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Amperometric sensor for ethanol based on one-step electropolymerization of thionine–carbon nanofiber nanocomposite containing alcohol oxidase[☆]

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Abstract

Thionine had strong interaction with carbon nanofiber (CNF) and was used in the non-covalent functionalization of carbon nanofiber for the preparation of stable thionine–CNF nanocomposite with good dispersion. With a simple one-step electrochemical polymerization of thionine–CNF nanocomposite and alcohol oxidase (AOD), a stable poly(thionine)–CNF/AOD biocomposite film was formed on electrode surface. Based on the excellent catalytic activity of the biocomposite film toward reduction of dissolved oxygen, a sensitive ethanol biosensor was proposed. The ethanol biosensor could monitor ethanol ranging from 2.0 to 252 μM with a detection limit of 1.7 μM. It displayed a rapid response, an expanded linear response range as well as excellent reproducibility and stability. The combination of catalytic activity of CNF and the promising feature of the biocomposite with one-step non-manual technique favored the sensitive determination of ethanol with improved analytical capabilities.

Keywords: Amperometric sensor; Electropolymerization; Carbon nanofiber; Thionine; Ethanol

1. Introduction

In recent years, the unique properties and potential technological applications of various nanomaterials, particularly carbon nanomaterials, have attracted increasing interest [1,2]. Carbon nanomaterials possess good electrical conductivity, unique structural and catalytic properties, high loading of biocatalysts, good mechanical and chemical stability, and excellent adsorption and penetrability [3–6]. Their functionalization has been a powerful strategy to improve the solubility or wettability, realize their potential in a wide range of application, and prepare novel materials with tailor-made structures and properties [7–9]. However, the covalent functionalization can often destroy the sp^2 structures thereby diminishing their pristine mechanical and electronic properties [10,11]. Thus, non-covalent functional strategy seems to be a more friendly and effective alternative for this purpose through effective π–π stacking [12], which has been used to facilitate the solubility of carbon nanotubes (CNTs) [13–17]. Aromatic compounds such as methylene blue and toluidine blue have been used for the non-covalent functionalization of CNTs [12,13]. This work proposed a thionine–functionalized carbon nanofiber (CNF) for preparation of biosensors by electrochemical copolymerization of the functionalized CNF and oxidase.

CNF has cylindrical nanostructure with graphene layers arranged as stacked cones, cups, or plates [18]. The mechanical strength and electric properties of CNF are similar to CNTs [19]. This work showed that CNF also had a strong interaction with thionine molecule like CNTs. Non-covalent functionalization of CNF with the thionine molecule was very simple and useful to facilitate the solubility. Thionine–CNF nanocomposite could form a thin polymeric film on an electrode surface with an electropolymerization process, which could effectively incorporate enzyme into the film [20,21]. The electropolymerization process was easily controlled [22], and the film could provide a biocompatible surface to maintain the activity of entrapped enzyme [23]. With the incorporation of alcohol oxidase (AOD), an ethanol biosensor was thus prepared based on the electrocatalytic response of CNF toward the reduction of dissolved oxygen [24].

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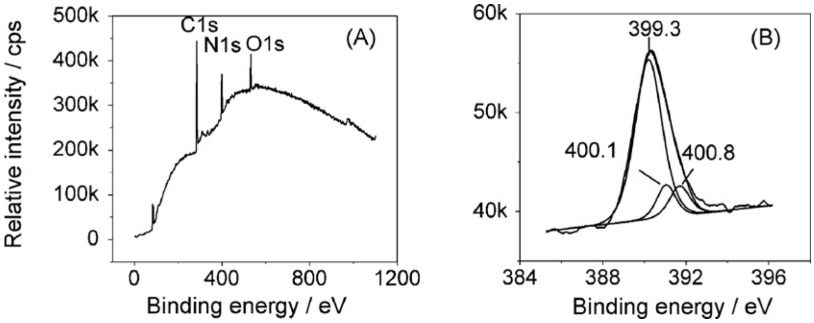

Fig. 1. (A) XPS spectrum and (B) N1s XPS spectrum of poly(thionine)–CNF film.

The quantitative detection of ethanol plays an important role in the quality control of alcoholic beverages during the fermentation process and clinical analyses of human breath and blood [25,26]. Ethanol amperometric biosensors are commonly based on either alcohol dehydrogenase (ADH) or alcohol oxidase (AOD) [27–30]. ADH based enzyme biosensor has the disadvantage that NAD^+ has to be added to the assay. The ethanol biosensor based on AOD are based on the monitoring of O_2 consumption or H_2O_2 formation, thus only dissolved oxygen is required as a co-substrate [31]. CNF has been used for the electrochemical detection of dissolved oxygen [32,33]. This work made use of the electrocatalytic reduction of CNF toward dissolved oxygen and the O_2 consumption at poly(thionine)–CNF/AOD film for ethanol detection. The proposed biosensor showed rapid response and excellent reproducibility and stability.

2. Experimental

2.1. Chemicals

CNF was a gift from WPI (Sarasota, USA). AOD from *Hansenula polymorpha* with an activity of 20–40 U/mg was purchased from Sigma. Thionine was from the Shanghai Biochemical Reagent (China). Other reagents were of analytical reagent grade. All solutions were prepared with twice-distilled water. The buffer for the assay was 0.2 M phosphate buffer saline (PBS), prepared by mixing stock standard solution of K_2HPO_4 and KH_2PO_4.

2.2. Apparatus

Electrochemical measurements were performed on a CHI 660 electrochemical analyzer (Co. CHI, USA) with a conventional three-electrode system comprised of platinum wire as auxiliary electrode, saturated calomel electrode as reference and modified glassy carbon electrode (GCE) as working electrode. The cyclic voltammetric and amperometric experiments were performed in a thermostated electrochemical cell at 25 °C. Amperometric experiments were carried out in a stirring air-saturated 0.2 M pH 7.0 PBS by applying a potential step of −0.4 V.

UV–vis absorbance spectroscopy was performed using a UV–vis-3100-NIR recording spectrophotometer (Shimadzu, Japan). X-ray photoelectron spectrum (XPS) was recorded on an ESCALAB MKII X-ray photoelectron spectrometer.

2.3. Construction of ethanol biosensor

The GCE was successively polished to a mirror finish using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by rinsing thoroughly with twice-distilled water. After successive sonication in 1:1 nitric acid, acetone and twice-distilled water, the electrode was rinsed with twice-distilled water and allowed to dry at room temperature. 0.5 mg CNF was added into 1.0 mg/mL thionine solution and sonicated at room temperature for 1 h to obtain a well-dispersed thionine–CNF nanocomposite solution.

The electropolymerization was performed in the mixture of 4 mL thionine–CNF nanocomposite solution and 1 mL 0.2 M at +1.5 V for 300 s. The poly(thionine)–CNF film was then grown in the same solution by potential cycling between −0.5 and 0.0 V at 50 mV/s for 50 cycles. The poly(thionine)–CNF/AOD modified electrode was prepared through the same process in presence of 0.2 mg/mL AOD.

3. Results and discussion

3.1. XPS and UV–vis characterization of poly(thionine)–CNF film

XPS was used to investigate the surface composition of the polymer film prepared under optimal experimental conditions. The intense signals for C1s at 285 eV, N1s at 398 eV and O1s at 531 eV were observed on the XPS survey spectrum of the polymer film (Fig. 1A). The N1s spectrum could be well resolved into three peaks at 400.1, 399.3 and 400.8 eV (Fig. 1B), indicating that three chemical states of nitrogen atoms existed in the polymer film, which were in accordance with the poly(thionine) film [34]. The peak at 399.3 eV was corresponding to the amino nitrogen atoms, while the peak at 400.8 eV was ascribed to the heterocyclic nitrogen atom. The peak at 400.1 eV was ascribed to the bonding via an amino nitrogen atom in one thionine molecule to a carbon atom.

The atomic ratio of carbon to nitrogen could be used to estimate the surface composition of the polymer film. The results showed the polymer film contained 75.1 atomic% carbon and 14.6 atomic% nitrogen, thus the calculated weight percents of thionine was 84.2% and CNF was 15.8%. The weight ratio of

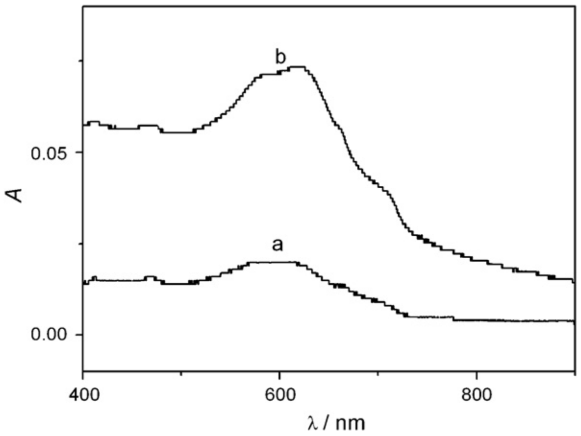

Fig. 2. UV–vis spectra of (a) poly(thionine) and (b) poly(thionine)–CNF composite.

thionine to CNF was higher than that in electropolymerization solution, which indicated that more thionine molecules were participated in the electropolymerization.

Fig. 2 shows the UV–vis spectra of poly(thionine) and poly(thionine)–CNF film. Poly(thionine) showed an absorption peak in the visible region around 600 nm, which showed a little red shift observed in poly (thionine)–CNF, indicating that CNF was incorporated into the film and interacted with thionine molecules.

3.2. Electropolymerization of thionine–CNF nanocomposite

The preparation of poly(thionine)–CNF film was performed using the method reported for the electropolymerization of thionine in neutral aqueous solution [34]. The growth of poly(thionine)–CNF film was dependent on the preanodization operation at a constant potential of +1.5 V carried out on a bare GCE in pH 7.0 PBS containing thionine–CNF nanocomposite. A large amount of positive charge could be accumulated to create the thionine cation radical during this process. After the preanodization, the poly(thionine)–CNF film was gradually formed with successive cyclic sweeps, as indicated in Fig. 3. During the process of electropolymerization, a pair of reversible redox peaks were observed at −0.268 and −0.246 V, which were attributed to the oxidation and reduction of thionine unit. Both the cathodic and anodic peak currents increased gradually and trended towards the stable values. The peak shape also became better, indicating the gradually increasing coverage of thionine unit. Finally, a stable cyclic voltammogram with almost symmetric shape was obtained, which was stable in pH 7.0 PBS upon cyclic sweep at 50 mV/s over the same potential range.

Fig. 4 shows the cyclic voltammograms of the poly(thionine)–CNF modified GCE at various scan rates. Both the anodic and cathodic peak currents were proportional to the scan rate in the range from 10 to 400 mV/s, indicating a surface-controlled electrode process. The surface coverage was calculated from the peak areas to be $(4.42 \pm 0.15) \times 10^{-9}$ mol/cm^2.

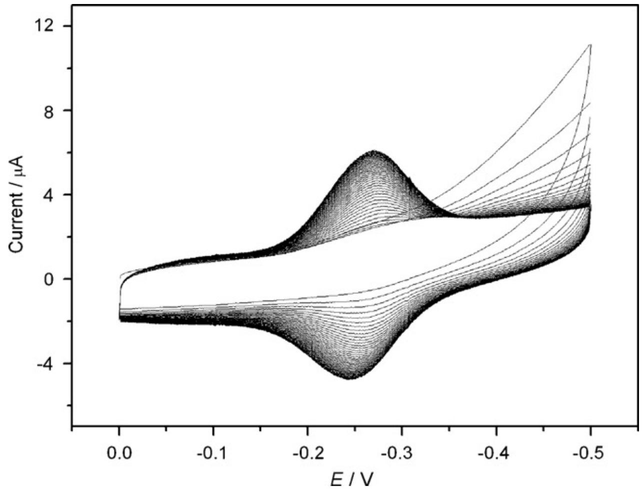

Fig. 3. Cyclic voltammogram for electropolymerization of CNF–thionine nanocomposite on pretreated GCE after preanodization at +1.5 V for 300 s in pH 7.0 PBS containing thionine–CNF nanocomposite.

3.3. Electrocatalysis of poly(thionine)–CNF toward reduction of dissolved oxygen

Fig. 5 displays the cyclic voltammograms of poly (thionine)–CNF modified GCE in nitrogen- and air-saturated 0.2 M pH 7.0 PBS. In the potential window from 0 to −0.5 V, the poly(thionine)–CNF modified GCE exhibited a pair of small redox peaks at −0.251 and −0.271 V at 50 mV/s in nitrogen-saturated PBS. In air-saturated PBS, the reduction peak current increased from 5.80 to 7.78 μA and the oxidation peak current decreased, indicating a typical electrocatalytic reduction process toward dissolved oxygen. The poly(thionine) modified electrode did not show observable electrocatalytic activity toward the reduction of dissolved oxygen, so the electrocatalytic activity should be ascribed to the CNF whose electrocatalytic property had been reported in [24].

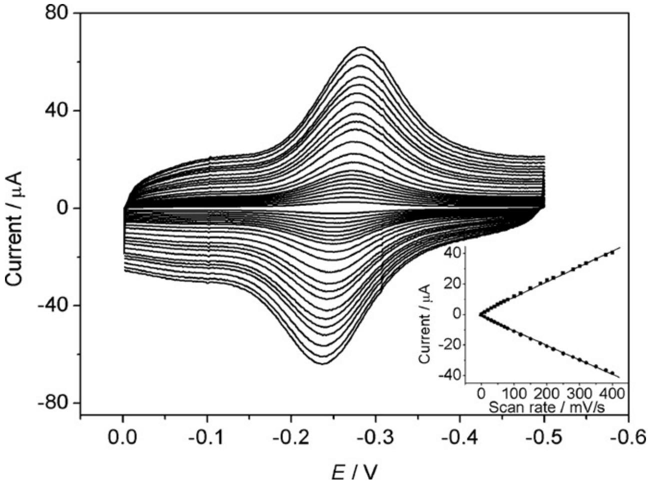

Fig. 4. Cyclic voltammograms of poly(thionine)–CNF modified GCE in 0.2 M pH 7.0 PBS at 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 150, 180, 200, 220, 250, 280, 300, 320, 350, 380 and 400 mV/s. Inset: plots of peak currents vs. υ.

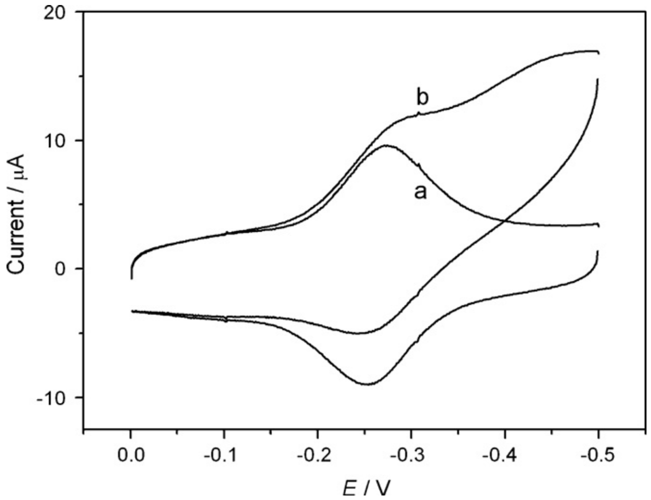

Fig. 5. Cyclic voltammograms of poly(thionine)–CNF modified GCE in 0.2 M pH 7.0 nitrogen: (a) and air-saturated (b) PBS.

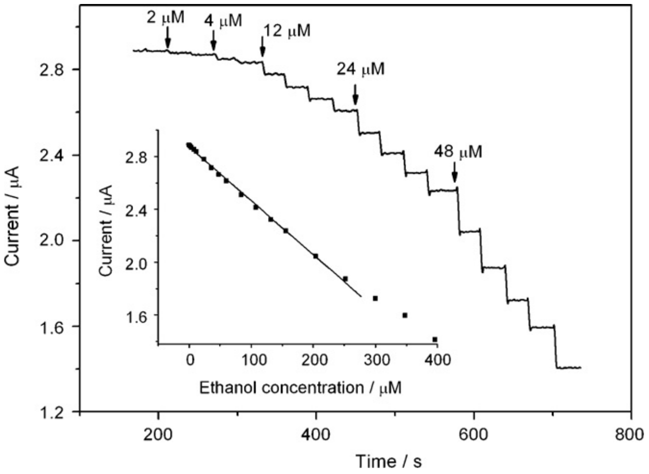

Fig. 7. Successive amperometric responses of the poly(thionine)–CNF/AOD modified GCE to ethanol in pH 7.0 PBS at −0.4 V. Inset: plot of amperometric response vs. concentration of ethanol.

3.4. Electropolymerization of thionine–CNF nanocomposite containing AOD

The isoelectric point of AOD is 6.1. In pH 7.0 PBS, the enzyme was negatively charged. Since the thionine–CNF nanocomposite was polymerized as a polycation of thionine, the AOD could be incorporated into the growing film via electrostatic affinity interaction. The cyclic voltammogram for electrochemical polymerization of thionine–CNF/AOD nanocomposite film in 0.2 M pH 7.0 PBS containing 0.2 mg/mL AOD after preanodization is shown in Fig. 6A. During the electropolymerization process, the peak currents of a pair of reversible redox peaks at −0.288 and −0.231 V increased. In comparison with Fig. 2, the peak-to-peak separation was larger, indicating that AOD was incorporated in the polymer film and blocked the electron transfer.

The concentration of AOD in electropolymerization solution was an important parameter for substrate detection. With the increasing concentration of enzyme in electropolymerization solution, the response to ethanol increased and then tended towards a constant value. At the enzyme concentration of 0.2 mg/mL, the current reached a maximum value, indicating a saturation of enzyme in the electropolymerized film. When the concentration was larger than this value, excess AOD could not be incorporated in the polymer film and would block the electron exchange. Thus, the concentration of 0.2 mg/mL AOD was used for preparation of poly(thionine)–CNF/AOD film.

3.5. Amperometric biosensing of ethanol

AOD is an oligomeric flavoprotein with eight identical subunits arranged in a quasi-cubic orientation, each containing a non-covalently bound flavin adenine dinucleotide molecule (FAD) as a cofactor [26]. AOD can catalyze the oxidation of ethanol by O_2 into the corresponding aldehydes with the concomitant production of H_2O_2 [26]. During this reaction, the AOD cofactor (FAD) is first reduced to its hydrogenated form ($FADH_2$) and re-oxidized to its native form [35]. Ethanol biosensors are generally based on the detection of the oxidation signal of H_2O_2 or the reduction signal of O_2. The poly(thionine)–CNF/AOD based biosensor retained the catalytic activity of the CNF for the efficient reduction of dissolved oxygen, allowing fast and sensitive ethanol quantification.

As shown in Fig. 6B, in the potential window from 0 to −0.5 V, the poly(thionine)–CNF/AOD modified GCE exhibited

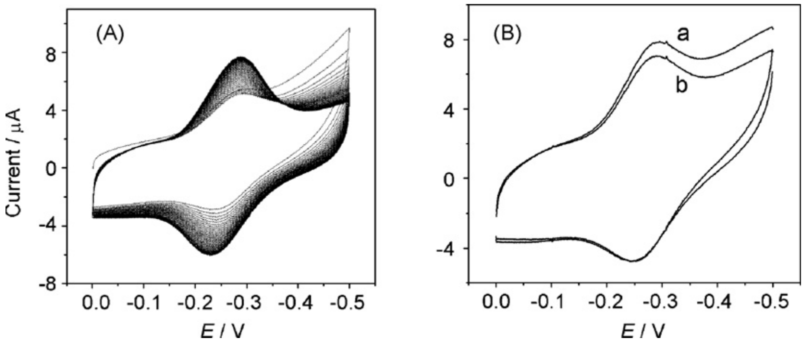

Fig. 6. Cyclic voltammograms for (A) coimmobilization of AOD and CNF-thionine nanocomposite on pretreated GCE after preanodization at +1.5 V for 300 s in pH 7.0 PBS containing thionine–CNF composite and 0.2 mg/mL AOD and (B) poly(thionine)–CNF/AOD modified GCE in pH 7.0 air-saturated PBS in absence (a) and presence (b) of 10 mM ethanol.

a pair of redox peaks at −0.249 and −0.292 V at 50 mV/s in air-saturated PBS. Upon addition of ethanol to the air-saturated PBS, the reduction current response of poly(thionine)–CNF/AOD modified GCE decreased from 4.01 to 3.56 μA due to the consumption of dissolved oxygen.

Fig. 7 shows the amperometric response of the poly(thionine)–CNF/AOD modified GCE at −0.4 V to the successive addition of ethanol in 0.2 M pH 7.0 PBS. Immediately after the addition of ethanol, the current decreased and reached a steady state within 5 s. The response displayed a linear range from 2.0 to 252 μM with a correlation coefficient of 0.999 and a slope of 4.1 nA/μM. The detection limit is 1.7 μM, which was much lower than that of 6.26 mM (3.5×10^{-2}%, v/v) based on AOD immobilized within electrochemically deposited resydrol film [36]. The sensitivity was 6.8 times higher than that of 0.6 nA/μM at a trienzyme graphite–Teflon–glucose oxidase–AOD–horseradish peroxidase–ferrocene based biosensor [37].

The coefficients of variation for intra-assay with this method were 5.9% and 5.0% at the ethanol concentrations of 8.0 and 80 μM, respectively. In addition, the relative standard deviation of current signals for the measurement of 100 μM ethanol at five independently prepared biosensors was 8.2%, which proved good reproducibility of the biosensor preparation.

The stability of the poly(thionine)–CNF/AOD modified electrode was investigated when stored in 0.2 M pH 7.0 PBS at 4 °C. After 4 weeks the response current was still retained at 96% of the initial response, implying that the three-dimensional structure of the poly(thionine)–CNF structure was very efficient for retaining the bioactivity of AOD.

4. Conclusions

A nanocomposite of thionine–CNF with good dispersion in water can be prepared through π–π electronic and hydrophobic interaction of thionine and CNF. With a one-step electrochemical polymerization two nanocomposite films of poly(thionine)–CNF and poly(thionine)–CNF/AOD have been successfully immobilized on an electrode surface. The presence of CNF leads to excellent catalytic activity of the nanocomposite films to the reduction of dissolved oxygen, producing a sensitive ethanol biosensor. The proposed ethanol biosensor possesses excellent characteristics and performance such as low detection limit, fast response and good stability, manifesting that the as-prepared thionine–CNF nanocomposite and this electrochemical polymerization method are suitable for enzyme immobilization and biosensor construction.

Dear Professor Wang,

It is a great honor and a tremendous pleasure for me to congratulate you on your 60th birthday!

When I was a postdoc in your lab at NMSU, you helped shepherd me through, and I will never forget the encouragement and guidance you gave me. Your life has been an inspiration for many scholars, but it touched mine in very special ways. You inspired me at a turning point in my life. There are so many ways that I learned from your example, but perhaps the most important lesson was your belief in the importance of what you were doing. You always tell us “no pain, no gain”. I only hope I can repay some of my debt to you by passing on some of the lessons I learned to my students and colleagues who have not had the good fortune of leaning from you and knowing you. Most are admired because they move others to follow their example . . . only a very few are special enough to encourage others to find their own unique path. Just by being who you are, you have sparked me to be who I am. And for that, if nothing else, I call you my inspiration.

Happy Birthday, Professor Joseph Wang! May your bright light shine on! May it be for you a wonderful, flourishing, exciting decade! (By Xueji Zhang).

Dear Joe,

Congrats and best wishes on your 60th birthday, another milestone another success. As your collaborator, I wish your health be great and your birthdays. In oriental philosophy, 60 years means the completion of one full life cycle and the beginning of a new cycle. Hence, a 60th birthday is a very significant event and a time for great celebrations. On your 60th anniversary, I wish to extend my special congratulations to you, a leader in the development of many chemical and biological sensors, striving as always for further advances in our field. Your important works on behalf of Electroanalysis and Sensors have been benefited many people a great deal, both within and outside academia. You will probably never know how many people like me deeply appreciate you and celebrate your creativity and wisdom.

I am sure that anyone has lived those 60 years in a manner as remarkable and distinguished as you have done is indeed a cause for celebration. Happy Birthday to you Joe! (by Huangxian Ju).

Acknowledgements

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