Beer's- Lambert Law and Standard Curves

BCH 312 [practical]

- The spectrophotometer: it can be used to measure the amount of light absorbed by a solution. How?

- It consist of two parts spectrometer and photometer.

- 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.

So, the more concentrated solution → high absorbance value.

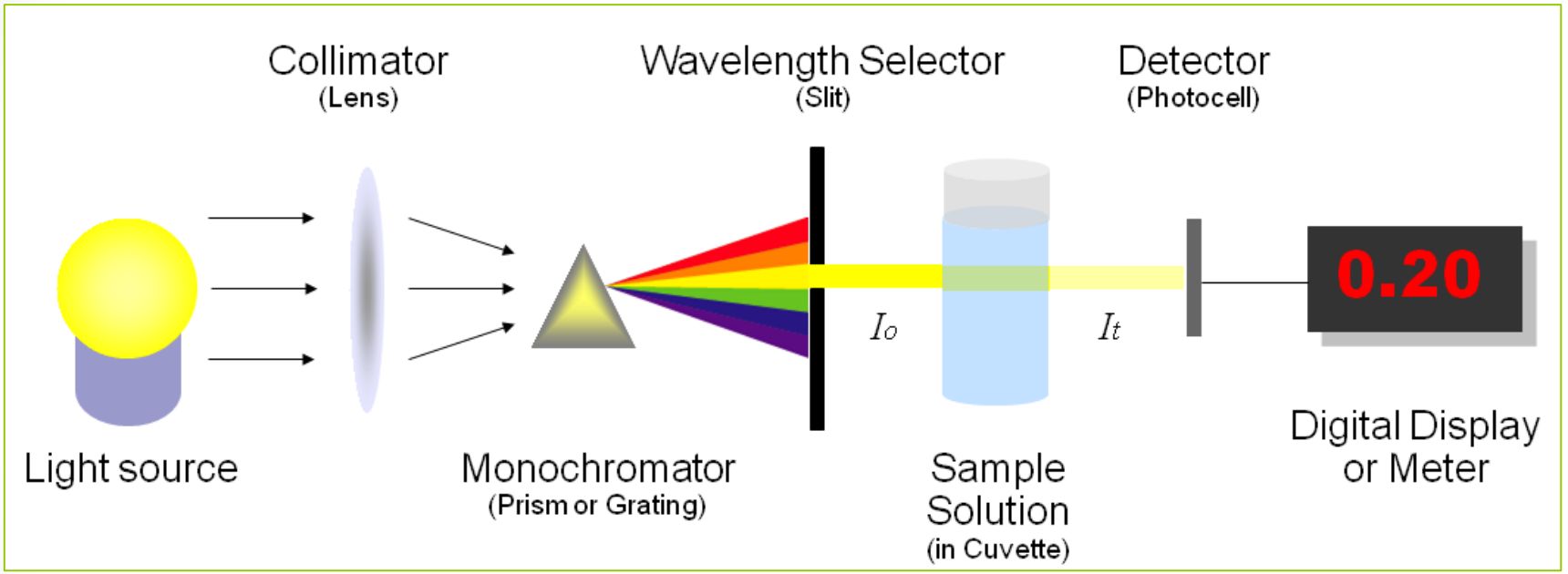
And Less concentrated solution → less absorbance value.

-Wavelength in this instrument divided into:

1. Invisible range-ultraviolet (from 100 to 360 nm).

2. Visible range (above 360 nm -700 nm)

- **Blank:** contain every thing expect the substance that we want to measure.



How a spectrophotometer works

- 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.

$A = a_m \times c \times l$

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.

From this law we observe :

1- There are direct proportional between C and Ab.

2- Also, there is direct proportional between l and Ab.

- So, what does standard curve of concentrations mean?

- A 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 wave length.
- If we plot absorbance against concentration, we get a straight line.

-Standard curve are most commonly used to determine the concentration of a substance, using serial dilution of solutions of known concentrations.

-So, what is the principle of a standard curve???

- What is standard solutions???

- Standard solution:

- It is a solution containing a precisely known concentration of an element or a substance.

- A series of known standard solutions can be prepare by diluting the stock known solution.

- We should calculate the concentration of mixture after diluted by the formula: $C1 \times V1 = C2 \times V2$

- Notes:

1- The Ab of solution with “unknown concentration” preferred to be lower than highest Ab value in the standard Curve.

[the absorbance of solution with “unknown concentration”, is within the rang of absorbance values of solution with “known concentration solutions”].

2- If your unknown sample had an absorbance higher the highest absorbance recorded by standard [**out of the rang**].

- How will you determine its concentration correctly?

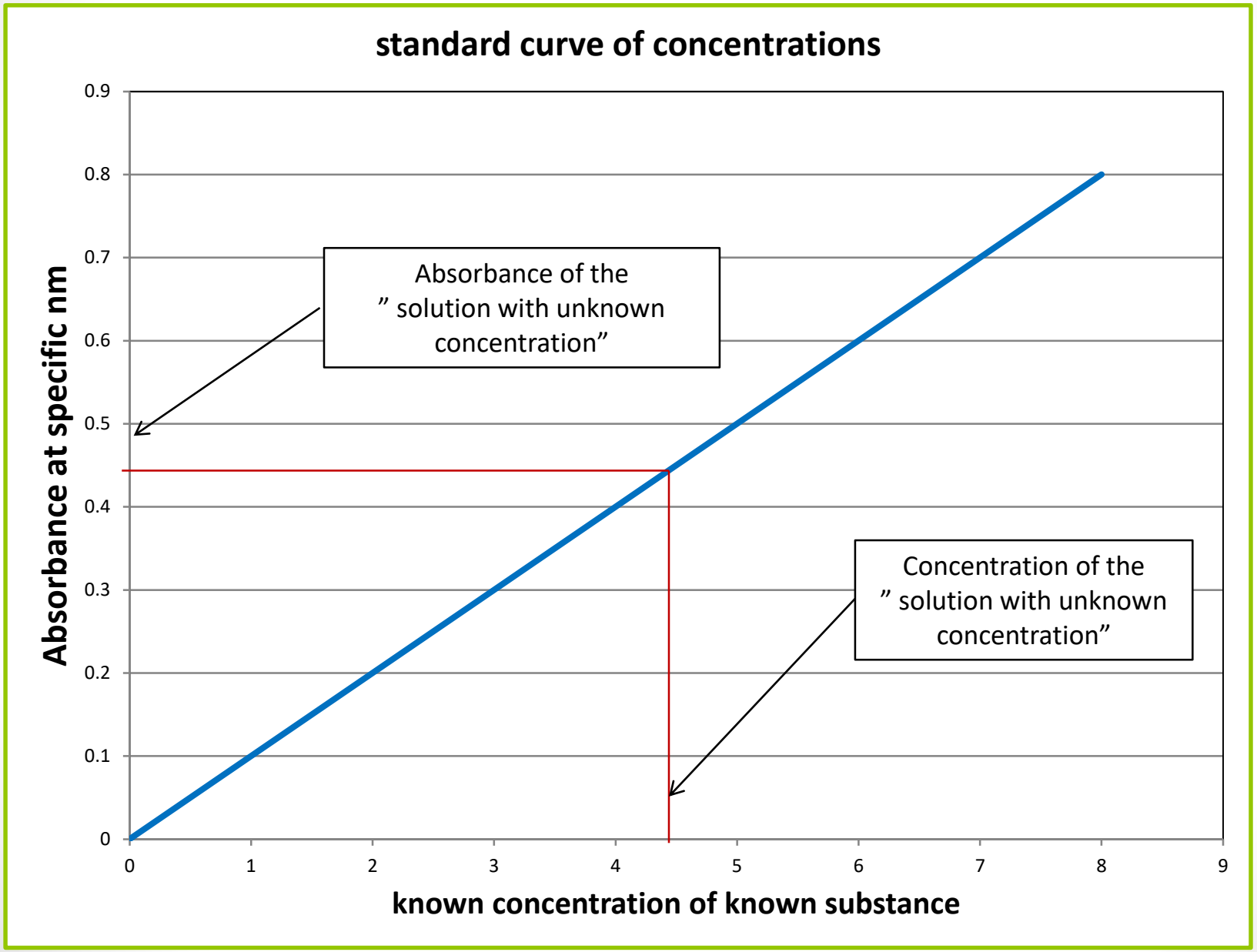
- I have two choice :

1- Increase the concentration of standard solution.

2- Dilute the solution with “unknown concentration” →measure the Ab → After dilution then determine its concentration from the curve →then multiply the value by Dilution factor.

How to determine unknown concentration of a solution with known absorbance value?

- If the unknown concentration of a solution has absorbance value =0.45, the conc. From the curve will be .....??



- To determine the concentration of a solution with "an unknown concentration":

- From standard curve:

- Measure the absorbance of the "solution with unknown concentration" in order 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: $A = \varepsilon lc$
- Note: "ε" will changed when the weave length changed.

Practical part

- Objectives:

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

- Method:

- 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".

- Method:

- Set up 8 test tubes, as following table:

Tube	0.1M Copper Sulfate Standard Solution (ml)	d.H2O(ml)	Solutions with unknown concentration (ml)
Blank	-	10 ml	-
A	2ml	8ml	-
B	4ml	6ml	-
C	6ml	4ml	-
D	8ml	2ml	-
E	10ml	-	-
Solution "A"	-	-	10 ml
Solution "B"	-	-	10 ml

↓

Mix the contents using the vortex.

- **Measure the absorbance of each tube at 600 nm against the blank [……].**

- Result:

- Calculate the concentrations of the series of known standard solutions.
- Plot the standard curve (Absorbance vs. Concentration), determine the concentration of unknown from graph.
- Determine the concentration of Solution "A" and "B".

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