THE BIOSENSING PERFORMANCE AND ELECTROCHEMICAL PROPERTIES OF CARBONISED ELECTROSPUN NANOFIBER

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Abstract-

Biosensor is one of the most important analytical devices to be used in analysing analyte where the analyte is referring to any substance whose chemical constituents are being identified and measured. There are several compulsory components to make up a fully functional biosensor. By principle, a biosensor must consist of sample, analyte, bioreceptor and transducer. Transducer plays an important role in biosensing as it converts physical quantitative and qualitative measurement and convert those values into electrical signal. Transducer is also known as sensing platform. In order transducer to become biologically capable in detecting the presence of analyte, bioreceptors or biosensing elements are required to be integrated with the support of transducer. In this research, our main concern is to find the best type of electrospun nanofiber that is used as the support for the transducer of the glucose monitoring biosensor. Thus, there were several PAA/PAN-based electrospun nanofibers used and each of the nanofibers has different associated nanoparticle fabricated together with the PAA/PAN polymer. The nanofibers were used to be immobilised with (GOx) enzyme. The biosensing performance were analysed by using several analyses mainly electro impedence spectroscopy (EIS), capacitance-voltage profiling (CV-plot), scan rate dependent study and chemical concentration dependent study. From this research, we have summarized that the presence of reduce Graphene oxide nanoparticles (rGO NPs) in Sample 2 improved conductivity of the electrospun nanofiber.

Keywords – biosensor, support, transducer, electrospun nanofiber, immobilisation, glucose monitoring.

I. INTRODUCTION

Undeniably, as biological technology is becoming advance on par with current globalized health issues, lots of sophisticated health monitoring devices were developed and one of them is biosensor. Biosensor is basically a type of sensor which implemented the functionality of biological element or bio-receptor such as enzyme and antibody in order to detect analyte such as glucose and living microorganism. Monitoring glucose in Malaysia is already becoming a common routine for people either healthy or with diabetic disease. Nowadays, diet pattern illustrates the unhealthy lifestyle that contributed such huge number of diabetic patients.

According to International Diabetes Federation, a study revealed about 387 million people worldwide who are diagnosed with diabetes disease on 2014. Unfortunately, 2 million of them are Malaysians [1]. Furthermore, people who are in obesity society are those who prone to have diabetes mellitus disease as human body mass index has strong relationship with diabetes and insulin resistance [2].

Furthermore, people who are in obesity society are those who prone to have diabetes mellitus disease as human body mass index has strong relationship with diabetes and insulin resistance [2]. Diabetes mellitus defines a metabolic disorder type of disease which is involving carbohydrate, fat and protein metabolism as disturbances [3]. As result, it defects in insulin secretion. Insulin in fact is a type of protein or hormone made by pancreas that helps in human digestive system which has role in converting glucose, a simple sugar form into glycogen, complex form of sugar. Diabetic patients are normally face difficulty in regulating their blood glucose level due to the distortion of insulin secretion that results in increasing sugar level in blood.

Over the years, due to the increment of people with diabetic diseases, glucose biosensor was developed. In glucose monitoring biosensor, glucose oxidase is a common enzyme used in biosensing glucose. This biosensor work by tracking number of electrons that passes through the enzyme. Glucose that will be analysed by the biosensor will undergo oxidation process catalysed by (GOx) glucose oxidase enzyme to produce H_2O_2 and Gluconic Acid [4]. The presence if GOx enzyme tend to speed up the oxidation reaction. Thus, the production of H_2O_2 then causes it to oxidise and electron transfer occurs during the process will be detected by electrode of the biosensor. There is a special unit in the biosensor which is called transducer that will perform the detection and send signal to be converted into either quantitative or qualitative readable form of readings. In order to integrate the usage of GOx into the biosensor, the GOX enzyme in the first place need to be immobilized with the electrode of the biosensor. There are several types of electrode or support used which can be in the form of polymer, metal and glass. The most common polymer used as the biosensor supports are (PAA) Polyacrylic acid, (PAN) Polyacrylonitrile and (PVC) Polyvinyl Chloride. Polymer-type support is normally fabricated in a form of thin nanofiber that is produced from an electrospinning process which produces fibre size of micrometre up to nanometre scale.

The performance of glucose biosensor is depending on its precision and accuracy upon performing its detection. The lesser time required for the biosensor to perform detection, the better performance of biosensor will be considered. There are few methods to amplify the sensitivity of biosensor. One of those methods is by carbonising the electrospun nanofiber [5]. The main concern in this research is to develop highly sensitivity of glucose monitoring biosensor. As the glucose monitoring biosensor plays in a crucial part in monitoring blood glucose level, it is important to have a type of biosensing platform which is highly sensitive and perform significantly precise and accurate detection. In order to solve the problem, sensor could perform detection of glucose even in small amount concentration is present. Hence, we use a carbonised polymer-based electrospun nanofiber as the support of the biosensor that will be immobilized with GOx enzyme. A better glucose biosensor must be responsive and conductive to allow the flow of electron occurs efficiently. The capability of the sensing support to allow the flow of electron has relation with the material's electrochemical properties. Hence, a good biosensing support must have good electrochemical properties.

II. METHODOLOGY

2.0 List of Samples

Electrospun nanofibers that were used for this research is PAA/PAN type of polymer) with several type of nanoparticles associated with the nanofiber composition (refer Table 1). PAA stands for Polyacrylic acid polymer and PAN refers to Polyacrylonitrile polymer. All nanofiber samples were provided by University of Edinburgh.

Table 1: Samples

Sample	Type of Electrospun Nanofiber
1	PAA/PAN electrospun nanofiber (Bare)
2	PAA/PAN electrospun nanofiber with Reduced Graphene
C	PAA/PAN electrospun nanofiber with Gold nanoparticles
G	PAA/PAN electrospun nanofiber with Ferric Oxide

2.1 Chemicals and Stock Solution Preparation

2.1.1 Glucose Oxidase Enzyme Solution

This solution was used for immobilization process. The stock GOx SIGMA-ALDRICH with (E.C number of 1.1.3.4) is present in powdered form. Hence, 0.75g of GOx powder was dissolved in 25ml of phosphate buffer solution 0.1M, 7.4pH to produce 30mg/ml GOx solution.

2.1.2 Glucose Solution

Glucose solutions of 0, 2, 4, 6, 8 and 10mM. were prepared by using 0.072g SIGMA-AIDRICH glucose powder with 40ml ,0.1M, pH 7.4 Fisher Bioreagent™ phosphate buffer solution as the solvent.

2.1.3 Pyrenebutyric Acid Solution

The 1-Pyrenebutyric acid was used to functionalise carbonised-electrospun nanofiber samples. The 1-pyrenebutyric acid was dissolved in dimethylformamide (DMF) solvent. 100mM concentration of 1-Pyrenebutyric Acid Solution by AnaSpec Inc was prepared.

2.1.4 Ethyl-3-(3-Dimethylaminopropyl)carbodiimide Solution

The 1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide solution or DMF was used to treated the carbonised-electrospun nanofiber samples. 200mMconcentration of DMF solution was prepared by dissolving it with PBS solvent pH of 7.4.

2.2 Carbonisation

The PAA/PAN electrospun nanofiber nanofiber samples went through carbonisation process in order to make the nanofiber becomes carbonised. Before carbonisation process, there is another pre-treatment required. The process is called stabilisation. Electrospun nanofiber samples went through carbonisation in the first place where the samples were heated inside the furnace (model number: GSL-1100X) by MTI CORP. Right after 12 hours of stabilization by using the furnace, carbonisation is required by using the same GSL-1100X furnace with different parameter settings were changed. The samples were carbonised under temperature of 750°C for 75 minutes of holding time with nitrogen gas continuously flowed through the combustion compartment of the furnace along the process. This stabilisation and carbonisation process were done by the following settings (refer Table 2):

Table 2: GSL-1100X Furnace Setting for Stabilisation and Carbonisation

Settings	Set Point Value (SP) for Stabilisation	Set Point Value (SP) for Carbonisation
Initial Temperature / (°C)	25.0	25.0
Heating Time / (min)	10.0	75.0
Target Temperature / (°C)	200.0	750.0
Holding Time / (min)	750.0	60.0
Final Temperature / (°C)	200.0	750.0
Nitrogen gas flowrate /(m^3/s)	0.0	4.0

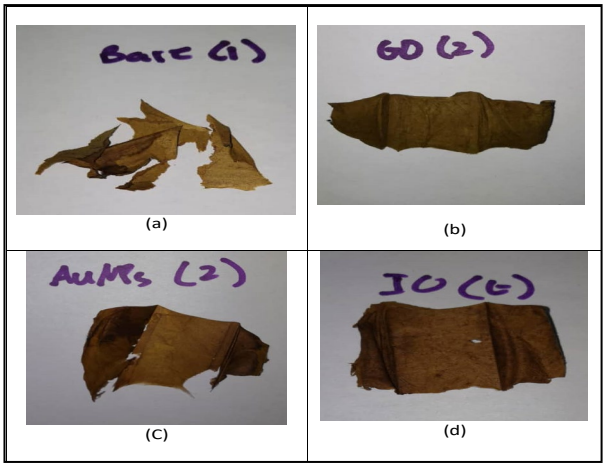


Figure 1: Electrospun nanofiber samples after stabilisation process (a) Sample 1 PAA/PAN electrospun nanofiber (Bare), (b) Sample 2 PAA/PAN electrospun nanofiber with Reduced Graphene, (c) Sample C PAA/PAN electrospun nanofiber with Gold nanoparticles, (d) Sample G PAA/PAN electrospun nanofiber with Ferric Oxide

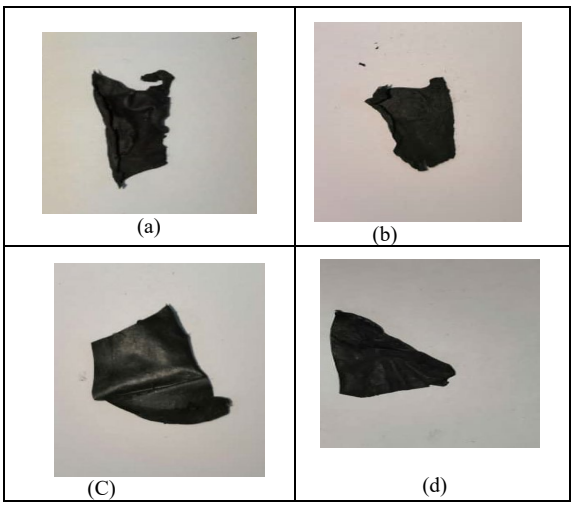


Figure 2: Electrospun nanofiber samples after carbonisation process (a) Sample 1 PAA/PAN electrospun nanofiber (Bare), (b) Sample 2 PAA/PAN electrospun nanofiber with Reduced Graphene, (c) Sample C PAA/PAN electrospun nanofiber with Gold nanoparticles, (d) Sample G PAA/PAN electrospun nanofiber with Ferric Oxide

2.3 Immobilisation for Carbonised Electrospun Nanofiber

The carbonised electrospun nanofibers were cut into circular shape with 3mm diameter which was about the same size of the circular carbon on screen printed carbon electrode (SPCE). The carbonised electrospun nanofibers were went through functionalisation

(non-covalent method) process. Samples went through adsorption process with 20ml of 100mN 1-pyrenebutyric acid in 98.9% of dimethylformamide (DMF) for 3 hours in room temperature and shake under 50rpm. The carbonised electrospun nanofibers were washed with methanol followed by distilled water Samples were incubated in 1.5ml oh 200mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in aqueous buffer of 50 mM phosphate buffer, pH6.5 for 30 min at room temperature shaking condition of 50rpm for 30 minutes. The incubated carbonised electrospun nanofiber samples were then immobilised with 3ml of 10mg mL^{-1} GOx solution for 30 minutes. Then samples were glued on SPCE for analysis [6].

2.4 C-V plot Analysis

This cyclic voltammetry (CV) analysis was divided into two part of studies. First is scan rate dependent study and followed by concentration dependent study. The scan rate and concentration dependent study were purposely to analyse the nanofibers' electrochemical behaviour when different scan rate and concentration of electrolyte were applied toward the nanofibers through SPCE electrode. **Figure 3** shows the SPCE that will be used for the CV analysis. On the glued sample (refer **Table 1**) should be the sample that will be used for the analysis.

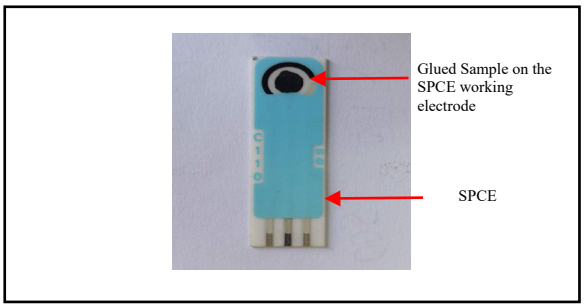


Figure 3: Typical graphene-based electrochemical sensor used, scanning Printed Carbon Electrode (SPCE)

III. RESULT AND DISCUSSION

3.1 The Presence of Graphene Oxide in Sample 2 Justification

3.1.1 Scanning Electron Microscopy

This analytical study was conducted in order to verify the presence of reduce graphene oxide in the electrospun nanofiber **Sample 2** by referring to **Table 1**. Graphene oxide has chemical formula of $C_{140}H_{42}O_{20}$[7]. From the graphene oxide chemical formula illustrates that Carbon element dominates the overall chemical composition of graphene oxide. Reduced graphene oxide or (RGO) is another form of graphene oxide that is chemically and thermally processed to lower down the oxygen content. As the result, reduced graphene oxide relatively has lower oxygen content than ordinary graphene oxide [8].

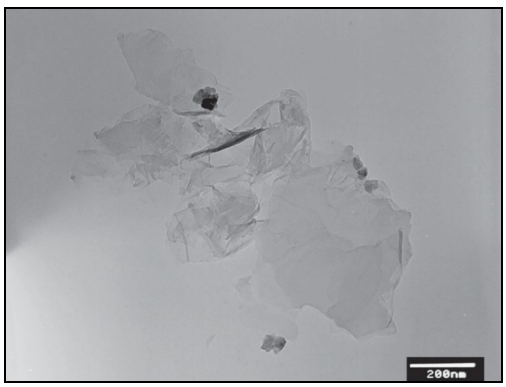


Figure 4: Scanning Electron Microscope Image of Reduce Graphene Oxide [8]

Figure 4 shows the actual microscopic image of reduced graphene oxide retrieved from a study conducted upon the study of Structural analysis of reduced graphene oxide. Graphene seemed to have sheet-like shape and has a small degree of transparency. The same analytical study was performed toward **Sample 2** in order to detect the similar shape that represents reduced graphene oxide.

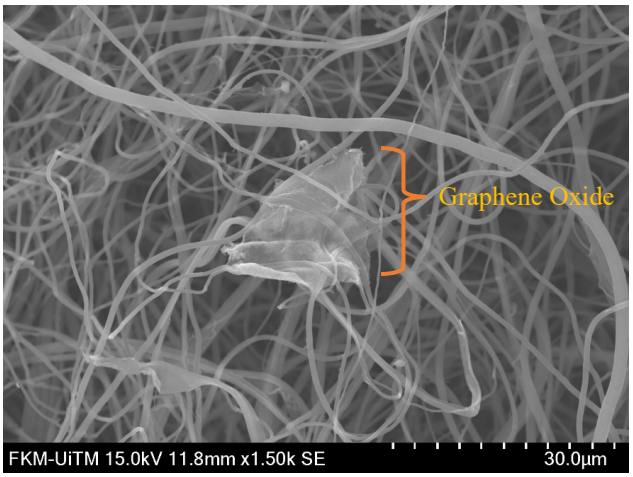


Figure 5: Field Emission Scanning Electron Microscopy Image of **Sample 2**

Figure 5 above shows the footage from (FESEM) that was used to scan **Sample 2** which is the PAA/PAN electrospun nanofiber with reduced graphene oxide. The same sheet-like structure of graphene was found within the electrospun fibres justified the presence of reduce graphene oxide associated with the PAA/PAN electrospun nanofiber.

3.1.2 Energy Dispersive Spectroscopy

Another graphene oxide presence justification was made by using energy dispersive spectroscopy (EDS), an analytical device that uses X-ray spectroscopy for elemental analysis and chemical characterisation. This analytical strategy was also used to authenticate the presence of graphene oxide within the electrospun nanofiber of **Sample 2** (refer **Table 1**).

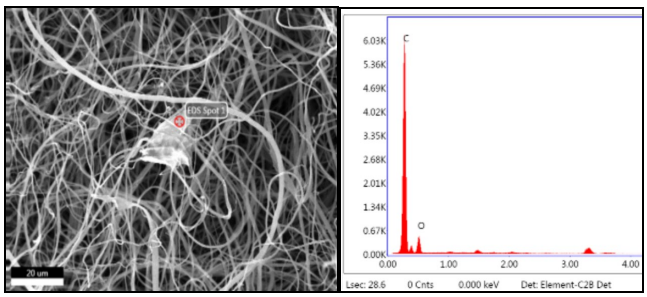


Figure 6: EDS Analytical Region and Data Obtained

Another further analysis by using EDS as elemental analysis was performed to justify the composition at the desired region. From the EDS result that was conducted, figure 3.3 shows the spotted region on the partially transparent sheet-like structure that was initially expected to be the graphene oxide has rich in carbon [9]and oxygen content. The carbon element that has been found on that region is up to 84.5% atomic composition and 15.5% atomic composition for oxygen element. This concludes that the desired area where the sheet-like structure was located is the graphene oxide as graphene compound has high number of carbon element in its composition.

4.0 Pre-treatment of Untreated Scanning Printed Carbon Electrode (SPCE)

All the samples that were involved in this research were used with SPCE that already went through pre-treatment process as the process helps to eliminate contaminants and unwanted constituents on the surface of the SPCE working electrode. The pre-treatment was done by treated the working electrode with 0.1M sulphuric acid solution to increase the electrode's surface roughness and its functionality [9].

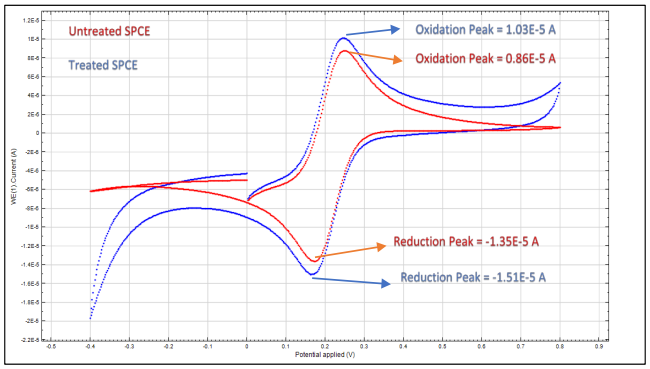


Figure 7: CV-plot of bare Treated and Untreated SPCE Electrode at Scan Rate of 0.01V/s

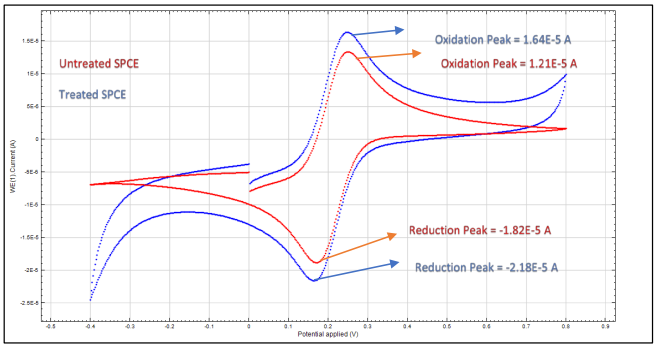


Figure 8: CV-plot of bare Treated and Untreated SPCE Electrode at Scan Rate of 0.02V/s

To activate the SPCE and make the electrode functionally optimised, cyclic voltammetry in 0.05M sulphuric acid solution was performed toward the SPCE electrodes. SPCE that already went through this process is considered as treated SPCE. This treated SPCE electrodes were then ready to be used for further analysis with the real samples glued on top of the SPCE's working electrode.

To verify this study, one treated SPCE electrode and one untreated SPCE electrode were used to differentiate the oxidation and reduction peak values when tested the electrodes with ferrocyanide solution. The justification was conducted by using 2 different scan rates of 0.01V/s and 0.02V/s **(See Figure 7 and Figure 8)**. For both scan rates show the significant values for oxidation and reduction. SPCE that went through pre-treatment shows higher value for oxidation and reduction of ferrocyanide solution in comparison with untreated SPCE. This concludes that the treated SPCE gives more reliable results than the untreated SPCE. The treated SPCE was then used for further analytical study.

5.0 Scan Rate Dependent Study

The scan rate dependent study was conducted toward the treated SPCE electrodes (refer Section 4.0) with the real electrospun nanofibers (refer **Table 1**) that were glued on top of the working electrodes to analyze the CV responses in fixed 5mM of hydrogen peroxide solution that was diluted in 0.1M pH 7.4 phosphate buffer solution (PBS) and 2mM ferrocyanide solution that was dissolved in 0.1M KCl solution. The scan rates that were used in this study were 0.02, 0.04, 0.08, 0.1 and 0.2 v/s. One of the reason why hydrogen peroxide solution was used in this analysis is to comply with the theoretical scientific fact of glucose oxidation process where the hydrogen peroxide is involved [10].

$$\text{Glucose} + \text{GOx} + \text{FAD} \rightarrow \text{Glucolactone} + \text{GOx} + \text{FADH}_2$$

$$\text{GOx - FADH}_2 + \text{O}_2 \rightarrow \text{GOx} - \text{FAD} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}$$

5.1 Scan Rate Dependent Study with Potassium Ferrocyanide Solution

Figure 9: Scan Rate Dependent Study of **Sample 1** (a) CV responses plot analysis (b) Peak current vs square root of scan rate

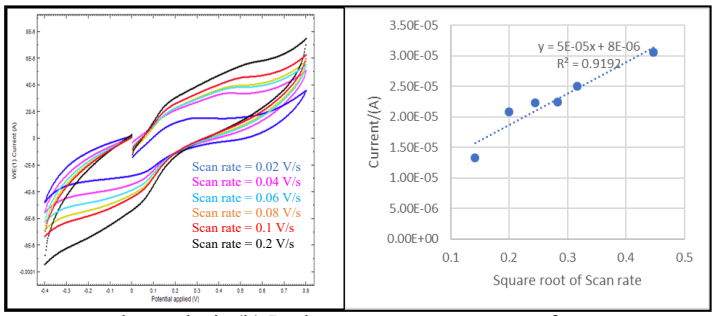


Figure 10: Scan Rate Dependent Study of **Sample 2** (a) CV responses plot analysis (b) Peak current vs square root of scan rate

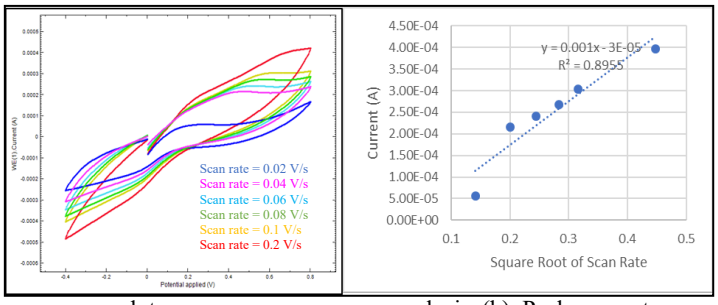
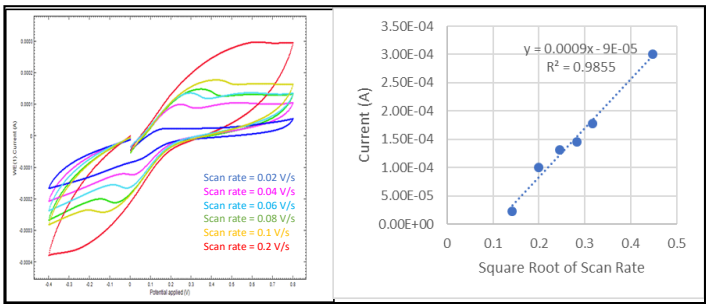
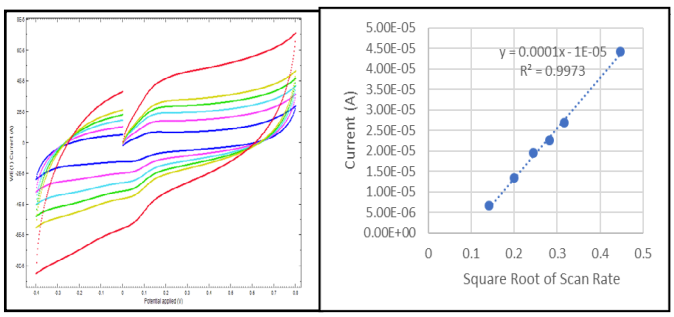


Figure 11: Scan Rate Dependent Study of **Sample C** (a) CV responses plot analysis (b) Peak current vs square root of scan rate





Results obtained from **Figure(a)** which is the (current vs scan rate) were used to construct graphs of (peak current vs square root of the scan rate) in **Figure(b)**. This scanning rate dependent study was conducted by using 2mM potassium ferrocyanide solution that was dissolved in 0.1M KCl solution. Ferrocyanide chemical compound from the potassium ferrocyanide solution has excellent oxidising property [11]. By referring to the CV plots from **Figure 9(a), 10(a), 11(a)** and **12(a)**, oxidation and reduction peaks are visible on every of each line plotted. This concludes that all samples were having capability to catch up signal caused by the electron flow from the oxidation process.

Discussing further about this study, by referring to **Figure 9(b), 10(b), 11(b)** and **12(b)** that represent the redox pattern of oxidation peaks vs the square root of the scan rate values. The oxidation peak should be increased as the scan rate applied toward the samples increases. From the results, all samples were compensating positive output. **Sample 1** to **sample G** redox peaks were following the scan rates that were supplied respectively. This concludes that **sample 1**, **2, C** and **G** were scan rate dependable[12] .

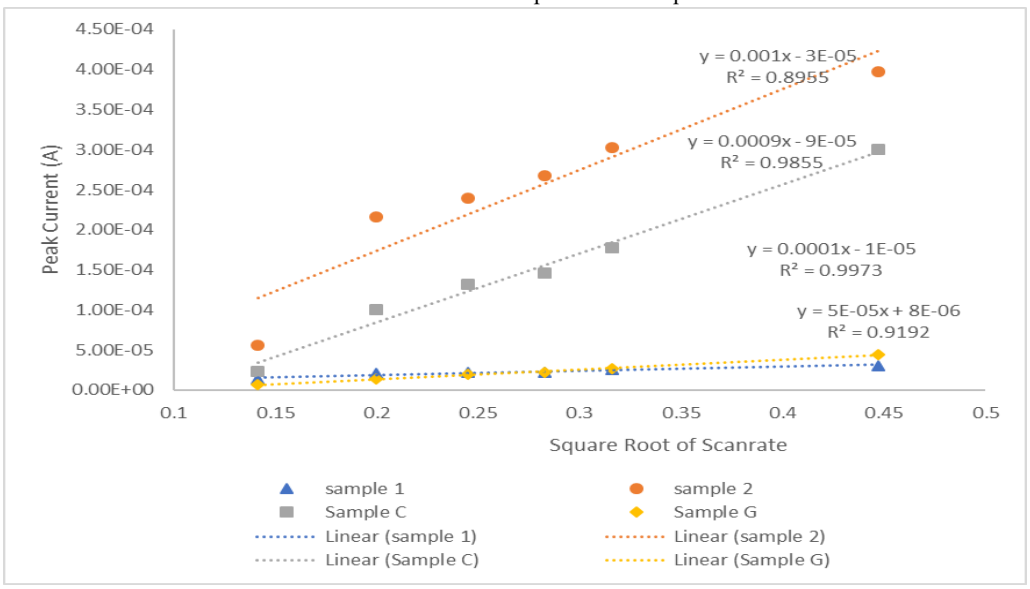


Figure 13: Oxidation Peak Responses for Sample 1, 2, C and G on Potassium Ferricyanide Solution

Peak current vs square root of scan rate plotted on **Figure 9(b), 10(b), 11(b)** and **12(b)** were merged together to form graph on **Figure 13**. Therefore, **Figure 13** can be used to differentiate the differences between all samples' conductivity and sensitivity. This figure shows that sample 2 had the highest sensitivity by according to its slope of (0.001 A $V^{-1/2}s^{-1/2}$) and had the highest conductivity due to its highest peak current values in comparison with other remaining samples. Sample 1 had the least sensitivity of (0.00005 A $V^{-1/2}s^{-1/2}$). According to **Table 1**, **Sample 2** was made up of PAA/PAN polymer associated with reduced graphene oxide (rGO) nanoparticles nanofiber that was fabricated through electrospinning process. The presence of (rGO) nanoparticles provide more conductivity to the nanofiber nanofiber. In the recent years, graphene oxide has attracted many attention due to its superior electrical conductivity [13]. **Sample 1** in other hand is the electrospun nanofiber that has no nanoparticle associated within the nanofiber. Hence it has the lowest conductivity and sensitivity. **Sample C** was the electrospun nanofiber associated with gold nanoparticles while **sample G** used was the electrospun nanofiber with iron oxide nanoparticle. **Figure 13** shows that **sample C** has sensitivity of (0.0009 A $V^{-1/2}s^{-1/2}$) which is significantly higher than **sample G** with sensitivity of (0.00005 A $V^{-1/2}s^{-1/2}$). This is duo to the metallic properties of gold from the gold nanoparticles which had increased the nanofiber's sensitivity and conductivity. Nonetheless, iron from the iron oxide nanoparticles also has good conductivity but lesser performance than gold nanoparticles [14] .

5.2 Scan Rate Dependent Study with Hydrogen Peroxide Solution

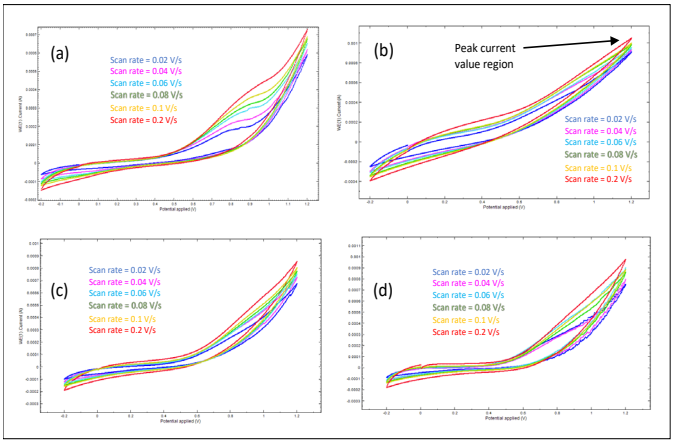


Figure 14: CV response toward 5mM H_2O_2 solution (a) Sample 1, (b) Sample 2, (c) Sample C and (d) Sample G

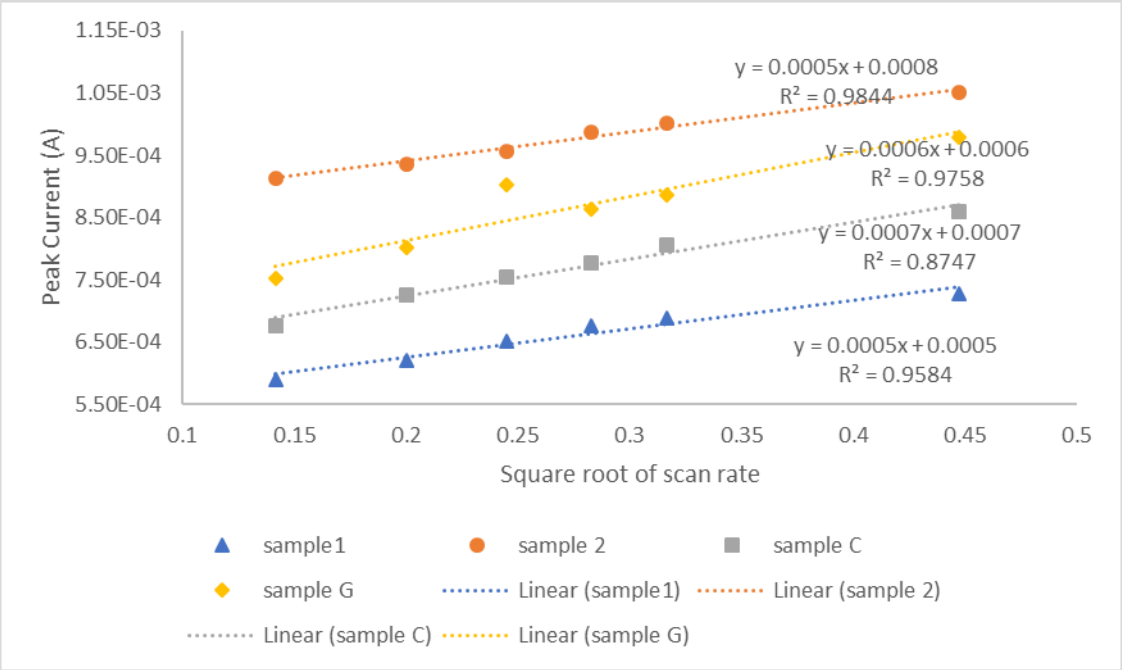


Figure 15: Oxidation Peak Responses for Sample 1, 2, C and G on Hydrogen Peroxide Solution

Unlike the CV responses that were obtained on **section 5.1**, the CV responses of the samples toward hydrogen peroxide solution had lesser visible oxidation and reduction peaks due to the hydrogen peroxide properties that has lesser degree of oxidation in comparison with potassium ferrocyanide chemical compound. However, the response peak values were taken at the edge of the CV plotted line (refer to **Figure 14(b)**) of the peak current value region.

Figure 15 reveals the different responses from **sample 1, 2, C and G** (refer **Table 1**) toward 5mM hydrogen peroxide solution. In this figure, same as the result obtained from **Figure 13** where **Sample 2** shows the highest in conductivity as we compared with the rest of the samples. **Sample 2** which is the PAA/PAN electrospun nanofiber nanofiber that was fabricated together with graphene oxide nanoparticles shows its superior conductivity properties. However, in term of sensitivity, **sample C** shows the highest conductivity of (0.0007 A $V^{-1/2}s^{-1/2}$) according to its slope from the graph. **Sample C** contains gold nanoparticles. The presence of gold element in **sample C** also promotes better electrons flowability. **Sample 1**, the bare electrospun nanofiber again shows the least conductivity and sensitivity compared to other samples.

IV CONCLUSION

From the SEM footage (refer **Figure 5**) shows the presence of thin layer and partially invisible sheet structure which verified the presence of graphene oxide nanoparticle associated within the **Sample 2** PAA/PAN electrospun nanofiber. **Figure 6** of the energy dispersive spectroscopy (EDS) was giving the same outcome as carbon and oxygen elements were found the most within the thin layer sheet structure. Hence, from EDS analysis, the scanned area was referring to graphene oxide. **S**can rate dependent study of the samples (refer **Table 1**) with potassium ferrocyanide solution concluded that **Sample 2** that is the PAA/PAN electrospun nanofiber with reduced graphene oxide nanoparticles showed the highest in conductivity and sensitivity followed by **Sample C**, **Sample G** and **Sample 1** where Sample 1 is the nanofiber without additional nanoparticle or bare sample. Hence, it has the least performance in sensitivity and conductivity. Another CV analysis of scan rate dependent study with hydrogen peroxide solution was also concluding the same outcome for **Sample 2** where it showed that **Sample 2** ranked as the highest in conductivity followed by sample G, Sample C and Sample 1 (refer Figure 15). From this research, we have summarized that **Sample 2** had the highest sensitivity and conductivity which makes it suitable to be fabricated as the support or transducer of the glucose biosensor in comparison with other samples Furthermore, the presence of reduce Graphene oxide nanoparticles (rGO NPs) in Sample 2 has significantly improvised the nanofiber's conductivity and sensitivity .

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor, Dr. Tan Huey Ling for the continuous support and numerous of helps toward this research.

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Oxidation Peak = 1.64E-5 A

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