CHAPTER I

THEORY OF SPECTROPHOTOMETRY

THEORY OF SPECTROPHOTOMETRY

Spectrophotometric method is often preferred for the determination of trace amounts of metal ions. New analytical methods are based on instrumental technique. Atomic emission, flame photometry, polarography, potentiometry, radiochemical techniques etc. are commonly used. Spectrophotometric techniques are partially useful when insufficient sample is present for gravimetric and volumetric methods. In spectrophotometric method, trace analysis depends on sensitivity of the colour reaction. The sensitivity of the colour reaction may be defined as the smallest weight of the substance that can be detected in a column of solution of unit cross section. The sensitivity is based on Lambert-Beer's law. The spectrophotometric method is highly selective, sensitive and rapid for analysis of variety of materials. The absorption of ultra-violet and visible radiation is measured in spectrophotometric 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.

Spectrophotometric determinations are based on the measurement of the ratio of the radiant power of the light entering a sample to that emerging from it.

Although visual photometry is possible, a photoelectric detector replaces the eye in current analytical practice. Photometric methods employing white light are feasible, but light of restricted wavelength region is used almost exclusively. Where restricted several regions are secured by filters, the instrument may be termed, a filter photometer and measurements employing it as filter photometry, where light of a narrow wavelength range is obtained by using a portion of a prism or grating spectrum, the instrument is known as a spectrophotometer and measurements employing it as spectrophotometry where a distinction between filter photometry and spectrophotometry is unnecessary, the general terms photometer, photometry, and photometric determination are appropriate.

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 existance 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 = Energy in ergs

ν = Frequency in cycles per second and

h = Planck's constant

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

$$\nu = \frac{C}{\lambda}$$

where λ = the wavelength in centimeter

C = the velocity of the radiant energy in cm/Sec.

The equivalent expression involving wavelength is

$$E = \frac{h\ c}{\lambda}$$

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 10-200nm and the near ultraviolet region which extends from 200 - 380 nm. 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 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, 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 quantity of energy, the probability of occurrence for a

particular 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 a given species, its absorption spectrum is also unique as 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 to the ground state.

FUNDAMENTAL LAWS OF PHOTOMETRY :

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 I_o, that of the absorbed light by I_a and that of the transmitted light by I_t and that of the reflected light by I_r, then,

$$I_o = I_a + I_t + I_r$$

The reflected light (I_r) is usually eliminated by the use of a control, such as a comparision 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 is 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

$$-\frac{dI}{dl} = k\,I$$

where

I = Intensity of the incident light of wavelength 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 light falling upon an absorbing medium of thickness l

I_t = Intensity of the transmitted light

k = constant for the wavelength and the absorbing medium used.

By changing from natural to common logarithms we obtain

$$I_t = I_o . 10^{-0.434\,kl}$$

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

Where K = k/2.3026 and is usually termed the absorption coefficient or extinction coefficient is generally defined as the reciprocal of the thickness (1 cm) required to reduce the light to $1/10^{th}$ of its 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 constituents in solution upon the light transmission or absorption. He found the same relation between transmission and concentration as Lambert had discovered the relation 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

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

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

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

where c = concentration, k' and K' are constants. Combining equations for Lambert and Beer's law, we have

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

or $$\text{Log} \frac{I_o}{I_t} = acl$$

This is the fundamental equation of spectrophotometry and is often spoken of as the Beer-Lambert's 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 (formally

molar absorption 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 $I_t = I_o\, 10^{-\epsilon}$ 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 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 superscript. 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 $D = \epsilon cl$

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 term transmittancy $T_=$, 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.

$$D = \text{Log } (1/T) = - \text{Log } T$$

The scales of spectrophotometers are often calibrated to read directly optical densities, and frequently also the percentage transmittance. It will be noted that the extinction coefficient is the optical density for unit path length

$$K = D/t \qquad \text{or} \qquad I_t = I_o \, 10^{-kl}$$

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

$$Es = D/Cl \qquad \text{or} \qquad I_t = I_o . \, 10^{-Eslc}$$

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

$$\epsilon = D/cl$$

APPLICATIONS OF BEER'S LAW :

Let us consider the case of two solutions of coloured substance with concentration 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 the same colour intensity

$$I_{t1} = I_o\ 10^{-\epsilon l_1 c_1}$$

$$I_{t2} = I_o\ 10^{-\epsilon l_2 c_2}$$

Here l_1 and l_2 = lengths of the coloumn of solution with concentration c1 and c2 respectively.

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

$$l_1 c_1 = l_2 c_2$$

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 unknown concentration c_2 of a coloured solution by comparison with a solution of known concentration c_1. It must be emphasized 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 solution 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 Lambert-Beer's 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

$$Cx = \frac{\log (I_o/I_t)}{\epsilon\, l}$$

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 approximation 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's law may be written

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

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

or

$$= B \log T$$

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, which passes through the point c = 0, T = 100 percent. This calibration line may then be used to determine unknown concentrations of solutions of the same materials after measurement of absorbances.

DEVIATIONS FROM LAMBERT-BEER'S LAW :

An absorbance versus concentration calibration curve is usually a straight line at a sufficiently low concentration range. At some higher concentration, however, the curve starts to deviate from straight line and may bend either toward the ordinate or the abscissa. Various factors may operate singly or together to produce a deviation. Frequently, however, the deviation is not caused by a true failure of the law, but is, rather, due to the fact that the conditions prevailing do not confirm to the premises of the law.

The Lambert-Beer's law is strictly valid only for monochromatic light. Strict monochromacity is not attainable with a practical photometer, where a filter is used as the monochromating device. The limitations are usually quite pronounced and for a given filter little

can be done to improve the situation. Consequently, with a filter photometer deviations are often encountered even at a relatively low concentration. In a spectrophotometer the prism or gratings produce a continuous spectrum from which a small wavelength region is selected by a slit. Obviously the degree of monochromacity attained is the greater the narrow the slit. However, as the slit width is progressively decreased, the radiant power of the beam passed decreases and eventually a point is reached where the instrument can no longer be adjusted to 100% T with solvent in the beam. Consequently, monochromacity is also limited here. Insufficient monochromacity is the most frequent reason for deviation from Beer's law and always causes the absorbance versus concentration curve to bend towards the concentration axis. Fortunately, the requirement of strict monochromacity can often be relaxed in practical photometry.

Sometimes it is held that deviations from the law are the result of chemical effects, especially where dissociation - association equilibria are involved, since the position of the equilibrium will depend on the concentrations of the participating species. In many cases, however, the deviations are not true ones but rather result from inappropriate plotting of the calibration curve.

Finally, it may be seen that deviations (usually erratic ones) from a straight line plot may result from temperature changes during the measurements. Variations in temperature cause expansion or contraction of the solution and thereby changes in concentration. The novice sometimes fails to appreciate that the solution under measurement is exposed to a light beam of considerable intensity and the radiant power absorbed by the solution is converted to heat. Thus, significant warming can occur when the solution is allowed to remain in the cell compartment of the instrument for a protracted period of time. Such warming can result in evaporation losses, especially if volatile nonaqueous solvents are employed. Additionally the warming frequently causes the formation of gas bubbles due to the release of absorbed gases (commonly air). The bubbles cling to the cell walls, and if they are in the light path, highly erratic analytical results are obtained. Temperature effects become especially serious when the absorbing species participate in a temperature - sensitive equilibrium.