Instrumental Analysis

Fundamentals of Spectrophotometry

- ملاحظات من الدكتور (الريكورد)

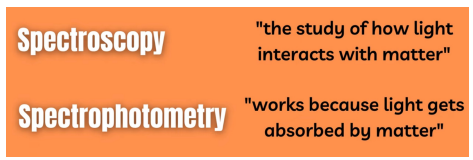
- سنوات + اسئلة ✯

By raneem Al-syouf

you got this !!

https://youtu.be/pxC6F7bK8CU?si=oz4WarIi4hdZvPUz

nice start



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Objectives

- **Fundamentals of Spectrophotometry**
  - **Properties of light**
  - **Regions of the electromagnetic spectrum**
  - **Absorption of light**
- **What happens when molecules absorb light?**
  - **Electronic energy states**
  - **Vibrational and rotational energy states**
- **Beer's Law**
- **Limitations and Deviations from Beer's Law.**

Spectroscopy is any procedure that uses the interaction of Electromagnetic Radiation (EMR) with matter to *identify* and/or to *estimate* an analyte.

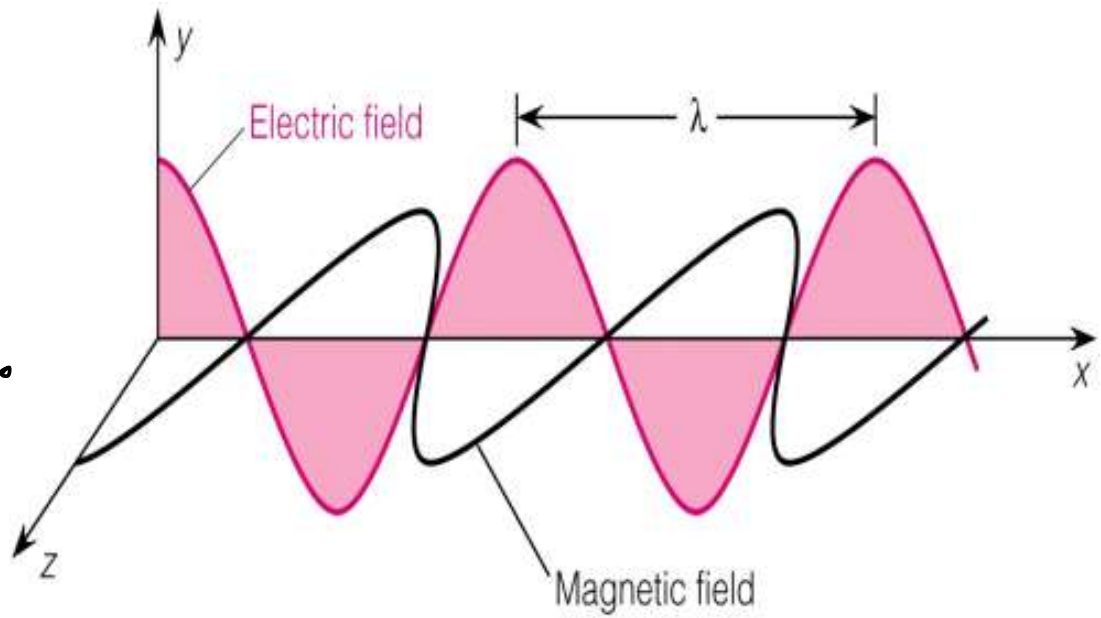
Quantitative Analysis

molecules	solid
ions	liquid
atoms	gas
Mixtures	solutions

Qualitative Analysis

Electromagnetic radiation (light)

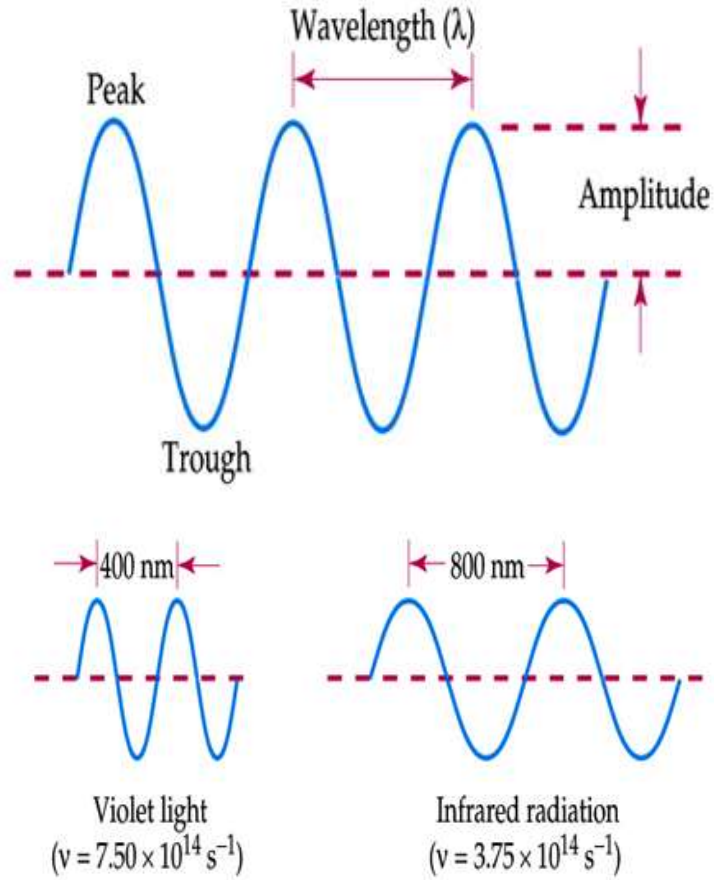
- EMR can be described in terms of both particles and waves (Dual nature of light)
- Light waves consist of متذبذبه perpendicular and oscillating electric and magnetic fields



Light waves can be characterized By:

Wavelength (λ, ***Greek lambda***):
Distance from one wave peak to the next.

Units: m, cm, μm, nm or Å



Frequency (υ, ***Greek nu***):
Number of peaks that pass a given point per second.

Units: Cycles/second or s^{-1} or Hertz (Hz)

Wavenumber
Number of waves per cm.

$$\text{wave number } \bar{\upsilon} = \frac{1}{\lambda}$$ cm^{-1}

الأنعكاس انكسار

Wave nature of light can explain phenomena such as reflection, refraction and diffraction.

حيود

سؤال سنوات

(1)

3. The term "wavenumber"

(a) refers to the number of waveform cycles traveled by a photon in a fixed time.

(b) refers to a quantity often expressed in cm^{-1}.

(c) refers to the product of frequency and Planck's constant.

(d) refers to the distance corresponding to one cycle of a waveform.

يجيب منا 2-3
كمساعدة إلنا

Wave Calculation

✓ **The wavelength of a laser pointer is reported to be 663 nm. What is the frequency of this light?**

= 10^{-9}

$$\upsilon = \frac{c}{\lambda}$$

c speed of light (3×10^{8} m/s)

$$\lambda = 663\,\text{nm} \times \frac{10^{-9}\,\text{m}}{\text{nm}} = 6.63\times10^{-7}\,\text{m}$$

$$\upsilon = \frac{3.00\times10^{8}\,\text{m/s}}{6.63\times10^{-7}\,\text{m}} = 4.52\times10^{14}\,\text{s}^{-1}$$

or Hz

λ

Calculate the wavelength of light, in nm, of light with a frequency of $3.52 \times 10^{14}\ s^{-1}$.

$$\lambda = \frac{c}{\upsilon}$$

$V = \frac{c}{\lambda}$

$\lambda = \frac{3 \times 10^{8}}{3.52 \times 10^{14}}$

$$\lambda = \frac{3.00 \times 10^{8}\ \text{m/s}}{3.52 \times 10^{14}\ \text{s}^{-1}} = 8.52 \times 10^{-7}\ \text{m}$$

$$\lambda = 8.52 \times 10^{-7}\ \text{m} \times \frac{10^{9}\ \text{nm}}{\text{m}} = 852\ \text{nm}$$

9. The wavelength of a laser pointer is 265 nm. What is the frequency?

(a) $1\times10^{6}\ s^{-1}$

(b) $6.6\times10^{7}\ s^{-1}$

(c) $500\ s^{-1}$

(d) $1.14\times10^{15}\ s^{-1}$

سؤال سنوات

2

متفرقة

- Electromagnetic radiation consists of discrete packets of energy, which we call photons.
- Photons are the particles of light or the quanta of light.
- Each photon carries the energy, E (Joule).

$$E = h\upsilon$$

where h is the Planck's constant (=6.626x10^{-34} J.s) حفظ

- The all characteristics of light can be related as follows:

$$E = h\upsilon = h\frac{c}{\lambda} = hc\bar{\upsilon}$$

من القانون نقدر نعرفهم

The greater the energy, the higher the frequency and wavenumber and the shorter the wavelength

The particle nature can explain phenomena like absorption and emission of light.

Planck's Equation: The relationship between frequency ν of light and energy E,

$$E = h\nu$$

h = Planck's constant = 6.6×10^{-27} $cm^2.g.\ s^{-1}$ = 6.6×10^{-34} joule.sec

In vacuum, velocity of light = $c = \nu\lambda = 3 \times 10^{10}$cm/s which gives, $\nu = c/\lambda$

$E = h(c/\lambda) = hc\bar{\nu}$ (where, $\bar{\nu} = 1/\lambda$ = wavenumber)

Energy directly proportional to wavenumber

$E = hc\bar{\nu}$

Calculate the energy (in joules) of a photon with a wavelength of 700.0 nm

$E = h\nu = \frac{hc}{\lambda}$

$$\lambda = 700.0\,\text{nm} \times \frac{10^{-9}\,\text{m}}{\text{nm}} = 7.00 \times 10^{-7}\,\text{m}$$

$$\upsilon = \frac{3.00 \times 10^{8}\,\text{m/s}}{7.00 \times 10^{-7}\,\text{m}} = 4.29 \times 10^{14}\,\text{s}^{-1}$$

$$E = (6.63 \times 10^{-34}\,\text{J} \cdot \text{s})(4.29 \times 10^{14}\,\text{s}^{-1})$$

$$E = 2.84 \times 10^{-19}\,\text{J}$$

b. A photon has an energy of 5.68×10^{-19} J. What is its wavelength?

3

(1) 250 nm

(2) 350 nm

(3) 450 nm

(4) 550 nm

$$E = h\nu = \frac{hc}{\lambda}$$

$$\lambda = \frac{hc}{E} = \frac{6.626 \times 10^{-34} \times 3 \times 10^{8}}{5.68 \times 10^{-19}}$$

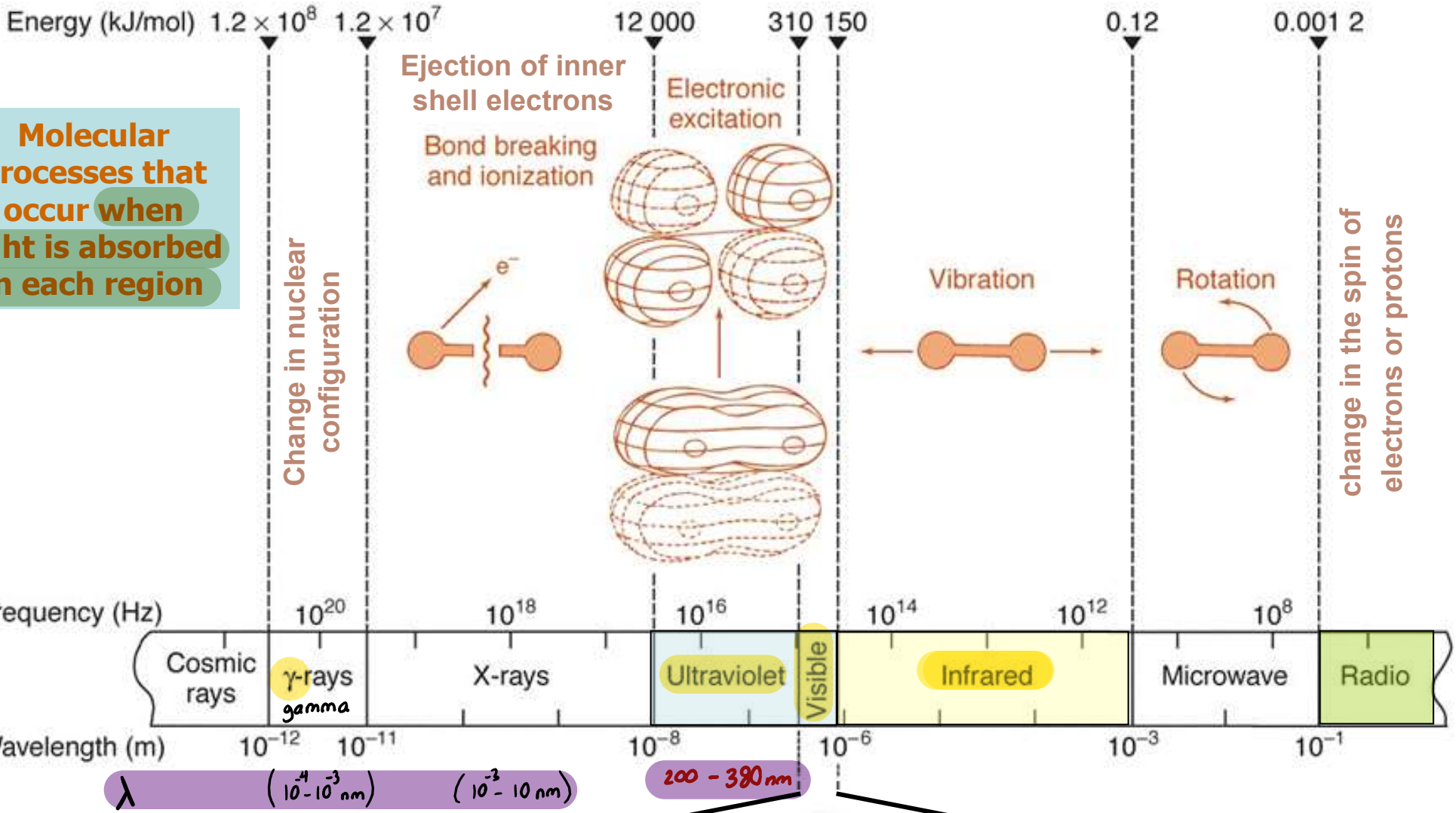
$$\lambda = 350 \times 10^{-9}$$

$$\lambda = 350 \text{ nm}$$

2

Regions of electromagnetic radiation

Molecular processes that occur when light is absorbed in each region



A near UV light 200 - 380

B far UV light 10 - 200

! مهم

Memorize

Highest energy / frequancy

lowest wavelength

gamma → Radio

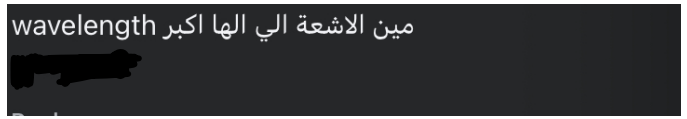
Y-rays

$\lambda = 10^{-12}$

$\lambda = 10^{-1}$

lowest energy / frequancy

highest wavelength



- **gamma rays**
  - high energy
  - change in nuclear configuration

$\lambda\ (10^{-12} - 10^{-11})\ m$

- **X-rays**
  - ejection of inner shell elcctrons
  - Bond breaking and ionization

$\lambda\ (10^{-11} - 10^{-8})\ m$ + short λ UV light

Ultraviolet (UV)
- Electronic excitation

(200 - 380 nm)

Visible Light

380 - 780 nm

$3.8 \times 10^{-7}\ m - 7.8 \times 10^{-7}\ m$

Infard (IR)
- bond vibrations
  stretching and bending

$\lambda\ (10^{-6} - 10^{-3}\ m)$

only qualitative

فقط لنوع

microwave
- Radiation

$\lambda\ (10^{-3} - 10^{-1}\ m)$

Radio

change in the spin of electrons or photons

سنوات

3 بماذا يتم رؤية الهيموغلوبين visible

4 لما تكون العينة غير ملونة شو بنسخدم ؟ uv

5 لما يصر امتصاص بال ir شو يصير انتقال من مدار اقل لاعلى

انه الred sample مثل الHemoglobin بأي radiation ينشاف

all of the above

4

9. Which of the following lists correctly ranks electromagnetic radiation (EMR) from low to high wave length *
(1 Point)

يسار → يمين

radio → gamma

- Gamma, x-rays, ultra-violet (UV), visible, infra-red (IR), microwaves
- UV, visible, IR, gamma, microwaves, x-rays
- Visible, UV, IR, gamma, x-rays, microwaves
- Microwaves, IR, visible, UV, x-rays, gamma
- None of the other options is right

...titative and quantitative analysis *

A photon with a frequency of 6×10^{14} cycles/sec is

(a) an ultraviolet photon.

(b) a visible photon.

(c) an infrared photon.

(d) none of the above.

سؤال سنوات (5)

$\lambda = \frac{c}{v} = \frac{3 \times 10^8}{6 \times 10^{14}}$

$\lambda = 5 \times 10^{-7}$

b

8. Which arrangement is correct in order of increasing energy?

(a) visible < infrared < ultraviolet

(b) visible < infrared < ultraviolet

(c) infrared < visible < ultraviolet

(d) None of the above

اعطانا انه ال frequency بتساوي 2×13^10 وين بتكون موجودة (7)

1300 Hz

1)UV
2)IR
3)Visible
4)non

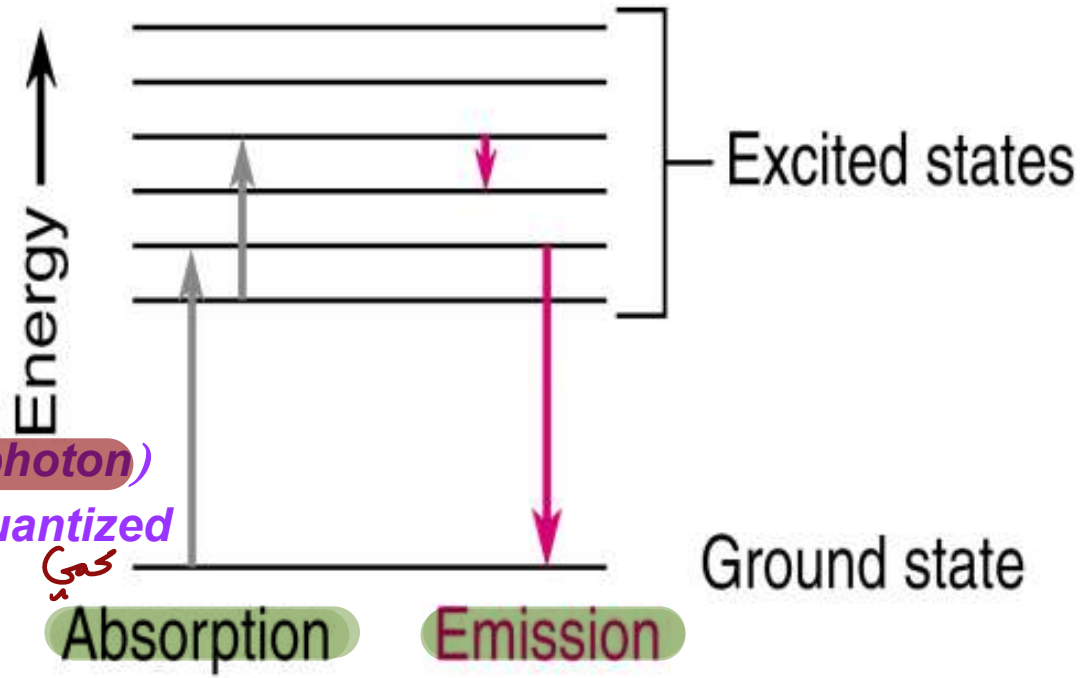
$\lambda = \frac{c}{v} = 230.8 \times 10^{3}$

radio

Absorption of light

A **molecule** that absorbs light photons will end up with increased energy. The molecule will be promoted to an **excited state**. Microwave energy will cause rotation of compounds. IR energy is high enough to promote bond stretching. UV/Vis energy promotes electrons into higher orbitals. Short-λ UV and X-rays can ionize molecules or even break bonds.

Excitation



$\Delta E\,(molecule) = E\,(photon)$

absorbed light is quantized

Because only certain discrete levels exist. Electrons cannot absorb just any amount of energy.

كمي

λ_{max} هو الطول الموجي الذي يعطي finger print الـ

Most excited molecules relax again to the ground state emitting the excess energy in the form of heat.

j. Infrared photons

(1) can only be absorbed by molecules with a bond dipole.

(2) can only be absorbed when the photon frequency matches a vibrational frequency of the molecule.

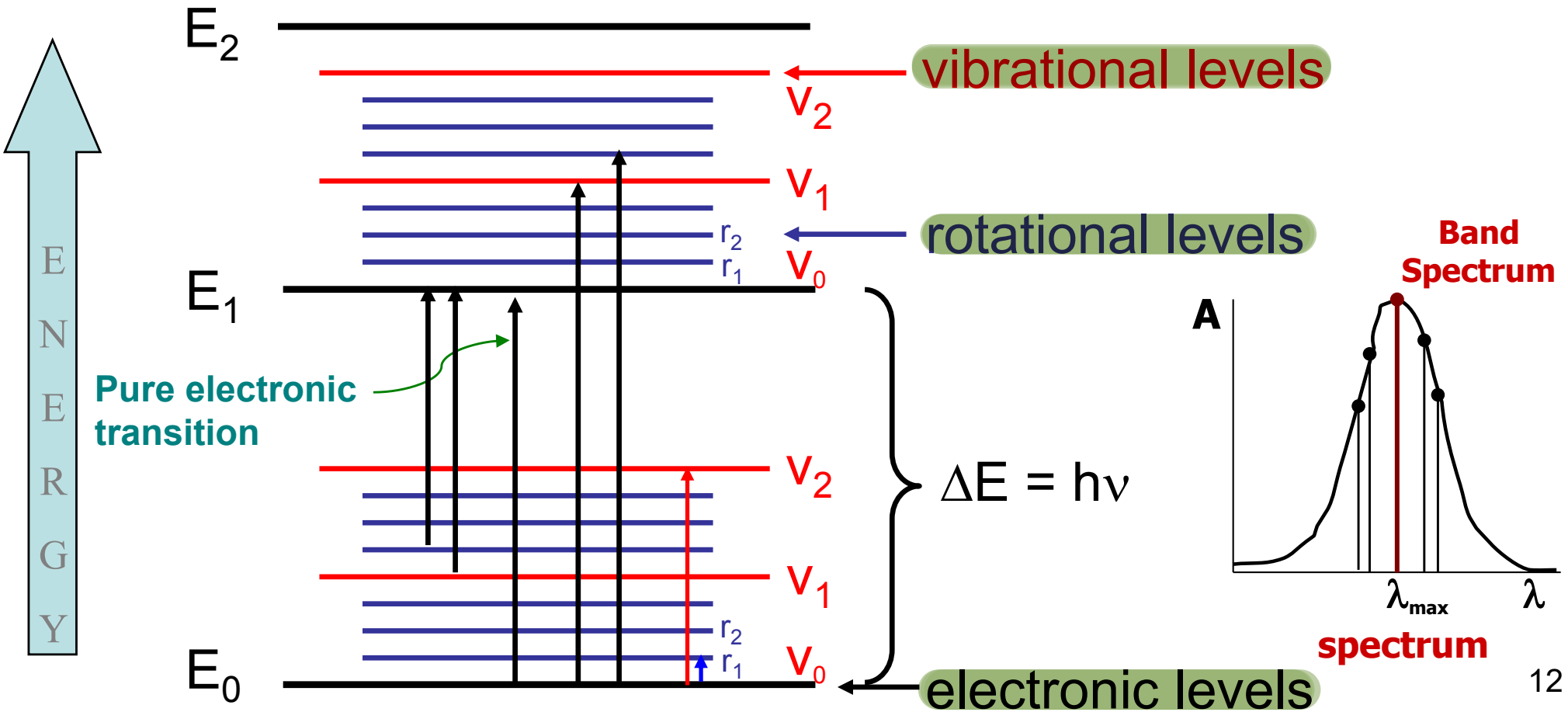
(3) can only be absorbed when their energy matches the difference in energy between two vibrational states.

(4) all of the above.

Ultraviolet-Visible Spectrophotometry

What happens when a molecule absorbs UV-Visible radiations?

- When a molecule absorbs light having sufficient energy (e.g. UV-Vis radiation) to cause an electronic transitions, (additional vibration and rotation transitions also occur)
- Molecule can absorb one photon of just the right energy to cause the following simultaneous changes:



A molecule can only absorb a photon if the photon's energy exactly matches the energy gap between the initial and final energy levels.

Mathematically:

$$E_{\text{photon}} = \Delta E = hf = \frac{hc}{\lambda}$$

This is why energy levels are said to be **quantized**.

electronic level كبير UV-vis اسقطنا طاقة على منطقة

vibration

rotational

rotational + vibrational IR (infrared) " " " لو

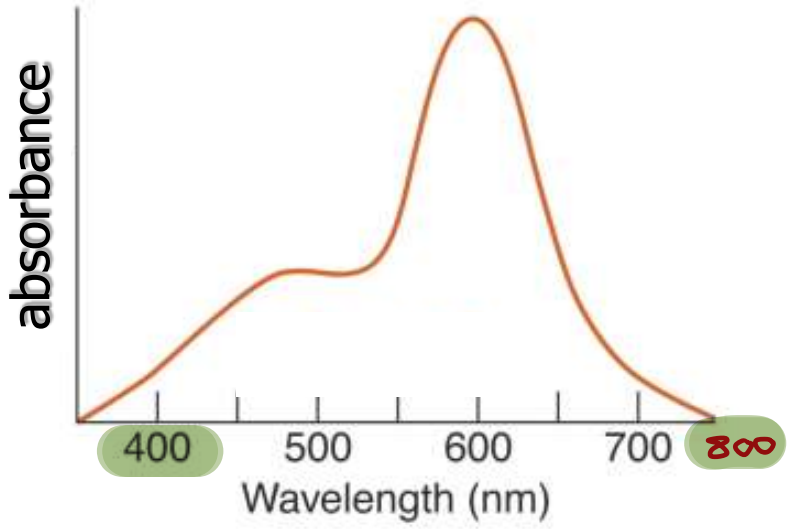
low energy no electronic

$(n_1 \rightarrow n_2)$ rotational microwave " " " "

1. A transition from the ground electronic state E_0 to the E_1 excited electronic state
2. A change in the vibrational energy from the ground vibrational state of E_0 to an excited vibrational state of E_1
3. A transition from one rotational state of E_0 to a different rotational state of E_1
4. All the above transitions are quantized which means that they required certain exact amount of energy
5. Thus, total energy absorbed = $E_{elec} + E_{vib} + E_{rot}$

$$\Delta E_{elec} >> \Delta E_{vib} >> \Delta E_{rot}$$

As a result, a large numbers of photons of certain wavelengths are absorbed by a molecule. These individual wavelengths are too numerous and too close to each other and a spectrum of broad bands of absorbed wavelengths are obtained



Spectrum (a graph that shows how absorbance varies with wavelength)

quartz plastic

6. Which statement is false regarding absorption of photons by molecules?

(a) Absorption of a photon results in a change in the energy state of the molecule.

(b) There is a correspondence between photon energy and the energy difference between initial and final states.

(c) Electronic transition energy is greater than vibrational transition energy.

(d) None of the above.

سنوات

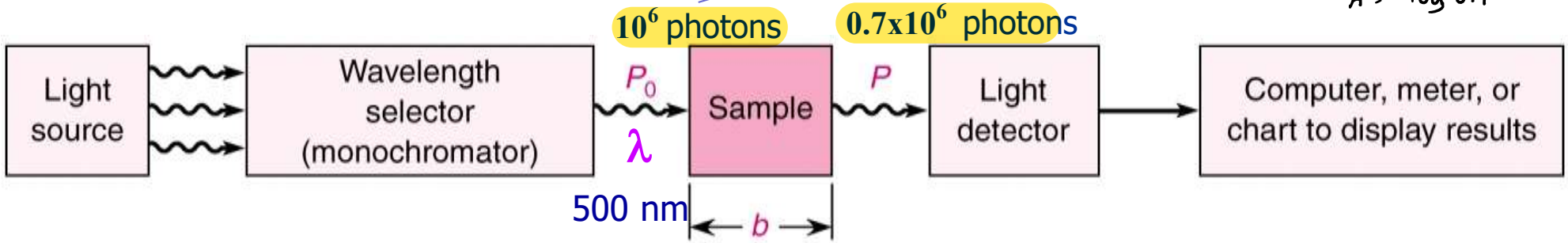
a

d

Transmittance and Absorbance

T = 0.7, %T = 70%, A = 0.155

A = -log 0.7



There are two quantities that relate the change in the intensity or radiant power of EMR before, P_0, and after, P, interaction with matter.

incident transmitted

1. **Transmittance, T, is simply defined as "the fraction of light that reaches a detector after passing through a sample"**

"نافذ"

transmitted ← $T = \frac{P}{P_\circ}$ → incident

العنوء الخارج

العنوء الساقط

$0 \leqslant T \leqslant 1$

100 photons enter
70 leave
30 absorbed

$T = \frac{70}{100} = 0.7$

$A = -\log 0.7 = 0.155$

The percent transmittance, %T, is simply 100 T

$$\%T = \frac{P}{P_\circ} \times 100 \qquad 0 < \%T < 100$$

As transmittence ↑
Absorbance ↓

2. **Absorbance, defined as:**

$$A = -\log T \longrightarrow A = -\log \frac{P}{P_\circ} \longrightarrow A = \log \left(\frac{P_\circ}{P}\right)$$

What is the relationship between **absorbance** and **transmittance** of light through a sample in a spectrophotometer?

(a) an increase in transmittance usually results in an increase in absorbance

(b) a decrease in transmittance usually results in a decrease in absorbance

(c) a decrease in transmittance usually results in an increase in absorbance

(d) transmittance and absorbance should remain equal

Which of the following equations is INCORRECT regarding the relationship between Absorbance (A) and Transmittance (T or $\%T$)?

A) $A = -\log T$

B) $A = 2 - \log(\%T)$

C) $A = 1 - \log(\%T)$

D) None of the above (all are correct)

سنوات (١١)

$\%T = T \times 100$

$T = \frac{\%T}{100}$

خذ log - لطرفينا

$-\log T = -\log \frac{\%T}{100}$

$A = -(\log \%T - \log 100)$

$A = 2 - \log \%T$ ✓✓✓ (C)

absorbabce = 1 سؤال معطيني ال
percentage if transmition وبده

$A = 1 \quad T = 10^{-1}$

$A = -\log T$

$\%T = 10\%$

For purpose of chemical analysis

Absorbance is directly proportional to:

1. **concentration, c, of absorbing species in the sample ($A \alpha c$)**
2. **path length of light, b, through the sample ($A \alpha b$)**

Beer's law

$$A = \varepsilon bc$$

The previous equation is the heart of spectrophotometry as applied to analytical chemistry, it is called Beer-Lambert law or simply Beer's law

1- Concentration of the analyte is given in unit mol/L (M)

2-The path length, b, in cm

3-ε, is called the molar absorptivity or molar absorption coefficient

"Absorbance of 1 M solution measured in a cell of 1 cm path length"

سؤال سنوات ← no unit

$$\varepsilon = \frac{A}{bc} = \frac{1}{\frac{mol}{L}\,cm} = L\,mol^{-1}cm^{-1} = M^{-1}cm^{-1}$$

none شو وحدتها

How strongly a substance absorbs light

شو وحدة ال molar absorption ؟

كل مركب إله epsilon خاصة فيه عند طول موجي معين

وهاي epsilo لنفس المركب تتغير بتغير الطول الموجي

ε, is characteristic for <u>each substance</u> at a <u>particular wavelength</u>, λ.

---

e. A 1.0×10^{-4} M solution is placed in a 1.00 cm pathlength cell and the absorbance is measured as 0.113 at 400 nm and 0.166 at 450 nm. Given that the concentration and pathlength are identical, why does the absorbance change?

12

Absorbance is the product of absorptivity, path length and concentration. Absorptivity is a property of the analyte and changes as a function of wavelength (i.e., the probability that the analyte absorbs varies with wavelength).

...In the lab I have you checked for noise when an instrument had an absorbance reading of 2.5. How much light is being transmitted through the sample when it has this absorbance?

(a) 0.22%
(b) 1%
(c) 0.99%
(d) 0.32% *(circled)*

$A = 2.5$

$A = -\log T$

$\% T = 10^{-A} \times 100$

$T = 0.32\%$

d

سنوات

13

5. The Beer–Lambert Law

(a) states that transmittance is linearly related to concentration.

(b) requires that the product of absorptivity and path length be known before absorbance can be related to concentration.

(c) requires that absorptivity be independent of wavelength.

(d) cannot be used if more than one absorbing species is present in a sample.

14

b

2. Compound X has an absorptivity of 703 L g^{-1} cm^{-1}. The transmittance of a solution of X is measured in a 1.00 cm path length cell and found to be 20.6%. Compute the concentration of the solution in g/L.

(a) 81.732×10^{-3} g/L

(b) 0.4732×10^{-3} g/L

(c) 1.213×10^{-4} g/L

(d) 9.76×10^{-4} g/L

$\varepsilon = 703\ g^{-1}.cm^{-1}.L$

$b = 1\ cm$

$T = 20.6\% = 0.206$

$A = -\log T = -\log 0.206$

$A = \varepsilon b c$

$c = \frac{A}{\varepsilon b} = \frac{-\log 0.206}{703\ cm^{-1}.g^{-1}.L \times 1\ cm}$

$= 9.76 \times 10^{-4}\ g/L$

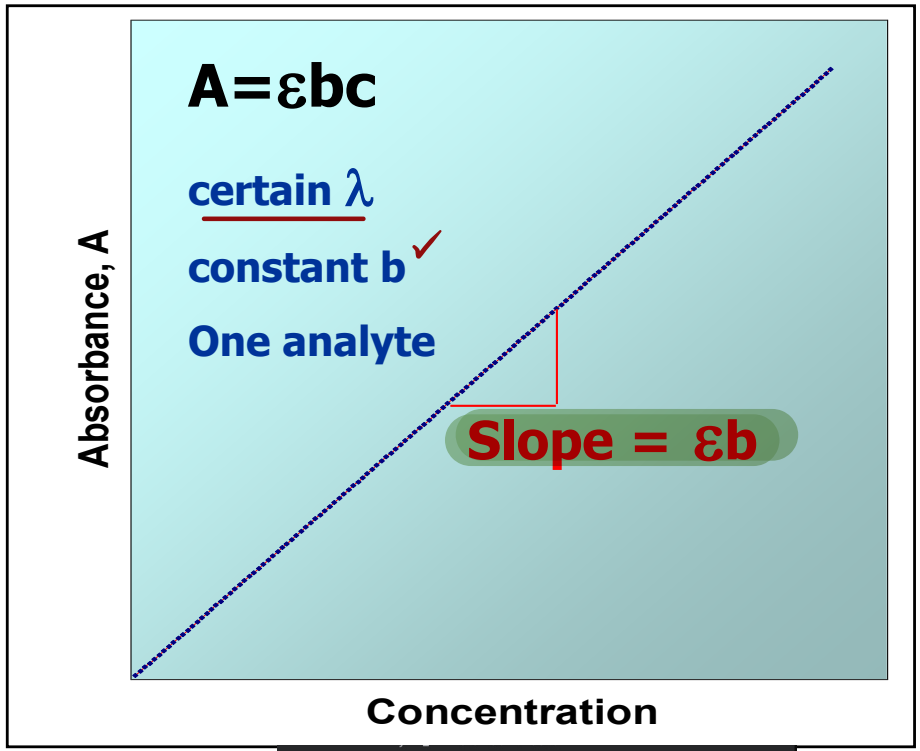
15

d

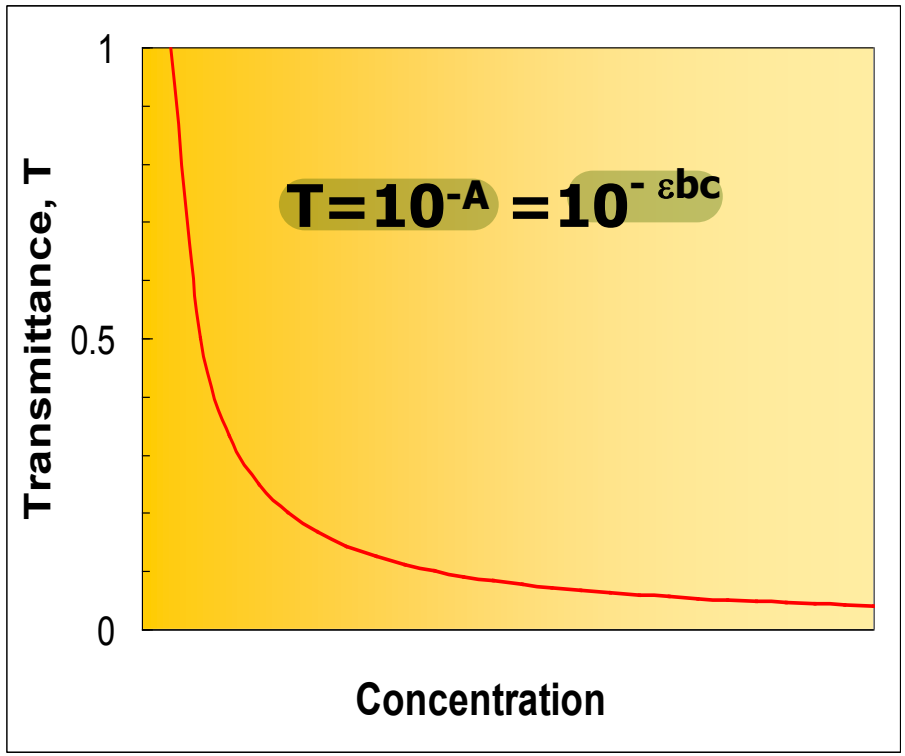
According to the Beer-Lambert Law, on which of the following does not affect the absorbance? *
(1 Point)

16

- (●) Color of the solution.
- ( ) Distance that the light has travelled through the sample
- ( ) Absorbtivity coefficient of the sample
- ( ) Solution concentration



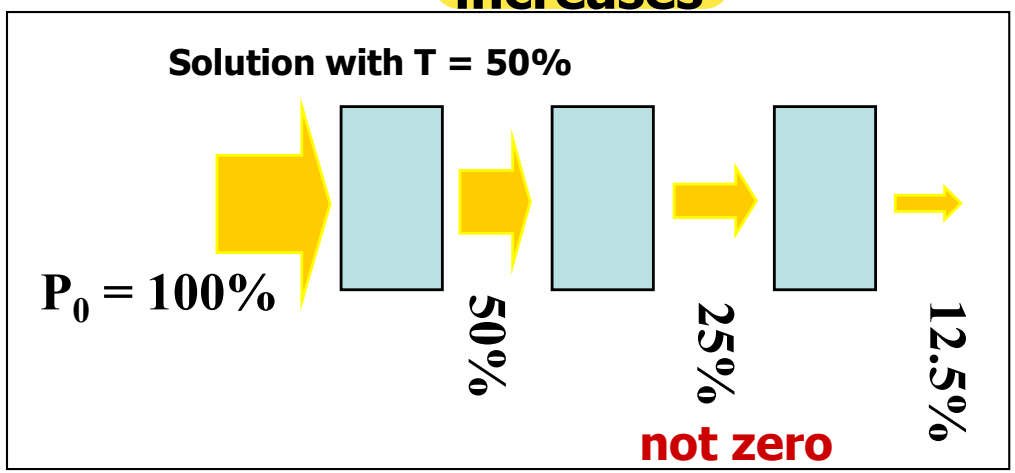
كان سؤال انه الابيسيلون ضرب ال path length بال calbration curve.
شو بتعني ، مش متأكده بس حطيتها slop



Beer's law is a relation between absorbance and concentration which is a straight line passes by origin at constant pathlength, b, and at certain wavelength, λ.

Beer's law is obeyed for monochromatic light

Transmittance decreases exponentially as concentration increases



9 wich of the following is obtained from beer law?
Obtained only from monochromatic light

4. An instrument used to measure transmittance

(a) must have a monochromator.

(b) must have a detector for measuring light intensity.

(c) must have the ability to ratio the light intensities measured with and without the sample.

(d) all of the above.

Beer-Lambert's law proves a direct correlation between the absorbance (A) of a molecule to the concentration (c) and the path length (b).

انحرافات

Derivation of Beer Lambert Law.

This relationship is a linear for the most part. However, under certain circumstances the Beer relationship gives a non-linear relationship.

These deviations from the Beer Lambert law can be classified into three categories:

Real Deviations - These are fundamental deviations due to the limitations of the law itself.

Chemical Deviations- These are deviations observed due to specific chemical species of the sample which is being analyzed.

Instrument Deviations - These are deviations which occur due to how the absorbance measurements are made.

1- Real Deviation

Beer law and Lambert law is capable of describing absorption behavior of solutions containing relatively low amounts of solutes dissolved in it (<0.01 M or 10 mM). works best at low concentration (below 0.01)

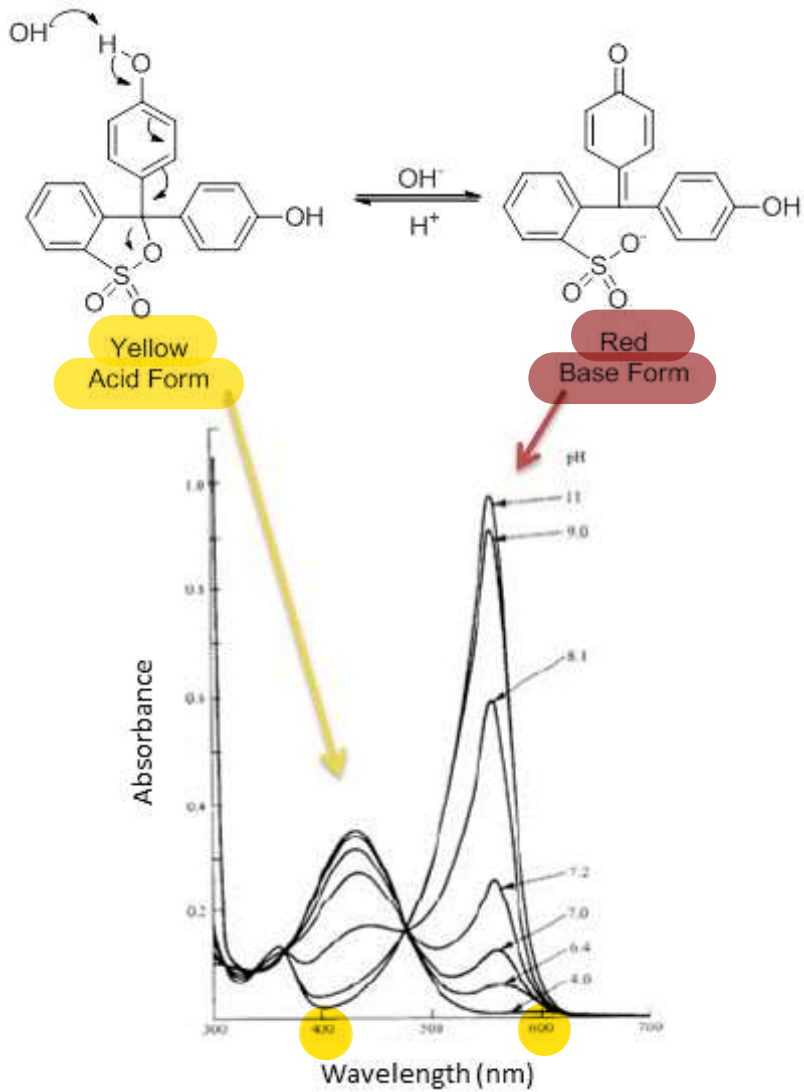
When the concentration of the analyte in the solution is high (> 0.01 M or 10 mM), the analyte begins to behave differently due to interactions with the solvent and other solute molecules and at times even due to hydrogen bonding interactions.

It is also possible that the concentration is so high, that the molecules create a screen for other molecules thereby shadowing them from the incident light.

2- Chemical Deviations

Chemical deviations occur due to chemical phenomenon involving the analyte molecules due to association, dissociation and interaction with the solvent to produce a product with different absorption characteristics. change in PH

For example, phenol red undergoes a resonance transformation when moving from the acidic form (yellow) to the basic form (red). Due to this resonance, the electron distribution of the bonds of molecule changes with the pH of the solvent in which it is dissolved.



3- Instrumental Deviations

A] Due to Polychromatic Radiation

(Beer's law assumes only monochromatic (single wavelength) light

Beer-Lambert law is strictly followed when a monochromatic source of radiation exists. In practice, however, it is common to use a polychromatic source of radiation with continuous distribution of wavelengths along with a monochromators to create a monochromatic beam from this source.

each wavelength has different ε

B] Due to Presence of Stray Radiation

↳ unwanted light that reaches the detector

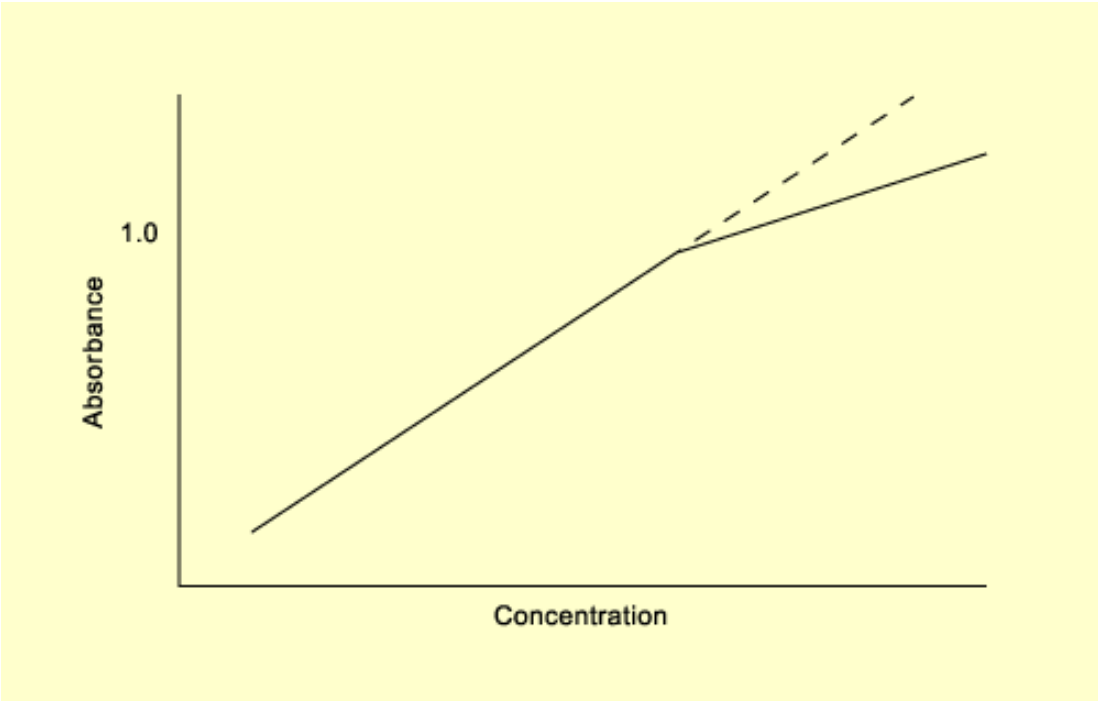
Stray radiation or scattered radiation is defined as radiation from the instrument that is outside the selected wavelength band selected.

Usually, this radiation is due to reflection and scattering by the surfaces of lenses, mirrors, gratings, filters and windows. If the analyte absorbs at the wavelength of the stray radiation, a deviation from Beer-Lambert law is observed similar to the deviation due to polychromatic radiation.

C] Due to Mismatched Cells or Cuvettes

$A = \varepsilon b c$

If the cells holding the analyte and the blank solutions are having different path-lengths, or unequal optical characteristics, it is obvious that there would be a deviation observed in Beer-Lambert law.



Deviations from Beer's Law

High-Yield Summary

Memorize these key points:

- Total absorbed energy = Electronic + Vibrational + Rotational.
- Energy order: Electronic > Vibrational > Rotational.
- UV spectra show broad bands because many vibrational and rotational transitions accompany each electronic transition.
- Transmittance: $T = P/P_0$.
- Percent transmittance = $100 \times T$.
- Absorbance: $A = -\log T = \log(P_0/P)$.
- Beer-Lambert law: $A = \varepsilon bc$.
- Absorbance increases linearly with concentration and path length.
- The slope of an absorbance vs. concentration graph is εb.
- Transmittance decreases exponentially as concentration increases.
- Beer-Lambert law is valid for monochromatic light.
- Three types of deviations:
  1. Real: high concentration and molecular interactions.
  2. Chemical: changes in the chemical form of the analyte.
  3. Instrumental: polychromatic light or stray light.