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ORIGINAL RESEARCH PAPER

Investigation of the antimicrobial effect of silver doped Zinc Oxide nanoparticles

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ABSTRACT

Objective(s): The antimicrobial effect of metal nanoparticles such as zinc oxide and silver nanoparticles has been taken into great consideration separately during recent years. The useful application of these nanoparticles in the areas of medicine, biotechnology, and professional prevention of microbes motivated us. The aim of this study was to evaluate antibacterial activity properties of silver doped zinc oxide nanoparticles (ZnO: Ag) by synthesizing them.

Materials and Methods*:* The silver doped zinc oxide nanoparticles (ZnO:Ag) were provided with wet chemical method in an aqueous solution, and mercaptoethanol. The physical properties of the sample were investigated with UV, XRD, and TEM techniques. Then, the antibacterial activity of 50 to 3.12 concentrations of the silver doped zinc oxide nanoparticles (ZnO:Ag) was investigated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* by well diffusion method. Moreover, the MIC and MBC values of these nanoparticles were assessed by microdilution method.

Results: The size of the nanoparticles was obtained as between 12 and 13 nanometers in average. The optical study of the nanoparticles demonstrated that the band gap of the silver doped nanostructures is higher than that of the pure sample. The zone of inhibition diameter in the presence of 50 mg/ml density was 19, 15 and 8 mm against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively.

Conclusion*:* The results showed that silver doped zinc oxide nanoparticles prevented *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, but did not affect *Enterococcus faecalis*. The zone of inhibition diameter increases as the density of the nanoparticles does.

Keywords: *Antibacterial activity, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*

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INTRODUCTION

Recent advances in the field of nanotechnology, in particular, the ability of nanoparticle synthesis in different shapes and sizes, have led to the production of a wide range of antimicrobial agents. Materials in nanoscale have a higher surface to volume ratio than larger particles with the same chemical composition, and this makes them biologically more active [1]. Nanoparticles are concerned by scientists due to their unusual optical, chemical, electrical, and photoelectrochemical properties.

It is known that many heavy metals kill bacteria in very low concentrations. Damaging DNA and degradation of proteins and cell wall are the main mechanisms of the effect of nanoparticles on bacteria [10]. Antimicrobial activity of silver compounds and zinc oxide (ZnO) is known from the very distant past and has many applications in disinfecting medical devices, water purification, and wound healing, creams, lotions, and antibacterial creams [3]. Zinc oxide is one of the nanoparticles which is used in an

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industrial scale in many countries. The mechanism of action of zinc oxide is similar to other nanoparticles, but it acts mostly through destruction of bacterial walls. Given this feature, zinc oxide nanoparticles have been widely used against Gram-positive and Gram-negative bacteria [10]. Silver nanoparticles possess also these properties for many Gram-positive and Gram-negative bacteria and do not damage human and mammals cells [10]. According to research, silver nanoparticles have more antibacterial activity compared to other metal nanoparticles.

Although various theories have been proposed as the mechanism of silver nanoparticles anti-microbial activity, it is widely believed that they penetrate into the membrane of bacterial cell and result in the leakage of intracellular material and ultimately in bacterial cell death. It is reported that the antibacterial effects of silver nanoparticles are influenced by the shape of particles and the type of organisms [7].

Many studies have been performed on the synthesis of ZnO:Ag in different ways. In this study, the chemical inhibitor method was used to produce high purity products at room temperature. The method is based on inhibiting the prevention of the particles growth and agglomeration during the reaction through creating an envelope around them. The antibacterial effect of these nanoparticles was also investigated against four standard strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*.

MATERIALS AND METHODS

Materials

Zinc chloride, silver nitrate, sodium hydroxide, and mercaptoethanol were used as inhibitors to synthesize nano-ZnO:Ag [2]. *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* were prepared from the Iranian Research Organization for Science and Technology. Solid Mueller Hinton Agar (MHA) and liquid Muller Hinton Broth (MHB) culture media were used for microbial testing.

Silver doped ZnO nanoparticles

Silver doped ZnO nanoparticles (ZnO:Ag) were developed through the wet chemical method as follows, $ZnCl_2$ was stirred in distilled water in a triple neck flask. Then the doping substrate solution was dripped into the flask through a Soxhlet. The substrate in this study was $AgNO_3$. As the inhibitory factor, mercaptoethanol was dripped to the main solution and finally, NaOH was added. Upon completion of synthesis, the solution was washed several times with distilled water and then centrifuged to remove the alkaline metal salt. The obtained precipitate was poured into a plate and incubated in a 40 °C oven for 24 hours to completely remove moisture and produce nano-ZnO:Ag containing white powder [5].

Determining the structure of silver doped ZnO nanoparticles

The prepared sample was examined with UV-visible spectroscopy (UV-Vis) (UV-2600 model TCC-240A), and X-ray diffraction (XRD) (Phillips PW3040, Netherland) was performed on nanoparticles. To ensure the formation of nano-ZnO:Ag nanoparticles, the sample was studied with transmission electron microscope (TEM) [1, 8].

Microbiological tests

Standard strains of *E. coli* (ATCC: 25922), *S. aureus* (ATCC: 25923), *P. aeruginosa* (PTCC: 1555), and *E. faecalis* (ATCC: 29212) were prepared from the Iranian Research Organization for Science and Technology. The bacteria were cultured for 24 hours, and a concentration equivalent to 0.5 McFarland (1.5×10^8) was prepared and diluted at a ratio of 1/9 in MHB culture medium to obtain a turbidity equal to 1.5×10^7 bacteria per mL. The bacterial suspension was then cultured on MHA medium using a sterile swab through the sweeping method [9].

The desired concentrations of ZnO:Ag nanoparticles were prepared by serial dilution with distilled water and were injected into the wells created on agar surface. Then the plates were incubated at 37 °C and the diameter of inhibition zone was measured (12). Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the nanoparticles were measured using the microdilution method.

Statistical analysis

All experiments were repeated at least three times and the obtained data were analyzed with SPSS. The Kruskal-Wallis test was used to compare the bacterial growth inhibitory zone between different concentrations, and $p>0.05$ was considered significant.

RESULTS AND DISCUSSION

UV-Visible Results

Fig. 1 shows the results of UV-Vis spectroscopy of zinc oxide doped with 10% silver.

RESULTS

X-ray diffraction

Fig. 2 depicts the X-ray diffraction pattern for the sample of 10% silver doped zinc oxide nanoparticles. The nanoparticles size was calculated using the Debye-Scherrer equation (1) which is:

1. $D = (0.9\lambda)/\beta\cos\theta$

Where D is the crystallite size, λ the wavelength of copper X-ray corresponding to 1.504 A°, θ Bragg angle, and β the width at half maximum peak height. It was found less than 5nm for all samples.

Results of imaging by transmission electron microscopy

Fig. 3 shows the results of analysis of transmission electron microscopy (TEM) for 10% silver doped zinc oxide nanoparticles.

It can be seen that, there is a uniform size distribution approximately in whole of the photograph. It means that the synthesis manner has been suitable. The size of the particles was determined around 12 nm from TEM photograph.

Microbial test results

Table 1 shows the results of impact of silver doped zinc oxide nanoparticles against *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* through the well diffusion method.

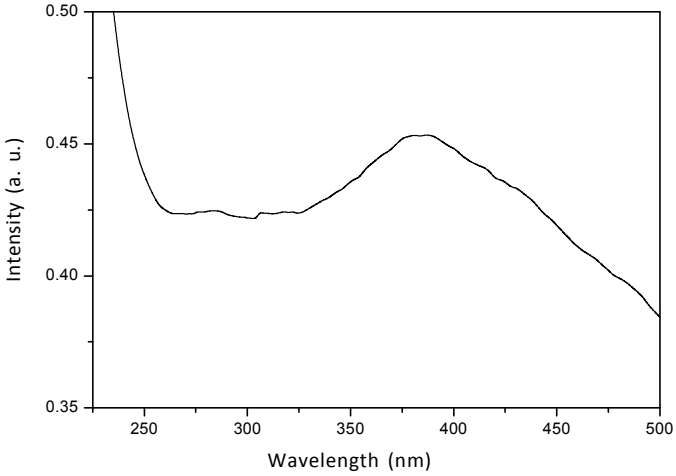

Fig. 1. Results of UV-Vis of 10% silver doped zinc oxide nanoparticles

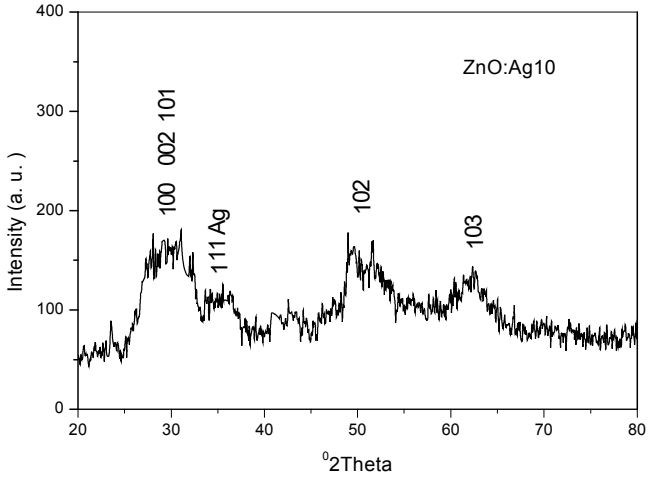

Fig. 2. XRD results for 10% silver doped zinc oxide nanoparticles

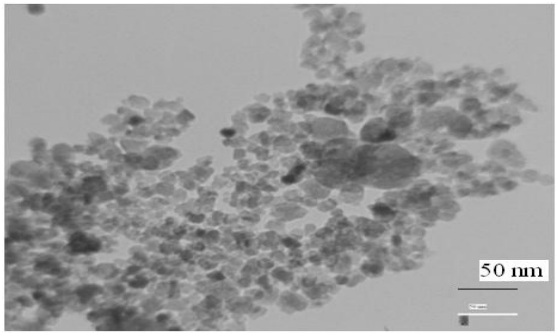

Fig. 3. Image of transmission electron microscope (TEM)

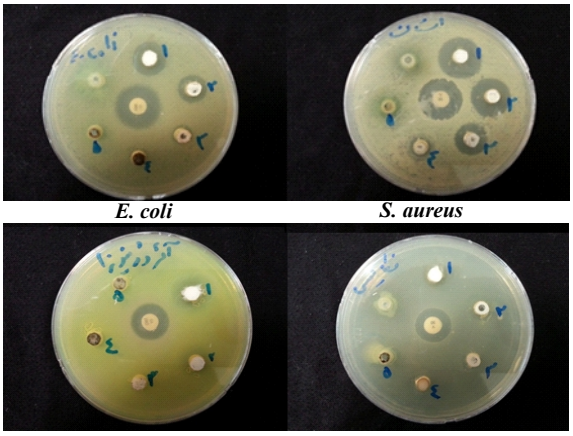

Fig. 4. shows the inhibition zone of ZnO:Ag nanoparticles

Fig. 4. Anti-bacterial feature of nano-ZnO:Ag on *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecalis*

Table 2 shows the results of determination of MIC and MBC of nano-ZnO:Ag up to 100 mg/mL against the desired bacteria using the microdilution method.

Results of statistical analysis

Table 3 shows the results of descriptive indices for values of inhibition zone diameter at different concentrations of ZnO:Ag nanoparticles for the studied bacteria.

Based on the results presented in Table 3, the mean inhibition zone diameter was reduced by reducing the concentration, so that the highest and the lowest diameter were seen in 50 mg/mL and 3.12 mg/mL, respectively.

The results of Bonferroni adjusted Mann-Whitney Post hoc test showed no significant difference between the diameter of inhibition zone in 50 mg/mL

Table 1. The diameter of inhibition zone of the studied bacteria at different concentrations of ZnO:Ag Nanoparticles (mm)

Concentration (mg/mL)	50	25	12.5	6.25	3.12	Control*
E. coli	15	14	11	6	6	16
S. aureus	19	18	14	11	6	14
P. aeruginosa	8	6	6	6	6	13
E. faecalis	6	6	6	6	6	13

* Vancomycin (V) and gentamicin (GM) were used as controls for Gram-positive and gram-negative bacteria, respectively.

Table 2. MIC and MBC of ZnO:Ag nanoparticles(mg/mL)

	MIC	MBC
E. coli	3.12	3.12
S. aureus	3.12	3.12
P. aeruginosa	6.25	25
E. faecalis	6.25	-

Table 3. Mean of inhibition zone diameter of the studied bacteria at different concentrations of nano ZnO:Ag(mm)

Nanoparticles concentrations (mg/mL)	*E. coli*	*S. aureus*	*P. aeruginosa*	*E. faecalis*
50	15.00±0.00	19.33±0.58	8.67±2.31	8.67±3.06
25	13.33±0.58	18.33±0.58	6.00±0.00	6.00±0.00
12.5	11.67±0.58	15.67±1.53	6.00±0.00	6.00±0.00
6.25	6.00±0.00	13.00±2.00	6.00±0.00	6.00±0.00
3.12	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
Positive control	17.00±2.65	15.33±2.31	13.33±1.53	14.67±2.08

E. coli and the positive control group (p>0.05) and the inhibition zone diameter was significantly higher at 25 mg/mL and 12.5 mg/mL compared to 6.25 mg/mL and 3.12 mg/mL.

Results for *S. aureus* showed a significant difference between inhibition zone values in different concentrations (p<0.05).

The diameter of inhibition zone at nanoparticles concentrations of 50 mg/mL and 25 mg/mL had no significant difference (p<0.05).

The inhibition zone diameter at concentration 50 mg/mL was significantly higher than those of 12.5, 6.25, and 3.12 mg/mL and the positive control group. The results were identical for *P. aeruginosa* and *E. faecalis* and significant differences existed between the values of inhibition zone between different concentrations (p<0.05). The results of Bonferroni adjusted Mann-Whitney Post hoc test showed that the inhibition zone diameter in all concentrations was significantly less than the diameter of the positive control group (p<0.05).

CONCLUSION

ZnO:Ag nanoparticles were prepared by wet chemical synthesis and mercaptoethanol was used as an inhibitor. The average size of nanoparticles crystallites was obtained 4-5 nm using the Debye-Scherrer equation [1]. Optical studies of nanoparticles showed that the band gap of silver doped nanostructures was bigger than the pure sample. According to transmission electron micrographs of silver doped zinc oxide in Fig 3, the particle size was 12-13 nm. The study also showed that 10% silver doped zinc oxide nanoparticles can reduce the growth of *E. coli*, *S. aureus*, and *P. aeruginosa* and has no effect on *E. faecalis*. With increasing concentrations of nanoparticles, the growth of bacteria is dramatically inhibited.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript.

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