

# Antimicrobial And Antifungal Activity On Glutathione Stabilized Silver Nanoparticles – An In-Vitro Study

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**Abstract:** Silver nanoparticles were obtained by chemical reduction of silver nitrate in water with sodium borohydride (NaBH<sub>4</sub>) in the presence of glutathione (a tripeptide - biomolecule) as a stabilizer. The synthesized silver nanoparticles were characterized by XRD, UV-Visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The antibacterial and antifungal activity of glutathione stabilized silver nanoparticles (GSH-AgNPs) was tested by using Gram-negative and Gram-positive bacteria (*Bacillus sp.*, *E.coli*, *Streptococcus sp.*, *Pseudomonas sp.*, and *Rhizobium sp.*) and fungal agents (*Aspergillus niger* and *Candida albicans*). The silver nanoparticles, whose bacterial activity was dependent on the aggregation degree between particles, exhibited bacterial activity against *Bacillus sp.* and similarly for fungal activity against *C. albicans*. The maximum zone of inhibition (mm) was shown by *Bacillus sp.* (gram negative bacteria) for antibacterial activity and for antifungal, maximum activity shown by *C. albicans* for 100µg of the sample.

**Key words:** GSH-AgNPs, Antibacterial activity, Antifungal activity.

## I INTRODUCTION

In recent years noble metal nanoparticles have been the subjects of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties that are

significantly different from those of bulk materials [1]. These special and unique properties could be attributed to their small sizes and large specific surface area. For these reasons metallic nanoparticles have found uses in many applications in different fields as catalysis, electronics, and photonics. A variety of preparation routes have been reported for the preparation of metallic nanoparticles [2, 3], notable examples include, reverse micelles process [4,5], salt reduction [6], microwave dielectric heating reduction [7], ultrasonic irradiation [8], radiolysis [9,10], solvothermal synthesis [11], electrochemical synthesis [12,13], etc. In recent years nanoparticles of silver have been found to exhibit interesting antibacterial activities [14, 15].

Presently, the investigation of this phenomenon has regained importance due to the increase of bacterial resistance to antibiotics, caused by their overuse. Recently, silver nanoparticles exhibiting antimicrobial activity have been synthesized [16]. Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization on prostheses [17], catheters [18, 19], vascular grafts [20], dental materials [5], stainless steel materials [21] and human skin [5, 22]. The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed to eliminate microorganisms on textile fabrics [12, 23] or they can be used for water treatment [24]. 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 [14]. Therefore, in developing routes of synthesis, an emphasis was made to control the size of silver nanoparticles. Silver nanoparticles have been produced using different methods: electrochemical method [25-27], thermal decomposition [28], laser ablation [29], microwave irradiation [30] and sonochemical synthesis [31]. The simplest and the most commonly used bulk-solution synthetic method for metal nanoparticles is the chemical reduction of metal salts [32, 33]. In fact, production of nanosized metal silver particles with different morphologies and sizes [34] using chemical reduction of silver salts has been reported [35]. This synthetic method involves reduction of an ionic salt in an appropriate medium in the presence of surfactant using various reducing agents [36]. The dispersions of silver nanoparticles display intense colours due to the plasmon resonance absorption. The surface of a metal is like the plasma, having free electrons in the conduction band and positively charged nuclei. Surface Plasmon resonance is a collective excitation of the electrons in the conduction band; near the surface of the nanoparticles. Electrons are limited to specific vibrations modes by the particle's size and shape. Therefore, metallic nanoparticles have characteristic optical absorption spectrums in the UV-Vis region [37].

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include: Diffusion (*Kirby-Bauer* and *Stokes*), Dilution (Minimum Inhibitory Concentration) and Diffusion & Dilution (E-Test method). Antimicrobial susceptibility testing in the clinical laboratory is most often performed using the disc diffusion method. The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The Kirby-Bauer method was originally standardized by Bauer et al. (the so called Kirby-Bauer method). This method is well documented and standard zones of inhibition

have been determined for susceptible and resistant values [38, 39]. The antibacterial characteristics of silver nanoparticles produced have been demonstrating by directly exposing bacteria to colloid silver particles solution [40].

In the present work on the preparation of nanosized silver nanoparticles from aqueous solution of silver nitrate, in which sodium borohydride act as a reductant and glutathione was employed as a stabilizer and checked for its antibacterial and antifungal activity.

## II EXPERIMENTAL

### A. Materials and methods for synthesis of GSH-AgNPs

The glutathione-stabilized silver nanoparticles (GSH-Ag NPs) were synthesized using modified creighton's method [48] and it is sensed with different metal ion solutions. All chemicals used are of the highest purity available. All solutions were prepared with Millipore water. AgNO<sub>3</sub>, NaBH<sub>4</sub> and Glutathione were purchased from SD Fine Chemicals Ltd, India. NaOH and HCl were used to adjust the pH of the solutions. The chemicals were used as received without further purification.

### B. Characterization techniques

The synthesized GSH-AgNPs were subjected to various characterization techniques like XRD, SEM, FT-IR, UV-Vis spectrophotometer, Zeta potential measurements.

### C. Antimicrobial Study

#### 1. Antibacterial Activity:

For the antibacterial assay the Agar Well Diffusion Method were used. The bacterial pathogens used were *Bacillus sp.*, *E.coli*, *Streptococcus sp.*, *Pseudomonas sp.*, and *Rhizobium sp.*

Aliquot of 100  $\mu$ L spores suspension ( $1 \times 10^6$  spores/mL) of each tested isolate was streaked in

radial patterns on the surface of Luria Broth Agar (LBA) complete media plates. Disc of 3 mm in diameter were performed in the media, and then each disc was loaded with certain concentration (500  $\mu\text{g}$ ). The GSH-AgNPs treated plates act as positive control and only the medium act as the negative control. The cultured plates were incubated at 28°C for 48hrs. The radius for the zone of inhibition was measured [41].

## 2. Antifungal Activity:

The antifungal assay had been done by Agar Well Diffusion Method. The fungal pathogens used were *Aspergillus niger* and *Candida albicans*.

Aliquot of 100 $\mu\text{l}$  spores suspension ( $1 \times 10^8$  spores/mL) of each tested isolate was streaked in radial patterns on the surface of Sabourand Dextrose Agar (SDA) complete media plates. Disc of 6 mm in diameter were performed in the media, and then each disc was loaded with certain concentration (100  $\mu\text{g}$  and 50  $\mu\text{g}$ ) of each tested material. 50% DMSO was used as negative control. Fluconazole 28  $\mu\text{g}$ /disc was used for positive control. The cultured plates were incubated at 28°C for 3-5 days. The radius for the zone of inhibition was measured in two directions at right angles to each other. Experiments were carried out with three replicates per treatment and each treatment was repeated at least twice [41].

The standard dilution micromethod, determining the minimum inhibitory concentration (MIC) leading to inhibition of bacterial growth is under way.

## III RESULTS AND DISCUSSION

### A. Synthesis & Characterization of GSH-AgNPs:

The glutathione stabilized silver nanoparticles (GSH-AgNPs) were synthesized using modified creighton's method and pale yellow colour colloidal solution was obtained. Characterizations like XRD spectrum confirmed the crystalline structure of silver nanoparticles,

FTIR suggests that GSH is modified onto the surface of silver nanoparticles via the thiol group from the cysteine moiety of GSH, SEM analysis proves that the GSH-AgNPs was found to be almost spherical in its structure and the average diameter of the particles was found 50nm [46].

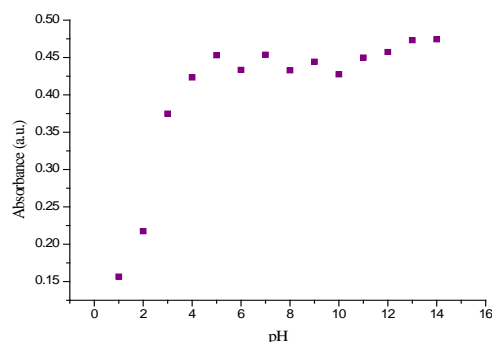
### B. Zeta Potential Analyzer:

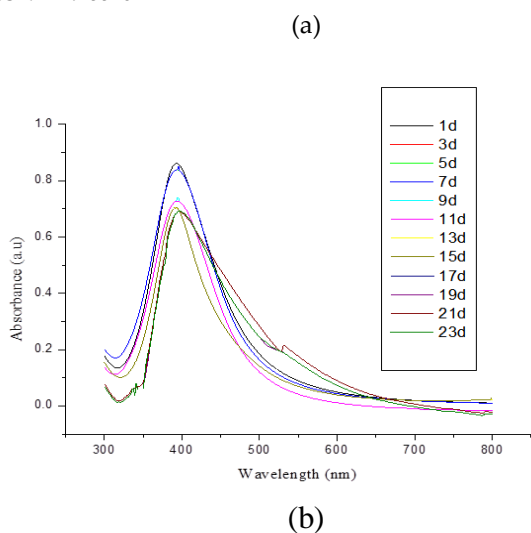
A lower level of the zeta potential results (0 to  $\pm 30$  mV) in a smaller electrostatic repulsion between the particles, maximizing aggregation/flocculation. Zeta potential measurements of the as-prepared samples yielded values of  $-3.05$  mV for the GSH-AgNPs, which is usually considered as the lowest limit of detection for colloidal solutions [42]. The values of the Z potential reflect that the stability of the GSH-AgNPs is compromised for the synthesis of colloidal suspensions.

### C. UV-Visible Spectroscopy:

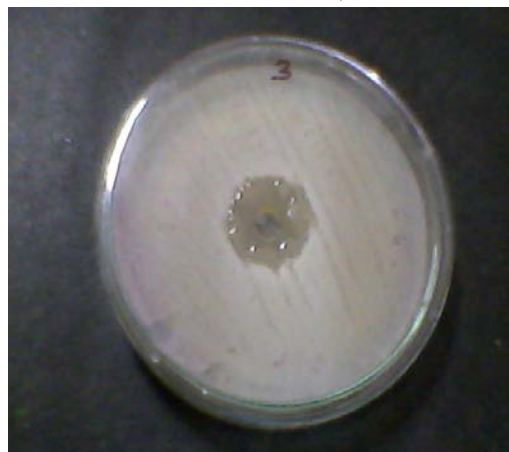
Effect of pH and time on the stability of GSH-Ag NPs

From the Fig 4 (a), it was observed that the synthesized nanoparticles are extremely stable in the pH range 3-14, probably due to the strong interparticle electrostatic repulsion between the carboxylate anion of glutathione capping on the nanoparticles surface [43]. In Fig 4 (b), there was no significant change in the shape, position and symmetry of the absorption peak during the initial 23 days, except a little decrease in the absorbance intensity. This result proves that the synthesized GSH-Ag NPs are relatively stable.





(b) *Streptococcus sp.*



(c) *E.coli*



(d) *Pseudomonas sp.*



(e) *Rhizobium sp.*

Fig 4: UV-Vis spectra of GSH-AgNPs effect on (a) pH and (b) Time

#### D. Antibacterial Activity



(a) *Bacillus sp*



Fig 4: Photographic images of bacterial inhibition zones against various pathogens GSH-AgNPs



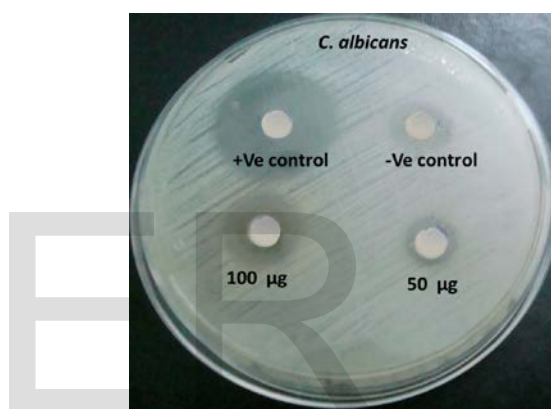
Table 2: Zone of inhibition of GSH-AgNPs with different concentrations against bacterial pathogens

Bacterial Pathogens	Zone of inhibition (mm)
<i>Bacillus sp.</i>	30
<i>Streptococcus sp.</i>	25
<i>E. coli</i>	24
<i>Pseudomonas sp.</i>	28
<i>Rhizobium sp.</i>	25

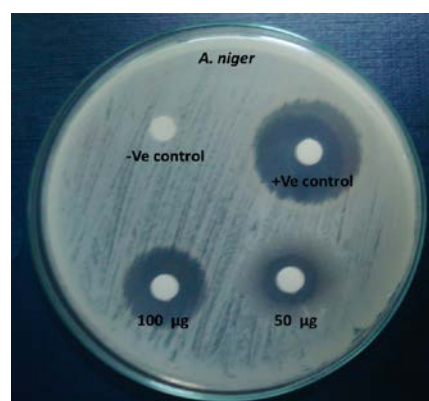
The antibacterial activity was assayed against four common bacterial species namely *Bacillus sp.*, *Streptococcus sp.*, *E.coli*, *Pseudomonas sp.*, and *Rhizobium sp.* (Fig 4). The results reflect the innate and diverse morphological, physiological and metabolic characteristics diversity of different kinds of bacteria. It is noteworthy here that in the case of *Bacillus sp.* Gram positive, the Ag NPs are more effective as antibacterial agents as compared to the other four bacteria; namely: *Streptococcus sp.*, (gram positive) and *E. coli*, *Rhizobium sp.* and *Pseudomonas sp.* (Gram negative). Due to the difference between outer wall if Gram positive and Gram negative bacteria is given by the degree of permeability, with exclusion limit for substances with a molecular weight of more than approximately 600Da for gram negative cells. Outer wall of the Gram negative act as permeability barrier due to the presence of a lipopolysaccharide layer that is able to exclude macromolecules and hydrophilic substance, therapy, being responsible for the intrinsic resistance of gram negative bacteria. It has been generally believed that the mechanism of the antibacterial effects of silver ions  $Ag^+$  involves their absorption and accumulation by the bacterial cells that would lead to shrinkage of the cytoplasm membrane or its detachment from the cell wall [44]. But our synthetic silver nanoparticles were more effective on both gram

negative and gram positive bacterium due to our belief that the inhibition of the growth of microorganisms is not related to penetration of outer cell wall but is also dependent on the size of silver and depend on the capping material as well. The growth inhibitory concentration measurements were measured and tabulated. From the Table 2, it was found that the maximum activity was 30, shown by *Bacillus sp.* for 500  $\mu$ g of the sample which is a gram positive bacteria and *Pseudomonas sp.*, shown the activity of 28 which is a gram negative bacteria. Hence, silver nanoparticles show maximum antibacterial activity on both types of bacteria.

### E. Antifungal Activity



(a)



(b)

Fig 6: Photographic images of fungal inhibition zones against (a) *C. albicans* and (b) *A. niger*

produced by GSH-AgNPs with different concentrations

Table 3: Zone of inhibition of GSH-AgNPs with different concentrations against fungal pathogens

Concentrations of GSH-AgNPs	Fugal pathogens Zone of inhibition (mm)	
	<i>C. albicans</i>	<i>A. niger</i>
Control (Fluconazole)	31	29
100 µg	15	16
50 µg	9	10

The colloidal silver nanoparticles were tested to evidence their antifungal properties. The antifungal properties of silver nanoparticles against two fungal species are shown in figure 5. It is our belief that the inhibition of the growth of microorganisms is not related only to the penetration of outer cell wall but is also dependent on the size of silver. In this work, glutathione was used as a capping material for silver nanoparticles to investigate the fact that some of the fungal species are highly resistant towards some polymer such as chitosan since those species contain biopolymer in their cell wall. Also the antifungal activity of silver nanoparticles may be attributed to the disruption of transmembrane energy metabolism. In addition to the disruption of the membrane electron transport chain may be disrupted by formation of insoluble compounds in the cell wall. The formation of insoluble compound may be due to the inactivation of cell wall sulphhydryl group. On the other hand, silver ions can create mutation in fungal DNA by displacing the

hydrogen bonds. The interaction of the surface modified nanoparticles with the peptide glycol layer of the cells has a remarkable effect on the inhibition of growth of microorganisms. The growth inhibitory concentration measurements were measured and tabulated. From the Table5, it was found that the maximum activity was 16, shown by *C. albicans* for 100 µg of the sample [45].

#### IV CONCLUSION

Silver nanoparticles were successfully prepared by reducing AgNO<sub>3</sub> aqueous solution with sodium borohydride in the presence of glutathione as stabilizer. The obtained particles were spherical with an average size of 20-30 nm. The diameter of the particles showed no obvious change with increasing NaBH<sub>4</sub>/AgNO<sub>3</sub> molar ratios. The silver nanoparticles formed were highly stable and well dispersed in solutions. The obtained glutathione stabilized silver nanoparticles inhibited the growth of the Gram-positive Gram-negative bacteria. The maximum zone of inhibition (mm) was shown by *Bacillus sp.* (gram negative bacteria) for antibacterial activity and it was found to be 30 and for antifungal, maximum activity was 16, shown by *C. albicans* for 100µg of the sample.

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