



Original Research Article

Evaluating the Antimicrobial Efficacy of Chemically Synthesized Silver Nanoparticles

Vandana Singh and Archana Tiwari*

School of Sciences, Noida International University, Greater Noida, U.P, 201301, India

*Corresponding author

ABSTRACT

Rapid increase in antibiotic-resistant microorganisms are making essential to synthesize new antimicrobial agents. "Silver nanoparticle", has apprehended the attention of the researchers because of its acknowledged antimicrobial activity. This study is mainly emphasized on chemical based synthesis of silver nanoparticles and its evaluation as antibacterial agent against different pathogenic bacterial strains. The antimicrobial abilities were tested against Gram Positive bacteria, *Staphylococcus aureus*, *Bacillus cereus* and Gram negative bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. All strains were inoculated consistently onto sterile Muller-Hinton Agar petri plates (lawn inoculation method). Wells of 06 mm diameter were bored into the agar medium by using sterilized borer. 100 µl silver nanoparticles solution (200ppm) was added into each well and after incubation of 24hrs at 37°C and different levels of inhibition zones were measured. Tetracycline assisted as positive control. The determinations were done in triplicate and the mean values ± SD were presented in graphical form. The inhibition zones (diameters in mm) measured for Gram positive bacteria, *Staphylococcus aureus* was 27.7 ± 0.62 mm and *Bacillus cereus* was 24.1 ± 0.80 mm where as for Gram negative bacteria, *Pseudomonas aeruginosa* was 15.8 ± 0.41 mm, *Klebsiella pneumoniae* was 28.2 ± 1.07 mm and *Escherichia coli* was 29.4 ± 0.56 mm. Against tetracycline 26mm, 24mm, 26mm, 28 mm, and 27 mm respectively. Silver nanomaterials are appreciated as key in the future era to many technological, medical and environmental challenges in the field of catalysis, medicine, water treatment etc. Our study reveals that silver nanoparticle shows a moral antibacterial activity in contrast to other antimicrobial agents.

Keywords

Silver nanoparticles, Antibacterial, Zone of inhibition, Chemical synthesis, Sodium borohydride

Introduction

Nano derives from a Greek word 'nanos' which means 'dwarf' or 'extremely small' ranging from 1-100 nm in size they consist of molecular materials in which the active ingredients (drugs or biologically active

material) is dissolved, entrapped, or attached. (Johnston *et al.*, 2010; Dong *et al.*, 2012). Currently nanoparticles are in inordinate consideration due to their rare and interesting properties than their majority counterparts.

There are an enormous number of physical, chemical, biological, and hybrid methods available to synthesize different types of nanoparticles (Tolaymat *et al.*, 2010; Balu *et al.*, 2012; Slistan Grijalva *et al.*, 2008). The physical and chemical approaches are more popular in the production of nanoparticles. The particles generated by these procedures have higher catalytic reactivity, greater specific surface area, and a better contact between the enzyme and metal salt in bacterial carrier matrix (Kheybari *et al.*, 2010; Xiangqian *et al.* (2011).

A multitude of chemical reduction approaches have been used to produce stable and several forms of silver nanoparticles in water by the practice of diverse reducing agents such as ascorbic acid, hydrazine, ammonium formate, dimethylformamide and sodium borohydride. The shape and size of AgNPs intensely depends on the strong and weak tendency of organic substrates used to reduce (Hussain *et al.*, 2011; Ericka *et al.*, 2013). These nanoparticles have been used in a variety of applications including drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science, and magnetic resonance imaging (MRI) (Mohan raj and Chen, 2006; Dhritika Patel *et al.*, 2013). The usage of silver nanoparticles as antibacterial agent is moderately fresh. Due to their high reactivity it plays a vital role in inhibiting bacterial growth in aqueous and solid media. Silver comprising materials can be used to eradicate microbes on textile fabrics (Sukdeb *et al.*, 2007; Mohan and Chen 2006) or they can also be employed for treatment of waste water (Tiwari *et al.*, (2008). Contrary to bactericide effects of ionic silver, the antimicrobial activity of colloid silver particles are influenced by the dimensions of the particles the smaller the particles, the greater antimicrobial effect (Hussain *et al.*, 2011; Maribel G. Guzmán *et*

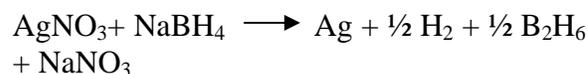
al., 2013). The tamest and the furthestmost usually used solution synthetic technique for silver nanoparticles is the chemical reduction of metal salts (Dong *et al.*, 2012).

Silver nanoparticles are very much in limelight due to their advantages in various fields such as food packaging materials and food supplements, odor-resistant textiles, electronics, household appliances, cosmetics and medical advices, water disinfectants and room sprays (Tiwari *et al.*, 2008; Wijnhoven *et al.*, 2009). The determination of this study is to synthesize silver nanoparticles evaluation of chemically and its antimicrobial sensitivity against Gram positive and Gram negative bacteria (Taayde *et al.*, 2012; VibhaSaklani *et al.*, 2012).

Material and Methods

A. Synthesis of AgNPs

Silver nanoparticles were prepared by reducing aqueous silver nitrate in the presence of NaBH₄. Briefly, an aqueous solution of NaBH₄ (0.002 M) was added to a flask. The solution was stirred and cooled for another 20 min. 2.0 ml of 0.001 M AgNO₃ was dropped into the stirred NaBH₄ solution at approximately 1 drop per second. Stirring the solution was stopped as soon as all of the AgNO₃ was added. Particles produced by this method were called borohydride-AgNPs (Anna Zielinska *et at.* 2009). The chemical reaction for sodium borohydride reduction of silver nitrate:



B. Antimicrobial efficacy

Preliminary analysis of antimicrobial activity was conducted by using Agar well diffusion assay. Bacterial inoculums was prepared in peptone broth and incubated for 4 hour. Then

0.1 ml of inoculums was homogeneously inoculated on Petri plates containing MH Agar medium then wells of 6mm diameter were punctured on it by using sterile cork-borer. 50 μ l of biologically Chemically synthesized Ag-Nps were poured in the wells using sterilized micropipettes. Plates were incubated for 24 hours at 37°C. Inhibition Zones was determined (Kheybari *et al.*, 2010; Xiangqian *et al.*, 2011; Balu *et al.*, 2012).

Characterization of silver nanoparticles

UV–vis spectroscopy

The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at different time intervals after diluting a small aliquot of 100 μ L of the sample with 1 ml deionized water. UV-Vis spectral analysis will be done by using a Perkin-Elmer Lamda-25 spectrophotometer (Hussain *et al.*, 2011).

Transmission Electron Microscopic (TEM)

Analysis was done by using a Techni G2 300 kV. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid was allowed to dry by putting it under a mercury lamp for 5 min (Hussain *et al.*, 2011).

AAS (atomic absorption spectroscopy)

Silver ion concentration was analyzed by AAS which showed the conversion of Ag⁺ ions into Ag⁰ nanoparticles.

XRD (X-ray diffraction)

X-ray diffraction was carried out to confirm the crystalline nature of the silver nanoparticles (Hussain *et al.*, 2011).

Results and Discussion

Synthesis and characterization of AgNPs

The AgNPs synthesized from aqueous solution of NaBH₄, were yellowish-brown color in appearance (Figure 1). TEM and SEM divulge the spherical-cluster of 40 nm in size. The crystallite dimensions were confirmed by using X-ray diffraction pattern.

Antimicrobial efficacy

The analysis of antimicrobial efficacy was examined by using Agar well diffusion assay. After incubation at 37°C for 24hrs, different levels of inhibition zones were measured. Tetracycline served as positive control for antimicrobial activity (Table 1). The determinations were done in triplicate and the mean values \pm SD were presented.

The diameters of bacterial inhibition zones (mm) measured for Gram positive bacteria, *Staphylococcus aureus* were 27.7 ± 0.62 mm, *Bacillus cereus* were 24.1 ± 0.80 mm (figure 2). For Gram Negative bacteria, *Pseudomonas aeruginosa* were 15.8 ± 0.41 mm, *Klebsiella pneumonia* were 28.2 ± 1.07 mm and *Escherichia coli* were 29.4 ± 0.56 mm (Figure 3) whereas against tetracycline 26mm, 24mm, 26mm, 28 mm, and 27 mm respectively.

Table.1 Evaluation of Gram negative and Gram positive in terms of sensitivity against silver nanoparticles and tetracycline as positive control

Test micro-organisms	Tetracycline (Dia in mm)	AgNps (Dia in mm)
<i>Staphylococcus aureus</i>	26	27.7± 0.62
<i>Bacillus cereus</i>	24	24.1± 0.80
<i>Pseudomonas aeruginosa</i>	26	15.8± 0.41
<i>E. coli</i>	27	29.4± 0.56
<i>Klebsiella pneumoniae</i>	28	28.2± 1.07

Figure.1 The AgNPs synthesized from aqueous solution of NaBH₄, were yellowish-brown color in appearance



Silver nanoparticles were prepared by reducing aqueous silver nitrate in the presence of NaBH₄

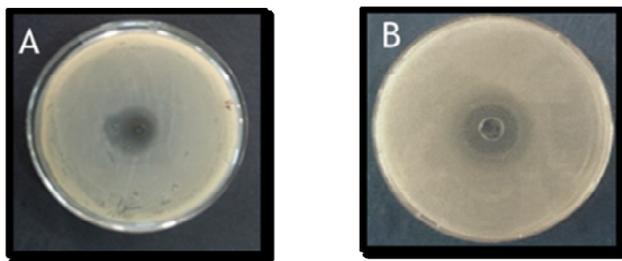


Figure 2: The diameters of bacterial inhibition zones (mm) measured for *Staphylococcus aureus* were 27.7 ± 0.62mm (A), *Bacillus cereus* were 24.1 ± 0.80 mm (B), as Gram positive bacteria

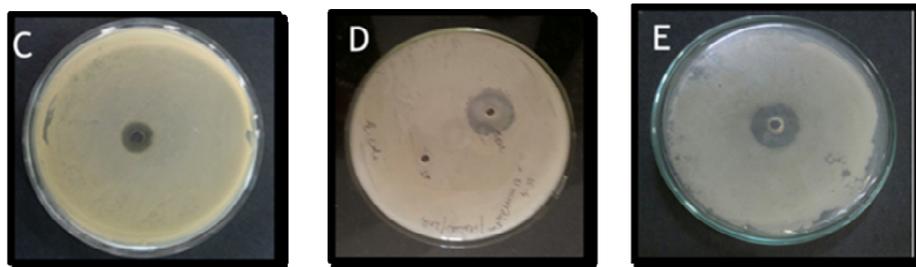
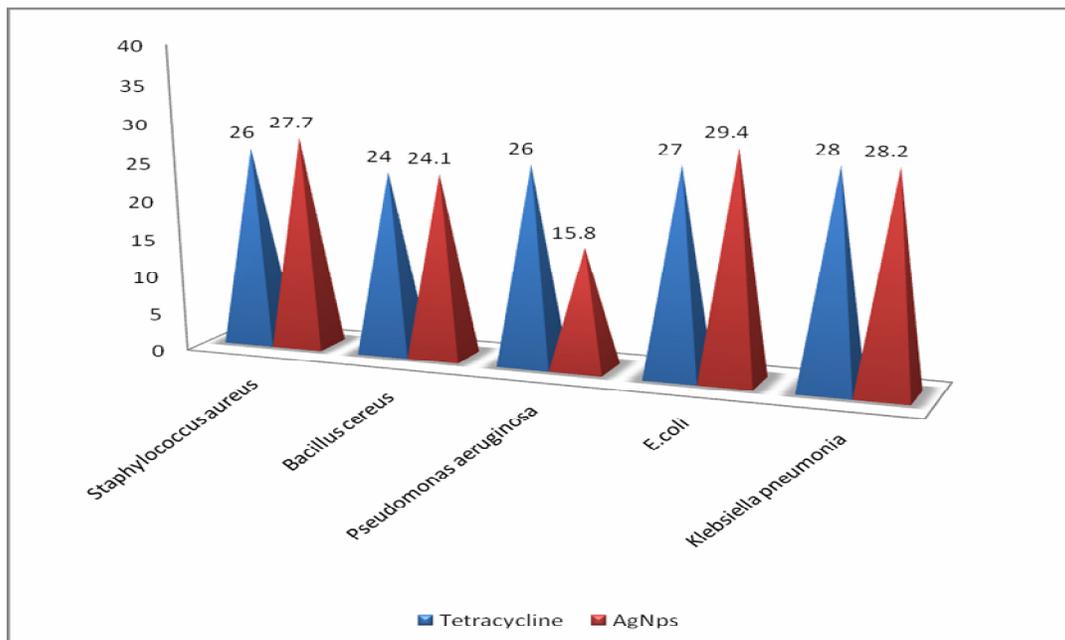


Figure 3: The diameters of bacterial inhibition zones (mm) measured for *Pseudomonas aeruginosa* were 15.8 ± 0.41 mm, *Escherichia coli* were 29.4 ± 0.56mm and *Klebsiella pneumoniae* were 28.2 ± 1.07 mm

Graph.1 Graphical representation of antimicrobial efficacy of silver nanoparticles



In Comparison to Gram positive the Gram negative bacteria are more sensitive to AgNPs (Graph 1).

Silver nanoparticle has always been an excellent antimicrobial agent. The exclusive physical and chemical properties of silver nanoparticles always intensify the efficacy of silver. Although there are numerous of mechanisms which credited to the antimicrobial activity shown by silver nanoparticles, but still the actual mean is not fully known. Chemical and physical approaches of silver nanoparticle synthesis were being used over the eras, but they are found to be costly and sometime slightly toxic too. The applications of silver nanoparticles are diverse and numerous, but the most anticipated characteristic is their antimicrobial and anti-inflammatory efficacy. The remarkably strong antimicrobial activity is the major direction for expansion of nano-silver products. Examples are food packaging materials and food supplements, odor-resistant textiles,

electronics, household appliances, cosmetics and medical advices, water disinfectants and room sprays.

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References

- Anna Zielinska, EwaSkwarekb, Adriana Zaleska, Maria Gazda, Jan Hupkaa, 2009. Preparation of silver nanoparticles with controlled particle size. *Procedia Chem. (Published by Elsevier)*, 1: 1560–1566.
- Balu, S.S. *et al.* 2012. Synthesis of silver nanoparticles by chemical reduction and their antimicrobial activity. *Int. J. Eng. Res. Technol.*, 1(6): 1–5.
- Dhrutika Patel, *et al.* 2013. Silver nanoparticles: Biosynthesis and its

- antimicrobial activity. *Cibtech J. Bio-Prot.*, 2(1): 50–57.
- Ericka Rodríguez-León, *et al.* 2013. Synthesis of silver nanoparticles using reducing agents obtained from natural sources (*Rumex hymenosepalus* extracts). *Nanoscale Res. Lett.*, 8: 318.
- Helinor J. Johnston, Gary Hutchison, Frans M. Christensen, Sheona Peters, Steve Hankin, Vicki Stone. 2010. A review of the in vivo and in vitro toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. *Crit. Rev. Toxicol.*, 40(4): 328–346.
- Hussain Javed Ijaz, Kumar Sunil, Hashmi Athar Adil, Khan Zaheer. 2011. Silver nanoparticles: preparation, characterization, and kinetics, *Adv. Mat. Lett.*, 2(3): 188–194.
- Kheybari, S., Samadi, N., Hosseini, S.V., Fazeli, A., Fazeli, M.R. 2010. Synthesis and antimicrobial effects of silver nanoparticles produced by chemical reduction method. *DARU J. Pharm. Sci.*, 18(3): 168–172.
- Maribel G. Guzmán, Jean Dille, Stephan Godet. 2013. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *Int. Res. J. Pharm.*, 4(10).
- Mohan raj, V.J., Chen, Y. 2006. Nanoparticles – A review. *Trop. J. Pharm. Res.*, 5(1): 561–573.
- Pham Van Dong, Chu Hoang Ha, Le Tran Binh, Jörn Kasbohm. 2012. Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. *Int. Nano Letters*, 2(9).
- Slistan Grijalva, R., Herrera-Urbina, J.F., Rivas-Silva, M., A´valos-Borja, F.F., Castillo´n-Barraza, A., Posada-Amarillas. 2008. Synthesis of silver nanoparticles in a polyvinylpyrrolidone (PVP) paste, and their optical properties in a film and in ethylene glycol. *Mater. Res. Bull.*, 43: 90–96.
- Sukdeb Pal, Yu Kyung Tak, and JoonMyong Song. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol. (Am. Soc. Microbiol.)*, 73(6): 1712–1720.
- Susan W.P. Wijnhoven *et al.* 2009. Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology*, 3(2): 109–138.
- Taayde, P.S. *et al.* 2012. Antibacterial potential of silver nanoparticle produced from lonar lake bacilli. *Biosci. Disc.*, 3(3): 351–354.
- Thabet M. Tolaymat, Amro M. El Badawy, Ash Genaidy, Kirk G. Scheckel, Todd P. Luxton. 2010. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. *MakramSuidan Sci. Total Environ.*, 40(8): 999–1006.
- Tiwari, D.K. *et al.* 2008. Application of nanoparticles in waste water treatment. *World Appl. Sci. J.*, 3(3): 417–433.
- Vibha Saklani, *et al.* 2012. Microbial synthesis of silver nanoparticles: A review. *J. Biotechnol. Biomater.*, 13: 1–3.
- Xiangqian Li, *et al.* 2011. Biosynthesis of nanoparticles by microorganisms and their applications. *J. Nanomater.*, 2011: 1–16