Analytical Biosensing of Hydrogen Peroxide on Brilliant Cresyl Blue/Multiwalled Carbon Nanotubes Modified Glassy Carbon Electrode

*Ya-Yu Peng, A. K.Upadhyay, Shen-Ming Chen**

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No.1, Section 3, Chung-Hsiao East Road , Taipei 106, Taiwan
*e-mail: smchen78@ms15.hinet.net

Received: July 20, 2009
Accepted: October 29, 2009

Abstract
A cationic quinine-imide dye brilliant cresyl blue (BCB) and horseradish peroxidase (HRP) were co-immobilized within ormosil on multiwalled carbon nanotubes modified glassy carbon electrode for the fabrication of highly sensitive and selective hydrogen peroxide biosensor. The presence of epoxy group in ormosil as organic moiety improves the mechanical strength and transparency of the film and amino group provides biocompatible microenvironment for the immobilization of enzyme. The presence of MWCNTs improved the conductivity of the nanocomposite film. The surface characterization of MWCNT modified ormosil nanocomposite film was performed with scanning electron microscopy (SEM) and atomic force microscopy (AFM). Cyclic voltammetry and amperometry measurements were used to study and optimize the performance of the resulting peroxide biosensor. The apparent Michaelis–Menten constant was determined to be 1.5 mM. The proposed H_2O_2 biosensor exhibited wide linear range from 3×10^{-7} to 1×10^{-4} M, and low detection limit 1×10^{-7} M ($S/N = 3$) with fast response time < 5 s. The probable interferences in bio-matrix were selected to test the selectivity and no significant response was observed in the biosensor. This biosensor possessed good analytical performance and long term storage stability.

Keywords: Multiwalled carbon nanotubes, Brilliant cresyl blue, Ormosil, Horseradish peroxidase, Biosensors, Nanotubes

DOI: 10.1002/elan.200900366

1. Introduction

Now days, carbon nanotubes are frequently using for the fabrication of electrochemical biosensors due to their excellent and unique physical and chemical properties. Due to their nanometric size, higher electrical conductivity, strong adsorptive ability, good mechanical strength, excellent biocompatibility, minimization of surface fouling, lack of toxicity and good electrocatalytic properties, currently, CNTs have received enormous attention in the biosensor field [1–5]. MWCNTs modified biosensor offer substantially greater signals especially at low potential, reflecting the excellent electrocatalytic activity of MWCNTs. Such low-potential operation of MWCNTs based biosensor results in a wide linear range and fast response time.

The highly sensitive, selective and accurate analytical detection of hydrogen peroxide is of great relevance, because it is not only a by-product of several highly selective oxidases, but also an essential mediator in food, pharmaceutical, clinical, industrial, environmental analysis and many other fields [6, 7]. Several analytical techniques such as fluorimetry [8], titrimetry [9], spectrometry [10], chemiluminescence [11] and electrochemical methods [12–14] have been developed to monitor H_2O_2. The first four methods suffer from a variety of drawbacks including interferences, long pre-treatments of the sample and in many cases these methods require the utilization of toxic or expensive reagents. Electrochemical methods, such as amperometric biosensors based on simple and economical immobilized redox proteins or enzymes modified electrodes have been extensively employed for determination of H_2O_2 for their simplicity, highly selective and intrinsic sensitivity [15–17]. Nowadays, horseradish peroxidase (HRP) is being widely used in the fabrication of ormosil based amperometric biosensor for the detection of H_2O_2 due to its high purity, sensitivity, low cost and easy availability and high affinity to encapsulate into the ormosil film.

A series of water soluble organic dyes such as methylene green [18], meldola blue [19], Prussian blue [20] and celestine blue [21] have been used for the modification of electrode surface and they exhibit excellent mediating ability for the electrocatalytic reduction of H_2O_2. We used brilliant cresyl blue (BCB) dye in our investigation due to its higher sensitivity and mediating ability toward H_2O_2 determination. The leaching out of the biomolecules/dyes from the electrode surface is a crucial problem in the fabrication of electrochemical biosensor. To overcome this issue, we used MWCNTs modified organically modified sol-

gel glasses (ormosil) as a biocompatible matrix to the immobilization of HRP and BCB. Ormosils are the unique matrices to encapsulate the biomolecules [22, 23], due to their inert chemical nature, high mechanical strength, excellent optical properties, tunable porosity, strong adhesion property to its surface support and ease of modification. Ormosils are more biocompatible and it increases the long term stability of the developed biosensor. The intersection of ormosil with multiwalled carbon nanotube provides better nanoporous matrix with high electrical conductivity for the immobilization of enzymes in its native state. The ormosil/ multiwalled carbon nanotubes modified electrodes possess large potential window, low and almost constant background current over a large potential window and fast kinetics for a large number of electrochemical mediators.

Several numbers of MWCNTs modified amperometric peroxide biosensors have been fabricated based on MWCNT/thionine/Nafion modified PIGE [24], Carbon nanotube/Chitosan/HRP/SG modified GCE [25], MWCNT/HRP/gold modified electrode [26], poly(TB)/HRP/MWCNT/CHIT modified electrode [27], MWCNT/BSA/HRP/Ferrocene modified electrode [28], MWCNT/HRP/Methylene blue modified electrode [29] and MWCNT/TTF-TCNQ/HRP modified gold electrode [30].

In present article, for the first time we co-immobilized a cationic quinine-imide dye brilliant cresyl blue (BCB) with horseradish peroxidase (HRP) into the multiwalled carbon nanotubes modified nanoporous ormosil matrix (3-aminopropyltrimethoxysilane, phenyltrimethoxysilane and 2(3,4-epoxycyclohexyl) ethyltrimethoxysilane) for the development of an amperometric hydrogen peroxide biosensor. The widely present amino groups in nanocomposite ormosil matrix provide a hydrophilic environment, which is compatible with the biomolecules [31]. The experimental results showed that the MWCNT/BCB/HRP modified GCE exhibited excellent electrocatalytic activity towards the reduction of H_2O_2. The proposed hydrogen peroxide biosensor exhibited better wide linear range and low detection limit toward to H_2O_2, compare to the other biosensors earlier reported in the literature.

2. Experimental

2.1. Reagents and Materials

Peroxidase from horseradish (POD, EC 1.11.1.7 type VI) was obtained from Sigma; brilliant cresyl blue (BCB) was purchased from Fluka. 3-Aminopropyltrimethoxysilane, 2-(3,4-epoxycyclohexyl) ethyltrimethoxysilane, phenyltrimethoxysilane *N*,*N*-dimethylformamide (DMF) and multiwalled carbon nanotubes (10 – 15 nm diameter) were purchased from Aldrich Chemical Co. Hydrogen peroxide (30% w/v solution) was purchased from Wako Pure Industrial Co., Japan. The concentrations of more diluted hydrogen peroxide solutions were determined by titration with cerium (IV) to a ferroin end point. Phosphate buffer solutions (PBS) of various pH were prepared with 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4 with supporting electrolyte 0.1 M KCl. All other chemicals employed were of analytical grade. All the solutions were prepared with doubly distilled water.

2.2. Apparatus

The electrochemical measurements were performed with a computer controlled CHI750A (TX, USA) electrochemical system. Cyclic voltammetry and amperometric measurements were done with a three electrode system comprising the MWCNT/BCB/HRP/GCE as a working electrode, an Ag/AgCl reference electrode and platinum wire as counter electrode. All electrochemical measurements were carried out in 5 mL (0.1 M, pH 7.0) phosphate buffer solution (PBS). All experimental solutions were thoroughly deoxygenated by bubbling nitrogen through the solution for at least 15 min. Hitachi scientific instruments (Japan) Model S-3000H scanning electron microscope and CPSM4000 model (China) atomic force microscope were used for surface characterization of MWCNT/BCB/HRP/ormosil nanocomposite film.

2.3. Fabrication of MWCNT/BCB/HRP Modified Glassy Carbon Electrode for H_2O_2 Biosensor

A glassy carbon electrode (GCE, 3 mm in diameter) was polished with 1.0, 0.3 and 0.05 μm Al_2O_3 slurry successively followed by rinsing thoroughly with double distilled water until a mirror-like surface was obtained. Then it was washed ultrasonically in 1 : 1 nitric acid, absolute ethanol and double distilled water, each for 5 min, and allowed to dry at room temperature. For the electrode modification, MWCNT modified ormosil nanocomposite has been prepared by the following method. About 50 μL of aminopropyltrimethoxysilane with 20 μL of brilliant cresyl blue in 175 μL double distilled water were mixed in a cell for 5 min. This above prepared mixture was added with 40 μL of MWCNTs (dissolved in DMF) and 30 μL of HRP. Following the MWCNTs and HRP addition, 10 μL of EPTMOS and 10 μL HCl have also been added in the solution to complete the hydrolysis. Then the whole mixture was stirred for 10 min to get homogenous solution of MWCNT modified ormosil nanocomposite. After the nanocomposite preparation, about 10 μL of prepared homogeneous solution was dispersed on the surface of glassy carbon electrode and was dried at room temperature for 6 h. The simple sol-gel method was used for the fabrication of modified electrodes. This method has some advantages than other process in three aspects (1) It is very simple and convenient process (2) no involvement of organic solvents (3) the amino and epoxy groups present in ormosil provide better microenvironment for the encapsulation of enzymes [26]. In the following text, the MWCNT/BCB/HRP/ormosil nanocomposite film modified GCE will be shortly referred as MWCNT/BCB/HRP/GCE.

3. Results and Discussion

3.1. Surface Characterization of MWCNT/BCB/HRP Modified Electrode

The surface morphology of MWCNT/BCB/HRP/GCE modified electrode was investigated using atomic force microscopy (AFM) and scanning electron microscopy (SEM).The SEM image showed that the BCB/ormosil film was highly porous with a three dimensional open framework (Fig. 1A). The nano-structured ormosil network increases the electron kinetic in the matrix and enhance the sensitivity of the biosensor. Figure 1B, showed the SEM image of MWCNT/BCB/ormosil modified film with small globular shaped particles of HRP. Incorporation of MWCNT into the ormosil matrix increase the electron transfer property and porosity of the nanocomposite film, which will lead to fast diffusion of the target analyte. Fig. 2 showed the AFM image of BCB/HRP/ormosil and MWCNT/BCB/HRP/ormosil on ITO glass, respectively. The spherical shaped bright particles of HRP were shown in Figure 2A, and were uniformly distributed into the ormosil film. The thicknesses of the BCB/HRP/Ormosil and MWCNT/BCB/HRP/ormosil hybrid films were found to be ~27 and ~300 nm, respectively (estimated by AFM image). Film thickness was higher for hybrid film due to incorporation of MWCNTs onto the electrode surface. The surface thickness increase 1.36 nm to 30.4 nm for hybrid film indicated that MWCNTs were successfully incorporated into ormosil to improve the film conductivity. The height difference between the bright region and the dark ground was about 7 nm and 50 nm for BCB/HRP/Ormosil and MWCNT/BCB/HRP/Ormosil hybrid film, respectively. In Figure 2 B, the bright globular shaped particles of HRP were shown with MWCNTs and were equally distributed in the ormosil nanocomposite film. However a uniform, stable and regularly patterned compact structure was observed on MWCNT/BCB/HRP/Ormosil hybrid film.

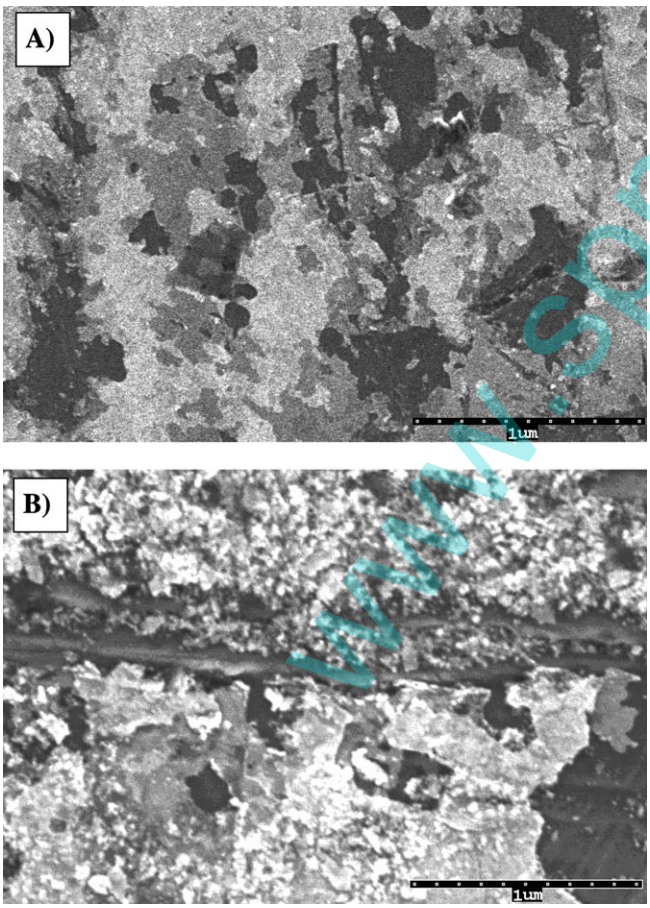

Fig. 1. Typical SEM images of BCB/ormosil nanocomposite (A) and MWCNT/BCB/HRP ormosil nanocomposite film (B) on the surface of ITO glass.

3.2. Electrochemical Response of MWCNT/BCB/HRP Modified Electrode

The redox mediator brilliant cresyl blue modified-MWCNT electrode was electrochemically characterized by cyclic voltammetry. Figure 3 shows the cyclic voltammograms of MWCNT/BCB/HRP/GCE in 0.1 M PBS (pH 7.2) at different scan rate in potential range -0.10 V to -0.40 V. Figure 3 A shows the symmetrical and good redox behavior of brilliant cresyl blue in ormosil matrix and contributing to the significance of MWCNTs. Multi walled carbon nanotubes not only increased the current response, they also improved the reversible character of redox mediator brilliant cresyl blue in ormosil nanocomposite film because it increases the effective surface area of modified electrode and provides the sufficient space for fast electrochemical phenomenon and maximum enzyme loading on the modified electrode surface. The cathodic peak potential and anodic peak potential obtained at MWCNT/BCB/HRP/GCE were -300 and -250 mV, respectively at the scan rate of 50 mVs^{-1}. The formal potential $[E^{o\prime} = (E_{pa} + E_{pc})/2]$ was -275 mV. The peak separation (ΔE_p) was ~50 mV at the scan rate of 50 mV/s and the ratio of anodic peak currents and cathodic peak currents were almost unity ($I_{pa}/I_{pc} \approx 1$). With the increasing scan rates corresponding anodic peaks shifted toward positive potential but there was no change in the position of cathodic peaks. Figure 3 B shows that the peak currents increased linearly with the scan rate between 10 to 200 mVs^{-1} as expected for a surface confined process.

Surface coverage (Γ) for the electroactive species was estimated by using

$$\Gamma = Q/nFA \quad (1)$$

Where A (0.70 cm^2) is the area of the working GCE, n = the number of electron per reactant molecule, Q (1.62×10^{-6} C) the charge obtained by integrating the anodic peak at low voltage scan rate (10 mV s^{-1}), and F is the Faraday constant. In the present case, the calculated surface coverage for the electroactive species was 2.39×10^{-11} mol cm^{-2}.

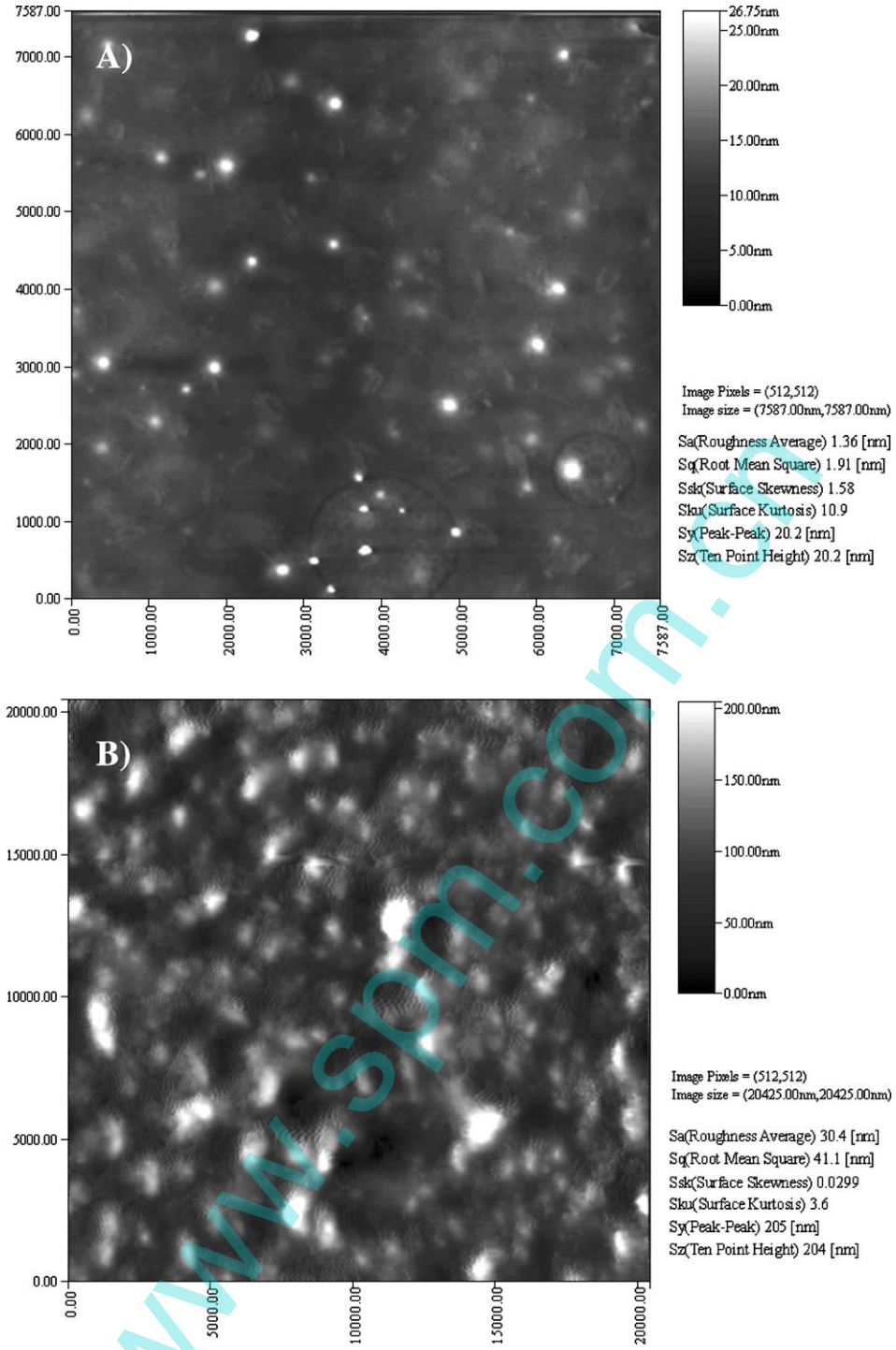

Fig. 2. AFM images of BCB/HRP/ormosil (A) and MWCNT/BCB/HRP/ormosil nanocomposite film (B) on the surface of ITO glass.

3.3. Electrocatalysis of H_2O_2 on MWCNT/BCB/HRP Modified Electrode

Figure 4 illustrates the cyclic voltammograms of bare GCE, without addition of H_2O_2 (curve a) and with addition of H_2O_2 (curve b), and MWCNT/BCB/HRP/GCE in the absence (curve c) and presence (curve d) of 0.1 mM H_2O_2 in 0.1 M PBS (pH 7.2) at the scan rate of 30 mV s^{-1}. It can be seen that bare GCE had not significant response on the addition of H_2O_2 at potential range -0.05 V to -0.45 V in the presence 0.1 mM H_2O_2 of but MWCNT/BCB/HRP/GCE modified electrode showed remarkable increase in the cathodic current in the same amount of H_2O_2. The electrochemical reduction of hydrogen peroxide on MWCNT/BCB/HRP/GCE modified electrode started at -0.20 V and obvious catalytic reduction peak appeared at the potential of -0.32 V. Figure 5 presents the CVs of addition of various concentration of hydrogen peroxide at the scan rate of

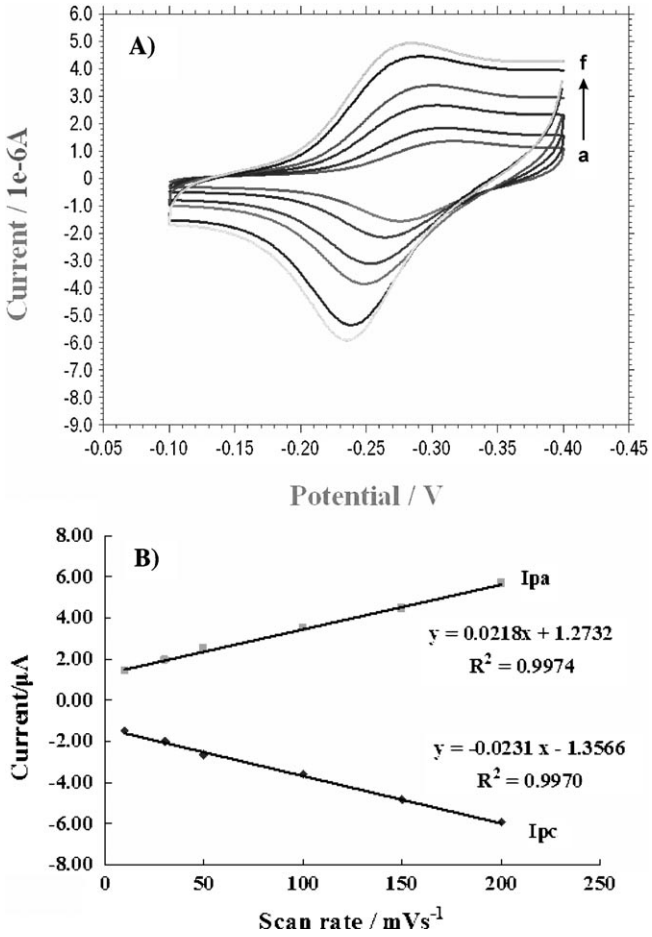

Fig. 3. (A) Cyclic voltammogarms of brilliant cresyl blue immobilized into ormosils on the MWCNT modified GCE in 0.1 M PBS (pH 7.2) at the scan rate of (a) 10, (b) 30, (c) 50, (d) 100, (e) 150 and (f) 200 mVs^{-1}. (B) Show plots of peak currents vs. scan rate.

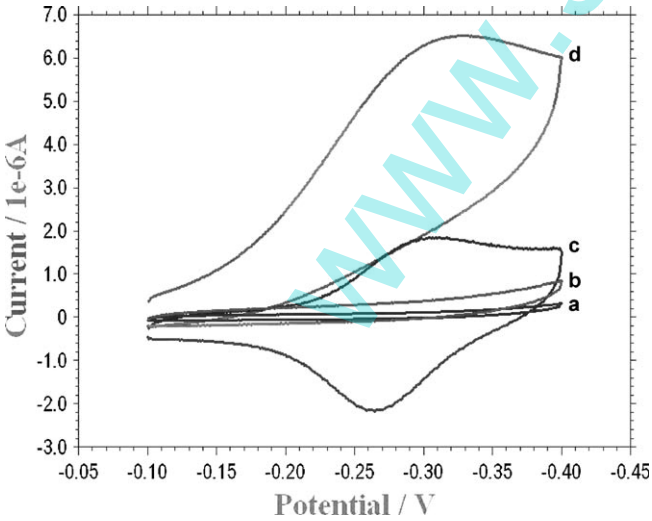

Fig. 4. Cyclic voltammogarms of (a) bare GCE (b) bare GCE in presence of 0.1 mM H_2O_2 (c) and (d) CVs of MWCNT/BCB/HRP/GCE in the absence and presence of 0.1 mM H_2O_2 in 0.1 M PBS (pH 7.2) at the scan rate of 30 mV s^{-1}, respectively.

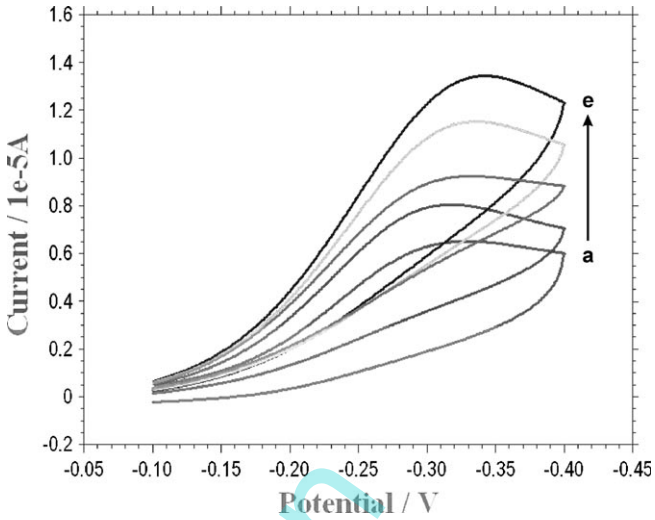

Fig. 5. Cyclic voltammogarms of MWCNT/BCB/HRP/GCE in the presence of different concentration of H_2O_2: (a) 0.1, (b) 0.3, (c) 0.6, (d) 1.0 and (e) 1.3 mM in 0.1 M PBS (pH 7.2) at the scan rate of 30 mV s^{-1}.

30 mVs^{-1} in (0.1 M, pH 7.2) PBS for MWCNT/BCB/HRP/GCE modified electrode. The cathodic peak current was dramatically increased and anodic peak current disappeared, indicating the fast electrocatalytic reduction of peroxide on the MWCNT modified GCE electrode.

3.4. Optimization of Experimental Parameters

Amperometric measurements were further performed to study the influence of various experimental parameters on the sensitivity of MWCNT/BCB/HRP/GCE. The applied potential has an important influence over the sensor response, because the applied potential contributes to the sensitivity and selectivity of the system [32]. Amperometric responses of the proposed biosensor to H_2O_2 at different potentials were examined in 1.0 M, PBS (pH7.2) (figure not shown). The steady-state current increased slowly with applied potential decreasing from -0.2 V to -0.4 V which can be attributed to the increasing driving force for the fast reduction of compounds. The current approaches a maximum value at -0.32 V and then started to decline. To avoid interferences and reduction of oxygen at high negative applied potential, -0.32 V was selected as the applied potential for amperometric measurement.

The dependence of the biosensor response on pH of the measurement solution was investigated at the applied potential -0.32 V, using 0.1 mol L^{-1}, PBS (pH 7.2) in the presence of (0.1 mM) H_2O_2 (figure not shown). The pH of the solution ranged from 4.0 to 9.0. The current response increased from pH 4.0 and reached the maximum at pH 7.2. The current and potential was of the peak depending on the solution pH. Then the current response decreased from pH 7.0 to 9.0. Therefore, the suitable pH with the maximum activity of the immobilized HRP was at pH 7.2, which was in

agreement with that reported for soluble HRP [33].Thus the optimum pH for the further studies was selected at 7.2.

The effect of temperature on the sensor had been examined between 15 to 60 °C. The response signal of the H_2O_2 sensor increased as the temperature varied from 15 to 35 °C. But at temperature lower than 20 °C, the activity of the enzyme was rather lower and the response time was relatively longer. On the other hand, at temperatures higher than 35 °C, the activity of enzyme decreased rapidly due to the partial denaturation of the enzyme. Taking both the lifetime and response time into consideration, 25 °C was the selected temperature for the present work.

The amount of the enzyme in composite is a vital factor affecting the analytical sensitivity of the biosensor. The amount of HRP used in the fabrication of MWCNT/BCB/HRP/GCE has a profound influence on the electrochemical behavior of electrode towards hydrogen peroxide. The response current increases with increase in the loading of HRP (figure not shown). However, a substantial decrease in current response was observed at higher enzyme loading (>1.5 mg mL^{-1}). This could be due to the decrease in electron relaying capacity by higher enzyme loading and its insulation effect.

3.5. Kinetic Analysis

The apparent Michaelis–Menten constant (K_M), a reflection of the enzyme affinity, was evaluated from the electrochemical version of the Lineweaver–Burk equation,

$$1/I_{ss} = 1/I_{max} + K_M/I_{max} \times 1/C$$

Where I_{ss} is the steady-state current after the addition of substrate, C is the bulk concentration of the substrate and I_{max} is the maximum current measured under saturated substrate condition. The K_M value was determined by analysis of the slope and intercept for the plot of the reciprocals of the cathodic current versus H_2O_2 concentration. The K_M value of the H_2O_2 sensor was determined by steady-state amperometric response and found to be 1.5×10^{-3} M. This value was lower than those of 4.51×10^{-3} M for HRP on AuNP/CHIT/SPCE [34], 4.04×10^{-3} M for HRP on TTF/TCNQ/MWCNT modified electrode [30], 2.5×10^{-3} M for HRP immobilized on a ferrocene containing polymer electrochemically deposited onto a Pt electrode [35], 2.1 mM for sol-gel/nafion/MG electrode [18], 4.6×10^{-3} M for HRP immobilized in sol-gel/hydrogel modified electrode [36], 2.0×10^{-3} M for HRP immobilized on FMC-BSA/MWCNT ormosil composite-modified GC electrode [28] and was close to 1.3×10^{-3} for PDDA/HRP/DNA-Ag/Au modified electrode [37]. The lower value of K_M means that HRP immobilized into the MWCNT/BCB/ormosil nanocomposite retains well its natural bioactivity with high affinity to H_2O_2.

3.6. Analytical Performances of Fabricated H_2O_2 Biosensor

The amperometric response of the MWCNT/BCB/HRP/GCE biosensor was tested in a stirred cell containing 0.1 M PBS (pH 7.2) under the optimized operating electrode potential of -0.32 V. Figure 6 presents the typical amperometric response of MWCNT/BCB/HRP/GCE modified electrode in the presence of 0.1 mM H_2O_2. With increasing H_2O_2 concentration, the amperometric response of the enzyme electrode increased and reached a maximum value within 5s, indicating a fast response of the fabricated biosensor. The faster response of biosensor was mainly due to the biocompatible microenvironment, good porosity and well conductive properties of the MWCNTs modified ormosil nanocomposite [38]. Figure 6 Inset shows calibration curve of prepared biosensor under the optimum conditions. The linear response range of the H_2O_2 biosensor was 5×10^{-7} to 1.8×10^{-5} with a correlation coefficient of 0.9973 and a detection limit of 1×10^{-7} M at a signal to noise ratio of 3. After analyzing the analytical performances of the H_2O_2 biosensor, it can be seen that the developed MWCNT/BCB/HRP/GCE biosensor offered excellent linear range for hydrogen peroxide detection and the detection limit was lower than that of some reported literatures. The results indicated that MWCNT/BCB/HRP/GCE film modified electrode was relatively stable and can be conveniently used for the analytical detection of hydrogen peroxide as a biosensor.

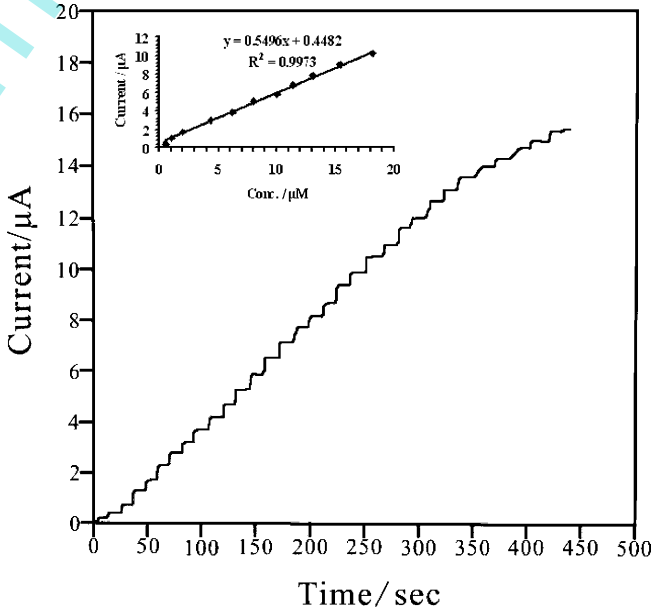

Fig. 6. (A) Amperometric response of MWCNT/BCB/HRP/GCE in a stirred 0.1 M PBS (pH 7.2) after successive hydrogen peroxide additions at applied potential -0.32 V vs. Ag/AgCl. Inset: Linear calibration plot of catalytic currents vs. hydrogen peroxide concentrations for MWCNT/BCB/HRP/GCE.

3.7. Stability, Reproducibility and Selectivity of the Developed H_2O_2 Biosensor

The developed biosensor showed a good reproducibility for the determination of H_2O_2 in its linear range. The concentration of 0.10 m M H_2O_2 was measured consecutively for 10 times, and a relative standard deviation (*RSD*) of 2.4% was obtained. The stability of the proposed biosensor was also investigated. The current response of prepared biosensor was tested every 3 days. The response of the biosensor to 0.10 mM H_2O_2 only decreased 5% of its initial value after a storage period of 21 days, and 12% after a storage period of 45 days. When not in use, the electrode was stored by suspending it above 0.1 M PBS (pH 7.2) at 4 °C in a refrigerator. Such a good stability of the biosensor was attributed to the excellent film forming ability of ormosil/MWCNTs nanospheres and a favorable microenvironment of ormosil/MWCNTs nanospheres for retaining the biological activity of enzymes.

The selectivity and anti-interference ability of the biosensor was investigated by chronoamperometric method. Table 1 shows the amperometric response of the biosensor to the consecutive injection of 0.10 mM H_2O_2 and 0.20 mM interfering species including dopamine, ascorbic acid, NADH, glucose and uric acid. It was evident that the influence of interfering species tested on the H_2O_2 response was negligible, indicating a high selectivity of the proposed biosensor.

Table 1. Interferences studies.

Interferents	Current ratio
Dopamine	0.98
Ascorbic acid	1.00
Glucose	1.00
NADH	1.03
Uric acid	0.99

4. Conclusions

We had introduced a simple strategy to fabricate MWCNT based amperometric hydrogen peroxide biosensor at lower potential in this article. HRP had been successfully co-immobilized on MWCNT modified glassy carbon electrode with redox dye BCB. The developed H_2O_2 biosensor based on the MWCNT/BCB/HRP/GCE possessed high sensitivity, quick response time, low detection limit and excellent selectivity towards hydrogen peroxide. The attractive electrochemical and structural properties of MWCNT suggested the potential application of MWCNT for electrocatalysis and biosensor. This approach provides a simple method to develop a new class of electrochemical biosensor for determining H_2O_2 with the merit of simple preparation, convenient operation, low quantity of enzyme and keeping good activity of enzyme.

5. Acknowledgement

This work was financially supported by The National Science Council of Taiwan.

6. References

[1] Y. M. Yan, O. Yehezkeli, I. Willner, *Chem. Eur. J.* **2007**, *13*, 10168.
[2] Y. Liu, J. Lei, H. Ju, *Talanta* **2008**, *74*, 965.
[3] L. Q. Rong, C. Y. Q. Y. Quan, X. H. Xia, *Talanta* **2007**, *72*, 819.
[4] Z. Song, J. Huang, B. Wu, Z. Zhao, L. Huang, Q. Chen, *Sens. Actuators B* **2006**, *115*, 626.
[5] X. Yu, B. Munge, V. Patel, G. Jensen, A. Bhirde, J. D. Gong, S. N. Kim, J. Gillespie, J. S. Gutkind, F. Papadimitrakopoulos, J. F. Rusling, *J. Am. Chem. Soc.* **2006**, *128*, 11199.
[6] X. H. Shu, Y. Chen, H. Y. Yuan, S. F.Gao, D. Xiao, *Anal. Chem.* **2007**, *79*, 3695.
[7] L. Wang, E. Wang, *Electrochem. Commun.* **2004**, *6*, 225.
[8] J. Li, P. K. Dasgupta, G. A. Tarver, *Anal. Chem.* **2003**, *75*, 1203.
[9] N. V. Klassen, D. Marchington, H. C.E. McGovan, *Anal. Chem.* **1994**, *66*, 2921.
[10] A. Lobnik, M. Cajlakovuc, *Sens. Actuators B* **2001**, *74*, 194.
[11] S. H. Chen, R. Yuan, L. Y. Zhang, N. Wang, X. L. Li, *Biosens. Bioelectron.* **2007**, *22*, 1268.
[12] L. Gorton, G. Jonsson, *J. Mol. Catal.* **1986**, *38*, 157.
[13] J. Wang, M. Gu, J. Di, Y. Gao, Y. Wu, Y. Tu, *Biopro. Biosyst. Eng.* **2007**, *30*, 289.
[14] W. Li, R. Yuan, Y. Chai, L. Zhou, S. Chen, Na Li, *J. Biochem Biophys. Meth.* **2008**, *70*, 830.
[15] Y. Xiao, H. X. Ju, H. Y. Chen, *Anal. Chem. Acta* **1999**, *391*, 73.
[16] C. X. Lie, S. Q. Hu, G. L. Shen, R. Q. Yu, *Talanta* **2003**, *59*, 981.
[17] Y. Song, L. Wang, C. Ren, G. Zhua, Z. Li, *Sens. Actuators B, Chem.* **2006**, *114*, 1001.
[18] B. Wang, S. Dong, *Talanta* **2000**, *51*, 565.
[19] A. Vasilescu, S. Andreescu, C. Bala, S. C. Litescu, T. Naguer, J. L. Mart, *Biosens. Bioelectron.* **2003**, *18*, 781.
[20] A. Lupu, P. Lisboa, A. Valsesia, P. Colpo, F. Rossi, *Sens. and Actua. B: Chemical* **2009**, *137*, 56.
[21] A. Noorbakhsh, A. Salimi, E. Sharifi, *Electroanalysis* **2008**, *20*, 1788.
[22] D. Avnir, O. Lev, J. Livage, *J. Mater. Chem.* **2006**, *16*, 1013.
[23] R. Ciriminna, L. M. Ilharco, A. Fidalgo, S. Campestrinic, M. Pagliaro, *Soft Matter* **2005**, *1*, 231.
[24] D. R. S. Jaikumari, S. Ramaprabhu, S. S. Narayanan, *Carbon* **2007**, *45*, 1340.
[25] X. Kang, J. Wang, Z. Tang, H. Wu, Y. Lin, *Talanta* **2009**, *78*, 120.
[26] Y. Yang, G. Yang, Y. Huang, H. Bai, X. Lu, *Engineering Aspects* **2009** in press.
[27] Y. Liu, J. Lie, H. Xu, *Talanta* **2007**, *74*, 965.
[28] V. S. Tripathi, V. B. Kandimalla, H. Ju, *Biosens. Bioelectron.* **2006**, *21*, 1529.
[29] J. Xu, J. Zhu, Q. Wu, Z. Hu, H. Chen, *Electroanalysis* **2003**, *15*, 219.
[30] Z. Cao, X. Jiang, Q. Xie, S. Yao, *Biosens. Bioelectron.* **2008**, *24*, 222.
[31] A. F. Groboillot, C. P. Champange, G. D. Darling, D. Poneelet, R. J. Neufild, *Biotech. Bioeng.* **1993**, *42*, 1157.
[32] B. Wang, J. J. Zhang, Z. Y. Pan, X. Tao, H. Wang, *Biosens. Bioelectron.* **2009**, *24*, 1141.

[33] X. Yang, Y. Lu, Y. Ma, Z. Liu, F. Du, Y. Chen, Biotech. Lett. **2007**, *29*, 1775.
[34] M. P. G. Armada, J. Losada, I. Cuadrado, B. Alonso, B. González, C. M. Casado, J. Zhang, *Sens. and Actua. B* **2004**, *101*, 143.
[35] B. Wang, B. Li, Z. Wang, G. Xu, Q. Wang, S. Dong, *Anal. Chem.* **1999**, *71*, 1935.
[36] T. Tangkuaram, C. Ponchio, T. Kangkasomboon, P. Katikawong, W. Veerasai, *Biosen. Bioelectro.* **2007**, *22*, 2071.
[37] L. Ma, R. Yuan, Y. Chai, S. Chen, *J. Mole. Cata. B* **2009**, *56*, 215.
[38] B. Q. Wang, B. Li, Q. Deng, S. J. Dong, *Anal. Chem.* **1998**, *70*, 170.

