Screen-printed biosensor chips with Prussian blue nanocubes for the detection of physiological analytes

Danfeng Jiang, Zhenyu Chu**, Jingmeng Peng, Wanqin Jin*

State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemistry and Chemical Engineering, Nanjing Tech. University, 5 Xinmofan Road, Nanjing 210009, PR China

A R T I C L E   I N F O

Article history:
Received 21 September 2015
Received in revised form 4 January 2016
Accepted 18 January 2016
Available online 21 January 2016

Keywords:
Bioensor chip
Screen-printing
Nanocubic Prussian blue
High sensitivity

A B S T R A C T

A Prussian blue (PB) biosensor chip with the nanocubic crystals was fabricated in batch by a screen printing technique for the general detection of various physiological substances. Through a low-speed chemical synthesis approach, the nanostructure of PB slurry was well controlled to be a 100 nm nanocube by the synthesis temperature and reactant concentration. Then, PB slurry was screen-printed as the working electrode to construct a microchip by the integration of the printed reference and counter electrodes. Due to the high electrocatalytic activity attributed by the regular nanostructure of PB electrode, the as-prepared chips exhibited the generally high sensitivities of 83.404, 31.642 and 6.379 μA mM \(^{-1}\) cm \(^{-2}\) for the respective detections of glucose, glutamate and lactate, as well as the excellent selectivity, reproducibility and stability under the low work potential of \(-0.05\) V.

1. Introduction

Enzymatic biosensor is an essential analytic tool in the fields of clinic assay, environment monitor and food safety due to its fast response, accuracy and low-cost. Since Clark and Lyons proposed the first enzyme electrode for glucose detection [1], it has aroused tremendous attention of scientific research due to the close relations to humans' health. Its work principle is mainly depended on the signal change induced by the enzymatic reaction. It is well known that hydrogen peroxide is a common production generated from an enzymatic reaction [2,3]. Prussian blue (PB), which is famed by its high electrocatalysis to \(\text{H}_2\text{O}_2\), has been widely served as the electron transfer mediator in the enzyme biosensor [4–6]. The direction of electron transfer in enzyme biosensor is from the support electrode to target solution and it can be explained according to the following reactions: [7]

\[
\text{Fe}_4(\text{II})[\text{Fe}(\text{II})(\text{CN})_6]_3 + 4e^- + 4K^+ \rightarrow K_4\text{Fe}_4(\text{II})[\text{Fe}(\text{II})(\text{CN})_6]_3 + 2\text{H}_2\text{O}_2
\]

\[
\text{GO}_x + \text{O}_2 \rightarrow \text{GDL} + \text{H}_2\text{O}_2
\]

\[
\text{Fe}_4(\text{II})[\text{Fe}(\text{II})(\text{CN})_6]_3 + 2\text{H}_2\text{O}_2 \rightarrow \text{Fe}_4(\text{III})[\text{Fe}(\text{II})(\text{CN})_6]_3 + 4\text{OH}^- + 4\text{K}^+
\] (3)

Currently, more and more research results reveal that the structure, especially in nanoscale, can strongly affect the catalysis and chemical stability of materials [8,9]. However, due to the rapid synthesis reaction rate, the morphology of PB can hardly be controlled to form the regular nanostructure by the traditional synthesis methods, which always affects the performance of fabricated biosensors. Hence, PB material was rarely used for mass production of biosensors. Recently, the screen printing technique was widely served as a large-scale production technology in the industrialization production of computer circuit, solar battery and electronic medicine due to its remarkable advantages of low cost, time saving and good repeatability. In this preparation route, features of the obtained production are mainly determined by the properties of the applied printing slurry. If a kind of PB slurry composed by the regular nanocrystals can be synthesized, the mass manufacture of PB biosensor with high performance will be expected through the screen printing method.

In this work, we have designed and fabricated a new type of enzymatic biosensor chip to realize the versatile detection of various physiological substances by a screen printing technique. PB nanocubes with 100 nm size, which were synthesized in batch by a low-speed chemical synthesis approach, were adopted as the main

** Corresponding author. Fax: +86 25 8317 2292.
* Corresponding author.
E-mail addresses: zychu@njtech.edu.cn (Z. Chu), wqjin@njtech.edu.cn (W. Jin).

http://dx.doi.org/10.1016/j.snb.2016.01.076
0925–4005/© 2016 Elsevier B.V. All rights reserved.
ingredient of the printing slurry through the control of temperature and reactant concentration. Through the screen printing method, PB slurry, AgCl and carbon inks were respectively served as the work, reference and counter electrodes to construct a function-integrated biosensor microchip. By immobilization of different enzymes, the as-prepared chips exhibited the versatility to the detections of glucose, lactate and glutamate with the excellent sensitivities, selectivity and stability. This high-performance minisize biosensor is meaningful for the support of the further development toward the product manufacture in medical diagnosis.

2. Experimental

2.1. Reagents and apparatus

Potassium ferrocyanide trihydrate ($K_3[Fe(CN)_6] \cdot 3H_2O$), iron(III) chloride hexahydrate (FeCl$_3 \cdot 6H_2O$), glucose oxidase (GO$_x$) from Aspergillus niger (EC.1.1.3.4, 180200U/g), Lactate oxidase (LO$_x$) from Pediococcus sp. (EC1.1.12.4, 20U mg$^{-1}$), and glutamate oxidase (GMO$_x$) from Streptomyces sp. (EC1.4.3.11, 5U mg$^{-1}$) were purchased from Sigma–Aldrich. Hydrochloric acid, potassium carbonate and glutaraldehyde 25% (v/v) were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (China). Sodium lactate and sodium glutamic acid monosodium salt monohydrate were purchased from Alfa-Aesar. Hydrogen peroxide (H$_2$O$_2$, 30%, w/v, solution), glucose, uric acid (UA) and ascorbic acid (AA) were received from Sinopharm Chemical Reagent Co., Ltd. (China). Carbon ink and silver chloride ink were bought from Yingman Nano Technology Jiangsu Co., Ltd.

Screen-printed electrode (SPE) was produced with a 245 DEK (Weymouth, UK) screen-printing machine. The morphology of PB surface and an energy-dispersive X-ray (EDX) of working electrode were observed by field emission scanning electron microscopy (FESEM) (Hitachi, ModelS–4800II, Japan). The spectroscopy of carbon ink and mixed ink were both investigated with Fourier-transform infrared (FTIR) (Thermo Electron, Nicolet-8700, USA). The X-ray diffraction (XRD) of PB, carbon ink and mixed ink was measured on an X-ray diffractometer (D/MAX 2500V/PC) with a Cu-Ka line (0.15419 nm). All of electrochemical measurements were carried out in a 0.05 M phosphate buffered saline (PBS, pH 6.5) containing 0.1 M KCl by using electrochemical workstation (CHI 660E, Shanghai Chenhua Instrument Co., Ltd., China). The glucose level in blood was analyzed by a glucometer (ACCU-CHEK® Performa, Roche Diagnostics GmbH, Germany) to calibrate the results obtained by the as-prepared device.

2.2. Preparation of PB slurry

The synthesis of PB was based on the chemical reaction between $K_3[Fe(CN)_6]$ and FeCl$_3$. Their solutions were prepared as follows: solution A, 0.01 M $K_3[Fe(CN)_6]$ + 0.1 M KCl + 0.1 M HCl, and solution B, 0.01 M FeCl$_3$ + 0.1 M KCl + 0.1 M HCl. After solution A and solution B were poured into the syringe, syringe was fixed on the injection pump with the injection speed of 1000 µL/min. At the same time, a beaker at the heating magnetic stirrer was used for reaction vessel, in which the beaker contained distilled water before the start of the reaction. To control the synthesis temperature, a constant temperature water-bathing was employed here. The distilled water was firstly heated to the desired temperature in the heating magnetic stirrer. The synthesis temperature and reactant concentration were regulated to get the PB under different conditions. The PB slurry was obtained by centrifuging PB solution at the beaker, and the mixed ink was formed by mixing the PB slurry with the carbon ink.

2.3. Screen printing biosensor chips

A commercial PVC plate was served as the substrate for the screen-printing. The mixed ink self-made was used to prepare the working electrode. Carbon ink was used to obtain the counter electrodes and connector, and silver chloride ink was used to print the reference electrode.

2.4. Enzyme immobilization

In order to immobilize enzyme, the enzyme solution contained 0.25% glutaraldehyde (v/v), in which 1.8 U/µL GO$_x$, 5 U/µL LO$_x$ and 1 U/µL GMO$_x$ were included, respectively. Then, 4 µL of enzyme solutions were dropped evenly onto the center of the working electrode of biosensor chip, followed by drying at 4 ℃. Finally, PB based biosensor chip was stored at 4 ℃ when not in use.

3. Results and discussion

3.1. Nanostructure control of PB crystals for the slurry preparation

The performance of the screen-printed product mainly depends on the properties of applied slurry. Here, the synthesis of PB slurry was realized by a low-speed chemical synthesis approach which depended on the chemical reaction between the adding FeCl$_3$ and $K_3[Fe(CN)_6]$ solutions under a low flowing rate. In our previous work, it was found that the synthesis temperature was an essential parameter for the crystallization behavior of PB [10], as well as the reactant concentration also determines the initial amount of crystal nucleuses which strongly affect the further crystal growth process. Therefore, the effects of temperature and reactant concentration on the PB structure were respectively investigated. As shown in Fig. 1, the FESEM images showed the morphology evolution of PB slurries prepared under the increasing synthesis temperature from 25 to 40 ℃. At a lower temperature, PB slurry was composed by lots of irregular particles with the size of ca. 30 nm (Fig. 1a), and the particles tended to aggregate together. When the temperature increased to 30 ℃, the morphology of PB crystal was close to cube, although on some edge sites the deficiencies still existed (Fig. 1b). Till the 35 ℃, the regular PB nanocubes were successfully formed to reach the 100 nm size, and each crystal kept the independent existence with rare aggregation (Fig. 1c). Nevertheless, further increase of synthesis temperature, the structure of PB crystal turned to be amorphous with a heavy intergrowth and aggregation, and the size of single particle exhibited the very small size (Fig. 1d). This may be attributed to the overly rapid reaction rate caused by the high temperature. More crystal nucleuses formed at its initial crystallization stage with the increased temperature, hence, the growth competition will hinder the absolute growth of PB crystal to form the regular structure.

In our previous work, it was found that regular nanostructures can benefit the material performance [10]. Therefore, the electrochemical redox property of PB slurries synthesized by different temperatures was investigated by the cyclic voltammetry (CV) technique (Fig. 2). Accompanying with the scanning, a couple of well-defined redox peaks were produced to indicate the electron transfer during the conversion between PB and Prussian white (PW). The absolute value of peak currents continuously increased with the raise of the synthesis temperature from 25 to 35 ℃. But the peak current value showed a sudden decrease at 40 ℃. Above results indicated that PB slurry prepared at 35 ℃ can produce more electrocatalytic electrons to possess the higher catalysis activity which was due to the regular nanocube structure. Besides, among these five temperatures, the potential difference
$\Delta E$ of PB slurry synthesized by 35 °C presented the highest value which indicated the stronger resistance for electron transfer. Above result was attributed to the formation of the regular PB nanocubes which exhibited an isolated distribution feature to possess rare connection sites between each cube. In this case, there were few continuous channels which were provided for the electron transfer from one crystal to another. Differently, other temperatures caused the obvious intergrowth of crystals to benefit the electron transportation. Consideration of both CV and FESEM results, it was found that the more regular structure of PB crystals, the lower transfer resistance and catalytic activity can be obtained together. Consequently, the further performance characterization should be required to determine the optimum temperature condition.

PB slurries synthesized at different temperatures were modified on the graphite electrodes to investigate the electrocatalytic performance of $\text{H}_2\text{O}_2$. All slurries showed the excellent linear relation between the response current and $\text{H}_2\text{O}_2$ concentration in Fig. 3. The sensitivities were calculated as 381.21, 498.92, 580.46, 355.99 and 328.47 $\mu\text{A}\text{mM}^{-1}\text{cm}^{-2}$ for the preparation temperatures of 25, 30, 35, 40 and 45 °C, respectively. As expected, the highest performance was obtained for the PB slurry fabricated at 35 °C, which was mainly attributed to the plenty of strongly active cites provided by the regular cubic nanostructure [10]. Although the regular crystals
could lead to the increase of transfer resistance, the sensitivity was mainly determined by the electrocatalytic ability. Hence, 35 °C was selected as the optimum temperature for the PB slurry synthesis. Because the PB slurry was prepared by the chemical reaction between the FeCl₃ and K₄[Fe(CN)₆] solutions, the reactant concentration was another key parameter for the crystal growth. For optimum, the concentrations of above two reactants were always kept the same. The features of four PB slurry samples prepared by the 0.005, 0.01, 0.03 and 0.05 M of reactant concentrations were respectively investigated. The FESEM images in Fig. 4 illustrate that the reactant concentration was strongly affect the formed structure of PB crystals. If the concentration was beyond 0.01 M, most of crystals exhibited the irregular morphology with various sizes. This may be attributed to the rapid formation of huge amount of nucleuses under the high concentration. Over-large amount of nucleuses can cause the space competition to limit the sufficient growth of each crystal. Therefore, the aggregation behavior was so heavy. Compared with the crystal structure synthesized under the 0.005 M, most of crystals presented the well-defined nanocube shape with the average size of 100 nm for 0.01 M of preparation condition. Although low concentration can provide the free growth, far fewer reactants were not enough to support the sufficient crystallization [11]. Hence, the shape of crystal is just similar with cube, but not possessed the regular edges of cube.

In order to decide the optimum condition of reactant concentration, the electrocatalytic performance of these PB slurries was also characterized with the target of H₂O₂. Fig. 5 shows the calibration diagrams of the response current to H₂O₂ concentration obtained from the data of the chronoamperometry tests. It was found that with the increase of the reactant concentration from 0.005 to 0.01 M, the response signal occurred an obvious enhancement for the same H₂O₂ concentration. However, this tendency was
Because mixture, (4) synthesized JCPDS structure, characterized 0), and 4 PB, pure crystal 0), the PB crystals were uniformly distributed in the working electrode. This uniform distribution can possess two advantages for the biosensing performance: one is the increase of the contact area of PB crystals to the target, and another is the decrease of the resistance for electron transfer due to the prevention of the aggregation on partial sites.

3.3. Electrochemical characterization of biosensor chip

CV was applied to evaluate the electrochemical properties of the prepared biosensor chip. As shown in Fig. 8a, a quasi reversible electrochemical process can be proposed according to the increase of the peak potential separation (ΔEp) with the scan rate. Furthermore, it showed that the observed anodic and cathodic peak currents were directly proportional to square root of the scan rate (υ1/2). Such a behavior implied that the redox process is controlled by linear diffusion of the electroactive species [13,14]. In order to detect the physiological substances, the biosensor chip requires the immobilization of the enzyme on the working electrode. PB owns a high electrocatalytic activity to H2O2 which is a common product of the enzyme oxidation. According to this mechanism, the as-prepared biosensor chip can be applied in the detection of various targets by the loading of the matching enzymes. GOx, G-MOx, and LOx were respectively loaded on the chip to confirm the versatile functions in application. The electro-

![Fig. 5](http://example.com/image5.png) The chronoamperometry results of the screen-printed PB electrodes with different reactant concentration in 0.05 M PBS (a) Calibration curves for H2O2 detection; (b) the dependence of sensitivities on the reactant concentration.

![Fig. 6](http://example.com/image6.png) XRD patterns of mixed ink, carbon ink and PB.

opposite to decrease with the further increase of reactant concentration. The related sensitivities of PB slurries were calculated as 411.67, 580.46, 375.25 and 372.16 μA mM⁻¹ cm⁻² for the reactant concentration of 0.005, 0.01, 0.03, and 0.05 M, respectively. Association with the above results of temperature characterization, the preparation at 35 °C with the 0.01 M reactant concentration can produce the optimum PB slurry for the further printing.

3.2. The screen-printed fabrication of biosensor chip

In order to fulfill the screen-printing for biosensor chip preparation, the SPE ink usually met two requirements which were the suitable viscosity and conductivity. Due to not realizing the two requirements in the case of only PB slurry, the commercial carbon ink was introduced to mix with PB slurry for the satisfaction. Because PB crystals were the main electroactive material for the sensing application, the stability of PB had been examined after the mixture of carbon ink. Pure PB slurry, carbon ink, and mixed ink were characterized by the XRD patterns to investigate the crystal structure stability. As shown in Fig. 6, for pure PB slurry, the peaks located at 17.3°, 24.2°, 35.2°, 39.4°, 50.6°, 53.8° and 57.3° respectively represented the PB crystal planes of (200), (220), (400), (420), (440), (600) and (620) [12]. Above was consistent with the JCPDS card of standard PB crystal (No. 73-0687), indicating that the synthesized crystals were an integrated face-centered-cubic phase of Prussian blue. Moreover, the shape of peak was sharp and narrow, and the full width at half maximum peak was small, which illustrated that the good crystallinity of PB crystals in PB slurry. After the mixing of PB slurry into carbon ink, all above characteristic peaks of PB remained to be existed and obvious, as well as no new peaks generated and no original peaks vanished. This result suggested the lattice structure of PB was still complete, and not damaged by organics and other chemical substances in the carbon ink.

The mixed ink, carbon ink and AgCl ink were screen-printed as to respectively serve as working, counter and reference electrodes which were integrated as the biosensor chip (shown in Fig. 7b). The working and counter electrodes were both characterized by FTIR. As shown in Fig. 7a, after the mixture of PB slurry, the working electrode exhibited a strong absorption peak at 2090 cm⁻¹ which belonged to the stretching absorption band of the CN group in the Fe⁵⁺-CN-Fe⁺⁺ section of PB. The transfer resistance and electrocatalysis of screen-printed chip will be strongly affected by the PB distribution in the working electrode. Therefore, the working electrode surface was scanned by EDX which employed the iron element as the label of PB location (Fig. 7c). The result illustrated that the PB crystals was uniformly distributed in the working electrode. This uniform distribution can possess two advantages for the biosensing performance: one is the increase of the contact area of PB crystals to the target, and another is the decrease of the resistance for electron transfer due to the prevention of the aggregation on partial sites.
chemical behaviors of the as-prepared biosensors before/after the immobilization of different enzymes were investigated by CVs. As shown in Fig. 9a, after the immobilization of GOx on the PB film, both current values of redox peaks showed an obvious decline. This may be attributed to the poor conductivity of enzyme layer, which hindered the electron transfer toward the electrode surface. Furthermore, after the injection of glucose into PBS solution, the oxidation current increased obviously, as well as the decrease of reduction current. In addition, the investigations on GMO and LOx showed the similar results in Fig. 9b and c. Under the potential of −0.05 V, the current responses of these biosensor chips were recorded with the continuous addition of the same concentration targets by the chronoamperometry test. The calibration lines of the fabricated biosensor chips for the detections of glucose, lactate and glutamate are shown in Fig. 9d, which sensitivities were respectively calculated to be 83.40, 31.64 and 6.38 μA mM⁻¹ cm⁻². In

---

**Table 1** Performance comparisons of reported glucose, lactate and glutamate biosensors.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Modifiers</th>
<th>Potential (V vs. Ag/AgCl)</th>
<th>Sensitivity (mAM⁻¹ cm⁻²)</th>
<th>Detection limit (μM)</th>
<th>Linear range (mM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>GOx/PB nancubes</td>
<td>−0.05</td>
<td>83.404</td>
<td>10</td>
<td>0.01–1.3</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>GOx/Chit/IL/PB/Pt</td>
<td>−0.05</td>
<td>37.8</td>
<td>5.0</td>
<td>0.01–4.2</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Nafion/CHIT/GOx/Pt/PtNCs/Pt</td>
<td>0.2</td>
<td>35.92</td>
<td>0.5</td>
<td>0.001–5.0</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Au(MWCNT/PVP/PB</td>
<td>0.1</td>
<td>38</td>
<td>2</td>
<td>0.01–0.7</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>GOx/PD/AuNP3.5/GOx</td>
<td>0.3</td>
<td>52.1</td>
<td>0.024</td>
<td>0.1–10</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>Gr/PANI/AuNPs/GOx/SPCE</td>
<td>NA</td>
<td>20.32</td>
<td>0.1</td>
<td>0.2–11.2</td>
<td>[19]</td>
</tr>
<tr>
<td>Glutamate</td>
<td>GOx/PB nancubes</td>
<td>−0.05</td>
<td>31.642</td>
<td>10</td>
<td>0.01–1.0</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>GS-GMOD/PB/Pt</td>
<td>−0.1</td>
<td>12.36</td>
<td>NA</td>
<td>NA</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>Pt/Ta-C/APTES/GLOx</td>
<td>0.6</td>
<td>2.9</td>
<td>10</td>
<td>0.01–0.5</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>CNT</td>
<td>−0.1</td>
<td>0.71</td>
<td>2</td>
<td>NA</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>EDC/TGA</td>
<td>0.005</td>
<td>20.75</td>
<td>0.089</td>
<td>0.0001–10</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>MWCNT/GLOx/Chit</td>
<td>0.3</td>
<td>28</td>
<td>5.4</td>
<td>0.01–3.495</td>
<td>[24]</td>
</tr>
<tr>
<td>Lactate</td>
<td>GOx/PB nancubes</td>
<td>−0.05</td>
<td>6.379</td>
<td>10</td>
<td>0.01–0.5</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>GS-LOD/PB/Pt</td>
<td>−0.1</td>
<td>4.5</td>
<td>NA</td>
<td>NA</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>LOD-FSM8.0/Naf/CoPC-SPCE</td>
<td>0.45</td>
<td>4.54</td>
<td>18</td>
<td>0.02–1.5</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>FeOx/MWNTs/LDH/NAD+/GCE</td>
<td>0</td>
<td>7.67</td>
<td>5</td>
<td>0.005–0.5</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Fpolymer/Lox/Pt</td>
<td>0.25</td>
<td>2.14</td>
<td>2.1</td>
<td>0–0.6</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>0.6</td>
<td>5.8</td>
<td>4</td>
<td>Up to 1.2</td>
<td>[28]</td>
</tr>
</tbody>
</table>
addition, wide linear ranges from 0.01 to 1.3 mM in glucose detection with the very short response time (within 5 s), 0.01–1.0 mM in glutamate and 0.01–0.5 mM in lactate were obtained. Compared with the performance of reported biosensors, the sensitivities of as-prepared biosensor are much higher than the others (Table 1) and the LODs are also higher. In this work we only adopted PB as the single electrocatalytic material instead of composite film for performance complementation. In this case, the performance limit of PB material on LOD was presented. However all in all, the as-prepared biosensor certainly represented a good balance between excellent analytical performance and reproducible mass production. Moreover, till now, rare biosensors can be generally qualified to the detection of various targets with high sensitivity by using a single material. In order to solving the problem of higher limitation in the future, the materials owning with high catalytic activity may be added. This special character will be benefited to widen the application field for the more substances analysis with low cost, which can be promising to accelerate the steps for production transformation.

The accuracy of biosensor is an essential factor for the application in the real system. Such in the blood analysis, huge amount of physiological activators were coexisted to easily produce the redox reaction which could cause the interference signal to affect the accuracy. AA and UA are usually regarded as the most common electrochemical interfering species because of the low oxidation potential (normally higher than 50 mV), therefore, above two substances were served as the simulative interferents to investigate the selectivity of prepared chips. As shown in Fig. 10, 0.1 mM AA and 0.1 mM UA were respectively added into the system after 0.1 mM glucose was injected. It can be seen that the current responses produced no evident variations both in the presence of AA and UA, which was mainly attributed to the low operation potential and the barrier effect of the enzyme immobilized at the surface of working electrode. This result suggested that the proposed enzyme biosensor chip based on Prussian blue-modified screen-printed electrodes possessed sensor selectivity.

Besides, the reproducibility of the enzyme biosensor chips was tested with the parallel experiments, in which ten electrodes were examined in each experiment. The relative standard deviation (RSD) of 7.46% was observed in the detection of glucose, suggesting

Fig. 9. Each diagram showed the CV curves of PB film, enzyme loaded working electrode and the addition of the 15 mM target in 0.05 M PBS containing 0.1 M KCl. (a) to (c) were CV experiments of the as-prepared glucose, glutamate and lactate biosensors. Scan rate of CV was 0.05 V s⁻¹. (d) Linear calibration curves for the current responses of glucose, lactate and glutamate biosensors respectively.

Fig. 10. The selectivity test of AA and UA on the responses of the glucose biosensor in 0.05 M PBS.
that the fabricated enzyme biosensors chips possessed the satisfactory reproducibility. Moreover, in order to determine the stability of enzyme biosensor chips, they were carefully stored in the refrigerator at 4 °C for 3 weeks, and then examined after that. From the experiment result, it is showed that the fabricated enzyme biosensor chips still retained 74.1% of their initial sensitivities in glucose, indicating an excellent usage stability of the fabricated enzyme biosensor chips [29,30]. Also, in order to study the performance in real target system, the chip was employed to detect the blood glucose amount of the rabbit whole blood. Adding 400 μL rabbit serum into the PBS solution, the blood glucose concentration was analyzed to be 10.04 mM, which is close to the detected value 10.2 mM by the commercial glucometer. As calculation, the deviation of detection was 1.57%.

4. Conclusions

In this work, we have successfully designed a new screen-printing strategy to fabricate a PB nanocubes based biosensor chips for the general detection of various physiological substances. The printing PB slurry was synthesized in batch to possess a nanoucubic structure by a low-speed chemical synthesis approach. This PB slurry was screen-printed to construct a biosensor chip with the integration of the reference and counter electrodes. Under the low working potential of −0.05 V, the as-prepared chips can be ultrasensitive, 83.404, 31.642 and 6.379 mA·M⁻¹·cm⁻², to the respective detections of glucose, lactate and glutamate with the excellent accuracy, reproducibility and stability. Our designed biosensor chip can be expected to probably apply in the analysis of more physiological targets. The low-cost and mass production route would be promising in the construction of more advanced biosensors for the product development.

Acknowledgements

This work was supported by the Jiangsu Province Natural Science Foundation for the Youth (No. BK20140931), the Innovative Research Team Program by the Ministry of Education of China (No. IRT13070) and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References


Biographies

Wanqin Jin is Professor of chemical engineering at Nanjing Tech University and currently researches on mixed-conducting membranes for oxygen separation and catalytic membrane reactors, organic/inorganic composite membranes for pervaporation, and biosensors. He was an Alexander von Humboldt Research Fellow (2001), and a visiting Professor at Arizona State University (2007) and Hiroshima University (2011, JSPS invitation fellowship). He has published over 130 internationally refereed research papers and edited several books on materials-oriented chemical engineering. He serves as an editorial board member for several journals and is a council member of the Aseanian Membrane Society.
Danfeng Jiang received his B.Sc. degree in chemical engineering from the Department of Chemistry and Chemical Engineering, Nantong University, China in 2014. Currently he is undertaking the master research at Nanjing Tech University.

Zhenyu Chu received his B.Sc. and Ph.D. degree in chemical engineering from the Department of Chemistry and Chemical Engineering, Nanjing Tech University, China in 2008 and 2013. He studied in Institute of Technology Tallaght, Dublin, Ireland for 3 months as a collaborative visitor in 2011. He is now a lecturer at Nanjing Tech University.

Jingmeng Peng received her B.Sc. degree from the Department of Chemistry and Chemical Engineering, Changchun University of Science and Technology, China in 2012. She is undertaking the master research at Nanjing Tech University.