

*Full Paper*

## Electrochemical Behavior and Determination of Carbidopa on Modified Graphite Screen Printed Electrode

Afsaneh Hajializadeh,<sup>1,\*</sup> Shohreh Jahani,<sup>2,\*</sup> Somayeh Tajik<sup>2</sup> and Hadi Beitollahi<sup>3</sup>

<sup>1</sup>Department of Natural Resources, Sirjan Branch, Islamic Azad University, Sirjan, Iran

<sup>2</sup>NanoBioElectrochemistry Research, Bam University of Medical Science, Bam, Iran

<sup>3</sup>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](mailto:a.hajializadeh@yahoo.com); [jahanishohre@gmail.com](mailto:jahanishohre@gmail.com)

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**Abstract-** Electrochemical behaviors of carbidopa at the surface of the graphite screen printed electrode (SPE) modified with Cu(II) nanocomplex, [CuCl<sub>2</sub>(salophen)]. H<sub>2</sub>O (salophen=o-phenylenediaminebis(salicylidenaminato)) were studied. The oxidation peak potential of the carbidopa at a surface of Cu/SPE appeared at 360 mV that was about 100 mV lower than the oxidation peak potential at the surface of the traditional SPE under similar condition. On other hand, the oxidation peak current was increased for about two times at the surface of Cu/SPE compared to SPE. The linear response range and detection limit were found to be 0.5-700 μM and 0.1 μM, respectively. The proposed sensor was successfully applied for the determination of carbidopa in real samples.

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

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### 1. INTRODUCTION

The catecholamines are a group of compounds bearing a dihydroxyphenyl moiety known as main neurotransmitters, and have been employed as markers of neuroblastoma, stress condition and other autonomic nervous system disorders [1-3]. Carbidopa (CD) is one of the important catecholamine and it is used in the treatment of Parkinson's disease. Carbidopa is a drug given to people with Parkinson's disease in order to inhibit peripheral metabolism of

levodopa. Carbidopa does not cross the blood-brain barrier (BBB) and contributes to the production of effective brain concentrations of dopamine from lower doses of levodopa by inhibiting the peripheral decarboxylation of levodopa to dopamine. In addition, the reduced peripheral formation of dopamine decreases the peripheral side effects such as nausea, vomiting and cardiac arrhythmia [4]. To release the fluctuating symptoms in clinical, the most commonly used method is to administrate with controlled-release levodopa/carbidopa tablet, which is developed to prolong the therapeutic plasma level of levodopa [5,6]. Therefore, it is very important to establish a selective and sensitive method for detection of carbidopa in pharmaceutical formulations and human serum, because of the coexistence of levodopa and carbidopa in pharmaceutical formulations and the rapid fluctuations of plasma drug concentration of levodopa. However, the accurate and sensitive analysis of carbidopa is challenging due to their poor stability, low molecular mass, and high polarity [7-10]. Different techniques have been employed for the determination of carbidopa in pharmaceutical formulations [11,12]. Among of these methods, electrochemical techniques offer the opportunity for portable, economical, sensitive and rapid methodologies [13-19].

In recent years, screen-printed carbon electrode (SPE) has been widely applied in electrochemical detections [20-22]. It challenges the conventional three-electrode system due to its inherent advantages including simple fabrication, low cost, small size, fast response, disposability, portability and easy mass-produced. However, the use of bare conventional electrode materials is sometimes not always satisfactory, due to the fouling character of the adsorption [23-25].

More recently, chemically modified electrodes (CMEs) have attracted much interest in the electrocatalytic oxidation/reduction of important redox systems. Modified electrodes can be prepared by deposition of various compounds such as conducting polymers, metal complexes, transition metals and metal oxides on various electrodes [26-35]. The operation mechanism of such electrodes depends on the properties of the modified materials used to promote selectivity and sensitivity toward the target analytes [36-42]. This kind of electrode is inexpensive and possesses many advantages such as low background current, wide range of potential windows (in both cathodic and anodic region), easy fabrication, and rapid surface renewal. One of the most important properties of CMEs has been their ability to catalyze the electrode process via significant decrease of the overpotential and increase of the electron transfer kinetics with respect to the unmodified electrode [43-50].

Thus, the combination of inorganic nanoparticles and sensors is one of the most exciting areas in modern analytical detection development because they offer excellent prospects for designing highly sensitive and selective sensors. 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 [51-53].

According to the previous points, it is important to create suitable conditions for analysis of carbidopa in biological fluids. In this study, we describe application of novel Cu(II) nanocomplex as a nanostructure sensor for voltammetric determination of carbidopa. The proposed sensor showed good electrocatalytic effect on carbidopa. The modified electrode shows advantages in terms of sensitivity. Eventually, we evaluate the analytical performance of the suggestion sensor for carbidopa determination in real 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. Carbidopa 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 °C) synthesis route at atmospheric pressure via reaction of salophen ligand, copper chloride under reflux. Typically, Cu(Cl)<sub>2</sub>·6H<sub>2</sub>O (1 mmol), salophen ligand (1 mmol) and methanol (20 ml) were mixed and sonicated (2 h, 60 °C). 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 °C 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<sub>2</sub>O suspension solution was casted on the carbon working electrodes, and waiting until the solvent was evaporated in room temperature.

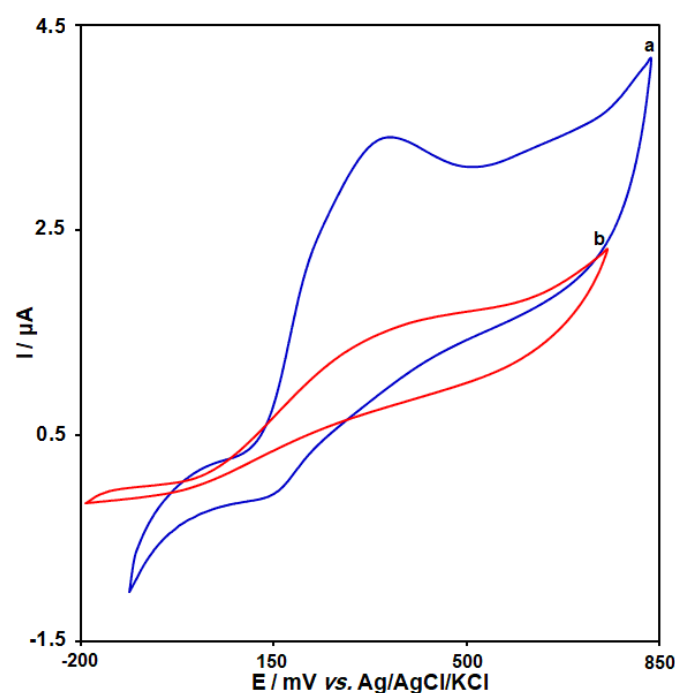
## 2.4. Preparation of real samples

The urine specimens were kept in a refrigerator after sampling. To prepare the test samples 10 millilitres of these were taken or centrifuged at 2000 rpm for 15 min. After filtering the supernatant with a 0.45  $\mu\text{m}$  filter, different volumes of it were diluted in 25 mL volumetric flasks using PBS (pH=7.0). The diluted urine sample was spiked with different amounts of carbidopa.

## 3. RESULTS AND DISCUSSION

### 3.1. Electrocatalytic oxidation of carbidopa at a Cu/SPE

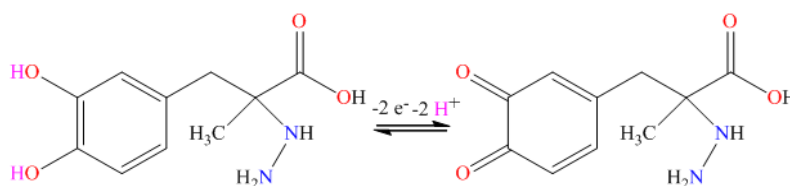
The electrochemical behavior of carbidopa is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of carbidopa. Thus the electrochemical behavior of carbidopa was studied in 0.1 M PBS in different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



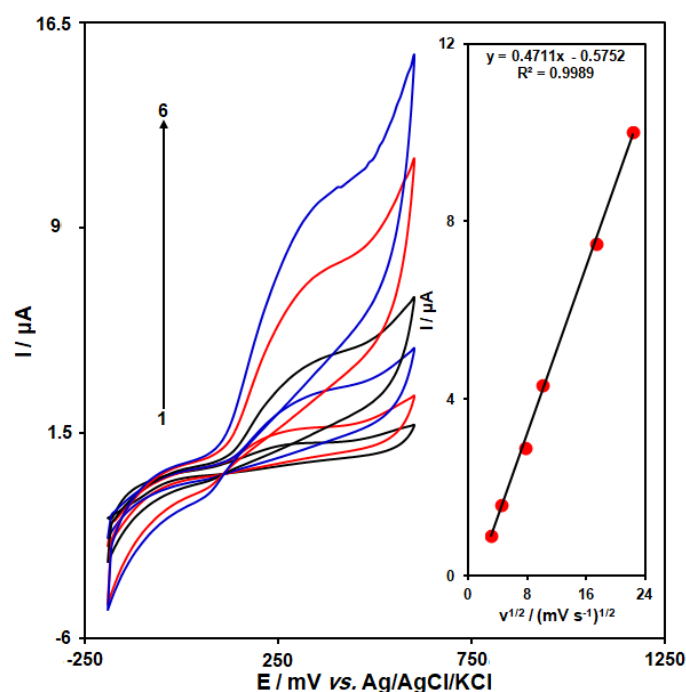
**Fig. 1.** Cyclic voltammograms of (a) Cu/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 700.0  $\mu\text{M}$  carbidopa at the scan rate  $50 \text{ mVs}^{-1}$

Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of carbidopa oxidation at the surface of Cu/SPE. Fig. 1 depicts the cyclic voltammetric responses for the electrochemical oxidation of 700.0  $\mu\text{M}$  carbidopa at Cu/SPE (curve a) and bare SPE (curve

b). The anodic peak potential for the oxidation of carbidopa at Cu/SPE (curve a) is about 360 mV compared with 460 mV for that on the bare SPE (curve b). Similarly, when the oxidation of carbidopa 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 carbidopa oxidation signal. The mechanism of electro-oxidation of carbidopa is shown in Fig. 2.



**Fig. 2.** Mechanism of electro oxidation of carbidopa

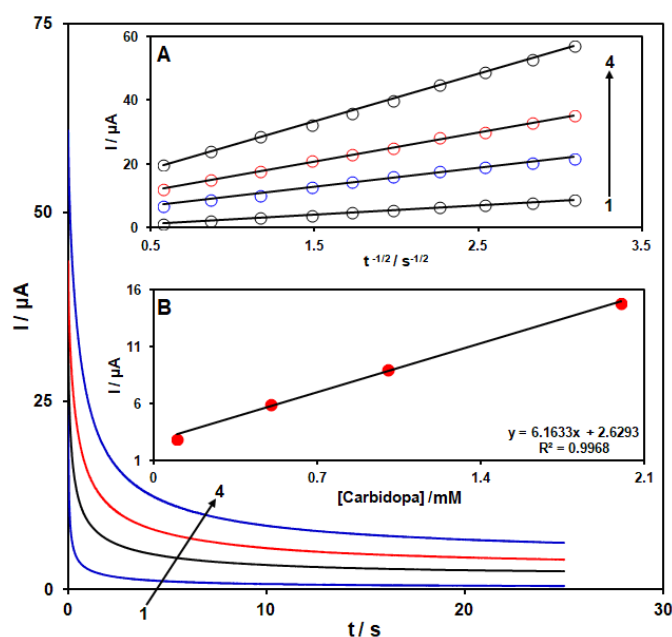


**Fig. 3.** CV of Cu/SPE in 0.1 M PBS (pH 7.0) containing 700.0  $\mu\text{M}$  carbidopa at various scan rates; numbers 1-6 correspond to 10, 20, 60, 100, 300 and 500  $\text{mV s}^{-1}$ , respectively. Inset: variation of anodic peak current vs.  $v^{1/2}$

The effect of potential scan rates on the oxidation current of carbidopa has been studied (Fig. 3). 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 10 to 500  $\text{mV s}^{-1}$ .

### 3.2. Chronoamperometric measurements

Chronoamperometric measurements of carbidopa at Cu/SPE were carried out by setting the working electrode potential at 0.4 V for the various concentration of carbidopa in PBS (pH 7.0) (Fig. 4). For an electroactive material (carbidopa 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 [55].



**Fig. 4.** Chronoamperograms obtained at Cu/SPE in 0.1 M PBS (pH 7.0) for different concentration of carbidopa. The numbers 1–4 correspond to 0.1, 0.5, 1.0 and 2.0 mM of carbidopa. Insets: (A) Plots of  $I$  vs.  $t^{-1/2}$  obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against carbidopa concentration.

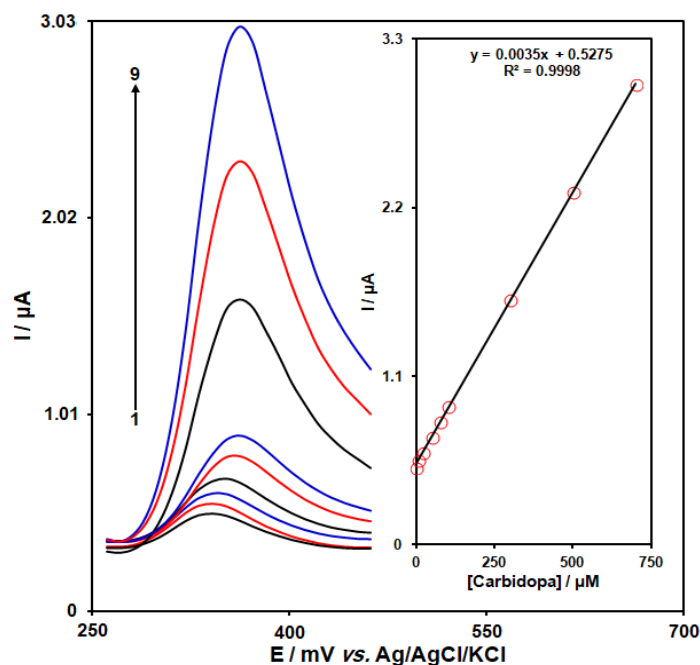
$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

where  $D$  and  $C_b$  are the diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ ) and the bulk concentration ( $\text{mol cm}^{-3}$ ), respectively. Experimental plots of  $I$  vs.  $t^{-1/2}$  were employed, with the best fits for different concentrations of carbidopa (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. carbidopa concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the  $D$  was found to be  $3.2 \times 10^{-6} \text{ cm}^2/\text{s}$ .

### 3.3. Calibration plot and limit of detection

The peak current of carbidopa oxidation at the surface of the modified electrode can be used for determination of carbidopa in solution. Therefore, differential pulse voltammetry (DPV) experiments were done (Initial potential=0.26 V, End potential=0.46 V, Step

potential=0.1 V, Modulation amplitude=0.02505 V) for different concentrations of carbidopa (Fig. 5). The oxidation peak currents of carbidopa at the surface of a modified electrode were proportional to the concentration of the carbidopa within the ranges 0.5 to 700.0  $\mu\text{M}$ . The detection limit ( $3\sigma$ ) of carbidopa was found to be  $1.0 \times 10^{-7}$  M.



**Fig. 5.** DPVs of Cu/SPE in 0.1 M (pH 7.0) containing different concentrations of carbidopa. Numbers 1–9 correspond to 0.5, 5.0, 20.0, 50.0, 75.0, 100.0, 300.0, 500.0 and 700.0  $\mu\text{M}$  of carbidopa. Inset: plot of the electrocatalytic peak current as a function of carbidopa concentration in the range of 0.5-700.0  $\mu\text{M}$ .

### 3.4. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of carbidopa in urine sample. The results for determination of carbidopa in urine sample are given in Table 1.

**Table 1.** The application of Cu/SPE for determination of carbidopa in urine sample (n=5). All concentrations are in  $\mu\text{M}$

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	0	-	-	-
	7.5	7.4	98.7	2.1
	12.5	12.7	101.6	2.4
	17.5	18.1	103.4	3.2
	22.5	22.4	99.6	1.9

Satisfactory recovery of the experimental results was found for carbidopa. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

#### 4. CONCLUSION

In the current study, a Cu(II) nanocomplex [CuCl<sub>2</sub>(salophen)].H<sub>2</sub>O (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 carbidopa. A wide linear range and low detection limit, suggest that this electrode will be an attractive candidate for practical applications.

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