Int. J. Electrochem. Sci., 6 **(2011)** 2223 - 2234

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Fabrication of a Cholesterol Biosensor Based on Cholesterol Oxidase and Multiwall Carbon Nanotube Hybrid Composites

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Received: 2 May 2011 / *Accepted:* 22 May 2011 / *Published:* 1 June 2011

A cholesterol biosensor of ChOx/MWCNTs containing multiwall carbon nanotubes (MWCNTs) and cholesterol oxidase (ChOx) has been successfully synthesized on glassy carbon electrode (GCE). The presence of MWCNTs not only enhances the surface coverage (Γ) but also exhibits a promising enhanced electrocatalytic activity to hydrogen peroxide. It is stable, pH-dependent, and electroactive as produced on electrode surface. The average surface coverage (Γ) was estimated about 1.86×10^{-11} mol cm^{-2} higher than both of MWCNTs and ChOx modified electrodes. Using the equation $E_p = K - 2.303(RT/nF)\log v$ and the two electrons transferred for ChOx a charge transfer coefficient 0.22 was obtained. An apparent surface electron transfer rate constant (K_s) 0.0269 s^{-1} was estimated for the redox peaks. Compared with the SEM images of the MWCNTs/GCE and ChOx/GCE, the ChOx/MWCNTs film shows the significant surface morphology. The electrocatalytic responses of ChOx/MWCNTs were investigated with various analytes by cyclic voltammetry (CV) and amperometry (AMP). Compared with bare electrode and ChOx/GCE, the ChOx/MWCNTs/GCE has better electrocatalytic current response to cholesterol. This sensor shows excellent performance with a sensitivity of 1261.74 μA mM^{-1} cm^{-2}, a linear range of 4.68×10^{-5} – 2.79×10^{-4} M, and a detection limit of 4.68×10^{-5} M.

Keywords: Multiwall carbon nanotubes, cholesterol oxidase, modified electrodes, electrocatalysis, cholesterol.

1. INTRODUCTION

Cholesterol is essential as it has many major functions in all animal life. For example, cholesterol is an important compound in cell membranes as it can regulate the membrane over a range of physiological temperatures [1]. Even though cholesterol is important for life, elevated levels in circulation are associated with atherosclerosis. Therefore, the estimation of cholesterol quantity is of interest to both the biological science and food industries [2]. Cholesterol oxidase (ChOx) is one such

water-soluble enzyme that is catalytically active at the membrane interface from which cholesterol, the substrate, is accessed [3]. Cholesterol is oxidized to cholest-5-en-3-one by the flavin cofactor. The reduced cofactor is recycled by oxygen to form hydrogen peroxide. This product is the basis of the serum cholesterol assays, because hydrogen peroxide can be coupled to colorimetric assays using horseradish peroxidase (HRP). However, the cholest-5-en-3-one intermediate is not particularly stable [1-4]. Various studies have been performed using ChOx to precisely monitor the cholesterol level in its biological environment [4]. Although, some studies have been achieved success in this direction, there have been no reports revealing the direct electron transfer (DET) of ChOx until now. Immobilization of enzymes to solid electrode surface is a key step for the design, fabrication and performance of the biosensor, since it is well known that some enzymes retain their activity when they are immobilized [5].

Carbon nanotubes (CNTs) can be described as a sheet of carbon atoms rolled up into a cylinder held together by van der Waals interactions [6-10]. Multiwall carbon nanotubes (MWCNTs) consist of concentric cylindrical shells of graphene sheets arranged around a central hollow area. Since their discovering [6], they have gained considerable interest due to their unique structural, mechanical and chemical properties [6-10]. The first attempt to use CNTs for developing an electrochemical sensor based on CNTs was performed by Britto et al. [11] who demonstrated the excellent electrocatalytic properties of single wall carbon nanotubes (SWCNTs) dispersed within bromoform. Since then, the interest of using CNTs for developing modified electrodes has largely increased due to their known advantages connected with the high surface area, favorable electronic properties and electrocatalytic effects [12-16]. Compton et al. [17-19] have demonstrated that the interesting electrocatalytic properties of CNTs are mostly due to the presence of defects like the edge planes of pyrolytic graphite located mainly at the end of the tubes. The electrochemical detection of hydrogen peroxide has been thoroughly studied at CNT ensemble networks with a range of parameters explored to observe how these may affect the electrochemical performance [20]. Recently researches, the CNT hybrid composites are also wildly studied in chemical sensors [21] and biosensors [22].

In the present work, multiwall carbon nanotubes/ChOx film modified glassy carbon electrode (ChOx/MWCNTs/GCE) was prepared and its electrocatalytic behavior towards cholesterol was investigated using cyclic voltammetry. The modified electrode showed electrocatalytic activity only towards cholesterol. We have also attempted to utilize ChOx/MWCNTs/GCE electrode to measure the cholesterol.

2. EXPERIMENTAL

2.1. Reagents and chemicals

Multiwall carbon nanotubes (Aldrich) was used as received. Cholesterol oxidase (ChOx), Cholesterol (99%), N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), were obtained from Sigma. All other chemicals used were of analytical grade and used without

further purification. The phosphate buffer solution (0.1 M PBS) was used as supporting electrolyte. All aqueous solutions were prepared using doubly distilled deionized water.

2.2. Apparatus

All electrochemical experiments were carried out with a single compartment cell, in which a BAS (Bioanalytical Systems, West Lafayette, IN) glassy carbon electrode (area = 0.07 cm^2) was used as working electrode and a platinum wire served as counter electrode. An Ag|AgCl (saturated KCl) reference electrode was used to monitor the cell potentials. Cyclic voltammetry (CV) and amperometry (AMP) experiments were performed with CH Instruments. Amperometric measurements were carried out under stirred condition. All the experiments were carried out at room temperature (≈ 25°C). The morphological characterization of composite films was examined by means of SEM (Hitachi S-3000H) and AFM (Being Nano-Instruments CSPM-5000).

2.3. Preparation of ChOx/MWCNTs/GCE modified electrodes

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 hours. Prior to modification, GCE electrode was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. The cleaned GCE surface was dipped with 2 μL of the prepared MWCNTs solution and then dried out at room temperature. Then, it was dipped with 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 hours. This electrode was further immersed in fresh phosphate solution containing 1 mg mL^{-1} ChOx. The modified electrode was rinsed with supporting electrolyte to be used for the further studies.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of ChOx/MWCNTs film

ChOx is covalently attached to MWCNTs modified electrode by activating -COOH group using EDC as the coupling agent and NHS as activator. The EDC-NHS-activated -COOH group binds with $-NH_2$ group of ChOx resulting in the formation of a covalent amide bond (CO-NH). For covalent attachment, MWCNTs electrode is first dipped in 0.1 M PBS containing 0.4 M EDC and 0.1 M NHS at room temperature for 4 h to activate -COOH group present on the functionalized MWCNT [22]. The film formed on the electrode surface can be expressed as the Scheme 1.

This film modified electrode was electrochemically characterized in 0.1 M PBS (pH 7.0) by cyclic voltammetry. Fig. 1 shows the voltammograms of (a) bare GCE, (b) MWCNTs/GCE and (c) ChOx/MWCNTs/GCE, respectively. The corresponding cyclic voltammograms have been examined at 100 mV s^{-1} in the potential range of 0 – -0.9 V. Both bare GCE and MWCNTs/GCE show 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 flavin adenine dinucleotide (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. [23]. The MWCNTs/ChOx/GCE is well known for an obvious redox couple ($E^{0'}$ = -0.4 V) which is involving the FAD/$FADH_2$ redox process of ChOx. Further, it has been observed that the presence of MWCNTs increases the overall back ground current. These results are evident with the active surface coverage (Γ = Q/nFA) given in Table 1. The surface coverage was calculated in 3.53×10^{-12} mol cm^{-2}, 1.58×10^{-11} mol cm^{-2}, and 1.86×10^{-11} mol cm^{-2} for ChOx/GCE, MWCNTs/GCE, and ChOx/MWCNTs/GCE, respectively. This means that the overall surface coverage of ChOx/MWCNTs/GCE is increasing in the presence of MWCNTs.

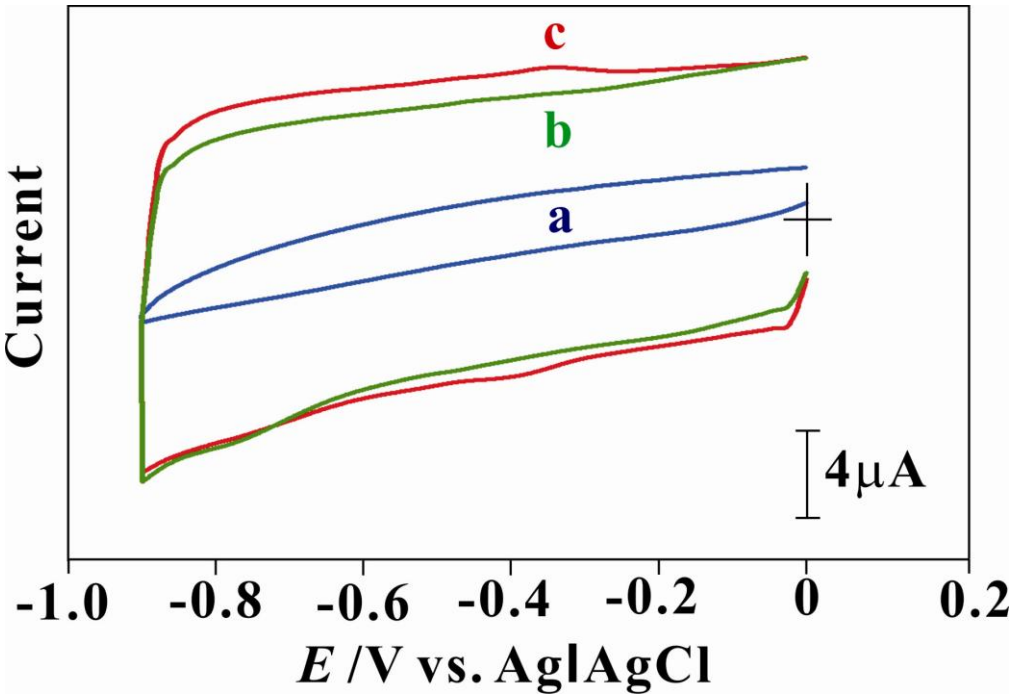


Figure 1. Cyclic voltammograms of (a) bare GCE, (b) MWCNTs/GCE, and (c) ChOx/MWCNTs/GCE examined in 0.1 M PBS (pH 7.0), scan rate = 100 mV s^{-1}.

Table. 1. Active surface coverage concentration (Γ) of ChOx at different types of modified and unmodified electrodes using CV technique in PBS (pH 7.0).

Types of modified electrodes	Γ (mol cm^{-2})
ChOx/GCE	3.53×10^{-12}
MWCNTs/GCE	1.58×10^{-11}
ChOx/MWCNTs/GCE	1.86×10^{-11}

In the present paper, MWCNTs and ChOx hybrid film (ChOx/MWCNTs) was firstly modified onto the electrode surface to study the electrocatalytic reaction of cholesterol. The electrochemical properties of ChOx/MWCNTs modified GCE were studied with various scan rates and pH solutions by cyclic voltammetry. Fig. 2 shows the cyclic voltammograms of the resulting electrode obtained with various scan rates in 0.1 M PBS (pH 7.0). The electrochemical response of ChOx/MWCNTs/GCE exhibits one stable redox couple, in which can be attributed to the electron transformations between MWCNTs and ChOx in the hybrid film. Fig. 2 shows the cyclic voltammetric response of the ChOx/MWCNTs/GCE examined at different scan rates (10 to 3000 mV/s) in 0.1 M PBS (pH 7.0). Inset (a) of Fig. 2 exhibits that the linearity between peak current (anodic and cathodic peak current) and the scan rate. The ratio of I_{pa}/I_{pc} is closed to 1 has demonstrated that the redox process has not been controlled by diffusion. However, the ΔE_p of each scan rate reveals that the peak separation of composite redox couple increases as the scan rate is increased.

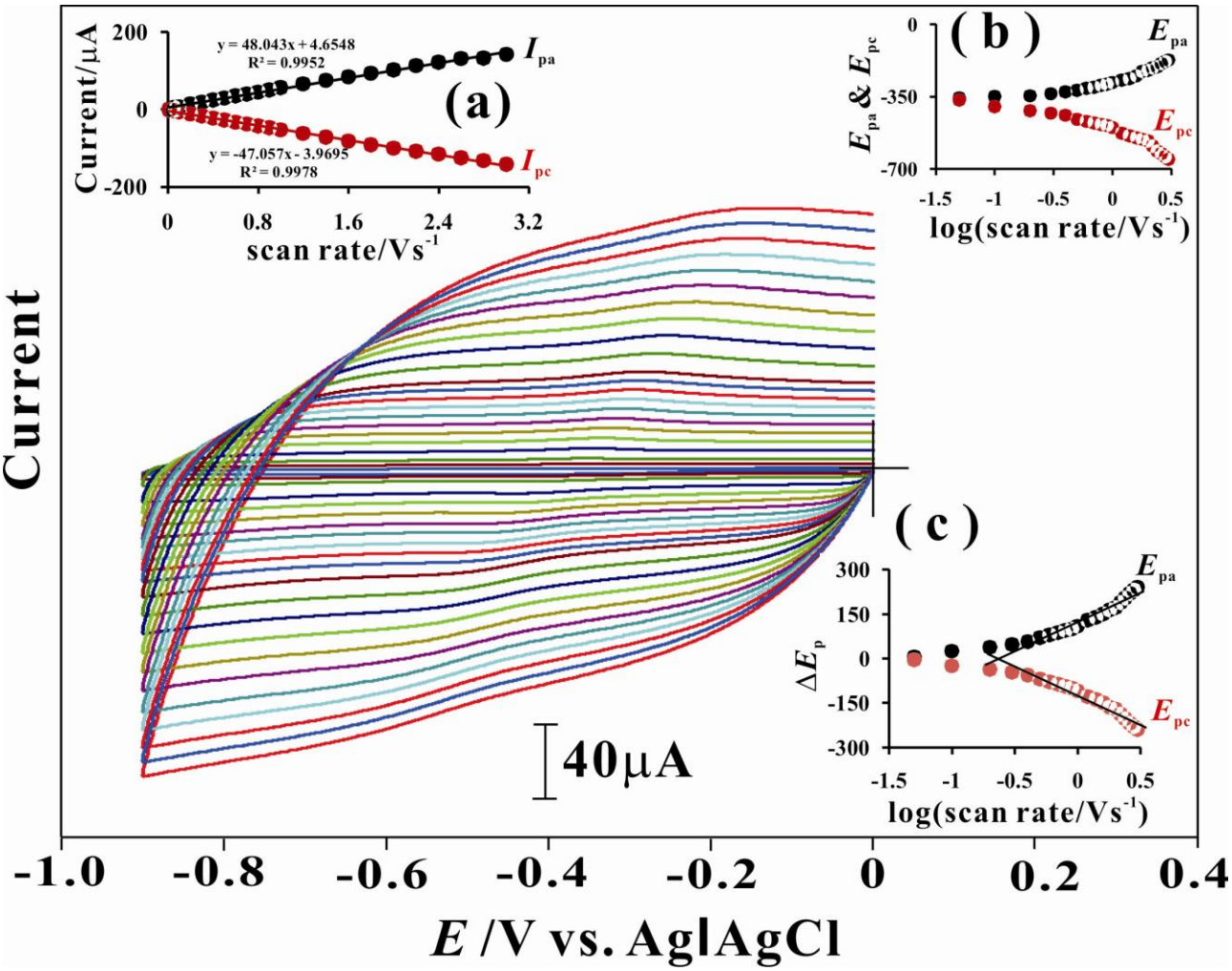


Figure 2. Cyclic voltammograms of ChOx/MWCNTs/GCE examined in 0.1 M PBS (pH 7.0) with various scan rates of 10 – 3000 mV s^{-1}, respectively. Insets: (a) plot of peak current (I_{pa} & I_{pc}) vs. scan rate (v) and (b) plot of peak separation (ΔE_p) vs. logarithmic scan rate (log(v)).

The dependence of the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) of the ChOx/MWCNTs/GCE on the log(v) in pH 7 PBS is depicted in the inset (b) of Fig. 2. At lower scan rates, E_{pa} and E_{pc} remained almost unchanged with the increase of scan rate. However, E_{pa} positively and E_{pc} negatively shifted at higher scan rates. At higher scan rates, the electron transfer coefficient (α) and the apparent surface electron transfer rate constant (K_s) can be obtained from Laviron theory [24]. The peak-to-peak potential separation ($\Delta E_p = E_{pa} - E_{pc}$) is about 60 mV for ChOx/MWCNs redox peaks obtained below 100 mV s^{-1}, suggesting facile charge transfer kinetics over these scan rates. Based on Laviron theory [25], the electron transfer rate constant (K_s) and charge transfer coefficient (α) can be determined by measuring the variation of peak potential with scan rate. The values of peak potentials were proportional to the logarithm of the scan rate for scan rates higher than 200 mV s^{-1} (inset (c) of Fig. 2). The slope of ΔE_p versus log(v) was about 398 mV for ChOx redox peaks. Using the equation $E_p = K - 2.303(RT/nF)\log(v)$ and the two electrons transferred for ChOx a charge transfer coefficient 0.22 was obtained. Introducing these values in the equation [26] as following:

$$\log K_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log(RT/nFv) - \alpha (1 - \alpha)nFE/2.3RT \qquad (1)$$

An apparent surface electron transfer rate constant (K_s) 0.0269 s^{-1} was estimated for reversible redox peaks in ChOx/MWCNTs/GCE.

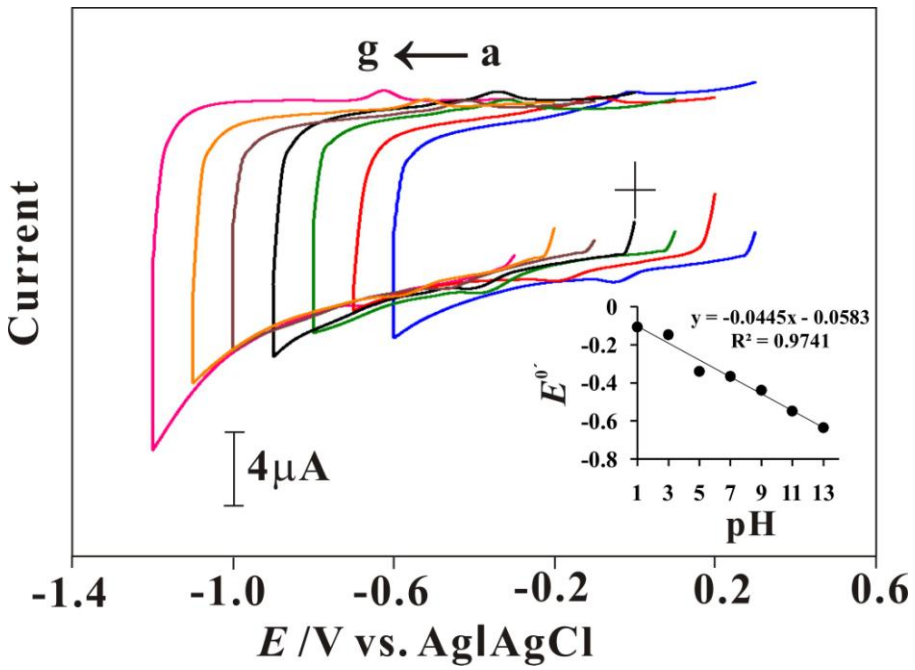


Figure 3. Cyclic voltammograms of ChOx/MWCNTs/GCE examine with various pH solutions of pH 1 – 13. Inset: the plot of formal potential ($E^{0'}$) vs. pH.

Fig. 3 shows the cyclic voltammograms of ChOx/MWCNTs/GCE examined in various pH solutions. This shows that the film is highly stable in the pH range of pH 1–13. The formal potential depends on the pH value of the buffer solution. Inset of Fig. 3 shows the plot of formal potential of

ChOx/MWCNTs versus pH value of the buffer solutions. It shows linearity with a slope of -44.5 mV pH^{-1}. This close to that expected from calculations using Nerstian equation. The phenomenon indicates that the number of electrons and protons is the same. In our case, two electrons and two protons were involved in the ChOx redox couple. The above results show that the ChOx/MWCNTs hybrid film is stable and electrochemically active in the aqueous buffer solutions.

3.2. Morphology study of ChOx/MWCNTs by SEM

The surface morphology of the sensing area of ChOx/MWCNTs was examined using SEM. The ChOx/MWCNT was prepared on ITO electrode to study.

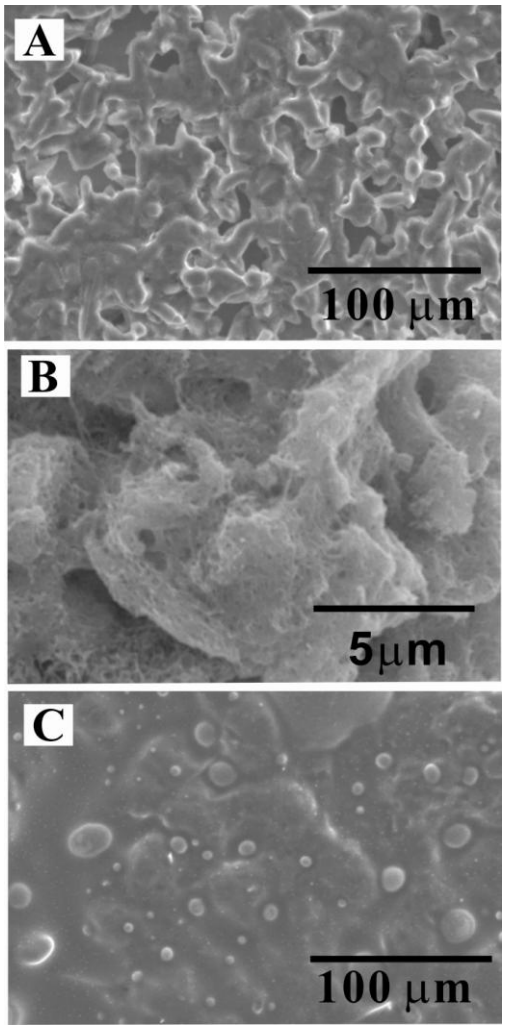


Figure 4. SEM images of (A) ChOx/ITO, (B) MWCNTs/ITO, (C) ChOx/MWCNTs/ITO.

Fig. 4 shows the SEM images of (A) ChOx/ITO, (B) MWCNTs/ITO, and (C) ChOx/MWCNTs/ITO, respectively. These three films display obvious different morphology. From the ChOx/ITO image (shown in Fig. 4A), it looks like that the aggregation of macro enzyme cluster. From

the MWCNTs/ITO image (shown in Fig. 4B), it looks like that the aggregation of the MWCNTs lead to the porous structure. Particularly, the ChOx/MWCNTs/ITO image (shown in Fig. 4C) shows the significant difference from both ChOx and MWCNTs. It looks like that the ChOx cover over the MWCNTs. This morphological difference might also indicate that the activity of the modified electrode surface might be different from both MWCNTs and ChOx. They were further discussing in the electrocatalytic reaction of cholesterol in the section 3.3.

3.3. Electrocatalytic reaction of cholesterol by ChOx/MWCNTs/GCE

The electrocatalytic reaction of cholesterol was examined at ChOx/MWCNTs modified electrode with the potential range of 0 – -0.9 V and the scan rate of 100 mV s^{-1} in 0.1 M PBS (pH 7.0).

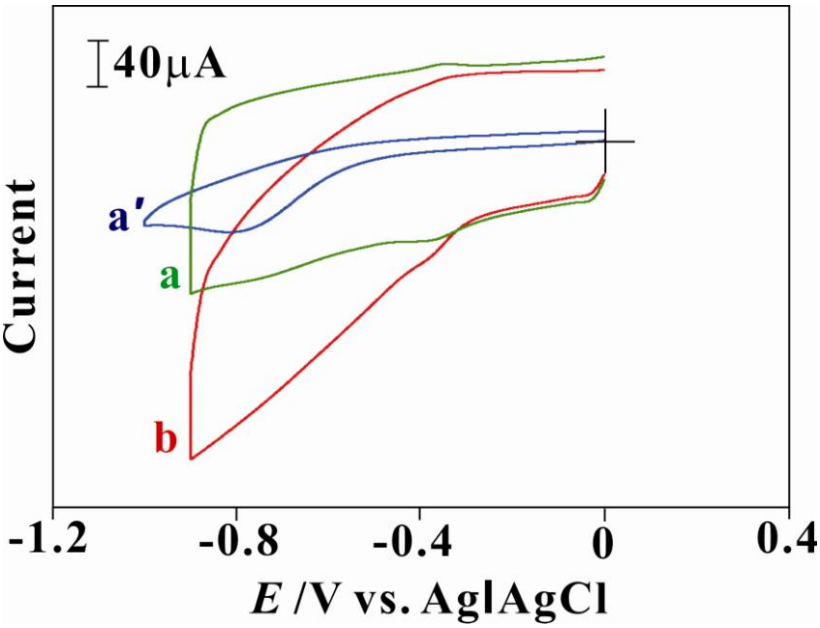
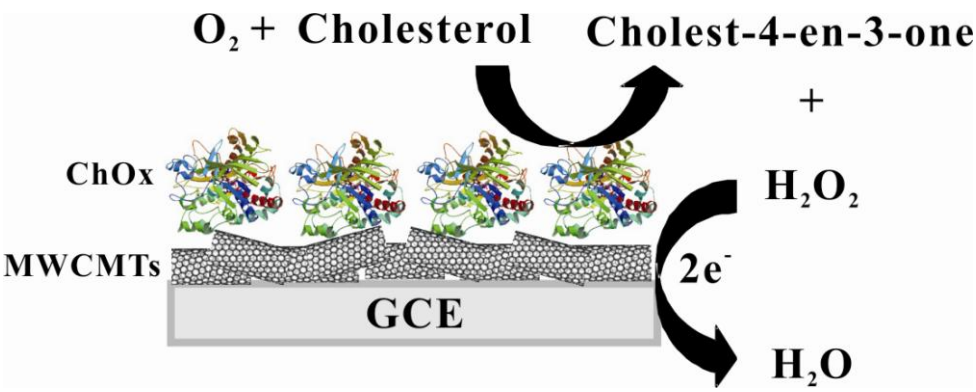


Figure 5. Cyclic voltammograms of (a') bare GCE and (b) ChOx/MWCNTs/GCE examined in 0.1 M PBS (pH 7.0) containing 28 mM cholesterol, scan rate = 100 mV s^{-1}. (a) Cyclic voltammogram of ChOx/MWCNTs/GCE in the absence of cholesterol.



Scheme 1. Electrocatalytic reaction of cholesterol by ChOx/MWCNTs modified electrodes.

Fig. 5 shows the cyclic voltammogram of ChOx/MWCNTs 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 ChOx/MWCNTs/GCE 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 as following equation:

$$\text{Cholesterol} + O_2 \text{ (in the presence of ChOx)} \rightarrow \text{Cholest-4-en-3-one} + H_2O_2 \qquad (2)$$

Since the H_2O_2 is the byproduct of cholesterol oxidation, the electroreduction current of H_2O_2 can be further detected by the ChOx/MWCNTs/GCE electrode as shown in Scheme 1.

Furthermore, the catalytic current response can be directly proportional to cholesterol concentration if increasing the cholesterol content in the system. As the result, this electrode is found stable and electroactive for the electrocatalytic reduction of cholesterol. Further quantifying cholesterol was studied by amperometry (in the section 3.4).

3.4. Amperometric response of ChOx/MWCNTs/GCE for cholesterol determination

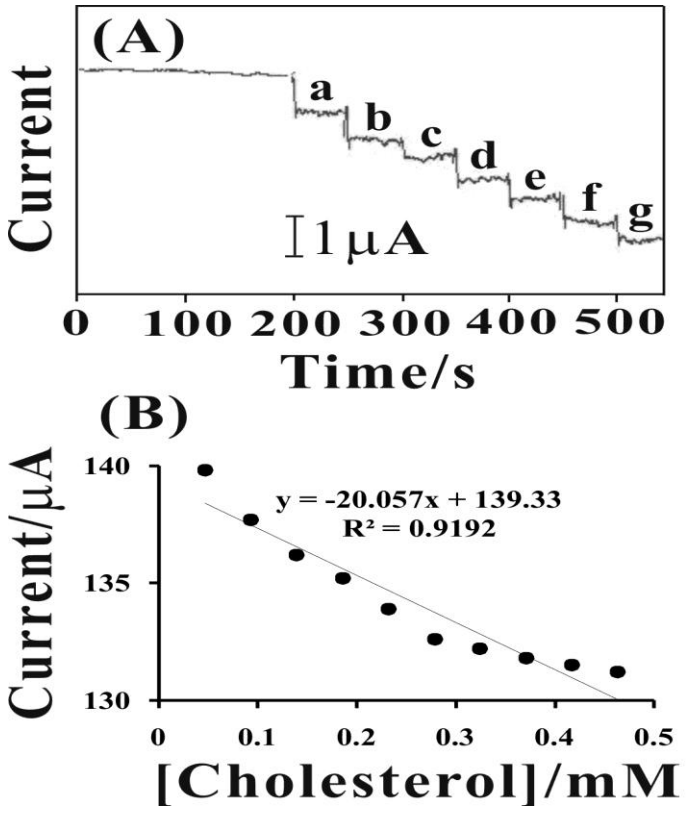


Figure 6. (A) Amperometric current responses of ChOx/MWCNTs/GCE examined in 0.1 M PBS (pH 7.0) containing [cholesterol] = (a) 4.68×10^{-5} M, (b) 9.36×10^{-5} M, (c) 1.40×10^{-4} M, (d) 1.87×10^{-4} M, (e) 2.34×10^{-4} M, (f) 2.81×10^{-4} M, and (g) 3.28×10^{-4} M, respectively. Applied potential (E_{app}) at -0.8 V. Electrode rotation rate = 1000 rpm. (B) Plot of catalytic current vs. cholesterol concentration.

Table. 2. Performance comparison of various cholesterol biosensors based on cholesterol oxidase.

Immobilization matrix	Transducer method	E_{app} (V)	Detection limit (μM)	Linearity (μM)	Sensitivity (μA mM^{-1} cm^{-2})	Ref.
Polyaniline/ChOx	Spectrophotometry	–	643	$72 – 1\times10^3$	3.016 (Abs M^{-1} cm^{-2})	[27]
Carbon nanotube–chitosan–Pt/ChOx	Amperometry	0.4	4.9	–	44	[28]
Polyaniline - MWCNT/ChOx	Amperometry and Spectrophotometry	0.28	-	$1.26\times10^3 – 1.293\times10^4$	6.8	[29]
NanoZnO–chitosan/ChOx	Amperometry	0.3	128.65	0.129 – 771.9	21945	[30]
Chitosan–$NanoCeO_2$/ChOx	Cyclic voltammetry	0.25	128.65	$25.73 – 1\times10^3$	18288	[31]
ZnO/ChOx	Amperometry	0.355	0.37	$1 – 5\times10^3$	23.7	[32]
PEDOT/ChOx	Amperometry	0.7	400	–	10	[33]
ZnO nanorods/ChOx	Potentiometry	0.22	–	$1 – 1\times10^4$	35.2 (mV $decade^{-1}$)	[34]
AuPt–chitosan–ionic liquid/ChOx	Amperometry	-1	10	$50 – 6.2\times10^3$, $6.2\times10^3 – 1.12\times10^4$	90.7	[35]
Au-dithiol-AuNPs/ChOx	Cyclic voltammetry	0.45	34.6	40 – 220	45.9	[36]
MWCNT/ChOx	Amperometry	-0.8	46.8	48.6 – 279	1261.7	This work

Fig. 6A shows the amperomograms of MWCNTs/Cox/GCE for cholesterol determination with micro-injection of cholesterol (4.68×10^{-5} M per 50 s).

The electrode potential was applied at -0.8 V, the current response was directly proportional to the cholesterol concentration. The sensor achieves 95% of steady-state current in less than 5 s. Such a short response time indicates fast mass transfer across the film and also fast electron exchange between mediator and analyte.

As shown in Fig. 6B, the calibration curve was found linear correlation between electrocatalytic current and cholesterol concentration. The steady-state reduction current varies linearly with cholesterol concentrations in the entire concentration range between 4.68×10^{-5} and 2.79×10^{-4} M. The sensitivity of ChOx/MWCNTs electrode was 1261.74 μA mM^{-1} cm^{-2} with a correlation coefficient

of 0.9707. This sensor has competitive performance when compared with other ChOx based cholesterol biosensors (Table 2).

4. CONCLUSION

We fabricate a cholesterol biosensor based on the combination of ChOx and MWCNTs. It is stable, pH-dependent, and electroactive by the study of electrochemistry and SEM morphology. The presence of MWCNTs not only enhances the surface coverage (Γ) but also exhibits a promising enhanced electrocatalytic activity to hydrogen peroxide. High sensitivity, stability, and very easy preparation of ChOx/MWCNTs electrode make it a promising candidate for cholesterol determination.

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

References

1. Z.Y. Chen, K.Y. Ma, Y. Liang, C. Peng, Y. Zuo, *J. Funct. Foods* 3 (2011) 61.
2. D.S. Fredrickson, R.I. Levy, *The Metabolic Basis of Inherited Disease*, McGraw-Hill, New York (1972) 545.
3. N.S. Sampson, S. Kwak, *ESCEC* (2007) Germany.
4. A.A. Abdelwahab, M.S. Won, Y.B. Shim, *Electroanalysis* 22 (2010) 21.
5. A.Salimi, R. Hallaj, S. Soltanianb, *Electroanalysis* 21 (2009) 2693.
6. S. Ijima, *Nature* 354 (1991) 56.
7. K. Balasubramanian, M. Burghard, *Small* 1 (2005) 180.
8. H. Dai, *Acc. Chem. Res.* 35 (2002) 1035.
9. P.M. Ajayan, *Chem. Rev.* 99 (1999) 1787.
10. H. Dai, *Surf. Sci.* 500 (2002) 218.
11. P.J. Britto, K.S.V. Santhanam, P.M. Ajayan, *Bioelectrochem. Bioenerg.* 41 (1996) 121.
12. M. Trojanowicz, M. Szewczynska, *Trends Anal. Chem.* 24 (2005) 92.
13. J.J. Gooding, *Electrochim. Acta* 50 (2005) 3049.
14. A.Merkoci, M. Pumera, X. Llopis, B. Perez, M. del Valle, S. Alegret, *Trends Anal. Chem.* 24 (2005) 826.
15. J. Wang, *Electroanalysis* 17 (2005) 7.
16. G. Rivas, M.D. Rubianes, M.C. Rodr_guez, N. Ferreyra, G.L. Luque, M.L. Pedano, C. Parrado, *Talanta* 74 (2007) 291.
17. C.E. Banks, A. Croosley, C. Salter, S. J. Wilkins, R.G. Compton, *Angew. Chem. Int. Ed.* 45 (2006) 2533.
18. C.E. Banks, T.J. Davies, G.G. Wildgoose, R.G. Compton, *Chem. Commun.* (2005) 829.
19. C.E. Banks, R.G. Compton, *Analyst* 131 (2006) 15.
20. J. Kruusma,V. Sammelselg,C.E. Banks., *Electrochem. Commun.* 10 (2008) 1872.
21. H.R. Zare, N. Nasirizadeh, *Int. J. Electrochem. Sci.*, 4 (2009) 1691.
22. P. Norouzi, F. Faridbod, E. Nasli-Esfahani, B. Larijani, M.R. Ganjali, *Int. J. Electrochem. Sci.,* 5 (2010) 1008.
23. Z. Matharu, P. Pandey, M.K. Pandey,V. Gupta, B.D. Malhotraa, *Electroanalysis* 21 (2009) 1587.

24. J.R. Harris, *Cholesterol Binding and Cholesterol Transport Proteins*, first ed., Springer, UK (2010) 137-148.
25. E. Laviron, *J. Electroanal. Chem.* 101 (1979) 19-28.
26. E. Laviron, *J. Electroanal. Chem*. 52 (1974) 355-393.
27. C. Dhand, S.P. Singh, Sunil K. Arya, M. Datta, B.D. Malhotr, *Anal. Chimi. Acta* 602 (2007) 244.
28. Y.C. Tsai, S.Y. Chen, C.A. Lee, *Sens. Actuators B* 135 (2008) 96.
29. C. Dhand, S.K. Arya, M. Datta, B.D. Malhotra, *Anal. Biochem.* 383 (2008) 194.
30. R. Khan, A. Kaushik, P.R. Solanki, A.A. Ansari, M.K. Pandey, B.D. Malhotra, *Anal. Chimi. Acta* 616 (2008) 207.
31. B.D. Malhotra, A. Kaushik, *Thin Solid Films* 518 (2009) 614.
32. A.Umar, M.M. Rahman, M. Vaseem, Y.B. Hahn, *Electrochem. Commun.* 11 (2009) 118.
33. Ö. Türkarslan, S.K. Kayahan, L. Toppare, *Sens. Actuators B* 136 (2009) 484.
34. M.Q. Israr, J.R. Sadaf, M.H. Asif, O. Nur, M. Willander, B. Danielsson, *Thin Solid Films* 519 (2010) 1106.
35. A.Safavi, F. Farjami, *Biosens. Bioelectronics* 26 (2011) 2547.
36. U. Saxena, M. Chakraborty, P. Goswami, *Biosens. Bioelectronics* 26 (2011) 3037.