Journal of Pharmacological Biomolecules and Therapeutics

ETERNO PRESS

Review

Study of the Antibacterial, Antifungal and Photodegradation Performance of Zinc Oxide and Mg/Ag-Zinc Oxide Nanoparticles

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Abstract

Microbial infectious diseases remain a persistent threat to global public health. In this context, Nanotechnology has emerged as a powerful platform for contemporary technology. In this study, we synthesized pure zinc oxide (ZnO) and magnesium-silver co-doped ZnO nanoparticles (Mg/Ag-ZnO NPs) and evaluated their antibacterial and antifungal activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Candida albicans* using the broth microdilution method to determine minimum inhibitory concentrations (MICs). Additionally, the photocatalytic performance of NPs was assessed by monitoring the degradation of methylene blue (MB) dye under UV irradiation. Results demonstrated that Mg/Ag-ZnO NPs exhibited significantly enhanced photocatalytic activity compared to undoped ZnO, achieving 85.6% MB degradation after 180 min, attributable to the synergistic effects of Ag and Mg doping, which promote charge separation and reactive oxygen species generation. Antimicrobial assays revealed that co-doping markedly improved efficacy, MIC values for *Staphylococcus epidermidis* were 160, 80, 40, and 40 µg/mL, and for *Staphylococcus aureus* were 80, 40, 40, and 20 µg/mL, corresponding to ZnO, Mg/Ag1-ZnO, Mg/Ag2-ZnO, and Mg/Ag4-ZnO, respectively. Against the more resistant *Pseudomonas aeruginosa* and *Candida albicans*, MICs decreased from >5120 µg/mL (ZnO) to 320 µg/mL and 1280 µg/mL, respectively, with the highest doping level (Mg/Ag4-ZnO). These findings indicate that silver plays a dominant role in inhibiting Gram-positive bacteria, while combined Ag/Mg doping progressively enhances activity against Gram-negative bacteria and fungi, likely through intensified oxidative stress.

Keywords:

Zinc oxide nanoparticles, Photodegradation, Antifungal, MIC.

Article History

Received: 6 August 2025 Revised: 16 December 2025

Accepted: 23 December 2025 Available Online: 4 January 2026

1. Introduction

Nanotechnology offers promising solutions across multiple fields, including medicine, where nanomaterials exhibit potent antimicrobial activity. When materials are engineered at the nanoscale (1-100 nm), their physical and chemical properties change dramatically, differing significantly from those of their bulk counterparts [1]. These unique properties are particularly valuable in addressing the persistent challenge of infectious diseases and the growing crisis of antibiotic resistance, a leading cause of global mortality [2]. Consequently, metal nanoparticles have attracted considerable research interest as potential antimicrobial agents.

Nanomaterials, defined by having at least one dimension between 1-100 nm, exhibit strong antibacterial activity against both Gram-positive and Gram-negative bacteria. For instance, zinc oxide (ZnO) nanoparticles can suppress *Staphylococcus aureus*, while silver (Ag) nanoparticles act effectively against *Escherichia coli* and *Pseudomonas aeruginosa* in a concentration-dependent manner [3]. Although their precise antimicrobial mechanisms are not fully understood, they are generally attributed to three synergistic processes: oxidative stress, metal ion release, and non-oxidative pathways [4-6]. A key mechanism is the generation of reactive oxygen species (ROS), which overwhelms bacterial antioxidant defenses and damages cell membranes. Recent studies consolidate the action of nanoparticles activity into four primary modes of mechanisms: 1) cell membrane disruption, 2) ROS generation, 3) direct penetration of the membrane, and 4) intracellular targeting of vital components such as DNA and proteins [7].

Water pollution poses a severe global threat to human health and ecosystems. A major contributor is industrial effluent, which often contains persistent and toxic synthetic dyes. The textile industry utilizes over 10,000 different dyes, with an estimated 10-15% discharged untreated into waterways. These pollutants block sunlight, disrupting aquatic photosynthesis, and pose health risks such as skin irritation and organ damage. Their stability and resistance to biodegradation make conventional wastewater treatment challenging, necessitating advanced degradation strategies. Heterogeneous photocatalysis, which employs semiconductor nanomaterials such as metal oxides to degrade pollutants under light irradiation, has emerged as a highly effective method for this purpose [8,9]. This study focuses on the degradation of methylene blue (MB), a common and hazardous textile dye, highlighting the potential of nanotechnology-driven approaches for water purification.

Methylene blue (MB), a cationic dye extensively used in the textile industry, contributes to health issues like nausea and eye irritation when present in effluents [10]. Therefore, the effective removal of MB from industrial wastewater is critical for environmental protection. Advanced heterogeneous photocatalysis is considered a highly promising method for this purpose due to its exceptional efficacy in dye degradation. This approach commonly employs of semiconductor metal oxide nanomaterials has found extensive application as photocatalysts, particularly when subjected to natural sunlight and/or ultraviolet light. These materials are recognized for their ability to facilitate various chemical reactions under light exposure, making them valuable in environmental remediation and energy conversion processes [11-13].

Among them, zinc oxide (ZnO) is a widely studied material due to its high biocompatibility, potent antimicrobial properties, and cost-effectiveness. Historically utilized in disinfection, water purification, and wound healing [14,15], its photocatalytic and antibacterial efficacy can be further enhanced through doping with elements such as silver [16-18]. ZnO is a crucial element found in numerous enzymes, sunscreens, and topical ointments designed to alleviate pain and itching. The microcrystals of ZnO serve as highly effective light absorbers within the UVA and UVB spectral ranges, attributed to their broad band gap. The biological and chemical activity of ZnO is dependent on several physicochemical factors, including its shape, size of the particles, length of time it is present, concentration levels, acidity or alkalinity (pH), and how compatible it is with biological systems. Metal oxide nanoparticles have antimicrobial activity against *B. subtilis*, *B. megaterium*, *P. aeruginosa*, *S. aureus*, *S. lutea*, *E. coli*, *C. albicans*, *P. vulgaris*, and *Aspergillus*. These natural preservatives are commonly used in various applications and are often incorporated into polymer-based packaging materials. Their primary purpose is to prevent microbial spoilage of food products, thereby extending food shelf life and ensuring consumer safety [19].

While the precise antimicrobial mechanism of nanostructures is still unclear, but many studies indicate that their most significant toxic effect is linked to oxidative stress. ROS are central mediators of this process. ZnO nanostructures can lead to oxidative stress in target cells, activating apoptotic pathways. The ROS generated by ZnO nanoparticles can impair mitochondria and trigger apoptotic signaling, leading to cell death. Nanostructures may promote ROS production by directly stimulating mitochondria or releasing ions. The mitochondrial electron transport chain is a major source of ROS, and due to their small size, nanostructures can penetrate mitochondria, causing damage that disrupts this chain and further amplifying ROS production [20]. Supporting this mechanistic understanding, a study of Rafael O. Trevisan et al demonstrated that Ag-doped ZnO/AgO/TiO_2 nanocomposites exhibit potent, synergistic antibacterial activity against multidrug-resistant bacteria, including MRSA and KPC. The 50Ti/50ZnAg composite was particularly effective, significantly inhibiting bacterial growth and disrupting pre-formed biofilms by generating ROS and the release of bactericidal silver ions.

The urgent need to address multi-drug-resistant pathogens, coupled with the economic imperative for cost-effective antimicrobial solutions, has driven significant research into nanotechnology-based strategies. While previous studies focused on single-metal doping of ZnO, leaving a gap in understanding the synergistic effects of co-doping with two

different metals (Mg and Ag) on both antimicrobial and photocatalytic performance. Therefore, this study aims to synthesize of Mg/Ag-doped ZnO nanoparticles via a green synthesis route and comprehensively evaluate their antibacterial, antifungal, and photocatalytic activities in direct comparison with pure ZnO. The novelty of this work lies in the green synthesis of dual-doped (Mg/Ag) ZnO nanoparticles and the demonstration of their enhanced, multi-functional efficacy for both dye degradation and broad-spectrum antimicrobial action.

2. Materials and Methods

2.1 Synthesis of ZnO and Mg/Ag-ZnO NPs

The undoped and silver/magnesium-doped ZnO nanoparticles (NPs) were synthesized via a green approach using an aqueous extract of *Salvadora persica* as the reducing and stabilizing agent, following a methodology adapted from Hamidian et al. [21]. Doping was designed to incorporate silver and magnesium at distinct molar ratios (Mg:Ag of 1:1, 1:2, and 2:4), yielding the respective samples designated as Mg/Ag1-ZnO, Mg/Ag2-ZnO, and Mg/Ag4-ZnO, respectively. This co-doping strategy aimed to synergistically modify the properties of the ZnO lattice. The resulting precipitates were subjected to a calcination process at 600 °C for 2 hours to ensure complete crystallization.

2.2 Photodegradation Assay

The study focused on the photo-degradation of methylene blue (MB) dye to assess the catalytic performance of ZnO and Mg/Ag4-ZnO NPs. This investigation was conducted over various time intervals, specifically at 0, 30, 60, 90, 120, 150, and 180 minutes, to determine how effectively these nanoparticles can degrade the dye under UV exposure. Solutions of MB (10 mg L^{-1}, pH 7) were utilized as model contaminants. The experiment was conducted in a semi-manual reactor to guarantee uniform distribution of nanoparticles within the antibiotic solution. Initially, the samples were incubated in darkness condition for 30 minutes to make an adsorption-desorption equilibrium. Photocatalytic degradation was initiated using a UV lamp (11 W) as the irradiation source. A control experiment consisted of an MB solution without catalyst under irradiation. Samples were collected every 30 minutes to evaluate degradation efficiency, which was determined using the following equation:

$$\eta = (C_0 - C/C_0) \times 100\ \% \quad (1)$$

C_o represents the absorbance of MB in absence of the catalyst, while C indicates the absorbance of MB when exposed to the catalyst after undergoing UV irradiation for a specified duration.

2.3 Microorganisms

This study employed standard strains of the bacteria *Staphylococcus epidermidis* ATCC6538P and *Pseudomonas aeruginosa* ATCC15442, as well as the standard strain of the fungus *Candida albicans* ATCC1677. These specific microbial strains were obtained from the microbial collection maintained by the Scientific and Industrial Research Organization of Iran, ensuring their reliability and consistency for the research conducted. Noting that *Staphylococcus epidermidis* is common Gram-positive pathogens, *Pseudomonas aeruginosa* is a model for resilient Gram-negative bacteria, and *Candida albicans* is a prevalent fungal pathogen. This selection allows for a broad evaluation of the NPs' efficacy across different microbial types.

2.4 Assessment of MIC and MBC of NPs

2.4.1 Preparation of Culture Medium

To determine the minimum inhibitory concentration (MIC) of the NPs, Mueller Hinton Broth (MHB) was used. The culture medium was heated on a magnetic stirrer, which ensured it was continuously mixed during the heating process. The medium was heated while being stirred continuously until fully dissolved. It was subsequently sterilized by autoclaving at 121°C for 15 minutes.

2.4.2 MIC Method

A stock suspension of each nanoparticle type was prepared at 4 mg/mL by dispersing 8 mg of NPs in 2 mL of sterile Mueller Hinton Broth. Subsequently, a series of serial dilutions were prepared, with concentrations ranging from 1 µg/mL to 5120 µg/mL, to assess the effects of varying NPs concentrations on microbial activity. Following the preparation of the dilutions, 10 µL of a specific microbial suspension, standardized to a concentration of 0.5 McFarland, equivalent to approximately 10^8 CFU/mL, was added to each of the tubes containing the nanoparticle solutions. The prepared tubes were located in an incubator for 24 hours at 37 °C. Afterwards, a volume of 10 µL from each tube was carefully diluted in 15 mL of physiological serum. A portion of this dilution was sub-cultured onto Mueller Hinton agar plates using a sterile spreader. The plates were incubated at 37°C for 24 hours to assess bacterial viability. Then, growth of bacterial colonies in plate was examined and the MIC and MBC of NPs were determined. The concentration at which 99.9% of microbes were killed was chosen as the MBC and the clear tube as the MIC.

3. Results and Discussion

Morphological characteristics, analyzed via FESEM, were obtained from previously published synthesis protocol reported by Hamidian et al [21]. The FESEM analysis revealed that ZnO NPs are hexagonal in shape and in the case of doped nanoparticles, they have taken on a rod shape with the growth of the particles. The average particle sizes, determined from size distribution analysis, were 36.44 nm for pure ZnO, and 36.89, 47.92, and 51.45 nm for the three doped variants, respectively (Figure 1). This progressive increase in particle size correlates directly with the elevated doping concentrations of magnesium and silver, as detailed in the source study [21].

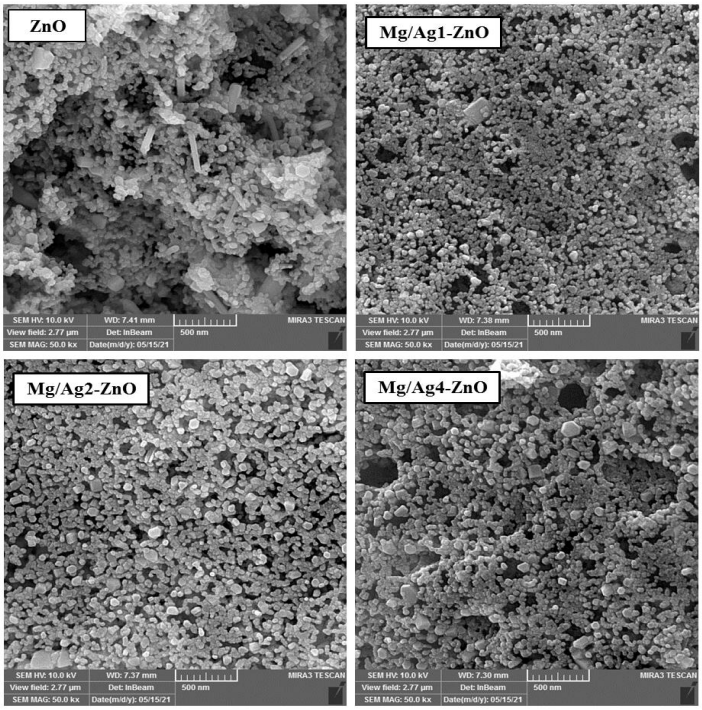

Figure 1. FESEM image of ZnO and Ag/Mg-ZnO NPs [21].

Figure 2a depicts the degradation rates of MB when exposed to UV light conditions via ZnO and Ag/Mg-ZnO NPs. The degradation rates obtained were 72.82% and 85.56% for MB utilizing ZnO and Mg/Ag4-ZnO NPs catalysts at the 180-minute, respectively. Figure 2b and 2c showed that the photodegradation of MB follows a first-order reaction model of ZnO and Ag/Mg-ZnO NPs, with R^2 constants of 0.9944 and 0.9922, respectively.

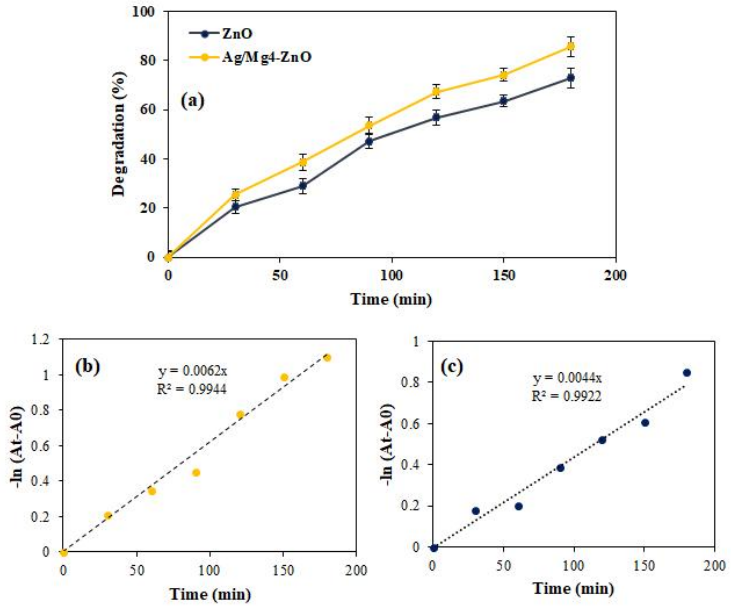

Figure 2. Comparison of photocatalytic activity and degradation kinetics of methylene blue (MB) under UV light for pure ZnO and Mg/Ag co-doped ZnO (Mg/Ag4-ZnO) nanoparticles. (a) Temporal UV-Vis spectral changes of MB. Corresponding first-order kinetic plots for (b) ZnO NPs and (c) Mg/Ag4-ZnO NPs.

The photocatalytic mechanism of ZnO and Mg/Ag4-ZnO NPs begins when UV or visible light generates electron-hole pairs. Excited electrons jump to the conduction band, leaving holes in the valence band to drive oxidation-reduction reactions on the catalyst surface. Reactive oxygen species decompose MB into less toxic intermediates. As illustrated in Figure 3, when dissolved oxygen is present, electrons in the conduction band play a crucial role in the reduction of molecular oxygen (O_2). This process results in the formation of hydrogen peroxide (H_2O_2) as well as hydroxyl radicals (•OH), which are highly reactive species. The interaction between these conduction band electrons and oxygen molecules initiates a series of chemical reactions that ultimately yield these important ROS. Potent oxidative capability of these radicals is attributed to the effective separation of electron-hole pairs. This separation is facilitated by surface oxygen vacancies, which act as electron traps, thereby suppressing charge carrier recombination and enhancing the yield of reactive oxygen species. Furthermore, a synergistic effect between adsorption and photocatalysis increases process efficiency by concentrating pollutant molecules on the catalyst surface for effective interaction with the reactive species [22-25]. Suwannaruang et al. proposed that during the photodegradation process, The nanoparticles generate photo-induced electrons and holes, which act as traps for the electron-hole pairs. These traps play a crucial role in preventing the recombination of charge carriers by significantly improving the spatial separation between the electron-hole pairs. As a result, this enhanced separation leads to higher degradation efficiency, allowing for more effective utilization of the generated charge carriers in various applications [26].

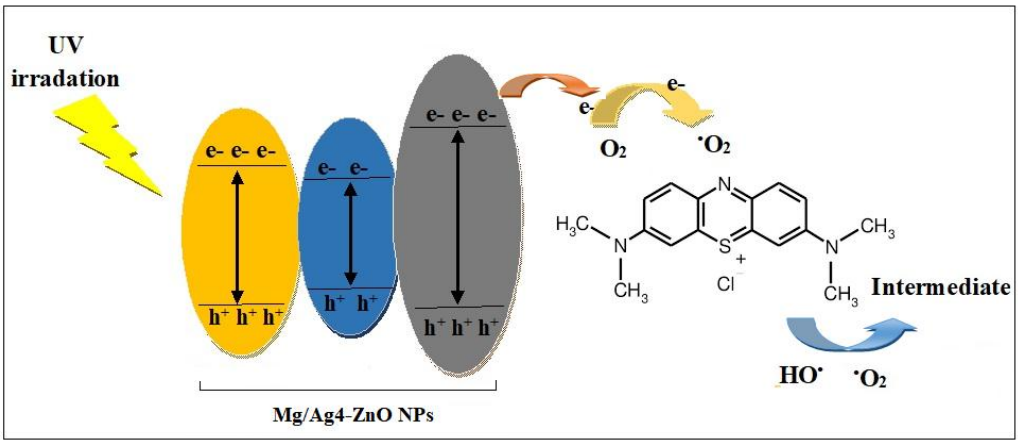

Figure 3. Suggestion mechanism of degradation process by Mg/Ag4-ZnO NPs on MB under UV irritation.

The results of the MIC and MBC study of nanoparticles by broth dilution method are presented in Table 1 and Table 2. Based on the table, the most significant inhibitory activity of NPs was noted against *S. epidermidis* and *S. aureus* bacteria. However, it is important to note that antimicrobial activity was also recorded against *P. aeruginosa* and *C. albicans*, but this was only at higher concentrations of the NPs.

Table 1. MIC study of NPs on *S. epidermidis, S. aureus, P. aeruginosa,* and *C. albicans.*

	MICs outcomes (µg/mL)				
	ZnO	**Ag/Mg1-ZnO**	**Ag/Mg2-ZnO**	**Ag/Mg4-ZnO**	***gentamicin***
S. epidermidis	160 ± 2.3	80 ± 2.1	40 ± 1.8	40 ± 2.2	12.5 ± 1.5
S. aureus	80 ± 1.5	40 ± 1.8	40 ± 1.7	20 ± 1.9	6.25 ± 1.2
P. aeruginosa	>5120 ± 2.1	640 ± 2.3	320 ± 1.9	320 ± 2.2	6.25 ± 1.2
C. albicans	>5120 ± 2.3	2560 ± 2.4	2560 ± 2.1	1280 ± 1.8	25 ± 1.8

Table 2. MBC study of NPs on *S. epidermidis, S. aureus, P. aeruginosa,* and *C. albicans.*

	MBCs outcomes (µg/mL)				
	ZnO	**Ag/Mg1-ZnO**	**Ag/Mg2-ZnO**	**Ag/Mg4-ZnO**	***gentamicin***
S. epidermidis	640 ± 1.8	320 ± 1.9	160 ± 2.1	160 ± 1.6	50 ± 1.1
S. aureus	320 ± 2.3	160 ± 2.2	160 ± 1.9	80 ± 1.5	50 ± 1.8
P. aeruginosa	-*	-	5120 ± 2.2	5120 ± 1.9	50 ± 1.8
C. albicans	-	-	-	-	100 ± 2.1

* No results

Zinc oxide nanoparticles (ZnO NPs) are the focus of extensive research due to their high biocompatibility, antimicrobial properties at neutral pH, and cost-effective production [14]. Their antibacterial mechanism of the nanoparticles involves the disruption of microbial cell membrane integrity, and accumulating in the cytoplasm, which inhibits essential cellular processes. Furthermore, ZnO NPs induce oxidative stress, damaging vital cellular components like lipids, proteins, and DNA. This damage subsequently triggers apoptosis, leading to cell death. The addition of silver ions to zinc oxide significantly enhances its antibacterial properties. This action stems from the electrostatic attraction between the positive metal ions and the negatively charged bacterial cell membrane. This binding interacts with thiol proteins,

triggering membrane oxidation. This process generates reactive oxygen species (ROS) that induce oxidative stress, leading to lipid peroxidation and the consequent loss of cell membrane integrity. The damage hinders essential functions like permeability and cellular respiration, leading to bacterial cell death [16-18].

Azam et al. (2012) examined the antimicrobial properties of ZnO nanoparticles against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) as well as Gram-positive bacteria (*B. subtilis*). The findings indicated that the antimicrobial effectiveness improves as the particle size decreases and the surface area to volume ratio increases [27]. In an investigation, Aflatonian et al. (2017) examined the antimicrobial properties of nanoparticles in vitro by employing the broth microdilution technique to determine the MIC and MBC for two bacterial strains. Their findings indicated that both Gram-positive and negative bacteria exhibit sensitivity to ZnO nanoparticles, with a notably greater sensitivity observed in Gram-negative bacteria [28]. Jafari et al. (2011) studied the antibacterial effects of silver-doped zinc oxide nanoparticles on *P. aeruginosa*, *B. subtilis*, *S. gallinarum*, *E. coli*, and *S. aureus*. They found that *E. coli*, *Salmonella,* and *Pseudomonas* were more sensitive to the nanoparticles than *Staphylococcus* and *Bacillus*. Additionally, the nanoparticles improved bactericidal efficacy [29]. The findings from our investigation into the antimicrobial properties of nanoparticles suggest that the inclusion of silver within the nanoparticle structure enhances their bactericidal effectiveness.

As a result of the findings from this study, it is evident that both ZnO and Mg/Ag-ZnO NPs possess notable antimicrobial properties. The data obtained through the broth dilution method for the bacteria *S. epidermidis* and *S. aureus* revealed that the presence of silver significantly boosts the ability to inhibit these bacterial strains. Additionally, for the gram-negative bacterium *P. aeruginosa* and the fungus *C. albicans*, it has been observed that increasing the concentrations of silver and magnesium doping leads to a stronger inhibitory effect of the nanoparticles against these pathogens. The high MIC/MBC values for *P. aeruginosa* and *C. albicans* indicate limited efficacy against these strains and that such high concentrations may not be clinically practical for therapeutic use, but could be relevant for surface coatings or environmental applications.

Mg/Ag-ZnO NPs offer a key advantage over traditional photocatalysts like TiO_2 and g-C_3N_4 by combining enhanced dye degradation with strong intrinsic antimicrobial activity. While TiO_2 is highly stable but mostly UV-active [22,26,27], and g- C_3N_4 is visible-light active but has weak antimicrobial action [9], the co-doped ZnO provides a dual-functionality that is promising for applications like self-cleaning surfaces and water purification. So, there is a synergy between antimicrobial and photodegradation properties, which is found in the increased generation of ROS resulting from photodegradation, which simultaneously enhances antimicrobial activity by elevating oxidative stress on microbial cells. This dual functionality renders the nanoparticles effective for both environmental remediation and microbial suppression.

4. Conclusion

This study demonstrates that the eco-friendly synthesis of magnesium and silver co-doped zinc oxide (Mg/Ag-ZnO) nanoparticles establishes a synergistic effect that significantly enhances material functionality. The primary conclusion is that this strategic dual-doping establishes a significant synergy, greatly improving the functionality of the material beyond that of pure ZnO. The photocatalytic performance saw a remarkable enhancement, with the optimal Mg/Ag4-ZnO nanoparticles achieving an 85.6% degradation rate of methylene blue dye under UV light, attributed to improved charge separation. Concurrently, the antimicrobial activity experienced a substantial increase, reducing the MIC for resistant pathogens such as *P. aeruginosa* from over 5120 µg/mL to 320 µg/mL, showcasing a strong combined effect that elevates oxidative stress across a wide range of microorganisms. The broader implications suggest that these dual-doped nanoparticles could serve as promising multi-functional agents. Their improved photocatalytic ability positions them as excellent candidates for wastewater treatment aimed at degrading organic pollutants. Future research should prioritize translational development, focusing on bridging the gap between laboratory proof-of-concept and real-world application, particularly through in vivo toxicity evaluations and the creation of immobilized, reusable catalytic systems for sustainable water treatment and self-sterilizing surfaces.

5. Limitations and Future Perspectives

This study presents compelling in vitro evidence regarding the improved functionality of Mg/Ag-ZnO NPs; however, it is not without its limitations. The main limitation is the high effective concentrations necessary to inhibit resilient pathogens such as *P. aeruginosa* and *C. albicans*, which could create challenges for systemic biomedical applications. Additionally, the research is limited to laboratory environments and a specific selection of microbial strains. Future investigations will expand on these findings to connect laboratory proof-of-concept with practical applications. Important directions include: Assessments of *in vivo* toxicity and efficacy to determine biocompatibility and therapeutic potential within a biological framework. The immobilization of NPs on solid substrates (such as polymers and ceramics) to create reusable, stable catalytic systems for ongoing water treatment and self-sterilizing surfaces. Evaluation under solar irradiation to substitute for energy-intensive UV light, thereby improving the economic feasibility and environmental sustainability of the photocatalytic process for practical applications.

Conflict of Interest

The authors affirm that there are no conflicts of interest.

Data Availability Statement

The information that underpins the conclusions of this research can be obtained from the corresponding author upon a reasonable request.

Generative AI Statement

The authors declare that no generative artificial intelligence technologies were used when preparing this manuscript.

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