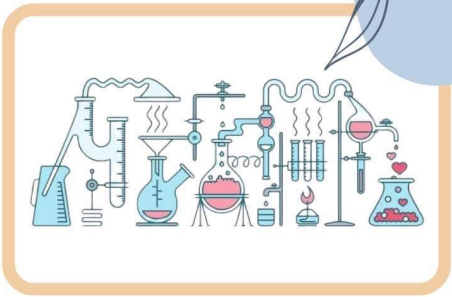
تفريغ تحليل آلي



اسم الموضوع:

إعداد الصيدلاني/ـة: عادل الحطيط

Instrumental Analysis

Fundamentals of Spectrophotometry

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.**

main source of Light Sun

نستخدمه عشان نتأكد كيف الضوء يتفاعل مع المواد reaction

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

الهدف

Quantitative Analysis

molecules	solid
ions	liquid
atoms	gas
Mixtures	solutions

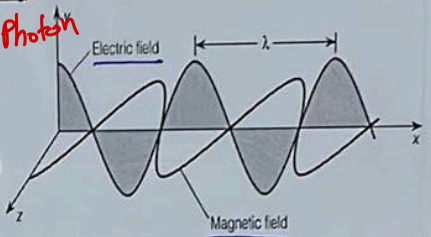
Qualitative Analysis

Electromagnetic radiation (light)

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

Photon

ثابتة



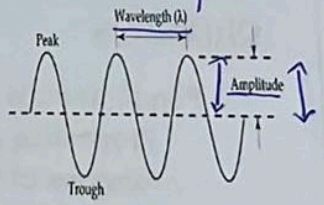
التغير بأثر عبوضي

2 Peak ← قمة وقمة distance بين

Light waves can be characterized By:

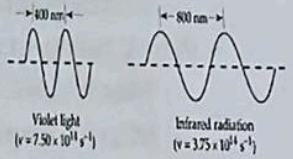
Wavelength (λ, *Greek lambda*):
Distance from one wave peak to the next.
Units: m, cm, μm, nm or Å

طول



Frequency (υ, *Greek nu*):
Number of peaks that pass a given point per second.
Units: Cycles/second or s^{-1} or Hertz (Hz)

عدد الموجات داخلة بنقطة معينة



Wavenumber
Number of waves per cm.

$$\bar{\upsilon} = \frac{1}{\lambda} \quad \text{cm}^{-1}$$ length مقلوب

Wave nature of light can explain phenomena such as reflection, refraction and diffraction.
الانعكاس الانحراف
الانكسار
الظواهر الموجية بتفسرهم.

4

لما تدخل موجات كتير في نقطة معينة خلال ثانية معناه distance بين الموجات قليلة (distance بين قمة وقمة قليل).

↑ wavenumber · ↓ wavelength ↑ frequency

↑ Wn ↑ F
↓ WL ↑ F
↓ WL ↑ Wn

Wave Calculation

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

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

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

c → velocity

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

سرعة الضوء

$$\upsilon = \frac{3.00 \times 10^{8}\,\text{m/s}}{6.63 \times 10^{-7}\,\text{m}} = 4.52 \times 10^{14}\,\text{s}^{-1}$$

Calculate the wavelength of light, in nm, of light with a frequency of 3.52 x 1014 s-1.

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

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

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

- 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)

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

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

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 □ of light and energy E,

ثوابت

$$E = h\square$$

h = Planck's constant = 6.6 x 10-27 cm2.g. s-1 = 6.6 x 10-34 joule.sec

In vacuum, velocity of light = c = □□ = 3 x 1010cm/s which gives, □ = c/□

E = h(c/□) = hc□ (where, □ = 1/□ = wavenumber)

Energy directly proportional to wavenumber

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

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

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

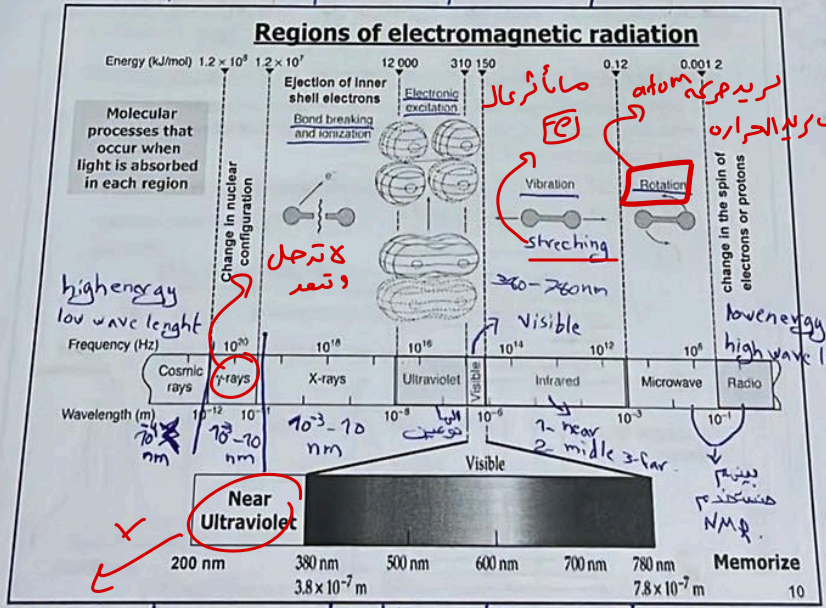
$$E = (6.63 \times 10^{-34}\,\text{J} \cdot \text{s})(4.29 \times 10^{14}\,\text{s}^{-1})$$

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

حالقاوں

من الأقل WL و أعلى E

CO-R (cosmic rays)	Gamma (γ-rays)	X-Rays	Far-uv	Near-uv
$< 10^{-4}$	$10^{-4} - 10^{-3}$	$10^{-3} - 10$	10-200	200-380

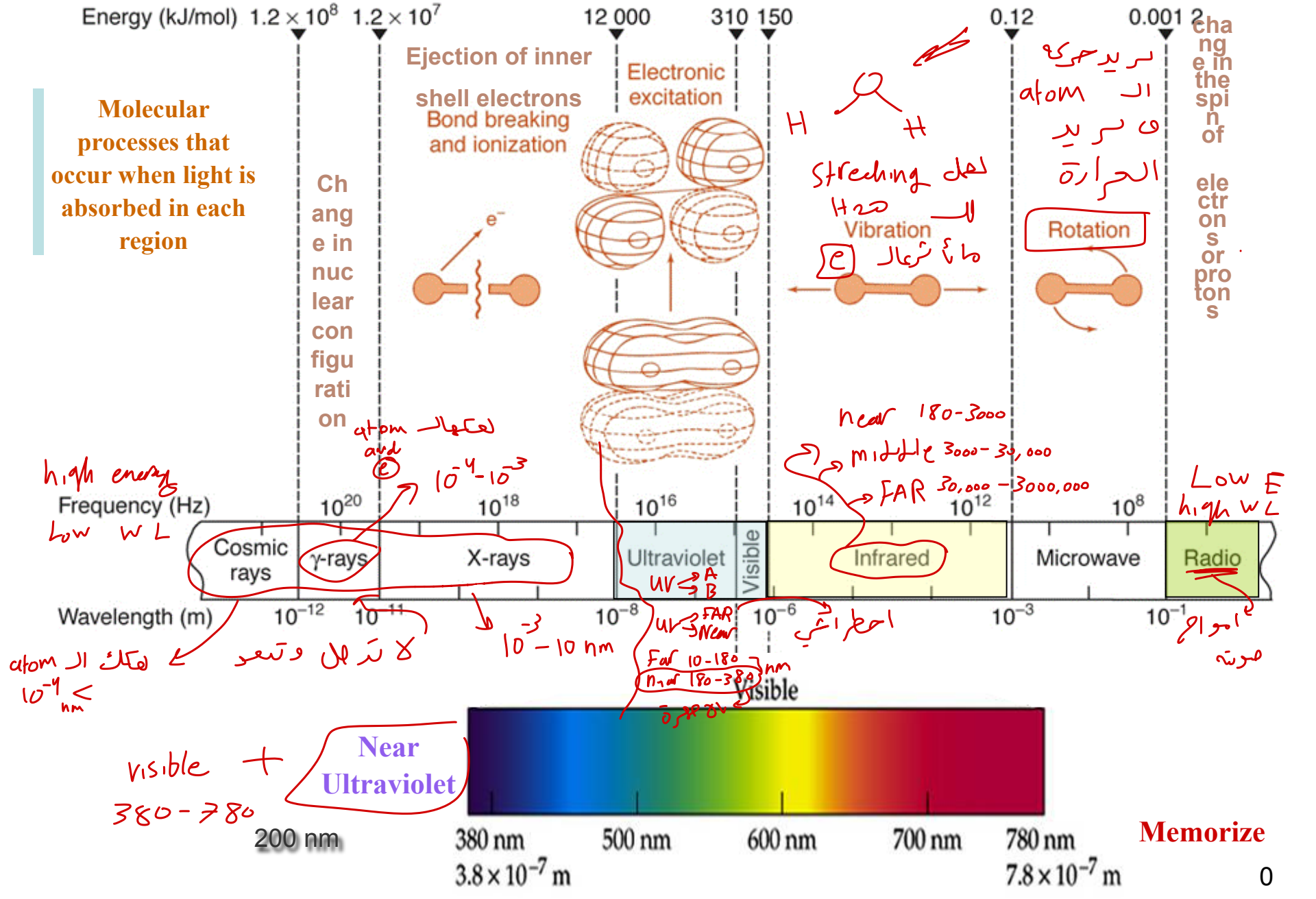


Visible	Near-IR	Middle-IR	Far-IR	Microwave
380-780	780-3000	3000-30000	30000-300000	$300000 - 10^9$

من الأطول WL و أقل E

Regions of electromagnetic radiation

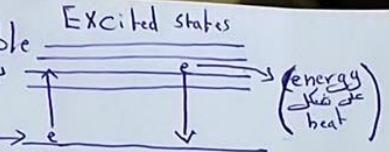
Molecular processes that occur when light is absorbed in each region



Memorize

UV / Visible

electron يعمل excitation

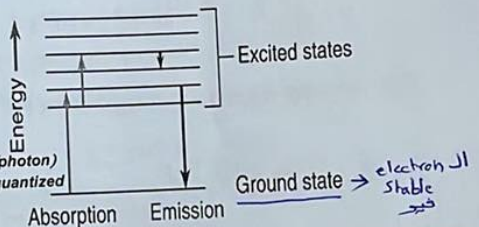


لما اسلط الضوء الازرق photon على electron راح يمتص الطاقة electron تبعي راح photon راح يصير متحمس ويطلع لـ excited state

Absorption of light

A **molecule** that absorbs light photons will end up with increased energy. The molecule will be promoted to an **excited** state. ① Microwave energy will cause rotation of compounds. ② IR energy is high enough to promote bond stretching. ③ UV/Vis energy promotes electrons into higher orbitals. ④ Short-λ UV and X-rays can ionize molecules or even break bonds.

vibration ←



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

1
1

electron عشان يرجع stable راح يعمل emission على شكل heat عشان يتخلص من هاي الطاقة ويرجع لـ ground state

Absorption → radiation على شكل

emission → heat على شكل

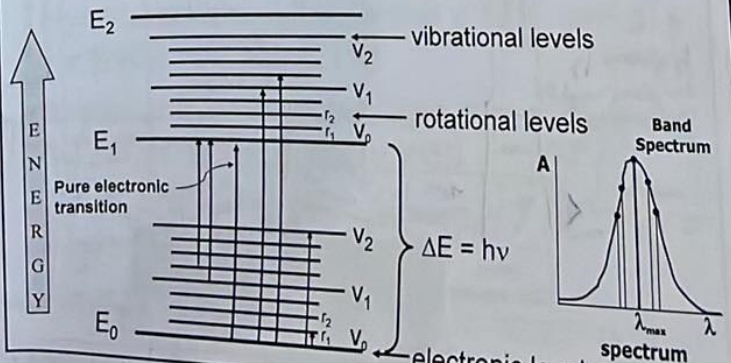
electron transition ← هاي العملية كلها اسمها

الهدف منها: اعرف نوع المادة (أشوف تركيب المادة) Qualitative.

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:**



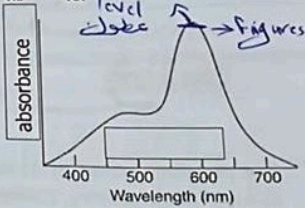
12

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

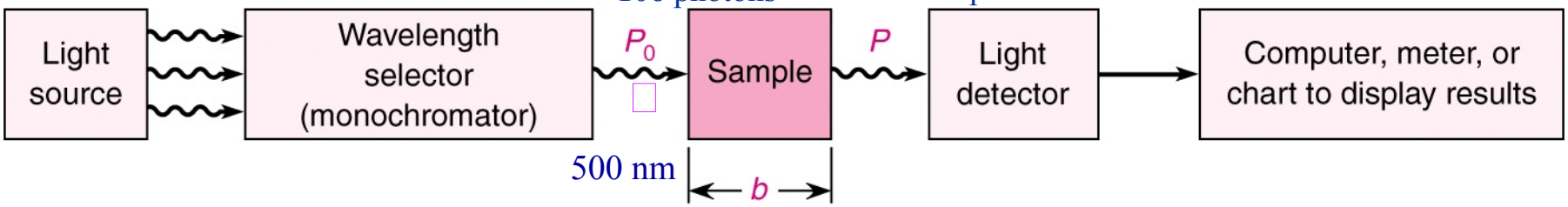
Qualitative



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

Transmittance and Absorbance

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



There are two quantities that relate the change in the intensity or radiant power of EMR before, P0 , and after, P, interaction with matter.

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

$$T = \frac{P}{P_{?}} \qquad 0 \leq T \leq 1$$

The percent transmittance, %T, is simply 100 T

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

2. **Absorbance, defined as:**

$$A = \square \log T \qquad A = \square \log \frac{P}{P_{?}} \qquad A_{?} = \log(\quad)$$

$$\frac{P_{?}}{P} \qquad 0$$

2 way

For purpose of chemical analysis

Absorbance is directly proportional to:

- **concentration, c, of absorbing species in the sample (A□□ c)**
- **path length of light, b, through the sample (A□□ b)**

Beer's law

$$A = \epsilon bc$$

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 calibration $y = mx + b$

② beers law $A = \text{molar absorption} * b * c$

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

2-The path length, b, in cm

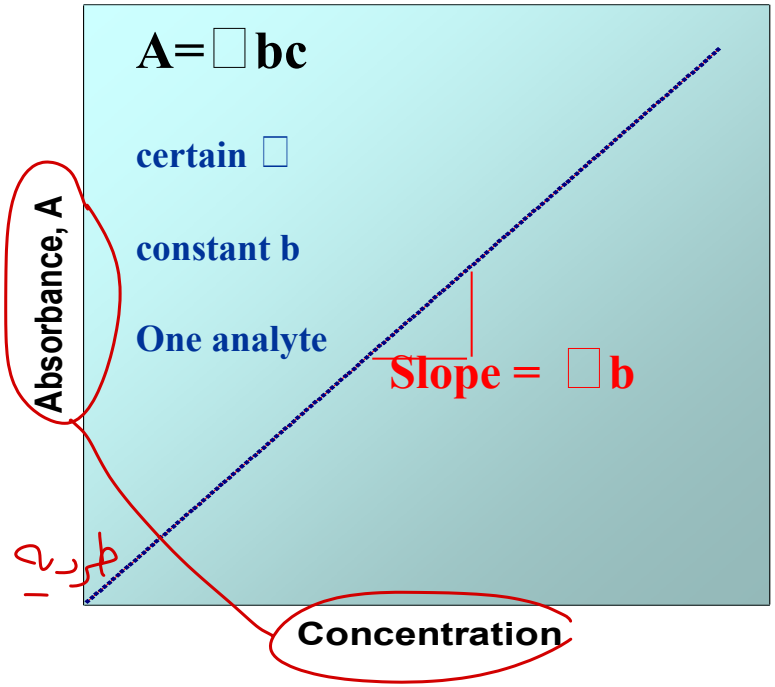
3- □, is called the molar absorptivity or molar absorption coefficient

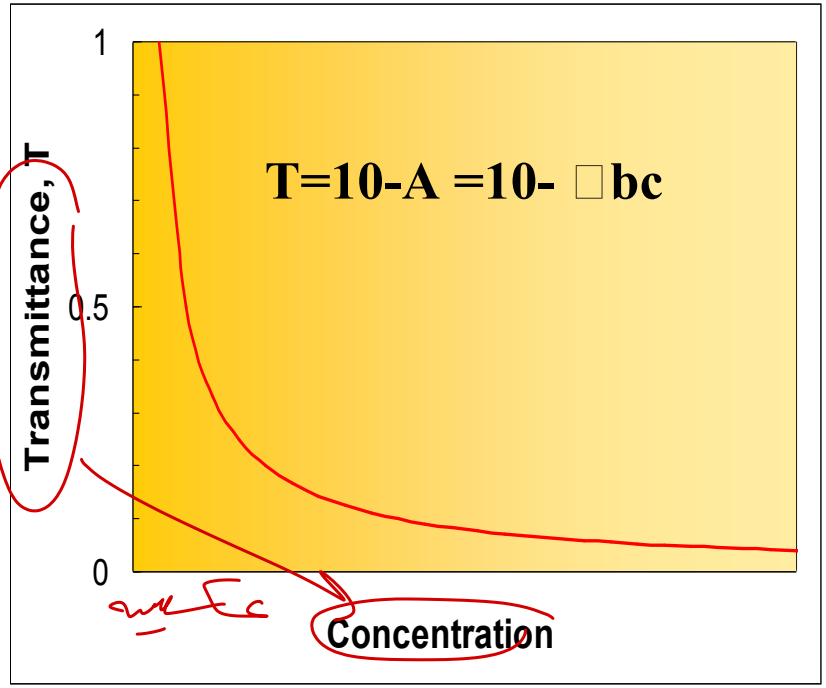
"Absorbance of 1 M solution measured in a cell of 1 cm path length"

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

وحدتها
من الاشتقاق

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

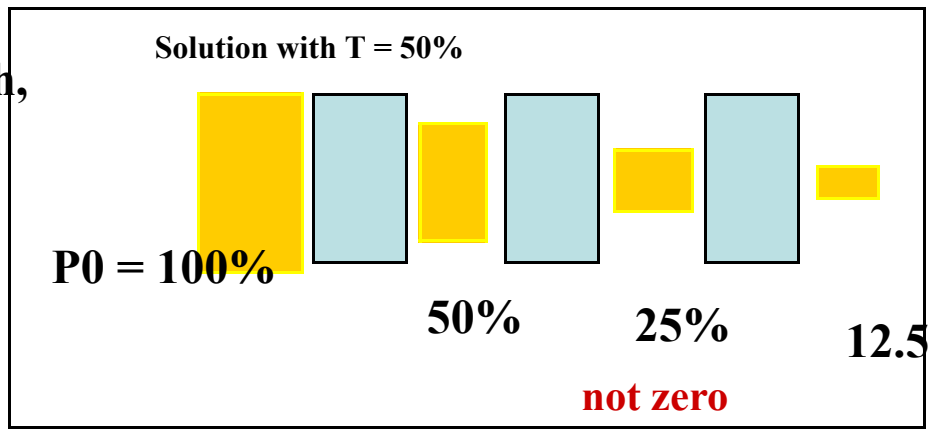




Beer's law is a relation between absorbance and concentration which is a straight line passes by origin at constant pathlength, b, and at certain wavelength, .

Beer's law is obeyed for monochromatic light

Transmittance decreases exponentially as concentration increases




temperature control
sample compartment

blank
sample
shutter
source
chopper
λ_1 λ_2 λ_3
monochromator
sample
P_T
mirror
signal processor
blank
P_0
mirror
semitransparent mirror
detector

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

Derivation of Beer Lambert Law.

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

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

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

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

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

1- Real Deviation

كل انحراف مشروح بالتفصيل

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).

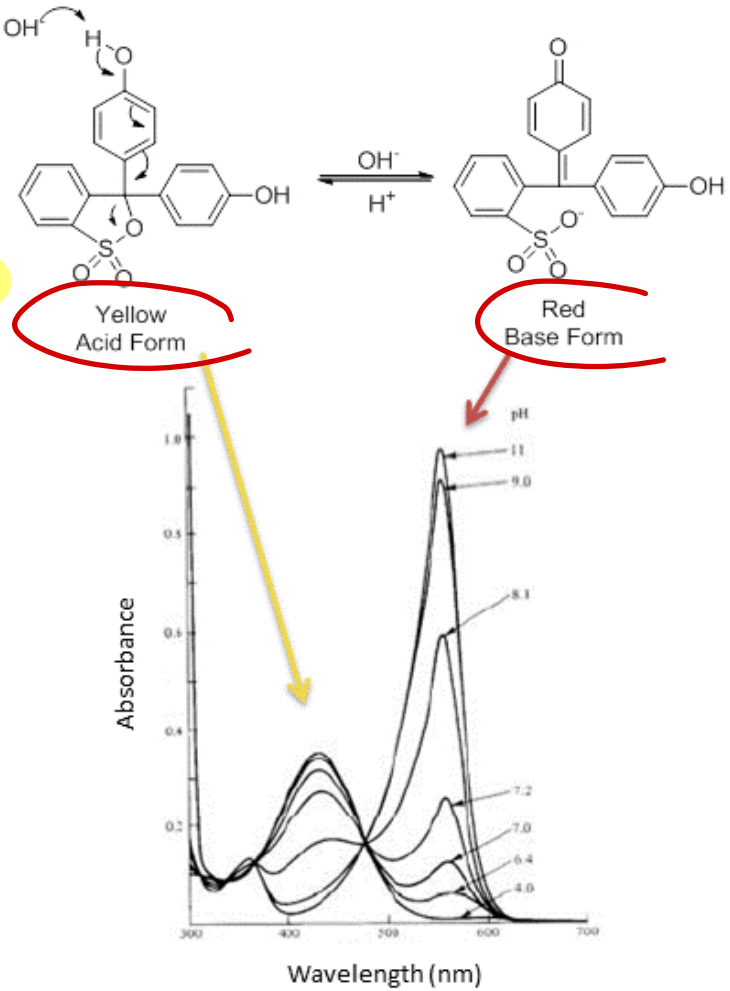
When the concentration of the analyte in the solution is high (> 0.01 M or 10 mM), the analyte begins to behave differently due to interactions with the solvent and other solute molecules and at times even due to hydrogen bonding interactions.

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.

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-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.

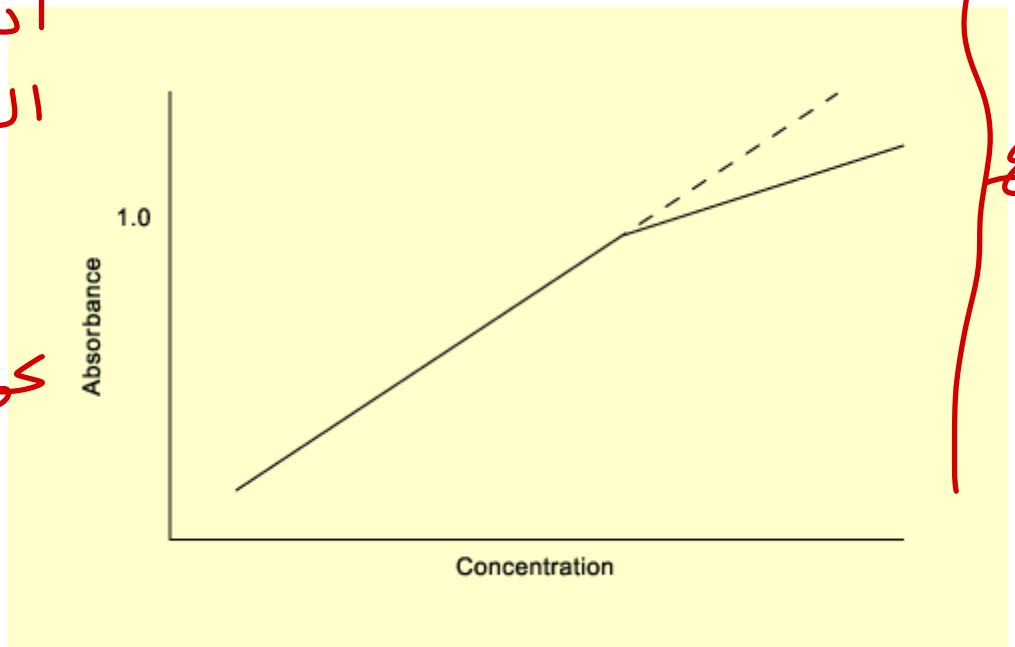
الاحمره الحديثه
تخلصت من جاي الخطأ

B] Due to Presence of Stray Radiation

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

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