Principle and Use of UV-Visible Spectroscopy

Principle:

UV-Visible spectroscopy is based on the **absorption of ultraviolet (UV) and visible light** by molecules, leading to electronic transitions from the ground state to an excited state.

1. **Beer-Lambert Law**: The absorbance (A) of a solution is directly proportional to its concentration (c) and path length (l):

$$A = \epsilon \cdot c \cdot l$$

   where:
   - A = Absorbance (no units)
   - ϵ = Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)
   - c = Concentration (mol/L)
   - l = Path length (cm)
2. **Electronic Transitions**:
   - When molecules absorb UV-Visible light (190–800 nm), electrons transition between energy levels (e.g., π→π*, $n \rightarrow \pi$).
   - Conjugated systems (e.g., aromatic compounds, dyes) absorb strongly in this region.

Uses of UV-Visible Spectroscopy:

1. **Quantitative Analysis**:
   - Determining the concentration of unknown solutions (e.g., drugs, biomolecules).
2. **Qualitative Analysis**:
   - Identifying functional groups (e.g., aldehydes, ketones, conjugated dienes).
3. **Chemical Kinetics**:
   - Monitoring reaction progress by tracking absorbance changes.
4. **Pharmaceutical & Biochemical Applications**:
   - DNA/RNA analysis, protein quantification (e.g., Bradford assay).
5. **Environmental Analysis**:
   - Detecting pollutants (e.g., heavy metals, organic contaminants).

Schematic Diagram of a UV-Visible Spectrophotometer:

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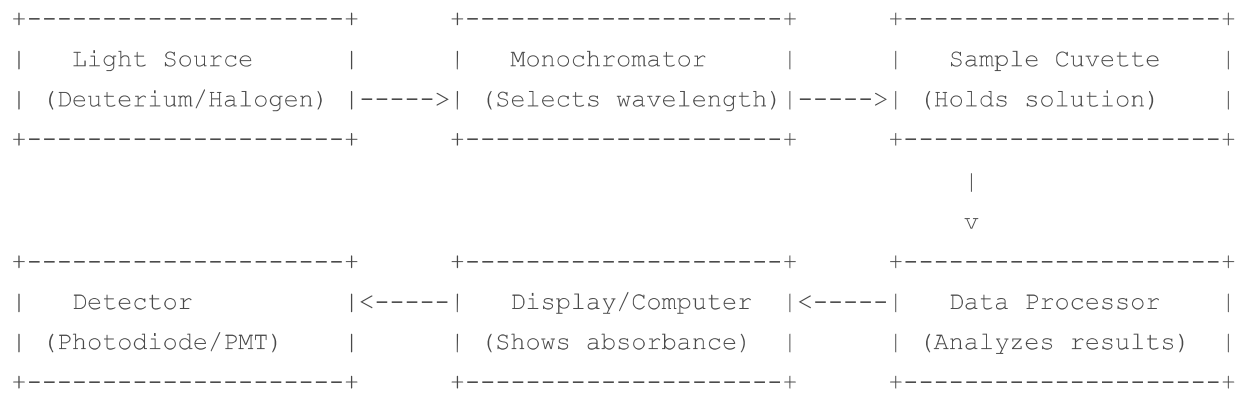

Key Components:

1. **Light Source**: Emits UV (Deuterium lamp) and visible (Tungsten/Halogen lamp) light.
2. **Monochromator**: Selects a specific wavelength.
3. **Sample Cuvette**: Holds the sample solution (usually quartz for UV, glass/plastic for visible).
4. **Detector**: Measures transmitted light (Photodiode or Photomultiplier Tube).
5. **Data System**: Displays absorbance/transmittance spectra.

This technique is widely used due to its **simplicity, accuracy, and broad applicability** in chemistry, biology, and environmental science.