Antibacterial activity of a malodor neutralizer containing silver nanoparticles

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Abstract

In this study bactericidal activity of a malodor neutralizer containing silver nanoparticles manufactured in Iran has been tested. For this purpose different concentrations of the product encounter with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* then the activity were evaluated in different contact times. The products containing at least 200 ppm of silver nanoparticles were effective on all of bacterial strains and higher exposure time increased its antibacterial activity**.** Bactericidal activity against spore of *Bacillus cereus* was less obvious in comparison with other bacteria. It seems that silver nanoparticle is a valuable antibacterial agent even in presence of aromatic fragments and could be applied as disinfectant in many situations.

Key words: silver nanoparticle, bactericidal, aromatic, disinfectant

Introduction

Since ancient times, it has been known that silver and its compounds are effective antimicrobial agents (Klasen, 2000). Because of the recent advances on metal nanotechnology, silver nanoparticles have received renewed attention as a possible antimicrobial agent (Lee et al., 2008; Melaiye et al., 2005; Sondi and Salopek-Sondi, 2004; Landsdown, 2002).The great interest arouse when recently silver nanoparticles claim as new antibacterial compound which rarely develop resistant bacteria (Landsdown, 2002; Baker et al., 2005; Lock et al., 2007). It has been shown that the LD50* of silver nanoparticles either by ingestion or injection is very high (1266 mg/kg & 284 mg/kg respectively) and inhalation of a high doses (1.32 x 10(6) particles/cm3, 61 microg/m3) of silver nanoparticles is safe, so it can be classified as a non-toxic substance (Ji et al., 2007; Fu et al., 2006). This and some other properties make silver nanoparticles very suitable for disinfecting usage.

The acceptability of each product for a defined purpose cannot be determined without valid tests. Therefore, the product should be subjected to relevant tests in order to evaluate their activity under conditions recommended for their intended use (Zhao and Stevens, 1998; ISIRI 10504, 2008). The aim of this study was to evaluate basic bactericidal activity of a fragrant product containing silver nanoparticles.

Nanotechnology deals with preparation of uniform nanosized silver particles with specific requirements in terms of size, shape, and physical nanomaterials which are very useful both in scientific and commercial applications (Sondi and Salopek-Sondi, 2004; Landsdown, 2002).

Material and Methods

For assessment of bactericidal activity of this product and determination of minimum inhibitory concentration, we prepared serial dilutions of products containing 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 ppm of silver nanoparticles (Nanocid, Iran). Size of silver nanoparticles that we used was around 4.5 nanometers.

Each dilution of product encountered with 1.5×10^8 CFU of bacteria prepared in Muller Hinton broth (Merck, Germany). Bacterial strains that we used in this study were *Staphylococcus aureus* (PTCC[*]1112) as an indicator of Gram positive bacteria and *Pseudomonas aeruginosa* (PTCC 1073) as a Gram negative and and resistant bacteria. *Bacillus cereus* (PTCC 1014) was also included in this study as a spore forming bacteria.

In this study contact time was 5, 15, 30 minutes and 24 hours and the temperature during the exposure was 25^oc. After each contact 10 µl of bacterial suspension and the disinfecting product have been

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[*] Lethal dose for 50 percent of subjects

[*] Persian Type Culture Collection

taken and inoculated on Muller Hinton agar medium and incubated at 37°c for 24 hours. Then each plate was inspected and the results were reported. We used distilled water without any disinfectant as negative control for each suspension. Also Carrier test is performed for evaluation of antibacterial activity of this substance which is to apply on surfaces. This test mimics a condition similar to the real application of disinfecting agent. In this test, 1.5×10^7 CFU of *E. coli* (PTCC: 1389) was applied to a sterile surface. Then three dilutions of disinfecting agent which contained 200, 100 and 50 ppm of silver nanoparticles were sprayed on this surface. Samples were taken by sterile swabs from this surface after 5 minutes and inoculated on to Muller Hinton agar medium (Merck, Germany). After 24 hours incubation in 37°c, the plates were inspected for bacterial growth.

Results

The bactericidal activity of this disinfecting agent on *S. aureus* was presented in table 1. The product with concentrations greater than 12.5 ppm of active ingredient demonstrated at least a 5 decimal log reduction in bacterial population when tested and there is a significant difference in bacterial number between before and after usage of this product with corresponding concentrations ($p<0.01$).

Table 1: Number of remaining bacteria after exposure with different concentration of silver nanoparticles which presents bactericidal activity on *S.aureus*

Concentration / time	200	100	50	25	12.5	6.25	3.12	1.56	0.78
5 min	0	0	0	0	0	$5.7*10^3$	$27*10^3$	$44*10^3$	$>10^5$
15 min	0	0	0	0	0	$3.3*10^3$	$19*10^3$	$30*10^3$	$>10^5$
30 min	0	0	0	0	0	0	$15*10^3$	$21*10^3$	$>10^5$
24 h	0	0	0	0	0	0	$0.3*10^3$	$4*10^3$	$>10^5$

Results of bactericidal activity of different concentration of this disinfecting agent on *P. aeruginosa* were presented in Table 2. The product with concentrations greater than 100 ppm of active ingredient and more than 5 minutes exposure demonstrated at least a 5 decimal log reduction in bacterial population when tested and there is a significant difference in bacterial number between before and after usage of this product with corresponding concentrations ($p<0.01$).

Table 2: Number of remaining bacteria after exposure with different concentration of silver nanoparticles which presents bactericidal activity on P. aeruginosa

Concentration / time	200	100	50	25	12.5	6.25	3.12	1.56	0.78
5 min	$0.3*10^3$	$14*10^3$	$21*10^3$	$>10^4$	$>10^4$	$>10^5$	$>10^5$	$>10^5$	$>10^5$
15 min	0	0	$19*10^3$	$24*10^3$	$>10^4$	$>10^5$	$>10^5$	$>10^5$	$>10^5$
30 min	0	0	$2.3*10^3$	$3*10^3$	$7*10^3$	$14.6*10^3$	$15.3*10^4$	$>10^4$	$>10^5$
24 h	0	0	0	0	0	0	0	$6.5*10^3$	$28.5*10^3$

Results of bactericidal activity of different concentration of this disinfecting agent on *B.cereus* were presented in Table 3. No tested concentration of the product demonstrated a 5 decimal log reduction in bacterial population although there is a significant difference in bacterial number between before and after usage of this product ($p<0.01$).

Table 3: Number of remaining bacteria after exposure with different concentration of silver nanoparticles which presents bactericidal activity on *B. cereus*

Concentration / time	200	100	50	25	12.5	6.25	3.12	1.56	0.78
5 min	$24*10^3$	$33*10^3$	$>10^4$	$>10^4$	$>10^5$	$> 10^5$	$> 10^5$	$> 10^5$	$> 10^5$
15 min	$33*10^3$	$40*10^3$	$43*10^3$	$>10^4$	$>10^4$	$> 10^5$	$> 10^5$	$> 10^5$	$> 10^5$
30 min	$41*10^3$	$43*10^3$	$54*10^3$	$>10^4$	$>10^4$	$> 10^5$	$>10^5$	$>10^5$	$> 10^5$
24 h	$25*10^3$	$30*10^3$	$>10^4$	$>10^4$	$> 10^4$	$> 10^5$	$> 10^5$	$> 10^5$	$> 10^5$

Carrier test show that 200 ppm of silver nanoparticles destroyed all bacteria so has good disinfecting property when were sprayed on surfaces

Discussion

This product which contains silver nanoparticles (200 ppm), perfume (0.02 -0.05%), FOT (1.5% - 2%) and water has good bactericidal activity on Gram positive bacteria like *S.aureus*. The longer contact time has additive effect on bactericidal activity of this product. This fact is also true for Gram negative bacteria like *P. aeruginosa*.

In this study, although higher concentrations of silver nanoparticles seem to decrease the number of *B. cereus*, but longer contact time doesn't seem to cause significant decrease in the number of this bacterium so it sounds that this product is not an effective disinfectant on spores. Our previous study shows that presence of FOT as a malodor neutralizer in composition of this product have slight negative effect on antibacterial activity of silver nanoparticles. Optimal concentration that is a minimum concentration which demonstrates a five log reduction in the test conditions were 12.5, 100 and 200 ppm of silver nanoparticles for *S.aureus, P. auerogenosa* and *B. cereus*, respectively. The silver nanoparticles are stable in environment, thus concentration of it will increase in environment after each usage.

It is believed that silver nanoparticles destroyed bacteria by two mechanisms: ion mechanism and catalectic mechanism (Jia et al., 2008; Kim et al., 2008; Kim et al., 2008). In ion mechanism, silver nanoparticles gradually radiate Ag+ ions. These ions during replacement reaction change HS- bands in microorganism's membrane and enzymes into Ag-S bands, in this way the nano-silver suppresses respiration, basal metabolism of electron transfer system, and transport of substrate in the microbial cell membrane. The result of this reaction, are denaturation and wasting of the microorganism (Landsdown, 2002; Panacek et al., 2006).

In Catalectic mechanism, silver nanoparticles were put on semi conductor bases, such as TiO_2 or SiO_2. In this case particle acts like an electro chemical pile which produces O_2^- radicals and OH ion that both are active bases that are amoung the strongest antibacterial agent. (Lok et al., 2007, Jia et al., 2007; Jeon et al., 2003)

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