CHAPTER 2
METHODS OF DETERMINATION OF THE
STABILITY CONSTANTS OF COMPLEXES

A. pH-METRIC METHOD

B. CONFIRMATION OF COMPLEX FORMATION BY
SPECTROPHOTOMETRIC MEASUREMENTS
GENERAL CONSIDERATIONS

Most of the numerical and graphical methods developed for the calculations of equilibrium constants are based on or related to the quantity called the complex formation function.

Complex formation involves disappearance of certain ions which form a complex compound. Metal complex formation may be considered to be due to the displacement of a proton from the ligand causing a drop in the pH values of the solution. Irving and Rossotti\(^1\) have given a method for calculation of stability constants of complexes by potentiometric technique. A general technique, used by Bjerrum\(^2\), is followed. Martell and Calvin\(^3\) have done potentiometric study of metal complexes of a large variety of polycarboxylic acids, oximes, phenols etc.

Various experimental methods\(^4\) are used to determine the composition and stability constants of the complexes; these are (i) measurement of colligative properties, (ii) optical methods (spectrophotometric study of systems with two & three or more components), (iii) NMR & ESR methods, (iv) calorimetry, (v) determination of equilibrium constants via kinetic measurements, (vi) extraction methods, (vii) solubility studies, (viii) study of ion exchange equilibria, (ix) potentiometry & pH-metric equilibrium studies, (x) polarography and (xi) other methods which include measurement of conductivity, polarimetry, Infrared spectrophotometry, Raman spectrophotometry, equilibrium study of metal complexes of anionic ligands (Wallace R.M., 1967), polarographic diffusion current, chronopotentiometry, cyclic voltammetry, gas chromatography, density measurement, magnetic susceptibility measurement, emission titration, measurement of self-diffusion coefficient, positronium annihilation and pressure measurement in the case of gaseous ligands. The possibilities for the application of other methods are limited as they each relate only to a well-defined group of equilibrium systems.

2. Methods of determination of the stability constants of complexes........15
2A. pH-METRIC METHOD

The method most frequently applied for the study of complex equilibria is pH-metric titration\textsuperscript{4}. One of the reasons for this is that pH measurements with a reproducibility within ± 0.005 pH unit can be performed virtually with a good - quality glass electrodes and modern, high-accuracy but relatively inexpensive pH-meters. Under carefully chosen and maintained experimental conditions, it is possible to attain a reproducibility better than this by even an order of magnitude. Another fundamentally important reason for the widespread use of the method is that practically all equilibrium processes that take place in water or in water containing solvent mixtures respond sensitively, either directly or indirectly, to a change in the pH.

The stepwise formation of the mononuclear binary complexes can be described by a set of equilibrium constants. Concentration changes caused by a complex formation are reflected in the potential of well-chosen electrodes. For pH-metric measurements an electrode must be selected, the potential of which is a well defined function of the concentration of the ion to be determined and also a reference electrode. It is a prerequisite of the application of pH-metric method that the electrode reaction must be reversible. According to Bjerrum, Martell and Calvin\textsuperscript{2,3}, the formation of complex $ML_N$ is, in general, a stepwise process and one has to deal with a series of equilibria of the type:

\[
\begin{align*}
M + L & \rightleftharpoons ML \quad \text{.........1(a)} \\
ML + L & \rightleftharpoons ML_2 \quad \text{.........1(b)} \\
ML_2 + L & \rightleftharpoons ML_3 \quad \text{.........1(c)} \\
ML_{N-1} + L & \rightleftharpoons ML_N \quad \text{.........1(d)}
\end{align*}
\]

(The charges on a metal ion are neglected)

The corresponding stepwise formation constants are then given by:

\[
\begin{align*}
K_1 &= \left\{ \frac{a_{ML}}{a_M \cdot a_L} \right\} \\
K_2 &= \left\{ \frac{a_{ML_2}}{a_{ML} \cdot a_L} \right\} \\
K_3 &= \left\{ \frac{a_{ML_3}}{a_{ML_2} \cdot a_L} \right\} \\
K_N &= \left\{ \frac{a_{ML_N}}{a_{ML_{N-1}} \cdot a_L} \right\} \quad \text{.........1(e)}
\end{align*}
\]

2. Methods of determination of the stability constants of complexes \ldots .16
A. pH-metric method

The stability constant \( \beta_N \) for the over all equilibrium process,
\[
M + NL \rightleftharpoons ML_N \quad (2)
\]
is the product of various stepwise formation constants and may be written as:
\[
\beta_N = K_1 \cdot K_2 \cdot K_3 \ldots K_N = \frac{a_{ML_N}}{a_M a_L} \quad (3)
\]
Writing in a similar fashion, for ligand equilibria

the general equation can be written as:
\[
LH_i + H = LH_i \quad (4)
\]
where LH\(_i\) is the ligand acid. The proton-ligand stability constant for such a reaction is given by:
\[
K_i^H = \frac{a_{LH_i}}{a_{LH_{i-1}} a_H} \quad (5)
\]
where \( K_i^H \) is called the ith thermodynamic proton-ligand stability constant and is the reciprocal of thermodynamic dissociation constant of the acid LH\(_i\) dissociating as:
\[
LH_i = LH_{i-1} + H \quad (6)
\]
The pK value is given by:
\[
pK_i = \log \frac{a_{LH_{i-1}}}{a_{LH_i}} \quad (7)
\]
For monobasic ligand pK\(_i^H\) = pK\(_i\) in magnitude.

For polybasic acids (H\(n\)A):
\[
pK_1 = pK_1^H, \quad pK_2 = pK_2^H, \quad \ldots, \quad pK_n = pK_n^H
\]
Bjerrum introduced the concept of degree of formation or ligand number (\( \bar{n} \)), which he defined as the average number of ligand bound per metal ion present in whatever form i.e.
\[
\bar{n} = \frac{\sum_{i=0}^{N} i [ML_i]}{\sum_{i=0}^{N} [ML_i]} \quad (8)
\]
Substituting for the values of ML\(_i\) from equation 1(e) applied to equilibria 1(a) to 1(d) and eliminating M, we have:
\[
\bar{n} = \frac{K_1 [L] + 2K_1 K_2 [L]^2 + \ldots + NK_1 K_2 \ldots K_N [L]^N}{1 + K_1 [L] + K_1 K_2 [L]^2 + \ldots + K_1 K_2 \ldots K_N [L]^N} \quad (9)
\]
which can also be written using equation (3) as:
\[
\bar{n} = \left\{ \frac{\sum_{i=0}^{N} i \beta_i [L]^i}{\sum_{i=0}^{N} \beta_i [L]^i} \right\} \quad (where \( \beta_0 = 1 \)) \quad (10)
\]
Equation (10) is called the formation function of the system.

A similar function for proton – ligand complexes is given by:
\[
\bar{n}_A = \left\{ \frac{\sum_{i=0}^{N} i \beta_i^H [H]^i}{\sum_{i=0}^{N} \beta_i^H [H]^i} \right\} \quad (where \( \beta_0^H = 1 \)) \quad (11)
\]
where \( \bar{n}_A \) is the mean number of proton bound per noncomplex bound ligand molecule.
Since the total amounts of the metal salt and the chelating agent introduced into the solution are known, we have

\[ T_L = \sum_{i=0}^{N} i [LH_i] \]  \hspace{1cm} (12)

\[ T_M = \sum_{i=0}^{N} [ML_i] \]  \hspace{1cm} (13)

The concentration of complex \( (T_{ML}) \) is given by expression:

\[ T_{ML} = [M] \sum_{i=0}^{N} \beta_i [L]^i \]  \hspace{1cm} (14)

The extent of complex formation is characterised by the ligand number (\( \bar{n} \)) given as:

\[ \bar{n} = \frac{[ML] + 2[ML_2] + \cdots + N[ML_N]}{[M] + [ML] + [ML_2] + \cdots + [ML_N]} \]  \hspace{1cm} (15)

Or

\[ \bar{n} = \left\{ \frac{T_L - [L]}{T_M} \right\} \]  \hspace{1cm} (16)

where \( T_L \) is the concentration of ligand in all forms, \( [L] \) is the concentration of free chelating species and \( T_M \) is the total concentration of metal ion, bound or free.

2 A.1 Determination Of Stability Constants

Determination of the stability constants from the experimental data consist of three steps:

1) the determination of the formation curve of the system expressed as a plot of \( \bar{n} \) against \( pL = \log \frac{1}{[L]} \)

2) the calculation of values of \( K_1, K_2 \) by solving the formation function of the system

3) the conversion of the stoichiometric constants into the thermodynamic functions

Irving and Rossotti, Hearson and Gilbert, Wilkins and Lewis, & Rossotti and Rossotti have described general methods for the determination of stability constants. These authors made use of potentiometric titration technique first used by Calvin and Melchior and now known as Calvin-Bjerrum titration technique. The method of Irving and Rossotti has been employed in present investigation.

The experimental procedure involves titrations of:

(i) Nitric acid (\( A \))
(ii) Nitric acid + Ligand acid (\( A + L \))
(iii) Nitric acid + Ligand acid + Metal salt (\( A + L + M \))

2. Methods of determination of the stability constants of complexes........18
A. pH-metric method

with standard solution of sodium hydroxide. The ionic strength of each solution is kept constant at 0.10M by addition of 1.00M potassium nitrate solution.

Calculation of $\bar{n_A}$, $\bar{n}$ And pL.

The experimental data obtained from the titration curves help in calculation of $\bar{n_A}$, $\bar{n}$ and pL by applying following relationship:

(i) Proton-ligand formation number ($n^*_A$) for different pH values can be obtained from expression:

$$\bar{n_A} = \gamma - \frac{(V_2 - V_1)(N + E^0)}{(V^0 + V_1)T_L^0}$$

where $V$ is the initial volume of the solution, $E^0$, $T_L^0$ are the total concentrations of the mineral acid and ligand respectively, $V_1$ and $V_2$ are the volumes of alkali of a given normality required during the acid and ligand titrations respectively at a given pH, $\gamma$ is the number of replaceable hydrogen ions and N is the normality of NaOH.

(ii) The metal-ligand formation number ($\bar{n}$) can be calculated from the expression:

$$\bar{n} = \frac{(V_3 - V_2)(N + E^0)}{(V^0 + V_2)\bar{n_A}T_M^0}$$

(iii) The values of pL = - Log L can be calculated with the help of following expression:

$$pL = \log \left( \frac{[H^+]^2}{K_1K_2 + \frac{[H^+]}{K_1}} \right) \left( \frac{T_L^0 - T_M^0}{\bar{n}} \right)$$

This relation expresses $\bar{n}$ as a function of pL which is represented as the formation curve. The number of complexes formed in the reaction can be deduced from the formation curve and the values of stability constants can be determined.

Different workers have used different mathematical techniques for obtaining stoichiometric stability constants. All these methods are based on (a) solution of the formation function (b) the graphical representation of data and (c) the use of numerical integration of curve fitting procedure.

Irving and Rossotti have calculated $K_1$ and $K_2$ from the formation curve with the help of following methods:

(i) Successive approximation method
(ii) Solution of simultaneous equations
(iii) Interpolation of half $n$ values
(iv) Interpolation at various $n$ values
(v) Schwarzenbach's graphical method
(vi) Use of midpoint slope
(vii) Method of least squares.

The method of least-squares is the most reliable and accurate because it makes use of all experimental data and avoids subjective smoothening of the data accidental to plotting the best straight line.

2A 2. Measurement Of pH In Mixed Solvent Media

Irving and Rossotti have taken into consideration the use of the organic solvent-water mixtures, especially, dioxane-water mixtures, which have been employed by several workers to bring the water insoluble metal chelates into a homogeneous medium. As the glass electrode used with the commercially available direct reading pH- meters, is the most convenient means of determining pH values, it has been employed in these solvent mixtures.

The measurement of $H^+$ ion concentration in mixed solvents is associated with a number of difficulties. These are:

(i) glass electrode potential changes in going from an aqueous to mixed solvents.
(ii) liquid junction potential of uncertain magnitude which increases with the increase in the percentage of the organic component may vitiate the results appreciably.
(iii) solubility of an electrolyte decreases when the percentage of the organic solvent in the mixed medium becomes high.
(iv) sensitivity of glass electrode decreases as the percentage of organic component increases, and
(v) activity coefficients calculated on the basis of Debye-Huckel equation may differ considerably from the true values. The constants are, therefore, determined at various low values of ionic strength and extrapolated to $m = 0$ (where $m$ = ionic strength of solution)
For measuring the acidity in solvent-water mixtures Van Uitert and coworkers\textsuperscript{12,13} calibrated the glass electrode and related the hydrogen ion concentration $[H^+]$ with pH-meter reading $B$ by expression:

$$-\log [H^+] = \left\{ B + \log \frac{1}{Y} + \log U_H^{0}\right\}$$

where $Y$ is the activity coefficient of hydrogen ion in the solvent mixtures & $U_H^{0}$ is the correction factor at zero ionic strength and its value in water is unity.

The correction factor for measurement of pH in different dioxane-water mixtures proposed by Van Uitert\textsuperscript{12,13} alongwith the correction factors for other mixed solvents are given by Aditya et al.\textsuperscript{14} Chakraborthy and Patel\textsuperscript{15} have investigated the complexes of transition metal ions with monofunctional bidented Schiff base and monodented neutral ligands in non-aqueous and aqueous media.

\section*{2A. 3 EXPERIMENTAL}

\subsection*{2A.3.1 Solvents}

**Distilled water**

'Distilled water prepared' may contain small quantities of dissolved ammonia and carbon dioxide\textsuperscript{16}.

Distilled water used in investigation was obtained by redistilling twice the 'distilled water prepared' from alkaline potassium permanganate; the head and tail fractions were rejected to remove the dissolved ammonia\textsuperscript{16}. The dissolution of carbon dioxide was prevented during distillation by connecting a potassium hydroxide trap in the receiver of distillate. The resulting distillate was boiled to expel oxygen and then it was cooled in a well stoppered glass bottle ('Distilled water free from ammonia and carbon dioxide' had pH 7.00).

**Dioxane**

Dioxane contains small quantities of acetaldehyde and appreciable amount of glycol acetal together with some water\textsuperscript{17}. Upon keeping the acetal tends to undergo hydrolysis and the liberated acetaldehyde leads to some peroxide formation.
A. pH-metric method

Purification: (1) 1-litre dioxane ((1,4-Dioxan Extrapure s.d.fine - CHEM Ltd)), 14 ml concentrated hydrochloric acid and 100 ml distilled water were heated in a fume cupboard under reflux for 12 hours whilst a slow stream of nitrogen was bubbled through the solution to remove acetaldehyde formed. The cold solution was treated with excess potassium hydroxide pette with shaking until some remained undissolved and the strongly alkaline aqueous layer was run off; the residual water was removed by keeping the dioxane over fresh potassium hydroxide pellets for 24 hours. This treatment was followed by heating the decanted solvent under reflux over excess sodium for 12 hours (i.e. until reaction ceased and the sodium remained bright). Finally, the dioxane was distilled from sodium into a receiver encased in black paper; it was stored out of contact with air and in the dark.

(2) Distillation of the purified dioxane was carried out from lithium aluminium hydride to ensure the removal of any peroxide which may develop on storage.

(Dioxane had b.p. 101°C)

Acetone

Impurities in acetone are methanol & acetic acid (organic impurities) (less than 0.1 percent) and water (as high as 1 percent)\(^7\). Purification: One hundred grams of finely powdered sodium iodide were dissolved under reflux in 440 g of boiling acetone (E. Merck (India) Ltd.) and the solution was cooled in a mixture of ice and salt (\(-8^\circ C\)). The crystals were filtered off and quickly transferred to a dry distilling flask, connected to condenser and to a receiver cooled in ice. Upon gentle warming, the acetone distilled rapidly.

(Acetone had b.p. 56°C)

Ethanol

Rectified spirit is the constant boiling point mixture which ethanol forms with water and usually contains 95.6 percent of ethanol by weight\(^7\). Purification: (1) Ethanol of a 99.5 percent purity may be prepared by the dehydration of rectified spirit with calcium oxide.

A mixture of 250 g of calcium oxide (freshly ignited) and 1-litre of rectified spirit taken into a 2-litre round-bottomed flask fitted with a

2. Methods of determination of the stability constants of complexes........22
double surface condenser carrying a calciumchloride guard-tube was allowed to cool. The mixture was refluxed gently for a 6 hours and allowed to stand overnight. The ethanol was distilled gently discarding the first 20 ml of distillate into a receiver flask with side arm receiver adapter protected by means of a calciumchloride guard-tube. The absolute ethanol (99.5%) was preserved in a glass bottle with a well fitting stopper.

(2) 'Super-dry' ethanol : Very high grade ethanol (99.8 percent purity or higher) may be prepared from the product of dehydration of rectified spirit with calcium oxide by the method of Lund and Bjerrum. The method depends upon the reactions:

\[ \text{Mg} + 2C_2H_5OH \rightarrow \text{H}_2 + \text{Mg(OC}_2\text{H}_5)_2 \]  

(1)

\[ \text{Mg(OC}_2\text{H}_5)_2 + 2\text{H}_2\text{O} \rightarrow \text{Mg(OH)}_2 + 2C_2\text{H}_5\text{OH} \]  

(2)

Reaction (1) proceeds readily provided the magnesium is activated with iodine and the water content does not exceed 1 percent. Subsequent interaction between magnesium enolate and water gives highly insoluble magnesium hydroxide; only a slight excess of magnesium is, therefore, necessary.

5 g of clean dry magnesium turnings and 0.5 g of iodine, followed by 75 ml absolute ethanol were taken in a 2-litre round-bottomed flask fitted with a double surface condenser and a calcium chloride guard-tube. The mixture was warmed until iodine had disappeared. Heating was continued until all magnesium was converted into enolate; then 900 ml absolute ethanol was added and the mixture was refluxed for 30 minutes. The ethanol was distilled off using an apparatus similar to that described for the dehydration of rectified spirit.

(Ethanol had b.p. 78°C)

Methanol

A purity of 99.85 percent with not more than 0.1 percent by weight of water and not more than 0.02 percent by weight of acetone is claimed in methanol. Purification: (1) Most of the water was removed from 1-litre of methanol (Methanol Extrapure (s.d. fine-CHEM Ltd.)) by distillation through fractionating column.

(2) Anhydrous methanol was obtained from the fractionally distilled solvent by treatment with magnesium metal using the procedure given for 'super-dry' ethanol.
A. pH-metric method

(3) Small proportion of acetone was removed by the following procedure (Morton and Mark, 1934):

A mixture of 500 ml of methanol, 25 ml of furfural and 60 ml of 10 percent sodium hydroxide solution was refluxed in a 2-litre round-bottomed flask, filled with a double surface condenser, for 12 hours. A resin was formed which carried down all the acetone present. The alcohol was then fractionated, the first 5 ml of which containing a trace of formaldehyde being rejected.
(Methanol had b.p. 65°C)

2A. 3.2 Chemicals

All the chemicals used were of Anala R grades.

Nitricacid

Nitric acid (B.D.H.) is used for preparation of stock solution. Stock solution of 0.10M was prepared by diluting a suitable quantity of original commercial acid. Its exact molarity was calculated by titrating against sodium hydroxide solution.

Potassiumnitrate

The stock solution of 1.00M potassium nitrate was prepared by dissolving the requisite amount in distilled water.

Peptides

All the peptides used for the present investigation, were called from Germany. Stock solution of 0.01M of each peptide was prepared by dissolving the requisite quantities in distilled water.

Metalsalts

All the metal ions \{Mg(II), Co(II), Ni(II), Cu(II), Fe(III), Cr(III), Al(III), Ce(III), Nd(III), UO_2(II) and Th(IV)\} are used in the form of their nitrates to avoid the possibility of complex formation of the metal ion with anions. Stock solution of 0.01M of each metal salt was prepared by dissolving the requisite quantities in distilled water. The concentration of metal ions in solution are estimated by titrating against sodium salt of EDTA solution by procedure of Schwarzenbatch et al.°

Nitrolotriaceticacid

The stock solution of 0.01M nitrolotriacetic acid (B.D.H.) was prepared by dissolving the requisite quantity in distilled water.
A. pH-metric method

Sodium hydroxide

The solution of sodium hydroxide (E. Merck) was prepared free from carbonate by allowing the solution to stand for a long time till any carbonate, if present, precipitated. The solution was filtered through a sintered glass crucible and kept in a pyrex vessel free from carbon dioxide and was used as the titrant for the pH-metric titrations. The solution of sodium hydroxide was standardised by titrating against standard oxalic acid solution before starting an experiment.

2A. 3.3 pH-Meter And pH-Measurements

All the pH-measurements and titrations are carried out with pH-Meter Model L1-10T (Elico make) (accuracy ±0.01) with a glass and calomel electrode assembly. The instrument could read pH in the range 0.00 to 14.00 in steps of 0.05. This pH-meter has a built in internal electronic voltage stabiliser for ±10% fluctuations in voltage supply with temperature compensator covering the range 0.00 to 100.00°C. The pH-meter was switched on half an hour before starting the titrations for the initial warm up of the instrument. Before making any measurement with the meter the electrodes were washed with distilled water and dried with filter paper. The reading were recorded only when the instrument registered a steady value for at least one minute. The pH-meter was standardised before each titration with a buffer solution of pH = 4.01, 7.00 and 9.15.

All the glassware used in these experiments were of pyrex quality. The consumption of alkali during the titration was from a microburette giving an accuracy of 0.01 ml. The microburette which was graduated to 0.01 ml. was calibrated by the method described by Vogel. The micropipettes were similarly calibrated and the other apparatus like measuring flasks were checked with a standard burette. The alkali solution taken in the burette was protected against carbon dioxide from air by the sodalime guard-tube attached to burette.

Maintenance Of Glass Electrode

The glass electrode, is always kept in distilled water when not in use. After each titration during which it was exposed to alkali, the
A. pH-metric method

glass electrode was washed with 2N hydrochloric acid and then with distilled water. In order to prevent glass electrode from developing any asymmetry potential, it was occasionally kept in 2N hydrochloric acid for a period of 12 hours and then repeatedly washed with distilled water. All the precautions necessary for smooth working of a glass electrode were taken. These were in accordance to suggestions of Bates, Albert and Serjeant.

Calibration Of pH-Meter Scale

It is necessary to calibrate a pH-meter scale over the entire range as the pH of a solution in the titration vessel can vary continuously between 2.00 and 11.00 during titration. A calibration check was made at pH 4.01 and 9.15 only. Standard buffer solutions were prepared by dissolving Cambridge buffer tablets in distilled water and making the volume to 100ml. The exact pH of the buffer depends upon the temperature as indicated on the buffer label. The actual value of pH of the buffer solution at temperature 26.5°C (and evaluation of thermodynamic parameters at 25°C, 35°C & 45°C) at which the titrations are carried out in the present investigation is calculated from the following formula supplied by manufacturers:

(1) \( \text{pH}_{t} = \left\{ \text{pH}_{15^\circ C} - 0.0085 \left( t-15 \right) \right\} \)

for buffer solution of pH 9.15

(2) \( \text{pH}_{t} = \left\{ \text{pH}_{15^\circ C} - \left( \frac{0.5 \left( t-15 \right)^2}{100} \right) \right\} \)

for buffer solution of pH 4.01

After the instrument warmed up, freshly prepared buffer solution was taken in the titration vessel and the electrodes were immersed in it. The temperature compensator was set to the temperature of the buffer and the range switch was turned to obtain the appropriate range. The standardisation control was used to set the meter needle to the pH of the buffer. The meter was standardised by both the buffer solutions. The standardisation with buffer solutions was done once before starting the pH-metric titrations and then again after completion of a series of titrations in a day.

Inert atmosphere

pH-metric titrations are carried out in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrodes in order to prevent atmospheric
oxidation. Nitrogen gas served to expell dissolved oxygen and carbondioxide from the solution. An arrangement of a magnetic stirrer was made for rapid mixing of the solution in beaker.

**Calvin-Bjerrum Titrations**

The experimental procedure involves pH-metric titrations of solutions of (i) free acid i.e. HNO$_3$ (A) (ii) free acid + ligand (A+L) and (iii) free acid + ligand + metal ions (A+L+M) against standard sodiumhydroxide solution. The ionic strength of each solution was first adjusted to a constant value of 0.10M by addition of appropriate amount of 1.00M potassiumnitrate solution.

The titrations were carried out in a 100 ml. pyrex glass beaker kept in a water bath maintained at a constant temperature 26.5°C (and at 25°C, 35°C & 45°C for studies in thermodynamic parameters). The titrating solution was allowed to attain the bath temperature before the commencement of the titration. Nitrogen gas was bubbled continuously during the titration, the pH-meter readings were taken only after the gas bubbling and magnetic stirring were stopped. At the point, when the meter reading rose suddenly i.e. at the neutralisation point of HNO$_3$, the rate of bubbling was increased to allow the reading to become steady more quickly. Normally, it took about one hour to complete one titration.
2B. CONFIRMATION OF COMPLEX FORMATION
BY SPECTROPHOTOMETRIC MEASUREMENTS

One of the most spectacular effects of complex formation is the change of spectral properties. The reasons for light absorption by the complexes are as follows:

i) the excitation of the electrons of both the metal ions and the ligand is influenced by their interaction.

ii) the electrons of transition metal ions are easily excited and consequently absorbed in the visible region i.e. these ions give coloured compounds.

iii) the electronic systems of non-transition metal ions and of the ligands are more stable; excitation of the electrons requires greater energy, and thus these species absorb radiation in the ultraviolet range of spectrum.

iv) owing to interaction of the central metal ion and the ligand, a charge transfer from the ligand to metal ion may occur on irradiation; this phenomenon is the reason for the so called charge transfer spectra in the visible and near ultraviolet region.

Beer's Law

It is possible to ascertain the concentration of a given specie in solution if it absorbs light of a particular wavelength and obey Beer's Law.

This law can be regarded as an idealised behaviour of a specie strictly towards monochromatic radiation. The formation constant of a complex can be calculated by taking known amounts of a metal & a ligand and measuring the optical densities of the metal, ligand & metal-ligand mixture separately.

Selection Of Wavelength

It is first necessary to select the wavelength at which a complex specie present has a greater absorbance than either the free metal ion or the free ligand. For finding this absorption, spectra of a metal ion & the ligand are taken separately and compared with the spectra obtained after mixing the metal ion & ligand. One of the complex species absorb light predominantly at the wavelength selected.
B. Confirmation of complex formation by spectrophotometric measurements

2B. 1 Selection Of pH

For deciding the optimum pH for studying complex formation, a preliminary study of spectra of (i) the metal ion (ii) the ligand and (iii) the metal ion + ligand, is to be made over a large range of pH upto the pH of hydrolysis.

If more than one complex is formed at different pH conditions, their existence can be decided by this type of study. The pH, at which the absorption due to a particular complex specie is far greater than that of metal-ion or the ligand alone, is selected for the study of that specie. The pH is kept constant for a particular system as the complex formation is a function of pH. Similarly ionic strength is maintained constant throughout by adding an appropriate volume of 1.00M potassium nitrate. A constant pH can be maintained by using a proper buffer, provided the buffer does not interfere with the complex formation, and does not itself show any absorption at wavelength where complex specie shows maxium absorption.

For complexes which show different colours with a change in pH, a plot of optical density against the wavelength over a large range of pH gives a number of curves representing absorption spectra. The points at which the curves corresponding to a number of solutions having equal formal concentration of metal and ligand intercept are called the isobestic points. The number of complexes are, given by Vareille to be, equal to the number of isobestic points plus one. The method is applied by him to Ferric(III)-phenolate complexes. Same method is applied under the similar conditions by Narwade for Ferric(III)-sulphonic acid complexes.

2B. 2 The Composition Of The Complex

Before determination of stability constant of a given complex spectrophotometrically, it is essential to ascertain the number of the ligand groups attached to the metal ion. The composition of the complex can be determined by:

i) Job's method of continuous variation,
ii) method of mole ratio,
iii) method of slope ratio,
iv) method of isobestic point.

Job's Method Of Continuous Variation

Job's method consists of mixing equimolar solutions of metal and ligand in varying proportions in such a manner that the total concentration of the metal and the ligand is...
B. Confirmation of complex formation by spectrophotometric measurements

constant in the resulting mixtures. The absorbance of a series of mixtures is measured at a suitable wavelength. If x mole/litre of L are added to (1 - x) mole/litre of M and if \( C_1, C_2 \), and \( C_3 \) are the equilibrium concentrations in moles/litre of M, L and \( ML_n \) respectively, the following relations hold good:

\[
C_1 = \left\{ (1 - x) - C_3 \right\} \\
C_2 = \left\{ x - nC_3 \right\} \\
C_3 = C_1 \cdot C_2^n
\]

It can be seen that the number of ligand 'n' attached to metal ion can be easily determined from the position of the maximum or minimum in the graph. If the complex species are coloured and the metal ion and the ligand are colourless, the plots of optical density versus composition will give a maximum at the composition of the complex.

Job's method is ordinarily applicable to systems in which only one complex is present. Robert Gould and Vosburgh have, however, shown that even if a second equilibrium exists in addition to the main reaction, Job's condition is obeyed. The method is applicable to systems which obey Beer's Law.

2B.3 Calculation Of Stability Constants

The conditional stability constants of a complex can be determined from Job's curves. The two methods usually adopted are as follows:

From Extinction Coefficient Data:

Optical density of solution prepared by taking a large excess of ligand (metal to ligand ratio 1:25) can be considered as first approximation, to be due to the complex. Knowing the optical density, one may determine values for the extinction coefficient of the complex with the help of Beer's Law. From this value, it is possible to calculate the concentration of the complex in various mixtures and obtain the value of stability constant.

Method Of Corresponding Solutions:

A solution containing metal and ligand in 1:1 ratio can be diluted at constant ionic strength, until solutions are obtained having the same optical densities (correction for the absorption for the metal and ligand is made) as those at other ratios. In this manner pairs of
solutions may be prepared which have the same optical density (from the complex) and thus contain equal concentrations of the complex but different total concentrations of the metal and ligand.

If \( \bar{D}_1 \) & \( \bar{D}_2 \) are two such values which represent the concentrations \([ML]_1\) & \([ML]_2\) respectively obtained from the observed solutions (i) & (ii) and since \( \bar{D}_1 = \bar{D}_2 \) we may write \([ML]_1 = [ML]_2\) and the formation constant \(K^f\) for this reaction is given by:

\[
K^f = \left\{ \frac{[ML]_1}{[ML]_1 + [L]_1} \right\} = \left\{ \frac{[ML]_2}{[ML]_2 + [L]_2} \right\}
\]

Since

\([ML]_1 = [ML]_2 = [ML]\)

\[
K^f = \left\{ \frac{[ML]}{T_{M1} - [ML] T_{L1} - [ML]} \right\} = \left\{ \frac{[ML]}{T_{M2} - [ML] T_{L2} - [ML]} \right\}
\]

where \(T_{M1}\) & \(T_{L1}\) correspond to the concentrations of total metal & total ligand respectively in solution (i) and \(T_{M2}\) & \(T_{L2}\) correspond to the concentrations of total metal & total ligand, respectively in solution (ii). As the values of \(T_M\) and \(T_L\) are known, the above equation can be solved to obtain the value of \(K^f\).

The real stability constant \(K\) has been shown to be related to the conditional stability constant \(K^f\) by the following expression proposed by Banks and Singh:

\[
\log K = \log \left\{ \frac{[H^+] + [H^+]}{K_1 \cdot K_2} \right\} \cdot K^f
\]

In this expression \([H^+]\) represents the hydrogen ion concentration of the solution, \(K_1\) & \(K_2\) are the first and second dissociation constants of the ligand acids.

2B. 4 EXPERIMENTAL

2B.4.1 Reagents

The reagents used in the present investigation are of the same quality as that used in pH-metric technique.

2B. 4.2 Instruments

Spectrophotometer

The optical density of some peptides solutions and their metal-complexes have been measured by UV-VIS Spectrophotometer, Model 108, (Sistronics). The spectral range of the instrument is from 200 nm. to 900 nm. i.e. UV, Visible and Nearinfrared. Blue and red photocells...
B. Confirmation of complex formation by spectrophotometric measurements

are used above 360 nm and 580 nm, respectively. Both the cells are in circuit when the instrument is in operation and the change from one photocell to the other is effected by wavelength knob.

Pyrex glass cells of 1.00 cm. path length were used for the study of spectra in the 340 nm. to 750 nm. range. First, one of the cells was filled with water as a reference and the other was filled with experimental solution and the absorbance values were noted. The same cells were used for all the measurements.

2. Methods of determination of the stability constants of complexes
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