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MWCNT–cysteamine–Nafion modified gold electrode based on myoglobin for determination of hydrogen peroxide and nitrite

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ABSTRACT

In this work, a novel amperometric biosensor of hydrogen peroxide (H_2O_2) was developed based on the immobilization of myoglobin (Mb) on the surface of the multi-walled carbon nanotube (MWCNT) –Nafion–cysteamine (CA) modified gold electrode (Au) and its electrocatalytic activity was used for the determination of nitrite (NO_2^-). In the optimization studies, the best MWCNT and myoglobin amount were investigated. It was discovered at the experiments for the optimization of the working conditions that the buffer at this study as 50.0 mM, pH 7.0 phosphate buffer (PBS) and working temperature as 30 °C for the H_2O_2 biosensor. It was determined at the characterization studies on the biosensor that linear results are obtained between the ranges of 0.1 μM to 70.0 μM for H_2O_2 concentration and 1–250 μM for NO_2^-. The reproducibility of the biosensor was determined both H_2O_2 and nitrite. From the experiments, average value, standard deviation (SD) and coefficients of variation (CV%) were calculated to be 10.02 ± 0.43 μM, and 4.29% for 10.0 μM H_2O_2 ($n = 6$) and 52.0 ± 2.1 μM, and 3.89% for 50.0 μM nitrite ($n = 8$), respectively. At the same time the sample was analyzed for NO_2^- in drinking and mineral waters.

1. Introduction

The accurate determination of hydrogen peroxide (H_2O_2) is of great importance because it is an essential mediator in food, pharmaceutical, clinical, industrial and environmental analyses [1]. Nitrite (NO_2^-) is an important precursor in the formation of N-nitrosamines, many of which have been shown as potent carcinogens in human bodies [2,3]. It also exists widely in the environment, beverages, and food products as a preservative [4]. Various techniques have been developed to determine H_2O_2 and nitrite, such as spectrophotometry [5,6], chromatography [7,8] capillary electrophoresis [9,10], chemiluminescence [11,12] and electrochemistry [13–15]. Especially, the potential low cost and portability of electroanalytical devices provide a number of attractive options. The sensors based on the electrochemical methods are favorable with high sensitivity, relatively good selectivity and fast response. Myoglobin (Mb) is known to have some intrinsic peroxidase activity due to its close similarity to peroxidase. Therefore, it might be possible to employ Mb containing a heme group that serves as the active center to catalyze the reduction of H_2O_2. However, electron transfer between Mb and bare solid electrodes is usually slow and the protein is irreversibly denatured [16]. Thus, it is necessary to search for a way to develop a Mb-modified electrode with well-behaved electrochemistry and good stability.

Carbon nanotubes (CNTs) [17] have attracted much attention due to their high chemical stability, high surface area, unique electronic properties, and relatively high mechanical properties. As electrode materials, CNTs can be used for promoting electron-transfer between the electroactive species and electrode and provide a novel method for fabricating chemical sensor or biosensor [18–21]. The ability of CNTs-based electrode to electrocatalytic activity and to minimize surface fouling has been reported [22]. Nafion has good electrical conductivity, good biocompatibility, excellent film forming and adhesion ability, high chemical stability and ability to resist interferences from anions and biological macromolecules, which make it a good matrix for biomolecule immobilization [23].

The aim of this study is to develop a cysteamine (CA)–myoglobin (Mb)–MWCNT–Nafion-nanobiocomposite film for the bioanalytical application. Self assembled monolayers are formed on gold electrode surface with using CA. By the combination of MWCNT, Nafion, and myoglobin an electroanalytical nanobiocomposite film was produced by simple solvent casting processes. MWCNTs act as efficient conduits for electrons transfer; Nafion is an electrochemistry promoting polymeric binder as backbone, and Mb is a biological catalyst that facilitates the translation of substrate into product by lowering the activation energy. The potential application of the Au/CA/MWCNT–Nafion–Mb as an electrochemical biosensor to detect H_2O_2 was explored. In addition, the catalytic ability of the modified electrode to NO_2^- was also studied, and a simple as well as novel NO_2^- biosensor with high selectivity based on Au/CA/MWCNT–Nafion–Mb was fabricated.

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2. Experimental

2.1. Apparatus

In the experiments PalmSens potentiostat (Netherlands), a three-electrodes system from CH Instruments (USA) that contains a CHI 101 model Au working electrode (2 mm diameter), a CHI 111 model Ag/AgCl reference electrode and a CHI 115 model platinum wire counter electrode, Isolab P100 and P1000 automatic pipettes (Germany), Yellow-Line magnetic stirrer (Germany) and Nuve model thermostat (TR) were used.

2.2. Chemicals and reagents

Horse heart myoglobin (Mb No. M1882), potassium ferricyanide, hydrogen peroxide (30%, w/v, solution), sodium nitrite, cysteamine, and Nafion (5% in a mixture of lower aliphatic alcohols and water), not any pretreatment multiwalled carbon nanotube (>99% purity and diameter in the range of 7.0–15.0 nm, 0.5–10 μM length, product number: 412988) chemicals were purchased from Sigma Chemical. All the other chemicals were obtained from Riedel-de-Haen. All solutions were prepared with twice-distilled water.

2.3. Electrode fabrication

Prior to coating, Au electrode (Au) surface was polished with alumina slurries on microfiber cloth to obtain a mirror surface. After that, it was thoroughly rinsed with double distilled water and sonicated first in absolute ethanol and then in double distilled water for 10 min to remove undesired adsorbed particles. In the next step, the electrode was cleaned by five successive cyclic voltammetric sweeps between −1.0 and +1.0 V in the 0.1 M HNO_3.

The Au/CA modified electrode was formed by immersing the cleaned electrode into 50 mM CA aqueous solution at room temperature in darkness for 4 h. After that, for removing the physically adsorbed CA, the electrode was thoroughly rinsed with water and dried with nitrogen gas stream. Secondly, the Au/CA modified electrode was immersed into 10 mg/mL myoglobin solution which is containing 50 mM PBS (pH:7) to form Au/CA/Mb. At the same time, a 1.0 wt.% Nafion solution was prepared by diluting 5 wt.% Nafion solution with ethanol. A 5 mg MWCNTs were added to 1 ml 1.0 wt.% Nafion solution with the aid of ultrasonic agitation for 1 h to form a homogeneous MWCNT–Nafion solution [24]. Finally 6 μL of this solution dropped onto Au/CA/Mb to form a stable film and dried under room temperature in the air. The stepwise formation of the biosensor is displayed in Scheme 1.

2.4. Principle of measurements

Measurements were carried out in a phosphate buffer (50.0 mM, pH 7.0) for amperometric measurements. Amperometric detection was made under potential of −0.3 V for H_2O_2 and 0.6 V for NO_2^-.

3. Results and discussion

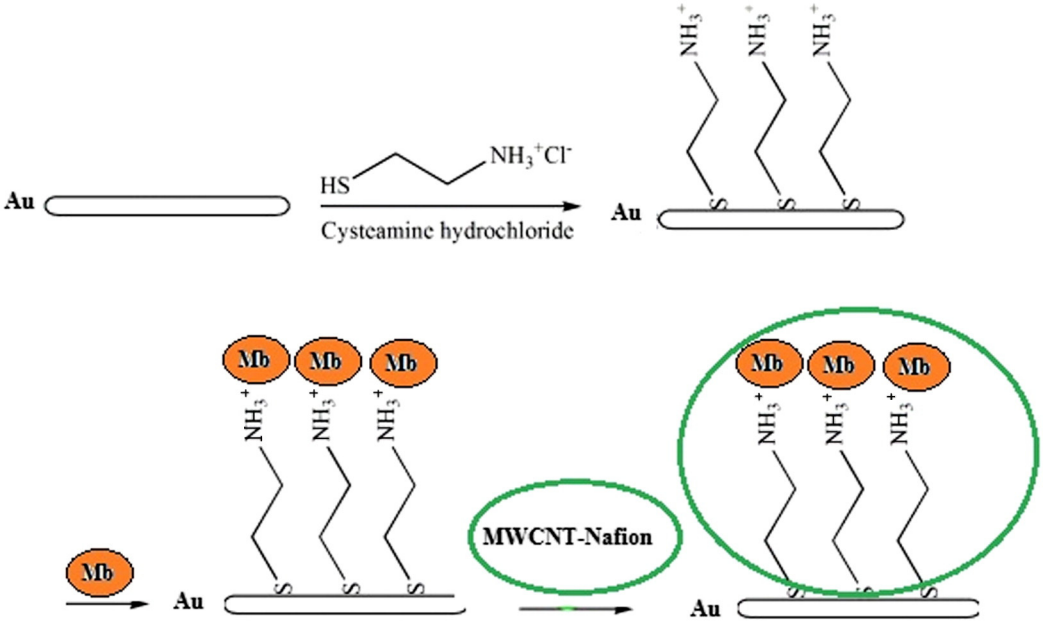
3.1. Immobilization

Cyclic voltammograms were carried out at a potential range between −0.6 and 0.6 V in a phosphate buffer (50.0 mM, pH 7.0) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ by using bare electrode, cysteamine–Mb modified electrode (Au/CA/Mb) and Au/CA/Mb–MWCNT–Nafion for establishing the formation of SAM, immobilization of enzymes and MWCNT–Nafion composite film. A pair of dominant redox peaks was observed on bare electrode (Fig. 1, curve a). When cysteamine–myoglobin monolayer formed on the electrode a decrease was observed at cathodic (at 0.1 V) and anodic (at 0.2 V) peak current (Fig. 1, curve b). After the formation of MWCNT–Nafion certain cathodic and anodic peak currents were observed which are attributed to the good conductive properties of the Au–CA–MWCNT–Nafion–Mb modified surface (Fig. 1, curve c). Cyclic voltammograms showed that immobilization of MWCNT–Nafion and enzymes brought about prominent oxidation and reduction peaks which facilitated the monitoring of enzyme activity.

3.2. Electrocatalytic reduction of H_2O_2 at Au/CA/Mb–MWCNT–Nafion

Electrocatalytic reaction of hydrogen peroxide at Au/CA/Mb–MWCNT–Nafion was examined by CVs. Fig. 2 shows the cyclic voltammograms of the Au/CA/Mb–MWCNT–Nafion in a 50.0 mM PBS (pH 7.0) in the absence (curve a) and presence (curves b and c) of H_2O_2 at a scan rate of 50 mV s^{-1}. When H_2O_2 was added to a pH 7.0 PBS, an increase in reduction peak was seen at about −0.30 V.

The mechanism of catalytic reduction of hydrogen peroxide on the Au/CA/Mb–MWCNT–Nafion is most probably similar to that of the



Scheme 1. Showing the stepwise preparation of Au/CA/Mb–MWCNT–Nafion biosensor.

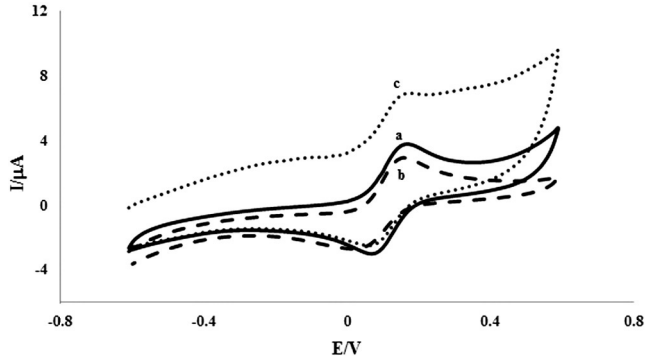


Fig. 1. Cyclic voltammograms of the biosensor at different stages obtained from the experiments. (a) Bare Au electrode; b) Au/CA/Mb; (c) Au/CA/Mb–MWCNT–Nafion in phosphate buffer; pH 7.0, 50.0 mM containing 5.0 mM potassium ferricyanide; *T*: 30 °C. Scan rate is 50 mV s^{-1}; Mb: 10 mg ml^{-1}; MWCNT: 5 mg ml^{-1}.

horseradish peroxidase (HRP) film system [25], since Mb and HRP are all heme proteins and have similar electrochemical properties. The electrocatalytic process can be expressed as follows [26].

$$\text{Mb heme Fe(III)} + H^+ + e^- \rightleftharpoons \text{Mb heme Fe(II)}$$

$$H_2O_2 + 2H^+ + 2\ \text{Mb heme Fe(II)} \rightarrow 2\ \text{Mb heme Fe(III)} + 2H_2O$$

$$\text{Mb heme Fe(III)} + e^- + H^+ \rightarrow \text{Mb heme Fe(II)}$$

In the presence of H_2O_2, Mb heme Fe(II) was efficiently converted to its oxidized form, MbFe(III). Consequently, MbFe(III) was reduced at the electrode surface by the direct electron transfer via MWCNT.

Amperometric response of the Au/CA/Mb–MWCNT–Nafion modified electrode to H_2O_2 was investigated at different applied potentials according to Fig. 2 (curves b and c) between −0.1 and −0.6 V. The most suitable and highest responses were obtained at −0.3 V.

3.3. Optimization of bioactive layer

3.3.1. Comparison of the modified electrodes

Amperometric responses of the different modified electrodes to the addition of H_2O_2 in 50.0 mM PBS (pH 7.0) were recorded and shown in Fig. 3. For every 20 s, aliquots of H_2O_2 were successively injected into the supporting electrolyte. All the biosensors except Au/CA/MWCNT–Nafion responded when H_2O_2 was added to the stirring PBS and steady-state current could be obtained. However, no similar amperometric response corresponding to the reduction of H_2O_2 can be observed at an electrode modified with CA/MWCNT–Nafion under the same conditions. This results show that biosensor responses are due to catalytic

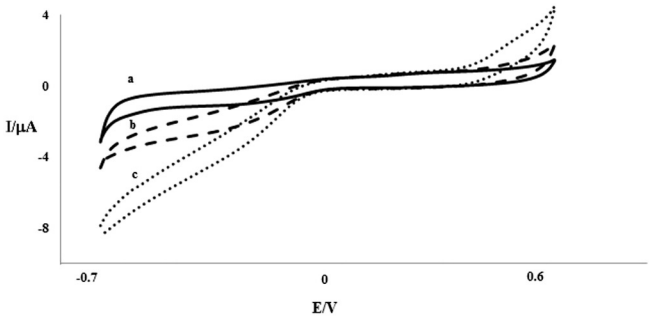


Fig. 2. Cyclic voltammograms of (a) in the absence; (b and c) in the presence of 25.0 and 50.0 μM H_2O_2 in 50.0 mM phosphate buffer solution (pH 7.0) at the Au/CA/Mb–MWCNT–Nafion. Scan rate: 50 mV s^{-1}; Mb: 10 mg ml^{-1}; MWCNT: 5 mg ml^{-1}.

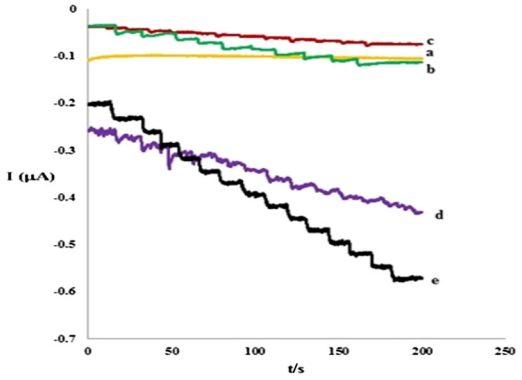


Fig. 3. Amperometric responses of different modified electrodes upon successive addition of 1.5 mM H_2O_2 into pH 7.0 50.0 mM 15 mL PBS solution. (a) Au/CA/MWCNT–Nafion; (b) Au/CA/Mb–Nafion; (c) Au/CA/Mb; (d) Au/Mb–MWCNT–Nafion; (e) Au/CA/Mb–MWCNT–Nafion. Applied potential: −0.3 V (vs. Ag/AgCl); Mb: 10 mg ml^{-1}; MWCNT: 5 mg ml^{-1}, Nafion: 1%.

ability of Mb. The amperometric responses of Au/CA/Mb–MWCNT–Nafion electrode (curve e) is much higher than other Mb modified electrodes preparing without MWCNT (curves b, c) which can be contributed to the promoted electron transfer. When comparing to the two electrodes prepared with MWCNT, Au/CA/Mb–MWCNT–Nafion (curve e) showed higher and more stable amperometric responses than Au/Mb–MWCNT–Nafion (curve d). This result shows that using cysteamine offer binding more Mb which was adhered better and more regular. Moreover, the CA/Mb–MWCNT–Nafion structure also played an important role in the good performance of the biosensor, owing to high specific surface area for enzyme immobilization.

3.3.2. Detection of the effect of the amount of Mb on biosensor responses

Depending on the configuration used for the fabrication of the MWCNT–Nafion–Mb nanobiocomposite-based biosensor, the myoglobin loading within the MWCNT–Nafion nanobiocomposite film was selected for investigation. Different concentrations of myoglobin (5.0, 10.0, 15.0 mg mL^{-1}) within Au/CA/Mb–MWCNT–Nafion nanobiocomposite modified electrode were studied. The response currents improved by raising the concentration of myoglobin from 5.0 to 10.0 mg mL^{-1}. Higher enzyme contents, 15.0 mg mL^{-1}, gave a biosensor with a substantially reduced response. By increasing the myoglobin concentration in the MWCNT–Nafion–Mb nanobiocomposite, the myoglobin surrounding the MWCNTs becomes denser and could influence the conductor phase within the matrix. This makes the lower response

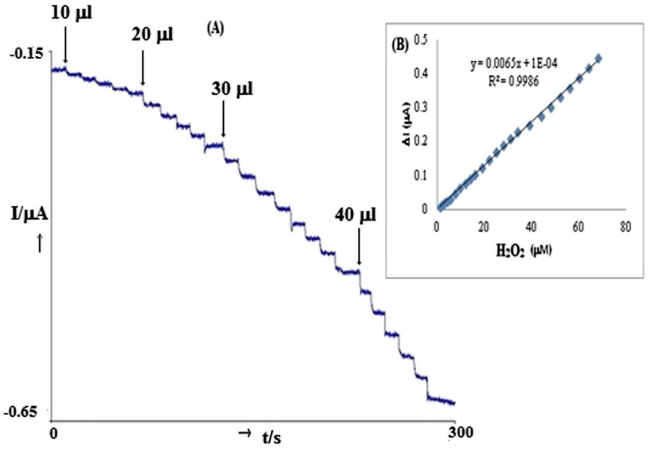


Fig. 4. Amperometric current–time response curves of Au/CA/MWCNT–Nafion–Mb upon successive addition of 1.5 mM H_2O_2 into pH 7.0 50.0 mM 15 ml PBS solution. Applied potential: −0.3 V (vs. Ag/AgCl); Mb: 10 mg mL^{-1}; MWCNT: 5 mg mL^{-1}. Inset: calibration curve of steady-state currents vs. H_2O_2 concentration at Au/CA/MWCNT–Nafion–Mb.

Table 1
Comparison of H_2O_2 and NO_2^- biosensors.

Electrode	Substrate	Linear range	Response time(s)	Detection limit	Method	Working potential	Mediator	Reference
GCE/FBCS	H_2O_2	35–2000 μM	20	15 μM	Amperometric	+0.15 V vs Ag/AgCl	Ferrocene	[28]
GCE/P(GMA-co-VFc)	H_2O_2	2–30 mM	4	2.6 μM	Amperometric	+0.35 V vs Ag/AgCl	Ferrocene	[29]
Au/SA–HRP	H_2O_2	7–4100 μM	–	1.8 μM	Amperometric	−0.3 V vs SCE	No mediator	[30]
GCE/DDAB–HRP	H_2O_2	1–4 mM	5	–	Amperometric	−0.2 V vs SCE	No mediator	[31]
CCE/nanoAu–HRP	H_2O_2	12–1100 μM	<8	6.1 μM	Amperometric	−0.17 V vs SCE	Hydroquinone	[32]
Au/CA/Mb/MWCNT–Nafion	H_2O_2	0.1–70 μM	<2	0.01 μM	Amperometric	−0.3 V vs Ag/AgCl	No mediator	This work
Cytc/1-Cys/P3MT/MWCNT/GCE	NO_2^-	10–100 μM	<5	0.5 μM	Amperometric	+0.9 V vs Ag/AgCl	No mediator	[33]
Hb–ZnO–Nafion/GC	NO_2^-	10–2700 μM	<5	4.0 μM	Amperometric	−0.675 V vs Ag/AgCl	No mediator	[34]
Nafion-$BMIMPF_6$/Mb/ClLE	NO_2^-	10–8400 μM	<5	5.0 μM	Cyclic Voltammetry	−0.2 & −0.8 V vs SCE	No mediator	[35]
Mb/LaF_3-DP-CeO_2/IL-CPE	NO_2^-	5–4650 μM	<5	2.0 μM	Amperometric	0.8 V vs Ag/AgCl	No mediator	[27]
Au/CA/Mb/MWCNT–Nafion	NO_2^-	1–280 μM	<2	0.1 μM	Amperometric	0.7 V vs Ag/AgCl	No mediator	This work

GCE, glassy carbon electrode; P(GMA-co-VFc) poly(glycidyl methacrylate-co-vinylferrocene); FBCS, ferrocene branched chitosan; PEGDGE: polyethylene glycol diglycidylether; SA, sodium alginate; CCE, carbon ceramic electrode; HRP, horseradish peroxidase; DDAB, didodecyldimethyl ammonium bromide; and SCE, saturated calomel electrode.

signals at higher myoglobin content. In the case of 10 mg mL^{-1} myoglobin, the biosensor exhibits a detection limit of 0.01 μM, and a response time of less than 2 s.

3.3.3. Investigation of MWCNT concentration on biosensor responses

Different MWCNT concentrations were prepared and used, for the determination of the effect of MWCNT concentration on the biosensor response. For this purpose three biosensors that contained 2.5, 5.0 and 10.0 mg mL^{-1} MWCNT were constructed.

In the case of using 10.0 mg mL^{-1} MWCNT the biosensor responses were similar with a biosensor that contained 5.0 mg mL^{-1} MWCNT. Although similar biosensor responses were observed, biosensor preparing with 5.0 mg mL^{-1} has better linearity and sensitivity. Also using less MWCNT is more economical for biosensor preparation. The lowest biosensor responses were obtained by using 2.50 mg mL^{-1} MWCNT. Thus, due to not only better biosensor responses but also accuracy and low cost 5.0 mg mL^{-1} MWCNT was chosen to be the most suitable amount in the construction of the biosensor.

3.4. Optimization of working conditions

3.4.1. Working temperature and pH effect on the biosensor responses

Experiments were carried out at 15, 20, 25, 30 and 35 °C for the determination of temperature effect on biosensor responses (see Supporting information Fig. 1).

According to the results, optimum temperature was found to be 30 °C. As a result of the increase in temperature, some defects and deformations occurred on modified electrode surface as well as on the activities of the Mb, thus 30 °C was chosen to be the working temperature for the biosensor.

In order to investigate the pH effect on biosensor responses different buffer solutions were prepared. For this purpose 50.0 mM phosphate buffers at different pH values (pH 6.0, 6.5, 7.0, 7.5 and 8.0) were prepared and used (see Supporting information Fig. 2).

In our case, the optimum pH for enzymatic biosensor was shifted towards the acidic side compared to that of free enzymes. The tertiary structure of an enzyme is produced by intramolecular interactions that include hydrogen bonding and electrostatic interactions. Among these interactions, electrostatic interactions are sensitive to the pH of the surrounding environment where pH changes can make variations in the pattern of charges on the enzyme. These differences in enzyme pattern can result with a change in the tertiary structure of the enzyme which can affect the active site in such a way that an increase or decrease in the biocatalytic activity is obtained. Moreover, immobilization can change the enzyme's micro-environment and this can cause a shift in optimum pH of the enzyme. According to the data, it could also be stated that CNT–Nafion modification did not affect the pH optima of these systems [27]. As a result, further studies were conducted at pH 7.0.

3.5. Analytical characteristics of biosensor

3.5.1. Linear range for hydrogen peroxide

In this study, we have used the amperometry technique to evaluate the performance of the developed Au/CA/Mb-MWCNT–Nafion biosensor. During the amperometric current–time measurements, the electrode potential was held at −0.3 V (from the CV electrocatalysis) and the N_2-saturated PBS (pH 7.0) was constantly stirred at 1600 rpm. This low applied potential is beneficial for efficient H_2O_2 detection because the matrix effect caused by the common interference species can be minimized and the oxygen reduction current can be limited. For every 20 s, aliquots of H_2O_2 were successively injected into the supporting electrolyte. Fig. 4A shows the amperometric current–time response obtained at Au/CA/Mb–MWCNT–Nafion on various H_2O_2 concentration additions. The response time of the Au/CA/Mb–MWCNT–

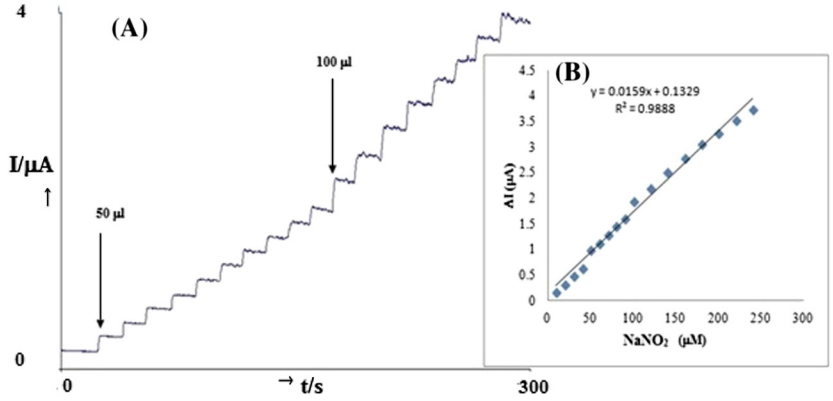


Fig. 5. Amperometric current–time response curves of Au/CA/MWCNT–Nafion–Mb upon successive addition of 1.5 mM $NaNO_2$ into pH 7.0 50.0 mM 15 mL PBS solution. Applied potential: 0.6 V (vs. Ag/AgCl); Mb: 10 mg mL^{-1}; MWCNT: 5 mg mL^{-1}. Inset: calibration curve of steady-state currents vs. $NaNO_2$ concentration at Au/CA/MWCNT–Nafion–Mb.

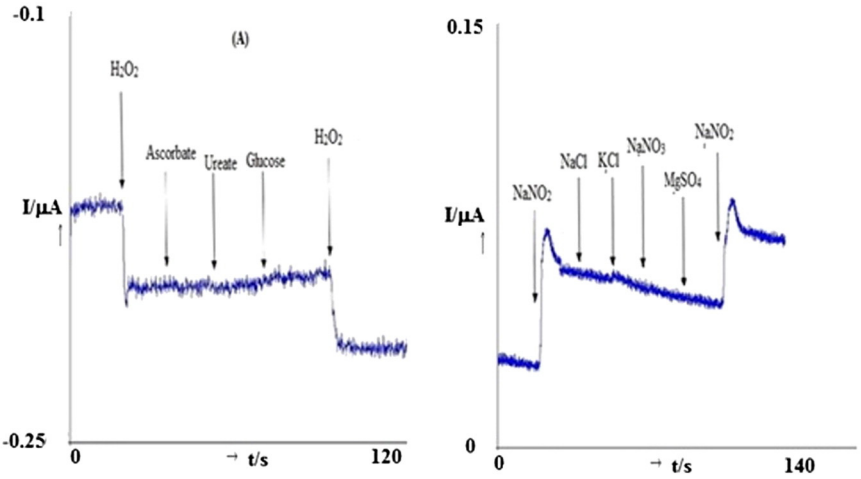


Fig. 6. Interference ability of Au/CA/MWCNT–Nafion-Mb. (A) for H_2O_2; (B) for $NaNO_2$. Concentration of all substances is 10 μM for (A); concentration of all substances is 10 μM for (B). Applied potential: −0.3 V for (A); 0.6 V for (B) (vs. Ag/AgCl); Mb: 10 mg ml^{-1}; MWCNT: 5 mg ml^{-1}; PBS pH:7 (50 mM).

Nafion composite film toward H_2O_2 was less than 2 s, validating the rapid catalytic reduction process occurring at the composite film surface, which was faster than that of other hydrogen peroxide biosensors (Table 1), and its overall performance was quite comparable. The response current increased linearly between 0.1 and 70.0 μM H_2O_2 (Fig. 4B, lower inset). The limit of detection, determined as 3 times the standard deviation of the single for buffer (blank) was 0.01 μM.

3.5.2. Electrocatalytic determination of nitrite at the Au/CA/Mb–MWCNT–Nafion

Fig. 5A displayed the amperometric responses of the Au/CA/Mb–MWCNT–Nafion upon successive addition of $NaNO_2$ to 50.0 mM pH 7.0 PBS at an applied potential of 0.6 V under stirring. The biosensor exhibited a very rapid and sensitive response to the changes of NO_2^- concentration, the steady-state current being reached in 2 s. Under these conditions, a calibration graph was constructed for nitrite (inset in Fig. 5), with a linear range between 1.0 μM and 250.0 μM ($R = 0.9991$). The limit of detection, determined as 3 times the standard deviation of the single for buffer (blank), was 0.1 μM of nitrite. Compared with other NO_2^- biosensors listed in Table 1, the biosensor based on Au/CA/Mb–MWCNT–Nafion had wider linear range, lower detection limit. The prominent electrocatalytic ability of Au/CA/Mb–MWCNT–Nafion might be attributed that the Au/CA/MWCNT–Nafion composite provided more sites for protein binding and also has electrocatalytic ability and a short diffusion distance for NO_2^-.

3.5.3. Reproducibility

The reproducibility of the biosensor was determined for 10.0 μM H_2O_2 ($n = 6$). From the experiments, average value, standard deviation (SD) and coefficients of variation (CV%) were calculated to be 10.02 ± 0.43 μM, and 4.29%, respectively.

The reproducibility of the biosensor was also determined for 50.0 μM nitrite ($n = 8$). Average value, standard deviation (SD) and coefficients of variation (CV%) were calculated to be 52.0 ± 2.1 μM, and 3.89%, respectively.

Table 2
Results for NO_2^- determination in real samples using the biosensor and the reference method.

Sample	Biosensor (n = 5)	Reference method [36]	Reference values
Spring water	Not detected	Not detected	0
Tap water	2.55 ± 0.11 μM	2.50 ± 0.10 μM	<2.041 μM
Mineral water	5.43 ± 0.23 μM	5.50 ± 0.24 μM	0
Mineral water 2. trade	Not detected	Not detected	0

3.5.4. Interference effects of some compounds on biosensor responses

Fig. 6A shows the amperometric current–time responses obtained at the Au/CA/Mb–MWCNT–Nafion modified electrode for each successive addition of 10.0 μM ascorbic acid, glucose and uric acid at regular intervals (20 s once) into N_2-saturated PBS (pH 7.0). It is evident that each addition of the electroactive interfering species brought out a hardly discernible current response, whereas a notable response was observed for 10.0 μM H_2O_2. These results revealed that the prepared H_2O_2 biosensor exhibits high selectivity toward H_2O_2, and it has the ability to reduce the interference from the electroactive species, which is more helpful for practical applications.

Possible interference for the detection of nitrite on Au/CA/Mb–MWCNT–Nafion modified electrode was investigated by the addition of various species into 50.0 mM pH 7.0 PBS containing 10.0 μM NO_2^-. The interference study showed that 10 μM of NaCl, KCl, $NaNO_3$, and $MgSO_4$ did not interfere with the detection of 10.0 μM of NO_2^- (Fig. 6B). These results should make the biosensor an ideal analytical tool for sensitive detection of NO_2^- in the environment.

3.6. Stability and reproducibility of the biosensor

To investigate the storage stability of the Au/CA/Mb–MWCNT–Nafion biosensor to H_2O_2, the modified electrode was stored in N_2-saturated PBS (pH 7.0) at 4 °C, and its amperometric response was monitored periodically in fresh supporting electrolyte. The modified electrode retained approximately 93% and 85% of its initial sensitivity after 30 and 60 days, respectively, indicating the excellent storage stability of the composite film. The good biocompatibility of the MWCNT–Nafion composite and the strongly bounded Mb via CA are plausible reasons for the high stability.

3.7. Real sample analysis

The developed biosensor was used for NO_2^- detection in tap water, spring water and mineral water samples. The 0.3 mL real sample was mixed with 15 mL 50.0 mM pH 7.0 PBS for the determination NO_2^- of with the proposed procedure [36]. Recovery studies were completed on samples by adding NO_2^- standard solution. The obtained results were displayed in Table 2.

4. Conclusions

In this paper, we developed a novel amperometric biosensor for hydrogen peroxide and nitrite by Au/CA/Mb–MWCNT–Nafion biosensor. The biosensor has great capability in catalyzing the reduction of

hydrogen peroxide and can be used as an amperometric sensor for the determination of hydrogen peroxide. The developed biosensor exhibits a fast response and satisfactory determination to H_2O_2. In comparison with other methods used to fabricate biosensors by heme proteins, besides the excellent electrocatalytic function, another notable advantage of the present biosensor is that the fabrication method is quite simple and convenient. The reason is that MWCNT can work as electron-conducting pathways between the prosthetic groups of the Mb and the electrode surface and therefore can facilitate the electron transfer process. In addition, the catalytic ability of the modified electrode to NO_2^- was also studied, and a simple as well as novel NO_2^- biosensor with high selectivity based on Au/CA/Mb–MWCNT–Nafion was fabricated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bioelechem.2014.09.001.

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