Electrochemical Behavior of Cu^{2+}-Histidine Complexes on a Glassy Carbon Electrode

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The electrochemical behavior of Cu^{2+}-L-histidine complexes on a glassy carbon electrode with various coordination environments in aqueous solution has been investigated. The Cu^{2+}-histidine complexes are more easily reduced and oxidized at low pH (pH = 3 $\sim$ 4) than at high pH (pH = 8 $\sim$ 10). Both reduction and oxidation reactions of the Cu^{2+}-histidine complexes are controlled by mass transfer at medium (pH = 5 $\sim$ 7) and high pH (pH = 8 $\sim$ 10) solutions. Even if the molar ratio of histidine to Cu^{2+} ions is as high as 100 : 1 at low pH of 4, the complexes are easily reduced to form Cu metal directly on the electrode surface. Glassy carbon rotating disk electrode experiments have shown that the electron transfer of the reduction reaction of the Cu^{2+}-histidine complexes is close to 2.

Key words: Cu^{2+}-L-Histidine, Copper Complexes, Glassy Carbon Electrode, Electrochemistry, Histidine Complexes

Introduction

Cu^{2+}-L-histidine complexes have attracted vast interest due to their biochemical and pharmacological properties, as well as their rich coordination geometries. Cu^{2+}-histidine species were discovered in 1966 in human blood, and since then extensive research has been carried out to determine their role in copper transport [1]. The L-histidine ligand has three potential sites for coordination including the amino nitrogen (pK_a = 9.18), the imidazole nitrogen (pK_a = 6.0), and the carboxylate oxygen atoms (pK_a = 1.8) which become all available to coordination as the pH increases [2]. Thus, the composition of the predominant complexes of Cu^{2+}-histidine in aqueous solution strongly depends upon the pH value, the metal ion to ligand ratio and the temperature. There are at least eight different configurations of Cu^{2+}-histidine complexes as a function of pH including MHL, ML, MH_2L_2, MHL_2, ML_2, $MH_{-1}L_2$, $MH_{-1}L$ and $M_2H_{-2}L_2$ (M: Cu^{2+}, L: histidine, H: proton of histidine) [2]. Some proposed structures of Cu^{2+}-histidine complexes [2–4] are shown in Fig. 1. Results of detailed titration studies have indicated that among all these species, the major Cu^{2+}-histidine species are MHL, MHL_2, and ML_2.

Many studies have investigated the structures and binding in copper-histidine complexes [1–10]. In contrast, comparatively less attention has been devoted to their redox properties that are important for the understanding of the activity inside a biological cell [11–15]. Conflicting reports exist on the mechanism of the electroreduction of Cu^{2+} complexes with histidine at mercury electrodes [11–14]. Davis and Bordelon [11] investigated the electrochemical behavior of Cu^{2+}-histidine and Cu^{+}-histidine complexes by polarography. They reported that Cu^{2+}-histidine complexes are directly reduced to Cu metal by a simple two electron reduction reaction at pH = 8. They also found that Cu^{+} ions in a solution at pH = 8 containing excess histidine are not stable and easily disproportionate at the mercury surface, typical for uncomplexed Cu^{+}. Perez and co-workers [12] obtained two oxidation waves in alkaline solution at pH = 9.58 and suggested that these can be attributed to two different structures of complexes resulting from the zwitterionic properties of histidine. On the other hand, a Cu^{2+} to Cu^{+} reduction mechanism for the Cu^{2+}-histidine complex were proposed by Pena and Lopez [13] on the basis of polargraphic voltammetric results. Bilewicz [14] further obtained evidence of two consecutive steps with an intermediate Cu^{+} complex stabilized by adsorption at the mercury surface using dc, cyclic, normal pulse, and reverse pulse voltammetry. The solution used by the author was a borate buffer at pH = 7.2.

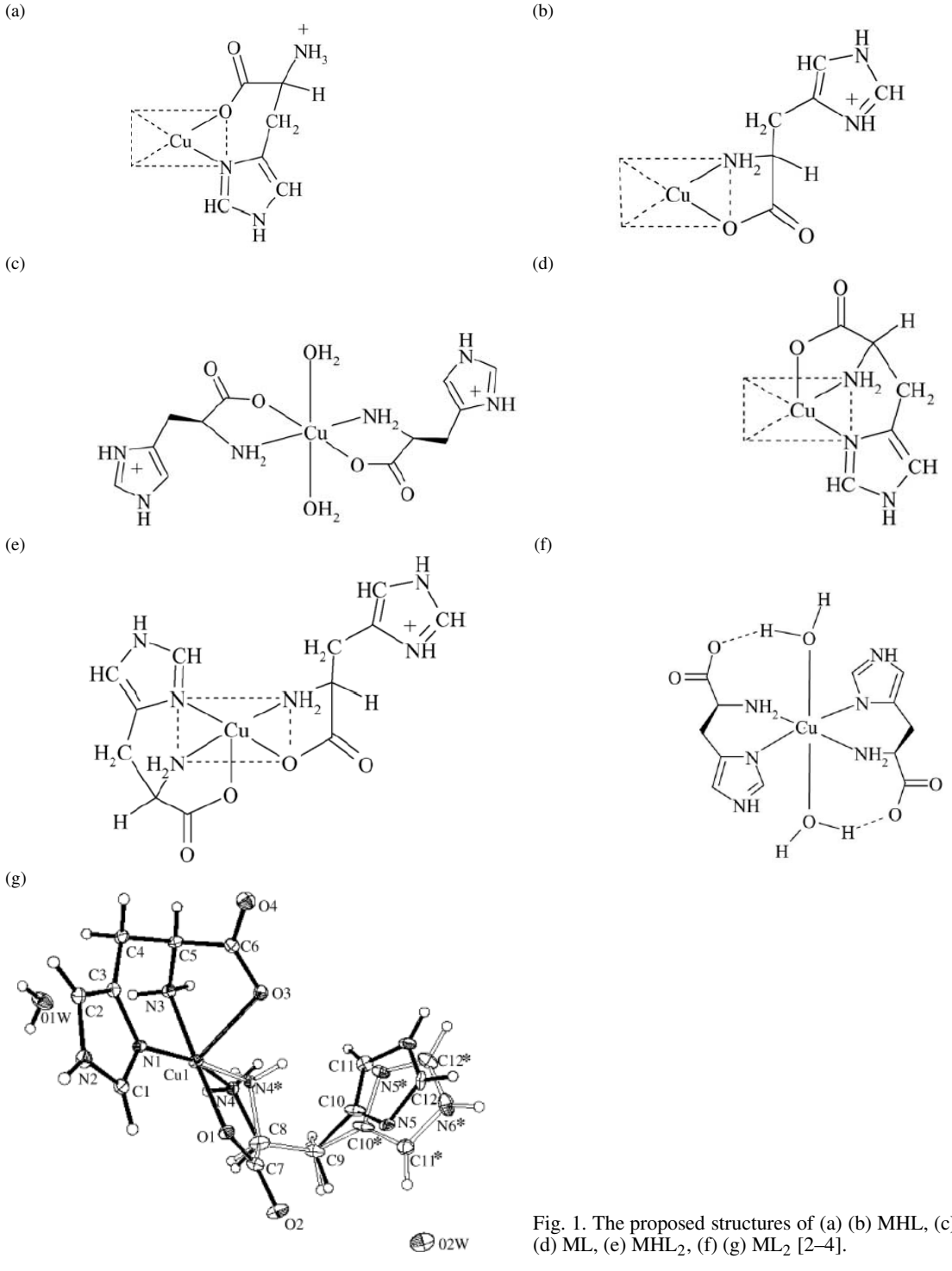


Fig. 1. The proposed structures of (a) (b) MHL, (c) MH_2L_2, (d) ML, (e) MHL_2, (f) (g) ML_2 [2–4].

Instead of using mercury electrodes, Daniele and Pena [15] studied the reduction of Cu^{2+}-histidine complexes at a solid electrode (platinum) in aqueous solution by cyclic voltammetry. They discovered that a

Cu^{+}-histidine complex is stable in aqueous solutions; however, no evidence supports its formation during electrochemical reduction of the Cu^{2+}-histidine complex. Thus, they have suggested that Cu^{2+}-histidine complexes undergo a two electron reduction process to metallic copper on the platinum electrode in solutions containing SO_4^{2-} and ClO_4^{-} at pH = 6.

Glassy carbon (GC) electrodes are frequently used as working electrode because of their excellent mechanical and electrical properties, wide potential window, chemical inertness and widely reproducible performance [16]. In this work, the electrochemical behavior of the Cu^{2+}-histidine complexes has been examined on a glassy carbon electrode in aqueous solution. The effect of the variables for the electrochemical behavior of the Cu^{2+}-histidine complexes, including the pH value, the scan rate, and the molar ratio of Cu^{2+} to histidine, is discussed in detail. Furthermore, the electron transfer number of the reduction reaction of Cu^{2+}-histidine complexes has been determined by the glassy carbon rotating disk electrode.

Experimental Section

Doubly distilled water and analytical reagent grade chemicals were used for all experiments without further purification. The Cu^{2+}-histidine complexes were prepared by mixing freshly weighed portions of $CuSO_4$ and histidine into water. The pH was adjusted using a phosphate buffer (0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4) for the pH range $3 \sim 11$. The pH value was checked by means of a S20-K SevenEasy™ pH-meter. The solutions were deaerated using nitrogen before each experiment.

Absorption spectra and circular dichroism (CD) spectra were recorded on a Varian Cary 50 conc UV/Vis spectrophotometer and an AVIS 62DS spectropolarimeter, respectively, in the 900 – 300 nm range. Cyclic voltammograms (CVs) were obtained in a three electrode cell with a glassy carbon disk (3 mm in diameter) as working electrode and a carbon strip as counter electrode. All the potentials reported in this study were referred to an Ag/AgCl reference electrode. The working electrode was pretreated with acetone in an ultrasonic bath and thoroughly rinsed with doubly deionized water before testing. Electrochemical experiments were controlled with a potentiostat-galvanostat (PAR, VERSASTAT^3) under computerized control (PAR, VERSASTAT^3 Software). The rotating disk electrode experiments were also performed using a standard three-electrode cell configuration, which was the same as the above electrochemical system with the sole difference of transforming the glassy carbon disk working electrode into a commercial glassy carbon rotating disk electrode.

Results and Discussion

Spectroscopic studies

The ultraviolet visible and circular dichroism (CD) spectra were used to identify the formation of the Cu^{2+}-histidine complexes. Histidine showed no absorption wave in the UV/Vis range, and aqueous Cu^{2+} solutions exhibited a maximum absorption at about 800 nm. UV/Vis spectra of Cu^{2+}-histidine complexes in PBS at various pH values are shown in Fig. 2. Cu^{2+} and histidine began to form a complex (MHL) at pH = 2 [2]. The absorption waves appeared at 685 and 758 nm at pH = 3 implying that the Cu^{2+}-histidine complexes and unbound Cu^{2+} coexisted in PBS. At pH = 4, only one absorption wave located at 628 nm indicated that all Cu^{2+} ions were coordinated with histidine. The predominant species at pH = 4 is MHL_2 [2]. At pH = 5, the major Cu^{2+}-histidine complex was also MHL_2. As the pH increased further, ML_2 was formed, the dominant form in the pH range 6 – 10 [2]. The absorption wave of ML_2 appeared at 640 nm. As the pH increased from pH = 6 to pH = 9, the intensity of the absorption wave increased from 0.38 to 0.45. Above pH = 9, $MH_{-1}L_2$ species were gradually formed, and the intensity of the absorption wave at 640 nm decreased due to a decrease of ML_2.

CD spectroscopy is a powerful technique to examine the conformational changes of optically active molecules based on the differential absorption of left- and right-handed circularly polarized light. Fig. 3 illustrates the CD spectrum of Cu^{2+}-histidine complexes

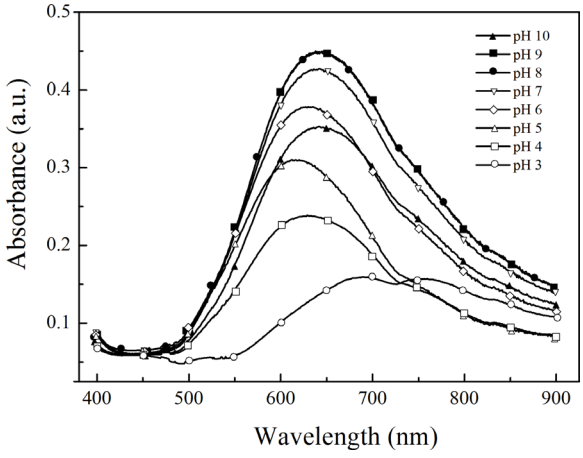
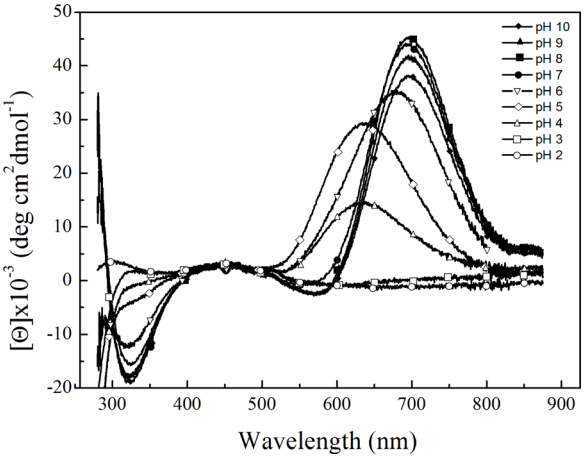


Fig. 2. UV/Vis spectra of Cu^{2+}-histidine complexes at various pH values. Testing conditions: histidine to Cu^{2+} molar ratio 10 (0.03 M histidine and 0.003 M $CuSO_4$ in 0.1 M PBS); temperature 25 °C.



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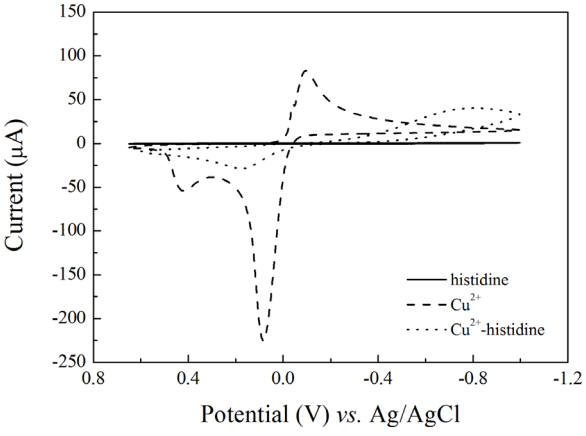

Fig. 4. CVs of the glassy carbon electrode in the presence of histidine, Cu^{2+}, and Cu^{2+}-histidine complexes, in 0.5 M Na_2SO_4 at pH = 6.

in a wide pH range from 2 to 10. Although the formation of the MHL complex started around pH = 2, no CD bands were observed until pH = 4. The protonated MHL_2 complex dominated at pH = 4 ∼ 5 was supported by the appearance of the positive band at 635 nm. On further increase of the pH value from 5 to 7 a rearrangement of the coordination took place leading to ML_2 species, the positive band shifting from 653 to 696 nm. The CD band decreased in intensity with an increase of the pH value from 8 to 10. Both UV/Vis and CD spectroscopic data have thus demonstrated that the stepwise formation of a variety of Cu^{2+}-histidine complexes is a function of the pH value.

Cyclic voltammograms of histidine, Cu^{2+} and Cu^{2+}-histidine complexes on a glassy carbon electrode

Initial electrochemical studies were performed by establishing, cyclic voltammograms for histidine, Cu^{2+} and Cu^{2+}-histidine complexes in 0.5 M Na_2SO_4 at pH = 6 on a glassy carbon electrode as shown in Fig. 4. It is obvious that histidine at pH = 6 exhibits no electroactivity. There are four different protonated forms of histidine, abbreviated as H_3L, H_2L, HL, L^-, as a function of pH. Their pK_a values are 1.8, 6.0 and 9.18, respectively [2]. The electrochemical analysis of these four forms showed no electrochemical activity. The CV of Cu^{2+} at a scan rate of 50 mV s^{-1} (dashed line of Fig. 3) showed a reduction of Cu^{2+} to Cu metal at the cathodic peak potential, E_{pc}, of −0.09 V. The Cu deposits on the electrode were stripped upon the reverse scan with an anodic peak potential, E_{pa}, of 0.083 V. For the Cu^{2+}-histidine complexes, the reduction current decreased, and the reduction peak potential was shifted to a more negative potential at −0.776 V. In the reverse scan, unlike the stripping peak in the free Cu^{2+} ions solutions, the oxidation wave of the Cu^{2+}-histidine complexes appeared at 0.178 V.

Effect of pH

The effect of pH on the CVs of the Cu^{2+}-histidine complexes is shown in Fig. 5, where pH = 3 ∼ 4, pH = 5 ∼ 7, pH = 8 ∼ 11 are given in Figs. 5(a), (b), (c), respectively. The concentration of histidine was 1×10^{-2} M, and the histidine to Cu^{2+} molar ratio was adjusted to 10 : 1. When the Cu^{2+}-histidine complexes are in an environment of pH = 2 and below, complexes are dissociated into free metal ions and ligands. The content of non-bonded Cu^{2+} is up to 83 % and above, so that the electrochemical behavior of the Cu^{2+}-histidine complexes at pH = 2 is similar to that of free Cu^{2+} ions in the solution. When the pH is adjusted to 3, the quantity of free Cu^{2+} ions in the solution decreases, and more complexes were formed. The Cu^{2+}-histidine complexes include the types of MHL, ML, MHL_2, and MH_2L_2 with the proportions of 43, 22, 20, and 8 %, respectively [2]. The reduction peak of the Cu^{2+}-histidine complexes at pH = 3 appears at −0.313 V, while the oxidation peak appears at 0.11 V as shown in Fig. 5(a). When comparing the redox behavior with that of free Cu^{2+}

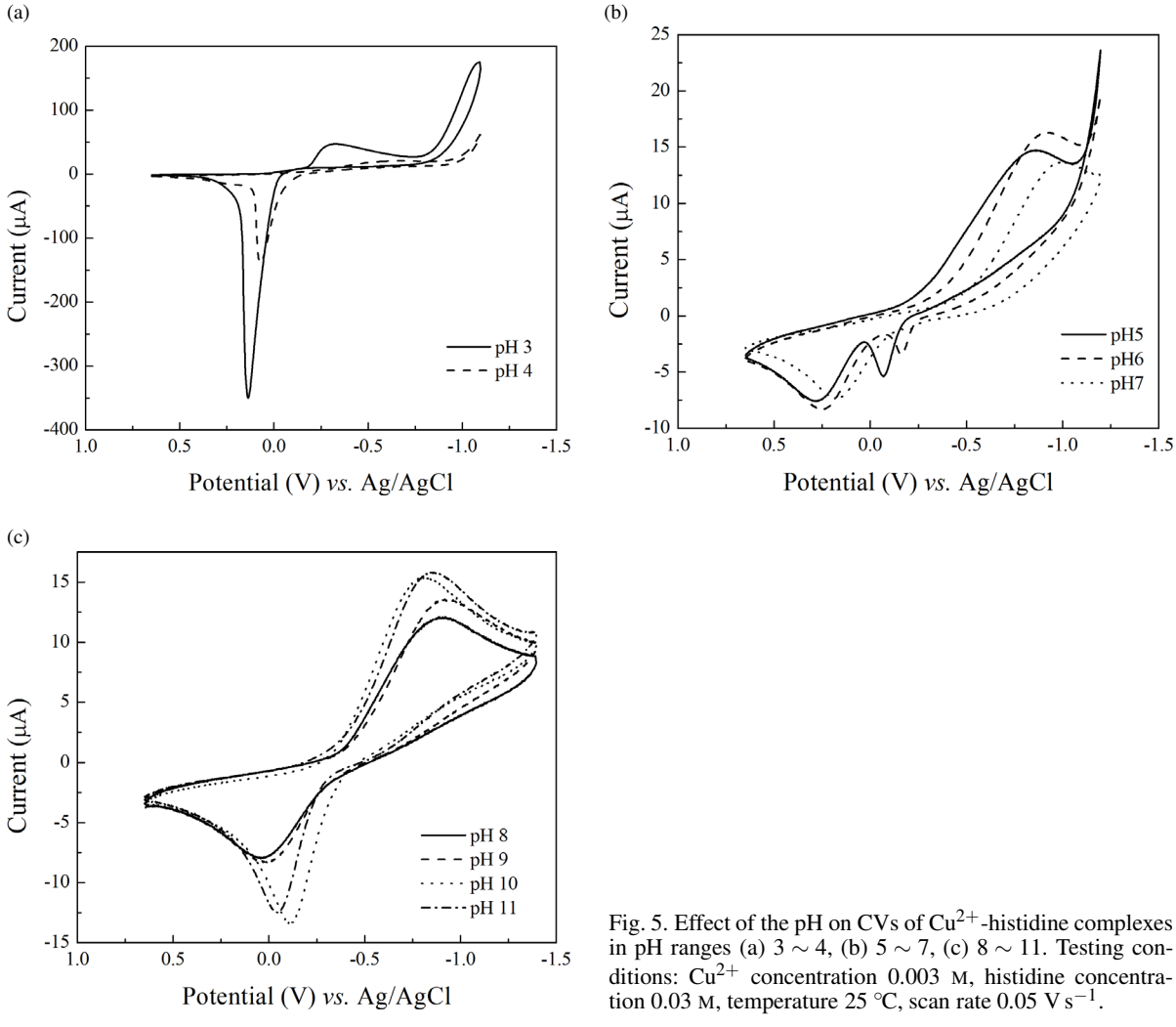

Fig. 5. Effect of the pH on CVs of Cu^{2+}-histidine complexes in pH ranges (a) $3 \sim 4$, (b) $5 \sim 7$, (c) $8 \sim 11$. Testing conditions: Cu^{2+} concentration 0.003 M, histidine concentration 0.03 M, temperature 25 °C, scan rate 0.05 V s^{-1}.

ions, we found that the reduction potential of the Cu^{2+}-histidine complexes is shifted towards a more negative direction while the oxidation peak of the Cu^{2+}-histidine complexes is at the same potential as when Cu metal is stripped from the electrode surface, showing that at pH = 3 the Cu^{2+}-histidine complexes are directly reduced to Cu metal on the electrode surface. At pH = 4, Cu^{2+} ions form complexes with histidine completely The reduction peak at -0.585 V and two oxidation peaks at 0.074 and 0.21 V were observed. The first oxidation peak is contributed by the Cu stripping while the following oxidation peak is provided by oxidation of the Cu^{2+}-histidine complexes. Although the Cu^{2+} ions completely form complexes with histidine at pH = 4, they are easily reduced to Cu metal on the electrode surface. Both the reduction and the oxidation currents of Cu^{2+}-histidine complexes are higher at pH = 3 than those at pH = 4. The redox behavior of the Cu^{2+}-histidine complexes is more irreversible at pH = 4 than at pH = 3.

Fig. 5(b) shows the comparison of cyclic voltammograms of Cu^{2+}-histidine complexes in solution at pH = 5, 6 and 7. In pH = 5 solution, the reduction peak of the Cu^{2+}-histidine complexes appears at -0.87 V, while two oxidation peaks appear at -0.06 and 0.28 V in the reverse scan. The oxidation peak potential positions and shapes suggest that the first oxidation peak is a stripping peak of Cu metal while the second oxidation peak indicates oxidation of the Cu^{2+}-histidine complexes When scanning towards a more negative

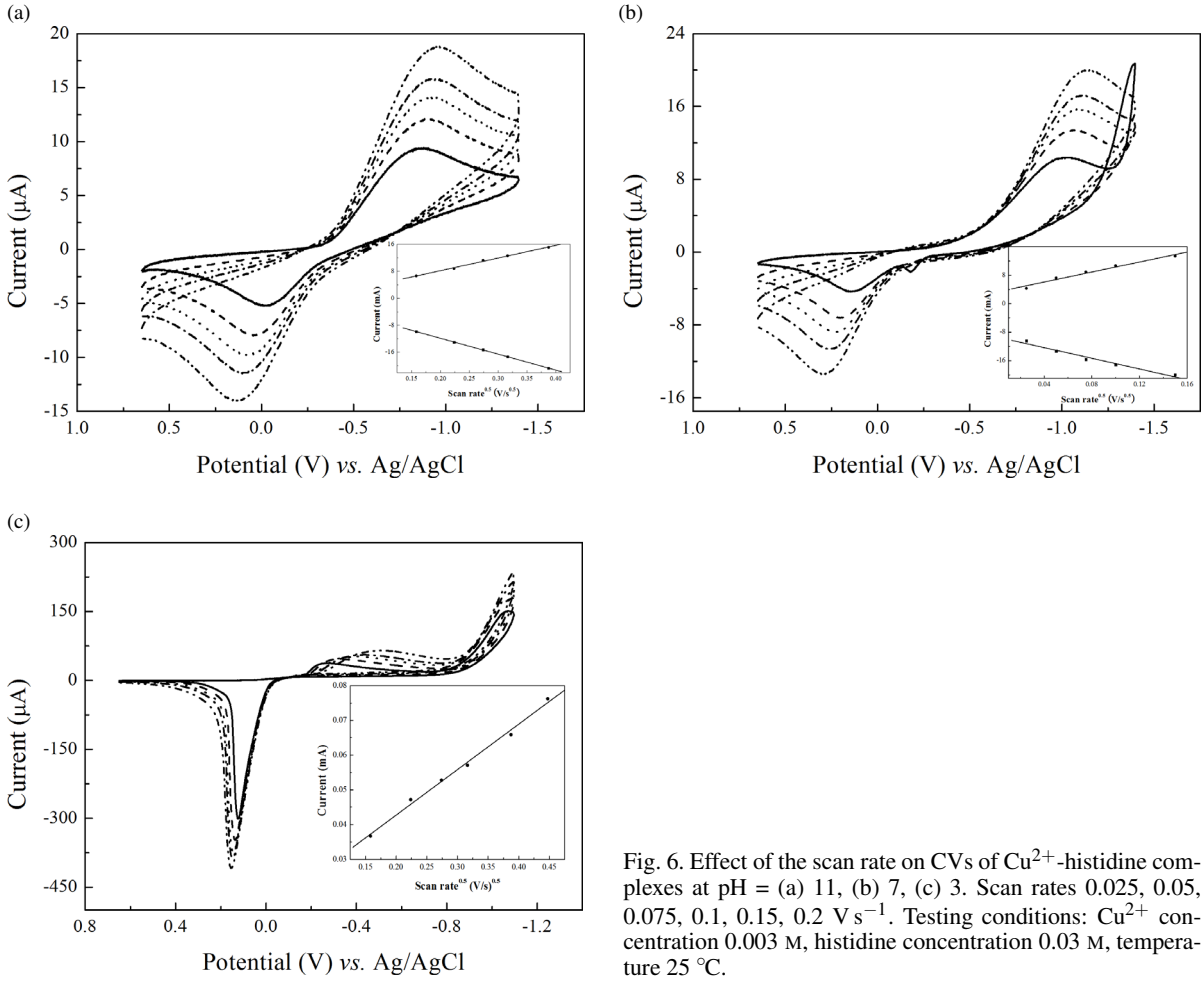

Fig. 6. Effect of the scan rate on CVs of Cu^{2+}-histidine complexes at pH = (a) 11, (b) 7, (c) 3. Scan rates 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 V s^{-1}. Testing conditions: Cu^{2+} concentration 0.003 M, histidine concentration 0.03 M, temperature 25 °C.

potential above −1.2 V, a higher stripping peak was observed, indicating that the formation of a Cu deposit on the electrode surface benefits from hydrogen evolution. The reduction peak of the Cu^{2+}-histidine complexes was shifted to more negative potential when the pH value increased from 5 to 7. In addition, an increase of the pH value resulted in a decrease of the stripping peak Sarkar *et al.* reported that the complex structure of the Cu^{2+}-histidine complexes in the pH = 5 solution includes the types of ML, MHL_2, and ML_2 [2]. When the pH value increases to pH = 6, the proportion of ML_2 continuously increases, while that of the complex MHL_2 decreases. Since the stability constant of the ML_2 structure is larger than that of ML and MHL_2 [2–4] it is reasonable that the Cu^{2+}-histidine complexes at pH = 6 are more difficult to reduce than those at pH = 5, which results in a significant shift of the reduction peak to more negative potential and a decrease of the stripping peak in the reverse scan.

Fig. 5(c) displays the cyclic voltammograms of the Cu^{2+}-histidine complexes in the pH = 8, 9, and 10 solutions, where the reduction peaks appear in the range from −0.8 to −0.9 V while the oxidation peaks are located in the range from 0.03 to −0.11 V. Both the reduction and oxidation peaks at high pH values (pH = 8 ∼ 10) are shifted towards more negative potentials than those of medium pH (pH = 5 ∼ 7) solutions. Furthermore, hydrogen evolution takes place at a much more negative potential in high-pH solutions, and a stripping peak exists in the reverse scan only when a very negative potential is scanned above −1.4 V in the forward scan. The reduction current in high-pH solutions is less than that in medium-

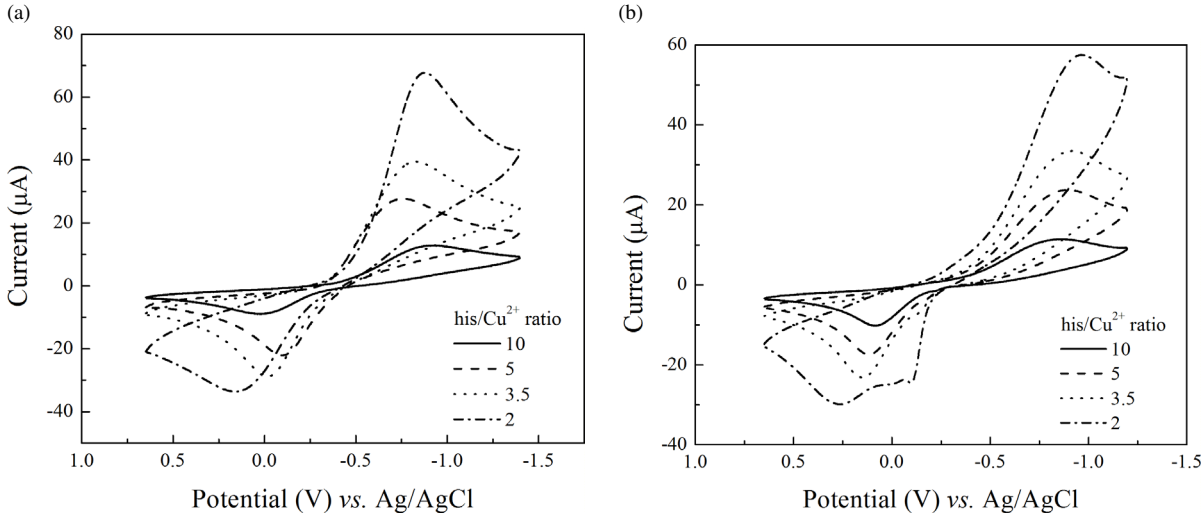

Fig. 7. Effect of the metal ion to ligand ratio on CVs of Cu^{2+}-histidine complexes with a fixed concentration of Cu^{2+} at pH = (a) 10, (b) 7. Testing conditions: Cu^{2+} concentration 0.003 M, temperature 25 °C, scan rate 0.05 V s^{-1}.

and low-pH solutions, indicating that the complexes in high-pH solutions are more stable than those in medium- and low-pH solutions. There are merely ML_2 and $MH_{-1}L_2$ complex types existing in the solution of pH = 8 and above. With an increase of pH from 8 to 10, ML_2 decreases while $MH_{-1}L_2$ increases, and the stability constants of ML_2 and $MH_{-1}L_2$ are larger than those of other types [2]. The influence of the pH value on the electrochemical behavior of the Cu^{2+}-histidine complexes on the glassy carbon electrode thus strongly depends on the nature of the complexes.

Effect of scan rate

The effect of the scan rate on the electrochemical behavior of the Cu^{2+}-histidine complexes at pH = 11, 7, and 3 is shown in Fig. 6. Fig. 6(a) shows the cyclic voltammograms of the Cu^{2+}-histidine complexes in pH = 11 solution with different scan rates. When the scan rate increases from 0.025 to 0.2 V s^{-1}, the reduction and oxidation peaks of the Cu^{2+}-histidine complexes shift towards more negative and more positive potential, respectively. Drawing the graph of the square root of the scan rate *vs.* the reduction and oxidation peak currents, linear relations are found indicating that the reduction and oxidation reactions of the Cu^{2+}-histidine complexes are controlled by mass transfer.

With a different scan rate for the pH = 7 solution, as shown in Fig. 6(b), the stripping peak in the reverse scan is obtained only at the low scan rate of 25 mV s^{-1}, suggesting that the Cu^{2+}-histidine complexes at a low scan rate of 25 mV s^{-1} are reduced more completely on the electrode surface. The subsequent hydrogen evolution reaction further enhances the decomposition of the reduced state of the complexes to form a Cu deposit on the electrode surface. Similarly, the graph of the square root of the scan rate *vs.* the reduction and oxidation peak current of the Cu^{2+}-histidine complexes at pH = 7 shows a linear relation, indicating that both the reduction and oxidation reactions of the complexes are controlled by mass transfer in the pH = 7 solution. Nonetheless, changing the scan rate in the pH = 11 solution does not lead to an oxidation stripping peak as in the pH = 7 solution, showing that the complexes present in high-pH solutions are more stable than in medium-pH solutions.

When the pH value is adjusted to 3, Fig. 6(c), the oxidation and reduction current is higher than that in the pH = 11 and pH = 7 solutions. Since free Cu^{2+} ions exist in the pH = 3 solution, the electrochemical behavior is similar to that when Cu^{2+} ions exist alone. Moreover, a higher scan rate leads to a higher reduction current of the Cu^{2+}-histidine complexes resulting in a higher oxidation stripping peak current in the reverse scan. In the pH = 3 solution, the relation of the scan rate to the reduction peak current of the Cu^{2+}-histidine

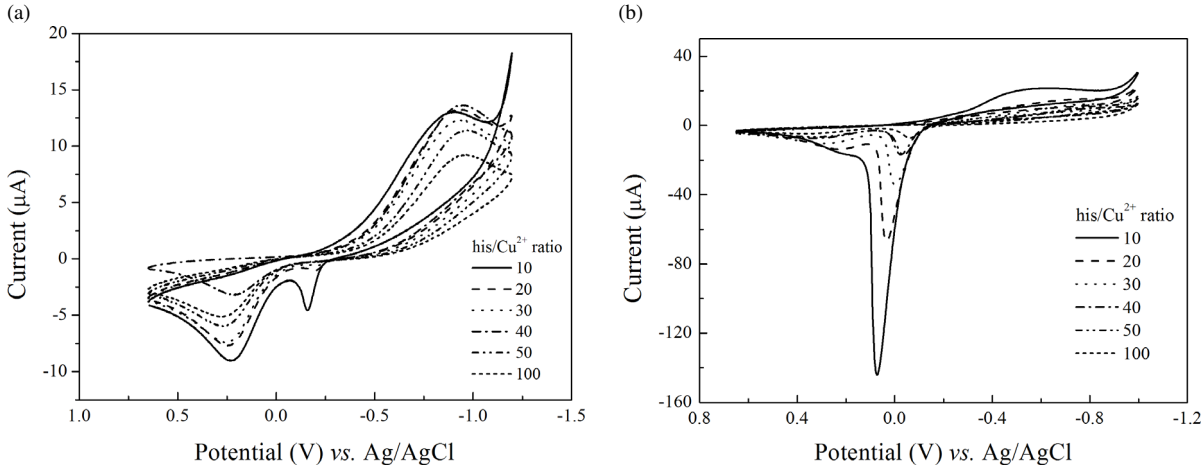

Fig. 8. Effect of the metal ion to ligand ratio on CVs of Cu^{2+}-histidine complexes with a fixed concentration of histidine at pH = (a) 6, (b) 4. Testing conditions: histidine concentration 0.03 M; temperature 25 °C, scan rate 0.05 V s^{-1}.

complexes was further analyzed. The results show a linear relation between the reduction peak current of the Cu^{2+}-histidine complexes and the square root of the scan rate, indicating that the reduction of the Cu^{2+}-histidine complexes is controlled by mass transfer.

Effect of the metal ion to ligand ratio

Figs. 7(a) and 7(b) illustrate the cyclic voltammograms for a fixed histidine concentration changing the concentration of the Cu^{2+} ions in the pH = 10 and pH = 7 solutions, respectively. As proposed in the literature [2], when the molar ratio of histidine to Cu^{2+} ions (his/Cu^{2+}) is larger than 2, Cu^{2+} ions in the solution are completely chelated by histidine. Fig. 7(a) shows that the concentration of the Cu^{2+}-histidine complexes increases when the concentration of the Cu^{2+} ions increases; this further results in larger reduction and oxidation currents of the Cu^{2+}-histidine complexes. Besides, in the pH = 10 solution, the complexes do not give oxidation stripping peaks upon an increase of the Cu^{2+} ion concentration, showing that the complexes are more stable in the high-pH solution. However, with the increase of the Cu^{2+} ion concentration in the pH = 7 solution, the oxidation stripping peak in the reverse scan becomes more apparent in addition to the increase of the reduction and oxidation peak current of the Cu^{2+}-histidine complexes. These results indicate that the Cu^{2+}-histidine complexes in the pH = 7 solution are less stable than those in the pH = 10 solution and more easily form a Cu deposit on the electrode surface during the reduction process.

Figs. 8(a) and 8(b) show the cyclic voltammograms of a fixed Cu^{2+} ion concentration and a changing histidine concentration in the pH = 6 and pH = 4 solutions, respectively. When the molar ratio of his/Cu^{2+} is 10, Fig. 8(a), not only hydrogen evolution reactions occur at a high potential above −1.1 V, but the oxidation stripping peak in the reverse scan is also obvious. When the concentration of histidine is continuously increased, the hydrogen evolution potential moves to more negative values, and the oxidation stripping peak current in the reverse scan decreases. After increasing the concentration of histidine, the complexes in the pH = 6 solution become more stable. Nonetheless, in the pH = 4 solution, Fig. 8(b), even though the ratio of molar concentrations of histidine to Cu^{2+} ions has increased from 10 to 100, the complexes are still easily reduced to form a Cu deposit on the electrode surface during the reduction process that results in an appearance of the oxidation stripping peak, showing that the complexes in the pH = 4 solution are relatively unstable.

Electron transfer number

A glassy carbon rotating disk electrode was applied to obtain the electron transfer number of the reduction reaction of the Cu^{2+}-L-histidine complexes. The Koutecky-Levich Equation (Eq. 1) describes the relation between the current and the angular velocity in the

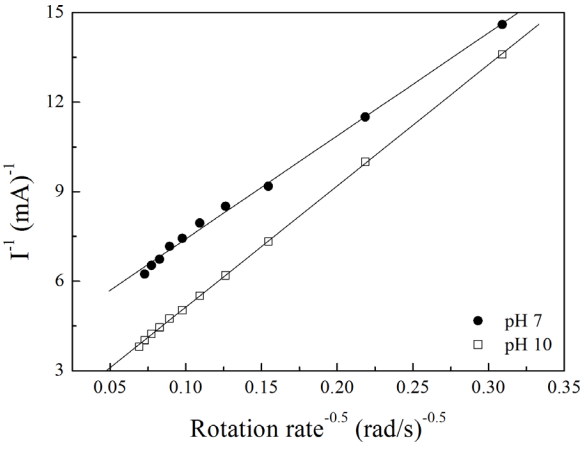

Fig. 9. Plot of i^{-1} against $\omega^{-0.5}$ for a glassy carbon electrode for a 0.1 M phosphate solution containing 0.003 M Cu^{2+}-histidine complexes at pH = 7 and pH = 10.

rotating electrode system:

$$\frac{1}{i} = \frac{1}{i_k} + \frac{1}{i_l} = \frac{1}{i_k} + \frac{1}{0.62nFC_bD^{\frac{2}{3}}v^{-\frac{1}{6}}w^{\frac{1}{2}}} \qquad (1)$$

where n is the number of electrons transferred per Cu^{2+}-histidine complex, F the Faraday constant (96487 C mol^{-1}), C_b the bulk concentration of the Cu^{2+}-histidine complexes, D the diffusion coefficient of the Cu^{2+}-histidine complexes (5.69×10^{-6} cm s^{-1}) [14], v the kinematic viscosity (0.01 cm^2 s^{-1} for the solvent), ω the electrode rotation rate, i_l the limiting current and i_k the charge transfer current. Typical i^{-1} against $\omega^{-0.5}$ plots at -0.9 V are shown in Fig. 9. The electron transfer number can be obtained from the slope. In the pH = 7 and 10 solutions, the n value of the Cu^{2+}-histidine complexes is 1.65 and 1.68, respectively. Thus, reduction of the Cu^{2+}-histidine complexes on the glassy carbon electrode can be regarded as a two electron transfer process.

Conclusions

The electrochemical behavior of Cu^{2+}-L-histidine complexes on a glassy carbon electrode is closely related with the nature of the complexes. The Cu^{2+}-histidine complexes that possess a high stability constant in high-pH solutions (pH = 8 $\sim$ 11) are not easily reduced to Cu metal on the electrode surface directly, while the complexes in medium-pH solutions (pH = 5 $\sim$ 7) are easily affected by a hydrogen evolution reaction accelerating the generation of a Cu deposit on the electrode surface. On the other hand, the complexes present in the low-pH solutions (pH = 3 $\sim$ 4) are very unstable. The complexes are easily reduced to produce large amounts of Cu metal on the electrode surface. The reduction and oxidation peak currents of the Cu^{2+}-histidine complexes show a linear relation with the square root of the scan rate. When changing the molar ratio of histidine to Cu^{2+} ions, it was found that the oxidation and reduction peak currents of the Cu^{2+}-histidine complexes increase with the decrease of the his/Cu^{2+} molar ratio in high-pH solutions; the oxidation stripping peak appears more readily upon decreasing the his/Cu^{2+} molar ratio in medium-pH solutions; the complexes in low-pH solutions are less stable. Even when the his/Cu^{2+} molar ratio is increased from 10 to 100, the complexes are still easily reduced to form Cu metal on the electrode surface.

Acknowledgements

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[1] B. Sarkar, T. P. A. Kruck in *Biochemistry of Copper*, (Eds: J. Peisach, P. Aisen, W. Blumberg), Academic Press, New York **1966**, pp. 183.
[2] P. Deschamps, P. P. Kulkarni, M. Gautam-Basak, B. Sarkar, *Coord. Chem. Rev.* **2005**, *249*, 895 – 909.
[3] T. P. A. Kruck, B. Sarkar, *Can. J. Chem.* **1973**, *51*, 3549 – 3554.
[4] T. P. A. Kruck, B. Sarkar, *Can. J. Chem.* **1973**, *51*, 3563 – 3571.
[5] L. Casella, M. Gullotti, *J. Inorg. Biochem.* **1983**, *18*, 19 – 31.
[6] B. Evertsson, *Acta Crystallogr.* **1969**, *B25*, 30 – 41.
[7] B. A. Goodman, D. B. McPhail, H. K. J. Powell, *J. Chem. Soc., Dalton Trans.* **1981**, 822 – 827.
[8] T. Szabo-Planka, A. Rockenbauer, L. Korecz, D. Nagy, *Polyhedron* **2000**, *19*, 1123 – 1131.
[9] P. Deschamps, P. P. Kulkarni, B. Sarkar, *Inorg. Chem.* **2004**, *43*, 3338 – 3340.
[10] N. Camerman, J. K. Fawcett, T. P. A. Ktuck, B. Sarkar, A. Camerman, *J. Am. Chem. Soc.* **1978**, *100*, 2690 – 2693.

[11] D. G. Davis, W. R. Bordelon, *Anal. Lett*. **1970**, *3*, 449 – 456.
[12] A. S. Perez, F. L. Conde, J. H. Mendez, *J. Electroanal. Chem.* **1976**, *74*, 339 – 346.
[13] M. I. Pena, V. Lopez, *An. Quim.* **1986**, *82*, 28 – 33.
[14] R. Bilewicz, *J. Electroanal. Chem.* **1989**, *267*, 231 – 241.
[15] S. Daniele, M. J. Pena, *Electrochim. Acta* **1993**, *38*, 165 – 174.
[16] J. Wang, *Analytical Electrochemistry*, 3rd Edition, John Wiley, Hoboken, New Jersey **2006**, pp. 131.