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Preparation and Characterization of Polymer Nanocomposites for Biosensors Applications

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Preparation and Characterization of Polymer Nanocomposites for Biosensors Applications

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Abstract. Biosensors are advanced devices that bridge biology and technology and act as detectors of chemical compounds through physical and chemical means. Different types of sensors, such as chemical and biological sensors, play a crucial and useful role in our daily lives. In this work, we developed a biosensor device to detect blood glucose levels. The biosensor was manufactured in an easy and inexpensive way from accessible materials using uncomplicated techniques. The production of silver nanoparticles with a grain size ranging from 5 to 30 nanometers and a purity level of up to 99.9% took place in a laboratory by dissolving silver nitrate in a solution of okra plant extract. A semiconductor material from the okra plant was mixed with polyaniline in a 1:1 ratio to make the glucose sensor. It also had 10 weight percent silver nanoparticles and a glucose enzyme added to it. The sensor demonstrated the ability to detect low amounts of glucose, as low as 0.25 mg/100 ml. The sensor circuit output current was 50 µA at 2.6 V and 397 µA at 2.6 V at the highest concentration of 100 mg/100 mL. The sensor operates at a constant voltage of 2.6 V. Glucose sensors have properties such as linearity, sensitivity, and selectivity. Multiple chemicals were scanned with the sensor, but no current was detected. Likewise, it did not record any data in the air. The water was tested, and the reading was 42 microamps, which is much lower than the sensor readings. It is also environmentally beneficial, as it is manufactured from natural ingredients that are not toxic to the environment. Therefore, it can be considered as a suggested alternative to the sensors used. The examination was also carried out with an SEM device, through which the nanosized size of the nanoparticle was determined, as well as the homogeneity of the mixture of the semiconducting material with the okra plant.

Key Words: Polyaniline, semiconductive, glucose sensing, AgNPs, H_2O_2.

1. INTRODUCTION

Over the past 10 years, there has been a significant increase in the development and use of electrochemical biosensors in various fields such as nanoscience, nanotechnology, medicine, environmental monitoring, and food. Advanced technology has allowed the development of precise, specific, adaptable, and portable sensors for measuring glucose levels [1,2]. Glucose biosensors are essential for detecting and managing diabetes, a worldwide health concern associated with increased risks of heart disease, renal failure, eye impairment, surgical infections, and wound infections (3). Seven million people are diagnosed with diabetes each year, and the disease kills one person every 10 seconds worldwide. Glucose sensors are now widely used to monitor analyte levels in blood, saliva, tears, and sweat owing to their rapid speed, precision, and straightforwardness [4-6]. Combining expertise in electrochemistry, materials science, polymer and enzyme synthesis, and biochemistry is necessary to create small enzyme biosensors, which can range in size from a few millimeters to a few micrometers [7,8]. Nanomaterials have opened horizons in biosensor applications, such as gold nanoparticles (AuNPs)(7), silver nanoparticles (AgNPs) (8), Carbon nanotubes (9) and metallic oxides (10), Silver nanoparticles, particularly, have a strong interaction with certain sections of protein molecules, causing the proteins to adhere to the surface of the nanoparticles. When the redox active sites of the proteins are in close proximity to the surface of the nanoparticles, electron transfer becomes more effortless (11). Due to their biocompatibility and high conductivity, AgNPs are capable of catalyzing the oxidation of H_2O_2 By aiding in the transport of acceptor electrons to the electrode, it improves the sensitivity and stability of biosensors (12).

The use of conductive polymers like polyaniline and polypyrol can enhance electrical transmission. These polymers have a continuous electronic system that is influenced by the induction process, resulting in a change in their electrical conductivity. This change is directly proportional to the interaction between the glucose oxidase enzyme and glucose, as well as the production of hydrogen peroxide. Consequently, the conductivity of the polymer is altered, allowing for the reception of an electrical signal that corresponds to the concentration of glucose [15,16].

Sh. Li *et al.* Advancements in nanotechnology, microfluidics, and biomarker research in the last ten years have resulted in the creation of small diagnostic devices for testing entire blood. Electrochemical biosensors are a type of developing technology used for detecting targets in complicated environments such as whole blood or the human body. They are valued for their quick, sensitive, small-scale, reagent-free, and washing-free features. This paper provides a detailed overview of the advancements made in the last ten years in creating electrochemical sensors for analyzing whole blood. It also discusses the obstacles and possibilities for incorporating these sensing platforms (15).

N. Germa et al. The work focused on enhancing the analytical characteristics of biosensors for detecting blood glucose levels using conducting polymer nanocomposites containing embedded GOx and 6 nm diameter AuNPs. Short chain polymer nanocomposites (PANI-AuNPs (6 nm)-GOx and Ppy-AuNPs (6 nm)-GOx) were developed for their excellent sensitivity, low limit of detection, wide linear range, good reproducibility, and acceptable storage stability (16).

R Batool et al. This study examined the integration of several nanomaterials into the development of electrochemical biosensors for detecting glucose. Nanomaterials encompass metal/metal oxide, carbon-based nanoparticles, and composite nanoparticles. Nanomaterials aim to enhance analytical performance by offering rapid reaction time, cost-efficient transducer surface development, and long-term stability (17).

Z. Chen *et al*. A paper-based electrical sensor with molecularly imprinted glucose recognition sites has been created to reduce the expenses of glucose sensors for diabetes patients globally. The sensor, equipped with a sensing electrode made of a conductive polymer, is capable of detecting different glucose levels in bovine blood solutions. The sensor successfully detected glucose concentrations between 2.2 and 11.1 mM in cow blood samples. The non-enzymatic glucose sensor might save healthcare expenses and increase the availability of glucose sensor test strips to marginalized regions, possibly decreasing the cost of glucose strips (18).

Á Terán-Alcocer *et al.* This work explores the creation of electrochemical biosensors designed to selectively and flexibly detect various physiologically significant organic and inorganic substances. The most commonly utilized monomers are pyrrole derivatives, aniline, ethylenedioxythiophene, and its conjugates. Organic dyes are used to create conductive polymers that have a high capacity, broad surface area, and improved electron transfer kinetics, resulting in increased electrical activity of the sensor. Furthermore, these polymers, together with materials like graphene, carbon nanotubes, enzymes, and metallic nanoparticles, can enhance analyte absorption, sensitivity, and biocompatibility due to their electrical conductivity (19).

In this study, a semiconducting material was extracted from the okra plant and mixed with polyaniline (PANI), with the addition of silver nanoparticles in certain proportions and the addition of the glucose oxide enzyme as a suitable composite material for developing biosensors for glucose monitoring. Conjugated polymer nanocomposites in biosensors offer superior sensing capabilities by facilitating effective interaction between the enzyme and the electrode. Furthermore, these biosensors are more resilient to some interfering substances (20).

1.2 Materials Used

Since the sensors consist of several materials, polyaniline in powder form and from Jiangsu Xfnano Materials Tech Co., Ltd. with high purity (99.8%), natural semi conductive extract from the okra plant, and nano silver prepared in the lab, Glucose oxidase enzyme from AGAPPE Diagnostics Switzerland Gmbh.

1.3 Preparation of Semiconductive Material

A semiconducting material was extracted from the okra plant. Okra sticks are collected, washed well, cut into small pieces, then boiled in 500 ml for 4 h at a temperature of 70°C, after which the mixture is filtered with a cloth to obtain a gelatinous substance, and dried in the oven at a temperature of 30-40 °C. For one hour, the dried material is then ground to obtain a fine semiconductor powder as shown in. figure 1 (21).

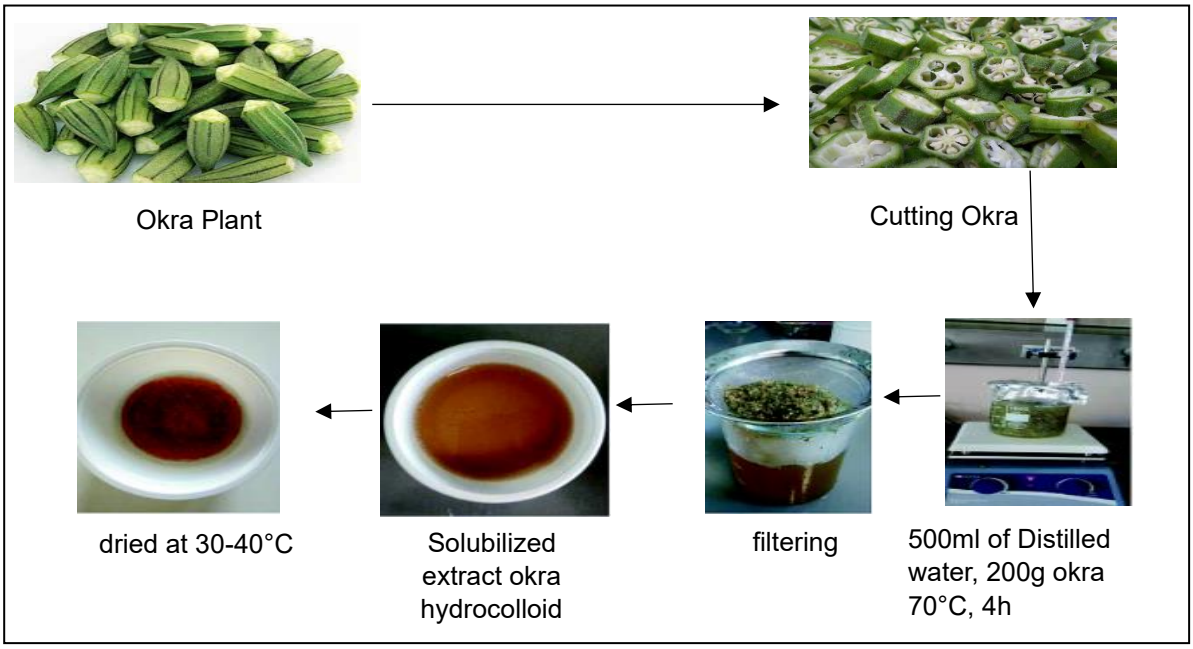


Figure 1: Steps to prepare the semiconductor material.

1.4 Preparation of Nano Silver

Silver nanoparticles were prepared by dissolving silver nitrate at a concentration of 1 millimolar in 50 ml distilled water and adding 5 ml of semi-conductive substance from the okra plant at a concentration of 1% wt with continuous stirring at a temperature of 70 °C for a period of 20 minutes (22).

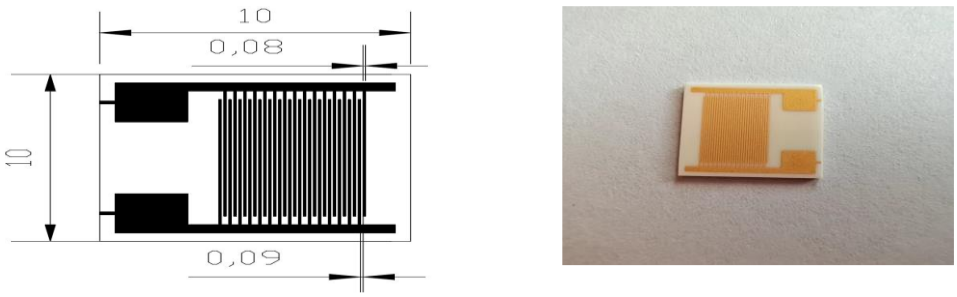
A brown solution of silver nanoparticles was formed in the form of a transparent colloidal suspension at a concentration of 32 mg/ml, as shown in the. figure 2. As in the following chemical equation:

$$AgNO_3 + \text{(semi-conducting material have Na)} \longrightarrow Ag + NaNO_3$$

Figure 2: Pure silver nanoparticles.

1.5 Integrated Sensor

An integrated sensor, specified with dimensions (10 * 10 * 0.635 mm), working temperature range of -50℃ to 500℃, and constructed with key materials like alumina ceramics and gold, was acquired from CHANGCHUN MEGA BORUI TECHNOLOGY CO., LTD. in China. The main parameters of the sensor include an electrode spacing of (0.09 mm), electrode width of (0.08 mm), carbon film thickness ranging from (9 to 11 μm), and a total of 15 electrode pairs. figure 3. shown the Sketch and integrated.



(a) **(b)**

Figure 3: (a) Sketch (b) the integrated sensor.

1.6 Preparation Samples

Sample preparation includes two categories, as illustrated below:

1.6.1 Preparation of films from polyaniline and semi-conductive substance

Preparation of polyaniline and semiconducting material film using okra plants at varying concentrations, as shown in Table 1. The semiconducting material and polyaniline were dissolved using a few drops of dimethyl sulfoxide (DMSO) because DMSO has the ability to dissolve both substances using ultrasound for (15 min) at amplitude (60%).

The prepared mixture is poured onto an integrated sensor by pouring 2 ml, distributing it by hand, and then drying it at 40°C for 24 hours to obtain the biosensor figure 4.

Table 1: Percentages of samples.

No. of samples	PANI (g)	semi-conductive substance (g)
F1	2.5	2.5
F2	1	4
F3	4	1

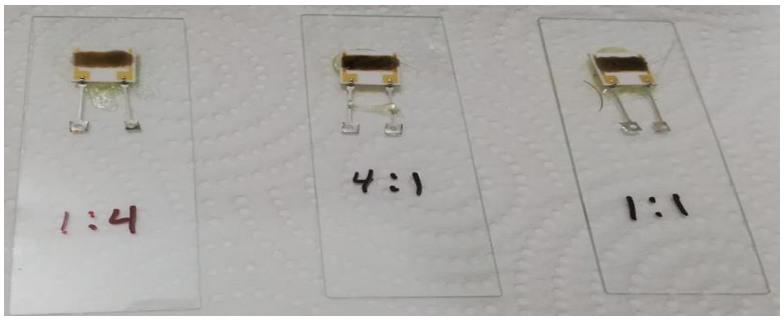


Figure 4: prepared sensor.

1.6.2 Preparation of films from polyaniline, semi-conductive substance and silver nanoparticles.

Polyaniline and semi-conductive substance were dissolved by Dimethyl sulfoxide (DMSO) at concentration F1, by ultrasonic for 15 min at 60% amplitude.

And then add the silver nano colloidal solution to the mixture of polyaniline and the semi-conducting material at different concentrations (1%, 5% and 10%) wt, and the mixture is mixed by magnetic stirrer at 25 °C for 6 hours.

The prepared blend is casting on integrated sensor by cast 2 ml and spread it by hand, then dried in 40C^{o} for 24 h, to get biosensor.

1.7 Result and Discussion

1.7.1 I-V Curve Measurement

The voltage was applied to the two ends of the gold trace using a DC power supply. The voltage ranged from 1.2 to 3.2 V with increments of 0.2 V. The I-V curve measurement was conducted in the presence of air, water, H_2O_2, and glucose. Table2. displays the current readings of three sensors at various voltages in the air. The chart indicates that the air has no effect on the sensors at different voltages.

Table 2. show different current with voltage for three sensors in air.

Volt	F1 µA	F2 µA	F3 µA
1	0	0	0
2	0	0	3
4	1	2	14
6	2	2.5	29
8	3	3	42
10	3.7	3.2	53
12	5	4-5	63
14	7.2	5.2	64
16	9	6	70

In the presence of distilled water, the voltage-current relationship of the sensors (F1, F2, and F3) was measured before adding silver nanoparticles. When a voltage difference is applied to the sensor from one end, a voltage difference of (1.2-3.2) volts is applied. This means, urging electrical charges to move and this will lead to the flow of electrical current. Therefore, at low voltages, we find stability in the electrical current, but in the case of increasing voltages we get the highest conductivity and the highest stability in the electrical current, and this means that the sensor is crossing a non-specific current. The material may have been destroyed or an insulator breakdown occurred, which is that the material is insulating or semi-conducting that gives off a current. It is linearly stable with voltage. That is, when a high voltage is applied to it, the current leaks. This is a characteristic of materials, especially polymers, so it appears in this figure 5. It was found that the applied voltage at 2.6 is the best due to the wide range of currents (135,71,81) µA. Which has the ability to detect high and low concentrations, and by studying the relationship between them it was found that the best sample is F1 due to the regularity between the potential difference (volts) and the current. While F2 the range of currents is small which means that a stable current is not obtained below dielectric breakdown, as it only senses low concentrations. While F3, the insulation breakdown, was found to rise in a way, as it does not distinguish between high and low ultra-low concentration. This means that high and low concentration give the same signal and the same current, and this is unreasonable. Therefore, the flow of charges does not depend on the concentration of the added substance.

Through these measurements in the presence of water, a range of sensitivity to a change in current is obtained. This sensor, in any area, senses any voltage applied to it from 1.2 to 2.6 volts. I found that 2.6 volts is the best range of sensitivity, as above it a breakdown occurs. The sensitivity range is very small, and this ensures correct readings of the sensor, so that a difference appears between them.

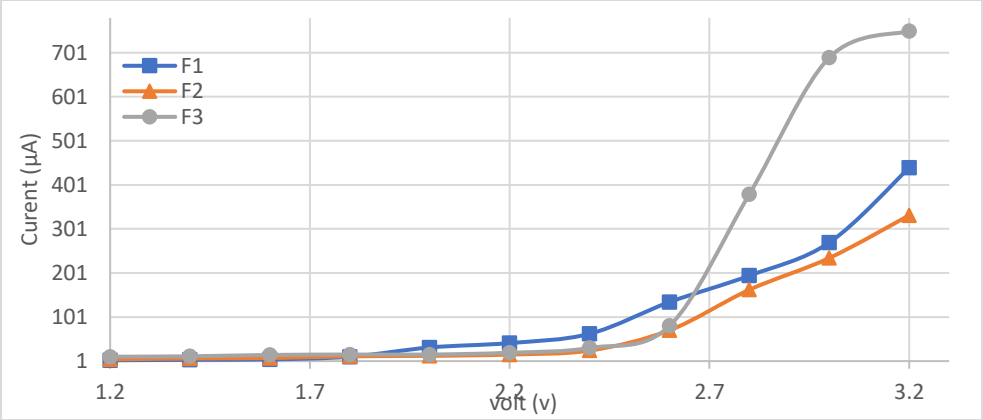


Figure 5: Show different current with voltage for three sensors in Distilled water.

When glucose in the blood combines with the glucose enzyme, hydrogen peroxide is made. Hydrogen peroxide is also a very reactive chemical because the links between peroxide atoms are not stable. So, $H2O_2$ usually breaks down in a step where two hydroxyl ions or an ion and a radical are made(23)(24) therefore, the interaction between hydrogen peroxide (H_2O_2) and the (semiconductive material + PANI) mixture sensor leads to the degradation of H_2O_2 and formation of a complex molecule, accompanied by the release of electrons, thereby inducing conductivity.

In the Table 3-4 and 5, the change in the current passing through the sensors with different voltages at different concentrations of H_2O_2. In Sample (F_2), show the instability of the current in the sample with different concentration of H_2O_2 also in different voltage at same concentration of H_2O_2 as shown in the table 4. In the sample (F3), show that there is a slight effect in the current during the change in concentration at Constant voltage as shown in Table 5, but in sample (F1) show that there is stability of increase in the current passing through the sensor at different concentrations of H_2O_2 at the same voltage as shown in Table 3.

Table 3. show different current with voltage in H_2O_2 for sample (F_1)

Volt	IµA at concentration $1x10^{-5}$mg/ml	IµA at concentration $1x10^{-4}$ mg/ml	I µA at concentration $8x10^{-4}$ mg/ml	I µA at concentration $3.2x10^{-2}$ mg/ml
2.2	3	6	70	120
2.4	19	14	80	160
2.6	25	34	99	312
2.8	56	63	139	440
3	86	95	160	610
3.2	126	144	250	1151

Table4. show different current with voltage in H_2O_2 for sample (F_2)

Volt	I µA at concentration $1x10^{-5}$mg/ml	I µA at concentration $1x10^{-4}$ mg/ml	I µA at concentration $8x10^{-4}$ mg/ml	I µA at concentration $3.2x10^{-2}$ mg/ml
2.2	5	4	-	176
2.4	10	6	-	280
2.6	15	8	3	300
2.8	-	11	6	360
3	18	15	18	445
3.2	31	42	56	600
3.4	744	92	85	850

Table 5. show different current with voltage in H_2O_2 for sample (F_3)

Volt	I µA at concentration $1x10^{-5}$mg/ml	I µA at concentration $1x10^{-4}$ mg/ml	I µA at concentration $8x10^{-4}$ mg/ml	IµA at concentration $3.2x10^{-2}$ mg/ml
1.4	4	4	17	18
1.6	5	5	26	25
1.8	8	7	43	42
2.2	12	16	32	44
2.6	34	39	53	57
2.8	44	67	94	130
3	95	130	140	205

Hence, the sensor needs to identify a variation in current when the concentration of H_2O_2 changes. The Tables 3, 4, and 5 indicate that sample (F_1) is the most effective due to its clear impact and consistent current increase with rising H_2O_2 concentration at a specific voltage, contributing to sensor stability.

Also, in Figure 6 illustrates that the current exhibits a proportional increase in response to concentration of H_2O_2 on the sensor, at different voltage of (2.2-3.2 V). The relationship between the current measured from the electrical circuit and the concentration of H_2O_2 was observed to be linear at (2.6 v) with the exception of a sudden surge in current. at a concentration of (8×10^{-4}). At this concentration, the recorded current was (99 µA), whereas at a concentration of (3.2×10^{-2}), the measured current (312 µA). The increasing of the recorded current suggests an anomaly in the expected pattern with varying concentrations of H_2O_2, which can be attributed to the catalysis of hydrogen peroxide degradation, occurs at the active sites on the sensor surface, leading to the generation of OH radicals. In this process, the radicals acquire electrons from the active material, reverting to hydroxyl anions and desorbing as water molecules. This mechanism is a prevalent detection system in electrochemical sensors (25).

To enhance the sensor's sensitivity and boost the produced current, nano silver was included into the blend material in sensor (F_1) at three concentrations (1%, 5%, and 10%) wt., in Figure 7. illustrates that the addition of silver nanoparticles (1 wt. %) to the blend. The results in a discernible sensitivity in the measured currents at voltage (2.6 V). Additionally, a linear correlation between the concentration of H_2O_2 and the measured currents is observed, but a sudden increase in currents occurs at a concentration of (3.2×10-2) where measured current reached (680 µA), whereas at a concentration of (8×10-4), the measured current was (190 µA). The adding of silver nanoparticles led to significant increasing in the recorded current which attributed to the hydroxyl ions and radicals produced by hydrogen peroxide have the potential to undergo chemical reactions with the active materials (Ag nanoparticles) the existing in sensor. In contrast to the sensing mechanism involving noble metals, hydroxyl ions are not only adsorbed but actively engaged with the active sites of transition metal-based structures before being released, usually carrying hydrogen atoms along with them (26).

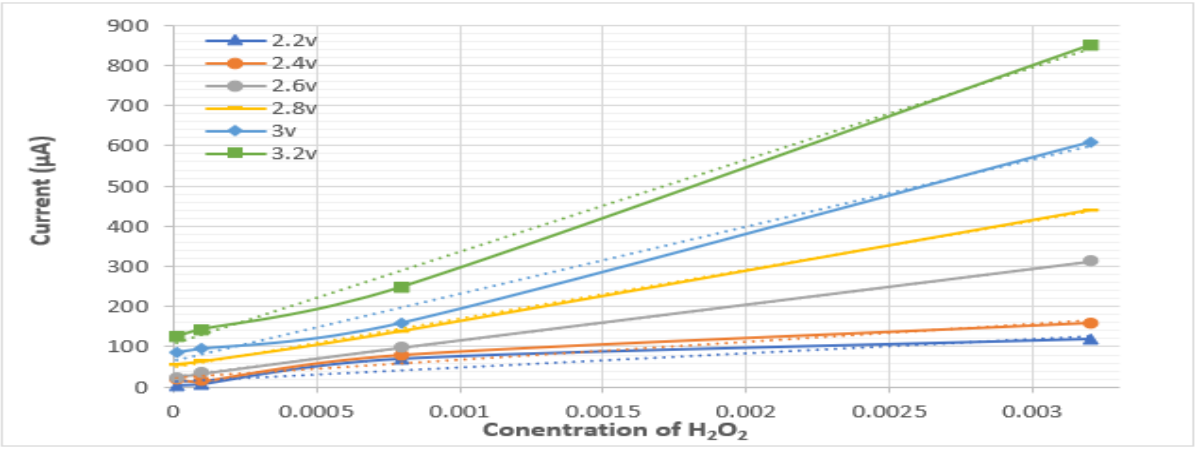


Figure 6: show different current with voltage in H_2O_2 for sample (F_1)

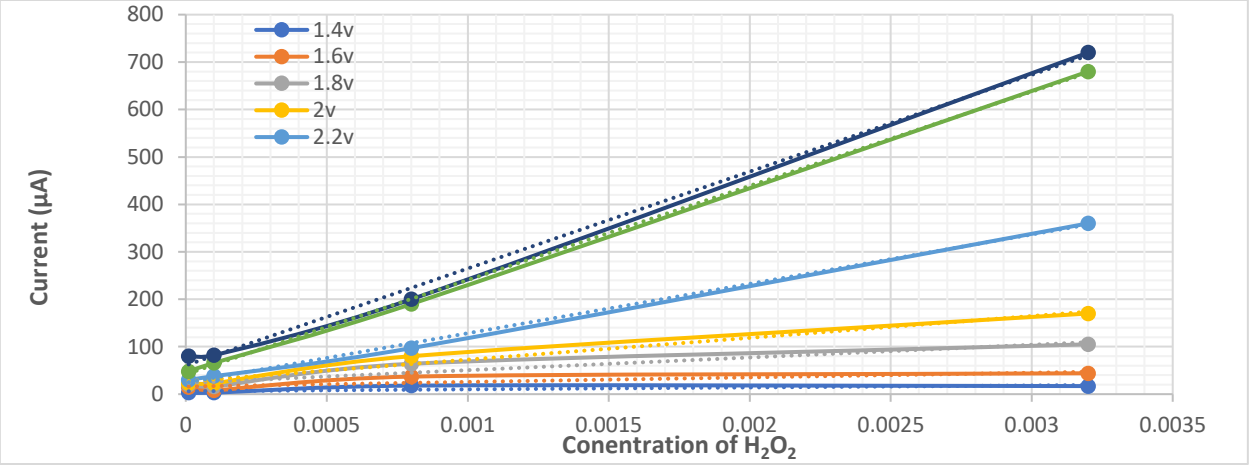


Figure 7: Show different current with in H_2O_2 for sample (F_1) with the silver nanoparticles concentration (1 wt. %).

When increase silver nanoparticles concentration to (5 wt. %), figure 8, illustrates the current exhibits a proportional increase in response to concentration of H2O2 on the sensor, at different voltage of (1.4 - 3 V). But there is no evident linear correlation between the concentration of H2O2 with current at different voltages. Therefore, the concentration of silver was raised to 10%.

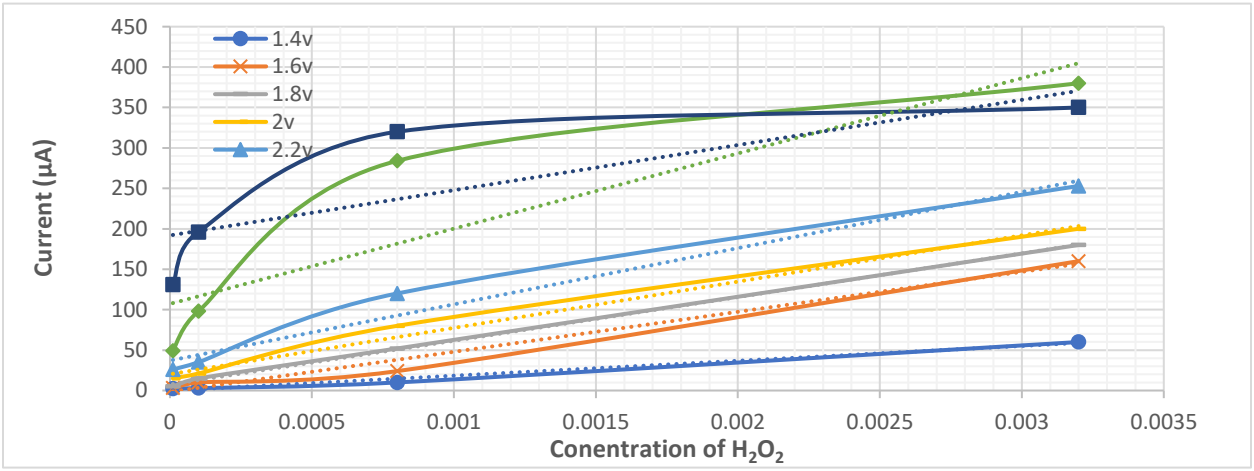


Figure 8: show different current with in H2O2 for sample (F1) with the silver nanoparticles concentration (5 wt. %).

Figure 9. illustrates a distinct linear correlation between the concentration of H_2O_2 and the current at a voltage of 2.6 V. where the Current increase gradual with concentration increase of H_2O_2, at concentration of 10wt % nano silver.

The Figure 7,8 and 9 demonstrate that at a voltage of 2.6 V, the current exhibited a nearly linear relationship with H_2O_2. Additionally, with a 10%wt nano silver in the sensor, the current gradually increased with higher H_2O_2 concentration. Also, at concentration of ($1*10^{-5}$) for H_2O_2, the current was (56 µA), above the current generated by the sensor in the presence of water, which was (40 µA), and this meets with the sensitivity property requirement. This indicates that the sensor from blend (OKRA+PANI) with the addition of silver nanoparticles at concentration (10 wt. %), give both properties linearity and sensitivity which makes it as a proposed sensor for H_2O_2. Furthermore, when other liquids were added on the sensor, did not yield any current. Also, this measurement conducted in the air, also did not yield any current, this demonstrating the selectivity property (27).

The reduction of H_2O_2 is accelerated in the presence of a higher content of silver nanoparticles (10 wt. %) on the surface of the (PANI+Okra+Ag) sensor. and the reaction between the H2O2 and the Ag nanoparticles goes toward more O_2 generation. Subsequently, the generated O_2 serves as the detection signal on the prepared modified electrode (28). On the other hand, the detection mechanism in the sensor depends on the nature of the conductive material (Ag nanoparticles), where the interaction with the hydrogen peroxide H_2O_2 leads to change the electrical conductivity (29).

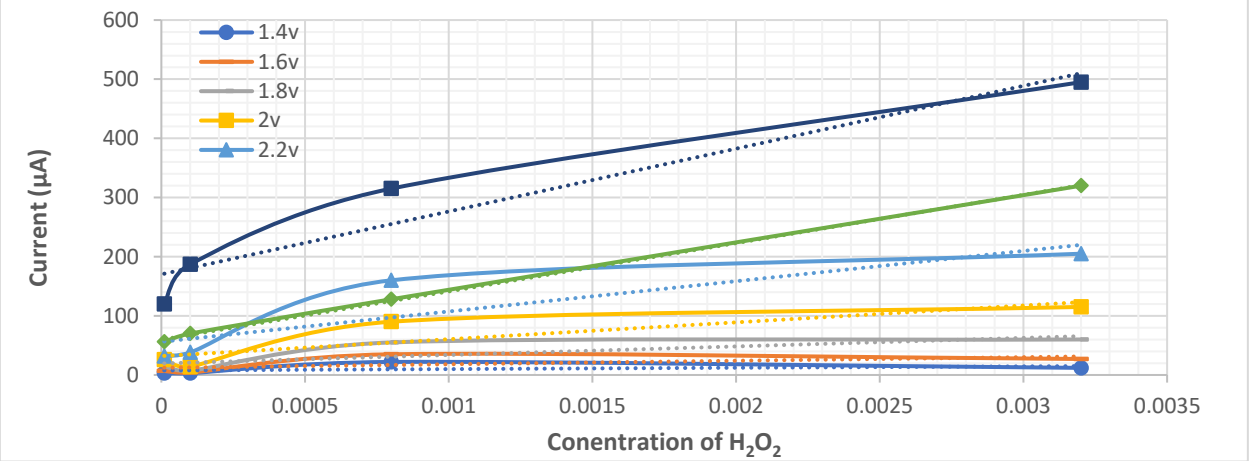


Figure 9: Show different current with in H2O2 for sample (F1) with the silver nanoparticles concentration (10 wt.%).

The I-V Curve Measurement was repeated with varying concentration of glucose and nano silver for F1 sample at a constant voltage of 2.6V, as Shown in figure 10. The best ratio was of nano silver is 10 %wt, will show linear relationship between concentration of glucose and current. Also, several chemicals, such as sucrose and other compounds, were applied to the sensor to demonstrate its selectivity, but no current was detected.

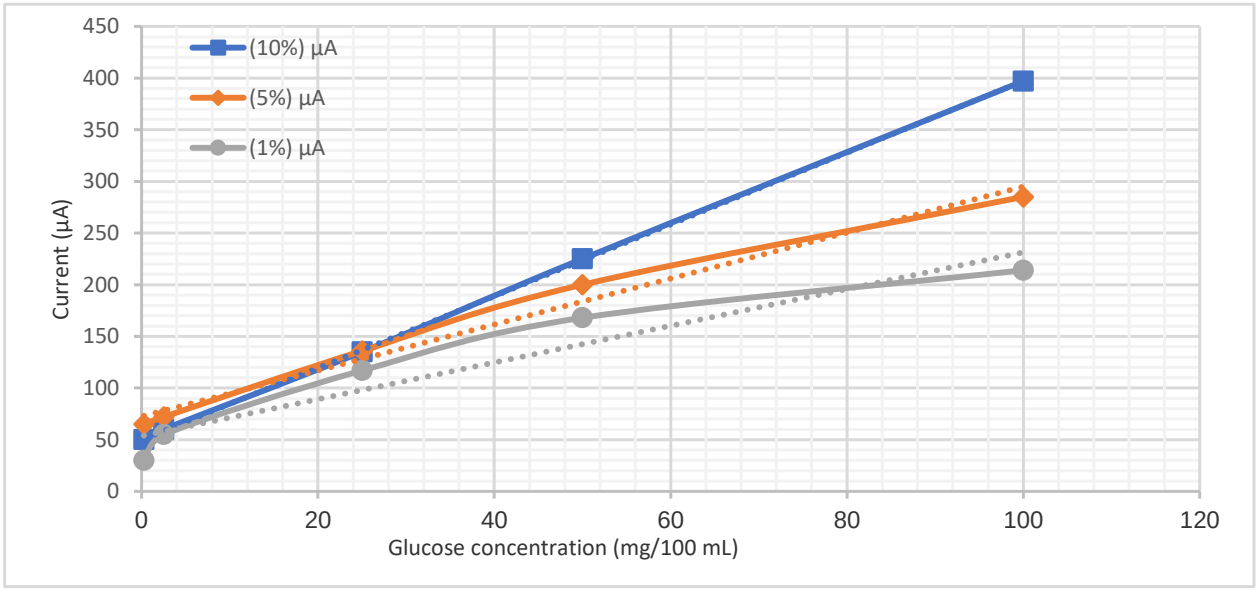


Figure 10: show different current with in Glucose for sample (F1) with the different concentration of silver nanoparticles.

The results demonstrate that the sensor made of okra and PANI in a 1:1 ratio with 10% nano silver exhibits properties selectivity, sensitivity, and linearity. it could serve as a potential glucose sensor for blood.

1.7.2 Scan Electron Microscopy (SEM)

Nanoparticle silver was synthesized in the laboratory and its particle size was determined by Scan Electron Microscopy (ASTM E 986) analysis, as depicted in figure 10. The particles were discovered to have a size range of 5-30 nm[3,32].

Figure 11 shows the SEM image of the polymer blend has pores. These pores represent the crystalline region in the blend. Further, the uniform distribution of these pores over the total surface of the blend indicates that the (PANI/ Okra plant) blends form a homogeneous structure due to polymer- okra plant interactions which confirm that the okra plant is miscible with PANI, that led showed a rough surface[33,34].

The addition of nano silver to the mixture of okra with PANI resulted in a homogeneous dispersion of the blend across the sensor surface, along with the presence of cellular regions in the sensor, as depicted in figure 12. This elucidates the precision of the currents produced by the sensor(34)

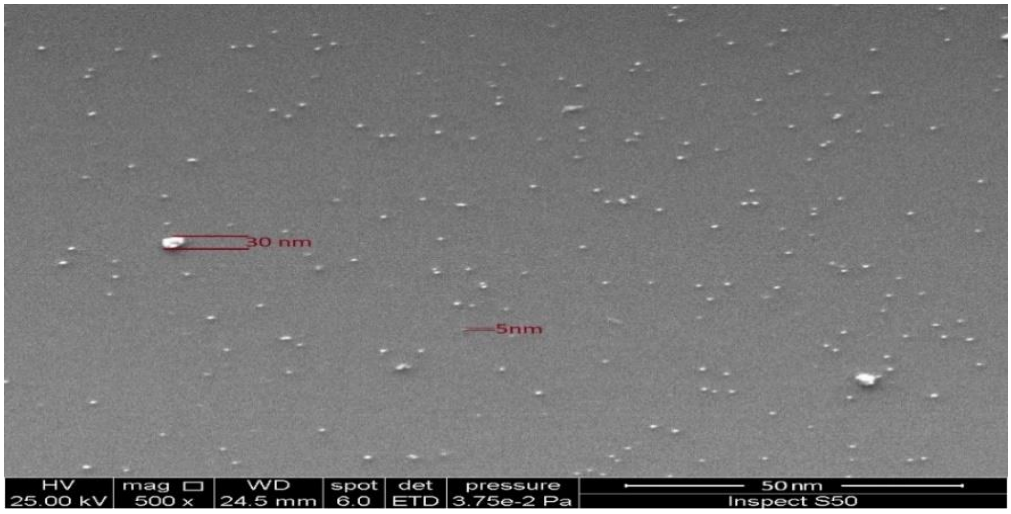


Figure 10: Show the particles size of nano silver.

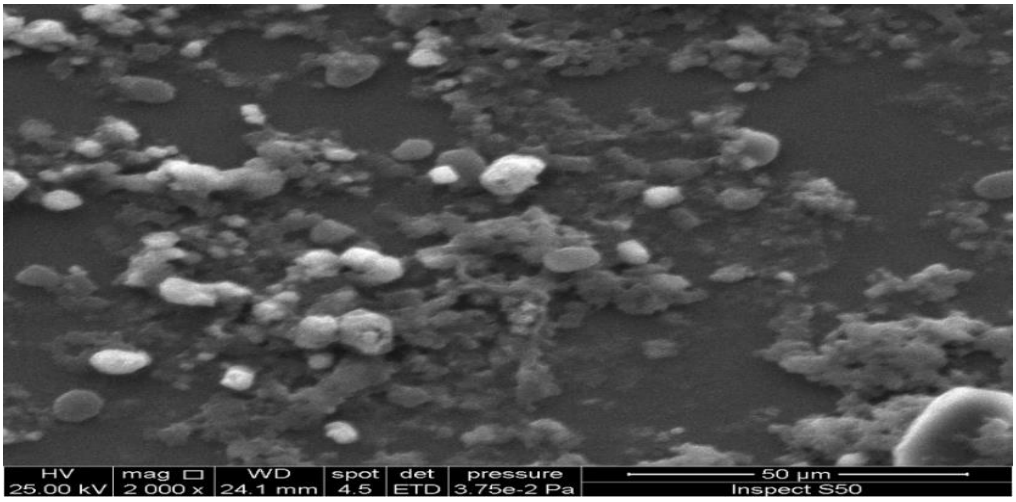


Figure 11. Show the microscopic structure of the blend of okra Plant with PANI

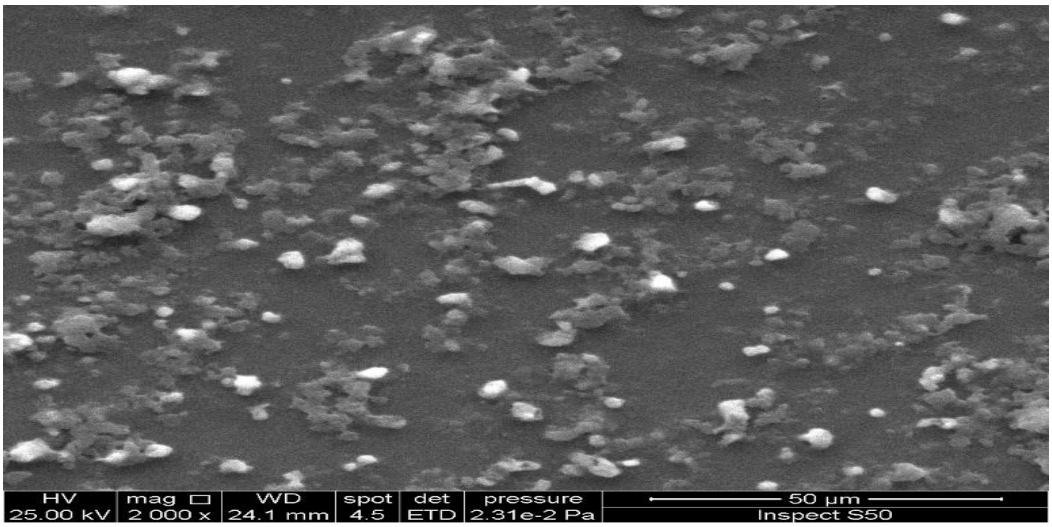


Figure 12. Shows the microscopic structure of the blend of okra + PANI with 10%wt nano silver.

1.8 Conclusions

The results obtained from this study lead to the proposal of a composite material that acts as a sensor to detect blood glucose. This material includes a compound extracted from a semiconducting material extracted from the okra plant, and mixed with polyaniline. Experimental results showed that using polyaniline at a ratio of 50:50 with okra extract produces a current of 0.25 mg/100 ml. The sensor demonstrated the ability to detect low amounts of glucose as low as 0.25 mg/100 ml. The sensor circuit current output was 50 µA at 2.6 V, and 397 µA at 2.6 V at the highest concentration of 100 mg/100 mL. This means the sensor's ability to detect low and high concentrations.

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