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Potentiometric Studies of Binary and Ternary Nickel(II) Complexes Involving Ethylenediamine-N,N′-Diacetic Acid, Amino Acids and Peptides

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The formation equilibria of binary and ternary complexes of nickel(II) with ethylenediamine-N,N′-diacetic acid (EDDA) as primary ligand and amino acid or peptide (HL) as secondary ligand have been investigated. Stoichiometries and stability constants of the complexes were determined at 25°C and 0.1 M $NaNO_3$ ionic strength. The hydrolysis of the Ni (EDDA) complex and the deprotonation of the amide residue in the peptide complexes are discussed in relation to physiological conditions.

Key words: Potentiometric, Binary, Ternary, Nickel(II), Complexes, Ethylene diamine-N,N′-diacetic acid, Amino acids, Peptides.

INTRODUCTION

It is well known that amino acids and peptides are important ligands for transition metal ions in many biological systems[1–4]. The ionized amide residue of the peptide (—CONH—) is an important ligating group, in for example the antibiotic bleomycin. The strongest ligation of peptide to nickel(II) is through the ionized amide group[5]. In continuation of our current studies on metal complexes of biological significance[6–8], we have investigated the formation and stabilities of binary and ternary Ni(II) complexes involving ethylenediamine-N,N′-diacetic acid (EDDA) whose metal complexes have been shown to possess anti-inflammatory activity[9]. The present investigation describes the formation equilibria of binary and ternary complexes involving Ni(II), EDDA and amino acids or peptides.

EXPERIMENTAL

Glycine, alanine, serine, iso-leucine, lysine, methionine, proline, valine, threonine, 2-amino-*n*-butyric acid, mercaptoethylamine, mercaptopropionic acid, glycinamide, glutamine, glycylleucine, glycylglycine and glycylglycylglycine were provided by B.D.H. Ethylenediamine-N,N′-diacetic acid was obtained from Sigma. Nickel(II) nitrate was received from Merck. The NaOH solution used for the titrations was determined by titration with potassium hydrogen phthalate (Merck). A solution of nickel nitrate was prepared and estimated complexometrically[10]. All solutions were prepared in deionized water.

The potentiometric pH titration was carried out on a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland). The electrode was calibrated with standard buffer solutions prepared according to NBS specifications[11]. The electrolytic conductance was measured using a WTW LBP conductivity bridge (Germany).

The following mixtures (A–D) were prepared for equilibrium constant determinations:

(A) 0.005 M EDDA, amino acid or peptide (10 mL) + 0.13 M $NaNO_3$ (30 mL)

(B) 0.005 M nickel(II) ion (10 mL) + 0.005 M EDDA (10 mL) + 0.20 M $NaNO_3$ (20 mL).

(C) 0.005 M nickel(II) ion (10 mL) + 0.015 M amino acid or peptide (10 mL) + 0.20 M $NaNO_3$ (20 mL).

(D) 0.005 M nickel(II) ion (10 mL) + 0.005 M EDDA (10 mL) + 0.005 M amino acid or peptide (10 mL) + 0.4 M $NaNO_3$ (10 mL).

The following mixture (E) was prepared and titrated conductometrically with 0.05 M NaOH.

(E) 0.005 M nickel(II) ion (10 mL) + 0.005 M EDDA (10 mL) + 0.005 M glycine (10 mL).

Mixtures A–D were titrated potentiometrically against standard NaOH solution (0.05 M) at 25°C in an atmosphere of purified nitrogen. The formation constants of the binary complexes formed in solution were determined by titrating mixture B. The nickel(II) amino acid or peptide concentration ratio was 1 : 3 to allow the formation of 1 : 1 and other complexes. The stability constants of the ternary complexes were determined by titration of mixture D, utilizing the data obtained in the pH range corresponding to the complete formation of [Ni(EDDA)] complex. The calculations were performed using the computer program MINIQUAD-75.[12] The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models for the systems studied. The model selected was that which gave the best statistical fit and which was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere[12]. Table-1 lists the stability constants together with their standard deviations and the sum of the squares of residuals as obtained from MINIQUAD-75 calculations.

RESULTS AND DISCUSSION

Protonation Equilibria: The protonation equilibria of ethylenediamine-N,N′-diacetic acid (EDDA) were investigated previously[6]. The protonation constants used in the calculation of the stability constants of nickel(II)-EDDA complexes are given in Table-1.

Nickel(II)-EDDA Equilibria: The potentiometric titration curves of the nickel(II)-EDDA system are given in Fig. 1. The titration curve of Ni(II)-EDDA complex is lowered from that of the free EDDA, indicating formation of nickel(II) complex by displacement of protons. Different equilibrium models have been used to try to fit the experimental potentiometric data of nickel(II)-EDDA complexes. The model that gave the best statistical fit plausibly consists of the 1 : 1 complex species, Ni(II)-EDDA. After complete formation of the Ni(II)-EDDA complex, the titration curve drifts due to the formation of the hydroxo complex $[Ni(EDDA)(OH)]^-$. The concentration distribution diagram of the Ni(II)-EDDA system is given in Fig. 2.

TABLE-1
FORMATION CONSTANTS OF THE SPECIES Ni_l $(EDDA)_p$(AMINO ACID OR PEPTIDE)$_q H_r$ AT 25°C AND I = 0 1 M $NaNO_3$

System	l	p	q	r	log β^a	S^b	Δ log k
EDDA	0	1	0	1	9.97 (0.006)	1.7 E-8	
	0	1	0	2	16.57 (0.01)		
	0	1	0	3	19.32 (0.02)		
	0	1	0	4	21.26 (0.08)		
	1	1	0	0	11.75 (0.04)	4.5 E-8	
	1	1	0	1	15.81 (0.08)		
	1	1	0	−1	6.63 (0.10)		
Glycine	0	0	1	1	9.45 (0.00)	1.1 E-9	−2.68
	1	0	1	0	5.89 (0.01)	2.2 E-9	
	1	0	2	0	10.81 (0.03)		
	1	0	3	0	14.19 (0.01)		
	1	1	1	0	3.21 (0.02)	2.1 E-7	
Alanine	0	0	1	1	9.59 (0.00)	7.7 E-7	−2.17
	1	0	1	0	5.44 (0.05)	1.4 E-8	
	1	0	2	0	10.03 (0.09)		
	1	0	3	0	13.79 (0.01)		
	1	1	1	0	3.27 (0.07)	1.6 E-6	
Proline	0	0	1	1	10.49 (0.00)	3.8 E-8	−1.79
	1	0	1	0	6.12 (0.06)	6.8 E-8	
	1	0	2	0	10.19 (0.02)		
	1	0	3	0	14.53 (0.02)		
	1	1	1	0	4.33 (0.01)	7.8 E-9	
Valine	0	0	1	1	9.41 (0.01)	1.2 E-7	−2.72
	1	0	1	0	5.32 (0.02)	7.6 E-9	
	1	0	2	0	9.83 (0.01)		
	1	0	3	0	12.31 (0.05)		
	1	1	1	0	2.60 (0.04)	3.4 E-7	
Iso-leucine	0	0	1	1	9.52 (0.00)	3.7 E-8	−1.92
	1	0	1	0	5.55 (0.06)	8.2 E-8	
	1	0	2	0	10.28 (0.01)		
	1	0	3	0	13.22 (0.03)		
	1	1	1	0	3.63 (0.10)	1.1 E-6	

System	l	p	q	r	log β[a]	S[b]	Δ log k
2-Amino-*n*-butyric acid	0	0	1	1	9.25 (0.01)	2.1 E-7	–1.93
	1	0	1	0	5.38 (0.05)	2.8 E-9	
	1	0	2	0	9.83 (0.03)		
	1	0	3	0	13.45 (0.02)		
	1	1	1	0	3.45 (0.02)	3.01 E-7	
Threonine	0	0	1	1	8.98 (0.00)	1.6 E-8	–2.25
	1	0	1	0	5.59 (0.04)	3.4 E-9	
	1	0	2	0	10.45 (0.01)		
	1	0	3	0	13.40 (0.02)		
	1	1	1	0	3.34 (0.03)	3.7 E-7	
Methionine	0	0	1	1	9.12 (0.00)	1.6 E-8	–2.02
	1	0	1	0	5.27 (0.01)	2.2 E-8	
	1	0	2	0	9.80 (0.06)		
	1	0	3	0	12.15 (0.09)		
	1	1	1	0	3.25 (0.04)	5.5 E-7	
Serine	0	0	1	1	8.77 (0.00)	4.7 E-8	–2.86
	1	0	1	0	5.53 (0.02)	1.2 E-8	
	1	0	2	0	10.26 (0.03)		
	1	0	3	0	13.46 (0.09)		
	1	1	1	0	2.67 (0.03)	7.2 E-8	
Lysine	0	0	1	1	10.65 (0.00)	1.3 E-8	–0.24
	0	0	1	2	19.85 (0.00)		
	1	0	1	0	6.00 (0.03)	4.7 E-9	
	1	0	2	0	10.76 (0.08)		
	1	1	1	0	5.76 (0.07)	4.7 E-8	
	1	1	1	1	15.44 (0.03)		
Mercaptoethylamine	0	0	1	1	10.37 (0.01)	3.7 E-8	–2.3
	0	0	1	2	18.64 (0.02)		
	1	0	1	0	9.87 (0.06)	5.6 E-7	
	1	0	2	0	17.88 (0.03)		
	1	1	1	0	7.57 (0.09)	2.6 E-7	
	1	1	1	1	17.92 (0.09)		

System	l	p	q	r	log β[a]	S[b]	Δ log k
Mercaptopropionic acid	0	0	1	1	9.88 (0.02)	4.5 E-7	–1.08
	0	0	1	2	13.77 (0.05)		
	1	0	1	0	8.16 (0.05)	9.0 E-7	
	1	0	2	0	15.88 (0.03)		
	1	1	1	0	7.08 (0.02)	6.8 E-9	
	1	1	1	1	12.48 (0.04)		
Glycinamide	0	0	1	1	7.88 (0.00)	4.5 E-8	–0.10
	1	0	1	0	5.33 (0.01)	7.4 E-8	
	1	0	2	0	9.52 (0.03)		
	1	0	1	–1	–3.42 (0.03)		
	1	1	1	0	5.23 (0.02)	5.1 E-9	
	1	1	1	–1	–2.63 (0.02)		
Glycylglycine	0	0	1	1	7.97 (0.00)	4.0 E-8	–0.11
	1	0	1	0	4.09 (0.04)	9.4 E-8	
	1	0	2	0	7.53 (0.01)		
	1	0	3	0	9.62 (0.06)		
	1	0	1	–1	–5.52 (0.07)		
	1	1	1	0	3.98 (0.07)	6.2 E-8	
	1	1	1	–1	–4.10 (0.05)		
Glycylglycylglycine	0	0	1	1	7.94 (0.004)	7.9 E-10	–0.20
	1	0	1	0	3.80 (0.02)	9.2 E-8	
	1	0	2	0	6.92 (0.03)		
	1	0	3	0	8.46 (0.09)		
	1	0	1	–1	–4.95 (0.07)		
	1	1	1	0	3.60 (0.08)	1.9 E-7	
	1	1	1	–1	–5.03 (0.04)		
Glycylleucine	0	0	1	1	8.13 (0.00)	4.1 E-8	–0.33
	1	0	1	0	4.45 (0.01)	5.5 E-8	
	1	0	2	0	8.08 (0.06)		
	1	0	1	–1	–3.76 (0.04)		
	1	1	1	0	4.12 (0.02)	2.3 E-8	
	1	1	1	–1	–4.42 (0.02)		
Glutamine	0	0	1	1	8.98 (0.00)	1.0 E-8	–0.55
	1	0	1	0	6.73 (0.03)	5.9 E-8	
	1	0	2	0	10.03 (0.05)		
	1	0	1	–1	–3.76 (0.05)		
	1	1	1	0	6.18 (0.02)	3.1 E-8	
	1	1	1	–1	–2.6 (002)		

[a]Standard deviations are given in parentheses. [b]Sum of squares of residuals.

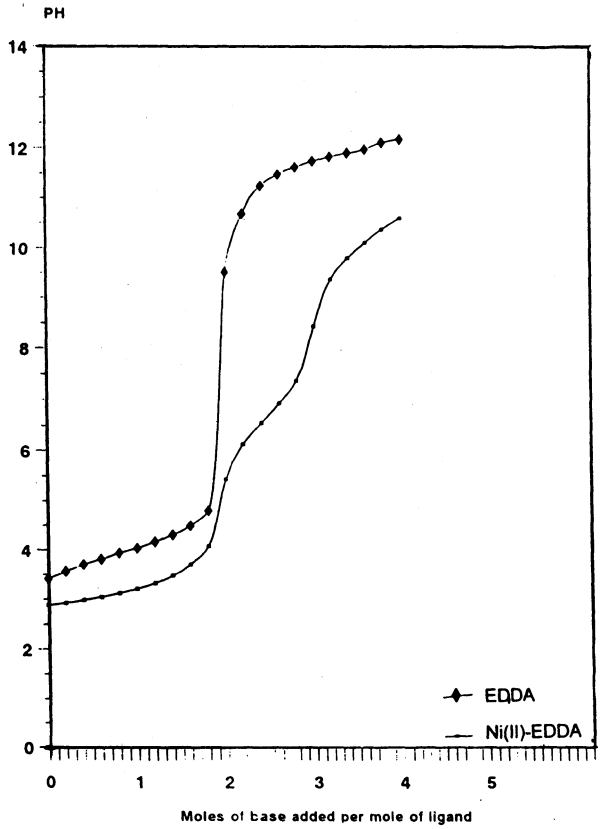

Fig. 1. Potentiometric titration curves of Ni(II)-EDDA system.

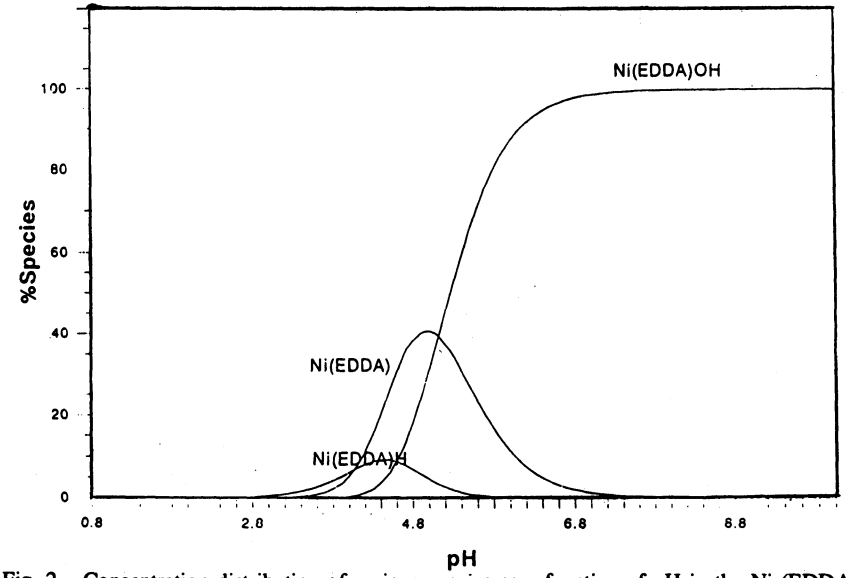

Fig. 2. Concentration distribution of various species as a function of pH in the Ni-(EDDA) system

The concentration of the $[Ni(EDDA)OH]^-$ species increases with increasing pH and attains a maximum concentration of 100% at pH 7.2. This reveals that in the physiological pH range, the hydroxo complex is the predominant species.

Nickel(II)-Amino Acids or Peptides Equilibria: It is noteworthy that previous studies on complexes of Ni(II) with amino acids and peptides were rather ambiguous and were carried out under very different conditions, which did not allow meaningful comparison of the reported stability constants and definite stoichiometries. For example, it was argued that Ni(II) might form a tris complex in stepwise formation, but bis-complexes were considered in a number of literature reports[13]. In the present study, it has been planned to redetermine the complex formation constants of Ni(II)-amino acids and peptides under carefully specified conditions used to study the ternary complexes.

Ternary Nickel(II) Complexes: The potentiometric titration curve of the ternary system Ni(II)-EDDA-glycine (as a representative) coincides with the 1 : 1 Ni(II)-EDDA curve in the region $0 \leq a \leq 2$ (Fig. 3). In this region Ni-EDDA complex (1 : 1) is formed firstly. Beyond a = 2, the formation of a ternary complex was ascertained by comparison of the mixed ligand titration curve with the composite curve obtained by the graphical addition of glycine titration data to that of the nickel(II)-EDDA titration curve, indicating the formation of a ternary complex. The amino acids are assumed to bind through the amino and carboxylate groups. With lysine, the protonated complex is formed together with the

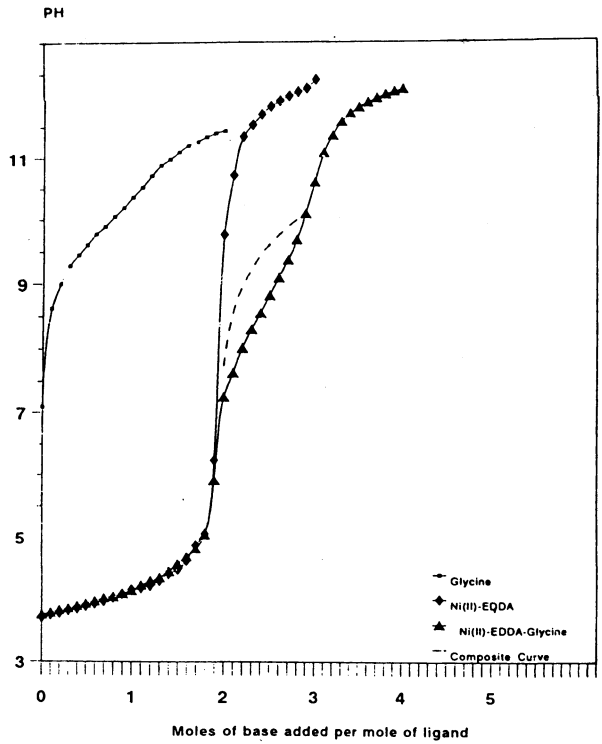

Fig. 3. Potentiometric titration curves of Ni(II)-EDDA-Glycine system.

deprotonated complex. The acid dissociation constant of the protonated complex is given by the following equation (1):

$$pK^{(H)}_{Ni(EDDA)(L)(H)} = \log K^{Ni(EDDA)}_{Ni(EDDA)(L)(H)} - \log K^{Ni(EDDA)}_{Ni(EDDA)(L)} \quad \ldots (1)$$

The value of pK^H is 9.68 for lysine ternary complex. This value compares favourably with the acid dissociation constant of the ω-amino group. This indicates that one amino group is bound to Ni(II) ion in ternary complex formation and the ω-amino group is free.

The complex-formation equilibria involving peptides (HL) were characterized by fitting their potentiometric data to various models. The best model was found to be consistent with the formation of the complexes with stoichiometric coefficients 110 and 11-1, according to the following scheme, (Eq. (2), (3)):

$$[Ni(EDDA)(H_2O)_2] + L^- \underset{(110)}{\overset{K}{\rightleftharpoons}} [Ni(EDDA)L]^- + 2H_2O \quad \ldots (2)$$

$$[Ni(EDDA)L]^- - H^+ \underset{(11\text{-}1)}{\overset{K_a'}{\rightleftharpoons}} [Ni(EDDA)(LH_{-1})]^{2-} \quad \ldots (3)$$

$$pK_a' = \log \beta_{110} - \log \beta_{11\text{-}1} \quad \ldots (4)$$

The pK_a' is determined using Eq. (4). The complexes are formed by coordination of the amino and carboxyl groups of the peptides. On deprotonation of the amide group, the coordination sites switch from carboxyl oxygen to amide nitrogen. Such changes in coordination centres are now well-documented[14]. It is interesting to note that the pK_a' value for the glycinamide complex is lower than those for the other peptides. This can be explained on the assumption that the more bulky substituent group on the peptide may serve to hinder the structural changes in going from the protonated to the deprotonated complexes. The pK_a' of the glutamine complex is markedly higher that those of the other peptide complexes. This is ascribed to the formation of a seven-membered chelate ring, which would probably be more strained and therefore less favoured.

The relative magnitudes of the pK_a' value of the nickel(II) complex with peptides have interesting biological implications. Under normal physiological conditions (pH *ca.* 7.4), the peptides would coordinate with $[Ni(EDDA)(H_2O)_2]$ in entirely different fashions: glutaminate would exist solely in the protonated form, whereas the other peptides would be present entirely in the deprotonated form. In addition, the slight difference in the side chains of the peptides produces dramatic differences in their behaviour toward the nickel species.

In order to assess the significance of the stability of the ternary complex species in relation to those of the parent binary complex[15], the parameter Δ log K, the difference in stability between the binary and ternary complexes was calculated:

$$\Delta \log K = \log K^{Ni(EDDA)}_{Ni(EDDA)L} - \log K^{Ni}_{Ni\,L}$$

In general one expects to observe negative values for Δ log K, because more coordination positions are available for bonding of amino acid or peptide in the binary complex than in the ternary complex. Based on our study, Δ log K values are invariably negative. This means that the amino acids or peptides form more stable complexes with the Ni(II) ion than with the [Ni(EDDA)] complex.

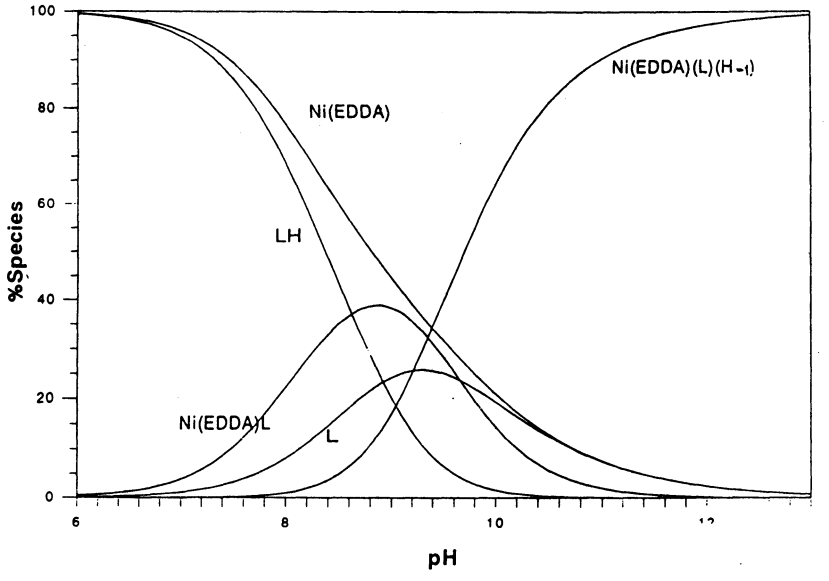

Fig. 4. Concentration distribution of various species as a function of pH in the Ni(II)-(EDDA)-Glycylglycine system.

The concentration distribution diagram for Ni(EDDA)-glycylglycine system, (as a representative) is given in Fig. 4. The [Ni(EDDA)L$^-$] starts to form at

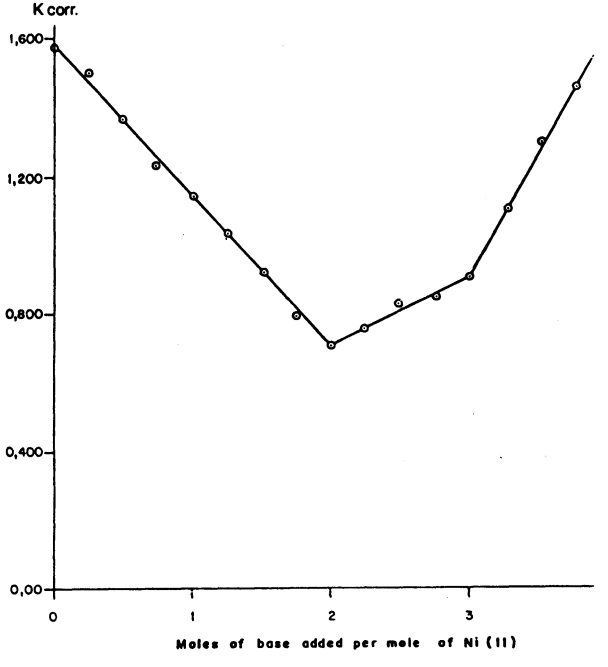

Fig. 5. Conductometric titration of Ni(II)-EDDA-glycine system.

pH *ca.* 6 and with increasing pH its concentration increases, reaching a maximum of 39% at pH = 8.8. A further increase of pH is accompained by a decrease in $[Ni(EDDA)L]^-$ concentration and an increase of the $[Ni(EDDA)(LH_{-1})]^{2-}$ complex, reaching a maximum of 99% at pH = 12.2.

The conductomertic titration curve for the ternary complex of Ni(II) with EDDA and glycine (Fig. 5) shows an initial decrease and an inflection at a = 2 which probably corresponds to the neutralization of H^+ ions resulting from the formation of Ni(II)-EDDA complex. In the $3 \geq a \geq 2$ range, the conductance increases slightly, supposedly due to the formation of a ternary complex associated with the release of a H^+ ion from glycine. Beyond a = 3, the conductance increases appreciably due to the presence of an excess of NaOH.

REFERENCES

1. S. Hyman, J.S. Gatmaitan and E. Patterson, *Biochemistry*, **13**, 4486 (1974).
2. B. Sarkar and Y. Wigfield, *Canad. J. Biochem.*, **46**, 601 (1968).
3. S. Lau and B. Sarkar, *Canad. J. Chem.*, **53**, 710 (1975).
4. J. Laussac and B. Sarkar, *Canad. J. Chem.*, **58**, 2055 (1980).
5. G. Brookes and L.D. Pettit, *J. Chem. Soc. Dalton Trans.*, 2106 (1975).
6. E.M. Shoukry, *Annali di Chim. (Rome)*, 91 (2001).
7. E.M. Shoukry, M.M. Shoukry, A.E. Mahgoub and H.M. Galal, *Annali di Chim. (Rome)*, 90 (2000).
8. M.M. Shoukry, E.M. Shoukry and S.M. El-Medani, *Monatsh Fur Chem.*, **126**, 909 (1995).
9. Katz and B. March, *Diss. Abstr. Int.*, **46B**, 4245 (1986).
10. F.J. Welcher, The Analytical Uses of Ethylenediamine Tetraacetic Acid, 4th Edn., Van Nostrand, Princeton (1965).
11. R.G. Bates, Determination of pH Theory and Practice, 2nd Edn., Wiley-Interscience, New York (1975).
12. P. Gans, A. Sabatini and A. Vacca, *Inorg. Chim. Acta*, **18**, 237 (1976).
13. D.D. Perrin, Stability Constants of Metal-Ion Complexes, Part B, Pergamon Press, Oxford (1979).
14. M.C. Lim, *J. Chem. Soc., Dalton Trans.*, 15 (1977).
15. R.B. Martin and R.J. Prados, *J. Inorg. Nucl. Chem.*, **36**, 1665 (1974).

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