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Interaction of Cu(II) with Cytosine in Britton-Robinson Buffer Solution - A Cyclic Voltammetric Study

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Abstract

Electrochemical redox behavior of Cu(II) at different pH in Britton-Robinson (BR) buffer solution has been investigated using cyclic voltammetry at glassy carbon electrode (GCE). Cu(II) shows a cathodic peak and a hump-like peak, and an intense anodic peak at pH, 0.59, 1.59, 3.01 and 4.08. Although at lower pH (0.59 and 1.59) a cathodic peak, a hump and an anodic peak are observed, at higher pH (3.01 and 4.08) the hump-like cathodic peak disappeared. Cyclic voltammetry has also been used to observe the interaction of Cu(II) with cytosine in BR buffer solution at various pH. A reasonably strong interaction between Cu(II) and cytosine at different Cu(II)/cytosine molar ratio is observed at all the studied pH. However, maximum interaction occurs at 1:4 molar ratio at pH 4.08, this is perhaps the most suitable condition for Cu(II)-cytosine interaction.

Keywords: Cytosine; Britton-Robinson buffer; Glassy carbon electrode; Peak potential

I. Introduction

Cytosine is one of the five main nitrogenous bases used in storing and transporting genetic information within a cell[1]. It is a pyrimidine derivative, with a heterocyclic aromatic ring and two substituent attached (an amine group at position 4 and a keto group at position 2). The nucleoside of cytosine is cytidine[2]. DNA methylation involves the addition of a methyl group to the 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring (cytosine and adenine are two of the four bases of DNA). DNA methylation also plays a crucial role in the development of nearly all types of cancer[3]. DNA methylation involves the addition of a methyl group to DNA, for example, to the number 5 carbon of the cytosine pyrimidine ring, in this case with the specific effect of reducing gene expression. DNA methylation at the 5 position of cytosine has been found in every vertebrate examined. Cytosine plays a vital role in pairing, through hydrogen bonds with the guanine base of guanosine and deoxyguanosine. Cytosine is involved in the genetic codon of 17 amino acids and controls the essential features of life[4].

Although cytosine is a base it can act as a ligand because of the presence of unpaired electrons on nitrogen and oxygen which are available to donate in complexation reaction. Cyclic voltammetric behaviour of cytosinato bridged complexes of ruthenium(II) and platinum(II) with 1-alkyl-2-(arylazo)imidazoles were investigated[5]. Electrochemical properties of the complexes have been examined by cyclic voltammetry in CH_3CN in presence of $[n\text{-}Bu_4N][ClO_4]$ as supporting electrolyte using a Pt-disc working electrode at the scan rate of 50 mV/s. Although several groups[6-8] have tried to investigate the electrochemical interaction of metal-cytosine with different electrodes, our aim is to investigate the interaction of Cu(II) with cytosine in BR buffer medium maintaining various pH at GCE using cyclic voltammetry.

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II. Experimental

Materials

Analar grade $Cu(NO_3)_2.3H_2O$ was purchased from Merck, Germany and used without further purification. Analytical grade cytosine (BDH, UK) was used in this study. Acetic acid (BDH, UK), phosphoric acid (Merck, Germany), and boric acid (Merck, Germany) were also used for the preparation of BR buffer. Sodium hydroxide (Merck, Germany) and perchloric acid (BDH, UK) were used as it is available for maintaining the pH of the solution. All aqueous solutions were prepared in doubly distilled water obtained from a Milli-Q water purification system. The experiments were carried out at room temperature.

Equipments

Three electrodes system consisting of a GCE as the working electrode, Ag/AgCl (satd. KCl) as the reference electrode and a 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. Solution pH was measured with a pH meter (Microprocessor pH meter, model pH 211, HANNA Instruments).

Methods

Preparation of Britton-Robinson buffer solution

For the preparation of BR buffer solution, 0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M CH_3COOH solutions were prepared separately, and the three solutions were then mixed together with same volume ratio. By adding 0.2 M NaOH or $HClO_4$ solution the desired pH values (pH values are 0.59, 1.59, 3.01 and 4.08) were adjusted.

Preparation of metal ion and cytosine solution

Metal ion solution of 0.5 mM Cu(II) was prepared using BR buffer. For the complexation study, 0.5, 1.0, 1.5 and 2.0 mM cytosine solutions were also prepared using BR buffer solution.

Preparation of working electrode

GCE was polished with fine alumina powder of 0.3 micron on a wet polishing cloth. For doing so a part of the cloth was made wet with deionized water, and alumina powder was sprinkled over it. The GCE was then polished on this surface by pressing softly the electrode against the polishing surface for about 10 minutes. A shiny black mirror like electrode surface was then thoroughly washed with deionized water.

First of all, the cell was filled with desired volume of the experimental solution and the Teflon cap was placed on the cell. The purging glass tube together with reference electrode was inserted through the holes. Under computer controlled stirring, experimental solution was deaerated by purging for at least 10 minutes with 99.9977% pure nitrogen gas. Thus traces of dissolved oxygen were removed from the solution.

III. Results and Discussion

Cyclic voltammetric investigation of the redox behavior of Cu(II) in BR buffer solution, and its interaction with cytosine in an identical voltammetric condition has been performed. The effect of pH for this study was also examined. The results are presented below:

Voltammetric response of Cu(II) in BR buffer solution

Cyclic voltammetric study of 0.5 mM Cu(II) in BR buffer at different pH values (0.59 to 4.08) has been investigated at GCE within the potential window of 500 to -500 mV. At more positive or negative potential values only non-faradic current is observed. Figure 1(a) shows a cyclic voltammogram of 0.5 mM Cu(II) in BR buffer solution with scan rate of 100 mVs^{-1} at pH 1.59. In the forward scan two cathodic peaks i_{pc1} (a hump like peak) and i_{pc2} at about 9.84 mV and -84.96 mV respectively, and in the reverse scan an intense anodic peak i_{pa2} at about 67.60 mV are observed. The cathodic peaks are due to the reduction of Cu(II) to Cu(I) and Cu(I) to Cu(0), while the intense anodic peak is for the oxidation of Cu(0) to Cu(II). However, cyclic voltammogram of cytosine in an identical experimental condition shows no peak (Figure 1(b)).

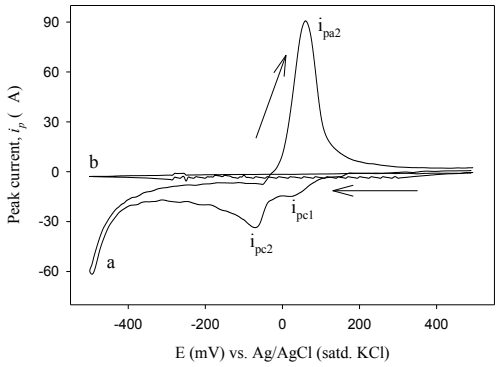


Fig. 1. Cyclic voltammograms of (a) 0.5 mM Cu(II) and (b) 0.5 mM cytosine in Britton-Robinson buffer at pH 1.59 with scan rate of 100 mVs^{-1} at GCE.

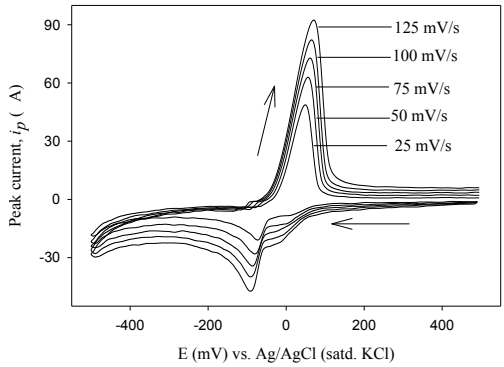


Fig. 2. Cyclic voltammograms of 0.5 mM Cu(II) in Britton-Robinson buffer solution with scan rate of 25, 50, 75, 100 and 125 mVs^{-1} at pH 1.59.

The effect of the scan rate on the electrochemical response of Cu(II) at pH 1.59 is also examined by gaining the cyclic voltammograms of Cu(II) with scan rate of 25, 50, 75, 100 and 125 mVs^{-1} under identical condition. The voltammograms are shown in Figure 2.

Table 1. Data obtained from the cyclic voltammograms of 0.5 mM Cu(II) in Britton-Robinson buffer with different scan rate at pH 1.59.

Scan rate, v (mVs^{-1})	Cathodic peak current, i_{pc} (A)		APC, i_{pa} (A)	Cathodic peak potential, E_{pc} (mV)		APP, E_{pa} (mV)	PCR i_{pa2}/i_{pc2}	$E_p = E_{pa2} - E_{pc2}$ (mV)
	i_{pc1} (-)	i_{pc2} (-)	i_{pa2}	E_{pc1}	E_{pc2} (-)	E_{pa2}		
25	13.25	20.96	48.57	9.84	79.88	50.00	2.31	130
50	8.57	27.87	63.33	5.04	82.32	57.60	2.27	140
75	12.16	28.24	73.30	7.86	82.32	58.40	2.59	140
100	13.94	34.65	82.85	9.84	84.96	67.60	2.39	152
125	20.07	47.75	92.41	2.40	92.40	72.00	2.01	164

APC= Anodic peak current, APP= Anodic peak potential, PCR= Peak current ratio

The recorded voltammograms are analyzed and various parameters such as anodic and cathodic peak current, peak potential separation and peak current ratio are gathered as shown in Table 1. It is found that with the increase of scan rate, both the cathodic and anodic peak currents are increased (Figure 2). This observation suggests that the electrode process is under diffusion controlled in BR buffer medium. The anodic (E_{pa2}) and cathodic (E_{pc2}) peak potential separation (130-164 mV) and anodic (i_{pa2}) and cathodic (i_{pc2}) peak current ratio (2.01-2.59) reveal that the redox process of copper system is quasi-reversible, in agreement with the observations on the shape of the cathodic and anodic peaks[9].

The cathodic peak is slightly shifted towards negative potential while the anodic peak is moved a little towards positive potential with the incease of scan rate. The shifting of the peak potential at various scan rates also indicates that the redox process is shifted from quasi-reversible to irreversible direction.

Figure 3 apparently shows that the peak current for the redox behavior of copper system in BR buffer bears a linear relationship with square root of scan rate and it passes through the origin. The ratio of the oxidation peak current to its corresponding reduction counterpart, i_{pa2}/i_{pc2} is about 2.59-2.01. It is found (Figure 4) that the peak current ratio is decreased with the increase of scan rate. It is again in favor of the fact that the electrode process is diffusion-controlled. It is also in good agreement with the previous study[10].

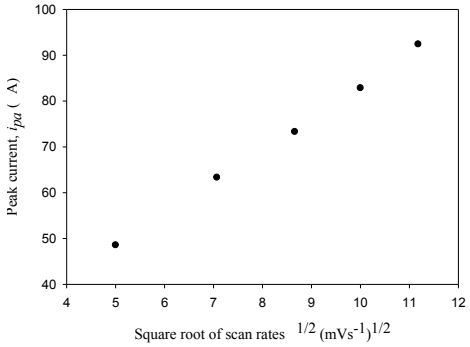


Fig. 3. Variation of peak current with square root of scan rate for Cu(II) in Britton-Robinson buffer at pH 1.59.

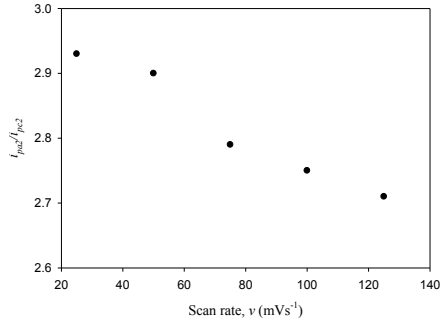


Fig. 4. Peak current ratio (i_{pa2}/i_{pc2}) dependence on scan rate of 0.5 mM Cu(II) in Britton-Robinson buffer medium at pH 1.59.

The peak potential separation, E_p is in between 130-164 mV and it is increased with the increase of scan rate (Figure 5). It is evident that the redox process is quasi-reversible rather than a reversible.

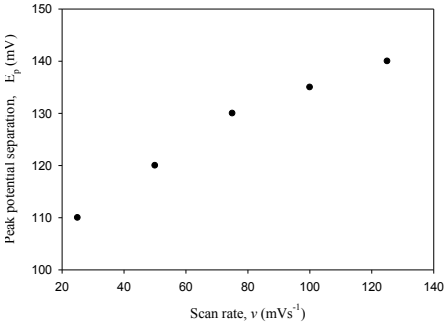
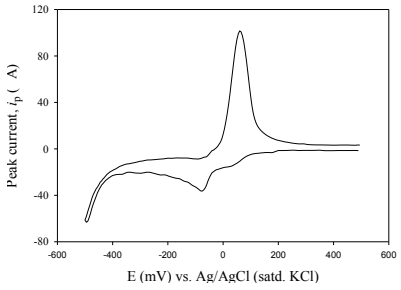


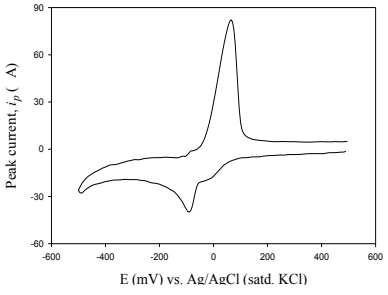
Fig. 5. Variation of peak potential separation with scan rate of 0.5 mM Cu(II) in Britton- Robinson buffer at pH 1.59.

Effect of pH on the redox behavior of Cu(II)

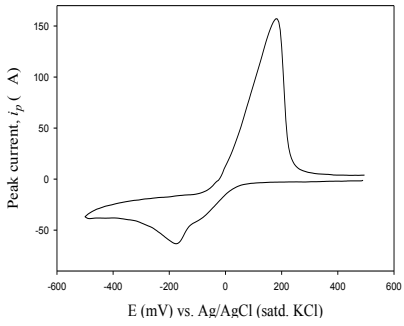
The effect of pH on redox behavior of Cu(II) in BR buffer has also been investigated at GCE. Voltammograms for Cu(II) in BR buffer at pH 0.59, 1.59, 3.01 and 4.08 are recorded. A series of voltammograms at different pH of the solution are shown in Figure 6.



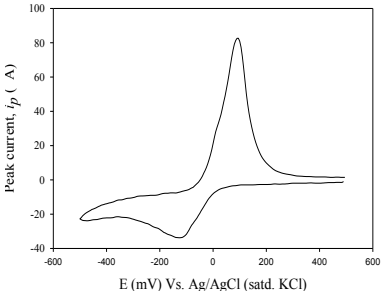
a



b



c



d

Fig. 6. Cyclic voltammograms of 0.5 mM Cu(II) in Britton-Robinson buffer at different pH: (a) 0.59, (b) 1.59, (c) 3.01 and (d) 4.08 at GCE.

At low pH ranging from 0.59 to 3.08, a cathodic peak and a hump-like peak, and an intense anodic peak are appeared. With the increase of pH, the first cathodic peak current gradually decreases and finally disappears at pH 4.08. In general, both cathodic and anodic peak currents are decreased with the increase of pH, as given in Table 2. However, both the anodic and cathodic peaks are identical in shape and the peak potential separation is comparable. At low pH limit $[Cu(H_2O)_6]^{2+}$ and $[Cu(H_2O)_{6-x}]^{+}$ ions can be assumed to be present in significant amount while at high pH (~ 4.08), $[Cu(OH)(H_2O)_5]^{+}$ ion is understood to be the only electroactive species[11,12]. It has been mentioned before that Cu(I) as the solvated ion is stable in a number of nonaqueous solvents such as acetonitrile[13], nitromethane[12]. In such solvents, the stability of Cu(I) is due to the fact that these solvents solvate Cu(II) less strongly than does water and they solvate Cu(I) more strongly than does water.

Table 2. Data obtained from the voltammogram of Cu(II) at different pH.

Solution pH	Peak current, i_p (□A)		Peak potential, E_p (mV)		Peak potential separation, □E_p
	i_{pc2} (-)	i_{pa2} (+)	E_{pc2} (-)	E_{pa2} (+)	
0.59	35.94	100.97	80.16	54.96	134
1.59	34.23	81.94	95.04	64.80	159
3.01	62.45	155.53	180.00	179.76	359
4.08	34.21	80.52	125.04	90.00	215

Voltammetric response of Cu(II) in presence of cytosine in BR buffer solution

The voltammograms of Cu(II) in presence of cytosine in BR buffer at various Cu(II)/cytosine molar ratio, 1:1, 1:2, 1:3 and 1:4 have been recorded at pH 0.59, 1.59, 3.01 and 4.08 at GCE within the same potential window.

The recorded voltammograms of Cu(II), and at various Cu(II)/cytosine molar ratio, 1:1, 1:2, 1:3 and 1:4 at pH 0.59 is shown in Figure 7. At this pH the cathodic peak is significantly reduced and a large reduction of the anodic peak height is observed in the voltammograms and the peak currents are relatively lower than that of Cu(II) alone. Moreover, the heights of the peaks are apparently erratic with respect to cytosine concentration. The lowest peak current is found at 1:4 molar ratio of Cu(II) to cytosine concentration suggesting the maximum interaction occurs at this molar ratio. It is noted that the voltammogram of cytosine in identical condition exhibits no anodic or cathodic peak (Figure 1(b)) within the studied potential window.

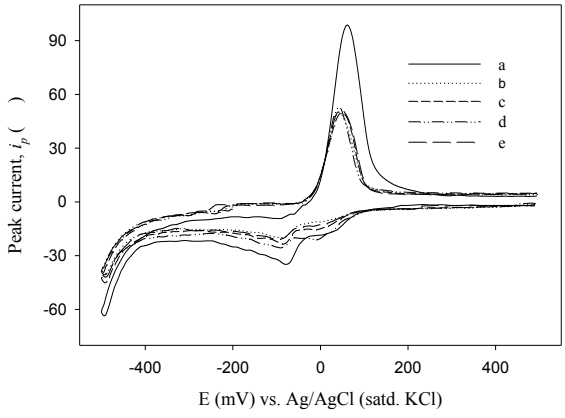


Fig. 7. Cyclic voltammograms of (a) Cu(II) (solid), and Cu(II) and cytosine with different molar ratio (b) 1:1 (dotted), (c) 1:2 (short dash), (d) 1:3 (dash-dot-dot) and (e) 1:4 (long dash) in Britton-Robinson buffer at pH 0.59.

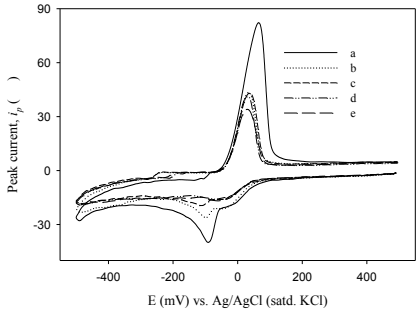


Fig. 8. Cyclic voltammograms of (a) Cu(II) (solid), and Cu(II) and cytosine with different molar ratio (b) 1:1 (dotted), (c) 1:2 (short dash), (d) 1:3 (dash-dot-dot) and (e) 1:4 (long dash) in Britton-Robinson buffer at pH 1.59.

Figure 8 shows the voltammograms of Cu(II), and Cu(II) in presence of cytosine with different molar concentration at pH 1.59. A significant change of the shape of voltammograms is observed. The cathodic peak completely disappeared (at 1:3 and 1:4 molar ratio) while the anodic peak current considerably decreased compared to only Cu(II). The anodic peak potentials also shifted towards negative direction. Furthermore, the heights of the peaks are apparently inconsistent with respect to cytosine concentration. Since the lowest peak current (Table 3) is found for 1:4 molar ratio of Cu(II) to cytosine, the maximum interaction, is indeed, occurred at this molar ratio at pH 1.59.

In presence of cytosine, Cu(II) displays almost identical electrochemical behavior at pH 3.01 and 4.08. In most of the cases the cathodic peak disappears completely. The behaviors that are common in both cases, (i) significant decrease of the peak currents of Cu(II), (ii) slight shifting of peak potentials, (iii) inconsistent respond of peak current with respect to Cu(II)/cytosine molar ratios, and (iii) a maximum interaction of Cu(II) with cytosine at their 1:4 molar ratio. The voltammograms recorded at pH 3.01 and at 4.08 are shown in Figures 9 and 10 respectively.

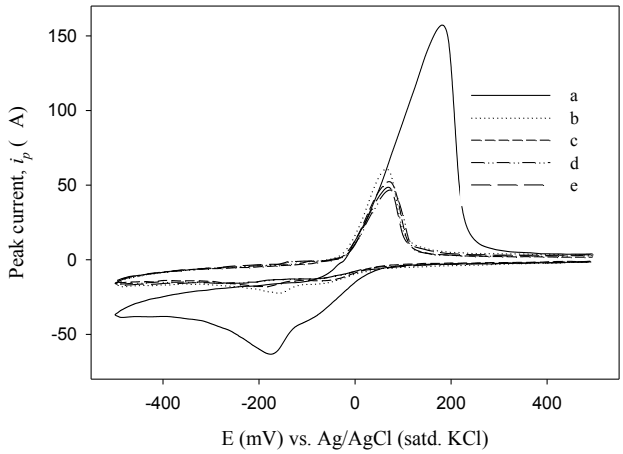


Fig. 9. Cyclic voltammograms of (a) Cu(II) (solid), and Cu(II) and cytosine with different molar ratio (b) 1:1 (dotted), (c) 1:2 (short dash), (d) 1:3 (dash-dot-dot) and (e) 1:4 (long dash) in Britton-Robinson buffer at pH 3.01.

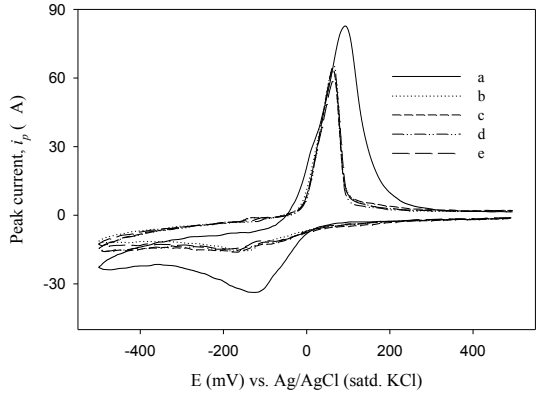


Fig. 10. Cyclic voltammograms of (a) Cu(II) (solid), and Cu(II) and cytosine with different molar ratio (b) 1:1 (dotted), (c) 1:2 (short dash), (d) 1:3 (dash-dot-dot) and (e) 1:4 (long dash) in Britton-Robinson buffer at pH 4.08.

From the above observation it can be concluded that Cu(II) interacts strongly with cytosine at molar ratio of 1:4 at all studied pH in BR buffer medium. However, the maximum interaction occurs at pH 4.08. The data for the different voltammograms recorded for Cu(II)/cytosine molar ratio of 1:4 at various pH is gathered in Table 3.

Table 3. The data for the voltammograms recorded for 1:4 molar ratio of Cu(II)/cytosine at different pH at GCE with scan rate of 100 mVs^{-1}.

Solution pH	Peak current, □A		Peak potential, mV		Peak current ratio (i_{pa}/i_{pc})	Peak potential separation, □E_p
	i_{pc} (-)	i_{pa} (+)	E_{pc} (-)	E_{pa} (+)		
0.59	21.00	49.63	93.00	43.00	2.36	136.00
1.59	16.15	34.16	70.08	30.00	2.11	100.08
3.01	-	51.63	-	43.00	-	-
4.08	-	51.00	-	63.00	-	-

- indicates no significant value

IV. Conclusion

Cyclic voltammogram of Cu(II) in BR buffer exhibits an intense cathodic peak and a hump-like peak, and a strong anodic peak at all studied pH. However, at high pH, only a cathodic and an anodic peak are found. In presence of cytosine the cathodic peak current of Cu(II) disappeared significantly or completely while the anodic peak height is reduced appreciably with the negative shifting of peak potential. This observation indicates that the interaction between Cu(II) and cytosine occurs in BR buffer medium at acidic pH. Among the studied pH region maximum interaction is found at pH 4.08 when the molar ratio of Cu(II)/cytosine is 1:4.

Acknowledgement

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