Effect of Silver Nanoparticles on Beneficial Microbes in the Environment

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The silver nanoparticles were synthesized by chemical reduction of silver nitrate using sodium borohydride. The synthesized nanoparticles were characterized by UV visible spectroscopic analysis, Fourier transform infrared spectroscopic analysis and scanning electron microscope. The antimicrobial activity of chemically synthesized silver nanoparticles was determined against two bacteria (Azotobacter chroococcum and Pseudomonas fluorescens) and two fungi (Aspergillus niger and Penicillium chrysogenum) by well diffusion method. Our study shows that silver nitrate as the bulk material behind the synthesis of silver nanoparticles is toxic whereas silver nanoparticles are less toxic to beneficial microbes in the environment.

Keywords: Silver nanoparticles, Characterization, Antibacterial activity, Antifungal activity, Beneficial Microbes

Introduction

The route of synthesizing nanoparticles can varies from physical to chemical methods1. Among all those methods chemical method is used to synthesize nanoparticles in large scale. The properties of the materials change as their size approaches the nano scale and the percentage of atoms at the surface of a material becomes significant2. Different reducing agents have been used to form, assembly of metallic silver (Ag) nanoparticles. The nanoparticles easily increase their antibacterial efficiency. We studied and reported the effect of chemically synthesized silver nanoparticles on the beneficial microbes of the environment.

Materials and methods

Chemical synthesis of silver nanoparticles

Took 30 ml of 0.002 M sodium borohydride (NaBH₄) in an Erlenmeyer flask. To that added a magnetic stir bar and placed the flask on a stir plate. Dripped 2 ml of 0.001M silver nitrate (AgNO₃) into the stirring NaBH₄ solution at approximately 1 drop per second. The addition of a few drops of 1.5 M sodium chloride (NaCl) solution causes the suspension to turn darker yellow, then grey as the nanoparticles aggregate3. Stirred and cooled the liquid for about 20 minutes. And the solution was centrifuged for 20 minutes at 4000 rpm. After centrifugation supernatant was discarded and obtained pellet were separated and dried in hot air oven for 8 hours at 60°C. The resultant sample was collected and smashed in a mortar and pestle so as to get a finer nature for characterization of silver nanoparticles and stored in air tight container for further use.

Characterization studies of silver nanoparticles

UV-Visible spectroscopy analysis

In order to study the formation of silver nanoparticles, the UV-Visible (UV-Vis) absorption spectrophotometer is used. UV-visible spectral analysis was performed using Perkin Elmer lambda 35 model spectrophotometer. The UV-Vis spectrum was recorded at room temperature using the quartz cuvette with a UV-visible spectra wavelength range 200-800 nm.

FTIR analysis

FTIR measurement was carried out using Perkin Elmer Spectrum RX-1 FT-IR Spectrometer to investigate possible bio reducing agents present in the silver nanoparticles. FTIR analysis was performed to classify the bio molecules in the sample which are responsible for reduction of the metals and for the stabilization of nanoparticles. The functional group responsible for the silver nanoparticles was also analyzed in the wavelength range 4000–400 cm⁻¹.
SEM analysis

SEM analysis was carried out using ZEISS EVO 18 SEM machine. SEM was used to record the micrograph images of synthesized AgNPs. Thin films of the sample were prepared on a carbon coated copper grid by just dropping small amount of the sample on the grid and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5min. The size and morphology of nanoparticles were examined.

Isolation of Microorganisms

Isolation of Aspergillus niger from soil by serial dilution method

Soil sample was collected normally at the depth of 15 cm and transferred to clean containers and the soil was taken from in and around Tirupattur. The collected soil sample was used to isolate microorganisms by the serial dilution method using aseptic techniques. After that each of serial dilution was transferred into SDA plate by using spread plate method. 0.1ml of an appropriately diluted culture was spreaded over the surface of agar using sterile glass spreader.

Isolation of Penicillium chrysogenum from air by open plate method

SDA plates were prepared and placed in open air. Allowed for few minutes and plates were closed. Finally plates were incubated for few days. Fungal growth was seen visually. Then the Colonies were selected and sub cultured.

Collection of Bacterial sample

Microbial cultures of Azotobacter chroococcum and Pseudomonas fluorescens has been purchased commercially from University of Agricultural Sciences, Bangalore. They were sub cultured in our laboratory for the antibacterial efficacy.

Antimicrobial activity

The Chemically synthesized silver nanoparticle was tested for antimicrobial activity by the disc diffusion method using the suspension of bacteria (Azotobacter chroococcum and Pseudomonas fluorescens) and fungi (Aspergillus niger and Penicillium chrysogenum) over Muller Hinton agar. Dipped the swab into the broth culture of the organism. Gently squeezed the swab against the inside of the tube to remove excess fluid. Used the swab to streak on the Muller Hinton agar plates for a lawn of growth. The Chemically synthesized silver nanoparticles was dissolved in 5 % DMSO and then poured into the well. Then the plates were incubated at 37˚C for 24 hours. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

Results and Discussion

Chemical Synthesis of silver nanoparticles

The reduction of Ag⁺ into AgNPs during the addition of Sodium borohydride was able to be followed by the color change. However, after the addition of AgNO₃ the color changes in aqueous solution occur. The result obtained in this study is interesting because it turn darker yellow to grey color as soon as the nanoparticles aggregate.

UV-Visible spectroscopy analysis

UV-Visible spectroscopy analysis of chemically synthesized silver nanoparticles is carried out by Perkin Elmer lambda 35 model spectrophotometer. Maximum absorption peak was observed at 458 nm. Most of the papers reported that the absorbance bands between 400-460 nm for silver nanoparticles. In this study the absorbance band were located at 458 nm which confirms the presence of silver nanoparticles and it has been previously reported. Even Kanniah Paulkumar etal also reported the UV absorbance of Silver nanoparticles at 460nm.

FTIR analysis

FTIR measurements were carried out in order to identify the presence of various functional groups in biomolecules responsible for the bioreduction of Ag ions and capping/stabilization of silver nanoparticles. In FTIR, the bands are observed at 3422cm⁻¹, 2921cm⁻¹, 2856cm⁻¹, 1743cm⁻¹, 1631cm⁻¹, 1450cm⁻¹, 1377cm⁻¹, 1240cm⁻¹, 1043cm⁻¹ and 596 cm⁻¹ indicating the presence of capping agent with the nanoparticles.

![Fig. 1 — UV-Vis spectra Analysis](image-url)
SEM analysis

SEM studies were revealed to visualize the shape of silver nanoparticles which was shown in figure 2. In this present study, the morphology of the synthesized silver nanoparticles was found to be spherical in shape. It was observed that the produced silver nanoparticles that present in samples were scattered as well as in aggregates of varying sizes less than 100nm. It was observed that produced silver nanoparticles were spherical, pseudo spherical and of undefined morphology with traces of agglomeration. The results on the present studies on the surface morphology were in accordance with the experimental findings of Payal and Agarwal.

Antimicrobial efficacy

The antimicrobial activity of chemically synthesized silver nanoparticles was determined against two bacteria (Azotobacter chroococcum and Pseudomonas fluorescens) and two fungi (Aspergillus niger and Penicillium chrysogenum) by well diffusion method showed in Table 1. On reducing the silver nitrate to its nanosized form (i.e. as Silver nanoparticles) showed no zone formation against those beneficial microorganisms which are present in the environment.

Conclusion

Silver nanoparticles has different properties which have been made it has a nanoparticle suitable for many field in science. Silver nanoparticles have been synthesized by many methods which include biological and chemical methods. Silver nanoparticles have been used as an antimicrobial agent for a prolonged period of time. It has been reported that silver nanoparticles has been found to have antimicrobial efficacy against pathogens, but less studies has been carried out on the effect of silver nanoparticles on beneficial microbes in the environment. According to our studies silver nanoparticles are less toxic to beneficial microbes in the environment.

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Conflict of Interest

All authors have no conflict of interest to report.

References


