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Pseudo-bi-enzyme glucose sensor: ZnS hollow spheres and glucose oxidase concerted catalysis glucose†

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This work creatively uses peroxidase-like ZnS hollow spheres (ZnS HSs) to cooperate with glucose oxidase (GOx) for glucose determinations. This approach is that the ZnS HSs electrocatalytically oxidate the enzymatically generated H_2O_2 to O_2, and then the O_2 circularly participates in the previous glucose oxidation by glucose oxidase. Au nanoparticles (AuNPs) and carbon nanotubes (CNTs) are used as electron transfer and enzyme immobilization matrices, respectively. The biosensor of glucose oxidase–carbon nanotubes–Au nanoparticles–ZnS hollow spheres–gold electrode (GOx–CNT–AuNPs–ZnS HSs–GE) exhibits a rapid response, a low detection limit (10 μM), a wide linear range (20 μM to 7 mM) as well as good anti-interference, long-term longevity and reproducibility.

Introduction

Diabetes mellitus, which can cause death and disability, is a worldwide public health problem. The complications of diabetes are numerous, including high risks of heart disease, kidney failure and blindness.[1,2] If blood glucose can be personally controlled within the normal range of 80–120 mg dL^{-1} (4.4–6.6 mM) by tight monitoring, these complications would be greatly reduced.[2] In the past decades, glucose sensors have been widely researched. Vast numbers of articles on glucose detection in different ways have been published, including electrochemiluminescence,[3] photoluminescence,[4,5] phosphorescence,[6] fluorescence,[7,8] colorimetric methods[9,10] and electroanalysis.[11–13] Thereinto, electroanalysis has the merits of simple operation, low cost and energy consumption. Hence, based on glucose oxidase (GOx), amperometric glucose biosensors have been playing a leading role in the blood sugar test.

The reaction mechanisms by which glucose is catalyzed by glucose oxidase are extensively researched.[1,2] In 1936, Hugo Theorell showed that the reaction of the reduced "yellow enzyme" with O_2 produced H_2O_2.[14] Hence, plenty of research on the detection of glucose through H_2O_2 testing as been reported. Therein, a bi-enzyme which takes full advantage of the bi-enzymes–GOx and horseradish peroxidase (HRP) seems particularly conspicuous.[15–19] Briefly, HRP acts as an amperometric transducer for the detection of its substrate, H_2O_2, which is *in situ* generated by the GOx-catalyzed oxidation of glucose. However, the activity and stability of the enzymes may be weakened while enzymes are immobilized on the surface of the electrode. At the same time, the costs will be increased. To remedy these problems, HRP is replaced by composite materials[20–26] in biosensors with good sensitivity and a wide detection range and excellent stability, such as Prussian blue, thionin, Pt nanoparticles, and so on. But, these materials reduce hydrogen peroxide to water irreversibly, making the oxygen a one-time consumable, which will not only decrease the sensitivity but also increase the costs. So our work makes up for this drawback.

In this work, we use ZnS HSs to replace HRP. The ZnS HSs catalyze H_2O_2 to produce O_2, and then the generated O_2 continues to attend to the reaction. As inorganic semiconductors, ZnS HSs can amplify the electrical signals because of their good electrical conductivity and unique structure. More than that, a strong chemical bond is formed between ZnS HSs and the surface of the gold electrode, which can make the biosensor more stable. Besides, there would not be an activity decline of the ZnS HSs. But their micron sizes limit the efficacy on specific areas, so we add AuNPs to expand the reaction surface and to enhance the adsorption efficiency of subsequent materials and enzymes. In addition to these, carbon nanotubes (CNTs) can make electron transfer possible, due to their exceptional structural, electronic and mechanical properties. Carbon nanotubes (CNTs) decorated by polydiallyldimethylammonium chloride (PDDA) are used as enzyme immobilization matrices. Through electrostatic adsorption, negatively charged GOx (isoelectric point is about 4.2) are tightly fastened onto these positively charged CNTs.

Materials and methods

Instruments and chemicals

The electrochemical measurements were performed on a CHI604 electro-chemical work station (Shanghai, China). The

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conventional three-electrode system included a gold electrode as the working electrode, a saturated calomel as the reference electrode (SCE), and a platinum wire as the auxiliary electrode. Transmission electron microscopy (TEM) observations were performed on a JEOL (JEM 2100) transmission emission microscope operated at a 200 kV accelerating voltage. XRD patterns were taken using X-ray diffraction (XRD-3D, PuXi, Beijing, China).

Glucose oxidase (GOx), chloroauric acid ($HAuCl_4$), carbon nanotubes (CNTs) and polydiallyldimethylammonium chloride (PDDA) were all purchased from Aladdin (Shanghai, China). Glucose, sodium citrate, $ZnSO_4 \cdot 7H_2O$, $Na_2S_2O_3 \cdot 5H_2O$ and all other chemicals were of analytical grade. All solutions were prepared with double-distilled water. Phosphate buffer (PB, 0.1 M) solutions with various pH values were prepared by mixing stock standard solutions of K_2HPO_4 and KH_2PO_4.

Synthesis of ZnS HSs, AuNPs and CNT@PDDA

ZnS HSs. 2.9 g $ZnSO_4 \cdot 7H_2O$ and 2.48 g $Na_2S_2O_3 \cdot 5H_2O$ were loaded into a 50 mL Teflon-lined stainless-steel autoclave, which was then filled with distilled water up to 90% of the total volume. The autoclave was sealed and maintained at 200 °C for 4 h. The resulting white solid products were filtered off, washed with absolute ethanol and distilled in water for several times, then dried in a vacuum at 60 °C for 6 h.[27]

AuNPs. A 50 mL sample of aqueous $HAuCl_4$ (0.25 mM) was prepared in a 100 mL flask, adjusting the pH of the solution to 7.5 with 0.5 M NaOH. The solution was brought to the boil while being stirred, and 0.45 mL of 5% aqueous sodium citrate was added. The reaction was allowed to run until the solution reached a wine red color.[28]

CNT@PDDA. 10 mg CNTs were mixed with 50 mL of 1% PDDA aqueous solution, followed by circulation reflux for 5 h. The solution was centrifuged and washed with water in order to remove the excessive PDDA. The products were filtered off, washed with distilled water for several times, and then dried in a vacuum at 60 °C.[29] Hereafter, CNT is short for CNT@PDDA.

Preparation of the modified electrode

The assembly process of the electrode is described in Scheme 1. Prior to the modification, a bare gold electrode was sequentially polished to a mirror-like surface with a 0.3 and 0.05 μm alumina slurry, respectively. Then the electrode was successively sonicated in anhydrous ethanol and doubly distilled water (each for 5 min) and dried at room temperature.

ZnS HSs were fastened by cycling the potential between −1.2 and 1.2 V at a scan rate of 100 mV s^{-1} in a 1 mg mL^{-1} ZnS HSs solution for 20 consecutive cycles, then the electrode was washed with distilled water for several times. Secondly, the electrode was dipped into a AuNPs aqueous solution for 1 hour. It was rinsed with distilled water and dried in a nitrogen atmosphere. Thirdly, 5 μL of the 0.1 mg mL^{-1} CNT was dropped onto the surface of the previous electrode and allowed to dry at ambient temperature. Lastly 5 μL of the 1 mg mL^{-1} glucose oxidase PB solution (pH 7.5) was dropped as before and allowed to dry. Distilled water was used to wash in order to remove redundant glucose oxidase. The same procedure was employed to fabricate other modified electrodes.

Results and discussion

Characterization of ZnS HSs and CNT

The morphological features of ZnS HSs and CNT are investigated by TEM. From Fig. 1A, the hollow spheres are rather uniform with an average diameter of about 1 μm with a shell thickness of about 50–100 nm. Some peculiar hollow spheres are also seen in the TEM image (Fig. 1B) because the shell of the microspheres is relatively weak and easy to crack under collision. Consequently, it can be seen that the cracked microspheres also tend to form an

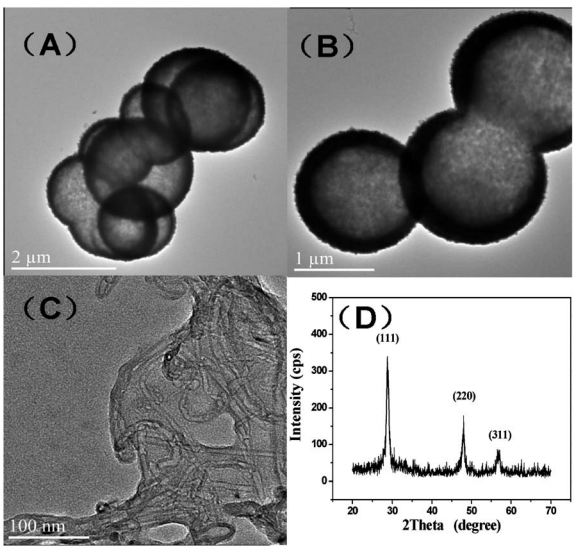
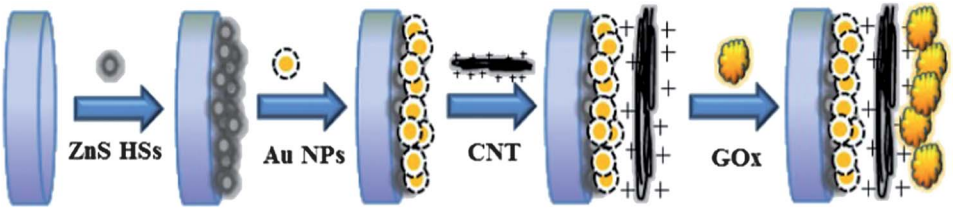


Fig. 1 TEM images of the ZnS HSs (A and B) and CNT (C), and the XRD pattern of ZnS HSs (D).



Scheme 1 The illustration of the stepwise fabrication process of the biosensor.

"8" configuration. Fig. 1D is a typical XRD pattern of the ZnS HSs. There are three diffraction peaks at 28.70°, 47.16° and 56.62°, which match well with the (111), (220) and (311) planes of the cubic ZnS.[30] Fig. 1C displays the morphology of the CNT by TEM. It can be seen that the hollow CNT stick together because they are modified by PDDA.

Electrochemical oxidation of H_2O_2 on ZnS HSs–GE

The cyclic voltammogram (CV) of ZnS HSs–GE in a PB solution (0.1 M, pH 7.4) is shown in Fig. 2A (curve a). Upon addition of H_2O_2, the shape of the redox couple changes significantly which is displayed in the characterization with the enhanced height of the anodic peak and the decreased height of the cathodic peak (curve b and c), which proves that H_2O_2 is oxidated by ZnS HSs. Fig. 2B, which is the amperometric response of ZnS HSs–GE to the successive addition of H_2O_2, indicates that ZnS HSs have a high catalytic activity towards H_2O_2.

Electrocatalytic oxidation of glucose on GOx–CNT–AuNPs–ZnS HSs–GE

CVs of differently modified electrodes in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ are shown in Fig. 3A. Compared with the bare gold electrode (curve a), the response current of the ZnS HSs electrode (curve b) is amplified due to the relatively favorable conductivity of ZnS HSs. After the adsorption of AuNPs, the current (curve c) increases slightly which indicates that AuNPs can improve the electron transfer between the electrode and the modified membrane. After dripping CNT, the response current of the electrode (curve d) decreased. Perhaps because CNT modified with PDDA lessen the electroconductibility of the electrode. As GOx are protein molecules which hamper electron transfer. The current of the electrode (curve e) decreases correspondingly, which proves that GOx is fastened onto the electrode successfully.

In order to study the reaction mechanism of the biosensor, CVs are used for investigating the electrocatalytic activity of the GOx–CNT–AuNPs–ZnS HSs–GE. Fig. 3B presents the CV responses of an as-prepared electrode in a 0.1 M PB solution (pH 7.4) with and without 0.5 mM glucose. Upon the addition of glucose, an increase of the anodic peak current and a little decrease of the cathodic peak current are obtained, which

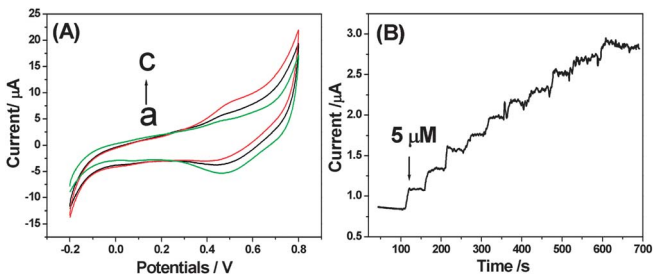


Fig. 2 (A) The CVs of the ZnS HSs–GE in a PB solution (0.1 M, pH 7.4) in the absence (a) and presence of 0.1 (b) and 0.2 mM H_2O_2 (c). The scan rate: 50 mV s^{-1}. (B) Amperometric responses of ZnS HSs–GE to the successive addition of H_2O_2 in a PB solution (0.1 M, pH 7.4) at an applied potential of 500 mV.

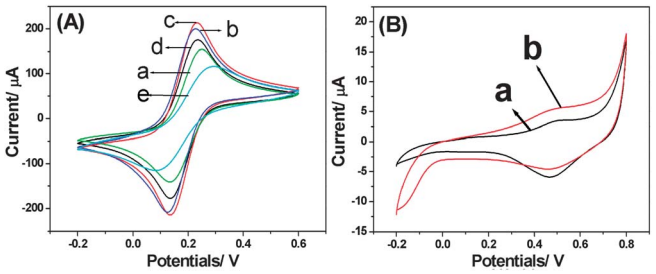


Fig. 3 (A) The CVs of (a) bare GE, (b) ZnS HSs–GE, (c) AuNPs–ZnS HSs–GE, (d) CNT–AuNPs–ZnS HSs–GE and (e) GOx–CNT–AuNPs–ZnS HSs–GE in 5.0 mM $[Fe(CN)_6]^{3-/4-}$. (B) The CVs of GOx–CNT–AuNPs–ZnS HSs–GE in a PB solution (0.1 M, pH 7.4) with (curve b) and without (curve a) 0.5 mM glucose. Scan rate: 50 mV s^{-1}.

demonstrates that the biosensor exhibits excellent electrocatalytic activity towards the oxidation of glucose.

The working principle of the modified electrode towards the detection of glucose is illustrated in Scheme 2. Glucose transfers from the bulk solution to the surface of the modified electrode through diffusion. GOx in the presence of the natural co-substrate O_2 converts glucose to gluconic acid and O_2 to H_2O_2 (eqn (1)). The H_2O_2 are oxidated to O_2 by ZnS HSs, and then O_2 sequentially participates in the reaction (eqn (2)). ZnS HSs would be recycled at the electrode surface.[1,31,32] In some of the literature, H_2O_2 is reduced to H_2O, as with Pt nanoparticles.[21] The mechanism of the biosensor is summarized as follows:

$$\text{Glucose} + O_2 + 2e^- + 2H^+ \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2 \quad (1)$$

$$2H_2O_2 \xrightarrow{\text{ZnS HSs}} O_2 + 2H_2O + 2e^- \quad (2)$$

Analytical performances of the GOx–CNT–AuNPs–ZnS HSs

The pH of the PB solution, which affects the activity and electrochemical behavior of GOx, is essential to the sensitivity of biosensors. As shown in Fig. 4A, it turns out that the optimum pH would be 7.5. Given the physiological pH (pH 7.4), the PB solution with pH 7.4 is chosen as the optimal condition for glucose detection.

The choice of the applied potential is necessary to achieve the lowest detection limit and to avoid interference from the electrochemical species. Fig. 4B displays the relationship between the response current of the electrode and the applied potential. We found that the current increases very slowly when the potential is below 0.4 V. While the potential exceeds 0.5 V, the current tends to be gentle. Thus, a detection potential of 0.5 V is selected in this work.

Scheme 2 Bio-electro-catalytic reaction catalyzed by GOx and ZnS HSs for the detection of glucose on the GOx–CNT–AuNPs–ZnS HSs modified electrode.

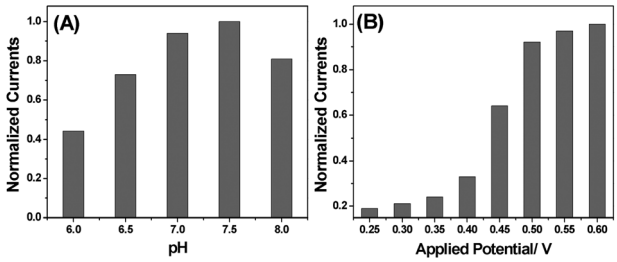


Fig. 4 The response current of the biosensor varies with pH value (A) and the applied potential (B). For a better comparison, the currents are normalized by the highest response at each condition.

The amperometric responses of different glucose biosensors are displayed in Fig. 5A. As the figure shows, the GOx–CNT–AuNPs–ZnS HSs–GE (curve c) has better linearity and a higher sensitivity compared with GOx–ZnS HSs–GE (curve a) and GOx–AuNPs–ZnS HSs–GE (curve b) under the same conditions. That is because the decorated CNT can connect the catalytic active site of GOx to the electrode surface to increase the biosensor sensitivity. AuNPs can expand the specific surface area and increase the adsorption of the subsequent materials and enzyme. Therefore, CNT and AuNPs not only have the ability to promote the electron transfer reaction when used as an electrode modification material, but also are promising as enzyme immobilization matrices due to their fine biological compatibility.

Fig. 5B is a typical amperometric response of the glucose biosensor after the addition of successive glucose to a PB solution (pH 7.4) at an applied potential of 0.5 V. As shown in Fig. 5B, a well-defined amperometric response with the response time less than 5 s is observed, which can demonstrate that the biosensor exhibits a rapid and sensitive response to the change of glucose concentration. The corresponding calibration curve of the fabricated amperometric glucose sensor is shown in inset of Fig. 5B, which exhibits a linear response in the concentration range from 0.02 mM to 7 mM with a low detection limit of 10 μM ($S/N = 3$). The equation is $I\,(\mu A) = 0.04 + 0.3\,[c_{glucose}]\,(mM)$ with a correlation coefficient of 0.9954. The high sensitivity may depend on the O_2 which participates in the reaction circularly and the special structure of the CNT which is beneficial for getting access to the active center of the enzyme (as shown in Table S1 in the ESI,† the analytical performance of the GOx–CNT–AuNPs–ZnS HSs–gold electrode was compared with other glucose biosensors).

Anti-interference, long-term longevity and reproducibility of the biosensor

In real samples, there are some coexisting electroactive species that may potentially interfere with glucose detection. The interferences selected for the test are ascorbic acid (AA), uric acid (UA) and L-cysteine (L-Cys). As shown in Fig. 6, the current responses to 0.5 mM of AA, UA and L-Cys are negligible compared to 0.5 mM of glucose. The result reveals the excellent anti-interference ability of the GOx–CNT–AuNPs–ZnS HSs electrode.

The long-term longevity of the biosensor was proved by continuous monitoring every day. After the daily detection of glucose for 21 days in a 0.1 M PB solution (pH 7.4), the biosensor showed a good stability and a 13% loss of activity (see the ESI, Fig. S1†). Thus, GOx is immobilized tightly by positively charged CNT, which can retain the electrocatalytic activity of GOx.

Five electrodes were fabricated independently, which showed an acceptable reproducibility with a R.S.D. of 4.3% for the current determined in a 0.5 mM glucose–PB solution.

Determination of glucose in a practical serum sample

In order to investigate the possible application of this new biosensor in clinical analysis, the modified electrode was also tested in real serum samples. Given that the biosensor is designed for non-diabetic blood sugar monitoring, we chose blood serum of a non-diabetic. A rapid and stable amperometric response was acquired at 0.5 V with the direct addition of 10 μL of samples to 10 mL of a 0.1 mol L^{-1} PB solution. The content of glucose in the samples was calculated by the calibration curve and the obtained results are shown in Table 1. Herein the concentration of glucose in the sample was also measured by clinical detection. The values given by the analyzer were in good agreement with our results, which demonstrated that the

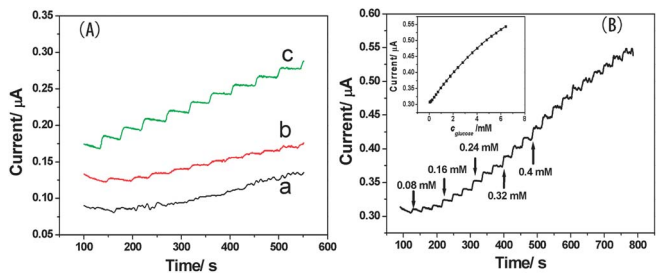


Fig. 5 (A) Amperometric responses of the GOx–ZnS HSs–GE (a), GOx–AuNPs–ZnS HSs–GE (b) and GOx–CNT–AuNPs–ZnS HSs–GE (c) to the successive addition of glucose under the same conditions; (B) amperometric responses of the GOx–CNT–AuNPs–ZnS HSs–GE to the successive addition of glucose to the PB solution (0.1 M, pH 7.4) at an applied potential of 500 mV. The inset shows the calibration curve for the response of the biosensors under optimal conditions.

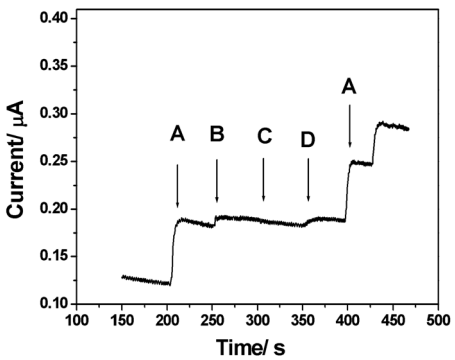


Fig. 6 Effect of interfering species to the response of the biosensor. (A) glucose (0.5 mM), (B) ascorbic acid (0.5 mM), (C) uric acid (0.5 mM) and (D) L-cysteine (0.5 mM).

Table 1 The glucose concentrations (mmol L^{-1}) measured with the GOx–CNT–AuNPs–/ZnS HSs–GE biosensor and clinical detection (average values of 3 measurements)

Sample no.	Determined by the biosensor	Clinical detection
1	4.33	4.25
2	6.79	6.82
3	5.25	5.30

as-prepared biosensor was effective and sensitive for the determination of glucose in actual samples. So it indicates a promising practicability for determining blood glucose in real biological samples.

Conclusions

In this work, ZnS HSs reveal an excellent electrical catalytic activity as they oxidate H_2O_2 to O_2 and then the O_2 participates in the reaction circularly. AuNPs and treated CNT exhibit favorable electrical conductivities and biocompatibilities for facilitating electron transfer between a protein and the electrode surface. Due to the remarkable synergistic effects of ZnS HSs, CNT, AuNPs and GOx, the biosensor exhibits a fast response, a broad linear range, a low detection limit and satisfactory stability and repeatability, which would have a great potential application in the determination of glucose.

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