

Electrochemical Immobilization of Ascorbate Oxidase in Poly(3,4-ethylenedioxythiophene)/Multiwalled Carbon Nanotubes Composite Films

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ABSTRACT: Immobilization of ascorbate oxidase (AO) in poly(3,4-ethylenedioxythiophene) (PEDOT)/multiwalled carbon nanotubes (MWCNTs) composite films was achieved by one-step electrochemical polymerization. The PEDOT/MWCNTs/AO modified electrode was fabricated by the entrapment of enzyme in conducting matrices during electrochemical polymerization. The PEDOT/MWCNTs modified electrodes were investigated by cyclic voltammetry and electrochemical impedance spectroscopy. The experimental results showed that the composite films exhibited better mechanical integrity, electrochemical activity, higher electronic and ionic conductivity, and larger redox capacitance compared with pure PEDOT films, which would be beneficial to the fabrication of PEDOT/MWCNTs/AO electrochemical

biosensors. The scanning electron microscopy studies revealed that MWCNTs served as backbone for 3,4-ethylenedioxythiophene (EDOT) electropolymerization. Furthermore, the resulting enzyme electrode could be used to determine L-ascorbic acid successfully, which demonstrated the good bioelectrochemical catalytic activity of the immobilized AO. The results indicated that the PEDOT/MWCNTs composite are a good candidate material for the immobilization of AO in the fabrication of enzyme-based biosensor. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 122: 1142–1151, 2011

Key words: ascorbate oxidase; poly(3,4-ethylenedioxythiophene); multi-walled carbon nanotubes; electrochemical immobilization; conducting polymer

INTRODUCTION

Enzyme-based biosensors are very useful tools in satisfying analytical requirements, particularly in biochemistry, pharmacology, industry, and environmental sciences.¹ One of the key steps in successfully fabricating an enzyme-based biosensor is the

simple and reliable procedure for immobilizing and stabilizing reactive enzymes on the electrode. As several methods reported for the immobilization of enzyme,^{2–5} the electrochemical polymerization method has been used as an effective technique for immobilization of an enzyme on the electrode surface.⁶ The amount of enzyme that is immobilized can readily be adjusted via the controlled electrochemical polymerization step. Another challenge of using enzymes in enzyme-based biosensors is the need to find the support materials to be compatible with the enzyme and to achieve rapid electron transfer at the electrode surface. The excellent and unique properties of electronically conducting polymers (ECPs), such as high electrical conductivity, superior stability, good chemical and electrochemical properties, make them extremely attractive materials for such applications in enzyme-based biosensor.^{7,8}

In recent years, ECPs/ascorbate oxidase (AO) biosensors have been extensively studied. Wang et al.⁹ reported the electrochemical immobilization of AO in polyaniline films; Uchiyam et al.¹⁰ reported polypyrrole (PPy) being the choice of matrix coated

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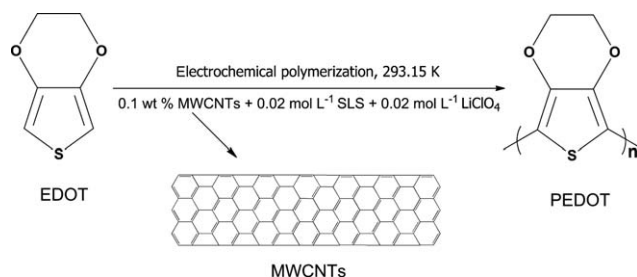
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Scheme 1 The structure of EDOT, PEDOT, and MWCNTs, together the electrochemical polymerization of EDOT to PEDOT.

carbon felt films for AO immobilization. In our groups, poly(3,4-ethylenedioxythiophene) (PEDOT) have been studied by electrochemical immobilization of AO in PEDOT films for the fabrication of PEDOT/AO electrochemical biosensor.¹¹ However, the biosensors suffered from long-term storage instability due to the easy leakage of enzymes from supports during washing and/or reaction process.

Because of the remarkable mechanical, structural, electrical, and thermal properties, carbon nanotubes (CNTs; Scheme 1) have attracted considerable attention since the discovery by Iijima in 1991.^{12,13} The application of CNTs in biological and medical fields, particularly in electrochemical biosensors, has been expanding rapidly.^{14–17} It has been shown that incorporating CNTs into a polymer matrix enhances the mechanical and electrical properties of the original polymer.^{18–20} Notably, CNTs have been demonstrated as a good candidate material for the immobilization of enzyme in enzyme-based biosensor fabrication.^{21,22} However, there are no articles concerning the electrochemical immobilization of AO in PEDOT/MWCNTs composite films have appeared.

In our present work, a novel enzyme electrode was successfully fabricated by the electrochemical entrapment of AO in PEDOT/MWCNTs composite films in phosphate buffered-aqueous solution (PBS) containing AO, MWCNTs and amino acid-based surfactant sodium *N*-lauroylsarcosinate (SLS). The electrochemical properties of PEDOT/MWCNTs composite films were studied using cyclic voltammetry (CV). The PEDOT/MWCNTs/AO composite films were also investigated using electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM). The bioelectrochemical catalytic activity of the PEDOT/MWCNTs/AO modified electrode towards L-ascorbic acid (AA) was investigated by chronoamperometry.

EXPERIMENTAL

Chemicals

AO (162 U mg⁻¹, EC 1.10.3.3) from cucumber (*Cucurbita* sp.), MWCNTs dispersed were purchased from

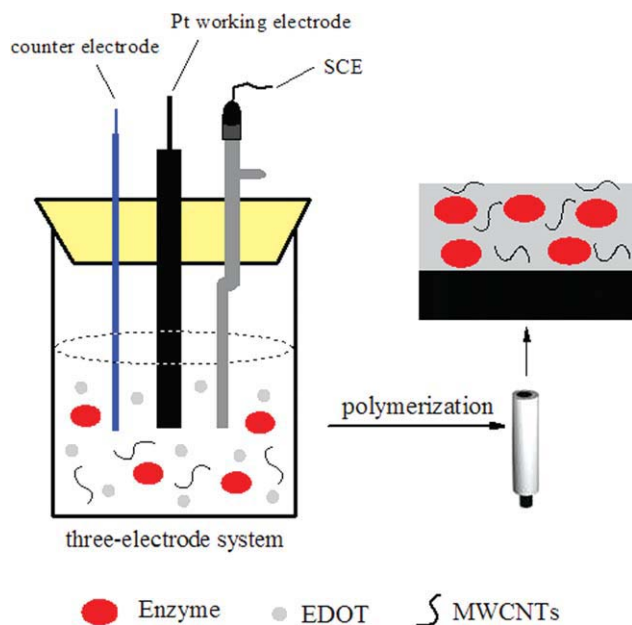
Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences (purity of MWCNTs 4.39 wt %, out diameter 50–100 nm, length 5–15 μm). AA was purchased from Bio Basic Inc. EDOT (99%; Aldrich), SLS (95%; Aldrich), a 0.05 mol L⁻¹ phosphate buffer solution (PBS; pH = 6.5) was prepared using Na₂HPO₄ and NaH₂PO₄. Lithium perchlorate trihydrate (LiClO₄; Sinopharm Chemical Reagent Co., Ltd.) of analytical reagent grade was used as received. Double-distilled deionized water was used as the solvent.

Electrochemical measurements and characterizations

The electrochemical polymerization of EDOT in aqueous solution containing different contents of MWCNTs were performed in a one-compartment cell using a potentiostat-galvanostat (Model 263A, EG and G Princeton Applied Research) under computer control. For electrochemical tests, a 3-mm diameter platinum (Pt) disc electrode served as the working electrode and 1 mm diameter stainless steel wire used as the counter electrode. The reference electrode was a saturated calomel electrode (SCE). The three electrodes were placed 5 mm apart during the measurements. The Pt working electrode was polished with alumina (Al₂O₃, 0.05 μm), rinsed with distilled water and cleaned ultrasonically. The stainless steel wire was carefully polished with abrasive paper (1500 mesh), and cleaned successively with distilled water and acetone, then air-dried before each experiment. All aqueous solutions were deaerated by bubbling with a dry argon stream for 10 min, prior to electrochemical experiments. All experiments were carried out under a slight argon overpressure. The typical solution used was aqueous solution with different MWCNTs contents of 0, 0.1, 0.4, 1.2 wt % (weight percent with respect to solution), containing 0.02 mol L⁻¹ EDOT, 0.02 mol L⁻¹ SLS, and 0.02 mol L⁻¹ LiClO₄. The PEDOT/MWCNTs composite films were grown potentiostatically at 1.1 V versus SCE and their thickness was controlled by the total charge passing through the cell, which was read directly from the current–time (*I*–*t*) curves by computer. PEDOT/MWCNTs composite films were washed repeatedly with double-distilled deionized water and acetone to remove the excess electrolyte, EDOT, and MWCNTs.

Preparation of PEDOT/MWCNTs/AO electrode

Immobilization of AO in PEDOT/MWCNTs composite films was performed in a typical three-electrode cell by applying constant potential of 1.1 V at 293.15 K (Scheme 2). To avoid denaturing the enzyme during deposition it is generally desirable to carry out the electropolymerization from aqueous solution close to neutral pH. This process is controlled by the electrode potential and it is therefore



Scheme 2 Illustration of the fabrication process of PEDOT/MWCNTs/AO modified electrode. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

possible to control the polymer films thickness (through the total charge passed) and hence the amount of enzyme entrapped in close proximity to the electrode surface. For the preparation of enzyme electrode, the phosphate buffer system (PBS, pH = 6.5) consists of 0.02 mol L⁻¹ EDOT, 0.02 mol L⁻¹ SLS, 0.4 wt % MWCNTs, 0.05 mg mL⁻¹ AO, 0.02 mol L⁻¹ LiClO₄. After electrolysis, electrode was washed with distilled water to remove excess supporting electrolyte and unbound enzymes.

Electrochemical impedance spectroscopy and scanning electron microscopy

EIS measurements were performed in 0.1 mol L⁻¹ LiClO₄ solution by using an Autolab Frequency Response Analyzer System (AUT30; FRA2-Autolab, Echemie, B.V.) connected to a conventional one-compartment three-electrode electrochemical cell. The impedance spectra were recorded in the frequency range from 100 kHz down to 0.1 Hz, using an alternating current (ac) signal amplitude of 10 mV at a direct current (dc) bias. The data were recorded after the dc potential being applied for 10 min because the composite films on the electrodes need a period to reach an electrochemical steady state. SEM measurements were taken with a Hitachi S3000N scanning electron microscope.

Electrocatalytic determination of ascorbic acid

The electrochemical detection of different concentrations of AA (0, 0.1, 0.5, 1, 2, 3, 4, 5, 6, and 7 mmol L⁻¹) was carried out by applying a constant poten-

tial of 0.4 V versus SCE for 100 s at 293.15 K. The supporting electrolyte was 0.05 mol/L⁻¹ air-saturated PBS (pH = 6.5).

RESULTS AND DISCUSSION

Electrochemical synthesis of PEDOT/MWCNTs composite films

The successive cyclic voltammograms (CVs) between -0.7 V and 1.15 V versus SCE of 0.02 mol L⁻¹ EDOT in 0.02 mol L⁻¹ SLS + 0.02 mol L⁻¹ LiClO₄ aqueous solution containing different MWCNTs contents on a Pt electrode are shown in Figure 1. This mixture was essentially a MWCNTs stabilized emulsion of EDOT droplets in water and was sufficiently stable to allow successful electropolymerization of PEDOT/MWCNTs composite films. In our previous work,²³ the introduction of environmentally friendly amino acid-based surfactant SLS into the electrolyte not only enhanced the solubility of EDOT but also lowered its onset oxidation potential. Furthermore, SLS can facilitate the biocompatibility of PEDOT with biologically-active species. It is clear that, in Figure 1(A), on the first CV cycle the current densities on the reverse scan are higher than that on the forward scan. The formation of this loop can be explained as characteristics of nucleation process.²⁴⁻²⁶ The redox peaks at 0.6 and -0.04 V were attributed to the doping/dedoping processes of PEDOT films formed in previous scans. As the CV scan continued, conducting polymer films were formed on the working electrode surface, which could be taken out of the electrolytic solution after CV. The increase in the redox wave current density implied that the amount of polymer on the electrode was increasing.

The nucleation process and homogenous polymer film deposition can also be observed when different MWCNTs contents were used [Fig. 1(B-D)]. The trend of the CVs of EDOT solutions containing different contents of MWCNT was similar. The redox peaks are at 0.6 and -0.04 V for 0.1 wt % MWCNTs, 0.6 and -0.04 V for 0.4 wt % MWCNTs solution while 0.2 and -0.2 V for 1.2 wt % MWCNTs solution. It was noticed that the polymerization current densities recorded during the backward potential scan for PEDOT/MWCNTs composite films were larger than that of pure PEDOT films. This results from the high conductivity and high surface area of MWCNTs, and more reaction sites become available on the formed composite films than on the original Pt electrode surface. More importantly, the CNTs themselves acted as both the charge carriers in solution and also the charge balancing counter ions in the polymerization. This not only is beneficial to the formation of uniform composite films, but also compromises the polymerization rate because of the

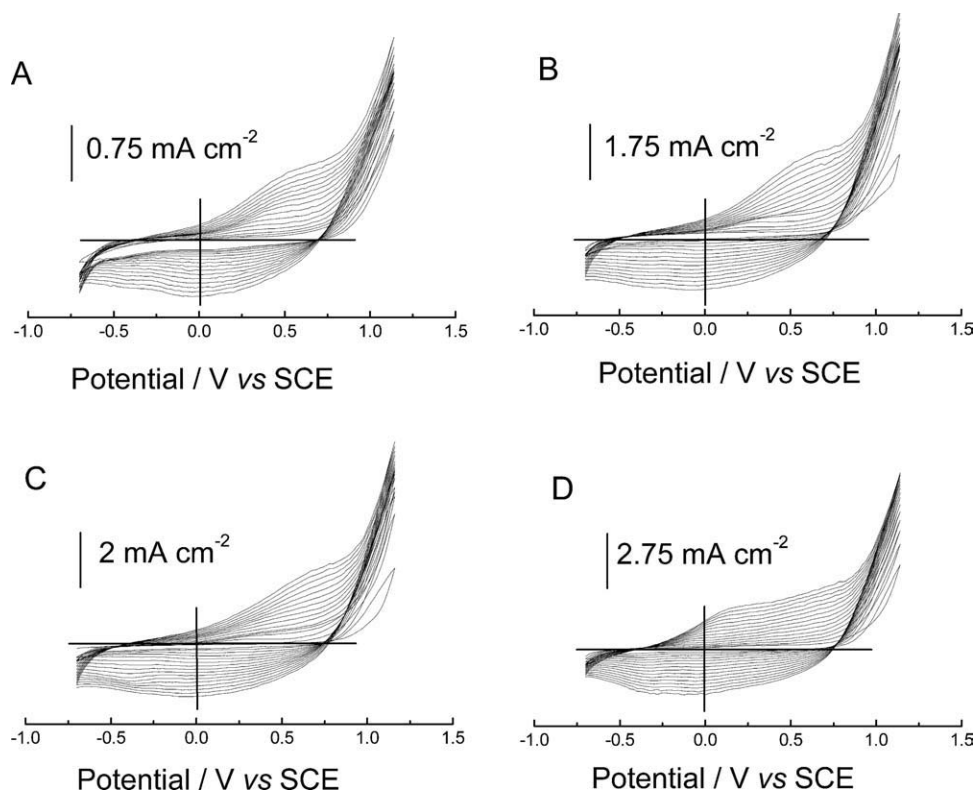


Figure 1 CVs obtained on Pt disc electrode in 0.02 mol L^{-1} EDOT + 0.02 mol L^{-1} SLS + 0.02 mol L^{-1} LiClO₄ aqueous solutions containing: (A) no MWCNTs, (B) 0.1 wt % MWCNTs, (C) 0.4 wt % MWCNTs, (D) 1.2 wt % MWCNTs. Potential scan rates: 50 mV s^{-1} .

relatively lower ionic conductivity of CNTs.²⁷ In addition, the slopes of cathodic peak current densities against numbers of scan were calculated at about -0.04 V , as illustrated in Table I. These values can be used to evaluate the polymerization rates of EDOT in different media. In comparison with no MWCNTs in aqueous solution [Fig. 1(A)], the introduction of MWCNTs increased the polymerization rate of EDOT. This behavior is consistent with a higher electrochemical polymerization reaction yield, and hence with a better electrode covering, as increasing the MWCNTs content. However, a large thickness of the polymer films can reduce the overall conductivity of the composite films, then decrease the MWCNTs contribution to the charge transfer.²⁸ As observed in this work, under similar electropolymerization conditions, the thin PEDOT films were navy blue. Clearly, the black color of the composite films was due to the presence of MWCNTs. The PEDOT/MWCNTs composite films were robust mechanically and could not be peeled-off easily. As expected, the composite films showed good mechanical integrity, which would be beneficial to extend the lifetime of the enzyme biosensor.

Figure 2 shows a set of current transients during electropolymerization of 0.02 mol L^{-1} EDOT in 0.02 mol L^{-1} SLS and 0.02 mol L^{-1} LiClO₄ aqueous

solution containing different MWCNTs contents at different applied potentials on a Pt working electrode. Two or more stages were clearly observed at different MWCNTs contents [Fig. 2(A–D)]. The initial stage of electrodeposition was a combination of instantaneous two-dimensional and three-dimensional mechanisms since electrodeposition was under charge transfer control than diffusion; in the later stages, layer-by-layer growth mode was in accordance with the Stran-ski-Krastanov mode.^{29,30} At applied potential below 0.9 V , polymerization does not occur on the electrode surface. Once the applied potential reaches the threshold value, all the electro-synthetic current densities initially experience a sharp decrease and then keep constant as a result of uniform deposition of the polymer films on the electrode surface. All the $I-t$ curves conducted in aqueous micellar solution are smooth, suggesting uniform electrodeposition of PEDOT/MWCNTs composite films.

TABLE I
The Slopes of Peak Current Densities Against Cycle Number During the CVs of EDOT in Aqueous Solution Containing Different MWCNTs Contents

	No MWCNTs	0.1 wt %	0.4 wt %	1.2 wt %
Cathodic	0.0325	0.1035	0.1185	0.1435

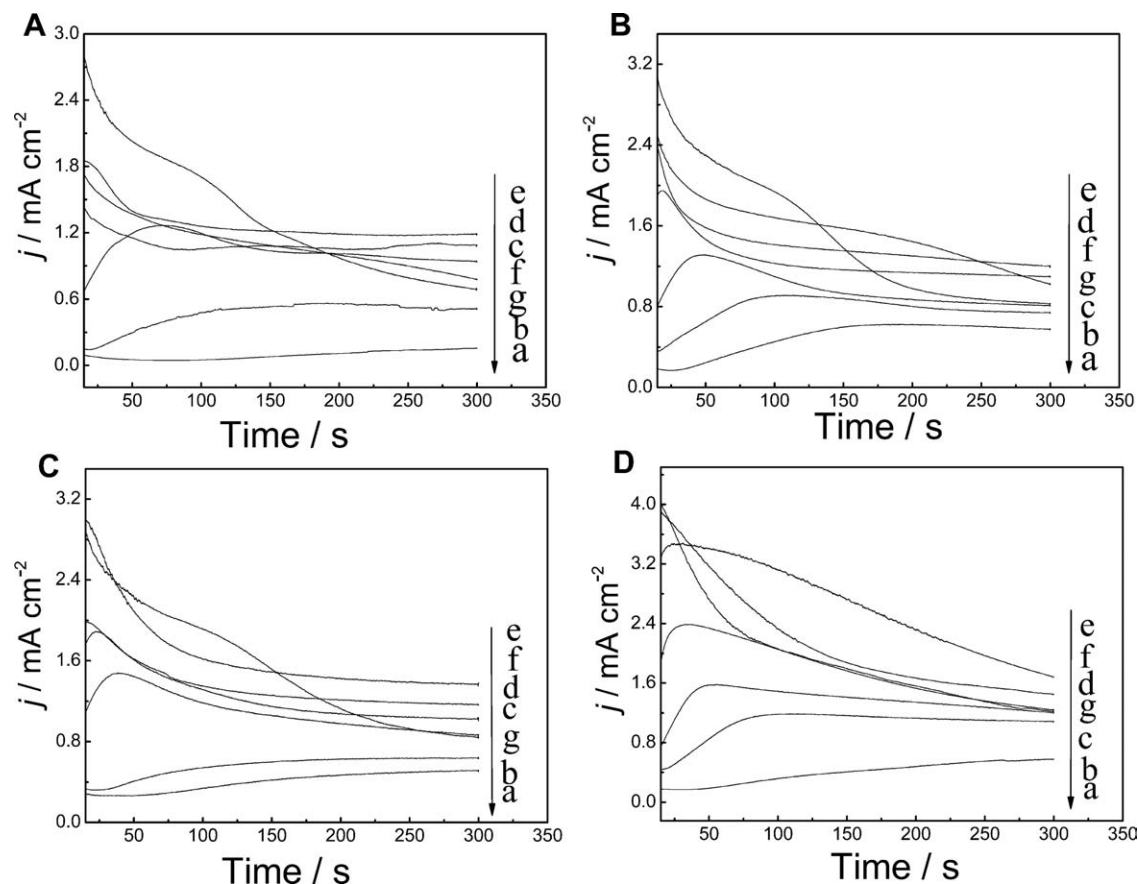


Figure 2 Chronoamperograms of 0.02 mol L^{-1} EDOT + 0.02 mol L^{-1} SLS + 0.02 mol L^{-1} LiClO₄ aqueous solutions in the presence of different MWCNTs contents. (A) no MWCNTs, (B) 0.1 wt % MWCNTs, (C) 0.4 wt % MWCNTs, (D) 1.2 wt % MWCNTs on a Pt disc electrode at the applied potentials of (a) 0.9 V, (b) 0.95 V, (c) 1.0 V, (d) 1.05 V, (e) 1.1 V, (f) 1.15 V, (g) 1.2 V.

Moreover, the current density increased at the Pt electrode with increased polymerization potential (E) from 0.9 V to 1.15 V (Fig. 2) when the E was >1.15 V, the current density dramatically decreased. So 1.15 V seems a turning point, at $E > 1.15$ V, the plateau currents decreased gradually, which might be ascribed to overoxidation of PEDOT at higher potential during electropolymerization.^{31,32} In the range from 0.9 V to 1.15 V, the overoxidation of PEDOT is negligible and the electropolymerization rate mainly depends on E . So increased E leads to increased plateau current. Simultaneously, overoxidation of PEDOT coexists with electropolymerization at $E > 1.15$ V. Under this circumstance, the electropolymerization and overoxidation of the deposited PEDOT take place simultaneously. The higher the applied potential, the more serious is the overoxidation of PEDOT.

The current density increased when MWCNTs were introduced. The influence of MWCNTs contents on current density is shown in Figure 3. This may be attributed to the high conductivity and high surface area of MWCNTs, which has been discussed above that more reaction sites become available on the formed composite films than on the original Pt

electrode surface. The presence of the MWCNTs can interact with the polymer and some of the formed polymer-MWCNTs aggregates can reduce the ion intercalation distance, thus facilitate the charge transfer and increase the conductivity of the polymer films.²⁸

Electrochemistry of PEDOT/MWCNTs composite films

To elucidate the effect of MWCNTs on the properties of PEDOT films, electrochemical performance of composite films was evaluated by carrying out CV measurements in monomer-free aqueous solutions (Fig. 4). The films were prepared from the different growth solutions containing 0, 0.1, 0.4, and 1.2 wt % MWCNTs, respectively, which can be cycled repeatedly without obvious decomposition. In Figure 4(A), the CVs of the films showed a couple of broad oxidation and reduction waves. In addition, the large capacitance-like currents are characteristic of PEDOT. The similar voltammograms of films prepared in different contents MWCNTs aqueous micellar solution all showed significant differences between the first cycle and those following, which

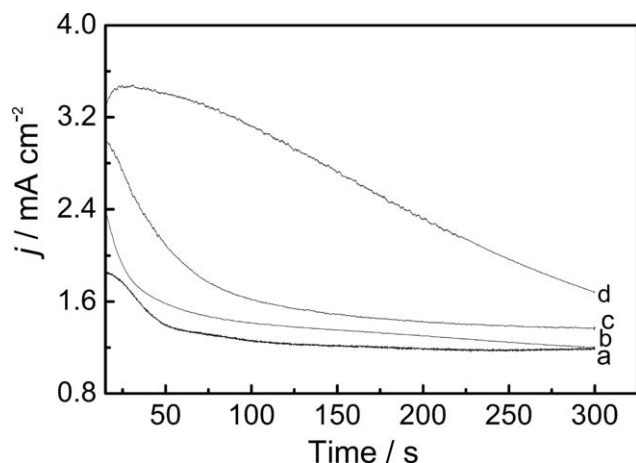


Figure 3 Chronoamperograms of 0.02 mol L^{-1} EDOT + 0.02 mol L^{-1} SLS + 0.02 mol L^{-1} LiClO₄ in the presence of different MWCNTs contents aqueous micellar solutions. (a) no MWCNTs, (b) 0.1 wt % MWCNTs, (c) 0.4 wt % MWCNTs, (d) 1.2 wt % MWCNTs on a Pt disc electrode at the applied potential of 1.1 V vs SCE.

indicated that the ionic redox exchanges played an important role in the electroactivity of the composite films.³³ All these results indicated that the composite films obtained from aqueous solution at different

MWCNTs contents had good redox activity and stability. When the MWCNTs content is 0.1 wt % [Fig. 4(B)], the prepared composite films showed greater redox activity compared with pure PEDOT films. The values of anodic and cathodic current density were 3.65 and -3.68 mA cm^{-2} , much higher than those of pure PEDOT films (1.68 and -1.35 mA cm^{-2} , respectively). When higher MWCNTs contents were used, only a slight increase in the values of anodic and cathodic current density was observed. This difference could be explained that as increasing the content of MWCNTs, the thickness of the composite films also increases, which reduce the contribution of MWCNTs to the composite films. It is therefore plausible to suggest that the incorporation of relatively low content of MWCNTs had significantly improved the electrochemical activity and capacitive property of pure PEDOT films. In addition, not only the anodic and cathodic peak current densities in the insert of Figure 4(B) were proportional to the scanning rates, but also the two curves were almost overlapping, which further indicated the better reversible redox behavior of the composite films prepared in 0.1 wt % MWCNTs aqueous solution compared with others. Hence, 0.1 wt % MWCNTs was selected in the subsequent experiments.

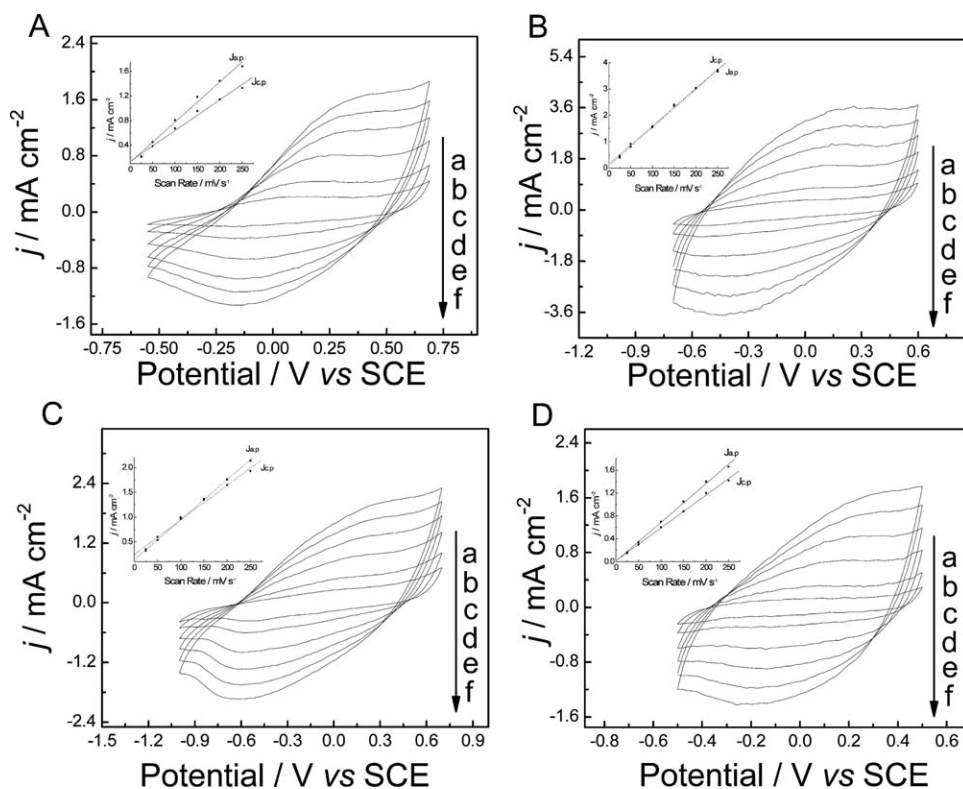


Figure 4 CVs of PEDOT/MWCNTs composite films on a Pt disc electrode in monomer-free aqueous solution at potential scan rates of (a) 25 mV s^{-1} , (b) 50 mV s^{-1} , (c) 100 mV s^{-1} , (d) 150 mV s^{-1} , (e) 200 mV s^{-1} , and (f) 250 mV s^{-1} . These PEDOT/MWCNTs composite films were synthesized electrochemically in 0.02 mol L^{-1} EDOT + 0.02 mol L^{-1} SLS + 0.02 mol L^{-1} LiClO₄ containing different MWCNTs contents aqueous micellar solutions: (A) no MWCNTs, (B) 0.1 wt % MWCNTs, (C) 0.4 wt % MWCNTs, (D) 1.2 wt % MWCNTs at a constant potential of 1.1 V vs SCE.

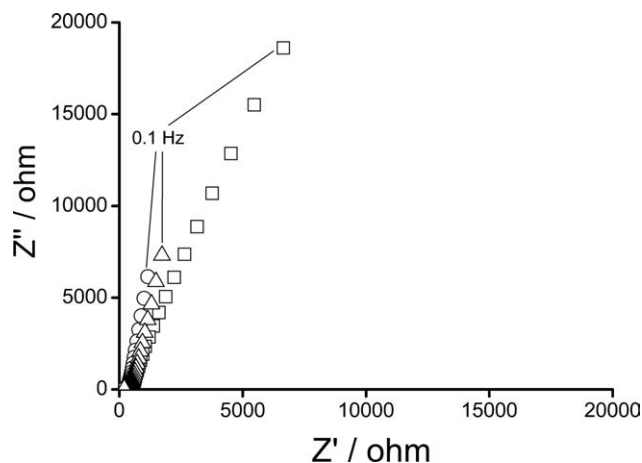


Figure 5 Impedance spectra of the PEDOT (Δ), PEDOT/MWCNTs (\circ), and PEDOT/MWCNTs/AO modified electrodes (\square) recorded in $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$ aqueous solution. The spectra were recorded at a potential of 0 V , and $\Delta E_{\text{ac}} = 10 \text{ mV}$. The frequency range was $100 \text{ kHz} - 0.1 \text{ Hz}$.

Electrochemical impedance spectroscopy of PEDOT/MWCNTs/AO composite films

EIS is an effective method for probing the features of surface-modified electrodes.³⁴ Therefore, the composite and pure PEDOT films were studied by EIS. The resulting Nyquist plots are shown in Figure 5. The shape of the impedance spectra was typical for PEDOT films in an aqueous electrolyte.³⁵ The imaginary part of impedance at low frequencies was almost perpendicular to real part, which verified a good capacitance behavior. As can be seen from the low-frequency part of the impedance spectra of the PEDOT/MWCNTs composite films and pure PEDOT films, the imaginary part of impedance was lower for the PEDOT/MWCNTs composite films when compared with the pure PEDOT films. This low-frequency region was related to the bulk redox capacitance of composite films.³⁶ The redox capacitance was estimated using the equation $C_{\text{LF}} = 1/(2\pi fZ'')$, where f is the lowest frequency used to record the spectra (0.1 Hz), and Z'' is the imaginary part of the impedance at the frequency. The calculated C_{LF} was 2.68 and 2.12 mF for PEDOT/MWCNTs composite films and pure PEDOT films, respectively. These results were in good agreement with the results obtained by CVs where the PEDOT/MWCNTs composite films showed a higher redox capacitance than pure PEDOT films. The higher redox capacitance of the composite films resulted obviously from the contribution of the embedded MWCNTs which can exert a number of effects: (1) the provision of interconnected pathways for electrons through the MWCNTs and ions through the pore network, regardless of the conductivity of the polymer; (2) the thinness of the polymer

layer on each MWCNT, minimizing barriers both to ion transfer across the polymer/electrolyte interface and ion transport within the polymer phase; (3) direct interaction between the delocalized electrons on polymer chains and the CNTs.^{37–39}

The real impedance at low frequencies, where the capacitive behavior dominates, is an indication of the combined resistance of the electrolyte and the films including both electronic and ionic contributions.^{40,41} The values of the real impedance at 0.1 Hz were given in Table II for all the three films. It can be seen that the PEDOT/MWCNTs composite was lower in resistance than its pure polymer. The MWCNTs inside the polymer matrix may lead to a faster electron transport in the bulk-films and charge transfer in the parallel PEDOT films/solution interface and MWCNTs/solution interface, compared with that in the originally single PEDOT/solution interface.⁴² This fact suggested that the presence of MWCNTs makes the composites have more active sites for faradic reactions and a larger redox capacitance than pure PEDOT films. It can also be seen that AO immobilized composite films offered much lower overall conductivity compared with the other two films. This result might be caused by the hindrance of the macromolecular structure of AO to the electron-transfer and ion-transport, and it also confirmed the successful immobilization of AO.

Surface morphologies of PEDOT/MWCNTs/AO composite films

Figure 6(a) shows SEM micrograph of the surface of the pure PEDOT films. As can be seen, PEDOT films prepared from aqueous solution were regular and smooth, presenting dense, compact and homogeneous structure, unlike the rough and porous surface obtained from organics.³³ This can be explained as a consequence of the relatively high concentration of doping anions (LS^-) of SLS can stabilize with EDOT^+ during the electropolymerization process. This may lead to morphological changes of the coating giving rise to a more regular and compact structure. But the morphology of PEDOT films changed greatly by the introduction of $0.1 \text{ wt } \%$ MWCNTs. In Figure 6(b,c), the SEM images of the PEDOT/MWCNTs composite films showed a three-

TABLE II
EIS Results for the PEDOT, PEDOT/MWCNTs and PEDOT/MWCNTs/AO Modified Electrodes

	PEDOT	PEDOT/ MWCNTs	PEDOT/ MWCNTs/AO
Z' at 0.1 Hz (Ω)	1850	1020	6545
Z'' at 0.1 Hz (Ω)	7520	5940	18,650
C_{LF} (mF)	2.12	2.68	0.85

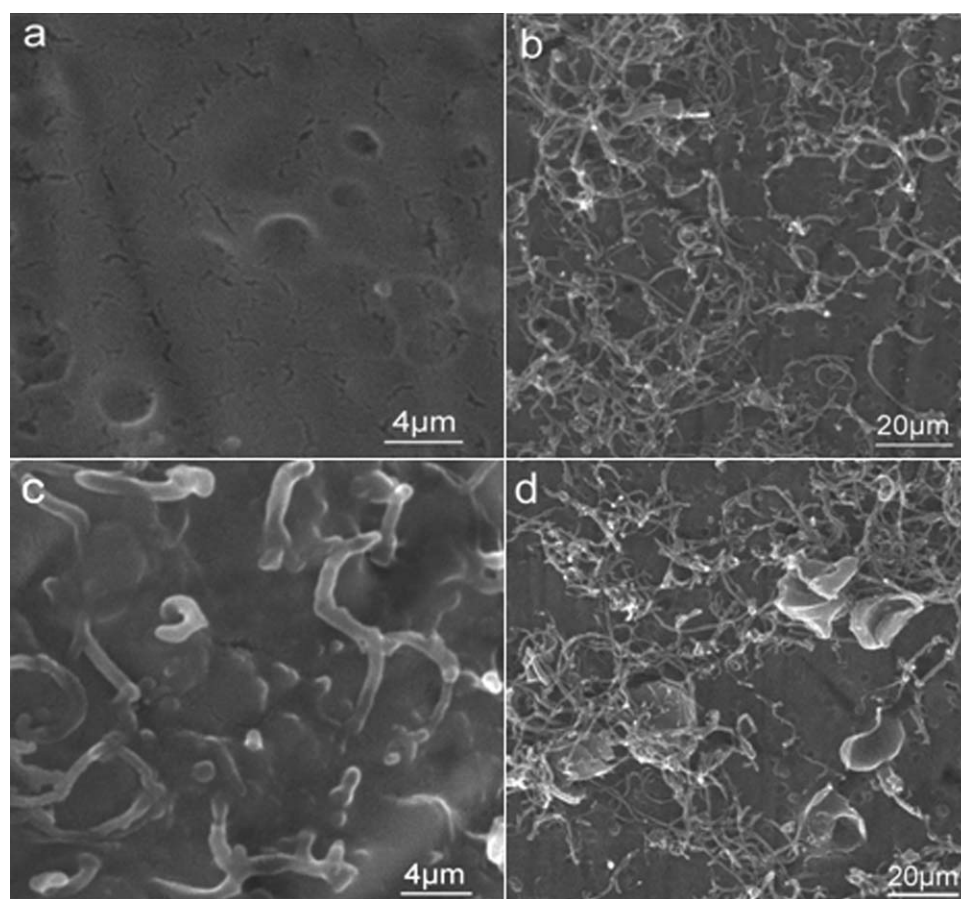


Figure 6 SEM images of the surfaces of (a) PEDOT, (magnification: 50000 \times); (b) PEDOT/MWCNTs composite films, (magnification: 10000 \times); (c) PEDOT/MWCNTs composite films, (magnification: 50000 \times); (d) PEDOT/MWCNTs/AO composite films, (magnification: 10000 \times).

dimensional network composed of interconnected fibers with similar diameters. The diameter of the MWCNTs was about 50 to 100 nm, while the fibrils in the composites were of 0.5 to 2 μm in width. This difference implied that the fibers formed may serve as condensation nuclei during the growth process and therefore they were coated with a considerable amount PEDOT to form such a fiber materials which may give rise to conductive passways and lead to high conductivity. In addition, the MWCNTs formatted three-dimensional network which was conducive to decrease the contacting chance between the PEDOT and the electrolyte, can increase more sites of the composite films for faradic reaction than pure PEDOT films. It can be then concluded that the three-dimensional network was formed with the MWCNTs serving as the backbone, thus greatly enhancing the mechanical properties of the composite films. It has been shown that incorporating MWCNTs into PEDOT improve not only its mechanical and electrical properties, but also the electrochemical performance. Figure 6(d) shows the morphology of AO immobilized PEDOT/MWCNTs composite films. The three-dimensional network

structure which is composed of interconnected MWCNTs not only provides an excellent template for the polymerization of EDOT, but also allows the immobilization of enzyme in the resulting PEDOT/MWCNTs composite during the electrochemical polymerization progress. The three-dimensional network of composite films could be beneficial to the immobilization of enzyme due to the large surface area and high absorption ability of MWCNTs.^{42–46} Therefore, This morphology and structure may improve the stability of PEDOT/MWCNTs based AO biosensors.

Catalytic activity of PEDOT/MWCNTs/AO modified electrode

AO is a multicopper enzyme that catalyzes the oxidation of AA to dehydroascorbic acid and water in the presence of oxygen. The bioelectrochemical catalytic activity of PEDOT/MWCNTs/AO modified electrode towards AA was investigated by chronoamperometry. Figure 7 shows the current-time plots of PEDOT/MWCNTs/AO electrode in 0.05 mol L^{-1} PBS containing AA with various

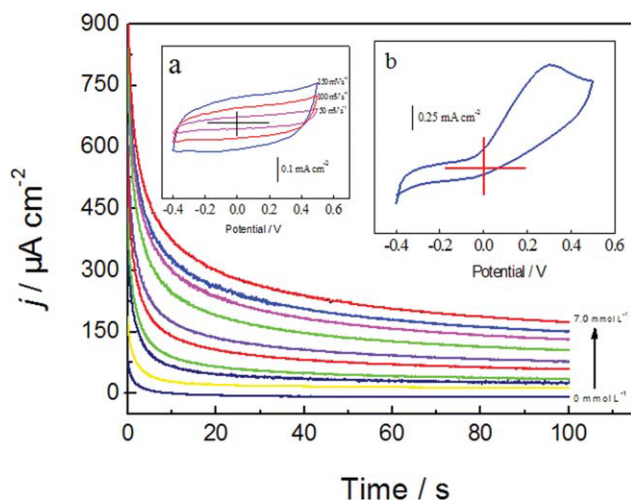


Figure 7 Current-time plots of PEDOT/MWCNTs/AO modified electrode in 0.05 mol L^{-1} PBS containing AA with various concentrations (0, 0.1, 0.5, 1, 2, 3, 4, 5, 6 and 7 mmol L^{-1}) at a detection potential of 0.4 V . Inset (a): CVs of PEDOT/MWCNTs/AO modified electrode in 0.05 mol L^{-1} PBS containing 0.02 mol L^{-1} LiClO_4 at potential scan rates of 50 mV s^{-1} , 100 mV s^{-1} and 150 mV s^{-1} ; (b) CV of PEDOT/MWCNTs/AO modified electrode in 0.05 mol L^{-1} PBS containing 0.02 mol L^{-1} LiClO_4 and 1 mmol L^{-1} AA at a potential scan rate of 100 mV s^{-1} . [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

concentrations ($0\text{--}7 \text{ mmol L}^{-1}$) at a detection potential of 0.4 V . Obviously, the response current increased with the increase of AA concentration and showed a short response time. The cyclic voltammogram of PEDOT/MWCNTs/AO modified electrode to 0.05 mol L^{-1} PBS containing 1 mmol L^{-1} AA at the scan rates of 100 mV s^{-1} was revealed in Figure 7(b). A clean oxidation peak was observed at around $+0.4 \text{ V}$, which was different from the cyclic voltammograms of PEDOT/MWCNTs/AO modified electrode to 0.05 mol L^{-1} PBS without AA. This fact also suggested that the enzyme electrode could catalyze the oxidation of AA to dehydroascorbic acid. These results indicated that the AO entrapped in PEDOT/MWCNTs composite films possesses a good bioelectrochemical catalytic activity for the oxidation of AA.

CONCLUSIONS

The PEDOT/MWCNTs composite films were synthesized successfully by electropolymerization of EDOT in aqueous solution containing different MWCNTs contents. AO has been successfully immobilized in the PEDOT/MWCNTs composite films using the one-step electrochemical polymerization method. Both CV and EIS studies revealed obvious improvement in the electrochemical response of PEDOT matrix after the incorporation of MWCNTs. Moreover, the PEDOT/MWCNTs/AO modified

electrode was used to determine AA successfully and showed good bioelectrochemical catalytic activity. Hence, our work proved that PEDOT/MWCNTs composite material is a promising enzyme immobilization platform for the fabrication of enzyme-based biosensor.

References

1. Ghindilis, A. L.; Atanasov, P.; Wilkins, E. *Electroanalysis* 1997, 9, 661.
2. Manza, L. L.; Stamer, S. L.; Ham, A. J. L.; Codreanu, S. G. C. *Proteomics* 2005, 5, 1742.
3. Wang, X.; Watanabe, H.; Uchiyama, S. *Talanta* 2008, 74, 1681.
4. Wei, L. M.; Zhang, W.; Lu, H. J.; Yang, P. Y. *Talanta* 2010, 80, 1298.
5. Tan, Y. M.; Deng, W. F.; Chen, C.; Xie, Q. J.; Lei, L. H. *Biosens Bioelectron* 2010, 25, 2644.
6. Bartlett, P. N.; Birkin, P. R. *Synth Met* 1993, 61, 15.
7. Nie, G. M.; Qu, L. G.; Xu, J. K.; Zhang, S. S. *Electrochim Acta* 2008, 53, 8531.
8. Bobacka, J.; Lewenstam, A.; Ivaska, A. *J Electroanal Chem* 2000, 489, 17.
9. Wang, H. Y.; Mu, S. L. *J Electroanal Chem* 1997, 436, 43.
10. Uchiyama, S.; Hasebe, Y.; Tanaka, M. *Electroanalysis* 1997, 9, 176.
11. Wen, Y. P.; Lu, B. Y.; Yue, R. R.; He, H. H.; Xu, J. K. *Talanta*, Submitted.
12. Iijima, S. *Nature* 1991, 354, 56.
13. Balasubramanian, K.; Burghard, M. *Anal Bioanal Chem* 2006, 385, 452.
14. Ahammad, A. J. S.; Lee, J. J.; Rahman, M. A. *Sensors* 2009, 9, 2289.
15. Tsai, Y. C.; Li, S. C.; Liao, S. W. *Biosens Bioelectron* 2006, 22, 495.
16. Huang, J.; Yang, Y.; Shi, H.; Song, Z.; Anzai, J.; Osa, T.; Chen, Q. *Mater Sci Eng C* 2006, 26, 113.
17. Liu, G.; Lin, Y. *Electrochem Commun* 2006, 8, 251.
18. Schadler, L. S.; Giannaris, S. C.; Ajayan, P. M. *Appl Phys Lett* 1998, 73, 3842.
19. Lota, K.; Khomenko, V.; Frachowiak, E. *J Phys Chem Solids* 2004, 65, 295.
20. Wu, T. M.; Lin, Y. W. *Polymer* 2006, 47, 3576.
21. Wang, S. G.; Zhang, Q.; Wang, R.; Yoon, S. F.; Ahn, J.; Yang, D. J.; Tian, J. Z.; Li, J. Q.; Zhou, Q. *Electrochem Commun* 2003, 5, 800.
22. Gómez, J. M.; Romero, M. D.; Fernández, T. M. *Catal Lett* 2005, 101, 275.
23. Wen, Y. P.; Xu, J. K.; He, H. H.; Lu, B. Y.; Li, Y. Z.; Dong, B. *J Electroanal Chem* 2009, 634, 49.
24. Downard, A. J.; Pletcher, D. *J Electroanal Chem* 1986, 206, 139.
25. Nofle, R. E.; Pletcher, D. *J Electroanal Chem* 1987, 227, 229.
26. Randriamahazaka, H.; Noel, V. *J Electroanal Chem* 1999, 472, 103.
27. Peng, C.; Jin, J.; Chen, G. Z. *Electrochim Acta* 2007, 53, 525.
28. Agüí, L.; Farfal, C. P.; Sedeño, P. Y.; Pingarrón, J. M. *Electrochim Acta* 2007, 52, 7946.
29. Randriamahazaka, H.; Noel, V.; Chevrot, C. *J Electroanal Chem* 1999, 472, 103.
30. Venables, J. A.; Spiller, G. D. T.; Hanbucken, M. *Rep Prog Phys* 1984, 47, 399.
31. Zhang, S. S.; Hou, J.; Zhang, R.; Xu, J. K.; Nie, G. M.; Pu, S. Z. *Eur Polym Mater* 2006, 42, 149.
32. Du, X.; Wang, Z. *Electrochim Acta* 2003, 48, 1713.
33. Sakmeche, N.; Aeiyaich, S.; Aaron, J. J.; Jouini, M.; Lacroix, J. C.; Lacaze, P. C. *Langmuir* 1999, 15, 2566.
34. Zhao, H. T.; Ju, H. X. *Anal Biochem* 2006, 350, 138.

35. Bobacka, J.; Lewenstam, A.; Ivaska, A. *J Electroanal Chem* 2000, 489, 17.
36. Mousave, Z.; Bobacka, J.; Lewenstam, A.; Ivaska, A. *J Electroanal Chem* 2009, 633, 246.
37. Chen, G. Z.; Shaffer, M. S. P.; Coleby, D.; Dixon, G.; Zhou, W.; Fray, D. J.; Windle, A. H. *Adv Mater* 2000, 12, 522.
38. Wu, M.; Snook, G. A.; Gupta, V.; Shaffer, M.; Fray, D. J.; Chen, G. Z. *J Mater Chem* 2005, 15, 2297.
39. Peng, C.; Snook, G. A.; Fray, D. J.; Shaffer, M. S. P.; Chen, G. Z. *Chem Commun* 2006, 44, 4629.
40. Albery, W. J.; Chen, Z.; Horrocks, B. R.; Mount, A. R.; Wilson, P. J.; Bloor, D.; Monkman, A. T.; Elliott, C. M. *Faraday Discuss* 1989, 88, 247.
41. Chen, Z. PhD Thesis. University of London Imperial College: London, 1992.
42. Guo, D. J.; Li, H. L. *J Solid State Electrochem* 2005, 9, 445.
43. Nau, V.; Nieman, T. A. *Anal Chem* 1979, 51, 424.
44. Ying, L.; Kang, E. T.; Neoh, K. G. *J Membr Sci* 2002, 208, 361.
45. Zheng, H.; Xue, H. G.; Zhang, Y. F.; Shen, Z. Q. *Biosens Bioelectron* 2002, 17, 541.
46. Rubtsova, M. Y.; Kovba, G. V.; Egorov, A. M. *Biosens Bioelectron* 1998, 13, 75.