Research Article

Equilibrium Studies on Mixed Ligand Complexes of Copper (II) ion with Drug Fluoxetine. HCl and Glycine Oligopeptides using Potentiometric Titration Technique

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ABSTRACT

A potentiometric titration technique has been used to determine the stability constants for the various complexes of Cu(II) with drug fluoxetine hydrochloride (FLX) as primary ligand and glycine oligopeptides (L) as secondary ligands. The formation constants of the complexes formed in aqueous solutions and their concentration distributions as a function of pH were evaluated at 25°C and ionic strength 0.1 M $NaClO_4$. The relative stabilities of the ternary and corresponding binary complexes were also studied.

Keywords: Mixed-ligand, Cu(II) complexes, Potentiometric, Stability constants.

INTRODUCTION

Although medicinal chemistry was almost exclusively based on organic compounds and natural products, during the past three decades metal complexes have gained a growing interest as pharmaceuticals for the use as diagnostic agents or as chemotherapeutic drugs[1-7]. There is no doubt that the discovery of cisplatin, *cis*-diamminedichloroplatinum (II), represents one of the most significant events for cancer chemotherapy in the 20th century[6]. In metal-based drugs, the metal can coordinate ligands in a precise three-dimensional configuration, thus allowing the tailoring of the molecule to recognize and interact with a specific molecular target. This is further enhanced by different chemical modifications of ligands. Moreover, metal complexes easily undergo redox reactions and ligand substitution which allow them to participate in biological redox chemistry and interact with biological molecules. It is remarkable that investigations in this area are focused on the use of biologically active complexes formed by essential ions, such as copper[8]. Oligopeptides are molecules of low symmetry and with three or more functional groups, which are able to associate with protons. Cu (II) forms very stable complexes with simple oligopeptides. The modes of coordination of copper with simple oligopeptides have been studied in detail[9,10]. The mixed ligand complexes have been studied extensively because of their potential role in biological processes and can manifest themselves as enzyme-metal ion substrate complexes[11-17]. In view of the growing interest in the ternary complexes, it is thought worthwhile to study the ternary complexes of number of Oligopeptide and drug fluoxetine. HCl with copper (II). Fluoxetine Hydrochloride, ***Fig 1***, is an anti-depressant drug, chemically called as Benzenepropanamine, N-methylgamma-[4-(trifluoromethyl) phenoxy]-, Hydrochloride, or (+-)-N-Methyl-3-phenyl-3 [(alpha, alpha, alpha-trifluoro-ptolyl) oxy] propylamine hydrochloride. Although the metal complexes of fluoxetine have been investigated because of the application in medicine[18], less attention has been paid in solution system to its coordinative behaviour and the stability. Of particular relevance, in the present investigation, efforts have been made to study the stability constants of copper (II) complexes with fluoxetine. HCl as a primary ligand and glycine oligopeptides as secondary ligands *via* potentiometric titrations in aqueous media, having ionic strength (*I*) of 0.10 mol/L $NaClO_4$ at ambient temperature. The reactions associated with formation of these complexes have been undertaken in different pHs. Species distribution in solution over a wide range of pHs was also evaluated.

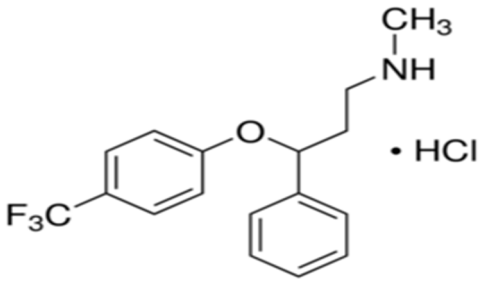


Figure 1: *Structure of Fluoxetine hydrochloride.*

Experimental

MATERIALS AND REAGENTS

All chemicals used were of guaranteed grade and used without further purification. Fluoxetine, HCl and glycine, glycinamide, diglycine, triglycine and tetraglycine were obtained from the Sigma Chem. Co. All stock solutions of $Cu(ClO_4)_2$, sodium perchlorate and perchloric acid (analytical reagent grade, Merck) were prepared in deionized water. Stock solution of $Cu(ClO_4)_2$ was standardized by EDTA titrations[19]. Furthermore, no supporting electrolyte was used in these mixed solvents. Carbonate-free sodium hydroxide solution was prepared and standardized against a potassium hydrogen phthalate

solution. The ionic strength of each solution was adjusted to 0.10 mol/L by addition of $NaClO_4$. Acid solutions prepared from perchloric acid were titrated against standardized sodium hydroxide[20].

Apparatus

Potentiometric titrations were performed at (25°C±0.1°C) in a double-walled glass vessel using a Griffin pH J-300-010 G Digital pH meter. The temperature was controlled by circulating water through the jacket, from a constant temperature bath. The electrode system was calibrated in terms of hydrogen-ion concentrations instead of activities.

Procedures

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 ml) solution (1.25×10^{-3} M) of constant ionic strength 0.1 M, adjusted with $NaClO_4$. The stability constants of the binary complexes were determined by titrating 40 ml of a solution mixture of metal ion (1.25×10^{-3} M), the ligand (2.5×10^{-3} M) and 0.1 M $NaClO_4$. The stability constants of mixed ligand complexes were determined by titrating 40 ml of solution containing Cu(II), FLX and peptides, all of concentration (1.25×10^{-3} M) and 0.1 M $NaClO_4$. The above solutions were titrated against 0.1 mol/L NaOH in an atmosphere of pure N_2 gas. For all the titrations, $HClO_4$ solution was added, so that they were fully protonated at the beginning of the titrations.

The overall stability constants β_{lpqr} defined by Eq. (1).

$$l(Cu) + p(FLX) + q(L) + r(H) \rightleftharpoons (Cu)_l (FLX)_p (L)_q (H)_r$$

$$\beta_{lpqr} = \frac{[Cu_l (FLX)_p (L)_q (H)_r]}{[Cu^l (FLX)^p (L)^q (H)^r]} \quad (1)$$

(charges are omitted for simplicity)

Where l, q, q and r are the numbers of copper(II) ion, fluoxetine. HCl (FLX), peptides (L) and proton, respectively, in the complex $Cu_l\ FLX_p\ L_q\ H_r$. and used as fixed parameters for the refinement of the stability

Data processing

The stoichiometries and stability constants of the complex species formed in solution were determined by examining various possible composition models for the systems studied. About 110 to 150 experimental data points were available for evaluation in each run. All the dissociation and the complex formation constants were determined using the HYPERQUAD program[21] and the speciation as a function of pH using the HYSS program[22].

RESULTS AND DISCUSSION

Acidity Constants

The proton dissociation constants of FLX and glycine Oligopeptides are given in *Table 1*. FLX contain at least one site that can reversibly disassociate a proton (a hydrogen ion) to form a negatively charged anion. FLX can release one proton from amine group according following deprotonation equilibria:

$$H(FLX) \rightleftharpoons H^+ + FLX^-$$

The pK_a value of protonated FLX is 8.8 means that is predominantly present in the ionized form at a physiologic pH.

Table 1: Acidity constant of ligands at 25 °C and I = 0.10 M $NaClO_4$.

Ligand	pK_{a1} (COOH)	$pK_{a2}(NH_3^+)$
FLX	$pK^{H}_{H(FLX)} = 8.8(0.03)$	
Diglycine	3.21(0.05)	8.13(0.03)
Triglycine	3.27 (0.03)	7.96 (0.02)
Tetraglycine	3.24 (0.03)	7.97 (0.01)

Standard deviations are given in parentheses.

Binary copper (II) complex formation equilibria with FLX

FLX was titrated in the presence and absence of Cu(II) ion. The titration curve of the Cu(II)-FLX complex is lowered from that of the free FLX curve, indicating formation of Cu(II) complex by displacement of protons. The formation constants were determined by fitting potentiometric data on the basis of possible composition models. The selected model with the best statistical fit was found to consist of Cu(FLX) (110) and Cu(FLX)H (111) complexes. The stability constants of their complexes are given in Table (2). The pK_a of the protonated form $pK_a = log\ \beta^{Cu}_{Cu(FLX)H} - log\ \beta^{Cu}_{Cu(FLX)}$ is 2.13. The lower value of pK_a can be attributed to coordination of FLX with the Cu(II) ion. Species distribution diagram of Cu(II)-FLX system is shown in **Fig. 2**. The concentration of the Cu(II)-FLX species increases with increasing pH, attaining a maximum of 86.3% at pH 6.0. Further increase in pH is accompanied by a decrease in the concentration of the Cu(II)-FLX species and an increase in the concentration of the Cu(II)-$(FLX)_2$ species. Cu(II)-FLX(H) complex species has been found to be most favored at lower pH values.

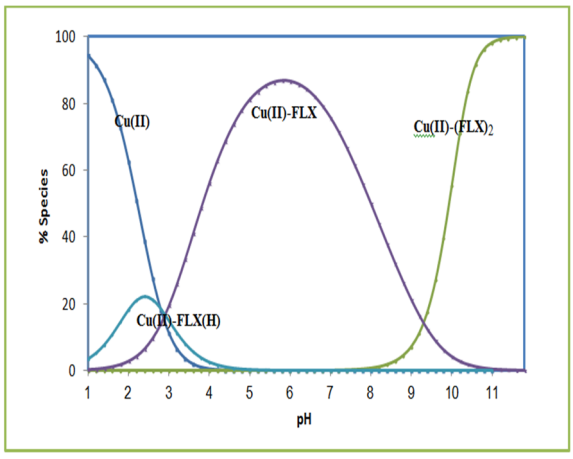
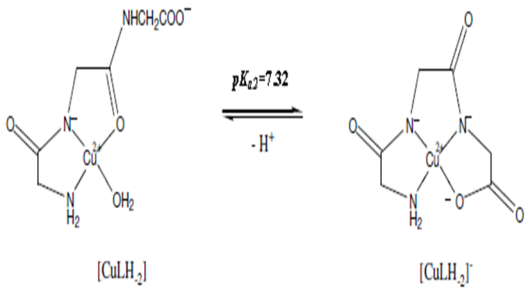


Figure 2: Variation of complex species concentration with pH in the binary system Cu(I1)- FLX system.

Binary copper (II) complex formation equilibria with oligopeptides

In the complexation between oligoglycines and copper (II), the complex formation process involves the successive formation of 1N-, 2N, 3N, or 4N-coordinated species with an increasing pH to form the chelate rings. The chelation starts at the amino end of the molecule, with the assistance of carbonyl oxygen, and continues with the sequential deprotonation and coordination of the amide groups[23,24]. At low pH the dominant species is $[CuL]^+$, being formed between copper and diglycine with bidentate ligand [(NH_2, CO); H_2O; H_2O]. Towards pH 5, as the pK_a diglycine value is 4.28 ***(Table 2)***, the deprotonation of peptidic hydrogens is possible to give another complex species $[CuH_{-1}L]$, where H_{-1} indicates the dissociation of hydrogen; and also the formation of a new five-membered chelate ring. The bonded donor groups to copper are amide-N, the C-terminal carboxylate group via O and the N- terminal amino group *via* N[25,26]. Triglycine and tetraglycine continue with the amide deprotonation process and the further formation of extra five-membered chelate rings around Cu(II). $[CuH_{-1}L]$ binding groups of triglycine are [(NH_2, N-, CO); H_2O]. The pKa_1 value of amide deprotonation is 5.12, higher than diglycine **(Table 2)**. When pH increase around the pK_{a2} of triglycine (7.32), the new conformation will appear $[CuH_{-2}L]^-$ **(Scheme1)**. The binding ligands of monohydroxylic complex ([CuH-2L]-) are [NH2, N-, CO, COO-]. This type of coordination prevents the hydrolytic processes, which occur only in basic solution (pH>12)[27,23].



Scheme 1: Triglycine complexes with Cu(II). Axial water molecules are omitted

Tetraglycine follows the same process but with little differences in the *pKa* values of the amide deprotonation. Tetraglycine can continue the process to form a tetradentate ligand complex which occupies the four equatorial positions of Cu(III) ion with the amino –N and three deprotonated amide-N-. When the pH is around 9, there is again a deprotonation of amide group with the formation of dihydroxo complex or $[CuLH_{-3}]^{2-}$ [27,23].

Stability constants of ternary complexes

Ternary complex formation may proceed either through a stepwise or a simultaneous mechanism depending on the chelating potential of FLX and other ligands. The formation constants of the 1:1 Cu (II) complexes with FLX and those of glycine oligopeptides, cited in Table 2, are of the same order. Consequently, the ligation of FLX and glycine oligopeptides will proceed simultaneously. The validity of this model was verified by comparing the experimental potentiometric date with the theoretically calculated (simulated) curve. Fig. 3 presents such a comparison for the Cu- FLX -diglycine system, taken as a representative one. The potentiometric data of the Cu(FLX) L system were fitted by various models. The most acceptable model was found to be consistent with the formation of the complexes with stoichiometric coefficients 1110 ([Cu(FLX)(L)]) and 111-1([Cu(FLX)(LH_{-1})]). In the 1110 case, L is bound through the amino and carbonyl oxygen groups. On increasing the pH, the coordination sites should switch from the carbonyl oxygen to the amide nitrogen. Such a change in coordination centers is now well documented[28,29]. The amide groups undergo deprotonation and the [Cu(FLX)(LH_{-1})] complexes are formed. The *pKa* values are calculated by the following equation:

$$pK_a = log\beta_{1110} - log\beta_{111-1} \qquad (2)$$

the *pKa* a value for the glycinamide complex is lower than those for other oligoglycines (*see* **Table 2**). This can be explained on the premise that the more bulky substituent group on the peptide may hinder the structural changes when going from the protonated to the deprotonated complexes. The *pKa* a of the glutamine complex is exceptionally higher than those of the other peptide complexes. This is due to the formation of a seven membered chelate ring which is more strained and therefore less favoured. The distribution diagram of the diglycine complex is given in **Fig. 3**. The mixed ligand species [Cu (FLX)L](1110) starts to format pH at ~2 and, with increasing pH, its concentration increases reaching a maximum of 88% at pH = 5.1. A further increase of pH is accompanied by a decrease in the 1110 complex concentration and an increase in [Cu (FLX) LH_{-1}] (111-1)] complex formation.

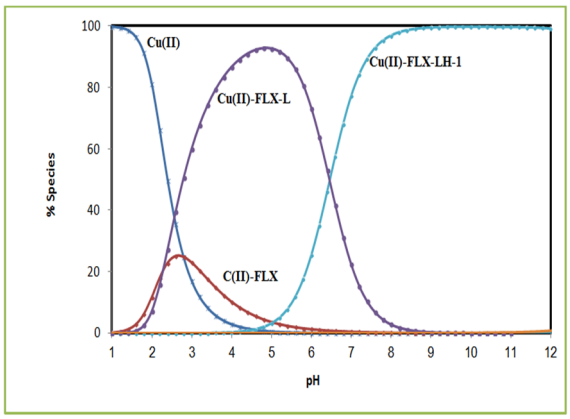


Figure 3: Variation of complex species concentration with pH in the ternary Cu(II)- FLX- diglycine system.

Table 2: Formation constants of the binary and ternary complexes in the Cu (II)- FLX- peptides systems at 25 °C and I = 0.10 M $NaClO_4$.

System	l	p	q	r[a]	Log β[b]	Δlog K
Fluoxetine hydrochloride (FLX)	1	1	0	0	6.32(0.02)	
	1	2	0	0	10.33(0.01)	
	1	1	0	1	8.45(0.05)	
Diglycine	1	0	1	0	5.63(0.01)	
	1	0	1	-1	1.35(0.01)	
	1	0	1	-2	-7.76(0.02)	
	1	0	2	-1	4.46(0.01)	
	1	1	1	0	11.03(0.01)	-0.92
	1	1	1	-1	4.61(0.01)	-3.06
Triglycine	1	0	1	0	5.45(0.01)	
	1	0	1	-1	0.33(0.01)	
	1	0	1	-2	-6.99(0.02)	
	1	0	1	-3	-17.24(0.02)	
	1	0	2	-1	3.67(0.03)	
	1	1	1	0	11.45(0.02)	-0.32
	1	1	1	-1	4.10(0.03)	-2.55
Tetraglycine	1	0	1	0	5.32(0.01)	
	1	0	1	-1	-0.47(0.01)	
	1	0	1	-2	-7.47(0.02)	
	1	0	1	-3	-16.78(0.03)	
	1	1	1	0	11.60(0.01)	-0.04
	1	1	1	-1	3.87(0.05)	-1.98
Glutamine	0	0	1	1	8.95(0.01)	
	1	0	1	0	8.50(0.01)	
	1	0	1	-1	-1.58(0.02)	
	1	1	1	0	14.69(0.01)	-0.13
	1	1	1	-1	2.67(0.001)	-2.07
Glycinamide	0	0	1	1	7.61(0.01)	
	1	0	1	0	4.72(0.01)	
	1	0	1	-1	1.48(0.02)	
	1	0	1	-2	-5.50(0.03)	
	1	0	2	-1	2.60(0.01)	
	1	1	1	0	10.78(0.00)	-0.26
	1	1	1	-1	6.78(0.02)	-1.02

[a]l, p, q and r are the stoichiometric coefficient corresponding to Cu(II), FLX, peptides (L) and H^+, respectively. [b]standard deviations are given in parentheses.

The tendency towards ternary complex formation can be evaluated in various ways. *Δlog K* has been widely accepted and used for many years[30] and the advantages in using *Δlog K* in comparing the stabilities of ternary and binary complexes have been reviewed. The *Δlog K* value for protonated and deprotonated ternary complexes formed through simultaneous mechanism are given by Eqs. (3) and (4) where as those of the induce deprotonated peptide complex can be calculated using Eq. (5):

$$\Delta logK = log\beta_{1111} - log\beta_{1100} - log\beta_{1011} \quad (3)$$

$$\Delta logK = log\beta_{1110} - log\beta_{1100} - log\beta_{1010} \quad (4)$$

$$\Delta logK = log\beta_{111-1} - log\beta_{1100} - log\beta_{101-1} \quad (5)$$

The negative Δ*logK* values (**Table 2**) of this system indicates that the ternary complex is less stable than binary complex. This is in accordance with statistical considerations. The negative value of Δ*logK* is due to the higher stability of its binary complexes, reduced number of coordination sites, steric hindrance, electronic consideration, difference in bond type, geometrical structure[31-33].

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