# Chapitre 3 :

# Introduction aux principales catégories de techniques d’analyse

# I. chromatographic techniques

**Chromatography** is a general term used to define separation methods based on the distribution of a solute between two phases, one of which is mobile (a gas or a liquid), and the other is stationary (a solid or a liquid).

## I.1. Definition of Chromatography

Chromatography is a technique used to separate the components of a mixture. It allows purification, identification, and quantification of compound.

**Uses in pharmaceutical labs:**

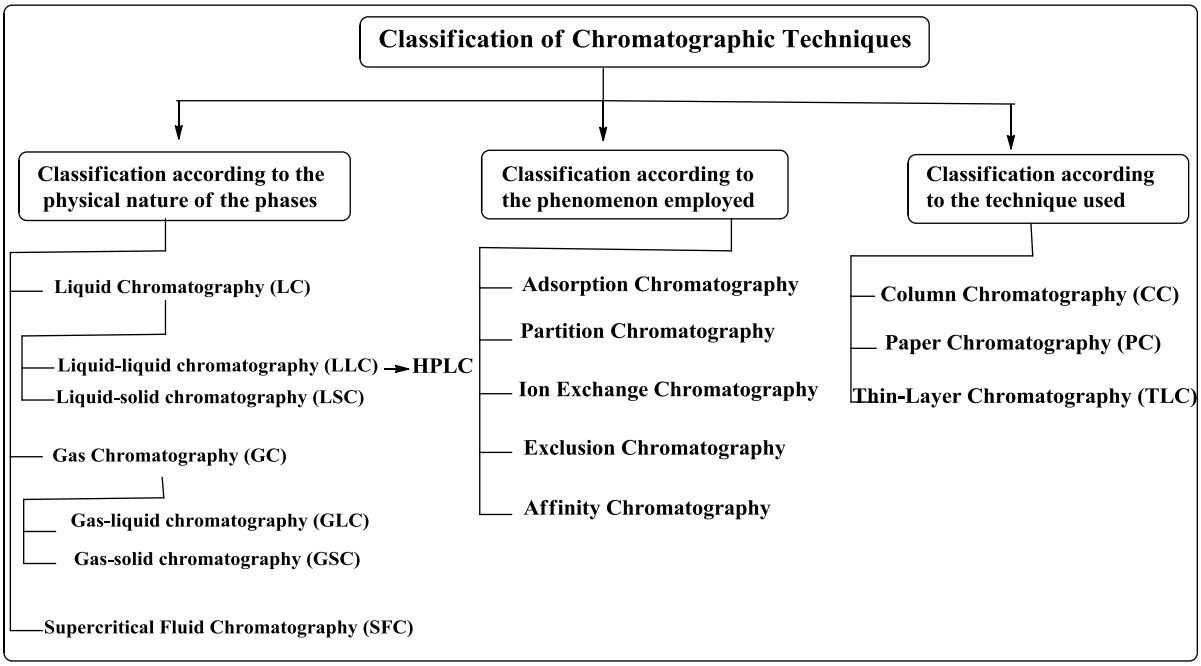
- Check the purity of raw materials and products.
- Follow the progress of a chemical reaction.
- Isolate and purify drug substances.

## I.2. Classification of Chromatographic Techniques

Chromatography can be classified depending on how separation occurs. Several factors can influence separation: solubility, polarity, molecular size/shape, electrical charge, and specific interactions.

Under the term chromatography, a wide range of different techniques is grouped, which can be classified into three categories based on:

- Classification according to the physical nature of the phases.
- Classification according to the phenomenon employed.
- Classification according to the technique used.




### I.2.1. Classification According to the Physical Nature of the Phases

In this classification:

#### I.2.1.1. Liquid Chromatography (LC)

If the mobile phase is a liquid, the stationary phase can either be a finely powdered solid or a liquid immobilized on a solid phase. The combination of these possibilities allows for the distinction of two types of chromatography:

- **Liquid-liquid chromatography (LLC).**
- **Liquid-solid chromatography (LSC).**

**I.2.1.2. Gas Chromatography (GC)** If the mobile phase is a gas (carrier), the stationary phase can either be a porous solid, reserved for the analysis of low-boiling gas mixtures, or a liquid immobilized on a solid support through impregnation or grafting. This leads to the distinction of two types of chromatography:

- **Gas-liquid chromatography (GLC).**
- **Gas-solid chromatography (GSC).**

#### I.2.1.3. Supercritical Fluid Chromatography (SFC)

SFC represents an intermediate case between **LC** and **GC**, as supercritical fluids possess properties that are between those of liquids and gases.

### I.2.2. Classification According to Chromatographic Phenomena

The phenomena leading to separation depend on the nature of the stationary phases, and we consider:

**I.2.2.1. Adsorption Chromatography**

The stationary phase is a finely divided solid on which molecules adhere through both physisorption and chemisorption. The relevant physicochemical parameter is the adsorption coefficient.

**I.2.2.2. Partition Chromatography**

The stationary phase is a liquid immobilized on an inert solid support: either impregnated into a porous solid (risk of leaching) or grafted onto the solid (grafted phase). The separation is based on the partition coefficient of the solute between the two liquid phases.

**I.2.2.3. Ion Exchange Chromatography**

The stationary phase consists of macromolecules (resins) bearing acidic or basic functional groups that allow for the exchange of counter-ions with ions of the same charge from the sample. The separation relies on ionic distribution coefficients.

**I.2.2.4. Exclusion Chromatography, or Gel Permeation, or Molecular Sieve Chromatography**

The stationary phase is a porous solid: large particles are excluded from it, while small particles are included and diffuse into the pores of the gel, thus being delayed.

**I.2.2.5. Affinity Chromatography**

The stationary phase is an inert substrate onto which an "effector" is grafted that has an affinity for a solute from the sample to be analyzed (enzyme-substrate affinity, ligand-receptor, and antibody-antigen).

**I.2.3. Classification According to the Procedure Used**

Based on the immobilization of the stationary phase, we distinguish:

**I.2.3.1. Column Chromatography (CC)**

The stationary phase is contained within a cylindrical column made of glass or metal.

**I.2.3.2. Paper Chromatography (PC)**:

A cellulose surface, considered as a support, maintains a liquid stationary phase by imbibition.

**I.2.3.3. Thin-Layer Chromatography (TLC)**: In this case, the stationary phase is retained on a flat surface (glass, plastic, or aluminum foil) covered with a thin layer of 0.2 to 0.3 mm thick silica gel, cellulose, alumina, or even ion-exchange resin grains.

# I. 3. Gas Phase Chromatography (GPC)

*(Also called Gas Chromatography, GC)*

Here is a **typical structured course summary** suitable for undergraduate (L3) or Master level students in chemistry.

**1. Introduction**

Gas Phase Chromatography (GPC), commonly known as **Gas Chromatography (GC)**, is an analytical separation technique used to separate, identify, and quantify **volatile and thermally stable compounds**.

It is widely used in organic chemistry, pharmaceutical analysis, petrochemistry, environmental analysis, and quality control laboratories.

**2. Principle of Gas Chromatography**

GC is based on the distribution of analytes between:

- **Mobile phase:** an inert carrier gas
- **Stationary phase:** a liquid or solid material inside a column

**Separation Mechanism**

1. The sample is vaporized in the injector.
2. The carrier gas transports the vaporized analytes through the column.
3. Components interact differently with the stationary phase.
4. Compounds elute at different **retention times (tR)**.
5. A detector records the signal, producing a **chromatogram**.

Separation depends mainly on:

- Volatility (boiling point)
- Polarity
- Interaction with the stationary phase

**3. Instrumentation**

A typical GC instrument consists of:

**3.1 Carrier Gas System**

Common gases:

- Helium (He)
- Nitrogen ($N_2$)
- Hydrogen ($H_2$)

Requirements:

- Chemically inert

- High purity
- Constant flow rate

**3.2 Injection System**

The injector:

- Vaporizes the sample instantly
- Operates at high temperature
- Allows split or splitless injection (for concentration control)

**3.3 Column**

The column is the heart of the GC system.

**Types of Columns:**

1. **Packed Columns**
    - Filled with coated solid particles
    - Lower efficiency
2. **Capillary (Open Tubular) Columns**
    - Narrow internal diameter
    - High efficiency
    - Most commonly used

**Stationary Phases:**

- Non-polar (e.g., dimethylpolysiloxane)
- Polar (e.g., polyethylene glycol)

**3.4 Oven**

- Controls column temperature.

Two modes:

- **Isothermal:** constant temperature
- **Temperature programming:** gradual temperature increase (better for complex mixtures)

**3.5 Detectors**

Common detectors:

**◆ Flame Ionization Detector (FID)**

- Most widely used
- Sensitive to organic compounds
- High sensitivity and wide linear range

**◆ Thermal Conductivity Detector (TCD)**

- Universal detector
- Less sensitive than FID

◆ **Electron Capture Detector (ECD)**

- Highly sensitive for halogenated compounds

◆ **GC–MS (Mass Spectrometry coupling)**

- Structural identification
- Very powerful analytical tool

**4. Types of Gas Chromatography**

**4.1 Gas–Liquid Chromatography (GLC)**

- Stationary phase is a liquid coated on a solid support
- Most common type

**4.2 Gas–Solid Chromatography (GSC)**

- Stationary phase is a solid adsorbent
- Used mainly for permanent gases

**5. Important Chromatographic Parameters**

- **Retention time (tR)**
- **Dead time (tM)**
- **Capacity factor (k')**
- **Selectivity (α)**
- **Resolution (Rs)**
- **Number of theoretical plates (N)**
- **Height Equivalent to a Theoretical Plate (HETP)**

**6. Applications**

- Purity analysis of organic compounds
- Residual solvent analysis (pharmaceutical industry)
- Petrochemical analysis
- Environmental monitoring
- Food and flavor analysis
- Forensic toxicology

**7. Advantages**

- High separation efficiency
- High sensitivity
- Fast analysis

- Small sample quantity required
- Good reproducibility

**8. Limitations**

- Only suitable for volatile and thermally stable compounds
- Not ideal for large biomolecules
- Sometimes requires derivatization

## I.4. Column Liquid Chromatography (CLC)

*(Also called Liquid–Solid Column Chromatography)*

Here is a **typical structured course** suitable for undergraduate or Master students in chemistry.

**1. Introduction**

Column Liquid Chromatography is a separation technique in which compounds are separated based on their different interactions with:

- A **liquid mobile phase**
- A **solid stationary phase** packed inside a column

It is widely used in:

- Organic synthesis laboratories
- Natural product isolation
- Pharmaceutical purification
- Reaction monitoring

It is considered the classical form of liquid chromatography and the precursor to HPLC.

**2. Principle of Separation**

The separation is based on **adsorption and partition phenomena**.

When a mixture is introduced at the top of the column:

1. The mobile phase flows through the stationary phase.
2. Each component interacts differently with the adsorbent.
3. Weakly adsorbed compounds move faster.
4. Strongly adsorbed compounds move slower.
5. Components elute separately and are collected in fractions.

Separation depends on:

- Polarity of compounds
- Nature of stationary phase

- Polarity of mobile phase

**3. Components of the System**

**3.1 Column**

Usually a glass column packed with an adsorbent.

Characteristics:

- Length
- Diameter
- Particle size of stationary phase

**3.2 Stationary Phase**

Most commonly used adsorbents:

- **Silica gel ($SiO_2$)** → polar (most common)
- **Alumina ($Al_2O_3$)** → polar
- Cellulose (less common)

Silica gel is slightly acidic and very suitable for separating organic compounds.

**3.3 Mobile Phase (Eluent)**

Organic solvents or mixtures such as:

- Hexane
- Petroleum ether
- Dichloromethane
- Ethyl acetate
- Methanol

The choice depends on polarity.

**4. Types of Elution**

**4.1 Isocratic Elution**

Single solvent or constant solvent mixture.

**4.2 Gradient Elution**

Gradual increase in solvent polarity to improve separation.

Example:

Hexane → Hexane/Ethyl acetate mixture → Pure ethyl acetate

**5. Types of Column Chromatography**

**5.1 Normal Phase Chromatography**

- Polar stationary phase (silica)
- Non-polar mobile phase
- Non-polar compounds elute first

**5.2 Reverse Phase Chromatography**

- Non-polar stationary phase (C18 modified silica)
- Polar mobile phase
- Polar compounds elute first

(Reverse phase is more common in HPLC.)

**6. Procedure**

1. Packing the column (wet or dry packing method)
2. Equilibrating with solvent
3. Loading the sample
4. Eluting with mobile phase
5. Collecting fractions
6. Monitoring fractions (usually by TLC)
7. Evaporating solvent to recover purified compounds

**7. Key Factors Affecting Separation**

- Polarity of solvent
- Particle size of adsorbent
- Column length
- Flow rate
- Sample size

**8. Applications**

- Purification of reaction products
- Isolation of natural products
- Separation of isomers
- Removal of impurities
- Preparation of analytical standards

**9. Advantages**

- Simple and inexpensive
- Suitable for preparative scale
- Flexible solvent selection

**10. Limitations**

- Time-consuming
- Large solvent consumption
- Lower efficiency compared to HPLC
- Manual and operator-dependent

**11. Comparison with HPLC**

| Column Chromatography | HPLC |
|---|---|
| Low pressure | High pressure |
| Manual | Automated |
| Large particle size | Small particle size |
| Preparative scale | Analytical & preparative |

## I.5. High-Performance Liquid Chromatography (HPLC)

**1. Introduction**

**High-Performance Liquid Chromatography (HPLC)** is an advanced analytical technique used to separate, identify, and quantify components in a liquid mixture.

Unlike classical column chromatography, HPLC uses:

- **High pressure**
- **Small particle size stationary phases**
- **Automated systems**

It is widely used in:

- Pharmaceutical analysis
- Organic chemistry
- Biochemistry
- Environmental analysis
- Food chemistry

**2. Principle of HPLC**

HPLC is based on differential distribution of analytes between:

- **Mobile phase** (liquid solvent or solvent mixture)
- **Stationary phase** (solid particles packed inside a column)

**Separation Mechanism**

1. The sample is injected into the mobile phase.
2. A high-pressure pump forces the mobile phase through the column.
3. Components interact differently with the stationary phase.
4. Each compound elutes at a specific **retention time (tR)**.
5. A detector records the signal, producing a chromatogram.

Separation depends on:

- Polarity
- Hydrophobicity

- Ionic interactions
- Molecular size (in some modes)

**3. Instrumentation**

A typical HPLC system consists of:

**3.1 Solvent Reservoirs**

Contain mobile phase solvents.

The mobile phase must be:

- Filtered
- Degassed (to remove air bubbles)

**3.2 Pump**

- Generates high pressure (up to 400–600 bar in conventional HPLC)
- Delivers constant flow rate
- Allows isocratic or gradient elution

**3.3 Injector**

- Manual injector or autosampler
- Introduces precise sample volume (typically 5–20 µL)

**3.4 Column**

- The heart of the system.

Typical characteristics:

- Length: 10–25 cm
- Internal diameter: 2–4.6 mm
- Particle size: 3–5 µm

Smaller particles → higher efficiency → higher pressure required.

**3.5 Detector**

Common detectors include:

**◆ UV–Visible Detector**

- Most widely used
- Suitable for compounds with chromophores

**◆ Diode Array Detector (DAD)**

- Records full UV spectrum
- Useful for peak identification

**◆ Fluorescence Detector**

- Very sensitive

- For fluorescent compounds

◆ **Refractive Index (RI) Detector**

- Universal detector
- Less sensitive

◆ **HPLC–MS**

- Structural identification
- Highly sensitive and selective

**4. Modes of HPLC**

**4.1 Normal Phase HPLC**

- Polar stationary phase (silica)
- Non-polar mobile phase
- Non-polar compounds elute first

**4.2 Reverse Phase HPLC (RP-HPLC)**

- Non-polar stationary phase (C18, C8)
- Polar mobile phase (water + methanol or acetonitrile)
- Most commonly used mode
- Polar compounds elute first

**4.3 Ion-Exchange Chromatography**

- Separation based on charge
- Used for ionic compounds and biomolecules

**4.4 Size Exclusion Chromatography (SEC)**

- Separation based on molecular size
- Large molecules elute first

**5. Types of Elution**

**Isocratic Elution**

- Constant mobile phase composition

**Gradient Elution**

- Composition changes during analysis
- Improves separation of complex mixtures

**6. Important Chromatographic Parameters**

- Retention time (tR)
- Dead time (tM)
- Capacity factor (k')

- Selectivity (α)
- Resolution (Rs)
- Number of theoretical plates (N)
- Plate height (HETP)

**7. Applications**

- Drug quality control
- Assay of active pharmaceutical ingredients (API)
- Stability studies
- Bioanalysis
- Natural product analysis
- Food additives analysis
- Environmental pollutant detection

**8. Advantages**

- High resolution
- High sensitivity
- Good reproducibility
- Suitable for non-volatile compounds
- Automated operation

**9. Limitations**

- Expensive instrumentation
- High solvent consumption
- Requires method development optimization

**10. Comparison with GC**

| HPLC | GC |
|---|---|
| Liquid mobile phase | Gas mobile phase |
| Suitable for non-volatile compound | Suitable for volatile compounds |
| No need for vaporization | Requires vaporization |
| Moderate temperature | High temperature |

# I.6. Thin Layer Chromatography (TLC)

*(Planar Chromatographic Technique)*

**1. Introduction**

**Thin Layer Chromatography (TLC)** is a simple, rapid, and inexpensive analytical technique used to separate, identify, and monitor compounds in a mixture.

It is widely used in:

- Organic synthesis laboratories
- Reaction monitoring
- Purity testing
- Natural product analysis
- Preliminary identification of compounds

TLC is a form of **planar chromatography**, where separation occurs on a flat plate instead of inside a column.

**2. Principle of TLC**

TLC is based on differential adsorption and partition of compounds between:

- **Stationary phase:** thin layer of adsorbent coated on a plate
- **Mobile phase:** solvent (eluent) moving upward by capillary action

**Separation Mechanism**

1. The sample is spotted near the base of the plate.
2. The plate is placed in a developing chamber containing solvent.
3. The solvent rises by capillary action.
4. Components move at different speeds depending on their interaction with the stationary phase.
5. Separation occurs as distinct spots.

Compounds with:

- Weak interaction with stationary phase → travel further
- Strong interaction → remain closer to the origin

**3. Components of TLC**

**3.1 TLC Plate**

Usually made of:

- Glass
- Aluminum
- Plastic

Common stationary phases:

- **Silica gel ($SiO_2$)** → polar (most common)
- **Alumina ($Al_2O_3$)**
- Cellulose

Silica gel is slightly acidic and very suitable for organic compounds.

**3.2 Mobile Phase (Eluent)**

Organic solvent or solvent mixture, such as:

- Hexane
- Ethyl acetate
- Dichloromethane
- Methanol

The polarity of the solvent strongly affects separation.

**3.3 Developing Chamber**

Closed container saturated with solvent vapor to ensure reproducible development.

**4. Rf Value (Retention Factor)**

The main quantitative parameter in TLC is the **Rf value**:

Rf=Distance traveled by compoundDistance traveled by solvent frontRf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}Rf=Distance traveled by solvent frontDistance traveled by compound

Characteristics:

- $0 \leq Rf \leq 1$
- Characteristic for a compound under fixed conditions
- Used for identification (comparison with standard)

**5. Visualization Methods**

Since many compounds are colorless, visualization is required.

**Common Methods:**

- **UV light (254 nm or 365 nm)**
- Iodine vapor
- Chemical staining (e.g., ninhydrin for amino compounds)
- Anisaldehyde stain
- $KMnO_4$ stain

**6. Types of TLC**

**6.1 Normal Phase TLC**

- Polar stationary phase (silica)

- Non-polar mobile phase
- Non-polar compounds move further

**6.2 Reverse Phase TLC**

- Non-polar stationary phase
- Polar mobile phase

**7. Applications**

- Monitoring reaction progress
- Checking product purity
- Selecting solvent system for column chromatography
- Identifying compounds (comparison with standards)
- Detecting impurities

**8. Advantages**

- Simple and fast
- Low cost
- Minimal sample required
- Multiple samples analyzed simultaneously
- No complex instrumentation

**9. Limitations**

- Lower resolution compared to HPLC
- Semi-quantitative
- Reproducibility depends on conditions
- Limited sensitivity

**10. Comparison with Other Techniques**

| TLC | Column Chromatography | HPLC |
|---|---|---|
| Planar system | Column system | High-pressure column |
| Fast & simple | Preparative | Highly precise |
| Semi-quantitative | Purification | Quantitative |

# II. Spectral techniques

# II.1. UV–Visible Spectroscopy and Its Applications

**1. Introduction**

UV–Visible spectroscopy is an analytical technique based on the absorption of ultraviolet (200–400 nm) and visible (400–800 nm) radiation by molecules.

It is widely used in:

- Organic and inorganic chemistry
- Pharmaceutical analysis
- Drug research
- Quantitative analysis using the Beer–Lambert law

Its major application is **quantitative determination of concentration**.

**2. Energy Domain**

- The theoretical UV range: **110–800 nm**
- Practical working range: **200–800 nm**
- Most instruments do not measure below 200 nm.

**UV Regions:**

- **UV-C (210–280 nm):** Bactericidal
- **UV-B (280–315 nm):** Vitamin D synthesis
- **UV-A (315–400 nm):** Skin pigmentation

**3. Electronic Transitions**

Absorption in UV–Vis corresponds to **electronic transitions** in molecules.

Electrons involved:

- **σ (sigma)** → strong single bonds
- **π (pi)** → double bonds
- **n (non-bonding)** → lone pairs

**Main Types of Transitions:**

| Transition | Energy | Intensity | Typical Region | Practical Use |
|---|---|---|---|---|
| σ → σ* | Very high | Strong | < 200 nm | Not practical |
| n → σ* | High | Medium | ~200 nm | Limited use |
| π → π* | Moderate | Strong | 200–400 nm | Most important |
| n → π* | Low | Weak | 270–300 nm | Limited use |

✔ The **π → π*** transition is the most useful in analysis because:

- High sensitivity
- Moderate energy
- Occurs in conjugated systems

**4. Chromophores and Auxochromes**

**Chromophore**

- The part of a molecule responsible for absorption.

  Examples:

  C=C, C=O, N=N

Characterized by:

- λmax (maximum absorption wavelength)
- εmax (molar absorptivity)

**Auxochrome**

- A group that modifies absorption when attached to a chromophore.

  Examples: –OH, $–NH_2$, $–NO_2$, halogens

They can:

- Shift λmax
- Increase or decrease intensity

**5. Spectral Effects**

- **Bathochromic shift:** Shift to longer wavelength (red shift)
- **Hypsochromic shift:** Shift to shorter wavelength (blue shift)
- **Hyperchromic effect:** Increase in intensity
- **Hypochromic effect:** Decrease in intensity

Increasing conjugation → increases λmax → may reach visible region.

**6. Perception of Color**

- The observed color is the **complementary color** of the absorbed radiation.
- A compound absorbing in the visible region appears colored.
- If no absorption occurs in the visible range → compound is colorless.

**7. Beer–Lambert Law**

The fundamental equation for quantitative analysis:

$$\mathbf{A = \varepsilon \cdot l \cdot C}$$

Where:

- A = Absorbance
- ε = Molar absorptivity
- l = Path length

- C = Concentration

Also:

$$A = log \frac{I_0}{I}$$

**Conditions for Validity:**

- Monochromatic light
- Dilute solutions
- No scattering
- No chemical changes with concentration

Absorbance is additive for mixtures.

**8. Instrumentation**

Main components of a UV–Vis spectrophotometer:

1. **Source** (200–800 nm)
2. **Monochromator** (prism or grating)
3. **Sample and reference cells**
    - Quartz (UV region)
    - Glass (visible region)
4. **Detector** (photoelectric effect)
5. **Recorder / display**

The spectrum obtained is:

$$\mathbf{A = f(\lambda)}$$

**9. Spectrum Interpretation**

Important parameters:

- $\lambda_{max}$ (position of peak)
- Absorbance intensity
- Band shape (broad or narrow)

Key factors influencing spectrum:

- Conjugation
- Substituents
- Solvent polarity (solvatochromism)
- Charge-transfer transitions

**10. Applications**

**Qualitative Analysis**

- Identification of chromophores

- Limited structural information

**Quantitative Analysis**

- Determination of concentration
- Pharmaceutical quality control
- Stability studies
- Reaction monitoring

If the analyte does not absorb:→ Derivatization with a chromophore reagent.

## II.2. Infrared (IR) Spectroscopy

**1. Introduction**

- Molecules vibrate constantly; covalent bonds act like **springs**.
- IR spectroscopy measures **absorption of infrared photons** that match molecular vibrational energies.
- Provides information about **functional groups** and **molecular fragments**, not complete structure.
- Useful for **organic and inorganic molecules** identification.

**2. Spectral Range**

- IR absorption range: **4000 – 400 $cm^{-1}$** (mid-IR).
- Energy corresponds to **1–11 kcal/mol** → affects molecular vibrations and rotations, **not electronic transitions**.

**3. Vibrational Theory**

- Molecules vibrate like **harmonic oscillators**:

$$E_0 = \frac{1}{2}\left(\frac{h}{2\pi}\sqrt{\frac{k}{\mu}}\right)$$

- μ = reduced mass of the two atoms: $\mu = (m_1 \times m_2)/(m_1+m_2)$
- Energy levels: $E_v=(v+1/2)h\nu$
- Most molecules at room temperature are in **v = 0** (ground vibrational state).

**4. Vibrational Modes**

**4.1 Stretching Vibrations**

- Change bond lengths along bond axis.
- **Symmetric**: atoms move together.
- **Asymmetric**: atoms move in opposite directions.
- Asymmetric stretches → stronger absorption bands.

### 4.2 Bending Vibrations

- Change bond angles.
- Less intense than stretching bands.
- Occur **in-plane** or **out-of-plane**.
- Help identify substituent positions in aromatic rings.

### 4.3 Number of Vibrational Modes

- Diatomic: 1 stretching
- Linear molecule: 3n−5
- Non-linear molecule: 3n−6
- Example: $H_2O$ → 3 vibrations (symmetric stretch, asymmetric stretch, bending)

### 5. IR Band Characteristics

- **Band intensity:** strong (s), medium (m), weak (w)
- Only **polar bonds** are IR active.
- **Band shapes:** narrow (sharp) or broad (e.g., O-H)
- **Fingerprint region:** 600–1400 $cm^{-1}$; complex, used for comparison

### 6. Functional Group IR Absorption

| $\bar{\nu}$ ($cm^{-1}$) | Structure | $\bar{\nu}$ ($cm^{-1}$) | Structure |
|---|---|---|---|
| **1050-1400** | C-O (ether, alcool et ester | **2700-2800** | H-C=O (C-H du l'aldéhyde) |
| **1150-1360** | $SO_2$ (dérivé sulfoniques) | **2500-3000** | O-H (acide carboxylique) |
| **1315-1475** | C-H (alcanes, vibration de déformation) | **3000-3100** | C-H (fait partie d'un cycle aromatique |
| **1340-1500** | $NO_2$ (dérivés nitrés) | **3300** | C-H (fait partie d'un acétylène $H-C\equiv C$ |
| **1450-1600** | C=C (cycle aromatiques) | **3020-3080** | C-H (fait partie d'un acétylène $H-C=C$ |
| **1620-1680** | C=C (alcènes insaturés) | **2800-3000** | C-H (alcanes) |
| **1630-1690** | C=O (amides) | **3300-3500** | N-H (amides et amines) |
| **1690-1750** | C=O (aldéhyde cétone et ester) | **3200-3600** | O-H (R-OH et Ar-OH avec liaisons hydrogènes) |
| **1700-1725** | C=O (acide carboxylique) | **2100** | O-D (composés deutérés) |
| **1770-1820** | C=O (Chlorure d'acide) | **690-710** | C-H (monosubstitution) |
| **2100-2200** | $C\equiv C$ (alcynes) | **735-770** | Ortho (di substitution) |
| **2210-2260** | $C\equiv N$ $(Nitriles)$ | **780-810** | Méta (di substitution)) |
| **2500** | S-H (thiol) | **810-840** | Para (di substitution) |

### 7. Analysis Strategy

1. Determine **degree of unsaturation**:

$$\boldsymbol{i = \frac{2n_c - n_H + n_N - n_X + 2}{2}}$$

2. Examine spectrum from **high wavenumbers** downward.
3. Identify **characteristic bands** using reference tables.
4. Avoid over-interpreting the **fingerprint region (<1400 $cm^{-1}$)**.

**8. Sample Presentation**

- **Solids:** KBr pellet (0.2–2%), Nujol mull
- **Liquids:** Pure or solution; thin cells (0.005–1 mm)
- **Gases:** Larger IR cell
- Solvents must be **anhydrous and transparent in IR**, e.g., $CCl_4$, $CHCl_3$, $CS_2$

**9. Instrumentation**

- **Source (S):** Globar (SiC) or Nernst filament → emits IR
- **Monochromator (M):** prism or grating separates wavelengths
- **Cells ($C_1$, $C_2$):** reference & sample; made of NaCl or KBr
- **Detector (D):** converts radiation to signal
- **Recorder (E):** captures spectrum

**10. Summary of Applications**

- Identify **functional groups**
- Compare molecules using **fingerprint region**
- Determine **molecular fragments**
- Distinguish **aldehydes vs ketones, alcohols, amines, acids, etc.**

**Limitations:**

- Does not provide full structural info
- Must be combined with other techniques (e.g., NMR, MS) for complete analysis

## II.3. ATOMIC ABSORPTION AND EMISSION PHOTOMETRY

*(Atomic Spectrometry)*

**1. Introduction**

Atomic spectrometry is a group of analytical techniques used for the **qualitative and quantitative determination of elements**, especially metals.

These techniques are based on the interaction between:

- Free atoms in the gaseous state
- Electromagnetic radiation

The two main techniques are:

- **Atomic Absorption Spectrometry (AAS)**

- **Atomic Emission Spectrometry (AES)**

Both require the transformation of the sample into **free atoms**, usually in a flame or plasma.

## 2. Fundamental Concepts

### 2.1 Atomic Energy Levels

Atoms possess **discrete electronic energy levels**.

- Ground state → lowest energy level
- Excited state → higher energy levels

When an atom absorbs energy:

$$\mathbf{E = h\nu = \frac{hC}{\lambda}}$$

Where:

- h = Planck constant
- ν= frequency
- λ = wavelength

Each element has a **unique set of energy levels**, giving a characteristic spectrum.

### 2.2 Resonance Lines

The most important transitions in atomic spectrometry are:

Ground state → First excited state

These transitions produce **resonance lines**, which are:

- Very intense
- Highly specific
- Used for quantitative analysis

## 3. Atomic Absorption Spectrometry (AAS)

### 3.1 Principle

In AAS:

- Free atoms in the ground state absorb radiation.
- A monochromatic light beam passes through the atomic vapor.
- The decrease in intensity is measured.

$$\boldsymbol{A = log\frac{I_0}{I}}$$

Where:

- $I_0$ = incident intensity

- I = transmitted intensity
- A = absorbance

According to **Beer–Lambert law**, absorbance is proportional to concentration:

$$\mathbf{A = k\,C}$$

### 3.2 Instrumentation of AAS

Main components:

**1. Radiation Source**

Usually a **Hollow Cathode Lamp (HCL)** specific to each element.

**2. Atomizer**

- Flame (air–acetylene, $N_2O$–acetylene)
- Graphite furnace (higher sensitivity)

**3. Monochromator**

Selects the analytical wavelength.

**4. Detector**

Usually a photomultiplier tube.

### 3.3 Types of AAS

- **Flame AAS (FAAS)**
- Temperature: 2000–3000 K
- Fast
- Moderate sensitivity
- **Graphite Furnace AAS (GFAAS)**
- Very high sensitivity
- Small sample volume
- Used for trace analysis

### 3.4 Advantages of AAS

✔ High selectivity

✔ Good sensitivity

✔ Relatively simple instrumentation

### 3.5 Limitations

✘ Usually single-element analysis

✘ Chemical and spectral interferences

## 4. Atomic Emission Spectrometry (AES)

Atomic Emission Spectrometry (AES) is a sensitive, multi-element analytical technique used to determine elemental composition (qualitative) and concentration (quantitative) by measuring light emitted from excited atoms. It utilizes high-energy sources—typically ICP (inductively coupled plasma), arcs, or sparks—to excite atoms, which emit characteristic wavelengths upon relaxation, enabling detection in environmental, biological, and material science fields.

### 4.1 Principle

In AES:

- Atoms are excited thermally.
- They emit radiation when returning to lower energy states.
- Emitted light intensity is measured.

Emission intensity ∝ number of excited atoms ∝ concentration.

### 4.2 Excitation Sources

- **Flame Emission**

Used mainly for:

- Na
- K
- Li

- **Plasma (ICP)**

The most powerful source is:

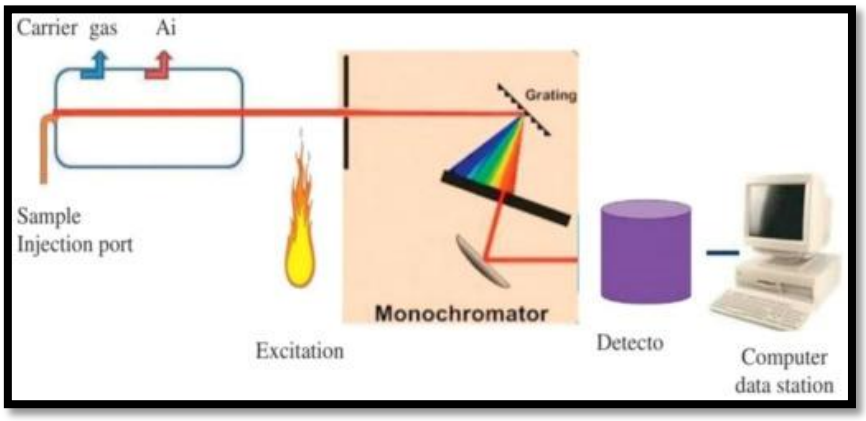
**Inductively Coupled Plasma (ICP)**

- Temperature: 6000–10000 K
- Almost complete atomization and excitation
- Multi-element analysis

### 4.3 Instrumentation of ICP-OES

Components:

1. Nebulizer
2. Plasma torch
3. Optical system
4. Detector (CCD or photomultiplier)




### 4.4 Advantages of AES

✔ Multi-element analysis

✔ Very high sensitivity (ICP)

✔ Wide linear range

### 4.5 Limitations

✘ Expensive equipment

✘ Higher operational cost

## 5. Comparison between AAS and AES

| Parameter | AAS | AES |
|---|---|---|
| Process | Absorption | Emission |
| Source of radiation | External lamp | Sample itself |
| Multi-element analysis | Limited | Yes (ICP) |

| Parameter | AAS | AES |
|---|---|---|
| Sensitivity | High | Very high (ICP) |
| Cost | Moderate | High |

## 6. Interferences

### 6.1 Spectral Interference

Overlapping lines.

### 6.2 Chemical Interference

Formation of stable compounds.

### 6.3 Ionization Interference

Loss of neutral atoms at high temperature.

### 6.4 Matrix Effects

Effect of other components in sample.

## 7. Applications

• Clinical analysis ($Na^+$, $K^+$, $Ca^{2+}$ in blood)

• Water quality control

• Heavy metal analysis

• Food analysis

• Geological samples

• Pharmaceutical quality control

## 8. Analytical Procedure (Typical Steps)

1. Sample preparation (digestion if necessary)
2. Calibration curve preparation
3. Measurement
4. Validation (precision, accuracy)

# IV. Classical Titrimetric Techniques

Titrimetry (or titration analysis) encompasses a group of quantitative analytical methods based on measuring the amount of a reagent of known concentration required to react completely with an analyte present in a solution of known volume.

The reagent used in titrimetry may be:

- **A standard solution** (a solution whose concentration is accurately known) → *Volumetric titration*
- **A solid reagent of known purity** → *Gravimetric or precipitation titration*
- **An electric current of known intensity applied for a measured time** → *Coulometric titration*

Titrations are classified according to the type of chemical reaction involved:

- Precipitation reactions
- Acid–base (neutralization) reactions
- Complexation reactions
- Oxidation–reduction (redox) reactions

## IV.1. Precipitation Titration

**Principle**

Precipitation titration is based on the quantitative formation of a sparingly soluble compound through the reaction between the analyte and the titrant.

This method aims to achieve the complete separation of a cation or an anion (in aqueous solution) by selective precipitation as an insoluble salt under controlled experimental conditions.

For a precipitation titration to be suitable for analytical purposes, the reaction must be:

- **Complete** (quantitative)
- **Rapid**
- **Selective (specific)**
- **Well-defined stoichiometry**

### IV.1.1. Argentimetry (Argentometry)

Argentimetry refers to precipitation titrations that involve **silver ions ($Ag^+$)** as the titrating species.

The titrant is typically a standard solution of **silver nitrate ($AgNO_3$)**.

Argentimetric methods are mainly used for the determination of certain anions, including:

- Halides ($Cl^-$, $Br^-$, $I^-$)
- Thiocyanate ($SCN^-$)
- Cyanide ($CN^-$)

### IV1.1.1. Determination of Halide Ions by the Mohr Method

**Principle**

In the Mohr method, halide ions ($X^-$) are directly titrated with a standard silver nitrate solution. The reaction leads to the formation of a precipitate of silver halide:

$$\mathbf{Ag^+_{(aq)} + X^-_{(aq)} \rightarrow AgX_{(s)}}$$

To detect the equivalence point, **potassium chromate ($K_2CrO_4$)** is used as an indicator. After all halide ions have reacted, the first excess of silver ions reacts with chromate ions to form **silver chromate ($Ag_2CrO_4$)**:

$$\mathbf{2\,Ag^+_{(aq)} + CrO_4^{-2} \rightarrow Ag_2CrO_{4(S)}}$$

Silver chromate has a characteristic **brick-red color**, which signals the endpoint.

**Experimental Conditions**

For accurate results, the Mohr method must be carried out:

- In a **neutral medium** ($6.5 < pH < 7.5$)
- At **room temperature**
- In the absence of interfering ions

If the medium is too acidic, chromate ions are converted to dichromate; if too basic, silver hydroxide may form.

### IV1.1.2. Charpentier–Volhard Method

The Volhard method is an **indirect (back) titration** method used primarily for chloride determination.

**Step 1: Precipitation**

An excess known amount of silver nitrate is added to the chloride-containing solution, forming a white precipitate of silver chloride:

$$\mathbf{Ag^+ + Cl^- \rightarrow AgCl_{(s)} \downarrow}$$

**Step 2: Back Titration**

The excess (unreacted) silver ions are titrated with a standard solution of **potassium thiocyanate (KSCN)**:

$$Ag^{+} + SCN^{-} \rightarrow AgSCN_{(s)}$$

**Endpoint Detection**

The endpoint is detected using **ferric ammonium sulfate** (commonly called ferric ammonium alum), containing $Fe^{3+}$ ions.

At equivalence, once all $Ag^{+}$ ions have reacted, the first excess of thiocyanate reacts with $Fe^{3+}$ to form a **blood-red complex**:

$$Fe^{3+} + SCN^{-} \rightarrow [FeSCN]^{2+}$$

The appearance of this persistent red coloration indicates the endpoint.

**Experimental Conditions**

The Volhard method must be carried out:

- In a **strongly acidic medium (pH < 2)**
- In the presence of **nitric acid ($HNO_3$)**

The acidic medium:

- Prevents formation of metal hydroxides
- Avoids side precipitation reactions
- Ensures clear visualization of the endpoint

**6. Direct vs Indirect Titration**

**Direct Titration**

- A titration in which the analyte reacts directly with the titrant in a single reaction step to reach equivalence.

**Example:** Mohr method.

**Indirect (Back) Titration**

A titration involving two reaction steps:

1. The analyte reacts with an excess of a standard reagent.
2. The excess reagent is titrated with a second standard solution to determine the amount that reacted.

**Example:** Volhard method.

# IV.2. Acid–Base (Neutralization) Titrations

### 1. Principle

Acid–base titrations are based on a **neutralization reaction** between an acid and a base, leading to the formation of salt and water.

The general reaction is:

$$\textbf{Acid + Base} \rightarrow \textbf{Salt + Water}$$

For example:

$$\mathbf{HCl + NaOH \rightarrow NaCl + H_2O}$$

At the **equivalence point**, the number of moles of acid equals the number of moles of base according to the reaction stoichiometry.

For a monoprotic acid and a monobasic base:

$$\mathbf{n_{acid} = n_{base}}$$

$$\mathbf{C_aV_a = C_bV_b}$$

Where:

- $\mathbf{C_a.C_b}$ **= concentrations**
- $\mathbf{V_a.V_b}$ **= volumes**

### 2. Requirements for a Valid Acid–Base Titration

For accurate analytical determination, the reaction must be:

- Fast
- Complete
- Stoichiometric
- Without significant side reactions

### 3. Types of Acid–Base Titrations

Acid–base titrations are classified according to the strength of the acid and the base involved:

#### 3.1 Strong Acid – Strong Base

**Example:**

HCl titrated by NaOH

- Equivalence point at **pH = 7**
- Sharp and steep pH jump
- Many indicators are suitable

Curve characteristics:

- Initial low pH
- Sudden vertical jump at equivalence
- Final basic pH

### 3.2 Strong Acid – Weak Base

Example:

HCl titrated by $NH_3$

- Equivalence point at **pH < 7**
- Solution contains acidic salt at equivalence
- Suitable indicator: methyl orange

### 3.3 Weak Acid – Strong Base

Example:

$CH_3COOH$ titrated by NaOH

- Equivalence point at **pH > 7**
- Formation of a basic salt
- Suitable indicator: phenolphthalein

### 3.4 Weak Acid – Weak Base

- No sharp pH jump
- No suitable visual indicator
- Rarely used in classical titrimetry

## 4. The Equivalence Point vs Endpoint

**Equivalence Point**

- The theoretical point at which the stoichiometric amount of titrant has reacted completely with the analyte.

**Endpoint**

The experimental point detected by:

- A color change (indicator)
- A pH meter
- Conductometric measurement

The endpoint should be as close as possible to the equivalence point.

## 5. Acid–Base Indicators

### Principle

Acid–base indicators are weak organic acids or bases whose protonated and deprotonated forms have different colors.

General equilibrium:

$$\mathbf{HIn \rightleftharpoons H^+ + In^-}$$

- HIn = acidic form (color 1)
- $In^-$ = basic form (color 2)

The color depends on the pH of the solution.

### Common Indicators

| Indicator | pH Range | Color Change |
|---|---|---|
| Methyl orange | 3.1 – 4.4 | Red → Yellow |
| Bromothymol blue | 6.0 – 7.6 | Yellow → Blue |
| Phenolphthalein | 8.2 – 10 | Colorless → Pink |

Indicator choice depends on the expected pH at equivalence.

## 6. Titration Curve

A titration curve represents:

$$\text{pH} = f\,(\text{Volume of titrant added})$$

It allows:

- Determination of equivalence point
- Selection of suitable indicator
- Study of buffering regions (in weak acid/base titrations)

For weak acids, the curve shows a **buffer region** before equivalence, where:

$$\mathbf{pH = pKa \text{ at half-equivalence}}$$

## 7. Applications of Acid–Base Titrations

- Determination of acidity in food products
- Pharmaceutical quality control
- Water analysis
- Determination of purity of acids and bases
- Industrial process monitoring

# IV.3. Complex Formation Titrations (Complexometry)

## 1. Introduction

Complexometric titration allows simple, rapid, and accurate volumetric determination of a large number of metal ions through the formation of coordination complexes.

A complex-forming reaction can be used as a titration reaction provided that it is:

- Unique (selective)
- Complete (quantitative)
- Rapid

Complexometric titrations became reliable analytical methods after the introduction of chelating agents by **G. Schwarzenbach (1945)**, particularly ethylenediaminetetraacetic acid (EDTA).

## 2. Definition of a Complex

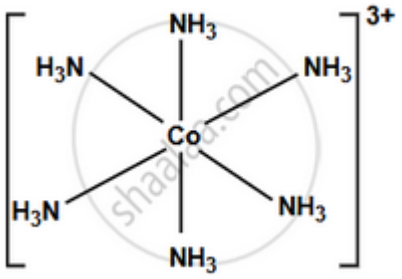
A complex (coordination compound) is a polyatomic structure consisting of:

- A central metal atom or metal cation
- Surrounding molecules or anions called ligands (or coordinands), bonded through coordinate (dative covalent) bonds

The formula of a complex is written in square brackets.

Examples:

- Neutral complex: $[Cr(CO)_6]$
- Cationic complex: $[Co(NH_3)_6]^+$
- Anionic complex: $[AlF_6]^{3-}$




**Co : central metal atom**

**N: donor atom**

**$NH_3$: ligands (or coordinands)**

In ionic complexes, counter-ions are present outside the brackets:

- $[Co(NH_3)_6]Cl$

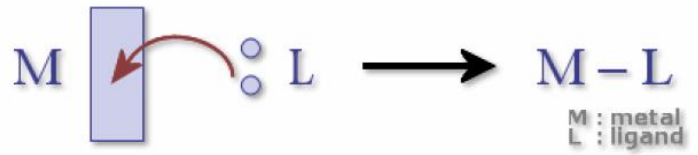
- $Na_3[AlF_6]$

### 3. Coordination and Lewis Acid–Base Concept

Complex formation is a Lewis acid–base reaction:

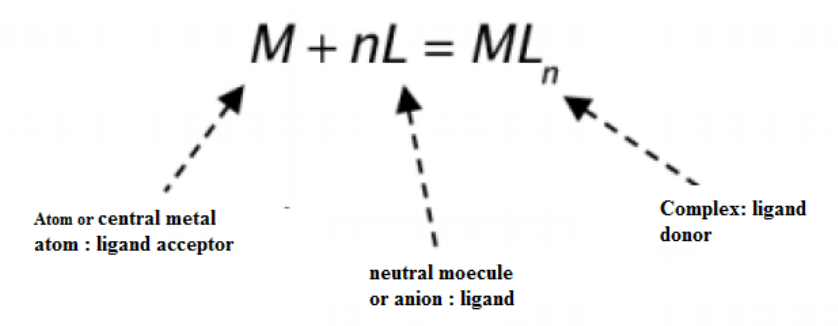
- The central metal ion is a **Lewis acid** (electron pair acceptor).
- The ligand is a **Lewis base** (electron pair donor).

The bond formed is a coordinate covalent (dative) bond.




### 4. Ligands

Ligands used in metal ion titrations must **possess** at least **one nonbonding** (lone) pair of electrons available for coordination.

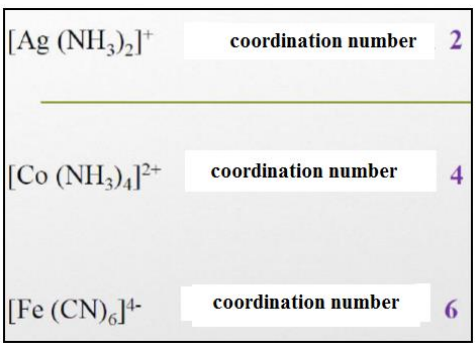



**Coordination Number**

The number of donor atoms bonded to the central metal ion is called the **coordination number**.

Common coordination numbers are:

- 2
- 4
- 6



**Stability of Complexes**

The stability of a complex depends on:

- Nature of the metal ion
- Nature of the ligand
- pH of the medium
- Solvent
- Presence of interfering ions

Chelating ligands (multidentate ligands) form particularly stable complexes due to the **chelate effect**.

**EDTA (Complexon III)**

The most widely used titrant in complexometry is **EDTA (ethylenediaminetetraacetic acid)**, also called **Complexon III**.

EDTA is a hexadentate ligand capable of binding metal ions in a 1:1 stoichiometric ratio:

$$M^{n+} + EDTA^{4-} \rightarrow [M - EDTA]^{(n-4)}$$

This 1:1 stoichiometry greatly simplifies calculations.

**5. Types of Complexometric Titrations**

**1) Direct Titration**

**Principle**

This is the simplest and most common method.

The procedure is as follows:

- The metal ion solution is buffered to an appropriate pH.
- A suitable metallochromic indicator is added.
- The EDTA standard solution is added dropwise.

Before the endpoint:

- The solution is colored due to the metal–indicator complex.
- EDTA reacts first with free metal ions in solution.

Near equivalence:

- Both metal–EDTA and metal–indicator complexes coexist.

At equivalence:

- A slight excess of EDTA displaces the indicator from the metal–indicator complex because the metal–EDTA complex is more stable.

- The indicator is released, producing a color change.

**Applications**

- Determination of $Ca^{2+}$ using murexide or calcon indicator
- Determination of $Mg^{2+}$ using Eriochrome Black T (EBT)

**2) Displacement (Replacement) Method**

This method is used when the metal–indicator complex is insufficiently stable, resulting in poor endpoint detection.

**Example: Calcium Determination with EBT**

The Ca–EBT complex is weak and gives an imprecise endpoint.

To improve sensitivity:

- A small amount of magnesium–EDTA complex is added.
- Magnesium forms a more stable complex with the indicator (Mg–EBT).

During titration:

- Free $Ca^{2+}$ ions are first complexed by EDTA (Ca–EDTA is more stable).
- At equivalence, EDTA displaces magnesium from the Mg–indicator complex.
- The released indicator changes color, marking the endpoint.

**3) Back (Indirect) Titration**

This method is used when:

- No suitable indicator exists,
- The complexation reaction is too slow,
- The metal ion forms insoluble hydroxides at the working pH.

Examples include the determination of:

- Lead ($Pb^{2+}$)
- Mercury ($Hg^{2+}$)
- Manganese ($Mn^{2+}$)

**Procedure**

1. A known excess of EDTA is added to the analyte.
2. After complete complexation, the remaining (excess) EDTA is titrated with a standard $Mg^{2+}$ or $Zn^{2+}$ solution.
3. Eriochrome Black T is used as indicator.

**Applications of Complexation in Therapeutics**

Complexation phenomena are important in pharmacology and drug interactions.

Certain drugs form complexes with divalent metal ions (especially $Ca^{2+}$ and $Mg^{2+}$), which may reduce their bioavailability and therapeutic activity.

Examples include:

- Tetracyclines
- Aminoglycosides
- Furosemide
- Sodium edetate
- D-penicillamine

The formation of poorly absorbable complexes in the gastrointestinal tract can lead to partial drug inactivation.

## IV.4. Titration by Oxidation–Reduction Reactions (Electrochemical Methods)

It is possible to use electrical energy to produce a chemical reaction: this is called **Electrolysis**.

**Electrolysis Phenomenon**

The electrolysis phenomenon can be considered from two aspects:

- **Classical electrolysis**

This involves redox phenomena leading to the deposition of a metal on a cathode.

- **Microelectrolysis**

This consists of carrying out electrolysis on a dilute solution for a very short time, using a very low-intensity current and small electrodes.

The measurement of:

- **I (current intensity)**
- **E (potential)**

Developed during this microelectrolysis can be used for analytical purposes.

**Instrumental Electrochemical Methods**

The main electrochemical instrumental methods are:

- Potentiometry
- Conductimetry
- Amperometry
- Coulometry (Polarography)

**1) Potentiometry**

Analytical methods based on potential measurements are called **potentiometric methods**.

Potentiometry makes it possible to:

- determine thermodynamic parameters
- evaluate complexation constants
- determine solubility products
- monitor industrial preparation processes
- determine the equivalence point of a titration reaction

**2) Amperometry**

Amperometry makes it possible to follow, during titration, the concentration variations of the analyte through measurement of the electric current passing through the electrodes.

The equivalence point appears as a singular point on the curve representing current variation as a function of the volume of reagent added.

**3) Coulometry**

**Principle**

Coulometric methods are based on measuring the quantity of electricity (charge) required to quantitatively modify the oxidation state of the analyte.

**Applications**

- Electrolytic titration and synthesis of organic compounds

  **Example**: Trichloroacetic acid
- Mercury is the most commonly used cathode, on which approximately 25 different metals can be deposited.

**4) Conductimetry**

The conductance of a solution is linearly related to the concentrations of ions present in solution.

Conductimetry, or conductance measurement, therefore makes it possible in principle to determine concentrations.

A solution containing ions is called an **electrolytic solution**.

In the absence of ions, a liquid conducts almost no electric current and behaves nearly as an insulator.

For an electrolytic solution to conduct electric current two conductive electrodes must be immersed in the solution and an electrical voltage applied.

**In an electrolytic solution crossed by direct current:**

- cations migrate toward the electrode connected to the negative pole
- anions migrate toward the electrode connected to the positive pole

**Field of Application of the Method**

In principle, all reaction types may be suitable:

- oxidation–reduction reactions
- acid–base reactions
- complex formation
- precipitation
- ion exchange
- extraction

The only condition is that ions must participate in the reaction.

The method is also suitable for dilute media, down to: $\mathbf{10^{-4}\ M}$