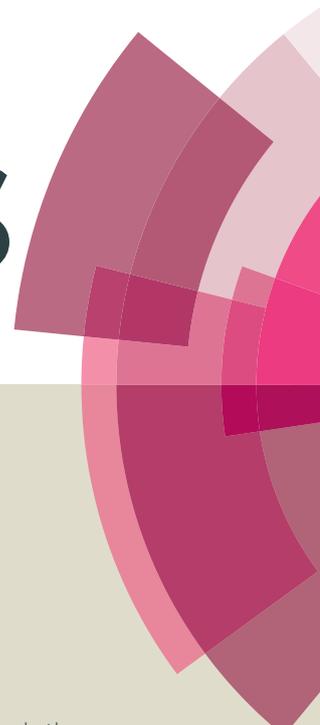


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One-step Electrochemical Fabrication of Nickel Oxide Nanoparticles/Polyaniline Nanowire/Graphene Oxide Hybrids on Glassy Carbon Electrode for a Non-enzymatic Glucose Biosensor

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- 10 We propose here a novel non-enzymatic glucose biosensor based on nickel oxide nanoparticles/polyaniline nanowire/graphene oxide modified glassy carbon electrode (NiONPs/PANiNW/GO/GCE). The composite, prepared by mixing aniline with graphene oxide (GO) together, was transferred to the surface of bare glassy carbon electrode (GCE). The modified electrode was then immersed into deoxygenated 50 mM NiCl₂ solution and electrodeposited at -0.8V for 400s to obtain NiONPs/PANiNW/GO/GCE. We characterized the morphology and electrochemical performance of the modified electrode using scanning electron microscopy (SEM) and cyclic voltammetry (CV), respectively. We found that NiONPs/PANiNW/GO/GCE exhibited higher electrocatalytic activity to glucose oxidation than nickel oxide nanosheets/graphene oxide modified glassy carbon electrode (NiONS/GO/GCE) in alkaline solution. The sensitivity of the sensor towards glucose oxidation was 376.22 μA mM⁻¹ cm⁻² with a linearity range of 2 μM - 5.560 mM and a detection limit of 0.5 μM (S/N = 3). The sensor selectively detected glucose in presence of the common interfering species such as ascorbic acid, uric acid, and dopamine. Furthermore, we examined the applicability of this modified electrode as a sensing probe for the detection of the glucose concentrations in the fetal bovine serum. We conclude that the highly selective and sensitive NiONPs/PANiNW/GO/GCE based non-enzymatic glucose sensor has potential to be applied for accurate measurement of glucose level for various practical purposes such as clinical diagnosis, food analysis, etc.

1. Introduction

Accurate measurement of glucose level is essential for various purposes, especially in clinical diagnosis^[1], chemical testing^[2], and food analysis^[3], etc. Meanwhile, diabetes, one of the worst diseases, is considered a serious threat to human health worldwide in the 21st century^[4]. Not only diabetes, glucose level is an important indicator of other health conditions such as trauma, stroke and other acute conditions requiring intensive care management^[5]. A simple, inexpensive, portable and easy-to-use method for regular monitoring of blood glucose will be a boon to diabetic patients. Although there are several glucose measuring kits, for an example glucometer based on glucose oxidase, available commercially, maintaining activity is a difficult task due to enzymatic nature property. Designing a glucose biosensor having high sensitivity, selectivity, accuracy and low sample-consumption is a priority.

Glucose oxidase (GOx) based biosensors lack accuracy because of the unstability of bioenzyme at altered temperatures^[6]. However, a non-enzymatic glucose biosensor may overcome such limitations and perform sensitively and selectively^[7]. In the past decade, many non-enzymatic glucose biosensors have been developed^[8-10].

Non-enzymatic electrochemical glucose biosensors are always attractive alternatives to enzymatic biosensors because of the stability^[11]. Noble metal based materials, such as Pt^[12], Pd^[13], Au and their metal alloys^[14], have been explored as catalysts for non-enzymatic glucose detection; however, high cost associated with noble metal based materials has limited its application. In recent years, several types of nanomaterials, e.g., CuO^[15], MnO₂^[16], NiO_x^[17], and CoO_x^[18], have been extensively explored as the sensing materials for developing non-enzymatic glucose biosensors. Among them, nickel oxide (NiO) nanostructures based materials have been widely used in non-enzymatic glucose sensor due to its excellent electrocatalytic properties and chemical stability^[19,20]. Graphene-NiO nanoparticles (NiONPs)^[21,22], graphene-Ni(OH)₂^[23], and graphene oxide (GO)-electrospun NiO nanofibers^[24] composites are known for their exceptional thermal, chemical and mechanical properties, high specific surface area, and excellent conductivity. To improve the performance of electrochemical non-enzymatic glucose sensor, the above nanomaterials have been functionalized by conductive

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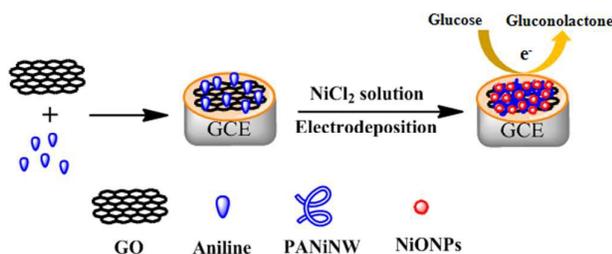
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polymer in general, such as polyaniline (PANI), polypyrrole, polythiophene, and etc.^[25,26]

PANI has been used as a typical conducting polymer in glucose biosensor^[27]. Owing to conjugation in PANi molecule, electrons possess a high degree of transferability^[28]. Altering PANi's electronic structure by "mixing" with other materials, one can improve its magnetic properties, optical properties, electrical conductivity, and structural features remarkably^[29]. Lee et al.^[30] reported electrochemical enzymatic glucose sensor by modifying GOx with functionalized PANi and a multiwalled carbon nanotube with high sensitivity (4.34 $\mu\text{A mM}^{-1}$), reproducibility, and the detection limit of 0.11 μM (S/N = 3).

This study reported an electrochemical non-enzymatic glucose sensor based on three-dimensional (3D) NiO nanoparticles (NiONPs) and PANi nanowire (PANiNW). We one-step electrochemically fabricated the NiONPs/PANiNW at the surface of GO modified glassy carbon electrode (GCE) to develop a non-enzymatic glucose sensor (NiONPs/PANiNW/GO/GCE). The modified electrode was characterized using scanning electron microscope (SEM), elemental mapping, X-ray diffraction, and elemental analysis. The new sensor possessed excellent selectivity and sensitivity with good results. Furthermore, the NiONPs/PANiNW/GO/GCE demonstrated glucose detection in real sample - glucose level in fetal bovine serum.



Scheme 1 Schematic illustration of the enhanced electrochemical detection strategy for glucose based on NiONPs/PANiNW/GO modified on the GCE.

2. Experimental

2.1 Reagents

Graphene oxide (GO) was purchased from Nanjing XFNano Materials Technology Company (Nanjing, China). Glucose, uric acid (UA), ascorbic acid (AA), dopamine (DA), and nickel(II) chloride hexahydrate were purchased from Sigma (Shanghai, China) and used as supplied. All other chemicals used were of analytical reagent grade, and deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was used for all experiments.

2.2 Apparatus

Electrochemical measurements were performed using a computer-controlled electrochemical analyzer (CHI 832,

Chenhua, Shanghai, China) composed of a conventional electrochemical cell containing one as-prepared glassy carbon (GC) electrode as the working electrode, one platinum wire as the counter electrode, and an Ag/AgCl electrode (KCl-saturated) as the reference electrode. The morphology of various modified GC electrodes was directly examined by field emission scanning electron microscope (SEM) (S-4800, Hitachi, Japan) using a special GC electrode (3 mm) for SEM. The X-ray diffraction (XRD) analysis was conducted on a Rigaku D/max 2500 X-ray diffractometer with Cu K α radiation ($\lambda = 1.54 \text{ \AA}$) at a scan rate of $5 \text{ degree min}^{-1}$. All experiments were conducted at room temperature.

2.3 Preparation of NiONPs/PANiNW/GO/GCE

GO aqueous dispersion (5 mL , 1 mg mL^{-1}) was mixed with aniline (5 mL , 5 mM), ultrasonicated for 60 min , centrifuged for 10 min at 10000 rpm ($12,300 \times g$), and subsequently, redispersed in water. Prior to modification, the bare GCE was polished first using emery paper and then with aqueous slurries of alumina powder ($0.3 \mu\text{m}$ and $0.05 \mu\text{m}$) on a polishing cloth, and finally rinsed with doubly distilled water under an ultrasonic bath for 10 min . Next, the GC electrode surface was casted with $5 \mu\text{L}$ aniline/GO hybrid and dried in the atmosphere to fabricate the aniline/GO/GCE electrode. The NiONPs/PANiNW/GO/GCE was structured by an electrodeposition method: (i) the aniline/GO/GCE was first electrodeposited in a deoxygenized 50 mM NiCl_2 solution with 0.1 M KCl using chronoamperometric technique at 0.8 V for 400 s . The polymerization of the aniline was completed during the chronoamperometry process; (ii) the electrode was then rinsed with doubly distilled water for 30 s . To compare the performance of the NiONPs/PANiNW/GO/GCE, NiONS/GO/GCE was used as the control and fabricated in the same way mentioned above.

2.4 Electrochemical measurement

The sensing strategy for glucose is illustrated in Scheme 1. Cyclic voltammetry (CV) was performed to characterize the different modified electrodes in 0.1 M NaOH solution containing different concentrations of glucose at a scan rate of 100 mV s^{-1} . The chronoamperometric responses of NiONPs/PANiNW/GO/GCE were recorded at $+0.6 \text{ V}$ by continuously injecting glucose solution into a $10 \text{ mL } 0.1 \text{ M NaOH}$ solution. The measurements were conducted under stirring at room temperature.

3. Results and discussion

3.1 characterization of the modified electrode

We examined the morphology of the electrodes with SEM. Fig. 1 presented the SEM images of GO/GCE (A), NiONS/GO/GCE (B), and NiONPs/PANiNW/GO/GCE (C, D). The surface of GO/GCE presented wrinkle structure, the typical morphology of GO. As shown in Fig. 1, the structures of NiO nanomaterials deposited on GO/GCE and aniline/GO/GCE surface are different. On the GO/GCE surface, a 2D structure of NiONS

(with ~ 200 nm width and ~ 20 nm thickness) was deposited, while a 3D dendritic structure of PANiNW (25 ± 4 nm in width as shown in Fig 1D, $n = 10$) with dense NiONPs were generated on the surface of GO/GCE. We found that the 3D structure of NiONPs/PANiNW/GO/GCE was more homogeneous and smaller, because the presence of PANiNW in the hybrids made NiONPs more regular in shape and more compact. Besides, the element mapping, as shown in Fig. S1, described the element distribution at the surface of the modified electrodes. Compared to the NiONS/GO/GCE, the Ni element was more homogeneous and the N element existed at the surface of the NiONPs/PANiNW/GO/GCE, implying the PANiNW was formed at the surface. Furthermore, the XRD pattern in Fig. S2 showed

the PANi and NiO crystalline structure, and the broad peak was observed at $2\theta = 20.31^\circ$ for PANi, and the peaks at $2\theta = 43.85^\circ$ and 79.49° were corresponding to the (2 0 0), and (2 2 2) planes of the cubic phase of NiO, respectively [31]. According to the above results, the two components in the hybrids, PANiNW and NiONPs, had metal chelation interaction, resulting in a stable existence of NiONPs within the PANiNW. In addition, it was worth noting that *van der Waals* force of interaction between PANiNW and NiONPs was an important factor that made it possible to manipulate the nanoparticles into a close-packed and ordered array with much larger active surface area than that of NiONS/GO/GCE; this makes NiONPs/PANiNW/GO/GCE more suitable for electrocatalysis.

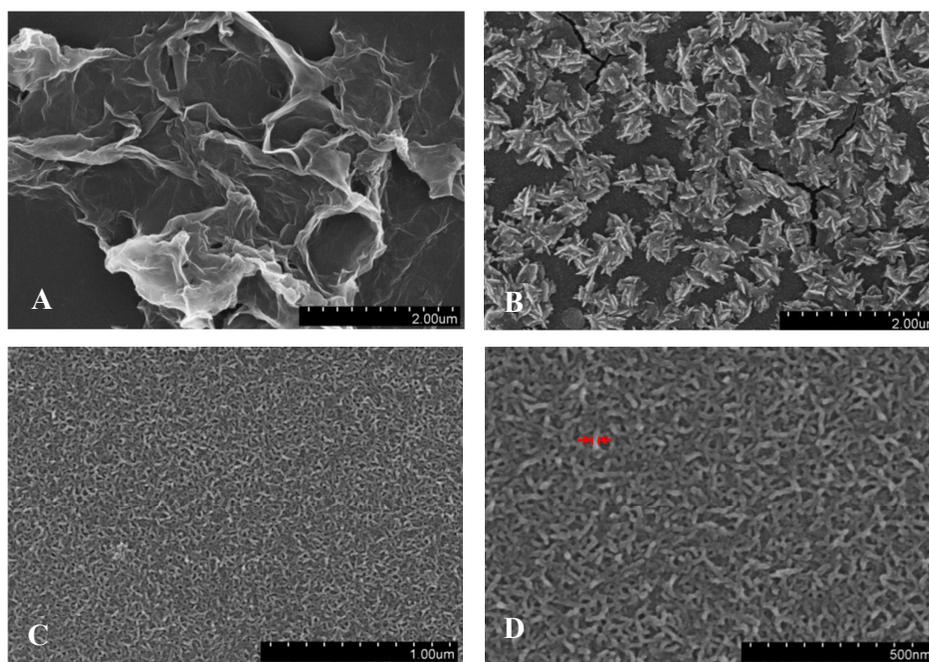


Fig. 1 Typical SEM images of GO/GCE (A), NiONS/GO/GCE (B), and NiONPs/PANiNW/GO/GCE (C, D).

Table 1. Elemental analysis of modified electrode by EDS.

Modified Electrode	Element	Atomic% ^a
NiONS/GO/GCE	C	76.64
	O	17.33
	Ni	6.02
NiONPs/PANiNW/GO/GCE	C	67.44
	N	17.66
	O	13.40
	Ni	1.51

^a Atomic% was adjusted with the exclusion of substrate.

Fig. S3 and table 1 presented the elemental analysis of the hybrids deposited on graphene. The result implied the presence of N in the hybrids of NiONPs/PANiNW/GO/GCE (17.66%), suggesting abundance of PANiNW on the surface of

the modified electrode. Based on the energy-dispersive X-ray spectroscopy (EDS) analysis, we found the atomic proportions of Ni in the hybrids of NiONS/GO/GCE and NiONPs/PANiNW/GO/GCE as 6.02% and 1.51%, respectively. Evidently, both the electrodes contain less Ni with NiONPs/PANiNW/GO/GCE having the least, indicating that the nanoparticles deposited on the surface of the modified electrode would act as Ni nanocatalysts.

3.2 Electrochemical behaviour

Fig. 2 illustrated the cyclic voltammograms (CVs) of GO/GCE (a), PANi/GO/GCE (b), NiONS/GO/GCE (c), and NiONPs/PANiNW/GO/GCE (d) in 0.1 M NaOH, respectively. As evident from the figure that there is no oxidation or reduction peaks for the GO/GCE (curve a), and the PANi/GO/GCE in alkaline solution (curve b); however, NiONS/GO/GCE (curve b) has a couple of reversible redox peaks - peak currents were

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198.33 μA and 58.48 μA , respectively. Curve d, corresponding to NiONPs/PANiNW/GO/GCE, shows a pair of well-defined redox peaks with the peak currents at 603.23 μA and 292.12 μA , which were several times than that of NiONS/GO/GCE. The electrochemically active surface area (ESA) could be calculated based on the Rendles-Sevcik formula:

$$i_p = 0.4463 n^{1.5} F^{1.5} A D v^{0.5} C / RT$$

n : the number of electrons transferred in the redox reaction; F : Faraday constant; A : ESA; D : diffusion coefficient; v : scan rate; C : concentration; R : perfect gas constant; T : temperature. The ESA was calculated as 0.131 and 0.378 cm^2 for NiONS/GO/GCE and NiONPs/PANiNW/GO/GCE, as compared to the geometric area of 0.0707 cm^2 (0.3 cm diameter). This result indicated that the NiONPs/PANiNW/GO/GCE was more electrochemically active than NiONS/GO/GCE under the same process of electrochemical deposition, because of the existence of PANi.

The electrochemical reaction mechanism of NiO in basic solution has been well studied. The redox peaks, shown in Fig. 2, are attributed to the transition between NiO and NiOOH [32, 33].



Therefore, we can infer that NiO could retain its redox properties even after immobilizing on GCE surface with PANiNW.

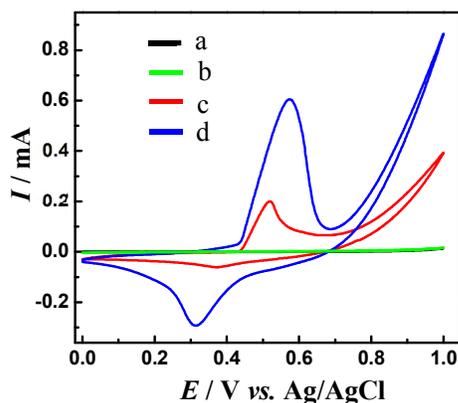


Fig. 2 CVs of GO/GCE (a), PANi/GO/GCE (b), NiONS/GO/GCE (c), and NiONPs/PANiNW/GO/GCE (d) in 0.1 M NaOH at a scan rate of 100 mV s^{-1} .

3.3 Amperometric detection of glucose

To check whether NiONPs/PANiNW/GO/GCE electrochemically catalyzes the oxidation of glucose, we recorded CVs of the tested electrodes in presence of 2 mM glucose in 0.1 M NaOH. As shown in Fig. 3A, no catalytic currents were observed for GO/GC electrode (curve a) and PANi/GO/GCE (curve b) upon addition of 2 mM glucose in 0.1 M NaOH. However, we could observe catalytic characteristics achieved with an increase of the anodic current and a disappearance of the cathodic current at NiONS/GO/GCE (curve b), and NiONPs/PANiNW/GO/GCE (curve c). The increase in the anodic current mainly originated from the doped nickel in the modified electrode during glucose oxidation. Importantly, we found that the catalytic current of glucose oxidation by NiONPs/PANiNW/GO/GCE

was two times as big as that by NiONS/GO/GCE, indicating the former possessed higher catalytic activity with lower nickel content.

To investigate the applicability of the NiONPs/PANiNW/GO/GCE as a sensing element in non-enzymatic glucose detection, we recorded the CVs of NiONPs/PANiNW/GO/GCE in 0.1 M NaOH containing glucose of varying concentrations (Fig. 3B). We observed that the oxidation peak current corresponding to the transformation from NiO to NiOOH increased significantly with the increase in glucose concentration, while the reduction peak current disappeared accordingly. The reason could be ascribed to more NiOOH consumption with increasing glucose concentration (Equation 2), which in turn accelerates the oxidation of NiO to NiOOH (Equation 1), suggesting excellent electrocatalytic activity of the hybrids for glucose oxidation [32].

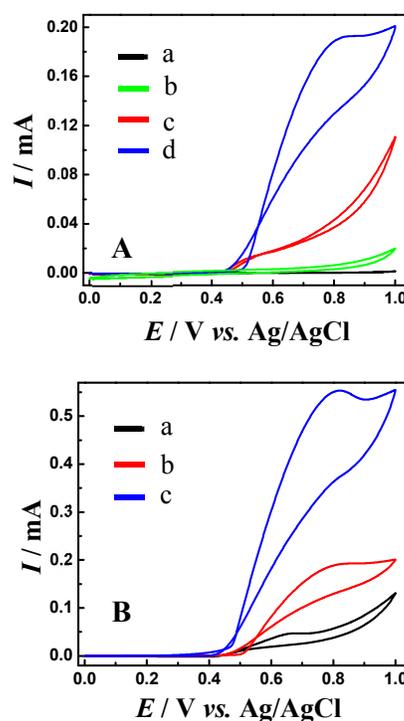


Fig. 3 (A) CV of GO/GCE (a), PANi/GO/GCE (b), NiONS/GO/GCE (c), and NiONPs/PANiNW/GO/GCE (d) in 0.1 M NaOH containing 2 mM glucose at a scan rate of 100 mV s^{-1} . (B) CV of NiONPs/PANiNW/GO/GCE in 0.1 M NaOH containing glucose of varying concentrations at a scan rate of 100 mV s^{-1} : (a) 1 mM; (b) 2 mM; (c) 5 mM.

3.4 Sensitivity study

Next, we monitored chronoamperometric responses of the NiONPs/PANiNW/GO/GCE to successive additions of glucose to the stirred 0.1 M NaOH at an applied potential of +0.6 V (Fig. 4A). We found that the non-enzymatic glucose sensor responded quickly to the additions of glucose and the oxidation current reached to its steady-state within 5s. The oxidation current increased with increasing concentration of glucose in the range from 2 μM to 960 μM with linear equation: $y = 0.03596x + 3.676$ ($R^2 = 0.9936$), and from 2 960 μM to 5.560

mM with linear equation: $y = 0.02553x + 13.39$ ($R^2 = 0.9939$), respectively. The detection limit was calculated to be $0.5 \mu\text{M}$ (signal-to-noise ratio of 3). The sensitivity exhibited by our sensor to glucose oxidation was $376.22 \mu\text{A mM}^{-1} \text{cm}^{-2}$. The lower value of the detection limit, compared with some previous reports^[34,35], suggests the ability of the new sensor to detect low level of glucose in samples, thus confirming the improved sensitivity of NiONPs/PANiNW/GO/GCE based non-enzymatic glucose sensor accompanying a wide linear range of detection.

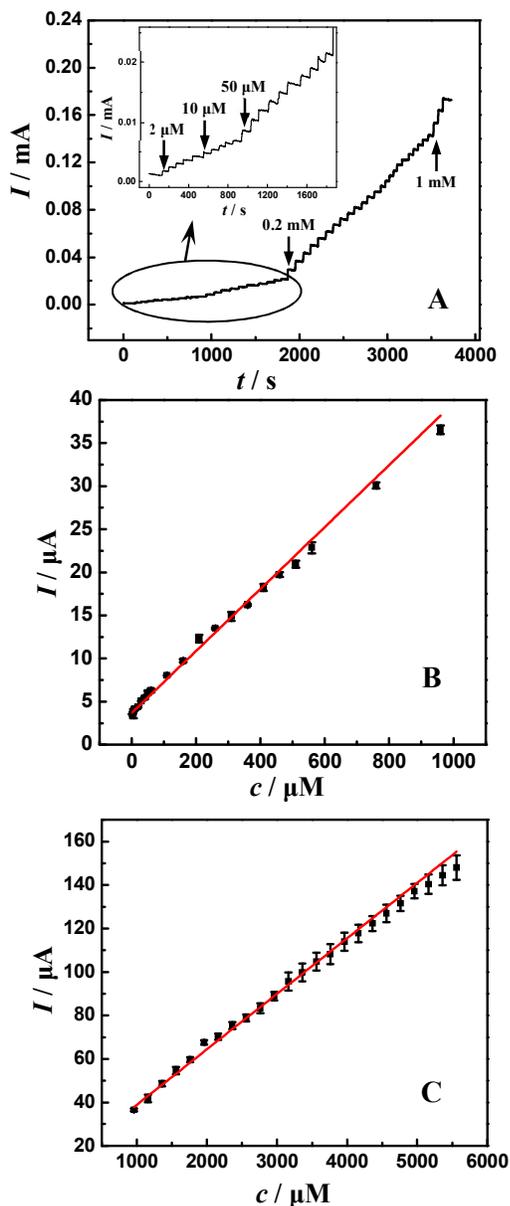


Fig. 4 (A) Chronoamperometric responses of NiONPs/PANiNW/GO/GCE to successive additions of glucose to 0.1 M NaOH at $+0.6 \text{ V}$. Inset: amplified calibration plot corresponding to the lower concentration range. Calibration plots between the current and glucose concentration in the range from $2 \mu\text{M}$ to $960 \mu\text{M}$ (B), and from $960 \mu\text{M}$ to 5.560 mM (C), respectively.

3.5 Selectivity study

Interfering reagents often pose challenge in glucose detection. To examine the selectivity of the NiONPs/PANiNW/GO/GCE, we subjected the sensor to glucose determination at $+0.6 \text{ V}$ in presence of interfering reagents such as AA, DA, and UA (Fig. 5). We found that the NiONPs/PANiNW/GO/GCE produced negligible current signal ratios upon addition of 0.1 mM interfering reagents in the test solution containing 0.1 mM glucose, suggesting excellent selectivity (or anti-interference effect) of the NiONPs/PANiNW/GO/GCE. The excellent selectivity could be attributed to high isoelectric point (10-11) of NiO. Due to higher isoelectric point, the surface of NiONPs remain negatively charged in NaOH solution, and the interfering reagents (UA, AA, and DA) become negatively charged in NaOH solution due to proton loss^[36]. Consequently, the negatively charged NiONPs/PANiNW/GO surface strongly repels the negatively charged molecules, thus reducing the electrooxidation of interfering reagents and resulting in improved selectivity.

Furthermore, we investigated other influences from common co-existing substances and found that most ions and common substances at high concentration caused negligible change: Na^+ , K^+ , Cl^- , NO_3^- , SO_4^{2-} (>300 fold); Ca^{2+} , Zn^{2+} , Mg^{2+} (150 fold). Therefore, NiONPs/PANiNW/GO/GCE exhibited enhanced selectivity for glucose detection.

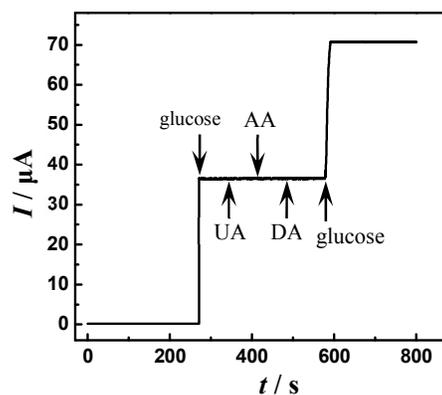


Fig. 5 Amperometric responses of NiONPs/PANiNW/GO/GCE to successive additions of 0.1 mM glucose, 0.1 mM UA, 0.1 mM AA, 0.1 mM DA and then 0.1 mM glucose at $+0.6 \text{ V}$ in 0.1 M NaOH .

3.6 Application to real samples

We investigated the applicability of the proposed method in real sample analysis by directly analyzing glucose level in the fetal bovine serum. The ability in amperometric determination of glucose concentration in the fetal bovine serum, based on the repeated responses ($n = 5$) of the diluted analytes, was measured; the samples were spiked with a specified concentration of glucose. Using the standard addition method, we measured the glucose concentration in the pharmaceutical preparations as well as the recovery rate of the spiked samples; Table 2 listed the results.

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Table 2. Method recoveries for the determination of glucose in the fetal bovine serum (n = 5).

Spiked (mM)	Found (mM)	Recovery (%)	RSD (%)
0.50	0.502	100.3	1.3
2.0	1.97	99.2	1.9
5.0	5.2	100.4	1.7

Conclusions

In summary, we have developed a novel electrochemical non-enzymatic glucose sensor based on the NiONPs/PANiNW/GO/GCE probe with high sensitivity and improved selectivity. The new sensor exhibited selective detection of glucose in a linear concentration range of 2 μM - 5.560 mM at a tolerance limit of 0.5 μM . Furthermore, the NiONPs/PANiNW/GO/GCE exhibited enhanced ability to suppress the background current from common physiological interferents. To check the applicability of the proposed sensor, the NiONPs/PANiNW/GO/GCE was subjected to detect glucose level in the fetal bovine serum; the sensor performed satisfactorily. Based on our findings, we can conclude that the NiONPs/PANiNW/GO/GCE based non-enzymatic glucose sensor would be a promising analytical platform for glucose detection in real samples.

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Tables of Contents Entry



A non-enzymatic glucose biosensor was developed by one-step electrodeposition fabrication of nickel oxide nanoparticles/polyaniline nanowire hybrids on graphene oxide/glassy carbon electrode.