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Synergic Effect of Cu(II) Nanocomplex for the Fabrication of Highly Sensitive Voltammetric Sensor for Levodopa Determination

Afsaneh Hajializadeh,[1,*] Somayeh Tajik[2,*] Shohreh Jahani[2] and Hadi Beitollahi[3]

[1]*Department of Natural Resources, Sirjan Branch, Islamic Azad University, Sirjan, Iran*
[2]*NanoBioElectrochemistry Center, Bam University of Medical Science, Bam, Iran*
[3]*Environment Department, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran*

*Corresponding Author, Tel.: +98 3426226613; Fax: +98 3426226617
E-Mail: a.hajializadeh@yahoo.com; Tajik_s1365@yahoo.com

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Abstract- A novel modified graphite screen printed electrode (SPE) is prepared as an electrochemical sensor for determination of levodopa. The experimental results suggest that a screen printed electrode (SPE) modified with Cu(II) nanocomplex, $[CuCl_2(salophen)].H_2O$ (salophen= *o*-phenylenediaminebis(salicylidenaminato)), accelerates the electron transfer reactions of levodopa. The fabricated sensor revealed some advantages such as convenient preparation, good stability and high sensitivity. The DPV data in 0.1 M phosphate buffer solution (PBS) (pH 7.0) allowed a method to be developed for the determination of levodopa concentrations in the ranges 5.0–900.0 μM, with the detection limits of 0.9 μM. The proposed method was successfully applied to determinations of levodopa in urine samples.

Keywords- Levodopa, Cu(II) nanocomplex, Graphite screen printed electrode, Voltammetry

1. INTRODUCTION

Parkinson's disease is a neurodegenerative disorder of the central nervous system and symptoms of this disease result from loss of neurons in the substantia nigra, a region of the

midbrain. Loss of these neurons leads to a deficiency of dopamine neurotransmitter in the brain that may lead to Parkinson's disease. Symptoms of this disease can be alleviated by oral administration of levodopa, the biological precursor to dopamine [1-5]. Levodopa is a naturally occurring dietary supplement and psychoactive drug found in certain kinds of herbs and food and is synthesized from the essential amino acids l-phenylalanine and l-tyrosine in the brain and mammalian body. Following oral administration, levodopa exhibits a significant first pass effect with substantial enzymatic decarboxylation, giving rise to dopamine. After high dose administration, elevated levels of systemic dopamine may cause side effects such as nausea, vomiting, and cardiac arrhythmias [6-8].

According to the above points, many attempts have been made to determine levodopa in biological and pharmaceutical conditions [9-12]. Numerous analytical methods have been developed to determine of levodopa in different sample matrices. Nevertheless, each technique has often suffered from diverse disadvantages with regard to cost and selectivity, the use of organic solvents, complex sample preparation procedures, or long analysis time. Electrochemical methods provide useful alternatives since they allow faster, cheaper, and safer analysis [13-18].

Screen-printed electrodes (SPEs) have allowed the electrochemical determination of a wide range of substances since the 1990s [19-21]. This technology has offered the high-volume production of inexpensive electrochemical (bio)sensors. Quite simply, SPEs are electrical devices that reproduce at least one small electrochemical cell with working, counter and reference electrodes, using conductive inks imprinted on a ceramic/plastic sheet. Their architecture allows one drop of a few microliters of sample to be placed onto these electrodes. The easy modification to develop different sensing surfaces is one of the best advantages of such electrodes manufactured with carbon, metals, nanotubes or conducting polymer inks [22-24].

There are four principle enhancement techniques for voltammetric and amperometric modified electrodes, namely selective preconcentration, permselectivity, selective recognition and electrocatalysis [25-31]. Electrocatalysis at chemically modified electrodes is widely utilized for the determination of many drugs and biosubstrates. Various inorganic and organic materials have been used to fabricate modified electrodes which can enhance the electron transfer rate and reduce the overpotential for the oxidation of substrates [32-38]. The chemical modification of electrodes using electron transfer mediators is an interesting field in analytical chemistry [39-44]. One of the most important effects of any mediator is a reduction of the overpotential required for electrochemical reaction, which enhances the sensitivity (current) and selectivity of the method [45-51].

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 [52-55].

According to the previous points, it is important to create suitable conditions for analysis of levodopa in biological fluids. In this study, we describe application of novel Cu(II) nanocomplex as a nanostructure sensor for voltammetric determination of levodopa. The proposed sensor showed good electrocatalytic effect on levodopa. The modified electrode shows advantages in terms of selectivity, reproducibility and sensitivity. Eventually, we evaluate the analytical performance of the suggestion sensor for levodopa 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. Levodopa 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 [54]. Cu(II) nanocomplex is prepared by a facile low-temperature (<100 ºC) 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 ºC). 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 ºC for 2 h prior to a further analysis or use.

2.3. Preparation of real samples

Urine samples were stored in a refrigerator immediately after collection. Ten millilitres of the samples were centrifuged for 15 min at 2,000 rpm. The supernatant was filtered out by using a 0.45 μm filter. Next, different volumes of the solution was transferred into a 25 mL

volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine samples were spiked with different amounts of levodopa. The levodopa contents were analysed by the proposed method by using the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Electrocatalytic oxidation of levodopa at a Cu/SPE

The electrochemical behavior of levodopa 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 levodopa. Thus the electrochemical behavior of levodopa 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 levodopa 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 levodopa oxidation at the surface of Cu/SPE.

Fig. 1 depicts the cyclic voltammetric responses for the electrochemical oxidation of 900.0 µM levodopa at Cu/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of levodopa at Cu/SPE (curve a) is about 355 mV compared with 455 mV for that on the bare SPE (curve b). Similarly, when the oxidation of levodopa 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.

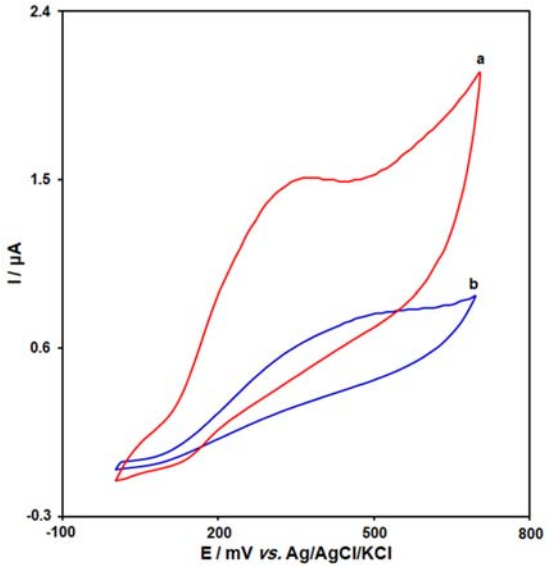

Fig. 1. Cyclic voltammograms of (a) Cu/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 900.0 µM levodopa at the scan rate 50 mVs^{-1}

In other words, the results clearly indicate that the Cu nanocomplex improve the levodopa oxidation signal.

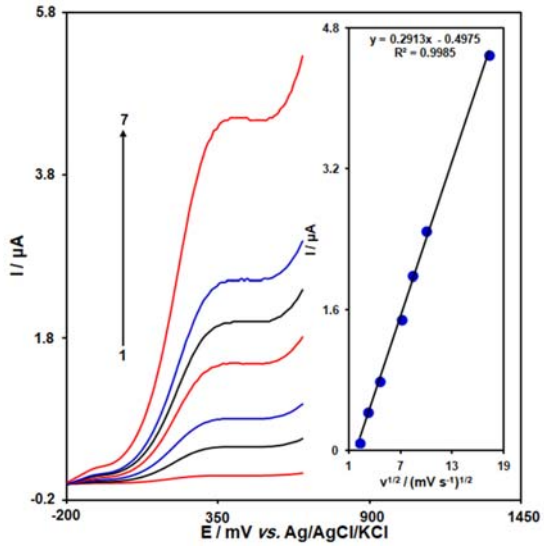

Fig. 2. LSV of Cu/SPE in 0.1 M PBS (pH 7.0) containing 900.0 µM levodopa at various scan rates; numbers 1-7 correspond to 5, 10, 20, 50, 70, 100 and 300 mV s^{-1}, respectively. Inset: variation of cathodic peak current *vs*. $v^{1/2}$

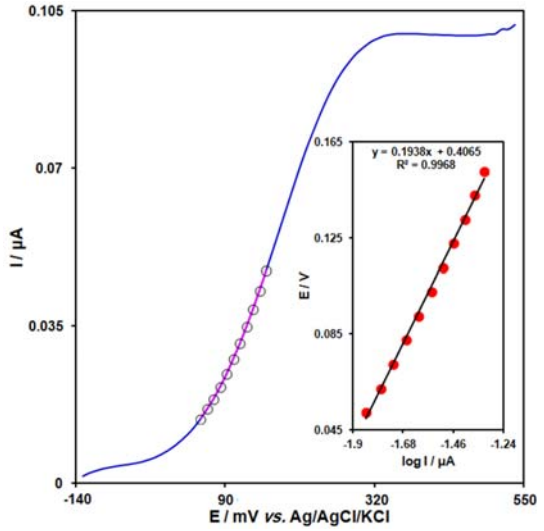

Fig. 3. LSV (at 5 mV s^{-1}) of electrode in 0.1 M PBS (pH 7.0) containing 900.0 µM levodopa the points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV

The effect of potential scan rates on the oxidation current of levodopa 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 300 mV s^{-1}.

Further Tafel curve was plotted using the data from the rising section (i.e. the Tafel region) of the current–voltage curve obtained at 5 mVs^{-1} (Fig. 3). The Tafel region of the current potential curve is influenced by the electron transfer kinetics of the electrode reactions. The results showed Tafel slope of 0.1938 V, which indicates a one electron (Fig. 3) rate determining step (RDS) for the electrode process [56] for charge transfer coefficient (α) of 0.69.

3.2. Chronoamperometric measurements

Chronoamperometric measurements of levodopa at Cu/SPE were carried out by setting the working electrode potential at 0.4 V for the various concentration of amitriptyline in PBS (pH 7.0) (Fig. 4). For an electroactive material (levodopa 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 [56].

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

where D and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively.

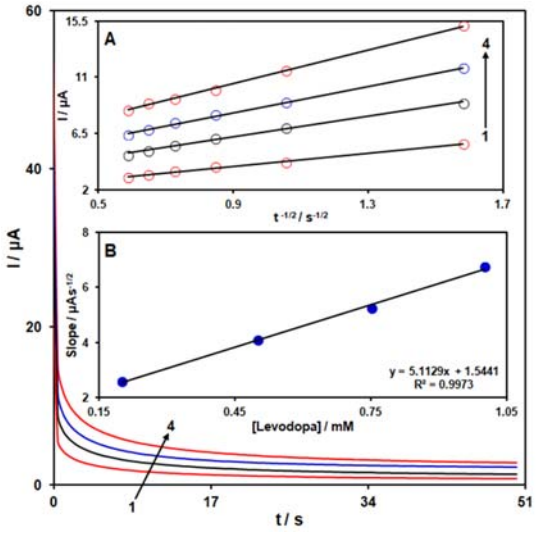

Fig. 4. Chronoamperograms obtained at Cu/SPE in 0.1 M PBS (pH 7.0) for different concentration of levodopa. The numbers 1–4 correspond to 0.2, 0.5, 0.75 and 1.0 mM of levodopa. Insets: (A) Plots of I *vs*. $t^{-1/2}$ obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against levodopa concentration

Experimental plots of I *vs*. $t^{-1/2}$ were employed, with the best fits for different concentrations of levodopa (Fig. 4A). The slopes of the resulting straight lines were then plotted *vs*. levodopa concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the D was found to be 2.2×10^{-6} cm^2/s.

3.3. Calibration plot and limit of detection

The peak current of levodopa oxidation at the surface of the modified electrode can be used for determination of levodopa in solution. Therefore, differential pulse voltammetry (DPV) experiments were done for different concentrations of levodopa (Fig. 5). The oxidation peak currents of levodopa at the surface of a modified electrode were proportional to the concentration of the levodopa within the ranges 5.0 to 900.0 µM. The detection limit (3σ) of levodopa was found to be 9.0×10^{-7} M.

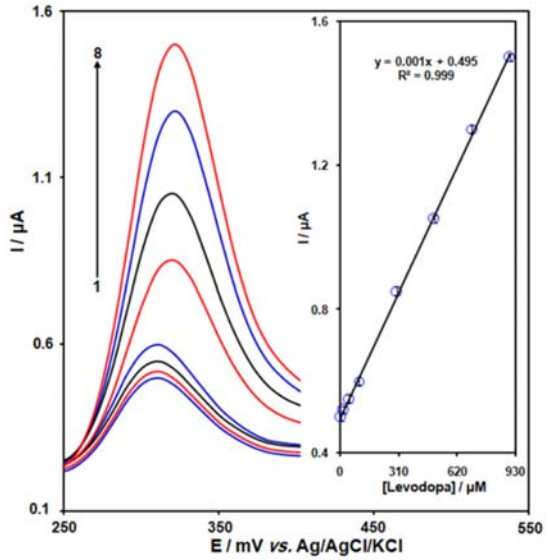

Fig. 5. DPVs of Cu/SPE in 0.1 M (pH 7.0) containing different concentrations of levodopa Numbers 1–8 correspond to 5.0, 20.0, 50.0, 100.0, 300.0, 500.0, 700.0 and 900.0 µM of levodopa. Inset: plot of the electrocatalytic peak current as a function of levodopa concentration in the range of 5.0-900.0 µM

3.4. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of levodopa in urine sample. The results for determination of levodopa in urine sample are given in Table 1. Satisfactory recovery of the experimental results was found for levodopa. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 1. The application of Cu/SPE for determination of levodopa in urine sample (n=5). All concentrations are in μM

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	0	-	-	-
	5.0	4.9	98.0	3.2
	10.0	10.3	103.0	2.1
	15.0	14.9	99.3	1.8
	20.0	20.2	101.0	2.4

4. CONCLUSION

In the current study, a Cu(II) nanocomplex [$CuCl_2$(salophen)].H_2O (salophen= *o*-phenylenediaminebis(salicylidenaminato)) modified graphite screen printed electrode was fabricated. The Cu(II) nanocomplex show the characteristics of large surface area, good dispersing properties and fast electron transfer. Due to the co-contribution of SPE and modifiers on the electrode surface, the resulting electrode exhibited a good electrocatalytic performance to trace determination of levodopa. A wide linear range and low detection limit, suggest that this electrode will be an attractive candidate for practical applications.

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