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Nanoparticles of copper and copper oxides: Synthesis and Determination of antibacterial activity

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Abstract

In the present study, nanoparticles of copper and copper oxides were synthesized and their antibacterial activity was evaluated and compared with silver nanoparticles. The nanoparticles were synthesized using facile chemical reactions, and then characterized using field emission scanning microscopy. The nanoparticles were stable for at least two weeks. The antibacterial activity of the nanoparticles against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterococcusfaecalis* and *Escherichia coli* was investigated based on inhibition zone in disk diffusion assay. The minimum inhibitory concentration and minimum bactericidal concentration of the nanoparticles were also reported. Antibacterial activity of the nanoparticles showed better inhibitory activity against gram positive bacteria.

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Introduction

The synthesis of metal nanoparticles attracts increasing interests due to different characteristics of them as compared with those of macroscopic phase. Metal nanoparticles have attractive applications in various fields such as nano medicine, biotechnology, optics, catalysis and electro catalysis (1, 2). For centuries, bactericidal activity of silver has been well-known, and it is an effective bactericidal metal because it is safe to animal cells and very toxic to microorganisms such as *Escherichia coli* and *Staphylococcus aureus* (3).Several mechanisms for its bactericidal effects have been proposed (3). It is believed that the mechanism of the bactericidal effect of metallic ions involves their absorption and accumulation by bacterial cells and ruptures of the cytoplasm membrane or detachment of the cell wall (4). As a result, DNA molecules become condensed and lose their ability to replicate upon the infiltration of metallic ions. The metallic ions also interact with thiol functional groups of the proteins, and inactivate them (3, 4).

Nanoparticles have the properties of high surface to volume ratio, small size, and high dispersion which allows them to interact with bacterial surface in one hand, and they can release (bactericidal) metal ions, on the other hand (3, 4). The antibacterial ability of metal and metal oxide

nanoparticles are attributed to their small size, which is >300 times smaller than a bacterium. This facilitates their adherence to the cell wall of the microorganisms causing its destruction and consequently cell death (5). Up to now, bactericidal activity of different nanoparticles has been investigated (6), but there are a few studies about the antibacterial properties of copper species (7-9).

In the present study, nanoparticles of copper, cuprous oxide and cupric oxide were synthesized and their bactericidal activity against some gram positive and gram negative bacteria was evaluated.

Materials and methods

Synthesis of copper nanoparticles

In a typical procedure, solutions of 5 mL of 0.30 Mpolyvinylpyrrolidone (PVP, 40000, Sigma, USA) dissolved in anhydrous ethylene glycol (EG, Schalau, Spain), 3 mL of 0.10 Mcopper sulfate (Schalau, Spain) dissolved in EG and3 mL of 0.25 Mascorbic acid (Schalau, Spain) in EG were firstly prepared. The PVP solution was heated to 140°C, and the solutions of copper sulfate and ascorbic acid were simultaneously injected drop-wise with a rate of ~0.25 mL min-1. Immediately after the initial injection, the reaction mixture turned to red, indicating the formation of copper seeds. Upon addition of further solutions, the reaction system gradually became turbid with a dark red wine color. Heating and stirring were continued until obtaining a reddish brown solution. The resultant solution was aged for approximately one hour at 80°C in order to promote the growth of nanoparticles. The sample was then washed with distilled water.

Synthesis of Cu2O nanoparticles

A mixture of 10mL of 0.0 09 Maqueous ascorbic acid solution and 16 mL of 0.11 Maqueous sodium hydroxide solution were added into 16 mL of 0.005 Maqueous copper acetate solution under stirring. The mixed solution was then stirred vigorously for 30 min until the solution became yellow and turbid, indicating the formation of Cu2O nano particles. The sample was then washed with distilled water.

Synthesis CuO nanoparticles

2 mL of 0.02 Maqueous copper acetate solution and 3 mL of 0.13 Maqueous PVP solution were added into a 50 mL round-bottomed flask. Then 10 mL of a mixed solution of 0.08 M sodium hydroxide + 0.05 mMcetyltrimethylammonium bromide (CTAB, Merck, Germany) dissolved in EG was added to the flask, to keep the molar ratio of copper acetate/sodium hydroxide/PVP equal to1/2/10. The mixture was heated in microwave oven (900 W) for 120 s and a dark brown colloid was obtained. The sample was then washed with distilled water.

Morphology of the nanoparticles

The size, morphology and structure of the nanoparticles were evaluated using field emission electron microscopy (FESEM) using a Zeiss, Sigma-IGMA/VP instrument (Germany).

Antibacterial studies

Antibacterial activity of the synthesized nanoparticles against gram negative bacteria of *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (PTCC 1430), and gram positive bacteria of *Bacillus subtilis* (PTCC 1023), *Enterococcusfaecalis* (PTCC 1237) and *Staphylococcus aureus* (PTCC 1431) were evaluated. The stains were taken from Pasteur Institute of Iran. The antibacterial activity of the nanoparticles was evaluated using disc diffusion method. Nutrient agar powder was dissolved in boiling water, sterilized, cooled and poured into petri dishes with almost equal agar thickness (~2.5 mm). The bacteria were seeded in agar plates by the spread plating technique. Sterilized paper

discs were dipped in the nanoparticle suspensions and dried at room temperature. The disks were then introduced into the petri dishes and incubated at 37 ºC for 24 h. Mean and standard deviation (SD) were reported for each nanoparticle and with each microbial strain based on four replicates.

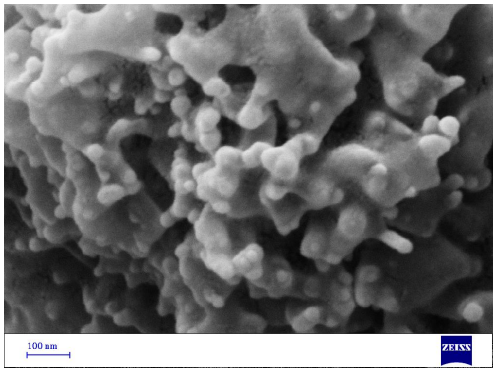
The minimum inhibitory concentration (MIC) of an antibacterial agent for a particular bacterium is defined as its concentration in the growth medium which causes complete inhibition of bacterial growth without cell killing even after overnight incubation. On the other hand, the minimum bactericidal concentration (MBC) of an antimicrobial substance is defined as the concentration for which overnight incubation with a bacterial population causes 99.9% cell killing. Sterile multi well ELISA plates, each well containing 180 µL nutrient broth, were inoculated with 20 µL of the freshly prepared bacterial suspension in order to maintain initial bacterial concentration 10^3-10^4 CFU mL^{-1}, and the suspensions were mixed. Negative controls (wells containing inoculum and nutrient media without nanoparticles) were also prepared. The plates were kept at 37ºC until the colonies appeared. Then, suspected wells were cultured on the petridish with the pour plate technique for ensuring that colonies did not exist in well, and the concentration of nano structures was assumed as MIC or MBC. All the experiments were carried out in triplicate.

Statistical analysis

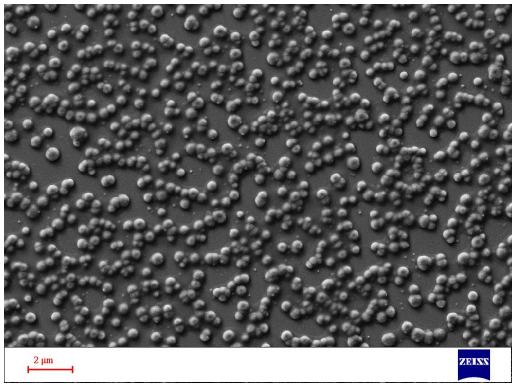
The values of MIC, MBC and zone of inhibition were measured in triplicate, and the means and standard deviations were reported.

Results and discussion

Fig 1 Shows FESEM images of the nanoparticles of copper and copper oxides. Based on the results, copper and copper (I) oxide had a spherical morphology with a mean size of 35 and 65 nm, respectively. On the other hand, copper (II) oxide had a cubic structure with a mean size of 85 nm.



(A)



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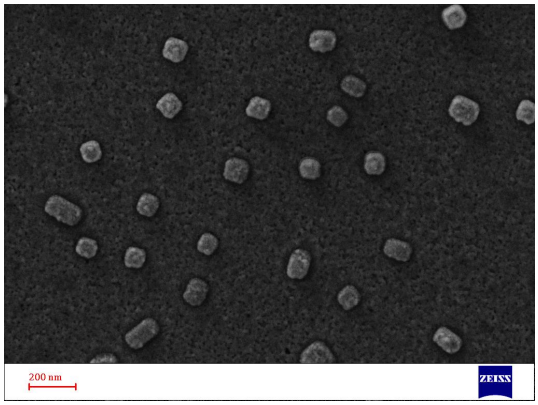

(C)

Fig 1. FESEM images of copper (A), copper(I) oxide (B), and copper(II) oxide (C).

The antibacterial activity of the synthesized nanoparticles against five types of different species of bacteria was studied. Negative control shows no zone of inhibition, and the results for the nanoparticles are presented in Tables 1-3.The values of MIC and MBC for the nanoparticles are also reported in Tables 1-3. Based on the results, copper nanoparticles had bactericidal activity against all the bacteria species, copper (I) oxide nanoparticles only affected *Escherichia coli* and *Bacillus subtilis*, and copper (II) oxide nanoparticles had bactericidal activity against gram positive bacteria. On the other hand, copper had the highest antibacterial activity against *Bacillus subtilis*, and the lowest effect on *Enterococcusfaecalis*.

Table 1. The values of MIC and MBC of copper nanoparticles for various microorganisms

Strain	Zone of inhibition / mm	MIC / µg mL-1	MBC / µg mL-1
Escherichia coli	5±0.7	220±18	360±17
Bacillus subtilis	10±0.9	260±19	440±25
Pseudomonas aeruginosa	2±0.2	360±12	780±37
Staphylococcus aureus	5±0.2	180±12	360±24
Enterococcusfaecalis	1±0.1	740±29	740±30

Table 2. The values of MIC and MBC of copper (I) oxide nanoparticles for various microorganisms

Strain	Zone of inhibition / mm	MIC / µg mL-1	MBC / µg mL-1
Escherichia coli	1±0.1	600±31	800±18
Bacillus subtilis	2±0.1	500±27	700±16
Pseudomonas aeruginosa	-	800±24	900±23
Staphylococcus aureus	-	800±15	800±16
Enterococcusfaecalis	-	-	-

Table 3. The values of MIC and MBC of copper (II) oxide nanoparticles for various microorganisms

Strain	Zone of inhibition / mm	MIC / µg mL-1	MBC / µg mL-1
Escherichia coli	-	280±10	420±12
Bacillus subtilis	2±0.3	200±9	280±14
Pseudomonas aeruginosa	-	280±17	480±13
Staphylococcus aureus	1±0.1	240±14	420±12
Enterococcusfaecalis	3±0.2	-	-

The different sensitivity of gram positive and gram negative bacteria toward the nanoparticles can be related to their cell structure, physiology, metabolism and their inter action with the charged nano particles. Copper, copper (I) oxide and copper (II) oxide are all positively charged particles in the working conditions of bacteria culture(10).The antibacterial effect of these nanoparticles on microorganisms may be held through the electrostatic at traction of positive charged nano particles and negative charged cell surface of the bacteria. On the other hand, small nano particles are also able to penetrate inside the bacteria, and cause damage, lose cell activity and death. Moreover, the nanoparticles can release copper ions, which can have an additional contribution to the antibacterial activity. Therefore, nanoparticles can represent antibacterial activity through different manners of cell surface attachment, destruction of cellular components, and release of reactive oxygen species.

Conclusion

A simple chemical synthesis was developed for the synthesis of copper, copper (I) oxide and copper (II) oxide nanoparticles. Bactericidal action of the synthesized nanoparticles was examined against gram positive and gram negative pathogens. The antimicrobial activity of copper nanoparticles showed better inhibitory activity against gram positive bacteria than negative bacteria.

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References

1. Nalwa HS. Encyclopedia of Nanoscience and Nanotechnology. vols 1-10. USA, California: American Scientific Publishers; 2004.
2. Rahi A, Karimian K, Heli H. Nanostructured materials in electro analysis of pharmaceuticals. *Anal Biochem* 2016; 497, PP 39-47.
3. Rizzello L, Pompa PP. Nano silver-based antibacterial drugs and devices: mechanisms, methodological drawbacks, and guidelines. *Chem Soc Rev* 2014; 43(5): 1501-18.
4. Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. *Adv Drug Deliv Rev* 2013; 65(13-14): 1803-15.
5. Chwalibog A, Sawosz E, Hotowy A, Szeliga J, Mitura S, Mitura K, Grodzik M, Orlowski P, Sokolowska A. Visualization of interaction between inorganic nanoparticles and bacteria or fungi. *Int J Nanomedicine* 2010; 5: 1085-94.
6. Hajipour MJ, Fromm KM, Ashkarran AA, Jimenez de Aberasturi D, de Larramendi IR, Rojo T, Serpooshan V, Parak WJ, Mahmoudi M. Antibacterial properties of nanoparticles. *Trends Biotechnol* 2012; 30(10): 499-511.
7. Effenberger F.B, Sulca M.A, Machini M.T, Couto R.A, Kiyohara P.K, Machado G, Rossi L.M. Copper nanoparticles synthesized by thermal decomposition in liquid phase: the influence of capping ligands on the synthesis and bactericidal activity. *Journal of Nanoparticle Research* 2014; 16: 2588.
8. Jeziorska R, Zielecka M, Gutarowska B, Zakowska Z. High-density polyethylene composites filled with Nano silica containing immobilized Nano silver or Nano copper: thermal, mechanical, and bactericidal properties and morphology and interphase characterization. *International Journal of Polymer Science* 2014; pp. 1-13.
9. Zain NM, Stapley AG, Shama G. Green synthesis of silver and copper nanoparticles using ascorbic acid and chitosan for antimicrobial applications. *Carbohydr Polym* 2014; 112: 195-202.
10. Parks G.A. the isoelectric points of solid oxides, solid hydroxides, and aqueous hydroxo complex systems. *Chemical Reviews* 1965; 65(2): 177-198.