CHAPTER-II

PART-I
METHODS OF DETERMINATION OF PROTON-LIGAND AND METAL-LIGAND STABILITY CONSTANTS

PART-II
EXPERIMENTAL
a) POTENTIOMETRIC MEASUREMENT.
b) SPECTROPHOTOMETRIC MEASUREMENT.
CHAPTER-II

PART-I

METHODS OF DETERMINATION OF PROTON-LIGAND & METAL-LIGAND STABILITY CONSTANTS.

* General consideration.
* Stability constants determination.
* pH measurement in mixed solvent media.
* Limitation of Calvin-Bjerrum titration technique.
* Methods used for confirmation of complex formation and stability constants determination by spectro-photometric measurements.
  * Beer’s Law.
  * Job’s method of continuous variation.
  * Calculation of stability constants.

PART-II

EXPERIMENTAL

a) POTENTIOMETRIC TITRATION.
  * Reagents
  * Instrument
  * Maintenance of glass electrodes.
  * Inert atmosphere.

b) SPECTROPHOTOMETRIC MEASUREMENTS.
  * Instruments.
  * Reagents
  * Conductivity water.
  * References.
Methods of determination of proton-ligand stability constant by the use of potentiometric technique.

General Consideration

Equilibrium constants are calculated by the numerical and graphical developed methods. Irving and Rossotti\(^1\) have given a method for calculation of stability constants of complexes by potentiometry. Calvin & Bejerrum\(^2\) followed the general technique for potentiometric study of metal complexes.

The stepwise formation of the mononuclear of large variety of polycarboxylic acids, oximes, phenols etc studied by Martell\(^3\). Binary complexes can be described by a set of equilibrium constants. The concentration changes caused by a complex formation are reflected in the potential of well-chosen electrodes. For studying the complex equalibria, potentiometric measurements are frequently applied. An electrode, the potential of which is well defined function of the concentration of the ion to be determined and a reference electrode must be selected for potentiometric measurement. In fact, most of the recorded stability data is determined by potentiometric methods. It is a pre-requisite of the application of potentiometric methods that the electrode reaction must be reversible.

The concentration relation of the reactants and products in every reversible chemical reaction can be strictly determined by applying the law of mass action. The numerical values of the equalibrium constants depend on the coencentration scale applied.

According to Bjerrum\(^4\) and Martell etal\(^5\), the formation of complex \(ML_N\) is in general a stepwise process and one has to deal a series of equalibria of the type

\[
M + L \rightleftharpoons ML - - - - - - (a)
\]
\[ ML^+ + L \rightleftharpoons ML^2 \quad \text{(b)} \]
\[ ML^2^+ + L \rightleftharpoons ML^3 \quad \text{(c)} \]
\[ ML_{N-1}^+ + L \rightleftharpoons ML_N \quad \text{(d)} \]

( Neglected the charges on a metal ion )

Applying the law of mass action

\[ K = \frac{[ML]}{[M][L]} \quad \text{(e)} \]

Where \( K \) - Equilibrium constant

\( [M] \) - Concentration of the metal

\( [L] \) - Concentration of the ligand

\( [ML] \) - Concentration of the complex.

The information of a complex or a chelate \( ML_N \) in solution, takes place in steps and can be described by the following set of equilibrium constants.

\[ K_1 = \frac{[ML]}{[M][L]} \quad \text{(f)} \]
\[ K_2 = \frac{[ML_2]}{[ML_1][L]} \quad \text{(g)} \]
\[ K_3 = \frac{[ML_3]}{[ML_2][L]} \quad \text{(h)} \]
\[ K_N = \frac{[ML_N]}{[ML_{N-1}][L]} \quad \text{(i)} \]

These equilibrium constants characterize the stability of complexes and are usually called as stability constants. The law of mass action is strictly valid only when activities are used instead of concentrations, because the activity of species is equal to the product of its concentration and the activity coefficients. The complex is governed by \( N \) equilibrium constants, each defined as -

\[ k_{im} = a_{ML_i} / (a_{ML_{i-1}} a_L) \quad \text{(j)} \]
\[ k_{im} = k_1 r_{ML_{i-1}} r_L / r_{ML_i} \quad \text{(k)} \]

Where,

\( k_{im} \) - \( i \)th thermodynamic metal - ligand stability constant or formation constant.
a - activity

r - activity coefficient of the constituents

The overall or cumulative formation constants \( \beta \) is obtained by the product of individuals stability constants

\[
\beta_1 = k_1 = \frac{[ML]}{[M][L]} \quad \text{(l)}
\]

\[
\beta_2 = k_1 \times k_2 = \frac{[ML_2]}{([M][L]^2)} \quad \text{(m)}
\]

\[
\beta_N = k_1 \times k_2 \cdots k_N = \frac{[ML_N]}{([M][L]^N)} \quad \text{(n)}
\]

In general \( \beta_N = \prod_{i=1}^{N} k_i \)

A stoichiometric overall stability constant \( \beta_i \) for a given system is defined as

\[
\beta_i = \frac{[ML_i]}{([M][L]^i)} \quad \text{(m)}
\]

\[
= \prod_{i=1}^{N} k_i \quad \text{(m)}
\]

In a similar fashion the equations (a),(b),(c) for ligand equilibria can be written in the following general form.

\[
LH_{i-1} + H \rightleftharpoons LH \quad \text{--------------------------- (o)}
\]

Where \( LH_i \) is the ligand acid. The proton - ligand stability constant for such a reaction is given

\[
K_{im}^{H} = \frac{a_{H}L_{i}}{(a_{L}H_{i-1} \times a_{H})} \quad \text{------------------ (p)}
\]

Where \( K_{im}^{H} \) is called as the thermodynamic proton - ligand stability constant and is reciprocal of the thermodynamic dissociation constant of acid. \( LH_i \) dissociating as

\[
LH_i \rightleftharpoons LH_{i-1} + H
\]

The \( pk_i \) value is given by

\[
pk_i = a_{L}H_{i-1} \times a_{H} \div a_{L}H_i
\]

For monobasic ligand
\[ pk_i H = pk_i \text{ in magnitude} \]

While for polybasic acids \((H_n A)\),

\[ pk_1 = pk_n H, pk_2 = pk_{n-1} \text{H}, \]
\[ pk_n = pk_H \]

The degree of formation or ligand number expressed by Bjerrum as

\[ \bar{n} = \frac{\sum_{i=0}^{N} ([ML])}{\sum_{i=0}^{N} ([ML])} \]

Substituting the values of MLi from equation (I) applied to equalibria (a) to (d) and eliminating M we have

\[ \bar{n} = \frac{k_i[L]+2k_1xk_2[L]^2+\ldots\cdot NK_1K_2\ldots\cdot K_N[L]^N}{1+k_1[L]+k_1xk_2[L]^2+\ldots\cdot k_1xk_2\ldots\cdot K_N[L]^N} \]

Which also can be written by using equation (N) as

\[ \bar{n} = \frac{\sum_{i=0}^{N} \beta_i [L]^2}{\sum_{i=0}^{N} \beta_i [L]^2} \]

as follow.

\[ \bar{n}_A = \frac{\sum_{i=0}^{j} \beta_i [H]^i}{\sum_{i=0}^{j} \beta_i [H]^i} \]

Where \( \bar{n}_A \) - mean number of protons bound per non-complex bound ligand molecule

The total concentration \( T_m \) of the metal ion M is the sum of concentration of the different species containing is as:

\[ T_m = [M] + [ML] + \ldots + [ML_N] \]

= \[ \sum_{i=0}^{N} [ML_i] \]

Similarly, the total concentration of ligand is the weighed sum of concentrations of the species containing it as

\[ T_L = [L] + [ML] + 2[ML_2] + \ldots + N[ML_N] \]

= \[ [L] + \sum_{i=0}^{N} i[ML_i] \]
The total concentrations of Tm and TL are given by expression

\[ T_m = [M] \sum_{i=0}^{N} \beta_i [L]^i \]

\[ T_L = [L] + [M] \sum_{i=1}^{N} i\beta_i [L]^i \]

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

\[ \bar{n} = \frac{[ML] + 2[ML_2] + \cdots + N[ML_N]}{[M] + [ML] + [ML_2] + \cdots + [ML_N]} \]

\[ \bar{n} = \frac{T_L - [L]}{T_m} \]

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

**Stability constants determination**

From the experimental data, stability constants can be determined by following three steps -

(i) The construction of the formation curve of the system. This is expressed as a plot of \( \bar{n} \) against \( P^t = \log(1/[L]) \).

(ii) By solving the formation curve, the values of \( k_1, k_2 \) values are calculated.

(iii) The conversion of the stoichiometric constants in the thermodynamic function.

Rossotti & Rossotti\(^6\), Wilkins and Lewis\(^7\), Irving and Rossotti\(^8\), Hearson and Gilbert\(^9\) have described general methods for the determination of stability constants. These authors made use of the potentiometric titration technique which is first used by Calvin and Melchior\(^10\). Now this technique is known as Calvin - Bjerrum titration.
technique. In present investigation, the method of Irving and Rossotti has been employed.

The experimental procedure involves titration of

(I) Nitric acid (A)

(II) Nitric acid + Ligand peptide (A+L)

(III) Nitric acid + Ligand peptide + Metal salt (A+L+M)

With standard solution of NaOH. By the addition of calculated amount of 1M KNO₃ solution, ionic strength of each solution is maintained constant at 0.1M.

Calculations of $\bar{n}_A$, n and pH$

The values $\bar{n}_A$, n are calculated with the help of experimental data obtained from the titration curves. Proton-ligand formation number ($\bar{n}_A$) for different pH values can be obtained by applying the following equation.

\[
\bar{n}_A = \gamma \times \frac{(N+E^0)(V_2-V_1)}{T_L^0(V_0+V_1)}
\]

Where

$V_0$ - Initial volume of the solution.

$E^0$ - Concentrations of the mineral acid

$T_L^0$ - Concentrations of the ligand.

$V_1$ - Volume of alkali required during acid titration.

$V_2$ - Volume of alkali required during ligand titration.

$\gamma$ - Number of replaceable hydrogen ion.

N - Normality of NaOH.

Similarly, the metal-ligand formation number ($\bar{n}$), can be calculated by using the following expression.

\[
\bar{n} = \frac{(N+E^0)(V_3-V_2)}{T_m^0(V_0+V_2)}
\]
Where

\( Tm^0 \) - Total concentrations.

\( V_3 \) - Volume of alkali required to obtained the same \( p^H \) as the ligand and acid titration.

The value of \( P^L = - \log L \) can be calculated with the help of following expression.

\[
P^L = \log \left\{ \frac{[H^+]/Ka}{Tl^0 - Tm^0 \times \bar{n}} \right\} \times \left( \frac{V_0 + V_3}{V_0} \right)
\]

The equation express \( \bar{n} \) as a function of \( P^L \) which is represented as the formation curve. The number of complexes formed in the reaction can be deducted from the formation curve and the values of stability constants can be determined.

Stiochiometric stability constants are obtainrd by different workers using different mathematical techniques. 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 filling procedure.

Irving and Rossotti have calculated \( k_1 \) and \( k_2 \) from the formation curve with the help of following methods.

(1) Solution of simultaneous equation.
(2) Successive approximation method.
(3) Schwarzenbach's graphical method.
(4) Interpolation at various \( \bar{n} \) values.
(5) Interpolation of half \( \bar{n} \) values.
(6) Method of least - square
(7) Use of mid point slope.

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

**pH measurement in mixed solvent media**

Irving and Rossotti have taken into consideration the use of organic solvent - water mixtures, especially, dioxane - water mixture, which have been employed by several workers to bring the water insoluble metal chelates into a homogenous 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 relation between glass electrodes reading B in dioxane-water medium and stoichiometric hydrogen ion concentration of the same has been studied by Van Uitert and workers in mixtures of different compositions at different ionic strength and they have shown that -

\[- \log[H^+] = B + \log \frac{1}{\gamma} + \log U^0_H\]

Where \(\gamma\) is the activity coefficient of the hydrogen ions in the solvent mixtures under consideration at the same temperature and ionic strength. \(U^0_H\) is the correction factor at zero ionic strength, depend only on composition of the solvent mixtures and temperature. The value of \(U^0_H\) in water is unity. The use of this relation has been extended to pure ethanol.

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

(I) Solubility of an electrolyte decreases when the percentage of organic solvent in the mixed medium becomes high.

(II) Glass electrode potential changes in going from an aqueous to mixed solvent.
(III) Liquid junction potential of uncertain magnitude which increases with in the increase in the percentage of the organic component may vitiate the result appreciably.

(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\(^\text{14}\). The constants are therefore determined at various low values of ionic strength and extra plotted to \(\mu=0.1\text{M}\) (where \(\mu=\text{ionic strength of solution}\)).

**Important precautions in the determination of reliable & accurate values of stability constants.**

In presence of hydrolysis, metal ion releases protons and the stability constants obtained in the \(p^H\) range in which hydrolysis occurs will be much lower than the true values. Therefore measurement should be restricted to the range of \(p^H\) where hydrolysis is minimum. Hydrolysis curve in a medium of 50% dioxane-water (v/v) have been obtained by Freiser, Charles and Johnation\(^\text{15}\) by titrating the metal salt solutions with sodium hydroxide and have concluded that for these metal ions calculations made in the \(p^H\) range 3 to 6 are least vitiated by metal ion hydrolysis. In order to lower the pH range of complex formation and for minimising the effect of hydrolysis, several workers suggested to use of higher ligand to metal ratio. Charles and Freiser used a ratio as high as 100:1.

The anions other than ligand ions can not form complexes with the metal. Schwarenbach\(^\text{16}\) has given special attention to this point in his stability measurements.
For avoiding anion complexing, usually dilute solutions of potassium nitrates are used. Metal perchlorates can also be used.

**Limitation of Calvin - Bjerrum titration technique**

Most widely, this method is used for determination of metal chelate stability, becomes non applicable in the presence of very strong or very weak complexes. In the former case the concentration of the ligand required for formation of the complex is so small that the complex formation is completed even at very low pH values irrespective of whether the ligand is a free base or the anion of an acid. The effect of the protons released on the pH values of the solution under this conditions would be too small to be detected on any potentiometer. Such systems have been studied recently by employing the method which uses the principles of

(a) Replacement of one metal by another and

(b) Replacement of one ligand by another.

**Methods used for confirmation of complex formation and stability constants determination by spectrophotometric measurements.**

Considerable research work is going on all over the world using spectrophotometric technique. The basic principle of this technique deals with the study of the measurement of interactions between electrons of substance and radiation energy. This technique is an analytical method which is commonly used in chemical laboratory for estimating the concentration of metal ion in liquid solution.

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

2) The excitation of electrons of both the metal ion and ligand is influenced by their interaction.

3) The electron systems of non transition metal ions and of the ligands are much more stable; excitation of the species absorb radiation in the ultraviolet range of the spectra.

4) Owing to interaction of the central metal ion and the ligand, a charge transfer from the ligand to metal ion may occur on irradiation. The 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 species in solution if it absorbs light of a particular wavelength and obeys Beer's law. This law can be regarded as an idealized behaviour of the species toward strictly monochromatic radiation. The formation constant of a complex can be calculated by taking known amount of metal and ligand & measuring the optical densities of the metal ligand and the metal-ligand mixture separately.

**Selection of wavelength**

It is most necessary to select a wavelength at which the complex species present has a greater absorption than either the free metal ion or the free ligand.

For finding these absorption, spectra of the metal ion and the ligand are taken separately and compared with the spectra obtained after mixing the metal ion and ligand. It is also important that at
a wavelength selected, only one complex species should predominately absorb light. As KNO₃ ions have no absorption in the spectral range 250 to 1800 nm. KNO₃ solution will show absorption of light by metal ions alone.

**Selection of pH**

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

By this type of study if more than one complex is formed at different pH conditions, their existence can be decided. The pH at which the absorption due to a particular complex species is far greater than that of metal ion or ligand alone is then selected for the study of species. pH is to be kept constant for a particular system as the complex formation is a function of pH.

By addition of an appropriate volume of 1M KNO₃, ionic strength is maintained constant. 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 species show maximum absorption. The complexes which show different colour 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 point at which the curves corresponding to a large number of solutions having equal formal concentration of metal and ligand intercept are called the isobestic points.
According to Varillel, the number of complexes are to be equal to the number of isobestic points plus one. This method is applied by him to Fe(III) phenolate complexes. Under the similar conditions, same method is applied by Narwade et al. for Fe(III) sulphonic acid complexes. Sawwalakhe for Fe(III) diketone complexes and Mandakmare for UO2(II) coumarin complexes.

**The Composition of the complexes**

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

(i) Job's method of continuous variation

(ii) The slope ratio method

(iii) The mole ratio method

The details of job's method are given as this particular method is used in the present work.

**Job's method of continuous variation.**

Job's method consists of mixing equinormal solutions of metal and ligand in varying proportions in such a manner that the total concentration of the metal plus the ligand is constant in the resulting mixtures. The absorbance of a series of mixture 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 $M_{LN}$ respectively, the following relations hold good.
\[ \begin{align*}
  c_1 &= (1-x)c_3 \\
  c_2 &= (x-nc_3) \\
  c_3 &= c_1 \times c_2^n
\end{align*} \]

From the position of the maximum or minimum in the graph, the number of ligand "n" attached to a metal ion can be easily determined. 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.

The systems in which only one complex is present Job's method is applicable to such a system: Gould and Vosburg\textsuperscript{21} 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.

**Calculation of stability constants**

From Job's curve, the stability constants of a complex can be determined. Usually, following two methods are adopted.

1) **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. The concentration of the complex may be taken equal to that of the metal ions as all the metal ions are presented in the form of a 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.
2) **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. In this manner pairs of solution may be prepared which have the same optical density and this contain equal concentrations of the complex but different total concentrations of the metal and ligand.

If \( \bar{D}_1 \) and \( \bar{D}_2 \) are two such values which represent the concentrations \([ML]_1\) and \([ML]_2\) respectively obtained from the observed solutions (I) and (II). Since \( \bar{D}_1 = \bar{D}_2 \), we may write \([ML]_1 = [ML]_2\) and the formation constant \(K\) for this reaction is given by:

\[
K = \frac{[ML]_1}{[M][L]_1} = \frac{[ML]_2}{([M][L]_2)}
\]

Further, since \([ML]_1 = [ML]_2 = [ML]\)

\[
K = \frac{[ML]}{(TM_1 - [ML]_1TL_1 - [ML])} = \frac{[ML]}{(TM_2 - [ML]_2TL_2 - [ML])}
\]

Where

- \(TM_1\) ------ concentration of total metal in solution (I)
- \(TL_1\) ------ concentration of total ligand in solution (I)
- \(TM_2\) ------ concentration of total metal in solution (II)
- \(TL_2\) ------ concentration of total ligand in solution (II)

As the values of TM and TL are known, the above equation can be solved to obtain the value of \(K\).

The real stability constant \(K\) has been shown to be related to the apparent or conditional stability constant \(K\) by the following expression proposed by Franks and Singh.
\[
\log k = \log \left\{ \frac{[H^+]^2}{k_1 k_2} + \frac{[H^+]}{k_2} \right\} \times K
\]

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

Kolekar et al.\textsuperscript{22} have investigated extractive spectrophotometric determination of selenium(IV) using pyridine-2-Thiol from alloys and pharmaceutical sample. The study of synthesis and characterization of some complexes of Co(II) and Ni(II) with Dimethyl pyridine has been done by Renu Sachar et al.\textsuperscript{23}.
Part II

Experimental

A) Potentiometric titrations

Double distilled water free from carbon dioxide was used. Distilled water obtained in a steel container was again redistilled over alkaline permagnate in a glass quick fit sep’up and was used always a fresh. Before using it, nitrogen gas was bubbled through it. The pH of this water was found to be 6.8

Metal perchlorates

To avoid the possibility of complex formation of the metal ion with anions, transition metal ions were used in the form of their perchlorates. The metal perchlorates was prepared from the metal nitrate (Anala R quality) by the standard procedure.\(^\text{15}\)

(The metal perchlorate were prepared by dissolving the corresponding metal nitrate or oxide in a known volume of standard perchloric acid which was prepared by diluting 66% acid supplied by E-Merck. To a small volume of (5ml) of this acid solutions, the transition metal nitrates were added in a small quantity each time and the solution was heated gently until no more oxide or nitrate dissolved)

The solution of metal perchlorate was made up to known volume by adding distilled water (0.01M). The concentrations of metal ions in solution were estimated by titrating them against sodium-EDTA solution by procedure of Schwarzenbach.\(^\text{16}\)

Copper nitrate, Nickel nitrate & cobalt nitrate

Copper nitrate, Nickel nitrate & cobalt nitrate (Anala R quality / Romali quality) were used. The solutions were prepared in standard perchloric acid. The metal content in the solution was estimated gravimetrically.\(^\text{24}\)
**Potassium nitrate**

Potassium nitrate (E-merck) was distilled in carbon dioxide free distilled water. 0.1M potassium nitrate solution was used in present investigation.

**Sodium hydroxide**

The solution of sodium hydroxide (E-merck, Anala R quality) 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 pyrex glass crucible and kept in a pyrex vessel free from carbon dioxide and was used as the titrant for the pH titrations. Before starting an experiment, the solution was standardized by titrating it with standard oxalic acid solution (Anala R) before starting an experiment.

**Perchloric acid**

Perchloric acid of Riedel (Germany / Quilgen) was used for preparation of stock solution. Its exact normality was calculated by titrating against standard sodium hydroxide.

**Dioxane**

Dioxane (B.D.H Anala R / E-merck) was purified by the method described by vogel\(^2\). One litre of Dioxane was refluxed with 14 ml of concentrated Hydrochloric acid and 100ml of distilled water for about 9 to 10 hours, while a slow stream of nitrogen, freed from traces of oxygen by passing through alkaline paragallol solution, was bubbled through it. The mixture which was pale yellow in colour was allowed to cool and KOH (E-merck) pallets were added with shaking till some were left undissolved. The clear colourless supertant dioxane was separated from the aqueous layer and then transfered to another
container to stand over KOH pallets for 24 hours. If any water separated, it was removed and the dioxane was allowed to remain over fresh KOH pallets for some more time. It was then refluxed over excess sodium, in the form of wire for 9 to 10 hours till the reaction ceased and sodium remained bright in appearance. Fresh clean sodium wire was introduced in the solution and the liquid was distilled at constant temperature to 90°C at the atmospheric pressure (72 cm). The distillate was collected in a receiver fitted with CaCl₂ guard tube. The pure liquid was stored out of contact with air.

**Amino acids/ peptides**

The solutions of the ligands (Amino acids / peptides) of 0.01 M were prepared by dissolving requisite quantities in pure distilled water. All these solutions of ligands were always used a fresh in present investigation.
INSTRUMENTS

**Measurement of pH and pH meter.**

All the $p^H$ measurements and titrations were carried out with Elico-LI-12T pH meter (accuracy ± 0.01) with a glass and calomel electrode assembly. The instrument could read pH in the range of 0 -14.0 in steps of 0.005. The $p^H$ meter has an inbuilt internal electronic voltage stabilizer for ±10% fluctuations in voltage supply with temperature compensator covering the range from 0 -100°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 $p^H$ meter, the electrodes were washed with distilled water and dried with filter paper. When the instrument registered a steady value for at least one minute, then only the readings were recorded.

The standardization of $p^H$ meter was done, before each titration by a buffer solution of potassium hydrogen phthalate in acidic range (N/20 with a $p^H$ value of 4.01 at 30°C) and checked by titrating 0.025 M HClO$_4$ against NaOH. The $p^H$ values measured and calculated from the analytical concentration of HClO$_4$ and activity coefficient were agreed within ± 0.02 $p^H$ unit.

$p^H$ meter was standardized in alkaline range using 0.01M solution of borax (Na$_2$B$_4$O$_7$·10H$_2$O). Appropriate $p^H$ of the buffer solution was considered at different temperatures.

Pyrex quality all glass wares used throughout the experiments. 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 describe by Vogel 24.
The micropipette were similarly calibrated and other apparatus like measuring flask 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 burett.

**Maintenance of glass electrodes.**

The glass electrodes was always kept in a distilled water when not in use. Since it was exposed to alkali during the titration, it was washed with 2NHCl and then with distilled water after each titration. In order to prevent glass electrode from developing any asymmetry potential, it was occasionally kept in 2N HCL for a period of 12 hours and then repeatedly washed with distilled water. For smooth working of a glass electrodes, all necessary precautions are taken. These were in accordance to suggestions of Butes\textsuperscript{25}, Albert and Serjeant\textsuperscript{26}.

In order to prevent the formation of chloro complexes, the calomel electrode was not dipped directly in the experimental vessel but it was kept in saturated solution of KNO\textsubscript{3} in a beaker and then was connected to the titrating vessel through a salt bridge formed by setting a mixture of a saturated KNO\textsubscript{3} and agar-agar (4%). The presence of saturated KCL solution in calomel electrode was checked from time to time.

**Calibration of pH meter scale.**

It was necessary to calibrate the pH meter scale over the entire range as the pH of a solution in the titration vessel could vary continuously between 2.0 to 11.0 during titration. A calibration check was made at 4.01 and 9.11 only. Standard buffer solutions were prepared by dissolving cambridge buffer tablet 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 the pH

of the buffer solution of temperature 27°C at which the titrations were
carried out in the present investigation; was calculated from the follow­
ing formula supplied by the manufactures.

1) \( \text{pH}_{15°C} = \text{pH}_{15°C} - 0.0085 \times (t-15) \) for buffer solution of pH 9.11
2) \( \text{pH}_{15°C} = \text{pH}_{15°C} - 0.5 \times (t-15)^2 / 100 \) for buffer solution of pH 4.01

After warming up the instrument, freshly prepared buffer so­
lution was taken in the titration vessel and the electrodes were im­
mersed in it. The temperature compensator was set upto the tempera­
ture of the buffer and the range switch was turned to obtain the ap­
proximate range. The standardization control was used to set the me­
ter needle to the pH of the buffer. The meter was standardized by both
the buffer solutions. The buffer standardization was done once before
starting the potentiometric titration and then again after completion of
series of titrations in a day.

**Experimental vessel**

Pyrex glass beaker(capacity 100ml) was used as an experi­
mental vessel. A side tube of which the height is more than beaker,
was attached at the lower end of beaker. This arrangement permitted
the bubbling of nitrogen gas through the solution in experimental vessel.
The mouth of experimental vessel was covered with a rubber bung in
which an arrangement was made for inserting the glass electrode, one
limb of the salt bridge and the stem of the microburette. The rubber
stopper had another hole, through which a glass stirrer could be in­
serted for stirring the titration solution. This hole also served as an exit
for nitrogen. The glass stirrer was constructed by connecting a glass
rod, having a glass pedal of its lower end, to the axial steel rod attach­
ed to a 9 volt D.C. motor. The speed of glass stirrer was controlled by feeding the current to the motor through a stepdown transformer attached in an eliminator.

**Inert-atmosphere**

In an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrodes in order to prevent atmospheric oxidation, potentiometric titration were carried out. The nitrogen gas served to expel dissolved oxygen and carbon dioxide from the solution and also stir it. Nitrogen gas supplied by Indian Oxygen and Acetylene company Ltd; Bombay; contained a small percentage of oxygen (0.5%) which was removed by passing the gas through alkaline pyragallol (15 gms in 100ml of 50% KOH solution). The purified nitrogen was presaturated with 0.1N sodium perchlorate solution, before passing it through the test solution.

**Calvin-Bjerrum titration**

The experimental procedure involved potentiometric titration of solutions of -

1) Free HNO₃ (A)

2) Free HNO₃ + ligand (A+L)

3) Free HNO₃ + ligand + metalion (A+L+M) against standard NaOH solution

The titrations were carried out in a 100ml pyrex glass beaker in a water bath maintained at a constant temperature 27°C±0.1°C. 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 reading were taken only after the gas bubbling and magnetic stirring were stoped. At the point where the meter reading rose suddenly i.e.- at the neutralization point of HNO₃.
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.

b) **Spectrophotometric measurements**

The same quality of reagent used in this present investigation as that was used in potentiometry.

**Instruments**

**Spectrophotometer**

By using UV-visible spectrophotometer, model 108, systronic (accuracy = 0.005) used for the measurement of optical density of some amino acid/peptides.

The spectral range of the instrument was from 200 nm° to 900 nm° i.e. UV, visible and near infra-red. Blue and red photocells are used above 340 nm° and red 610 nm° respectively. Both the cells are in circuit when the instrument is in operation and the change from one photocell to other is effected by the wave length knob.

One cm path length pyrex glass cells were used for the study of spectra in the 340 nm° to 900 nm° range. First, one of the cell 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.

**pH-meter**

The pH meter (Ellico - LI - 12 T ) was used for the measurements of the PH of the experimental solutions.

**Conductivity water**

The conductivity water was prepared by redistillation of a good distilled water with the addition of alkaline potassium permanganate in all glass apparatus. It was always used a fresh. The conductivity of such a water was 10⁻⁶ mhos.
**REFERENCES**


2) Bjerrum. J. and Calvin,M. : "Metal Amine formation in Aqueous solution", P-Haas and son, Copenhagen (1941), reprinted in 1957


4) Bjerrum, J. : "Metal Amine formation in aquious solutions".P-Haas and Son Copenhagen(1941), Reprinted in 1957


13) Lahiri, S.C and Aditya, S.  
14) Robinowitch, E and Stockmayer, W-H  
15) Freiser, H, Charles, R.G and Johnston, W.D.  
16) Schwarzenbatch, G.  
17) Vareille, L  
18) Narwade, M.L.  
19) Sawwalakhe P.D. and Narwade M.L.  
20) Mandakemare, A.U. and M.L. Narwade  
21) Robert, Gloud, K. and Vosburg, W.C  
22) Kolekar, G.B. and Anse, M.A.  
23) Renusachar, Anju, Anand and Yash Paul  
24) Vogel, A.I.  

: J. Am. Chem. Soc., 64, 335 (1942)  
: "Complexometric titrations" Methuen and co. Ltd., Landon pp-69, 76, 79 and 82  
: J. Am Chem. Soc., 64, 1630 (1942)  