A novel biosensor based on the direct electrochemistry of horseradish peroxidase immobilized in the three-dimensional flower-like Bi₂WO₆ microspheres

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Abstract

Three-dimensional flower-like Bi₂WO₆ microspheres (3D-Bi₂WO₆ MSs) have been synthesized through a simple hydrothermal method. The morphology and structure of 3D-Bi₂WO₆ MSs were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). The 3D-Bi₂WO₆ MSs subsequently were used to immobilize horseradish peroxidase (HRP) and fabricate a mediator-free biosensor for the detection of H₂O₂. Spectroscopic and electrochemical results reveal that 3D-Bi₂WO₆ MSs constitute an excellent immobilization matrix with biocompatibility for enzymes. Meanwhile, due to unique morphology of the flower-like microspheres, the direct electron transfer of HRP is facilitated and the prepared biosensors display good performances for the detection of H₂O₂ with a wide linear range, including two linear sections: 0.5~100 μM (R²=0.9983) and 100~250 μM (R²=0.9981), as well as an extremely low method detection limit of 0.18 μM.

Keywords: Biosensor, Enzyme immobilization, Bi₂WO₆, Three-dimensional flower-like microspheres, hydrogen peroxide

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1. Introduction

Recent researches show that nanomaterials modified electrode has special effect on electrocatalysis and electrochemical determination [1-4]. Especially in system contained many kinds of similar substrate, some electrodes have selective detection or simultaneously detection [5-8]. Meanwhile, nanomaterial can play an important and unique role in direct electrochemical biosensor [9-14]. The researches of direct electron transfer (DET) of enzymes or redox proteins can not only help to understand the intrinsic kinetic and thermodynamic properties of proteins but also establish a foundation for fabricating mediator-free biosensors [15, 16]. However, it is difficult for proteins to realize the DET process on the bare electrode, due to the deep burying of electroactive center in the protein structure and the denaturation of proteins on the bare electrode surface [17, 18]. Recent studies show that a series of nanomaterials with good biocompatibility and special surface effect can be used to immobilize enzymes and fabricate mediator-free biosensors [18, 19]. Among them, three-dimensional (3D) structured nanomaterials, such as 3D nanostructured NiO [19], ZnO [20], TiO₂ [21], MnO₂ [17] microspheres and Co(OH)₂ macroporous film [22], have received increasing attention for their unique structure and morphology. It has been shown that the structure and morphology of nanomaterials have a significant effect on the property of the immobilized enzyme, which determines their efficacy in biosensors [23]. So finding new nanomaterials with special morphology and structure possessing high biosensor performance is still a challenge.

To the best of our knowledge, 3D structured Bi₂WO₆ nanomaterial as a support for constructing mediator-free biosensor has rarely been reported. Here, three-dimensional flower-like Bi₂WO₆ microspheres (3D-Bi₂WO₆ MSs) were synthesized by a simple hydrothermal method. This kind of 3D-Bi₂WO₆ MSs possess many advantages as a support for immobilization of enzyme. As shown in Scheme 1, numerous nanosheets, all pointing toward the center of the sphere, constitute the 3D-Bi₂WO₆ MSs which own a large surface area available for enzyme entrapment. And the trumpet-shaped pores among the nanosheets

can become tunnels to the interior of the microsphere for enzymes. Furthermore, the nanosheets are formed from numerous smaller nanosheets (less than 100 nm in length) which can greatly enhance the active surface area for adsorbing enzymes (Fig. S1). Meanwhile, the substrate has easy access to the enzymes immobilized in the microspheres through the trumpet-shaped pores. Concentration of substrate and enzyme in this confined region will increase the chance of effective collisions between them [24]. Hence, such kind of 3D-Bi₂WO₆ MSs are able to become a promising support for enzyme immobilization and have potential applications in biosensors.

In this study, horseradish peroxidase (HRP) is selected as a model redox protein for construction of the biosensor. Meanwhile, to highlight the advantages of the 3D-Bi₂WO₆ MSs, Bi₂WO₆ nanoparticles (Bi₂WO₆ NPs) are also used as a support to immobilize HRP.

2. Experimental

2.1 Materials and apparatus

HRP and Nafion were purchased from Sigma (St. Louis, MO, USA). Other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., China. All reagents are analytical grade. In all experiments, deionized water was used.

Field-emission scanning electron microscopy (FE-SEM) was performed using a Hitachi S-4800 & Hiroba energy dispersive X-ray electron microscope (Hitachi, Japan). X-ray diffraction (XRD) patterns were recorded on a Rigaku D/max 2200pc diffractometer (Rigaku, Japan) using Cu K α radiation of wavelength $\lambda = 0.15418$ nm at 40 kV and 40 mA. UV-vis spectra were recorded using a PerkinElmer Lambda-950 spectrophotometer (PerkinElmer, America). FT-IR spectra were obtained on a Bruker TENSOR27 instrument (Bruker, Germany). Zeta potential was performed by a Malvern Nano-Z system (Malvern, England). All electrochemical experiments were performed with a CHI 660E electrochemical workstation (CH Instruments, China). A conventional three-electrode system, which consisted of a platinum wire as the counter electrode, an Ag/AgCl/3M KCl as the reference electrode and a 3-mm diameter modified glassy carbon electrode (GCE) as the working electrode, was

used in all electrochemical experiments. Unless otherwise noted, 0.1 M phosphate buffered saline (PBS; pH 7.0) was used as the supporting electrolyte in all experiments. The buffer was purged with highly purified nitrogen for at least 30 min and a nitrogen atmosphere environment was kept in electrochemical measurements.

2.2 Synthesis of 3D-Bi₂WO₆ microspheres

The 3D-Bi₂WO₆ microspheres were synthesized by a facile hydrothermal reaction. In a typical synthesis, the starting materials of Na₂WO₄·2H₂O and Bi(NO₃)₃·5H₂O with the stoichiometric ratio were added into 30 mL deionized water with stirring for 10 min, respectively. Then the Na₂WO₄·2H₂O solution was added with drop by drop to the Bi(NO₃)₃·5H₂O suspension. After stirring for 30 min, the obtained white suspension was loaded into a 100 ml Teflon-liner autoclave and then treated for 12 h at 180°C. The products were washed with deionized water and absolute ethanol to remove any ionic residue then dried at 60°C.

2.3. Preparation of enzyme electrode

Prior to use, a glassy carbon electrode (GCE) was polished with 0.3 and 0.05 μm alumina slurry and sonicated in ethanol and water successively. The cleaned GCE was dried using a purified nitrogen stream. The enzyme electrode was prepared by a simple casting method. Firstly, 1 mL of 2 mg mL⁻¹ 3D-Bi₂WO₆ suspension and 0.5 mL of 10 mg mL⁻¹ HRP PBS were mixed and stirred for 20 min. Secondly, 0.5 mL of Nafion (5%) was added to the mixture and then stirred for 10 min. Finally, 4 μL of the mixture was applied to the surface of a freshly polished GCE to prepare the Nafion/HRP/3D-Bi₂WO₆/GCE and then stored in refrigerator prior to use. To compare, Bi₂WO₆ nanoparticles (Bi₂WO₆ NPs) were used for the fabrication of Nafion/HRP/Bi₂WO₆ NPs/GCE with similar procedures as described above. In the control experiment, Nafion/HRP/GCE and Nafion/3D-Bi₂WO₆/GCE were also prepared. Before electrochemical measurements, all the as-prepared film electrodes were immersed in pH 7.0 PBS for 30 min to remove residual components.

3. Results and discussions

3.1. Characterization of 3D-Bi₂WO₆ MSs

X-ray diffraction (XRD) is firstly used to determine the structure and crystallinity of the as-prepared products. Fig. 1 shows the XRD pattern of the products. The peaks in this diffraction pattern correspond to the orthorhombic Bi₂WO₆ crystal phase (JCPDS No. 73-2020). The XRD pattern also indicates that the prepared Bi₂WO₆ sample has a high crystallinity.

As shown in Fig. 2A and B, the 3D-Bi₂WO₆ MSs with the diameter of about 4 μ m are composed of numerous nanosheets pointing toward the center of the sphere. The trumpet-shaped pores among the nanosheets can become tunnels to the interior of the microsphere for enzymes. Furthermore, from the higher-magnification SEM image (the inset of Fig. 2B), the nanosheets are formed from numerous smaller nanosheets (less than 100 nm in length). This special structure can greatly enhance the active surface area for enzymes adsorption.

Fig. 2C depicts the microstructure image of Nafion/HRP/3D-Bi₂WO₆ composite film. It is noted that 3D-Bi₂WO₆ MSs is discernible on the surface of modified electrode. The 3D-Bi₂WO₆ MSs is embedded in the Nafion to form a stable composite film which is essential for the stability of the prepared enzyme electrode. Moreover, because of the high surface area of 3D-Bi₂WO₆ MSs, the effective surface area of the modified electrode improves greatly (Fig. 2D).

Fig. S1 gives the typical SEM images of 3D-Bi₂WO₆ MSs (A) and HRP/3D-Bi₂WO₆ MSs (3D-Bi₂WO₆ MSs immobilizing HRP) (B). It can be clearly seen from the comparison that the special three-dimensional flower-like structure and the rough surfaces of Bi₂WO₆ microspheres can greatly enhance the active surface area for enzymes adsorption.

3.2. Encapsulation of HRP in the of Nafion/3D-Bi₂WO₆ composite film

Zeta potential measurement shows that the prepared 3D-Bi₂WO₆ MSs carry negative charges with a zeta potential of -15.3 mV. The isoelectric point (pI) of enzymes decides the surface electrical property of enzymes in solution. As we known, the pI of HRP (pI = 8.9 [25]) is larger than pH 7.0. Hence, when pH (solution) ≤ 7.0 , the surface of HRP carries positive

charge. Thus, positively charged HRP molecules in pH 7.0 PBS can be attached onto the 3D-Bi₂WO₆ MSs surface by the electrostatic interaction.

UV-vis spectroscopy is an effective method to monitor the structure change of heme proteins. The Soret absorption bands of heme proteins provide information about the conformational integrity of the enzymes [26, 27]. As shown in Fig. 3, the Soret absorption band of HRP immobilized on Nafion/HRP/3D-Bi₂WO₆ (storage at 4 °C for four weeks) is located at 407 nm (curve a), which is close to that of native HRP at 409 nm (curve b), indicating that HRP retained the essential features of its conformational integrity. This reveals that Nafion/3D-Bi₂WO₆ composite film has biocompatibility and do not suffer significant enzymes denaturation.

3.3. Direct electrochemistry behavior of Nafion/HRP/3D-Bi₂WO₆/GCE

Fig. 4 depicts the typical cyclic voltammograms (CVs) of different modified electrodes in 0.1 M PBS (pH 7.0) at a scan rate of 0.1 V s⁻¹. No redox peak is observed at Nafion/3D-Bi₂WO₆/GCE (curve a), which indicates that Nafion/3D-Bi₂WO₆/GCE is electroinactive in the potential window. The Nafion/HRP/3D-Bi₂WO₆/GCE (curve d) exhibits a couple of stable and well-defined redox peaks at -0.367 V and -0.323 V vs. Ag/AgCl, which could be ascribed to direct electron transfer between HRP and the underlying electrode. According to Li et al. [28], the electron transfer of HRP occurs between the Fe^{III} and Fe^{II} site within HRP. The formal potential ($E^0 = (E_{p,a} + E_{p,c})/2$) of HRP is -0.345 V vs. Ag/AgCl is in agreement with -0.335 V, -0.362 V and -0.369 V vs. Ag/AgCl reported for the HRP(Fe^{III/II}) redox couple in HRP-ZrP, HRP-graphene-Nafion and HRP-GO-Nafion films [28-30]. The potential difference (ΔE_p) between the anodic and cathodic peak potential is about 44 mV. Such a small ΔE_p value reveals a fast and quasi-reversible electron-transfer process, and then indicates that the 3D-Bi₂WO₆ MSs can provide a favorable microenvironment for HRP to undergo a facile electron-transfer reaction. Compared with that of the Nafion/HRP/3D-Bi₂WO₆/GCE, almost no redox peaks are observed at the Nafion/HRP/GCE (curve b, in Fig. 4), suggesting an enhanced electron transfer between HRP molecules and the surface of the Nafion/HRP/3D-

Bi₂WO₆/GCE. Meanwhile, due to the deep burying of electroactive center in the protein structure and the denaturation of proteins on the bare electrode surface, it is difficult for proteins to realize the DET process on the bare electrode [31]. It can be clearly seen from the comparison that direct electron transfer between HRP molecules and GCE is greatly enhanced at the Nafion/HRP/3D-Bi₂WO₆/GCE. For comparison, Nafion/HRP/Bi₂WO₆NPs/GCE has also been carried out (curve c, in Fig. 4). The reduction peak of the electrode is 45.8% smaller than that of Nafion/HRP/3D-Bi₂WO₆/GCE. The ΔE_p is 68 mV, which is larger than that of Nafion/HRP/3D-Bi₂WO₆/GCE (44 mV), indicating faster electron transfer rate of HRP on the electrode than on Nafion/HRP/Bi₂WO₆NPs/GCE. It can be concluded that 3D-Bi₂WO₆ MSs have better effect on the facilitation of electron transfer, which may result from unique morphology and property of the 3D-Bi₂WO₆ MSs.

Fig. 5A shows the cyclic voltammograms of Nafion/HRP/3D-Bi₂WO₆/GCE at 0.1 M PBS (pH 7.0) with the scan rate increasing from 0.1 to 0.8 V s⁻¹. With the increasing of the scan rate, the cathodic and anodic peak currents of HRP increase simultaneously. Meanwhile, the cathodic and anodic peak potentials show a slight shift respectively lead to the peak to peak separation slightly enlarged. Fig. 5B shows that the cathodic (i_{pc}) and anodic (i_{pa}) peak currents increase linearly with scan rates from 0.1 to 0.8 V s⁻¹. This reveals that the electrode reaction corresponds to a surface-controlled quasi-reversible process. By using the Laviron method for a surface-controlled electrochemical system [29], the apparent heterogeneous electron transfer rate constant (k_s) of HRP immobilized on Nafion/HRP/3D-Bi₂WO₆/GCE is estimated to be about 3.4 s⁻¹, which is higher than the value reported for HRP immobilized β -cyclodextrin hybrid film (2.87 s⁻¹) [32], flowerlike ZnO-gold nanoparticle-Nafion nanocomposite (1.94 s⁻¹) [33], suggesting a faster electron-transfer process. This means that the 3D-Bi₂WO₆ MSs facilitate the direct electron transfer of HRP. The average surface concentration of electroactive HRP (Γ^* , mol cm⁻²) can be estimated from the charge integration of the reduction peak of the CV using the formula:

$$Q = nF\Gamma^* \quad (1)$$

where F is Faraday constant, Q is the charge (C) and can be obtained by integrating the reduction peak of HRP, n and A stand for the number of electron transferred and the geometrical surface area of the electrode, respectively. According to this method, the surface concentration of electroactive HRP (Γ^*) at Nafion/HRP/3D-Bi₂WO₆/GCE is calculated as $1.13 \times 10^{-10} \text{ mol cm}^{-2}$, which is much greater than the theoretical value for monolayer coverage ($1.89 \times 10^{-11} \text{ mol cm}^{-2}$) [34]. This means that multilayers of HRP entrapped in the Nafion/HRP/3D-Bi₂WO₆/GCE participates in the electron-transfer process.

Fig. 6 shows the cyclic voltammograms of 0.1 M PBS of different pH at a Nafion/HRP/3D-Bi₂WO₆/GCE. Stable and well-defined CVs are observed in the pH range 6.0~8.0. With the increasing of pH value, both the cathodic and anodic peaks shift to the negative (Fig. 6). This can be contributed to the involvement of proton transfer in the HRP(Fe^{III})/HRP(Fe^{II}) redox couple [35]. The $E^{0'}$ value of HRP varies linearly in the range of pH 6.0~8.0, with a slope of -52.5 mV pH⁻¹ (the inset of Fig. 6). This value is very close to the theoretical value of the transfer of one proton and electron in a reversible reduction (-58mV pH⁻¹ at 25°C) [36].

3.4 Electrocatalytic properties of the Nafion/HRP/3D-Bi₂WO₆/GCE.

By using H₂O₂ as a probe, the electrocatalytic properties of the Nafion/HRP/3D-Bi₂WO₆/GCE were investigated. As shown in Fig. 7, the reduction peak increases dramatically with the addition of H₂O₂, accompanied by a decrease and disappearance of the oxidation peak, displaying the obvious electrocatalytic behavior of HRP for the reduction of H₂O₂ [27]. A possible reaction mechanism of H₂O₂ catalyzed by the HRP-based electrode is postulated as follows [28]:

With the H₂O₂ concentration increasing, the current value reaches a saturation limit (the inset of Fig. 7) and saturation behavior is characteristic of enzyme-based catalysis. The apparent Michaelis-Menten constant (K_M^{app}) can be obtained by the electrochemical-version of Lineweaver-Burk equation [37]

$$\frac{1}{I_{ss}} = \frac{K_M^{app}}{I_{max}C} + \frac{1}{I_{max}} \quad (4)$$

where I_{ss} is the steady-state current after the addition of substrate, C is the bulk concentration of the substrate, and I_{max} is the maximum current measured under saturated substrate conditions. The K_M^{app} value for Nafion/HRP/3D-Bi₂WO₆/GCE is estimated to be 153 μ M. This value is smaller than those ever reported values of 262 μ M [26] and 684 μ M [30], indicating that HRP immobilized on Nafion/HRP/3D-Bi₂WO₆/GCE retains higher bioactivity.

3.5 Biosensor performance of the Nafion/HRP/3D-Bi₂WO₆/GCE

The biosensor performance for the detection of H₂O₂ of the Nafion/HRP/3D-Bi₂WO₆/GCE was also investigated by amperometry. Fig. 8 gives the amperometric responses of the modified glassy carbon electrodes at -0.35 V after adding H₂O₂ in stirred PBS (pH 7.0). Fig. 8A demonstrates that, on Nafion/HRP/3D-Bi₂WO₆/GCE, the response time (achieving 95% of the steady-state current) is less than 3 s. Fig. 8B shows two linear sections in the calibration plots. The first section is from 0.5 to 100 μ M with a correlation coefficient of 0.9983 ($N = 14$), and the other is from 100 to 250 μ M with a correlation coefficient of 0.9987 ($N = 16$). The method detection limit is estimated to be 0.18 μ M (the signal to noise ratio is 3). Performances of various H₂O₂ biosensors are listed in Table S1. It is clear that the performance of Nafion/HRP/3D-Bi₂WO₆/GCE is significantly improved. Furthermore, compared with Nafion/HRP/Bi₂WO₆NPs/GCE, the 3D-Bi₂WO₆ based biosensor has better performance (Fig. S2). There are some reasons why the Nafion/HRP/3D-Bi₂WO₆/GCE displays both a lower method detection limit and a wider linear range. Firstly, this kind of three-dimensional flower-like Bi₂WO₆ microspheres can greatly enhance the active surface area for enzymes' adsorption. More importantly, the substrate can simply be entrapped by 3D-Bi₂WO₆ MSs with larger specific surface area and the special flower-like structure, and have easy access to the enzymes immobilized on the surface of 3D-Bi₂WO₆ MSs. There is an increased chance of effective collisions between substrate and redox protein leading to improved performance of this sensor [24].

3.6 Stability and reproducibility of the biosensor

The long-term stability of the fabricated biosensors was studied by examining its current response after storage at 4°C. The biosensors can retain 93.1% of the initial response to H₂O₂ after 30-day storage, demonstrating good long-term stability (Fig. S3). The good long-term stability can be contributed to the good biocompatibility of 3D-Bi₂WO₆ MSs which can provide a favorable microenvironment for HRP to retain its bioactivity.

The reproducibility of the biosensor was investigated by determining 5 µM H₂O₂ in PBS (pH 7.0). The relative standard deviation (R.S.D) is 3.2% for 5 successive measurements. Five biosensors prepared under the same conditions independently give a R.S.D. of 3.8% for the determination of 5 µM H₂O₂ in PBS (0.1 M, pH 7.0), indicating an acceptable electrode-to-electrode reproducibility.

3.7 Analysis of real samples

In order to evaluate the analytical utility of the electrode in practice, the Nafion/HRP/3D-Bi₂WO₆/GCE is applied for determination of real contact lens care solution. Before experiments, the real samples are diluted 5000 times with doubly distilled water and without any other pretreatments. Recovery tests and KMnO₄ titration method were used to examine the reliability and accuracy of this method. The H₂O₂ content of different samples and recoveries of added analyte are evaluated, and the results confirm that determining the H₂O₂ concentration in real samples by using the proposed method is reliable (Table 1)."

Conclusion

In summary, three-dimensional flower-like Bi₂WO₆ microspheres have been synthesized through a simple hydrothermal method and subsequently are used to fabricate a mediator-free biosensor for the detection of H₂O₂. Due to unique morphology of the flower-like microspheres, the Nafion/HRP/3D-Bi₂WO₆/GCE biosensor displays a low method detection limit of 0.18 µM and a wide linear range from 0.5 to 250 µM for H₂O₂.

Acknowledgments

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Figure Captions:

Scheme 1 Schematic illustration of the 3D-Bi₂WO₆ MSs immobilizing HRP.

Fig. 1 XRD pattern of the 3D-Bi₂WO₆ MSs.

Fig. 2. SEM images of typical as-synthesized 3D-Bi₂WO₆ MSs (A and B) and Nafion/HRP/3D-Bi₂WO₆ composite film (C and D).

Fig. 3 UV-vis spectra of Nafion/HRP/3D-Bi₂WO₆ composite film (a) and HRP (b).

Fig. 4 Cyclic voltammograms of Nafion/3D-Bi₂WO₆/GCE (a), Nafion/HRP/GCE (b), Nafion/HRP/Bi₂WO₆NPs/GCE (c), Nafion/HRP/3D-Bi₂WO₆/GCE (d), in 0.1 M pH 7.0 PBS with a scan rate of 0.1 V s⁻¹.

Fig. 5 CVs of Nafion/HRP/3D-Bi₂WO₆/GCE in 0.1 M pH 7.0 PBS with increasing scan rates from 0.1 to 0.8 Vs⁻¹ (A), plot of cathodic and anodic peak current vs. scan rate (B).

Fig. 6 Cyclic voltammograms of Nafion/HRP/3D-Bi₂WO₆/GCE in 0.1 M PBS with different pH values from 6 to 8. Inset: plot of cathodic and anodic peak current vs. pH values.

Fig. 7 CVs of the Nafion/HRP/3D-Bi₂WO₆/GCE in 0.1 M pH 7.0 PBS containing 0 (a), 10 (b), 30 (c), 50 (d), 70 (e), 110 (f), 150 (g), 190 (h), 230 (i) μM H₂O₂ at a scan rate of $v = 0.1$ V s⁻¹. Inset: plot of the relationship between catalytic reduction current and the concentration of H₂O₂.

Fig. 8 Amperometric response curves of the Nafion/HRP/3D-Bi₂WO₆/GCE to H₂O₂. Applied potential: -0.35 V (A). Plot of steady-state response currents against concentrations of H₂O₂ (B).

Table 1 Determination results of H₂O₂ in real samples.

Scheme 1

Scheme 1 Schematic illustration of the 3D-Bi₂WO₆ MSs immobilizing HRP.

Figure 1

Fig. 1 XRD pattern of the 3D-Bi₂WO₆ MSs.

Figure 2

Fig 2. SEM images of typical as-synthesized 3D-Bi₂WO₆ MSs (A and B) and Nafion/HRP/3D-Bi₂WO₆ composite film (C and D).

Figure 3

Fig. 3 UV-vis spectra of Nafion/HRP/3D-Bi₂WO₆ composite film (a) and HRP (b).

Figure 4

Fig. 4 Cyclic voltammograms of Nafion/3D-Bi₂WO₆/GCE (a), Nafion/HRP/GCE (b), Nafion/HRP/Bi₂WO₆NPs/GCE (c), Nafion/HRP/3D-Bi₂WO₆/GCE (d), in 0.1 M pH 7.0 PBS with a scan rate of 0.1 V s⁻¹.

Figure 5

Fig. 5 Cyclic voltammograms of Nafion/HRP/3D-Bi₂WO₆/GCE in 0.1 M pH 7.0 PBS with increasing scan rates from 0.1 to 0.8 Vs⁻¹ (A), plot of cathodic and anodic peak current vs. scan rate (B).

Figure 6

Fig. 6 Cyclic voltammograms of Nafion/HRP/3D-Bi₂WO₆/GCE in 0.1 M PBS with different pH values from 6 to 8. Inset: plot of cathodic and anodic peak current vs. pH values.

Figure 7

Fig. 7 Cyclic voltammograms of the Nafion/HRP/3D-Bi₂WO₆/GCE in 0.1 M pH 7.0 PBS containing 0 (a), 10 (b), 30 (c), 50 (d), 70 (e), 110 (f), 150 (g), 190 (h), 230 (i) μM H₂O₂ at a scan rate of $v = 0.1 \text{ V s}^{-1}$. Inset: plot of the relationship between catalytic reduction current and the concentration of H₂O₂.

Figure 8

Fig. 8 Amperometric response curves of the Nafion/HRP/3D- Bi_2WO_6 /GCE to H_2O_2 . Applied potential: -0.35 V (A). Plot of steady-state response currents against concentrations of H_2O_2 (B).

Table 1 Determination results of H₂O₂ in real samples

Samples	KMnO ₄ titration (mM)	This work (mM)	Added (mM) ^a	Found (mM) ^b	Recovery (%)
1	0.17	0.17	0.10	0.26	96.3
2	0.13	0.14	0.10	0.25	104.1
3	0.14	0.15	0.10	0.24	96.0

^a Standard H₂O₂ solution added to the samples.

^b The total concentrations of H₂O₂ after addition of standard H₂O₂ solution to the samples.

Graphical abstract