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METHIONINE - GLYCINE - COPPER (II) MIXED COMPLEXES. A CYCLIC VOLTAMMETRIC STUDY

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SUMMARY

Cyclic voltammetric studies on the reduction of the title complexes were carried out in acetic acid-sodium acetate buffer at the HMDE. The mechanism of these processes is discused. The complex species in solution of this system have been determined, depending on methionine and glycine concentrations, pH values and potential scan rate.

INTRODUCTION

Mixed amino acid peptide copper (II) complexes have received considerable attention because of their ocurrence and involvement in the transport of copper (II) ions in biological systems (1). Several ternary amino acid or peptides complexes of copper (II) have been described in both solution and solid state (2-5).

Multiligand-multimetal equilibria are quite common in biological systems since millions of potential ligands compete for metal ions found in vivo (6-14).

Copper is one of the most important trace elements for plants and animals (15-21). In a number of biological processes copper (II) is involved in mixed-ligand complex formation and ligand catalysed complex formation reactions (22).

The major copper-amino acid complex found in the serum ultrafiltrate is copper-L-histidine. Along with this complex, several ternary complexes involving amino acids are present in human serum. Copper bound to albumin is in rapid equilibrium with tissue copper and is hence considered to be the inmediate

transport form of copper in blood. Various equilibria governing this transport can be represented as:

Copper + Amino acid $\rightleftharpoons$ Copper - Amino acid

Copper-Amino acid+Albumin $\rightleftharpoons$ Albumin-Copper-Amino acid

Albumin-Copper-Amino acid $\rightleftharpoons$ Albumin-Copper+Amino acid

The above mechanism could play an important role in the exchange of copper between a macromolecule and a low-molecular-weight substance, which in turn may help its transport across the biological membrane. Kinetic analysis did show that the ternary complex involving aminoacids plays an important role in the exchange and transfer of copper (II) between amino acid and albumin (23).

Except for a short report on the reduction of some histidine containing mixed amino acid copper (II) complexes by polarography, we have no report about the redox behaviour of these complexes. In view of the above facts, studies on mixed ligand complexes of copper (II) involving amino acids are of great importance in biological processes (24).

This report describes studies on the reduction of methionine containing ternary copper (II) glycine complexes by cyclic voltammetry.

INSTRUMENTATION

Voltammograms have been recorded using a Amel 563 polarograph coupled with a Hewlett-Pachard 7047 A X-Y recorded. The three electrode cell was equipped with a hanging mercury drop electrode as working electrode and a platinum auxiliary electrode. The reference electrode is a satured calomel electrode to which all the potentials are referred. Experiments have been carried out at 25° C ± 0.1° C in a cell Thermostated by a Colora M.B. Ultrathermostat. pH are measured using a Beckman Expandomatic SS-2.

REAGENTS

All the reagents used were of analytical grade.

Supporting electrolytes, made of 0.25 M sodium perchlorate and 0.1 M acetic acid-sodium acetate buffer, are prepared in double distilled water. Oxygen is removed from the solution by bubbling oxygen-free nitrogen before each measurement.

RESULTS AND DISCUSSION

I- RESULTS FROM SOLUTION OF DIFFERENT pH VALUES

I-a) Cu(II) (Methi) (Glyc) solutions in unbuffered medium

Cyclic voltammetry (CV) of these ternary complexes is performed in aqueous solutions a pH= 5.5 using $NaClO_4$ as supporting electrolyte.

Figure 1 represents the CV behaviour of the reduction of methionine containing mixed amino acid Cu(II) complex, Cu(Methi) (Glyc).

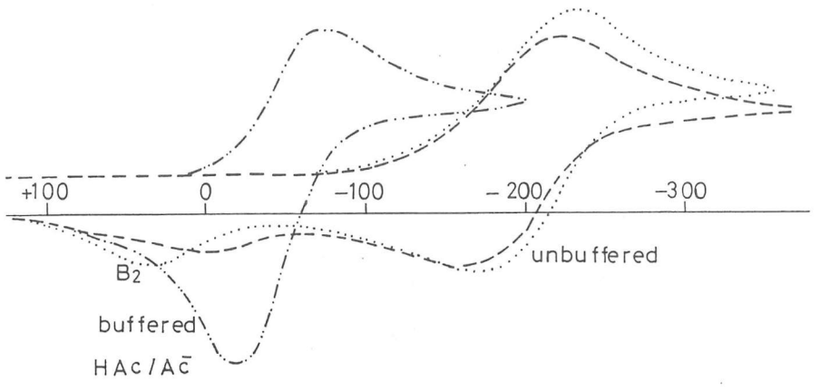


Fig. 1.- Voltammograms of Cu(II) 5.10^{-4} M in $NaClO_4$ at pH=5.5
A(---)Methi=8.10^{-3} M. Glyc=8.10^{-3} M-. B(....)Glyc=8.10^{-3}
C(..-..)Methi=8.10^{-3} M. Glyc=8.10^{-3} M

I-b) Cu(II)(Methi) (Glyc) solutions in buffered medium

When the pH value of solutions containing Cu(II) (Methi) (Glyc) is fixed with acetic acid and sodium acetate, it is first noted that peak B_2 (Fig.1) disappears. The oxidation peak B_2 remains and becomes more evident at pH values close to the methionine isoionic point. The appearance of this oxidation peak implies a decreases the i_p value corresponding to the first oxidation peak. The experimental conditions and the results obtained are shown in Table I. Fig. 2 shows the voltammograms at different pH values.

TABLE I

Voltammetric results from solutions at different pH values:

a) With excess methionine

Methi=2.10^{-3} M

Glyc= 10^{-3}M Cu(II)=5.10^{-4} M and $NaClO_4$=0.25 M

Buffered solution :AcH/Ac^-

pH	i_{pc}/ A	i_{pa}/ A	i_{pa}/i_{pc}	E_{pc}/mV	E_{pa}/mV	ΔE_p
4.2	5.7	9.3	1.6	-12	+38	50
4.5	4.7	7.8	1.6	-14	+36	50
5.1	5.3	7.8	1.4	-40	+16	56
5.5	5.1	6.4	1.2	-62	0	62
5.7	5.3	6.7	1.2	-58	+6	64
6.2	4.5	5.5	1.2	-90	-12	78

b) With excess glycine

Mehti=2.10^{-3} M

Glyc=8.10^{-3} M

4.5	5.6	9.4	1.6	-32	+24	56
5.1	7.0	10.3	1.4	-62	0	62
5.3	7.1	10.5	1.4	-66	0	66
5.7	5.9	8.5	1.4	-86	-22	64
6.2	5.4	8.1	1.5	-122	-46	76

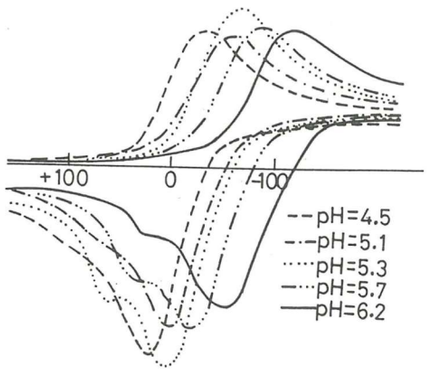


Fig.2.- Voltammograms of Cu(II) 5.10^{-4} M + Methi + Glyc in $NaClO_4$ at different pH values fixed with HAc/Ac^-.

It is observed that there is a shift of E_p to more negative values, when the methionine or glycine concentrations increase.

In a pH range between 4.2-5.2 there is no change in the peak form for both the cathodic and the anodic peaks.

ΔE_p values are close to 55 mV and the i_{pa}/i_{pc} ratio is greater than one. The E_p values in this pH range are of the same magnitude as those for Cu(II)/Cu redox process in this medium without complexing agent.

In a pH range between 5 and 6.2, E_{pc} values shift to more negative values when the methionine or glycine concentrations increase. (Fig. 3).

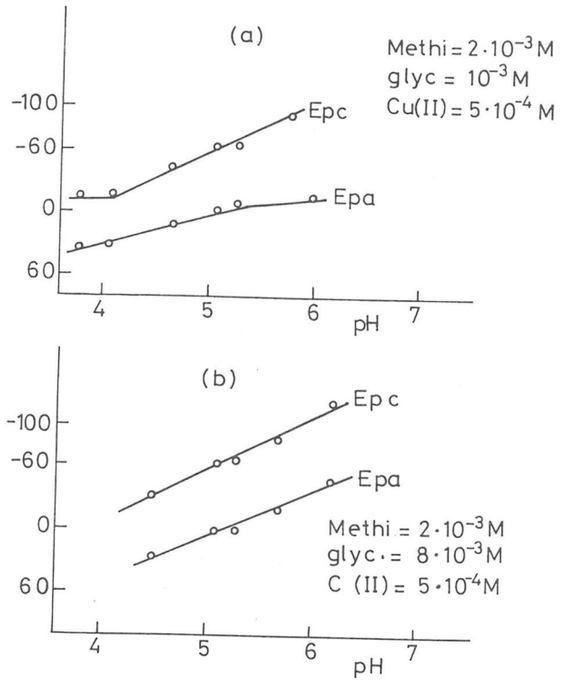


Fig.3.- Variation of E_p values versus pH.
a) With excess of Methi; b) with excess of Glyc.

In a pH range between 4.5 and 5.0 a linear variation of E_{pc} and E_{pa} was found (Fig. 3). The slopes of these plots are

about 48 mV. Around pH 5.3 - 6.2 the i_{pa}/i_{pc} ratio decreases slightly and a second oxidation peak is obtained at more positive potentials.

Owing to the fact that when the OH^- concentration is increased the voltammograms shape is changed, the second oxidation peak either does not appear, or is very small, and a big shift of the potential values is observed. (Fig. 2).

In view of the above facts, the bond between Cu(II) and methionine-glycine is labile in and HAc/Ac^- medium and OH^- ions qare able to compete with the amino acid molecules joining to Cu(II).

II- VARIATION OF METHIONINE, GLYCINE CONCENTRATIONS

It is known that for the $Cu(Glyc)_2$ type complexes, the overall electrode processes have been indentified as resulting from the following reactions sequences (26):

$$Cu(II)(Glyc)_2 \overset{+1\bar{e}}{\rightleftharpoons} Cu(I)(Glyc)_2 \qquad (1)$$

$$Cu(I)(Glyc)_2 \longrightarrow Cu^{\circ}\ (Hg) + 2Glyc \qquad (2)$$

$$Cu^{\circ}\ (Hg) \overset{-2\bar{e}}{\rightleftharpoons} Cu(II)\ aq. \qquad (3)$$

The important step in these reactions is the production of the intermediate Cu(I) species, which can either be reoxidised to Cu(II) or undergo chemical reactions to generate $Cu^{\cdot}$ (Hg) which subsequentely can undergo a two -electron oxidation. The presence of peaks (22) corresponding to equation (1) and (3) shows that a certain fraction of the Cu(I) species is reoxidised while the other fraction undergoes chemical reactions (22). The Peaks of oxidation and reduction D and C result from the redox process described in equation (1), while peaks B and A result from the process shown in equation (3) (26).

II-a) With excess methionine

I.E. curves were obtained with different methionine concentrations, from solutions with the same Cu(II) and glycine concentrations at same pH values.

The relevant cyclic voltammetric data for Cu(II) (Methi)(Glyc) are given in Table II and Fig. 4(a).

TABLE II

Voltammetric results from solution at different methionine and glycine concentration values.

a) With excess methionine

Glyc=10^{3} M

Cu(II)=5.10^{4} M.$NaClO_4$=0.25 M.AcH/Ac

	Methi	i_{pa}/ A	i_{pc}/ A	E_{pa}/mV	E_{pc}	i_{pa}/i_{pc}	ΔE_p	$\delta E_p/\delta$ log.Methi.
pH=5,5	$2x10^{-3}$M	6.2	4.8	-12	-68	1.2	56	
	$4x10^{-3}$M	7.0	5.3	-8	-76	1.3	58	
	$6x10^{-3}$M	6.1	4.5	-28	-86	1.3	58	
	$8x10^{-3}$M	5.7	4.7	-36	-94	1.2	58	-29 mv.
	10^{-2}M	5.8	5.0	-38	-94	1.1	56	
	$1,2x10^{-2}$M	4.5	4.5	-30	-82	1.0	62	
pH=6,2	10^{-3}M	6.8	5.1	+10	-86	1.3	96	
	$2x10^{-3}$M	5.5	4.4	-26	-104	1.2	78	
	$3x10^{-3}$M	5.2	4.0	-36	-116	1.3	80	-42 mv.
	$4x10^{-3}$M	5.3	4.3	-44	-112	1.2	68	
	$6x10^{-3}$M	4.7	4.0	-54	-118	1.1	64	

b) With excess glycine

Methi.=10^{-3}M

pH=5,5	$2x10^{-3}$M	7.8	5.4	-2	-60	1.4	58	
	$4x10^{-3}$M	7.6	5.0	-14	-80	1.5	66	
	$6x10^{-3}$M	8.0	5.7	-24	-88	1.4	64	-31 mv.
	$8x10^{-3}$M	8.3	5.9	-22	-86	1.4	64	
	10^{-2}M	8.0	5.5	-26	-86	1.4	60	
	$1,6x10^{-2}$M	8.6	5.9	-32	-90	1.4	58	
pH=6	$2x10^{-3}$M	6.3	4.5	-12	-86	1.4	74	
	$4x10^{-3}$M	7.8	5.6	-30	-104	1.3	74	
	$6x10^{-3}$M	6.9	4.9	-42	-114	1.4	72	-50 mv.
	$8x10^{-3}$M	7.3	5.1	-42	-110	1.4	68	
	10^{-2}M	7.8	5.8	-54	-124	1.3	70	
	$1,6x10^{-2}$M	7.2	5.2	-64	-132	1.3	68	

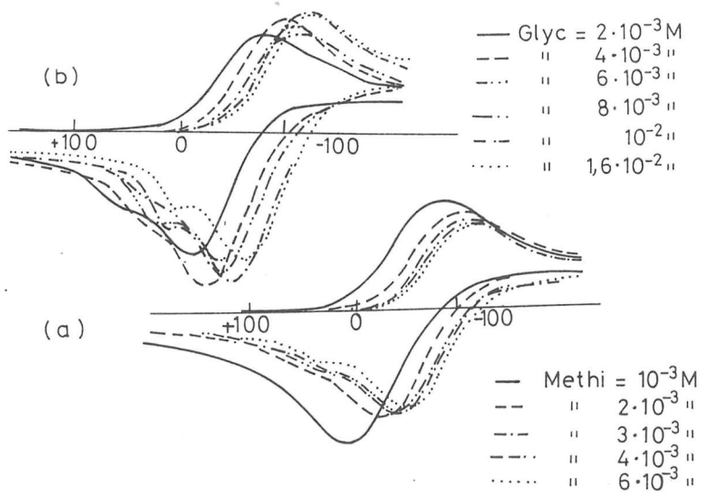


Fig.4.- Voltammograms of Cu(II) 5.10^{-4}:a) at different Methi concentration and Glyc=10^{-3}M; b) at different Glyc. concentration and Methi=2.10^{-3}M

At pH= 4.5 the E_p values become more negative as the methionine concentration increases, with constant Cu(II) concentrations and pH value.

The plot of E_p versus log(Methi) is a straight line and the slope value is -30 mV and about -60 mV when the pH reaches 5.0.

For the complex species, the slope values could be in agreement with a coordination number, between zero and one, if the process is assumed to be as described below (25):

$$Cu(II)(X)_p \xrightarrow{+2\bar{e}} Cu + pX;\ X = \text{ligand}$$

If the reduction process is:

$$Cu(II)(X)_p \xrightarrow{+1\bar{e}} Cu(I)(X)_q + (p-q)X$$

zero and one should be (p-q) values.

At pH 5.5-6.2 the plot E_p versus log(Methi) is a straight line (Slopes -29 mV and -42 mV).

The ΔE_p value increases with the pH and is approximately constant with the methionine concentration.

At pH value close to 6.2, E_p values shift to more negative values, but general behaviour is similar to that observed at lower pH's (Slope -62 mV).

The voltammograms at different methionine concentrations for all pH's (Fig. 4 (a)) also show the second oxidation peak, which appears when the methionine concentration and pH values increase. It is in these conditions that the Cu(II)(Methi)(Glyc) complex becomes more stable.

All the results lead to the conclusion (25) that the Cu(I)(Methi)(Glyc) complex must be considered as an intermediate compound in the electrochemical reduction of Cu(II)(Methi)(Glyc) to copper metal. Other authors have reached this same conclusion for other ligands (26-28). This Cu(I)(Methi)(Glyc) complex is unstable and dismutation steps will take place.

II-b) With excess glycine

I-E curves were obtained with different glycine concentrations from solutions with the same Cu(II) and methionine concentrations and pH value.

Voltammetric results are given in Table II (b) and Fig. 4(b).

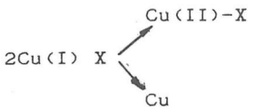
When an amino acid is added to the solution containing Cu(II) and methionine, the E_p shifts to a more negative value, which indicates the formation of the ternary complexes (20). At all pH's values the E_p values become more negative when the glycine concentration increases. These E_p values are higher at pH between 6 - 6.5.

The plot of E_{pc} against log(Glyc) is a straight line and the slope increase with the pH value. These slopes are: -31 mV to pH= 5.5 and -50 mV to pH= 6.

The ΔE_p value increases with the pH but is practically the same when the glycine concentration increases (Table II(b)) at a potential scan rate of 20 mV/s. On the other hand, i_{pa}/i_{pc} rations were higher than one, under equal experimental conditions.

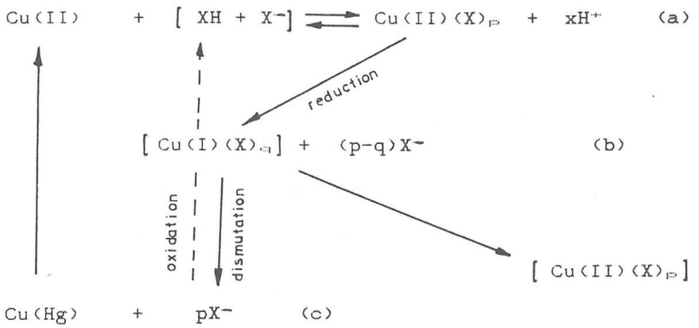
The voltammograms at different glycine concentrations, for all pH's values, also show the second oxidation peak wich appears when both glycine concentration and pH increase (Fig. 4(b)). In a previous paper (25) we have concluded that:

the $Cu(I)(Methi)_q$ complex must be considered as an intermediate compound in the electrochemical reduction of $Cu(II)(Methi)_p$ to copper metal. This $Cu(I)(X)_q$ complex is unstable and a dismutation step takes place:



Three steps can be considered for the reduction and oxidation of the copper - methionine system in HAc/Ac⁻ medium and pH values between 4.5 and 6.5.

The process should be similar (25) for the Cu(II)(Methi)(Glyc) complex:



At low potential scan rates, step a-b is favoured by increasing amino acids concentration and pH.

The second sharp oxidation peak appearing in the voltammograms could either correspond to the oxidation of copper metal and amino acids adsorbed at the electrode to Cu(I)-(X) (step c-b) or to step (b-a), which is the oxidation of Cu(I)-(X)(ad) to Cu(II)-(X). In others papers, other investigators have reached to the same conclusion for other ligands (26-28).

When the scan rate is increased this second oxidation peak disappears and the curves are different.

On the other hand, the ΔE_p values increases with the pH and the amino acids concentrations (Table III). The process is less reversible and, under these conditions, the redox process that takes place is step(a-c) and conversely (c-a).

Since the deprotonated form of the amino acids increases with the pH, the number of protons in the process (x) changes from 1 to zero. Likewise, the (p-q) value changes from 1 to zero when the pH increases, as shown by the experimental results.

In the pH range between 4.5-6 in low potential scan rate the first oxidation waves corresponds to the oxidation of Cu(Hg)→Cu(II) X and the i_{pa}/i_{pc} ratio is higher than one, this

value decrease when increase the pH and amino acids concentration. In fact, these are the best conditions for Cu(I) X stability.

The process at low potential scan rate at pH 4.5 - 6 is reversible and quasi reversible when pH= 6 - 6.5.

III- VARIATION OF POTENTIAL SCAN RATE

In order to confirm steps a,b,c assumed before, a study at different potential scan rate, v, was carried out.

III-a)- At low potential scan rate: 2-20 mV/s

At pH 4.5 - 6.1 i_{pc} and i_{pa} are practically constant or there is a slight increase with the scan rate. $i_{pc}/ v^{1/2}$ is independent of v and $i_{pa}/ v^{1/2}$ decreases when v increases.

These results are in agreement with the reduction process controlled by diffusion and the oxidation of Cu(Hg). Therefore, taking into account the previously presented process at this pH value it can be deduced that the first oxidation peak belongs to a C.E. process (27,28).

On the other hand, the CV for the ternary Cu(II)(Methi)(Glyc) complexes (Table III(a), fig.5(a)) in the pH range between 4.5 and 6 at low potential scan rate, shows a first peak of oxidation that increases at low scan rate.

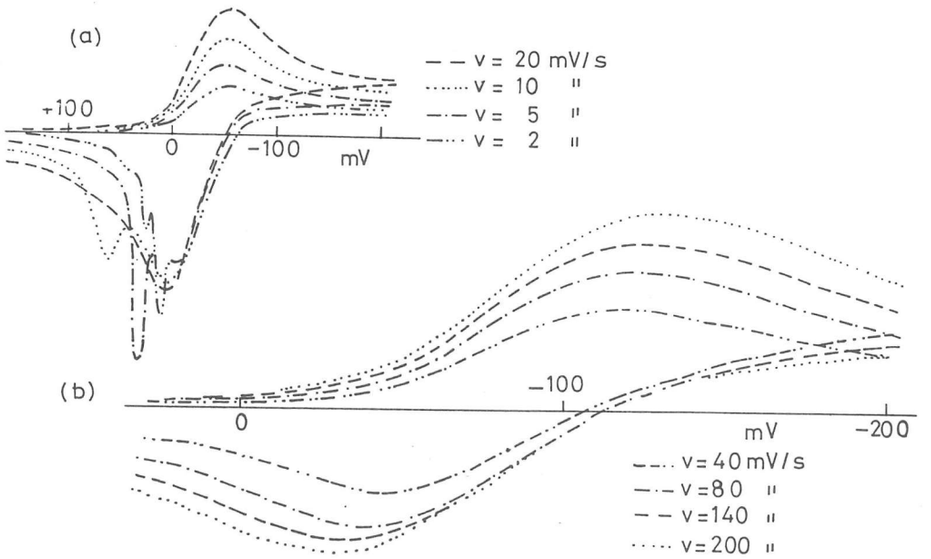


Fig.5.- Voltammograms of Cu(II) 5.10^{-4}M. Methi.=8.10^{-3}M. Glyc.=2.10^{-3}M
a) Influence of the potential scan rate v= 2-20 mV/s.
b) Influence of the potential scan rate v= 40-200 mV/s.

TABLE III

Influence of potential scan rate in different pH values.
Methi.=2.10^{-3}M ;Cu(II) =5.10^{4} M ;$NaClO_4$ =0.25 M

Glyc. =10^{-3} M.

a) v =2-20 mV/s.

	v/mV/s	i_{pc}/ A	$i_{pc}/v^{1/2}$	E_{pc}/mV	i_{pa}/ A	$i_{pa}/v^{1/2}$	E_{pa}/mV	i_{pa}/i_{pc}	ΔE_p/mV
pH=5	2	1.9	1.3	-62	7.6	5.3	-8	4.0	48
	5	2.7	1.2	-60	7.5	3.3	-10	2.7	50
	10	4.0	1.2	-60	7.9	2.5	-8	1.9	52
	20	5.0	1.1	-62	7.8	1.7	-8	1.5	54
pH=6,2	2	1.9	1.3	-112	4.8	3.4	-38	2.5	74
	5	2.7	1.2	-110	5.0	2.2	-36	1.8	74
	10	3.7	1.1	-110	5.6	1.7	-40	1.5	70
	20	5.2	1.1	-112	6.1	1.3	-40	1.1	72

b) v= 40- 200 mV/s. Methi.=2.10^{-3} M; Glyc.=8.10^{-3}M.

pH=5	40	3.3	0.51	- 56	3.2	0.51	+20	0.9	66
	60	3.8	0.49	- 57	3.5	0.45	+26	0.9	70
	80	4.2	0.46	- 61	3.6	0.40	+30	0.8	76
	120	4.8	0.44	- 69	3.7	0.34	+32	0.7	85
	140	5.4	0.45	- 74	4.1	0.34	+30	0.7	89
	180	6.1	0.45	- 75	4.2	0.31	+36	0.7	91
	200	6.7	0.47	- 77	4.6	0.32	+56	0.7	93
pH=6,1	40	3.0	0.47	- 120	2.6	0.41	-90	0.8	75
	60	3.5	0.45	- 123	3.0	0.38	-88	0.8	79
	80	4.3	0.48	- 124	3.5	0.39	-80	0.8	84
	100	4.4	0.44	- 126	3.6	0.36	-78	0.8	87
	120	4.6	0.42	- 129	3.7	0.33	-70	0.8	94
	140	5.1	0.43	- 130	3.9	0.33	-62	0.7	99
	180	5.8	0.43	- 135	4.2	0.31	-56	0.7	107
	200	6.0	0.42	- 136	4.4	0.31	-52	0.7	110

III-b)- At potential scan rate: 40-200 mV/s.

The relevant CV data for the complexes are given in Table III(b), fig.5(b). When the potential scan rate was 40-200 mV/s., all the results lead to confirm that the process under consideration was a-c in the whole pH interval.

For the highest scan rates, as is well known, at higher scan rates the amount diffusing material is relatively small when compared with the adsorbed material reduced at the electrode.

So adsorption is predominant in relation to the diffusion mechanism. This behaviour is characteristic of weak adsorption when there is reversible charge transfer, the rate of adsorption-desorption is sufficientely fast in terms of the time scale of the technique, and the initial potential is at least 200/n mV more positive than the first peak(30). The ratio i_{pa}/i_{pc} decreases with the scan rate. For the range between 40-200 mV/s. the potential of the peak E_{pc} shifts cathodically by about 25-35 mV for the complexes with methionine-glycine. On the other hand, the difference $E_{pc}-E_{pa}$ = ΔE_p tends to a increase with the scan rate. For these complexes the cathodic peaks are perturbed depending on the

time of contact of the electrode specially in terms of width since they are broader. So, in this case, although there is probably adsorption superimposed on a kinetic mechanism, the first effect is more pronounced.

If the amino acids can be adsorbed as electroinactive substances, they may influence the rate of the reduction process by a steric effect. There is also the possibility of adsorption of the complexes themselves on the electrode. A competition between the two mechanisms is also possible.

Several authors have studied adsorption of amino acids (30-32) and have noticed that when there are short hydrocarbon chains as, for example, in glycine, the adsorption near the potential of zero charge p.z.c. is negligible and there is an increase of capacity at both ends of the curve (Cs vs E) (33), as opposed to what happens with the amino acids with long hydrocarbon chains where there is a decrease in capacity near the p.z.c.

At higher positive or negative potential the amino acid molecule is adsorbed with the zwitterion group more or less oriented, with the positive end (at negative potentials) or its negative end (at positive potentials) toward the electrode surface.

It has also been noticed that the increase of the polar character of amines or acids results in a decrease of their surface activity. So generally we can conclude that for organic compounds the neutral species are more adsorbed than the charged ones, since mercury is an hidrophobic substance uncharged species have more affinity for it. On the other hand, physical forces of an electrostatic character have to be considered since the electrode has a charge and the molecules have positive or negative groups.

It has been noticed (33) that the amino-acids are adsorbed mainly on the positive side of the maximum of the electrocapillary curve, which is consistent with general adsorption behaviour, that is, the anions are preferentially adsorbed. However, with the exception of methionine and other amino acids the capacitance curves (33) cannot be distinguished from each other within the experimental errors. This shows that the amino acids either of HL or HL^{-} type are weakly adsorbed on the mercury, as was also observed by Bangh and Parsons (31), unless there is a sulphur group in the structure (methionine) or the bonds (histidine) or a longer hydrocarbon chain. The complexes Cu(II)(Methi)(Glyc) are not strongly adsorbed on mercury since they are charged (33). However, there is a weak but net adsorption probably due to

the adsorption of the ligand itself trough the sulphur group of methionine.

The adsorption of the ligand itself can influence the reduction process of copper ion from dissociation of the complex by a steric effect, or, as the amino acids group is directed to the aqueous solution, the copper complex can be formed directly reduced on the mercury.

On the other hand, when potential scan rate was 40-200 mV/s. the ΔE_p could correspond to a quasi reversible two-electron process.

CONCLUSIONS

The acetic acid inhibits the formation of chelate (31) type complexes between amino acids and Cu(II). This is due to the fact that a bond can be formed through a hydrogen bridge, between the acetate ion and the nitrogen of amino acids.

The reduction process of Cu(II) and methionine-glycine are adsorbed on the mercury electrode. An oxidation peak, due to the adsorbed amino acids, is observed.

The reduction process of Cu(II)(Methi)(Glyc) in HAc/Ac⁻ medium, to copper metal, ,is through an intermediate Cu(I) amino acids. This species involves a dismutation step (27-28).

At low potential scan rate the system Cu(II)(Methi)(Glyc) exhibits a reversible process, while at higher values of potential scan rate, the ΔE_p values elucidate a quasi reversible two-electron process.

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