Lecture 1 Introduction to Spectroscopy Dr. Ruba Fahmi Abbas

Spectroscopy is concerned with studying the **interaction** between **matter** and **electromagnetic radiation**, which ranges from **highly energetic gamma rays** to **very low energetic radio waves**, passing through **X-rays**, **microwaves**, **ultraviolet (UV)**, **visible**, and **infrared (IR) radiation**.

Term	Define	Example
Spectroscopy	The **general field of science** dedicated to **studying** how energy and matter interact.	**HNMR Spectroscopy** studies molecular structure using radio waves and a magnetic field.
Spectrometry	The **practical process of measurement** used to obtain quantifiable data from a spectrum.	**Mass Spectrometry (MS)** is the technique used to measure the mass-to-charge ratio of molecular fragments.
Spectrometer	The **physical instrument or device** that separates and measures the components of a spectrum.	A **UV-Vis Spectrometer** is the lab machine that measures how much light a sample absorbs.

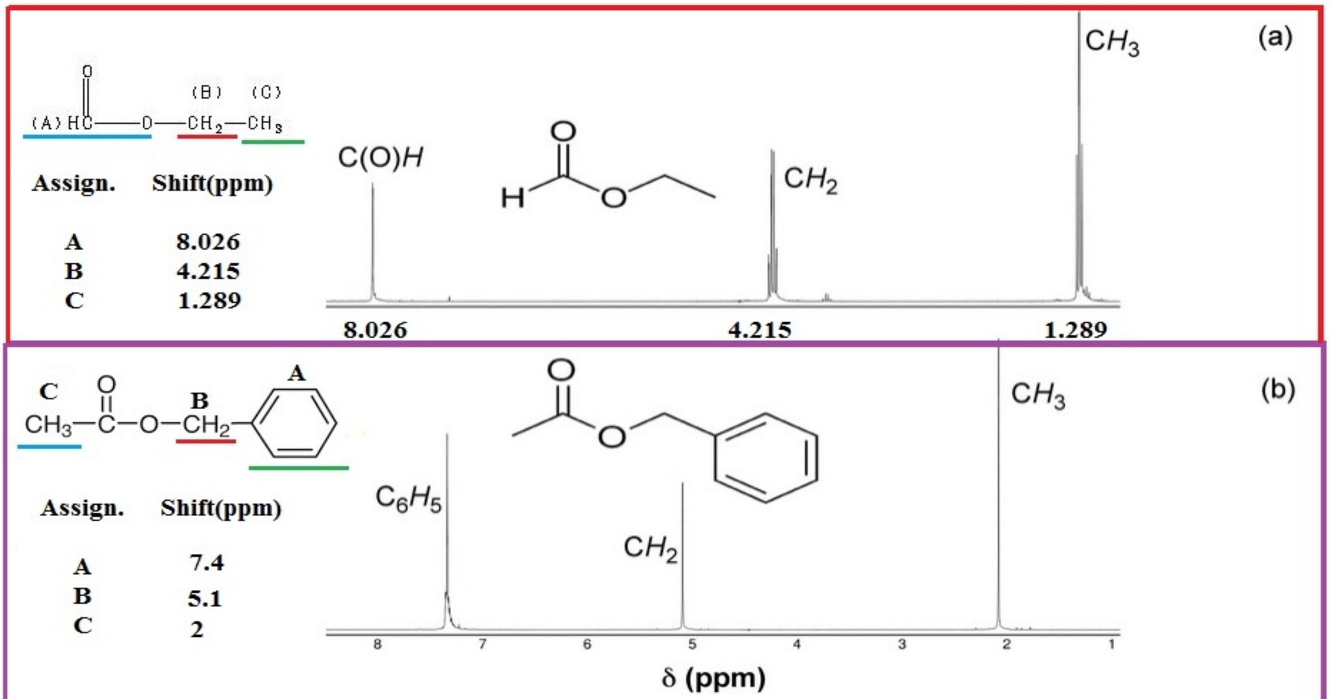


Figure \ ^{1}H NMR spectra of (a) ethyl formate and (b) benzyl acetate.

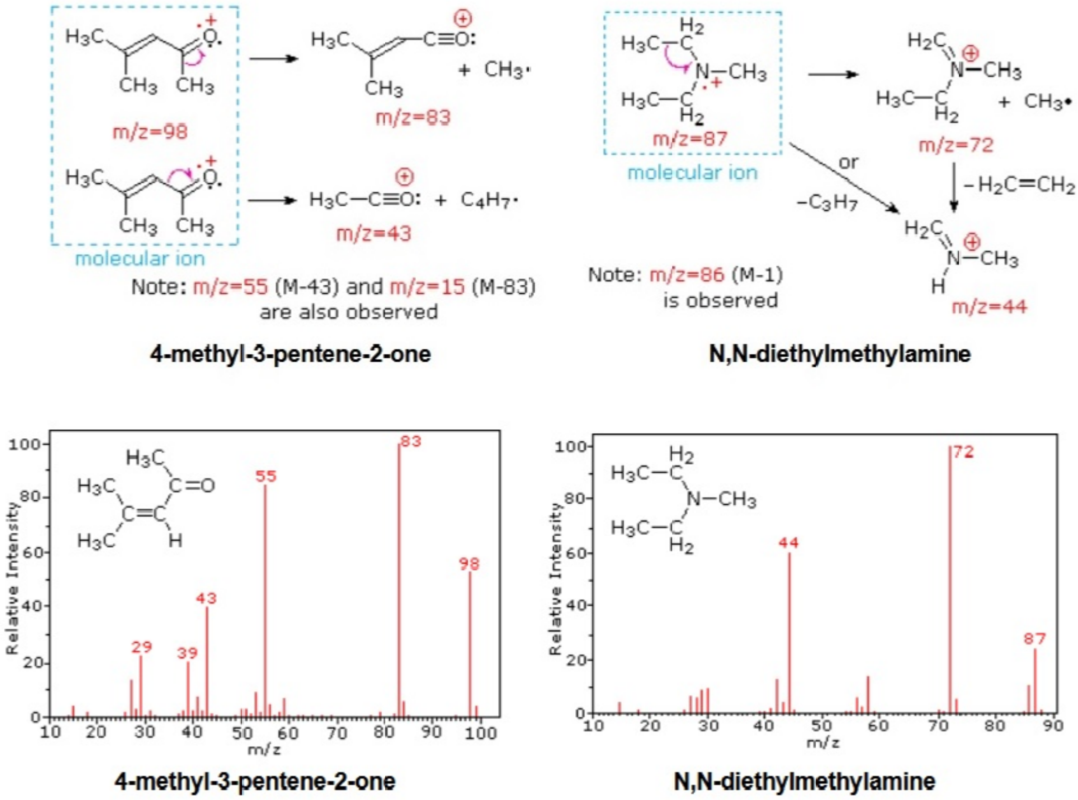


Figure 2/ Mass Spectrometry (MS)(GC-Mass) spectrum

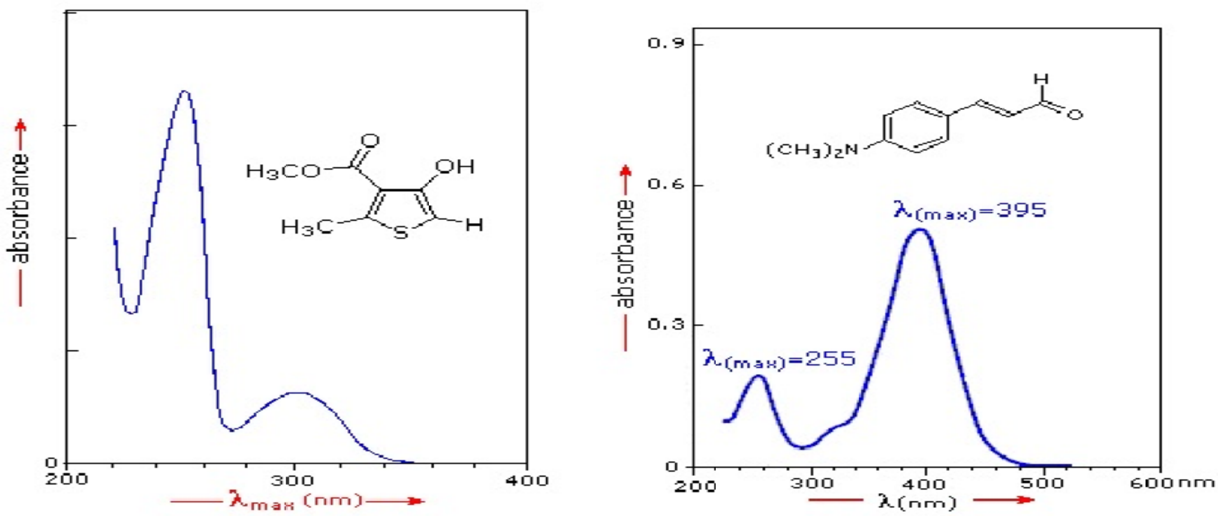


Figure 3/ spectrum of UV-Vis Spectrometer

Electromagnetic Radiation (EMR): EMR is a form of energy that behaves both as a wave (having wavelength λ and frequency v) and as a particle (**photon**). The energy (E) of a photon is directly proportional to its frequency, defined by the Planck-Einstein relation:

E=h×v

where h is Planck's constant ≈ 6.626×10^{-34} J·s (Joule.seconds).

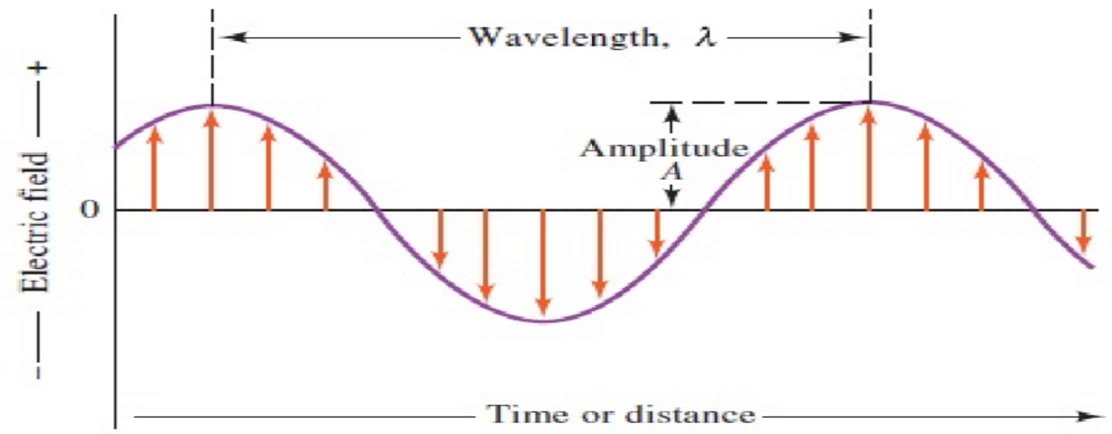


Figure 4/ Wave nature of a beam of single frequency electromagnetic radiation

Wavelength (λ) is the distance between two consecutive peaks. Since wavelength is a distance, its SI unit is the meter (m). In spectroscopy, it is often measured in nanometers (nm), particularly for UV and visible light.

Frequency (v) is the number of waves (*cycles*) per second that pass a given point in space, the standard unit is the Hertz (Hz= s^{-1} or cycles per second).

Period (P) is the time (seconds) required for one complete wave cycle to pass a given point in space.

Amplitude (A) is the maximum distance moved by a point on a wave.

Wave Number $\tilde{\nu}$: is the reciprocal of the wavelength (λ). It is measured by cm^{-1}.

$$\tilde{\nu} = \frac{1}{\lambda}$$

Velocity or Speed:

v (Velocity)	c (Speed of Light)
This is the general symbol for wave speed, and it is used when a wave (or light) is traveling through any **medium** (like air, water, or glass). In a medium, the velocity of light (v) is less than c. SI Unit Meters per second **(m/s)**.	This special symbol is used specifically for the speed of electromagnetic radiation (EM) radiation in a **vacuum**. **≈3.00×10^8 m/s**
السرعة v : هذا هو الرمز العام لسرعة الموجة، ويُستخدم عند انتقال الموجة (أو الضوء) عبر أي وسط (مثل الهواء أو الماء أو الزجاج). في هذا الوسط، تكون سرعة الضوء (v) أقل من c	سرعة الضوء c: يُستخدم هذا الرمز الخاص تحديدًا لسرعة الإشعاع الكهرومغناطيسي في الفراغ ≈ **3.00×10^8 m/s**
$v=\lambda\times v$	**$c=\lambda\times v$**
v =is used if the wave is in a **medium**	C= is used if the wave is in a **vacuum**.

The Direct Relationship with Energy

The fundamental equation linking energy and light is Planck's equation:

$E=h\times v$ .....(1)

Where, frequency (v), wavelength (λ)

$v= \frac{c}{\lambda}$ ....(2)

We can substitute this into Planck's equation:

$E= h\times\frac{c}{\lambda}$

when $\tilde{\nu} = \frac{1}{\lambda}$, then

$E= h\times \tilde{\nu} \times c$

Question: What is the energy (E) of a single photon of green light that has a wavelength (λ) of **510 nm**?

Solution:

$m = 10^9 nm$

$\lambda = \frac{510}{10^9} = 51\times10^{-8}$ m

$E = h\times\frac{c}{\lambda}$

$$\mathbf{E} = \frac{(6.626\times10^{-34}\ \text{J}\cdot\text{s})(3\times10^{8}\ \text{m/s})}{51\times10^{-8}\ \text{m}}, \qquad \mathbf{E = 3.90\times10^{-19}\ J}$$

Question: What is the energy (E) of a single photon of **UV-C light** that has a wavelength (λ) of **254 nm**?

Question: What is the energy (E) of a single photon of Red Light **that has a wavelength (λ) of 700 nm?**

Question: What is the energy (E) of a single photon of Blue Light that has a **wavelength (λ) of 450 nm?**

Question: What is the energy (E) of a radio wave with a frequency (v) of 1.00×10^8 Hz?

Solution:

E=h×v

$E = (6.626\times10^{-34}\ \text{J}\cdot\text{s})\times(1.00\times10^{8}\ \text{s}^{-1})$

$E = 6.63\times10^{-26}$ J

Question: A bond requires 8.0×10^{-19} J of energy to break. What is the longest wavelength of light (λ) that can break this bond?

Solution:

$$E= h\times\frac{c}{\lambda}$$

$$8.0\times10^{-19}\ J = \frac{(6.626\times10^{-34}\ J{\cdot}s)(3\times10^{8}\ m/s)}{\lambda}$$

$$\lambda = 2.485\times10^{-7}\ m$$

$m= 10^{9} nm$

$$\boldsymbol{\lambda = 2.485\times10^{-7} \times10^{9} = 248\ nm}$$

Question: What is the energy (E) of a single X-ray photon that has a **frequency (v) of 3.00×10^{17} Hz**?

Question: What is the energy (E) of a single microwave photon used in a microwave oven, given its common operating **frequency (v) of 2.45×10^{9} Hz?**

Question: Infrared (IR) spectroscopy often measures wavelengths in micrometers (μm). What is the energy (E) of a single photon with a **wavelength (λ) of 10.0 μm (micrometers)?**

1 μm=10^{-6} m

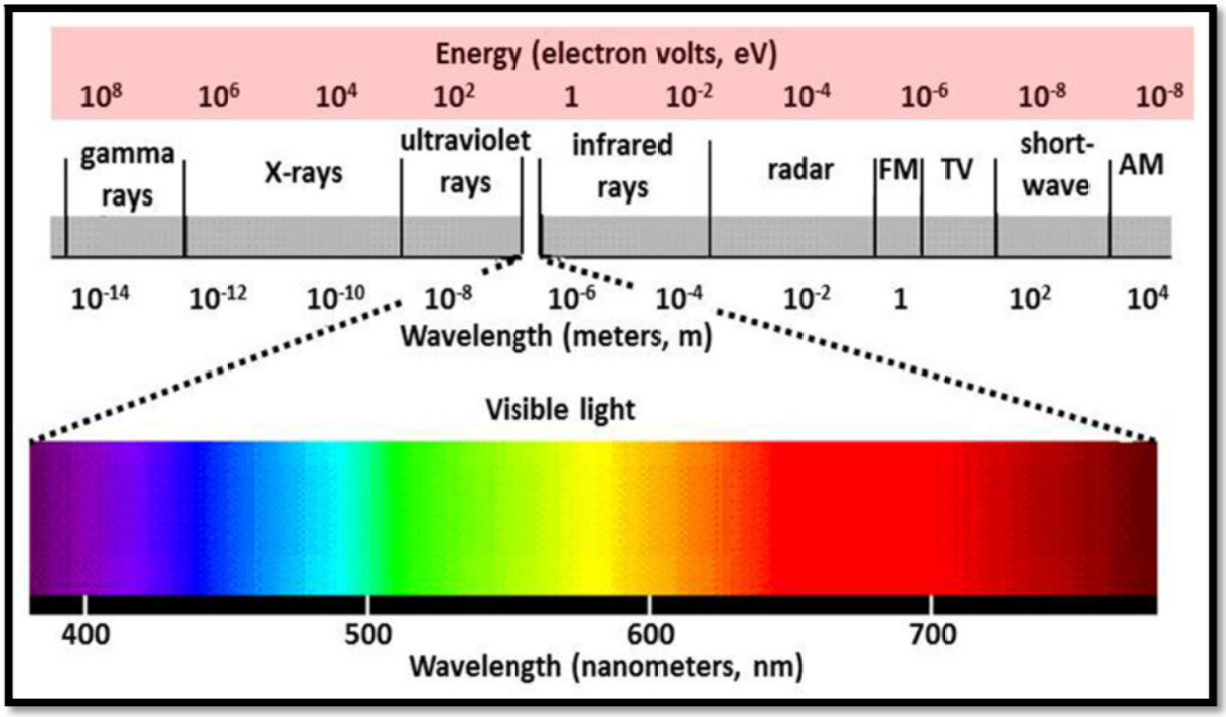
Question: What is the energy (E) of a single Gamma Ray photon that has an extremely high **frequency (v) of 3.00×10^{2} Hz?**

The Electromagnetic Spectrum

The EM spectrum is the full range of all types of electromagnetic radiation, ordered according to their energy, frequency, and wavelength.

- The shorter the wavelength (λ), the greater the energy (E) and the higher the frequency (v). (e.g., Gamma and X-rays).
- The longer the wavelength (λ), the lower the energy (E) and the lower the frequency (v) (e.g., Radio waves).

$$E = h \times \frac{c}{\lambda} \quad , \quad E = h \times v$$



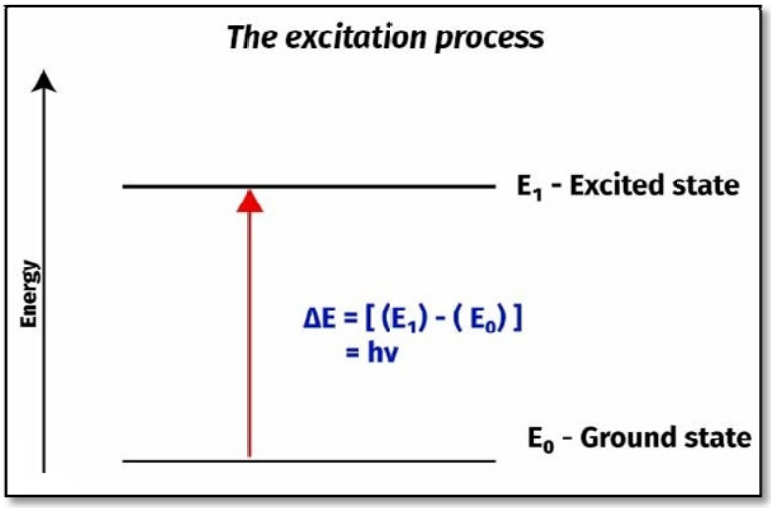
The Spectrum of Electromagnetic Radiation

Fundamentals of UV Visible Spectroscopy

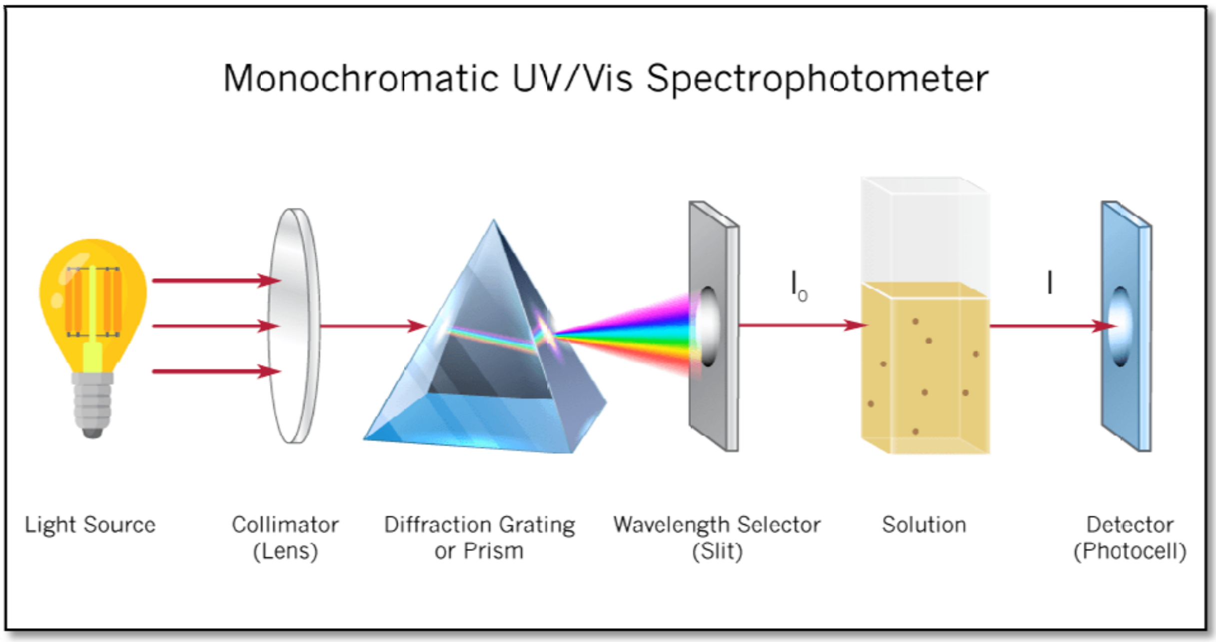
The majority of the organic molecules and functional groups are transparent to ultraviolet (UV) and visible light. UV-visible spectroscopy (ultraviolet-visible spectroscopy) uses this electromagnetic radiation ranges to generate a spectrum and gain other information about molecules. UV-visible spectroscopy is mostly applied in analyzing organic compounds.

The wavelength range of UV	10nm – 400nm
The wavelength range of visible light	400nm - 750nm

So, molecules have the ability to absorb those transmitted radiations from those wavelengths and use them to excite their electrons to a higher energy level from the ground state.

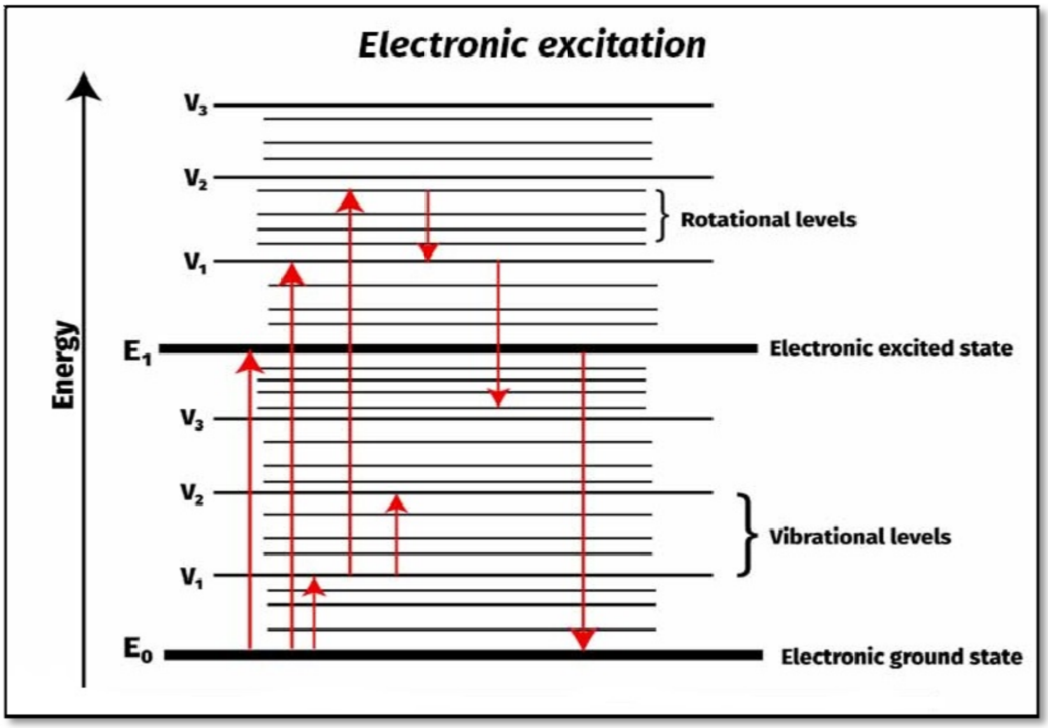


When radiation is transmitted through an organic molecule, a portion of that radiation can be absorbed by the organic molecule. If the transmitted radiation passes through a prism, a spectrum can be observed. This is called as "**Absorption spectrum**".



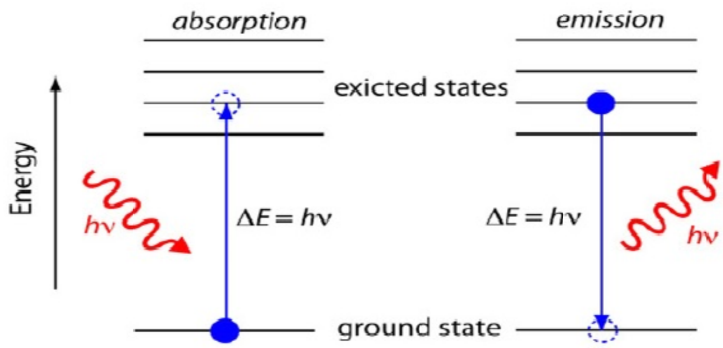
The organic molecules or atoms use that absorbed energy to excite their electrons to an excited state (higher energy level) from the ground state (lower energy level). The energy difference between the ground and the excited states is exactly equal to the amount of radiation absorbed.

In UV-Vis spectroscopy, radiation from ultraviolet and visible light excites electrons of the molecules from a ground state to an excited state. In this process, electrons were excited from the occupied molecular orbital to the unoccupied molecular orbital.



Question What is the difference between absorption and emission?

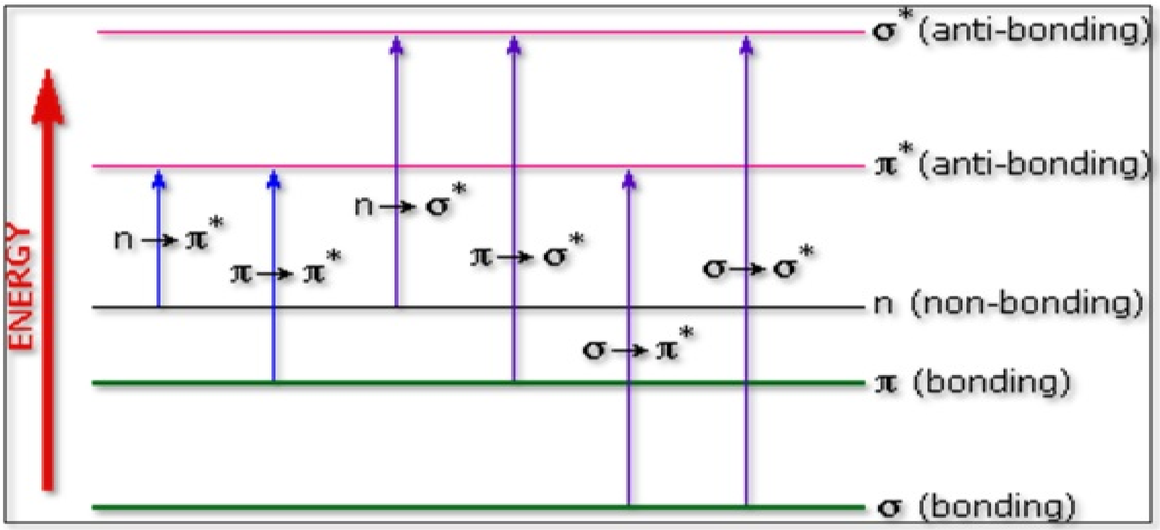
Answer :



In organic molecules, there are three primary types of electrons, which dictate how the molecule interacts with UV/Vis light:

Electron Type	Description & Bond Type	Energy Required for Excitation	UV/Vis Absorption
σ Electrons (σ bonds)	Involved in **single, saturated bonds** (C-C, C-H).	Excitation Energy: The energy required to excite σ bonding electrons is much greater than the energy of UV radiation.	Absorption: saturated compounds do not absorb UV/Vis light in this region. For this reason, saturated compounds are often used as **good solvents** in UV spectroscopy.
π Electrons	Involved in **double or triple bonds** (unsaturated bonds). These compounds typically contain both σ and π bonds.(such as benzene or hexatetraene.)	Moderate energy. (such as benzene or hexatetraene.)	Absorption: These electrons require less energy for excitation and readily absorb UV/Vis light.
n Electrons (Nonbonding)	**ion pairs** on hetero atoms such as Nitrogen (N), Oxygen (O), Sulfur (S), and Halogens in organic compounds.	Moderate energy.	Absorption: Molecules containing n electrons can absorb UV or Visible light.
For a molecule to be analyzed by UV-Vis spectroscopy (or be colored), it must contain **π bonds** (unsaturation) or **n electrons** (lone pairs on N, O, S, etc.), as these electrons require the lower energy of UV/Vis light for excitation.			
UV or Visible light if it contains a heteroatom (like nitrogen, oxygen, halogen, or sulfur) with nonbonding electrons (n) or if it contains an unsaturated bond (π bond). The molecular group responsible for this absorption is called a **chromophore.**			

Electronic Transitions



Electronic Transition Diagram

Electronic transitions in the UV (Ultraviolet) and Visible regions are categorized into **six main types** based on the orbitals involved:

1. **σ→σ***	2. **n→σ***	3. **π→π***
4. **n→π***	5. **π→σ***	6. **σ→π***

The last two transitions, **π→σ*** and **σ→π***, are **theoretically defined but not practically observed**. The remaining four transitions are commonly observed and analyzed in UV/Vis spectroscopy.

1. **σ→σ* Transition :** Present in **all organic compounds** (e.g., alkanes, alkenes, and all other organic molecules).

Energy/Wavelength: This transition involves the highest energy/shortest wavelength, as the σ bond is the most stable.

2. **n→σ* Transition:** Occurs in compounds containing a **heteroatom** (like Oxygen, Nitrogen, Sulfur, or Halogens). These atoms possess non-bonding electrons (n electrons). **(Examples:** Alcohols, amines, alkyl halides, and ethers). These compounds show both **σ→σ*** and n→σ* transitions.

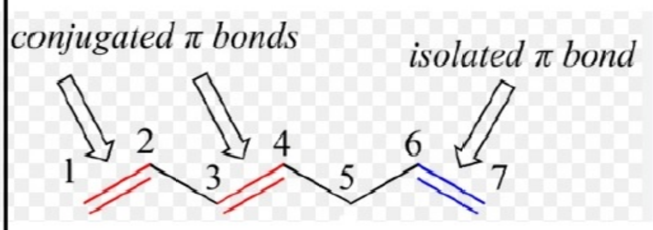
3. π→π* Transition: Occurs in compounds that contain a π bond **(double or triple bonds).** (Examples: **Alkenes, alkynes, carboxylic acids and their derivatives, carbonyl compounds).** These compounds show both **σ→σ*** and **π→π*** transitions.

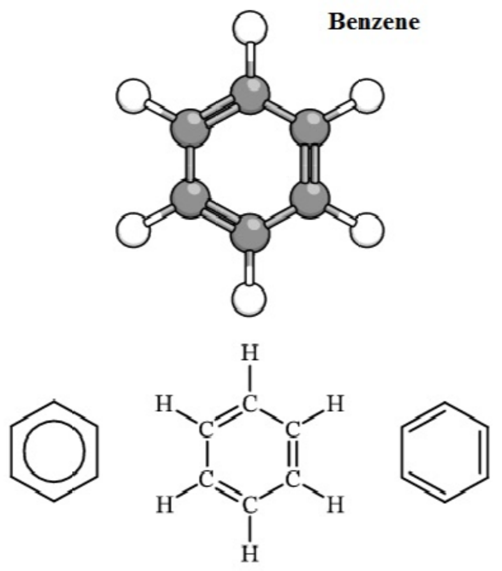
- **K-Band (Conjugation Band):** This band appears due to **π→π*** absorption in **conjugated systems** (alternating single and double bonds), such as butadiene.
- **B-Band (Benzenoid Band):** This **π→π*** absorption is characteristic of **aromatic rings** (like benzene) substituted with chromophoric groups (e.g., styrene, acetophenone).

Benzene Transitions (B-Band)

Benzene has three π molecular orbitals (occupied by six electrons) and three unoccupied π∗ molecular orbitals. The π→π*transitions result in three main absorption bands:

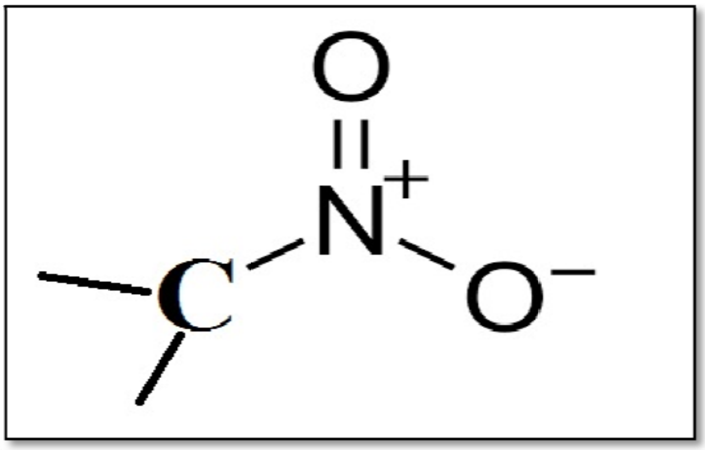
- **Absorption 1 (B-Band):** High intensity at 184 nm.
- **Absorption 2 (2E-Band):** At 204 nm.
- **Absorption 3 (1E-Band):** Appears as a broad absorption band with multiple peaks in the near-UV region, around 230−270 nm.

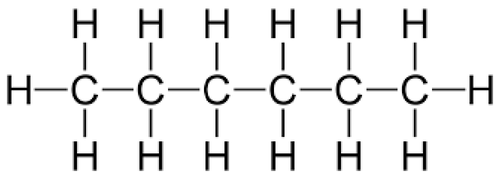
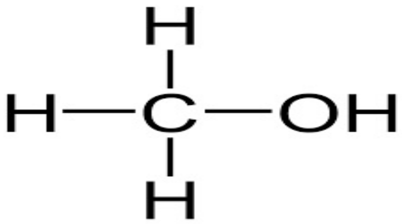
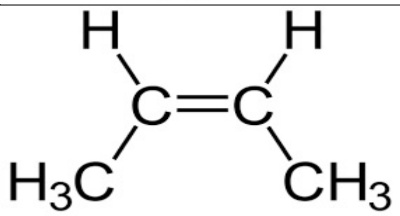
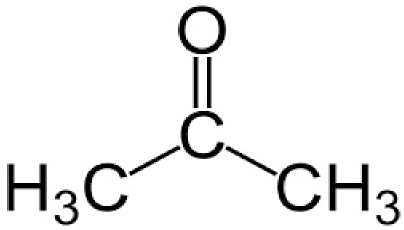




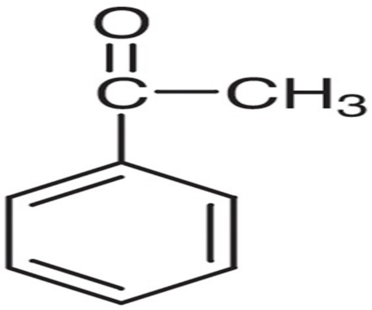
4. n→π* Transition: Occurs in compounds that contain both **a π bond and a heteroatom** with lone pairs (n electrons). (**Examples:** Carboxylic acids, their derivatives, and carbonyl compounds). **These compounds show all four practical transitions**: **σ→σ*, σ→ π*** , **π→π***, and **n→π***

- **R-Band (Radical Band):** This absorption, resulting from the **n→π*** transition, is characteristic of unsaturated chemical groups (chromophores) such as the **nitro group (O-N=O)** and the **carbonyl group (C=O)**.



Compound	**Structure**	**Electron Types Present**	**UV/Vis Transitions**
Hexane		**σ only**	**σ→σ* (High Energy)**
Methanol		**σ,n**	**σ→σ*(High Energy)** **n→σ* (Medium Energy)**
1-Butene		**σ,π**	**σ→σ*(High Energy)** **π→π*(Medium Energy)**
Acetone		**σ,π,n**	**σ→σ*(High Energy)** **n→σ*(Medium Energy)** **π→π*(Medium Energy)** **n→π* (low Energy)**

Q/ What are electronic transitions in Uv-Visible spectroscopy for following compound?

Compound	**Electronic transitions**
 **Acetophenone**	**π→π*** **n→π*** **σ→σ***
Methane, Hexane, Acetone, Ethylene, Triethylamine (Et3N), **1,3-Butadiene,** **1-Butene,** **Methanol,** **CH_3COOH, Nitrobenzene, Nitromethane, Benzaldehyde, Ethyl Acetate**	

The Solvent and Its Role in Uv-Vis Spectrophotometry

The solvent plays a crucial role in UV measurements because all samples measured must be in a liquid state and must not be turbid (a clear solution). The presence of suspended particles (turbidity) causes the radiation passing through the solution to scatter, which interferes with the measurement.

Solvents are classified into two main types:

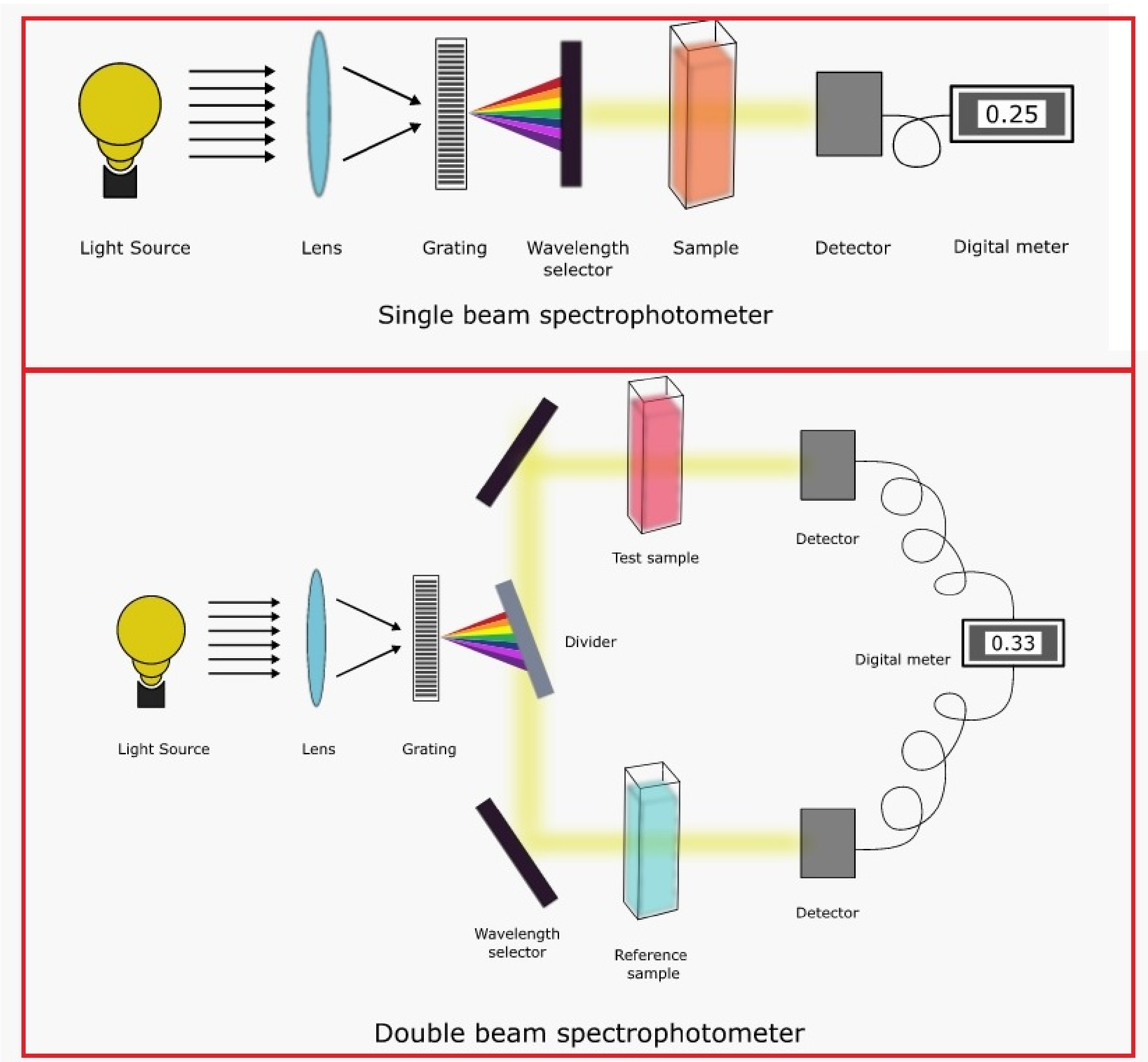
1. **Polar Solvents:** These solvents have a high dielectric constant and permanent dipole moments. Examples: Water, 95% Ethanol, and Dioxane.

2. **Nonpolar Solvents:** These solvents have low dielectric constants and no permanent dipole moments. Examples: Hexane, Cyclohexane, and Chloroform.

UV/Vis Spectrophotometer Instruments

These spectrophotometers come in two main types:

1. **Single-beam Spectrophotometer**: Requires **manually switching** the sample solution and the reference (blank) solution for **each wavelength** and performing a new zero adjustment (calibration).
2. **Double-beam Spectrophotometer**: The light beam is **split** using a **beam splitter** (a half-transparent mirror). One part falls on the sample cell, and the second part falls simultaneously on the reference (blank) solution.



The Beer-Lambert Law

The UV/Vis. domain has been widely exploited in quantitative analysis and the visible region for a particularly long time. The measurements are based upon the Lambert–Beer law links the absorption of the light to the concentration of a compound in solution.

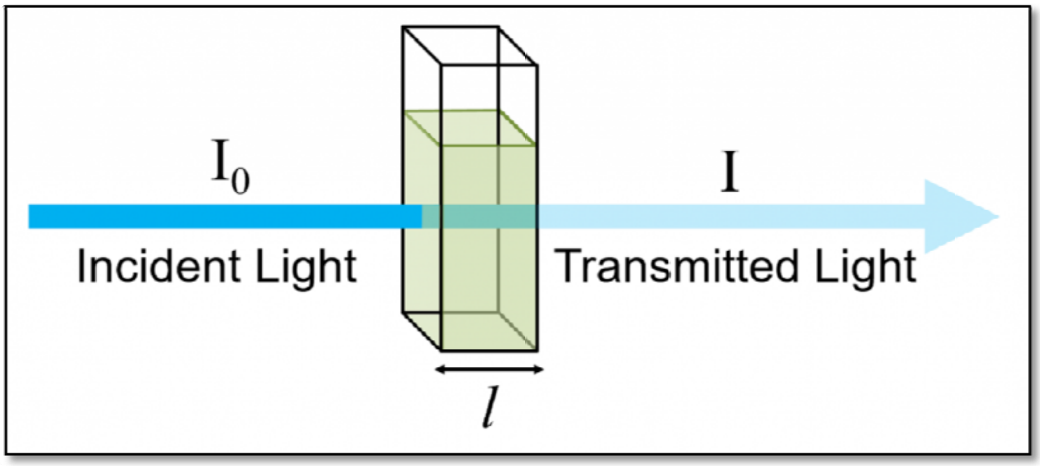
$$A = \log \frac{I_o}{I} = -\log T$$

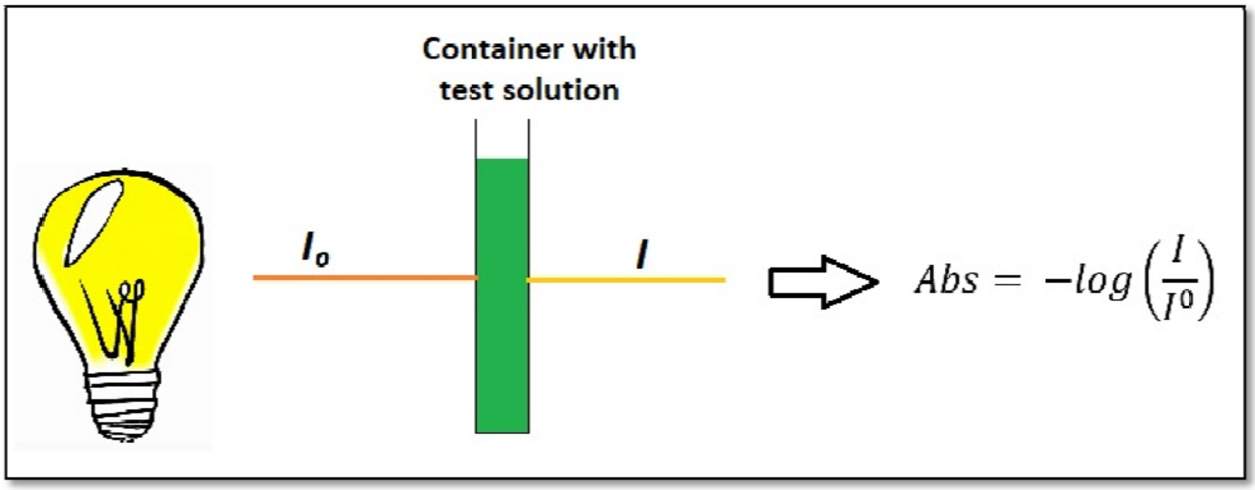
A = Absorbance

T=transmittance

I= transmitted intensity

I_o= incident intensity



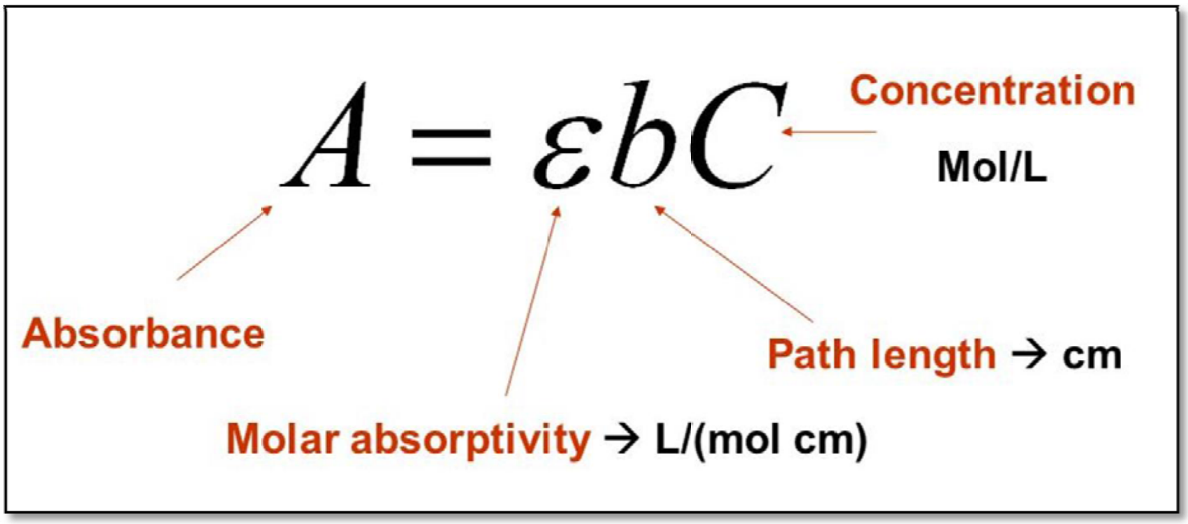


$$A = \varepsilon\ b\ c$$

ε = The molar absorptivity with unit $(\frac{L}{mol.cm})$

b = path length with unit (cm)

c = concentration of solution with unit $(\frac{mol}{L})$



Q/ What is the concentration of the solution if its molar absorptivity is 1500 $\frac{L}{mol.cm}$ and the measured absorbance in a 1.00 cm cuvette is 0.742?

Solution:

$$A = \varepsilon\ b\ c$$

$$0.742 = 1500\ \frac{L}{mol.cm} \times 1\ cm \times c$$

$$c = 4.95 \times 10^{-4}\ (\frac{mol}{L})$$

Q/ The measured absorbance of a sample in a 1.00cm cuvette is 0.544. If the concentration is 1.40 x $10^{-3}(\frac{mol}{L})$, what is the molar absorptivity for the solution?

Solution:

$$\mathbf{A = \varepsilon\ b\ c}$$

$$0.544 = \varepsilon \times 1\ \text{cm} \times 1.40 \times 10^{-3}(\frac{mol}{L})$$

$$\varepsilon = 389 \frac{L}{mol.cm}$$

Q3: Calculate the expected absorbance (A) of a solution if its concentration is 5.00×10^{-5} $(\frac{mol}{L})$, its molar absorptivity (ϵ) is 12,500 $L\cdot mol^{-1}\cdot cm^{-1}$, and it is measured in a cuvette with a path length (b) of 2.00 cm.

Q4: A solution with a known concentration of 2.50×10^{-4} $(\frac{mol}{L})$ and a molar absorptivity (ϵ) of 850 $L\cdot mol^{-1}\cdot cm^{-1}$ shows an absorbance of 0.298. What is the path length (b) of the cuvette used?

Complementary colors of spectrophotometer

A spectrophotometer uses the principle of complementary colors to determine the color of a substance.

When white light (which contains all visible wavelengths: Red, Orange, Yellow, Green, Blue, Indigo, Violet) shines on the solution, the colored molecules (chromophores) absorb specific wavelengths of this light.

If the solution appears Red to us, it means its molecules absorbed its Complementary Color. The complementary color to Red on the color wheel is Green.

The concept of complementary color is key to understanding the relationship between the perceived color (human eye perceives) and the absorbed light by spectrophotometer.

- The Complementary Color (Green) is the color or wavelength that the solution absorbs most strongly.
- The Color We See (human eye perceives)(Red) is the color that is reflected or transmitted (the light that was not absorbed).

Color We See=Color That Is Transmitted	اللون الذي نراه=اللون الذي لم يُمتص
Complementary Color=Color That Is Absorbed Most Strongly by spectrophotometer	اللون المتمم=اللون الذي تم امتصاصه بشدة بواسطة الجهاز الطيفي

When you analyze the red solution with the instrument:

- The UV-Vis instrument will scan different wavelengths of light through the solution.
- It will record the Maximum Absorbance (λmax) at the wavelength corresponding to the Green light (typically around 500–560 nm), which is the complementary color.