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STUDY ON THE REDOX BEHAVIOR OF BIOLOGICAL IMPORTANT METAL ION (CU^{2+}) AND ITS INTERACTION WITH METRONIDAZOLE DRUG IN AQUEOUS SOLUTION

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ABSTRACT

The redox studies of biologically active compounds are gaining importance for various purposes. Such redox phenomena are close to the natural redox processes occurring in human and other living organisms. This study involves the investigation of redox behavior of Cu^{2+} ion and its electrochemical nature when interacts with pharmaceutical drugs like metronidazole. This research carried out by very sophisticated and popular cyclic voltammetry (CV), chronoamperometric (CA) and chronocoulometric (CC) techniques. The change of electrochemical properties, reversibility and adsorption properties with the variation of pH also observed.

Keywords: *Chronoamperometric, Chronocoulometric, Cyclic voltammetry, Electrochemical properties, redox processes.*

1. INTRODUCTION

Copper is one of the relatively small groups of metallic elements which are essential to human health (Araya *et al.* 2006). These elements, along with amino and fatty acids as well as vitamins, are required for normal metabolic processes (Harris, 2001; Groff *et al.,* 1995; Bonham *et al.,* 2002). On the other hand metronidazole is a common medicine used by human being (Tally *et al.,* 1972; Tally *et al.,* 1975).

Chemical reaction occurring in living systems are numerous and complex. Many of them are redox reaction. Introduction of other biologically active compounds such as, medicinal compounds may alter the pathway of the redox reaction mechanism occurring at the biological system (Ross *et al.* 2006; Cox *et al.,* 2010; Autreaux *et al.,* 2007). The complexion capacity of biologically active organic compounds with selected metals plays an important role in metabolism in biological systems. This capacity may be significantly changes with the introduction of the medicinal compounds. They may also form complexes that are non-toxic, soluble in biological system and readily eliminated from body, saving it from toxic effect (Chen *et al.,* 2003). Simultaneously, excess use of the drugs also causes drastic changes charge transfer in the in the biological system. Therefore, study of metal-medicinal compounds is very much important (Scheiber *et al.,* 2009).

Investigation of mechanism of electrochemical interaction between the essential trace element like Cu and metronidazole in biological system is necessary. Among all the techniques, the cyclic voltammetry is extremely popular in electrochemical research, because it can provide useful information about redox reactions (Heinze and jurgel 1984).

2. EXPERIMENTAL

2.1 Chemicals and reagents

Chemicals used in the experiment were Copper Sulphate (MERCK, Germany) Metronidazole (Aldrich Chemical Co. Ltd., Gillingham, Dorset, England) and Potassium Chloride (MERCK, Germany). The buffers were prepared using sodium acetate (MERCK, Germany) and acetic acid (Sigma-Aldrich Labor chemikaline, GmbH). Cleaning of the electrodes and all the solutions were prepared using de-ionized water. 99.997 % Nitrogen (Bangladesh Oxygen, Ltd.) was used for purging purpose.

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2.2 Equipments

This study was carried out using an Epsilon Electroanalyser developed by Bioanalytical System, Inc., USA. A glassy carbon electrode was used as working electrode, which was cleaned by polishing on cloth using alumina powder. Ag/AgCl electrode and Pt wire were used as reference and counter electrodes, respectively. An AGE (VELP SCIENTIFICA) magnetic stirrer with a teflon coated magnetic bar and a pH meter (METTLER, TOLEDO) was employed for stirring and measuring of the pH of the solutions, respectively. All glass wares used in the preparation of solutions were made of pyrex glass.

3. RESULT AND DISCUSSION

3.1 Electrochemical study of Cu^{2+} and its interaction with ligand

3.1.1 Cyclic voltammetric study of Cu^{2+} ion

The redox behavior of 1 mM solution of uncoordinated Cu^{2+} was studied in 0.1M KCl as supporting electrolyte at room temperature (25 ˚C) using cyclic voltammetric technique with potential window from 1500 mV to -600 mV at glassy carbon electrode. A series of cyclic voltammograms of the uncoordinated Cu^{2+} in KCl electrolyte at different scan rates are shown in Figure 1 and the parameters are listed in Table 1.

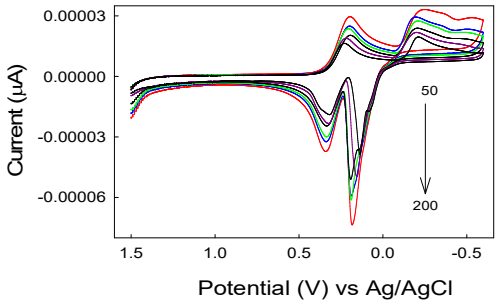

Figure 1: CVs of Cu^{2+} in 0.1 M KCl solution. Scan rates 50, 70, 90, 120, 140 and 200

Table 1: The effect of scan rate on the peak potential and peak current of cyclic Voltammograms of Cu^{2+} in 0.1M KCl solution at GCE.

Scan rate,ν (Vs^{-1})	SQRT of scan rate $\nu^{1/2}$	Anodic peak potential (V)	Cathodic peak potential (V)	Anodic peak current (µA) (-)	Cathodic peak current (µA)	Peak potential separation $\Delta E_p=E_{pa}-E_{pc}$ (V)	Peak current ratio i_{pa}/i_{pc}
		E_{pa1}	E_{pc1}	$i_{pa1} \times 10^{-5}$	$i_{pc1} \times 10^{-5}$	ΔE_{p1}	i_{pa1}/i_{pc1}
0.050	0.2236	0.3180	0.2320	1.900	1.6268	0.0860	1.17
0.070	0.2645	0.3238	0.2205	2.317	1.8887	0.1033	1.23
0.090	0.3000	0.3352	0.1975	2.453	2.0403	0.1377	1.22
0.120	0.3464	0.3352	0.2090	2.990	2.3623	0.1401	1.26
0.140	0.3742	0.3410	0.1975	3.229	2.5020	0.1435	1.27
0.200	0.4472	0.3410	0.1975	3.724	2.9519	0.1455	1.29
		E_{pa2}	E_{pc2}(-)	$i_{pa2} \times 10^{-5}$	$i_{pc2} \times 10^{-5}$	ΔE_{p2}	i_{pa2}/i_{pc2}
0.050	0.2236	0.1344	0.209	3.862	1.9507	0.3434	1.98
0.070	0.2645	0.1516	0.198	4.974	2.1765	0.3496	2.29
0.090	0.3000	0.1918	0.204	5.071	2.3877	0.3958	2.13
0.120	0.3464	0.1861	0.204	6.092	2.7466	0.3901	2.22
0.140	0.3742	0.1861	0.198	5.916	2.9407	0.3841	2.02
0.200	0.4472	0.1803	0.244	7.293	3.3155	0.4243	2.47

From the Table 1, we can see that for the 1st anodic peak, the peak potentials are gradually increased and for the 2nd the increase rate is irregular as the scan rate increased. For 1st cathodic peak, initially the peak potentials are decreased and then become nearly stable and for the 2nd peak, the potentials are irregular as the scan rate increased. In both cases, the increasing and decreasing rate of potential are small. This behavior can be described by slower charge propagation, probably due to difference in salvation and or permeability (Bard *et al.,* 2000; Conway, 1965; Nicholson, 1965; Du Vall *et al.,* 1999).

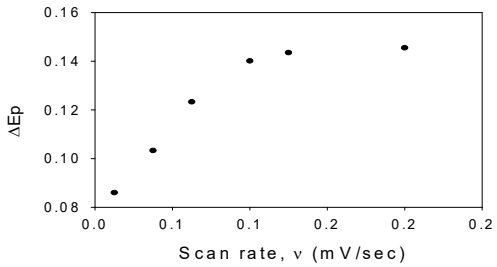

Figure 2: Variation of peak potential separation (ΔEp) against scan rate for Cu^{2+}

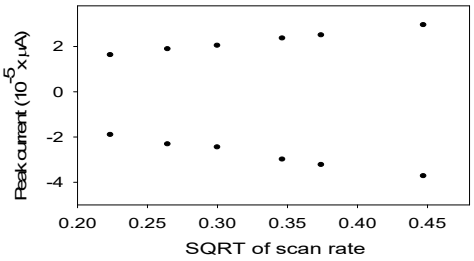

Figure 3: Variation of peak current against SORT of scan rate

Figure 2 represent the peak potential separation increases with the increase of scan rate. This is because of the effect of iR drop. Figure 3 shows the peak current of both cathodic and anodic are increases as the SQRT of scan rate increase, giving the information that the process is adsorption controlled (Conway, 1965; Nicholson, 1965).

Table 1 also illustrates that the peak current of both the cathodic and anodic peaks are increases as the scan rate increases. This can be rationalized by considering the size of the diffusion layer and the time taken to record the scan. We also observe that as the scan rate increases the peak current ratios (i_{pa}/i_{pc}) for all peaks were increased. The values of the peak current ratio indicate that the charge transfer process for this system is not reversible (Nicholson *et al.,* 1964; Bond *et al.,* 1998).

3.1.2 Interaction with metronidazol

The CV of Cu^{2+} ion was studied in presence of metronidazol to understand whether there is any interaction between the metal and the drug molecule. The Figure 4 presents the comparison of CVs of Cu^{2+} ion, metronidazol and Cu^{2+} in presence of the ligand. It shows the peak numbers and their positions in the CV of interaction is different from that of the CVs of Cu^{2+} ion and metronidazol, which gives the indication for the Cu^{2+} ion of being complexed. The CVs of interactions of Cu^{2+} and metronidazol for 1:1 ratio at different scan rates are given in Figure 5.

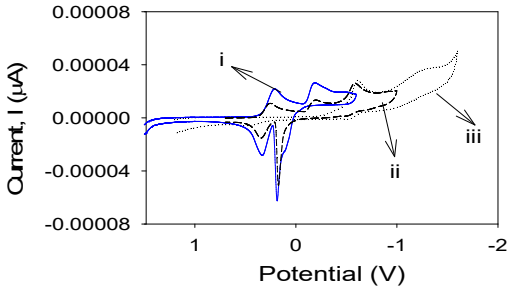

Figure 4: Comparison of CVs of (i) Cu^{2+} ion, (ii) interection with metronidazol and (iii) metronidazol in aqueous KCl solution

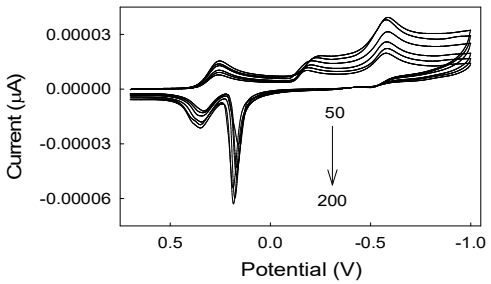

Figure 5: Scan rate variation (200,160,140, 90, 70, 50) for the interaction of (Cu:Meetro) = (1:1)

From the Figure 5 we observe that the CVs of the interaction between the Cu^{2+} and metronidazol in different scan rates are more or less similar. For different ratios are also gives nearly similar CVs. i.e. their electrical properties are similar type. The data are recorded in Table 2.

Both Figure 6 and Figure 7 shows the potential separation increases with the increase of scan rate for both peaks. This is same as Cu^{2+} ion itself (Figure 2). So it also indicates there is a limitation due to charge transfer kinetics or ohmic potential (iR) drop (Nicholson *et al.,* 1964; Nicholson, 1965; Bond *et al.,* 1998). The 1st peak current of both

cathodic and anodic peaks are increases as the scan rate increases (Figure 8). Similar trend observe in table 2 for 2nd pair of peak current.

As the scan rate increases the peak current ratios (i_{pa1}/i_{pc1}) for both the 1st and 2nd peaks are nearly constant and the values are higher than one (Table 2). So we can say that this redox system is not reversible.

Table 2: Current-potential data for the interaction of Copper and Metronidazol (1:1) at different scan rates

Scan rate,ν (Vs^{-1})	SQRT of scan rate $\nu^{1/2}$	Anodic peak potential (V)	Cathodic peak potential (V)	Anodic peak current (μA) (-)	Cathodic peak current (μA)	Peak potential separation ΔE_p (V)	Peak current ratio i_{pa}/i_{pc}
		E_{pa1}	E_{pc1}	$i_{pa1} \times 10^{-5}$	$i_{pc1} \times 10^{-5}$	ΔE_{p1}	i_{pa1}/i_{pc1}
0.050	0.2236	0.3331	0.2587	1.181	0.7787	0.0744	1.52
0.070	0.2645	0.3377	0.2587	1.291	0.9127	0.0790	1.41
0.090	0.3000	0.3470	0.2541	1.464	1.0275	0.0929	1.42
0.140	0.3742	0.3423	0.2541	1.782	1.2833	0.0882	1.40
0.160	0.4000	0.3516	0.2587	1.957	1.3785	0.0929	1.42
0.200	0.4472	0.3516	0.2541	2.110	1.5584	0.0975	1.35
		E_{pa2}	E_{pc2} (-)	$i_{pa2} \times 10^{-5}$	$i_{pc2} \times 10^{-5}$	ΔE_{p2}	i_{pa2}/i_{pc2}
0.050	0.2236	0.1612	0.182	2.971	1.0257	0.3432	2.90
0.070	0.2645	0.1658	0.187	4.263	1.1755	0.3528	3.63
0.090	0.3000	0.1705	0.201	4.703	1.3446	0.3715	3.50
0.140	0.3742	0.1751	0.205	5.939	1.5654	0.3801	3.79
0.160	0.4000	0.1844	0.224	5.350	1.7207	0.4084	3.11
0.200	0.4472	0.1844	0.247	6.254	1.8431	0.4314	3.39

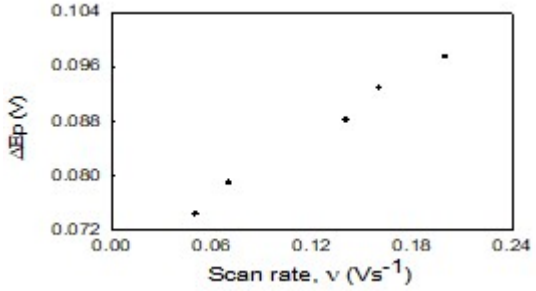

Figure 6: Variation of 1st peak potential separation against scan rate for interaction of Cu^{2+} and Metronidazol (1:1)

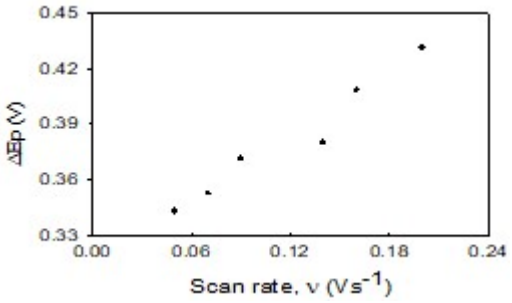

Figure 7: Variation of 2nd peak potential separation against scan rate for interaction of Cu^{2+} and Meronidazol (1:1)

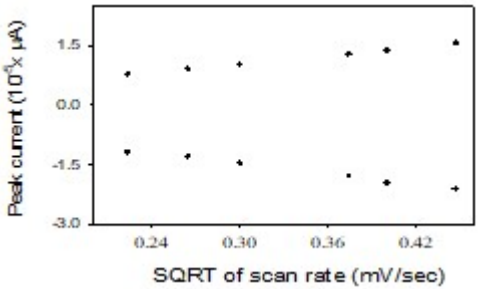

Figure 8: Variation of 1st peak current against SQRT of scan rate

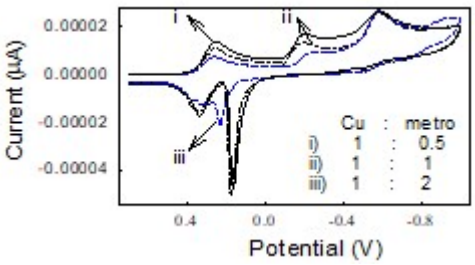

Figure 9: Comparison of CVs for different ratios

3.1.3 Effect of Concentration of Ligand

The effects of ligand concentration on CV are given in the following Figure 9 and the data are shown in Table 3. The table shows that both the anodic peak current and cathodic peak current decreases as the mole fraction of ligand increases. i.e. the electrochemical process or the redox reaction of the Cu^{2+} ion in presence of ligand (metronidazol) is decreases due to the decrease of the number of electroactive species (Bard *et al.,* 2000; Conway, 1965; Nicholson, 1965; Du Vall *et al.,* 1999).

Table 3: Current and peak current ratio of the voltammogram of the interaction of Cu^{2+} with ligand at different ratio at 200 mVs^{-1} scan rate at GCE

Cu^{2+}: Metronidazol (ligand)	Mole fration of ligand	Anodic Peak current (i_{pa1} x 10^{-5}) μA (-)	Cathodic Peak current (i_{pc1} x 10^{-5}) μA	Peak Current ratio ($i_{pa1/ipc1}$)
1:0.5	0.33	2.699	1.9605	1.38
1:1	0.50	2.110	1.5584	1.35
1:2	0.66	1.425	1.0449	1.36

3.1.4 Effect of pH

The redox behaviour of uncoordinated Cu^{2+} in KCl solution and in acetate buffer (pH 4.5) is shown in Figure 10 and observes large deviation of CVs for two different media. In buffer media there is one anodic peak and one cathodic peak. On the other hand two pairs of anodic and cathodic peak in KCl electrolyte. That means Cu^{2+} system has a large effect on buffer. A series of CVs of the uncoordinated Cu^{2+} in acetate buffer at different pH are shown in the Figure 11.

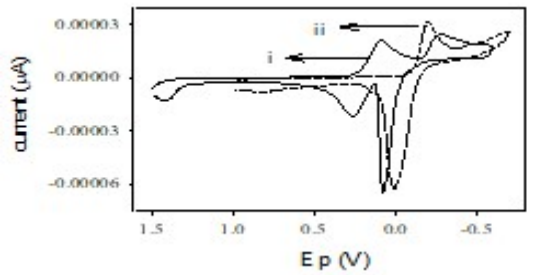

Figure 10: Comparison of CVs of Cu^{2+} (i) in KCl and (ii) in acetate buffer at pH 4.5

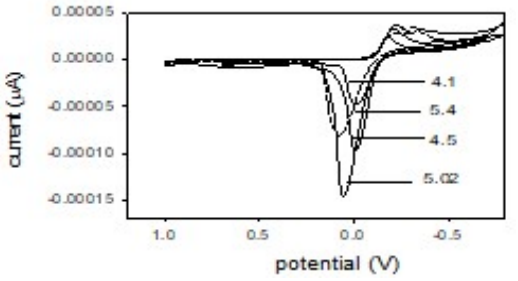

Figure 11: CVs of Cu^{2+} solutions in buffer of pH (i) 4.1 ii) 4.5 (iii) 5.02 and (iv) 5.4 at scan rate 70 mVs^{-1}

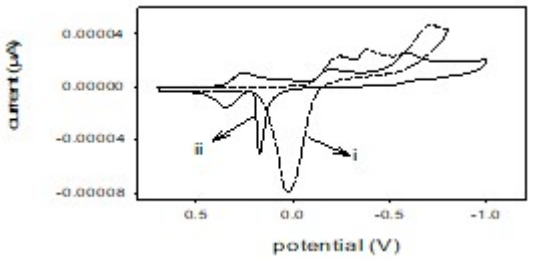

Figure 12: Interaction of Cu^{2+} and metronidazole (1:1) in (i) acetate buffer and (ii) KCl colution

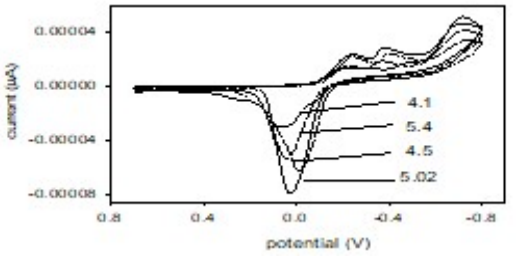

Figure 13: Interaction of Cu^{2+} and metronidazol in acetate buffer of pH (i) 4.1 (ii) 4.2 (iii) 5.02 and (iv) 5.4 at scan rate 100 mVs^{-1}

3.1.5 Chronoamperometric and chronocoulometric study of Cu^{2+}

We observed when the pH of the buffer is increase, the current also increase and reaches maximum at pH 5.02. After that the current again decrease as the pH increase (Figure 11). i.e. at around pH 5.02 the electrolysis rate is higher (Kolthof *et. al.* 1959; Shaikh *et. al.* 2011).

From the Figure 12 we see the CVs of interaction of Cu^{2+} and metronidazol in KCl solution and in buffer solution. This figure express that the redox behavior for the interaction in two media is quite different. The peak position and the size of the peak have been changed. Figure 13 shows a series of CVs at different pH values, which have the similar trend as uncoordinated metal ion. It also noticed that the interaction of drug is maximum at pH 4.1. i.e. at lower pH value.

Chronoamperometric study of Cu^{2+} was also conducted in presence of metronidazol. It shows that the spike height after interaction with metronidazol is decreased compared to that of Cu^{2+} in the absence of metronidazol (Figure 14). This means that after interaction the rate of electrolysis has been decreased, which support the CV experiment.

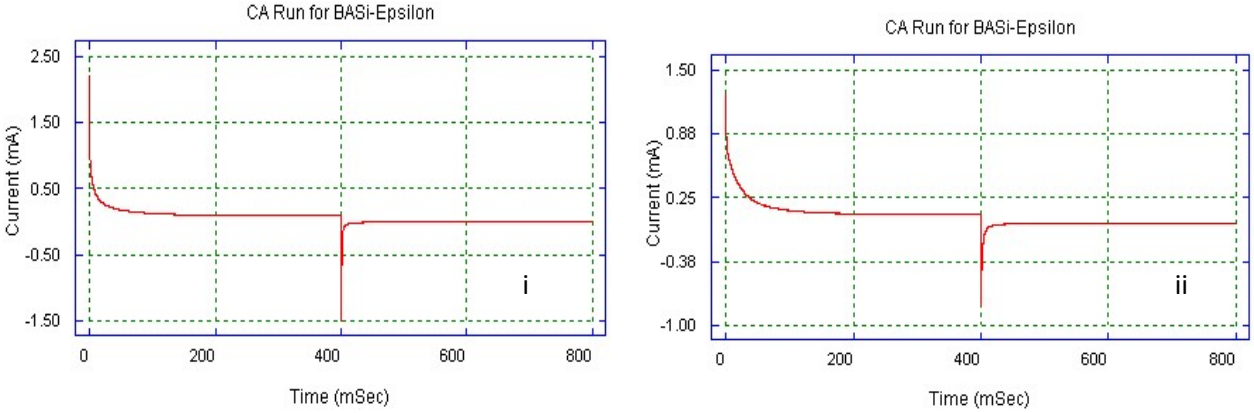

Figure 14 : Current responses for (i) uncoordinated Cu^{2+} and (ii) coordinated with Metronidazol.

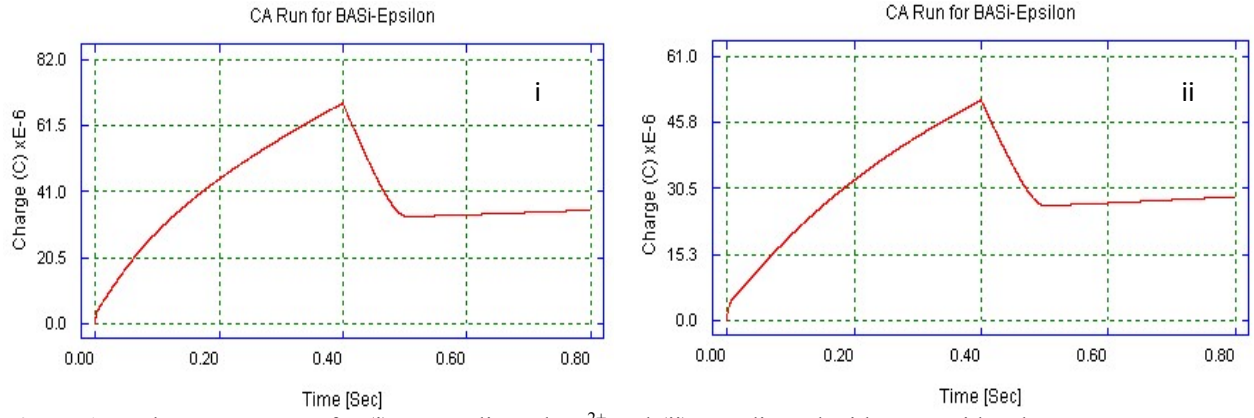

Figure 15 : Charge responses for (i) uncoordinated Cu^{2+} and (ii) coordinated with Metronidazol.

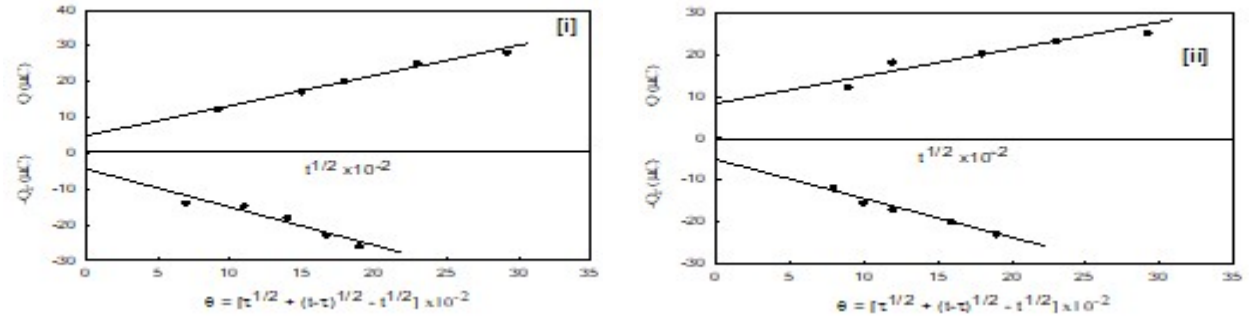

Figure 16 : Plots of Q vs $t^{1/2}$ and $-Q_r$ vs θ for (i) uncoordinated Cu^{2+} and (ii) coordinated with Metronidazol

Chronocoulometric response shows that the charge at τ is decreased after interaction with metronidazol. It was 66.12 μC in the absence of metronidazol and 50.32 μC in presence of metronidazol, as shown in the figure 15.

Figure 16 represents the plots of Q vs $t^{1/2}$ and $-Q_r$ vs θ on the same graph for the uncoordinated and coordinated Cu^{2+} with metronidazole. The two plots do not intersect at Q=0 axis as well as they do not have equal slope. Therefore, in both of these cases adsorption occurs (Conway, 1965; Nicholson, 1965).

Chronoamperometric and chronocoulometric study of uncoordinated and coordinated Cu^{2+} in buffer system was also studied. All the CA and CC experiments support the CV experiments. The plots of Q vs $t^{1/2}$ and $-Q_r$ vs θ on the same graph for every experiments also confirmed that the all redox system in this experiment occurs by adsorption (Conway, 1965; Nicholson, 1965).

4. CONCLUSION

From the CV experiment we observed that the peak current decreases after the interaction of metal and ligand. CA and CC also gave the same result. These can be concluded that the electrochemical process or the redox reaction after the interaction is decrease due to the decrease of the number of the electro active species.

Peak potential separation increases with the increase of scan rate and the peak current ratio (i_{pa}/i_{pc}) is not stable or not near the value of unity. So, from the diagnostic criteria of CV experiment we can clearly say that the redox systems are quasi-reversible or irreversible.

pH variation also gives the important indication for all systems. We observed that for both uncoordinated and coordinated system pH of the medium largely affects the redox system. From the pH variation we noticed that maximum interaction occurs at pH 4.1.

We also observed that the peak current of both cathodic and anodic peaks are increases as the SQRT of scan rate increases. So we can concluded that the processes are adsorptive controlled. This is confirmed from the CA and CC experiment.

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