A bienzyme channeling glucose sensor with a wide concentration range based on co-entrapment of enzymes in SBA-15 mesopores

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Abstract

A novel bienzyme-channeling sensor was constructed by entrapping glucose oxidase (GOD) and horseradish peroxidase (HRP) in the mesopores of well-ordered hexagonal mesoporous silica structures (SBA-15). The SBA-15 mesoporous materials accelerated the electron transfer between the entrapped HRP and electrode. Both HRP and GOD retained their catalytic activities in the bienzyme-entrapped SBA-15 film. In presence of glucose the enzymatic reaction of GOD-glucose-dissolved oxygen system generated hydrogen peroxide in the bienzyme-entrapped mesopores, which was immediately reduced at −0.40 V by an electrocatalytic reaction with the HRP entrapped in the same mesopore to lead to a sensitive and fast amperometric response. Thus the bienzyme channeling could be used for the detection of glucose with excellent performance without the addition of any mediator. Optimization of the experimental parameters was performed with regard to pH, operating potential and temperature. The detection limit was down to 2.7 × 10^{-7} M with a very wide linear range from 3.0 × 10^{-6} to 3.4 × 10^{-2} M. The constructed bienzyme channeling provided a strategy for amperometric detection of oxidase substrates by co-entrapping the corresponding oxidase and HRP in the mesoporous materials.

Keywords: Glucose oxidase; Horseradish peroxidase; Enzyme-channeling sensor; SBA-15; Glucose

1. Introduction

During the past 30 years numerous attempts have been made to assemble enzymes or proteins on suitable supports for biosensing purpose. Owing to the clinical significance of measuring blood glucose levels, many sensitive, selective and low-cost amperometric biosensors for glucose have been fabricated by immobilizing glucose oxidase (GOD) in different matrices (D’Orazio, 2003; Malhotra and Chaubey, 2003; Poscia et al., 2003; Forrow and Bayliff, 2005). These biosensors show practical advantages such as operation simplicity, low fabrication cost and suitability for real-time detection, etc. (Crouch et al., 2005; Shan et al., 2006). Most of them are based on measuring the increase of the anodic current during the oxidation of hydrogen peroxide (H2O2) produced from the oxidation of glucose by dissolved oxygen in presence of GOD (Guilbault and Lubrano, 1973) or the decrease of the cathodic current during the reduction of dissolved oxygen due to its consumption in the enzymatic reaction (Clark and Lyons, 1962; Updike and Hicks, 1967). H2O2 is usually detected at anodic potentials more positive than +0.6 V (vs. SCE) (Tian and Zhu, 2002) at which many oxidasable species such as ascorbic acid (AA), uric acid (UA) and acetaminophen (AP) will interfere with its detection. Thus a series of methods have been developed to suppress the interferences by reducing its oxidation overpotential (Yu et al., 2003; Liu et al., 2005). The amperometric biosensors based on the reduction of dissolved oxygen often suffer from the interference of H2O2 reduction (Liu and Ju, 2003; Huang et al., 2005), resulting in the narrow linear range and low sensitivity.

One attractive alternative approach to suppress the interferences and improve the biosensor performance has been performed by enzyme-channeling assays (Niculescu et al., 2000; Perez and Neto, 2001; Shi et al., 2003; Cosnier et al., 2006). The construction of bienzymatic peroxidase/oxidase amperometric biosensors has been applied for glucose detection (Ferri...
et al., 1998; Cosnier et al., 2000; Delvaux et al., 2005). In such configurations, \( \text{H}_2\text{O}_2 \) produced by the oxidase-substrate-dissolved oxygen system is detected through its horseradish peroxidase (HRP) mediated reduction, which permits the detection of glucose at substantially milder potential and can avoid the interference of the coexisting electroactive substances. However, these biocatalyst channeling based biosensors cannot detect glucose at high concentration. To meet the challenges posed by complex clinical samples and the need for on-line determination of serum glucose, which needs a wide detectable range of glucose up to more than 20 mM, this paper developed a novel biocatalyst-channeling sensor based on the co-entrapment of GOD and HRP in the mesopores of well-ordered hexagonal mesoporous silica structures (SBA-15). The preparation of biocatalyst channeling biosensors is inexpensive and can be performed in batches with tiny amounts of enzymes.

Recently, the unique structural, catalytic and biodegradation resistant properties of porous materials for constructing an electrochemical/electron transfer environment have attracted considerable attention (Walcarius, 2005). A series of porous materials such as micromachined silicon (Piechotta et al., 2005), titania sol–gel (Yu et al., 2003), gold nanoparticles microporous (Zhang et al., 2006) and electrically conductive polypyrrole composite (Li et al., 2005) have been proven to be promising as the immobilization matrices and applied in constructing glucose biosensors. Mesoporous molecular sieves have a large specific surface area, excellent mechanical, thermal, and chemical stability, good adsorption and penetrability. Thus it is a good matrix for protein immobilization through physical or chemical action (Xiao et al., 2003). Furthermore, it can reduce the insulating property of the protein and facilitate the electron transfer. This work made use of these advantages of mesoporous molecular sieves to present a novel approach for design of amperometric biosensors by assembling a biocatalyst channel on an electrode surface. The co-entrapment of HRP molecules in the mesopores avoided the aggregation of proteins on electrode, and thus accelerated the direct electron transfer between the heme in HRP and the electrode. The co-entrapment of HRP and GOD in the same mesopores made the generated hydrogen peroxide is immediately reduced by an electrocatalytic reaction with the HRP, leading to a sensitive amperometric response with a wide linear range in four orders of magnitude up to \( 3.4 \times 10^{-2} \) M. The proposed biosensor for glucose showed excellent performance such as short analysis time, high sensitivity, good reproducibility and acceptable stability for flow injection analysis (FIA). Therefore it could be extensively used to monitor glucose in food, environmental, clinic especially diabetic samples.

2. Experimental

2.1. Materials and reagents

GOD (EC 1.1.3.4, 35.3 units mg\(^{-1}\), Type II from Aspergillus niger), HRP (EC 1.11 1.7, RZ > 3.0, >250 units mg\(^{-1}\)) and \( \beta \)-D-(+)-glucose were purchased from Sigma and used as received. \( \text{H}_2\text{O}_2 \) (30% w/v solution) was purchased from Shanghai Biochemical Regent Co. (China). Nafion (10% in methanol with equivalent weight of about 1100) was obtained from Aldrich and was diluted to 5% with \( \text{H}_2\text{O} \) before use. Phosphate buffer solutions (PBS, 0.1 M) with various pH values were prepared by mixing stock standard solutions of \( \text{K}_2\text{HPO}_4 \) and \( \text{KH}_2\text{PO}_4 \) and adjusting the pH with \( \text{H}_3\text{PO}_4 \) or NaOH. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with doubly distilled water.

2.2. Assembly of biocatalyst channeling

SBA-15 was synthesized following the method reported previously (Zhao et al., 1998). In brief, 2 g of EO\(_{20}\)PO\(_{70}\)EO\(_{20}\) (P123) were dissolved in 60 mL of 2 M HCl, followed by the addition of 4.25 g of tetraethyl orthosilicate (TEOS). The mixture was stirred at 35 °C for 5 h, and then transferred into an autoclave and heated at 100 °C for 24 h. After filtration, the obtained particles were dried in air, and calcined at 550 °C for 5 h to remove the templates.

Thirty milligrams of SBA-15 was dispersed in 10 mL of water to obtain a suspension of SBA-15. Glassy carbon electrode (GCE, 3 mm in diameter) was polished to a mirror-like finish with 0.3 and 0.05 \( \mu \)m alumina slurry (Beuhler, USA) followed by rinsing thoroughly with doubly distilled water. Then it was successively sonicated in acetone and doubly distilled water, and allowed to dry at room temperature. The real area of the pretreated GCE was 0.092 cm\(^2\), which was determined from the slope of the plot of the anodic peak current of 1.0 mM K\(_3\)[Fe(CN)\(_6\)] in 0.1 M KCl at the GCE versus the square root of scan rate. The GOD–HRP/SBA-15 modified GCE were prepared by dropping 2 \( \mu \)L SBA-15 suspension, 2 \( \mu \)L of the mixture of 3 mg mL\(^{-1}\) GOD and 3 mg mL\(^{-1}\) HRP (v:v = 1:1) and 2 \( \mu \)L of 5% Nafion solution, respectively, and allowed to dry under ambient conditions for 3 h. When not in use the electrode was stored in 0.1 M p\( \text{H} \) 7.0 PBS at 4 °C.

2.3. Apparatus and measurements

Cyclic voltammetric and amperometric measurements were performed on CHI 660 electrochemical workstation (CH Instruments, USA). All electrochemical experiments were carried out in a cell containing 5.0 mL of 0.1 M PBS at room temperature (25 ± 2 °C) and using the modified electrode as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference against which all potentials were measured. The amperometric experiments were carried out by applying a potential of −0.40 V on a stirred cell. The sensor responses were measured as the difference between total and residual currents. The flow injection system comprised of two peristaltic pumps (BT100–1J, Baoding Longer Precision Pump Co. Ltd., Baoding, China), an eight-port rotary injection valve and a homemade thin-layer flow cell, which was cuboid with the dimension of 3 cm \( \times \) 1 cm \( \times \) 0.15 cm. The total volume of the flow cell including tubing was about 500 \( \mu \)L. Polytrafluoroethylene tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow rate was optimized to be 1.20 \( \mu \)L min\(^{-1}\). Nitrogen adsorption isotherms were obtained using an ASAP 2000 instrument (Micromeritics, Norcross, GA).
Before adsorption measurements, the samples were degassed for 2 h at 150 °C.

3. Results and discussion

3.1. Immobilization of HRP and GOD in mesopores

The specific surface area and pore volume obtained by the N₂ adsorption isotherms and calculated by the Brunauer–Emmett–Teller (BET) method (Barrett et al., 1951) were 710 m² g⁻¹ and 0.58 cm³ g⁻¹, respectively. The pore diameter of the SBA-15 was 8.0 nm derived from the adsorption and desorption branches by the Broekhoff and deBoer (BdB) model. The mesopore diameter of the SBA-15 was larger than the dimensions of both GOD and HRP. Thus, enzymes should be entrapped in the pores of SBA-15 by simply immersing SBA-15 in the enzyme solution (Diaz and Balkus, 1996). To verify this appearance, the N₂ adsorption isotherms before and after loading of enzymes were examined. As shown in Fig. 1, the pore volume of SBA-15 decreased upon the loading, which was 65% of that of SBA-15 due to the presence of GOD and HRP in the mesopores by their adsorption on the internal wall of SBA-15 (Takahashi et al., 2001). Therefore when SBA-15 suspension and the mixture of GOD and HRP were dropped on the electrode surface, both enzymes could be entrapped in the mesopores of SBA-15 on the electrode surface.

3.2. Direct electrochemistry of entrapped enzymes

Similar to GCE, no response of SBA modified GCE was observed in the nitrogen-saturated 0.1 M pH 7.0 PBS. SBA-15 was electroinactive in the potential window. Both GOD and GOD/SBA-15 modified GCEs did not show any detectable redox peak in this solution, while the HRP modified GCE showed a small irreversible peak corresponding to the reduction of HRP. The reduction peak enhanced and a small oxidation peak was observed at HRP/SBA-15 modified GCE. As observed in Fig. 2 for GOD–HRP and HRP–GOD/SBA-15 modified electrodes, the GOD–HRP modified electrode showed a couple of redox peaks at −0.228 and −0.348 V with the formal potential of −0.288 V at 0.1 V s⁻¹, and the HRP–GOD/SBA-15 modified GCE showed greater responses at the potentials of −0.252 and −0.342 V. This couple of redox peaks was attributed to the direct electrochemistry of the immobilized HRP. Obviously the presence of SBA-15 accelerated the electron transfer between the heme in HRP and the electrode and led to a smaller peak-to-peak separation of HRP–GOD/SBA-15 modified GCE than that of HRP–GOD modified GCE. Although SBA-15 was not conducting, it prevented the aggregation of proteins on electrode, thus was in favor of the electron transfer, due to the entrapment of a single protein molecule in the pore. With an increasing scan rate the redox peak currents of the entrapped HRP increased linearly, indicating a typical surface-controlled electrode process. From the integration of the reduction peak of HRP–GOD/SBA-15 modified GCE at different scan rates, an average surface coverage of enzymes was calculated to be 5.5 × 10⁻¹⁰ mol cm⁻². The coverage was much larger than 5.0 × 10⁻¹¹ mol cm⁻² for a fully packed monolayer of HRP (Li and Dong, 1997) and 5.1 × 10⁻¹¹ mol cm⁻² reported in the DNA film (Chen et al., 2000), indicating that more enzyme molecules were assembled on the bienzyme co-entrapped SBA-15 film modified electrode.

The electron transfer rate constant kₛ of the entrapped HRP was estimated with the formula

\[ kₛ = \frac{m n F v}{RT} \]

where the peak-to-peak separation was less than 200 mV (Laviron, 1979) where m is a parameter related to the peak-to-peak separation. The peak-to-peak separation was 81, 90, 110 and 128 mV at 0.05, 0.10, 0.20 and 0.30 V s⁻¹, respectively, producing an average kₛ value of 1.78 ± 0.13 s⁻¹ that was much larger than that of HRP/hexagonal mesoporous silica (HMS) (another mesoporous silica) modified GCE (0.92 ± 0.03 s⁻¹) (Dai et al., 2005).

3.3. Electrocatalytic reduction of glucose using bienzyme channel

Upon addition of H₂O₂ to the nitrogen saturated PBS, the shape of the cyclic voltammogram for the direct electron transfer of HRP at HRP/SBA-15 modified electrode changed dramatically with an increase of the reduction current and a decrease
of the oxidation current, and the cathodic peak shifted to a more negative potential, displaying an obviously electrocatalytic behavior of the immobilized HRP to the reduction of \( \text{H}_2\text{O}_2 \). No apparently electrocatalytic current was observable at bare SBA-15 or GOD/SBA-15 modified GCEs when \( \text{H}_2\text{O}_2 \) was added to the nitrogen-saturated PBS. Furthermore, the reduction current increased with an increasing \( \text{H}_2\text{O}_2 \) concentration. Thus, the entrapped HRP could retain its good bioactivity.

It was noted that at HRP–GOD/SBA-15 modified GCE, upon addition of glucose to pH 7.0 PBS, the shape of cyclic voltammogram also changed dramatically with an increase of reduction current and shift in cathodic peak potential in negative direction, displaying an pronounced electrocatalytic behavior of the immobilized HRP to the reduction of \( \text{H}_2\text{O}_2 \). With the increasing concentration of glucose the electrocatalytic response obviously increased. It should be pointed out that the cathodic response shown in Fig. 3 contained the signals produced from the direct electrochemical response of HRP and the electrocatalytic response of \( \text{H}_2\text{O}_2 \) formed not only in the enzymatic cycle but also from the electrochemical reduction of oxygen at electrode surface. The latter could be observed from the difference between the cyclic voltammograms of HRP–GOD/SBA-15 modified GCE in presence and absence of the dissolved oxygen in pH 7.0 PBS and did not affect the measurement of the electrocatalytic response resulted from the GOD-glucose-dissolved oxygen system, which led to a greater reduction peak after combining the current decrease of the dissolved oxygen due to its consumption in the enzymatic reaction. The mechanism can be shown as Eqs. (1)–(5).

\[
\begin{align*}
glucose + \text{GOD}(\text{FAD}) & \rightarrow \text{gluconolactone} + \text{GOD}(\text{FADH}_2) \\
\text{GOD}(\text{FADH}_2) + \text{O}_2 & \rightarrow \text{GOD}(\text{FAD}) + \text{H}_2\text{O}_2 \\
\text{HRP}_{\text{red}} + \text{H}_2\text{O}_2 & \rightarrow \text{HRP}_{\text{ox}} + \text{H}_2\text{O}
\end{align*}
\]

In total:

\[
\text{glucose} + \text{O}_2 + 2\text{H}^+ + 2\text{e} \rightarrow \text{gluconolactone} + 2\text{H}_2\text{O} \tag{5}
\]

At HRP/SBA-15 and GOD/SBA-15 modified electrodes, the addition of glucose to air saturated PBS did not change their responses (not shown). Although the HRP–GOD modified GCE was responsive to glucose (inset in Fig. 3), the electrocatalytic response was very low due to the low efficiency of the direct electron transfer of the immobilized HRP and the diffusion of \( \text{H}_2\text{O}_2 \) to bulk solution. Thus, SBA-15 provided the advantages for preparation of biosensor and improvement of sensitivity. \( \text{H}_2\text{O}_2 \) generated in the bienzyme-entrapped mesopores increased the electrocatalytic response.

In the range of scan rate from 0.050 to 0.20 V s\(^{-1}\), the electrocatalytic response was proportional to the square root of the scan rate, indicating a diffusion-controlled process. Thus the response of the bienzyme-channeling sensor was controlled by the diffusion of glucose from solution to the electrode surface.

### 3.4. Effecting factors

The effect of applied potential on the amperometric response of the biosensor to glucose was firstly examined. The amperometric response to glucose was observed at around −0.20 V. Upon decreasing applied potential from −0.20 to −0.60 V, the steady-state current increased due to the increased driving force for the fast reduction of \( \text{H}_2\text{O}_2 \) at low potentials. Considering the interference of many coexisted foreign species at too negative potential, this work selected −0.40 V as the working potential for detection of glucose.

The pH value of the target solution was one important parameter affecting the response of the biosensor to glucose since the biosensor involved two enzymatic reactions in the bienzyme channel. Fig. 4A illustrates the effect of pH on the amperometric response of 1.0 mM glucose. With an increasing solution pH from 4.0 to 9.0 the electrocatalytic activity increased and reached a maximum value at around pH 7.0. At the pH values less than 4.0, the response decreased steeply. This resulted from the influence of pH on protein denaturation. A high pH value was disadvantageous with the electrochemistry of HRP\(_{\text{ox}}\). Therefore pH 7.0 PBS was used as the supporting electrolyte for glucose detection.

With an increasing temperature from 15 to 40 °C the activity of the immobilized enzymes increased, leading to an increasing amperometric response (Fig. 4B). When the temperature was higher than 40 °C the amperometric response decreased. The immobilized enzymes showed activity even at 60 °C. It was evident that the immobilized enzymes had a good thermal stability because of the unchangeability of its microenvironment and native structure upon temperature change. For practical application, the amperometric detection of glucose was performed at 25 °C (room temperature), at which the response was 81% of that at 40 °C.
3.5. Amperometric response of bienzyme-channeling sensor

The amperometric response of glucose at −0.40 V increased with an increasing concentration at HRP–GOD/SBA-15 modified GCE. Fig. 5 illustrates a typical current–time plot for successive additions of 5.0 μL of 0.4 M glucose to 5.0 mL of 0.1 M pH 7.0 PBS at an applied potential of −0.40 V. When an aliquot of glucose was added into the buffer solution, the reduction current rose steeply to reach a stable value, and achieved 95% of steady-state current in less than 10 s, indicating this mesoporous matrix created a fast diffusion of substrate from the bulk solution to the enzyme. When the concentration of glucose was over 34 mM, the amperometric response trended to a constant value. This was typical of enzymatic reaction kinetics.

Under the optimized experimental conditions the bienzyme-channeling sensor showed a linearly increased amperometric response to glucose ranging from $3.0 \times 10^{-6}$ to $3.4 \times 10^{-2}$ M with a relative coefficient of 0.9994 ($P < 0.0001$, $n = 21$, inset A in Fig. 5). For illustrating the linear response at low glucose concentrations inset B in Fig. 5 shows the linear plot in the range from 3.0 to 240 μM, which gives a relative coefficient of 0.9997 ($P < 0.0001$, $n = 11$). The average relative standard deviation (RSD) of the plots was 3.6%. This linear range of four orders of magnitude was much wider than the ranges of 0.5–7 mM for GOD in microemulsions of dioctyl sulfosuccinate sodium salt (AOT) in nonpolar organic solvents (Shipovskov et al., 2005), 1.0–10 mM for a ribbon-like mass-sensitive magnetoelastic sensor (Cai et al., 2004) and 2.5 μM to 0.5 mM in the enzyme-immobilized magnetic micoreactor for the flow injection analysis (Nomura et al., 2004). It had a high sensitivity of 90 μA cm$^{-2}$ mM$^{-1}$, which was much higher than those of 50 μA cm$^{-2}$ mM$^{-1}$ in perfluorosulfonated ionomer membranes (Karyakin et al., 2002) and 2.98 μA cm$^{-2}$ mM$^{-1}$ for GOD in microemulsions of AOT in nonpolar organic solvents (Shipovskov et al., 2005). Although the similar upper limits of detection for glucose have been reported to be 20, 30 and 35 mM at GOD immobilized carbon nanotubes (Wang and Musameh, 2003; Rubianes and Rivas, 2003; Wang et al., 2003; Lin et al., 2004), our bienzyme-channeling sensor with an upper limit of 34 mM can detect glucose down to 3.0 μM, which is much lower than 2.0, 0.08 and 0.6 mM at these carbon nanotubes (Wang et al., 2003; Lin et al., 2004; Rubianes and Rivas, 2003). This illustrated that the designed bienzyme-channeling sensor was suitable for sensing of glucose in practical samples of diabetic patients. The detection limit of 0.25 μM at a signal-to-noise ratio of 3 was also much lower than 180 μM based on GOD adsorbed mesoporous composite material of no. 41 (MCM-41) modified GCE (Dai et al., 2007) and 20 μM at a ultrasmall platinum (Hrapovic and Luong, 2003). Obviously the co-entrapment of GOD and HRP in the same mesopores of SBA-15 greatly increased the sensitivity, widened the linear range and deduced the detection limit.

3.6. Interferences

The amperometric responses for relevant physiological levels of glucose, ascorbic acid, acetaminophen, and uric acid at the GOD–HRP/SBA-15 modified electrode at −0.40 V were compared. The amperometric responses were obtained by adding 0.5 mM interfering species in the solution containing 1.0 mM glucose, respectively. 0.5 mM uric acid and 0.5 mM p-acetaminophenol did not cause any observable interference to the sensor response to glucose. However, ascorbic acid could diffuse through the porous structure of mesoporous molecular sieves film to the electrode surface to produce a weak response. The relative response of 0.5 mM ascorbic acid was about 6.0%,
indicating these species coexisting in the sample matrix did not affect the determination of glucose.

3.7. Stability and reproducibility

SBA-15 membrane imparted the bienzyme-channeling sensor a good long-term stability. The storage stability of the glucose biosensor in 0.1 M pH 7.0 PBS or air at 4 °C was examined by checking periodically their relative responses (the ratios of the catalytic currents detected at different times to the initial current value) in 0.1 M pH 7.0 PBS containing 1.0 mM glucose. The sensors could retain 90% of their initial activity to glucose within a storage period of 60 days in 0.1 M pH 7.0 PBS at 4 °C (curve a, Fig. 6A), while only 60% of activity to glucose was retained when stored in air at 4 °C (curve b, Fig. 6A). Thus, SBA-15 nanoparticles were very efficient for retaining the bioactivity of GOD and HRP and preventing them from leaking out of the biosensor.

The fabrication reproducibility of six electrodes, made independently, showed an acceptable reproducibility with a RSD of 5.1% for the currents determined at a glucose concentration of 1.0 mM. The detection reproducibility of the developed sensor in the on-line assay for glucose was studied by establishing a FIA system with a thin-layer cell. An optimal flow rate of 1.2 mL min⁻¹ was obtained by evaluating the analytical performance of the biosensor, peak width and the measurement reproducibility. The reproducibility ascertained by monitoring the current response for 10 replicate injections of 1.0 mM glucose with an applying potential of −0.40 V was shown in Fig. 6B. The RSD was 4.5%, indicating a good reproducibility of the biosensor for FIA, suggesting both enzymes did not leak out of the film in the process of FIA, and the biosensor could repeatedly be used for FIA or on-line determination of glucose.

3.8. Practical sample determination

FIA of glucose in a serum sample was performed on the biosensor utilizing a calibration method. The sample was diluted to its half concentration by mixing it with 0.1 M pH 7.0 PBS. Five parallel determinations were carried out. The glucose level was determined to be 8.45 mM with a RSD of 5.2% for five measurements, close to 8.62 mM by spectrophotometry. The glucose level was determined to be 8.52 mM with a RSD of 3.9% for five measurements when the experiments were performed without dilution, showing a good accuracy. The Clark Error Grid analysis showed that 100% of data were within zones A and B with 65% in zone A. The recoveries for the assays of 1.0–8.0 mM glucose were between 98 and 101% for 10 measurements, indicating good practicability of the biosensor in clinic laboratory.

4. Conclusion

This work develops a new bienzyme-channeling sensor by co-entrapping GOD and HRP in the mesopores of SBA-15 for glucose detection without addition of any mediator. The porous structure of SBA-15 results in a high catalytic activity of the immobilized enzymes and accelerates the direct electron transfer between the heme in HRP and the electrode. The immobilized HRP shows electrocatalytic behavior toward the reduction of hydrogen peroxide produced from the enzymatic reaction of GOD-glucose-dissolved oxygen system in the same mesopore, leading to a fast and sensitive amperometric response to glucose with a wide linear range in four orders of magnitude from 3.0 × 10⁻⁶ to 3.4 × 10⁻² M. The proposed biosensor shows excellent performance and good stability for flow injection analysis of serum glucose level. The designed bienzyme-channeling sensor provides a promising strategy to construct fast, sensitive, stable and anti-interferential amperometric biosensors for oxidase substrate with wide concentration range by co-entrapping the corresponding oxidase and HRP in the mesoporous materials.

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