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Fabrication and Characterization of Cytochrome C Modified Poly(3-Aminobenzoic Acid) Thin Film

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The electropolymerized poly(3-aminobenzoic acid) (PABA) film on indium tin oxide electrode was used for fabrication of cytochrome c (Cyt c) modified PABA thin film upon immobilization of Cyt c onto the PABA surface. The obtained Cyt c modified PABA thin film was characterized by UV-vis spectroscopy, XPS, AFM, and SEM-EDX techniques. Shifts of the UV-vis absorption peaks were observed from Cyt c modified PABA thin film compared with Cyt c solution and PABA thin film. The formation of the covalent amide bond between the carboxylic groups of PABA and amine groups in Cyt c was observed from XPS results.

Keywords Poly(3-aminobenzoic acid); Cytochrome c; Electropolymerization; Thin film; Characterization

1. Introduction

Conducting polymers, which showed specific redox properties, have attracted considerable for biological sensing application since the property change during biological interaction can be easily detected [1–2]. The functionalized conducting polymers especially polyanilines and polypyrroles such as carboxylated [3–5] and aminated [6–7] polyanilines and polypyrroles have become the interesting materials for biosensor applications. The functional group serves as a matrix material to immobilize with biomolecules such as antibody, antigen, protein and DNA. The self-doping functionalized polyaniline derivatives had recently been employed in the applications of biosensors and immunosensor [3, 4, 6]. Cytochrome C (Cyt c) is one of the redox heme proteins which involving the electron transfer in mitochrondia [8–9]. The development of Cyt c modified electrodes had been recently reported for use as superoxide biosensor [10], nitric oxide biosensor [11], electrochemical biosensor [12–13], and bioelectronic devices [14].

The development of biosensors based on functionalized conducting polymer which modified with redox proteins had been intensively studied in the last decade. Therefore, the

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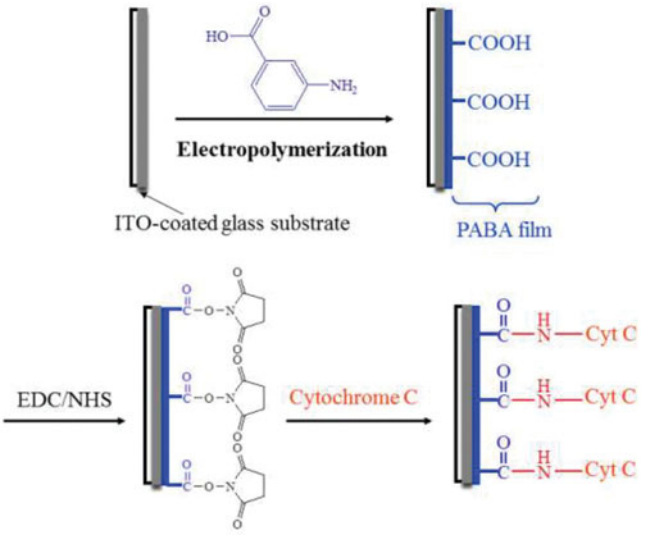

Figure 1. Schematic diagram for fabrication of Cyt c modified PABA thin film.

main aim of this study is to fabricate and characterize the Cyt c modified PABA (PABA/Cyt c) thin film for future use as biosensor for detection of biomolecules. The carboxylic group of PABA molecule serves as a matrix material for immobilization of Cyt c molecule via covalent bonding with amine group of Cyt c. Moreover, from the previous work [4], PABA molecule showed different properties, i.e. surface morphology and binding behavior, under different applied potentials. We therefore used the PABA for construction of the Cyt c modified PABA thin film biosensor.

2. Experimental Details

2.1. Materials

All chemicals including 3-aminobenzoic acid (ABA; Merck), cytochrome c from horse heart (Fluka), 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide (EDC; Fluka), *N*-Hydroxysuccinimide (NHS; Sigma-Aldrich) and phosphate buffer saline tablet (Sigma-Aldrich) were used as received. An indium tin oxide (ITO)-coated glass substrate (Sigma-Aldrich) was ultrasonic cleaned with absolute ethanol and deionized water, respectively, prior to use.

2.2. Electropolymerization of PABA Thin Film and Fabrication of Cyt c Modified PABA Thin Film

The electropolymerization process for fabrication of PABA thin film was performed prior to use for fabrication of PABA/Cyt c thin film. The electropolymerization on ITO working electrode was carried out using 50 mM ABA monomer in 0.5 M H_2SO_4 aqueous solution by applying the potential range of 0 – 1.2 V for 2 cycles at scan rate of 20 mV/s

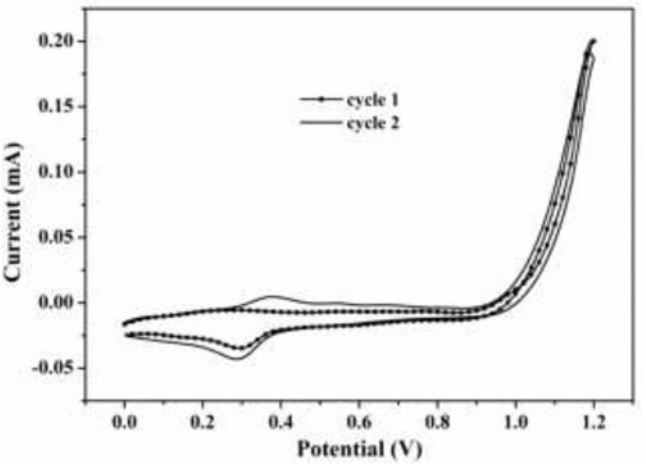

Figure 2. CV traces during electropolymerization of PABA for 2 cycles.

[4]. The counter electrode was platinum wire and reference electrode was Ag/AgCl aqueous electrode. All potentials reported in this study are relative to this reference electrode. The cyclic voltammogram of electropolymerization process was recorded using eDAQ: ED410 e-corder 410. The formation of the film was further confirmed by UV-vis measurement.

The obtained PABA thin film was used for fabrication of PABA/Cyt c thin film by firstly immersing the PABA thin film in an aqueous mixture of EDC/NHS (1:1) for 15 h. The thin film was then washed with PBS buffer solution (pH 7.4) and immobilized with 0.4 mg/mL Cyt c in PBS solution at 4°C for 51 h [14]. Finally, the obtained PABA/Cyt c thin

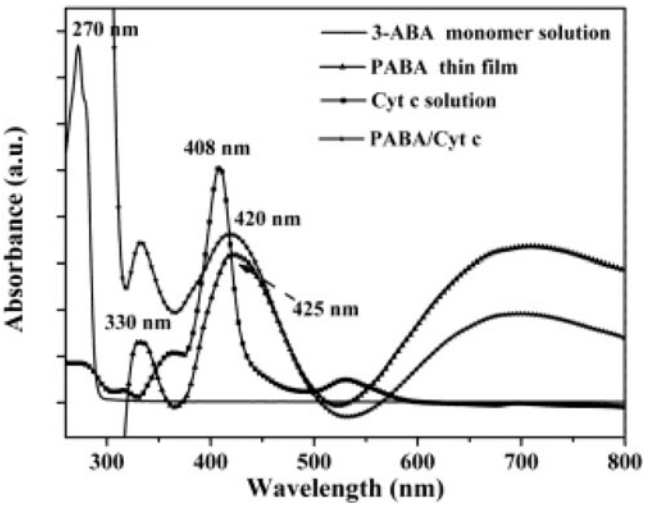

Figure 3. UV-vis spectra of ABA monomer solution, Cyt C solution, PABA thin film, and PABA/Cyt c thin film.

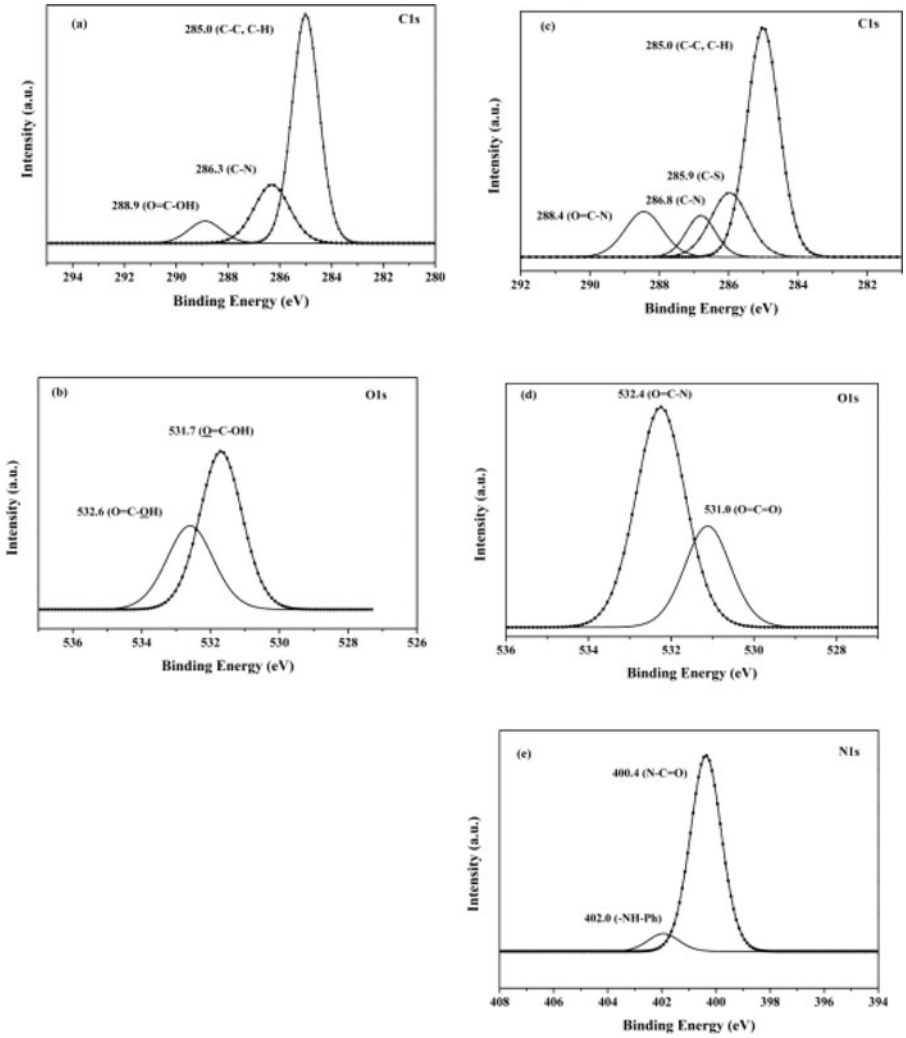

Figure 4. XPS spectra of PABA film in (a) C1s region (b) O1s region and PABA/Cyt c thin film in (c) C1s region (d) O1s region (e) N1s region.

film was washed with PBS solution. A schematic diagram for construction of PABA/Cyt c thin film is shown in Fig. 1.

2.3. Characterization of Cyt c Modified PABA Thin Film

The PABA/Cyt c thin film was characterized by UV-vis absorption spectroscopy (Hewlett Packard: 8452A), scanning electron microscopy-energy dispersive X-ray spectrometry (SEM-EDX; Quanta200 3D Dual Beam: FP2022/31), X-ray photoelectron spectroscopy (XPS; AXIS ULTRADLD, Kratos analytical, Manchester UK., Light source; Al $K_{\alpha 1,2}$ radiation at 1.4 keV) and atomic force microscopy (AFM; Veeco: Nanoscope IIIA). In UV-vis measurement, the UV-vis spectrum of PABA/Cyt c thin film was compared with the UV-vis spectra of PABA thin film and Cyt c solution. The AFM

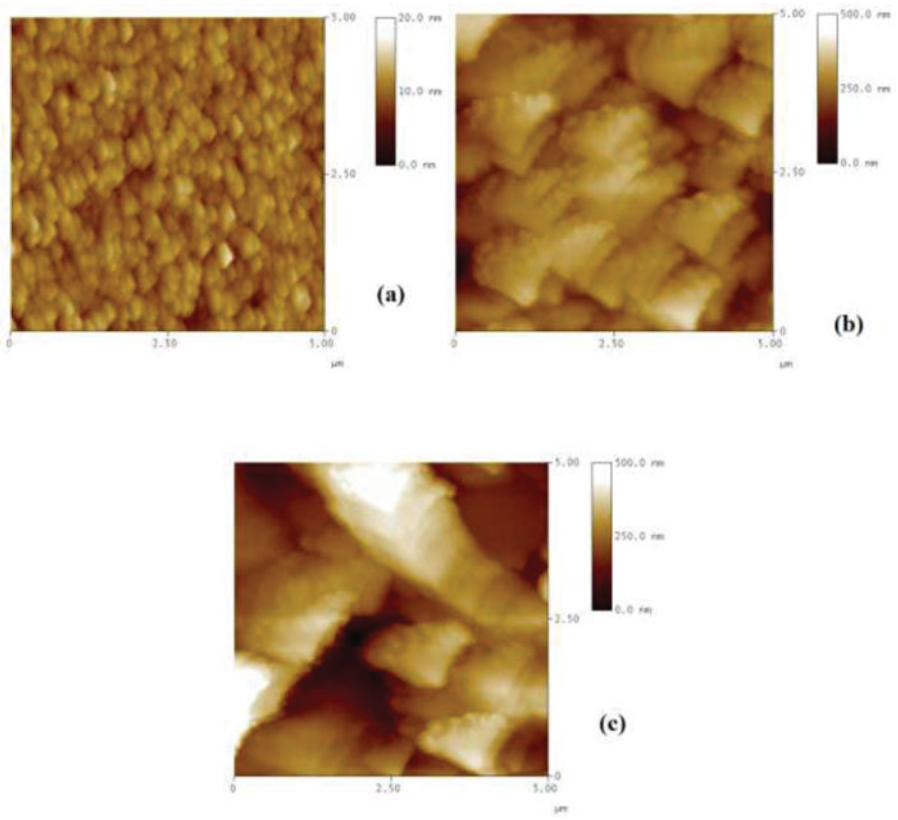

Figure 5. AFM images of (a) ITO substrate (b) PABA film and (c) PABA/Cyt c thin film.

images and XPS spectra of the PABA thin film and PABA/Cyt c thin film were compared.

3. Results and Discussion

3.1. Electropolymerization of PABA Thin Film and Fabrication of Cyt c Modified PABA Thin Film

The PABA thin film was firstly prepared through electropolymerization process of ABA monomer on ITO-coated glass substrate which also used as working electrode. The cyclic voltammetry (CV) was performed with potential range of 0 - 1.2 V for 2 cycles at scan rate of 20 mV/s using Ag/AgCl as reference electrode and platinum wire as counter electrode. The cyclic voltammogram in Fig. 2 showed a pair of reversible peak at about 370/280 mV (oxidation peak/reduction peak) versus Ag/AgCl. The obtained PABA thin film was then used for construction of PABA/Cyt c thin film as previously shown in Fig. 1. The carboxylic acid group of the PABA thin film was activated to an ester group using an aqueous mixture of EDC/NHS [4]. The amide bond was then formed between the activated ester group and an amine group of Cyt c [14].

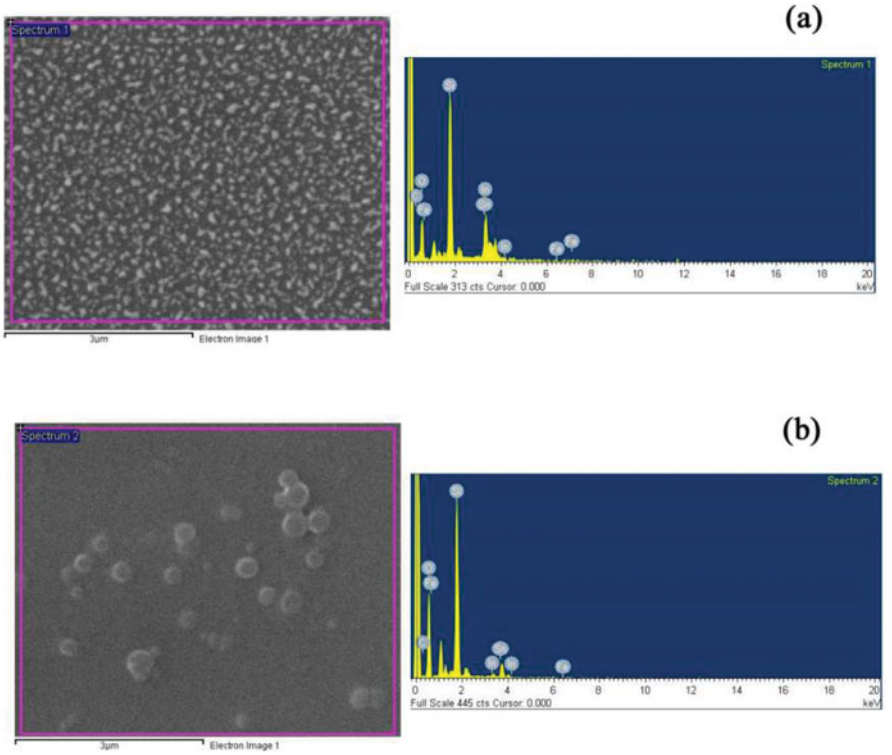

Figure 6. SEM images of (a) PABA film and (b) PABA/Cyt c thin film with corresponding EDX spectra.

3.2. Characterization of Cyt c Modified PABA Thin Film

To study the formation of Cyt c modified PABA thin film, we studied the UV-vis absorption property of the ABA monomer solution, PABA thin film, Cyt c solution and PABA/Cyt c thin film as shown in Fig. 3. The UV-vis spectrum of ABA monomer solution showed an absorption peak at about 270 nm. The UV-vis spectrum of PABA thin film showed further absorption peaks at about 330 and 425 nm which correspond to π-π^* transition in polymer structure [6]. The absorption peak at about 700 nm is relative to the longer conjugation length of PABA film comparing with the monomer molecule. The UV-vis spectra of PABA/Cyt c thin film showed a similar absorption peak at about 330 nm with the PABA film. The absorption peak at about 425 nm of the PABA thin film was slightly shifted to shorter wavelength at about 420 nm in PABA/Cyt c thin film whereas the Cyt c showed the UV-vis absorption peak at about 408 nm. This could indicate the formation of PABA/Cyt c thin film.

The XPS measurement was performed for further supporting the formation of PABA/Cyt c thin film. The XPS spectra (C1s, O1s, and N1s spectra) of the PABA thin film and PABA/Cyt c thin film were compared as shown in Fig. 4. The C1s spectra of PABA film in Fig. 4(a) is composed of three main peaks at 285.0 eV for C-C and C-H bonds, 286.3 eV for C-N bond, and 288.9 eV for C=O bond of carboxylic acid group, respectively [15]. A new peak of C-S bond in Cyt C molecule was observed at 285.9 eV for PABA/Cyt c thin film as shown in Fig. 4(c). In addition, the C-N bond was slightly

shifted to higher energy (286.4 eV) while the C=O bond was slightly shifted to lower energy (288.4 eV) which corresponding to the C=O bond of amide bond formation between carboxylic acid group of PABA and amine group of Cyt C [16]. In Fig. 4(b), the O1s spectra of carboxylic acid group of PABA film was observed at 531.7 eV for C=O bond and 532.6 eV for C-O bond, respectively. The formation of amide bond on the PABA/Cyt c thin film was again confirmed by the O1s peak of C=O bond of amide bond at 532.4 eV in Fig. 4(d). The C=O bond of carboxylic acid group of PABA/Cyt c thin film at 531.0 eV is probably due to carboxylic acid group in Cyt C molecule. For N1s spectra as shown Fig. 4(e), only N1s spectra of the PABA/Cyt c thin film was observed at 400.4 eV for N-H bond of amide bond and 402.0 eV for N-H bond in the PABA structure [17]. However, the N1s spectra of PABA film could not be observed probably due to a trace amount of N-H bond in PABA film could not be detected.

The surface morphology of both PABA thin film and PABA/Cyt c thin film was studied for further confirmation the immobilization of Cyt C onto PABA surface. The surface roughness of the films could be obtained from the AFM images as shown in Fig. 5. The roughness of the PABA film in Fig. 5(b) was about 39.4 nm comparing with the ITO substrate of 1.1 nm in Fig. 5(a) which indicating the formation of the PABA film on ITO substrate. The rough surface was then observed after immobilization of Cyt C onto the PABA film as shown in Fig. 5(c). The roughness of PABA/Cyt c thin film was about 74.8 nm. The AFM results could again confirm the successfully fabrication of the PABA/Cyt c thin film.

The SEM images as shown in Fig. 6 with the corresponding EDX spectra showed the different morphological surfaces of PABA thin film and PABA/Cyt c thin film. After immobilization of Cyt c, the cluster form of PABA/Cyt c was observed in Fig. 6(b) comparing with the uniform distribution of PABA in Fig. 6(a). These results also corresponded to the results from AFM measurement.

4. Conclusion

In this study, we successfully fabricated the Cyt c modified PABA thin film. The PABA thin film was firstly fabricated by electropolymerization of ABA monomer on ITO-coated glass substrate which also used as working electrode. The obtained PABA thin film was then used for fabrication of the Cyt c modified PABA thin film. The obtained Cyt c modified PABA thin film was finally studied by UV-vis absorption, XPS, AFM and SEM-EDX techniques which confirmed the formation of amide bond between carboxylic acid group of PABA and amine group of Cyt c. This film should be useful for use as biosensors in further experiments.

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