Chapter - 7

Principles of Spectrophotometry
The methods based on the absorption of electromagnetic radiation are the most important of all the instrumental methods of analysis. Spectrophotometry is one of the most widely employed physico-chemical techniques which is remarkable for its sensitivity and precision. The popularity of the spectrophotometric technique is mainly due to the easiness with which the instruments can be operated and their wide availability.

Spectrophotometry in the visible region of electromagnetic spectrum, generally considered to extend from 380nm to 780nm, is often referred to as colorimetry. 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 trace concentrations of metal ions, non metals and organic substances is perhaps the main reason for the wide utilization of photometric methods of analysis.

In spectrophotometric analysis the radiant power of a beam of radiation transmitted by an absorbing medium placed between the radiant energy source and the detector is measured. A plot between the radiant power transmitted or absorbed and the wavelength is called the absoroption spectrum.
and is characteristic of the absorbing species and provides the basis for qualitative analysis.

The radiant power of a beam of radiation absorbed or transmitted is also a measure of concentration and forms the basis for the quantitative analysis.

Laws of photometry

When a beam of radiant energy impinges upon 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

Lambert in 1760 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 expressed in the logarithmic form as

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

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1.1
Where

\[ P_0 = \text{Power of incident radiation} \]
\[ P = \text{Power of transmitted radiation} \]
\[ T = \text{Transmittance} \]
\[ k = \text{a constant} \]

(2) Beer’s law

In 1852 Beer\(^2\) stated that the radiant power of a beam of parallel monochromatic radiation decreases in a similar fashion as the concentration (C) of the light absorbing constituent increases. Thus,

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

Where \( k \) is a proportionality constant. The two laws can be combined employing a new proportionality constant ‘a’ and written as

\[ \log \frac{P_0}{P} = abc \]

The ratio \( \log \frac{P_0}{P} \) is known as transmittance (T) which is dimensionless quantity. As, ‘abc’ is a logarithmic quantity, it is a pure number. Thus equation 1.3 can be written as

\[ A = abc \]

where \( A = \log \frac{1}{T} = \log \frac{P_0}{P} \) and is called absorbance, ‘a’ is the absorptivity, a constant dependent upon the wave length of the radiation and the nature of the absorbing species, whose concentration \( C \) 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,

\[ A = \varepsilon bc \]  

Equation 1.5 is the fundamental law governing the process of 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**

According to Beer's Law (eq. 1.5) a linear relationship between the absorbance and the concentration of the absorbing species should exist, provided the path length (b) is maintained constant. Deviations from this 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. A nonlinear 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 Seiller\(^3\), \( \varepsilon \) 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 $\kappa$ by $\kappa n/(n^2 + 2)^2$ in Eq. (1.5).

Deviations are observed in systems exhibiting scattering or fluorescence. Presence of inert salts cause deviations even at low concentration 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. Discrepancies also occur due to non-monochromatic nature of the radiation used.

**Ringboom's plot**

Ringboom in 1939 developed a new method of plotting spectrophotometric data which helps in obtaining practical range of concentration.

In this method a plot of percent absorptance versus logarithm of concentration is constructed. The term absorptance is defined as

$$\text{Absorptance} = 1 - \text{transmittance} = 1 - T$$

$$\% \text{Absorptance} = 100 - \% T$$

An S-shaped or sigmoid curve is always obtained when the spectrophotometric data is plotted by Ringboom's method. According to Ringboom 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 0.4543.
There are two important advantages of Righboom's Plot.

(1) At a glance it represents the concentration range in which the analysis error is minimum. This corresponds to the nearly linear portion of the Ringboom's plot and

(2) In any concentration range the accuracy of the analysis can be easily evaluated. If the system under consideration does not obey Beer's law over the entire range of concentration, the Ringboom's plot has a sigmoid curve. The curve will have a different slope and the inflection point occurs 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

Generally sensitivity 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 can be numerically expressed by the "molar absorptivity" at the wavelength of maximum absorbance and is given as

\[ \varepsilon = \frac{A}{bc} \]

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 trimol\(^{-1}\) mm\(^{-1}\). According to quantum theory the value of \( \varepsilon \) cannot exceed \( \approx 1.5 \times 10^5 \). Higher values are only possible in some indirect methods such as amplification methods. Methods with \( \varepsilon > 1 \times 10^4 \) are referred to as sensitive while those with \( \varepsilon < 1 \times 10^3 \) as less sensitive.
The term specific absorptivity \((a)\) is sometimes used to denote the sensitivity of a given method and it is defined as

\[
a = \frac{\epsilon}{AtWt \times 1000}
\]

the units of \(a\) are ml gm\(^{-1}\) cm\(^{-1}\).

Sandell\(^6\) 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 ug 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 ortho 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 methods depends on

1. the nature of the reagent used
2. the oxidation state of the element
3. the pH of the medium
4. the nature of the reagent used to mask the interfering ions and
5. the temperature.

Determination of the composition of metal complexes:

Spectrophotometry is one of the most versatile 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 the purpose are

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

1. Job's continuous variation method

In 1928, Job described a method for the determination of the composition of the complex. The complex equilibrium can be represented by the general equation.
\[ M + nL \rightleftharpoons ML_n \] \[ \text{1.11} \]

where \( M \) and \( L \) represents the metal and the reagent.

Solutions of the metal ions 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 made with the volume fraction, \( \frac{V_M}{V_M + V_L} \) (where \( V_M \) and \( V_L \) are the volumes of the metal ion solution and the reagent solution respectively 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,

\[ \frac{V_M}{V_M + V_L} = \frac{V_M}{V_M + V_L} \] \[ \text{1.12} \]

Corresponding to the maximum or minum 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, 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. 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 then be concluded that a single complex is formed and the Job’s method can be successfully employed for the elucidation of the composition of the complexes. 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 suggests the formation of a single complex species and Job's method can be used at the wavelength.

2. Mole ratio method:

Yoe and Jones\textsuperscript{11} in 1944 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:

The 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

\begin{equation}
mM + IL \rightleftharpoons M_m L_i \tag{1.13}
\end{equation}

the following equation can be written when 'L' is present in very large excess

\begin{equation}
(M_m L_i) = F_{M/m} \tag{1.14}
\end{equation}
If Beer's law is obeyed

$$A_m = \varepsilon b (M_m L_l) = \varepsilon b \frac{F_M}{m} \tag{1.15}$$

and a plot of 'A' versus $F_M$ will be linear. The slope of the line

$$S_m = \frac{\varepsilon b}{m} \text{ (When 'M' is in excess with respect to L)} \tag{1.16}$$

and $A_1 = \varepsilon b [M_m L_l] = \varepsilon b \frac{F_L}{l} \tag{1.17}$

The plot of 'A' against 'F_M' will be linear. Combining ratio between L and M is obtained from the ratio of the two slopes

$$\frac{S_m}{S_1} = \frac{\varepsilon b}{m} = \frac{1}{m} \tag{1.18}$$

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 determined from the spectrophotometric data obtained in Job's continuous variation method.

$$mM + nL = M_m L_n \tag{1.19}$$

by using the following mathematical relation

$$b = \frac{A/A_m}{m^n n^m [1-(A/A_m)]^m + n [C]^{m+n-1}} \tag{1.20}$$

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

\[ \frac{A_m - A}{A_m} = \alpha = \text{degree of dissociation} \]

1.21

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 facilitates the detection of poorly resolved absorption peaks arising from admixtures of impurities in solution or for structural reasons and it enables the exact determination of \( \lambda_{\text{max}} \) of the particular analyte species and increases the sensitivity of the spectrophotometric method. The influence of Rayleigh scattering, constant background absorbance, non-selective absorption of the matrix of accompanying components is removed and the resolution of signal to noise are enhanced.

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.

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 wavelength. The first or the second derivative is easily accessible instrumentally. The higher order 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
components. It is also useful for the multicomponent analysis or identification of
components with high spectral similarity. Thus the first or the second order
derivatives can eliminate unwanted effects such as various scatterings,
instrumental affect, differences by replacing cells, base line shifts caused by
continuous background, which allow a more accurate quantitative evaluation of
data.

If Beer's - Lambert's law is 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's law are given as

\[
\frac{dA}{d\lambda} = -0.434 \frac{1}{l} \frac{dl}{d\lambda} = \frac{dF}{d\lambda} Cl
\]

\[
\frac{d^2A}{d\lambda^2} = 2.303 C^2 \lambda^2 (\frac{dF}{d\lambda})^2 - 0.434 \frac{1}{l} \frac{dl^2}{d\lambda^2} = \frac{d^2F}{d\lambda^2} = Cl
\]

assuming \(\frac{dl_0}{dl} = 0\)

The amplitude which is least affected by variation in the matrix and
gives the best calibration statistics is selected for quantitative evaluations. 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, \(D_s\) are proportional
to the analyte concentration. The measurement becomes more complicated for
higher order derivatives.
Fig 1.1 Various kinds of quantitative evaluation of the first-derivative spectrum

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