

# Graphene Synthesis from Empty Palm Oil Bunch for Modifying Screen Printed-Carbon Electrode Glucose-Biosensor

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**Abstract:** Glucose is a monosaccharide that is the energy source and controls vital activities in the body. Therefore, continuous glucose level monitoring is needed to know the representation of the body's condition. A commonly used method for monitoring is the SPCE biosensor for practical and economical reasons. The effectiveness of SPCE biosensor performance is determined by its constituent materials, so this research was conducted to determine how the graphene synthesis process from empty oil palm bunches (EOPB) and its application to SPCE can affect the performance of glucose biosensors. Graphene was chosen based on its superior characteristics, while EOPB was used as raw material because of its content such as lignin and cellulose, and its abundant availability. This research is quantitative-experimental with the object, EOPB. Graphene synthesis was carried out using the liquid phase exfoliation (LPE) method while electrode printing on the biosensor using the drop-casting method. The results of the characterization of graphene synthesis with SEM-EDS show a layer of graphene sheets with a single-layer hexagonal planar structure with an average distribution size of small particles,  $0.023 \pm 0.019 \mu\text{m}$ . The distribution of elements in graphene synthesis is dominated by carbon, which is 52.1%, indicating the existence of carbon allotrope, namely graphene. The performance of the SPCE biosensor was tested using amperometry technique with three variations of graphene concentration (P1, P2, and P3) and two variations of glucose solution concentration (L1 and L2). The test results showed a positive relationship between increasing the concentration of graphene and glucose solution with the strong electric current produced, indicating that the SPCE biosensor is stable and good at reading electric current.

**Keywords:** Biosensor, Empty Oil Palm Bunch, Glucose, Graphene Synthesis, Liquid Phase Exfoliation.

**Abbreviations:** Screen Printed Carbon Electrode (SPCE), Empty Oil Palm Bunch (EOPB), Liquid Phase Exfoliation (LPE), Solution Concentration (L-n), Variation of Graphene Concentration (P-n)

## Introduction

Glucose is a monosaccharide sugar carbohydrate that is the source of energy as well as controlling vital activities such as the body's metabolism. Monitoring glucose concentration in the body can give an indicator of the body's condition. If the glucose levels in the body are indicated to be high for a long time, then the body has a risk of contracting various diseases such as diabetes and kidney disorders (Umar, Syahrir, and Alyah 2022). In 2021, based on the calculations of the International Diabetes Federation, as many as

19,465.1 million people in Indonesia has diabetes, where it is estimated that in 2045 the number of people with diabetes in Indonesia can touch 28,569.9 million people (IDF 2024).

It is important to monitor glucose levels regularly. Measurement of glucose concentration in the body can be done using several techniques and methods, one of which is a biosensor. Biosensors in the medical world have been used as an alternative to biomolecule measurement (Azis 2011). Biosensor is an instrument measuring chemical and biological reactions where the biosensor will produce a signal that is in accordance with the

concentration of the analyte in the reaction (Azis 2011). According to the concentration of the analyte in the reaction (Bhalla et al. 2016). The components in the biosensor itself is the analyte, bioreceptor, transducer, electronic device, and display screen.

The use of graphene in biosensors itself has been studied and is quite common due to the characteristic properties of graphene material that match the criteria of the biosensor material itself, such as having strong mechanical strength, a large and thin surface, and good electrical conductivity (Najati 2023). Graphene is becoming a more frequently studied allotrope of carbon due to its high mechanical strength, thermal conductivity, and good elasticity, presenting facilities for direct electron transfer processes thus improving its performance as an anode (Pareek, Shanthi Sravan, and Venkata Mohan 2019). Many studies have been conducted on the synthesis of graphene by various methods (Mbayachi et al. 2021) where the synthesis method used this time is Liquid Phase Exfoliation (LPE). Graphene synthesis with this method (LPE) produces similar characteristics as actual graphene (Aziz and Tjahjono, 2022).

Empty Oil Palm Bunch (EOPB), can then be used as a basic raw material in graphene synthesis because of its cellulose, lignin, and hemicellulose content (Hardi et al. 2021) where this biomass is widely found, especially in South Sumatra which in 2024 will produce 3,361,940.00 tons of oil palm (BPS 2023). The results of graphene synthesis research are pegged to have superior characteristics so that existing needs can be met (Gadipelli and Guo 2015).

The use of electrode-based biosensors in the medical sector is more practical compared to using other methods of testing, which can make the the general community is able to do self check-ups (Suwandi et al. 2013). SPCE is one of one type of practical biosensor base that functions to detect certain biomolecules. The working principle of SPCE uses a three-electrode configuration, i.e. the working electrode, reference electrode, and comparison electrode (Deviyana, 2023).

In glucose biosensors, the measurement of glucose levels is done by observing the result of glucose interaction with one of three enzymes: hexokinase, glucose oxidase, 3 or glucose-1-

dehydrogenase. In this research, the enzyme glucose oxidase was used because of its relatively high selectivity for glucose detection, easy to obtain, and better resistance to extreme temperatures than other enzymes (Yoo and Lee 2010).

By considering the various aspects previously described, this research needs to be continued to develop biosensors. This research aims to determine whether graphene synthesis using the EFB-based LPE method can be accomplished, which will then be studied for its application as a material implemented in the SPCE biosensor for glucose control.

## Materials and Methods

### Materials and tools

The tools used are digital balance, laboratory oven, 100 mesh sieve, blender, magnetic stirrer, GT R9 UC9L sonicator, hitachi centrifuge CT6E, Scanning Electron Microscope (SEM), dropping pipette, beaker glass, mortar and pestle, dry oven Maskot OV 43, and basic meter 90. Then the materials are isopropanol, dimethyl sulfoxide (DMSO), cellulose methyl ether, distilled water, AgCl powder, NaOH, KCl, acetic acid, glucose oxidation enzyme, and glucose powder.

### Procedures

#### Material Preparation

1 kg of EOPB was obtained from a local resident's oil palm plantation in the Ogan Komering Ilir area then cut into smaller parts and cleaned, dried in the sun for 5 hours, followed by drying again using a dry oven at 50°C for 6 hours to reduce the water content before burning process.



**Figure 1.** The EOPB preparation process includes: EOPB collecting (1 and 2); EOPB washing (3); EOPB drying (4); EOPB weighing (5); EOPB drying (6).

### Combustion Process

A total of 1 kg of empty palm bunches were sliced into smaller pieces and then cleaned, dried in sunlight for 5 hours, and again using a dry oven at 50°C for 6 hours to reduce the water content before burning.



**Figure 2.** Combustion results using oven (1); charcoal crushing process (2); EOPB charcoal powder (3).

### Graphene Synthesis Process

Graphene synthesis was carried out using the LPE (Liquid Phase Exfoliation) method, which is dispersion in the liquid phase. The EOPB charcoal powder was first sieved using a 100 mesh sieve to obtain a uniform particle size. Then, EOPB carbon powder was dissolved into a liquid solvent (isopropanol and distilled water in a ratio of 80:20), stirred using a magnetic stirrer for 5 minutes to make the solution homogeneous, sonicated for 4 hours with a frequency of 40 kHz, then dEOPsited overnight. After that, the solution was separated from the sediment and centrifuged for 5 minutes to separate the graphene from the solution. Afterward, the centrifuged precipitate was dried using an oven to obtain graphene in powder form. After graphene synthesis, SEM (Scanning Electron Microscope) and EDS characterization were carried out to see the morphology, composition, and elemental composition of the synthesized graphene.



**Figure 3.** The synthesis process includes: sieving (1); dissolving and stirring (2); sonication (3); centrifugation (4); centrifuged sediment (5); drying graphene to powder (6).

### SPCE Printing

The SPCE printing process is carried out in 3 stages, first is the printing of the working electrode. The working electrode was printed using graphene paste of varying concentrations of 0.25 g (P1), 0.5 g (P2), and 0.75 g (P3) then diluted in 1.2 ml of dimethyl sulfoxide (DMSO) solvent and 0.07 ml of thickening agent. Next, the paste is dripped using a pipette or by drop-casting method on a PCB mold following the existing pattern and then in the oven at 90°C for 15 minutes to accelerate the drying of the solution. The process continued with the molding of the reference electrode using AgCl paste prepared by crushing 2 g of AgCl powder into smaller particles then dissolving the powder with 2 ml of dimethyl sulfoxide and stirring for 10 minutes. Afterward, the drop-casting method was used again following the pattern for the reference electrode and put into a dry oven at 40°C for 30 minutes. The last step was specific enzyme coating. The preparation process began by diluting 0.2 g of glucose oxidase enzyme using 1 ml of NaOH and CH<sub>3</sub>COOH buffer solution with pH 5.5 which was then dripped as much as 0.05 ml on each SPCE working electrode and then incubated for 7 hours in a Petri dish at room temperature.



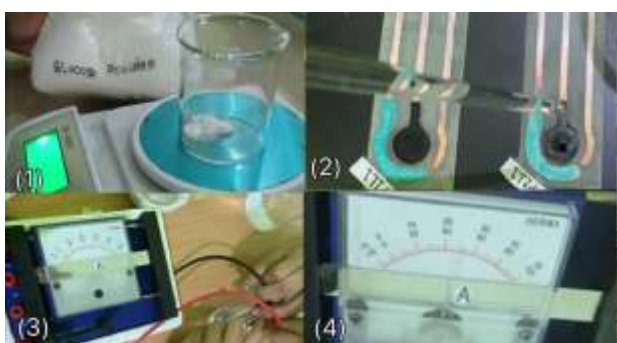
**Figure 4.** PCB pattern design (1); Calculation of graphene mass variation (2); graphene drop-casting (3); graphene concentration variation (4), SPCE working electrode (5).



**Figure 5.** AgCl particle reduction(1); AgCl paste preparation(2); drop-casting of reference electrode(3); enzyme solvent buffer pH 5.5(4); glucose oxidase enzyme(5); enzyme incubation(6).

### Sample Preparation

Samples were prepared for testing the performance of graphene as a working electrode in SPCE. Sample preparation was carried out by diluting glucose powder in water with two concentration variations, namely 2 g/dL (L1) and 4 g/dL (L2) then, as much as 0.05ml of glucose solution was dripped on the working electrode that had been given the enzyme glucose oxidase. Furthermore, the biosensor that has been dripped with glucose is immersed in 2 M KCL solution as an electrolyte and connected using a current measuring device (basic meter 90).



**Figure 6.** Preparation of glucose solution(1); treatment(2); current measurement(3); zoom detail of current measurement(4). basic meter 90(5).

### Data analysis

#### Graphene Characterization with SEM-EDX

Graphene characterization was carried out to validate the suitability of the synthesized material and the original material. By analyzing using SEM (Scanning electron microscope) and additional EDX (energy dispersive x-ray) methods, the surface morphology, elemental arrangement, and composition of the synthesized graphene material can be determined.

#### Performance Test of Synthesized Graphene as SPCE Transducer

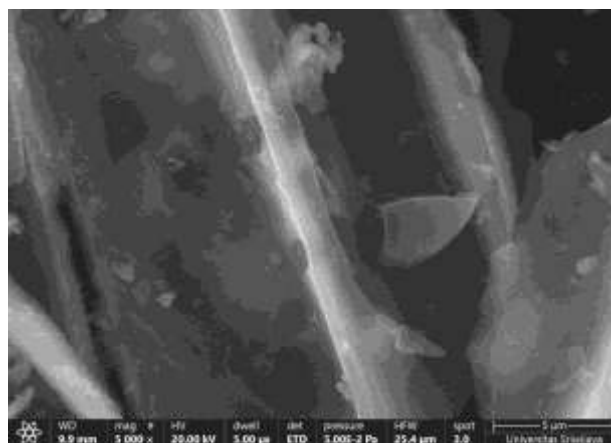
A basic meter is used to measure the amount of electric current flowed by synthesized graphene as a working electrode in SPCE to test its sensitivity to analyte samples that form electrochemical reactions after reacting with bioreceptors.

## Results and Discussion

### Results

#### Graphene Characterization Result with SEM-EDS

The characterization test of the graphene synthesized material using the SEM-EDS (Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy) method was carried out to determine the surface morphology, which this time was calculated on a micrometer scale ( $\mu\text{A}$ ) (Falcone et al. 2024) so that it could be validated that the material was graphene. The sample was made in powder form to facilitate the characterization process. From the tests that have been carried out, the following data were obtained:



**Figure 7.** SEM results with 5000 times magnification.

**Table 1.** Data count map.

Element	Wt. %
C	52.1
O	35.0
N	8.3
Ca	1.2
Si	1.1
Mg	0.8
Al	0.6
K	0.4
P	0.3
S	0.2

**Result of Biosensor Performace**

Tests were carried out using amperometric analysis techniques. The prepared biosensor was inserted into 40 ml of 2 M KCl suspension solution then connected to an ammeter using a connecting cable and monitored for current movement. After that, repetition was repeated using three SPCEs with different graphene concentrations and two different concentrations of glucose solution, i.e. 2000 mg/dL (L1) and 4000 mg/dL (L2). Based on the research conducted, the following data were obtained:

Table 2. Biosensor test results with ammeter.

Treats	Electric current (µA)		The difference between L2-L1 (µA)
	L1	L2	
P1	4	38	34
P2	9	42	33
P3	16	49	33

**Discussion**

**SEM-EDS results**

Where it is seen that the layers of graphene particles consist of one planar hexagonal structural layer which makes it look transparent [17]. SEM results processed with the help of ImageJ software, showed a standard deviation of ± 0.019 with a mean of 0.023, so the average distribution size of small particles is 0.023 ± 0.019 µm. In Table 1. The count map of the EDS results shows the wt.% levels of C, O, and N in order of 52.1%, 35.0%, 8.3%, as well as other elements such as Al and Si which are very low in number. It appears that the element carbon is highly ranked, indicating the existence of an allotrope of carbon, which is

graphene. The presence of oxygen (O) will make graphene more easily dispersible and nitrogen (N) can improve the conductivity properties of graphene as an electrode.

**SPCE Performance Test Results**

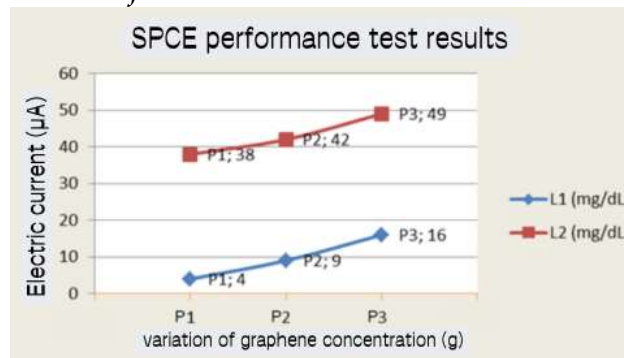


Figure 8. SPCE Performance Test Results

The results of SPCE performance testing showed several results, the first is the proof that graphene concentration can affect the electric current generated by SPCE in detecting glucose in a concentration solution. This can be seen from the current generated in each treatment in sequence, namely P1-L1 (graphene concentration 0.25 g - glucose solution 2000 mg/dL) of 4 µA, P2-L1 (graphene concentration 0.5 g - glucose solution 2000 mg/dL) of 9 µA, and P3-L1 (graphene concentration 0.75 g - glucose solution 2000 mg/dL) of 16 µA. The data presented in Table 2 and depicted in Figure 1 show an increase in electric current along with an increase in graphene concentration. This is in accordance with the statement that the increase in graphene concentration, the bigger the electric current that is read. This is due to the increase in the number of active surfaces involved in the electrochemical reaction resulting in more molecules converted into electrons during the glucose detection process (Insani and Herdyastuti 2013).

Then, a significant increase in electric current strength also occurred in the variation of glucose solution concentration (L1 and L2). The data trend shows that increasing the concentration of the sample dissolved in water also increases the electric current generated. In P1-L1 (graphene concentration 0.25 g - glucose solution 2000 mg/dL), an electric current of 4 µA was generated, while P1-L2 (graphene concentration 0.25 g -

glucose solution 4000 mg/dL) showed a current of 38  $\mu$ A. P2-L1 (graphene concentration 0.5 g - glucose solution 2000 mg/dL) produced a current of 9  $\mu$ A then P2-L2 (graphene concentration 0.5 g - glucose solution 4000 mg/dL) of 42  $\mu$ A, then P3-L1 (graphene concentration 0.75 g - glucose solution 2000 mg/dL) of 16  $\mu$ A and P3-L2 (graphene concentration 0.75 g - glucose solution 4000 mg/dL) of 49  $\mu$ A.

The increase in current that is directly proportional to the increase in glucose solution concentration indicates that the graphene biosensor was sensitive to glucose concentration changes. This happens because the higher amount of glucose dissolved, the more molecules that can be identified and bind to the enzyme glucose oxidase as a biorecognition agent or bioreceptor in accordance with the working principle of the glucose biosensor (Yoo and Lee 2010). In addition, the data also shows the difference in the increase in current numbers from L1-L2 (glucose solution 2000 mg/dL - glucose solution 4000 mg/dL) in each variation of graphene concentration and shows almost uniform numbers ranging from 33-34  $\mu$ A. This indicates that the graphene in the three concentrations has high stability in measuring the electric current.

### Conclusions

Based on the synthesis of graphene from empty palm oil bunches (EOPB) using the Liquid Phase Exfoliation (LPE) method, the graphene sheets produced were identified through SEM analysis. These sheets have a planar hexagonal structure with a single layer that appears transparent, with a standard deviation 0.019  $\mu$ m and mean value 0.023  $\mu$ m. In addition, EDS analysis identified carbon as the dominant element, followed by oxygen which facilitates the graphene dispersion process, and nitrogen which increases the conductivity of graphene when used as an electrode. Performance test showed that the higher the concentration of graphene on the working electrode of the screen printed-carbon electrode (SPCE) biosensor for glucose, the higher the electric current produced. The biosensor also showed good sensitivity to

variations in dissolved glucose concentration, with stable electric current readings between solutions L1 (glucose 2000 mg/dL) and L2 (glucose 4000 mg/dL), proving the reliability of the biosensor.

This shows that the application of graphene synthesis material from EOPB has been optimal as a working electrode in the glucose biosensor. In addition, the opportunity to implement graphene from EOPB in other fields, such as electronic device materials or chemical sensors, is still widely open, considering its good conductivity and stability characteristics (Karim Kakaei, 2019).

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