

Research Article

A CV Study of Copper Complexation with Guanine Using Glassy Carbon Electrode in Aqueous Medium

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Received 22 February 2014; Accepted 18 March 2014; Published 2 April 2014

Academic Editors: H. Karimi-Maleh and B. Lakard

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Voltammetric behaviors of Copper (II) nitrogen bearing nucleobases, such as Guanine ($C_5H_4N_5O_2$) was studied in electro analyzer 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, variation of scan rate, variation of step height, variation of pH values, and variation of supporting electrolyte as $(NH_4)_2SO_4$, KCl, and NaCl. It was observed that Copper and Guanine forms a 1 : 2 ratio complex. The work reflects that 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 sharp. Anodic and cathodic current increases linearly with decreasing step height. For the complex mixture the complexation occurs maximum at a pH of 2.3–7.0 and is badly restricted in the slightly alkaline medium and the complexing order of the supporting electrolyte showed a trend as $(NH_4)_2SO_4 > NaCl > KCl$.

1. Introduction

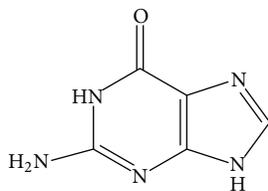
The complexation of organic compounds with selected metal ion has a wide variety of application in medicinal chemistry, surface chemistry, and analytical chemistry. Complexation of medicinal substances with ions influence the bioavailability of drugs in the body and the biological action that affects 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. The studies of redox behavior biologically and biochemically important compound are gaining importance because such redox phenomena are close to natural processes occurring in human and living organism.

Toxic metals are generally more important than abundant metals, in terms of environmental pollution, because of their 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 would not, above the threshold level, generate toxicity with lethality at the end. At least 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. However, despite universal toxicity at high concentrations, the Cu^{2+} ion at lower concentrations is an essential trace nutrient to all higher plant and animal life. In animals, including humans, it is found widely in tissues, with concentration in liver, muscle, and bone. It functions as a co-factor in various enzymes and in copper-based pigments.

Nucleobases are nitrogen-containing biological compounds found within DNA, RNA, nucleotides, and nucleosides. They are also termed as nitrogenous bases or simply



SCHEME 1: Chemical structure of guanine.

bases due to their ability to form base-pairs and to stack upon one another leading directly to the helical structure of DNA and RNA. The primary Nucleobases bases are cytosine, guanine (Scheme 1), adenine (DNA and RNA), thymine (DNA), and uracil (RNA), abbreviated as C, G, A, T, and U, respectively.

In DNA, guanine is paired with cytosine. With the formula $C_5H_5N_5O$, guanine is a derivative of purine, consisting of a fused pyrimidine-imidazole ring system with conjugated double bonds. Being unsaturated, the bicyclic molecule is planar. Guanine has the C-6 carbonyl group that acts as the hydrogen bond acceptor, while a group at N-1 and the amino group at C-2 act as the hydrogen bond donors.

Metals in the ionic forms have essential catalytic physiological functions to perform in living systems, including human [7]. 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 guanine have been used to form complex with the free metal ion and to study how it can be made non-toxic 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 [8, 9].

Guanine is a critical component of DNA; it has extra duties to perform in the cell, including defying the characteristics of the living body. In other words, they function 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, either that produced by our cells or carcinogenic in our environment.

Thus researches on synthesis and properties of various metal-amino acid complexes are 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 guanine in order to gain greater insight in the metal interaction with guanine, which could help to understand the role of metal ions and Nucleobases in the biological process. Not too much research work was carried out with metal-ligand complex in searching their electrochemical behavior.

Electrochemical techniques, based on electrochemical sensors, are the most versatile analytical tool to study the

redox behavior and to diagnose electro-active species in the field of chemistry, biochemistry, pharmacy, food science [10], and environmental science. Electrochemical sensors [11] are mostly based on the redox behavior of the analytes, for example, gas molecules, ions, and drugs. Sensitivity to a wide range of chemical species has made electrochemical sensor an essential tool for analysis and monitoring of pollutants, medicine, narcotics, neurotransmitters, and so forth.

In this present work we are interested in investigating the electrochemical behavior of those complexes and their influence in our body system. Hence, the study, the redox behavior of guanine at GCE electrode and its complexation with Cu, has been investigated in aqueous medium by using electrochemical methods. Cyclic voltammetric studies of copper (II) and its complex with guanine to reveal the redox behavior in aqueous environment. Thus a systematic approach with the mode of formation and structural relationship with properties of this new compound may be derived from such study.

2. Experimental

2.1. Materials. Analytical grade $CuSO_4 \cdot 5H_2O$ was purchased from E. Merck, Germany. Guanine ($C_5H_5N_5O$) was purchased from China. Extra pure acetic acid (BDH, England) and phosphoric acid (BDH, England) were procured for buffer.

2.2. Measurements. $CuSO_4 \cdot 5H_2O$ was dissolved in deionized water to prepare 0.5–2.0 mM solutions. Guanine solutions were prepared in slightly acidic medium (dil. HCl) upon warming. The sodium acetate buffer solution was prepared by taking 0.1 M CH_3COONa , and 0.1 M CH_3COOH of solutions at different ratios. The phosphate buffer solution was prepared by mixing 0.1 M di-sodium hydrogen phosphate, 0.1 M HCl, and 0.1 M NaOH solutions in different ratios. Milli-Q deionized water used throughout the experiment.

GCE 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 99.9977% pure nitrogen gas before performing every experiment. Traces of dissolved oxygen gas were thus removed from the solution. Thus interference due to molecular oxygen dissolved in the solutions was eliminated from the Cyclic Voltammograms.

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 developed by Advanced Analytics, USA.

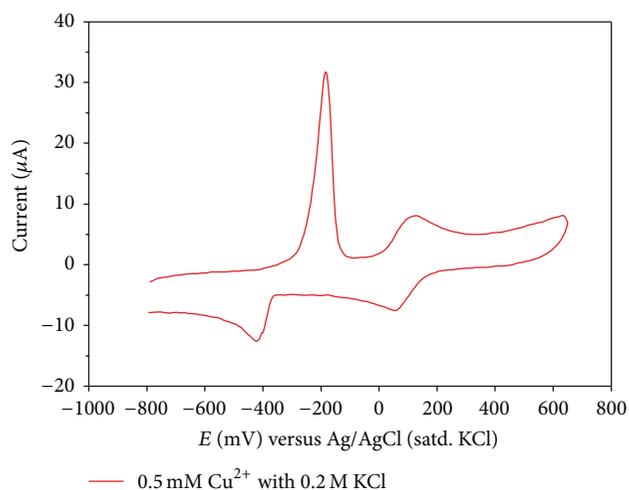


FIGURE 1: Cyclic Voltammogram of 0.5 mM Cu²⁺ in presence of 0.2 M KCl at -800 mV to 650 mV potential window, 5 mV step height and 100 mV s⁻¹ scan rate.

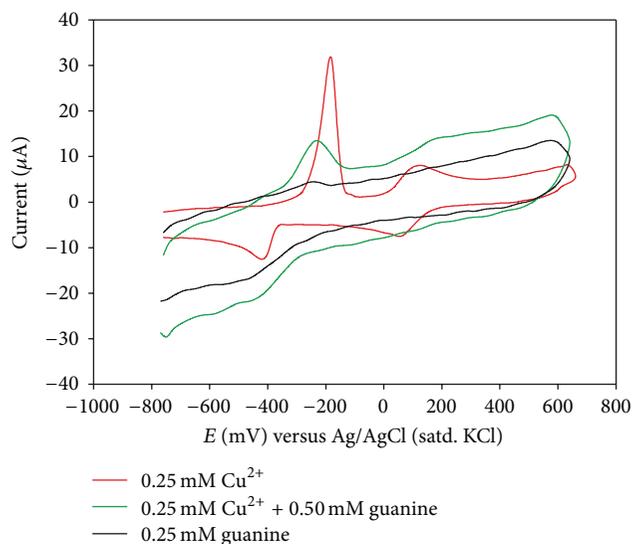


FIGURE 3: Cyclic Voltammograms of copper ion, copper-guanine complex and guanine in presence of 0.2 M KCl.

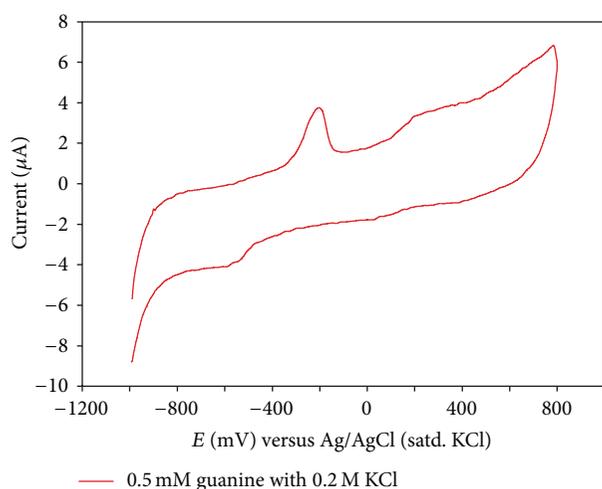
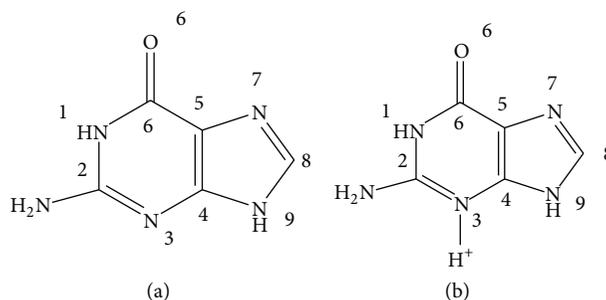


FIGURE 2: Cyclic Voltammogram of 0.5 mM guanine in presence of 0.2 M KCl at -1000 mV to 800 mV potential window, 5 mV step height and 100 mV s⁻¹ scan rate.

3. Results and Discussion

The redox behavior of Cu (II) in copper (II) sulphate was examined in 0.2 M KCl by Cyclic Voltammetric technique on glassy carbon electrode at room temperature.

From Figure 1 it is observed that Cu²⁺ solution is electroactive as it gives two cathodic and two anodic peaks. First anodic peak current is 8.0760 µA and potential is 125 mV. Second anodic peak current is 31.7697 µA and potential is -185 mV. First cathodic peak current is 7.55 µA and potential is 55 mV. Second cathodic peak current is 12.60 µA and potential is -425 mV. This implies that the Cu²⁺ system is a two electron reversible system.



SCHEME 2: Structure of guanine: (a) non-protonated, (b) protonated.

3.1. Characterization of Copper-Guanine Complex. Cyclic Voltammogram of 0.5 mM guanine, in presence of 0.2 M KCl at -1000 mV to 800 mV potential window, 5 mV step height and 100 mVs⁻¹ scan rate was taken.

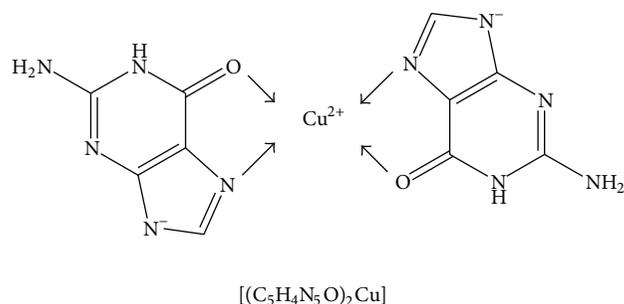
From Figure 2 it is observed that it has an anodic peak at -200 mV potential having peak current of 3.7511 µA. It proves that guanine is an electroactive compound and an irreversible system [12]. The complexation ratio of copper-guanine is 1 : 2, confirmed by Jobs Continuous Variation Method and Mole Ratio Method.

3.1.1. Proposed Structure of the Copper-Guanine Complex. In Figure 3 a comparison has been depicted to understand the behavior of changing peak current.

Figure 3 illustrates that due to complex formation, peak current of copper ion decreases while that of guanine increases.

In acidic medium, ring nitrogen N(3) of guanine is protonated (Scheme 2).

The guanine complexes of several metal ions, such as Al(III), Co(II), Cu(II), Zn(II), Pt(II), and Pd(II) have been reported earlier [13, 14]. It is found from the literature [15, 16]



SCHEME 3: Proposed structure of copper (II) and guanine complex.

that guanine binds with metal ions through carbonyl oxygen O(6) and/or imidazolyl nitrogen, N(7). Early transition metal ions show a preference for the O(6) position, while later ones prefer N(7). However, the information available in this regard is very limited to understand the potential binding sites of guanine and the possible mechanism of interaction between guanine and the various metal ions.

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 Scheme 3 could be proposed as the structure of copper (II) and guanine complex [17, 18].

3.1.2. Variation of Cu^{2+} Concentration. Cyclic Voltammogram of 0.25 mM guanine at -800 mV to 650 mV potential window and 20 mV step height and 100 $mV\ s^{-1}$ 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 that as the concentration of the Cu^{2+} increases, more reactions take place [19] and more electrons are transferred so anodic and cathodic currents increase. As the complex is formed the anodic and cathodic potential shifts their position to more positive direction.

3.1.3. Variation of Guanine Concentration. Cyclic Voltammogram of 0.25 mM Cu^{2+} at -800 mV to 650 mV potential window and 20 mV step height and 100 $mV\ s^{-1}$ scan rate in presence of 0.2 M KCl as supporting electrolyte and different guanine concentrations (0.25 mM, 0.50 mM, 0.75 mM, 1.00 mM, and 1.25 mM) reflects that as the concentration of guanine increases the more reactions take place [20] 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 guanine is a least electroactive substance, the addition of guanine increases less amount of current compared to the addition of Cu^{2+} in guanine.

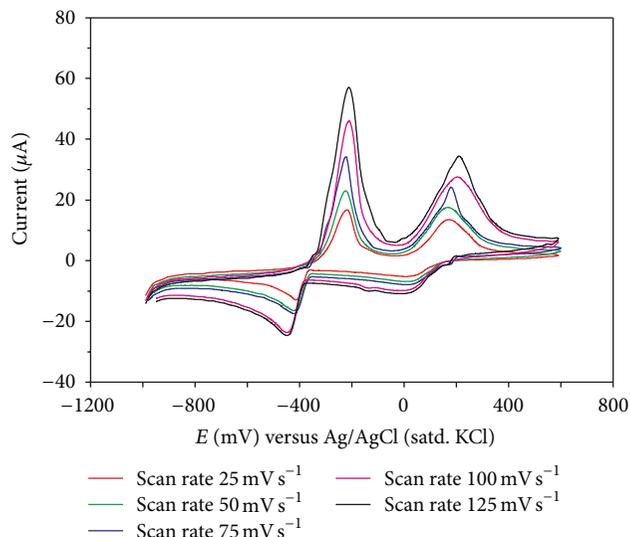


FIGURE 4: Cyclic Voltammogram of 0.6 mM Cu^{2+} and 1.2 mM guanine at -1000 mV to $+600$ mV potential window and 20 mV step height in presence of 0.2 M KCl as supporting electrolyte and different scan rates (25 , 50 , 75 , 100 , and 125 $mV\ s^{-1}$).

3.1.4. Variation of Scan Rate. Cyclic Voltammogram of 0.6 mM Cu^{2+} and 1.2 mM guanine at -1000 mV to $+600$ 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 , and 125 $mV\ s^{-1}$) was taken (Figure 4).

The intensity for both the anodic and cathodic peaks is controlled by the following equation:

$$i_p = 2.69 \times 10^5 \cdot n^{3/2} \cdot A \cdot D^{1/2} \cdot C \cdot v^{1/2}, \quad (1)$$

where, i_p = peak current in ampere, n = electron stoichiometry, A = area of the electrode in cm^2 , D = diffusion coefficient in cm^2/s , C = concentration of the species in mol/cm^3 , and v = scan rate in volts/s.

Two plots of anodic peak current versus square root of the scan rate and cathodic peak current versus square root of the scan rate are shown in the Figure 5, which depict the comparison of their increasing and decreasing trend, respectively.

The electrochemical processes are diffusion controlled which can be explained from the graph of i_p versus $v^{1/2}$ as shown in Figure 4. First anodic current increases from 13.58 μA to 34.64 μA and second anodic current increases from 16.43 μA to 57.13 μA . First cathodic current increases from 4.27 μA to 11.06 μA and second cathodic current increases from 12.84 μA to 24.26 μA . From Figure 4 it can be explained that as the scan rate of the complex mixtures increases, the corresponding current increases but anodic and cathodic potential shift very little [21, 22].

3.1.5. Variation of Step Height. Cyclic Voltammogram of 0.25 mM Cu^{2+} and 0.50 mM guanine at -800 mV to $+600$ mV potential window at 100 mV/S scan rate at different step height (20 , 25 , 30 , 35 , and 40 mV) was taken. It is observed

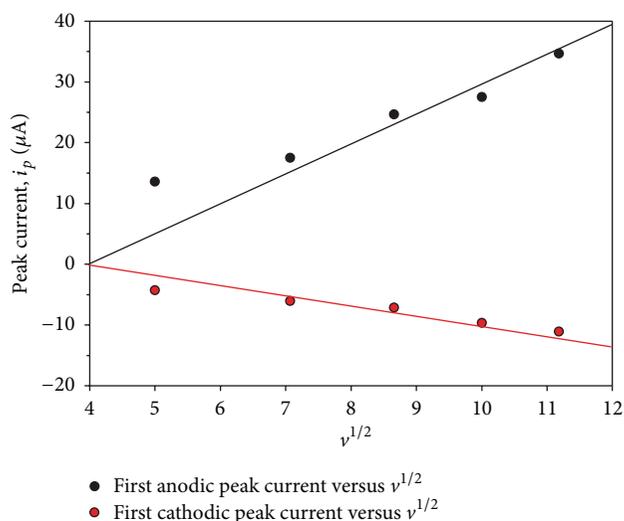


FIGURE 5: Peak current versus square root of the scan rate for copper and guanine complex.

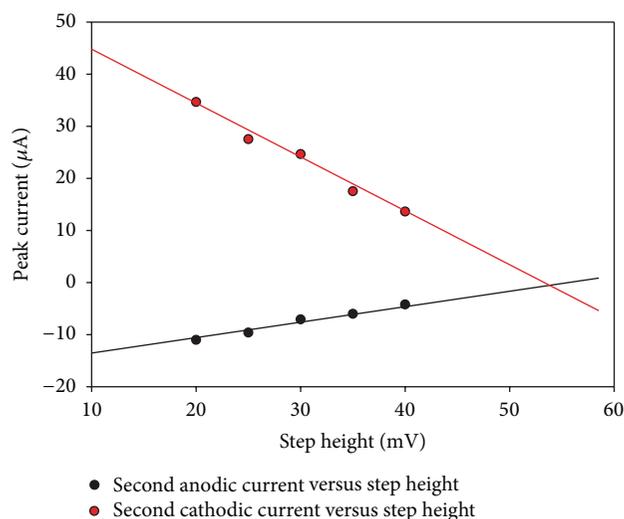


FIGURE 6: Peak current versus step height for Cu^{2+} and guanine complex.

as 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 decrease and consequently anodic and cathodic current decreases with increasing step height [23].

Plotting peak current versus step height Figure 6 is obtained, and it shows a straight line with negative slope, which indicates that the increase in step height decreases the peak current.

3.1.6. Variation of pH. Cyclic Voltammogram of 0.25 mM Cu^{2+} and 0.50 mM guanine at -800 mV to $+600$ mV potential

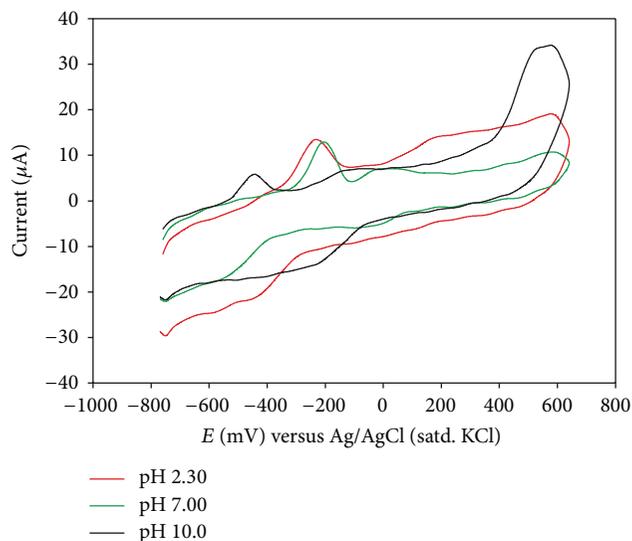


FIGURE 7: Cyclic Voltammogram of 0.25 mM Cu^{2+} and 0.50 mM guanine at -800 mV to $+600$ mV potential window at 100 mV/S scan rate and 20 mV step height at different pH values (2.30, 7.00, and 10.0).

window at 100 mV/S scan rate and 20 mV step height at different pH values (2.30, 7.00, and 10.0) was taken (Figure 7) 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 guanine. There was a little increase in peak current of Cu^{2+} guanine complex species below pH 7.0. From pH 7.0 onwards, the peak current increased sharply, the optimum pH range appears to be between pH 2.3 and 7.0.

3.1.7. Variation of Supporting Electrolyte. Figure 8 illustrates the effect of variation of supporting electrolyte on the electrochemical behavior of copper complexation with guanine.

Any species that compete with metal ion for the surface of the electrode would inevitably interfere in the adsorption of metal thymine complex species hence leading to a decrease in the increase in current effect [24]. This explains why the supporting electrolyte such as $(\text{NH}_4)_2\text{SO}_4$, which is weakly complexing, produce higher current as compared to NaCl and KCl. More over in case of NaCl and KCl both are Chloride containing Supporting electrolyte but NaCl is weakly complexing compared to KCl produces more current in the anodic and cathodic peak. So the order of the anodic and cathodic current enlargement is $(\text{NH}_4)_2\text{SO}_4 > \text{NaCl} > \text{KCl}$.

4. Conclusions

The results indicated that guanine is electroactive at -1000 mV to 800 mV potential window and 100 mV s^{-1} scan rate as it has an anodic peak at -200 mV potential having peak current of $3.7511 \mu\text{A}$ in the Cyclic Voltammogram. Cu

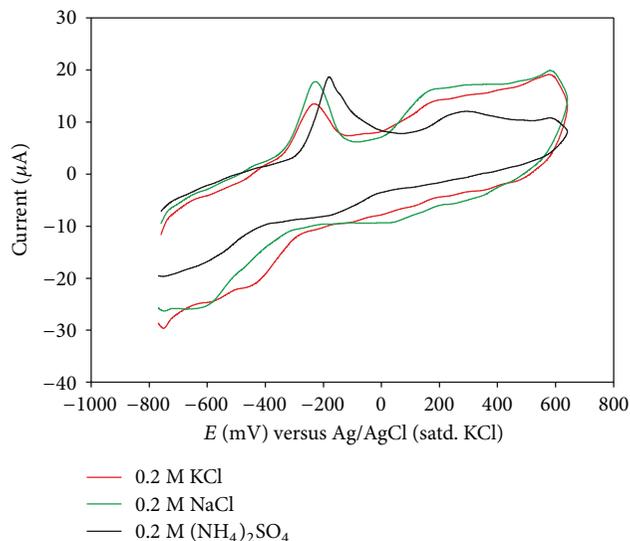


FIGURE 8: Cyclic Voltammogram of 0.25 mM Cu^{2+} and 0.50 mM guanine at -800 mV to $+600$ mV potential window at 100 mV/S scan rate and 20 mV step height in presence of different Supporting electrolytes (0.2 M KCl, 0.2 M NaCl, and 0.2 M $(\text{NH}_4)_2\text{SO}_4$).

(II) is electroactive as it gives two cathodic and two anodic peaks. Two anodic peaks are at 125 mV and -185 mV and two cathodic peaks at 55 mV and -425 mV which indicate that Cu (II) is two electron processes.

Complex formation of metal ion and ligands were observed according to Jobs Method and Mole Ratio Method. Cu (II) forms $1:2$ complexes with guanine. Probable structures of the complexes of copper with guanine have been proposed, which is being supported by the previous works.

As the concentration of any component increases, interaction between the metal and ligand increases, more complexes are formed, more electrons are transferred, and consequently current increases. Scan rate was varied for all the metal and ligand pairs which indicates the increase in scan rate increases the number of time of current scanning in the system per second increases. Hence the corresponding current also increases. Plot of the peak current versus square root of scan rate gives straight line with positive slope passing through origins which indicate that the peak current is directly proportional to the square root of the scan rate.

Step height was varied from 20 mV to 40 mV for copper and guanine complex which implies that the corresponding anodic and cathodic current decreases with increasing step height, it is due to less interaction between the metal and ligand ions and fewer complexes are formed.

The pH value of the complex mixture was varied from 2.3 to 10.0 for all metal and ligand complex mixture by using acetate and phosphate buffers. At a lower pH the H^+ ion compete with metal ions and less complexes are formed, less current is obtained, increasing pH value from 2.3 to 10.0 , and current increases and becomes maximum and then decreases rapidly with increasing pH value. That implies the complex is favored to slightly acidic medium and slightly alkaline

medium. The optimum pH range can be considered as 2.3 to 7.0 for the complexes of copper with guanine and thymine.

The supporting electrolytes were used as $(\text{NH}_4)_2\text{SO}_4$, NaCl, and KCl for all metal and ligand pair complex ratio. More interaction occurs when SO_4^{2-} containing supporting electrolyte $(\text{NH}_4)_2\text{SO}_4$ is used compared to chloride containing supporting electrolyte 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 $(\text{NH}_4)_2\text{SO}_4 > \text{NaCl} > \text{KCl}$.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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