Sarkar [1] discussed the therapeutic problems for using chelating agents in treatment of Wilson’s diseases due to Cu(II) toxicity. In order to design a drug from small organic ligands for inactivate of a metalloenzyme, a widespread studies on matrix metalloproteinases have been established [2,3]. Because the enzymes containing zinc lead to various diseases like cancer and arthritis, many drug improvement approaches interested in an inhibition of zinc active site. Definitely, the variety of structures and catalytic characters of enzymatic zinc, make it an attractive objective [4,5]. Albert [6] revealed that there is a drugs whose role is to kill cells act as chemotherapeutic agents and drugs whose achievement must be basically reversible and/or short-lived acting by a pharmacodynamic mechanism. The differences between these two drug types in addition to the recent and future uses must be study and covered [6]. Various studies revealed the great consideration for importance role of the metal complex formation using drugs and ligands in the therapeutic and biological field in the last decade [7-11]. Literature survey demonstrations that in order to recognize the suitable dose of drugs, drug effects on all blood stream components and to determine the metal ligand bonds strength, a stability constant of metal-drugs complexes must be estimated [12]. The drugs complexes has advanced value than parent drugs [13]. With the purpose of interpreting the action mechanism of drugs, the studies of metal ion-drugs complex equilibria is essential [14]. In this work, a potentiometric pH titration results were used to determine the stability constant of both ternary and binary complexes, Cu(II)-oxprenolol (as primary ligands) and Cu(II)-DNA constituents (as secondary ligands), at 25 °C and 0.1 M of NaClO₄, respectively. Furthermore, species distribution in solution within a wide range of pH was also assessed. The chemical structure of oxprenolol HCl (Oxp) was depicted in Fig. 1.

The oxprenolol-Cu(I) complexes have been used as DNA constituents (L) and antihypertensive drug. In this study, the formation constants of these complexes, which formed in H₂O/MeOH (80:20) solution and their concentration distributions were assessed at ionic strength of 0.1 mol/L NaClO₄ at 25 °C. Stability constants and the values of Δ log K for the targeted complexes have also been evaluated depending on pH meter.

**Keywords:** Oxprenolol HCl, Mixed-ligand, Stability constants, Potentiometric titration.

**EXPERIMENTAL**

All the reagents used were of AR grade. Oxprenolol HCl was purchased by Sigma Chem. Company. DNA unit components investigated are inosine, thymine, uracil, adenine, uridine and thymidine. These materials were provided by BDH-Biochemicals Ltd. EDTA titrations was used for standardization of Cu(ClO₄)₂ stock solution [15]. A prepared solution of sodium hydroxide (carbonate free) was standardized with potassium hydrogen phthalate solution. An adjusted ionic
strength of 0.1 mol/L of all solutions was attained by addition of NaClO₄.

A Griffin pH J-300-010 G Digital pH meter was used for potentiometric titrations in a double-walled glass vessel. Before the pH measurements started, a calibrated electrode was prepared using buffer solutions at pHs (4 and 10) and the temperature was fixed at 25 ± 0.1 °C by flowing water through the jacket. In order to measure the pH of the free [H⁺], the pH results were corrected by titrating an identified concentration of standard HCl solution with standard NaOH solution using the same experimental conditions. The pKₐ was evaluated using methanol-water (20 %) solutions at ionic strength equal 0.1 mol/L to be 13.78 (0.05). A magnetic stirrer was used for solution stirring and all titrations were achieved in triplicate. For studying both of the formation constant of possibly binary or ternary complexes formed and the acid dissociation constants of the ligands, three mixture solutions were prepared and titrated potentiometrically with 0.05 mol/L NaOH solution.

(A) 40 mL of a solution containing 0.005 mol/L ligands and 0.1 mol/L NaClO₄.

(B) 40 mL of a solution containing 0.005 mol/L Cu(II) solution, 0.01 mol/L oxprenolol HCl or L and 0.1 mol/L NaClO₄.

(C) 40 mL of a solution containing 0.005 mol/L Cu(II) solution, 0.005 mol/L (oxprenolol HCl), 0.005 mol/L (L) and 0.1 M mol/L NaClO₄.

Titration of mixture (A) gave the protonation constants of oxprenolol HCl (or L). While the formation constants of Cu(II)-Oxp and Cu(II)-L complexes were determined by titration of mixture (B) and titration of mixture (C) provided the stability constants of the mixed-ligand complexes. Before titrations started, all titrated solutions were fully protonated by adding of HClO₄ solution. By studying of several possible conformation simulations for the system, the stability and stoichiometric constants of the complex species formed in aqueous solution were evaluated. Both of the complex formation and dissociation constants were measured using the computer program HYPERQUAD [16]. Whereas the computer program HYSS was used to determine the speciation as a function of pH [17].

TABLE-1

<table>
<thead>
<tr>
<th>System</th>
<th>l</th>
<th>p</th>
<th>q</th>
<th>r⁻</th>
<th>log β⁻</th>
<th>Δ log</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(H₂O)₂⁺</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>-2</td>
<td>-7.44 (0.03)</td>
<td>15.09 (0.02)</td>
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<tr>
<td>Oxprenolol HCl</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>10.36 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>-2.55 (0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>-2</td>
<td>9.64 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uricil</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>8.89 (0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4.63 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymine</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>8.89 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4.66 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymidine</td>
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<td>1</td>
<td>1</td>
<td>9.04 (0.02)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5.73 (0.01)</td>
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<tr>
<td>Inosine</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>8.32 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>9.34 (0.01)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11.56 (0.03)</td>
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<td></td>
</tr>
<tr>
<td>Adenine</td>
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<td>1</td>
<td>1</td>
<td>9.38 (0.01)</td>
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<td></td>
</tr>
<tr>
<td></td>
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<td>1</td>
<td>2</td>
<td>13.78 (0.02)</td>
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<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5.54 (0.01)</td>
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</tr>
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</table>

*The values l, p and q are the stoichiometric coefficients corresponding to Cu(II), oxprenolol HCl or DNA units and H⁺, respectively. Standard deviations are given in parentheses.

The titration procedure was performed for oxprenolol HCl alone and for oxprenolol HCl in presence of Cu(II). The titration results reveal that the Cu(II)-Oxp complex curve is dropped than that of the free oxprenolol HCl curve. This means that Cu(II) complex formation was carried out by displacement in methanol is higher than in water solutions. The calculated acid dissociation constant (pKₐ) values of the investigated oxprenolol HCl ligand are given in Table-1. The oxprenolol HCl ligand can form a negatively charged anion since it has at least one site that can reversibly dissociate a proton, so oxprenolol HCl can release one proton from amine group according following deprotonation equilibria:

\[ H(Oxp) \rightleftharpoons H^+ + Oxp^- \] (1)

The pKₐ value of protonated oxprenolol HCl equal of 9.15, this means that it is mainly present in the ionized form at a physiologic pH.

Copper(II) complex formation constants of oxprenolol HCl: The titration procedure was performed for oxprenolol HCl alone and for oxprenolol HCl in presence of Cu(II). The titration results reveal that the Cu(II)-Oxp complex curve is dropped than that of the free oxprenolol HCl curve. This means that Cu(II) complex formation was carried out by displacement
of protons. In order to determine the formation constant, the potentiometric results was fitted based on promising composition simulations. The Cu(Oxp) and Cu(Oxp)$_2$ complexes were found to be the model with superlative statistical acceptable and oxprenolol HCl acts as a bidentate fashion over the amino nitrogen and hydroxyl oxygen. The concentration distribution for Cu(Oxp) complex system is given in Fig. 2. The Cu(Oxp) species concentration increases as pH increase, attaining a maximum concentration of 92.7% at pH (4.8). For more increase in pH values, the Cu(Oxp) species concentration need to be decreased, while the Cu(Oxp)$_2$ species concentration increased.

Conclusion

In this study, the formation equilibria of Cu(II), (oxprenolol HCl) and DNA constituents has been estimated. The results reveal that the complexes are formed by simultaneous mechanism. Consequently, the (Cu(II)-Oxp) and (Cu(II)-DNA) complexes, which can be calculated by eqn. 4:

$$\Delta \log K = \log \beta_{\text{CuOxp}} - \left( \log \beta_{\text{CuOxpL}} + \log \beta_{\text{CuL}} \right)$$

Because of the higher stability of the binary complexes, the value of the $\Delta \log K$ is negative and this lead to reduce in steric hindrance, number of coordination sites, difference in bond type, geometrical structure and electronic consideration [22,23].

Studies of species distribution: Species distribution curves have been plotted as a function of pH using HYSS program at $I = 0.10$ M NaClO$_4$ and $T = 25$ °C in order to clarify the equilibria nature and to estimate the stability constant calculated of Cu(II)-Oxp-DNA constituents (L) ternary complexes [17].

As pH values increase, the ternary complexes concentration increase in all of the species distributions. The complex reached to the maximum concentration (about 30%) at a pH about 9 is the ternary complexes formed with DNA constituents. The results revealed that the complexes of Cu(II) (oxprenolol HCl) and DNA constituents has been estimated. The results reveal that the complexes are formed by simultaneous mechanism. Consequently, the (Cu(II)-Oxp) and (Cu(II)-DNA) complexes, which can be calculated by eqn. 4:

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For indication of the relative stability for the ternary complexes formed by simultaneous mechanism, the $\Delta \Delta \log K$ values are used, comparing to corresponding binary complexes, which can be calculated by eqn. 4:

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As pH values increase, the ternary complexes concentration increase in all of the species distributions. The complex reached to the maximum concentration (about 30%) at a pH about 9 is the ternary complexes formed with DNA constituents.
unites) complexes formation constants have been found to be of the same demand. Both of the concentration distribution and the stability constants of complexes in solution have been evaluated. Studying the pH-ranges of different systems, obviously show that the ternary complexes concentration increases as pH value increase. Reducing the steric hindrance electronic and the number of coordination sites can be reached by the negative value of $\Delta \log K$ due to the higher stability of its binary complexes.

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