UNIT-4

Separation techniques-1

Syllabus: 9 hour

Solvent extraction- Principle and theory, Batch extraction technique, application of batch extraction in the separation of organic compounds from mixture- acid & neutral, base &neutral. Chromatography – Principle and theory, classification, types of adsorbents, eluents, Rf values, and factors affecting Rf values.Thin layer chromatography - principle, experimental procedure, advantages and applications.

Solvent Extraction: Overview

Solvent extraction (also known as liquid-liquid extraction) is a technique used to separate compounds based on their solubilities in two immiscible liquidsA, typically water and an organic solvent. It is widely used in chemical analysis and industrial processes for the purification and concentration of products. In solvent extraction, a compound is transferred from one liquid phase to another because of its higher solubility in the second liquid.

This process is utilized in a variety of applications, including the extraction of metals, organic compounds, pharmaceuticals, and natural products.

Principle and Theory of Solvent Extraction

Principle:

The basic principle of solvent extraction is the partitioning of a compound between two immiscible liquid phases. These two liquids do not mix, forming separate layers. When a compound is present in a mixture and is more soluble in one of the phases, it will transfer from the phase in which it is less soluble to the phase in which it is more soluble.

This principle is governed by the distribution law (or partition law), which states that at equilibrium, the ratio of the concentrations of a solute in the two phases is constant at a given temperature, provided the solute exists in the same molecular form in both phases.

$K_D = \frac{Concentration\ of\ solute\ in\ solvent\ 1}{Concentration\ of\ solute\ in\ solvent\ 2}$ ***Where K_D is the distribution coefficient, which indicates how much of the solute will be extracted into the solvent.***

Theory:

- *Distribution Coefficient (K_D): The solute will distribute itself between two immiscible solvents according to its solubility in each solvent. The distribution ratio is a key parameter for the extraction efficiency.*
- *Multiple Extractions: If the distribution coefficient is not favorable for complete extraction in a single step, multiple extractions can be performed. In each extraction, a portion of the solute will move into the extracting solvent until equilibrium is achieved.*
- *Nernst Distribution Law: For a solute distributed between two immiscible liquids, the Nernst distribution law helps predict the distribution of the solute across the two phases. The extraction efficiency depends on this equilibrium and can be maximized through optimization of the solvent's nature, concentration, and temperature.*

Types of Solvent Extraction

1. Batch Extraction

Batch extraction is the simplest form of solvent extraction where the mixture is extracted in discrete stages. In this method, a fixed quantity of the feed (the phase containing the solute to be extracted) is mixed with the solvent for a set period of time. The two phases are then allowed to separate, and the solute is transferred from the feed phase into the extracting solvent.

Steps in Batch Extraction:

1. *Mix the feed with the solvent in a suitable container.*
2. *Allow the phases to separate based on density differences.*
3. *Remove the solvent phase, which contains the extracted solute.*
4. *Repeat the process if multiple extractions are required for efficient recovery.*

- *Advantages: Simple setup, suitable for small-scale operations, and can be repeated for better efficiency.*
- *Disadvantages: Labor-intensive, less efficient compared to continuous methods for large-scale extractions, and can result in incomplete recovery of the solute in a single operation.*

2. Continuous Extraction

In continuous extraction, the solvent is continuously passed over the feed material, ensuring that fresh solvent is always in contact with the solute. This method is more efficient for large-scale operations and industrial processes, as it allows the extraction to proceed without interruption.

- *Equipment: Continuous extractors like Soxhlet extractors are commonly used. In such devices, the solvent is continuously heated, condensed, and recycled over the feed material, ensuring complete extraction of the solute over time.*
- *Advantages: Increased efficiency, automation of the process, and better yield due to the continuous nature of the solvent contacting the feed.*

Disadvantages: Higher initial cost for equipment, more complex setup, and requires a continuous supply of solvent.

Application of batch extraction in the separation of organic compounds from mixture Acid & neutral & Base&neutral.

Batch Extraction – Separation of Organic Compounds

Principle

- *Solvent extraction (liquid–liquid extraction) is based on the different solubilities of compounds in two immiscible solvents (commonly water and an organic solvent like ether).*
- *Batch extraction means performing the extraction stepwise (multiple portions of solvent) to increase efficiency.*
- *Acidic, basic, and neutral organic compounds can be separated by taking advantage of their acid–base properties:*
- *Acids → converted into water-soluble salts with alkali.*
- *Bases → converted into water-soluble salts with acid.*
- *Neutral compounds → remain dissolved in organic layer.*

Applications

1. Separation of Acid + Neutral compound

- *Example mixture: Benzoic acid + Naphthalene.*
- *Mixture is dissolved in ether (organic layer).*
- *Shaken with aqueous NaOH:*
- *Benzoic acid → sodium benzoate (water-soluble, goes to aqueous layer).*
- *Naphthalene remains in ether.*
- *Acidify aqueous layer with HCl → benzoic acid precipitates.*
- *Ether layer on evaporation gives naphthalene.*

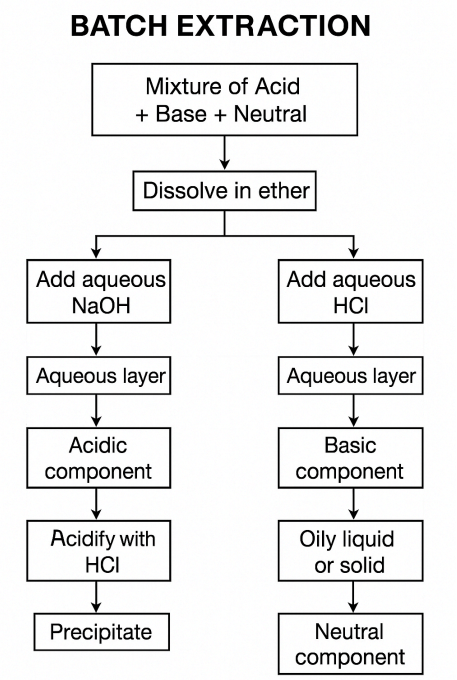
2. Separation of Base + Neutral compound

- *Example mixture: Aniline + Naphthalene.*

- *Mixture is dissolved in ether.*
- *Shaken with dilute HCl:*
- *Aniline → anilinium chloride (water-soluble, aqueous layer).*
- *Naphthalene remains in ether.*
- *Make aqueous layer alkaline with NaOH → free aniline separates.*
- *The Ether layer gives naphthalene on evaporation.*

3. Separation of Acid + Base + Neutral

- *Example mixture: Benzoic acid + Aniline + Naphthalene.*
- *Dissolve mixture in ether.*
- *Shake with dil. NaOH:*
- *Benzoic acid → sodium benzoate (aqueous layer).*
- *Separate and acidify → recover benzoic acid.*
- *Shake remaining ether layer with dil. HCl:*
- *Aniline → anilinium chloride (aqueous layer).*
- *Separate and basify → recover aniline.*
- *Ether layer → neutral compound (naphthalene).*



General Experimental Procedure -Requirements

- *Separating funnel*
- *Organic solvent (diethyl ether / chloroform)*
- *Aqueous NaOH, HCl solutions*
- *Mixture containing acid, base, and neutral compound*
- *Beakers, glass rod, filter paper, etc.*

Procedure

- *Preparation of mixture solution*

- *Dissolve the given mixture (acid + base + neutral) in ether (~20 mL).*
- *Pour it into a separating funnel.*

Separation of Acidic Compound

- *Add 10 mL of dilute NaOH (10%), shake gently, allow layers to separate.*
- *Aqueous (lower) layer contains sodium salt of acid.*
- *Ether layer contains base + neutral.*
- *Collect aqueous layer, acidify with dilute HCl → acid precipitates (filter & dry).*

Separation of Basic Compound

- *To ether layer, add 10 mL of dilute HCl (10%), shake and separate.*
- *Aqueous layer contains salt of base.*
- *Ether layer now contains neutral compound.*
- *Basify aqueous layer with NaOH solution → free base separates out.*

Isolation of Neutral Compound

- *Wash ether layer with water.*
- *Evaporate ether on a water bath → neutral compound is obtained.*

Observations

- *On acidifying NaOH extract → precipitate of acid confirms acidic component.*
- *On basifying HCl extract → oily liquid or solid base confirms basic component.*
- *Neutral solid remains after evaporation of ether.*

Result

- *The given mixture is separated into:*
- *Acidic component (e.g., Benzoic acid)*
- *Basic component (e.g., Aniline)*
- *Neutral component (e.g., Naphthalene*

Chromatography:

Principle and Theory

Chromatography is a powerful technique used for the separation of components in a mixture based on their differential distribution between two phases: the stationary phase and the mobile phase. The basic principle of chromatography involves the movement of a mixture dissolved in the mobile phase through a stationary phase. The components in the mixture move at different rates due to their varying affinities for the stationary and mobile phases, leading to their separation.

Principle:

- *Partitioning: The separation is based on the differential partitioning between the two phases. Components that interact more strongly with the stationary phase move slower, while those that interact more with the mobile phase move faster.*
- *Adsorption: In some forms of chromatography, such as adsorption chromatography, the principle relies on the adsorption of components onto the surface of the stationary phase.*
- *Size exclusion: In size exclusion chromatography, separation is based on the size and shape of molecules, where larger molecules move faster than smaller ones through the stationary phase.*

Theory:

The theory of chromatography is based on two major models: plate theory and rate theory.

- *Plate Theory: Proposes that the chromatographic column consists of a series of hypothetical discrete layers or "plates," where equilibrium between the mobile and stationary phases is achieved.*
- *Rate Theory: Takes into account the finite rate of mass transfer between the stationary and mobile phases. The Van Deemter equation explains the relationship between the flow rate of the mobile phase and the efficiency of the separation, describing how factors such as diffusion, eddy diffusion, and resistance to mass transfer affect band broadening.*

Classification of Chromatography

Chromatography can be classified based on the nature of the mobile phase, the stationary phase, and the separation mechanism:

1. *Based on the Physical State of the Mobile Phase:*
   - *Gas Chromatography (GC): Mobile phase is a gas.*
   - *Liquid Chromatography (LC): Mobile phase is a liquid.*
   - *Supercritical Fluid Chromatography (SFC): Mobile phase is a supercritical fluid.*

2. *Based on the Mechanism of Separation:*
   - *Adsorption Chromatography: Separation is based on the adsorption of solutes on the stationary phase.*
   - *Partition Chromatography: Separation is based on the partitioning of solutes between two liquid phases.*
   - *Ion-Exchange Chromatography: Separation is based on the exchange of ions between the stationary phase and the sample.*
   - *Size-Exclusion Chromatography (Gel Filtration/Permeation): Separation is based on the size of the solutes.*
   - *Affinity Chromatography: Separation is based on specific interactions between an analyte and a ligand attached to the stationary phase.*
3. *Based on the Technique Employed:*
   - *Column Chromatography: The stationary phase is packed in a column.*
   - *Thin-Layer Chromatography (TLC): The stationary phase is a thin layer on a glass, plastic, or aluminum plate.*
   - *Paper Chromatography: The stationary phase is paper.*
   - *High-Performance Liquid Chromatography (HPLC): A highly efficient form of liquid chromatography using high pressure to push solvents through the column.*

Types of Adsorbents

Adsorbents are materials used in chromatography to attract and hold molecules on their surface:

1. *Silica Gel: One of the most common adsorbents used in chromatography. It has a polar surface and is mainly used for separating polar compounds.*
2. *Alumina (Aluminum Oxide): Another polar adsorbent, used primarily for separating non-polar to moderately polar compounds.*
3. *Activated Carbon (Charcoal): Used for adsorption of non-polar compounds.*
4. *Cellulose: Commonly used in paper chromatography.*
5. *Ion Exchange Resins: Used in ion-exchange chromatography for separating ionic compounds.*

Eluents

Eluents are the solvents or mixtures of solvents used as the mobile phase in chromatography. The choice of eluent depends on the nature of the stationary phase and the compounds being separated. A good eluent should effectively move the compounds through the stationary phase without causing excessive band broadening.

- *Polar Eluents: Water, methanol, ethanol, acetonitrile.*
- *Non-Polar Eluents: Hexane, toluene, chloroform.*
- *Gradient Elution: In HPLC, the composition of the eluent can be changed during the separation process to improve the efficiency of separation.*

Rf Values (Retention Factor)

The Rf value is a measure used in thin-layer and paper chromatography to describe the relative position of a compound on the chromatogram. It is the ratio of the distance traveled by the compound to the distance traveled by the solvent front.

Formula for Rf Value:

$$Rf = \frac{Distance\ traveled\ by\ the\ compound}{Distance\ traveled\ by\ the\ solvent}$$

- *Rf values are always less than 1.0 and are specific to the compound, the stationary phase, and the mobile phase.*
- *Compounds with higher affinity for the stationary phase will have lower Rf values, and those with higher affinity for the mobile phase will have higher Rf values.*

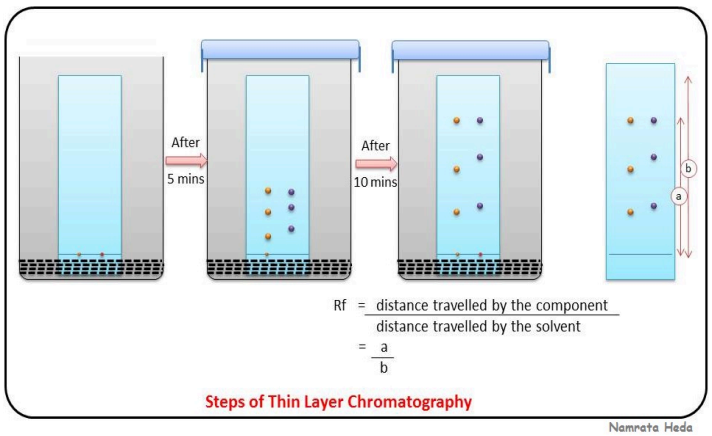
Factors Affecting Rf Values

1. *Nature of the Solvent (Eluent): Polar solvents may increase the Rf value of polar compounds, while non-polar solvents may increase the Rf value of non-polar compounds.*
2. *Type of Adsorbent (Stationary Phase): The interaction between the compound and the stationary phase influences the Rf value. Polar stationary phases (e.g., silica gel) tend to retain polar compounds longer, resulting in lower Rf values.*
3. *Temperature: An increase in temperature generally increases the rate of movement of solutes, leading to higher Rf values.*
4. *Thickness of the Stationary Phase Layer: A thicker stationary phase can lead to greater retention of compounds, reducing their Rf values.*
5. *Solvent Saturation: The degree of saturation of the solvent vapor in the chamber can affect the movement of the solvent and the compound.*
6. *Amount of Sample Applied: Overloading the stationary phase with too much sample can cause tailing and distortion of the Rf values.*

These factors should be carefully controlled to achieve consistent and reproducible Rf values.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a widely used separation technique in analytical chemistry to separate non-volatile mixtures. It is based on the principle of differential adsorption of substances on the surface of the stationary phase (typically a thin layer of adsorbent) and their movement through a mobile phase (solvent). It is commonly used for



the identification, purification, and quantification of compounds.

Principle of Thin Layer Chromatography

TLC operates on the principle of adsorption chromatography. In TLC, a thin layer of adsorbent, such as silica gel, alumina, or cellulose, is spread uniformly over a plate (commonly made of glass, aluminum, or plastic). The sample mixture is applied as small spots near the bottom of the plate. When the plate is placed in a developing chamber containing a suitable solvent or solvent mixture (mobile phase), the solvent travels up the plate by capillary action.

- *Differential Affinity: Different components of the sample mixture have different affinities for the stationary phase (adsorbent) and the mobile phase (solvent). Components that have a stronger interaction with the stationary phase move slowly, while those with a stronger interaction with the mobile phase move faster. This difference in movement leads to the separation of the components on the TLC plate.*
- *Partition and Adsorption: Separation can occur due to adsorption (interaction between the sample and the surface of the adsorbent) or partition (interaction between the sample and the mobile phase). The exact mechanism depends on the nature of the stationary phase and the solutes.*
- *Rf Value (Retention Factor): The movement of each compound is characterized by its Rf value, which is the ratio of the distance traveled by the compound to the distance traveled by the solvent front.*

$$Rf = \frac{Distance\ traveled\ by\ the\ compound}{Distance\ traveled\ by\ the\ solvent}$$

Experimental Procedure of Thin Layer Chromatography

1. Preparation of TLC Plate:

- *Adsorbent Layer: A thin layer of an adsorbent material (usually silica gel, alumina, or cellulose) is coated onto a solid support like a glass, aluminum, or plastic plate. Pre-coated plates are often used.*
- *Activation: If necessary, the TLC plate can be activated by heating it in an oven to remove any adsorbed moisture.*

2. Sample Application:

- *Spotting the Sample: Using a capillary tube, small spots of the sample mixture are applied near the bottom edge of the TLC plate. The distance from the bottom is typically about 1-2 cm, and the spots are small to prevent spreading.*
- *Drying: After application, the sample spots are allowed to air-dry before the plate is placed in the developing chamber.*

3. Development of the TLC Plate:

- *Developing Chamber Preparation: A developing chamber is prepared by placing a small amount of solvent or solvent mixture (the mobile phase) at the bottom. The solvent level should be below the level of the sample spots.*
- *Chromatographic Development: The TLC plate is then placed upright in the developing chamber. The chamber is covered to allow the solvent to rise up the plate by capillary action. As the solvent moves, it carries the components of the sample mixture along the stationary phase.*
- *Solvent Front: The development is allowed to proceed until the solvent front reaches near the top of the plate. The plate is then removed from the chamber, and the solvent front is marked immediately with a pencil.*

4. Visualization of Spots:

- *Staining or Spraying: If the separated compounds are not visible under normal light, the plate can be visualized using a UV lamp (many organic compounds fluoresce under UV light). Alternatively, a variety of chemical stains or reagents (like iodine vapors, ninhydrin, or sulfuric acid) can be used to visualize the separated spots.*
- *Rf Calculation: Once the spots are visible, the distance traveled by each compound and the solvent front is measured, and the Rf values for each component are calculated.*

5. Documentation: The TLC plate is documented either by photographing or drawing a diagram of the spots with their respective Rf values.

Advantages of Thin Layer Chromatography

- *Speed and Efficiency: TLC is a quick and efficient method for separating small amounts of compounds. Development times are generally short (a few minutes to an hour).*
- *Simplicity and Low Cost: The experimental setup is simple and inexpensive. It does not require sophisticated instrumentation, making it accessible for routine use in many labs.*
- *Versatility: A wide range of adsorbents and solvent systems can be used to suit different types of compounds, including polar and non-polar substances.*
- *Easy Visualization: Separated compounds can be easily visualized under UV light, using staining reagents, or chemical sprays, allowing qualitative analysis.*
- *Semi-Quantitative: TLC allows for the semi-quantitative analysis of compounds by comparing spot intensities with known standards.*

Applications of Thin Layer Chromatography

1. Qualitative Analysis:

- *Identification of Compounds: TLC is commonly used to identify compounds by comparing their Rf values and appearance (under UV light or after staining) with known reference compounds.*

- *Purity Check: TLC is used to assess the purity of a sample by detecting the presence of impurities. If additional spots appear, it indicates the presence of impurities in the sample.*

2. *Quantitative Analysis:*
   - *Quantitative Estimation: Although less accurate than other chromatographic techniques like HPLC, TLC can be used for the quantitative estimation of compounds based on spot intensity and size.*
3. *Separation of Mixtures:*
   - *Separation of Multicomponent Samples: TLC can separate complex mixtures of compounds, such as plant extracts, pharmaceuticals, or reaction mixtures. It is commonly used in organic synthesis to monitor the progress of reactions.*
4. *Pharmaceutical and Clinical Applications:*
   - *Drug Analysis: TLC is used to test the composition of pharmaceutical products, detect adulterants, and analyze metabolites in biological fluids.*
   - *Toxicology: It can be employed in forensic science to detect drugs and poisons in body fluids.*
5. *Environmental and Food Analysis:*
   - *Pesticide Residue Analysis: TLC is utilized for the detection of pesticide residues in food and water.*
   - *Food Additives: It can be used to analyze dyes, preservatives, and contaminants in food products.*
6. *Natural Product Research:*
   - *Plant Extracts: TLC is often used to analyze plant extracts for alkaloids, flavonoids, and other natural products.*
7. *Biochemistry:*
   - *Separation of Amino Acids and Sugars: TLC is commonly used in biochemistry for the separation of amino acids, sugars, and other biomolecules from complex mixtures.*