



Modification Strategy of Screen-Printed Carbon Electrode with Functionalized Multi-Walled Carbon Nanotube and Chitosan Matrix for Biosensor Development

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Biosensor is a rapid technique to determine the analyte based on the incorporation of bioactive materials with physio-chemical transducing element. In this work, multi-walled carbon nanotube (MWCNT) was functionalized with carboxylic groups and mixed with chitosan (CS) prior to drop casting on screen-printed carbon electrode (SPCE). The acid treatment of multi-walled carbon nanotube by using mixture of H₂SO₄ and HNO₃ in 3:2 ratio was better compared to treatment by piranha solution, in terms of carboxylic group attachment and capacitance effect on the current response. The functionalized carbon nanotubes were characterized by using fourier transform infrared spectroscopy, field emission scanning electron microscopy and cyclic voltammetry analysis. Few modification parameters have been optimized to maximize the performance of working electrode including ratio of MWCNT:CS, coating volume and drying method. The addition of MWCNT/CS matrix on the screen-printed carbon electrode greatly enhanced the electron transfer rate and increased the electrode active surface area for further immobilization of bioactive components.

Keywords: Screen-printed carbon electrode, Multi-walled carbon nanotube, Chitosan, Biosensor.

INTRODUCTION

In electrochemical biosensor, the stability of sensor surface or transducer is very crucial for the immobilization of biological substances. This is highly contributed to the sensitivity of the developed sensor. The transducer is responsible to measure the current produced from the oxidation and reduction reaction of the electroactive components. It is usually made from conductive materials such as mercury, noble metals (gold and platinum), carbon and polymeric conductors. The working electrode should offer great signal-to-noise properties and also a reproducible response [1]. Besides that, the electrical conductivity, mechanical properties, potential window, surface reproducibility, cost, availability and toxicity are also need to be considered.

The transducer, screen-printed carbon electrode alone is already able to provide a suitable and convenient working site for the biochemical reactions. However, various attempts have been done to improve the performance, as well as the sensitivity of the sensors including integrating nanomaterials [2]. For example, nanoparticles, nanowires as well as nanotubes offer outstanding prospects for interfacing biological recognition events with electric signal transduction [3]. The incorporation

of these materials is believed to enhance the current produced from the reaction. The work presented in this paper describes the usage of multi-walled carbon nanotube and chitosan as the modification materials for screen-printed carbon electrode.

Carbon nanotube is widely being used in biosensor application compared to others. Carbon nanotube (CNT) can be referring to a hexagonal network or sheet of carbon atoms rolled up into a tube with a diameter of around tens of nanometers [2] or can be simply known as a layer of graphite rolled-up into a cylinder. Carbon nanotubes have advantages over their small size, high strength, high electrical and thermal conductivity and high specific surface area [4].

Furthermore, carbon nanotubes need to be functionalized prior to application in biosensor because of the lack of solubility in aqueous media. Therefore, solubilization and functionalization have been modified the surface of nanotube by change it from hydrophobic to hydrophilic which can be done by chemical, electrochemical, thermal, plasma oxidation treatment, adsorption, electrostatic interaction or covalent bonding of different molecules [5,6].

In this study, screen-printed carbon electrode was modified using multi-walled carbon nanotube and chitosan before proceed to development of a sensitive immunosensor

to detect toxic compounds in foods. This modification is believed to provide a convenience and suitable matrix for the immobilization of biological elements, aiming at improving their state of interactions with macromolecules and optimize the final properties of the hybrid nanomaterials [7]. Immobilization method of antibodies is very crucial prior to analysis to ensure the biological activity of antibody and produce efficient binding [8]. The physical adsorption of antibody immobilization on solid phase is reliant on the non-specific interaction between antibody and solid phase such as hydrogen bonding. Antibody immobilization was greatly improved due to contact between hydrophilic antibody and hydrophilic carbon nanotube and nanodiamond, subsequently promoting the antigen binding [9].

EXPERIMENTAL

Multi-walled carbon nanotube (> 98 %) and medium molecular weight chitosan were purchased from Sigma. All chemicals and reagents were used without further purification including sulphuric acid, nitric acid, hydrogen peroxide, sodium chloride, potassium chloride, sodium phosphate dibasic (Na_2HPO_4) and anhydrous potassium phosphate monobasic (KH_2PO_4).

Buffer solutions: 10 mM phosphate buffered saline (PBS), pH 7.4, was used as a supporting electrolyte with the addition of 5 mM potassium ferricyanide. Piranha solution was prepared by mixing H_2SO_4 and H_2O_2 at ratio of 3:2, respectively.

Electrochemical measurements were performed at room temperature, using a computer-controlled system, Autolab Electrochemical Analyzer ($\mu\text{3Aut71052}$) with GPES software. The screen-printed carbon electrode were purchased from DropSense (Metrohm Malaysia Sdn. Bhd). Each screen-printed carbon electrode consisted of three printed electrodes; working (carbon, 4 mm diameter), counter electrode (carbon) and reference electrode (silver). Fourier transform infrared spectroscopy (FTIR) and field emission scanning electron microscopy (FESEM) at Institute of Advanced Technology, UPM were used for characterization of multi-walled carbon nanotube.

Purification and Functionalization of multi-walled carbon nanotube: In order to purify multi-walled carbon nanotube, a non-oxidative treatment using HCl has been applied to remove the impurities and amorphous carbon presence in pristine multi-walled carbon nanotube. In this step, 1 g of multi-walled carbon nanotube was mixed with 200 mL of concentrated HCl. The mixture was stirred using magnetic stirrer for 2 h. Then, the suspension was diluted in water, filtered, washed with deionized water to remove acid traces until the pH was neutral and dried at 40 °C for overnight.

The purified multi-walled carbon nanotube was further treated with two types of acid treatment to modify the surface of nanotubes by adding $-\text{COOH}$ and $-\text{OH}$ functional groups. The first treatment was chemical oxidation by piranha solution. For this treatment, as described by Datsyuk *et al.* [10], 0.3 g of the purified multi-walled carbon nanotubes was dispersed in 25 mL of piranha solution and the solution was kept for 5 h under stirring. Then, the suspension was diluted in water, filtered and washed up to neutral pH. The treated multi-walled carbon nanotubes were then dried at 40 °C, overnight.

The second treatment was done by using the mixture of H_2SO_4 and HNO_3 at ratio of 3:2, respectively. The purified multi-walled carbon nanotube (0.5 g) was dispersed in the solution and stirred at room temperature for 24 h. Then, the suspension was diluted in water, filtered and washed up to neutral pH. The treated multi-walled carbon nanotubes were then dried at 40 °C overnight.

Characterization of functionalized multi-walled carbon nanotube: Pristine and functionalized multi-walled carbon nanotube samples were characterized by several techniques to identify the presence of carboxylic functional groups ($\text{O}=\text{C}-\text{OH}$ and $\text{C}-\text{OH}$) on the surface of treated surface. Fourier transform infrared spectroscopy was used to estimate the chemical composition and surface groups on the multi-walled carbon nanotube before and after treatment. The structure and morphology of functionalized multi-walled carbon nanotube were studied by field emission scanning electron microscopy (FESEM). Finally, cyclic voltammetry analysis was used to determine the electrochemical behaviour and evaluate the active surface area after modification process.

Optimization of casting conditions: The functionalized multi-walled carbon nanotube was added in 0.5 % of chitosan to form a MWCNT/CS solution. The solution was then homogenized in ultrasonic water bath for 1 h to form a well black suspension. Drop casting was the crucial procedure in the modification of screen-printed carbon electrode. The produced MWCNT/CS suspension was then dropped manually using micropipette onto the surface of working electrode, as the site of modification. Three parameters were tested to optimize the casting condition of the MWCNT/CS matrix including MWCNT:CS ratio, volume of droplet and drying condition. In addition, effect of pH of supporting electrolyte and scan rate were also studied.

RESULTS AND DISCUSSION

FTIR analysis: FTIR is generally used as a qualitative technique for estimation of functional group. Fig. 1 shows the attenuated total reflection infrared spectra of pristine multi-walled carbon nanotube (a), functionalized multi-walled carbon nanotube treated with piranha solution (b) and $\text{H}_2\text{SO}_4 + \text{HNO}_3$ (c). The resulted spectra were in a range of $4000\text{--}500\text{ cm}^{-1}$. It has been shown that there is a broad peak at about 3445 cm^{-1} refers to the O-H stretching of the hydroxyl group from carboxyl groups ($\text{O}=\text{C}-\text{OH}$ and $\text{C}-\text{OH}$). The presence of carboxyl groups on the surface of pristine multi-walled carbon nanotube may be due to the partial oxidation during purification by the manufacturer [11].

Two new noticeable peaks at 1715 cm^{-1} and 1056 cm^{-1} (Fig. 1c) appeared in multi-walled carbon nanotube oxidized by the mixture of H_2SO_4 and HNO_3 . The occurrence of these bands are contributed to the existence of $\text{C}=\text{O}$ and $\text{C}-\text{O}$ stretching vibrations of the carboxylic groups ($-\text{COOH}$), respectively [12]. This formation was caused by the oxidation of some carbon atoms on the surface of the multi-walled carbon nanotube by strong acids. Based on these spectra, a decreasing relative intensity of the $-\text{OH}$ band at 3445 and 1635 cm^{-1} can be observed when compared with the pristine and functionalized multi-walled carbon nanotubes, this is related to the associated water

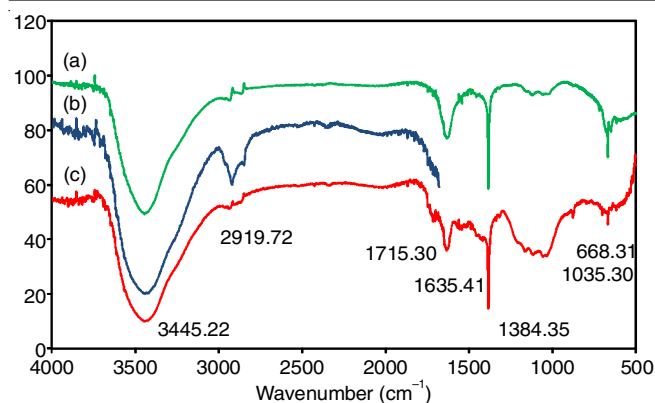


Fig. 1. FTIR spectra of pristine multi-walled carbon nanotube (a), functionalized multi-walled carbon nanotube treated with piranha (b) and functionalized multi-walled carbon nanotube treated with $\text{H}_2\text{SO}_4 + \text{HNO}_3$ (c)

[13] and stretching of the carbon nanotube backbone [14]. Based on Fig. 1(b), the peak at 3745 cm^{-1} is ascribed to the free hydroxyl groups [15], meanwhile peak at 2919 cm^{-1} is attributed to the C-H stretch modes of H-C=O in the carbonyl group and indicate the presence of a long-chain alkyl molecule [11].

This study was done to compare two types of treatment in terms of their effect on introducing carboxylic groups on the surface of carbon nanotubes, as well as improving the properties of carbon nanotube. Based on the FTIR result, multi-walled carbon nanotubes which were oxidized by the mixture of H_2SO_4 and HNO_3 successfully added carboxylic groups (-COOH) to the wall of carbon nanotube. This is supported by the presence of a peak at 1715 cm^{-1} which represent C=O and C-O stretching vibrations. Meanwhile, a noticeable peak at 2919 cm^{-1} for the multi-walled carbon nanotube oxidized by piranha solution is also attributed to the C-H stretch modes in the carbonyl group.

Surface morphology: FESEM: The surface morphology of multi-walled carbon nanotube after functionalization was observed using FESEM. Fig. 2 shows images of pristine and functionalized multi-walled carbon nanotube under 25000X magnification. Based on this observation, the walls of those tubes are not fully damaged although after treated with strong acid. The characteristics of pristine multi-walled carbon nanotube (Fig. 2a) are longer in length, smooth surfaces and they were attached tightly together in a bundle. However, after functionalization with strong acids, the nanotubes appeared shorter, curled and tangled. Carbon nanotube was observed to be equally dispersed and no longer in a bundle (Fig. 2b). Strong acid solution is believed to attack the present destructions site of multi-walled carbon nanotube and consumed the oxidized vacancies to produce shorter nanotube, by means increasing reaction will greatly produce shorter tubes [10]. Carbon nanotube is made up of carbon sheet called graphene that are rolled at specific and discrete angle. They are held together by van der Waals forces which are relatively weak. Therefore, by using strong acid in functionalization, it will break some C=C double bond and subsequently make the tube shorter.

Fig. 3 shows the diameter of carbon nanotube at magnification of 200000X. The diameter of functionalized nanotubes was observed to be increased compared to pristine nanotubes. The diameter of pristine multi-walled carbon nanotubes are distributed from 5 to 15 nm meanwhile, the diameter of functionalized multi-walled carbon nanotube (Figs. 3b and 3c) are in the range of 15 to 30 nm due to the additional layer of functional groups attached at the carbon nanotube walls.

Cyclic voltammetry analysis: Cyclic voltammetry analysis was used to characterize the surface of working electrode on screen-printed carbon electrode in the presence 5 mM of potassium ferrocyanide in supporting electrolyte. Based on the result of Fig. 4, higher peak separation between the anodic and cathodic peak was observed for the modified electrode

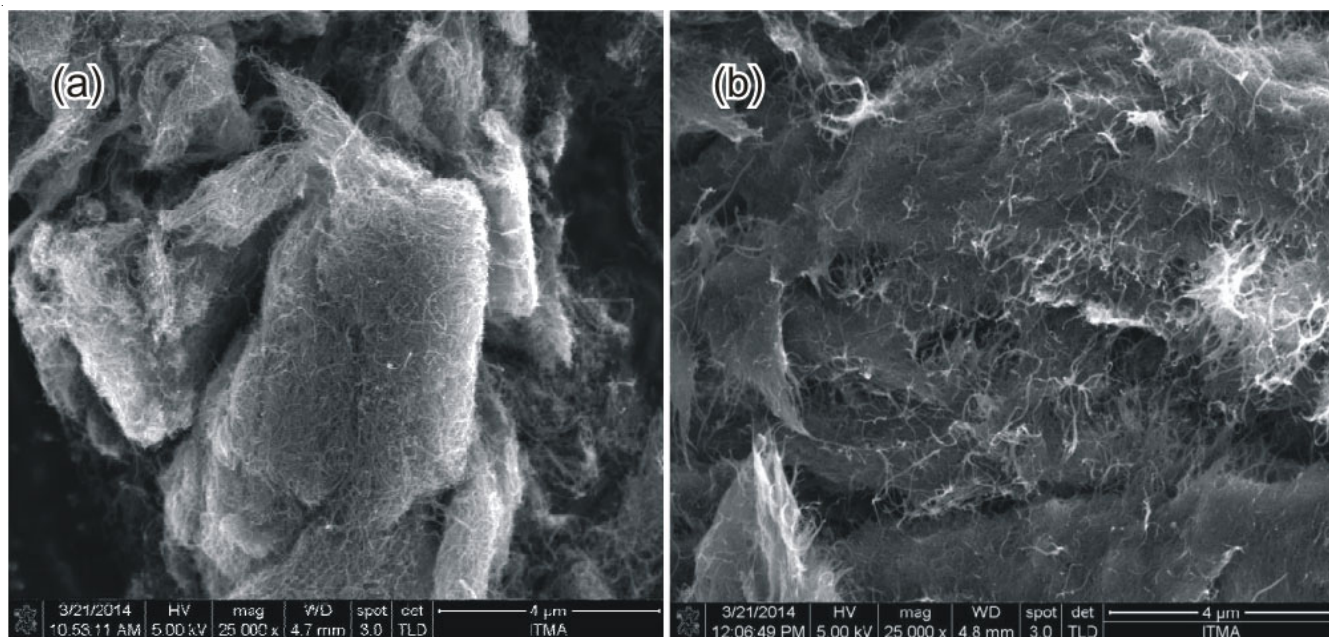


Fig. 2. FESEM image of pristine multi-walled carbon nanotube (a) and functionalized multi-walled carbon nanotube (b) under 25000X magnifications

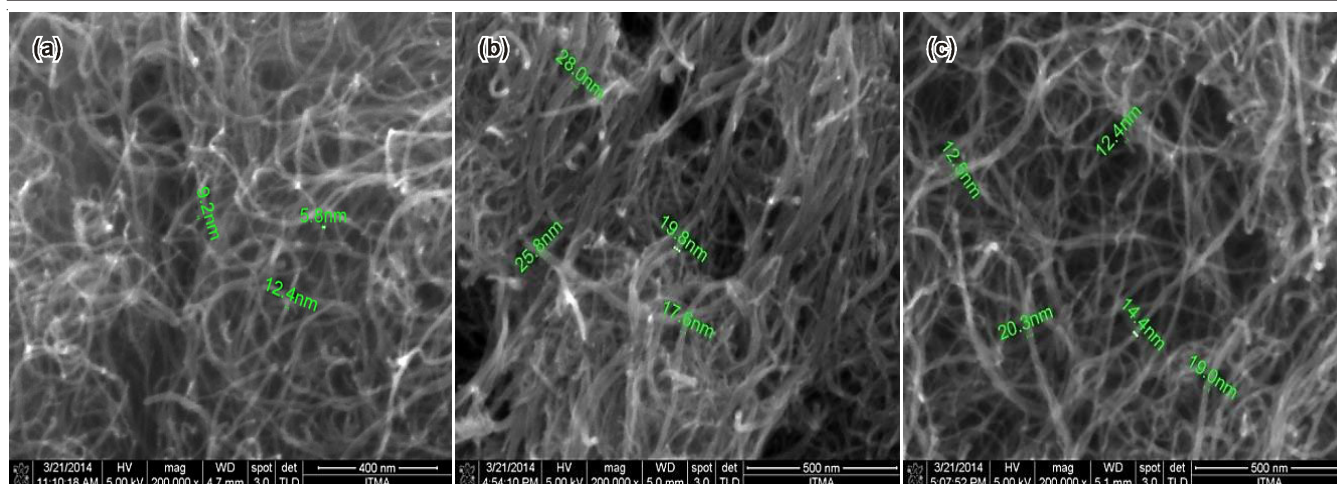


Fig. 3. FESEM image of carbon nanotube under 200 000 X magnification; Pristine multi-walled carbon nanotube (a) Functionalized multi-walled carbon nanotube by $\text{H}_2\text{SO}_4 + \text{HNO}_3$ (b) and functionalized multi-walled carbon nanotube by Piranha solution (c)

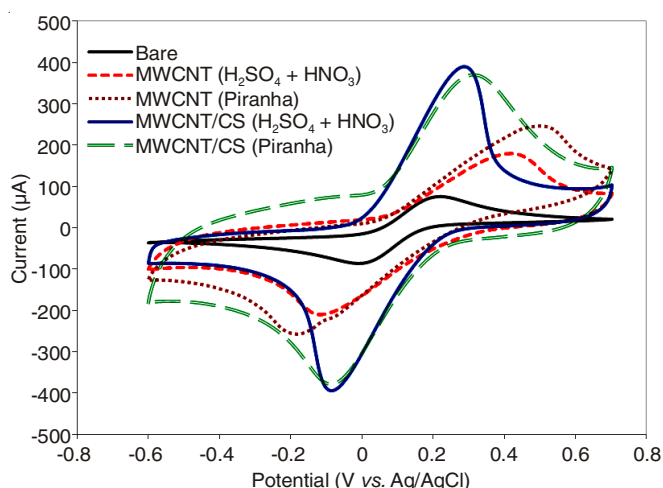


Fig. 4. Cyclic voltammogram of bare and modified screen-printed carbon electrode in 0.1 M PBS with 5 mM of $\text{K}_3[\text{Fe}(\text{CN})_6]$ at a scan rate of 0.1 V/s. Scan was set to 3 cycles from -0.6 to 0.7 V (vs. Ag/AgCl)

compared to the bare screen-printed carbon electrode. The peak separation can be used to determine the number of electrons transferred, as a criterion for a Nernstian behaviour [1]. Cyclic voltammogram shows the improvement of anodic and cathodic currents for both multi-walled carbon nanotube treated with $\text{H}_2\text{SO}_4 + \text{HNO}_3$ and piranha solution. However, multi-walled carbon nanotube treated by piranha produced a big capacitance effect at the reverse and forward edges. The active surface area of screen-printed carbon electrode was calculated based on Randles-Sevcik equation:

$$i_p = (2.69 \times 105) n^{3/2} ACD^{1/2} v^{1/2} \quad (1)$$

where n is the number of electrons, A is the electrode area (cm^2), C is the concentration (mol cm^{-3}), D is the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and v is the scan rate (V s^{-1}).

Based on eqn. 1, the real active surface area of the electrode was calculated by substitute $n = 1$ [$\text{Fe}(\text{CN})_6^{3-}$ system], $A = \pi r^2 = (22/7) (0.2 \text{ cm})^2 = 0.126 \text{ cm}^2$, $C = 5.0 \times 10^{-3} \text{ mol L}^{-1}$, $D = 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and $v = 0.1 \text{ V s}^{-1}$. The geometrical surface area of the electrode is 0.126 cm^2 and as expected, the real electrochemical surface area is much larger. Active surface area of bare screen-printed carbon electrode was calculated as 0.295 cm^2 while it was 1.879 and 2.063 cm^2 after modification with multi-walled carbon nanotube treated with ($\text{H}_2\text{SO}_4 + \text{HNO}_3$) and Piranha solution (Table-1), respectively.

Meanwhile, in this study, chitosan biopolymer was prepared in 1 % acetic acid solution. The integration of multi-walled carbon nanotube and chitosan was confirmed by coating a screen-printed carbon electrode with multi-walled carbon nanotube in 1 % acetic acid in the absent of chitosan. As shown in Fig. 4, the peak currents of SPCE/MWCNT without chitosan were lower and shifted to more oxidation/reduction potentials. This proved that chitosan enhances the stability and conductivity of the developed sensor.

Mass transfer process of MWCNT-CS modified electrode: Electroactive marker, potassium ferrocyanide can evaluate the reproducibility of the modified sensors. Thus, the electrochemical behaviour was tested at various scan rates in the range of 10 to 500 mV s^{-1} . During scan under different scan rate, the higher peak separation between the anodic and cathodic peak was observed as shown in Fig. 5.

TABLE-1
ELECTROCHEMICAL PARAMETERS OBTAINED FROM VOLTAMMOGRAMS IN Fig. 4.

Electrode	E_{pc} (V)	ΔE_p (V)	i_{pa} (μA)	i_{pc} (μA)	A (cm^2)	Current density ($\mu\text{A}/\text{cm}^2$)
Bare	-0.0104	0.324	75.5	-86.3	0.295	255.93
MWCNT/CS (Piranha)	-0.1129	0.518	481.0	-486.0	1.879	255.99
MWCNT/CS ($\text{H}_2\text{SO}_4 + \text{HNO}_3$)	-0.1514	0.547	528.0	-566.0	2.063	255.94
MWCNT (Piranha)	-0.1129	0.394	246.1	-210.2	0.961	256.09
MWCNT ($\text{H}_2\text{SO}_4 + \text{HNO}_3$)	-0.1813	0.340	179.7	-257.5	0.702	255.13

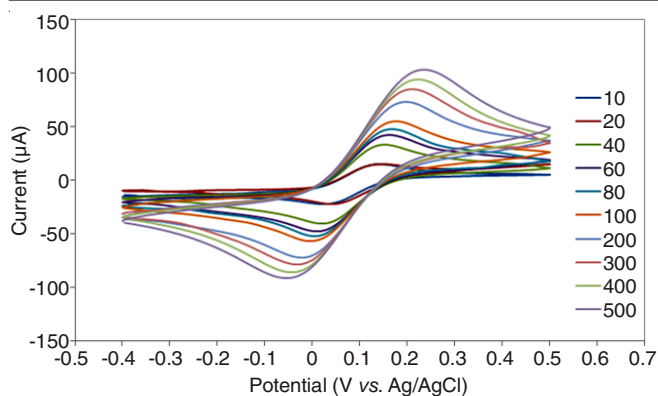


Fig. 5. Cyclic voltammograms of SPCE/MWCNTs/CS ($\text{H}_2\text{SO}_4 + \text{HNO}_3$) in $\text{K}_3[\text{Fe}(\text{CN})_6]$ and 0.1 M PBS at different scan rates (10-500 mV/s)

Optimization of screen-printed carbon electrode modified with functionalized multi-walled carbon nanotubes

Study on the ratio of multi-walled carbon nanotube and chitosan (MWCNT/CS): The functionalized carbon nanotube was mixed with chitosan as an electroactive biopolymer. Chitosan has great advantages for biosensor development due to its excellent film-forming ability, mechanical strength, biocompatibility, non-toxicity and cost-effectiveness [16]. Entrapment of multi-walled carbon nanotube in chitosan was being optimized for its immobilization stability and active surface area.

The experiment was carried out at six ratios of MWCNT:CS including 1:5, 1:2, 1:1, 2:1, 5:1, 10:1 (mg:mL), respectively. As it has shown in Fig. 6, the highest sensitivity was at ratio of 5:1. At optimum ratio of 5:1, the multi-walled carbon nanotube was well entrapped homogeneously without interrupting the carbon nanotube structure and properties either chemically or physically. Based on the result, as the ratio increased up to 10:1, the peak current decreased drastically. This may be due to the high density of carbon nanotube and low porosity for substrate to reach the active site of the electrode. Higher concentration of multi-walled carbon nanotube affected the system to be unstable and flaked away easier [17]. Therefore, the formation of multi-walled carbon nanotube matrix in chitosan onto the electrode was carried out at ratio of 5:1 for next studies.

Drop coating volume of MWCNT/CS matrix: Another key parameter for optimization of working electrode is the casting volume. This is to maximize the response and at the same time minimize the production cost. Based on result in Fig. 7, the current response increased as the volume increased up to 10 μL . Therefore, 10 μL of MWCNT/CS suspension was chosen as the optimal casting volume. This could be due to the adequate amount of MWCNT/CS at the electrode surface which consequently increased the active surface area for the electron transfer. Diameter of the working electrode is 4 mm, therefore increasing volume more than 10 μL causes overflowing on the surface of electrode.

Drying condition: Drying condition of the casted screen-printed carbon electrode has also been analyzed by comparing three different conditions which are at room temperature for overnight, oven drying at 40 and 80 $^\circ\text{C}$ for 30 and 10 min, respectively with the optimal MWCNT:CS ratio (5:1) and

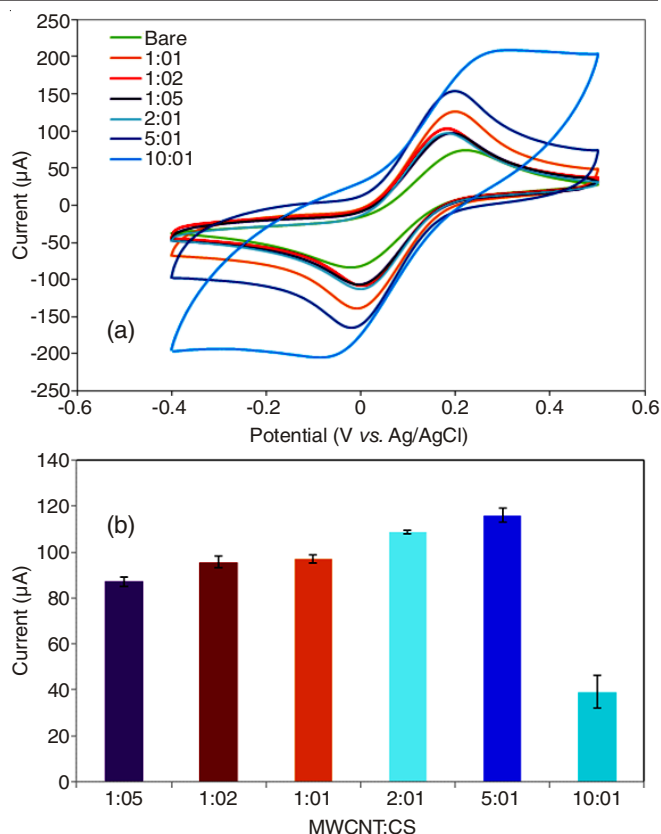


Fig. 6. Cyclic voltammetry of $\text{K}_3[\text{Fe}(\text{CN})_6]$, 5 mM in different ratios of MWCNTs:CS (a). The variation of peak current at different ratios of MWCNTs:CS (b). (mean \pm SD, $n = 3$)

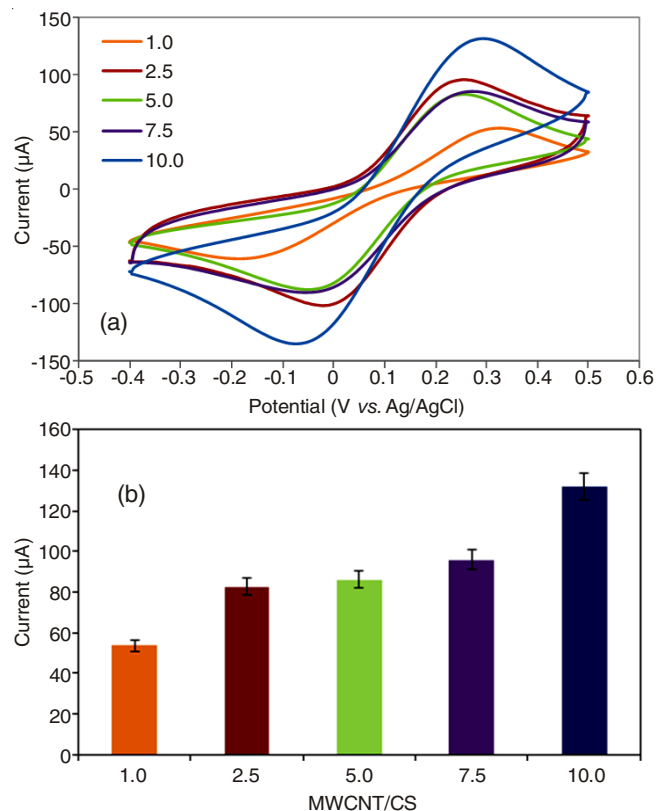


Fig. 7. Cyclic voltammetry studies of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 5 mM at different drop casting volumes (1-10 μL) (a). Study on the drop coating volume of MWCNT:CS suspension onto the working (b) (mean \pm SD, $n = 3$)

coating volume (10 μL). Based on the result in Fig. 8, there was no significant difference between those three different drying conditions. The current produced was almost the same for each drying method which proved that the oven drying does not effect the properties of multi-walled carbon nanotube and chitosan. This result is supported by one of the carbon nanotubes characteristic which is highly heat stable, up to 250 $^{\circ}\text{C}$ [4]. Since the preparation time is also a crucial parameter to be considered for a simple yet sensitive biosensor, therefore, oven drying at 80 $^{\circ}\text{C}$ for 10 min was an optimal condition to dry up the coating matrix.

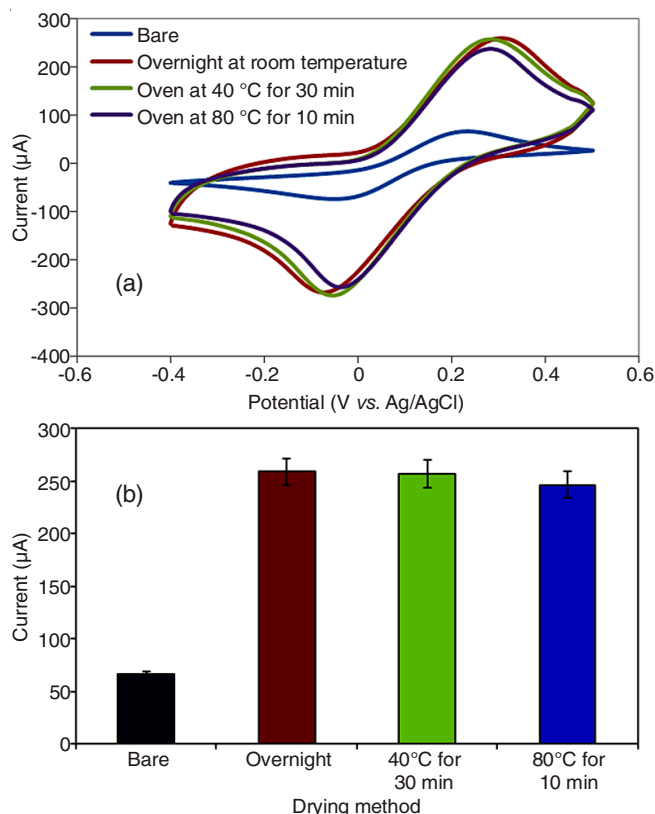


Fig. 8. Cyclic voltammetry studies of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 5 mM at different drying methods (a) (a: bare; b: overnight at room temperature; c: 40 $^{\circ}\text{C}$ for 30 min; d: 80 $^{\circ}\text{C}$ for 10 min) (b) The effect of drying method on the sensitivity of MWCNT/CS modified electrode (mean \pm SD, $n = 3$)

Precision of electrochemical sensor: The reproducibility of the modified electrode was studied by fabrication of five modified electrodes to determine the oxidation peak current of ferrocyanide under optimum conditions. The result yielded a relative standard deviation (RSD %) of 3.36 % which shows that the proposed fabricated sensor is reproducible. The repeatability of the fabricated sensor was reported by preparing one electrode and used for five measurements ($n = 5$) of $\text{K}_3[\text{Fe}(\text{CN})_6]$.

The result gives the RSD of 4.68 %, suggesting that the electrode is good for five repeated measurements.

Conclusion

In this work, we have optimized a convenient method to modify the screen-printed carbon electrode for further development of immunosensor as well as enhancing the conductivity. Multi-walled carbon nanotube functionalized with mixture of sulphuric acid and nitric acid provides more hydrophilic environment and carboxylic groups subsequently provides a better baseline for the attachment of bioactive component in further development of biosensor. The optimal condition for deposition of MWCNT/CS matrix onto the screen-printed carbon electrode was found at 5:1 ratio with 10 μL drop casting volume onto the 4 mm diameter working electrode and dried at 80 $^{\circ}\text{C}$ for 10 min. This optimized modification of screen-printed carbon electrode with carbon nanotube and chitosan improved its stability and active surface area.

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REFERENCES

1. J. Wang, *Analytical Electrochemistry*, John Wiley & Sons, New York, edn 2 (2000).
2. J.V. Veetil and K. Ye, *Biotechnol. Prog.*, **23**, 517 (2007).
3. J. Wang, *Analyst*, **130**, 421 (2005).
4. N. Sinha, J. Ma and J.T.W. Yeow, *J. Nanosci. Nanotechnol.*, **6**, 573 (2006).
5. D. Akiladevi and S. Basak, *Int. J. Adv. Pharm. Sci.*, **1**, 187 (2010).
6. Y. Tzeng, T.S. Huang, Y.C. Chen, C. Liu and Y.K. Liu, *Carbon Technol.*, **14**, 193 (2004).
7. Y.R. Shin, I.-Y. Jeon and J.-B. Baek, *Carbon*, **50**, 1465 (2012).
8. E. Alocilja and Z. Muhammad-Tahir, in eds.: M. Zourob, S. Elwary and A.P.F. Turner, *Label-Free Microbial Biosensors Using Molecular Nanowire Transducers*, In: *Principles of Bacterial Detection Biosensors, Recognition Receptors and Mocosystems*, Springer, New York, pp. 377-413 (2008).
9. T.S. Huang, Y. Tzeng, Y.K. Liu, Y.C. Chen, K.R. Walker, R. Guntupalli and C. Liu, *Diamond Rel. Mater.*, **13**, 1098 (2004).
10. V. Datsyuk, M. Kalyva, K. Papagelis, J. Parthenios, D. Tasis, A. Siokou, I. Kallitsis and C. Galiotis, *Carbon*, **46**, 833 (2008).
11. F.A. Abuilawi, T. Laoui, M. Al-Harathi and M.A. Atieh, *Arab. J. Sci. Eng.*, **35(1C)**, 37 (2010).
12. C. Velasco-Santos, A.L. Martínez-Hernández, F.T. Fisher, R. Ruoff and V.M. Castano, *Chem. Mater.*, **15**, 4470 (2003).
13. G. Trykowski, S. Biniak, L. Stobinski and B. Lesiak, *Acta Phys. Pol. A*, **118**, 515 (2010).
14. S. Goyanes, G.R. Rubiolo, A. Salazar, A. Jimeno, M.A. Corcuera and I. Mondragon, *Diamond Rel. Mater.*, **16**, 412 (2007).
15. J.E. Huang, X.H. Li, J.C. Xu and H.L. Li, *Carbon*, **41**, 2731 (2003).
16. A. Kaushik, P.R. Solanki, M.K. Pandey, S. Ahmad and B.D. Malhotra, *Appl. Phys. Lett.*, **95**, 173703 (2009).
17. L. Daling, S. Yi, Y. Dongsheng and Z. Jing, *Chinese Online Thesis*, **5**, 1 (2007).