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AUTHOR'S AFFILIATIONS

Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5, 65145, Malang, Indonesia^{1,2,4,5,6}

Laser Center, Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, Skudai, Johor, 81310, Malaysia³

CORRESPONDING AUTHOR

Ahmad Taufiq, Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5, 65145, Malang, Indonesia

E-mail:

ahmad.taufiq.fmipa@um.ac.id

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Original Research Article

INTRODUCTION

Diabetes is a chronic disease that is currently a global problem because it continues to increase and has the potential to cause death (Hacker, 2024). In 2024, the Diabetes Atlas reported that the number of people with diabetes is projected to rise to approximately 853 million by 2050 (Genitsaridi et al., 2026). A key characteristic of diabetes is abnormally high blood sugar levels (Rama Rao & Tejomurtula,

Preliminary Electrochemical Characterization of Fe₃O₄/AC-GOx Nanocomposite-Modified Electrodes as Glucose Biosensor Candidates

Bulkis Kanaya¹, Dea Berliana Ramadhani², Muhammad Safwan Abd Aziz³, Nurul Hidayat⁴, Markus Diantoro⁵, Ahmad Taufiq^{6*}

Abstract

Background: Diabetes is a chronic disease and a major global health problem, characterized by elevated blood glucose levels and potentially leading to serious complications and death. Therefore, accurate and efficient blood glucose monitoring is essential. **Objective:** This study aimed to develop a glucose biosensor based on a Fe₃O₄/AC-(GOx) nanocomposite using an electrochemical method. **Methods:** The Fe₃O₄/AC nanocomposite was synthesized using the coprecipitation method, with natural iron sand and coconut-shell-derived carbon as the main precursors. The synthesized nanocomposite was characterized using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and magnetic characterization. The Fe₃O₄/AC-GOx nanocomposite was then applied to a screen-printed carbon electrode (SPCE) for electrochemical evaluation. **Results:** XRD and FTIR characterization confirmed the formation of the Fe₃O₄/AC nanocomposite and the presence of characteristic Fe–O and carbon-based functional groups. SEM analysis showed an irregular morphology with a tendency to agglomerate and an average particle size of 48.05 nm. The nanocomposite exhibited a saturation magnetization of 9.26 emu/g, with low coercivity and remanent magnetization. The Fe₃O₄/AC-GOx-modified SPCE showed a higher current response than the bare SPCE, indicating improved electrochemical activity. However, glucose-dependent responses and analytical performance parameters have not yet been evaluated. **Conclusion:** The Fe₃O₄/AC-GOx-modified SPCE shows potential for further development as a glucose biosensor, although systematic analytical validation is still required.

Keywords: Coconut shell, Electrochemical, Fe₃O₄/AC-(GOx) nanocomposite, Glucose biosensor, Iron sand

2024), even though the human body relies on blood sugar as a primary source of energy (Shourie & Trivedi, 2025). Therefore, accurate and efficient blood sugar monitoring is crucial for diagnosis, glucose level management, and the prevention of complications. Currently, blood glucose monitoring still relies on conventional methods, one of which often involves drawing blood samples from a patient's finger using a needle (Xue et al., 2022). Therefore, this study developed a biosensor to detect and monitor blood sugar levels using the enzyme-based electrochemical method, Glucose Oxidase (GOx) to improve the selectivity, effectiveness, and performance of biosensors.

In general, electrochemical biosensors for detecting blood sugar use a single enzyme, GOx as the main component (Mohamad Nor et al., 2022). The use of GOx enzyme-based electrochemical biosensors is attractive because GOx has bioreceptors that can detect blood sugar levels in the body more sensitively and selectively. Furthermore, GOx has been thoroughly investigated for use in biosensors due to its relatively low cost and high bioactivity, selectivity, and stability (Lin et al., 2022). However, the active sites of GOx are located deep within the non-conductive protein layer, thus not supporting electron transfer between the enzyme and the electrode. Furthermore, GOx enzymes are susceptible to conformational changes caused by different immobilization methods, thus affecting their catalytic performance in biosensors. Therefore, GOx needs to be modified with supporting materials that have high conductivity, good biocompatibility, and a large specific surface area to optimally immobilize GOx (Bi et al., 2023).

In this study, electrode modification is offered by adding Fe₃O₄ nanoparticles. These Fe₃O₄ nanoparticles have the potential to improve the performance of glucose biosensors because they have activity that resembles that of the enzyme peroxidase. Thus, the nanoparticles can facilitate the electrochemical reaction of H₂O₂ resulting from the oxidation of glucose by GOx (Bilalis et al., 2022). In addition, these nanoparticles have superparamagnetic properties, thus facilitating the process of separation, purification, and stabilization of enzymes immobilized on their surface (Gama Cavalcante et al., 2024). However, Fe₃O₄ has low electrical conductivity, is easily agglomerated, and has limited enzyme binding capacity (Kesici-Meco & Unur-Yilmaz, 2024; Paul et al., 2025). To overcome this problem, Fe₃O₄ is composited with Activated Carbon (AC), which is known to have high electrical conductivity (Sujiono et al., 2022). This characteristic allows for more efficient electron transfer between the enzyme and the electrode, resulting in a stronger signal and higher sensitivity to changes in blood sugar concentration. Modifying the GOx enzyme with Fe₃O₄/AC nanocomposites could be a potential solution to improve the selectivity, effectiveness, and stability of enzyme-based blood sugar biosensors. Interestingly, this study used natural materials such as iron sand and coconut shells as the primary precursors for the Fe₃O₄/AC nanocomposite. Therefore, the use of these natural materials offers potential for developing environmentally friendly biosensors and reducing production costs.

MATERIALS AND METHODS

The synthesis of Fe₃O₄/AC nanocomposites begins with the preparation of Fe₃O₄ nanoparticles using the coprecipitation method with iron sand precursors, where the synthesis steps follow previous research (Berliana Ramadhani et al., 2025). Iron sand was reacted with 12M HCl to produce FeCl₂ and FeCl₃ solutions, which was then titrated with 6.5M NH₄OH to produce a black precipitate. The resulting precipitate was then washed to a neutral pH and dried at 100°C to obtain Fe₃O₄ powder. The second stage was the synthesis of AC using coconut shell precursors (Mufandi et al., 2020), where the coconut shell is burned and calcined at a temperature of 400°C to obtain carbon powder.

The activated carbon powder was washed with 2M KOH until the pH was neutral. The resulting powder was dried at 100°C to obtain AC powder. Furthermore, the Fe₃O₄ nanoparticles and Activated Carbon (AC) were mixed in a 1:1 ratio with 18 ml of deionized (DI) water at pH 7, then stirred for 10 minutes at 720 rpm. Subsequently, the mixture was filtered to collect the precipitate. The resulting precipitate was then dried in an oven at 100 °C (Zainullah et al., 2025). The obtained nanocomposite was then used for the immobilization of the enzyme glucose oxidase (GOx). A total of 0.1 g of the Fe₃O₄/AC nanocomposite was dispersed into 2 ml of PBS (pH 7) and sonicated for 30 minutes to obtain a homogeneous suspension. Separately, 0.1 g of GOx was dissolved in 1 ml of PBS (pH 7) and homogenized using a vortex mixer for 10 minutes. Next, 1 ml of the Fe₃O₄/AC nanocomposite

suspension in PBS was mixed with the vortex-homogenized GOx solution. The mixture was then incubated in a chiller for 24 hours to allow for the immobilization of the GOx enzyme onto the nanocomposite surface. Upon completion of the immobilization process, 4 μ L of Fe₃O₄/AC-GOx was evenly applied to the surface of the working electrode (WE) and allowed to dry at room temperature. Subsequently, 2 μ L of Nafion was applied over the Fe₃O₄/AC-GOx layer and again dried at room temperature for 2 hours until a stable layer formed on the electrode surface (Zhou et al., 2022).

Fe₃O₄/AC nanocomposite process was carried out using a conventional mixing method, following previous research (Zainullah et al., 2025). The preparation of the Fe₃O₄/AC-GOx nanocomposite sample began by mixing Fe₃O₄/AC and GOx nanocomposite powders, each dispersed in PBS. The mixture was then incubated for 24 hours in a refrigerator. Afterward, 4 μ L of Fe₃O₄/AC-GOx was dropped onto the SPCE working electrode and coated with Nafion (Zhou et al., 2022). The modified electrode was then dried at room temperature for 2 hours and subjected to a cyclic voltammetry (CV) test. Furthermore, CV measurements were performed using 50 mL of phosphate-buffered saline (PBS) at pH 7 mixed with 0.9 g of glucose, corresponding to a glucose concentration of approximately 100 mM, as the electrolyte. The integrated reference electrode of the SPCE was used as a pseudo-reference electrode during the electrochemical measurement.

Next, the nanocomposite was characterized using X-Ray diffraction (XRD) to determine its crystal structure and phases. Furthermore, the functional groups contained in the nanocomposite were analyzed using Fourier Transform Infrared Spectroscopy (FTIR). The morphology and particle size were also analyzed using a Scanning Electron Microscope (SEM). Furthermore, the magnetic properties of the sample were determined from Vibrating Sample Magnetometer (VSM) characterization. Meanwhile, the biosensor performance was analyzed using electrochemical responses from Cyclic Voltammetry (CV) characterization.

RESULTS

In this study, XRD testing was carried out, which produced a diffraction pattern of Fe₃O₄/AC nanocomposite in Figure 1. The diffraction peaks of Fe₃O₄ nanoparticles appeared at 2θ around 30.1°, 35.4°, 43.1°, 53.5°, 57.0°, and 62.6°. The AC material was detected at its peak broadening around 2θ = 25°, which was not clearly visible.

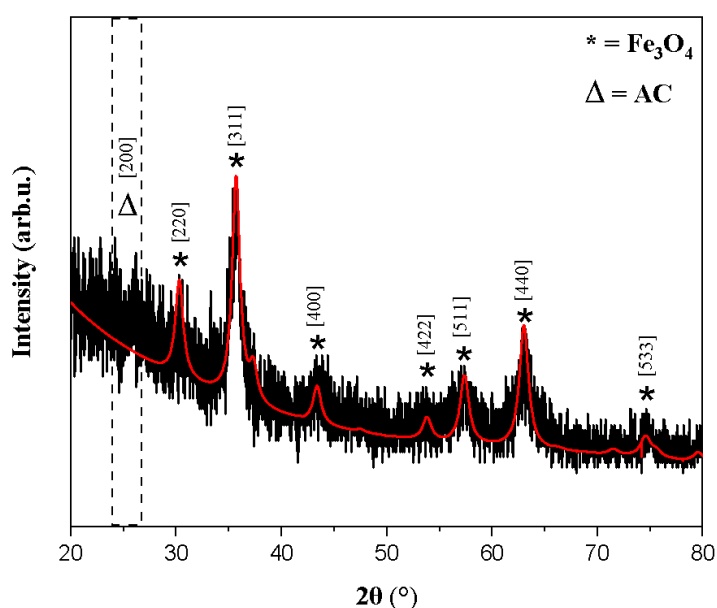


Figure 1. Diffraction pattern of Fe₃O₄/AC nanocomposite

Then, Figure 2 shows the morphology and size distribution of the $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposite. The nanoparticles produce an irregular shape on the AC surface with a size distribution of 48.05 nm.

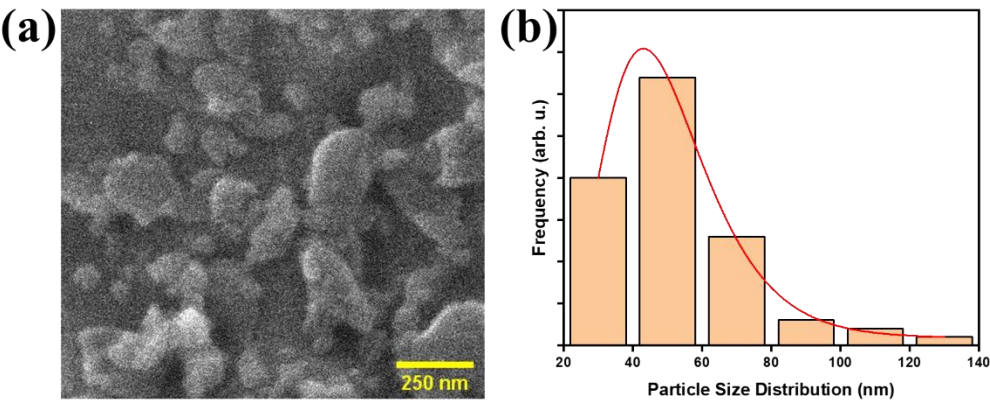


Figure 2. (a) morphology and (b) size distribution of $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposites

The FTIR characterization results of $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposite are shown in Figure 3 with a wavenumber range of $4000\text{--}400\text{ cm}^{-1}$. Based on the figure, the absorption peaks of O–H, C=C, C=O, C–H, C–O, Fe–O octahedral, and Fe–O tetrahedral have appeared.

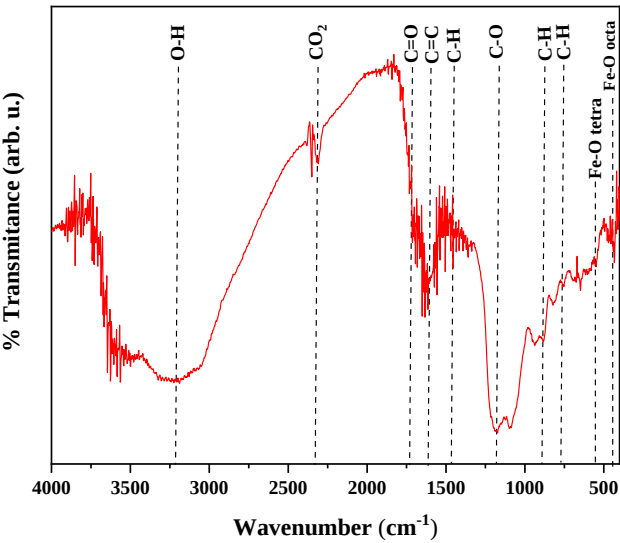


Figure 3. FTIR results of $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposite

Furthermore, the VSM characterization results of the $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposite are shown in Figure 4, which was carried out at room temperature. Based on the figure, an S-shaped curve is obtained, with saturation magnetization (M_s), coercivity (H_c), and remanent magnetization (M_r) values of 9.26 emu/g, 0.0021 T, and 0.0044 emu/g, respectively.

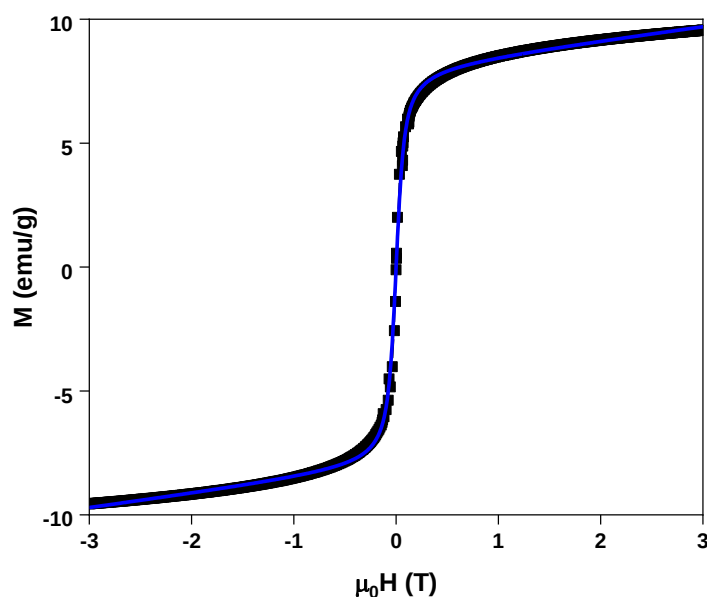


Figure 4. Hysteresis curve of Fe₃O₄/AC nanocomposite

Based on Figure 5, the electrochemical responses of a bare SPCE and an SPCE modified with Fe₃O₄/AC–GOx were evaluated using cyclic voltammetry (CV) over a potential range of –1.0 V to +1.0 V for three cycles, employing a phosphate-buffered saline (PBS) solution mixed with glucose as the electrolyte at pH 7. The reference electrode integrated into the screen-printed carbon electrode (SPCE) served as a pseudo-reference electrode during electrochemical measurements. The test results reveal that the bare SPCE (unmodified) exhibited a relatively low current response of 10^{-6} A. In contrast, the SPCE modified with the Fe₃O₄/AC–GOx nanocomposite demonstrated a measurable current response, with the cathodic current reaching approximately -6.3×10^{-5} A and the anodic current reaching approximately 3.0×10^{-5} A. Therefore, the nanocomposite modification on the SPCE surface is shown to yield a higher current response compared to the bare SPCE alone. Furthermore, this modification enhances the electrochemical activity at the electrode interface

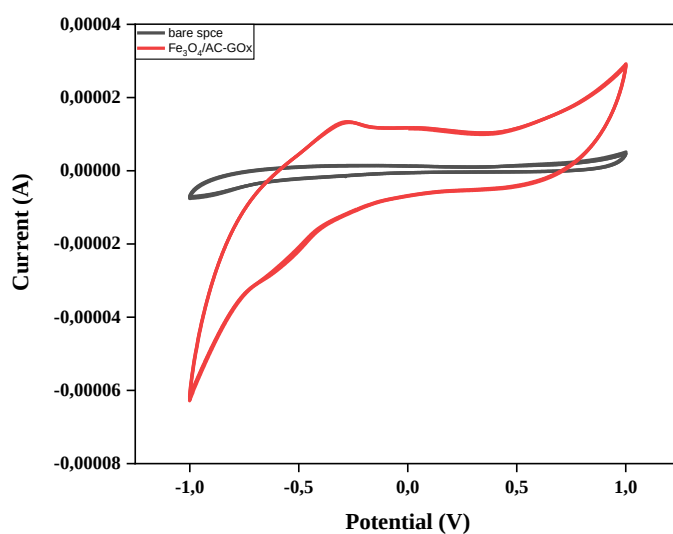


Figure 5. Cyclic voltammogram of bare SPCE and Fe₃O₄/AC-GOx nanocomposite

DISCUSSION

Based on the XRD results, it is known that the diffraction peaks of Fe_3O_4 nanoparticles appear at 2θ around 30.1° , 35.4° , 43.1° , 53.5° , 57.0° , and 62.6° which correspond to the hkl planes (220), (311), (400), (422), (511), (440), and (533) with the lattice parameters $a = b = c = 8.360 \text{ \AA}$. The value of this lattice parameter is within the standard range of Fe_3O_4 nanoparticles reported in previous studies, which is $8.358\text{--}8.393 \text{ \AA}$ ($a = b = c$) (Hariyanto et al., 2026). The position of the peak of diffraction is in accordance with the model data (AMCSD 0007423), which has been analyzed using the Rietveld method. These results show that Fe_3O_4 nanoparticles have a cubic spinel crystal structure and a face-centered cubic (FCC) atomic arrangement, where Fe^{2+} and Fe^{3+} ions are distributed at tetrahedral and octahedral sites in the crystal lattice (Nikmah et al., 2021; Tatarchuk, 2024). Furthermore, the AC material was detected by the presence of a broad peak around $2\theta = 25^\circ$ (002), which is characteristic of the amorphous phase structure. However, this amorphous peak is not clearly visible because it is mostly convergent and covered by the Fe_3O_4 crystalline material (Vali et al., 2024).

Based on the analysis results, the crystallite size of the Fe_3O_4 nanoparticles was determined to be 10.46 nm . This nanometer-scale crystallite size yields a larger specific surface area, thereby providing more active sites that have the potential to enhance the material's catalytic activity (Naikoo et al., 2021).

Furthermore, the morphology of the resulting $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposite is irregular in shape with Fe_3O_4 nanoparticles tending to clump on the AC surface (Figure 2(a)). This agglomeration is caused by the interaction phenomenon between magnetic particles due to the presence of Van der Waals forces (Wolf et al., 2023). Furthermore, the particles had a size distribution of 48.05 nm (Figure 2(b)). In this study, the particle size distribution was analyzed using ImageJ software and then a log-normal fitting model. This result is in line with previous research, which reported that the coprecipitation synthesis method was able to produce nanoparticles with crystallite sizes in the range of $10\text{--}20 \text{ nm}$ and that they were distributed relatively evenly on the AC surface (Juang et al., 2018). The distribution of particles on the AC surface is generally influenced by the interaction between the AC surface functional groups and iron ions (Hakim & Sedyadi, 2020). Based on the particle size results from the SEM analysis, it showed a greater value than the crystal size results obtained from the XRD analysis. This is because the SEM results provide the overall particle size observed on the sample surface, while the XRD results show the size of the crystallite domain. With that in mind, a single particle of the SEM results can consist of a collection of crystallite domains, so that the particle size is greater than the crystal size in XRD (Basak et al., 2022; Geetha et al., 2024).

The formation of the $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposite was also confirmed through functional group analysis, with absorption peaks shown in Figure 3. An absorption peak corresponding to O–H stretching vibration was detected at a wavenumber of 3209 cm^{-1} , consistent with previous studies indicating O–H bond vibrations in the $3000\text{--}3600 \text{ cm}^{-1}$ range. This bond confirms the existence of an organic source of coconut shell as an AC precursor (Taufiq et al., 2020). In this regard, the existence of an O–H bond has the potential to support the interaction with the GOx enzyme in the performance of glucose biosensors (Xiao et al., 2024). In addition, the carbon structure of activated carbon in the form of C=O and C=C bonds has been identified at the wavenumbers 1700 cm^{-1} and 1607 cm^{-1} , as reported in previous studies in the wavenumber range $1900\text{--}1700 \text{ cm}^{-1}$ (Awodi et al., 2026). Both bonds also have the potential to strengthen surface interactions with biomolecules (Fu et al., 2024). Furthermore, AC also forms a C–O bond at the wavenumber 1174 cm^{-1} (Dirgayanti et al., 2021), as well as the presence of C–H bonds at 1454 cm^{-1} , 882 cm^{-1} , and 759 cm^{-1} , indicating an aromatic structure in AC (Awodi et al., 2026; Dirgayanti et al., 2021; Hussain et al., 2023). Then, the emergence of Fe_3O_4 is characterized by the presence of octahedral and tetrahedral Fe–O bonds at wavenumbers of 440 cm^{-1} and 554 cm^{-1} (Taufiq et al., 2020; Zainullah et al., 2025). The sample also showed CO_2 at wavenumber 2371 cm^{-1} (Agista et al., 2025).

Furthermore, the magnetic properties of $\text{Fe}_3\text{O}_4/\text{AC}$ nanoparticles are illustrated by the hysteresis curve in Figure 4. The analysis reveals an S-shaped curve, with coercivity (H_c) and remanent magnetization (M_r) values of 0.0021 T and 0.0044 emu/g , respectively, close to zero. This confirms that the nanocomposite is superparamagnetic, in line with previous studies (Fi Ramadhani et al., 2025).

Based on its magnetic properties, it can contribute to better dispersibility of nanoparticles, which have the potential to maintain surface area in enzyme immobilization (Gama Cavalcante et al., 2024). Meanwhile, the saturation magnetization value produced in this study was 9.26 emu/g, which is lower than that of pure Fe₃O₄ (68.10 emu/g). This decrease in magnetization value is due to the addition of non-magnetic materials in the form of AC materials, where in this study the addition of AC to nanocomposites is in a ratio of 1:1 (Fe₃O₄:AC) (Astuti et al., 2022).

The performance of the biosensor is evaluated through cyclic voltammetry (CV) with the addition of Nafion. Nafion serves as a proton exchange membrane to improve enzyme stability and accelerate ion transfer during electrochemical processes. Nafion also acts as a protective matrix that prevents the release of enzymes from the electrode surface, thereby enhancing the biosensor's reusability (Mohammadzadeh Kakhki, 2024). Figure 5 shows a cyclic voltammetry graph of the CV test results, showing the electrochemical response between the modified SPCE of the Fe₃O₄/AC-GOx nanocomposite and the unmodified SPCE. These results show that the modified SPCE results in a higher current response compared to the unmodified SPCE. This current response is characterized by an anodic feature at a relatively low potential of approximately -0.3 V, followed by a response in the higher potential range of 0.5–1.0 V.

The emergence of this current response is attributed to the presence of the Fe₃O₄/AC-GOx nanocomposite modification on the SPCE surface. The Fe₃O₄ nanoparticles themselves facilitate an electron transfer process involving the Fe²⁺/Fe³⁺ redox couple and provide electroactive sites (Park, 2026). Furthermore, the performance of the Fe₃O₄ nanoparticles is enhanced by the addition of AC, which possesses a relatively large surface area. This increases the electrochemically accessible surface area of the electrode and provides additional active sites and oxygen-containing functional groups to support electrochemical reactions (Zhang et al., 2022). Additionally, the response observed at higher potentials is influenced by the successful immobilization of GOx on the electrode surface, as this is expected to trigger the oxidation of H₂O₂ generated by the GOx enzymatic reaction. However, since measurements across varying glucose concentrations have not yet been performed, it cannot be definitively confirmed that the response observed in the 0.5–1.0 V range stems from glucose oxidation. Nevertheless, surface modification using the Fe₃O₄/AC-GOx nanocomposite yields a measurable response and demonstrates potential as a biosensor candidate compared to the unmodified state. This behavior can be attributed to the favorable electrochemical properties of Fe₃O₄ nanoparticles and AC, combined with the glucose-recognition capability of GOx (Gupta & Tai, 2024; Mohamad Nor et al., 2022; Xiong et al., 2026).

Moreover, the incorporation of GOx into the Fe₃O₄/AC electrode is crucial for glucose biosensing, as GOx acts as the biorecognition element and catalyzes the oxidation of β-D-glucose into D-glucono-δ-lactone and H₂O₂ (Alatzoglou et al., 2023; Kesici-Meco & Unur-Yilmaz, 2024). Immobilizing GOx on the surface of a nanostructured electrode can provide an interface that facilitates the link between the enzymatic reaction and the electrochemical response (Mohamad Nor et al., 2022). This situation is like the previous study by Jędrzak et al. on Fe₃O₄-GOx, which demonstrated promising glucose detection performance, reporting a linear range of 0.2–24 mM, a sensitivity of 97.34 μA mM⁻¹ cm⁻², and an LOD of 6.1 μM (Jędrzak et al., 2022). However, the present study is limited to preliminary electrochemical characterization, focusing on the potential of the Fe₃O₄/AC-GOx nanocomposite as a biosensor candidate. Consequently, analytical performance parameters such as selectivity, reproducibility, repeatability, storage stability, reusability, and validation using real samples have not yet been experimentally evaluated. Selectivity of potential interferants, such as ascorbic acid, uric acid, paracetamol, and fructose, needs to be studied to ensure a specific response to glucose. Similarly, nafion's contribution to stability and reusability cannot be ascertained in the absence of comparative testing. In addition, because the tests were conducted in a buffer solution, the application of electrodes to blood or serum samples, including matrix effect evaluation, recovery, and accuracy still requires further research. Therefore, advanced testing needs to be carried out to ensure the analytical performance of such electrodes as biosensors.

CONCLUSION

A modified $\text{Fe}_3\text{O}_4/\text{AC-GOx}$ nanocomposite on the electrode as a candidate for a blood glucose detection biosensor has been successfully synthesized. The success of the nanocomposite formation was confirmed through the results of XRD, SEM, and FTIR analysis. These nanocomposites exhibit superparamagnetic properties that are able to contribute to better nanoparticle dispersibility, so that they have the potential to maintain surface area for enzyme immobilization. In addition, the existence of potential as a glucose biosensor was confirmed through the results of the initial electrochemical characterization of electrochemistry using CV, which showed an increase in current in the $\text{Fe}_3\text{O}_4/\text{AC-Gox}$ nanocomposite electrode compared to the SPCE electrode without any modification. This is due to the combination of $\text{Fe}_3\text{O}_4/\text{AC}$ nanocomposites that are able to produce an effective surface area and are supported by the addition of GOx enzymes to effectively catalyze reactions so as to strengthen electrochemical signals.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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