Saudi Journal of Biological Sciences (2012) 19, 465–471

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Bionanotechnology approach for FAD-dependent enzymes with nanomaterials sensor

Ying Li [a], Shen-Ming Chen [a,*], Cheng-Yu Yang [a], M. Ajmal Ali [b], Fahad M.A. AlHemaid [b]

[a] Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei 106, Taiwan, ROC
[b] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Received 29 April 2012; revised 29 June 2012; accepted 4 July 2012
Available online 13 July 2012

KEYWORDS
Bionanotechnology;
Multiwall carbon nanotube (MWCNTs);
FAD-dependent enzymes;
Biosensor;
Modified electrode

Abstract We have reported the modification of biomolecule with nanomaterials. In this paper, the electrochemical response of different FAD-dependent enzymes at carbon nanomaterials modified electrode. The modified electrode also exhibits a promising enhanced electrocatalytic activity toward the oxidation of substrate. Different methods were used for fabrication of modified electrode. The presence of nanomaterials enhances the enzyme loading and stability. Cyclic voltammograms (CVs) were used for the determination of substrate and the apparent coefficient values for these compounds at different electrodes. Finally, we have studied the surface morphology of the modified electrode using scanning electron microscopy (SEM), which revealed that enzyme is coated on nanomaterials.

1. Introduction

Biomolecule-based modified electrodes are attracting attention rapidly partially due to the promising advances reported recently (Li et al., 2009; Yogeswaran et al., 2010). This paper pinpointed the FAD-dependent enzymes such as FAD (Kumar et al., 2010; Lin et al., 2011), Cholesterol oxidase (ChOx) (Yang et al., 2010; Zhang et al., 2012) and Glucose oxidase (GOx) (Li et al., 2011b; Deng et al., 2012; Tasviri et al., 2012). However, there are issues to be addressed before biosensor or biofuel cells become competitive in practical applications. Some critical issues are enzyme lifetime and specific activity, both of which are related to enzyme stability, electron transfer rate, and enzyme loading. Recent progress in nanobiocatalysis opens the possibility to improve in these aspects. Many nanostructured materials, such as mesoporous media (Pie et al., 2012), nanoparticles (Cheng et al., 2011; Rajkumar et al., 2011; Thiagarajan et al., 2011; Tsai et al., 2011a,b), nanofibers, and nanotubes (Li et al., 2011b,d,e; Prakash et al., 2009; Shie et al., 2009; Upadhyay et al., 2009a,b), have been demonstrated as efficient hosts of enzyme immobilization.

Nanomaterials have been used as hosts for enzymes immobilization to improve the performance of enzymes in various biocatalytic processes. In particular, carbon materials have attracted a lot of attention due to their controlled porosity, high

* Corresponding author. Tel.: +886 2270 17147; fax: +886 2270 25238.
E-mail address: smchen78@ms15.hinet.net (S.-M. Chen).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

1319-562X © 2012 King Saud University. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.sjbs.2012.07.001

loading volume (Tapas et al., 2012), and large surface area (Ting et al., 2011). It is evident that, when the nanostructure of conductive materials is used, the large surface area of these nanomaterials can increase the enzyme loading and facilitate reaction kinetics, and thus improving the biocatalytic processes of biosensor (Lin et al., 2011). In addition, research efforts have also been made to improve the activity and stability of immobilized enzymes by using nanostructures. Initially, most enzymes immobilization in carbon materials was performed via the conventional approaches of simple adsorption, crosslinking (Thangamuthu et al., 2009; Tsai et al. 2011a,b), electropolymerization and covalent attachment (Li et al., 2011a,c), but they did not take long for more diverse approaches to be developed and reported. Recently, various nanobiocatalytic approaches have been gaining more and more attention due to their successful results in biomolecule stabilization. It appears to be reasonable to us to expect that progress in nanostructured biocatalysts will play a critical role in overcoming the major obstacles in the development of biosensor.

This paper discusses the electrochemical response to substrate composed of enzymes and nanomaterials on various electrodes, and the enhancement of oxidation current by nanomaterials modification of the electrode surface. It was interesting to study the electrochemical oxidation of substrate in different carbon nanomaterials and conditions. In addition, the observed behavior of enzymatic and electrochemical reactions from enzymes has been estimated by digital simulation of cyclic voltammograms.

2. Experimental

2.1. Materials

Multiwall carbon nanotube (MWCNTs) (OD = 10–20 nm, ID = 2–10 nm and length = 0.5–200 μm), Anilien, Flavin adenine dinucleotide (FAD) and Acetaminophen were obtained from Aldrich. Cholesterol oxidase (ChOx), Glucose oxidase (GOx), Cholesterol, Glucose, Cetrimonium bromide (CTAB), Hydroquinone (HQ), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Sigma. All other chemicals used were of analytical grade and used without further purification. pH 7.0 (0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4) Phosphate buffer solutions (PBS), solutions were prepared from 1 M $HClO_4$ for the pH 2.28 aqueous solution. Aqueous solutions were prepared using doubly distilled deionized water and then deaerated by purging with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements.

2.2. Apparatus

Cyclic voltammetry (CVs) was performed in an analytical system model CHI-1205A and CHI-627 potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode was used for the electrochemical measurements. The working electrodes were gold electrode (area 0.03 cm^2) and glassy carbon electrode (GCE; area 0.07 cm^2). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films were examined by scanning electron microscopy (SEM) (Hitachi S-3000H). Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements. All the experiments were carried out at room temperature (≈25 °C).

2.3. Preparation of different modified electrodes

Before starting each experiment, working electrodes were polished by BAS polishing kit with 0.05 μm alumina slurry rinsed and then ultrasonicated in double distilled deionized water. In this paper we fabricated three types of modified electrodes:

2.3.1. Type 1

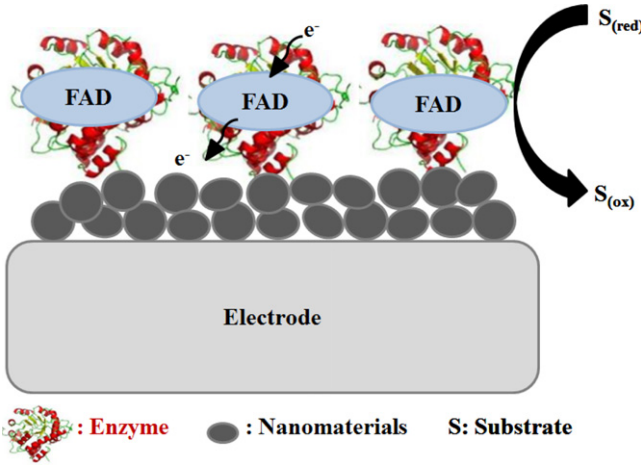
This functionalization and treatment process of MWCNTs by following the previous studies were done to get hydrophilic nature for the homogeneous dispersion, in water. The gold electrodes studied were uniformly coated with 50 μg cm^{-2} of MWCNTs and then dried at 35 °C. The electropolymerization of aniline with FAD to form Poly-aniline-FAD was performed using the mixture 50 mM aniline and 1 mM FAD present in pH 2.28 aqueous solution by consecutive CVs over a suitable potential region of −0.35 to 1.0 V.

2.3.2. Type 2

The MWCNTs solution was obtained by mixing 1.0 mg of MWCNTs and 1.0 mL of phosphate buffer solutions (PBS) with sonication for 6 h. Prior to modification, GCE electrode was polished by the same steps. The cleaned GCE surface was dipped in 2 μL of the prepared MWCNTs solution and then dried out at room temperature. Then, it was dipped in 2 μL of the mixing solution of 0.1 M PBS containing 0.4 M EDC and 0.1 M NHS and dried out at room temperature for 4 h. This electrode was further immersed in fresh phosphate solution containing 1 mg/ml ChOx.

2.3.3. Type 3

MWCNTs (dispersed in the solution of 0.1% CTAB) was ultrasonicated for 6 h to get a uniform dispersion. The cleaned GCE was coated with 2 μL of MWCNTs and the solvent



Scheme 1 Schematic of nanomaterials with FAD-dependent enzymes reaction.

was allowed to evaporate at room temperature. The GOx was immobilized onto the MWCNTs modified electrode surface by adsorption attachment. 2 μL GOx solution (5 mg/mL in pH 7.0 PBS) was pipetted onto the electrode and the surface was dried for approximately 1 h at room temperature. The resulted modified electrode was stored in a refrigerator at 4 °C for use. All modified electrodes formed on the electrode surface can be expressed as Scheme 1.

3. Results and discussions

3.1. Electrochemical characterizations of FAD-dependent enzymes modified electrode

The electrochemical properties of FAD-dependent enzymes (FAD, ChOx and GOx) with nanomaterials (3 types of MWCNTs) modified electrode were investigated using cyclic voltammetry in aqueous solutions having pH values between 2.28 and 7.0. Fig. 1 (A) represents the electrochemical redox signals of Poly-aniline, Poly-aniline-FAD and MWCNTs/Poly-aniline-FAD modified gold electrodes present in pH 2.28 aqueous solution; scan rate 20 mV s^{-1}. Among these films, Poly-aniline (curve a) has a redox couple at $E^{0'}$ = 0.51 V vs. Ag/AgCl, whereas for Poly-aniline-FAD (curve b) two new redox couples appear at $E^{0'}$ = 0.2 and 0.72 V along with the enhancement in the redox couple's peak current. Similarly in MWCNTs/Poly-aniline-FAD (curve c), the presence of MWCNTs abruptly enhances the Poly-aniline's redox couple.

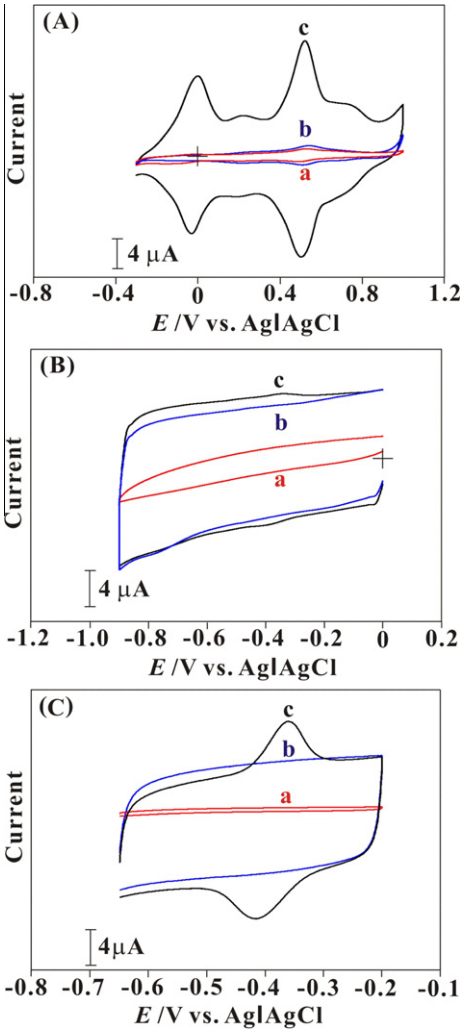


Figure 1 (A) Repetitive CVs of different modified gold-electrode from 50 mM aniline with 1 mM FAD present in pH 2.28 aqueous solution, scan rate 100 mV s^{-1}. Comparison of (a) Poly-aniline, (b) Poly-aniline-FAD and (c) MWCNTs/Poly-aniline-FAD. (B) CVs in 0.1 M PBS (pH 7.0) for different modified glassy carbon-electrodes: (a) only ChOx, (b) MWCNTs and (c) MWCNTs/ChOx, scan rate = 100 mV s^{-1}. (C) CVs in 0.1 M PBS (pH 7.0) for different glassy carbon-electrodes: (a) only GOx, (b) MWCNTs and (c) MWCNTs/GOx, scan rate = 100 mV s^{-1}.

Fig. 1 (B) showed of bare GCE, MWCNTs and MWCNTs/ChOx, respectively. The corresponding CVs have been examined at 100 mV s^{-1} in the potential range of 0 to −0.9 V. Both bare GCE (curve a) and MWCNTs (curve b) showed no obvious redox peaks. Particularly, a redox couple is significantly found at about $E^{0'}$ = −0.4 V ChOx (curve c). This is because that the enzyme (ChOx) is a large family of flavin-specific oxidoreductases and is found in two different forms: one where the FAD cofactor is covalently linked to the protein and one where the cofactor is non-covalently bound to the protein. Here, the redox process is involving the FAD/$FADH_2$ redox process of ChOx (Manjunatha et al., 2012). Further, it has been observed that the presence of MWCNTs increases the overall background current.

Fig. 1 (C) showed different modified electrodes only GOx, MWCNTs and MWCNTs/GOx. The corresponding cyclic voltammograms have been obtained at 100 mV s^{-1} scan rate in the potential range of −0.65 to −0.2 V. The redox peak current at formal potential $E^{0'}$ = −0.39 V represents the redox peak for GOx (curve c). Curve (a) of figure showed of GOx modified electrode. As can be seen, there is no obvious peak in the potential region studied. Curve (b) only MWCNTs modified electrode showed broad background current. From this figure, comparing curve (a) and curve (c), it is found that the presence of MWCNTs showed the currents increased, and showed the catalytic effect on GOx redox peak currents. The above results showed that the presence of MWCNTs in the film enhances the redox reaction (Jacobs et al., 2012).

3.2. Electroanalytical response of acetaminophen, cholesterol and glucose

The MWCNTs/Poly-aniline-FAD has been synthesized on gold electrode at similar conditions as given in experimental section. Then the MWCNTs/Poly-aniline-FAD modified electrode has been washed carefully in deionized water and transferred to pH 2.28 aqueous solution for the electrocatalysis of acetaminophen. Fig. 2 (A) showed the electrocatalytic oxidation of acetaminophen (60 μM) at modified and unmodified gold electrodes (curve a′); scan rate 20 mV s^{-1}. The CVs for MWCNTs/Poly-aniline-FAD exhibit reversible redox couples in the absence of acetaminophen (curve a), and upon addition of acetaminophen a new growth in the oxidation peak of acetaminophen appeared at E_{pa} = 0.72 V (curve b). This above said anodic peak showed that the electrocatalytic oxidation of acetaminophen takes place at Poly-aniline-FAD co-polymer's redox couple $E^{0'}$ = 0.718 V. More specifically, the enhanced electrocatalytic activity of MWCNTs/Poly-aniline-FAD can be explained in terms of both lower overpotential and higher peak current than bare gold electrode. The anodic

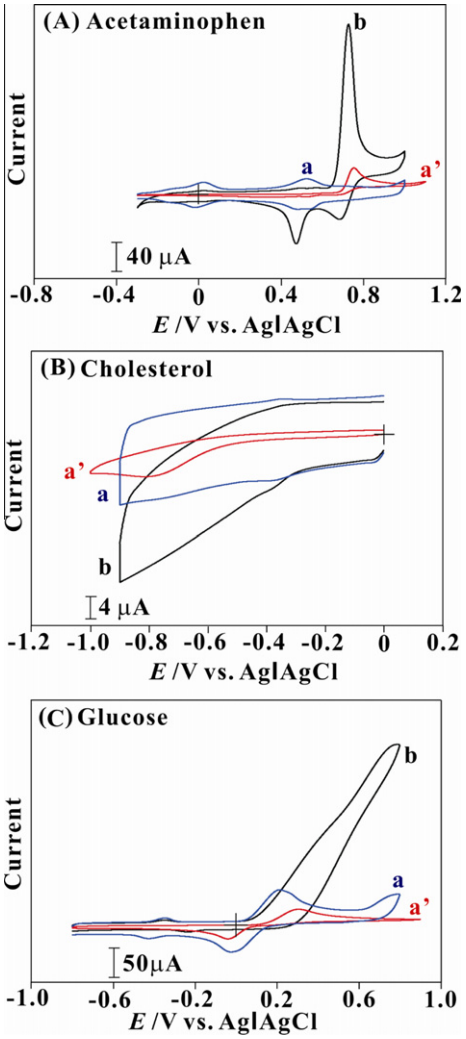


Figure 2 (A) CVs of acetaminophen (60 μM) at (a′) bare gold electrode and (b) MWCNTs/Poly-aniline-FAD in pH 2.28 aqueous solution at 100 mV s^{-1}, (a) MWCNTs/Poly-aniline-FAD was shown with the absence of acetaminophen. (B) CVs of (a′) bare GCE and (b) MWCNTs/ChOx examined in 0.1 M PBS (pH 7.0) containing 28 mM cholesterol, (a) MWCNTs/ChOx in the absence of cholesterol at100 mV s^{-1}. (C) CVs of different electrodes in pH 7.0 PBS containing 1 mM HQ with 5 mM glucose, scan rate = 100 mV s^{-1}: (a′) bare GCE and (b) MWCNTs/GOx; (a) MWCNTs/GOx in the absence of glucose.

peak potential is lowered by MWCNTs, and the peak current is enhanced by both MWCNTs and Poly-aniline-FAD.

The electroanalytical response in Fig. 2 (B) showed the cyclic voltammogram of MWCNTs/ChOx examined in the absence (curve a) and presence (curve b) of the 28 mM cholesterol. Compared with bare electrode (curve a′) examined in the same concentration of cholesterol, the MWCNTs/ChOx exhibited a redox couple ($E^{0'} = -0.4$ V) and a higher cathodic current response to cholesterol. This cathodic current response occurs due to the well-known reaction mechanism of cholesterol oxidation and the determination of H_2O_2. The enzymatic reaction in the use of ChOx as a receptor can be described by following equation:

$$\text{Cholesterol} + O_2 \quad (\text{in the presence of ChOx}) \rightarrow \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2 \qquad (1)$$

The MWCNTs/GOx film was synthesized on GCE at similar conditions. Fig. 2 (C) showed the electrocatalytic oxidation of glucose. Bare GCE (curve a′) and MWCNTs/GOx (curve b); MWCNTs/GOx (curve a; absence of glucose) in pH 7.0 PBS containing 1 mM hydroquinone (HQ) with 5 mM glucose, scan rate = 100 mV s^{-1} in the potential range of −0.5 to 0.5 V. Upon addition of glucose a new growth in the oxidation peak at 0.78 V of respective analytes has appeared at the current values. Oxidation of glucose by the biocatalyst yields the reduced $FADH_2$ redox site. The hydroquinone (HQ) mediates oxidation of $FADH_2$ and further electron transfer to the electrode. As the quinone is exposed to the electrode surface, its rapid oxidation by the conductive support allows the cyclic electrochemically induced oxidation of glucose. The observations at bare electrode (curve a′) clearly indicate the fouling effect of the electrode surface with the oxidation obtaining the weak single peak for glucose. From all these above results it is clear that MWCNTs/GOx film is more efficient and exhibits enhanced functional properties compared to that of bare alone. The enzymatic reaction in the use of GOx as a receptor can be described as follows:

$$\text{FAD} + \text{glucose} \xrightarrow{\text{GOx}} FADH_2 + \text{Gluconolactone(Enzymatic)} \qquad (2)$$

$$FADH_2 \xrightarrow{\text{HQ}} \text{FAD} + 2H^+ + 2e^- \text{(Electrochemical)} \qquad (3)$$

3.3. Sensitivities and correlation coefficient of different modified electrodes

In these above electrocatalysis experiment, we have been calculated in Fig. 3. Figure (A) an increase in the concentration of 6–60 μM acetaminophen simultaneously produces a linear increase in the oxidation peak current of acetaminophen with good film stability at MWCNTs/Poly-aniline-FAD. It is obvious that the MWCNTs/Poly-aniline-FAD showed higher electrocatalytic activity for acetaminophen than bare gold

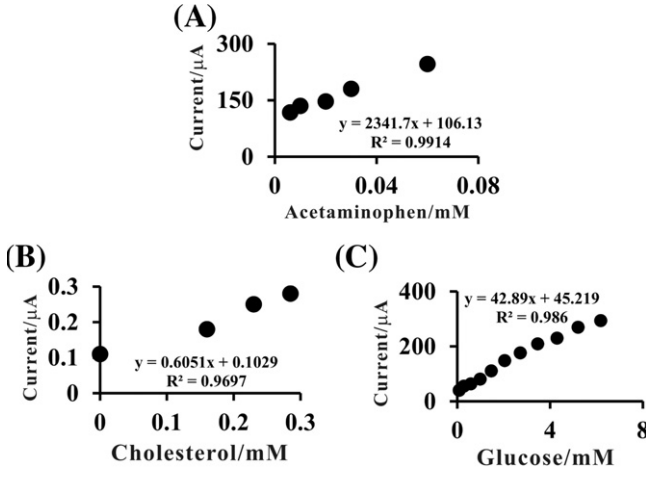


Figure 3 Showed current vs. concentration plot of (A) acetaminophen at MWCNTs/Poly-aniline-FAD, (B) cholesterol at MWCNTs/ChOx and (C) glucose at MWCNTs/GOx modified electrode.

electrode. Where, the increase in peak current and decrease in overpotential; both are considered as the electrocatalytic activity. From the slopes of linear calibration curves, the sensitivities of MWCNTs/Poly-aniline-FAD modified gold electrodes and correlation coefficient were 78.05 mA mM^{-1} cm^{-2} and 0.9914. It is obvious that the sensitivity of MWCNTs/Poly-aniline-FAD is higher for acetaminophen when comparing Poly-aniline-FAD and MWCNTs films. The overall view of these results reveals that MWCNTs/Poly-aniline-FAD was efficient for acetaminophen analysis.

As shown in Figure (B), the calibration curve was found to have linear correlation between electrocatalytic current and cholesterol concentration. The steady-state reduction current varies linearly with cholesterol concentrations in the entire concentration range between 0.02 and 0.28 × mM. The sensitivity of MWCNTs/ChOx electrode was 8.64 μA mM^{-1} cm^{-2} with a correlation coefficient of 0.969. This sensor has competitive performance when compared with other ChOx based cholesterol biosensors.

Figure (C) showed a current vs. concentration plot of glucose at MWCNTs/GOx electrode. An increase in concentration of glucose, simultaneously produced a linear increase in the oxidation peak currents of the glucose. The growth of the cyclic voltammogram current exhibit a potential of $E^{0'} = 0.78$ V (vs. Ag/AgCl). The sensitivities of MWCNTs/GOx modified electrodes and correlation coefficient were 612.71 μA mM^{-1} cm^{-2} and 0.986. The response current increased in concentration range between 0.01 and 6.19 mM. These results showed the enhanced functional property in the presence of MWCNTs.

3.4. Morphological characterization of modified electrode

SEM is a very useful technique that can image conductive nanomaterials as well. It can also be used for imaging the surface of polymer membranes if a thin layer of conductive material is sputter coated on the surface. The surface morphology of different types of modified electrode has been examined using SEM. Here the SEM studies could furnish the comprehensive information about the surface morphology of nanostructure on the ITO surface. Prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. Further, three types of modified electrodes have been prepared on ITO electrode and were characterized using SEM. From Fig. 4, it is significant that there are morphological differences between both the types. It is a well known fact that the prolonged exposure to the electron beam will damage the biomolecule (such as FAD, enzyme, DNA, etc.) so an utmost care was taken to measure these images. Comparison of (A′) and (A) SEM images reveals significant morphological difference between Poly-aniline-FAD and MWCNTs/Poly-aniline-FAD. The top views of nanostructures showed grains of Poly-aniline-FAD deposited on the electrode. The MWCNTs/Poly-aniline-FAD showed that the plateaus of Poly-aniline-FAD deposited over MWCNTs to form MWCNTs /Poly-aniline-FAD. Second

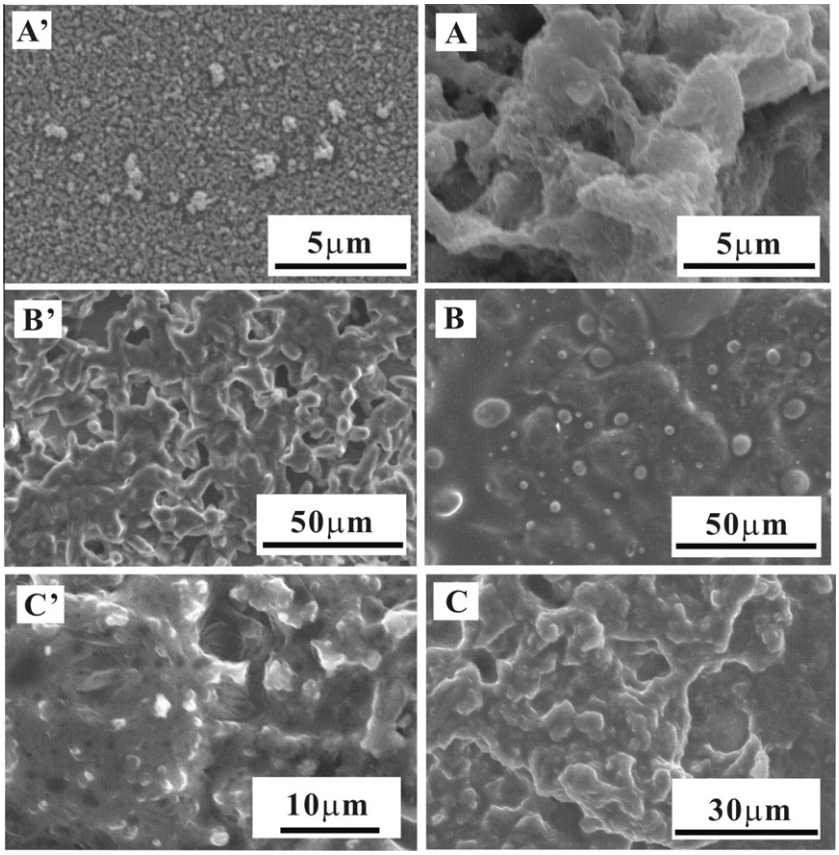


Figure 4 SEM images of (A′) Poly-aniline-FAD and (A) MWCNTs/ Poly-aniline-FAD; (B′) ChOx and (B) MWCNTs/ ChOx; (C′) GOx and (C) MWCNTs/GOx on ITO electrode.

type modified electrode (B′) ChOx and (B) MWCNTs/ ChOx on the ITO electrode surface showed uniformly deposited homogeneously dispersed MWCNTs on this electrode. The MWCNTs/ChOx reveals that the ChOx had covered the entire MWCNTs. Comparison of (B′) only ChOx and (B) MWCNTs/ChOx reveals, these results could be explained as the increase in deposition of ChOx on MWCNTs. We can clearly see that the immersed MWCNTs/ChOx has been gathered together. Typical SEM images of the (C′) GOx and (C) MWCNTs/GOx surfaces are shown. In the SEM analysis the GOx was exhibited as an irregular surface. At the same time, after modification the MWCNTs are shown in (C). It indicates that the surface modified the MWCNTs and can assist in loading more substrate and uniform coating.

4. Conclusions

We have demonstrated the application of enzyme modified electrode for determination of substrate. This feature provides a favorable clinical diagnosis for the electrocatalytic oxidation of substrate at modified electrode. High sensitivity and stability together with very easy preparation modified the electrode as promising candidate for constructing simple electrochemical biosensor. The experimental methods of CVs with biosensor integrated into the modified electrode which are presented in this paper, provide an opportunity for qualitative and quantitative characterization, even at physiologically relevant conditions. The SEM results have showed the difference between enzymes and nanomaterials/enzymes films morphological data. Therefore, this work establishes and illustrates, in principle and potential, a simple and novel approach for the development of a voltammetric sensor.

Acknowledgement

This work was supported by the National Science Council of the Taiwan (ROC).

References

Cheng, C.Y., Thiagarajan, S., Chen, S.M., 2011. Electrochemical fabrication of AuRh nanoparticles and their electroanalytical applications. Int. J. Electrochem. Sci. 6, 1331–1341.

Deng, C., Chen, J., Nie, Z., Si, S., 2012. A sensitive and stable biosensor based on the direct electrochemistry of glucose oxidase assembled layer-by-layer at the multiwall carbon nanotube-modified electrode. Biosens. Bioelectron. 26 (1), 213–219.

Jacobs, C.B., Peairs, M.J., Venton, B.J., 2012. Review: carbon nanotube based electrochemical sensors for biomolecules. Anal. Chim. Acta 662 (2), 105–127.

Kumar, S.A., Chen, S.L., Chen, S.M., 2010. Electrochemical Sensing of H_2O_2 at flavin adenine dinucleotide/chitosan/CNT nanocomposite modified electrode. Electrochem. Solid-State Lett. 13 (10), K83–K86.

Li, Y., Yogeswaran, U., Chen, S.M., 2009. Multiwalled carbon nanotubes with poly (NDGAChi) biocomposite film for the electrocatalysis of epinephrine and norepinephrine. Anal. Biochem. 388, 288–295.

Li, Y., Chen, S.M., Chen, W.C., Li, Y.S., Ali, M.A., AlHemaid, F.M.A., 2011a. Platinum nanoparticles (PtNPs) – laccase assisted biocathode reduction of oxygen for biofuel cells. Int. J. Electrochem. Sci. 6, 6398–6409.

Li, Y., Chen, S.M., Sarawathi, R., 2011b. Membraneless enzymatic biofuel cells based on multi-walled carbon nanotubes. Int. J. Electrochem. Sci. 6, 3776–3788.

Li, Y., Wei, J.X., Chen, S.M., 2011c. Platinum nanoparticles (PtNPs) with poly (tyramine) – choline oxidase (ChOx) film for the electrocatalysis of choline. Int. J. Electrochem. Sci. 6, 3385–3398.

Li, Y., Yang, C.Y., Chen, S.M., 2011d. Photoelectrocatalysis of hydrogen peroxide at functionalized multi-walled carbon nanotubes (*f*-MWCNT) with brilliant blue modified electrode. Int. J. Electrochem. Sci. 6, 4829–4842.

Li, Y., Yang, S.Y., Chen, S.M., 2011e. Biosensing approach for glutathione detection using glutathione reductase (GR) with multi-walled carbon nanotubes on gold electrode. Int. J. Electrochem. Sci. 6, 3982–3996.

Lin, K.C., Yin, C.Y., Chen, S.M., 2011. An electrochemical biosensor for determination of hydrogen peroxide using nanocomposite of poly(Methylene Blue) and FAD hybrid film. Sens. Actuators, B. Chem. 157, 202–210.

Manjunatha, R., Nagaraju, D.H., Suresh, G.S., Melo, J.S., D'Souza, S.F., Venkatesha, T.V., 2012. Direct electrochemistry of cholesterol oxidase on MWCNTs. J. Electroanal. Chem. 651 (1), 24–29.

Pie, P., Krishna, V., Shalini, P., 2012. Nanoporous impedemetric biosensor for detection of trace atrazine from water samples. Biosens. Bioelectron. 32 (1), 155–162.

Prakash, P.A., Yogeswaran, U., Chen, S.M., 2009. Direct electrochemistry of catalase at multiwalled carbon nanotubes-nafion in presence of needle shaped DDAB for H_2O_2 sensor. Talanta 78, 1414–1421.

Rajkumar, M., Thiagarajan, S., Chen, S.M., 2011. Electrochemical fabrication of Rh-Pd particles and electrocatalytic applications. J. Appl. Electrochem. 41, 663–668.

Shie, J.W., Yogeswaran, U., Chen, S.M., 2009. Haemoglobin immobilized on nafion modified multi-walled carbon nanotubes for O_2, H_2O_2 and CCl_3COOH sensors. Talanta 78, 896–902.

Tapas, K., Saswata, B., Partha, K., Ananta, K.M., Nam, H.K., Joong, H.L., 2012. Recent advances in graphene-based biosensors. Biosens. Bioelectron. 26 (12), 4637–4648.

Tasviri, M., Rafiee-Pour, H.A., Ghourchian, H., Gholami, M.R., 2012. Amine functionalized TiO_2 coated on carbon nanotube as a nanomaterial for direct electrochemistry of glucose oxidase and glucose biosensing. J. Mol. Catal. B: Enzym. 68 (2), 206–210.

Thangamuthu, R., Wu, Y.C., Chen, S.M., 2009. Silicomolybdate-incorporated-glutaraldehyde-cross-linked poly-L-lysine film modified glassy carbon electrode as amperometric sensor for bromate determination. Electroanalysis 21 (14), 1655–1658.

Thiagarajan, S., Tsai, T.H., Chen, S.M., 2011. Electrochemical fabrication of nano manganese oxide modified electrode for the detection of H_2O_2. Int. J. Electrochem. Sci. 6, 2235–2245.

Ting, S.W., Prakash, A.P., Chen, S.M., Saraswathi, R., 2011. Direct electrochemistry of catalase immobilized at electrochemically reduced graphene oxide modified electrode for amperometric H_2O_2 biosensor. Int. J. Electrochem. Sci. 6, 4438–4453.

Tsai, T.H., Lin, K.C., Chen, S.M., 2011a. Electrochemical synthesis of poly(3,4-ethylenedioxythiophene) and gold nanocomposite and its application for hypochlorite sensor. Int. J. Electrochem. Sci. 6, 2672–2687.

Tsai, T.H., Wang, S.H., Chen, S.M., 2011b. Anthraquinonedisulfonate doped glutaraldehyde cross-linked poly-L-lysine modified electrode for $S_2O_8^{2-}$, IO_3^- and oxygen reduction. Int. J. Electrochem. Sci. 6, 1655–1668.

Upadhyay, A.K., Peng, Y.Y., Chen, S.M., 2009a. Immobilization of horseradish peroxidase and nile blue into the ormosil nanocomposite for the fabrication of hydrogen peroxide biosensor based on MWCNT modified glassy carbon electrode. Sens. Actuators, B 141, 557–565.

Upadhyay, A.K., Ting, T.W., Chen, S.M., 2009b. Amperometric biosensor for hydrogen peroxide based on coimmobilized horse-

radish peroxidase and methylene green in ormosils matrix with multiwalled carbon nanotubes. Talanta 79, 38–45.

Yang, J.Y., Li, Y., Chen, S.M., Lin, K.C., 2010. Fabrication of a cholesterol biosensor based on cholesterol oxidase and multiwall carbon nanotube hybrid composites. Int. J. Electrochem. Sci. 6, 2223–2234.

Yogeswaran, U., Li, Y., Chen, S.M., 2010. Characterization of MWCNTs-PMel composite film and its application in simultaneous determination of DOPA and serotonin. J. Electrochem. Soci. 157 (9), K187–K193.

Zhang, M., Yuan, R., Chai, Y., Chen, S., Zhong, H., Wang, C., Cheng, Y., 2012. A biosensor for cholesterol based on gold nanoparticles-catalyzed luminol electrogenerated chemiluminescence. Biosens. Bioelectron. 32 (1), 288–292.