Effects of pH and concentration on antibacterial activity of ZnO nanofluids against Staphylococcus aureus

R. Jalal[1,2,3], M. Saliani[1], E. K. Goharshadi[1].
[1]Depatment of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad.91779, Iran.
[2]Research and Technology Center of Biomolecules, Ferdowsi University of Mashhad, 91779, Iran
[3]Institute of Biotechnology, Ferdowsi Univesity of Mashhad, Mashhad 91779, Iran
razieh@ferdowsi.um.ac.ir(rsjalal@yahoo.com

Abstract:
Nanoparticle metal oxides represent new classes of important materials that are increasingly being developed for use in research and health-related applications. Highly ionic metal oxides are interesting not only for their wide variety of physical and chemical properties but also for their antibacterial activity. Although the in vitro antibacterial activity and efficacy of regular zinc oxides have been investigated, little is known about the antibacterial activity of nanoparticles of ZnO. In this work, the antibacterial activity of ZnO nanofluids was tested against Staphylococcus aureus (ATCC:25923) at different concentration and broad range of pH. Antibacterial tests were performed by measuring the growth curve of S.aureus in the TSB broth medium in the presence of nanofluids containing 1.5, 0.75, 0.375, 0.187,and 0.09 2g/ml of ZnO nanoparticles (ZnO NP) and pH range of 7-10. Antibacterial activity increased with increasing nanoparticle concentration . Also, it seems that basic pH improves antibacterial activity of ZnO NP.

Keywords: ZnO nanoparticles, Antibacterial activity, pH, Staphylococcus aureus.

Introduction:

The re-emergence of infectious diseases and the continuous development of antibiotic resistance among a variety of disease-causing bacteria pose a serious threat to public health worldwide . Despite antimicrobial therapy, morbidity and mortality associated with bacterial infections remain high, partially as a result of the ability of these organisms to develop resistance to virtually all antibiotics. New strategies are therefore needed to identify and develop the next generation of drugs or agents to control bacterial infections.

Recent advances in the field of nanotechnology, particularly the ability to prepare highly ordered nanoparticulates of any size and shape, have led to the development of new biocidal agents. Several studies have indicated that nanoparticulate formulations can be used as effective bactericidal materials[1].

Nanoparticles can be naturally occurring or they can be manufactured, the latter can be classed into several categories including the following:

1. Metal nanomaterials, such as gold and silver nanoparticles
2. Metal oxide nanomaterials, such as titanium dioxide and zinc oxide
3. Carbon nanomaterials such as fullerenes and nanotubes
4. Quantum dots such as cadmium telluride and cadmium selenide[2].

Amoung manufactured nanoparticles, nanoparticle metal oxides represent new classes of important materials. Sawai and others[3,4,5] have evaluated the antibacterial activity of 26 ceramic powders, and 10 of them were found to inhibit bacterial growth. Among these active powders, MgO, CaO, and ZnO exhibited strong antibacterial activity. It was found that the treatment with ZnO formulation caused a net reduction in bacterial cells of 78% and 62% in the case of treated cotton and cotton/polyester fabrics respecectively. Our previous report also showed the antibacterial properties of ZnO nanofluids against E.coli DH5?[6]. ZnO is one of the five zinc compounds that are currently listed as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). However, there are few data that demonstrate antimicrobial efficacy of ZnO in foods[7].

In this study, combined effect of pH and concentration of ZnO NP is investigated on Staphylococcus aureus (ATCC:25923) as gram positive bacteria. Our results showed the antimicrobial efficacy of ZnO NP against S.aureus. Antibacterial activity increased with increasing nanoparticle concentration. Also it seems that basic pH improves antibacterial activity of ZnO NP. So optimization of inviromental factors in order to achieve maximum activity of ZnO NP is very important.

Experimental:

ZnO NP used for antibacterial assay were prepared by thermal decomposition method[6].

Bacterial strain and culture medium:

To investigate the effect of pH on antibacterial activity of ZnO nanoparticles, we used Staphylococcus aureus (ATCC:25923) as gram positive bacteria.The bacteria is taken from Dr. Mohsenzadeh (Veterinary faculty, Ferdowsi University of Mashhad).TSB(triptic soy broth) is used as culture media.

Effect of pH and Zno concentration:

Concentration of 10_5 CFU/ml of bacterial suspension from logarithmic phase was added to different broth medium with concentrations of 1.5, 0.75, 0.375, 0.187,and 0.092g/ml of ZnO NP. After 24 hours the visible turbidity of different cultures were compared with positive control (culture without ZnO NP). Since, no visible growth of bacterial cells was observed at concentrations of 1.5, 0.75, and 0.375 2g/ml, MIC (minimum inhibitory concentration) of ZnO NP against S. aureus is 0.375 2g/ml. Hence concentrations of 0.375, 0.187, and 0.09 2g/ml of ZnO NP were choosed to investigate the combined effect of pH and concentration on the antibacterial activity. The concentration of 10_5 CFU/ml from logarithmic phase bacterial cells were added to different broth medium with concentrations of 0.375, 0.187, and 0.09 2g/ml of ZnO NP and pH range from 7-10. Two negative controls were used:i)-Broth medium with pH range of 7-10 and concentrations of 1.5, 0.75, 0.375, 0.187, and 0.09 2g/ml of ZnO NP.ii)-Broth medium with pH conditions from 7-10.

Different cultures with pH range of 7-10 were used as positive controls. Finally, after preparation all of the samples, 1502l of each one was transferred to every well of 96-well microplate. Then the microplate was located in shaker incubator and OD600 was readen by Elizareader in exact times and growth curve of each sample was drawn. Decreased growth of bacterial cells in the presence of ZnO NP was compared with the normal growth of bacterial cells in absence of ZnO NP.

Results:

Bacterial growth was inhibited in the presence of ZnO NP at concentrations of 0.375 and 0.0187 2g/ml (as compared with positive control) with the lowest growth in 0.375 2g/ml. But there was no significant difference at bacterial growth between concentration of 0.09 2g/ml and positive control (Fig. 1).

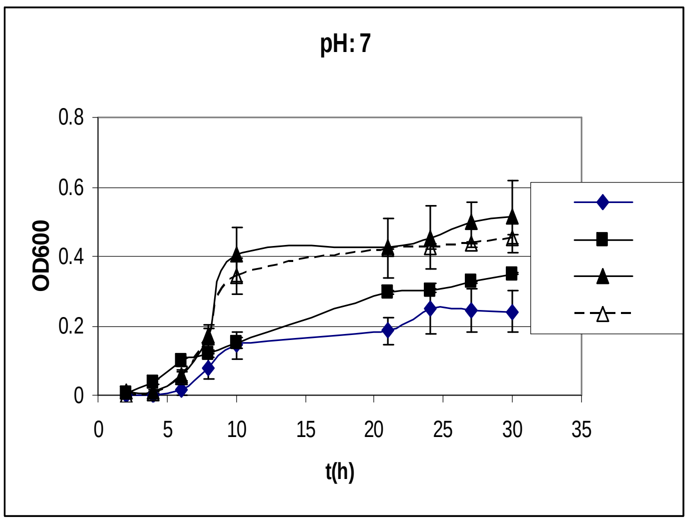

Fig. 1: Effect of ZnO NP concentration on the growth curve of S.aureus at pH:7. D) 0.3752g/ml, E) 0.1872g/ml, F) 0.092g/ml and G) positive control

Bacterial growth was completely inhibited in concentration of 0.375 2g/ml of ZnO NP in pH 8. Also bacterial growth is reduced in concentration of 0.187 2g/ml in comparison with positive control. There was a delay time about 8 hours in growth curve of bacteria in concentration of 0.187 2g/ml. When the concentration of ZnO NP eas 0.09 2g/ml, bacterial growth reduced in 8 and 10 hours after cultivation in comparison with positive control (Fig.2).

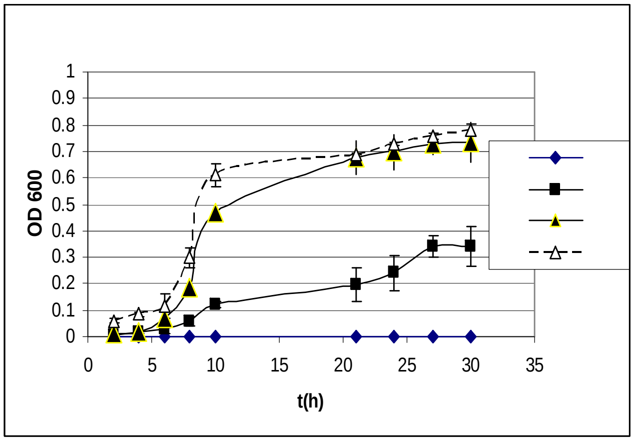

Fig. 2: Effect of ZnO NP concentration on the growth curve of S.aureus at pH:8. D) 0.3752g/ml, E) 0.1872g/ml, F) 0.092g/ml and G) positive control

ZnO NP inhibited the bacterial growth completely at concentration of 0.375 and at pH 9. Also, bacterial growth reduced in concentration of 0.187 2g/ml in comparison with positive control. The delay time of the growth curve of bacteria in pH 9 was about 10 hours, which was more than the amount at pH 8. There was no significant difference for bacterial growth between concentration of 0.09 2g/ml and positive control (Fig. 3).

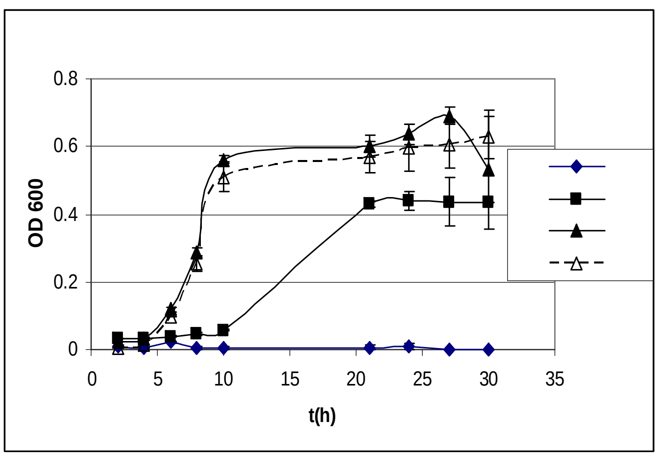

Fig. 3 : Effect of ZnO NP concentration on the growth curve of S.aureus at pH:9. D) 0.3752g/ml, E) 0.1872g/ml, F) 0.092g/ml and G) positive control

At pH 10 and the 0.375 2g/ml of ZnO NPs, 100% inhibition of bacterial growth was observed. At concentration of 0.187 2g/ml, reduction of bacterial growth and a delay time of about 10 hours was elicited.At 0.09 2g/ml, bacterial growth reduced in pH:10 in comparison with positive control after 6 hours of cultivation. Also, the delay time of about 10 hours was observed at concentration of 0.09 2g/ml and at pH:10 (Fig. 4).

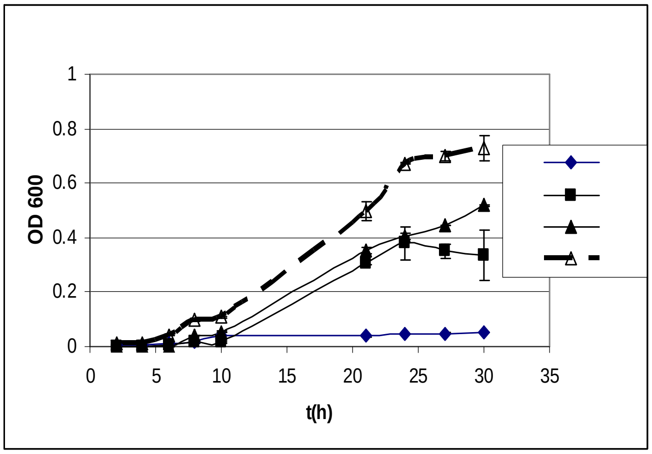

Fig. 4: Effect of ZnO NP concentration on the growth curve of S.aureus at pH:10. D) 0.3752g/ml, E) 0.1872g/ml, F) 0.092g/ml and G) positive control

Discussion:

Our results showed as the concentration of ZnO NPs increased, the antibacterial activity improved (Fig. 1-4). According to the growth curves of S.aureus at 0.3752g/ml of ZnO NPs, no bacterial growth was observed at pH range of 8-10, however bacterial cells grew at pH:7. At the concentration of 0.187 2g/ml of ZnO NP, the delay time increased as the pH enhanced. Also, at 0.09 2g/ml, more reduction of bacterial growth was observed as the pH increased, so as at pH 10 after 6 hours, significant difference of bacterial growth between positive control and growth curve of bacteria was observed.

So, it seems that in basic pH, the antibacterial activity of ZnO NP increases. Zinc oxide is a photocatalyst; once it is illuminated by light with energy higher than its band gaps, the electrons in ZnO will jump from the valence band to the conduction band, and the electron (e^-) and electric hole (h+) pairs will form on the surface of the photocatalyst. The negative electrons and oxygen will combine into O_{2-}; the positive electric holes and water will generate hydroxyl radicals. Since both are unstable chemical substances, when the organic compound falls on the surface of the photocatalyst it will combine with O_{2-} and $OH_{\cdot}$ respectively, and turn into carbon dioxide (CO_2) and water (H_2O)[7]. As the pH increases, the concentration of hydroxyl anione increased too. So these anions and positive electric holes combine into hydroxyl radicals, so the antibacterial activity of ZnO NPs enhances at basic pH.

Coclusions:

Overall, the findings suggest that ZnO nanoparticles can be used externally to control the spreading of bacterial infections. It seems that optimization of environmental factors, could change the biocidal properties of ZnO NP against S.aureus. Therefore, in the future, ZnO nanoparticles containing formulations may be utilized for external uses as antibacterial agents in ointments, lotions, mouthwashes, and surface coatings on various substrates to prevent microorganisms from attaching, colonizing, spreading, and forming biofilms in indwelling medical devices.

Refferences:

[1] N. Jones, B. Ray, K. T. Ranjit2 & A. C. Manna, Antibacterial activityof ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett 279 (2008) 71–76.
[2]Toxicological Review of Nano Zinc Oxide. Prospect global nanomaterial safety. 8 September 2009.
[3]Sawai J, Igarashi H, Hashimoto A, Kokugan T, Shimizu M. Evaluation of growth inhibitory effect of ceramic powder slurry on bacteria by conductance method. J Chem Eng Jpn 28 (1995) 288–93.
[4] Sawai J, Shoji S, Igarashi H, Hashimoto A, Kokugan T, Shimizu M, Kojima H. Hydrogen peroxide as an antibacterial factor in zinc oxide powder slurry. J Ferment. 86 (1998) 521-22.
[5] Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J Microbiol Methods 54 (2003) 177–82.
[6] R. Jalal, E. K. Goharshadi, M. Abareshia, M. Moosavi, A. Yousefi, P. Nancarrow, ZnO nanofluids: Green synthesis, characterization, and antibacterial activity. Materials Chemistry and Physics 121 (2010) 198–201.
[7] T. Jin, D. Sun, J.Y. SU, H. Zhang, H.-J. Sue. Antimicrobial Efficacy of Zinc Oxide Quantum Dots against Listeria monocytogenes, Salmonella Enteritidis, and Escherichia coli O157:H7. J Food Sci. 74(2009) M46-52.
[8] Y. W. H. Wong, C. W. M. Yuen, M. Y. S. Leung, S. K. A. Ku1, and H. L. I. Lam. Selected Applications of Nanotechnology in Textiles. AUTEX Research Journal, 6 (2006) 1-8.