C H A P T E R - IV

FORMATION CONSTANTS OF SOME MIXED - LIGAND COMPLEXES INVOLVING DIPEPTIDES AND CATECHOL OR SUBSTITUTED CATECHOL

INTRODUCTION

Tyrosinase is an enzyme widely distributed in nature mainly involved in the biosynthesis of melanins and other polyphenolic compounds [1,2]. It catalyses both the o-hydroxylation of monophenols and the oxidation of o-diphenol to o-quinones. The two enzymic activities are commonly referred to as cresolase or monophenolase and catecholase or diphenolase activities, respectively. The involvement of copper as the prosthetic group in tyrosinase was firmly established long ago [3,4].

Laccase an enzyme catalysing the oxidation of p-dihydroxy phenols to quinones, contains one molecule of type I, one molecule of type II copper and two molecules of antiferromagnetically coupled type III copper [5]. Thus the importance of metal ion in the catalytic activity of phenol oxidase is evident.

The enzymic oxidation of catechols to quinones by molecular oxygen with tyrosinase and laccase is similar in some respects to the Cu (II) - ion catalysed oxidation of catechols. Hence binary and ternary copper (II) complexes of catechols have been studied extensively [6-12] and these complexes have been used as catalysts for phenol oxidation.

A model enzyme study [13] of the oxidation of pyrocatechol and ditertiary butylpyrocatechol by oxygen in the presence of various divalent metal chelates (ML) showed the formation of an intermediate LM - pyrocatechol-oxygen complex. The order of catalytic activities of metal ions investigated is Mn(II) $>$ Co(II) $>$ Fe(II) $>$ Cu(II) $>$ Ni(II).

In copper containing proteins, exhibiting polyphenol oxidase activity, there is intermediate formation of ternary complexes of Cu(II) containing phenolate and protein. Hence it was thought of interest to study in the present chapter a closer oxidase model i.e. ternary systems involving dipeptides and substituted catechols.

Gergely and co-workers [14] studied the stability constants of mixed ligand complexes of manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) ions with L-dopa, dopamine, L-adrenaline, L-noradrenaline as ligand A and L-alanine, L-histidine, glycylglycine and adenosine - 5' - triphosphate (ATP) as ligand B. The relationship of the complexes have been discussed by taking into account the difference between the $\log K_1/K_2$ of the parent complexes.

The formation constants of the complexes MLL' where M = Cu(II) or Ni(II), L = catechol, pyrogallol, aminophenol or orthophenylene diamine and L' = amino acids with uncoordinated side groups such as tryptophan, phenylalanine and tyrosine were studied [15]. It was concluded that the stabilisation of the above ternary complexes is mainly, due to the non-coordinating side groups of L'occupying a position in the vicinity of the less hydrophilic (CuL) complex. Additional stabilisation may be because of electrostatic hydrogen bonding interaction between the side groups of L'and the ligand L.

Present chapter deals with the study of mixed-ligand complexes of the type CuAL where A = dipeptides gg, ga and gl and L = catechol (cat), pyrogallol (pyr), 1,2 - dihydroxybenzene - 3,5 -

disulphonic acid (tiron) and 2, 3 - dihydroxynapthalene (nap) in aqueous medium with initially constant ionic strength 0.2 M $NaClO_4$ and temperature being 30°C.

EXPERIMENTAL

The metal perchlorate, sodium perchlorate, sodium hydroxide and perchloric acid solutions were prepared and standardised in the same way as detailed in chapter IIA. The dipeptides used were of the same grade as mentioned in chapter II. The ligands pyrocatechol (De Riedel), pyrogallol (BDH), 1,2 - dihydroxybenzene - 3,5 - disulphonic acid (tiron) (BDH) and 2,3 - dihydroxy napthalene (Fluka AG) were of Analar grade.

The titrations were carried out in aqueous medium using a pH - meter DIGICHEM 8201 with an accuracy of ±0.01. The values of the proton-ligand formation constant, the binary copper(II) complex formation constant and the mixed ligand formation constant were refined using the computer program SCOGS, as detailed in chapter IIA.

The values used for the proton-ligand formation constant and formation constant of copper (II) - dipeptide binary complexes were same as given in chapter IIB. In case of catechol and substituted catechols, proton-ligand formation constants and formation constants of binary complexes with copper (II) were also refined under identical conditions. These values are tabulated in Table 4.1 and are found to be in good agreement with those reported earlier [16]. These refined values were used as fixed parameters for the refinement of the formation constants of the mixed-ligand complexes.

For the determination of the formation constants of the ternary complexes, the following sets of solutions (50 cc) having Cu : A : L in 1 : 1 : 1 and 1 : 1 : 2 ratio were prepared and titrated against standard alkali solution :

i) 0.02 M $HClO_4$, 0.004 M ligand A, 0.004 M ligand L, 0.004 M metal perchlorate and 0.168 M $NaClO_4$.

ii) 0.02 M $HClO_4$, 0.004 M ligand A, 0.008 M ligand L, 0.004 M metal perchlorate and 0.164 M $NaClO_4$.

Titrations of each set were carried out twice to check the reproducibility of the data. The mixed-ligand formation constants were evaluated by taking into account the species H_2A, HA, A, Cu, [CuA], $[CuA_{-H}]$, H_2L, HL, L, $[CuL_2]$ and the mixed-ligand species [CuAL] and $[CuA_{-H}L]$. The formation constants for the ternary species, determined by using the SCOGS computer program, are presented in Table 4.2.

The titration data has been shown in figs. 4.1 to 4.4. The distribution of various binary and ternary species formed, during the course of titration has been shown in fig. 4.5, as a function of pH.

Table - 4.1 : Proton-ligand and copper(II) complex formation constants of catechol and substituted catechols in aqueous media at 0.2 M $NaClO_4$ and 30°C with standard deviations ($\sigma\beta$) in parentheses.

Ligand	pK_1^H	pK_2^H	$\log K_{CuL}^{Cu}$	$\log K_{CuL_2}^{CuL}$
Catecholate (Cat)	13.06	8.94	13.66	11.30
	(0.05)	(0.04)	(0.04)	(0.05)
Pyrogallolate (Pyr)	10.86	9.17	12.80	-
	(0.02)	(0.01)	(0.06)	
1,2 - dihydroxybenzene - 3,5 - disulphonate(Tiron)	11.97	7.69	13.82	11.19
	(0.01)	(0.01)	(0.04)	(0.04)
2,3 - dihydroxy - napthalene (Nap)	10.90	8.34	11.78	9.08
	(0.05)	(0.08)	(0.03)	(0.04)

Table - 4.2 : Formation constants of mixed - ligand complexes and Δ logK in aqueous media, I = 0.2 M ($NaClO_4$) at 30°C Standard deviation ($\sigma\beta$) are given in parentheses.

Complexes	$\log K^{Cu}_{CuAL}$	$\Delta \log K$	$\log K^{CuA}_{CuAL} - H^{L}$
Cu-gg-cat	18.15 (0.24)	-1.29	8.12 (0.13)
Cu-gg-pyr	17.68 (0.02)	-0.90	8.56 (0.03)
Cu-gg-tiron	18.34 (0.05)	-1.26	8.75 (0.08)
Cu-gg-nap	16.56 (0.20)	-1.00	8.70 (0.12)
Cu-ga-cat	18.26 (0.20)	-1.24	8.17 (0.11)
Cu-ga-pyr	17.79 (0.02)	-0.85	8.57 (0.02)
Cu-ga-tiron	18.43 (0.05)	-1.23	8.88 (0.08)

Table 4.2 (Continued)

Cu-ga-nap	16.40	-1.22	8.28
	(0.32)		(0.13)
Cu-gl-cat	18.38	-1.21	8.37
	(0.14)		(0.12)
Cu-gl-pyr	17.81	-0.92	8.66
	(0.04)		(0.04)
Cu-gl-tiron	18.40	-1.35	8.78
	(0.05)		(0.08)
Cu-gl-nap	16.49	-1.22	8.59
	(0.15)		(0.10)

Fig. 4.1 Potentiometric titration curves of aqueous solutions containing metal ions, gg, ga or gl and cat (each 4.0×10^{-3} M).

(1) Cu^{2+} + gg + cat.

(2) Cu^{2+} + ga + cat.

(3) Cu^{2+} + gl + cat.

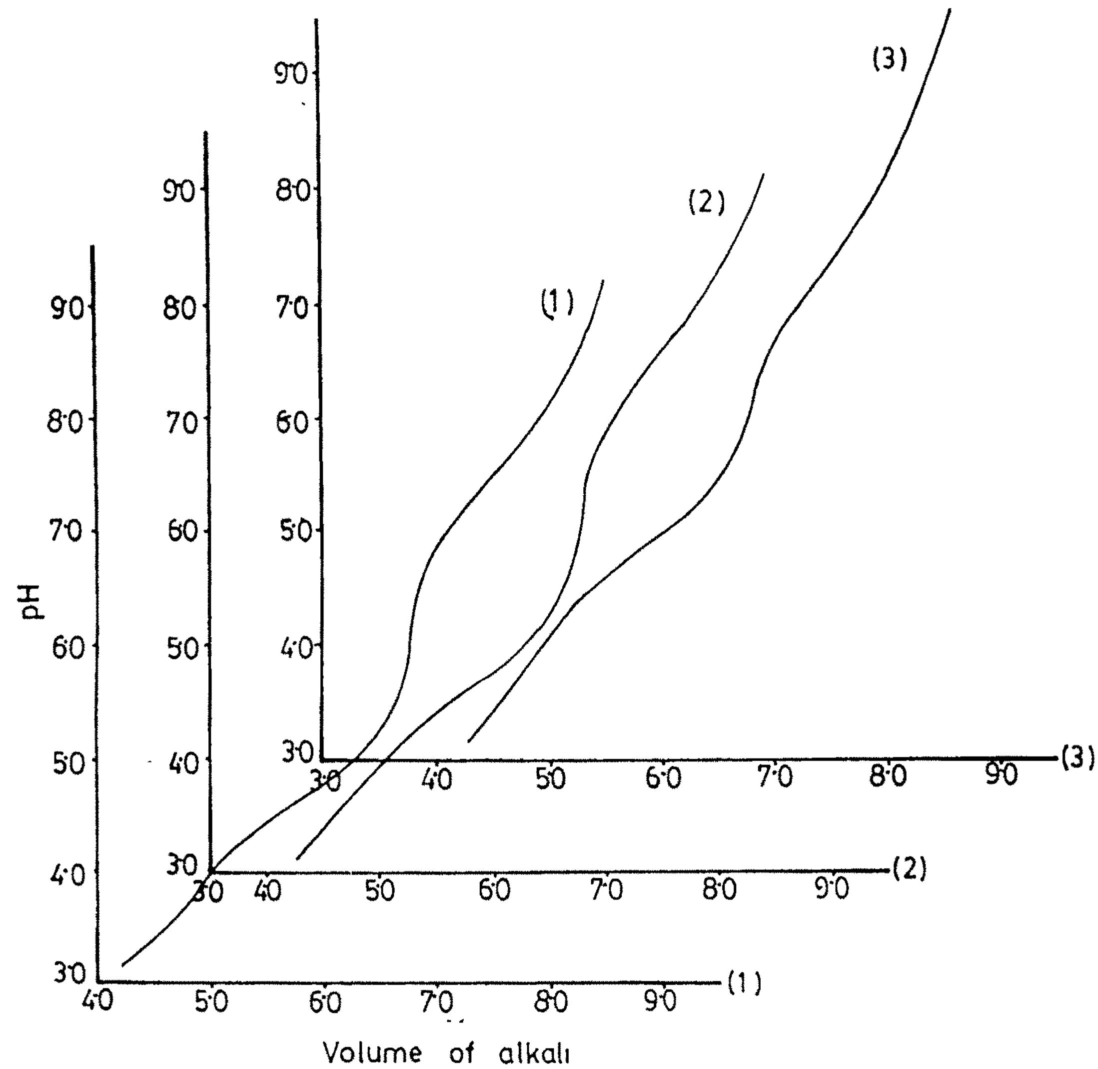


Fig. 4.2 Potentiometric titration curves of aqueous solutions containing metal ions, gg, ga or gl and pyr. (each 4.0 x 10^{-3} M).
(1) Cu^{2+} + gg + pyr.
(2) Cu^{2+} + ga + pyr.
(3) Cu^{2+} + gl + pyr.

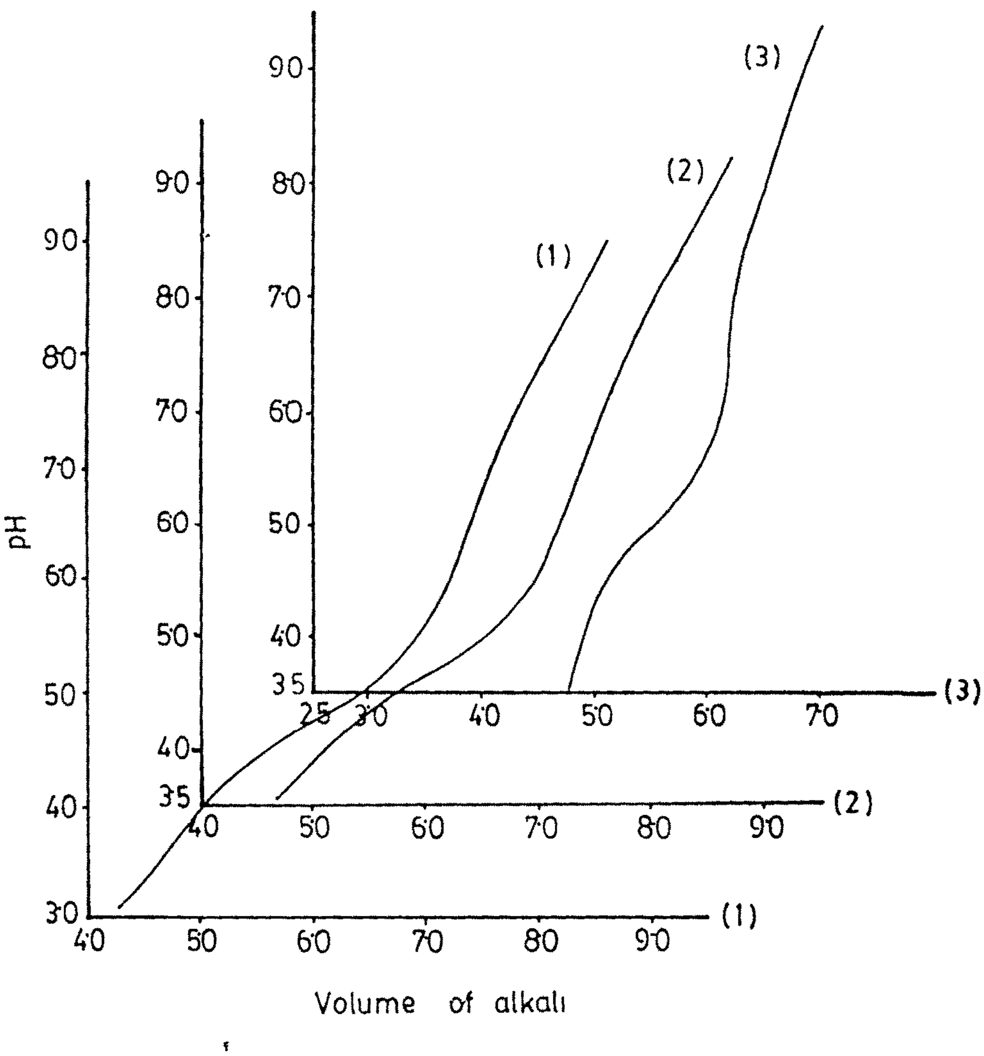


Fig. 4.3 Potentiometric titration curves of aqueous solutions containing metal ions, gg, ga or gl and tiron (each 4.0 x 10^{-3} M).
(1) Cu^{2+} + gg + tiron.
(2) Cu^{2+} + ga + tiron.
(3) Cu^{2+} + gl + tiron.

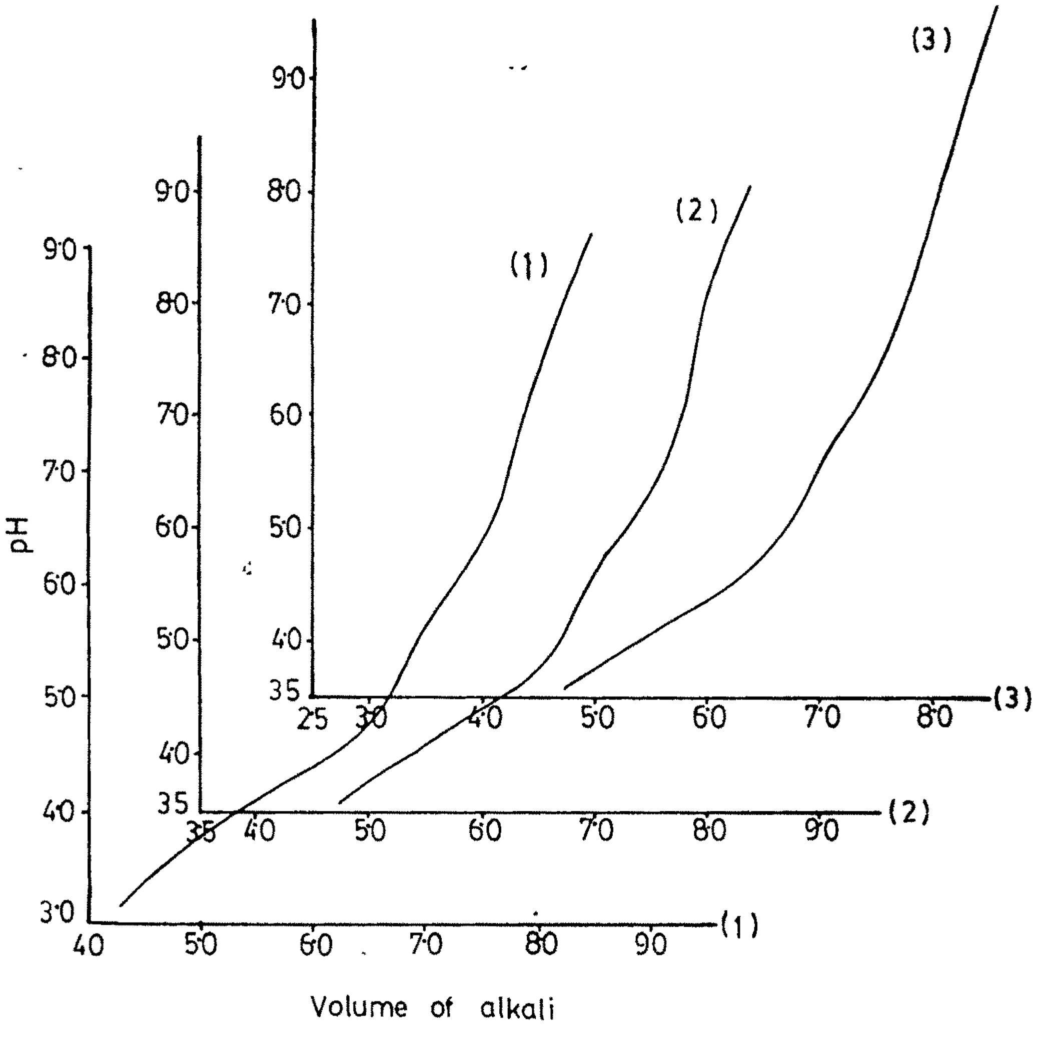
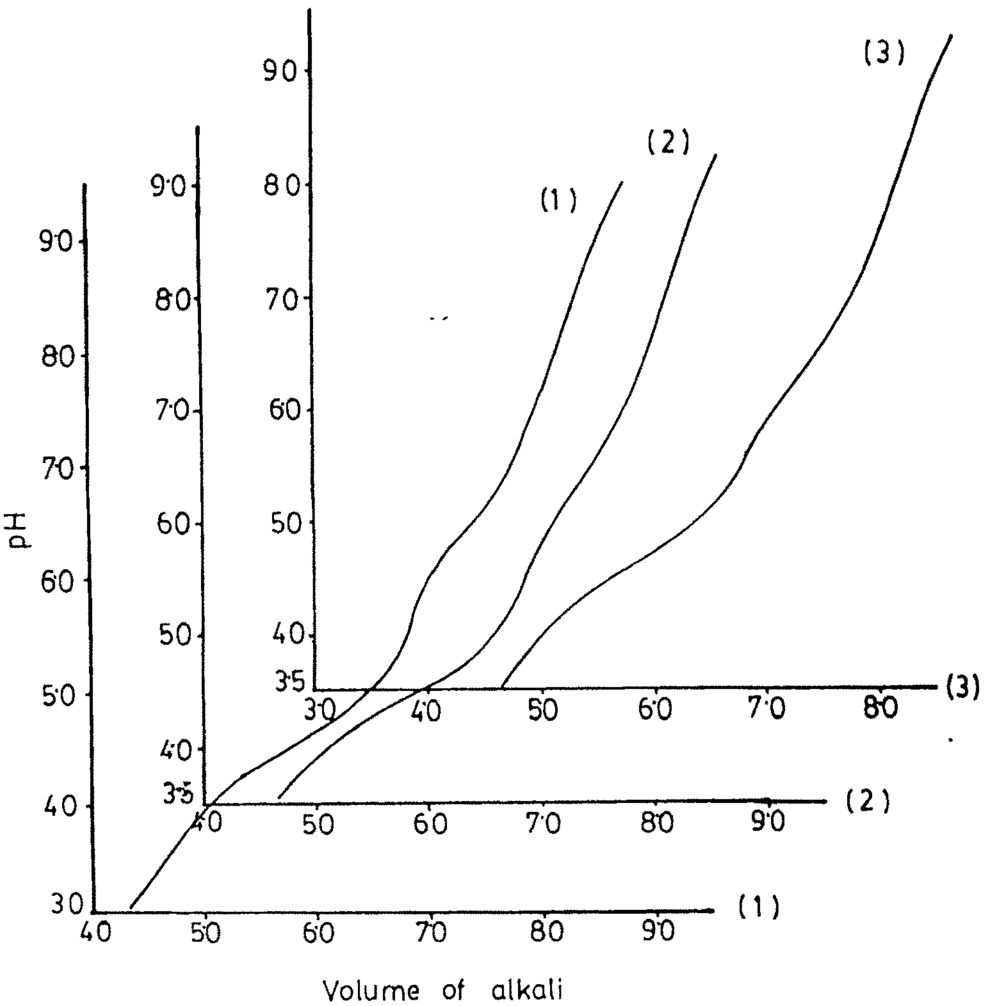


Fig. 4.4 Potentiometric titration curves of aqueous solutions containing metal ions, gg, ga or gl and Nap. (each 4.0 x 10^{-3} M).

(1) Cu^{2+} + gg + Nap.

(2) Cu^{2+} + ga + Nap.

(3) Cu^{2+} + gl + Nap.



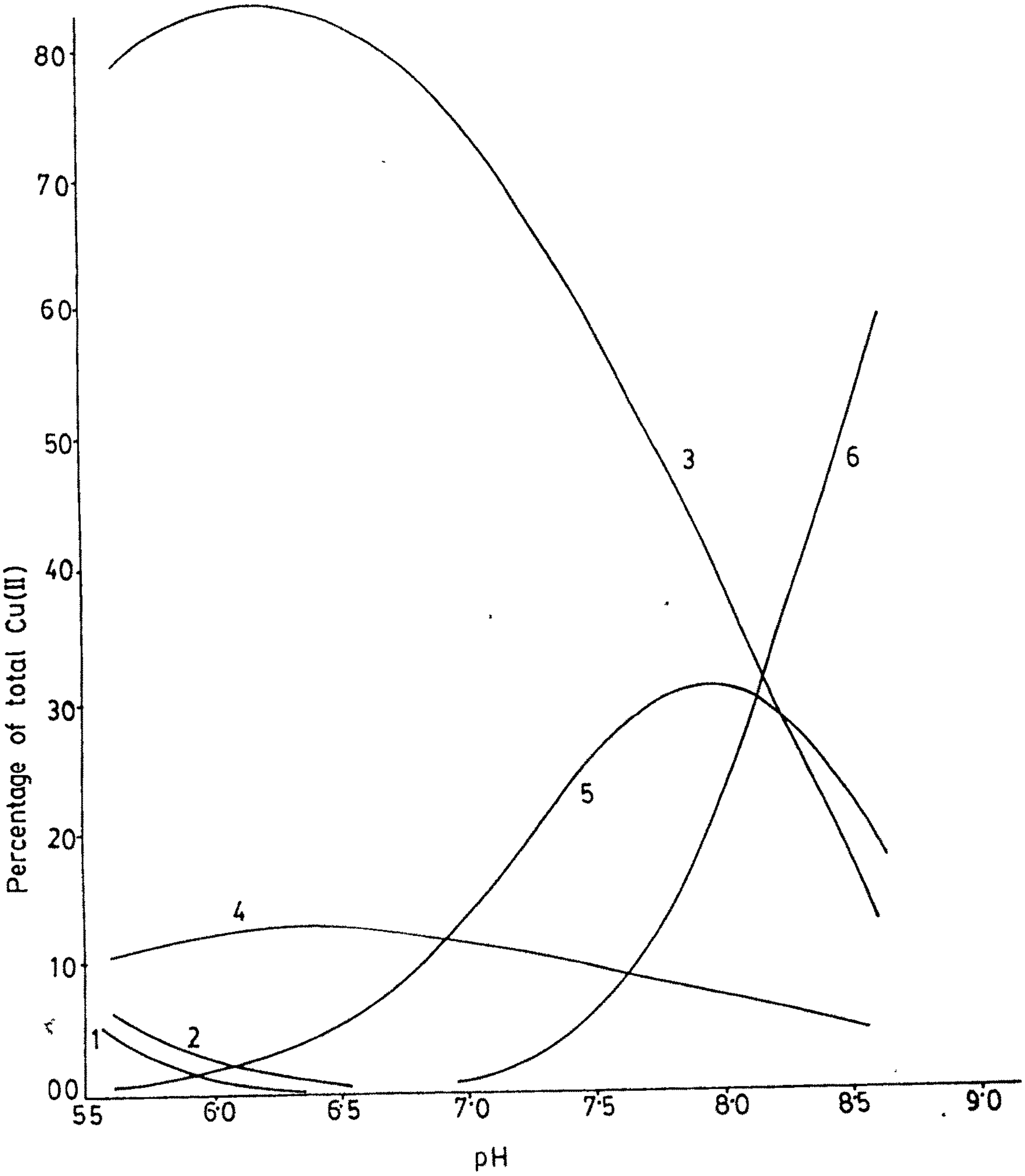


Fig. 4.5 Species Distribution of Copper(11) - Glycylglycine(A) - Catechol (L) Ternary system at Metal : A : L ratio of 1:1:1 ; (1) Unbound Copper(11), (2) [CuA], (3) [CuA_{-H}], (4) [CuL], (5) [CuAL] and (6) $CuA_{-H}L$.

RESULTS AND DISCUSSION

The species distribution curves (fig.4.5) show that the two ligands A and L coordinate simultaneously to form the mixed-ligand species CuAL. This ternary species starts forming at a pH $\sim$ 5.5 and attains a maximum concentration at pH $\sim$ 7.8. The formation of the ternary complex $CuA_{-H}L$, with the dipeptide coordinated from the deprotonated N^- end, starts at a pH $\sim$ 7.0 and its concentration increases steadily with the increase in pH.

Formation and Stability of CuAL Complexes :

In the formation of the ternary complex CuAL the dipeptide coordinates through its amino nitrogen and amide oxygen, the carboxylate group remaining free. Catechol and its derivatives coordinate through their phenolate oxygen sites. The values of Δ logK, in the complexes studied, are of the order of - 1.20. In the complex [CuAL] one of the ligands i.e. the dipeptide is a monoanion and the other ligand i.e. catechol or its derivative is a dianion, and hence from electrostatic consideration ΔlogK should be expected to be intermediate between ($\log K_2 - \log K_1 = -1.69$) where A = salicylaldehydate with two monoanions, and ($\log K_2 - \log K_1 = -2.34$) where A = catecholate with two dianions. However, the value of Δ logK in the present CuAL complex (-1.20) is less negative than expected from only electrostatic considerations.

A similar less negative value has been observed for ($\log K_2 - \log K_1$) in case where A = alaninate (= - 1.09). A probable reason for high stability of [Copper (alaninate)$_2$] complex is intramolecular hydrogen bonding between $-NH_2$ group of one alanine and carboxylate group of another in the bis complex (fig.4.6). Similar explanation can also be extended for copper (II) - dipeptide - catechol complexes, considering intramolecular hydrogen bonding between phenolate oxygen of catechol and hydrogen of amino or peptide group. However, direct hydrogen bonding between phenolate O^- and -NH or $-NH_2$ group of a dipeptide in the above complexes is less likely from the consideration that the ligands are far apart. The greater stabilization of the present CuAL complexes and less negative $\triangle$logK value may be due to hydrogen bonding between the two ligands through a solvent water molecule (fig.4.7). Such hydrogen binding has been suggested in ternary copper (II) complexes involving tertiary amine like phenanthroline [17].

Formation and Stability of $CuA_{-H}L$ Complex :

In the case of the ternary complex $CuA_{-H}L$, it was observed that tendency of the dipeptide A to get deprotonated is reduced in the ternary complex, compared to binary complex CuA_{-H}. This is evident from the fact that deprotonation of the dipeptide occurs at low pH ($\sim$ 4.5) in the case of binary complex, whereas it occurs at high pH ($\sim$ 7.0) in the case of ternary complexes. This can be explained by considering the following two factors : (i) electrostatic repulsion between the two ligands, (ii) Jahn - Teller distortion. The dipeptide, on deprotonation of the -NH of peptide group forms a dianion. There is a strong repulsion between the dipeptide dianion and the catechol dianion, which inhibits the coordination of the dipeptide from the peptide nitrogen end and hence reduces the deprotonation. In the binary copper (II) dipeptide complex at high pH, the peptide group is deprotonated and the species $[CuA_{-H}]$ is formed, where coordination involves the amino nitrogen, peptide nitrogen and carboxylate oxygen, bound in equatorial positions. However, in the ternary complex [CuA_{-H}] the tridentate A_{-H} ligand has to occupy two equatorial and one axial position or the ligand L has to occupy one equatorial and one axial position (fig. 4.8). Occupation of the axial position by either ligand will bring a strain in the ligand due to Jahn-Teller distortion and hence the ternary complex is destabilised. Thus the two factors inhibit the formation of [$CuA_{-H}L$] complex and hence deprotonation.

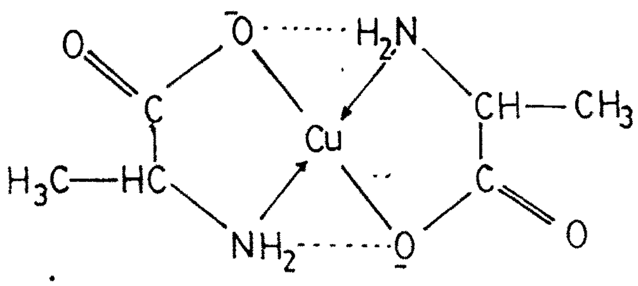


Fig. 4.6

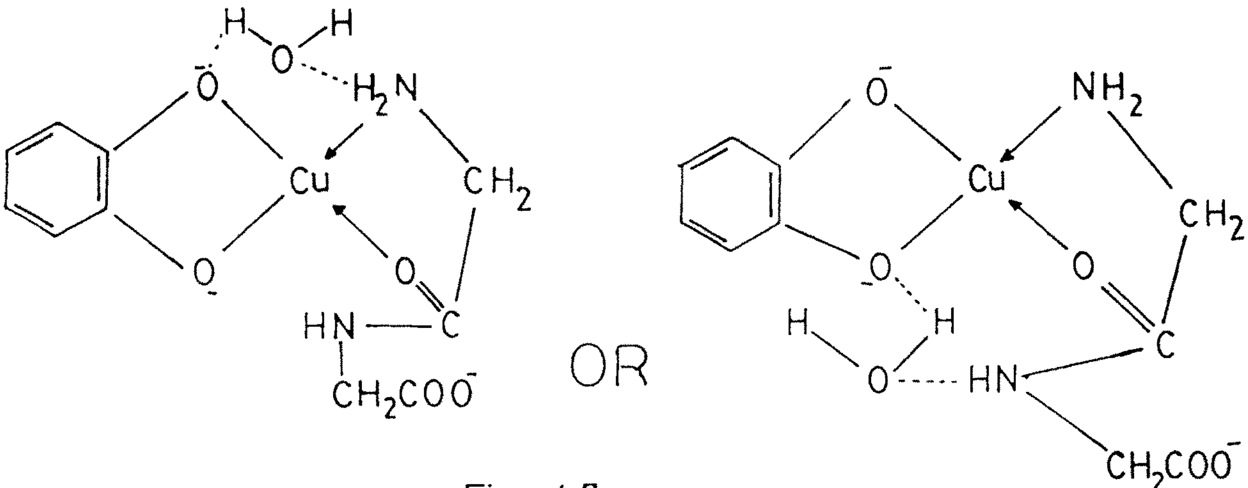


Fig. 4.7

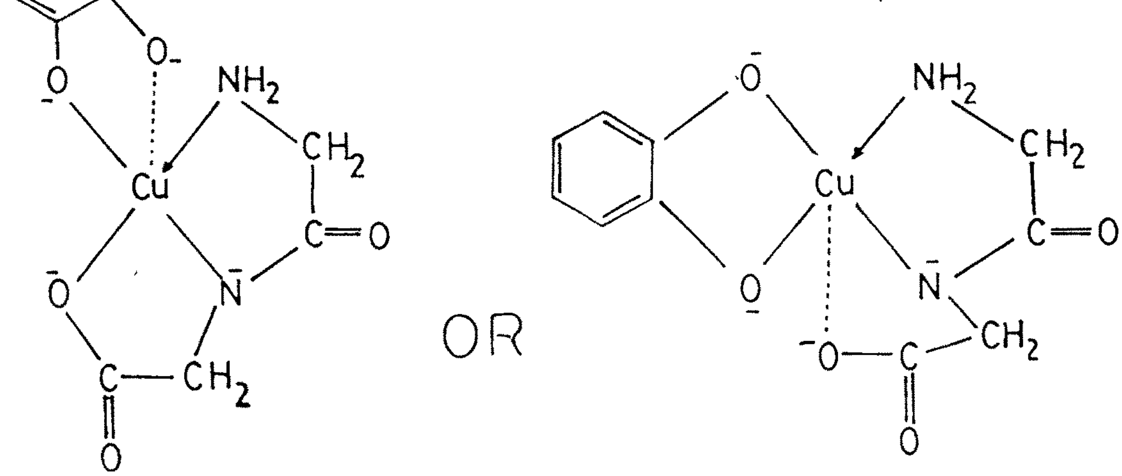


Fig 4 8

REFERENCES

1. R.A. Nicolaus, in Melanins, Hermann, Paris, 1968.

2. E.Küster in Enzyme Chemistry of Phenolic Compounds (J.B. Pridhan, ed.) Pergamon Press, Oxford, 1962.

3. F.Kubowitz, Biochem. Z., **299**, 32, 1938.

4. D.Keilin and T. Mann, Proc. Roy.Soc., Ser.B., **125**, 187, 1938.

5. R.W. Hay, ' Bio-Inorganic Chemistry', Ellis Horwood, Chichester, P.145, 1984.

6. R. Freimund and L. Alan, Inorg. Chem.,**5**, 1542, 1966.

7. R.F. Jameson and W.F.S. Neillies, J. Inorg. Nucl. Chem., **27**, 2623, 1965.

8. V.T. Athavale, L.J. Prabhu and D.G. Vartak, J.Inorg.Nucl. Chem., **28**, 1237, 1968.

9. Y. Murakami, K. Nakamura and M. Tokunage, Bull. Chem. Soc. Jpn., **B6**, 669, 1963.

10. A. Sinha, Proc. Nat. Acad. Sci., India Sect., **28A**, 342, 1959.

11. D.C. Patel and P.K. Bhattacharya, J. Inorg. Nucl. Chem., **33**, 529, 1971.

12. D.C. Patel and P.K. Bhattacharya, Ind. J. Chem., 835, 1970.

13. C.A. Tyson and A.E. Martell, J.Am. Chem. Soc., **94**, 939, 1972.

14. T.Kiss, G.Deak and A. Gergely, Inorg. Chım. Acta, **91**, 269, 1984.

15. V.Manjula and P.K. Bhattacharya, J. Inorg. Biochem., **41**, 63, 1991.

16. H. Sigel, Inorg. Chem.,**10**, 1138, 1971.

17. M.G.B. Drew, A.H. Bin Othman, S.G. McFall and S.M. Nelson, J.Chem. Soc. Chem. Comm., 558, 1977.