



Fe₃O₄ magnetic nanoparticles/reduced graphene oxide nanosheets as a novel electrochemical and bioelectrochemical sensing platform



Hazhir Teymourian^a, Abdollah Salimi^{a,b,*}, Somayeh Khezrian^a

^a Department of Chemistry, University of Kurdistan, 66177-15175, Sanandaj, Iran

^b Research Center for Nanotechnology, University of Kurdistan, 66177-15175, Sanandaj, Iran

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ABSTRACT

We have developed Fe₃O₄ magnetic nanoparticles/reduced graphene oxide nanosheets modified glassy carbon (Fe₃O₄/r-GO/GC) electrode as a novel system for the preparation of electrochemical sensing platform. Decorating Fe₃O₄ nanoparticles on graphene sheets was performed via a facile one-step chemical reaction strategy, where the reduction of GO and the in-situ generation of Fe₃O₄ nanoparticles occurred simultaneously. Characterization of as-made nanocomposite using X-ray diffraction (XRD), transmission electron microscopy (TEM) and alternative gradient force magnetometry (AGFM) clearly demonstrate the successful attachment of monodisperse Fe₃O₄ nanoparticles to graphene sheets. Electrochemical studies revealed that the Fe₃O₄/r-GO/GC electrode possess excellent electrocatalytic activities toward the low potential oxidation of NADH (0.05 V vs. Ag/AgCl) as well as the catalytic reduction of O₂ and H₂O₂ at reduced overpotentials. Via immobilization of lactate dehydrogenase (LDH) as a model dehydrogenase enzyme onto the Fe₃O₄/r-GO/GC electrode surface, the ability of modified electrode for biosensing lactate was demonstrated. In addition, using differential pulse voltammetry (DPV) to investigate the electrochemical oxidation behavior of ascorbic acid (AA), dopamine (DA) and uric acid (UA) at Fe₃O₄/r-GO/GC electrode, the high electrocatalytic activity of the modified electrode toward simultaneous detection of these compounds was indicated. Finally, based on the strong electrocatalytic action of Fe₃O₄/r-GO/GC electrode toward both oxidation and reduction of nitrite, a sensitive amperometric sensor for nitrite determination was proposed. The Fe₃O₄/r-GO hybrid presented here showing favorable electrochemical features may hold great promise to the development of electrochemical sensors, molecular bioelectronic devices, biosensors and biofuel cells.

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1. Introduction

Graphene has been the major focus of recent research to exploit an sp² hybrid carbon network in applications such as capacitors, cell images, sensors, devices, drug delivery, and solar cell (Wang et al., 2008) due to its unique electronic, mechanical and thermal properties (Li et al., 2008). In addition, graphene is an ideal material for electrochemistry (Pumera, 2009; Navaee et al., 2012; Zhou et al., 2009) because of its very large 2-D electrical conductivity (550 S cm⁻¹), large surface area (2630 m² g⁻¹) and a large number of electrochemically favorable edge carbons per mass of graphene which facilitate electron transfer between molecules to an electrode substrate with a low overpotential.

Besides the applications of graphene oxide (GO) and reduced graphene oxide (r-GO), integration of nanoparticles (NPs) and

graphene into nanocomposites has recently become a hot topic of research due to their new and/or enhanced functionalities that cannot be achieved by either component alone, and therefore holds great promise for a wide variety of applications in catalysis, optoelectronic materials, surface enhanced Raman Scattering, bio-medical fields, and so on (Si and Samulski, 2008; Cong et al., 2010; He et al., 2010). Among them are nanocomposites of magnetic NPs (MNPs), especially iron oxide (either Fe₃O₄ or Fe₂O₃), and graphene oxide (GO) (Fe₃O₄/GO), for potential applications in enhanced optical limiting, MRI, drug delivery, energy storage and removal of contaminants from wastewater (Cong et al., 2010; He et al., 2010; He and Gao, 2010; Chandra et al., 2010; Su et al., 2011). Thus far, although a number of researchers have proposed methods for the preparation of magnetic graphene finding applications in various fields, only a few of them have managed to apply these hybrids for electrochemical sensing or biosensing purposes (Tang et al., 2011; He et al., 2011; Ye et al., 2012).

Herein, via applying different kinds of important compounds as representative examples, the usefulness of the magnetic graphene hybrid for sensing and biosensing purposes will be

* Correspondence to: University of Kurdistan, Department of Chemistry, Pasdaran Street, 66177-15175, Sanandaj, Iran. Tel.: +98 871 662 4001; fax: +98 871 662 4008.

E-mail addresses: absalimi@yahoo.com, absalimi@uok.ac.ir (A. Salimi).

strictly demonstrated. At first, the Fe₃O₄/r-GO nanocomposite was synthesized through a simple approach in which the reduction of GO to graphene and the in situ formation of Fe₃O₄ NPs on graphene sheets are accomplished in a one-step reaction. The as-prepared nanocomposite possesses both the electrically conductive and superparamagnetic properties as well as a good dispersibility in polar solvents. After the nanocomposite has been well characterized and the stable formation of Fe₃O₄ NPs on graphene has been confirmed, the potential applicability of the nanocomposite modified GC electrode as an electrochemical sensing platform has been investigated. The electrocatalytic behaviors of different kinds of important electroactive compounds at the Fe₃O₄/r-GO/GC electrode were assessed. In summary, a better electrochemical performance and higher electrocatalytic behavior towards reduction of O₂ and H₂O₂ was obtained at the Fe₃O₄/r-GO/GC electrode compared with r-GO/GC or Fe₃O₄/GC electrodes. In addition, the electrochemical experiments revealed the great ability of Fe₃O₄/r-GO/GC electrode to catalyze β-nicotinamide adenine dinucleotide (NADH) electrooxidation at a very low potential (0.05 V vs. Ag/AgCl) after which with the successful confinement of L-lactate dehydrogenase (LDH) as a model NAD⁺-dependent dehydrogenase enzyme, an effective lactate biosensor was developed. The electrochemical oxidation behaviors of AA, DA and UA at the Fe₃O₄/r-GO/GC electrode were also investigated. Finally, relying on the strong electrocatalytic action of Fe₃O₄/r-GO/GC electrode toward both oxidation and reduction of nitrite, a sensitive amperometric sensor for nitrite determination has been reported.

2. Experimental

The details of experiments are given in Supporting Information (SI). In brief, GO was synthesized by using the modified Hummers method (Hummers and Offeman, 1958) through oxidation of graphite powder. The Fe₃O₄/r-GO nanocomposite was synthesized according to a previously described procedure with a slight modification (Chandra et al., 2010) using ammonia solution (30%) and hydrazine hydrate at a temperature of 90 °C and pH=10. Reduced graphene oxide nanosheets (r-GO) for control experiments were obtained by reduction of GO with hydrazine as described previously (Wang et al., 2009). Fe₃O₄ nanoparticles for AGFM and control electrochemical experiments were prepared using Massart's method (Massart, 1981).

3. Results and discussion

3.1. Characterization of Fe₃O₄/r-GO

TEM was used to reveal the internal structure of the nanocomposite. The low- and high- magnification TEM images of e₃O₄/r-GO hybrid are shown in Fig. 1 A and B. It is evident that two-dimensional r-GO sheets are well decorated by a large quantity of spherical Fe₃O₄ nanostructures (with average size of ~20 nm and rather good size distribution) and both the outline of r-GO and Fe₃O₄ nanoparticles can be clearly observed. The Fe₃O₄ nanoparticles are not simply mixed up or blended with r-GO; rather, they are entrapped inside the r-GO sheets.

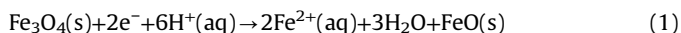
Fig. 1C shows the X-ray diffraction (XRD) patterns of the Fe₃O₄/r-GO nanocomposite. As can be seen, the pattern of Fe₃O₄/r-GO displayed obvious diffraction peaks of Fe₃O₄, and the peak positions and relative intensities match well with the standard XRD data for magnetite (JCPDS card, file No. 75-0033). The very weak and broad peak around 23.9° corresponds to r-GO (Zhu et al., 2010) and derived from the short range order in stacked graphene sheets, indicating the reduction of GO during reaction process.

The magnetic properties of Fe₃O₄ nanoparticles and Fe₃O₄/r-GO nanocomposite were examined with AGFM. Fig. 1 D gives the room temperature magnetization hysteresis loops with an applied magnetic field sweeping from -5 to +5kOe. For both profiles of the magnetization curves of Fe₃O₄ and Fe₃O₄/r-GO, the magnetic remanences are nearly zero. This result indicates that there is almost no remaining magnetization when the external magnetic field is removed which is characteristic of superparamagnetic behavior. The Fe₃O₄/r-GO nanocomposite showed a decrease in saturation magnetization compared to the Fe₃O₄ nanoparticles that is explained by decrease in the amount of magnetic component in the nanocomposite. Our results are in good agreement with the results previously reported by other groups (Chandra et al., 2010; Su et al., 2011).

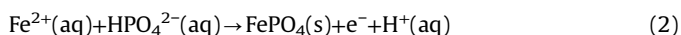
3.2. Electrochemical behavior of Fe₃O₄/r-GO/GC

The effective surface area (A) for different electrodes of GC, Fe₃O₄/GC, r-GO/GC and Fe₃O₄/r-GO/GC was determined from cyclic voltammograms of 0.1 mM K₄[Fe(CN)₆] in PBS (pH 7). The values of A for different electrodes were determined as Fe₃O₄/r-GO/GC (0.0442 cm²) > r-GO/GC (0.0428 cm²) > GC (0.0314 cm²) > Fe₃O₄/GC (0.0308 cm²). Therefore, after GCE was modified with Fe₃O₄/r-GO, the electroactive surface area increased relative to that modified with r-GO, indicating that the introduction of the magnetic graphene nanohybrid provide more conduction pathways for the electron-transfer of Fe(CN)₆^{3-/4-}. In addition, the electrode modified with Fe₃O₄ nanoparticles possesses the least electroactive surface area between the above electrodes which is mostly attributed to the repulsion between Fe(CN)₆^{3-/4-} probe molecule and negative surface charges of nanoparticles as well as relatively agglomerated nature of nanoparticles themselves. However, integrating them with graphene nanosheets leads to greatly increase the electrochemical activity, indicating that graphene nanosheets play an important role in enhancing Fe₃O₄ activity. Electrochemical impedance spectroscopy (EIS) is also an efficient tool for studying the interface properties of surface-modified electrodes. Thus, the electron transfer capability of these different electrodes was investigated by electrochemical impedance technique (Fig. 1E). After fitting the data, the values of charge-transfer resistance (R_{ct}) for different electrodes were obtained in the following order: Fe₃O₄/GC (336.0 Ω) > GC (126.1 Ω) > r-GO/GC (54.9 Ω) > Fe₃O₄/r-GO/GC (37.6 Ω). Based on these results, it may be speculated that the best electrochemical behavior among the studied electrodes should be observed at Fe₃O₄/r-GO/GC electrode. The inset of Fig. 1E shows the electrochemical impedance spectrum of LDH/Fe₃O₄/r-GO/GC electrode. It can be seen that for LDH/Fe₃O₄/r-GO/GC, the value of R_{ct} (4900.0 Ω) significantly increases compared to the GC, suggesting that LDH enzyme was successfully immobilized onto the electrode surface.

The electrochemical redox behavior of the Fe₃O₄/r-GO/GC electrode was further studied by cyclic voltammetry. The cyclic voltammograms of this modified electrode in a deaerated PBS (pH 7.0) at various potential scan rates were recorded (Fig. S1). As can be seen, there exist an anodic peak of ca. 0.0 V as well as a cathodic peak of ca. -0.15 V. The peak potentials separation (ΔE_p) is about 120 mV and the ratio of cathodic to anodic peak current *i*_{pc}/*i*_{pa} nearly equals to unity. The observed electrochemical behavior for Fe₃O₄/r-GO modified electrode could be attributed to the iron phosphate redox system. At first, Fe₃O₄ nanoparticles directly reduced at the electrode surface according to the following reaction:



Then, Fe²⁺ ions produced could combine with phosphate ions in the buffer solution:



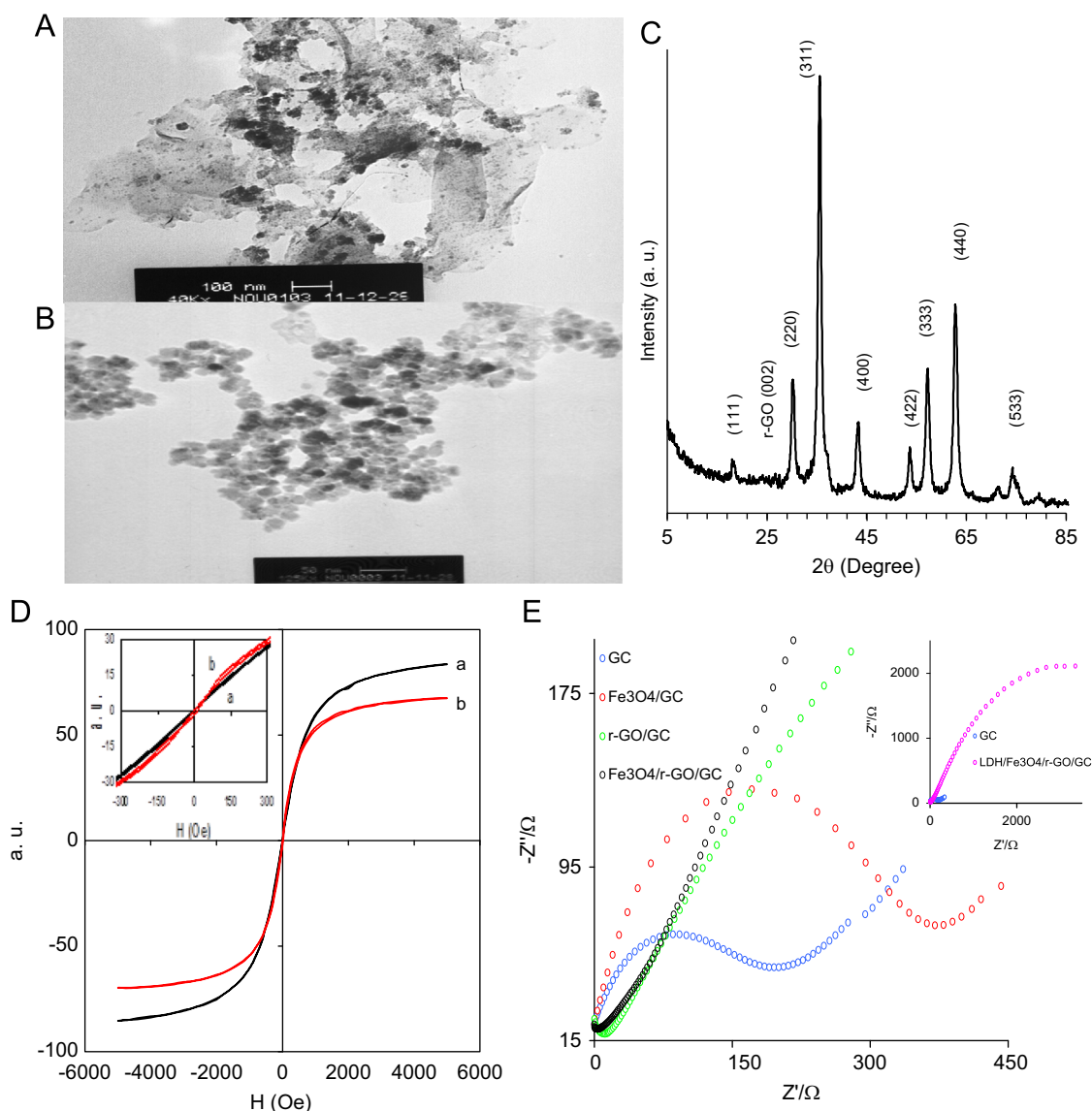


Fig. 1. (A) Low and (B) high magnification TEM images of $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite. (C) XRD pattern of $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposites. (D) Room temperature magnetization curves of (a) Fe_3O_4 nanoparticles and (b) $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposites. (E) EIS plots of $1.0 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl recorded at different electrodes of GC, $\text{Fe}_3\text{O}_4/\text{GC}$, $\text{r-GO}/\text{GC}$ and $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$. The inset of Fig. 1E shows the EIS plots of $\text{LDH}/\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ and GC electrodes.

Here after, the solid FePO_4 at the surface of electrode could be responsible for observed redox behavior (McKenzie and Marken, 2001; Teymourian et al., 2012). The plot of anodic and cathodic peak currents vs. scan rate was recorded (inset A of Fig. S1). As can be seen, the peak currents were directly proportional to the scan rate in the whole range studied supported the idea of a surface-confined redox process. Moreover, the plotted E_p vs. $\log$ of scan rate (inset B of Fig. S1) shows that at high sweep rates, peak separations begin to increase indicating limitation due to the charge transfer kinetics. Based on the Laviron theory (Laviron, 1979), the heterogeneous electron transfer rate constant (k_s) and charge transfer coefficient (α) were obtained as 31.62 s^{-1} and 0.26, respectively. The large value of k_s indicates high ability of r-GO as an electron carrier for promoting electron transfer between Fe_3O_4 nanoparticles and electrode surface.

3.3. Electrocatalysis of O_2 and H_2O_2 at the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode

At first, H_2O_2 and oxygen were selected to study the electrocatalytic behavior of $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite. Fig. 2(A and B) compares the cyclic voltammograms (CVs) for different electrodes

of $\text{Fe}_3\text{O}_4/\text{GC}$, $\text{r-GO}/\text{GC}$ and $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ in the absence and presence of O_2 and H_2O_2 . An obvious reduction wave of O_2 at the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ could be observed at -0.3 V with onset potential of 0.0 V that was more positive than ones obtained for $\text{Fe}_3\text{O}_4/\text{GC}$ and $\text{r-GO}/\text{GC}$ electrodes (Fig. 2A). Furthermore, according to the Fig. 2B, while both $\text{Fe}_3\text{O}_4/\text{GC}$ and $\text{r-GO}/\text{GC}$ electrodes hardly responded for such concentration of H_2O_2 , the electrode loaded with $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite exhibited significantly enhanced currents for both the oxidation and reduction of H_2O_2 starting around $+0.45 \text{ V}$ and $+0.05 \text{ V}$, respectively. It was believed that while the Fe_3O_4 nanoparticles are mainly responsible for observed electrocatalytic behavior at $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite modified electrode, however, the existence of graphene nanosheets as a carbon support with excellent electronic conduction features is essential to the dispersion of these nanoparticles so as to fully utilize their catalytic properties and in fact, the superior electrocatalytic activity of the $\text{Fe}_3\text{O}_4/\text{r-GO}$ film modified electrode can be attributed to the good synergistic coupling effects between the Fe_3O_4 nanoparticles and r-GO nanosheets. Thus, the $\text{Fe}_3\text{O}_4/\text{r-GO}$ modified electrode not only improves the redox currents but also decreases the overvoltage potential for the O_2 reduction as well as

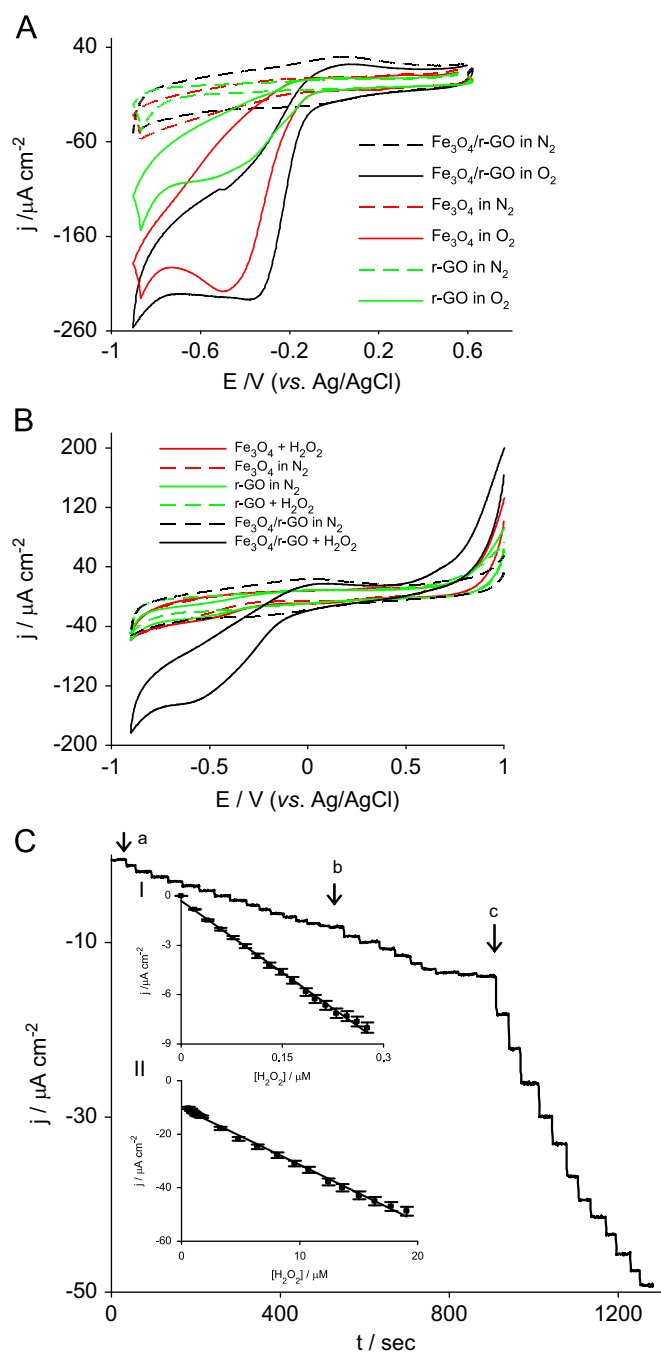


Fig. 2. (A) CVs of $\text{Fe}_3\text{O}_4/\text{GC}$, $\text{r-GO}/\text{GC}$ and $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrodes in 0.1 M PBS at pH 7.0 at various conditions (scan rate 0.02 V s^{-1}). (B) CVs of $\text{Fe}_3\text{O}_4/\text{GC}$, $\text{r-GO}/\text{GC}$ and $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrodes in deaerated 0.1 M PBS at pH 7.0 in the absence and presence of 0.4 mM H_2O_2 . (C) Current-time curve for $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode with successive H_2O_2 additions of 0.02 μM (a), 0.2 μM (b) and 2 μM (c). Electrolyte: 0.1 M PBS (pH 7.0). Applied potential: -0.3 V . (Insets I and II are the correspondent calibration curves for lower and higher concentration ranges, respectively).

for the oxidation and reduction of H_2O_2 , allowing convenient low-potential amperometric detection. The amperometric responses of the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode to the successive additions of different concentrations of H_2O_2 at applied potential of -0.3 V along with the resulting calibration plot are given in Fig. 2(C). As can be seen from this amperogram, well-defined and fast amperometric signals are observed from H_2O_2 additions, generating steady-state signals within 1–2 s. Referring Table 1A that summarizes the response characteristics of the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode obtained for different analytes, the linear range for H_2O_2 is 0.02–19 μM at

the proposed nanocomposite modified electrode. Also the detection limit of H_2O_2 was calculated as 6 nM ($S/N=3$). This result is lower than those at other H_2O_2 sensors, such as CNTs/chitosan modified electrode (10.3 μM at -0.2 V) (Qian and Yang, 2006), reduced graphene oxide/ferro-ferric nanocomposite modified electrode (3.2 μM at -0.3 V) (Ye et al., 2012), chemically reduced graphene oxide/Ag nanoparticles modified electrode (31.3 μM at -0.3 V) (Liu et al., 2011), Au/graphene/HRP/chitosan modified electrode (1.7 μM at -0.3 V) (Zhou et al., 2010), graphene/Au NPs/chitosan (180 μM at -0.2 V) (Shan et al., 2010) and silver nanoparticles capped in a PVA film modified Pt electrode (1.0 μM at -0.5 V) (Guascito et al., 2008), indicating that $\text{Fe}_3\text{O}_4/\text{r-GO}$ can serve as an excellent choice for the enhanced electrochemical sensing. Additionally, very good reproducibility was obtained using the same $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode for 10 repetitive amperometric analyses of 1.0 μM H_2O_2 at applied potential of -0.3 V resulting in a relative standard deviation of 3.8%. In order to study the reproducibility of the electrode modification, five modified electrodes were prepared independently. The RSD for determining 10 μM H_2O_2 was 6.3%. The $\text{Fe}_3\text{O}_4/\text{r-GO}$ -based H_2O_2 sensor also exhibited a good long-term stability. The catalytic current response can maintain about 95% of its initial value even after one month.

3.4. Electrocatalytic oxidation of NADH and lactate biosensing

The electrochemical oxidation of NADH to the corresponding oxidized form (NAD^+) in aqueous solution has received considerable interest, owing to its significance both as a substrate for dehydrogenase enzymes and also to the design of the novel biosensors, because NAD^+/NADH -dependent dehydrogenases constitute the largest group of redox enzymes known today (Bartlett et al., 2002). In Fig. 3A, CVs for NADH oxidation at different electrodes were compared. As can be seen, in the case of the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode, the anodic potential of NADH shifted negatively to 0.0 V and exhibited highly increased current signal in comparison with $\text{r-GO}/\text{GC}$ and $\text{Fe}_3\text{O}_4/\text{GC}$ electrodes. Here, the excellent electrochemical behavior of $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite-based system toward electrooxidation of NADH may be attributed to the promising mediator-like electrocatalytic activity of Fe_3O_4 NPs as well as high electron transfer kinetics on graphene nanosheets. In this system, Fe_3O_4 plays the role of an electrocatalyst for NADH oxidation while r-GO acts as an electron carrier. The mechanism of the electrochemical NADH oxidation in

Table 1A

Response characteristics of different analytes on $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite modified electrode.

Analyte	Applied potential (V)	Linear range (M)	Detection limit (M)	Sensitivity ($\text{A M}^{-1} \text{ cm}^{-2}$)
H_2O_2	-0.3	2×10^{-8} – 2.8×10^{-7}	6×10^{-9}	29.18
NADH	$+0.05$	2.8×10^{-7} – 1.9×10^{-5}	–	2.262
		2×10^{-6} – 1.5×10^{-5}	4×10^{-7}	0.113
Lactate	$+0.1^a$	1.5×10^{-5} – 1.9×10^{-4}	–	0.034
	$+0.01^a$	2×10^{-4} – 2.2×10^{-3}	2×10^{-5}	0.0226
	$+0.01^a$	1.6×10^{-4} – 7.2×10^{-3}	2×10^{-5}	0.0335
	$+0.16^a$	4×10^{-7} – 3.5×10^{-6}	8×10^{-8}	38.8
UA	$+0.33^a$	3.5×10^{-6} – 1.6×10^{-5}	–	12.9
	$+0.33^a$	4×10^{-6} – 2×10^{-5}	5×10^{-7}	4.50
Nitrite	$+0.7$	2×10^{-5} – 2.1×10^{-4}	–	1.22
	-0.3	1×10^{-6} – 9.2×10^{-5}	3×10^{-7}	0.226
	-0.3	1×10^{-5} – 1.6×10^{-4}	1.2×10^{-6}	0.158

^a The potential at which the electrocatalytic current was reported.

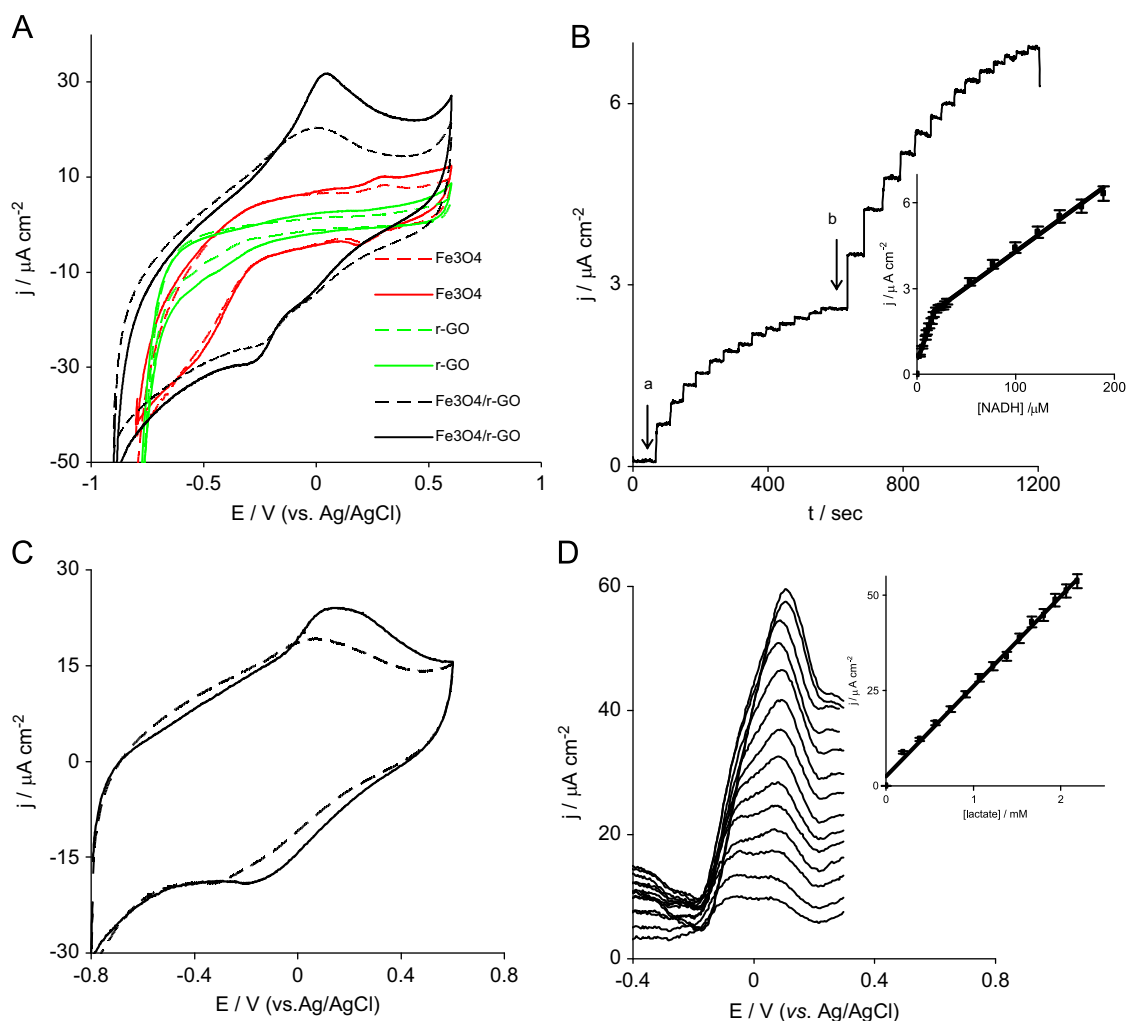
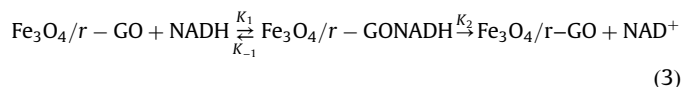


Fig. 3. (A) CVs of $\text{Fe}_3\text{O}_4/\text{GC}$, $\text{r-GO}/\text{GC}$ and $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrodes in 0.1 M PBS (pH 7.0) and scan rate 20 mV s^{-1} in the absence (dashed line) and presence (solid line) of 0.5 mM NADH. (B) Chronoamperometric response of $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in PBS (0.1 M, pH 7.0) on successive addition of different NADH concentrations of 2.5 μM (a) and 25 μM (b) at working potential of 0.1 V vs. Ag/AgCl. Inset is the plot of amperometric response vs. NADH concentration. (C) CVs of LDH/ $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in 0.1 M PBS (pH 7.0) containing 5 mM NAD^+ and scan rate 20 mV s^{-1} in the absence (dashed line) and presence (solid line) of 1.0 mM lactate. (D) DPVs of LDH/ $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in 0.1 M PBS (pH 7.0) containing 5 mM NAD^+ at different lactate concentrations, (from inner to outer) 0.2, 0.38, 0.57, 0.74, 0.91, 1.07, 1.23, 1.38, 1.52, 1.67, 1.8, 1.93, 2.06 and 2.19 mM. Inset is the plot of peak currents vs. lactate concentration (data obtained were the averages of three measurements).

the suggested system is as follows:



where NADH diffuses and adsorbs on the electrode surface and then it is electrochemically oxidized by an ECE mechanism (Eq. (3)) (Moiroux and Elving, 1980). According to the ECE mechanism, the first step of NADH electrochemical oxidation is an irreversible heterogeneous electron transfer. In this step, one electron is lost and a cation radical NADH^{++} is produced (Eq. (4)). The neutral radical NAD^+ was produced through a first-order deprotonation reaction of NADH^{++} (Eq. (5)). A continual reaction for electron transfer from NAD^+ occurred through a second heterogeneous electron transfer (Eq. (6)).

Fig. 3B presents the amperometric response of the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode at +0.05 V to the successive additions of NADH. Immediately after the addition of NADH, the anodic current increased and reached a steady state within $< 5 \text{ s}$. The sensor response displayed

two linear concentration ranges (as in Table 1A); one from 2 to 15 μM ($R^2 = 0.994$) with sensitivity of $0.113 \text{ A M}^{-1}\text{cm}^{-2}$ and another one from 15 to 190 μM ($R^2 = 0.996$) with a sensitivity of $0.034 \text{ A M}^{-1}\text{cm}^{-2}$. The limit of detection of this sensing system was determined as $0.40 \mu\text{M}$ ($S/N = 3$). The analytical parameters for NADH detection at the proposed $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite modified electrode are comparable to other previously reported results (Huang et al., 2007; Yang and Liu, 2009; Kim et al., 2010). The kinetic parameters of this reaction can be estimated by plotting the NADH concentration vs. the current difference (inset of Fig. 3B). This plot showed the kinetics of a heterogeneous second-order reaction type, hence, the affinity of NADH for the electrode ($k_{-1} + k_2/k_1$ in Eq. (3)) can be estimated by a Michaelis–Menten constant (K_M) calculation; the value was $52.8 \mu\text{M}$, deduced from a Lineweaver–Burk plot. The obtained K_M is lower compared to our previously reported value of $95 \mu\text{M}$ (Teymourian et al., 2012), that obviously implies the higher affinity of $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode toward NADH when compared to $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{GC}$ electrode. Also this value is lower than other reported ones such as 3.04 mM (Kim et al., 2010), 0.54 mM (Kim and Yoo, 2009), 0.212 mM (Santos Alvarez et al., 2005) and 0.8 mM (Gligor et al., 2009). Moreover, the reproducibility of the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode for NADH detection was examined. The chronoamperometric signals produced by a series of five successive measurements of 10 and

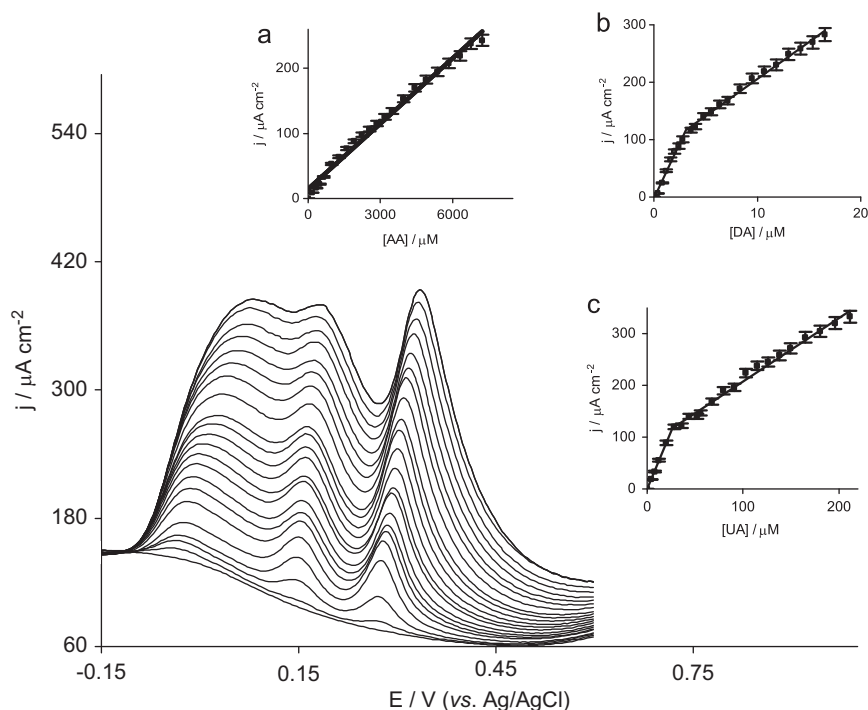


Fig. 4. DPVs of $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in PBS (0.1 M, pH 7.0) containing mixed concentrations of the AA, DA and UA mixture. [AA]: 0.0, 0.3, 0.48, 0.64, 0.96, 1.28, 1.59, 1.91, 2.23, 2.54, 2.86, 3.17, 3.49, 3.96, 4.43, 4.90, 5.37, 5.83, 6.30, 6.76 and 7.22 mM. [DA]: 0.0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.39, 2.79, 3.59, 3.98, 4.78, 5.57, 6.36, 7.15, 8.33, 9.51, 10.68, 11.86, 13.03, 14.2, 15.36 and 16.52. [UA]: 0.0, 4.0, 8.0, 11.98, 19.96, 27.92, 35.87, 43.81, 51.73, 55.69, 67.54, 79.36, 91.16, 102.93, 114.67, 126.38, 138.07, 149.72, 165.22, 180.68, 196.08, 211.43. Insets are the plots of oxidation peak currents vs. AA (a), DA (b) and UA (c) concentrations.

100 μM NADH yielded good reproducibilities with the relative standard deviations of 4.8 and 5.2%, respectively.

Using LDH as the model biorecognition element, we further demonstrated the use of the $\text{LDH}/\text{Fe}_3\text{O}_4/\text{r-GO}$ nanobiocomposite as an electronic transducer for the development of an electrochemical biosensor for lactate. Fig. 3C shows the CVs of $\text{LDH}/\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode containing 5 mM NAD^+ cofactor in the absence and presence of 1.0 mM lactate. As indicated, the anodic peak current increased in the presence of lactate with the onset potential of 0.0 V reaching the maximum current at 0.15 V. DPVs of $\text{LDH}/\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode for successive additions of lactate were recorded (Fig. 3D). This observation clearly supports that the increasing in peak observed at +0.1 V is due to the oxidation of enzymatically produced NADH during lactate oxidation. The calibration plot drawn between the response current and the concentration of lactate (0.2–2.2 mM) is found to be linear with sensitivity of $0.0226 \text{ A M}^{-1}\text{cm}^{-2}$, and the detection limit was calculated as 20 μM (Table 1A). The data obtained here further confirm the efficiency of $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite both as a mediatorless electrocatalyzing material and also as an excellent platform for immobilization of enzymes and construction of biosensing devices.

3.5. Electrochemical detection of AA, DA and UA at $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode

Ascorbic acid (AA), dopamine (DA), and uric acid (UA) are electroactive compounds that have similar electrochemical properties, which complicate their electrochemical identification (Sun et al., 2011). Fig. 4 depicts the DPV recordings at various concentrations of AA, DA and UA at $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode. As can be seen, three well-defined separated anodic peaks corresponding to oxidation of AA, DA and UA are observed at potentials of approximately 0.01, 0.16 and 0.33 V, respectively. The oxidation peak current of these three molecules

linearly increases with their concentrations. For AA (Fig. 4A), the linear regression equation is calibrated as $j_{\text{AA}} (\mu\text{A cm}^{-2}) = 14.833 + 0.0335[\text{AA}] (\mu\text{M})$ ([AA]: 160.0–7227.1 μM , $R^2 = 0.9993$). For DA (Fig. 4B), two linear regression equations were obtained which are expressed as $j_{\text{DA}} (\mu\text{A cm}^{-2}) = -2.8705 + 38.771[\text{DA}] (\mu\text{M})$ ([DA]: 0.4–3.5 μM , $R^2 = 0.9992$) and $j_{\text{DA}} (\mu\text{A cm}^{-2}) = 77.213 + 12.844 [\text{DA}] (\mu\text{M})$ ([DA]: 3.5–160.0 μM , $R^2 = 0.9995$). In the case of UA (Fig. 4C), two linear regression equations were also obtained which are calculated as $j_{\text{UA}} (\mu\text{A cm}^{-2}) = -0.1579 + 4.474 [\text{UA}] (\mu\text{M})$ ([UA]: 4.0–20.0 μM , $R^2 = 0.9998$) and $j_{\text{UA}} (\mu\text{A cm}^{-2}) = 86.052 + 1.224 [\text{UA}] (\mu\text{M})$ ([UA]: 20.0–212.0 μM , $R^2 = 0.9996$). The detection limits ($S/N=3$) for the determination of AA, DA and UA were evaluated as 20.0 μM , 0.08 μM and 0.50 μM , respectively (Table 1A). The lowest detection limits obtained here for simultaneous determination of AA, DA and UA are comparable or in some cases better than previously reported values such as 50, 0.02 and 0.2 μM at mesoporous carbon nanofiber modified pyrolytic graphite electrode (Yue et al., 2012), 20, 0.5 and 0.4 μM at ordered mesoporous carbon/naion composite film (Zheng et al., 2009) and 3, 0.09 and 0.3 μM at iron(III)-porphyrine functionalized MWCNTs (Wang et al., 2012).

3.6. Electrocatalytic determination of nitrite at $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode

In order to further demonstrate the usefulness of the proposed $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite-based platform, its ability toward sensing nitrite as an important inorganic analyte has been surveyed. Fig. 5A presents the CVs for $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in the absence and presence of different concentrations of nitrite. As can be seen, the $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite exhibited significantly enhanced currents for both the oxidation and reduction of nitrite starting around +0.5 V and +0.3 V, respectively. Such a high electrocatalytic behavior of the $\text{Fe}_3\text{O}_4/\text{r-GO}$ modified electrode for the oxidation and reduction of nitrite can be exploited to

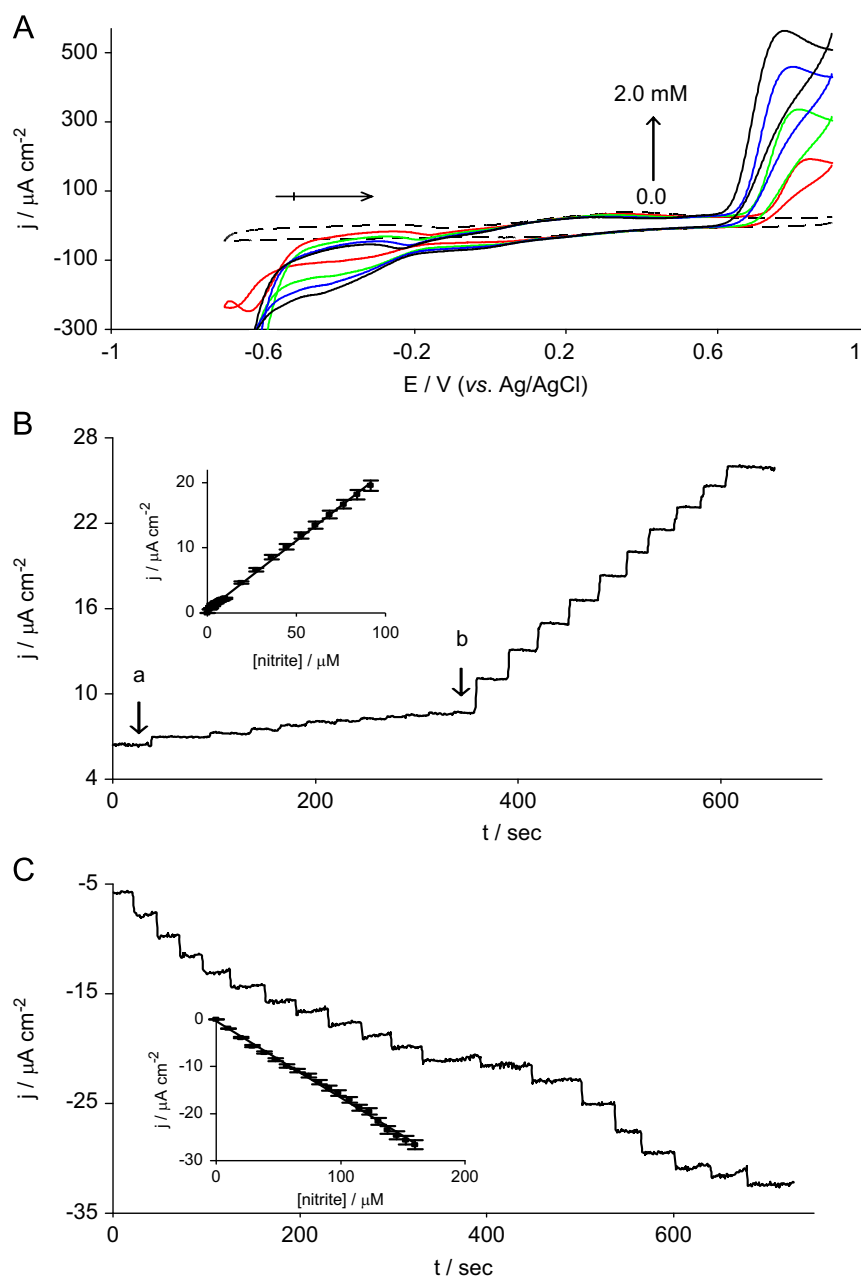


Fig. 5. (A) CVs of $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in 0.1 PBS pH 2.0 at scan rate 20 mV s^{-1} with increasing nitrite concentration of 0.0, 0.5, 1.0, 1.5 and 2.0 mM. (B, C) Current–time responses at $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode in 0.1 PBS with successive additions of 1.0 (a) and 10.0 μM (b) in B and 10.0 μM in C (Applied potentials: +0.7 V in B and –0.4 V in C). Insets show the relationship between oxidation and reduction currents with the concentration of nitrite.

convenient low-potential amperometric detection of nitrite. The amperometric responses of the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode to the successive additions of different concentrations of nitrite at applied potentials of +0.7 V and –0.4 V along with the resulting calibration plots are given in Fig. 5 (B and C), respectively. As can be seen from these amperograms, well-defined and fast amperometric signals are observed from nitrite additions, generating steady-state signals within less than 3 s. The linear ranges for nitrite (Table 1A) were obtained as 1–92 μM and 10–160 μM based on its oxidation and reduction at the proposed nanocomposite modified electrode, respectively. Also the detection limits of nitrite at the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode were calculated as 0.3 μM (at +0.7 V, based on $S/N=3$) and 1.2 μM (at –0.3 V, based on $S/N=3$). The analytical performances obtained above are comparable to or better than those of the other nitrite sensors (Dreyse et al., 2011; Mani et al., 2012).

Table 1B

Determination of nitrite in sausage samples by using $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite modified electrode.

Sample no.	Amount found ^a (ppm)		Added (ppm)	Found (ppm)	Recovery (%)
	Griess method	Proposed sensor			
1	71.09 ± 2.06	72.85 ± 3.69	8	83.37	103.12
2	95.33 ± 1.64	98.19 ± 3.20	8	108.91	102.56

^a Mean value for triplicate measurements.

In order to further demonstrate the good performance of the proposed sensing system, its applicability was evaluated by the analysis of nitrite as a model analyte in real samples. The obtained results were given in Table 1B. It could be seen that there is a good

agreement between the values obtained by our proposed methodology and the spectroscopic reference method and also between the spiked and found values for detection of nitrite. These results clearly indicate that the system presented here to be valid for the real samples analysis.

4. Conclusion

In this paper, a facile one-step synthetic route (a chemical reaction strategy including the reduction of GO and the in-situ generation of Fe_3O_4 nanoparticles) was used to produce $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite. The new nanohybrid material combines the unique and attractive electronic behavior of r-GO nanosheets with excellent catalytic properties of Fe_3O_4 nanoparticles. The resulting nanocomposite was investigated by various characterization methods, including TEM, XRD, AGFM and EIS. $\text{Fe}_3\text{O}_4/\text{r-GO}$ nanocomposite modified GC electrode was reported as a novel electrode system for the preparation of electrochemical sensing and biosensing platform and the electrocatalytic behaviors of different kinds of important electroactive compounds (H_2O_2 , NADH, nitrite, neurotransmitters (dopamine (DA)), and other biomolecules (ascorbic acid (AA) and uric acid (UA)) at the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode were assessed. Electrochemical studies verified that the $\text{Fe}_3\text{O}_4/\text{r-GO}/\text{GC}$ electrode possess excellent electrocatalytic activities toward all these analytes because of the synergistic integration of the two nanomaterials. As demonstrated here, r-GO sheets are uniquely advantageous to serve as a conductive support to uniformly anchor Fe_3O_4 magnetic nanoparticles with well-defined size and shapes. Agglomeration, the common phenomenon of metallic oxide preparations, is no longer an issue. Above all, the $\text{Fe}_3\text{O}_4/\text{r-GO}$ hybrid could be an extremely promising candidate applicable for a wide range of electrochemical sensing and biosensing applications.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2013.04.034>.

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