Experiment 4
UV - spectroscopy

quantitative analysis

الكلام اللي تحت تركيز الـ Sample اي عندي

Aim of the experiment

- To be able to determine the concentration of a pharmaceutical compound " paracetamol" using UV spectroscopy.
- To be able of determining the percentage of label of a pharmaceutical dosage form using UV spectroscopy.

Unknown Sample

paracetamol in any dosage form // percentage of Concentration

assay *

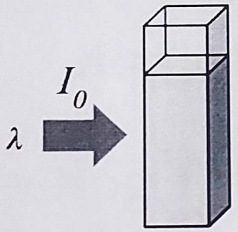
λ_{max} : absorbance at maximum wavelength.

Different compounds may have very different absorption maxima and absorbance.

What are transmittance and absorbance?

Consider monochromatic light transmitted through a solution; with an incident intensity of I_0 and a transmitted intensity of I.

$I < I_0$



شدة الضوء الخارج من العينة أقل من الداخل عليها لان المادة امتصت منه داخل الـ sample

القيمة بين 0 - 1

The transmittance, T, of the solution is defined as the ratio of the transmitted intensity, I, over the incident intensity, I_0 and takes values between 0 and 1.

initial intensity

$$T = \frac{I}{I_0}$$

fraction of I/I_0

* اذا T=1 الضوء مخرج الذي طلع صاوي للي دخل $I_0 = I$

* اذا T=0 العينة امتصت كل الضوء وما طلع ضوء $I = 0$

However, it is more commonly expressed as a percentage transmittance:

$$T(\%) = 100\frac{I}{I_0}$$

$\frac{I}{I_0}$ * 100 فقط بضرب

What are transmittance and absorbance?

- The absorbance, *A*, of the solution is related to the transmittance and incident and transmitted intensities through the following relations:

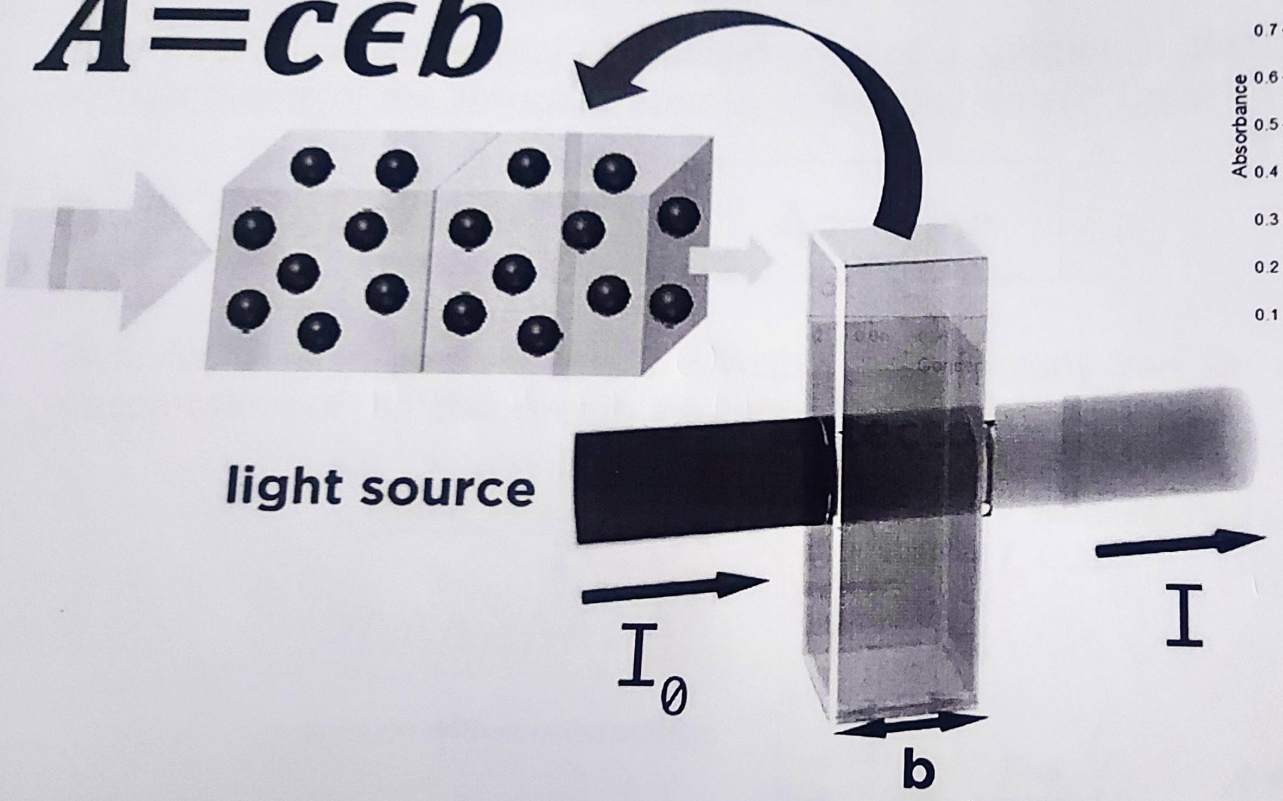
$$A = \log_{10} \frac{I_0}{I}$$

$$A = -\log T = \log \frac{1}{T}$$

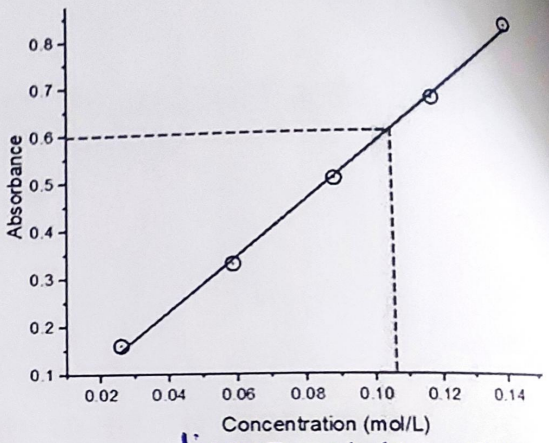
- The absorbance has a logarithmic relationship to the transmittance; with an absorbance of 0 corresponding to a transmittance of 100% and an absorbance of 1 corresponding to 10% transmittance

- Intensely absorbing compounds must be examined in dilute solution, so that significant light energy is received by the detector, and this requires the use of completely transparent (non-absorbing) solvents in the same UV region.

$$A = c\epsilon b$$



path length.



linear relationship.

What is the Beer-Lambert Law?

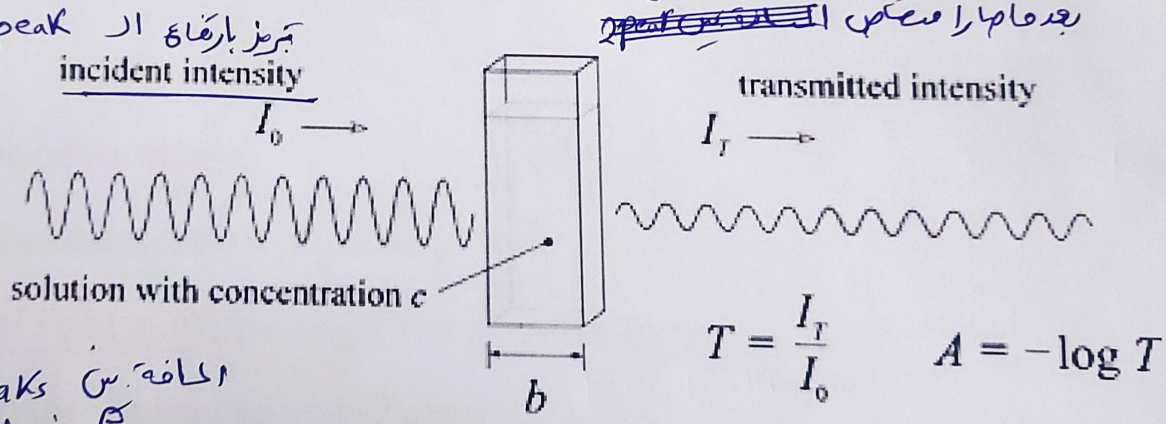
The Beer's law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length.

ε و b ثوابت
اذاً العلاقة بين A و C

$$A = \varepsilon bc$$

ε = epsilon

Thus, for a fixed path length, UV/Visible spectroscopy can be used to determine the concentration of the absorber in a solution.



peak تمثل بارتفاع ال

الارتفاع قل

2peaks تمثل ثابتة

frequency ثابت

intensity قل كانت جزء منها امتصت الاشعة

اذا بين تركيزها بال molar تكون العلاقة بين A و C (molar) طردية

C ← A علاقة طردية مع

- Because the absorbance of a sample will be proportional to its molar concentration in the sample cuvette, a corrected absorption value known as the **molar absorptivity** (ε) is used when comparing the spectra of different compounds. It is a physical constant of the compound at a particular wavelength. This is defined as:

ثابت لكل مادة وبوجه reference

$A = \varepsilon bc$

* اذا كان 1molar = C

$A = \varepsilon$

Corrected absorbance

if C = 1 molar.

Molar Absorptivity; $\varepsilon = A/(c*b)$

Where

A= absorbance (unitless)

c = sample concentration in moles/liter (molar unit ما يزيد فيه)

b = length of light path through the cuvette in cm (diameter of Cuvette) (اذا قلت المسافة الذي يقطعها الـ light داخل العينة)

ε = molar absorptivity in L mol^{-1} cm^{-1}

بعد تعويض الوحدات بالقانون

L/mol.Cm

* اذا عندي 2samples الهم نفس التركيز constant C .
وحطيتهم بنفس Cuvette الهم نفس b (path b Constant)
معناها كل ما زادت قراءة ε بزداد A
معناها عينة ε عندها اعلى تمتص ال light (A) اعلى من عينة الثانية اي ε قليلة الها

Absorbance	**A**, a measure of the amount of radiation that is absorbed
Chromophore	Structural unit responsible for the absorption (مسؤول عن امتصاص ال light)
Molar absorptivity	ε, Parameter defining how strongly a substance absorbs light at a given wavelength per molar concentration ε↑ → A↑
Path length	***b***, the length of the sample cell in cm
Beer's Law	$A = \varepsilon bc$
$\boldsymbol{\lambda}_{max}$	The wavelength at which maximum absorbance occurs
$\boldsymbol{\varepsilon}_{max}$	The molar absorbance at $\boldsymbol{\lambda}_{max}$

- The value of **ε** is usually given by the pharmacopeia in pharmacopoeial procedure or else it can be determined practically in the lab.

- The Beer's law (A= εbc) represents a linear equation; (y = ax + b), where the intercept (b) in the case of Beer's law is zero and the slope (a) is εb. **As "b" is fixed = 1cm, the slope will be equal to the molar absorptivity "ε".**

- In order to be able to obtain the value of **ε**, a reference standard material of the analyte should be available; if so prepare a series of standard solution of the material and measure them properly at the desired wavelength. • Plot the obtained values of absorbance against corresponding concentrations. The slope of the obtained linear line should represent **ε**.

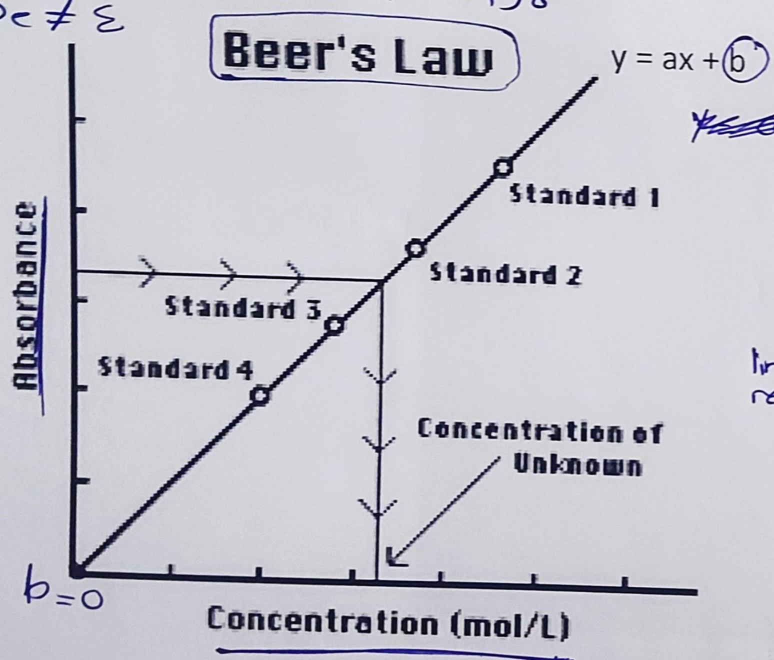
* العلاقة بين Absorbance و Concentration تقدر اطلع منها Molar absortivity ε

وممكن عشان اعرف C_{Molar} اذا عندي Absorbance

If we plot absorbance against concentration, a straight line passing through the origin (zero) should be obtained and a linear relationship is observed as it obeys Beer's law.

لازم لما اطبقه يكون عندي b=0 لأن اذا غير هيك معناها

slope ≠ ε



intercept = zero

linear relationship

y = ax + b
y= A= absorbance
X= molar concentration
b= 0
a=slop = εb
b(path length) = 1
a= ε
A= εx

1 = ثابتة

اذا عندي عينة مجهولة

absorbance = ε C (Molar Concentration)

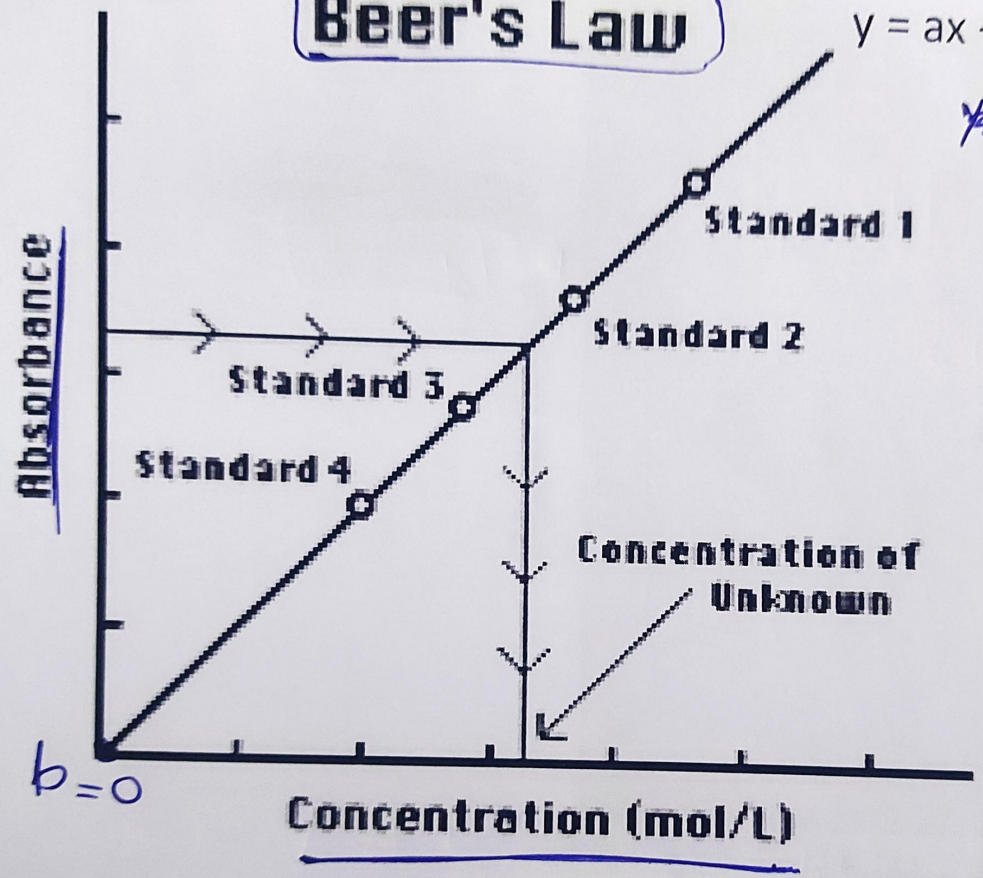
* اذا عندي عينة وقست absorbance وبدي استنتج كم Concentration برجع على ال Curve وبشوف ال Absorbance اي

تركيز ال Concentration منه

او باخذ absorbance وبعوض بالمعادلة $A = \varepsilon X$ وبدي ε بعطي X Molar Conc.

obtained and a linear relationship is observed as it obeys Beer's law.

لازم هاا الطبعة يكون عندي b=0 لانه اذا غير هيك معناها slope ≠ ε

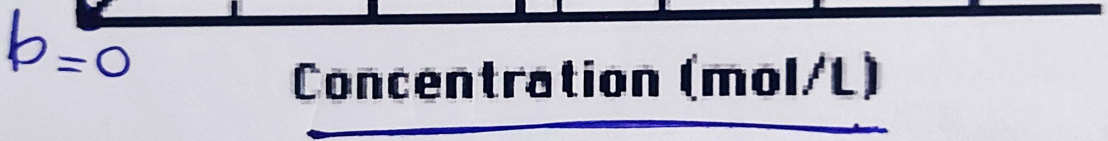


linear relationship

y = ax + b
y= A= abs
X= molar
b= 0
a=slop =
b(path l
a= ε
A= εx

absorbance =

* اذا عندي عينة وقست absorbance وبدي اسوي كم Concentration برجع على ال Curve وبيطلعلي Absorbance اي عندي كم Concentration عند ال absorbance وبعوضهم بالمعادلة $A = \varepsilon X$ وبدي ε بطلع $\frac{X}{\text{Molar Conc.}}$



* اذا عندي عينة وقست absorbance وبدي اشوف كم

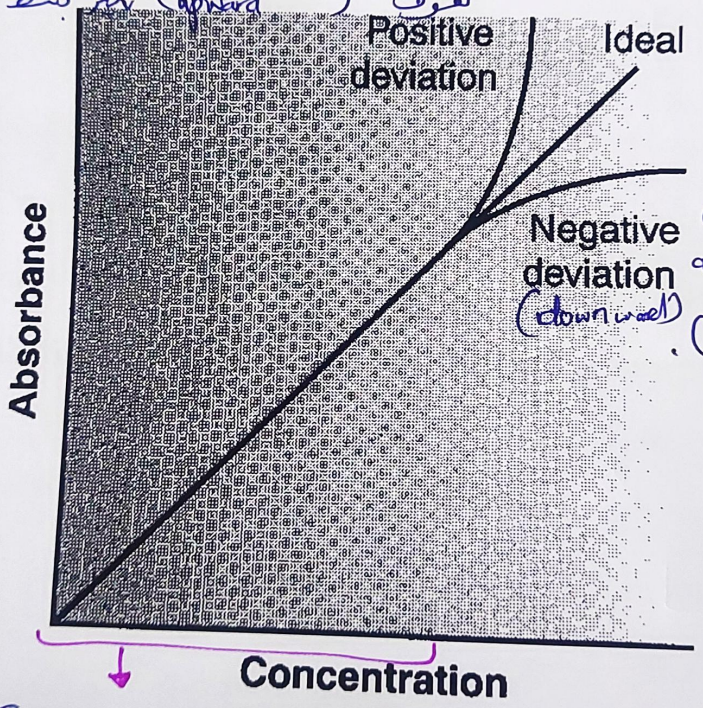
ab

Concentration برجع على ال Curve وبشوف ال Absorbance الي

عندي كم ال Concentration عنه

او باخد ال absorbance وبعوض بالمعادلة $A = \varepsilon X$ وبدي ε بطلع $\frac{X}{\text{Molar Conc.}}$

But yet there are some factors that lead to deviate from a linear relationship between concentration and absorbance and a subsequent apparent failure of Beer's law. Deviation from Beer's law are reported as positive or negative; according to whether the curve is concave upward or concave downward.



متى يصبح صعب استخدام beers law

Deviation from Beer's law may occur due to the following factors:

Sample has impurities → تعمل امتصاص للـ light

1) Presence of impurities that absorb at the same absorption wavelength.

light of one wavelength

2) If monochromatic light is not used. *I_0 ↓ اختلاف في Incident Intensity*

معناها اذا الـ Slit بيتغير الـ width باستمرار (مرة ضيق ومرة واسع) الـ I التي تدخل للعينة لا تكون ثابتة. / الضوء يمر بأكثر من wavelength

3) If width of slit is not proper, therefore allows undesirable radiations to fall on the detector.

physical structure المركب

4) If the solution species undergoes polymerization, ionization, dissociation, association or any other chemical change during analysis. *المركب في العينة له chemical structure معينة فاذا تغير نتيجة تغيره او (unstable) تكسير، تفاعل مع مركب آخر، bi-bond معناها تغير λmax ، intensity*

5) If the sample concentration is high (> 0.001M) as the distance between particles decreases and allows for interaction between the absorbing particles such that the absorption characteristics of the analyte are affected.

زي ما حكينا

تركيز العينة يجب ان يكون diluted عشان الـ light يمر مرة وحدة وما يمر بأكثر particle لذلك حكينا تركيز العينة يجب ان يكون اقل من 0.001 M

اما اذا تركيز العينة > 0.001 M (واكبر منه).

distance بين particle قليلة

ممكن يصير interaction بين particle او بين particle و light absorbed

فرح يأثر على قراءة الـ absorbance. / تركيز العينة عالي ممكن يصير deviation on beer's law

deviation ممكن يكون very high زي الصفحة اللي قبل حكينا الـ deviation هون يكون على higher conc. (positive deviation)

لذلك المناطق تركيزها قليل صعب يصير deviations.