Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.810356

52 (2014) 4969–4976
July

Taylor & Francis
Taylor & Francis Group

Antimicrobial potential of synthesized zinc oxide nanoparticles against gram positive and gram negative bacteria

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Received 3 August 2012; Accepted 7 May 2013

ABSTRACT

Antimicrobial potential of synthesized zinc oxide (ZnO) nanoparticles against four bacteria strains (*Escherichia coli ATCC* 25922 and *Pseudomonas aeruginosa ATCC* 27853, as gram negative bacteria, *Staphylococcus epidermidis PTCC* 1114 and *Staphylococcus aureus ATCC* 25923 as gram-positive bacteria) in liquid and solid phases was studied in this work. Firstly, nanoparticle ZnO was prepared by the chemical method with an organic chemical inhibitor. The detail characterization of the nanoparticles was carried out using UV–vis spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) analysis. From SEM image analysis, the average particle size was found to be 50 nm. Also, the mean surface area was determined as $90\,m^2\,g^{-1}$ by Brauner–Emmet–Teller (BET) analysis. We studied the antibacterial assay, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and disk diffusion method as per CLSI recommendations and time–kill studies were performed. Disk diffusion studies revealed greater effectiveness for *P. aeroginosa*. In vitro time–kill studies were performed for one or two times; the MICs and its results showed that the efficiency of particles increases with rising particle dose in suspension and treatment time. From this work, it is possible to suggest that ZnO nanoparticles are excellent antibacterial agents.

Keywords: Antimicrobial characteristic; ZnO nanoparticle; MIC; MBC; Time–kill

1. Introduction

Nanoparticles (1–100 nm), comparable to the size range of biological molecules and their structures, are very attractive for using in many sciences [1]. By reducing particle sizes to the nanoscale, their surface reactivity increases. For example, Nel and colleagues reported that by reducing particle sizes to 30, 10, and 3 nm, the average number of atoms represented on the

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particle surface changed from 1% in bulk samples to 10, 20, and 50%, respectively [2].

The unique properties of nanoparticles have led to their dramatic use [3]. Some nanoparticles such as Ag, copper oxide, and zinc oxide (ZnO) can have numerous physical, biological, biomedical, and pharmaceutical applications [4–6]. The toxicity of nanoparticles is generally superior to larger particles of the same materials, even for materials with relatively low toxicity [7,8]. Therefore, developing nanoparticles with antimicrobial properties is of considerable interest.

Antibacterial agents are categorized as organic and inorganic according to their chemical composition. Inorganic antimicrobial materials are made of heavy metal ions that have biocidal action (such as TiO_2, MgO, CaO, and ZnO) [9,10]. The stability of organic antibacterial materials is lower at high temperatures or pressures compared with inorganic antibacterial agents [9,10]. Therefore, ZnO is a strong antibacterial agent in formulating microscale and nanoscale systems for therapeutic applications [1,11].

ZnO particles have bactericidal effects on both gram-positive and gram-negative bacteria, and have antibacterial activity against spores which are resistant to high temperatures and pressures [11]. The surface area and concentration play an important role in the antibacterial activity of ZnO nanoparticles and have a direct relationship with it. However, the crystalline structure and the particle shape have little effect on antibacterial activity [3]. Moreover, smaller ZnO particles have higher antibacterial activity.

The factors distinguishing ZnO from other materials is its semiconductor characteristic, with a wide band gap (3.3 eV), large excision binding energy, its abundance, as well as being environmentally friendly [1,6]. Therefore, it can have a variety of applications and can be used in solar cells, optical coatings, photocatalysts, electrical devices, as well as having antibacterial properties and as active medium in UV semiconductor lasers and gas sensors [12,13]. Because of the recent development of some resistant bacterial strains against antibiotics, researchers are investigating the antibacterial activity of nonmaterial.

The bactericidal properties of ZnO nanoparticles result from the electrostatic interaction between the nanoparticles and the cell surface [3]. This increased the interaction and, therefore, cell damage. As a result of the production of reactive oxygen species, metabolic processes are disrupted because of the toxic effects of nanoparticle channels and their bind with different organelles. Thus, ZnO nanoparticles act as strong antimicrobial agents [14,15]. Investigations regarding the enhancement of antimicrobial potential of synthesized ZnO nanoparticles were reported previously [16–19]. However, limited information is available for the removal efficiency and antimicrobial potential of synthesized ZnO nanoparticles.

The present study investigated the antimicrobial potential of synthesized ZnO nanoparticles against gram-positive and gram-negative bacteria. In the present study, we tried the report to antibacterial activity of synthesized by using an easy, economical and environment-friendly method. In addition, prepared nanoparticles were used against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. Most *E. coli* strains are not pathogenic, but some serotypes can cause serious food poisoning in humans. Metal oxide is used as a food preservative agent instead of organic compounds. *P. aeruginosa* is a common bacterium that can cause disease in animals, including humans. *S. epidermidis* is one of the leading pathogens of nosocomial infections, particularly associated with foreign body infections. *S. aureus* is a common cause of skin infections, respiratory disease, and food poisoning.

2. Experimental details

All chemical reagents such as zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$, 99.5%; Merck company, Germany), NaOH, HCl, KOH, 2-merceptoetanol, and culture medium were of analytical grade (Merck company) and were used without further treatment.

2.1. Synthesis of nanoZnO

Synthesis was done similar to Shirzad Siboni and colleagues' methods [20] using zinc sulfate and sodium hydroxide as precursors (Fig. 1). So a stock solution (pH 5.3) of 0.2 M zinc sulfate ($ZnSO_4$) was prepared by the dissolution of zinc sulfate

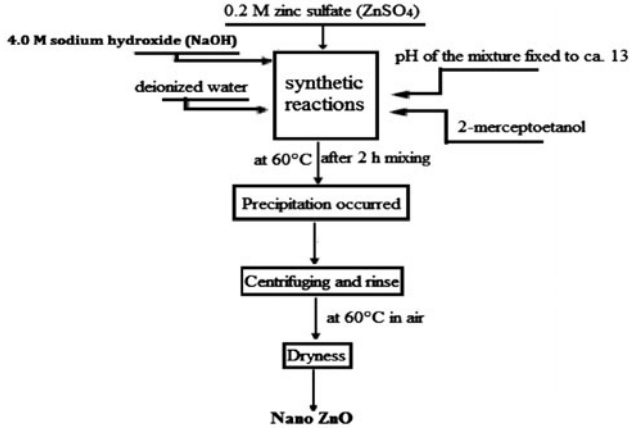


Fig. 1. Schematic diagram of the preparation of ZnO nanoparticles.

heptahydrate ($ZnSO_4{\cdot}7H_2O$, 99.5%; Merck Company) into deionized water. An alkaline stock solution (pH 13.8) of 4.0 M sodium hydroxide (NaOH) was prepared by the dissolution of NaOH into deionized water. The synthetic reactions were done by stirring the stock solution of $ZnSO_4$, the alkali solution of NaOH, and a certain amount of deionized water together. The final pH of the mixture was fixed to Ca. 13. Precipitation occurred at 60˚C after mixing the solutions for 2 h. The produced white product was separated by centrifugation, washed with deionized water, and then dried at 60˚C in air. We used 2-merceptoetanol, because it can prevent particle growth and we could obtain a uniform distribution of particle size [21,22].

2.1.1. Nanocharacterization

Metallic particles were characterized through different techniques. The optical property of the nanoparticle was surveyed at 200–800 nm scanning range using a UV–vis spectrophotometer. Baseline correction of the spectrophotometer was carried out using a blank reference. A nano product sample was studied using X-ray diffraction technique (XRD, Siemens D5000) using CuKα radiation. In this study, the SERON- ATS-2,100 model of SEM was used to characterize mean particle size and morphology of the nanoparticles. In order to determine specific surface area the BET isotherm and Belsorb software (Version 5) (BEL, Japan, Inc) of Karaj Energy and Material Research institute was used. Nitrogen isotherm for the produced ZnO nanoparticle was done at 77 K (boiling temperature of N_2 in 1 atmospheric pressure) and in a relative pressure of 0.001–1 from N_2.

2.1.2. Ion release test

0.1 g of synthesized ZnO nanoparticles was placed in polypropylene bottles, which were filled with 20 mL of tryptone soya broth (TSB) medium and shaken for 24 h at 37˚C. Zn(II) concentrations in the solutions were then measured by ICP-AES spectrometry (Perkin–Elmer In- ICP-AES) at a wavelength of 213.9 nm.

2.1.3. pH_{zpc} determination

In order to determine pH_{ZPC}, the equilibrium technique was applied in a batch system [23]. This technique is based on estimating H + and OH− which are the ions determining the electric potentials. About 0.1 g of ZnO nanoparticle was mixed in the incubator machine with 20 mL of KNO_3 solution in a sealed container for 24 h. Primary pHs were adjusted using the 0.1 M KOH and HNO_3 in the pH range of 2–12. The final pH of each container was measured using the pH meter (Hach Co., USA) and their amounts were drawn in the primary pH.

2.2. Preparation of nanoparticle suspensions

The synthesized ZnO NANOPARTICLES were added to 100 mL of double distilled water to obtain a final concentration of 10 g/L and shaken vigorously and dispersed by ultrasonication.

2.3. The bactericidal experiments

The bactericidal experiments were carried out with gram-negative (*E. coli ATCC* 25922 and *P. aeruginosa ATCC* 27853) and gram-positive (*S. epidermidis PTCC* 1114 and *S. aureus ATCC* 25923) bacteria in nutrient media. Bacterial strains were obtained from the collection of the Department of Microbiology at Hamedan University of Medical Sciences.

2.3.1. Disk diffusion test

Bacterial sensitivity to antibiotics is commonly tested by disk diffusion method, using antibiotic impregnated disks [3]. We used similar test with nanoparticle laden disks. Nanoparticle suspension at serial dilution concentrations (11 dilutions from 10,000 μg/mL) was sonicated and subsequently filtered through a membrane filter. The nanoparticle laden filter paper was dried in an oven for 1 h and small disks of uniform size (6 mm diameter) were punched out and stored in a desiccator at room temperature. The bacterial suspension (10^8 CFU mL^{-1}) was applied uniformly on the surface of a Muller–Hinton agar plate before placing the disks on the plate (four per plate). The plates were incubated at 35˚C for 24 h, after which the average diameter of the inhibition zone surrounding the disk was measured with a ruler with up to 1 mm resolution. The mean and standard deviation (SD) reported for each type of nanoparticle and with each microbial strain were based on three replicates.

2.3.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The MIC is defined as the minimum inhibitory concentration of antibiotic sufficient to prevent bacterial growth in vitro. The MICs were determined using

the broth dilution technique as recommended by CLSI [24]. Briefly, serial dilutions of ZnO nanoparticle (10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125, 39.0625, 19.53, 9.765, and 9.883 μg/mL) in 2 mL TSB broth were prepared. Bacterial suspensions were then added to each tube to achieve final inoculums of 8 logs CFU/mL. Tubes containing growth media alone or bacterial culture without ZnO nanoparticle suspension were included as negative and positive controls, respectively. The cultures were inspected for bacterial growth after incubation at 37°C for 24 h and the MICs of ZnO nanoparticle suspension were recorded for each bacterial strain. The MBC was defined as the lowest concentration of ZnO nanoparticle suspension that resulted in more than 99.9% reduction of the initial inoculums.

2.3.3. Time–kill study

A bacterial concentration of 8 log colony-forming units (CFU/mL) was used in the time–kill experiments. Test tubes containing TSB with ZnO nanoparticle suspension at 1 and 2 × MICs were inoculated with overnight cultures of each bacteria strain. The cultures were then incubated in a shaker incubator at 37 °C for 1, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min. At the end of each time period, ten-fold serial dilutions were prepared with PBS and were plated onto Mueller–Hinton agar plates in triplicate. The CFU for each strain at different time points were counted after 18 h. Plates with 30–300 colonies were used for CFU counts.

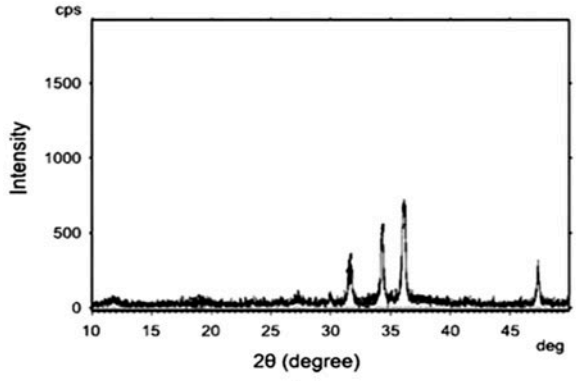
Data were expressed as mean ± SD. Data were analyzed using analysis of variance (ANOVA) using SPSS software, version 16. Significance was expressed at the 5% level.

3. Results and discussion

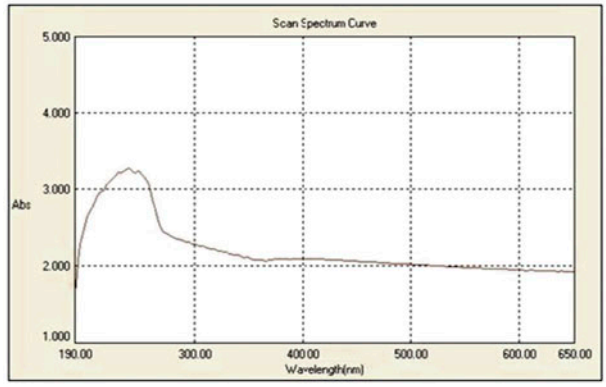
3.1. Characteristics of the ZnO nanoparticles

SEM micrograph and typical XRD of the nanoZnO are shown in Figs. 2(a) and (c). As shown, the particles were found to be almost spherical. XRD analysis of the samples showed that the particles had no impurity.

The mean surface area was calculated as $90\,m^2\,g^{-1}$. A mean particle size can then be estimated if the particles are assumed to have the same spherical shape and size dimensions. The average particle diameter (D) can be calculated by the equation used by Rena et al. [25]: $D = 6/(S \times \delta)$, where S = surface area and δ = density (the density of ZnO is 5,606 $kg\,m^{-3}$). Thus, the ZnO nanoparticle size was on average



(a)



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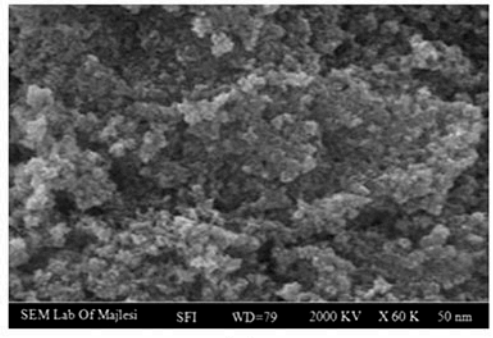

(c)

Fig. 2. (a) XRD patterns of ZnO nanoparticles, (b) UV–vis spectra of the ZnO nanoparticles, and (c) SEM micrograph of ZnO nanoparticles synthesized chemically and used in this study.

11.89 nm. [$D = 6/(90 \times 1{,}000 \times 5{,}606) = 11.89$ nm]. This was smaller than obtained from SEM analysis (50 nm). It is believed that these larger particles are composed of van der Waals clusters of smaller entities [26].

Fig. 2(b) shows UV–vis absorption spectra of ZnO nanoparticles, immediately after ablation, prepared by different laser fluencies. The absorption spectra have a semi sharp peak centered approximately at 245 nm

with a long tail towards a long wavelength. This peak is the characteristic of ZnO formation. The tail may be caused by the scattering of a range of particle sizes and some type of Urpach effect because of the intergrain depletion regions [27] that confirms the nanocrystalline character of the particles and the low degree of their polydispersity. The monodispersity of the synthesized particles is confirmed by the sharp absorption edges and the well-developed excitonic peaks [28].

Experimental results of the pH_{PZC} determination are illustrated in Fig. 3. They are given as pH values of filtered solutions after equilibration (pH_f) with ZnO as a function of initial pH values (pH_i) of the solution. It can be seen that PZC of ZnO nanoparticles is at pH 7.51.

The Zn^{2+} ion released into the aqueous phase was estimated for nanoparticles suspended in nutrient media similar to the antibacterial assay. According to the results, fractions of released zinc ions of ZnO nanoparticle have an average of 1.75%. The nutrient media might have expedited Zn^{2+} most probably because of the oxide layer on the ZnO nanoparticles and its reaction with the nutrient media [3]. The presence of nanoparticles in the suspension is important because it would continuously release ions into the nutrient media and increase the antibacterial activity of the ZnO nanoparticle because it attacks the bacterial cell wall and ruptures it [26]. On the other hand, the antibacterial activity of the nanoZnO may be related to attachment of both zinc ions and nanoparticles to the cell wall.

3.2. Antibacterial activity assay

In our study, the antibacterial activity of ZnO suspensions of particles towards gram-positive and negative bacteria was studied quantitatively by disk diffusion and time–kill study, and qualitatively in terms of MIC and MBC. Bacterial growth was studied by visually inspecting the broth medium for turbidity. If the material being tested does not kill but instead inhibits the growth of bacteria (bacteriostatic agent), the bacteria will grow when it is removed from the solution containing the material, and colonies will be observed when plating an aliquot. If the material being tested is bactericidal, the absence of bacterial colonies will be observed upon plating. To establish whether the suspensions were bacteriostatic or bactericidal, 100 μl aliquots were taken from the incubated broth, each containing ZnO and bacteria, and were placed on nutrient agar plates and incubated for 18–20 h. The results are summarized in Table 1.

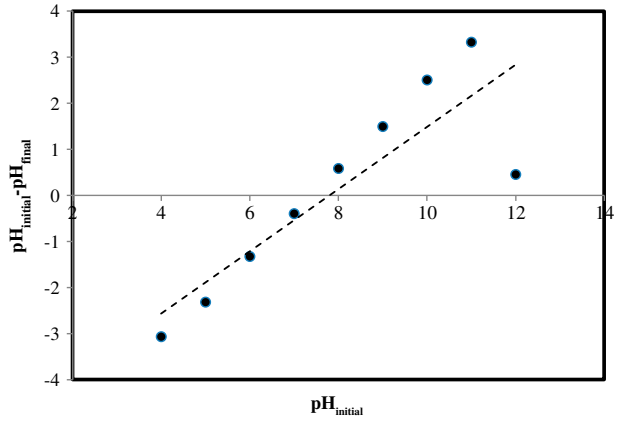

Fig. 3. Determination of pH_{ZPC} of nano ZnO.

The MIC results show that the highest maximum inhibition concentration of ZnO nanoparticles was against *P. aeruginosa* and the lowest was against *E. coli* and *S. aureos* (156.25 vs. 1,250 μg/mL). The lowest bactericidal concentration (MBC) of ZnO was for *P. aeruginosa* (312.5 μg/mL). The concentration of 156.25 μg/mL was the lowest MIC against a bacterium that corresponds to higher antibacterial effectiveness. It is suggested that the MIC of gram-positive bacteria is higher than gram-negative bacteria. Since particles may be settled at the bottom of the tube with a high concentration of ZnO nanoparticles, the test performed with highest concentration (10,000 and 5,000 μg/mL) would not show the real antibacterial activity of the ZnO nanoparticle suspension.

The antibacterial activity of ZnO nanoparticles was compared for various micro-organisms using the diameter of inhibition zone in disk diffusion test. ZnO exhibited remarkable antibacterial activity against all tested bacterial strains. Gentamicine and ampicillin were used as reference drugs (HiMedia Laboratories Pvt. Limited). As shown in Table 2, as the concentration of ZnO nanoparticles increased the antibacterial activity of ZnO nanoparticles also increased for all strains. According to ANOVA test, there was a significant difference between the values of diameter of inhibitions zones for different concentrations of ZnO nanoparticles ($p < 0.001$). Among the studied

Table 1
Minimum bactericidal and Inhibitory concentrations (MBCs and MIC) of the ZnO nanoparticle

Bacteria	MBC (μg/mL)	MIC (μg/mL)
E. coli ATCC 25922	1,250	1,250
S. epidermidis PTCC 1114	2,500	625
S. aureos ATCC 25923	5,000	1,250
P. aueroginosa ATCC 27853	312.5	156.25

bacteria, ZnO nanoparticles showed maximum activity against *Pseudomonas aeroginosa* at lower concentrations with 13.01 ± 1.04 mm D (156.25 μg/mL) and the minimum activity was against *S. aureus* with 16.75 ± 0.6 mm D (1,250 μg/mL). Statistical analysis showed no significant difference between the antibacterial effect of the nanoparticles and the studied bacterial strains ($p = 0.291$).

The diameter of inhibition zone (DIZ) reflects magnitude of susceptibility of the micro-organism. The strains susceptible to disinfectants exhibit larger DIZ at the same concentration, whereas resistant strains exhibit smaller DIZ. The size of inhibition zone was different according to the type of bacteria. The DIZ for *P. aeruginosa* in two concentrations of ZnO nanoparticles (10,000 and 5,000 μg/mL) was almost 4–7% greater than that observed with the Gentamicine-impregnated disks. Similarly, for *S. aureus*, *S. epidermidis*, and *E. coli* the ZnO nanoparticle-impregnated disks were effective in inhibiting bacterial growth. However, the method illustrates the potential biocidal effect of the ZnO nanoparticle suspension on different microbial strains.

3.2.1. Time–kill study

A bactericidal effect is defined as a 3 log decrease in the CFU/mL or a 99.9% kill over a specified time [29]. The definition of kill for this study has been used based on the National Committee for Clinical Laboratory Standards (1992) accompanied by modifications based on Handwerger and Tomasz's recommendation that a kill can be determined at 6 h treatment time [30,31]. A constant logarithmic rate of kill has been assumed during a time–kill. A 90% kill at 6 h is equivalent to a 99.9% kill at 24 h [31]. In this study, the kill measurement was determined by the actual reduction in viable counts at 6 h for each bacterial strain. Data from the time–kill studies are shown in Figs. 4 and 5.

At 1 × MIC, a large initial drop (4–7 log CFU/mL) in colony counts was seen after 6 h, which was followed by bacterial regrowth for *P. aeruginosa* and *S. aureus* at 180- and 300-min treatment time, respectively.

At 2 × MIC, of nanoZnO suspension caused complete bacterial eradication after 6 h and lowers it. Fig. 5 demonstrates the time–kill curves for two times of MIC of all strains. As shown, the killing time for *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. epidermidis* at 2 × MIC were 150, 360, 180, and 240 min, respectively. At 2 × MIC, compared with 1 × MIC, no regrowth was found when the culture was treated with higher concentrations of ZnO nanoparticle. Bacterial killing at the initial 100-min treatment time was faster than others, so maybe agglomeration effect enhanced by the presence of salts in the nutrient media and increasing agglomeration over this change was not investigated but it has been reported by some researchers[3]. The agglomeration effect may have affected the bactericidal efficiency and killing time as also suggested by Gan and colleagues [32].

The number of surviving bacteria with treatment time, strains of bacteria, simultaneous bacteria strain and treatment time, and concentration of ZnO nanoparticle in the suspension had significant differences ($p < 0.001$).

It has been reported that the positive charge on metallic ions is an important factor for its antibacterial

Table 2
Zone of Inhibition of Antibacterial test of ZnO nanoparticles

ZnO nanoparticles concentration (μg/mL)	Gram-negative bacteria		Gram-positive bacteria	
	P. aeruginosa ATCC 27853	*E. coli ATCC* 25922	*S. aureus ATCC* 25923	*S. epidermidis PTCC* 1114
10,000	21 ± 0.44a	19 ± 0.44	21.25 ± 0.77	15.66 ± 0.94
5,000	20.43 ± 0.4	18.5 ± 1.06	20.5 ± 0.35	15 ± 1.24
2,500	18.8 ± 0.61	18 ± 0.65	18.65 ± 0.16	14.5 ± 0.84
1,250	15.8 ± 0.54	17.75 ± 0.28	16.75 ± 0.6	15.16 ± 0.14
625	14.2 ± 0.34	ND	ND	14 ± 0.7
312.5	15.1 ± 0.94	ND	ND	ND
156.25	13.01 ± 1.04	ND	ND	ND
78.125	ND	ND	ND	ND
Negative control (without nano ZnO)	0	0	0	0
Positive control (Gentamicine and Ampicillin)	19.6 ± 0.9	21.8 ± 0.25	23.8 ± 0.34	16 ± 0.13

a = mean ± SD. ND = No diameter for inhibition zone.

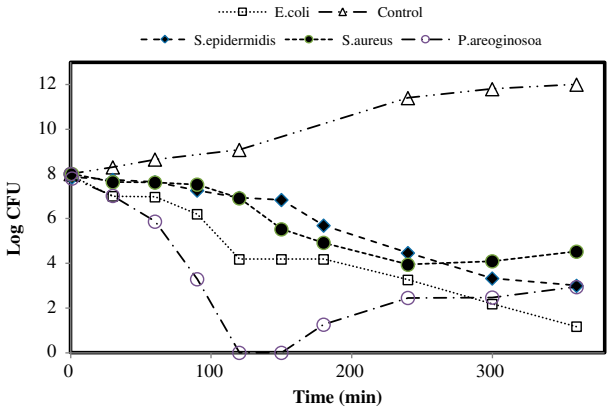

Fig. 4. Time–kill curves for *P. aeruginosa ATCC* 27853, *E. coli ATCC* 25922, *S. aureus ATCC* 25923 and *S. epidermidis PTCC* 1114 strains exposed to 1 time the minimum inhibitory concentrations (1XMICs).

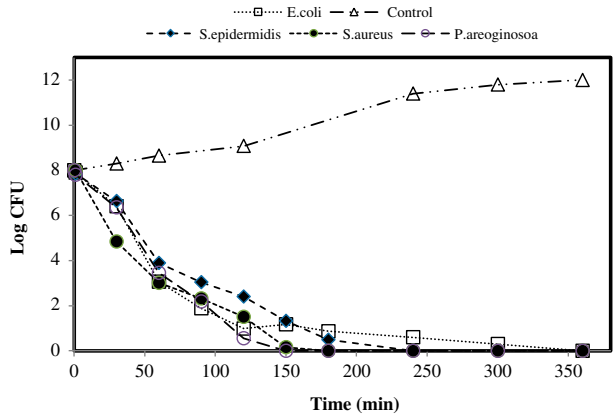

Fig. 5. Time–kill curves for *P. aeruginosa ATCC* 27853, *E. coli ATCC* 25922, *S. aureus ATCC* 25923 and *S. epidermidis PTCC* 1114 strains exposed to 2 times the minimum inhibitory concentrations (2XMICs).

nature, through electrostatic interaction between the negatively charged cell membrane of the micro-organisms and positively charged nanoparticles [26,33]. pH_{ZPC} is one of the most important characteristics of the nanoparticles, because it determines the scattering state charge on the particles' surface. This parameter indicates which dominant kind of (+/−) charge the surface of the nanoparticles has at different pH values. So at a pH lower than the pH_{ZPC} of nanoparticles, the nanoparticle surface would be positively charged and if the pH is above the pH_{ZPC}, the surface of the nanoparticle had negative electric charge [23]. Note that the value of this parameter for the ZnO nanoparticles is 7.51 and the average pH of the suspension of bacteria-ZnO nanoparticles was 8.1. We can conclude that ZnO nanoparticles in the culture medium had been negatively charged. It is proposed that the repulsive force might be an inhibiting factor for the interaction between the nanoparticles and the bacteria.

Primarily gram-positive and gram-negative bacteria are categorized on the basis of their cell wall structure. The outside layer of gram-negative bacteria has a lipopolysaccharide, and there is a thin layer of peptidoglycan under this layer. Lipopolysaccharides are not as rigid as peptidoglycan, because of the covalent linkage between the lipid and polysaccharide [3]. Lipopolysaccharides contain negative charge, and repel negatively charged ZnO nanoparticle. On the other hand, gram-positive bacteria have a thick layer (20–80 nm) of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides to form a three-dimensional rigid structure [3]. Because of the rigid structure and extended cross-linking, the cell wall has fewer anchoring sites for ZnO nanoparticles, which makes it difficult to penetrate. Therefore, we cannot conclude which bacterial group the ZnO nanoparticles are more toxic are to. However, it is clear that the nanoparticles anchor the cell at several sites and cause damage at various sites in the membrane, which could result in cell lysing. If the mechanism of ZnO nanoparticles disrupts the outer membrane components such as porin and lipopolysaccharide and considering that gram-positive bacteria do not have an outer membrane, then we can conclude the rate of cell destruction should be less severe compared to that of gram-negative bacteria.

Akiyama and coworkers reported the effectiveness of ZnO, which inhibited the fibrin formation by coagulase of *S. aureus*, as a treatment for atopic dermatitis related to *S. aureus* [26]. This suggests that the effectiveness of ZnO for atopic dermatitis may depend on the strong relationship between ZnO and *S. aureus*.

H_2O_2 generated from the surface of ZnO can easily penetrate the bacterial cell wall. From BET measurement, the specific surface area of the prepared ZnO nanoparticle was about $90\,m^2\,g^{-1}$. Since H_2O_2 generates from the surface of ZnO, the generation of H_2O_2 should be dependent on the high specific surface area of ZnO, i.e. increase of H_2O_2 with increasing surface area [1]. By comparing similar studies, we found that the difference in antibacterial activity of the prepared nanoZnO with different methods may be related to the different specific surface areas [1,13,26,34].

4. Conclusion

The major findings of this study are as follows: the analysis of XRD, UV–vis, and SEM showed that particles of all samples are of nano size. Our study showed the antibacterial effect of ZnO nanoparticles prepared using chemical methods. Based on the results of antimicrobial tests, the antimicrobial efficiency of the

ZnO nanoparticle depends on the suspension concentration, treatment time, as well as the strain of microorganism. Based on the results, the ZnO nanoparticle has a high potential for decreasing bacteria population, so it may be used as an antibacterial agent. *P. aeruginosa* population at the treatment time decreased faster than others. Also, the results showed that ZnO could be a highly effective disinfectant for controlling a wide range of bacteria.

Acknowledgments

This work linked to Msc thesis of Mr. Edris Hoseinzadeh that was supported by Vice-Chancellor for Research and Technology of Hamadan University of Medical Sciences.

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