



Received: 18 January 2015
Accepted: 07 January 2016
First Published: 10 February 2016

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Reviewing editor:
Mark Russell StJohn Foreman, Chalmers University of Technology, Sweden

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MATERIALS CHEMISTRY | RESEARCH ARTICLE

The improvements in the size distribution of zinc oxide nanoparticles by the addition of a plant extract to the synthesis

A. Samzadeh-Kermani^{1*}, F. Izadpanah² and M. Mirzaee¹

Abstract: Recently, the progress in using the plant extracts in the synthesis of nanoparticles (NPs) has occupied a lot of attention. In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized in the presence and without the presence of a plant extract. The morphology and the size of ZnO NPs synthesized using these two methods were studied by scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis, and Fourier transform infrared spectroscopy. XRD results were shown the wurtzite hexagonal type structure for the NPs and the average size of prepared NPs was found to be smaller, in the presence of plant extract. SEM images were confirmed the XRD results. Finally, the antibacterial activity of prepared NPs was evaluated against two bacteria of gram-positive *staphylococcus aureus* and gram-negative *Escherichia coli*.

Subjects: Bioscience; Engineering & Technology; Physical Sciences

Keywords: ZnO nanoparticles; plant extract; electron microscopy; infrared spectroscopy; antibacterial

1. Introduction

The use of plant extracts in the synthesis techniques makes the use of moderately pollutant free chemicals to synthesize nanomaterials and increase the use of eco-friendly solvents such as water. Green chemistry seeks reducing pollution at the source (Tundo & Anastas, 2000). Green chemistry

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PUBLIC INTEREST STATEMENT

The objective of this study is to synthesize ZnO nanoparticles comparatively using *Bougainvillea glabra* leaf extract and chemically and then the evaluation of bactericidal property of prepared ZnO nanoparticles.

encompasses all aspects and types of chemical processes that reduce negative impacts to human health and the environment (Hjeresen, Boese, & Schutt, 2000). This principle focuses on choosing reagents that encounter the least risk and generate only benevolent byproducts. Though physical and chemical methods are trendier for the synthesis of nanoparticles (NPs), the biogenic fabrication is a better choice due to eco-friendliness (Clark & Macquarrie, 2002). Because of the need of environmental-friendly NPs, researchers are using green methods for the synthesis of various NPs for pharmaceutical applications (Awwad, Salem, & Abdeen, 2012). The biological approaches of using micro-organisms and plants or plant extracts for the synthesis of NPs have been suggested as valuable alternatives to chemical methods. Several biological systems including bacteria, fungi, and yeast have been used in the synthesis of NPs (Vidya et al., 2013). NPs due to their smaller size and large surface exhibit remarkable novel properties and methodical applications in the field of biotechnology, sensors, medical, catalysis, optical devices, DNA labeling, drug delivery (Fan & Lu, 2005) and they are rewardingly treated as a bridge between bulk material and atomic and molecular structures. It is well known that ZnO is an important metal oxide semiconductor (Baruah et al., 2009). ZnO NPs have found fabulous applications in biomolecular detection, diagnostics, and micro electronics (Gnanasangeetha & Thambavani, 2013). Among different nanomaterials, ZnO NPs possess a special place due to their own merits such as high specific surface area, optical transparency, biocompatibility, non-toxicity, chemical and photochemical stability, ease of fabrication, high-electron communication features, and electrochemical activities. Even having versatile properties, the biosensor applications of ZnO NPs are very rare. There are only two reports on ZnO NPs-based cholesterol biosensor (Umar, Rahman, Vaseem, & Hahn, 2009). It displays interesting properties at nanoscale, and has significant applications as antibacterial and antifungal agents. It also holds importance in biomedical sciences (Eskandari, Haghghi, Ahmadi, Haghghi, & Mohammadi, 2011; Sharma, Rajput, Kaith, Kaur, & Sharma, 2010). ZnO NPs inhibited the growth of gram-positive bacteria more strongly than gram-negative bacteria (Sawai, Igarashi, Hashimoto, Kokugan, & Shimizu, 1995). Current research was focused on the synthesis of ZnO NPs as antibacterial agents to prevent the growth of bacteria. Recently, there have been several reports regarding the antibacterial activity of ZnO NPs (Tankhiwale & Bajpai, 2012). *Bougainvillea glabra* is a lush evergreen subtropical vine (Ali, Baloch, & Chachar, 2013). *B. glabra* belongs to the family Nyctaginaceae. The family has 30 genera and 300 species. It is native to Latin America (Brazil), commonly grown in gardens, porches, and boundary walls (Shah, Zamir, Muhammad, & Ali, 2006). Parts of the plant which have been used for medicinal purposes are the leaves and fruits (Iwu, 1993). Among different species of ornamental plants, *B. glabra* is a well-known medicinal plant which grows on various lands and climates (Gillis, 1976). In this study, ZnO NPs were synthesized from $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ using leaf extract of *B. glabra* an ornamental plant and an eco-friendly agent (method 1), or chemically (method 2). Then, the size distribution ranges and antibacterial activity were investigated.

2. Materials and methods

2.1. Materials

Zinc acetate dehydrate powder and sodium hydroxide pellets (both 99% purity) were used as starting materials which purchased from Merck Company (Darmstadt, Germany). Fresh leaves of *B. glabra* were prepared and washed thoroughly with double-distilled water, grinded, filtered, and used for this work.

2.2. Micro-organisms

The antibacterial activity of ZnO NPs prepared using two methods was evaluated against two different bacterial strains including gram-positive *Staphylococcus aureus* (*Staph.*) and gram-negative *Escherichia coli* (*E. coli*). All bacterial strains were provided from the faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran.

2.3. Preparation of ZnO NPs

ZnO NPs were prepared using two different methods. In the method 1 (using plant extract), an aqueous zinc acetate solution (50 mL of 0.02 M) was prepared and added to the aqueous leaf extract of *B. glabra* (100 mL of 50% because of better size distribution of ZnO NPs) at room temperature. The resulting mixture was stirred for 10 min. Then sodium hydroxide solution (ca. 8 mL of 2.0 M NaOH) was added dropwise to increase the pH to 12. The resulting pale creamy mixture was then stirred (2 h). The white precipitate was then collected by filtration, it was washed with distilled water and then with ethanol (96%) to remove the impurities. The resulting white powder of ZnO NPs was dried in a vacuum oven (at 70°C 12 h). The yield of the NPs was 98%. In the method 2 (chemical), a similar procedure was accomplished, 15 mL of 2.0 M NaOH was added in order to adjust the pH to 12, and the white powder of ZnO NPs was obtained in 97% yield. In this process, a nearly complete conversion of Zn(OH)_2 to ZnO NPs was taken place after drying (Gnanasangeetha & Thambavani, 2013).

2.4. Characterization

The synthesized Zinc Oxide NPs was analyzed using a Fourier transform infrared (FT-IR) spectrometer (Bruker Optics Ft Tensor 27, Germany). The structure and the size distribution of NPs were determined using the Bruker - D8 Advanced (Germany) X-ray diffractometer and Hitachi S4160 (Germany) Field emission scanning electron microscope. For SEM analysis, thin films of samples were prepared on graphite adhesives, and then the surface of samples was coated by gold powder using sputter hummer instrument.

2.5. Antibacterial activity

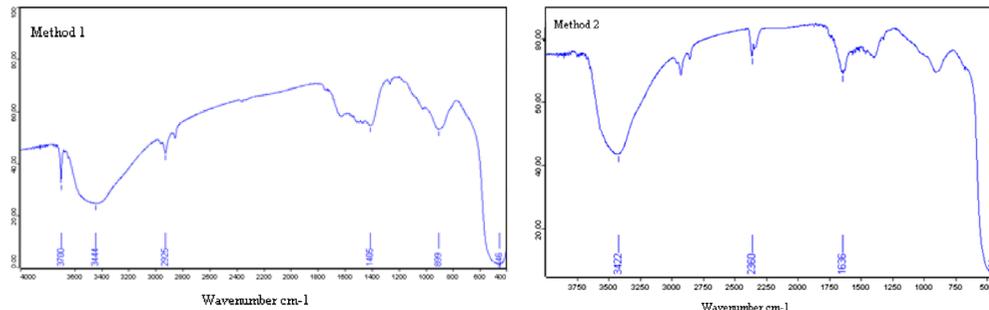
The antibacterial activity of ZnO NPs was investigated against *Staph.* and *E. coli* micro-organisms by disk diffusion method in accordance with the procedure described by Hwang and Ma (2012). This method is a mean of measuring the effects of an antimicrobial agent on bacteria growth in a culture. Muller-Hinton Agar (MHA) powder was used as a culture medium for bacteria growth. To prepare the culture medium, 19 g of agar was dissolved into 500 mL of distilled water, and then a transparent brown solvent was achieved via boiling the solution. The MHA medium (15 mL) was sterilized at 120°C for about 1 h in autoclave, cooled to room temperature, and then poured into sterilized Petri dishes (10–90 mm). After cooling over 24 h, the bacteria are swabbed uniformly across the culture plate. Filter-paper disks were placed on the surface of agar. To evaluate the antibacterial activity, 40 μL of each of the samples was dropped moderately on disks' surface using a sampler. If the samples are effective against the bacteria at a certain concentration, then no colonies would grow and the concentration in agar is greater than or equal to the effective concentration. This region is called inhibition zone. The size of inhibition zone measures the efficiency of sample. A more effective sample produces a larger clear area around the disk. All tests were done under laminar flow hood. Finally, all Petri dishes containing bacteria and antibacterial reagents were incubated at 37°C for 24 h. At the end of incubation period, the diameters of inhibition zones formed around disks were determined and presented in mm. The results concerning bactericidal activity were expressed as strong activity (>13 mm), moderate activity (6–12 mm), weak activity (5 mm), or no activity (inhibition zone < 5 mm).

3. Results and discussion

3.1. FT-IR spectroscopy

Figure 1 shows the FT-IR spectrum of ZnO NPs synthesized using method 1. Strong absorption peaks in the region 430–520 cm^{-1} were attributed to Zn–O stretching band at 432 cm^{-1} and to the oxygen vacancy in ZnO at 503 cm^{-1} (Hong et al., 2009). The broad and intense band at 3,416–3,617 cm^{-1} was assigned to OH stretching. It is suggested that trace amounts of Zn(OH)_2 were produced. Asymmetric mode of vibration for C=O was also observed about 1,490 and 1,550 cm^{-1} (Sharma et al., 2010). FT-IR spectrum for ZnO NPs prepared using method 2 was shown a broadband near 3,360 cm^{-1} , which was attributed to the hydrogen bonded O–H stretching vibration (Thirumavalavan, Huang, & Lee, 2013). The bands at 2,956 and 2,921 cm^{-1} were ascribed to the symmetric and asymmetric stretching vibrations of CH in alkyl groups (Ma & Zhang, 2009). The band at 540–417 cm^{-1} was assigned to ZnO NPs (Li, Deng, Deng, Liu, & Xin, 2010).

Figure 1. FT-IR spectra of the ZnO NPs prepared by using methods 1 and 2.



3.2. X-ray diffraction Analysis

X-ray diffraction (XRD) pattern for ZnO nanocrystals synthesized using method 1 showed sharp and intense peaks around 2θ values of 30.78, 35.13, 37.28, 46.38, 56.8, 61.9, 67.4, and 68.38 which were equivalent to 100, 002, 101, 102, 110, 103, 112, and 201 planes, respectively. Similarly, the XRD pattern for ZnO nanocrystals synthesized using method 2 showed 2θ values of 31.8, 34.4, 36.2, 47.5, 56.6, 62.8, 67.90, and 68.5 which were equivalent to 100, 002, 101, 102, 110, 103, 112, and 201 planes, respectively (Figure 2). These results were in a good agreement with those for wurtzite hexagonal type structure of ZnO NP (Guo, Diao, & Cai, 2005). The average crystallite size of ZnO NPs was estimated to be at the range 19.88 ± 1.83 nm for method 1, while found to increase to 28.23 ± 2.78 nm for method 2. Measurement of the average crystallite size for obtained ZnO NPs was accomplished (mainly for 2θ values of 30.78, 35.13, 37.28 and 56.8 in method 1 and for 31.8, 34.4, 36.2 and 56.6 in method 2) using Scherrer's Equation:

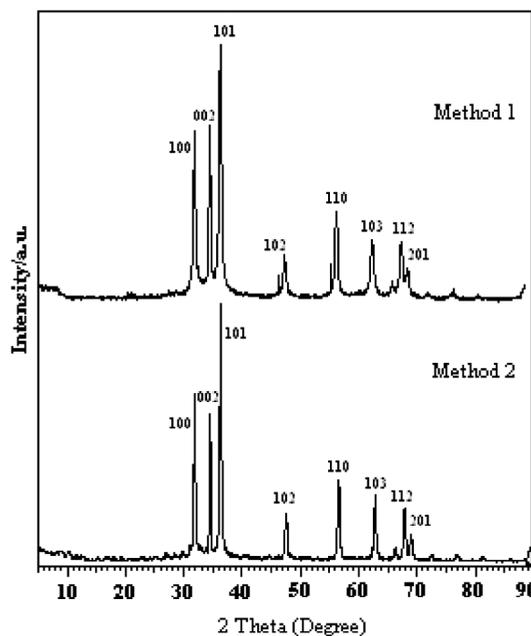
$$D = \frac{K\lambda}{\beta \cos \theta}$$

where D is the crystallite size, k is the shape factor that assumes a value of 0.89 for ZnO, λ is the X-ray wavelength (1.5418 Å), β is the half height width of XRD peak, and θ is the diffraction angle (Talebian, Amininezhad, & Doudi, 2013).

3.3. FESEM analysis

SEM images of ZnO NPs prepared using the two methods were shown in Figure 3. The particle size and the external morphology of ZnO NPs were convinced the XRD results. The two images were

Figure 2. XRD spectra of ZnO NPs prepared by using methods 1 and 2.



shown cubic structures composed of quite a lot of individual small NPs for ZnO. The crystals are formed as clumps for both methods. Also the results were shown that, ZnO NPs have smaller size when prepared using leaf extract of *B. glabra* (method 1). As the size determination in accordance with the SEM analysis results was difficult, so the size determination of NPs was accomplished using XRD results. The size distribution of NPs in the method 1 was smaller than that in the method 2. The size distribution of ZnO NPs prepared using method 1 was at the range of 100–200 nm with the average size of 172 nm and the size distribution of ZnO NPs prepared using method 2 was at the range of 130–250 nm with the average size of 191 nm. This large size for ZnO NPs is due to the aggregation of nanocrystallites.

3.4. Antibacterial properties of ZnO NPs

ZnO NPs synthesized using the two methods were shown strong antibacterial activity against two bacterial strains including *E. coli* and *staph*. The growth inhibition zone values were calculated and summarized in the Table 1. According to the results, the extract was not shown antibacterial effect. ZnO NPs synthesized via method 1 were shown greater antibacterial activity than method 2, because the NPs' size was smaller in the method 1. ZnO NPs were shown to have a wide range of antibacterial activities against both gram-positive and gram-negative bacteria, including major foodborne pathogens like *E. coli* and *Staph*. (Jones, Ray, Ranjit, & Manna, 2008; Liu et al., 2009). Some studies suggest that the primary cause of the antibacterial function might be from the disruption of cell membrane activity (Brayner et al., 2006). Another possibility could be the induction of intercellular reactive oxygen species, including hydrogen peroxide (H_2O_2), a strong oxidizing agent harmful to bacterial cells (Jones et al., 2008; Sawai, 2003). It has also been reported that ZnO can be activated by UV and visible light to generate highly reactive oxygen species such as OH^- , H_2O_2 , and O_2^{2-} . The negatively charged hydroxyl radicals and superoxides cannot penetrate into the cell membrane and are likely to remain on the cell surface, whereas H_2O_2 can penetrate into bacterial cells (Padmavathy & Vijayaraghavan, 2008).

4. Conclusion

ZnO NPs were synthesized using *B. glabra* leaf extract (method 1), and chemically (method 2). The XRD study was revealed that the average size was 19.88 ± 1.83 nm in the method 1, while found to increase to 28.23 ± 2.78 nm in the method 2. Though both methods were appropriate for the

Figure 3. SEM images of ZnO NPs prepared by using methods 1 and 2.

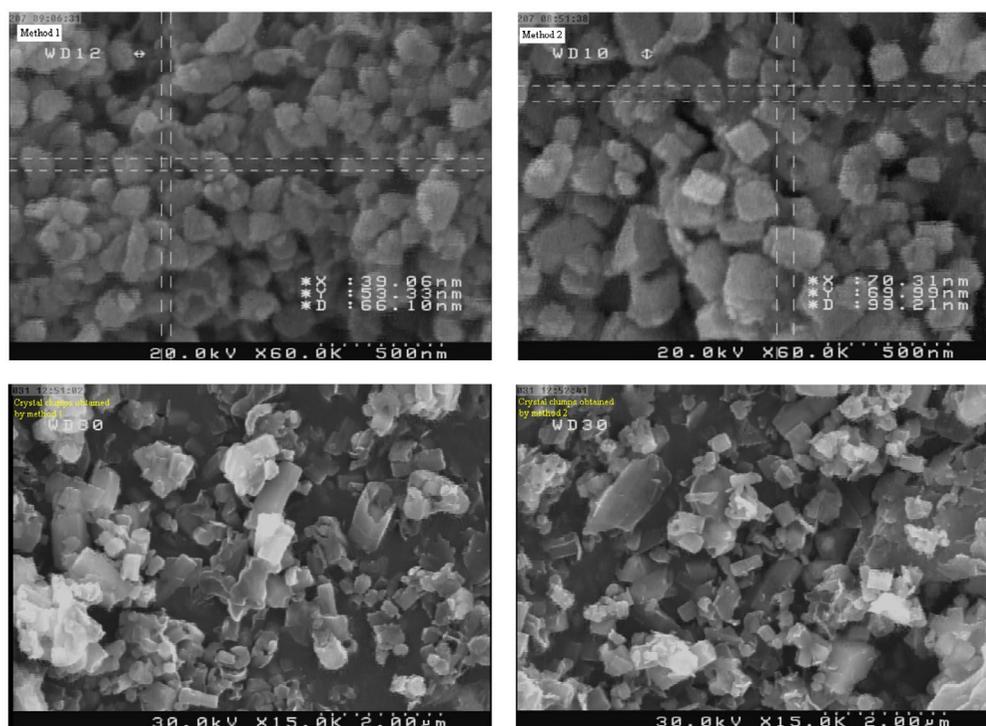


Table 1. The average inhibition zone for the ZnO NPs prepared by using method 1 and 2.

Sample	Inhibition zone diameter (mm)	
	<i>Escherichia coli</i> (<i>E. coli</i>)	<i>Staphylococcus aureus</i> (<i>Staph.</i>)
ZnO (method 1)	20	21
ZnO (method 2)	11	14
Bgle ^a	0	0

^aBougainvillea glabra leaf extract.

synthesis of ZnO NPs, the biogenic one (method 1) is a better choice due to eco-friendliness. Moreover, the XRD analysis peaks for ZnO NPs were in good agreement with those of hexagonal wurtzite structure for both methods. SEM images were shown a proper size distribution of ZnO NPs in the method 1, and also smaller sizes of ZnO NPs were observed. The size distribution in accordance with the SEM results was reliable with that of the XRD results. The antibacterial activity of the prepared NPs was shown a strong antibacterial activity against gram-positive and gram-negative bacteria. These results were shown that the antibacterial resistance of ZnO NPs prepared using method 1 was modified according to their smaller size distribution with respect to chemical method.

Funding

This research is accomplished under financial support of the Department of Chemistry, University of Zabol and cordially laboratorial collaboration of the Faculty of Pharmacy, Zabol University of Medical Sciences, which should be appreciated.

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Citation information

Cite this article as: The improvements in the size distribution of zinc oxide nanoparticles by the addition of a plant extract to the synthesis, A. Samzadeh-Kermani, F. Izadpanah & M. Mirzaee, *Cogent Chemistry* (2016), 2: 1150389.

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