Sec (Chemistry) Unit → 2

Chromatographic Techniques :→ (Seperation Technique)
↳ (Related to colour)

Chromatography is a biophysical seperation Technique, primarily employed to analyse complex mixtures both Qualitatively and Quantatively. It is a powerful technique that enables seperation, Identification and purification of the components of a complex mixture. It was first discovered by Russian Botanist M. S. Tsvet, which was further developed by Martin and Synge.

provided by: SM Mazidul

Stationary phase: is a phase that may be either a solid or liquid which remains fixed in a place, either in a column or in a planar surface.

Mobile phase :→

Mobile phase is a chromatography, refers to the solvent or mixture that moves through the stationary phase, due to capillary action carrying the components of the mixture being analyzed. The mobile phase can be liquid (in liquid chromatography) or gas (in gas chromatography.)

Paper chromatography :→

It is an analytical method used to seperate coloured chemicals or substance. It is useful for seperating complex mixtures of compounds having similar polarity. eg:- Amino Acids. The setup has three components. The mobile phase is a solution that travels up ~~solvent mixture~~ the stationary phase due to capillary action. The mobile phase is generally an alcohol solvent mixture, while the stationary phase is a strip of chromatography paper, also called chromatogram. The stationary phase is the water trapped between the cellulose fibres of the paper.

The mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it.

Rf values (Retention factor) :->

The Retardation factor (Rf) may be defined as the ratio of the distance travelled by the substance/compound to the distance travelled by the solvent.

$$Rf = \frac{\text{distance Travelled by compound}}{\text{distance Travelled by solvent}}$$

TLC (Thin Layer chromotography) :->

It is a technique used to seperate and identify compound in a mixture. It is commonly used in organic chemistry, bio-chemistry and plant-sciences for the analysis of small quantities of substances. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity and speed of seperation.

TLC functions on the same principle as all Chromotography. A compound will have different affinities for the mobile and stationary phases and this affects the speed at which it migrates.

Classification of chromatography :->

Chromatography is a versatile technique used to seperate mixtures into individual components. It can be classified based on different components.

(i) Based on Physical state of Mobile phase :->

- Gas chromatography (GC) :->

The mobile phase is a gas. This method is often used for volatile compounds.

- Liquid chromatography (LC) :->

The mobile phase is a liquid. It's commonly used in both analytical and preparative seperations.

- Supercritical fluid chromatography :-> (SFC)

A supercritical fluid is used as the mobile phase, often carbon-dioxide above its critical temperature and pressure.

(ii) Based on the nature of stationary phase :->

- Column Chromatography :->

The stationary phase is a column packed with solid particles.

- Thin-Layer chromatography :-> (TLC)

The stationary phase is a thin layer of adsorbent material (eg:- silica or alumina) spread on a glass or plastic plate.

- Paper chromatography :->

The stationary phase is a sheet of paper, usually filterpaper.

(iii) Based on the seperation Mechanism :->

- Adsorption chromatography :->

Separation is based on the adsorption of solutes on the surface of the stationary phase. (eg:- TLC, column chromatography)

- Partition chromatography :->

Separation is based on partitioning of solutes between two immiscible liquids (eg:- paper chromatography)

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- **Ion-Exchange chromatography :->**
Separation is based on the exchange of ions between the solute and the charged stationary phase.
- **Size-Exclusion chromatography :->**
Separation is based on molecular size, with larger molecules eluting faster than smaller ones. (eg:- gel filtration chromatography.)
(breaking)
- **Affinity chromatography :->**
This is based on specific interactions between a solute and the stationary phase. (eg:- antibody-antigen interactions.)

(iv) **Based on Mode of chromatography :->**
- **Planar Chromatography :->**
Includes paper chromatography and Thin layer chromatography, where the stationary phase is flat.
- **Column chromatography :->**
The stationary phase is packed into a tube or column.

Principle of chromatography :->

The principle of chromatography is based on the separation of components in a mixture due to differences in their affinities for a stationary phase and a mobile phase. The components move at different rates through the stationary phase, resulting in their separation.
The two main components principles that drive this technique are :->

(i) **Adsorption or partition :->**
- In adsorption chromatography the components of the mixture are adsorbed to the surface of the stationary phase. The stronger the adsorption, the slower the movement.
- In partition chromatography, the components dissolve in stationary phase (usually a liquid) and their partitioning between the stationary and mobile phases determines their movement.

(ii) Differential Migration :->

The mobile phase (a liquid or gas) moves through or over the stationary phase (a solid or liquid). Components that have a stronger attraction to the mobile phase move faster, while those with a stronger attraction to the stationary phase move slower, leading to Seperation.

The extent of seperation depends on :->

- The solubility of the components in the mobile phase.
- The interaction between the components and the stationary phase.

-> common types of chromatography include paper chromatography, thin layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC).

Efficiency of chromatographic Techniques :->

The Efficiency of chromatographic Technique refers to how well it can seperate different components in a mixture. Several factors determine the efficiency of chromatography, including —

(i) Column Efficiency :->

This is often measured by the number of theoretical plates (N) in a column. A higher number of theoretical plates indicates better seperation. It depends on factors such as column length and particle size of the stationary phase.

(ii) Selectivity :->

The ability to distinguish between different components in a mixture. It depends on the chemical nature of both the stationary and mobile phases, as well as the interactions between the analytes and these phases.

(iii) Resolution (Rs) :->

The degree to which two peaks (representing different components) are seperated in the chromatogram. High resolution means better seperation, which leads to higher efficiency.

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(iv) Flow Rate :->
The speed at which the mobile phase passes through the stationary phase. An optimal flow rate increases efficiency by improving interaction time, but too high a rate may cause poor seperation.

(v) Temperature :->
In technique like gas chromatography, temperature affects the volatility of components and can either improve or reduce efficiency.

(vi) Column Length :->
Longer columns may provide better seperation but also lead to longer analysis times.

PYQ Mechanism of Seperation :- (1) Adsorption :->
Adsorption seperation is a process where certain components from a mixture are selectively retained on a solid surface based on differences in their affinity for the adsorbent. This technique is widely used in purification, gas-seperation, and chemical processing.

PYQ Mechanism :->
(i) Adsorbent and Adsorbate :->
The substance that accumulates on the surface is called the adsorbate, while the solid material that provides the surface for adsorption is called adsorbent.

(ii) Surface Interaction :->
Adsorption occurs due to physical forces (Vander Waals forces) or chemical interaction (covalent bonding) between the adsorbent and adsorbate. The stronger the affinity of the adsorbate to the adsorbent, the more it will be retained.

(iii) Equilibrium :->
Adsorption continues until equilibrium is reached, where the rate of adsorption equals the rate of desorption. This equilibrium determines how much of the target substance will remain adsorbed on the surface.

(iv) Selective Retention :->
Different components in a mixture have different affinities for the adsorbent, allowing selective retention of one or more components while the others pass through.

(v) Desorption :->
The absorbed substances can later be removed from the adsorbent by changing conditions such as :- Temperature, or pressure (known as desorption)

Factors affecting Adsorption Seperation :->

(i) Surface Area :->
Larger surface areas increases the capicity for adsorption.

(ii) Pore Size :->
The adsorbent should have pores that match the size of the molecules being Separated.

(iii) Polarity :->
Polar molecules are more likely to adsorb onto polar adsorbents.

(iv) Temperature :->
Increasing temperature generally reduces adsorption in physical adsorption.

(v) Pressure :->
Higher pressure favor adsorption in gases.

Applications :->

(i) Gas Seperation :->
Seperating oxygen from air using zeolites.

(ii) Water purification :->
Removing organic pollutants using activated carbon.

(iii) Chromatography :->
Separating complex ~~molecules~~ mixtures of molecules.

(2) Partition :-

Partition Seperation is a method based on the solubility of componente between two immiscible phases typically liquid phases, such as an aqueous (water-based) and an organic solvent (non-polar). The seperation occurs due to the varying affinities of the solutes for each of the phases.

Mechanism of Partition Seperation :-

(1) Two Immiscible phases :-

The process begins with two immiscible liquids that form distinct layers when mixed. These are commonly a polar aqueous phase and a non-polar organic solvent, such as hexane, ether or chloroform.

(2) Partitition coefficient :-

When a solute is added to the system, it distributes itself between the two phases based on its solubility in each. This distribution is qualified by the partition coefficient (k) which is expressed as :-

$$K = \frac{\text{Concentration of solute in Organic phase}}{\text{Concentration of solute in aqueous phase}}$$

(3) Selective Solubility :-

Each component in the mixture will have a different partition coefficient, mean they will dissolve more readily in one phase than the other. for instance, polar compounds are more soluble in the aqueous phase, while non-polar compounds are more soluble in the organic phase.

(4) Equilibrium Distribution :-

Over time, the solutes distribute themselves between the two phases until an equilibrium is reached, where the rate of transfer of solutes between the phases is equal. The equilibrium condition determines the concentration of each solute in both phases.

(5) Extraction :-

Once equilibrium is reached, the two phases can be physically seperated, typically using a seperation funnel.

The Solutes are effectively partitioned between the two phases, allowing for their seperation based on their Solubility differences.

(6) Multiple Extraction :→

To improve the purity or recovery of the Solute, multiple extractions can be performed. Each extraction enhances the seperation by redistributing Solutes more effectively between the phases.

Factors Affecting Partition Seperation :→

(i) Nature of the Solvent :→

The choice of the Organic and aqueous solvents greatly affects the seperation, as different Solutes will have varying Solubilities in different Solvents.

(ii) Temperature :→

Changes in temperature can affect the Solubility of Substances in the Solvents, altering the partitioning behavior.

(iii) PH of the aqueous phase :→

For weak acids or bases the PH of the aqueous phase can influence the Solutes ionization State, affecting its Solubility and partition coefficient.

(iv) Polarity :→

More polar substances tend to stay in the aqueous phase, while non-polar Substances prefer the organic phase.

Applications of partition coefficient :→

(i) Liquid-Liquid extraction :→

Used to extract organic compounds from aqueous mixtures.

(ii) Chromatography :→

In this techniques like paper chromatography and thin-layer chromatography, Substances partition between a stationary liquid phase and a mobile phase.

(iii) Pharmaceuticals :→ Used in drug purification and Isolation.

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Development of chromatograms :-

① Frontal :-

Frontal development of chromatograms is a technique used in Paper or thin-layer chromatography. In this method the solvent moves horizontally or vertically across the chromatographic medium (such as-paper or a thin layer of adsorbent) carrying the sample components with it. The components of the mixture ~~seperated~~ seperate based on their differential affinities for the stationary phase (Paper or adsorbent) and the mobile phase (solvent). Frontal development in chromatography is useful in specific scenarios mainly when bulk seperation is required over analytical precision.

- A breakdown of the frontal development process :-

① Principle :-

Frontal development is based on differential migration of the components of a mixture under the influence of a solvent (mobile phase) across a stationary phase. The components seperate based on their solubility and adsorption characteristics. This technique is often used when the sample needs to be continuously applied to the system, such as in the purification of large quantities of material.

② Procedure :-

- Continuous sample Application :-

Instead of spotting the sample in one position (as in conventional paper or thin-layer chromatography) a mixture of the sample is continuously introduced at one end of the chromatographic medium.

- Mobile phase Movement :-

The solvent (mobile phase) is passed through the stationary phase (eg:- Paper or thin layer) in a continuous stream. The components of the mixture get carried along with the mobile phase based on their solubility and interaction with the stationary phase.

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- **Seperation Zones:→**

As the solvent moves forward, the components of the mixture begin to seperate into different zones or fronts, depending on their affinity for the mobile phase and the stationary phase. The mobile components travel further, while less mobile components remain closer to the origin.

- **Frontal Elution:→**

In this type of chromatography, one cannot see distinct spots as in other methods but rather continuous zones that overlap slightly. Each zone represents a component of the mixture being seperated.

③ **Visualization:→**

After the development the seperated zones can be visualized using different methods, such as UV light, chemical staining, or radioactive tracers, depending on the nature of the components being seperated.

- **Applications:→**

(i) Frontal chromatography is particularly useful for bulk-seperations or preparative chromatography, where large quantities of material are being processed.

(ii) It is less common for analytical purposes where small, discrete amounts of components need to be identified, as it doesnot produce sharp, distinct spots but rather continuous fronts.

- **Advantages:→**

→ Ideal for purifying larger quantities of material.
→ Can be used for both qualitative and quantitative seperations.

- **Disadvantages:→**

→ The overlapping zones can make it harder to achieve high-resolution seperations.
→ Not as precise for analytical work compared to other methods.

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(ii) Elution :→

Elution development is a widely used method in chromatography where a solvent (mobile phase) moves through the stationary phase, carrying the components of the mixture with it. These components seperate based on their interaction with the stationary phase and the solvent allowing for their identification or purification. Unlike frontal development, elution development results in discrete spots or bands representing each component of the mixture.

Elution development in chromatography is a highly effective method for seperating the components of a mixture based on their differential movement through a stationary phase under the influence of a mobile phase. It's a versatile technique used in both analytical and preparative applications.

- A breakdown of elution development in chromatography :→

① Principle :→

Elution refers to the process where the mobile phase moves through the stationary phase, carrying different components of the sample along with it. The components are seperated based on their relative affinities for the mobile and stationary phases. Those with a higher affinity for the mobile phase elute faster, while those with a higher affinity for the stationary phase elute slower.

② Procedure :→

(i) Sample Application :→

A small concentrated sample of the mixture is applied at the starting point (usually near the bottom edge) of the chromatographic medium (paper or TLC plate).

(ii) Mobile phase Introduction :→
A suitable solvent (mobile phase) is introduced and it moves through the stationary phase by capillary action or through other driving forces (gravity, Pressure etc.) The mobile phase dissolves the sample and carries it upward (or downward in descending chromatography.)

(iii) Seperation :→
As the mobile phase moves through the stationary phase, the sample components begin to separate based on their differing solubilities in the ~~bot~~ mobile phase and their interactions (adsorption or partitioning) with the stationary phase.

(iv) Elution and spot formation :→
The separated components moves at different speeds, creating discreate spots (for paper and thin-layer chromatography) or bands (for column chromatography) Each spot or band corresponds to a different component of the mixture.

- Fast Eluting compounds :→
  components with a higher affinity for the mobile phase travel further or elute faster.
- Slow Eluting compounds :→
  Components with a higher affinity for the stationary phase travel more slowly and stay closer to the origin.

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③ Types of Elution :→

- Isocratic Elution :→ The composition of the mobile phase remains constant throughout the seperation process. This is typically used when the components of the mixture have similar polarities.
- Gradient Elution :→ The composition of the mobile phase is gradually changed during the development process. For example :- In HPLC or TLC the polarity of the solvent system may be increased to help elute more strongly retained components. This is useful for seperating complex mixtures with wide range of polarities.

④ Visualization :→

After the mobile phase has moved a sufficient distance, the chromatogram is removed from the development chamber, dried and visualized. The seperated components can be observed directly (if coloured) or visualized by spraying with reagents, using UV light, or other detection methods, depending on the nature of the compounds.

⑤ Rf Values :→

In paper or thin-layer chromatography, the seperation is qualified using Rf (retention factor) values, calculated by the ratio of the distance travelled by the component to the distance travelled by the solvent front. The Rf value helps in identifying compounds based on their elution behaviour.

$$Rf = \frac{\text{Distance travelled by the compound.}}{\text{Distance travelled by the solvent front.}}$$

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(6) Applications :->

Analytical purposes :->

Elution development is widely used in both qualitative and quantitative analysis in various fields, including chemistry, biology, pharmaceuticals and environmental science.

Purification :->

This method is also used to purify compounds in preparative chromatography (eg :- column chromatography.)

High-performance Liquid chromatography (HPLC) :->

In HPLC, elution is key to seperating complex mixtures based on polarity, size or charge.

- **Advantages :->**
  - -> Produce distinct, easily identifiable spots or bands.
  - -> Suitable for both small-scale analytical work and large-scale preparative chromatography.
  - -> Allows for a clear seperation of complex mixtures.

- **Disadvantages :->**
  - -> Requires careful choice of solvent system to optimize seperation.
  - -> Some compounds may not separate cleanly, resulting in overlapping spots or bands.

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(iii) Displacement Methods :->

In displacement chromatography, the development of chromatograms involves the use of displacer, which forces the seperation components on a stationary phase. This method is particularly effective for obtaining sharp and well-separated peaks in a chromatogram, especially in cases where high-purity seperation is required.

The displacement method is a powerful technique for developing chromatograms, providing high-purity seperations with sharp, well-defined peaks or bands by using a displacer that pushes components off the stationary phase.

Principle of Displacement chromatography :->

The principle behind the displacement method in chromatography relies on introducing a substance (displacer) that has a stronger affinity for the stationary phase than the sample components. As the displacer moves through the stationary phase, it competes for binding sites and displaces the sample components based on their affinity for the stationary phase. This results in the sample components being separated and eluted in a specific order, with the weakest retained component being displaced or eluted first.

Steps in the Development of chromatograms by Displacement method :->

(i) Sample introduction :-> The sample mixture containing various components is applied to the stationary phase, which can be a column or a thin-layer plate.

(ii) Initial Migration :->

The mobile phase begins to move through the stationary phase and sample components starts seperating based on the interactions with the stationary phase. However, the sample components remain ~~extent~~ absorbed to a certain extent.

(iii) Displacer Introduction :->

A displacer which has a higher affinity for the stationary

phase than the sample components, is introduced into the mobile phase.

(iv) Component Displacement :→

As the displacer moves through the stationary phase, it begins to displace the components of the sample. The component with the weakest interaction with the stationary phase is displaced first, followed by components with stronger affinities.

(v) Elution and Chromatogram :→

The displaced components move out of the stationary phase in a distinct sequence, with well-separated bands or peaks. The chromatogram is developed as the components elute, one by one, from the system.

Characteristics of Displacement chromatography :→

(i) High Resolution :→

The displacement method results in very sharp and concentrated bands or peaks in the chromatogram because the components are actively pushed off the stationary phase by the displacer.

(ii) Sequential Elution :→

The components are eluted in the order of their affinity to the stationary phase. Components with weaker binding elute first, while those with stronger affinities elute later.

(iii) Purification of Biomolecules :→

In protein or peptide purification, displacement chromatography can be used to obtain pure samples for preparative processes.

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Applications :->

→ preparative chromatography :->
Displacement chromatography is often used in large-scale separations, especially in pharmaceutical and biochemical industries, to purify large amounts of substances.

→ Separation of complex mixtures :->
It is particularly useful when dealing with complex mixtures where high resolution is needed to separate closely related compounds.

→ Purification of Biomolecules :->
In protein or peptide purification, displacement chromatography can be used to obtain pure samples in preparative processes.

Advantages of Displacement chromatography :->

(i) High-purity :-> The technique can produce highly pure fractions, making it ideal for applications requiring exact separations.

(ii) Sharp peaks :-> Since the displacer actively displaces the components, the chromatographic bands or peaks are narrow and well-resolved.

(iii) Concentration :-> The displacement of components results in their concentration, which can enhance the sensitivity and detectability of the components.

Limitations :->

(i) Displacer Selection :->
A suitable displacer must be chosen carefully to ensure it has a stronger affinity for the stationary phase than the sample components but does not interfere with detection.

(ii) complexity :->
This method can be more complex to setup and operate, compared to other chromatographic techniques, as the displacer must be carefully chosen and introduced at the right stage.

Rf Value :→ PYQ

The Rf Value (retention factor or retardation factor) is a measurement used in thin-layer chromatography (TLC) to describe how far a compound travels on the stationary phase (such as a TLC plate) relative to the solvent front. It helps in identifying compounds and comparing them with known substances.

formula to calculate Rf Value :→

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

- **Rf Value characteristics :→**

(i) The Rf value is always a fraction (less than or equal to 1) because the compound can never more further than the solvent front.

(ii) High Rf values (close to 1) indicate that the compound is less polar and ~~indicates~~ interacts weakly with the stationary phase, allowing it to travel further.

(iii) Low Rf values (closer to 0) indicate that the compound is more polar and interacts more strongly, with the stationary phase, moving less.

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- **Importance of Rf Value :→**

~~(i) Comparison :→~~

(i) By comparing the Rf values of unknown compounds to those of known standards, one can help in identifying substances.

~~(ii) Reproducibility :→~~

(ii) Rf values are consistent under identical experimental conditions, allowing for reproducibility and comparison across experiments.

(iii) ~~(iii) Polarity and Interaction~~ :→ Rf values given an idea indication of the compounds polarity and its interaction with the stationary and mobile phases.

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• Limitations :→

(i) Not-absolute :→ Rf values can vary depending on the solvent system, the type of stationary phase, temperature, and other experimental conditions.

(ii) Non-unique identification :→ Multiple compounds can have similar or identical Rf values, so Rf alone cannot confirm identity without further analysis.

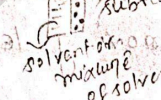
* Important chromatographic methods of Analysis :→

(i) Paper chromatography :→ [definition written in previous pages.]

Principles :→ Paper chromatography is a technique used to separate and identify mixtures of substances. Its principles includes :→

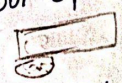
(i) Stationary phase :→ The paper serves as the stationary phase, typically made of cellulose.

(ii) Mobile phase :→ A solvent or mixture of solvents acts as the mobile phase, carrying the substances to be seperated.



Process :→

(i) Sample Application :→ A small spot of the mixture is applied near one end of the paper.



(ii) Development :→ The paper is placed upright in a container with a solvent. The solvent travels up the paper via capillary action, carrying the components of the mixture with it.

(iii) Seperation :→ Different substances move at different rates, resulting in separation based on factors like - solubility and affinity for the stationary phase.

(iv) Visualization :→ After the solvent front has moved a certain distance, the paper is removed, and the spots can be visualized using techniques such as UV light, staining or by observing color differences.

Applications :->

(i) Analyzing pigments in plants, food coloring and pharmaceuticals.

(ii) Identifying components in mixtures, such as amino acids and sugars.

Advantages and Disadvantages :->

Simple, inexpensive, and effective for separating small quantities.

Limited Resolution and less effective for complex mixtures compared to other methods like : High-performance liquid chromatography (HPLC).

(ii) TLC :- (defination given Previous pages.)

Thin Layer chromatography (TLC) is a widely used technique ~~- is based on principles of adsorption and partition of Stationary and Mobile phase~~.

(i) ~~Stationary phase~~ :-> based on separation of compounds through their differential interactions with a Stationary and mobile phase.

(i) Stationary phase :-> A thin layer of adsorbent material (commonly silica gel or alumina) is coated on a plate. This layer remains fixed and interacts with the compounds being analyzed.

(ii) Mobile phase :-> A solvent or solvent mixture moves up the plate by capillary action. This mobile phase carries the compounds along with it.

Process :-> PYQ

(1) Sample Application :-> A small spot or line of the sample mixture is applied near one end of TLC plate.

(2) Development :-> This plate is placed upright in a developing chamber containing a small amount of solvent. The solvent travels up the plate by capillary action.

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(3) Separation :→ Different components of the mixture travel at different rates based on their interactions with the stationary phase and solubility in the mobile phase.

(4) Visualization :→ After the solvent has moved a certain distance, the plate is removed, and the spots can be visualized using UV light, iodine staining or other visualization techniques.

Applications :→

(i) Identifying compounds in Organic chemistry.
(ii) Monitoring reactions and purity checking.
(iii) Seperating pigments, drugs and various small molecules.

Advantages and Disadvantages :→

Quick, simple, low-cost and requires minimal sample size.

Limited resolution for very complex mixtures and not suitable for large-scale separations.

(iii) Pre Column Chromatography :→

Column chromatography is a technique used to separate and purify individual components from a mixture. It is particularly useful in organic and analytical chemistry. It involves the use of a column filled with a stationary phase through which the mobile phase (solvent) moves, carrying the sample components with it.

PYQ
Principle:→ Column chromatography is a seperation technique based on key concepts:→

(i) Separation:→ As the mobile phase flows through the column, components separate based on their differential affinities for the stationary and mobile phases.

(ii) Elution:→ The process of washing out the separated compounds from the column is called elution. Different solvents or gradients can be used to optimize separation.

Process:→

(i) Column packing:→ The stationary phase is packed into a vertical column, ensuring uniformity and avoiding air bubbles.

(ii) Sample Application:→ A mixture is loaded onto the top of the column.

(iii) Elution:→ The mobile phase is added to the column. As it flows through different components of the mixture interact differently with the stationary phase, causing them to elute at different rates.

(iv) Collection:→ Fractions of the elute are collected at regular intervals, allowing for the separation of components based on their affinity for the stationary phase.

PYQ
Applications:→

(i) purification of organic compounds, natural products and pharmaceuticals.

(ii) Separation of proteins, nucleic acids, and other biomolecules in biochemistry.

(iii) Analytical applications in quality control and research.

Advantage:→ Versatile, scalable for large-scale separations, and suitable for a wide range of sample types.

Disadvantage:→ Can be time-consuming and requires careful optimization of conditions for effective separation.

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(iv) HPLC (High performance liquid chromatography) :→

High Performance liquid chromatography (HPLC) is a powerful analytical technique used to separate, identify, and components in a liquid mixture. It is widely employed in various fields, including pharmaceuticals, biochemistry, and environmental analysis.

The principle of HPLC is based on the partitioning of compounds between a stationary phase and a mobile phase :→. (Principle) :→

Mobile phase :→ A liquid solvent that carries the sample through the system.

Stationary phase :→ A solid or liquid coating on the inside of the column where separation occurs.

- Process :→

(i) Sample injection :→ A small volume of the sample mixture is injected into the HPLC system.

(ii) Separation :→ The mobile phase flows through the column. As components interacts differentially with the stationary phase, they elute (come out) at different times.

(iii) Detection :→ As the components exit the column, they are detected by various detectors (like UV, florescence or mass spectrometry) which provide information on their identity and concentration.

(iv) Data Analysis :→ The data is processed to generate chromatograms, displaying the separation of components.

Application :→

(i) Pharmaceuticals :→ purity testing and formulation analysis.

(ii) Environmental Science :→ Analyzing pollutants in water and soil.

(iii) Food industry :→ Testing for additives, contaminants and nutritional contents.

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PYQ Advantages and Disadvantages :->

Advantages: High resolution, speed (faster analysis), Quantitative accuracy, and sensitivity; suitable for a wide range of compounds.

Disadvantages: Requires expensive equipment and can be complex to operate.

PYQ Solutions :->

Unit -> II

(8) Locating agents :->

Locating agents are substances used in chromatography, particularly in thin-layer chromatography (TLC) to visualize spots of separated compounds on a plate. They help to identify and differentiate the components of a mixture that may be colorless or otherwise not easily seen.

Types :->

- UV Active Dyes.
- Chemical Stains.
- Spray Reagents.
- Heat.
- Enzyme Reagents.

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