Int. J. Electrochem. Sci., 11 **(2016)** 8193 – 8203, doi: 10.20964/2016.10.56

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Selective and Reliable Electrochemical Sensor Based on Polythionine/AuNPs Composites for Epinephrine Detection in Serum

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Received: 19 July2016/ *Accepted:* 22 August 2016 / *Published:* 6 September 2016

This work used a highly selective electrochemical sensor based on polythionine/AuNPs composites (PTh/AuNPs) modified glassy carbon electrode (GCE) to detect epinephrine (EP). An elevated response of the oxidative behavior of EP was obtained on the PTh/AuNPs modified GCE in phosphate buffer saline (pH 6.0), which allowed sensitive sensing of EP using the differential pulse voltammetry (DPV). The electrochemical sensor can selectively measure EP in a wide linear range from 1 to 40 mg/L with a detection limit of 0.3 mg/L (S/N = 3) and a high reproducibility in PBS. The developed electrochemical sensor can be favorably applied to detect EP in a diluted serum. Furthermore, the good linear regression between the EP concentrations and the currents in various diluted serum implied the potential of the proposed method as a promising candidate approach for complex clinical conditions.

Keywords: Epinephrine; Polythionine/AuNPs composites (PTh/AuNPs); Selective detection; Differential pulse voltammetry; Electrochemical sensor

1. INTRODUCTION

Epinephrine (EP), also known as adrenalin or adrenaline, an important neurotransmitter in the mammalian central nervous systems [1], exists as organic cations in the nervous tissue and body fluids [2]. In a series of nervous chemical processes and other biological reactions, such as contracting blood vessels and increasing heart rate [3], EP transmits nerve impulses to other organs and controls the performance of the corresponding organs [4−5]. Abnormal EP levels in human nervous tissue and body fluids results in several chronic diseases, such as Parkinson's disease [6], Alzheimer's disease [7], and schizophrenia [8]. Furthermore, EP acts as medication in several common emergencies, including anaphylaxis [9] and cardiac arrest [10], as well as in the effective and accurate monitoring of the

significance of EP metabolism and variability concentration change for clinical efficacy. Thus, developing a method for monitoring EP concentration in complex biological conditions is a vitally significant analytical agenda to further study of EP pharmacokinetics.

At present, numerous methods, such as high performance liquid chromatography (HPLC) [11], chemiluminescence [12], capillary electrophoresis [13], fluorescent method [14], and spectrophotometry [15] have been developed for EP detection. Although these methods are effective in the determination of EP, the abovementioned quantitative methods require expensive instrumentation with typical experimental conditions and cumbersome sample preparations. An electrochemical method [4, 16−17] has recently attracted extensive attention in the analytical fields, especially for sensing small molecules, because of its intrinsically advantageous rapid response, excellent selectivity, and perfect sensitivity. An increasing number of electrochemical analytical methods based on functional materials modified electrode have been developed in recent years to detect EP [18-20]. Apetrei et al. developed an electrochemical sensor based on tyrosinase immobilized single-walled carbon nanotube to detect EP with a low detection limit [21]. Zhou et al. prepared a multiporous imprinted electrochemical sensor that combined a molecularly imprinted polymers (MIPs) film, silica nanoparticles (SiO_2NPs), and multiwalled carbon nanotubes (MWNTs) to specifically recognize and detect EP [22]. However, the reported sensors involved complicated preparation processes, and their application for EP detection in biological samples, such as serum, was unreported. Thus, fabricating some effective electrochemical sensor that can be applied to accurately detect EP in complex biological samples is necessary.

Conducting polymers (CPs), such as polyanilines [23−24] and polypyrrole [25] are effective alternative choices for the fabrication of electrochemical sensors owing to their conformability, stability, biocompatibility, and activity. Compared with recently developed CPs, they are stable and excellent in conducting polymer of phenothiazine derivatives; furthermore, polythionine and its composites provide a promising route for creating flexible and significant electrochemical sensors with competitive properties [26]. In this work, we simply synthesized polythionine/AuNPs composites (PTh/AuNPs) through a one-pot synthesis method. After Au nanoparticles mixed into polythionine (PTh), PTh/AuNPs displayed some novel and enhanced electrochemical properties [27, 28]. Herein, PTh/AuNPs were prepared and applied to modify GCE to form an electrochemical sensor for the unique and sensitive catalytic activity for EP. The electrochemical sensor for EP demonstrated outstanding properties, such as a wide detection range, high sensitivity, fine selectivity, and low detection limit. Moreover, it was used to detect EP in a biological sample with a satisfactory result.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Epinephrine Hydrochloride (EP) injection (1 mL : 1 mg), obtained from Fujian Medical University Union Hospital, was used directly for the sensing. Thionine, uric acid (UA), dopamine (DA), and bovine serum albumin (BSA) were purchased from Sigma–Aldrich Company (China). N,N-dimethylformamide (DMF), chloroauric acid ($HAuCl_4$•$3H_2O$), 30% H_2O_2 solution, iron chloride hexahydrate ($FeCl_3$•$6H_2O$), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium hexacyanoferrate

trihydrate ($K_4[Fe(CN)_6]•3H_2O$), sodium chloride (NaCl), potassium chloride (KCl), glucose, and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water (18.2 MΩ•cm) purified through the Millipore water purification system was used throughout the experiments. Unless otherwise specifically defined, the electrolyte for EP detection was phosphate buffered saline (PBS) with pH 6.0. Under the informed consent, the clinical human serum samples collected from the Affiliated Union Hospital of Fujian Medical University were used as biological samples. All other reagents were of analytical grade and used as received, without any purification. Phosphate buffer saline (PBS, pH=6.0) acted as the testing solution containing 0.1 mol/L Na_2HPO_4–NaH_2PO_4 and 0.1 mol/L NaCl, and was adjusted its pH with 50 mmol/L H_3PO_4 and NaOH. All solutions were stored in a refrigerator (4 °C) when not in use.

The cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometric i-t curve were measured at the CHI 660C electrochemical workstation (Shanghai ChenHua Instruments Co., China) in PBS. The conventional three-electrode system for electrochemical detection consisted of the working electrode (glassy carbon electrode (GCE, Φ=3 mm), reference electrode (Ag/AgCl electrode with saturated KCl), and auxiliary electrode (platinum wire). Transmission electron microscopy (TEM) was obtained by JEOL JEM-1400 Transmission Electron Microscope with high voltage of 100 kV. The Fourier Transform Infrared Spectroscopy (FTIR) was performed using Nicolet 380 FT-IR. All experiments were carried out at room temperature.

2.2. Preparation of polythionine/AuNPs composites (PTh/AuNPs)

PTh/AuNPs were synthesized through a one-step synthesis method according to the reported literature [28−29]. For the modified electrode preparation, 10 mg of PTh/AuNPs was gently added into 2 mL of DMF and was subjected to ultrasonic oscillation for 10 min to obtain a PTh/AuNPs suspension.

2.3. Preparation of electrochemical sensor for EP

Prior to preparation, GCE was polished successively with 0.3 and 0.05 μm alumina powder suspensions on the microcloth pad, and then adequately sonicated in HNO_3 solution (the volume ratio of water to concentrated HNO_3 was 1:1), ethanol (95%), and deionized water for 3 min accordingly. Once the pretreated GCE was dried under nitrogen, 5 μL of PTh/AuNPs suspension was dropped on its surface and dried at room temperature. The prepared electrode was named PTh/AuNPs modified GCE. Different specific volumes of EP injection were added in PBS, and then the corresponding DPV curves were measured with PTh/AuNPs modified GCE.

2.4. Specificity of the sensing of EP

The specificity of the sensing of EP was carried out with amperometric i-t. Using PTh/AuNPs modified GCE, the potential was set at 0.4 V, then 10 mg/L of EP (5 times), interferences (20 μM DA,

50 μM UA, 1 mM glucose, 1 mM Ca^{2+}, 1 mM Zn^{2+}, 1 mM ethanol and 10 mg/L of BSA) and again 10 mg/L of EP were successive injected in the same PBS to record the continuous amperometric response.

3. RESULTS AND DISCUSSION

3.1.Characterization of PTh/AuNPs composites

Figure 1 showed the FTIR spectrum (Fig. 1A) of PTh/AuNPs and the TEM image (Fig. 1B) of PTh/AuNPs. The PTh/AuNPs FTIR result displayed that the molecular structure of the thionine monomer was nearly maintained during the polymerization preparation of PTh [20]. The molecular stretching vibration of N-H at about 3500 cm^{-1} was the amino moieties in the polymer skeleton of polythionine. In addition, the presence of the aromatic C–H stretching vibration at 2900 cm^{-1} illustrated the successful preparation of PTh/AuNPs [31]. PTh/AuNPs preparation was also reflected by the appearance obtained through the camera. The PTh/AuNPs aggregation illustrated a dark purple to a black powder, as shown in the inset of Fig.1A. Furthermore, the PTh/AuNPs morphology obtained through TEM distinctly revealed the main composition of PTh/AuNPs as the nano-network and the polythionine fiber structure with the embedding of Au nanoparticles with the uniform and average particle size of 80 nm. The organic structure as a whole body of the prepared PTh/AuNPs can initiate enhanced properties.

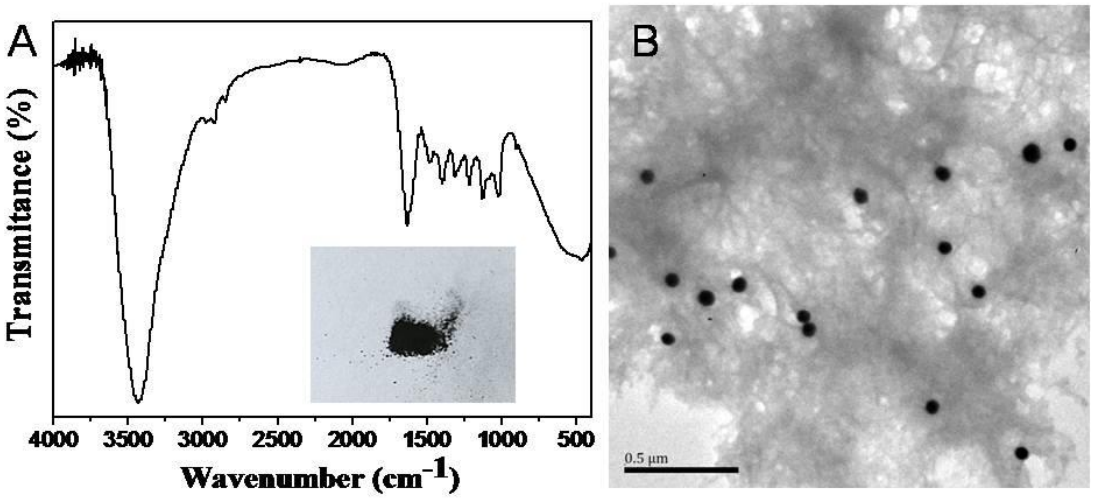

Figure 1. FTIR spectrum (A) and TEM image (B) of PTh/AuNPs.

3.2. Electrochemical behaviors of EP on PTh/AuNPs modified GCE

The CV and DPV were effective tools for characterizing the electrochemical behaviors of EP on PTh/AuNPs modified GCE.

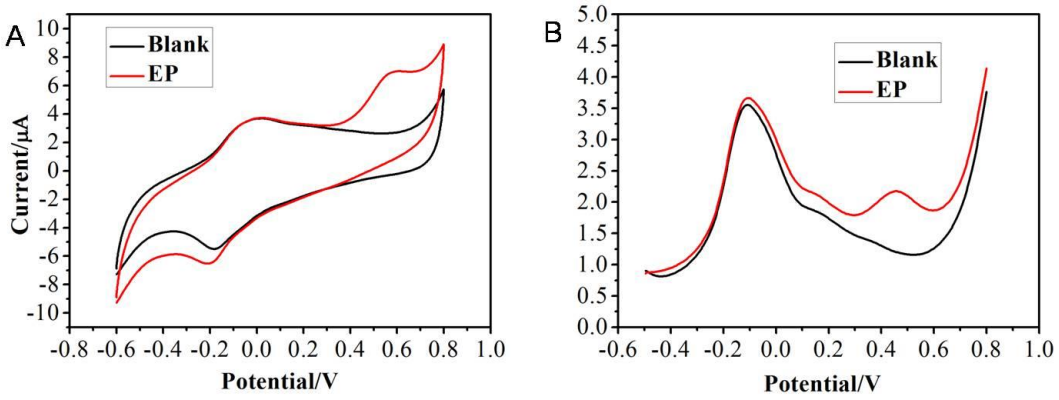

Figure 2. CVs (A) and DPVs (B) of PTh/AuNPs modified GCE in PBS (pH=6.0) without or with 2 mg/L of EP.

Fig. 2 shows the measured CV and DPV in PBS (pH = 6.0) without or with 2 mg/L of EP. The CV of the PTh/AuNPs modified GCE without EP indicated an evident redox peak at around -0.03 V assigned to the internal electrochemical response of PTh/AuNPs [28], whereas no other peak was found. Correspondingly, the CV of PTh/AuNPs modified GCE generated an obvious anodic peak at 0.6 V with EP, as well as the pre-existing redox peak at -0.03 V. In addition, the scan rate influence on EP oxidation was investigated by CV, as shown in Fig. 3.

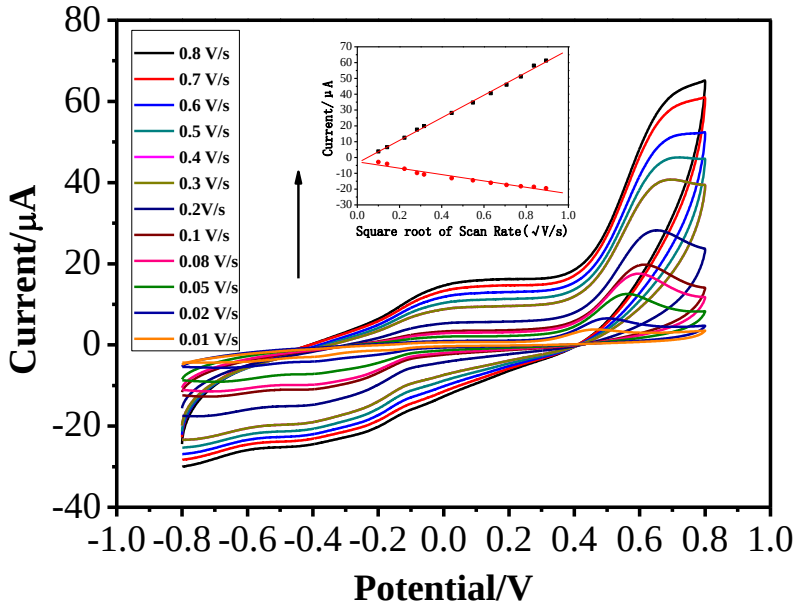

Figure 3. CVs of PTh/AuNPs modified GCE in PBS solution with 2 mg/L of EP at various scan rates (from inside to outside: 0.01, 0.02, 0.05, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 V/s), inset showed the linear relation of oxidative and redox peak currents and the square root of scan rate.

Increased redox currents were obtained in the scan range of 0.01 to 0.8 V/s. Both oxidative and reduced currents exhibited excellent linearity with the square root of the scan rate, which suggests that

the EP reaction on PTh/AuNPs modified GCE was a diffusion-controlled process. At the same time, DPV was further utilized to discuss the sensing possibility of EP using PTh/AuNPs modified GCE, as shown in Fig. 2B. With the addition of EP, PTh/AuNPs modified GCE displayed two oxidative peaks at -0.1 and 0.48 V. In addition, compared with the pure DPV behavior of PTh/AuNPs modified GCE without EP, the anodic peak at -0.1V served as the response of PTh/AuNPs [28], and the distinct anodic peak at 0.48 V was the electrochemical response of EP. Furthermore, compared with the DPV response of EP on the bare GCE shown in Fig. 4, the improved and sharp oxidative current peak of EP was obtained using PTh/AuNPs modified GCE, indicating the increased electrocatalytic activities of PTh/AuNPs to achieve the sensitive detection of EP.

The influence of the pH of the testing PBS was optimized to obtain the best performance. The current response of EP on PTh/Au modified GCE was initially compared in PBS without AA and DA, as shown in Fig. 5A. The EP potentials and currents on PTh/Au modified GCE were changed in PBS with different pH values, and the maximum oxidative peak current was achieved at pH 6.0 and 6.5 without considering the unsuitable acidic condition with pH 3.5. Furthermore, the pH optimization with AA and DA was also carried out because of the possible interfering effects of AA and DA in the real biological sample [32], as shown in Fig. 5B. As the pH changed, the electrochemical behavior of EP on PTh/Au modified GCE with AA and DA also varied. Compared with Fig. 5A, the peak currents and the oxidative potentials of EP changed as the pH differed with AA and DA, except for the condition of the pH 6.0.. The results suggested that the interference of AA and DA differed for sensing EP in PBS with variable pH values [33]. Compared with the EP response curves in pH 6.0 PBS with and without AA and DA in Fig. 5, the oxidative peak currents and potentials remained approximately constant. Furthermore, the current of EP reached its maximum in the condition of pH 6.0 in the presence of AA and DA. In summary, the solution with pH 6.0 was selected as the most appropriate condition for sensing EP.

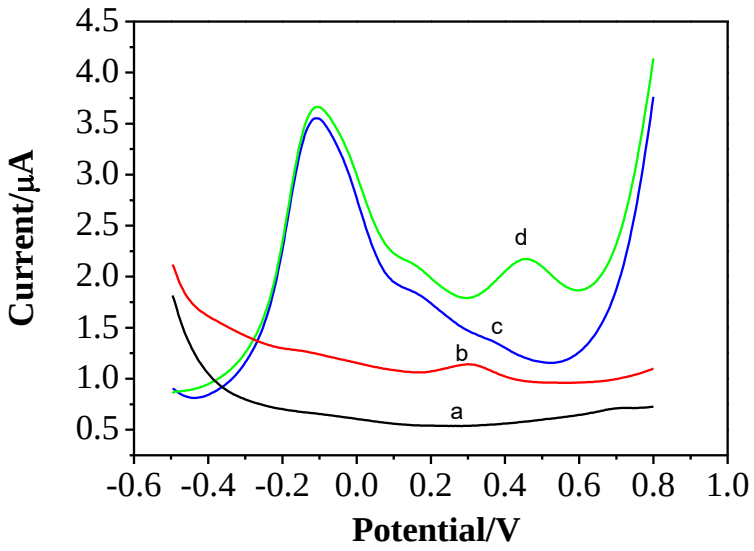

Figure 4. DPVs of bare GCE (a, b) and PTh/AuNPs modified GCE (c, d) in PBS (pH=6.0) with (b, d) or without (a, c) 2 mg/L of EP.

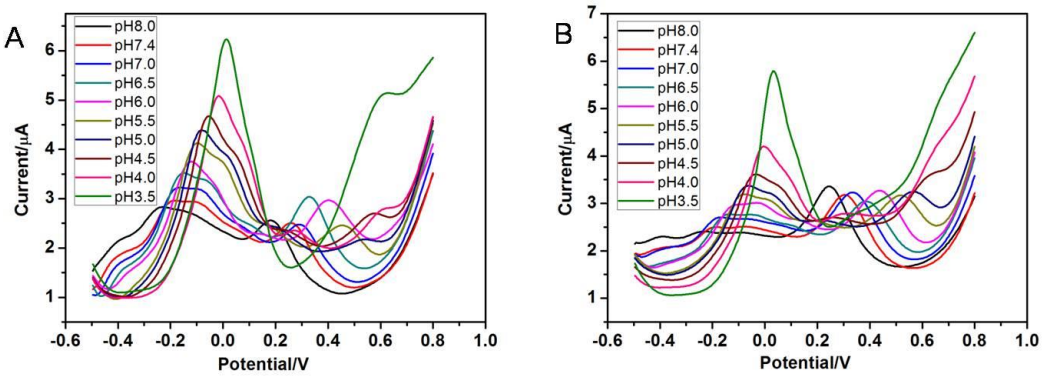

Figure 5. DPVs of PTh/AuNPs modified GCE in PBS of different pH values containing 2 mg/L EP without (A) or with (B) 100 μM AA and 20 μM DA.

3.3. Identification of EP on PTh/AuNPs modified GCE

The specific molecular recognition of PTh/AuNPs modified GCE for EP is an extremely important factor in detecting practical samples in body fluids. Common coexisting electrochemical active substances, such as DA, UA, and glucose, which may seriously affect EP detection, were discussed. The amperometric responses under the set potential of PTh/AuNPs modified GCE for interferents were tested in this work.

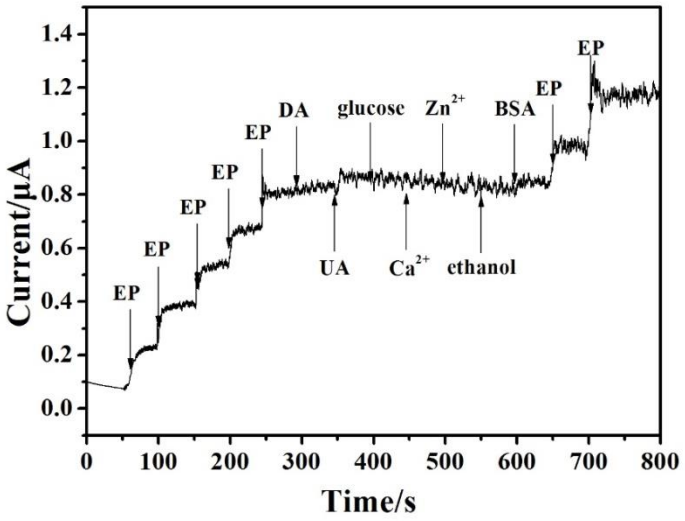

Figure 6. Amperometric response of PTh/AuNPs modified GCE for successive addition of EP and several interferents in stirring PBS (pH=6.0).

The amperometric responses of PTh/AuNPs modified GCE immediately increased and reached the platform when EP was added, as shown in Fig. 6. Correspondingly, the amperometric responses

were stably maintained with the successive injection of DA, UA, glucose, Ca^{2+}, Zn^{2+}, and ethanol, as well as BSA in the same PBS. Furthermore, the amperometric responses indicated no evident change for the detection system with EP in the mixture of several interferents. Moreover, after the injection of interferents, the instantaneous addition of EP obviously increased the current again, as shown in Fig. 6. These results implied that the developed electrochemical sensor possessed outstanding specificity for EP detection.

3.4. Performance of PTh/AuNPs modified GCE for EP

At first, the real content of the EP injection obtained from the local hospital was determined and confirmed using the standard HPLC method according to the protocol of Chinese Pharmacopoeia (2015). The result illustrated that the average content of the EP injection was 0.992 mg/mL, which was in accord with the labeled content (1 mL : 1 mg). According to the results in Fig. 2, DPV was chosen as the detecting technique for the calibration curve of different EP concentrations. A series of different EP concentrations were measured under optimized pH condition by the electrochemical sensor. With the successive addition of different EP concentrations, the DPV anodic peaks current at about 0.4 V gradually increased as the EP concentration was amplified, as shown in Fig. 7A. Fig. 7B revealed that the amperometric currents of DPV linearly increased as the EP concentration rose. In the concentration range from 1 (3.9 μM, with a 255 EP molecular weight) to 40 mg/L (156.7 μM), the linear fitting equation of the EP and the current was I (μA) =2.443 + $0.097C_{EP\ (mg/L)}$ with R^2 = 0.9930, as shown in Fig. 7B. Furthermore, the limit of detection (LOD) for EP was calculated at 0.3 mg/L (1.18 μM) based on S/N = 3. The linear range and LOD were comparable with that of the reported electrochemical methods for EP sensing based on metal nanomaterials [34−35], as shown in table 1.

The repeatability of the proposed method was evaluated by intra-assay relative standard deviation (RSD) values for the successive testing of EP (n=7) under similar conditions. Intra-assay RSDs of 6.4% and 3.6% were derived in EP sensing at 2 and 10 mg/L, respectively, which illustrated the excellent reproducibility of the prepared electrochemical sensor based on PTh/AuNPs modified GCE.

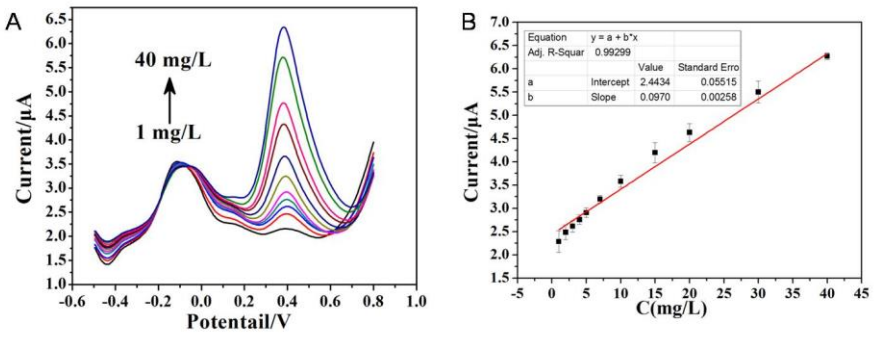

Figure 7. DPVs (A) and the plot of anodic peak values at 0.4 V vs. the concentration of EP (B) for the sensing of EP in the range of 1~40 mg/L in PBS (pH=6.0) .Error bars represent the SDs from three independent determinations.

Table 1. Comparison of the detection of EP using different modified electrodes

Electrode	Linear range (μM)	LOD (μM)	Sesnsing method	Reference
Nanoporous spongelike Au-Ag films	10-100	5.05	DPV	34
Ordered nanoporous thin Au films	20-100	2.42	DPV	35
Three dimensional molecularly imprinted polymer arrays	1-10 and 10-800	Not mentioned	DPV	36
Graphene/poly (brilliant cresyl blue) nanocomposite	1-1000	0.24	DPV	37
Polythionine/AuNPs Composites	3.9-156.7	1.18	DPV	This work

3.5. Real application of PTh/AuNPs modified GCE

The drug metabolism process usually monitors the concentration of drug or its metabolite in the body. A number of methods should be developed to assess drug change in serum. Different diluted serums were used in simulating complicated systems to examine the sensing EP performance in order to investigate the feasibility of the proposed method for EP monitoring in the body. Fig. 8 shows the detection of different EP concentrations in 10% and 20% serums using PTh/AuNPs modified GCE. Successions of DPV curves were obtained, and the anodic peak currents of DPV increased gradually as the EP concentration increased in different diluted serum solutions. Although the EP response currents were somewhat reduced compared to the EP sensing in pure PBS results, the anodic peak currents were still in good accord with the variable concentrations of EP. The linear relations were obtained between the anodic peak values of DPV and EP concentrations in the range of 3 to 40 mg/L. The linear regression equations were $I\ (\mu A) = 2.587 + 0.0898 C_{EP\ (mg/L)}$ with R = 0.9967 and $I\ (\mu A) = 2.111 + 0.0572 C_{EP\ (mg/L)}$ with R = 0.9987 in 10% and 20% serums, respectively. The small error bars indicated the acceptable reproducibility and reliability of the proposed method for EP sensing in diluted serum. The results adequately indicated the use of the electrochemical sensor for EP detection in complicated systems, which imply future clinical application of the current method.

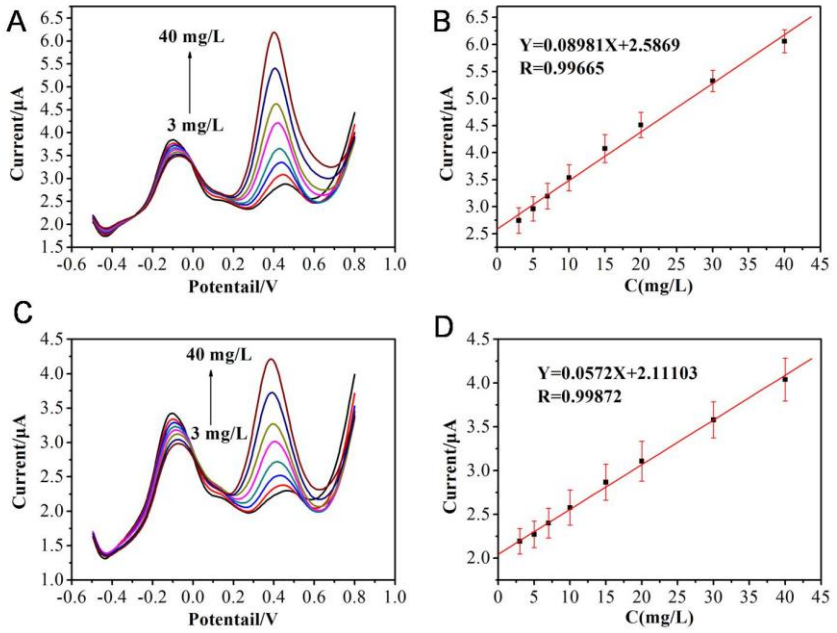

Figure 8. DPVs (A, C) and the plot of anodic peak values at 0.4 V vs. the concentration of EP (B, D) for the sensing of EP in the range of 3~40 mg/L in 10% serum (A, B) and 20% serum (C, D). Error bars represent the SDs from three independent determinations.

4. CONCLUSIONS

PTh/AuNPs that showed good electrochemical properties were synthesized through a one-step synthesis method. The PTh/AuNPs modified GCE exhibited satisfactory electrocatalytical activity for EP in PBS (pH = 6.0). The electrochemical sensor can effectively detect EP in PBS without the effect of other interferents. Furthermore, the electrochemical sensor can sensitively detect EP in a wide linear range with low LOD. Different EP concentrations can also be measured accurately in diluted serums, which were usually used to simulate complex biological samples. Therefore, the sensor indicated promising prospects in clinical applications. Owing to its excellent electrochemical properties, the PTh/AuNPs composites can be hopefully applied to other research fields.

ACKNOWLEDGEMENTS
This work is supported from the National Science Foundation of Fujian Province (2015J01043), and the Innovation and Entrepreneurship Training Programs for College Students of Fujian Province.

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