قسم الكيمياء الحيوية
Biochemistry Department
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BCH 312
Experiment (7)

Beer's- Lambert Law and Standard Curves

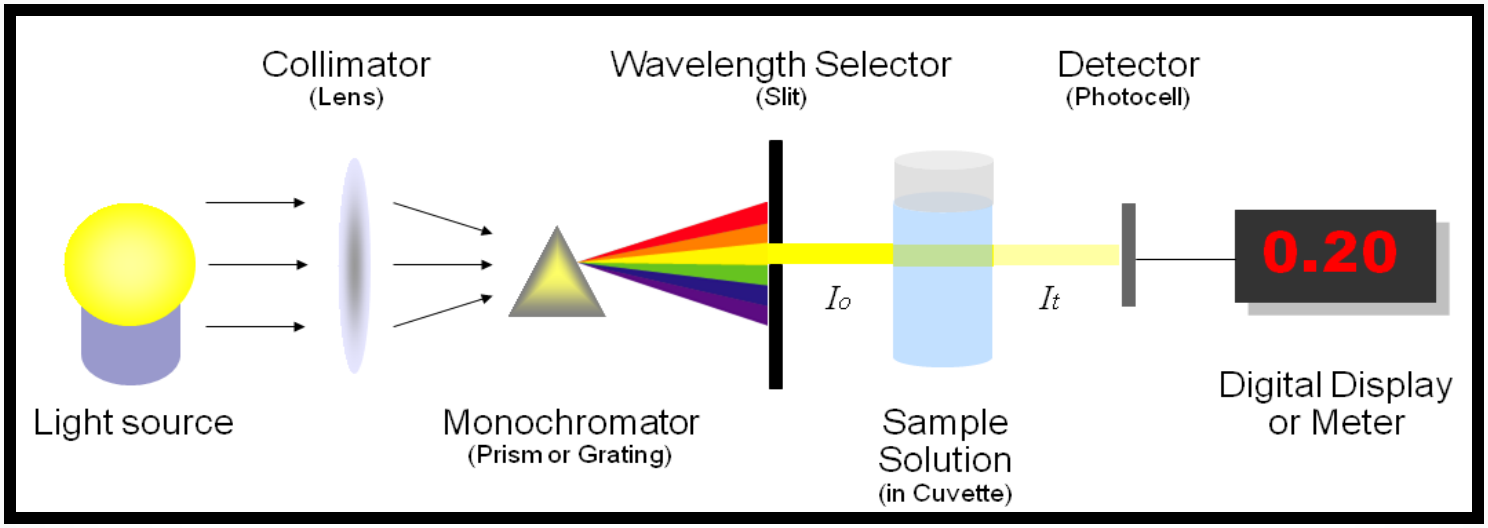
Objectives

1. To understand the concept of Beer-Lambert law and its application.

2. To get introduced to standard curves, their applications, and to learn how to design protocols for the creation of a standard curve.

Spectrophotometer

- Spectrophotometer is instrument used to measure the intensity of light at a given wavelength that is transmitted or absorbed by a sample.



Wavelength in this instrument is divided into:

-Invisible range (ultraviolet) from 100 to 360 nm **[Quartz cuvette are used]**

-Visible range (400 -700 nm) **[Glass or plastic cuvette are used]**

Blank solution: Is a solution that contains everything except the compound to be measured.

Beer's Lambert Law :

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

Where:

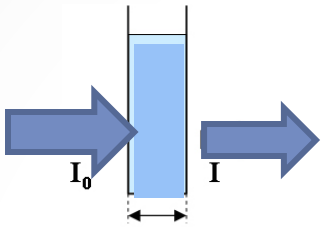
- **A** = the absorbance of the solution.
- a_m= the molar extinction coefficient.
- **C** = the concentration of the absorbing substance.
- **l** = the length of the light path.

Molar extinction coefficient

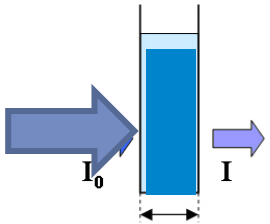
- The **molar extinction coefficient**, is a measurement of how strongly a chemical species absorbs light at a given wavelength.

- It is different for different substances and wave lengths.

- The extinction coefficient has units of reciprocal concentration and path length.
- If the concentration is in molarity and length of light path is in cm, then the molar extinction coefficient will be in **(M^{-1} cm^{-1})**

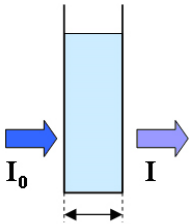


Lower concentration
Lower absorption

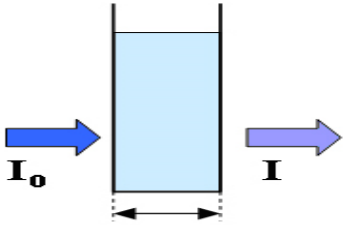


higher concentration
higher absorption

A α C



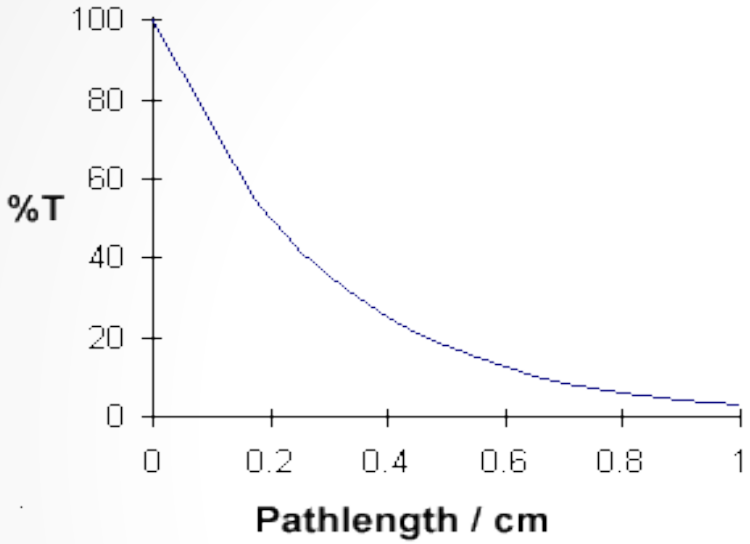
Shorter pathlength
Lower absorption



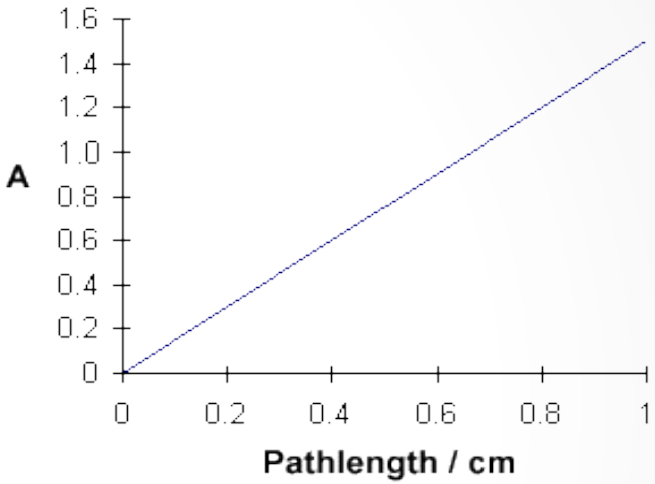
longer pathlength
higher absorption

A α l

Transmittance



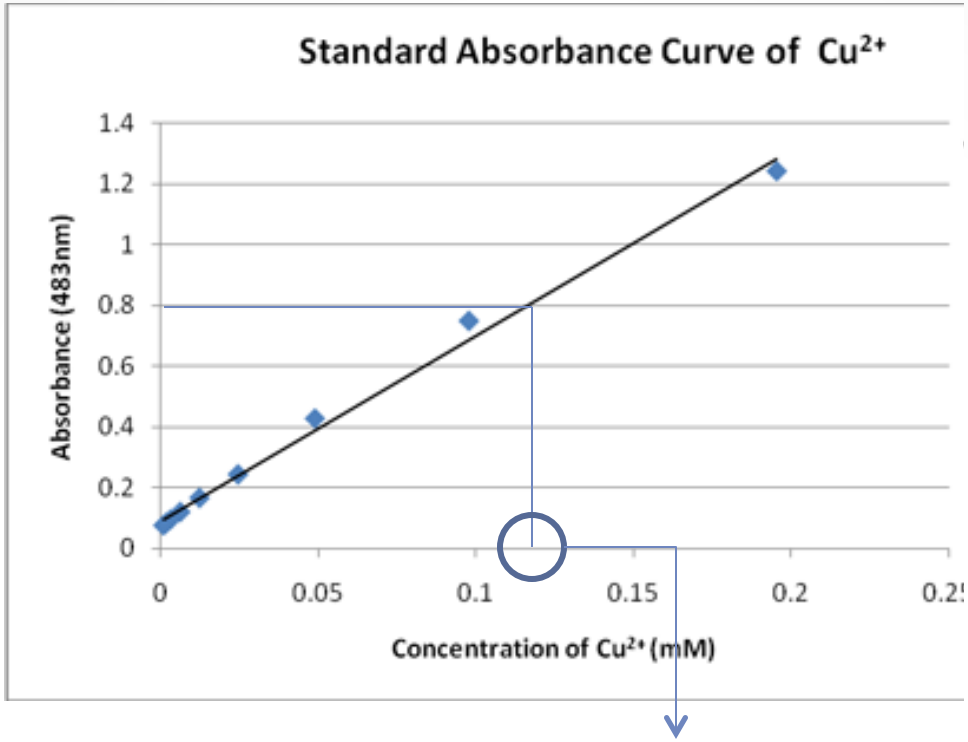
Absorbance



The relationship between C and absorbed light (A) is **directly proportional**, while the relationship between C and transmitted light (T) is **indirectly proportional.**

Standard curve

- A standard curve is a type of graph used as a quantitative research technique.
- They are most commonly used to determine the concentration of a substance, using serial dilution of solutions of known concentrations (standard solutions)
- If the type of substance and the path length are constant, then the absorbance is proportional to the concentration (in molarity) of the substance in the solution
- Standard curve accuracy increases by using a large number of standards.



Concentration of unknown sample

The absorbance of the unknown must fall within the line of the standard curve, preferably within the linear region, you should not extrapolate your line beyond the highest concentration standard you have.

Some points to consider

1. Absorbance has no units, it is read off of the spectrophotometer, the wavelength is often specified along with the absorbance ,such as A540 = 0.3.
2. The path length is usually in cm and if not specified **is assumed to be 1cm.**
3. Lots of things can interfere with the spectrophotometer reading:
   - If the cuvette is scratched, light will be scattered rather than absorbed by the solution.
   - If there is insufficient volume the light may pass over the solution instead of going through it.

Method

- Set up 8 test tubes ,clean and label test tubes A,B,C,D,E, for standard solution ,blank and two unknown solution.
- Prepare a series of known standard solutions by diluting the stock solution following the protocol in the table .

Tube	0.1 M Copper sulfate standard	H_2O	Unknown(1)	Unknown(2)
A	2	8	------	
B	4	6	-----	
C	6	4	------	
D	8	2	-----	
E	10	----	-----	
Unknown (1)	-----	----	10	
Unknown (2)	---------	----	-------	10
Blank	-------	10	------	

Mix contents, measure the absorbance of each tube at 600 nm against the blank ,and record your results

Results

Tube	Absorbance at 600 nm	Concentration
A		
B		
C		
D		
E		
Unknown(1)		
Unknown(2)		

Plot the standard curve (Absorbance vs. Concentration), determine the concentration of unknown solutions from graph.