CHARACTERIZATION OF REDUCED GRAPHENE OXIDE CONDUCTIVE POLYMER COMPOSITES TOWARDS THE DEVELOPMENT OF NON-INVASIVE GLUCOSE BIOSENSORS

BY

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ABSTRACT

Graphene and conductive polymers had gain great deal of interest in various fields, such as in energy, environmental, and biomedical application, owing to their outstanding chemical and physical properties compared to conventional metal. Thus, in this study a reduced graphene oxide (rGO)-poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS)-based electrode was fabricated via a simple two-step, physical drop-cast method followed by subsequent electrochemical reduction. Cyclic voltammetry (CV) was used to characterize the redox capability of the composite layer on the electrode surface. Electrochemical deposition of rGO-PEDOT:PSS composites with a 1:1 ratio gives the highest anodic peak current I_{pa} of 1.184 mA and the largest effective surface area of 12.9 mm^2, compared to rGO alone electrodes (I_{pa} = 0.552 mA, A=12.9 mm^2). The rGO-PEDOT:PSS composites were characterized by scanning electron microscopy (SEM), transmission electron microscope (TEM), RAMAN Spectroscopy, Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). SEM results show the composite to have an open edge with a rougher surface morphology compare to rGO alone and TEM implies PEDOT:PSS is encapsulated by rGO. RAMAN spectroscopy shows the D band/G band intensity ratio of the composite films was increased from 1.12 (GO-PEDOT-PSS) to 1.25 (rGO-PEDOT:PSS) after electrochemical reduction, suggesting reduction of defects in graphene. Furthermore, XRD shows that the interlayer distance of rGO was increased from 0.37 nm (for rGO film) to 0.47 nm for rGO-PEDOT-PSS, confirming the effective intercalation of PEDOT/PSS in between rGO layers. Finally, glucose biosensor with rGO-PEDOT:PSS as transducer was successfully fabricated, and the sensitivity, sensitivity per electrode area, and limit of detection were 2.03 µA/mM, 15.74 µA/mM cm^2, and 0.08 µM, respectively. The results show rGO-PEDOT:PSS composite is an excellent precursor for the development of redox active transducer that can result in highly sensitive non-invasive glucose sensor.

Keywords: *graphene, reduced graphene oxide, cyclic voltammetry, PEDOT:PSS, glucose sensor*

خلاصة البحث

في السنوات الأخيرة ، حَظِيتْ المواد النانوية والبوليمرات الموصلة بقدرٍ كبيرٍ من الاهتمام في مجالات مختلفة ، مثل الطاقة والبيئة والتطبيقات الطبية الحيوية ، بسبب خصائصها الكيميائية والفيزيائية المتميزة مقارنة بالمواد المعدنية التقليدية. ولذلك في هذه الدراسة، تمَّ تصنيع قطب منخفض الجرافين-poly(3,4)(rGO) ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS)-based من خلال قطرة بسيطة مبدئية على الأقطاب تليها تخفيضات كهربية كيميائية لاحقة من الأقطاب القطنية. تمَّ استخدام مقياس الفولتية الحلقي(CV) لتوصيف قدرة الأكسدة للطبقة المركبة على سطح القطب. الترسيب الكهروكيميائي لـ rGO-PEDOT:PSS مركب بنسبة 1:1 يعطي أعلى ذروة تيار Ipa بمقدار 1.184 مللي أمبير وأكبر مساحة سطح فعلية تبلغ 12.9 مليمتر مربع، بالمقارنة مع أقطاب rGO الكهربائية المكشوفة. تتميز مركبات rGO-PEDOT:PSS أيضاً بمسح المجهر الإلكتروني (SEM) ، المجهر الإلكتروني النافذ (TEM)، مطيافية رامان (RAMAN Spectroscopy)، FTIR وانحراف الأشعة السينية(XRD). تظهر نتائج SEM حافة مفتوحة مع سطح مورفولوجي خشن تمت ملاحظته على سطح التركيبة. الـTEM يُظْهِر أن rGO غُلِفَتْ بواسطة PEDOT: PSS. ومن ناحية أخرى، يوضح مطياف رامان أن نسبة كثافة D/G للأغشية المركبة زادت من 1.12 (GO-PEDOT-PSS) إلى 1.25 (rGO-PEDOT: PSS) بعد التخفيض الكهروكيميائي مما يشير إلى تقليل العيوب في GO. في حين تُظْهر نتائج XRD أن المسافة البينية من rGO قد زادت من 0.37 نانومتر (للفيلم rGO النقي) إلى 0.47 نانومتر (للrGO-PEDOT: PSS)، مما يؤكد على التداخل الفعال لPSS / PEDOT بين طبقات الـ rGO. يبدو أن مركبات rGO-PEDOT: PSS يمكن أن تكون مولداً ممتازاً لتطوير قطب أو محول طاقة الأكسدة والاختزال والذي يمكن أن ينتج أجهزة عالية الحساسية قائمة على الكهروكيميائية. وأخيراً ، تمّ تصنيع جهاز الاستشعار البيولوجي للجلوكوز المعتمد على محول طاقة الrGO-PEDOT:PSS بنجاح ، والحساسية، والحساسية في منطقة القطب الواحد، والحد من الكشف عن 2.03 µA/mM،و15.74 µA/mM cm^2 ، و 0.08 µM على التوالي. لذلك فإن مركبات الـ-rGO PEDOT:PSS سوف تكون مولداً ممتازاً لتطوير محول نشط للأكسدة والاختزال والذي ينتج عنه أجهزة استشعار حيوية شديدة الحساسية للاستخدام غير الغازي.

كلمات مفتاحية: الجرافين ، انخفاض أكسيد الجرافين ، الجرافين المركّب النانوي، مقياس الفولتية الحلقي، PEDOT:PSS.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion; it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science (Biotechnology Engineering).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Thanks to Allah for granting me patience and perseverance to accomplish this thesis successfully. Indeed, without His Help and will, nothing is accomplished.

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LIST OF ABBREVIATIONS

CE	Counter electrode
CPs	Conductive polymers
CV	Cyclic voltammetry
DC	Drop-casting
SPGCE	Screen printed glassy carbon electrode
ME	Modified electrode
MNPs	Metal nanoparticles
MWCNTs	Multi-walled carbon nanotubes
RE	Reference electrode
WE	Working electrode
PBS	Phosphate Buffer Solution
Ppy	Polysterene
FPtE	Flat platinum electrode
EPD	Electropolymerisation deposition

LIST OF NOTATIONS

A	area of the electrode (cm^2)
A	effective surface area of the electrode after modification.
C^b	solution concentration (mole/l)
D	diameter (cm)
D	diffusion coefficient in (cm^2/s)
i	amperometric sensitivity (μA/mM) and
I_p	peak current
I_{pa}	anodic peak current
Ipc	cathodic peak current
K	current density (μA/mM cm^2),
n	number of moles of electrons transferred per mole
r	radius
v	scan rate of the potential (V/s)
ΔI	current difference

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LIST OF SYMBOLS

H_2O_2	Hydrogen peroxide
O_2	Oxygen
H^+	Hydrogen ion
GO	Graphene Oxide
rGO	Reduced Graphene Oxide
EDOT	3,4-Ethylenedioxythiophene
PEDOT	Poly (3, 4 Ethylenedioxythiophene)
GOx	Glucose oxidase
AuNPs	Gold nanoparticles
PEDOT:PSS	Poly (3,4 ethylenedioxythiophene): poly(styrenesulfonate)
PSS	Polystyrenesulfonic acid
$K_4[Fe(CN)_6]$	Potassium ferrocyanide
NaCl	Sodium chloride
KCl	Potassium chloride
HCl	Hydrochloric acid
Na_2HPO_4	Disodium phosphate
KH_2PO_4	Monopotassium phosphate
H_2SO_4	Sulfuric acid

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Diabetes Mellitus (DM) is a chronic disease, in which the person loses ability to produce or respond to hormone insulin (Pickup and Crook, 1998). International Diabetes Federation (IDF) estimated around 415 million of world population was diagnosed with diabetes in 2015. The number is expected to shoot up to 642 million by 2040. In addition, the IDF also estimated by end of 2015, diabetes will cost between USD 673 billion and USD 1, 197 billion in healthcare spending and have caused 5.0 million deaths (Jaacks et al., 2016). Early detection and continuous monitoring are the most effective approach to hinder serious complications, such as stroke, nerve degeneration, blindness, cardiovascular diseases and kidney diseases in diabetic patients (Nathan et al., 2013).

The control of blood glucose level relies on blood glucose measurement. Diabetic patients are advised to measure their blood glucose level several times for a better management of the diseases. Currently, the widely used method to check blood glucose level is a finger-prick glucose sensor (Hortensius et al., 2013). The semi-invasive glucose monitoring device is shown in Figure 1.1 and is always related with pain, complication, and skin infection. Other than that, it is also too painful and aggressive for certain age group of patients (Budda and Addi, 2014).

Figure 1.1 Commercially available –semi-invasive glucose biosensor or commonly known as the glucose meter.

Thus, there is a huge need for a painless and stress-free glucose monitoring method. Non-invasive glucose biosensor refers to the measurement of blood glucose level without drawing blood, puncturing the skin, or causing pain or trauma (Yan, Chan and Tang, 2017). It uses bodily fluids such as sweat, saliva or tears to measure blood glucose level. In the past years, many extensive campaigns have been made to develop a non-invasive glucose monitoring biosensor (Yan, Chan and Tang, 2017).

Cygnus Inc. successfully introduced a wearable GlucoWatch device measuring the glucose electro osmotically extracted across skin in 2002. Later on, OrSense Ltd. have introduced their glucose meter called OrSense NBM, a device that detects blood glucose

concentration via an optical method called ‘‘occlusion spectroscopy’’ (Bandodkar and Wang, 2014). Unfortunately, both devices failed at market valuation due to low level of accuracy, poor sensitivity and poor spectral signal-to-noise level. Following these failures, considerable efforts have been made to develop non-invasive glucose sensor. However, dependable non-invasive glucose measuring tool is still not available because of several challenges in accuracy, usability, and sensitivity. Biosensors based of nanomaterials have great potential to solve the aforementioned challenges.

Biosensor is a bioanalytical device that converts a biological response into a measurable electrical signal to detects and report specific analytes. Figure 1.2 shows biosensor to consist of three main components: the first component is the biological recognition element that detects the target molecules in the presence of various chemicals, the second component is a transducer that converts the biorecognition element into a measurable signal, and the third component is a signal processing system that converts the signal into a readable form (Yogeswaran & Chen, 2008). Out of these three components, the transducer is the most important component as improvements in the transducer layer can enhance sensor overall performance parameter. The incorporation of nanomaterials as transducers can be used to detect lower concentration of analyte, such as glucose, for non-invasive glucose biosensor purpose.

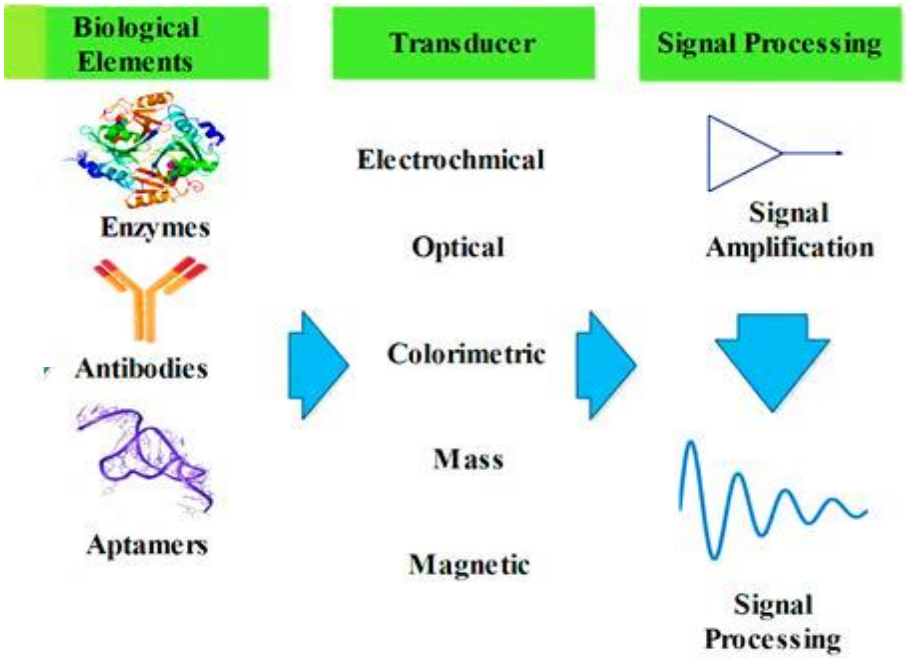

Figure 1.2 Three main components of a biosensor namely the biological element, the transducer, the signal processing and examples of transduction (Reprinted from Luka et al., 2015, licensed under CC BY 4.0).

A biosensor can be categorized into several types: resonant biosensors, optical biosensors, thermal biosensors, and electrochemical biosensors; depending on its transduction mechanism. Electrochemical biosensor were preferred over the others due to their electrodes can detect the materials which are present within the host without causing any damage to the host system (Malhotra and Chaubey, 2003; Kuila et al., 2011).

The performance of an electrochemical biosensor is measured based on several parameters such as sensitivity, selectivity, detection limit, dynamic linear range, response time, and stability. Sensitivity of a biosensor is a change of measured signal per analyte concentration unit, i.e., the slope of a calibration curve; selectivity refers to the characteristics that determine whether a sensor can respond selectively to different types

of analytes or specifically to a single analyte and with reduced interference from other analytes; detection limit is the lowest concentration of analyte that can be sensed by the sensor; dynamic linear range of a sensor is the relative deviation of an experimentally determined calibration graph from an ideal straight line; response time is the time needed for sensor to respond to a step concentration change from zero to a certain concentration value; stability refers to the ability of a sensor to provide reproducible results for a certain period of time (Thévenot et al.,2001). A non-invasive glucose biosensor should possessed high sensitivity, selectivity, stability, and shorter response time (Bochenkov and Sergeev, 2010). Thus, previous research focuses on optimizing the transducer component with nanomaterials for enhancing sensor performance (Devi, 2015).

The incorporation of nanomaterial such as carbon nanotube (CNT), graphene, metal nanoparticles (MNPs) and conductive polymers (CPs) can contribute in enhancing biosensors' performance parameters (Nambiar & Yeow, 2011). In addition, incorporating nanomaterial in transducer layer can increase the effective surface area of the electrode for redox reactions contributes to higher electron transfer from the bioreceptor and subsequently leads to improvements in signal detection (Salim et al., 2017). Graphene is one of the many available nanomaterials; it is a monolayer sheet of sp^2-bonded pure carbon (Novoselov et al., 2004). Graphene is the thinnest (only 1 atom thick), lightest (only 0.77 mg m^{-2}) and strongest (tensile strength of 1130 GPa) nanomaterial ever discovered and is a promising material for many applications from drug delivery to water filtration (Georgakilas et al., 2016). Graphene has excellent electrical conductivity, excellent thermal conductivity (approximately 5300 $W \cdot m^{-1} \cdot K^{-1}$), and it comes with increased light

absorption (Novoselov et al., 2012). Graphene also exhibits high structural stability and energy storage capability due to the increased surface area (Singh et al., 2011).

Being an unrolled carbon nanotube, graphene and its derivatives are suitable materials as transducers for the development of non-invasive glucose biosensor, as graphene-based biosensor can function without a mediator (e.g. ferrocene), as well as providing higher sensitivity and shorter response time towards molecule detection owing to its large surface area (Putzbach and Ronkainen, 2013). However, graphene alone tends to stack together owing to strong van der Waals and π-π forces, which results in decreased surface area and pore size, and inhibition of effective ion exchange between the electrode material and the electrolyte (Mercante et al., 2017). To overcome this, graphene has been reported to undergo modification namely covalent and non-covalent functionalization. Noncovalent functionalization with nanoparticles or conductive polymer such PEDOT:PSS were more preferred in graphene modification as this type of functionalization was found to not disrupt the sp2 -hybridized bond which is required for good electron conduction (Seekaew et al., 2014).

Poly (3, 4-ethylenedioxythiophene), often abbreviated, as PEDOT), is a derivative of polythiophene; it belongs to a family of conductive polymer (CP). PEDOT is formed from the monomer (3, 4-ethylenedioxythiophene) (EDOT); one of the many ways to prepare PEDOT is by electropolymerization of the EDOT monomer in polystyrenesulfonic acid (PSS) solution (Yang et al., 2013). PEDOT:PSS is a promising material in non-covalent functionalization of graphene. The composite of graphene and PEDOT:PSS possesses high electrical conductivity, good stability, good processability, and biocompatibility; all are important characteristics in biosensor development (Jiang, et al., 2014). Thus, in this

research, a highly sensitive and conductive transducer layer using reduced graphene oxide (rGO) and poly (3,4-ethylenedioxythiophen)–poly(styrenesulfonate) (PEDOT:PSS) composite for application in non-invasive glucose biosensor was developed.

1.2 PROBLEM STATEMENT

Glucose molecules are tough molecules; it is colourless, small, and normally can be converted to gluconic acid for measurement of glucose concentration. Table 1.1 shows the amount of glucose present in bodily fluid such as blood, saliva, sweat, and tear. From the table, it can be concluded that bodily fluids such as saliva, sweat or tear have lower concentration of glucose compare to blood. Thus, most of currently available non-invasive glucose biosensor for glucose detection did not meet the required accuracy and sensitivity, and failed at the global market (Vashist, 2013).

Table 1.1 Bodily fluid glucose concentrations in healthy and diabetic patients (Salam et al., 2016)

Bodily Fluid	Biomarker	Healthy Patients (Concentration mM)	Diabetic Patients (Concentration mM)
Blood	Glucose	4.9–6.9	2–40
Saliva	Glucose	0.23–0.38	0.55–1.77
Sweat	Glucose	0.06–0.11	0.01–1
Tear	Glucose	0–3.6	4.7

In addition, currently available non-invasive glucose biosensor has poor glucose specificity and sensitivity. Bodily fluids like saliva have plenty of other molecules such as glycoproteins, enzymes (e.g. amylase and lipase) and water, thus selecting glucose signal from background noise resulting from other molecules, is one of the main challenges of developing a non-invasive glucose sensor (Abhilash and Augustine, 2014). Several studies of non-invasive biosensor indicate that non-invasive assays need an increase of the glucose

signal to background noise ratio (Salam et al., 2016). Finally, GlucoWatch was withdrawn from market, 2008 due to the 15 minutes longer warm up time compare to finger-pricking reading meters (Tamar et al., 2017).

1.3 RESEARCH SIGNIFICANCE

This research aims to synthesize a reduced graphene oxide- poly (3,4-ethylenedioxythiophen)–poly(styrenesulfonate) (rGO-PEDOT:PSS) composites for fabrication of a biosensor transducer for glucose sensing. The modified rGO-PEDOT:PSS based transducer aims to solve issues of accuracy, sensitivity, and response time, which are the main functional issues to be solved in the development of non-invasive glucose monitoring device. Owing to availability of new nanomaterials, improvements of biosensors with graphene and conductive polymer transducer can be made, and can lead the way to the successful commercialization of non-invasive glucose sensor technology.

1.4 RESEARCH OBJECTIVES

To overcome the limitations of non-invasive glucose biosensor, this project aims to fabricate a graphene- and conductive polymer-based transducer on screen-printed glassy carbon electrode (SPGCE) surfaces for application in glucose sensing.

1. To analyze graphene and graphene-conductive polymer PEDOT:PSS- based composites anodic/cathodic peak current and effective surface area via cyclic voltammetry and surface characterization techniques.
2. To immobilize glucose oxidase (GOx) on the composite with the highest anodic/cathodic peak current and to confirm successful immobilization via surface