CHAPTER - 1

PRINCIPLES OF SPECTROPHOTOMETRY

Spectroscopy is a powerful tool used for quantitative and qualitative analysis of samples, and is the most important of all the instrumental methods of analysis. It is a branch of science dealing with the study of interaction of electromagnetic radiation with matter. Spectrophotometry is one of the most widely applied physico-chemical technique, which is remarkable for its sensitivity and precision. Two reasons for the popularity of the spectrophotometric technique are the instrumentation is fairly easy to operate and widely available. The chief advantage of spectrophotometric method is that they provide a simple means for determining minute quantities of substances.

Spectrophotometry in the visible region of electromagnetic spectrum generally considered to extend from 380nm to 780nm, is often called colorimetry. Colorimetry, is defined as a technique of analysis based on comparing the colour intensity of the unknown with that of standard solution.

Spectrophotometry is defined as a technique which involves provisions for continuously varying wavelength of the central band and therefore, measuring the absorption of radiation with wavelength by the sample. This is a convenient and direct technique which does not effect

changes in composition and properties of the experimental solutions. The applicability of the spectrophotometric technique to the determination of traces of metals, non-metals and organic compounds is one of the main reasons for the wide utilization of photometric methods of analysis.

In spectrophotometric analysis, the intensity of radiation, transmitted by an absorbing medium placed between the light source and the detector is measured as a function of wavelength. The graph between the intensity of radiation transmitted or absorbed and the wavelength is called the absorption spectrum and is characteristic of the absorbing component and provides the basis for qualitative analysis.

The intensity of radiation absorbed or transmitted is also a measure of concentration and this forms the basis for the quantitative analysis. The upper limit of spectrophotometric methods is, in general, the determination of constituents which are present in quantities of less than 1 or 2 percent.

The sensitivity can, however, be improved if the technique of derivative spectrophotometry is employed.

LAWS OF PHOTOMETRY :

When a beam of electromagnetic impinges on matter

several types of interactions such as reflection, refraction, diffraction, interference, absorption etc., may occur. Of these interactions, absorption in which certain frequencies are selectively removed by matter is of unique importance. The energy thus absorbed is transferred to atoms or molecules in matter. As a result, these atoms or molecules in the ground state are excited.

There are two fundamental laws governing the fraction of incident radiation absorbed by matter. They are

    1. Lambert's law   and

    2. Beer's law

1. LAMBERT'S LAW :

The relationship between the absorption of radiation and the path length of the absorbing medium was first formulated by Bouguer (1729) and later by Lambert[1] (1760) which is mathematically expressed in the logarithmic form as

$$\log T = \log P/P_o = -Kb \qquad \qquad \text{... 1.1}$$

Where

P_o	=	Radiant power of incident radiation
P	=	Radiant power of transmitted radiation
T	=	Transmittance
K	=	is a constant.

Lambert's law though exact has only limited applications in chemistry.

2. BEER'S LAW :

Beer[2] in 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 absorbing layer. The intensity of a beam of monochromatic radiation decreases exponentially as the concentration of the absorbing substance increases arithmetically. This may be written in the form

$$\text{Lot } T = \text{Log } P/P_O = - K^1 c \qquad \qquad \text{... 1.2}$$

Where K^1 is a proportionality constant.

The two laws are combined using a new proportionality constant 'a' and written as

$$\log (P/P_O) = abc \qquad \qquad \text{... 1.3}$$

The ratio of P/P_O is known as transmittance (T) which is dimensionless quantity. As, 'abc' is a logarithmic quantity, it is a pure number. The above equation can be written as

$$A = abc \qquad \qquad \text{... 1.4}$$

Where

$$A = \log 1/T = \log P_o/P.$$

and is called absorbance and the constant 'a' called absorptivity, where the concentration is expressed in grams/litre. If the concentration expressed in moles/litre, the constant 'a' is replaced by ' ε ' called molar absorptivity.

Thus,

$$A = \varepsilon bc \qquad \qquad \ldots 1.5$$

The equation (1.5) is a fundamental law governing the absorption of all types of electromagnetic radiation and applies not only to solutions but also to gases and solids as well.

DEVIATIONS FROM BEER'S LAW :

No exceptions are known for the linear relationship between absorbance and path length at a fixed concentration of absorbing system. Deviations from the direct proportionality between measured absorbance and concentration, where 'b' is constant, occur as a result of chemical and instrumental factors. The obedience of Beer's law is tested experimentally by plotting a graph between absorbance and concentration. A straight line plot passing through the origin indicates conformation to Beer's law. A non-linear plot indicates the deviation from the Beer's law.

Deviations are termed positive and negative depending on the observed curve is above and below the theoretical straight line respectively.

According to Kortum and Seiler[3] the Beer's law is fundamentally a limiting law and is valid and obeyed at low concentrations, but the law does not hold good for solutions of higher concentration. The deviations may be attributed to factors, such as reflection of radiation by the test solution[4], pure solvent, transmission, non-use of monochromatic radiation, florescence or scattering, change of structure of the coloured species with concentration, reaction of electrolytes with the coloured complex when present in large amount, temperature, ionization, association or dissociation of coloured solutes in solution, inert salts when present even in very low concentration due to the influence of electrostatic interactions.

It is still possible to apply spectrophotometry in quantitative analysis with marked success, provided that necessary precautionary measures are taken to control the experimental conditions.

RINGBOM'S PLOT :

There is an element of uncertainty in measurements due to the working of the instrument even in the range of

obedience of Beer's law. A small random error in the reading of percent transmittance (T) causes a large relative error in concentration when 'T' is either very small or very large. The practical range of concentration can be determined spectrophotometrically with the help of Ringbom's plot.

In Ringbom's[5] plot, a graph is drawn between per cent absorptance against logarithm of the concentration, where a 'S' shaped or sigmoid curve is obtained. The term absorptance is defined as

Absorptance   = 1 - transmittance = 1 - T   ...1.6

% Absorptance = 100 - % T   ... 1.7

Deviation based on Beer's law, lead Ringbom to arrive at the conclusion that the accuracy is the greatest when $\Delta P/(\Delta c/c)$ reaches a maximum value i.e., at the point of steepest slope (inflection point) of the curve between P and log C. Thus if a system obeys Beer's law, the inflection point occurs at 36.8 % transmittance or 62.2 % absorptance and the corresponding absorbance value is 0.4343. The Ringbom plot has two advantages.

1.   It shows at a glance the concentration range in which the analysis error is minimum. This is the concentration range corresponding to the nearly linear portion of the Ringbom plot and

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2. The accuracy of the analysis at any concentration range can be easily evaluated.

If the system under study does not obey Beer's law over the entire range of concentration, the ringbom plot takes a sigmoid shape. The curve will have a different slope and the inflection point appears at a value other than 63.2% absorptance. However, the curve still shows the optimum concentration range at a glance and the relative analysis error can be calculated from the slope of the curve.

SENSITIVITY OF SPECTROPHOTOMETRIC METHOD :

Sensitivity of a spectrophotometric method is defined as the least amount of the determined species that can be determined spectrophotometrically under experimental conditions. It refers to the calibration curve obtained between concentration and absorbance values. The sensitivity is numerically expressed as molar absorptivity 'ε'

$$\varepsilon = A/bc \qquad \ldots 1.8$$

Where 'A' is absorbance, 'b' is the pathlength expressed in cm and 'c' is concentration in moles per litre. The units for molar absorptivity are litre $mole^{-1}$ cm^{-1}. Quantum mechanical evaluation revealed that ε value can not exceed 1.5×10^5. Spectrometric methods with $> 1 \times 10^4$

are referred to as sensitive while those with $< 1 \times 10^3$ as less sensitive.   Sensitivity is sometimes expressed in terms of specific absorptivity denoted by 'a' and is given by the expression.

$$a = \frac{\varepsilon}{At. \ Wt. \ X \ 1000} \qquad \dots 1.9$$

The unit for specific absorptivity 'a' are $ml \ gm^{-1}cm^{-1}$ and its value corresponds to the absorbance of 1 $\mu g/ml$ (1ppm) solution of the determined in a cuvette with an optical path length of 1 cm. very often the sensitivity is expressed in terms of sensitivity index (S) given by Sandell[6]. According to Sandell, sensitivity is the amount of obsorbing species in microgram per ml. of the solution (1 ppm), with an absorbance of 0.001 for a path length of 1 cm.

The units of 'S' are $\mu g \ cm^{-2}$ and is given by

$$S = 10^3/a \qquad \dots 1.10$$

where 'a' is specific absorptivity.

SELECTIVITY :

A reagent which reacts only with a limited number of elements is considered a selective reagent.

To achieve the desired degree of selectivity in analytical work, it is usually necessary to exploit difference in two or more physical and chemical properties.

The selectivity of a colour reaction and the

corresponding spectrophotometric method depends on

1. The nature of the reagent

2. The oxidation state of the element

3. The pH of the medium

4. The nature of the reagent and to mask the interfering ions.

5. The temperature.

In dilute mineral acid solution Zr and Hf (also Ti, Th, Sn and Al) form intensely coloured but sparingly soluble layer with alizarin. Insertion of sulphonic group into alizarin confers water solubility to few metal complexes without otherwise affecting the complexation reaction. Thus a change in the structure of the reagent imparts some selectivity.

In the determination of niobium by the thiocyanate method, iron does not interfere if reduced to iron (II). Thus exemplifies achieving selectivity by changing the oxidation state of an interfering element, while chelating agent do not exhibit absolute specificity in their reaction with metal ions. They may exhibit varying degree of selectivity depending upon the experimental conditions. 8-Hydroxy-quinoline (Oxine) forms water insoluble precipitates with about thirty different metal ions. This number can be reduced by controlling the pH of the solution

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from which precipitation take place. A still greater degree of selectivity may be achieved by using masking or sequestering reagents. Thus oxine precipitates very few metal ions from a buffer solution acid containing EDTA as a sequestering agent. Masking consists in transforming the interfering ion into a stable complex so that it can neither react with spectrophotometric reagent nor otherwise interfere in the colour reaction of interest.

SPECIFICITY :

A reagent which reacts with only one element under the specified conditions is usually called a specific reagent. However it seems most unlikely that a reagent will be found that will react with only one metal and no other. Absolute specificity in this sense is unachievable. This does not mean that there is no such thing as specific test where the specificity resides not in the reagent but in the product of the test. Dimethylglyoxime, which combines readily with a number of metals, forms a highly insoluble red chelate with one metal only namely nickel. The specificity of this reaction lies in the colour of the product. Other metal like palladium and platinum form insoluble chelates with the dioxime which are yellow and blue respectively but nickel is the only metal which forms a water insoluble red compound.

Cuproine for copper (II) and

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bathophenanthorline for iron (II) may be mentioned as examples to spectrophotometric specific reagents.

DETERMINATION OF THE COMPOSITION OF THE METAL COMPLEXES :

Spectrophotometry is one of the most powerful tools for elucidating the composition of complex ions in solution and for determining their formation constants due to the fact that quantitative absorption measurements can be performed without fear of disturbing the equilibrium under consideration. The three important methods that are widely employed for this purpose of the metal complexes are

1. Job's continuous variation method

2. Mole ratio method

3. Slope ratio method

1. JOB'S CONTINUOUS VARIATION METHOD :

In 1928, Job[8] described a method for determination of the composition of complexes. The complex equilibrium can be represented by the general equation.

$$M + nL \rightleftharpoons MLn \qquad \qquad ... \; 1.11$$

Where M and L represents the metal ion and the reagent with identical formal concentrations are mixed in different volume ratios, keeping the total volume of the mixture constant. The absorbance of each mixture is measured at a

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3. SLOP RATIO MTHOD :

suitable wavelength and corrected for any absorbance if no reaction occurred. A plot is then constructed with the volume fraction $V_M/V_M + V_L$ (where V_M and V_L are the volume of the metal ion solution and the reagent solution respectively) and the corrected absorbance. A curve with a maximum is obtained (or minimum if the complex absorbs less than the reactants). The composition of the complex is then given by the volume fraction ratio.

$$\frac{V_M}{V_M + V_L} : 1 - \frac{V_M}{V_M + V_L} \qquad \ldots\ldots\ldots\ldots \quad 1.12$$

corresponding to the maximum or minimum point.

The composition bears a simple relation to 'n' and is independent of equilibrium constant if equiformal solutions are used. The position of the maximum depends on 'n' as well as on the equilibrium constant 1 if the formalities are not equal. While Job's method gave satisfactory results for several complexes. instances of inconclusive results are reported in the literature[7-9]. This method is not found generally applicable when more than one complex is formed in the concentration range of study under the experimental conditions.

Cooper et al[10]. suggested a method to establish this fact. According to copper's modified procedure the absorbance is measured at various wavelengths covering the

entire range instead of measuring it at the wavelength of maximum abosrbance. If the same conclusions are obtained at all the wavelengths, it can be assumed that a single complex is formed and the Job's method can be successfully employed for the elucidation of the composition of the complex, In another modification, the absorption spectra of solutions containing different integral ratios of the metal ion and the reagent are recorded. If the maximum absorbance obtained at the same wavelength in each case, it indicates the presence of a single complex species and Job's method can be successfully used at this wavelength.

2. MOLE RATIO METHOD

In 1944 "Yoe and Jones" introduced this method for the study of complex formation by spectrophotometric technique. In this method a series of solutions is prepared in which the formal concentration of one of the reactants (often the metal ion) is held constant while that of the other is varied. A plot of the absorbance versus mole ratio of the reactants is then prepared. If the formation constant is reasonably high, two straight lines of different slopes are obtained, the intersection occurs at a mole ratio corresponding to the combining ratio of the complex.

This method is proposed by Harvey and Manning[12] and is particular useful for weak complexes. It is applicable if oly a single complex is formed. The method assumes that the complex formation reaction can be forced to completion in the presence of a large excess of either of the reactants and that Beer's law is followed under these circumstances.

For the reaction

$$mM + nL \xrightleftharpoons{} M_m + L_n \qquad \ldots 1.13$$

The following equation can be written, when 'c' is present in very large excess ....

$$[Mm \ Ln] = F_M/m \qquad \ldots 1.14$$

If Beer's law is obeyed

$$A_m = \varepsilon b \ [Mm \ Ln] = \varepsilon b \ F_M/m \qquad \ldots 1.15$$

and plot of 'A' with respect to F_M will be linear. The slope of the lines $S_M = \varepsilon b/m$

when 'M' is very large with respect to L.

$$[Mm \ Ln] \xrightarrow{} F_L/n \qquad \ldots 1.16$$

and

$$Al = \varepsilon b \ [M_m \ L_n] = \varepsilon b \ F_L/n \qquad \ldots 1.17$$

The plot of 'A' against F_L will be linear .

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The combining ratio between L and M is obtained from the ratio of these slopes

$$S_m/S_n = \frac{\varepsilon b/m}{\varepsilon b/n} = n/m \qquad \ldots 1.18$$

EVALUATION OF STABILITY CONSTANT OF A METAL COMPLEX :

The stability constant of the complex $M_m L_n$ given by the following reaction can be evaluated from the spectrophotometric results obtained in Job's continuous variation method.

$$mM + nL \rightleftharpoons M_m L_n \qquad \ldots 1.19$$

by using the following mathematical relation

$$\beta = \frac{A/A_m}{m^n n^n [(I-A/A_m)]^{m+n} [C]^{m+n-1}} \qquad \ldots 1.20$$

where

A_m = Absorbance corresponding to the point of intersection of the extrapolated lines

A = Observed absorbance at concentration 'c'

c = Concentration corresponding to the point of intersection.

β = Stability constant

$$\frac{A_m-A}{A_m} = \alpha = \text{degree of dissociation} \qquad \ldots 1.21$$

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DERIVATIVE SPECTROPHOTOMETRY

The differentiation of absorption spectra has many advantages for spectrophotometry in the UV and visible regions. It is the key for the potential enhancement of resolution of overlapping bands. It facilities the detection of poorly resolved absorption peaks arising from admixtures of impurities in solution or for structural reasons. It enables the exact determination of λ_{max} of the particular analyte species and increases the sensitivity of the spectrophotometric procedures. In addition, it is an excellent back ground elimination technique. The influence of Rayleigh scattering, constant background absorbance, non-selective absorption of the matrix of accompanying components is removed and the resolution of signal noise ratios are improved.

Derivative spectrophotometry enables detection of the presence of small amounts of impurities in samples, a more accurate determination of a minor component in the presence of bulk components and its combination with spectrophotometric mutlicomponent analysis. With the development of micro computer technology, derivative spectra are developed almost instantly. Therefore the analytical use of derivative spectrophotometry has became very popular
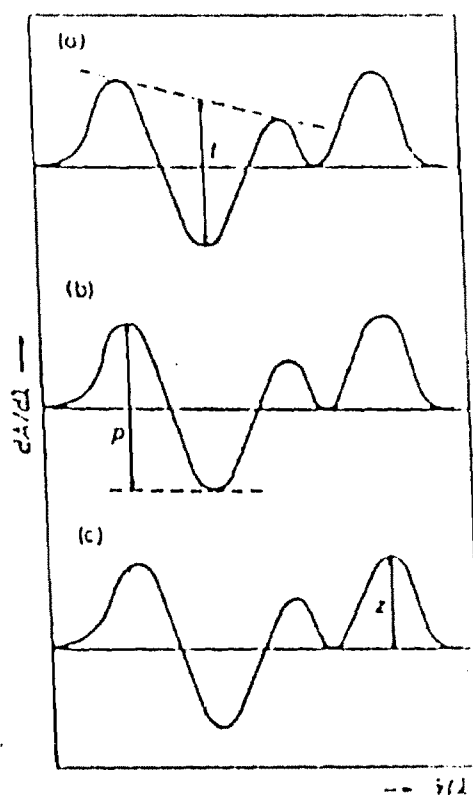
quantitative evaluation of derivative plots.

The first, second etc. derivatives of the absorption spectrum of an analyte is defined as the first, second etc. derivative of the absorbance as a function of wave number or wave length. The first or the second derivative is easily accessible instrumentally. The higher derivative are calculated on the basis of computer generated functions. The first derivative spectrum is useful for the determination of the components more accurately in presence of matrix component. It is also useful for the multicomponent analysis or identification of derivative with high spectral similarity. Thus the first or the second order derivatives can eliminate unwanted effects such as various scatterings, instrumental effect, differences by replacing cells of base line.

Assuming that the Beer's - Lambert's law are obeyed for the Zero order spectrum, various amplitudes of the derivative are proportional to analyte concentration in a similar way as is the absorbance of the primary absorption peak.

The first and second derivatives of Beer'slaw

$$dA/d\lambda = -0.434 \frac{1}{I} \frac{dI}{d\lambda} = \frac{d\varepsilon}{d\lambda} CI \qquad \ldots\ldots 1$$

Fig. 1. Various kinds of quantitative evaluation of the first derivative spectrum. (a) The tangent method; (b) the peak-peak method, (c) the peak-zero method (according to the IUPAC-report *Molecular Absorption Spectroscopy* UV/VIS, April 1985, by Laqua, K., Melhuish W. H. and Zander, M.)

$$\frac{d^2 A}{d\lambda^2} = 2.303 \; c^2 \; l^2 \left(\frac{d\varepsilon}{d\lambda}\right)^2 - 0.434 \; \frac{1}{I} = \frac{d^2 I}{d\lambda^2} = \frac{d\varepsilon^2}{d\lambda^2} \; CI$$

$$\dots \; (2)$$

$$\text{assuming} \quad \frac{dI_o}{d\lambda} = 0$$

The amplitude which least affected by variation in the matrix and gives the best calibration statistics is selected for quantitative measurements. The procedures used for quantitative evaluation are (a) tangent, (b) peak - valley and (c) peak-zero line methods which are shown in fig. 1.1.

In principle both the peak-to-valley amplitude D_s D_L and base line - to - valley distance, D_B, are proportional to the analyte concentration. The measurement is more complicated for higher derivatives. In the present study the peak-zero method is adopted for measuring the peak heights.

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