Int. J. Bio-Inorg. Hybr. Nanomater., 4(4): 197-202, Winter 2015

Graphene-manganase oxide nanocomposite as a hydrogen peroxidase sensor

M. Ramezani; Kh. Eskandari; M. Kamali*

Nanobiotecnology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran

Received: 16 July 2015; Accepted: 20 September 2015

ABSTRACT: A feasible and fast method to fabricate hydrogen peroxide sensor was investigated by graphene-manganase nanocomposite carbone paste electrode. In the present work, in first step, the graphene was synthesized by chemical method and in second step, manganese oxide nanoparticle was doped on graphene. graphene-manganase nanocomposite was characterized by FTIR and SEM. The nanocomposite shows a high conductive and sensitivity for hydrogen peroxide determination as shown by electrochemical impedance spectroscopy. Graphene-manganase nanocomposite carbone paste electrode was used for sensing of hydrogen peroxide by choronoamperometry with a potential of 250 mV (Ag/AgCl), in 0.1 M phosphate buffer solution at pH= 7. The linear concentration rang of sensor is 8.8-263.3 μM, and with detection limit of 1.04 μM. Also, the life time of sensor is infinite.

Keywords: *Electrochemistry; Graphene; Hydrogen peroxide; Manganese oxide nanoparticle; Sensor*

INTRODUCTION

In the recent years, graphene has great interest in the fields of materials science, physics, chemistry and biology. This allotrope of carbon comprises layers of six-atom rings in a hexagonal configuration with atoms bonded by sp^2 bonds (Chen, *et al.*, 2012). As a basic building block of other carbon allotropes, graphite can be wrapped to generate 0D fullerenes, rolled up to form 1D carbon nanotubes, and stacked to produce 3D graphite (Wang, *et al.*, 2011).

Nowadays, Graphene can be produced relatively easy by mechanical exfoliation of graphite, or by heating SiC, or by the reduction of graphene oxide (Gasnier, *et al.*, 2013). Graphene has unique properties, such as high surface area, high electrical conductivity wide potential windows, fairly inert electrochemistry and good electrocatalytic activity for many redox reactions, low cost and strong mechanical strength. Also, in comparison with CNTs, graphene has many advantages, as follows: i) no metallic impurity, ii) cheap and easy production (Ting, *et al.*, 2011). These properties have caused that, the graphene to be ideal candidates for electrochemistry investigation and chemical sensors and biosensors fabrication. Recently, much attention was reported for protein immobilization on graphene and hybrid of graphene (Wu, *et al.*, 2009) was reported. Recent years have witnessed the establishment of vari-

✉ Corresponding Author - e-mail: kheskandari@alumni.ut.ac.ir

ous synthetic routes for graphene, including chemically derived graphene from graphite oxide (graphene oxide, GO), chemical vapor deposition (CVD) of graphene on transition metal films, and epitaxial growth of graphene resulted from the high temperature reduction of silicon carbide. Nanocomposites have attracted great attention because of their unique and novel properties as structural or functional materials. With appropriate designs, nanocomposites can exploit the superlative properties of parent constituents, producing a desired material with improved performance. In this context, intensive efforts have been made to elaborating organic–inorganic nanocomposites for different implementations such as optoelectronics, sensors, biology. Catalysis, Organic components mainly include synthetic polymers and biomacromolecules, while inorganic components typically include nanoparticles, nanotubes, and layered inorganic materials (Kang, *et al.*, 2009). Hydrogen peroxide is an integral part of invironmental chemistry and biological systems. In the atmosphere, H_2O_2 is produced from the combination of hydroperoxyl radicals and their hydrated form it is an oxidant that. In biological systems, H_2O_2 is produced in reactions catalyzed by numerous bio-enzymes (Gregory, *et al.*, 2011). different methods have been developed for H_2O_2 detection, such as Colorimetric, electrochemistry, biosensor and so on (Keihan, *et al.*, 2013).

In this study, graphene-manganase nanocomposite, recently synthesized in our laboratories, was used as a substrate modifier for hydrogen peroxide detection. Manganese nanoparticles is sensitive and selective substrate for hydrogen peroxide detection, and the other hand, graphene is very conductive for electron transferring of hydrogen peroxide. Therefore, we prepared graphene-manganase nanocomposite as a high conductive substrate and selective hydrogen peroxide sensor.

EXPERIMENTAL DETAILS

Reagents

Graphite powder was purchased from Fisher (Chemical Scientific grade #38). Paraffin oil, $NaBH_4$, hydrogen peroxide, dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck. A 0.1 M phosphate buffer solution pH 7.4 was employed as supporting electrolyte. Ultrapure water from a Millipore-MilliQ system was used for preparing all solutions. All the reagents were used as received, without further purification and all experiments were carried out at room temperature (25±2°C).

Apparatus and measurements

Electrochemical experiments were performed with an Autolab potentiostat (PGSTAT 101). A working glassy carbon electrode with a diameter of 3 mm, a silver/silver chloride (Ag/AgCl) reference electrode, containing 3 M, KCl and a platinum rod auxiliary electrode were used from metrohm. Electrochemical studies were performed using a single-compartment conventional three-electrode cell (volume 300 μL). A carbon paste electrod was used as working electrode. Saturated silver/silver chloride (Ag/AgCl) reference electrode, containing 3 M KCl and a platinum rod auxiliary electrode were used. All potentials were measured and reported versus the Ag/AgCl reference electrode. Cyclic voltammetry experiments were performed at 0.1 V/s. The amperometric experiments were carried out by applying the desired potential and allowing the transient current to reach the steady-state value prior to the addition of the analyte and the subsequent current monitoring.

The morphology of the synthesized nanocomposite was obtained using a SEM Model LEO 440i, UK. The electrochemical behavior of nanocomposite and detection of hydrogen peroxide was carried out in an air-saturated solution for similarity of in vivo usage by Autolab potentiostat (PGSTAT 101).

Graphene synthesis

Graphene oxide (GrO) was synthesized from graphite using the Hummers method (Tang, *et al.*, 2009) and reduction graphene (RGr) was obtained by reduction of GrO with $K_2S_2O_8$. Briefly, graphite, sodium nitrate and potassium permanganate were added to concentrated sulfuric acid. After heating at 35°C for 30 min, the reacted mixture turned greenish and pasty. Then, the reaction was carefully quenched by the slow addition of water. The paste was kept at 100°C for 15 min

and turned brownish. After further dilution with water it was allowed to cool to 30°C for 30 min, during which it turned yellow. Hydrogen peroxide was carefully added to form colorless soluble manganese sulfate. The resulting GrO was isolated while still warm by filtration and the yellow-brown filter cake was washed with warm 5% diluted hydrochloric acid and finally with water. The resulting stable and brownish GrO aqueous solution was reduced by 1:1 Gr/$NaBH_4$ mass ratio, at room temperature, overnight. The graphene black precipitate was filtrated, washed with water. The different steps of the synthesis were evaluated by FT-IR spectroscopy.

Graphene-manganase nanocomposite preparation

The recognized graphene was homogenized with potassium permanganate, then 0.1% of hydrogen peroxide was added by droppwish on the mixture until to change color to brownwish. Then, graphene-manganase nanocomposite centrifuge in 10000 rpm and wash three times by ultrapure water.

Electrode preparation

In the first step, 200 mg of graphite was mixed by 10 mg of graphene-manganase nanocomposite, and it was added to 10 µL of paraffin oil. The mixture of nanocomposite was fixed on syringe electrode and washed with the PBS three times. Finally, the prepared electrode was stored at 4°C before use.

RESULTS AND DISCUSSION

Graphene-manganase nanocomposite characterization

Characterization of graphene-manganase nanocomposite is presented in Fig. 1. It shows that, manganese nanoparticles was synthesized successfully on graphene and graphene-manganase nanocomposite was made. The size of manganese nanoparticles are approximately 82 nm.

FT-IR Spectroscopic characterization of Graphene

Fig. 3 displays the spectroscopic FT-IR characterization of GrO and RGr. The IR spectrum of graphene

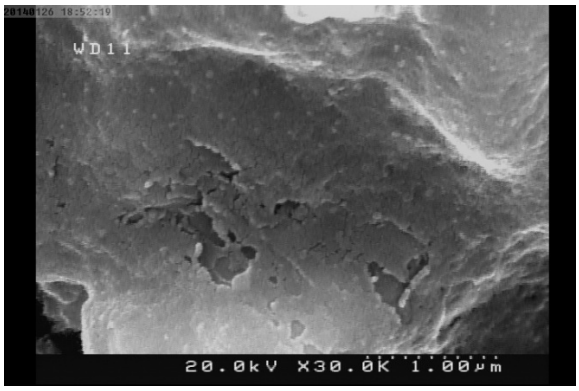

Fig. 1: SEM image of graphene-manganase nanocomposite

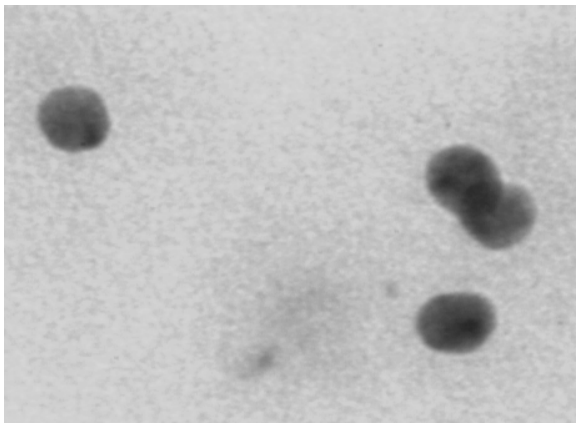

Fig. 2: HRTEM image of graphene-manganase nanocomposite.

oxide show bands attributed to oxygen containing groups, which confirmed the successful oxidation of graphite. These bands are assigned to (O-H) stretching vibration mode of intercalated water (3400 cm^{-1}); (C-O) stretching (1730 cm^{-1}); (CO epoxy) stretching (1170 cm^{-1}); and (CO alkoxy) stretching vibration (1014 cm^{-1}) (Wan, *et al.* 2011). It is obvious that, the intensity of the absorption peaks for reducing graphene were decreased. This figure proves that, the

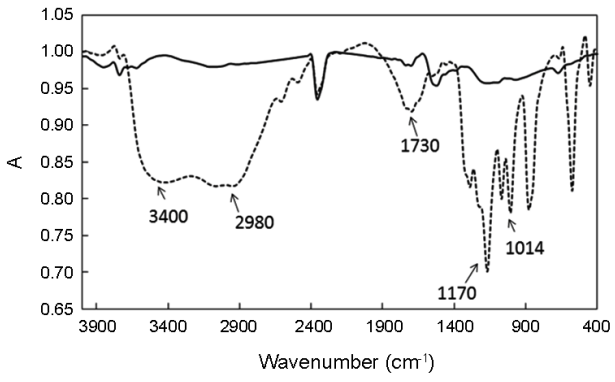

Fig. 3: FTIR spectra of graphene oxide (---) graphene (—).

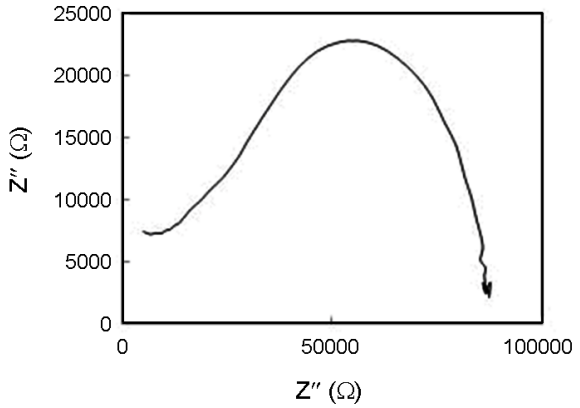

Fig. 4: EIS of Cp electrode in 3 M K3[Fe(CN)6] in PBS solution (pH= 7.4).

GrO was successfully reduced to RGr.

The investigation of conductivity by electrochemical impedance spectroscopy (EIS)

The electrochemical study results are presented as Nyquist plots in Figs. 4 and 5. According to the Figs. 4 and 5 it is evident that all the Nyquist plots showed a simple semicircle that revealed a deposited layer on electrode surfaces. The nanocomposite plot showed the minimum radius related to low charge transfer resistance and high conductivity of the electrode surface

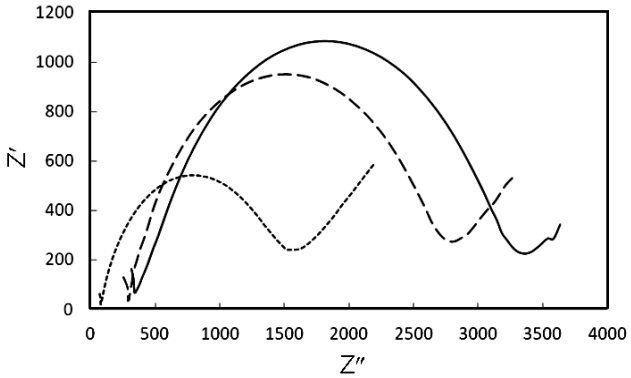

Fig. 5: EIS of graphene-manganase nanocomposite electrode in different ratio, 1:10 (….), 1:1 (—), 10:1 (----) nanoparticle to graphene as same condition of Fig. 4.

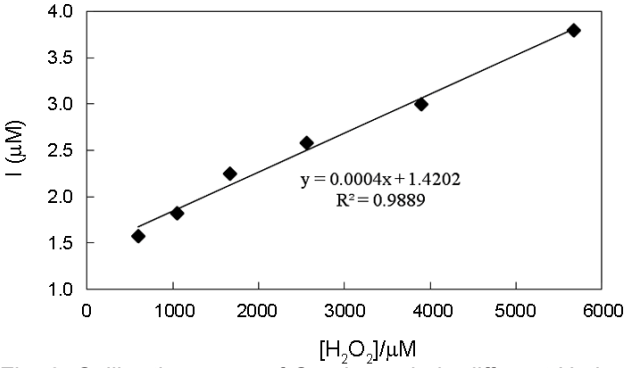

Fig. 6: Calibration curve of Cp electrode in different Hydrogen peroxide concentrations in PBS solution (pH= 7.4).

layer referred to attend of graphene-manganase nanocomposite on surface and concerned to its conductivity nature (Fig. 4) (Gao, *et al.*, 2012). The graphit plot showed the maximum semicircle radius related to maximum charge transfer resistance and minimum conductivity (Fig. 5).

Determination of hydrogen peroxide by nanocmposite

Fig. 5 displays the chronoamperometric response of the sensor to different concentrations of hydrogen peroxide for Cp electrode (Fig. 6) and graphene-man-

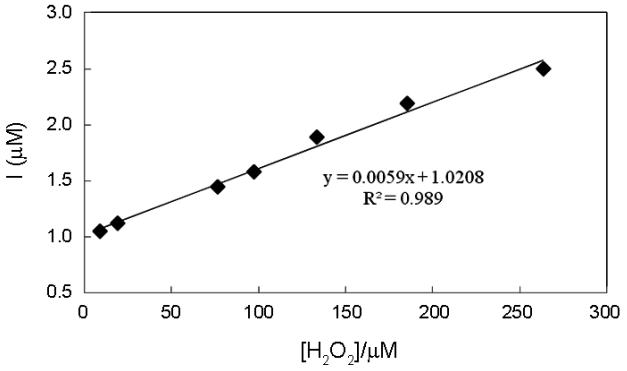

Fig. 7: Calibration curve of graphene-manganase nanocomposite electrode in different Hydrogen peroxide concentrations in same condition of Fig. 5.

Table. 1: Comparison of the analytical parameters obtained in the present work with those reported in the literature.

Sensing materials	L. R. (M)	D. L. (M)	Ref.
Hb/Au@Fe_3O_4	3.4×10^{-6}~4.0×10^{-3}	6.7×10^{-7}	(Yu, *et al.*, 2009)
HRP-nano-Au	1.2×10^{-5}~1.1×10^{-3}	6.1×10^{-6}	(Lie, *et al.*, 2004)
HRP/AuNPs	8.0×10^{-6}~3.0×10^{-3}	2.0×10^{-6}	(Wang, *et al.*, 2009)
Gr/Mn	8.8×10^{-6}~2.6×10^{-4}	1.0×10^{-6}	This work

ganase nanocomposite Cp electrode (Fig. 7). Subsequent addition of H_2O_2 to the solution resulted in an apparent increase in the reduction current. The linear concentration range of sensor for Cp electrode is 0.6-5.7 mM with detection limit of 0.06 mM (A) and the linear concentration rang of graphene-manganase nanocomposite Cp electrode (B) is 8.8-263.3 μM, and with detection limit of 1.04 μM. Present work is caparable with other Hydrogen peroxide sensors (Table 1).

CONCLUSIONS

Nowadays, the use of nanomaterial with having of biomimetic effect is extentive. Because they are cheep, simple to synthesize, high abundance and high life time. Therefore, We synthesize graphene-manganase nanocomposite as a peroxides biomimetice for hydrogen oxide detection. The graphene-manganase nanocomposite carbone paste electrode is a fast, easy and renewable electrochemical sensor. The proposed sensor have good characteristics such as; low detection limit of 1.04 μM, wide concentration range from (8.8-263.3 μM), infinite life time, fast response time and good selectivity coefficient. In this nanocomposit manganese oxide nanoparticle is a good catalyst for hydrogen peroxide. On the other hand, graphene is very conductive for electron transferring of chemical reaction. Thus, this synergetic effect caused that, the linear concentration rang of biosensor to be low.

ACKNOWLEDGMENT

Financial supports provided by the Research Council of the Baqiatallah University of Medical Science are gratefully appreciated.

REFERENCES

Chen, L., Hernandez, Y., Feng, X., Mullen, K., (2012). From Nanographene and Graphene Nanoribbons to Graphene Sheets: Chemical Synthesis. Angew. Chem. Int. Ed., 51; 7640-7654.

Wang, Y., Li, Z., Wang, J., Li, J., Lin, Y., (2011). Graphene and graphene oxide: biofunctionalization and applications in biotechnology. Trends Biotech., 29; 205-212.

Gasnier, A., Pedano, M.L., Rubianes, M.D., Rivas, G.A., (2013). Graphene paste electrode: electrochemical behavior and analytical applications for the quantification of NADH. Sens. Actuat. B, 176; 921-926.

Ting, S.W., Periasamy, A.P., Chen, S.M., Saraswathi, R., (2011). Direct electrochemistry of
catalase immobilized at electrochemically reduced graphene oxide modified electrode for
amperometric H_2O_2 biosensor. Int. J. Electrochem. Sci., 6; 4438-4453.

Wu, J.F., Xu, M.Q., Zhao, G.C., (2010). Graphene-based modified electrode for the direct electron transfer of cytochrome c and biosensing. Electrochem. Communications, 12; 175-177.

Kang, X., Wang, J., Wu, H., Aksay, I.A., Liu J., Lin Y., (2009). Glucose biosensor based on immobilization of glucose oxidase in platinum nanoparticles/graphene/chitosan nanocomposite film. Biosen. Bioelectron., 25; 901-905.

Su, G., Wei, Y., Guo M., (2011). Direct Colorimetric Detection of Hydrogen Peroxide Using 4-Nitrophenyl Boronic Acid or Its Pinacol Ester. Amer. J. Anal. Chem., 2; 879-884.

Keihan, A.H., Sajjadi, S., (2013). Improvement of the electrochemical and electrocatalytic behavior of Prussian blue/carbon nanotubes composite via ionic liquid treatment. Electrochimica Acta, 113; 803-809.

Tang, L., Wang, Y., Li, Y., Feng, H., Lu, J., Li, J., (2009). Preparation, structure, and electrochemical properties of reduced graphene sheet films. Adv. Funct. Mater., 19; 2782-2789.

Wan, L., Song, Y., Zhu, H., Wang, Y., Wang, L., (2011). Electron transfer of co-immobilized cytochrome c and horseradish peroxidase in chitosan-graphene oxide modified electrode. Inter. J. Electrochem. Sci., 10; 4700–4713.

Gao, Z., Liu, N., Wu D., Tao, W., Xu, F., Jiang, K., (2012). Graphene–CdS composite, synthesis and enhanced photocatalytic activity. Appl. Surf. Sci., 258; 2473-2478.

Yu ,CM., Guo, JW., Gu, HY., (2009) Direct electrochemical behavior of hemoglobin at surface of Au@Fe_3O_4 magnetic nanoparticles. Microchim Acta. 166; 215-220.

Lei, C.X., Hu, SQ., Gao, N., Shen, G.L., Yu, R.Q., (2004). An amperometric hydrogen peroxide biosensor based on immobilizing horseradish peroxidase to a nano-Au monolayer supported by sol–gel derived carbon ceramic electrode. Bioelectrochemistry, 65; 33–39.

Wang, J.W., Wang, L.P., Di, J.W., Tu, Y.F., (2009). Electrodeposition of gold nanoparticles on indium/tin oxide electrode for fabrication of a disposable hydrogen peroxide biosensor. Talanta, 77; 1454-1459.

AUTHOR (S) BIOSKETCHES

Manizheh Ramezani, M.Sc., Nanobiotecnology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran

Khadijeh Eskandari, Assistant Professor, Nanobiotecnology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran, *E-mail: kheskandari@alumni.ut.ac.ir*

Mehdi Kamali, Assistant Professor, Nanobiotecnology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran