Improved Biosensing Efficiency of Glucose Immobilized on ZnO Nanoparticle-Decorated Multiwalled Carbon Nanotubes - An InVitro Study

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Background

Glucose biosensors play a crucial role in diabetes management. Multiwalled carbon nanotubes (MWCNTs) enhance biosensor performance due to their high conductivity and surface area. Zinc oxide (ZnO) nanoparticles improve enzyme immobilization, but their combined effect on glucose detection remains underexplored. This study aim for an Improved Biosensing Efficiency of Glucose Immobilized on ZnO Nanoparticle-Decorated Multiwalled Carbon Nanotubes

Methods

ZnO nanoparticles were synthesized via a hydrothermal method and integrated with MWCNTs. The composite was characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), and Field Emission Scanning Electron Microscopy (FESEM). Fluorescence intensity measurements were conducted for glucose detection.

Results

XRD confirmed high crystallinity, while TEM and FESEM revealed uniform ZnO distribution on MWCNTs. Fluorescence measurements showed a direct correlation between glucose concentration and biosensor response, with a peak at 100 mg/mL glucose.

Conclusion

MWCNTs decorated with ZnO nanoparticles exhibited enhanced glucose biosensing performance. This study highlights their potential for highly sensitive and selective glucose detection. Future research should focus on optimizing synthesis for improved stability and reproducibility.

Key Words: Biosensors, Glucose, Nanotubes, Nanomaterials,

Introduction

Biosensing plays an essential role in medical diagnostics, particularly in glucose monitoring, which is critical for managing diabetes. Diabetes is a global health concern that affects millions of people, requiring continuous monitoring of blood glucose levels to prevent complications such as kidney failure, cardiovascular diseases, and neuropathy [1]. Glucose biosensors help patients and healthcare professionals monitor glucose levels in real time, allowing for timely medical interventions and better disease management [2].

Glucose biosensors work based on enzymatic reactions, mainly facilitated by glucose oxidase (GOx). This enzyme catalyzes the oxidation of glucose, producing gluconolactone and hydrogen peroxide, which are then detected by the biosensor [3]. Several types of biosensors, such as electrochemical (EC) sensors, photo-electrochemical (PEC) sensors, and immunosensors, have been developed for glucose detection [4]. Among these, electrochemical biosensors are widely used because of their ability to detect biomolecules such as glucose, lactate, cholesterol, and DNA with high sensitivity and specificity [5].

Recent advancements in nanotechnology have improved biosensor performance by incorporating nanomaterials [6]. Multiwalled carbon nanotubes (MWCNTs) are widely used in biosensors because of their excellent electrical conductivity, high mechanical strength, and large surface area [7]. These properties make MWCNTs suitable for enzyme immobilization, enhancing biosensor efficiency [8]. However, despite these advantages, MWCNTs also have some drawbacks. Their poor dispersibility in aqueous solutions and limited ability to fine-tune electrical and thermal properties affect sensor performance [9]. Additionally, inhalation of carbon nanotubes may pose health risks, potentially affecting the lungs, liver, and kidneys [10].

To address these challenges, researchers have explored combining MWCNTs with other nanomaterials [11]. Zinc oxide (ZnO) nanoparticles have attracted significant attention due to their biocompatibility, high isoelectric point, and catalytic activity [12]. Zinc is an essential trace element in the human body, playing a crucial role in enzyme regulation, hematopoiesis, and cellular redox balance. The integration of ZnO nanoparticles with MWCNTs increases the surface area, enhances enzyme immobilization, and improves biosensor performance [12]. Despite these promising features, previous studies have not fully explored the combined effect of ZnO nanoparticles and MWCNTs on glucose biosensing efficiency. This study aims to improve the biosensing efficiency of glucose immobilized on MWCNTs decorated with ZnO nanoparticles. By combining these nanomaterials, we seek to enhance glucose detection by offering a more effective solution for diabetes management.

Materials and Methods

Zinc nitrate hexahydrate (Zn(NO3)2.6H2O), sodium hydroxide (NaOH), multi-walled carbon nanotubes (MWCNTs), ethanol, and acetone

Method

The hydrothermal technique was used to synthesize zinc oxide (ZnO) nanoparticles. First, 3 g of zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) was dissolved in 30 ml of distilled water. This solution was stirred continuously for 20 minutes to ensure complete dissolution. In a separate beaker, sodium hydroxide (NaOH) was dissolved in 30 ml of distilled water and stirred for another 20 minutes. The prepared NaOH solution was then slowly added drop by drop to the zinc nitrate solution. As a result, a white precipitate started forming, indicating the formation of ZnO nanoparticles. The mixture was then stirred for 2 hours at room temperature to allow the reaction to proceed fully.

After this, 100 mg of multi-walled carbon nanotubes (MWCNTs) was added to the solution. The solution was stirred for another 3 hours to ensure proper mixing of the MWCNTs with the ZnO nanoparticles. Next, the entire solution was transferred into a Teflon-lined stainless steel autoclave. The autoclave was placed in an oven and heated at 180°C for 12 hours to facilitate the hydrothermal reaction. Once the reaction was complete, the solution was allowed to cool naturally to room temperature. The resulting precipitate was then collected and washed several times using distilled water, ethanol, and acetone to remove any impurities or unreacted materials.

The purified precipitate was then dried in a hot air oven at 80°C for 24 hours. Finally, the dried sample was calcined at 500°C for 3 hours to improve its crystallinity and enhance its structural properties. This process resulted in the successful synthesis of ZnO-decorated MWCNTs, which were then used for further biosensing applications.

Results

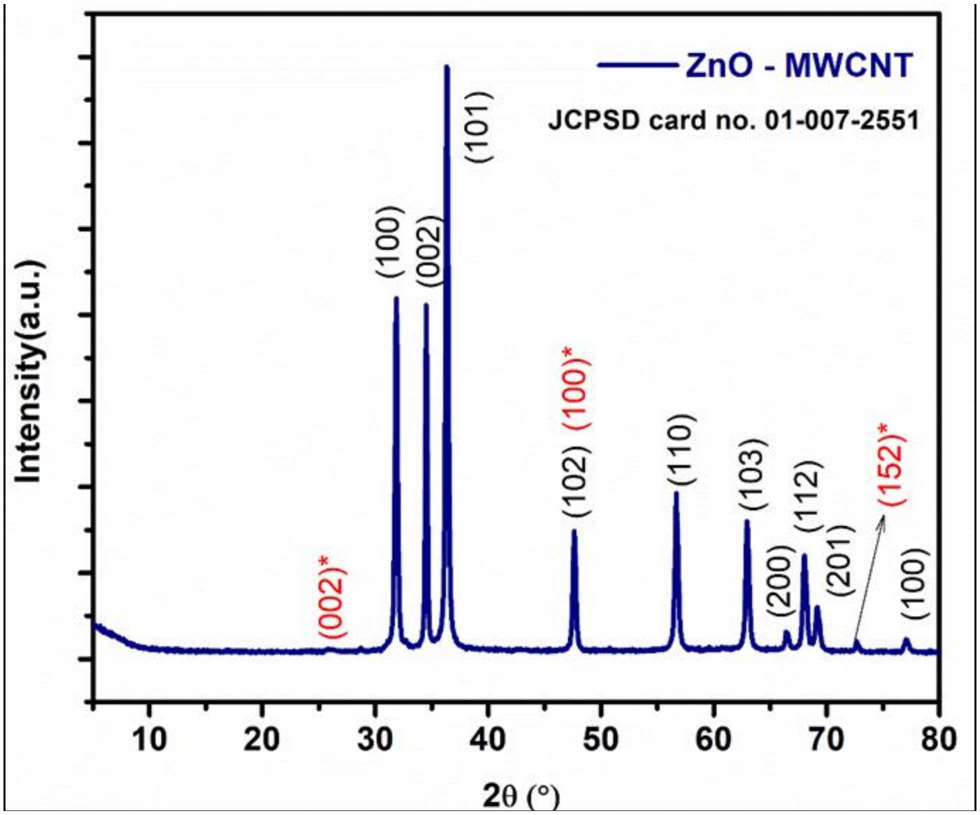

Figure 1:. XRD analysis of MWCNTs decorated with ZnO nanoparticles

The MWCNTs decorated with ZnO nanoparticles showed distinct diffraction peaks at 2θ values of 30.23°, 34.6°, and 37.5°. These peaks matched the (100), (002), and (101) crystal planes of monoclinic ZnO, aligning well with the standard JCPDS card number 14-0699. The XRD pattern of multi-walled carbon nanotubes (MWCNTs) is shown in Figure 1. A strong diffraction peakappeared at 2θ = 37°, along with additional peaks at 48°, 57°, 64°, and 68°. These peaks corresponded to the (102), (110), (103), and (112) crystal planes of standard graphite, indicating that the MWCNTs had a high degree of graphitization.

The interlayer spacing in the MWCNT structure was observed at 2θ = 26°, which corresponds to the (002) plane of graphitic carbon. This peak confirmed that the graphene layers in the MWCNTs were properly aligned. Additionally, no impurity peaks were detected for ZnO, indicating that the prepared material was pure and did not contain any unwanted secondary phases. By analyzing the distinctive peaks of both MWCNTs and ZnO, we understood their interaction and integration. Examining their intensities and shifts was important for designing the composite material for specific applications. These findings are presented in Figure 1.

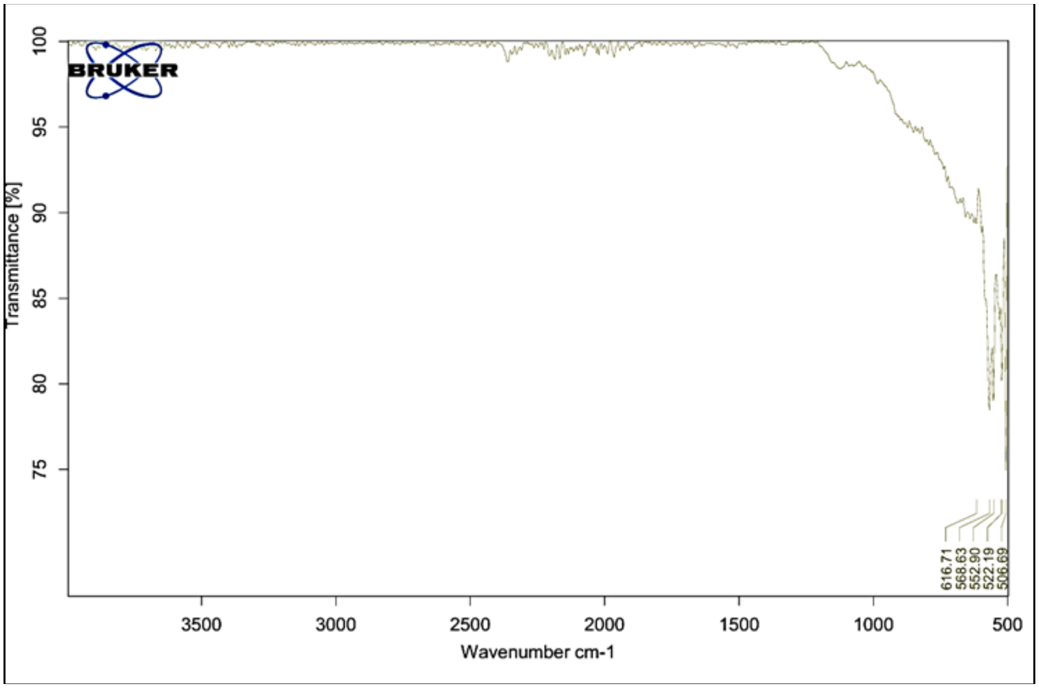

Figure 2: FTIR analysis of ZnO-MWCNT

The MWCNTs showed characteristic carbon bond peaks. The graphitic structure was confirmed by the C=C stretching, which appeared at around 1587 cm^{-1}. The presence of bismuth oxide (Bi_2O_3) was identified through its Bi-O stretching vibrations, which were observed in the 450-600 cm^{-1} range. The O-Bi-O bond bending was found at approximately 1391 cm^{-1}.

If the composite spectra showed changes in the MWCNT and Bi_2O_3 signature peaks, it suggested that the two materials were interacting. A shift in the C=C peak indicated that MWCNTs and Bi_2O_3 had some electronic contact or charge transfer. Additionally, changes in intensity or the appearance of new peaks could mean the formation of new bonds or strong interactions between their surfaces (Figure 2).

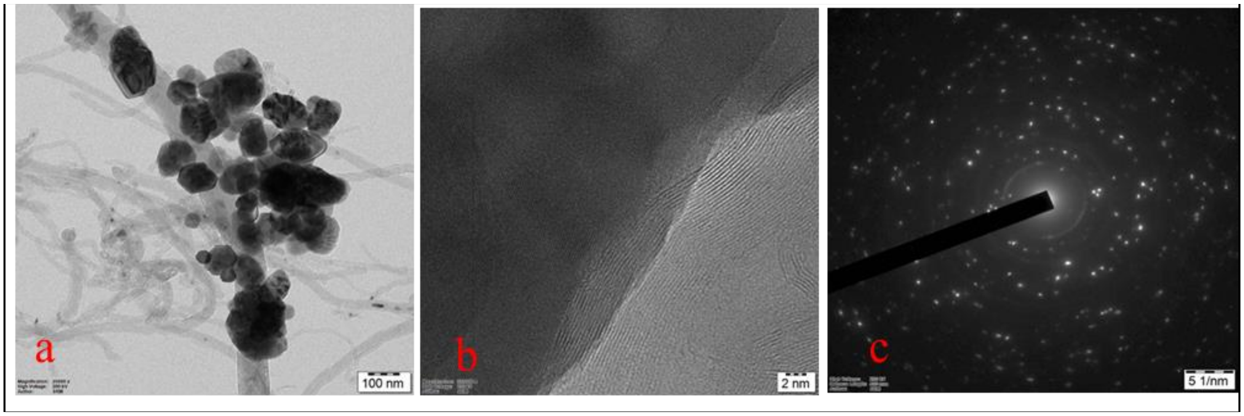

Figure 3: TEM images of MWCNTs decorated with ZnO nanoparticles (a)100 nm (b) HRTEM image at 2 nm (c) SAED pattern at 5 1/nm.

The TEM images showed that ZnO nanoparticles were evenly spread across the surfaces of MWCNTs. The ZnO particles appeared to have a strong interaction with the carbon nanotubes, as they were often seen surrounding or sticking to them (Figure 3a). In the TEM images, MWCNTs appeared as long, tube-like structures, revealing multiple graphene layers (Figure 3b). The size of these structures was measured in nanometers (nm). In most cases, the ZnO sheets had a layered and plate-like morphology, which was different from the cylindrical shape of the MWCNTs. The TEM images displayed the MWCNTs as thin, flat structures with distinct borders. The interaction between ZnO sheets and MWCNTs was essential for the functionality of the composite. The TEM images also helped visualize how the ZnO sheets attached to the MWCNTs, possibly revealing chemical bonds or van der Waals interactions.

The good crystallinity of the ZnO sheets, observed through clear fringes, was important for properties such as catalytic activity. The TEM analysis also helped identify structural defects, such as dislocations or vacancies, in the ZnO nanoparticles or MWCNTs, which could affect the composite's properties. Some ZnO defects could enhance catalytic sites, while MWCNT defects might influence electrical conductivity.

Selected Area Electron Diffraction (SAED), a useful TEM technique, provided information about the crystallographic orientation and structure of the material. The SAED patterns of the composite showed different features of both components when α-Bi_2O_3 sheets were examined on MWCNTs (Figure 3c). The SAED pattern of the composite displayed overlapping diffraction spots from ZnO and MWCNTs. The sharp ZnO spots in the SAED pattern confirmed that the ZnO sheets had high crystallinity. In contrast, diffuse rings or dots could indicate the presence of amorphous regions or structural defects. Additionally, interactions at the interface of the composite could cause strain, which appeared as slight shifts or distortions in the diffraction spots.

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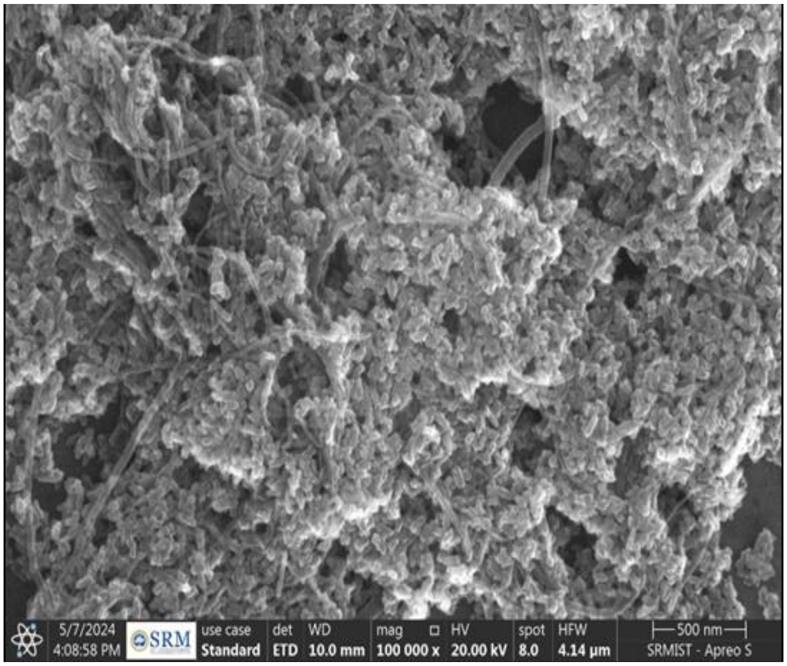

Figure 4: FESEM image of MWCNTs decorated with ZnO Nanoparticles at 500nm

The FESEM analysis provided important information about the morphology and structural interactions of the ZnO/MWCNT composites. The images showed that the ZnO sheets were evenly distributed and firmly attached to the MWCNTs, demonstrating the composite's potential for advanced applications. Understanding these morphological features was important for adjusting the material's properties to meet specific performance needs. The formation of ZnO sheets on the MWCNT surface was clearly visible (Figure 4).

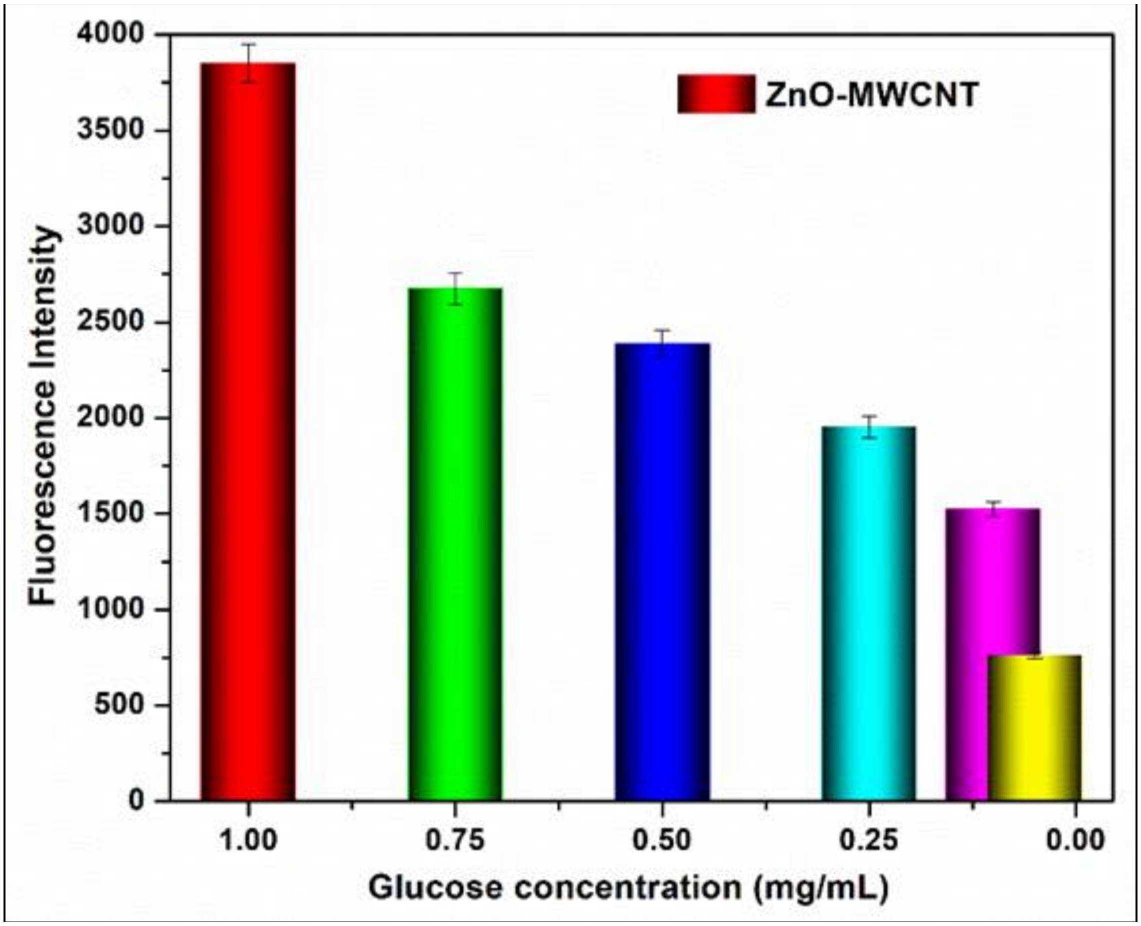

Figure 5: BSA-Biosensor sensitivity of MWCNTs decorated with ZnO Nanoparticles

The ability of ZnO-MWCNT sensors to detect glucose was carefully studied. Fluorescence intensity measurementswere taken at different glucose concentrations to evaluate the sensor's performance. The highest detection was observed at a glucose concentration of 100 mg/mL, where the fluorescence intensity reached 3800 units. The results also showed that as the glucose concentration increased, the fluorescence intensity increased consistently. This indicated a direct relationship between glucose levels and fluorescence response (Figure 5).

Discussion

The study of MWCNTs decorated with ZnO nanoparticles provided valuable information about their structural and functional properties, especially for glucose sensing applications. The X-ray diffraction (XRD) patterns showed distinct peaks at 2θ values of 30.23°, 34.6°, and 37.5°, which corresponded to the (100), (002), and (101) crystal planes of monoclinic ZnO. These values matched the JCPDS card number 14-0699 standard, confirming the high purity and crystallinity of the synthesized ZnO nanoparticles. This finding was consistent with previous research by Noman et al., who also reported similar diffraction patterns for ZnO [13]. The absence of impurity peaks indicated the successful synthesis of pure ZnO nanoparticles, without any unwanted secondary phases, which was essential for maintaining the composite's properties.

The XRD pattern of MWCNTs showed a strong peak at 2θ = 37°, along with additional peaks at 48°, 57°, 64°, and 68°, corresponding to the (102), (110), (103), and (112) diffraction planes of standard graphite. This result confirmed a high degree of graphitization, which was important for improving the electrical conductivity of MWCNTs. Similar patterns were also reported by Liu et al., who emphasized that graphitization played a key role in enhancing the electrochemical properties of MWCNT-based composites [14]. Additionally, the interlayer spacing at 2θ = 26° matched the (002) plane of graphitic carbon, confirming the proper alignment of graphene layers within the MWCNTs. This structural feature was crucial for ensuring efficient electron transport in the composite.

The study also examined the interactions between ZnO and MWCNTs using different analytical techniques. The presence of C=C stretching at around 1587 cm^{-1}, which is characteristic of graphitic structures, along with Bi-O stretching vibrations in the 450-600 cm^{-1} range, indicated possible interactions between the two components. These interactions suggested electronic contact or charge transfer between ZnO and MWCNTs, as shown by the shift in the C=C peak. This observation was similar to the findings of Wang et al., who reported spectroscopic shifts in ZnO/MWCNT composites, highlighting the importance of these interactions in modifying the electronic properties of the material [15].

The Transmission Electron Microscopy (TEM) images showed that ZnO nanoparticles were evenly spread across the MWCNT surfaces, with the nanoparticles closely attached to the nanotubes. This uniform distribution and strong adhesion were important for ensuring consistent performance, especially in applications like glucose sensing. The TEM images also displayed long, tube-like structures and distinct graphene layers, similar to the findings of Chen et al., who highlighted the role of these structures in improving the sensitivity and selectivity of MWCNT-based sensors [16]. Additionally, defects in ZnO nanoparticles, which could act as catalytic sites, and MWCNT flaws, which might influence electrical conductivity, further emphasized how structural features impacted the composite’s performance.

The Selected Area Electron Diffraction (SAED) patterns confirmed the high crystallinity of the ZnO nanoparticles, as seen from sharp diffraction spots, which indicated a well-ordered structure [17]. This high crystallinity was essential for applications that required stable catalytic activity, ensuring the reliability of catalytic sites. Similar results were reported by Xu et al., who found that high ZnO crystallinity in MWCNT composites played a key role in achieving better electrochemical properties [18]. The Field Emission Scanning Electron Microscopy (FESEM) analysis provided more details about the morphology of the ZnO/MWCNT composites. The images showed that the ZnO sheets were evenly distributed and firmly attachedto the MWCNTs. This feature was important for advanced applications like biosensing. The FESEM results were similar to the findings of Kumar et al., who demonstrated that uniform nanoparticle distribution was key to improving the performance of nanocomposites in sensor applications [19]. In the future, the use of nanoparticles is expected to grow due to cost-effective biomodification techniques. Because of their unique properties, nanoparticles are widely used in various scientific fields, including biomedicine, materials science, engineering, electronics, and food science [20].

The process of synthesizing ZnO nanoparticles and decorating MWCNTs with these nanoparticles was complex and required precise control over reaction conditions. Achieving consistent synthesis of MWCNTs decorated with ZnO nanoparticles was challenging. Even small variations in the synthesis process could lead to differences in nanoparticle size, distribution, and interaction between ZnO and MWCNTs, which could affect the biosensor’s performance. Future research could focus on surface modifications or functionalization techniques to improve synthesis consistencyand enhance sensor performance.

Conclusion

The study findings are consistent with previous literature, demonstrating the potential of MWCNTs decorated with ZnO nanoparticles for advanced applications such as glucose sensing. The structural and morphological characteristics, along with the electronic interactions between the components, play crucial roles in determining the composite's performance. Future research should focus on optimizing these properties to further enhance the sensitivity, selectivity, and stability of ZnO/MWCNT-based sensors.

Acknowledgement
The authors are thankful to Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, and Saveetha University for providing a platform to conduct the study.

Conflict of interest
All the authors declare that there is no conflict of interest in the present study.

Source of funding
The present project is funded by
- Saveetha Institute of Medical and Technical Sciences,
- Saveetha Dental College and Hospitals and Saveetha University
- Saveetha University

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