

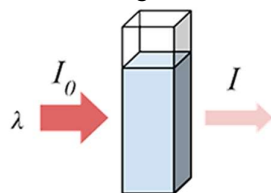
## EXPERIMENT 3: Ultraviolet-Visible Spectroscopy – Quantitative Analysis



### INTRODUCTION

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  $I$ .



**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).

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

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

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

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

$$A = \log_{10} \frac{I_0}{I}$$
$$A = -\log T = \log \frac{1}{T}$$

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.

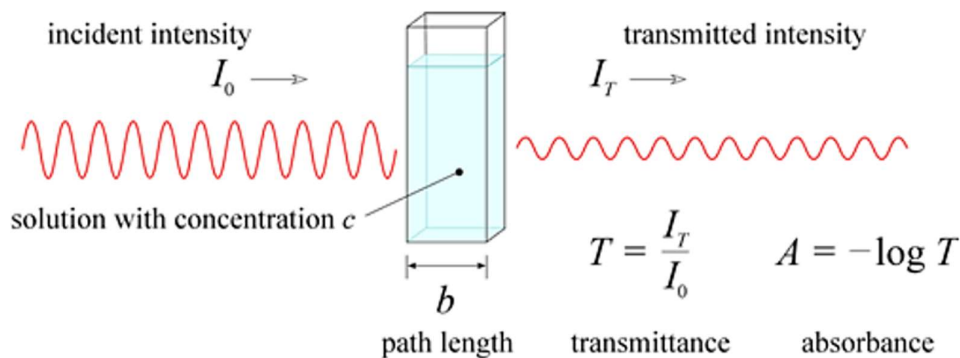
## Beer-Lambert Law

$$A = \epsilon bc$$

|                                    |                                                                                                                                                                                         |
|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Absorbance</b>                  | <b>A</b> , a measure of the amount of radiation that is absorbed ( <b>unitless</b> )                                                                                                    |
| <b>Molar absorptivity</b>          | <b><math>\epsilon</math></b> , Parameter defining how strongly a substance absorbs light at a wavelength per molar concentration ( <b><math>L \cdot mol^{-1} \cdot cm^{-1}</math></b> ) |
| <b>Path length</b>                 | <b>b</b> , the length of the sample cell ( <b>cm</b> )                                                                                                                                  |
| <b><math>\lambda_{max}</math></b>  | The wavelength at which maximum absorbance occurs                                                                                                                                       |
| <b><math>\epsilon_{max}</math></b> | The molar absorptivity at <b><math>\lambda_{max}</math></b>                                                                                                                             |

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.



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 ( $\epsilon$ ).

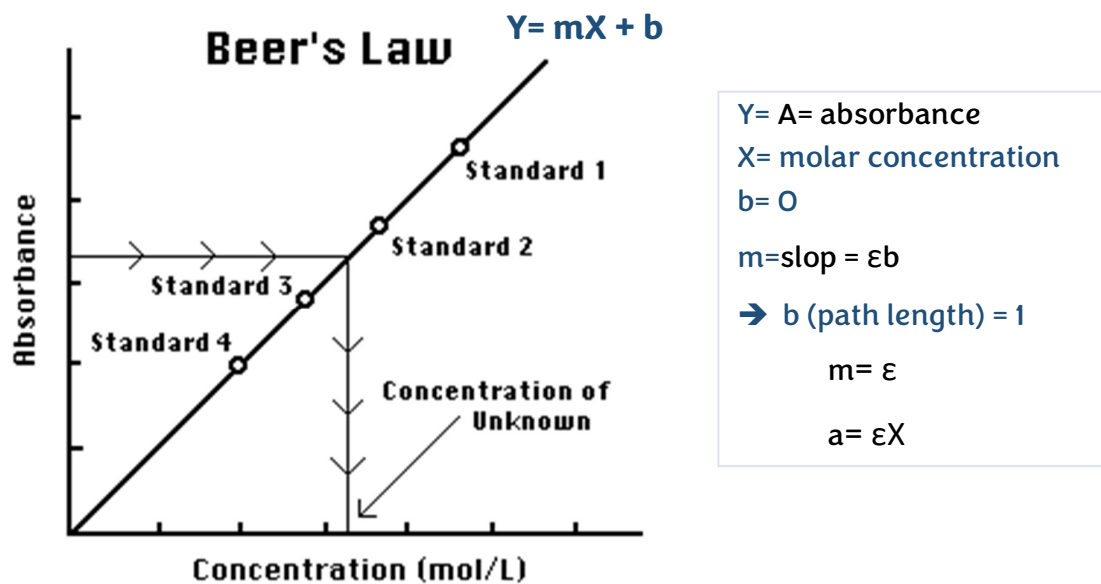
$$\epsilon = A / (c \cdot b)$$

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

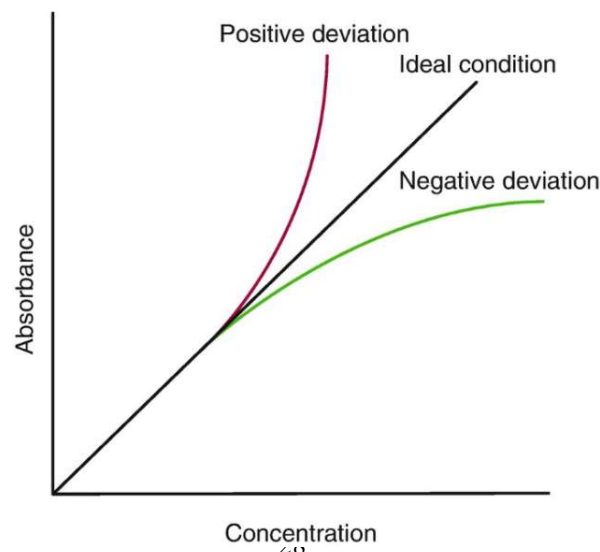
The Beer's law represents **a linear equation**: ( $Y = mX + b$ ), where the intercept (**b**) in the case of Beer's law is **zero** and the slope (**m**) is  $\epsilon b$ .

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

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.



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.



### 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 ( $>0.01\text{M}$ ) as the distance between particles decreases and allows for interaction between the absorbing particles such that the absorption characteristics of the analyte are affected.

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

## 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 THE 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.

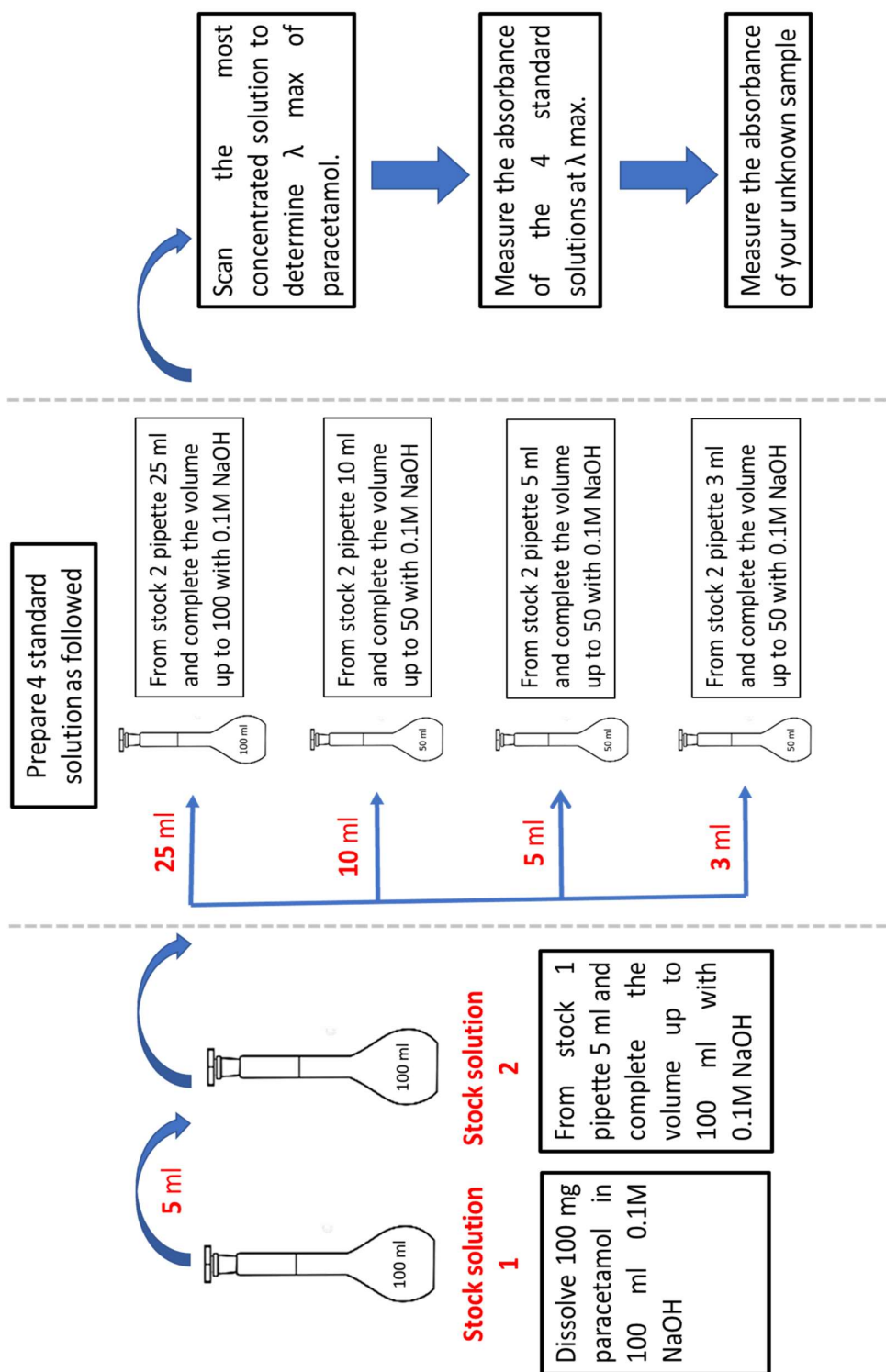
### PROCEDURE

#### 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  $\lambda_{\text{max}}$ .
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  $\lambda_{\text{max}}$  → Construct a calibration curve → Calculate the molar absorptivity  $\epsilon$

## PART 1 PROCEDURE DIAGRAM



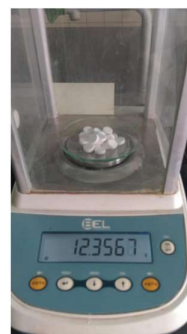


**Part 2: Measure the concentration of an unknown sample:**

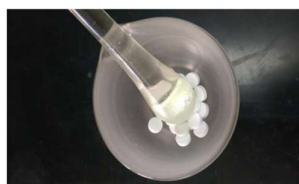
Measure the absorbance of the unknown sample → Calculate the concentration of the unknown sample using Beer's law → Calculate the concentration of the unknown sample using calibration curve equation.

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

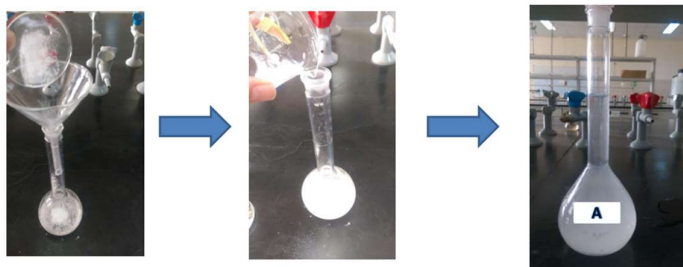
1. Weigh 20 tablets of paracetamol.



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

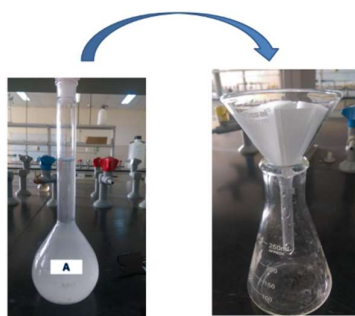


3. Weigh accurately a quantity of the powdered tablets equivalent to 0.15g of paracetamol in a 200ml volumetric flask and record the weight in the report sheet.
4. To the flask, add 150 ml of 0.1 M NaOH, and shake for 15 min, then complete the volume up to 200ml with water to get solution A.

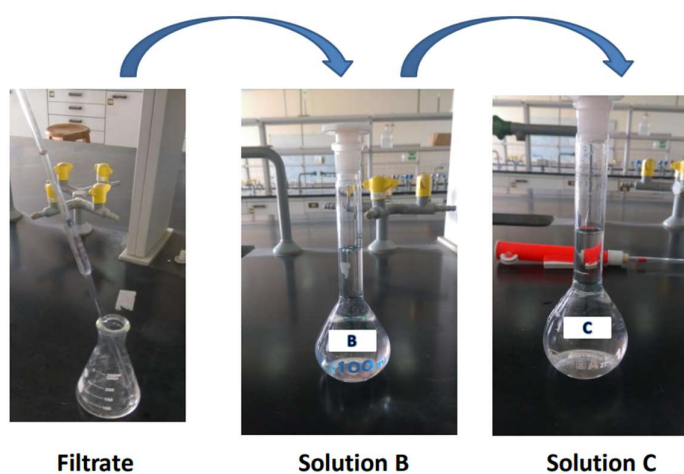


**Solution A**

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.