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Kinetics and mechanism of complexation of Cu (II) with γ -L-glutamyl-L-cyteinyglycine

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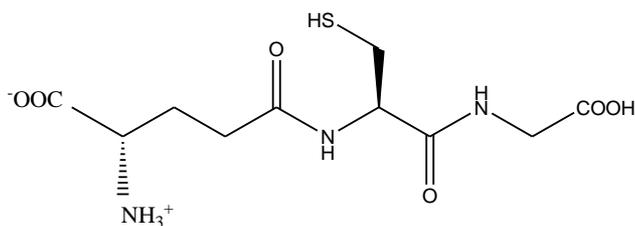
Abstract

The kinetics of the complexation of Cu(II) with γ -L-glutamyl-L-cyteinyglycine (IUPAC name: (2S)-2-Amino-4-[[[(1R)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl]butanoic acid) has been investigated at 25, 30, 35 and 40 \pm 0.05 $^{\circ}$ C in the pH range 2.51-3.53 using Aminco Morrow Stopped Flow Spectrophotometer. The ionic strength was maintained at 0.1 M KNO₃. No indicator was used for this complexation reaction because transmittance changes were large enough to be monitored directly. The anionic form of γ -L-glutamyl-L-cyteinyglycine is more reactive and the protonated form interacts only to a small extent. Kinetic results indicate that the ligand is not involved in the rate determining step which is, in fact, associated with the release of a water molecule from the shell of the metal ions prior to complexation with the ligand. Enthalpy, entropy of activation, energy of the molecule and heat of formation were also calculated. A mechanism consistent with the kinetic data has been suggested.

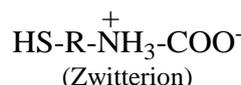
Keywords: Kinetics, complexation, metal ions, biochemical ligand, rate constant, activation parameters, energy of the molecule, heat of formation

Introduction

γ -L-glutamyl-L-cyteinyglycine (glutathione) is involved in numerous cellular processes. These processes include the protection of cellular membranes and its constituents from oxidation. It is involved in transport of amino acids and peptides across cell membranes via the glutamyl cycle and play a role in determining the behaviour of metal ions in the body. It is an important antioxidant in plants, animals, fungi, and some bacteria. It also inhibits the damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals. The predominant form of glutathione in solution is



This can be represented in an abbreviated form as



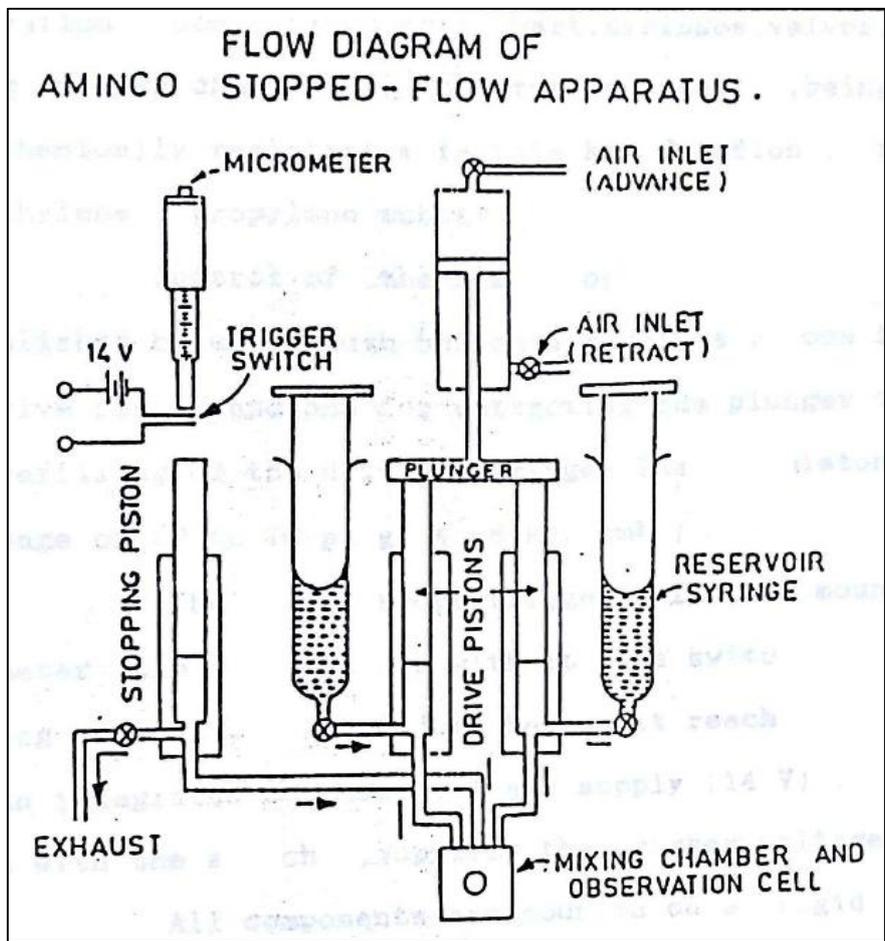
In order to understand the steps of metal binding to the glutathione it was thought desirable to carry out a comprehensive kinetic study on the complexation of copper (II) with glutathione. Therefore, the kinetics of complexation of metal ions Cu (II) by γ -L-glutamyl-L-cyteinyglycine would no doubt give important information regarding the types of interaction of these ions with γ -L-glutamyl-L-cyteinyglycine and the clearance of γ -L-glutamyl-L-cyteinyglycine or metal ions in the form of metal – ligand complex through the animal body [1-8]. With the hope that these anomalies might have rational explanation and to understand the biological processes clearly, a comprehensive kinetic study has been undertaken on the reaction of Cu (II) with γ -L-glutamyl-L-cyteinyglycine.

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Such investigations are further desired for determining some important parameters, e.g. binding steps, rate constants corresponding to the binding steps K_{os} (outer sphere complex formation constant), k_o (Rate constant of water exchange) [9] and activation parameters corresponding to the interaction of various reactive forms of the glutathione.

Materials and Methods

The complexation of Cu(II) with γ -L-glutamyl-L-cysteinyglycine (IUPAC name: (2S)-2-Amino-4-[[[(1R)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl]butanoic acid) has been investigated at 25, 30, 35 and $40 \pm 0.05^\circ\text{C}$ in the pH range 2.51-3.53 using Aminco Morrow Stopped Flow Spectrophotometer..



γ -L-glutamyl-L-cysteinyglycine(B.D.H.), KNO_3 (Sarabhai, G.R.) were used as such. Other chemicals used were of A.R. grade. The pH of ligand solution and metal ion solution was adjusted to same value using 2, 6-lutidine (Merck Schuchardt) and HCl. However, a slight change in pH value (~ 0.05 units) was observed after mixing of two solutions. The final pH was recorded from Radiometer pH meter, pH M26. pH's reported are those of reaction mixtures. The temperature of the system was maintained by immersion type thermostat (German NBE model). The kinetic runs were made under pseudo first order conditions, i.e., $[\text{M(II)}] \gg [\gamma\text{-L-glutamyl-L-cysteinyglycine}]$ ($M = \text{Cu}$) at 600nm. Copper nitrate, 10^{-2}M , buffer 10^{-2}M Lutidine and, $\mu = 0.1\text{M}$ NaClO_4 was mixed with ligand solution and the traces from the oscilloscope gave excellent first order plots, from which second order rate constants (k_{obs}) were computed by the relation.

$$k_{obs} = k'_{obs}[\text{M(II)}] \text{----- (1)}$$

where k'_{obs} is the pseudo first order rate constant.

Results and Discussion

Cu(II)- γ -L-glutamyl-L-cysteinyglycine Complexation:

The kinetics of complexation of Cu(II) – γ -L-glutamyl-L-cysteinyglycine was found to be of first order in copper ion, which was taken in large excess over ligand to ensure pseudo-first order conditions and complete formation of mono complex only [8-15]. As protonated form of the ligand predominates in the pH range 2-4 (pKa: $\text{pK}_1 = 2.12$, $\text{pK}_2 = 3.53$, $\text{pK}_3 = 8.66$, $\text{pK}_4 = 9.12$), the kinetic study of interaction has been made in the pH range 2.51-3.53 at ionic strength 0.1M KNO_3 and at temperatures 25, 30, 35 and $40 \pm 0.05^\circ\text{C}$, under the condition $[\text{Cu(II)}] \gg [\gamma\text{-L-glutamyl-L-cysteinyglycine}]$. Oscilloscope traces of voltage versus time were used to determine the values of pseudo-first order rate constants (k'_{obs}), and these were further utilized to evaluate the values of second order rate constants (k_{obs}), using equation (1). These rate constants are tabulated in Table 1.

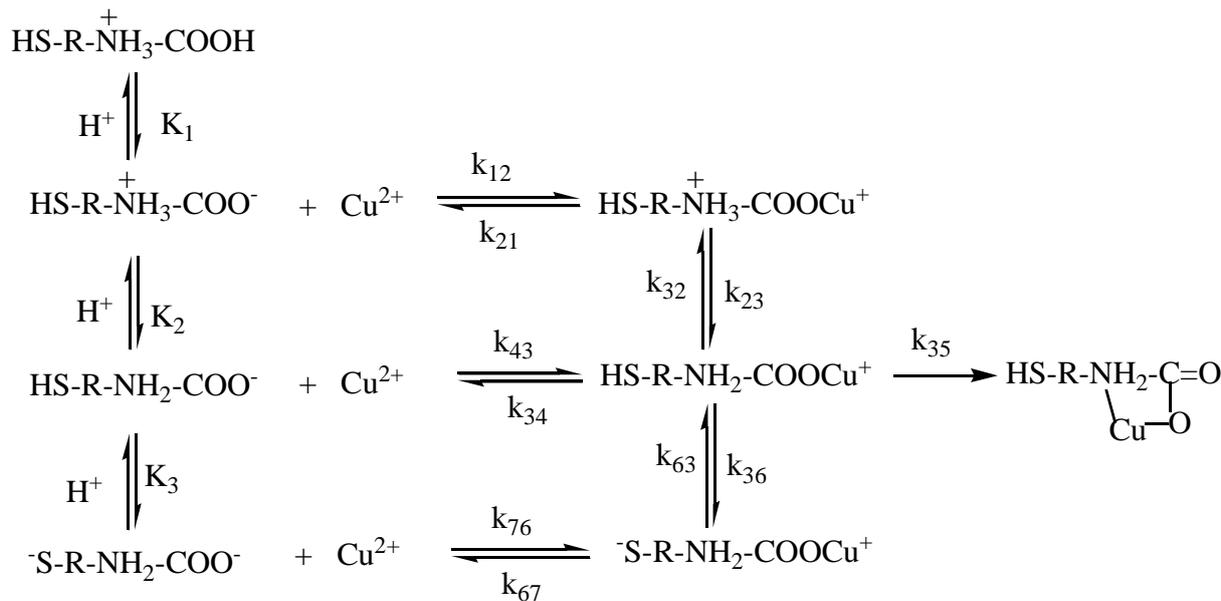
Table 1: First order and Second order rate constants for the complexations of Cu(II) with γ -L-glutamyl-L-cyteinylglycine at different pH's and temperatures
 I = 0.10 M KNO₃
 [Cu(II)] = 1.78 x 10⁻² M
 [γ -L-glutamyl-L-cyteinylglycine] = 1.26 x 10⁻³ M

Temp. ($\pm 0.05^\circ\text{C}$)	pH	$k'_{\text{obs}} \times 10^4 (\text{s}^{-1})$	$k_{\text{obs}} \times 10^{-1} (\text{M}^{-1} \text{s}^{-1})$	Temp. ($\pm 0.05^\circ\text{C}$)	pH	$k'_{\text{obs}} 10^4 (\text{s}^{-1})$	$k_{\text{obs}} 10^{-1} (\text{M}^{-1} \text{s}^{-1})$
25	2.51	2.33	1.31	35	2.51	4.14	2.33
25	2.83	2.70	1.52	35	2.81	6.21	3.49
25	2.99	5.30	2.98	35	2.95	7.92	4.45
25	3.21	8.47	4.76	35	3.20	13.1	7.39
25	3.34	10.7	6.05	35	3.34	17.0	9.58
25	3.52	15.3	8.63	35	3.53	25.4	14.3
30	2.51	3.23	1.82	40	2.58	6.15	3.46
30	2.82	4.80	2.70	40	2.83	8.33	4.68
30	2.98	6.83	3.84	40	2.99	10.7	6.03
30	3.21	9.98	5.61	40	3.21	15.5	8.74
30	3.33	13.2	7.45	40	3.35	21.7	12.2
30	3.51	19.2	10.8	40	3.47	27.9	15.7

The dissociation of glutathione can be represented as



These forms react with Cu(II) stepwise as presented in Scheme 1.



Scheme-1

The rate equation for Cu(II) – γ -L-glutamyl-L-cyteinylglycine interaction can be written as

$$\begin{aligned}
 \text{Rate} &= -d/dt[\text{Cu(II)}] \\
 &= -d/dt[\gamma\text{-L-glutamyl-L-cyteinylglycine}] \\
 &= k_{\text{obs}} [\text{Cu(II)}] [\gamma\text{-L-glutamyl-L-cyteinylglycine}] \quad \text{----- (2)} \\
 &= k'_{\text{obs}} [\gamma\text{-L-glutamyl-L-cyteinylglycine}] \quad \text{----- (3)}
 \end{aligned}$$

Where $k'_{\text{obs}} = k_{\text{obs}} [\text{Cu(II)}]$
 Rate can be written as

$$\text{Rate} = k'_{\text{obs}} \{ [\text{HS-R-NH}_3^+\text{-COOH}] + [\text{HS-R-NH}_3^+\text{-COO}^-] + [\text{HS-R-NH}_2\text{-COO}^-] + [\text{S-R-NH}_2\text{-COO}^-] \} \quad \text{----- (4)}$$

Where $[\text{HS-R-NH}_3^+\text{-COOH}]$, $[\text{HS-R-NH}_3^+\text{-COO}^-]$, $[\text{HS-R-NH}_2\text{-COO}^-]$, $[\text{S-R-NH}_2\text{-COO}^-]$ represent the triprotonated form, diprotonated, monoprotonated and deprotonated form of the ligand respectively.

The rate from scheme 1 can be given as

$$\text{Rate} = k_{35} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] \tag{5}$$

Using steady state approximation for the intermediates

$$\begin{aligned} \left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right], \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right], \left[\text{S-R-NH}_2\text{-COOCu}^+ \right] \\ \frac{d}{dt} \left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right] = k_{12} \left[\text{HS-R-NH}_3^+\text{-COO}^- \right] \left[\text{Cu}^{2+} \right] - k_{21} \left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right] \\ + k_{32} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] - k_{23} \left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right] \left[\text{H}^+ \right] = 0 \end{aligned} \tag{6}$$

$$\begin{aligned} \frac{d}{dt} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] = k_{43} \left[\text{HS-R-NH}_2\text{-COO}^- \right] \left[\text{Cu}^{2+} \right] - k_{34} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] \\ + k_{23} \left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right] + k_{63} \left[\text{S-R-NH}_2\text{-COOCu}^+ \right] \\ - k_{32} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] - k_{35} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] = 0 \end{aligned} \tag{7}$$

$$\begin{aligned} \frac{d}{dt} \left[\text{S-R-NH}_2\text{-COOCu}^+ \right] = k_{76} \left[\text{S-R-NH}_2\text{-COO}^- \right] \left[\text{Cu}^{2+} \right] - k_{67} \left[\text{S-R-NH}_2\text{-COOCu}^+ \right] \\ + k_{36} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] \left[\text{H}^+ \right] - k_{63} \left[\text{S-R-NH}_2\text{-COOCu}^+ \right] = 0 \end{aligned} \tag{8}$$

From Scheme 1, it can be written as

$$\left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right] = \frac{k_{32}}{k_{23}} \left[\text{H}^+ \right] \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] \tag{9}$$

$$\left[\text{S-R-NH}_2\text{-COOCu}^+ \right] = \frac{k_{36}}{k_{63}} \left[\text{H}^+ \right] \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] \tag{10}$$

Adding equations (6), (7) and (8) and substituting the values of $\left[\text{HS-R-NH}_3^+\text{-COOCu}^+ \right]$ and $\left[\text{S-R-NH}_2\text{-COOCu}^+ \right]$ from equations (9) and (10) and on rearrangement, we get

$$\begin{aligned} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] = \frac{k_{23}k_{63}k_{12} \left[\text{HS-R-NH}_3^+\text{-COO}^- \right] \left[\text{Cu}^{2+} \right] + k_{23}k_{43}k_{63} \left[\text{HS-R-NH}_2\text{-COO}^- \right] \left[\text{Cu}^{2+} \right]}{k_{21}k_{32}k_{63} \left[\text{H}^+ \right] + k_{23}k_{34}k_{63}} \\ + \frac{k_{23}k_{63}k_{76} \left[\text{S-R-NH}_2\text{-COO}^- \right] \left[\text{Cu}^{2+} \right]}{k_{23}k_{35}k_{63} + k_{23}k_{36}k_{67}} \end{aligned} \tag{11}$$

From the dissociation of glutathione, the concentration of $\left[\text{HS-R-NH}_3^+\text{-COO}^- \right]$, $\left[\text{HS-R-NH}_2\text{-COO}^- \right]$ and $\left[\text{S-R-NH}_2\text{-COO}^- \right]$ can be written as

$$\left[\text{HS-R-NH}_3^+\text{-COO}^- \right] = K_1 \left[\text{HS-R-NH}_3^+\text{-COOH} \right] / \left[\text{H}^+ \right] \tag{12}$$

$$\left[\text{HS-R-NH}_2\text{-COO}^- \right] = K_1K_2 \left[\text{HS-R-NH}_3^+\text{-COOH} \right] / \left[\text{H}^+ \right]^2 \tag{13}$$

$$\left[\text{S-R-NH}_2\text{-COO}^- \right] = K_1K_2K_3 \left[\text{HS-R-NH}_3^+\text{-COOH} \right] / \left[\text{H}^+ \right]^3 \tag{14}$$

Substituting equations (12), (13) and (14) into equation (11) and on rearrangement, we get

$$\begin{aligned} \left[\text{HS-R-NH}_2\text{-COOCu}^+ \right] = \frac{k_{23}k_{63}k_{12}K_1 \left[\text{H}^+ \right]^2 + k_{23}k_{43}k_{63} K_1K_2 \left[\text{H}^+ \right] + k_{23}k_{63}k_{76} K_1K_2K_3}{k_{21}k_{32}k_{63} \left[\text{H}^+ \right] + k_{23}k_{63} (k_{34} + k_{35}) + k_{23}k_{36}k_{67}} \\ \times \frac{\left[\text{HS-R-NH}_3^+\text{-COOH} \right] \left[\text{Cu}^{2+} \right]}{\left[\text{H}^+ \right]^3} \end{aligned}$$

Now substituting in equation (5), we get

$$\text{Rate} = \frac{k_{35} k_{23} k_{63} k_{12} K_1 [H^+]^2 + k_{23} k_{43} k_{63} K_1 K_2 [H^+] + k_{23} k_{63} k_{76} K_1 K_2 K_3}{k_{21} k_{32} k_{63} [H^+] + k_{23} k_{63} (k_{34} + k_{35}) + k_{23} k_{36} k_{67}} \times \frac{[HS-R-NH_3^+-COOH] [Cu^{2+}]}{[H^+]^3} \quad \text{----- (15)}$$

Substituting $[HS-R-NH_3^+-COO^-]$, $[HS-R-NH_2-COO^-]$ and $[S-R-NH_2-COO^-]$ from equations (12), (13) and (14) respectively in equation (4), we obtain :

$$\text{Rate} = k_{obs} \{ [H^+]^3 + K_1 [H^+]^2 + K_1 K_2 K_3 \} \times \frac{[HS-R-NH_3^+-COOH] [Cu^{2+}]}{[H^+]^3} \quad \text{----- (16)}$$

Comparing equations (15) and (16) assuming that $k_{35} \gg k_{34}$ which is usually the case for normal substitution as reported by Letter and Jordan [8-9] and at high pH's

$k_{21} k_{32} k_{63} [H^+] \ll k_{23} k_{63} (k_{34} + k_{35})$ and

$k_{23} k_{36} k_{67} \ll k_{23} k_{63} (k_{34} + k_{35})$, we get

$$k_{obs} \{ 1 + K_3/[H^+] \} = k_{43} + k_{67} K_3 / [H^+] \quad \text{----- (17)}$$

Linear plots of $k_{obs} \{ 1 + K_3/[H^+] \}$ versus $[H^+]^{-1}$ at temperatures 25,30,35 and 40°C are shown in Fig 1.

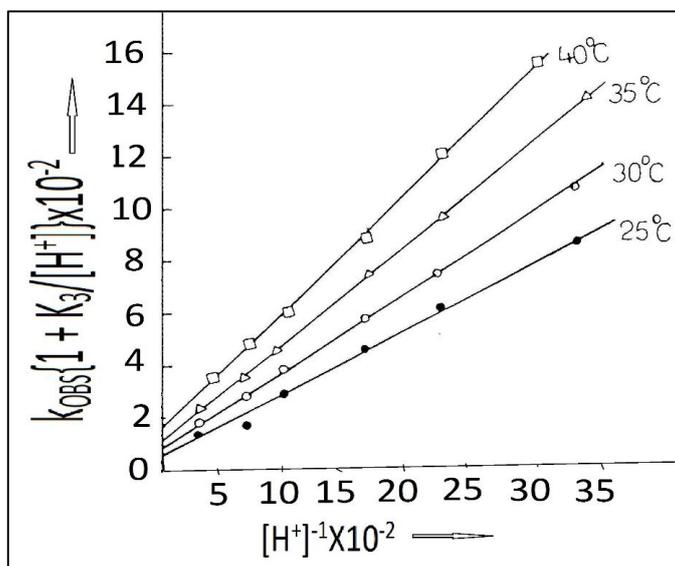


Fig 1: Variation of $k_{obs} \{ 1 + K_3 / [H^+] \}$ versus $[H^+]^{-1}$ for Cu(II) γ -L-glutamyl-L-cyteinylglycine interaction at different temperatures

The values of k_{43} and k_{76} were evaluated from intercept and slope respectively of this plot (Table 2). The value of K_3 was taken from the literature[9]. At the temperature of our investigation, the corrected values of K_3^T were evaluated using the thermodynamic relation:

$$pK_a^T = \{ \Delta H (T_2 - T_1) / 4.576 T_2 T_1 \} + pK_a^{25 \text{ } ^\circ C} \quad \text{----- (18)}$$

Values of energies of activation corresponding to specific rate constants k_{43} and k_{76} were obtained from linear plot of $\log k$ versus $1/T$ and those of entropies and enthalpies of activation corresponding to k_{43} and k_{76} were calculated from linear plot of $\log k/T$ versus $1/T$ (Fig 2 and 3) These values are given in Table 3.

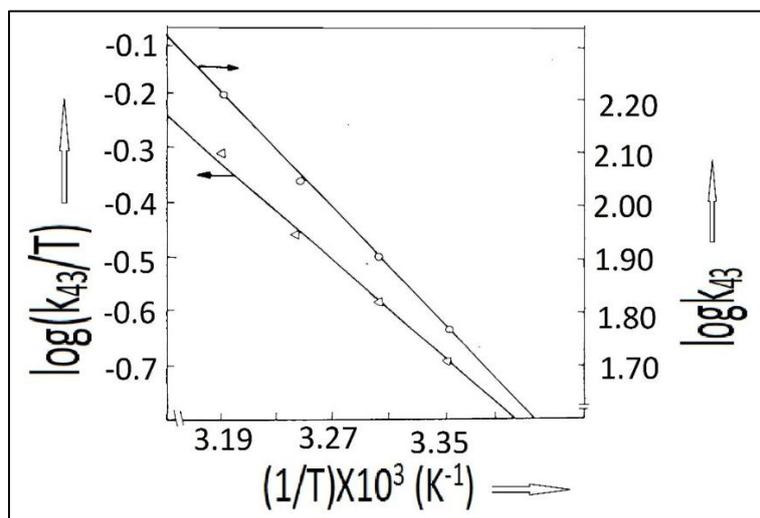


Fig 2: Variation of $\log k_{43}$ and $\log k_{43} / T$ versus $(1/T)$ for the interaction of Cu(II)- γ -L-glutamyl-L-cyteinylglycine

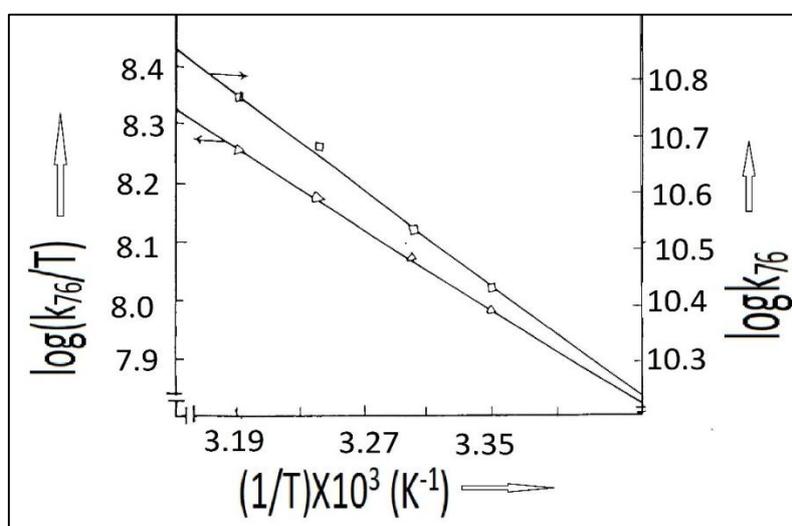


Fig 3: Variation of $\log k_{76}$ and $\log k_{76} / T$ versus $(1/T)$ for the interaction of Cu (II) - γ -L-glutamyl-L-cyteinylglycine

Table 2: Values of k_{43} and k_{76} and k_0 for the complexation of Cu (II) with γ -L-glutamyl-L-cyteinylglycine

Temp.($\pm 0.05^\circ\text{C}$)	$(k_{43} \text{ (M}^{-1}\text{s}^{-1}))$	$k_{76} \times 10^{-10} \text{ (M}^{-1}\text{s}^{-1})$	$k_0 \times 10^{-10} \text{ (s}^{-1})$
25	60.0	2.94	1.48
30	80.0	3.69	1.86
35	101	4.68	2.36
40	160	5.71	2.88

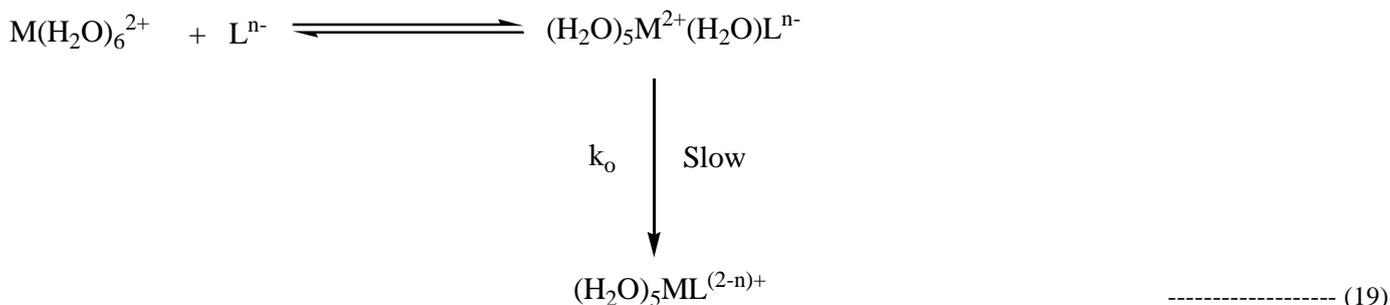
The high values of ΔH^\ddagger corresponding to k_{43} step confirm that the monoprotonated form of γ -L-glutamyl-L-cyteinylglycine is less reactive whereas low value of ΔH^\ddagger corresponding to k_{76} supports the high reactivity of deprotonated form of γ -L-glutamyl-L-cyteinylglycine. The negative value of entropy corresponding to k_{76} can be attributed to the fact that the transition state for this complex is highly charged and clearly shows that the reaction is between two oppositely charged ions (Table 3).

Table 3: Values of activation parameters corresponding to k_{43} and k_{76} for the complexation of Cu(II) with γ -L-glutamyl-L-cyteinylglycine

	k_{43}	k_{76}
$\Delta H^\ddagger \text{ (kJmol}^{-1})$	47.8 ± 1.3	32.3 ± 0.8
$\Delta E^\ddagger \text{ (kJmol}^{-1})$	51.4 ± 1.4	34.7 ± 1.1
$\Delta S^\ddagger \text{ (JK}^{-1}\text{mol}^{-1})$	-80.9 ± 2.1	-32.9 ± 0.9

Calculation of Water Exchange Rate Constant (k_0)

The rate of substitution on metal ion is controlled by the rate of water exchange and rate determining step is the loss of water molecule from the inner coordination sphere, i.e.



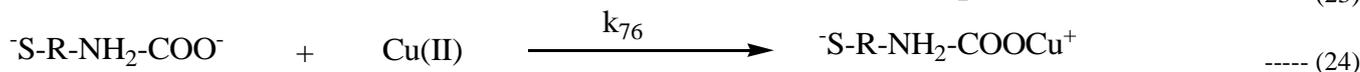
The rate law in terms of outer sphere complex formation (K_{os}) and rate constant of water loss (k_o) from equation (19) be written as

$$\begin{aligned}
 \frac{d}{dt} (H_2O)_5ML^{(2-n)+} &= k_o [(H_2O)_5M^{2+}(H_2O)L^{n-}] \\
 &= k_o K_{os} [M(H_2O)_6^{2+}] [L^{n-}]
 \end{aligned}
 \tag{20}$$

So the rate law in terms of outer sphere complex formation (K_{os}) for Cu(II)- γ -L-glutamyl-L-cyteinylglycine can be written as

$$\text{Rate} = \frac{d}{dt} [Cu(II)L] = K_{os} k_o [Cu(II)] [L]
 \tag{21}$$

Diprotonated, monoprotated and deprotonated forms of the ligand can be considered to react with Cu(II) in the following ways :



Using these three equations, rate can be written as

$$\begin{aligned}
 \text{Rate} &= k_{12} [HS-R-NH_3^+-COO^-] [Cu^{2+}] + k_{43} [HS-R-NH_2-COO^-] [Cu^{2+}] \\
 &+ k_{76} [^-S-R-NH_2-COO^-] [Cu^{2+}]
 \end{aligned}
 \tag{25}$$

In equation (25), k_{12} appears with $K_1 [H^+]^2$ which is very small, so the first term from equation (25) will disappear. As can be seen from Table 2 that $k_{43} \ll k_{76}$, therefore equation (25) reduces to

$$\text{Rate} = k_{76} [^-S-R-NH_2-COO^-] [Cu^{2+}]
 \tag{26}$$

On comparing equations (21) and (26), we get,

$$k_{76} = K_{os} k_o [^-S-R-NH_2-COO^-]
 \tag{27}$$

being the only reactive form can be taken as the concentration of ligand L.

In order to calculate the value of k_o , we must know the value of K_{os} . The value of K_{os} was calculated using the equation which was given by Fuoss[10] on statistical grounds,

$$K_{os} = 4\pi N a^3 \cdot e^{-u/KT} / 3000
 \tag{28}$$

Where $u = Z_1 Z_2 e_o^2 / aD - Z_1 Z_2 e_o^2 x / D (1 + xa)$

And $x^2 = 8\pi N e_o^2 u / 1000DKT$

where

N = Avogadro's number

a = Distance of closest approach of two ions

K = Boltzmann's constant

e_o = Charge of an electron in esu units

D = Bulk dielectric constant

u = Ionic strength

Z_1Z_2 = Charge of reactants

Distance of closest approach of two ions often taken 5\AA for reactions of two aqua cations with ordinary ligands. After substituting the values of all the terms in equation (28), the value of K_{os} can be approximated to 1.98 mol dm^{-3} at all temperatures. The values of k_o are reported in Table 2.

Mechanism

Based on Scheme 1, following mechanism for the complexation of Cu(II) with γ -L-glutamyl-L-cysteinyglycine has been suggested

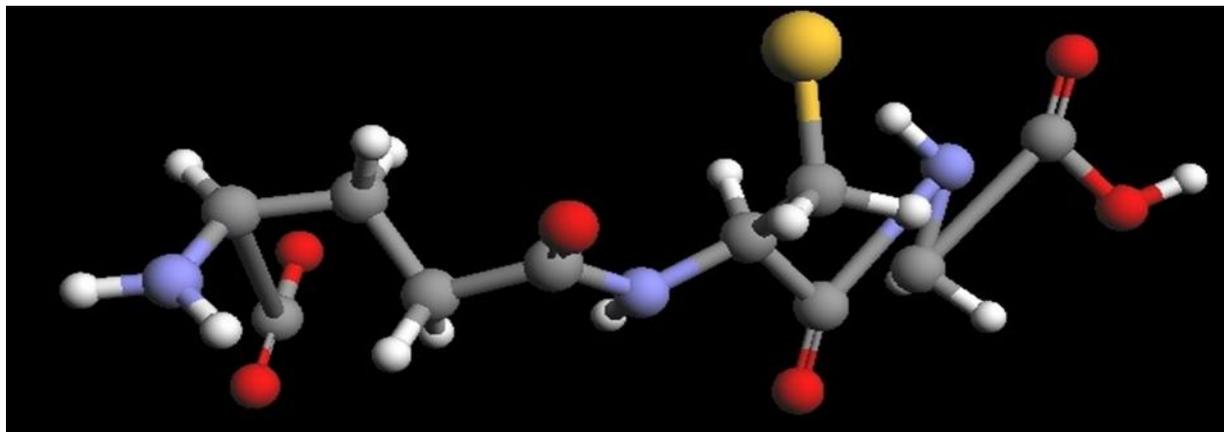


Fig 4: Deprotonated form of γ -L-glutamyl-L-cysteinyglycine
Final Geom Energy = $-88725.355\text{ cal/mol}$
Heat of Formation = -132.57 kcal/mol

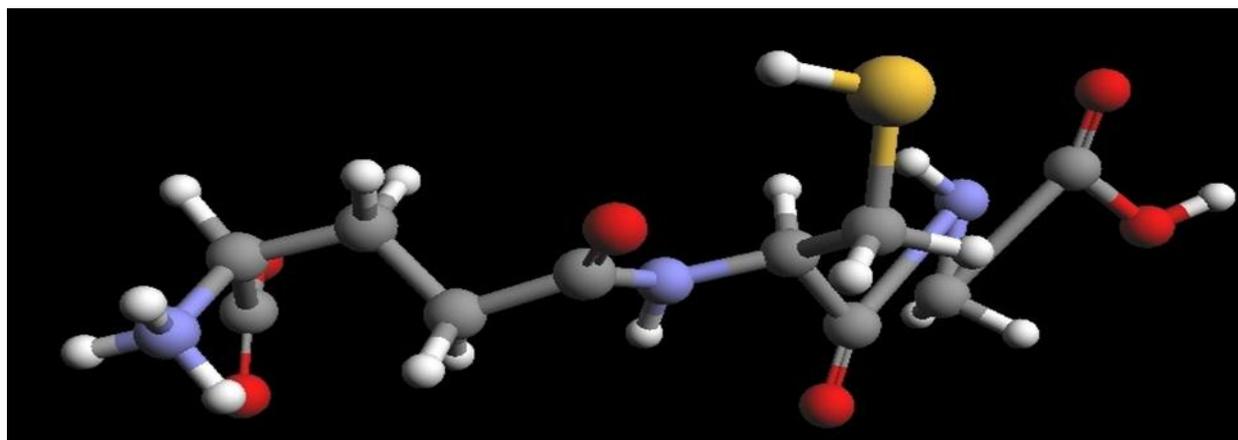


Fig 5: Zwitterionic form of γ -L-glutamyl-L-cysteinyglycine
Final Geom Energy = $-89515.33\text{ kcal/mol}$
Heat of Formation = -215.59 kcal/mol

The deprotonated form react with Cu (II) in two ways (i) Ring formation via nitrogen and (ii) Ring formation via oxygen. It is evident from Table 3 that deprotonated form will react more rapidly with Cu (II) as compared to the monoprotated form. Due to strong electrostatic interaction between the positive charge of Cu (II) and negative charge on the oxygen and sulphur of γ -L-glutamyl-L-cysteinyglycine, it is inferred that k_{76} is greater than k_{43} . The value of activation parameters corresponding to k_{76} further confirm that the deprotonated form is more reactive than the zwitterionic form of the ligand. This mechanism is further confirmed by the values of energy of activation and entropy of activation. This mechanism is further confirmed from the molecular modelling method in which the energy as well as the heat of formation of the zwitterionic form and deprotonated form has been calculated [16-20]. Values of the energies as well as heat of formation were calculated after optimizing the geometry of the molecules. These values are given in Fig 4 and 5. It has been found that the deprotonated form is more reactive than the protonated form.

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