



Highly Sensitive Amperometric Sensor Based on Gold Nanoparticles Polyaniline Electrochemically Reduced Graphene Oxide Nanocomposite for Detection of Nitric Oxide

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PAPER INFO

Paper history:

Received 11 December 2017

Received in revised form 01 January 2018

Accepted 04 January 2017

Keywords:

Nitric Oxide Sensor

Reduced Graphene Oxide

Gold Nanoparticle

Polyaniline

Cyclic Voltammetry

ABSTRACT

A sensitive electrochemical sensor was fabricated for selective detection of nitric oxide (NO) based on electrochemically reduced graphene (ErGO)-polyaniline (PANI)-gold nanoparticles (AuNPs) nanocomposite. It was coated on a gold (Au) electrode through stepwise electrodeposition to form AuNPs-PANI-ErGO/Au electrode. The AuNPs-PANI-rGO nanocomposite was characterized by Field Emission Scanning Electron Microscopy (FESEM) and UV-vis. Electrochemical behavior of modified electrode was analyzed by cyclic voltammetry (CV) and chronoamperometry (CA) techniques. CVs of AuNPs-PANI-ErGO/Au, PANI-ErGO/Au and ErGO/Au electrodes showed that conductivity of AuNPs-PANI-ErGO/Au was higher than others. Nafion was used to improve selectivity of modified electrode. Nafion/AuNPs-PANI-ErGO/Au electrode represented favorable electrochemical and electrocatalytic behavior towards NO oxidation. The resultant electrode exhibited a high sensitivity of $0.113 \mu\text{A}/\mu\text{M}$ over a wide linear range from 0.8×10^{-6} to 86×10^{-6} M with a low detection limit of 2.5×10^{-7} M (S/N=3). In addition, the sensor had excellent stability, as well as reproducibility and selectivity, which makes it possible to detect NO quickly and accurately.

doi: 10.5829/ije.2018.31.02b.01

1. INTRODUCTION

Nitric oxide (NO) has been studied extensively due to its effects on air pollution, and especially on biological systems. It is involved in many physiological and pathological processes [1]. The abnormal production of NO affects on some important biological activities and leads to many diseases [2]. Under-production or overproduction of NO can lead to arteriosclerosis, hypertension, stroke, Parkinson's disease, Alzheimer's disease and diabetes (types I and II) [3, 4]. In industrial, biochemical and medical perspectives, it is important to quantify NO produced in normal and abnormal tissues, including in-vivo or in-vitro measurements [5]. Therefore, identification of NO is a vital necessity for these areas. Since the NO, a hydrophobic free radical, has automatic chemical reactivity with a half-life of a

few seconds (about 6 seconds), the quantification of NO is difficult [2, 6].

Among UV-visible spectroscopy, chemiluminescence, electron spin resonance spectroscopy, electrochemical and fluorescence methods [7], electrochemical technique is often used due to its superior features such as fast response, high sensitivity, low cost, and particularly its applicability for both in vivo and in vitro detection [8-11]. Recently, extensive research has been conducted on improving the sensitivity of electrochemical sensors through electrode modification by nanomaterials [12]. Among them, graphene due to its high loading efficiency, excellent conductivity, supreme biocompatibility, interesting electronic transfer property and extremely high specific surface area [13, 14], has attracted considerable attention in electrochemical NO sensors [15, 16]. Graphene is synthesized by chemical and electrochemical methods. In a chemical method, a dangerous chemical reagent such as hydrazine is used

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for reduction of graphene oxide (GO) to graphene. In comparison to this method, the electrochemical method is more accurate and environmental-friendly process [17]. Recently, graphene-metal nanocomposites has been investigated for electrochemical sensors [18-20]. One of the most important metals is gold nanoparticles (AuNPs); that is due to high surface area, stability in physiological conditions and catalytic potential makes it very interesting for NO sensors [15, 16].

Polyaniline (PANI) is unique among various conducting polymers, because of its easy synthesis, good conductivity, stability and reasonable cost [21, 22]. PANI based electrochemical sensors have been reported for detection of many substrates [23-25].

Herein, a sensitive and stable NO sensor was developed based on electrochemically reduced graphene oxide (ErGO)-PANI-AuNPs modified Au electrode. The rGO-PANI-AuNPs nanocomposite was characterized by Field Emission Scanning Electron Microscopy (FESEM) and UV-vis spectroscopy. A Nafion film was coated on the modified electrode to prevent interfering species penetration. Electrochemical and electrocatalytic response of the resultant Nafion/ AuNPs-PANI-rGO/Au electrode was investigated using cyclic voltammetry (CV) and chronoamperometry (CA) techniques. It was found that the modified electrode exhibited high performance towards NO oxidation.

2. EXPERIMENTAL

2.1. Reagents Gold chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$ trace metals basis), potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$, 99%), trisodium citrate dehydrate, and Nafion (5wt% solution in a mixture of lower aliphatic alcohols and water) were purchased from Sigma Chemicals (Saint Louis, MO, USA). The other reagents such as hydrazine hydrate solution (80% solution in water), pyrogallol (ACS reagent, $\geq 99\%$), ammonium peroxydisulfate (extra pure), graphite fine powder extra pure and aniline were purchased from Merck Company (Darmstadt, Germany). A series of phosphate buffer saline solutions (PBS, 0.1 M, pH 7.4) were prepared by dissolving 80 g NaCl, 2 g KCl, 14.4 g Na_2HPO_4 and KH_2PO_4 in one liter distilled water and used as supporting electrolyte [2]. All solutions were prepared with double distilled water (DDW) purified by a Millipore-Q System (18.2 $\text{M}\Omega$ cm).

2. 2. Analytical Measurements All electrochemical measurements were carried out with an electrochemical workstation (VSP model, BioLogic Science Instruments Co., Paris, France) at room temperature. A conventional three-electrode cell was used with an Ag|AgCl reference electrode, a Pt counter electrode and a modified gold working electrode.

Freshly prepared 10 mL of 0.1 M PBS (pH 7.0) was used as electrolyte solution. UV-vis measurements were executed using a SPEKOL 1500 spectrophotometer (Analytik Jena AG-Germany) with subtraction from blank solutions. Field emission scanning electron microscopic (FESEM) images were acquired using TESCAN-MIRA3.

2. 3. Preparation of NO Standard Solutions and Colloidal Gold Nanoparticles

NO was generated by dropwise addition of 2 M H_2SO_4 into a saturated NaNO_2 solution. Then, the produced gas was continuously passed through 5% (w/v) pyrogallol, saturated and 10% (w/v) potassium hydroxide solutions, respectively. The filtered gas was injected to 10 mL of deoxygenated PBS (pH 7.0) containing 0.1 M NaCl for 30 min to generate a 1.8 mM NO solution. Colloidal gold nanoparticles (AuNPs) were synthesized through sodium citrate reduction of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$. The resultant AuNPs had diameters in the range of 12-48 nm. The produced solutions were stored in a refrigerator until further use [26]. Details of these processes were discussed in our previous published work [2].

2. 4. Preparation of PANI-rGO and PANI-rGO-AuNPs nanocomposites

GO was synthesized from graphite by Hummers' method [27]. The PANI-rGO nanocomposite was prepared using chemical oxidative polymerization method [28]. Briefly, 2 mg of aniline, 10 mg of GO and 0.2 mL of 1 M HCl were added into 17 mL of DDW with stirring for 30 min at room temperature. Then, 10 mg of ammonium peroxydisulfate was added into the suspension in continuous stirring condition for 4 h. Finally, the suspension was autoclaved at 120 °C for 3 h. To prepare PANI-rGO-AuNPs nanocomposite, 1.2 ml of 25 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was added to 20 ml of previously prepared PANI-rGO solution followed by stirring at room temperature for 2 h. Then, 1.8 mL of 0.1 M NaBH_4 aqueous solution was added and the reaction mixture was continuously stirred for another 2 h. The reaction resulted in a complete reduction of the ions of Au and GO.

2. 5. Fabrication of Nafion/AuNPs- PANI- ErGO /Au electrode

A gold wire (0.75 mm in diameter) with plastic cover was used as working electrode. Electrode cleaning was accomplished by mechanical (Al_2O_3 powder) and chemical (ethanol) methods [2]. The cleaned electrode was ethanol ultrasonicated and allowed to dry at room temperature. GO solution was diluted to 0.75 mg/mL and used for electrodeposition. A volume of 15 μL of GO solution was dropped on the polished Au electrode surface, and the electrode was dried in ambient air at room temperature to form a GO modified electrode (GO/Au electrode).

The GO/Au electrode was dipped into 10 mM PBS (pH7.2) and GO film was electrochemically reduced to ErGO film by CV scanning (0 to -1.3 V at 50 mV/s scan rate, for 10 cycles). Electrodeposition of PANI on ErGO/Au electrode was performed at room temperature and potentiostatically 1.0 V vs. Ag/AgCl for 130 s in aqueous solution containing 50 mM aniline and 1 M HCl. The obtained PANI-ErGO/Au electrode was washed with DDW and dipped into a 0.1 M NaOH solution for over-oxidation of PANI film by CV scanning (-0.2 to 1.4 V at 100 mV/s scan rate).

AuNPs were electrodeposited on the PANI-ErGO/Au electrode by immersing into AuNP solution and scanning the potential between -0.1 V and +1.5 V vs. Ag/AgCl electrode for 90 cycles. Finally, the obtained electrode was washed with DDW and denoted as AuNPs-PANI-ErGO/Au electrode. The AuNPs-PANI-ErGO/Au electrode was coated by 3 μ L of in 1 wt% Nafion solution (diluted with ethanol) and subsequently dried at 4°C for 12 h.

3. RESULTS AND DISCUSSION

3. 1. Spectroscopic Analysis UV-vis measurement was used to characterize the formation of rGO-PANI-AuNPs nanocomposite. Figure 1 shows UV-vis spectra of GO, rGO-PANI and rGO-PANI-AuNPs nanocomposite. This figure illustrates, the GO (solid line) have two characteristic absorption peaks at 230 nm and 300 nm, which are respectively related to the π - π^* transition of aromatic C=C bonds and the $n \rightarrow \pi^*$ transition of C=O groups [29]. As can be seen, the maximum absorption peak is found to red shift towards 270 nm in the case of rGO-PANI (dotted line) as compared to that of GO (solid line). This shift indicates the refreshed electronic conjugation in graphene sheets. The possible reduction mechanism is that the released electrons in the polymerization of aniline are transferred

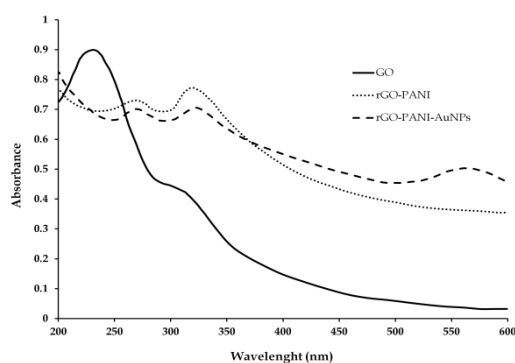


Figure 1. UV-vis spectra of GO, rGO-PANI and rGO-PANI – AuNPs

to the nearby oxygen-containing species on GO [28]. In addition, a peak is observed at 320 nm, indicating the formation of PANI. Similarly, the absorption spectra of rGO-PANI- AuNPs nanocomposite (dashed line) showed two peaks at 270 nm and 320 nm correlating to the presence of GO and PANI in the rGO-PANI-AuNPs nanocomposite, and the other peak at 550 nm corresponds to the surface plasmon resonance absorption of AuNPs [15].

3. 2. Surface Characterization FESEM was used for morphological characterization of the different modified electrodes (ErGO/Au, PANI-ErGO /Au and AuNPs-PANI-ErGO/Au electrodes), as shown in Figure 2. The stepwise modification of Au electrode can be seen clearly from these images. Figure 2A confirms the formation of rGO sheets on bare Au electrode. Therefore, it can be concluded that GO was reduced electrochemically to rGO (graphene) when the potential decreased to a negative value. As can be seen in Figure 2B, the PANI layer was clearly deposited electrochemically on rGO sheets. In preparation of PANI-ErGO electrode as discussed before (see section 2.5): PANI-ErGO electrode was prepared via electrochemical oxidative polymerization method. The ErGO electrode was oxidized electrochemically in aniline solution; as shown in this figure a layer of PANI deposited on the electrode. Morphology of AuNPs-PANI-ErGO/Au electrode can be seen in Figure 2C which shows well distribution of spherical AuNPs on ErGO-PANI composite. From this figure, it can be concluded that the AuNPs-PANI-ErGO nanocomposite has a high surface area that enables it to significantly improve electron transfer.

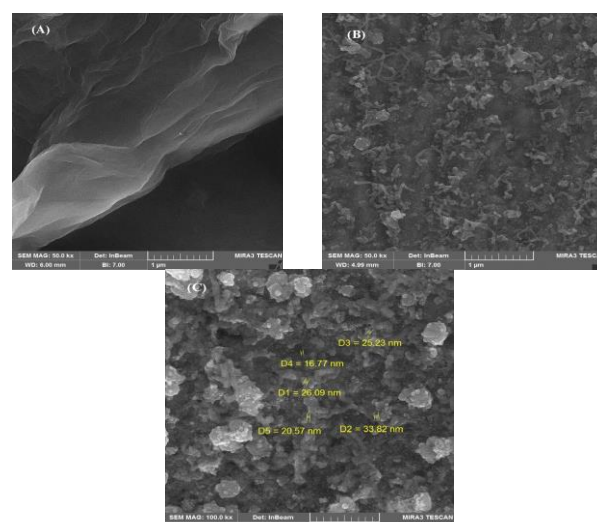


Figure 2. FESEM images of ErGO film (A), PANI-ErGO film (B) and AuNPs-PANI-ErGO film (C)

3. 3. Electrochemical Characterization of AuNPs-PANI-ErGO/Au Electrode

The electrochemical cyclic voltammograms response of ErGO/Au, PANI-ErGO/Au and AuNPs-PANI-ErGO/Au electrodes have been investigated in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1M KCl and the results are shown in Figure 3. The anodic peak current in the case of PANI-ErGO/Au electrode (dashed line) were found to be lower than ErGO/Au electrode (dotted line) due to the deeply entrapped redox active sites of rGO into the macromolecular structure of the PANI, leading to blockage in the electron transfer [29]. However, the anodic peak potential is shifted towards a higher potential (i.e. from 0.3 to 0.35 V) due to lower electrical conductivity of the of PANI-ErGO/Au electrode [30]. The maximum anodic peak potential belongs to AuNPs-PANI-ErGO/Au electrode (solid line) which is due to high surface area and facile heterogeneous electron transfer properties of the AuNPs. In fact, AuNPs-PANI-ErGO/Au electrode shows highest conductivity which allows $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to reach the electrode surface easily.

3. 4. Effect of Nafion Coating and Interferences

NO sensor used in biological systems should have high selectivity. For electrochemical detection of NO in biological systems, many species of interferences, such as nitrite (NO_2^-), ascorbic acid (AA) and uric acid, can easily be oxidized at anodic potential. NO_2^- is a main source of interference in the biological detection of NO because it is one of the products of NO oxidation and can be oxidized at approximately the same potential [31]. In order to improve the selectivity of the NO sensor, Nafion, a cation-exchange film, does not act as a barrier to discriminate against anions, such as nitrite, ascorbate and uric acid, but also prevents the electrode from fouling due to the non-specific adsorption of proteins and other materials [32].

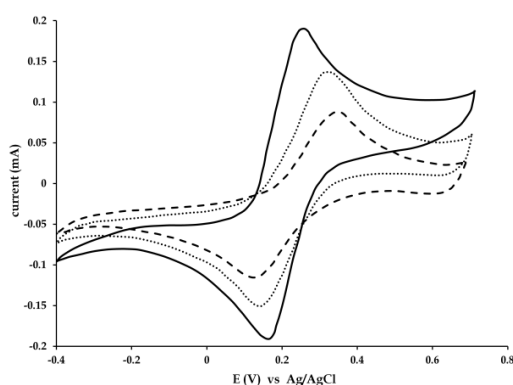


Figure 3. Cyclic voltammograms of different modified electrodes: ErGO/Au electrode (dotted line), PANI-ErGO/Au electrode (dashed line) and AuNPs-PANI-ErGO/Au electrode (solid line) in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with 0.1 M KCl

The positive charge of Nafion makes it possible for neutral NO molecules to diffuse freely through the membrane. Obviously, increasing the thickness of the membrane will increase the response time and reduce the sensor's sensitivity. Thus, for best sensitivity and selectivity, optimization of the Nafion's thickness is essential. Figure 4 shows the effect of Nafion coating on the NO and NO_2^- responses of peak current. As can be seen in this figure, after coating of 1 μL of 1.0% Nafion onto surface of AuNPs-PANI-ErGO/Au electrode, the NO response current (curve (a)) decreased to 52% of the initial response, while the NO_2^- response (curve (b)) decreased to 37% of the initial response. It means that the selectivity ratio of NO to NO_2^- had increased. When 2 μL or more of 1.0% Nafion was coated on the electrode surface, the response of NO slightly changed while the NO_2^- response decreased clearly. According to Figure 4, it can be concluded that 3 μL of 1.0% Nafion coating on the AuNPs-PANI-ErGO/Au electrode surface is sufficient to get the best selectivity of NO towards NO_2^- .

3. 5. Electrochemical Behavior and Amperometric Response of NO on Nafion/AuNPs-PANI-ErGO/Au Electrode

Figure 5 shows cyclic voltammograms of the Nafion/AuNPs-PANI-ErGO/Au electrode in absence and presence (different concentrations) of NO in oxygen-free 0.1 M PBS (pH 7.0) solution with a scan rate of 100 mVs^{-1} . According to this figure, the modified electrode displayed oxidation peak current in +0.8 V in the presence of NO while did not show any response in the absence of NO based on applied voltage range (0 to 1 V), PBS does not show any redox peak at pH 7.0. Significant increase in oxidation peak implies electrocatalytic oxidation process of NO as compared to that in the absence of NO.

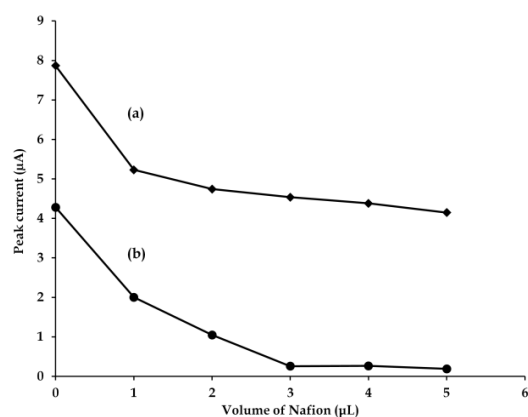


Figure 4. Effect of Nafion coating on the NO and NO_2^- response at the AuNPs-PANI-ErGO/Au electrode, 60×10^{-6} M NO (a) and 75×10^{-6} M NO_2^- (b)

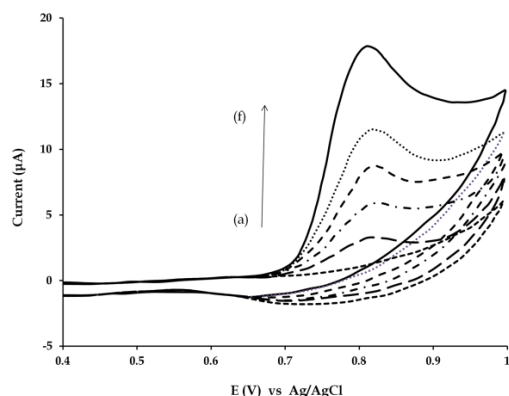


Figure 5. Cyclic voltammograms of Nafion/AuNPs-PANI-ErGO/Au electrode in 0.1 M PBS (pH 7.0) in varying NO concentrations 0 μM (a), 20 μM (b), 50 μM (c), 80 μM (d), 120 μM (e) and 250 μM (f) at the scan rate of 100 mVs^{-1}

Based on the results thus obtained, chronoamperometry (CA) experiments were performed at an applied potential of +0.8 V versus reference electrode.

The CA analysis of NO oxidation was investigated with Nafion/AuNPs-PANI-ErGO/Au electrode for different concentrations of NO in 0.1 M PBS (pH 7.0) solution at a regular time interval of 50 s (Figure 6A). In each injection of NO, the oxidation current steeply decreased to reach a steady-state current (I_{ss}) within 4 s (Less than half-life of NO in biological systems, 6 seconds). This fast response is due to high electrical conductivity of AuNPs-PANI-ErGO film. With an increase in concentration of injected NO to the electrochemical cell, the current response also increased. Figure 6B shows the calibration curve of response current against NO concentration for fabricated NO sensor. The limit of detection (LOD) was estimated to be 0.25 μM in the linear range of 0.8-86 μM ($R=0.999$). The sensitivity of the sensor was found to be 0.113 $\mu\text{A}/\mu\text{M}$ based on the criterion of a signal-to-noise ratio of 3 ($S/N=3$). Such performance is superior compared to the previously reported NO sensors with modified electrodes based on Nafion/rGO [33], Nafion/multi-walled carbon nanotubes (MWNTs)-chitosan (CS) -AuNPs [34], AuNPs-polyelectrolyte hybrid film [35] and AuNPs/sulphide-functionalized polydopamine (S-PDA) [36].

3. 6. Repeatability, Reproducibility, Stability and Selectivity of the Nafion/AuNPs-PANI-ErGO/Au Electrode The repeatability of the Nafion/AuNPs-PANI-ErGO/Au electrode was checked by repetitive (five time) amperometric measurements of 1 mM NO. A relative standard deviation value (RSD) of 2.1 % was obtained for the five successive assays.

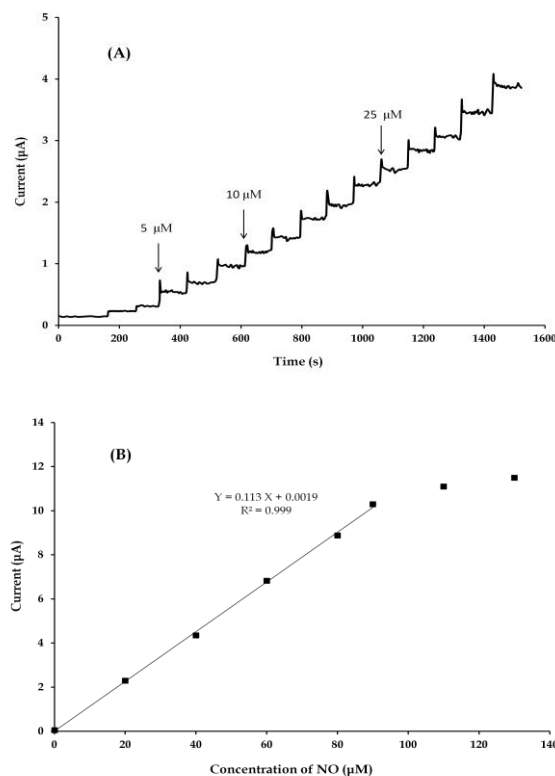


Figure 6. (A) Amperometric response of the Nafion/AuNPs-PANI-ErGO/Au electrode to successive injection of NO into 10 mL of stirring 0.1 M PBS (pH 7.0) at an applied potential of +0.8 V (vs. Ag/AgCl). (B) Calibration curve of current to NO concentration. Applied potential +0.8 V (vs. Ag/AgCl), supporting electrolyte 0.1 M PBS (pH 7.0)

In order to examine reproducibility of the same modified electrode, current response of five different modified electrodes was recorded in 0.1 M PBS (pH 7.0) containing 1 mM NO. The RSD was 3.2 % for these tests. After 4 weeks, the current response to 1 mM NO only decreased by 4.2 % from the original value. These result showed that the Nafion/AuNPs-PANI-ErGO/Au electrode has good stability and reproducibility.

Selectivity of Nafion/AuNPs-PANI-ErGO/Au electrode for NO detection was evaluated by injections of different interfering species, i.e. ascorbic acid (AA), nitrite (NO_2^-) and hydrogen peroxide (H_2O_2) into the stirring 0.1 M PBS (pH 7.0) solution as shown in Figure 7. To evaluate the current response of Nafion/AuNPs-PANI-ErGO/Au electrode towards NO even after injection of 50-fold higher concentrations of interfering species, NO was again introduced to the cell. Each addition was introduced at an interval of 60 s. As it was observed in this figure, no response was shown for interfering species during detection of NO; while NO injection showed a considerable current response.

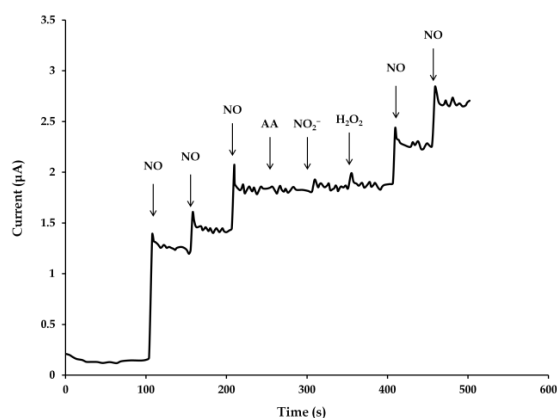


Figure 7. Amperometric response recorded by the Nafion/AuNPs-PANI-ErGO/Au electrode in a 0.1 M PBS (pH 7.0) at an applied potential of +0.8 V (vs. Ag/AgCl) to which 10 μM NO, 500 μM AA, 500 μM NO_2^- , 500 μM H_2O_2 and 10 μM NO were added, respectively

Thus, Nafion significantly eliminated the effect of these species, especially NO_2^- , during NO detection. Accordingly, it can be concluded that this sensor had specific sensing for NO even in the presence of interfering species.

4. CONCLUSION

In summary, we have successfully fabricated a selective and sensitive NO sensor based on AuNPs-PANI-ErGO hybrid. The interferences species (NO_2^- , AA and H_2O_2) were eliminated by Nafion coating. The Nafion coating volume was optimized (3 μL of 1.0% Nafion). The AuNPs-PANI-ErGO electrode exhibited an enhanced electron transfer activity and wider active area for substrate adsorption, compared to ErGO and PANI-ErGO electrodes. The sensor represented a supreme electrochemical performance in NO oxidation. A good linear response towards NO concentrations of 0.8-86 μM was obtained with a low detection limit of 0.25 μM ($S/N=3$). In addition, this fabricated sensor exhibits high sensitivity (0.113 $\mu\text{A}/\mu\text{M}$) and stability, fast response time (4 s) and good selectivity for NO detection even in the presence of interfering species. The approach proposed in this work may provide a platform for further study on highly conductive polymer-graphene nanocomposites and their application in sensors and biosensors to improve the accuracy and speed of detection of NO in biological systems.

5. ACKNOWLEDGMENTS

The authors are thankful to Biotechnology Research Center, Fuel Cell Research Center and Nanotechnology

Research Institute, Noshirvani University of Technology, Babol, Iran for their technical supports provided to accomplish present research.

6. REFERENCES

- Moncada, S., Palmer, R. and Higgs, E., "Nitric oxide: Physiology, pathophysiology, and pharmacology", *Pharmacological Reviews*, Vol. 43, No. 2, (1991), 109-142.
- Pashai, E., Darzi, G.N., Jahanshahi, M., Yazdian, F. and Rahimnejad, M., "An electrochemical nitric oxide biosensor based on immobilized cytochrome c on a chitosan-gold nanocomposite modified gold electrode", *International Journal of Biological Macromolecules*, Vol. 108, (2018), 250-258.
- Pluth, M.D., Tomat, E. and Lippard, S.J., "Biochemistry of mobile zinc and nitric oxide revealed by fluorescent sensors", *Annual Review of Biochemistry*, Vol. 80, (2011), 333-355.
- Kavya, R., Saluja, R., Singh, S. and Dikshit, M., "Nitric oxide synthase regulation and diversity: Implications in parkinson's disease", *Nitric Oxide*, Vol. 15, No. 4, (2006), 280-294.
- Li, C.M., Zang, J., Zhan, D., Chen, W., Sun, C.Q., Teo, A.L., Chua, Y., Lee, V. and Moochhala, S., "Electrochemical detection of nitric oxide on a swcnt/rtil composite gel microelectrode", *Electroanalysis*, Vol. 18, No. 7, (2006), 713-718.
- Wink, D.A. and Mitchell, J.B., "Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide", *Free Radical Biology and Medicine*, Vol. 25, No. 4, (1998), 434-456.
- Taha, Z.H., "Nitric oxide measurements in biological samples", *Talanta*, Vol. 61, No. 1, (2003), 3-10.
- Shibuki, K., "An electrochemical microprobe for detecting nitric oxide release in brain tissue", *Neuroscience Research*, Vol. 9, No. 1, (1990), 69-76.
- Haruyama, T., Shiino, S., Yanagida, Y., Kobatake, E. and Aizawa, M., "Two types of electrochemical nitric oxide (no) sensing systems with heat-denatured cyt c and radical scavenger pto", *Biosensors and Bioelectronics*, Vol. 13, No. 7, (1998), 763-769.
- Fan, C., Chen, X., Li, G., Zhu, J., Zhu, D. and Scheer, H., "Direct electrochemical characterization of the interaction between haemoglobin and nitric oxide", *Physical Chemistry Chemical Physics*, Vol. 2, No. 19, (2000), 4409-4413.
- Brovkovich, V., Stolarczyk, E., Oman, J., Tomboulian, P. and Malinski, T., "Direct electrochemical measurement of nitric oxide in vascular endothelium", *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 19, No. 1, (1999), 135-143.
- Wen, W., Chen, W., Ren, Q.-Q., Hu, X.-Y., Xiong, H.-Y., Zhang, X.-H., Wang, S.-F. and Zhao, Y.-D., "A highly sensitive nitric oxide biosensor based on hemoglobin-chitosan/graphene-hexadecyltrimethylammonium bromide nanomatrix", *Sensors and Actuators B: Chemical*, Vol. 166, (2012), 444-450.
- Chen, D., Feng, H. and Li, J., "Graphene oxide: Preparation, functionalization, and electrochemical applications", *Chemical Reviews*, Vol. 112, No. 11, (2012), 6027-6053.
- Muthoosamy, K., G Bai, R. and Manickam, S., "Graphene and graphene oxide as a docking station for modern drug delivery system", *Current Drug Delivery*, Vol. 11, No. 6, (2014), 701-718.
- Bai, R.G., Muthoosamy, K., Zhou, M., Ashokkumar, M., Huang, N.M. and Manickam, S., "Sonochemical and sustainable synthesis of graphene-gold (g-au) nanocomposites for enzymeless and selective electrochemical detection of nitric

- oxide", *Biosensors and Bioelectronics*, Vol. 87, (2017), 622-629.
16. Ting, S.L., Guo, C.X., Leong, K.C., Kim, D.-H., Li, C.M. and Chen, P., "Gold nanoparticles decorated reduced graphene oxide for detecting the presence and cellular release of nitric oxide", *Electrochimica Acta*, Vol. 111, (2013), 441-446.
 17. Casero, E., Alonso, C., Vazquez, L., Petit-Domínguez, M., Parra-Alfambra, A., De La Fuente, M., Merino, P., Álvarez-García, S., De Andrés, A. and Pariente, F., "Comparative response of biosensing platforms based on synthesized graphene oxide and electrochemically reduced graphene", *Electroanalysis*, Vol. 25, No. 1, (2013), 154-165.
 18. Lian, W., Liu, S., Yu, J., Li, J., Cui, M., Xu, W. and Huang, J., "Electrochemical sensor using neomycin-imprinted film as recognition element based on chitosan-silver nanoparticles/graphene-multiwalled carbon nanotubes composites modified electrode", *Biosensors and Bioelectronics*, Vol. 44, (2013), 70-76.
 19. Wang, R., Yan, K., Wang, F. and Zhang, J., "A highly sensitive photoelectrochemical sensor for 4-aminophenol based on cds-graphene nanocomposites and molecularly imprinted polypyrrole", *Electrochimica Acta*, Vol. 121, (2014), 102-108.
 20. Tan, X., Hu, Q., Wu, J., Li, X., Li, P., Yu, H., Li, X. and Lei, F., "Electrochemical sensor based on molecularly imprinted polymer reduced graphene oxide and gold nanoparticles modified electrode for detection of carbofuran", *Sensors and Actuators B: Chemical*, Vol. 220, (2015), 216-221.
 21. Schoch, K., Byers, W. and Buckley, L., "Deposition and characterization of conducting polymer thin films on insulating substrates", *Synthetic Metals*, Vol. 72, No. 1, (1995), 13-23.
 22. Lin, Y. and Cui, X., "Electrosynthesis, characterization, and application of novel hybrid materials based on carbon nanotube-polyaniline-nickel hexacyanoferrate nanocomposites", *Journal of Materials Chemistry*, Vol. 16, No. 6, (2006), 585-592.
 23. Batra, B., Lata, S., Rani, S. and Pundir, C., "Fabrication of a cytochrome c biosensor based on cytochrome oxidase/nio-nps/cmwcnt/pani modified au electrode", *Journal of Biomedical Nanotechnology*, Vol. 9, No. 3, (2013), 409-416.
 24. Sha, R., Komori, K. and Badhulika, S., "Graphene-polyaniline composite based ultra-sensitive electrochemical sensor for non-enzymatic detection of urea", *Electrochimica Acta*, Vol. 233, (2017), 44-51.
 25. Lata, S., Batra, B., Karwasra, N. and Pundir, C.S., "An amperometric H₂O₂ biosensor based on cytochrome c immobilized onto nickel oxide nanoparticles/carboxylated multiwalled carbon nanotubes/polyaniline modified gold electrode", *Process Biochemistry*, Vol. 47, No. 6, (2012), 992-998.
 26. Omidi, M., Amoabediny, G., Yazdian, F. and Habibi-Rezaei, M., "Hydrogen sulfide detection using a gold nanoparticle/metalloprotein based probe", *Chinese Physics Letters*, Vol. 31, No. 8, (2014), 088701.
 27. Marcano, D.C., Kosynkin, D.V., Berlin, J.M., Sinitskii, A., Sun, Z., Slesarev, A., Alemany, L.B., Lu, W. and Tour, J.M., "Improved synthesis of graphene oxide", Vol. 4, No. 8, (2010), 4806-4814.
 28. Xu, X., Zheng, Q., Bai, G., Song, L., Yao, Y., Cao, X., Liu, S. and Yao, C., "Polydopamine induced in-situ growth of au nanoparticles on reduced graphene oxide as an efficient biosensing platform for ultrasensitive detection of bisphenol a", *Electrochimica Acta*, Vol. 242, (2017), 56-65.
 29. Srivastava, S., Kumar, V., Ali, M.A., Solanki, P.R., Srivastava, A., Sumana, G., Saxena, P.S., Joshi, A.G. and Malhotra, B., "Electrophoretically deposited reduced graphene oxide platform for food toxin detection", *Nanoscale*, Vol. 5, No. 7, (2013), 3043-3051.
 30. Li, Q., Cheng, K., Weng, W., Du, P. and Han, G., "Highly sensitive hydrogen peroxide biosensors based on tio 2 nanodots/ito electrodes", *Journal of Materials Chemistry*, Vol. 22, No. 18, (2012), 9019-9026.
 31. Wang, Y. and Hu, S., "A novel nitric oxide biosensor based on electropolymerization poly (toluidine blue) film electrode and its application to nitric oxide released in liver homogenate", *Biosensors and Bioelectronics*, Vol. 22, No. 1, (2006), 10-17.
 32. Moussy, F. and Harrison, D.J., "Prevention of the rapid degradation of subcutaneously implanted ag/agcl reference electrodes using polymer coatings", *Analytical Chemistry*, Vol. 66, No. 5, (1994), 674-679.
 33. Wang, Y.-L. and Zhao, G.-C., "Electrochemical sensing of nitric oxide on electrochemically reduced graphene-modified electrode", *International Journal of Electrochemistry*, Vol. 2011, (2011).
 34. Deng, X., Wang, F. and Chen, Z., "A novel electrochemical sensor based on nano-structured film electrode for monitoring nitric oxide in living tissues", *Talanta*, Vol. 82, No. 4, (2010), 1218-1224.
 35. Yu, A., Liang, Z., Cho, J. and Caruso, F., "Nanostructured electrochemical sensor based on dense gold nanoparticle films", *Nano Letters*, Vol. 3, No. 9, (2003), 1203-1207.
 36. Ananthi, A. and Phani, K.L., "Self-assembly of gold nanoparticles on sulphide functionalized polydopamine in application to electrocatalytic oxidation of nitric oxide", *Journal of Electroanalytical Chemistry*, Vol. 764, (2016), 7-14.

Highly Sensitive Amperometric Sensor Based on Gold Nanoparticles Polyaniline Electrochemically Reduced Graphene Oxide Nanocomposite for Detection of Nitric Oxide

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PAPER INFO

چکیده

Paper history:

Received 11 December 2017

Received in revised form 01 January 2018

Accepted 04 January 2017

Keywords:

Nitric Oxide Sensor

Reduced Graphene Oxide

Gold Nanoparticle

Polyaniline

Cyclic Voltammetry

در این پژوهش یک حسگر (سنسور) الکتروشیمیایی برای شناسایی حساس و انتخاب پذیر نیتریک اکسید (NO) بر مبنای نانوکامپوزیت گرافن اکسید کاهش یافته الکتروشیمیایی (ErGO) - پلی آنیلین (PANI) - نانوذرات طلا (AuNPs) ساخته شد. این نانوکامپوزیت از طریق الکترودیپوزیشن گام به گام روی یک الکتروود طلا (Au) پوشش داده شد تا الکتروود AuNPs-PANI-ErGO/Au تشکیل شود. مشخصه‌ی نانوکامپوزیت AuNPs-PANI-rGO به وسیله تکنیک‌های UV-vis و FESEM تعیین شد. رفتار الکتروشیمیایی الکتروود اصلاح شده به وسیله ولتامتری چرخه‌ای (CV) و کرومیاومپرومتری (CA) ارزیابی شد. نمودارهای ولتاگرام الکتروودهای PANI-ErGO/Au, AuNPs-PANI-ErGO/Au, and ErGO/Au نشان داد که هدایت AuNPs-PANI-ErGO/Au از دو مورد دیگر بیشتر بود. از نفیون برای بهبود انتخاب پذیری الکتروود اصلاح شده استفاده شد. الکتروود Nafion/AuNPs-PANI-ErGO/Au در مورد اکسیداسیون NO رفتار الکتروکاتالیتیکی و الکتروشیمیایی مطلوبی از خود نشان داد. در شرایط بهینه، الکتروود ساخته شده حساسیت بالای $0.113 \mu\text{A}/\mu\text{M}$ در محدوده‌ی خطی وسیع $0.8-86 \mu\text{M}$ با حد تشخیص پایین $0.25 \mu\text{M}$ از خود نشان داد. علاوه بر این، این حسگر پایداری، تکرارپذیری و انتخاب پذیری بالایی داشت که باعث می‌شود بتوان از آن در تشخیص سریع و دقیق NO استفاده کرد.

doi: 10.5829/ije.2018.31.02b.01