

SEPARATION TECHNIQUES

◇ Introduction to Chromatography

Chromatography is a separation technique based on the differential distribution of components between two phases:

- Stationary phase: fixed (solid or liquid on solid support)
- Mobile phase: fluid that moves over the stationary phase

Separation occurs because components have different affinities for each phase.

◇ 1. Column Chromatography

Principle:

Separation based on differential adsorption of compounds on a stationary solid phase (usually silica or alumina) and elution by a liquid mobile phase (solvent).

Instrumentation/Setup:

- Column packed with adsorbent
- Sample applied at the top
- Solvent (eluent) flows down
- Compounds elute at different rates

Applications:

- Separation of organic mixtures
 - Isolation of plant pigments, alkaloids, amino acids
 - Purification of synthesized compounds
-

◇ 2. Thin Layer Chromatography (TLC)

Principle:

Separation occurs due to adsorption of solutes on the thin layer (stationary phase) and movement with the solvent (mobile phase).

Procedure:

- Stationary phase: silica gel/alumina coated on glass/plastic plate
- Spot sample at base line
- Place in solvent chamber → solvent rises by capillary action
- Calculate R_f value = Distance moved by solute / Distance moved by solvent

Applications:

- Purity check

- Identification of compounds
 - Monitoring reaction progress
-

◇ 3. Paper Chromatography

Principle:

Based on partition of components between water (stationary phase trapped in paper fibers) and solvent (mobile phase).

Types:

- Ascending
- Descending
- Circular

Applications:

- Separation of amino acids, sugars, pigments
 - Forensic analysis (ink, dyes)
-

◇ 4. Gas Chromatography (GC)

Principle:

Separation depends on partition between a stationary liquid phase and a mobile gas phase (carrier gas).

Components:

- Carrier gas (He, N₂, H₂)
- Injector → Column → Detector (e.g., FID, TCD)
- Recorder for chromatogram

Applications:

- Analysis of volatile organic compounds
 - Environmental pollutants
 - Drug testing and food flavour analysis
-

◇ 5. High-Performance Liquid Chromatography (HPLC)

Principle:

Separation based on adsorption or partition under high pressure using liquid mobile phase and solid/liquid stationary phase.

Types:

- Normal phase (polar stationary, nonpolar mobile)
- Reverse phase (nonpolar stationary, polar mobile)

Applications:

- Pharmaceuticals (drug purity and stability)

- Biochemical compounds (proteins, amino acids)
 - Food analysis and forensic applications
-

THERMOANALYTICAL TECHNIQUES

These techniques study the change in physical or chemical properties of substances as a function of temperature or time.

◇ 1. Thermogravimetric Analysis (TGA)

Principle:

Measures weight change of a sample as temperature increases.
Used to study thermal stability and composition.

Graph:

Plot of weight (%) vs temperature (°C).

Applications:

- Decomposition studies
- Moisture and volatile content determination
- Oxidation or reduction analysis

- Polymer and metal oxide analysis
-

◇ 2. Differential Thermal Analysis (DTA)

Principle:

Measures temperature difference between a sample and an inert reference as both are heated simultaneously.

Endothermic/exothermic reactions are detected as peaks.

Applications:

- Phase transitions
 - Melting point determination
 - Dehydration, oxidation, decomposition studies
 - Identifying exothermic/endothermic reactions
-

◇ 3. Differential Scanning Calorimetry (DSC)

Principle:

Measures the heat flow required to maintain both sample and reference at the same temperature as they are heated.

Provides quantitative enthalpy data.

Graph:

Heat flow (mW) vs temperature (°C)

Applications:

- Determination of melting point, glass transition, crystallization
- Polymer characterization
- Purity determination
- Protein unfolding and stability studies

◇ Comparison of Thermal Techniques

Technique	Measures	Output	Key Use
TGA	Weight change	Weight vs Temp	Thermal stability
DTA	Temp difference	ΔT vs Temp	Phase transitions
DSC	Heat flow	Heat vs Temp	Enthalpy, purity, transitions