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Electrogenerated chemiluminescence biosensor for glucose based on poly(luminol–aniline) nanowires composite modified electrode

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ABSTRACT

Poly(luminol–aniline) nanowires composite (PLANC) was synthesized on the surface of graphite electrode by electro-oxidizing the mixture of luminol with aniline in the H_2SO_4 acidic medium. The properties of the nanowires composite were characterized by transmission electron microscopy, electrogenerated chemiluminescence and electrochemical impedance spectroscopy. The investigating results showed that: firstly, while the luminol alone was electro-oxidized on the surface of the graphite electrode with the proposed synthetic method, only the polyluminol film was formed on electrode surface. However, while a suitable amount of the aniline was present in the luminol solution, the PLANC could be formed on the electrode surface and this PLANC presented better ECL properties for H_2O_2 compared with pure polyluminol film; secondly, since the PLANC modified electrode not only provided a larger surface area for higher glucose enzyme loading but also formed the nano-structured interface on the electrode surface to improve the analytical performances of the electrogenerated chemiluminescence (ECL) biosensor for glucose, the resulting ECL biosensor presented good response to glucose and a novel ECL biosensor for glucose was proposed.

1. Introduction

As an analytical technique, electrogenerated chemiluminescence (ECL) has been paid more and more attention by the analysts since it not only retains the advantages of chemiluminescence (CL) sensor, such as excellent sensitivity, wide dynamic concentration response range, but also has some additional advantages (Richter, 2004; Zu et al., 2001; Qiu et al., 2009). Luminol is considered as an efficient system in ECL measurement for the detection of hydrogen peroxide and one of the most commonly used ECL luminophores or labels especially in the field of bioanalytical application such as enzyme biosensors (Borisov and Wolfbeis, 2008; Qiu et al., 2009; Blum et al., 1989). In combination with an appropriate enzymatic reaction, some efficient ECL systems with improved detection limits and linear ranges were reported for glucose, lactate or choline measurements (Tsafack et al., 2000; Marquette and Blum, 1999; Zhu et al., 2002a,b), which allowed the development of low-cost optical biosensors involving oxidases. Efficient and highly sensitive detection of glucose is of great interests from several points of view ranging from medical applications in blood glucose sensing to ecological applications. Various methods are available for glucose determination, especially enzymatic methods (Sun et al., 2001; Willner, 2003; Ye et al., 2004; Chen et al., 2009). Luminol-based ECL systems have been developed to construct the ECL biosensors for glucose because of its low oxidation potential, inexpensive reagent consumption and high emission yields (Qin et al., 1998; Wang et al., 2002; Lin and Chen, 2006). Generally, according to the literature, the enzyme can be immobilized on a proper support, such as sol–gel film (Zhu et al., 2002a,b; Liu et al., 2008), polymer (Marquette and Blum, 1999; Tsafack et al., 2000) and nafion film (Lin et al., 2008; Qiu et al., 2009), which were immobilized on the electrode.

In order to develop easy-to-use reagentless systems that avoid conventional luminophore addition, the immobilization of luminol on a transducer surface appears attractive. Based on the structural analogy between aniline and luminol, polyluminol has been rapidly studied recently. Polyluminol film has been formed on graphite, glassy carbon, platinum, gold, screen printed and indium tin oxide electrodes in strongly acid and near-neutral solutions (Zhang and Chen, 2000; Lin and Chen, 2006; Chen and Lin, 2002; Wang et al., 2002, 2005; Sassolas et al., 2008, 2009a,b). Moreover, hybrid polyluminol films, such as poly(luminol–aniline) films, hybrid films composed of poly(luminol) and nanometer-sized clusters of polyoxometalate were also formed on electrode surface, which provide

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improved chemical properties over pure films (Lin and Chen, 2006; Chang et al., 2005; Ferreira et al., 2008a,b). Of them, the ECL sensors based on polyluminol film on various electrodes have gained much attention. This polyluminol-based ECL sensor offered a great potential towards the preparation of various more elaborate oxidase based ECL reagentless biosensors (Zhang and Chen, 2000; Wang et al., 2002; Sassolas et al., 2008; Sassolas et al., 2009a,b). However, the enzyme loading on the surface of pure polyluminol film modified electrode is low due to the simplex structure of polyluminol film and the electron transfer to and from enzymes is limited due to the poor electroactivity of polyluminol in basic medium.

The emergence of nanotechnology has opened new horizons for the application of nanomaterials in bioanalytical chemistry. Recent advances in nanotechnology offer exciting prospects in the field of bioelectronics (Wang, 2008). This could be attributed to the unique chemical and physical properties of nanoparticles (Cao et al., 2002). Nanocomposite involves the multiphase material where at least one of the constituent phases has one dimension less than 100 nm. Nanocomposite materials have attracted much attention because of their potential applications in catalysis, microelectronics, biomedicine and photovoltaics (Li et al., 2008; Wang et al., 2004a,b; Willner and Shipway, 2001; Chuang and Yang, 2008; Guico et al., 2004). As one of the most promising conducting materials, polyaniline (PANI) has a wide range of interesting electrical, electrochemical and optical characteristics along with excellent environmental stability (Khor et al., 1990; Ameen et al., 2008; Yan and Xue, 1999; MacDiarmid, 2001), and polyaniline-based composites exhibit broadened applications such as sensing and electrocatalysis (Huang et al., 2003; Zhang and Wan, 2003; Rajesh et al., 2009). Polyaniline nanowires have the properties of mechanical flexibility, high surface area, chemical specificity, tunable conductivity and easy processing, which make the conducting polymer nanowires the promising sensing material for ultrasensitive, trace-level biological and chemical nanosensor. Up to now, polyaniline nanowires have been synthesized using various techniques. As a simple and cost effective way, electrochemical polymerization is a direct way to deposit polyaniline nanowires on the desired substrate for a particular application (Gupta and Miura, 2005a,b; Zhu et al., 2006; Wang et al., 2004a,b), and the electrochemically fabricated polyaniline nanowires have been used in the development of electrochemical biosensor based on its large surface area and covalent binding site (Horng et al., 2009; Zhu et al., 2006). Although polyaniline nanowires have been used for immobilization of enzyme, and polyluminol has also been used to the development of ECL-based biosensor via immobilization of enzyme on electrode surface, ECL-based biosensor based on the composite of polyluminol and polyaniline nanowires for tuning the ECL properties of polyluminol and biosensor has not been reported previously, to our knowledge.

In this paper, we explored the feasibility of preparing nanowires composite containing polyluminol on electrode surface directly and nanowires composite-based ECL biosensor. It was found that poly(luminol–aniline) nanowires composite (PLANC) could be synthesized on the surface of graphite electrode by electrochemical oxidation of the mixing solution of aniline and luminol in acidic medium. The PLANC presented better ECL properties compared with polyluminol film alone, and the PLANC on the electrode surface provided a higher surface area for higher enzyme loading on electrode surface. Glucose oxidase (GOD) is chosen as the model enzyme for the fabrication of ECL-based glucose biosensor. Accordingly, an ECL biosensor for glucose was developed based on the immobilization of GOD and PLANC by their cross-linking with glutaraldehyde on the electrode surface. The proposed ECL-based biosensor exhibited high sensitivity, easy operation, low-cost and simple fabrication.

2. Experimental

2.1. Materials

Luminol stock solution (5×10^{-2} mol L^{-1}) was prepared by dissolving 0.886 g luminol (>95%, Shaanxi Normal University, Xi′an, China) in 100 mL of 0.10 mol L^{-1} NaOH. Glucose oxidase (GOD) (Type X-S from *Aspergillus niger*, 185 U mg^{-1}) was obtained from Sigma (St. Louis, MO, USA). Aniline sulfate, 30% H_2O_2 and 25% glutaraldehyde, was purchased from Xi′an Reagent Plant (Xi′an, China). All aqueous solutions were prepared exclusively in deionized distilled water.

2.2. Apparatus

The cyclic voltammetry was performed with a conventional three-electrode cell linked to a CHI660b Electrochemistry Working Station (CH Instruments, Inc.). The ECL was performed by a MPI-A ECL analytical system (Xi′an Remax Electronic Science Tech. Co., Ltd., Xi′an, China) with a R456 photomultiplier (PMT) (Xi′an Remax Electronic Science Tech. Co., Ltd., Xi′an, China) for transforming ECL emission into electrical signals. The PMT was operated at −800 V, and the ECL cell was placed in front of the PMT. The cell was made of a microbeaker (high: 35 mm, i.d.: 25 mm). A graphite electrode (Shanghai, 12.6-mm^2 surface area) was used as the basal working electrode. A Pt flake (7 mm × 7 mm) and Ag/AgCl (saturated KCl solution) were used as auxiliary electrode and reference electrode, respectively. SEM images were taken using a Quanta 200 Scanning Electron Microscopy (FEI Company, The Netherlands).

2.3. Preparation of the PLANC-based ECL sensor

The graphite electrode was carefully polished on rough and fine sandpapers successively, and the electrode was then subjected to be sonicated to remove adsorbed particles and rinsed with double-distilled water several times.

Three electrolytic modes were chose to prepare PLANC. The first one, potentiostatic technique was applied on the electropolymerization of aniline. The deposition of PLANC was carried out at the constant potential of 0.75 V for several minutes with electrolyte solution of 0.5 mol L^{-1} H_2SO_4 + 0.025 mol L^{-1} aniline + 0.001 mol L^{-1} luminol; The second method: PLANC was fabricated on electrode surface using galvanostatic method by the following three step electrochemical deposition procedure (Zhu et al., 2006). In the first step, a large current density was applied on the electrode in an aqueous solution containing 0.025 mol L^{-1} aniline, 0.001 mol L^{-1} luminol and 0.5 mol L^{-1} H_2SO_4. The initial stage was followed by continuous polymerization with reduced current density. A typical procedure involves: 0.06 mA cm^{-2} for 0.5 h, followed by 0.03 mA cm^{-2} for 3 h, which was then followed by another 3 h at 0.015 mA·cm^{-2}; The Final method: repetitive cycle voltammetry was applied on the electrode in an aqueous solution containing 0.025 mol L^{-1} aniline, 0.001 mol L^{-1} luminol and 0.5 mol L^{-1} H_2SO_4 at a scan rate of ν = 200 mV s^{-1} in the interval of potential between −0.2 V and 1.0 V for 50 repetitive cycles. Then, the electropolymerization was carried out continuously at a scan rate of ν = 100 mV s^{-1} for 50 repetitive cycles, which was then followed by another 50 repetitive cycles with scan rate of ν = 50 mV s^{-1}. Finally, the obtained modified electrode was immersed in 0.5 mol L^{-1} H_2SO_4 for continuous cyclic scanning to made it stable.

The enzyme electrode was prepared as follows: The PLANC modified electrode was firstly immersed in PBS (pH 7.4) for continuous cyclic scanning, and then the electrode was put into 2.5% glutaraldehyde (GA) phosphate buffer (pH 7.4) solution for 6 h. Then, the electrode was washed with distilled water, and ten microliters of 5.00 g L^{-1} GOD solution was spread evenly onto the surface

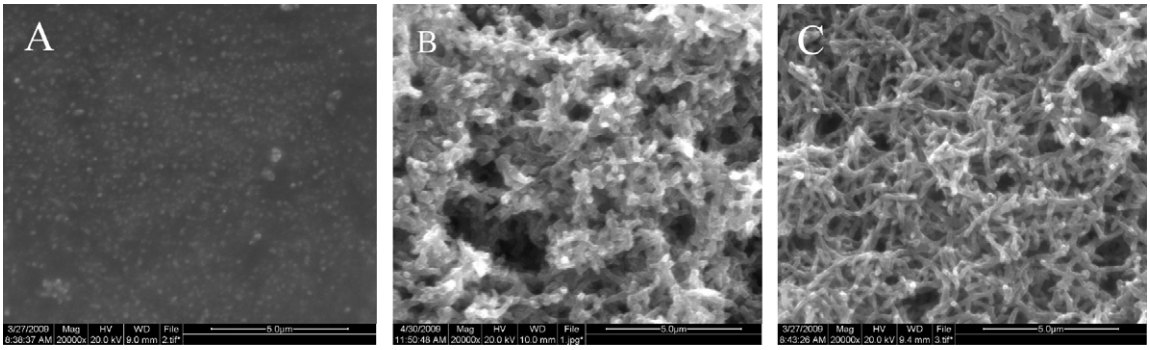

Fig. 1. Scanning electronic micrographs of bare graphite electrode surface (A), polyluminol film modified electrode surface (B), poly(luminol–aniline) nanoparticles composite modified electrode surface (C) and PLANC modified electrode surface (D, E).

of the obtained electrode with a microsyringe. After the electrode was dried at the room temperature and rinsed with water, the PLANC–GOD modified electrode was obtained through cross-linking of glutaraldehyde. (The fabricating process is showed in Supporting Information Scheme S1).

3. Results and discussion

3.1. Preparation of the PLANC modified electrode

Luminol has been electropolymerized on electrode surface to generate polyluminol film, which offered an easy way both to fabricate reagentless-based ECL sensor and to broaden the application fields of ECL-based sensor. Nanomaterials provide an ideal platform for enzyme immobilization: minimum diffusional limitation, maximum surface area per unit mass, and high effective enzyme loading. Therefore, for fabricating a proper platform for the enzyme immobilization, the preparation of nano-structured polyluminol was investigated using electrochemical way. We found that polyluminol film could be prepared on graphite electrode. However, pure nano-structured polyluminol could not be obtained by the proposed electrochemical way (see Supporting Information Fig. S1). For establishing a nano-structured polyluminol platform for the fabrication of ECL-based biosensor, nano-structured composites containing polymeric luminol was investigated. Nano-structured polyaniline, with different morphologies, has been synthesized using various techniques, and poly(luminol–aniline) composite films could be generated on electrode surface by electropolymerization. Therefore, it is possible to synthesize nano-structured poly(luminol–aniline) composite on electrode surface, which may presents ECL activity and provides a better microenvironment for enzyme immobilization.

For copolymerizing luminol in polyaniline nanowires effectively, the preparation of the PLANC was studied in detail. 1×10^{-3} mol L^{-1} luminol and 0.025 mol L^{-1} aniline were chosen in the preparation of the composite nanowires. Three electropolymerization modes were applied on the preparation of PLANC, including galvanostatic method, potentiostatic technique and cyclic voltammetry. For applying these modified electrodes on ECL sensor very well, we examined the copolymerization effect of aniline and luminol using ECL performance. In order to know the microstructure of the electropolymerized polymer obtained by the above three method, the obtained film was observed under the scanning electron microscope. The surface morphologies of the three kinds of electropolymerized polymer films generated on electrode surface are shown in Fig. 1A–C. It was found that nanoparticles were formed on the electrode surface with electropolymerization of galvanostatic method, and nanowires were generated with electropolymerization of potentiostatic technique or cyclic voltammetry under the optimal conditions. The diameters of the nanoparticles and nanowires are about 150 nm. The nanowires network is highly porous and the PLANC are interconnected. On the other hand, the electrochemistry characteristic of PLANC coating electrode has been investigated using $K_3Fe(CN)_6$ as a electrochemical probe (see Supporting Information Fig. S2), and the result shows that the PLANC modified electrode exhibits larger background current and electrochemical active response when compared with the bare electrode. It indicates that the PLANC modified electrode modified have a larger electrode effective surface area than bare electrode (Zhu et al., 2006).

On the other hand, the ECL of the three modified electrodes obtained with three polymerization modes were investigated, and the results demonstrated that the ECL emission of the PLANC modified electrode prepared by cyclic voltammetry with three steps is better than that of the other two PLANC electrodes modified by potentiostatic method and galvanostatic method, indicating that PLANC could be generated on electrode surface effectively under CVs with the other selected conditions. When the scan rate of the CV is 200 mV s^{-1}, the polymer was deposited on the electrode surface as small particles, which served as seeds for the growth of nanoframeworks during the following electropolymerizing procedures (Zhu et al., 2006). After two continued polymerization with reduced scan rate was performed, a layer of composite nanowires was formed on electrode surface.

3.2. ECL characteristics and response to H_2O_2 of the PLANC modified electrode

The ECL of the PLANC modified electrode was investigated. The electrode was immersed in 0.05 mol L^{-1} borax–0.2 mol L^{-1} borate solution (pH 9). The ECL response of the PLANC modified electrode was investigated under continuous potential scanning, and the result showed that the PLANC modified electrode displayed strong and stable ECL signal (see Supporting Information Fig. S3). The result suggests that luminol could be copolymerized with aniline, and the PLANC could present stable ECL emission when continuous CV scanning was applied on the PLANC modified electrode.

Furthermore, the I_{ECL}/E curves of the PLANC modified electrode were investigated. Fig. 2 shows I_{ECL}/E curves in 0.05 mol L^{-1} borax–0.2 mol L^{-1} borate solution with different modified electrodes. It can be seen that all three electrodes modified with the polyluminol film, poly(luminol–aniline) composite nanoparticles or PLANC exhibited ECL emission when electrolytic potential was applied on the modified electrode. However, new ECL peaks appeared at about 0.2 V and 0.5 V under CV scanning with the poly(luminol–aniline) composite nanoparticles modified electrode

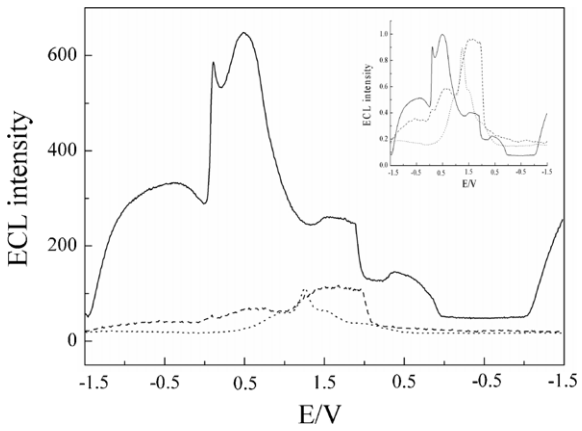

Fig. 2. I_{ECL}/E curves of pure polyluminol film modified electrode (dot line), poly(aniline–luminol) nanoparticles composite modified electrode (dash line) and PLANC modified electrode (solid line) in 0.05 mol L^{-1} borax–0.2 mol L^{-1} borate solution under CV of −1.5 V to 1.5 V.

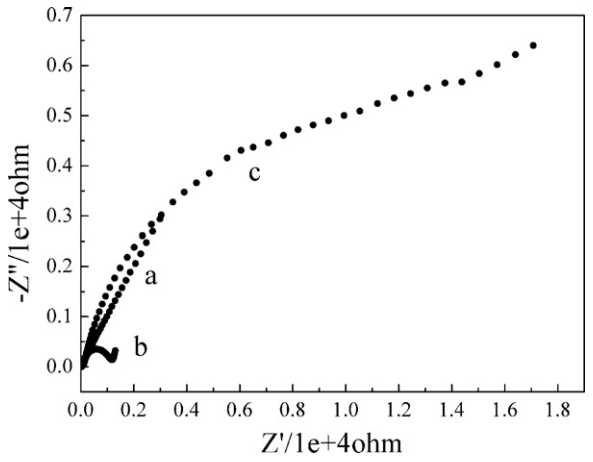

Fig. 3. Nyquist plots of (a) bare graphite electrode, (b) PLANC modified graphite electrode, (c) PLANC–GOD modified graphite electrode in 1 mol L^{-1} $K_3Fe(CN)_6/K_4Fe(CN)_6$/1 mol L^{-1} NaCl solution with frequency of 0.05–100 kHz.

and the PLANC modified electrode compared with that of the pure polyluminol film modified electrode. Moreover, the ECL response of the PLANC modified electrode under CV was stronger than that of the polyluminol film modified electrode and poly(luminol–aniline) composite nanoparticles modified electrode. On the positive scan of the potential from -1.5 V, obvious oxidation ECL peaks appear at about 0.2 V and 0.5 V on the PLANC modified electrode. However, a new ECL peak appeared at the potential of −0.55 V from the second CV scanning, suggesting that electrogenerated species of polymeric luminol produced in the positive scanning could react with the electrochemically reduced species at the surface of the PLANC electrode. On the negative san of the CV, the corresponding ECL response of the PLANC modified electrode presents two wide ECL peaks from 1.5 V to 1.0 V and 0.6 V. For eliminating the difference coming from the amount of the polymeric luminol on electrode surface, the I_{ECL}/E curves of the PLANC modified electrode, poly(luminol–aniline) composite nanoparticles modified electrode and pure polyluminol film modified electrode were normalized (as shown in Fig. 2, inset). It is obvious that the ECL intensity at 0.2 V and 0.5 V increases comparing to the polyluminol film modified electrode. It can be seen from above results that new ECL peaks of the polymeric luminol appear at about 0.2 V and 0.5 V when aniline is polymerized with luminol, and the ECL response of the polymeric luminol in PLANC is stronger than that in pure polyluminol film and poly(luminol–aniline) nanoparticles at low potential. The result indicates that the polymeric aniline has an important effect on the ECL properties of polymeric luminol in PLANC. The change of polymeric luminol′s ECL properties originates from the polymeric aniline in PLANC. The polymeric aniline in PLANC may be more favorable to the generation of active species produced at low potential, and provide a more proper hydrophobic microenvironment for the ECL reaction at the PLANC modified electrode surface (Li et al., 2009). Furthermore, the nanowires composite is more favorable to the ECL reaction of polymeric luminol at low potential due to the rapid electron transfer at the nano-structured interface.

It is well known that the presence of H_2O_2 can increase the ECL intensity of luminol and polyluminol. So we also tested the effect of the H_2O_2 on the ECL intensity of PLANC. The ECL intensity can be enhanced by H_2O_2, and the ECL response to H_2O_2 was linear in the concentration range of 3.0×10^{-9} to 1.0×10^{-4} mol L^{-1}. Based on the analysis of H_2O_2 developed above, glucose can be analyzed by coupling the GOD with the composite nanowires at the electrode surface.

3.3. Fabrication of biosensor and ECL sensing of glucose

The performant sensor for the H_2O_2 detection can be used to detect many other oxidase–substrate compounds. In this work, the GOD was chosen as the model enzyme.

Covalent bonding may be used to achieve the immobilization of enzyme on a membrane matrix. In this system, glutaraldehyde was used as a cross-linking agent to immobilize GOD on the PLANC modified electrode surface by means of Schiff base reaction between the aldehyde groups of glutaraldehyde and the free amino sites of the GOD and PLANC. The large area of the PLANC deposited on the electrode surface provides more sites for the immobilization of GOD. In the experiment, PLANC modified electrode was fabricated with GOD by cross-linking of glutaraldehyde, and electrochemical impedance spectrum (EIS) of $Fe(CN)_6^{4-/3-}$ were used to monitor the procedure. Electrochemical impedance spectroscopy is one of the most powerful and sensitive techniques for investigating the features of surface-modified electrodes. Fig. 3 shows the impedance spectra of the electrode in each step. The response of electrochemical impedance spectroscopy at the bare electrode is a straight line (Fig. 3a), revealing there is almost no heterogeneous charge-transfer resistance on bare electrode surface. After the bare electrode was modified with PLANC, the *R*ct increased (Fig. 3b), while the impedance response at the GOD modified electrode (Fig. 3c) is much larger, illustrating the successful immobilization of GOD on the PLANC modified electrode surface. On the other hand, the electrochemical impedance spectrum of the polyluminol film modified electrode and polyluminol–GOD film modified electrode was investigated. As shown in Fig. 4, the impedance response of the polyluminol–GOD film modified electrode changed little compared with the polyluminol film modified electrode. The above results indicate that the PLANC provides a more ideal platform for the GOD immobilization due to the larger surface area and more active sites than polyluminol film alone.

Standard glucose solutions (1.0×10^{-6} mol L^{-1}, 3.0×10^{-6} mol L^{-1}, 5.0×10^{-6} mol L^{-1}, 7.0×10^{-6} mol L^{-1}, and 1.0×10^{-5} mol L^{-1}) were measured continuously with this ECL biosensor (as showed in Fig. 5). Under the selected conditions given above, the calibration graph of emission intensity versus glucose concentration was linear in the range of 1.0×10^{-7} to 5.0×10^{-5} mol L^{-1}, and the detection limit was 3×10^{-8} mol L^{-1} (3σ). The regression equation was $I = 16.83C \times 10^6 + 18.72$ (where C is the glucose concentration, mol L^{-1}) with a correlation coefficient of 0.9952. The relative

Table 1
Results analysis of glucose in serum.

	Determined by hospital (mM)	This method (mM)[a] (% R.S.D.)	Added (mM) (%)	Detected (nM)	Recovery
1	3.25	3.35 (3.60)	0.5	3.83	96.4
2	2.87	2.66 (2.15)	1.0	3.63	97.3
3	2.54	2.42 (2.34)	1.0	3.45	103.4

[a] Average of three determinations.

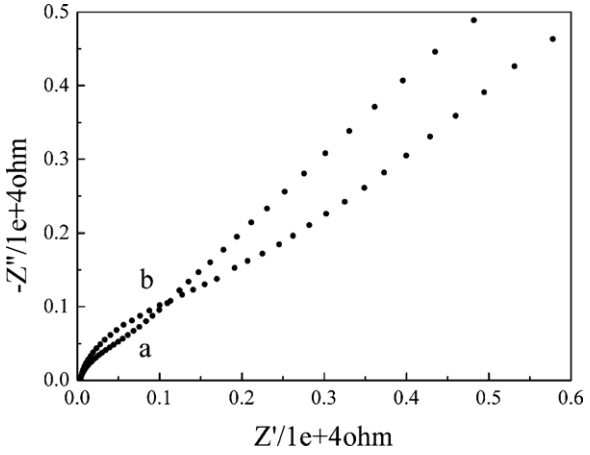

Fig. 4. Nyquist plots of polyluminol film modified electrode (a) and polyluminol–GOD film modified electrode (b) 1 mol L^{-1} KCl + 1×10^{-3} mol L^{-1} $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution with frequency of 0.05–100 kHz.

standard deviation of the biosensor response to 1.0×10^{-6} mol L^{-1} glucose was 4.3% for eleven successive measurements.

The stability of proposed biosensor was tested over a 1-month period. When the PLANC modified electrode was stored in the dry state at room temperature, ECL intensity decreased less than 5% compared with the initial steady state value after 1 month of storage. Because the sensor was used in water solution in this experiment, the stability of the sensor kept in 0.05 mol L^{-1} borax–0.2 mol L^{-1} borate solution (pH 9.0) was also investigated. The ECL intensity only decreased 9% compared with the initial steady state value. Furthermore, the operational stability of the biosensor was investigated by continuously measuring the ECL intensity by repeated injection of 1 μM glucose, in which two ECL signal was recorded for every injection. The response of the biosensor kept quite stable for the first 50 injections and a relative standard deviation not more than 10% and then gradually decreased. The ECL stability of the proposed biosensor is likely due

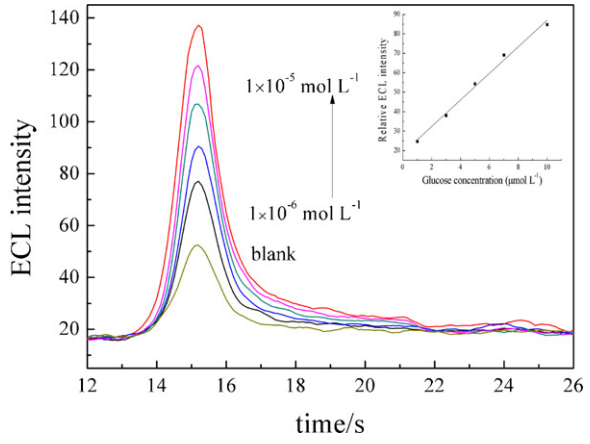

Fig. 5. ECL response of the biosensor to glucose with concentration of 1×10^{-6} mol L^{-1}, 3×10^{-6} mol L^{-1}, 5×10^{-6} mol L^{-1}, 7×10^{-6} mol L^{-1}, 1×10^{-5} mol L^{-1}.

to the little exhausting of polymeric luminol by the lower concentration of H_2O_2 and the effective electropolymerization of PLANC on electrode surface. However, the lifetime of the biosensor was short when the concentration of H_2O_2 is high. The proposed biosensor still has a limited lifetime because of the irreversibility of the polymeric luminol's electrochemical oxidation.

Some species that commonly existed and possibly interfered with determination of glucose in biological samples were tested. Increasing amounts of foreign species were added into the solution containing 1.0×10^{-6} mol L^{-1} of glucose. A foreign specie was considered not to interfere if it caused a relative error <5% during the measurement of the glucose sample. It was found that 15 times of uric acid and 10 times of phenylformic acid, ascorbic acid, 5 times of sucrose, lactose, maltose, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+} and 0.1 times of Co^{2+} has no interference with the determination of glucose.

3.4. Optimization of ECL detection system

The electrochemical parameters would obviously affect the ECL emission intensity for the determination of glucose. The effect of the electrolytic potential waveform, such as cyclic voltammetry, square wave, triangular wave and pulse wave, were examined, respectively. The results showed that the strongest ratio of the ECL signal to the noise was given by the use of cyclic voltammetry. The effect of the final potential on the enhancing ECL signal indicated that, when the final potential was 0.8 V, the greatest enhanced ECL signal of glucose could be easily observed. So 0.8 V was selected for the subsequent studies.

In this ECL system, the buffer solution is the medium of enzyme reaction and also is the medium of the ECL reaction. The influence of pH on the enzyme activity and the effect of pH on the generated ECL signal both determine the overall response of the biosensor. In view of the nature of polyluminol ECL reaction, which is more favored under basic conditions (pH 10–11), an alkaline medium would improve the sensitivity of the system. However, the optimal pH value for GOD is 6–7. In order to obtain better ECL sensing to glucose, the effect of medium pH on the biosensor was investigated. The biosensor showed strongest ECL response to glucose at pH 9 (see Supporting Information Fig. S4). As such, the reaction was performed at pH 9.0 throughout this study.

3.5. Sample analysis

To illustrate the feasibility of the PLANC–GOD modified electrode in practical analysis, it was used to detect glucose in human urine samples. Fresh human urine samples were first analyzed in a local hospital by spectrophotometric method. Then the samples were diluted 100 times by water to yield testing sample solutions and assayed with this ECL glucose sensor. The satisfied recovery could be found in Table 1, which showed the dependability of the proposed biosensor for the determination of glucose.

4. Conclusion

In this paper, a facile method for fabrication of PLANC and its application to ECL-based biosensor were demonstrated. PLANC can be successfully synthesized directly on electrode surface using

electrochemical polymerization. The PLANC displayed better ECL properties compared with polyluminol film alone. Furthermore, because of the large specific surface area, excellent conductivity and high porosity, PLANC easily provides larger effective surface area to immobilize GOD. Moreover, the interface of the PLANC with specific properties allows rapid electron transfer and hence enhances the glucose response. Based on these findings, a new concept for the fabrication of biosensor in ECL was proposed, and it can be readily extended to other biosensor systems based on the ample selection of ECL-active nano-structured composites and enzymes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.07.003.

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