Lab: 1

Spectrophotometry (colorimetery)

- A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.
- Spectrophotometer techniques are used to measure the concentration of solutes in solution
- Most instruments used filter called filter photometer while those used prisms or gratings called spectrophotometer.

Nature of light:

• Electromagnetic waves are characterized by their frequency and wavelength.

• Light is a spectrum of different wavelengths which the eye recognizes as "white" but can be isolated into different colors

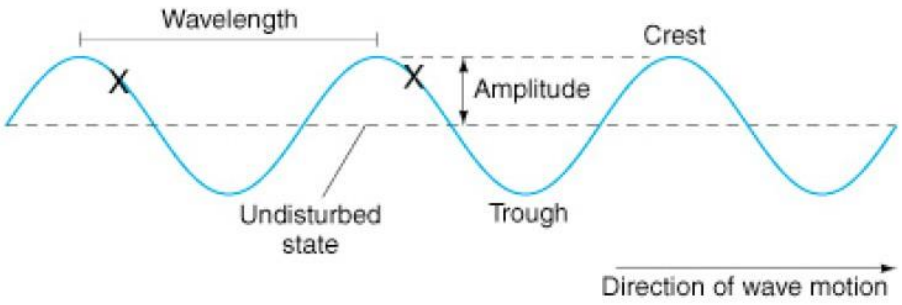
• Human eye responds to radiant energy between 380 and 750nm, but modern instruments can measure shorter wavelengths (UV) and longer (IR) ones.

Table 18-1 Colors of visible light

Wavelength of maximum absorption (nm)	Color absorbed	Color observed
380—420	Violet	Green-yellow
420—440	Violet-blue	Yellow
440—470	Blue	Orange
470—500	Blue-green	Red
500—520	Green	Purple
520—550	Yellow-green	Violet
550—580	Yellow	Violet-blue
580—620	Orange	Blue
620—680	Red	Blue-green
680—780	Red	Green

The term of **light** is used to describe radiant energy with different wave length that the human eye recognizes as (White).

Wavelength: Is the distance between two peaks of wave of light, expressed in nm.



Main components of a spectrophotometer including:

1) Light source

The function of the light source is to provide a sufficient of light which is suitable for marking a measurement

- **Types**

- Tungsten Lamp is the most common light source used in spectrophotometer for the visible region.
- Hydrogen / Deuterium Lamps For the ultraviolet region

2) Lenses

3) Monochromator

consists of three parts:

I) Entrance slit
II) Exit slit
III) Dispersion device

Types of dispersion devices :

1) Prism is used to isolate different wavelength .
2) Filters separate different parts of the electromagnetic spectrum.

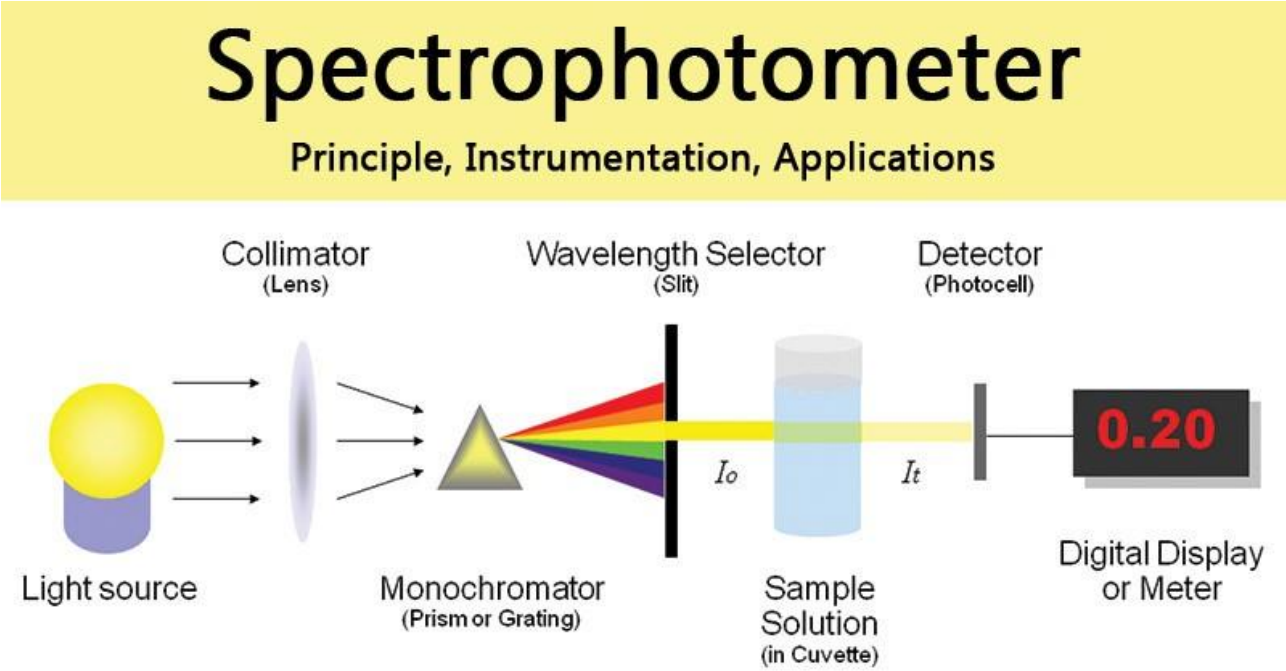
4) Cuvettes

are chosen for transparency in the spectral wavelengths of interest.

- **Types**

1- Glass cuvette for measurements in the visible region
2- quartz cuvette is more expensive and used for wavelengths below 350nm

5) **Photocell**: Converts light into electrical current
6) Galvanometer: To record the electrical current



Beer's Lambert Law:

Is relationship between the intensity of an incident light passing through a colored solution of a compound that absorbed light of certain wavelength and the concentration of that solution

- The base of Colorimetery is the measurement of the concentration of a colored substance.
- Absorbance (A) of a colored solution will be directly proportional to its concentration (C) multiplied by the thickness of solution (length path of light) (b) .

$$A \alpha bc \rightarrow A = abc$$

a) a = Proportional constant.
b) b = thickness of solution.
c) c = Molar concentration.

So when (C) is increased the absorbance (A) is increased we find that transmittance (T) varies inversely and logarithmically with Concentration (C) , When the (C) is increased the transmittance (T) is decreased .

$$A = -\log T\%$$

Information when we use Beer's Lambert's Law:

1. The solution must be colored.
2. The solution must be in low conc.
3. The solution must be clear.

We can compare the conc of the test solution with standard solution measured in the same way:

$$Ct = \frac{At}{As} \times Cs \quad \text{Or} \quad Ct = \frac{At - AB}{As - AB} \times Cs$$

Solution required for photometric measurements:

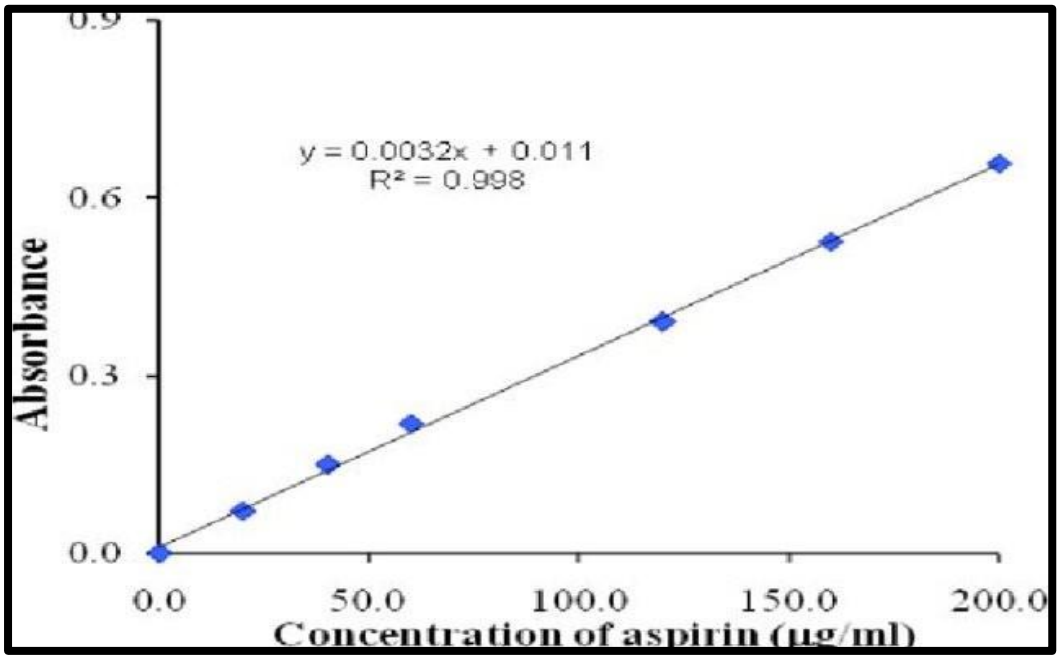
1. **Test** : made from any unknown specimen (blood, plasma and serum)
2. **Blank** : contain all reagents used except the substance.
3. **Standard** : made from a known quantity of the substance.
4. **Control** : make in enzyme assay.

Calibration (standard) curve

A calibration curve is a general method for **determining the concentration of a substance in an unknown sample** by comparing the unknown to a set of standard samples of known concentration.

The operator prepares a series of standards across a range of concentrations near the expected concentration of analyte in the unknown. The concentrations of the standards must lie within the working range of the technique (instrumentation) they are using. Analyzing each of these standards using the chosen technique will produce a series of measurements. For most analyses a plot of instrument response vs.

concentration will show a linear relationship. The operator can measure the response of the unknown and, using the calibration curve, can interpolate to find the concentration of analyte.



Example

Conc. of ca^{+} (mg/dl)	
Absorbance (A)	**Conc. Of std**
0.3	**50**
0.4	**60**
0.6	**62**
0.65	**65**
0.7	**68**
0.9	**70**
1	**80**

What the concentration of calcium if absorbance is 0.8