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Syntheses of fully sulfonated polyaniline nano-networks and its application to the direct electrochemistry of cytochrome *c*

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Abstract

Fully sulfonated polyaniline nano-particles, nano-fibrils and nano-networks have been achieved for the first time by electrochemical homopolymerization of orthanilic acid using a three-step electrochemical deposition procedure in a mixed solvent of acetonitrile (ACN) and water. The diameter of the uniform nano-particles is about 60 nm, and the nano-fibrils can be organized in two-dimensional (2D) or three-dimensional (3D) non-periodic networks with good electrical contact. Average distance between contacts is about 850 and 600 nm for a 2D and 3D system, respectively. The details of the poly(orthanilic acid) (POA) nano-structure were examined with a field emission scanning electron microscope (SEM). The structure and properties of POA were characterized with FTIR, UV–vis and electrochemical methods. The 3D POA nano-networks coated platinum electrode gave a direct electrochemical behavior of horse heart cytochrome *c* (Cyt *c*) immobilized on this electrode surface, a pair of well-defined redox waves with formal potential ($E^{\circ\prime}$) of −0.032 V (versus Ag/AgCl) was achieved. The interaction between Cyt *c* and POA makes the formal potential shift negatively compared to that of Cyt *c* in solution. Spectrophotometric and electrochemical methods were used to investigate the interaction of Cyt *c* with POA. The immobilized Cyt *c* in the nano-networks POA film maintained its activity, showing a surface-controlled electrode process with the electron transfer rate constant (k_s) of 21 s^{-1} and a of 0.53, and could be used for the electrocatalytic reduction of hydrogen peroxide. The quantitative determination of Cyt *c* by differential pulse voltammetry (DPV) using the fully sulfonated 3D POA nano-networks film coated platinum electrode was also studied.

Keywords: Fully sulfonated polyaniline; Nano-networks; Cytochrome *c*; Direct electron transfer; Hydrogen peroxide

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1. Introduction

Among the conducting polymers, polyaniline (PAN) has attracted significant interests for its chemical, electrical and optical properties. These properties are favorable to its applications in rechargeable batteries (Oyama et al., 1995) and electrocatalysis (Kanungo et al., 2003; Karyakin et al., 1999; Rajendra Prasad and Munichandraiah, 2002). However, PAN shows electrochemical activity only in acidic conditions, normally pH < 4 (Diaz and Logan, 1980; Ohsaka et al., 1984). This greatly restricts its applications, especially in bioelectrochemistry, which normally requires a neutral pH environment. Thus, in recent years, many efforts have been focused on adaptation of PAN to a higher pH. Following the first introduction of sulfonic acid group into PAN backbone by Yue and Epstein (1990) to get so-called ‘self-doped’ PAN, which can maintain its electroactivity in neutral pH, many research groups have 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). However, all these approaches cannot obtain polymers

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with higher degrees of sulfonation and higher conductivities. Recently, chemical (Chan et al., 1998) and electrochemical (Krishnamoorthy et al., 2002) homopolymerizations of metanilic acid were carried out successfully for the first time to yield fully sulfonated polyaniline, which is soluble in water and organic solvents and electrical conducting.

With the intensive studies to conducting polymers, some applications including sensors or electronic devices may eventually require nano-sized polymer wires or dots. For this reason, 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; Yang and Wan, 2002), liquid crystals (Huang et al., 2002), thiolated cyclodextrins (Choi and Park, 2000; Park et al., 2001), and polyacids (Liu and Yang, 1991) and template-free procedures (Langer et al., 2001). Recently, Liu and co-workers (Liang et al., 2002; Liu et al., 2003) reported the electrochemical syntheses of large arrays of uniform and oriented nano-wires on Pt surface without using a supporting template. Kaner's group (Huang et al., 2003) reported a facile chemical route to prepare PAN nano-fibers using aqueous/organic interfacial polymerization in the presence of camphorsulfonic acid (CSA), and proposed that CSA was most likely tightly incorporated as anions within the PAN backbone during the in situ polymerization of aniline in CSA solution. Therefore, up to now, there has been no report on the homopolymerized nano-structures of ring sulfonated aniline. In this paper, we report, for the first time, the syntheses of fully sulfonated polyaniline nano-particles, nano-fibrils and nano-networks by homopolymerization of orthanilic acid in a mixed solvent of acetonitrile (ACN) and water (8:1) using a three-step electrochemical deposition procedure. And, a large arrays of poly(orthanilic acid) (POA) nano-fibrils standing upright on Pt surface and POA micro-rings structures have also been synthesized using cyclic voltammetry and potentiostatical electrolysis procedures, respectively. Since the homopolymer of orthanilic acid is electrochemical active in organic solvents, electrochemical n-doping is possible. The chemical and electrochemical properties of POA were also investigated.

Since POA is electrochemical active and stable in co-solvent of ACN and aqueous solution of 10 mM PBS (pH 7.0), it is possible to be used as biosensor for the determination of biomolecules. As an example of the potential applications of the new polymer with nano-networks structure, the direct electrochemical behavior of Cyt *c* was explored at the POA nano-networks coated platinum electrode and found that POA film gave a pair of well-defined redox waves with the formal potential of −0.032 V, a negative shifting occurs compared to that of Cyt *c* in solution. This is due to the electrostatic and intercalation interactions between positively charged Cyt *c* and negatively charged 3D POA networks film. And, it is also intended to know the electroactivity of Cyt *c* immobilized in the nano-networks POA flim and its pseudo peroxidase activity.

2. Experimental

2.1. Chemicals and solutions

Orthanilic acid was purchased from Sigma and used as received. Horse heart cytochrome *c* (Sigma), whose concentration was determined by UV–vis spectroscopy using $\varepsilon_{410} = 10{,}600\,\mathrm{M^{-1}\,cm^{-1}}$, was prepared in the mixture of ACN and 10 mM PBS (pH 7.0) with volume ratio of 8:1. ACN (99.5%) was from Beijing Chemical Reagents Company. Lithium perchlorate ($LiClO_4{\cdot}3H_2O$, 99.5%) was from Shanghai Chemical Reagents Company and dried at 95 °C for 12 h prior to use. 0.01 M phosphate buffer solution (PBS) was prepared from KH_2PO_4 (Sigma) and K_2HPO_4 (Sigma). All other chemicals used in this investigation were of analytical grade and used as received.

All experiments were made at room temperature (≈18 °C) unless stated otherwise. The solutions were thoroughly deoxygenated by bubbling highly purified nitrogen and a nitrogen atmosphere was maintained over the solutions.

2.2. Apparatus

All electrochemical experiments were performed using a computer-controlled CHI 660A electrochemical analyzer (USA) in a conventional three-electrode electrochemical cell using POA nano-networks coated platinum disk (Ø3-mm) as working electrode, twisted platinum wire as auxiliary electrode and Ag/AgCl (in saturated KCl) as reference electrode.

UV–vis absorption spectra were performed on a Cary 500 UV–vis–NIR spectrophotometer (Varian Co., USA). FTIR spectra were obtained by using a Nicolet 520 (Nicolet Co., USA) spectrophotometer. A JXA 840 field emission scanning electron microscope (JEOL, Japan) was used to examine the morphologies of the polymer films.

2.3. Electrode preparation

The platinum working electrode was polished carefully with alumina powder (Buehler, 1.0, 0.5 and 0.03 μm, successively) on a soft polishing cloth (Buehler). After sonicating in absolute ethanol, then in water for 10 min, successively, they were treated with cyclic scanning in the potential range of −0.2 to 1.2 V at $100\,\mathrm{mV\,s^{-1}}$ in 0.1 M H_2SO_4 until the cyclic voltammogram characteristics for a clean platinum electrode was obtained. The clean Pt electrode was then coated with POA nano-networks by the following three-step electrochemical deposition procedure. After polymerization, the POA nano-networks coated platinum electrode was sufficiently rinsed with monomer-free solution to remove unreacted orthanilic acid, and then was stored in monomer-free solution for use.