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Investigation of the Formation of Copper(II)-Tryptophane-Aspirin Ternary Complexes

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Formation constants and the stoichiometric composition of the potentially therapeutic complex of Cu-tryptophane-aspirin were determined potentiometrically, at 25 ºC (± 1) and I = 0.11. The formation constants of binary Cu(II)-aspirin (1:2) complex, on which few and many information exist in literature, were also investigated. Distribution of species was calculated for various pH values. Cu(II)-Tryptophan-aspirin ternary complex formation was confirmed through UV and IR spectrometry and qualitative metal analysis in the precipitates. The stability constants of ternary complexes of Cu(II)-Tryp-ASA were reported, which is important for further studies on this therapeutically important ternary complex, both from the point of synthesis and commenting on its pharmacokinetics.

Key Words: Copper ternary complex, Aspirin, Tryptophane, Stability constant, Spectroscopy.

INTRODUCTION

Use of Cu(II) complexes of different antiinflammatory agents has been shown to increase the effects of these drugs and reduce their gastrointestinal toxicity[1,2]. To understand the possible interactions of copper(II), amino acids and aspirin, one should consider a whole picture comprised of the synergistic capability of aspirin[3], the antiinflammatory activity of Cu(II)[4], the synergistic effect of tryptophan-aspirin combination in migraine and in diseases which cause immune activation[5,6], the stronger analgesic, antiinflammatory and antithrombotic effect of Cu(II)-aspirinate than aspirin[7-9], decreased gastrointestinal toxicity of Cu(II)-aspirinate than aspirin because of increased selectivity in cyclooxygenase inhibition[10] and the stronger analgesic and antiinflammatory effects of some Cu(II)-amino acid complexes than Cu(II)-aspirinate[1]. Thus, the investigation of formation of the Cu(II)-tryptophan-aspirin ternary complex was carried out to provide chemical data for the synthesis and stability of a new potentially therapeutic Cu(II) complex. The formation (stability) constants and the conditional formation constants of this complex at various pH

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would also be important parameters in the estimation of their pharmacokinetic behaviour. To our best of knowledge, no study has been reported on the investigation of formation of this ternary complex in literature. Also, since there is few and contradictory information on the stoichiometric ratios and formation constants of Cu(II)-aspirin, this study contributes to the literature by providing data on this binary complex. In two different potentiometric studies, the constants of Cu(II)-aspirin complex were determined as log β_1 = 1.264 and log β_2 = 3.033[11] and as log β_1 = 1.565[4]. The experimental conditions in each literature is quite different from the conditions in present study.

There is just a single study report available in the literature[4] on the investigation of formation of ternary aspirin complexes of Cu(II) with an amino acid. Brumas *et al.*[4] found the overall formation constant (log β_{pqrs}) of Cu(II)-histidine-aspirin as 11.75 for 1:1:1 and as 19.89 for 1:1:2 stoichiometric ratios, at 37 ºC and I = 0.15. The authors have used MINIQUAD program, where these formation constants logarithmically represent the sum of the binding constants of both ligands to the metal (as in HYPERQUAD), which differs from the original Irving-Rossotti approach where log β represents the binding constants of the secondary ligands (aspirin) to the existing metal-ligand (here Cu(II)-Tryp) binary system. Both approaches are utilized in this study. In literature studies, other ligands were used instead of aspirin[12-14]. Tryptophan is an important amino acid in the physiological transport of Cu(II) and in Cu(II)-binding of certain proteins *via* their tryptophan residues. In this study, formation of Cu(II)-tryptophan-aspirin was investigated using potentiometric methods of Calvin-Bjerrum and Irving-Rossotti[15-17], spectrometric methods of UV and FTIR. By the potentiometric method used, the standard formation and conditional formation constants of the complex (log β) which actually display the binding constants of aspirin to the binary Cu(II)-tryptophan complex at various pH were determined. The overall formation constant (log β_{pqrs}) of the ternary complex was used to plot the distribution of the species. Thus, this study is important from the viewpoint of showing that the above mentioned ternary complex can favourably form.

EXPERIMENTAL

Radiometer Titralab TIM 800 Titration manager, Radiometer ABU 901 titrator, HI 1131 pH glass electrode, Shimadzu UV-2100 S spectrophotometer and Shimadzu-8300 FTIR spectrometer. $NaClO_4$, $HClO_4$, $Cu(NO_3)_2$, 1,4-dioxane (Merck), aspirin (ASA) (Acros), L-tryptophan (Tryp) (Sigma) were used. All reagents were of analytical grade and solutions were prepared freshly before each study, using deoxygenated and double distilled water. Potentiometric titrations were carried out under N_2 atmosphere and repeated at least three times. The exact calibration of pH-meter was performed before each study, using commercial buffer solutions (Merck) of pH 4.0 and 7.0. Potentiometric calculations were performed in a calculation program formed in Excel, according to Calvin-Bjerrum and Irving-Rossotti method and species distribution diagrams were plotted using the program HySS.

Potentiometric Investigation of Cu(II)-Tryp-ASA, Cu(II)-ASA and Cu-Tryp complexes

The following solutions, each having a volume of 50.0 mL and compositions below, were titrated potentiometrically with NaOH (0.1000 N) under N_2 atmosphere, at 25 (± 1) ºC and I = 0.11 ionic strength using $NaClO_4$. The formation constants of Cu(II)-Tryp-$(ASA)_2$, Cu(II)-Tryp, Cu(II)-$(Tryp)_2$ and Cu(II)-$(ASA)_2$ were calculated, using the NaOH volumes spent for titration of the mixtures below:

(a): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M)

(b): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + ASA (4.00×10^{-3} M)

(c): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + Cu(II) (2.00×10^{-3} M) + ASA (4.00×10^{-3} M)

(d): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + Cu(II) (2.00×10^{-3} M) + ASA (4.00×10^{-3} M) + Tryp (2.00×10^{-3} M)

(e): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + Tryp (2.00×10^{-3} M)

(f): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + Cu(II) (2.00×10^{-3} M) + Tryp (2.00×10^{-3} M)

(g): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + Tryp (4.00×10^{-3} M)

(h): $HClO_4$ (1.00×10^{-2} M) + $NaClO_4$ (1.00×10^{-1} M) + Cu(II) (2.00×10^{-3} M) + Tryp (4.00×10^{-3} M)

The procedure for ternary complex formation (above) is given for 1:1:2 Cu(II): Tryp:ASA ratio, which gave the best results in accord with the conditional formation constant. The same procedures were also performed for 1:1:1, 1:2:1 and 1:2:2 ratios.

Qualitative UV spectrophotometric investigation of Cu(II)-Tryp-ASA complexes

After potentiometric determination of the stability constant of the ternary complex in solution, the formation of the same complex was investigated by UV spectrophotometry in 1:1:1 and 1:1:2 ratios, at pH = 5.50, where the complex formation did not exactly reach a maximum in clear solution (without the initiation of precipitation). Three different concentrations were studied for each system and parallel results were obtained for each.

Equimolar (1.20×10^{-3} M) Cu(II), Tryp and ASA stock solutions were prepared and mixed in 1:1:1 and 1:1:2 ratios, also keeping a 2.0 mL blank volume for pH adjustment to 5.50 with NaOH, such that the final concentration of the Cu(II) was 2.00×10^{-3} M after completion of the final volume of each solution to 12.0 mL, using distilled water at pH = 5.50. It was also ensured that the final pH of the solutions remain at pH 5.50. Seven solutions in each set included the order: Cu(II), Tryp, ASA, Cu(II) + Tryp, Cu(II) + ASA, Tryp + ASA, Cu(II) + Tryp + ASA.

In order to comparatively investigate the interactions pertaining to the 1:1:1 and 1:1:2 ternary complexes, each solution was diluted (to 6.00×10^{-5} M, 8.00×10^{-5} M and 1.00×10^{-4} M concentrations), using distilled water adjusted to pH =

5.50. As a result, total 6 sets were studied to investigate the formation of the complexes. The spectra of each mixture was recorded in respect to its reference solution which was prepared in the same way, by adding the same NaOH volume and diluted as in the sample.

Investigation of Cu(II)-Tryp-ASA complex using FTIR spectrometry

Tryp (2.40×10^{-3} M), Cu(II) (7.20×10^{-3} M) and NaOH (0.10 N) aqueous solutions and ASA solutions in dioxane (4.80×10^{-3} and 2.40×10^{-3} M) were prepared. The experimental procedure was as follows:

10.0 mL Cu(II) + 30.0 mL ASA + 30.0 mL Tryp
+ ...mL NaOH (pH adjustment) + ...mL dioxane

According to the procedure above, which was performed for 1:1:1 and 1:1:2 ratios, dioxane volumes were calculated, regarding NaOH volumes used for pH adjustment and final percentages of dioxane targeted. The pH was adjusted to 7.20, at which the conditional complex formation curve showed its maximum in the potentiometric study.

Each solution was shaken for 5 min after pH adjustment and addition of calculated dioxane volumes. Precipitates obtained from 43-50 % (v/v) dioxane-including aqueous mixtures were filtered under vacuum and dried in vacuum dessicator for 3 days. Precipitates in KBr pellets were analyzed using FTIR spectrometry. The same procedure was applied for Cu(II)-Tryp and Cu(II)-$(ASA)_2$ complexes and the spectra of ternary and binary complexes were compared using the frequencies of the coordinating and ester sites of ASA, since ASA was the weaker ligand.

RESULTS AND DISCUSSION

In this study, the formation constants of binary and ternary complexes of Cu(II) with aspirin (ASA) and L-tryptophan (Tryp) ligands were obtained potentiometrically. The formation of the ternary complex was confirmed using UV spectrometry in aqueous solutions and IR spectrometry of the precipitates obtained in dioxane/ water solutions.

Potentiometric results: The protonation and dissociation constants of the ligands and the formation constants of the binary and ternary complexes were found with the help of Calvin-Bjerrum and Irving-Rossotti methods, as tabulated in Table-1 and compared with literature values[11,18]. The formation constants of the binary complex of Cu(II)-Tryp agreed with relevant literature values, while of the ternary complex reported for the first time in this work and Cu(II)-ASA (on which contradictory informations exist in literature) was also determined at 25 (± 1) ºC, I = 0.11 M ionic strength. The titration curves are given in Fig. 1.

The reason that the formation constants of Cu-$(ASA)_2$ differs from the values in two different publications[4,11] is that the experimental conditions in each literature is quite different from the reagent concentrations and conditions in the present study.

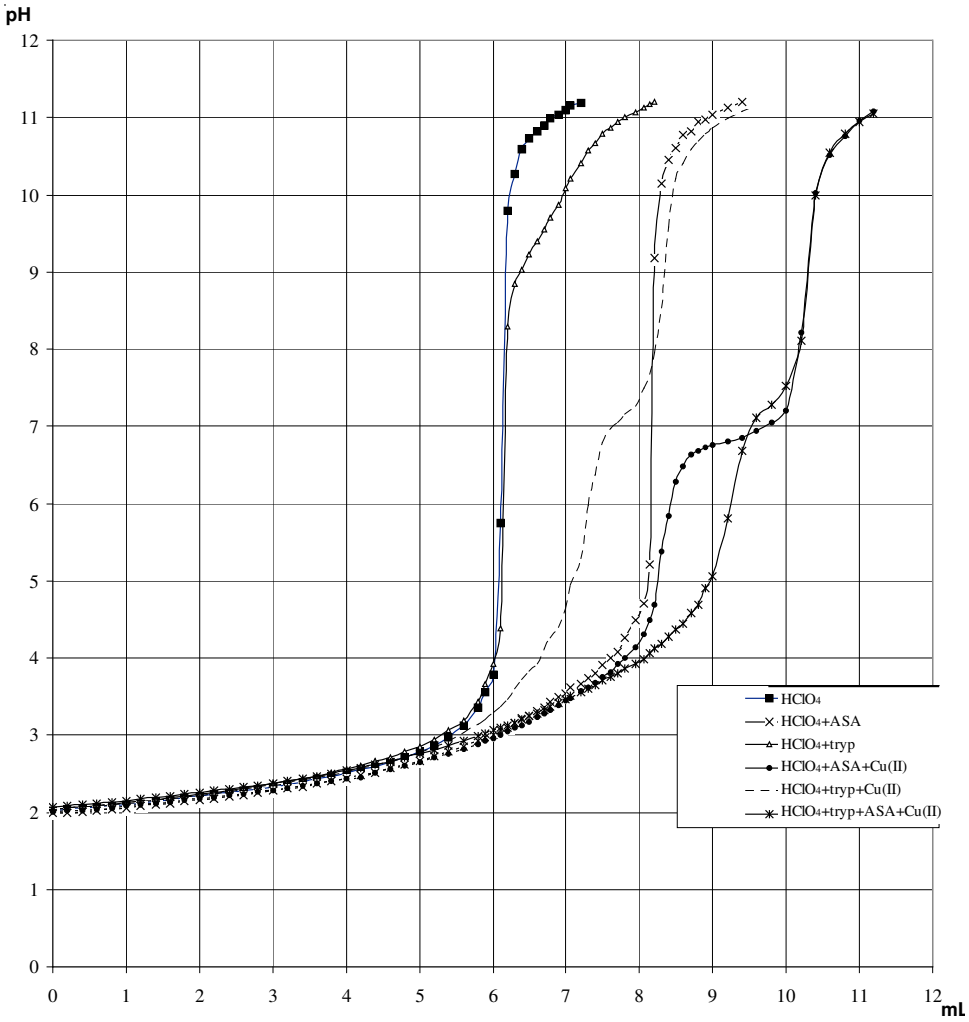

Fig. 1. Potentiometric titration curves of $HClO_4$, $HClO_4$ + ASA, $HClO_4$ + Tryp, $HClO_4$ + Cu(II) + Tryp, $HClO_4$ + Cu(II) + ASA and $HClO_4$ + Cu(II) + ASA + Tryp (I = 0.11; T = 25 (± 1) ºC)

While investigating the ternary complex, a basic assumption of the Irving-Rossotti data treatment protocol where metal-ligand binary system (*i.e.*, Cu(II)-Tryp, which has the greater formation constant) behaved as the metal ion and the secondary ligand (ASA) which binds more weakly to the existing metal-ligand binary system behaved as the ligand, was applied here as in previous potentiometric studies[19]. It should be noted that log K values of the ternary systems (found here) are the subsequent binding constants of ASA to the Cu(II)-Tryp system, which is different from the HYPERQUAD or MINIQUAD results. While drawing the speciation diagrams, since HySS program required the overall formation constants (log β_{pqrs}) which represents the sum of the logarithmic binding constants of both ligands to

the metal for ternary complexes, the values obtained from Irving-Rossotti method were adopted for use in HySS as log K of Cu(II)-Tryp (treated as metal) added up to log β (the binding constants of ASA to Cu(II)-Tryp system) and the summed value was used as log β_{pqrs} of Cu(II)-Tryp-$(ASA)_2$. Cu(II), Tryp and ASA were mixed in 1:1:1 and 1:1:2 molar ratios and titrated to investigate the formation of the ternary complex. Since Cu(II)-Tryp ratio was maintained as 1:1 in the mixture, the other coordination sites of Cu(II) could remain available for a second ligand (aspirin).

In the calculation of formation constants using the Irving-Rossotti method[16,17]; $\bar{n}_A$ values were found using V_1 and V_2 from the potentiometric titration curves of $HClO_4$ and ligand (eqn. 1). The protonation constant of the secondary ligand, aspirin was used in eqns. 2 and 3, in calculation of $\bar{n}_L$ and pL values of the ternary complex. log K values were calculated from the $\bar{n}_L$ = f(pL) graph, plotted using the $\bar{n}_L$ and pL values.

$$\bar{n}_A = y + \frac{(V_1 - V_2)(N + E^o)}{(V^o + V_1)T^o{}_L} \quad (1)$$

$$\bar{n}_L = \frac{(V_3 - V_2)\left\{N + E^o + T^o{}_L(y - \bar{n}_A)\right\}}{(V^o + V_2).\,\bar{n}_A T^o{}_M} \quad (2)$$

V^o = 50.0 mL, N = 0.1000 N, $T^o_L = 4.00 \times 10^{-3}$ M, $T^o_L = 2.00 \times 10^{-3}$ M, E^o = exact molarity of $HClO_4$, y = 1 (protons that can be released by the ligand)

$$pL = \text{Log}\frac{1 + \beta_1[H^+]}{T^o_L - \bar{n}_L T^o_M} \quad \beta_1 = 3.45 \text{ (for one of the repeated titrations of ASA)} \quad (3)$$

log K values of tryptophane which was used as the first ligand, log K values of aspirin (secondary ligand), the formation constant of Cu(II)-Tryp (1:1) and the formation constants of Cu(II)-$(ASA)_2$ (Table-1) were used to calculate the variation of conditional formation constant (eqn. 4) of the ternary complex as a function of pH, also comprising the formation of hydroxo complexes (plotted in Fig. 2). In the formation of ternary complexes, where formation of hydroxo complexes (hydrolysis behaviour) cannot be neglected at the studied pH range, the formation constants of the hydroxo complexes should also be used in α_M (eqn. 5) in the calculation of conditional formation constant.

$$K_{cond} = K_{Cu(II)-Tryp}.\alpha_L^n.\alpha_M \quad (4)$$

$$\alpha_M = \frac{1}{1 + K_{MY}[Y] + K_{MY}.K_{MY_2}[Y]^2 + \ldots + K_{MY}\ldots.K_{MY_n}[Y]^n + K_{MOH}[OH^-] + K_{M(OH)_2}[OH^-]^2 + \ldots + K_{MOH}\ldots.K_{M(OH)_n}[OH^-]^n} \quad (5)$$

The first formation constant of Cu(II)-Tryp complex (log K_1 = 8.42) was used in the equation, because one tryptophane and two aspirin molecule was forced to bind to Cu(II) in the ternary complex. During the titration of Cu(II):Tryp:ASA (1:1:2) ternary mixture, a light blue colour appeared at pH = 3.3-3.4 and precipitation started at pH ≈ 7.2. The binding constant of ASA (log β = log K_1 + log K_2) of 1:1:2

ternary complex found experimentally was in accordance with the calculated constant obtained from the conditional formation graph comprising the hydroxo species. Because of this, the complex formed was identified as Cu(II)-Tryp-$(ASA)_2$. Small discrepancies between the experimental results and calculated conditional formation constants (Table-1, Fig. 2) are thought to arise from some binary and/or hydroxo complexes coexisting with the ternary complex in solution (Fig. 2).

TABLE-1
PROTONATION CONSTANTS OF LIGANDS AND FORMATION CONSTANTS OF BINARY AND TERNARY COMPLEXES (T = 25 (±1) ºC and I = 0.11)

Ligand/Complex	Reference values	Experimental results
L-Tryp	$\log K_1 = 9.39 \rightarrow pKa_2$ $\log K_2 = 2.38 \rightarrow pKa_1$ 18]	$\log K_1 = 9.65 \pm 0.15 \rightarrow pKa_2$ (n = 10) $\log K_2 = 2.46 \pm 0.17 \rightarrow pKa_1$ (n = 10)
ASA	$\log K = 3.49 \rightarrow pKa$ [18]	$\log K = 3.41 \pm 0.16 \rightarrow pKa$ (n = 17)
Cu(II)–ASA (1:2)	$\log K_1 = 1.26$ $\log K_2 = 1.77$ $\log \beta_2 = 3.03$ [11]	$\log K_1 = 2.74 \pm 0.06$ $\log K_2 = 3.11 \pm 0.07$ $\log \beta_2 = 5.85 \pm 0.09$ (n = 3)
Cu(II)–Tryp (1:2)	$\log K_1 = 8.31$ $\log K_2 = 7.15$ $\log \beta_2 = 15.46$ [18]	$\log K_1 = 8.42 \pm 0.05$ $\log K_2 = 7.62 \pm 0.10$ $\log \beta_2 = 16.04 \pm 0.11$ (n = 3)
Cu(II)–Tryp-$(ASA)_2$ (1:1:2)	No reference	$\log K_1 = 2.80 \pm 0.05$ $\log K_2 = 3.17 \pm 0.03$ $\log \beta = 5.97 \pm 0.06$ (n = 3) $^*\log \beta_{pqrs} = 14.39 \pm 0.08$

$^*\log \beta_{pqrs} = \log K_{1(Cu(II)\text{-}Tryp)} + \log K_{1(Cu(II)\text{-}Tryp\text{-}ASA)} + \log K_{2(Cu(II)\text{-}Tryp\text{-}(ASA)2)}$

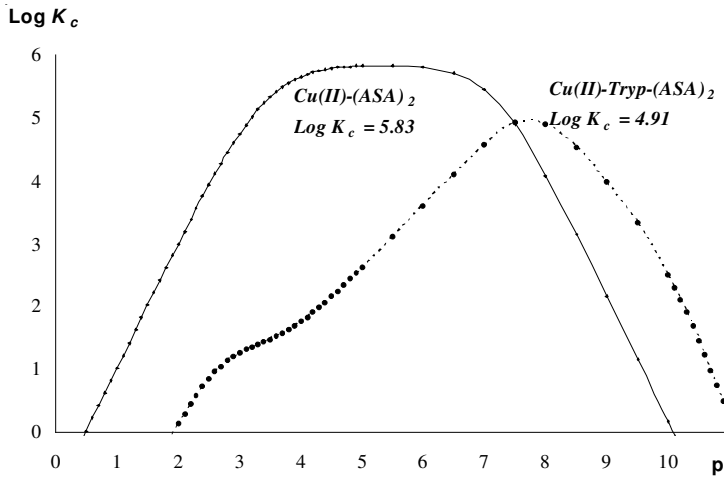

Fig. 2. Conditional formation curves of Cu(II)-Tryp-$(ASA)_2$ and Cu(II)-$(ASA)_2$ complexes (I = 0.11; T = 25 (± 1) ºC) and the binding constants of aspirin (ASA) to Cu(II) and Cu(II)-Tryp complex

For Cu(II)-$(ASA)_2$ and Cu(II)-Tryp-$(ASA)_2$ binary and ternary complex systems, respectively, the binding of the second ASA molecule had a somewhat greater stepwise stability constant than that of the first ASA molecule ($\log K_2 > \log K_1$, Table-1) as an indication of the weak binding of aspirin, which is a rare phenomena as seen in some other studies[11,16,18,20].

The species distribution diagrams (Fig. 3) were plotted using the program HySS[21], where the overall formation constant ($\log \beta_{pqrs}$ = log K of Cu(II)-Tryp (treated as metal) + log β (the binding constant of aspirin to Cu(II)-Tryp system)) was used.

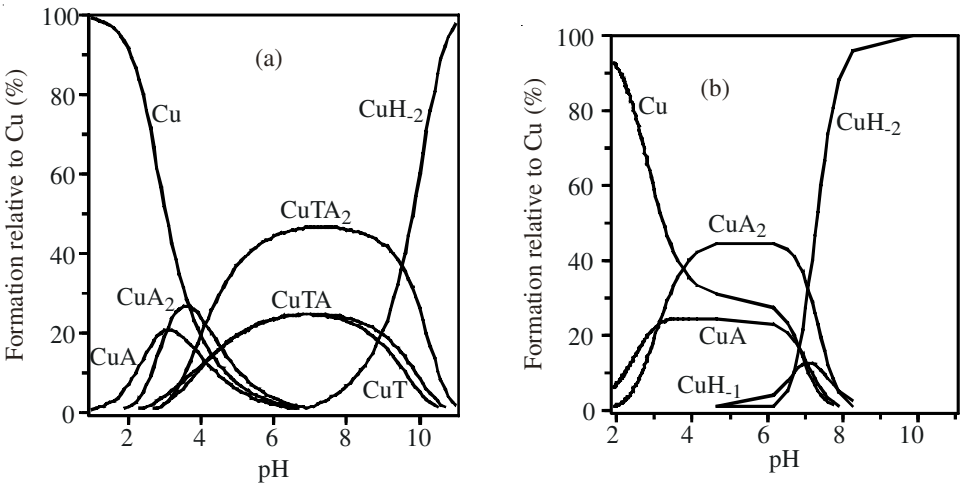

Fig. 3. Species distribution curves as a function of pH values for (a) Cu(II):ASA binary system at 1:2 molar ratio; (b) Cu(II):Tryp:ASA ternary system at 1:1:2 molar ratio; $c_{Cu(II)} = 2.00 \times 10^{-3}$ M, $c_{tryp} = 2.00 \times 10^{-3}$ M, $c_{ASA} = 4.00 \times 10^{-3}$ M (I = 0.11; T = 25 (± 1) ºC)

UV spectrometric results

The formation of the complexes were investigated qualitatively within the wavelength range of 190-320 nm at pH = 5.50, where none of the complexes started to precipitate. For both binary and ternary complexes, the significant deviations of the absorbances of Cu(II)-containing mixtures from the additive absorbances of ligands + metal or complex + ligand, confirmed the formation of binary and ternary complexes in aqueous solution. For each concentration studied, deviation of absorbances of binary and ternary mixtures from the sum of absorbances of individual constituents forming the mixture solutions pointed out to a significant interaction best observed at 210 nm (Fig. 4). The interaction in 1:1:2 mixture was stronger than that in 1:1:1 mixture. In Fig. 4, the spectra obtained for the investigation of formation of 1:1:2 complex are given. Even when Cu(II) and ligand concentrations in the mixtures were diluted to relatively low concentrations (for example: 8.00×10^{-5} M for Cu(II)

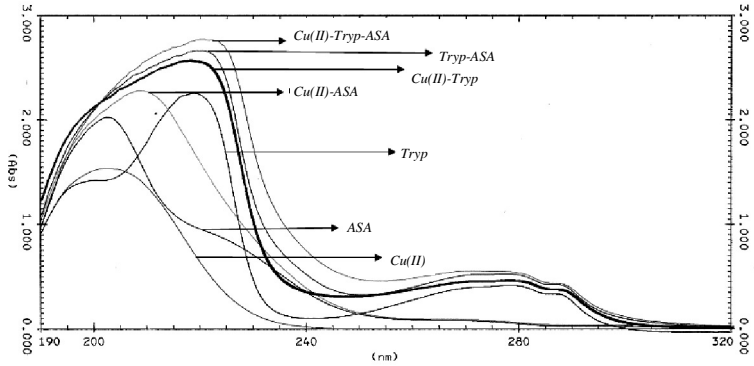

Fig. 4. Spectra pertaining to the comparative investigation of formation of Cu(II)-Tryp-ASA (1:1:2) complex (pH = 5.50). The interaction was best observed at 210 nm, at 25 ºC. Conc: Cu(II) = 8.00×10^{-5} M ; Tryp = 8.00×10^{-5} M; ASA = 1.60×10^{-4} M

and tryptophane, 1.60×10^{-4} M for aspirin in 1:1:2 mixture), interaction was still significant. In the investigation of Cu(II)-ASA 1:2 mixture, a clear shift in maximum wavelength was observed. The additivity of absorbance values of different solutions were investigated in comparison with the absorbance due to the ternary mixture as given in Table-2. Besides the interaction pertaining to Cu(II)-Tryp-ASA ternary complex formation, the deviation of ASA-Tryp mixture from additivity may also be an evidence for a ligand-ligand interaction inside the ternary complex.

TABLE-2
INVESTIGATION OF THE DEVIATIONS FROM ADDITIVITY OF ABSORBANCES OF THE SOLUTIONS AND MIXTURES, IN THE UV SPECTROMETRIC INVESTIGATION OF TERNARY COMPLEX FORMATION, AT 210 nm. THE MIXTURES ARE DENOTED BOLD AND IN BRACKETS

Compared mixtures and solutions	Total absorbance*	Deviation from additivity*
[Cu(II) - Tryp -ASA]	2.573	-2.049
Cu(II) + ASA + Tryp	4.622	
[Cu(II) - Tryp -ASA]	2.573	-1.479
[Cu(II) - ASA] + Tryp	4.052	
[Cu(II) - Tryp -ASA]	2.573	-1.331
[Cu(II) - Tryp] + ASA	3.904	
[Cu(II) - Tryp -ASA]	2.573	-1.283
Cu(II) + **[ASA-Tryp]**	3.856	
[ASA-Tryp]	2.488	-0.766
ASA + Tryp	3.254	

*pH = 5.50, t = 25 ºC.

IR spectrometric results

After confirmation of the formation of the ternary complex in aqueous solution, the complexes were precipitated *via* increasing ion association by decreasing the dielectric constant of the aqueous solution with dioxane. The comparison of the

IR-spectra of precipitates obtained from Cu(II):Tryp:ASA 1:1:1 and 1:1:2 ternary mixtures (in 45 and 44 % dioxane/water (v/v) solution at pH = 7.20) with those of the precipitates of Cu(II)-Tryp (Fig. 5) and Cu(II)-ASA binary complexes showed that aspirin peaks contributed to the IR spectrum of ternary complexes. The appearance of ester peaks of aspirin was observed more clearly in the IR spectrum of the precipitate from 1:1:2 mixture (Fig. 6). However, since the formation constant of the ternary complex was relatively low, a significant amount of Cu(II)-Tryp binary complex may have also existed in the precipitates. The IR results pertaining to ternary complex were evaluated and interpreted according to literature[22-24], in comparison with the spectra of binary complexes of Cu(II) with Tryp and ASA.

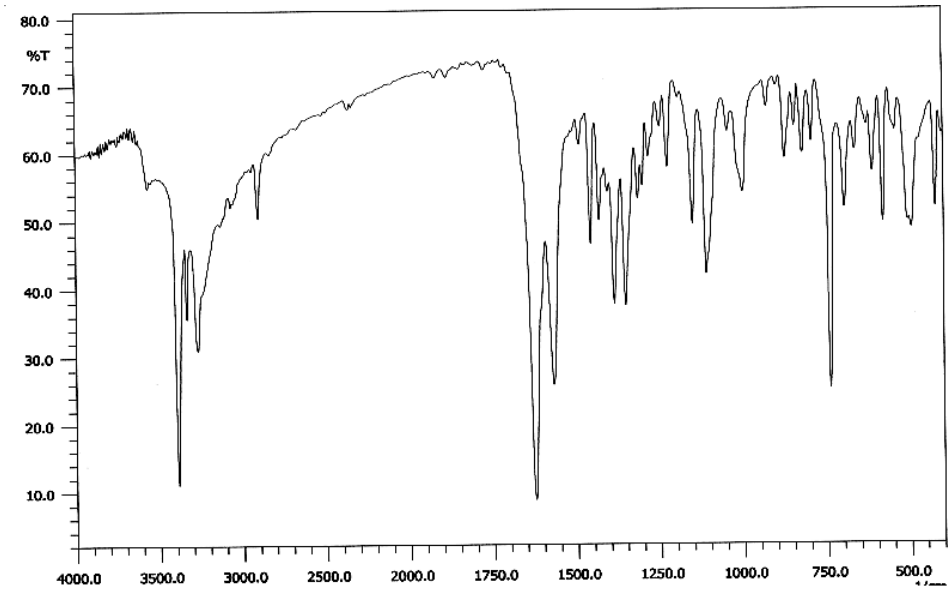

Fig. 5. IR spectrum of Cu(II)-Tryp (1:1) complex, precipitated at pH = 7.20

All precipitates investigated by IR spectrometry were dissolved in aqueous HCl and presence of Cu(II) was confirmed through qualitative systematic analysis. Reactions specific to Cu(II) identification were observed clearly with distinct colours in each step.

Aspirin dissolves freely in dioxane. It was also confirmed that the peaks of aspirin do not arise because of the precipitation of free aspirin, through performing the same experimental procedure for IR, this time by only using aspirin, as a blank reaction: No precipitate was observed. Since the formation constant of Cu(II)-Tryp (1:1) was greater than that of Cu(II)-ASA (1:1); Cu(II) prefers tryptophan at pH = 7.50, where the formation of ternary complex is maximum and the aspirin ligand binds to Cu(II)-Tryp as a secondary ligand at the preset composition of the mixture solution. This phenomena and the potentiometric titration results clearly showed that the

precipitate could not be Cu(II)-ASA complex either. In the light of the related literature[22-25], the band assignments of the observed IR peaks of Cu(II)-Tryp-ASA (1:1:2) complex (obtained in 44 % dioxane-containing aqueous mixture) are given below (Fig. 6).

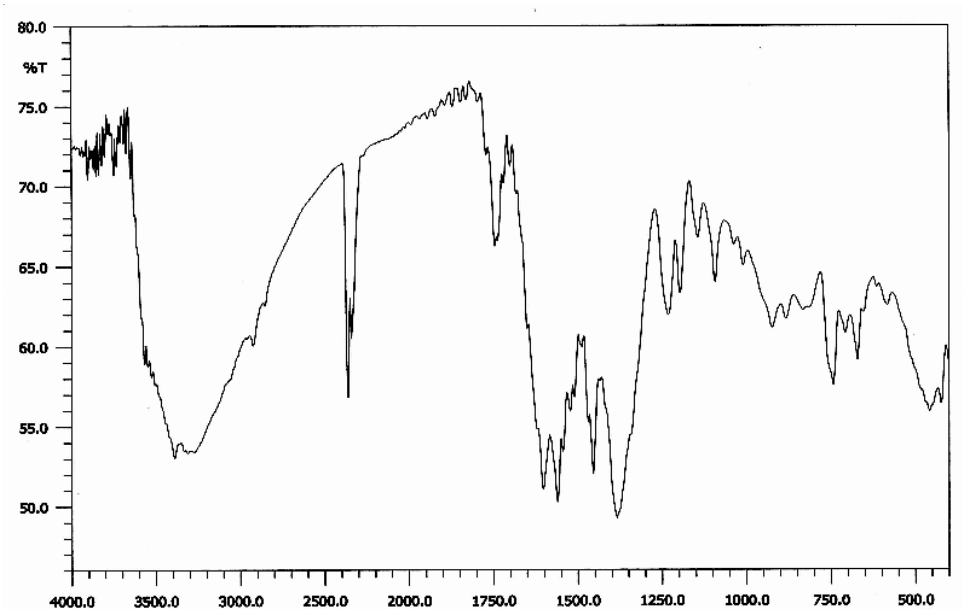

Fig. 6. IR spectrum of the precipitate of Cu(II):Tryp:ASA (1:1:2) mixture (precipitated at pH = 7.20)

Strong -NH stretching peak of indole nitrogen (Tryp) at 3388 cm^{-1} has shifted to lower frequency (3470-3450). The characteristic amino acid band at 2075 cm^{-1} (2200-2000 cm^{-1}), which is a combination of NH_3^+ deformation around 1600 cm^{-1} and NH_3^+ torsion around 500 cm^{-1}, has disappeared because of complex formation (binding on nitrogen of Tryp).

In ester complexes, ν(C=O) shifts to lower frequencies by complex formation. When these shifts are dependent on the metal ions, shifts follow the well known Irving-Williams order: Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II). So the maximum shifts appear with Cu(II)[26]. Although the ester band seems to be shifted mostly to lower frequencies and contribute to the bands around 1618-1600, there still seems to exist some uncoordinated ester having a C=O stretching band at 1745 cm^{-1} (1765-1720 cm^{-1}) showing the incorporation of aspirin ligand more obviously.

A new wide and intense peak appearing in the frequency range between 1352 and 1387 cm^{-1} was thought to arise from a -COO- stretching band (1440-1350 cm^{-1}) showing that aspirin binds to Cu(II) through its oxygen atom. Appearance of a wide

shoulder around 1220 cm^{-1} pointed to the fact that a peak related to tryptophan at 1230 cm^{-1} and >C-O stretching band related to phenolic ester at 1220 cm^{-1} were combined (1220-1190). C-O-C antisymmetric stretching of ester (1280-1150) at 1197 cm^{-1} in the spectrum of the ternary mixture did not exist in the spectrum of Cu(II)-Tryp complex. The -CH out-of-plane deformation related to *o*-disubstituted benzene and 5 membered ring at 740 cm^{-1} could be attributed to a combination with the *o*-disubstituted benzene peak that appeared in the same region. This peak was widened and a shoulder appeared at this frequency. The two peaks have presumably overlapped. Finally, M-O band was observed at 420 cm^{-1} frequency region[27].

Conclusion

When potentiometric and UV spectrophotometric data in aqueous solution and IR data of precipitates from aqueous dioxane mixtures were evaluated together, it was seen that Cu(II)-Tryp-$(ASA)_2$ complex formed at the preset suitable ratios of constituents. The results suggest that; when Tryp and Cu(II) are present at 1:1 molar ratio in the ternary mixture, tryptophane preferentially binds as a bidentate ligand to Cu(II) and 2 molecules of aspirin can bind to the same copper center as monodentate and/or bidentate ligands, according to the experimental conditions.

The extent of complex formation was less in aqueous media and at lower pH, but more at higher pH in an aqueous dioxane solution of decreased dielectric constant. Therefore, pH, solution polarity and formation constants of the complex will be important parameters when future synthesis of this potentially therapeutic complex is considered. The stability constants of Cu(II)-Tryp-ASA ternary complex were reported for the first time in this work and this finding will be important for further studies on this therapeutically important ternary complex, both from the point of synthesis and commenting on its pharmacokinetics. Since the potentiometric method used actually displays the binding constant of aspirin to the binary Cu(II)-Tryp complex, this study is important from the point of showing that this ternary complex can favourably form.

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