Unit-IV
Separation techniques

Solvent Extraction:

Solvent extraction, also known as liquid-liquid extraction, is a separation technique used to isolate and purify substances based on their relative solubilities in two immiscible liquids, usually an aqueous phase and an organic solvent.

Principle of Solvent Extraction

The principle of solvent extraction is based on the distribution of a solute between two immiscible liquids according to Nernst's Distribution Law.

Nernst's Distribution Law

When a solute is added to a system containing two immiscible solvents that do not mix, it distributes itself between the two solvents in a definite ratio at equilibrium, provided the solute has the same molecular form in both layers.

$$K = \frac{C_2}{C_1}$$

where:

C_1= Concentration of solute in organic phase

C_2 = Concentration of solute in aqueous phase

K = Distribution coefficient (or partition coefficient)

A higher K value indicates that the solute prefers the organic solvent.

A lower K value indicates that the solute prefers the aqueous phase.

Batch Extraction Technique:

Batch extraction is a technique where a solute is transferred from one immiscible liquid layer to another by shaking the two layers together until equilibrium is reached and then allowing the layers to settle for separation.

Batch extraction is especially advantageous when the distribution ratio is large, meaning the solute strongly prefers one phase over the other, and small quantities of solute need to be separated quickly and effectively.

Process of Batch Extraction

1. **Contacting the Layers:** The aqueous solution containing the solute and the organic solvent is placed in a separating funnel. The two liquids must be immiscible, meaning they do not mix (e.g., water and ether).
2. **Shaking and Mixing:** The funnel is gently shaken to create maximum surface contact between the two liquids. This allows the solute to transfer from one phase to the other, depending on its solubility.
3. **Equilibrium Stage:** The process continues until equilibrium is achieved, where the solute distribution between the two liquids becomes constant.
4. **Settling:** After shaking, the funnel is kept undisturbed to allow the two layers to separate by gravity.
    - *Top Layer* → Usually organic solvent (lighter, less dense)

- *Bottom Layer* → Usually aqueous solution (heavier, more dense)

5. **Sampling and Separation:** The layers are drained carefully, and the desired solute is collected from the layer where it is most concentrated.

Advantages

1. This technique is simple and cost-effective.
2. This technique requires minimal equipment and basic laboratory skills.
3. This technique is beneficial for both small-scale separations and academic purposes.
4. This process can be repeated multiple times to achieve higher extraction efficiency.

Application of batch extraction in the separation of organic compounds from mixture- acid & neutral, base & neutral.

Batch extraction is widely used to separate organic compounds based on their acidic, basic, or neutral properties. This separation is possible because acids and bases react with aqueous solutions of bases or acids, forming water-soluble salts, while neutral compounds remain in the organic layer.

1. Separation of Acid and Neutral Compounds

Ex: Benzoic acid (acidic) and naphthalene (neutral).

Step-by-Step Procedure:

1. Dissolve the mixture in an organic solvent like ether or dichloromethane.
2. Add aqueous NaOH solution and shake well:
   - Benzoic acid reacts with NaOH to form sodium benzoate (water-soluble salt).
   - Naphthalene remains unchanged in the organic layer.
3. Allow the layers to settle:
   - *Aqueous layer* → Contains sodium benzoate.
   - *Organic layer* → Contains naphthalene.
4. Separate the two layers using the separatory funnel.
5. Acidify the aqueous layer with HCl to convert sodium benzoate back to benzoic acid, which can be collected by filtration.

$$C_6H_5COOH+NaOH \rightarrow C_6H_5COONa+H_2O$$
$$C_6H_5COONa+HCl \rightarrow C_6H_5COOH+NaCl$$

2. Separation of Base and Neutral Compounds

Ex: Aniline (basic) and naphthalene (neutral).

Step-by-Step Procedure:

1. Dissolve the mixture in an organic solvent.
2. Add aqueous HCl solution and shake:
   - Aniline reacts with HCl to form anilinium chloride (water-soluble salt).
   - Naphthalene remains in the organic layer.
3. Allow the layers to separate:
   - *Aqueous layer* → Contains anilinium chloride.
   - *Organic layer* → Contains naphthalene.
4. Separate the two layers.
5. Add aqueous NaOH to the aqueous layer to free aniline by converting the salt back to the base, which can be separated as an oily layer or distilled.

$$C_6H_5NH_2+HCl \rightarrow C_6H_5NH_3Cl$$
$$C_6H_5NH_3Cl+NaOH \rightarrow C_6H_5NH_2+NaCl+H_2O$$

Principle and Theory of Chromatography

Definition: Chromatography is a separation technique used to separate the components of a mixture based on their differential distribution between a stationary phase and a mobile phase.

Principle of Chromatography

When a mixture is introduced into a chromatographic system, its individual components distribute themselves between two phases: a stationary phase and a mobile phase. The extent of interaction with each phase determines their rate of movement:

- Components with a stronger attraction toward the stationary phase migrate more slowly.
- Components with a stronger attraction toward the mobile phase migrate more quickly.

This difference in migration speeds causes the components of the mixture to separate from one another.

The separation mechanism can be based on:

- Adsorption, when the stationary phase is a solid.
- Partition, when the stationary phase is a liquid.

Other intermolecular forces such as van der Waals interactions, hydrogen bonding, dipole–dipole interactions, ion-exchange, and size exclusion may also govern the separation process.

Classification of Chromatography

Chromatography can be classified in different ways:

(A) Based on Physical State of the Phases

1. **Gas Chromatography (GC)**
    - The mobile phase is an inert gas (e.g., helium, nitrogen, hydrogen).
    - The stationary phase is either a solid adsorbent (Gas–Solid Chromatography) or a liquid coated on a solid support (Gas–Liquid Chromatography).
2. **Liquid Chromatography (LC)**
    - The mobile phase is a liquid.
    - The stationary phase may be a solid (adsorption) or a liquid supported on an inert solid (partition).

(B) Based on Separation Mechanism

1. **Adsorption Chromatography**
    - Separation occurs due to differences in the adsorption of solutes on a solid stationary phase.
    - Molecules bind via physical forces (e.g., van der Waals, hydrogen bonding).
2. **Partition Chromatography**
    - Based on differential solubility of components between two immiscible liquid phases (one stationary, one mobile).
3. **Ion-Exchange Chromatography**
    - Uses a stationary phase with charged groups that attract oppositely charged analytes.

- Cation-exchange resins attract positively charged ions, while anion-exchange resins attract negatively charged ions.

4. **Size-Exclusion (Gel Filtration) Chromatography**
    - Separation is based on molecular size and shape.
    - Large molecules are excluded from pores of the stationary phase and elute first, while small molecules enter pores and elute later.

(C) Based on Technique/Development

1. **Column Chromatography**
    - The stationary phase (adsorbent like silica/alumina) is packed into a column, and the mobile phase flows through it.
    - Different components migrate at different rates, resulting in separation.
2. **Thin Layer Chromatography (TLC)**
    - A thin layer of adsorbent (silica, alumina) is coated on a glass, plastic, or aluminum plate.
    - The sample moves up by capillary action when dipped in the solvent, separating components based on affinity.
3. **Paper Chromatography**
    - Uses filter paper as the stationary phase (water trapped in fibers acts as partition medium).
    - The mobile phase (organic solvent) carries solutes upward by capillary action.
    - Common in teaching labs for identifying amino acids and pigments.
4. **High-Performance Liquid Chromatography (HPLC)**
    - An advanced form of liquid chromatography using high-pressure pumps to push solvents through packed columns.
    - Offers high resolution, sensitivity, and speed.
    - Widely used in pharmaceuticals, food, and environmental analysis.
5. **Gas Chromatography (GC)**
    - Repeated here under technique: involves separation of volatile compounds using a gaseous mobile phase.
    - Detection is highly sensitive (e.g., Flame Ionization Detector, Mass Spectrometry).

3. Types of Adsorbents (Stationary Phase)

Adsorbents are solid materials used in adsorption chromatography. The choice depends on the polarity of the compounds:

- **Silica gel** – Polar, acidic sites; separates a wide range of compounds.
- **Alumina (Al_2O_3)** – Polar, basic sites; effective for separating weakly polar to moderately polar compounds.
- **Cellulose, Starch, Kieselguhr** – Used in paper chromatography.
- **Charcoal** – Used for adsorption of colored substances.
- **Modified silica/alumina** – Functionalized for special separations (e.g., reverse phase).

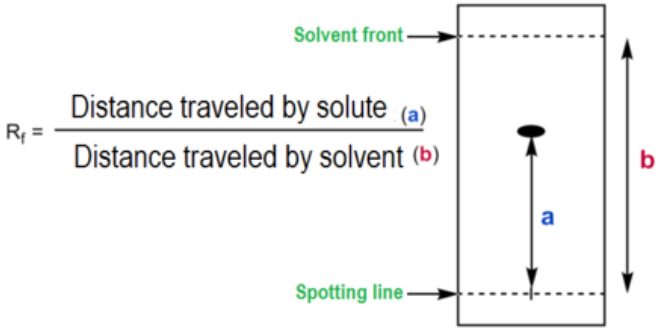
4. Types of Eluents (Mobile Phase)

- **Eluent**: A solvent or mixture of solvents used to carry analytes through the stationary phase.
- **Types**:
  - **Non-polar solvents** – e.g., hexane, petroleum ether.
  - **Polar solvents** – e.g., methanol, ethanol, water.
  - **Intermediate polarity solvents** – e.g., chloroform, ethyl acetate, acetone.
- **Polarity Gradient**: Often eluents are applied in increasing polarity (gradient elution) to separate components of varying polarity effectively.

5. Retardation Factor (R_f Value)

Definition:

The ratio of the distance travelled by the solute to the distance travelled by the solvent front in chromatography.



R_f value is a dimensionless number in chromatography that is always less than 1. It is a characteristic physical constant for a specific compound under a given set of conditions, which allows for the identification of substances by comparing their R_f values to those of known standards.

6. Factors Affecting R_f Values

1. **Chromatographic System:** The nature of the adsorbent (stationary phase) and the polarity of the eluting solvent (mobile phase) are the primary determinants of a compound's migration and R_f value.
2. **Sample Properties:** The size, concentration, and specific chemical interactions of the applied sample can cause tailing or altered mobility, leading to inaccurate R_f measurements.
3. **Experimental Conditions:** Experimental conditions like temperature, humidity, the thickness of the adsorbent layer, and the duration of development significantly influence solvent evaporation and solute mobility.
4. **System Equilibration:** Inadequate saturation of the chamber or insufficient development time prevents the establishment of a stable environment, resulting in inconsistent and unreliable R_f values.

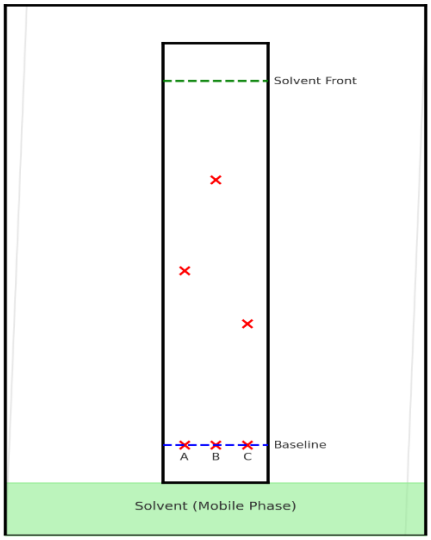
Thin Layer Chromatography (TLC)

1. Principle

Thin Layer Chromatography (TLC) separates components of a mixture based on their differential adsorption on a stationary phase and solubility in a mobile solvent. Substances with

stronger adsorption move slowly, while those more soluble in the solvent move faster, leading to separation.

2. Experimental Procedure



1. **Preparation of Plate** – A thin layer (~0.2–0.5 mm) of adsorbent (silica/alumina) is coated on a plate and dried/activated by heating.
2. **Sample Application** – Small spots of the sample solution are applied near the bottom of the plate (baseline).
3. **Development of Chromatogram** – Plate is placed vertically in a closed chamber containing a shallow layer of solvent (mobile phase). Solvent rises by capillary action, carrying the components.
4. **Detection of Spots** – After development, plate is removed, dried, and spots are visualized under UV light, iodine vapours, or by spraying with detecting reagents.

3. Advantages of TLC

1. Simple, quick, and cost-effective method requiring only small sample quantities.
2. Fast separation compared to paper or column chromatography.
3. High sensitivity, capable of detecting very small amounts (nanogram level).
4. Versatile technique – allows choice of different adsorbents and solvents for various compounds.
5. Multiple analyses at once, enabling simultaneous testing of several samples and providing qualitative or semi-quantitative results

4. Applications of TLC

1. **Identification of compounds:** Unknown samples can be identified by comparing their R_f values with those of known standards under the same conditions.
2. **Purity testing:** Helps in detecting impurities or confirming the completion of a chemical reaction by showing single or multiple spots.
3. **Separation of mixtures:** Useful for separating and analyzing mixtures such as amino acids, sugars, alkaloids, dyes, and plant pigments.
4. **Monitoring chemical reactions:** Provides a quick method to track the progress or endpoint of a reaction by observing spot changes.
5. **Pharmaceutical analysis:** Applied in drug testing for purity, quality control, and detection of adulterants.
6. **Food industry:** Used to identify preservatives, coloring agents, sweeteners, and contaminants in food products.

7. **Forensic science:** Important in the analysis of drugs, poisons, inks, dyes, and explosives, aiding crime investigation.