

Fabrication of Chiral Poly(3,4-ethylenedioxythiophene) Derivatives Modified Glassy Carbon Electrodes for Ascorbic Acid Determination

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Abstract—Two chiral poly(*N*-(*tert*-butoxycarbonyl)-*L*-phenylalanyl (3,4-ethylenedioxythiophene-2'-yl)methylamide) (PEDOT-Boc-*L*-Phe) and poly(*L*-phenylalanyl (3,4-ethylenedioxythiophene-2'-yl)methylamide) (PEDOT-*L*-Phe) were synthesized by electrochemical polymerization, and their properties as an immobilization platform for biosensor application were explored. After covalent immobilization of ascorbate oxidase (AO) on the polymeric matrixes, their applications for ascorbic acid (AA) biosensing were investigated in detail. Importantly, optimized biosensors showed very good linear relationships between [AA] and the steady-state current response (*I*) in the concentration range of 0.03 μM to 7 mM ($y = 0.08972x + 0.5785$, $r = 0.9954$, (PEDOT-Boc-Phe)) and 0.07 μM to 7 mM ($y = 0.0397x + 0.59381$, $r = 0.9928$ (PEDOT-Phe)), respectively. Satisfactory results implied that the as-formed polymer films were realized the immobilization of biologically active species and could be candidates in the development of biosensing performance.

Keywords-chiral poly(3,4-ethylenedioxythiophene); electropolymerization; amperometric biosensor; ascorbic acid

I. INTRODUCTION

Chiral polymers have attracted much attention because of their chiroptical activity [1], asymmetric separation [2] and recognition properties [3], and various types of chiral structure. Based on these advantages, it is now desirable to cultivate novel chiral polymers to achieve multifunctional and high-performance requirements. Chiral poly(3,4-ethylenedioxythiophene) (PEDOT) is a representative of chiral polymers, which has attracted considerably widespread interest due to its versatile fascinating properties such as high electrical conductivity, low band gap, good redox activity, thermal stability, and excellent transparency in the doped state [4]. To date, there are several methods to obtain chiral PEDOTs. One of the most effective ways to induce such chiral structures in polymers is to introduce chiral moieties into the polymer structures [5]. Therefore, a series of new-generation chiral PEDOTs have been designed and synthesized to explore their promising potential applications. Importantly, to the best of our knowledge, the

study of using the property of chiral PEDOT for the detection of chiral drugs still remains blank.

Vitamin C or ascorbic acid (AA), a water-soluble vitamin, is the chiral enolic form of an α -ketolactone, which is widely present in many biological systems and in multivitamin preparations. AA plays a key role in the metabolism of cholesterol by increasing its elimination and thereby assisting lowering blood cholesterol [6]. Its deficiency leads to the development of a well-known syndrome called scurvy. It is administered in the treatment of many disorders, including Alzheimer's disease, atherosclerosis, cancer, and infertility as well as some clinical manifestations of HIV infections [7, 8]. There are various methods available for determination of AA such as colorimetry [9], high performance liquid chromatography [10], spectrophotometric methods [11], and sequential injection spectrophotometry [12]. However, the operation of colorimetry and high performance liquid chromatography methods is more complex, and the cost is relatively high. Spectrophotometric and chromatographic methods usually require sample pretreatment (e.g., extraction, complex formation) that is laborious and time-consuming. To overcome these defects, electrochemical methods are used extensively for the elegant and sensitive properties such as selectivity, simplicity, and reproducibility of this approach.

In this work, two novel PEDOT derivatives, poly(*N*-(*tert*-butoxycarbonyl)-*L*-phenylalanyl (3,4-ethylenedioxythiophene-2'-yl)methylamide) (PEDOT-Boc-*L*-Phe) and poly(*L*-phenylalanyl (3,4-ethylenedioxythiophene-2'-yl)methylamide) (PEDOT-*L*-Phe), were electropolymerized (Scheme 1). A simple and fast procedure was used for the fabrication of electrodes modified with chiral PEDOT-Boc-*L*-Phe (PEDOT-Boc-*L*-Phe/AO/GCE) and PEDOT-*L*-Phe (PEDOT-*L*-Phe/AO/GCE). The obtained electrodes for determination of AA by steady-state current response in phosphate buffer solution as the supporting electrolyte was described. Based on the effective electrocatalytic activity of the modified electrodes toward these species, a sensitive electrochemical sensor for determination of AA was established. Finally, the analytical

performance of this sensor for determinations of AA in some commercial pharmaceutical samples was evaluated.

II. EXPERIMENTAL

A. Materials

N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC HCl, 99%), and N-hydroxysuccinimide (NHS, 99%) were purchased from Aladdin Chemistry Co. Ltd. Ascorbate oxidase (AO, 400 U mg⁻¹) was purchased from J&K. Phosphate-buffered solution (PBS, pH 7.0) was prepared from 0.1 M sodium dihydrogen phosphate dihydrate (NaH₂PO₄•2H₂O, Sinopharm chemical reagent Co., Ltd.) and 0.1 M disodium hydrogen phosphate dodecahydrate (Na₂HPO₄•12H₂O, Sinopharm chemical

B. Fabrication of biosensor

The glassy carbon electrode (GCE) electrode with a diameter of 3 mm served as the working electrode, a platinum wire with a diameter of 0.5 mm was used as an auxiliary electrode and a saturated calomel electrode (SCE) was used as the reference electrode. Then, the counter electrode and the GCE was ultrasonically cleaned in turn with deionized distilled water, ethanol, and deionized distilled water each for 5 min, respectively. Finally, they were dried in air before the experiment. Three electrodes in the cell were placed 5 mm apart during electrochemical measurements. The PEDOT-Boc-L-Phe film was performed by cyclic voltammograms (CVs) from -0.5 V to 1.5 V in CH₂Cl₂ and 0.1 M tetrabutylammonium hexafluorophosphate system (CH₂Cl₂-Bu₄NPF₆(0.1 M)) with a speed of 100 mV s⁻¹ at room temperature. The PEDOT-L-Phe film was performed by CVs from -0.2~0.8 V in CH₂Cl₂-Bu₄NPF₆ (0.1 M) containing 3% TFA with a speed of 100 mV s⁻¹ at room temperature. The obtained PEDOT-L-Phe modified GCE electrode was washed repeatedly with double-distilled deionized water to remove the electrolyte and monomer, and dried in air.

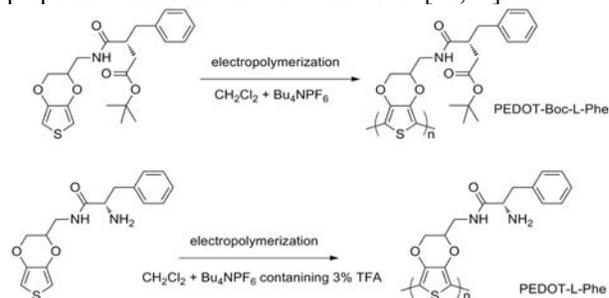
In bioactive platform preparation, EDC and NHS were used as cross linkers to obtain a covalent binding between amino groups of the polymer and carboxylic acid groups of the enzyme molecules [15-17]. The enzyme solution that contained 3 μL (3.33 U) AO, 3 μL 0.4 M EDC and 3 μL 0.1 M NHS was dip-coated on the surface of the polymer coated electrode [18]. The modified electrodes were stored in a refrigerator (4 °C).

III. RESULTS AND DISCUSSION

Amperometric detection of AA

The amperometric response of the PEDOT-L-Phe/AO/GCE to successive adding AA was further evaluated under the optimized experimental conditions. Fig. 1 showed the typical current-time dynamic response of PEDOT-Boc-L-Phe/AO/GCE (A) and PEDOT-L-Phe/AO/GCE (B) towards AA. The concentration of O₂ was kept constantly by air-saturating buffer solutions because O₂ was a co-substrate

reagent Co., Ltd.). Ascorbic acid (AA) was purchased from Bio Basic Inc. All chemicals were used without any further purification. EDOT-Boc-L-Phe and EDOT-L-Phe were prepared in accordance with Duan et al [13,14].



Scheme 1 Electropolymerization of EDOT-Boc-L-Phe and EDOT-L-Phe

of AO catalytic reaction. The steady-state current response increased with the increasing concentrations of AA revealing that AA could be detected successfully by the fabricated biosensor. The linear range of the steady-state current response (I) and the concentration of AA ([AA]) were defined at lower concentration.

The biosensor responses for varying AA concentrations were recorded under optimized conditions using 0.38 V as the working potential, in 50 mM O₂-saturated PBS (pH=7.0) under stable stirring condition at room temperature. The results indicated that this biosensor exhibited an excellent current response for AA with short response time (within 10 s). Good linear relationship was observed between [AA] and

I in the concentration range of 0.03 μM to 7 mM ($y = 0.08972x + 0.5785$, $r = 0.9954$, (Fig. 1A)) and 0.07 μM to 7 mM ($y = 0.0397x + 0.59381$, $r = 0.9928$ (Fig. 1B)), respectively. The sensitivity (from the slope of the linear part in Fig. 1B) was 0.5785 μM and 0.59381 μM, respectively. The detection limits were estimated to be 0.01 μM and 0.023 μM (based on S/N=3) for AA. In addition, the higher concentration of AA was not studied owing to the consumption of the dissolved O₂ amounts in surrounding enzymes resulting in a gradual deviation from linearity according to our previous reports [19,20]. The performances of the fabricated sensor were compared with different AA sensors and the results indicated that this method was reasonable for detecting the unknown [AA] and could determine the content of AA in plants within the narrow limitations of 2×10^{-3} to 2.5×10^{-2} M [21].

IV. CONCLUSION

In this study, chiral PEDOT-Boc-L-Phe/AO/GCE and PEDOT-L-Phe/AO/GCE electrodes were prepared by electrochemical polymerization method and used as a novel sensing platform for electrochemical detection of AA. The GCE electrodes modified with PEDOT-Boc-L-Phe and PEDOT-L-Phe compositing with AO showed high electrocatalytic activity toward the determination of AA and exhibited good linear dependence and selectivity to AA concentration change. These results indicated that the new

composite was scientifically interesting and had great potential for use in sensors. This can be a promising approach for detection of AA and may open new opportunities for facile, biocompatible, sensitive and robust chiral assays in biochemical applications.

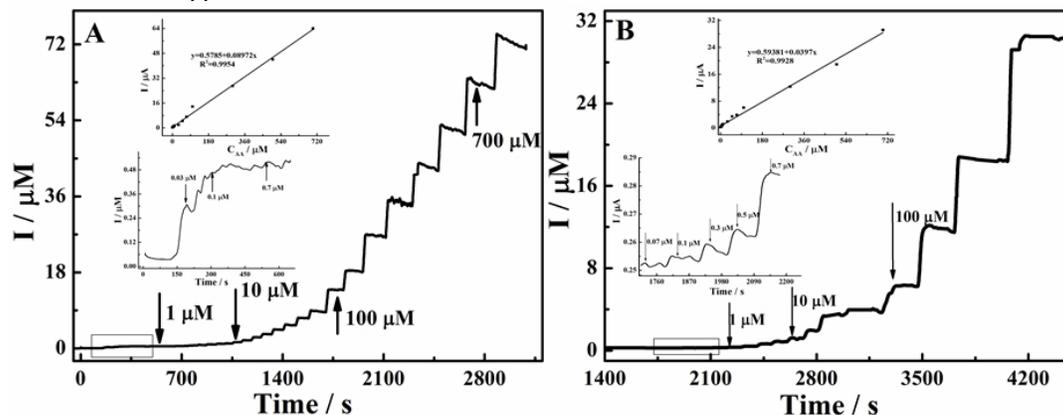


Figure 1. The constant potential amperometric response curves of AA at 0.38 V in oxygen saturated PBS under stable stirring condition at room temperature.

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