# EXPERIMENT 4: Ultraviolet-Visible Spectroscopy – Quantitative Analysis

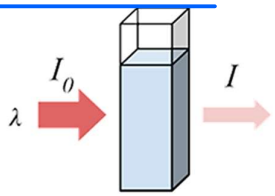
## INTRODUCTION

قد يكون للمركبات المختلفة أطوال موجية قصوى مختلفة جدًا وامتصاصية مختلفة.

Different compounds may have very different absorption maxima and absorbance.

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

لنفترض ضوءًا أحادي اللون ينتقل عبر محلول:
بشدة سقوط $I_0$ وشدة نفاذ I.




**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).

الخارج على الداخل

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

تُعرَّف نفاذية المحلول، T، بأنها نسبة شدة النفاذية، I، إلى شدة السقوط، 100، وتأخذ قيمًا بين 0 و1.

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

ومع ذلك، يُعبَّر عنها عادةً كنسبة مئوية للنفاذية:

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

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

يرتبط الامتصاص، A، للمحلول بالنفاذية وشدة الضوء الساقط والمنفذ من خلال العلاقات التالية:

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

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

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.

يرتبط الامتصاص بالنفاذية بعلاقة لوغاريتمية، حيث يتوافق امتصاص 0 مع نفاذية 100%، ويتوافق امتصاص 1 مع نفاذية 10%.

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.

يجب فحص المركبات شديدة الامتصاص في محاليل مخففة، بحيث يستقبل الكاشف طاقة ضوئية كبيرة، وهذا يتطلب استخدام مذيبات شفافة تمامًا (غير ماصة) في نفس منطقة الأشعة فوق البنفسجية.

الامتصاصية A، مقياس لكمية الإشعاع الممتص (بدون وحدة)

الامتصاص المولي ε، وهو معامل يحدد مدى قوة امتصاص المادة للضوء عند طول موجي معين لكل تركيز مولاري (لتر. مول. سم).

طول المسار b، وهو طول خلية العينة (سم)

الطول الموجي الذي يحدث عنده أقصى امتصاص

الامتصاصية المولية عند max

## *Beer-Lambert Law*

at the intercept= 0

$y = m x$

$A = \varepsilon b c$

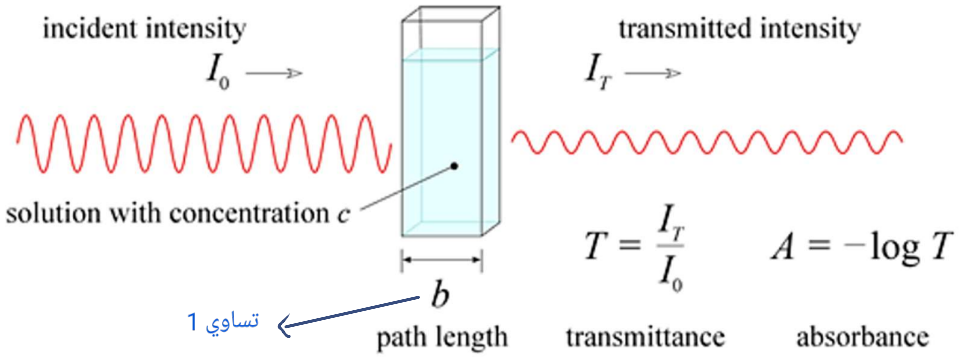
| | |
|---|---|
| **Absorbance** | **A**, a measure of the amount of radiation that is absorbed (**unitless**) |
| **Molar absorp** | **ε**, Parameter defining how strongly a substance absorbs light at a wavelength per molar concentration (**L . mol$^{-1}$ . cm$^{-1}$**) |
| **Path length** | ***b***, the length of the sample cell (**cm**) |
| $\lambda_{max}$ | The wavelength at which maximum absorbance occurs |
| $\varepsilon_{max}$ | The molar absorbance at $\lambda_{max}$ |

ينص قانون بير على أن امتصاص المحلول يتناسب طرديًا مع تركيز المادة الماصة في المحلول وطول المسار.

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.

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

وبالتالي، بالنسبة لطول مسار ثابت، يمكن استخدام مطيافية الأشعة فوق البنفسجية/المرئية لتحديد تركيز المادة الماصة في المحلول.




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 is used when comparing the spectra of different compounds.

This is defined as Molar Absorptivity (ε).

نظرًا لأن امتصاصية العينة تتناسب طرديًا مع تركيزها المولي في خلية العينة، تُستخدم قيمة امتصاص مصححة تُعرف باسم معامل الامتصاص المولي عند مقارنة أطياف المركبات المختلفة. يُعرَّف هذا المعامل بمعامل الامتصاص المولي (ε).

$$\varepsilon = A / (c*b)$$

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



→ عادةً ما تُعطى قيمة ε من قِبل دستور الأدوية في إجراءات دستور الأدوية، أو يمكن تحديدها عمليًا في المختبر.

يُمثل قانون بير معادلة خطية: (Y = mX + b)، حيث يكون التقاطع (b) في حالة قانون بير صفرًا، والميل (m) هو εb.

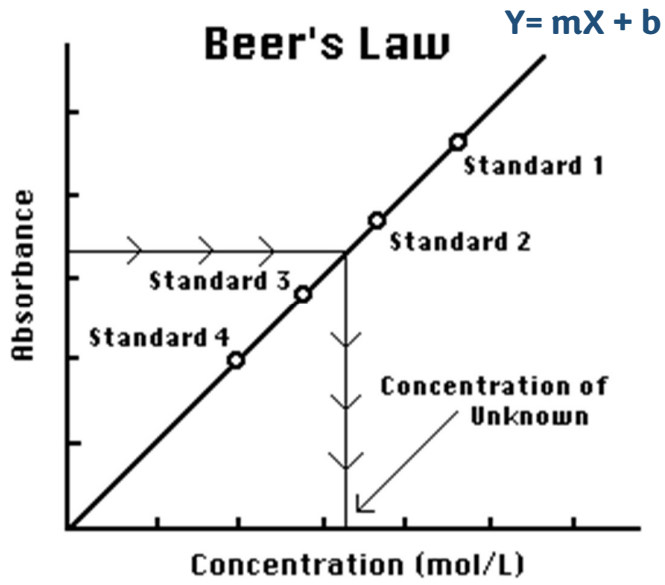
The Beer's law represents ***a linear equation***; (**Y = mX + b**), where the intercept (**b**) in the case of Beer's law is **zero** and the slope (**m**) is **εb**.

→ As **"b" is fixed = 1cm** → the slope will be equal to the molar absorptivity "**ε**".

← بما أن "b" ثابت = 1 سم ← فإن الميل سيكون مساويًا لمعامل الامتصاص المولي "ε".

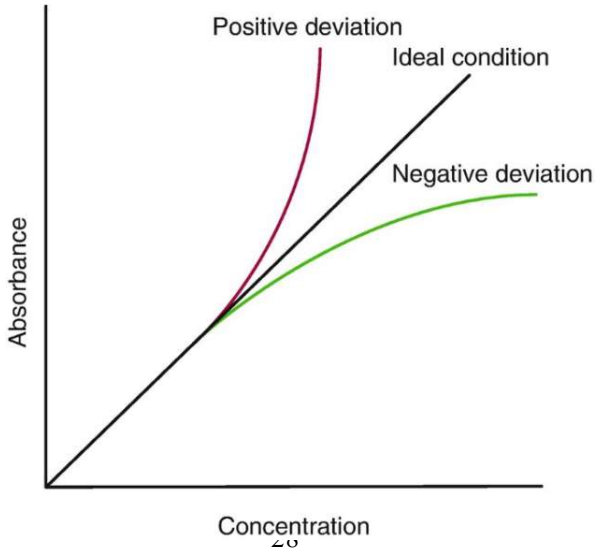
If we plot absorbance against concentration, a straight line passing through the origin (zero) should be obtained and a linear relationship is observed as it obeys Beer's law.

إذا رسمنا الامتصاصية مقابل التركيز، فسنحصل على خط مستقيم يمر بنقطة الأصل (الصفر)، وستلاحظ علاقة خطية لأنها تخضع لقانون بير.




Y= A= absorbance
X= molar concentration
b= O
m=slop = εb
➔ b (path length) = 1
m= ε
a= εX

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




ومع ذلك، هناك بعض العوامل التي تؤدي إلى الانحراف عن العلاقة الخطية بين التركيز والامتصاص، وما يترتب على ذلك من فشل واضح لقانون بير. يُبلغ عن الانحراف عن قانون بير على أنه موجب أو سالب وفقًا لما إذا كان المنحنى مقعرًا لأعلى أو مقعرًا لأسفل.

قد يحدث انحراف عن قانون بير بسبب العوامل التالية:
1)وجود شوائب تمتص عند نفس طول موجة الامتصاص.
2) في حالة عدم استخدام ضوء أحادي اللون.
3) إذا لم يكن عرض الشق مناسبًا، فإنه يسمح بالتالي بسقوط إشعاعات غير مرغوب فيها على الكاشف.

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

1) Presence of impurities that absorb at the same absorption wavelength.
2) If monochromatic light is not used.
3) If width of slit is not proper, therefore allows undesirable radiations to fall on the detector.
4) If the solution species undergoes polymerization, ionization, dissociation, association, or any other chemical change during analysis.
5) If the sample concentration is high (>0.01M) as the distance between particles decreases and allows for interaction between the absorbing particles such that the absorption characteristics of the analyte are affected.

4) إذا خضعت أنواع المحلول لعملية بلمرة أو تأين أو تفكك أو ارتباط أو أي تغيير كيميائي آخر أثناء التحليل.

٥) إذا كان تركيز العينة مرتفعًا (>٠,٠١ مولار)، فإن المسافة بين الجزيئات تقل، مما يسمح بتفاعل الجزيئات الماصة، وبالتالي تتأثر خصائص امتصاص المادة المراد تحليلها.

***Note:*** *Beer's law cannot be applied to suspensions.*

ملاحظة: لا يمكن تطبيق قانون بير على المعلقات.

# PRACTICAL PART

| GLASSWARE | CHEMICALS |
|---|---|
| Volumetric flasks 50 & 100 ml | Paracetamol powder |
| Beakers | Distilled water |
| Quartz cuvette | Paracetamol tablets |
| Volumetric pipette 10, 5, 3 & 25 ml | 0.1 M NaOH |

## AIM OF THIE EXPERIMENT

- *To determine the concentration of a pharmaceutical active ingredient "paracetamol" in an unknown sample using UV spectroscopy.*
- *To determine the percentage assay of label of a pharmaceutical dosage form using UV spectroscopy.*

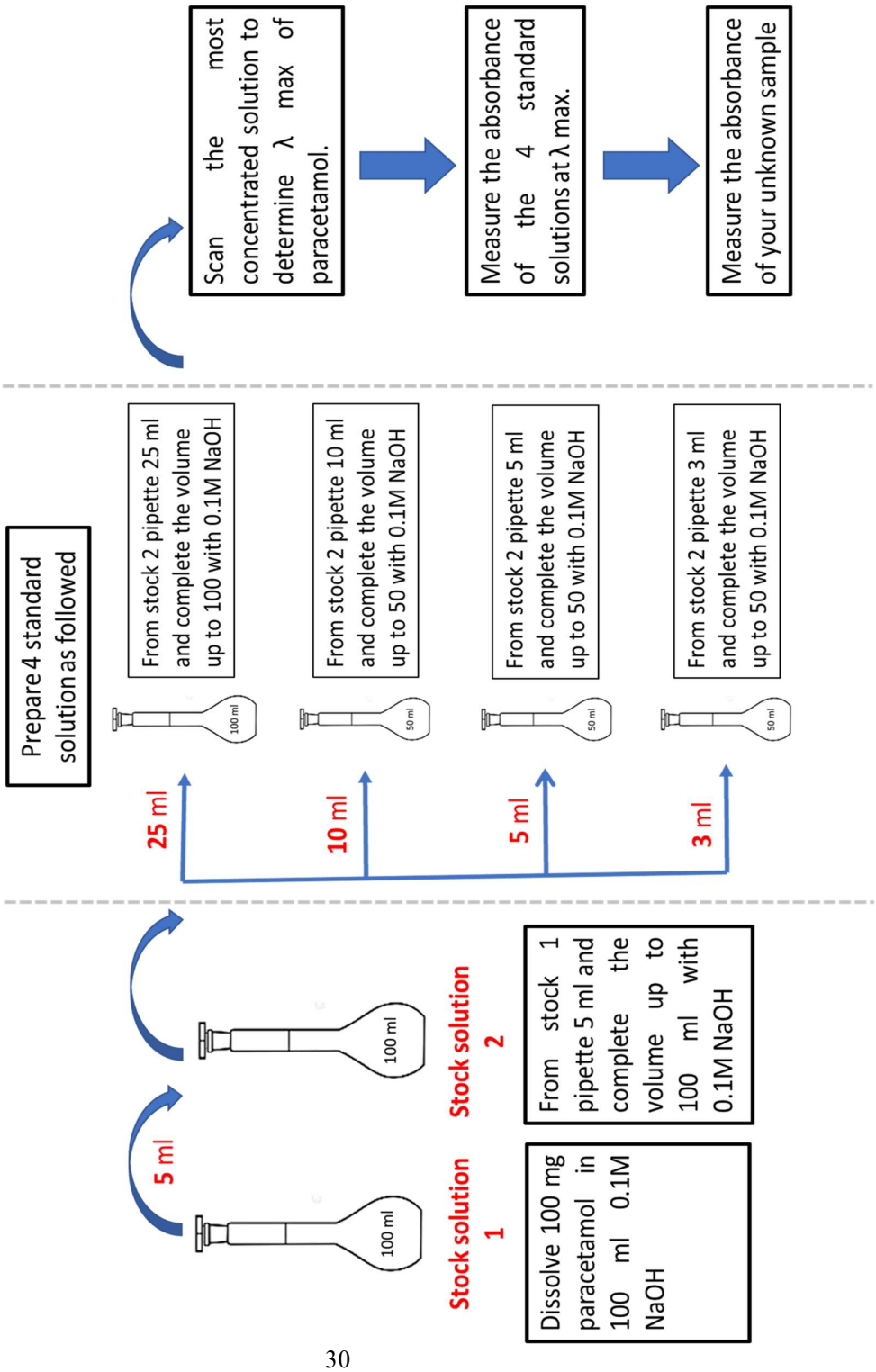
## PROCEDURE

### Part 1: Preparation of standard dilutions:

1. Weigh accurately 100mg of paracetamol pure powder in a 100ml volumetric flask and record the weight on the report sheet. Add 100ml of 0.1M NaOH up to the volume to prepare stock soln.1.
2. Take 5ml of the above solution and dilute up to 100ml with 0.1M NaOH to prepare stock soln.2.
3. Prepare 4 standard dilutions from the stock soln.2 by diluting 25ml to 100ml 0.1M NaOH, 3ml, 5ml, 10ml to 50ml with 0.1M NaOH.
4. Determine the maximum wavelength using the highest conc. Of the standards.
5. Run these 4 standard dilutions for absorbance against blank at $\lambda_{max}$.
6. Refer to the report sheet to tabulate the results and plot calibration curve between absorbance vs. concentration as requested in the report sheet.

Prepare standard solutions → Measure their absorbance at λmax → Construct a calibration curve → Calculate the molar absorptivity **ε**

## PART 1 ROCEDURE DIAGRAM

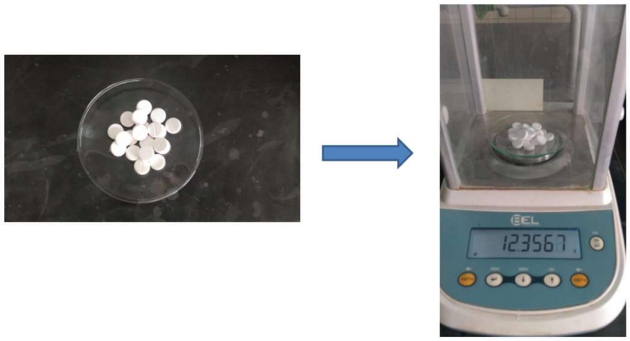



**Part 2: Measure the concentration of an unknown sample:**

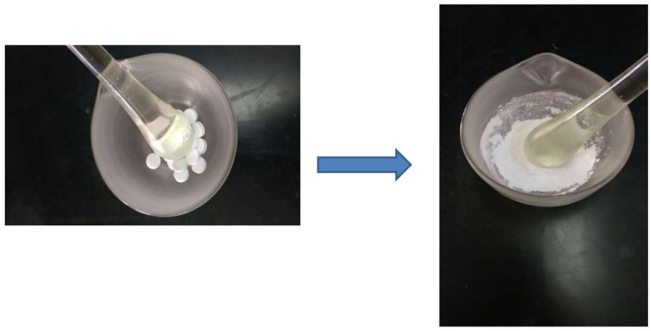
Measure the absorbance of the unknown sample → Calculate the concentration of the unknown sample using Beer's law → Calculate the concentration of the unknown sample using calibration curve equation.

**Part 3: Assay of Paracetamol tablets:**

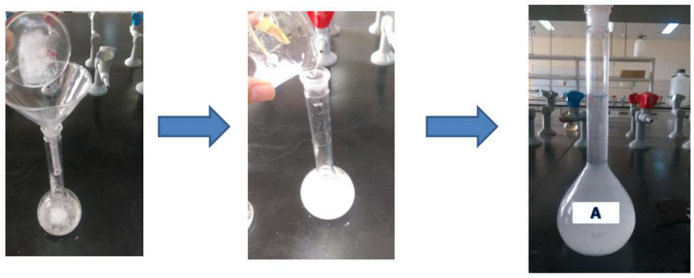
1. Weigh 20 tablets of paracetamol.




2. Crush the 20 tablets with the help of mortar and pestle.

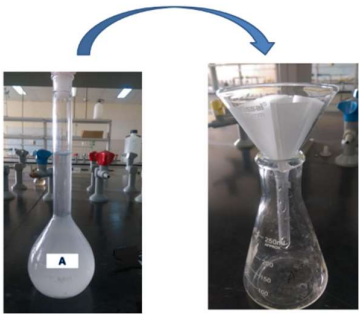


3. Weigh accurately a quantity of the powdered tablets equivalent to 0.15g of paracetamol in a 200ml volumetric flask and record the weight in the report sheet.
4. To the flask, add 150 ml of 0.1 M NaOH, and shake for 15 min, then complete the volume up to 200ml with water to get solution A.

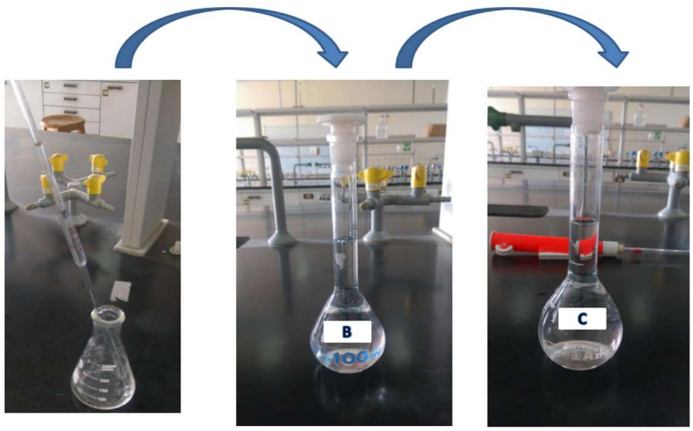



Solution A

5. Mix well and filter the resulting solution.




6. Take 10ml of the filtered solution and dilute to 100ml with distilled water to prepare solution B.
7. Take 10ml of solution B and dilute to 100ml with distilled water and mix well to prepare solution C.




Filtrate Solution B Solution C

8. Measure the absorbance of the resulting solution at 257nm against blank solution.
9. Refer to the report sheet to fill in the results and calculations.

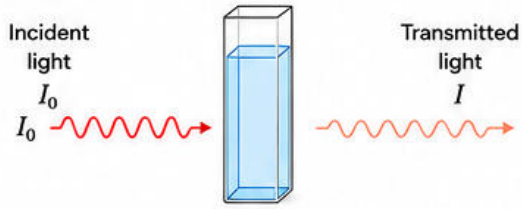
# EXPERIMENT 3

# ULTRAVIOLET–VISIBLE SPECTROSCOPY

## – QUANTITATIVE ANALYSIS –

## 1. INTRODUCTION

UV-Vis spectroscopy measures how much light is absorbed by a solution at a particular wavelength.
Different compounds may have very different absorption maxima and absorbance.




**Transmittance (T):**

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

$(0 \le T \le 1)$

**% Transmittance**

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

**Absorbance (A)**

$$A = \log_{10}\frac{I_0}{I} = -\log T$$

- A = 0 → T = 100% (no absorption)
- A = 1 → T = 10% (strong absorption)
- Intensely absorbing compounds must be examined in dilute solution using non-absorbing solvents in the same UV region.

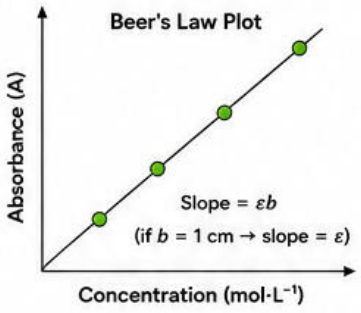
## 2. BEER–LAMBERT LAW

$$A = \varepsilon bc$$

**Where:**

A = Absorbance (unitless)
$\varepsilon$ = Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)
b = Path length of cuvette (cm)
c = Concentration ($mol \cdot L^{-1}$)

- Beer's law: Absorbance is directly proportional to the concentration of the absorbing species and path length.
- For a fixed path length ($b$ = 1 cm), $A = \varepsilon c$
- Plotting absorbance (A) vs. concentration (c) gives a straight line through the origin (Beer's law).



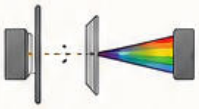

| Important Terms | |
|---|---|
| $\lambda_{max}$ | Wavelength at which maximum absorbance occurs |
| $\varepsilon_{max}$ | Molar absorptivity at $\lambda_{max}$ |
| A | Absorbance (unitless) |
| b | Path length of cuvette (cm) |
| $\varepsilon$ | Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$) |

## 3. DEVIATION FROM BEER'S LAW

Deviation from linearity may be positive (curve bends upward) or negative (curve bends downward).

**1. Impurities**
Presence of impurities that absorb at the same wavelength.

**2. Non-monochromatic light**
If monochromatic light is not used.



**3. Improper slit width**
Width of slit is not proper, allowing unwanted radiations to reach the detector.

$$HA \rightleftharpoons H^+ + A^-$$

**4. Chemical changes**
Polymerization, ionization, dissociation, association, or other chemical changes during analysis.

**5. High concentration (> 0.01 M)**
Interparticle interactions affect absorption characteristics.

Note: Beer's law cannot be applied to suspensions.

## 4. PRACTICAL PART

### PART 1: Preparation of Standard Dilutions

1. Weigh accurately 100 mg of paracetamol pure powder in a 100 mL volumetric flask. Add 100 mL of 0.1 M NaOH to the mark to prepare Stock solution 1.


2. Take 5 mL of the above solution and dilute to 100 mL with 0.1 M NaOH to prepare Stock solution 2.


3. Prepare 4 standard dilutions from Stock solution 2:


4. Determine $\lambda_{max}$ using the highest concentration standard.
5. Measure absorbance of the 4 standards against blank at $\lambda_{max}$.
6. Tabulate results and plot calibration curve (Absorbance vs. Concentration).

### PART 2: Measure the Concentration of an Unknown Sample


Calculate concentration in two ways:

1. Using Beer–Lambert law

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

(if $\varepsilon$ is known, $b$ = 1 cm)

2. Using calibration curve equation

(From the line: $A = mc + b$)

**Result:**
Concentration of the unknown sample

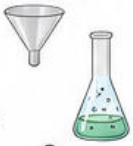
### PART 3: Assay of Paracetamol Tablets

1. Weigh 20 tablets of paracetamol.


2. Crush the 20 tablets with mortar and pestle.

3. Weigh an amount of the powder equivalent to one tablet.
4. Transfer to 100 mL volumetric flask, add some 0.1 M NaOH, sonicate/shake to dissolve, and dilute to mark.

5. Filter if necessary.



6. Prepare appropriate dilutions (if needed), measure absorbance at $\lambda_{max}$, and calculate the amount of paracetamol per tablet and % assay.

### GLASSWARE

- Volumetric flasks (50 & 100 mL)
- Beakers
- Quartz cuvette
- Volumetric pipettes (10, 5, 3 & 25 mL)

### CHEMICALS

- Paracetamol powder
- Paracetamol tablets
- 0.1 M NaOH
- Distilled water


### OVERALL WORKFLOW