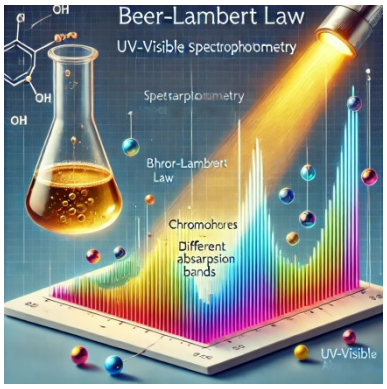
Chapter 2:
UV-Visible Absorption Spectroscopy



1. Introduction:

Light absorption by matter is a fundamental phenomenon in spectrophotometry, widely used in chemical analysis for the characterization and quantification of substances. This chapter explores the fundamental principles governing light absorption, highlighting the Beer-Lambert law, the pillar of UV-Visible spectrophotometry.

We will begin with a study of the general principles of absorption and the different interactions between light and matter, before examining the spectral domains concerned. Particular attention will be paid to chromophores, these functional groups responsible for absorption in the UV-Visible, which make it possible to identify and analyze many molecules of interest.

Finally, we will discuss the analytical applications of the Beer-Lambert law, highlighting its central role in determining the concentrations of colored or transparent solutions in the laboratory. This chapter will thus provide the essential foundations for an effective use of UV-Visible spectrophotometer in quantitative analysis.

2. Spectral domain

The UV-visible range extends from approximately 800 to 10 nm:

- Visible: 800 nm (red) - 400 nm (indigo)
- Near-UV: 400 nm - 200 nm
- Far UV: 200 nm - 10 nm.

2. Principle of UV-Visible Spectroscopy

UV-Visible spectroscopy is an analytical technique based on the absorption of light in the ultraviolet (UV) and visible wavelength range by a substance in solution. This absorption provides information on the concentration and electronic structure of molecules.

2.1 Steps of the UV-Visible Analysis Process

(a) Sample preparation

- The sample is usually a solution containing the compound to be analyzed.
- It must be dissolved in a solvent transparent to the wavelengths used (example: water, ethanol, acetone).
- In some cases, complexing agents or coloring reagents are added to enhance absorption.

(b) Choice of wavelength

- Each molecule has a characteristic absorption at certain wavelengths, defined by its chromophores.
- Absorption is measured in the **UV (200-400 nm)** and **visible (400-800 nm) region** .

(c) Passage of light through the sample

- monochromatic light (of a specific wavelength) passes through the sample.
- The sample absorbs part of this light according to the Beer-Lambert law:

$$A = \varepsilon \times C \times l$$

Or :

- A is the absorbance,
- ε is the molar absorption coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$),
- c is the concentration of the sample (mol / L),
- l is the length of the tank (in cm, usually 1 cm).

(d) Detection and obtaining the spectrum

- A detector measures the intensity of the transmitted light after absorption.
- Absorbance is recorded as a function of wavelength.
- We obtain a **UV-Visible absorption spectrum** , which is a curve representing A as a function of λ.

3. Interpretation of Results

3.1. UV-Visible Spectrum

- **Position of the absorption peak** : It provides information on the electronic structure and the presence of chromophores (e.g. conjugated double bonds, aromatic rings).
- **Absorption intensity** : The stronger the absorbance, the higher the concentration.

3.2. Type of analysis: Quantitative or Qualitative?

(a) **Quantitative method**

- Since absorbance is proportional to concentration (Beer-Lambert law), this technique is widely used to determine the concentration of a substance in solution.
- Example: Determination of the concentration of proteins, DNA, dyes.

(b) Qualitative method

- UV-Visible also allows certain substances to be identified thanks to specific absorption wavelengths.
- Example: Identification of chromophores in an organic molecule.

Conclusion

UV-Visible spectroscopy is a **quantitative and qualitative technique** that allows both the measurement of the concentration of a substance and the obtaining of information on its molecular structure.

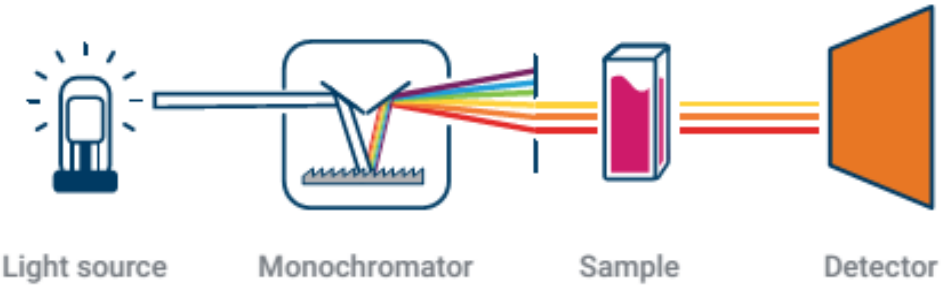


Fig. 1: Schematic of the main components in UV-vis spectrophotometer

4. Molecular orbitals (MO)

Valence electrons (the electrons involved in bonding) are usually found in one of three types of orbitals:

- σ orbital (ensuring single bonds);
- π orbital (ensuring double or triple bonds);
- Non-bonding n orbital (loose electron pairs).

(a) Bonding and antibonding σ orbital (σ and σ*)

Formed from two s-type atomic orbitals (AOs) or from one s-type AO and one p-type AO, or from two p-type AOs (Pz) having their axes of symmetry collinear and in this case it is called σz.

(b) Bonding and antibonding π orbital (π and π*)

Formed from two p-type OAs with side overlap. This type of orbital occurs in double or triple bonds.

(c) n orbital (non-bonding)

Located around a heteroatom like O, N, S... This type of orbital has an almost atomic character (p-type).

4.1 Energy order of molecular orbitals

According to quantum mechanics, the energy of molecular orbitals is quantized. Each orbital is defined by its own energy (energy level) which differentiates it from others (**see Fig. 2).**

- The σ molecular orbital is the least energetic orbital (electrons belonging to this type of orbital are in a stable state).
- The σ* molecular orbital is the most energetic orbital.

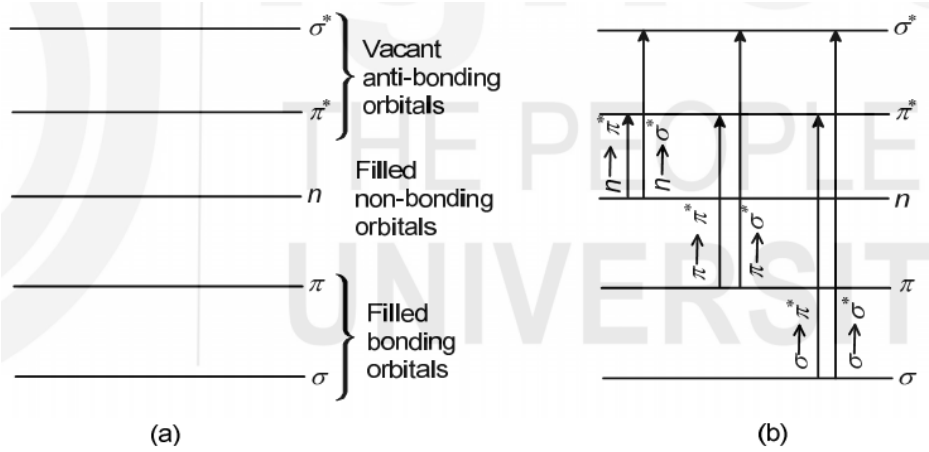


Fig.2 : Schematic diagram of (a) order of molecular orbital energies and (b) possible electronic transition

5. Different types of electronic transitions

Electronic transitions correspond to the passage of electrons from filled bonding or nonbonding molecular orbitals to unfilled antibonding molecular orbitals:

Electronic transitions are allowed if $\Delta l = \pm 1$ and $\Delta S = 0$, that is, there is a transition between orbitals of the same spin and different symmetry.

The allowed transitions are:

(σ ------ σ*)

(n-------σ*)

(n-------π*)

(π-------π*)

The electrons that participate in the formation of a bond between atoms are the σ and π electrons. And the electrons of the non-bonding pairs are the n electrons.

The absorption of a photon in the UV-visible range corresponds to electrons belonging to small groups of atoms called **chromophores** (C=C, C=O, C=N, C ≡C, C ≡N…). The absorption wavelength depends on the nature of the orbitals involved.

5.1 σ → σ* Transition The high stability of σ bonds in organic compounds means that the transition of an electron from a σ-bonding OM to an σ*-antibonding OM requires a lot of energy. The corresponding band is intense and located in the far-UV, around 130 nm.

5. 2 n →π* Transition This transition results from the passage of an electron from a non-bonding OM n to an anti-bonding OM π*. This type of transition occurs in the case of molecules containing a heteroatom carrying free electron pairs belonging to an unsaturated system. The corresponding band is weak because the transition is forbidden.

These are weak transitions, encountered in the case of molecules containing an atom with a non-bonding doublet belonging to an unsaturated system. The absorption coefficient is between 10 and 100 $L.mol^{-1}.cm^{-1}$.

5. 3 n → σ* transition The transfer of an electron from the n doublet of a heteroatom (O, N, S, Cl..) to a σ * **level** is observed for alcohols, ethers, amines as well as for halogenated derivatives. This transition gives a band of average intensity which is located at the extreme limit of the near-UV.

They correspond to wavelengths between 150 and 250 nm. The absorption coefficient varies from 100 to 5000 $L.mol^{-1}.cm^{-1}$. This transition is located around 180 nm for alcohols, 190 nm for ethers and 220 nm for amines.

5.4- π →π* Transition The electronic transition in compounds having an isolated double bond leads to a strong absorption band around 165-200 nm. These transitions are strong with an absorption coefficient ranging from 1000 to 10000 $L.mol^{-1}.cm^{-1}$.

5.5 d → d Transition This type of transition does not belong to the purely molecular transitions mentioned above. Such transitions occur in certain atoms of transition metals or rare earths. In fact, when such atoms are in the presence of a crystalline field (created by a complexing agent), the 5 d orbitals are therefore not degenerate and transitions can occur within the same d orbital.

5.6. Transition due to charge transfer: The transfer of electrons from a donor to an acceptor requires energy which is manifested by the absorption of a quantum of energy, like the transitions which occur between a metal (atom) and a ligand in a complex. Two cases are considered:

- Metal to ligand electron transfer (MLCT)
- Ligand to metal electron transfer (LMCT)

6. Certain terms and definitions:

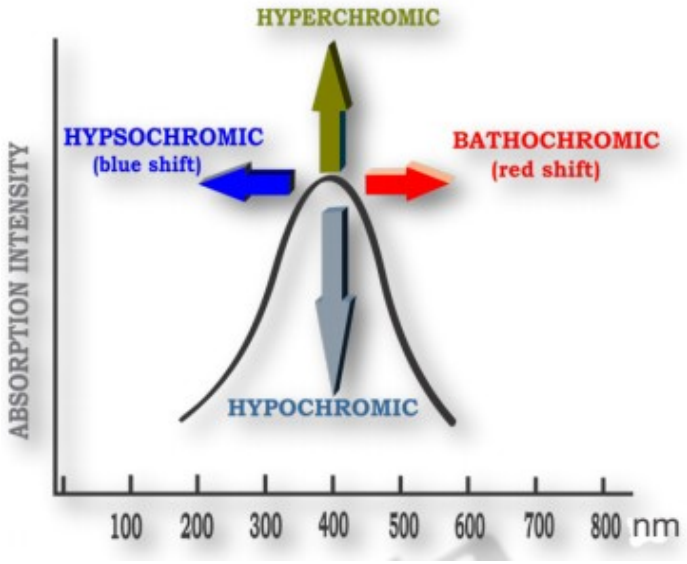


Fig.3: Effect of substances on the position and intensity of an absorption band

- **Chromophore group**: An unsaturated functional group such as C=C, C=O, C≡C, C≡N, N=N. Otherwise, a chromophore is the part of the molecule that contains the electrons involved in a transition giving rise to an absorption.

- **Auxochrome group :** An auxochrome group does not absorb light by itself, unless it is attached to a chromophore group. In this case, it can modify either the position of the absorption (λmax) or the intensity of the absorption (I max). This results from the interactions that are established between the free electron pairs carried by an atom of the auxochrome group (N, O, S...) and the electrons forming the π bond of the chromophore group (generally increasing the resonance). As auxochrome groups, we find -OH, $-NH_2$, $-NO_2$.

(Saturated group linked to a chromophore → modifies wavelength and intensity of absorption).

- **Bathochromic effect:** Shift of absorption bands towards long wavelengths.
- **Hypsochromic effect** Shift of absorption bands towards short wavelengths.
- **Hyperchromic effect** Increase in absorption intensity.
- **Hypochromic effect** Decrease in absorption intensity.

7. Effect of environment on UV-vis absorption

The environment is understood to be everything that surrounds the chromophore group or everything that surrounds the molecule itself. Therefore, we can consider two types of environment:

- Intrinsic environment (conjugation, substitution, etc.);
- Extrinsic environment (nature of the solvent, etc.).

7.1 Effect of conjugation: The increase in conjugation causes a bathochromic effect. Indeed, the delocalization of electrons reflects the ease of these electrons to move along the molecule, and it is accompanied by a rapprochement of the energy levels.

7.2 Effect of substitution: The donor inductive effect causes a bathochromic effect, this is the case of the presence of alkyl groups on the double bonds .

7.3 Effect of the solvent

UV-vis analysis of a compound with different solvents of different polarity often results in a difference in intensity, position of absorption maximum and shape of the absorption band. High purity, non-polar solvents such as saturated hydrocarbons do not interact with the solute molecules either in their ground or excited states and the absorption spectrum of a compound in such solvents is similar to that recorded in the gaseous state. However, polar solvents such as water, alcohols etc. can stabilize or destabilize the molecular orbitals of a molecule either in their ground or excited states and the spectrum of a compound in these solvents can vary considerably from that recorded in a non-polar solvent (**see Fig. 4**).

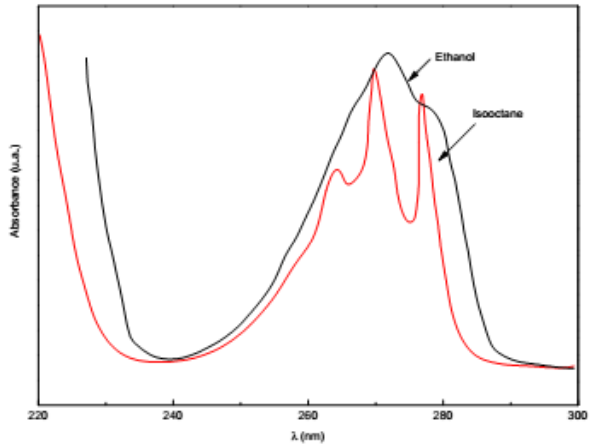


Fig. 4: Effect of the solvent

The molar absorption for an n→π* transition is quite low (10 to 100 mol/l.cm) and the energy required for the transition is affected by the polarity of the solvent. In the presence of a polar solvent, nonbonding electrons interact with polar solvents to form hydrogen bonds. Solvation of the n electrons reduces their energy (energy of the n orbitals). A partial

stabilization of the polar π* orbital is also observed, but to a lesser extent than the n electrons. Therefore, a net increase in the energy required for an n→π* transition is thus observed in polar solvents, such as in water or alcohols. An increase in energy reflects a decrease in the wavelength of the transition, or what is called a hypsochromic shift or blue shift as shown in **Fig. 5**.

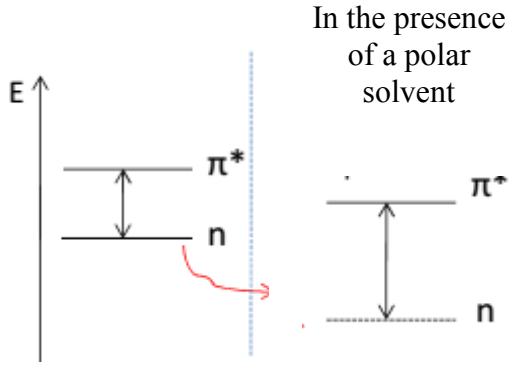


Fig.5: Effect of solvent polarity on the transition n - π^*

8. Beer-Lambert law (absorbance and concentration)

When light hits a homogeneous medium of length l (optical path), part of this incident light, denoted $\mathbf{I_0}$, is absorbed by the medium and the rest, denoted I , is transmitted. The fraction of incident light absorbed by a substance of concentration C contained in a tank of length l is given by the Beer-Lambert law:

$$\mathbf{A = \log(I_0/I) = \varepsilon \times l \times C}$$

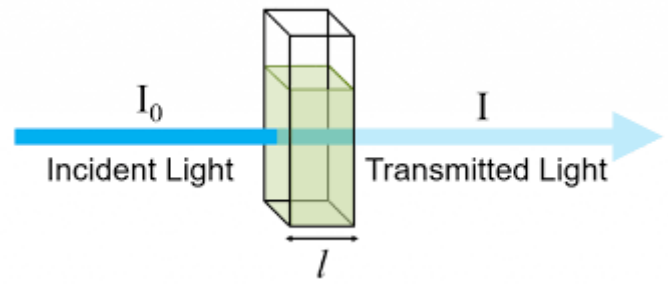


Fig.6 : Beer-Lambert law principle

- **A:** absorbance formerly called optical density (OD)
- l: thickness of the tank expressed in centimeters
- **ε**: extinction coefficient . This is a characteristic quantity of the compound. If the concentration is in grams per liter, ε is called the specific extinction coefficient. If the concentration is in moles per liter, ε is called the molar extinction coefficient.

Transmission **T is also defined** as the ratio of transmitted intensity to incident intensity: $\mathbf{T = I / I_0}$

$$\mathbf{\log (1/T) = A}$$

The percentage of transmission (%T) is the **transmittance.**

8.1 Validity of Beer-Lambert's law

- Monochromatic light

- Low concentrations
- The solution must be neither fluorescent nor heterogeneous (bubbles, precipitate, etc.)
- The solution is not the seat of a photochemical reaction.

8.2 Additivity of absorbances

The absorbance A of a mixture of n absorbing species is the sum of the absorbances of each species (the individual absorbances (Ai) are additive). This is valid if there are no interactions between the species in the mixture to be analyzed.

$$A = \sum_{i=1}^{n} A_i = \sum_{i=1}^{n} (\varepsilon_i \times l \times c_i)$$

For this, we measure the absorbance of the solution at different wavelengths (at λmax of each species).

9. Determination of the concentration of a solution by calibration:

From **Beer Lambert 's law** , it is possible to determine the concentration of a species by measuring its absorbance. To do this, we can follow the following experimental protocol:

- We determine the wavelength corresponding to the absorption maximum λmax.
- A series of solutions at different concentrations ci are prepared, and the absorbance A_i of each of these solutions at λmax is measured.
- We plot the calibration curve $A_i=f(c_i)$.
- We measure the absorbance A of our solution of unknown concentration at λmax. From the curve we can read the concentration c of our solution of absorbance A.

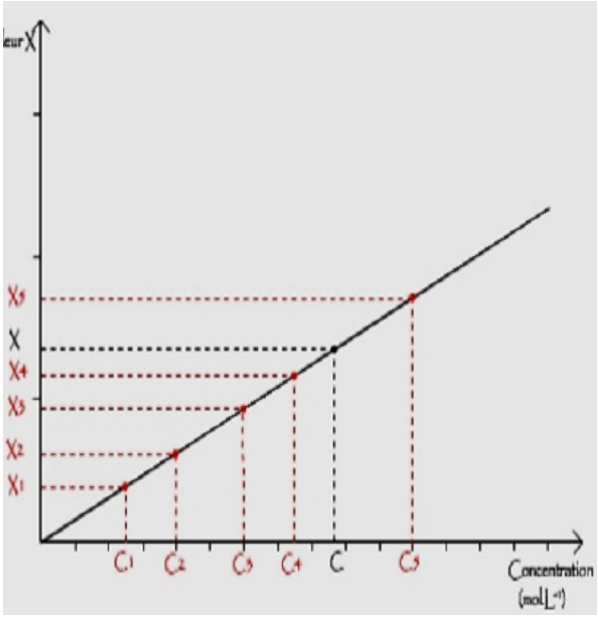


Fig.7: Calibration curve

Notes :

- A fluorescent solution is a solution containing molecules capable of absorbing light at a certain wavelength and re-emitting part of this energy in the form of fluorescence. Examples **:**

✓ **Fluorescein** : Used in medicine for eye examinations.

✓ **Rhodamine B** : fluorescent dye used in biochemistry.

✓ **Quinine** : present in tonic water, emits blue fluorescence under UV.

- A photochemical reaction is a chemical reaction initiated by the absorption of light. It often involves the formation of excited states followed by chemical transformations. Examples **:**

✓ **Photosynthesis** : conversion of solar energy into chemical energy by plants.

✓ **Photodegradation of polymers** : degradation of plastics under the effect of UV.

✓ **Formation of ozone in the atmosphere**: dissociation of O_2 under UV and recombination into O_3.

10. Energy transitions

The frequency resulting from the passage of the molecule from the energy level E_i to the higher energy level E_f by absorbing the radiation is given by:

$$h\upsilon_{i-f} = E_f - E_i \qquad E_f > E_i$$

The states E_i and E_f are characteristic of a level of:

- Rotation;
- Vibration;
- Electronics.

Three types of transitions can be considered:

a) Only the rotation quantum number J varies (this is a pure rotation transition, its frequency is in the microwaves or far infrared ;

b) The vibration quantum number V can also vary (this is a vibrational transition. Its frequency is in the infrared;

c) The electronic quantum number can also vary (this is then an electronic transition). Its frequency is in the visible or ultraviolet range .

Its energy levels E_f can be populated without the input of photonic energies (electric discharge, temperature, etc.): the return to the equilibrium state will be done by the emission of a photon h_{i-f}; this is emission spectroscopy.

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