

Article

Polymerization of PEDOT/PSS/Chitosan-Coated Electrodes for Electrochemical Bio-Sensing

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Received: 15 May 2017; Accepted: 4 July 2017; Published: 6 July 2017

Abstract: Poly(3,4-ethylenedioxythiophene) (PEDOT) doped with poly (styrene sulfonate) (PSS) has a variety of chemical and biomedical applications. Additionally, chitosan has been extensively used in industrial and medical fields. However, whether chitosan could be incorporated into conducting polymers of PEDOT/PSS is not clear. In this study, the PEDOT/PSS/chitosan coatings were electrochemically polymerized on the surface of 0.5 mm platinum (Pt) electrodes and the properties of electrochemical cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) of the PEDOT/PSS/chitosan-coated electrodes were investigated. Furthermore, PEDOT/PSS/chitosan-coated electrodes used for electrochemical bio-sensing, using dexamethasone (Dex) as a model bio-sensing material, were examined. The results demonstrated that PEDOT/PSS/chitosan-coated electrodes were stable in phosphate-buffered saline (PBS) solution. The electrochemical CV curve areas, reflecting the charge delivery capacity, and the EIS of the PEDOT/PSS/chitosan-coated electrodes were sensitive to Dex, and the good linearity can be obtained between CV curve areas, the EIS and the concentration of Dex. In addition, electrochemical sensitivity of the PEDOT/PSS/chitosan-coated electrodes to Dex was much higher than ultraviolet (UV) spectroscopy detection. All these results revealed that the PEDOT/PSS/chitosan-coated electrodes can be electrochemically polymerized and used for electrochemical bio-sensing.

Keywords: poly(3,4-ethylenedioxythiophene) (PEDOT); chitosan; electrochemical polymerization; bio-sensing; dexamethasone

1. Introduction

Poly(3,4-ethylenedioxythiophene) (PEDOT) is a highly-promising conducting polymer owing to its extraordinary electrical properties, long-term stability, and biocompatibility [1]. Wide application for PEDOT in various electronic products have been known, such as antistatic coatings, photovoltaic devices, and organic thin-film transistors [2,3]. Recent findings of the application of PEDOT in bioengineering and medical fields, such as conductive coatings for neural microelectrodes [4,5], drug delivery system [6,7], biosensors [8,9] and tissue engineering [10], have attracted attention. All these studies have shown that PEDOT can be electrochemically polymerized of 3,4-ethylenedioxythiophene (EDOT) monomer [11] or chemical polymerization of EDOT in the presence of oxidants [12]. Furthermore, during these polymerization processes, a variety of dopants can be incorporated into the PEDOT polymers and may provide the doped PEDOT polymers some specific properties and applications. Biomedical molecules, such as, peptide and neurotrophic growth factors, have been reported to be incorporated into the PEDOT/PSS polymers [6,13,14].

Chitosan is the most abundant natural amino polysaccharide and it can be used in agricultural, industrial, and medical fields. Due to its non-toxic nature, excellent film forming ability, good mechanical strength, high permeability, and cost-effectiveness, chitosan is regarded as one of the

most widely used biopolymers for sensor applications [15,16]. Chitosan can favor the immobilization of biomolecules over its surface. It has been reported that chitosan has an excellent property of immobilization and protection of the bio-sensing elements, such as for DNA probes [17,18] and tonic metal ions [19] and, thus, can be used for monitoring cancer-susceptible genes and metal ions. Even though chitosan is a non-conductive biomaterial it can, however, be modified or doped into conducting polymers, such as polyaniline [20,21] and polypyrrole [19,22], and these conducting polymer/chitosan coatings can be used for electrochemical sensing detections.

Electrochemical detection approaches are sensitive, quantitative, and dynamic and, therefore, provide a simple, accurate, and inexpensive platform for bio-sensing [23,24]. Electrochemical techniques, such as traditional cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), have been widely used for bio-sensing purposes [25–27]. Although electrochemical biosensor systems based on polyaniline/chitosan [20,21] and polypyrrole/chitosan [19,22] have been investigated, however, polymers of PEDOT/chitosan for bio-sensing has not been reported. In this study, EDOT, poly(styrene sulfonate) (PSS) (acting as count ions), and chitosan were subjected to electrochemical reactions and the PEDOT/PSS/chitosan coatings were electrodeposited on the surface of platinum (Pt) electrodes. Using these PEDOT/PSS/chitosan-coated electrodes, dexamethasone (Dex), served as a model bio-sensing material, and was detected by traditional CV and EIS techniques.

2. Materials and Methods

2.1. Chemicals

3,4-Ethylenedioxylthiophene (EDOT > 97%) monomer, poly(styrene sulfonate) (PSS, 70,000 M_W) and dexamethasone (Dex) were purchased from Sigma-Aldrich Co., LLC. (St. Louis, MO, USA). A 0.5-mm diameter platinum (Pt) electrodes and Pt foil were purchased from Sinopharm Chemical Reagent Co., (Shanghai, China). All other chemicals were of analytical grade and were used as received.

2.2. Electrochemical Polymerization of PEDOT/PSS/Chitosan Coatings on Pt Electrodes

Electrochemical polymerization of PEDOT/PSS/chitosan coatings on 0.5 mm diameter Pt electrodes, as working electrodes, was carried out using an electrochemical workstation (CHI 660, CH Instruments, Inc., Austin, TX, USA). The electrochemical reactions were performed in a three-electrode cell. A Pt foil served as counter-electrode and a saturated calomel electrode (SCE) was used as the reference electrode. The length of the PEDOT/PSS/chitosan coatings on 0.5 mm diameter Pt electrodes was 10 mm, and the actual electrode area, which was covered by the coatings was, thus, ~16 mm². The same diameter (0.5 mm) Pt electrodes and the same actual electrode area which coated or uncoated by PEDOT/PSS/chitosan polymers were used in the subsequent experiments.

For the polymerization of PEDOT/PSS/chitosan coatings on Pt electrodes, the monomer electrodepositing solution (50 mL) contained 15 mM EDOT monomer, 20 mM PSS and 1% (*w/v*) chitosan in a 1% acetic acid solution, in aqueous 5 mM potassium phosphate-buffered saline (PBS, deionized water with 9 g NaCl, 0.8 g Na₂HPO₄·2H₂O and 0.14 g KH₂PO₄ added per liter). The polymerization of PEDOT/PSS/chitosan on Pt electrodes was carried out at the constant anodic potential (1.0 V) under potentiostatic conditions at room temperature. The amount of PEDOT/PSS/chitosan polymers coated on the electrodes was controlled by the polymerization time. After the polymerization, electrodes with PEDOT/PSS/chitosan coatings were washed and soaked in deionized water for 24 h to leach out any possible excess monomer, dopants, or process contaminants.

2.3. Electrical Properties of PEDOT/PSS/Chitosan-Coated Pt Electrodes Characterized by Electrochemical Cyclic Voltammetry (CV)

Electrochemical measurements were performed with the CHI 660 workstation using a three-electrode cell configuration in 0.1 M PBS (pH = 7.0). The PEDOT/PSS/chitosan-coated or

the uncoated Pt electrodes were used as the working electrode, while a Pt foil and an SCE were used as the counter electrode and the reference electrode, respectively.

For characterizing the electrical properties of the PEDOT/PSS/chitosan-coated and the uncoated Pt electrodes, CV was performed by sweeping the voltage of the working electrodes at 0.05 V/s from −1.0 to 1.0 V vs. SCE. CV measurements were used to evaluate the electrochemical reactions in each solution and to calculate the cathodic or anodic charge storage capacity of the films by integrating the currents enclosed by each phase of the CV.

2.4. Electrical Properties of PEDOT/PSS/Chitosan-Coated Pt Electrodes Characterized by Electrochemical Impedance Spectroscopy (EIS)

Impedance characteristics of the PEDOT/PSS/chitosan-coated and the uncoated Pt electrodes were measured by EIS using an electrochemical workstation (CHI 660) with associated frequency response analyzer. The potentiostat was configured to sequentially inject 5 mV sine waves at 60 frequencies logarithmically spaced from 1 Hz to 100 kHz. EIS measurements were made by immersing the PEDOT/PSS/chitosan-coated or the uncoated Pt electrodes, as working electrodes in 0.1 M PBS buffer (pH = 7.0). A Pt foil was used as the counter electrode and an SCE was used as the reference electrode.

2.5. Ultraviolet (UV) Spectroscopy

Dex in deionized water was detected using UV spectrophotometers (UV-759, Cangy Precision Instruments Co., Ltd., Shanghai, China). The readings were taken from 200 to 400 nm. The characteristic absorbance band range of Dex is 242 nm. Deionized water was used as a blank.

2.6. Statistical Analysis

All values were taken as the mean ± SEM. For comparison between different groups, statistical analysis was performed by analysis of variance (ANOVA) using the general linear model procedure followed by separation of means using the least significant difference. The criterion for significance was set at $p < 0.05$.

3. Results

3.1. Electrochemical Polymerization of PEDOT/PSS/Chitosan Coatings on Pt Electrodes

Polymerization of PEDOT/PSS/chitosan coatings on the 0.5 mm diameter Pt electrode was performed at the constant anodic potential of 1.0 V relative to the counter. The amount of PEDOT/PSS/chitosan film on Pt electrodes could be controlled by the polymerization time. During the electrochemical polymerization of PEDOT/PSS/chitosan coatings on Pt electrodes, the currents as a function of time were recorded. As showed in Figure 1, immediately after the polymerization, the currents were decreased rapidly (within 500 s) and then decayed with the polymerization time. Figure 1 showed that at the polymerization time of 1800 s, the currents were ~1 μ A. At the polymerization time of 3600 and 5400 s, the currents were decreased to 0.72 and ~0.58 μ A, respectively. The polymerization time of 1800, 3600 and 5400 s was chosen, at which different PEDOT/PSS/chitosan coatings were polymerized on the Pt electrodes. Electrical properties of CV, EIS, and stabilities of these PEDOT/PSS/chitosan-coated Pt electrodes were examined and were compared.

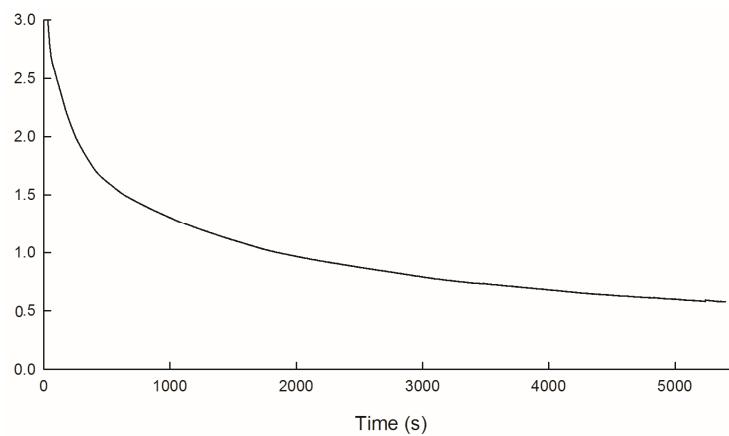


Figure 1. Currents as a function of time during the electrochemical polymerization of PEDOT/PSS/chitosan coatings on Pt electrodes.

3.2. Electrochemical Stability of PEDOT/PSS/Chitosan-Coated Pt Electrodes in PBS

Electrochemical stabilities of several types of PEDOT/PSS/chitosan-coated electrodes, which were prepared at the different polymerization time of 1800, 3600 and 5400 s, were examined. CV and EIS of these electrodes in 0.1 M PBS solution at different time points were measured, respectively. A typical CV spectrum at the scan rate of 0.05 V/s of the PEDOT/PSS/chitosan-coated Pt electrodes at the time points of 0, 0.5, 1, 1.5, 2 and 48 h, respectively, in PBS was shown in Figure 2A. Background subtracted CV curve areas represent the total amount of stored charge. Each background subtracted CV curve areas were calculated and the percentages to 0 h were presented in Figure 2B. As shown, the percentages were all around 100% and no difference was found among the different time points ($F(5,18) = 2.48$, $p > 0.05$). Similarly, EIS of the PEDOT/PSS/chitosan-coated electrodes was measured at the different time points of 0, 0.5, 1, 1.5, 2 and 48 h, respectively, in 0.1 M PBS solution. The EIS data in the form of Bode plot (the EIS data in the form of Nyquist plot presented in Supplementary Materials Figure S1) showed that in the frequency range of 1–100 kHz, the impedance of the PEDOT/PSS/chitosan-coated Pt electrodes was decreased as a function of frequency (Figure 2C). Furthermore, a similar pattern of EIS data was observed at different time points of electrodes in PBS (Figure 2C). Impedance of the electrodes at 1 kHz has been used as a standard measurement of the coated electrode quality [7]. At different time points, the impedance at 1 kHz was calculated and the percentage to 0 h in PBS was presented (Figure 2D). As shown, the average percentage was all ~100% and no significant difference was found among different time points ($F(5,18) = 2.33$, $p > 0.05$). For all the PEDOT/PSS/chitosan-coated electrodes, which prepared at the polymerization time of 1800, 3600 or 5400 s, CV and EIS in PBS were stable, implying that the PEDOT/PSS/chitosan coatings were deposited on the surface of Pt electrodes.

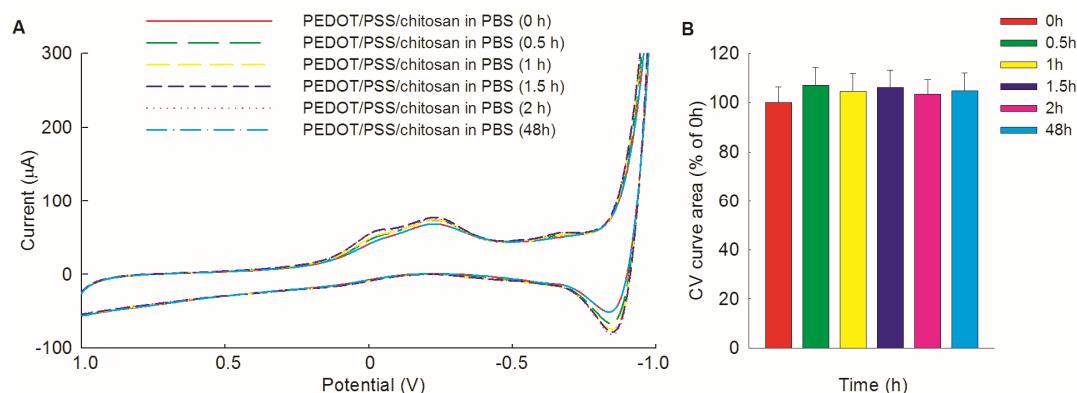


Figure 2. Cont.

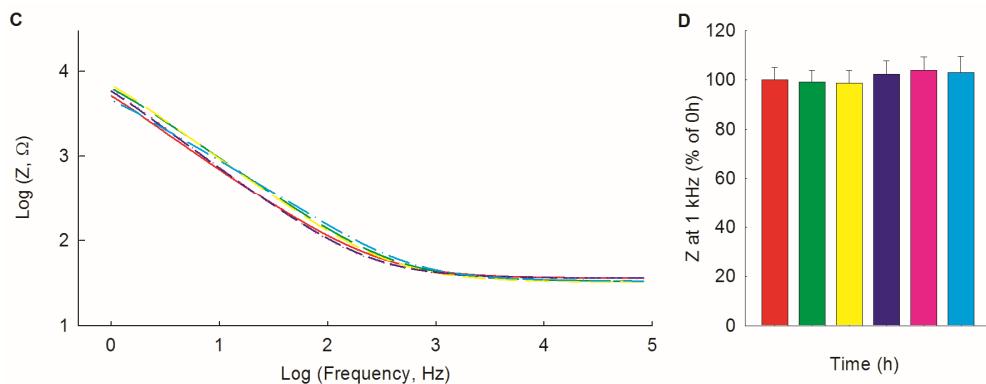


Figure 2. (A) A typical electrochemical cyclic voltammetry (CV) spectrum of the PEDOT/PSS/chitosan-coated Pt electrodes in PBS at different time points of 0, 0.5, 1, 1.5, 2 and 48 h. (B) The percentage of the mean values of the CV curve areas of the PEDOT/PSS/chitosan-coated electrodes in PBS at the time points of 0.5, 1, 1.5, 2 and 48 h, respectively, relative to those at 0 h in PBS. (C) A typical electrochemical impedance spectroscopy (EIS) in the form of Bode plot of the PEDOT/PSS/chitosan-coated electrodes in PBS at different time points of 0, 0.5, 1, 1.5, 2 and 48 h. (D) The percentage of the mean impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes in PBS at the time points of 0.5, 1, 1.5, 2 and 48 h, respectively, relative to those at 0 h in PBS.

3.3. Possibility of the PEDOT/PSS/Chitosan-Coated Pt Electrodes for Dex Bio-Sensing

To investigate the possibility of PEDOT/PSS/chitosan-coated Pt electrodes for bio-sensing, the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 1800, 3600 or 5400 s, respectively, were immersed in 10 mM saturated Dex water solution. The length of the PEDOT/PSS/chitosan-coated electrodes immersing in Dex was 10 mm, the same actual electrode area as the PEDOT/PSS/chitosan coatings. At different time points, 0, 0.5, 1, 1.5 and 2 h after electrode immersion in Dex solution, electrochemical CV and EIS were tested. A typical CV spectrum of the PEDOT/PSS/chitosan-coated electrode in Dex was showed in Figure 3A. Each background subtracted CV curve areas of the PEDOT/PSS/chitosan-coated electrodes were calculated and percent to mean values of 0 h in Dex. As shown in Figure 3B, for the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 1800, 3600 or 5400 s, 0.5, 1, 1.5 and 2 h immersion of Dex significantly increased the CV curve areas relative to 0 h (100%) ($F(1,6) \geq 9.66, p < 0.05$). Furthermore, relative to 0.5 h immersion in Dex, CV curve areas of 1 h, 1.5 h and 2 h immersion in Dex were significantly enhanced ($F(1,6) \geq 6.63, p < 0.05$) and no significant difference in CV curve areas was found among 1, 1.5 and 2 h immersion in Dex solution ($F(1,6) \leq 2.02, p > 0.05$). In addition, for the magnitude of increases in CV curve areas, the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 3600 s was up to 320%–330%, whereas the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 1800 and 5400 s was ~170% and ~200%, respectively (Figure 3B). These findings demonstrated that immersion of the PEDOT/PSS/chitosan-coated electrodes, especially the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 3600 s in Dex, could increase CV curve areas, the CV curve areas increasing with time at first and then became stable after the immersion of 1–2 h.

Likewise, EIS of several types of PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 1800, 3600 or 5400 s, in Dex solution at the different time points was examined. A typical EIS Bode plot (the Nyquist plot presented in Supplementary Materials Figure S2) of PEDOT/PSS/chitosan-coated electrodes in Dex was showed in Figure 3C. As shown, with the time of Dex immersion, impedance of the PEDOT/PSS/chitosan-coated electrodes was decreased within the whole frequency range of 1–100 kHz (Figure 3C). The impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes at different time points in Dex was calculated. As shown in Figure 3D, for all the PEDOT/PSS/chitosan-coated electrodes, Dex immersion of 0.5, 1, 1.5 and

2 h significantly decreased the impedance at 1 kHz relative to 0 h in Dex ($F(1,6) \geq 7.55, p < 0.05$). Furthermore, relative to 0.5 h immersion in Dex, the impedance at 1 kHz of 1, 1.5 and 2 h immersion in Dex was significantly decreased ($F(1,6) \geq 9.45, p < 0.05$) and no significant difference was found among 1, 1.5 and 2 h immersion in Dex solution ($F(1,6) \leq 3.18, p > 0.05$). For the magnitude of decreases in the impedance at 1 kHz, the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 3600 s was down to ~25%, whereas the PEDOT/PSS/chitosan-coated electrodes with the polymerization of 1800 s and 5400 s were ~65% and ~55%, respectively (Figure 3D).

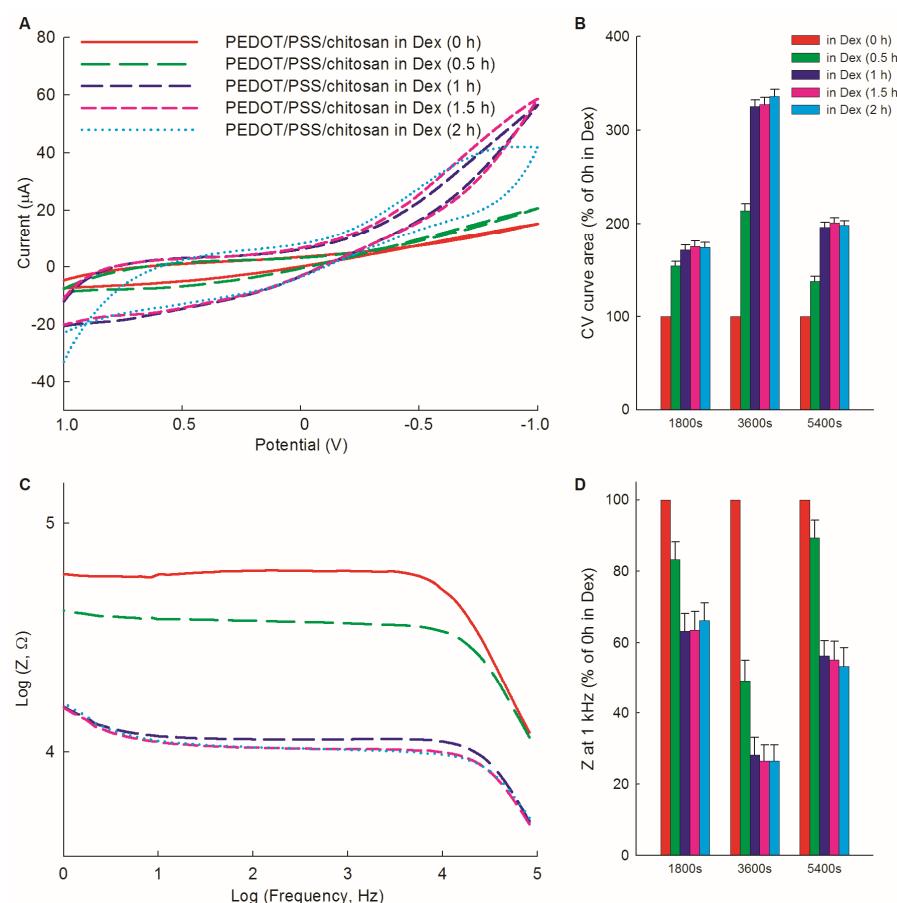


Figure 3. (A) A typical electrochemical CV spectrum of the PEDOT/PSS/chitosan-coated Pt electrodes in 10 μ M Dex solution at different time points of 0, 0.5, 1, 1.5 and 2 h. (B) The percentage of the mean values of the CV curve areas of the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 1800, 3600 and 5400 s, respectively, at the time points of 0.5, 1, 1.5 and 2 h in 10 μ M Dex solution, relative to those at 0 h in Dex. (C) A typical EIS in the form of Bode plot of the PEDOT/PSS/chitosan-coated electrodes in 10 μ M Dex solution at different time points of 0, 0.5, 1, 1.5 and 2 h. (D) The percentage of the mean impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 1800, 3600 and 5400 s, respectively, at the time points of 0.5, 1, 1.5 and 2 h in 10 μ M Dex, respectively, relative to those at 0 h in Dex.

These results demonstrated that the CV curve areas and EIS could be altered by immersing of the PEDOT/PSS/chitosan-coated electrodes into Dex solution, implying that the PEDOT/PSS/chitosan-coated electrodes could be used for electrochemical Dex bio-sensing. Considering the sensitivities of the PEDOT/PSS/chitosan-coated electrodes to Dex bio-sensing, the polymerization time of 3600 s was chosen for synthesis of PEDOT/PSS/chitosan coatings on the surface of Pt electrodes. Thus, the PEDOT/PSS/chitosan-coated electrodes with the polymerization time of 3600 s were selected in the subsequent experiments.

3.4. Electrochemical Evaluation of PEDOT/PSS/Chitosan-Coated Pt Electrodes

The PEDOT/PSS/chitosan-coated Pt electrodes could be used for Dex bio-sensing. To fully evaluate properties of the PEDOT/PSS/chitosan-coated electrodes (the polymerization time: 3600 s), electrochemical CV and EIS were examined before, during and after Dex bio-sensing. CV and EIS of uncoated (bare) Pt electrodes were tested as controls. Since the CV and EIS values of the PEDOT/PSS/chitosan-coated electrodes in Dex became stable 1–2 h after Dex immersion (Figure 3), thus, the PEDOT/PSS/chitosan-coated electrodes immersed in Dex solution for 2 h were chosen for evaluation. Correspondingly, to rule out any possible Dex absorption or excess Dex on the surface of the PEDOT/PSS/chitosan-coated electrodes after Dex bio-sensing, these electrodes were firstly washed in deionized water for 2 h and then tested in PBS. A typical CV spectrum of bare electrode in PBS, the PEDOT/PSS/chitosan-coated electrode in PBS before Dex bio-sensing, in Dex solution for 2 h, and in PBS after Dex bio-sensing was showed in Figure 4A. Background-subtracted CV curve areas were calculated and the values were normalized to those of bare electrodes in PBS. As shown (Figure 4B), relative to the bare electrodes in PBS, CV curve areas of the PEDOT/PSS/chitosan-coated electrodes in PBS before Dex bio-sensing and after Dex bio-sensing were significantly increased ($F(1,6) \geq 20.1, p < 0.05$), whereas, CV curve areas of the PEDOT/PSS/chitosan-coated electrodes in Dex were significantly decreased ($p < 0.05$). Furthermore, CV curve areas of the PEDOT/PSS/chitosan-coated electrodes in PBS after Dex biosensing were similar to those in PBS before bio-sensing ($F(1,6) = 1.24, p > 0.05$).

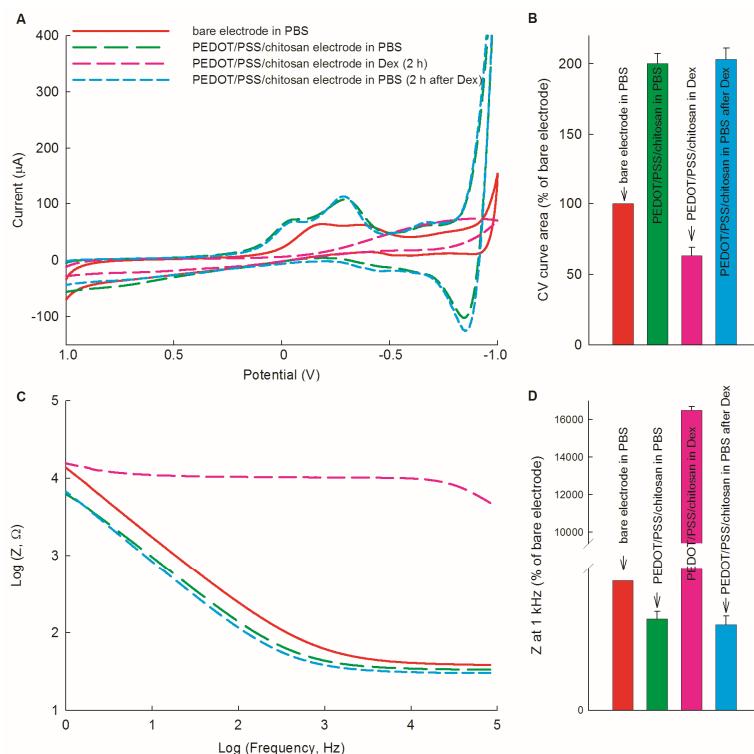


Figure 4. (A) A typical electrochemical CV spectrum of the bare (uncoated) Pt electrode in PBS, the PEDOT/PSS/chitosan-coated electrodes (polymerization time: 3600 s) in PBS, in 10 μ M Dex for 2 h, and in PBS 2 h after in Dex, respectively. (B) The percentage of the mean values of the CV curve areas of the PEDOT/PSS/chitosan-coated electrodes in PBS, in 10 μ M Dex for 2 h, and in PBS 2 h after in Dex, respectively, relative to those of the bare electrodes in PBS. (C) A typical EIS in the form of Bode plot of the bare Pt electrode in PBS, the PEDOT/PSS/chitosan-coated electrodes in PBS, in 10 μ M Dex for 2 h, and in PBS 2 h after in Dex, respectively. (D) The percentage of the mean impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes in PBS, in 10 μ M Dex for 2 h, and in PBS 2 h after in Dex, respectively, relative to those of the bare electrodes in PBS.

Likewise, EIS of the bare electrodes, the PEDOT/PSS/chitosan-coated electrodes before, during and after Dex bio-sensing, was also examined and a typical Bode plot of EIS (Nyquist plot of EIS presented in Supplementary Materials Figure S3) was showed in Figure 4C. Values of impedance at 1 kHz were calculated and were normalized to those of bare electrodes in PBS (Figure 4D). As shown, relative to the bare electrodes, the impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes in PBS before Dex bio-sensing and after Dex bio-sensing was significantly decreased ($F(1,6) \geq 6.73, p < 0.05$). Whereas, the impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes in Dex solution was extraordinarily enhanced relative to the bare electrodes, with the ratio of ~165. In addition, the impedance of the PEDOT/PSS/chitosan-coated electrodes in PBS after Dex bio-sensing was close to those before Dex bio-sensing ($F(1,6) = 2.25, p > 0.05$).

These results revealed that relative to the bare electrodes, the PEDOT/PSS/chitosan-coated electrodes increased the charge delivery capacities and decreased the impedance. For the PEDOT/PSS/chitosan-coated electrodes, bio-sensing of Dex was reflected by decreasing the charge delivery capacities and increasing the impedance. Furthermore, the properties of the PEDOT/PSS/chitosan-coated electrodes in CV and EIS before and after Dex bio-sensing were comparable, implicating that the PEDOT/PSS/chitosan-coated electrodes were stable for Dex bio-sensing and might be used for Dex bio-sensing repetitively.

3.5. Sensitivity of PEDOT/PSS/Chitosan-Coated Pt Electrodes for Dex Bio-Sensing

Electrochemical Dex bio-sensing of the PEDOT/PSS/chitosan-coated electrodes was represented by the decreases in CV curve areas and the increases in the impedance. To investigate the sensitivity and the linearity of the PEDOT/PSS/chitosan-coated electrodes for Dex bio-sensing, the PEDOT/PSS/chitosan-coated electrodes were immersed in 1, 5, 10, 50, 100, and 500 nM Dex solutions for 2 h, and electrochemical CV and EIS were measured, respectively. CV and EIS of the PEDOT/PSS/chitosan-coated electrodes in PBS were examined as controls. Background-subtracted CV curve areas and impedance at 1 kHz of the PEDOT/PSS/chitosan-coated electrodes in different concentrations of Dex and in PBS were calculated and all values in Dex solution were normalized to those in PBS. As showed in Figure 5A, the CV curve areas were negatively correlated with the concentration of Dex. Linear regression analysis yielded the equation of $y = 2.8292x + 44.1495$, where y is the CV curve areas (percent to values in PBS), x is the concentration of Dex (log(nM)). The impedance at 1 kHz was positively correlated with the concentration of Dex (Figure 5B). Analysis obtained the linear regression equation of $y = -1.3068x + 87.4236$, where y is the impedance at 1 kHz (ratios to values in PBS) and x is the concentration of Dex (log(nM)). Both CV curve areas (Figure 5A) and the EIS (Figure 5B) vs. the concentration of Dex revealed high correlation coefficients ($R^2 = 0.9936$ for CV curve areas and $R^2 = 0.9984$ for impedance at 1 kHz). All these results demonstrated good linearity for the relationship between the CV curve areas, the impedance, and Dex concentration, confirming that the PEDOT/PSS/chitosan-coated electrodes could be used for Dex bio-sensing.

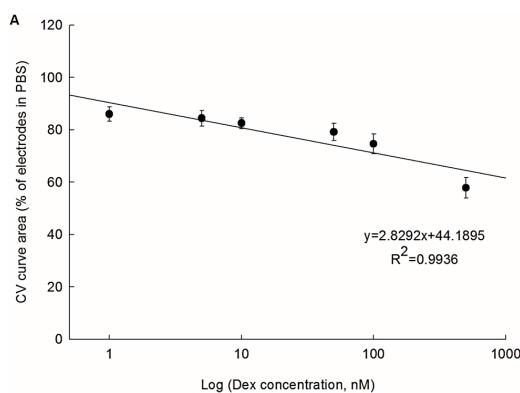


Figure 5. Cont.

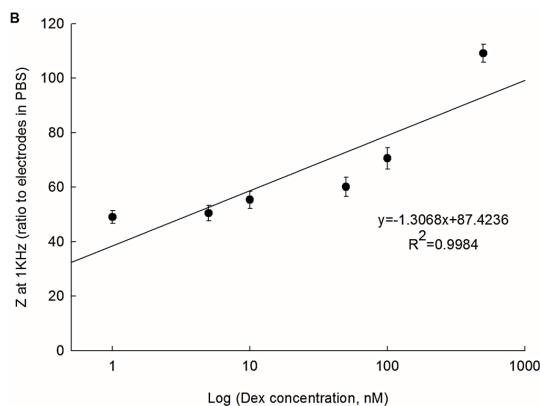


Figure 5. The standard curves of (A) CV curve areas (the percentage of the PEDOT/PSS/chitosan-coated Pt electrodes in Dex solution to those in PBS); (B) the impedance at 1 kHz (the ratio of the PEDOT/PSS/chitosan-coated Pt electrodes in Dex solution to those in PBS), vs. Dex concentration (log(nM)).

To compare the sensitivity of Dex bio-sensing between the PEDOT/PSS/chitosan-coated electrodes and traditional UV spectroscopy, different concentrations of Dex, ranging from 1 to 500 μM were examined by UV spectroscopy. As showed in Figure 6A, characteristic Dex absorbance spectra at 242 nm could be observed in 100, 125, 250 and 500 μM Dex solutions, however, no Dex absorbance spectrum was observed in 1–10 μM Dex solution. Furthermore, the UV spectra absorbent values were positively correlated with the concentration of Dex at the range of 100–500 μM and the correlation coefficient equal to 0.9539 (Figure 6B).

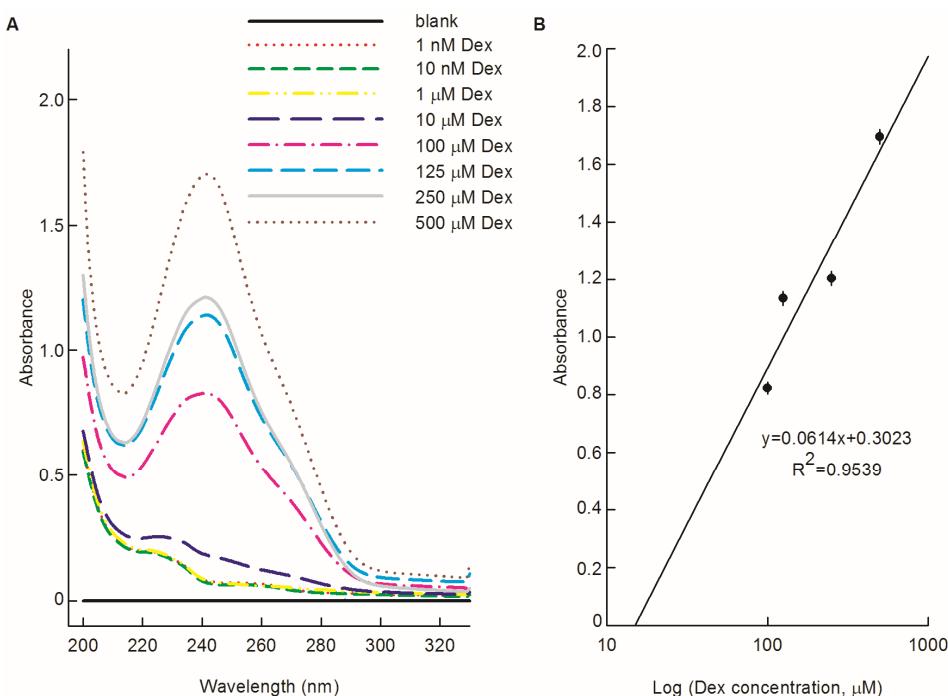


Figure 6. (A) Typical UV spectroscopy spectra of different concentrations of Dex, ranging from 1 to 500 μM . The characteristic Dex absorbance bands at 242 nm are observed in 100–500 μM Dex solution. (B) The standard curves of the absorbance at 242 nm of the UV spectroscopy spectra vs. Dex concentration (log(μM)).

These results, together with the electrochemical measurements of the PEDOT/PSS/chitosan-coated electrodes for Dex bio-sensing, revealed that the PEDOT/PSS/chitosan-coated electrodes possess the higher sensitivity for Dex bio-sensing than traditional UV spectroscopy.

4. Discussion

In the present study, the PEDOT/PSS/chitosan coatings were electrochemical polymerized on the surfaces of 0.5 mm Pt electrodes. Constant anodic potential of 1.0 V relative to the counter and polymerization time of 3600 s was selected for the polymerization of PEDOT/PSS/chitosan-coated electrodes. Electrochemical CV and EIS properties of the PEDOT/PSS/chitosan-coated electrodes in PBS and in Dex solutions were investigated. The results demonstrated that the PEDOT/PSS/chitosan-coated electrodes increased the charge delivery capacities and decreased the impedance relative to the bare electrodes. The PEDOT/PSS/chitosan-coated electrodes exhibited stable CV and EIS properties in PBS solution. In Dex solution, the PEDOT/PSS/chitosan coatings could absorb Dex, resulting in the CV and EIS changing with the time at first and then becoming stable in Dex solution. In addition, the PEDOT/PSS/chitosan-coated electrodes could be used for Dex bio-sensing based on some evidence. First, CV and EIS properties could be altered by Dex. Second, good linearity for the relationship between CV and Dex concentration, and between EIS and Dex concentration, can be obtained. Third, after Dex bio-sensing and thorough cleaning, CV and EIS value of the PEDOT/PSS/chitosan-coated electrodes were close to those before bio-sensing. Fourth, the sensitivity of the PEDOT/PSS/chitosan-coated electrodes for Dex detection was better than traditional UV spectroscopy.

Numerous previous studies have revealed that conductive PEDOT can be polymerized by electrochemical polymerization of EDOT monomer and during this polymerization process, a variety of dopants can be incorporated into the PEDOT polymers [28,29]. In the present study, EDOT monomer, PSS, and chitosan were placed into the electrolyte solution and subjected to electrochemical polymerization. Even though chitosan is a non-conducting biomaterial and the exact mechanism of electrochemical interactions during the polymerization of PEDOT/PSS/chitosan was not fully understood, however, it was thought to involve ionic interactions with dopant anions, hydrophobic interactions with either dopants molecules or monomer or physical entrapment of the biomedical molecule [21]. The exact process and mechanism of electrochemical copolymerization of PEDOT/PSS/chitosan needs further investigation, such as by Fourier transform infrared spectra characterization. In this study, it might be assumed that chitosan was incorporated into the PEDOT/PSS polymers and the PEDOT/PSS/chitosan coatings were deposited on the surface of Pt electrodes, since the characteristic current-time curve during the polymerization process was detected (Figure 1) and the PEDOT/PSS/chitosan-coated electrodes were consistent in electrochemical properties in PBS solution (Figure 2). During the polymerization of PEDOT/PSS/chitosan coatings, the currents were decreased as the function of time might be explained by competition between increases in the amount of conducting PEDOT/PSS polymers and surface areas, and the increases in the incorporation of chitosan.

Chitosan is one of the most widely used biopolymers for sensor applications due to several qualities of chitosan, such as excellent film forming ability and high permeability. In this study, the immersion of the PEDOT/PSS/chitosan-coated electrodes into Dex solution, the electrochemical properties of the PEDOT/PSS/chitosan-coated electrodes were altered with time at first, then stay at a constant level. Chitosan facilitating the immobilization of molecular or ions [19,30] could result in the permeability of Dex into the PEDOT/PSS/chitosan coatings increasing with time at first. The electrochemical properties of the PEDOT/PSS/chitosan-coated electrodes reached constant levels, probably due to the saturation of the binding sites available on the PEDOT/PSS/chitosan coatings. As to the mechanism of the PEDOT/PSS/chitosan coatings binding Dex, it was hypothesized that the physical binding may dominate since the electrochemical properties of the PEDOT/PSS/chitosan-coated electrodes could be resumed after Dex bio-sensing and thorough cleaning.

In the present study, the findings have demonstrated that the PEDOT/PSS/chitosan coatings could be deposited on the surface of Pt electrodes and could be used for electrochemical bio-sensing, reflected by the alterations in CV curve areas and impedance of EIS. In this study, Dex was served as a model testing materials. It was mostly possible that the PEDOT/PSS/chitosan-coated electrodes could be used for many biomaterials or chemical materials testing, such as toxic metal ions or compounds. As a matter of fact, previous studies have revealed that several conducting polymers/chitosan coatings or films can be used for electrochemical bio-sensing. For example, polypyrrole/chitosan conducting polymer composite was used for toxic metal Pb^{2+} and Hg^{2+} ion detection [19]. Polyaniline/chitosan hybrid conducting polymers were used for detection of Ochratoxin-A, one of a mycotoxin [30], and a breast cancer susceptible gene [21]. Our present results, collected with these previous studies, suggested that conducting polymers/chitosan coatings or films be one of the most promising electrochemical biosensors. These biosensors, including the PEDOT/PSS/chitosan coatings, are low-cost since the whole detection process does not require specific sample labeling. Furthermore, these electrochemical bio-sensing are sensitive and Dex as low as nM can be detected in this study, four orders lower than UV spectroscopy detection. It is, thus, concluded that the PEDOT/PSS/chitosan-coated electrodes can provide simple, inexpensive, and sensitive electrochemical detection. It should be addressed that, in this study, any element in the electrolyte, which could be immobilized on the surface of the PEDOT/PSS/chitosan-coated electrodes, might influence the electron-transfer kinetics and affect the electrochemical properties of the PEDOT/PSS/chitosan coatings. It, thus, cannot be denied that the PEDOT/PSS/chitosan-coated electrodes for traditional electrochemical detection may not have specific selectivity. However, immobilization of specific probes onto the PEDOT/PSS/chitosan-coated electrodes will increase the electrochemical selectivity [21].

5. Conclusions

In this study, the PEDOT/PSS/chitosan coatings were electrochemically polymerized on the surface of Pt electrodes. Electrochemical properties of the PEDOT/PSS/chitosan-coated electrodes in CV curve areas and EIS were sensitive to Dex, a model bio-sensing material in this study. Linear regression analyses revealed good linearity for the relationship between the electrochemical properties of the PEDOT/PSS/chitosan-coated electrodes and the concentrations of Dex, and the sensitivity of these electrodes to Dex was much higher than the UV spectroscopy detection. Based on this evidence, it could be concluded that the PEDOT/PSS/chitosan-coated electrodes can be used for electrochemical bio-sensing. The remarkable features of this kind of biosensor include ease of polymerization and high sensitivity.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2079-6412/7/7/96/s1>. Figure S1: A typical electrochemical impedance spectroscopy (EIS) in the form of Nyquist plot of the PEDOT/PSS/chitosan-coated electrodes in PBS at different time points of 0, 0.5, 1, 1.5, 2 and 48h; Figure S2: A typical EIS in the form of Nyquist plot of the PEDOT/PSS/chitosan-coated electrodes, with the polymerization time of 1800, 3600 and 5400s, in 10 μ M Dex solution at different time points of 0, 0.5, 1, 1.5 and 2h, respectively; Figure S3: A typical EIS in the form of Nyquist plot of the bare Pt electrode in PBS, the PEDOT/PSS/chitosan-coated electrodes in PBS, in 10 μ M Dex for 2h, and in PBS 2h after in Dex, respectively.

Acknowledgments: This work was supported by Program of Development of Science and Technology of University of Shanghai for Science and Technology (16KJFZ107 and 2017KJFZ160).

Author Contributions: Li Sui conceived and designed the experiments; Bingshu Zhang and Jun Wang performed the experiments; Ainan Cai analyzed the data; Li Sui wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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