Preparation, Characterization of Polypyrrole Encapsulated Prussian Blue Nanocomposite and Its Application for Biosensing

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Polypyrrole (PPy) encapsulated Prussian Blue (PB) composite was prepared by one-step chemical method for the first time. After mixed with multi-walled carbon nanotubes (MWNTs), the PPy@PB/MWNTs nanocomposite shows improved electrochemical performance. A glucose electrochemical biosensor was prepared based on the PPy@PB/MWNTs nanocomposite substrate. The biosensor shows excellent amperometric response to glucose with linear range from 1.0 to 13.0 mM at 0.0 V. The lowest detection limit is 0.01 mM. Furthermore, the biosensor displays high sensitivity, good reproducibility and selectivity. The PPy@PB/MWNTs system possesses enhanced electrochemical activity and good stability, which is promising for immobilizing different oxidase enzymes for other bioelectrochemical applications.

**Keywords:** Glucose biosensor; Carbon nanotubes; Polypyrrole; Prussian Blue.

1. INTRODUCTION

Prussian blue (PB) is a prototype of metal hexacyanoferrates with well-known electrochemical, electrochromic, and magnetic properties and potential analytical applications. Owing to its excellent electrocatalysis, PB has been widely used as a low-potential electron mediator in the electrochemical biosensors [1-3]. Thus, the interference from the coexisting biomolecules such as ascorbic acid and acetaminophen, which are easily oxidized at high potentials, can be inhibited. The traditional method for the preparation of PB film is based on electrodeposition of metal ions and the corresponding metalcyanide ions in solution with supporting cations [4]. The disadvantage of this method is that the PB layer is easily loss of its catalytic effect because of its rapid desorption from the electrode surface.
Therefore, the biosensor stability is poor, in some cases a matter of hours [5]. Moreover, the reduced redox state of PB is thermodynamically unstable in neutral or alkali solution [6,7].

To overcome this limitation, attempts have been made to improve the stability of PB film. So far, many methods, such as microemulsion [8], polymer protection [9, 10], and template imprint [11], and the use of a biomolecular matrix as a chemically and spatially confined environment, have been proposed for the synthesis of PB. Zhang et al. provide an alternative electrochemical approach to the formation of a PB nanocluster layer from an acidic solution of ferricyanide [12]. Xia reported a preparation method of PB nanoparticles by dropping FeCl₃ solution mixed with a slight excess of H₂O₂ into a K₃Fe(CN)₆ solution [13]. Recently, Polymer encapsulated PB have been gained much attention. Kitagawa described the formation of well-dispersed PB nanoparticles controlled poly(diallyldimethylammonium chloride) as a protecting polymer [14]. Yakhmi and Sun have reported the synthesis PB nanoparticles encapsulated by conducting polymer polyaniline [15, 16]. Among the conducting polymers, polypyrrole (PPy) has been considered as one of the most attractive materials because of its excellent conductivity, good stability and biocompatibility even in neutral pH solution. Vidal et al. have reported PPy encapsulated PB film by electrodeposition method. However, to the best of our knowledge, the synthesis PPy@PB nanoparticle by a chemical method in one-step has not yet been reported [7].

On the other hand, carbon nanotubes (CNTs) have been received considerable interest because of their unique chemical structure, good conductivity and high surface area. These features are expected to be employed in biosensors to lower the detection limit, decrease the response time, and improve the sensitivity. Recently, the synergistic effect between CNTs and other materials have gained growing attention. Materials for such purposes include conducting polymers, redox mediators and nanoparticles. For example, coupling CNTs with toluidine blue resulted in significant improvement of the electrocatalytic activity of the composite materials toward β-nicotinamide adenine dinucleotide through synergistic effect [17]. Yang et al reported the synergy effect between CNTs and cobalt hexacyanoferate nanoparticles (CoNP) with the substantial improvement of electrochemical activity of CoNP [18].

In the present paper, PPy encapsulated PB composite was prepared by a one-step chemical method. After mixed with multi-walled carbon nanotubes (MWNTs), the PPy@PB/MWNTs system shows synergy between the PPy@PB and MWNTs. PPy@PB/MWNTs nanocomposite was modified on the surface of a glassy carbon electrode and applied to construct a glucose biosensor. The resulted biosensor exhibits excellent performance.

2. EXPERIMENTAL

2.1. Reagents

Pyrrole (≥99.5%, Shenyang Lianbang reagent Factory, Shengyang, China) was distilled before experiments. MWNTs (95% 20–60 nm) were obtained from Shenzhen Nanotech. Port. Co., Ltd. (Shenzhen, China). The MWNTs were treated with established procedure [19]. GOD (from Aspergillus niger; 300,000 unit g⁻¹) was purchased from Sanland (America). D-glucose was used without further
purification and the solutions were stored overnight at room temperature before use. All other chemicals were of analytical grade and used as received. 0.1 M phosphate buffer solution (PBS) was prepared with Na₂HPO₄ and NaH₂PO₄ and used as supporting electrolyte.

2.2. Apparatus and measurements

The infrared (IR) spectra were obtained on Bruker Equinox 55 Fourier transform infrared spectrometer (FTIR). The morphology was measured using a transmission electron microscope (TEM, JEM-2000EX).

Electrochemical measurements were performed using an IM6e electrochemical workstation (Zahner-Elektrik, Kronach, Germany). Conventional three-electrode cell assembly was employed. The working electrode was the Bioanalytical Systems (BAS) cavity glassy carbon electrode (3 mm in diameter) (GCE). An Ag/AgCl (saturated with NaCl) electrode was used as reference electrode and a platinum wire was used as a counter electrode. The rotating disk electrode (RDE) experiments were carried out by BAS rotator system in conjunction with an IM6e. The rotating rate is 3000 rpm when detect H₂O₂ and glucose unless stated otherwise. All electrochemical experiments were carried out at 25 ± 1 °C.

2.3. Preparation of the PPy@PB composites

PPy@PB composites were prepared by wet chemical method. Briefly, to an solution of 0.1 M pyrrole 50 mL, an aqueous of 0.002 M FeCl₃ +0.002 M K₃Fe(CN)₆ +0.1 M HCl solution 20 mL was dropwise added with a vigorous stirring. A strong dark color was observed after the addition. The reaction was stirred further for another 24 h and the solid was filtered and washed thoroughly with 0.1 M HCl, water and ethanol, respectively. The material was dried under vacuum for 12 h before characterization.

2.4. Glucose biosensor construction

The PPy@PB/MWNTs modified electrode was prepared as follows: 1.5 mg of purified MWNTs was dispersed in 5 ml dimethylformamide (DMF) with the aid of ultrasonic agitation. Then 15 mg of PPy@PB nanoparticle was dispersed in the MWNTs suspension by 30 min sonication. A suspension (5 μL) of the PPy@PB/MWNTs in DMF was dropped onto the GCE surface by the spinning method and then dried under an infrared lamp. GOD solution was obtained by dissolving 10 mg of GOD in 2 mL of 0.1 M PBS (pH 6.5). For the immobilization of enzyme, 3 μL of GOD solution was dropped onto the PPy@PB/MWNTs modified electrode surface carefully. Then the electrode was dried for 60 min at 4 °C, a mixture (3 μL) of glutaraldehyde, BSA and Nafion was placed on the enzyme surface as protective film [20]. The enzyme electrode was then stored under damp conditions at 4 °C for 24 h before used.
3. RESULTS AND DISCUSSION

3.1. PPy@PB composite synthesis

Conventionally, PB is synthesized through electrochemical reduction in ferric-ferricyanide solution. In the present work, PB was chemically prepared by the spontaneous redox reaction in the FeCl$_3$-K$_3$[Fe(CN)$_6$] and the pyrrole solution. It has been demonstrated that the solution of FeCl$_3$-K$_3$[Fe(CN)$_6$] in acidic media is a very strong oxidant [19,20]. On the other hand, PPy can be polymerized by a wide range of chemical oxidants, such as (NH$_4$)$_2$S$_2$O$_8$ [21], FeCl$_3$ [22]. It is proven that pyrrole can also be oxidized by acidic FeCl$_3$-K$_3$[Fe(CN)$_6$] solution and then polymerized to PPy. PPy is electrical conductive and environmental stable, which offers potential applications in the domain of composite materials, tissue engineering, and biosensor et al. In this study, PPy@PB composite was firstly prepared based on the spontaneous redox reaction in FeCl$_3$+K$_3$[Fe(CN)$_6$] and pyrrole solution.

Fig. 1 shows the IR transmission spectra of PPy@PB composite. The IR spectra shows a strong absorption band around 2086 cm$^{-1}$, corresponding to the CN stretching in the Fe$^{2+}$-CN- Fe$^{3+}$ of PB [7]. For PPy, the characteristic absorptions peaks were observed for the ring fundamental vibration appeared at 1564 cm$^{-1}$, and the C-H in-plane vibration and C-N stretching vibration appeared at 1310 and 1045 cm$^{-1}$, and at 1184 cm$^{-1}$, respectively [22]. These results indicate the PPy@PB composite were successfully prepared through the chemical redox reaction method.

![Figure 1. IR spectra of the PPy and PPy@PB composite.](image-url)
Figure 2. TEM image of the PPy@PB and PPy@PB/MWNTs hybrid composites, (a) PPy@PB, (b) PPy@PB/MWNTs.

The morphology of the PPy@PB and PPy@PB/MWNTs composites were imaged by a TEM and shown in Fig. 2. It is clear that the PB nanoparticles were encapsulated in the polymer. After introduction with MWNTs, the PPy@PB nanocomposite was well decorated on the surface of MWNTs. The distribution of carbon nanotubes in the PPy@PB composite could preserve the three-dimensional bulky structure of the composite, which would lead to increased surface area and facilitate electron transfer.

3.2. Electrochemical behavior of PPy@PB/MWNTs composite

To investigate the stability of the PPy@PB/MWNTs composite film, consecutive CVs were recorded in 0.1 M PBS +0.1 M KCl (pH 6.5) solution. As shown in Fig.3, after scanned for 50 cycles, no obvious peak current decrease was observed. The decrease of the current signal was only about 10% of the initial value after 250 cycles. These results indicated that the PPy@PB/MWNTs possessed good stability. The good stability of the hybrid composites is probably due to preparation method. PPy has good chemical stability and conductivity, which prevents the PB film from solubility [7,19]. Doping PPy@PB with MWNTs is also considered an effective way to improve their electrochemical performance because of the combined effect of the two materials [19].
The electrochemical characterization of PPy@PB/MWNTs composite was investigated by CV. The effect MWNTs on the electrochemical performance of PPy@PB/MWNTs composite was investigated by change the amount of MWNTs in the composite from 0.1 to 0.4 mg mL⁻¹. As shown in Fig.4, after introduction of 0.1 mg mL⁻¹ of MWNTs in the 3 mg mL⁻¹ of PPy@PB suspension, the redox peak current increased greatly and the separation of the anodic and cathodic peak potentials (ΔEₚ) decreased. The peak current did not increase when the content of MWNTs beyond 0.3 mg mL⁻¹. The optimized amount of MWNTs is found to be 0.3 mg mL⁻¹, which has been selected for preparation of the PPy@PB/MWNTs modified electrodes.

**Figure 3.** Consecutive CVs of PPy@PB/MWNTs composite film modified GC electrode for 250 cycles in pH 6.5 0.1 M PBS +0.1 M KCl, scan rate, 50 mV s⁻¹.

**Figure 4.** CVs of PPy@PB/MWNTs composite modified electrode with in the 0.1 M PBS +0.1 M KCl (pH 6.5), scan rate: 50 mV/s, (a) PPy@PB, (b) PPy@PB/ 0.1mg mL⁻¹ MWNTs, (b) PPy@PB/ 0.2mg mL⁻¹ MWNTs, (c) PPy@PB/ 0.3mg mL⁻¹ MWNTs, (d) PPy@PB/ 0.4mg mL⁻¹ MWNTs.
3.3. Current response of PPy@PB/MWNTs modified electrode toward H$_2$O$_2$

The current response of PPy@PB/MWNTs modified electrode toward H$_2$O$_2$ was investigated by increasing the level of H$_2$O$_2$ in 0.25 mM step at potential of 0.0 V in a 0.1 M PBS+0.1 M KCl (pH 6.5) solution. As shown in Fig.5, the PPy@PB modified electrode gives slight response signal to the addition of H$_2$O$_2$. The current response increased for the first several additions. The current response did not increase at higher concentration. It indicated that the PPy@PB modified electrode was easy to be saturated [23,24]. In contrast, the PPy@PB/MWNTs modified electrode respond quickly to the changes of the H$_2$O$_2$ concentration and reached to a steady-state current within 5 s. Furthermore, the PPy@PB/MWNTs electrode shows a much higher sensitivity than that of PPy@PB. It has demonstrated that the CNTs also shows excellent electrocatalytic activity toward H$_2$O$_2$, while for the PPy@PB/MWNTs, the current response was further increased. This was probably because the nanocomposite combined the electrocatalytic activity of both MWNTs and PPy@PB nanoparticles toward H$_2$O$_2$. Repeated use of the PPy@PB/MWNTs modified electrode did not affect its performance. For instance, 0.5 mM H$_2$O$_2$ was measured for five times, and a relative standard deviation (R.S.D.) of 4.7% was obtained.

![Figure 5](image_url)

**Figure 5.** Current–time curves obtained at the PPy@PB/MWNTs (b) and PPy@PB (a) composite upon increasing the concentration of H$_2$O$_2$ in steps of 0.25 mM at 0.0 V in 0.1 M PBS +0.1 M KCl (pH 6.5); rotating rate: 3000 rpm.

3.4. Amperometric determination of glucose at the PPy@PB/MWNTs modified electrode

Glucose oxidase is a typical model enzyme in the development of new biosensing materials. Owing to its good electrocatalytical activity of PPy@PB/MWNTs composite film to H$_2$O$_2$, in this study, a glucose biosensor was further constructed based on PPy@PB/MWNTs. Fig. 6 shows the current–time curve for the glucose biosensor upon the successive addition of 1 mM glucose at 0.0 V. The time required for reaching the 95% of the steady-state current is less than 5 s, indicating its fast
response of the PPy@PB/MWNTs based biosensor. The inset of Fig. 6 shows the calibration curve of glucose concentration versus current. A linear range from 1-13 mM (R=0.999) and a sensitivity of 11.25 μA mM⁻¹ cm⁻² were obtained on the enzyme electrode. The sensitivity is much higher than the reported 0.98 μA mm⁻¹ cm⁻² [18] and 5.6 μA mM⁻¹ cm⁻² [27]. The detection limit is 0.01 mM at the S/N ratio of 3. When the glucose concentration is higher than 13 mM, a plateau current was observed, showing the characteristics of Michaelis–Menten kinetics [25]. The apparent Michaelis–Menten constant (kₘ) and the maximum current density (iₘₐₓ) was estimated to be 4.9 mM and the iₘₐₓ is 173.9 μA mM⁻¹ cm⁻², respectively. The value of kₘ is much lower than the reported 20.3 mM [26] and 6.6 ± 0.5 mM in solution phase [23]. The good performance of the enzyme electrode can be attributed to the excellent electrocatalytic activity of PPy@PB/MWNTs nanocomposite. The synergistic effect between MWNTs and PPy@PB may be preferable for the biosensor and improves the performances of the biosensor [19].

![Graph showing the calibration curve of the biosensor as a function of glucose concentrations.](image)

**Figure 6.** Amperometric response of the glucose biosensor for successive addition of 1 mM glucose (pH 6.5 0.1M PBS+0.1 M KCl), applied potential: 0.0V, rotating rate: 3000 rpm. Inset: calibration curve of the biosensor as a function of glucose concentrations.

3.5. Interference study

The selectivity and anti-interference ability of the glucose biosensor was also examined. The most common electrochemical interfering species such as ascorbic acid and acetaminophen were evaluated. Injection of 0.2 mM ascorbic acid, 0.2 mM acetaminophen to 1.0 mM glucose did not produce observable interference in the biosensor response. That may be due to the low applied potential was used. Furthermore, Nafion polymer is as an effectively perselective barrier, which can prevent the entry of anionic biological interferences [19].
3.6. Reproducibility and stability of glucose biosensor

The reproducibility of glucose biosensor was estimated from the response to 5 mM glucose at five enzyme electrodes prepared under the same conditions. The results are shown in Fig. 7. It revealed that the biosensor has a satisfied reproducibility with a R.S.D. of 5.3 %. The operational stability of the enzyme electrode was also examined at 0.0 V in 0.10 M PBS+0.1 M KCl containing 5 mM glucose. As shown in Fig.8, the relative deviation is less than 2.7 % for five times continuous determinations of the same sample, which indicates that the biosensor is reliable.

![Figure 7](image1)

**Figure 7.** Current response toward 5 mM glucose with five electrodes prepared under the same conditions.

![Figure 8](image2)

**Figure 8.** Current response toward 5 mM glucose with the same glucose biosensor for five times.

The storage stability of the PPy@PB/MWNTs based glucose biosensor was also studied. The biosensor was stored at 4 °C when not in use. The response to 5 mM glucose was studied every 2 days. The results are shown in Fig. 9 and indicate that the steady-state current response only decreases by 10% after 30 days (15 times) measurements, which shows that the enzyme electrode is very stable.
4. CONCLUSIONS

A novel method for fabrication PPy@PB composite material is proposed in this study. With the incorporation of MWNTs, a glucose biosensor based on PPy@PB/MWNTs composite was further constructed. The resulting glucose biosensor displayed wide linear range from 1 μM to 13 mM with a high sensitivity of 11.25 μA mM$^{-1}$ cm$^{-2}$. The enzyme electrode also demonstrated excellent stability and non-interferences. The enhanced electrochemical performances of PPy@PB/MWNTs hybrids could be mostly attributed to their unique nanostructure, high surface area of the MWNTs as well as unique features of the PPy@PB film. These results indicate that PPy@PB/MWNTs composite held great potential application for construction of a variety of electrochemical biosensors.

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References


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