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Cyclic voltammetric studies of copper and manganese in the presence of L-leucine using glassy carbon electrode

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Abstract

Cyclic voltammetric studies were used to investigate the interaction behavior of the metal ions such as Cu(II) and Mn(II) with L-leucine. The interaction studies have been carried out in variation of metal ion concentration, leucine concentration, pH and scan rate. The results indicated that Cu(II)/Cu(I) and Mn(I)/Mn(0) were reversible but Cu(I)/Cu(0) and Mn(II)/Mn(I) were quasi-reversible. Moreover, the results indicated that both the cathodic and anodic peaks were shifted and developed new peaks upon the addition of the leucine. The development of the new peaks suggested the formation of metal-leucine complex. The intensities of the new peaks were increased with increasing both the concentrations of metal ions as well as leucine. This is due to the formation of more metal-leucine complex that enhanced the peak intensities. The increasing trend of the peak intensities of the metal-leucine complex as a function of pH is due to the formation of more metal-leucine complex. This is because the coordination sites of the leucine molecules were increased with increasing pH that favored the formation of more metal-leucine complex. The intensities of the peak current were also increased with increasing scan rate that has been explained by the Randles-Sevcik equation. The voltammograms for both the metal ions showed two pairs of signals for both the cathodic and anodic modes and every step was single electron process. A linear behavior of i_p vs. $\nu^{1/2}$ plot indicated that the electrochemical processes were diffusion controlled.

Keywords: Cyclic voltammetry; divalent metal ions; L-leucine; Metal(I)/(II)-leucine complex

Introduction

Corresponding to the rapid development of molecular biology, amino acids have become an important class of organic compounds that are helpful in understanding biological functions of macromolecules like peptides, proteins. However, some free amino acids especially branched-chain amino acids play important role in many physiological activities of the human body. L-leucine is one of them that cannot be manufactured in the body. Several years ago the branched-chain amino acids (BCAAs) created some interest in the neurological research community when a pilot study indicated that amyotrophic lateral sclerosis (ALS) patients showed symptomatic improvement when given large doses of BCAAs [1]. Branched-chain amino acids are sometimes used in enteral and parenteral feedings in the management of hepatic encephalopathy [2].

Leucine is an essential amino acid, which cannot be manufactured in the body and is a part of the three branched chain amino acids. It helps with the regulation of blood sugar levels, the growth and repair of muscle tissue, the growth hormone production as well as wound healing. It can also assist to prevent the breakdown of muscle protein that some-times occur after trauma or severe stress. The inherited deficiencies of enzymes involved in its metabolism result in a group of disorders referred to as “leucine metabolism disorder”. In these cases, the body is not able to breakdown one several metabolites of the essential amino acid

leucine, resulting in toxic levels of that metabolite in the body. Approximately 60 cases of leucine metabolism disorders have been reported. These generally result in "metabolic acidosis", which can lead to neurological damage, coma and death if not corrected.

Leucine containes $-NH_3^+$ and –COOH groups, which have good ability to coordinate to metal ions and often involve in forming hydrogen bond networks [3], therefore, the interactions between leucine and the metal ions may be expected. It is well known that essential elements such as Na^+ and K^+ exist in human body. They all play the important roles in our life and may interact with many biological molecules. It is helpful to understand the physiological function of leucine by studying the coordination structure between leucine and the metal ions. However, the study in this field has been neglected [4, 5].

Studies of metal complexes of amino acids and small peptides have been carried in order to obtain a better understanding of transition metal complexes in proteins. In particular Cu(II) complexes were quite often investigated due to the possibility of applying different spectroscopic techniques and also to the widespread occurrence of copper proteins, for instance blue proteins [6]. Metal ions can bind to peptides in several ways, depending on the amino acid residues, pH and binding stoichiometry. In general several species coexist in solution and the involvement of oxygen from carboxylate groups has been demonstrated [6]. The coordination is often completed with oxygen atoms from water molecules [7]. It has been reported that electrochemical behaviors of some amino acids and transition metal ions studied on Pt electrode [8, 9].

In the present study, attempts have been made to investigate the interaction behavior of Cu(II) and Mn(II) with L-leucine using glassy carbon electrode (GCE) in cyclic voltammetric mode.

Experimental section

Reagents

L-Leucine (99.9%), sodium acetate, acetic acid were purchased from E. Merck (Germany); hydrated copper(II)sulfate, hydrated manganese(II) sulfate were purchased from E. Merck (India); potassium chloride was purchased from BDH (UK).

Methods

Stock solution of 4mM Cu(II) and Mn(II) was prepared from their respective sulfate salts in two separate 100 mL volumetric flasks. A standard solution of L-leucine of 4mM was prepared in a 100 mL volumetric flask. A solution of 1M KCL was also prepared in a 100 mL volumetric flask. Finally, acetate buffer solution was prepared as follows: 0.2M CH_3COONa and 0.2M CH_3COOH were prepared separately in two 100 mL volumetric flasks and then the buffer solutions of desired pH were prepared by mixing requisite volume of the solutions, individually. Then preparation of buffer has been done by mixing of these two solutions at different ratio and then measured the pH of the buffer solution using a pH meter. A pH meter (Model, TOA, HM-16S) was used throughout the experiment. Milli-Q deionized water was used throughout the experiment.

The glassy carbon electrode was polished with fine alumina powder (0.3 micron or lower) on a wet polishing cloth. To do so a part of the cloth was made wet with deionized water and alumina powder was sprinkled on it. The glassy carbon electrode was then polished on this surface by pressing softly the electrode against the polishing surface in the end for 5-10 minutes. The electrode was then thoroughly washed with deionized water. At this point the electrode surface would look like a shiny black mirror.

First of all, the cell and the Teflon top (with the counter electrode) were cleaned with deionized water, any excess water was wiped off with a tissue paper. A small magnetic stir-bar was put in the cell. The cell was filled with desired volume of the analyte solution and the Teflon cap was placed on the cell. The reference electrode and the purging tube were inserted through the holes. The purging tube should be halfway inside the solution. Then the solution was flushed by nitrogen gas (99.9977%) for 5 min under computer controlled stirring of the solution to expel the dissolved oxygen. A range of both the metal and

leucine concentrations (0.40 to 1.60 mM) was used. Solution pH and scan rate were varied from 2.0 to 4.5 and 20 to 300 mV/s respectively.

Apparatus

Three electrodes system comprising were Glassy carbon (as working electrode); Ag/AgCl (satd. KCl) (as the reference electrode) and Platinum wire (as the counter electrode). A computerized electrochemistry system (Model HQ 2040) was used to investigate the interaction behavior of Cu(II) and Mn(II) with L-leucine.

Results and discussion

Effect of metal ion concentration on the voltammograms in the presence of leucine.

Figure 1 shows the cyclic voltammograms of different concentrations of the metal ions (e.g., Cu(II) and Mn(II)) in the presence of 0.8mM leucine at 100 mVs^{-1} scan rate. Figure 1(p) shows the effect of copper concentration on the voltammograms of Cu(II) in the presence of leucine at 100 mVs^{-1}. The results show that the current intensity of the first anodic (Cu(0) to Cu(I), i_{pa2}) peak (-120 mV) is decreased upon the addition of leucine and shifted at the positive direction (-5.23 mV). The development of the new peak suggests the formation of Cu(I)-leucine complex. The intensity of the new peak increases with increasing of Cu(II) concentration. This may be due to the formation of more Cu(I)-leucine complex with increasing of Cu(II) concentration. Moreover, the same phenomena are observed in the case of the second cathodic (Cu(II) to Cu(I)) peak. The shifting of the peak potential of the i_{pc2} from -390 to -430 mV also suggests the formation of Cu(I)-leucine complex. On the other hand, the second anodic peak (i_{pa1}, 209 mV) shifts a little bit at 213 mV which indicates that the interaction between Cu(II) and leucine not significant but the increasing trend of the intensity may be due to the presence of excess Cu(II) ions.

Figure 1(q) shows the effect of manganese concentration on the voltammograms of Mn(II) in the presence of leucine at 100 mVs^{-1}. The figure shows that the current intensity of the first cathodic (Mn(II) to Mn(I), i_{pc1}) peak (862 mV) is decreased upon the addition of leucine and developed a new peak slightly at negative direction (653 mV). The development of the new peak suggests the formation of Mn(II)-leucine complex. The intensity of the new peak increases with increasing of Mn(II) concentration. This is due to the formation of more Mn(II)-leucine complex.

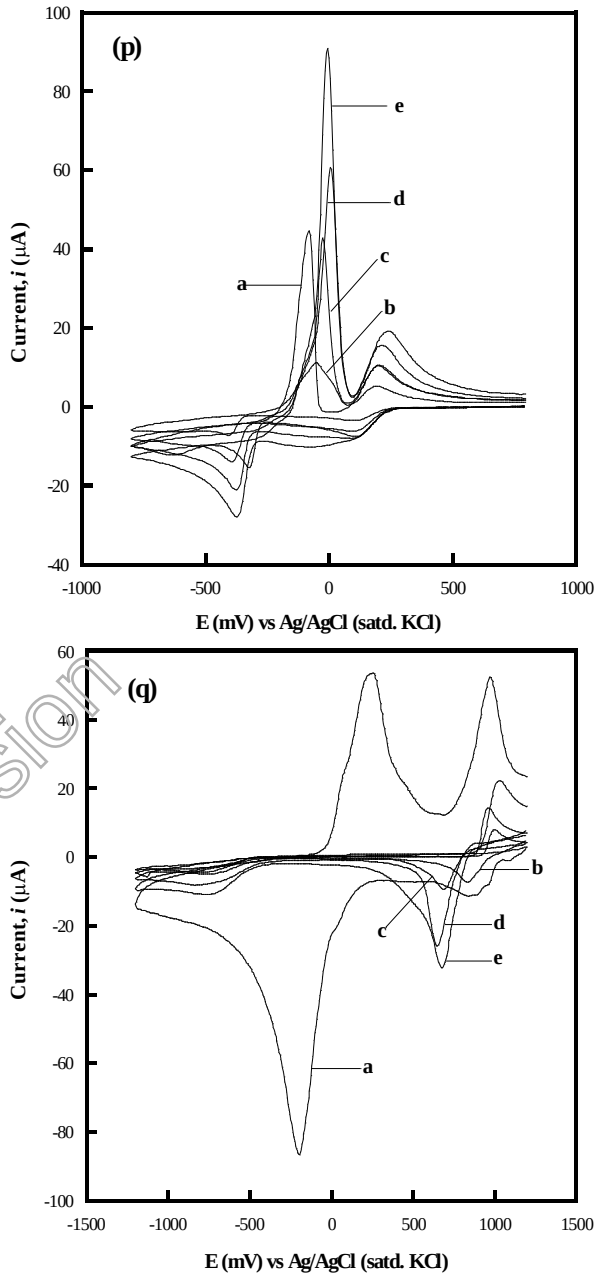


Fig. 1. Cyclic voltammograms of different concentrations of (p) Cu(II) and (q) Mn(II). The concentrations were (a) 0.8 mM of metal ions without leucine, (b) 0.4 mM metal ions + 0.8 mM leucine, (c) 0.8 mM metal ions + 0.8 mM leucine and (d) 1.6 mM metal ions + 0.8 mM leucine. Scan rate and pH were maintained at 100 mVs^{-1} and 3.32, respectively.

Similar results are observed for the second cathodic peak (i_{pc2}, Mn(I) to Mn(0)), but this peak (-200 mV) is shifted at more negative potential (Mn(I)-leucine complex at -801 mV) and the peak height was reduced significantly compared to the first one.

During the reverse scan the peak potential of the Mn(I)-leucine complex shifted towards more positive potential (for i_{pa2} free Mn(I) at 235 mV and Mn(I)-leucine complex at about 1000 mV). Therefore, the results suggested that the trend of Mn(I)-leucine complex formation is favored under the present experimental condition.

Effect of leucine concentration on the voltammograms of Cu(II) and Mn(II).

Figure 2 shows the effect of leucine concentration on the voltammogram of the metal ions. The Fig. 2(p) shows that the first anodic peak (i_{pa2}, -120 mV) for the transformation of Cu(0) to Cu(I) is shifted to -5.23 mV upon the addition of lecucine which indicates the formation of Cu(I)-leucine complex. The intensity of the peak at -5.23 mV increases with increasing of leucine through the formation of more Cu(I)-leucine complex. In addition, the intensity of the second anodic peak (i_{pa1}, 209mV) decreases with increasing of leucine that suggests that the availability of Cu(I) for the oxidation to Cu(II) is limited through the formation of the complex, Cu(I)-leucine. On the other hand, the significant decreasing trend of the both the cathodic peaks (i_{pc1} and i_{pc2}) suggests that Cu(II) also from complex at high concentration of leucine.

Figure 2(q) shows the effect of leucine on the voltammogram of manganese. These results show that the first cathodic peak (i_{pc1}, 862 mV) for the transformation of Mn(II) to Mn(I) is shifted to 653 mV upon the addition of lecucine which indicates the formation of Mn(II)-leucine complex. The intensity of the peak at 653 mV increases with increasing of leucine. This indicates the formation of more Mn(II)-leucine complex with leucine concentration. In addition, the intensity of the second cathodic peak (i_{pc2}, 209mV) decreases with increasing of leucine that suggests that the availability of Mn(II) for the reduction to Mn(I) is limited through the formation of the Mn(II)-leucine complex.

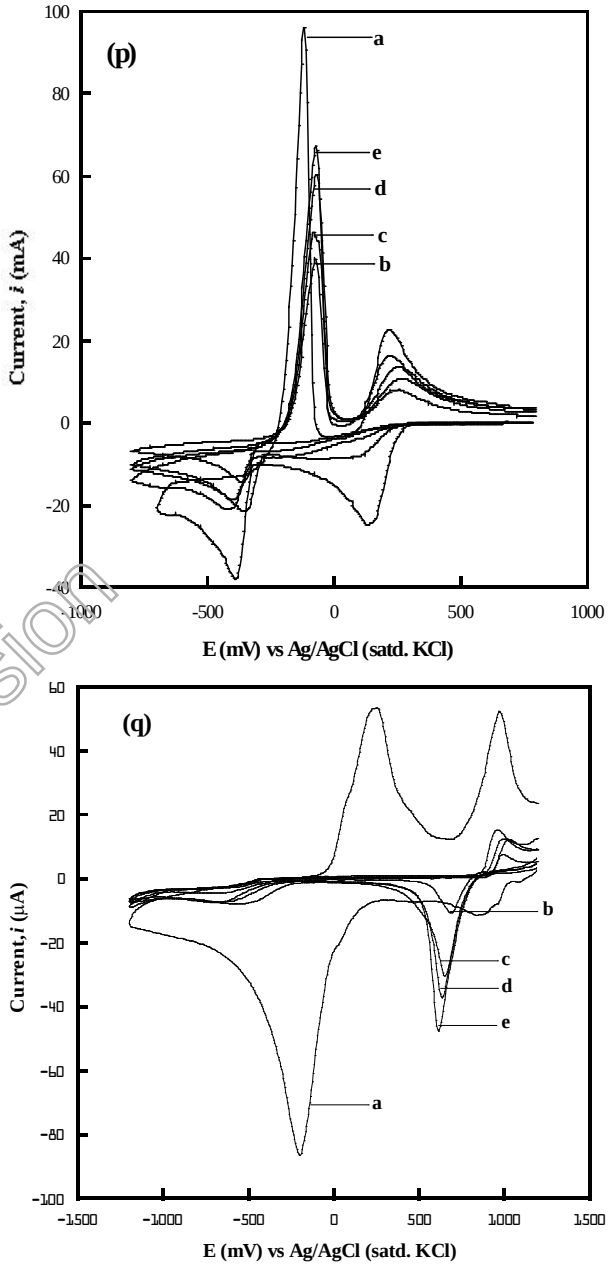


Fig. 2. Cyclic volammograms of (p) Cu(II) and (q) Mn(II) in the presence of leucine at (a) 0, (b) 0.4, (c) 0.8, (d) 1.2 and (e) 1.6 mM at pH = 3.32. Concentration of Cu(II) and Mn(II) was maintained at 0.8 mM. Scan rate was adjusted at100 mVs^{-1}.

Effect of pH on the voltammograms of Cu(II) and Mn(II) in the presence leucine.

Figure 3 shows the effect of pH on the voltammograms of the metal ions in the presence

of leucine. Figure 3(p) shows that the first anodic peak (i_{pa2}, -120 mV) for Cu(0) to Cu(I) is shifted to -5.23 mV in presence of lecucine which indicates the formation of Cu(I)-leucine complex. The intensity of the peak at -5.23 mV increases with increasing of pH. The trend of increasing intensity indicates the formation of more Cu(I)-leucine complex. The formation of more Cu(I)-leucine complex with increasing pH is due to the increasing of coordination sites of the amino group of the leucine molecule through deprotonation.

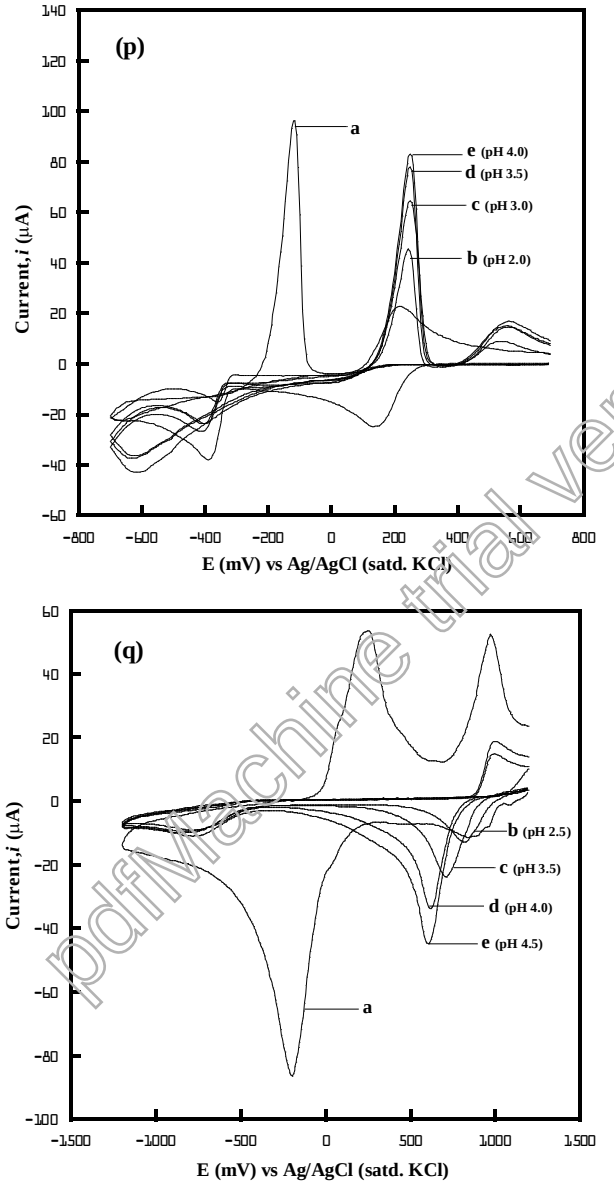


Fig. 3. Cyclic voltammograms of (p) Cu(II) and (q) Mn(II) in the presence of 0.8 mM leucine at different pH (a) 3.32 (without leucine), (b) 2.0, (c) 3.0, (d) 3.5 and (e) 4.0. Concentration of the metal ions was maintained at 0.8 mM. Scan rate was adjusted at 100 mVs^{-1}.

Figure 3(q) shows the effect of pH on the voltammogram of manganese. The results show that the first cathodic peak (i_{pc1}, 862 mV) for the Mn(II) to Mn(I) is shifted to 653 mV upon the addition of lecucine which indicates the formation of Mn(II)-leucine complex. The intensity of the peak at 653 mV increases with increasing of pH through the formation of more Mn(II)-leucine complex. The reason is described in above.

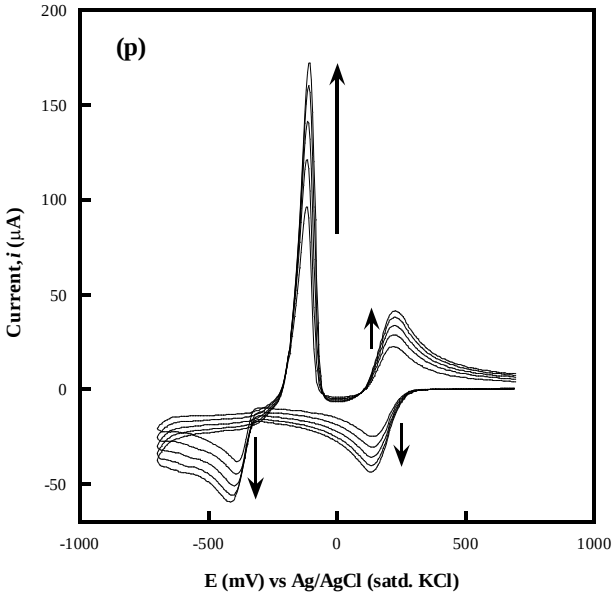
Effect of scan rate on the voltammogram of Cu(II).

Figure 4 shows the effect of scan rate on the voltammograms of Cu(II) and Mn(II). The intensity for both the anodic and cathodic peaks are controlled by the following Randles-Sevcik equation,

$$i_p = 2.69 \times 10^5 . n^{3/2} . A . D^{1/2} . C . \nu^{1/2}$$

Where, i_p = peak current in ampere
n = electron stoichiometry
A = area of the electrode in cm^3
D = diffusion co-efficient in cm^2 /s
C = concentration of the species in mol/ cm^3
ν = scan rate in volts /s

The electrochemical processes are diffusion controlled that can be explained from the graph of i_p vs $\nu^{1/2}$ as shown in Figure 5.



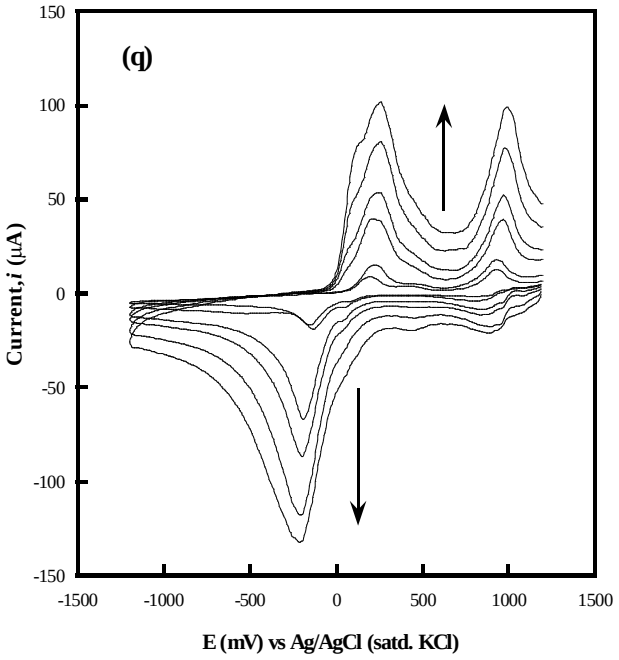


Fig. 4. Effect of scan rate on the voltammograms of (p) Cu(II) and (q) Mn(II). The scan rates were maintained at 20, 50, 75, 100, 150 and 200 mVs^{-1}. Concentration of the metal ions and pH were maintained at 0.8 mM and 3.32, respectively.

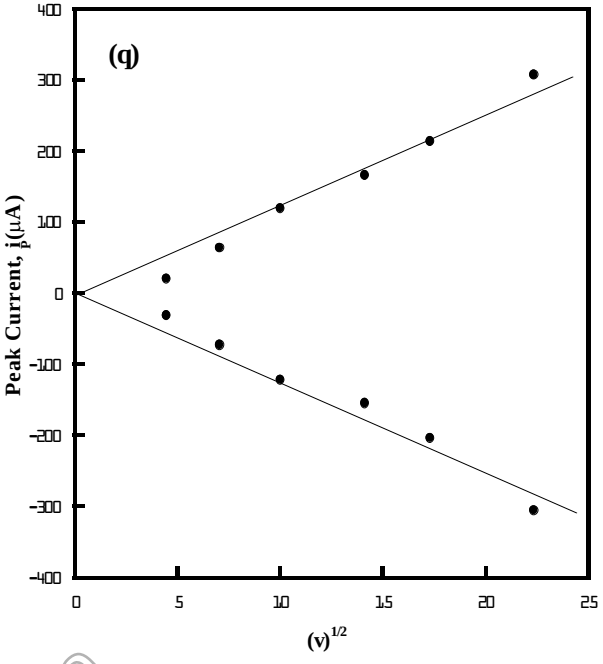
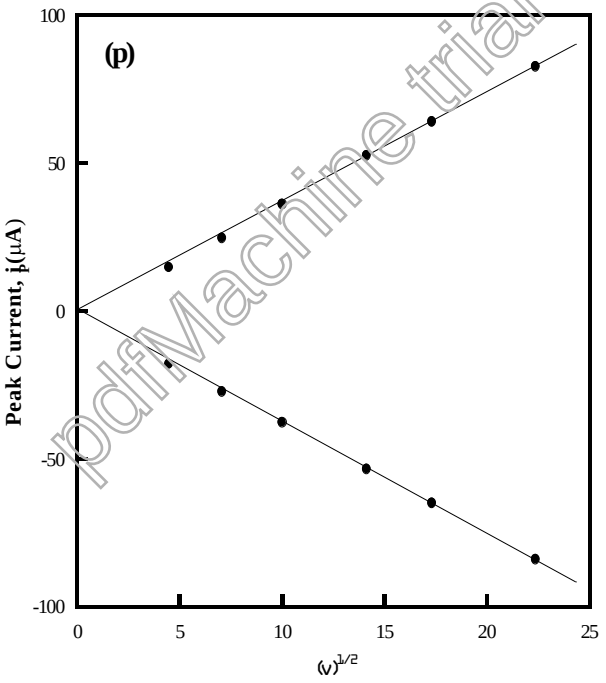


Fig. 5. Plots of peak current (i_P) vs. square root of scan rate $(\upsilon)^{1/2}$ for (p)Cu(II) and (q)Mn(II).



Conclusion

The cyclic voltammograms of the divalent metal ions such as Cu(II) and Mn(II) showed two pairs of signals for the both cathodic and anodic mode and every step is single electron process. The results indicated that both the Cu(II)/Cu(I) and Mn(I)/Mn(0) are reversible but Cu(I)/Cu(0) and Mn(II)/Mn(I) are quasi-reversible. In the presence of L-Leucine, both of the cathodic peaks are shifted towards negative potential and the anodic peaks are shifted towards positive potential. The intensities of all the peaks are decreased compared to those of free metal ions upon the addition of leucine. The development of the new peaks suggests the formation of metal-leucine complex. The intensities of the new peaks are increased with the increase of metal as well as leucine concentration. This may be due to the formation of more metal-leucine complex.

The increasing trend of the peak intensities of the metal-leucine complex with pH is due to the formation of more metal-leucine complex. This is because the coordination sites of the leucine molecules are increased (due to deprotonation) with increasing pH that favors the formation of

metal-leucine complex. The intensities of the peak current are also increased with increasing scan rate according to the Randles-Sevcik equation. A linear behavior of i_p vs. $\nu^{1/2}$ plotted indicated that the electrochemical processes are diffusion controlled.

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