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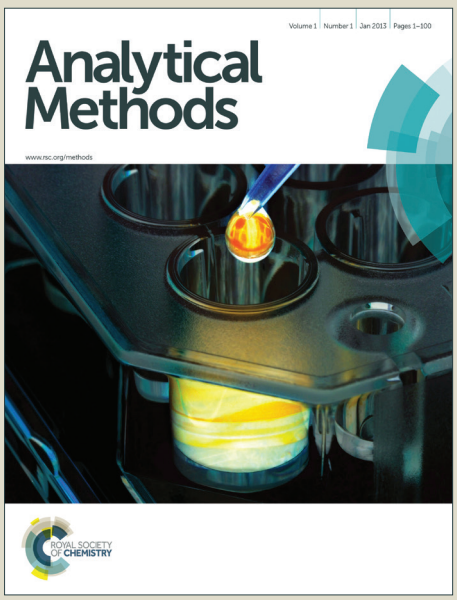

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Preparation of poly(*N*-acetylaniline)-Prussian blue hybrid composite film and application to hydrogen peroxide sensing

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Abstract

In this work, the poly(*N*-acetylaniline)-Prussian blue (PNAANI-PB) hybrid composite film has been prepared by co-electrodeposition for the first time. With the spontaneous redox reaction, electrochemical preparation of the PNAANI-PB hybrid film was carried out by cyclic voltammetry (CV) in electroplating bath containing low concentration of *N*-acetylaniline. Its surface morphology revealed that PB nanoparticles in the hybrid film have smaller size than that in pure PB film. Due to the existence of electrostatic interaction between PNAANI and PB particles, the PNAANI-PB hybrid film shows wonderful synergistic effect which can remarkably enhance sensitivity, expand linear range and broaden acidic adaptability for detection of hydrogen peroxide (H_2O_2). The hybrid film displays good stability in neutral solution contrast to pure PB film, with a linear range from 10^{-6} M to 10^{-3} M and a high sensitivity of 507.29μA·mM^{-1}·cm^{-2} for H_2O_2 detection. An amperometric glucose biosensor was further constructed by immobilizing glucose oxidase (GOD) on the hybrid film. The biosensor also exhibited excellent response to glucose with the linear range from 10^{-5} M to 10^{-3}M and a high sensitivity of 62.45μA·mM^{-1}·cm^{-2}. Furthermore, the glucose biosensor possesses rapid response, good reproducibility, long-term stability and free of interference from other co-existing electroactive species.

Keywords: hydrogen peroxide; hybrid film; Prussian blue; poly-N-acetylaniline; glucose biosensor

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1 Introduction

The detection of hydrogen peroxide (H_2O_2) is an important topic for environmental control and clinical diagnosis, due to it is not only a product of industry but also a risk factor for some diseases. Commonly, the direct electrochemical detection of H_2O_2 is accomplished with its oxidation on noble metal electrodes (Pt[1, 2], Au[2], Pd[3] etc.) or carbon electrodes (glassy carbon[4–8], graphite paste[9], carbon fiber[4] etc.), although the required voltage is often high enough to oxidize other interfering compounds or organic molecules, such as ascorbate, bilirubin, urate, etc., which may be present in bio-samples.

Prussian Blue (PB), known as an artificial peroxidase, has been extensively used for H_2O_2 detection and been a fundamental material for possible mass production of biosensors, due to its excellent catalysis for H_2O_2 reduction at low potentials compared to noble metal, and a relatively cheap and stable electro-catalyst compared to enzyme[10-12]. PB can facilitate the electron transfer between H_2O_2 and the base electrode, which causes the operating potential reduced around 0.0 V (*vs* Ag/AgCl) [8, 13]. The potential is so low that the current contribution from all the most common interferences mentioned above is avoided or greatly reduced. Moreover, the peculiar morphology of the PB molecules seems to be the cause for an effective electrochemical selectivity. Molecules with a molecular weight higher than H_2O_2, such as ascorbic acid, bilirubin and urate, cannot penetrate the PB lattices to give a catalytic redox reaction[14, 15]. In addition, PB is low cost and can be easily deposited on the surface of electrode. All these promising advantages have been used to obtain a sensitive and interference-free probe for H_2O_2 detection.

However, the primary problem of using PB as catalyst for detection of H_2O_2 is the poor operational stability[4, 16, 17]. The current response decreases quickly at neutral and alkaline solutions. The operational stability of PB seems to be dependent on the modified methods of the PB layer. PB was deposited on electrode based on the screen printing technology, the product has a good stability even in alkaline pH[18, 19]. Borisova[20] reported on interfacial synthesis of Prussian blue with organic polymers. Besides these methods, protective polymer is also a choice to enhance the reproducibility and stability, such as cellulose acetate[21], poly(-odiaminobenzene)[22,23], poly(vinylpyrrolidone)[24–26], poly(o-phenylendiamine)[27, 28], oaminophenol[29], poly[4,4'-bis(butylsulfanyl)-2,2'-bithiophene][30] and dendrimers [31–33]. The protective polymer films are prepared simply, but lack of mechanical stability, and shed easily due to swelling in aqueous solution.

Recently, hybrid film, which based on integration of PB and some other materials, has both properties of the individual components with the synergistic effect[34]. Materials for such purpose include conducting polymers, redox mediators and metal nanoparticles. Among them, conducting polymers have been considered as a new class of advanced materials to fabricate the hybrid films, owing to their unique electronic and mechanical properties, as well as biocompatibility[35]. Polyaniline (PANI), one of the most promising conducting polymers, has been extensively investigated because of its high conductivity, homogeneity of film formation, ease of preparation, and good stability[36,37]. However, as pH of the media increased above 4 the polymer would lose its electrochemical activity, the applications of PANI in bio-electrochemistry are limited in neutral aqueous solutions [38].

By now, large kinds of conducting polymer/PB composite films, especially composites of PB and PANI[39-43] have been wildly studied. However, conducting hybrid composites using PB and polyaniline derivatives are seldom studied. On the other hand, many reports were focused on the

avenue of step by step for deposition of PANI/PB composites, but the procedure of co-deposition have rarely been reported on this field[44]. Herein, a novel conductive polymer PNAANI-PB hybrid film was prepared by one step co-electrodeposition in electroplating bath simultaneously containing low concentration of *N*-acetylaniline and $FeCl_3$-$K_3[Fe(CN)_6]_4$. The structure and electro-activity of the PNAANI-PB hybrid film were evaluated. The effects of potential scanning cycles and the concentration of *N*-acetylaniline on the formation of the hybrid composite film were investigated. The hybrid film demonstrated rapid response, good reproducibility and long-term stability. A glucose biosensor was further fabricated by immobilization of glucose oxidase on the hybrid film and applied to detect glucose in human serum samples.

2 Experimental

2.1 Reagents

All chemicals used were of analytical grade. The 0.1M phosphate buffer solution (PBS), which was made from K_2HPO_4 and KH_2PO_4, was always employed as the supporting electrolyte. *N*-acetylaniline (Shanghai Chemical works, China) was redistilled before use. Potassium ferricyanide ($K_3Fe(CN)_6$), potassium chloride (KCl), ferric chloride ($FeCl_3$), *L*-cysteine, *L*-cystine, *L*-histidine and hydrogen peroxide (H_2O_2,30% wt%) were all purchased from GUOYAO group Co. (Shanghai, China). The exact concentration of hydrogen peroxide was determined by titration with potassium permanganate. Uric acid, chitosan (CS) and ascorbic acid were from Shanghai Chemical Reagents (Shanghai, China). Dopamine and glucose oxidase (GOD, EC1.1.3.4, lyophilized powder, 245U mg1) from Shanghai YUANJU Co. (Shanghai, China) were produced by Sigma. 0.5% chitosan solution was prepared by dissolving 0.5g CS in100ml 2% acetic acid under stirring. GOD was used without further purification and glucose solution (0.1M) were stored overnight at room temperature before use.

2.2 Apparatus

All electrochemical experiments were performed using an LK98B Electrochemistry Work Station (LANLIKE Co. Ltd., Tianjin, China). A conventional three-electrode cell was employed. The glassy carbon electrode (GCE, 3mm in diameter) was used as the substrate electrode. Prior to each experiment, the GCE was first polished with 0.3μm alumina powder, rinsed thoroughly with double distilled water, then ultra-sonicated successively in 1:1 nitric acid, acetone and double distilled water, finally dried at room temperature. A saturated calomel electrode (SCE) was used as the reference electrode, and a platinum wire was used as the counter electrode. All experiments were performed at room temperature.

The scanning electron microscopy (SEM) was performed on a JEOLJSM-6700F SEM system to characterize the morphologies of the PB film and PNAANI-PB hybrid film modified electrodes. The X-ray photoelectron spectra (XPS) were recorded on a VG ESCALAB MKII X-ray photoelectron spectrometer, using nonmonochromated Mg Kα radiation as the excitation source.

2.3 Fabrication of the PNAANI-PB hybrid film electrode

Electrolyte preparation: Take 1mL 0.02M $K_3[Fe(CN)_6]$ solution, added into 5mL 0.2M HCl solution (containing 0.2M KCl), then 1mL 0.02M $FeCl_3$ solution was added slowly, subsequently 2mL double distilled water was added under vigorous stirring. Finally, 1mL 1M $HClO_4$ solution containing 0.1M *N*-acetylaniline was rapidly added to the above mixed solution.

The PNAANI-PB hybrid film was electrochemically prepared via cyclic voltammetry(CV) in a three-electrode cell. The film was obtained on the working electrode by potential cycling between -0.2V and 0.9V (*vs*. SCE) at a scan rate of 0.05V·s^{-1} for 10 cycles. After electrodeposition, the electrode was rinsed with pure water and immersed into a solution containing 0.1M KCl and 0.1M HCl, subsequently the electrode potential was scanned between -0.05V and 0.35V for 25 cycles at a scan rate of 0.05 V·s^{-1}, until a stable voltammetric response was obtained. The modified electrode was then rinsed with deionized water and dried in air.

2.4 Fabrication of the GOD-CS/ PNAANI-PB biosensor

For immobilization of enzyme, 10mL 5mg·mL^{-1} GOD-CS was dropped onto the PNAANI-PB hybrid film surface carefully. After drying for 60min at 4□ in the refrigerator, the sandwich-like enzyme biosensor was accomplished. The enzyme electrode was then kept in damp conditions at 4□ before use.

2.5 Sample source and measurement

Serum samples were obtained from patients of health examination centre of the hospital, the reference data were provided by the hospital's clinical laboratory, and all data were feedback to the patients. Additionally, decontamination of wastes was carried out by pouring the serum samples and residues inside a container within a NaClO solution (40 g L^{-1} of active chlorine) after the measurement. All experiments were performed in compliance with the relevant laws and institutional guidelines.

3 Results and discussion

3.1 The co-deposition of PNAANI and PB nanoparticles

The PNAANI-PB hybrid film has been synthesized by cyclic voltammetry. The typical cyclic voltammograms for the electrochemical co-deposition of Prussian blue nanoparticles and poly-*N*-acetylaniline are illustrated in Fig. 1. As we can see, there are three pairs of redox peaks that can be distinguished at potentials around +0.15V, +0.45V and +0.65V respectively, which indicate that PNAANI-PB hybrid composite film is of common voltammetric responses of PNAANI and PB. The first peak can be explicated the overlap of the oxidation peaks of the Prussian blue and the *N*-acetylaniline. At the same time, the first and the third pairs have been ascribed to the polaron and bipolaron forms of the PNAANI. The origin of the second peak is much more complex and it has been attributed to different intermediates and degradation products.

Fig. 1 is here.

The incorporation of PB into conducting polymer can lead to new composite materials possessing the properties of each component with a synergistic effect. From Fig.1, it can be seen that with the number of scan cycles increased, the peak current at +0.15V increased gradually, which means the continuous growth of well-conductive Prussian blue nanoparticles. In other words, the PB nanoparticles and the poly-*N*-acetylaniline can be grown simultaneously and to form the hybrid composite film. Furthermore, the peak current at +0.15V is higher than the sum of the peak currents of PNAANI and PB alone, and the difference between anodic and cathodic peak potentials is also smaller, which can be seen in the inset of Fig. 1. This shows that in the composite

film there probably exist strong electrostatic interaction between the spin central Fe atoms in PB film and the nitrogen atoms of *N*-acetylaniline in PNAANI. Due to the electrostatic attraction between the electronegativity of PB nanoparticles and the electropositivity of PNAANI, the PNAANI-PB hybrid composite film can get more stable structure to potentially overcome the weakness they maintain severally, and their synergistic interaction might be favor of electrocatalytic action to analytes.

3.2 Physicochemical characteristics of the PNAANI-PB hybrid film

The surface morphologies of the hybrid films were investigated with scanning electron microscopy (SEM). The morphological images of pure PB and PNAANI-PB hybrid film on GCE are illustrated in Fig. 2(A,B). As can be seen, the actual size and distribution of the hybrid film grains is markedly different in comparison to pure PB, and the PNAANI-PB hybrid film shows a much denser structure than the pure PB film. The improvement of the film can be ascribed to PNAANI skeleton providing active centers for PB residing in and growth. Owing to electrostatic interaction between the positively charged PNAANI backbone and the negatively charged PB nanoparticles, it is feasible to form a dense robust film in which the content of functional redox centers is higher and the free space between the grains is minimized by introduction of conducting polymer matrix, so that PB particles become smaller and uniform. The SEM images also reveal that the PNAANI-PB hybrid film was constructed by the accumulation of spherical particles which assembled homogeneously and well distributed in the PNAANI matrix. The porous structure of PNAANI has large surface area to provide an ideal location for the distribution of PB particles. This composite structure is very beneficial for the modified electrode's performance, because most of the well-dispersed hybrid spherical particles are electrochemically accessible.

Fig. 2 is here.

The surface microstructure of the hybrid film was also characterized by X-ray photoelectron spectrum (XPS). The XPS N1s spectra of PNAANI-PB hybrid film is compared with that of the PNAANI and pure PB in Fig. 2(C). The XPS N1s signal of the PNAANI film can be deconvolved into three components: imine N (398.0eV), amine N (399.8eV) and positively charged N (402.3eV)[45]. The XPS N1s signal of the pure PB can only be deconvolved into one component: positively charged N (402.3eV). However, from the XPS N1s signal of the PNAANI-PB hybrid film, the binding energy of N1s is changed to 397.3eV for imine N, 398.7eV for amine N, and 402.3eV for positively charged N, respectively. Besides, the peak areas of N1s in PNAANI-PB hybrid film became smaller than that in PNAANI and pure PB film. Therefore, the XPS data has shown to a great extent that there is an interaction between PNAANI and PB.

In order to further prove this mechanism, the XPS Fe2p spectra of PB and PNAANI-PB films was also illustrated and compared in Fig. 2(D). The XPS data of Fe2p showed two iron peaks at 708.8eV (Fe2p3/2) and 722.0eV (Fe2p1/2) respectively for PB and PNAANI-PB films[46]. Besides, the peak areas of Fe2p in PNAANI-PB film also became smaller than that in PB film. Hence, the XPS signal strength of Fe2p was weakened due to the interaction of electrons in PNAANI with the spin centers in PB.

Fig. 3 is here.

The PNAANI-PB hybrid composite film modified electrodes were studied using cyclic voltammetry in a potential range of -0.05 V to +0.35V at different scan rates. Peak currents (both anodic and cathodic) vary linearly with the scan rate in a range between 10 and 200mV·s^{-1}, as shown in the inset of Fig.3(A). This indicates that the electrode reaction becomes electrochemically reversible and surface-controlled process at scan rates below 200 mV·s^{-1}. When the scan rate is increased, the cathodic peak potential (E_{pc}) shifted negatively and the anodic peak potential (E_{pa}) shifted positively. When the scan rate is higher than 200 mV·s^{-1} , the wave shape is distorted severely (ΔE_p>200mV). This indicates that the electrode reaction becomes electrochemically irreversible at higher scan rates. Besides, the peak-to-peak separation was almost constant with different scan rates, indicating a fast electron transfer rate. According to the Laviron's theory[47] and tabulated value(m value), the electron transfer rate constant k_s was calculated to be 4.50±0.56 s^{-1}(degree of confidence t=95%), a considerable value which can certainly be in favor of enhancing the electron transfer rate between PNAANI-PB composite and target substances.

The stability of the hybrid film was also evaluated by using cyclic voltammetry, according to the decrease of the redox peak currents after 200 cycles in the buffer solutions with different acidity. As is shown in Fig. 3(B), the results showed that the PNAANI-PB hybrid film, contrast to pure PB, still have good stability after 200 scans in various solutions with different acidity. The decrease of the peak current of PB/PNAANI film is at the level not exceeding 20% even in neutral solution, while the decrease of the peak current of PB film exceeds 80%. This is because there is a micro acidic environment in the PNAANI-PB hybrid film which is provided by the protonation of poly(*N*-acetylaniline), that seems to be favorable to stabilization of PB particles and avoiding decomposition of PB crystals in neutral medium.

3.3 Optimization for coelectrodeposition of PNAANI-PB hybrid film

Both the effects of the concentration of *N*-acetylaniline in the electroplating bath and the potential scanning cycles of cyclic voltammetry on codeposition of PNAANI-PB hybrid film were investigated. The influences were evaluated from the performances of the resulting modified glassy carbon electrodes towards H_2O_2 reduction.

The concentration of *N*-acetylaniline in the electrolyte influences remarkbaly on the performance of PNAANI-PB hybrid composite film. Fig. 4(A) illustrates the amperometric responses and sensitivities of different PNAANI-PB film modified GCEs towards reduction of hydrogen peroxide. It is obviously that with the concentration of *N*-acetylaniline increasing from 2mM to 10mM, the sensitivity increased. However, the sensitivity decreased sharply with the concentration of *N*-acetylaniline beyond 10mM. Therefore, 10mM *N*-acetylaniline was selected for co-electrodeposition of PNAANI-PB hybrid film.

Fig. 4 is here.

The potential scanning cycles of cyclic voltammetry is another important influencing factor. Obviously, with the scanning cycles increasing, the thickness of the hybrid composite film is increased, whereas it will influence on the current response sensitivity of the modified electrode.

Also the stability (or mechanical stability) of the hybrid film on GCE is affected by its thickness, and then the operability. When the hybrid film is thinner, the current response is quicker, also the sensitivity is higher, whereas the modified electrode is less stable. From Fig. 4(B), with the increasing of scan cycles, the current sensitivity was increased. While the scanned cycles were beyond 10, the current sensitivity was decreasing gradually. To get a relatively good stability and high sensitivity, 10 scan cycles was selected to prepare the PNAANI-PB modified electrodes.

As a key parameter, the applied potential at the working electrode is fundamental to achieve the lowest detection limit and to avoid interfering species. From Fig. 4(C), the effect of applied potential on the current response of H_2O_2 showed that the sensitivity of the electrode response increased linearly with the applied potentials ranging from 0.2 to 0.0V, however almost unchanged with the applied potentials under 0.0V. Therefore, the applied potential was chosen as 0.0V, in order to operate stably and avoid interferences.

3.4 Amperometric detection of H_2O_2 using the PNAANI-PB hybrid film modified electrode

According to literature [48], the catalytic mechanism of Prussia blue towards H_2O_2 reduction could be described as follows:

$$\underset{\text{Prussian blue}}{KFe^{III}[Fe^{II}(CN)_6]} + e^- + K^+ \rightarrow \underset{\text{Prussian white}}{K_2Fe^{II}[Fe^{II}(CN)_6]} \quad (1)$$

$$\underset{\text{Prussian white}}{2K_2Fe^{II}[Fe^{II}(CN)_6]} + H_2O_2 + 2H^+ \rightarrow \underset{\text{Prussian blue}}{2KFe^{III}[Fe^{II}(CN)_6]} + 2H_2O + 2K^+ \quad (2)$$

The electrocatalytic activity of the pure PB film and PNAANI-PB hybrid film was compared by their amperometric responses through ten times of successive addition of 0.1mM H_2O_2 under the optimal conditions, as shown in Fig 5(A). Obviously, the response of H_2O_2 at PNAANI-PB hybrid film is about three times as much as that at PB film, which is probably due to the nano-size effect of the smaller Prussian blue nanoparticles in the PNAANI matrix. The synergistic effect between the PNAANI and PB improved the stability of composite film and enhanced the current response sensitivity of H_2O_2. Therefore, it is obvious that the PNAANI-PB hybrid film takes on the characteristics of PB, which can preferably electrocatalyze the reduction of hydrogen peroxide, and that the hybrid film modified electrode is provided with higher stability and better electrocatalytic activity than pure PB film modified electrode.

Fig. 5 is here.

The inset in Fig.5（A）shows the cyclic voltammograms obtained at the PNAANI-PB hybrid film modified GCE in 0.025M PBS+0.1M KCl(pH=6.86) solution in absence and in presence of H_2O_2, respectively. Without H_2O_2, the modified electrode gives the classic redox peaks of PB. With successive addition of 0.4mM H_2O_2 into the solution, it was observed that the reduction currents were dramatically increased after the cathodic peaks of PB emerged at about 0.17V. It indicated the electrocatalytic activity of the PNAANI-PB hybrid film towards H_2O_2 reduction.

Fig.5（B）shows the calibration curve of the amperometric response at the PNAANI-PB

modified electrode towards H_2O_2 detection, and the inset of Fig.5（B）give the amperometric response of PNAANI-PB hybrid film in the concentration of H_2O_2 ranging from 1.0×10^{-6} M to 1.0×10^{-5} M (a), 1.0×10^{-5} M to 1.0×10^{-4} M (b), and 1.0×10^{-4} M to 1.0×10^{-3} M (c). All the measurements were preformed in 0.1M PBS+0.1M KCl (pH6.86) solution and at an applied potential of 0.0V. At the optimal conditions, the response time, detection limit, linearity range, current sensitivity and the reproducibility for H_2O_2 detection were investigated. The calibration curves obtained from the PNAANI-PB hybrid film modified electrode shows a very wide linearity in the range of 1.0×10^{-6}M to 1.0×10^{-3}M with a correlation coefficient of 0.9991, it spanned three orders of magnitude of H_2O_2 concentration. The detection limit was found to be 68 nM at signal to noise ratio of 3. The response time needed to reach 90% of the steady state response was less than one second. In particular, the current sensitivity was 507.3μA·mM^{-1}·cm^{-2}. Also, the repeatability and reproducibility of the PB/PNAANI modified electrode were investigated. For six replicated measurements in 5.0×10^{-4} M H_2O_2 solution using the same modified electrode, the relative standard deviation (R.S.D) was 1.8%, and a R.S.D of 4.0% was obtained using six different modified electrodes in the same solution mentioned above.

3.5 Application to glucose biosensor

Depending on the excellent electrocatalytic activity of PNAANI-PB hybrid film towards H_2O_2 reduction, the glucose biosensor was fabricated. The chitosan membrane containing GOD was immobilized on the PNAANI-PB hybrid film modified electrode to construct the GOD/PNAANI-PB biosensor. The inset A and B in Fig.6 show the typical current-time plot for the biosensor upon the successive addition of 0.1mM glucose at an applied potential of 0.0V, and the current response of the resulting biosensor increased along with glucose concentration. The biosensor reached 90% of the steady-state current within 3s. Fig.6 shows the calibration curve of glucose at the enzyme electrode. The enzyme electrode gave a linear response to glucose in the concentration range from 1.0×10^{-5}M to 1.0×10^{-3}M (also a relatively wide linear range) with a correlation coefficient of 0.9961 and the sensitivity was 60.86μA.$mM^{-1}cm^{-2}$. The electrode had a low detection limit of 1.2μM at signal-to-noise ratio of 3.

Fig. 6 is here.

The number of the interfering species depends on the working potential and the nature of the sample. The most common electrochemical interfering species such as ascorbic acid and *L*-cysteine were evaluated. As shown in the inset C in Fig.6, there is no significant change in current response to be observed by adding six common interferences into glucose test solution indicating that thus-fabricated biosensor has good anti-interference ability.

The reproducibility of the enzyme electrode was evaluated from the responses of six different enzyme electrodes prepared under the same conditions to 0.5mM glucose solution. The results revealed that this glucose biosensor has a satisfied reproducibility with a R.S.D of 5.6%. The repeatability was of a relative deviation 3.6% for six times successive determinations of the same sample solution, which indicates that the biosensor has a good operational stability. The steady-state response current of 0.5mM glucose was determined every 5 days. When not in use, the biosensor was stored in a refrigerator at 4□ dryly. The results showed that the steady-state

response current only decrease by 10% for 15 days, which indicates that the enzyme electrode was considerably stable. The stability of the PB in neutral solution and the leakage of the enzymes are two main factors affecting the stability of the PB-based biosensors. In the present work, the hybrid film provides a good microenvironment for stabilization of GOD and PB, which ensures the long-term stability of the biosensor.

The fresh human serum samples, which were provided by a local hospital, were assayed to demonstrate the practical application of thus-fabricated glucose biosensor by standard addition method. These samples were firstly analyzed with a standard clinical assay based on glucose dehydrogenase electrode method. As shown in Table 1, a good agreement between the two sets of data allows us to ascertain the practical utility of the biosensor and apply this biosensor to determine glucose in real samples. Besides, within the error limit, there appears to be no statistically significant difference between these two methods (significance level 0.05).

Table 1 Results of the analysis of the human serum samples with different glucose concentrations

Serum sample no	Clinical assay result / mM	Test result* /mM	Deviation / mM
Sample 1	6.72	6.92	+0.2
Sample 2	9.32	8.91	-0.43
Sample 3	15.35	15.75	+0.4

*: the average value for three measurements

4 Conclusions

A novel route, one step co-electrodepostion, for preparation of poly(*N*-acetylaniline) and Prussian blue hybrid composite film was proposed for the first time. The PNAANI-PB hybrid composites film modified electrode exhibited high sensitivity, fast response, wide linear range, excellent stability and free of interference towards reduction of H_2O_2. The microporous structure of the hybrid film can provide a protective environment to improve the operational stability of Prussian blue. SEM showed that the PB particle is smaller in the polymeric net-matrix than in pure PB film and combined tightly with the backbone of polymer, and the micro hole allows H_2O_2 go-through and being reduced at the electrode catalyzed by Prussian blue. The good performance can be attributed to the synergistic effect between poly(*N*-acetylaniline) and PB. A glucose biosensor based on PNAANI-PB hybrid film was further constructed and illustrated an excellent performance. It is demonstrated that the PNAANI-PB hybrid film may be served as a platform for detection of H_2O_2 to develop a variety of bio-electrochemical sensors or devices.

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References

[1] A. A. Karyakin, Electroanalysis 2001, 13, 813.
[2] I. L. de Mattos, L. Gorton, Truzgas, Biosens. Bioelectron. 2003, 18, 193.
[3] D. X. Cao, L. Sun, G. L. Wang, Y. Z. Lv, M. L. Zhang, J. Electroanal. Chem. 2008, 621, 31.
[4] X. Zhang, J. Wang, B. Ogorevc, U. C. Spichiger, Electroanalysis 1999, 11, 945.

[5] W. Hou, J. Wang, Anal. Chim. Acta 1992, 257, 275.
[6] W. Lu, G. G. Wallace, A. A. Karyakin, Electroanalysis 1998, 10, 472.
[7] A. A. Karyakin, O. V. Gitelmacher, E. E. Karyakina, Anal. Chem. 1994, 27, 2861.
[8] A. A. Karyakin, O. V. Gitelmacher, E. E. Karyakina, Anal. Chem. 1995, 67, 2419.
[9] S. F. White, A. P. F. Turner, U. Bilitewski, J. Bradley, Electroanalysis 1994, 6, 625.
[10]Karyakin, A.A., Karyakina, E.E., Gorton, L., 2000. Analytical Chemistry 72, 1720–1723.
[11]Liu, Z.L., Liu, B.H., Zhang, M., 1999. Anal. Chim. Acta 392, 135–141.
[12]Karyakin, A.A., Karyakina, E.E., Gorton, L., 1996. Talanta 43, 1597–1606.
[13] V. D. Neff, J. Electrochem. Soc. 1978, 125, 886.
[14] K. Itaya, T. Ataka, S. Toshima, J. Am. Chem. Soc. 1982, 104, 4767.
[15] F. Ricci, G. Palleschi, Biosens. Bioelectron. 2005, 21, 389.
[16] R. Garjonyte, A. Malinauskas, Sens. Actuators B 1999, 56, 93.
[17] R. Garjonyte, A. Malinauskas, Sens. Actuators B 1998, 46, 236.
[18] F. Ricci, A. Amine, C. S. Tuta, A. A. Ciucu, Anal. Chim. Acta. 2003, 485, 111.
[19] F. Ricci, A. Amine, G. Palleschi, D. Moscone, Biosens. Bioelectron. 2003, 18, 165.
[20] A. V. Borisova, E. E. Karyakina, S. Cosnier, A. A. Karyakina, Electroanalysis 2009, 21, 409.
[21] S. Wu, J. Liu, X. Bai, W. Tan, Electroanalysis 2010, 22(16), 1906-1910.
[22] V. Lukachova, E. A. KotelBnikova, D. DBOttavi, Bioelectrochem. 2002, 55, 145.
[23] X. Zhang, R. Yuan, Y. Chai, J. Dai, Y. Liu, D. Tang, Anal. Lett. 2005, 38, 1085.
[24] T. Uemura, S. J. Kitagawa, J. Am. Chem. Soc. 2003, 125, 7814.
[25] Y. Xian, Y. Zhou, Y. Xian, L. Zhou, H. Wang, L. Jin, Anal. Chim. Acta 2005, 546, 139.
[26] Y. Miao, J. Chen, X. Wu, K. Tang, A. Jia, J. Liu, J. Nanosci. Nanotechnol. 2007, 7, 2877.
[27] Garjonyte, A. Malinauskas, Sens. Actuators B 1999, 56, 85.
[28] Garjonyte, A. Malinauskas, Sens. Actuators B 2000, 63, 122.
[29] D. Pan, J. Chen, L. Nie, W. Tao, S. Yao, Electrochim. Acta. 2004, 49, 795.
[30] S. Lupu, C. Mihailciuc, L. Pigani, Electrochem. Commun. 2002, 4,753.
[31] E. Bustos, J. Manr_quez, G. Orozco, Luis A. God_nez, Langmuir. 2005, 21, 3013
[32] E. Bustos, T.W. Chapman, F. Rodr_guez-Valadez, Electroanalysis 2006, 18, 2092.
[33] S. Wu, T. Wang, C. Wang, Z. Gao, C. Wang, Electroanalysis 2007, 19, 659.
[34]Wilson, G.S., Hu, Y., 2000. Chem. Rev. 100, 2693–2704.
[35]M. Angelopoulos, in The Handbook of Conducting Polymers, 2nd ed. (Eds.: T. Skotheim, R. Elsenbaumer, L. D. Reynolds),Marcel Dekker, New York 1997; pp. 9021 ± 944.
[36] J. C. Chiang, A. G. MacDiarmid, Synth. Met. 1986, 13, 193.
[37]Y. Cao, S. Li, Z. Xue, D. Guo, Synth. Met. 1986, 16, 305.
[38] T. Hirai, S. Kuwabata, H. Yoneyama, J. Chem. Soc. FaradayTrans. I 1989, 85, 969.
[39]N.S. Shilpa, B. Nitin, H. Subramanian, and J.V. Yakhmi, 2004. Philos. Mag., 84, 2127
[40] D.M. Delongchamp and P.T. Hammond, 2004. Chem. Mater., 16,4799
[41] B.P. Jelle and G. Haghen, 1998. J. Appl. Electrochem., 28, 1061.
[42]K. Ogura, K. Nakaoka, and M. Nakaoka, 2000. J. Electroanal.Chem., 486, 119 .
[43]P.J. Kulesza, M. Krzysztof, C. 2001. Electrochim. Acta, 46, 4065.
[44]Liu G.,Wu Sg.,2012, Biosensors and Bioelectronics, 38, 289-294
[45]T.R.I. Cataldi, R. Guascito, and A.M. Salvi, 1996. J. Electroanal.Chem., 417, 83.
[46] R.P. Pant, S.K. Dhawan, N.D. Kataria, and D.K. Suri, 2002. J.Magn. Mater., 252, 16
[47]Laviron, E., 1979. J. Electroanal. Chem. 101, 19–28.

[48]Garjonyte, R., Malinauskas, A., 1998. Sensors and Actuators B: Chemical 46, 236–241.

Figure captions

Figure 1:

Cyclic voltammograms for the co-electrodeposition of PNAANI-PB hybrid film. Conditions: 10 consecutive scans in the potential range from -0.20 to +0.90V at a scan rate of 50 mVs^{-1}. Inset compares the peak current of the last cycle for the PNAANI-PB film and pure PB film respectively.

Figure 2:

SEM images of (A) PB and (B) PNAANI-PB film electrodeposited on glass carbon slice, XPS of (C) the N1s region for the PNAANI (a) , PB (b) and PNAANI-PB (c) film , and (D) the Fe2p region for PB (a) and PNAANI-PB (b) (CPS:counts per second; BE: binding energy).

Figure 3:

(A) Cyclic voltammograms of the PNAANI-PB hybrid film modified electrode at the san rate(v) of 20,40,60,80,100,120,140,160,180,200mVs^{-1} respectively (from internal to external) in 0.025M PBS + 0.1 M KCl. The inset of A shows the calibration plot of the peak current *vs* v.

(B) Electrochemical stability of the PNAANI-PB hybrid film (a) and the pure PB film (b) at different pH solutions evaluated from CV peak currents. Conditions: supporting electrolyte, 0.025 M PBS + 0.1 M KCl with different pH; potential scan rate, 100 mVs^{-1}; scan cycles, 200. The inset of B shows the CV curves at pH 7.0, (c) and (d) represent the first and the 200th cycle, respectively.

Figure 4:

Dependence of amperometric response of H_2O_2 in 0.025 M PBS + 0.1 M KCl at PNAANI-PB hybrid film on (A) the concentration of N-acetylaniline, (B) the scanning cycles during the co-deposition, and (C) the working potential.

Figure 5:

(A) Amperometric response of PNAANI-PB (a) and PB (b) film to 10 times addition of 0.1mM H_2O_2 at the working potential of 0.0V. The inset of A is the cyclic voltammograms of PNAANI-PB at various concentration of H_2O_2 in 0.025 M PBS + 0.1M KCl at a potential scan rate of 50 mVs^{-1}.

(B) The calibration curve of the amperometric response at the PNAANI-PB hybrid film as a function of H_2O_2 concentration. The inset of B gives the amperometric response of PNAANI-PB hybrid film in the concentration of H_2O_2 ranging from 1.0×10^{-6} M to 1.0×10^{-5} M(a), 1.0×10^{-5} M to 1.0×10^{-4} M(b),and 1.0×10^{-4} M to 1.0×10^{-3} M(c). The applied potential was 0.00V.

Figure 6:

The calibration curve of the amperometric response of biosensor as a function of glucose concentration.

Inset give the amperometric responses of the biosensor in the concentration of glucose ranging from 2.0×10^{-3}M to 8.0×10^{-3} M (b) and 1.0×10^{-4} M to 1.0×10^{-3} M(a) respectively. The applied potential was 0.0V. Inset c shows chronoamperometric response to the injection of 0.70 mM glucose and six common interferences: 0.20mM uricacid(UA), 0.20mM dopamine(DA), 0.20mM ascorbicacid(AA), 0.20mM L-histidine, 0.20mM L-cysteine and 0.20mM L-cystine. The applied potential was 0.00V.