POTENTIOMETRIC STUDIES OF BINARY AND TERNARY COMPLEXES INVOLVING CADMIUM(II) AND NITRILO-TRIS(METHYL PHOSPHONIC ACID) WITH AMINO ACIDS, PEPTIDES AND DNA CONSTITUENTS.

Azza A. SHOUKRY and Mohamed M. SHOUKRY(°)

Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt.

Summary - The complexing properties of nitrilo-tris(methylphosphonic acid) (NTP) with cadmium(II) were investigated pH-metrically at 25°C and at ionic strength of 0.1 mol dm^{-3} ($NaNO_3$). Stoichiometry and stability constants for the complexes formed are reported. Cadmium (II) forms $Cd(NTP)^{4-}$ and the corresponding hydroxy complexes. The ternary complexes are formed in a stepwise mechanism whereby NTP binds to cadmium(II), followed by coordination of amino acids, peptides or DNA. The concentration distribution of the various complex species has been evaluated.

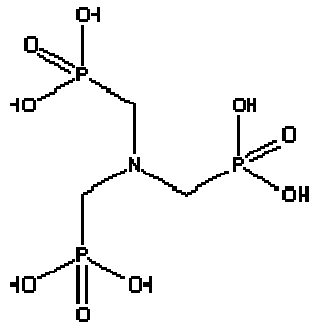
INTRODUCTION

The toxic effects of cadmium in the form of Cd^{II} are well documented. Cd^{II} has been found to induce various pathological conditions, some of which may be fatal, e.g. the 'Itai-Itai disease in northwestern Japan[1]. Other conditions include cardiovascular diseases [2,3], hypertension [4] and cancer.[5] The toxicology of cadmium(II) was found to be governed by its interaction with an abundance of certain potential ligands in biological systems [6-10]**.** For example**,** in animals, Cd^{II} accumulates mainly in the liver and kidney, where it is largely bound to thionine, a sulfur-rich protein[11-13]. In red blood cells, Cd^{II} is complexed by glutathione and hemoglobin[14]. Mixed ligands are also important in the biological chemistry of Cd^{II}. For example it has been suggested that a ternary complex of Cd^{II}, hemoglobin and glutathione is formed in red blood cells.

In conjunction with our research program[15-23] directed to the study of the metal complexes of biological significance, the present investigation presents the results of potentiometric studies of the formation of binary and ternary complexes involving Cd^{II}, NTP and some selected bio-relevant ligands. NTP was chosen as it forms a highly stable 1:1 complex with Cd^{II} in aqueous solution and has a coordination sites that resemble those of nucleotides. Also, this class of ligands has interesting biological activity. They include a variety of herbicides, plant growth regular antibiotics and inhibitors of metalloenzymes. For example, N, N-(diphosphenomethyl) glycine is known as plant growth regulator[24], whereas N-(phosphenomethyl) glycine is an active ingredient of popular herbicides[25, 26]. As potent metal binders, aminophosphonate could be involved in interactions

(°) Corresponding author. E.mail: shoukrym@hotmail.com

relevant for the fate of metal ions in the natural environment or biological systems[27,28]. Therefore, numerous studies were aimed to understand the chelating properties of this class of ligands and determining the stability of the complexes found [29, 30]



Scheme 1. - NTP

EXPERIMENTAL

Materials and reagents

All the reagents were of the analytical grade. Nitrilotris-methyl phosphonic acid were obtained from Aldrich Chem. Co. The amino acids: glycine, alanine, proline, methylamine, threonine, methionine, cysteine, lysine, L- histamine 2HCl, L-histidine.HCl, L-ornithine, imidazole, ethanolamine.HCl mercaptopropionic acid were provided by the Sigma Chem. Co. The peptides used were glycylglycine, glycylleucine, glutamine, aspargine and glycineamide, also provided by Sigma Chem. Co. The DNA constituents uracil, uridine, thymine, thymidine, inosine, inosine 5'-monophosphate were supplied by BDH-Biochemicals Ltd. $CdCl_2.2H_2O$ was provided by DBH. Carbonate free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized water.

Apparatus and measuring techniques

Potentiometric measurements were made using a Metrohm 751 Titrino. The titroprosessor and electrode were calibrated with standard buffer solutions prepared according to NBS specifications[31]. The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.01M), the ionic strength of which was adjusted to 0.1M with $NaNO_3$, with standard base (0.10M) at 25°C. The pH is plotted against p[H]. The relationship pH - p[H] = 0.05 was observed. $[OH^-]$ value was calculated using a pK_w value of 13.997[32]. All potentiometric titrations were carried out at 25 ± 0.05°C, in a double-walled glass cell of 50 ml capacity. The temperature of all solutions was maintained at 25 ± 0.05°C by circulation of thermostated water through the outer jacket of the cell. The solutions were stirred with a magnetic stirrer, and all titrations were performed in triplicate at an ionic strength of 0.1M ($NaNO_3$).

Equilibrium measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 cm^3) solution (1.25×10^{-3} M) of constant ionic strength 0.1M, (adjusted with $NaNO_3$). The stability constant of the Cd-NTP complex was determined by titrating 40 cm^3 of a solution mixture of Cd^{II} (1.25×10^{-3}M), NTP ligand (2.5×10^{-3}M) and $NaNO_3$ (0.1M). The

formation constant of the mixed ligand complexes were determined by titrating solution mixtures containing equivalent amounts of Cd^{II} (1.25×10^{-3} M), NTP and the other ligands in molar concentration ratio 1:1:1 for amino acids and peptides and 1:1:2 for the DNA constituents. All titrations were performed in a purified N_2 atmosphere using aqueous 0.05 M NaOH as titrant.

The stability constants of

$$K^{Cd(NTP)}_{Cd(NTP)L}$$

where HL is amino acid, peptide, DNA or amino acid ester, were determined using the data obtained within the pH range corresponding to the complete of Cd-NTP complex. Hence, in calculation only complex formation between $Cd(NTP)^{4-}$ and (HL) is considered and each of these systems could be treated as a binary one. The equilibrium constants evaluated from the titration data (summarized in Table 1) are defined by equations (1) and (2), where M, L and H stand for the $[Cd(NTP)]^{4-}$, ligand and proton respectively.

$$pM+pL+rH \rightleftharpoons M_pL_qH_r \qquad (1)$$

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[(M]^p[L]^q[H]^r} \qquad (2)$$

The calculations were obtained from ca. 100 data points in each titration using the computer program[33] MINIQUAD-75. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere[33]. The results obtained are shown in Table 1. The concentration distribution diagrams were obtained using the program SPECIES[34] under the experimental condition used.

RESULTS AND DISCUSSION

Nitrilotris(methyl phosphonic acid) (NTP) differs from nitrilotiacetic acid (NTA) in the replacement of carboxylic by phosphonic groups. NTP is provided with three phosphonic groups (H_6A). NTP is more basic than NTA due to the electron repelling effect of the dinegative charge on the phosphonic functions. The most basic donor for NTP is the tertiary amino group and the next most basic groups are the phosphonates which protonate in the pH range 4.9-7.2. The remaining phosphonic group is very weakly basic ($\log K_a$ value is around 2), which can be determined pH-metrically only with rather high uncertainties. Five of the six protons are titrable and can be measured by pH potentiometry. The protonation constants listed in Table 1 are in reasonable good agreement with earlier reports[35-37]. The acid dissociation constants of amino acids, peptides and DNA constituents have been reported. We redetermined them under the experimental conditions used for determining the stability constants of the mixed-ligand complexes. The results obtained are in a good agreement with the literature values[38] and are reported in Table 2.

TABLE 1.- Formation constants of $Cd_p(NTP)_qH_r$ species at 25°C and I = 0.1M $NaNO_3$.

System	p	q	r[a]	log β[b]	S[c]
Cd-NTP	0	1	1	11.00(0.01)	5.0E-8
	0	1	2	18.18(0.02)	
	0	1	3	24.28(0.02)	
	0	1	4	28.95(0.03)	
	0	1	5	30.85(0.06)	
	1	0	-1	-9.11(0.04)	1.4E-7
	1	1	0	11.10(0.03)	8.0E-8
	1	1	1	18.25(0.03)	
	1	1	2	24.01(0.03)	
	1	1	3	28.54(0.03)	

[a] p, q and r are the stoichiometric coefficients corresponding to Cd^{2+}, NTP and H^+; [b] Standard deviations are given in parentheses; [c] Sum of square of residuals.

Different equilibrium models have been tried to fit the potentiometric data of Cd-NTP solution mixture. The model selected giving the best statistical fit consists of 110, 111, 112, and 113 complex species but neither a 120 species. The validity of this model is given in Fig. 1, where an excellent fit can be observed between the potentiometric titration data and the theoretical curve calculated from the protonation constants of NTP and the formation constants of the corresponding complex species. The formation of the 1:2 complex seems to be hindered because NTP ligand is a tetradentate ligand. Also the electrostatic repulsion of the negatively charged phosphonate groups precludes coordination of the second ligand. In the 1:1 complex $Cd(NTP)^{4-}$ the ligand is tetradentate with nitrogen and phosphate donor groups. After complete formation of the $Cd(NTP)^{4-}$ complex, the titration curve drifts due to the formation of the hydroxo-complexes. The data in this region are fitted considering the formation of $Cd(NTP)(OH)_n$ species, where n = 1 and 2. The concentration distribution diagram for $Cd(NTP)^{4-}$ complex is shown in Fig. 2. The protonated complexes are predominated in the acidic pH range. Both 110 and 111 complex species prevail in the physiological pH range. This indicates that the Cd^{II} complex can interact with bio-ligands as amino acids, peptides and DNA constituent.

The potentiometric data in the range of formation of mixed ligand complexes of amino acids, peptides, and DNA constituents is fitted assuming the formation of the complexes species with stoichiometric coeffients 110 and 11-1. The validity of this model is tested by comparing the experimental titration data with the theoretically simulated curve.

Ternary complex formation may proceed either through a stepwise or simultaneous mechanism depending on the chelating potential of NTP and the other ligand (L) [L= amino acid, peptide or DNA]. The formation constant value of Cd-NTP complex is higher than those of 1:1 Cd^{II}- Ligand (L) complexes (Table 1). It is reasonable to propose that in presence of both ligands, one molecule of NTP is coordinated to Cd^{II} ion, with subsequent coordination of the secondary ligand (L). This assumption was supported by potentiometric data. A representative set of pH titration curves for Cd^{II}-NTP-glycine system is shown in Fig. 3. The Cd^{II}-NTP(1:1) solution mixture titration curve has a sharp inflection at a = 6 (a= number of moles of base added per mole of ligand), corresponding to complete formation of the 1:1 complex. In this respect the Cd^{II}-NTP complex is formed first due to its greater stability compared to Cd^{II}-L complex (Table 1). Beyond a

= 6, the formation of ternary complex was ascertained by comparison of the mixed-ligand titration curve with the composite curve obtained by graphical addition of glycine titration data to the Cd^{II}-NTP titration curve.

TABLE 2. - Formation constants of $(Cd\text{-}NTP)_pL_qH_r$ species at 25°C and I = 0.1M $NaNO_3$.

System	p	q	r[a]	logβ[b]	S[c]	$Log\ K_{CuL}^{Cu}$[d]	ΔlogK	
Cd(NTP)-OH	1	0	-1	-10.48(0.02)	8.8E-8			
	1	0	-2	-22.09(0.04)				
Glycine	0	1	1	9.60(0.01)	1.5E-7	4.62	-0.79	
	0	1	2	11.92(0.03)				
	1	1	0	3.83(0.005)	5.3E-9			
	1	1	-1	-6.66(0.003)				
Alanine	0	1	1	9.69(0.01)	9.2E-8	3.98		-0.81
	0	1	2	11.88(0.02)				
	1	1	0	3.17(0.03)	1.4E-7			
	1	1	-1	-7.77(0.05)				
Proline	0	1	1	10.52(0.01)	7.9E-9	4.27		-0.28
	0	1	2	12.03(0.02)				
	1	1	0	3.99(0.02)	1.3E-7			
	1	1	-1	-7.54(0.05)				
Threonine	0	1	1	9.06(0.01)	7.9E-9	3.9		-0.66
	0	1	2	11.03(0.02)				
	1	1	0	3.24(0.02)	9.8E-8			
	1	1	-1	-6.45(0.01)				
Methionine	0	1	1	9.10(0.01)	1.9E-9	3.68		-0.95
	0	1	2	11.08(0.02)				
	1	1	0	2.73(0.01)	2.2E-8			
	1	1	-1	-6.86(0.008)				
Imidazole	0	1	1	7.04(0.01)	2.6E-9	3.09		-0.63
	1	1	0	3.46(0.08)	5.0E-7			
	1	1	-1	-5.35(0.02)				
Methylamine	0	1	1	10.55(0.004	8.9E-9	5.6		-1.8
	1	1	0	3.32(0.03)	3.9E-8			
	1	1	-1	-7.55(0.07)				

TABLE 2 – (continued)

Histidine	0	1	1	9.53(0.01)	1.6E-7	5.66		-1.25
	0	1	2	15.81(0.03)				
	0	1	3	17.81(0.06)				
	1	1	0	4.41(0.01)	7.4E-8			
	1	1	-1	-5.84(0.01)				
Histamine	0	1	1	9.82(0.01)	2.4E-8	4.75		-0.77
	0	1	2	15.97(0.01)				
	1	1	0	3.98(0.08)	3.3E-7			
	1	1	-1	-6.22(0.07)				
Lysine	0	1	1	10.52(0.01)	1.6E-7	6.29	-2.07	
	0	1	2	19.65(0.03)				
	0	1	3	21.91(0.04)				
	1	1	0	4.22(0.007)	1.4E-8			
	1	1	1	13.99(0.01)				
	1	1	-1	-7.43(0.03)				
Ornithine	0	1	1	10.57(0.01)	1.6E-7			
	0	1	2	19.43(0.03)				
	0	1	3	21.38(0.02)				
	1	1	0	4.04(0.08)	4.0E-8			
	1	1	1	13.64(0.02)				
	1	1	-1	-6.77(0.01)				
Mercapto-propionic acid	0	1	1	9.88(0.01)	5.9E-8			
	0	1	2	13.77(0.01)				
	1	1	0	4.72(0.008)	1.7E-8			
	1	1	-1	-6.22(0.01)				
Cysteine	0	1	1	10.63(0.01)	4.7E-8			
	0	1	2	19.18(0.02)				
	0	1	3	21.62(0.05)				
	1	1	0	6.04(0.08)	5.4E-8			
	1	1	1	15.21(0.06)				
Ethanolamine	0	1	1	9.62(0.01)	4.6E-8			
	1	1	0	3.21(0.01)	1.6E-8			
	1	1	-1	-6.98(0.008)				
Glycinamide	0	1	1	7.88(0.01)	4.6E-8			
	1	1	0	2.42(0.06)	1.2E-7			
	1	1	-1	-5.88(0.01)				
Glycylglycine	0	1	1	7.97(0.01)	4.6E-8			
	1	1	0	2.20(0.07)	8.1E-8			
	1	1	-1	-6.04(0.01)				

TABLE 2 – (continued)

Glycylleucine	0	1	1	8.13(0.01)	4.6E-8
	1	1	0	2.22(0.04)	2.7E-8
	1	1	-1	-6.04(0.008)	
Glutamine	0	1	1	9.09(0.01)	4.5E-8
	1	1	0	3.15(0.01)	2.6E-8
	1	1	-1	-6.43(0.007)	
Aspargine	0	1	1	8.55(0.01)	5.9E-8
	1	1	0	2.22(0.02)	5.1E-8
	1	1	-1	-6.64(0.004)	
Inosine	0	1	1	8.43(0.01)	5E-9
	1	1	0	1.67(0.05)	5.0E-9
	1	1	-1	-6.55(0.004)	
5`-IMP	0	1	1	9.21(0.01)	2.4E-8
	0	1	2	15.21(0.01)	
	1	1	0	3.38(0.01)	3.3E-8
	1	1	-1	-6.09(0.007)	
Uridine	0	1	1	9.01(0.01)	1.1E-7
	1	1	0	1.94(0.05)	1.3E-8
	1	1	-1	-7.20(0.009)	
Uracil	0	1	1	9.28(0.01)	1.1E-7
	1	1	0	3.13(0.03)	4.0E-7
	1	1	-1	-4.94(0.03)	
Thymidine	0	1	1	9.50(0.01)	8.7E-8
	1	1	0	2.76(0.01)	7.8E-9
	1	1	-1	-7.45(0.007)	
Thymine	0	1	1	9.58(0.006)	8.1E-8
	1	1	0	3.53(0.009)	1.1E-8
	1	1	-1	-7.12(0.007)	

[a] p, q and r are the stoichiometric coefficients corresponding to Cd(NTP), amino acids, peptides or DNA units and H^+; [b] Standard deviations are given in parentheses; [c] Sum of square of residuals.[d]

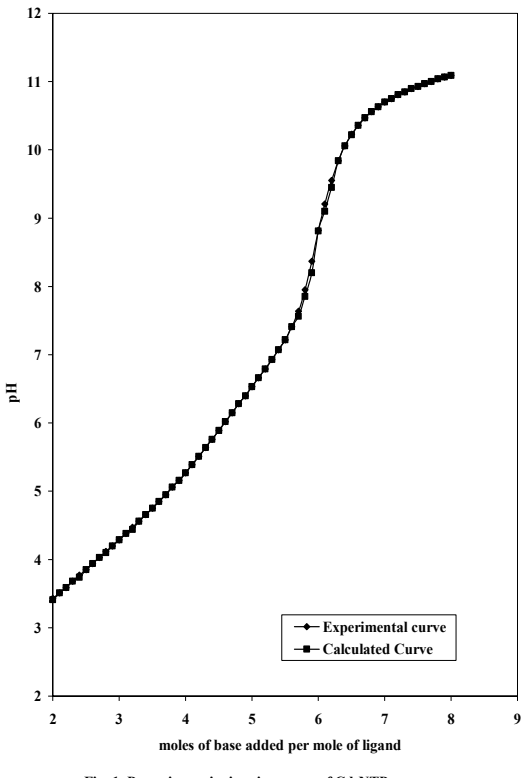


FIGURE 1. – Potentiometric titration curve of Cd-NTP system.

The mixed ligand system was found to deviate considerably from the resulted composite curve above pH 8.2 where the $(Cd\text{-}NTP)^{4-}$ is completely formed and the corresponding protonated complex is not of significant contribution (Fig. 2). This indicates the formation of the ternary complex. Also, this is further evidenced by comparing the experimental titration curve of proline taken as a representative with the theoretically simulated one calculated by the formation constant of the complexes. This was visualized in Fig. 4. The good coincidence supported the validity of the complex formation model. Based on the above finding, it seems evident that in presence of both ligands NTP is primarily ligated to Cd^{II} ion, occupying four coordination positions of the octahedral environment. This is followed by the ligation of the secondary ligands occupying the remaining coordination position.

The $(Cd\text{-}NTP)^{4-}$ complex in the pH region 8.2-9.5 is susceptible to hydrolysis. The experimental data of the mixed ligand complex of amino acids detected the species 11-1 . In the later species NTP is coordinated as tertradentate and the amino acid is coordinated as monodentate by carboxylate group as reported previously[39] while the sixth position is occupied by the hydroxyl group. Then after, this species undergoes ring closure where the amino acid coordinates by both the carboxylate and the amino group. This argument was supported by finding that the stability constant value of monodentate methylamine complex is slightly lower than that of glycine. This indicates that glycine most likely coordinates with $Cd(NTP)^{4-}$ as a bidentate rather than as a monodentate ligand.

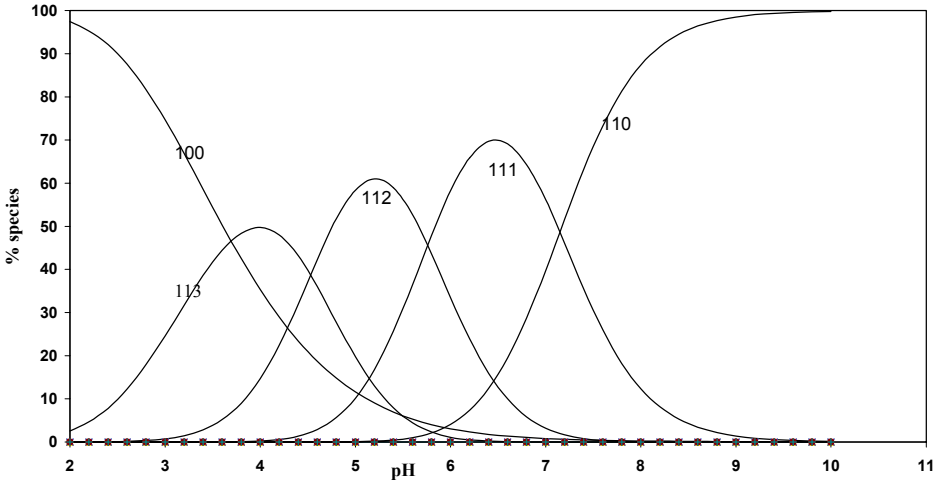


FIGURE 2. – Concentration distribution of various species as a function of pH in the Cd-NTP system (at a concentration of 1.25 $mmol^{-1}$).

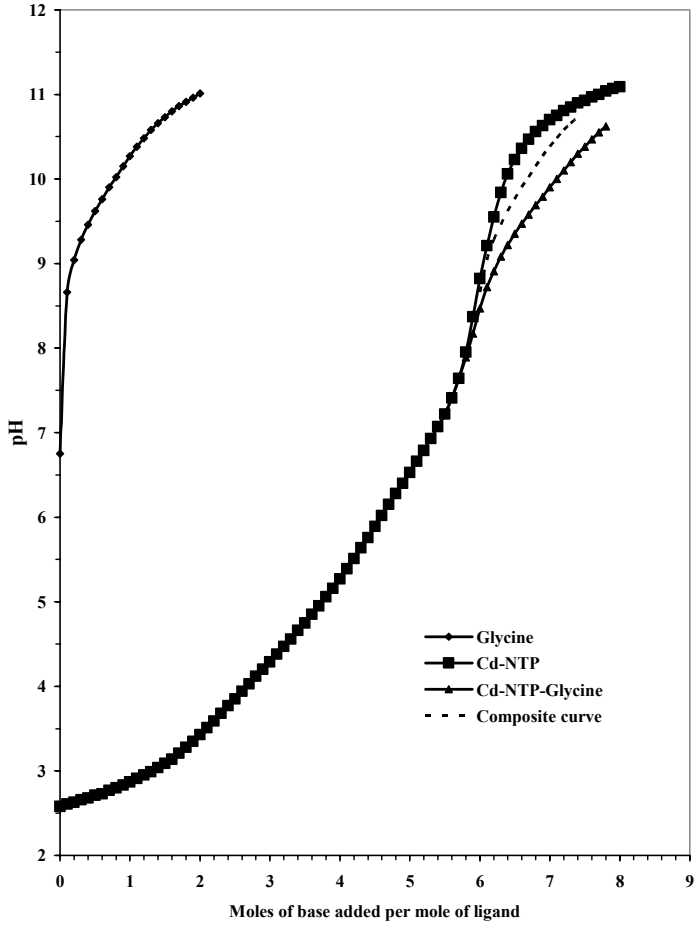


FIGURE 3. – Potentiometric Titration curve of Cd-NTP-Glycine system.

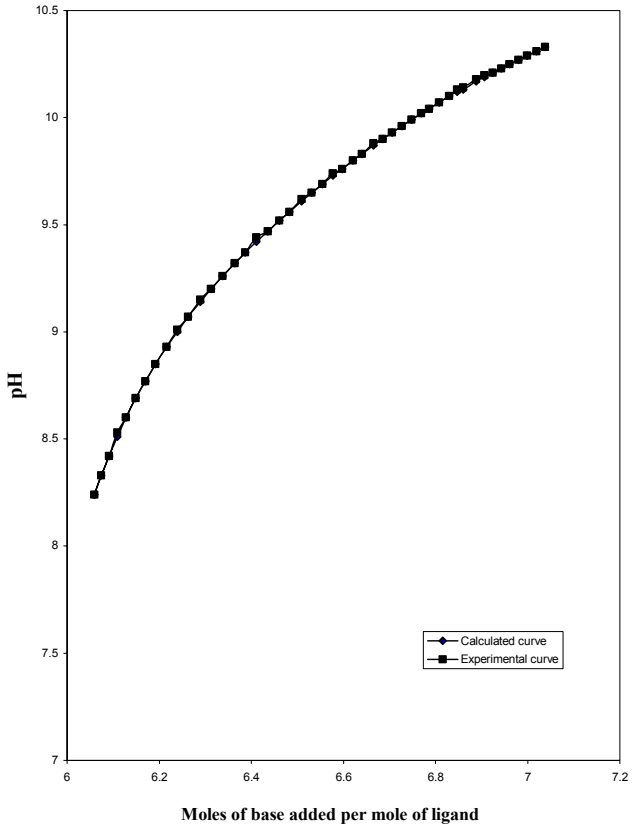


FIGURE 4. – Potentiometric Titration curve of Cd-NTP-Proline system.

Histidine is a tridentate ligand and may coordinate in a glycine-like or a histamine-like way. The stability constant value of the histidine complex is higher than those of α-amino acids and near to that of histamine complex. This indicates that histidine is coordinating to $Cd(NTP)^{4-}$ complex in a histamine-like way. If histidine coordinates as tridentate, the complex produced will be significantly more stable than that of histamine which is not the case obtained.

Imidazole forms a more stable complex than that of methylamine, although imidazole is less basic than methylamine. This may be due to enhanced stability involving π- back donation from the negatively charged NTP^{6-} ion to the π-system of the imidazole. This will contribute to the stabilization of the formed complex.

The stability constants of the ternary complexes formed with lysine and ornithine are higher than those of α-amino acids, the extra stability may be due to either the high basisity of the α-amino group or the possible hydrogen bonding formed between the extra -amino group and one of the phosphate oxygen atoms of NTP.

Cysteine and mercaptopropionic acid have various binding sites viz. amino, carboxylic and sulfhydryl groups. The stability constants of their ternary complexes are higher than those of lysine and ornithine. This indicates that these sulfhydryl-containing amino acids are most likely coordinated to $Cd(NTP)^{4-}$ through amino and sulphur groups (as N,S-donor set).

The stability of the mixed ligand complexes was compared with those of Cd-amino acid complexes. The stability of the mixed ligand complexes is quantified in relation to the corresponding binary complexes using Eq. 3

$$\Delta\log K = \log K^{Cd(NTP)}_{Cd(NTP)L} - \log K^{Cd}_{CdL} \qquad (3)$$

Based on our experimental data, ΔLog K values are invariably negative. This means that the amino acids form more stable complexes with Cd^{II} than with $(Cd\text{-}NTP)^{4-}$ complex.

The amide residue of the peptide ,[-CONH-], behaves as an important ligating group, and coordinates to metal ions as Cu(II), Pd(II), and Ni(II), through binding with the ionized amide group[40]. The potentiometric data reported for the peptide complexes reveals the formation of 110 and 11-1 complex species. Since the stability constant value of the 11-1 species is in fair agreement with those of amino acids, where there is no induce ionization, therefore it is assumed that the 11-1 species is formed through coordination of the hydroxyl group forming the hydroxo-complex.

The pyrimidines uracil, uridine, thymine and thymidine are protonated at (N_3) site. They form mixed-ligand complex at high pH and they do not form protonated complex. Consequently, the pyrimidines coordinate through the basic nitrogen (N_3). Inosine may become protonated at N_7 atom with formation of [N_1H-N_7H] monocations. In the present study, the pK_a of N_1H is only determined since the pK_a of N_7H is too low to be detected by the potentiometric technique. The potentiometric data of the mixed ligand complex involving inosine showed the formation of the Cd(NTP)L species, where L is the monoanion of inosine. In the acidic pH range, N_1 atom remains protonated, while the metal ion is attached to N_7 atom. The gradual change from N_7-binding to N_1-binding has been frequently documented by ^{1}H-NMR[41] and EPR[42] spectroscopic measurements. Consequently, it is proposed that N_1 serves as a coordination site in the mixed ligand complex of inosine at higher pH values.

Estimation of equilibrium concentrations of various complex species as a function of pH provides a useful picture of metal ion binding in the biological fluids. In all the species distribution the concentration of the complex increases with increasing pH, thus making the complex formation more favoured in the physiological pH range. The protonated ternary complexes are dominated at lower pH values. The species distribution pattern for Cd-NTP-Ornithine system, taken as a representative of amino acids is given in Fig. 5. The ternary complexes of α-amino acids starts to form at pH ≈ 8 and reaches a maximum concentration of ca. 50% at pH around10.3.

CONCLUSION

The present investigation may have important biological implications. The high stability of $(Cd\text{-}NTP)^{4-}$ complex may encourage the use of NTP as an antidote to release Cd^{II} ion. Thiol-containing amino acids, as cysteine, considered as sulphur-rich protein model is competing with NTP for interaction with Cd^{II} ion. However, Simple amino acids, peptides and DNA constituent do not compete. From combination of stability constant data of the mixed ligand complexes, it should in principle be possible to calculate the equilibrium distribution of Cd^{II} forms in biological fluids.

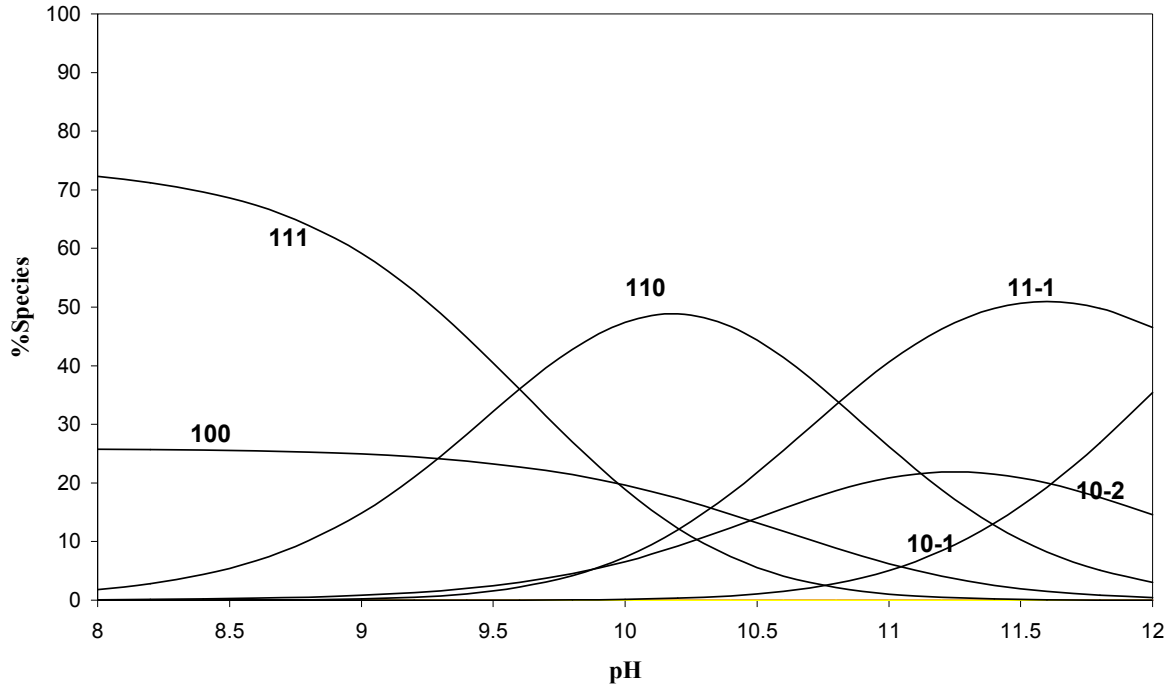


FIGURE 5. – Concentration distribution of various species as a function of pH in the Cd-NTP-Ornithine system (at a concentration of 1.25 $mmol^{-1}$ for each).

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