CHAPTER - I

THEORY OF SPECTROPHOTOMETRY

C H A P T E R - I

THEORY OF SPECTROPHOTOMETRY

INTRODUCTION

Spectrophotometric method is often preferred for the determination of trace amount of metal ions. New analytical methods are based on instrumental technique. Atomic emission, flame photometry, polarography potentiometry, radiochemical techniques etc. are commonly used. Spectophotometric techniques are partially useful when insuffecient sample is present for gravimetric and volumetric methods. In colorimetric or spectrophotometric, trace analysis depends on sensitivity of the colour reaction. The sensitivity of the colour reaction may be defined as the smallest weight of substance that can be detected in a column of solution of unit cross section. The sensitivity is based on law of Lambert-Beer. The spectrophotometic technique is highly selective, sensitive and rapid for analysis of variety of materials. The absorption of Ultra-violet and visible radiation is measured in spectophotometric determination. The upper limit of the method is, in general, the determination of constituents which are present in the quantities of less than 1 or 2 percent.

The variation of the colour of the system with change in concentration of some component forms the bases of 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 disired constituent itself. The intensity of the colour may then be compared with that obtained by treating a known amount of substance in same manner.

Colorimetry is concerned with the determination of the concentration of substance by measurment of the relative absorption of light with respect to a known concentration of the substance. In visual colorimetry, natural or artificial white light is generally used as a light source, and determinations are usually made with a simple instrument termed a colorimeter or colour comparator. When the eye is replaced by a photoelectric cell the instrument is termed as photoelectic 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 photoelectic spectrophotometer. Spectrophotometer is an instrument for measuring the intensity of the light of various wavelengths transmitted (or reflected) by a solution (or other medium). It consists essentially of a device (usually a monochromator or a diffraction gratting) for furnishing "monochromatic light", one or more absorption cells for holding the sample and reference solutions and a photometric arrangement for the subjective or objective measurment of the

intensity of transmitted light.

Higher precision can be obtained in photoelectic specrophotometry than in ordinary colorimetry. More over, when many deteminations are to be made, objective spectophotometic methods are more rapid and less fatiguing than the subjective colorimetric methods.

In spectrochemical analysis we make use of spectrum of electromagnetic radiation to analyse chemical species and study their interactions 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= 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 λ of a beam of elecromagnetic

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

$$\nu = C/\lambda \text{ where,}$$

λ = the wavelength in centimeter

C = the velocity of the radiant energy in centimeter per second

The equivalent expression invoving wave length is

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

Visible light, a very small part of the electromagnetic spectrum is generally concered 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 10-200nm and the near ultraviolet region which extends from 200-380nm. To state that a solution coloured 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 wavelegths 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, colour is attributed to the selective absorption of incident radiant energy of certain wavelengths.

Absorption in the ultra-violet 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 quntity of energy, the probability of occurence for a perticular transition is greatest when the photon absorbed supplies precisely this quantity of energy.

The energy is required for these transitions. 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, ultraviolet, 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 energies required for various processes responsible for absorption are unique for agiven species, its absorption spectrum is also unique as a consequence absorption spectra are often helpful for qualitative identification purposes.

Irrespective of amount of energy absorbed an excited species tends spontaneously to return to its unexcited or ground state. To accomplish this the energy of 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 proceeds to return the ground state

Theory of Spectophotometry and Colorimetry :

When light (monochromatic or heterogeneous) falls upon a homogeneous medium, a portion of 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, and that of the transmitted light by I_t and that of the reflected light by I_r, then ;

$$I_o = I_a + I_t + I_r$$

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

$$I_o = I_a + I_t$$

Lambert (1760) investigated the relation between I_o and I_t. Beer (1852) extended the experiments to solutions. Spectrophotometry and colorimetry are based upon Lambert's and Beer's laws.

Lambert's law : This law states that when monochromatic light passes through a transparent medium, the rate of decrease in intensity with 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 the differential equation

$$-\frac{dI}{dl} = kI$$

Where I = Intensity of the incident light of wave length λ

l = thickness of the medium

k = proportionality factor

Integrating the above equation and putting

$I = I_o$ when, $l = 0$

$$\ln \frac{I_o}{I_t} = kl$$

or in other terms

$$I_t = I_o . e^{-kl}$$

Where, I_o = intensity of the incident ligh falling up on an absorbing mediun of thickness l

I_t = = Intensity of the transmitted light

k = cosnstant for the wave length and the absorbing medium used.

By changing from natural to common logarithms we obtain

$$I_t = I_o.10^{-0.4343kl}$$

$$=I_o.10^{-kl}$$

Where, $K = k_{/2.3026}$ and is usually termed the absorption coeffcient or extinction coefficient. The absorption coefficient is generally defined as the reciprocal of the thickness (1 cm) required to reduce the light to $1_{/10}$ of it's intensity.

$$I_{t/I_o} = 0.1 = 10^{-kl}$$

or, $Kl = 1$ and $K = 1/l$

The ratio I_{t/I_o} is the fraction of the incident light transmitted by a thickness l of the medium and is termed the transmittance T. 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

$$D = \log \frac{I_o}{I_t}$$

Thus 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 the 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 :

$$I_t = I_o.e^{-k'c}$$

$$= I_o.10^{-0.4343k'c}$$

$$= I_o.10^{-K'c}$$

where c = concentration

k' and K' are constants

Combining equations for Lambert's and Beer's law, we have

$$I_t = I_o.10^{-acl}$$

or

$$\log \frac{I_o}{I_t} = acl$$

This is the fundamental equation of colorimetry and spectro photometry, and is often spoken of as the 'Beer-Lambert law'.

The value of 'a' will clearly depend upon the method of expression of the concentration. If C is expressed in gram mole per litre and l in centimeters, then 'a' is given the symbol ε and is called the molar absorption coefficient or molar absorptivity (formarly molar extinction coefficient) or molar absorbancy index. The later is equal to the reciprocal value of the thickness in centimeters of a molar solution (c=1) at which

$$I_t = 0.1 \times I_o$$

Since $I_t = I_o.1^{-}0^{\varepsilon}$ 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 cocentration.

Where the molecular weight of substance is not definitely known, it is obviously not possible to write dawn the molecular extiction 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.

$$D = E = A = \log (I_o/I_t)$$

or $$I_t = I_o \times 10^{-D}$$

D is obviously related in a simple way to the molecular extinction coefficient,

Since : $D = \varepsilon c l$

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 1 gram mole per litre.

The terrm 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 measurments Io is usually understood as the intensity of the light transmitted by the pure solvent or the intensity of the light entering the solution. It is the intensity of the light emerging from the solution or transmitted by the solution. It will be noted that : the extinction coefficient is the optical density for unit path length.

$$k = \frac{D}{t}$$

or

$$I_t = I_o \cdot 10^{kl}$$

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

$$E_s = \frac{D}{cl}$$

or

$$I_t = I_o \cdot 10^{-Escl}$$

The molar extiction coefficient is the specific extinction coeffecient for a concentration of 1 gm mole per litre and a path length of 1 cm.

$$\varepsilon = {}^{D}/_{Cl}$$

Application of Beer's Law :-

Let us consider the case of two solutions of a coloured substance with concentrations C_1 and C_2. These are placed in an instrument in which the thickness of the layers can be altered and measured easily, and which also allows a comparison of the transmitted light when the two layers have to the same colour intensity

$$I_{t1} = I_o \cdot 10^{-\varepsilon l_1 C_1}$$

$$I_{t2} = I_o \cdot 10^{-\varepsilon l_2 C_2}$$

Here l_1 and l_2 = lengths of the coloumn of solutions with concetration C_1 and C_2 respectively

when the system is optically balanced. Hence under these conditions and when Beer's law holds;

$$l_1C_1 = l_2C_2$$

A colorimeter can therefore be employed in a dual capacity:

a) to investigate the validity of Beer's law by varying C_1 and C_2 and noting whether above equation applies and

b) for the determination of an uknown concentration C_2 of a coloured solution by comparison with a solution of known concentation C1. It must be emphasised that the equation is valid only if Beer's law obeyed over the concentration range employed and the instrument has no optical defects.

When a spectrophotometer is used it is unnecessary to make comparison with solutions of known concentration. With such an instrument the intensity of the transmitted light or, better the ratio I_t/I_o (the transmittance) is found directly at a known thickness l. By varying l and c the validity of the Lombert-Beer law, equation can be tested and the value of ε may be evaluated. When the latter is known the concentration C_x of an unknown solution can be calculated from the formula

$$C_x = \frac{\log I_o/I_t}{\varepsilon l}$$

Attention is directed to the fact that the extinction coefficient ε depends upon the wavelength of the incident

light, the temperature and the solvent employed. In general, it is best to work with light of wavelength approximating to that of for which the solution exhibits a maximum selective absorption (or minimum selective transmittance) : the maximum sensitivity is thus attained. For matched cells (i.e. l constant) the Lambert-Beer law may be written

$$C = A \log \frac{I_o}{I_t}$$

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

or $$C = B \log T$$

where A and B are constants : the concentration is therefore, proportional to the logarithm of its transmittance. Hence by plotting log 1/T (ordinates) against concentration (abscissa) a straight line will be obtained, and this will pass through the point C= 0, T= 100 percent. This calibration line may then be used to determine unknown concentrations of solutions of the same material after measurement of absorbances.

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 compounds, do not usually affect the light absorption, large amount of the 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 ionises, dissociates or associates in solution, since the nature of species in solution will vary with the concentration. The law does not hold when the coloured solute forms complexes, the composition of which depends upon the concentrtion. Also discrepancies may occur when monochromatic light is not used. The behaviour of a substance can always be tested by plotting log I_o/I_t or log T against the concentration : a straight line passing through the origin indicates conformity 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 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 be met with in the actual comparison.