OPTIMISATION OF CARBON NANOTUBES GROWTH CONDITIONS BY CVD AND ITS APPLICATION IN AMPEROMETRIC GLUCOSE BIOSENSOR

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DEDICATION

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ABSTRACT

This thesis presents the fabrication of glucose biosensor by modifying the surface of the glassy carbon electrode (GCE) using optimized carbon nanotubes (CNTs). Chemical vapor deposition (CVD) method was utilized to grow vertically aligned carbon nanotubes (VACNTs) with various aspect ratios. Field emission scanning electron microscopy (FESEM) images coupled with Raman spectroscopy results highlighted the high aspect ratio as well as uniformity of the high crystalline carbon nanotubes. Transmission electron microscopy (TEM) images of the grown CNTs confirm the successful synthesis of multiwall carbon nanotube (MWCNTs) due to larger outer diameter of the CNTs. Furthermore, to increase the graphitic ratio of synthesized CNTs, sequential experimental strategies based on response surface methodology (RSM) was employed to investigate the crystallinity model of CNTs. In the next step, glucose oxidase (GOx) was immobilized on the optimized multiwall carbon nanotubes/gelatin (MWCNTs/Gl) composite using the entrapment technique to achieve enzyme-catalyzed oxidation of glucose at anodic potentials, which was drop-casted onto the GCE. Cyclic voltammetry (CV) results coupled with the chronoamperometric response obtained from modified GCE indicates that, GOx/MWCNTs/Gl/GC electrode can be utilized as a glucose biosensor with high direct electron transfer rate (8.42 s^{-1}) between GOx and MWCNTs/Gl in a wide linearity range (8.9 mM) to glucose. The detection limit of the fabricated biosensor recorded was 0.59 mM by keeping its initial stability of 75.4% after 25 days. The performance of the fabricated biosensor as an electronic tongue was also investigated by designing a frequency based circuit attached to the electrochemical cell. The resistivity alteration of GOx/MWCNTs/Gl/GCE was recorded after each drop of glucose in the electrochemical cell. The oscilloscope results clearly showed that, by adding glucose to the circuit design, the output oscillation frequency changed and the square wave frequency reached a new stable value. These results indicated that, the modified GCE with the GOx/MWCNTs/Gl showed potential application in the determination of glucose in human serum samples as well as voltammetric based electronic tongue.

ABSTRAK

Tesis ini memerihalkan penghasilan sebuah penderia glukosa dengan mengubahsuai permukaan elektrod karbon berkaca (GCE) menggunakan nanotiub karbon (CNT) yang telah dioptimumkan. Kaedah wap pelupusan kimia (CVD) telah digunakan untuk menghasilkan susunan nanotiub karbon secara menegak (VACNT) dengan pelbagai aspek nisbah. Hasil pemerhatian imej dari pelepasan bidang imbasan microskop elektron (FESEM) yang digandingkan dengan keputusan *Raman spectroscopy* menunjukkan aspek nisbah yang tinggi, selain daripada keseragaman susunan nanotiub karbon yang berkristal tinggi. Imej dari penghantaran electron mikroskop (TEM) pula membuktikan penghasilan nanotiub karbon pelbagai-dinding (MWCNT), disebabkan oleh diameter luar CNT yang lebih tebal. Di samping itu, bagi tujuan menambah nisbah grafit CNT yang terhasil, strategi eksperimen berangkai berdasarkan kaedah gerak balas permukaan (RSM) telah digunakan untuk mengkaji model pengkristalan CNT. Dalam langkah yang seterusnya, oxidase glukosa (GOx) telah dikakukan di atas komposit nanotiub karbon pelbagai-dinding/gelatin (MWCNTs/G1) menggunakan teknik pemerangkapan bagi mencapai pengoksidaan glukosaa berkatalisis dengan enzim pada anod, di mana ia dijatuh-acuankan ke atas GCE. Keputusan voltametri siklik yang digandingkan dengan kesan *chronoamperometric* yang didapati daripada GCE menunjukkan. elektrod GOx/MWCNTs/G1/GC boleh digunakan sebagai penderia glukosa dengan kadar pemindahan elektron secara terus yang tinggi (8.42 s^{-1}) di antara GOx dan MWCNTs/G1 dalam kawasan kepekatan secara linear dan luas (8.9 mM). Had pengesanan bagi penderia yang dihasilkan adalah 0.59 mM dengan mengekalkan kestabilan asal iaitu 75.4% selepas 25 hari. Penderia yang dihasilkan turut diuji keberkesanannya sebagai lidah elektronik dengan mereka-bentuk litar elektrik berasaskan frekuensi yang dilekatkan kepada sebuah sel elektrokimia. Perubahan kerintangan bagi GOx/MWCNTs/G1/GC telah direkodkan selepas setiap titisan glukosa ke dalam sel elektrokimia tersebut. Keputusan osiloskop menunjukkan dengan jelas bahawa frekuensi ayunan keluaran telah berubah, dan frekuensi gelombang petak mencapai nilai baru yang stabil, apabila glukosa ditambahkan ke dalam rekaan litar elektrik. Semua keputusan ini menunjukkan GCE yang diubahsuai dengan GOx/MWCNTs/G1 mempunyai potensi untuk diaplikasikan sebagai alat penderia glukosa di dalam sampel serum manusia, dan juga sebagai lidah elektronik yang berasaskan kaedah voltametri.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

CVD	-	Chemical vapour deposition
CE	-	Counter electrode
CMEs	-	Chemically modified electrodes
CV	-	Cyclic voltammetry
DET	-	Direct electron transfer
DI water	-	Deionized water
DWCNTs	-	Double walled carbon nanotubes
E	-	Potential
EDAX	-	Energy dispersive X-ray
ET	-	Electronic Tongue
FAD	-	Flavin adenine dinucleotide
FESEM	-	Field emission scanning electron microscopy
FTIR	-	Fourier transform infrared spectroscopy
GCE	-	Glassy carbon electrode
GL	-	Gelatine
GLU	-	Glucose
GNR	-	Graphenenanoribbon
GOx	-	Glucose oxidase
GPES	-	General purpose electrochemical system
HRTEM	-	High resolution transmission electron microscopy
I	-	Current
LDR	-	Linear dynamic range
LOD	-	Limit of detection
MWCNT	-	Multi walled carbon nanotubes
PBS	-	Phosphate buffered saline
R	-	Resistance
RE	-	Reference electrode
RSD	-	Relative standard deviation
SWCNTs	-	Single walled carbon nanotubes

TTF	-	Tetrathiafulvalene
WE	-	Working electrode

LIST OF APPENDIX

CHAPTER 1

INTRODUCTION

1.1 Introduction

Over the past few decades, a number of reports have highlighted the growing importance of nanotechnology-based devices. Many types of nano-devices have been employed in engineering, fundamental sciences, and biology. One of the most valuable applications of nanotechnology is in the development of novel and effective biosensors that circumvent the limitations of current molecular diagnostics, provide accurate diagnosis, and leading to the development of personalized treatments (Lu et al., 2012). Recent advances in nanotechnology that combines sensors with nanomaterials introduced efficient and simple devices that can be used to measure the analytes' concentration (Chen et al., 2012; Hasan et al., 2014; Jianrong et al., 2004). Researchers have exploited a variety of bio-sensing techniques using miniaturized and portable devices to optimize, observe, and control biological reactions on micro or nano-surfaces. Electrochemical device is one of the promising method in biosensor development, due to their simplicity, low costs, accuracy, and sensitivity towards medical diagnosis (Ahuja and Kumar, 2009). The electrochemical biosensor converts biological recognition events to useful and understandable electrical signals. Electrochemical biosensors rely on nanomaterials, especially nanostructures (Kumar, 2007). Nanostructures differ from bulk materials, due to their small size and unique structure, exhibiting unique physical, chemical, and electronic properties (Wu et al., 2012). They can be used to construct novel materials to improve biosensors. Generally, nanostructures such as carbon nanomaterials (for example, fullerenes, nanotubes, and graphene) possess excellent electrical properties and conductivity, rendering them suitable for electrochemical sensors (Hu et al., 2010). Carbon nanotube (CNTs) is a novel type of nanomaterials that have significantly different properties compared to its counterparts. Rolled graphene sheets are known as CNTs with capped ends, which could be single-walled carbon nanotube (SWCNTs), double-walled carbon nanotube (DWCNTs), or multi-walled carbon nanotubes (MWCNTs) (Odom et al., 2000). Although techniques such as arc discharge or laser ablation has been traditionally employed to build CNTs, currently, low temperature techniques (<800°C), such as chemical vapor deposition (CVD), have been developed to produce CNTs that are touted as the most popular synthesizing method of CNTs, due to the possibility of synthesizing CNTs at different lengths and diameters, uniform orientation, and extra high purity at low costs (Shahriary et al., 2014).

1.2 World Demand of Electrochemical Glucose Biosensor

Glucose monitoring is one of the main targets of the bio-sensing studies, as 5% of the population of developed countries are suffering from diabetes (Meetoo et al., 2007). Diabetes mellitus is a common metabolic disorder that results in insulin deficiencies and hyperglycemia. This causes the blood glucose levels to fluctuate within 80-120 mg/dL (4.4-6.6 mM). Diabetes is a major cause of death or disabilities to people across the world, and it also increases the risks of heart disease, kidney failure, and blindness. These complications can be prevented, or at least decreased, by an accurate personal control of blood glucose levels (Malhotra and Chaubey, 2003). Therefore, an accurate monitoring of the blood glucose level seems to play a major role in the diagnosis and management of diabetes mellitus (Cooper and Cass, 2004).

Beside glucose monitoring for diabetes, the biosensors array for electronic tongue (ET) has also become an important area of research. Generally, an electronic tongue is a single or multisensory device dedicated to the automated analysis of complex composition samples and recognizes their characteristic taste properties. Recently, the number of fabricated electronic tongues has significantly increased due to the increase of the electrochemical detection of biomolecules. However, several possible architectures of electronic tongues were proposed, such as potentiometric, voltammetric, embracing mass, and optical-sensors.

Hence, the reliability of electrochemical biosensors and precise recognition process prompted researchers to fabricate electrochemical glucose sensors in the simplest and most reliable scheme for multiple applications. Biosensors, as a specific kind of chemical modified electrodes (CMEs), is the most common detection method for glucose concentration, due to advantages such as simplicity, reliability, and sensitivity. A tremendous trend to the development of glucose biosensors based on carbon nanostructures such as CNTs can be observed during the past decade due to the excellent physical and chemical properties of CNTs (Trojanowicz, 2006). In fact, CNTs display unrivaled properties in electrochemical biosensors, due to its high surface area for sensing interaction, as well as excessive sensitivity to chemical-doping effects during the interplay with diverse biological molecules (Cai and Chen, 2004). Interaction between the nanotube and other entities such as solvents, polymers, and biopolymer matrices, or other nanotubes can be tailored by means of chemical bonds via covalent and non-covalent functionalization (Casey et al., 2012). The basis of the covalent functionalization is the covalent link of functional entities onto the nanotube's carbon scaffold, while a non-covalent functionalization relies on supramolecular complexation utilizing a variety of sorption forces like van der Waals' and pi-stacking interaction (Holzinger et al., 2001). Generally, the total charge carrier of CNTs will be changed by the adsorbent biomolecules and the alteration of the conductance, making CNTs-based biosensors capable of powering a full device in continuous monitoring of biological molecules (Hanna Varghese et al., 2010). Latest advances of electrochemical glucose biosensors is indicative of the fact that well-optimized aligned CNTs play an important role towards the incorporation of nanotechnology in biosensor to obtain a higher linear range and advisable sensitivity for CNTs based electrochemical biosensors (Zhu et al., 2012). CNTs-based paste electrodes (Shahriary et al., 2014), electrodes modified by CNTs (Mani et al., 2013), metallic nanoparticles modified CNTs-based electrodes (Yu et al., 2014), and CNTs-based electrodes with immobilized enzymes (Xu et al., 2015) are some of the recent techniques being implemented for CNTs-based electrochemical biosensors. According to previous reports, functionalization via enzymes is the most effective approach in modifying the surface of CNTs to fabricate electrochemical biosensors (Wang and Lin, 2008; Yang et al., 2007). In this method, enzymes can be attached onto the outer surface of functionalized CNTs due to hydrophobic and electrostatic interactions (Mittal, 2014; Saifuddin et al., 2012).The inner channel of the opened nanotubes also entraps small proteins via simple adsorption (Wong et al., 2013).

The speed of electron-transfer between enzymes' active sites and the electrochemical transducer is crucial towards biosensor designs (Luo et al., 2005), due to the fact that it is promotable via CNTs (Barsan et al., 2012). Polymeric entrapment with covalent immobilization methods also enhances the direct and fast electron transfer between enzymes and CNTs, which is rapidly emerging as a new research area in terms of functionalizing CNTs (Ahuja et al., 2007). Gelatin is a known natural polymer in electrochemical biosensors, obtained from collagen, which is widely used to immobilize matrices to prepare biosensors (Khadka and Haynie, 2012). A great gel forming ability, high biocompatibility with extremely heterogeneous polymer networks, different sizes of polypeptides, and molecular weight distribution render gelatin ideal for the preparation of electrochemical biosensors (Guiseppi-Elie, 2010). Taking into account previous reports on the advantageous properties of gelatin in electrochemical biosensors (Ozdemir et al., 2010; Sarma et al., 2009), the CNTs gelatin matrix is a promising form of nanocomposite to improve the direct electron transfer processes in biosensors via hydrophobic–hydrophobic interactions, forming stable dispersions of CNTs.

1.3 Problem Statement

It is reported that 5% of the population of developed countries suffers from diabetes; thus, a tight monitoring of glucose level is urgent for preventing and managing diabetes mellitus. Due to constraints in current glucose detection methods such as low stability and sensitivity, high costs, and complex understanding of the mechanisms, the implementation of an effective approach is essential in designing modern glucose biosensors, such as the electrochemical approach. Electrochemical detecting of glucose is mostly owing a wide dynamic detection range and continuous determination of glucose both in diabetic and electronic tongue approaches. CNTs demonstrate better electrochemical reactivity due to the hollow core being suitable for storing enzyme and promoting electron-transfer reactions of enzymes, such as glucose oxides. Unfortunately, there are some limitations in the immobilization of enzyme on the electrode’s surface, coupled with the poor electron transfer between the enzymes’ active site and the electrode from enzyme leaching and short lifetime of enzyme. Based on our knowledge, several methods have been developed to increase the biological function, stability, and efficacy of enzyme immobilization in electrochemical method by increasing the surface area of the CNTs. Therefore; the synthesis of optimized CNTs and fabricating CNTs-modified electrodes for electrochemical glucose biosensor is extremely attractive in improving the detection range, sensitivity, and the selectivity of the glucose biosensor.

1.4 Research Objectives

This thesis mainly focuses on the synthesis, optimization, and the utilization of carbon nanotubes composite materials to develop amperometric glucose biosensor. Amperometric glucose biosensors would be employed as the model system to compare the effects of different components on the performance of the biosensors. The objectives are:

- To synthesize and optimize vertically aligned carbon nanotubes using chemical vapor deposition method.
- To fabricate chemically modified glassy carbon electrode using optimized carbon nanotubes composite.
- To investigate the performance of fabricated nanocomposite-based electrode in electrochemical glucose biosensor and electronic tongue.

1.5 Scopes of Research

The central theme of this dissertation highlights the critical role of optimized MWCNTs to achieve enhanced performance and miniaturization of modified glassy carbon electrode applied in fabrication of glucose biosensor and electronic tongue. With the intention of achieving the above-mentioned objective, a series of scopes was carefully designed. The scopes are listed as:

i. Vertically aligned carbon nanotubes were synthesized by using chemical vapor deposition method. Camphor oil and ferrocene was used to compose the vaporized CNTs, and different CVD conditions were applied to obtain the best morphology of vertically aligned CNTs.

ii. The optimized MWCNTs were mixed with gelatin to produce MWCNTs/gelatin composite to entrap glucose oxidase and then drop casted on the surface of glassy carbon electrode. The modified glassy carbon electrode was examined under the different conditions to ensure the successful immobilization of glucose oxidase on the modified glassy carbon electrode.

iii. The modified glassy carbon electrode was used for electrochemically detection of glucose in the buffer solution. The polymeric entrapment and covalent immobilization of enzyme enhances the direct and fast electron transfer in continuous monitoring of glucose. The glucose biosensor was attached to a brain-like circuit to fabricate electronic tongue which consisted of two different layers: In the first layer, the alteration of current obtained by glucose detection of biosensor was transduced to a spiking pattern. The spiking pattern was conducted to the second layer via paracrine-like optical transmission, and induced synchronization of neural spiking activity in the second layer.

1.6 Significance of the Study

The development of an authentic, economical, and convenient glucose bio-sensing system for diabetes diagnosis is essentially important to monitor and treat diabetes. This study is significant, since optimized vertically aligned carbon nanotubes can maximize enzyme immobilization on the surface of the electrode, which can further promote electron transfer rates between the enzymes' active site and the modified electrode, resulting in an amprometric glucose biosensor with high stability and sensitivity.

1.7 Organization of Thesis

Chapter 1 of this thesis introduces the synthesis, optimization, properties, and electrochemical applications of carbon nanotubes. Chapter 2 describes a comprehensive review on the synthesis, characterization, optimization of carbon nanotubes, and glucose biosensor based on carbon nanotube composites. Chapter 3 describes the materials and methods of synthesis, characterization, optimization of carbon nanotubes, and modification methods of glassy carbon electrode (GCE) via carbon nanotube/gelatin composite. The fabrication method of glucose biosensor and electronic tongue will also be discussed in this chapter. Chapter 4 describes the results and discussion of all experiments, including the synthesis, characterization, and optimization of carbon nanotubes, as well as glucose biosensor and electronic tongue based on modified glassy carbon electrodes. Chapter 5 concludes the experiments conducted during the course of this work.

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