COLORIMETRY

Shehreen Akhtar
Department of Biochemistry, MGMCH

"Colorimetry is the measurement of intensity of color in a solution"

Thus, the concentration of **colorless biochemical compounds and metabolites** can be estimated if they are converted into **colored compounds** using chemical reactions under defined conditions **by measuring the intensity of color which is proportional to concentration of the compound.**

Principle of Colorimetry

The principle of Colorimetry is based on two fundamental laws:

- **Beer's Law**
- **Lambert's Law**

Beer's Law

Beer's law states that when a parallel beam of monochromatic light is passed through a solution, the **absorbance (A)** of the solution is directly proportional to the **concentration (C) of the solute in the solution**.

$$A \propto C$$

or

$$A = KC$$

Lambert's Law

According to **Lambert's law**, the **absorbance (A)** is directly proportional to **path length of light (l) which is the thickness of solution**.

$$A \alpha l$$

or

$$A = Kl$$

Combining the two laws, we get,

$$A = KCl$$

Where K is proportionality constant. It is also called **extinction coefficient** or **absorption coefficient** and is fixed for a given substance at specific concentration and wavelength.

- If the length of light path is also made constant. Then absorbance will be directly proportional to the concentration of chromogen. In a colorimeter length of the light path is kept constant by using a fixed diameter cuvette.
- In routine Colorimetry used in laboratories, absorption coefficient is not used. Instead concentration of the unknown is calculated by comparison of its absorbance with the absorbance of the solution of known concentration (standard).

If C_t and C_S are the concentration of same substance (e.g. urea) in the test sample and standard sample then:

Absorbance of test sample (A_t) = KC_tl

Absorbance of standard (A_s) = KC_sl

For the same substance and at constant path length under same conditions, K and l are constant.

$$A_t = C_t$$

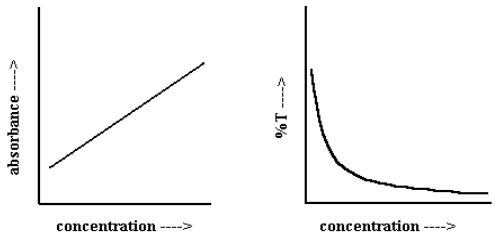
And $A_s = C_s$

$$A_t/A_s = C_t/C_s$$

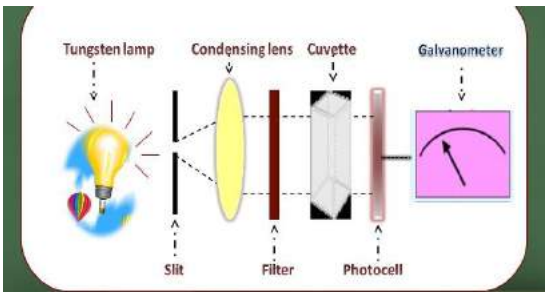
$$C_t = A_t/A_s \times C_s$$

- A **standard solution or Calibrator** refers to the solution of the same substance as of the test but of known concentration. It is therefore, representative of test compound with known concentration and absorption.
- Ideally a series of standards of known concentration should be used and their absorption plotted against concentration to obtain the standard curve or calibration curve to know the range of concentration over which the Beer's Law is obeyed.
- **A blank** containing all the solvents and reagent but not the test compound should also be used to exclude the absorbance contributed by them.

Transmittance v/s Absorbance



Components of Colorimeter



Light source

It is usually a tungsten lamp emitting light in the visible range only.

Filters/monochromators

Colored glass filters are used in simple instruments. They absorb most of the light and permit light of the corresponding color only with sufficiently narrow wave length. A suitable wavelength of light is selected by using a specific filter of a known wavelength provided in the instrument. The use of different filters depends on the color of the resultant solution. Complimentary color filters are used because the absorbance is maximum of the corresponding light.

Cuvettes

These are glass tubes of usually 1 cm diameter and uniform thickness in which absorbance is measured. A cuvette must be optically transparent, thoroughly clean, devoid of any scratch and free from any contamination.

Photosensitive detectors

Photosensitive detectors are used to convert the transmitted light into electrical energy. In photocell, a metal plate is coated with a thin layer of photosensitive element such as selenium. This in turn, is coated with a thin transparent layer of a metal such as gold or copper.

Measuring devices

- The photo detector response can be measured by:
- Galvanometers
- Ammeters
- Recorder
- Digital readout.
- The signal may be transmitted to computer or printout devices.

Applications of Colorimeter

- It is widely used in hospitals and laboratory for estimation of biochemical samples like plasma, cerebrospinal fluid, urine.
- It is specially used for quantitative estimations of serum components like glucose, proteins and various other biochemical components.

Advantages

- It is easily transportable.
- It is cost effective.
- It is well applicable for quantitative analysis of colored compounds.

Disadvantages

- Similar colors from interfering substances can produce errors in results.
- Can not be used for colorless compounds, does not work in UV and IR ranges.
- Matrix interferences can produce bad results in uncontrolled situations.

Thank You