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A simple method for determination of D-penicillamine on the carbon paste electrode using cupric ions

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ABSTRACT

The interaction of D-penicillamine (PA) with copper at the carbon paste electrode (CPE) in the presence of cupric ions (Cu^{2+}) was used for the determination of PA at very low potential (0.1 V vs. Ag/AgCl) without applying of any modifier. The electrochemical response of copper is changed considerably in the presence of negligible amount of PA. In this report some important parameters, such as pH effect, Cu^{2+} concentration and scan rate are studied, which the selected conditions were acetate buffer (pH = 6) and 1 mM Cu^{2+}. The linear range for PA was from 1.0×10^{-6} to 1.0×10^{-4} M with an experimental detection limit of 1.0×10^{-7} M. The relative standard deviation for 6 measurements was 3.8%. The interfering effects of some important inorganic ions were investigated, which there was no significant effect on the PA measurements. Also three organic interferences including ascorbic acid (AA), uric acid (UA) and L-cysteine (Cys) were examined, which the effect of AA was not notable, the interference of UA was moderate and for Cys was significant, but moderate at the levels which fined in the urine samples. This method was applied successfully for the determination of PA in urine sample.

1. Introduction

D-penicillamine (PA) with the synonyms of 3,3-dimethyl-D-cysteine, 3-mercapto-D-valine and D-(−)-2-amino-3-mercapto-3-methylbutanoic acid, is the characteristic acid degradation product of β-lactam antibiotics [1]. It is a chelating agent which is used to aid the elimination of copper in the treatment of hepatolenticular degeneration (Wilson's disease). It has been used in cystinuria, in heavy metal poisoning and for the treatment of rheumatoid arthritis [2]. It is also used as an antifibrotic agent to treat scleroderma [3], and as an antirheumatic drug to treat patients with active rheumatoid arthritis [4].

Due to its biological importance there have been many attempts made to determine PA, thus several methods have been reported for its analysis in both pharmaceutical and biological samples. Some of these methods include colorimetry, fluorimetry [5], chromatography [6], flow injection analysis [7], electrophoresis [8], potentiometry [9], voltammetry [10,11], PMR spectrometry [12] and NMR spectrometry [13].

Among these methods, electroanalytical methods have attracted more attention due to their accuracy, sensitivity, lower cost, and simplicity. It is well known that the direct electrochemical oxidation of many materials at the surface of bare electrodes such as glassy carbon or carbon paste electrodes is irreversible, and a high overpotential is required for their oxidation [14], therefore heterogeneous [15] or homogeneous [16] mediators are usually used.

In this study PA was determined electrochemically at very low potentials using the interaction of cupric ions with PA at a bare CPE. The effects of some important parameters were optimized. This procedure was used successfully for the determination of PA in a urine sample.

2. Experimental

2.1. Apparatus

Electrochemical studies were carried out using a Metrohm computer-controlled electroanalyzer model 797 VA Computrace. The instrument software for signal analyzing was 797 VA Computrace (version 1.2). The three-electrode system consists of the bare CPE as working electrode, Ag/AgCl (3 M KCl) as reference electrode and a Pt wire as the counter electrode. The body of the working electrode was a Teflon cylinder (2.0 mm i.d.), which was tightly packed with carbon paste. A stainless steel rod was inserted into the Teflon tube containing carbon paste to establish the electrical contact [14].

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2.2. Chemicals

Spectroscopic grade paraffin oil (Uvasol®) and graphite fine powder (extra pure, particle size ≤50 μm) were used for paste preparation, and obtained from Merck. D-penicillamine was purchased from Sigma, and copper (II) nitrate three hydrate was obtained from LobaChemie. All materials and reagents were analytical grade and used without further purification. All solutions were prepared with double distilled water.

2.3. Electrode preparation

A carbon paste electrode (CPE) was prepared by carefully hand mixing the graphite powder with paraffin oil (70:30) to obtain a uniform paste. This paste was packed into the end of the Teflon cylinder hole, and then polished on a smooth paper. Before each measurement for the elimination of any memory effects, the electrode surface was easily and rapidly renewed, and a few potential cycles (vs. Ag/AgCl) within the range of −0.5 to 0.5 V were applied in the buffer solution to obtain a constant background current. The RSD% of this procedure was smaller than 5% which is acceptable.

3. Results and discussion

3.1. Electrochemical determination of PA

PA has a large over voltage on the bare CPE, inset of Fig. 1 shows that 10 μM PA has no obvious peak up to 1000 mV (vs. Ag/AgCl) on the CPE. In Cu^{2+} solution 2 pairs of redox peaks, which may be related to Cu^0/Cu^I and Cu^I/Cu^{II}, are seen. In this study the influence of PA on the peak currents of Cu^{2+} in solution was used for an indirect determination of PA. For obtaining the best results some important parameters were optimized. Fig. 1 illustrates the influence of PA on the redox signals of Cu^{2+} at pH 6. As this figure shows the presence of a very low concentration of PA (10 μM) causes considerable change in the copper oxidation peak currents (I_{pa}) and potentials (E_{pa}), which indicates a major interaction between PA and Cu. Given the low concentration of PA relative to the copper ions in solution, it can be concluded that a solution interaction is not the main reason for the copper peak current changes in the presence of PA. There are many reports, and also recently a comprehensive review, on promising drug applications in corrosion inhibition [17]. One mechanism for drug inhibition is the adsorption of drug molecules on the metal surface at low concentration. When solution pH is close to drug pK_a the adsorption is strongest [17]. In the present study, it is likely that the adsorption of PA on copper plated on the electrode surface causes the copper redox peaks to change, especially at pH 6, where deprotonation of the two functional groups of PA is more probable.

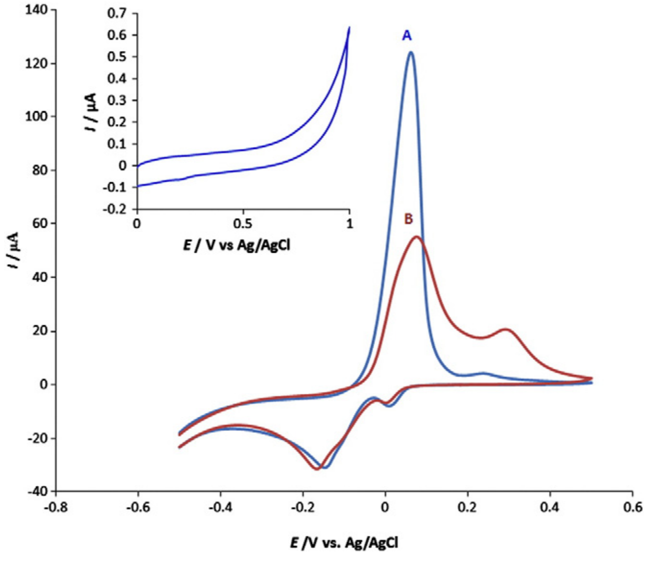

Fig. 1. Cyclic voltammograms of 1 mM of Cu^{2+} (A) and 1 mM of Cu^{2+} containing 10 μM of PA (B) on CPE. Inset is the CV of 10 μM PA on CPE. Conditions: acetate buffer (pH = 6) and scan rate of 100 mV s^{-1}.

3.2. pH optimization

It well known that the electrochemical behavior of PA is dependent on the pH of the solution [15]. Also, PA has three pK_a values related, respectively, to $-COOH$ (1.8), $-NH_2$ (7.9) and $-SH$ (10.5) groups [18]. Therefore, pH can influence the interaction between PA and Cu^{2+} by changing the PA charge. For this study, acetate buffer of various pH values ($3 \leq pH \leq 6$) was used as supporting electrolytes, and the response of the electrode to 1 mM Cu^{2+} solution in the presence and absence of 10 μM PA was determined. It should be noted that at pH greater than about 6, Cu^{2+} may precipitate.

Acetate buffer is most commonly used in the pH range 3.5–5.5. Acetate forms complexes with Cu(II) by the stepwise equilibria; the equilibrium constants for the formation of $Cu(Ac)^+$, $Cu(Ac)_2$, $Cu(Ac)_3^-$ and $Cu(Ac)_4{}^{2-}$ are 50, 10, 2.5, and 0.6, respectively [19]. This indicates that the apparent potential for Cu^{2+} reduction will be significantly decreased by the presence of acetate ion. Although no experimental data have been located for the reduction of Cu(II)–acetate complexes, a redox potential value has been reported for the reduction of the 1: 1 Cu(I) complex with acetate [20]:

$$Cu(Ac) + e^- \leftrightarrow Cu(s) + Ac^- \qquad E^0 = +0.276\,V. \tag{1}$$

This potential indicates that the K_{sp} value for Cu(Ac) is about 7.5×10^{-5}. Thus, any electrochemical studies on copper that are conducted in the presence of acetate buffer will presumably be affected by the formation of acetate complexes [21].

Acetate is not the only buffer that causes difficulties when used in studies on copper systems. All Brønsted bases are also Lewis bases and, therefore, potential complexing agents [21].

In this study the interaction of acetate buffer with copper ions was used for shifting Cu^{2+} reduction potential to more negative potentials, and hence the overlap probability of interfering peak potentials decreased.

Table 1 summarized the Δi_{pa} and ΔE_{pa} (vs. Ag/AgCl) for the first oxidation peak after addition of PA. The results show that the maximum current difference is obtained at pH 6, therefore, this pH value was chosen for the rest of the experiments. At this pH the process is different, and instead of increasing current after addition of PA as at lower pH values, a decrease is observed. This may be related to deprotonation of the amine group at pH 6, and increase of the negative charge of PA.

3.3. Influence of the scan rate

Fig. 2 shows the effect of scan rate (υ) on the cyclic voltammograms of the first oxidation peak of 1 mM Cu^{2+} in the presence of 10 μM PA in 0.1 M acetate buffer solution at pH of 6 (ABS6). The inset of this figure illustrates the plot of $\sqrt{\upsilon}$ vs. i_{pa} in the range of 20–150 mV s^{-1} which

Table 1
The first oxidation peak current and potential for 1 mM Cu^{2+} in the presence and absence of 10 μM PA (penicillamine) (E_{pa} vs. Ag/AgCl).

pH	E_{pa} (mV)		i_{pa} (μA)		ΔE_{pa}	$\lvert\Delta i_{pa}\rvert$
	Cu^{2+}	Cu^{2+}+PA	Cu^{2+}	Cu^{2+}+PA		
6	61.5	73.4	124.00	47.10	11.9	76.9
5	45.6	115.0	77.90	128.00	69.4	50.1
4	113.0	177.0	103.00	133.00	64.0	30.0
3	89.3	177.0	63.10	114.00	87.7	50.9