CHAPTER V

SUMMARY
Summary

The physico-chemical study on metal-vitamin complexes has been the subject matter of the present thesis. The first chapter deals with the discussion on role of the vitamins in biochemical processes specially in presence of various metal ions. The complex formation between the vitamins and metal ions affects the reactivity of the vitamins and other biologically active organic compounds. The survey of literature reveals that the main areas of research on metal-vitamin complexes are: (1) preparation of metal-vitamin complexes, (2) the use of the vitamins in quantitative estimation of metal ions, (3) kinetic studies on metal-vitamin complexes and (4) the physico-chemical studies on metal-vitamin complex systems to evaluate the thermodynamic stability constants. The absence of adequate data on thermodynamic stabilities necessitated a systematic physico-chemical investigation of various metal-vitamin systems. The methods applied for such studies have been based on potentiometry, spectrophotometry and solubility techniques.
The second chapter deals with the determination of the stabilities of simple complexes of ascorbic acid, para-aminobenzoic acid, folic acid and glutamic acid with various metal ions in aqueous solutions using pH titration technique. The titrations were carried out at 25°C in presence of known excess of an electrolyte under the inert atmosphere of nitrogen. Precautions against aerial oxidation were taken especially for ascorbic acid titrations, where the weighed amount of the solid vitamin was added directly to the reaction vessel in rigorous absence of air. Folic acid is found to be soluble in alkali and thus, the titrant used for its titrations was a standard solution of an acid. From the volume of titrant and pH data, the titration curves were plotted and the functions i.e. $\bar{n}_H$, $\bar{n}$ and $p_L$ calculated. From the plot of $\bar{n}$ versus $p_L$ Log $K_{ML}$ was evaluated by Bjerrum's half $\bar{n}$ method. Where necessary the values of stability constants were also calculated by graphical methods. The significant features of these studies are:

1. The stability of copper (II)-ascorbic acid complexes is not obtainable because of reduction of copper (II) to copper (I) by ascorbic acid.
2. For the $d^5-d^{10}$ ions of first transition series, iron(III) and iron (II) form the strongest complexes.

3. In the cases of other metal ions, beryllium (II) and uranium (VI) complexes (excepting para-aminobenzoic acid complexes) exhibit greater stability than the other complexes.

The order of stabilities of different metal ions of various metal-vitamin systems is as follows:

**Metal-ascorbic acid system**: 

$\text{Be}^{2+} > \text{Pb}^{2+} > \text{Fe}^{2+} > \text{Co}^{2+} = \text{Ni}^{2+} > \text{Mn}^{2+}$

**Metal-para-aminobenzoic acid system**: 

$\text{Fe}^{3+} > \text{Zn}^{2+} > \text{Th}^{4+} > \text{Zr}^{4+} > \text{Be}^{2+} > \text{UO}_2^{2+}$

**Metal-folic acid system**: 

$\text{Fe}^{2+} > \text{Zr}^{4+} > \text{Be}^{2+} > \text{Mn}^{2+} > \text{UO}_2^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+}$

**Metal-glutamic acid system**: 

$\text{UO}_2^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$
The determination of stabilities of some mixed ligand complexes including ascorbic and glutamic acids are described in chapter third. The individual metal-vitamin/amino acid systems were studied in presence of another ligand which is either 2,2'-dipyridyl or ethylenediamine. Bjerrum-Calvin's titration technique as modified by Irving-Rossotti was employed and the stability constants evaluated at 25°C in presence of 0.1M electrolyte. In these mixed ligand systems, 2,2'-dipyridyl was considered to be the primary ligand, whereas ethylenediamine was taken as the secondary ligand. The values of the stability constants for simple and the mixed ligand complexes are given in a graphical form in Fig. 45 (C, D) where the \( \log K \) is plotted against atomic number of the metal ions studied.

From the study of Fig. 45 (C, D) the following conclusions are drawn:

1. Excepting iron (II)-ascorbic acid-ethylenediamine system the other M (II)-ascorbic acid-ethylenediamine/2,2'-dipyridyl systems having nitrogen as donor sites, exhibit greater stability than the simple aquated metal-ligand complexes.
2. The stability increments are particularly large for iron (II)-2,2'-dipyridyl-ascorbic acid complex and copper (II)-mixed ligand complexes with ethylenediamine and 2,2'-dipyridyl. The simple copper(II)-ascorbic acid system has very low stability and is susceptible to quick disproportionation.

3. The metal-glutamic acid-ethylenediamine/2,2'-dipyridyl complexes in general have lower stabilities than the M(II)-ascorbic acid-ethylenediamine/2,2'-dipyridyl complexes. This is probably due to lower pKₐ for glutamic acid as compared to ascorbic acid. In fact, the M(II)-glutamic acid-ethylenediamine systems have slightly higher stability than the simple M(II)-glutamic acid systems, whereas the M(II)-2,2'-dipyridyl-glutamic acid complexes have slightly lower stabilities as compared to simple metal-ligand systems (with the exception of iron (II)-2,2'-dipyridyl-glutamic acid which has higher value for stability constant). The lowering of stability constants for copper (II)-2,2'-dipyridyl-glutamic acid complex is probably due to steric hindrance caused in the complex by the bulkier 2,2'-dipyridyl molecule.
4. The stability increments for mixed ligand complexes of ascorbic acid are much higher than the corresponding glutamic acid complexes. This is basically due to the fact that ascorbic acid has only O as donor site, whereas glutamic acid has both O as well as N donors. Consequently, the presence of second ligand having nitrogen donor causes greater stability difference for the mixed ligand complexes of ascorbic acid as compared to that of glutamic acid.

The fourth chapter incorporates the spectrophotometric studies on copper (II)-ligand, mixed ligand complexes, preparation, analysis and spectrophotometric study on copper (II)-thiamine hydrochloride complex and solubility studies on silver (I) and copper (II)-nicotinates. Copper (II)-glutamic acid system was studied by Job's continuous variation method and limiting logarithmic method. The value of stability constant, Log $K_2$, thus determined, tallies well with the value evaluated by pH titration technique. The electronic absorption spectra of various copper (II)-mixed ligand systems were studied at pH 5.6 and 10.0 for the reaction mixtures containing the
reactants in required proportions. From the results on electronic absorption spectra, it is observed that the progressive substitution of water by N of ethylenediamine or 2,2' -dipyridyl causes the shift of \( \lambda_{\text{max}} \) from higher wave length to lower wave length. The probable complex species and their \( \lambda_{\text{max}} \) are as given below:
The copper(II)-ascorbic acid-ethylenediamine system is found to be very unstable in absence of nitrogen and the reaction mixtures precipitate cuprous oxide immediately. For copper (II)-ascorbic acid-2,2'-dipyridyl system the oxidation is pH dependant. At pH 10.0 the oxidation of ascorbic acid is immediate and at pH 2.5, it is very slow. At pH 5.6, copper (II)-ascorbic acid-2,2'-dipyridyl appears to be formed and the absorption curve exhibits a \( \lambda_{\text{max}} \) at 660 m\( \mu \). The complex slowly breaks at room temperature to give copper (I) and dehydroascorbic acid.

The copper (II)-thiamine hydrochloride complex was prepared by taking the stoichiometric ratio of copper(II) chloride and the vitamin in methanol. The yellow crystalline complex so obtained was analyzed for its metal content and the composition of the complex is found to be

\[ \text{CuCl}_2 \cdot (\text{C}_{18}\text{H}_{12}\text{Cl}_2\text{N}_4\text{O}_4\text{S})_2. \]

It can be concluded, that the yellow coloured Cu(II)L\text{2} complex isolated from methanol is probably four coordinate (Planar or tetrahedral) and the \( \lambda_{\text{max}} \) at 380 m\( \mu \) is probably a charge transfer type indicating the coordination through chloride ions. The
spectrophotometric studies of copper (II)-thiamine hydrochloride system in aqueous solutions using Job's continuous variation method indicate the formation of (1:2) as well as (1:4) complexes. The solubility technique was used to determine the solubility product of precipitated silver (I) and copper (II)-nicotinates at a temperature of 25°C and ionic strength of 0.2M(KNO₃) in an aqueous solution. These insoluble metal-nicotinates were isolated, dried and analyzed for their metal content. The analysis showed that the precipitates had the composition (C₅H₄CO₂⁻Ag) and (C₅H₄CO₂⁻)₂Cu for silver (I) and copper (II)-nicotinates, respectively. The average of solubility product for

\[ [C₅H₄CO₂⁻] x[Ag⁺] \text{ and } [C₅H₄CO₂⁻] x[Cu²⁺] \text{ at } 25°C \] is 6.78 x 10⁻⁶ and 5.32 x 10⁻⁹, respectively.

The variation of stability with electronegativity for d⁵–d⁹ ions

The plot of stability constant values (Log K₁) for the first row d⁵–d⁹ ions against electronegativity of the respective metal ions is given in Fig. 45(A,B). A perusal of Fig. 45 indicates that generally the value of Log K₁ increases with the increase in electronegative character, the only exception being iron (II) whose stability is found to be much greater.
FIG. 1A

FIG. 1B

VOLUME OF KOH (0.23 M) (ml)

VOLUME OF KOH (0.24 M) (ml)

PH-TITRATION CURVES FOR ASCORBIC ACID-METAL ION SYSTEMS.

NICKEL (II)

MANGANESE (II)

LIGAND (AS2H2)

ACID
FIG. 2. pH-TITRATION CURVES FOR ASCORBIC ACID-METAL ION SYSTEMS.
FIG. 4. PH-TITRATION CURVES FOR PARA-AMINOBENZOIC ACID-METAL ION SYSTEMS.

- ZIRCONIUM(IV) ligand $CrH_2$
- LIGAND $CrH_2$
- Acids

Vol. of KOH (0.1557 M) (ml)

Vol. of KOH (0.235 M) (ml)

Vol. of KOH (0.127 M) (ml)

Vol. of KOH (0.12 M) (ml)

Vol. of KOH (0.08 M) (ml)

Vol. of KOH (0.08 M) (ml)

Vol. of KOH (0.04 M) (ml)

Vol. of KOH (0.04 M) (ml)

Vol. of KOH (0.02 M) (ml)

Vol. of KOH (0.02 M) (ml)

Vol. of KOH (0.01 M) (ml)

Vol. of KOH (0.01 M) (ml)

Vol. of KOH (0.005 M) (ml)

Vol. of KOH (0.005 M) (ml)
FIG. 5

PH-TITRATION CURVES FOR PARA-AMINOACID-METAL ION SYSTEMS.

- ZINC(II)
- BERYLLIUM(II)
- LIGAND (PabH2)
- ACID

Vol. of KOH (0.1551 M) (ml)

pH

FIG. 5(A)

FIG. 5(B)
FIG. 6 (A) FIG. 6 (B)

$\text{PH}$

VOLUME OF KOH (0.1536 M) (mL)

$\Delta$ URANIUM($\text{UO}_2^{2+}$)
○ LIGAND ($\text{PabH}_2$)
△ ACID

$\Delta$ THORIUM(IV)
○ LIGAND ($\text{PabH}_2$)
△ ACID

FIG. 6. $\text{pH}-\text{TITRATION CURVES FOR PARA-AMINOBENZOIC ACID-METAL ION SYSTEMS.}$
FIG. 7. PH-TITRATION CURVES FOR FOLIC ACID-METAL ION SYSTEMS.
FIG. 8. PH-TITRATION CURVES FOR FOLIC ACID-METAL ION SYSTEMS.

- NICKEL (II)
- COBALT (II)
- LIGAND (FLH2)
- ACID

VOLUME OF HClO4 (0.05 M) (ML)
FIG. 9. PH-TITRATION CURVES FOR FOLIC ACID-METAL ION SYSTEMS.

COPPER (II)
LIGAND (FLH₂)
ACID

BERYLLIUM (II)
LIGAND (FLH₂)
ACID

VOLUME OF HClO₄ (0.0566 M) (mL)

VOLUME OF HClO₄ (0.0566 M) (mL)
FIG. 10. pH-TITRATION CURVES FOR FOLIC ACID-METAL ION SYSTEMS.
FIG. II. pH-TITRATION CURVES FOR GLUTAMIC ACID-METAL ION SYSTEMS.
FIG. 12. pH-TITRATION CURVES FOR GLUTAMIC ACID-METAL ION SYSTEMS.
FIG. 13. PH-TITRATION CURVES FOR GLUTAMIC ACID-METAL ION SYSTEMS.

A: Copper (II) Ligand (GLH₂) Acid
B: Chromium (III) Ligand (GLH₂) Acid

Volume of KOH (0.1562 M) (mL)
FIG. 14. PH-TITRATION CURVES FOR GLUTAMIC ACID-METAL ION SYSTEMS.
FIG. 15. PH-TITRATION CURVES FOR GLUTAMIC ACID-METAL ION SYSTEMS.
FIGURE 16. PROTONATION CURVE—GLH$_2$ SYSTEM.

FIGURE 16. PROTONATION CURVE—AsH$_2$ SYSTEM.
FIG. 17. COMPUTATION OF Log $K_1^H$ AND Log $K_2^H$ FOR (Pab H$_2$) SYSTEM BY SPEAKMAN METHOD.
FIG. 18(A) PROTONATION CURVE FOR $\text{FH}_2$ SYSTEM.

FIG. 18(B) GRAPHICAL EVALUATION OF PROTONATION CONSTANTS (SPEAKMAN METHOD)

$\frac{(2-n_H) a^2}{n} \times 10^{-13}$

$\frac{c(n_H-1)a}{n} \times 10^{-8}$
FIG. 19. FORMATION CURVES FOR ASCORBIC ACID–METAL ION SYSTEMS.
FIG. 20. COMPUTATION OF Log $K_1$ AND Log $K_2$ FOR METAL-PARA-AMINOBENZOIC ACID COMPLEXES.
FIG. 21. FORMATION CURVES FOR FOLIC ACID–METAL ION SYSTEMS.
FIG. 22. FORMATION CURVES FOR GLUTAMIC ACID-METAL ION SYSTEMS.
FIG. 23. FORMATION CURVES FOR GLUTAMIC ACID—METAL ION SYSTEMS.
FIG. 24. pH-TITRATION CURVES FOR METAL IONS-(Dipy)-(AsH₂) SYSTEMS.
FIG. 25. pH-TITRATION CURVES FOR METAL IONS-(DiPy)-(AsH₂) SYSTEMS.

VOLUME OF KOH SOLUTION (0.2030M)
FIG. 26. pH-TITRATION CURVES FOR METAL IONS-(Dipy)-(GLH₂) SYSTEMS.
FIG. 27. PH-TITRATION CURVES FOR METAL IONS - (ASH2) - (en) SYSTEMS.
FIG. 28. pH-TITRATION CURVES FOR METAL IONS - (AsH₂) - (en) SYSTEM.
FIG. 29. pH-TITRATION CURVES FOR METAL IONS -(AsH₂) -(en) SYSTEMS.
FIG. 30. PH-TITRATION CURVES FOR METAL ION - (AsH2) - (en) SYSTEMS.
FIG. 3. PH-TITRATION CURVES FOR METAL IONS-(G,LH2)-\(\text{en}\) SYSTEMS.

VOLUME OF KOH SOLUTION (0.243 M)

Table: KOH Solution

<table>
<thead>
<tr>
<th>Metal Ions</th>
<th>Volume (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (II)</td>
<td>0.1905 M</td>
</tr>
<tr>
<td>Manganese (II)</td>
<td>0.1905 M</td>
</tr>
</tbody>
</table>

Graphs show pH titration curves for metal ions with KOH solution.
Fig. 32. pH-TITRATION CURVES FOR METAL IONS - (GLH₂) - (en) SYSTEMS.
FIG. 33. pH-TITRATION CURVES FOR METAL IONS - (GLH₂) - (en) SYSTEMS.
FIG. 34. pH-TITRATION CURVES FOR (GLH₂)-(en)-METAL ION SYSTEMS.
FIG. 35. FORMATION CURVES FOR METAL IONS-(Dipy)-(AsH₂) SYSTEMS.
FIG. 36. FORMATION CURVES FOR METAL IONS-(DiPy)–(GlH₂) SYSTEMS.
FIG. 37. FORMATION CURVES FOR METAL IONS-(AsH₂)-(en) SYSTEMS.
FIG. 38. FORMATION CURVES FOR METAL IONS-(GLH₂)-(en) SYSTEMS.
FIG. 39. ABSORPTION SPECTRA OF COPPER (II)-G1H2 / MIXED LIGAND SYSTEMS.
FIG. 4. CONTINUOUS VARIATION CURVES FOR COPPER (II) - GLH₂ SYSTEM.
FIG. 42 (B)
Limiting Logarithmic Curve
Copper (II)-GLH₂ System.

Overall ligand conc. = 0.035M
Total volume = 50.0 ml
λ = 620 mμ
pH = 5.6

0.50
-1.00
-1.50
log absorbance

-2.00
-2.50
-3.00
-4.00
-4.25

FIG. 42 (A)
Effect of Varying pH on Absorbance
Copper (II)-GLH₂ System.

Overall copper (II) conc. = 0.005M
Overall ligand conc. = 0.025M
Total volume = 50.0 ml
λ = 620 mμ

0.40
0.36
0.32
0.28
0.24
0.20
0.16
0.12

pH
absorbance
FIG. 43. ABSORPTION SPECTRA OF COPPER (II) - TH. HCL. SYSTEMS.
FIG. 44(A)

$[Cu^{2+} : \text{Th. HCl}]$
- $[0.0024 M : 0.0056 M]$
- $[0.0016 M : 0.0064 M]$
- $pH = 5.0$

FIG. 44(B)

Overall Th. HCl conc. = 0.02 M
Overall copper (II) conc. = 0.02 M
$pH = 5.0$
- $\lambda = 380 \text{ m\textmu} $
- $\Delta \lambda = 360 \text{ m\textmu} $

FIG. 44. EFFECT OF pH ON ABSORPTION CURVE.

JOB'S CONTINUOUS VARIATION CURVES.
FIG. 45. LOG $K_1$ VS. ELECTRONEGATIVITY (A AND B) / ATOMIC NUMBER (C AND D).