International Journal of Nano and Material Sciences, 2016, 5(1): 1-8

Modern Scientific Press

International Journal of Nano and Material Sciences **ISSN: 2166-0182**

Journal homepage: www.ModernScientificPress.com/Journals/ijnanos.aspx *Florida, USA*

Article

Evaluation of Minimum Inhibitory Concentration of Silver Nanoparticles against Gram Positive and Gram Negative Bacteria

Richa Peeyush Karnani[1,*], Shashikant Vaidya[1] and Abhay Chowdhary[2]

[1]*Department of Clinical Pathology, Haffkines Institute of Training, Research and Testing, Acharya Donde Marg, Parel, Mumbai, Maharashtra 400 012, India*

[2]*Department of Microbiology, J.J Hospital, Mumbai, Maharashtra 400 008, India*

* Author to whom correspondence should be addressed; E-Mail: richa.l.karnani@gmail.com, abhaychowdhary@gmail.com, abhaychowdhary@gmail.com

Article history: Received 17 June 2016, Received in revised form 19 July 2016, Accepted 25 July 2016, Published 28 July 2016.

Abstract: A comparative evaluation of synthesized silver nano particles using two different procedures (biological by *Tylophora indica* plant and chemical reduction methods) and effect of these synthesized nanoparticles against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Eschericia coli* were investigated. The synthesized nanoparticles were characterised using UV–visible absorption spectrophotometry and shape and size was determined by Transmission electron microscopy (TEM). Biologically synthesized silver nanoparticles (BAgNPs) were observed at 423 nm with particle sizes of 20 nm. The chemically synthesized silver nanoparticles (CAgNPs) showed a maximum peak at 422 nm with particle sizes of 10 nm. Both type of nanoparticles showed the most pronounced antibacterial potency against gram negative compare to gram positive bacteria as denoted by the zone of inhibition (ZoI). The microdilution method evaluated allows the testing of silver nanoparticles in multiple concentrations to determine the minimum inhibitory concentration. The current study therefore reported that the synthesized AgNPs were efficient against bacteria at lower concentration which can be comparable to standard ampicillin drug.

Keywords: Silver Nanoparticles, Zone of Inhibition, Broth Dilution Minimum Inhibitory Concentration.

1. Introduction

In the recent times, Nanotechnology plays an important role in many key technologies. The effective range within 1 to 100 nanometres of nanoscience and nanotechnology which has lots of application of nanoscale and nano-structure materials. Nanomaterials may provide solutions to technological and environmental challenges in the area of optical, electrical, magnetic, and catalytic properties of metal nanoparticles[1]. Still an extensive research is required to control the size and shape of metal nanoparticles, which is crucial to tune and optimize their physical, chemical, and optical properties[2]. Many chemical and physical methods have been reported to prepare metal nanoparticles, including chemical reduction[3,4,5,6], electrochemical reduction[7,8] photochemical reduction[9,10] and heat evaporation[11, 12, 13]. Unfortunately, nanoparticle production by chemical method is generally released some toxic compound and pollute the environment when large-scale nano particles are produced[1]. Therefore, simple and eco-friendly procedures are needed for the synthesis of silver nanoparticles (Ag NPs) which do not emit large quantities of toxic chemicals in solid, liquid, and gaseous forms in the environment[14].

Major factor for the development of new microbe inhibitory agents is microbial resistance towards the available antimicrobial agents. Silver has antimicrobial effects with distinctive properties of conduction, stability and activity[15]. Silver nanoparticles possess inhibitory and bactericidal effects have a high surface area to volume ratio along with high fraction of surface atoms that elicits elevated antimicrobial activity compared to the silver metal as a whole [16]. It predicts that silver species have an effect at the molecular, metabolic or membrane level of the microorganism [17]. Taking these studies as an initiative, we have synthesized AgNPs for analyzing their antibacterial activity against gram positive and gram negative bacteria. This attempt is hence an effort to revitalize the use of ethical knowledge of plants for ailments that can be treated with traditional medicine applying modern techniques at nano-scale.

Figure 1. Synthesis of Silver nanoparticle by Biological Reduction Method (a)*Tylophora Indica plant (b)1 mM AgNO3 without extract and Silver nitrate with Sodium borohydride*

2. Materials and Methods

2.1. Synthesis of Silver Nanoparticles

Tylophora indica leaves were thoroughly washed in distilled water, dried, cut into fine pieces and was crushed into sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 μm).10ml of1mM Silver nitrate solution was prepared and added into 100ml plant extract. For chemical reduction method, we used ice cooled 15ml of sodium borohydride (2 mM) and stir vigorously with the help of a magnetic stirrer.1mM silver nitrate solution (5 ml) was then added dropwise.

$$AgNO_3 + NaBH_4 \rightarrow Ag + ½\ H_2 + ½\ B_2H_6 + NaNO_3$$

2.2. Characterization of Siler Nanoparticles

Formation of AgNPs is confirmed by using Ultraviolet-Visible (UV-vis) Spectrophotometry and size was determined by Transmission Electron Microscopy. Samples for transmission electron microscopy (TEM) were prepared by drop-coating the AgNPs solution into the carbon-coated copper grid, and their size and morphology were characterized by TEM (JEOL 2000).

2.3. Antibacterial Activity of Silver Nanoparticles by Disc Diffusion

The antibacterial activity of AgNPs was tested against the following microorganism by disc diffusion method: *Escherichia coli* (MTCC40), *Staphylococcus aureus* (MTCC3160) was obtained from the Haffkines Institute of Training, Research and Testing, Mumbai. Pure cultures of bacteria to be tested were grown in nutrient broth for 24 h at room temperature and sterile nutrient agar plate was prepared. Inocula were spread over the agar plate using sterile spreader and using a sterile well-cutter with the diameter of 6.0 mm 5 wells were made in each agar plate, one for control and the other for test. Different concentrations of nanoparticles in culture media was prepared and put into the wells and then incubated at 25°C for 24 h, measured the diameter of inhibitory zones in mm after incubation. The diameter of the clearing zones was measured in mm using the ruler scale and compared with standard Ampicillin disc (positive control).

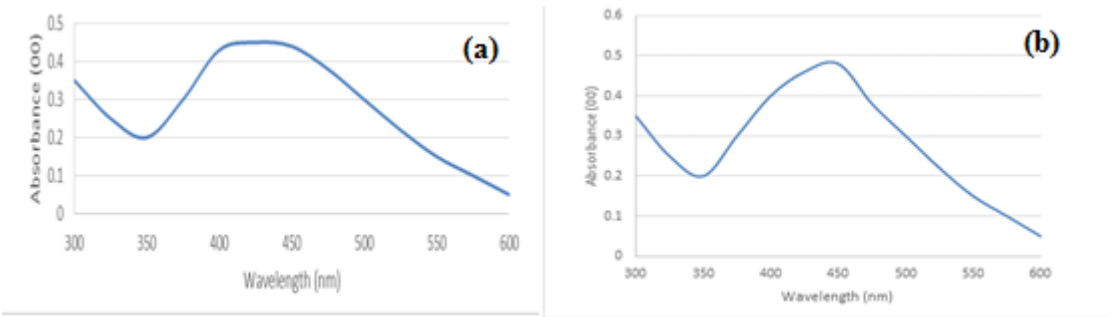

Figure 2. UV spectrophotometer of Silver nanoparticles, BAgNPs (a) and CAgNPs (b).

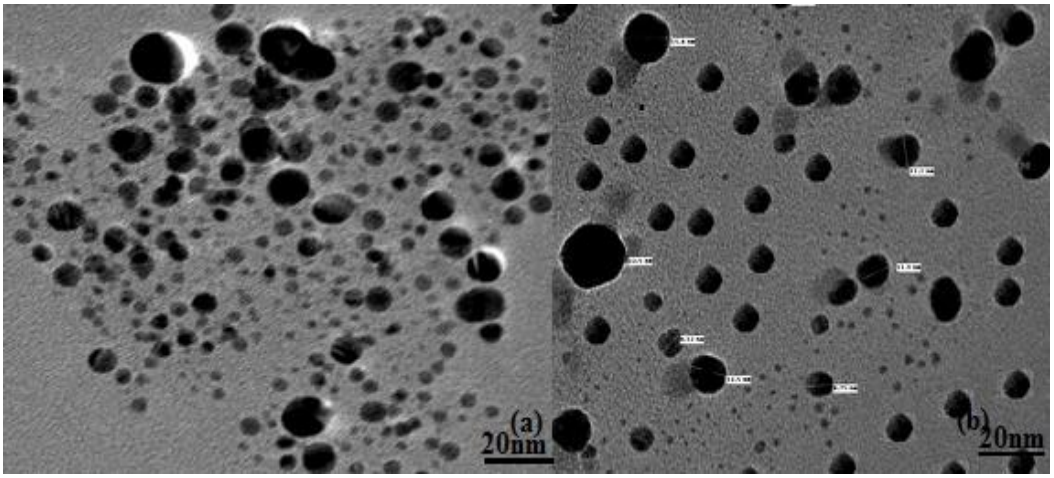

Figure 3. TEM observation of BAgNPs (a) and CAgNPs (b)

2.4. Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) of silver nanoparticles was checked by Broth Dilution Method against bacteria such as *Staphylococcus aureus* and *Escherichia coli* using Luria Bertania (LB) media on 96 micortire well plate. Silver nanoparticles were dissolved in culture broth to a stock concentration of 200ug/mL. Now for primary screening following 100, 90, 80, 70, 60, 50, 40 and 30μg/mL concentrations of the synthesized nanoparticles were taken against the tested strains. After primary screening, both bacteria showed a Minimum Inhibitory concentration at different concentration. On the basis of Antibacterial activity by agar diffusion and primary screening we prepared a different six concentration for *E.coli* and *S.aureus* bacteria for secondary screening and100 μL of each dilution were distributed in 96-well plates, as well as a sterility control and a growth control (containing culture broth, without silver nanoparticles).

3. Results and Discussions

Synthesis of AgNPs by *Tylophora indica* leaf extract and by Sodium borohyride reduction method was carried out. Antibacterial activity against gram positive and gram-negative bacteria, on the basis of it minimum inhibitory concentration were reported from this work. Figure 1 shows flasks containing the silver nanoparticles from Chemical and Biological reduction method. The change in color of both solutions were noted by visual observation. The excitation spectra of the AgNPs samples were characterized by UV-vis spectroscopy plotted in Figure.2. The technique outlined above has proved to be very useful for the analysis of nanoparticles[18]. The strong surface Plasmon resonance centered at ~423 nm for BAgNPS and 430nm for CAgNPs clearly showed an increase in intensity after 24 hours of reaction. TEM technique used to visualize size and shape of the synthesized AgNPs is

shown in figure 3. In case of CAgNPs, the morphology of nanoparticles is highly variable, with uniform spherical shape with diameter ranged from 10-20nm and in case of BAgNps, not exact spherical, non-uniform, size ranged from 20-40nm nanoparticles were observed on micrographs. Nanoparticles synthesized by two different reduction method was investigated against gram-positive and gram-negative bacteria using the agar diffusion method. The diameter of the inhibition zone (mm) around the different antibiotic disks with and without AgNPs against test strains is shown in figure 4. The minimum inhibitory concentrations (MIC) of extracellular biosynthesized AgNPs on gram-positive and gram-negative bacteria were determined by broth dilution method[19]. The observed MIC values for AgNPs were 30µg/ml and 80µg/ml for *E. coli* and *S. aureus* respectively as shown in figure 5.

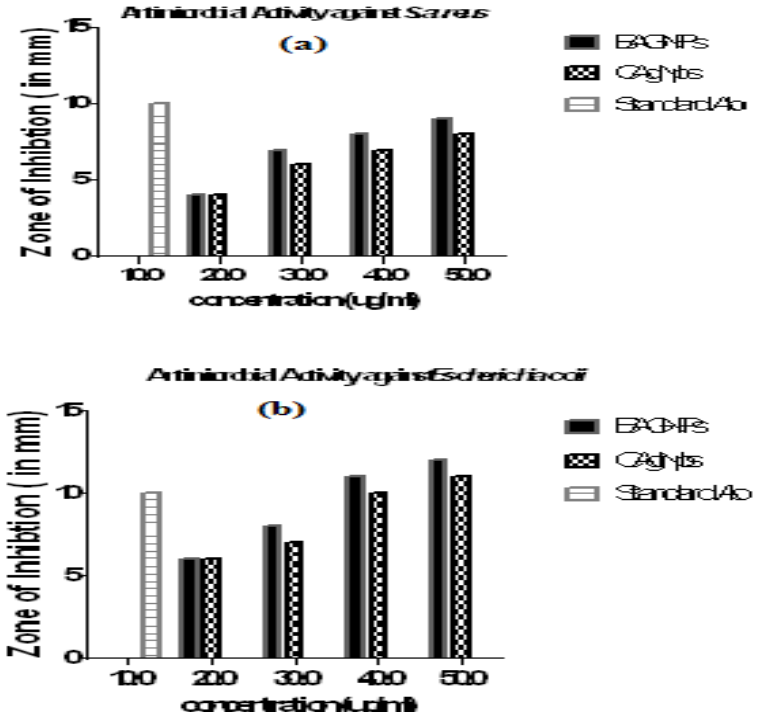

Figure 4. Comparative evaluation of Antibacterial activity of Chemical and Biological reduced silver nanoparticles by Agar diffusion (a)Against *S.aureus (b)*Against *Escherichia coli*

The research on biosynthesis of nanomaterials gives a valuable contribution to nano biotechnology and investigated as an alternative to chemical and physical ones. In this regard *Tylophora indica* proves to be an important biological component for extracellular biosynthesis of stable AgNPs and reduction of the Ag+ ions can be easily followed by visual observation and UV-vis spectroscopy. It is well known that BAgNPs show a yellowish brown color and CAgNPs shows yellow colour in aqueous solution; this color arises from excitation of surface plasm on vibrations in the metal

nanoparticles[19]. It is observed from TEM micrographs that most of the AgNPs are spherical and are in the range of 5–40 nm in size. The particle size distribution histogram determined from TEM is shown in Figure 3. From this histogram it is observed that there is variation in the particle size; almost 85% of the particles are in the 5 to 20nm range. The TEM micrograph shows that the particles are poly-dispersed and are mostly spherical. The MIC of biogenic AgNPs against test strains shows that AgNPs have a less significant effect on growth of gram-positive bacteria than on gram-negative bacteria. This is due to the structural difference in cell wall composition of gram-positive and gram-negative bacteria. The gram-negative bacteria have a layer of lipopolysaccharides at the exterior, followed underneath by a thin (~7–8 nm) layer of peptidoglycan[20]. Although the lipopolysaccharides are composed of covalently linked lipids and polysaccharides, there is a lack of strength and rigidity. The negative charges on lipopolysaccharides are attracted toward the weak positive charge available on AgNPs However, in the current studies the presence of negatively charged AgNPs was confirmed by zeta potential measurement[21]. These negatively charged AgNPs can attack the gram-negative bacteria by metal depletion, as suggested by others. On the other hand, the cell wall in gram-positive bacteria is principally composed of a thick layer (~20–80 nm) of peptidoglycan consisting of linear polysaccharide chains cross-linked by short peptides to form a three-dimensional rigid structure[22].

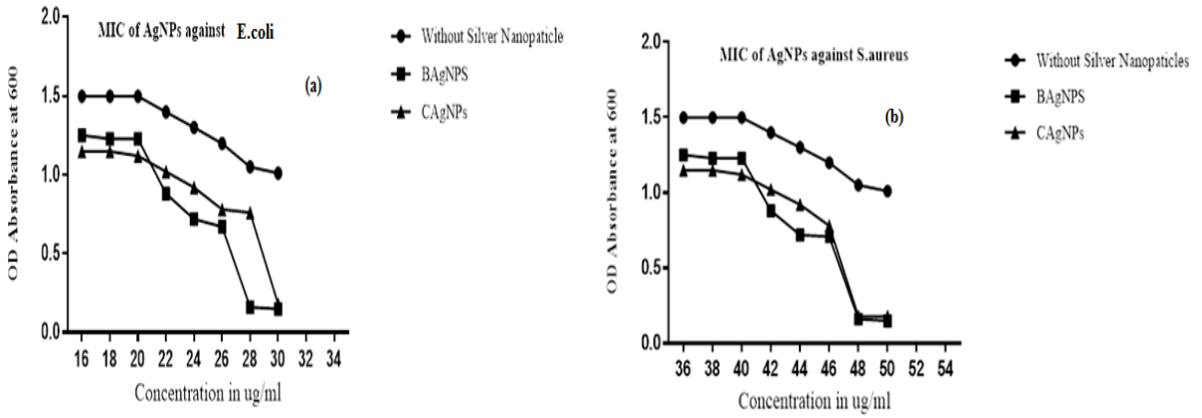

Figure 5: Minimum Inhibitory concentration of silver nanoparticle against *E.coli* and *S.aureus*

4. Conclusion

It can be concluded that among the different antimicrobial agents, silver has been most extensively studied and used since ancient times to fight infections and prevent spoilage. Antibacterial potential of both BAgNPs and CAgNPs nanoparticles as a function of nanoparticles concentration was tested against two different bacteria like *Escherichia coli* (MTCC40) and *Staphylococcus aureus* (MTCC3160), *Corynebacterium diptheria*, *Micrococcus lylae*. From the results, it can be concluded

the silver nanoparticles has a good antimicrobial activity at low concentration. The microorganisms are unlikely to develop resistance against silver as compared to antibiotics as silver attacks a broad range of targets in the microbes. The silver nanoparticles with their unique chemical and physical properties are proving it can be as an alternative for the development of new antimicrobial agents. There are some questions, which need to be mentioned, such as, the exact mechanism of interaction of silver nanoparticles with the bacterial cells, how the surface area of nanoparticles influence its killing activity, use of animal models and clinical studies to get a better understanding of the antimicrobial efficiency of silver dressings, the toxicity if any of the silver dressings, etc.

Acknowledgments

Author gratefully acknowledges Haffkines Institute for Training, Research and Testing, Mumbai for their financial support.

References

[1] Bar H, Bhui DKr, Sahoo GP, Sarkar P, De SP, Misra A, Green synthesis of silver nanoparticles using latex of Jatrophacurcas. *Colloid Surface A*, 2009, 339:134–139

[2] Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP, Semiconductor nanocrystals as fluorescent biological labels. *Science*, 1998, 281:2013–2016

[3] Petit C, Lixon P, Pileni MP, In situ synthesis of silver nanocluster in AOT reverse micelles. *J Phys Chem*, 1993 97:12974–12983

[4] Vorobyova SA, Lesnikovich AI, Sobal NS. Preparation of silver nanoparticles by interphase reduction. *Colloid Surface A*. 1999, 152: 375–379

[5] Tan Y, Wang Y, Jiang L, Zhu D., Thiosalicylic acid-functionalized silver nanoparticles synthesized in one-phase system. *J Colloid Interf Sci*, 2002, 249:336–345

[6] Yu DG, Formation of colloidal silver nanoparticles stabilized by Na+–poly(γ-glutamic acid)–silver nitrate complex via chemical reduction process. *Colloid Surface B*, 2007, 59:171–178

[7] Sandmann G, Dietz H, Plieth W, Preparation of silver nanoparticles on ITO surfaces by a double-pulse method. *J Electroanal Chem*, 2000, 491:78–86

[8] Liu YC, Lin LH, New pathway for the synthesis of ultrafine silver nanoparticles from bulk silver substrates in aqueous solutions by sonoelectrochemical methods. *Electrochem Commun*, 2004, 6:1163–1168

[9] Keki S, Torok J, Deak G, Daroczi L, Zsuga M, Silver nanoparticles PAMAM-assisted photochemical reduction of Ag+. *J Colloid Interf Sci*, 2000, 229:550–553

[10] Mallick K, Witcombb MJ, Scurrella MS, Self-assembly of silver nanoparticles in a polymer solvent: formation of a nanochain through nanoscale soldering. *Mater Chem Phys*, 2005, 90:221–224

[11] Bae CH, Nam SH, Park SM, Formation of silver nanoparticles by laser ablation of a silver target in NaCl solution. *Appl Surf Sci*, 2002, 197:628–634

[12] Smetana AB, Klabunde KJ, Sorensen CM , Synthesis of spherical silver nanoparticles by digestive ripening, stabilization with various agents, and their 3- and 2-D super lattice formation. *J Colloid Interf Sci*, 2005, 284:521–526

[13] Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M, Synthesis of gold nanotriangles and silver nanoparticles using Aloevera plant extract, *Biotechnol Prog*, 2006, 22:577–583

[14] Dubeya SP, Lahtinen M, Sillanpaa M., Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of Rosa rugosa. *Colloid Surface A*, 2010, 364:34–41

[15] Jin Y, Zhao X, *Cytotoxicity of photoactive nanoparticles*. In Safety of Nanoparticles: From Manufacturing to Medical Applications. Edited by Webster T. Springer Science + Business Media, LLC, 233 Spring St.: New York, NY 10013, 2008:19-31.

[16] Kundu S, Liang H, Polyelectrolyte-mediated non-micellar synthesis of monodispersed 'aggregates' of gold nanoparticles using a microwave approach. *Colloids Surf A Physicochem Eng Asp*, 2008, 330:143–150.

[17] Abate, G., R. N. Mshana, and H. Miorner, Evaluation of a colorimetric assay based on 3 (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) for rapid detection of Rifampicin resistance in Mycobacterium tuberculosis. *Int. J. Tuberc. Lung Dis* 1998, 2:1011–1016.

[18] Muvaney P., Surface plasm on spectroscopy of nanosized metal particles. *Langmuir* 1996, 12:788-800.

[19] Shrivastava S, Tanmay BE, Roy A, Singh G, Rao PR, Dash D., Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology,* 2007, 18:1-9.

[20] Madigan M, Martinko J. Brock, *biology of microorganisms*. 11th ed. Englewood Cliffs, NJ: Prentice Hall; 2005.

[21] Sui ZM, Chen X, Wang LY, Xu LM, Zhuang WC, Chai YC, et al., Capping effect of CTAB on positively charged Ag nanoparticles. *Physica E*, 2006, 33: 308-14.

[22] Amro NA, Kotra LP, Wadu-Mesthrige K, Bulychev A, Mobashery S, Liu G., High resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability. *Langmuir*, 2000, 16:2789-96.

[23] Baron S., *Medical microbiology*. 4th ed. Galveston: University of Texas Medical Branch; 1996.