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Synthesis of silver nanoparticles and their antibacterial activity

ABSTRACT

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In study, spherical Silver nanoparticles (SNPs) were synthesized by chemical reduction method from a metal precursor silver nitrate in presence of an anionic surfactant and strong reducing agent. In this experimental work, SNPs are synthesized in presence of different concentration of stabilizing agent and effect of stabilizing agent on size distribution of SNPs have been observed. Further antibacterial activities of the SNPs prepared under different concentration of sodium lauryl sulphate (SDS) carried out on gram negative and gram positive bacteria showed that as concentration of sodium lauryl sulphate increases antibacterial activity decreases.

Keywords: *Chemical reduction; Silver; Colloidal spherical silver nanoparticles; Sodium lauryl sulphate; Antibacterial activity; Gram positive and Gram negative bacteria.*

INTRODUCTION

Noble metallic nanoparticles have drawn a great deal of attention due to their unique and unusual physical and chemical properties which are completely different from their bulk properties [1]. These unique physical and chemical properties of nanoparticles are due to their small sizes and high surface area to volume ratios (specific surface area) [2-3] which are important for applications such as catalysis, electronics and photonics etc [4]. The use of silver nanoparticles as antibacterial agent is relatively new and presently the investigation of this phenomenon has gained importance due to the increase of bacterial resistance to antibiotics. Various methods for synthesis of silver nanoparticles like electrochemical method [5-7], thermal decomposition [8], laser ablation [9], sonochemical synthesis [10] etc. have been reported in the literature. The most simple and widely used method is chemical reduction method [11, 12] in which a metal precursor (ionic salt) in an appropriate medium is reduced by a reducing agent and stabilized by a surfactant.

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Antibacterial susceptibility testing can be done by Diffusion (Kirby-Bauer and Stokes), Dilution (Minimum Inhibitory Concentration) or Diffusion & Dilution (E-Test method).

In the present work, SNPs are synthesized by chemical reduction method. The antibacterial activity of colloidal SNPs prepared has been demonstrated by direct exposure of pathogenic bacteria.

EXPERIMENTAL

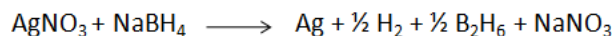
Materials

Silver nitrate (AgNO_3 , 99%) was obtained from Fisher Scientific Pvt. Ltd. Sodium lauryl sulphate (SDS, 90%) and sodium borohydride (NaBH_4 , 99%) were purchased from Merck Specialties Pvt. Ltd. Agar and Peptide was purchased from HiMedia Laboratories Pvt. Ltd. Beef extract was obtained from Qualigens Fine Chemicals. All chemicals were used as such without further purification.

General procedure for preparation of spherical SNPs

SNPs were prepared by chemical reduction method at room temperature wherein silver nitrate was dissolved in 100 ml double distilled water in an Erlenmeyer flask. Separately, the NaBH_4 solution was prepared by dissolving required amount of NaBH_4 and SDS in 50 ml double distilled water for 45 mins. SNPs were obtained by dropping AgNO_3 solution into NaBH_4 solution containing SDS slowly (approx. 1 drop/sec) under constant stirring. After all solution was added, the complete mixture was stirred for 60 mins. Various spherical SNPs were synthesized at different molar ratios (NaBH_4 : AgNO_3 ; 0.5, 1, 2 and 10).

Reaction:



Antibacterial Activity of SNPs

Inhibition zone method was used for evaluation of antibacterial activity of silver nanoparticles. Four different bacteria, gram positive (*S. aureus*, *B. subtilis*, *B. megaterium*) and gram negative (*E. coli*) were used for testing antibacterial activity of SNPs. Bacteria-inoculated agar plate

plates were prepared by adding nutrient agar containing ca. 10^5 colony-forming units (CFU)/ml of bacteria poured in a petri dish and then solidifying at 60°C for 60 mins. Four wells of diameter 8 mm were made on each bacterium inoculated nutrient plate and silver nanoparticles were dropped into corresponding wells using a sterile micropipette and the plates were inoculated at 37°C for 24 hours.

Characterization of silver nanoparticles

- **Visual inspection**

Formation of SNPs was roughly monitored by visual inspection of the solution by color change.

- **UV-Vis Spectroscopy**

Optical absorption spectra of SNPs suspensions were recorded using double beam Bioera UV-visible spectrophotometer.

- **Particle size analyzer**

The average particle size was determined by using size distribution analyzer DelsaTMNano C which uses photon correction spectroscopy.

RESULTS AND DISCUSSION

SNPs were prepared by the method described in the previous section. The weight ratios for SDS: AgNO_3 (10) was kept constant throughout the experiment. On mixing two transparent colorless solutions, the color of the solution changed to faint yellow to darker red color indicating formation of SNPs [13-14]. SNPs pale yellow, wine red, dark wine red and dark brown red color for NaBH_4 : AgNO_3 molar ratios of 0.5, 1, 2 and 10 respectively are shown in Figure 1.

UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles which is well documented in large number of papers [15]. The absorption spectrum of solution in Figure 1 (a), (b), (c) and (d) showed a Surface Plasmon absorption band with a maximum of 422 nm, 425 nm, 420 nm and 410 nm respectively throughout the reaction period suggesting well dispersed SNPs capped by SDS in aqueous solution.

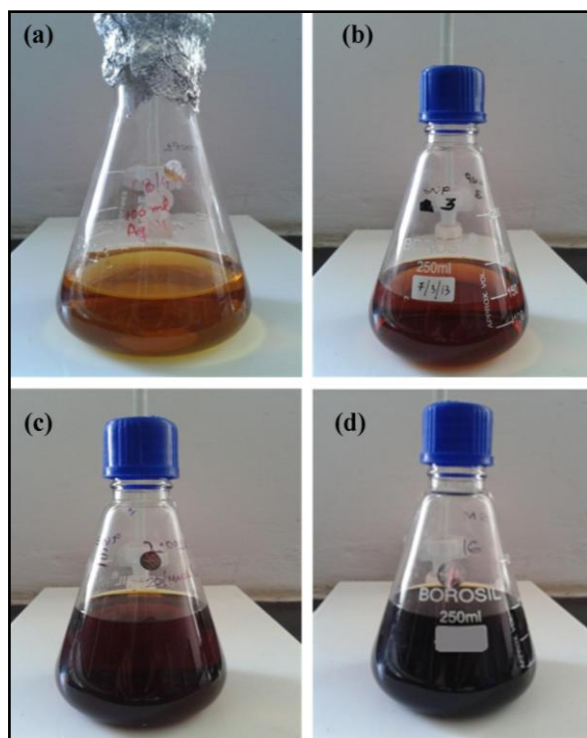


Fig. 1. Colors of synthesized SNPs of molar ratio $\text{NaBH}_4:\text{AgNO}_3$; 0.5 (a), 1(b), 2(c), 10(d) stabilized by SDS

Size distribution analysis showed average particle size of 8 nm, 15 nm, 18.7 nm and 21.9 nm for 0.5, 1, 2, and 10 $\text{NaBH}_4:\text{AgNO}_3$ molar ratios respectively.

Antibacterial activity

The activity of SNPs is tabulated in Table 1 for different molar ratios of $\text{NaBH}_4:\text{AgNO}_3$ and the images of inhibition zone of various bacteria are shown in Figure 2(a-d).

Table 1. Zone of Inhibition against different pathogenic bacteria at different $\text{NaBH}_4:\text{AgNO}_3$ molar ratios

| Bacterial Strains | Molar ratio ($\text{NaBH}_4:\text{AgNO}_3$) | | | |
|----------------------|--|-----|-----|-------------|
| | 0.5 | 1.0 | 2.0 | 10 |
| | Activity in mm (inhibition zone diameter- well diameter) in mm | | | |
| <i>E. coli</i> | 26 | 21 | 24 | No activity |
| <i>B. megaterium</i> | 14 | 11 | 07 | |
| <i>S. aureus</i> | 25 | 20 | 20 | |
| <i>B. subtilis</i> | 18 | 12 | 08 | |

E. coli showed maximum inhibition zone among the various bacteria used and next to it, *S. aureus* showed maximum activity among the Gram-positive bacteria. Highest activity was observed for molar ratio ($\text{NaBH}_4:\text{AgNO}_3$) 0.5 for all bacteria's and minimum activity was observed for higher molar ratio of ($\text{NaBH}_4:\text{AgNO}_3$). For molar ratio ($\text{NaBH}_4:\text{AgNO}_3$) 10, no antimicrobial activity was observed. The antibacterial activity observed is due to attachment of SNPs on to the surface of the cell membrane, disrupting its function then SNPs penetrates bacteria and at last releases silver ions [16-18].

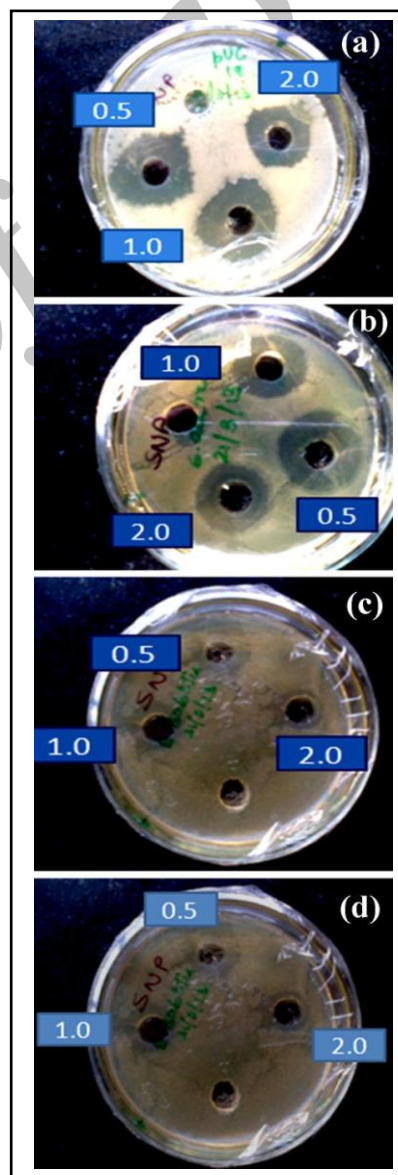


Fig. 2. Images of antibacterial activity of Ag-SNPs solution stabilized with SDS against *E. coli* (a) *S. aureus* (b) *B. subtilis* (c) *B. megaterium* (d)

CONCLUSIONS

In summary, uniform SDS capped stable SNPs have been prepared through chemical reduction method at room temperature in aqueous medium. The SDS capped SNPs showed strong antibacterial activity. The significance of these results demonstrates that SNPs solutions have good antibacterial activity against Gram-positive as well as Gram-negative bacteria.

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