Full Paper

Application of Cu(II) Nanocomplex Modified Graphite Screen Printed Electrode to Improve the Sensitivity and Selectivity for Epinephrine Detection

Faezeh Soofiabadi,[1] Asghar Amiri[1,]* and Shohreh Jahani[2]

[1]*Department of Chemistry, Payame Noor University, P.O.BOX 19395-3697 Tehran, Iran*
[2]*Bam University of Medical Science, Bam, Iran*

*Corresponding Author, Tel.: +98 2184234114; Fax: +98 2188948994

E-Mail: a.amiri@pnu.ac.ir

Abstract- A Cu(II) nanocomplex, [CuCl$_2$(salophen)].H$_2$O [salophen=o-phenylenediaminebis(salicylidenaminato)], was synthesized. The electrochemical properties of the as-prepared Cu(II) nanocomplex modified graphite screen printed electrode (Cu/SPE) were investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Moreover, as a nanosensor for the determination of epinephrine the Cu/SPE exhibited excellent electrocatalytic activity for the oxidation of epinephrine with a faster electron-transfer rate. The DPV technique was used for the trace determination of epinephrine. The dependence of current *vs*. concentration was linear from 10.0 to 600.0 μM with a regression coefficient of 0.9975, and the detection limit of epinephrine was 2.5±0.05 μM. Finally, the method was applied to the selective and precise analysis of epinephrine in epinephrine injection.

1. INTRODUCTION

In the human organism, catecholamines affect almost all tissues, exert many important cardiovascular, metabolic, endocrine and neuronal effects, and affect the intestinal barrier and

the immune system [1]. Epinephrine (adrenaline) is the best known catechol amine, which constitute a group of compounds with a terminal amine chain attached to a benzene ring with two hydroxyl groups [2,3]. It has a key role in the functioning of central nervous system (CNS), renal, hormonal, and cardiovascular system [4]. Normal epinephrine concentration in plasma is 0-900 pg/ml, although the usual plasma concentration most often is 2385 pg/ml. However, during or following stress, the catecholamine levels rise very sharply (often 5- to 20-fold) [5-8]. The existence of abnormal levels of epinephrine causes several diseases such as phaeochromocytoma, hypoglycaemia and myocardial infarction [9]. Concentrations of 177 pg/mL of epinephrine were found in patients undergoing endotracheal tube and proseal laryngeal mask airway. In patients with postoperative pain, the epinephrine concentration was 235 pg/ml. In patients with severe traumatic brain injury undergoing a "neurological wake-up test", the levels of epinephrine increased to 750 pg/ml [10-12]. Therefore, the detection of trace amounts of epinephrine in biological fluids gives valuable information in clinical medicine on its physiological function and the diagnosis of certain diseases [13]. For this purpose, numerous attempts have been made to detect epinephrine sensitively and selectively. Among these attempts, electrochemical methods have several advantages over other methods such as their simple procedures, low cost, high selectivity and sensitivity [14-23].

Screen-printed electrodes (SPE) have attracted considerable attention in recent years because they generally offer beneficial attributes over the traditional electrodes, such as they are portable, field-based size and cost-effective sensors which offer true potential for application in-the-field [24-26]. SPE are inexpensive to manufacture which allows them to be disposable. This aspect is clearly important when testing biological samples and thus avoids surface fouling complications [27]. In addition SPE are reliable, simple to operate with high sensitivity, selectivity and are highly reproducible [28]. Unfortunately, the bare electrode usually suffer from a slow response and low reproducibility. This is probably due to relatively low electron-transfer rates are obtained at the surface of such electrodes because of the low diffusion coefficient of the analytes in the electrode [29-38]. Hence, it is significantly important to develop new materials with excellent properties and suitable designs to gain modified electrode owning superior performance. Compared with single component, the nanoparticles have certain synergistic effects such as good signal-to-noise ratio, fast electron transport and larger surface area, et al [39-50].

In the past decades, the investigations on the direct electrochemistry and electrochemical applications of metal complexes have aroused considerable interest in analytical chemistry and bioinorganic chemistry. Among many metal complexes, Cu complexes with various nitrogen donor ligands have attracted considerable attention due to diversities in their structural chemistry and their potential applications in catalysis, electrical conductivity, luminescent and biology [51,52].

According to the previous points, it is important to create suitable conditions for analysis of epinephrine in biological fluids. In this study, we describe application of novel Cu(II) nanocomplex as a nanostructure sensor for voltammetric determination of epinephrine. The proposed sensor showed good electrocatalytic effect on epinephrine. The modified electrode shows advantages in terms of selectivity, reproducibility and sensitivity. Eventually, we evaluate the analytical performance of the suggestion sensor for epinephrine determination in drug sample.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode.

All solutions were freshly prepared with double distilled water. Epinephrine and all other reagents were of analytical grade and were obtained from Merck chemical company (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

2.2. Synthesis of Cu(II) nanocomplex

Salophen ligand was synthesized similar to a previously described method [53]. Cu(II) nanocomplex is prepared by a facile low-temperature (<100 ℃) synthesis route at atmospheric pressure via reaction of salophen ligand, copper chloride under reflux. Typically, $Cu(Cl)_2.6H_2O$ (1 mmol), salophen ligand (1 mmol) and methanol (20 ml) were mixed and sonicated (2 h, 60 ℃). The obtained green solid was further purified by two-step processes using double solvent extraction with water and methanol. The solid was finally dried in a vacuum desiccator at 80 ℃ for 2 h prior to a further analysis or use.

2.3. Preparation of modified electrode

The bare graphite screen printed electrode was coated with Cu(II) nanocomplex as follows. A stock solution of Cu(II) nanocomplex in 1 ml aqueous solution was prepared by dispersing 1 mg Cu(II) nanocomplex with ultrasonication for 1 h, and a 5 µl aliquot of the Cu(II) nanocomplex/H_2O suspension solution was casted on the carbon working electrodes, and waiting until the solvent was evaporated in room temperature.

2.4. Preparation of real samples

One milliliter of a epinephrine ampoule (Caspian tamin Company, Iran, contained 200 mg in 5 ml of epinephrine) was diluted to 10 ml with 0.1 M PBS (pH 7.0); then, different volume of the diluted solution was transferred into each of a series of 25 ml volumetric flasks and diluted to the mark with PBS. The epinephrine content was analyzed by the proposed method using the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Electrocatalytic oxidation of epinephrine at a Cu/SPE

The electrochemical behavior of epinephrine is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of epinephrine. Thus the electrochemical behavior of epinephrine was studied in 0.1 M PBS in different pH values (2.0<pH<9.0) at the surface of Cu/SPE by CV. It was found that the electrocatalytic oxidation of epinephrine at the surface of Cu/SPE was more favored under neutral conditions than in acidic or basic medium. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of epinephrine oxidation at the surface of Cu/SPE.
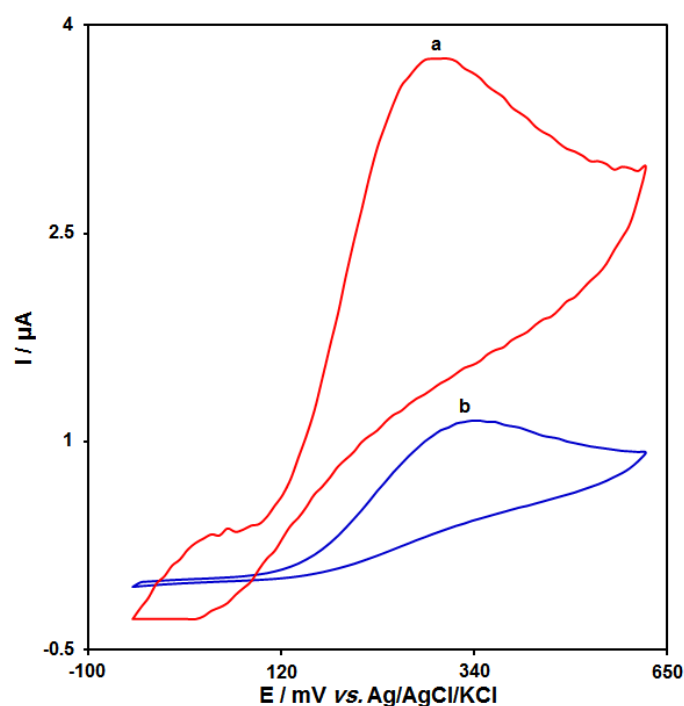

Fig. 1. Cyclic voltammograms of (a) Cu/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 600.0 μM epinephrine at the scan rate 50 mVs[-1]

Fig. 1 depict the cyclic voltammetric responses for the electrochemical oxidation of 600.0 μM epinephrine at Cu/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of epinephrine at Cu/SPE (curve a) is about 280 mV compared with 330 mV for that on the bare SPE (curve b). Similarly, when the oxidation of epinephrine at the Cu/SPE (curve a) and bare SPE (curve b) are compared, an extensive enhancement of the anodic peak current at Cu/SPE relative to the value obtained at the bare SPE (curve b) is observed. In other words, the results clearly indicate that the Cu nanocomplex improve the epinephrine oxidation signal.

The effect of potential scan rates on the oxidation current of epinephrine has been studied (Fig. 2). The results showed that increasing in the potential scan rate induced an increase in the peak current. In addition, the oxidation process is diffusion controlled as deduced from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) over a wide range from 5 to 900 mV s^{-1}.
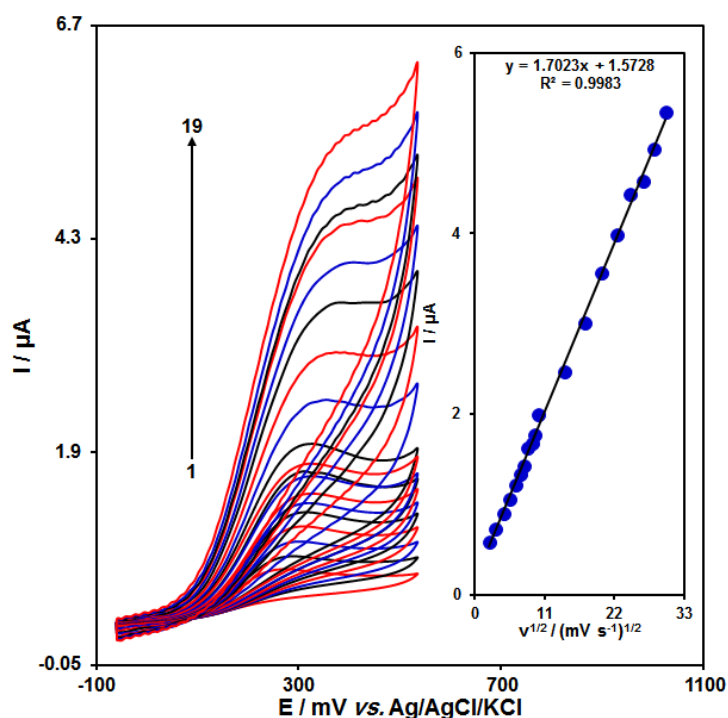

Fig. 2. Cyclic voltammograms of Cu/SPE in 0.1 M PBS (pH 7.0) containing 200.0 μM epinephrine at various scan rates; numbers 1-19 correspond to 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800 and 900 mV s^{-1}, respectively. Inset: variation of cathodic peak current *vs.* v$^{1/2}$

3.2. Chronoamperometric measurements

Chronoamperometric measurements of amitriptyline at Cu/SPE were carried out by setting the working electrode potential at 0.35 V for the various concentration of amitriptyline

in PBS (pH 7.0) (Fig. 3). For an electroactive material (epinephrine in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [54].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

Where D and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs.* t$^{-1/2}$ were employed, with the best fits for different concentrations of epinephrine (Fig. 3A). The slopes of the resulting straight lines were then plotted *vs.* amitriptyline concentration (Fig. 3B). From the resulting slope and Cottrell equation the mean value of the D was found to be 2.0×10^{-6} cm^2/s.
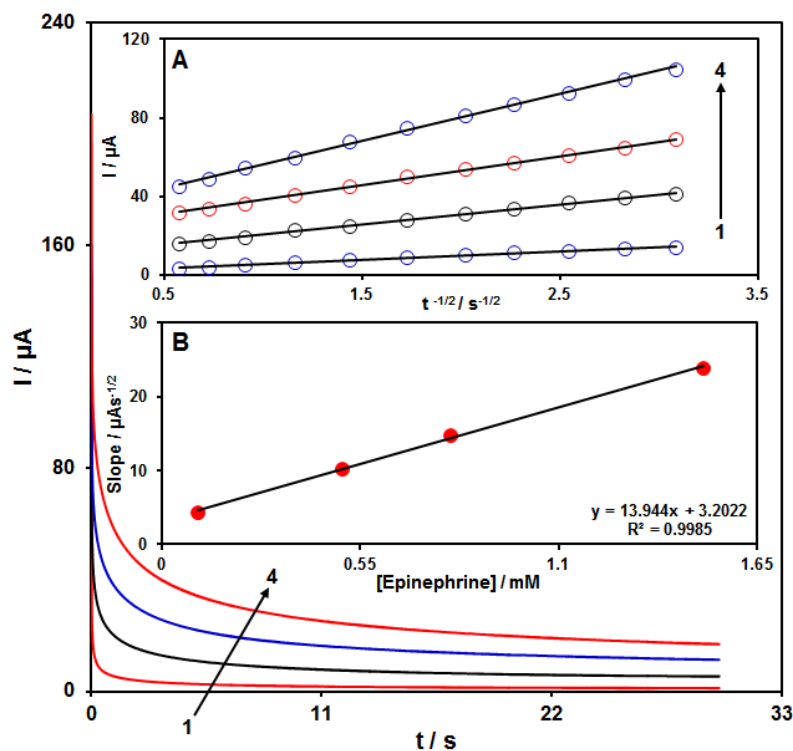

Fig. 3. Chronoamperograms obtained at Cu/SPE in 0.1 M PBS (pH 7.0) for different concentration of epinephrine. The numbers 1–4 correspond to 0.1, 0.5, 0.8 and 1.5 mM of epinephrine. Insets: (A) Plots of I *vs.* t$^{-1/2}$ obtained from chronoamperograms 1–4; (B) Plot of the slope of the straight lines against epinephrine concentration

3.3. Calibration plot and limit of detection

The peak current of epinephrine oxidation at the surface of the modified electrode can be used for determination of epinephrine in solution. Therefore, differential pulse voltammetry (DPV) experiments were done for different concentrations of epinephrine (Fig. 4).
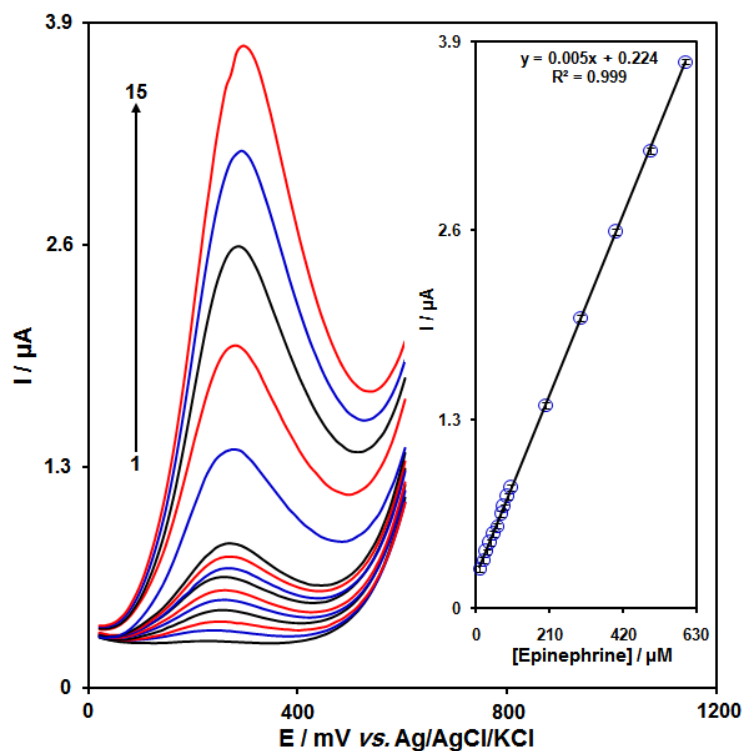
Fig. 4. DPVs of Cu/SPE in 0.1 M (pH 7.0) containing different concentrations of epinephrine Numbers 1–15 correspond to 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 200.0, 300.0, 400.0, 500.0 and 600.0 μM of epinephrine. Inset: plot of the electrocatalytic peak current as a function of epinephrine concentration in the range of 10.0-600.0 μM

Table 1. Comparison of the efficiency of electrochemical methods used in detection of epinephrine

Method	Modifier	LOD	LDR	Ref.
Electrochemical	SnO_2/graphene nanocomposite	0.017 μM	0.5-200.0 μM	[9]
Electrochemical	Poly(ionic liquids) and polypyrrole nanotubes	298.9 nM	35.0-960.0 μM	[55]
Electrochemical	Nanoporous thin Au films	2.42 μM	25.0-500.0 μM	[56]
Electrochemical	Graphene and poly(brilliant cresyl blue)	0.24 μM	1.0-1000.0 μM	[57]
Electrochemical	AuPt alloy and graphene	0.9 nM	0.0015-900.0 μM	[58]
Electrochemical	Nanoporous Au-Ag	5.05 μM	25.0-300.0 μM	[59]
Electrochemical	Multi-walled carbon nanotubes	0.029 μM	0.5-100.0 μM	[60]
Electrochemical	Polyserine and multi-walled carbon nanotubes	0.6 μM	0.5-400.0 μM	[61]
Electrochemical	Ag ion irradiated multi-walled carbon nano tube	2.0 nM	0.1-105.0 μM	[62]
Electrochemical	Cu(II) nanocomplex	2.5 μM	10.0-600.0 μM	This work

The oxidation peak currents of epinephrine at the surface of a modified electrode were proportional to the concentration of the epinephrine within the ranges 10.0 to 600.0 μM. The detection limit (3σ) of epinephrine was found to be $(2.5\pm0.05)\times10^{-6}$ M. These values are comparable with values reported by other research groups for the determination of epinephrine at the surface of modified electrodes (see Table 1).

3.4. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of epinephrine in epinephrine injection. The results for determination of epinephrine in real samples are given in Table 2. Satisfactory recovery of the experimental results was found for epinephrine. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 2. The application of Cu/SPE for determination of epinephrine in epinephrine injection (n=5). All concentrations are in μM

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Epinephrine injection	0	10.0	-	3.4
	2.5	12.3	98.4	2.7
	5.0	15.5	103.3	1.6
	7.5	17.7	101.1	2.9
	10.0	19.8	99.0	3.1

4. CONCLUSIONS

A Cu(II) nanocomplex [CuCl$_2$(salophen)].H$_2$O [salophen=o-phenylenediaminebis(salicylidenaminato)] was synthesized. The Cu(II) nanocomplex coated on the surface of graphite screen printed electrode, and the as-prepared modified Cu/SPE electrode was used to detect epinephrine in aqueous solutions, thus demonstrating the electroanalytical application of the Cu(II) nanocomplex. The Cu/SPE showed a faster electron transfer rate and better electrocatalytic oxidation abilities towards epinephrine than the bare graphite screen printed electrode. The detection limit of epinephrine could be as low as 2.5±0.05 μM, with a linear range from 10.0 to 600.0 μM. Finally, the method was applied to the selective and precise analysis of epinephrine in commercial injection.

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