TOPIC: Colorimetry

- **Colorimetry is the field of determining the concentration of a coloured compound in a solution. A colorimeter, also known as a filter photometer, is an analytical machine that acts as the tool quantify a solutions concentration by measuring the absorbance of a specific wavelength of light.**
- Colorimeters are used for a wide range of applications across the chemical and biological fields including, but not limited to, the analysis of blood, water, nutrients in soil and foodstuffs, determining the concentration of a solution, determining the rates of reaction, determining the growth of bacterial cultures and laboratory quality control.

Colorimeter Principles

- Colorimeters are used to detect colour and determine the solutions concentration, i.e. when a wavelength is passed through a sample, some of the light is absorbed and some passes through. It is the wavelengths of light that pass through that are detected
- By knowing which wavelengths have passed through the detector can also work out which colored wavelengths were absorbed.
- If the solution to be tested is colourless, a common procedure is to introduce a reagent that reacts with the solution to produce a coloured solution. The results are compared against known standards.
- The colorimeter uses the Beer-Lambert law to detect the absorbance of the wavelength. Beer-Lamberts law is commonly written as:

$$A = \varepsilon cl$$

- Where, A is the absorbance, ε (epsilon) is the molar absorptivity, c is the concentration of the solution and l is the length that the light passes through (also known as the mean free path).
- Aside from this, if there is a continual changing of the solution, i.e. it is a reaction, then % of transmittance against time is generally used.

What is Colorimeter?

- A Colorimeter is a light and sensitive device used to measure the transmittance and absorbance of light that passes through a liquid sample.
- The colorimeter device also measures the intensity or color concentration that develops upon introducing a particular reagent into a solution.

These are Divided into Two Types.

- **Color densitometers,** which measures the density of primary colors,
- **Color photometers,** which measures transmission and color reflection.
- Usually, the Colorimeter is used to measure the known solute concentration in a given solution with the help of Beer-Lambert law. **The Colorimeter was invented by Louis J Duboscq, in the year 1870.**

Principle of Colorimeter

Let us discuss the principle of colorimeter. A photometric is a technique which states that when a beam of incident light of intensity I_o passed through a solution, it occurs the following.

A part is reflected which is denoted as I_r

A part is absorbed which is denoted as I_a

The remaining light is transmitted, which is denoted as I_t

Thus, $I_o = I_a + I_r + I_t$

- To determine Ia, the measurement of It and Io is sufficient. Therefore, I_r is eliminated and the amount of light reflected is kept as constant to measure It and Io.
- The Colorimeter is based on two fundamental photometry laws. Let us discuss them briefly.

Beer's law

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

$$\text{Log10 } I_o/I_t = a_s c$$

Where c is the concentration of the solution, and as is absorbency index.

Lambert's law

$$A = \log 10\ I_o/I_t = a_s b$$

Where, as is the standard absorbance and A is the test absorbance of the test, and b is the thickness/length of the solution.

Working of Colorimeter

As discussed in the Colorimeter's different principles, let us look at the working of colorimeter.

Step 1

Before going to start the experiment, it is essential to calibrate the Colorimeter. It can be done with the help of the standard solutions of the known solute concentration that has to be determined. Then, fill the standard solutions in the cuvettes and place it in the cuvette holder of the Colorimeter.

Step 2

A light ray of a particular wavelength specific for the sample is in the direction of the solution. The light travels through a series of various filters and lenses. The colored light, then navigates by taking the help of lenses, and the filter allows the split of a beam of light into different wavelengths allowing only the required wavelength to pass through and reach the standard test cuvette.

Step 3

As the light beam reaches the cuvette, it is transmitted, reflected, and absorbed by the solution. The transmitted ray falls on the photodetector system, where the intensity of transmitted light is measured. Now, the photodetector system converts the beam into the electrical signals and sends it to the galvanometer.

Step 4

The electrical signals that are measured by the galvanometer displays in the digital form.

Step 5

Formula to determine the substance concentration in the test solution is,

$$A = \in cl$$

Where $\in$ and l are constant for the standard and test solutions,

$$A_T = C_T \text{ ---- (i)}$$

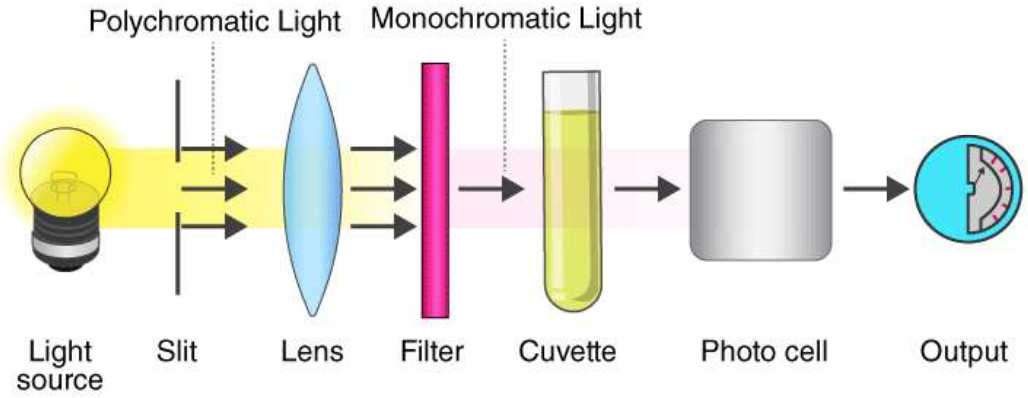
$$A_S = C_S \text{ ---- (ii)}$$

From the above two equations, we get the colorimeter formula as,

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

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

Where A_T is the optical density/absorbance of the test solution, A_S is the absorbance / optical density of the standard solution, C_T is the concentration of the test solution, and C_S is the standard concentration.



- To measure concentrations, the amount of light absorbed is dependent upon the amount of solute (also known as the analyte as it is the species being measured) in the solution- a higher concentration of dissolved solute means that more light will be absorbed, and vice versa, hence, the concentration can be backed out from the absorption of specific wavelengths.

The Colorimeter Itself

- A colorimeter is composed of many parts. Aside from using a known standard solution, alongside either known concentrations and unknown concentrations, there are many vital components to a colorimeter.
- As the principles are based around light, a light source is required and usually takes the form of a filament lamp.
- Other components include an adjustable aperture to let the light through, coloured filters to filter specific wavelengths of light, a cuvette to hold the solution (commonly made of quartz), a photodetector to measure the transmitted light and a meter to quantify the values into a readable output.
- The coloured filters are chosen to select the wavelength in which the dissolved solute will absorb the most.
- For most experiments the common wavelength range is between 400 and 700 nm, but when some analytes absorb in the ultraviolet range (less than 400 nm) then modification of the colorimeter is generally required.
- This normally takes the form of removing the filament lamp and replacing it with light-emitting diode(s) of a specific colour.
- The output can be either analogue or digital in nature and, depending of the principle used, will give either an absorbance (0-infinity logarithmic output) or a %transmittance (0-100%) readout. The ideal output for an absorbance measurement is between 0 and 2, but it is desirable to have a reading between 0 and 1, as above 1 the results can become unreliable due to the scattering of light. The readout is usually in the form of a spectrum.
- Most calorimeters will require calibration, which is the solvent alone and not the measurable contents with the solvent- i.e. a standard or 'blank' solution.
- The calibration allows the absorbance of the solvent to be measured, also known across many instruments as the background noise.

- Once measured, the solvent absorption values are removed from any future readings, allowing the absorbance (or %transmittance) to be calculated (and plotted on a spectrum) for the desired analyte(s) without noise interference.
- There are a wide variety of colorimeters out there, where some colorimeters are large machines and generally used for a wide-range of laboratory analyses, but some colorimeters are now hand-held and can be used for on-site analyses such as the determination of *in-situ* water and soil samples. In the case of handheld colorimeters, a numerical readout is the common procedure as opposed to a spectrum found on the larger laboratory machines.

Colorimeter Uses

- Colorimeter device is used to test the water quality by screening chemicals such as chlorine, cyanide, fluoride, dissolved oxygen, iron, zinc, hydrazine, and molybdenum.
- Colorimeters are widely used to monitor bacterial growth or yeast culture.
- They provide highly accurate and reliable results when used for the assessment of color in bird plumage.
- They are also used to monitor and measure the colour in various foods and beverages, including sugar and vegetable products.
- It is used in hospitals and medical laboratories to estimate biochemical samples, including plasma, urine, cerebrospinal fluid, serum, and a few more.
- Besides, it helps in the identification of counterfeit and substandard drugs.
- Most of the food industries use this device.
- Paints and textile manufacturers use a colorimeter.
- This device often checks the strength and durability of the colours in paints and fabrics to ensure a similar quality