تفريغ تحليل آلي

اسم الموضوع: *fundamental of Spectrophotomety*

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ان الدُّفعات

Instrumental Analysis

Fundamentals of Spectrophotometry

الضوء✨✨✨

Qualitative & Quantitative

finger print ↵

λ max

↳ Absorbance

hybridization

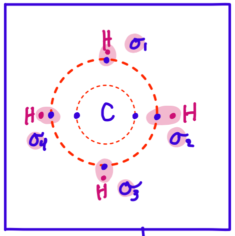
التهجين

sp³ sp² sp

(المدار الأخير) Valence

CH4

light



molecules

↓

Bonds + Atomes

- → σ
- → π
- → $\equiv$

noble مابتكون موجودة في ال Gases

الباقي بعمل molecules مشان

بصير يشبه الغازات النبيلة ويستقر

عشان يكون في π لازم يكون في σ

وعشان يكون في $\equiv$ لازم يكون في $\pi + \sigma$

بأثر فقط على Valence

بمنطقة موجة واحدة

طول موجي معين والـ طاقة محددة

fingerprint of matter

بتكون كافية لحتى تهيج e^- التكافؤ

Objectives

- Fundamentals of Spectrophotometry
  - Properties of light (Particle, energy)
  - 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

عدد الموجات لهذا الطول

شدة الضوء intensity ← يعني كم موجة امتصت

باقي العناصر ← **molecules**

ions

Nopel Gases only ← **atoms**

Mixtures

solid

liquid

gas

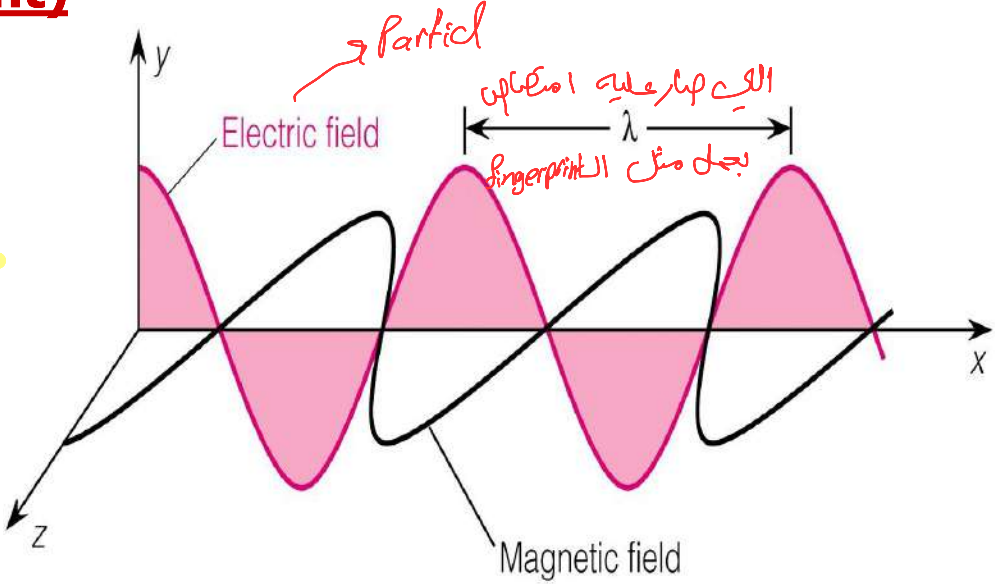
solutions

Qualitative Analysis

على مبدأ الـ fingerprint

Electromagnetic radiation (light)

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



Particl

الي صار عليه امتصاص

بعمل مثل الـ fingerprint

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

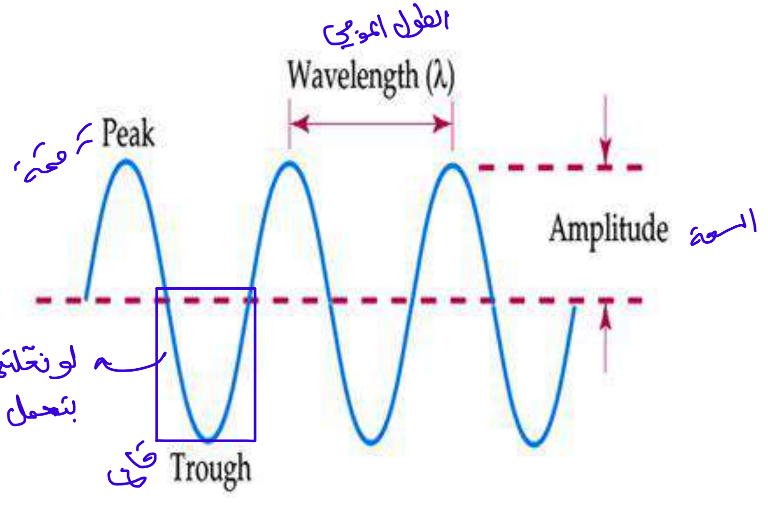
Å = 10^{-10}

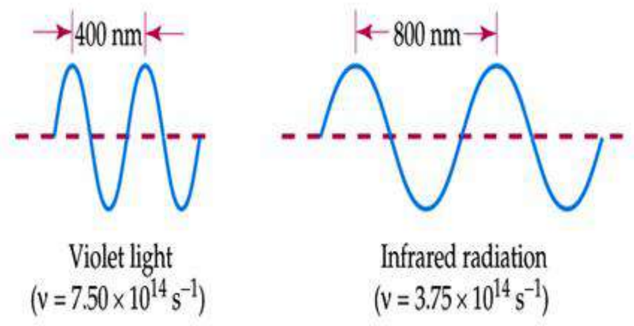
Frequency (υ, ***Greek nu***): التردد
Number of peaks that pass a given point per second.

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

Wavenumber → راح نتعامل معو IR
Number of waves per cm.

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





انعكاس انكسار الضوء

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

علاقة الضوء بالمواد

انحراف الضوء

Wave Calculation

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

λ

ν

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

$C = \nu \cdot \lambda$

3×10^8 m/s = سرعة الضوء

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

C = ثابت لجميع

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

λ

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

$\nu =$

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

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

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

كل طول موجي اله قيمة من الطاقة " طاقة متحركة "

- 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).

light ⟿ molecule → افذ الطاقة من الـفوتون

$\nu, \bar{\nu}, \lambda$ الـ → energy

$$\text{energy} \leftarrow \underline{\underline{E}} = h\,\upsilon$$

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

light ⟿ نعمل Zoom على المدارات

CH_3

↑↓	↑	↑
sp^3	sp^2	sp

$\boxed{↑↓}$ S_1 core

بأثر على الـValence مش الـcore

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

$$E = h\upsilon = h\frac{c}{\lambda} = hc\boxed{\bar{\upsilon}} \quad \frac{1}{\lambda}$$

ومتى بتكون الطاقة كبيرة ؟

- لما λ تكون قليلة ← عدد الـ () Cycles اكثر

يعني عندي كمية موجات → بالتالي ν كبيرة

كبيرة بتأثر على الـ e^- في وقت قصير

← وتكسبه طاقة عالية

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

مش wavenumber ($\frac{1}{\lambda}$) بتكون العلاقة طردية

$E \propto c$

$E \propto \nu$

$E \propto \bar{\nu}$

$E \propto \frac{1}{\lambda}$

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,

Planck constant

$$E = h\nu$$

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

In vacuum, velocity of light = c = $\nu\lambda$ = 3 x 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

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

λ

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

$\nu = \frac{3\times 10^{8}}{7\times 10^{-7}}$

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

$= 4.29 \times 10^{14}\ \frac{1}{s}$

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

$E = h\nu$

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

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

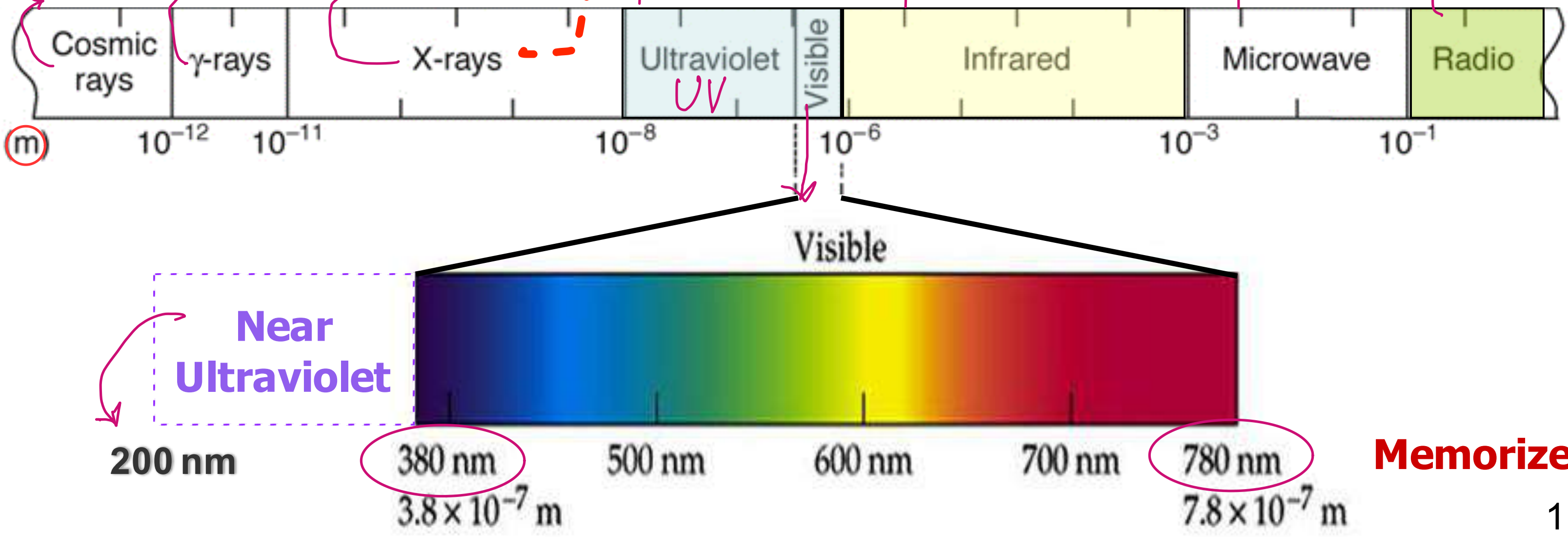
Regions of electromagnetic radiation

السلايد هاد جزئين
لأنه في معلومات كثير عليها

العظم فاتح

الباقي غامق

لأنه العظم كثيف فبعكس الاشعة بينما العضلات والسوائل كثافتها قليلة فبتمرر الاشعة



λ (nm)

$>10^{-4}$

$10^{-4} - 10^{-3}$

$10^{-3} - 10$

UVA far
طاقة عالية
10 – 200 nm

UVB near
200-880 nm

UV

IR near
780- 3000 nm

IR middle
8000–30,000 nm → mid الجهاز نعطاء ال

IR far
30,000 – 3M nm

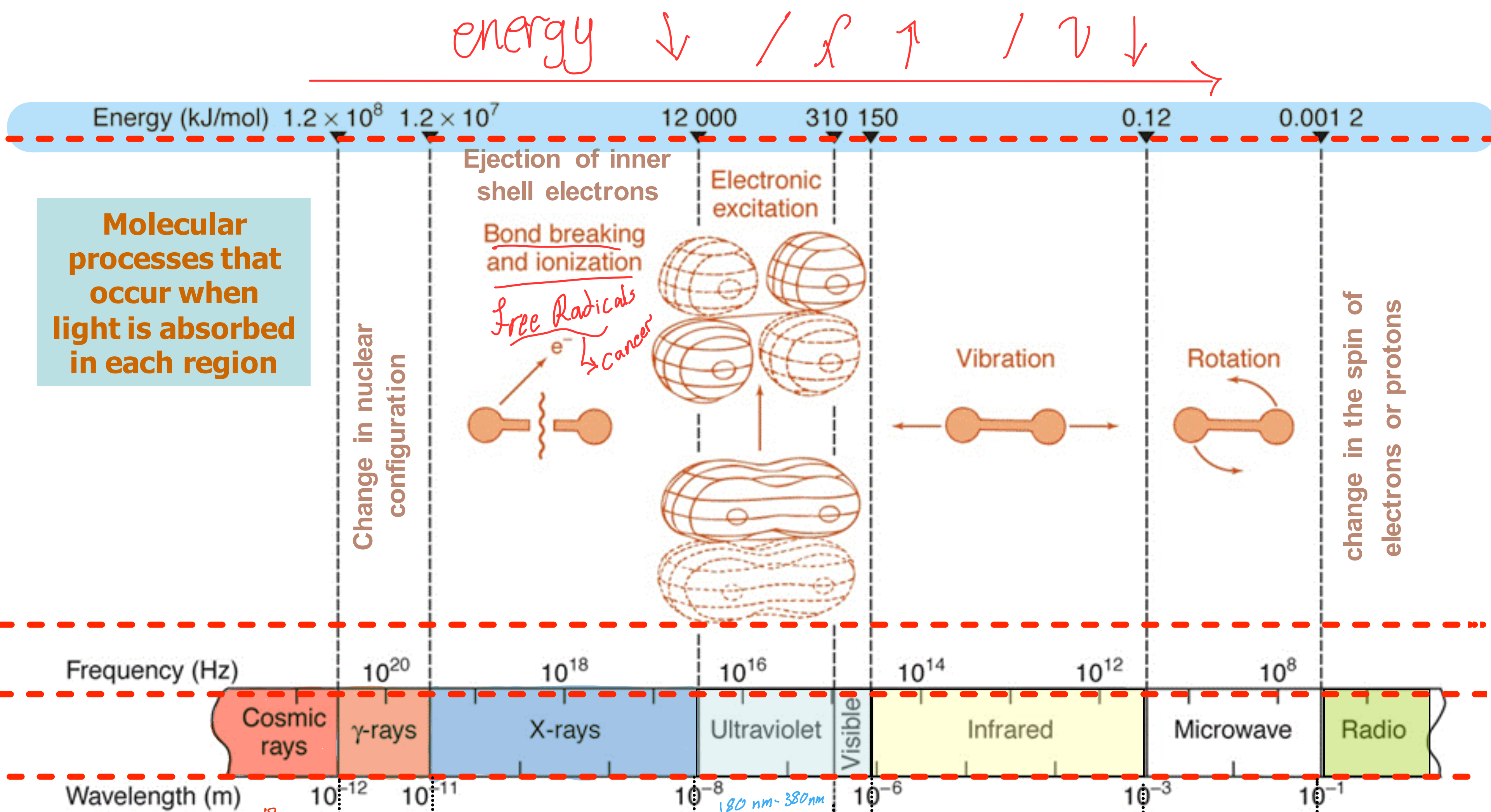
$3M - 10^9$

$>10^9$

Memorize

energy ↓ / λ ↑ / ν ↓

Molecular processes that occur when light is absorbed in each region



$> 10^{-12}$

* أعلى طاقة بينعم، اقل λ

* إذا بتخطى المادة بتفتتها

* بيتجاوز الـ valence وبوصل للـ Core و الـ nucleuse

* بترجعها لـ ($n^{\pm}$, p^+, e^-)

* تستخدم بالأسلحة، تأتي من الشمس ويعكسها الأوزون

* بتفكك الـ Valence والـ Core بس ما بتأثر على النواة

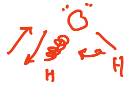
الموجودة داخل المستشفيات (للعظام والاسنان) بتفكك الـ Bonds

بس عند الـ Valence ما بتوصل الـ Core

بقدر استخدمها لمرة واحدة خلال مدة زمنية

ما بفكك أي Bond بس بهيج الـ e^- وبنتقله للـ next Level وبرجع لمكانه

الـ Bond بتعمل Stretching & Bending



بزيد الطاقة الحركية للجزيئات

، حركة الـ molecules داخل الـ Solids صعبة عكس الـ liquids

- بتأثر على الجسم على المدى البعيد

بتنتقل بالفضاء وما إلها تأثير على الجسم

UV-Vis

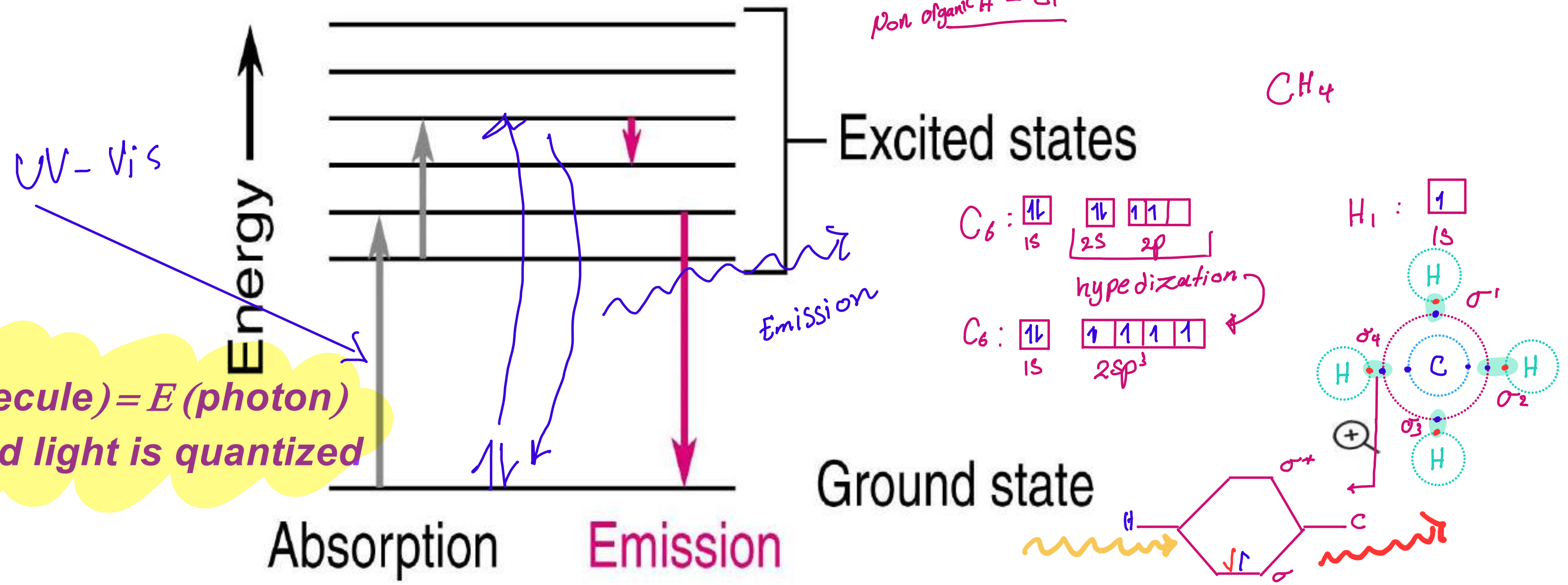
Absorption of light

on covalent Bond
Not work in ionic Bond

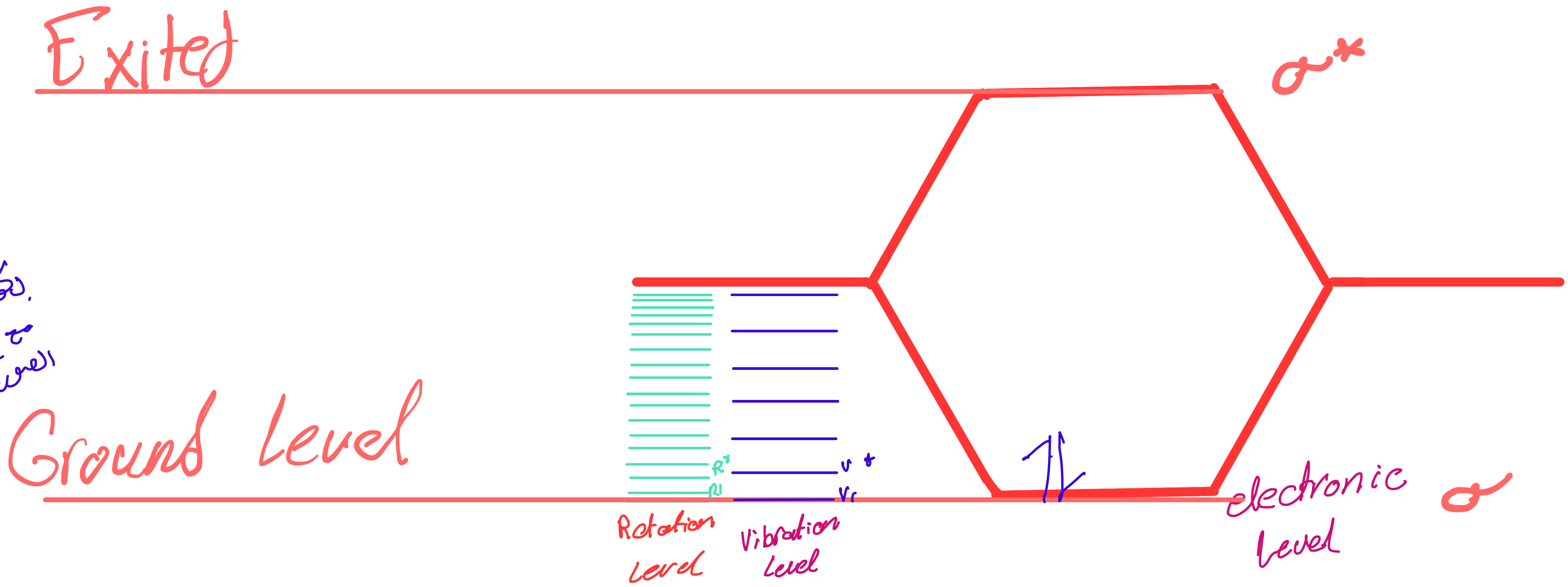
A **molecule** that absorbs light photons will end up with increased energy. The molecule will be promoted to an **excited** state. Microwave energy will cause rotation of compounds. IR energy is high enough to promote bond stretching. UV/Vis energy promotes electrons into higher orbitals. Short-λ UV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

Covalent
Non organic H – Cl

ΔE (molecule) = E (photon)
absorbed light is quantized



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



بكونوا متلاصقين مع بعض زي الصورة تحت

$E \downarrow$

المسافة بين الـ levels بتقل فالطاقة اللازمة للانتقال بينهم بتقل

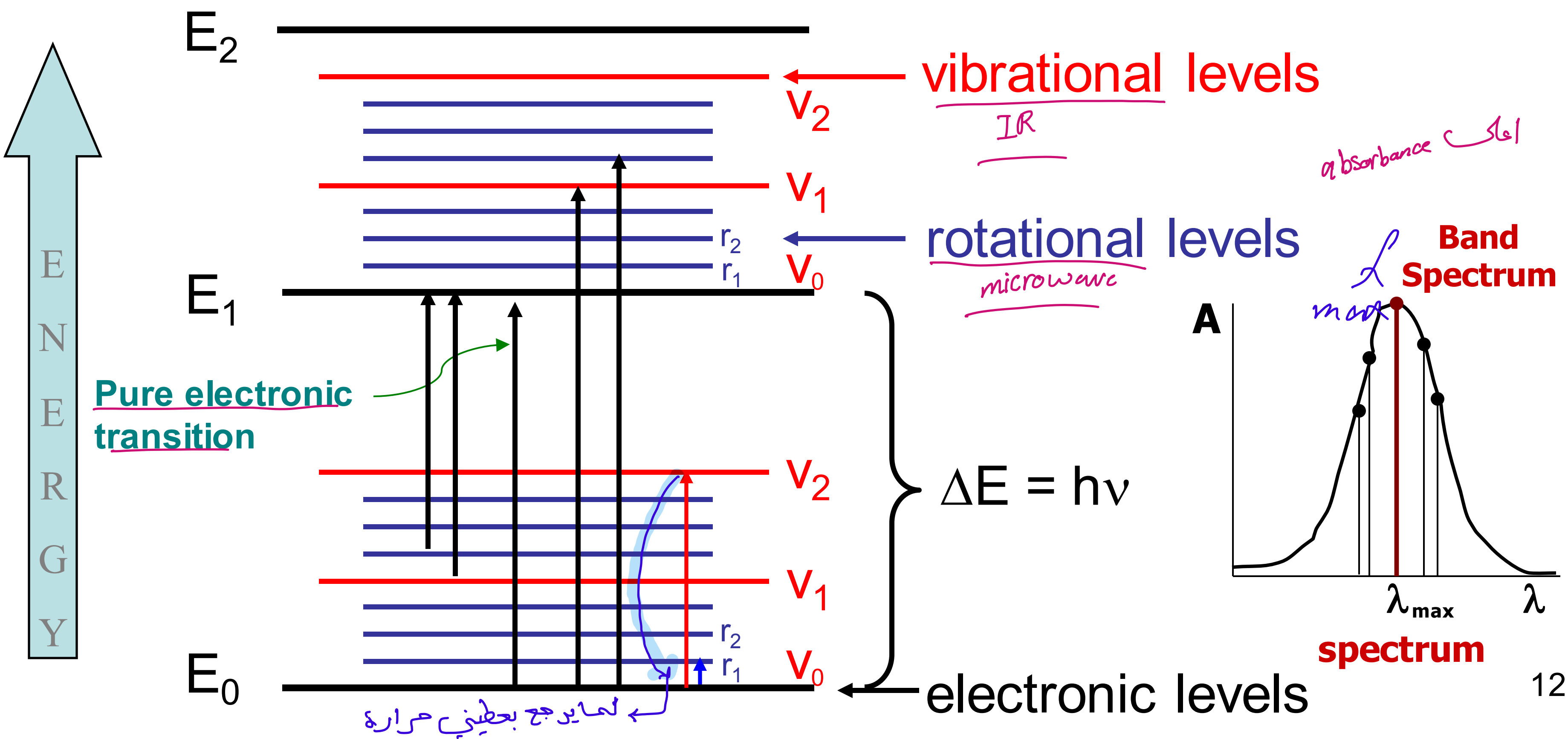
$E_{etl} \gg E_{vib} > E_R$

UV-Vis	$E^{\circ} \rightarrow E^{*}$ $R \rightarrow V \rightarrow E$	Light
IR	$V^{\circ} \rightarrow V^{*}$ $R \rightarrow V$	heat
Microwave	$R^{\circ} \rightarrow R^{*}$	heat

Ultraviolet-Visible Spectrophotometry

What happens when a molecule absorbs UV-Visible radiations?

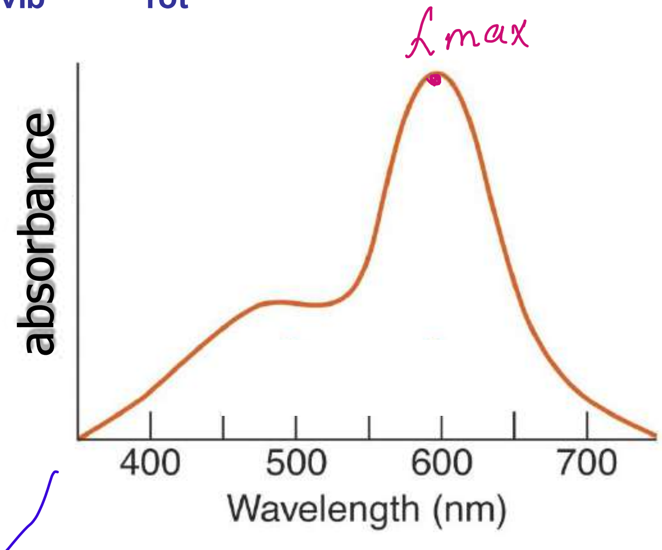
- When a molecule absorbs light having sufficient energy (e.g. UV-Vis radiation) to cause an electronic transitions, (additional vibration and rotation transitions also occur)
- Molecule can absorb one photon of just the right energy to cause the following simultaneous changes:



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 = $E_{elec} + E_{vib} + E_{rot}$

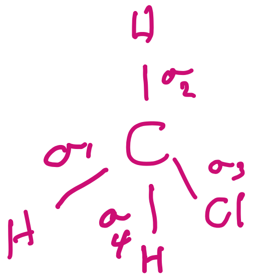
$$\Delta E_{elec} >> \Delta E_{vib} >> \Delta E_{rot}$$

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)

مش ا



2 types of Bond

C – H

C – Cl

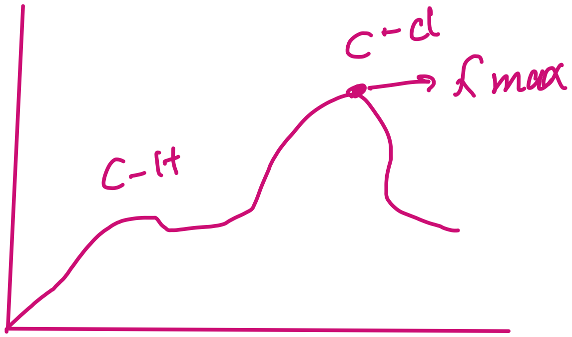
فَ لما يسقط الضوء

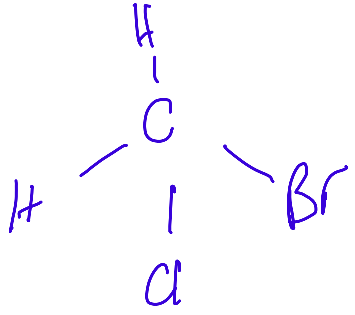
C – H بتمتص موجة

الـ λ_1

و

C – Cl بتمتص موجة الـ λ_2





3 types of Bonds

C – H

C – Cl

C – Br

fingerprint → λ max

pharmacopiea بتكون موجودة في

الـ molecule كامل

مبدأ عمل الجهاز

2 holders →  مكان الـ Cuvette

1) فيها الـ Sample + Solvent

2) فيها فقط الـ Solvent

↳ Blank "

عندي mirrors وظيفتها تعكس الضوء و

توزعه بطريقة متساوية من مصدر الضوء

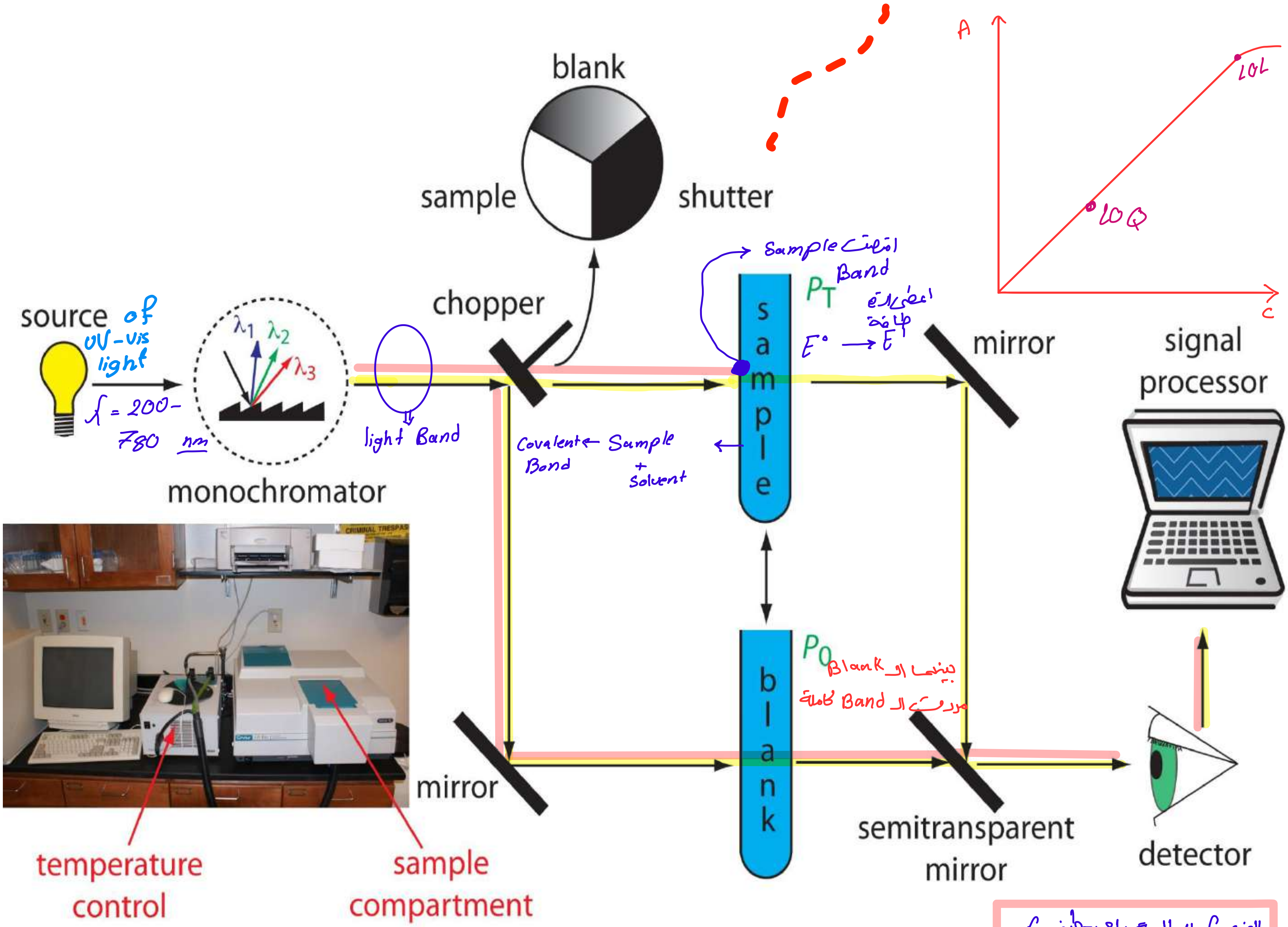
أول اشي : بدخل حزمة كاملة من الضوء

وبحدد ايش الطول الموجي يلي ما تم

امتصاصه ← fingerprint

ثاني خطوة : بدخل λ الي امتصتها

العينة وبحسب الـ $\underline{A}$



الضوء الأحمر المطلوب راح يعطيني λ_{max}

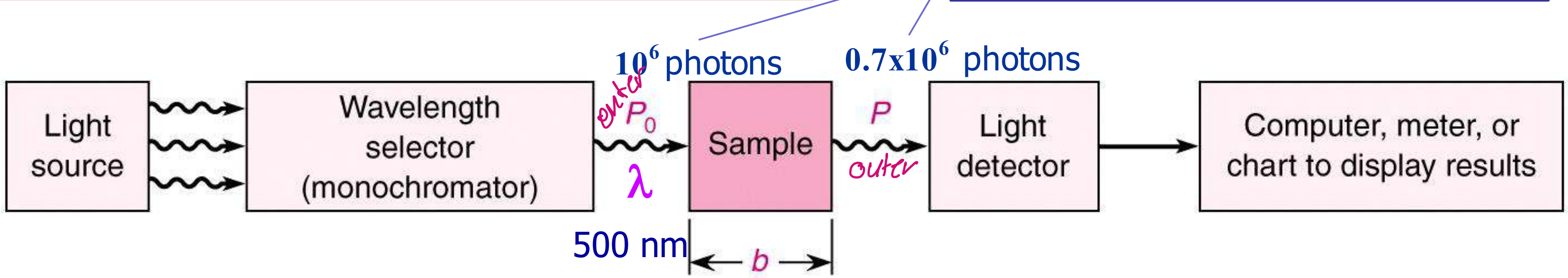
الضوء كامل - الضوء المطلوب

يارب تكون واضحة

غيرت
ترتيب السلايد

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, P_0, 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"**

$$T = \frac{P}{P_{\circ}}$$

$$\boxed{0 \leqslant T \leqslant 1}$$

The percent transmittance, %T, is simply 100 T

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

$\frac{P}{P_0}$ = الضوء الذي تم

امتصاصه Transparent

$P = P_0$ يعني العينة ما

$T = \frac{1}{1} = \boxed{1}$ امتصت اي ضوء

$P = 0$ يعني ان العينة امتصت

الضوء كله وما خرجت اي ضوء

$T = \frac{0}{1} = \boxed{0}$

$0 \geqslant T \geqslant 1$

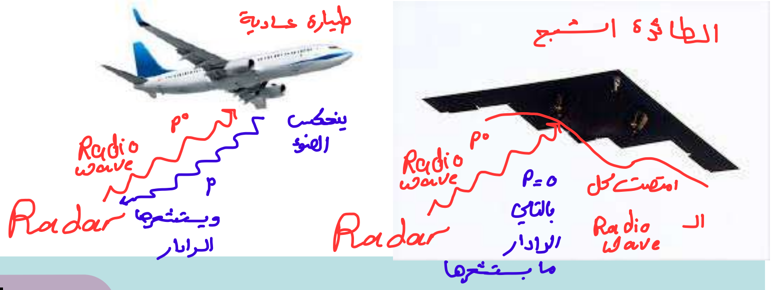
زجاج الطائرات $T\% = 98\%$

2. **Absorbance, defined as:**

Unitless

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

For purpose of chemical analysis



Absorbance is directly proportional to:

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

بستخدم excel
وبخلي intercept
يساوي صفر
فبعطيني معادلة جديدة

$y = mC$ ± intercept

$y = mC$
slope
جديد

Beer's law

$$\boxed{\overset{y}{A} = \underbrace{\varepsilon}_{\text{molar absorbativity}}\overset{\text{constant}}{b}\underbrace{c}_{\text{concentration}}}$$

قيمة عملية

بيجي مع الجهاز
الـ Cuvette
صارت ثابتة

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

$A = y$ بعد التخيل

$\varepsilon \underset{1=}{b} \cancel{C} = m \cancel{C}$

$\varepsilon = \frac{m}{b}$
$b \rightarrow = 1$ cm

$\varepsilon = m$

في حالة intercept = 0

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

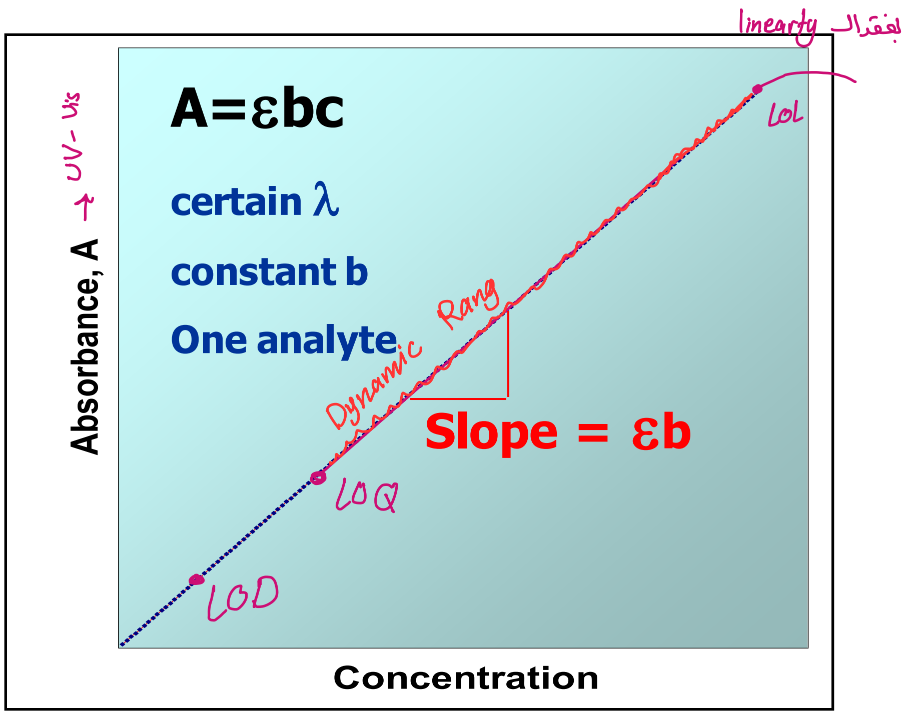
"Absorbance of 1 M solution measured in a cell of 1 cm path length"

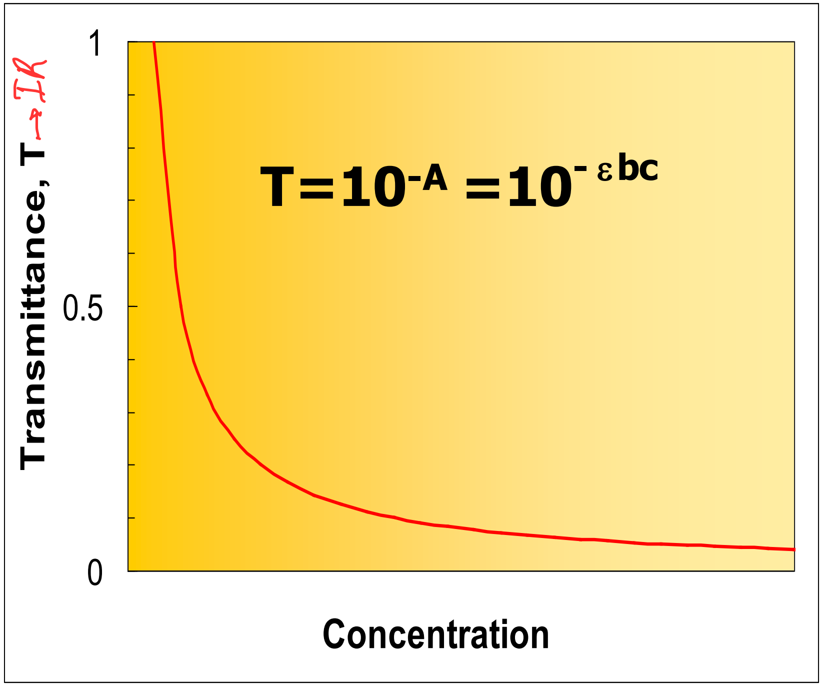
unitless

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

ε, is characteristic for each substance at a particular wavelength, λ.

العلاقة بين A & C وبين C & T

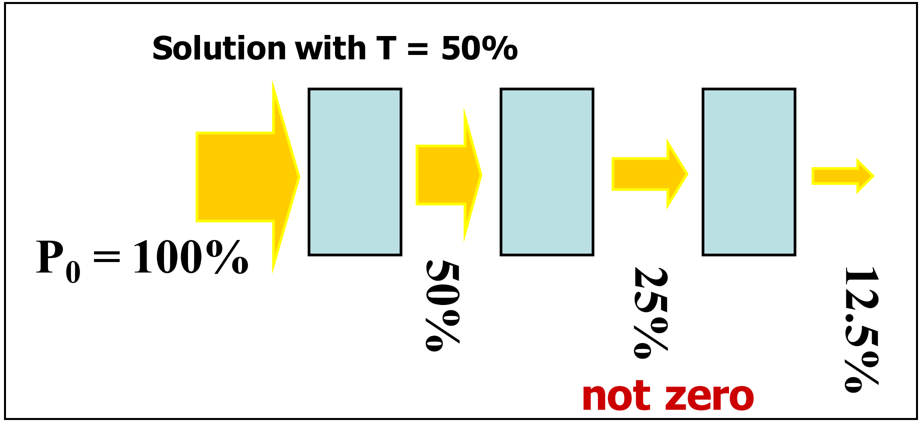




Beer's law is a relation between absorbance and concentration which is a straight line passes by origin at constant pathlength, b, and at certain wavelength, λ.

Transmittance decreases exponentially as concentration increases

Beer's law is obeyed for monochromatic light



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.

موانع تطبيق Beer's law

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

ظروف

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

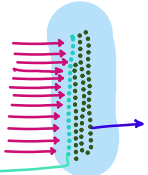
Beer law and Lambert law is capable of describing absorption behavior of solutions containing relatively low amounts of solutes dissolved in it (<0.01 M or 10 mM).

لازم 10 mM
او اعلى

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.

يعني قراءة الجهاز كانت قليلة وغير مطابقة للعينة بسبب انه الجزيئات ما وصلها ضوء حتى تعطي قيمة Absorbance حقيقية

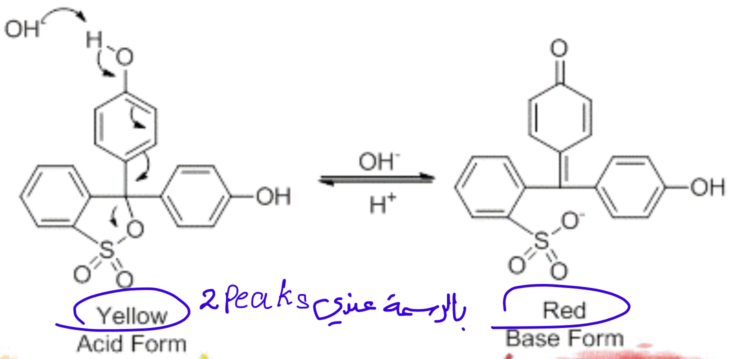


2- Chemical Deviations

ما في وضع منيح ووضع خطأ

لكن لازم اعمل optimize بحيث تكون كل المادة in one form

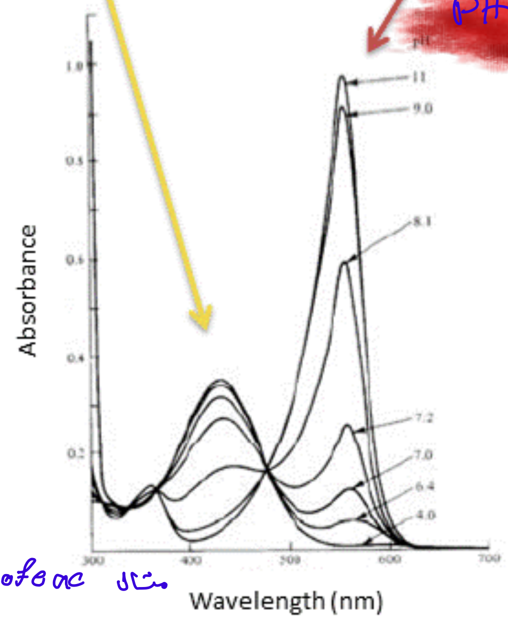
Chemical deviations occur due to **chemical phenomenon** involving the analyte molecules due to association, dissociation and interaction with the solvent to produce a product with different absorption characteristics.



2 Peaks بالرسمة عندي

بتقلل ال PH

برفع ال PH



indecator → Acid - Base titration

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.

المادة لما بدي احللها لازم تكون one type, one form
one molecule

إذا صارت 2 types, 2 molecules واجيت اشوف ال peak راح تكون الكمية مش حقيقية

لأنه جزء من المادة صار In another form

مثال Diclofenac

organic → salt

$DCF-H \rightarrow DCF^{-}N^{+}$

سام ← log p = 4.1

دوا log p = 2.1

بتراكم داخل الخلية لحدما يصير قاتل

بصير عليه metabolism وبطلع من الخلية عادي

الموجود داخل عينة البول هو Salt form فبنقدره على انه Salt

3- Instrumental Deviations

mono = 1

poly > 1

chromatic

يعني لون

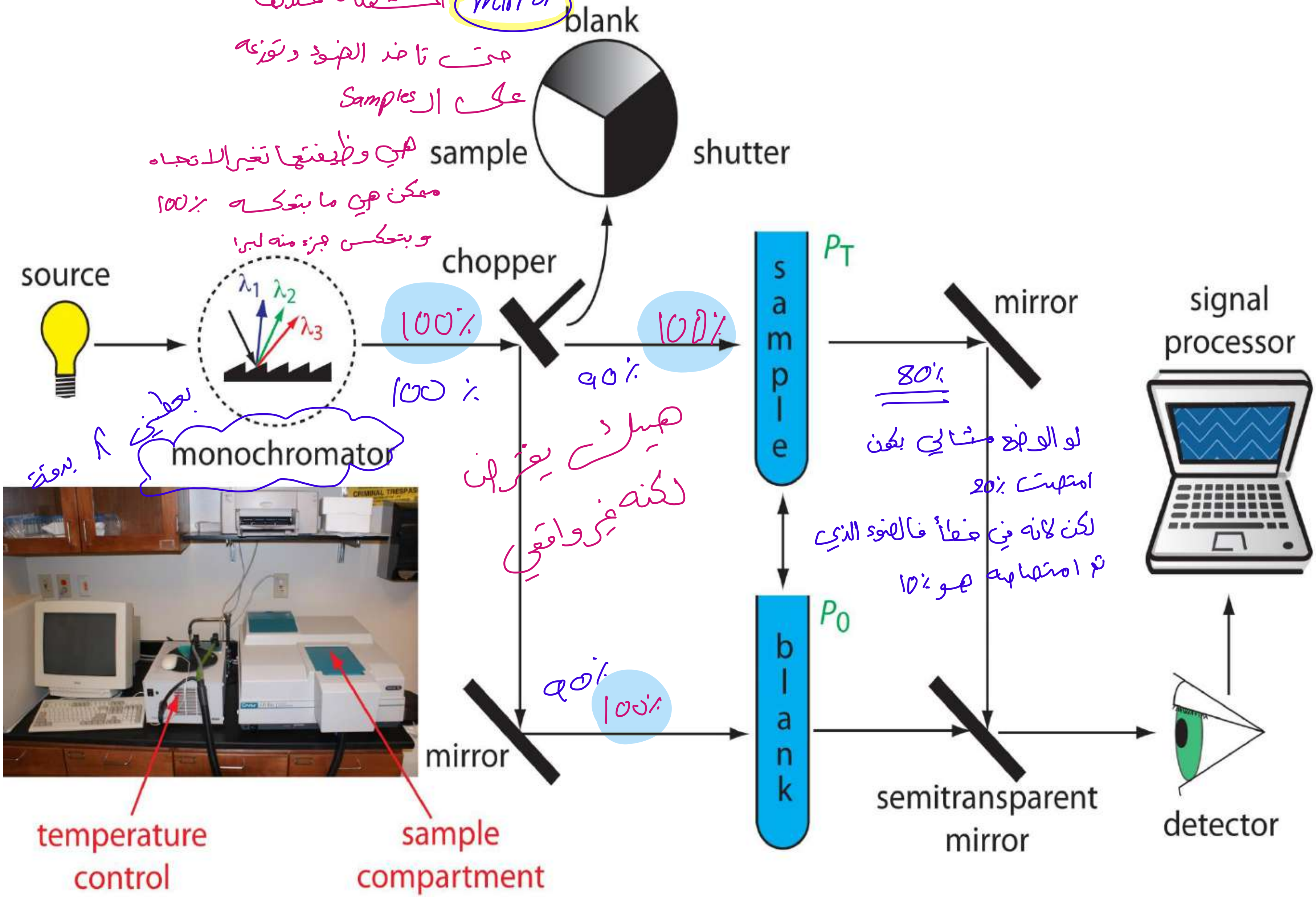
A] Due to Polychromatic Radiation

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.

B] Due to Presence of Stray Radiation

بعض الأشعة الضائعة

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.



mirror المشكلة عندها
حتى تاخذ الضوء وتوزعه
على الـ Samples

هي وظيفتها تغير الاتجاه
ممكن هي ما بتعكسه 100%
وبتعكس جزء منه لبرا

بعطيني λ بدقة

100%

100 %

90%

100%

هيك بيعتبروا
لكنه غير واقعي

80%

لو الوضع مثالي بكون
امتصت 20%
لكن لانه في خطأ فالضوء الذي
تم امتصاصه هو 10%

90%

100%

كررت السلايد
عشان الدكتور شرح عليه

$A = \varepsilon \underline{b} C \rightarrow$ ما يزيد عن

0.01 M

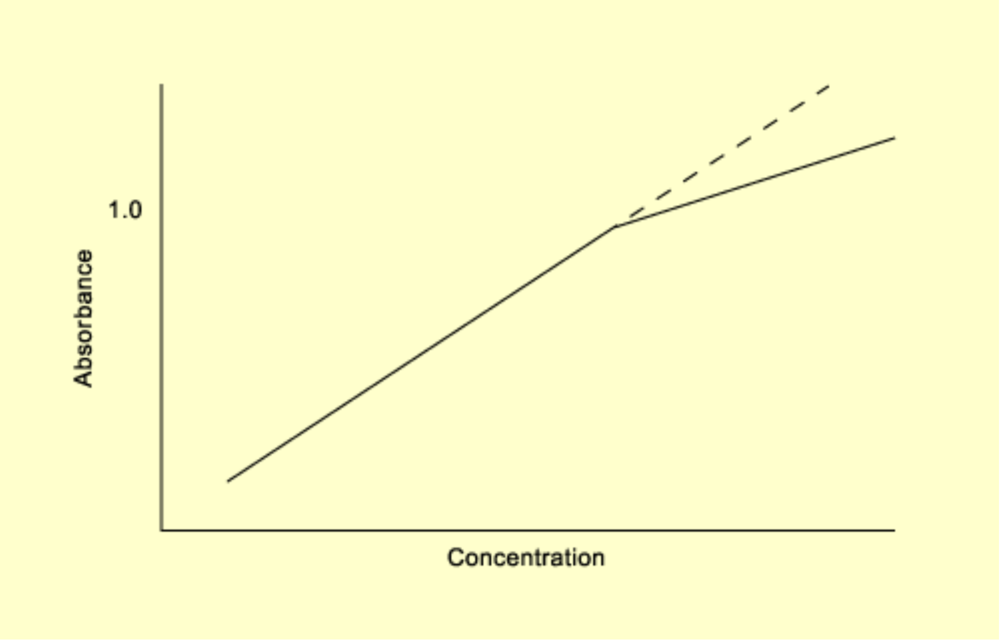
او

10 mM

$b \rightarrow$ ثابتة

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