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Nanobiocomposite Electrochemical Biosensor Utilizing Synergic Action of Neutral Red Functionalized Carbon Nanotubes

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Abstract: An amperometric hydrogen peroxide biosensor using a nanobiocomposite based on neutral red modified carbon nanotubes and co-immobilized glucose oxidase and horseradish peroxidase is reported. Modification of the nanobiocomposite electrode with neutral red resulted in a sensitive, low-cost and reliable H_2O_2 sensor. The use of carbon nanotubes, as the conductive part of the composite, facilitated fast electron transfer rates. The biosensor was characterized for the influence of pH, potential and temperature. A remarkable feature of the biosensor is the detection of H_2O_2 at low applied potentials where the noise level and interferences are minimal. The sensor has a fast steady-state measuring time of 10 s with a quick response (2 s). The biosensor showed a linear range from 15 nM to 45 mM of H_2O_2 and a detection limit of 5 nM. Nafion, which is used as a binder, makes the determination free from other electroactive substances. The repeatability, reproducibility, stability and analytical performance of the sensor are very good.

Keywords: Multiwalled carbon nanotubes; Neutral red; Glucose oxidase; Horseradish peroxidase; Nafion; Nanobiocomposite biosensor

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Introduction

Nanotechnology offers the potential to increase biosensor sensitivity, response speed and selectivity. A wide variety of nanomaterials have been explored for their application in biosensors due to their unique chemical, physical, and optoelectronic properties [1,2]. For example, incorporation of carbon nanotubes (CNT) and fullerenes has greatly increased biosensor sensitivity and response speed due to their high chemical stability, high surface area, and unique electronic properties [3].

The irruption of carbon nanotubes has constituted a significant milestone in modern analytical sciences. Their unique properties [4] have led their applications in many fields such as electronics, medicine, aerospace industry, etc., which has also prompted the need of analytical methodologies to characterize and control the quality of these nanomaterials. Electrode modification with CNTs gives electrocatalytic activity towards the electro-oxidation of molecules such as NADH or H_2O_2 [5]. This property led to the use of these nanomaterials for the preparation of dehydrogenase or oxidase based electrochemical biosensors [6-10]. Further, an increased electrode active surface area, which gives rise to enhanced electrochemical response, and a demonstrated

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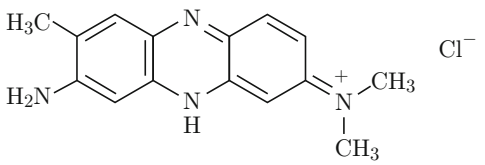
anti-fouling capability of electrode surface upon modification with CNTs, are other important advantages that have promoted a large number of significant applications in electroanalytical chemistry, including electrochemical sensors [11-13].

Direct electron transfer between the electrode and the redox enzyme is very important for fundamental studies and construction of biosensors [14-16]. However, the direct electron transfer between the enzyme and unmodified electrode is usually slow or prohibited due to shielding of the redox active sites by the protein shells [17, 18]. Therefore, several studies have been made to enhance the rate of electron transfer. Mediators are widely used to access the redox center of an enzyme and act as the charge carriers. Mediators also minimize the effects of interferences by lowering the operating potential of the electrodes, and improve the linear response range and sensitivity of the sensor [19].

Redox dyes of the azine, phenoxazine and phenothiazine types appear to show great promise for the construction of mediated amperometric biosensors due to their excellent stability, low cost and special electrocatalytic processes. Among the water soluble dyes, neutral red (NR) is an azine dye, which is found to be a convenient redox mediator for electrochemical investigations of biological system. NR which is similar to other planar dyes in the chemical structure belonging to the acridine, thazine and xanthene groups due to the heteroatom, which is nitrogen instead of divalent oxygen or sulfur [20]. It has a much lower redox potential than analogous phenothiazine and phenoxazine dyes. Due to significant mechanical strength, excellent electrical conductivity and good chemical stability of carbon nanotubes are promising platforms for immobilization of these electron transfer mediators [21,22].



Chemical structure of Neutral red

The determination of hydrogen peroxide is of practical importance in chemical, biological, clinical, and many other fields. Extensive techniques have been developed for this purpose, amongst which enzyme electrodes have been reported as the sensor for hydrogen peroxide [23, 24]. Among these, the amperometric sensors based on electron transfer between an enzyme and the electrode [25] are promising in fabricating sensitive and linearly responding devices. Though a direct electron transfer is possible between an electrode and a peroxidase catalyzing the reduction of hydrogen peroxide, this is generally a slow process on common electrode materials. An appropriate electron donor can mediate the electron transfer between peroxidase and an electrode [26] and hence such a mediator is expected to improve the performance of a peroxidase-based hydrogen peroxide sensor.

Nafion encapsulation of enzyme is a common practice to prepare biosensors. Nafion is a sulfonated tetrafluorethylene copolymer that has been widely used as a proton conductor for proton exchange membrane, in fuel cells [27, 28] and biosensor applications [29]. The main advantages of Nafion in biosensor applications are its biocompatibility, excellent thermal and mechanical stability and antifouling properties. Pioneering work by Wang, et al. showed that Nafion was an effective solubilizing agent for carbon nanotubes that yielded CNT-based biosensors exhibiting both the efficient electrocatalytic action of CNT toward hydrogen peroxide and the antifouling/discriminative properties of Nafion films.

The aim of the present work is the development and characterization of improved electrochemical biosensors for hydrogen peroxide by using a bienzymatic strategy with neutral red functionalized carbon nanotubes for bioanalytical application. The sensor employs NR functionalized multiwalled carbon nanotubes (MWNTs) as electrocatalyst, and GOx and HRP as bio-electrocatalysts. By the combination of NR functionalized MWNTs, Glucose oxidase, HRP and Nafion, a nanobiocomposite film was produced by simple solvent casting process. This system serve to "electrically wire" the enzyme, facilitating an easy flow of electrons from the enzyme to the electrode. The biosensor showed high sensitivity and good stability and also exhibited good analytical performance towards the quantification of hydrogen peroxide.

Experimental

Materials and Reagents

MWNTs were produced by a chemical vapour deposition method by the catalytic decomposition of acetylene over a Ni/Cr hydrotalcite-type anionic clay catalyst [30] and the purity of the MWNTs sample was about 95%. MWNTs samples are usually 10∼25 nm in diameter and many micrometres in length, but they are entangled together in the solid state to form a dense, robust, network structure which is difficult to disperse in organic or polar media. Before the MWNTs sample was used, it was further purified according to the literature [31]. GOx (E.C.1.1.3.4, activity 250 EU.mg^{-1}, from Aspergillus niger) and HRP (E.C.1.11.1.7, activity 90 EU/mg) were from Sigma Chemical Co. (St. Louis, MO), and were used without further purification. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide

hydrochloride (EDAC) and N-hydroxy sulfosuccinimide (sulfo-NHS) were obtained from Himedia and neutral red was from SD-fine chemicals. H_2O_2 (30% w/v solution) was purchased from Merck. Nafion (5 wt% in ethanol) was purchased from Aldrich. Phosphate buffer solutions (PBS, 0.1 M) with various pHs were prepared by mixing stock standard solutions of K_2HPO_4 and KH_2PO_4 and adjusting the pH with H_3PO_4 or NaOH. H_2O_2 solutions were calibrated by titration with $KMnO_4$ solution and fresh solutions of H_2O_2 were prepared daily. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with doubly distilled water.

Instrumentation

All electrochemical measurements were performed with a CHI 660B electrochemical workstation (CH Instruments, USA). Electrochemical experiments were carried out using a conventional three-electrode system with a GCE (ϕ=3 mm) as the working electrode, a Pt wire as the auxiliary electrode, and saturated calomel electrode (SCE) as the reference electrode. All experiments were performed at room temperature in a conventional electrochemical cell. Electrochemical impedance spectra (EIS) measurements were performed in 0.1 M phosphate buffer solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and plotted in the form of complex plane diagrams (Nyquist plots) with a frequency range of 0.1 Hz to 100 kHz. The amplitude of the applied sine wave potential was 5 mV, whereas the formal potential of the system was set at -0.35 V. CV experiments were carried out in quiescent solution at a scan rate of 20 mV/s. The current–time curves were recorded in a stirred cell with successive addition of glucose standard solution to the cell at an operating potential of −0.35 V.

Functionalization of MWNTs with NR

Purification of MWNTs was done by heating the as prepared MWNTs (50 mg) at 400℃ for 30 mins and then dispersed in conc. HCl for 1 h under ultrasonic agitation, centrifuged, washed until the pH of the supernatant was neutral and then dried in air at room temperature. The MWNTs sample was then treated with HNO_3 (12.8 M) and refluxed for 12 h. Then the mixture was diluted with water to about three times of its original volume and subjected to high-speed centrifugal sedimentation to separate the oxidized MWNTs powder. The powder was washed with distilled water until no residual acid was present and then dried in a vacuum oven at 80℃ for 4 h [31].

The resulting MWNTs-COOH was added to a freshly prepared 100 ml aqueous solution of EDC (10 mg/ml) and 300 mg of NHS, and allowed to react, for 2 h under stirring, at room temperature, and then, for another 22 h at room temperature. Finally, 50 mg of NR was added and allowed to react further for 2 h, at room temperature and then the MWNTs were washed thoroughly with water and filtered to remove the excess NR [32].

Also, a blank reaction was performed in the absence of EDC and NHS to verify if the NR is adsorbed on the MWCNTs-COOH backbone.

Preparation of the bienzyme nanobiocomposite

The GOx/HRP bienzyme nanobiocomposite was prepared as follows: 4 mg of GOx and 3 mg of HRP were dissolved in 0.3 ml of phosphate buffer (pH=7.0). Enzyme immobilization with NR-functionalized MWNTs (1 mg) was achieved by immobilizing with 20 μl of the enzyme mixture by stirring. The enzyme-MWNTs mixture was then dispersed in 1ml of 0.5 wt% Nafion solution with the aid of ultrasonic agitation for 5 min to form a homogeneous nanobiocomposite colloidal solution. For comparison, the bienzyme electrode with mere MWNTs was also prepared.

Fabrication of MWNTs/NR/GOx/HRP/Nf nanobiocomposite biosensor

To prepare the biosensor, a GCE was polished with emery paper followed by alumina (0.1 and 0.5 m) and then thoroughly washed with double-distilled water. Then the electrode was placed in 1:1 nitric acid solution, alcohol and redistilled water, sequentially, and subjected to sonication to remove adsorbed particles. Bienzyme nanobiocomposite electrodes were prepared by casting 10 μl of MWNT/NR/GOx/HRP/Nf or MWNT/GOx/HRP/Nf nanobiocomposite colloidal solutions on the surface of GC electrode followed by air drying for about 2-3 h, rinsed with water several times before use. When not in use all the modified electrodes were stored at 4℃. Before utilization, the electrodes were soaked into the solution, at room temperature for 30 min, to restore the enzyme activity.

Results and discussion

Structural and morphological characterizations of the nanobiocomposite

The SEM image of the MWNTs/NR/GOx/HRP/Nf nanobiocomposite is given in Fig. 1(a) and the enzyme immobilized NR/MWNTs were confirmed with Fourier transform infrared spectroscopy (FTIR) spectra (Fig. 1(b)).

EIS characterization

EIS measurements give information on the impedance changes of the electrode surface. The high-

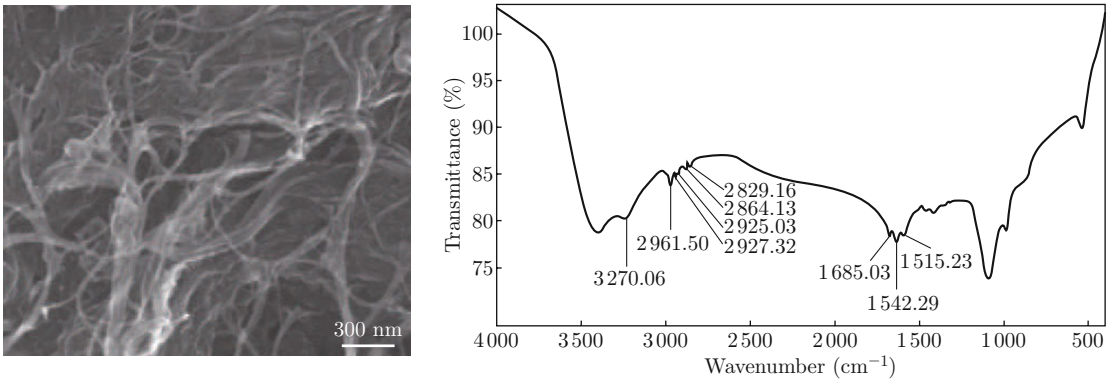


Fig. 1 (a) SEM image of MWNT/NR/GOx/HRP/Nf nanobiocomposite. (b) FTIR spectra of NR functionalized MWNTs immobilized with GOD and HRP.

frequency region contains information of kinetics of the faradaic process, while the low-frequency region gives information concerning the diffusion of species to electrode surface [33]. The semicircle diameter of well conducting substrates equals the electron transfer resistance, R_{ct}. If the surface is covered by a film with some ohmic resistance, R_f, the diameter of the semicircle will be dependent on that resistance. The EIS behaviors of the modified surfaces prepared in this study are shown in Fig. 2. The larger semicircle in the high-frequency region of MWNTs/Nf represents slower electron-transfer kinetics and more blocking behavior for the redox couple (curve b) due to the presence of COO^- group on the surface, whereas the bare GC electrode (curve a) exhibits an almost straight line that is characteristic of a diffusion limiting electron-transfer process. Upon covalent attachment of NR with MWNTs (curve c), the R_{ct} decreased dramatically due to the decrease in the negative charges, making it easier for the electron transfer to take place. With further immobilization of monoenzymes, GOx and HRP (curve d and e) separately over the surface of the MWNTs, which provides a hydrophobic insulating layer on the electrode surface, introduces a barrier to the electron transfer and hence the resistance increases. With bienzyme electrode, the enzymes with negative charges also blocked the access of the probe molecules to the electrode surface, resulting in further increase of the electron transfer resistance (curve f). However, the value of R_{ct} was less than that of MWNTs/Nf modified electrode. These results show that presence of GOx and HRP blocked the electron transfer at the electrode surface. Therefore, it can be concluded that MWNTs/NR/GOx/HRP/Nf nanobiocomposite has successfully been adsorbed on the surface of GC electrode and formed a tunable kinetic barrier.

Electrochemical performance of biosensor towards hydrogen peroxide

The electrocatalytic activity of biosensor toward H_2O_2 means that it can provide a signal transduction in the fabrication of biosensors because H_2O_2 is a product of a number of oxidase-based enzyme reactions. To evaluate the catalytic effect of the nanobiocomposite on the enzymatic reaction between HRP and H_2O_2, the reduction of H_2O_2 by nanobiocomposite biosensor was examined by cyclic voltammetry.

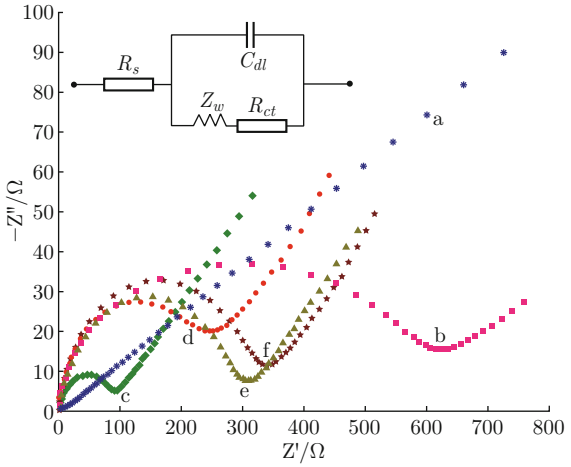


Fig. 2 Nyquist diagram (Z' vs Z'') for the Electrochemical impedance measurements in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ after the different steps of modification: (a) bare GC electrode, (b) MWNT-Nf modified (c) MWNT-NR-Nf modified (d) MWNT-NR-GOx-Nf modified (e) MWNT-NR-HRP-Nf modified and (f) MWNT-NR-GOx-HRP-Nf modified GC electrodes. All data were recorded in 0.1 M PB, pH 7.4. The electrode potential was −0.35 V vs SCE. The inset is the Randles equivalent circuit for the modified electrodes.

The cyclic voltammograms of MWNTs/NR/GOx/HRP/Nf nanobiocomposite before and after addition of H_2O_2 are shown in Fig. 3. Without hydrogen peroxide two typical reversible redox waves are observed for the biosensor (Fig. 3 (curve a)). Nevertheless, the addition of hydrogen peroxide to the solution brings about a significant increase in the cathodic peak current and an almost complete disappearance of the anodic peak (curve b). Comparison of the voltammograms with and

without hydrogen peroxide indicates that NR incorporated with MWNTs effectively enhances electron transfer between immobilized enzymes and the glassy carbon electrode.

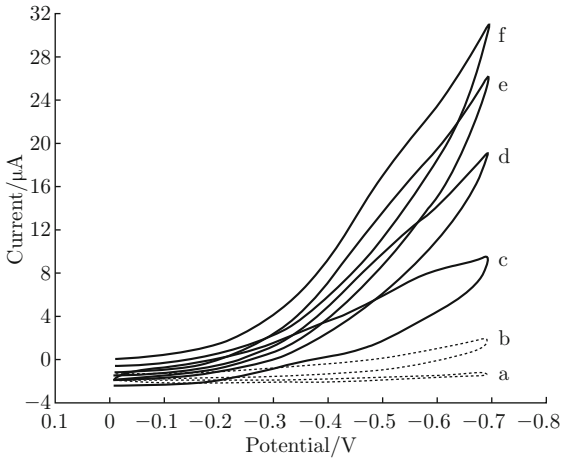


Fig. 3 Cyclic voltammograms of bare GCE (a) without H_2O_2 (b) with 3.0 μM H_2O_2 and (c, d, e, f) MWNTs/NR/GOx/HRP/Nf modified GCE in 0.1 M 7.4 PBS containing 0, 1.0, 2.0 and 3.0 M H_2O_2 at 0.20 V/s.

The cyclic voltammograms of a mediatorless biosensor with and without hydrogen peroxide was also studied (the figure not shown here). In the absence of H_2O_2 the mediatorless biosensor was observed. There was a small increase in cathodic current at the mediatorless biosensor upon addition of hydrogen peroxide which indicates that direct electron transfer from a glassy carbon electrode to the enzyme occurs only to a small extent. Comparison of the NR mediated biosensor with the mediatorless one shows that the NR mediated biosensor displays 500-fold higher sensitivity to hydrogen peroxide than the mediatorless one, which indicates that electron transfer via NR functionalized MWNTs is more efficient in the bioelectrocatalytic reduction of hydrogen peroxide. The mechanism of the bioelectrocatalytic reaction between hydrogen peroxide and HRP has been widely studied [34-36]. In a first two electron step, hydrogen peroxide is involved in the oxidation of the ferriheme prosthetic group of HRP (Fe^{3+}), producing an unstable intermediate HRP-I consisting of a π cation radical of heme with Fe(IV) to which an oxygen atom is co-ordinated ([Fe(IV)=O]):

$$\mathrm{HRP}(Fe^{3+}) + H_2O_2 \longrightarrow \text{HRP-I} + H_2O \quad (1)$$

The reduction of HRP-I to HRP (Fe^{3+}) can be achieved through two successive one-electron steps via an electron transfer mediator or by direct electron transfer from the electrode to the heme site of the HRP in intimate contact to the conducting surface [34]:

$$\text{HRP-I} + NR_{red}(\text{or } e^-) \longrightarrow \text{HRP-II} + NR_{ox} \quad (2)$$

$$\text{HRP-II} + NR_{red}(\text{or } e^-) \longrightarrow \mathrm{HRP}\ (Fe^{3+}) + NR_{ox} \quad (3)$$

where HRP-II, an intermediate state, possesses a heme with Fe (IV) to which OH is coordinated ([Fe (IV) OH]). NR_{ox} and NR_{red} represent oxidized and reduced forms of neutral red, respectively. Oxidized mediator (NR_{ox}) is reduced at the electrode, bringing about a reduction current.

$$NR_{ox} + 2H + 2e^- \longrightarrow NR_{red}(\text{at the electrode}) \quad (4)$$

Therefore, the detection of hydrogen peroxide via a NR functionalized MWNTs mediated biosensor is based on the measurement of the amperometric response due to the electrochemical reduction of the NR_{ox} ion generated from the enzymatic reaction at −0.35 V (vs. SCE).

Condition optimization for H_2O_2 sensing

Various experimental parameters which affect the amperometric determination of H_2O_2 such as the pH of the solution, temperature and applied potential were studied. Because the activities of GOx/HRP and the stability of MWNT-NR are pH-dependent, the influence of pH is very important to the sensitivity of the biosensors [37, 38]. Figure 4 (curve a) shows the effect of the pH on the detection of 1.0 M H_2O_2 solution. The optimum response was achieved in the pH range 6.5∼7.5, which is close to the optimum pH 7.4 observed for free GOx and HRP and which is also near the physiological environment [39, 40]. To ensure better sensitivity and stability of the biosensor, 0.1 M PBS (pH=7.4) was chosen for the determination of H_2O_2.

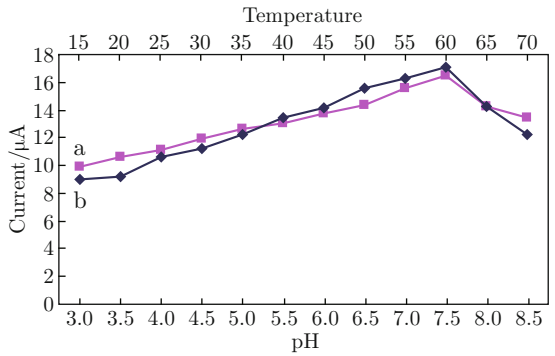


Fig. 4 Amperometric response of the MWNT-NR-GOx-HRP-Nf modified GCE for 1.0 M H_2O_2 solution (a) at different pHs and (b) at different temperatures.

It is well known that the analytical performance of the enzyme-immobilized materials is highly sensitive to variations of temperature. The study of the effect of the temperature on bienzyme biosensor experiments were carried out over the temperature range 15∼70℃. Curve b of Fig. 4 shows the effect of temperature on the MWNT/NR/GOx/HRP/Nf modified electrode. The immobilized enzymes showed activity even at 60℃ and beyond this temperature, the response dropped sharply. Although the response of the sensor was highest at 60℃,

for practical reasons room temperature is recommended in order to simplify the experimental procedure and prolong the lifetime of the biosensor.

The amperometric response of the sensor depends on the applied potential. Cyclic voltammograms were recorded at MWNT/NR/GOx/HRP/Nf biosensor in the presence of various concentrations of H_2O_2 (as shown in Fig. 3(d)-(f)). MWNT/NR/GOx/HRP/Nf biosensor exhibits significant electrocatalysis to the reduction of H_2O_2 starting around −0.2 V. In order to optimize potential for the biosensor operation, hydrodynamic voltammetric studies were carried out in 0.1 M PBS (pH=7.4). The potential of the working electrode was varied between 0 and 0.7 V and the currents were noted. Figure 5 compares the HDVs of (a) bare GC electrode (b) MWNT/GOx/HRP/Nf modified and (c) MWNT/NR/GOx/HRP/Nf modified GC electrode in the presence of 3.0 M H_2O_2. As expected no response was observed at the bare GC electrode in the entire potential range studied. For the MWNT/GOx/HRP/Nf modified electrode the reduction started at −0.3 V, then the current increased slowly until −0.45 V. In contrast the voltammetric response of the MWNT/NR/GOx/HRP/Nf modified GC in the presence of 3.0 M H_2O_2, the electrode showed a sharp increase around −0.2 V and leveled off above −0.35 V. Such a potential dependence profile is in agreement with the cyclic voltammogram results shown in Fig. 3. An operating potential of −0.35 V (vs. SCE) was chosen for further experiments to demonstrate the applicability of the biosensor electrode towards the detection of H_2O_2.

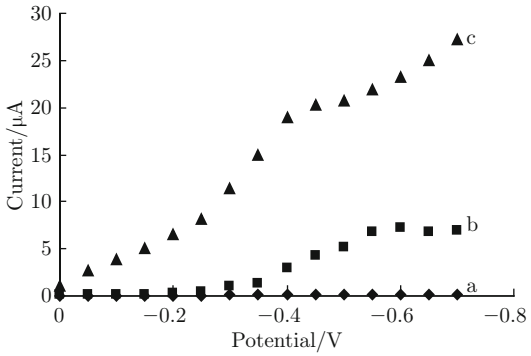


Fig. 5 Hydrodynamic voltammograms for 3.0 M H_2O_2 in 0.1 M phosphate buffer solution (pH=7.4) at (a) unmodified (b) MWCNT-GOx-HRP-Nf modified and (c) MWCNT-NR-GOx-HRP-Nf modified GC electrodes.

Amperometric determination of H_2O_2 with the biosenor

Figure 6 shows the amperometric responses of MWNT/NR/GOx/HRP/Nf modified electrode at −0.35 V (vs. SCE) for the successive 0.1 M addition of H_2O_2. The time required to reach 95% of the maximum steady-state current was 2 s. The electrode exhibits a rapid and sensitive current response for the changes of H_2O_2 concentration and indicates the excellent electrocatalytic behavior of the electrode A linear response to current is noticed for a wider concentration range of H_2O_2 (15 nM to 45 mM) at MWNT/NR/GOx/HRP/Nf biosensor. The linear regression equation was $I(A) = 0.5326[H_2O_2] + 4.9947$ (A is the correlation coefficient of 0.9994). The lower detection limit, 5 nM, was calculated as the H_2O_2 concentration giving a signal equal to the blank signal yB (intercept) plus three standard deviations of y-residuals sy/x.

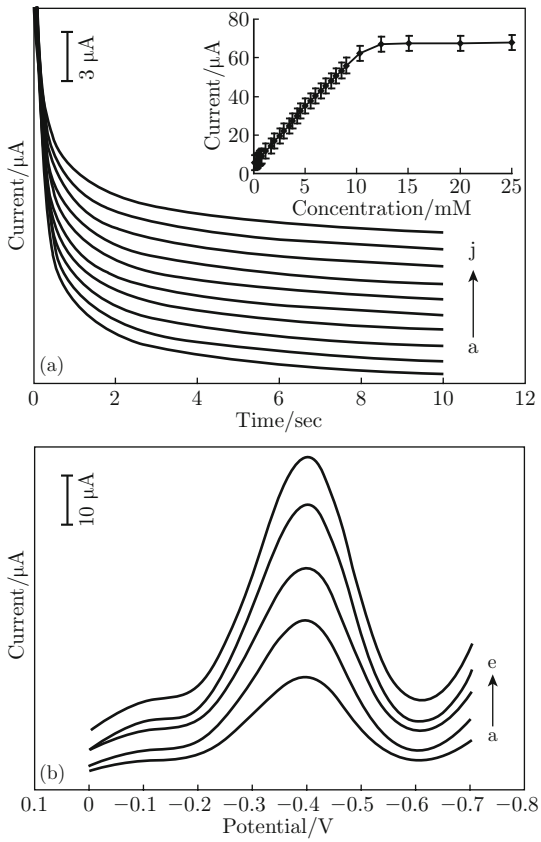


Fig. 6 (a) Amperometric response of MWCNT-NR-GOx-HRP-Nf biocomposite electrode (a-j) for the successive addition of 0.1 M H_2O_2 in 0.1 M PB (pH=7.4); Inset shows the linear calibration plot. (b) Differential pulse voltammograms of nanobiocomposite biosensor (a-e) at successive addition 1.0 M H_2O_2.

Differential pulse voltammetric experiments were also carried out for the determination by varying H_2O_2 concentrations (Fig. 6(b)). The sensor showed a linear relationship for H_2O_2 from 15 nM to 45 mM.

Selectivity and stability

The practical usefulness of an amperometric biosensor often rests upon the selectivity, in another word,

the interference level from electroactive species. This aspect is of particular concern in the present case since it is known that carbon nanotubes show catalytic property for electrochemical oxidation of ascorbic acid (AA), uric acid (UA) and acetaminophen (AP), which are the common interferents in hydrogen peroxide determination [41]. The effect of the possible interfering substances on the response of the biosensor was evaluated at the operation potential of −0.35 V. It was discovered that the addition of 0.4 mM UA and 0.2 mM AP to 1 M H_2O_2 solution caused no interference on the response of the biosensor. H_2O_2 could be determined in the presence of a 1:10 molar ratio of sodium chloride, potassium bromide, potassium iodide, sodium sulfate and nicotinamide which is shown in Fig. 7.

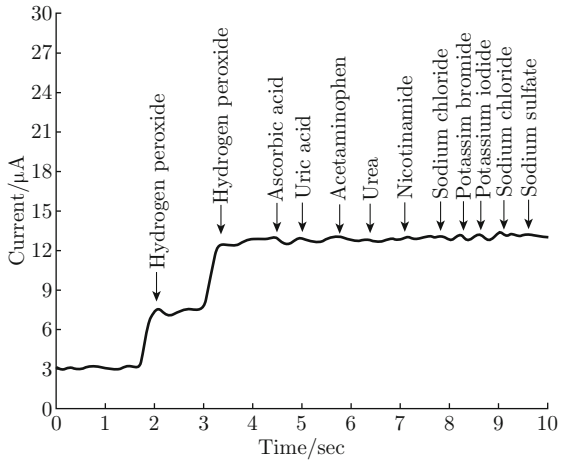


Fig. 7 Influence of electroactive interferences in 1.0 M H_2O_2 determination.

The operational and long-term stability (shelf lifetime) of the bienzymatic biosensor were studied. The MWNT/NR/GOx/HRP/Nf electrode was incorporated in the electrochemical cell stirring with 5 M H_2O_2 solution to study its operation stability under continuous use for 10 h. The response decreased by about 1% within the first 3 h, and about 3% within 10 h, which indicated that the bienzymatic biosensor has a good operational stability and can be used continuously. The storage stability of the bienzymatic biosensor was also investigated by performing triplicate measurements with 5 M H_2O_2 solution in phosphate buffer daily. No significant change in the current response was observed over the 6 months period study, when biosensors were stored and desiccated at 4℃ when not in use. The highly stable nature of this system was attributed to the strong interaction between NR immobilized MWNTs and the enzymes which are not affected by the changes of pH and temperature. Finally, the good biocompatibility of NR immobilized MWNTs maintains the biological activity of the enzymes immobilized on the electrode.

Sample analysis

To assess the possible application to the assay of hydrogen peroxide, the proposed biosensor was applied to pharmaceutical formulations. The results showed an acceptable correlation between the claimed and measured values. The acceptable quantitative recoveries from 98.9 to 102.8% were obtained when a known amount of hydrogen peroxide was added to the sample.

Conclusions

A new simple and reliable method was demonstrated for building a CNT-based amperometric biosensor using a bienzymes consisting of GOx and HRP using neutral red functionalized MWNTs for hydrogen peroxide. The enzymes were well immobilized within the electrode matrices and retained satisfactory enzymatic catalytic activities. The functionalized CNTs markedly influenced the interfacial property of the modified electrode and played an important role in the biosensor response. The proposed method where a mediator transfers electrons between the enzyme and electrode reduced the problem of interferences by other electroactive species. It shows high performance characteristics with a broad detection range, a short measuring time, and a simple operation. Thus, we provide a new analytical approach for the determination of hydrogen peroxide that is specific, sensitive and fast and the immobilization platform is very promising. The method presented here can be easily extended to other biosensor devices by using other enzymes and proteins.

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