

THE OPTIMIZATION OF MULTI-WALLED CARBON
NANOTUBES SURFACE MODIFICATION VIA NITRIC
ACID OXIDATION FOR DNA IMMOBILIZATION

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UNIVERSITI MALAYSIA PERLIS

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**The Optimization of Multi-Walled Carbon Nanotubes
Surface Modification via Nitric Acid Oxidation for DNA
Immobilization**

by

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
Ag/AgCl	Argentum/Argentum Chloride
ANOM	Analysis of Mean
ANOVA	Analysis of Variance
Au	Aurum
CNTs	Carbon Nanotubes
COOH	Carboxylic Group
CV	Cyclic Voltammetry
CVD	Chemical Vapor Deposition
DC	Direct Current
DDA	Dodecylamine
DF	Degree of Freedom
dI	Deionized Water
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
DPV	Differential Pulse Voltammetry
DOE	Design of Experiment
dsDNA	Double Stranded DNA
DTG	Derivative Thermogravimetric Analysis
EDC	1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride
EDS	Energy Dispersive X-ray Spectroscopy
ETR	Electron Transfer Rate
FET	Field Effect Transistor

FTIR	Fourier Transform Infra Red Spectroscopy
H ₂ O ₂	Hydrogen Peroxide
H ₂ SO ₄	Sulphuric Acid
HCl	Hydrochloric Acid
HNO ₃	Nitric Acid
I _D	Defect state intensity
I _G	Graphitic state intensity
IPA	Iso-Propyl Alcohol
ISFET	Ion Sensitive Field Effect Transistor
K ₃ Fe(CN) ₆	Potassium Hexacyanoferrate III
KBr	Potassium Bromide
KCl	Potassium Chloride
KH ₂ PO ₄	Mono Potassium Phosphate
KMNO ₄	Potassium Permanganate
LTB	Larger the Better
MOSFET	Metal Oxide Semiconductor Field Effect Transistor
MWCNTs	Multi-Walled Carbon Nanotubes
NADH	Nicotinamide Adenine Dinucleotide
NHS	N-hydroxysuccinimide
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PEI-b	Poly Ethyl-Eneimine Branched
Pt	Platinum
RBM	Radial Breathing Mode
S/N	Signal to Noise

SEM	Scanning Electron Microscopy
SiO ₂	Silicone Oxide
SNR	Signal to Noise Ratio
SQC	Statistical Quality Control
ssDNA	Single Strand DNA
SWCNTs	Single Walled Carbon Nanotubes
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analyzer
XRD	X-Ray Diffraction

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**PENGUBAHSUAIAN OPTIMUM KE ATAS PERMUKAAN TIUB NANO
KARBON DINDING BERGANDA DENGAN PENGOKSIDAAN ASID NITRIK
UNTUK APLIKASI IMOBILISASI DNA**

ABSTRAK

Tesis ini membincangkan tentang pengubahsuaian optimum ke atas permukaan tiub nano karbon dinding berganda (MWCNTs) dengan menggunakan kaedah pengoksidaan asid nitrik untuk aplikasi imobilisasi DNA. Selepas menjalani pengoksidaan asid, bendasing yang terdapat di dalam MWCNTs seperti partikel karbon dan pemangkin unsur logam telah berjaya dikurangkan seperti yang dilaporkan oleh ujian Pembelauan Elektron Sinar-X (EDS), Serakan Sinar-X (XRD) dan Analisis Thermo-Gravimetrik (TGA). Pengoksidaan asid akan memberi kesan kepada pembukaan hujung MWCNTs dan menyebabkan kecacatan pada struktur MWCNTs hasil daripada serangan asid. Kumpulan unsur oksigen terutama kumpulan karbosilik (COOH) telah terhasil pada pembukaan hujung MWCNTs dan juga pada ruangan kecacatan struktur yang mana mampu untuk berinteraksi dengan molekul-molekul lain, seperti di dalam kes ini, prob amine-DNA. Keputusan ujian Spektroskopi Transformasi Jelmaan Infra-Merah (FTIR) dan Spektroskopi Raman telah menunjukkan bahawa jumlah COOH yang terhasil bergantung kepada kadar kecacatan struktur yang terbentuk. Sementara itu, keputusan ujian Voltametri Pusingan (CV) telah menunjukkan perhubungan terus antara arus imobilisasi DNA dengan jumlah COOH. Akan tetapi, kecacatan struktur pada MWCNTs turut memberikan kesan kepada arus imobilisasi DNA apabila nisbah I_D/I_G semakin meningkat. Parameter pengoksidaan asid perlu dioptimumkan supaya jumlah COOH yang terhasil dapat dimaksimumkan di samping meminimumkan kecacatan struktur. Maka dengan itu matlamat utama untuk mendapat arus imobilisasi yang maksimum bakal tercapai. Rekabentuk eksperimen L9 Taguchi telah digunakan

untuk mengoptimalkan parameter pengoksidaan asid. Keputusan menunjukkan bahawa kepekatan asid nitrik pada 5 M, suhu rawatan pada 120 °C, dan masa rawatan selama 6 jam adalah kombinasi parameter pengoksidaan asid yang optimum. Peratusan pengaruh bagi setiap faktor utama yang digunakan adalah masing-masing 46%, 35% dan 18% untuk kepekatan asid, suhu rawatan dan masa rawatan. Sebanyak 11.6% peningkatan turut dilaporkan bagi arus imobilisasi DNA hasil daripada pengubahsuaian optimum ini.

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THE OPTIMIZATION OF MULTI-WALLED CARBON NANOTUBES SURFACE MODIFICATION VIA NITRIC ACID OXIDATION FOR DNA IMMOBILIZATION

ABSTRACT

This thesis discussed on the optimization of MWCNTs surface modification via nitric acid oxidation for DNA immobilization. After acid oxidation treatment, the impurities in multi-walled carbon nanotube (MWCNTs) such as carbonaceous and metal catalyst particles are successfully reduced as has been analyzed by energy dispersive x-ray spectroscopy (EDS), x-ray diffraction (XRD) and thermogravimetric analyzer (TGA). Acid oxidation will caused to the opening of MWCNTs tips and structural defects formed on the MWCNTs surface due to the acid attack. Oxygen containing functional groups, mainly, carboxylic group (COOH) has been introduced on the MWCNTs opened tips and at the defect sites which are useful to interact with other molecules, in this case, aminated-ssDNA probe. The results from fourier transform infrared spectroscopy (FTIR) and Raman Spectroscopy have shown that the COOH amount is depended on the MWCNTs structure defects. Meanwhile, cyclic voltammetry (CV) results have indicated that the immobilization current is directly proportional to the COOH amount. However, structure defect will affect to the immobilization current when I_D/I_G ratio is increased. The acid oxidation parameter should be optimized, thus the amount of COOH can be increased with the minimal structure defect. Therefore, the main goal to have a maximum immobilization current can be achieved. L9 Taguchi orthogonal array has been used to optimize the acid oxidation parameters. From the result, 5 M of nitric acid concentration, 120 °C of treatment temperature and 6 hours of treatment time are selected as the most optimum combination of acid oxidation parameters. The percentage influence of each main factor is also calculated to be 46%,

35% and 18% for nitric acid concentration, treatment time and treatment temperature, respectively. The improvement is happened to be 11.6% of increment in the immobilization current.

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CHAPTER 1

BACKGROUND

1.1 Introduction

Unlike detecting the glucose levels for diabetes patients which only required a drop of blood directly onto the biosensor, DNA biosensor requires more complex procedures. Starting from extracting DNA from the sample until detecting the hybridization of DNA, all procedures need to be done in the laboratory because special expensive equipments like the real time PCR machine is required. Nowadays, there are companies that suggest to their customers to extract their own samples by using a DNA kit but the extracted DNA must be sent to the nearest laboratory for at least two to seven days in order to obtain the results. Moreover, the analysis takes longer time due to classical approaches such as the mass spectrometry and the bio-labeled fluorescence techniques that have elongated procedures before the biomolecules are detected (Mickelson et. al, 1999). These techniques are very time consuming as compared to the biosensor which utilizes electrical testing equipments such as dielectric analyzer and potentiostat that are a lot simpler and faster.

The biosensor, also known as the electrochemical sensors, has been under intensive research worldwide. They offer great advantages such as simplicity, rapid detection, low-cost and high sensitivity (Shu-Feng et. al, 2005). In order to increase the accuracy and sensitivity, many scientists have conducted research to find the best material to be used as the transducer to improve the signal detected from the DNA

hybridization. Although gold and other metals can be used as the sensitive material of sensors, CNTs show great potential that ensured it can be used in electrochemical sensor too (Shu-Feng et. al, 2005; Kannan et. al, 2006; Christopher et. al, 2010). CNTs are promising for sensing applications due to several intriguing properties such as high electrical conductivity which is similar to copper, high chemical stability, extremely high mechanical strength, good electronic transfer properties, thermal conductivity better except diamond, and can carry much higher current (Arben et. al, 2005). In particular, their large length-to-diameter aspect ratios (which can be up to 34,000,000:1) also provide high surface-to-volume ratios (Wang et. al, 2009). Moreover, CNTs have an outstanding ability to mediate fast electron-transfer kinetics for a wide range of electroactive species, such as hydrogen peroxide or NADH (Kannan et. al, 2006).

There are three types of CNTs which are Single-Walled Carbon Nanotubes (SWCNTs), Double-Walled Carbon Nanotubes (DWCNTs) and Multi-Walled Carbon Nanotubes (MWCNTs) (Veena et. al, 2011; Thomas, 2007). Figure 1.1 shows the all three types of CNTs. While, the ends of the tubes in Figure 1.2, shows the incomplete fullerene structure which contained pentagons (Antonio et. al, 2008).

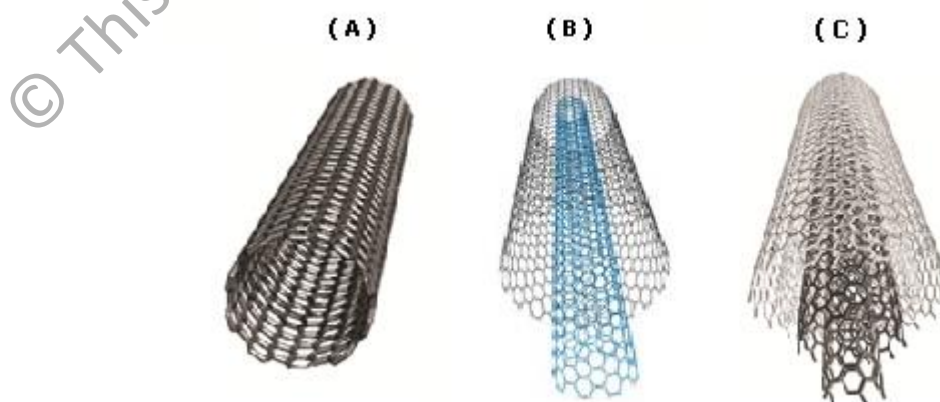


Figure 1.1: Types of CNTs, (A) SWCNTs, (B) DWCNTs, and (C) MWCNTs (Veena et. al, 2011; Thomas, 2007).

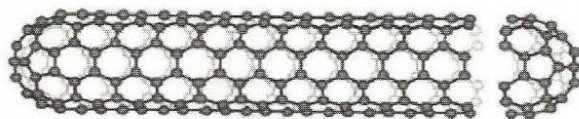


Figure 1.2: The incomplete fullerene structure of CNTs caps (Antonio et. al, 2008).

The difference between those is the number of their wall. There is only one layer of graphitic rolled in SWCNTs and double layers for DWCNTs. Meanwhile in MWCNTs, there are three or more layers of graphitic rolled inside each other. Having multiple layers of wall in one single strand of MWCNTs is actually an advantage if the end tips of MWCNTs are open. Therefore, many electrons can flow through the multi layer at one time. This characteristic can enhance electron transfer rate compared to SWCNTs. Besides, SWCNTs with smaller diameter could cause them to be easily lost in extreme oxidation of CNTs (Yu et. al, 2004).

As-synthesized CNTs usually consist of impurities such as carbonaceous and metal catalyst. These impurities can affect the CNTs electrical performance if they are not removed. Therefore, the purification process has to be done in order to remove the impurities. There are three methods of purification namely the physical, the chemical and the multi-step purification. Of all the three methods of purification, the chemical purification has been widely used since the method not only can successfully remove the impurities but also help to overcome the hydrophobicity issue of CNTs by introducing oxygenated functional groups at the opened end tips and along the CNTs walls (Hou et. al, 2008). The open ends of CNTs tip has resulted in an increased surface area while the oxygenated functional groups that are attached to the open ends have increased the electron transfer rate of CNTs (Alison et. al, 2005; Porro et. al, 2006). Since chemical purification technique can provide oxygenated functional groups

attached onto the CNTs surfaces, this technique is justifiable to be used as one of the method to surface modify the CNTs.

Surface modification of CNTs is a method where CNTs surfaces are being modified through addition of any types of functional groups to improve the interaction between the CNTs and other molecules (Andreas et. al, 2005). Initially, surface modification of CNTs is designed to overcome the hydrophobicity of as-synthesized CNTs. Nowadays, surface modification of CNTs is essential for it to be used in various potential applications such as biosensor. The most common and easiest method used to modify the surface of CNTs is by using the acid oxidation. It is widely used due to some advantages they possess such as non-toxic, simplicity and low cost. Hydrochloric acid (Djordjevic et. al, 2006), nitric acid (Faraj et. al, 2010; Stobinski et. al, 2010), mixture of sulfuric acid and nitric acid (Matthew et. al, 2006; Ramin et. al, 2011; Alison et. al, 2005) are the example of acids that are commonly used as the oxidizing agent during the acid oxidation modification process. Datsyuk et. al, (2008) has reported that nitric acid can cause structural damage to CNTs due to the strong oxidants attack. However, nitric acid is still convenient to be used as oxidizing agent because of its mild oxidation, non-toxic, low cost and can provide more oxygenated species, especially carboxylic groups as long as precautions are taken in controlling the main parameters such as the nitric acid concentration, treatment temperature and treatment time to minimize the structural damage of CNTs (Faraj et. al, 2010).

In the application of DNA immobilization, the amount of carboxylic groups (COOH) that are introduced to the open tips and the wall surfaces of CNTs are very important as it might affect the number of DNA strands that will possibly be

immobilized onto the CNTs. During the immobilization process, the COOH functional groups on CNTs will bind with amine-ssDNA probes to form stable amide bonds as shown in Figure 1.3.

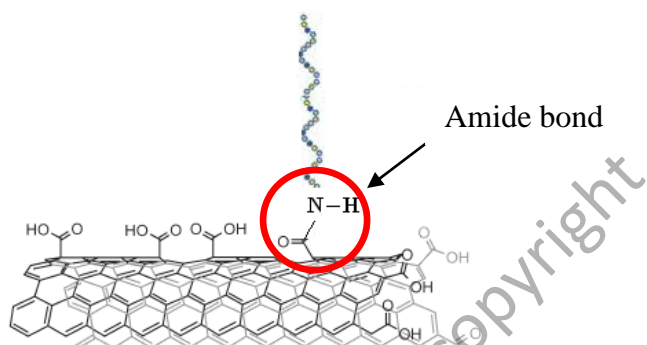


Figure 1.3: The formation of amide bond during the immobilization of DNA.

It is assumed in this research that the larger the amount of COOH introduced to the CNTs surfaces, the higher the amount of ssDNA can be immobilized onto the surface of CNTs. COOH group can be increased by using the stronger oxidants or increasing the oxidation time. However, the CNTs structure could be damaged if they are treated with stronger oxidants for a longer time. Structural damage could affect the electrochemical performance of CNTs because they are losing their original graphene structure and their special electrical characteristic. This defect can be controlled by optimizing the three main parameters which are the acid concentration, treatment temperature and treatment time. Therefore, figuring out the optimum parameter to modify the surface of MWCNTs via acid nitric oxidation for DNA immobilization has become the aim of this research.

1.2 Problem Statement

DNA biosensor is an electrochemical device that utilizes the ability of two complementary strands of nucleic acids to form a double helix (Kerman et. al, 2009). Traditional DNA biosensor has been prepared by using a glassy carbon electrode (GCE) where the ssDNA probes are immobilized onto the electrode with random orientation due to the phosphate backbone and hydrophobic nitrogenous bases, which can have multiple interactions with the carbon surfaces (Lucarelli et. al, 2008). However, the performance of the DNA biosensor is dependent on the orientation of the immobilized ssDNA probes (Christopher et. al, 2010; Cai et. al, 2003). Therefore, this issue has become a major concern in producing DNA biosensor with high sensitivity and selectivity. By using CNTs as the sensing element, the performance of DNA biosensor can be improved. The capability of CNTs to be surface modified with the addition of various oxygenated functional groups through acid oxidation has enabled them to selectively immobilize ssDNA probe with proper orientation. In addition, the unique properties of CNTs such as higher current capacity, faster electron transportation and high surface-to-volume ratio also contribute towards improving the DNA biosensor performance (Kannan et. al, 2006).

In order to increase the selectivity and sensitivity of the CNTs based DNA biosensor, oxygenated functional groups such as -OH, -C=O, and -COOH are recommended to be added onto the CNTs surfaces by surface modification through the use of acid oxidation treatment (Cher at. al, 2008; Andreas et. al, 2005; Porro et. al, 2006; Faraj et. al, 2010). Carboxylic groups will act as extra hands to interact with amine-ssDNA probe to form stable amide bonds during the immobilization process. The