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AN OPTIMIZED GLUCOSE BIOSENSOR AS A POTENTIAL MICRO-FUEL CELL

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ABSTRACT

Glucose oxidase (GOx), a flavoprotein enzyme with the nomenclature of E.C.1.1.3.4, is widely employed in the different sectors such as an implantable fuel cell. In this paper, optimal conditions for the size of carbon paste, GOx volume, and glutaraldehyde concentrations for fabrication of polyaniline nanofiber modified carbon paste electrodes (GOx/MCPE) were investigated. The optimum parameters of the carbon paste size, GOx volume, and glutaraldehyde concentration were found to be 3 mm x 3 mm, 5 µL and 0.5%, respectively. The maximum current in carbon paste size (3 mm x 3 mm), GOx volume (5 µL) and glutaraldehyde concentration (0.5%) were estimated to be 5.39 mA, 4.12 mA, and 7.10 mA, respectively. This GOx/MCPE showed great potential in fuel cell application.

Keywords: Biosensor, Carbon paste, Electrode, Glucose oxidase, Polyaniline nanofiber.

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INTRODUCTION

Glucose oxidase (GOx), the name of β-D-glucose: oxygen 1-oxidoreductase (GOD) and also called glucose aero dehydrogenase, is a flavoprotein enzyme with the nomenclature of EC. 1.1.3.4.[1] Molecular oxygen (O_2) as an electron acceptor in the GOx-catalyzed oxidation of β-D-glucose to D-glucono-σ-lactone and hydrogen peroxide (H_2O_2)[2] according to the following reaction:

$$\text{Glucose} + O_2 \rightarrow H_2O_2 + \text{Gluconic acid}$$

Electrochemically, the amount of hydrogen peroxide was measured quantitatively to determine the amount of glucose used indirectly[3], where the glucose produced is proportional to the hydrogen peroxide concentration. The reaction is shown below:

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

GOx is a highly specific enzyme and the most widely studied of all amperometric based enzymes for biosensors, such as in clinical diagnostics and biotechnology[4, 5], in food[6], pharmaceutical[7], environmental analyses[8], and fuel cells.[9] In recent years, the glucose measurements are based on immobilization of GOx as complicated systems for detecting H_2O_2 concentration which is quick and accurate.[10-12] Ambarsari *et al.*[13] reported a glucose biosensor utilizing nanofiber-polyaniline/glutaraldehyde as an immobilization matrix for GOx in an enzymatic fuel cell system. GOx has been isolated from a local fungus collection of *Aspergillus niger* (IPBCC.08.610) with yield and activity was 72.86% and 27.77 U/mg, respectively.[14]

In this research, a novel carbon paste electrode using the nanofiber-polyaniline was prepared and GOx was immobilized by cross-linking with glutaraldehyde to modified carbon paste electrode (MCPE). The

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optimum working conditions of the biosensor were based on the size of the carbon paste, GOx volume, and glutaraldehyde concentration.

EXPERIMENTAL

Instrumentation and Chemicals

The electrochemical measurement was carried out using an eDAQ potentiostat-galvanostat. The working electrode was a carbon paste electrode in diameter of 3 mm and length of 3-10 mm glass capillary. The Pt wire and Ag/AgCl used as a counter and reference electrodes, respectively. Glucose oxidase was purified from *Aspergillus niger* (IPBCC.08.610) with the activity of 27.77 U/mg. Glucose and glutaraldehyde purchased from Sigma and all other chemicals were analytical grades.

Preparation of Modified Carbon Paste Electrodes

The modified carbon paste electrode (MCPE) was made with 2 mg nanofiber-polyaniline by thoroughly mixing in mortar 100 µL paraffin with 0.15 g of graphite powder according to method Ambarsari *et al*[13] with slight modification. For the preparation of MCPE capillary glass (diameter of 3 mm) the tubes were filled which carbon paste with the size of 3, 5, 7 and 10 mm. Furthermore, Pt wire made the electric a contact. The electrode surface was smoothed with oil-paper to produce a reproducible working surface.

Preparation of Glucose oxidase/Modified Carbon Paste Electrode (GOx/MCPE)

Immobilization of GOx into MCPE was done with glutaraldehyde as a crosslinker according to a previous method.[13,14] Briefly, the volume (of 5, 10, 15, and 20 µL) of GOx were mixed with 1 mg BSA, 50 µL of 0.1 M acetic buffer (pH 4.5) and 30 µL glutaraldehyde (of 0.5, 1.0, 1.5, 2.0, and 2.5%). The mixture was placed into micro tubes and then shake slowly. Furthermore, the mixture was dipped upon the MCPE. The electrode was then transferred to an ice bath (approx. 4°C) and washed with 0.1 M acetic buffer (pH 4.5) several times to remove the non-immobilized excess enzyme and glutaraldehyde. The electrode was kept in a refrigerator at 4°C in the acetic buffer when not in use.

Electrochemical Measurements

In-room temperature, a traditional three-electrode configuration containing the working electrode (GOx/MCPE), a counter electrode (Pt), and a reference electrode (Ag/AgCl) were performed for electrochemical measurements. These electrodes were placed into a solution that contained three mL potassium chloride as a supporting electrolyte and then connected to a potentiostat. To each cell was added 100 mM K[Fe(CN)6] aqueous solution containing 15 mM glucose as the substrate and the system was stirred. The currents and voltages obtained were recorded.

RESULTS AND DISCUSSION

In this research, a novel modified carbon paste electrode using nanofiber-polyaniline was prepared. The parameters which are affecting the performance of the biosensor and optimum working condition, such as the size of carbon paste, GOx volume, and glutaraldehyde concentration, were investigated.

Optimization of carbon paste size on GOx/MCPE

The current and voltage responses of the GOx/MCPE were determined for the different size of carbon paste. A shown in Table-1 and Fig.-1, when the size of 3x3 mm^2 of carbon paste was used, current differences were obtained higher than another size of carbon paste.

Table-1: Current and voltage optimization size of carbon paste on GOx/MCPE

Size of carbon paste (mm^2)	Maximum voltage (V)	Maximum current (mA)
3 x 3	1.25	5.39
3 x 5	1.22	3.20
3 x 7	1.67	4.58
3 x 10	1.40	3.56

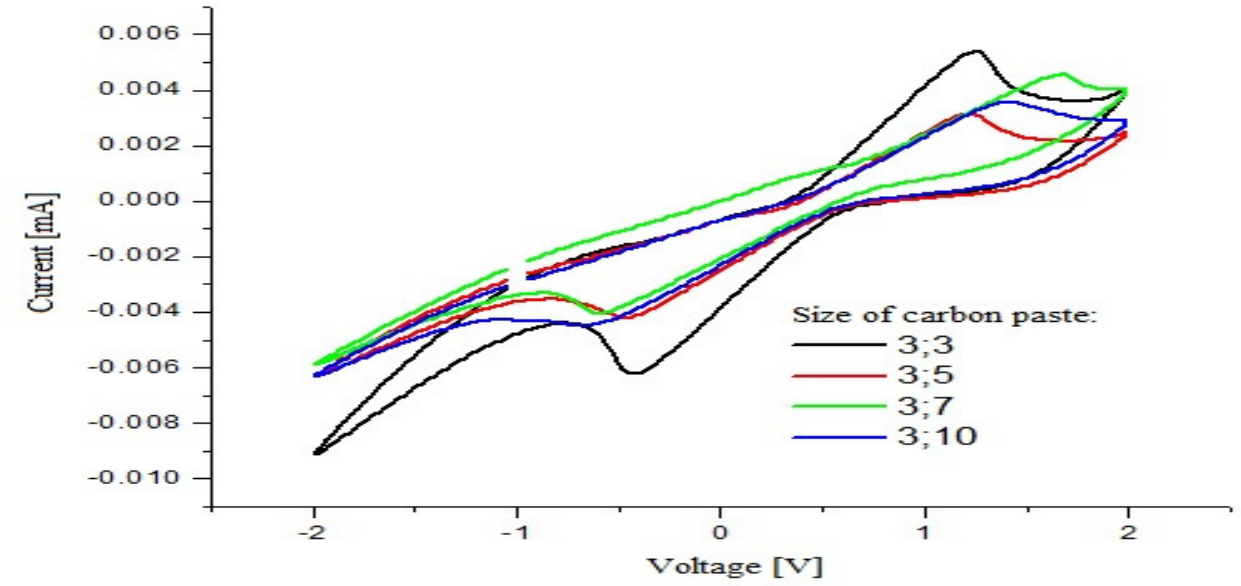

Fig.-1: Cyclic voltammograms of MCPE between -2 and 2 V of the applied size of carbon paste with a scan rate of 100 mV/s at room temperature.

The maximum currents were shown by the greatest difference between the top and bottom lines of oxidation picks of the cyclic voltammograms. This result indicated that the modification of the size of carbon paste was effective in increasing the electroconductivity of GOx/MCPE. In this research, a carbon paste electrode modified with nanofiber-polyaniline was used as the working electrodes because they are easily changed, inexpensive, and easily regenerated[15]. Maximum current was 5.39 mA (carbon paste size of 3x3 mm^2), which was better than that reported in our previous studies.[13, 14]

Optimization of GOx volume on GOx/MCPE

The effect of the applied GOx volume on the current response of the GOx/MCPE was examined (Table-2 and Fig.-2). As shown in Table-2 and Figure-2, when the volume of 5 and 10 µL of GOx was used, current differences were obtained higher than another GOx volume with a value of 4.12 and 4.25 mA, respectively. This result indicated that the variation of the GOx volume was useful in increasing the electroconductivity of GOx/MCPE, which was better than that reported in our previous studies.[13, 14] Therefore, the volume of 5 µL of GOx was used in future studies.

Table-2: Current and voltage of optimization GOx volume on GOx/MCPE

GOx volume (µL)	Maximum voltage (V)	Maximum current (mA)
5	1.94	4.12
10	1.74	4.25
15	1.63	2.99
20	1.30	1.44

Optimization of glutaraldehyde concentration on GOx/MCPE

GOx was immobilized onto MCPE via glutaraldehyde cross-linking. This immobilization was aimed at stabilizing the enzyme and thus render it less sensitive to the external environment. The effect of the applied glutaraldehyde concentration on the current response of the GOx/MCPE was investigated (Table-3 and Fig.-3). The current decreased as the glutaraldehyde concentration increased from 0.5% v/v to 1.5% v/v and the current increased slightly as the glutaraldehyde concentration increased from 2% v/v to 2.5% v/v (Table-3). Additionally, the increase in the glutaraldehyde concentration caused a decrease in the current, showing deactivation of the enzyme. This result was in agreement with that reported by Wang et al.[16] and

Ang *et al.*[2] who found deactivation of the enzyme by a high concentration of glutaraldehyde. Thus, the concentration of 0.5% v/v glutaraldehyde was used for subsequent enzyme immobilization.

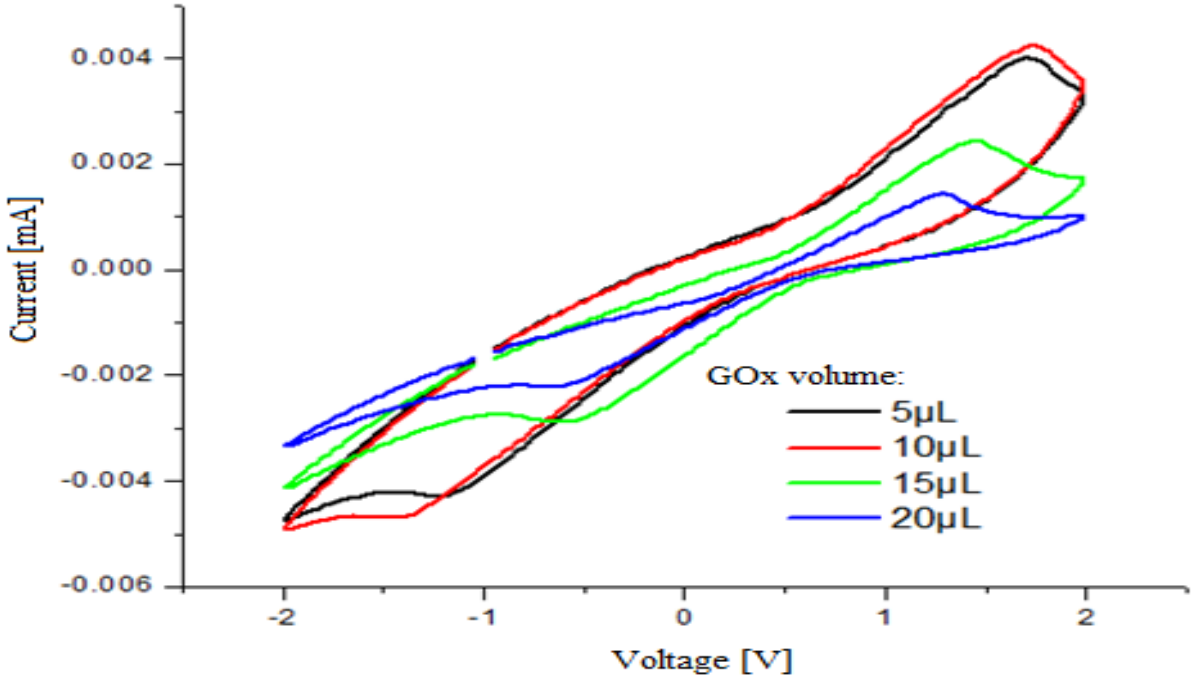

Fig.-2: Cyclic voltammograms of MCPE between -2 and 2 V of applied of GOx volume with a scan rate of 100 mV/s at room temperature.

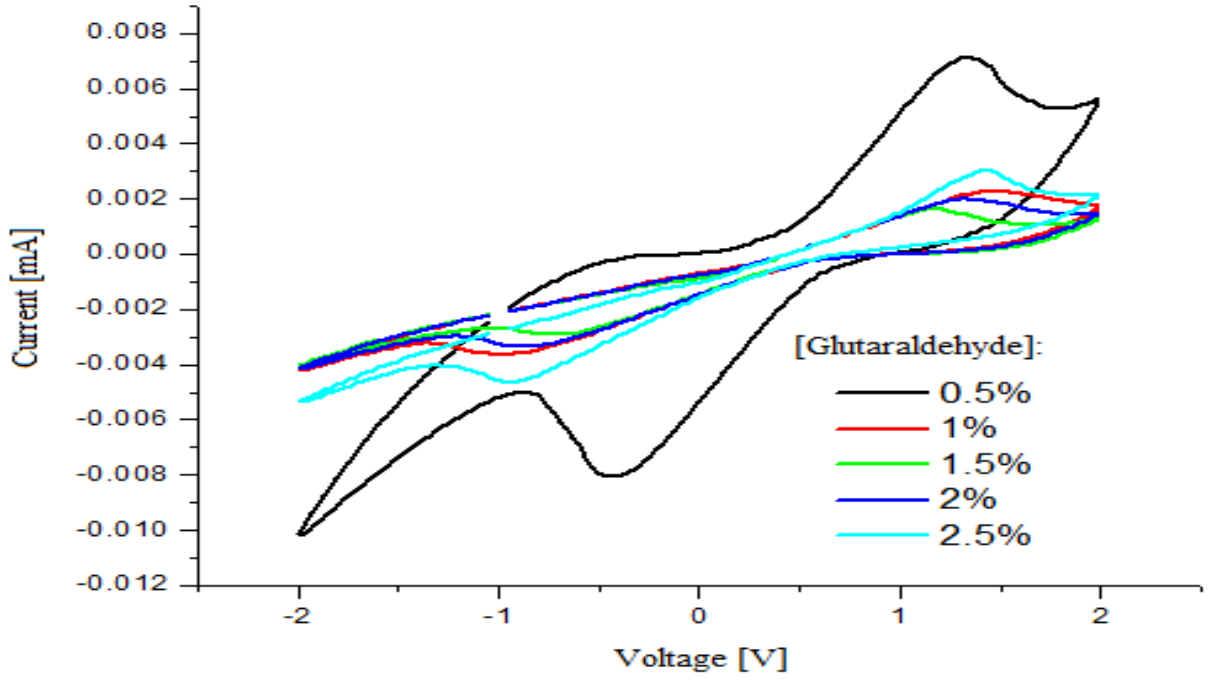

Fig.-3: Cyclic voltammograms of MCPE between -2 and 2 V of applied of glutaraldehyde concentration with a scan rate of 100 mV/s at room temperature.

Table-3: Current and voltage of optimization glutaraldehyde concentration on GOx/MCPE

[Glutaraldehyde] (%)	Maximum voltage (V)	Maximum current (mA)
0.5	1.35	7.10
1	1.44	2.29
1.5	1.17	1.64
2	1.31	2.00
2.5	1.42	3.04

CONCLUSION

Glucose oxidase/ a modified carbon paste electrode (GOx/MCPE) using glucose a substrate have been fabricated and optimized for the size of carbon paste, the volume of glucose oxidase, and the concentration of glutaraldehyde. The optimal GOx/MCPE had a size of carbon paste of 3 mm x 3 mm, a GOx volume of 5 μL and glutaraldehyde concentration of 0.5% v/v.

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