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Formation Constants of Mixed Ligand Complexes of Anti-Inflammatory Drug Piroxicam and Some Bioligands with Copper(II)

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The formation constants of various complexes of copper(II) with anti-inflammatory drug piroxicam (P) as primary ligand and some bioligands such as L-serine, L-tyrosine, L-threonine as secondary ligand have been determined pH metrically at 25 ºC and I = 0.1 M $NaNO_3$. The results suggest that the formation of Cu(P)L and Cu(P)(LH-1) species in the pH range of 5-12. The values of $\Delta \log_{10} K$, percentage of relative stabilization and log X were evaluated and discussed.

Keywords: Potentiometric titration, Mixed ligand complexes, Amino acids, Piroxicam.

INTRODUCTION

Piroxicam (P), is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class used to relieve the symptoms of painful inflammatory conditions like arthritis [1]. The chemical name of piroxicam is 4-hydroxyl-2-methyl-*N*-2-pyridinyl-2*H*-1,2,-benzothiazine-3-carboxamide-1,1-dioxide. NSAIDs are clinically important [2] but have unpleasant adverse effects [3] and tolerance and dependence induced by opiates, use of these drugs have not been successful in some cases. Alternatives to NSAIDs and opiates are needed. Significant interest has arisen regarding the anti-inflammatory role of mineral ions and their synergistic action when combined with common NSAIDs [4-7]. It has long been emphasized that copper complexes of inactive substances exert anti-inflammatory activity and that copper complexes of NSAIDs are more active than these drugs themselves. Based on these observations, it was suggested that the copper complexes of NSAIDs show synergistic activity. It has been reported that Cu (II) complexes have anti-inflammatory activities to reduce inflammation associated with rheumatoid arthritis [8-10]. Piroxicam complexes of metal(II) have also been described in solid state [11-13]. In the literature, there is no available information on the complex tendencies of peroxicam with metal(II) and amino acids in solution. The studies of complex equilibria of metal ions with drugs are useful in elucidating the mechanism of action of drugs [14]. This study focuses on the reactions of Cu(II) with inflammatory drug piroxicam and amino acids *e.g.*, L-serine, L-tyrosine and L-threonine in an aqueous medium at 25 ºC and an ionic strength 0.1 M ($NaNO_3$) using glass electrode potentiometry. The concentration distributions of various species formed in solution were also evaluated as a function of pH.

EXPERIMENTAL

All chemicals used in this investigation including piroxicam, L-serine, L-tyrosine, L-threonine, $Cu(NO_3)_2$, KOH, HNO_3 and KNO_3 were provided by Sigma-Aldrich.

The pH measurements were found using a Griffin pH meter at 25°C in a double-walled glass cell through which water was circulated in the outer jacket from a constant temperature bath. The autoprotolysis of water ($2H_2O = H_3O + + OH^-$, K_w) at 25 ºC and ionic strength of 0.1 M of $NaNO_3$ was 13.97.

For the equilibrium constant determination, the potentiometric titrations were carried out in aqueous medium in total volume 50 ml at the constant ionic strength (I) = 0.1 M (KNO_3) under a nitrogen atmosphere. The following reaction mixtures (**a-d**) containing proton and/or Cu(II) and the ligands at ratios (1:1) and (1:2) in binary systems and (1:1:1) in ternary systems, were titrated through incremental additions of CO_2-free (0.05 M) KOH solution as titrant.

(a) 40 mL of a solution containing 0.005M ligands and 0.1M KNO_3 (for the determination of the proton association constants of piroxicam and bioligands).

(b) 40 mL of a solution containing 0.005M Cu(II) solution, 0.01M (T) or (L) and 0.1 M KNO_3 (for the determination of the formation constants of binary complexes).

(c) 40 mL of a solution containing 0.005 M Cu(II) solution, 0.005 M (T), 0.005 M (L) and 0.1 M KNO_3. (for the determination of the formation constants of ternary complexes).

A solution of HNO_3 was added for all the titrations, so that they were fully protonated at the beginning of titrations. Proton association constants and the complex formation constants were determined by using the computer program HYPERQUAD [15]. The program use least-square refinements to reduce the differences between calculated and experimental data to get the best model that gives the best fit. The sum of square of residuals between experimental and calculated values is normally very small; it is typically between 10^{-6} and 10^{-9}. Titrations were performed up to pH ≈ 12. Repeat titrations were done to check the reproducibility of the titrations. Distribution curves of the binary and ternary systems were drawn by HySS computer program [16].

RESULTS AND DISCUSSION

Proton association constants of ligands: The proton association constants of the ligands were re-examined under the same experimental conditions of ionic strength and temperature used to study the binary and the ternary complexes (Table-1). The results obtained are in well agreement with the literature data [17,18]. The pK_{a_1} values are related to the attachment of H^+ to phenolic oxygen in tyrosine and attachment of H^+ to $–NH_2$ group in serine or threonine. The pK_{a_2} value corresponds to the attachment of a proton to -NH_2 and -COOH groups in tyrosine and serine or threonine, respectively. The pK_{a_3} values are the smallest and are thought to correspond to the protonation of carboxyl groups. Piroxicam exists as ampholytic in water [19]. It exhibits a weakly acidic 4-hydroxyproton (pK_{a_1} 5.08) and a weakly basic pyridyl nitrogen (pK_{a_2} 1.86) (**Scheme-I**).

TABLE-1
PROTONATION CONSTANTS OF LIGANDS IN AQUEOUS MEDIA [Temp. = 25 °C and I = 0.1 M $NaNO_3$]

System	pK_{a_1}	pK_{a_2}	pK_{a_3}
Piroxicam (P)	5.08 (0.01)	1.86 (0.03)	–
L-Serine (Ser)	9.17 (0.01)	2.19 (0.01)	–
L-Tyrosine (Tyr)	10.10 (0.01)	9.13 (0.01)	2.23 (0.01)
L-Threonine (Thr)	9.06 (0.01)	2.33 (0.02)	–

Note: pK_{a_1} = corresponds to 11 species (*i.e.*, $L^- + H^+ \rightleftharpoons LH$); pK_{a_2} corresponds to 12 species (*i.e.*, $LH + H^+ \rightleftharpoons LH_2^+$). Standard deviations are given in parentheses.

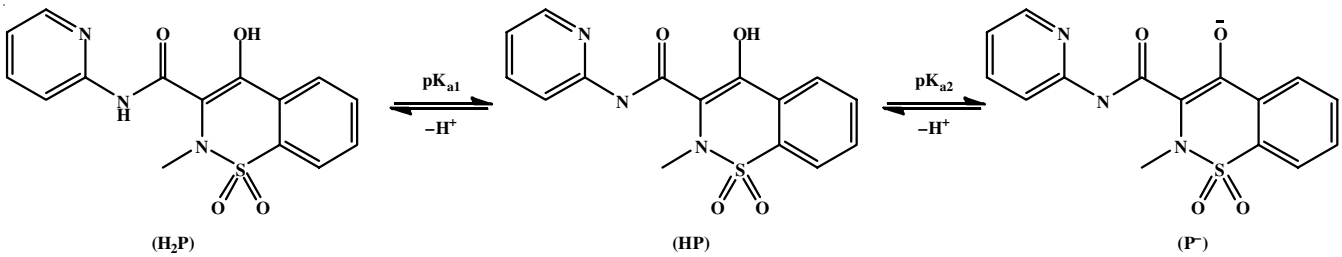
Formation equilibria of binary complexes: The formation constants of Cu(II) chelates with piroxicam or amino acid L-serine or L-tyrosine or L-threonine were calculated from the titration graphs in which the metal to ligand ratio was 1:2 are given in Table-2. Piroxicam and bioligands were titrated in the presence and absence of Cu(II) ion. The pH titration curve of Cu(II) complex is lowered from that of the free ligands curves. This indicates a complex formation associated with release of hydrogen ions. In binary system of Cu(II)-P, the selected model with the best statistical fit was found to consist of Cu(P) 1100 and $Cu(P)_2$ 1200 species. Piroxicam acts as a bidentate chelating ligand coordinated to the metal ions *via* pyridyl nitrogen and amide oxygen [20]. For binary systems with biolignads, equilibrium analyses confirmed the formation of the species: Cu(L) 1010, $Cu(L)_2$ 1020 and Cu (LH-1) 101-1 (L, any amino acid). LH-1 complex is shaped by induced ionization of β-alcohol group as reported in the literature [21]. The speciation graphs in Fig. 1 show species formed by the complexation of drug (P) with Cu(II). The concentration of Cu(P) 1100 species increases with increasing pH, attaining a maximum of 97.6 % at pH 6.8. Further increase in pH is accompanied by a decrease in the concentration of 1100 species and an increase in the concentration of 1200 species. Therefore, species Cu(P) predominates in the physiological pH range.

Formation equilibria of mixed ligand complexes: The stability constants of mixed-ligand complexes giving the best fit of pH-metric titration curves are listed in Table-3. The stability constants of 1:1 Cu(II) complexes with piroxicam (P) or bioligands (L) are of the same order of magnitude (Table-2). As a result, the ligation of P and L will proceed simultaneously

TABLE-2
FORMATION CONSTANTS OF THE BINARY COMPLEXES [Temp. = 25 °C AND I = 0.1 M $NaNO_3$]

System	l	p	q	r*	$\log_{10}\beta$**
Cu(OH)n	1	0	0	-1	-7.29 (0.001)
	1	0	0	-2	-13.33 (0.01)
Piroxicam (P)	1	1	0	0	6.29 (0.01)
	1	2	0	0	12.12 (0.02)
L-Serine (Ser)	1	0	1	0	7.30 (0.01)
	1	0	2	0	13.45 (0.02)
	1	1	0	-1	0.49 (0.02)
L-Tyrosine (Tyr)	1	0	1	0	7.89 (0.01)
	1	0	2	0	14.15 (0.01)
	1	1	0	-1	1.25 (0.01)
L-Threonine (Thr)	1	0	1	0	7.01 (0.04)
	1	0	2	0	13.25 (0.01)
	1	1	0	-1	2.34 (0.01)

*l, p, q and r are the stoichiometric coefficient corresponding to Cu(II), P, L and H^+, respectively. **Standard deviations are given in parentheses. Coefficient–1 reflects proton loss.



Scheme-I: Protonation of piroxicam as ligand

TABLE-3
STABILITY CONSTANTS AND PARAMETERS OF TERNARY (MIXED CuPL) COMPLEXES
[Temp. = 25 °C AND I = 0.1 M $NaNO_3$]. % R.S. IS THE PERCENTAGE RELATIVE STABILIZATION VALUE

System	l	p	q	r*	$\log_{10}\beta^{CuP}_{Cu(P)L}$	$\log_{10} K^{Cu(P)}_{Cu(P)L}$	$\log_{10} K^{Cu(L)}_{Cu(P)(L)}$	$\Delta\log_{10} K$	% R.S.	$\log_{10} X$
Serine (Ser)	1	1	1	0	14.23 (0.01)	7.94	6.93	0.64	8.77	2.89
	1	1	1	-1	4.22 (0.01)					
Tyrosine (Tyr)	1	1	1	0	15.33 (0.3)	9.04	7.44	1.15	14.58	4.39
	1	1	1	-1	4.41 (0.01)					
Threonine (Thr)	1	1	1	0	14.05 (0.03)	7.76	7.04	0.75	10.70	2.63
	1	1	1	-1	4.41 (0.01)					

*l, p q and r represents stoichiometric constants corresponding to Cu(II), P, L and H^+, respectively. Standard deviation presented in parentheses.

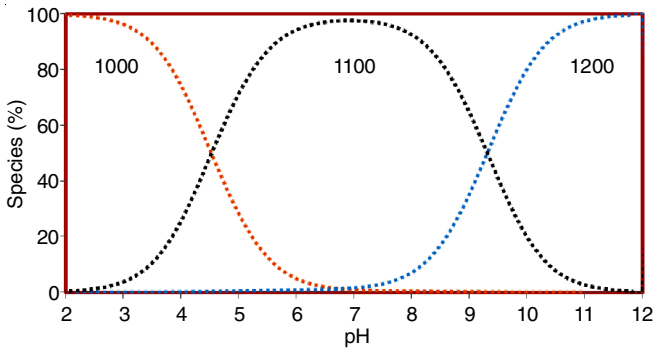


Fig. 1. Concentration distribution of various species as a function of pH in Cu(II)–piroxicam complex

according to eqn 1. According to the earlier reports [22-25], the correct choice of the model is confirmed by overlapping of experimental titration curves obtained from the equilibrium study with the theoretically calculated (simulated) curve. The model that best fits the potentiometric data was found to consist of 1110 [Cu(P)(L)] and 111-1[Cu(P)(LH-1)]. [Cu(P)(LH-1)] complex is formed through induced ionization of β-alcohol group. The stability constants of ternary Cu(II) complexes with P and L as given in Table-3 are in order: [Cu(P)(Try)] = 15.33 > [Cu(P)(Ser)] = 14.23 > [Cu(p)(The)] = 14.05.

$$Cu + P + L + H \rightleftharpoons [Cu(P)(L)(H)] \quad (1)$$

(charges are omitted for simplicity)

The pK_a values of coordinated alcohol group in Cu(II) ternary complexes ($\log_{10}\beta_{1110} - \log_{10}\beta_{111\text{-}1}$) obtained with Ser, Try and The are 10.01, 10.92 and 9.64, respectively. These values obtained in the current study are supported by the observation that in basic solutions Cu(II) promotes the ionization of alcoholato-group of threonine with pK_a value of 10.3 [26].

The distribution curve of serine mixed ligand system, taken as a representative, is given in Fig. 2. The ternary species 1110 starts to form at pH ~ 3.0 and with increasing pH, its concentration increases reaching a maximum of 35.7 % at pH = 8.8. A farther increase of pH is accompanied by a reduction in the concentration of 1110 complex and an increase in [Cu(P)LH_{-1}] (111-1) complex formation.

$\log_{10} K^{Cu(P)}_{Cu(P)(L)}$ and $\log_{10} K^{Cu(L)}_{Cu(P)(L)}$ formation constants were calculated using eqns. 2 and 3 (Table-3) for each mixed ligand and compared with each other in order to decide which one of the ligands was contributing to formation of the mixed ligand complexes, and which one is acting as the primary or secondary ligand. The results showed that drug acts as the primary ligand in all systems and amino acids act as secondary ligands.

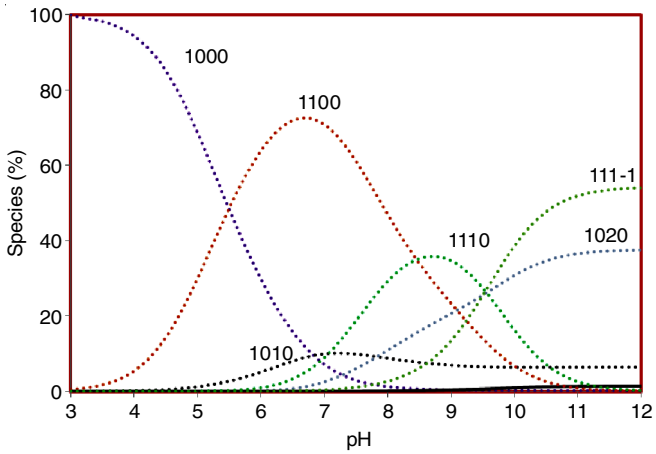


Fig. 2. Concentration distribution of various species as a function of pH in Cu(II)–piroxicam–Serine complexes

$$\log_{10} K^{Cu(P)}_{Cu(P)(L)} = \log_{10}\beta^{Cu}_{Cu(P)(L)} - \log_{10}\beta^{Cu}_{Cu(P)} \quad (2)$$

$$\log_{10} K^{Cu(L)}_{Cu(P)(L)} = \log_{10}\beta^{Cu}_{Cu(P)(L)} - \log_{10}\beta^{Cu}_{Cu(L)} \quad (3)$$

Comparison of formation constant of mixed ligand complexes with binary complexes: The relative stability of a mixed ligand complex, as compared to that of a binary complex, can be quantitatively expressed in different ways [27-29]. The most suitable comparison is in terms of $\log_{10}$. The values of $\Delta\log_{10}$ for Cu(P) L complexes are defined by eqns. 4 and 5.

$$Cu(P) + Cu(L) \rightleftharpoons Cu(P)L + Cu \quad (4)$$

$$\Delta\log_{10} K = \log_{10}\beta^{CuP}_{Cu(P)L} - \left(\log_{10}\beta^{Cu}_{Cu(P)} + \log_{10}\beta^{Cu}_{Cu(L)}\right) \quad (5)$$

This is a measure of difference in the strength of binding of ligand to free metal ion and to the metal ion already bound to another ligand. For Jahn-Teller distorted tetragonal coordination sphere of Cu^{2+}, theoretical value of $\Delta\log K_{Cu}$ should be - 0.9 [30]. However, in the present complexes, ligands having side groups, it was noted that $\Delta\log_{10} K$ values are more positive than expected statistical considerations (Table-3). Positive values are considered as evidence of enhanced stability as a result of intermolecular ligand-ligand interactions, hydrogen bonding, the π-back donation effect and/or hydrophobic effects. The $\Delta\log_{10} K$ value for ternary complex of tyrosine is more positive. This can be explained by the promise that the non-coordinated side group hydroxyphenyl ring of tyrosin comes over the aromatic moiety of drug and hence non-covalent hydrophobic interaction is possible. This intramolecular inter-ligand interaction stabilizes the mixed ligand complex, leading the more positive $\Delta\log_{10} K$ value.

The second way to characterize the formation of a tendency of Cu(II):mixed ligand complexes is $\log_{10} X$ (non-proportional dissociation constant) values [31]. This parameter is calculated by using eqns. 6 and 7:

$$Cu(P)_2 + Cu(L)_2 \rightleftharpoons 2Cu(P)(L)$$

$$X = \frac{[Cu(P)(L)]^2}{[Cu(P)_2][Cu(L)_2]} \quad (6)$$

$$\log X = 2\log\beta^{Cu}_{Cu(P)L} - \left(\log\beta^{Cu}_{Cu(P)_2} + \log\beta^{Cu}_{Cu(L)_2}\right) \quad (7)$$

The values of $\log_{10} X$ are higher than that expected on a statistical basis (0.60) [32]. This means that the formation of mixed ligand complexes is favoured in these systems. This is due to π back donation from Cu(II) ion to the aromatic moiety [33] in addition to the hydrophobic interaction between the moieties of drug and amino acids.

The quantitative stabilization of ternary complexes can also be expressed in terms of percent relative stabilization (% R.S., %) [34] as defined by eqn. 8:

$$\%\ R.S. = \left(\frac{\left(\log K^{Cu(P)}_{Cu(P)L} - \log\beta^{Cu}_{Cu(L)}\right)}{\log\beta^{Cu}_{Cu(L)}}\right)\times 100 \quad (8)$$

The values of % R.S. have been calculated and for all systems, the parameter % R.S. is positive. Positive values of % R.S. agree with the $\Delta\log_{10} K$ values (Table-3).

Conclusion

This work presents potentiometric investigations of Cu(II) complexes involving piroxicam (P) as anti-inflammatory drug ligand and some amino acids as bioligands *viz*., L-serine, L-tyrosine and L-threonine. From the results, it may be concluded that Cu(II) can form binary and ternary complexes with piroxicam and bioligands at various combinations when these compounds are present as mixed ligand systems in an aqueous solution through a simultaneous mechanism. The mixed-ligand complexes are formed in the physiological pH range. The positive value of $\Delta\log_{10} K$ is attributed to the extra stability of the ternary complexes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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