Synthesis of ZnO Nanoparticles and Their Antibacterial Effects

Ali Karami, and Saber Imani

Abstract—The zinc oxide nanoparticles, with the average particle size of about 30 nm, were synthesized by the chemical technique and their properties were studied with the help of scanning electron microscope and the X-ray diffraction. The aim of this study was to detect the antibacterial properties of 0.01, 0.5 and 1% nano-ZnO against E. coli. E. coli was cultured in liquid and agar nutrient medium to evaluate the antibacterial effects of 0.01, 0.05 and 1% of ZnO via the optical density (OD) and log CFU/ml measurements. Non-significant effect was seen for 0.01% of ZnO nano-particles. 0.05 and 1% of nanoparticles showed considerably decreased bacterial number. A 4.385 and 2.04 times decrease in the OD value was found in the presence of 1 and 0.5%/nano-ZnO, respectively (P<0.001) as compared to control. In the second study, 6.3 log CFU/ml of E. coli were present in the cultures treated with 1% nano-ZnO at 4 ºC in water. Control E. coli cells survived for 12 days while complete cell death was seen when 1% nano-ZnO was applied for 24 hours. In the third study, E. coli was grown in the agar medium with and without nanoparticles and suppressed growth (8.56 times; P<0.001) was seen in the presence of 1% nano-ZnO.

Keywords—ZnO-Nanoparticle, Antibacterial, Bactericidal, E. coli.

I. INTRODUCTION

Nanomaterials are being used in many branches of science such as harmful microorganisms, recognition and treatment of various diseases [2]. Nanotechnology has also invaded engineering, biology, chemistry, medicine physics etc. Metallic nanoparticles have different functions such as antibacterial characteristics [3]. Metallic nanoparticles are continuously being used in the manufacture of manufacture of bactericides, but unfortunately the application in these processes reduces the antibacterial characteristics of nanoparticles. However, in the meantime, inorganic nanoparticles seem to have been good bactericides because of their tolerance to high temperatures [4].

Metal nano particles have various functions that are not observed in bulk phase [1-2]. Antimicrobial agents, used in textile industry, are divided into two parts: the organic and inorganic matters. The organic antibacterial materials have been used as insecticides and bactericides for many years. Unfortunately, high temperatures in manufacturing process reduce their antibacterial properties. However, inorganic antibacterial agents show excellent resistance against the bacterial and thermal stability [3]. Over the past few decades, inorganic nanoparticles, that structures exhibit significantly novel and improved physical, chemical, and biological properties and functionality due to their nano-scale size, have elicited much interest. Nano-structured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications [4-6].

At present, the use of nano-structured materials is becoming more widespread and a major advantage over either organic or inorganic nanoparticles offers many possibilities of applications in the areas of physics, chemistry, pharmacy, surface coating agents, textile sizing, agriculture, biochemistry and so on [4-9]. It has been demonstrated that specially formulated metal oxide nanoparticles have good antibacterial activity, and antimicrobial formulations comprising nanoparticles could be effective bactericidal materials [10]. Nano-materials are called “a wonder of modern medicine”. It is stated that antibiotics kill perhaps a half dozen different disease-causing organisms but nano-materials can kill some 650 cells [11]. Resistant strains fail to develop if we apply nanoparticle-based formulations in their culture media. In laboratory tests with nanoparticles, the bacteria, viruses, and fungi are killed within minutes of contact. The effect of nanoparticles on bacteria is very important since they constitute the lowest level and hence enter the food chain of the ecosystems [5-8]. Recent studies have demonstrated that specifically formulated nanoparticles demonstrate good antibacterial activity and constitute the antimicrobial formulations [12-14]. As, nano-silver has been used for imparting antibacterial properties [14-15], this study aimed to investigate the potent long-lasting antibacterial activity of nano-ZnO toward the gram-negative bacterium E. coli, which is known as diarrhea-causing organism.

II. EXPERIMENTAL

A. Chemicals, growth media and bacterial Strain

E. coli (ATCC 25922) was used for the present experiment. Nutrient Broth (BD234000; Becton Dickinson & Company, MD, USA) was used in growing and maintaining the bacterial cultures as per supplier’s protocol. zinc nitrate, sodium hydroxide (NaOH), ethanol, potassium nitrate (KNO3), dihydrogen potassium phosphate (K2HPO4), potassium hydrogen phosphate (KH2PO4), acetic acid (CH3COOH), sodium acetate (CH3COONa), was purchased from Sigma and

http://dx.doi.org/10.17758/IAAST.A0614086
Merck Co.. The chemicals such as; ascorbic acid, sodium citrate tribasic dehydrate, ammonium sulphate, ethanol and cetyltrimethyl ammonium bromide (CTAB) were purchased from Sigma and were of the highest purity available. These reagents were used as received without further purification. Materials such as all solutions were prepared with double distilled water.

B. Synthesis of ZnO nanoparticles

To prepare ZnO Nanoparticles, in a typical experiment, a 0.45 M aqueous solution of zinc nitrate (Zn(NO$_3$)$_2$.4H$_2$O) and 0.9 M aqueous solution of sodium hydroxide (NaOH) were prepared in distilled water. Then, the beaker containing NaOH solution was heated at the temperature of about 55 ºC. The Zn(NO$_3$)$_2$ solutions were added drop wise (slowly for 1h) to the above heated solution under high speed stirring. The beaker was sealed at this condition for 2 h. The precipitated ZnO nanoparticles were cleaned with deionized water and ethanol then dried in air atmosphere at about 60 ºC. The scanning electron microscopic image of the synthesized ZnO nanoparticles was taken by a scanning electron microscope Model XL30- Philips Company, operated at 30 KV.

C. Bacterial susceptibility to nanoparticles

To examine the susceptibility of E. coli to nano-ZnO, three different estimation methods were used with three tiles repetition.

D. Bacterial growth in the presence of nano-ZnO in liquid medium

In the first method, the bacteria were grown in nutrient broth (NB). To start the growth, 2 mL of the overnight-cultured E. coli stock was added to 100 mL NB, containing 0.12% glucose with and without 0.01, 0.5 and 1% nano-ZnO. The bacteria were aerobically cultured at 30 ºC for 24 hours. The bacteria were washed and re-suspended in distilled water, reaching a final concentration of 6.3 log CFU/ml in each of the sample flasks and incubated at 4 ºC. The final concentration of the E. coli suspensions was made in 100 ml distilled water. Different amounts of nano-ZnO (0.01, 0.5 and 0.1%) were then separately added to the bacterial suspensions to keep in contact with the bacterial cells and shaken at 4 ºC for 48 hours. Optical density (OD) was measured to monitor the bacterial concentration.

E. Bacterial killing in the presence of nano-ZnO in liquid medium

In the second method, the culture solution was centrifuged and the cells were washed and re-suspended in distilled water, reaching a final concentration of 6.3 log CFU/ml in each of the sample flasks and incubated at 4 ºC. The final concentration of the E. coli suspensions was made in 100 ml distilled water. Different amounts of nano-ZnO (0.01, 0.5 and 0.1%) were then separately added to the bacterial suspensions to keep in contact with the bacterial cells and shaken at 4 ºC for 48 hours. Optical density (OD) was measured to obtain the results. Aliquots of 0.1 ml of the growth mixtures (water+bacterial cells+ nanoparticles) were sampled every two hour. The number of resulting bacterial cells was noted after every two hours of incubation. Bacterial number was determined by measuring the optical density (OD) at 600 nm. The OD values were converted into the E. coli concentration as log CFU/ml [13].

F. Bacterial growth in the presence of nano-ZnO in agar medium

In the third method, the same bacteria strain was grown on a solid NB containing 0.12% glucose, 2% agar (control plates) alone or in the presence of 1% nano-ZnO. Bacterial cells were grown at 30 ºC for 48 hours. Afterwards, the plates were visually estimated and bacterial colonies counted. The pictures were taken by an Olympus C2020Z digital camera. The data obtained in all tests were compared with the control. Student’s t-test was used to evaluate the significance of experimental results (P<0.05).

III. RESULTS

A. Size distribution of synthesized nanoparticles by SEM and XRD

The Figure 1 shows the scanning electron microscope (SEM) image of the synthesized ZnO nanoparticles. SEM provided images of the particles by magnification of about one million times greater. The assembly was attached with a computer software programming to analyze the mean size of the particles in sample. It should be noted that the particle diameter is always overestimated due to the distortion of SEM images [15].

Fig. 1. Scanning Electron Microscope image of synthesized ZnO nanoparticles for corresponding sample 2 hr-160 ºC -2-180 ºC.

Figure 2 (a and b) demonstrates the XRD patterns of the synthesized ZnO nanoparticles. The x-ray diffraction data were recorded by using Cu Kα radiation (1.5406 A$^\circ$). The intensity data were collected over a 20 range of 20-80$^\circ$. The average grain size of the samples was estimated with the help of Scherrer equation using the diffraction intensity of (101) peak. X-ray diffraction studies confirmed that the synthesized materials were ZnO with wurtzite phase and all the diffraction peaks agreed with the reported JCPDS data and no characteristic peaks were observed other than ZnO.
The lattice parameters calculated were also in agreement with the reported values. The reaction temperature greatly influences the particle morphology of as-prepared ZnO powders. The results of nanoparticle size measurement of samples by XRD and SEM indicate that the size of the ZnO nanoparticles was about 30 nm.

**B. UV–visible absorption spectra of ZnO nanoparticles**

The UV–visible absorption spectra of ZnO nanoparticles are shown in Figure 3. Although, the wavelength of our spectrometer is limited by the light source, the absorption band of the ZnO nanoparticles shows a blue shift due to the quantum confinement of the excitations present in the sample as compared with the bulk ZnO particles. This optical phenomenon indicates that these nanoparticles have a quantum size effect [16].

Fig 3. UV-Visible absorption spectra for ZnO nanoparticles.

**C. Effect of nano-ZnO on the growth of E. coli in liquid medium**

In the first study, we investigated the effect of different concentrations of nanoparticles in liquid culture of E. coli. The optical density of the medium was investigated as the number of bacteria after contact with the nanoparticles. Figure 4 shows the effect of different 0.01, 0.5 and 1% nano-ZnO on the in growth and killing of E. coli. As demonstrated by the figure, 0.01% nano-ZnO did not have antibacterial efficiency on E. coli but the concentrations of 0.5 and 0.1% nano-ZnO inhibited the bacterial growth. Figure 4 shows that 0.5% nano-ZnO showed 2.04 times decrease the optical density of bacterial cultures (P<0.05) as compared to the control. While, in the presence of 1% nano-ZnO, the optical density of E. coli cultures decreased 4.385 times as compared to the control experiment.

Fig 4. E.coli concentration dependence upon different concentrations of nano-ZnO in the culture medium.

**D. Bactericidal effect of nano-ZnO on E. coli in liquid medium**

In the second study, estimation of the number of viable E. coli cells in contact with 1% nano-ZnO was carried out in water at 4 ºC for different contact time intervals. Our result showed the reduction of E. coli cells from 6.3 log CFU/ml to undetectable levels after 12 days (Data have not been shown). While, upon the addition of these nano-materials to the bacterial culture showed decreased survival rate within 2 days as compared to that of 12-day experiment for control group. Figure 5 represents the number of viable E. coli cells in contact with 1% nano-ZnO, suspended in water at 4 ºC for different contact times. From the figure, it can be clearly observed that nano-ZnO exhibited different antibacterial properties. After the E. coli were suspended in water along with ZnO, the number of microbial cells reached zero after 24 hours. These results demonstrate that nano-ZnO have a high antibacterial efficiency against E. coli.

Fig 5. killing kinetics of 1% nano-ZnO on the E.coli cultures.

**IV. DISCUSSION**

The antibacterial activities of different concentrations of nano-ZnO were investigated during the recent analysis. E. coli (ATCC 25922) was used as the test organism during the experiments. Good growth-inhibition results were observed when the bacterial cells were incubated with nanoparticles during the liquid and solid cultures. The quantitative examination of bacterial activity was estimated by the survival
ratio as calculated from the number of viable cells, which formed colonies on the nutrient agar plates [23]. The present data demonstrate that a formulation made with the biologically stabilized ZnO nanoparticles can be useful in the treatment of infectious diseases caused by E. coli. A strong binding of nanoparticles to the outer membrane of E. coli causes the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis, which finally leads to cell lysis as was seen for E. coli during the present study. Such effective and less-time consuming formulations can be useful in the clinical practices where E. coli causes urinary tract infections (UTIs). It has been known that nano-materials exhibit strong inhibitory effects towards a broad spectrum of bacterial strains [25].

During the present study, different concentrations of nanoscale ZnO were tested to find out the best concentration that can have the most effective antibacterial property against the E. coli culture. Our data is in accordance with the previous studies; dealing with the antibacterial effects of nano-materials [26–28]. Several investigations have suggested the possible mechanisms involving the interaction of nano-materials with the biological macromolecules.

It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an “electromagnetic” attraction between the microbe and treated surface. Once the contact is made, the microbe is oxidized and dead instantly. Generally, it is believed that nano-materials release ions, which react with the thiol groups (-SH) of the proteins present on the bacterial cell surface. Such proteins protrude through the bacterial cell membrane, allowing the transport of nutrients through the cell wall. Nano-materials inactivate the proteins, decreasing the membrane permeability and eventually causing the bacterial death. Nano-materials also retard the bacterial adhesion and bio-film formation [26].

Antimicrobial modification to prevent the growth of detrimental microorganisms is a highly desired objective. Microbial cell growth and colonization result in the formation of a compact bio-film matrix, capable of protecting the underlying microbes from antibiotics and host defense mechanisms. Microbial infestation can result in serious infection [27]. Such infections are also implicated in food spoilage, spread of food-borne diseases, and bio fouling of materials [28].

V. CONCLUSION

Hence, there is a significant interest in the development of antimicrobial materials and surfaces for applications in the health, biomedical, and food and personal-hygiene industry. The nanomaterials based on the metal ions, exhibit broad-spectrum biocides activity towards different bacteria, fungi, and viruses. Nanomaterials are known to deactivate cellular enzymes and DNA by coordinating to electron-donating groups such as thiols, carboxylates, amides, imidazoles, inooles, hydroxyls, and so forth. They cause pits in bacterial cell walls, leading to increased permeability and cell death.

REFERENCES

http://dx.doi.org/10.1039/b702887c

http://dx.doi.org/10.1016/j.cplett.2004.09.077


http://dx.doi.org/10.1088/0957-4484/17/20/008

http://dx.doi.org/10.1021/jp0502196

http://dx.doi.org/10.1111/j.1525-1594.2007.00530.x


http://dx.doi.org/10.1155/MBD.1994.467

http://dx.doi.org/10.1021/es803450f

http://dx.doi.org/10.1016/S0924-8579(99)00120-X


http://dx.doi.org/10.17758/IAAST.A0614086

66