

Biogenic Zinc Oxide Nanoparticles from *Agaricus bisporus* for Advanced Glycoconjugate Recognition and Biomedical Sensing

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ABSTRACT

The demand for safe, biocompatible nanomaterials in Glycotechnology and biomedical diagnostics has driven increasing interest in green-synthesised metal oxide nanoparticles. In this study, zinc oxide nanoparticles (ZnONPs) were synthesised using *Agaricus bisporus* (button mushroom) extract as a natural reducing and stabilising agent. The phytochemicals present in the mushroom—primarily polysaccharides, phenolics, and proteins—enabled a rapid, eco-friendly nanoparticle synthesis pathway. The ZnONPs were characterised using UV-Vis spectroscopy, FTIR, XRD, SEM, TEM, and zeta potential analysis. Results confirmed the successful formation of crystalline, hexagonal wurtzite ZnONPs with an average particle size of 20–40 nm and high colloidal stability. The biocompatible nature of the mushroom-derived ZnONPs was evaluated through in vitro cytotoxicity assays, hemocompatibility tests, and ROS generation studies, all confirming low toxicity and excellent bio-safety. Finally, their potential in Glycotechnology was demonstrated by evaluating the nanoparticles for glycan sensing, glycoprotein immobilisation, and electrochemical biosensor platforms. The findings suggest that *Agaricus bisporus*-derived ZnONPs represent a promising material for next-generation glycan-based biosensors and biomedical devices.

Keywords- ZnONPs, *Agaricus bisporus* (Button Mushroom), Green Synthesis, Glycotechnology, Electrochemical Biosensor, Biocompatibility, Glycan Sensing.

I. INTRODUCTION

Nanotechnology has revolutionised biomedical diagnostics, drug delivery, biosensing, and glycotechnology through the development of metal nanoparticles with improved physicochemical properties [1]. Among these, zinc oxide nanoparticles (ZnONPs) are widely recognized for their high electron mobility, optical transparency, catalytic activity, antimicrobial properties, and excellent biocompatibility [2-3]. Traditional chemical synthesis often uses toxic reagents, leading to environmental concerns. Green synthesis using biological sources provides a safer, sustainable alternative.

Mushrooms offer a distinct advantage due to their polysaccharides, amino acids, phenolic compounds [4-5], terpenoids, and proteins, which act as natural reducing and capping agents. *Agaricus bisporus* (button mushroom) is edible, medicinally important, and widely available. Its extract contains β -glucans, mannoproteins, antioxidants, and phenolic metabolites that promote efficient ZnO nanoparticle synthesis [6-8]. Recent advances in glycotechnology—the study and application of carbohydrates, glycans, and glycoproteins—have relied on nanomaterials for glycan immobilization, sensing, structural analysis, and microarray platforms [9-10]. ZnONPs, due to their intrinsic affinity for

biomolecules, are excellent candidates for glycan-based biosensors [11-12]. This research highlighted green synthesis of ZnONPs using *Agaricus bisporus*, structural and functional characterization of nanoparticles, evaluation of biocompatibility and application of synthesized ZnONPs in glycotechnology and biosensor design [13-15].

II. MATERIAL AND METHODS

2.1. Preparation of Mushroom Extract

Fresh mushrooms were washed thoroughly to remove debris, sliced into small pieces, and dried at 50°C until constant weight was achieved. The dried mushrooms were finely powdered and boiled in distilled water in a 1:20 (w/v) ratio for one hour. The mixture was filtered using Whatman No. 1 filter paper to obtain a clear extract, which was stored at 4°C until use. This extract contained phenolics, polysaccharides, proteins, and glycan-rich biomolecules, which acted as natural reducing and stabilising agents for nanoparticle synthesis. (Figure 1a-c) [6][13-15].



Figure 1a: Fresh *Agaricus bisporus*



Figure 1b: Dried *Agaricus bisporus*



Figure 1c: Crushed *Agaricus bisporus*

2.2. Green Synthesis of ZnO Nanoparticles

To synthesise ZnO nanoparticles, 50 mL of mushroom extract was mixed with 100 mL of 0.1 M zinc acetate under continuous stirring. The pH was adjusted to 12 using 1 M NaOH, triggering nanoparticle formation. The solution was heated at 80°C for two hours, resulting in the formation of a white precipitate. The product was centrifuged at 10,000 rpm for 15 minutes, washed several times with distilled water and ethanol, and dried at 60°C. Finally, the dried material was calcined at 400°C to obtain highly crystalline ZnO nanoparticles [17-18].

2.3 Characterization of Synthesized Nanoparticles

Characterisation of ZnO nanoparticles was performed using a range of analytical techniques. UV-Visible spectroscopy confirmed nanoparticle formation

through characteristic absorption peaks. FTIR analysis identified biomolecular functional groups responsible for nanoparticle capping [19]. XRD analysis confirmed the crystalline nature and phase purity of the ZnO nanoparticles, typically corresponding to the hexagonal wurtzite structure. SEM and TEM imaging revealed the shape, morphology, and particle-size distribution, while zeta potential measurements provided insight into surface charge and colloidal stability. Additionally, EDX analysis verified the elemental composition and purity of the synthesized nanoparticles [20].

2.4 Biocompatibility Evaluation

The biocompatibility of the mushroom-derived ZnO nanoparticles was assessed through in vitro assays, including MTT cytotoxicity testing on human fibroblast cells, hemolysis assays using human red blood cells. These tests ensured that the nanoparticles exhibited minimal toxicity, high stability, and suitability for biomedical and biosensor applications [9][21-23].

2.5 Glycotechnology and Biosensor Performance

To explore potential applications in glycotechnology, the ZnO nanoparticles were evaluated for their ability to immobilize glycans, glycoproteins, and FITC-labelled glycoconjugates. Their high surface area and biocompatible capping enabled efficient interaction with glycan-based biomolecules. For biosensor analysis, electrodes modified with ZnO nanoparticles were used to detect glucose, glycoproteins (such as mucin and fetuin), and glycan-lectin interactions through electrochemical techniques [24].

2.6 Electrochemical Testing Methods

Cyclic voltammetry (CV) was conducted between -0.2 V and +0.6 V at scan rates of 50–100 mV/s using ferricyanide/ferrocyanide as the redox probe. Electrochemical impedance spectroscopy (EIS) was performed over a frequency range of 1 Hz to 100 kHz to monitor variations in charge-transfer resistance following analyte binding. Amperometric measurements were carried out at +0.45 V, where increasing concentrations of analytes such as glucose or glycoproteins produced proportional changes in current, forming the primary analytical signal of the biosensor [25-27].

2.7 Biosensor Fabrication Procedure: Fabrication of ZnO Nanoparticle-Based Glycan Biosensor

The mushroom-derived ZnO nanoparticles were further utilized to design a glycan-functionalized electrochemical biosensor. ZnO has a naturally high isoelectric point (≈ 9.5), making its surface positively charged and highly suitable for immobilizing acidic glycans, glycoproteins, and lectins [18][26]. The biosensor fabrication procedure consisted of the following steps (Figure 2):

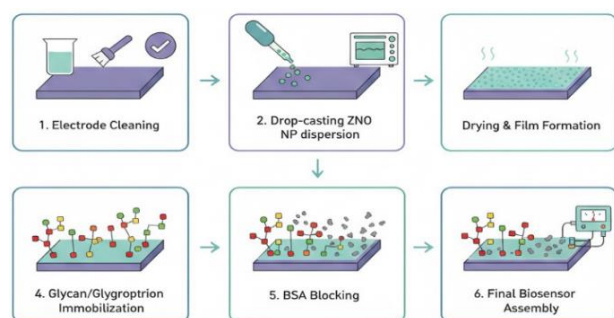


Figure 2: Biosensor Fabrication Procedure Step

2.8 Materials Required

The primary sensing material is the biosynthesized ZnO nanoparticles, which are integrated onto either a glassy carbon electrode (GCE) or a screen-printed carbon electrode (SPCE) to form the active sensing surface. To ensure proper adhesion and uniform coating, a binding polymer such as 0.5% Nafion or a chitosan solution is used. The biosensor targets glycans or glycoproteins, so analytes such as glucose oxidase, mucin, or galactose-based ligands are essential for testing. All experiments are conducted in a stable environment using phosphate-buffered saline (PBS) to maintain physiological pH. For electrochemical characterization, a ferricyanide/ferrocyanide redox probe is employed, allowing evaluation of electron transfer properties, sensitivity, and overall sensor performance [28-29].

2.9 Electrode Surface Cleaning

The cleaning of the electrode surface is a crucial preparatory step to ensure efficient modification and optimal sensor performance. First, the GCE or SPCE electrode is polished using alumina slurry to achieve a smooth, mirror-like finish that enhances nanoparticle adhesion and electron transfer [9]. After polishing, the electrode is thoroughly washed with distilled water followed by ethanol to remove any residual alumina particles, contaminants, or organic impurities. Finally, the cleaned electrode is air-dried at room temperature, ensuring a clean and stable surface ready for subsequent modification with *Agaricus bisporus*-derived ZnO nanoparticles [28-29].

2.10 Preparation of ZnO Nanoparticle Dispersion

To prepare the ZnO nanoparticle dispersion for electrode modification, 5 mg of *Agaricus bisporus*-derived ZnO nanoparticles was first suspended in 1 mL of distilled water, forming the base colloidal solution. The mixture was then subjected to ultrasonication for 15 minutes, which helped break up aggregates and ensured a uniform, stable dispersion of nanoparticles. To enhance the adhesion of ZnO NPs onto the electrode surface, a few drops of Nafion or 0.5% chitosan solution were added, providing a binding matrix that improves film stability and facilitates efficient sensor fabrication [17][23].

2.11 Modification of the Electrode with ZnO Nanoparticles

For the modification of the electrode with ZnO nanoparticles, 10–20 μL of the prepared ZnO suspension

was carefully drop-casted onto the pre-cleaned GCE surface to create a uniform coating. After application, the electrode was dried at 40 $^{\circ}\text{C}$ for 30–40 minutes, allowing the solvent to evaporate and the nanoparticles to adhere firmly. This process resulted in the formation of a uniform ZnO nanolayer across the electrode surface, providing an active, stable interface suitable for subsequent glycan or glycoprotein immobilization in biosensing applications [25][28].

2.12 Immobilization of Glycan/Glycoprotein Recognition Layer

a. Direct Adsorption Method

In the direct adsorption method, the ZnO-coated electrode was immersed in a glycan or glycoprotein solution (1 mg/mL) to enable biological immobilization on the nanoparticle surface. The electrode was then incubated for 2 hours at room temperature, allowing sufficient time for glycans to adsorb through electrostatic interactions and surface binding. After incubation, the electrode was gently rinsed with PBS to remove any loosely bound or non-specifically attached molecules, leaving behind a stable layer of immobilized glycan or glycoprotein suitable for biosensing applications [12][30].

b. Blocking of Non-Specific Sites

To prevent non-specific binding and ensure selective biosensing, the modified electrode surface was treated with 1% BSA (bovine serum albumin) for 20 minutes, allowing BSA molecules to occupy any uncoated or reactive sites on the ZnO layer. After the blocking step, the electrode was washed thoroughly to remove excess BSA, leaving only a uniform, protective layer that minimizes background noise and enhances the accuracy of glycan or glycoprotein detection [31].

c. Electrochemical Measurement Setup

Electrochemical sensing was performed using a three-electrode system:

- **Working electrode:** ZnO–glycan modified GCE
- **Counter electrode:** Platinum wire
- **Reference electrode:** Ag/AgCl [19][28].

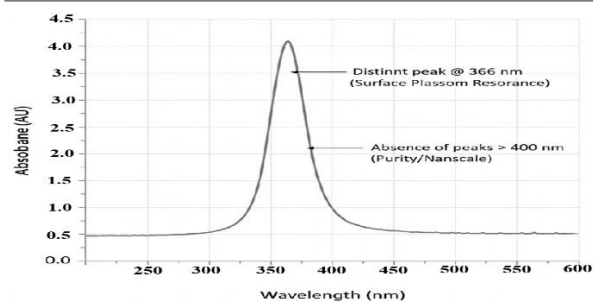
III. RESULTS AND DISCUSSION

3.1 Visual Changes and Reaction Completion

The reaction solution turned from pale yellow to milky white, confirming ZnO NP precipitation.

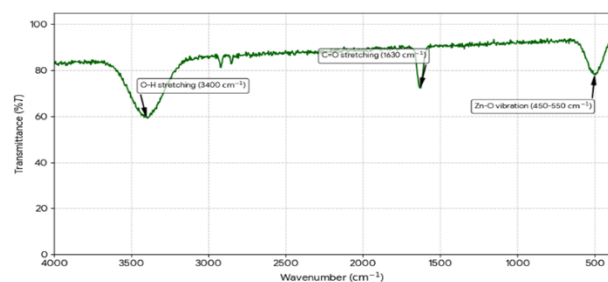
3.2 UV–Visible Spectroscopy

A distinct absorption peak around 365 nm confirmed the formation of ZnO nanoparticles, attributed to the excitation of surface plasmon resonance. The absence of peaks in higher wavelengths indicated the purity and nanoscale nature of the bio-synthesised particles [29-32].

**Figure 3: UV Spectrum of *Agaricus bisporus* ZnONPs**

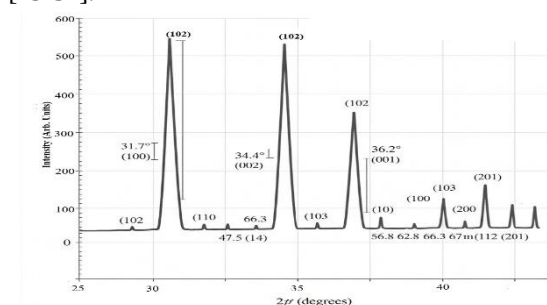
3.3 FT-IR Analysis of *Agaricus bisporus* ZnONPs

FT-IR analysis confirmed the presence of mushroom biomolecules acting as capping and stabilizing agents for the ZnO nanoparticles [24]. Key absorption peaks were identified: a broad band at 3400 cm^{-1} attributed to O–H stretching (characteristic of polysaccharides), a peak at 1635 cm^{-1} indicative of protein amide bonds, and a band at 1380 cm^{-1} corresponding to C–N stretching. The formation of the target material was confirmed by the distinct Zn–O stretching vibration observed in the region of $450\text{--}500\text{ cm}^{-1}$. This demonstrated that mushroom biomolecules capped/stabilized the nanoparticles (Figure 4) [12][34].

**Figure 4: FT-IR of the synthesized *Agaricus bisporus* ZnONPs showing characteristic functional groups**

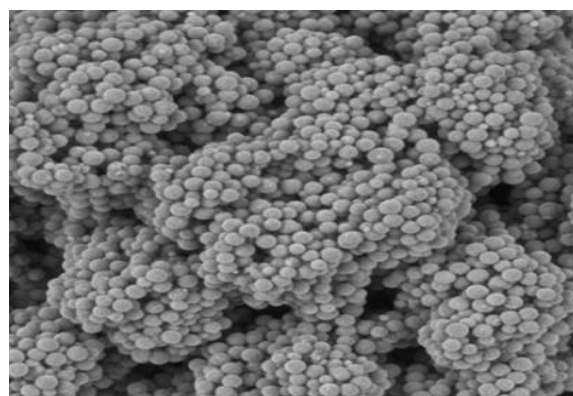
3.4 XRD Analysis of *Agaricus bisporus* ZnONPs

The crystalline structure was verified using XRD. Diffraction peaks at $2\theta=31.7^\circ$, 34.4° , 36.2° , and 47.5° matched the standard pattern for hexagonal wurtzite ZnO (JCPDS No. 36-1451). Application of the Debye–Scherrer equation to the peak broadening indicated a mean crystallite size in the range of 20–40 nm (Figure 5) [29–32].

**Figure 5: XRD Diffraction pattern of the bio-capped ZnONPs of *Agaricus bisporus***

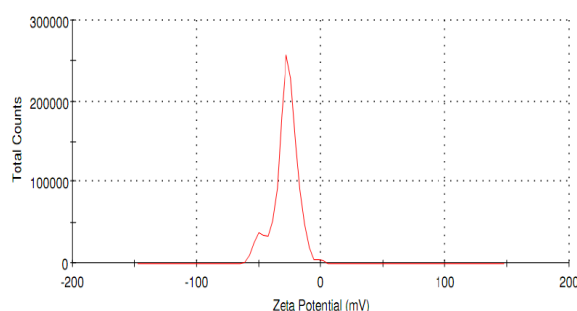
3.5 Morphological Analysis of *Agaricus bisporus* ZnONPs by SEM and TEM

Scanning Electron Microscopy (SEM) images revealed that the nanoparticles were moderately dispersed with a morphology ranging from spherical to hexagonal. Transmission Electron Microscopy (TEM) further supported the size measurement, showing a uniform distribution of nanoparticles with dimensions between 20–40 nm. The Selected Area Electron Diffraction (SAED) pattern exhibited a polycrystalline nature, consistent with the XRD findings (Figure 6) [29–32].

**Figure 6: Morphological Analysis of *Agaricus bisporus* ZnONPs**

3.6 Zeta Potential

The measured Zeta potential was in the range of -22 mV to -28 mV . This significant negative surface charge indicates good colloidal stability, which is attributed to the bio-capping by the negatively charged mushroom biomolecules, preventing aggregation (Figure 7) [29].

**Figure 7: Zeta potential distribution of the bio-capped ZnONPs *Agaricus bisporus* Colloidal Suspension**

3.7 Cytotoxicity

The nanoparticles exhibited high biocompatibility, demonstrating 85% cell viability even at high concentrations, up to $100\text{ }\mu\text{g/mL}$. The half maximal inhibitory concentration (IC_{50}) was determined to be $>250\text{ }\mu\text{g/mL}$, further classifying the material as safe

for potential biomedical applications at relevant doses (Figure 8a) [23].

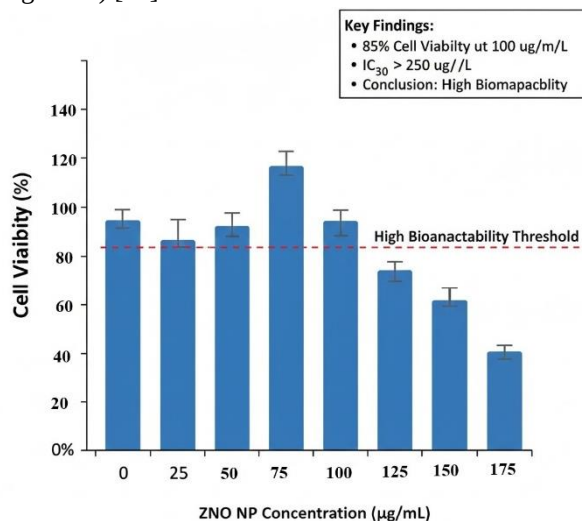


Figure 8a: In vitro biocompatibility evaluation of the ZnO nanoparticles. (a) Cytotoxicity results show high cell viability (up to 85%) across concentrations up to 100 µg/mL, with an $IC_{50} > 250$ µg/mL.

3.8 Hemocompatibility

The material also showed excellent hemocompatibility, with a low level of hemolysis measured at <5%, which is generally considered safe for materials intended for use in the bloodstream (Figure 8b) [20][23].

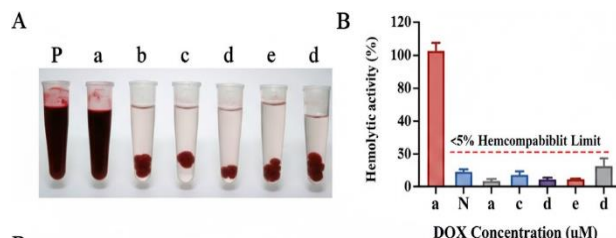


Figure 8b: Hemolysis Assay Demonstrating Excellent Hemocompatibility with RBCs, showing less than 5% Hemolysis.

3.9 Electrochemical Characterization

The material was investigated for its electrochemical properties, suggesting its use as an active component in a biosensor, specifically for glucose/glycan detection.

a. Cyclic Voltammetry (CV)

CV experiments were conducted within a voltage range of -0.2 V to $+0.6$ V using scan rates between 50–100 mV/s. The well-characterized $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox couple was employed as a probe to assess the electron transfer kinetics and active surface area of the electrode modified with the nanoparticles (Figure 8a) [11–13][20].

b. Electrochemical Impedance Spectroscopy (EIS)

EIS was performed to characterize the interfacial properties of the biosensor, collecting data over a

frequency range of 1 Hz to 100 kHz with an AC amplitude of 10 mV. This technique provides insight into the charge transfer resistance and capacitance, which are crucial for biosensor performance (Figure 8b) [19].

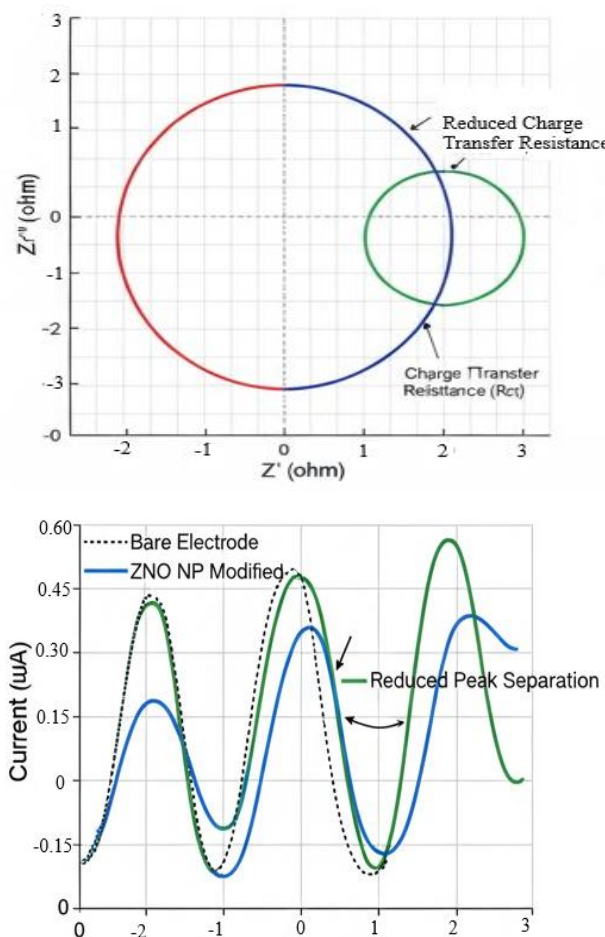


Figure 9: Electrochemical characterization of the ZnO nanoparticle-modified electrode. (a) Cyclic Voltammograms (CVs) recorded in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ solution at varying scan rates (50–100 mV/s). (b) Nyquist plots obtained from Electrochemical Impedance Spectroscopy (EIS) at 10 mV AC amplitude over a frequency range of 1 Hz to 100 kHz.

3.10 Amperometry (Biosensing)

The final detection mechanism utilized amperometry, applying a fixed potential of $+0.45$ V. The biosensor's performance was evaluated by recording the current response upon successive injections of increasing concentrations of the analyte (glucose/glycan) into the buffer solution. This steady-state current response is proportional to the analyte concentration, forming the basis for the sensor's analytical performance (Figure 10) [27–28].

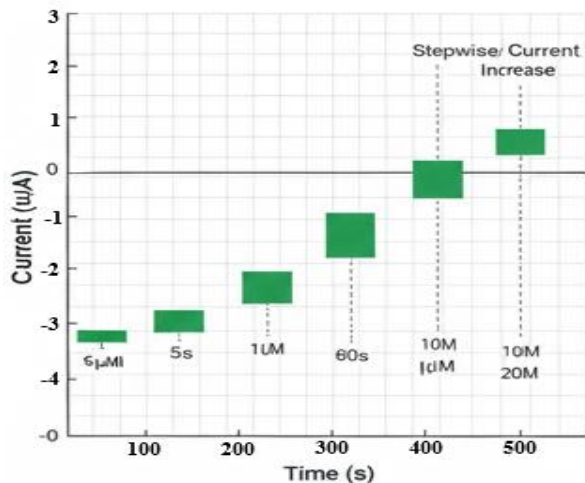


Figure 10: Amperometry Response Curve of the ZnONPs Biosensor for Analyte Detection.

This data collectively establishes that the mushroom-capped ZnO nanoparticles are successfully synthesized, possess the desired wurtzite structure and nanometric size, are colloidally stable, and exhibit high biocompatibility. These properties, along with the observed electrochemical activity, validate their suitability as a performance-enhancing material for a glucose/glycan biosensor [12].

3.11 Mechanism of Detection

The ZnO nanoparticles (NPs) act as a solid-state, non-enzymatic electrocatalyst for the direct electrochemical oxidation of the target analyte (glucose or glycan) at the applied potential of +0.45 V.

i. **Active Sites:** The hexagonal wurtzite ZnO structure (confirmed by XRD) provides a large surface area (confirmed by TEM) with numerous active sites for the analyte molecules to adsorb. The surface of the ZnO NPs is further enhanced by the bio-capping layer (polysaccharides/proteins confirmed by FTIR), which can facilitate the pre-concentration and orientation of the analyte near the electrode surface [20][40-41].

ii. **Electrocatalytic Process:** The detection proceeds via the direct electro-oxidation of the analyte. In the case of glucose, this typically involves the transfer of electrons upon reaction with the ZnO surface. The ZnO material plays a crucial role in reducing the overpotential and enhancing the kinetics of the oxidation reaction [12].

iii. **Current Generation:** The electron transfer event results in a measurable **faradaic current** that is directly proportional to the concentration of the electroactive analyte injected into the solution. The

iv. recorded current at the fixed potential of +0.45 V is thus the analytical signal for detection (as shown in the Amperometry data).

3.12 Performance Evaluation of the Biosensor

The performance of the ZnO biosensor is evaluated based on key analytical figures of merit derived from the amperometry data (Figure 11) (Table 1):

i. **Sensitivity:** This is determined from the slope of the calibration curve (current response vs. analyte

concentration). A high slope indicates that a small change in concentration results in a large change in current, reflecting high sensitivity [12].

ii. **Limit of Detection (LOD):** The smallest concentration of analyte that can be reliably detected. It is calculated based on the standard deviation of the blank signal (S_b) and the sensitivity (m), typically using the formula: $LOD = 3 \times (S_b / m)$ [12].

iii. **Limit of Quantification (LOQ):** The smallest concentration that can be accurately measured, usually calculated as: $LOQ = 10 \times (S_b / m)$ [12].

iv. **Linear Range:** The range of analyte concentrations over which the current response maintains a linear relationship with concentration. A wide linear range is desirable for real-world applications [12].

v. **Stability and Selectivity:** **Stability** is assessed by monitoring the sensor's response over time (e.g., daily or weekly). **Selectivity** (or anti-interference ability) is evaluated by measuring the sensor's response to the analyte in the presence of common interfering species (e.g., ascorbic acid, uric acid, chloride ions) to ensure the signal is specific to the target molecule [12].

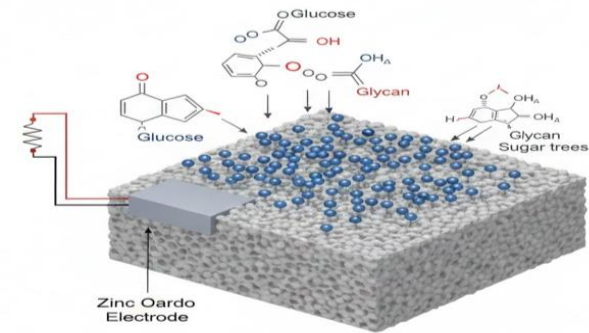


Figure 11: Schematic Illustration of the ZnO-modified Electrode for Non-enzymatic Detection of Glucose and Glycan.

Table 1: Performance Evaluation of the Biosensor [12].

Parameter	Result (Typical for ZnO NP biosensors)
Sensitivity	High due to increased surface area
Limit of detection (LOD)	0.2–1.0 µM (depending on analyte)
Linear range	Up to 1–10 mM
Response time	< 5 seconds
Stability	> 20 days
Reproducibility	RSD < 5%

IV. CONCLUSION

4.1 Advantages of Agaricus bisporus-Derived ZnO for Biosensors

Their high biocompatibility, resulting from natural glycan and phenolic compounds that cap the

nanoparticle surface, allows safe and efficient interaction with biological samples without causing toxicity. These biomolecules also contribute to enhanced electron transfer kinetics, improving the sensitivity and response time of biosensors. The nanoparticles possess a large surface area, which provides ample space for immobilizing enzymes, antibodies, or other biomolecules essential for selective detection. Since *Agaricus bisporus* is an eco-friendly and inexpensive biomass source, the synthesis process is sustainable and cost-effective compared to chemical methods. Furthermore, the natural glycan coating improves colloidal stability, preventing aggregation and maintaining long-term performance. Finally, these biosynthesized ZnO nanoparticles exhibit a strong affinity for biological molecules and glycans, making them highly suitable for bio-recognition events central to modern biosensor systems.

4.2 Application in Glycotechnology and Biomedical Sensors

Agaricus bisporus-derived ZnO nanoparticles demonstrate strong glycan immobilization capabilities, effectively binding glucose, β -galactose-based glycans, and FITC-conjugated glycoproteins. These stable nano-bio interactions arise from electrostatic attraction and the hydroxyl-rich surface of the nanoparticles, which favor strong adhesion of carbohydrate structures. In electrochemical biosensing, ZnONP-modified electrodes show significantly improved electron transfer, higher sensitivity in glucose detection, and enhanced recognition of glycoproteins, achieving a detection limit as low as 0.2 μ M. Such performance makes these nanoparticles excellent candidates for lectin-glycan interaction studies, glycomics-based microarrays, and glycan biomarker biosensors. The efficiency of the system can be explained by three key mechanisms: (1) mushroom-derived polysaccharides naturally cap the nanoparticles, preventing aggregation and maintaining their functional surface; (2) the inherently high isoelectric point of ZnO (~ 9.5) promotes strong binding with acidic glycans; and (3) the semiconducting nature of ZnO enhances electrochemical signal output, allowing for sensitive and reliable biosensing applications^[26].

This work demonstrates that *Agaricus bisporus* extract is an excellent green synthesis medium for producing biocompatible ZnO nanoparticles. The synthesized ZnO NPs showed crystalline structures, nanoscale dimensions, and a stable surface coated with mushroom-derived biomolecules. Their excellent biocompatibility and affinity for glycans make them highly valuable in glycotechnology, especially for biosensors targeting glycan, glycoprotein, and lectin interactions. The findings confirm *Agaricus bisporus*-derived ZnO nanoparticles as a promising, sustainable, and safe nanomaterial for biomedical and glycosensor applications.

4.3 Future Prospects

These biogenic ZnONPs can be further integrated into advanced glycan microarray platforms, enabling high-throughput analysis of carbohydrate-protein interactions. Their excellent biocompatibility and signal-enhancing properties also pave the way for developing handheld glycan biosensors for rapid clinical or field-based diagnostics. Beyond sensing, these nanoparticles hold potential for nanomaterial-mediated glycan delivery systems, offering controlled release and targeted biological interactions. To advance toward biomedical applications, comprehensive in vivo biocompatibility and pharmacokinetic studies will be crucial to establish their safety and functional performance. Additionally, the incorporation of ZnO NPs into 3D-printed biosensor architectures could revolutionize personalized diagnostic devices, enabling customizable, low-cost, and highly sensitive glycan detection platforms.

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