

Research Article

Electrochemical Sensing of Nitric Oxide on Electrochemically Reduced Graphene-Modified Electrode

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Received 31 January 2011; Revised 29 March 2011; Accepted 29 March 2011

Academic Editor: Vinod Kumar Gupta

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Graphene-modified electrode was prepared through electrochemically reducing graphene oxide on the surface of a glassy carbon electrode in PBS solution. The as-prepared electrode owns higher stability and stronger catalytic activity towards the oxidation of nitric oxide (NO). At the electrode, an oxidation peak of NO can be observed at about 1.05 V (versus Ag/AgCl), and the electrode reaction of NO is controlled by diffusion process. Under the optimum conditions, the peak currents are dependent linearly on NO concentrations in the range from 7.2×10^{-7} to 7.84×10^{-5} M with a limit of detection of 2.0×10^{-7} M. The response time of the as-prepared electrode to NO is less than 3 s, and the sensitivity is about 299.1 μ A/mM, revealing that the electrode can be used as an excellent sensor for the determination of NO. With further modification of Nafion, the determination is free from the interference of nitrite and some other biological substances. This investigation provides an alternate way for the determination of NO.

1. Introduction

Graphene, as a “rising star” of carbon material, has attracted a lot of interest from both the theoretical and experimental scientific communities since it was discovered and successfully isolated from bulk graphite just a few years ago. Graphene is a one-atom-thick planar sheet of sp^2 -bonded carbon atoms arranged in a honey comb crystal lattice, the difference of whose structure from graphite's is shown in Figure 1 [1, 2]. Graphene can be wrapped up into zero-dimensional fullerenes, rolled into one-dimensional nanotubes, or stacked into three-dimensional graphite [3]. Hence, the two-dimensional carbon material, graphene, is considered as a building block for graphitic materials of all other dimensionalities [4]. Furthermore, owing to its extraordinary fast electron transportation, high surface area, unique graphitized basal plane structure, and low manufacturing cost, numerous novel applications of graphene nanosheets have been investigated, including graphene-based field-effect transistors [5, 6], batteries [7], and supercapacitors [8]. Particularly, because of its excellent conductivity (1.43×10^4 S·m⁻¹) [9] and high electrocatalytic activities,

graphene-based electrochemical sensors and biosensors have recently received increasing attention in the field of electrochemistry [10, 11], such as direct electrochemistry of enzymes [12–14] and small biomolecules detection [15–17].

Nitric oxide as an important biological messenger and effective molecule [18] plays an important physiological role in the *in vivo* physiological and pathological process of broad participation [19]. NO is known to be one of the signaling functions existed in the central and peripheral nervous system and in vascular endothelium, while the result of the mussy level of NO will be devastating, which may lead to inflammatory and degenerative diseases, immune response, septic shock, and Parkinson's and Alzheimer's diseases [20–22]. Due to the importance of NO in biology, it is important to develop the methods for detecting NO. One of the most promising methods of NO monitoring is electrochemical detection using different modified electrodes. A variety of modified electrodes have been developed in recent years [23–25].

In this paper, the graphene oxide (GO) on glassy carbon electrode surface was electrochemically reduced to form graphene-modified electrode, noted as ERG electrode. The

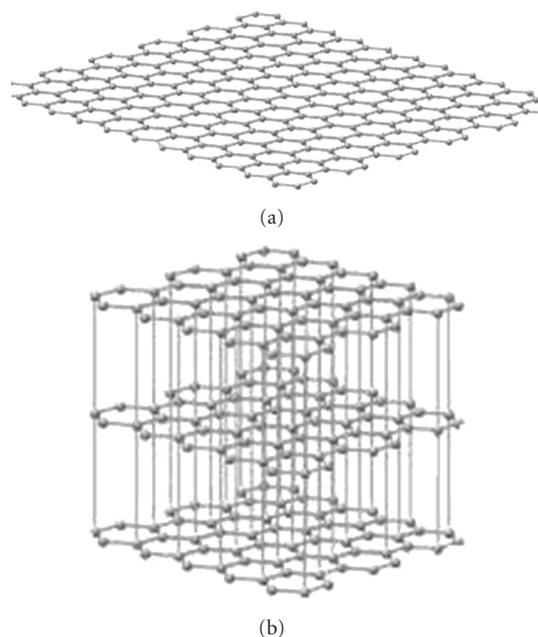


FIGURE 1: The structure of sp^2 carbon materials: graphene (a) and graphite (b).

properties of the modified electrode and its electrocatalytic oxidation of NO in aqueous solution were investigated in detail.

2. Experimental

2.1. Instruments and Reagents. Electrochemical experiments were performed with CHI 660A electrochemical analyzer (CHI, USA) with a conventional three-electrode system. The working electrode was an ERG electrode. A saturated calomel electrode (SCE) and a platinum electrode were used as the reference and the auxiliary electrode, respectively. Water used in all experiments was double distilled with a quartz apparatus. S-4800 scanning electron microscopy (Hitachi, Japan) was used for the characterization of graphene. High-purity nitrogen gas was used for deaeration in all electrochemical experiments.

Graphene oxide was synthesized from graphite according to Hummers' method [26]. Graphite was purchased from Shanghai Chemical Reagent Co. Nafion was purchased from Sigma. Saturated NO solutions were prepared as the previous literature. In detail, the double-distilled water was bubbled with high-purity nitrogen for 30 minutes to remove oxygen, and then the water was bubbled with pure NO gas for 30 minutes to prepare an NO-saturated solution. Aliquots of this saturated solution were used to prepare serial solutions of known NO concentration, using a value of 1.8×10^{-3} M as its saturation concentration at room temperature.

2.2. The Preparation of ERG Electrode. ERG electrode was prepared through electrochemically reducing graphene oxide on the surface of GC electrode. The detail process can

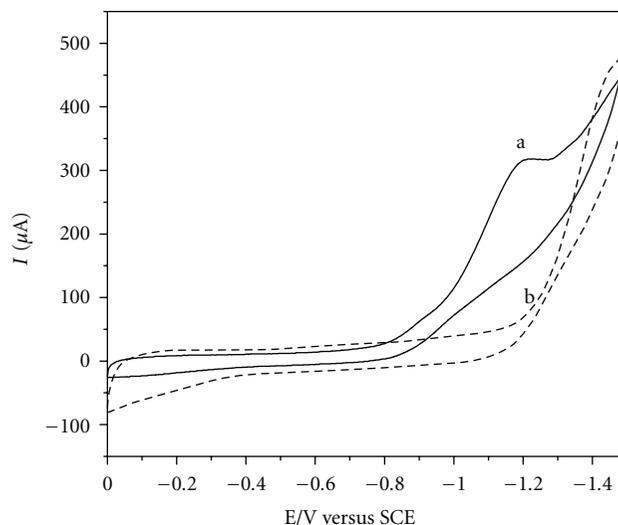


FIGURE 2: CVs of electrochemical reduction of graphene oxide on GC electrode in 10 mM PBS (pH 5.0) with a scan rate of 50 mV/s for (a) the first cycle and (b) the fourth cycle.

be described as follows. The synthesized graphene oxide was dispersed in water to create a 0.125 mg/mL aqueous solution. The bare glassy carbon electrode was polished with $0.3 \mu\text{m}$ alumina slurry, then thoroughly rinsed with double-distilled water, and sonicated in ethanol, acetone, and water (each for 5 minutes), respectively. A volume of $10 \mu\text{L}$ of graphene oxide aqueous solution was dropped on the GC electrode surface, and the electrode was dried in ambient air at room temperature to form a graphene oxide-modified electrode, noted as GO/GC electrode. The obtained electrode was dipped into 10 mM, pH 5.0 phosphate buffer solution, and five cyclic potential scans were performed in the potential range of 0.0 ~ -1.5 V. In this process, the cyclic voltammograms (CVs) were recorded, and the typical CVs were shown in Figure 2. In the first cyclic, a broad cathodic current peak at -1.2 V with a starting potential of -0.8 V (Figure 2(a)) was observed, which corresponded to the reduction of C-O bond in graphene oxide [27]. After four cycles, the cathodic peak at -1.2 V disappeared (Figure 2(b)), suggesting that the electrochemical reduction was fulfilled and the graphene-modified electrode was obtained, noted as ERG/GC electrode.

3. Results and Discussion

3.1. Electrochemical Characterization of ERG Electrode. The redox couple of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is close to an ideal quasireversible system and it was usually used to characterize the property of modified electrode. Figure 3 shows the CVs obtained by a bare GC electrode, GO/GC, electrode, and ERG electrode in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 M KCl. As shown in line a of Figure 3, a couple of well-defined redox peaks are observed for the bare GC electrode, which corresponds to the quasireversible one-electron redox behavior of ferricyanide ion. In contrast, for GO/GC electrode,

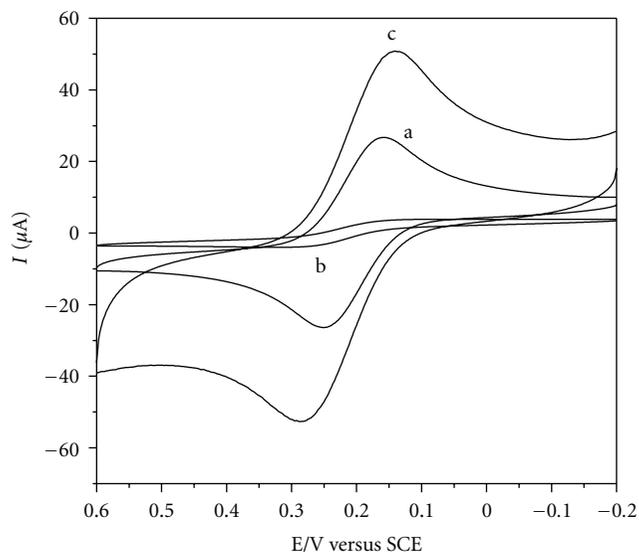


FIGURE 3: Typical CVs of different electrode in 1 mM $K_3Fe(CN)_6$ solution containing 0.1 M KCl. (a) Bare GC electrode, (b) GO/GC electrode, and (c) ERG/GC electrode.

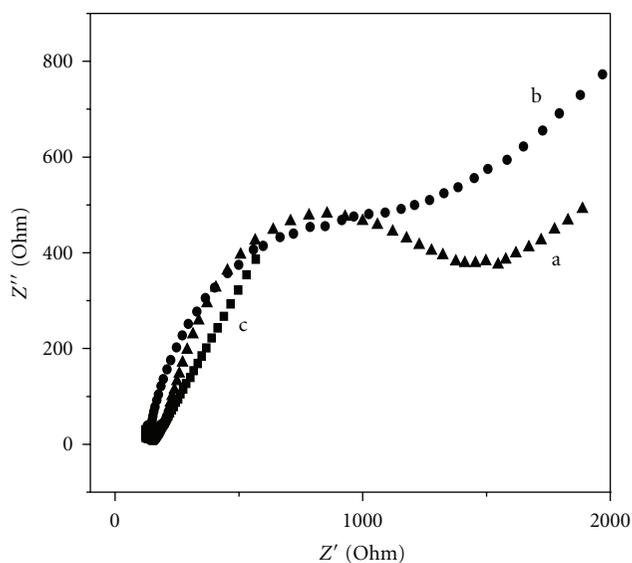


FIGURE 4: The Nyquist plots of different electrode recorded in the presence of 5 mM $Fe(CN)_6^{3-/4-}$ (1:1) by applying an AC impedance with 5 mV amplitude in a frequency range from 0.1 Hz to 100 kHz under open-circuit potential conditions. (a) GO/GC electrode, (b) ERG/GC electrode, and (c) bare GC electrode.

cathodic and anodic peak currents dramatically decrease compared with that of bare GC electrode, as shown in line b of Figure 3. The reason may be that graphene oxide has low conductivity as a result of the existence of C–O bond, and the negative charge interface of graphene oxide largely blocked interfacial charge transfer between ferricyanide ion and electrode. Once the graphene oxide on the electrode surface was electrochemically reduced into graphene, thus formed an ERG/GC electrode, an obvious increase of both cathodic

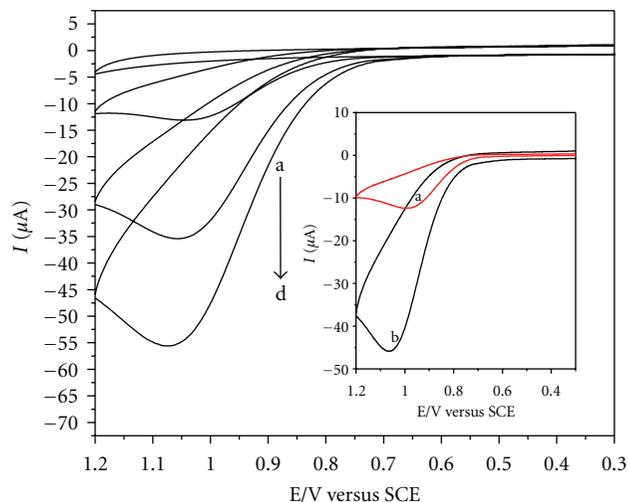


FIGURE 5: CVs of different NO concentrations at ERG/GC electrode in 0.1 M, pH 7.0 phosphate buffer solution. From a to d, the concentration of NO is 0, 7.2, 21.6, and 36.0 μM , respectively. Inset: CVs of 28.8 μM NO at bare GC electrode (a) and ERG/GC electrode (b) in the same condition.

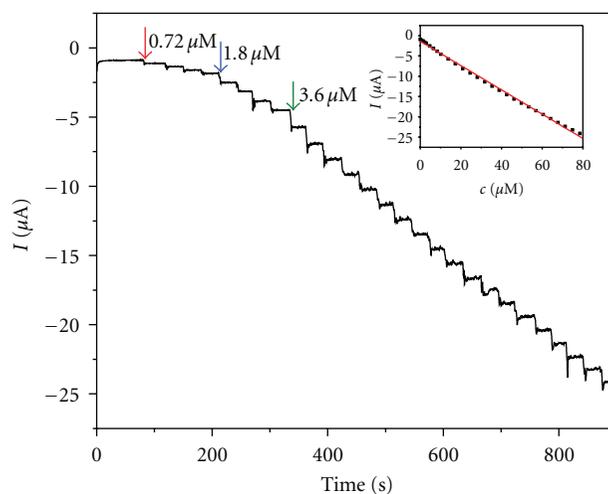


FIGURE 6: Amperometric response of ERG/GC electrode to NO. Conditions: a 1.05 V constant potential modulated with 50 mV pulse in the time intervals of 0.5 s, successive additions of NO to 0.1 M, pH 7.0 phosphate buffer solution.

and anodic peak currents is observed as in line c of Figure 3, indicating that the high electroactive graphene interface was formed. Compared with the bare GCE, increased redox peak currents are observed on ERG/GCE mainly due to the high conductivity of graphene.

Electrochemical impedance spectroscopy (EIS) can provide the information on the impedance changes of the electrode surface during the modification progress. Figure 4 reveals the typical EIS of different electrodes. As can be seen, when graphene oxide is modified onto the GC electrode surface, the semicircle dramatically augments as compared to the bare GC electrode, suggesting that the graphene

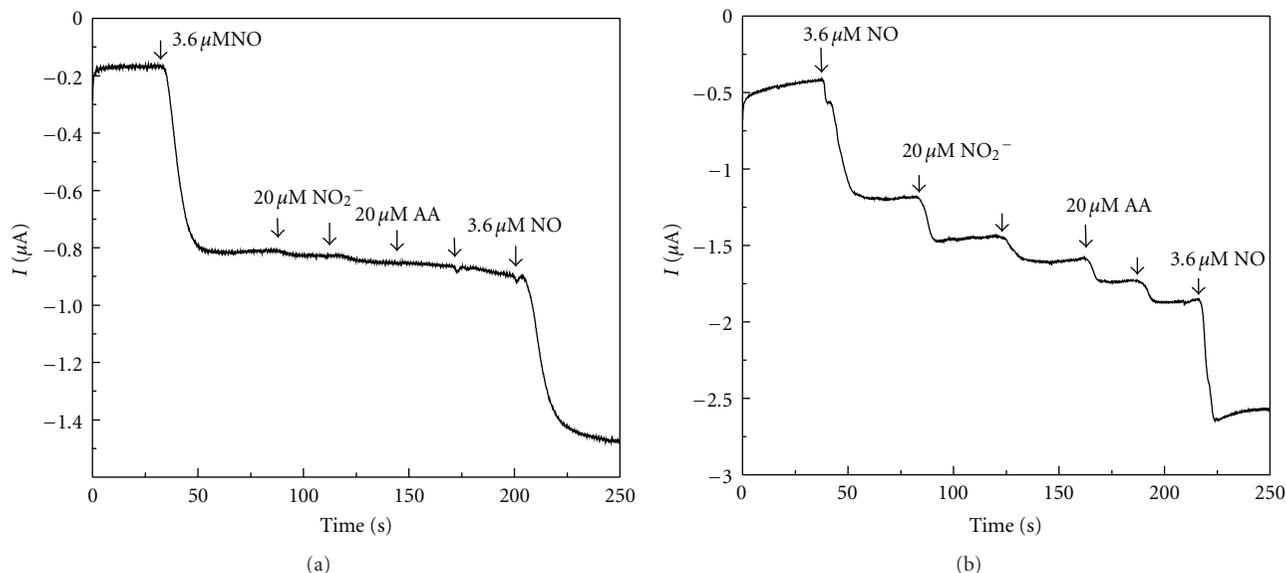


FIGURE 7: Amperometric response of the modified electrode after (a) and before (b) coated with Nafion to NO and interferences, NO_2^- and AA.

oxide acts as a low conductive layer which makes the interfacial charge transfer difficult and the surface negative charges of the graphene oxide repel the access of ferricyanide and ferrocyanide ions to the electrode surface for electron communication as well. After the graphene oxide is electrochemically reduced on the electrode, the semicircle decreases distinctively, indicating that graphene film has higher electrical conductivity, and it can obviously accelerate electron transfer between the probe $\text{Fe}(\text{CN})_6^{3-/4-}$ and the electrode surface.

3.2. Electrooxidation Behavior and Amperometric Response of NO on the ERG/GC Electrode. The CVs of nitric oxide at the modified electrode in the oxygen-free 0.1 mol/L phosphate buffer solution (pH 7.0) was demonstrated in Figure 5. With the addition of NO into the solution, an obvious anodic peak was observed at about 1.05 V (versus Ag/AgCl), and the peak currents are dependent linearly on the NO concentration. Compared to bare GC electrode, the anodic peak current corresponding to the oxidation of NO increased significantly at modified electrode, as shown in the inset of Figure 5. These suggest that the ERG can act as a modifier to realize the electrochemical oxidation of NO. The anodic peak current is proportional linearly to the square root of scan rates in the range from 0.2 to 0.16 Vs^{-1} with a correlation coefficient of 0.9996, while the peak potential slightly shifted to positive direction. The results suggested that the oxidation of NO was undergoing a diffusion-controlled process in low scan rates.

Figure 6 demonstrates a typical current-time curve of ERG/GC electrode-applied potential of +1.0 V for successive additions of NO to pH 7.0 phosphate buffer solution. With the addition of NO, the steady-state currents can be reached less than 2 s and keep stable, suggesting a fast response time and high stability. The linear relationship between the

catalytic current and the concentration is shown in the inset of Figure 6. As can be seen, the linear range is from 7.20×10^{-7} to 7.84×10^{-5} M with a correlation coefficient of 0.9986. The limit of detection is 2.0×10^{-7} M at a signal-to-noise ratio of 3 with a sensitivity of $-299.1 \mu\text{A}/\text{mM}$. We also summarized some other carbon-related materials NO sensors in Table 1 with respect to the linear range and the detection limit. It can be seen that the performance of the developed sensor is comparable to most of NO sensors in literature in one or more categories. Meanwhile, the proposed sensor is simpler in preparation.

In order to eliminate anion interferences like NO_2^- , Nafion was selected and modified on the surface of ERG/GC electrode. Nafion, a good cation exchanger, is widely used as a modifier to modify electrodes in electrochemistry. The negatively charged Nafion has a high degree of permeability for nitric oxide. But it prevents the diffusion of anions like NO_2^- to the modified electrode surface [30]. In this work, 10 μL of a 0.5% (w/v) Nafion solution in ethanol was cast on the prepared electrode surface to form a Nafion/ERG/GC electrode. The Nafion film can prevent anion interferences like NO_2^- and ascorbic acid from responding at the electrode. Five times relative to NO concentration of anion interferences did not disturb the detection of NO. As shown in Figure 7, amperometric response of the electrode after and before coated with Nafion to interferences, NO_2^- and AA, was compared. In the presence of Nafion, no response to NO_2^- and AA can be observed, as shown in Figure 7(a). Under the same conditions, in the absence of Nafion, the response of the electrode to NO_2^- and AA is obvious, as in Figure 7(b). So, Nafion obviously improves the selectivity of the electrode over other interfering anions. On the other hand, the electrode can be used for long time. Even after six weeks, the response signal of electrode can keep unchanged.

TABLE 1: Summary of some other carbon-related materials NO sensors.

No.	Electrode	Linear range	Detection limit	Reference
1	Hemoglobin adsorbed on the gold colloids-modified carbon paste electrode	9.0×10^{-7} to 3.0×10^{-4} M	1.0×10^{-7} M	[25]
2	Hemoglobin/montmorillonite/polyvinyl alcohol at a pyrolytic graphite electrode	1.0×10^{-6} to 2.5×10^{-4} M	5.0×10^{-7} M	[28]
3	Multiwall carbon nanotubes film-modified carbon fiber ultramicroelectrode	2.0×10^{-7} to 8.6×10^{-5} M	2×10^{-8} M	[29]
4	Electrochemically reduced grapheme-modified electrode	7.2×10^{-7} to 7.8×10^{-5} M	2.0×10^{-7} M	This work

4. Conclusion

In summary, we have prepared a grapheme-modified electrode through electrochemical reducing of graphene oxide on the glassy carbon electrode in this work. The graphene electrode gave a significant activity towards the electrochemical oxidation of nitric oxide. Interference effects of NO_2^- and some other biological substances are virtually eliminated because of the Nafion film. The prepared electrode shows fast response to NO and high sensitivity and stability and can be applied to the determination of nitric oxide. The preparation of graphene electrode through direct electrochemical reducing of graphene oxide on the substrate electrode surface can provide a new way to construct electrochemical sensing platform based on graphene.

Acknowledgment

This research was supported financially by the National Nature Science Foundation of China (20975001).

References

- [1] C. N. R. Rao, A. K. Sood, K. S. Subrahmanyam, and A. Govindaraj, "Graphene: the new two-dimensional nanomaterial," *Angewandte Chemie—International Edition*, vol. 48, no. 42, pp. 7752–7777, 2009.
- [2] S. Niyogi, E. Bekyarova, M. E. Itkis, J. L. McWilliams, M. A. Hamon, and R. C. Haddon, "Solution properties of graphite and graphene," *Journal of the American Chemical Society*, vol. 128, no. 24, pp. 7720–7721, 2006.
- [3] A. K. Geim and K. S. Novoselov, "The rise of graphene," *Nature Materials*, vol. 6, no. 3, pp. 183–191, 2007.
- [4] J. D. Fowler, M. J. Allen, V. C. Tung, Y. Yang, R. B. Kaner, and B. H. Weiller, "Practical chemical sensors from chemically derived graphene," *ACS Nano*, vol. 3, no. 2, pp. 301–306, 2009.
- [5] C.-K. Tzahi, Q. Qing, Q. Li, Y. Fang, and C. M. Lieber, "Graphene and nanowire transistors for cellular interfaces and electrical recording," *Nano Letters*, vol. 10, no. 3, pp. 1098–1102, 2010.
- [6] H. E. Romero, N. Shen, P. Joshi et al., "n-type behavior of graphene supported on Si/SiO₂ substrates," *ACS Nano*, vol. 2, no. 10, pp. 2037–2044, 2008.
- [7] P. Guo, H. Song, and X. Chen, "Electrochemical performance of graphene nanosheets as anode material for lithium-ion batteries," *Electrochemistry Communications*, vol. 11, no. 6, pp. 1320–1324, 2009.
- [8] F. Jiang, Y. Fang, Q. Xue, L. Chen, and Y. Lu, "Graphene-based carbon nano-fibers grown on thin-sheet sinter-locked Ni-fiber as self-supported electrodes for supercapacitors," *Materials Letters*, vol. 64, no. 2, pp. 199–202, 2010.
- [9] S. J. An, Y. Zhu, S. H. Lee et al., "Thin film fabrication and simultaneous anodic reduction of deposited graphene oxide platelets by electrophoretic deposition," *Journal of Physical Chemistry Letters*, vol. 1, no. 8, pp. 1259–1263, 2010.
- [10] Y. Shao, J. Wang, H. Wu, J. Liu, I. A. Aksay, and Y. Lin, "Graphene based electrochemical sensors and biosensors: a review," *Electroanalysis*, vol. 22, no. 10, pp. 1027–1036, 2010.
- [11] M. Pumera, "Graphene-based nanomaterials and their electrochemistry," *Chemical Society Reviews*, vol. 39, no. 11, pp. 4146–4157, 2010.
- [12] J. Lu, L. T. Drzal, R. M. Worden, and I. Lee, "Simple fabrication of a highly sensitive glucose biosensor using enzymes immobilized in exfoliated graphite nanoplatelets nafion membrane," *Chemistry of Materials*, vol. 19, no. 25, pp. 6240–6246, 2007.
- [13] X. Kang, J. Wang, H. Wu, I. A. Aksay, J. Liu, and Y. Lin, "Glucose Oxidase-graphene-chitosan modified electrode for direct electrochemistry and glucose sensing," *Biosensors and Bioelectronics*, vol. 25, no. 4, pp. 901–905, 2009.
- [14] K. Liu, J. Zhang, G. Yang, C. Wang, and J. J. Zhu, "Direct electrochemistry and electrocatalysis of hemoglobin based on poly(diallyldimethylammonium chloride) functionalized graphene sheets/room temperature ionic liquid composite film," *Electrochemistry Communications*, vol. 12, no. 3, pp. 402–405, 2010.
- [15] M. Zhou, Y. Zhai, and S. Dong, "Electrochemical sensing and biosensing platform based on chemically reduced graphene oxide," *Analytical Chemistry*, vol. 81, no. 14, pp. 5603–5613, 2009.
- [16] Y. Wang, Y. Li, L. Tang, J. Lu, and J. Li, "Application of graphene-modified electrode for selective detection of dopamine," *Electrochemistry Communications*, vol. 11, no. 4, pp. 889–892, 2009.
- [17] S. Alwarappan, A. Erdem, C. Liu, and C. Z. Li, "Probing the electrochemical properties of graphene nanosheets for biosensing applications," *Journal of Physical Chemistry C*, vol. 113, no. 20, pp. 8853–8857, 2009.
- [18] D. R. Richardson and H. C. Lok, "The nitric oxide-iron interplay in mammalian cells: transport and storage of dinitrosyl iron complexes," *Biochimica et Biophysica Acta*, vol. 1780, no. 4, pp. 638–651, 2008.
- [19] H. Kosaka and A. Seiyama, "Physiological role of nitric oxide as an enhancer of oxygen transfer from erythrocytes to tissues," *Biochemical and Biophysical Research Communications*, vol. 218, no. 3, pp. 749–752, 1996.

- [20] K. K. Chung and K. K. David, "Emerging roles of nitric oxide in neurodegeneration," *Nitric Oxide*, vol. 22, no. 4, pp. 290–295, 2010.
- [21] E. Karpuzoglu and S. A. Ahmed, "Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: implications for immunity, autoimmune diseases, and apoptosis," *Nitric Oxide*, vol. 15, no. 3, pp. 177–186, 2006.
- [22] M. A. Titheradge, "Nitric oxide in septic shock," *Biochimica et Biophysica Acta*, vol. 1411, no. 2-3, pp. 437–455, 1999.
- [23] L. Zhang, Y. Ni, X. Wang, and G. Zhao, "Direct electrocatalytic oxidation of nitric oxide and reduction of hydrogen peroxide based on α -Fe₂O₃ nanoparticles-chitosan composite," *Talanta*, vol. 82, no. 1, pp. 196–201, 2010.
- [24] Y. C. Liu, S. Q. Cui, J. Zhao, and Z. S. Yang, "Direct electrochemistry behavior of cytochrome *c*/l-cysteine modified electrode and its electrocatalytic oxidation to nitric oxide," *Bioelectrochemistry*, vol. 70, no. 2, pp. 416–420, 2007.
- [25] Y. Xu, C. Hu, and S. Hu, "A reagentless nitric oxide biosensor based on the direct electrochemistry of hemoglobin adsorbed on the gold colloids modified carbon paste electrode," *Sensors and Actuators, B*, vol. 148, no. 1, pp. 253–258, 2010.
- [26] W. S. Hummers and R. E. Offeman, "Preparation of graphitic oxide," *Journal of the American Chemical Society*, vol. 80, no. 6, pp. 1339–1958, 1958.
- [27] H. L. Guo, X. F. Wang, Q. Y. Qian, F. B. Wang, and X. H. Xia, "A green approach to the synthesis of graphene nanosheets," *ACS Nano*, vol. 3, no. 9, pp. 2653–2659, 2009.
- [28] J. Pang, C. Fan, X. Liu, T. Chen, and G. Li, "A nitric oxide biosensor based on the multi-assembly of hemoglobin/montmorillonite/polyvinyl alcohol at a pyrolytic graphite electrode," *Biosensors and Bioelectronics*, vol. 19, no. 5, pp. 441–445, 2003.
- [29] Y. Wang, Q. Li, and S. Hu, "A multiwall carbon nanotubes film-modified carbon fiber ultramicroelectrode for the determination of nitric oxide radical in liver mitochondria," *Bioelectrochemistry*, vol. 65, no. 2, pp. 135–142, 2005.
- [30] G. A. Gerhardt, A. F. Oke, and G. Nagy, "Nafion-coated electrodes with high selectivity for CNS electrochemistry," *Brain Research*, vol. 290, no. 2, pp. 390–395, 1984.



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