Chapter ° 3: NUV-VIS Spectroscopy and Its Applications

I. Introduction

UV-Visible spectrometry is most often employed in quantitative analysis through the application of Beer-Lambert's law. This technique finds its application in both organic and inorganic domains, and has considerable importance as one of the spectroscopic methods supporting drug research in pharmaceutical analysis.

II. Energy Domain

The **UV-Vis** range extends approximately from **800** to **110 nm**. In practice, however, measurements are limited to the near UV-visible range (**200-800 nm**). Most standard devices operate starting from **200 nm**, making far UV measurements inaccessible under these conditions.
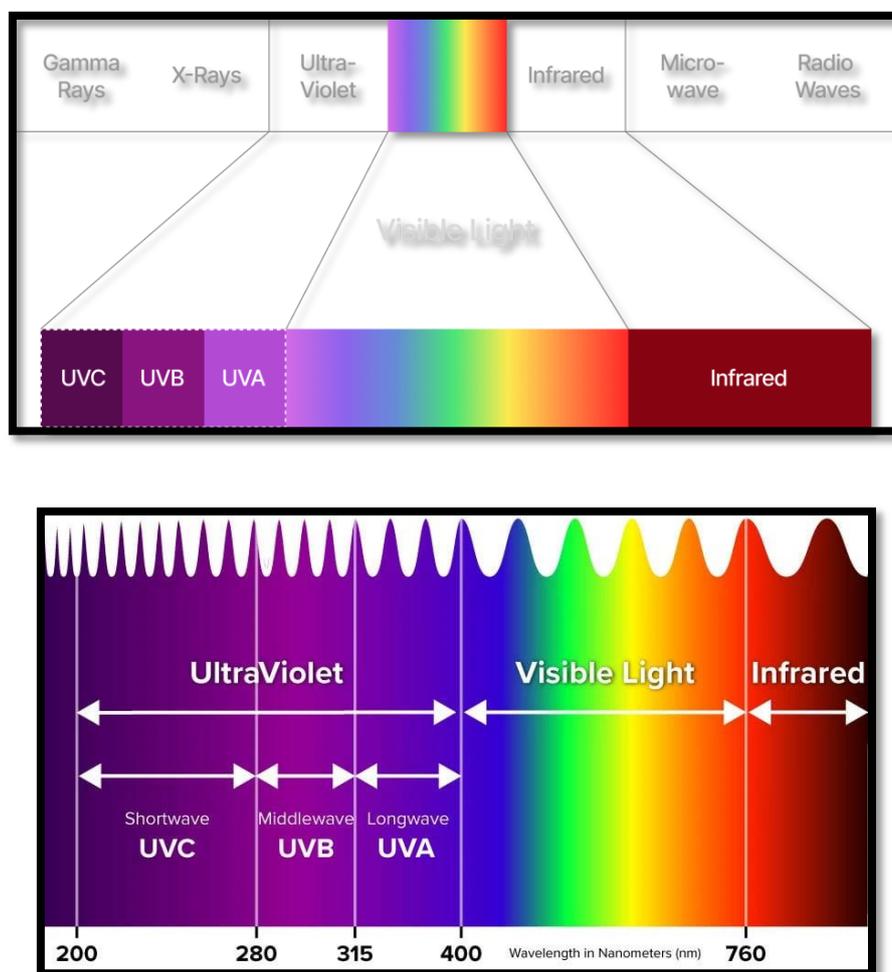
Fig 37. Electromagnetic spectrum

II.1. UV Absorption Properties

- **210-280 nm:** Bactericidal properties (UV-C, for disinfection).
- **280-315 nm:** Promotes vitamin D production, which is antirachitic (calcium absorption) (UV-B).
- **315-400 nm:** Promotes skin pigmentation (UV-A).

III.   UV/Visible Theory

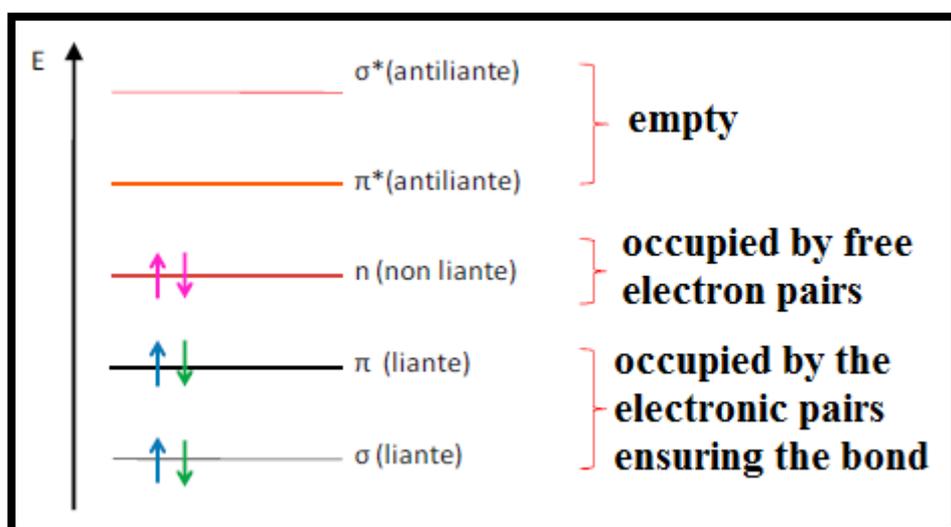A simplified molecular diagram depicts the electronic energy levels of a molecule.

Fig 38. Molecular diagram depicts the electronic energy levels

IV. Types of Possible Electronic Transitions

As in the case of atomic electronic transitions, molecular electronic transitions are governed by electronic transition rules. There are four main types of electronic transitions (among others!) that can occur within molecules when they absorb specific UV-visible radiation.

Types of Electrons Involved: σ, π, and n (non-bonding pair)

The characteristics of these electrons are as follows:

   σ electrons: Found in saturated bonds (strongly bound).

   π electrons: Found in unsaturated bonds (weakly bound).

n electrons: Non-bonding electron pairs.

IV.1. Transition σ→σ*

This transition requires a significant amount of energy due to the high stability provided by the σ orbital to its electrons. The corresponding absorption band is located in the far UV region (around 130 nm). Most organic compounds exhibit such transitions.
(Not usable with standard instruments as the energy is too high for wavelengths $\lambda < 200$ **nm**)
Example: Methane (CH_4) absorbs at around 125 nm.

IV.2. Transition n→σ*

This type of transition occurs in molecules containing a non-bonding electron pair (n) on a heteroatom, such as alcohols, amines, and halogenated derivatives. This transition results in an absorption band of medium intensity, located at the extreme limit of the near UV region (around 200 nm). (Usable with standard instruments for $\lambda \geq 200$ **nm**).
Example: Methanol (CH_3OH) absorbs at around **180 nm**.

IV.3. Transition π→π*

This type of transition occurs in molecules with double bonds. It results in an absorption band of high intensity (**165-200 nm**). The **π→π*** transition is highly tunable (bathochromic effect).

It becomes detectable for wavelengths $\lambda > 200$ nm when present in conjugated molecules, and such bands are referred to as K-bands (e.g., C=C-C=C systems).

IV.4. Transition n→π*

This transition occurs in molecules containing a heteroatom with free electron pairs (n) within an unsaturated system, resulting in absorption at **270-280 nm**.

It is detectable using standard instruments and originates from groups such as C=O, N=O, N=N, etc. This transition has a low absorption coefficient ε ($\varepsilon\_max < 100$) and is referred to as an R-band, regardless of the molecule type.

Example: Carbonyl group (C=O).

Study Example: Represent the σ→σ*, π→π*, and n→π* transitions for the following molecules: CH_4, CH_3Cl, C_2H_4, and $H_2C=O$.

Remarks

The σ→σ* and n→σ* transitions are not practical for technical reasons.

The **n→π*** transitions require relatively low energy, but their absorption is also weak. Additionally, the transition energy increases in the presence of polar solvents. Consequently, this transition is rarely used in quantitative analyses.

The most commonly used transition is the **π→π*** transition for the following reasons:

a. The absorption during the transition is relatively high (high sensitivity).

b. The required energy is moderate, much lower than the dissociation energy.

IV.5. Energy Order of Electronic Transitions

For an electronic transition to occur, energy must be supplied. This energy is specific to each type of transition.

Electronic Transition	Energy Difference
σ → σ*	Highest
n → σ*	
π → π*	
n → π*	Lowest

Fig 39. Decrease of electronic transition energy

IV.6. Perception of Colors

- The perceived color is complementary to the color that is absorbed.
- When a chemical species absorbs radiation only within a specific range of visible wavelengths, its color is the complementary color of the absorbed radiation.
- When a chemical species absorbs multiple ranges of visible wavelengths, its color results from the additive synthesis of the complementary colors of the absorbed radiation.
- A colorless species does not absorb any radiation within the visible spectrum.

Light absorbed			Perceived complementary (subtraction) color	
Wavelength	Color			
400-435	Violet		Green-yellow	
435-480	Blue		Yellow	
480-490	Green-Blue		Orange	
490-500	Blue-Green (cyan)		Red	
500-560	Green		Purple (magenta)	
560-580	Yellow-Green		Violet	
580-595	Yellow		Blue	
595-605	Orange		Green-Blue	
605-650	Red-orange		Blue-Green (cyan)	
650-750	Red		Green	

V. Properties of Transitions

V.1. Chromophore

In general terms, a chromophore is a characteristic feature of a given electronic transition, defined by its λ_{max} (maximum wavelength of absorption) and εmax (molar absorptivity). A chromophore can be either saturated or unsaturated, depending on the molecular structure.

In most cases, the chromophore is associated with unsaturation (detectable by standard instruments), for example: C=O, C=N, N=N, etc.

In other words, a chromophore is the part of a molecule that contains the electrons involved in a transition leading to absorption.

Molecule	λ_{max}(nm)	ε_{max} (l.mol^{-1}.cm^{-1})
CH_4	122 ($\sigma \rightarrow \sigma^*$)	strong
C_2H_4	170 ($\pi \rightarrow \pi^*$)	15000
C_6H_6	200 ($\pi \rightarrow \pi^*$)	8000
CH_3Br	204 (n $\rightarrow \pi^*$)	200
CH_3-C=O-CH_3	280 (n $\rightarrow \pi^*$)	15
	190 (($\pi \rightarrow \pi^*$)	1100
	156 ($\sigma \rightarrow \sigma^*$)	strong
Azo (-N=N-)	350	11

V.2. Auxochrome Group

An auxochrome group does not absorb light on its own unless it is attached to a chromophore. In this case, it can modify either the position of the absorption (λ_{max}) or the intensity of the absorption (I_{max}). This is due to the interactions between the free electron pairs on an atom in the auxochrome group (e.g., **N**, **O**, **S**) and the π-electrons of the chromophore (typically increasing resonance).

Examples of auxochrome groups: including **-OH**, **-NH$_2$**, **-NO$_2$**, and halogens (**-X**).

V.3. Bathochromic Effect

This effect refers to the shift of absorption bands toward longer wavelengths. Examples:

- **C=C** (174 nm)
- **C=C-C=C** (220 nm)
- **C=C-C=C-C=C** (258 nm).

The bathochromic shift is caused by electron delocalization, which facilitates electronic transitions by reducing the energy gap between levels. When the conjugation increases (e.g., **n > 10**), the absorption moves into the visible region.

V.4. Hypsochromic Effect (Opposite of the Bathochromic Effect)

This effect refers to the shift of absorption bands toward shorter (lower) wavelengths.

V.5. Hyperchromic Effect

This effect refers to an increase in absorption intensity.

V.6. Hypochromic Effect

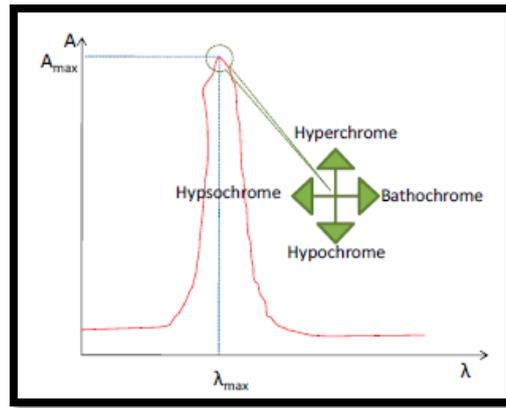This effect refers to a decrease in absorption intensity.

Fig 40. Diagram of transition

VI. Beer-Lambert Law

The absorbance of a solution at a given wavelength is proportional to its concentration and the path length of the radiation:

$$A(\lambda) = \varepsilon(\lambda) \times l \times C$$

Where:

- **C**: Concentration of the dissolved species in solution (analyte).
- **l**: Path length (width of the cuvette).
- **ε(λ)**: Molar absorptivity coefficient (characterizes the absorption intensity of the species at λ_{max}).
- **A**: Absorbance or optical density D_0.

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

Note: Relationship between Absorbance and Transmittance (T)

Transmittance is often expressed as a percentage:

$$A = -\log (T) \qquad ; \qquad T = \frac{I}{I_0}$$

Where:

- **I**: Transmitted light intensity.
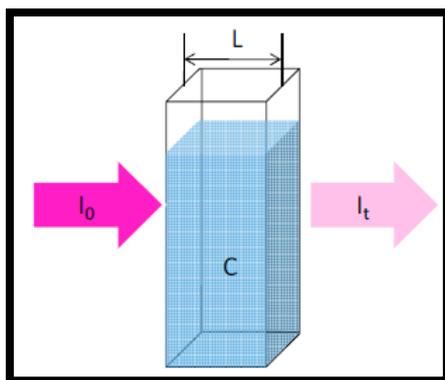- **I₀**: Incident light intensity.

Fig 41. Scheme illustrating the concept of transmittance

VI.1. Conditions for Applying the Beer-Lambert Law

- The following conditions must be met for the Beer-Lambert law to be valid:
- The light must be monochromatic.
- The solutions used must not be colloidal to avoid losses of radiation through reflection or scattering.
- The solutions must be dilute ($C < 10^{-2}$ M).
- The nature of the substance being analyzed must remain constant with concentration (i.e., no dissociation, polymerization, etc.).

VI.2. Additivity of the Beer-Lambert Law

If a mixture contains substances $C_1, C_2, ..., C_n$ and is exposed to monochromatic radiation, the total optical density (absorbance) of the mixture is equal to the sum of the partial optical densities (absorbance) contributed by each individual substance:

$(D_0)_T = (D_0)_1 + (D_0)_2 + (D_0)_3 + ...(D_0)_n$

$(D_0)_T = \varepsilon_1 C_1 l_1 + \varepsilon_2 C_2 l_2 + \varepsilon_3 C_3 l_3 + ... + \varepsilon_n C_n l_n.$

VII. Instrumentation

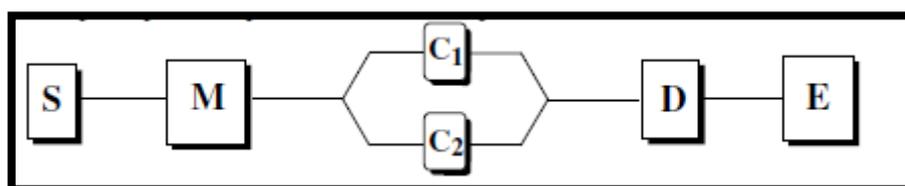The schematic principle of a UV-Vis spectrometer is as follows:

Fig 42. Schematic principle of UV-visible

S (Source): The source can operate in single-beam or double-beam mode and covers a wavelength range of 200 to 800 nm.

M (Monochromatic): A prism or grating is used to isolate specific wavelengths.

C_1 and C_2 (Cells):

C_1: Reference cell.

C_2: Sample (analysis) cell.

UV region: Quartz cells are used.

Visible region: Quartz or glass cells can be used.

D (Detector): Converts radiant energy into measurable electric current via the photoelectric effect.

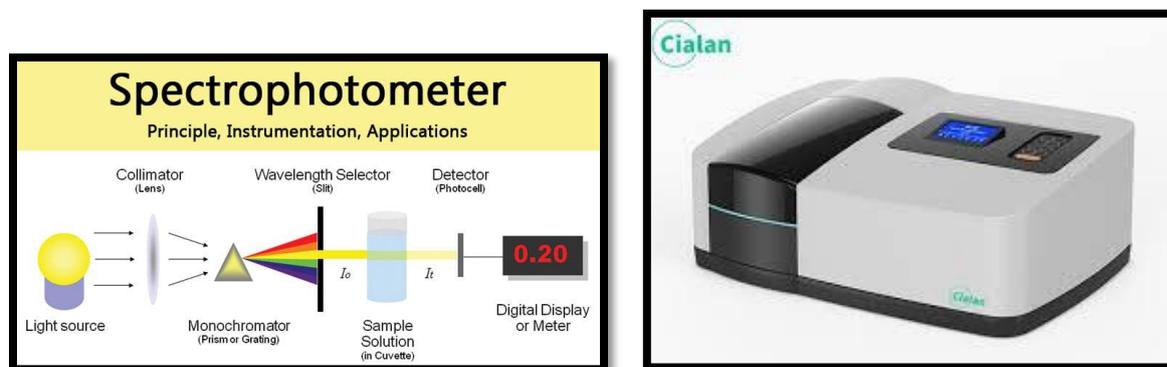**E (Recorder):** Records the spectrum.

Fig 43. Principle of UV/Vis Spectrophotometer

VII.1. Procedure and Presentation of Spectra

The sample to be analyzed is placed in a cuvette (liquid form) and subjected to monochromatic UV-visible radiation of wavelength λ, which can be either fixed (to measure absorbance at a specific wavelength) or variable (to plot a spectrum).

The instrument compares the intensities of two beams, I_t (transmitted light) and I_0 (incident light), and generates the UV-vis spectrum: $A = f(\lambda)$. It provides absorbance values and plots a graph.
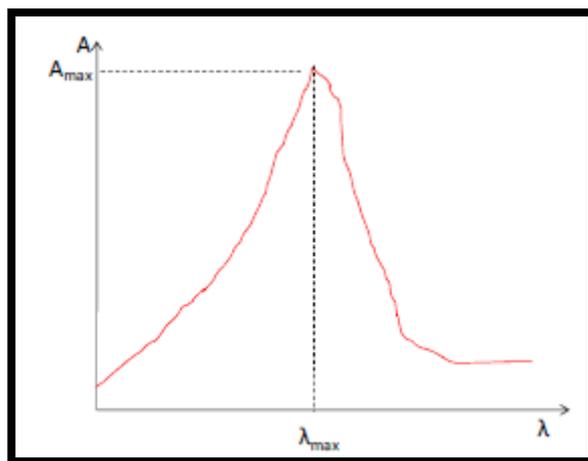
Fig 44. Diagram of determining λ_{max}

A UV-vis absorption spectrum is a graph with:

    X-axis: Wavelength (λ).

    Y-axis: Absorbance (A).

The spectrum may show one or more absorption bands, each characterized by:

- The wavelength at the peak maximum (λ_{max}).
- The molar absorptivity coefficient (ε_{max}) at the absorbance maximum (Amax).

VIII. Applications

VIII.1. Qualitative and Quantitative Analysis

VIII.1.1. Qualitative Analysis

UV-vis spectra typically provide limited information about the molecular structure of compounds.

VIII.1.2. Quantitative Analysis (Quantification)

UV-vis spectroscopy is extensively used for quantitative analysis due to the Beer-Lambert law:

"The concentration of an analyte can be determined by measuring its absorbance."

Two scenarios are possible:

a) The analyte absorbs in the UV-vis range.

b) The Analyte does not absorb in the UV-vis range. In this case, it can be reacted with a chromophore reagent to form an absorbing product. (This derivatization reaction must be quantitative).

Remarks

   UV-visible spectra often provide insufficient information about the compound's structure and are mostly obtained in the liquid phase (occasionally in the gas phase).

 Solvent: The choice of solvent is crucial and must:
   - Be inert with respect to the solute (or compound being analyzed).
   - Be transparent at the wavelength used.

Examples:
   - **Distilled water**: Transparent above **200 nm**.
   - **CH₃Cl**: Transparent above **230 nm**.

The solvent must be free of impurities before use.

IX. UV-Visible spectrophotometric study

IX.1. Interpretation of a UV-Visible absorption spectrum

   Interpreting a **UV-Visible** absorption spectrum involves analyzing the key features of the spectrum to gather information about the compound being studied. Here's a breakdown of how to interpret a UV-Visible absorption spectrum:

1. Wavelength (λ) vs. Absorbance (A)

   The spectrum typically plots absorbance (**A**) on the vertical axis and wavelength (λ) on the horizontal axis. The x-axis represents the wavelength of light passing through the sample, while the **y-axis** shows how much light is absorbed by the sample at each wavelength.

2. Absorption Peaks or Bands
   - **Absorption Bands:** These are broad features on the spectrum where absorbance increases. Absorption bands result from electronic transitions between molecular orbitals.
   - **Peaks:** Narrow peaks can also appear within the bands, but in many organic compounds, especially those with conjugated systems, the absorption appears as broad bands.

The location of these bands provides information about the electronic structure of the compound.

3. λ$_{max}$ (Wavelength of Maximum Absorption)

The **λmax** is the wavelength at which the absorbance reaches its highest point. This is a key value because it indicates the energy required for the transition in the molecule.

- For molecules with conjugated double bonds (like in aromatic compounds or compounds with extended **π-systems**), λmax typically appears in the **UV-Visible** region.
- The **λ$_{max}$** is sensitive to the molecular structure, especially the size and conjugation of the **π-system**.

4. Absorbance (A) and Beer-Lambert Law:

Absorbance is related to the concentration of the absorbing species and the path length through the sample, as described by the **Beer-Lambert law**:

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

High absorbance at a given wavelength suggests a high concentration of the absorbing species, whereas low absorbance suggests a low concentration or weak absorbance at that wavelength.

5. Electronic Transitions:

The UV-Visible region corresponds to electronic transitions, primarily from the ground state to excited states.

Charge Transfer Transitions (CT): Occur when an electron is transferred from one part of the molecule to another, often seen in complexes or molecules with metal ions.

6. Conjugation and Absorption:

- **Effect of Conjugation:** The more conjugated a system is (i.e., the more alternating double bonds), the lower the energy required for an electronic transition, which results in a longer **λ$_{max}$** (shifting towards the visible or lower **UV** region).
- **Effect of Substituents:** Substituents on an aromatic ring, for example, can either donate or withdraw electron density, which can shift the absorption bands (bathochromic shift for electron-donating groups, hypsochromic shift for electron-withdrawing groups).

7. Intensity of Absorption:

The intensity (height) of absorption peaks provides insight into the probability of a transition. Larger peaks indicate stronger absorption at that particular wavelength, which may relate to the concentration of the absorbing species or the molar extinction coefficient (ε).

8. Analysis of Broad vs. Narrow Bands

- **Broad Bands:** These often indicate that multiple transitions are taking place within a narrow energy range. Broad bands are typically associated with vibrational and rotational transitions happening along with the main electronic transition.
- **Narrow Peaks:** These indicate a more discrete transition between specific energy levels, which is often observed in simpler molecules or isolated electronic transitions.

9. Comparison with Reference Spectra

The spectrum of an unknown compound can be compared with known reference spectra to identify characteristic absorption bands. This is particularly useful in identifying functional groups, conjugated systems, or specific chromophores in the compound.

10. Solvent Effects:

The choice of solvent can influence the position of absorption bands. Solvents with different polarities can affect the energy levels of the electronic states, causing a shift in the absorption spectrum (called **solvatochromism**).

Example:

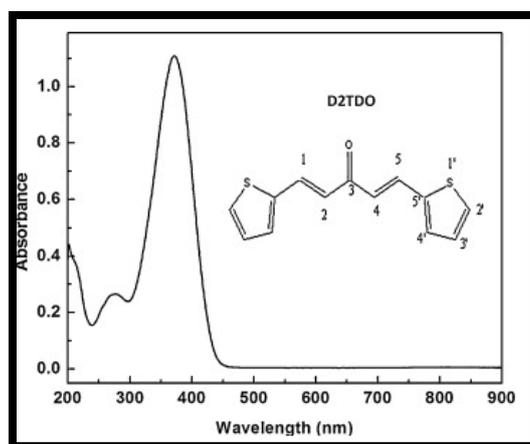For a compound like **D2TDO**, the UV-Visible spectrum will show:

Fig 45. UV-visible spectrum of (**D2TDO**) carried out at **25 °C** in acetonitrile

- A broad absorption band in the **UV** region, with a λ_{max} at around **360 nm**.
- The broadness of the band suggests that several closely related electronic transitions are taking place.
- If the spectrum is recorded in a solvent like acetonitrile, the observed λmax may shift compared to other solvents, reflecting the solvent's effect on the compound's electronic structure.