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A Cyclic Voltammetric Studies of Complexation of Copper (II) with Thymine Using Glassy Carbon Electrode in Aqueous Medium

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Abstract

Voltammetric behaviors of copper (II) complex with nitrogen bearing nucleobase, thymine ($C_5H_6N_2O_2$), was studied using cyclic voltammetry (CV) on a glassy carbon electrode. Assessment of the chemical and physical conditions that may favor optimum current enhancement was done by studying the effect of variation of concentration of metal and ligand ions, scan rate, step height, pH values and supporting electrolyte as $(NH_4)_2SO_4$, KCl and NaCl. It was observed that copper and thymine forms a 1:1 ratio complex and increasing the concentration of either metal ion or ligand ion increases the corresponding current. Increasing the scan rate increases the corresponding current linearly with the square root of the scan rate. As the step height decreases the peaks become sharper. Anodic and cathodic current increases linearly with decreasing step height. The highest degree of complexation was observed at pH ranging from 2.3 to 7.0. However, poor complexation was observed in slightly alkaline medium. The complexing order trend of supporting electrolytes was observed as $(NH_4)_2SO_4$>NaCl>KCl.

Keywords: Complexation; Nucleobase; Voltammetry; Electrolyte; Ligand.

Introduction

The complexation of organic compounds with selected metal ions has a wide variety of applications in medicinal chemistry, surface chemistry, and analytical chemistry. Complexation of medicinal substances with ions influence the bioavailability of drugs in the body and the biological action affects the stability of medicinal compounds since a large number of metals are taken into the body system either with drugs or in the form of diet. The complex formation has been suggested as one of the important mechanisms for certain drug action [1]. The metal chelating phenomena are used to reduce the toxic effect of drugs in human physiology.

Toxic metals are generally more important than abundant metals in terms of environmental pollution because of their adverse effects on living organisms. The study of trace metals toxicity on biological system [2, 3] indicates that an under supply would not yield steady growth and over supply above the threshold level would generate toxicity with lethality at the end. Approximately twenty metals are known to be toxic and half of these including As, Cd, Cr, Cu, Pb, Ni, Ag, Se, Mn and Zn are released into the environment in sufficient quantities to pose a risk to human health [4 - 6].

Copper (II) ions (Cu^{2+}) are soluble in water, where they function at low concentration as bacteriostatic substances, fungicides, and wood preservatives. In sufficient amounts, copper salts can be poisonous to higher organisms as well.

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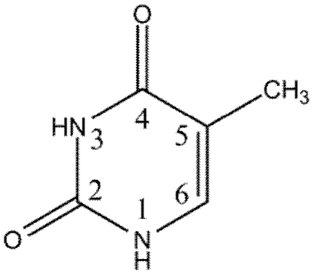
However, despite of universal toxicity at high concentrations, the Cu^{2+} ions at lower concentrations are an essential trace nutrient to all higher plant and animal life. In animals, including humans, it is found widely in tissues, liver, muscle, and bone. It functions as a co-factor in various enzymes and in copper-based pigments [7].

Nucleobases are nitrogen-containing biological compounds found DNA, RNA, nucleotides, and nucleosides. The primary nucleobases are cytosine, guanine, adenine (DNA and RNA), thymine (DNA) and uracil (RNA), abbreviated as C, G, A, T, and U, respectively (Scheme 1) is the structure of thymine.



Scheme 1: Structure of thymine

In DNA, thymine binds to adenine via two hydrogen bonds, thus stabilizing the nucleic acid structures. Metals in the ionic forms have essential catalytic physiological functions to perform in living systems, including human [8]. Excess metal ions in human system can damage specific organs. Free metal ions are more toxic than metal chelates. Thus, the chelating agents are used in medicine for the formation of soluble, easily extractable metal chelates by sequestering metal ions in the circulation of blood. In the present research thymine have been used to form complex with the free metal ion and to study how it can be made nontoxic in the body. Since chelation is the elimination of all the binding sites in the metal ion and as such chemical bonds to essential enzymes cannot be formed, lowering of toxicity by chelation of metal ions is ensured [9, 10].

Thymine is a critical component of DNA, has extra duties to perform in the cell, including defying the characteristics of the living body. In other words, it functions as character determiner. Interestingly, guanine functions by binding to phosphate group and form guanosine and takes part in cell mechanism with thymine. This later involves numerous enzymatic reactions necessary for sustaining life and protecting the cell from toxins, produced by our cells or carcinogenic in our environment [11, 12].

Thus research on synthesis and properties of various metal-amino acid complexes is important from the view point of chemistry, bio-chemistry and medical science. Attempts have been made in the present work to synthesize copper complex of thymine in order to gain greater insight in the metal interaction with thymine, which could help to understand the role of metal ions and nucleobases in the biological process. Limited research has been carried out on electrochemical behavior of metal ligand complex. In this present work we are interested in investigating the electrochemical behavior of this complex and its influence in our body system. Hence, the voltammetric behavior of thymine at glassy carbon electrode (GCE) electrode and its complexation with Cu has been investigated in aqueous medium.

Experimental

Materials

Analytical grade $CuSO_4.5H_2O$ was purchased from E. Merck, Germany. Thymine (99%) and hydrochloric acid (37%), were purchased from Sigma-Aldrich, USA. Extra pure acetic acid and phosphoric acid (BDH, England) were used to prepare buffer solutions.

Measurements

$CuSO_4.5H_2O$ was dissolved in deionized water to prepare 0.5-2.0 mM solutions. Thymine solutions were prepared in slightly acidic medium (0.1 M HCl) upon warming. Milli-Q water was used throughout the experiment.

GCE (QIS, Model QVMF-2070, 7.5 cm length x 6 mm OD) was polished with fine alumina powder of 0.3 and then 0.03 micron sized on a wet polishing lather surface. For doing so a part of the lather surface was made wet with deionized water and alumina powder was sprinkled over it. The GCE was then polished on this surface for about 10-20 minutes by pressing softly the electrode against the polishing surface. A shiny black mirror like electrode surface was then thoroughly washed with deionized water. Under computer controlled magnetic stirring, experimental solution was deaerated by purging for at least 10 minutes with 99.99% pure nitrogen gas. Three electrodes system consists of a GCE as the working electrode, Ag/AgCl (satd. KCl) as the reference electrode and platinum wire as the counter electrode was used. Cyclic voltammetric measurement was performed using Computerized Electrochemical System, Model HQ-2040 supplied by Advanced Analytics, USA.

Results and Discussion

The redox behavior of Cu (II) in copper (II) sulphate was examined in 0.2 M KCl by cyclic voltammetry on GCE at room temperature.

From (Fig. 1) it is observed that Cu^{2+} solution is electroactive as it gives two cathodic and two anodic peaks. First anodic peak current is 8.0760 µA and potential 125 mV. Second anodic peak current is 31.7697 µA and potential -185 mV. First cathodic peak current is 7.55 µA and potential 55 mV. Second cathodic peak current is 12.60 µA and potential -425 mV. This implies that the Cu^{2+} system is a two electron reversible system [13, 14].

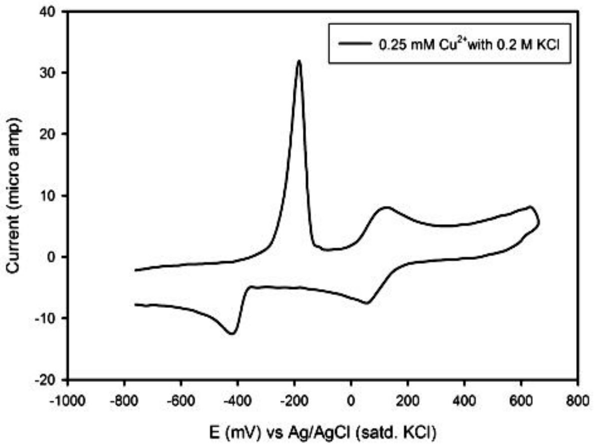


Figure 1. **Cyclic voltammogram of 0.5 mM Cu^{2+} in the presence of 0.2 M KCl at -800 mV to 650 mV potential window, 5 mV step height and 100 mV/s scan rate.**

Characterization of copper-thymine complex

Cyclic voltammogram of thymine in presence of 0.2 M KCl at -800 mV to 600 mV potential window, 20 mV step height and 100 mV/s scan rate, is shown in (Fig. 2).

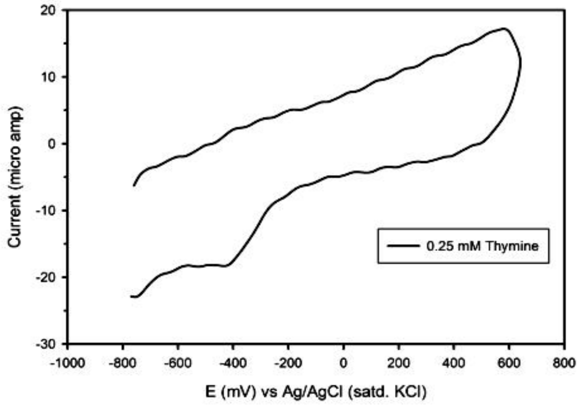


Figure 2. **Cyclic voltammogram of 0.25 mM thymine in the presence of 0.2 M KCl at -800 mV to 600 mV potential window, 20 mV step height and 100 mV/s scan rate.**

From (Fig. 2) it is observed there is no anodic peak or cathodic peak. It proves that thymine is an electro-inactive compound [15]. The complexation ratio of copper-thymine is 1:1, confirmed by Jobs continuous variation method, mole ratio method and Ardon's spectrophotometric method [16-18].

Proposed structure of the copper-thymine complex

In the (Fig. 3) a comparison of several cyclic voltammograms is depicted to understand the behavior of the system under investigation.

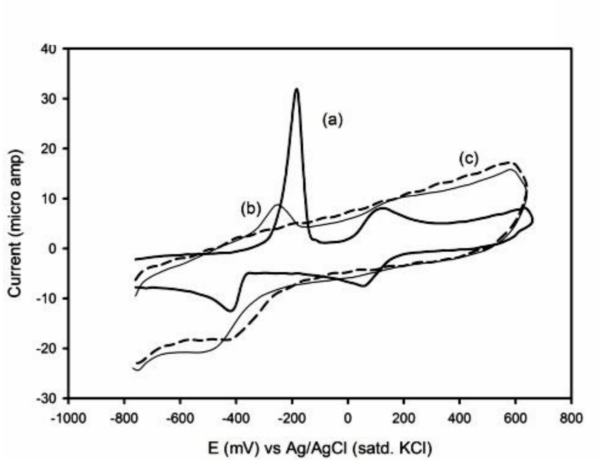
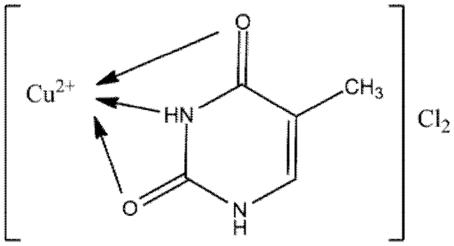


Figure 3. **Cyclic voltammograms of copper ion, copper – thymine complex and thymine in presence of 0.2 M KCl: (a) 0.25 mM Cu^{2+}, (b) 0.25 mM Cu^{2+} + 0.25 mM thymine and (c) 0.25 mM thymine.**

Fig. 2 illustrates that due to complex formation peak current of $^{\mathrm{Cu2+}}$ ion decreases while that of thymine increases.

The thymine complexes of several metal ions, such as Al(III), Co(II), Cu(II), Zn(II), Pt(II), and Pd(II) have been reported earlier [19,20]. It is found from the literature [21 - 23] that thymine binds with metal ions through carbonyl oxygen O(2) and O(4) and/or imidazolyl nitrogen, N(3). Early transition metal ions show a preference for the O(4) position, while later ones prefer N(3). However, the information available is very limited to understand the potential binding sites of thymine and the possible mechanism of interaction between thymine and the various metal ions.



$[Cu(C_5H_6N_2O_2)]Cl_2$

Scheme 2: Proposed structure of copper (II) and thymine complex

Attempts have been made to obtain the complex in solid form for further structural analysis but due to the instable nature of the complex as the individual components precipitate out with increasing pH it was not possible to accumulate more structural analysis data to confirm the structure of the complex.

However from our observations and literature review the above structure (Scheme 2) could be proposed as the structure of (Cu^{2+}) and thymine complex [21, 22].

Variation of Cu^{2+} concentration

Cyclic voltammogram of 0.25 mM thymine at -800 mV to 650 mV potential window and 20 mV step height and 100 mV/s scan rate in presence of 0.2 M KCl as supporting electrolyte and different Cu^{2+} ion concentrations (0.25 mM, 0.50 mM, 0.75 mM, 1.00 mM and 1.25 mM) reflects (Fig. 4) that as the concentration of the Cu^{2+} increases, the extant of reaction increases [24, 25], so that more electrons are transferred and both anodic and cathodic current increase. As the complex is formed the anodic and cathodic potential shifts their position to more positive direction.

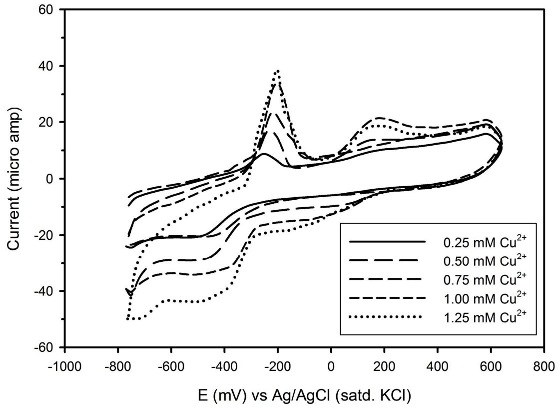


Figure 4. **Cyclic voltammogram of 0.25 mM thymine at -800 mV to 650 mV potential window and 20 mV step height and 100 mV/s scan rate in presence of 0.2 M KCl as supporting electrolyte and different Cu^{2+} ion concentrations (0.25 mM, 0.50 mM, 0.75 mM, 1.00 mM and 1.25 mM).**

Variation of thymine concentration

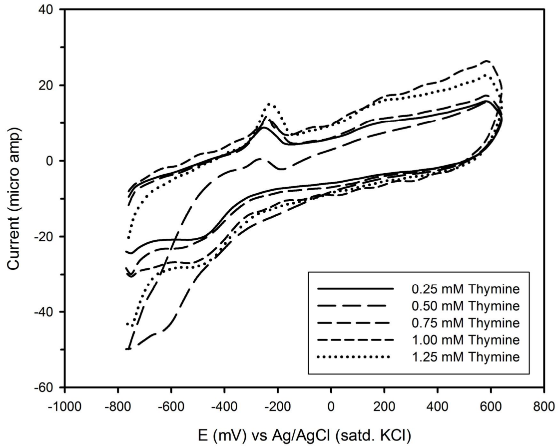


Figure 5. **Cyclic voltammogram of 0.25 mM Cu^{2+} at -800 mV to 650 mV potential window and 20 mV step height and 100 mV/s scan rate in presence of 0.2 M KCl as supporting electrolyte and different thymine concentrations (0.25 mM, 0.50 mM, 0.75 mM, 1.00 mM and 1.25 mM).**

Cyclic voltammogram of 0.25 mM Cu^{2+} at -800 mV to 650 mV potential window and 20 mV step height and 100 mV/s scan rate in presence of 0.2 M KCl as supporting electrolyte and different thymine concentrations (0.25 mM, 0.50 mM, 0.75

mM, 1.00 mM and 1.25 mM) reflects (Fig. 5) that as the concentration of thymine increases the more complex is formed [22], and more electrons are transferred so anodic and cathodic current increase. As the complex is formed the anodic and cathodic potential shifts their position to more negative direction. As thymine is an electro-inactive substance, the addition of thymine results in lower increase of current compared to the addition of Cu^{2+} to thymine.

Variation of scan rate

Cyclic voltammogram of 0.25 mM Cu^{2+} and 0.25 mM thymine at -800 mV to +650 mV potential window and 20 mV step height in presence of 0.2 M KCl as supporting electrolyte and different scan rates (25, 50, 75, 100, 125 and 150 mV/s) was taken.

The peak current [20] for both the anodic and cathodic peaks follows the equation:

$$i_p = 2.69\times10^5 .\ n^{3/2} .\ A .\ D^{1/2} .\ C .\ v^{1/2}$$

Where, i_p = peak current in ampere, n = number of exchanged electrons, A = area of the electrode in cm^2, D = diffusion coefficient in cm^2/s, C = concentration of the species in mol/cm^3, v = scan rate in volts/s.

Two plots of anodic peak current vs square root of the scan rate and cathodic peak current vs square root of the scan rate are shown in the (Fig. 6).

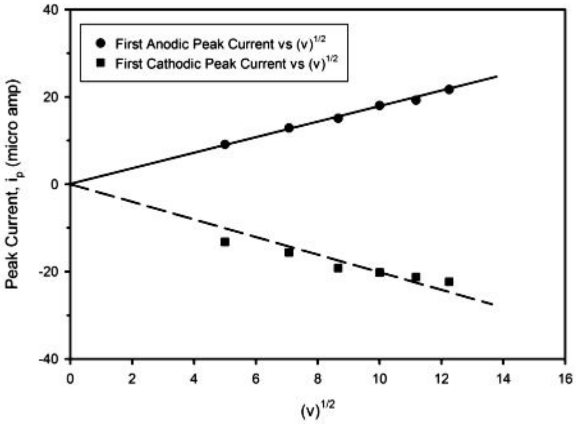


Figure 6. **Peak current vs square root of the scan rate for Cu^{2+} and thymine complex.**

The electrochemical processes are diffusion controlled which can be confirmed from the graph of i_p vs $v^{1/2}$ as shown in the (Fig. 6). From (Fig. 4) it can be seen that as the scan rate of the complex mixtures increases the corresponding current increases [26] but anodic and cathodic potential shift very little.

Variation of step height

Cyclic voltammogram of 0.25 mM Cu^{2+} and 0.25 mM thymine at -800 mV to +650 mV potential window at 100 mV/s scan rate at different step height (10,15, 20, 25, and 30 mV) was taken. It is observed as that the anodic and cathodic current decreases linearly with increasing step height.

Moreover in the lower step height the peak becomes sharper. This might happen as the interaction between the metal ions and ligand ions decreases with increasing step height and complex formation decreases and consequently anodic and cathodic current decreases with increasing step height.

Plotting peak current vs step height in (Fig. 7) shows a straight line with negative slope, which indicates that the increase in step height decreases the peak current.

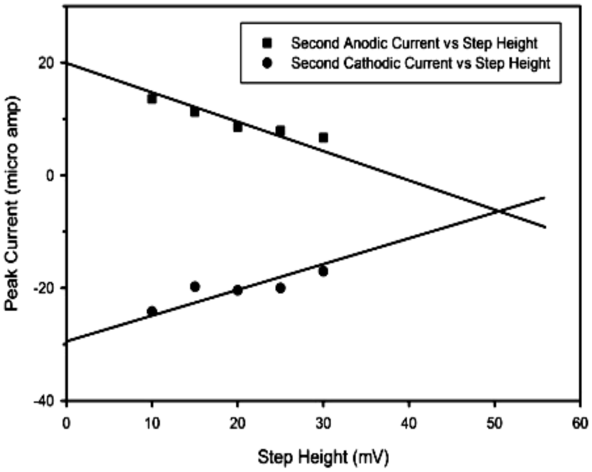


Figure 7. **Peak current vs step height for Cu^{2+} and thymine complex.**

Variation of pH

Cyclic voltammogram of 0.25 mM Cu^{2+} and 0.25 mM thymine at -800 mV to +650 mV potential window at 100 mV/s scan rate and 20 mV step height at different pH Values (2.3, 7.00 and 10.0) was taken to see the effect of pH on the complex.

At lower pH the H^+ ion can compete with Cu^{2+} ion for ligand in solution. But an increase in pH value will reduce the concentration of hydrogen ions which allows greater complex formation between Cu^{2+} and thymine. There was a little increase in the peak current (Fig. 8) of Cu^{2+} - thymine complex species below pH 7.0 [27]. From pH 7.0 onwards, the peak current increased sharply, the optimum pH range appears to be between pH 2.3 to 7.0.

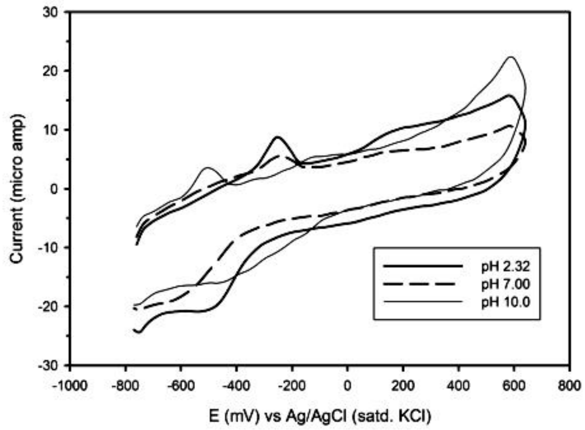


Figure 8. **Cyclic voltammogram of 0.25 mM Cu^{2+} and 0.25 mM thymine at -800 mV to +650 mV potential window at 100 mV/s scan rate and 20 mV step height at different pH values (2.3, 7.0 and 10.0).**

Variation of supporting electrolyte

Any species that competes with metal ion for the surface of the electrode would inevitably interfere in the adsorption of metal thyminate complex species hence leading to a diminishing (Fig. 9) in the increase of current effect [28]. This explains why the supporting electrolyte such as $(NH_4)_2SO_4$, which is weakly complexing produces higher current as compared to NaCl and KCl. More over in the case of NaCl and KCl both are chloride containing supporting electrolyte but NaCl is less complexing compared to KCl and thus produces lower current of the anodic and cathodic peak. So the order of the anodic and cathodic current enlargement is $(NH_4)_2SO_4 > NaCl > KCl$.

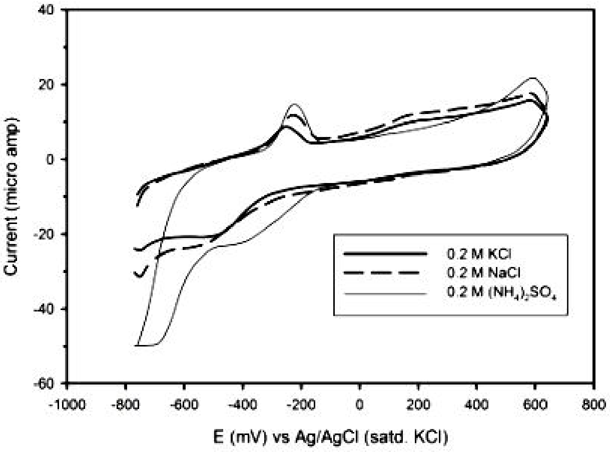


Figure 9. **Cyclic voltammogram of 0.25 mM Cu^{2+} and 0.25 mM thymine at -800 mV to +650 mV potential window at 100 mV/s scan rate and 20 mV step height in the presence of different supporting electrolytes (0.2 M KCl, 0.2 M NaCl and 0.2 M $(NH_4)_2SO_4$).**

Conclusion

The results showed that thymine is an electro inactive compound and Cu (II) is electro active and reduced in a two electron process. Cu (II) forms a 1:1 complex with thymine in aqueous medium. Probable structure of the complex of copper with thymine has been proposed, which is being supported by the previous works [21, 22]. The complex system showed an active response towards the variation of different parameters such as concentration, scan rate, step height, pH and supporting electrolytes. The complex formation is favored in acidic condition, as demonstrated from the variation of pH of the solutions. More interaction occurs when SO_4^{2-} containing supporting electrolyte $(NH_4)_2SO_4$ is used as compared to chloride containing supporting electrolyte i.e. KCl and NaCl. As Na is below K in the electrochemical series has higher tendency to be reduced compared to K, so less current is found for KCl than NaCl. So the complexation tendency of the supporting electrolytes follows the decreasing order $(NH_4)_2SO_4 > NaCl > KCl$.

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