CHAPTER - III

ELECTROCHEMICAL STUDIES OF MIXED - LIGAND COMPLEXES INVOLVING A DIPEPTIDE OR HISTIDINE AND ANOTHER AMINO ACID

INTRODUCTION

As discussed in the previous chapter copper containing protiens are widely distributed in both plants and animals mainly assisting electron transfer and oxidation processes. These activities are dependent on the redox potential of copper (II) centre in the protein, which in turn depends on the nature and flexibility of the coordinated ligands and the geometry of the metal complex. The copper ion in plastocyanin is in highly distorted tetrahedral environment with two sulphur donors from cysteine and methionine and two imidazole nitrogen donors from histidine [1]. The copper binding site in azurin is very similar to the corresponding site in the plastocyanins. In case of other copper proteins such as stellacyanin, umecyanin, rusticyanin and plantocyanin also, copper is bound to amino acid residues of the proteins.

In the biological systems, where copper (I) is found to be the stable state, the copper ion is generally bound to the soft sulphur donors or imidazole. Such coordination sites may be from more than one type of ligand as ternary coordination complexes are formed in various stages in biological processes. It was, therefore, thought of interest to study the redox potential of the ternary systems involving dipeptide and amino acids or mixed amino acids to understand the effect of ligand nature on the reduction potential of the metal centre and stabilization of the oxidation states.

It has been generally observed that the electrochemical reduction of Cu^{+2}_{aq} preferentially occurs by two electrons to form copper metal. If complexation stabilises copper(I) it can give rise to a one electron reduction of copper(II). Margerum etal [5] studied the reduction of copper(II) -

triglycine complex at a hanging mercury drop electrode (HMDE) at varying pH (7.0-10.0). They observed that the reduction of the above complex occurs through a two electron process in which no copper(I) - peptide complex is formed. However, study of the reduction of the ternary [Cu ($H_{-1}G_2$) dmp] (G_2 = diglycine, dmp = 2, 9 - dimethyl 1, 10-phenanthroline) complex [5] shows the formation of [Cu(1) ($H_{-1}G_2$)dmp] complex, which is a short lived species and rapidly gets converted to more stable [Cu(I) $(dmp)_2]^+$ complex.

Zacharius etal [6] studied the electrochemical behaviour of a series of copper(II) - dipeptide complexes of the general formula, Cu(II)L, (H_2L = H_3N^+ - CHR - CO - NH - CHR' - COO^-; R, R' = alkyl or H) at pH$\sim$7 revealing that the reduction proceeds through one electron proccess, generating copper(I) - dipeptide complex at the mercury electrode. Copper(I)L complex being an unstable species, part of it undergoes disproportionation, generating Cu(0) and Cu(II) in aqueous medium. The unchanged fraction of the copper(I) species is reoxidised to the copper(II) complexes. The Cu(0) generated undergoes a two electron oxidation to Cu(II), at a more positive anodic potential than the copper(I) complexes. The newly generated Cu(II) ion subsequently gets reduced at a less negative potential during the second scan. The study was further extended to ternary copper(II) amino acid complexes [7,8]. The complexes studied were Cu(Asp)(Lys), Cu(Asp)(Arg), Cu(Asp)(Orn), Cu(Glu)(Lys), Cu(Glu)(Arg) and Cu(Glu)(Orn). The electrochemical reduction of these species also leads to the formation of the corresponding Cu(I) species.

Zacharius etal [9] also studied the electrochemical behaviour of mixed -ligand complexes of the type Cu (BPY) (AA) (BPY = 2, 2' - bipyridyl, AA = amino acids such as glycine, serine, alanine, isoleucine, valine and

threonine by differential - pulse voltammetry (DPV) to show the generation of copper(I) species as an intermediate.

In the present chapter, electrochemical properties of ternary complexes involving dipeptide and amino acids or mixed amino acids have been investigated. The systems studied are as follows :

i) Copper(II) - glycylglycine (gg) - α-alanine (ala)

ii) Copper(II) - glycylglycine (gg) - tyrosine (tyr)

iii) Copper(II) - histidine (hist)

iv) Copper(II) - histidine - α- alanine

v) Copper (II) - histidine - phenylalanine (phe)

vi) Copper(II) - histidine - tyrosine

vii) Copper(II) - histidine - tryptophan (trp)

EXPERIMENTAL

The dipeptides and the amino acids were of same quality as described in chapter IIA and IIB. The pH of the solutions were changed by adding μl quantities of pure sodium hydroxide solution and determining the pH value by the pH meter used in earlier studies.

Cyclic voltammetric experiments were carried out on a EG & G PARC electrochemistry system, which included the 174A Polarographic Analyzer, 175 Universal Programmer, RE 0089 X-Y Recorder and a 303A Electrode system. The three electrode assembly consisted of a hanging mercury drop working electrode (HMDE), a platinum wire auxiliary electrode and a silver - silver chloride (Ag-AgCl) reference electrode.

Cyclic Voltammograms were recorded in the potential range +0.2 V to -0.6V in aqueous solution (ca. $1x10^{-3}M$) at different pH, with $NaClO_4$ as supporting electrolyte. The cyclic voltammograms for different complexes at different pH are shown in Figs. 3.1 to 3.3. The relevant CV data for all complexes are collected in Tables 3.1 and 3.2.

Table 3.1 : Cyclic Voltammetric data[a] on Copper(II) - dipeptide - amino acid (L) Complexes

COMPLEX	pH	E^1_{red}	E^2_{red}	E^1_{ox}	E^2_{ox}
Cu-gg-ala	3.0	-0.03	-	+0.045	-
	5.0	-0.06	-0.30	+0.045	b
	7.12	-0.02[c]	-0.36	+0.03	b
Cu-gg-tyr	3.0	-0.02	-	+0.06	-
	5.0	-0.03	-0.25	+0.05	b
	7.28	-0.03[c]	-0.37	+0.025	b

a. All potentials are in volts; Scan rate = $0.05Vs^{-1}$

b. Broad peak, difficult to measure precisely

c. Peak appears only in the second and subsequent scans.

Table 3.2 : Cyclic Voltammetric data[a] on Copper(II) - histidine and Copper(II) - histidine - amino acid complexes

COMPLEX	pH	E^1_{red}	E^2_{red}	E^3_{red}	E^1_{ox}	E^2_{ox}	E^3_{ox}
Cu-hist	3.0	-0.065	-	-	-0.01	-	-
	4.28	-0.13	-0.30	-	+0.05	-0.20	-
	6.10	-	-0.30	-0.44	-	b	-0.36
Cu-hist-ala	3.0	-0.03	-	-	-0.015	-	-
	4.50	-0.13	-0.30	-	+0.05	-0.20	-
	6.61	-	-	-0.45	-	-	b
Cu-hist-phe	3.10	-0.07	-	-	-0.02	-	-
	4.50	-0.14	-0.25	-0.44	+0.04	b	-0.34
	7.18	-	-	-0.46	-	-	b
Cu-hist-tyr	3.03	-0.07	-	-	-0.015	-	-
	4.44	-0.12	-0.25	-0.45	+0.03	b	-0.36
	7.20	-	-	-0.46	-	-	-
Cu-hist-trp	3.02	-0.09	-	-	-0.03	-	-
	5.01	-0.14	-0.30	-0.45	+0.02	b	-0.37
	7.50	-	-	-0.46	-	-	-0.35[b]

a. All potentials are in volts; Scan rate = 0.05 Vs^{-1}

b. Broad peak, difficult to measure precisely.

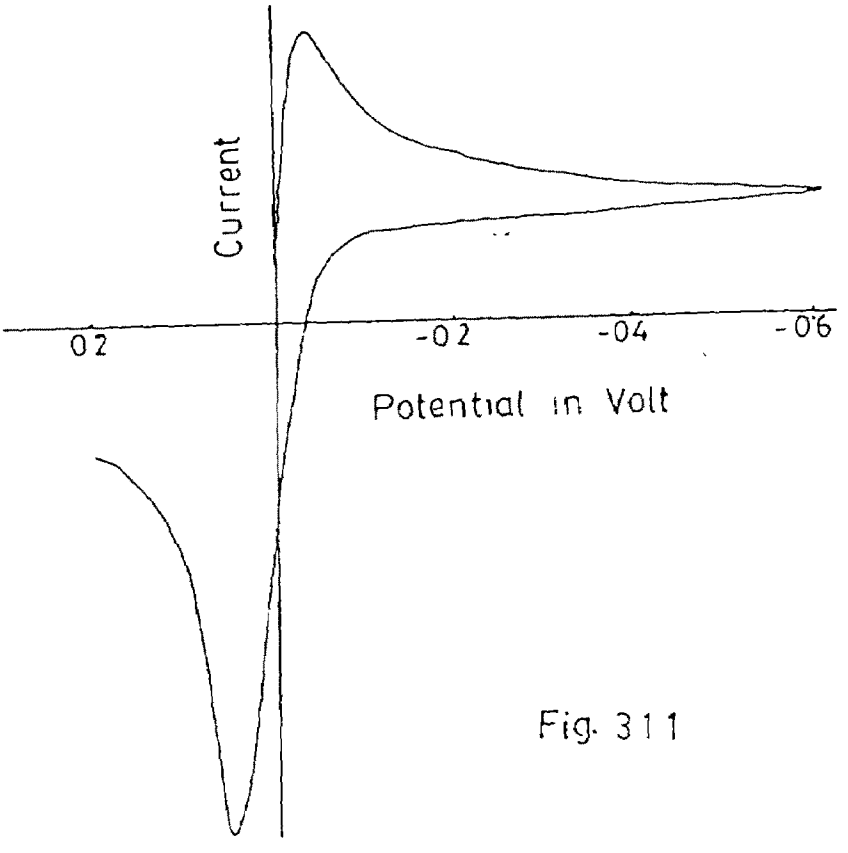

Fig. 311

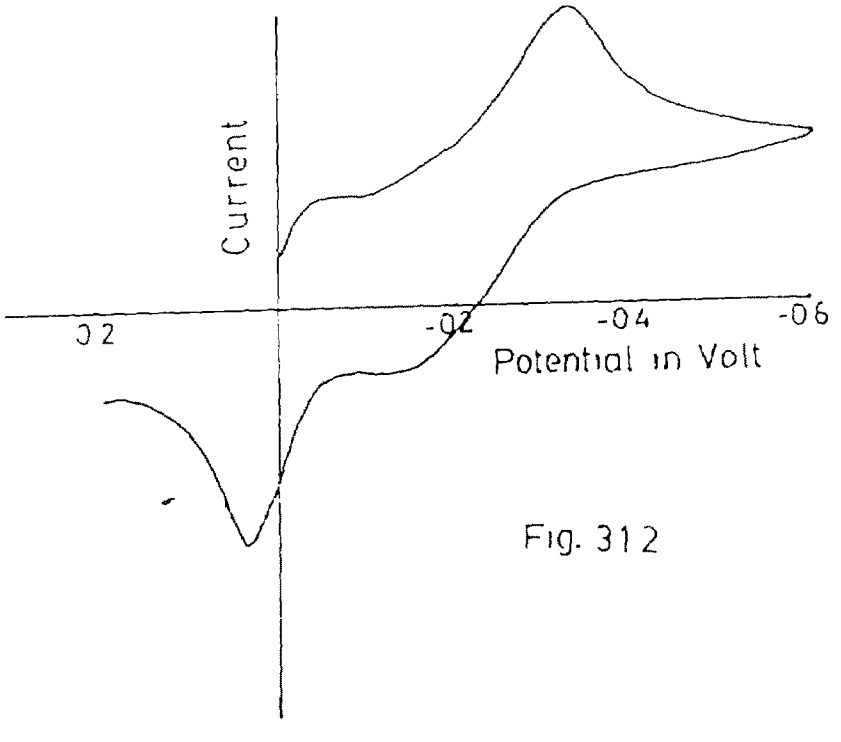

Fig. 312

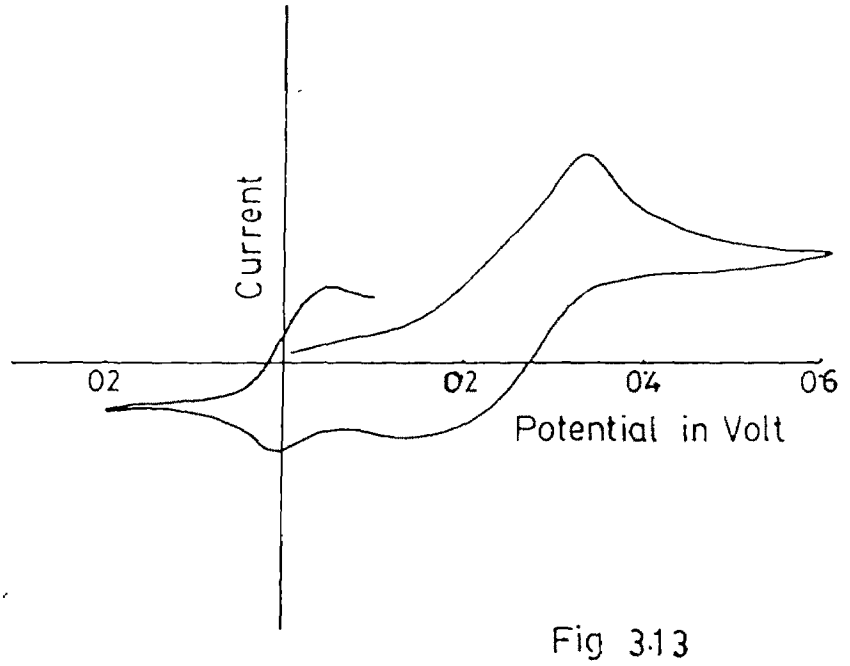

Fig 3.13

Fig. 3.1 Cyclic Voltammograms of Cu(II)gg ala Complex at scan rate = 0 05 Vs^{-1} and at various pH.

3.11) pH = 3·0 3·12) pH = 5·0 3·13) pH = 7·12

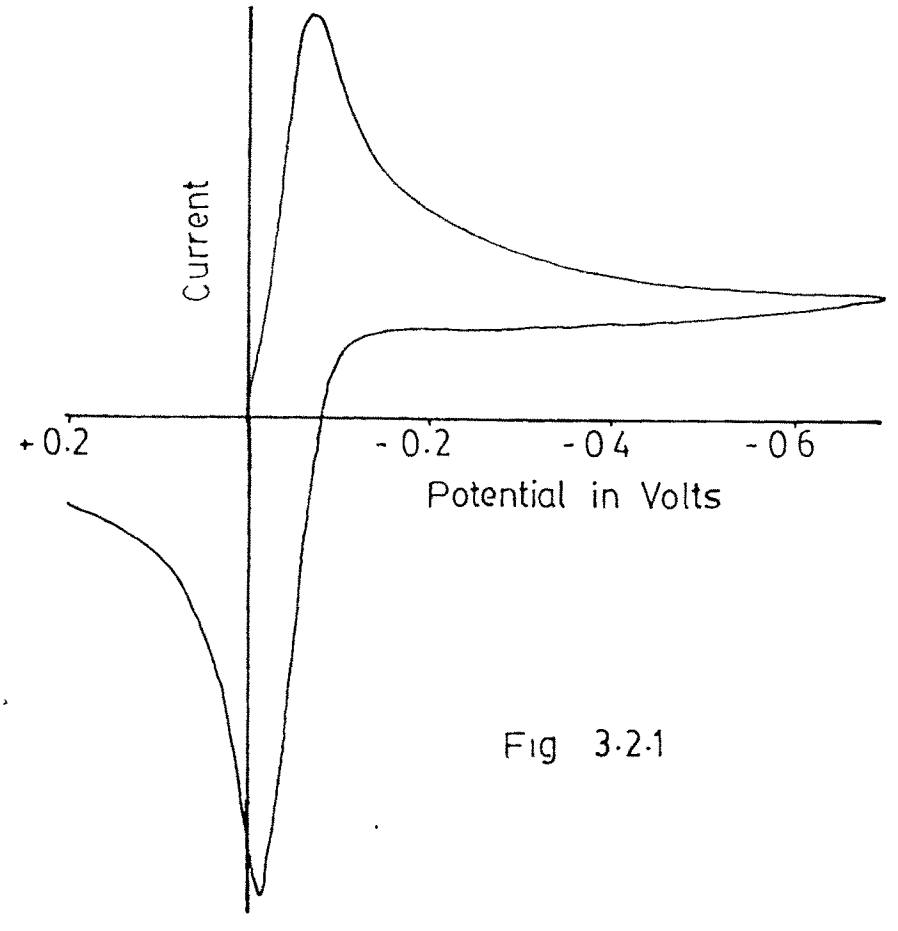

Fig 3·2·1

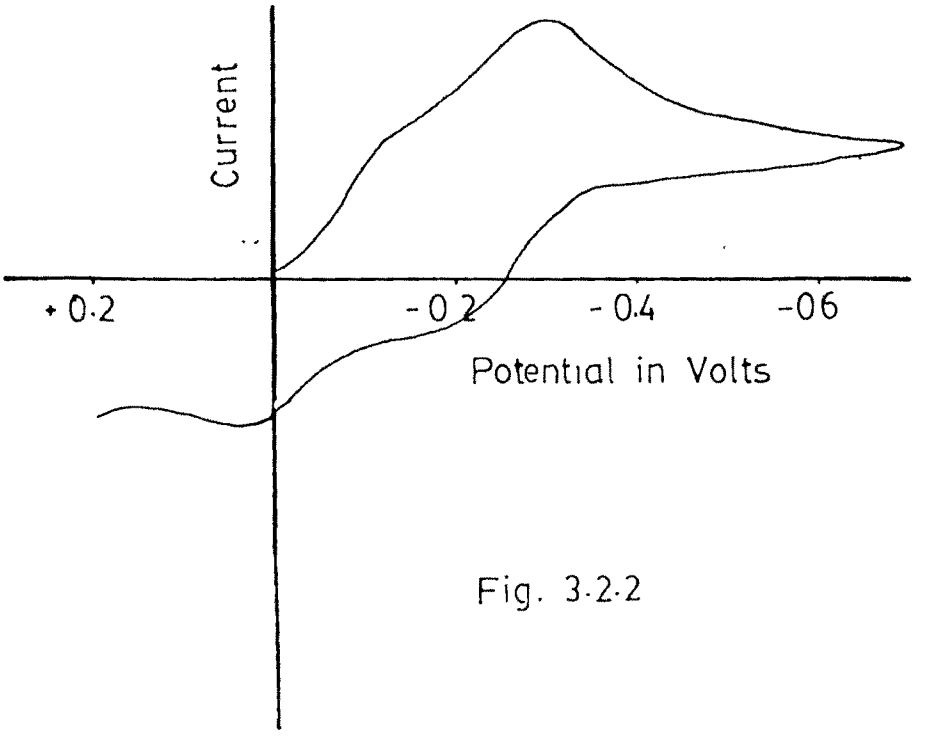

Fig. 3·2·2

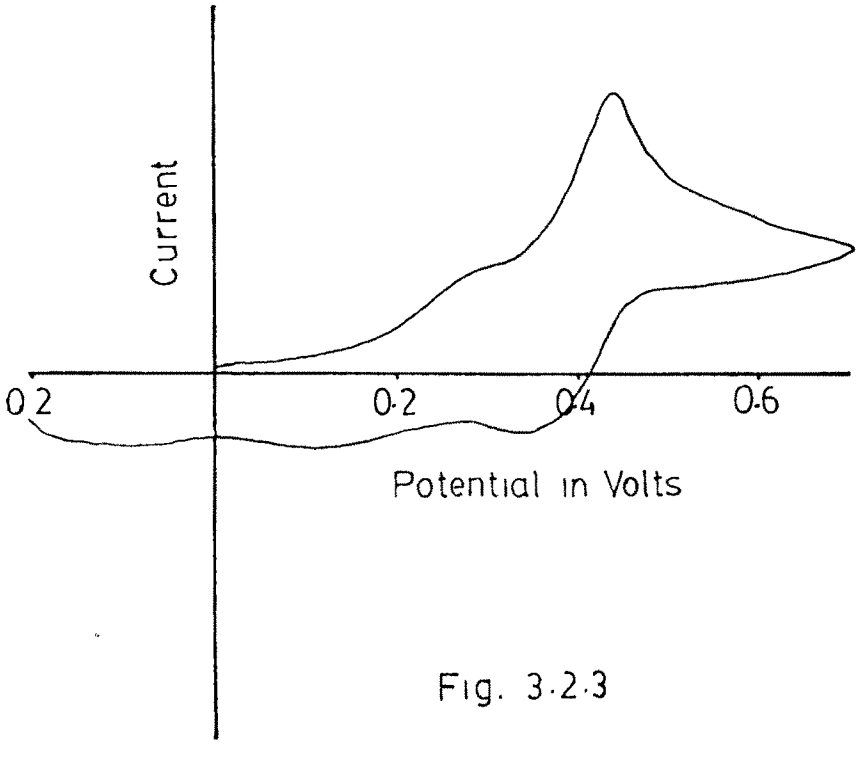

Fig. 3·2·3

Fig 3·2 Cyclic Voltammograms of Cu(II)hist complex at scan rate = 0·05 Vs^{-1} and at various pH

3·2·1) pH = 3·0 3·2·2) pH = 4·28

3·2·3) pH = 6·10

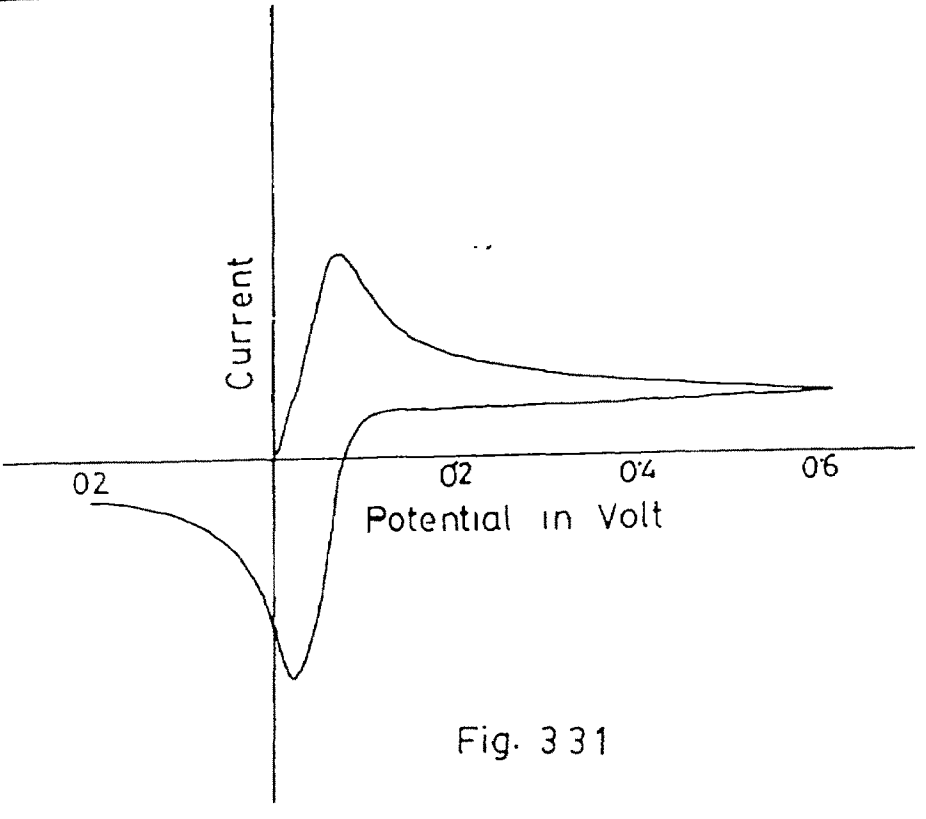

Fig. 3 3 1

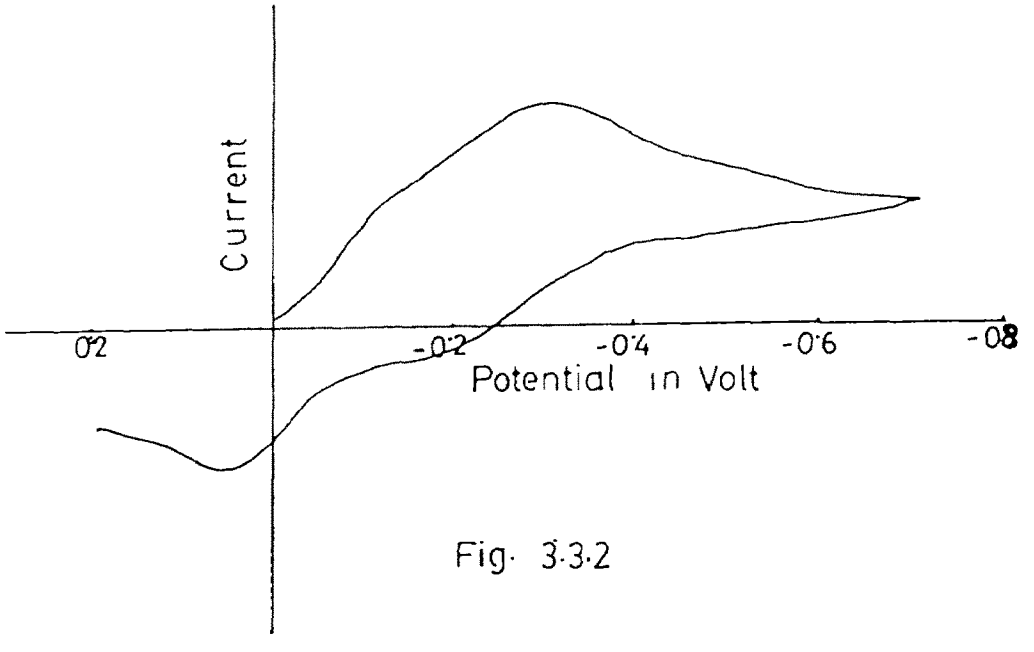

Fig. 3.3.2

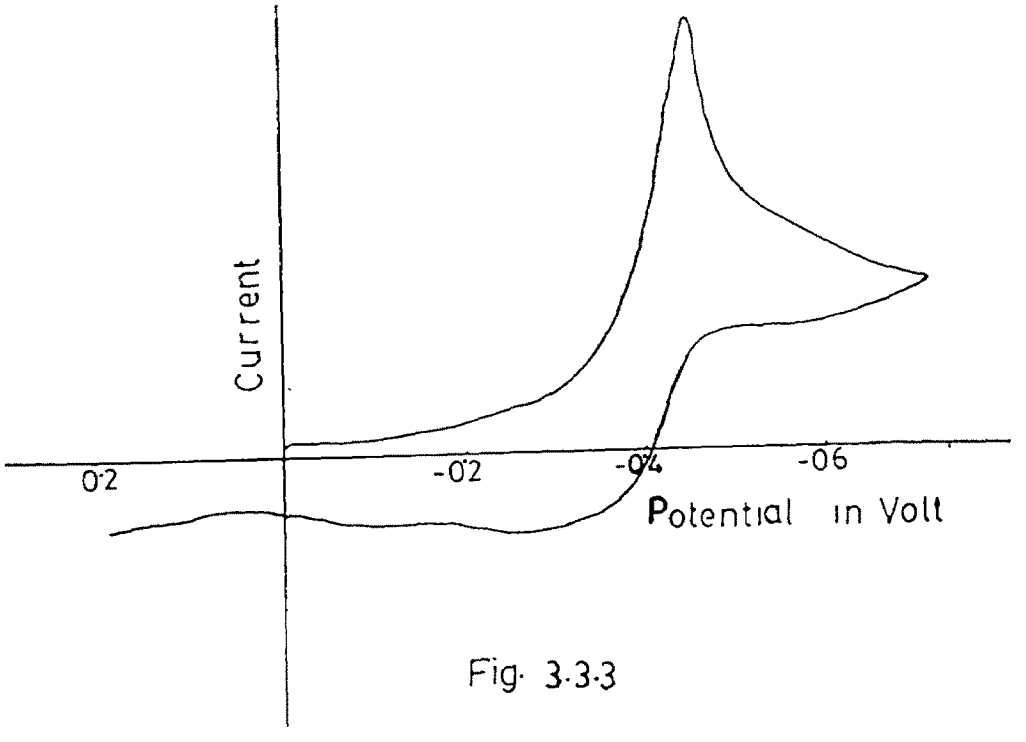

Fig. 3.3.3

Fig. 3.3 Cyclic Voltammograms of Cu(II)hist ala Complex at scan rate = 0·05 Vs^{-1} and at various pH

3.3.1) pH = 3.0 3.3.2) pH = 4.5 3.3.3) pH = 6.61

RESULTS AND DISCUSSIONS

I Copper(II) - glycylglycine (gg)-amino acids (L) Systems :

The dipeptides are known to be ambidentate in nature, coordinating through amino nitrogen and oxygen of the peptide C = O group at low pH and through amino nitrogen, deprotonated peptide nitrogen and carboxylate oxygen at higher pH [10-20]. It is also known that the dipetides retain their coordinating properties in ternary complexes also and hence two types of ternary species are generally formed at different pH. The formation constant of the above ternary complexes have been reported and formation of different species have been shown in species distribution curves (fig 2A.6) in chapter IIA earlier.

Neither the dipeptide nor the amino-acids used for this work show any peak in the absence of metal ions in the potential range +0.2V to -1.0V. The general features of the cyclic voltammograms are discussed below:

(i) At a low pH ($\sim$ 3.0) a cathodic peak at about -0.03V is observed in the first cathodic scan and a corresponding anodic peak at about +0.045V is observed during the reverse scan (fig. 3.1.1). Species distribution curves show that most of Cu(II) is present in the form of free ion at this pH. Hence the peak observed can be attributed to the electrode process:

$$Cu^{+2} \underset{-2e}{\overset{+2e}{\rightleftharpoons}} Cu^{\circ}$$

(ii) At an intermediate pH ($\sim$ 5.0) a broad cathodic peak at about -0.06V and a corresponding anodic peak at about +0.045V is observed. Another

cathodic peak at -0.30V is also observed during the cathodic scan. The corresponding reverse scan gives a very broad peak in the potential range -0.10V to -0.22V (fig. 3.1.2). Species distribution curves show the presence of number of binary Cu-gg, CuL, $Cugg_{-H}$, CuL_2 and ternary CuggL complexes in appreciable concentration at this pH. The cathodic peak at less negative potential and the corresponding anodic peak at a positive potential is attributed to the following oxidation and reduction processes :

$$Cu(II)gg/Cu(II)L \underset{-e}{\overset{+e}{\rightleftharpoons}} Cu(I)gg/Cu(I)L$$

The peak at comparatively higher negative potential is due to the electrode processes :

$$Cu(II)gg_{-H}/Cu(II)L_2/Cu(II)ggL \underset{-e}{\overset{+e}{\rightleftharpoons}} Cu(I)gg_{-H}/Cu(I)L_2/Cu(I)ggL$$

(iii) At a high pH ($>$ 7.0) a single cathodic peak is observed at about -0.36V during the first cathodic scan. In the reverse scan a broad anodic peak in the potential range -0.12V to -0.24V is observed (fig. 3.1.3). The cathodic peak is due to the reduction of the ternary species $Cu(II)gg_{-H}L$ to $Cu(I)gg_{-H}L$. This species is reduced at a higher negative potential, compared to Cu(II)ggL species. This is because in the $Cu(II)gg_{-H}L$ species there is coordination of strong σ-donor, the deprotonated peptide N^- of the dipeptide. This increases the electron density around the metal centre and hence reduction of Cu(II) to Cu(I) is difficult.

The anodic peak observed, should then be due to the oxidation of the above mentioned ternary species. However, the peak is very broad indicating that it must be due to overlapping of oxidation potentials of two or more species. A fraction of the $Cu(I)gg_{-H}L$ species may decompose into $Cu(I)gg_{-H}$

since Cu(I) will prefer coordination from tridentate gg_{-H} ligand rather than pentacoordination due to both gg_{-H} and L ligands. Overlapping of the oxidation potentials of Cu(I)gg_{-H}L and Cu(I)gg_{-H} species results in peak broadening.

Another oxidation peak at about +0.03V is also observed during the reverse scan. This corresponds to the oxidation of free Cu° to Cu^{2+} ions, though there is no free copper in the solution initially at such a high pH. A new cathodic peak at about -0.02V is apparent from second scan onwards. This can be explained by considering that the Cu(I)gg_{-H}L species formed during the first cathodic scan, being unstable, disproportionates into Cu(II) gg_{-H}L and Cu(o) species. The peak at +0.03V is due to oxidation of electrogenerated Cu(o) species to Cu^{+2} ions. The cathodic peak at -0.02V, apparent from second scan onwards, corresponds to reduction of the electrogenerated Cu^{+2} ions to Cu(o). The ternary species Cu(I)ggL formed at lower pH should also disproportionate into Cu(II)ggL and Cu(o). However, the reduction and the oxidation peaks of the electrogenerated Cu(o) species overlap with those of the binary Cugg and CuL species and hence electrogeneration of Cu(o) cannot be detected.

It is observed that disproportionation of Cu(I) species is scan rate dependent. As the scan rate becomes faster than the time scale of the disproportionation of Cu(I) complex, the extent of disproportionation goes down. This decreases the concentration of electrogenerated Cu(o) and hence the peak heights for both the backward and forward process Cu(o) (electrogenerated)$\longrightarrow Cu^{+2}$ decreases.

II. Copper(II) - Histidine (hist) Complexes:

Histidine is ambidentate in nature [21, 22]. A number of potentiometric studies by various group of workers have revealed that at low pH, histidine

coordinates via its amino nitrogen and carboxylate oxygen, whereas its imidazole nitrogen remains free, resulting in the formation of Cuhist H complex. At higher pH, coordination occurs through amino nitrogen and imidazole nitrogen with weak coordination through carboxylate oxygen, resulting in Cuhist species. The coordination sites of histidine was further confirmed by spectral studies [23]. It was thought of interest to study the coordinating properties of histidine and the formation of two types of binary species electrochemically.

The general features of cyclic voltammograms of binary copper(II) - histidine systems are as follows:

(i) At low pH ($\sim$3.0), the cathodic peak at about - 0.065V and an anodic peak at -0.01V during the reverse scan (fig.3.2.1) is attributed to the electrode processes :

$$Cu^{+2} \underset{-2e}{\overset{+2e}{\rightleftharpoons}} Cu^{\circ}$$

$$Cu(II)\ histH \underset{-e}{\overset{+e}{\rightleftharpoons}} Cu(I)\ hist\ H$$

(ii) At an intermediate pH ($\sim$ 4.28) a broad cathodic peak at about -0.13V and a corresponding anodic peak at +0.05V is observed. Another cathodic peak at a higher negative potential -0.30V is also observed during the first cathodic scan. A corresponding anodic peak at about -0.20V is obtained (fig.3.2.2). Species distribution curves [23] show the presence of the binary species CuhistH, Cuhist and CuhistHhist at this pH. The peaks at less negative or positive potentials is attributed to the redox processes:

$$Cu(II)histH/Cu(II)hist \underset{-e}{\overset{+e}{\rightleftharpoons}} Cu(I)histH/Cu(I)hist.$$

Whereas the redox peaks observed at more negative potential is attributed to the electrode process :

$$Cu(II)hist\ H\ hist \underset{-e}{\overset{+e}{\rightleftharpoons}} Cu(I)hist\ H\ hist$$

(iii) At a higher pH ($\sim$ 6.10), a cathodic peak is observed at -0.30V (fig.3.2.3) which is attributed to the reduction process Cu(II) hist H hist $\xrightarrow{+e}$ Cu(I) hist H hist. The anodic peak should be due to the reverse oxidation process. However, this peak is very broad, with a potential range -0.06V to -0.16V. This may be due to the fact that Cu(I) histHhist decomposes to Cu(I) histH and Cu(I) hist and overlapping of the oxidation potentials of these complexes results in peak broadening.

Another cathodic peak observed at about -0.44V is attributed to the reduction process $Cu(II)(hist)_2 \xrightarrow{+e} Cu(I)\ (hist)_2$. The corresponding anodic peak observed at about -0.36V is due to the oxidation of the Cu(I) $(hist)_2$ species to $Cu(II)(hist)_2$. The complexes [Cu(I)histHhist] or [Cu(I) $(hist)_2$], in which one or both the histidine molecules are coordinated through amino nitrogen and imidazole nitrogen , do not undergo disproportionation generating Cu(o) at the mercury electrode as observed in case of Copper(I) - amino acid or Cu(I) - dipeptide complexes. This is because imidazole coordination of histidine stabilises the Cu(I) state and hence there is no disproportionation.

III Copper(II)-Histidine-Amino Acids(L) Systems :

Potentiometric studies [24, 25] of ternary complexes of histidine with a variety of secondary liganda(L) have revealed that two types of ternary species are formed at different pH. [Cuhist HL] is formed at lower pH,

where histidine is coordinated through its amino acid end and the ternary species [CuhistL] is formed at higher pH, where histidine is coordidnated through histamine end. The general features of the cyclic voltammogram of the ternary complexes involving histidine and other aminoacids are discussed below :

(i) At a low pH ($\sim$ 3.0), the cathodic and the corresponding anodic peak obtained at about -0.03V and -0.015V, respectively, is due to the reduction and subsequent oxidation of free Cu^{+2} ions (fig. 3.3.1).

(ii) At an intermediate pH ($\sim$ 4.5) a broad reduction peak at about -0.13V and the corresponding oxidation peak at about +0.05V (fig.3.3.2) is observed. Species distribution curves [25] show the presence of different binary systems [CuhistH], [Cuhist], [CuL] and [CuL_2] and also the ternary species [CuhistHL] in appreciable concentration at this pH. The above mentioned peaks correspond to the redox processes.

$$\text{Cu(II)histH/Cu(II)hist/Cu(II)L} \underset{-e}{\overset{+e}{\rightleftharpoons}} \text{Cu(I)histH/Cu(I)hist/Cu(I)L}$$

Another broad reduction peak observed at -0.30V is attributed to the reduction processes

$$\text{Cu(II)}L_2\text{/Cu(II)histHhist/Cu(II)histHL} \underset{-e}{\overset{+e}{\rightleftharpoons}} \text{Cu(I)}L_2\text{/Cu(I)histHhist/Cu(I)histHL.}$$

The corresponding oxidation peak is observed at a potential range -0.12V to -0.20V. The peak broadening occurs due to the overlapping of the reduction and oxidation potentials of the different species present at that pH.

(iii) At a higher pH ($\sim$ 6.61) a cathodic peak corresponding to the reduction

processes

$$Cu(II)\ (hist)_2/Cu(II)\ hist\ L \xrightarrow{+e} Cu(I)\ (hist)_2/Cu(I)hist\ L$$

is observed, at about -0.44V. The oxidation peak for the reverse process is observed in the potential range -0.20V to -0.40V (fig. 3.3.3).

The oxidation and the reduction peaks for the process Cu° (electrogenerated) $\underset{+2e}{\overset{-2e}{\rightleftharpoons}} Cu^{+2}$ as observed in case of the ternary system Cu(II)ggL is not observed for Cu(II)histL systems. This is similar to that in case of Cu(II)hist complex and can be attributed to the imidazole coordination of histidine stabilising the copper(I) state. Hence, there is no disproportionation of Cu(I)histL species and no electrogeneration of Cu(o).

This is probably the reason that the imidazole bound type-III copper sites in the copper protein oxidases only involve Cu(II)/Cu(I) redox process.

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