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Cauliflower polyaniline/multiwalled carbon nanotube electrode and its applications to hydrogen peroxide and glucose detection*

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Abstract: Vertically aligned polyaniline (PANI) structures were prepared by controlling the deposition current density during a stepwise template-free electrochemical deposition process of aniline on a glassy carbon electrode (GCE). Scanning electron micrographs (SEMs) showed the formation of cauliflower PANI structures, each with a diameter of approximately 2–3 and 10 μm in length. The cauliflower-like PANI electrode was modified with multiwalled carbon nanotubes (cauliflower PANI/MWCNTs) and used as the working electrode for electrochemical detections where H_2O_2 and glucose were used as the models for the chemical sensor and biosensor, respectively. The sensor provided linearity in the range of 1.0 to 150 μM of H_2O_2 with the limit of detection (LOD) of 50 nM. This is 100-fold better than the LOD of the bare GCE. Moreover, this sensor exhibited remarkable operational stability, i.e., 50 μM H_2O_2 could be analyzed up to 140 times with a 2.7 % relative standard deviation (RSD). A glucose biosensor was prepared using the modified cauliflower PANI/MWCNT electrode. This had a 3.4 times higher sensitivity than an electrode modified with PANI film/MWCNTs. The regular size and high surface-to-volume ratio of the cauliflower PANI electrode will provide good opportunities for further biosensor applications.

Keywords: electrodes; glucose biosensors; hydrogen peroxide; multiwalled carbon nanotubes; nanostructured materials; oriented polyaniline; polymerization; sensors; template-free electropolymerization.

INTRODUCTION

Organic conducting polymers have emerged as promising materials in the development of chemical sensors, biosensors, and electronic devices owing to the considerable flexibility in their chemical structures in preparation, high surface areas, chemical selectivity, and their redox characteristics [1,2]. There are two methods for the preparation of conductive polymers. The first is classical chemical polymerization,

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which is generally applicable for all polymers. The second is electrochemical polymerization, which is an alternative method for the synthesis of conductive polymers because of their conductivity [3]. The electrochemical polymerization offers many advantages, including control of their thickness and morphology, good reproducibility, and a uniformity of the polymer film on electrode surfaces with more complex geometries based on the applied polymerization parameters [4]. Unlike conventional casting, the method has no limits in terms of geometry and area of the electrode surface [5].

Among the various conducting polymers, polyaniline (PANI) has received special recognition owing to its good stability, good conductivity, and excellent biocompatibility. PANI has been found to be an interesting material for sensor and biosensor interfaces since it acts as an effective mediator for electron transfer in redox or enzymatic reactions [6]. Furthermore, it is a suitable material for immobilization of biomolecules because its amine functional group can be utilized as a matrix for the cross-linking of various biomolecules [7]. In the past few years, nano- and submicron-scale structures of PANI have been developed in the form of rods [8], wires [9], fibers [10], and tubes [11] because they combine the advantages of organic conductors with low-dimensional systems. Electroactive and chemically active PANI with oriented structures have become an important class of active electrochemical materials [6]. Their high density and uniformity associated with aligned open nanostructures and orientations can usually establish a high capacity and high efficiency for the array devices. Their high porosity also allows a fast diffusion of molecules into the sensor structures that provides a higher sensitivity and faster response than non-oriented nanostructures [12].

Another important group of nanomaterials with attractive electronic, chemical, and mechanical properties is the multiwalled carbon nanotubes (MWCNTs). The high surface area and hollow geometry, combined with electronic conductivity and mechanical properties, help to promote electron-transfer reactions when MWCNTs are fabricated on electrodes for electrochemical reactions [13,14]. These MWCNT-modified electrodes have been shown to improve the sensitivity of electrocatalytic oxidation of various analytes such as bisphenol A [15], reduced nicotinamide adenine dinucleotide (NADH) [16], methanol [17], insulin [18,19], or other biomolecules [20]. In addition, the ability of MWCNTs to promote the electron-transfer reaction of H_2O_2 indicates great promise for oxidase-based amperometric biosensors. A highly sensitive, rapid, and reliable determination for H_2O_2 is practically important for many applications in different fields of food, pharmaceutical, environmental, industry, or clinical with a particular emphasis on biosensors based on oxidase enzymes [21,22].

This article reports the fabrication of an oriented structure of PANI to serve as a suitable matrix for enzyme immobilization. The PANI electrode was applied together with MWCNTs to take advantage of the catalytic activities of MWCNTs to act as an electrochemical sensor to detect H_2O_2. The usefulness of these polymer structures together with the MWCNTs is demonstrated with a chemical sensor for H_2O_2, the detection of which is widely investigated by biosensors. Finally, a glucose biosensor was studied to show its potential application to a wide range of oxidase enzyme biosensors.

EXPERIMENTAL

Apparatus

The electrodeposition of vertically aligned PANI on an electrode surface and its electrochemical studies were carried out using a μ-Autolab type III (Metrohm Autolab, B.V., The Netherlands) connected to a personal computer and driven by GPES 4.9 software (Eco Chemie, The Netherlands). An electrochemical cell composed of a working electrode, a glassy carbon electrode (GCE) (diameter = 2.0 mm) with or without surface modification, a Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode. All electrodes were held in a custom-built flow cell (ca. 10 μL). Scanning electron microscope (SEM) images were achieved with the JSM-5200 (JEOL, Japan) using an acceleration potential of 20 kV.

A flow-injection system was employed for the analysis of H_2O_2 and glucose. A peristaltic pump (0.5 mL min^{-1}) (Miniplus 2, Gilson, France) was used to deliver the carrier solution, which was 100 mM phosphate buffer solution (PBS) pH 6.0 containing 0.1 M KCl. A six-port valve (Valco, Houston, TX, USA) was used as the sample injector to control the exact sample volume (200 µL).

Materials and chemicals

All solutions were prepared with deionized water that was treated by a reverse osmosis system and purified with a Maxima ultrapure water instrument to obtain a resistivity of 18.2 MΩ (ELGA, England). H_2O_2 30 % was from Merck (Demstadt, Germany). Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4), D-glucose, and aniline (≥99.5 %) were from Sigma-Aldrich (Seelze, Germany). Aniline was distilled under reduced pressure prior to use. MWCNTs (95 % purity, 5–15 µm length, 40–60 nm i.d.) were kindly provided by Shenzhen Nanotech Port Co., Ltd. (Nanshan, Shenzhen, China). They were first sonicated for 12 h in 2.0 M nitric acid. All other chemicals and reagents were analytical grade.

Preparation of vertically aligned PANI-modified GCE

The experiment was carried out using 0.5 M aniline solution in 1.0 M perchloric acid with a GCE as the working electrode. The growth-oriented cauliflower PANI based on a template-free electrochemical method was obtained by separating the nucleation and the growth steps of the polymer. In the first step, a high current density was used to generate the necessary nucleation centers on the substrate surfaces. The current density was then reduced in two steps for the polymer to grow from the nucleation sites [23]. A current density of 0.08 mA cm^{-2} was applied for 0.5 h to create the nucleations. This was followed by 0.04 mA cm^{-2} for 3.0 h and another 3.0 h at 0.02 mA cm^{-2} for the growth of the polymer [24]. For comparison purposes, a similar surface was also prepared by applying a constant current density at 0.08 mA cm^{-2} for 3.0 h.

Fabrication of the hydrogen peroxide sensor

The cauliflower PANI electrode was used to support MWCNTs that functioned as the electroactive ingredient for H_2O_2 detection. The MWCNTs modified on both the cauliflower PANI electrode and the bare GCE were prepared by casting 3.0 µL of 1.0 % *w/v* MWCNTs in 0.05 % nafion on either the cauliflower PANI surface or the bare GCE. Before use, the modified electrode was cleaned in 100 mM PBS containing 0.1 M KCl by cycling the potential from 0 to +1.0 V at 50 mV s^{-1} for 20 cycles.

Fabrication of the glucose biosensor

Glucose oxidase was immobilized on the cauliflower PANI electrode by covalent binding using glutaraldehyde. First 10.0 µL of 0.1 % glutaraldehyde was spread onto the cauliflower PANI/MWCNT electrode, allowed to dry and any unbound glutaraldehyde molecules were removed by rinsing with deionized water. Then 10.0 µL of a 500 unit mL^{-1} glucose oxidase solution was cast on the electrode and kept for 4 h. The enzyme electrode was then electrochemically cleaned in PBS by cycling the potential between 0 and +1.0 V (20 cycles) and kept in PBS buffer at 4 °C until use. To compare the detection sensitivity, a glucose oxidase-modified PANI film/MWCNT electrode was also prepared by the same procedures.

RESULTS AND DISCUSSION

Scanning electron micrographs

Figures 1A and B show the SEM images of the GCE modified with PANI obtained by the stepwise current density electrodeposition. A large surface area with oriented PANI and a cauliflower-like structure was observed on the GCE surface. The cauliflower-shaped PANI had an approximate diameter of 2–3 μm and was 10 μm in length. This cauliflower PANI formation was obtained through controlled nucleation and growth during a stepwise electrochemical deposition process. This was similar to those reported earlier [23,24] where a large number of nuclei were first deposited on the substrate using a large current density. After the initial nucleation when the current density was reduced, horizontal and vertical growth occurred in combination to form a compact layer containing small vertical polymer rods. Lastly, growth occurred only in the vertical direction, and extended the polymer nodules to form rods. The resulting cauliflower PANI-modified GCE had an oriented and large surface area, which could make it very useful in the development of sensors and biosensors. In contrast, the morphology of the PANI obtained from applying a constant current density for an extended period was shown to have a film-like structure (Figs. 1C,D).

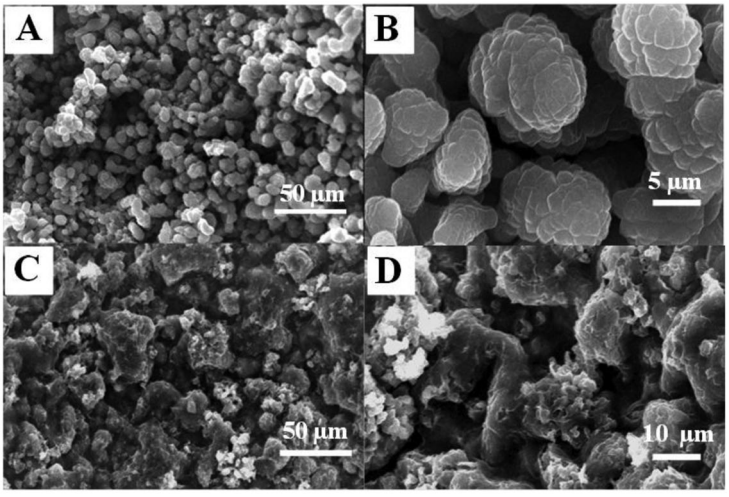

Fig. 1 SEM images of (A), (B) cauliflower PANI and (C), (D) PANI film modified on a GCE; accelerating voltage: 20 kV.

Electroactivity of modified electrode toward hydrogen peroxide

Cyclic voltammetric (CV) measurements were performed to evaluate the electroactivity of the modified surfaces toward the oxidation of H_2O_2. Figure 2 displays CVs at the various electrode surfaces in the absence and presence of 0.1 and 0.2 mM H_2O_2 (dotted, dashed, and solid lines, respectively). With the bare GCE (Fig. 1A), the anodic current of 0.1 and 0.2 mM H_2O_2 were very low when compared with the other three electrodes. The cauliflower PANI electrode had a higher response for H_2O_2 (Fig. 1B). However, the response was still lower than the GCE electrode modified with MWCNTs where a significant increase in the peak currents at potentials of H_2O_2 oxidation was observed (Fig. 1C). The highest response for H_2O_2 detection was from the cauliflower PANI/MWCNTs (Fig. 1D). Such an improved response is attributed to higher electroactive area effects. In this work, +0.6 V was selected as a detection potential for further measurements with all electrodes.

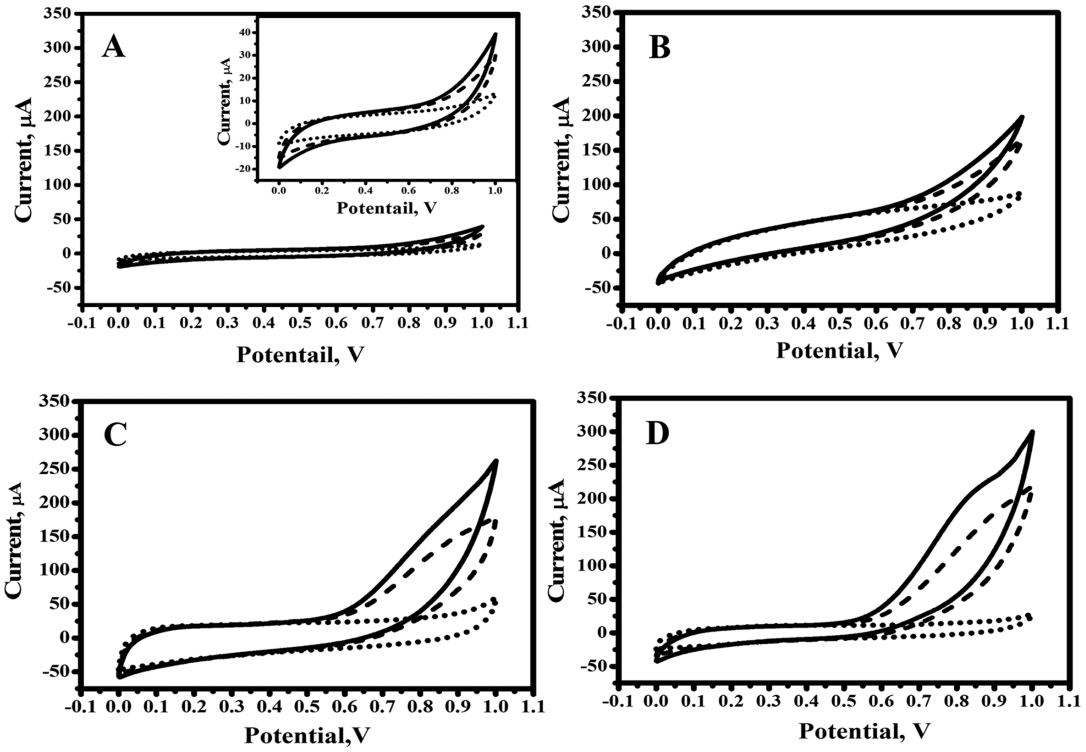

Fig. 2 CVs recorded for the oxidation of 0.1 mM (dash line) and 0.2 mM (solid line) H_2O_2 at (A) a bare GCE, (B) a cauliflower PANI electrode (C) a MWCNT electrode and (D) a cauliflower PANI/MWCNT electrode, dotted lines are for the blank (100 mM PBS, pH 6.0 containing 0.1 M KCl).

Flow-injection analysis of hydrogen peroxide

To investigate the impact of the fabricated material on the H_2O_2 sensor, H_2O_2 standard solutions were injected into the flow-injection system. Figure 3A shows the amperometric responses of cauliflower

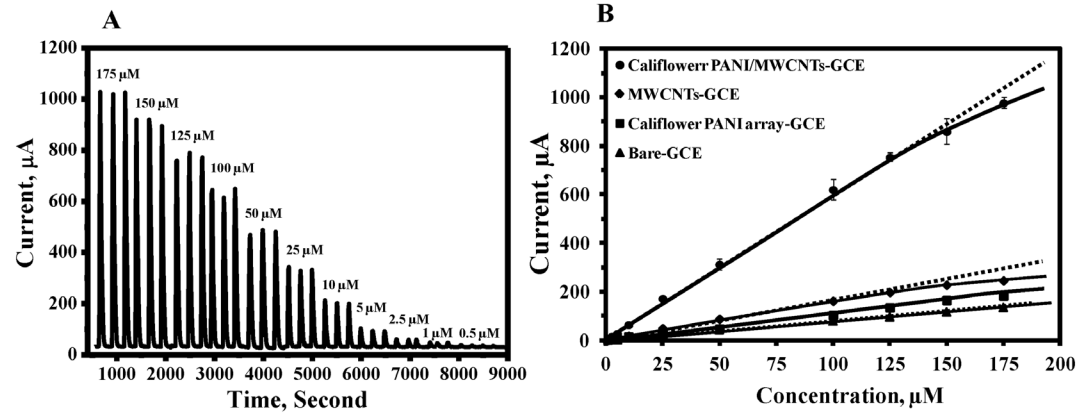

Fig. 3 (A) Amperograms obtained at cauliflower PANI/MWCNTs modified on GCE for the various concentration of H_2O_2 in 100 mM PBS pH 6 containing 0.1 M KCl; flow rate: 0.5 mL min^{-1}; sample volume 200 µL; potential: +0.6 V. (B) Calibration curve of various working electrodes to H_2O_2 at +0.6 V detection potential.

PANI/MWCNT electrode from 1.0 up to 175 μM H_2O_2 in 100 mM PBS pH 6.0 containing 0.1 M KCl. The response increases linearly with the concentration as indicated from the corresponding calibration plots (Fig. 3B). The performances of the various modified electrodes are shown in Table 1. The cauliflower PANI/MWCNT electrode gave a 7.2 times higher sensitivity (slope of the calibration plot) and a 100 times lower limit of detection (LOD) than by the bare GCE.

Table 1 Comparison of analytical performances of bare GCE, cauliflower PANI-GCE, MWCNT-GCE, and cauliflower PANI/MWCNT-GCE.

Type of electrode	Linear dynamic range (μM)	LOD (nM)	Sensitivity (μA μM^{-1})	Linear equation
Bare GCE	25–175	5000	0.88 ± 0.02	y = (0.88 ± 0.02)x + (1.6 ± 0.4); R^2 = 0.997
Cauliflower PANI-GCE	25–175	100	0.92 ± 0.05	y = (0.92 ± 0.05)x + (4 ± 1); R^2 = 0.994
MWCNT-GCE	0.1–150	50	1.79 ± 0.02	y = (1.79 ± 0.02)x + (0.8 ± 0.5); R^2 = 0.996
Cauliflower PANI/ MWCNT-GCE	1.0–150	50	6.4 ± 0.1	y = (6.4 ± 0.1)x + (1 ± 3); R^2 = 0.997

The high operational stability of the H_2O_2 amperometric determinations was another attractive feature of the cauliflower PANI/MWCNT electrode. The sensor was used to repeatedly measure 50 μM H_2O_2 in 100 mM PBS pH 6.0 containing 0.1 M KCl. Peak heights obtained from each injection were converted into a percentage response based on the 100 % response of the first injection. The first 140 analysis cycles yielded a 94 ± 3 % consistency with a relative standard deviation (RSD) = 2.7 % (Fig. 4). With this injection numbers, the responses are within ±10 % of the signal from the first injection.

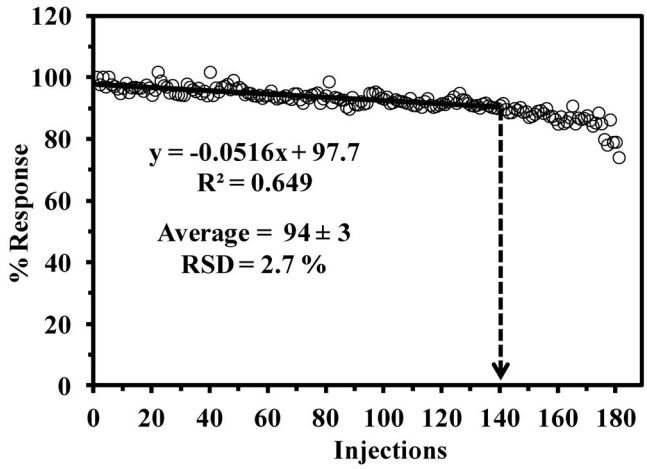

Fig. 4 The stability of cauliflower PANI/MWCNT-modified GCE electrode for the detection of 50 μM H_2O_2 in 100 mM PBS pH 6 containing 0.1 M KCl; E = +0.6 V; flow rate: 0.5 mL min^{-1}; sample volume 200 μL. The linear equation and % RSD were obtained from the first 140 injections.

To test the reproducibility of the electrode responses, three preparations of cauliflower PANI electrode modified with MWCNTs were used to analyze H_2O_2 at 10.0, 20.0, 30.0, 40.0, and 50.0 μM. The

slopes of the three electrodes were 5.9 ± 0.2 μA μM^{-1}, 6.1 ± 0.3 μA μM^{-1}, and 6.3 ± 0.4 μA μM^{-1}, respectively. To confirm that the differences between each preparation of the electrode were not significantly different, the slopes of the regression lines obtained from the three electrodes were tested using two-way ANOVA (analysis of variance) [25]. The results indicated that each preparation of the modified electrode provided good reproducibility ($P > 0.05$).

Potential application for glucose biosensor

The highly sensitive detection of H_2O_2 using the cauliflower PANI/MWCNT electrode indicated that this might be an ideal electrode to test for applications as a biosensor for oxidase-type enzyme applications. A glucose biosensor was prepared and tested in a flow-injection system. Figure 5 shows the calibration curves obtained from the amperometric detection of glucose at a detection potential of +0.6 V using glucose oxidase immobilized on cauliflower PANI/MWCNT electrode compared to the PANI film/MWCNT electrode. The enzyme electrode responded rapidly to glucose injection (analysis time = 3 min). The current changes led to a highly linear response to glucose concentrations of between 0.05 and 100 mM with a detection limit of 10.0 μM (S/N ≥ 3). The corresponding calibration plot has a slope of 4.73 ± 0.09 μA mM^{-1} which is 3.4 times higher than the PANI film/MWCNTs (1.39 ± 0.03 μA mM^{-1}). The high sensitivity of this biosensor is probably due to the open structure on the electrode surface, as the cauliflower-like formation PANI can be in contact with more enzyme molecules and thus be more efficient in assisting electron transfer from the enzyme to the electrode surface. Compared to some other glucose biosensors based on PANI or MWCNTs as one of the components using a matrix for enzyme immobilization (chitosan-coupled CNTs on PANI, a modified gold electrode [26], PANI-grafted MWCNTs/perfluorosulfonate ionomer-silica nanocomposite [27], sulfonated-PANI network grafted MWCNTs [28], CNTs coated with PANI/dendrimer-encapsulated Pt nanoparticles [29], or layer by layer of $\{GOx/Au\text{-}(SH)PANI\text{-}g\text{-}MWCNT\}_n$/ITO electrode [30]), the glucose oxidase immobilized on cauliflower PANI electrode provided a wider linear dynamic range and a lower or within the same range LOD. The good biocompatibility of the PANI nanostructure enables it to be applied as a simple and effective platform for the integration of proteins/enzymes and electrodes, providing analytical access to a large group of enzymes for a variety of bioelectrochemical applications.

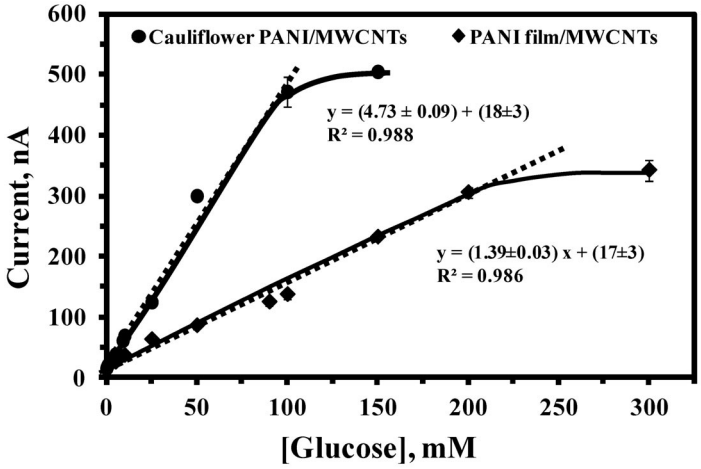

Fig. 5 Calibration curve of glucose biosensor using cauliflower PANI/MWCNT-modified and PANI film/MWCNT-modified GCE at the applied potential of +0.6 V. Carrier buffer: 100 mM PBS pH 6 containing 0.1 M KCl; flow rate: 0.5 mL min^{-1}; sample volume 200 μL.

CONCLUSIONS

A "cauliflower-like" PANI structure was prepared by a template-free electrochemical method using a stepwise current density on a GCE. The formation of PANI on GCE increased the electrode surface area. The cauliflower PANI together with MWCNTs provided high stability where over 140 analysis cycles were performed by one modified electrode. Compared to a bare GCE, cauliflower PANI/MWCNT-modified GCE had a 7.2 times higher sensitivity and 100 times better LOD than the bare GCE. The sensitive detection of H_2O_2, in particular the amperometric determination, is of great importance for biosensing based on oxidase-type enzymes. In conjunction with the immobilized glucose oxidase as a model, a glucose biosensor was tested and was more sensitive (3.4 times) than the one with the PANI film/MWCNT-modified GCE. The high sensitivity of the biosensor was most likely due to the oriented structure on the electrode surface, as the cauliflower-like structured PANI can contact more enzyme molecules. Because of this electrode's open structure together with the potential to be miniaturized and integrated, these results indicate that this sensor platform is very promising for development of oxidase-based biosensors.

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