FirstHope Notes | M.Pharm Semester 1

UV-Visible spectroscopy

Introduction to UV-Visible Spectroscopy

- UV-Visible spectroscopy is an analytical technique used to study how substances absorb light in the ultraviolet (UV) and visible regions of the electromagnetic spectrum.

  - **UV region:** 200–400 nm
  - **Visible region:** 400–800 nm
- When light passes through a substance, some wavelengths are absorbed while others are transmitted.
- The absorbed light provides information about the structure and concentration of the substance.

Basic idea:

- Molecules contain electrons.
- When light of suitable energy strikes the molecule, electrons get excited from a lower energy level (ground state) to a higher energy level (excited state).
- The amount of light absorbed is measured and used for analysis.

Key points:

- Works mainly for compounds with π electrons or non-bonding electrons (n electrons).
- Organic compounds with double bonds, aromatic rings, or conjugation absorb UV-visible light.
- The technique is widely used for qualitative (structure) and quantitative (concentration) analysis.

Example:

- Benzene absorbs UV light due to its conjugated π-electron system.

- Colored compounds absorb in the visible region, which is why they appear colored.

Theory of UV–Visible Spectroscopy

- UV–Visible spectroscopy is based on electronic transitions in molecules.

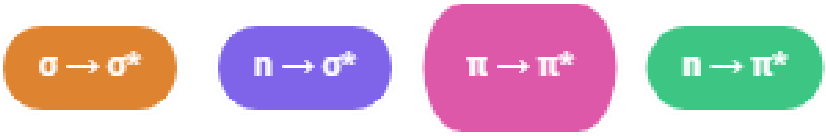
What Happens When Light Interacts with a Molecule?

- Molecules contain electrons in different energy levels
- **When UV/visible light hits the molecule:**
  - If the energy matches, electrons absorb energy
  - Electrons move from lower energy (ground state) to higher energy (excited state)

Electronic Energy Levels

- **Electrons in molecules exist in different types of orbitals:**
  - Sigma (σ) orbitals
  - Pi (π) orbitals
  - Non-bonding (n) orbitals
  - Upon absorption of UV/visible light, electrons move from lower to higher energy orbitals

Types of electronic transitions



1. **σ → σ***
   - Involves single bonds
   - Requires high energy (deep UV region)
   - Not very useful in routine analysis
2. **n → σ***
   - Involves lone pair electrons (e.g., oxygen, nitrogen)
   - Occurs in UV region
3. **π → π***
   - Involves double bonds
   - Common in organic compounds
   - Strong absorption
4. **n → π***
   - Lone pair to antibonding π orbital

- Lower energy than π → π*

Important terms

- **λmax (lambda max):** Wavelength at which maximum absorption occurs
- **Absorbance (A):** Amount of light absorbed
- **Transmittance (T):** Amount of light passing through
- **Bathochromic shift (red shift):** Shift to longer wavelength
- **Hypsochromic shift (blue shift):** Shift to shorter wavelength
- **Hyperchromic effect:** Increase in absorption intensity
- **Hypochromic effect:** Decrease in absorption intensity

Chromophores & Auxochromes

Chromophores

- Chromophores are the parts of a molecule that absorbs UV or visible light and are responsible for the color of the compound.
  - They contain π (pi) electrons or non-bonding (n) electrons
  - These electrons get excited when they absorb light

Examples:

- C=C (double bond)
- C=O (carbonyl group)
- N=N (azo group)
- NO_2 (nitro group)

Key point:

- Chromophores determine **where (at which wavelength)** absorption occurs.

Auxochromes

- Auxochromes are groups that do not absorb strongly on their own, but when attached to a chromophore, they modify its absorption.
  - They usually contain lone pair electrons
  - They increase intensity and shift absorption

Examples:

- –OH (hydroxyl)
- $–NH_2$ (amino)

- –OR (alkoxy)

Effects:

- Increase absorption intensity (hyperchromic effect)
- Shift absorption to longer wavelength (bathochromic shift)

Key point:

- Auxochromes **enhance and shift the absorption** of chromophores.

Beer–Lambert Law

- The Beer–Lambert Law relates the absorbance of light to the concentration of a substance and the path length through which light passes.

Basis of the Law

The Beer–Lambert Law is a combination of two laws:

1. **Lambert's Law**
   - Absorbance is directly proportional to the path length (b or l) of the sample
2. **Beer's Law**
   - Absorbance is directly proportional to the concentration (c) of the solution

Mathematical Expression

$$A = \varepsilon \times b \times c$$

- **Where:**
  - A = Absorbance
  - ε (epsilon) = Molar absorptivity (constant for a substance)
  - b (or l) = Path length (cm)
  - c = Concentration

Absorbance and Transmittance

- **Transmittance (T)**

$$T = \frac{I}{I_0}$$

- **Absorbance (A)**

$$A = \log\left(\frac{I_0}{I}\right)$$

- **Where:**
  - I_0 = Incident light intensity

- I = Transmitted light intensity

Key Points

- Absorbance is directly proportional to both concentration and path length
- Widely used for quantitative analysis of solutions
- The law is valid only under ideal conditions

Deviations / Limitations of Beer–Lambert Law

1. Chemical Deviations

- Association or dissociation of molecules
- Chemical interactions affecting concentration

2. Instrumental Deviations

- Use of non-monochromatic light
- Instrumental errors

3. Concentration Effects

- At high concentrations, interactions between molecules cause deviation

Derivation of Beer–Lambert Law

Step 1: Consider a beam of monochromatic light

- **Let:**
  - I_0= intensity of incident light
  - I= intensity after passing through solution
  - l= total path length of solution
- We divide the solution into very thin layers of thickness dx.

Step 2: Change in intensity in a thin layer

- When light passes through a very thin layer dx, a small amount of light is absorbed.
- **The decrease in intensity dI depends on:**
  - The intensity of light I
  - The thickness dx
  - The concentration c
- **So, we write:**

$$dI \propto I\, dx\, c$$

Step 3: Remove proportionality

- **Introduce proportionality constant k:**

$$dI = -k\,c\,I\,dx$$

- Negative sign indicates decrease in intensity.

Step 4: Rearranging the equation

$$\frac{dI}{I} = -kc\,dx$$

Step 5: Integration

- **Integrate both sides:**

$$\int_{I_0}^{I} \frac{dI}{I} = -kc \int_{0}^{l} dx$$

Step 6: Solve integrals

- **Left side:**

$$\int_{I_0}^{I} \frac{dI}{I} = \ln I - \ln I_0$$

- **Right side:**

$$-kc \int_{0}^{l} dx = -kcl$$

- **So:**

$$\ln I - \ln I_0 = -kcl$$

Step 7: Rearranging

$$\ln\left(\frac{I}{I_0}\right) = -kcl$$

$$\ln\left(\frac{I_0}{I}\right) = kcl$$

Step 8: Convert natural log to base 10

- **We know:**

$$\ln x = 2.303 \log x$$

- **So:**

$$\log\left(\frac{I_0}{I}\right) = \frac{k}{2.303}\,cl$$

Step 9: Define molar absorptivity

- **Let:**

$$\varepsilon = \frac{k}{2.303}$$

- **So, equation becomes:**

$$\log\left(\frac{I_0}{I}\right) = \varepsilon c l$$

Step 10: Define absorbance

- **Absorbance $A$is defined as:**

$$A = \log\left(\frac{I_0}{I}\right)$$

Final Beer–Lambert Equation

$$A = \varepsilon c l$$

Additional Relations

- **Transmittance**

$$T = \frac{I}{I_0}$$

- **Absorbance in terms of transmittance**

$$A = -\log T$$

Meaning of each term

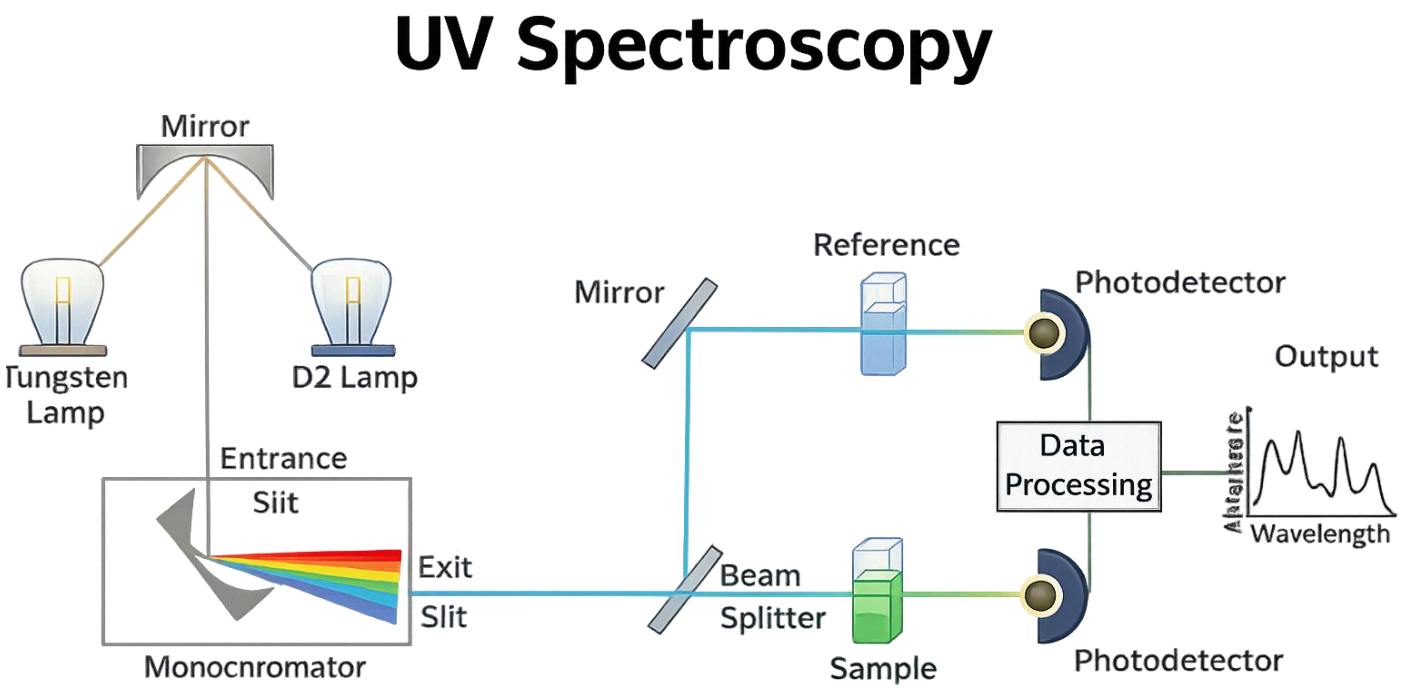
- A**:** Absorbance (no units)
- ε**:** Molar absorptivity (L mol $^{-1}$ cm $^{-1}$)
- c**:** Concentration (mol L $^{-1}$)
- l**:** Path length (cm)
- I_0**:** Incident light intensity
- I**:** Transmitted light intensity

Important idea in derivation

- Each thin layer absorbs a small fraction of light
- More layers (greater thickness) → more absorption
- More concentration → more absorbing particles → more absorption
- This leads to a logarithmic relationship between intensity and path length, which becomes linear in terms of absorbance

Instrumentation Associated with UV–Visible Spectroscopy

A UV–Visible spectrophotometer consists of several components:



1. Light Source

- Provides radiation in UV and visible regions
- **Types:**
  - **Deuterium lamp** → UV region
  - **Tungsten lamp** → Visible region

2. Monochromator

- Separates light into individual wavelengths
- **Components:**
  - Prism or diffraction grating
- Allows selection of specific wavelength

3. Sample Holder (Cuvette)

- Holds the sample solution
- **Materials:**
  - Quartz → for UV region
  - Glass → for visible region

4. Detector

- Measures intensity of transmitted light
- Converts light into electrical signal

- **Types:**
  - Photodiode
  - Photomultiplier tube

5. **Display/Recorder**
   - Shows absorbance or transmittance
   - Can produce spectra (graph of absorbance vs wavelength)

Types of instruments

1. **Single-beam spectrophotometer**
   - Measures sample separately
2. **Double-beam spectrophotometer**
   - Compares sample with reference simultaneously
   - More accurate

Choice of Solvents and Solvent Effects

- In UV-Visible spectroscopy, the solvent plays a very important role because it can influence both the **accuracy of measurement** and the **position of absorption peaks**.

Choice of Solvent

A good solvent should have the following properties:

1. **Transparent in the required region**
   - The solvent should not absorb UV or visible light in the region where the sample is being analyzed.
   - Each solvent has a **cut-off wavelength**:
     - Below this wavelength, the solvent absorbs strongly and interferes with measurement.
   - **Examples:**
     - Water: ~190 nm
     - Ethanol: ~205 nm
     - Methanol: ~205 nm
     - Hexane: ~201 nm
2. **Dissolves the sample completely**
   - The sample must be fully dissolved to avoid scattering of light.
3. **Chemically inert**

- The solvent should not react with the analyte.
- Reaction can change the structure and affect absorption.

4. **Stable under UV light**
   - Some solvents degrade when exposed to UV radiation, which can affect results.
5. **High purity**
   - Impurities can absorb light and cause errors.

Solvent Effects on Absorption

Solvents can influence absorption in several ways:

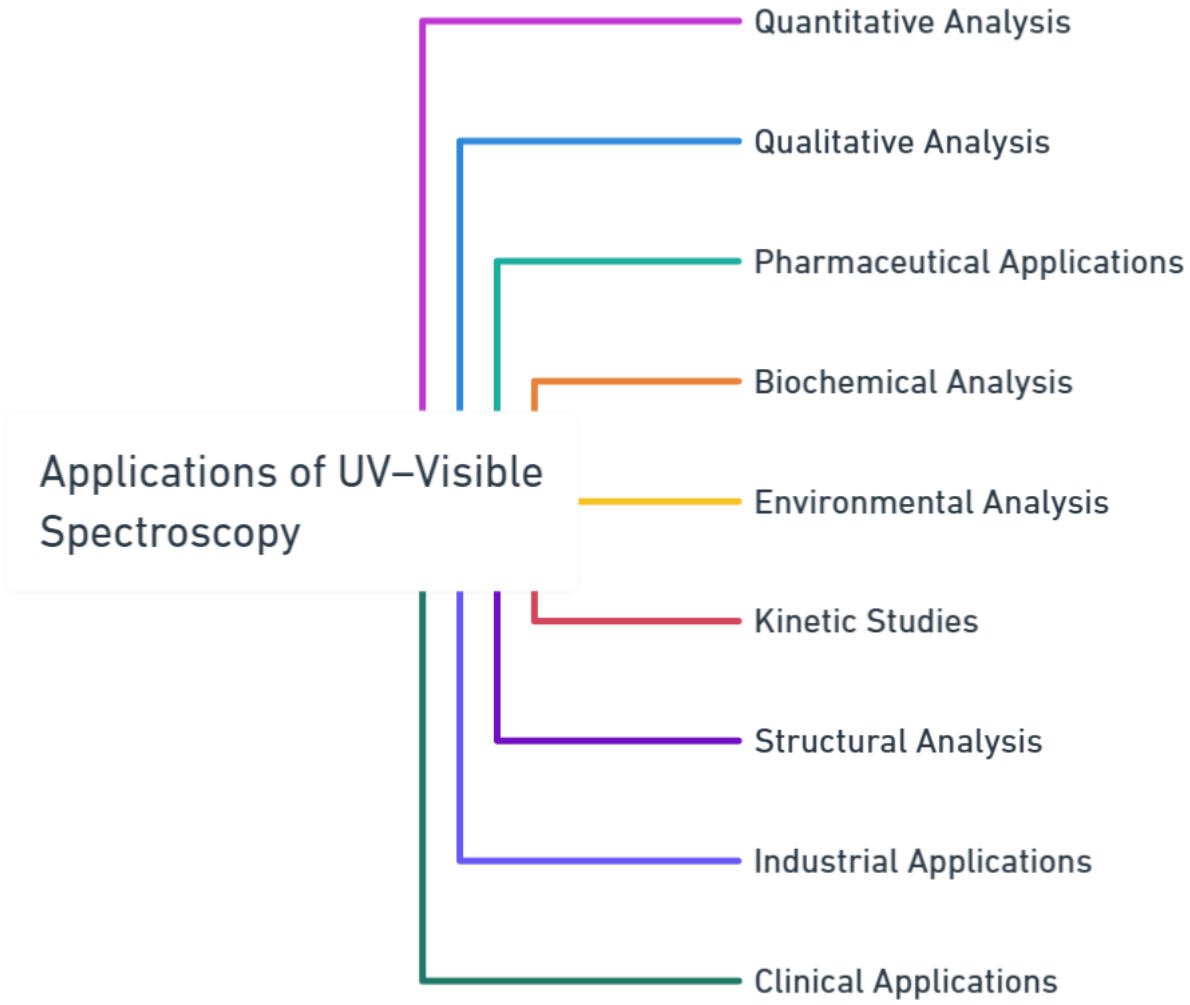
1. **Polarity Effects**
   - Polar solvents stabilize excited states differently
   - Causes shift in λmax (absorption wavelength)
2. **Hydrogen Bonding**
   - Alters energy levels
   - Changes absorption intensity
3. **Bathochromic Shift (Red Shift)**
   - Absorption shifts to longer wavelength
   - **Caused by:**
     - Increased conjugation
     - Polar solvent interactions
4. **Hypsochromic Shift (Blue Shift)**
   - Absorption shifts to shorter wavelength
   - **Caused by:**
     - Decreased conjugation
     - Less polar or strong solvent interactions
5. **Hyperchromic Effect**
   - Increase in absorbance intensity
6. **Hypochromic Effect**
   - Decrease in absorbance intensity

Why Solvent Matters

- Solvent polarity stabilizes different energy states
- This changes the energy gap

- Result: change in absorption wavelength and intensity

Applications of UV–Visible Spectroscopy



1. **Quantitative analysis**
   - Determination of concentration using Beer–Lambert law
   - Widely used in pharmaceuticals
2. **Qualitative analysis**
   - Identification of compounds based on λmax
   - Detection of functional groups
3. **Pharmaceutical applications**
   - Drug analysis and quality control
   - Dissolution studies
   - Stability testing
4. **Biochemical analysis**
   - Protein estimation

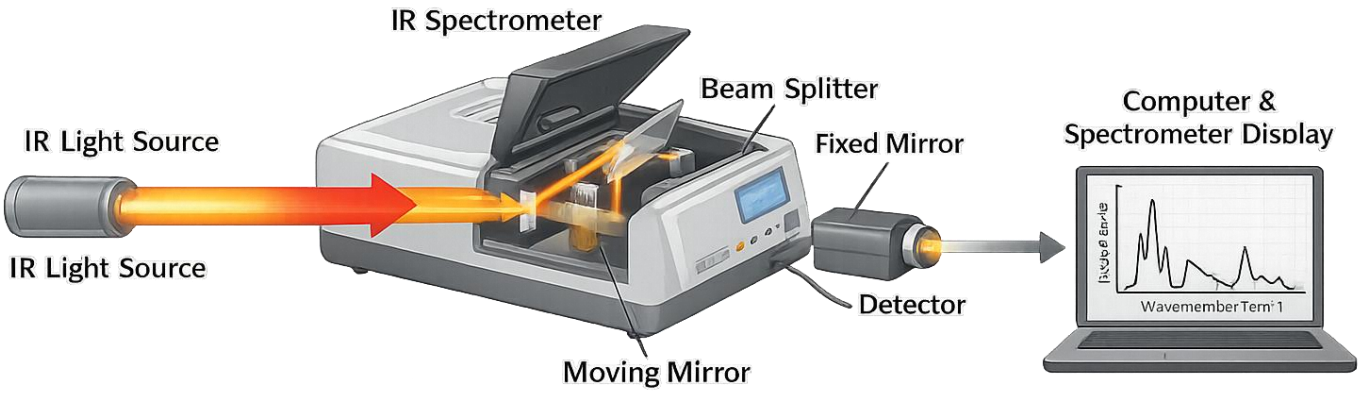
- DNA/RNA analysis
- Enzyme kinetics

5. **Environmental analysis**
   - Detection of pollutants in water and air
   - Measurement of metal ions
6. **Kinetic studies**
   - Monitoring reaction rates
   - Observing changes in absorbance over time
7. **Structural analysis**
   - Study of conjugation and electronic structure
   - Helps in understanding molecular behavior
8. **Industrial applications**
   - Dye and pigment analysis
   - Food quality control
   - Chemical manufacturing
9. **Clinical applications**
   - Blood analysis
   - Detection of biomolecules
   - Diagnostic testing

IR spectroscopy

Theory of IR Spectroscopy

- Infrared (IR) spectroscopy is based on the absorption of infrared radiation by molecules, which causes vibrational motion of chemical bonds.



Basic Concept

- Molecules are made of atoms connected by bonds
- These bonds behave like springs connecting atoms
- **When IR radiation is passed through a molecule:**
  - If the frequency matches, energy is absorbed
  - Bonds start vibrating

Vibrational Energy Levels

- Molecules have quantified vibrational energy levels
- **When IR radiation matches the energy gap:**
  - Absorption occurs
  - **Transition takes place:**
    - Lower energy level → Higher energy level

Condition for IR Absorption

- **A vibration absorbs IR radiation only if:**
  - There is a change in dipole moment during vibration

Dipole Moment (Important Concept)

- Dipole moment = separation of positive and negative charges
- If vibration changes this separation → IR absorption occurs
- **Examples:**
  - HCl → IR active (dipole changes)

- N_2, O_2 → IR inactive (no dipole change)

Hooke's Law (Approximation)

- **Vibration of a bond can be compared to a spring:**

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

- **Where:**
  - v = Vibrational frequency
  - k = Force constant (bond strength)
  - μ = Reduced mass

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

Important Points

- Stronger bonds → higher frequency
- Lighter atoms → higher frequency

IR Spectrum

- **Graph plotted as:**
  - Wavenumber (cm^{-1}) on x-axis
  - Absorbance or % Transmittance on y-axis

Important Regions

1. **Functional Group Region (4000–1500 cm^{-1})**
   - ➢ Shows characteristic peaks of functional groups
2. **Fingerprint Region (1500–400 cm^{-1})**
   - ➢ Unique for each compound

Wavenumber

- Unit used in IR spectroscopy
- **Defined as:**

$$\text{Wavenumber} = \frac{1}{\text{Wavelength}}$$

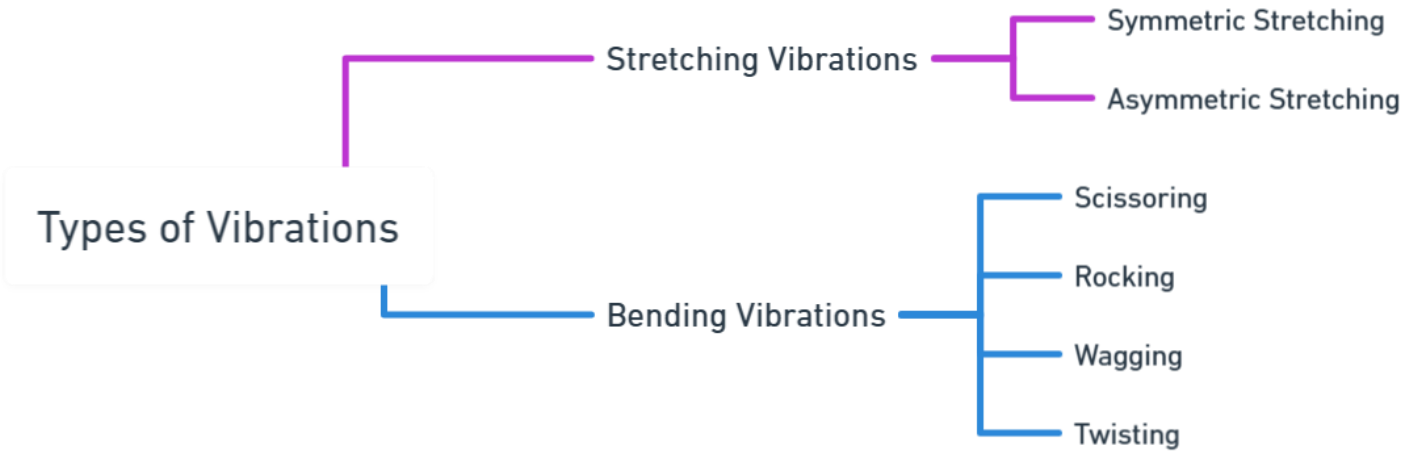
- Higher wavenumber → higher energy

Modes of Molecular Vibrations

- Molecules can vibrate in different ways depending on their structure and bonds.
- These are called vibrational modes.

Types of Vibrations

There are two main types:



A. Stretching Vibrations

- In stretching, the **bond length changes**
- **Types:**
  1. **Symmetric Stretching**
     - Both atoms move in and out together
     - Bond length increases and decreases uniformly
     - **Example:**
       - In CO_2, both oxygen atoms move simultaneously toward and away from carbon
  2. **Asymmetric Stretching**
     - Atoms move in opposite directions
     - One bond shortens while the other lengthens
     - Requires more energy than symmetric stretching

B. Bending Vibrations

- In bending, the **bond angle changes** instead of bond length
- **Types:**
  1. **Scissoring**
     - Two atoms move toward and away from each other
     - Motion resembles scissors
  2. **Rocking**

- Atoms move in the same direction
- Like a rocking motion

3. **Wagging**
   - Atoms move up and down together (out of plane)
4. **Twisting**
   - One atom moves up, the other moves down

Energy Comparison

- Stretching vibrations → higher energy → higher wavenumber
- Bending vibrations → lower energy → lower wavenumber

Degrees of Freedom

- A molecule with N atoms has 3N degrees of freedom
- **Types of motion:**
  - Translational
  - Rotational
  - Vibrational

Number of Vibrational Modes

- **Non-linear molecule:** 3N – 6
- **Linear molecule:** 3N – 5

Sample Handling

- Proper sample preparation is essential for accurate IR results, as IR radiation is easily absorbed by many substances.

Types of Samples

- Solid
- Liquid
- Gas
- Each type requires a different method.

A. Solid Samples

1. **KBr Pellet Method**
   - Sample is mixed with dry potassium bromide (KBr)
   - Ground into a fine powder

- Pressed into a thin, transparent pellet
- **Why KBr is used:**
  - Does not absorb IR radiation
  - Transparent in IR region

2. **Mull Method**
   - Sample is mixed with mineral oil (Nujol)
   - Forms a paste (mull)
   - Spread between two salt plates
3. **Thin Film Method**
   - Used for polymers or soft solids
   - Sample is melted or pressed into a thin film

B. Liquid Samples

1. **Neat Liquid Method**
   - A drop of liquid is placed between two salt plates (NaCl/KBr)
   - Forms a thin layer
2. **Solution Method**
   - Sample is dissolved in a suitable solvent
   - Solvent must not absorb IR radiation

C. Gaseous Samples

- Sample is placed in a gas cell
- Cell has a long path length due to low density
- Ensures better absorption

Important Precautions

- Avoid moisture (water strongly absorbs IR)
- Use clean and dry equipment
- Sample should be pure and homogeneous
- Avoid air bubbles in liquid samples
- Handle salt plates carefully (moisture sensitive)

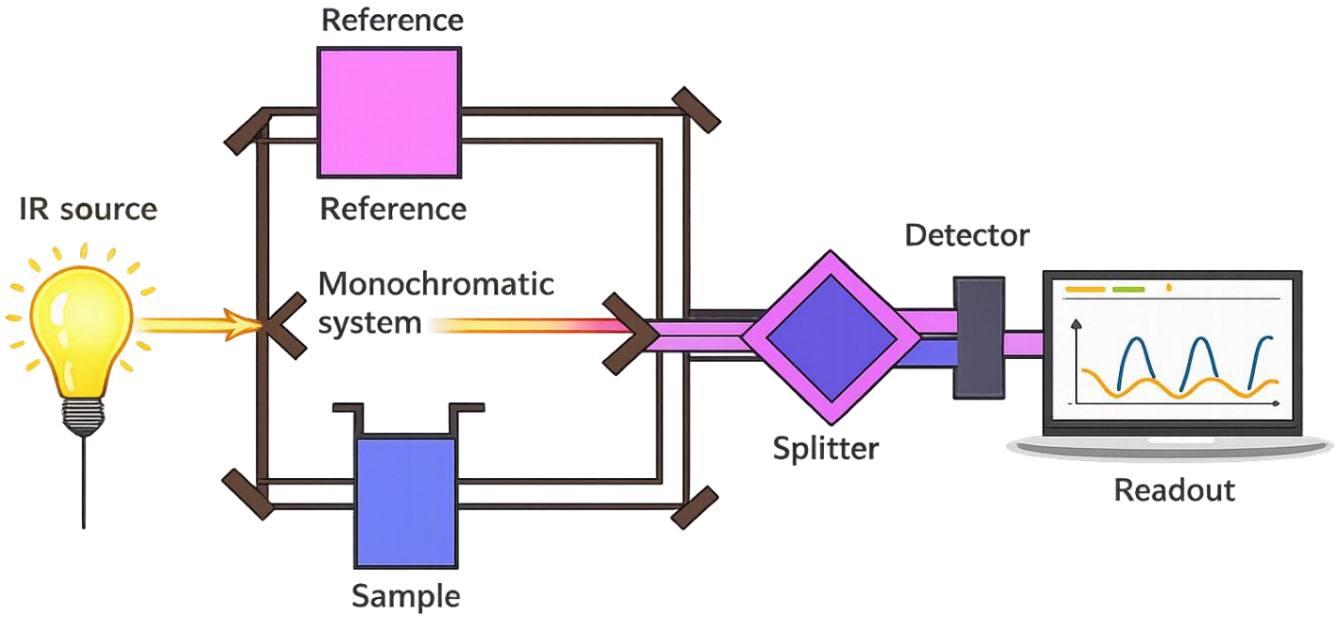
Instrumentation: Dispersive IR Spectrometer

- This is the traditional type of IR spectrometer.

Basic Principle

- Measures IR absorption one wavelength at a time
- Use a monochromator to separate wavelengths

Main Components



1. IR Radiation Source

- Produces continuous IR radiation
- **Examples:**
  - Globar (silicon carbide rod)
  - Nernst glower

2. Monochromator

- Separates radiation into individual wavelengths
- **Uses:**
  - Prism
  - Diffraction grating

3. Sample Holder

- Holds the sample in the path of IR beam

4. Detector

- Detects transmitted radiation
- Converts it into electrical signal
- **Examples:**
  - Thermocouple

- Bolometer

5. **Recorder**
   - Records the IR spectrum as a graph

Working

- IR radiation passes through monochromator
- A single wavelength passes through the sample
- Detector measures transmitted intensity
- Process repeats for all wavelengths

Limitations

- Slow (scans one wavelength at a time)
- Less sensitive
- Mechanical errors possible

Instrumentation: Fourier Transform Infrared (FTIR) Spectrometer

- FTIR is a modern and advanced technique.

Basic Principle

- Measures all wavelengths simultaneously
- Uses Fourier transform mathematics to generate spectrum

Key Component

- **Michelson Interferometer** → Core of FTIR instrument

Main Components

1. **IR Source**
   - Same as dispersive instrument
2. **Interferometer**
   - Core component of FTIR
   - **Parts:**
     - Beam splitter
     - Fixed mirror
     - Moving mirror
3. **Sample Holder**

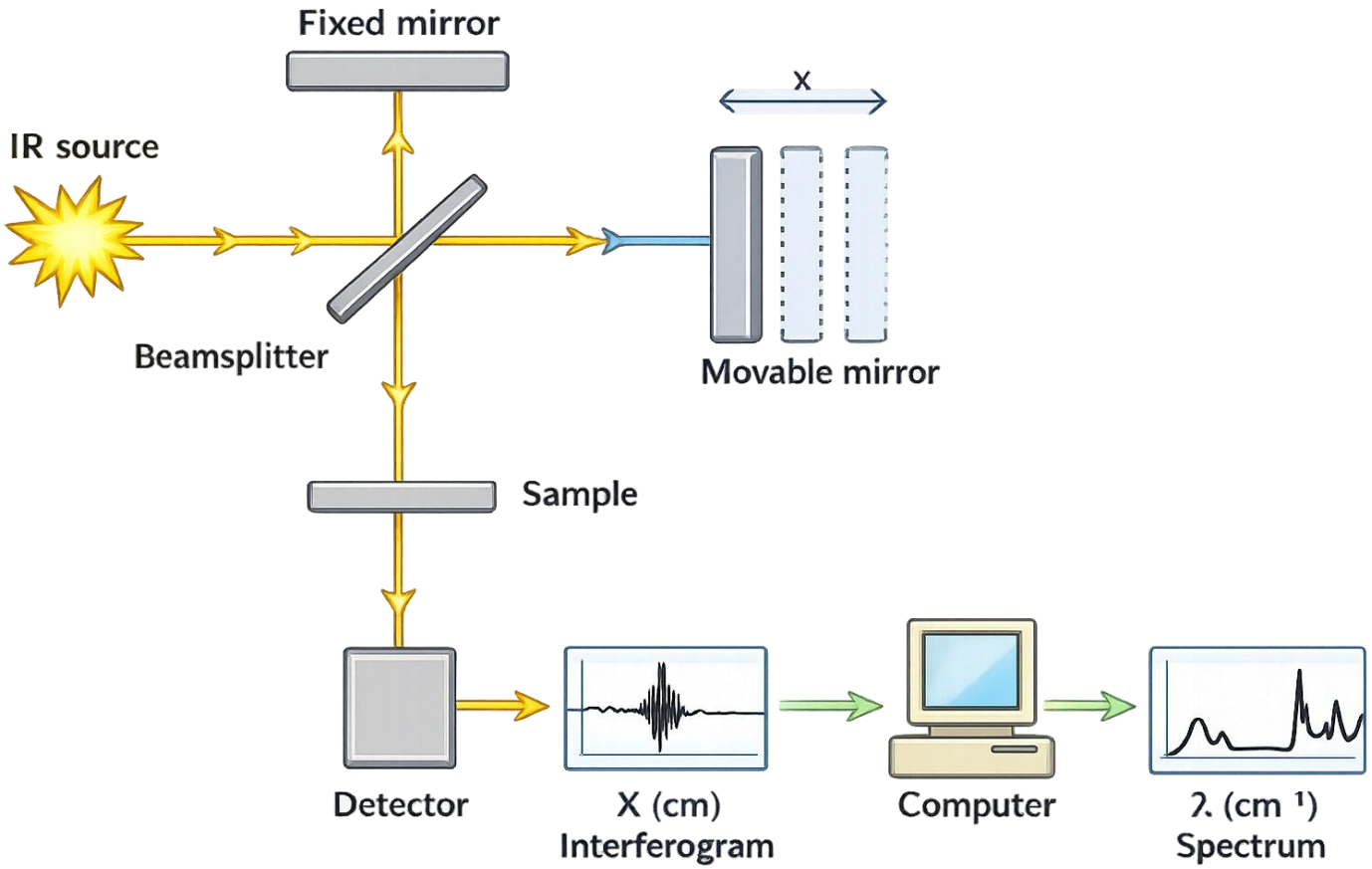
- Sample is placed in the beam path

4. Detector

- Detects the interferogram (signal)

5. Computer (Fourier Transform)

- Converts interferogram into spectrum using mathematical calculations



Working of Interferometer

- IR beam is split into two beams
- One reflects from fixed mirror
- Other reflects from moving mirror
- Beams recombine
- Produce an interference pattern (interferogram)
- Interferogram is converted into spectrum

Advantages of FTIR

- Very fast (all wavelengths measured at once)
- High sensitivity
- Better resolution
- Better signal-to-noise ratio

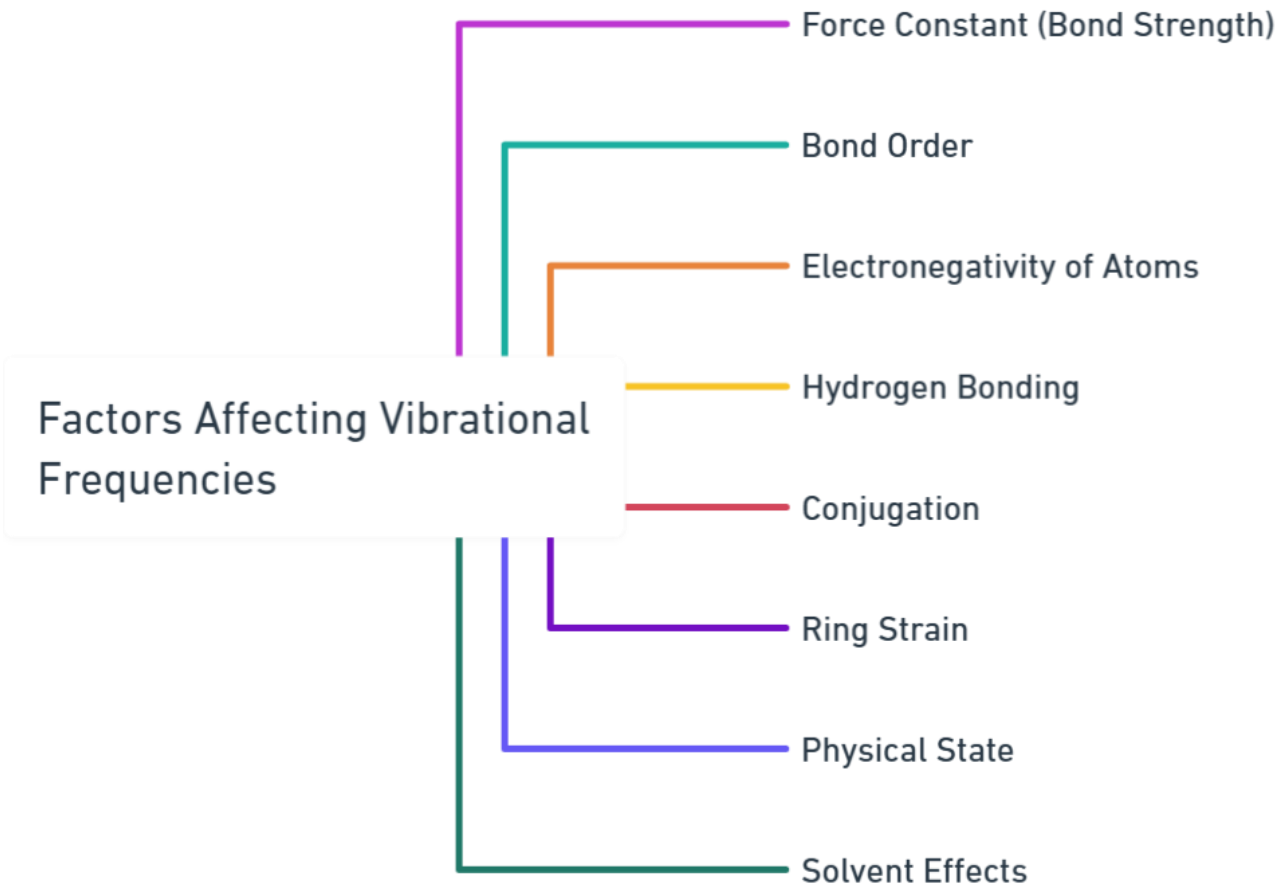
- Requires small sample

Important Concept

- FTIR collects all wavelength data simultaneously
- Then converts it into a spectrum using Fourier transform mathematics

Factors Affecting Vibrational Frequencies

- The position of absorption bands in an IR spectrum (i.e., vibrational frequencies) is not fixed.
- It depends on several factors related to the bond and the environment around it.



1. Force Constant (Bond Strength)

- **From Hooke's law:**

$$\nu \propto \sqrt{k}$$

- k= force constant (measure of bond strength)

Effect:

- Stronger bond → higher frequency
- Weaker bond → lower frequency

Examples:

- C≡C (triple bond) > C=C (double bond) > C–C (single bond)

- Triple bonds absorb at higher wavenumbers

2. Reduced Mass of Atoms

$$\nu \propto \frac{1}{\sqrt{\mu}}$$

- μ= reduced mass

Effect:

- Lighter atoms → higher frequency
- Heavier atoms → lower frequency

Examples:

- C–H > C–D (deuterium is heavier than hydrogen)

3. Bond Order

- Higher bond order means stronger bond

Effect:

- Single bond → lower frequency
- Double bond → higher
- Triple bond → highest

4. Electronegativity of Atoms

- More electronegative atoms pull electrons strongly

Effect:

- Increases bond strength → increases frequency

Example:

- C=O absorbs at higher frequency than C=C

5. Hydrogen Bonding

- Hydrogen bonding weakens bonds like O–H and N–H.

Effect:

- Decreases frequency
- Broadens the peak

Example:

- Free O–H: sharp peak
- H-bonded O–H: broad peak at lower frequency

6. Conjugation

- Conjugation means alternating double and single bonds.

Effect:

- Decreases bond strength slightly
- Shifts absorption to lower frequency

Example:

- Isolated C=O absorbs higher than conjugated C=O

7. Ring Strain

- In cyclic compounds, bond angles may be distorted.

Effect:

- Increases bond energy
- Increases frequency

8. Physical State

- Solid, liquid, or gas affects interactions

Effect:

- Hydrogen bonding in liquids/solids → lower frequency
- Gas phase → sharper peaks

9. Solvent Effects

- Solvent can interact with molecules

Effect:

- May shift peak positions
- Polar solvents can lower frequencies

Applications of IR Spectroscopy

IR spectroscopy is widely used to identify and study chemical substances.

A. Identification of Functional Groups

- Each functional group absorbs at a characteristic frequency.
- **Examples:**
  - O–H → around 3200–3600 cm^{-1}
  - C=O → around 1700 cm^{-1}
  - N–H → around 3300 cm^{-1}

B. Identification of Compounds (Fingerprint Region)

- Region: **1500–400 cm^{-1}**

- Each compound has a unique pattern
- **Used to:**
  - Confirm identity of a compound
  - Compare with known spectra

C. Detection of Impurities

- Extra peaks indicate impurities
- Useful in quality control

D. Study of Hydrogen Bonding

- Broad peaks indicate hydrogen bonding
- Helps understand intermolecular interactions

E. Monitoring Chemical Reactions

- Observe disappearance or appearance of peaks
- **Example:**
  - Reactant peak disappears
  - Product peak appears

F. Structural Analysis

- **Determines presence of:**
  - Double bonds
  - Triple bonds
  - Functional groups

G. Pharmaceutical Applications

- Drug identification
- Purity testing
- Checking formulation

H. Environmental Analysis

- Detection of pollutants
- Analysis of gases like CO, NO_2

I. Polymer and Material Analysis

- Identifies types of polymers
- Studies degradation and composition

J. Forensic Applications

- Identification of unknown substances
- Analysis of drugs, fibers, paints

Spectroflourimetry

Spectrofluorimetry

- Spectrofluorimetry is an analytical technique used to measure the fluorescence emitted by a substance after it absorbs light.

Basic Idea

- A molecule absorbs light (usually UV light).
- It becomes excited (higher energy state).
- After a very short time, it returns to a lower energy state.
- During this return, it emits light of lower energy (longer wavelength).
- This emitted light is called fluorescence.

Important Features

- The emitted light is always at a longer wavelength than the absorbed light.
- This difference is called the Stokes shift.
- Fluorescence is very sensitive, so even very small amounts of substance can be detected.

Process Steps

1. Absorption of light
2. Excitation of electrons
3. Relaxation (loss of some energy as heat)
4. Emission of fluorescence

Examples of Fluorescent Substances

- Aromatic compounds
- Quinones
- Fluorescein dye
- Proteins (tryptophan amino acid)

Theory of Fluorescence

- Spectrofluorimetry is based on fluorescence, where a substance absorbs light at one wavelength and emits light at a longer wavelength.

Basic Concept

- **When a molecule absorbs light (usually UV):**

- Electrons move from ground state → excited state

- The excited state is unstable
- The molecule returns to ground state
- Energy is released as light (fluorescence)

Electronic States

- **Molecules have different energy states:**
  - Ground state (S_0) → lowest energy
  - Excited singlet states (S_1, S_2)
  - Triplet state (T_1)

Steps in Fluorescence (Jablonski Diagram Concept)

1. Absorption

- Molecule absorbs UV light
- Electron moves from S_0 to higher excited state (S_1 or S_2)

2. Vibrational Relaxation

- Molecule loses some energy as heat
- Falls to lowest level of S_1

3. Fluorescence Emission

- Electron returns from S_1 to S_0
- Emits light

Important Point

- Emitted light has lower energy and longer wavelength than absorbed light

Stokes Shift

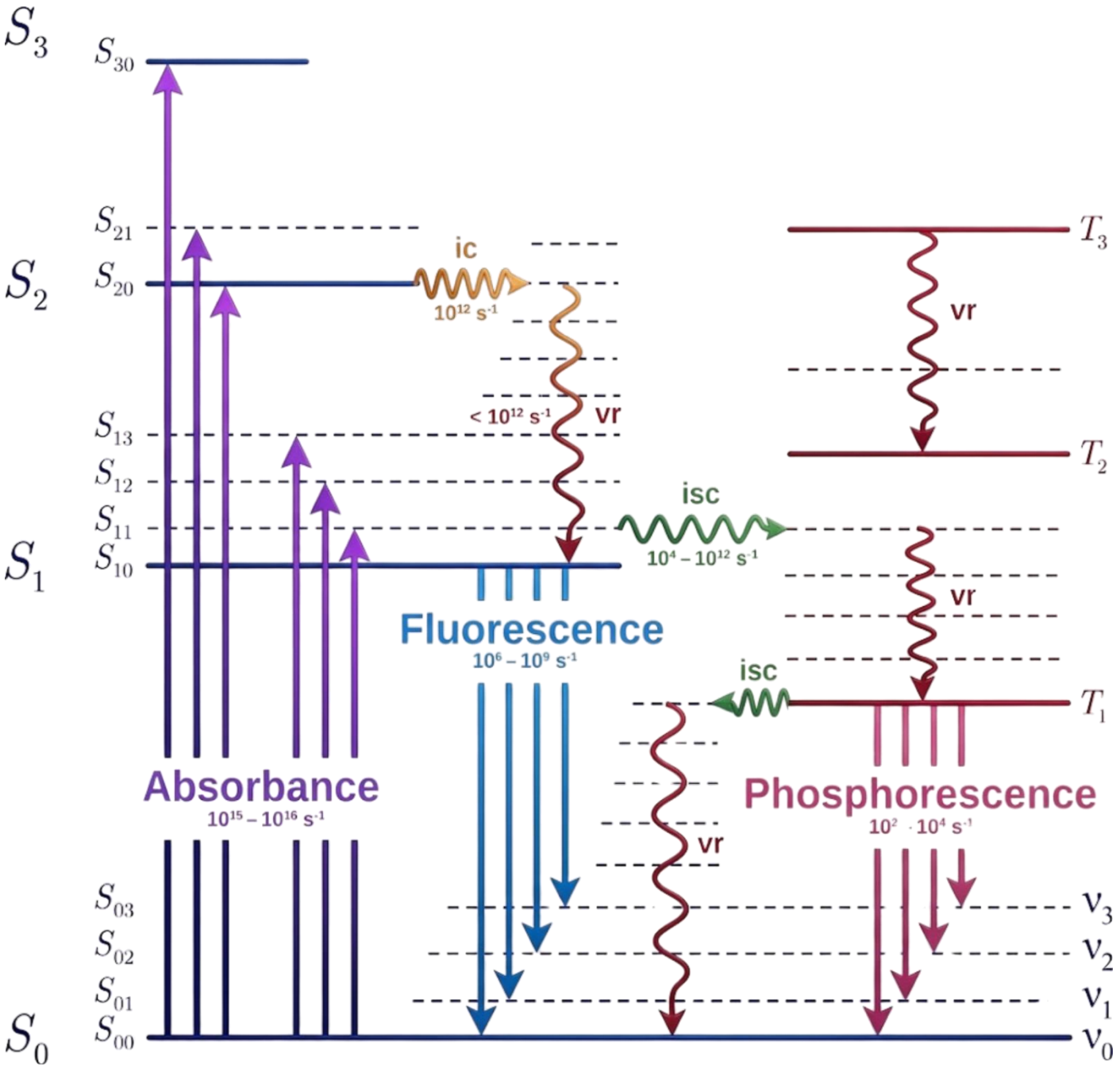
- Difference between absorption wavelength and emission wavelength

Key Points

- Fluorescence occurs very quickly (10^{-9} to 10^{-7} seconds)
- Only certain molecules show fluorescence
- **Usually seen in compounds with:**
  - Aromatic rings
  - Conjugated double bonds
  - Rigid structures

Jablonski Diagram (Conceptual Understanding)

- **It explains electronic transitions:**
  - Ground state (S_0)
  - Excited states (S_1, S_2)
- **Transitions:**
  - Absorption
  - Internal conversion
  - Fluorescence emission



Fluorescence vs Phosphorescence

Property	Fluorescence	Phosphorescence
Time	Very fast	Slow

Duration	Stops immediately	Continues after source removed
Transition	$S_1 \rightarrow S_0$ (Singlet → Singlet)	$T_1 \rightarrow S_0$ (Triplet → Singlet)
Visibility	Immediate	Delayed

Fluorescent Compounds

- **Compounds that show fluorescence usually have:**
  - Aromatic rings
  - Conjugated double bonds
  - Rigid structures

Factors Affecting Fluorescence

Fluorescence intensity and wavelength depend on several factors.

1. Molecular Structure

- Aromatic and conjugated systems show strong fluorescence
- Rigid structures increase fluorescence
- **Reason:**
  - Less energy loss → more emission

2. Substituents

- Electron-donating groups (–OH, $–NH_2$) increase fluorescence
- Electron-withdrawing groups ($–NO_2$) decrease fluorescence

3. Solvent Effects

- Solvent polarity affects energy levels
- **Effects:**
  - Can shift emission wavelength
  - Can increase or decrease intensity

4. pH of Solution

- Changes ionization of molecule
- **Effect:**
  - Alters fluorescence intensity

5. Temperature

- Higher temperature increases molecular motion
- **Effect:**
  - Decreases fluorescence (more energy lost as heat)

6. Concentration

- At low concentration → fluorescence increases
- At high concentration → decreases due to quenching

7. Presence of Oxygen

- Oxygen reduces fluorescence
- **Reason:**
  - Acts as a quencher

Quenchers

- Quenchers are substances that **reduce or suppress fluorescence**.

What is Quenching?

- Quenching is the process where fluorescence intensity decreases due to interactions with other molecules.

Types of Quenching

1. Dynamic (Collisional) Quenching

- Occurs when excited molecules collide with quencher
- Energy is lost without emission
- **Features:**
  - Depends on temperature and diffusion
  - More collisions → more quenching

2. Static Quenching

- Complex forms between fluorophore and quencher
- No fluorescence is produced

Common Quenchers

- Oxygen
- Halide ions (Cl^-, Br^-, I^-)
- Heavy metals

Stern–Volmer Equation

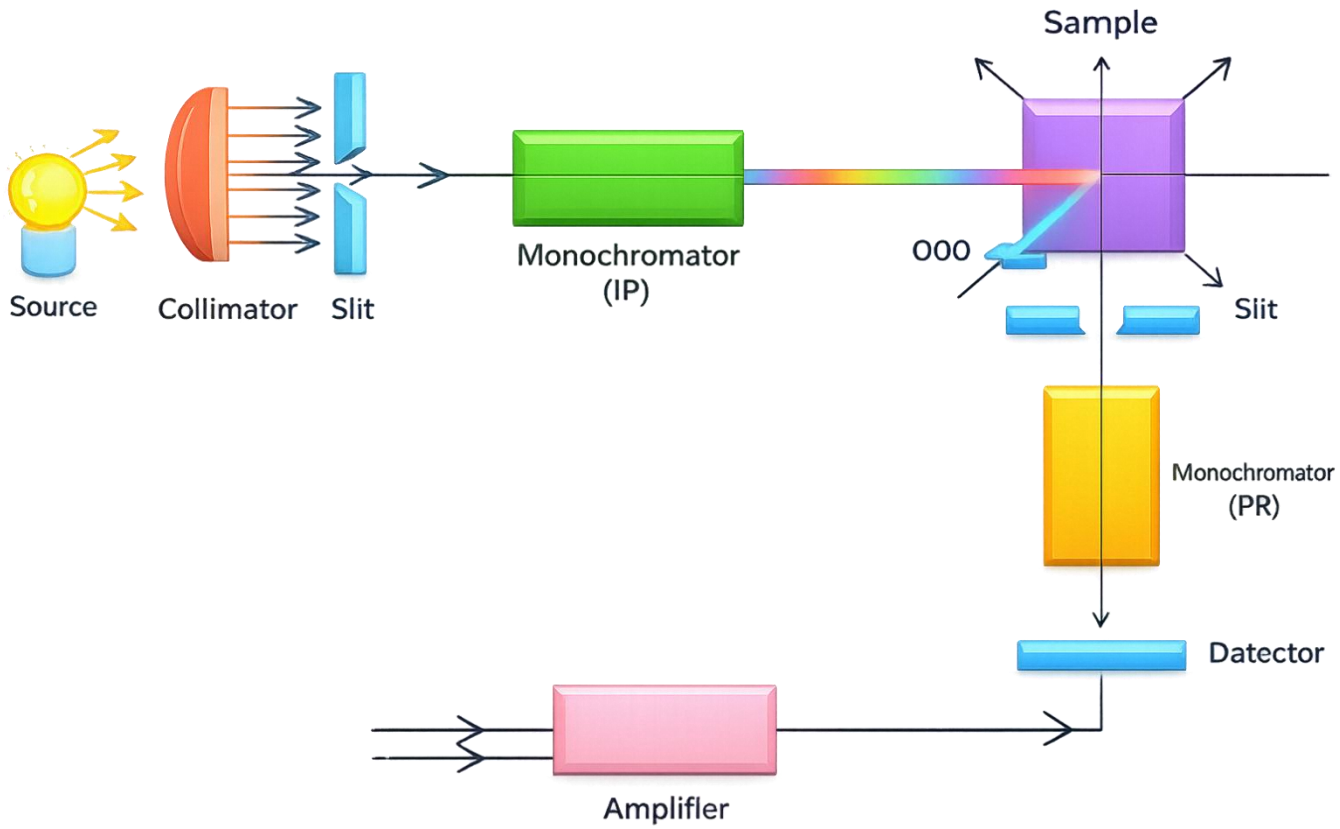
$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q]$$

- **Where:**
  - F_0= fluorescence without quencher
  - F= fluorescence with quencher
  - $[Q]$= quencher concentration
  - K_q= quenching constant
  - τ_0= lifetime

Instrumentation of Fluorescence Spectrophotometer

- A fluorescence spectrophotometer measures emitted light from a sample.

Main Components



1. Light Source

- Provides excitation light (usually UV)
- **Examples:**
  - Xenon lamp
  - Mercury lamp

2. Excitation Monochromator

- Selects specific wavelength for excitation
- Ensures only desired wavelength reaches sample

3. Sample Holder

- Contains the sample solution
- Usually quartz cuvette is used

4. Emission Monochromator

- Selects emitted light wavelength
- Removes scattered excitation light

5. Detector

- Detects emitted fluorescence
- Converts it into electrical signal
- **Examples:** Photomultiplier tube

6. Recorder/Display

- Displays fluorescence intensity
- Produces emission spectrum

Special Feature

- Detector is placed at **90° angle** to incident light
- **Reason:**
  - Minimizes interference from transmitted light
  - Measures only emitted fluorescence

Working Principle

1. Light source emits UV radiation
2. Monochromator selects wavelength
3. Sample absorbs light
4. Emits fluorescence
5. Emitted light is detected and recorded

Applications of Fluorescence Spectrophotometer

A. Quantitative Analysis

- Very sensitive method
- Detects very low concentrations
- **Examples:**
  - Vitamins
  - Drugs

B. Pharmaceutical Analysis

- Drug analysis
- Stability studies
- Impurity detection

C. Biochemical Applications

- Protein and DNA analysis
- Enzyme studies

D. Environmental Analysis

- Detection of pollutants
- Analysis of pesticides

E. Clinical Applications

- Detection of biomarkers
- Blood and urine analysis

F. Food Analysis

- Detection of additives and contaminants

G. Forensic Applications

- Detection of drugs and poisons
- Analysis of body fluids

H. Study of Molecular Interactions

- Binding studies
- Protein–ligand interactions

I. Imaging Techniques

- Used in fluorescence microscopy
- Cell and tissue imaging

J. Quality Control

- Used in industries to check purity and composition

Flame Emission Spectroscopy & Atomic Absorption Spectroscopy

Flame Emission Spectroscopy (FES)

Basic Principle

- When a solution containing metal ions is introduced into a flame, the heat of the flame converts the ions into free atoms.
- These atoms absorb energy from the flame and become excited.
- When they return to the ground state, they emit light of a specific wavelength.

Key Idea

- Each element emits light at a characteristic wavelength.
- The intensity of emitted light is proportional to the concentration of that element.

Steps in FES

1. Sample solution is sprayed into the flame
2. Solvent evaporates
3. Solid residue forms
4. Residue vaporizes into atoms
5. Atoms get excited
6. Atoms emit light while returning to ground state

Important Relation

$$\text{Emission intensity} \propto \text{concentration}$$

Example

- Sodium emits yellow light
- Potassium emits violet light

Atomic Absorption Spectroscopy (AAS)

Basic Principle

- Free atoms in the ground state absorb light of a **specific wavelength**.
- The absorbed light corresponds exactly to the energy required to excite the atoms.

Key Idea

- Each element absorbs light at a characteristic wavelength.
- The amount of light absorbed is proportional to the concentration of the element.

Steps in AAS

1. Sample is introduced into flame
2. Atoms are formed in ground state
3. Light from a source passes through these atoms
4. Atoms absorb specific wavelengths
5. Decrease in light intensity is measured

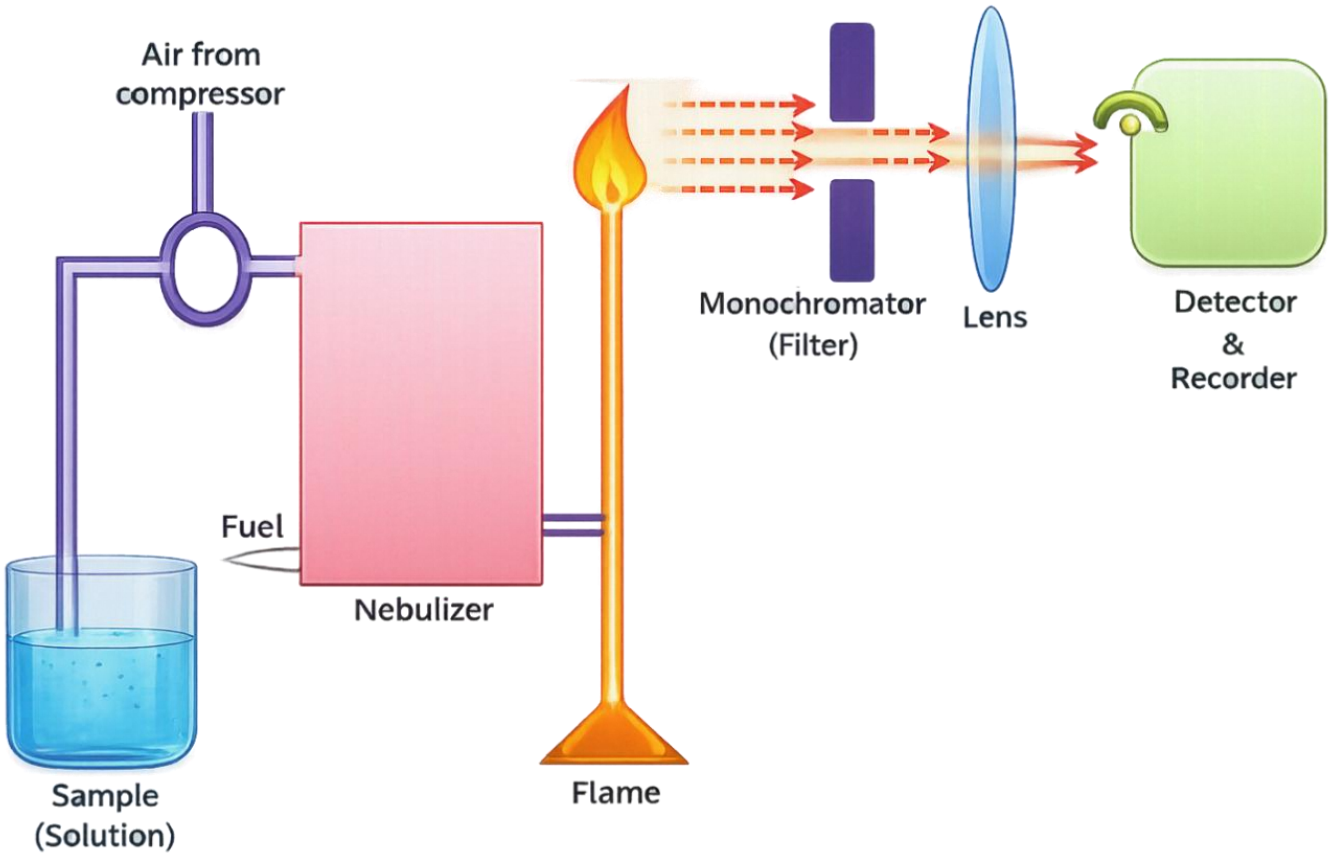
Important Relation (Beer–Lambert Law)

$$A \propto c$$

or

$$A = \varepsilon c l$$

Instrumentation of Flame Emission Spectroscopy



1. Nebulizer

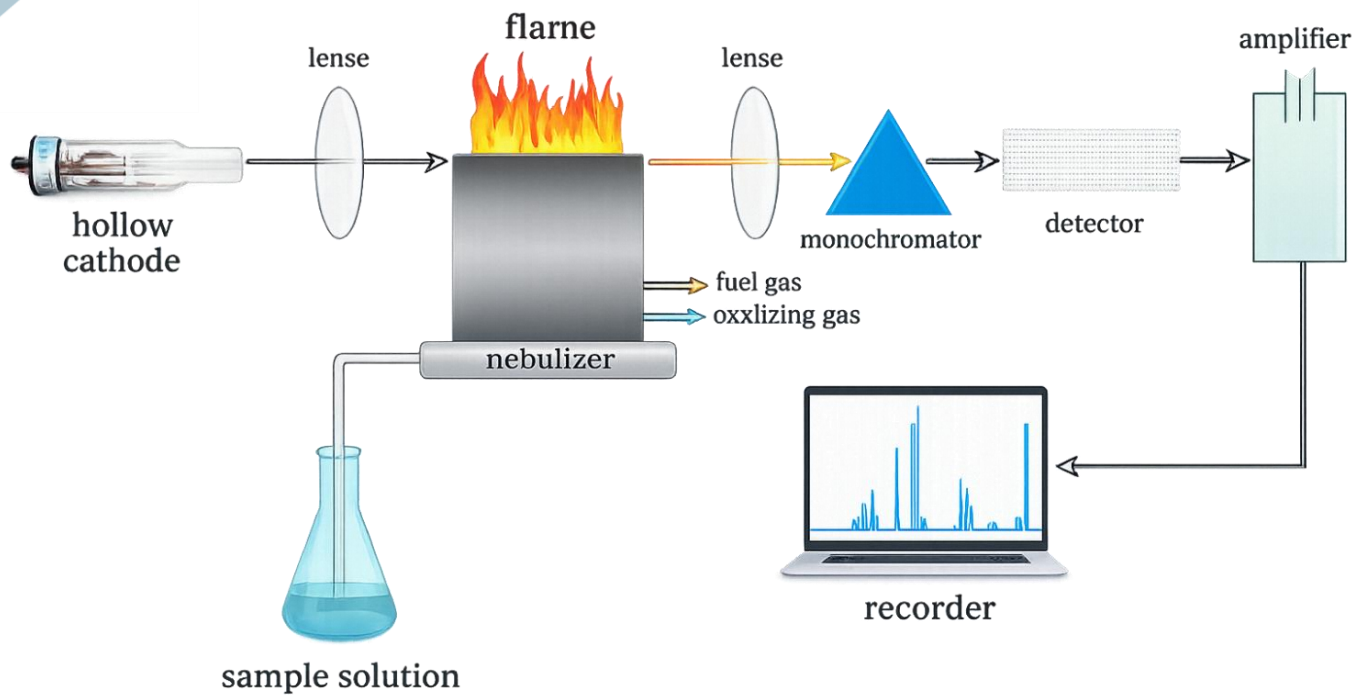
- Convert liquid sample into a fine mist (aerosol)
- Ensures uniform introduction into flame

2. **Burner (Flame Source)**
   - Provides heat for atomization and excitation
   - **Common flames:**
     - Air–acetylene
     - Oxygen–acetylene
3. **Atomizer**
   - Converts sample into free atoms
4. **Monochromator**
   - Separates emitted light into different wavelengths
   - Selects the required wavelength
5. **Detector**
   - Detects emitted light
   - **Examples:**
     - Photocell
     - Photomultiplier tube
6. **Readout System**
   - Displays intensity of emitted radiation

Working

1. Sample → nebulized
2. Enters flame → atoms excited
3. Atoms emit light
4. Monochromator selects wavelength
5. Detector measures intensity

Instrumentation of Atomic Absorption Spectroscopy



1. **Radiation Source (Hollow Cathode Lamp)**
   - Specific for each element
   - Emits characteristic wavelength
2. **Nebulizer**
   - Converts liquid sample into fine spray
3. **Atomizer (Flame or Graphite Furnace)**
   - Produces free atoms
   - **Types**:
     - Flame atomizer
     - Electrothermal (graphite furnace) atomizer
4. **Monochromator**
   - Isolates specific wavelength
5. **Detector**
   - Measures decrease in light intensity
6. **Readout System**
   - Displays absorbance or concentration

Special Feature of AAS

- Uses element-specific light source, making it highly selective

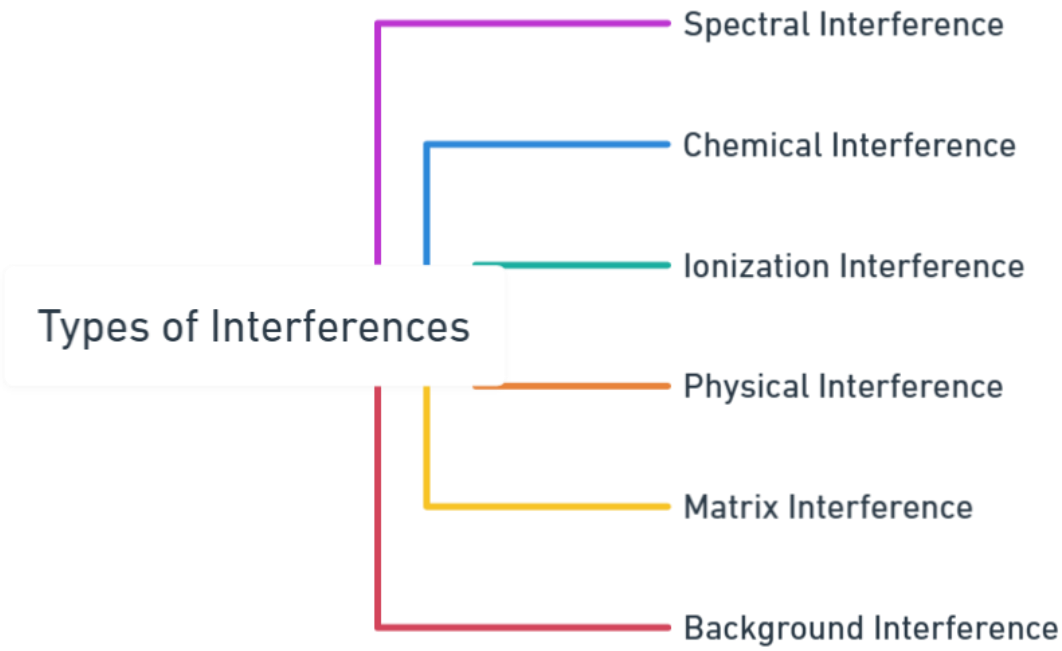
Working

1. Light from lamp passes through sample
2. Atoms absorb specific wavelength
3. Detector measures reduction in intensity
4. Absorbance is calculated

Interferences

- Interferences are factors that affect the accuracy of measurements.

Types of Interferences



1. Spectral Interference

- Occurs when other elements emit or absorb at similar wavelengths
- Leads to overlapping of spectral lines
- **Effect:**
  - Incorrect measurement

2. Chemical Interference

- Formation of stable compounds that do not atomize easily
- **Example:**
  - Formation of metal oxides

3. Ionization Interference

- At high temperature, atoms lose electrons and form ions
- **Effect:**

- Reduces number of neutral atoms
- Decreases signal intensity

4. Physical Interference

- **Due to differences in:**
  - Viscosity
  - Surface tension
  - Density
- **Effect:**
  - Affects nebulization and atomization (sample introduction)

5. Matrix Interference

- Other substances present in the sample affect measurement

6. Background Interference

- **Caused by:**
  - Flame emission
  - Scattering of light

Methods to Minimize Interferences

- Use of standard solutions / proper calibration
- Standard addition method
- Addition of releasing agents
- Use of ionization suppressors
- Use of higher temperature flames
- Background correction methods

Applications of Flame emission spectroscopy and Atomic absorption spectroscopy

A. Determination of Metal Ions

- **Detection of metals like:**
  - Na, K, Ca, Mg, Fe

B. Clinical Analysis

- **Measurement of electrolytes in blood:**
  - Sodium

- Potassium
- Calcium

C. Pharmaceutical Analysis

- Determination of metal impurities in drugs
- Quality control

D. Environmental Analysis

- **Detection of heavy metals in:**
  - Water
  - Soil
  - Air

E. Food Analysis

- Determination of mineral content
- Nutritional analysis

F. Agricultural Analysis

- Soil testing
- Fertilizer analysis

G. Industrial Applications

- Metal analysis in alloys
- Process control

H. Forensic Applications

- Detection of metals in samples
- Toxicology studies

I. Trace Element Analysis

- Highly sensitive detection of small quantities

J. Geological Analysis

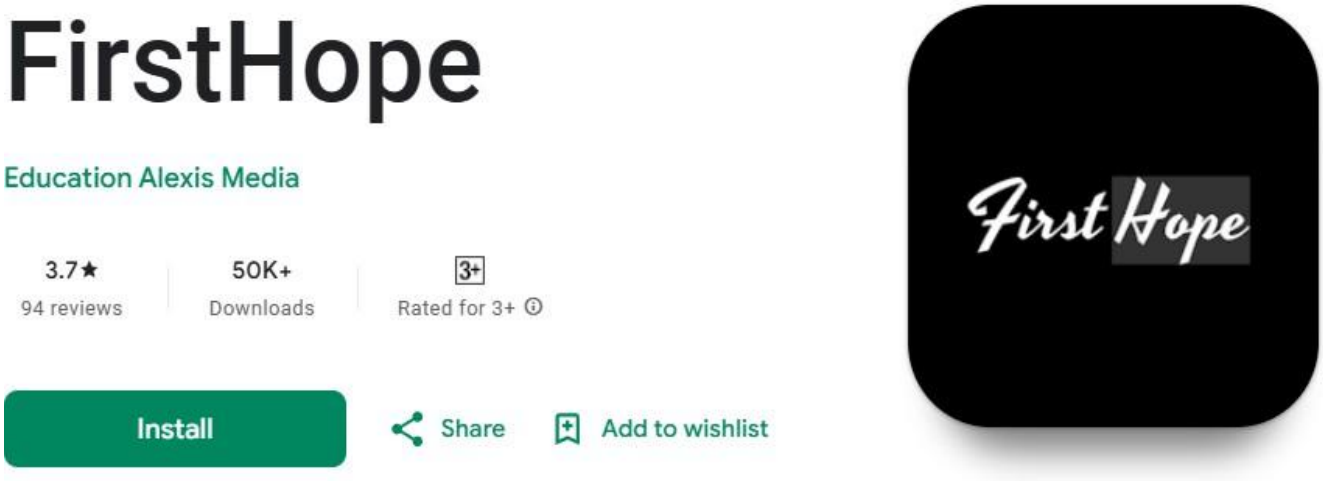
- Analysis of rocks and minerals

Key Understanding

- FES measures emitted light from excited atoms
- AAS measures absorbed light by ground state atoms
- Both are important for metal analysis and quantitative determination

First Hope

Easy Short Notes Designed to Help You Score Better



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