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Biosensing Applications of ZnO / Graphene on Glassy Carbon Electrode in Analysis of Tyrosine

Parviz Norouzi[1,2,*] , *Hadiseh Salimi*[3], *Somayeh Tajik*[4], *Hadi Beitollahi*[4,*],
Morteza Rezapour[5], *Bagher Larijani*[6]

[1] Center of Excellence in Electrochemistry, University of Tehran, Tehran, Iran
[2] Biosensor Research Center, Endocrinology & Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Chemistry, Graduate University of Advanced Technology, Kerman, Iran
[4] Environment Department, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran
[5] IP Department, Research Institute of the Petroleum Industry (RIPI), P.O. Box 14665-137, Tehran, Iran
[6] Diabetes Research Center, Endocrinology & Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[*]E-mail: norouzi@khayam.ut.ac.ir, h.beitollahi@yahoo.com

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A graphene-zinc oxide (ZnO/GR) nanocomposite film was fabricated on the glassy carbon electrode (ZnO/GR/GCE), and used for the measurement of tyrosine (Tyr) by differential pulse voltammetry (DPV). It was found that the ZnO/GR nanocomposites can improve the peak currents of tyrosine significantly due to the synergetic effect. The oxidation peak current was linearly related to tyrosine concentration in the range from 1.0 μM to 800.0 μM. The detection limit was calculated to be 0.5 μM (S/N = 3). Finally, the introduced electrode was successfully used for quantifying tyrosine in real samples.

Keywords: Tyrosine; ZnO/GR nanocomposite; Glassy carbon electrode; Cyclic voltammetry

1. INTRODUCTION

Tyrosine (Tyr), 4-Hydroxyphenylalanine, is one of the nonessential amino acid which can be a main target for radical's attack. It behaves as a singlet oxygen quencher. Tyr is the second highest in concentration species after ascorbic acid in human aqueous humor [1]. Tyrosine also is essential for the biosynthesis of some neurotransmitters like norepinephrine and dopamine. Tyrosine levels can be important factor in some disease such as Alzheimer atherosclerosis and lung diseases [2-4] Moreover, tyrosine is a precursor for synthesis of the thyroid hormones which are responsible for regulation of metabolism. The lack of tyrosine concentration in the body may cause albinism, alkaptonuria, depression, narcolepsy and other psychological diseases and a high level of tyrosine can leads to the increase of sister chromatid exchange. Hence, it is important to make a simple, accurate, rapid and inexpensive sensor for the determination of tyrosine [5-8].

There are some analytical methods which are able to determine the concentration of Try such as chromatographic and spectrometric techniques. However, they are somehow expensive, sophisticated, and awkward ones [9-11]. In contrast, Electroanalytical methods offers a new trends for the biological and environmental applications, because of thier simplicity, cost effectiveness, high sensitivity and the possibility of miniaturization [12-25]. These methods work based on the modified electrodes which lead to an active research field during past decade [26-40]. One of the key strategies to improve the electron transfer on the electrode surface in designing an electrochemical sensor or biosensor is application of new materials having highly electrocatalytic properties with highly conductive substances [41-48].

Electrochemical sensors based on nanomaterial have shown remarkable properties including higher sensitivity, selectivity, and signal to noise ratio, because of their high surface area and unique electrochemical nature [49-56]. Variety of metallic and non-metallic nanomaterials are used for electrode modification. Zinc oxide (ZnO) as one of them has attracted research interest in recent years due to its exclusive properties, as an example, wide direct band gap (3.37 eV at room temperature), large exciton binding energy (60 m eV) and a high isoelectric point (IEP) (~9.5), nontoxicity, biological compatibility, chemical and photochemical stability, high electrochemical activities and easy preparation together with porosity, large surface areas, and pore volumes, which allow better loading of reactive molecules per particle [57-63].

Graphene (GR), which is a closely packed honeycomb 2D lattice, has also aroused extensive research topics [64]. Owing to its unique properties, including superior electrical conductivity, excellent electrolytic permeability and wide potential window, graphene and its nanocomposites have been considered as promising modification materials for the design of electrochemical sensors. Furthermore, it has been verified that graphene nanosheets shows superior electrochemical activities over that of graphene oxides (GO) and reduced graphene oxides (RGO) [64-66]. Furthermore, graphene nanosheets provide high surface-to-volume ratio and faster electron-transfer rate. Thus, the integration of ZnO nanoparticles and exfoliated graphene could be expected to significantly improve the electrocatalytic properties of tyrosine [67].

Based on the previous points, it is important to create suitable conditions for analysis of tyrosine in biological fluids. In this study, we explain application of ZnO/GR nanocomposite as a nanostructure based sensor for voltammetric determination of tyrosine. The proposed sensor exhibited good electrocatalytic effect on tyrosine. Eventually, we examine the analytical ability of the fabricated sensor in the determination of Tyr in real samples.

2. EXPERIMENTAL SECTION

2.1. Apparatus and Reagents

The electrochemical measurements were performed with an Autolab Potentiostat/Galvanostat (the Netherland). An Ag/AgCl/KCl (3.0 M) electrode (as reference electrode), a platinum wire (as auxiliary electrode) and ZnO/GR/GCE (as working electrode) were used. ZnO/GR nanocomposite was synthesized in our laboratory [67].

All solutions were prepared freshly using double distilled water. Tyrosine and other reagents were of analytical grade obtained from Merck chemical company (Darmstadt, Germany). Buffer solutions were made from orthophosphoric acid or its salts in the pH range of 2.0-9.0.

2.2. Modification of the electrode

A bare glassy carbon electrode was coated with ZnO/GR nanocomposite according to the following procedure. A stock solution of ZnO/GR nanocomposite was made by dispersing 1 mg ZnO/GR nanocomposite in 1 mL water with ultrasonication for 1 h, and then a 5 µl aliquot of the ZnO/GR/H_2O suspension solution was casted on the glassy carbon electrode, and let the solvent was evaporated at room temperature.

2.3. Real samples Preparation

Urine samples were kept in a refrigerator directly after collection. Next, ten milliliters of the sample was centrifuged for 15 min at 2000 rpm. The supernatant was filtered out using a 0.45 µm filter. Then, 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 samples were spiked with altered amounts of tyrosine.

3. RESULTS AND DISCUSSION

3.1. Electrocatalytic oxidation of tyrosine at a ZnO/GR/GCE

Fig. 1 depict the cyclic voltammetric responses for the electrochemical oxidation of 400.0 µM tyrosine at ZnO/GR/GCE (curve a) and bare GCE (curve b). The anodic peak potential for the oxidation of tyrosine at ZnO/GR/GCE (curve a) is about 675 mV compared with 810 mV for that on the bare GCE (curve b). Similarly, when the oxidation of tyrosine at the ZnO/GR/GCE (curve a) and bare GCE (curve b) are compared, an extensive enhancement of the anodic peak current at ZnO/GR/GCE relative to the value obtained at the bare GCE (curve b) is observed. In other words, the results clearly indicate that the ZnO/GR nanocomposite improve the tyrosine oxidation signal.

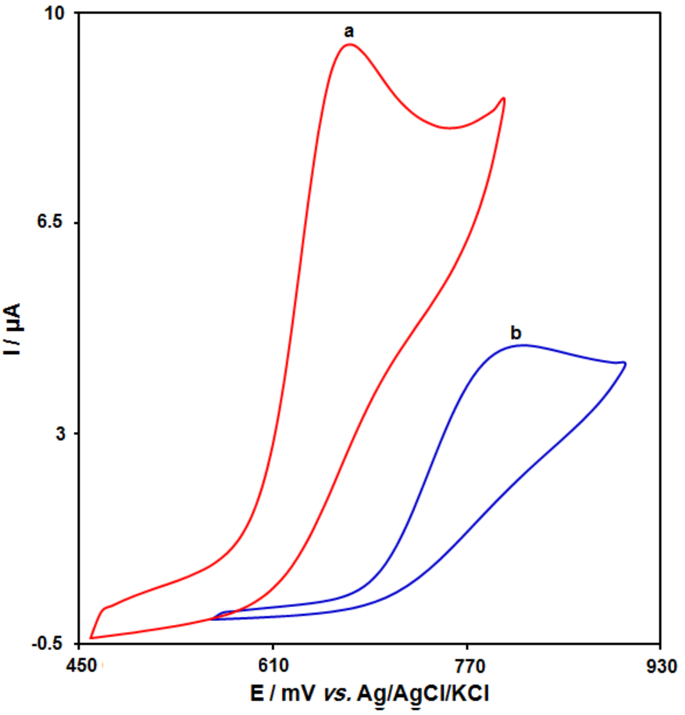

Figure 1. CVs of (a) ZnO/GR/GCE and (b) bare GCE in 0.1 M PBS (pH 7.0) in the presence of 400.0 µM tyrosine at the scan rate 50 mVs^{-1}.

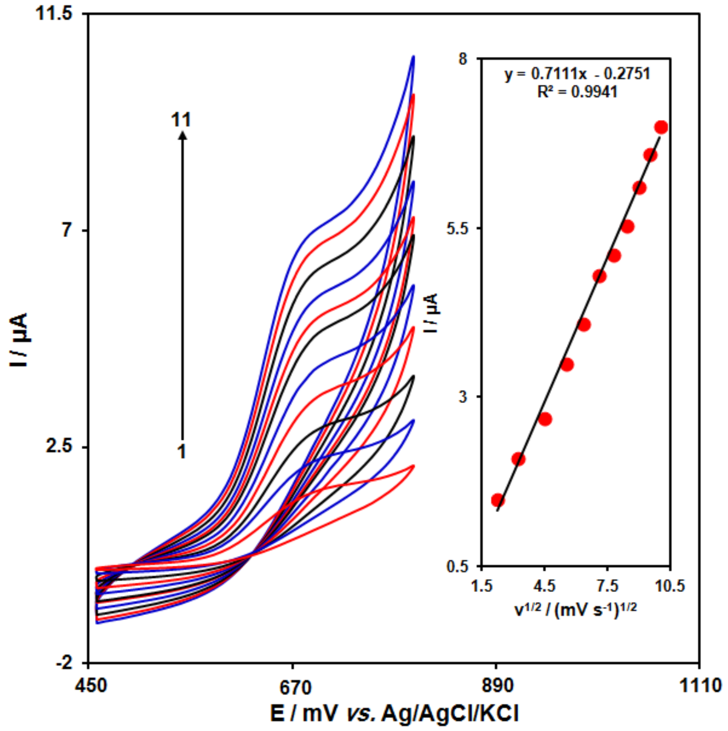

Figure 2. CVs of ZnO/GR/GCE in 0.1 M PBS (pH 7.0) having 150.0 µM tyrosine at various scan rates; numbers 1-11 correspond to 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV s^{-1}, respectively. Inset: variation of cathodic peak current vs. $\nu^{1/2}$.

In the next experiment, scan rate was changed on its effect on the oxidation current of Tyr was studied. The showed result in Fig. 2, states that increase of the potential scan rate causes an increase in the peak current. Furthermore, the oxidation process can be a diffusion controlled because there is a linearity between the anodic peak current (I_p) and the square root of the potential scan rate ($v^{1/2}$) over a wide range from 5 to 100 mV s^{-1}.

Fig. 3 show a Tafel plot that was drawn from points of the Tafel region of the CV. The Tafel slope of 0.0956 V obtained here corresponds well to the involvement of one electron in the rate determining step of the electrode process, suggesting a charge transfer coefficient of α=0.38 [68].

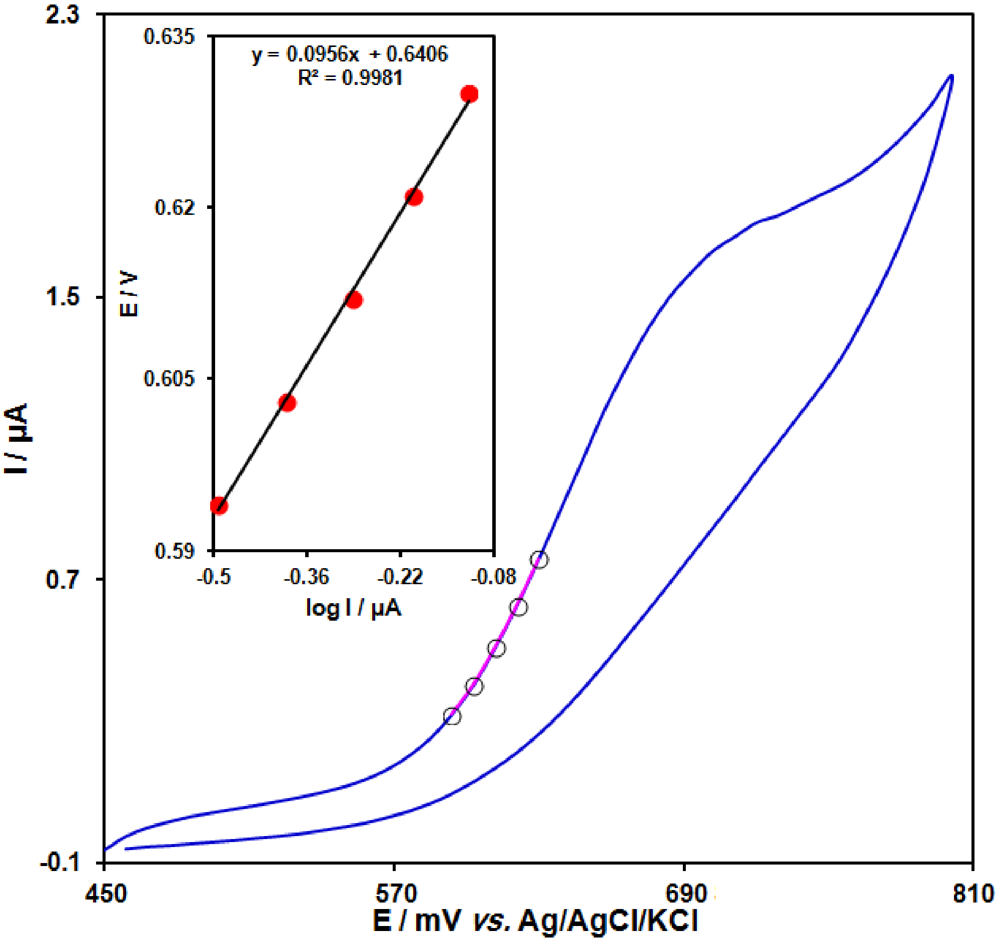

Figure 3. CV (at 5 mV s^{-1}) of electrode in 0.1 M PBS (pH 7.0) having 150.0 µM tyrosine. The points show the data used in drawing the Tafel plot. The inset is Tafel plot derived from the CV.

3.2. Chronoamperometric study

Chronoamperometric measurements of tyrosine at ZnO/GR/GCE were done by setting the potential at 0.75 V for the several concentration of tyrosine in PBS (pH 7.0) (Fig.4). In general, Cottrell equation is used for an electroactive material (tyrosine 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 [68].

$$I = \text{nFAD}^{1/2}\text{C}_\text{b}\pi^{-1/2}\text{t}^{-1/2} \qquad (1)$$

Where D and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}). The plots of I vs. $t^{-1/2}$ were drawn with the best fits for different concentrations of tyrosine (Fig. 4A).

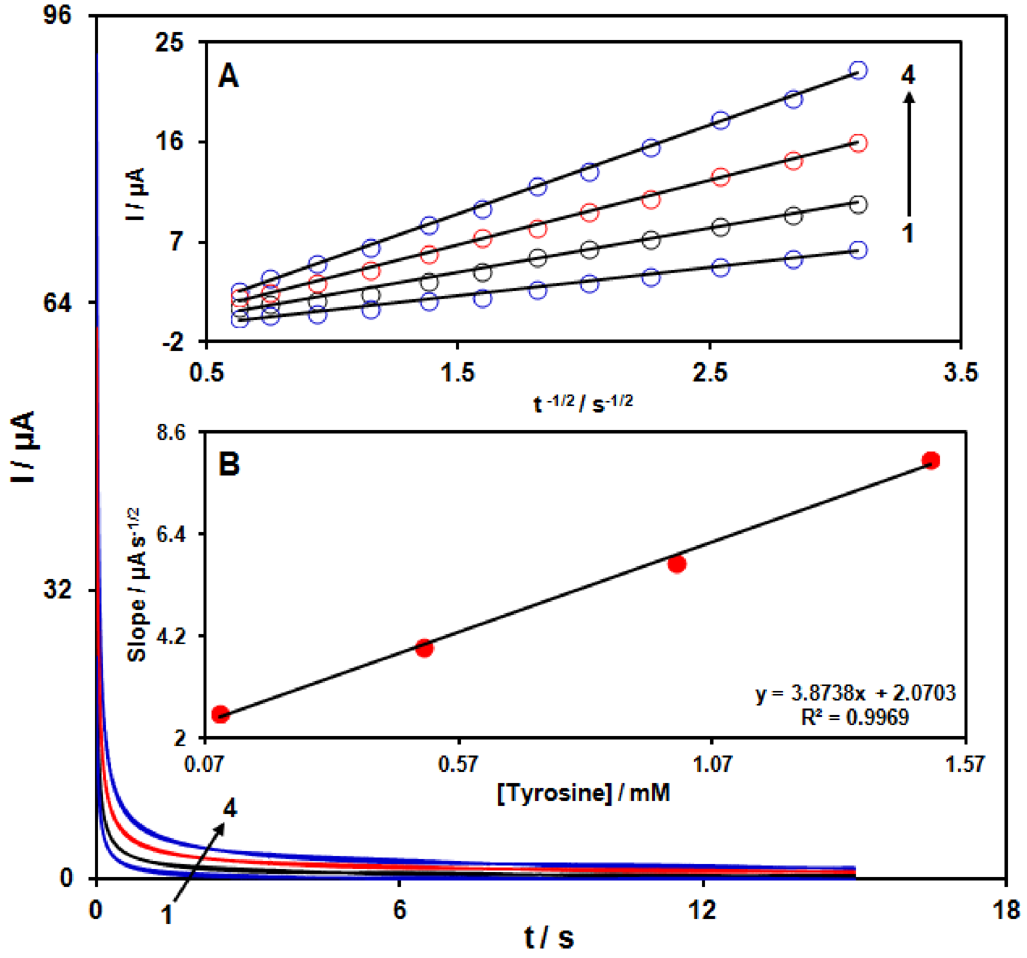

Figure 4. Chronoamperograms gotten for various concentrations of tyrosine at ZnO/GR/GCE in 0.1 M PBS (pH 7.0). The numbers 1–4 correspond to 0.1, 0.5, 1.0 and 1.5 mM of tyrosine. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against tyrosine concentration.

The slopes of the resulting straight lines were plotted vs. tyrosine concentration (Fig. 4B). From the obtained slope and Cottrell equation the mean value of the D was calculated to be 1.28×10^{-6} cm^2/s.

3.3. Calibration curve and detection limit

The oxidation peak current of tyrosine at the electrode surface can be used for determination of tyrosine in the solution. Thus, differential pulse voltammetry (DPV) experiments were done for different concentrations of tyrosine (Fig. 5). The oxidation peak currents of tyrosine at the surface of the modified electrode were related to the concentration of the tyrosine within the ranges 1.0 to 800.0

μM. Limit of detection (3σ) of tyrosine was calculated to be 5.0×10^{-7} M. These values are comparable with the values obtained by other research groups for determination of tyrosine (Table 1).

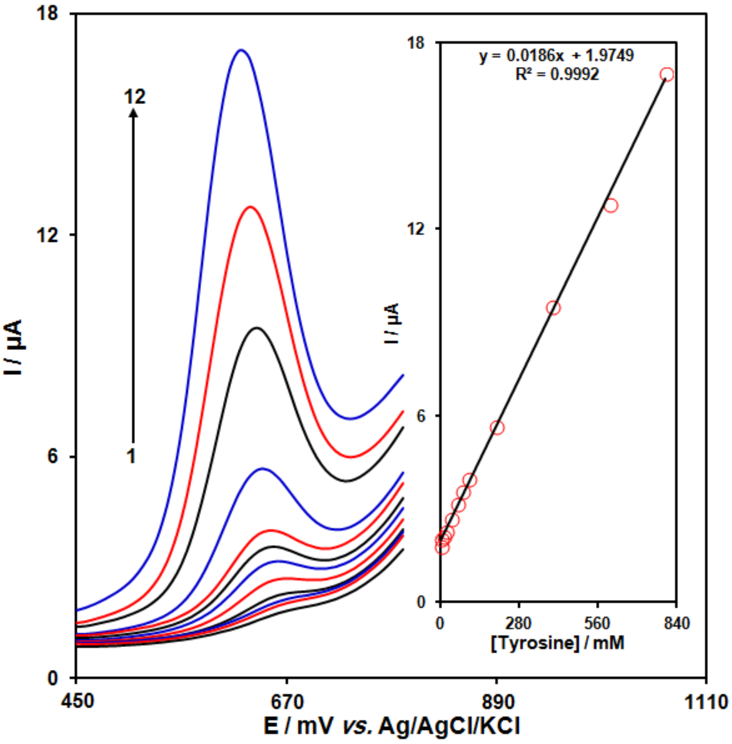

Figure 5. DPVs of ZnO/GR/GCE in 0.1 M (pH 7.0) containing different concentrations of tyrosine. Numbers 1–12 correspond to 1.0, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0, 200.0, 400.0, 600.0 and 800.0 µM of tyrosine. Inset: Plot of the electrocatalytic peak current as a function of tyrosine concentration in the range of 1.0-800.0 µM.

Table 1. Comparison of the efficiency of some modified electrodes used in the determination of tyrosine

Electrode	Modifier	Method	LDR (M)	LOD (M)	Ref.
Glassy carbon	Au-nanoparticles/poly(E)-4-(p-tolyldiazenyl)benzene-1,2,3-triol film	Voltammetry	1.0×10^{-5} – 5.6×10^{-4}	2.0×10^{-6}	[7]
Carbon paste	Silica nanoparticles	Voltammetry	5.0×10^{-7} -6.0×10^{-4}	1.5×10^{-7}	[8]
Glassy carbon	Gold nanoparticles/multiwalled carbon nanotube	Voltammetry	4.0×10^{-7} -8.0×10^{-5}	2.1×10^{-7}	[9]
Glassy Carbon	Zinc Oxide	Voltammetry	1.0×10^{-6} -8.0×10^{-4}	5.0×10^{-7}	This work

3.4. Urine sample analysis

The proposed method was applied to the determination of tyrosine in some urine samples successfully. The results for determination of spiked amount of tyrosine in urine samples are given in Table 2. Satisfactory recovery of the experimental results was obtained. The reproducibility of the method was stated by the mean relative standard deviation (R.S.D.).

Table 2. Application of ZnO/GR/GCE for determination of tyrosine in urine samples (n=5). The concentrations are in µM.

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	5.0	4.9	98.0	3.2
	10.0	10.3	103.0	2.8
	15.0	14.9	99.3	1.7
	20.0	20.3	101.5	2.4

4. CONCLUSIONS

Here, based on the ZnO/GR nanocomposite on a glassy carbon electrode, the electrochemical behavior of tyrosine were investigated in detail and a sensitive determination method was established synchronously. Differential pulse voltammetry (DPV) measurement revealed that the oxidation peak current increased linearly with tyrosine concentrations in the range of 1.0×10^{-6} M–8.0×10^{-4} M with detection limit of 5.0×10^{-6} M (S/N = 3). The obtained results allow concluding that the ZnO/GR/GCE could be promising alternative with a great potential for the quantitative measurement of tyrosine in real samples.

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