Paper M 604 unit 6.10 **Colorimetry**

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In physical and analytical chemistry, colorimetry or colorimetry is a technique used to determine the concentration of colored compounds in solution. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light (not to be confused with the tristimulus colorimeter used to measure colors in general). To use the colorimeter, different solutions must be made, including a control or reference of known concentration. With a visual colorimeter, for example, the Duboscq colorimeter illustrated, the length of the light path through the solutions can be varied while filtered light transmitted through them is compared for a visual match. The concentration times path length is taken to be equal when the colors match, so the concentration of the unknown can be determined by simple proportions. Nessler tubes work on the same principle.

There are also electronic automated colorimeters; before these machines are used, they must be calibrated with a cuvette containing the control solution. The concentration of a sample can be calculated from the intensity of light before and after it passes through the sample by using the Beer-Lambert law. Photoelectric analyzers came to dominate in the 1960s.

The color or wavelength of the filter chosen for the colorimeter is extremely important, as the wavelength of light that is transmitted by the colorimeter has to be the same as that absorbed by the substance being measured. For example, the filter on a colorimeter might be set to red if the liquid is blue.

A Colorimeter involves the measurement of Color and is the widely used method for finding the concentration of biochemical compounds. It measures absorbance and wavelength between 400 to 700 nm (nanometer) i.e. from the visible spectrum of light of the electromagnetic spectrum.

Absorption of light – Light falling on a colored solution is either absorbed or transmitted. A colored solution absorbs all the colors of white light and selectively transmits only one color. This is its own color.

Principle of Colorimeter

⇒ **A colorimeter** is based on the photometric technique which states that When a beam of incident light of intensity I_0 passes through a solution, a part of the incident light is reflected (I_r), a part is absorbed (I_a) and rest of the light is transmitted (I_t)

Thus,

$$I_0 = I_r + I_a + I_t$$

⇒ In colorimeter, (I_r) is eliminated because of the measurement of (I_0) and It is sufficient to determine the (I_a). For this purpose, the amount of light reflected (I_r) is kept constant by using cells that have identical properties. (I_0) & (I_t) is then measured.

⇒ The mathematical relationship between the amount of light absorbed and the concentration of the substance can be shown by the two fundamental laws of photometry on which the colorimeter is based.

Beer's Law

⇒ This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.

$$Log_{10}\ I_0/I_t = a_s c$$

where,

a_s = Absorbency index

c = Concentration of Solution

Lambert's Law

⇒ Lambert's law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$$A = \log_{10}\ I_0/I_t = a_s b$$

Where,

A = Absorbance of test

a_s = Absorbance of standard

b = length / thickness of the solution

The mathematical representation of the combined form of Beer-Lambert's law is as follows:

$$Log_{10}\ I_0 / I_t = a_s bc$$

If *b* is kept constant by applying Cuvette or standard cell then,

$$Log_{10}\ I_0/I_t = a_s c$$

The absorbency index as is defined as

$$a_s = A/cl$$

Where,

c = concentration of the absorbing material (in gm/liter).

l = distance traveled by the light in solution (in cm).

In simplified form,

The working principle of the colorimeter is based on Beer-Lambert's law which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

$$A \propto cl$$

Where,

A = Absorbance / Optical density of solution

c = Concentration of solution

l = Path length

$$A = \in cl$$

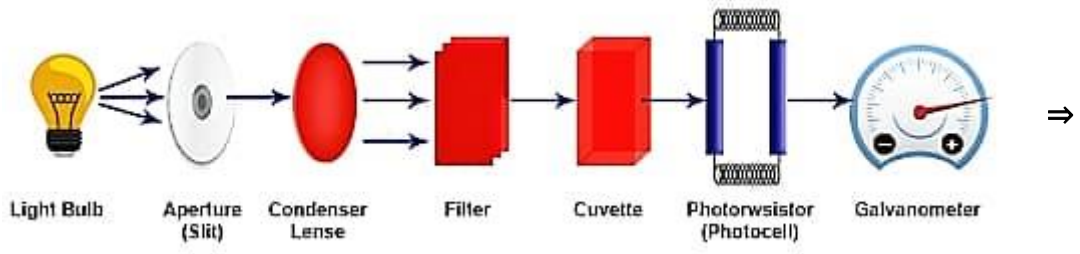
$\in$ = Absorption coefficient

Parts Of Colorimeter

There are 5 essential parts in a calorimeter……

⇒ **Light Source** – The most common source of light used in colorimeter is a tungsten filament.

⇒ **Monochromator** – To select the particular wavelength filter or monochromators are used to split the light from the light source.



⇒

Sample holder – Test tubes or Cuvettes are used to hold the color solutions they are made up of Glass at the visible wavelength.

⇒ **Photo Detector System** – when light falls on the detector system, an electric current is generated, this reflects the Galvanometer reading.

⇒ **Measuring device** – The current from the detector is fed to the measuring device, the Galvanometer, which shows the meter reading that is directly proportional to the intensity of light.

Working of the Colorimeter

⇒ When using a colorimeter, it requires being calibrated first which is done by using the standard solutions of the known concentration of the solute that has to be determined in the test solution. For this, the standard solutions are filled in the cuvettes and placed in the cuvette holder in the colorimeter.

⇒ There is a ray of light with a certain wavelength that is specific for the assay is directed towards the solution. Before reaching the solution the ray of light passes through a series of different filters and lenses. These lenses are used for navigation of the colored light in the colorimeter and the filter splits the beam of light into different wavelengths and allows the required wavelength to pass through it and reaches the cuvette containing the standard or test solutions. It analyzes the reflected light and compares it with a predetermined standard solution.

⇒ When the monochromatic light (light of one wavelength) reaches the cuvette some of the light is reflected, some part of the light is absorbed by the solution and the remaining part is transmitted through the solution which falls on the photodetector system. The photodetector system measures the intensity of transmitted light and converts it into the electrical signals that are sent to the galvanometer.

⇒ The galvanometer measures the electrical signals and displays them in digital form. That digital representation of the electrical signals is the absorbance or optical density of the solution analyzed.

⇒ If the absorption of the solution is higher than there will be more light absorbed by the solution and if the absorption of the solution is low then more lights will be transmitted through the solution which affects the galvanometer reading and corresponds to the concentration of the solute in the solution. By putting all the values in the formula given in the below section one can easily determine the concentration of the solution.

Applications of the Colorimeter

⇒ The colorimeter is commonly used for the determination of the concentration of a colored compound by measuring the optical density or its absorbance.

⇒ It can also be used for the determination of the course of the reaction by measuring the rate of formation and disappearance of the light-absorbing compound in the range of the visible spectrum of light.

⇒ By colorimeter, a compound can be identified by determining the absorption spectrum in the visible region of the light spectrum.

⇒Here is the formula used for determining the concentration of a substance in the test solution.

$$A = \in cl$$

⇒ For two solutions i.e. Test and standard,

$\in$ = Constant

l = Constant (using the same Cuvette or Standard cell)

$$A_T = C_T \qquad \text{….. (i)}$$

$$A_S = C_S \qquad \text{….. (ii)}$$

⇒ From (i) & (ii),

$$A_T \times C_S = A_S \times C_T$$

$$C_T = (A_T/A_S) \times C_S$$

Where,

C_T = Concentration of the Test solution

A_T = Absorbance/ Optical density of the test solution

C_S = Concentration of the standard

A_S = Absorbance / Optical density of the standard solution