromatography is a technique used to separate unknown substances by allowing solvents to flow on special filter paper.

Principle:

The separation occurs mainly through partitioning, not adsorption. The cellulose in the filter paper holds moisture, which acts as the stationary phase, while organic solvents or buffers serve as the mobile phase.

Paper Used:

The type of filter paper chosen depends on factors like thickness, flow rate, and purity. Common options include Whatman filter papers of different grades (No. 1, No. 2, No. 3MM, No. 17, etc.).

Modified Paper:

Some papers are treated with acid or base, or made from glass fiber. Hydrophilic papers are modified with substances like methanol or glycerol.

Applications:

- Identifying drugs and impurities
- Separating mixtures of polar and non-polar compounds
- Monitoring the purity of pharmaceuticals

Advantages:

- Highly sensitive: Can detect as little as 0.1 μg of a compound.
- Quick operation time.

Disadvantages:

- Not suitable for separating volatile substances like hydrocarbons and fatty acids.
- The minimum detection limit for most compounds is 1-5 µg.

GAS CHROMATOGRAPHY**Principle of Gas Chromatography**

Gas chromatography is a method used to analyze substances by separating them between two phases: a mobile phase (the carrier gas) and a stationary phase (the column material).

1. Process Overview:

- The sample is vaporized and carried through a column by the mobile gas.
- Components separate based on their vapor pressure and how much they interact with the stationary phase.

2. Distribution Constant (K_c):

- The separation is influenced by a property called the distribution constant (K_c), which indicates how much of a component is in the stationary phase compared to the mobile phase:

$$K_c = \frac{[A]_s}{[A]_m}$$

- Here, $[A]_s$ is the concentration in the stationary phase, and $[A]_m$ is the concentration in the mobile phase.

3. Separation Mechanism:

- Different components move through the column at different speeds due to their distribution constants, leading to their separation.
- Factors such as temperature and the type of stationary phase affect K_c, and thus, can be adjusted for better separation.

4. Equipment and Detection:

- A gas chromatograph consists of a column where the sample components travel at different speeds.
- As they exit the column at different times, they are detected electronically.

5. Key Parameters for Separation:

- Several factors can influence how quickly components move through the column, including:
- Flow rate of the carrier gas: Faster flow can reduce retention time.
- Length of the column: Longer columns can enhance separation.
- Temperature: Higher temperatures may help vaporize and separate components more effectively.

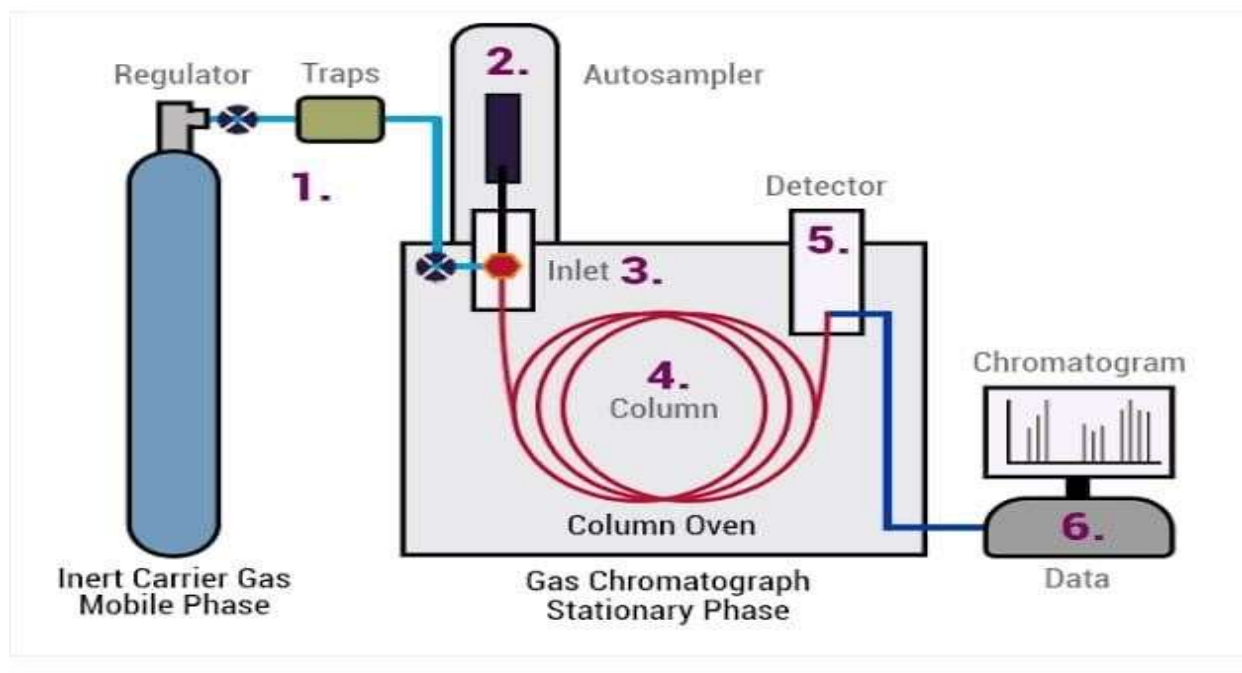
6. Sample Introduction:

- A known volume of the sample is injected into the column using a microsyringe.
- Components adsorb onto the stationary phase, affecting their movement through the column.

7. Detection and Analysis:

- As different components exit the column, their arrival time and quantity can be measured.
- This allows for identification based on retention time and concentration.

In summary, gas chromatography is a powerful analytical tool that separates and identifies chemical components in a mixture by exploiting their different interactions with a stationary phase.



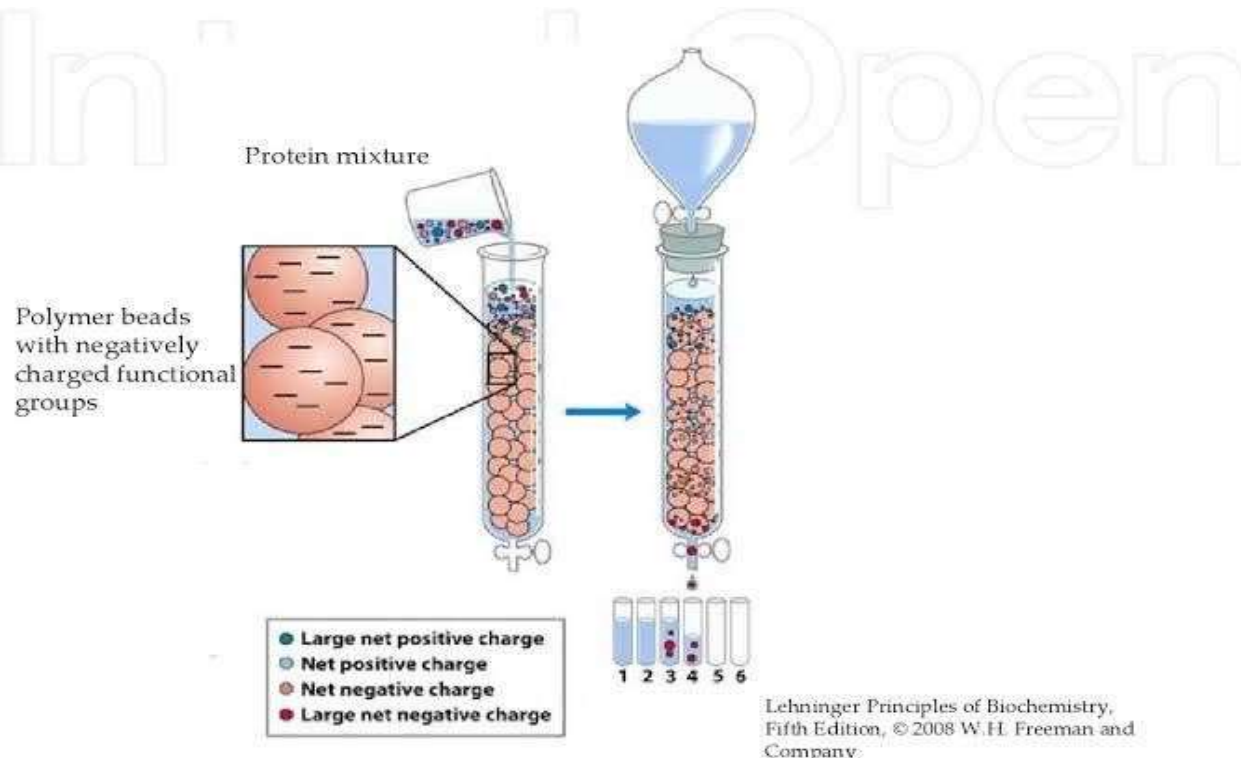
Advantages of Gas Chromatography

1. ***Reliable and Fast***: Gas chromatography provides quick and dependable results.
2. ***High Efficiency***: It offers excellent separation of components, resulting in high resolution.
3. ***Sensitive Detectors***: The technique uses very sensitive detectors to identify small amounts of substances.
4. ***Small Sample Size***: It requires only a small sample volume (less than 1 ml).
5. ***Non-Destructive***: The method can be combined with mass spectrometry, allowing for the analysis of samples without damaging them.
6. ***Quantitative Accuracy***: It delivers precise quantitative measurements of components.
7. ***Well-Established***: Gas chromatography has a vast amount of literature and is widely used in various applications.

Disadvantages of Gas Chromatography

1. ***Volatile Samples Only***: It is limited to analyzing volatile substances.
2. ***Not Suitable for Heat-Sensitive Samples***: It cannot be used for samples that degrade when heated.
3. ***Not Ideal for Preparative Purposes***: It's not designed for separating large amounts of substances for further use.
4. ***Need for Mass Spectrometry***: For detailed structural analysis, it typically requires mass spectrometry, as many non-MS detectors can damage the samples.

ION EXCHANGE CHROMATOGRAPHY



Ion Exchange Chromatography

Ion exchange chromatography is a method used to separate ions and polar molecules based on how strongly they stick to an ion exchanger. This technique can be applied to various charged molecules, including large proteins, small nucleotides, and amino acids. It can effectively separate both cations (positively charged ions) and anions (negatively charged ions).

Principle

The separation process involves a reversible exchange of ions between those in the solution and those in the ion exchange resin. This means that ions from the solution can swap places with ions attached to the resin, allowing for separation based on their affinity for the resin.

Applications

- ***Water Treatment***: Used for softening and demineralizing water.
- ***Inorganic Ion Separation***: Helps in isolating various inorganic ions.
- ***Biomolecule Separation***: Useful for separating sugars, amino acids, and proteins.
- ***Purification***: Effective in purifying solutions by removing ionic impurities.
- ***Enzyme Extraction***: Employed for extracting enzymes from tissues.
- ***HPLC***: Ion exchange columns are used in high-performance liquid chromatography (HPLC) for various analyses.

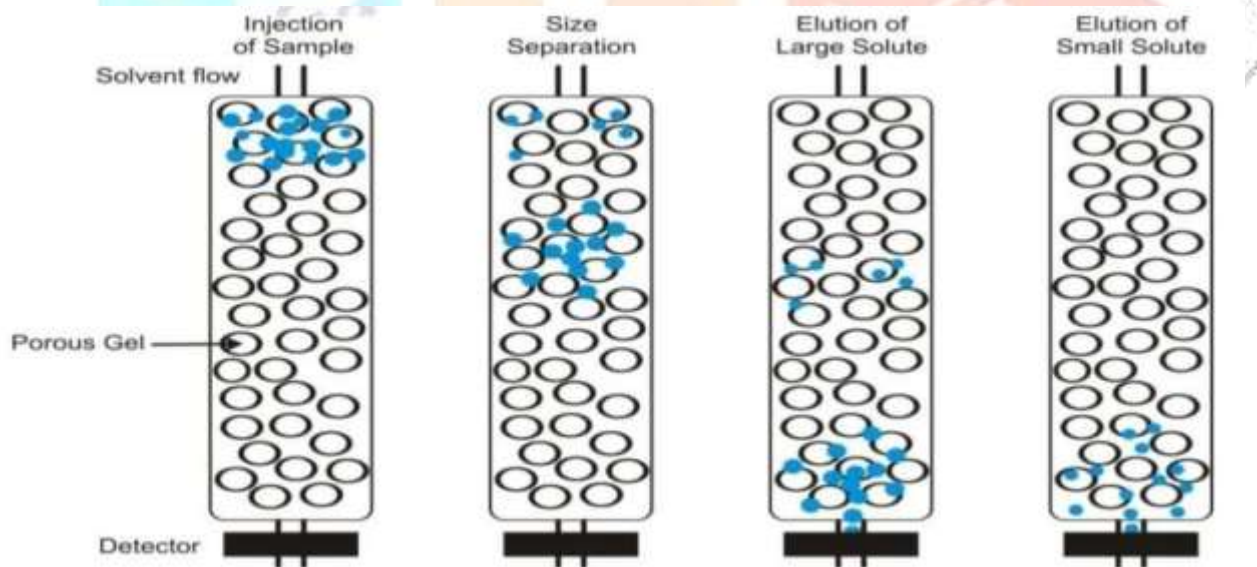
GEL FILTRATION CHROMATOGRAPHY

Principle of Gel Filtration Chromatography

Gel filtration chromatography separates molecules based on their size and shape using porous resin beads. Here's how it works:

1. ***Separation Process***: Large molecules cannot enter the pores of the beads, so they move quickly through the spaces between the beads and elute (come out) first. Smaller molecules can enter the pores and take longer to pass through the column, so they elute later.
2. ***Molecular Distribution***: The way a molecule is distributed between the space inside the beads and the space outside depends on the size of the molecule and the porosity of the beads.
3. ***Distribution Coefficient (K)***:
 - For large molecules that can't enter the beads, $K = 0$ (they are excluded).
 - For very small molecules that can fully access the space inside the beads, $K = 1$.
 - For medium-sized molecules, K will fall somewhere between 0 and 1.

This variation in K allows for effective separation of different molecules in a mixture based on their sizes.



Applications

1. **Protein Purification**: This method has been widely used to purify many proteins, including therapeutic proteins and enzymes used in food processing.
2. **Separation of Other Biomolecules**: It can also be used to separate carbohydrates and nucleic acids like DNA and RNA.
3. **Cell and Virus Separation**: Different sized cells and virus particles can be efficiently separated using this technique.
4. **Molecular Mass -Estimation**: Gel-filtration is useful for estimating the molecular weight of proteins based on their elution volumes.

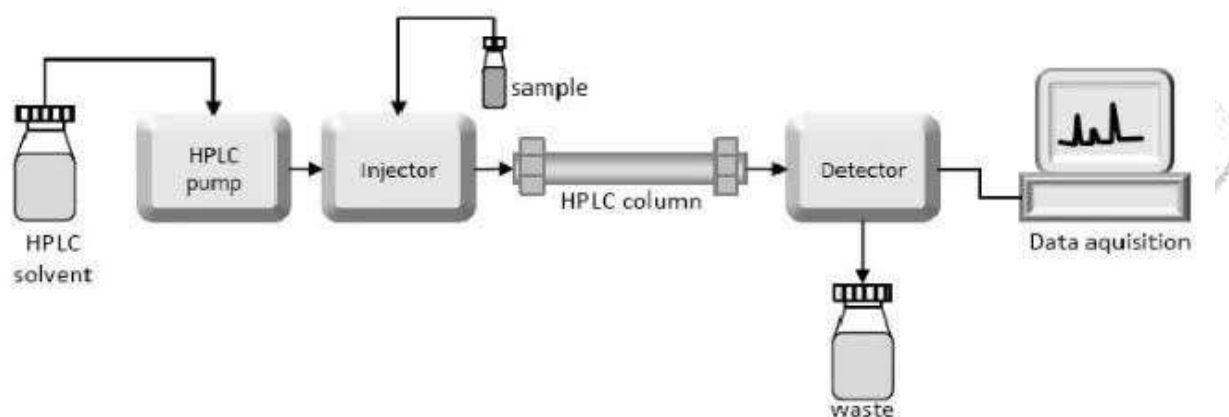
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-Performance Liquid Chromatography (HPLC), also called High-Pressure Liquid Chromatography, is a technique used to separate, identify, and measure the amounts of chemicals in a mixture. It is widely used in biochemistry and analytical labs.

In HPLC, solvents are forced through a column under high pressure—up to 400 times atmospheric pressure—allowing different components of a sample to separate based on how strongly they interact with the column's packing material (called the stationary phase).

The system includes a column filled with this packing material, a pump that pushes the liquid (mobile phase) through the column, and a detector that measures how long each component takes to pass through (retention time). This retention time depends on how the sample molecules interact with both the stationary phase and the solvents used.

Samples are introduced in small amounts into the mobile phase, where they are slowed down by interactions with the stationary phase. The choice of solvents, additives, and how the mobile phase composition changes during the analysis (gradient elution) all influence how well different components separate based on their affinity for the current mobile phase.



Applications

Pharmaceutical Applications:

- Identifying active ingredients in medication.
- Assessing the shelf life of pharmaceutical products.
- Measuring dopamine levels in levodopa treatments.

Environmental Applications:

- Detecting diphenhydramine in environmental samples.
- Monitoring pollutants in the environment.

Clinical Applications:

- Analyzing antibiotics in medical samples.
- Detecting natural neuropeptides in brain fluids.

Food Applications:

- Ensuring the consistency and quality of soft drinks.
- Analyzing natural contaminants, like mercury and phenol, in seawater.
- Monitoring drug stability and quality control.

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)***Ultra-Performance Liquid Chromatography (UPLC)***

UPLC is a more advanced form of liquid chromatography that improves on three main aspects: speed, resolution, and sensitivity. It uses particles smaller than 2 micrometers, allowing it to achieve better results in separating and analyzing substances compared to High-Performance Liquid Chromatography (HPLC).

CONCLUSION

In this study, we look at drug development using analytical techniques. Chromatography is a highly sensitive and effective separation method that has become an important innovation in recent years. While high-performance liquid chromatography (HPLC) was once limited to specialized analysts, it is now commonly used by students, chemists, biologists, production workers, and labs focused on research and quality control.

We also explore various spectroscopy techniques for measuring drugs, including UV-Visible spectroscopy, Mass Spectrometry, Infrared Spectroscopy, Nuclear Magnetic Resonance, Fluorimetry, and Phosphorimetry.

This review focuses on the principles, classifications, instruments, advantages, disadvantages, and applications of chromatographic and spectroscopic techniques. These methods enhance productivity by providing more reliable, robust, and sensitive results, which reduces the time needed to develop new techniques.

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