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Synthesis of Silver Nanoparticles by Microbial Method and Their Characterization

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ABSTRACT

The silver nanoparticles are synthesized by using microbial reduction of silver ions in the presence of fungus Aspergillus flavus . The fungus , Aspergillus flavus when challenged with silver nitrate solution accumulated silver nanoparticles on the surface of its cell wall in 96 hr . The silver nanoparticles synthesized are characterized by using UV-spectroscopy. An absorption peak at 420 nm in UV-visible spectrum corresponds to the Plasmon resonance of silver nanoparticles. The presence of protein as a stabilizing agent which surrounds the nanoparticles was confirmed by FTIR spectroscopy. The structural properties of silver nanoparticles were confirmed using XRD technique. The surface morphology of silver nanoparticles was studied using SEM. From SEM images grain size of silver nanoparticles is determined up to nano level.

Keywords: Aspergillus flavus, Microbial synthesis, Biosorption, Silver nanoparticles, UV, FTIR, XRD, SEM.

INTRODUCTION

Nanotechnology is the design, characterization, production & application of structures, devices & systems by controlling shape & size at nanometer scale. Many preparation ways of nanosilver have been reported. One of the most an ecofriendly process to develop silver nanoparticles is biosynthesis by some kind of fungus. To grow the nano-scale silver particle aspergillus flavus is one of the useful protective agents. The exotic properties of nanomaterials have been employed

in the applications in field of optoelectronics, catalysis, optosensors, electronic components, light emitters, optodevices [1].

Due to potential application of nanomaterials in technologies, many countries have launched major initiatives for the development of the fundamental & applied knowledge base in the area of nanotechnology [2].One major aspect of nanotechnology related with production of reliable experimental methods for synthesis of nanoparticles over range of chemical compositions, size & high monodispersity. Silver nanoparticles have potential application in selective coating for solar energy absorption & intercalation material. The particles should be chemically stable without under degradation such as partial oxidation [3].

Though various chemical & biochemical methods are being explored for silver nanoparticles production, micro-organisms are very much effective in this process [4]. The ability of some microorganisms such as bacteria & fungi to control the synthesis of metallic nanoparticles should be employed in the synthesis of new materials [5]. Some microorganism have inorganic material either intra or extracellularly [6]. The pseudomonas aeruginosa is able to produce gold nanoparticles [7]. A bacteria pseudomonas strutzeri from silver accumulates silver nanoparticles. Among various metal nanoparticles, silver nanoparticles have several important applications in the field of biolabelling [8], sensors, antimicrobial agents & filters [9], hence these are being intensively studied employing fusarium oxysporum [10], pseudomonas stutzer [11] Rhodococcus sp [12] , Thermomonospora [13] , phaenerochaete chrysosporium [14] .

In this paper, the synthesis & characteristics of silver nanoparticles is reported & discussed. In this study focus has been given to development of an extracellular process. Aspergillus flavus, fungi are found to be very good candidate for such processes since these biomasses are easy to handle.

MATERIALS AND METHODS

1. Instruments-Digital pH meter, uv-visible spectrophotometer (Shimadzu 1750) , Jasco FTIR spectrometer model No. 4100 unit was used for IR spectra , X-ray diffraction measurement was done on Philips model No. PW 1710 , Spray dryer model LU 222 Labultima , Scanning electron microscope VEGA MV 23000 T/40 model , magnetic stirred , Electrical shaker model No.
2. Filamentous fungi, such as Aspergillus flavus (NCIM650) was obtained from National chemical Laboratory , pune.
3. **Nutrient medium & growth conditions for A.F**.:
   Yeast extract malt dextrose both having composition dextrose 10 gm/L, Peptic digest of animal tissue 5 gm/L , yeast extract 3 gm/L , malt extract 3 gm/L , pH 6.2 ± 0.2 gm/Lit was used to grow A.F. The media used for the experimentation was purchased from Hi media & prepared in pure double distilled water each time.
4. **Preparation of fungal biomass**: The batch flask was the inoculated with loopful of fungal culture, flasks were incubated at 37^0 C on orbital shaker (200 rpm). After 72 h, the biomass was harvested by filtration through a plastic sieve & washed with pure distilled water to remove any medium component. 20 gm biomass was added to 100 ml of double distilled water for 72 h at 37^0 C in an Erlenmeyer flask & agitated as described earlier. After

incubation, biomasses passing it through Whatman filter paper No. 1 & millipore filter papers & mentioned as stock. 20 gm of wet biomass was added to 250 ml of 1 mM silver nitrate ($AgNO_3$) solution & kept on shaker (200 rpm) at room temperature. Prepared sample were removed periodically & subjected to uv-vissible analysis. Simultaneously, a negative control was maintained under identical conditions. Figure 1 (A) & (B).

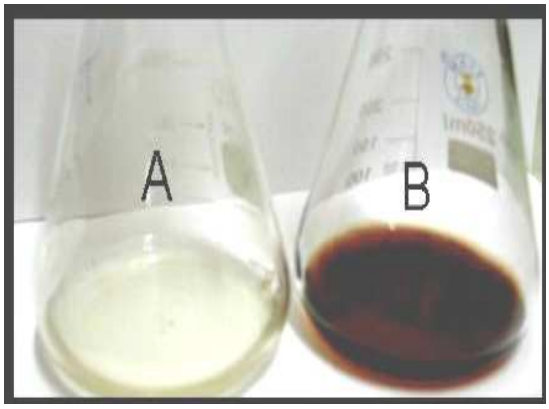

Figure 1 (A) Control flask with $AgNO_3$ (B) reaction mixture with 1mM $AgNO_3$ after 96 hours of incubation

RESULTS AND DISCUSSION

In this work we have investigated extracellular biosynthesis of silver nanoparticles using A.F. The synthesis process was quite effective & silver nanoparticles were formed within 96 hrs of silver ion coming in contact with the cell filtrate (After 96 hr, test sample was turned to dark red colour from pale yellow colour). Figure 1(a) shows the beginning reaction of cell filtrate of fungus incubated with silver ion & figure 1(b) test sample was turned to dark red colour from pale yellow colour.

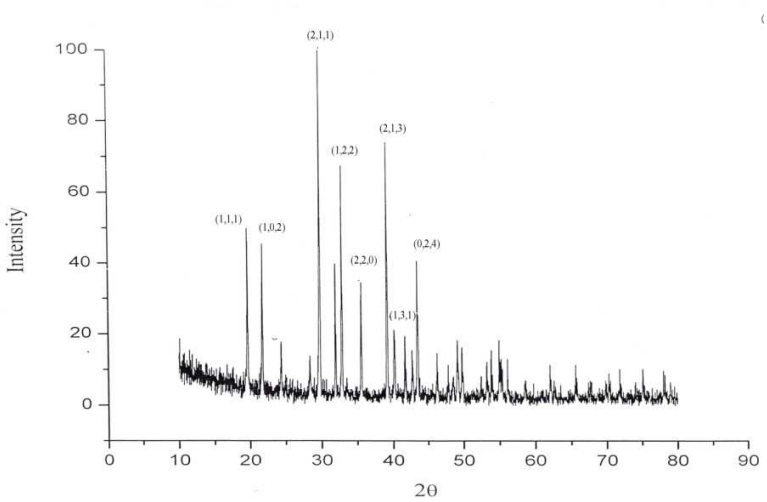

Figure 2 XRD pattern of silver nanoparticles

XRD Analysis: Figure 2 shows the XRD pattern obtained for silver nanoparticles synthesized using biological method (biosynthesis). A number of Bragg reflections corresponding to the (111), (102), (211), (122), (220), (213) sets of lattice planes are observed.

From the XRD studies the grain size was calculated by FWHM method. It is found to be (37.30) to (52.92) nm. These results are in good agreement with standard JCPDS (43-0649).

Scanning Electron Microscope: The SEM micrograph of silver nanoparticles is shown in figure 3.The SEM micrograph were used to calculate the grain size of silver nanoparticles by using Cottrell's method [15].

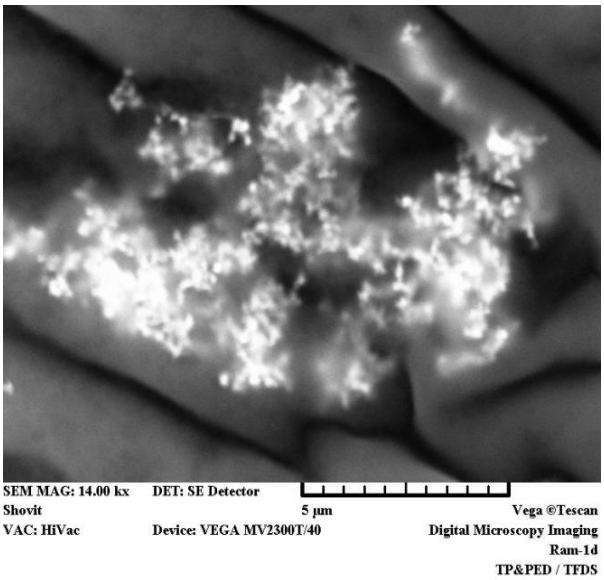

Figure 3 SEM view

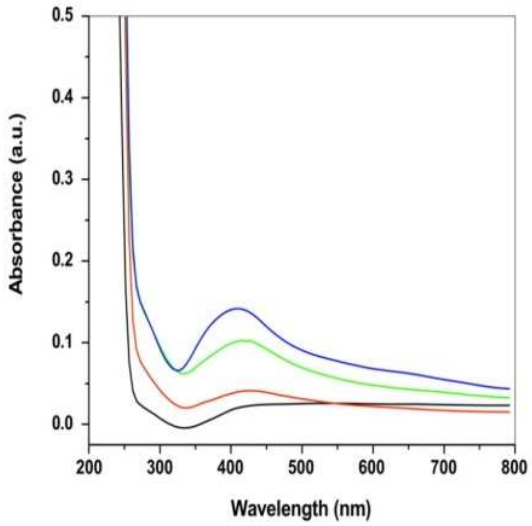

Figure 4 UV-visible spectra of aq. solution of 1mM $AgNO_3$ with the fungal biomass (*A. flavus* NCIM 650)

UV-visible spectrophotometric analysis: Figure 4 shows the uv-visible spectra of the silver nitrate solutions challenged with the fungus were taken to optimize to growth of nanoparticles. For this, test sample was analysis at 420 nm after interval of 24 hr corresponds to the surface Plasmon resonance of silver nanoparticles. Curve I corresponds to positive control while II , III & IV correspond to that of incubation with silver nitrate (1mM) after 24 hr, 48 hr, 72 hr & 96 hr respectively . After 96 hr of incubation , no change in intensity at 420 nm was observed indicating complete reduction of silver ions.

FTIR Spectroscopy-Figure 5 shows the FTIR spectrum recorded from the powder of silver nanoparticles, formed after 96 hr of incubation with the fungus A.F. The amide linkages between amino acid residues in proteins give a peak in infrared region of the electromagnetic spectrum.

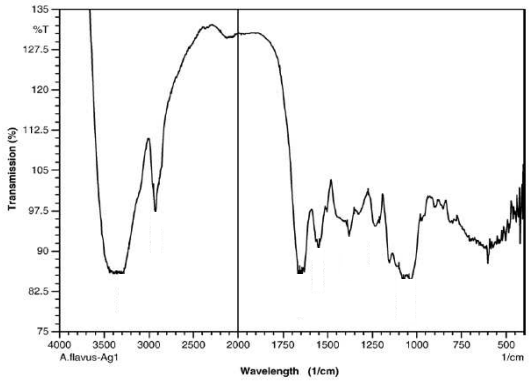

Figure 5 FTIR spectrum of silver nanoparticles synthesized by *A. flavus* after 96 h.

CONCLUSION

The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology today. One approach that shows immense potential is based on the biosynthesis of nanoparticles using biological microorganisms such as bacteria. Silver nanoparticles have been prepared through the reduction of silver ions by the fungus Aspergillus flavus. This is one of the simplest and cheapest processes for obtaining silver nanoparticles .UV-spectroscopy reveals the surface Plasmon property, while XRD analysis and SEM images reveal the nano nature of the prepared samples. Average size estimated from above studies is 44 nm. These silver nanoparticles are found to have characteristic absorption peak at 420 nm and emission peak at 553 nm.

Concluding, *Aspergillus flavus* is a good candidate for the synthesis of silver nanoparticles. Their formation proceeds via an extracellular mechanism .The most important feature of A. flavus is the fact they are widespread present in the waste biomass from pharmaceutical industry. Such cheap source of material, gives opportunity to cost-effective preparation of various silver-based nanostructures.

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