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Water disinfection by zinc oxide nanoparticle prepared with solution combustion method

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ABSTRACT

The removal of bacteria from water is a highly important process for drinking water and sanitation systems especially on growing outbreaks of water borne diseases. The aim of this study was to evaluate the water disinfection efficiency of ZnO nanoparticle synthesized by solution combustion method (SCM). The ZnO nanoparticle, as a disinfectant, was prepared by the SCM. The prepared disinfectant was characterized by scanning electron microscopy, X-ray diffraction, and Brunauer–Emmett–Teller. The disinfection efficiency of the synthesized ZnO nanoparticle was evaluated using *Escherichia coli* as an indicator organism by disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests. The results show that the synthesized ZnO nanoparticle showed an average size of 15 nm. The MIC and MBC of ZnO nanoparticle were 8 and 16 μg mL^{-1}, respectively. These results suggest that ZnO nanoparticle prepared by SCM could be used as an effective disinfectant, making this approach applicable to water-control systems.

Keywords: ZnO; Nanoparticle; Disinfection; *E. coli*; Solution combustion method

1. Introduction

Water-borne diseases remain as the leading cause of death in many developing countries. According to the WHO report, at least one-sixth of the world's population (1.1 billion people) lack access to safe water. Diarrhea is the main disease associated with unsafe water and sanitation, and is responsible for the deaths of 1.8 million people every year, mostly children under the age of five [1,2]. Generally, the water disinfection methods consist of chemical processes [3]. Chemical disinfectants such as chlorine, chloramines, and ozone, can react with various components in natural water to form harmful disinfection byproducts [4]. Furthermore, the resistance of some pathogens, such as *Cryptosporidium* and *Giardia*, to conventional chemical disinfectants requires extremely high disinfectant dosages, leading to the formation of more byproducts [5]. Therefore, there is an urgent need to reevaluate conventional disinfection methods and to consider innovative approaches that enhance the reliability of disinfection while avoiding the formation

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of byproducts. On the other hand, various natural and engineered nanomaterials have been shown to have antimicrobial properties [6,7]. Nanomaterials are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity. Unlike conventional chemical disinfectants, these antimicrobial nanomaterials are not strong oxidants and are relatively inert in water, and therefore, they are less likely to produce harmful byproducts. If properly incorporated into treatment processes, they have the potential to replace or enhance conventional disinfection methods [8]. The concept of decentralized or distributed water treatment systems has attracted much attention in recent years due to concerns about water loss and quality deterioration associated with aging distribution networks and the increasing energy cost of water transport. Another potential application of nanomaterials is their use in decentralized or point-of-use water treatment and reuse systems [9]. Recent advances in the field of nanotechnology, particularly the ability to prepare highly ordered nanoparticles of any size and shape, have led to the development of new antimicrobial agents. There are several methods for preparing nanosized ZnO powders, such as thermal decomposition [10], Chemical vapor deposition [11], sol–gel [12], spray pyrolysis [13], and precipitation [14]. Various methods yield different particles of ZnO depending on the type of precursor, the solvent, the pH, and the temperature of the reacting solution. The choice of method depends on the final application. To the best of our knowledge and based on the literature search, this is the first report that examined the water disinfection using ZnO nanoparticle prepared by solution combustion method (SCM). The objective of this study was to investigate the removal of *Escherichia coli* as a microbial indicator from water using ZnO nanoparticle prepared by SCM.

2. Materials and methods

2.1. Preparation and characterization of ZnO nanoparticle

In a normal combustion synthesis, the catalyst precursor is reacted with a fuel in solution [15,16]. The experiment was performed inside a stainless steel chamber for safety reasons. Zinc nitrate (Junsei, Japan) powder, as an oxidant agent, was used to prepare ZnO nanoparticle powder.

The zinc nitrate (5 g) was dissolved in 25 mL of distilled water in beaker. Liquid form of glycine (2.5 g), as a fuel, was then added to the starting solution. The solution mixture in the beaker was heated on a hot plate with stirring at a temperature of about 80–100˚C. The ZnO nanoparticle was heated at 400˚C for 1 h. As the distilled water was evaporated, the mixture solution was heated to be exploded and eventually combusted. The nitrate ions reacted with the fuel, and intense heat was generated (about 1,500–1,800˚C). This high temperature resulted in high pressure. The combustion was determined with an explosion at furnace. This method involves the release of a large volume of gases that leads to the high porosity and high surface area of the material. The crystalline phase of synthesized ZnO and solid structures were analyzed using a Bruker D8 Advance X-ray diffraction (XRD) equipped with a Cu Ka ($k = 1.54$ Å) source (applied voltage 40 kV and current 40 mA). The ZnO nanoparticle diameter D was calculated using the Debye–Scherrer formula $D = K\lambda/(\beta \cos \theta)$, where K is Scherrer constant, λ is the X-ray wavelength, β is the peak width of half maximum, and θ is the Bragg diffraction angle [17]. The morphology of the prepared ZnO nanoparticle was characterized by scanning electron microscopy (SEM) (Philips XL30). Surface area was determined from Brunauer–Emmett–Teller (BET) method using a Chem Bet-3000 (Quanta Chrome, USA) under N_2 flow. The synthesized ZnO was utilized as a disinfectant to remove the *E. coli* from water.

2.2. Preparation of E. coli inoculum

The *E. coli* (ATCC 25922) was obtained from reference laboratory of health, treatment and medicine education ministry (Tehran, Iran). The strain was maintained on slant nutrient agar at 4˚C. In each experiment, the *E. coli* inoculated from the stock media to fresh broth medium in a concentration of 0.5 McFarland standards, which corresponds to ~1.5×10^8 CFU mL^{-1}. Various bacterial concentrations were prepared with dilution of bacteria in phosphate buffered saline. Cell density of the liquid cultures was determined using optical density at 600 nm. The pH value was adjusted at the beginning of the experiments by the addition of NaOH or HCl (0.1 M).

2.3. Disk diffusion test

A modified disc diffusion method was applied to evaluate the antimicrobial activity of the prepared ZnO nanoparticle against *E. coli* [18]. Briefly, a suspension of the ZnO nanoparticle (2 mg mL^{-1}) was sonicated and subsequently peculated in a 47 mm diameter antibiotic disk (BBL Sensi-Disc, USA). The filter papers including the nanoparticles were dried in an oven for 1 h at 70˚C. Small disks of uniform size (6 mm diameter) containing nanoparticles were punched out and stored in a desiccator at room

temperature. *E. coli* was subcultured on to Muller Hinton agar (Merck, Germany), and was incubated under aerobic condition at 37˚C for 18 h. Following incubation, suspensions of pure cultures were prepared in broth medium, and were adjusted to give inoculums with an equivalent cell density to 0.5 McFarland turbidity standards (1.5×10^8 CFU mL^{-1}). Cell suspensions were inoculated onto Muller Hinton plates. On each 9 cm plate, ZnO nanoparticle disks were applied. Plates were incubated under aerobic conditions at 37˚C for 18 h. The inhibition zones for each disk were measured by ruler and were interpreted according to the guidelines recommended for bacteria by National Committee for Clinical Laboratory Standard. The mean and standard deviations were reported based on three replicates.

2.4. Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) were determined according to the microdilution method [19]. To determine the MIC, the ZnO nanoparticle suspensions with different concentrations of the ZnO nanoparticle were incubated with *E.coli* in aqueous Mueller Hinton broth for 18–20 h. The bacterial cells from the stock tubes were suspended in the phosphate buffer saline to a concentration of 0.5 McFarland standard, which corresponds to ~1.5×10^8 CFU mL^{-1}. The bacteria was added to triplicate wells of a 96-well plate containing 100 μL of prepared ZnO nanoparticle stock diluted in sterile phosphate buffer saline. The final ZnO nanoparticle concentrations were 0.5, 1, 2, 4, 6, 8, 16, 32, and 64 μg mL^{-1}. The plate was incubated for 18–20 h at 37˚C. Bacterial growth was studied by visually inspecting the Mueller Hinton broth for turbidity. The lowest concentration of ZnO nanoparticle that inhibited the growth of bacteria (unchanged optical density) was considered as the MIC. For MBC determination, a 10 μL sample of MIC was inoculated on Muller Hinton agar and examined for signs of growth. The positive control was given gentamicin and the blank control well only contained Muller Hinton agar. Growth of bacteria demonstrates the presence of the *E. coli* in the well. If the ZnO nanoparticle 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 upon plating an aliquot. If the ZnO nanoparticle being tested is bactericidal, the absence of bacterial colonies will be observed upon plating.

2.5. Statistical analysis

The MICs, MBCs, and disk diffusion tests were performed in triplicate and results are expressed as means ± the standard errors of the means. A student's *t*-test was used to compare these results. p Values lower than 0.05 were considered significant.

3. Results and discussion

Different methods of ZnO nanoparticles synthesis have been reported to have higher activities in various processes. Generally, the smaller particle size is required to obtain high activity because of their large surface area. In this study, we applied a SCM to prepare ZnO nanoparticle. The method has simple procedure for producing single-phase ZnO nanoparticle. Using glycine and zinc nitrate at the fuel/oxidant, the synthesized ZnO powder showed the best characteristics, including the highest XRD peak, an average grain size of 15 nm, and a specific surface area of 135 $m^2 g^{-1}$. This high quality ZnO powder results from defect removal by the high temperature and pressure generated during the synthesis process. These results agree with the previous findings reported in the literature [15]. The prepared ZnO nanoparticle was utilized as a disinfectant for the removal of *E. coli* from water.

3.1. Characterization of ZnO nanoparticles

Fig. 1 shows the XRD spectra of the prepared ZnO nanoparticle. The spectra show well-defined peaks typical of ZnO in the crystal structure of zincite, according to the Joint Committee on Powder Diffraction Standard card number 36–1451. The peaks are quite sharp indicating the crystalline nature of the particles, suggesting

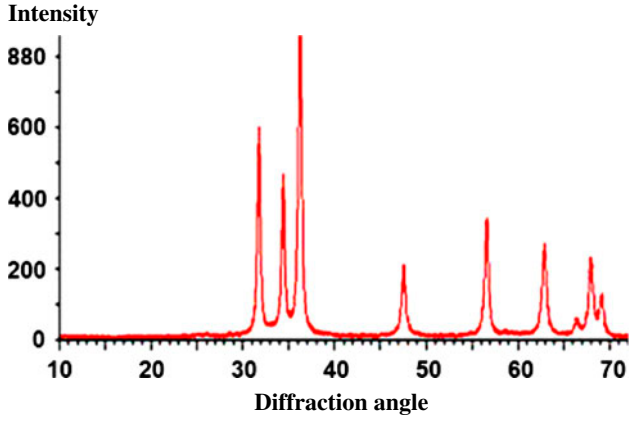

Fig. 1. XRD pattern of ZnO nanoparticle prepared with SCM.

that high-purity ZnO has been obtained. According to the Debye–Scherrer formula, the average particles sizes of ZnO nanoparticle is about 15 nm. SEM analyses were carried out to characterize the surface of ZnO nano-particle. The SEM image confirmed the ordered arrangement of pores on the surface of the ZnO nano-particle (Fig. 2). This is highly important to achieve the large active catalyst surface needed for adsorption. We used the BET method to determine the specific surface area of the prepared ZnO nanoparticle. Results indicate that the specific surface area of combustion synthesized ZnO is 135 $m^2 g^{-1}$.

3.2. Antibacterial assessment

The antibacterial activity of ZnO nanoparticle was assayed for *E. coli* as a bacterium model using the diameter of inhibition zone in disk diffusion as well as MIC, and MBC [20]. The methods involve placing an antibacterial agent on an inoculated agar plate where its antimicrobial activity diffuses into the surrounding agar and produces a "zone of inhibition" in which microbial growth does not occur. The diameter of the inhibition zone reflects magnitude of susceptibility of the microorganism. The disks with ZnO nanoparticles were surrounded by a 4 ± 0.5 mm inhibition zone. The method illustrates the potential biocidal effect of nanoparticles for different microbial strains. The MIC and MBC of ZnO nanoparticle is dependent on the concentration of nanoparticles and the initial bacterial concentration. As the concentration of Zno nanoparticle increased to the MIC of the bacterium, no growth was observed in the wells. Table 1 shows the MIC and MBC of prepared ZnO nanoparticle against *E. coli*. The MBC/MIC ratio is a parameter that reflects the bactericidal capacity of the analyzed compound. In our study, ZnO nanoparticle exerted a bacteriostatic effect against *E. coli* because the MBC/MIC ratio values were ≤2. We demonstrated the formation of large numbers of colonies when the MIC is less than 8 μg mL^{-1} ($p < 0.005$). This may be due to the possible presence of fewer Zn^{2+} ions that may act as a nutrient [21]. Although metals and metal oxides are known to be toxic at relatively high concentrations, toxicity is not expected at low concentrations. According to the literature, ZnO possess antimicrobial activity against a number of microorganisms. Penetration of the cell envelope and disorganization of bacterial membrane upon contact with the ZnO nanoparticle indicates the inhibition of bacterial growth [22]. In batch studies with *E. coli* and ZnO nanoparticles (concentration ranges from 0.01 to 100 Mmol), MIC was reported to be in the range of 0.8–8,000 μg mL^{-1} [23]. Due to the variation in the *E. coli* strain employed, and variation in the size of the ZnO nanoparticle and initial bacterial concentration, a direct comparison between the studies is not feasible. According to the obtained antibacterial results, it can be concluded that the prepared ZnO nanoparticle shows enhancing activity due to its large surface area to volume ratio (Fig. 3). The smaller particle size shows enhanced activity due to the large surface area to volume ratio, and the surface ZnO suspension in the lower concentration range exhibit less antimicrobial activity. The adjustment of the initial pH values in the range of 6.0–8.0 had no marked effect on the rate of *E. coli* inactivation. The detailed mechanism for the activity of ZnO is still under debate, though it has been suggested that cell wall rupture occurs as a result of surface activity of the ZnO nanoparticle in contact with the bacteria. Cell death is thought to be caused by the decomposition of the cell wall followed by the subsequent decomposition of the cell membrane. The damage to the cell membrane directly leads to the leakage of minerals, proteins, and genetic materials causing cell death.

Table 1
MICs and MBCs of ZnO nanoparticle against *E. coli*

Concentrations (μg mL^{-1})	MIC*	MBC**
0.5	+	+
1	+	+
2	+	+
4	+	+
8	+	+
16	−	+
32	−	−
64	−	−

*Minimum inhibitory concentration.
**Minimum bactericidal concentration.
Notes: +Positive growth; − Negative growth.

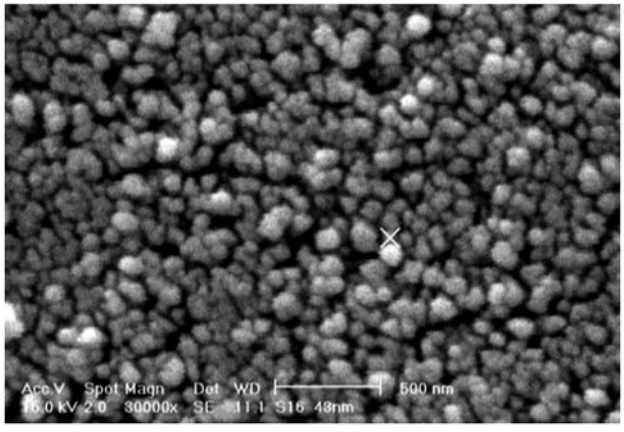

Fig. 2. The SEM micrograph of the ZnO nanoparticle.

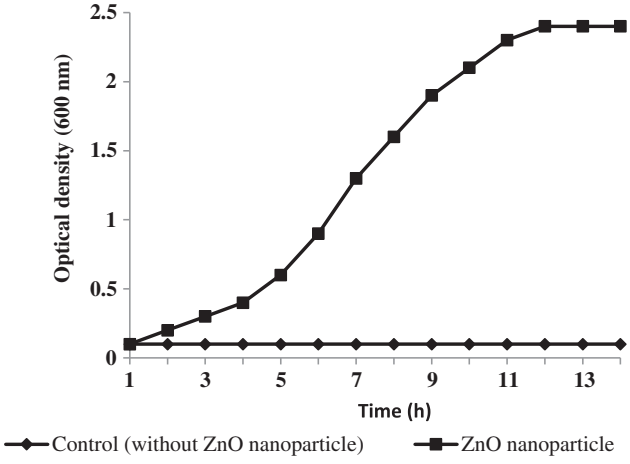

Fig. 3. Growth of *E. coli* in nutrient broth inoculated with 3×10^8 CFU mL^{-1} of *E. coli* in the presence of the ZnO nanoparticle.

Wang et al. [24] proposed that the orientation of ZnO can also affect bioactivity, with randomly oriented ZnO nanowires showing higher antibacterial activity than regularly oriented nanowires [24]. This suggests that different spatial arrangements of ZnO cause bactericidal activity.

4. Conclusion

The ZnO nanoparticle was synthesized through a SCM, and the nanoparticle was employed in a water disinfection process. The following conclusions can be drawn from our experiments and analyses:

(1) The synthesized ZnO powder has shown 15 nm average size and 135 $m^2 g^{-1}$ specific surface area.
(2) The ZnO nanoparticle has high bactericidal activity against *E.coli*.
(3) The ZnO nanoparticle treatment could maintain the antibacterial efficacy and may be useful in the development of antimicrobial materials.

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