

A New Scheme on Kinetics of Nickel(II)- γ -L-glutamyl-L-cysteinylglycine

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Kinetics of complexation of Ni^{II} by γ -L-glutamyl-L-cysteinylglycine have been investigated in the pH range 6.23–7.18, $I=0.15$ M KNO₃, and at temperatures 25, 30, 35 and 40° ($\pm 0.05^\circ$) using stopped flow spectrophotometer. The values of k_{obs} vary from 36.0 to 962 M⁻¹ s⁻¹. Kinetics of the complexation reaction has been studied in terms of a mechanism in which water loss from the metal ion is an important rate-determining step. Overall rate constants have been resolved into specific rate constants and there is good agreement between the calculated and experimentally obtained values for k_{obs} at various pH's. The values of activation parameters have been evaluated.

γ -L-Glutamyl-L-cysteinylglycine is involved in numerous cellular processes¹, in the transport of amino acids and peptides across cell membranes via the γ -glutamyl cycle². Because of its biological importance, it was thought desirable to investigate the kinetics of interaction of Ni^{II} with γ -L-glutamyl-L-cysteinylglycine, having NH₂, COOH and SH sites available for reaction. A number of kinetic studies³ of amino acids with metal ions have been carried out, but no clear information is available regarding the complexation of γ -L-glutamyl-L-cysteinylglycine with Ni^{II}. Letter and Jordan⁴ found the results irreproducible at higher pH's. Keeping this in view, a comprehensive kinetic study on interaction of Ni^{II} with γ -L-glutamyl-L-cysteinylglycine in the pH range 6.20–7.18 and at temperatures 25, 30, 35 and 40° ($\pm 0.05^\circ$) was carried out. It has been found by us that results were reproducible within 2% if freshly prepared solution is taken every time.

Experimental

γ -L-Glutamyl-L-cysteinylglycine (B.D.H.), KNO₃ (Sarabhai, G.R.), bromothymol blue (B.D.H.) and Ni(NO₃)₂·6H₂O (Merck) were used as such.

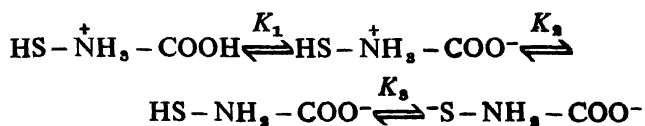
The pH of solution was adjusted using 2,6-lutidine (Merck-Schuchardt) and HCl (Glaxo, A.R.) and the final pH was taken on a Radiometer pHM 26 pH-meter.

The temperature of the system in the stopped flow assembly was maintained constant at the desired temperature. An Aminco Morrow stopped flow spectrophotometer along with matching electronic console was used for rate studies. The transmittance changes were monitored at 620 nm by pH indicator method⁵. The total transmittance change was kept small using linear log photometer so that relative voltage changes (ΔV) could be observed on the

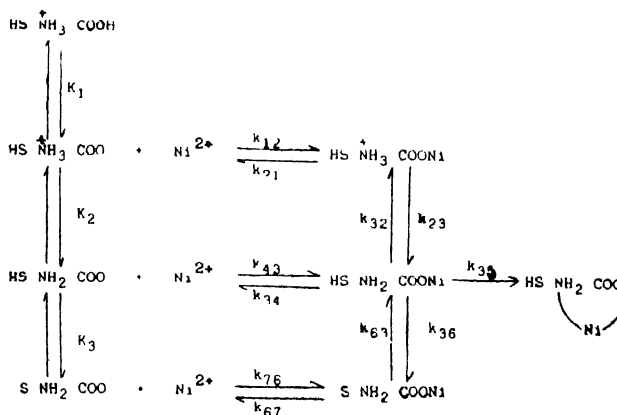
oscilloscope. Blank experiments in which (i) indicator and metal ion solution and (ii) indicator and ligand solutions were mixed showed no transmittance change to interfere with the results.

Results and Discussion

The dissociation of γ -L-glutamyl-L-cysteinylglycine can be represented as



These forms can react with Ni^{II} stepwise as presented in Scheme 1.



Scheme 1

The interaction of Ni^{II} with γ -L-glutamyl-L-cysteinylglycine follows the overall differential equation,

$$\text{Rate} = -d/dt [\text{Ni}^{II}] = -d/dt [\gamma\text{-L-glutamyl-L-cysteinylglycine}] \quad (1)$$

$$= k_{\text{obs}} [\text{Ni}^{II}] [\gamma\text{-L-glutamyl-L-cysteinylglycine}] = k'_{\text{obs}} [\gamma\text{-L-glutamyl-L-cysteinylglycine}] \quad (2)$$

$$\text{where } k'_{\text{obs}} = k_{\text{obs}} [\text{Ni}^{II}] \quad (3)$$

Rate can also be written as

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

where $[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COOH}]$, $[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-]$, $[\text{HS}-\text{NH}_2-\text{COO}^-]$ and $[-\text{S}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-]$ are triprotonated, diprotonated, monoprotated and deprotonated forms of the γ -L-glutamyl-L-cysteinylglycine respectively.

Rate from Scheme 1 can be given as

$$\text{Rate} = k_{35} [\text{HS}-\text{NH}_2-\text{COONi}] \quad (5)$$

Using the steady state approximation for the intermediates $[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}]$, $[\text{HS}-\text{NH}_2-\text{COONi}]$ and $[-\text{S}-\text{NH}_2-\text{COONi}]$,

$$d/dt [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}] = k_{12} [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-] [\text{Ni}^{II}] - k_{13} [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}] + k_{32} [\text{HS}-\text{NH}_2-\text{COONi}] - k_{23} [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}] [\text{H}^+] = 0 \quad (6)$$

$$d/dt [\text{HS}-\text{NH}_2-\text{COONi}] = k_{43} [\text{HSNH}_2-\text{COO}^-] [\text{Ni}^{II}] - k_{34} [\text{HS}-\text{NH}_2-\text{COONi}] + k_{23} [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}] + k_{76} [-\text{S}-\text{NH}_2-\text{COONi}] - k_{36} [\text{HS}-\text{NH}_2-\text{COONi}] - k_{32} [\text{HS}-\text{NH}_2-\text{COONi}] = 0 \quad (7)$$

$$d/dt [-\text{S}-\text{NH}_2-\text{COONi}] = k_{76} [-\text{S}-\text{NH}_2-\text{COO}^-] [\text{Ni}^{II}] - k_{67} [-\text{S}-\text{NH}_2-\text{COONi}] + k_{36} [\text{HS}-\text{NH}_2-\text{COONi}] [\text{H}^+] - k_{63} [-\text{S}-\text{NH}_2-\text{COONi}] = 0 \quad (8)$$

From the scheme it can be written that

$$[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}] = \frac{k_{32}}{k_{23}} [\text{H}^+] [\text{HS}-\text{NH}_2-\text{COONi}] \quad (9)$$

$$[-\text{S}-\text{NH}_2-\text{COONi}] = \frac{k_{36}}{k_{63}} [\text{H}^+] [\text{HS}-\text{NH}_2-\text{COONi}] \quad (10)$$

Adding equations (6), (7) and (8) and substituting the values of $[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COONi}]$ and $[-\text{S}-\text{NH}_2-\text{COONi}]$ from equations (9) and (10) and on

rearrangement we get,

$$[\text{HS}-\text{NH}_2-\text{COONi}] = \frac{k_{23} k_{63} k_{12} [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-] [\text{Ni}^{II}]}{k_{21} k_{32} k_{63} [\text{H}^+] + k_{23} k_{34} k_{63} + k_{23} k_{43} k_{63} [\text{HS}-\text{NH}_2-\text{COO}^-] [\text{Ni}^{II}] + k_{23} k_{36} k_{63} + k_{23} k_{36} k_{67} [-\text{S}-\text{NH}_2-\text{COO}^-] [\text{Ni}^{II}]} \quad (11)$$

From the dissociation of γ -L-glutamyl-L-cysteinylglycine, the concentration of $[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-]$, $[\text{HS}-\text{NH}_2-\text{COO}^-]$ and $[-\text{S}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-]$ can be written as

$$[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-] = \frac{K_1 [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COOH}]}{[\text{H}^+]} \quad (12)$$

$$[\text{HS}-\text{NH}_2-\text{COO}^-] = \frac{K_1 K_2 [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COOH}]}{[\text{H}^+]^2} \quad (13)$$

$$[-\text{S}-\text{NH}_2-\text{COO}^-] = \frac{K_1 K_2 K_3 [\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COOH}]}{[\text{H}^+]^3} \quad (14)$$

Substituting the value of equations (12), (13) and (14) into equation (11) and on rearrangement, the rate equation (5) becomes,

$$\text{Rate} = \frac{k_{63} k_{12} k_{23} k_{35} K_1 [\text{H}^+]^2}{k_{21} k_{32} k_{63} [\text{H}^+] + k_{23} k_{34} k_{63} (k_{34} + k_{35}) + k_{23} k_{36} k_{63} k_{76} K_1 K_2 K_3} \cdot \frac{[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COOH}] [\text{Ni}^{II}]}{[\text{H}^+]^3}$$

Substituting $[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COO}^-]$, $[\text{HS}-\text{NH}_2-\text{COO}^-]$ and $[-\text{S}-\text{NH}_2-\text{COO}^-]$ from equations (12), (13) and (14) respectively in equation (4) we obtain,

$$\text{Rate} = k_{\text{obs}} \{ [\text{H}^+]^3 + K_1 [\text{H}^+]^2 + K_1 K_2 [\text{H}^+] + K_1 K_2 K_3 \} \times \frac{[\text{HS}-\overset{+}{\text{N}}\text{H}_3-\text{COOH}] [\text{Ni}^{II}]}{[\text{H}^+]^3} \quad (16)$$

Comparing equations (15), (16), assuming that $k_{35} \gg k_{34}$ and at high pH's,

$$k_{21} k_{32} k_{63} [\text{H}^+] \ll k_{23} k_{63} (k_{34} + k_{35}) \text{ and } k_{23} k_{36} k_{67} \ll k_{23} k_{63} (k_{34} + k_{35}),$$

we get $k_{\text{obs}} \{1 + K_3/[\text{H}^+]\} = k_{43} + k_{76} K_3/[\text{H}^+] \quad (17)$

Plot of $k_{\text{obs}} \{1 + K_3/[\text{H}^+]\}$ vs $[\text{H}^+]^{-1}$ was found to be linear, and the values of k_{43} , k_{76} were evaluated from intercept and slope respectively of this plot (Table 2). The value of K_3 was taken from literature⁵. At the temperature of our investigation, the

corrected values of K_1^\ddagger were evaluated using the thermodynamic relation,

$$pK^{T_2} = \Delta H(T_2 - T_1)/4.6 T_1 T_2 + pK^{T_1} \quad (18)$$

The values of activation parameters are also reported in Table 2. The values of activation parameters corresponding to specific rate constants (k_{4s} and k_{7s}) were obtained from the linear plots of $\log k/T$ vs $1/T$ and these values are reported in Table 2.

TABLE 1—SECOND ORDER RATE CONSTANTS (k_{obs}) FOR THE COMPLEXATION OF Ni^{II} WITH γ -L-GLUTAMYL-L-CYSTEINYLGLYCINE AT DIFFERENT pH'S AND TEMPERATURES

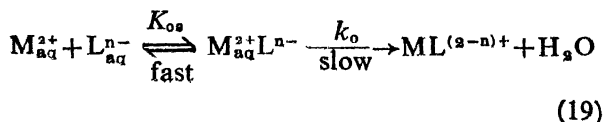
$I=0.15 M(KNO_3)$, $[Ni^{II}]=0.012 M$,
 $[\gamma\text{-L-Glutamyl-L-cysteinylglycine}]=1.26 \times 10^{-3} M$

pH	k_{obs}	$[H^+]^{-1} \times 10^{-5}$
Temp. = 25°		
6.33	36.0 ± 0.2	2.13
6.45	41.2 ± 0.1	2.18
6.67	82.5 ± 0.8	4.67
6.82	130 ± 1	6.60
7.18	270 ± 2	15.1
Temp. = 30°		
6.23	48.1 ± 0.2	1.69
6.36	57.7 ± 0.1	2.29
6.63	115 ± 1	4.26
6.79	144 ± 1	6.02
7.07	265 ± 2	11.7
Temp. = 35°		
6.20	64.1 ± 0.6	1.58
6.35	82.5 ± 0.5	2.23
6.54	144 ± 1	3.46
6.73	180 ± 2	5.87
7.09	385 ± 1	12.3
Temp. = 40°		
6.17	72.1 ± 0.2	1.47
6.31	115 ± 0.6	2.04
6.50	180 ± 0.8	3.16
6.69	240 ± 1	4.89
7.02	480 ± 2	10.4

TABLE 2—VALUES OF k_{4s} , k_{7s} AND k_0 FOR THE COMPLEXATION OF Ni^{II} WITH γ -L-GLUTAMYL-L-CYSTEINYLGLYCINE

Temp. °C	k_{4s} $M^{-1} s^{-1}$	k_{7s} $\times 10^{-4}$ $M^{-1} s^{-1}$	k_0 $\times 10^{-3}$ s^{-1}
25	0	1.33 ± 0.02	6.71
30	10 ± 0.5	1.39 ± 0.04	7.02
35	17 ± 0.1	1.46 ± 0.04	7.37
40	30 ± 0.1	1.50 ± 0.06	7.57
ΔH^\ddagger (kJ mol ⁻¹)	85.3 ± 1	3.57 ± 0.03	
ΔS^\ddagger (JK ⁻¹ mol ⁻¹)	24.7 ± 2	-184 ± 4	

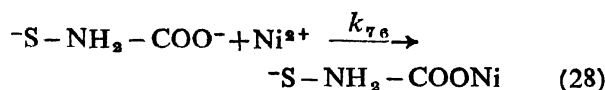
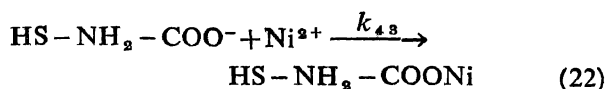
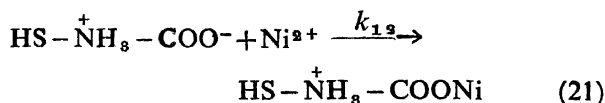
Calculation of water exchange rate constant (k_0) :
 The rate of substitution on metal ion is controlled by the rate of water-exchange, and rate-determining step is the loss of water molecule from inner coordination sphere⁶,



The rate law in terms of outer sphere complex formation constant (K_{os}) and the rate constant of water loss (k_0) can be written as

$$\text{Rate} = d/dt [Ni^{II}L] = K_{os} k_0 [Ni^{II}][L] \quad (20)$$

Diprotinated, monoprotinated and deprotonated forms of the ligand can be considered to react with Ni^{II} in the following ways :



Using these three equations, the rate can be written as

$$\text{Rate} = k_{1s} [HS - \overset{+}{NH}_3 - COO^-][Ni^{II}] + k_{4s} [HS - NH_2 - COO^-][Ni^{II}] + k_{7s} [-S - NH_2 - COO^-][Ni^{II}] \quad (24)$$

In equation (24), k_{1s} appears with $K_1[H^+]^2$ which is very small, so first term from equation (24) will disappear. As can be seen from Table 2 that $k_{4s} \ll k_{7s}$, therefore equation (24) reduces to

$$\text{Rate} = k_{7s} [-S - NH_2 - COO^-][Ni^{II}] \quad (25)$$

On comparing equations (20) and (25) we get,

$$k_{7s} = K_{os} k_0$$

$[-S - NH_2 - COO^-]$ being the only reactive form can be taken as the concentration of ligand L. In order to calculate the value of k_0 , we must know the value of K_{os} . The value of K_{os} was calculated using the equation which was given by Fuoss⁷ on statistical grounds,

$$K_{os} = 4\pi N a^3 \cdot e^{-u/K_b T / 3000}$$

$$u = z_1 z_2 e_0^2 / aD - z_1 z_2 e_0^2 x / D(1 + xa)$$

$$\text{where } x^2 = 8\pi N \cdot e_0^2 \mu / 1000 DKT$$

where a is distance of closest approach of ions and other symbols have their usual significance. If the value of a is taken as 5Å then the value of K_{os} can be approximated to 1.98 mol dm⁻³ at all temperatures. The values of k_0 are reported in Table 2. The high values of ΔH^\ddagger corresponding to k_{4s} further confirm that the monoprotinated form of γ -L-glutamyl-L-cysteinylglycine is non-reactive, whereas low value of ΔH^\ddagger corresponding to k_{7s} supports the high reactivity of deprotonated form of γ -L-glutamyl-L-cysteinylglycine. The negative value of entropy corresponding to k_{7s} 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.

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