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SYNTHESIS AND CHARACTERIZATION OF POLYANILINE CRYSTALLINE NANOCELLULOSE COMPOSITE FOR CHOLESTEROL BIOSENSOR APPLICATION

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By

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Chairman: Mahnaz M. Abdi, PhD
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A cholesterol biosensor was developed by the modification of Screen-Printed Electrode (SPE) with a polyaniline/crystalline nanocellulose (PANi/CNC) A thin layer of ionic liquid (IL) was used to enhance the electron transfer and cholesterol oxidase (ChOx) was immobilized on the substrate for biological recognition. Crystalline nanocellulose was prepared from Semantan bamboo (*Gigantochloa scortechinii)* via acid hydrolysis which was then used to synthesize the polyaniline/ crystalline nanocellulose (PANi/CNC) nanocomposite *via in situ* oxidative polymerization of aniline in the presence of crystalline nanocellulose. The electrochemical properties of the nanocomposite were studied using a modified PANi/CNC electrode *via* cyclic voltammetry (CV), and a higher current response was observed for the PANi/CNC-modified electrode compared to that for the modified electrode with PANi. The data obtained from EIS showed lower charge transfer resistance (Rct) values for the PANi/CNC-modified electrode, indicating that the incorporation of CNC into the PANi structure enhanced the electron transfer rate. The FTIR spectra of the nanocomposite revealed the characteristic peaks of PANi and CNC, however, an overlapping of some of the characteristic peaks of pure PANi and CNC resulted in a broad band at 3263 cm^{-1} that could be due to the interaction between the NH group of PANi and the OH group of CNC inside the nanocomposite structure. The XRD diffraction spectra indicated lowered crystallinity which was observed at 2-theta values of 22.6 and 16.1 for PANi/CNC nanocomposite compared to the corresponding values in CNC. The FESEM images showed no phase separation in the nanostructure composite, revealing the homogenous polymerization of the monomer on

the surface of the crystalline cellulose. Aggregation of PANi particles was observed with increasing aniline concentration.

Research surface methodology (RSM) was applied to optimize the parameters and conditions in order to maximise the performance and sensitivity of the biosensor. Differential Pulse Voltammetry (DPV) as a powerful electroanalytical technique was used for all electrochemical measurements. The PANi/CNC/IL/GAL/ChOx-modified electrode showed a comparable sensitivity value of 35.19 µA mM /cm^{-2} towards H_2O_2 and cholesterol. The biosensor also exhibited a high dynamic response ranging from 1 µM to 12 mM cholesterol with a low Limit of Detection (LOD) of 0.48 µM. The biosensor displayed an acceptable reproducibility and repeatability with low relative standard deviations (RSD) of 3.76 % and 3.31 %, respectively. The biosensor showed high selectivity by excluding the interfering species and current response was maintained at over 84% of the initial value after 19 days.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS DAN PENCIRIAN POLIANILINA/ SELULOSA NANO KRISTAL KOMPOSIT SEBAGAI KOLESTEROL BIOSENSOR

Oleh

RAWAIDA LIYANA BINTI RAZALLI

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Satu kolesterol biosensor telah dibangunkan dengan pengubahsuaian Elektrod skrin bercetak (SPE) bersama polianilina / nanoselulosa kristal (PANi/CNC). Lapisan nipis cecair ionik (IL) telah digunakan untuk meningkatkan pemindahan elektron dan kolesterol oksida (ChOx) telah dimobilkan pada substrat sebagai unsur pengenalan biologi. Nanoselulosa kristal telah disediakan dari buluh Semantan (*Gigantochloa scortechinii*) melalui hidrolisis asid dan telah digunakan untuk mensintesis polianilina/nanoselulosa kristal (PANi/CNC) nanokomposit melalui polimerian oksidatif anilin dengan kehadiran nanoselulosa kristal. Ciri-ciri elektrokimia nanokomposit telah dikaji dengan menggunakan elektrod yang diubahsuai dengan PANi/CNC melalui voltammetri berkitar (CV), dan tindak balas arus yang tinggi telah dilihat pada elektrod yang diubahsuai oleh PANi/CNC berbanding elektrod yang diubah dengan hanya PANi. Keputusan yang diperolehi daripada EIS menujukkan nilai rintangan pindah caj (Rct) adalah rendah untuk elektrod yang telah diubahsuai dengan PANi/CNC, menunjukkan bahawa penggabungan antara CNC ke dalam struktur PANi boleh meningkatkan kadar pemindahan elektron. Spektrum FTIR nanocomposit mendedahkan kedua-dua puncak ciri PANi dan CNC, bagaimanapun, pertindihan antara beberapa ciri-ciri puncak PANi dan CNC tulen telah menghasilkan peluasan pada 3263 cm^{-1} yang boleh disebabkan oleh interaksi antara NH dari PANi dan kumpulan OH CNC di dalam struktur nanokomposit. Selain itu, dalam pembelauan XRD, kristalografi yang lebih rendah telah diperhatikan pada nilai 2 theta iaitu 22.6 dan 16.1 untuk PANi/CNC berbanding dengan nilai-nilai yang ada dalam CNC. Mikrograf FESEM menunjukkan nanostruktur composite tanpa

pemisahan fasa, mendedahkan pempolimeran homogen monomer pada permukaan selulosa kristal. Pengagregatan zarah PANi telah diperhatikan dengan peningkatan kepekatan anilina.

Kaedah permukaan penyelidikan (RSM) telah digunakan untuk mengoptimumkan parameter dan keadaan yang membawa kepada pretasi dan kepekaan biosensor yang maksimum. *Differential Pulse Voltammetry* (DPV) sebagai teknik elektroanalitikal yang kuat telah digunakan untuk semua ukuran elektrokimia. Elektrod yang diubah suai dengan PANi/CNC/IL/GAL/ChOx menunjukkan kepekaan yang standing terhadap H_2O_2 dan kolesterol pada nilai 35.19 µA mM /cm^{-2}. Biosensor yang dicadangkan telah menunjukkan tindak balas dinamik yang tinggi dari 1 µM hingga 12 mM kolesterol dengan had pengesanan rendah (LOD) yang rendah iaitu 0.48 mM. Biosensor ini menunjukkan reproduktif dan kebolehulangan yang boleh diterima dengan sisihan standard relatif rendah (RSD) masing-masing sebanyak 3.76% dan 3.31%. Biosensor ini menunjukkan selektiviti yang tinggi dengan mengasingkan spesies gangguan dan mengekalkan tindak balas arus lebih dari 84% selepas 19 hari.

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I certify that a Thesis Examination Committee has met on 14 December 2017 to conduct the final examination of Rawaida Liyana binti Razalli on her thesis entitled "Synthesis and Characterization of Polyaniline Crystalline Nanocellulose Composite for Cholesterol Biosensor Application" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CPs	Conducting polymers
Ani	Aniline
PANi	Polyaniline
CNC	Crystalline nanocellulose
CV	Cyclic Voltammetry
DPV	Differential pulse voltammetry
FTIR	Fourier Transform Infrared Spectroscopy
EIS	Electrochemical Impedance Spectroscopy
FESEM	Field Emission Scanning Electron Microscopy
TEM	Transmission electron microscopy
XRD	X-Ray Diffraction (XRD)
TGA	Thermogravimetric analysis
RSM	Response Surface Methodology
Rct	Charge transfer resistance
Cdl	Double layer capacitance
CI	Crystallinity index
GAL	Glutaraldehyde
IL	Ionic liquid
RSE	Relative Standard Error
LOD	Limit of detection

CHAPTER 1

INTRODUCTION

1.1 Background

Cholesterol is a critical lipid in the cell membranes and steroid hormones where it helps to maintain the integrity of these membranes, and plays an important role in facilitating cell signalling. However, high cholesterol (LDL) levels can increase the risk of cardiovascular diseases such as heart-related issues for instance stroke and heart attack. Heart disease is the number one killer in Malaysia with 20% of the people prone to it. The cholesterol detection analysis has been found to rely heavily on spectroscopy (Osman & Chin, 2006), while these methods usually suffer of complexities, time consuming and costly (Mashkour *et al.,* 2017). Therefore, the development of an efficient, sensitive and low-cost cholesterol sensor was crucial. Numerous researcher have been reported on the various innovation of a desirable cholesterol sensor (Lin *et al.*, 2016, Ruecha *et al.*, 2014, Umar *et al.*, 2014) and studies have shown that the enzymatic biosensor displays a notable cholesterol detection compared to the chemical method, due to its wide selection and high specification features (Rahman *et al.*, 2014).

Of late, electrochemical biosensor has been more preferential for its wide range of selection, user-friendly application, immediate results, accuracy in resulted readings, and cost- effective characteristics (Ahmad *et al.*, 2014, Ruecha *et al.*, 2014, Umar *et al*., 2014). With regards to the enzymetic cholesterol biosensor system, electrochemical techniques operate by detecting oxygen consumption or production rate of hydrogen peroxide through the enzymatic reaction (Singh *et al.,* 2007). Tsai *et al.*, (2008) describe the following reactions as:

$$\text{Cholesterol} + O_2 \rightarrow \text{Cholest-4-en-3-one} + H_2O_2$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

In addition, the immobilization of enzymes on the electrode surface is a critical step to preserve the enzymatic activity. Therefore, to have a stable and long-life biosensor, a suitable material and metrics is necessary to hold the biomolecule and maintain the natural condition of enzymes without any denaturation (Soylemez *et al.,* 2015, Yildirimoglu *et al*., 2009).

Providing an appropriate platform for the enzyme immobilization is still a big challenge, however, conducting polymers (CPs) has recently been viewed as suitable metrics for enzyme loading (Arora *et al.*, 2006, Singh *et al*., 2007). Conductive polymers have unique electrical and thermophysical properties and possess interesting features that have enabled their use in a variety of commercial applications (Casado *et al*., 2014). Polyaniline (PANi) is one of the well-known conducting polymers that has been widely used in numerous applications. However, PANi has some flaws; for example, it has low dispersibility and solubility in most solvents, low electron transfer rates and conductivity in solutions with a higher pH which has limited its performance in biosensors and electrical devices (Bhadra *et al.,* 2009).

Recently much research has been carried out to address these limitations in particular, the preparation of PANi based nanocomposites formed by the addition of nanoparticles, inorganic materials or biopolymers such as nanocellulose. Liu *et al.,* (2014) engineered a flexible and electrically conductive nanocellulose-based polyaniline composite film. They reported that the composite film with a thickness of 50 µm could be bent up to 180 degrees without breaking. The synergistic effect of the high surface area of the nanocellulose and the good electronic conductivity of the conducting polymer made the nanocomposite compatible for applications such as electrochemically controlled ion-exchange, ultrafast all polymer-based batteries and sensors (Nystrom *et al*., 2009). In biosensor applications, crystalline nano-cellulose (CNC) was introduced into the polymer structure to provide a larger active surface area and higher specific strength (Peng *et al*., 2011).

In this research, the polymerization of aniline in the presence of nanocellulose in order to improve the physical and electrochemical properties of PANi is reported. Hydroxyl groups that cover the surface of cellulose allow cellulose to react well with a variety of materials including conducting polymers (Liu *et al.,* 2014, Mo *et al.,* 2009, Peng *et al.,* 2011). In this study, the nanocellulose used was prepared from Semantan bamboo (*Gigantochloa scortechini*) by acid hydrolysis and it was expected that CNCs will improve the conductivity and electron transferring of PANi even in the neutral medium. To the best of our knowledge, this is the first report on the preparation of CNC from Semantan bamboo for biosensor applications.

For biosensor applications, it is essential for the composite to be able to perform in neutral conditions as most enzymes work well in neutral pH. It has been known that PANi generates high conductivity only in acidic media; however, PANi composites have been used in neutral media as biosensors, in most cited researches. Ghosh *et al.,* (1992) stated that a certain amount of cellulose could produce enough net negative charges on the PANi cellulose composite due to the ionization of the acidic moieties such as carboxyl, sulphonic acid, or hydroxyl groups on the surface of the cellulose. To balance this excess charge, the composite undergoes protonation and the proton concentration inside the composite increased compared to the external solution. Thus, PANi in composites is protonated and hence, able to conduct even in a neutral solution (Ghosh *et al.,* 1992).

This study was aimed to develop a sensitive enzymatic cholesterol biosensor using the PANi/CNC nanocomposite followed by coating a thin layer of IL. Additionally, the sensitivity of the biosensor has been enhanced through the introduction of ionic liquid (IL) to the substrate. The electro catalytic behavior of the PANi/CNC/IL/ChOx-modified electrode towards the electrochemical oxidation of hydrogen peroxide has been presented and discussed. At length, the performance of this sensing substrate has been optimized through the application of Research Surface Methodology (RSM) to detect the cholesterol concentration in some standard solutions.

1.2 Problem Statement

Various cholesterol detection methods have been established, but the detection relying more on spectroscopy, chromatography and the calorimetric methods. However, these traditional methods were retained complexities such as time-consumption and exorbitant cost for high technology equipment purchases (Umar *et al.,* 2014). Therefore, innovative approaches to develop a reliable cholesterol detection method are still challenging. The objective of this research is to improvise an enzymatic cholesterol biosensor based on conducting polymers. The electrochemical biosensor was prepared based on polyaniline nanocomposite. As it was mentioned before, aniline suffers from low dispersibility and solubility in most solvents, low electron transfer rates and conductivity in solutions with a higher pH which has limited its performance in biosensors and electrical devices (Bhadra *et al.,* 2009). So that, in this research crystalline nanocellulose (CNC) has been used to improve the physical and electrochemical properties of PANi for the sensor application with regards to the development of a nanostructure and stable platform for enzyme immobilization. Ionic liquid (IL) with excellent ionic conductivity, and biomaterials compatibility was used to enhance electron transferring of nanocomposite.

1.3 Objectives

The ultimate goal of this thesis is to study the physical and electrochemical properties of the polyaniline/crystalline nanocellulose/ionic liquid nanocomposite for sensor application. The objectives of this research are:

1. To synthesize the Polyaniline/Crystalline nanocellulose/Ionic liquid nanocomposite by chemical preparation and characterize resulting nanocomposite using different chemical, physical and electrochemical methods.
2. To fabricate cholesterol sensing enzymatic electrode using polyaniline/crystalline nanocellulose/ionic liquid on Screen Printed Electrode (SPE)
3. To optimize the performance of the fabricated cholesterol biosensor with the aid of Research Surface Methodology (RSM).

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