B. Pharmacy 7th Semester Notes

UNIT I: Instrumental Methods of Analysis Notes

These notes cover the foundational concepts, instrumentation, and applications of UV Visible Spectroscopy and Fluorimetry.

UV Visible Spectroscopy

1. Introduction and Principle (Electronic Transition)

Ultraviolet (UV) and visible spectroscopy records the absorption of electromagnetic radiation (EMR) in the UV region (10–400 nm) and the visible region (400–800 nm). The UV region is subdivided into the near UV (quartz) region (200–400 nm) and the far or vacuum UV region (10–200 nm).

The absorption of EMR induces the excitation of an electron from a lower to a higher molecular orbital, hence it is often called electronic spectroscopy. Organic chemists use this technique for detecting conjugated multiple bonds or aromatic rings.

EMR waves are synchronized oscillations of electric and magnetic fields traveling at the speed of light ($3 \times 10^8 \text{ m/s}$). The distance between two crests or two troughs is termed the length of electromagnetic waves.

2. Types of Electrons and Transitions

Organic molecules contain σ (bonding), π (bonding), and n (non-bonding) electrons, and anti-bonding orbitals ($\sigma^$ *and* $\pi^$).

Transition Type	Energy Requirement	Region/Wavelength	Example Compound
$\sigma \rightarrow \sigma^*$	High energy	Vacuum UV region (150 nm)	Saturated hydrocarbons (e.g., methane, ethane)

$n \rightarrow \sigma^*$	Low energy (than $\sigma \rightarrow \sigma^*$)	Near UV region (150–250 nm)	Saturated compounds with heteroatoms (e.g., alcohols)
$\pi \rightarrow \pi^*$	Less energy (than $n \rightarrow \sigma^*$)	200–800 nm	Unsaturated compounds (e.g., alkenes, aromatic compounds)
$n \rightarrow \pi^*$	Lowest energy required	Nearly 290 nm	Unsaturated compounds with heteroatoms (e.g., aldehydes, ketone)

Based on energy and intensity, transitions are classified as High energy/Intense ($\sigma \rightarrow \sigma^*, $\pi \rightarrow \pi^*$) and Low energy/Weak ($n \rightarrow \pi^*$).

3. Chromophores and Auxochromes

- **Chromophore:** Any isolated covalently bonded group that shows characteristic absorption in the UV or visible region. It is the part of a molecule that absorbs light. A compound containing a chromophore is called a chromogen.
    - **Independent Chromophore:** Requires only one chromophore to impart colour (e.g., Azo group, -N=N-).
    - **Dependent Chromophore:** Requires more than one chromophore to impart colour (e.g., diacetyl).
- **Auxochrome:** A functional group of atoms with one or more lone pairs of electrons that, when attached to a chromophore, alters both the wavelength and intensity of absorption. If these groups are in direct conjugation with the pi-system, they intensify the absorption and may increase the wavelength at which light is absorbed.
    - **Types:** Acidic (-COOH, -OH) and Basic (-NH_2).

4. Absorption Bands

Absorption bands are classified into four types based on the electronic transition:

1. **K-Bands:** Seen in conjugated compounds (dienes, polyenes) due to $\pi \rightarrow \pi^*$ transitions. They are intense ($\text{E}_{max} > 10^4$).
2. **R-Bands:** Occur due to $n \rightarrow \pi^*$ transitions of single chromophore groups having at least one lone pair on a heteroatom. They are called **forbidden bands** due to low intensity ($\text{E} < 100$).
3. **B-Bands:** Occur due to $\pi \rightarrow \pi^*$ transition in aromatic or heteroaromatic compounds.
4. **E-Bands:** Occur in electronic transitions in the benzenoid system of three ethylenic bonds in closed cyclic conjugation. E_1 bands appear at lower wavelength (184 nm) and are more intense than E_2 bands (204 nm).

5. Spectral Shifts and Solvent Effects

The ultraviolet-visible spectrum is recorded as a plot of absorbance versus wavelength (λ). λ_{max} is the wavelength where maximum absorption occurs.

The choice of solvent is usually limited to nonconjugated solvents like methane (CH_3OH), ethanol, or water, as solvents like toluene are not suitable.

6. Beer and Lambert's Law and Deviations

- **Beer's Law:** The decrease in the intensity of monochromatic radiation is **directly proportional to the concentration (c)** of the solution ($A \propto c$).
- **Lambert's Law:** The decrease in the intensity of monochromatic radiation is **directly proportional to the thickness/pathlength (l)** of the absorbing medium ($A \propto l$).
- **Beer-Lambert's Law:** The decrease in the intensity of monochromatic radiation is **directly proportional to both pathlength (l) and concentration (c)**.
    - Equation: $A = \varepsilon \times c \times l$.
    - A is Absorbance ($\log I_0/I$); l is Optical path length (cm); c is Concentration (mol dm^{-3}); ε is Molar extinction coefficient.
    - For the law to be obeyed, the plot of absorbance versus concentration should be **linear**.

Derivation Summary

The derivation involves integrating the change in intensity (dI) with respect to length (dl), establishing the proportionality: $\frac{1}{I} \frac{dI}{dl} = -k \times C$. Integration yields $\log (I_0/I) = \varepsilon \times C \times l$.

Deviations from Linearity

Causes of nonlinearity include both chemical and instrumental factors:

- The radiation is **not monochromatic**.
- Deviation in absorptivity coefficients at **high concentrations** ($>0.01 \text{M}$) due to electrostatic interaction.
- **Scattering of light** due to particulates.
- **Fluorescence or Phosphorescence** of the sample.
- **Shifts in chemical equilibria** (e.g., dissociation or reaction with the solvent).
- Changes in refractive index at high analyte concentration.

7. Instrumentation

The essential parts of a spectrophotometer are the Radiation Source, Wavelength Selector, Cells/Cuvettes, Detector, Recording System, and Power Supply. Spectrophotometers are typically single-beam or double-beam.

Sources of Radiation

Sources must be stable, intense, and cover the spectrum range (180–360 nm up to 400 nm).

- **Hydrogen Discharge Lamp:** Hydrogen gas is stored under high pressure; an electric discharge produces UV radiation. High pressure causes the hydrogen to emit a continuum. They are stable and cover the range of 3500–1200 Å.
- The **Tungsten lamp** (400–800 nm) and the **D_2 lamp** (200–400 nm) are often used together, especially in double-beam systems.

Wavelength Selectors

1. **Filters:**
    - **Absorption Filters:** Produced in host materials (glass, plastic), they absorb shorter wavelengths and transmit longer wavelengths.

    - **Interference Filters (Fabry-Perot):** Based on optical interference. Consist of a dielectric spacer film (CaF_2, MgF_2, or SiO) sandwiched between two parallel, partially reflecting metal films (usually silver). They transmit a band of radiant energy.
2. **Monochromators (Prisms and Gratings):** Disperse radiation according to wavelength, requiring an entrance slit, a dispersing element, and an exit slit.

    - **Prisms:** Glass prisms absorb radiation between 2000–3000 Å. **Quartz and fused silica prisms** are transparent throughout the entire UV range and are widely used in UV spectrophotometers.
    - **Gratings:** Provide monochromatic light using parallel grooves on a reflecting surface, causing reflected light from neighbouring grooves to interfere. Grating monochromators are advantageous because their materials (like aluminium) are not attacked by moisture.

Sample Cells or Cuvettes

These must have windows transparent in the spectral region of interest. **Quartz or fused silica** is required for the UV region (wavelengths less than 350 nm). Silicate glass is ordinarily used for the 375–2000 nm region. The most common cell path length for studies in the UV and visible regions is **1 cm**.

Detectors

Detectors used are photometric detectors: Photo tubes, Photomultiplier Tubes (PMT), Photovoltaic cell, and Silicon Photodiode array detectors.

- **Photo Tubes or Photo-Emissive Cells:** Composed of an evacuated glass tube with a photocathode (coated with high atomic volume elements like caesium) and a collector anode. The flow of electrons produces a current proportional to light intensity.
- **Photomultiplier Tubes (PMT):** This is the **most sensitive** of all detectors, used in sophisticated instruments. The principle is the **multiplication of photoelectrons by secondary emission** using a photocathode and a series of up to 10 dynodes. An overall multiplication factor of 10^6 is achieved.
- **Photovoltaic Cell (Barrier-Layer Cells):** Operates **without the use of a battery**. Consists of a metal base plate, a semiconductor metal (like selenium), and a thin layer of silver or gold. Its use is generally limited to the visible region (450–650 nm).

- **Silicon Photodiode Array Detector:** Based on the internal photoelectric effect. Silicon photodiodes are advantageous due to their low cost but low sensitivity if light intensity is relatively large.

Spectrophotometer Types

1. **Single-beam UV spectrophotometer:** Measures the intensity of radiation reaching the detector. A problem is that it measures the total light, not the percentage absorbed, and the detector response varies significantly with the wavelength.
2. **Double-beam UV spectrophotometer:** Utilizes two sources and splits the beam to compare the sample cell against a reference cell.

8. Applications

- **Spectrophotometric Titrations:** Absorbance is plotted against the volume of titrant added; the equivalence point is found at the intersection of two linear segments. Advantages include application to a large number of **non-absorbing constituents** if only one absorber is present, and application to highly coloured solutions. Methods include Acid-Base, Oxidation-Reduction, Complexometric, and Precipitation Titrations. A dilution correction factor may be included in the calculation ($A_c = ((V_i + V_a)/V_i)(A_m)$).
- **Single Component Analysis Methods:**
    - **Direct Analysis:** Used for compounds containing conjugated double bonds or aromatic rings.
    - **Indirect Analysis:** Involves converting the analyte to a derivative by adding a reagent, especially if the analyte absorbs weakly in the UV region or if interference needs to be avoided.
- **Multicomponent Analysis Methods:** Used to minimize the cumbersome task of separating interferents, allowing for the determination of multiple analytes simultaneously, resulting in reduced analysis time and cost.

Fluorimetry

1. Theory and Principle

Fluorescence is the emission of radiation when molecules, excited by radiation, relax from the highly unstable **excited singlet state** back to the singlet ground state with the emission of light. **Fluorimetry** is the measurement of fluorescence intensity at a particular wavelength.

Electronic States and Conversions

- **Singlet state:** All electrons are paired ($\uparrow \downarrow$). The excited singlet state has unpaired electrons but opposite spin.
- **Doublet state:** Unpaired electrons are present ($\uparrow \downarrow$ or $\uparrow$).
- **Triplet state:** Unpaired electrons of the **same spin** are present ($\uparrow \uparrow$).
- **Collisional deactivation:** The entire energy is lost due to collision, and **no radiation is emitted**.
- **Internal conversion:** Intermolecular process where a molecule passes to a lower energy electronic state without emission of light.
- **External conversion:** Deactivation of an excited electronic state by interaction with the solvent or other solutes.
- **Intersystem crossing:** A process in which the spin of an excited electron is reversed, leading to a change in multiplicity (e.g., from singlet to triplet state).
- **Phosphorescence:** Emission of radiation resulting from a transition from the triplet state to the singlet ground state, typically involving intersystem crossing. It usually requires low temperature and the absence of oxygen.

2. Factors Affecting Fluorescence Intensity

1. **Concentration:** Fluorescence intensity is proportional to concentration only when the **absorbance is less than 0.02**.
2. **Quantum Yield (Φ):** The ratio of photons emitted to photons absorbed. It is always **less than 1.0** because energy is lost by radiationless pathways (e.g., Collisional deactivation).
3. **Intensity of incident light:** Increases in incident light intensity increase fluorescence intensity.
4. **Adsorption:** Adsorption of the sample onto the container walls can be a serious problem.
5. **Oxygen:** Oxidation of fluorescent species to non-fluorescent species quenches the substance.
6. **pH:** Alteration of pH significantly affects fluorescence (e.g., Aniline).

7. **Temperature & Viscosity:** Higher temperature increases collisional deactivation and reduces intensity. Higher viscosity reduces collision frequency and increases intensity.
8. **Photochemical decomposition:** Intense radiation can lead to photochemical decomposition.
9. **Quenchers:** Substances that reduce fluorescence intensity.
    - **Self-quenching:** Occurs at high concentrations of the fluorescent substance itself.
    - **Collisional quenching:** Collisions between the fluorescent substance and other molecules (e.g., halide ions) lead to reduction in intensity.
    - **Static quenching:** Occurs due to complex formation between the fluorescent molecule and other molecules (e.g., caffeine reducing riboflavin fluorescence).
10. **Scatter:** Scattering of incident light by colloidal particles decreases fluorescence intensity.

3. Instrumentation

- **Source of light:**
    - **Mercury vapor lamp:** Used in filter fluorimeters (high pressure gives intense lines, including at 254 nm).
    - **Xenon arc lamp:** Gives more intense radiation than the mercury vapor lamp; used in spectrofluorometers.
    - Tungsten lamps are used if excitation is in the visible region.
- **Filters and Monochromators:** Inexpensive filter fluorimeters use a **Primary filter** (transmits UV radiation for excitation) and a **Secondary filter** (transmits visible radiation for detection). Spectrofluorometers use **Excitation monochromators** and **Emission monochromators**.
- **Sample cells:** Usually made of quartz, cylindrical or rectangular.
- **Detectors:**
    - **Barrier layer/photovoltaic cell:** Employed in inexpensive instruments like filter fluorimeters, generating current proportional to incident light intensity.
    - **Photomultiplier tubes (PMT):** Highly sensitive, used in spectrofluorometers. They achieve high sensitivity by multiplying photoelectrons via secondary emission using dynodes. PMT can detect very weak signals, even 200 times weaker than photovoltaic cells, making them useful for fluorescence measurements.

Instrument Types

1. **Single beam (filter) fluorimeter:** Simple and cheap. Uses a secondary filter placed at a 90° angle (rather than 180° as in colorimetry) to separate emitted fluorescence from unabsorbed UV radiation.
2. **Double beam fluorimeter:** Similar to single beam but uses a single light source, passing beams separately through primary filters to the sample and a reference solution.
3. **Spectrofluorometer:** A double beam instrument where the primary filter is replaced by an **excitation monochromator** and the secondary filter is replaced by an **emission monochromator**.

4. Applications

Fluorimetric methods are primarily used in **quantitative analysis**.

- Determination of inorganic substances, such as Al^{3+}, Li^{+}, and Zn^{2+}.
- Determination of drugs like thiamine HCl and phenytoin.
- Determination of indoles, phenols, and steroids.
- Detection of impurities at the nanogram level, offering better results than absorbance spectrophotometers, with special emphasis on determining components of samples at the end of chromatographic columns.
- Determination of boron in steel by complex formed with benzoin.