

A Hybrid Nanocomposite of Poly(Neutral red) and Hemoglobin Codeposited on Multi-Walled Carbon Nanotubes for Determination of Hydrogen Peroxide

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Electrocodeposition of poly(neutral red) (PNR) and hemoglobin (Hb) can be easily prepared on electrode surface with multi-walled carbon nanotubes (MWCNT). Morphology of PNR-Hb-MWCNT hybrid nanocomposite is studied by SEM, AFM and electrochemical methods. It shows electrocatalytic activity to hydrogen peroxide (H_2O_2) with high current response and low overpotential. Amperograms presents a high sensitivity of $700.8 \mu\text{A mM}^{-1} \text{cm}^{-2}$ to H_2O_2 ($E_{\text{app.}} = -0.25 \text{ V}$). Linearity is estimated in a concentration range of 1×10^{-5} – $1.33 \times 10^{-3} \text{ M}$ with a detection limit of $5 \times 10^{-7} \text{ M}$ ($\text{S/N} = 3$). Hybridization and activity of PNR and Hb can be effectively enhanced by MWCNT. The hybrid composite can be applied for determination of H_2O_2 .

Keywords: Neutral red, Hemoglobin, Multi-walled carbon nanotubes, Hydrogen peroxide, Amperometry

1. INTRODUCTION

It is of great sense to monitor H_2O_2 accurately and rapidly. It is caused by not only its nature as a side product generated from many enzyme-involved reactions but also its important role as a signaling molecule in regulating various biological processes [1]. Developing the techniques of H_2O_2 determination is, therefore, important. Electrochemical detection of H_2O_2 provides some advantages over other techniques especially for convenience, sensitivity, selectivity, and immediate response.

Based on the direct electron transfer between redox proteins and the electrode for biomacromolecules, the electrochemical biosensors have been developed increasingly in the fields of

medicine, biotechnology, environmental monitoring, and so on [2–4]. Hemoglobin (Hb) is considered as an ideal model molecule for such study due to its known structure and commercial availability. However, as their redox centers are usually deeply buried within the protein molecules, it is difficult for electron to transfer between proteins and electrode. Therefore, it is a challenge to develop matrix to immobilize the redox protein, in which the protein can keep stability and its direct electron-transfer rate can be enhanced [5].

High chemical stability, high surface area, unique electronic properties, and relatively high mechanical properties are getting carbon nanotubes (CNTs) more and more attractive [6]. When CNTs are used as electrode materials, they can be used for promoting electron-transfer between the electroactive species and electrode and provide a novel method for fabricating chemical sensor or biosensor [7–10]. It is noticeable that the ability of CNTs-based electrode to electrocatalytic activity and to minimize surface fouling [6–11].

Neutral red (NR) a phenazine redox dye, with an amino group located on heteroaromatic phenazine ring, makes it amenable to facilitate electropolymerization [12]. Poly(neutral red) (PNR) pertains to a new kind of electroactive polymers derived from the neutral red monomers. The electrocatalytic reduction for H_2O_2 was also found while PNR/FAD-modified electrode and FAD-modified zinc oxide self-assembly film was studied in the absence/presence of Hb [13,14]. It's worthy to further investigate the electrocatalytic reduction of H_2O_2 with PNR, Hb, and their hybrid composite.

In this work, PNR, Hb, and CNTs are studied with their hybrid composite modified electrodes by simple electrochemical method. The formed PNR-Hb-MWCN hybrid nanocomposite can be easily prepared on electrode surface through electropolymerization of NR monomers using suitable arrangement of MWCNT as an active and steric template. The hybrid composites are electrochemically characterized and investigated with the electrocatalytic reduction of H_2O_2 .

2. MATERIALS AND METHODS

2.1. Reagents

Hemoglobin (Hb), neutral red (NR), and multi-walled carbon nanotubes (MWCNT) were purchased from Sigma-Aldrich (USA) and were used as-received. All other chemicals (Merck) used were of analytical grade (99%). Double-distilled deionized water ($> 18.1 \text{ M}\Omega \text{ cm}^{-1}$) was used to prepare all the solutions. All other reagents were of analytical grade and used without further purification. A pH 7 phosphate buffer solution (PBS) was prepared with 0.05 M Na_2HPO_4 and 0.05 M NaH_2PO_4 .

2.2. Apparatus

The PNR-Hb-MWCNT hybrid composite was characterized by cyclic voltammetry, UV–visible spectroscopy, SEM, AFM, and amperometry. A glassy carbon electrode (GCE) was purchased from Bioanalytical Systems (BAS) Inc., USA. All GCEs were used with diameter of 0.3 cm (exposed geometric surface area of $A = 0.0707 \text{ cm}^2$) for all electrochemical techniques. Electrochemical

experiments were completed by a CHI 1205a electrochemical workstation (CH Instruments, USA). A conventional three-electrode setup containing a GCE, an Ag/AgCl (3 M KCl) electrode, and a platinum wire were used as working, reference, and counter electrode, respectively. The buffer solution was entirely deaerated using nitrogen gas atmosphere. The morphological characterization of the hybrid composite was examined by means of SEM (S-3000H, Hitachi) and AFM. The AFM images were recorded with multimode scanning probe microscope (Being Nano-Instruments CSPM-4000, China). Indium tin oxide (ITO) substrates were used in morphological analysis for convenience.

2.3. Preparation of PNR-Hb-MWCNT modified electrode

The electropolymerization of neutral red (NR) to co-immobilize Hb was carried out in pH 7 BS containing 1×10^{-3} M NR and 10 mg mL^{-1} hemoglobin by consecutive cyclic voltammetry using a MWCNT-modified electrode. Electrochemical parameters were set in the potential range of $-1.2 \sim +0.8$ V with scan rate of 0.1 Vs^{-1} and 30 scan cycles.

The MWCNT-modified electrode was prepared by carboxylic functionalized MWCNT.¹⁵ The MWCNT was firstly well-dispersed in PBS (1 mg mL^{-1}) with sonication for 10 minutes. $10 \mu\text{l}$ of the solution was dropped on electrode surface and moved in the oven at $40 \text{ }^\circ\text{C}$. Then, the MWCNT-modified electrode was transferred to have hybrid immobilization of PNR and Hb in pH 7 PBS containing 1×10^{-3} M neutral red and 10 mg mL^{-1} hemoglobin by consecutive cyclic voltammetry. After these procedures, the PNR-Hb-MWCNT modified electrodes were prepared to study.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterization of PNR-Hb-MWCNT hybrid composite

PNR and Hb are designed to prepare the hybrid composite due to their activities towards H_2O_2 . However, the immobilization of huge Hb molecules on electrode surface might suffer some difficulties due to steric hindrance, weak affinity, and instability. In this work, one strategy is proposed to induce Hb coimmobilize on electrode surface by electropolymerization of NR using a high conductive MWCNT-modified electrode. Fig. 1 shows the consecutive cyclic voltammogram of a MWCNT/GCE examined in the presence of NR and Hb. Well current development of redox peaks indicates that the hybridization of PNR and Hb is performed successfully. The cyclic voltammogram depicts significant current development of NR electropolymerization at MWCNT/GCE. It exhibits two obvious redox couples with formal potential of $E^0_1 = -0.54 \text{ V}$ and $E^0_2 = 0 \text{ V}$, related to NR and PNR (Redox couple 1 and 2), respectively [14]. Both anodic and cathodic peak currents are increasing as the increase in the scan cycles. It indicates that MWCNT might effectively enhance the hybridization of PNR and Hb. However, no obvious peaks are observed for Hb, this phenomenon may be caused by that not enough amounts of Hb related to PNR in the hybrid composite. Therefore, it needs to have further test by UV-Visible spectroscopy.

Different materials prepared in aqueous solutions were examined by UV-Visible spectroscopy. Fig. 2A shows the UV-Vis spectra of different materials and mixtures prepared in pH 7 PBS. The UV-

Vis spectrum of Hb (curve a) shows soret band at 406 nm while NR (curve b) shows soret band at 524 nm. The spectrum of their mixture exhibits the related soret bands (406 nm and 524 nm) similar to their single spectrum [14,16-18]. These phenomena indicate that these materials can be mixed stably to prepare a stable hybrid composite on electrode surface. The materials are further immobilized on electrode surface to study. Fig. 2B shows the UV-Vis spectra of different materials and mixtures coated on ITO electrodes. Both PNR-Hb (curve c) and PNR-Hb-MWCNT (curve d) shows the similar spectrum related to Hb (curve a) and PNR (curve b). The result proves that Hb can be coimmobilized with PNR on both bare and MWCNT-modified electrodes. It also indicates that the film formation on electrodes is stable. This point is very important especially for hybrid composite. As the result, it proves that Hb can be coimmobilized by the electropolymerization of NR on MWCNT. One can conclude that the PNR-Hb-MWCNT can be easily prepared by our method.

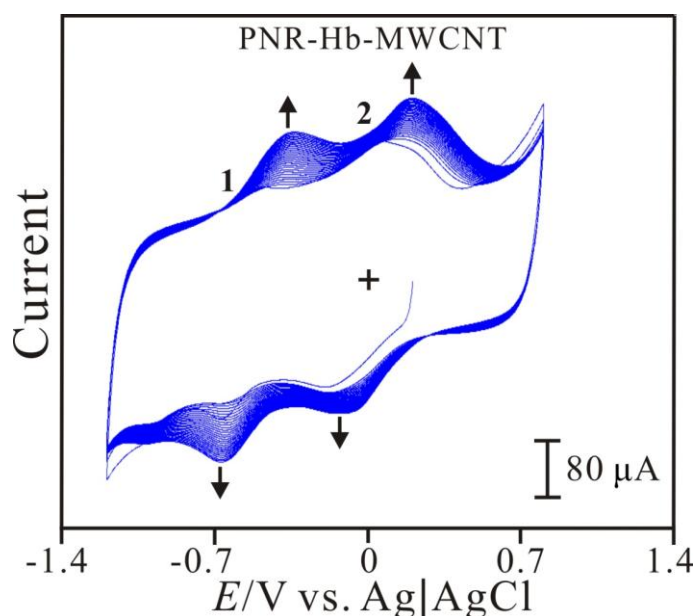


Figure 1. Consecutive cyclic voltammograms of a MWCNT/GCE examined in PBS (pH 7) containing 1×10^{-3} M neutral red and 10 mg mL^{-1} hemoglobin. Scan rate = 0.1 Vs^{-1} . Scan cycle = 30.

Different materials modified electrodes were prepared and studied by cyclic voltammetry. Fig. 3 shows the voltammograms of (a) Hb, (b) PNR, (c) PNR-Hb, (d) Hb-MWCNT, (e) PNR-MWCNT, and (f) PNR-Hb-MWCNT modified electrodes. Hb shows an obvious redox peak at $E^{0'}_1 = -0.347 \text{ V}$ while PNR shows two redox couples of $E^{0'}_1 = -0.552 \text{ V}$ and $E^{0'}_2 = +0.049 \text{ V}$. PNR-Hb shows significant redox couples estimated with formal potential of $E^{0'}_1 = -0.51 \text{ V}$ and $E^{0'}_2 = 0 \text{ V}$, related to NR, PNR (Redox couple 1 and 2), respectively [14,19]. Curve (d)-(f) shows the cyclic voltammograms of Hb, PNR, and PNR-Hb immobilized on MWCNT-modified electrodes. The redox peak currents are strongly enhanced with less peak shifting. It shows the redox peak current higher than other modified electrodes. One can conclude that the coimmobilization of PNR and Hb can be successfully performed and further enhanced by MWCNT. The obtained PNR-Hb-MWCNT hybrid composite can maintain the electrochemical characteristics with similar redox peaks and higher current response.

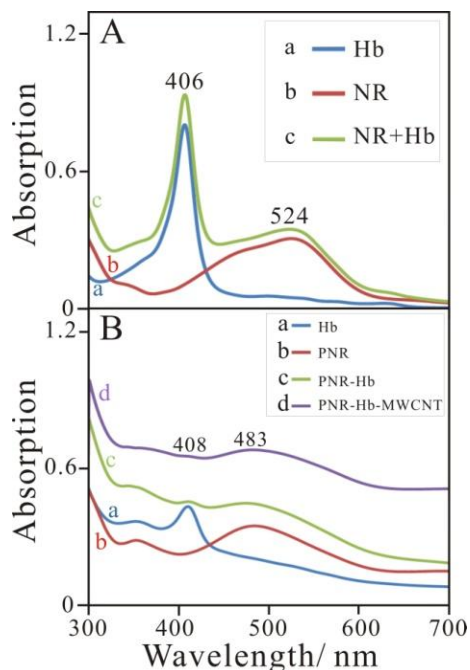


Figure 2. (A) Absorption spectra of (a) 1×10^{-3} M NR, (b) 10 mg ml^{-1} Hb, and (c) 1×10^{-3} M NR + 10 mg ml^{-1} Hb in 0.1 M PBS (pH 7) using quartz cell, path length = 1 cm. (B) Absorption spectra of (a) Hb, (b) PNR, (c) PNR-Hb, and (d) PNR-Hb-MWCNT coated ITO electrodes.

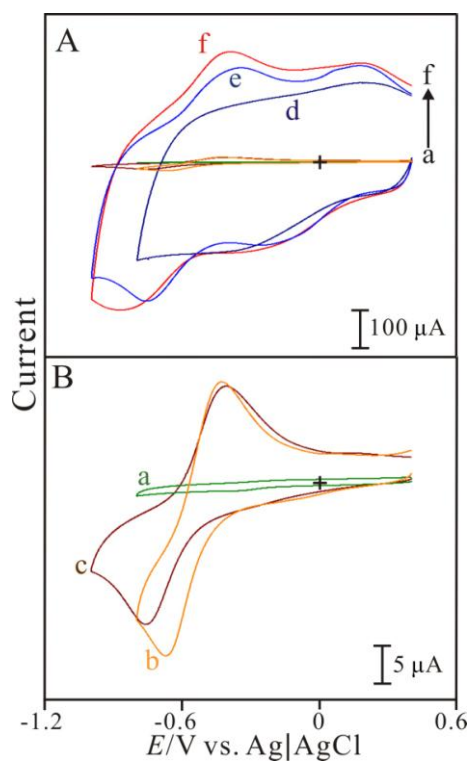


Figure 3. (A) Cyclic voltammograms of a modified GCE examined in pH 7 PBS with different modifiers including (a) Hb, (b) PNR, (c) PNR-Hb, (d) Hb-MWCNT, (e) PNR-MWCNT, and (f) PNR-Hb-MWCNT, respectively. Scan rate = 0.1 Vs^{-1} . (B) Scale-up cyclic voltammograms of (a) Hb, (b) PNR, and (c) PNR-Hb modified electrodes.

Surface morphology of these modified electrodes was studied by SEM and AFM. Fig. 4A–D shows the SEM images for (A) PNR, (B) Hb, (C) MWCNT, and (D) PNR-Hb-MWCNT, respectively. Fig. 4A–D exhibit single materials with unique structures of fine clusters (PNR), circular clusters (Hb), fiber-like clusters (MWCNT), and solid clusters (PNR-Hb-MWCNT), respectively. Fig. 4B shows large clusters indicating the aggregation occurs easily when Hb adsorbed on bare electrode. In this case, the MWCNT and NR are used to solve the problems. Because high conductive MWCNT can provide more space to load Hb molecules and NR monomers can be electropolymerized to bind Hb molecules on electrode surface during the preparation process. Fig. 4D shows the PNR-Hb-MWCNT image with specific solid cluster which is more compact when compared to Fig. A–C. These materials are further analyzed by AFM. Fig. 4A–D shows the AFM images for (A') PNR, (B') Hb, (C') MWCNT, and (D') PNR-Hb-MWCNT, respectively. They show average diameter of 17.6 nm, 71.2 nm, 60.0 nm, and 73.6 nm for PNR, Hb, MWCNT, and PNR-Hb-MWCNT, respectively. These modifiers are proved in nanocomposites with average roughness of 3.4 nm, 10.4 nm, 14.3 nm, and 16.6 nm, respectively. Similar morphological properties were observed in the results of SEM and AFM. One can conclude that the PNR-Hb-MWCNT nanocomposite can be easily prepared by that using a high conductive MWCNT and fine NR to immobilize Hb on electrode surface.

The PNR-Hb-MWCNT/GCE electrochemical response was studied with various scan rates and pH conditions. Fig. 5A shows the voltammograms of PNR-Hb-MWCNT examined with different scan rate of 10–100 mVs⁻¹. It shows obvious two redox couples with small peak-to-peak separation and higher current response. Obvious redox couples represents that PNR-Hb-MWCNT can be well immobilized on GCE and it shows stable current response in the scan rate of 10–100 mVs⁻¹. Both anodic and cathodic peak currents are directly proportional to scan rate ($I_{pc1}(\mu A) = -2.33v(mVs^{-1}) - 27.7$, $R^2 = 0.9993$; $I_{pc2}(\mu A) = -2.11v(mVs^{-1}) - 13.7$, $R^2 = 0.9988$), suggesting a surface controlled process in the electrochemical system. The observation of well-defined and persistent cyclic voltammetric peaks indicates that PNR-Hb-MWCNT/GCE exhibits electrochemical response characteristics of redox species confined on the electrode. The apparent surface coverage (Γ) was estimated by following equation:

$$I_p = n^2 F^2 v A \Gamma / 4RT \quad (1)$$

where, I_p is the peak current of the PNR-Hb-MWCNT composite electrode; n is the number of electron transfer; F is Faraday constant (96485 C mol⁻¹); v is the scan rate (mV s⁻¹); A is the area of the electrode surface (0.0707 cm²); R is gas constant (8.314 J mol⁻¹ K⁻¹); and T is the room temperature (298.15 K). Assuming a two-electron process for NR and PNR in the present case, the Γ was calculated in 8.77×10^{-9} mol cm⁻² and 7.95×10^{-9} mol cm⁻² for NR and PNR, respectively. These values are much higher than that published in previous report.¹⁴ The Γ of Hb in the PNR-Hb-MWCNT hybrid composite was calculated in 1.62×10^{-9} mol cm⁻² based on the current-scan rate relationship between PNR-Hb-MWCNT and PNR-MWCNT. The high surface coverage of PNR-Hb-MWCNT might indicate that the hybrid composite is more compact with active species on electrode surface.

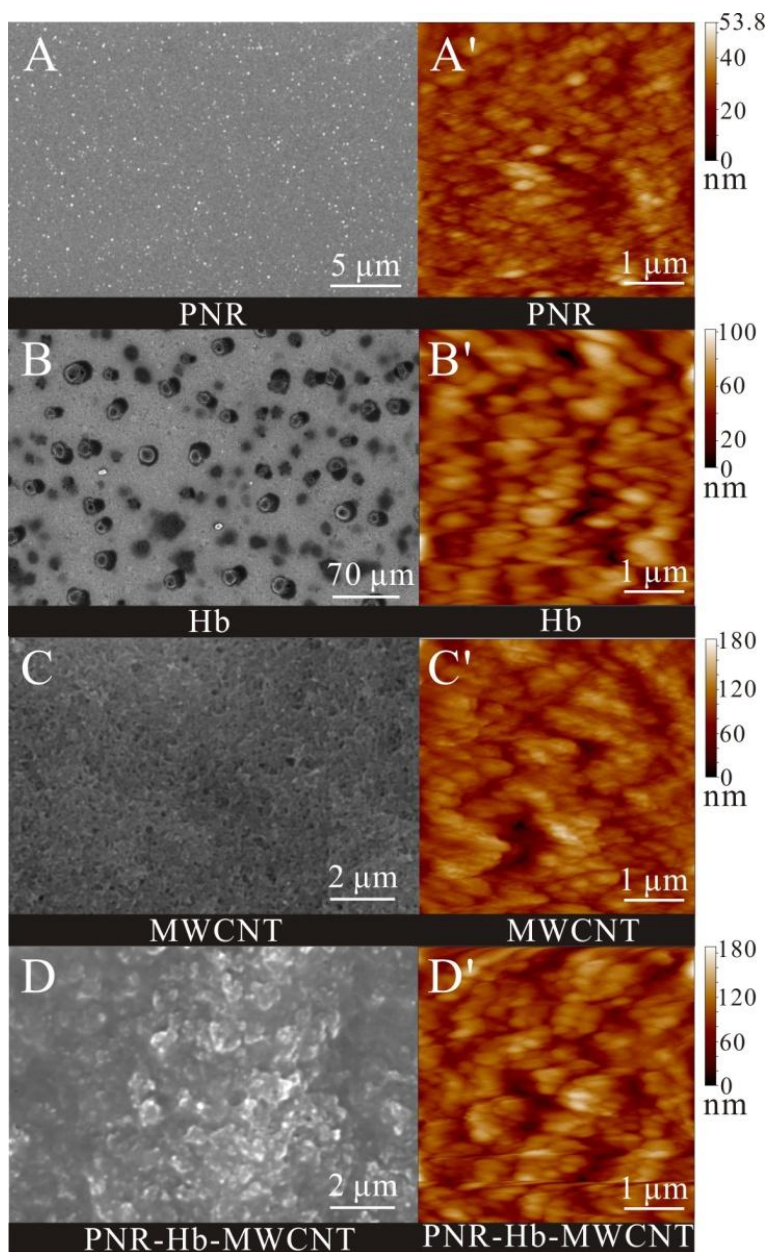


Figure 4. SEM and AFM images of (A) PNR, (B) Hb, (C) MWCNT, and (D) PNR-Hb-MWCNT coated ITO electrodes.

The PNR-Hb-MWCNT/GCE was examined with different pH solutions (pH 1 – 13). Fig. 5B presents the PNR-Hb-MWCNT redox peaks which are shifted to more negative potential as increasing pH value of solution. It shows stable redox peaks in various pH conditions. This result is the same even though PNR-Hb-MWCNT is repeatedly examined and change testing order of pH condition. It indicates that PNR-Hb-MWCNT hybrid composite is active and stable in wide pH condition. The characteristic PNR redox couples (with formal potential of $E^{0'}_1$ and $E^{0'}_2$) exhibit the significant slopes of -60.9 mV pH^{-1} ($y = -60.9x - 115.1$, $R^2 = 0.9977$) and -55.7 mV pH^{-1} ($y = -55.7x + 406.5$, $R^2 = 0.9999$) for redox couple 1 and 2, respectively. These slopes are close to that given by the Nernstian equation, suggesting a two-electron and two-proton transfer for NR and PNR redox processes [14]. As the result, the hybrid composites can be stable and electroactive in different scan rate and pH buffer

solutions.

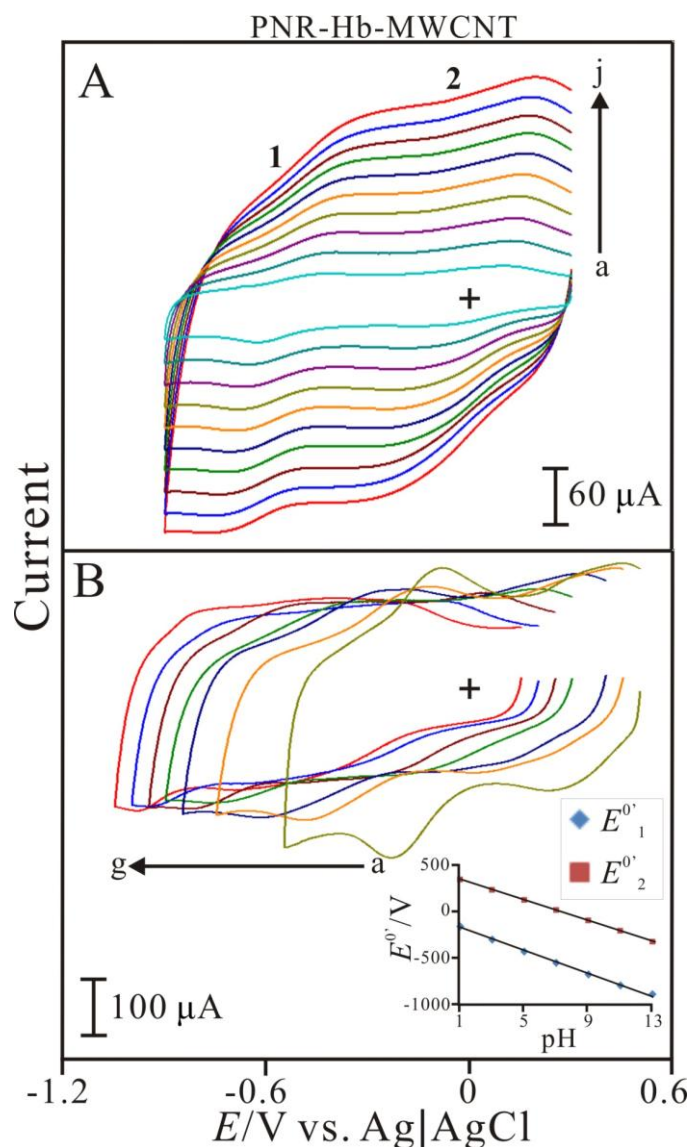


Figure 5. Cyclic voltammograms of PNR-Hb-MWCNT/GCE examined with (A) various scan rate = (a) 10 mVs^{-1} to (j) 100 mVs^{-1} ; and (B) various pH condition: (a) pH 1, (b) pH 3, (c) pH 5, (d) pH 7, (e) pH 9, (f) pH 11, and (g) pH 13, respectively. Scan rate = 0.1 Vs^{-1} .

3.2. Electrocatalysis of H_2O_2 and amperometric response at PNR-Hb-MWCNT electrode

The electrocatalytic activity of the PNR-Hb-MWCNT electrode was studied for the reduction of H_2O_2 in pH 7 PBS by cyclic voltammetry. Fig. 6 displays the voltammograms of PNR-Hb-MWCNT/GCE in pH 7 PBS with/without 3×10^{-3} M H_2O_2 . PNR-Hb-MWCNT/GCE shows two obvious reduction peaks at about -0.24 V and -0.81 V for H_2O_2 . These two characteristic reduction peaks are related to PNR and Hb redox processes indicating that PNR and Hb are active to H_2O_2 reduction.

The electrocatalytic activity of PNR-Hb-MWCNT hybrid composite was studied with different

modifiers. Fig. 7 shows the voltammograms of different modified electrodes examined in the absence/presence of 3×10^{-3} M H_2O_2 .

Fig. 7A – C represents the voltammograms for (A) PNR, (B) Hb, and (C) PNR-Hb, respectively. The electrocatalytic reduction peaks are observed for PNR (-0.675V, -0.063V), Hb (-0.347 V), and PNR-Hb (-0.748 V), respectively. One can see that the PNR-Hb shows higher current response for H_2O_2 . It means that the PNR-Hb hybrid composite is more active than that of their single species. The obvious electrocatalytic reduction peak is similar to that of single PNR. No obvious electrocatalytic reduction peak is observed for that of single Hb. This result indicates that related less amount of Hb was immobilized on electrode surface during the electrocodeposition of PNR and Hb using a bare electrode. Therefore, the redox peaks of Hb might be overlapping with that of PNR. These modifiers are further studied with MWCNT.

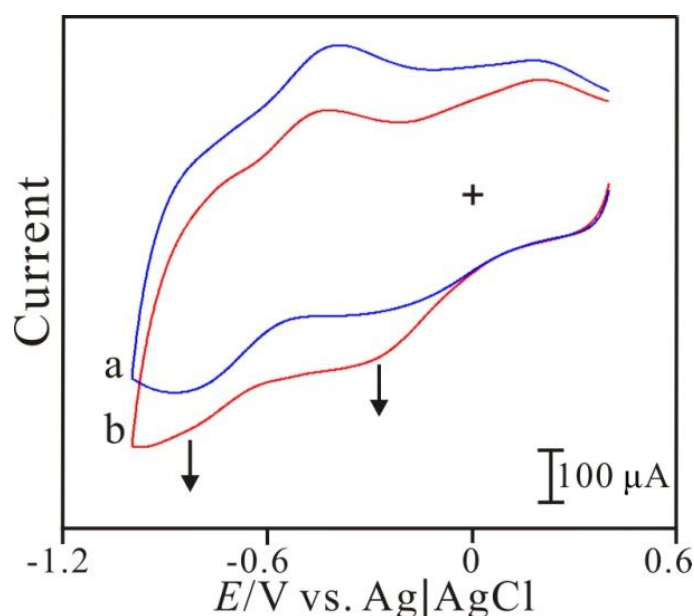


Figure 6. Cyclic voltammograms of PNR-Hb-MWCNT/GCE examined in PBS (pH 7) containing $[\text{H}_2\text{O}_2] =$ (a) blank and (b) 3×10^{-3} M, respectively. Scan rate = 0.1 Vs^{-1} .

Fig. 7A' – C' represents the voltammograms of MWCNT-composite incorporated with PNR, Hb, and PNR-Hb, respectively. One can see that the electrocatalytic reduction currents can be effectively enhanced by MWCNT. The PNR-Hb-MWCNT shows two significant electrocatalytic reduction peaks related to that of PNR and Hb, respectively.

Net current response and immobilized method is evaluated and summarized in Table 1. Except of Hb-MWCNT, the PNR-Hb-MWCNT shows much higher net current response for H_2O_2 compared to other modifiers. Although the Hb-MWCNT has higher net current response ($\Delta I_{\text{pc}} = 151.8 \mu\text{A}$) at $E_{\text{pc}} = -0.326 \text{ V}$, the PNR-Hb-MWCNT is still competitive due to lower overpotential ($E_{\text{pc}} = -0.241 \text{ V}$ with $\Delta I_{\text{pc}} = 106.2 \mu\text{A}$) and another high net current response ($\Delta I_{\text{pc}} = 315.5 \mu\text{A}$ at $E_{\text{pc}} = -0.814 \text{ V}$). When compared to the PNR-MWCNT, the PNR-Hb-MWCNT provides more net current response of $78.2 \mu\text{A}$ and $70.7 \mu\text{A}$ at cathodic peak 1 and 2. This result indirectly proves that the Hb has been coimmobilized on electrode surface by the proposed method. Particularly, the simple method of Hb coimmobilized

with PNR on MWCNT resulted in high net current response. One can conclude that the PNR-Hb-MWCNT is electroactive to be a bioelectrocatalyst for determination of H_2O_2 .

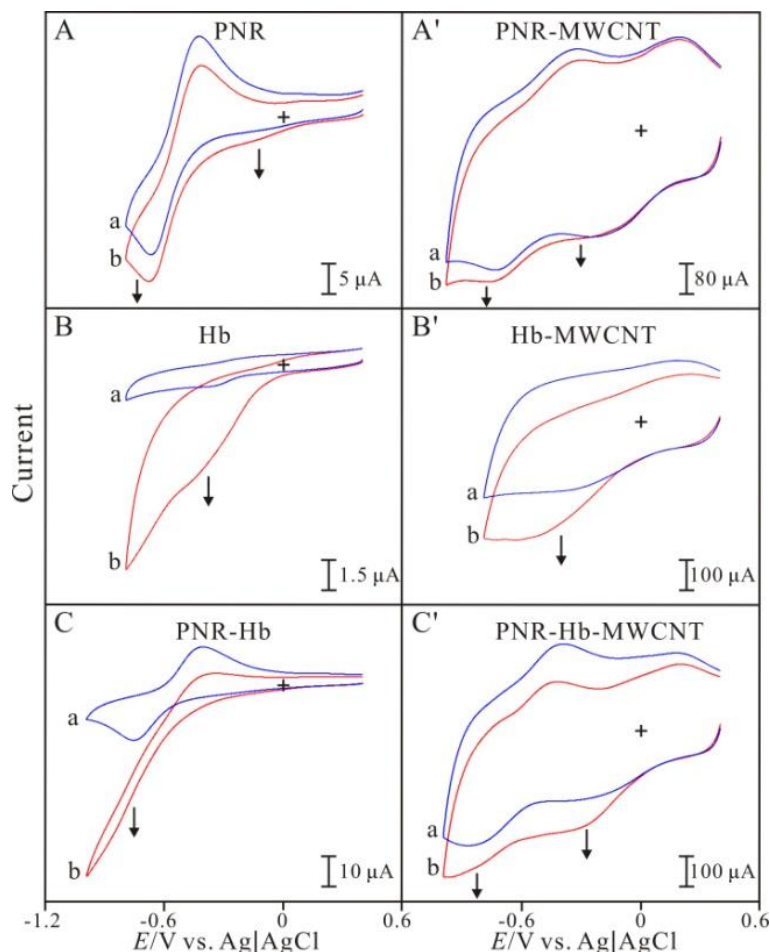


Figure 7. Cyclic voltammograms of different modified electrodes examined in PBS (pH 7) containing $[\text{H}_2\text{O}_2] =$ (a) blank and (b) 3×10^{-3} M, respectively. Scan rate = 0.1 Vs^{-1} .

Table 1. Net current response and immobilized method of the PNR-Hb-MWCNT related materials evaluated for 3 mM H_2O_2 .

Modifiers	Immobilized Method	$\Delta I_{pc1}/\mu\text{A}$	$\Delta I_{pc2}/\mu\text{A}$
PNR	electropolymerization	4.3	3.8
Hb	drop-casting	4.0	-
PNR-Hb	electrocodeposition	29.5	-
PNR-MWCNT	electropolymerization, drop-casting	28.0	244.8
Hb-MWCNT	drop-casting	151.8	-
PNR-Hb-MWCNT	electrocodeposition, drop-casting	106.2	315.5

Fig. 8A shows amperometric responses of PNR-Hb-MWCNT modified electrode with several additions of H_2O_2 . The electrochemical parameters were set with electrode rotation speed of 2000 rpm

and applied potential of -0.25 V, respectively. PNR-Hb-MWCNT/GCE shows significant amperometric responses for H_2O_2 additions spiked by micro-syringe. Inset (a) of Fig. 8A shows low detection limit of 5×10^{-7} M ($S/N = 3$) and response time of about 10 s. Inset (b) of Fig. 8A presents the calibration curve, it provides a linear concentration range of 1×10^{-5} – 1.33×10^{-3} M. The sensitivity is estimated in $700.8 \mu\text{A mM}^{-1} \text{cm}^{-2}$ with the regression equations of $I_{\text{H}_2\text{O}_2}(\mu\text{A}) = 49.55C_{\text{H}_2\text{O}_2}(\mu\text{M}) + 2.4$ ($R^2 = 0.9994$).

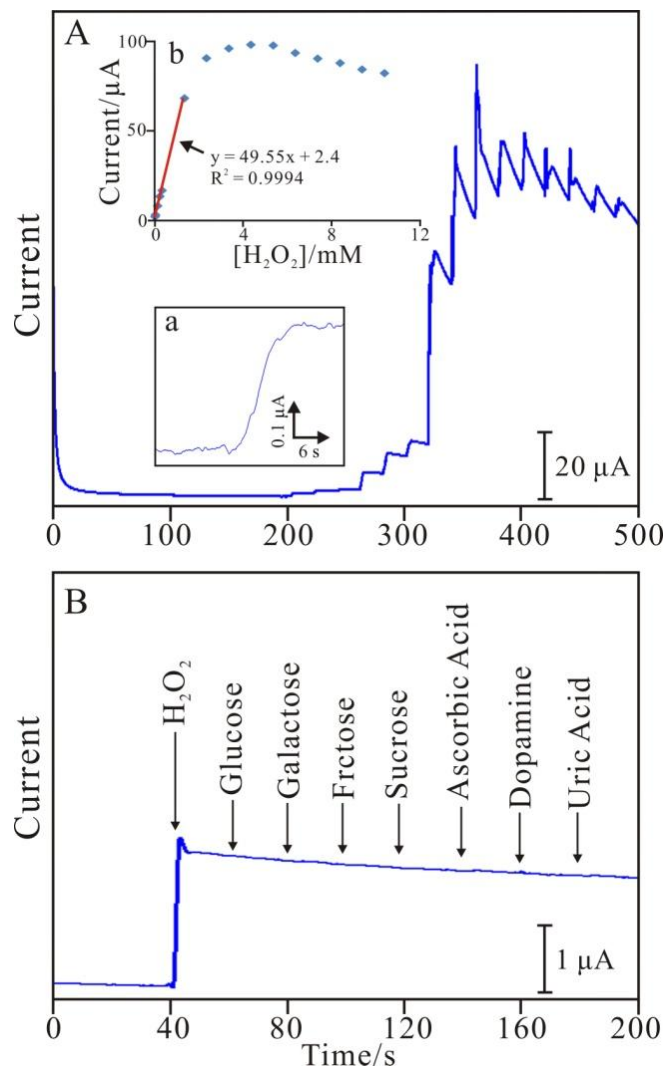


Figure 8. Amperograms of PNR-Hb-MWCNT/GCE examined in pH 7 PBS with several additions of (A) H_2O_2 and (B) interferents (each for 10^{-5} M). Electrode rotation speed = 2000 rpm. $E_{\text{app.}} = -0.25$ V. Insets: (a) the scale-up amperogram in the presence of $0.5 \mu\text{M}$ H_2O_2 and (b) the calibration curve.

The main performances of H_2O_2 sensors based on different modified materials have been summarized in Table 2. The performance of the PNR-Hb-MWCNT is competitive in applied potential, linear range, sensitivity, and limit of detection. Particularly, the preparation method is very simple due to the electrocodeposition of PNR and Hb on MWCNT. Good performance of the hybrid composite might be caused by the synergistic effect of PNR, Hb, and MWCNT. By the results, the active hybrid composite can be suggested as a H_2O_2 sensor.

Table 2. Performance comparison using various materials modified electrodes for H₂O₂ reduction.

Modifiers	E_{app}^a (V)	Linear range (μ M)	Sensitivity (μ A mM ⁻¹ cm ⁻²)	LOD ^b (μ M)	Ref.
Hb-graphene-chitosan	-0.4	6.5–230	445.9	0.51	[16]
Hb-Fe ₃ O ₄ -graphene	-0.3	1.5–585	33.9	0.5	[17]
Hb/graphene-ZnO	-0.3	1.8–2300	-	0.6	[18]
Au/SAM/CNT/Hb	-0.3	3–200	923.6	0.5	[20]
Hb/Au/GR-CS	-0.385	2–935	347.1	0.35	[21]
Au/CA/Mb/MW CNT-Nafion	-0.3	0.1–70	207	0.01	[22]
PNR-Hb-MWCNT	-0.25	10–1330	700.8	0.5	This work

^a E_{app} = Applied potential (V vs. Ag/AgCl).

^bLOD = Limit of detection.

3.3. Interference study of the PNR-Hb-MWCNT composite

The PNR-Hb-MWCNT modified electrode was studied with the interference effect for determination of H₂O₂ (E_{app} = -0.25 V). It was examined with potential interferents including glucose, galactose, fructose, lactose, ascorbic acid, dopamine, and uric acid. Fig. 8B shows the amperometric response of PNR-Hb-MWCNT with various interferants. No interference was observed in the amperograms. One can conclude that the modified electrode shows good selectivity, avoiding interference from most of interferents for determination of H₂O₂.

3.4. Reproducibility and stability study of the PNR-Hb-MWCNT modified electrodes

The reproducibility and stability of the sensor were evaluated. Five PNR-Hb-MWCNT electrodes were investigated at -0.25 V to compare their amperometric current responses. As shown in Table 3, three successive measurements of H₂O₂ on one PNR-Hb-MWCNT electrode yielded an average R.S.D. of 9.3% and an average recovery of 88.4%. This result indicates that the sensor is stable and reproducible. The long-term stability of the sensor was also evaluated by measuring its current response to H₂O₂ within a 7-day period. The sensor was stored at 4 °C and its sensitivity was tested every day. The current response of PNR-Hb-MWCNT electrode was approximately 90.1% of its original counterpart.

Table 3. Determination of H₂O₂ in neutral condition using PNR-Hb-MWCNT modified screen-printed electrodes.

No.	Spiked (μM)	Found ^a (μM)	R.S.D. ^b (%)	Recovery (%)
1	100	89	8.7	89
2	200	177	9.2	88.5
3	300	263	9.9	87.7

^aAverage of three measurements.

^bRelative standard deviation.

4. CONCLUSIONS

PNR and Hb hybrid nanocomposite can be successfully prepared on electrode surface and further enhanced by MWCNT. PNR-Hb-MWCNT shows specific nanostructure indicating that PNR and Hb species can be easily coimmobilized on MWCNT. The modified electrode presents good electrochemical property and specific nanocomposite morphology. It can be used as a novel H₂O₂ sensor due to attractive analytical features including high sensitivity, low overpotential, low detection limit, good stability, and good selectivity.

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