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An Assessment of the Antibacterial Activity of Ag-Doped TiO_2 and Ag-Doped ZnO Nanoparticles

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ABSTRACT

This work presents the antibacterial activity of Ag-doped TiO_2 and Ag-doped ZnO nanoparticles (NPs) that were synthesized using a sol-gel method and tested under visible light irradiation. The structural, morphological characteristics of synthesized nanoparticles were examined using X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy dispersive X-ray (EDX) spectrum. X-ray diffraction analysis indicated that Ag-doped TiO_2 comprised both rutile and anatase phases. The presence of the hexagonal wurtzite structure was detected in Ag-doped ZnO nanoparticles. The altered morphologies of TiO_2 and ZnO by the doping of Ag was demonstrated by the TEM. EDX analysis verified the presence of zinc (Zn), titanium (Ti), silver (Ag), and oxygen (O) components in the samples. Pathogenic bacteria, such as *Bacillus subtilis, Escherichia coli*, were used to determine the antibacterial activity of the synthesized nanoparticles. It was observed that both the synthesized nanoparticles Ag-TiO_2 and Ag- ZnO NPs exhibited antibacterial activity against gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli*, however Ag-ZnO NPs exhibited higher antibacterial activity compared to Ag-TiO_2 NPs.

Keywords Materials science, Nanotechnology, Materials chemistry, Pharmaceutical science.

INTRODUCTION

Nanoscale materials are currently regarded as the most cutting-edge category of materials, both in terms of scientific understanding and commercial utilization (Sahu *et al*. 2023). Inorganic nanoparticles (NPs), including silver, copper, titanium, and zinc, stand out as particularly intriguing NPs due to their diverse applications and beneficial effects on pathogenic microorganisms (Balderrama-González *et al.* 2021). Extensive research has been dedicated to NPs over the years, primarily because of their size-dependent physical and chemical attributes. Within the realm of

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NPs, significant emphasis has been placed on nano oxides. Nanotechnology has garnered substantial attention owing to their specific characteristics and properties, particularly in the realms of pharmaceuticals and biological applications. Laboratory experiments involving NPs have demonstrated the swift eradication of various microorganisms (Sharmin *et al.* 2021). The utilization of NPs against bacteria holds notable significance, considering the inclination of NPs to reside at the lower trophic levels and directly integrate into the food chain of the ecosystem.

In recent times, significant emphasis has been placed on Titanium Dioxide (TiO_2) and Zinc Oxide (ZnO), Nanoparticles (NPs) owing to their distinct optical, electrical, and chemical characteristics. TiO_2 serves as an exceptional photocatalyst, extensively employed for its antibacterial properties attributed to its elevated photosensitivity, remarkable efficiency, non-toxic attributes, potent oxidizing capabilities, cost-effectiveness, and chemical robustness (Gaur and Jagadeesan 2021). Similarly, ZnO exhibits promise as a photocatalyst and contributes significantly to antibacterial efficacy, characterized by its affordability, biocompatibility, high catalytic activity, and eco-friendly nature (Nawaz *et al*. 2024).

In order to augment the photocatalytic performance, extensive interdisciplinary investigations have been conducted on TiO_2 and ZnO. It is widely acknowledged that the photocatalytic effectiveness of nanoparticles is contingent upon factors such as their crystalline configuration, incorporation of dopants, surface area, and presence of hydroxyl groups (Nigussie *et al.* 2018). Presently, various scholars are dedicated to increasing the efficiency of photocatalysts through the utilization of metal additives like Ag, known for its exceptional stability and commendable electrical/thermal conductivity. Moreover, the introduction of Ag dopants onto the metal oxides' surface serves to boost photocatalytic activity by impeding rapid e- -h+ recombination processes (Hastir *et al.* 2017). Additionally, this mechanism may engender notable antibacterial characteristics. The present study involved the synthesis of Ag-doped TiO_2 and Ag-doped ZnO via the sol-gel approach, with a meticulous examination and discussion of their antibacterial properties.

MATERIALS AND METHODS

Synthesis of Ag-TiO_2 and Ag-ZnO nanoparticles

The Ag-TiO_2 nanoparticles were synthesised using sol gel method given by Akram *et al.* (2016). A 10 mL of silver nitrate and titanium dioxide (Ag/TiO_2 = 5% in mole ratio) were disseminated in 100 mL of ethanol. Subsequently, the mixture was vigorously stirred at room temperature for 30 minutes. The pH of the mixed solution was adjusted to approximately 4 by adding 3 mL of 2M nitric acid. Subsequently, the mixture was agitated for 30 minutes. The Sol was then dried at 80°C for 2 hours, and calcination at 450°C for 2 hrs. The nanoparticles will manifest as a greyish-white substance upon calcination, following which they were characterized. Ag-ZnO nanoparticles were synthesized using the technique given by Ariki and colleges with slight variations Al-Ariki *et al.* (2021). To prepare Ag-doped ZnO NPs, 0.45M of zinc acetate dihydrate and 0.05 M of $AgNO_3$ were mixed to 100 mL of distilled water. After that aqueous ammonia was added drop by drop until a pH of 7 was attained with continuous stirring for 30 min. The white gel produced was allowed to settle overnight. For the complete elimination of impurities, the precipitate formed were washed with deionized water and acetone followed by centrifugation at 3500 rpm. The precipitate was then kept in an oven at 80°C for 24 h to obtain a dry powder which was then subjected to calcination at 600°C for 2 hrs in a muffle furnace fitted with a PID temperature controller with the heating rate kept at 10°C min−1.

Characterizations of Ag-TiO_2 and Ag-ZnO nanoparticles

The characterization of nanoparticles using different sophisticated instruments allows for precise analysis of their size, shape, and surface properties, providing valuable insights into their potential applications. X-ray Diffraction (XRD) was used determine their crystal structure of the synthesized nanoparticle. Particle size and structural morphology of nanoparticles was characterized using Transmission Electron Microscopy (TEM). The chemical composition of nanoparticles was determined using Energy-Dispersive X-ray Spectroscopy (EDX).

Bacterial strain and culture

In the present study, two distinct bacterial strains were selected: The gram-positive *Bacillus subtilis* and the gram-negative *Escherichia coli*. The cultivation took place in 61748 LB Broth, a medium known as Luria Bertani Broth, within a humidified incubator set at 37°C with continuous agitation throughout the night. Subsequently, the microorganisms were incubated on a nutrient agar plate for a duration of 24 hrs under aerobic conditions at 37°C. Antibacterial activity of Ag-TiO_2 and Ag-ZnO nanoparticles.

Antibacterial activity of Ag-TiO_2 and Ag-ZnO nanoparticles

Nanoparticles antibacterial activity was evaluated using agar-well diffusion method (Goswami *et al.* 2024). The N-agar plates were prepared and two bacterial cultures (*B. subtilis* and *E. coli*) were poured on n-agar plate. Then a well of 6mm size were aseptically punched with the help of a sterile cork borer nanoparticles were autoclaved to create a 1000 ppm stock solution, sonicated for 20 minutes. Nano doses of 25, 50, 75, 100 ppm in 1ml of 10% dimethyl sulfoxide (DMSO) and were inoculated in plates. DMSO was used as a control to confirm non-antimicrobial properties. Azithromycin antibiotic selected as positive control. Antibacterial activity was assessed by measuring zone of inhibition after incubation.

RESULTS AND DISCUSSION

Material characterization

X-ray diffraction (XRD)

The diffraction angles of silver-doped titanium dioxide nanoparticles were observed at 27.46°, 38.09°, 54.34°, 56.73°, 62.80°, 69.02°, and 69.89° corresponding to the miller indices values of (101), (004), (105), (211), (204), (110), (220) respectively. Additionally, diffraction angles at 36.18°, 41.29°, 44.20°, 64.06°, 77.41° corresponding to (111), (200), (231), (220), and (311) miller indices values were observed for silver (Fig.1). The presence of both anatase and rutile phases in Ag-doped TiO_2 was confirmed through the JCPDS card number 4-783. Research conducted by Nigussie and colleagues highlighted that a mixture of anatase phase and rutile phases in Ag-doped TiO_2 demonstrates enhanced photocatalytic properties when compared to the pure anatase phase of TiO_2 (Nigussie *et al.* 2018). Moreover, the introduction of silver into titanium dioxide nanoparticles results into decrease in the crystallite size, estimated to be approximately 18.18 nm using the Scherrer's equation.

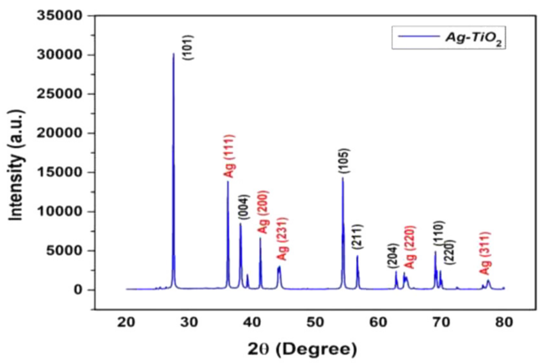

Fig. 1. Powder XRD graph of Ag-TiO_2 nanoparticles.

The XRD graph of Ag-ZnO nanoparticles (Fig. 2) illustrates prominent and intense peaks, suggesting the presence of a crystalline configuration. The observed peaks at 32.46˚, 34.95˚, 36.19˚, 46.42˚, 57.64˚, 59.23˚ and 79.19˚ correspond to specific miller indices values such as (100), (002), (101), (102), (110), (112), and (201), respectively, indicating the hexagonal wurtzite phase of ZnO nanocrystals with a dimension of 21.96 nm as determined through the application of the Scherrer equation. Furthermore,

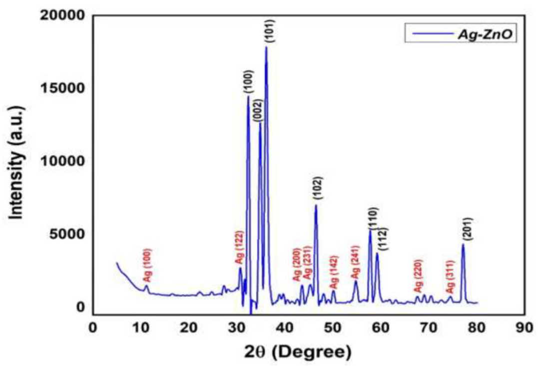

Fig. 2. Powder XRD graph of ZnO nanoparticles.

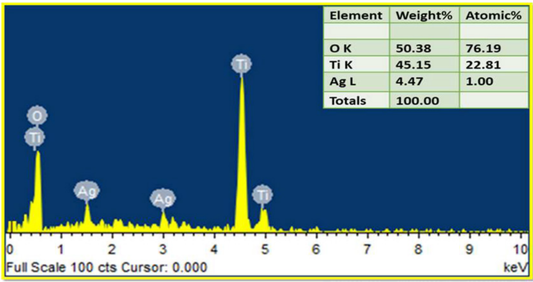

Fig. 3. EDAX spectra Ag-TiO_2 nanoparticles.

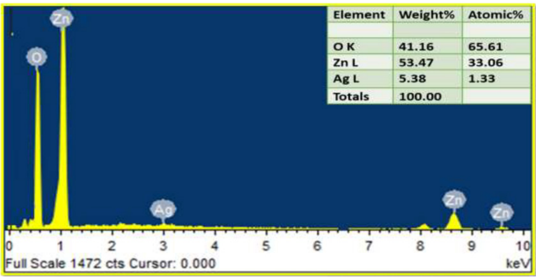

Fig. 4. EDAX spectra of Ag doped zinc oxide nanoparticles.

the identified peaks at 11.19°, 30.55°, 43.59°, 45.39°, 50.09°, 54.75°, 67.68°, and 74.67° are in alignment with the face-centered cubic (fcc) arrangement characteristic of Ag (ICDD card number 04-0783) (Primo *et al.* 2022).

Energy dispersive X-ray microanalysis (EDAX)

The investigation of the chemical compositions in terms of weight percentage (Wt%), atomic percentage (At%), and purity of Ag-TiO_2 and Ag-ZnO nanoparticles has been carried out through EDAX analysis. The presence of Ti (22.81%), O (76.19%), and Ag (1%) in high-intensity peaks in the EDAX spectra of Ag-TiO_2 nanoparticles (Fig. 3) confirms the existence of all three elements in a stoichiometric proportion of Ag-TiO_2 NPs without any impurities. Conversely, in the case of Ag-doped ZnO samples, there is a decrease in Zinc composition while the oxygen percentage increases. The EDAX analysis reveals the levels of transition metal ions present in the doped ZnO NPs, as depicted in Fig. 4 of Ag in the doped samples was determined to be 1.33%.

Transmission electron microscope (TEM) analysis

A Transmission Electron Microscopy (TEM) examination was undertaken to determine the internal morphology and dimensions of the nanoparticles. The study utilizing Transmission Electron Microscopy (TEM) serves as supplementary validation of the nanoparticle size established through X-ray diffraction (XRD). Depicted in Figs. 5–6 are Transmission Electron Microscopy (TEM) images illustrating Ag-TiO_2 and Ag-ZnO nanocrystals. The TEM images of titanium dioxide nanoparticles doped with silver revealed a hexagonal structure of titanium intertwined with a spherical structure of Silver nanoparticles, which were exceptionally small in size (3-4nm). Despite an extended duration, these particles displayed no inclination to amalgamate and form larger particles. It1is noteworthy that merely a small fraction of the particles exhibited a diameter exceeding 30 nm. The prominently visible lattice fringes of crystallites attested to the high crystallinity of the TiO_2 material. Furthermore, the TEM images of Ag-ZnO depicted a distinctly spherical structure varying in

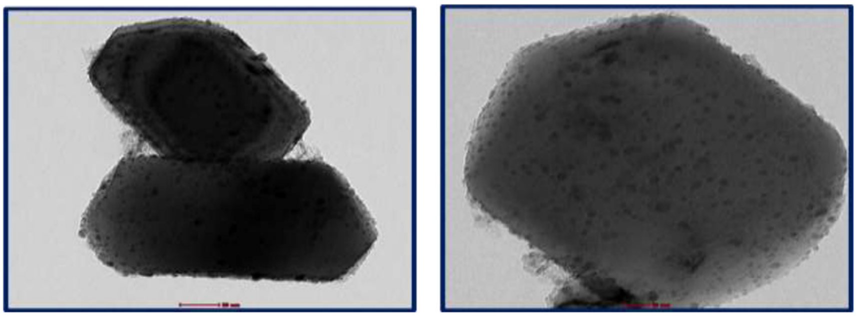

Fig. 5. TEM image of Ag-TiO_2 nanoparticles.

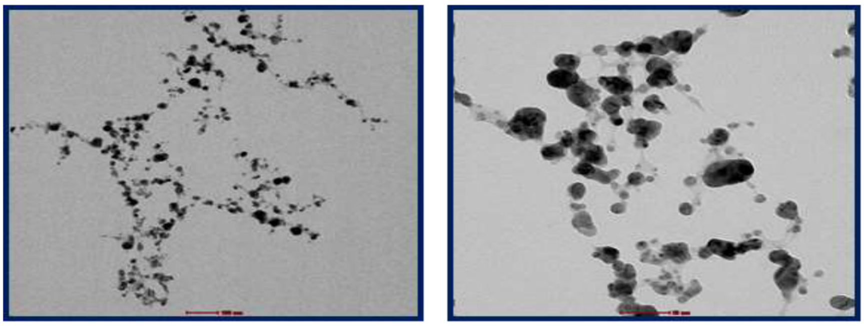

Fig. 6. TEM image of silver dopped zinc oxide nanoparticles.

size from 10 to 20 nm, adhering closely to the edge and the surfaces. Nonetheless, a slight inconsistency in particle sizes was noted between the assessments conducted through XRD analysis and TEM, findings that were similarly reported by Mallick *et al.* (2021). This discrepancy could potentially be ascribed to the relatively diminished precision of the effective mass estimation for minuscule nanoparticles.

Antibacterial activity of synthesized Ag-TiO_2 and Ag-ZnO nanoparticles against *Bacillus subtilis* and *Escherichia coli*

Antibacterial activity of synthesized nanoparticles against Bacillus subtilis

Both nanoparticles that were synthesized exhibited antibacterial properties, with a correlation observed between the inhibition zone and the concentration of nanoparticles ranging from 25 ppm to 100 ppm. The most significant antibacterial effect observed at a concentration of 100 ppm for Ag-ZnO NPs, which successfully inhibited a maximum microbial zone of 12 mm against *Bacillus subtilis* which is significantly very close to the standard antibiotic, this was followed by a zone of 10 mm at 75 ppm concentration, 9 mm at 50 ppm concentration, and the smallest zone of inhibition using Ag-ZnO NPs was recorded at 8.5 mm at a concentration of 25 ppm. Conversely, Ag-TiO_2 nanoparticles exhibited a maximum 8 mm zone of inhibition against *Bacillus subtilis* at a 100-ppm concentration, followed by zones of 7.8 mm, 7 mm, and 6.5 mm at 75 ppm, 50 ppm, and 25 ppm concentrations of Ag-TiO_2 NPs. It was evident that both Ag-ZnO and Ag-TiO_2 nanoparticles displayed heightened activity against the gram-positive bacteria *Bacillus subtilis* (Fig. 7).

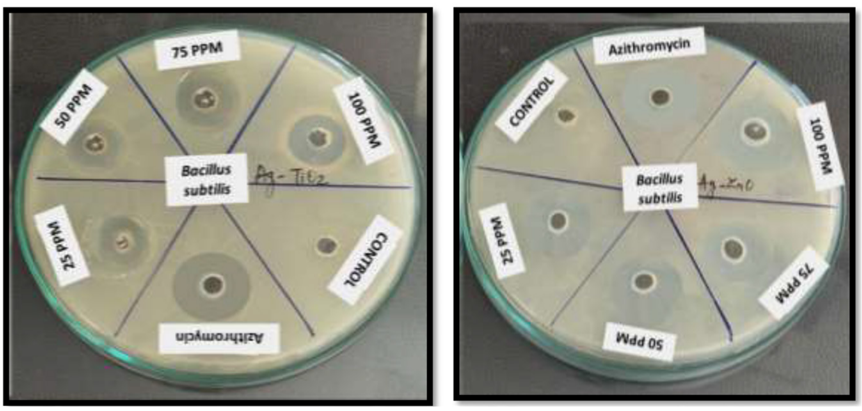

Fig. 7. Antibacterial activity of Ag-TiO_2 and Ag-ZnO nanoparticles against *Bacillus subtilis*.

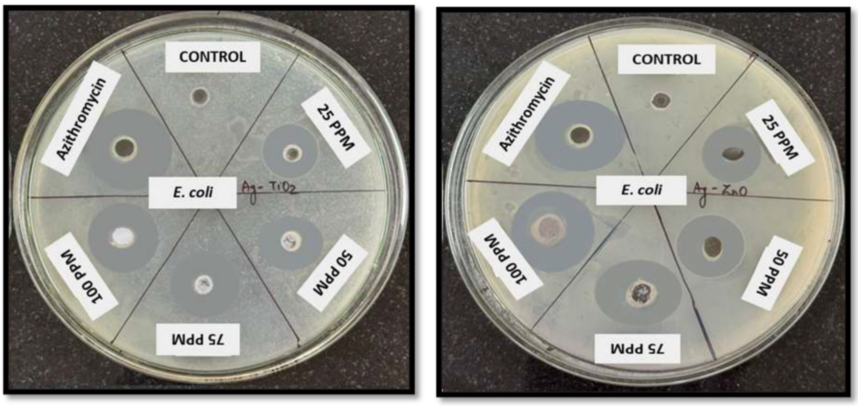

Fig. 8. Antibacterial activity of Ag-TiO_2 and Ag-ZnO nanoparticles against *Escherichia coli.*

Antibacterial activity of synthesized nanoparticles against Bacillus subtilis and Escherichia coli

Similarly to the trend observed in *Bacillus subtilis,* the Ag-ZnO nanoparticles demonstrated the ability to achieve a maximum microbial inhibition zone of 15 mm against *Escherichia coli* (Fig, 8), with a minimum inhibition zone of 9 mm at a dose of 25 ppm. In1the case of doses75 ppm and 50 ppm of Ag-ZnO nanoparticles, microbial inhibition zones of 12 mm and 10 mm were recorded, respectively. In comparison to Ag-ZnO nanoparticles, the activity of Ag-TiO_2 nanoparticles was comparatively lower. Specifically, at a concentration of 100 ppm, Ag-TiO_2 nanoparticles exhibited an inhibition zone of 12 mm, followed by a 10 mm zone at 75 ppm, an 8.8 mm zone at 50 ppm, and the smallest inhibition zone (7.5 mm) was observed at a concentration of 25 ppm.

CONCLUSION

In brief, Ag-TiO_2 and Ag-ZnO were fabricated utilizing the sol-gel technique. The synthesized nanoparticles underwent analysis using XRD, TEM, and EDX. This investigation has the potential to offer fresh perspectives on the formulation and production of nanomaterials, as well as the augmentation of antibacterial efficacy. When juxtaposed with alternative substances, the antibacterial outcomes displayed more preferable results in experiments carried out with identical species. Additionally, Ag-ZnO nanoparticles exhibited superior antibacterial effectiveness in comparison to Ag-TiO_2 nanoparticles. The synthesized Ag-TiO_2 and Ag-ZnO nanoparticles, possessing elevated thermal resistance and potent antibacterial properties, are anticipated to be utilized in pharmaceutical and nanocomposite domains. Our research presented a plausible approach for the advancement of nanomaterials with highly appealing attributes for utilization in antibacterial applications.

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