ROHINI
COLLEGE OF ENGINEERING AND TECHNOLOGY
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DEPARTMENT OF BIOMEDICAL ENGINEERING

BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.5 Colorimeter

A colorimeter is an analytical device used to determine the concentration of a colored compound in a solution by measuring the absorbance of a specific wavelength of light.

A basic colorimeter schematic is shown in Figure 5.5.1. Observe that light passes through an optical color filter, is focused by lenses on the reference and sample cuvettes, and falls on the reference and sample photodetectors. The difference in voltage between the two detectors is increased by a dc amplifier and applied to a meter.

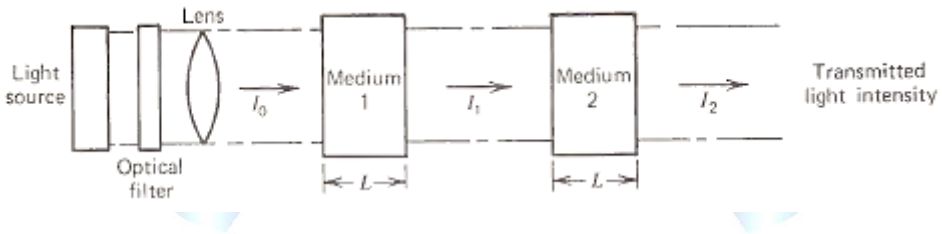

Fig 5.5.1 *Basic colorimeter analysis*

Basic colorimeter analysis (Figure 5.5.1) involves the precise measurement of light intensity. Transmittance is defined as:

$$T = \frac{I_1}{I_0} x100\ \%$$

$$I_2 = TI_1$$

$$I_2 = T^2 I_o$$

Io is initial light intensity

I_1 is first attenuated light intensity

I_2 is second attenuated light intensity

T is transmittance in percent

Absorbance (optical density) is defined as:

$$A = \log\frac{I_1}{I_0} = \log\frac{I}{T}$$

Where,

A is absorbance

If the path length or concentration increases, the transmittance decreases and the absorbance increases. Essentially, this phenomenon can be expressed by Beer's law: The operation 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 solution's concentration and the length of a light path through it.

A =aCL

where

A is absorbance

L is cuvette path length

C is concentration of absorbing substance

a is absorbtivity related to the nature of the absorbing substance and optical wavelength (known for a standard solution concentration)

Therefore, the concentration of the unknown solution can be found from the following relationship:

$$C_\mu = C_s \frac{A_u}{A_s}$$

where

C_u is unknown concentration

C_s is standard concentration (for calibration)

A_u is unknown absorbance

A_s is standard absorbance

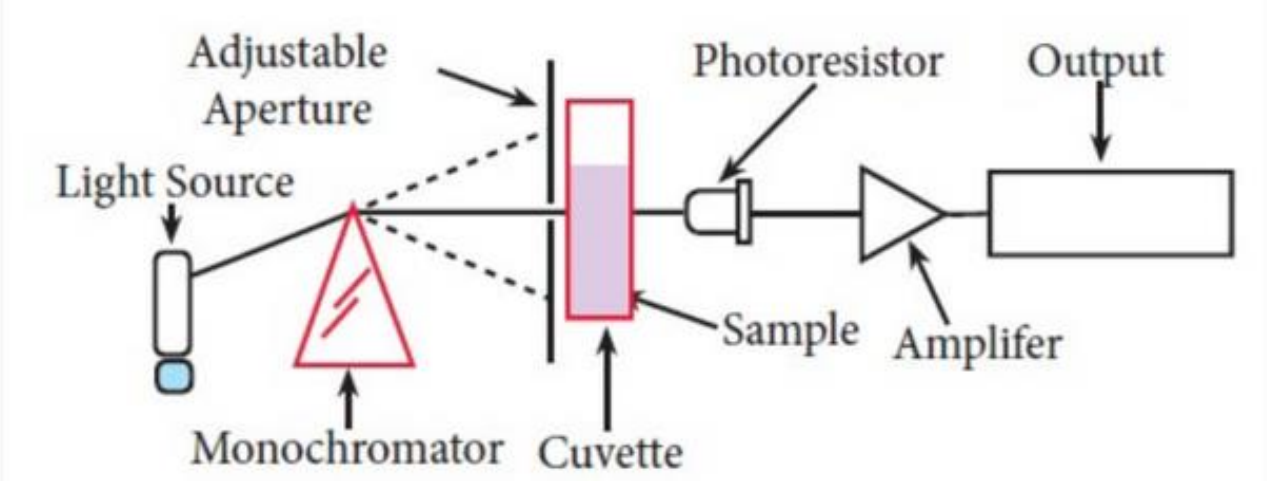

Fig 5.5.2 *Principle of Colorimeter*

Components of a Colorimeter:

1. **Light Source:** The source of light should produce energy with enough intensity to cover the entire visible spectrum (380-780 nm). Commonly, Tungsten lamps are used as a light source for measurement in the visible spectrum and near-infrared ranges. Halogen deuterium is suitable for measurement in the UV range (200-900 nm).
2. **Slit:** It reduces unwanted or stray light by allowing a light beam to pass through.
3. **Condensing lens:** Parallel beam of light emerges from condensing lens after the light passes through slit incidents on it.
4. **Monochromator:** It filters the monochromatic light from polychromatic light, which absorbs unwanted light wavelengths and permits only monochromatic light. These are of three types: prism, grating, and glass.
    a. **Prism:** It facilitates the refraction of light when it passes from one medium to another.
    b. **Glass:** It selectively transmits light in certain ranges of wavelengths.
    c. **Gratings:** These are made of graphite, which separates light in different wavelengths.

5. **Cuvette (Sample cell):** The monochromatic light from the filter passes through the colored sample solution placed in the cuvette. Their sizes range from square, and rectangle to round and have a fixed diameter of 1cm. These are of three types based on the substances these are made of: Glass, Quartz, and Plastic cuvette.
    a. **Glass cuvettes** are cheap and absorb light of 340 nm wavelength.
    b. **Quartz cuvettes** facilitate entry of both lights of UV and visible ranges.
    c. **Plastic cuvettes** are cheaper, easily scratched, and have shorter lifespans.
6. **Photocell (Photodetector**): These photosensitive devices measure light intensity by converting light energy into electrical energy.
7. **Galvanometer:** The electrical signal generated in a photocell is detected and measured by a galvanometer. It displays optical density (OD) and percentage transmission.

Working Principle:

1. **Light Emission**: The light source emits a beam of light that passes through the monochromator or filter. This component isolates the specific wavelength of light that corresponds to the maximum absorbance of the substance being measured.
2. **Light Transmission Through Sample**: The selected wavelength of light passes through the sample solution in the cuvette. If the sample contains a colored compound, it will absorb some portion of this light. The amount of light absorbed is proportional to the concentration of the colored compound in the solution.
3. **Detection**: The light that has not been absorbed passes through the sample and reaches the photodetector. The photodetector converts this light into an electrical signal.
4. **Measurement**: The electrical signal is processed and converted into a readable value, usually displayed as absorbance (A) or transmittance (%T).

Applications of Colorimeter:

- These are used in the food and ***food processing*** industries.
- It is frequently used in laboratories and hospitals to determine the ***biochemical composition*** of samples like blood, urine, cerebral spinal fluid, plasma, serum, etc.
- The instrument is also employed in cosmetology to measure the UV protection level of ***skin-care products***.
- They are used to evaluate the ***water's purity*** and screen for the presence of chemicals like cyanide, iron, fluorine, chlorine, molybdenum, etc.
- They are employed to evaluate the color contrast and brightness of ***screens on mobile devices***, computers, and televisions to give people the greatest viewing experience.
- A colorimeter is also employed in the ***pharmaceutical*** sector.
- Blood samples are tested using a colorimeter to determine the amount of ***hemoglobin*** present.

Advantages of Colorimeter:

1. User-friendly interfaces and minimal calibration steps make them accessible for routine analysis.
2. Colorimeters provide quick result.
3. Many colorimeters are portable and can be used in field settings, allowing for on-site analysis.

Disadvantages of Colorimeter:

1. Colorimeters generally have lower sensitivity and precision compared to spectrophotometers.
2. Colorimeters typically measure absorbance at a limited number of fixed wavelengths.
3. Colorimetric measurements can be affected by the presence of other colored substances or turbidity in the sample.
4. Accurate measurements depend on proper calibration using standard solutions.

5. Colorimeters are generally designed to measure one parameter at a time, unlike more advanced instruments that can analyze multiple parameters simultaneously.

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