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HIGH ANTIBACTERIAL ACTIVITY OF SILVER NANOBALLS AGAINST E.COLI MTCC 1302, S. TYPHIMURIUM MTCC 1254, B. SUBTILIS MTCC 1133 AND P. AERUGINOSA MTCC 2295

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In this present study, we report the preparation of silver nanoballs of mean average size of 12 nm, synthesized by wet-chemical reduction method and studying its antibacterial effect on various clinically important microorganisms. The size of these nanoballs were controlled with the help of Sodium Dodecyl Sulphate as capping agent. Conformation of nanoballs was done by using UV-Vis spectrophotometer and Dynamic light scattering (DLS). The morphology of the silver nanoballs was characterized by transmission electron microscopy (TEM). The effects of these nanoballs were studied on E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295. The antibacterial effect of nanoballs was measured by means of growth curves and colony forming unit (CFU) count. These nanoballs showed high antibacterial activity than any other nanomaterials because their chemical activity increases due to large surface to volume ratio. Silver nanoballs at concentration 40μg/ml were found to be effective bactericidal.

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Keywords: Antibacterial, Silver Nanoballs, E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295.

1. Introduction

Nanotechnology, which involves the manipulation of matter at nanometer length scales to produce new materials, structures and devices, has the potential to start the new industrial revolution of today [1]. The potential for new products leading to improvements in our lives is astounding. Nanoparticles often behave much differently than bulk samples of the same materials, resulting in unique electrical, optical, chemical, biological and mechanical properties [2-4]. Various particles, since long has been used as antimicrobial agent. Due to developments in nanotechnology, enabling us to form silver nanoparticle having surface area greater than their bulk form. Thus showing effective antibacterial properties and also very less amount of silver nanoparticle required for same antimicrobial effect that would have been produced by large amount of bulk counterparts [5-9].

Though the exact mechanism of antibacterial effect of silver nanoparticles is still unknown [10-12]. Here we have studied the effect of silver nanoballs on E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295. Silver nitrate as salt and hydrazine hydrate along with sodium citrate is used as reducing agent. Sodium Dodecyl Sulphate (SDS) acts as capping agent. By optimizing the above parameters nanoballs having average mean size 12nm were synthesized.

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Recently many publications have reported antibacterial effect of silver nanoparticle [5, 14-19] but in this research paper the effect of silver nanoballs have been studied on E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295.

2. Experimental details

2.1. Materials

Lyophilized cultures of E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295 were procured from the Microbial Type Culture Collection Center (MTCC) located at the Institute of Microbial Technology (IMTECH) Chandigarh, India. Luria bertani, Nutrient and Macconky media were used here and supplied by Hi-Media Laboratories. Sodium citrate, Silver nitrate, and hydrazine were obtained from Merck Limited and Sodium Dodecyl Sulphate was purchased from Central Drug House, New Delhi, India. All chemicals were used as received.

2.2. Synthesis of silver nanoballs

Silver nanoballs were synthesized by wet chemical method which is already reported in the literature [1, 12]. To synthesize silver nanoballs, an aqueous solution of silver nitrate (1.4mM) as metal salt precursor, a mixture of hydrazine hydrate and Citrate of sodium as reducing agent were used. Silver nitrate solution (1.4mM) and 9% (w/w) Sodium Dodecyl Sulphate (SDS) were used as metal salt precursor and stabilizing agent . Hydrazine hydrate solution 2.7mM and sodium citrate 1mM were made in deionized water. Add these solutions to the Silver nitrate and Sodium Dodecyl Sulphate (SDS) solution drop wise and observe the color change with constant stirring. Transparent colorless solution turned bright yellow indicating the formation of silver nanoballs. Centrifuge the solution at 3500rpm for 30min and wash it with deionized and ethanol several times to remove impurities.

2.3. Analysis of interaction of Silver nanoballs with Bacteria

2.3.1. Bacterial Growth Curve

To study growth of bacteria in broth media, inoculations were given from fresh colonies on agar media into 10ml broth (Luria Bertani). This media was supplemented with silver nanoballs ranges from 10-40 μg/ml and the bacterial cultures were incubated at 37^0C temperature with rapid shaking at 150rpm. The growth of E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295 in broth media was indexed by measuring the optical density (OD) at λ=600nm at regular intervals using UV-Vis Spectrophotometer. The control culture was treated in a similar fashion but without any exposure to the silver nanoballs. The growth curve was plotted between optical density and time.

2.3.2. Colony Forming Units (CFU)

Macconky agar media was used to count the numbers of bacterial colonies. The above media was autoclaved with various concentration ranges from 10-40μg/ml of silver nanoballs and then it allowed to cool in different petri-dishes .We have another Macconky agar media which does not have silver nanoballs, act as the control. For experimental results (10^4cell/ml) E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295 were inoculated for 24 hours to analyze the colony forming units (CFU). The viable cell number was recorded by counting the number of bacterial colonies grown on the media.

3. Results

3.1. UV-Vis Spectrophotometry

To understand why some compounds are colored and others are not, and to determine the relationship of conjugation to color, we must had an accurate measurements of light absorption at different wavelengths in and near the visible part of the spectrum. The silver nanoballs were characterized by using UV-Vis spectrophotometer (UV-1601 pc shimadzu spectrophotometer).

UV-Visible absorption spectra of silver nanoballs in the presence of SDS as capping agent is shown in Fig. 1. The Surface Plasmon band in the silver nanoballs solution remains close to 413 nm throughout the reaction period, suggesting that the nanoballs were dispersed in the aqueous solution with no evidence for aggregation in UV Spectrum.

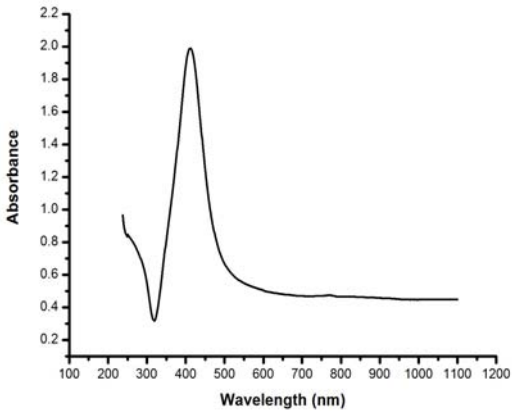

Fig. 1. UV-visible absorption spectra for silver nanoballs in the presence of SDS as capping agent.

3.2. Dynamic Light Scattering (DLS)

DLS is used to measure hydrodynamic sizes, poly-dispersities and aggregation effects of colloidal samples. DLS is used in the nanotechnology research for the accurate and fast size measurement of nanoparticles made of different materials. Here, dynamic Light scattering (Zetasizer, Malvern) technique was used to determine the size distribution profile of nanoballs in suspension. The mean average size of silver nanoballs comes out to 12 nm as shown as Fig. 2.

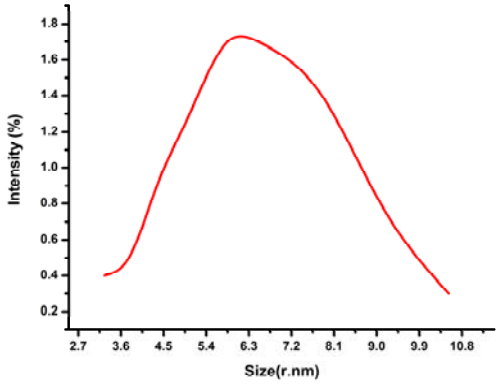

Fig. 2. DLS showing mean average size of silver nanoballs.

3.3. Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is a well known technique for imaging solid materials at atomic resolution. TEM (Carl Zeiss SMT - Nanotechnology Systems Division) image showed that the particles were agglomerated ball shaped that is spherical in shape and the mean size of the particles was found out to be 12nm as shown in figure 3. Image contrast is obtained by interaction of the electron beam with the sample. Several contrast effects play a role. In the resulting, TEM image denser areas and areas containing heavier elements appear darker due to scattering of the electrons in the sample.

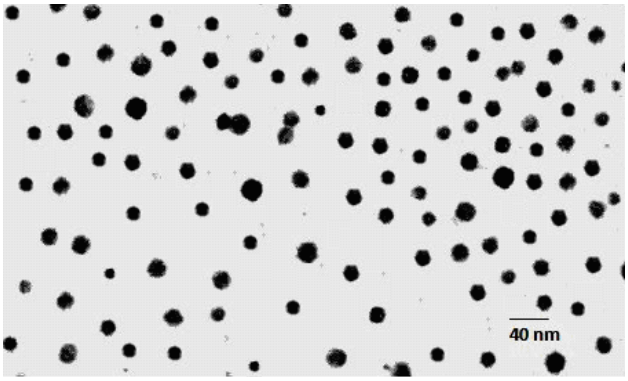

Fig. 3. TEM micrograph showing spherical silver nanoballs synthesized by wet chemical reduction process.

3.4. Analysis of Growth Curve

It was observed that optical density of bacterial growth decreases in comparison to the control with increasing concentration of silver nanoballs. It was observed that 40µg/ml and higher concentration of silver nanoballs act as effective bactericides and there was virtually no bacterial growth as optical absorption was insignificant. Optical densities were measured and plotted as a function of time for 25 hours at regular intervals with different concentration of silver nanoballs as shown in Figs. 4, 5, 6, and 7.

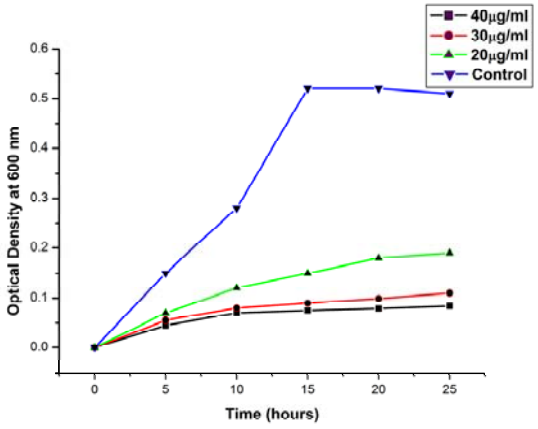

Fig. 4. Effect of silver nanoballs on E.coli MTCC 1302 growth rate.

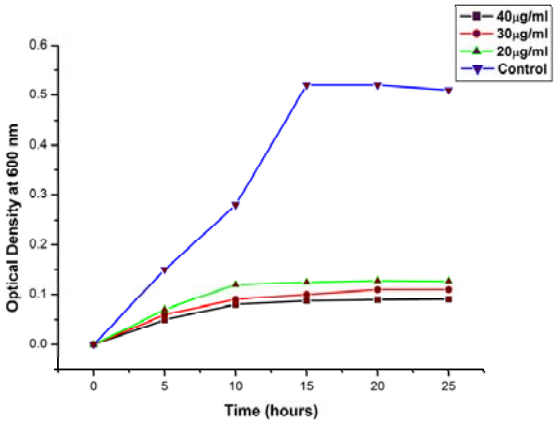

Fig. 5. Effect of silver nanoballs on Salmonella typhimurium MTCC 1254 growth rate.

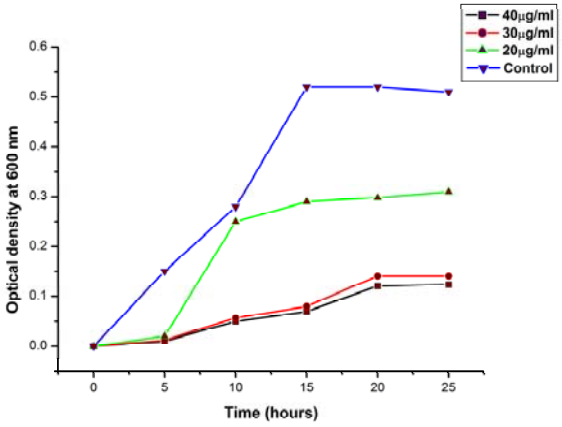

Fig. 6. Effect of silver nanoballs on Bacillus subtilis MTCC 1133 growth rate.

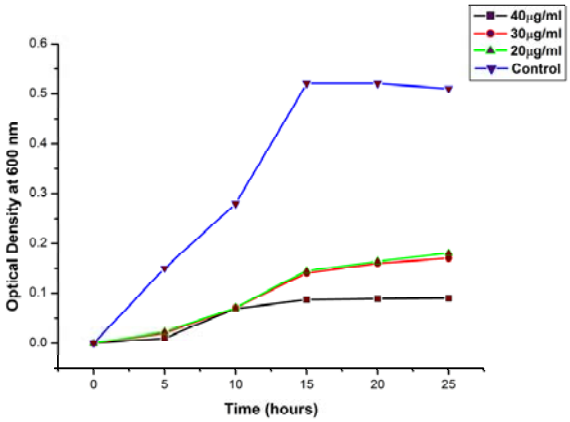

Fig. 7. Effect of silver nanoballs on Pseudomonas aeruginosa MTCC 2295 growth rate.

3.5. Analysis from colony forming units (CFU)

The bacterial colonies were counted by using colony forming unit (CFU). The graph was plotted between the numbers of bacterial colonies grown on media as the function of silver nanoballs with different concentration. As expected, the CFUs count decreases with increase in concentration of silver nanoparticle as shown in figures 8, 9, 10 and11.

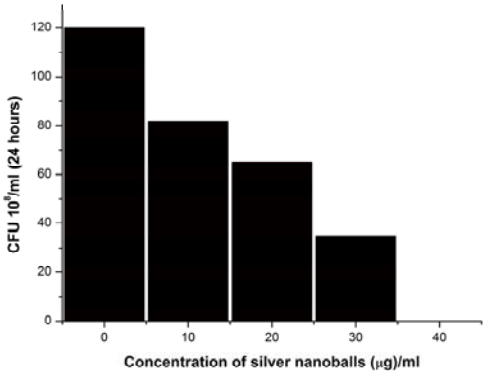

Fig. 8. Antibacterial characterization by CFU as a function of silver nanoballs concentration on E.coli MTCC 1302 after 24 hours incubation time on Macconky agar media.

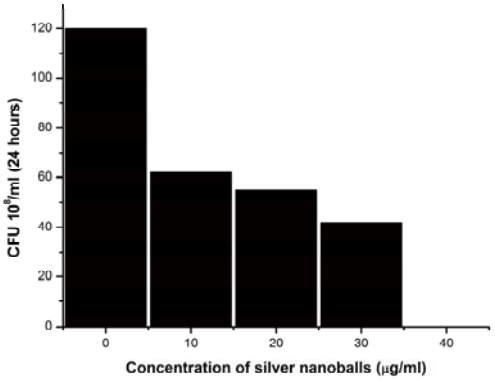

Fig. 9. Antibacterial characterization by CFU as a function of silver nanoballs concentration on Salmonella typhimurium MTCC 1254 after 24 hours incubation time on Macconky agar media.

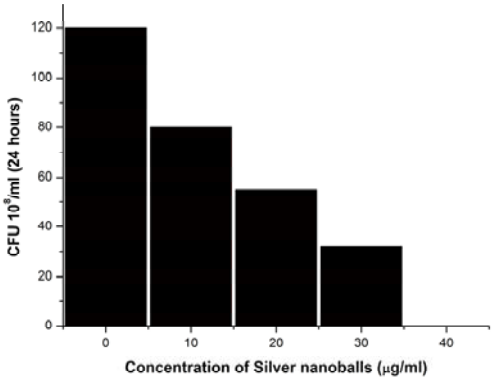

Fig.10. Antibacterial characterization by CFU as a function of silver nanoballs concentration on Bacillus subtilis MTCC 1133 after 24 hours incubation time on Macconky agar media.

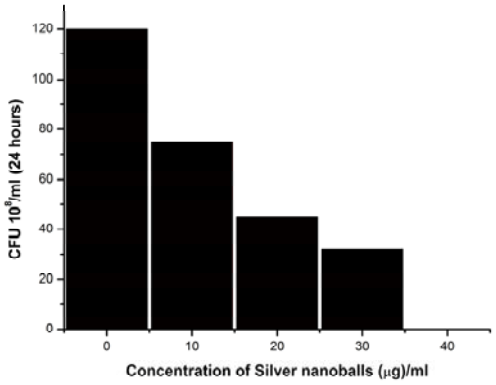

Fig. 11. Antibacterial characterization by CFU as a function of silver nanoballs concentration on Pseudomonas aeruginosa MTCC 2295 after 24 hours incubation time on Macconky agar media.

4. Discussion

Silver nanoballs of were synthesized using wet chemical reduction process and characterized by UV-Vis Spectroscopy, Dynamic light scattering and Transmission Electron Microscopy (TEM) .We found very effective antibacterial potency of silver nanoballs.

The Gram negative bacteria have a layer of lipopolysaccharides at the exterior that are composed of covalently linked lipids and polysaccharides; they lack strength and rigidity [12]. Negative charges on the lipopolysaccharides are attracted towards the positive charges available on silver nanoballs [20]. In simple words, the overall charge of bacterial cells at biological pH values is negative because of excess of number of carboxylic groups which upon dissociation makes the cell surface negative [21]. The opposite charges attract each other due to electrostatic forces. So once the nanoballs enter the bacteria it either inhibit the cell wall synthesis, damage the cytoplasmic membrane, inhibit nucleic acid and protein synthesis, inhibit specific enzyme systems which result in the inhibition of complete bacterial inhibition take place at a particular concentration of silver nanoballs about 40μg/ml. This shows that smaller particles having larger surface area for interaction and have efficient bactericidal effect. This antibacterial properties can been used to prevent and reduce the bacteria colonization on catheters [12, 22] prostheses vascular grafts, dental materials and human skin [12]. Thus, Antibacterial characteristics were showed against E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295. Silver nanoballs with concentration 40μg/ml demonstrate complete antibacterial properties against E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295.

5. Conclusion

Silver nanoballs having 12nm size show complete antibacterial characteristics against E.coli MTCC 1302, Salmonella typhimurium MTCC 1254, Bacillus subtilis MTCC 1133 and Pseudomonas aeruginosa MTCC 2295 at 40μg/ml. However, further studies can be conducted, to determine how nanoballs penetrate the cell wall and inhibit the cells divisions.

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