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RESEARCH ARTICLE

DISINFECTANT PROPERTY EVALUATION OF THREE DIFFERENT TYPES OF SILVER SULFIDE NANOPARTICLES AND COMPARING WITH SILVER NANOPARTICLES

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ABSTRACT

Silver nanoparticles and sulfur have been proved to possess significant antibacterial properties. In an attempt to improve nanosilver antibacterial properties, we prepared silver sulfide nanoparticles with sulfur component from three different sources including, thioacetamide (F_1), ammonium sulfide (F_2) and hydrogen sulfide gas (F_3). Silver sulfide nanoparticles have been evaluated by European standards (CEN TC 216) in order to find out whether they could be applied in industrial, domestic and institutional areas as a suitable disinfectant or not. Moreover, bactericidal activity of silver sulfide nanoparticles has been compared to silver nanoparticles. Silver sulfide nanoparticels made by hydrogen sulfide gas not only passed the standards, but also showed more bactericidal activity against silver nanoparticles.

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INTRODUCTION

Antimicrobial properties of silver nanoparticles (Devalapally *et al*., 2007; Xiaofeng and Linlin, 2005) and sulfur (Wang *et al*., 2005) have been broadly investigated by several researches throughout the last decade. So such nanoparticles which contained both silver and sulfur could improve silver nanoparticles bactericidal activity. (Chen and Schluesener, 2008, Hota and Jain, 2007). Silver sulfide nanoparticles as new surface disinfectant was studied in this article. Three different kinds of silver sulfide nanoparticles through their three different source of sulfur (thioacetamide (F_1), ammonium sulfide (F_2) and hydrogen sulfide gas (F_3)) were studied (Chen *et al*., 2008). Silver sulfide nanoparticle is such a precious target at delivering alternative methods of disinfection (Gupta, 2004) as the issue of antibiotic resistance has been raised (Xiaofeng and Linlin, 2005, Feng *et al.,* 2005 Shrivastava *et al*., 2007).

Escherichia coli (Devalapally *et al*., 2007, Wang *et al*., 2005, Duran *et al*., 2007), Staphylococcus aureus (Devalapally *et al*., 2007, Duran *et al*., 2007), methicillin resistant S. aureus (Sondi and Salopek-Sondi, 2004), S. epidermis (Feng *et al*., 2005), Bacillus subtilis (Feng *et al*., 2005) are among the targeted microorganisms used in different studies recommended by CEN TC 216. It has been concluded that silver sulfide nanoparticles which produced by hydrogen sulfide gas could prove to be simple, cost effective and suitable for formulation of new type of bacterial materials like silver nanoparticles and even more potent than nanosilver (Castellano *et al*., 2007), about one logarithmic unit decrease in number of CFU in bactericidal activity (Devalapally *et al*., 2007, Duran *et al*., 2007, Sondi and Salopek-Sondi, 2004). Therefore, it would be beneficial to set some specific standards through which bactericidal efficacy of silver-derivatives nanoparticles made by various synthesis methods could be assessed (Yuanhua and Bing, 2005, Zhang *et al*., 2005). Concerning the ever-growing application of silver-derivatives nanoparticles in various fields (Lee and El-Sayed, 2006, Lansdown, 2006), we managed to access CEN TC 216 standards relating to the evaluation of chemical disinfectants and antiseptics used in industrial, domestic and institutional areas.

MATERIALS AND METHODS

Bacterial strains and preparation

Bacterial strains included Pseudomonas aeruginosa (ATCC

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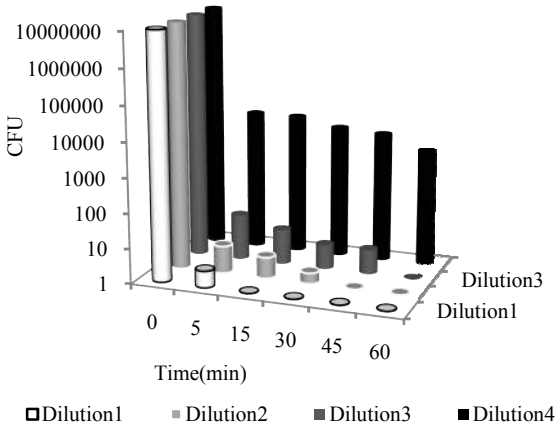
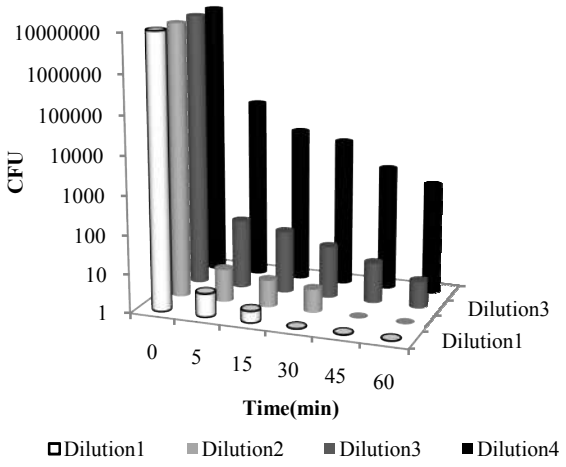
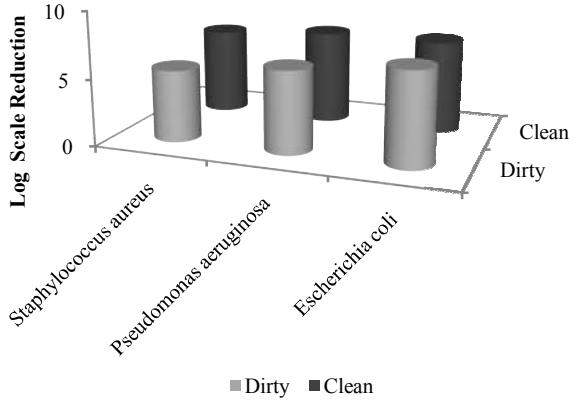
15442), Staphylococcus aureus (ATCC 6538) and Escherichia coli (ATCC 10536). The 24-hour cultured colonies were used to yield 10^7 CFU/ml of each bacterial strain in normal saline. Plate counts of 10^{-4}, 10^{-5}, 10^{-6} dilutions were done to confirm the initial bacterial load.



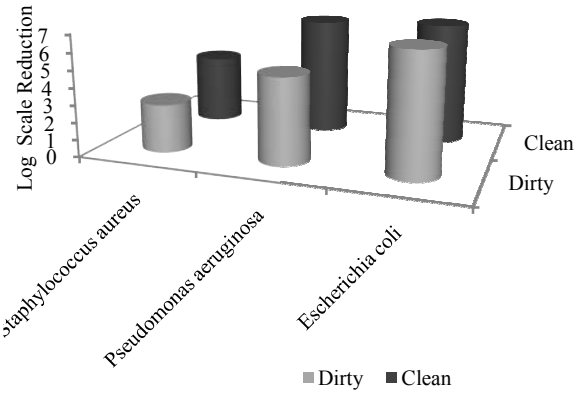
Graph 1 Staphylococcus aureus-nanosilver sulfide (F_3) (which made by H_2S gas) survivor count after 48h incubation at 37° C (phase 1)



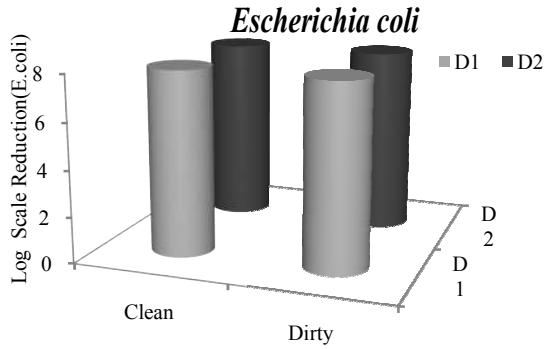
Graph 2 Pseudomonas aeruginosa –nanosilver sulfide (F_3) (which made by H_2S gas) survivor count after 48h incubation at 37° C (phase 1)

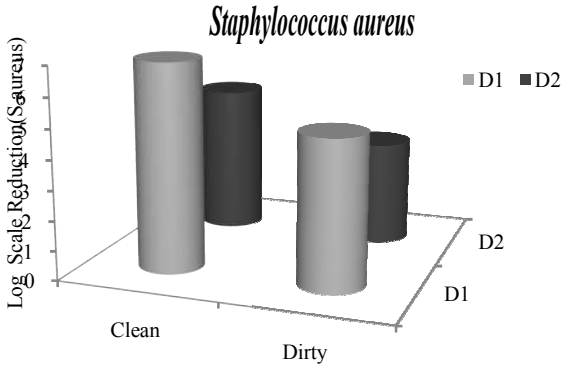


Graph 3 Reduction in the 10^7 inoculum for the three bacterial strains after exposure to the first dilution of nanosilver sulfide for 5 minutes followed by 48h incubation at 37° C (phase 2, step 1)

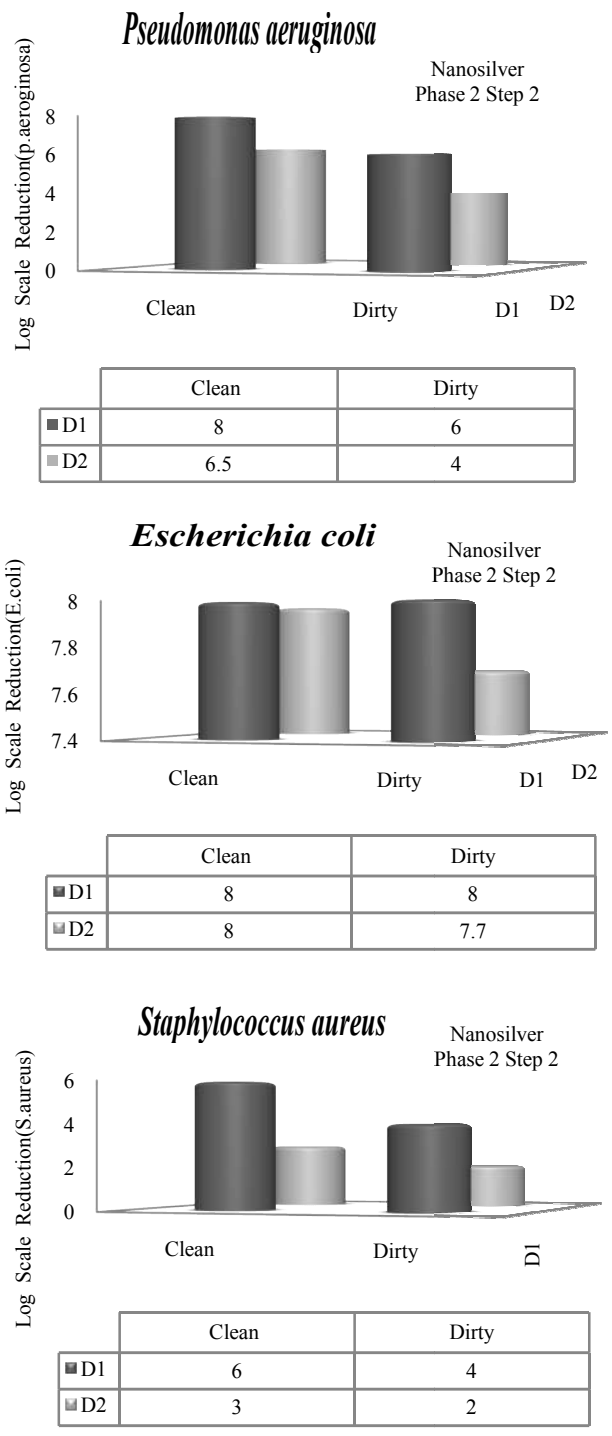


Graph 4 Reduction in the 10^7 inoculum for the three bacterial strains after exposure to the second dilution of nanosilver sulfide for 5 minutes followed by 48h incubation at 37° C (phase 2, step 1)





Graph 5 Reduction in the 10^8 inoculum of the three standard strains after exposure to the two dilutions of nanosilver sulfide in clean and dirty conditions (phase 2, step 2)



Graph 6 Reduction in the 10^8 inoculum of the three standard strains after exposure to the two dilutions of nanosilver in clean and dirty conditions (phase 2, step 2)

Bacterial susceptibility

The antibacterial potency of the synthesized silver and silver sulfide nanoparticles (Chopra, 2007) were analyzed according to the CEN TC 216. Available standard were a) EN 1040:1997 (phase 1) which is a quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics; b) EN 1276: 1997 (phase 2 step 1) which evaluates the bactericidal activity of chemical disinfectants and antiseptics used in industrial, domestic and institutional areas; c) WI 216028: CEN Enquiry 1999 (phase 2 step 2) which evaluates bactericidal activity on surfaces of chemical disinfectants and antiseptics used in industrial, domestic and institutional areas (Cremieux, A, J. Freney, A. Davin-Regli. 2001). Four dilutions of silver and silver sulfide nanoparticles were made (geometric range two) by using distilled water for phase 1 and hard water for phase 2. Standards indicate that dilutions should be prepared in a way that includes at least one in the active and one in the inactive range (two in the active range for phase 1). Nanoparticles were exposed to bacterial inoculums for 5, 15, 30 and 60 minutes at 20^o C in phase 1 and 5 minutes for phase 2. In phase 2, influence of interfering substances including bovine albumin (0.3% for 1^{st} step and 3% for the 2^{nd} step), skimmed milk (Himedia, India) and yeast extract (Fluka) in the efficacy of the disinfectants was assessed (dirty condition). Survivor count was done by inoculation in the trip tic soya agar culture media (TSA, Himedia, India) and incubation at 37^o C for 48 hours.

RESULTS

UV-visible spectroscopy revealed two surface plasmon absorption bands for F_1 at 262 and 206 nm and one absorption bands for F_2 and F_3 at 210 and 209 nm. Silver sulfide nanoparticles absorption band is about 200 nm (Kryukov *et al.*, 2004). Mean particle size for each of F_1, F_2 and F_3 was 193, 163 and 121 nm accordingly.

Phase 1

Graph (1-2) shows the number of log reduction in viability with in 60 minutes or less, with both microorganisms. The first two formulations caused about 3 to 4 log reductions but the third formulation showed about 7 log reductions in three dilutions with both microorganisms. Silver nanoparticles could pass this phase too but it is not as efficient as silver sulfide nanoparticles.

Phase 2, Step 1

First dilution of silver sulfide and silver nanoparticles showed about 5 to 7 log reduction in viability for all three microorganisms in two different condition (clean and dirty). But second dilution was not in active range for both nanoparticles (Graph 3-4). According to the results, again silver sulfide nanoparticles suppressed bacterial growth more than silver nanoparticles.

Phase 2, Step 2

Silver sulfide nanoparticles caused 4 to 8 log reduction in two dilutions for all microorganisms (Graph 5-6). But for silver nanoparticles, only the first dilution could be as potent as silver sulfide nanoparticles.

DISCUSSION

Phase 1

The results showed that the first two formulation of silver sulfide nanoparticles (F_1, F_2) could not passed the first step

(phase 1). According to the EN 1040 (phase 1) standard, 5-log reduction in viability within 60 minutes or less, with both microorganisms, is essential. A significant bactericidal effect by inhibiting the growth of both strains in all four dilutions was seen by the third formulation which produced by hydrogen sulfide gas (F_3). (Graph 1, 2). Silver nanoparticles could pass this phase too but a 1-log reduction in viability lower than silver sulfide nanoparticles.

Phase 2, Step 1

In this phase, 5-log reduction in viability is essential (Cremieux *et al*., 2001). Also other microorganism (Escherichia coli) was added in this phase. Dilution one in both silver sulfide (Graph 3,4) and silver nanoparticles passed the 1st step of 2nd phase with the minimum of 5-log reduction of Staphylococcus aureus and maximum of 7-log reduction of E.coli count. But silver sulfide nanoparticles were more efficient than silver nanoparticles about 0.5 to 1-log reduction. Second dilution of silver sulfide nanoparticles was in the active range except for S. aureus. But silver nanoparticles second dilution was in the inactive range.

Phase 2, Step 2

To pass this step, 4-log reduction in viability is needed. Two dilutions of silver sulfide nanoparticles passed this step completely (Graph 5, 6). But only the first dilution of nanosilver passed the 2nd step of phase 2, by displaying a minimum 4-log reduction of the 10^8 inoculum for S.aureus, yet inhibiting the growth of E.coli in both clean and dirty conditions. It was observed that among the three standard tested organisms, E.coli showed the highest sensitivity to both nanoparticles. On the other hand, S.aureus was the most stubborn among the three strains. The presence of interfering substances such as albumin was important in the efficacy of the bactericidal properties of nanosilver; it could be said that the more the concentration of interfering substances, the higher the concentration of the nanoparticles should be to provide the same bactericidal effect .

Silver sulfide nanoparticles produced in this study proved to have a prominent bactericidal activity and compared with silver nanoparticles is a competitive disinfectant and even more potent than it. Further standards could be applied to qualify nanosilver sulfide for specific uses in other areas such as medical and veterinary.

CONCLUSION

Silver sulfide nanoparticels made by hydrogen sulfide gas showed more bactericidal activity in comparison with silver nanoparticles

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