COLORIMETER

A colorimetric determination is one that involves the measurement of colour in the visual region of the electromagnetic spectrum (400-700nm).

In colorimeter, the sample is normally a liquid. The sample compartment of a colorimeter is provided with a holder to contain the cuvette, in which the liquid is examined.

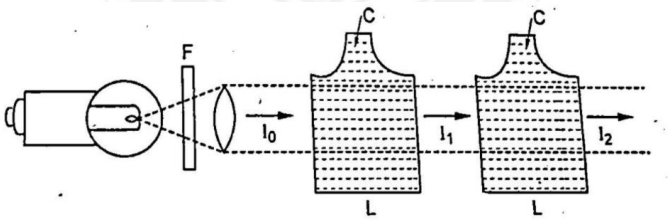
Principle of Colorimeter:

Blood cerum is a complex fluid that contains numerous substances in solution. The determination of the concentration of these substances is performed by specialized chemical techniques.

Although there are usually several different methods by which any particular analysis can be performed, more tests used are based on a chemical color reaction follows by a colorimetric determination of the concentration.

This principle gives that the chemical compounds in solution appear colored, with the saturation of the color depending on the concentration of the compound.

The way in which the light absorption can be used to determine the concentration of the substance is shown in the following figures.



In the above figure, it is assumed that a solution of concentration C is placed in a cuvette with a length of the light path L.

Light of an appropriate color or wavelength is obtained from a lump through filter F. The light that enters the cuvette has a certain intensity, I_0 with part of the light being absorbed in the solution, the light leaving the cuvette has a lower intensity I_1 the relation of I_0 and I_1 gives the transmittance T_1.

$$T = \frac{I_1}{I_0} \times 100\%$$

If a second cuvette with the same solution were brought into the light path behind the first cuvette, only a similar portion of the light entering this cuvette would be transmitted. The light intensity I_2, behind the second cuvette is

$$I_2 = T I_1. \text{ (or) } I_2 = T^2 I_0$$

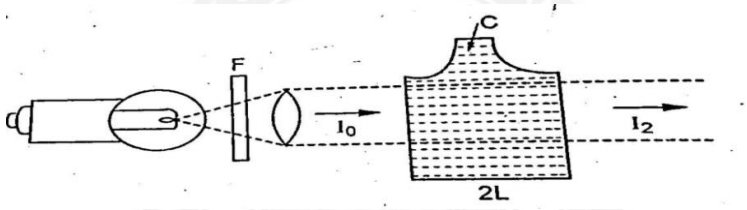
The light transmitted through successive cuvettes decreases in the same manner. The absorbance or optical density A can be measured as

$$A = -\log \frac{I_1}{I_0}$$

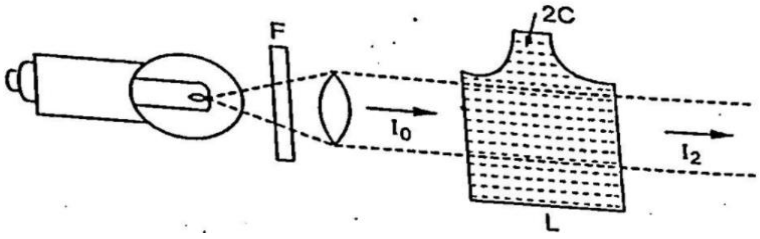
(or)

$$A = \log \frac{1}{T}$$

The total absorbance of the two cuvettes is the sum of the individual absorbances.



The amount of the light absorbed depends only on the number of molecules of the absorbing substance that can interact with the light. If instead of two cuvettes, each with path length L, one cuvette with path length 2L, were used the absorbance would be the same.



The absorbance is also the same if the cuvette has a path length L, but the concentration of the solution was doubled. This relation can be expressed by Beer's law.

Beer's Law:

The amount of light absorbed by a colored solution is directly proportional of the concentration of substance in the colored solution.

$$A=acL$$

Where

L – path length of the cuvette

c – concentration of the absorbing substance

a – absorbtivity

Filter Photometer:

A colorimeter or filter-photometer is used for measuring transmittance and absorbance of solutions. Following figure shows the principle of a colorimeter.

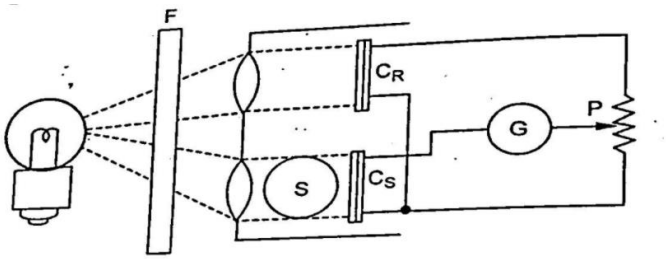


Fig: Colorimeter (Filter Photometer)

A filter F selects a suitable wavelength large from the light of a lamp. This light falls on two photoelectric cells, Reference cell C_R and Sample cell C_S.

Without a sample, the output of both cells is the same. When a sample is placed in the light path for the sample cell, its output is reduced and the output of C_R has to be divided by a potentiometer P until a galvanometer (G) shows a balance. The potentiometer can be calibrated in transmittance or absorbance units over a range of 1 to 100 percent transmittance corresponding to 2 to 0 absorbance units.

Flame Photometer:

A flame photometer is used to determine the concentration of sodium (Na), Potassium (K) and calcium (Ca). Lithium is used as a calibration substance in the analysis.

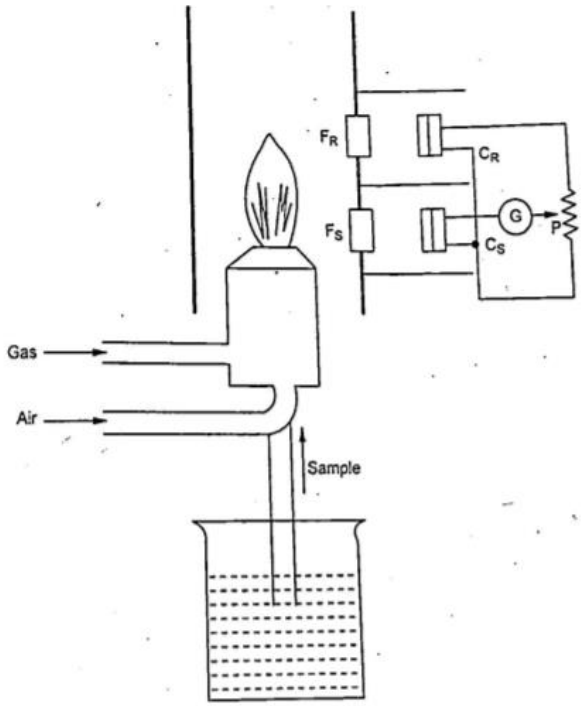


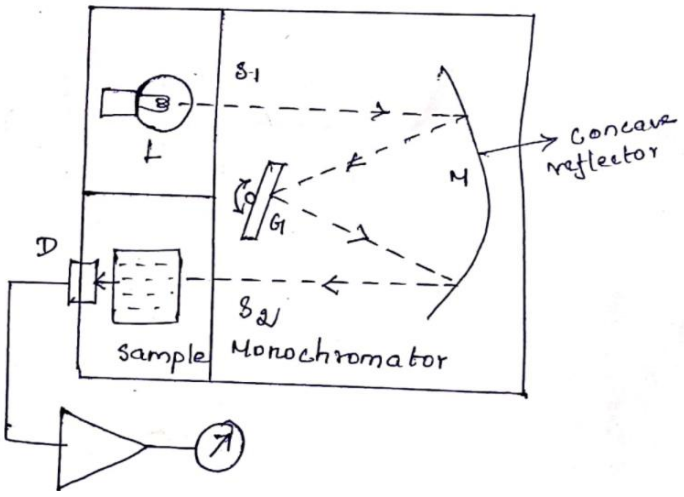
Fig: Flame Photometer

A colourless flame appears yellow for sodium and violet for potassium when their solutions are aspirated into the flame. Flame photometer is used in the analysis of blood or urine. A few drops of sample is aspirated into a glass flame that burns in a chimney, And a known amount of lithium salt is added to the sample, thus causing a red flame.

Filters are used to separate the red light produced by the lithium from the yellow or violet light emitted by the sodium or potassium. As in the colorimeter, the output from the sample cell C_S is compared with a fraction of the output from a reference cell C_R. Finally, the balance potentiometer P is calibrated directly in units of sodium or potassium concentration.

Spectro Photometer:

The blood serum is a complex fluid that contains a large amount of chemical substances dissolved in it. These substances have the property of absorbing emitting visible light. The nature of these substances can be analysed by analysing their absorbing and transmittance characteristics. Thus, the protein and iron levels in the blood can be measured with the help of photometer.



The figure shows the spectrophotometer here the light from a halogen lamp is passed through a entrance slit S_1 and incident on a concave reflector which focuses the light on a diffraction grating 'G'. The dispersed light from the grating directed on the reflector. From the reflector the light beam is directed to the sample through a narrow exit slit S_2. Photodetector detects the light and gives an electrical output corresponding to the intensity of the transmitted light.

The amplifier amplifies the output from the detector and finally the indicator indicates the concentration of the substance. By rotating the grating, the measurements can be made at different wavelengths.