

## Research Article

Antibacterial activity of Zinc Oxide and Ag doped Zinc Oxide Nanoparticles against *E.coli*

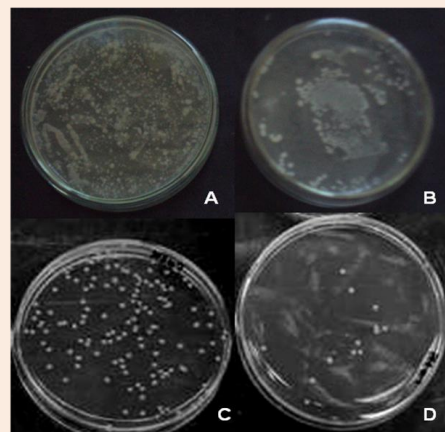
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**Abstract**

This work mainly focuses on the photocatalytic concert and antibacterial activity against *E. coli* using zinc oxide nanoparticles ( $ZnO_{nps}$ ) and silver (Ag) doped ZnO nanoparticles. The antibacterial activity of  $ZnO_{nps}$  was studied at different concentrations such as 0.005%, 0.015%, and 0.025% against *E.coli*. Different concentrations of Ag (1%, 2.5%, 5%, 7.5%, and 12.5%) were doped on ZnO nanoparticles. The antibacterial activity against *E.coli* was investigated under photocatalytic light setup equipped with 250watts halogen lamp. The results show that the ZnO nanoparticles have bacteriostatic activity against *E. coli*. But the Ag doped Zinc oxide nanoparticles showed better antibacterial activity than the ZnO nanoparticles. The overall percentage reduction of *E.coli* also increased in higher catalyst concentration (0.025 %) and 7.5% Ag doped ZnO nanoparticles. The antibacterial activity increases with increasing ZnO nanoparticles concentration.

**Keywords:** Zinc Oxide nanoparticles, *E.coli*, Ag doped ZnO nanoparticles, Antibacterial activity, Photocatalytic activity.

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**Introduction**

Nanomaterials are considered as excellent adsorbents, catalysts and sensors due to their large specific surface area and high reactivity. In recent years, the application of nanoparticles are expanded considerably in various fields such as cell labeling, drug targeting gene delivery, micro electronics, solar cells, electroluminescent devices, detergent and cosmetics. For instance nanoparticles have been examined for their ability to reduce microbial infections and to prevent bacterial colonization. Hence the nanoparticles are called “a wonder of modern medicine”.

Zinc oxide (ZnO) nanoparticles has been received considerable attention in recent years, because of their stability under harsh processing conditions and moreover they are safe materials to human beings and animals [1] [7]. Food and Drug Administration (US) recognized ZnO as safe” (GRAS). ZnO emerged to strongly resist microorganisms as well as spores which are highly resistant to temperature and pressure [6]. There are some reports on the considerable antibacterial activity of CaO, MgO and ZnO, which is attributed to the generation of reactive oxygen species on the surface of these oxides. The advantage of using these inorganic oxides as antimicrobial agents is that they contain mineral elements essential to humans and exhibit strong activity even when administered in small amounts [5].

Sawai *et al.* (1996) proposed that generation of hydrogen peroxide be a main factor for the antibacterial activity, while Stoimenov *et al.* (2002) explained that the binding of ZnO nanoparticles on the bacteria surface due to electrostatic forces could be a mechanism. ZnO can absorb light (UV or visible), which induces a separation of charge, generating a hole ( $h^+$ ) in the valence band and an electron ( $e^-$ ) in the conduction band. At the surface of the excited ZnO particle, the valence band holes attract electrons from water and/or hydroxyl ions, generating hydroxyl radicals ( $OH^\bullet$ ). In addition, electrons can reduce  $O_2$  to produce the super oxide anion  $O_2^{\bullet-}$ . The obtained  $OH^\bullet$  and  $O_2^{\bullet-}$  can induce lipid peroxidation in membranes, DNA damage due to strand breakage or oxidized nucleotides and oxidation of amino acids and protein catalytic centres. Another possibility is destruction of organic material in a direct reaction with positively charged ZnO particles. It was observed that ZnO shows bactericidal properties in the absence of light.

**Table 1** Characteristics of zinc oxide

Properties	ZnO
Appearance	White powder
Molecular Weight	81.39 AMU
Average Particle Size	24–71 nm
Density Bulk	0.25–0.4 g/cm <sup>3</sup>
Density True	5.606 g/cm <sup>3</sup>
Specific Surface Area	4.9–6.8 m <sup>2</sup> /g

Due to the increasing bacterial resistance to classic antibiotics, the investigations on the antibacterial activity of silver nanoparticles have increased [4]. It has been demonstrated that silver is non toxic to human cells at low concentration [8] [3]. Silver species release Ag<sup>+</sup> ions and they interact with the thiol groups in bacteria proteins, affecting the replication of DNA [2]. Based on above, this paper deals with the potent as well as long lasting antibacterial activity of ZnO nanoparticles and Ag doped ZnO nanoparticles towards the gram-negative bacterium *E. coli* under illumination as well as dark condition.

### Materials and Methods

ZnO nanoparticles ( $\geq 50$  nm) and Ag doped ZnO nanoparticles were purchased from Sigma-Aldrich Co. All Glass wares, Pipettes, Cotton swaps were purchased from Merck. All glass wares used in this test was washed with distilled water and then autoclaved.

### Bacterial isolation

The *E. coli* was isolated from drainage water present inside the Madurai Kamaraj University campus. Most Probable Number (MPN) method has most dominated method for identification of *E. coli* contamination in water samples. *E. coli* was further confirmed using selective media plating as well as serious of biochemical tests.

### Antibacterial test

The ZnO nanoparticles in different concentration 0.01, 0.015, 0.02, 0.025, 0.03% were used to find the optimum concentration to inhibit the bacterial growth. The appropriate concentration of ZnO nanoparticles was mixed in the sterilized nutrient broth and it was sonicated for 15 minutes in an ultrasonic bath. 100 $\mu$ l of 24 hrs *E. coli* culture was added to the sonicated broth and allowed to photocatalysis. Photo catalysis was performed in circular beam light setup equipped with 250 watts halogen lamp for 40 minutes. After 40 minutes the sample containing nutrient broth was taken out and serially diluted using sterile water. From the above diluted sample 50  $\mu$ l was inoculated in nutrient agar by spread plate method. These plates were incubated at 37°C for 24 hours. Then the plates were visually observed and bacterial colonies were counted by colony counter. Analyses were in duplicates and control runs were carried out each time under the same illumination conditions but without any photocatalytic materials. Similar tests were also performed in the dark in presence of the photocatalyst for comparison.

The experiments were also carried out for ZnO particles doped with different concentration of silver (Ag doped ZnO) in similar manner of both light and dark condition. From the obtained results antimicrobial activity was calculated using the formula.

Antimicrobial activity is defined as follows

$$R (\%) = (B - C) / B \times 100 \% \quad (1)$$

where,

**R** is considered as antimicrobial efficiency,

**B** is the average bacteria counts under dark condition

**C** is the average bacteria counts under illumination conditions

### Results and Discussion

#### Isolation and identification of *E. coli*

The sewage samples were collected from the drainage in Palkalainagar, Madurai Kamaraj University. *E. coli* contamination was identified in collected sewage water using Most Probable Number (MPN) method. Further the sewage samples were serially diluted and inoculated in the selective medium for *E. coli* known as Eosin Methylene

Blue (EMB). The metallic sheen colour colonies were observed confirming the presence of *E.coli*. For further confirmation, gram staining as well as biochemical tests was performed. Pink coloured rod shape morphology was appeared as results of gram staining. From the biochemical tests isolated bacterial strain was identified as *E.coli*.

**Table 2** Biochemical test for isolated bacterial strain

Test	Results
Indole test	Positive
Methyl red test	Positive
Voges Proscure test	Negative
Citrate utilization test	Negative
Catalase test	Negative
Urease test	Negative
Oxidase test	Negative

### Optimization of ZnO concentration

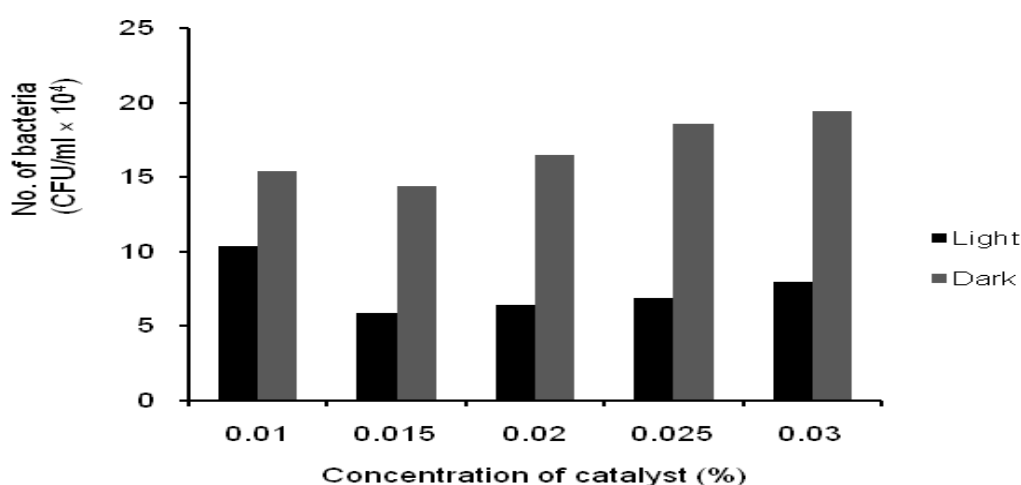
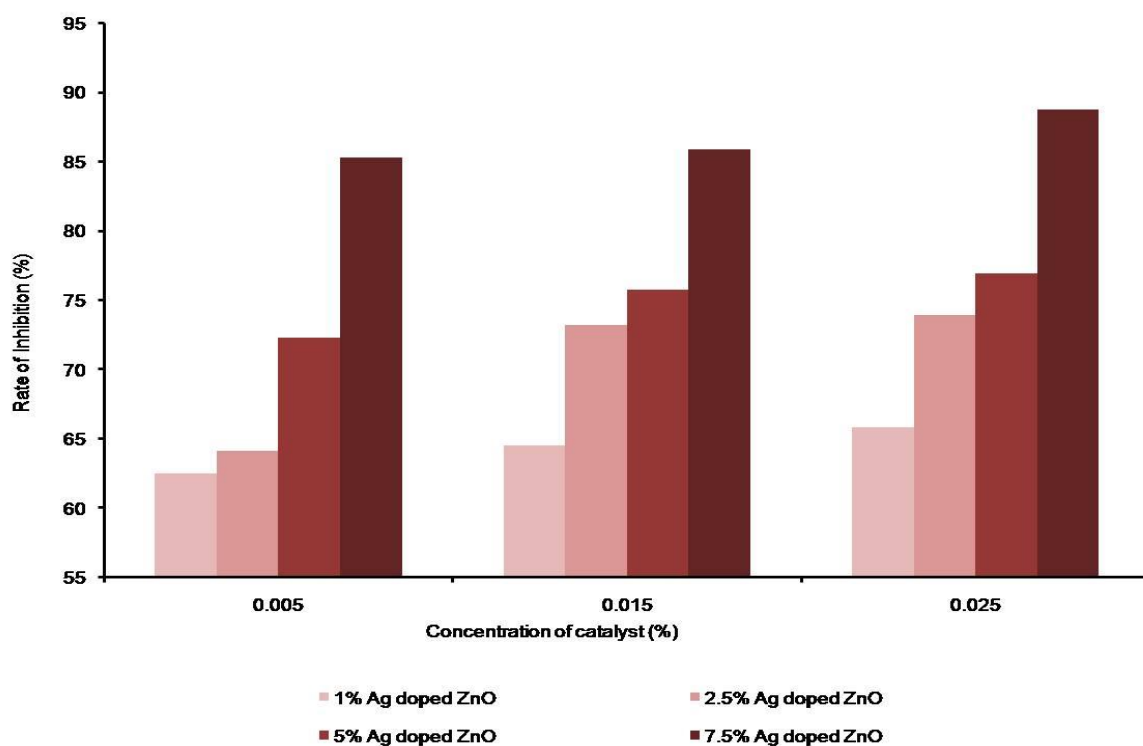


Figure. 1 Optimization of ZnO concentration for antimicrobial activity

Higher antibacterial activity range was observed between 0.005% and 0.025%, whose antibacterial efficiency range was from 59% to 62%. Generally, smaller concentration and higher surface area gives better antibacterial activity. The colony forming units (CFU) has been observed to reduce significantly with the increasing the Ag loading on the ZnO nanoparticles (Fig. 1).

### Doped ZnO nanoparticles

The silver nanoparticles were doped in the ZnO nanoparticles (Ag doped ZnO nanoparticles) in different concentration such as 1%, 2.5%, 5%, 7.5% and 12.5%. Antibacterial efficiency of five different Ag doped ZnO nanoparticles were tested for the above optimized concentrations such as 0.005%, 0.015% and 0.025% in both light and dark conditions. The results showed that 1% Ag doped ZnO nanoparticles had the considerable reduction in bacterial count over dark condition at higher catalyst concentration of 0.025%. The corresponding efficiency of antibacterial activity found between 62 and 65%. Similarly 2.5% of Ag doped ZnO nanoparticles exhibits reduction in bacterial count at their higher concentration (0.025%) as 1.8 CFU/ml in light and 6.9 CFU/ml in dark. The overall percentage reduction also increased to nearly 74 percent. In the same way, in 5% as well as 7.5% Ag doped ZnO nanoparticles exhibited improved reduction in the bacterial count at higher catalyst concentration when compared to lower concentration. In all cases higher antibacterial activity was observed under light condition than dark. The overall percentage reduction also increased to nearly 88% in higher catalyst concentration (0.025 %) and 7.5% Ag doped ZnO nanoparticles (Fig.2).

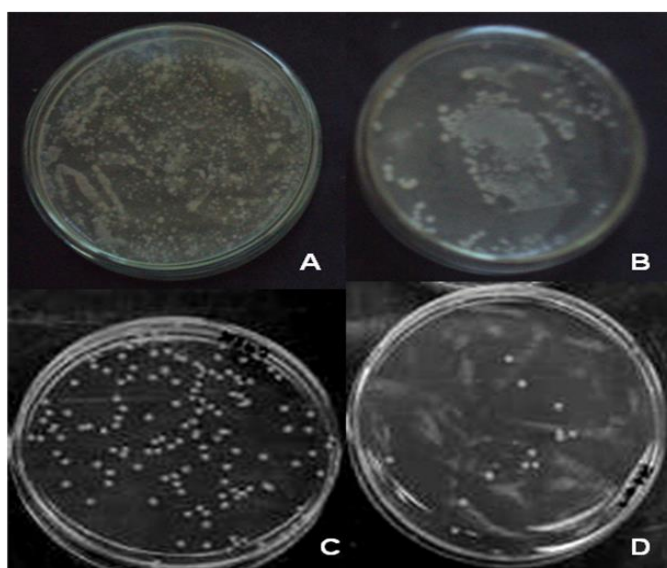


**Figure. 2** Antimicrobial effect of different concentration of Ag doped ZnO particles

**Table 3** Antimicrobial activity of 12.5% Ag doped ZnO nanoparticles

Sl. No.	Concentration of catalyst	CFU/ml (light condition)	CFU/ml (dark condition)
1.	0.005%	TLTC	$12.4 \times 10^4$
2.	0.015%	TLTC	$10.8 \times 10^4$
3.	0.025%	TLTC	$9.4 \times 10^4$

*TLTC – Too Low To Count*



**Figure. 3** Decrease in the number of feasible *E.coli* bacteria after exposure to different concentration of 7.5% Ag doped ZnO particles. A) Control, B) 0.005%, C) 0.015% , and D) 0.025%.

## Conclusion

The antibacterial activity of ZnO nanoparticles depends on its size, surface area and concentration. The inhibitory effects increase as the concentration of ZnO nanoparticles increased. The Ag doped ZnO nanoparticles have strong and great antibacterial activity when compared with ZnO nanoparticles against *E.coli*. The Ag (7.5%) doped ZnO nanoparticles inhibit the growth of *E.coli* more than 88%. From these studies it is concluded that the Ag doped nanoparticles effectively inhibit the *E.coli* than the ZnO nanoparticles.

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## Reference

- [1] Fu L, Liu Z, Liu Y, Han B, Hu P, Cao L, Zhu D (2005). Beaded Cobalt oxide nanoparticles along carbon nanotubes: towards more highly integrated electronic devices. *Advanced Materials*, Vol.17, pp., 217-221.
- [2] Marini M, Niederhausern N, Seppi RI, Bondi M, Sobia C, Toseli M, Pilati F (2007). Antibacterial activity of plastics coated with silver doped organic – inorganic hybrid coatings prepared by sol-gel processes. *Biomacromolecules*, 8:1246–1254.
- [3] Pal S, Tak Y, Song J (2007). 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* 73:1712–1720.
- [4] Panacek A, Kvitek L, Pucek R, Kolar M, Vecerova R, Pizurova N, Sharma V, Nevecna T, Zboril R (2006). Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J Phys Chem B* 110:16248–16253.
- [5] Sawai J and Yoshikawa T (2004). Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. *J Appl Microbiol* 96: 803.
- [6] Sawai J, Igarashi H, Hashimoto A, Kokugan T, Shimizu M (1996). Effect of Particle Size and Heating Temperature of Ceramic Powders on Antibacterial Activity of Their Slurry. *Journal of Chemical Engineering of Japan*, vol. 29: 288–93. *J Chem Eng* 29: 556.
- [7] Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ (2002). Metal oxide nanoparticles as bactericidal agents. *Langmuir* 18:6679–6686.
- [8] Zhang LL, Jiang YH, Ding YL, Povey M, York D (2007). Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). *J Nanopart Res*, 9, 479–489.

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## Publication History

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