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Short Communication

Potentiometric Determination of Stability Constants of Binary and Ternary Complexes of L-Tryptophan and Anti-Inflammatory Drugs with Zn(II)

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Formation equilibria of Zn(II) with tryptophan (L1) as a representative example of amino acids in presence of some carboxylic acid nonsteroidal anti-inflammatory drugs (NSAIDS) such as aspirin, ibuprofen and naproxen (L2) studied using the pH-metric titrations. The protonation constants of the ligands (pK_a) and their stability constants (log β) were determined in 50% (v/v) EtOH-H_2O solutions at 25±0.1ºC and I = 0.1 mol dm^{-3} $NaClO_4$. The stepwise formation of the complexes has been established in the pH region studied. The species distribution in solutions as a function of pH was determined using the HYSS program. The relative stabilities of the ternary complexes are compared with those of the corresponding binary complexes in terms of Δlog K and percentage of relative stabilization (% R. S.) values.

Keywords: Potentiometric titration, Ternary complexes, Stability constants, Anti-inflammatory drugs.

1. INTRODUCTION

Compounds with metals as therapeutic agents for various diseases states have been investigated [1–3]. The non–steroidal anti-inflammatory drugs (NSAIDs) is a drug class that groups together drugs that provide analgesic (pain-killing) and antipyretic (fever-reducing) effects, and, in higher doses, anti-inflammatory effects [4]. NSAIDs can be used as donor ligands for complexations of metals [5-10]. Coordinated metal ions with NSAIDs provide advantages over the drugs themselves. The use of metal-based drugs presents the most important strategy in the development of new anticancer and antimicrobial agents. It is well known that metal complexes accelerate the effect of the drug and the performance of

the therapeutic agent can be enhanced when coordinating with a metal ion [11, 12]. Moreover, the coordination residues can exert their own effects; for instance, anti-tumoral and anti-bacterial activities are expected in the cases the central atoms are Pt(II), Pt(TV), Bi(III), Sb(III), Ag(I), Sb(V), and Au(I) [6-8]. Ternary complexes have been extensively studied due to their potential role in biological processes and may manifest as metallic enzyme substrate complexes. The stability constant of metal complexes with drugs is useful for knowing the appropriate dose of a drug and its effect with all other components of the bloodstream as well as for measuring the strength of metal ligand bonds [13]. Here, we report the solution equilibrium studies of the ternary complexes including Zn(II) with tryptophan (L1) and some non-steroidal anti-inflammatory drugs (L2). Stability constants of ternary systems were determined by potentiometric method using the computer program named "HYPERQUAD" at 25±0.1ºC and I = 0.1 mol dm^{-3} $NaClO_4$ in 50% (v/v) EtOH-H_2O medium. The relative stabilities of the ternary complexes are compared with those of the corresponding binary complexes in terms of Δ log K and % R. S values.

2. EXPERIMENTAL

All chemicals and reagents were used as received without further purification (formulae of the ligands selected are provided in Figure 1). A prepared solution of NaOH (carbonate free) was standardized with potassium hydrogen phthalate (KHP) solution. Ionic strength was adjusted to 0.1 mol dm^{-3} by addition of $NaClO_4$. All the standard solutions were prepared using deionized water.

2.1. Apparatus and measuring techniques

Potentiometric pH-metric titrations have been carried out using a Metrohm 686 titroprocessor equipped with a 665 dosimat. The electrode was calibrated with standard buffer solutions (pH 4.0 and 10.0) before the pH measurements at 25±0.1ºC. The cell solution was stirred at constant speed during the titration using magnetic stirring system. The titration reaction was investigated in presence of purified N_2 atmosphere using standard solution of 0.05 mol dm^{-3} sodium hydroxide free from carbon dioxide. The ionic product (Kw = [H+][OH-]) was calculated at I= 0.10 mol dm-3 using $NaClO_4$ in 50% (v/v) EtOH-H_2O solutions based on measurements of [OH^-] and pH in several series of experiments. The pKw value obtained in this medium is 14.43. Total volume in all potentiometric titrations was adjusted to 40 cm^3. pK_a of the ligands were determined by titrating the ligand solution (1.0×10^{-3} mol dm^{-3}) of constant ionic strength 0.1mol dm^{-3} $NaClO_4$. log β of the binary complexes were determined by titrating a solution mixture of Zn(II) ($1.0x10^{-3}$ mol dm^{-3}) + (L1 or L2) ($1.0x10^{-3}$ and $3.0x10^{-3}$ mol dm^{-3}). log β of the ternary complexes were investigated using potentiometric data obtained from mixtures of Zn(II), L1 and L2 in the concentration ratio 1:1:1. For all the titrations, $HClO_4$ solution was added, so that they were fully protonated at the beginning of the titrations.

2.2. Data processing

pK_a and log β were calculated using the HYPERQUAD computer program [14]. For this purpose, a fitting criterion based on the minimization of the nonlinear least-squares sum defined by the difference between the calculated and the experimental data of the titration curves was used Eq.1:

$$X^2 = \Sigma \frac{(calculated - experimental)^2}{experimental} \quad (1)$$

The equilibrium can be written as follows (charges are omitted for simplicity).

$$a(M) + b(L1) + c(L2) + d(H) \rightleftharpoons [(M)_a(L1)_b(L2)_c(H)_d] \quad (2)$$

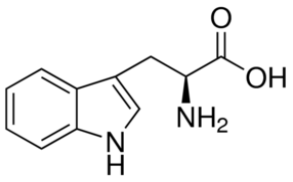
$$\beta_{abcd} = \frac{[Zn_aL1_bL2_cH_d]}{[Zn]^a\,[L1]^b[L2]^c\,[H]^d} \quad (3)$$

Where a, b, c and d are the numbers of Zinc(II) ion, amino acid (L1), drugs (L2) and proton, respectively, in the complex $Zn_a\,L1_b\,L2_c\,H_d$. The concentration distribution diagrams were obtained with the program HySS [15], which furnishes a variety of data presentations, including tables of concentrations of all species present in solutions in the selected pH ranges.

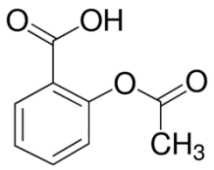
3. RESULTS AND DISCUSSION

3.1. Protonation constants of ligands

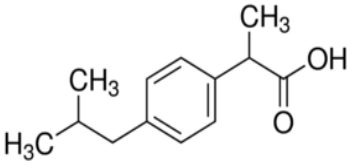
Protonation constants of tryptophan and anti-inflammatory drugs (aspirin, ibuprofen and naproxen) examined in this study were determined by potentiometric method at 25 ± 0.1°C and *I* = 0.1 mol dm^{-3} $NaClO_4$ in a medium containing 50% (v/v) EtOH-H_2O. *pK's* of the carboxylic acid group obtained with aspirin, ibuprofen and naproxen are 3.48, 4.89 and 4.22 3.49 respectively, which means that under nearly all physiological conditions, this group is almost entirely deprotonated. Two protonation constants for the tryptophan amino acid are pK_{a1}= 2.32: carboxylic acid and pK_{a2} = 9.21: primary amine. The results obtained are in good agreement with the literature data [16-18]. Protonation constants of all of the ligands determined in this study were listed in Table 1.



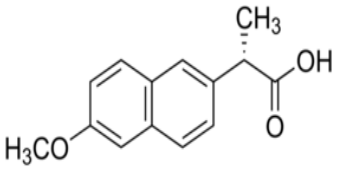
L-Tryptophan



Aspirin



Ibuprofen



Naproxen

Figure 1. The structural formula of ligands.

Table 1. The protonation constants of the ligands at 25 ± 0.1°C and I = 0.1 mol dm^{-3} $NaClO_4$ in 50% (v/v) EtOH−H_2O.

Ligands	pK_{a1}	pK_{a2}
Tryptophan (Tryp)	2.32 (0.001)	9.21(0.002)
Aspirin (Asp)	3.48 (0.001)	
Ibuprofen (Ibu)	4.89(0.002)	
Naproxen (Nap)	4.22(0.007)	

Note. pK_{a1}: corresponds to 11 species (i.e., $L^- + H^+ \rightleftharpoons LH$); pK_{a2} corresponds to 12 species (i.e., $LH + H^+ \rightleftharpoons LH_2^+$).Standard deviations are given in parentheses.

3.2. Binary complexes of Zn(II) ion

The titration procedure were performed for ligands (amino acid or drugs) alone, and in presence of Zn(II) ion potentiometrically. Analysis of potentiometric titrations indicated that binary complex formation curves are shifted to lower pH values than the free ligand solution curves. This shows that complex formation reactions proceed by releasing of protons from such ligands. The potentiometric titration curves of the Zn(II)-Aspirin (L2) system taken as representative drugs are given in Fig.1. The decrease in the pH value of the reaction mixture indicated that Zn(II)-Aspirin complexes form at 1 : 1 and 1 : 2 mol ratios as seen in Fig. 1, curve B for (1 : 1) and curve C for (1 : 2) binary complexes of Zn(II)-Aspirin, respectively. It can be seen that Zn(II) ion forms coordination complexes at 1 : 1, 1 : 2, and 1 : 3 mole ratios with tryptophan (L1). No precipitate was observed in the titration vessel which indicating that the possibility of $[Zn(OH)_2]$ formation can be excluded. Table 2 shows the logarithm of the formation constants for all types of complexes, which have been identified using potentiometric titration [19-23]. The following equilibrium reactions and equations ((4)–(6)) are given for binary complex systems which are thought that Zn(II) and the amino acids (L1) form in (1:1), (1:2), and/or (1:3) stoichiometry:

$$Zn + L1 \rightleftharpoons Zn(L1); \qquad \beta_1 = \beta^{Zn}_{Zn(L1)} = \frac{[Zn(L1)]}{[Zn][L1]} \qquad (4)$$

$$Zn + 2L1 \rightleftharpoons Zn(L1)_2; \qquad \beta_2 = \beta^{Zn}_{Zn(L1)_2} = \frac{[Zn(L1)_2]}{[Zn][L2]^2} \qquad (5)$$

$$Zn + 3L1 \rightleftharpoons Zn(L1)_3; \qquad \beta_3 = \beta^{Zn}_{Zn(L1)_3} = \frac{[Zn(L1)_3]}{[Zn][L2]^3} \qquad (6)$$

Table 2. Stability constants of binary complexes at 25 ± 0.1°C and I = 0.1 mol dm^{-3} $NaClO_4$ in 50% (v/v) EtOH−H_2O.

System	log β_1	log β_2	log β_3
Zn(II)- Tryp	5.52(0.04)	8.52(0.03)	12.87(0.005)
Zn(II)- Asp	2.58(0.01)	6.82(0.01)	
Zn(II)- Ibu	2.97(0.02)	6.83(0.01)	
Zn(II)-Nap	3.21 (0.01)	5.40(0.01)	

Numbers in parentheses are standard deviations.

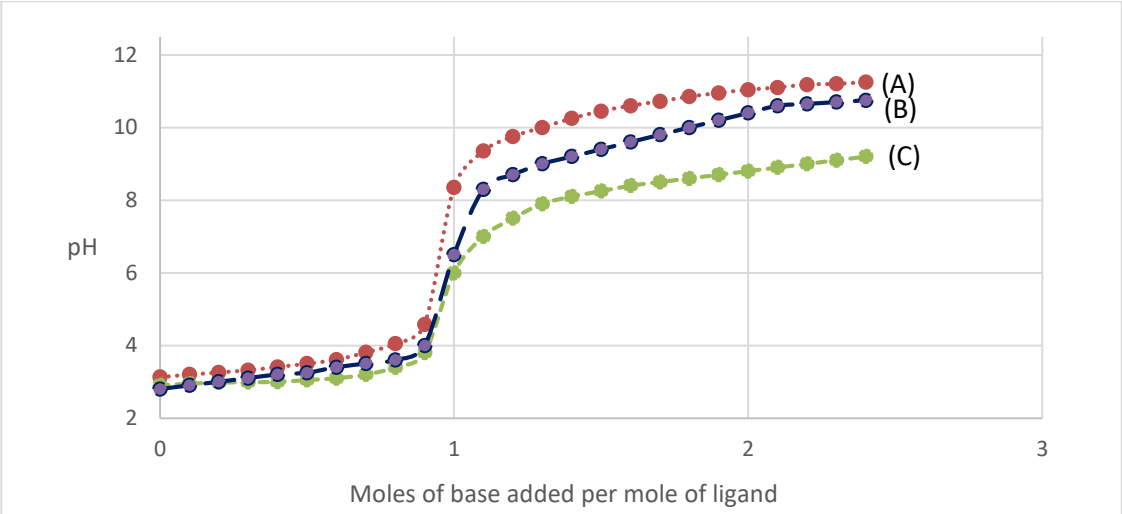


Figure 2. Potentiometric titration curves of binary complexes (at 25 ± 0.1°C and I = 0.1 mol dm^{-3} $NaClO_4$ in 50% (v/v) EtOH−H_2O). Curve A: Asp, curve B: Zn(II): Asp (1 : 1), and curve C: Zn(II): Asp (1 : 2).

3.3. Ternary complexes of Zn(II) ion

The stability constants of these complexes, which were formed, were calculated (according to (2) and (3)) with HYPERQUAD computer program [14] and given in Table 3. According to the method of calculation applied, the model of the best fit for the complexes under investigation has been found to be Zn(L1)(L2). The Zn(II) complex with amino acid is significantly more stable than the corresponding binary complexes with drugs (see Table 3). Consequently, in the presence of both ligands, tryptophan is primarily ligated to the Zn(II) ion, occupying four coordination position .This is followed by ligation of the drugs occupying the coordination positions remaining, i.e., the ternary complex formation could be considered in stepwise equilibria. The stability constants of the ternary systems in terms of secondary ligands follow this order [Zn(Tryp)(Nap)] = 9.77 > [Zn(Tryp) (Iub)] = 8.98 > [Zn(Tryp) (Asp)] = 8.33. The species distribution of Ibuprofen (Ibu), taken as a representative drug, is given in Fig. 3. The mixed ligand species [Zn(L1)(Ibu)] starts to form at pH ~3.4 and, with increasing pH, its concentration increases reaching a maximum of 98% at pH = 8.5.Therefore, the species Zn(L1)(Ibu)predominates in the physiological pH range. log $K^{Zn(L1)}_{Zn(L1)(L2)}$ and log $K^{Zn(L2)}_{Zn(L1)(L2)}$constants were calculated for ternary complexes according to equations (7 and 8) and compared with each other in order to decide which one of the ligands was contributing to formation of the ternary complexes, and which one is acting as the primary or secondary ligand [24]. The results obtained (Table 3) shown that tryptophan acts as the primary ligand.

$$logK^{Zn(L1)}_{Zn(L1)L2} = log\beta^{Zn}_{Zn(L1)L2} - logK^{Zn}_{ZnL1} \quad (7)$$

$$logK^{Zn(L1)}_{Zn(L1)L2} = log\beta^{Zn}_{Zn(L1)L2} - logK^{Zn}_{ZnL2} \quad (8)$$

The relative stability of the ternary complexes formed through stepwise mechanism, as compared to those of the corresponding binary complexes, is expressed in terms of ΔlogK as defined by the following equation [25]:

$$\Delta\, logK = logK^{Zn(L1)}_{Zn(L1)L2} - logK^{Zn}_{Zn(L2)} \quad (9)$$

The Δlog K values given in Table 3 are positive, this means that drugs form more stable complexes with [Zn(Tryp)] than with the free Zn(II) ion. The enhanced stability of ternary complexes in comparison to the binary complexes can also be explained by suggestion of inter-ligand interaction that exists between amino acid ligand and drugs.

Another parameter, which is percent relative stabilization (% RS) for quantifying the stability of a ternary complex, may be defined as [26]:

$$\%\ \text{R.S.} = \left[\frac{\Delta \log K}{logK^{Zn}_{Zn(L2)}}\right] \times 100 \quad (10)$$

For all systems, the parameter % R.S. is positive (Table 3). This may be considered as evidence for the occurrence of enhanced stabilities.

Table 3. Stability constants of ternary complexes at 25 ± 0.1°C and *I* = 0.1 mol dm^{-3} $NaClO_4$ in 50% (v/v) EtOH−H_2O.

System	$\log\beta^{Zn}_{Zn(L1)L2}$	$\log K^{Zn(L1)}_{Zn(L1)L2}$	$\log K^{Zn(L2)}_{Zn(L1)(L2)}$	Δlog K	% R.S.
Zn(II)- Tryp-Asp	8.33 (0.003)	2.81	5.75	0.23	8.91
Zn(II)- Tryp-Ibu	8.98 (0.001)	3.46	6.01	0.49	16.50
Zn(II)- Tryp- Nap	9.77 (0.01)	4.25	6.56	1.04	32.40

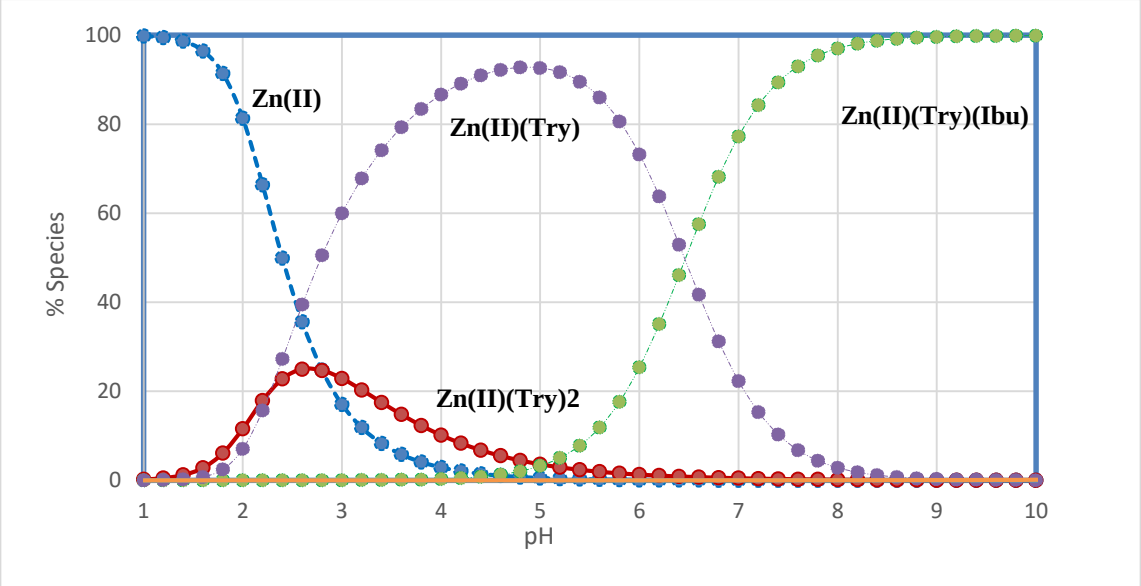


Figure 3. Distribution diagram of the species in the (1: 1: 1) Zn(II)-Try(L1)-Ibu(L2) ternary system (at 25 ± 0.1°C and I = 0.1 mol dm^{-3} $NaClO_4$ in 50% (v/v) EtOH−H_2O).

4. CONCLUSIONS

In summary, in our study we have determined the stability constants of binary and ternary complexes of Zn(II) with tryptophan and some anti-Inflammatory drugs (e.g., aspirin, ibuprofen and

naproxen) pH-metrically in 50% (v/v) EtOH−H_2O at 25 ± 0.1°C and I = 0.1 mol dm^{-3} $NaClO_4$. The possible formation of ternary complexes via stepwise mechanisms has been confirmed by comparison of the ternary titration curve with the composite curve obtained by graphical addition of L2 titration data to that of the Zn(II)-L1 titration curve.

The fact that the positive log*K* and % RS values were obtained from the mixed ligand complex systems Zn(II) shows that the stability of ternary complex systems is more dominant than that of the binary complex systems.

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