Chapter 1

Principles of Spectrophotometry
INTRODUCTION:

Absorption spectroscopy is a powerful tool employed for quantitative and qualitative analysis of samples. The technique is based on the absorption of radiation by matter and is the most important of all the instrumental methods of analysis. Spectrophotometry is one of the widely used physico-chemical techniques, which is remarkable for its sensitivity and precision. The reasons for the popularity of the spectrophotometric techniques are the low cost of the instruments and the easiness of their operation.

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. 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 utilisation of photometric methods of analysis.

In spectrophotometric analysis, the intensity of radiation, transmitted by an absorbing medium placed between the radiant energy source and the detector is measured. 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 is the basis for the quantitative analysis.
Laws of Photometry:

When a beam of monochromatic radiant power falls 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 radiant power absorbed by matter.

They are

1. Lambert's law and
2. Beer's law

1. Lambert's law:

In 1760 Lambert\(^1\) stated that when a beam of monochromatic radiation passes through a transparent medium, the radiant power of the transmitted radiation decreases exponentially as the thickness of the absorbing medium increases arithmetically.

The law is mathematically expressed in the logarithmic form as

\[
\log T = \log \frac{P}{P_0} = -K_b
\]

Where

- \(P_0\) = Power of incident radiation
- \(P\) = Power of transmitted radiation
- \(T\) = Transmittance
- \(K\) = a Constant.
2. Beer's law:

Beer\(^2\) in 1852 stated that the radiant power of a beam of parallel monochromatic radiation decreases exponentially as the concentration (C) of the absorbing substance increases arithmetically. Thus.

\[
\log T = \log \frac{p}{p_0} = -K'C
\]

Where 'K' is a proportionality constant.

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

\[
\log \left( \frac{p}{p_0} \right) = abc
\]

The ratio \( \log \frac{p}{p_0} \) is known as transmittance (T) which is a dimensionless quantity. As 'abc' is a logarithmic quantity, it is a pure number. The above equation can be written as

\[
A = abc
\]

Where

\[ A = \log \frac{1}{T} = \log \frac{p_0}{P} \]

is called absorbance and the constant 'a' is the absorptivity, where the concentration is expressed in grams/litre. If the concentration is expressed in moles/litre, the constant 'a' is replaced by '\( \varepsilon \)' called the molar absorptivity. Thus,

\[
A = \varepsilon bc
\]

The equation (1.5) is the 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 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 confirmation to Beer’s law. The non-linear plot indicates the deviations from Beer’s law. Deviations are termed positive and negative depending on the observed curve is above and below the theoretical straight line respectively.

Beer’s law is applicable for dilute solutions only. In this sense it is a limiting law. At high concentrations the average distance between the species responsible for absorption is diminished to the point where each affects the charge distribution of its neighbours. This interaction in turn can alter their ability to absorb a given wavelength of radiation. Because the degree of interaction is dependent upon concentration, the occurrence of this phenomenon causes deviations from Beer’s law.

According to Kortum and Seiler, $\varepsilon$ is dependent upon the refractive index ‘n’ of the solution. At low concentrations, ‘n’ is practically constant, and hence Beer’s law is obeyed. But at high concentrations the refractive index may vary appreciably and the system deviates from Beer’s law. A correction for this effect can be made by replacing $\varepsilon$ by $\varepsilon n/(n^2 + 2)^2$ in Eq(1.5).

Deviations are observed in systems exhibiting scattering or fluorescence. Presence of inert salts cause deviations even at low concentrations due to the influence of electrostatic interaction. Deviations are also encountered in cases where there is reflection of radiation by the test solution. Discrepancies are
usually found when the coloured solute ionise, dissociate or associate in solution, since the nature of the species in solution will vary with the dilution. Deviations also occur due to non-monochromatic nature of the radiation used.

**Ringbom's Plot**

A new method of plotting spectrophotometric data was developed by Ringbom in 1939. This helps in obtaining practical range of concentration.

A plot of percent absorption versus logarithm of concentration is constructed in this method. The term absorptance is defined as

\[
\text{Absorptance} = 1 - \text{Transmittance} = 1 - T \tag{1.6}
\]

\[
\% \text{Absorptance} = 100 - \% T \tag{1.7}
\]

An S-shaped or sigmoid curve is always obtained when the spectrophotometric data is plotted by Ringbom's method. According to Ringbom the accuracy of measurement is greatest when \(\Delta p/\Delta c/C\) reaches a maximum value i.e. at the point of steepest slope (the 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 63.2% 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's plot.
2. The accuracy of the analysis can be easily evaluated at any concentration range.
If the system under study does not obey Beer's law over the entire range of concentration, the Ringbom's 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 methods**

Sensitivity of spectrophotometric method means the least determinable concentration or amount of the species under consideration. In photometric analysis the steepness of the Beer's law plot is a measure of the sensitivity.

This is numerically expressed as molar absorptivity $\varepsilon$.

$$\varepsilon = \frac{A}{bc}$$ ................................................................. 1.8

Where $A$ is the absorbance, $b$ is the path length and $c$ is the concentration of the species in moles/l. If $b$ is expressed in centimeters the units of molar absorptivity are L mol$^{-1}$ cm$^{-1}$. In SI units $b$ is expressed in millimeters and thus the units of $\varepsilon$ are L mol$^{-1}$ mm$^{-1}$. According to quantum theory the value of $\varepsilon$ cannot exceed $\sim 1.5 \times 10^5$. Higher values are only possible in some indirect methods such as amplification methods. Methods with $\varepsilon > 1 \times 10^4$ are termed to as sensitive while those with $\varepsilon < 1 \times 10^3$ as less sensitive.

The term specific absorptivity ($a$) is some times used to denote the sensitivity of a given method and it is defined as

$$a = \frac{\varepsilon}{At \times 1000}$$ ................................................................. 1.9

The units of $a$ are ml gm$^{-1}$ cm$^{-1}$

Sandell expressed sensitivity of a method as

$$S = 10^{-3}/a$$
Where $S$ is called Sandell's sensitivity and '$a$' specific absorptivity.

Sandell sensitivity represents the number of micrograms of the species per ml of the solution having an absorbance of 0.001 for a path length of 1 cm. The units of '$S$' are \( \mu g \text{ cm}^{-2} \).

**Specificity**

A reagent which reacts with only one element under specified conditions is generally called a specific reagent. In practice it is highly unlikely to find a reagent that is specific. Absolute specificity in this sense is unattainable. Dimethyl glyoxime which combines readily with a number of metals, forms an insoluble red chelate with one metal namely nickel. The specificity of this reaction lies in the colour of the product. Other metals like Palladium and Platinum form soluble chelates with the dioxime which are yellow and blue in colour respectively but nickel is the only metal which forms a water insoluble red product. Cuproine for Copper(I) and otho phenanthroline for iron(II) can be cited as examples of spectrophotometric specific reagents.

**Selectivity**

A reagent is termed selective, if it reacts with a limited number of elements. By proper exploitation of the differences in two or more physical properties, the desired selectivity can be achieved in analytical work. The selectivity of a colour reaction and the corresponding spectrophotometric method depends on

1. the nature of the reagent used.
2. the oxidation state of the element.
3. the pH of the medium.
Determination of the composition of 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 equilibria under consideration. The three important methods that are widely employed for this purpose are

1. Job's continuous variation method
2. Mole ratio method and
3. Slope ratio method

1. **Jobs's continuous Variation method**

   Job in 1928 described a method for the determination of the composition of the complex. The complex equilibrium can be represented by the general equation.

   \[ M + nL \rightleftharpoons ML_n \]  

   Where M and L represents the metal and the reagent.

   Solutions of 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 suitable wavelength and corrected for any absorbance if no reaction occurred. A plot is then constructed with the volume fraction \( \frac{V_M}{V_M + V_L} \) and the corrected absorbance. A curve with a maximum is obtained (or a minimum if the complex absorbs less than the reactants). The composition of the complex is then given by the volume fraction ratio.
\[
\frac{V_M}{V_M + V_L} : 1 - \frac{V_M}{V_M + V_L} = 1.12
\]

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, 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 experimental conditions.

Cooper et al.\(^{10}\) suggested a method to establish this fact. According to Cooper's modified procedure the absorbance is measured at various wavelengths covering the entire range instead of measuring it at the wavelength of maximum absorbance. 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 is 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\(^{11}\) introduced a new 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 favourable, two straight lines of different slopes are obtained, the intersection occurs at a mole ratio corresponding to the combining ratio of the complex.

3. Slope ratio method

This method is proposed by Harvey and Manning\textsuperscript{12} and particularly useful for weak complexes. It is applicable, if only 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 reactant and the Beer's law is followed under these circumstances. For the reaction

\[ mM + nL = M_mL_n \]

The following equation can be written when \(L\) is present in very large excess

\[ [M_mL_n] = F_{M/m} \]

If Beer's law is obeyed

\[ A_m = \varepsilon b [M_mL_n] = \varepsilon b F_{M/m} \]

and a plot of \(A\) versus \(F_{M}\) will be linear. The slope of the line

\[ S_m = \varepsilon b / m \]

when \(M\) is in excess w.r.t \(L\)

\[ [M_mL_n] \approx F_{L/n} \]

and \(A_1 = \varepsilon b [M_mL_n] = \varepsilon b F_{L/n} \)

The plot of \(A\) against \(F_{M}\) will be linear with a slope.

\[ S_m = \varepsilon b / m \]

Combining ratio between \(L\) and \(M\) is obtained from the ratio of the two slopes.
Evaluation of stability constant of a metal complex

The stability constant of the complex $M_mL_n$ given by the following reaction can be determined from the spectrophotometric data obtained in Job's continuous variation method.

$$mM + nL \rightleftharpoons M_mL_n$$

by using the following mathematical relation.

$$b = \frac{A/Am}{m^n n^m [(1 - A/Am)]^{m+n} [c]^{m+n-1}}$$

Where

- $A_m$ = absorbance corresponding to the point of intersection of the extrapolated lines.
- $A$ = measured absorbance at concentration 'C'.
- $c$ = concentration corresponding to the intersection point.
- $\beta$ = stability constant.
- $A_m - A/Am = \alpha = \text{degree of dissociation}$.
Derivative spectrophotometry:

Derivative spectrophotometry has enjoyed extensive activity during the last ten years. Its dynamic development is due to the incorporation of electronic differentiators in most of the commercial spectrophotometers. These together with associated software, are able to compute derivatives up to n-th order of the spectrum. Today, this technique has become a standard in spectrophotometric laboratories. It has proved to be very useful, providing both qualitative and quantitative information derived from mathematical processing of UV and VIS spectra. Since, the principle of the derivative spectrophotometry are discussed, several reviews are published dealing both with theoretical aspects\textsuperscript{13-17} and practical\textsuperscript{18-23} problems.

Derivative spectrophotometry enables the detection of the presence of small quantities of impurities in samples, a more accurate determination of a minor component in the presence of bulk components and its combination with spectrophotometric multicomponent analysis. With the development of microcomputer technology, derivative spectra are developed almost instantly. Therefore the analytical use of derivative spectrophotometry has become very popular.

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 facilitates the detection of poorly resolved absorption peaks arising from admixtures or impurities in solution or for structural reasons and it enables the exact determination of $\lambda_{\text{max}}$ of the particular analyte species and increase the sensitivity of the spectrophotometric procedures. In addition, it is an excellent background elimination technique. The influence of Rayleigh scattering, constant
background absorbance, non-selective absorption of the matrix or accompanying components is removed and the resolution of signal-to-noise ratios improved.\textsuperscript{17,24-32}

The first, second etc derivative absorption spectrum of an analyte is defined as the first, second etc derivative of the absorbance as a function of wavelength. The first or the second derivative is easily accessible instrumentally. The higher derivatives are calculated on the basis of computer generated functions. The first derivative spectrum is useful for the determination of the component more accurately in presence of matrix component. It is also useful for the microcomponent analysis or identification of derivatives with high spectral similarity. The resolution of overlapping bands is considerably improved since band width of a Gaussian peak decrease to 51.41 or 34\% of the original bandwidth for the 2nd, 4th or 6th order derivatives respectively. Even order derivatives having a central peak of alternating sign narrower but coincident with the original peak may have greater analytical use than the odd functions.\textsuperscript{14,33}

Thus the first, second etc derivatives can eliminate unwanted effects such as various scattering instrumental effects, differences by replacing cells or base line shifts caused by continual back ground, which allow a more accurate quantitative evaluation of data.

**Quantitative evaluation of derivative plots :**

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

\[
\frac{dA}{d\lambda} = -0.434 \frac{1}{l} \frac{dl}{d\lambda} = \frac{de}{d\lambda} \text{ Cl} \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad 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Assuming $\frac{dl_0}{d\lambda} = 0$

The amplitude which is least affected by variation in the matrix and gives the best calibration statistics\textsuperscript{27} is selected for quantitative measurements. The procedures used for quantitative evaluation are the (a) tangent, (b) peak-valley (peak-peak), or (c) peak-zero line method which are shown in fig.1.1. In principle both the peak to valley amplitude, $D_S$, $D_L$ and the baseline-to-valley distance, $D_B$ are proportional to the analyte concentration. In fact, the situation is more complicated for higher derivatives, but the proportionality of the derived signal to the analyte concentration is often maintained.\textsuperscript{14,30}
Various kinds of quantitative evaluation of the first-derivative spectrum

a. The Tangent Method;
b. The Peak-Peak Method;
c. The Peak-Zero Method
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