

Voltammetric Determination of Dopamine Using Glassy Carbon Electrode Modified with ZnO/Al₂O₃ Nanocomposite

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Received: 11 October 2017 / Accepted: 4 January 2018 / Published: 5 February 2018

In this work, a new modification strategy was reported to modify a glassy carbon electrode (GCE) based on ZnO/Al₂O₃ nanocomposite. This modified electrode was designed in order to be used as a sensitive and selective sensor towards detection of trace amount of dopamine (DA). The effective parameters on the optimal performances of the electrode such as pH of the test solution and the applied scan rate during the electrochemical process were also studied. This sensor responded linearly towards detection of dopamine within a wide range of 5.0×10^{-6} - 7.0×10^{-4} M with a low detection limit of 2.0×10^{-6} M (pH=7.0), under the optimum conditions. Moreover, the electrode functioned in the determination of dopamine in real samples was satisfactory.

Keywords: Dopamine, Glassy carbon electrode, ZnO/Al₂O₃ nanocomposite, Modified electrode

1. INTRODUCTION

Dopamine (3,4-dihydroxyphenethylamine) is a chemical messenger released by nerve cells in the brain of human to send signals to other neurons. It belongs to the categories of catecholamine and

phenethylamine. This neurotransmitter plays some important roles in human and animals. It is synthesized by elimination of carboxylic group from the L-3,4-dihydroxyphenylalanine (L-DOPA) as its precursor [1]. One of the major functions of dopamine is in pleasure reward seeking behaviour [2]. In fact, the dopamine level is increased whenever people receive a reward. Even abuse of drugs in addicts, leads to increased dopamine neuronal activity. Other noteworthy functions of dopamine are in movement, learning, mood, sleep, behavior and cognition, attention, memory, control the releasing of various hormones, and inhibition the secretion of prolactine. As a chemical messenger it is synthesized locally, except for blood vessels, and affect around the vicinity of cells that release it. In addition, dopamine exerts its effect on the several internal organs such as blood vessels (as a vasodilator), kidney (increase of sodium excretion and urine), pancreas (reduce of insulin), digestive system (protect of intestinal mucosa), immune system (reduce of lymphocytes activity). It also plays a vital role in physiological events including central nervous system, hormonal and renal systems. Disorder in dopamine level either excess or deficiency is the root cause of some diseases such as Parkinson and drug addiction [3,4]. Thus, determination of dopamine molecule in biological fluids is of great importance.

In order to determine the drug in clinical samples, several techniques have been proposed such as ultra-high performance liquid chromatography/tandem mass spectrometry, chemiluminescence and spectrophotometry [5-7]. However, the electroanalytical methods are favor in terms of their high sensitivity, simplicity, low costs and uncomplicated equipment [8-14]. Furthermore, due to the important role of dopamine, sometimes, there is a need to online monitoring which can be capable through electrochemical methods. To have a selective and sensitive electrochemical signal for dopamine, the electrode modification is required. The application of modified electrodes in electrochemical method for the determination of drugs has been previously reported in the literature [15-30]. Among various potential electrodes in the electrochemical determinations, glassy carbon electrodes (GCEs) have demonstrated the stability and resistance. In electroanalytical methods, the redox of analyte requires a high over potential due to the slow rate of electron transfer at conventional electrodes [31-35]. Moreover, the conventional electrodes have demonstrated a poor performance in the determination of analytes which became the reason of increasing interest in the modification of these kinds of electrodes. According to the previous studies, the surface modification of electrodes not only causes a significant decrease in over potentials but also increases the electron transfer rate.

Nanotechnology has brought about tremendous changes in chemistry field. In nanomaterials, nanoparticles as particular have been used in various kinds of analytical processes [36-57]. Nanoparticles are now available in different sizes and compositions which expand and facilitate their electroanalytical applications. In this regard, the metal nanoparticles have presented excellent conductivity and catalytic activities which make them effective on increasing the rate of electron transfer in electrochemical reactions and promising candidate to act as "electronic wires" [58-61].

ZnO nanostructures due to wide band gap (3.37 eV), large excitation binding energy (60 eV), non-toxicity, biocompatibility, chemical and photochemical stability, and high electron communication features is preferred for the fabrication of effective sensors. In addition, ZnO is transparent to visible light and can be made highly conductive by doping. It is widely accepted that ZnO acts both as an electronic and structural promoter exhibiting a major influence on the catalytic activity, while alumina

or other refractory oxides mainly increase the long-term stability as structural promoter of the catalyst system. Eliminating any of the constituting components severely reduces the performance of the catalyst system. Consequently, the interest in ZnO/Al₂O₃ nanocomposite as catalytic materials remains very high [62-65].

In the present research, we benefit from the advantages of ZnO/Al₂O₃ nanocomposite in the modification of a glassy carbon electrode to study the electrochemical behavior of dopamine.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

An Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was employed to perform the electrochemical experiments and the system was controlled using a general purpose electrochemical system software.

A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and ZnO/Al₂O₃/GCE were used as the reference, auxiliary and working electrodes, respectively. pH was measured by a Metrohm 710 pH meter.

Dopamine and all other reagents were analytical grade, and were purchased from Merck (Darmstadt, Germany). For the preparation of buffers, the orthophosphoric acid and its salts were used to provide the pH range of 2.0-9.0.

2.2. Synthesis of ZnO/Al₂O₃ nanocomposite

The aluminum hydroxide was prepared by dissolving of 3 g of Al (NO₃)₃·9H₂O in 100 ml of distilled water. The pH of solution was set to 8 by ammonia solution and it was kept at 60 °C for 18 h. The precipitate was washed by ethanol and acetone three times, respectively. The Al(OH)₃ was prepared by aging of precipitate at 75 °C for 24 h.

A solution of zinc nitrate (0.3 M) was prepared in 80 ml of distilled water. The pH of solution was set to 9.5 by ammonium solution (25%) and the 0.13 g of aluminum hydroxide was added to the solution and the solution was mixed for 2h at room temperature. The solution was aged at 90 °C for 4 h at 250 rpm stirring rate. The precipitate of ZnO/Al₂O₃ nanocomposite was washed by ethanol and distilled water, respectively.

2.3. Preparation of modified electrode

The bare glassy carbon electrode was coated with ZnO/Al₂O₃ nanocomposite as follows. A stock solution of ZnO/Al₂O₃ nanocomposite in 1 mL aqueous solution was prepared by dispersing 1 mg ZnO/Al₂O₃ nanocomposite with ultrasonication for 1 h, and a 5 µl aliquot of the ZnO/Al₂O₃/H₂O suspension solution was casted on the GCE working electrodes, and waiting until the solvent was evaporated in room temperature.

2.4. Preparation of real samples

The dopamine injection was diluted by dilution factor of 500 with deionized water. Then, different volumes of the diluted solution was transferred into a 25 mL volumetric flask and diluted by PBS (pH 7.0) to the mark. Analysis the amount of dopamine in each sample was carried out by the proposed method using the standard addition method.

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 amount of dopamine. The dopamine content was analysed by the proposed method by using the standard addition method.

3. RESULT AND DISCUSSION

3.1. Electrochemical profile of the dopamine on the ZnO/Al₂O₃/GCE

To study the electrochemical behaviour of dopamine molecule which is a pH-dependent process, it is necessary to find the optimized pH value. By applied the modified electrodes at various pH values ranging from 2.0–9.0, it was found that the best results for electro-oxidation of dopamine occurred at pH=7. The obtained cyclic voltammograms in the presence of 100.0 μM dopamine using the ZnO/Al₂O₃/GCE (Curve a) and a bare GCE (Curve b) are shown in Fig. 1.

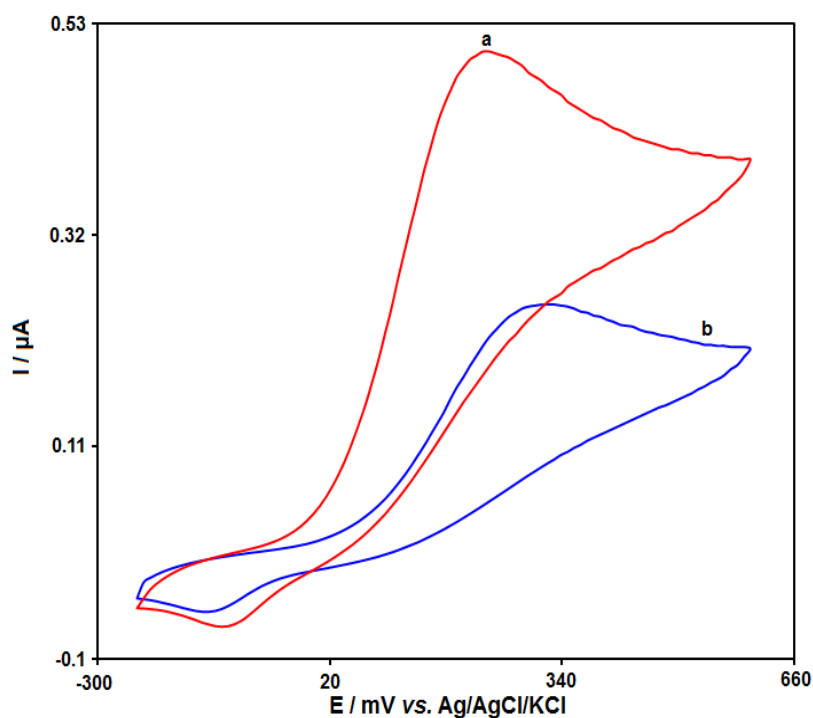


Figure 1. Cyclic voltammograms of (a) ZnO/Al₂O₃/GCE and (b) bare GCE in 0.1 M PBS (pH 7.0) in the presence of 100.0 μM dopamine at the scan rate 50 mVs^{-1} .

As it can be seen in Fig. 1, the new modifier can drastically enhance the oxidation peak of the dopamine. Also, according to CV results, the maximum oxidation of dopamine on the ZnO/Al₂O₃/GCE occurs at 230 mV which is about 80 mV more negative compared to unmodified (bare) GCE.

3.2. Effect of scan rate on the results

In the next experiment, the scan rate of the method was optimized to consider the response mechanism. Increasing in scan rate leads to enhancement of the oxidation peak current of dopamine. According to the obtained results from the study of the effect of potential scan rates on the oxidation currents of dopamine, Fig. 2, it can be concluded that there is a linear relationship between I_p and the square root of the potential scan rate ($v^{1/2}$). This demonstrates that the oxidation procedure of dopamine is a kind of diffusion control process.

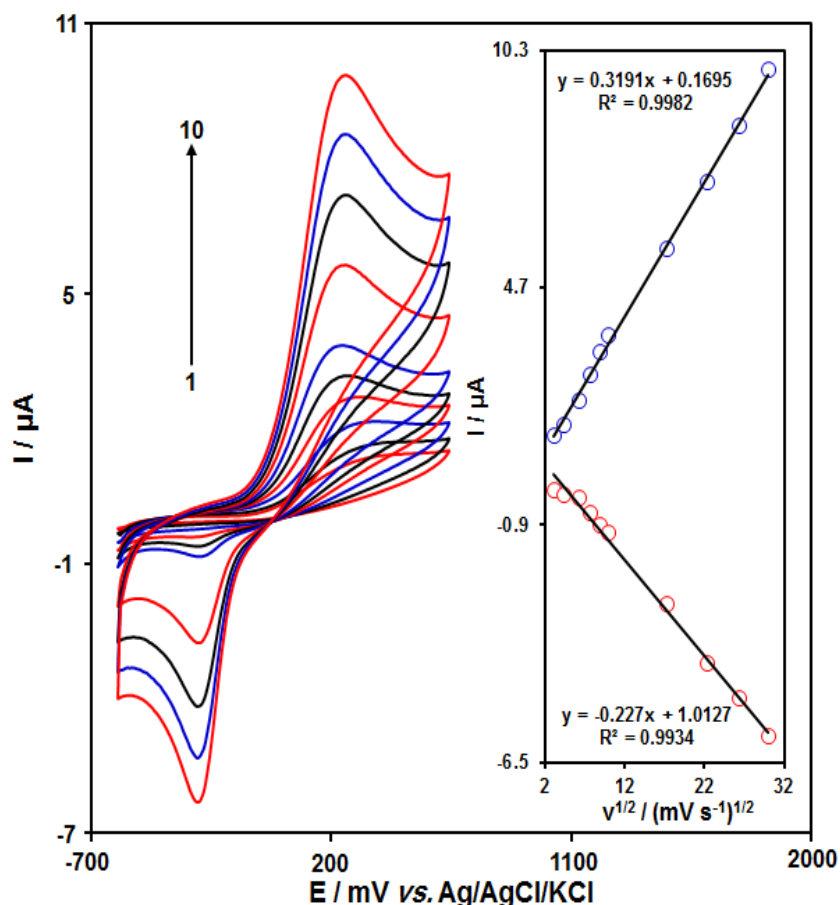


Figure 2. Cyclic voltammograms of ZnO/Al₂O₃/GCE in 0.1 M PBS (pH 7.0) containing 500.0 μ M dopamine at various scan rates; numbers 1-10 correspond to 10, 20, 40, 60, 80, 100, 300, 500, 700 and 900 mV s^{-1} , respectively. Inset: variation of anodic and cathodic peak current vs. $v^{1/2}$,

3.3. Chronoamperometric analysis

The analysis of chronoamperometry for dopamine samples was performed by use of ZnO/Al₂O₃/GCE vs. Ag/AgCl/KCl (3.0 M) at 0.3 V. The Chronoamperometric results of different concentrations of dopamine sample in PBS (pH 7.0) are demonstrated in Fig. 3. The Cottrell equation for chronoamperometric analysis of electroactive moieties under mass transfer limited conditions is as follow [66]:

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

Where D represents the diffusion coefficient (cm² s⁻¹), and C_b is the applied bulk concentration (mol cm⁻³). Experimental results of *I* vs. *t*^{-1/2} were plotted in Fig. 3A, with the best fits for different concentrations of dopamine. The resulted slopes corresponding to straight lines in Fig. 3A, were then plotted against the concentration of dopamine (Fig. 3B). The mean value of D was determined to be 1.2 × 10⁻⁶ cm²/s according to the resulting slope and Cottrell equation.

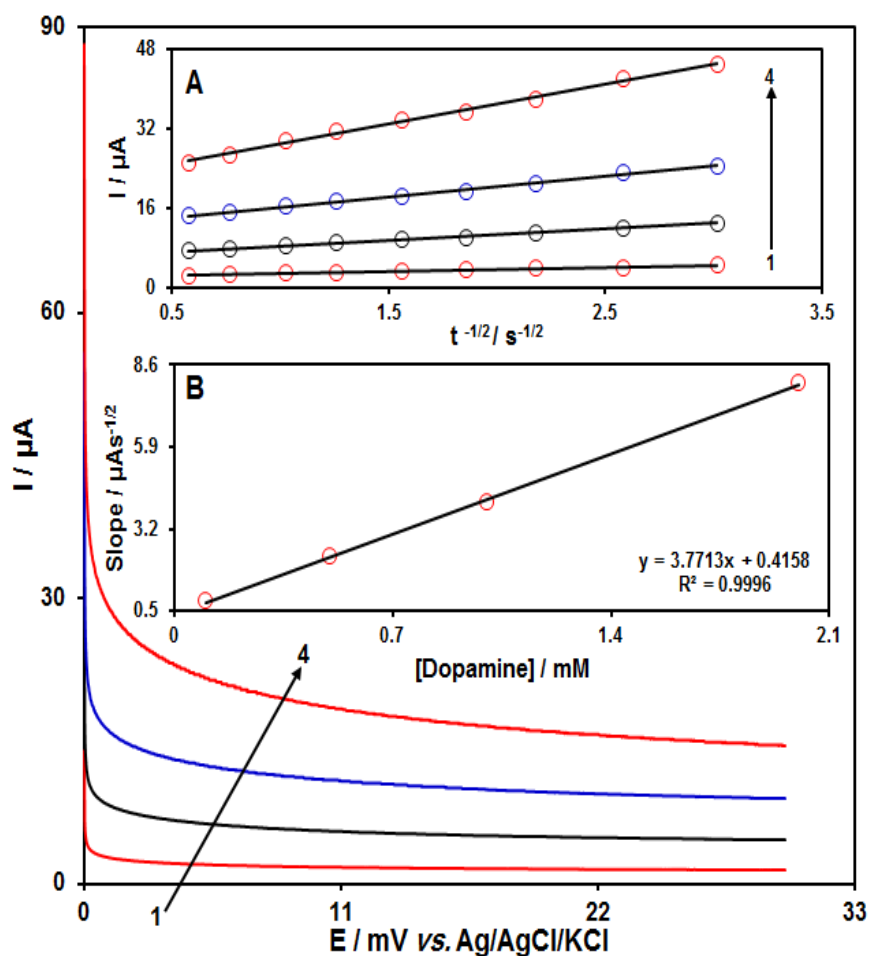


Figure 3. Chronoamperograms obtained at ZnO/Al₂O₃/GCE in 0.1 M PBS (pH 7.0) for different concentrations of dopamine. The numbers 1–4 correspond to 0.1, 0.5, 1.0, and 2.0 mM of dopamine. Insets: (A) Plots of *I* vs. *t*^{-1/2} obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against dopamine concentration.

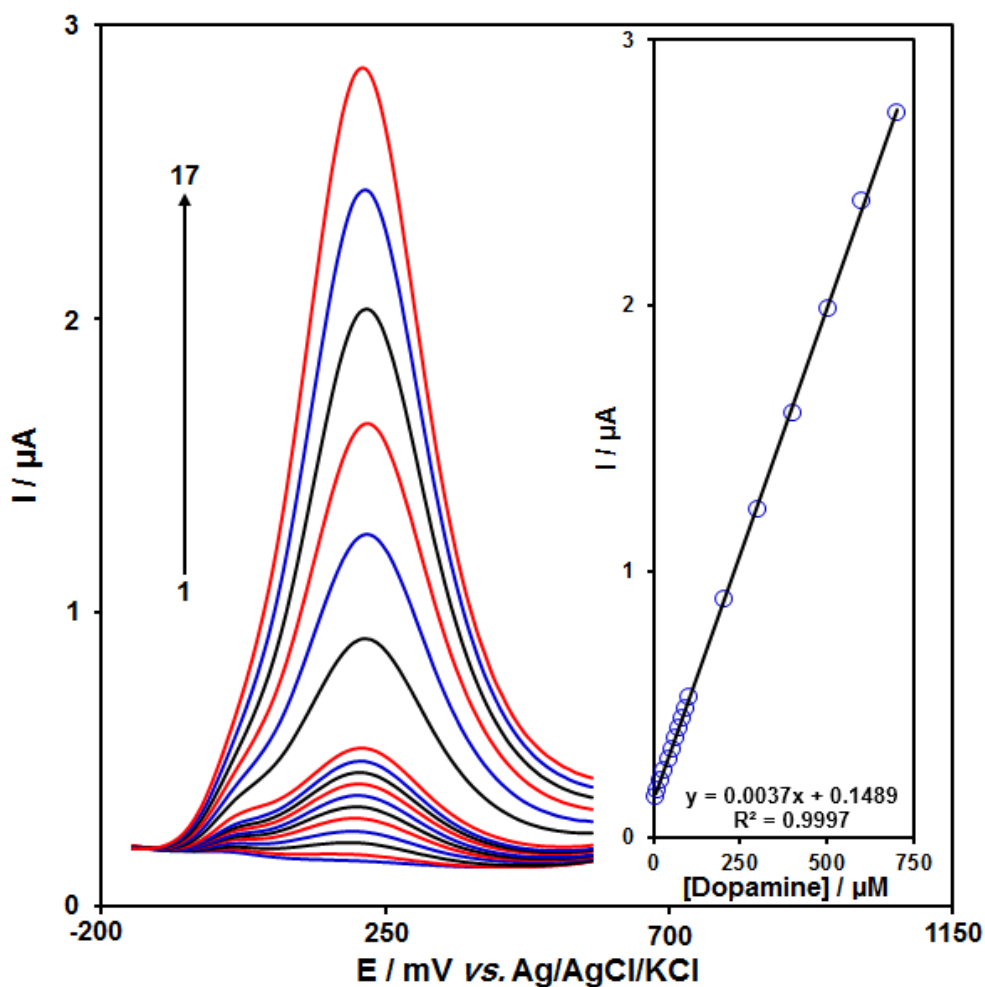


Figure 4. DPVs of ZnO/Al₂O₃/GCE in 0.1 M (pH 7.0) containing different concentrations of dopamine. Numbers 1–17 correspond to 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0 and 700.0 μM of dopamine. Inset: plot of the electrocatalytic peak current as a function of dopamine concentration in the range of 5.0-700.0 μM.

3.4. Calibration curves

Based on the resulting oxidation peak currents of dopamine using ZnO/Al₂O₃/GCE, the quantitative analysis were done in water solutions. The modified electrode (ZnO/Al₂O₃/GCE) as working electrode in the range of dopamine concentration in 0.1 M PBS was used in differential pulse voltammetry (DPV) due to the advantages of DPV including the improved sensitivity and better performance in analytical applications. According to the results, a linear relationship exists between the peak currents and concentrations of dopamine within the concentration range of 5.0-700.0 μM with the correlation coefficient of 0.9997. The detection limit was obtained 2.0 μM. Furthermore, Table 1 compares the results of the proposed modification with recently similar reported ones. As can be seen in Table 1, the values of this work are comparable with the values reported by other research groups for determination of dopamine through similar electrochemical methods (Table 1).

Table 1. Comparison of the efficiency of some recently modified electrodes used in the electro-oxidation of dopamine with the propose modified electrode in this work

Electrode	Modifier	Method	LOD (μM)	LDR (μM)	Ref.
Glassy carbon	l-tyrosine (l-Tyr) covalently functionalized graphene oxide (GO) composite	Differential pulse voltammetry	0.28	1.0–500.0	[67]
Glassy carbon	Carbon nanohorns/poly(glycine)	Differential pulse voltammetry	0.03	1.0–2800.0	[68]
Carbon fiber	Reduced graphene oxide	Differential pulse voltammetry	0.77	1.4–2240.0	[69]
Glassy carbon	Poly(l-leucine)/DNA composite film	Differential pulse voltammetry	0.04	0.1–100.0	[70]
Glassy carbon	Graphene nanosheets and ester-calix[n]arenes	Differential pulse voltammetry	0.2	0.5–400.0	[71]
Graphite screen printed	Graphene nanosheets and NiO nanoparticles	Differential pulse voltammetry	0.314	1.0–500.0	[72]
Glassy carbon	ZnO/Al ₂ O ₃ nanocomposite	Differential pulse voltammetry	2.0	5.0–700.0	This Work

3.5. Analysis of real samples

The applicability of this modified electrode in the determination of real samples was assessed through the determination of dopamine in dopamine injection and urine samples using the described method. In order to perform this analysis, standard addition method was employed and the results are listed in Table 2. Accordingly, the results of dopamine recoveries are satisfactory and the reproducibility of the results is proved by the mean relative standard deviation (R.S.D.).

Table 2. The application of ZnO/Al₂O₃/GCE for determination of dopamine in dopamine injection and urine samples (n=5). All concentrations are in μM .

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Dopamine injection	0	10.0	-	3.3
	5.0	15.1	100.7	2.4
	10.0	19.8	99.0	2.7
	15.0	24.3	97.2	1.9
	20.0	31.1	103.7	2.2
Urine	0	-	-	-
	5.0	4.9	98.0	1.8
	10.0	10.1	101.0	2.9
	15.0	14.9	99.3	3.4
	20.0	20.6	103.0	2.3

4. CONCLUSION

In order to achieve a sensitive analysis of dopamine, a new modified GCE was fabricated. For the modification, nanocomposite of ZnO/Al₂O₃ was applied (ZnO/Al₂O₃/GCE). According to the results, using nanocomposite of ZnO/Al₂O₃ is definitely effective on the improvement of electrode sensitivity towards detection of dopamine. In fact, the nanocomposite of ZnO/Al₂O₃ provides large specific surface area, excellent electrocatalytic activity and good conductivity which significantly increase the sensitivity of the electrode. In differential pulse voltammetry, the response of this modified electrode was linear in a wide range of 5.0-700.0 μM with detection limit as low as 2.0 μM, under the optimal conditions.

ACKNOWLEDGEMENTS

The authors are grateful to the Research Council of University of Tehran for the financial support of this work.

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