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# Electrochemical synthesis of polyaniline nano-network on α-alanine functionalized glassy carbon electrode and its application for the direct electrochemistry of horse heart cytochrome *c*

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ABSTRACT

Polyaniline (PAN) nano-particles, nano-fibrils, and nano-network have been synthesized via electrochemical polymerization of aniline using a three-step electrochemical deposition procedure on α-alanine (ALA)-monolayer functionalized glassy carbon electrode (GCE). The structure and properties of PAN nanostructures have been characterized using field emission scanning electron microscope (SEM), Fourier transform infrared spectra (FT-IR), and electrochemical techniques. The 3-dimensional (3D) PAN nanonetwork/ALA composite film coated GCE (PAN–ALA/GCE) leads to the direct electrochemistry of horse heart cytochrome *c* (Cyt *c*) immobilized on this electrode surface. The immobilized Cyt *c* maintains its activity, showing a surface-controlled electrode process with the electron transfer rate constant ($k_s$) of 21.9 $s^{-1}$ and the charge-transfer coefficient ($\alpha$) of 0.37, and could be used for the electrocatalytic reduction of hydrogen peroxide ($H_2O_2$). The steady-state current response increases linearly with $H_2O_2$ concentration from $2.5 \times 10^{-5}$ to $3.0 \times 10^{-4}$ mol $l^{-1}$. The detection limit ($3\delta$) is $8.2 \times 10^{-6}$ mol $l^{-1}$.



## 1. Introduction

Due to its chemical, electrical, and optical properties, PAN has been widely studied and used in rechargeable batteries (Oyama et al., 1995; Mu et al., 1993) and electrocatalysis (Rajendra Prasad and Munichandraiah, 2002; Kanungo et al., 2003; Zhang et al., 2006; Zhang and Lian, 2007; Zhang, 2007). However, the strict demands for medium acidity (pH < 4) limit the potential applications of PAN, especially in bioelectrochemistry, which normally requires a neutral pH environment. To develop the extensive application of PAN, many efforts have been focused on the adaptation of PAN to a higher solution pH. Following the first introduction of sulfonic acid group into PAN backbone to get the self-doped PAN (Yue and Epstein, 1990), which can maintain its electroactivity in neutral pH, many researchers tried to prepare sulfonated PAN including copolymerization of aniline with sulfonated aniline or organic acid or homopolymerization of ring sulfonated aniline by substituting with electron donating groups or by putting a spacer between the sulfonated group and the ring (Mav et al., 2000; Mu and Kan, 2002; Lukachova et al., 2003; Zhang and Dong, 2004; Zhang and Lian, 2007; Zhang, 2007). Recently, we have studied the synthesis of self-doped PAN via electrochemical copolymerization of aniline and o-aminobenzenesulfonic acid (Zhang, 2007) or o-aminophenol (Zhang and Lian, 2007) and their applications for the electrocatalytic oxidation of ascorbic acid.

With the intensive studies to conducting polymers and the potential application of nano-structures in practice, nano-sized conducting polymers fibrils have been prepared both chemically or electrochemically using "hard" or "soft" templates, such as track-etched polycarbonate (Martin, 1996; Parthasarathy and Martin, 1994), zeolite channels (Wu and Bein, 1994), anodized alumina (Wang et al., 2001), surfactants (Michaelson and McEvoy, 1994), micelles (Wei et al., 2002), liquid crystals (Huang et al., 2002), thiolated cyclodextrins (Park et al., 2001), and template-free procedures (Langer et al., 2001). Recently, large arrays of uniform and oriented PAN nano-wires have been synthesized on Pt surface without using a supporting template (Liang et al., 2002); and a facile chemical route to prepare PAN nano-fibres via aqueous/organic interfacial polymerization in the presence of camphorsulfonic acid (CSA) has been reported (Huang et al., 2003).

This report describes the syntheses of PAN nano-particles, nano-fibrils, and nano-network by direct electro-polymerization of aniline using a three-step electrochemical deposition procedure on ALA functionalized GCE for the first time. Due to the doping function of the carboxylic acid groups, the PAN shows good redox activity in neutral and even up to basic medium up to pH 9.0. Due to

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the porous nano-network structure and the electrochemical activity of PAN in neutral medium, the ALA-doped PAN network film modified electrode can be used as a sensor for detecting substances with redox activity, including of biomolecules. As an example of the applications of the PAN nano-network, the electrochemical behavior of Cyt *c* has been explored on the PAN nano-network coated GCE. Furthermore, the Cyt *c*-modified PAN–ALA/GCE exhibits electrocatalytic as well as good amperometric response towards $H_2O_2$, and a new $H_2O_2$ amperometric biosensor has been constructed.

## 2. Experimental

### 2.1. Chemicals and solutions

Aniline (99.5%, Shanghai) was distilled under vacuum prior to use and stored under high purified nitrogen in a refrigerator. Cyt *c* (Sigma, USA) was prepared in 10 mmol $l^{-1}$ phosphate buffer solution (PBS) pH 6.8. Lithium perchlorate ($LiClO_4 \cdot 3H_2O$, 99.5%, Shanghai) was dried at 95 °C for 12 h prior to use. ALA and ACN (99.5%) were from Sigma and used as received. 0.01 mol $l^{-1}$ PBS was prepared from $KH_2PO_4$ (Sigma, USA) and $K_2HPO_4$ (Sigma, USA). Sulfuric acid solutions (pH 1–3), 0.1 M acetate (pH 4–5), 0.1 M PBS (pH 6–8) and 0.1 M borate (pH 9) buffers were used. All experiments were made at room temperature (≈21 °C).

### 2.2. Apparatus

All electrochemical experiments were performed on a CHI 660B electrochemical analyzer (CHI, USA) in a three-electrode electrochemical cell using PAN–ALA/GCE and GCE (ø3-mm) as working electrodes, twisted platinum wire as auxiliary electrode, and Ag/AgCl as reference electrode.

FT-IR spectra were obtained by using a Nicolet 520 (Nicolet Co., USA) spectrophotometer. The morphologies of the polymer films were characterized using a JXA 840 field emission scanning electron microscope (SEM, JEOL, Japan). X-ray photo-electron spectroscopy (XPS) was recorded on an ESCALAB-MK spectrometer (VG Co., UK).

### 2.3. Electrode preparation

GCE was first polished with alumina powder (0.03 μm) on a polishing cloth. After being sonicated in absolute ethanol and water for 3 min, successively, the mirror-like GCE was dried with a fluid of highly purified nitrogen and treated by potential scanning between 0.5 and 1.7 V at 0.05 V $s^{-1}$ for 5 scans in ACN solution containing 0.001 mol $l^{-1}$ ALA and 0.1 mol $l^{-1}$ $LiClO_4$. To remove the physisorbed materials from electrode surface, the electrode was rinsed with ethanol and water and was sonicated for 2 min in 0.1 mol $l^{-1}$ PBS pH 6.8. Then, the ALA modified GCE (ALA/GCE) was obtained.

The ALA/GCE was then coated with PAN nano-network by the following three-step electrochemical deposition procedure in 0.1 mol $l^{-1}$ $H_2SO_4$ solution containing 0.01 mol $l^{-1}$ aniline. Firstly, a large current density was applied, the continuous polymerization was then performed by applying reduced current density. A typical procedure involves: 0.08 mA $cm^{-2}$ for 0.5 h, followed by 0.04 mA $cm^{-2}$ for 3 h, which was then followed by another 3 h at 0.02 mA $cm^{-2}$. After being rinsed with water, the electrode was electrolyzed again at 0.02 mA $cm^{-2}$ in solution containing only 0.1 mol $l^{-1}$ $H_2SO_4$ for 2 min to further polymerize the aniline monomer absorbed on/inside the film. Then, the PAN nano-network coated GCE (PAN–ALA/GCE) was obtained and stored in 0.1 mol $l^{-1}$ PBS pH 6.8 at 4 °C for use.

To immobilize Cyt *c* on/inside the ALA-doped PAN nano-network, the PAN–ALA/GCE was immersed into a 5 mmol $l^{-1}$ PBS pH 6.8 containing 0.5 mmol $l^{-1}$ Cyt *c* and kept in a refrigerator for 20 h. During this period the Cyt *c* was spontaneously adsorbed on/inside the PAN–ALA composite film due to the electrostatic and intercalation interaction between the negatively charged PAN nano-network and the positively charged Cyt *c*. After washing the PAN–ALA/GCE with water to get ride of the non-firmly adsorbed Cyt *c* molecules, the Cyt *c*-immobilized PAN–ALA/GCE (Cyt *c*-PAN–ALA/GCE) was then obtained.

## 3. Results and discussion

### 3.1. Electrode preparation

#### 3.1.1. Preparation of GCE

It is known that the GCE should be pre-treated via electro-oxidation in strong acid medium ($H_2SO_4$, $HClO_4$, etc), this process produces oxygen-containing functionalities on GCE surface and even inside the carbon substrate, such as, carbonyl, quinoid, carboxylate, and hydroxyl radical species, etc. These oxygen-containing functional groups show somewhat electrochemical catalytic effect to the redox of some electro-active substances (Cai et al., 1994). The aim of this report is to investigate the electrochemical activity and electro-catalysis of PAN doped by carboxylic groups, thus it is necessary to avoid the possible influence of these oxygen-containing functionalities on GCE surface; therefore, the GCE should not be pre-activated in strong acid medium. On the other hand, to avoid the exposing of GCE in air and thus the possible oxidation of carbon, after the polishing and washing, the GCE should immediately be dried with a fluid of highly purified nitrogen and immersed in solution of ALA for grafting.

#### 3.1.2. Covalent grafting of ALA on GCE surface

Fig. 1 shows the cyclic voltammograms (CVs) of GCE in ACN solution containing 0.001 mol $l^{-1}$ ALA and 0.1 mol $l^{-1}$ $LiClO_4$. As can be seen, there is a broad, irreversible anodic peak at 1.43 V in the first cycle, and no cathodic peak is observed on the reverse scan, indicating that the species obtained after the first electron transfer undergoes a chemical reaction. The one-electron oxidation of amino group turns into its corresponding cation radical, and then, these cation radicals form C–N covalent bonds at the carbon electrode surface (Deinhammar et al., 1994; Downard and Mohamed, 1999). On the 2nd cycle, the oxidation peak potential moves negatively to 1.32 V, and the corresponding current response decreases quickly. In the following potential cycles, the peak potential keeps

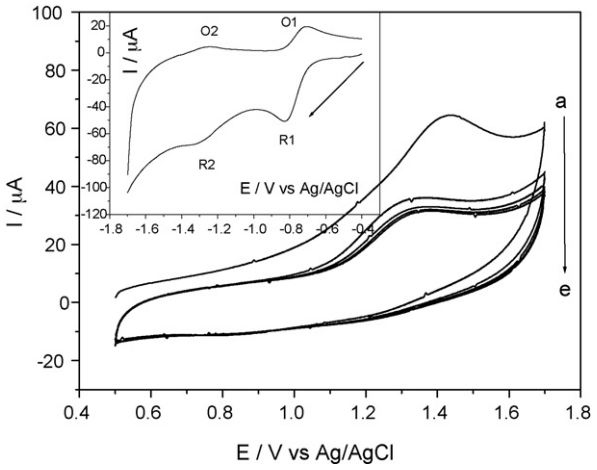


**Fig. 1.** Cyclic voltammograms of 0.001 mol $l^{-1}$ ALA at GCE in ACN containing 0.1 mol $l^{-1}$ $LiClO_4$; inset: cyclic voltammograms of ALA/GCE in ACN solution containing 0.1 mol $l^{-1}$ $LiClO_4$. Scan rates: 0.05 V $s^{-1}$.