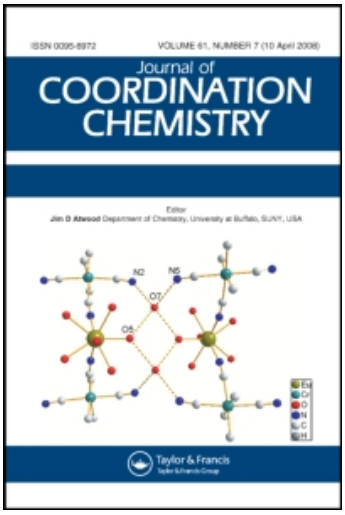

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Equilibrium investigation of complex formation reactions involving copper(II), nitrilo-tris(methyl phosphonic acid) and amino acids, peptides or DNA constitutents. The kinetics, mechanism and correlation of rates with complex stability for metal ion promoted hydrolysis of glycine methyl ester

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The complex formation reactions of $[Cu(NTP)(OH_2)]^{4-}$ (NTP = nitrilo-tris(methyl phosphonic acid)) with some selected bio-relevant ligands containing different functional groups, are investigated. Stoichiometry and stability constants for the complexes formed are reported. The results show that the ternary complexes are formed in a stepwise mechanism whereby NTP binds to copper(II), followed by coordination of amino acid, peptide or DNA. Copper(II) is found to form $Cu(NTP)H_n$ species with $n = 0$, 1, 2 or 3. The concentration distribution of the various complex species has been evaluated. The kinetics of base hydrolysis of glycine methyl ester in the presence of copper(II)-NTP complex is studied in aqueous solution at different temperatures. It is proposed that the catalysis of GlyOMe ester occurs by attack of OH^- ion on the uncoordinated carbonyl carbon atom of the ester group. Activation parameters for the base hydrolysis of the complex $[Cu(NTP)NH_2CH_2CO_2Me]^{4-}$ are, $\Delta H^{\pm} = 9.5 \pm 0.3\,kJ\,mol^{-1}$ and $\Delta S^{\pm} = -179.3 \pm 0.9\,J\,K^{-1}\,mol^{-1}$. These show that catalysis is due to a substantial lowering of $\Delta H^{\pm}$.

Keywords: Copper (II); Nitrilo-tris(methyl phosphonic acid); Amino acids; Peptides; DNA; Amino acid ester hydrolysis

1. Introduction

Aminopolyphosphonates are analogues of aminopolycarboxylates in which the carboxylate groups (CO_2^-) are substituted by phosphonate moieties (PO_3^{2-}). Natural and synthetic aminopolyphosphonic molecules are very effective ligands, in many cases with high specifity, for metal ions. This class of compounds and their derivatives has received considerable attention because of their interesting biological activity, including a variety of herbicides, plant growth regulator, antibodies and inhibitors of metallo-enzymes [1]. For example, N,N′-di(phosphenomethyl)glycine is known as a plant

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growth regulator [2], whereas N-(phosphenomethyl)glycine is an active ingredient of a popular herbicide [3, 4]. As potent metal binders, aminophosphonates could be involved in interactions relevant for the fate of metal ions in the natural environment or biological systems [5, 6]. Therefore, numerous studies were aimed at understanding the chelating properties of this class of ligands and determining the stability of the complexes formed [7, 8].

Investigation of formation equilibria of mixed ligand complexes involving aminophosphonic acid derivatives and some bio-relevant ligands provides information regarding the behaviour of this class of ligands in biological systems. In continuation of our published work on binary and ternary complexes of transition metal ions involving amino acids [9, 10], peptides [11, 12] and DNA units [13, 14], as well as the base hydrolysis of the mixed-ligand complexes with α-amino acid esters [15–17], we now describe the equilibria associated with the formation of binary and ternary complexes involving copper(II), nitrilo-tris(methyl phosphonic acid) and amino acid, peptides, DNA constutitents or amino acid ester. The study also includes base hydrolysis of glycine methyl ester in its ternary complex in aqueous solution at different temperatures.

2. Experimental

2.1. *Materials and reagents*

All the reagents were of Analar grade. Nitrilo-tris(methyl phosphonic acid) was obtained from Aldrich Chem. Co. The amino acids: glycine, alanine, threonine, ornithine dihydrochloride, histidine monohydrochloride as well as imidazole, histamine dihydrochloride and methylamine hydrochloride were provided by Sigma Chem. Co. The peptides used were glutamine and glycinamide, also provided by Sigma Chem. Co. The DNA constitutents, uridine, uracil and inosine, were supplied by BDH-Biochemicals Ltd. Glycine methyl ester hydrochloride was purchased from Fluka. $Cu(NO_3)_2 \cdot 3H_2O$ was provided by BDH. The copper content of solutions was determined by complexometric EDTA titrations [18]. Carbonate-free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized H_2O.

2.2. *Apparatus and measuring techniques*

Potentiometric measurements were made using a Metrohm 751 titrino. The electrode and titroprocessor were calibrated with standard buffer solutions prepared according to NBS specifications [19]. The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.01 M), the ionic strength of which was adjusted to 0.1 M with $NaNO_3$, with standard base (0.10 M) 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 [20]. The kinetics of hydrolysis were monitored by a pH-stat technique using the titroprocessor [15, 16, 21]. All potentiometric titrations were carried out at 25.0 ± 0.05°C, in a double-walled glass cell of 50 ml capacity. The temperature of all solutions was maintained at 25.0 ± 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 0.1 M ($NaNO_3$). Electronic spectra were recorded on a Shimadzu 3101-spectrophotometer.

2.3. *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 0.1 M, (adjusted with $NaNO_3$). The hydrolysis constant of Cu^{II} was determined by titrating Cu^{II} (40 cm^3) solution (1.25×10^{-3} M) in 0.1 M $NaNO_3$. The stability constant of the Cu-NTP complex was determined by titrating a solution (40 cm^3) of Cu^{II} (1.25×10^{-3} M), the NTP (2.5×10^{-3} M) and $NaNO_3$ (0.1 M). The stability constants of the ternary complexes were determined using potentiometric data obtained from mixtures (40 cm^3) of Cu^{II} (1.25×10^{-3} M), NTP and the bio-relevant ligand solutions in a concentration ratio 1 : 1 : 1 and $NaNO_3$ (0.1 M). All titrations were performed in a purified N_2 atmosphere, using aqueous 0.05 M NaOH as titrant.

The stability constants of Cu(NTP)L complexes, 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 formation of Cu-NTP complex. Hence, in calculation only complex formation between Cu(NTP) and ligand (HL) is considered and each of these systems could be treated as binary. 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 $[Cu(NTP)]^{4-}$, ligand and proton, respectively.

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \tag{1}$$

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

Calculations were performed using the computer program [22] MINIQUAD-75. The stoichiometries and stability constants of the complexes formed were determined

Table 1. Formation constants of $M_pL_qH_r$ species.

System	p	q	r[a]	logβ[b]	S[c]
Cu–NTP	0	1	1	11.08(0.01)	7.3E−8
	0	1	2	18.44(0.02)	
	0	1	3	24.60(0.02)	
	0	1	4	29.53(0.03)	
	0	1	5	31.19(0.08)	
	1	0	−1	−6.44(0.07)	
	1	0	−2	−12.99(0.02)	1.9E−7
	1	1	0	15.45(0.03)	
	1	1	1	21.78(0.04)	2.9E−7
	1	1	2	27.21(0.02)	
	1	1	3	31.01(0.01)	

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

by trying various possible composition models. 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 [22]. The concentration distribution diagrams are obtained using the program SPECIES (L. Pettit, personal communication) under the experimental conditions used. The results obtained are shown in table 1.

2.4. *Spectrophotometric measurements*

The samples utilized for spectrophometric measurements were prepared as follows:

(a) $5\,cm^3$ (0.01 M) + $5\,cm^3$ (0.01 M) NTP + amount of base required to neutralize the H^+ liberated from complex formation. The final volume was brought to $25\,cm^3$.
(b) $5\,cm^3$ (0.01 M) + $5\,cm^3$ (0.01 M) NTP + $10\,cm^3$ (0.01 M) Imidazole + amount of base required to neutralize the H^+ liberated from complex formation. The final volume was brought to $25\,cm^3$.

2.5. *Kinetic measurements*

The kinetics of hydrolysis of the complexed ester were investigated using aqueous solution ($40\,cm^3$) of a mixture of copper(II) (6.25×10^{-3} M), NTP (6.87×10^{-3} M), glycine methyl ester (1.25×10^{-3} M) and $NaNO_3$ (0.1 M). In this mixture the [Cu–NTP] : [ester] ratio was adjusted to 5 : 1, so as to maximize the amount of complexed ester present. A 10% excess of NTP over copper(II) was used to ensure coordination of all copper(II), which is itself an excellent catalyst. A 20% excess NTP gave the same rates as a 10% excess indicating free Cu^{2+} was not involved in the hydrolysis. In all cases the solutions were equilibrated at the desired temperature under a constant nitrogen flow. The ester solution was then added, and the pH brought to the desired value by addition of 0.05 M NaOH as described previously [15–17]. The data fitting was performed with the OLIS KINFIT set of programs [23] as indicated previously [13]. Values of the hydroxide ion concentration were estimated from the pH using $pK_w = 13.997$ and an activity coefficient of 0.772. For the variable temperature studies the following values of pK_w and γ were employed [24] at 20°C ($pK_w = 14.146$, $\gamma = 0.774$), at 30°C ($pK_w = 13.833$, $\gamma = 0.770$), at 35°C ($pK_w = 13.680$, $\gamma = 0.768$) and at 40°C ($pK_w = 13.535$, $\gamma = 0.766$). The activity coefficients were obtained using the Davies equation [25].

3. Results and discussion

3.1. *Equilibrium studies*

Nitrilo-tris(methyl phosphonic acid) (NTP) differs from nitrilotriacetic acid (NTA) in the stepwise 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 rang 4.9–7.2. The remaining phosphonic group has log K value arround 2, which can be determined pH-metrically only with rather high uncertainities. Five of the six protons are titratable and can be measured by pH potentiometry. The protonation constants listed in table 1 are in reasonably good agreement with earlier reports [26–28]. The acid dissociation constants of amino acids, peptides, DNA constitutents 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 [29].

The potentiometric data of Cu–NTP solution mixtures were fitted assuming the formation 110, 111, 112 and 113 species but not 120 species. The formation of the 1 : 2 complex seems to be generally hindered because NTP is a tetradentate ligand. Also the electrostatic replusion of the negatively charged phosphonate groups precludes coordination of the second ligand: the charge of the 1 : 2 complex would be −10. In the 1 : 1 complex $Cu(NTP)^{4-}$ the ligand is tetradentate with nitrogen and phosphate groups. A comparison between NTP and NTA complexes with copper(II) indicates that $Cu(NTP)^{4-}$ is more stable than the corresponding NTA complex (13.05) [30]. Thus substitution of carboxylate group by phosphonate group increases the stability of the complexes due to the higher basicities of the phosphonic functions.

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 of 1 : 1 Cu^{II}-NTP complex is larger than 1 : 1 Cu^{II}-Ligand (L) complex, (table 1). It is resonable to propose that in presence of both ligands, one molecule of NTP is coordinated to the copper(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 the Cu^{II}-NTP-glycine system is shown in figure 1. The Cu^{II}-NTP(1 : 1) 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 Cu^{II}-NTP complex is formed first due to its greater stability compared to the Cu^{II}-L complex (table 1). Beyond $a = 6$, the formation of a 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 that of the Cu^{II}-NTP titration curve. The mixed ligand system was found to deviate considerably from the resulting composite curve, indicating the formation of a ternary complex. Thus, formation of ternary complex can be described by the following stepwise equilibria (equations 3 and 4).

$$Cu + NTP \rightleftharpoons Cu(NTP) \tag{3}$$

$$Cu(NTP) + L \rightleftharpoons Cu(NTP)L \tag{4}$$

The stability constant value of the monodentate methylamine complex is slightly higher than that of glycine, indicating that glycine most likely coordinates with Cu(NTP) as a monodentate rather than a bidentate ligand. The extra stability of the methylamine complex may be due to higher basicity of its amino group than that of glycine amino group (as reflected by their pK_a values). This finding is further supported by the fact that the stability constant of Cu(ethylenediamine)-glycine

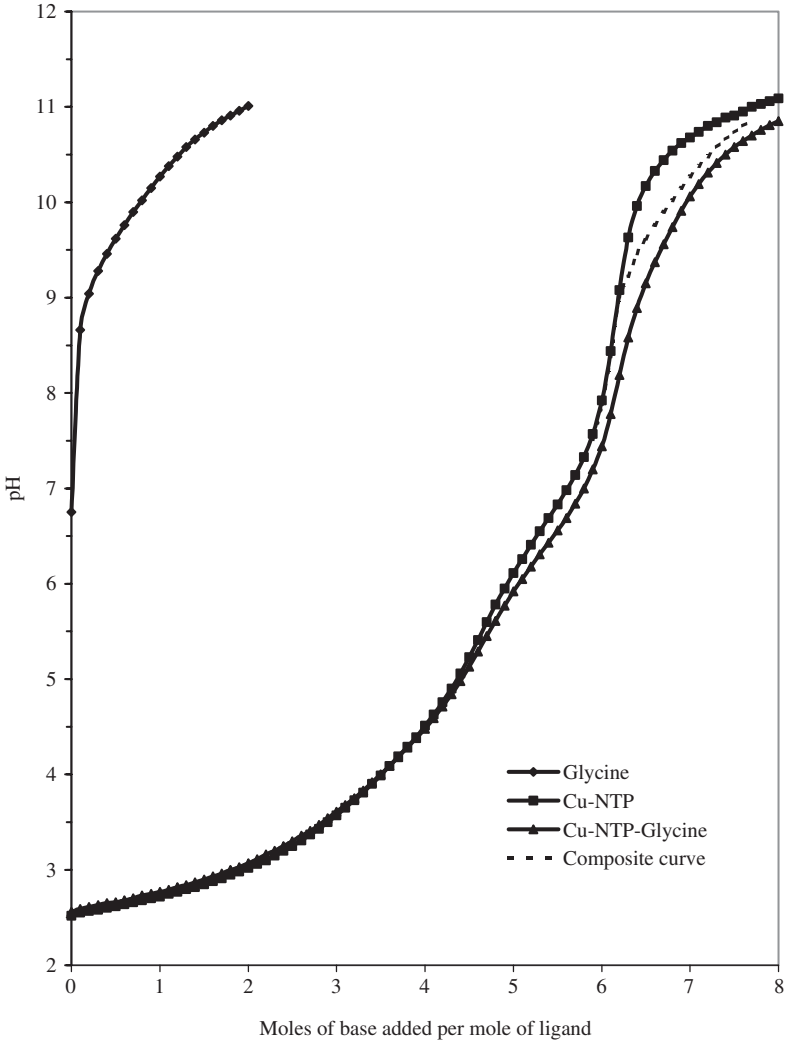


Figure 1. Potentiometric titration curves of Cu-NTP-Glycine system.

complex, is 7.47 [31], significantly higher than that of its corresponding complex with Cu(NTP) (2.84). This may be considered further evidence that glycine acts as monodentate ligand. Consequently, ring opening in Cu(NTP) complex, allowing for chelation of glycine as a bidentate ligand or chelation of glycine to equatorial and apical sites would be unlikely.

The β-alcoholato-group, in the side chain of the amino acids serine and threonine would play an essential role in the functions of a number of protolytic enzymes such as chymotrypsin and subtilisin [32] and previously [33] reported to bind to copper(II). The ionized amide residue of the peptide, [-CONH-], behaves as an important ligating group and coordinates to copper(II) through binding with the ionized amide group [11]. The potentiometric data reported for the peptides and threonine complexes reveals the formation of Cu(NTP)(L) species rather than $Cu(NTP)LH_{-1}$ supporting the view

that ring opening of Cu(NTP) and induced ionization of peptide hydrogen and β-alcoholato-group would be unfavored. This finding is in agreement with our previous investigation carried out on the Cu(II)-diethylenetriamine-peptide system [34].

The pyrimidines uracil, uridine, thymine and thymidine are protonated at the N_3 site. They form mixed-ligand complex at high pH and do not form protonated complex. Consequently, the pyrimidines coordinate through the deprotonated basic nitrogen (N_3). Inosine may become protonated at N(7) with formation of [N(1)H–N(7)H] monocations. In the present study, the pK_a of N(1)H is only determined since the pK_a of N(7)H is too low to be detected by the potentiometric technique. The potentiometric data of the mixed ligand complex involving inosine showed the formation of Cu(NTP)L, where L is the monoanion of inosine. In the acidic pH range, N(1) remains protonated, while the metal ion is attached to N(7). The gradual change from N(7)-binding to N(1)-binding with increasing pH has been frequently documented by ^{1}H NMR [35] and EPR [36] spectroscopic measurements. Consequently, it is proposed that N(1) serves as a coordination site in the mixed ligand complexes of inosine at higher pH values.

The relative stabilities of the ternary and binary complexes can be quantitatively expressed in a number of different ways. It has been argued that a comparison can best be made in terms of $\Delta\log K$ [37]. The relative stability of the ternary complexes formed through a stepwise mechanism, as compared to those of binary complexes, is expressed in terms of $\Delta\log K$ as defined by equation (5).

$$\Delta\log K = \log K^{Cu(NTP)}_{Cu(NTP)L} - \log K^{Cu}_{CuL} \tag{5}$$

One expects to obtain negative values for $\Delta\log K$ (table 1), since more coordination positions are available for bonding of ligand (L) in the binary than in the ternary complexes, indicating that the secondary ligand (L) amino acid, peptide or DNA forms more stable complexes with copper(II) alone than with Cu^{II}-NTP complex. It is to be noted that imidazole has the less negative $\Delta\log K$ value, perhaps evidence for enhanced stability involving π-back donation from the negatively charged NTP^{6-} ion to the π-system of the imidazole. The $\Delta\log K$ value of the mixed ligand amino acids complexes are more negative than that of methylamine. This may be described on the premise that the amino acid is a bidentate ligand while methylamine is a monodentate ligand and there is only one equatorial coordination site available in $Cu(NTP)^{4-}$. The negative values obtained for $\Delta\log K$, are of the order:- Tridentate- (as histidine) > bidentate- (as glycine) > monodentate- (as imidazole) ligand. This can be justified by the fact that the Cu-NTP complex provides only one available coordination site.

Estimation of the concentration distribution of the various species in solution provides a useful picture of metal ion binding. To illustrate the main features observed in the species distribution plots in these systems the speciation diagram obtained for the Cu-NTP-glycine system is shown in figure 2. The mixed ligand species [Cu(NTP)L] (110) starts to form at pH $\sim$6 and its concentration reaches the maximum of 28% at pH = 10.

3.2. *Visible electronic spectra*

Copper(II) complex geometries have been thoroughly characterized by means of visible spectra [38–40]. The visible spectra scanned in the region 500–900 nm reveal the d–d

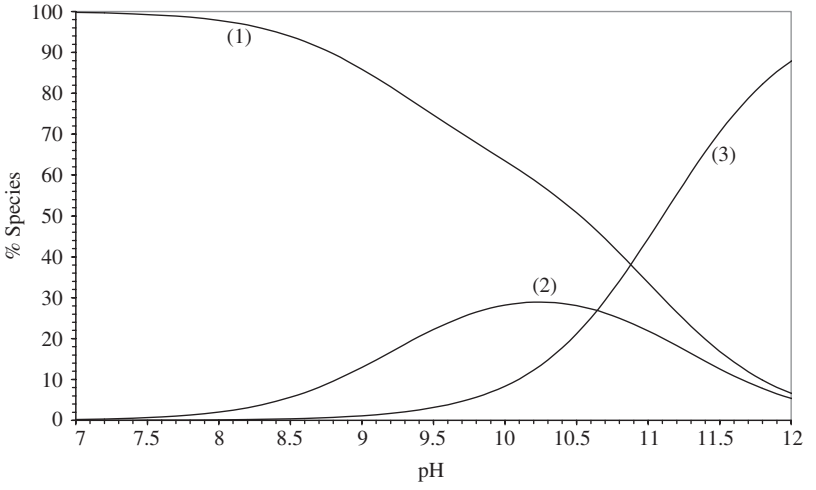


Figure 2. Concentration distribution of various species as a function of pH in the Cu-NTP-Glycine system. 1: $[Cu(NTP)]^{-4}$; 2: $[Cu(NTP)(glycinate)]^{-5}$; 3: $[Cu(NTP)(glycinate)(OH)]^{-6}$.

transition of the Cu(II) ion being dependent on the coordination geometry [41–42]. The UV/vis spectra of $[Cu(Me_6tren)(H_2O)]^{2+}$ and $[Cu(tren)(H_2O)]^{2+}$ were consistent with the published spectra [43–44] and were indicative of trigonal bipyramidal geometry since a maximum at 880 nm and a shoulder in the region of 700 nm for $[Cu(Me_6tren)(H_2O)]^{2+}$ and a maximum of 850 nm for $[Cu(tren)(H_2O)]^{2+}$ were observed. The X-ray structure of $[Cu(Me_6tren)(H_2O)]^{2+}$ supports this geometry [45]. The visible spectrum of [Cu-NTP], displayed in figure 3, shows an absorption maximum at 855 nm, suggesting that the [Cu-NTP] complex has such a geometry. Similarly the [Cu-NTP-Imidazole], as a representative of mixed ligand complexes, exhibits an absorption maximum at 900 nm and a shoulder in the region of 700 nm pointing toward trigonal bipyramidal geometry for the solution species.

3.3. *Hydrolysis kinetics*

Hydrolysis of the coordinated ester was monitored over the pH range (8.2–9.0). In this range, the rate of hydrolysis of MeGly is negligible in the absence of $Cu(NTP)^{4-}$. The kinetic data, the volume of base added to keep the pH constant versus time, could be fitted by one exponential as shown in figure 4. Various other kinetic models were tested without satisfactory fits of the data. The values of k_{obs} at different pH are given in table 3. Plots of k_{obs} versus the hydroxide ion concentration are linear, figure 5. The rate expression can therefore be given in the form of equation (6).

$$k_{obs} = k_o + k_{OH}[OH^-] \tag{6}$$

the k_o term arises from attack of water being expressed by the relation (7) [46].

$$k_{H_2O} = \frac{k_o}{55.5} \tag{7}$$

Table 2. Formation constants of $M_pL_qH_r$ species.

System	p	q	r[a]	log β[b]	S[c]	Log K_{CuL}^{Cu}[d]	Δlog K
Cu(NTP)-OH	1	0	−1	−10.88(0.01)	1.0E−8		
Glycine	0	1	1	9.60(0.01)	1.5E−7	8.19	−5.35
	0	1	2	11.93(0.03)	5.8E−9		
	1	1	0	2.84(0.04)			
Alanine	0	1	1	9.69(0.01)	9.2E−8	7.99	−5.20
	0	1	2	11.88(0.02)	1.3E−8		
	1	1	0	2.79(0.07)			
Threonine	0	1	1	9.06(0.01)	7.9E−9	8.22	−5.92
	0	1	2	11.03(0.02)	2.2E−7		
	1	1	0	2.30(0.05)			
Imidazole	0	1	1	7.04(0.01)	2.6E−9	4.15	−0.44
	1	1	0	3.71(0.08)	2.7E−7		
Methylamine	0	1	1	10.55(0.004	8.9E−9	6.82	−3.09
	1	1	0	3.73(0.02)	2.2E−8		
Histamine	0	1	1	9.88(0.01)	2.4E−8	9.5	−5.29
	0	1	2	15.97(0.01)	1.1E−7		
	1	1	0	4.21(0.09)			
Histidine	0	1	1	9.53(0.01)	1.6E−7	10.66	−6.80
	0	1	2	15.81(0.03)	5.5E−8		
	0	1	3	17.81(0.06)			
	1	1	0	3.86(0.07)			
Ornithine	0	1	1	10.58(0.00)	1.0E−8	11.9	−7.82
	0	1	2	19.43(0.02)	1.7E−7		
	0	1	3	21.38(0.02)			
	1	1	0	4.08(0.06)			
Glycinamide	0	1	1	7.88(0.02)	4.6E−8	4.7	−1.51
	1	1	0	3.19(0.09)	3.1E−9		
Glutamine	0	1	1	9.06(0.01)	4.5E−8	7.69	−5.22
	1	1	0	2.47(0.06)	1.3E−8		
Uracil	0	1	1	9.15(0.01)	5.0E−9	4.55	−1.06
	1	1	0	3.49(0.02)	3.9E−9		
Uridine	0	1	1	9.01(0.01)	1.1E−7	4.32	−1.34
	1	1	0	2.98(0.05)	7.6E−9		
Inosine	0	1	1	8.43(0.01)	4.1E−8	4.61	−1.89
	1	1	0	2.72(0.08)	1.0E−8		

[a]p, q and r are the stoichiometric coefficients corresponding to $Cu(NTP)^{4-}$, amino acids, peptides or DNA units and H^+; [b]Standard deviations are given in parentheses; [c]Sum of square of residuals. [d]These data, except that of $Cu-(NTP)^{4-}$ are taken from [16].

where 55.5 mol dm^{-3} is the molar concentration of water. The value of k_o can be determined from the intercept of figure 5, while the value of k_{OH} from the slope of the respective plot. The rate constants, k_{H_2O} and k_{OH}, are given in table 4.

The overall Cu^{2+}-catalyzed hydrolysis of MeGly proceeds via the following steps (8a–8c)

$$Cu^{2+} + A^{x-} \overset{K_f}{\rightleftharpoons} CuA^{2-x} \tag{8a}$$

$$CuA^{2-x} + GlyOMe \overset{K_x}{\rightleftharpoons} CuAGlyOMe^{2-x} \tag{8b}$$

$$CuAGlyOMe^{2-x} + OH^- \overset{k_{OH}}{\rightleftharpoons} CuAGly^{2-(X+1)} + MeOH \tag{8c}$$

Under the conditions used in this study almost all of the ester is coordinated to CuA, where $A = NTP$. Therefore, the observed rate law (equation 6) is the third step

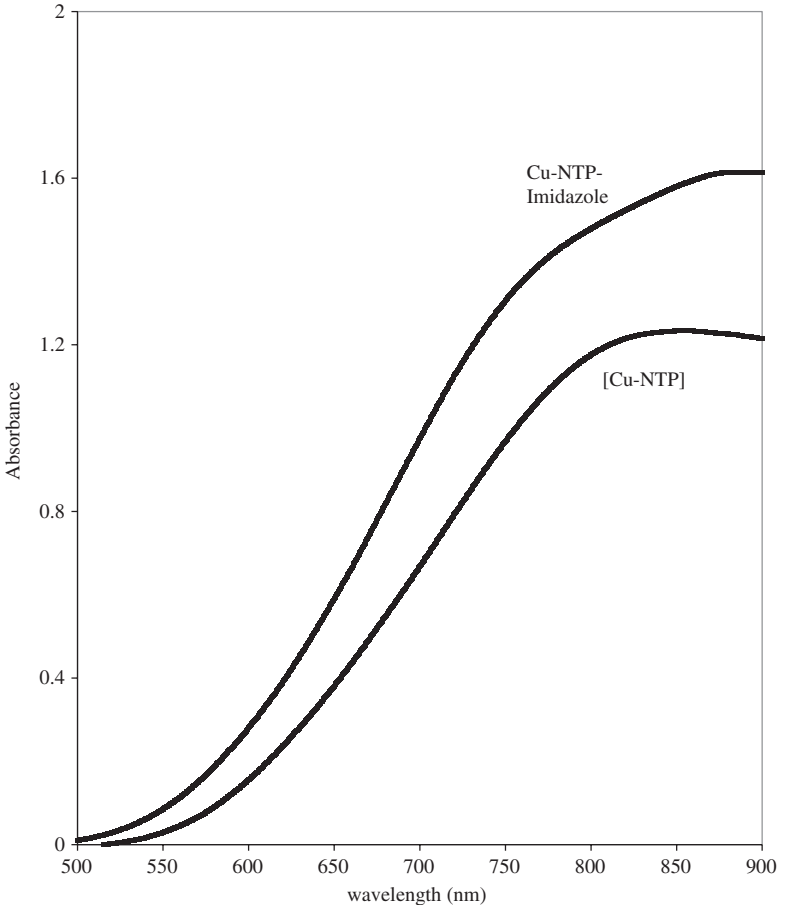
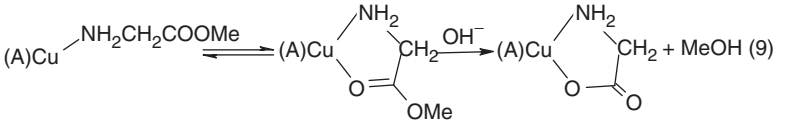
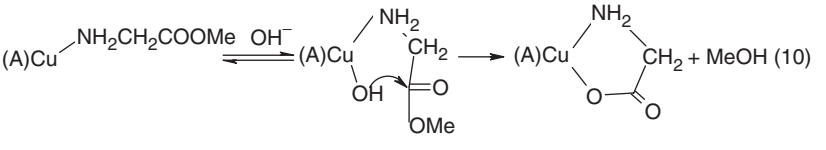


Figure 3. Room-temperature visible spectra of [Cu-NTP] and [Cu-NTP-Imidazole] complexes in water.

(k_{OH}) only. The first order dependence on OH^- concentration for this step may be accounted for by three general mechanisms [46–49]. One involves an initial rapidly established equilibrium in which the carbonyl oxygen of the ester group coordinates, followed by OH^- attack (equation 9).



The second mechanism involves rapid equilibrium formation of a M–OH complex, followed by intramolecular OH^- attack (equation 10).



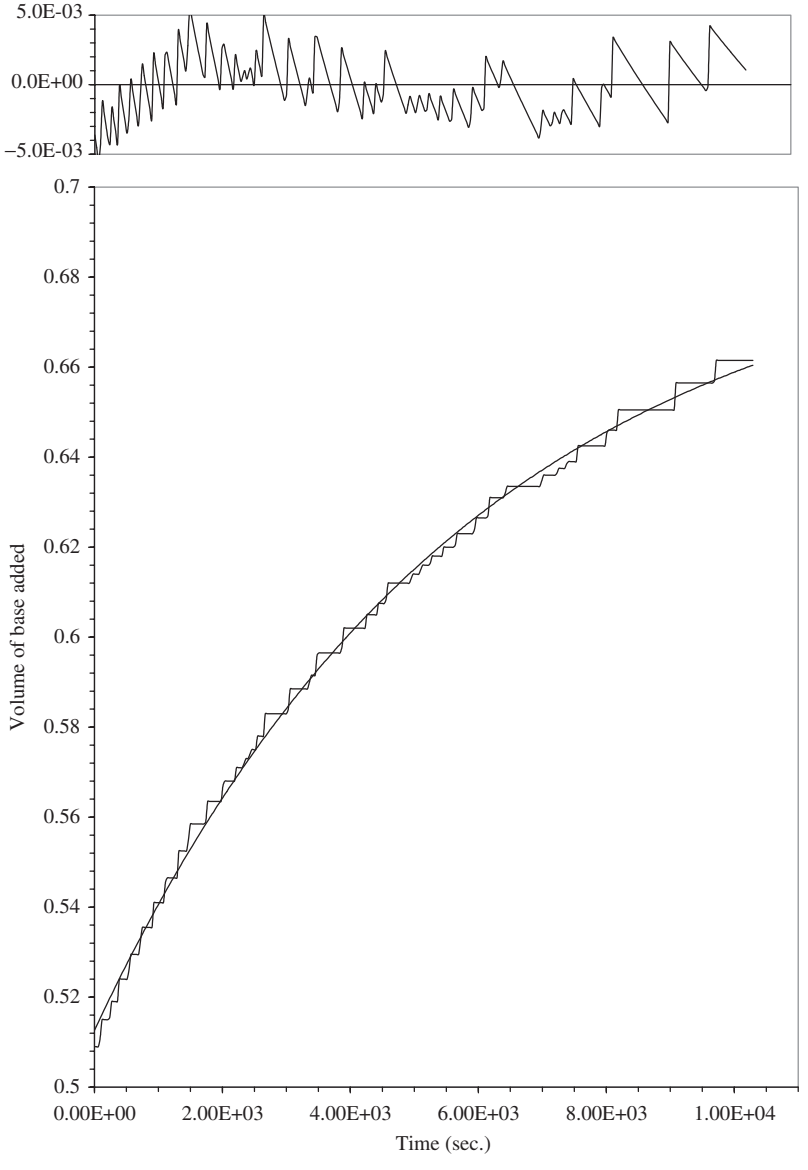


Figure 4. Typical volume of base added-time trace for the hydrolysis of Cu-NTP-GlyOMe ester fitted with one exponential function. The top of the figure shows the volume of base difference between the measured and calculated kinetic traces at $I = 0.1$ M; pH = 8.4 and T = 25°C.

The third mechanism inolves only OH^- attack on the uncoordinated carbonyl carbon of the ester group (equation 11).

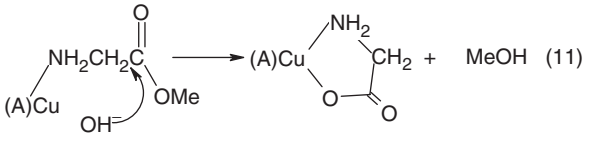


Table 3. Kinetics of hydrolysis of the coordinated glycine methyl ester at different temperatures in aqueous solution.

Temp.	pH	10^6 [OH][a]/mol dm^{-3}	10^4 k_{obs}/s^{-1}
20	8.20	1.46	0.80
	8.40	2.32	1.04
	8.60	3.67	2.05
	8.80	5.82	2.90
	9.00	9.23	4.70
25	8.20	2.06	1.30
	8.40	3.27	1.70
	8.60	5.19	2.75
	8.80	8.22	4.80
	9.00	13.04	7.18
30	8.20	3.02	2.05
	8.40	4.79	2.62
	8.60	7.59	4.82
	8.80	12.03	6.82
	9.00	19.02	11.53
35	8.20	4.31	2.50
	8.40	6.83	4.64
	8.60	10.83	7.14
	8.80	17.16	11.14
	9.00	27.20	17.40
40	8.20	6.03	3.67
	8.40	9.56	7.69
	8.60	15.16	9.97
	8.80	24.03	16.40
	9.00	38.08	26.30

[a] pK_w 14.146 at 20°C; 13.997 at 25°C, 13.833 at t 30°C, 13.68 at 35°C. and 13.535 at 40°C. These data were taken from [50].

The catalysis constant, $C = k_{OH}/k_{OH}^{ester}$ obtained for glycine methyl ester is 43. The formation of monodentate N-coordinated ester species would not lead to rate accelerations greater than ca. 10^2 [50] therefore, a rate enhancement ($C = 43$) is consistent with the third mechanism, equation (11) in which a monodentate ester coordination species is formed, while formation of bidentate ester complexes with copper (II) leads to rate accelerations of 10^5–10^6 [51–54]. On the other hand the plot of k_{obs} vs. the hydroxide ion concentration in the concentration range used is linear, which is inconsistent with the second mechanism (equation 10) [46]. Also it is unlikely that an OH^- ion would add to form a stable five-coordinate derivative since Cu^{II} is known to prefer four coordination [55]. Evidence for the formation of such complex species, in which the ester is coordinated by the amino group only, is also proved by the results of the potentiometric measurements. The amino acid is coordinated as a monodentate ligand by the amino group as it is unlikely that the carboxylate group occupies the apical site as a result of Jahn-Teller effect operative in copper(II), causing elongation of the bonds in the apical site about copper(II). Also, binding of negatively charged carboxylate to Cu^{II} already coordinated to hexanegatively charged $(NTP)^{6-}$ ion seems to be disfavored.

The activation parameters ($\Delta S^{\pm}$ and $\Delta H^{\pm}$) were determined for the hydrolysis of coordinated glycine methyl ester from the temperature dependence of the data in table 4. The thermodynamic parameters were obtained from the plot of $\ln(k_{OH}/T)$ versus $1/T$ using the Eyring equation [56] and displayed in figure 6. The slope of the plot

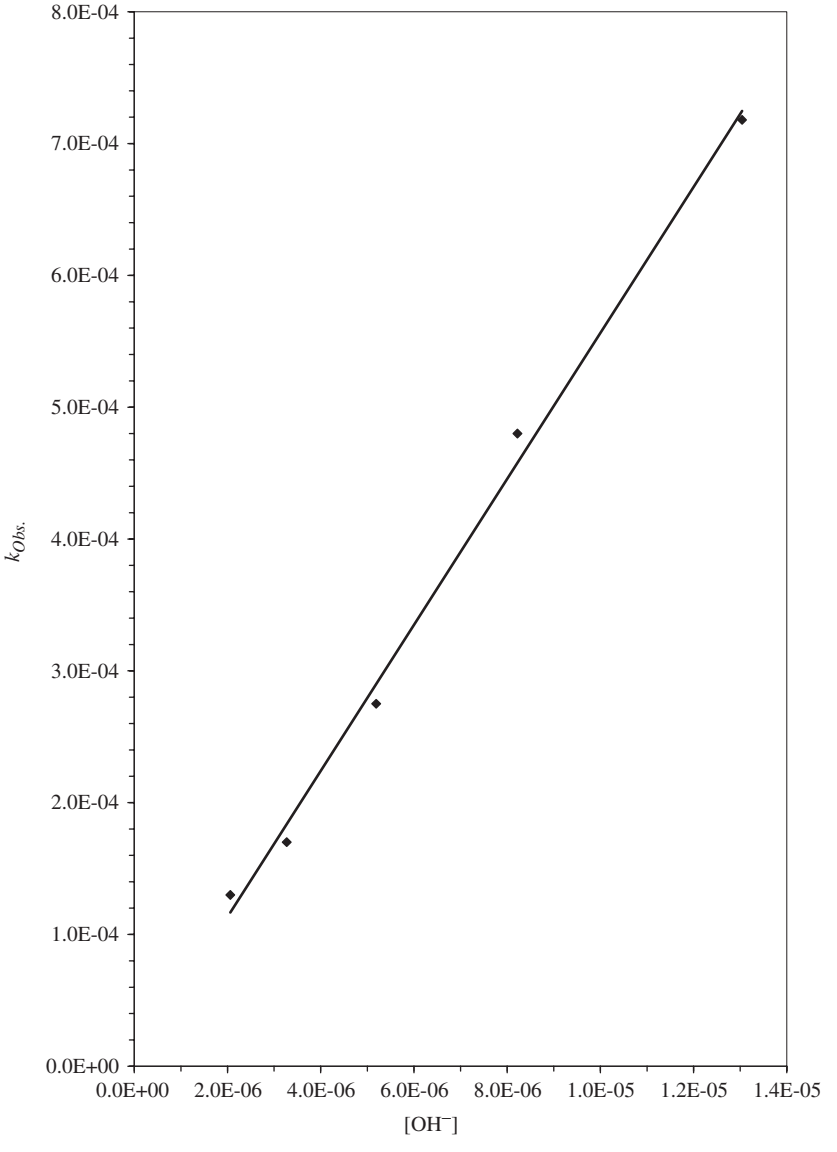


Figure 5. Kinetic plot of k_{obs} versus the hydroxide ion concentration for the hydrolysis of Cu–NTP–GlyOMe ester in water solutions at 25°C and $I = 0.1$ M.

Table 4. Rate constants (k/dm^{3}mol^{-1}s^{-1}) for base hydrolysis of coordinated glycine methyl ester at different temperatures.

Temp.°C	$10^{6}\ k_o$(s^{-1})	$10^{8}\ k_{H_2O}$(dm^3 mol^{-1} s^{-1})	$10^{-1}\ k_{OH}$(dm^3 mol^{-1} s^{-1})
20	1.79	3.23	5.06
25	2.71	4.87	5.54
30	3.59	6.48	5.95
35	5.10	9.18	6.42
40	6.25	11.26	6.86

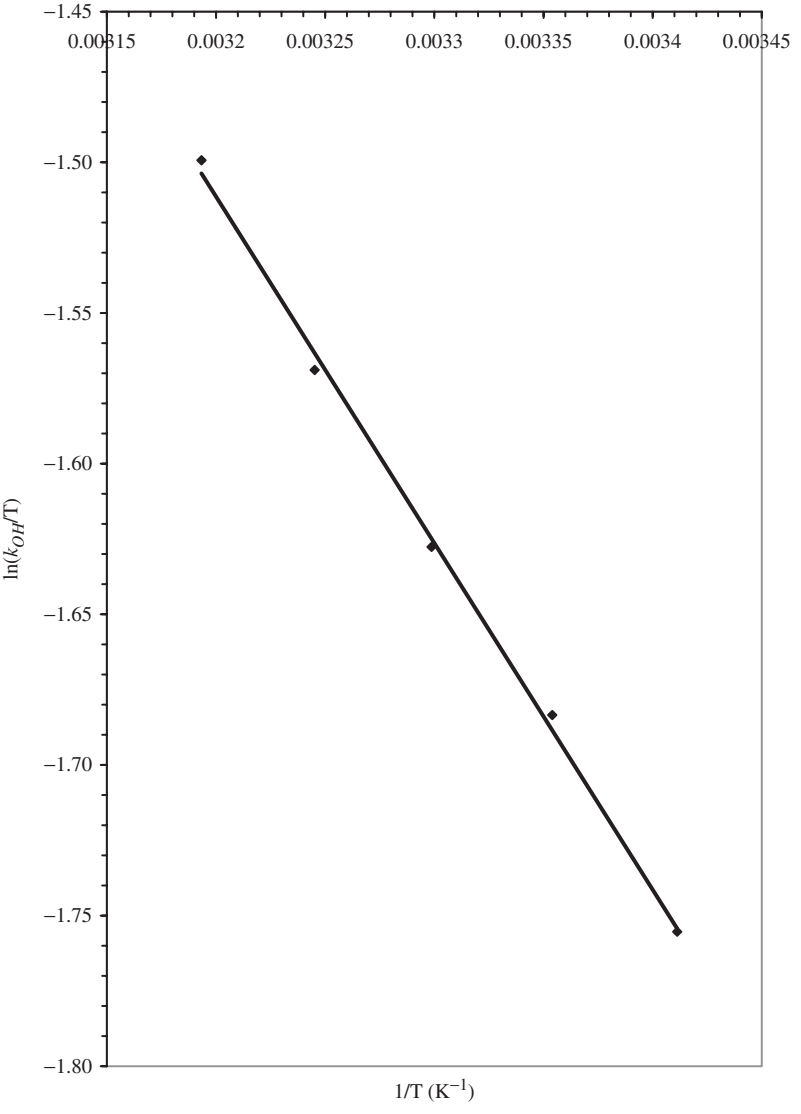


Figure 6. Eyring plot of $\ln(k_{OH}/T)$ versus $1/T$ for the base hydrolysis of the ester group in the complex Cu–NTP.

Table 5. Rate (k_{OH}) and equilibrium costants associated with Cu(II)–catalyzed hydrolysis of MeGly at 25°C.

CuL(MeGly)^n	k_{OH}(dm^3 mol^{-1} s^{-1})	Log K_x(ester)	Log K_f
Cu(EtGly)$^{2+}$	7.6×10^4 [a]	4.04[b]	–
Cu(Ida)(MeGly)	7.6×10^3 [c]	3.69[d]	10.63[e]
Cu(IdP)(MeGly)$^{2-}$	0.66×10^2[f]	3.18[f]	12.98[f]
Cu(NTP)(MeGly)$^{4-}$	0.54×10^2	2.92	15.45

[a] Although not reported, the rate for Cu(MeGly)$^{2+}$ would be somewhat faster(~2 times) than for Cu(EtGly)$^{2+}$ given above, [60]. [b] [61]. [c,d] [62]. [e] [63]. [f] [64].

is $\Delta H^{\pm}/R$ and the intercept is related to $\Delta S^{\pm}$ by equation (12) where K, h and R are the Boltzmann, Plank and gas constants, respectively.

$$\Delta S^{\pm} = [\text{intercept} - \ln(K/h)]R \tag{12}$$

The activation parameters were determined for the hydrolysis of [Cu(NTP)(GlyOMe)]$^{4-}$ at $I = 0.1$ M. The temperature dependences of the rate constants k_{OH} and k_{H_2O} are summarized in table 4. For k_{OH}, $\Delta H^{\pm} = 9.5 \pm 0.3$ kcal mol^{-1}, $\Delta S^{\pm} = -179.3 \pm 0.9$ cal K^{-1} mol^{-1}, while for k_{H_2O} $\Delta H^{\pm} = 45.3 \pm 3$ kcal mol^{-1}, $\Delta S^{\pm} = -232.8 \pm 9$ cal K^{-1} mol^{-1}. For base hydrolysis of free glycine methyl ester the activation parameters were found [57] to be $\Delta H^{\pm} = 39.7$ kJ mol^{-1}, $\Delta S^{\pm} = -117$ J K^{-1} mol^{-1}. The enhanced rate for base hydrolysis of the ester incorporated in the complex is therefore due to contributions from a decreased $\Delta H^{\pm}$. Attack of water on the [Cu(NTP)GlyOMe]$^{4-}$ complex as determined by k_{OH}/k_{H_2O} ratio is some 10^9 slower than that of hydroxide ion. This large difference in nucleophilicities between OH^- and H_2O is also observed for a variety of copper(II) complexes [57].

Previously, it was proposed [58] that the catalytic activity of metal chelates toward amino acid ester hydrolysis could be correlated with the formation constant of metal chelate (steps from a–c). Large formation constants (K_f) result in reduced Lewis acid character (K_x) of the metal chelate toward esters and, therefore, lower catalytic activities were observed for a series of Cu^{2+} chelates [59]. Metal chelates of the highest Lewis acidity are the most effective promoters of ester hydrolysis. According to the data in table 5, the Cu(II) complexes exhibit significant catalytic effects which decrease in the order Cu^{2+} > Cu(Ida) > Cu(Idp)$^{2-}$ > Cu(NTP)$^{4-}$, the same order observed for K_x (table 5) in the complexation of glycine esters by these Cu^{2+} complexes. The decrease in k_{OH} follows an increase in the formation constant of the metal chelate (K_f), but we can also interpret this trend in terms of the magnitude of positive charge on the metal chelates i.e. complexes with high positive charge yielded the highest k_{OH}.

4. Abbreviations

NTP : Nitrilo-tris(methyl phosphonic acid)
NTA : Nitrilotriacetic acid
Ida : Iminodiacetic acid
Idp : Iminodiphosphonic acid
Tren : 2,2′,2″-triaminotriethylamine
Me_3tren : 2,2′,2″-tris(monomethylamino)triethylamine
Me_6tren : 2,2′,2″-tris(dimethylamino)triethylamine
MeGly : Glycine methyl ester
EtGly : Glycine ethyl ester

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