

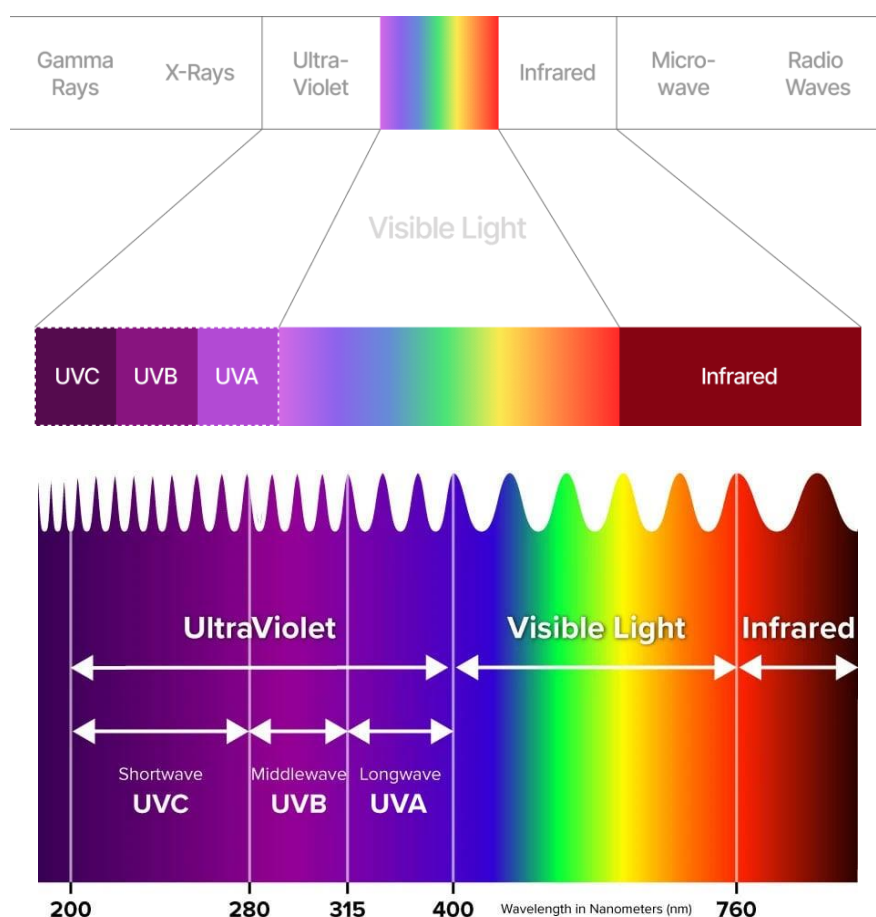
UV-Vis. Molecular Spectroscopy

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, making it valuable for pharmaceutical uses (e.g., determining active pharmaceutical ingredients).

II. Energy Domain

The **UV-Vis** range extends approximately from **800 to 10 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.

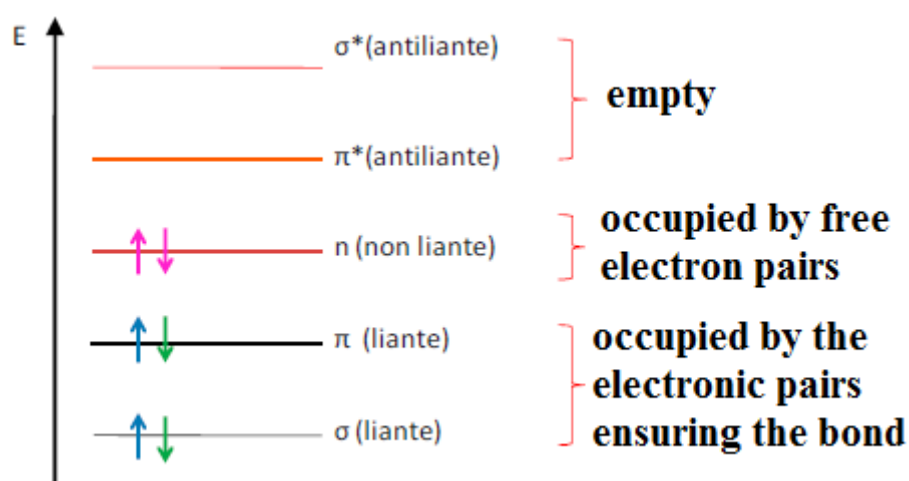


II.1. UV Absorption Properties

- **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.



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 $\sigma \rightarrow \sigma^*$

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 $n \rightarrow \sigma^*$

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 $\pi \rightarrow \pi^*$

This type of transition occurs in molecules with double bonds. It results in an absorption band of high intensity (165-200 nm). The $\pi \rightarrow \pi^*$ 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., $\text{C}=\text{C}-\text{C}=\text{C}$ systems).

IV.4. Transition $n \rightarrow \pi^*$

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 $\text{C}=\text{O}$, $\text{N}=\text{O}$, $\text{N}=\text{N}$, etc. This transition has a low absorption coefficient ϵ ($\epsilon_{\text{max}} < 100$) and is referred to as an R-band, regardless of the molecule type.

Example: Carbonyl group ($\text{C}=\text{O}$).

Study Example: Represent the $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, and $n \rightarrow \pi^*$ transitions for the following molecules: CH_4 , CH_3Cl , C_2H_4 , and $\text{H}_2\text{C}=\text{O}$.

Remarks

The $\sigma \rightarrow \sigma^*$ and $n \rightarrow \sigma^*$ transitions are not practical for technical reasons.

The $n \rightarrow \pi^*$ 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 $\pi \rightarrow \pi^*$ transition for the following reasons:

- The absorption during the transition is relatively high (high sensitivity).
- 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
$\sigma \rightarrow \sigma^*$	Highest ↑ Lowest
$n \rightarrow \sigma^*$	
$\pi \rightarrow \pi^*$	
$n \rightarrow \pi^*$	

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		Color	Perceived Complementary (Subtraction) Color
Wavelength (nm)			
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 ϵ_{\max} (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	$\lambda_{\max}(\text{nm})$	$\epsilon_{\max} (\text{l.mol}^{-1}.\text{cm}^{-1})$
CH ₄	122 ($\sigma \rightarrow \sigma^*$)	strong
C ₂ H ₄	170 ($\pi \rightarrow \pi^*$)	15000
C ₆ H ₆	200 ($\pi \rightarrow \pi^*$)	8000
CH ₃ Br	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. Properties of Transitions

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 include **-OH**, **-NH₂**, **-NO₂**, 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)

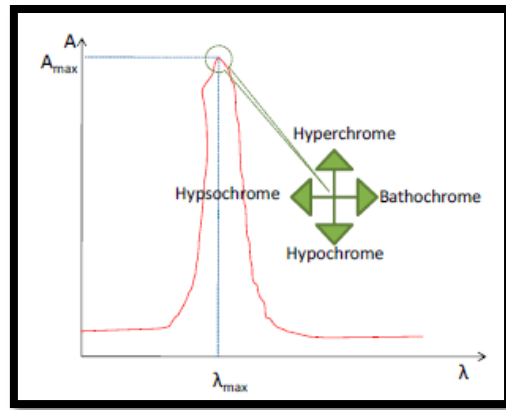
This effect refers to the shift of absorption bands toward shorter (lower) wavelengths.

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.



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).
- **$\varepsilon(\lambda)$** : Molar absorptivity coefficient (characterizes the absorption intensity of the species at λ_{\max}).
- **A**: Absorbance or optical density **D₀**.

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

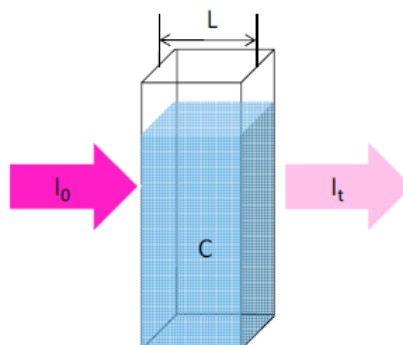
Note: Relationship Between Absorbance and Transmittance (T)

Transmittance is often expressed as a percentage:

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

Where:

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



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

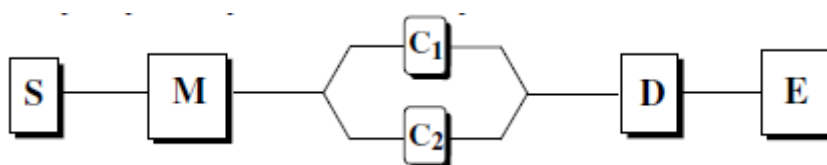
If a mixture contains substances C_1, C_2, \dots, C_n and is exposed to monochromatic radiation, the total optical density (absorbance) of the mixture is equal to the sum of the partial optical densities (absorbances) contributed by each individual substance:

$$(D_0)_T = (D_0)_1 + (D_0)_2 + (D_0)_3 + \dots + (D_0)_n$$

$$(D_0)_T = \epsilon_1 C_1 l_1 + \epsilon_2 C_2 l_2 + \epsilon_3 C_3 l_3 + \dots + \epsilon_n C_n l_n$$

VII. Instrumentation

The schematic principle of a UV spectrometer is as follows:



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

M (Monochromator): 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.

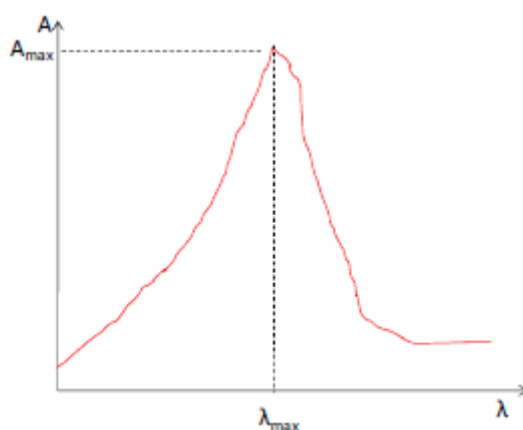
D (Detector): Converts radiant energy into measurable electric current via the photoelectric effect.

E (Recorder): Records the spectrum.

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.



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 (A_{max}).

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 chromophoric 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.