Notes by — Mulik S.V
Asst. Professor

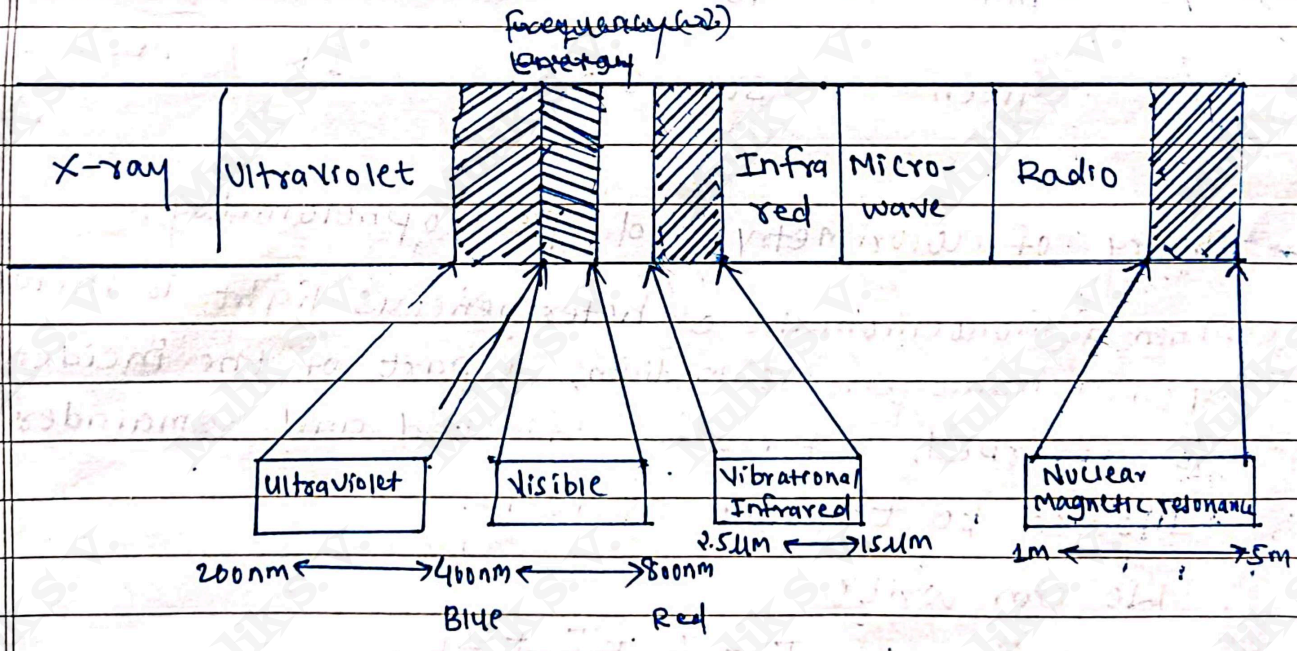
DATE

Colorimetry and Spectrophotometry

- Colorimetric analysis is based on the variation of colour of a system with change in concentration of some component.

- The colour is due to the formation of coloured compound by the addition of an appropriate reagent or the desired component may be coloured itself.

- Colorimetry is concerned with the determination of concentration of a substance by the measurement of relative absorption of light with respect to known concentration of the substance.



Light consists of radiation to which the human eye is sensitive. Waves of different wavelengths give rise to light of different colours, while a mixture of light of these wavelengths constitutes white light.

Colorimetry :- Visible region and near U.V. region

Spectrophotometry :- U.V (185-400nm), visible (400-760nm) and IR (0.76-15 mμ) region.

Approximate wavelengths of colours

Ultra-Violet	< 400nm	Yellow	570-590nm
Violet	400-450nm	Orange	590-620nm
Blue	450-500nm	Red	620-760nm
Green	500-570nm	IR	> 760 nm

* Theory of colorimetry and spectrophotometry :-

When a monochromatic or heterogeneous light is incident upon a homogeneous medium, a part of the incident is absorbed, a part is reflected and remainder is allowed to transmit as it is.

We can write

$$I_0 = I_a + I_r + I_t$$

I_0 = Intensity of incident light

I_a = —— absorbed light

I_r = —— reflected light

I_t = —— transmitted light

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(Assistant Professor)

* Lambert's Law:

When a beam of monochromatic radiation passes through a homogeneous absorbing medium, the rate of decrease of intensity of the radiation with thickness of absorbing medium, ~~the rate of decrease of~~ is proportional to the intensity of the incident radiation.

i.e.

$$\frac{-dI}{dx} = kI \qquad \ldots\ldots (3.3)$$

I is the intensity of the radiation after passing through a thickness 'x' of the medium.

dI = Infinitesimally small decrease in the intensity of the light radiation.

dx = Infinitesimally small thickness 'dx' of the medium

$\frac{-dI}{dx}$ = Rate of decrease of intensity of radiation with thickness of the absorbing medium.

k = Absorption coefficient (depends upon absorbing medium).

* Lambert - Beer's Law (or Beer's Law) :-

When a beam of monochromatic radiation passes through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional

to the intensity of incident radiation as well as to the concentration of the solution.

$$-\frac{dI}{dx} = K' \cdot I \cdot C \qquad \text{---------- (2)}$$

C = Concentration of solution in moles per dm^3.

If I_0 is the intensity of the radiation before entering the absorbing medium (i.e. $x=0$), then the intensity I, after passing through any finite thickness x of the medium can be obtained by

for Lambert's integrating equatn (1)

$$\int_{I_0}^{I} \frac{dI}{I} = -K \int_{X=0}^{X=x} dx$$

i.e. $\log \frac{I}{I_0} = -K \cdot x$

The exponential form of this equatn can be written as,

$$\frac{I}{I_0} = e^{-K \cdot x}$$

or. $I = I_0 \cdot e^{-Kx}$

for Lambert-Beer's (Beer's) Integrating equatn (2)

$$\int_{I_0}^{I} \frac{dI}{I} = -K' C \int_{X=0}^{X=X} dx$$

i.e. $\log \frac{I}{I_0} = -K' \cdot C \cdot x$ or $\log \frac{I_0}{I} = K' C x$

$$\frac{I}{I_0} = e^{-K' C \cdot x}$$

or $I = I_0 \cdot e^{-K \cdot x \cdot C}$

Changing natural logarithm to the base 10, we get

$I = I_0 \cdot 10^{-\varepsilon C x}$ or $\log_{10} \frac{I_0}{I} = \varepsilon C x$

$\therefore \varepsilon = \frac{K'}{2.303}$ called molar extinction coefficient)

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* Terms used in Colorimetry and Spectrophotometry.

(i) Transmittance or transmission (T): It is the fraction of incident light transmitted i.e. I/I_0

(ii) Opacity: It is the reciprocal of transmittance i.e. I_0/I or $1/T$.

(iii) Optical density (D) or Absorbance (A):

$$D = A = \log \frac{I_0}{I} = \varepsilon C x$$

(iv)

* Application of Beer's Law

Consider the case of two solutions of a coloured substance (same colour intensity) having concentrations C_1 and C_2 placed in the instrument having layer thickness x_1 and x_2, respectively, we can write.

$$I_1 = I_2$$

or

$$\text{i.e. } I_0 \cdot 10^{-\varepsilon C_1 x_1} = I_0 \cdot 10^{-\varepsilon C_2 x_2}$$

Where, System is optically balanced and Beer's law holds, then we can write,

$$\therefore C_1 x_1 = C_2 x_2$$

Therefore, a colorimeter can be used in dual capacity

* Deviations from Beer's law:-

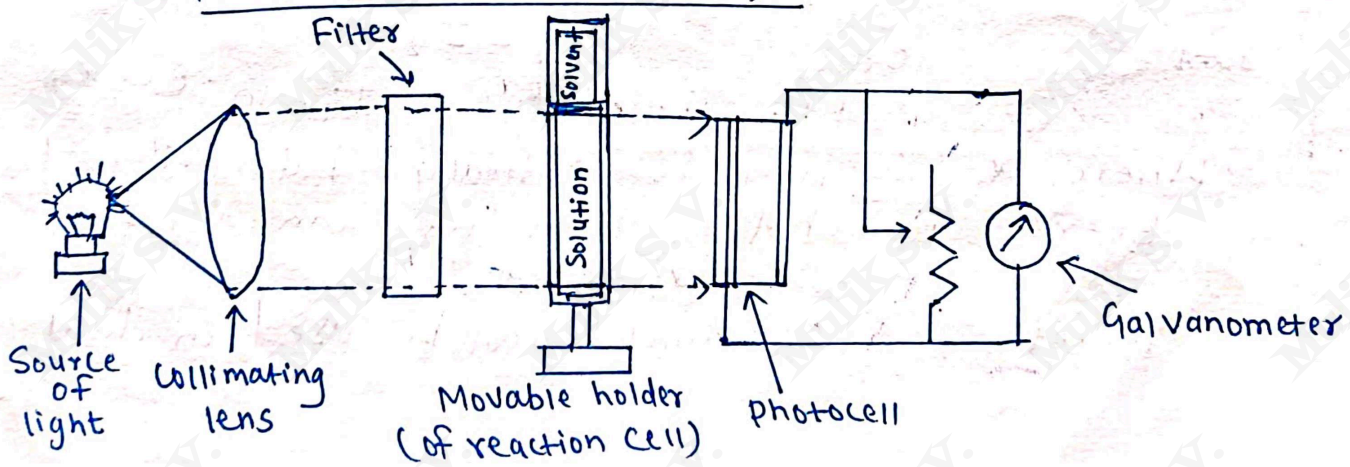
Beer's law may not hold good under following conditions.

1. When monochromatic light is not used as well as temperature is not kept constant
2. When colour of solution decomposes and its intensity decreases with certain period of time
3. When amount of electrolyte is varied with small amount
4. When coloured solute dissociates or associates in solution.
5. When coloured solute forms complexes.

* Classification of methods of "Color" measurement or comparison

(A) The quantitative comparison of these solutions may be carried out by one or more of the following six methods.

(A) Photoelectric colorimeter method (Single Beam photoelectric colorimeter)



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i) Light Source: Sunlight, arc light, mercury vapour lamp

ii) Filter (Monochromator):-

(a) Filters consist of coloured glass or coloured gelatin coated on glass and possess the property of transmitting light from a specified region of the spectrum.

(b) Monochromator allows light of suitable wavelength to pass through it.

(c) The best filter is which gives maximum absorption or minimum transmission for a given concentration of the absorbing substance.

(d) Less satisfactory methods include the use of a filter whose colour is as close as possible to the complementary colour of the solution.

Table: Complementary colours.

wavelength (nm)	Transmitted colour	Complementary colour
400-435	Violet	Yellowish-green
435-480	Blue	Yellow
480-490	Greenish-blue	Orange
490-500	Bluish-green	Red
500-560	Green	Purple
560-580	Yellowish-green	Violet
580-595	Yellow	Blue
595-610	Orange	Greenish-blue
610-750	Red	Bluish-green

(iii) A container for the solution (Cells or cuvettes)

Cuvettes are rectangular or cylindrical vessels with accurate dimensions.

Visible spectral region - Glass vessels are employed

U.V - optical parts made up of quartz

Light absorbed = [Light absorbed by empty cuvette or by cuvette filled by pure solvent] - [Light absorbed by solution under study]

(iv) A Barrier-layer Photocell [Photovoltaic or Photronic cell]

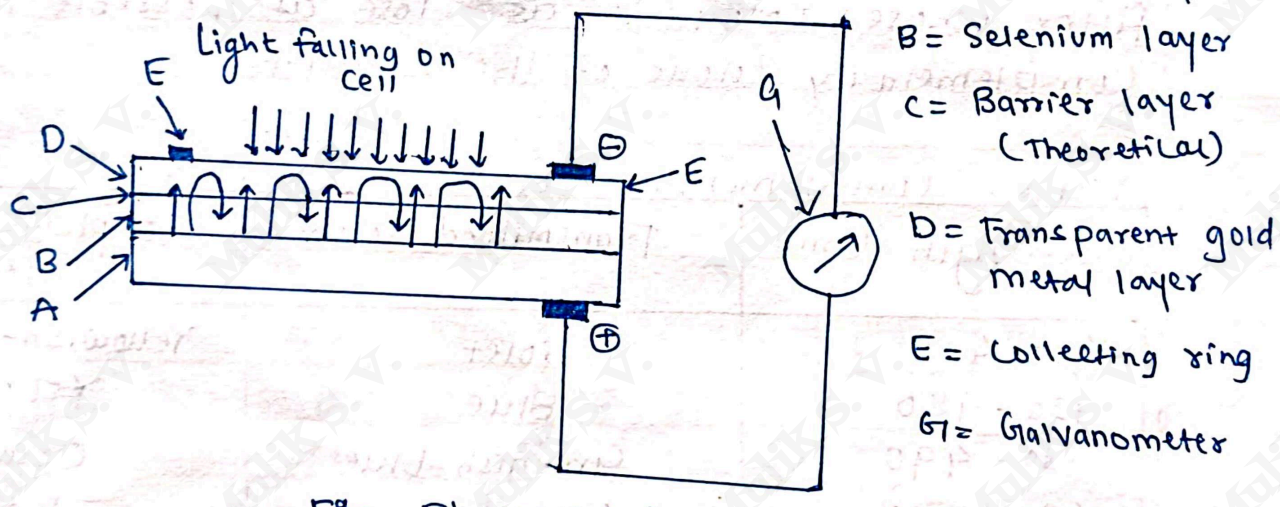


Fig. Photovoltaic cell.

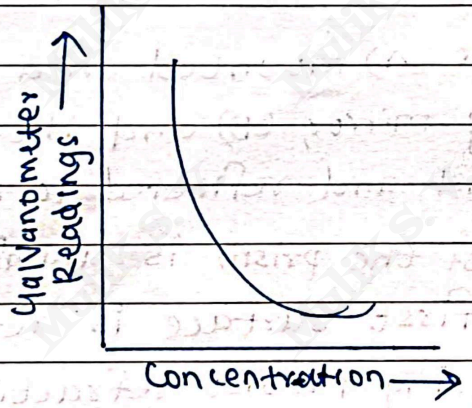
When light, Passing through the thin metal layer D falls upon selenium surface B, electrons are released which Penetrate a hypothetical barrier layer C, giving D layer negative charge

While the metal plate A acquires a positive polarity forming a cell leading to the flow of electrons due to generated potential difference

(v) Galvanometer:

The potential difference generated in photovoltaic cell is measured using galvanometer.

The flow of current will vary with intensity of the transmitted light from absorbing cell and galvanometer will give results accordingly.



→ Spectrophotometer Method (Single Beam Direct Reading Spectrophotometer)

This is the most accurate method for determination of the concentration of substances in solution. Spectrophotometer's are costlier.

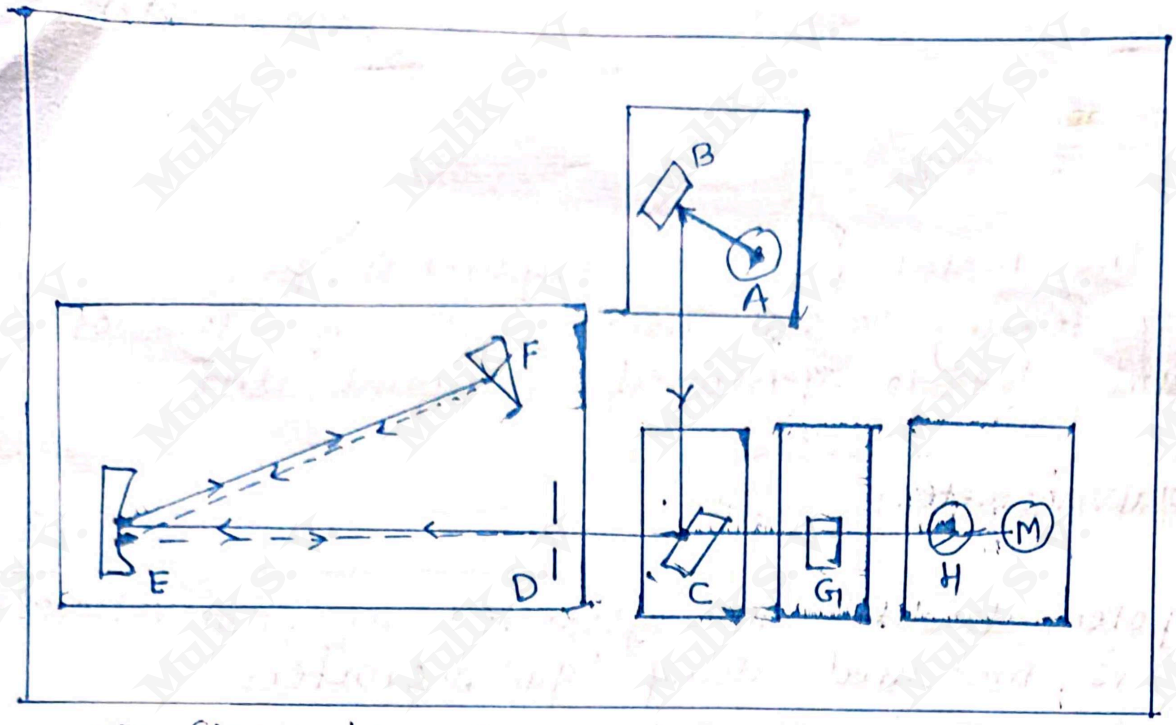


Fig. Single-beam spectrophotometer

A = Light source

B = mirror, C = diagonal mirror, D = entrance slit,

E = collimating mirror, F = quartz prism,

G = absorption cell, H = photocell, M = meter.

An image of the light source (A) mounted on a movable arm is focussed by the condensing mirror, (B) and the diagonal mirror (E) is rendered parallel and reflected to the quartz Prism (F). The back surface of the prism is aluminised, so that the light refracted at the first surface is reflected back through the prism, undergoing further refraction as it emerges from the prism. The collimating mirror focuses the prism in the plane of the slits (D) and light of the selected wavelength passes out of the monochromator through the exist (upper) slit, to the absorption cell (G) and then to the photocell (H), the response of which is amplified and is noted on the meter (M).

Light source (A) :-

(i) Tungsten-halogen lamp :- Visible & near U.V region

(ii) Deuterium lamp :- far-U.V

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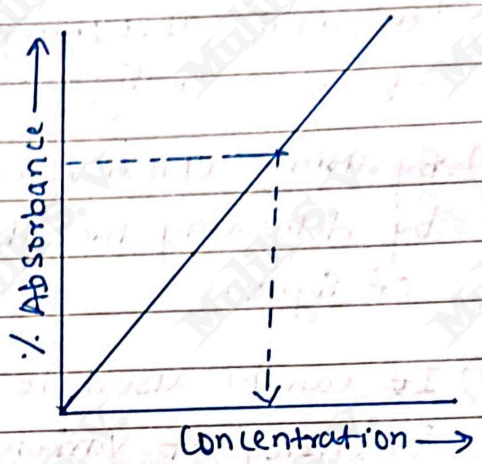
* Unknown Concentration by using Concentration - Absorbance plot.



* Applications of Colorimetry

(i) For the determination of the concentration of coloured compound by measuring the optical density or absorbance.

(ii) For the determination of the course of reaction by measuring the rate of formation and disappearance of the light absorbing compound in the range of visible spectrum of light.

(iii) By using colorimeter a compound can be identified by determing the absorption spectrum in the visible spectrum of light.

(iv) It can be used to measure the carol concentration and intensity of variety of materials in food ingradients, building materials, textile products beverages and chemical solutions.

(v) It is widely used in hospitals and laboratories for estimation of biochemical samples like plasma, serum, urine, cerebrospinal fluid (CSF).

* Applications of Spectrophotometry

(i) Spectrophotometry is used in molecular biology in measuring the growth of micro-organism like bacteria.

(ii) Spectrophotometry is used in forensic science.

(iii) Spectrophotometry is used in determination of molecular weight of molecule.

(iv) It is used for knowing the unknown concentration of the sample solutions.

(v) It is used in characterization of aromatic compound and in detection of composition.

(vi) for determining impurities present in the sample.