CHAPTER 2

BASIC PRINCIPLES OF SPECTROPHOTOMETRY
Spectrophotometry is one of the simplest analytical techniques based on absorption or emission of visible or UV radiations. There is a close relationship between the colour of the substance and its electronic structure. A molecule absorbs in the visible or UV region of the electromagnetic spectrum, raising the molecule from ground state to the excited state. The production or change of colour is related to change of electronic structure of the molecule. By measuring the amount of radiant energy absorbed or emitted by a substance in the form of spectrum, the substance can be analysed qualitatively and quantitatively. For qualitative analysis, absorption spectrum is used.

Basic laws of absorption spectrophotometry were first introduced by Bauguer, Lambert and Beer. In 1729 Bauguer established a relationship between the thickness of transparent medium and absorption by the medium. Lambert in 1760 devised a law based on the Bauguer’s suggestion.

**Lambert’s Law**

When a parallel beam of monochromatic radiation of intensity \( I_0 \) impinges upon a layer of a solution of thickness \( dl \), some radiant energy is absorbed. The fraction of radiation absorbed increases exponentially with linear
increase in the thickness of the layer. In other words, the intensity of the transmitted radiation \( I_t \) decreases exponentially as the thickness \( b \) of the absorbing medium increase arithmetically. This is given mathematically as

\[
I_t = I_0 e^{-K'b}
\]  

(1)

where \( I_0 \) is the intensity of the incident radiation of wavelength \( \lambda \), and \( K' \) is called proportionality constant.

The intensity of incident radiation \( I_0 \) is the sum of the fraction absorbed \( (I_a) \), fraction transmitted \( (I_t) \) and fraction reflected \( (I_r) \), through the medium.

Therefore,

\[
I_0 = I_a + I_t + I_r
\]  

(2)

In spectrophotometry, measurements are always made with respect to a reference solution taken in the identical containers (cells) of transparent material, as that of the sample solution. Therefore, reflection of incident radiation is neglected. Hence \( I_o \) is the sum of \( I_a \) and \( I_t \)

\[
I_o = I_a + I_t
\]  

(3)

Lambert's law is the relationship between \( I_o \) and \( I_t \).
**Beer's Law**

In 1852, Beer studied the effect of concentration of the coloured constituent in a solution upon the absorption or transmittance. When a parallel beam of monochromatic radiation of intensity \( I_o \) falls upon a layer of a homogeneous solution, some radiant energy is absorbed. The fraction of radiation transmitted decreases exponentially as the concentration of the absorbing species increases arithmetically. This is given mathematically as

\[
I_t = I_o e^{-K'c} \quad \ldots \quad (4)
\]

where
- \( I_o \) = intensity of incident radiation
- \( c \) = concentration
- \( I_t \) = intensity of transmitted radiation
- \( K \) = proportionality constant.

\[
I_t = I_o e^{-K' K'' b \cdot c} \quad \ldots \quad (5)
\]

or

\[
I_t = I_o e^{K \cdot bc} \quad \ldots \quad (6)
\]
Equation (6) can be expressed in the log form as

$$\log \left( \frac{I_o}{I_t} \right) = abc. \quad \ldots \ (7)$$

where

$$a = \frac{K}{2.303}$$

Log $I_o/I_t$ is termed as the absorbance $(A)$ of the substance.

Therefore,

$$A = abc \quad \ldots \ (8)$$

The term 'a' is called absorptivity or specific absorption coefficient when the concentration is expressed in g/lit and $b$ in cm. When the concentration is expressed in moles, liter$^{-1}$ and thickness of the medium $b$ in cm, we write $\varepsilon$ in place of $a$, known as molar absorptivity.

$$A = \varepsilon c b. \quad \ldots \ (9)$$

Equation (9) is the fundamental law governing the absorption of all types of electromagnetic radiations.

The absorbance, $(A)$ is the additive property. If there are more than one absorbing species present in the solution, the total absorbance is equal to sum of all the absorbance values of the components present in solution. It can be expressed as follows.
\[ A = [\varepsilon_{c_1} + \varepsilon_{c_2} + \varepsilon_{c_3} \ldots \ldots + \varepsilon_{n\cdot c_n}]b \]

\[ = b \sum_{i=1}^{n} \varepsilon_i c_i \quad \ldots \quad (10) \]

where the pathlength, \( b \) is kept constant.

Molar absorptivity (\( \varepsilon \)) is the intensive property. If there is a change in the concentration and thickness of the medium, the value of molar absorptivity will remain constant within the Beer's law range.

**Limitations of Beer's law**

A graph plotted between absorbance and concentration gives a straight line passing through the origin (\( A = 0, C = 0 \)) for a solution of species obeying Beer's law. In many cases absorbing species do not obey Beer's law.

**Deviations from Beer's law.** The law is not obeyed if a monochromatic radiation is not employed; reflection of the radiation by the test solution occurs due to difference in solvent composition in the test solution and blank, due to interionic forces and shifts in equilibria due to ionization of coloured solute.
Dissociation or association of complex of the absorbing species may cause deviation from the Beer's law. The experimental time and temperature, fluorescence of solute and poor solvent transmission also contributed to deviations from Beer's law.

Solutions not obeying Beer's law can be quantitatively analysed by preparing a calibration curve by plotting the absorbance of standard solution on ordinate against their concentrations. The concentration of the unknown is then obtained from the plot.

For a system that obeys Beer's law the molar absorptivity ($\varepsilon$) can be calculated from the slope of calibration plot. The unknown concentration is then calculated utilizing value of $\varepsilon$ and its measured value of absorbance under identical conditions.

General procedure for the development of spectrophotometric method

In quantitative analysis, spectrophotometric methods are used very frequently. Several new methods have been developed by making use of systematic procedures. These procedures are employed to know the effect of pH, maximum wavelength ($\lambda_{\text{max}}$), effect of reagent, verification of Beer's law and also the effect of foreign ions.
Sensitivity of Spectrophotometric Methods

Sensitivity is referred as the least determinable amount of species of interest. Sensitivity is known by molar absorptivity. This can be calculated through the slope of calibration plot prepared between absorbance and concentration of species.

\[ \varepsilon = \frac{A}{C \times l} \quad \ldots \quad (11) \]

where
- \( A \) = absorbance
- \( C \) = concentration of the absorbing species
- \( l \) = pathlength of the medium.

If \( l \) is in cm, the unit of \( \varepsilon \) is \( \text{L. mol}^{-1} \text{ cm}^{-1} \). If SI units are used \( l \) is expressed in mm and unit of \( \varepsilon \) is \( \text{L. mol}^{-1} \text{ mm}^{-1} \).

For sensitive spectrophotometric methods \( \varepsilon \) is greater than \( >1 \times 10^4 \text{ L. mol}^{-1} \text{ cm}^{-1} \) and the values of \( \varepsilon \) below \( 1 \times 10^3 \) correspond to less sensitive methods. The molar absorptivity cannot exceed \( 1 \times 10^5 \) according to quantum theory.

Specific absorptivity (a) is another way of expressing sensitivity of the method. This is obtained by dividing \( \varepsilon \) by the atomic weight of the element and 1000.
The number of micrograms (μg) of analyte estimated per ml of a solution having an absorbance of 0.001 with a pathlength of 1 cm. The Sandell's sensitivity (S) is expressed in μg. cm⁻² and is therefore equal to 10⁻³/α where α is known as specific absorptivity.

Specificity

Reagents which under definite experimental conditions are quite unambiguous to give reaction with only one component are termed as specific. In this sense absolute specificity is unattainable.

Even a non-specific reagent can be made specific by adopting suitable precautions to undergo a desirable limitation of its activity. Cuproine for Cu(I), and acyloin oximes for Cu(II) may be considered as examples for specific reagents.

Selectivity

A reagent which react with limited number of metal ions is termed as selective. The selectivity of a colour reaction depends on (1) the nature of the reagent, (2) the pH of the medium, (3) the oxidation state of the element, and (4) the nature of the masking agent.
Paradimethyl aminobenzidene rhodamine is highly selective reagent for Ag. Rhodamine group present in this reagent forms whitish yellow precipitate with Ag(I) in dilute HNO₃. It exhibits selective action in acid medium and this is lost in alkaline medium.

Methods used in the determination of composition and stability of complexes

Job's method

Job described a simple procedure for the identification of the complex formed by reaction of the components in solution. The reaction in which the two components are the metal ion (M) and the ligand (L) is represented as

\[ M + nL \rightleftharpoons ML_n \quad \cdots (13) \]

Solutions of metal ion and reagent having identical formal concentrations are mixed in varying volume ratios, keeping the total volume of the mixture constant. The absorbance of these mixtures is measured at suitable wavelength. A maximum is observed in the curve if the complex has higher absorption than the reactants, a minimum if the reverse is true. The mole fraction ratio at the maximum or minimum point on the curve gives the composition of the complex formed between metal ion and the ligand. This method gives inconclusive results, when there are more than one complex formed
between metal ion and ligand and the complex formed is not stable.

Vosburg and Cooper modified the Job's method. Their modification lies in first ascertaining in any particular case, whether or not more than one complex are formed from a pair of components. Solutions of metal ions and reagent having identical formal concentrations are mixed in different volume ratios, while maintaining the total volume of the mixture constant. For all these solutions absorption spectra are recorded. If the absorption maximum is obtained at the same wavelength for all these mixtures, it is concluded that there is only one complex formed and Job's method can be employed at that particular wavelength.

The stability constant of a complex may be represented by the following equation

\[ aA + bB \rightleftharpoons A_a B_b \]  \hspace{1cm} \ldots (14)

Using spectrophotometric data obtained in the Job's method, it is possible to determine the stability constant, the equation can be expressed as follows.

\[ \beta = \frac{A/A_a}{a^a b^b [1 - A/A_a]^a + b (c)^a + b - 1} \]  \hspace{1cm} \ldots (15)
where
\[ A_a \] absorbance corresponding to the point of intersection of extrapolated line
\[ A \] observed absorbance at concentration, C.
\[ \frac{A_N - A}{A_a} = \alpha \] degree of dissociation
\[ \beta \] stability constant.

Above equation may be simplified and written as

\[ \beta = \frac{(1 - \alpha)}{a^2 \cdot C} \] for M:L = 1:1 \( \ldots \) (16)

\[ \beta = \frac{1 - \alpha}{4 \cdot a^3 \cdot c^2} \] for M:L = 1:2 \( \ldots \) (17)

\[ \beta = \frac{1 - \alpha}{27 \cdot a^4 \cdot c^3} \] for M:L = 1:3 \( \ldots \) (18)

where
\[ \alpha \] degree of dissociation of the complex
\[ C \] concentration of the ligand at intersected point in the graph.

Molar Ratio Method

Molar ratio method is a counter part of Job's continuous variation method for spectrophotometric study of complex formation. This method has no firm theoretical background.
It is based on plotting the measured absorbances against molar ratio of any two components of a complex, when the concentration of one component is varied while the other is held constant. If the system forms a stable complex, a plot with sharp break is obtained. The molar ratio at the sharp break indicates the composition of the complex. However, if a weak complex with appreciable dissociation is formed, a curved plot is obtained. In such cases, the molar ratio method is applied by drawing convenient break points on such curved plots. Linear extrapolation of the curve is made where the curved plot becomes nearly parallel to the molar ratio axis, after an excess of the variable component is added. However, no theories justifies such extrapolation.

Slope Ratio Method

In a reaction

\[ mA + nB \rightleftharpoons A_m B_n \] ... (19)

if the concentration of B is constant and in excess to make the dissociation of the complex negligible, the equilibrium concentration of \( A_m B_n \) will be essentially proportional to the analytical concentration of A

\[ [A_m B_n] = \frac{C_A}{m} \] ... (20)
After applying Beer's law, the equation is rewritten as

\[ I = \varepsilon x b \frac{CA}{m} \]  \hspace{1cm} \text{... (21)}

where

- \( I \) = measured absorbance of solution
- \( \varepsilon \) = molar extinction coefficient of the complex
- \( b \) = thickness of the cell.

By plotting absorbance against analytical concentration of the metal a straight line is obtained. The slope of this straight line is given by \( \varepsilon x \frac{b}{m} \).

If the concentration of the metal is kept constant and in excess, and the concentration of \( B \) is varied, a plot of absorbance vs concentration of \( B \) will give another straight line with slope = \( \varepsilon b/n \).

From the ratio of the two slopes, \( (m:n) \) the composition of the complex is obtained.