COMPARATIVE STUDY OF ELECTROCHEMICAL TRANSDUCER FABRICATION METHODS WITH GLUCOSE OXIDASE AS RECOGNITION LAYER

BY

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ABSTRACT

Combination of reduced graphene oxide (rGO) with a conductive polymer, poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) is promising as transducer material for electrochemical biosensors. However, fundamental research into this composite behaviour is essential for generating the necessary scientific understanding to realize a non-invasive glucose monitoring approach. In this study, the rGO-PEDOT:PSS modified electrodes were fabricated with four different methods (method A, B, C, and D) where the fabrication parameters such as reduction cycles, sequence and glucose oxidase immobilisation techniques were varied. The aim is to elucidate how these fabrication parameters can affect the electrochemical reversibility, mass transport properties, heterogeneous electron transfer rate constant (k^0) and effective surface area (A_{eff}) of rGO-PEDOT:PSS materials in which can be determined from cyclic voltammetry (CV). This study also utilized machine learning algorithm to model the data from CV results where the most accurate model was used to analyse the interaction strength between the input and output data. From the electrochemical analysis, the ferri/ferrocyanide redox couple $[Fe(CN)_6]^{3-/4-}$ shows quasi-reversible and diffusion-controlled behaviour on rGO-PEDOT:PSS-modified SPCEs of all fabrication method. In terms of k^0, each fabrication methods generated different trend of k^0 value with increasing reduction cycles. Overall, the range of k^0 for rGO-PEDOT:PSS-modified SPCE are from 0.52 $x10^{-5}$ to 4 $x10^{-5}$ cm/s. We also found that the highest A_{eff} value with respect to fabrication method were obtained from different number of reduction cycles. Fabrication method A gave the highest A_{eff} when the composite was reduced for 5 reduction cycles (16.41 cm^2). For methods B and D, the highest A_{eff} obtained was for 30 reduction cycles (17.48 cm^2 for method B and 6.43 cm^2 for method D) while for method C, the highest A_{eff} value was obtained for 15 reduction cycles (21.41 cm^2). For SVM analysis, data from CV (ΔE_p and I_{pc}) and the fabrication parameters were used to construct a prediction model. Linear and non-linear kernels were compared, and the best performance was showed by radial basis function (RBF) kernel with a perfect accuracy of 100%. The RBF kernel was then used to measure the interaction between input and output variables in which the kernel model demonstrated the strongest interaction between reduction cycles and ΔE_p. In conclusion, this study reveals the effect of fabrication parameters on electrochemical characteristics of rGO-PEDOT:PSS-modified SPCEs and the capability of machine learning algorithm to model data and provide deeper insights on the fabrication process, opening a new route for development of non-invasive glucose biosensors.

خلاصة البحث

يُعد مركب أكسيد الجرافين المختزل (rGO) مع البوليمر الموصل بولي (٣،٤-إيثيلين ديوكسي ثيوفين): بولي (ستايرين سلفونات) (PEDOT: PSS) أحد المركبات الواعدة لكونها مادة محولة لأجهزة الاستشعار الحيوية الكهروكيميائية. ومع ذلك، فإن البحث الأساسي في سلوك هذا المركب ضروري لتوليد الفهم العلمي المطلوب لتحقيق نهج غير غازي لمراقبة الجلوكوز. تم تصنيع أقطاب rGO-PEDOT: PSS المعدلة في هذه الدراسة بأربع طرق مختلفة (الطريقة A و B و C و D) حيث اختلفت معاملات التصنيع مثل دورات الاختزال والتسلسل وتقنيات تثبيت أوكسيداز الجلوكوز. والهدف يكمن في توضيح كيفية تأثير عوامل التصنيع على ثابت معدل التفاعل (k^0) ومنطقة السطح الفعالة (A_{eff}) الخاص بمواد rGO-PEDOT:PSS التي يمكن تحديدها من المقياس الفولطي الدوري (CV). استخدمت هذه الدراسة أيضًا خوارزمية التعلم الآلي لنمذجة بيانات نتائج المقياس الفولطي الدوري حيث تم استخدام النموذج الأكثر دقة لتحليل قوة التفاعل بين بيانات الإدخال والإخراج. وبالنظر إلى التحليل الكهروكيميائي، يُظهر زوجا الأكسدة والاختزال فيري / فيروسيانيد $[Fe(CN)_6]^{3-/4-}$ سلوك شبه قابل للعكس والتحكم في الانتشار على rGO-PEDOT: PSS المعدلة من SPCE لجميع طرق التصنيع.أما من حيث k^0 ، فكل طرق التصنيع انتجت اتجاهات مختلفًا لقيمة k^0 مع زيادة دورات الإرجاع. بشكل عام ، يتراوح نطاق k^0 لـ rGO-PEDOT:PSS المعدل ما بين $0,52 \times 10^{-5}$ إلى 4×10^{-5} سم في الثانية. وقد وجدنا أيضًا أنه تم الحصول على أعلى قيمة لـ A_{eff} فيما يتعلق بطريقة التصنيع من عدد مختلف من دورات الاختزال. أعطت طريقة التصنيع A أعلى نسبة A_{eff} عندما تم تخفيض المركب إلى ٥ دورات إرجاع (١٦،٤١ سم^2). أما بالنسبة للطريقتين B و D ، فإن أعلى A_{eff} تم الحصول عليه مع ٣٠ دورة إرجاع (١٧،٤٨ سم^2 للطريقة B و ٦،٤٣ سم^2 للطريقة D) بينما بالنسبة للطريقة C ، فتم الحصول على أعلى قيمة A_{eff} مع ١٥ دورة إرجاع (٢١،٤١ سم^2). بالنسبة لتحليل شعاع الدعم الآلي، تم استخدام بيانات المقياس الفولطي الدوري (ΔE_p and I_{pc}) وعوامل التصنيع لبناء نموذج تنبؤ. تمت مقارنة النوى الخطية وغير الخطية، وأظهت نواة وظيفة الأساس الشعاعي (RBF) أفضل أداء بدقة مثالية بنسبة ١٠٠٪. ثم تم استخدام نواة RBF لقياس التفاعل بين متغيرات المدخلات والمخرجات حيث أظهر نموذج النواة أقوى تفاعل بين دورات الاختزال و ΔE_p. وفي النهاية، تكشف هذه الدراسة عن تأثير عوامل التصنيع على الخصائص الكهروكيميائية لـ rGO-PEDOT: PSS المعدلة SPCEs، وقدرة خوارزمية التعلم الآلي على نمذجة البيانات وتقديم رؤى أعمق حول عملية التصنيع ، وفتح طريق جديد لتطوير أجهزة الاستشعار الحيوية للجلوكوز غير الغازية.

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 thesis for the degree of Master of Science (Biotechnology Engineering).

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DECLARATION

I hereby declare that this thesis 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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LIST OF ABBREVIATIONS

CE	Counter electrode
CPs	Conductive polymers
CV	Cyclic voltammetry
GO	Graphene oxide
GOx	Glucose oxidase
PEDOT:PSS	Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate)
RBF	Radial basis function
RE	Reference electrode
rGO	Reduced graphene oxide
SPCE	Screen-printed carbon electrode
SVM	Support vector machine
WE	Working electrode

LIST OF SYMBOLS

A_{eff}	Effective surface area of the electrode (cm^2)
C	Solution concentration (mole/L)
c	Cost hyperparameter
E_p	Peak potential (V)
E_{pa}	Anodic peak potential (V)
E_{pc}	Cathodic peak potential (V)
D	Diffusion coefficient (cm^2/s^2)
I_p	Peak current (μA)
I_{pa}	Anodic peak current (μA)
I_{pc}	Cathodic peak current (μA)
k^o	Heterogeneous electron transfer rate constant (cm/s)
n	Number of electrons transferred per mole of electroactive species
v	Scan rate of the potential (V/s)
ΔE_p	Peak-to-peak potential separation (mV)
σ	Sigma hyperparameter
Ψ	Dimensionless parameter

CHAPTER ONE

INTRODUCTION

١,١ BACKGROUND OF THE STUDY

Electrochemical biosensors have become an attractive choice of detection service for industrial, environmental, and biomedical applications owing to its sensitivity towards analytes, low cost of construction, simple fabrication, and capability to measure a targeted analyte from a complex sample (Grieshaber, MacKenzie, Vörös & Reimhult, 2008; Hammond, Formisano, Estrela, Carrara, & Tkac, 2016). Among various types of electrochemical biosensors, glucose biosensors dominate consumer market since the concept of enzyme electrode was introduced in 1962. The market preference towards glucose biosensors is not only because of the reliability and rapid response in measuring glucose concentration from blood samples but is also due to the rise of diabetes prevalence around the world. In 2019, the global population of diabetes among adults is estimated to be 463 million, showing an increase of 62 % since the last decade (International Diabetes Federation, 2019).

Conventional glucose biosensors are considered as a gold-standard tool for diabetes monitoring. However, these biosensors involved finger-pricking which cause discomfort and pain. Thus, realization of a non-invasive glucose monitoring approach has become an ultimate goal under progress. The non-invasive approach aims for measuring glucose level in alternative bodily fluids such as tear, sweat, and saliva (Baca, Finegold, & Asher, 2007; Moyer, Wilson, Finkelshtein, Wong, & Potts, 2012; Jha, David, Saluja, Venkatesh, & Chaudhary, 2014; Gupta, Sandhu, Bansal, & Sharma, 2014; Jadoon et al., 2015;). While this approach is possible due to positive

correlation between glucose level in blood and the non-blood samples, the glucose level in the non-blood samples can be hundreds of times lower of that in blood (Makaram, Owens, & Aceros, 2014); thus, the sensing element must be sensitive enough to detect the low glucose concentration. To address the challenge to detect low glucose concentration of non-blood samples, research work on the electrode fabrication constitute the most important part where the transducer materials and enzyme immobilisation strategy greatly influenced the performance of the electrochemical biosensors.

In developing electrochemical biosensors, sensitivity and selectivity matters, hence, selection of a transducer material should aim for both a highly conductive material for sensitivity, and ability to the signal produced from the reaction between targeted analyte and biorecognition element selectively. The use of graphene with a conductive polymer, poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) as the transducer material for enzymatic glucose biosensor may improve biosensors' sensitivity owing to the synergistic interaction of both materials (Zhang, Yuan, Yao, Li, & Shi, 2014). Previous studies have been performed using graphene and conductive polymer composite for the detection of various analytes such as glucose (Wisitsoraat et al., 2013; Abd-Wahab, Abdul Guthoos, & Wan Salim, 2019), ammonia (NH_3) (Seekaew et al., 2014; Pasha, Khasim, Khan, & Dhananjaya, 2019), and iron (Fe) (Sundramoorthy, Premkumar, & Gunasekaran, 2016). The promising results from these studies have made the combination of graphene with PEDOT:PSS attractive as a transducer material for developing a non-invasive glucose biosensor. However, there is a need to investigate further on the composite's interaction and mechanism at the molecular level.

In this study, the rGO-PEDOT:PSS transducer layer was fabricated with four different fabrication methods in which two of the methods were adopted and modified from the previous studies by Abd Wahab et al. (2019) and Wisitsoraat et al. (2013). The fabrication parameters of GO reduction cycles (for removal of oxygen functional groups on GO surfaces), glucose oxidase (GOx) immobilisation sequence (either before or after reduction of GO), and immobilisation strategy were varied. The aim is to deduce how these fabrication parameters play their role to affect electrochemical reversibility, heterogeneous electron transfer rate constant (k^0), mass transport properties, and effective surface area (A_{eff}) of rGO-PEDOT:PSS-modified screen-printed carbon electrode (SPCE), all of which can be determined from cyclic voltammetry (CV).

Complex relationship between the fabrication parameters and CV measurements as input and output data may impede the progress of optimizing the fabrication parameters. Through recent advances in artificial intelligence (AI), meaningful information from biosensors data can be obtained by utilizing machine learning approaches. Support vector machine (SVM) is one of the machine learning algorithms that can be applied for data classification and regression. In present study, SVM was used to build a prediction model that can differentiate the different fabrication methods. The fabrication parameters and data obtained from CV were used as the dataset to build and test the model. Comparison between three SVM models with different characteristics known as linear, polynomial and radial-basis function (RBF) kernel was done to determine the best suited model for the biosensor's data. We also explored on how the fabrication parameters relate to the data from CV through variable interaction analysis using the best SVM model (Friedman &

Popescu, 2008; Molnar, 2018a). While SVM modelling on CV data for biosensor development has been reported previously, analysing the interaction between the input and output variables based on the model has not been done before. Therefore, this study provides new insights to advance the understanding on fabrication of rGO-PEDOT:PSS composite as transducer for biosensor development utilizing the SVM prediction model. The schematic illustration of this study is shown in Figure 1.1.

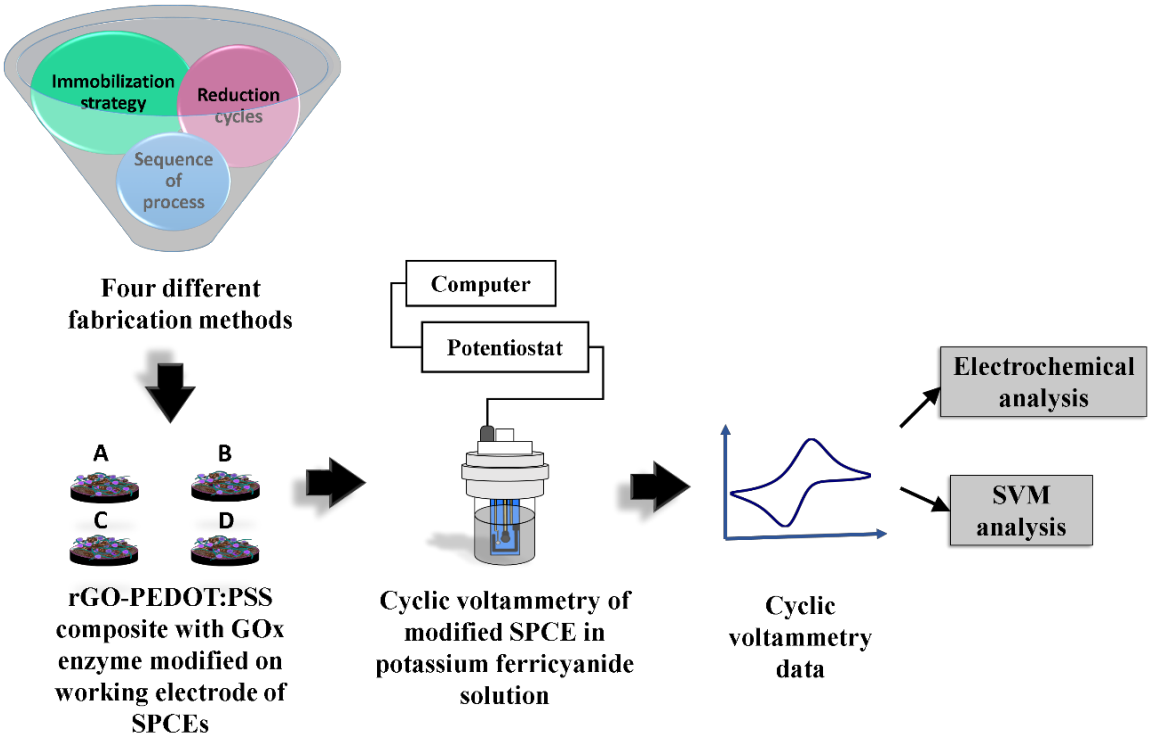

Figure 1.1 Schematic illustration of present study. The fabrication parameters were varied to generate four different fabrication methods of rGO-PEDOT:PSS-modified SPCE. The modified SPCEs were subjected to cyclic voltammetry in potassium ferricyanide and the data obtained were used for electrochemical and SVM analyses.

١,٢ PROBLEM STATEMENT

Diabetes is a lifelong condition that requires frequent self-monitoring of blood glucose level. Conventional glucose biosensors are partially invasive where a small, sharply pointed needle is used for blood sampling. The painful finger-pricking procedure is recommended up to seven times per day, thus, discouraging many diabetes patients from managing glucose level properly. Therefore, a non-invasive device for glucose monitoring utilizing alternative bodily fluids such as saliva, sweat, and tears, is in high demand where a better diabetes control can be achieved without the pain of finger-pricking.

Employing a composite of graphene with conductive polymer, PEDOT:PSS as transducer material is expected to improve the sensitivity of glucose biosensors and enable detection of glucose in alternative bodily fluids. Fundamental research into this composite behaviour is an essential prerequisite for generating the necessary scientific understanding in developing a non-invasive glucose biosensor. In this study, the fabrication parameters were varied to observe its effect on electrochemical reversibility, heterogeneous electron transfer rate constant (k^0), mass transport properties, and effective surface area (A_{eff}) via cyclic voltammetry (CV). Incorporating SVM analysis for mapping the input and output data from CV may uncover relationships and interactions between data that provide deeper insights on the fabrication process.