UNIT 2: INTRODUCTION TO SPECTROSCOPY

1. Principles of Spectroscopy

- Spectroscopy involves the study of the interaction between electromagnetic radiation and matter.
- It reveals the structure, composition, and dynamics of atoms and molecules.
- The basis is that atoms and molecules absorb, emit, or scatter light at specific wavelengths depending on their energy states.

2. Interaction of Light with Matter

- Key interactions include absorption, emission, scattering, reflection, and transmission.
- Absorption: Molecules absorb specific wavelengths, exciting electrons to higher energy levels.
- Emission: Excited molecules release energy as they return to lower energy levels, producing light.
- Scattering: Light is redirected in various directions by particles or molecules.

3. Absorption and Emission of Radiation

- Absorption Spectra: Unique for each substance, used for quantitative and qualitative analysis.
- Emission Spectra: Produced when excited atoms release energy, used in flame tests and elemental analysis.

4. Types of Spectroscopy

- UV-Visible Spectroscopy: Measures absorption in the UV and visible regions, widely used for chemical analysis.
- Fluorescence Spectroscopy: Measures emitted light from excited molecules, highly sensitive for biomolecule detection.
- Infrared (IR) Spectroscopy: Analyzes vibrational modes in molecules, useful for functional group identification.
- Atomic Absorption Spectroscopy (AAS): Measures free atom absorption,

widely used in metal analysis.

A) UV-Visible Spectroscopy

Principle -

- UV-Visible spectroscopy is based on the absorption of ultraviolet (200-400 nm) and visible (400-700 nm) light by molecules.
- When molecules absorb light in this range, their electrons get excited from lower energy ground states to higher energy excited states.
- The amount of light absorbed depends on the molecular structure and the environment of the absorbing species.
- The fundamental relationship governing this is **Beer-Lambert's Law**, which states that the absorbance (A) is directly proportional to the concentration (c) of the absorbing species and the path length (l) of the sample:

A = \varepsilon \cdot c \cdot l

- **A** = Absorbance (no unit)
- **ε** = Molar absorptivity or molar extinction coefficient (L mol^{-1} cm^{-1})
- **c** = Concentration of the sample (mol L^{-1})
- **l** = Path length of the cuvette (cm)

Instrumentation -

A typical UV-Vis spectrophotometer consists of the following components:

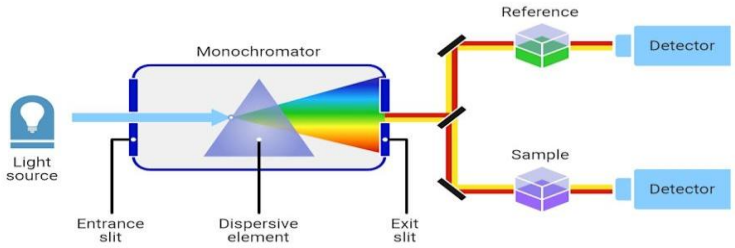
1. **Light Source**
    - Usually a combination of deuterium lamp (for UV region) and tungsten or halogen lamp (for visible region).
2. **Monochromator**
    - Splits light into individual wavelengths using prisms or diffraction gratings.
3. **Sample Holder (Cuvette)**
    - Made of quartz for UV measurements and glass or plastic for visible measurements.
4. **Detector**

- Converts transmitted light into an electrical signal (e.g., photodiodes, photomultiplier tubes).

5. **Signal Processor and Display**
   - Converts electrical signals into a readable spectrum, showing absorbance vs. wavelength.

Working -

- The light source emits a continuous spectrum.
- The monochromator isolates the desired wavelength.
- The selected wavelength passes through the sample in the cuvette.



- The detector measures the intensity of the transmitted light.
- The absorbance is calculated and displayed.

Applications -

- **Quantitative Analysis**
  - Determining concentrations of biomolecules like proteins, nucleic acids, and enzymes.
- **Qualitative Analysis**
  - Identifying substances based on their unique absorption spectra.
- **Kinetics Studies**
  - Monitoring reaction rates and enzyme activities.
- **Purity Testing**
  - Checking the purity of compounds in pharmaceuticals and chemicals.

B Fluorescence Spectroscopy

Principle

- Fluorescence spectroscopy is based on the phenomenon of **fluorescence**, where certain molecules absorb light at a specific (usually shorter) wavelength and then emit light at a longer wavelength.
- The process involves two main steps: **excitation** and **emission**.
- When a molecule absorbs a photon, its electrons are excited to a higher energy state. Upon returning to the ground state, the molecule releases energy as light (fluorescence).
- This emitted light has a longer wavelength (lower energy) than the absorbed light due to some loss of energy through non-radiative processes.
- **Stokes Shift**: The difference in wavelength between the absorbed and emitted light.

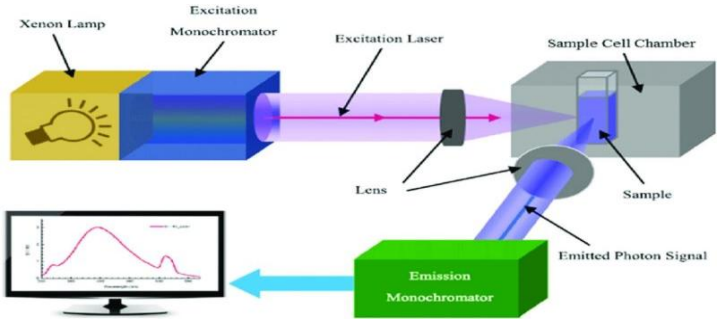
Instrumentation -

1. **Light Source**
    - High-intensity sources like xenon, mercury vapor, or lasers.
2. **Monochromator or Filter**
    - Selects the excitation and emission wavelengths.
3. **Sample Cell (Cuvette)**
    - Often made of quartz to avoid fluorescence interference.
4. **Detector**
    - Highly sensitive detectors like photomultiplier tubes (PMTs) or charge-coupled devices (CCDs).
5. **Data Processing and Display**
    - Converts signals into a readable fluorescence spectrum.

Working -

- Light from the source passes through an excitation monochromator, selecting the appropriate excitation wavelength.
- The selected light hits the sample, exciting the fluorophores.

- Emitted light is collected at a 90° angle to avoid interference from the incident light.
- The emission monochromator isolates the fluorescence signal, which



is then detected and processed.

Applications -

- **Biochemistry and Molecular Biology**
  - DNA sequencing, protein structure studies, enzyme activity assays.
- **Medical Diagnostics**
  - Immunoassays, biomarker detection, cancer diagnostics.
- **Environmental Science**
  - Pollutant detection, water quality analysis.
- **Pharmaceutical Analysis**
  - Drug testing, quality control, pharmacokinetics.
- **Forensic Science**
  - Detection of biological fluids, fingerprint analysis.

C) Infrared (IR) Spectroscopy

Principle

- Infrared spectroscopy is based on the **vibrational transitions** of molecules.

- When IR radiation (typically 4000-400 cm^{-1}) is passed through a sample, the molecules absorb specific frequencies corresponding to their natural vibrational modes.
- These vibrations include stretching, bending, and twisting of chemical bonds.
- The absorption pattern forms a unique **IR spectrum** that acts like a molecular fingerprint, allowing for the identification and analysis of compounds.

Instrumentation

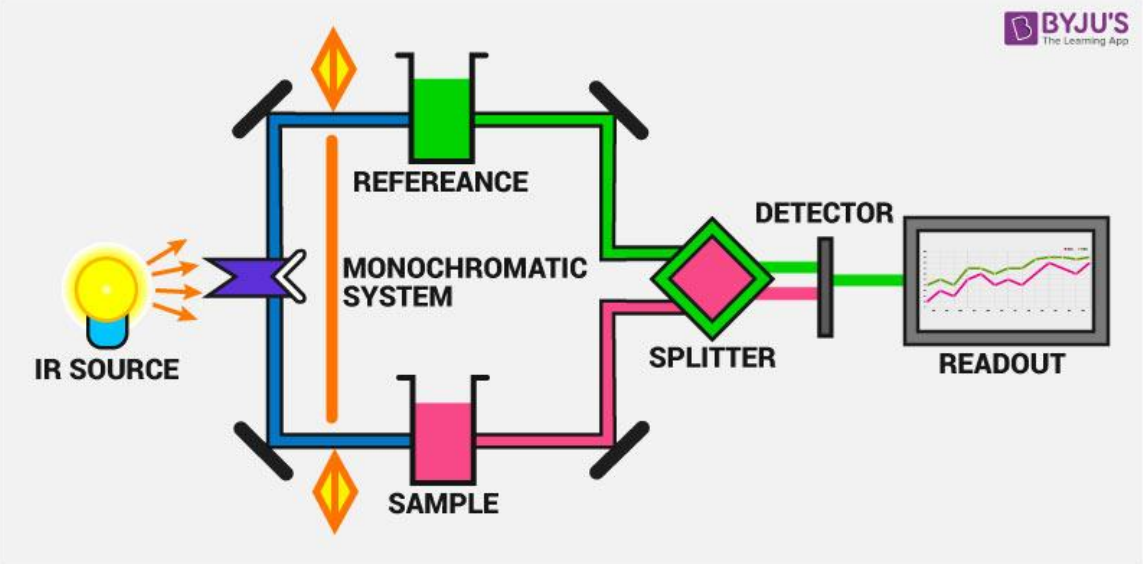
A typical IR spectrometer consists of the following components:

1. **IR Light Source**
   - Usually a **Globar (silicon carbide rod)** or **Nernst glower** for the mid-IR region.
2. **Sample Cell**
   - Can be solid, liquid, or gas. For solids, techniques like **KBr pellet** or **ATR (attenuated total reflectance)** are used.
3. **Monochromator or Interferometer**
   - Disperses the IR radiation into individual wavelengths (e.g., prisms, grating, or Michelson interferometer in FTIR).
4. **Detector**
   - Common types include **Pyroelectric detectors**, **Thermocouples**, or **Deuterated Triglycine Sulfate (DTGS)** detectors.
5. **Data Processing Unit**
   - Converts the detected signals into an IR spectrum.

Types of IR Spectroscopy

- **Dispersive IR Spectroscopy**
  - Uses a monochromator to separate IR light into individual wavelengths.
- **Fourier Transform Infrared (FTIR) Spectroscopy**
  - Uses an interferometer to measure all wavelengths simultaneously, offering high speed and sensitivity.

- **Near-IR (NIR) Spectroscopy**
  - 4000-14000 cm^{-1}, used for studying overtones and combination bands.
- **Mid-IR Spectroscopy**
  - 400-4000 cm^{-1}, commonly used for molecular structure analysis.
- **Far-IR Spectroscopy**
  - 10-400 cm^{-1}, used for heavy atoms and lattice vibrations.



Applications

- **Identification of Functional Groups**
  - Detects specific functional groups like -OH, -COOH, $-NH_2$, -C=O.
- **Structural Elucidation**
  - Helps determine molecular structure and bonding patterns.
- **Quality Control in Pharmaceuticals**
  - Purity testing, verification of drug formulations.
- **Polymer and Plastic Analysis**
  - Identification of monomers, additives, and degradation products.
- **Environmental Monitoring**
  - Detection of atmospheric gases and pollutants.
- **Forensic Analysis**

- Identification of unknown substances, residues, and paints.

D) Atomic Absorption Spectroscopy (AAS)

Principle

- **Atomic Absorption Spectroscopy** is based on the absorption of light by free, ground-state atoms.
- It measures the concentration of elements by detecting the **amount of light absorbed** at specific wavelengths characteristic of the element.
- When atoms absorb energy, their electrons are excited from the ground state to higher energy levels.
- The amount of absorbed light is directly proportional to the concentration of the element in the sample.

Beer-Lambert's Law is used to quantify the concentration:

$$A = \varepsilon \cdot c \cdot l$$

- **A** = Absorbance
- **ε** = Molar absorptivity
- **c** = Concentration of the analyte
- **l** = Path length of the light through the sample

Components of an AAS Instrument

1. **Radiation Source (Hollow Cathode Lamp - HCL)**
   - Contains a cathode made from the element being analyzed.
   - Emits sharp, characteristic atomic lines of the element.
2. **Atomizer**
   - Converts the sample into free atoms.
   - Types include:

        - **Flame Atomizers**: Use a fuel and oxidant (e.g., acetylene-air, acetylene-nitrous oxide).
        - **Graphite Furnace Atomizers**: Use electrically heated graphite tubes for higher sensitivity.
        - **Hydride Generation and Cold Vapor Systems**: Used for specific elements like arsenic and mercury.
3. **Monochromator**
    - Isolates the specific wavelength absorbed by the element of interest.
    - Ensures that only the characteristic line is detected.
4. **Detector**
    - Converts the absorbed light into an electrical signal (e.g., photomultiplier tube).
5. **Signal Processor and Data Display**
    - Amplifies and converts the electrical signal into readable data.

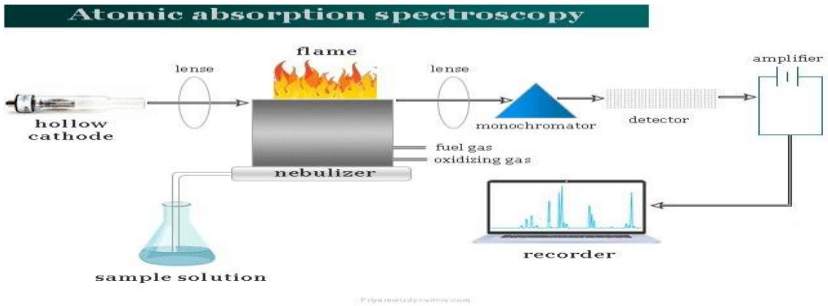
Working of AAS

1. The sample is introduced into the atomizer, converting it into a free atomic vapor.
2. The HCL emits the characteristic wavelength of the element being analyzed.
3. The light passes through the vaporized sample, where the free atoms absorb the specific wavelength.
4. The monochromator isolates the desired wavelength, eliminating background noise.
5. The detector measures the absorbance, which is then processed and displayed.

Types of AAS

1. **Flame Atomic Absorption Spectroscopy (FAAS)**
    - Simple and widely used.
    - Lower sensitivity but faster and cost-effective.
2. **Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)**
    - Higher sensitivity and lower detection limits.
    - Ideal for trace element analysis.
3. **Hydride Generation AAS (HGAAS)**
    - Used for elements like As, Se, Sb, and Hg.
4. **Cold Vapor AAS (CVAAS)**

- Specifically for mercury analysis



Applications

- **Environmental Analysis**
  - Detection of heavy metals in water, soil, and air.
- **Clinical and Biomedical Analysis**
  - Metal content in blood,