CHAPTER - I

THEORY OF SPECTROPHOTOMETRY

CHAPTER - I

THEORY OF SPECTROPHOTOMETRY

Spectrophotometry is the science which deals with the measurement of the relative capacity of Chemical systems to absorb incident radiant energy at specific wavelengths. The development of ultraviolet and visible absorption spectrometry as a discipline in modern analytical chemistry can be attributed primarily to the inherent utility of spectrophotometric methodology and the availability of reliable and inexpensive commerical spectrophotometers.

The colour is an important criteria for the identification of objects. The variation of the colour of a system component forms the basis of what the Chemist commonly terms colorimetric analysis. The colour is usually due to the formation of a coloured compound by the addition of an appropriate reagent, or it may be inherent in the desired constituent itself. The intensity of the colour may then be compared with that obtained by treating a known amount of the substance in the same manner.

Colorimetry is concerned with the determination of the concentration of a substance by measurement of the relative absorption of light with respect to a known concentration of of the substance. In visual colorimetry, natural or artificial

white light is generally used as a light source, and determinations are usually made with simple instrument termed a colorimeter or colour comparator. When the eye is replaced by a photoelectric cell, the instrument is termed a photoelectric colorimeter. In spectrophotometric analysis light of definite wavelength extending to the ultra-violet region of the spectrum constitutes the source of light, and thus necessitates the use of a more complicated, and consequently more expensive, instrument : the instrument has been named a spectrophotometer; or photoelectric spectrophotometer.

The chief advantage of colorimetric and spectrophotometric methods is that they provide a simple means for determining minute quantities of substances. The upper limit of colorimetric methods is in general, the determination of constitutents which are present in quantities of less than 1 or 2 percent.

In spectrochemical analysis we make use of spectrum of electromagnetic radiation to analyse chemical species and study their interaction with electromagnetic radiation. Electromagnetic radiation is a form of energy that can be described in terms of its wavelike properties. In contrast to sound waves, electromagnetic waves travel at extreme velocities and

do not require the existence of some supporting medium for propagation. Radiant energy is the energy associated with electromagnetic waves of different wavelengths. It consists of minute units of energy called quanta, or photons. The relationship between the energy of a photon and the frequency appropriate for the description of its propagation is -

$$E = h\nu$$

where E represents energy in ergs,
ν represents frequency in cycles, per second and
h is Planck's constant.

Radiant energy also can be thought of a continuous wave motion in which λ represents the interval between nodes in the wave pattern. The wavelength of a beam of electromagnetic radiation is the linear distance travelled by one complete wave cycle. The frequency ν is the number of cycles occuring per second. The relationship between wavelength and frequency is -

$\nu = c / \lambda$ where
λ - is the wavelength in centimeters and
c - is the velocity of the radiant energy in centimeters per second.

The equivalent expression involving wavelength is

$$E = \frac{hc}{\lambda}$$

Visible light, a very small part of the electromagnetic spectrum, is generally concerned to extend from 380 to 780 nm. The ultraviolet region of the electromagnetic spectrum is frequently subdivided into the far or vacuum ultraviolet region, approximately, 10-200 nm, and the near ultraviolet region which extends from 200-380 nm.

To state that a solution is colored means that of all wavelengths of white light incident upon the solution only selected wavelengths are absorbed, depending on the colour of the solution; the remaining wavelengths are transmitted. A red solution, for example, appears red because it absorbs the shorter wavelength of the visible region and transmits the larger wavelengths; therefore, color is attributed to the selective absorption of incident radiant energy of certain wavelengths.

Absorption in the ultraviolet region of the spectrum has been related to the presence of unsaturation and polarizability in ultraviolet absorbing materials. Saturated compounds are transparent in the ultraviolet region.

When an electromagnetic wave of a specific wavelength impinges upon a substance, the energy associated with that

wave may be altered by reflection, refraction, absorption and transmission processes, Reflection and refraction effects are generally negligible in the spectrophotometric analysis of solutions. Each of these transitions require a definite quantity of energy, the probability of occurence for a particular transition is greatest when the photon absorbed supplies precisely this quantity of energy.

The energy requirements for these transitions vary considerably. In general, promotion of electrons to higher levels requires greater energies than those needed to bring about vibrational changes. Alternation in rotational mode are likely to have the lowest energy requirements of all. Thus, absorptions observed in the microwave and far infrared regions will be due to shifts in rotational level since the energy of the radiation is insufficient to cause other type of transition. Changes in vibrational levels are responsible for absorptions in the near infrared and visible regions. Absorption due to promotion of an electron to some higher energy level takes place in the visible, ultra-violet, and x-ray regions of the spectrum.

The absorption of radiation by a system can be described by means of a plot of absorption as a function of wavelength; such a graph is called an absorption spectrum. In as much as the energies required for the various processes

responsible for absorption are unique for a given species, its absorption spectrum is also unique; as a consequence absorption spectra are often helpful for qualitative indentification purposes.

Irrespective of the amount of energy absorbed, an excited species tends spontaneously to return to its unexcited or ground, state. To accomplish this, the energy of the absorbed photon must somehow be given up, and this is ordinarily dissipated in the form of heat. In some instances, however transition to another excited state preceds return to the ground state.

Laws of Absorption :

When monochromatic light falls upon a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium, and the remainder is transmitted. If the intensity of the incident light is expressed by Io, that of the absorbed light by Ia, that of the transmitted light by It, and that of the reflected light by Ir, then :

$$Io = Ia + It + Ir$$

Ir is usually eliminated by the use of a control, such as a comparison cell, hence :

$$Io = Ia + It$$

Lambert (1760) investigated the relation between Io and It. Beer (1852) extended the experiments to solutions. Spectrophotometry and colorimetry are based upon Lambert's and Beer's law.

Lambert's Law :

This law states that when monochromatic light passes through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light. This is equivalent to stating that the intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically, or that any layer of given thickness of the medium absorbs the same fraction of the light incident upon it. We may express the law by differential equation :

$$-\frac{dI}{dt} = KI$$

where,

I is the intensity of the incident light of wavelength, t is the thickness of the medium, and K is a proportionality factor.

Integrating the above equation and putting

$$I = Io \quad \text{when } t = o$$

$$\text{In} \quad \frac{I_o}{I_t} = Kt$$

or in other terms $I_t = I_o \ e^{-Kt}$

Where I_o is the itensity of the incident light falling upon an absorbing medium of thickness t, I_t is the itensity of the transmitted light, and K is a constant, the absorption coefficient for the wavelength and the absorbing medium used. By changing from natural to Briggsian logarithms we obtain :

$$I_t = I_o.10^{-0.4343\ Kt} = I_o.10^{-kt}$$

where $k = K/2.3026$ and is usually termed the extinction coefficient. The extinction coefficient is generally defined as the reciprocal of the thickness (t cm) required to reduce the light to $\frac{1}{10}$ of its intensity.

$$\frac{I_t}{I_o} = 0.1 = 10^{-Kt} \text{ or } Kt = 1 \text{ and } K = 1/t$$

The ratio I_t/I_o is the fraction of the incident light transmitted by a thickness t of the medium, and is termed the transmission and transmittance I. Its reciprocal I_o/I_t is the opacity, and the optical density D of the medium, sometimes designated the extinction E or absorbance. A, is given by :

$D = \log I_o/I_t$.

Thus a medium with optical density 1 for a given wavelength transmits 10 percent of the incident light at the wavelength in question.

Beer's Law :

Beer (1852) studied the effect of concentration of the coloured constituent in solution upon the light transmission or absorption. He found the same relation between transmission and concentration as Lambert had discovered between transmission and thickness of the layer. i.e. the intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance increases arithmetically. This may be written in the form :

$$\begin{aligned} It &= I_o \cdot e^{K'C} \\ &= I_o \cdot 10^{-0.4343K'C} \\ &= I_o \cdot 10^{-K'C} \end{aligned}$$

Where,

C is the concentration, and K' and K are constants. Combining equations for Lambert's and Beer's law, we have

$$I_t = I_o\, 10^{-\epsilon Ct} \quad \text{or} \quad \log \frac{I_o}{I_t} = \epsilon\, Ct$$

This is the fundamental equation of colorimetry and spectrophotometry, and is often spoken of as the "Beer-Lambert Law." The value of C will clearly depend upon the method of expression of the concentration. If C is expressed in gram mols per litre and t in centimeters then C is the molecular extinction coefficient (also termed molar absorptivity or molar absorbancy index). The later is equal to the reciprocal value of the thickness in centimeters of a 1 molar solution (C = 1) at which,

$$I_t = 0.1 \times I_o$$

since $I_t = I_o . 10^{-\epsilon}$ when t = 1 and C = 1,

The specific extinction coefficient Es may be defined as the extinction or optical density per unit thickness and unit concentration.

Where the molecular weight of a substance is not definitely known, it is obviously not possible to write down the molecular extinction coefficient, and in such cases it is usual to write the unit of concentration as a subscript, and the unit of length as subscript. The optical density D, also called the extinction E or the absorbancy A, of a medium is the logarithmic ratio of the intensity of the incident light to that of the emergent light, i.e.

$$D = E = A = \log (I_o/I_t) \text{ or } I_t = I_o \times 10^{-D}$$

D is obviously related in a simple way to the molecular extinction coefficient, since : $D = \epsilon ct$.

The molecular extinction coefficient is, therefore, the optical density when the layer of solution is 1 cm thick and the concentration of the absorbing substance is 1g.mol per litre.

The transmission T is defined as the ratio of the intensity of the transmitted to that of the incident light thus $T = I_t/I_o$.

The term transmittancy Ts, is defined as the ratio of the transmittance of a cell containing the coloured solution to that of an identical cell containing the solvent or a blank solution.

The optical density D may, therefore, be put equal to the logarithm of the reciprocal of the transmission.

$$D = \log (1/T) = - \log T$$

The scales of spectrophotometers are often calibrated to read directly in optical densities, and frequently also in percentage transmittance. It may be mentioned that for

colorimetric measurements I_o is usually understood as the intensity of the light entering the solution. It is the intensity of the light emerging from the solution, or trasmitted by the solution. It will be noted that : the extinction coefficient is the optical density for unit path length,

$$K = D/t \text{ or } I_t = I_o . 10^{Kt}$$

the specific extinction coefficient is the optical density per unit path length and unit concentration

$$Es = D/cl \quad \text{or} \quad I_t = I_o . 10^{-Escl}$$

the molar extinction coefficient is the specific extinction coefficient for a concentration of 1g mol per litre and a path length of 1 cm.

$$\epsilon = D/Ct$$

Deviation from Beer's Law :

Beer's law will generally hold over a wide range of concentration if the structure of the coloured ion or of the coloured non-electrolyte in the dissolved state does not change with concentration. Small amounts of electrolytes, which do not react chemically with the coloured components, do not usually affect the light absorption; large amounts of

electrolytes may result in a shift of the maximum absorption, and may also change the value of the extinction coefficient. Discrepancies are usually found when the coloured solute ionizes, dissociates or associates in solution, since the nature of the species in solution will vary with the concentration. The law does not hold when the coloured solute form complexes, the composition of which depends upon the concentration. Also discrepancies may occur when monochromatic light is not used. The behaviour of a substance can always tested by plotting log $\frac{I_o}{I_t}$, E or log T against the concentration : a straight line passing through the origin indicates confirmity to the law.

For solutions which do not follow Beer's law, it is best to prepare a calibration curve using a series of standards of known concentration. Instrumental readings are plotted as ordinates against concentration. Instrumental readings are plotted as ordinates against concentrations in, say, mg per 100 ml or 1000 ml as abscissae. For the most precise work each calibration curve should cover the dilution range likely to met within the actual comparison.