Beer's- Lambert Law and Standard Curves

Spectrophotometer:

- Spectrophotometer can be used to measure the amount of **light absorbed or transmitted** by a solution. How?
- **It consist of two parts:** spectrometer and photometer.
  - **a.** **Spectrometer** is used for producing light of any selected color (wavelength)
  - **b.** **Photometer** is used for measuring the intensity of light.

- By using the spectrophotometer, we can **quantitatively measure absorbance**, and this information can be used to **determine the concentration of the absorbing molecule.**
- **More concentrated** solution will absorb **more light** and transmits less [why?]

➔So:

- The more concentrated solution ➔ **high** absorbance value.
- And less concentrated solution ➔ **less** absorbance value.

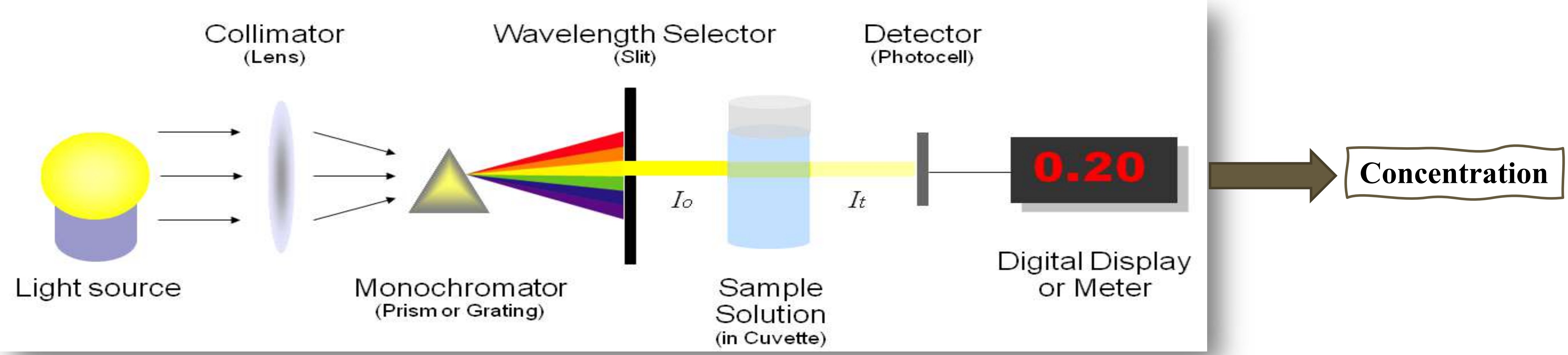
Spectrophotometer cont.:

- **Wavelength in this instrument divided into:**

1. Invisible range-ultraviolet- (from 100 to 360 nm)
2. Visible range (above 360 nm -700 nm)

- **What are the type of cuvettes used in each range ? Why ?**

- **Blank:** contain everything except the compound to be measured



Beer-Lambert law:

Principle:

The absorption of light by a solution is described by the Beer-Lambert law as:

- There is **linear** relationship between absorbance and concentration of an absorbing species.

$$A = a_m \times c \times l$$

Where:

- **A=** is the absorbance of the solution [Ab].
- a_m = the molar extinction (absorption) coefficient.
- **l** = length of the light path through the solution.
- **c** = concentration of the absorbing substance.

Beer-Lambert law cont.:

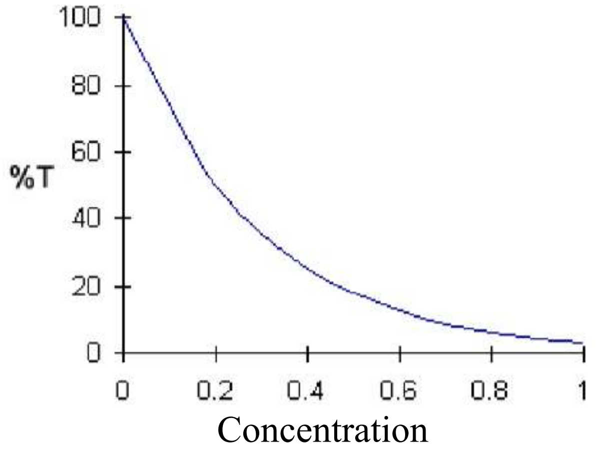
- **From the law we observe :**

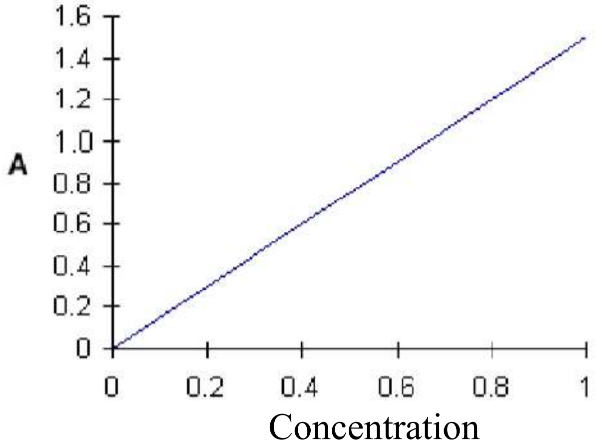
1. **Direct** relationship between C and A.
2. **Direct** proportional between I and A.

$$A = a_m \times c \times l$$

- If we plot absorbance against concentration, we get a straight line.
- The linear relationship between **concentration** and **absorbance** is both simple and straightforward.

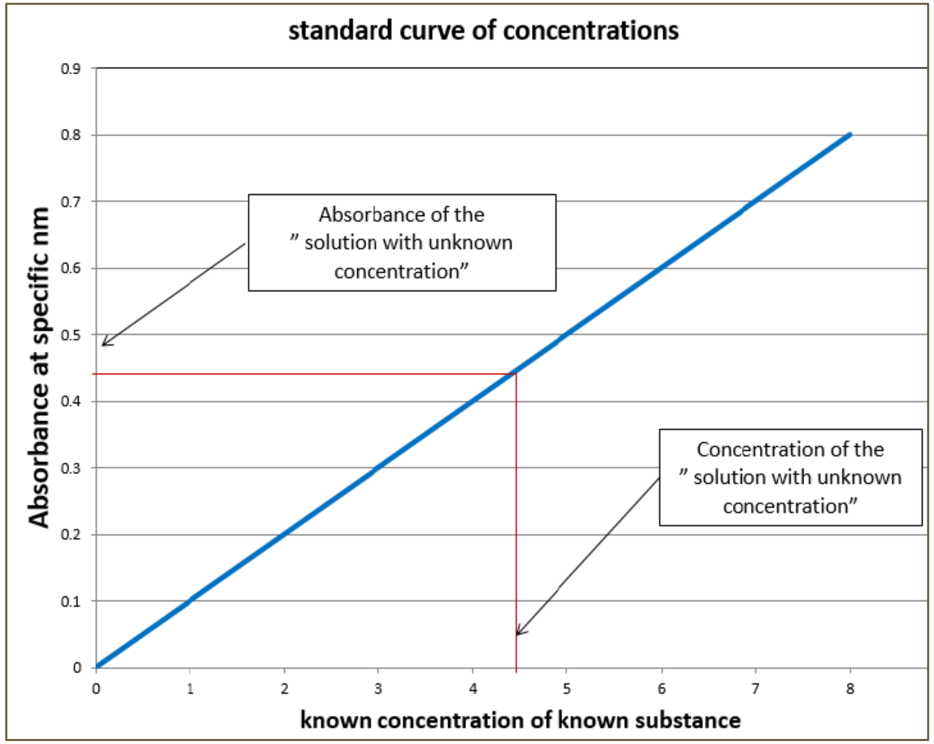
➔ Which is why we prefer to express the **Beer-Lambert law** using absorbance as a measure of the absorption rather than %T (% transmittance).





Standard curve for concentrations:

- It is a **graph** that shows the relationship between different known concentrations of a substance and the absorbance at a specific wavelength.
- **Standard curves** are most commonly used to determine the concentration of a substance, using serial dilution of solutions (standard solutions) of known concentrations.



➔What is a standard solution?

- Is a solution containing a **precisely known concentration** of an element or a substance.
- A series of known standard solutions can be prepared by diluting the stock known solution.

(We should calculate the concentration of the diluted solution by the formula: $C_1 X V_1 = C_2 X V_2$)

Some points to consider:

1. The absorbance of a solution with "unknown concentration" preferred to be **lower** than the highest Ab value in the standard curve.

   ➔ [the absorbance of solution with "unknown concentration", is within the range of absorbance values of solution with "known concentration solutions"].

2. If your unknown sample had an absorbance higher than the highest absorbance recorded by the standard [out of the range], **how would you determine its concentration correctly?**

   Two choices:

   I. **Increase** the concentration of standard solutions.

   II. **Dilute** the solution with "unknown concentration" → measure the Ab after dilution → determine its concentration from the curve → then multiply the value by the **dilution factor**.

3. If there is insufficient volume the light may pass over the solution instead of going through it.

4. Absorbance has no units.

Determining the unknown concentration of a solution by known absorbance value

From standard curve

- The standard is constructed by plotting the **absorbance** values vs **concentration** and solving for the best straight line which is given in terms of **y = mx + b**

  → where; y is absorbance, x concentration, b is the y-intercept and m is the slope.
- Measure the absorbance of the "solution with unknown concentration" to determine the concentration.

Beer-Lambert law

- Using available information of any standard solution to determine the "**ε**"
- Then using these information to get the unknown concentration using: **A = εlc**
- Note: "**ε**" will changed when the wavelength changed.

Example:

Determine the concentration of a solution with an absorbance value of **0.041** from the standard curve.

- **y = mx + b** (Where: **y** is absorbance, **x** concentration, **b** is the y-intercept and **m** is the slope)

Based on the curve, the slope (m) = 0.8 and (b) = 0

Then:

0.041 = 0.8(x) + 0

X = 0.041/0.8 = 0.051M

Or use the TREND formula

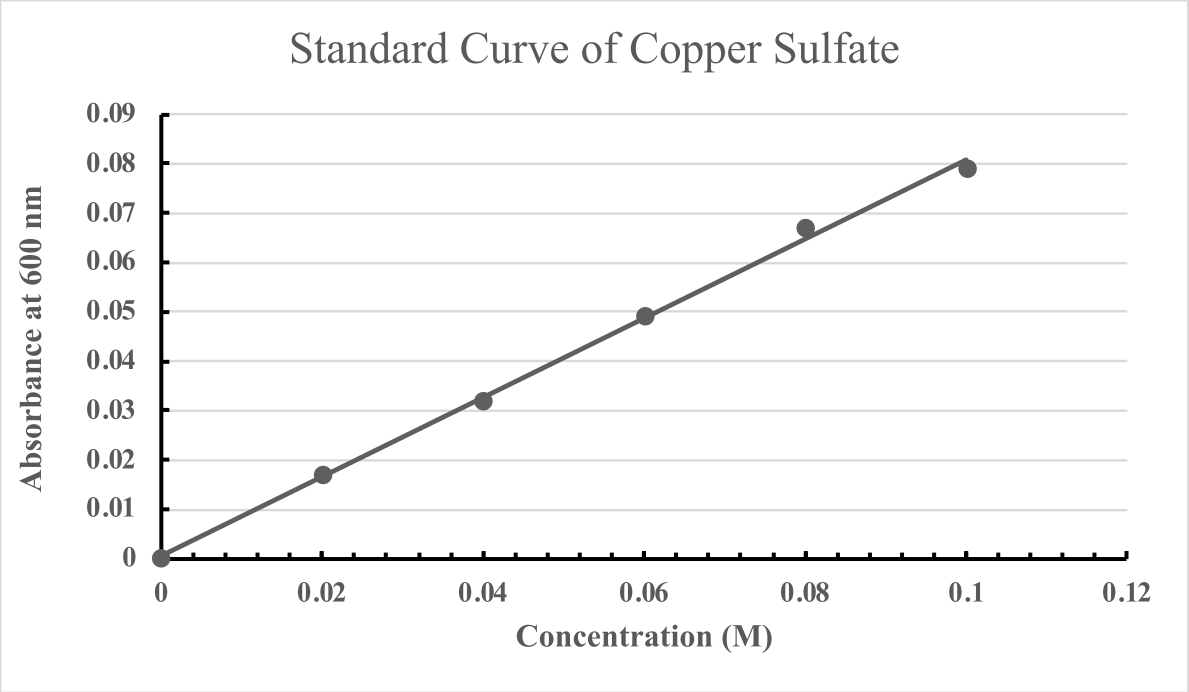


Figure 1. Standard curve of concentration of 0.1 M of copper sulphate

Practical Part

Objectives:

- To understand the concept of Beer-Lambert law and its application.
- Getting familiar with the standard curve.
- Determination of an unknown concentration for two solutions.

Method:

How to determine the **concentrations of the standard solutions ?**

- You are provided by:

1. Standard solution (Stock solution) of Copper Sulfate with 0.1 M [known concentration].
2. Solution with Unknown concentration "A".
3. Solution with Unknown concentration "B".

- Set up 8 test tubes, as following table:

Tube	0.1M Copper Sulfate Standard Solution (ml)	Distal water (ml)	Solutions with unknown concentration (ml)
Blank	-	5 ml	-
A	1ml	4 ml	-
B	2 ml	3 ml	-
C	3 ml	2 ml	-
D	4 ml	1 ml	-
E	5 ml		-
Solution "A"	-	-	2 ml
Solution "B"	-	-	2 ml

- Mix the contents using the vortex.
- Measure the absorbance of each tube at 600 nm against the blank.

Wear chemical splash goggles and chemical resistant clothing such as gloves and aprons when handling copper sulphate

Results:

- Calculate the concentrations of standard solutions (Tube A-E).
- Plot the standard curve (Absorbance vs. Concentration).
- Determine the concentration of Solution "A" and "B" from the standard curve.
- Calculate the concentration of Solution "A" and "B", from the law using (0.942 $M^{-1}cm^{-1}$).
- Calculate the extinction coefficient of your Copper Sulfate solution (using one of the known tubes).

Tube	Absorbance at 600nm	Concentration M
A		
B		
C		
D		
E		
Solution "A"		From the curve=
Solution "B"		From the curve=