

# Mycosynthesis and Characterization of Silver Nanoparticle

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**Abstract:** As silver nanoparticles (AgNPs) show effective antimicrobial activity against wide range of disease of silver nanoparticles have been demonstrated by *Fusarium oxysporum* fungus. I investigated the synthesis of silver nanoparticles through biological method. After synthesis, AgNPs were characterised by UV-Visible spectroscopy, X – Ray Diffraction (XRD), ZETA potential, FTIR and Transmission Electron Microscopy (TEM), These characterization techniques are required to assess the specific nanoparticles size, distribution of size, dispersion at nano level and deposition of those nanoparticles on particular substrate.

**Keywords:** *Fusarium oxysporum*, U. V. Vis, TEM, XRD, ZETA Potential

## 1. Introduction

Nanotechnology is interdisciplinary science dealing the smallest particles and is world-wide expanding in 21<sup>st</sup> century. Nanotechnology is defined as the science and engineering participating in the designing, manufacturing, characterization, and application of materials whose smallest functional organization is on the nanometer scale or one billionth of a meter. A particle with the size in the range of 1 nm to 100 nm, at least in one of the three dimensions is known as nanoparticles. There are two types of the nanoparticles generally used in nanotechnology are inorganic based nanoparticles like metal nanoparticles and the second one is the organic nanoparticles like carbon containing fullerenes (Tamasa, 2013).

Broadly chemical and biological methods for synthesis of nanoparticles have been developed. Chemical method has several drawbacks which chiefly include the use of toxic, hazardous and expensive chemicals limiting their consumption for synthesizing nanoparticles. The reducing agents used in chemical method are chemical solutions such as polyol, N<sub>2</sub>H<sub>4</sub>, NaBH<sub>4</sub>, sodium citrate and N, N-dimethylformamide; however in biological methods, only silver nitrate is generally used for synthesis of nanoparticles. Furthermore stabilizer is added to the solution to avoid agglomeration of silver nanoparticles under chemical approach, whereas in biological synthesis there is no need to add a stabilizing agent. Ultimately, environmental pollution is the major demerit of chemical synthesis unlike in biological synthesis. Also the optimization of size and shape of nanoparticles can't be adjusted as per the need when they are synthesized via chemical means; but using biological approach, one can do the same. So considering all these points, it is beneficial to use biological methods for synthesizing nanoparticles (Gudikandula, 2016).

## 2. Materials and Methods

Bacterial Cultures: *E. coli*, *F. oxysporum* Silver Nitrate, Sodium alginate – 1% W/V at 25%, Calcium chloride reagent Potato Dextrose Agar (PDB) Luria-Berteni Broth (LB)

Biological synthesis of silver nanoparticles:

### Procedure

#### Sub-culturing of fungi and bacteria:

The pure cultures of fungi were maintained on PDA slant by frequent sub culturing. These cultures were stored at 4°C for further experiments. The *Fusarium* species as mentioned above was cultured & slants were prepared on Potatoes Dextrose Agar.

#### Synthesis:

Different *Fusarium* species were subculture from the given stock using PDA slants, two slants each for 4 different stains & one tube was kept as control. Out of the two above kept slants, one for each, was use to inoculate 100 ml of sterile PDB & was incubated for 24 hrs. at 26 ± 2°C. The thick fungal mat (biomass) was allowed to grow on PDB. After 5-7 days of incubation, biomass was harvested by filtering grown culture through muslin cloth, followed by filtering through Whatman filter paper No.1. To remove the components of medium the biomass was rinsed 3 to 4 times with sterile distilled water. This provides the food stress condition to fungus, which induce the production of stress proteins. The rinsed biomass weighing approximately 20g were suspended in 100ml sterilised distilled water for 24 hours at room temperature and kept in orbital shaking incubator at 120 rpm. After incubation, the cell filtrate was obtained by passing the biomass suspension through muslin cloth and then Whatman filter paper No.1. The fungal filtrate was then centrifuged at 4000 rpm at 4°C for 20 minutes. The obtained supernatant was passed through the 0.2µ membrane filter to remove fungal spores, using filtration assembly. The filtrates were then treated with 1mM AgNO<sub>3</sub> and kept in sunlight for 1 minute to complete the reduction of silver ions to form silver nanoparticles. The solution was observed for colour change from colourless to yellowish brown. The fungal filtrate without treatment with 1mM AgNO<sub>3</sub> and 1 mM AgNO<sub>3</sub> were kept as positive and negative control respectively. All the experiments were performed in triplicate.



Figure 1: Fungal biomass grown on PDB

### Characterization of Silver Nanoparticle

#### Visual observation:

The synthesis of AgNPs can be primarily confirmed just by visual observation. The change in colour from yellowish to dark brown indicates the formation of AgNPs. This is due to the reduction of silver ions, which excites the surface plasmon in the U. V.-Vis range.

#### U. V spectra analysis:

Ultra violet spectroscopy is one of the fundamental techniques used to characterize the different colloidal solution. For silver nanoparticles absorption peak indicate that silver nanoparticles do not aggregates. Absorption peak usually shift towards red wavelength when size of particles increases. Aggregation of nanoparticles is avoided as silver colloidal particle get negatively charge due to adsorb citrate ion. The absorption peak of AgNPs ranges between 360-600nm.

#### FTIR Analysis:

FTIR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. Because the strength of the absorption is proportional to the concentration, FTIR can be used for quantitative analyses. The FTIR measurement can also be utilized to study the presence of a protein molecule in the solution, as the FTIR spectra in the 1400–1700  $cm^{-1}$  region provides information about the presence of "C=O" and "N-H" groups. The main goal of FTIR in this study is to determine the chemical functional groups in the sample.

#### TEM Analysis:

The morphology of the particle will have analyzed by TEM. The interactions between the electrons and the atoms will be used to observe features such as the crystal structure and features in the structure like dislocations and grain boundaries. In that chemical analysis will be performed. High resolution can be used to analyze the quality, shape, size and density of quantum wells, wires and dots.

#### XRD Analysis:

The determination of structure will be done by XRD. The study of crystal structures and atomic spacing will be observed by the X-ray diffraction it is common technique. By the cathode ray tube X-ray will be generated filtered to produce monochromatic radiation, collimated to concentrate. The interaction of the incident rays will be observed by Bragg's Law ( $n\lambda=2d \sin \theta$ ). Wavelength of electromagnetic radiation will be observed by using crystalline sample. Diffracted X-rays will detect, processed and counted. By scanning the sample diffraction directions will be observed.

#### ZETA potential:

Zeta Potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticles surface. This double layer of ions travels with the nanoparticle as it diffuses throughout the solution. The electric potential at the boundary of the double layer is known as the Zeta potential of the particles and has values that typically range from +100 mV to -100 mV. The magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticles with Zeta Potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to Vander Waal inter-particle attractions.

Zeta Potential is an important tool for understanding the state of the nanoparticle surface and predicting the long term stability of the nanoparticle. .

## 3. Results

#### Visual observation:

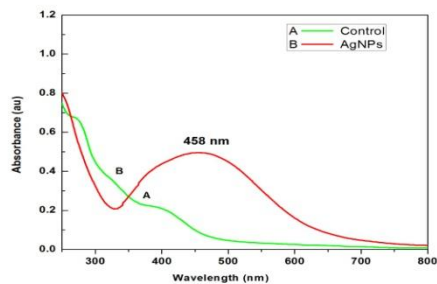
Within 10 minutes of exposure to the sunlight, the yellowish deep brown color was developed in the 1mM  $AgNO_3$ . Thus it can be said that AgNPs may be synthesized in the solution due to the reduction of silver ions.



Figure 2: Mycosynthesis of silver nanoparticles: Colour change from yellowish to deep brown

**U. V-Vis analysis:**

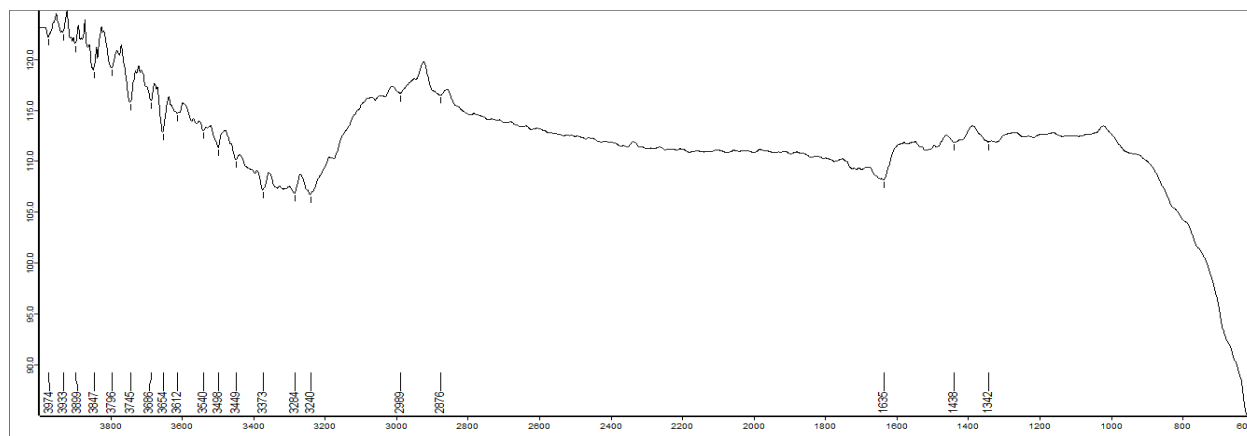
The U. V-Vis analysis of synthesized nanoparticle solution shown a sharp peak at 458nm, when subjected to analysis in the range of 300-800nm.



**Figure 3:** U. V.-Vis analysis of silver nanoparticles showing peak at 458 nm

**FTIR analysis:**

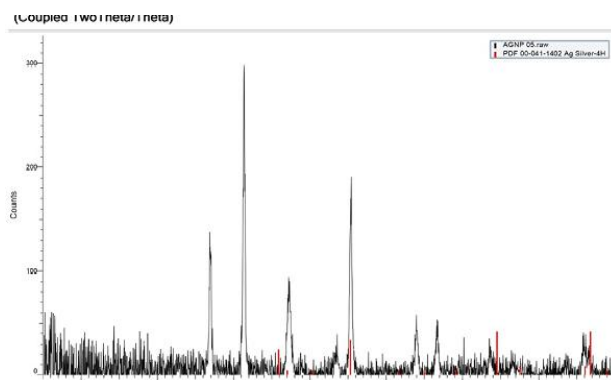
FTIR spectroscopy of synthesized AgNPs showed the vibration spectra of defined peak between 1011 and 1041 (i. e.1022, 1025, 1027). It indicated the presence of stretch vibrations typical of C-O-C bond. It suggested the possible presence of proteins capping the AgNPs.



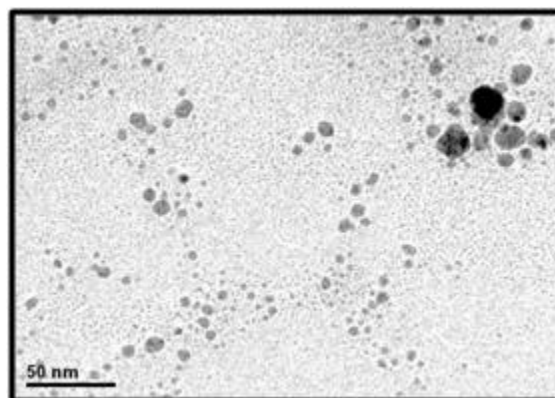
**Figure 4:** FTIR graph of AgNPs

**XRD analysis:**

X-ray diffraction peaks were observed at position 38°, 44°, 66°, 78° of 2θ showing the presence of (111), (200), (220) and (311) facets of faced centered cubic crystalline structures.



**Figure 5:** Spectra obtained by XRD spectroscopy of AgNPs.



**Figure 6:** TEM of protein capped AgNPs generated from *F. oxysporum*

**ZETA Potential:**

The zeta potential value-23, suggests that the synthesized nanoparticles were moderately stable. Synthesized AgNPs are moderately stable.

**TEM analysis:**

Transmission electron microscopy (TEM) analysis was carried out to understand the size and shape of AgNPs. The analysis indicated the synthesis of spherical shaped.

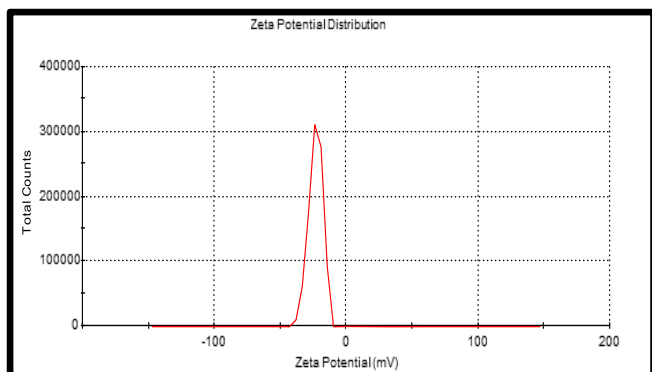


Figure 7: ZETA potential of AgNPs synthesized

#### 4. Discussion

The synthesis of AgNPs was primarily confirmed by change in colour of fungal filtrate after addition of silver nitrate solution to it. The peak of U. V. Vis analysis between range 360-600nm (Hussain., 2011) primarily confirm presence of AgNPs. Through FTIR analysis the role of protein in capping the AgNPs has been confirmed. XRD pattern also displayed the fcc crystal structure of AgNPs without any impurities. TEM analysis indicate particle size of AgNPs in the range of nanometers.

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