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Differential Pulse Voltammetric Determination of Levodopa in Pharmaceutical and Biological Samples using NiO/graphene Oxide Nanocomposite Modified Graphite Screen Printed Electrode

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Abstract- A chemically modified electrode was prepared by incorporating NiO/graphene oxide nanocomposite into graphite screen printed electrode (NiO/GO/SPE) to determine levodopa. Cyclic voltammetry, differential pulse voltammetry and chronoamperometry were used to investigate the electrochemical behavior of levodopa at the chemically modified electrode. According to the results, NiO/GO/SPE showed high electrocatalytic activity for levodopa oxidation, producing a sharp oxidation peak current at about 240 mV vs Ag/AgCl reference electrode at pH 7.0. The peak current was linearly dependent on levodopa concentration over the range of 0.8–700.0 µM with the detection limit (3σ) of 0.2 µM. The proposed method was successfully applied as a rapid, highly selective, simple, and precise one to determine levodopa in biological fluids.

Keywords- Levodopa, Graphene Oxide, NiO Nanoparticles, Drug Analysis

1. INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive movement disorder characterized clinically by bradykinesia, tremor, rigidity and gait dysfunction. The cause of PD is generally unknown, but arises when the level of dopamine in brain becomes low. Dopaminergic replacement therapy with the precursor levodopa is effective in ameliorating many signs and symptoms of early PD [1-6]. The unusual amino acid levodopa (3,4-dihydroxyphenylalanine, LD), is a drug that prevents this disease. Levodopa is produced from the amino acid L-tyrosine by the enzyme tyrosine hydroxylase. It is also the precursor for the monoamine or catecholamine neurotransmitters dopamine, noradrenaline and adrenaline. Dopamine is formed by the decarboxylation of levodopa. Levodopa crosses the protective blood–brain barrier, whereas dopamine itself cannot [7,8]. Thus, levodopa is used to increase dopamine concentrations in the treatment of PD and dopamine-responsive dystonia. But it's using in long-term make some side effects on human health such as gastritis, paranoia schizophrenia and dyskinesia. So, concentration determination of levodopa is very essential to control its dosage [9].

Hitherto, a variety of approaches are reported to detect the level of levodopa such as flow injection analysis, high-performance liquid chromatography (HPLC), capillary electrophoresis and spectrophotometry [10]. However, these listed methods have some limits, such as time-consuming, perform sophisticated equipment, and expensive [11]. But, electrochemical methods due to the advantages of relatively fast response, simple instrumentation, high sensitivity and selectivity, low cost, facile miniaturization and low power requirement have shown to be a powerful tool [11-15].

Screen-printed electrodes (SPEs) is an alternative material used instead of using the conventional electrodes based on economic substrate. Lately, SPE have been successfully used as the electrochemical sensor for various researches due to their disposability, simplicity of the apparatus, minimum sample preparation and obtaining of fast results. In addition, the main benefit of SPE is able to use only once and then is discarded [16]. However, the limitation of SPE is small surface area of working electrode leading to the lack of sensitivity. Hence, to overcome this problem electrode modification is necessary [17].

Electroanalytical methods have attracted more attention in recent years for environmental and biological compounds determination due to their sensitivity, accuracy, lower cost, and simplicity [18-48].

Many materials have been considered as the electrode modification. Among them, nickel oxide (NiO), a p-type semiconductor exhibiting many attractive advantages, such as low cost, easy availability, and high theoretical specific capacitance, has been extensively investigated. Unfortunately, the poor cycle stability and low electrical conductivity greatly hinder its extensive use. To overcome these problems, many researches had concentrated on synthesis of NiO-based composites with highly conductive materials [49]. Graphene, a two-

dimensional sheet of sp^2-bonded carbon atoms, with extraordinary thermal, mechanical, and electrical properties, has been introduced to improve the pseudo capacitive behaviors of NiO nanoparticles [50, 51].

The main target of this study is to develop a simple but sensitive electrochemical sensor for the determination of levodopa in blood serum and urine samples. In this work, a new electrochemical sensor fabricated based on a screen-printed electrodes modified with NiO/graphene oxide nanocomposite is developed for the sensitive detection of levodopa. The proposed modified electrode was tested by cyclic voltammetric techniques and was directed toward electroanalytical applications for determination of levodopa in biological fluid samples.

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. Preparation of modified electrode

The bare screen-printed electrode was coated with NiO/GO as follows. A stock solution of GO in 1 mL aqueous solution was prepared by dispersing 1 mg GO with ultrasonication for 1 h, and a 2 µl aliquot of the GO/H_2O suspension solution was casted on the carbon working electrodes, and waiting until the solvent evaporation in room temperature. Then the GO/SPE was immersed into 0.5 mM $Ni(NO_3)_2$ at a potentiostatic potential of -1.1 V for 7 s.

3. RESULT AND DISCUSSION

3.1. Electrochemical behavior of levodopa at a NiO/GO/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 NiO/GO/SPE by CV. It was found that the electrocatalytic oxidation of levodopa at the surface of NiO/GO/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 NiO/GO/SPE.

Fig. 1 depicts the cyclic voltammetric responses for the electrochemical oxidation of 500.0 µM levodopa at NiO/GO/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of levodopa at NiO/GO/SPE (curve a) is about 240.0 mV compared with 500.0 mV for that on the bare SPE (curve b). Similarly, when the oxidation of levodopa at the NiO/GO/SPE (curve a) and bare SPE (curve b) are compared, an extensive enhancement of the anodic peak current at NiO/GO/SPE relative to the value obtained at the bare SPE (curve b) is observed. In other words, the results clearly indicate that the combination of graphene and NiO nanocomposites improve the levodopa oxidation signal.

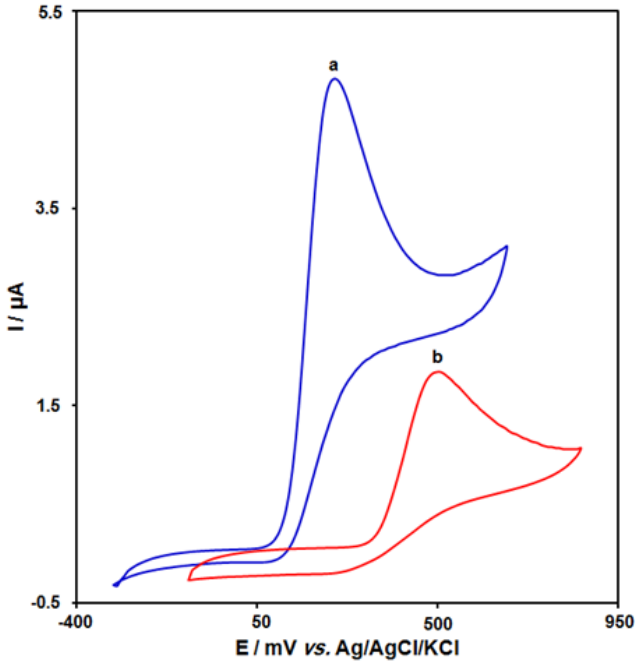

Fig. 1. Cyclic voltammograms of (a) NiO/GO/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 500.0 µM levodopa at the scan rate 50 mVs^{-1}

3.2. Effect of scan rate

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 10 to 300 mV s^{-1}.

Fig. 3 shows the LSV of a NiO/GO/SPE obtained in 0.1 M PBS (pH 7.0) containing 200.0 µM of levodopa, with a sweep rate of 10 mV s^{-1}. The points show the rising part of the voltammogram (known as the Tafel region), which is affected by the electron transfer kinetics between levodopa and NiO/GO/SPE. If deprotonation of levodopa is a sufficiently fast step, the number of electrons involved in the rate determining step can be estimated from the slope of the Tafel plot. The inset of Fig. 3 shows a Tafel plot that was drawn from points of the Tafel region of the cyclic voltammogram. The Tafel slope of 0.0789 V obtained in this case agrees well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of α=0.25.

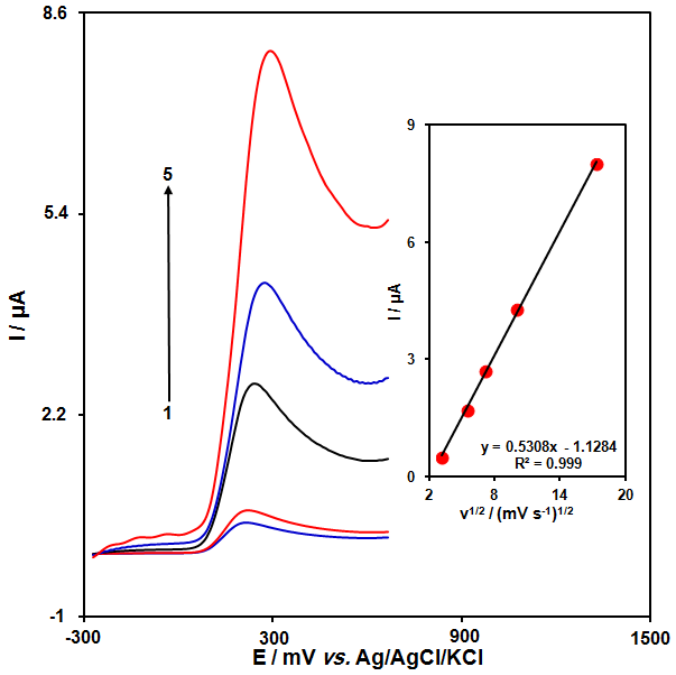

Fig. 2. Linear sweep voltammograms of NiO/GO/SPE in 0.1 M PBS (pH 7.0) containing 200.0 µM levodopa at various scan rates; numbers 1-5 correspond to 10, 30, 50, 100, and 300 mV s^{-1}, respectively. Inset: Variation of anodic peak current *vs*. square root of scan rate.

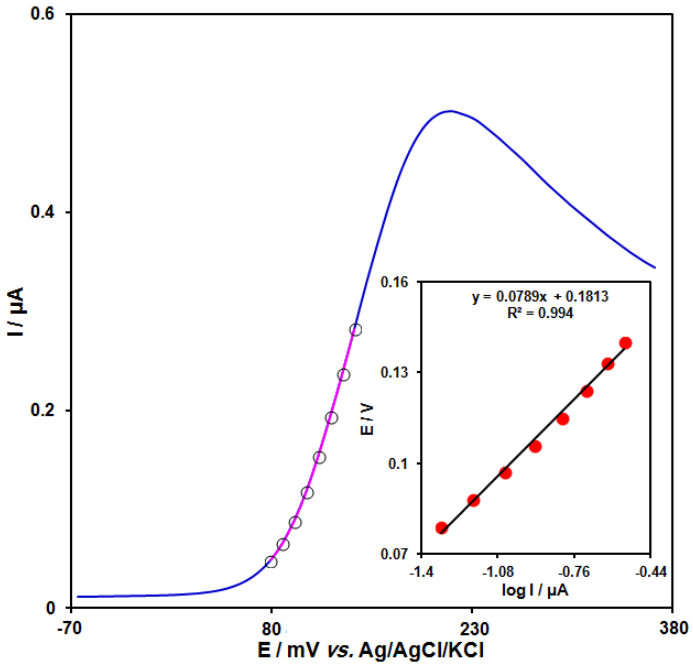

Fig. 3. LSV (at 10 mV s^{-1}) of a NiO/GO/SPE in 0.1 M PBS (pH 7.0) containing 200.0 µM levodopa. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV

3.3. Chronoamperometric measurements

Chronoamperometric measurements of levodopa at NiO/GO/SPE were carried out by setting the working electrode potential at 0.3 V vs. Ag/AgCl/KCl (3.0 M) for the various concentrations of levodopa 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 [52].

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

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 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 1.94×10^{-5} cm^2/s.

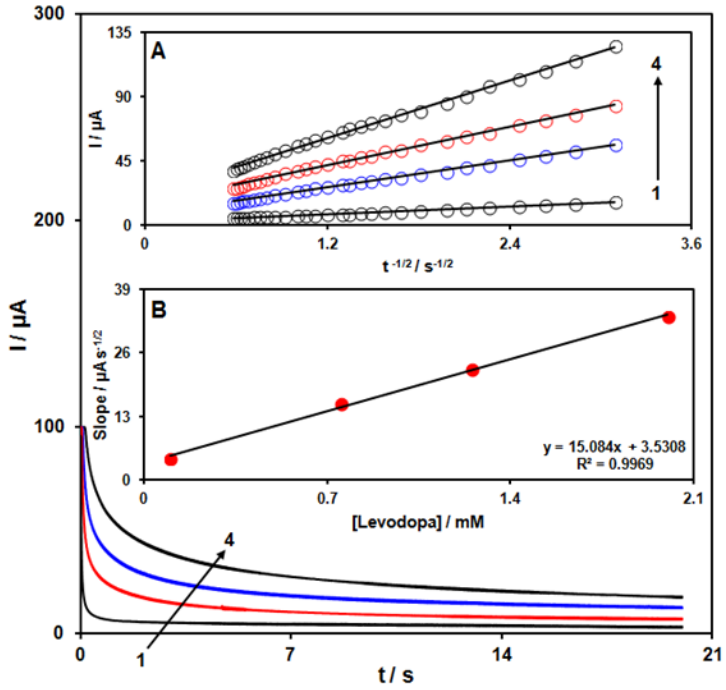

Fig. 4. Chronoamperograms obtained at NiO/GO/SPE in 0.1 M PBS (pH 7.0) for different concentration of levodopa. The numbers 1–4 correspond to 0.1, 0.75, 1.25 and 2.0 mM of levodopa. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against levodopa concentration

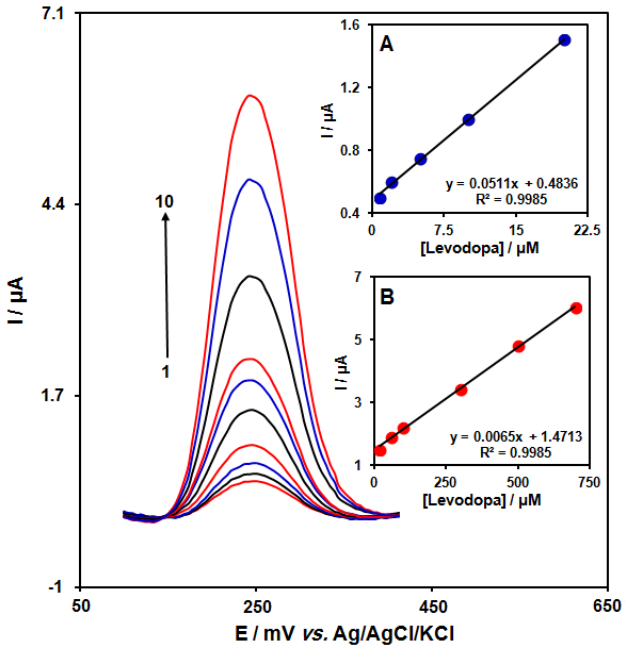

Fig. 5. DPVs of NiO/GO/SPE in 0.1 M PBS (pH 7.0) containing different concentrations of levodopa. Numbers 1-10 correspond to 0.8, 2.0, 5.0, 10.0, 20.0, 60.0, 100.0, 300.0, 500.0 and 700.0 µM of levodopa. Inset: (A) shows the plots of the peak current as a function of levodopa concentration in the range of 0.8-20.0 µM; (B) shows the plots of the peak current as a function of levodopa concentration in the range of 20.0-700.0 µM

3.4. 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, 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 8.0×10^{-7} to 7.0×10^{-4} M with detection limit (3σ) of 2.0×10^{-8} M.

3.5. 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 samples. The results for determination of this specie in real samples 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. Determination of levodopa in human urine sample. All the concentrations are in μM (n=5).

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	0	-	-	-
	7.5	7.6	101.3	2.3
	12.5	12.3	98.4	3.4
	17.5	18.1	103.4	2.1
	22.5	22.4	99.5	1.8

4. CONCLUSION

NiO/graphene oxide nanocomposite was used for modification of graphite screen printed electrode (NiO/GO/SPE) and and used for highly sensitive, selective and stable detection of levodopa. Under conditions optimized for the detection of levodopa, the linear range is from 0.8 to 700.0 μM, and the lower detection limit is 0.2 μM. Consequently the proposed electrode was successfully applied for analysis of levodopa in urine samples.

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