Electrosynthesis of polyaniline–mutilwalled carbon nanotube nanocomposite films in the presence of sodium dodecyl sulfate for glucose biosensing

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Abstract
Polyaniline–mutilwalled carbon nanotube (PANI–MWCNT) nanocomposites were electropolymerized in the presence of sodium dodecyl sulfate (SDS) onto interdigitated platinum-film planar microelectrodes (IDµE). The MWCNTs were first dispersed in SDS solution then mixed with aniline and H₂SO₄. This mixture was used to electro-synthesize PANI–MWCNT films with potentiostatic method at E = +0.90 V (versus SCE). The PANI–MWCNT films were characterized by cyclic voltammetry (CV) and scanning electron microscopy (SEM). The results show that the PANI–MWCNT films have a high electroactivity, and a porous and branched structure that can increase the specific surface area for biosensing application. In this work the PANI–MWCNT films were applied for covalent immobilization of glucose oxidase (GOx) via glutaraldehyde agent. The GOx/PANI–MWCNT/IDµE was studied using cyclic voltammetric and chronoamperometric techniques. The effect of several interferences, such as ascorbic acid (AA), uric acid (UA), and acetaminophen (AAP) on the glucosensing at +0.6 V (versus SCE) is not significant. The time required to reach 95% of the maximum steady-state current was less than 5 s. A linear range of the calibration curve for the glucose concentration lies between 1 and 12 mM which is a suitable level in the human body.

Keywords: polyaniline, carbon nanotubes, sodium dodecyl sulfate, glucose biosensing

Classification numbers: 2.04, 5.10, 5.11, 5.14, 6.09, 6.12

1. Introduction
Polyaniline (PANi) is one of the most intensively investigated conducting polymers due to its easy synthesis, well-behaved electrochemistry, good environmental stability, high conductivity, and strong biomolecular interactions [1, 2]. However, PANi expresses its conducting state only when it is in acidic medium, pH < 3.0 (emeraldine salt form) [3]. This pH condition is unfavorable for a biological system because most bioassays must be performed in neutral or slightly acidic conditions [2, 4]. Recent experimental studies have shown that the incorporation of carbon nanotubes (CNTs) into...
PANI matrix may significantly promote their mechanical and electrical conductivity both in reduced and neutral states [5]. Furthermore, PANi–CNTs composite has also been found to improve electrocatalytic activation of the redox enzymes via enhancing electrochemical transduction of biochemical processes [6, 7].

However, a major drawback is that CNTs are difficult to process, and they are insoluble in most solvents, which has limited the distribution of CNTs in the PANi matrix. These difficulties can be overcome using various surfactants such as sodium dodecyl benzenesulfonate (SDBS), dodecyltrimethyl-ammonium bromide (DTAB), hexadecyltrimethylammonium bromide (CTAB), octyl phenol ethoxylate (Triton X-100) and sodium dodecyl sulfate (SDS) [8]. These surfactants have been used not only for the dispersion of CNTs but also as a dopant for the synthesis process. Anionic surfactant SDS is widely used for this purpose [9–11].

In this study we synthesized the polyaniline–multiwalled carbon nanotubes (PANI–MWCNTs) nanocomposite in the presence of SDS [9, 12] on interdigitated platinum-film planar microelectrodes (IDµE) by potentiostatic technique. The electrochemical glucose sensor based on PANi–MWCNTs is also described.

2. Experimental

2.1. Chemicals and reagents

MWCNTs (with >95% purity) were purchased from Shenzhen Nanotech Port (China). Glucose oxidase (GOx) EC 1.1.3.4 (from Aspergillus niger), 25% glutaraldehyde and SDS were used as received (Sigma Aldrich). Aniline (Merck) was distilled under reduced pressure and stored below 0 °C before use. β-d-(-)-glucose, H₂SO₄, KCl, KH₂PO₄, K₂HPO₄ and other reagents were of analytical grade and used without further purification. The glucose stock solution was allowed to mutarotate for 24 h in order to reach the equilibrium of α- and β- anomers at room temperature prior to use. Phosphate buffered saline (PBS, 20 mM KH₂PO₄ + 20 mM K₂HPO₄ + 0.1 M KCl, pH 6.8) was used as supporting electrolytes. All of the solutions were prepared with doubly distilled water.

2.2. Apparatus

Electrochemical measurements were performed on an Autolab PGSTAT12 Electrochemical Analyser (EcoChemie, Netherlands) under the control of GPES version 4.9. The experiments were carried out using a conventional three-electrode system with interdigitated planar platinum-film microelectrodes (IDµE) as the working electrode, a Pt wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. Field-emission scanning electron microscope (FE-SEM) images of the films were recorded with Hitachi S-4800 (Japan).

2.3. Fabrication of interdigitated platinum-film planar microelectrodes

The interdigitated platinum-film planar microelectrodes (IDµE) were fabricated on a silicon wafer using a photolithographic metal lift-off process. Two pairs of the interdigitated electrodes were prepared by sputtering 50 nm Cr and 200 nm Pt on a layer of silicon dioxide (SiO₂) with a thickness of around 100 nm by means of dry thermal oxidation on top of the wafer. The microelectrode digit width was of 50 µm and the gap between them was 20 µm, and their length was around 1 mm (figure 1).

2.4. Electropolymerization of PANi–MWCNT films

Two milligrams of MWCNTs were dispersed in 0.1 mM of SDS aqueous solution and sonicated for 1 h [8, 13]. Then aniline monomer solution (0.01 M of aniline in 0.1 M of H₂SO₄) was dissolved in SDS and MWCNTs solution under ultrasonic stirring for 15 min at room temperature. The PANi–MWCNTs modified IDµE was fabricated by the potentiostatic method at a constant potential of +0.9 V versus SCE. After polymerization the electrode was rinsed with distilled water and placed into a PBS solution. For comparison, a PANi/IDµE was prepared under the same conditions.
2.5. Immobilization of glucose oxidase

Fresh solutions of glucose oxidase (GOx) were prepared in PBS and stored at 4 °C. The immobilization of GOx on the surface of the PANi–MWCNTs film was achieved by cross-linking through the glutaraldehyde agent. Firstly, the PANi–MWCNTs electrodes were placed in saturated glutaraldehyde vapor for 30 min then dried in air for 15 min at room temperature. Next, 8 µl of the GOx solution (2 U µl⁻¹) was spread on the PANi–MWCNTs surface using a drop method. The product, the formed GOx/PANi–MWCNT/Pt electrode, was rinsed thoroughly with PBS solution and stored at 4 °C when not in use.

3. Results and discussion

3.1. Voltammetric behavior of the PANi–MWCNTs electrode

Cyclic voltammetry (CV) was used to characterize the redox properties of PANi–MWCNTs modified IDµE. The cyclic voltammograms obtained for both PANi and PANi–MWCNTs composite electrodes at a scan rate of 50 mV s⁻¹ in the potential range of −0.20 to +1.0 V are shown in figure 2.

The current density of PANi–MWCNTs electrode is larger than that of the PANi electrode. Three identifiable anodic peaks with cathodic counterparts are observed for the PANi–MWCNTs electrode but not clearly for the PANi electrode. Here MWCNTs played an important role in increasing electroactivity and/or its specific surface area of the electrode. It significantly improves the overall biosensor performance.

3.2. Morphology

Figures 3(A) and (C) show the low magnification scanning electron micrographs obtained for PANi–MWCNTs and GOx/PANi–MWCNTs films on IDµE, respectively. The SEM image of PANi–MWCNTs shows spongy and porous morphology of polyaniline. The high magnification SEM of the PANi–MWCNT sample (figure 3(B)) indicates that carbon nanotubes dispersed in the polymer matrix and formed the homogeneous nanocomposites. It can be helpful in the entrapment of enzyme molecules. When GOx was added and allowed to covalently attach to PANi–MWCNT surface, the films became thicker and constituted a gel configuration (figure 3(D)). These small globules indicate the presence of enzyme (GOx) onto PANi–MWCNT surface.

3.3. Cyclic voltammograms of glucose on GOx/PANI–MWCNT/IDµE

Figure 4 shows the cyclic voltammograms (CV) of GOx/PANI–MWCNT/IDµE in the absence (curve a) and in the presence (curves b and c) of glucose in the PBS solution. The range of potential was from 0.0 to +0.8 V at a scan rate of 50 mV s⁻¹.

The CV curves of the electrodes corresponding to the addition of different glucose concentrations show an increase in anodic current when the potential varied from +0.2 to +0.8 V in comparison to that without glucose addition. It is worth noting that there are no changes in shape and position of the cyclic voltammograms when glucose concentrations were added. Here GOx has been successfully immobilized onto the
3.4. Selection of the applied potential

As can be seen in figure 4, the large response current was obtained within the range of +0.2 to +0.8 V. However, at high potential there may be interferences from other oxidable species such as ascorbic acid (AA), uric acid (UA), and acetaminophen (AAP). They commonly present in physiological samples of glucose and cause problems in the accuracy of the glucose determination. In this study the effect of the applied potential on amperometric response for glucose sensor was investigated.

The amperometric responses of AA, UA, AAP, and glucose (1 mM) in the potential range of +0.2 to +0.8 V are shown in figure 5. Commonly, the susceptibility to interference species is positively correlated to the potential of the sensor. As a sequence, the lower potential results in the lower susceptibility and the increase of the sensor selectivity. When the applied potential is higher, the response current increases, however, it is strongly influenced by the interferences. When the applied potential increased from +0.6 to +0.7 V, the sensor currents of AA, UA, and AAP increased from 4.7 to 8.8, 3.6 to 8.89, and 1.9 to 4.8 µA, respectively.

The errors in determination of 1 mM glucose at a potential of +0.6 V in solution caused by 1 mM AA, 1 mM UA, and 1 mM AAP are 22.38, 17.14 and 8.81%, respectively. Compared to the normal physiological levels that have been detected from blood samples, which are 0.13 mM for AA, 0.3 mM UA, and 0.125 mM for AAP [15], the biosensor exhibits essentially no response to such interfering species. Thus, the value of +0.6 V was chosen as applied potential of the GOx/PANI–MWCNT sensor.

3.5. Amperometric determination of glucose at GOx/PANI–MWCNT/IDµE

Figure 6 shows amperometric response at the GOx/PANI–MWCNT/IDµE in PBS at +0.6 V (versus SCE) for the successive addition 1 mM of glucose.

The bioelectrode exhibits a rapid and sensitive current response to each injection of glucose concentration. The time required to reach 95% of the maximum steady-state current was less than 5 s. The plot of current versus glucose concentration (inset, figure 3(C)) depicted a linear relationship over the range of glucose (1–12 mM) until saturation current at a high glucose-concentration (>12 mM). The linear regression equation was \( I (\mu A) = 1.07 \times C_{glucose} (mM) + 20.06 \) with the correlation coefficient of 0.99039. This linear range is clinically sufficient for monitoring of the glucose levels in normal human blood, 4.4–6.6 mM [16].

4. Conclusion

PANI–MWCNTs nanocomposites were successfully synthesized in the presence of SDS. SDS acts as an agent not only to improve the solubility of aniline monomer but also to gain the well-dispersion of MWCNTs, and as counter ions for PANi. FE-SEM images confirm that the uniform coating of PANi–MWCNTs has been deposited onto IDµE surface. The porous property of PANi–MWCNTs was used as an immobilization matrix and a transducer for glucose biosensing. It was found that the resulting glucose biosensor exhibited a fast response (<5 s) in a wide linear range. These features provide scope for utilizing the methodology.
proposed in the present study to immobilize other biomolecules in the process of fabricating novel biosensors.

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