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Complex Formation of Zinc(II) Ion with Glycine, N-acetyl- and N-benzoyl-glycine Anions in Aqueous and Ethanolic Solution by Polarographic Method

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The complex formation reactions of glycine, N-acetyl- and N-benzoyl-glycine with the Zn(II) ion were investigated using the polarographic method in aqueous and ethanolic solution to identify the number and type of complex species present in solution and to calculate their stability constants. In aqueous basic solution (pH 6–9), only the glycinate anion reacts with the metal ion forming three complexes of the type ZnL^+, ZnL_2 *and* ZnL_3^-. *On the contrary in ethanolic solution all the three amino acids react; the N-protected amino acid anions form four complexes of the type* ZnL^+, ZnL_2, ZnL_3^- *and* ZnL_4^{2-}, *while in the presence of the glycine anion two complexes only of the type* ZnL^+ *and* ZnL_2 *were identified. The results obtained for these systems in aqueous and ethanolic solution, discussed in comparison with those of the corresponding cadmium(II) systems, also suggest an amino acid coordination to the metal ion through different coordination sites in the solvents examined.*

Introduction

Although zinc and cadmium show some common physiochemical properties, they differ widely in biological systems. Zinc is an essential element, has a short biological half-time and is non-toxic, while cadmium is not essential, has an extremely long biological half-time and is toxic. While the chemical behavior of these metals is quite similar, cadmium is always present as an impurity in the zinc compounds and may give rise to interferences with zinc-dependent biological systems, especially with enzyme systems [1, 2].

In this paper we study the type and number of complexes present in solution, the properties connected with the electron-transfer process and the solvent effect on the zinc(II)–glycinate, –N-acetyl- and –N-benzoyl-glycinate systems in aqueous and ethanolic solutions and the results are compared with those previously found for the analogous cadmium(II) systems.

Experimental

The complexes were prepared as reported in refs. 3 and 4. All the other chemical products were of analytical reagent grade and used without further purification to investigate the systems in aqueous solution. For the study of the systems in ethanolic solution, anhydrous ethanol (C. Erba RSE) was used, and the salts were recrystallized from this solvent before use.

The polarographic solutions were prepared using zinc(II) perchlorate hexahydrate ($5 \cdot 10^{-4}$ *M*), $NaClO_4$ (0.1 *M*) was used as base electrolyte to keep the ionic strength constant ($\mu = 0.1$). The ethanolic solutions of the amino acids contained an equimolar amount of sodium hydroxide.

The polarographic measurements were carried out using an Amel Multipolarograph Model 471 at 25 ± 0.5 °C and at a drop-time of 2.0 sec. S.C.E. was used as a reference electrode in aqueous solution; a saturated Ag/AgCl, KCl electrode in ethanolic solution. However all the $E_{1/2}$ values were referred to S.C.E.

Results and Discussion

Zinc(II)–Amino Acid Systems in Aqueous Media

The polarographic findings of the zinc(II)–amino acid complexes in aqueous solution, as plotted in Fig. 1, show that only in the pH range of 6–9 and only the glycine forms complexes with zinc (II) ion. In fact the $E_{1/2}$ values of the N-protected amino acid systems correspond to the $E_{1/2}$ of the zinc(II) ion in the same experimental conditions and do not depend on the pH conditions.

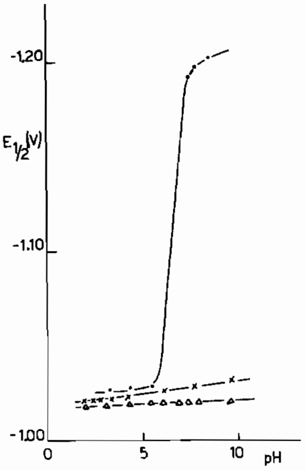

Fig. 1. Plot of pH *vs.* E for the Zn(II)–amino acid complexes in aqueous solution.

The analysis of the polarographic waves of the zinc(II)–glycine system at pH of 8.4 show that the $E_{1/2}$ values decrease when ligand concentration increases, that the reduction process is quasi-reversible and diffusion-controlled and involves two-electrons under any experimental conditions. By using the DeFord–Hume method [5, 6] number, type and stability constants of the complexes formed were determined (Table I). The presence of three complexes of the type ZnL^+, ZnL_2 and ZnL_3^- was pointed out, as previously also found in the cadmium(II)–glycine system [7], and their stability constants were in agreement with the literature data [8–10].

In aqueous solution the reaction of zinc(II) and cadmium(II) ions with glycine takes place because this amino acid coordinates through the oxygen and nitrogen atoms forming stable five-membered chelate rings. On the contrary the N-protected amino acids, which coordinate with the metal ions through the carboxylate group and have a ligand field strength similar to that of water [11, 12], are not able to substitute the solvent molecules, present in a great amount with respect to the amino acid molecules, in the coordination sphere of the metal ion.

The stability constants of zinc(II) complexes greater than those of the cadmium(II) complexes closely agree with the Irving–Williams sequence [13].

Zinc(II)-Amino Acid Systems in Ethanolic Media

The polarographic data show that the three zinc (II)–amino acid complexes have a different behavior when dissolved in ethanol (Table II): two waves are present in the reduction of $Zn(gly)_2$ and $Zn(NBzgly)_2$, while $Zn(NAcgly)_2$ shows only a wave. To characterize the species responsible for any wave, and to determine the type, number and stability constants of the complexes which may be formed and the overall reduction process, metal ion solution at increasing concentration of the three amino acid anions was investigated. The solutions examined were prepared by adding ethanolic solutions of the amino acids containing an equimolar amount of sodium hydroxide; in these conditions all the amino acid is present in the anionic form and shows the greatest complexation ability toward the metal ion.

TABLE II. Polarographic Data for the ZnL_2 Complexes in Ethanol.

	$-E'_{1/2}$ (V)	$-E''_{1/2}$ (V)	i'_l	i''_l
$Zn(gly)_2$	1.17	1.36	0.18	1.1
$Zn(Nacgly)_2$	–	0.95	–	1.2
$Zn(NBzgly)_2$	0.86	0.93	0.6	0.75
Zn^{2+}	0.77	–	–	–

a) Zinc(II)–glycine system. The polarographic analysis of the zinc(II)–glycine system shows two waves (Fig. 2) when the glycinate anion concentration is lower than $5\cdot10^{-4}$ *M*; the $E_{1/2}$ and i_l values of the first wave decrease at increasing ligand concentration, and at the same time the i_l value of the second wave increases but its $E_{1/2}$ value remains constant.

In the ligand concentration range from $5\cdot10^{-4}$ *M* to 10^{-3} a third wave appears, the $E_{1/2}$ value of which is constant and i_l value increases like the second wave;

TABLE I. Polarographic Data of the Zn^{2+}–Glycine System in Aqueous Solution [$C_{Zn^{2+}} = 5\cdot10^{-4}$ *M*; $C_{gly} = X\cdot10^{-3}$ *M*].

X	$-E_{1/2}$ (V)	$E_{1/2}$	F°	$F'\cdot10^{-4}$	$F''\cdot10^{-7}$	$F'''\cdot10^{-9}$
	1.01_6					
1	1.06_7	0.051	53.2	5.22	1.22	2.2
4	1.09_4	0.078	436.0	10.90	1.73	1.8
8	1.11_2	0.096	1772.7	22.20	2.28	1.6
10	1.12_5	0.109	4881.6	48.95	4.50	3.4
19.9	1.14_7	0.131	27104.4	136.19	6.64	2.8
29.8	1.15_9	0.143	69043.0	231.68	7.64	2.2
39.7	1.17_1	0.155	175873.3	443.00	11.06	2.5
	$\beta_1 = 4.40\cdot10^4$	$\beta_2 = 1\cdot10^7$	$\beta_3 = 2.4\cdot10^9$			

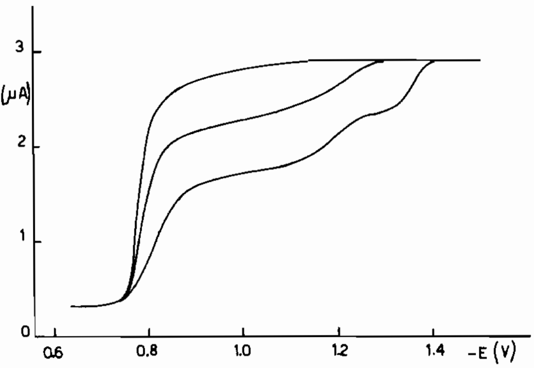

Fig. 2. Plot of E *vs.* i for the Zn(II)–glycine system.

the sum of the i_l values of the three waves corresponds to the i_d value of the free metal ion.

From $E_{1/2}$, i_l and i_l/i_{total} values of every wave, the concentrations of the different species simultaneously present in solution may be obtained (Table III).

When the anionic ligand concentration is about 10^{-3} *M* the two solution complex species have the same concentration. When the ligand concentration is greater than 10^{-3} *M*, a solid complex of stoichiometric formula $Zn_2L_3ClO_4$ precipitates. Thus it is possible to attribute the formulas ZnL^- and ZnL_2 to the two solution complexes that are reduced. Therefore the mechanism of the process may be represented as follows:

$$\begin{array}{lll} Zn^{2+} + 2e & \rightleftharpoons & Zn(Hg)\text{, first wave} \\ \big\downarrow\!\!\big\uparrow \; + L^- & & \\ ZnL^+ + 2e & \rightarrow & Zn(Hg) + L^-\text{, second wave} \\ \big\downarrow\!\!\big\uparrow \; + L^- & & \\ ZnL_2 \;\; + 2e & \rightarrow & Zn(Hg) + 2L^-\text{, third wave} \end{array}$$

The first wave is a reversible two-electron reduction wave, as demonstrated by the logarithmic analysis of the curve, and may be attributed to free metal ion reduction; its $E_{1/2}$ values decrease with increasing ligand concentration because the reduction process is kinetically-controlled by the reaction formation of the complexes [14]. The other two waves may be attributed to the reduction of the $Zn(gly)^+$ and $Zn(gly)_2$ complexes respectively and their $E_{1/2}$ values have almost the same value as those determined by solving the $Zn(gly)_2$ complex (Table II). Therefore in ethanolic solution only two complexes are present in equilibrium and the solid complex $Zn(gly)_2$ dissociates when dissolved in ethanol.

b) Behavior of Zn(II)–N-acetyl- and –N-benzoylglycinate systems. The zinc(II) ion-N-protected amino acid systems present two different behaviors if the anionic ligand concentration is lower or greater than 10^{-3} *M*. When the anionic ligand concentration is lower than 10^{-3} *M* the polarographic curve shows two waves not well resolved that behave like those of the Zn(II)–glycinate system in the same conditions and correspond to the reduction of zinc(II) ion and of one complex species, respectively (Table IV).

When the ligand concentration is increased above 10^{-3} *M* the first wave disappears, the $E_{1/2}$ value of the second wave decreases and its i_l value is constant and similar to i_d of the free metal ion.

In these conditions the logarithmic analysis applied to this wave shows that the reduction process is quasi-reversible and diffusion controlled. Therefore for any ligand concentration it is possible to obtain the corresponding reversible half-wave potential $E^r_{1/2}$ [15] and by applying again the DeFord–Hume method four complex species ZnL^+, ZnL_2, ZnL_3^- and ZnL_4^{2-} were identified as simultaneously present in solution. Their calculated stability constants are reported in Tables V and VI.

However the electrode reactions only involve the reduction of ZnL_2 complex [16], so the following mechanism may be proposed:

$$\begin{array}{l} M^{2+} + L^- \rightleftharpoons ML^+ + L^- \rightleftharpoons ML_2 + L^- \\ \qquad\qquad\qquad\qquad\quad \big\downarrow 2e \quad \rightleftharpoons ML_3^- + L^- \rightleftharpoons ML_4^{2-} \\ \qquad\qquad\qquad\qquad\quad M(Hg) + 2L^- \end{array}$$

The behavior shown by these systems is quite similar to that found for the corresponding cadmium (II) systems. In fact comparison of the results

TABLE III. Polarographic Data of Zn^{2+}–Glycine System in Ethanol ($C_{Zn^{2+}} = 5\cdot10^{-4}$ *M*; $C_{NaOH} = C_{gly} = X\cdot10^{-4}$ *M*).

X	$-E_{1/2}$ (V)			i (μA)			i_I/i_{tot}	i_{II}/i_{tot}	i_{III}/i_{tot}	$C_{free\ ion}$	C_{ZnL^+}	C_{ZnL_2}
	I	II	III	I	II	III				$\times10^4$ *M*		
2.5	0.79	1.19		1.94	0.63		0.75	0.24		3.75	1 25	
5.0	0.80	1.19		1.92	0.73		0.72	0.27		3.60	1.40	
6.5	0.82	1.19	1.34	1.46	0.52	0.44	0.60	0.22	0.18	3.00	1.10	0.90
7.93	0.83	1.19	1.34	1.31	0.54	0.45	0.57	0.23	0.20	2.85	1.15	1.00
9.79	0.84	1.19	1.34	1.02	0.58	0.58	0.47	0.27	0.27	2.30	1.35	1.35

TABLE IV. Polarographic Data for Zn^{2+}–N-acetylglycine and Zn^{2+}–N-benzoylglycine Systems in Ethanol at Anionic Ligand Concentration Lower than 10^{-3} *M* ($C_{Zn^{2+}} = 5 \cdot 10^{-4}$ *M*).

C_{NAcgly}	$-E'_{1/2}$ (V)	$-E''_{1/2}$ (V)	i'_e	i''_e	C_{NBzgly}	$-E'_{1/2}$ (V)	$-E''_{1/2}$ (V)	i'_e	i''_e
0	0.77		2.50		0	0.77		2.5	
$2.5 \cdot 10^{-4}$	0.83	0.95	1.72	0.62	$2.8 \cdot 10^{-4}$	0.80	0.92	1.9	0.6
$5.0 \cdot 10^{-4}$	0.85	0.95	1.56	0.87	$5.0 \cdot 10^{-4}$	0.83	0.92	1.62	0.8
$6.5 \cdot 10^{-4}$	0.86	0.95	1.41	0.94	$6.5 \cdot 10^{-4}$	0.85	0.92	1.6	0.9
$8.0 \cdot 10^{-4}$	0.86	0.95	1.25	1.20	$8.0 \cdot 10^{-4}$	0.85	0.92	1.4	1.1

TABLE V. Polarographic Data of Zn^{2+}–N-acetylglycine system in ethanol ($C_{Zn^{2+}} = 5 \cdot 10^{-4}$ *M*; $C_{NaOH} = C_{NAcgly} = X \cdot 10^{-3}$ *M*).

X	lg X	$-E_{1/2}$ (V)	$-(E_{1/2})^r_c$ (V)	$F_0 \cdot 10^{-5}$	$F_I \cdot 10^{-8}$	$F_{II} \cdot 10^{-11}$	F_{III} 10^{-14}	$F_{IV} \cdot 10^{-15}$
1.194	−2.923	0.95_5	0.94_0					
2.01	−2.697	0.96_3	0.94_3	4.843	2.405	0.522	0.110	
2.17	−2.66	0.96_6	0.94_5	5.66	2.608	0.557	0.118	
2.80	−2.55	0.97_2	0.95_2	9.765	3.487	0.745	0.158	3.15
3.56	−2.45	0.97_8	0.96_4	24.875	6.987	1.57	0.357	8.06
5.62	−2.25	0.99_8	0.98_1	93.55	16.64	2.71	0.429	6.39
5.96	−2.225	1.00_3	0.98_5	127.76	21.436	3.43	0.525	7.63
6.96	−2.157	1.00_8	0.99_3	238.30	34.24	4.72	0.635	8.1
8.00	−2.09	1.01_7	1.00_0	411.15	51.39	6.25	0.743	8.41
9.56	−2.019	1.04_1	1.01_0	896.19	93.74	9.66	0.979	9.50
	$\beta_1 = 1.4 \cdot 10^8$	$\beta_2 = 3 \cdot 10^{10}$	$\beta_3 = 8 \cdot 10^{12}$	$\beta_4 = 7.7 \cdot 10^{15}$				

TABLE VI. Polarographic Data of the Zn^{2+}–N-benzoylglycine System in Ethanol ($C_{Zn^{2+}} = 5 \cdot 10^{-4}$ *M*; $C_{NBzgly} = C_{NaOH} = X \cdot 10^{-3}$ *M*).

X	−lg X	$-E_{1/2}$ (V)	$-(E_{1/2})^r_c$ (V)	$F_0 \cdot 10^{-5}$	$F_I \cdot 10^{-7}$	$F_{II} \cdot 10^{-10}$	$F_{III} \cdot 10^{-13}$	$F_{IV} \cdot 10^{-15}$
1.00	3.0	0.94_0	0.92_2	0.94	9.42	3.83	1.33	
2.00	2.7	0.94_7	0.93_7	3.03	15.0	5.4	1.45	4.25
5.00	2.3	0.97_7	0.96_5	26.89	53.78	9.64	1.43	1.66
8.00	2.097	1.00_0	0.98_4	118.18	147.73	17.76	1.91	1.64
11.34	1.945	1.01_1	1.00_0	411.15	362.565	31.48	2.55	1.72
	$\beta_1 = 5.6 \cdot 10^7$	$\beta_2 = 2.5 \cdot 10^{10}$	$\beta_3 = 6 \cdot 10^{12}$	$\beta_4 = 2 \cdot 10^{15}$				

TABLE VII. $E_{1/2}$ Values of the Zn(II) and Cd(II) Complexes and the Substituent Parameters

	$-E_{1/2}$ (V)		R–CH_2COOH	σ'_{p-x}[a]	σ^+_{p-x}[b]	R–$NHCH_2COOH$	σ_{p-x}[c]
	M = Zn	M = Cd					
$M(gly)_2$	1.36	0.74	R = NH_2	−0.66	−1.3	R = H	0
$M(NAcgly)_2$	0.95	0.57	R = $NHCOCH_3$	−0.015	−0.6	R = $COCH_3$	0.53
$M(NBzgly)_2$	0.93	0.53	R = $NHCOC_6H_5$	0.08	−0.6	R = COC_6H_5	0.46

a) and c) see ref. 18; b) see ref. 19.

reported in Table VII shows that: *i*) the N-protected amino acids react with both the metals to form four complexes in equilibrium; *ii*) the polarographic reduction involves only the ML_2 compound; *iii*) the stability of N-acetylglycine complexes is greater than that of the N-benzoylglycine, but the stability of the zinc(II) derivatives is greater than that of cadmium (II), in agreement with the Irving–Williams sequence [13].

Conclusions

To quantify the substituent effect and to suggest a coordinative hypothesis for these ligands in ethanol, correlations of the $E_{1/2}$ values resulting from the reduction of zinc(II) and cadmium(II) complexes were tried with the same substituent parameters (σ'_{px} (R–CH$_2$COOH), σ^+_{px} (R–CH$_2$COOH) and σ_{px} (NHR–CH$_2$COOH)) [17]. Only the plots of $E_{1/2}$ versus σ'_{px}, that measures the polar electron-donating or electron-withdrawing effect of the substituent, are straight lines (Fig. 3). This confirms that the three complexes are reduced on the D.M.E. with the same mechanism and the $E_{1/2}$ values are determined by a prevalent polar effect of the substituent.

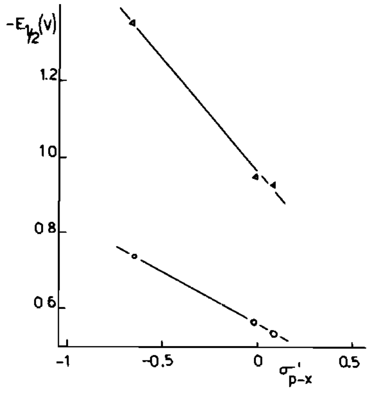

Fig. 3. Plot of E *vs.* σ for Zn(II) and Cd(II) complexes.

These conclusions suggest that the nitrogen atom does not directly participate in the reduction process and it agrees with the prevailing amino acid coordination through the carboxylate group.

Finally, since the reduction mechanism is the same in both the metal-systems, the different slopes of the two straight lines may also indicate a different interaction with the solvent.

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