



Ag nanoparticles self-supported on Ag₂V₄O₁₁ nanobelts: Novel nanocomposite for direct electron transfer of hemoglobin and detection of H₂O₂

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ABSTRACT

Nanocomposite of Ag nanoparticles self-supported on Ag₂V₄O₁₁ nanobelts, prepared by γ -ray irradiation treatments of Ag₂V₄O₁₁ nanobelts, was used to immobilize hemoglobin on glassy carbon electrode. Detailed electrochemical analysis was performed and it was found that Ag/Ag₂V₄O₁₁ nanocomposite significantly improved the direct electron transfer between hemoglobin and glassy carbon electrode, leading to the fabrication of a biosensor with a very sensitive detection of H₂O₂. The apparent surface concentration (Γ^*) of hemoglobin and the electron transfer rate constant (k_s) were calculated to be 1.6×10^{-10} mol/cm² and 2.6 s^{-1} , respectively. The linear detection range of H₂O₂ was 1.0–120 μM , with a detection limit of 0.3 μM .

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1. Introduction

Direct electrochemistry of redox proteins immobilized on nanomaterial-modified electrode has attracted much attention from electrochemical scientists in last few decades [1–5] since Eddowes and Hill [6] and Yeh and Kuwana [7] independently reported that the reversible electrochemistry was successfully realized via cytochrome *c* on gold electrode and on tin-doped indium oxide electrode respectively in 1977. The research on the direct electron transfer from protein to electrode surface can not only serve as a model to understand electron transfer mechanisms in biological systems that may elucidate the relationship between the structures and biological functions [8], but also establish a foundation for constructing the third-generation electrochemical biosensors [9], in which the redox mediators are no longer needed and the complications brought about by the mediators are avoided.

However, it is difficult for proteins to realize direct electron transfer on “bare” electrodes because of their easy denaturation, deeply embedded active centers, and frequently unfavorable orien-

tations [10,11]. To maintain the native structures and appropriate orientations of redox proteins and thus to acquire an increased electron transfer between the protein and electrode, various supporting matrices such as surfactant, sol-gel, polymers and nanomaterials have been studied [12,13]. Among these different kinds of matrices, nanosized materials offer many advantages due to their unique size and special physical, chemical and electrochemical properties [14,15].

Heme proteins are important redox proteins that contain the porphyrin complex of iron(II) or heme(III) as a prosthetic group. Among various heme proteins, hemoglobin (Hb) is an ideal model molecule for the study of electron transfer reactions of heme enzymes and also for biosensing and electrocatalysis [16] because of its known and documented structure, commercial availability and relatively inexpensiveness. Hemoglobin is a soft globular heme protein with a dimension of 5.3 nm \times 5.4 nm \times 6.5 nm and a molecular weight of approximately 64,500 g mol⁻¹. It consists of four subunits of polypeptide enzymes, every heme (iron porphyrin) group in each subunit acts as an active center [17].

Silver vanadium oxide (SVO) is a kind of potential cathode material for primary and secondary lithium ion batteries, and many efforts have been made in the preparation, characterization, and discharging and recharging behaviors of SVO [18–23]. Among numerous SVO phases that contain Ag, V, and O in a number of stoichiometric and nonstoichiometric ratios, Ag₂V₄O₁₁ has

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been investigated most extensively and was used as a commercial cathode material in primary lithium batteries for implantable biomedical devices [24]. $\text{Ag}_2\text{V}_4\text{O}_{11}$ can be easily synthesized by using different methods and starting materials. Recently, the hydrothermal method has also been introduced to the synthesis of SVO [25]. Compared with the traditional solid phase synthetic route, the hydrothermal process possesses many advantages such as low reaction temperature, short reaction time, easy post-treatment, energy saving, and environmental friendliness.

Silver nanoparticles are one kind of the most extensively investigated nanomaterials for their excellent performances such as chemical stability, catalytic activity, antibacterial ability, and non-linear optic property [26–35]. In fact, besides silver nanoparticles, nanocomposites with silver nanoparticles as one component has also been drawing more and more attention owing to its potential applications in many fields. In 2008, Li et al. developed a one-pot synthetic method to prepare Ag/SVO nanocomposite with Ag nanoparticles supported on AgVO_3 nanobelts [36]. Very recently, our group also applied a facile post-treatment method for the preparation of $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite with Ag nanoparticles self-supported on $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelts [37].

The rapid, convenient and accurate determination of hydrogen peroxide (H_2O_2) is required in many fields such as industry, clinical control, and environmental protection [38,39]. Compared with the traditional techniques such as titrimetry and spectrophotometry [40,41], the electrochemical method is fast and easy, and has obvious advantages for the determination of H_2O_2 [4]. Heme proteins have peroxidase activity and can be used to reduce H_2O_2 through electrochemical catalysis.

In this work, by modifying glassy carbon electrode with $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite, the direct electrochemistry of Hb was successfully realized, and a biosensor for the detection of H_2O_2 was constructed.

2. Experimental

2.1. Materials preparation

Bovine heart hemoglobin (Hb) was purchased from Sigma and used without further purification. Hydrogen peroxide (H_2O_2 , 30 wt% aqueous solution) was obtained from Shanghai Biochemical Reagent Company. All other reagents were of analytical grade and used as received.

$\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelt was synthesized according to the literature [37] through a hydrothermal method. In a typical process, 1 mmol AgNO_3 was first dissolved into 20 mL distilled water under magnetic stirring, then 1 mmol V_2O_5 was added and ultrasonic radiation was employed for 30 min to get a suspension. The obtained suspension was then transferred into a Teflon-lined stainless steel autoclave, heated to 170 °C and aged for 12 h. After being naturally cooled to room temperature, the final product was collected by centrifugation, washed with distilled water repeatedly until the pH value of the centrifugate was about 6. Then the obtained precipitate was washed with absolute ethanol, centrifuged and finally dried in air at room temperature and $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelt was thus obtained.

To obtain $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite, the obtained $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelt was irradiated by a certain dose of ^{60}Co γ -ray (Isotope Institute of Henan Academy of Sciences, the activity of the ^{60}Co γ -ray source is 7.4×10^{15} Bq), typically 20 kGy (1.0 g sample was sealed with PE film, located at the place where the dose rate is 200 Gy/min, and irradiated for 100 min). Under irradiation treatments, Ag nanoparticles were segregated at the surface of $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelts and hence $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite was formed [37].

For the construction of biosensor, 2 mg $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite was first dispersed in 2 mL double distilled water (abbreviated as dd H_2O), then 2 mL of the stock solution of Hb (2 mg/mL, 0.1 M phosphate-buffered solution [PBS], pH 7.0) was added and the mixture was equilibrated for 24 h at room temperature and centrifuged to collect the solid. The resulting $\text{Hb}/\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ composite was used for further testing.

Glassy carbon electrode (GCE) was first polished with 1.0 μm , 0.3 μm , and 0.05 μm alumina powder successively, followed by rinsing thoroughly with dd H_2O . The polished electrode was then sonicated in dd H_2O and acetone, and finally allowed to dry at room temperature.

The $\text{Hb}/\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ composite mentioned above was resuspended in 1 mL water, and 5 μL of this suspension was deposited onto the electrode surface. The electrode was then left to dry at 4 °C for at least 24 h. The sensor was stored under the same conditions when not in use.

2.2. Characterization and electrochemistry measurements

Powder X-ray diffraction patterns were obtained on a Philips X'Pert MPD Pro X-ray diffractometer operated with graphite-monochromatized high intensity Cu $\text{K}\alpha$ radiation at 50 kV. Transmittance electron microspectroscopy (TEM) images were recorded with a JEOL JEM-100S electron microscope at an accelerating voltage of 80 kV. Cyclic voltammetric experiments were conducted with a CHI660B workstation (Shanghai Chenhua). All experiments were carried out using a conventional three-electrode system, where GCE modified with $\text{Hb}/\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ was used as working electrode, a platinum wire was used as auxiliary electrode, and a saturated calomel electrode (SCE) was used as reference electrode. All solutions were deoxygenated by highly pure nitrogen before and during the measurements.

3. Results and discussion

3.1. XRD and TEM analysis

The powder XRD pattern of the as-prepared $\text{Ag}_2\text{V}_4\text{O}_{11}$ belt matches well with that reported previously [37]. A typical TEM image of $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite obtained by γ -ray treatment of $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelt is shown in Fig. 1. It can be seen that the silver nanoparticles are well dispersed on the surface of $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelts and their diameters vary from 10 nm to 50 nm.

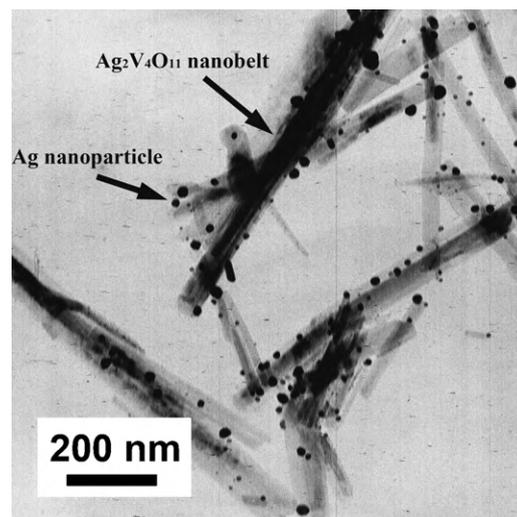


Fig. 1. TEM image of $\text{Ag}/\text{Ag}_2\text{V}_4\text{O}_{11}$ nanocomposite.

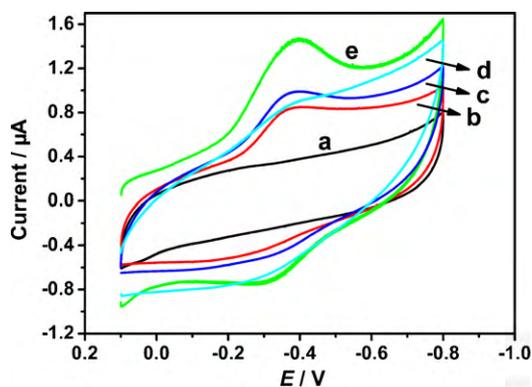


Fig. 2. Cyclic voltammograms of (a) Ag/Ag₂V₄O₁₁/GCE, (b) Hb/GCE, (c) Hb/Ag/GCE, (d) Hb/Ag₂V₄O₁₁/GCE, and (e) Hb/Ag/Ag₂V₄O₁₁/GCE in 0.1 M PBS (pH 7.0) at a scan rate of 100 mV/s.

3.2. Direct electrochemistry of Hb/Ag/Ag₂V₄O₁₁ modified electrode

The cyclic voltammograms (CVs) of different electrodes at a scan rate of 100 mV/s are given in Fig. 2. No peak appears at the electrode modified by Ag/Ag₂V₄O₁₁ nanocomposite, indicating that Ag/Ag₂V₄O₁₁ nanocomposite itself is not electroactive. When the electrode was modified with Hb, only a small irreversible reduction peak was observed and the reduction current was decreased with the number of cycles, suggesting the possible Hb denaturation and desorption over time on electrode surface as well as the non-reversible electrochemical reaction of Hb. However, at the Hb/Ag/Ag₂V₄O₁₁ modified electrode, a couple of well-defined redox peaks were observed at -0.372 V and -0.326 V, respectively, and the formal potential is calculated to be -0.349 V. The value of formal potential is quite close to that of Hb in agarose hydrogel film, -0.348 V [42], and ZnO-MWCNTs/Nafion film, -0.353 V [43].

For comparison, CVs of Hb/Ag modified GCE and Hb/Ag₂V₄O₁₁ modified GCE are also shown in Fig. 2. At the Hb/Ag modified GCE, the CV curve is almost the same as Hb modified GCE, but the reduction peak current is much bigger than the latter. Obviously, the co-existed Ag nanoparticles significantly improved the direct electron transfer between Hb and GCE. This can be explained by the high electronic conductivity and excellent biocompatibility of Ag nanoparticles [27,30]. However, at the Hb/Ag₂V₄O₁₁ modified GCE, although the reduction peak current is close to that of Hb modified GCE, its redox peaks are not as well-defined as the latter. In fact, it is very hard to identify its reduction peak. This phenomenon may be ascribed to the high electronic conductivity and poor biocompatibility of Ag₂V₄O₁₁ nanobelts [19].

Comparing all the CVs shown in Fig. 2, one can easily find that the Hb/Ag/Ag₂V₄O₁₁ modified GCE exhibits the best performance. This result presents strong evidence that the direct electron transfer between Hb and GCE can be greatly improved by the co-existence of Ag/Ag₂V₄O₁₁ nanocomposite.

A schematic diagram illustrating the microstructures of Hb modified GCE and Hb/Ag/Ag₂V₄O₁₁ modified GCE is shown in Fig. 3. On the surface of Hb modified GCE, in spite of the multilayer assembly of Hb molecules, only one or two layers of Hb which is very close to the electrode surface could realize direct electron transfer to and from the electrode and hence show their electrochemical activity (blue spheres emphasized with red circles), leaving most of the Hb molecules in the multilayers electrochemically nonactive (blue spheres).

However, in the case of Hb/Ag/Ag₂V₄O₁₁ modified GCE, the co-existed Ag/Ag₂V₄O₁₁ nanocomposites crossly contact with each other and thus a network which accommodates Hb molecules is formed. Because of the high electronic conductivity of Ag₂V₄O₁₁

[19] and Ag [27] as well as the biocompatibility of Ag [30], one can reasonably suppose that Ag/Ag₂V₄O₁₁ nanocomposite is both electronic conductive and biocompatible. Hence the network composed of Ag/Ag₂V₄O₁₁ nanocomposites could be a good matrix for the immobilization of biomolecules. Through the network matrix, Hb molecules that are directly contacted with Ag/Ag₂V₄O₁₁ nanocomposite are able to exchange electrons with GCE, showing electrochemical activity. Here Ag/Ag₂V₄O₁₁ nanocomposite plays the role of electronic bridge through which electrons are transferred from Hb to GCE or vice versa. Taking into account the much larger surface area of the network (compared with bare electrode), it is obvious that a great number of Hb molecules can transfer electrons to and from GCE indirectly and thus demonstrates their electrochemical activity.

In the above discussion, the orientations of Hb molecules are not taken into consideration. In fact, Hb molecules adopt random orientations on the surface of either GCE or Ag/Ag₂V₄O₁₁ nanocomposite. The possibilities of a favorable orientation of Hb on both surfaces are supposed to be equal because their space geometries are both global sphere referenced to a flat plane. Thus the specific value of the possibility is not the essential factor in the above discussion.

In summary, it is the conductive and biocompatible network of Ag/Ag₂V₄O₁₁ in which Hb molecules are accommodated that greatly improved the direct electron transfer between Hb and GCE. And the relatively lower electrochemical activity of Hb on Ag modified GCE and Ag₂V₄O₁₁ modified GCE can consequently be explained as follows. Ag nanoparticles are both conductive and biocompatible, but their small size and spherical shape make them difficult to form a network, hence the improvement effect of Ag nanoparticles is limited. On the other hand, Ag₂V₄O₁₁ nanobelts can construct a conductive network to accommodate Hb molecules, but their biocompatibility is poor. Thus Hb molecules may be denatured and the improvement effect of Ag₂V₄O₁₁ nanobelts is even more limited.

To further examine the direct electron transfer behavior between Hb and Ag/Ag₂V₄O₁₁ modified GCE, different scan rates were applied and the corresponding CVs are shown in Fig. 4. The electrode modified with Hb/Ag/Ag₂V₄O₁₁ shows well-defined peaks at different scan rates from 20 mV/s to 1000 mV/s. With the increase of scan rate, the redox peak currents of the Hb/Ag/Ag₂V₄O₁₁ modified electrode increase linearly, indicating a surface-controlled electrochemical process.

For thin-layer electrochemistry, the integration of CV peak gives the total amount of charge (Q) passed through the electrode for reduction or oxidation of electroactive species in the thin film. The apparent surface concentration (Γ^*) of electroactive species can be

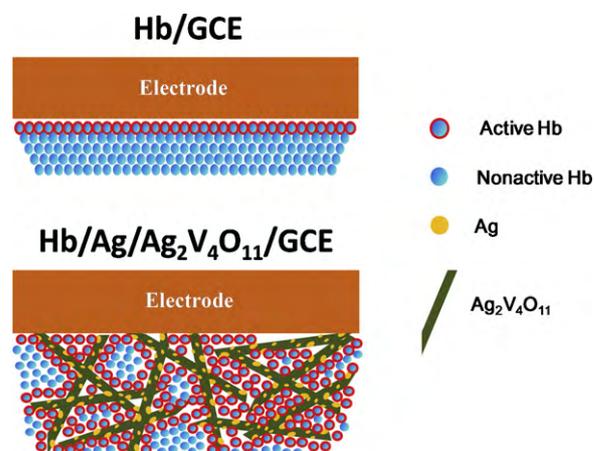


Fig. 3. A schematic diagram sketching the microstructures of Hb modified GCE and Hb/Ag/Ag₂V₄O₁₁ modified GCE.

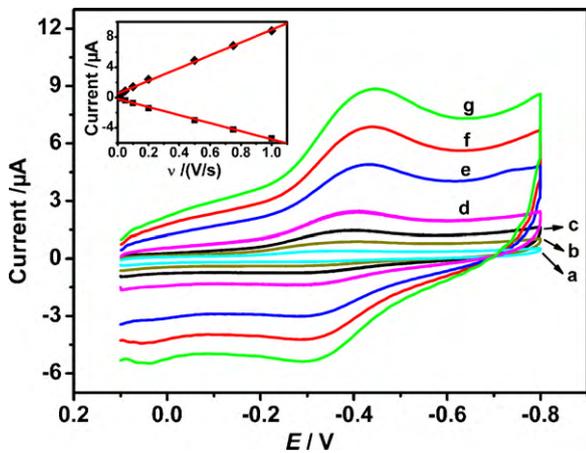


Fig. 4. Cyclic voltammograms of Hb/Ag/Ag₂V₄O₁₁ modified GCE in 0.1 M PBS (pH 7.0) at different scan rates: (a) 20 mV/s, (b) 50 mV/s, (c) 100 mV/s, (d) 200 mV/s, (e) 500 mV/s, (f) 750 mV/s, and (g) 1000 mV/s. Inset shows plot of peak current versus scan rate.

calculated from the following equation:

$$Q = nFA\Gamma^*$$

where n is the number of electrons transferred per biomolecule, F is Faraday's constant, and A is the electrode area. According to the equation, the average surface coverage of Hb was calculated to be 1.6×10^{-10} mol/cm² for the Hb/Ag/Ag₂V₄O₁₁ modified GCE. The value is more than 8 times of the theoretical monolayer coverage of Hb ($\sim 1.89 \times 10^{-11}$ mol/cm²) on the basis of the crystallographic dimensional structure of Hb and assuming that the biomolecule adopts an orientation with the long axis parallel to the electrode surface [44]. The striking big surface coverage can be ascribed to the co-existence of Ag/Ag₂V₄O₁₁ nanocomposites which play the role of electronic bridges and connect more Hb molecules with the electrode, indicating that multilayers of Hb participate in the electron transfer process. The crossly contacted Ag/Ag₂V₄O₁₁ nanocomposites form a matrix with a high electronic conductivity and greatly expand the surface area of the electrode, leading to a much larger interacting surface between the electrode and Hb multilayers.

A small peak-to-peak separation always indicates a fast electron transfer rate. The electron transfer rate constant k_s can be estimated by the Laviron equation [45]:

$$\log k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log \left(\frac{RT}{nFv} \right) - \frac{\alpha(1 - \alpha)nF\Delta E_p}{2.3RT}$$

where α is the charge transfer coefficient, R is the gas constant, T is the absolute temperature, ΔE_p is the peak potential separation, and v is the scan rate. A graph of the peak potential versus the logarithm of the scan rate yields a straight line (not shown), and from the slope of which a charge transfer coefficient of 0.45 was estimated for Hb. The peak-to-peak separations were 46 mV, 58 mV, 81 mV, and 87 mV at scan rates of 100 mV/s, 200 mV/s, 500 mV/s, and 750 mV/s respectively, giving an average k_s value of 2.6 s^{-1} . This value is much larger than the electron transfer rate constant of Hb in ZnO-MWCNTs/Nafion film (1.14 s^{-1}) [43], α -ZrP (1.85 s^{-1}) [46], silica-coated gold nanorods (0.83 s^{-1}) [47], and Au-SBA-15 (0.8 s^{-1}) [48], showing that the electron transfer between Hb in the matrix of Ag/Ag₂V₄O₁₁ nanocomposite and GCE is facile. This can be explained by the bridging effect of Ag/Ag₂V₄O₁₁ nanocomposite due to the excellent electronic conductivity, good biocompatibility and large surface area of Ag/Ag₂V₄O₁₁ nanocomposite network.

For comparison, CVs of Hb/Ag/GCE and Hb/Ag₂V₄O₁₁/GCE at different scan rates are shown in Figs. 5 and 6. From Fig. 5, the apparent surface concentration (Γ^*) of Hb and electron transfer constant (k_s)

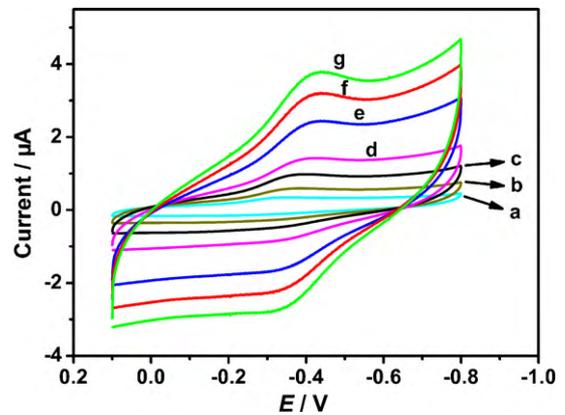


Fig. 5. Cyclic voltammograms of Hb/Ag modified GCE in 0.1 M PBS (pH 7.0) at different scan rates: (a) 20 mV/s, (b) 50 mV/s, (c) 100 mV/s, (d) 200 mV/s, (e) 500 mV/s, (f) 750 mV/s, and (g) 1000 mV/s.

are calculated to be 5.2×10^{-11} mol/cm² (about 2.8 times of monolayer coverage) and 1.4 s^{-1} , respectively. Both values are smaller than those for Hb/Ag/Ag₂V₄O₁₁/GCE, confirming that Ag/Ag₂V₄O₁₁ nanocomposite is a better matrix than Ag nanoparticles for Hb immobilization. In Fig. 6, redox peaks are not well-defined and both Γ^* and k_s are unable to be calculated, suggesting that Hb might be denatured and that Ag₂V₄O₁₁ nanobelt is not a good matrix for Hb immobilization.

3.3. Influence of solution pH on the direct electron transfer of Hb

In order to further study the electron transfer behavior of Hb/Ag/Ag₂V₄O₁₁ modified GCE, the influence of solution pH on the direct electron transfer of Hb was also examined. An increase of solution pH caused a negative shift of both cathodic and anodic peak potentials, and all changes in voltammetric peak potentials and currents with pH were reversible. For example, the CV for the Hb/Ag/Ag₂V₄O₁₁/GCE at pH 9.0 was reproduced after immersion in pH 4.0 buffer and then returned to the pH 9.0 buffer. The increase of pH also resulted in a negative shift of the formal potential (E^0) accordingly, in agreement with that reported previously. As shown in Fig. 7, the E^0 of the heme Fe(III)/Fe(II) redox couple for the Hb/Ag/Ag₂V₄O₁₁ modified electrodes has a linear relationship with pH in the range of 4.0–8.0 and a slope of -45.8 mV pH^{-1} . This value is much smaller than -57.8 mV pH^{-1} at 18°C for a reversible one-proton coupled single-electron transfer during electrochemical reduction. This may be due to the effect of the protonation states

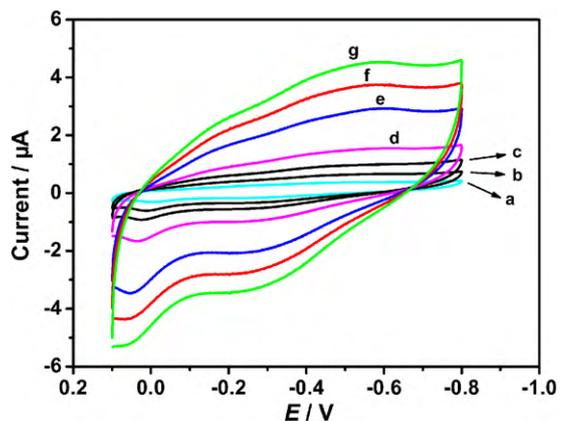


Fig. 6. Cyclic voltammograms of Hb/Ag₂V₄O₁₁ modified GCE in 0.1 M PBS (pH 7.0) at different scan rates: (a) 20 mV/s, (b) 50 mV/s, (c) 100 mV/s, (d) 200 mV/s, (e) 500 mV/s, (f) 750 mV/s, and (g) 1000 mV/s.

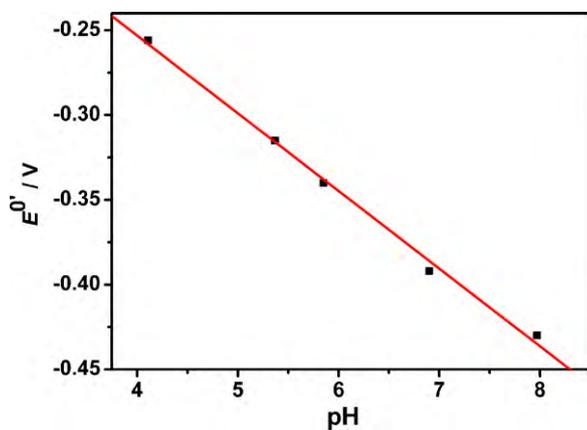
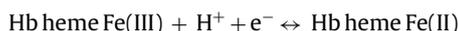


Fig. 7. Influence of solution pH on the formal potential of Hb/Ag/Ag₂V₄O₁₁ modified GCE.

of trans ligands to the heme iron and amino acids around the heme or to the protonation of water molecules coordinated to the center that may exist in different states under different pH values. Thus, the reaction equation for the electrochemical reduction of Hb may be described as follows:



3.4. Electrocatalysis of Hb/Ag/Ag₂V₄O₁₁/GCE to the reduction of H₂O₂

Heme proteins have peroxidase activity and can be used to reduce H₂O₂ through electrochemical catalysis. Based on the excellent electrochemical behaviors of Hb/Ag/Ag₂V₄O₁₁ modified GCE, it was applied to construct a biosensor for the detection of H₂O₂ in aqueous solution.

The CVs of Hb/Ag/Ag₂V₄O₁₁ modified electrode in 0.1 M PBS (pH 7.0) with different concentrations of H₂O₂ are shown in Fig. 8. The reduction peak current increased and the anodic peak current decreased dramatically with the addition of H₂O₂ on the Hb/Ag/Ag₂V₄O₁₁ modified electrode. Moreover, the currents of the reduction peaks increased with the increase of H₂O₂ concentration, indicating a typical electrocatalytic reduction process.

The reduction peak currents have a linear response to H₂O₂ concentration in the range of 1.0–120 μM with a detection limit of

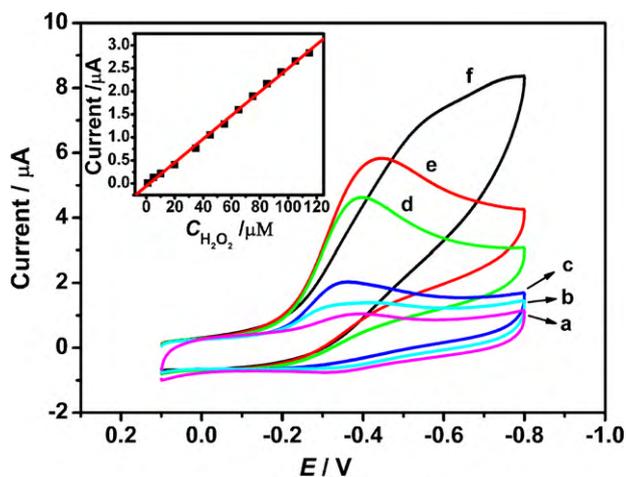


Fig. 8. Cyclic voltammograms of Hb/Ag/Ag₂V₄O₁₁/GCE with different H₂O₂ concentrations in 0.1 M PBS (pH 7.0) at a scan rate of 100 mV/s: (a) 0 μM, (b) 1 μM, (c) 10 μM, (d) 50 μM, (e) 100 μM, and (f) 200 μM. Inset shows plot of catalytic current versus H₂O₂ concentration.

Table 1

Linear range and detection limit of H₂O₂ for some Hb based electrodes.

Matrix	Linear range (μM)	Detection limit (μM)
Ag/Ag ₂ V ₄ O ₁₁ ^a	1.0–120	0.3
ZnO-MWCNTs/Nafion [43]	0.2–12	0.084
α-ZrP [46]	0.2–10.8	0.07
Carbon nanotube [49]	210–900	9

^a This work.

0.3 μM ($n = 13$, $R = 0.999$, inset in Fig. 5). The relative standard deviation (RSD) of the peak current in six successive determinations at an H₂O₂ concentration of 1.0 μM was 4.98% for Hb/Ag/Ag₂V₄O₁₁ modified GCE.

For comparison, other reported works of Hb immobilization on electrodes for H₂O₂ detection are listed in Table 1. Viewed from linear range and detection limit, the Hb/Ag/Ag₂V₄O₁₁ modified glassy carbon electrode is an excellent electrochemical biosensor for the determination of H₂O₂.

4. Conclusions

The direct electron transfer between hemoglobin and glassy carbon electrode can be greatly improved by the modification of Ag/Ag₂V₄O₁₁ nanocomposite. With the aid of Ag/Ag₂V₄O₁₁ nanocomposite, the apparent surface concentration (Γ^*) of Hb on the electrode surface is calculated to be 1.6×10^{-10} mol/cm², more than 8 times of the theoretical monolayer coverage of Hb, and the electron transfer rate constant k_s is estimated to be 2.6 s^{-1} , also much larger than previously reported values. In addition, the biosensor of Hb/Ag/Ag₂V₄O₁₁ modified GCE exhibits a good electrocatalytic response for monitoring H₂O₂ and has a large linear detection range from 1.0 μM to 100 μM with a detection limit of 0.3 μM in the pH 7.0 solution. The fast electron transfer rate and excellent catalytic ability to the reduction of H₂O₂ also show that the protein retains its bioactivity well. The excellent performances may be ascribed to the unique electronic conductivity of Ag₂V₄O₁₁ nanobelts and biocompatibility of Ag nanoparticles. The matrix of Ag/Ag₂V₄O₁₁ nanocomposite may provide a new platform for the realization of direct electron transfer of biomolecules and the construction of biosensors.

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References

- [1] X.H. Chen, J.Q. Hu, Z.W. Chen, X.M. Feng, A.Q. Li, Nanoplated bismuth titanate sub-microspheres for protein immobilization and their corresponding direct electrochemistry and electrocatalysis, *Biosens. Bioelectron.* 24 (2009) 3448–3454.
- [2] X. Chen, C.L. Fu, W.S. Yang, Graphite nanosheet-based composites for mediator-free H₂O₂ biosensor, *Analyst* 134 (2009) 2135–2140.
- [3] D. Shan, G.X. Cheng, D.B. Zhu, H.G. Xue, S. Cosnier, S.N. Ding, Direct electrochemistry of hemoglobin in poly (acrylonitrile-co-acrylic acid) and its catalysis to H₂O₂, *Sens. Actuators, B* 137 (2009) 259–265.
- [4] Q. Lu, T. Zhou, S.S. Hu, Direct electrochemistry of hemoglobin in PHEA and its catalysis to H₂O₂, *Biosens. Bioelectron.* 22 (2007) 899–904.
- [5] J.W. Shie, U. Yogeswaran, S.M. Chen, Haemoglobin immobilized on nafion modified multi-walled carbon nanotubes for O₂, H₂O₂ and CCl₃COOH sensors, *Talanta* 78 (2009) 896–902.
- [6] M.J. Eddowes, H.A.O. Hill, Novel method for the investigation of the electrochemistry of metalloproteins: Cytochrome c, *J. Chem. Soc., Chem. Commun.* 21 (1977) 771b–772b.
- [7] P. Yeh, T. Kuwana, Reversible electrode-reaction of cytochrome-c, *Chem. Lett.* 10 (1977) 1145–1148.

- [8] J. Wang, Electrochemical glucose biosensors, *Chem. Rev.* 108 (2008) 814–825.
- [9] J.B. Jia, B.Q. Wang, A.G. Wu, G.J. Cheng, Z. Li, S.J. Dong, A method to construct a third-generation horseradish peroxidase biosensor: self-assembling gold nanoparticles to three-dimensional sol-gel network, *Anal. Chem.* 74 (2002) 2217–2223.
- [10] A. Heller, Electrical wiring of redox enzymes, *Acc. Chem. Res.* 23 (1990) 128–134.
- [11] Y. Liu, M.K. Wang, F. Zhao, Z.A. Xu, S.J. Dong, The direct electron transfer of glucose oxidase and glucose biosensor based on carbon nanotubes/chitosan matrix, *Biosens. Bioelectron.* 21 (2005) 984–988.
- [12] F. Wang, X.X. Chen, Y.X. Xu, S.S. Hu, Z.N. Gao, Enhanced electron transfer for hemoglobin entrapped in a cationic gemini surfactant films on electrode and the fabrication of nitric oxide biosensor, *Biosens. Bioelectron.* 23 (2007) 176–182.
- [13] J.W. Li, C. Fan, F. Xiao, R. Yan, S.S. Fan, F.Q. Zhao, B.Z. Zeng, Influence of ionic liquids on the direct electrochemistry of glucose oxidase entrapped in nanogold-N, N-dimethylformamide-ionic liquid composite film, *Electrochim. Acta* 52 (2007) 6178–6185.
- [14] J.W. Di, S.H. Peng, C.P. Shen, Y.S. Gao, Y.F. Tu, One-step method embedding superoxide dismutase and gold nanoparticles in silica sol-gel network in the presence of cysteine for construction of third-generation biosensor, *Biosens. Bioelectron.* 23 (2007) 88–94.
- [15] D. Du, X. Huang, J. Cai, A.D. Zhang, Amperometric detection of triazophos pesticide using acetylcholinesterase biosensor based on multiwall carbon nanotube-chitosan matrix, *Sens. Actuators B* 127 (2007) 531–535.
- [16] C.X. Cai, J. Chen, Direct electron transfer and bioelectrocatalysis of hemoglobin at a carbon nanotube electrode, *Anal. Biochem.* 325 (2004) 285–292.
- [17] S.G. Peng, Q.M. Gao, Q.G. Wang, J.L. Shi, Layered structural heme protein magadiite nanocomposites with high enzyme-like peroxidase activity, *Chem. Mater.* 16 (2004) 2675–2684.
- [18] K.J. Takeuchi, A.C. Marschilok, S.M. Davis, R.A. Leising, E.S. Takeuchi, Silver vanadium oxides and related battery applications, *Coord. Chem. Rev.* 219 (2001) 283–310.
- [19] S.Y. Zhang, W.Y. Li, C.S. Li, J. Chen, Synthesis, characterization, and electrochemical properties of $\text{Ag}_2\text{V}_4\text{O}_{11}$ and AgVO_3 1-D nano/microstructures, *J. Phys. Chem. B* 110 (2006) 24855–24863.
- [20] F. Huang, Z.W. Fu, Q.Z. Qin, A novel $\text{Li}_2\text{Ag}_{0.5}\text{V}_2\text{O}_5$ composite film cathode for all-solid-state lithium batteries, *Electrochem. Commun.* 5 (2003) 262–266.
- [21] K.J. Takeuchi, R.A. Leising, M.J. Palazzo, A.C. Marschilok, E.S. Takeuchi, Advanced lithium batteries for implantable medical devices: mechanistic study of SVO cathode synthesis, *J. Power Sources* 119 (2003) 973–978.
- [22] S.J. Bao, Q.L. Bao, C.M. Li, T.P. Chen, C.Q. Sun, Z.L. Dong, Y. Gan, J. Zhang, Synthesis and electrical transport of novel channel-structured β - AgVO_3 , *Small* 3 (2007) 1174–1177.
- [23] G.Z. Shen, D. Chen, Self-coiling of $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelts into perfect nanorings and microloops, *J. Am. Chem. Soc.* 128 (2006) 11762–11763.
- [24] A.M. Crespi, S.K. Somdahl, C.L. Schmidt, P.M. Skarstad, Evolution of power sources for implantable cardioverter defibrillators, *J. Power Sources* 96 (2001) 33–38.
- [25] C.J. Mao, X.C. Wu, H.C. Pan, J.J. Zhu, H.Y. Chen, Single-crystalline $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelts: hydrothermal synthesis, field emission, and magnetic properties, *Nanotechnology* 16 (2005) 2892–2896.
- [26] S. Mohapatra, Y.K. Mishra, J. Ghatak, D. Kabiraj, D.K. Avasthi, Surface plasmon resonance of Ag nanoparticles embedded in partially oxidized amorphous Si matrix, *J. Nanosci. Nanotechnol.* 8 (2008) 4285–4289.
- [27] S.E. Kim, J.U. Kim, Y.H. Han, B.C. Lee, J.C. Lee, Size Controlled miniscale synthesis of silver nanoparticles using TEM electron beam, *J. Nanosci. Nanotechnol.* 8 (2008) 5212–5215.
- [28] L. Faucher, E.F. Borra, A.M. Ritchey, Use of thiols as protecting ligands in reflective surface films of silver nanoparticles, *J. Nanosci. Nanotechnol.* 8 (2008) 3900–3908.
- [29] Z.L. Wang, Oxide nanobelts and nanowires—growth, properties and applications, *J. Nanosci. Nanotechnol.* 8 (2008) 27–55.
- [30] R.D. Deshmukh, R.J. Composto, Surface segregation and formation of silver nanoparticles created in situ in poly (methyl methacrylate) films, *Chem. Mater.* 19 (2007) 745–754.
- [31] K. Esumi, T. Tano, K. Torigoe, K. Meguro, Preparation and characterization of bimetallic palladium-copper colloids by thermal decomposition of their acetate compounds in organic solvents, *Chem. Mater.* 2 (1990) 564–567.
- [32] A. Taleb, V. Rousier, A. Courty, M.P. Pilemi, Optical anisotropy of organized silver nanoparticles in 2D superlattice, *Appl. Surf. Sci.* 162 (2000) 655–661.
- [33] A. Henglein, Reduction of $\text{Ag}(\text{CN})_2^-$ on silver and platinum colloidal nanoparticles, *Langmuir* 17 (2001) 2329–2333.
- [34] P.C. Lee, D. Meisel, Adsorption and surface-enhanced Raman of dyes on silver and gold sols, *J. Phys. Chem.* 86 (1982) 3391–3395.
- [35] K.C. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, Preparation and characterization of Au colloid monolayers, *Anal. Chem.* 67 (1995) 735–743.
- [36] G.C. Li, K. Chao, C.S. Ye, H. Peng, One-step synthesis of Ag nanoparticles supported on AgVO_3 nanobelts, *Mater. Lett.* 62 (2008) 735–738.
- [37] C.L. Lu, L. Han, W.P. Ding, G. Yang, X.F. Guo, W.H. Hou, In-situ controllable synthesis of Ag nanoparticles: irradiation induced surface segregation of $\text{Ag}_2\text{V}_4\text{O}_{11}$ nanobelt, *J. Nanosci. Nanotechnol.* 9 (2009) 6554–6559.
- [38] S.W. Keller, H.N. Kim, T.E. Mallouk, Layer-by-layer assembly of intercalation compounds and heterostructures on surfaces: Toward molecular “beaker” epitaxy, *J. Am. Chem. Soc.* 116 (1994) 8817–8818.
- [39] P.N. Bartlett, P.R. Birkin, J.H. Wang, F. Palmisano, G. De Benedetto, An enzyme wwith employing direct electrochemical communication between horseradish peroxidase and a poly (aniline) film, *Anal. Chem.* 70 (1998) 3685–3694.
- [40] E.C. Hurdis, H. Romeyn, Accuracy of determination of hydrogen peroxide by cerate oxidimetry, *Anal. Chem.* 26 (1954) 320–325.
- [41] C. Matsubara, N. Kawamoto, K. Takamura, Oxo[5, 10, 15, 20-tetra(4-pyridyl)porphyrinato]titanium(IV): an ultra-high sensitivity spectrophotometric reagent for hydrogen peroxide, *Analyst* 117 (1992) 1781–1784.
- [42] S.F. Wang, T. Chen, Z.L. Zhang, X.C. Shen, Z.X. Lu, D.W. Pang, Direct electrochemistry and electrocatalysis of heme proteins entrapped in agarose hydrogel films in room-temperature ionic liquids, *Langmuir* 21 (2005) 9260–9266.
- [43] W. Ma, D. Tian, Direct electron transfer and electrocatalysis of hemoglobin in ZnO coated multiwalled carbon nanotubes and nafion composite matrix, *Bioelectrochemistry* 78 (2010) 106–112.
- [44] E. Laviron, General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems, *J. Electroanal. Chem.* 101 (1979) 19–28.
- [45] Y.Z. Xian, Y. Xian, L.H. Zhou, F.H. Wu, Y. Ling, L.T. Jin, Encapsulation hemoglobin in ordered mesoporous silicas: influence factors for immobilization and bioelectrochemistry, *Electrochem. Commun.* 9 (2007) 142–148.
- [46] Y.G. Liu, C.L. Lu, W.H. Hou, J.J. Zhu, Direct electron transfer of hemoglobin in layered α -zirconium phosphate with a high thermal stability, *Anal. Biochem.* 375 (2008) 27–34.
- [47] J.J. Zhang, Y.G. Liu, L.P. Jiang, J.J. Zhu, Synthesis, characterizations of silica-coated gold nanorods and its applications in electroanalysis of hemoglobin, *Electrochem. Commun.* 10 (2008) 355–358.
- [48] Y. Xian, Y. Xian, L. Zhou, F. Wu, Y. Ling, L. Jin, Encapsulation hemoglobin in ordered mesoporous silicas: influence factors for immobilization and bioelectrochemistry, *Electrochem. Commun.* 9 (2007) 142–148.
- [49] Y.D. Zhao, Y.H. Bi, W.D. Zhang, Q.M. Luo, The interface behavior of hemoglobin at carbon nanotube and the detection for H_2O_2 , *Talanta* 65 (2005) 489–494.

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