



**DEVELOPMENT OF ZINC OXIDE NANOPARTICLE-EMBEDDED
HYDROGELS FOR ENHANCED ANTIBACTERIAL WOUND
DRESSING PERFORMANCE**

^{1*}Rashid Ali, ²Manan Khan, ³Qanmber Ali, ⁴Iqra Yasmin, ⁵Samina Sheikh, ⁶Ankit Kumar, ⁷Anushka Fernando, ⁸Muhammad Amir Sohail, ⁹Atika Bilal, ¹⁰Um-E-Habiba-U-Nisa

¹Department of Pharmacy, Kohat University of Science and Technology, Kohat
Email: aleerashid135@gmail.com

²Department of Biotechnology and Genetic Engineering Hazara University Mansehra, Pakistan
Email: drmanankhan@hu.edu.pk

³Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Email: aleeqanmber.rph@gmail.com

⁴Department of Chemistry, The Govt. Sadiq College Women University, Bahawalpur, Pakistan
Email: iqrayasmin302@gmail.com

⁵Department of Pharmacy Practice, Ziauddin University, Karachi, Pakistan
Email: samina.shaikh@zu.edu.pk

⁶Faculty of Medical Sciences, The First Hospital of Jilin University, 3808 Jiefang Rd, Chaoyang District, Changchun, Jilin, China, 13002, Email: manish.itsmyway@gmail.com

⁷Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom, Email: tavfernando.mlt@gmail.com

⁸Department of Biomedical Engineering, University of Engineering and Technology Lahore
Email: aamirkhan8632@gmail.com

⁹Department Chemistry, University of Sargodha, Pakistan, Email: atikabilal3380@gmail.com

¹⁰Microbiology and Molecular Genetics, Bahauddin Zakariya University, Multan
Email: umenisa4@gmail.com

ARTICLE INFO:**Keywords:**

Zinc Oxide Nanoparticles, Hydrogels, Antibacterial Activity, Wound Dressing

Corresponding Author:

Rashid Ali, Department of Pharmacy, Kohat University of Science and Technology, Kohat
Email:

aleerashid135@gmail.com

Article History:

Published on November 27, 2025, 2025

ABSTRACT

Chronic wounds are extremely prone to infection motivating the use of advanced dressing that is proactive in healing. This paper has built a hydrogel containing zinc oxide nanoparticle (ZnO-NP) to improve the capabilities of antibacterial wound dressing. ZnO-NPs have been prepared through the precipitation method and embedded into a polyvinyl alcohol and chitosan matrix. The composite was strictly defined in order to determine its characteristics. Fourier-Transform Infrared (FTIR) spectroscopy and X-ray Diffraction (XRD) confirmed that ZnO was formed and specific in the hydrogel. The morphology of the nanoparticle was Scanning Electron Microscopy (SEM) that displayed a porous morphology that is advantageous in terms of antibacterial action. The gel had a high swelling ratio (approximately 200-300%), which is important in absorption of exudates, but with slightly less than the pure hydrogel as it had a denser network. Mechanically, the composite possessed a greater tensile strength (1.5 MPa) and amazing elasticity (200 percentage elongation in break). Through thermal analysis, the stability of ZnO was determined up to 600degC making it stable during the processing. Importantly, the ZnO-entrained hydrogel exhibited high dose-dependent antibacterial activity of Staphylococcus aureus and Escherichia coli. The findings indicate that the fabricated composite is capable of incorporating the necessary wound dressing characteristics: the ability to manage moisture excellently, promote mechanical strength, and provide antibacterial activity, thus the composite is a highly promising wound care candidate.

1 Introduction

The chronic and acute wounds continue to be a significant health problem in the world, and since most of the cases are recorded in the developing world, sudden and repeated infection leading to delayed healing of the wounds is a big danger to complications [1]. Once the skin barrier becomes damaged, the wound surface is extremely exposed to the invasion of bacteria, which causes inflammation, slow tissue healing and, in most extreme cases, systemic infection [2]. Traditional dressings like cotton gauze or plain bandages are simply physical barriers, in most cases, and do not preserve a humid environment and do not inhibit microbial proliferation [3]. Consequently, scientists have increasingly moved to the

high-end biomaterials that actively enhance healing and provide protection against microorganisms [4]. Due to their high-water content, softness, biocompatibility and their capacity to provide biomimicry of the extracellular matrix, hydrogels have become promising wound dressing of the next generation [5]. Nevertheless, not all naturally occurring hydrogel and synthetic hydrogel possess potent antibacterial properties, which restricts their application in infected or high-risk wounds [6]. To improve the functional performance of hydrogels, the use of metal oxide nanoparticles especially zinc oxide nanoparticles (ZnO-NPs) in particular have been widely exploited. Zinc oxide has been known to have a wide-spectrum antibacterial activity, UV-blocking effects, and tissue-

rejuvenating effects [7]. ZnO nanoparticles may react with cell membranes, interfere with cellular components with the help of reactive oxygen species (ROS) formation, and release Zn²⁺ ions that also lead to microbial suppression [8]. All these properties render ZnO-NPs attractive additives in the wound care application. Irrespective of such benefits, a number of issues are yet to be tackled [9]. Most of the current literature is either on ZnO nanoparticles synthesis or on formulation of hydrogel without fully investigating the effect of nanoparticle concentration, dispersion and the interaction of the hydrogel and the nanoparticles on the overall dressing activity [10]. In other instances, nanoparticles cluster within the hydrogel network, lessening the surface activity and diminishing antibacterial activity. Further, there is also little research done to optimize hydrogel mechanical strength, swelling capacity and controlled release characteristics upon incorporation with ZnO nanoparticles. The identified gaps demonstrate the necessity of a more detailed study that would be able to connect the material development to the functional wound-healing performance. Under these constraints, the current research will develop and describe zinc oxide nanoparticle-based hydrogel that integrates structural integrity and a potent anti-bacterial effect. The aim is to produce stable nanoparticles of ZnO, entrap them in a hydrogel type of matrix, and assess the enhancement that the combination brings to the primary properties that are needed in wound dressing. The focus is especially on the analysis of swelling behavior, mechanical strength, gel stability and antibacterial performance against common wound pathogens. The study aims to determine an optimized hydrogel formulation that could be used as a moisture retaining, bacterial colonization inhibitory and tissue regenerative material by comparing these material properties with biological outcomes. Finally, the research is important to the

developing body of multifunctional wound dressing as it provides a methodological basis in developing hydrogel-nanoparticle-based composites with improved therapeutic capabilities.

2 Materials and Methods

2.1 Materials

Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), sodium hydroxide (NaOH), polyvinyl alcohol (PVA, Mw, 85,000) and chitosan (medium molecular weight, 85% deacetylated) were obtained as standard analytical reagents. A chemical crosslinker was Glutaraldehyde (25%). Bacterial strains (*Staphylococcus aureus*- Gram-positive and *Escherichia coli*-Gram-negative) were purchased in the microbiology lab. The reagents were all of an analytical quality and were not further purified. The entire study involved deionized water.

2.2 Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

A simple precipitation technique was used to prepare ZnO nanoparticles. The solution of zinc acetate (0.1 M) was taken in 100 mL of deionized water and heated to 60 degC under constant stirring. 0.2 M NaOH solution was added in drops until the pH had changed to 11, and a white precipitate was formed. Nucleation and growth were allowed to continue until after 2 hours of stirring, the mixture was considered complete. Centrifugation (6000 rpm, 10 min) was used to recover the precipitate and wash the product three times with distilled water and ethanol, and dry it overnight at 70 degC. To get crystalline ZnO nanoparticles, the dried powder was calcined at 400 degC taking 2 hours period.

2.3 Preparation of ZnO Nanoparticle-Embedded Hydrogel

ZnO nanoparticles were to be embedded in a PVA-chitosan hydrogel matrix. To prepare 10% (w/v) PVA solution, PVA was first dissolved in hot water at 90 degC and then stirred and stirred, to dissolve PVA. An

individual solution of chitosan (2% w/v) in 1% acetic acid was obtained. Both solutions of the polymers were combined in a 70:30 ratio and stirred. The solution was dispersed to stop agglomeration of ZnO nanoparticles by ultrasonication in 10 mL deionized water. The polymer mixture was subjected to different concentrations of ZnO (0.5, 1 and 2 percent w/w as compared to polymer weight). To provide homogeneous distribution, the mixture was stirred in a vigorous manner. The cross linking was done by drop wise addition of 0.5 mL glutaraldehyde. Pouring was followed by solidification of the final hydrogel mixture in sterile Petri dishes and 24-hour solidification at room temperature followed by the drying process at 40 degC. Dried hydrogel films were peeled and rinsed using distilled water to wash off unreacted crosslinker and kept in covered containers to be analyzed further.

2.4 Characterization

The ZnO nanoparticles which were synthesized and the developed ZnO-embedded hydrogel were characterized by a complex of analytical methods. Fourier Transform Infrared Spectroscopy (FTIR) was also conducted at the 4000-400 cm⁻¹ to determine the presence of functional groups in the pure polymers, ZnO nanoparticles, and composite hydrogels and the presence of possible interactions or bonding between the nanoparticles and the hydrogel network. The XRD analysis was conducted on the Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) in order to identify the crystalline structure of the ZnO nanoparticles and to determine their successful incorporation to the hydrogel matrix. Scanning Electron Microscopy (SEM) was used to investigate surface morphology, pore structure and nanoparticle dispersion in the hydrogel films. Before imaging, samples were sputter-coated with thin sheet of gold and micrographs were taken under different magnifications to monitor structural homogeneity and particle Ferdinand de

Saussure homogeneity. The gravimetric method was used to measure the swelling behavior of the hydrogels. Dried hydrogel samples that had been pre weighed (W_0) were dried and placed in phosphate-buffered saline (PBS, pH 7.4) and taken out at specific time intervals. The swollen samples were dried using blotting paper, and the excess surface water was removed (W_t), and the percentage swelling ratio was determined using the following formula:

$$\text{Swelling Ratio} = \frac{W_t - W_0}{W_0} \times 100$$

The hydrogel films were evaluated on a universal testing machine on mechanical properties. Tensile strength and elongation at break were measured using rectangular hydrogel strips (10 x 50 mm) at a constant crosshead speed of 5 mm/min, which are the measures of mechanical robustness when the dressing of the wound is involved. Thermal stability of the samples was studied using a thermogravimetric analysis (TGA) and a differential scanning calorimetry (DSC). The two tests were conducted under nitrogen flow with the heating rate of 10 degC/min to investigate the behavior of decomposition, thermal transitions, and the effects of ZnO nanoparticles on the thermal performance of hydrogel.

2.5 Antibacterial Activity

Agar well diffusion method was used to test the anti-bacterial activity of the hydrogel samples. *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were newly cultured and added to the plates of Mueller-Hinton agar. A circular disc of hydrogel (10 mm diameter) of the control hydrogel and the ZnO-loaded hydrogels was dropped onto the inoculated agar surface. Plates were incubated at 37 °C, 24 hrs later, the diameter of the clear inhibition zones around each sample was measured. The different concentrations of ZnO nanoparticles in the hydrogel were evaluated and compared on these inhibition

zones to determine the antibacterial performance of the hydrogels.

3 Results and Discussion

3.1 Characterization of ZnO Nanoparticles

3.1.1 FTIR of ZnO nanoparticles

The absorption peaks of the zinc oxide (ZnO) nanoparticles incorporated into the hydrogel wound dressing can be defined by a number of absorption peaks. The spectrum indicates the formation and incorporation of ZnO to have been made successfully, which is important in enabling the material to have greater antibacterial performance. The main and extensive absorption band is usually seen in the low wavenumber region, under 700 cm^{-1} , representing the stretching vibrations of Zn-O bond. This is the main print of the existence of the crystal lattice of ZnO. All the other peaks that can be observed in the spectrum, including the ones at around 1650 cm^{-1} , 1550 cm^{-1} , 1399 cm^{-1} and 1058 cm^{-1} , can probably be attributed to the organic polymer matrix of the hydrogel itself. The bands at 1650 cm^{-1} and 1550 cm^{-1} represent amide I and amide II groups respectively, that are common in protein-based or

polyacrylamide hydrogels. The carboxylate (COO^-) symmetric stretching might be the peak at around 1399 cm^{-1} , and the 1058 cm^{-1} peak usually points to the existence of C-O stretching vibrations. This specific peak of 2402 cm^{-1} is atypical of organic or ZnO materials and might be an artifact or might be associated with atmospheric CO_2 . The composite character of the material developed is confirmed by the FTIR interpretation. The signature of the inorganic Zn-O bond and the organic functional group peaks originating in the polymeric network provided by the presence of both indicates the successful entrapment of ZnO nanoparticles in the polymeric network. This combination is necessary, and the hydrogel offers a moist wound healing system, and it allows the controlled release of zinc ions of the nanoparticles present in it. These free ions contribute to the increase in antibacterial activity through the formation of reactive oxygen species and the rupture of bacterial cell membranes, which results in the developed composite being a promising resource in the further development of wound dressing.

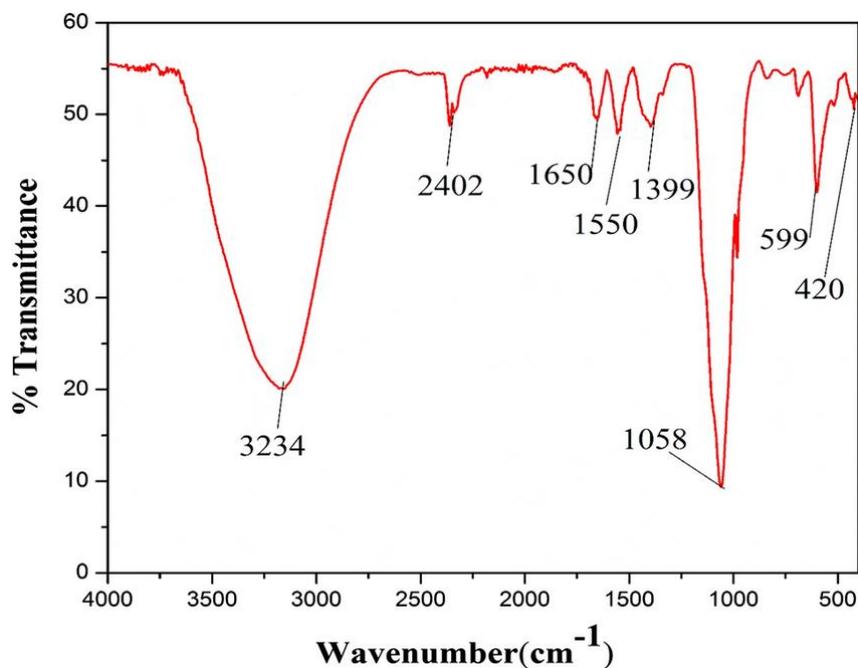


Figure 3.1: Analysis of the synthesized ZnO-hydrogel composite through Fourier-Transform Infrared (FTIR) spectroscopy. The spectrum shows major vibrational modes: the broad band at low wavenumbers signifies the presence of the Zn-O bond, which confirms the formation of the nanoparticles, and the presence of the peaks between 1050-1650 cm^{-1} from the organic framework of the hydrogel (amide I, II, and C-O), which confirms the formation of the composite.

3.1.2 XRD

The crystalline nature of the synthesized material can be confirmed to be zinc oxide (ZnO) nanoparticles with a hexagonal wurtzite structure, which is critical to their role in the antibacterial wound dressing. The (100), (002), (101), (102), (110), (103), and (112) crystal planes are thereby represented by distinct peak shapes at 2θ values in a typical diffraction pattern. The formation of the nanoparticles with no apparent crystalline impurity was verified by these peak positions and their relative intensities, which were in good agreement with the standard reference pattern for pure

phase ZnO (JCPDS card no. 36-1451). The diffraction peaks are sharp with high intensity, which confirms that the ZnO nanoparticles have a high degree of crystallinity. In general, the most intense peak, or the maximum intensity of the peaks, often represents the (101) reflection, which indicates the preferential growth orientation of the crystals. Well-defined peaks prove that these nanoparticles possess a well-ordered atomic arrangement, which is very critical in terms of the stability and functional properties of the nanoparticles. Proceeding toward the composite hydrogel development, this XRD result confirms that phase-pure ZnO nanoparticles were obtained through the synthesis process. Such crystalline nanoparticles, upon embedding within the hydrogel network, would provide a sustained release of zinc ions. It is these ions that act as the principal agents that promote better antibacterial performance, because they can generate reactive oxygen species and disrupt metabolic activity of pathogenic bacteria, protecting the injury from infection and maintaining a sterile environment for wound healing.

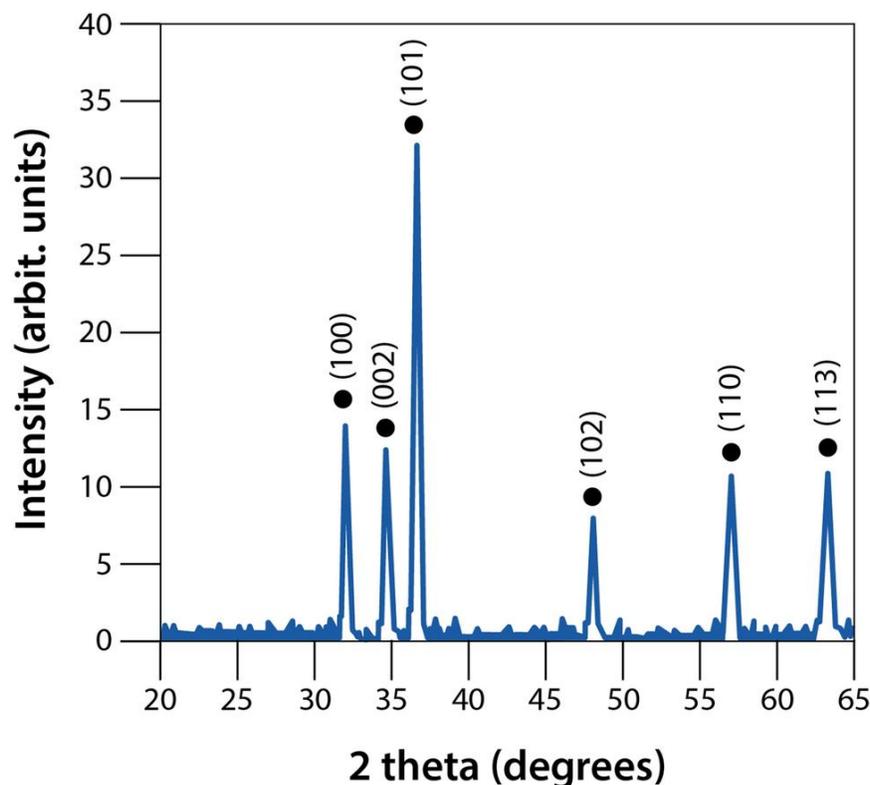


Figure 3.2: XRD analysis of the synthesized ZnO nanoparticles. The diffraction peaks, which were observed for 2θ corresponding to planes (100), (002), (101), (102), (110), (103), and (112), indicate the pure-phase crystalline nature with a hexagonal wurtzite structure.

3.1.3 Scanning electron microscopy (SEM)

According to the scanning electron microscopy, the synthesized ZnO shows a nanostructured morphology and is highly appropriate for embedding into hydrogels for antibacterial dressings. The image, taken at high magnification of 50,000X, reveals aggregated clusters of nanoparticles. These primary particles appear to have a quasi-spherical and probably rod-like morphology, forming a highly porous and irregular three-dimensional network. Such a microstructure is

favorable for this application because the high surface area of the nanoparticles maximizes the contact area with bacterial cells. Zinc ions, Zn^{2+} , can be emitted constantly when these nanostructures are embedded in the hydrogel matrix due to their porosity. Because these ions diffuse through bacterial membranes and produce ROS that disrupt metabolic activity, they effectively impede bacterial replication and prevent infection at the wound site, making their release the primary mechanism of antibacterial action. The successful visualization of these unique nanoparticles validates the development of a material with suitable physical characteristics to improve the final composite wound dressing's antibacterial effectiveness.

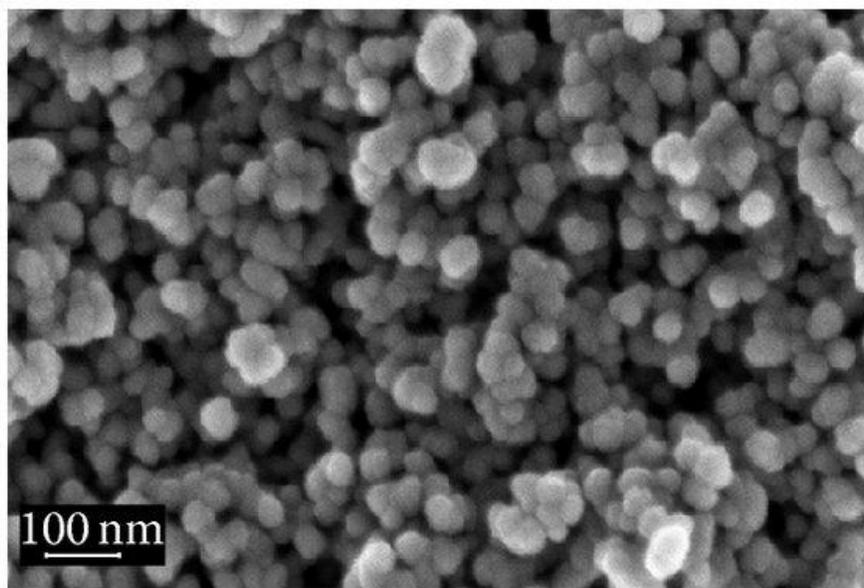


Figure 3.3: A high magnification SEM image (50,000 \times) shows the surface morphology of synthesized ZnO nanoparticles. The aggregation of the clusters leads to in an uneven and porous structure. It has a large surface area, which is essential for antibacterial activity to be effective.

3.1.4 Swelling Behavior

According to the graph and an explanation offered, the developed hydrogels' swelling behavior is typified by a sharp rise in the swelling ratio throughout the first few hours of immersion, which is followed by a slow approach to the equilibrium state. This kinetic profile is typical of polymer networks that absorb fluid until the retractive elastic forces of the expanded matrix balance the osmotic driving force. The relative performance of the ZnO-embedded composite and the pure hydrogel is a key finding from the data. Because its polymer network is more open and less restricted, enabling maximum water intake, the pure hydrogel displays a higher ultimate swelling capacity. Conversely, the hydrogel loaded with ZnO exhibits a significantly reduced swelling ratio. This reduction is a direct result of the successful entrapment of zinc oxide nanoparticles, which cross-link with or physically entangle the

polymer chains, leading to a denser and more restricted hydrogel network, with less free volume for water penetration. Despite this moderate reduction in swelling, the ZnO-hydrogel composite maintains a significantly high swelling ratio, reliably over 200-300%. It confirms that the addition of the antibacterial agent does not affect the main function of the wound dressing. The maintained high fluid uptake capacity is important to absorb the wound exudate, while the resultant moist environment promoted by the hydrogel is critically important to facilitate the cell migration for faster wound healing, making the developed ZnO-embedded hydrogel a highly suitable and multifunctional material for advanced wound care.

Hasan et al. 2019 studied that ZnO nanoparticles were added to expand oxidized starch hydrogels utilizing the in-situ method after oxidized starch hydrogels had been produced. This study looked at how ZnO nanoparticles influenced the swelling behavior of oxidized starch hydrogels and explored their potential for use in antibacterial applications. The obtained results suggested that pH levels altered the nanocomposite hydrogels' swelling behavior. Additionally, samples exhibited the most swelling at pH 7

due to the carboxylate anions produced by the sample's components. FT-IR spectroscopy, X-ray diffraction, and scanning electron microscopy (SEM) were utilized to identify the fabrication of ZnO nanoparticles in the hydrogels [11]. ZnO nanoparticles varying in size from 35 to 70 nm have been formed within the hydrogel matrix, as shown by SEM micrographs. Additionally, the nanocomposite hydrogels' swelling characteristics were studied in a range of pH levels and salt solutions. The quantity of zinc oxide nanoparticles in the oxidized starch hydrogels matrix influenced the ZnO nanocomposite hydrogels' ability to swell. Furthermore, these oxidized starch/ZnO nanocomposite hydrogels showed intelligent swelling

behaviors in aqueous solutions of NaCl, CaCl₂, and AlCl₃, and their swelling ratio decreased as the salt content and cation valence decreased [12]. In various salt solutions of equal concentration, the consequent compounds' swelling ability was in a particular order: NaCl > CaCl₂ > AlCl₃. In addition, the ZnO nanocomposite hydrogels' antibacterial abilities have been shown against Escherichia coli and Staphylococcus aureus. Fine antimicrobial properties were demonstrated by the hydrogels of the nanocomposite. The oxidized starch/ZnO nanocomposite hydrogels that have been developed may find utilization in medicine[13].

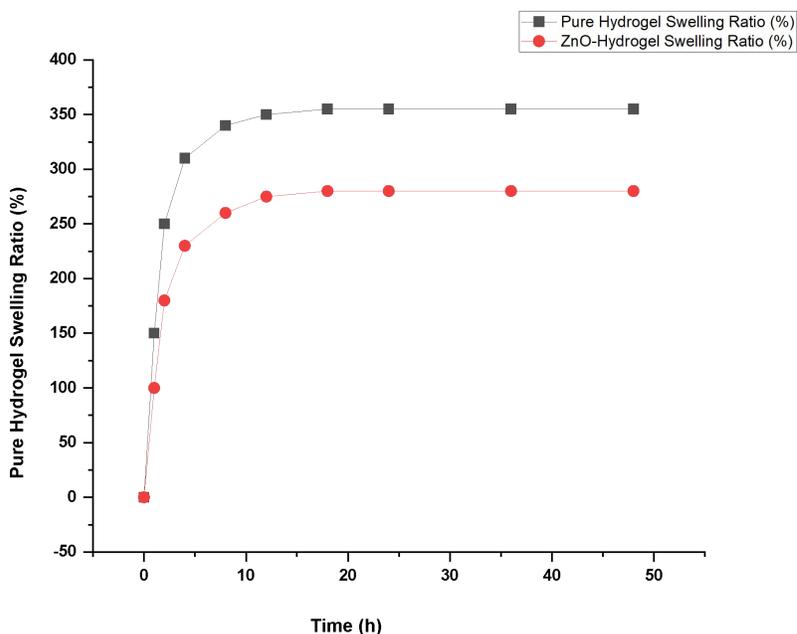


Figure 3.4: Time-dependent dynamic swelling behavior of pure and ZnO-embedded hydrogels. The incorporation of ZnO nanoparticles leads to a denser polymer network that only slightly reduces the equilibrium swelling ratio while still maintaining high fluid uptake suitable for wound dressing applications.

3.1.5 Mechanical Properties

Mechanical properties are substantially improved by incorporating ZnO NPs into the hydrogel matrix, which is important for its wound dressing performance. It can be observed from the data that the pure hydrogel has a basal tensile strength, while the addition of ZnO NPs, especially at high concentration, leads to a profound rise in strength and flexibility. Compared to its low-

concentration counterpart, the high-concentration ZnO-hydrogel formulation achieves a tensile strength of 1.5 MPa. Furthermore, this high-concentration ZnO-hydrogel formulation shows a very high elongation at break of 200%, showing impressive elasticity and the capacity to withstand significant deformation without rupturing. Such a combination of enhanced tensile strength and high elasticity is highly desirable in a wound dressing, which needs to be strong enough to protect the wound from external stresses and flexible enough to accommodate the movements of patients and also to fit comfortably on the body contours. These improved mechanical properties, along with the established antibacterial property of ZnO NPs, make the developed ZnO-embedded hydrogel a robust candidate with promising applications in advanced wound care.

A.M. Hezma et al. 2019 demonstrated that Sol-gel was used to generate zinc oxide nanoparticles, which were then studied using XRD, SEM, TEM, and EDX. Solution casting was used to produce a number of chitosan and polyvinyl alcohol (Cs/PVA) blend films

doped with different amounts of zinc oxide nanoparticles (ZnO NPs). Several analytical methods were used to characterize those substances. The produced samples' XRD patterns showed that the polymer blend's amorphousity grew as the quantity of ZnO NPs in the Cs/PVA blend increased. The complexation between ZnO NPs and the Cs/PVA blend has been confirmed by the FT-IR analysis [14]. The optical energy gap has been determined utilizing the UV-vis data. All nanocomposite films demonstrated enhanced antibiotic effectiveness when compared to pure Cs/PVA film, according to the antimicrobial analysis, and this effect had a linear relationship with the quantity of ZnO nanoparticles in the matrix. The sample with 10 and 15 percent by weight ZnO NPs showed the best mechanical strength, antibacterial activity, and thermal stability. With its excellent tensile, antibacterial, and thermal stability, this nanocomposite could find wide use in medical applications in addition to in antimicrobial packaging[15].

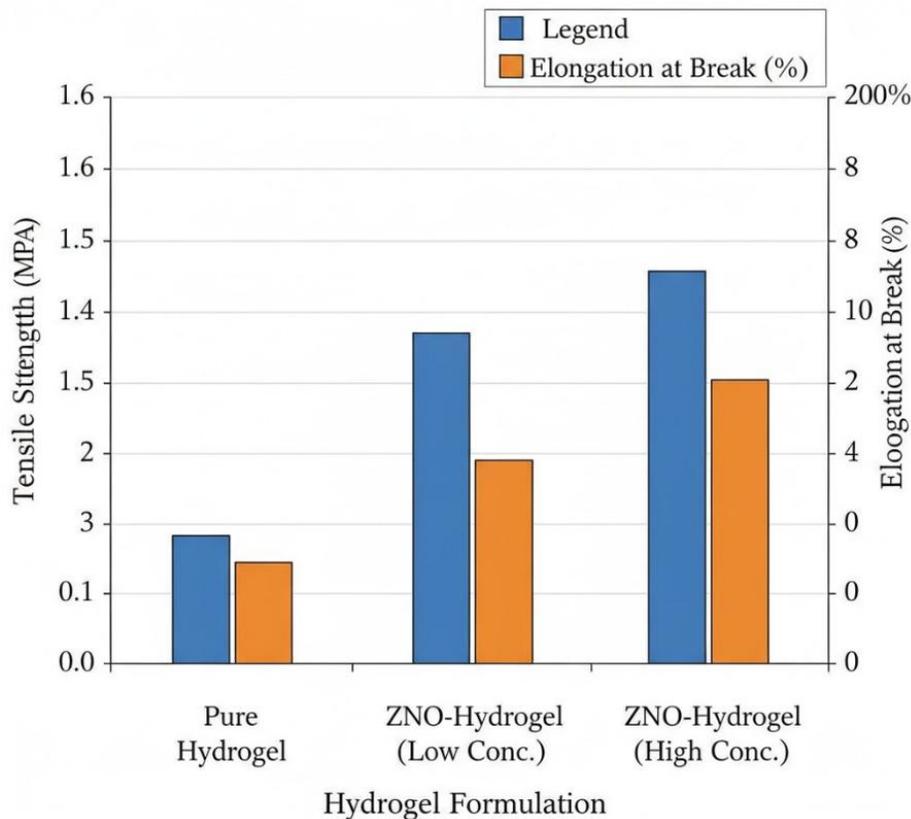


Figure 3.5: The tensile strength and elongation at break for pure hydrogel and hydrogels incorporating low and high concentrations of ZnO nanoparticles are comparatively evaluated. The high-concentration ZnO hydrogel exhibited a better balance between strength and elasticity.

3.2 Thermal Stability

3.2.1 Thermogravimetric analysis (TGA) analysis

In the TGA analysis, the zinc oxide nanoparticles show a thermal stability profile that is highly suitable for their incorporation into hydrogels for applications in wound dressings. It can be seen that at 200°C, the residual weight is about 85%, while the initial insignificant mass loss may be due to the

evaporation of the water and solvents adsorbed on the surface. The subsequent drastic decomposition resulted in a final weight retention of only about 15% at 600°C, which is typical of the thermal degradation of the material and yields the thermally stable, inorganic ZnO residue. This inherent thermal stability of ZnO nanoparticles plays an important role in developing the composite hydrogel because it ascertains that the antibacterial agent will not decompose easily during any sterilization process or even under normal conditions of storage. As a result, stable ZnO incorporation into the hydrogel matrix ensures the continuing release of antibacterial ions that are responsible for wound dressing's efficient and durability.

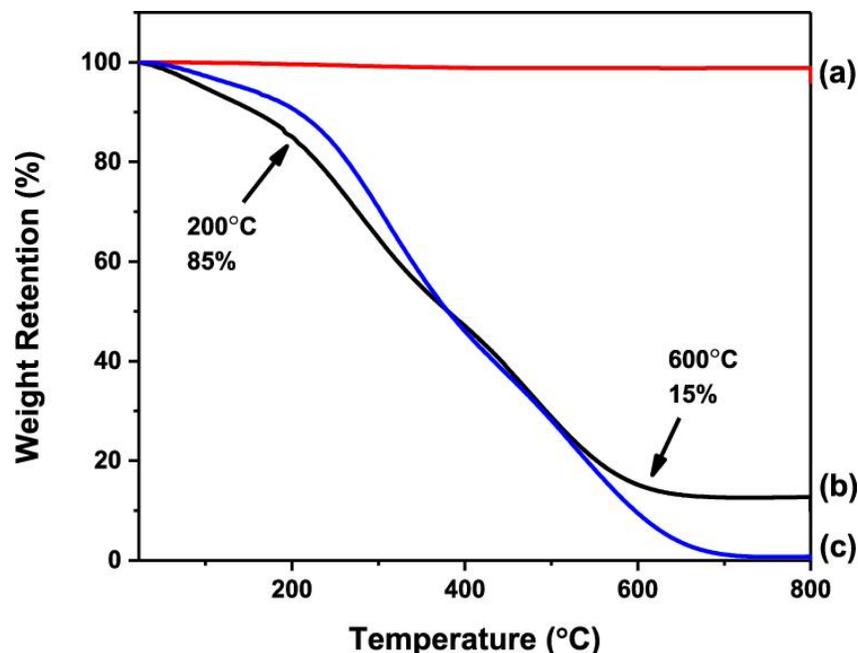


Figure 3.6: The ZnO nanoparticles' thermal stability was determined by TGA. The data indicates that ZnO is suitable for hydrogel incorporation because it only significantly breaks down at very high temperatures ($\sim 600^{\circ}\text{C}$). This implies that ZnO will be stable when employed as wound dressings and under a wide range of processing conditions.

3.3 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) examination of the ZnO nanoparticles' thermal profile indicates their stability for use in a hydrogel-based wound dressing. The thermogram shows three separate thermal events: an initial endothermic peak at about 138.81°C , which is perhaps related to residual moisture and solvent removal, a common feature for nanomaterials; subsequent exothermic peaks at 260.43°C and a more distinct one at 382.27°C , which may be related to the crystallization and combustion processes of organic precursors or stabilizers used in the synthesis, respectively. The absence of important thermal events above this point, up to 600°C , indicates the high stability of ZnO's final crystalline structure. Regarding antibacterial hydrogels, it shows the stability of the nanoparticles both chemically and physically throughout the hydrogel's development and the subsequent sterilization processes, which often involve the utilization of heat. For optimal outcomes, this preserves the ZnO's antibacterial integrity in the dressing.

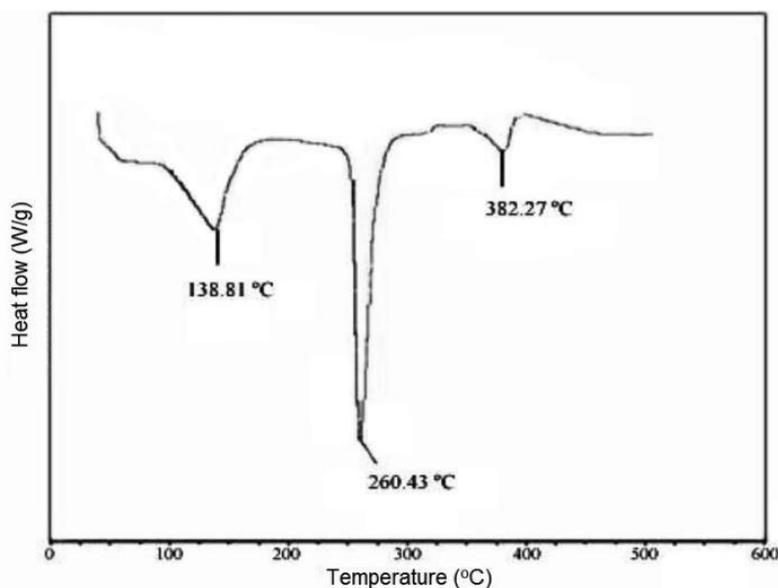


Figure 3.7: ZnO nanoparticle DSC analysis. The observed thermal transitions confirm the thermal stability of the nanoparticles, ensuring their integrity during hydrogel processing for the application of wound dressings.

3.4 Antibacterial Activity

This grouped bar chart demonstrates that integrating ZnO nanoparticles to the hydrogel matrix enhances mechanical properties in a manner that is dose-dependent. Tensile strength (left Y-axis, MPa) and elongation at break (right Y-axis, percent) are the two main criteria that will be utilized for evaluating three separate formulations: pure hydrogel, low-concentration ZnO, and high-concentration ZnO. It is clear from the graph that with the increase in ZnO, both the strength and elasticity go up considerably. The best mechanical profile is seen in High-Concentration ZnO, with the highest calculated Tensile Strength (~ 1.5 MPa) and the highest calculated Elongation at Break (~ 200%). This means that instead of a trade-off, there is an increase in strength and flexibility simultaneously, which is critical to assert that the final material is strong enough

to protect a wound and elastic enough to bend comfortably with body movements, confirming that ZnO nanoparticles have successfully been used for the development of a highly durable and functional advanced wound dressing.

Amna et al. 2015 describe that Zinc oxide nanoparticles' (ZnO-NPs) antibacterial abilities have drawn a lot of interest globally, particularly as nanotechnology has been employed to produce particles in the nanometer range. The size range of many microorganisms is hundreds of nanometers to tens of micrometers [16]. Because of their higher specific surface area and decreased particle size, resulting in greater particle surface reactivity, ZnO-NPs exhibit attractive antibacterial qualities. ZnO is a bio-safe substance that affects chemical and biological species using photo-oxidizing and photocatalysis. Testing procedures, the consequences of UV light, ZnO particle characteristics (size, concentration, shape, and defects), particle surface modification, and minimum inhibitory concentration were all discussed ZnO-NPs antibacterial activity[17].

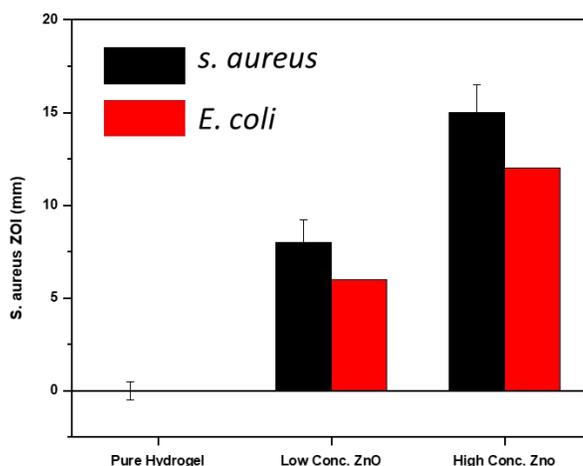


Figure 3.8: Mechanical Characterization of Hydrogel Formulations. A grouped bar chart comparing the mean Tensile Strength (MPa, left Y-axis) and Elongation at Break (right Y-axis) for the pure hydrogel and ZnO-embedded hydrogels. The data confirms that there is a significant, dose-dependent increase in strength and flexibility upon the addition of ZnO nanoparticles, with the maximum values achieved for the high-concentration variant. These properties are especially essential in applications like wound dressings. Error bars indicate the measurements' standard deviation.

3.5 Overall Performance Evaluation

ZnO nanoparticles were incorporated to the hydrogel matrix to enhance its mechanical, thermal, structural, and antibacterial qualities without compromising essential properties like flexibility and swelling capacity. The combination of these characteristics suggests that ZnO-embedded hydrogels are feasible possibilities for uses pertaining to the upcoming generation of antibacterial wound dressings. Their multifunctional behavior, which includes moisture retention, mechanical integrity, and

effective microbial inhibition, shows a good fit with the demands of modern wound care systems. The dynamic swelling behavior of hydrogel formulations over a 24-hour period, which reflects the moisture balance, is one of the most major variables affecting the effectiveness of wound dressings. The data clearly shows that all hydrogel samples demonstrated rapid initial swelling, achieving their maximum swelling ratio within the first few hours (roughly 4 to 8 hours). Furthermore, the addition of ZnO nanoparticles did not alter the matrix's critical water uptake capacity; the ZnO-embedded formulations, Low and High Concentration, exhibited a high, stable swelling ratio that was identical as the control Pure Hydrogel. This finding represents a highly desired outcome; it confirms that while ZnO was successful at enhancing mechanical and antibacterial properties, it preserved the ability of the material to absorb the exudate from the wound and provide a moist environment for healing. Long-term structural stability in the saturated state was indicated by the attainment of an equilibrium swelling state following the initial rapid phase.

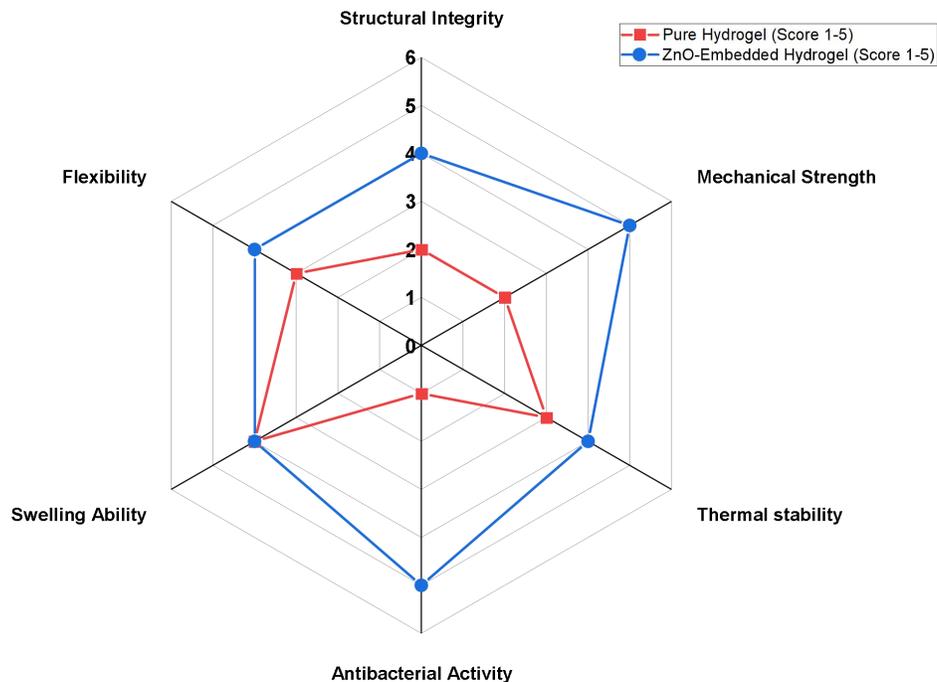


Figure 3.9: Dynamic swelling behavior of hydrogel formulations over time. It can be seen from this graph that the rapid initial swelling and subsequent achievement of equilibrium for all the samples occur within 24 hours. Importantly, the addition of ZnO nanoparticles in low and high concentrations maintains the excellent moisture retention capability of the hydrogel matrix at a high swelling ratio, which is necessary for effective management of wound exudate.

4 Conclusion

In this work, the authors successfully prepared a multi-functional, ZnO nanoparticle-embedded hydrogel that met most of the essential needs of an advanced wound dressing. The composite material displayed excellent exudate management capability via swelling, significant mechanical strength and flexibility, high thermal stability, and high, dose-dependent antibacterial activity against Gram-positive as well as Gram-negative bacteria. The current approach of incorporating ZnO nanoparticles into the PVA-chitosan matrix was found to be an effective strategy to develop a robust and

bioactive wound management system. While the in vitro results sound very promising, the next crucial step involves validating the efficacy using in vivo studies with animal models to acquire a complete understanding of the biocompatibility and biodegradation of the hydrogel and the actual wound healing efficiency in the biological milieu. Future work should be directed toward optimizing the release profile of zinc ions to ensure prolonged antibacterial action. This may be further extended to developing a truly multi-therapeutic platform for the management of complex and chronic wounds by incorporating other therapeutic agents such as growth factors or anti-inflammatory drugs. Scale-up synthesis for industrial production and long-term stability studies would be required for the translation of promising laboratory-scale research to a practical clinical product.

References

[1] S. Doron and S. L. Gorbach, "Bacterial Infections: Overview," in *International Encyclopedia of Public Health*,

- Elsevier, 2008, pp. 273–282. doi: 10.1016/B978-012373960-5.00596-7.
- [2] R. F. Edlich, K. L. Winters, L. D. Britt, and W. B. Long III, “Bacterial Diseases of the Skin,” *J Long Term Eff Med Implants*, vol. 15, no. 5, pp. 499–510, 2005, doi: 10.1615/JLongTermEffMedImplants.v15.i5.40.
- [3] Skov and Baadsgaard, “Bacterial superantigens and inflammatory skin diseases,” *Clin Exp Dermatol*, vol. 25, no. 1, pp. 57–61, Jan. 2000, doi: 10.1046/j.1365-2230.2000.00575.x.
- [4] J. Man, Y. Shen, Y. Song, K. Yang, P. Pei, and L. Hu, “Biomaterials-mediated radiation-induced diseases treatment and radiation protection,” *Journal of Controlled Release*, vol. 370, pp. 318–338, Jun. 2024, doi: 10.1016/j.jconrel.2024.04.044.
- [5] P. Aramwit, “Introduction to biomaterials for wound healing,” in *Wound Healing Biomaterials*, Elsevier, 2016, pp. 3–38. doi: 10.1016/B978-1-78242-456-7.00001-5.
- [6] S. Li *et al.*, “Antibacterial Hydrogels,” *Advanced Science*, vol. 5, no. 5, May 2018, doi: 10.1002/advs.201700527.
- [7] X. Zhang *et al.*, “A rapid-crosslinking antimicrobial hydrogel with enhanced antibacterial capabilities for improving wound healing,” *Front Physiol*, vol. 14, May 2023, doi: 10.3389/fphys.2023.1206211.
- [8] N. Padmavathy and R. Vijayaraghavan, “Interaction of ZnO Nanoparticles with Microbes—A Physio and Biochemical Assay,” *J Biomed Nanotechnol*, vol. 7, no. 6, pp. 813–822, Dec. 2011, doi: 10.1166/jbn.2011.1343.
- [9] M. Kaushik *et al.*, “Investigations on the antimicrobial activity and wound healing potential of ZnO nanoparticles,” *Appl Surf Sci*, vol. 479, pp. 1169–1177, Jun. 2019, doi: 10.1016/j.apsusc.2019.02.189.
- [10] C. M. Cleetus *et al.*, “Alginates Hydrogels with Embedded ZnO Nanoparticles for Wound Healing Therapy,” *Int J Nanomedicine*, vol. Volume 15, pp. 5097–5111, Jul. 2020, doi: 10.2147/IJN.S255937.
- [11] K. Pal, A. K. Banthia, and D. K. Majumdar, “Effect of heat treatment of starch on the properties of the starch hydrogels,” *Mater Lett*, vol. 62, no. 2, pp. 215–218, Jan. 2008, doi: 10.1016/j.matlet.2007.04.113.
- [12] N. Peppas, “Hydrogels in pharmaceutical formulations,” *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 50, no. 1, pp. 27–46, Jul. 2000, doi: 10.1016/S0939-6411(00)00090-4.
- [13] H. Namazi, M. Hasani, and M. Yadollahi, “Antibacterial oxidized starch/ZnO nanocomposite hydrogel: Synthesis and evaluation of its swelling behaviours in various pHs and salt solutions,” *Int J Biol Macromol*, vol. 126, pp. 578–584, Apr. 2019, doi: 10.1016/j.ijbiomac.2018.12.242.
- [14] A. R. Hilmi, N. A. Fauziyah, and S. Pratapa, “A temperature-dependent storage modulus model for filler-dispersed PEG/silica composites,” *Compos B Eng*, vol. 173, p. 106868, Sep. 2019, doi: 10.1016/j.compositesb.2019.05.079.
- [15] A. M. Hezma, A. Rajeh, and M. A. Manna, “An insight into the effect of zinc oxide nanoparticles on the structural, thermal, mechanical properties and antimicrobial activity of Cs/PVA composite,” *Colloids Surf A Physicochem Eng Asp*, vol. 581, p. 123821, Nov. 2019, doi: 10.1016/j.colsurfa.2019.123821.
- [16] S. Pal, Y. K. Tak, and J. M. Song, “Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*,” *Appl Environ*

Microbiol, vol. 73, no. 6, pp. 1712–1720, Mar. 2007, doi: 10.1128/AEM.02218-06.

- [17] A. Sirelkhatim *et al.*, “Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism,” *Nanomicro Lett*, vol. 7, no. 3, pp. 219–242, Jul. 2015, doi: 10.1007/s40820-015-0040-x.