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Low-Potential Amperometric Determination of Reduced Glutathione at Physiological pH with an Electrodeposited Cu Nanoparticles Modified Pt Electrode

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The electrodeposition of Cu nanoparticles was investigated at Pt electrode in 10 mM $Cu(NO_3)_2$ in the presence and absence of NH_4Cl. Pure Cu nanoparticles was synthesized in a solution containing NH_4Cl, and Cu/Cu_2O composite film was obtained without NH_4Cl. In addition, rare Cu decanedrons was electrochemically synthesized in 10 mM $Cu(NO_3)_2$+0.1 M $NaNO_3$. The electrodeposited Cu nanoparticles exhibits excellent electrocatalytic activity toward oxidation of reduced glutathione (GSH) at a super low potential and the electrocatalytic mechanism was discussed, which was first used for the electrochemical determination of GSH at physiological pH. Furthermore, the common interfering species, including ascorbic acid (AA), dopamine (DA), uric acid (UA), and glucose, were examined for the electrochemical detection of GSH, and the results showed that the oxidation from the interfering compounds was effectively avoided because of the super low detection potential.

Keywords: Cu nanoparticles; decanedron; electrodeposition; glutathione

1. INTRODUCTION

Reduced glutathione (γ-glutamyl-L-cysteinyl-glycine, GSH), the most abundant biological thiols, plays crucial roles in physiological and pathological processes [1]. It is considered to be one of the most important scavengers of reactive oxygen species, and its change in physiological concentrations has been directly linked to some diseases, such as Alzheimer's disease, Parkinson's disease, diabetes mellitus, atherosclerosis, arthritis, epilepsy, and numerous types of cancer [2]. It was reported that 8 fold increase [3] and 60–65% decrease [4] in the GSH level was observed from patients suffering from breast cancer and Parkinson's disease, respectively.

The methods for the detection of GSH have been mainly focused on liquid chromatography (LC) [5], capillary electrophoresis (CE) [6], fluorescence probe [7], and electrochemistry detection [2]. Electrochemical detection has attracted a lot of attention because of its simplicity, high sensitivity, high selectivity, and low instrumental cost. The electrochemical determination of thiols was relied on some mediators and materials. These mediators consist of cobalt phthalocyanine [8], pyrroloquinoline quinine [9], fluorone black [10], isoprenaline [11], and acetaminophen [12], while the materials include nickel oxide [13, 14], TiO_2 nanoparticles [15], copper hydroxide [16], $CuGeO_3$ [17], Ce-doped Mg-Al layered double hydroxide [18], poly-m-aminophenol [19], PtFeNi [20], PtNiCo [21], and carbon based electrode such as edge plane pyrolytic graphite [22], boron doped diamond electrode [23], fullerenes [24], carbon nanotubes [25], ordered mesoporous carbon [26], and graphene nanoribbon [27]. Recently, Compton [2] published a critical review, electrochemical determination of glutathione, and pointed out that electrochemical determination of GSH at low oxidation potential is promising because the common interfering species would not be oxidized at low potential. Electrodeposition is an effective method to prepare nanomaterials which was simultaneously modified on the surface of electrode. Herein, a novel electrochemical sensor based on electrodeposited Cu nanoparticles was presented for the determination of GSH at super low oxidation potential and physiological pH. To the best of our knowledge, electrochemical determination of GSH using electrodeposited Cu was first reported.

2. EXPERIMENTAL

2.1. Chemicals and solutions

GSH, glucose, uric acid (UA), ascorbic Acid (AA), dopamine (DA), and copper (II) nitrate were purchased from Aldrich (Milwaukee, WI, USA). All other chemicals used were of analytical reagent grade, and the aqueous solutions were prepared with doubly distilled water. 0.1 M pH 7.2 phosphate buffer solution (PBS) was used as the supporting electrolyte for the electrochemical experiments.

2.2. Apparatus

Electrochemical measurements were performed with a CH Instrument model 842C voltammetric analyzer (Austin, TX, USA) using a 1 mm Pt electrode as working electrode, a platinum coil as auxiliary electrode, and a Ag/AgCl electrode as reference electrode, respectively. The morphology of electrodes was directly characterized by field emission scanning electron microscope (SEM) (JSM-6701F, Jeol, Japan) using special Pt electrode (1 mm) for SEM.

2.3. Preparation of the Cu modified Pt (Cu/Pt) electrode

Prior to modification, the Pt electrode was polished to a mirror-like surface with 1, 0.3, and 0.05 μm alumina slurry, respectively. The Cu/Pt electrode was structured by electrodepositing Cu on

Pt electrode using amperometric i-t curve in a solution containing 10 mM $Cu(NO_3)_2$, 0.1 M NH_4Cl, and 0.05 M KCl, while Cu/Cu_2O film was prepared in a solution of 10 mM $Cu(NO_3)_2$ and 0.1 M $NaNO_3$.

3. RESULTS AND DISCUSSION

3.1 Electrodeposition of Cu

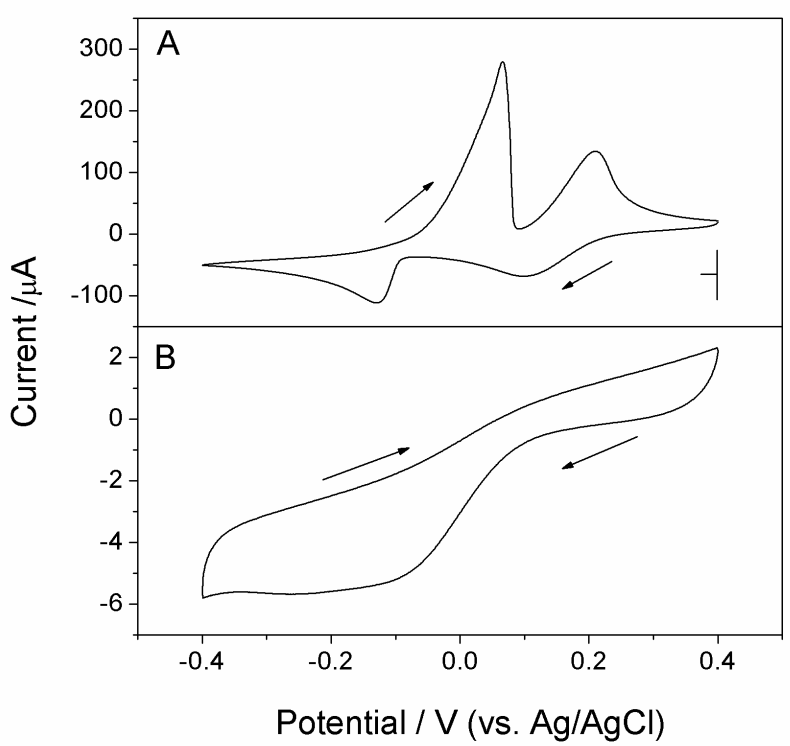

Figure 1. Cyclic voltammograms of Pt electrode in the presence of 10 mM $Cu(NO_3)_2$ (A) and 10 mM $NaNO_3$ (B). Supporting electrolyte: 0.1 M NH_4Cl+0.05 M KCl.

Figure 1 shows the cyclic voltammograms (CV) of Pt electrode in 0.1 M NH_4Cl+0.05 M KCl in the presence of 10 mM $Cu(NO_3)_2$ (A) and 10 mM $NaNO_3$ (B), respectively. It can be seen that a broad reduction peak was observed in the presence of 10 mM $NaNO_3$ at Pt electrode between 0.1 to -0.4 V (Figure 1-B), which was attributed to the reduction of NO_3^- and electrogenerated OH^- was produced according to reaction 1 [28]. The electrogenerated OH^- then reacted with NH_4^+ to form NH_3, as shown in reaction 2. The produced NH_3 will then coordinate with Cu^{2+} or Cu^+ [29]. Two pairs of redox peaks occurred on the CV at Pt electrode in the solution of 10 mM $Cu(NO_3)_2$+0.1 M NH_4Cl+0.05 M KCl (Figure 1-B). The first redox wave corresponds to reaction 3, and the second redox wave corresponds to reaction 4 [29].

$$NO_3^- + H_2O + 2\,e^- \rightarrow NO_2^- + 2OH^- \quad (1)$$

$$OH^- + NH_4^+ \rightarrow NH_3 \quad (2)$$

$$Cu(NH_3)_4^{2+} + e^- \leftrightarrow Cu(NH_3)_2^+ + 2NH_3 \quad (3)$$

$$Cu(NH_3)_2^+ + e^- \leftrightarrow Cu + 2NH_3 \quad (4)$$

3.2 SEM characterization of electrode

The morphology of Cu/Pt electrode at different electrodeposition potential was examined by SEM (Figure 2). As it can be seen in Figure 2, a single layer of Cu nanoparticles was synthesized at -0.25 V, and the diameter of particles was in the range of 50-200 nm. When -0.5 V was applied as the electrodeposition potential, several layers of Cu nanoparticles were electrodeposited on the surface of Pt electrode. The multi-layers nanostructures exhibit high active surface area, which may improve the electrocatalytic behavior.

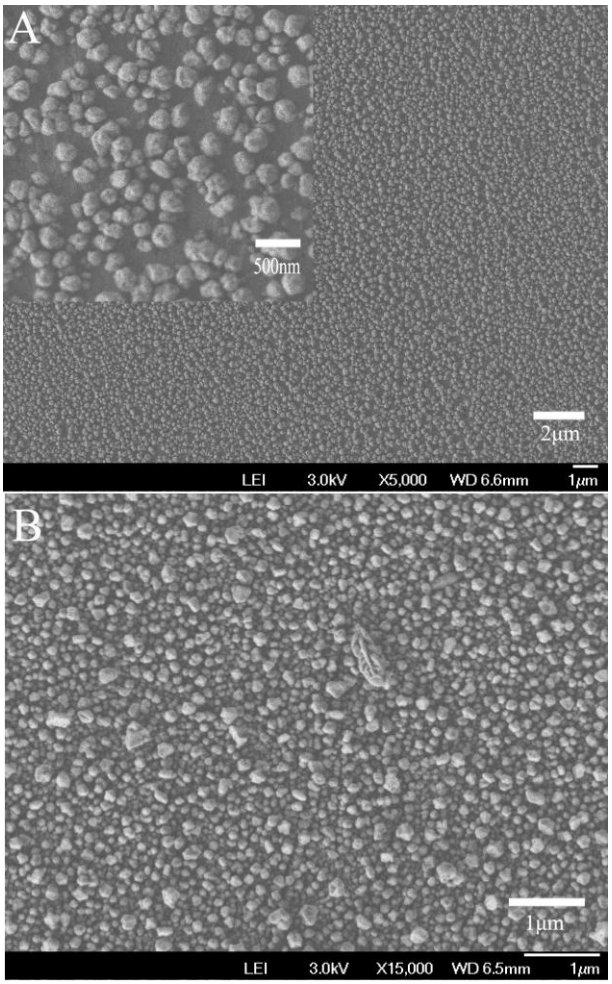

Figure 2. SEM images of Cu/Pt electrode electrodeposited in 10 mM $Cu(NO_3)_2$+0.1 M NH_4Cl+0.05 M KCl at -0.25 V (A) and -0.5 V (B).

The morphology of electrodeposited electrode in the solution of 10 mM $Cu(NO_3)_2$+0.1 M $NaNO_3$ was also investigated (Figure 3). Cu_2O can be formed by the electrochemical reaction between Cu^{2+} and electrogenerated OH^- due to the absence of NH_4^+. Therefore, under this condition, the Cu_2O/Cu composite was synthesized. We speculated that Cu_2O was at the bottom while Cu was on the top (Figure 3). In addition, rare Cu decahedron can be obtained (Figure 3-Inset).

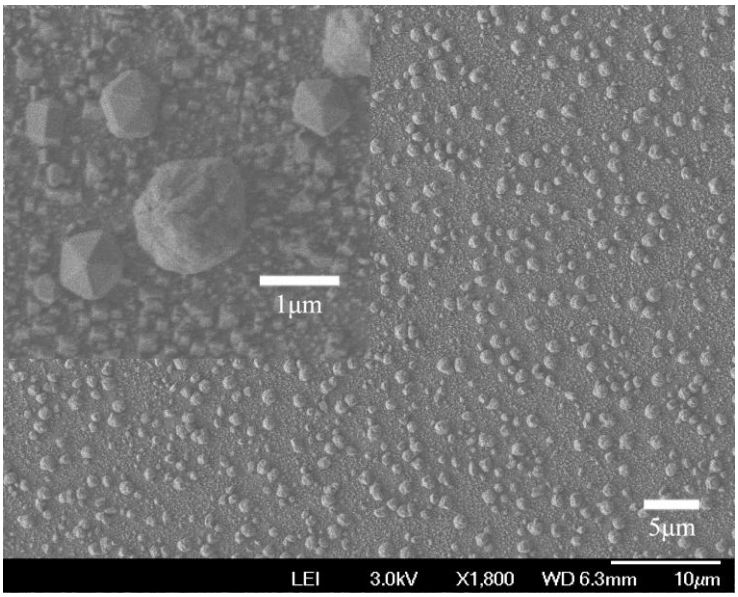

Figure 3. SEM images of $Cu/Cu_2O/Pt$ electrode electrodeposited in 10 mM $Cu(NO_3)_2$+0.1 M $NaNO_3$.

Herein, NH_4^+ plays an important part in the electrodeposition of Cu nanoparticles. It can react with OH^- to produce NH_3 which further coordinates with Cu^{2+} to prevent the formation of Cu_2O. Otherwise, Cu_2O can be obtained. It was reported that pure Cu_2O could be electrochemically synthesized in $NaNO_3$ when the temperature was about 65 ^{0}C [30]. Therefore, Cu_2O/Cu composite film was prepared in $NaNO_3$ at ambient temperature in our study.

3.3 Electrochemical detection of GSH

Figure 4 depicts the CVs of Cu/Pt electrode in the presence (solid line) and absence (dotted line) of 5 mM GSH in 0.1 M PBS (pH 7.2). Cu/Pt electrode shows a pair of redox peak in the background electrolyte, with a sharp reduction peak at 0.3V and an oxidation peak at 0.03 V, which correspond to the electrochemcial reactions between Cu (0) and Cu (Ⅱ). When GSH was added in the solution, the reduction peak decreased while the oxidation peak increased, indicating that GSH involved in the electrochemical reactions as reaction 5 and 6 [31]. Another reduction peak appeared at -0.16 V, which was attributed to the reduction of Cu^+ (reaction 7). No oxidation or reduction peak of GSH was observed at a pure Pt electrode (not shown). The super low oxidation potential of GSH might be caused by the interaction between Cu^{2+} and GSH. Furthermore, the Cu_2O/Cu also displayed the similar electrocatalytic property for GSH (not shown).

$$Cu \rightarrow Cu^{2+} + e^- \quad (5)$$

$$2Cu^{2+} + 6GSH \rightarrow 2Cu^+\text{-}[GSH]_2 + GSSG + 2H^+ \quad (6)$$

$$2Cu^+\text{-}[GSH]_2 + e^- \rightarrow Cu + 2GSH \quad (7)$$

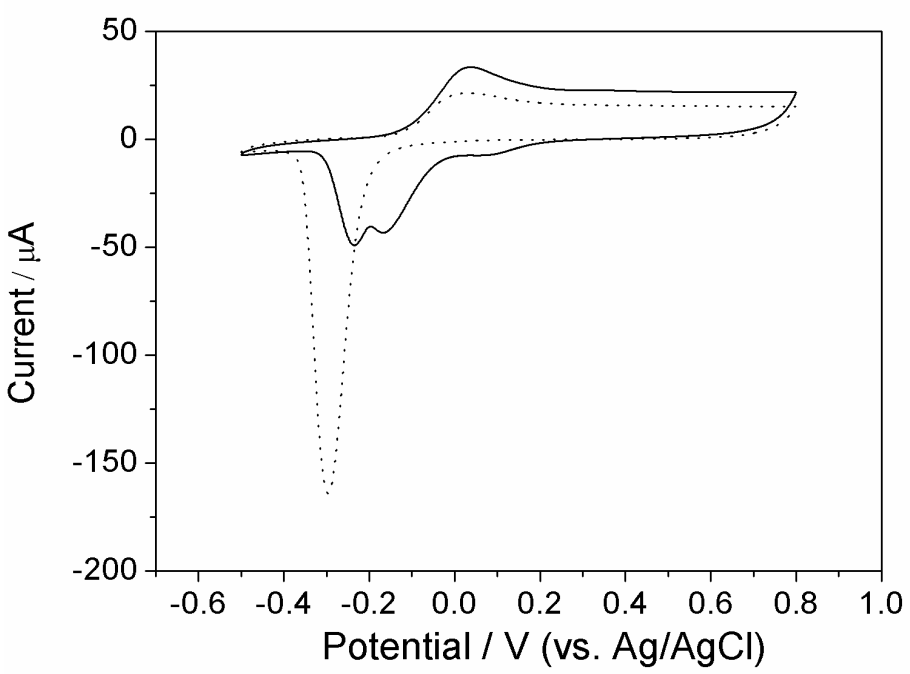

Figure 4. Cyclic voltammograms of Cu/Pt electrode in the presence (solid line) and absence (dotted line) of 5 mM GSH in 0.1 M PBS (pH 7.2).

3.4 Amperometric detection of GSH

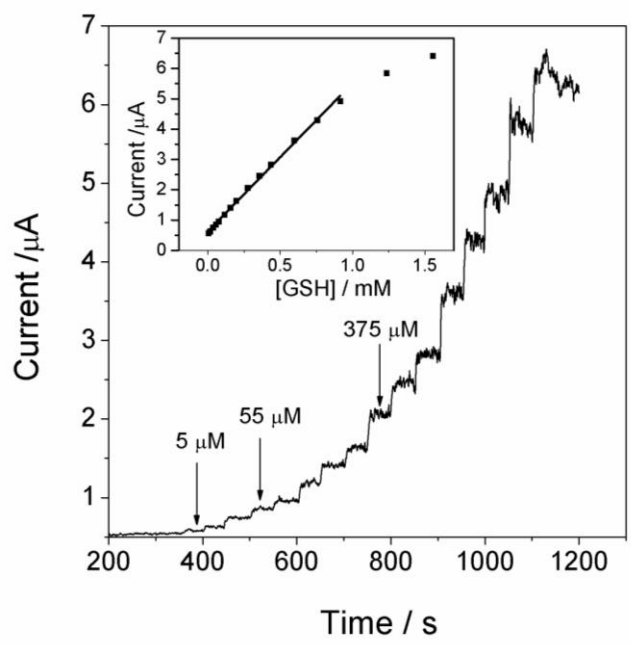

Figure 5. Amperometric sensing of GSH by successive addition of GSH at 0.03 V. Inset: (A), amperometric response with the concentration of GSH from 5 μM to 0.915 mM.

Figure 5 describes the amperometric sensing of GSH by successive addition of GSH at 0.03 V in pH 7.2 PBS. The Cu/Pt electrode showed fast response for the electrochemical detection of GSH. The linear range for GSH is from 5 μM to 0.915 mM (R^2=0.9965, n=14) with the detection limit of 5

μM and sensitivity of 71 μA mM^{-1} cm^{-2}, which is wider than that obtained at Pd-IrO_2 (10~800 μM) [32], poly-m-aminophenol (0.1~5.0 μM) [19], and NiO (0.2~6.0 mM) modified electrode [13], and is comparable to that obtained at porous NiO-CPE (0.01~6 mM) [14], but is inferior to that obtained at nanoscale copper hydroxide composite carbon ionic liquid electrode (1 μM~1.8 mM) [16].

The Cu/Pt electrode exhibited super-low detection potential, which is compared with other electrochemical methods based on other materials or mediators, as shown in Table 1.

Table 1. Electrochemical detection of GSH based on different materials or mediators

Materials/mediators	V [a]	T [b]	pH [c]	References
Cobalt phthalocyanine	0.1	*CV*	7.2	[8]
NiO microflower	0.4	*i-t*	5.0	[14]
Cobalt phthalocyaninetetrasulfonate	0.18	*i-t*	7.4	[33]
Pd-IrO_2	0.85			[32]
Mesoporous carbon	0.15	*i-t*	7.16	[26]
Fluorone black	0.42	*CV*	7.3	[10]
Pyrroloquinoline Quinone	0.5	*i-t*	3.45	[9]
Poly-m-aminophenol	0.5	*i-t*	4.0	[19]
NiO	0.6	*DPV*	7.2	[13]
Nanoscale Copper Hydroxide	0.15	*CV*	7.0	[16]
Nano-TiO_2/ferrocene carboxylic acid	0.75	*DPV*	7.0	[15]
Mesoporous carbon/CoO	0.25	*i-t*	4	[34]
Manganese dioxide	0.45	*i-t*	7.5	[35]
Isoprenaline	0.6	*i-t*	4.0	[11]
Acetaminophen	0.3	*DPV*	7.0	[12]
Electrodeposited Cu	0.03	*i-t*	7.2	Our work

[a] detection potential/ oxidation peak potential (V, vs. Ag/AgCl)
[b] electrochemical technology
[c] detection pH

It is significant to achieve the electrochemical determination of GSH at low detection potential and physiological pH because the common interfering species are easily oxidized at higher potential while applied in the real sample analysis, particular in blood samples. In addition, the feasible detection at physiological pH is promising in the living analysis.

3.5 Interference test

The effects of common interfering species on the detection of GSH, including AA, DA, UA, and glucose, were tested (Figure 6). As shown in Figure 6, 20 μM AA, 20 μM DA, 20 μM UA, and 20 μM glucose did not interfere on the measurement of 50 μM GSH because of the low detection potential.

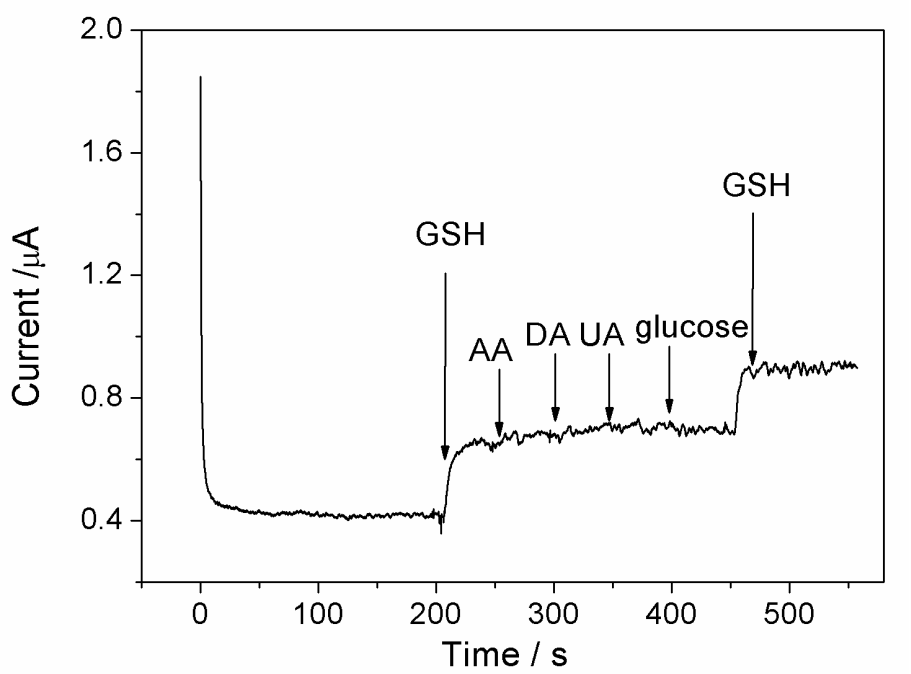

Figure 6. Interference test of Cu/Pt electrode in 0.1 M pH 7.2 PBS at +0.03 V with 50 μM GSH in the presence of 20 μM AA, 20 μM DA, 20 μM UA, and 20 μM glucose.

3.6 Reproducibility and stability

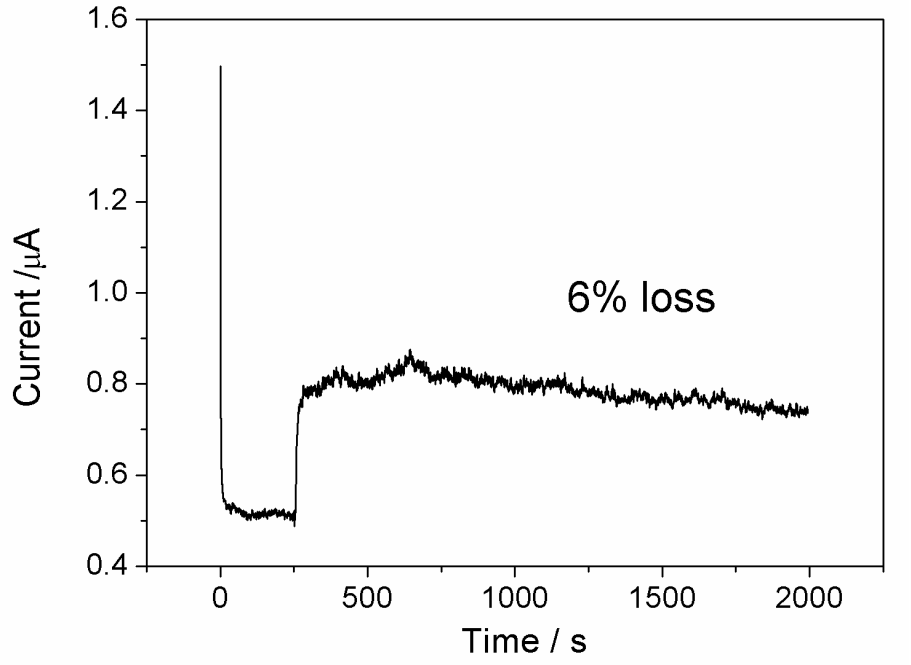

Figure 7. Stability test of Cu/Pt electrode with 50 μM GSH.

The stability and reproducibility of the GSH sensor was also investigated. The steady-state response current of the sensor retained 94% of its original current response to 50 μM GSH after 2000 s continuous measurement with magnetic stirring (Figure 7). Additionally, the long-term stability of the

sensor to GSH was also evaluated, and 9% loss on the amperometric current was observed after the Cu/Pt electrode was stored at dry N_2 atmosphere for 5 days.

3.7 Application

The Cu/Pt modified electrode could be applied in the analysis of GSH spiked in the urine sample (10 mM). The spiked urine sample was directly injected to the detection solution under stirring and analyzed without pretreatment. The detected value was 9.4 mM (RSD=3.15 %, n=3) with the recovery of 94%.

4. CONCLUSIONS

Uniform Cu nanoparticles were electrodeposited on the surface of Pt electrode by a simple electrochemical method, which was applied in the electrochemical determination of GSH at low potential and physiological pH. The results showed that the GSH sensor displayed good selectivity and stability.

ACKNOWLEDGMENTS
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