Digest Journal of Nanomaterials and Biostructures Vol. 4, No.2, June 2009, p. 349 – 355

# A NOVEL MICROBIAL ROUTE TO SYNTHESIZE SILVER NANOPARTICLES USING FUNGUS *HORMOCONIS RESINAE*

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Here we reported the synthesis of silver nanoparticles by previously unexploited fungus *Hormoconis resinae*. On treatment of aqueous solutions of silver with fungus, silver nanoparticles could be rapidly fabricated within an hour. These nanoparticles were characterized with UV-Vis spectroscopy, XRD, TEM and EDX analysis which revealed that the silver nanoparticles are polydisperse and of different morphologies ranging from 20 to 80 nm in size. X-ray diffraction (XRD) results reveal that these nanostructures exhibit a face-centered cubic crystal structure. To the best of our knowledge, this is the first report of silver nanoparticles synthesis using fungus *Hormoconis resinae.*

(Received April 26, 2009; accepted May 3, 2009)

*Keywords:* Microbes, Silver, Nanoparticles, Hormoconis resinae, Refinery pollutant

## 1. Introduction

Due to its unique properties, colloidal silver is widely used in catalysis [1] chemical sensing and biosensing [2,3] photonics [4] electronics [5,6] optics [7] DNA sequencing [8] surface-enhanced Raman spectroscopy [9,10] and pharmaceuticals [11,12] The diversity and importance of these applications has generated a great deal of interest in developing versatile methods to synthesize silver nanoparticles with well-defined and controlled properties. The list of approaches used to date includes reduction in solutions; chemical and photochemical reactions in reverse micelles; thermal decomposition of silver compounds; radiation-assisted, electrochemical, sonochemical and microwave-assisted processes; and, recently, biosynthesis using living plant systems. The reduction in homogeneous solutions has been for a long time the most attractive method for preparing highly dispersed metallic particles with the reducing agents most widely used being formaldehyde, alkali metals in ammonia, inorganic and organic borohydrides, ascorbic acid, free radicals, monoalcohols, polyols, acetonitrile, hydrazine, citrate, or ethylene di-amine tetra acetic acid. Because many of the reducing agents, solvents, and additives used in the reduction process often pose severe environmental and biological risks, it is important to develop "ecofriendly" precipitation processes especially for the large largescale production of these materials. Considering the rather limited choices of water-soluble precursor silver compounds, most attempts to provide "green" chemistries have been focused on selecting benign solvents, reductants, and stabilization agents.

Many biological organisms, both unicellular and multicellular, are known to produce inorganic materials either intra- or extra-cellularly [13,14] often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. Some well-known examples of bio-organisms synthesizing inorganic materials include magnetotactic bacteria (which synthesize magnetite nanoparticles) [15] and actinomycetes like extremophilic actinomycete, *Thermomonospora sp.* [16] and alkalotolerant actinomycete *Rhodococcus sp*. [17]. Both live microorganisms and dead

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microorganisms are gaining importance by virtue of their facile assembly of nanoparticles. Gold particles of nanoscale dimensions may be readily precipitated within bacterial cells by incubation of the cells with $Au^{3+}$ ions [18]. Bacterium *Pseudomonas stutzeri* AG259 isolated from a silver mine, when placed in a concentrated aqueous solution of $AgNO_3$, resulted in the reduction of the $Ag^+$ ions and formation of silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria [19]. Eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical compositions and sizes. A number of different genera of fungi have been investigated in this effort and it has been shown that fungi are extremely good candidates in the synthesis of gold [20,21], silver [22-24]. Several attempts of synthesis of metal nanoparticles have been made by researchers. Synthesis of gold nanoparticles within live alfalfa plants by gold uptake from solid media has been reported [25]. They also showed the biosynthetic procedure of silver nanoparticles by alfalfa sprouts [26]. It has been reported that the Au (III) ions are reduced in the solid media to Au (0) by the plant and then the atoms are absorbed into the plant where the nucleation and growth of gold nanoparticles takes place. This method can be very efficient in decontaminating soil polluted with heavy metal ions. The biosynthetic method employing plant extracts has received some attention as a simple and viable alternative to chemical procedures and physical methods synthesizing metal nanoparticles only in recent years. Sastry et al aforementioned attained the biosynthesis of metal nanoparticles by plant leaf extracts and their potential applications [27-29]. Recently J. Huang et al used novel sundried *C. camphora* leaf to synthesize silver and gold nanoparticles in aqueous solutions [30].

Here we report a novel method for the synthesis and characterization of silver nanoparticles synthesized by filamentous fungus *Hormoconis resinae*. On treatment of aqueous solutions of silver with fungus, silver nanoparticles could be rapidly fabricated. UV-Vis spectroscopy, XRD, EDX and TEM analysis revealed that the silver nanoparticles are monodisperse and with different morphologies as spherical to triangles ranging from 20 to 80 nm in size.

## 2. Experimental details

### 2.1. Preparation of silver nanoparticles

Fungal strain of *Hormoconis resinae* (Creosote fungus) was isolated from soil near Mathura refinery. It is one of the major blockage agent in the pipes due to its huge biomass production. Enumeration of microbes present in the soil was done by Serial dilution-agar plating method. Serial dilution of soil suspension was prepared upto $10^{-6}$ dilution. 1 ml of suspension from dilutions $10^{-3}$ to $10^{-6}$ was transferred to the petridishes containing Czapek's-dox (CD) agar (g/l $Na_2NO_3$, 2; $K_2HPO_4$, 1; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; Sucrose, 30; Agar, 15; pH 6.5) medium at 23 + 2°C for 6-8 days and growth was observed after two days. Experiment was set up in triplicate to minimize the deviations. Fungal biomass was cultivated in liquid medium using the shake flask method. Silver nitrate ($AgNO_3$) was purchased from Hi-media, and was used as received. In a typical synthesis for nanoparticles using *Hormoconis resinae*, the carefully weighted 0.5 gm biomass was added to 100 ml of 1 mM aqueous $AgNO_3$ solution, in conical flasks of 250 ml content at room temperature. The tubes were thereafter incubated at 26 ºC.

### 2.2. UV-Vis spectroscopic studies

The bioreduction of $Ag^+$ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring UV–vis spectra of the resulting diluents. UV–vis spectroscopy analyses of nanoparticles produced were carried out as a function of bioreduction time at room temperature on ELICO UV spectrophotometers at a resolution of 1 nm.

### 2.3. X-ray diffraction measurements

X-Ray diffraction (XRD) measurements of the bioreduced silver nitrate solution drop-coated onto glass substrates were done for the determination of the formation of Ag by an X'Pert Pro PANalytical X-ray diffractometer instrument with X'Pert high score plus software operating at a voltage of 45 kV and a current of 40 mA with Cu Kα radiation.

### 2.4. TEM observations

Samples of the aqueous suspension of silver nanoparticles were prepared by placing a drop of the centrifuged suspension on carbon-coated copper grids and allowing water to evaporate. TEM observations were performed on an H-600 electron microscope (Hitachi, Japan) operated at an accelerating voltage of 120 kV.

### 2.5. EDX observations

The biomass after reaction spontaneously precipitated at the bottom of the conical flasks in 1 h. After the precipitation, the suspension above the precipitate was sampled for TEM and SEM-EDX observations. SEM samples of the aqueous suspension of nanoparticles were fabricated by dropping the suspension onto clean electric glass and allowing water to completely evaporate. Samples were coated by carbon and SEM analyses were performed on a Zeiss EVO 40 Electron Microscope with Bruker EDX.

## 3. Results and discussion

Biological systems, masters of ambient condition chemistry, synthesized nanomaterials that are hierarchically organized from the nano- to the macroscale. In this work, a non-pathogenic fungus *Hormoconis resinae* was used for the extracellular synthesis of nanoparticles.

It is well known that silver nanoparticles exhibit reddish color in water; this color arises due to excitation of surface plasmon vibrations in the metal nanoparticles. The biomass incubated with deionized water (positive control) retained its original color i.e. yellow-green, while the silver nitrate treated biomass turned dark red (as shown in Fig.1a) after 6 hours due to the formation of silver nanoparticles extracellularly. This shows that it was a fast process. This color is primarily due to the surface plasmon resonance of silver nanoparticles. In case of negative control (silver nitrate solution alone), no change in color was observed and the silver nanoparticles analyzed by UV-Vis spectra and SEM were stable after 2 months (as shown in Fig.1c).

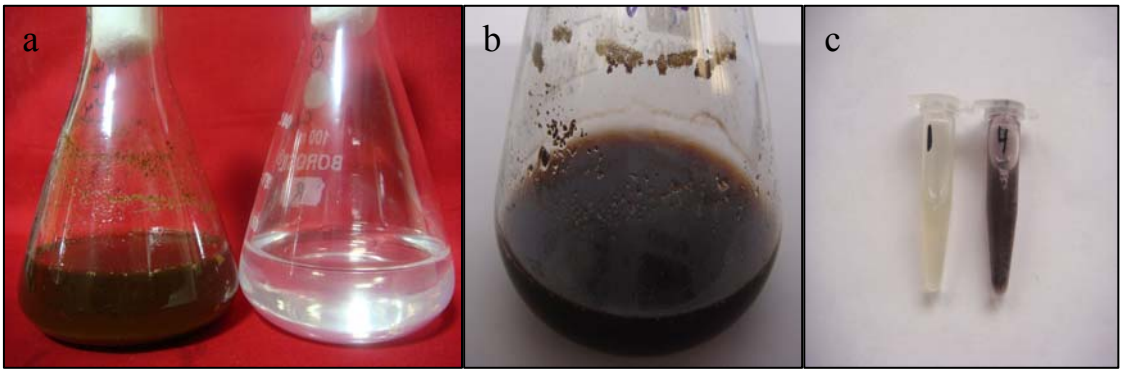


*Fig.1. Picture of flasks containing the Hormoconis biomass after incubation in an aqueous solution of $AgNO_3$ solution. (a) After 6 h with control, (b) After 24 h, (c) Tubes containing control and silver nanoparticles after 2 months.*

The surface plasmon resonance (SPR) band for spherical silver nanoparticles occurs in the range 380-440 nm. Graph shows the evolution of the absorbance spectra emanating from silver nanoparticles over time manifests increasingly sharp absorbance with increasing time of reaction at around 430 nm attributed to the surface plasmon resonance band (SPR) of the silver nanoparticles. While no absorption band was observed in both controls (Positive and Negative). After 24 h of incubation, no change in intensity at 430 nm was observed indicating the complete reduction of silver ions (as shown in Fig.1b).

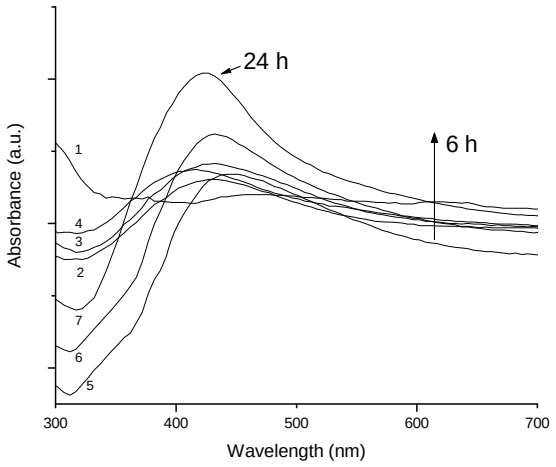


*Fig.2. UV-Vis spectra recorded as a function of time of reaction of 1 mM $AgNO_3$ aqueous solution.*

From fig.3, the particles were confirmed as elemental Ag (0) using XRD. In fig.3, a couple of Bragg's reflections are distinctly exhibited, which may be indexed on the basis of the face-centered cubic structure of silver. It exhibits a sharp and intense peak at ~38º and ~32º corresponding to diffraction from the (111) and (101) planes of silver with FCC lattice (JCPDS no. 04-0783).

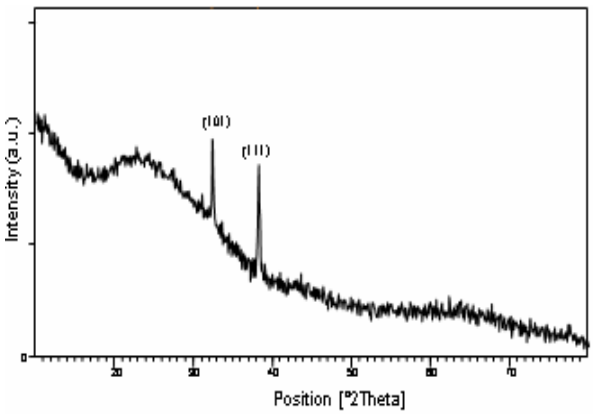


*Fig.3. XRD patterns recorded from drop-coated films of silver nanoparticles on glass substrates.*

The sharpening of the peaks clearly indicates that the particles are in the nanoregime. The average size of the silver nanocrystallites as estimated from the FWHM of the (111) peak (i.e.100%) using the Scherrer formula is ~42 nm.

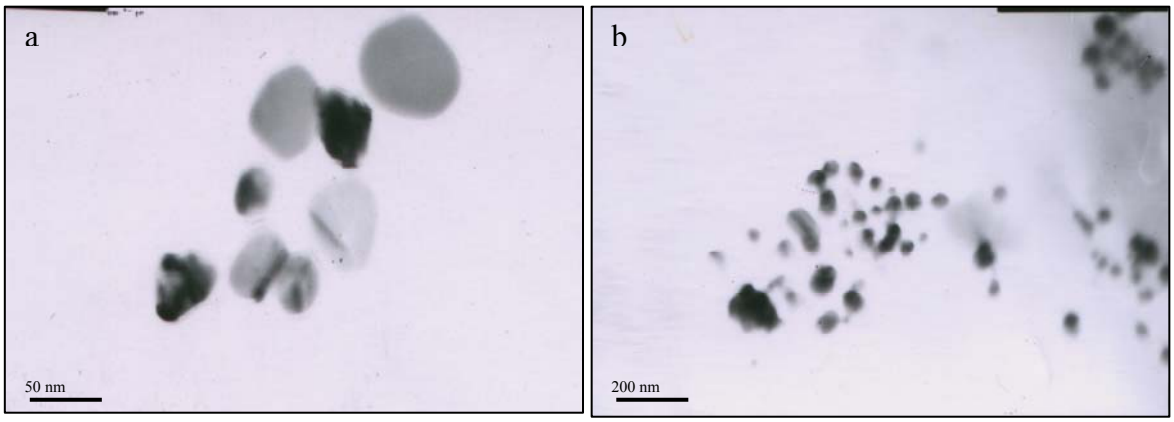


*Fig.4. TEM images of silver nanoparticles formed by reduction of $Ag^+$ ions.*

TEM picture (Fig.4) recorded from drop-coated films of the silver nanoparticles synthesized using *Hormoconis resinae* after reaction with silver nitrate solution for 24 h shows that the sample is composed of a large quantity of silver nanoparticles.

The silver nanoparticles formed were polydisperse, predominantly spherical with some nanotriangles in the range of 20-80 nm, some small particles in the regime of 10-20 nm are also present. It may be noted that the value obtained from TEM is in very good agreement with that obtained from XRD measurements. Under careful observation, it is noted that the some silver nanoparticles are present as clusters which explains the appearance of a longitudinal component in the UV-Vis spectra recorded from the silver nanoparticles solutions as discussed earlier (Fig. 2). These nanoparticles were not in direct contact even within the aggregates indicating stabilization of the nanoparticles by a capping agent. These are surrounded by a faint thin layer of other material, which we suppose is the capping organic material from *Hormoconis resinae* biomass. These may be tryptophan and tyrosine residues present in the proteins/enzymes present in the fungus. The assembly of the some silver nanoparticles

The particles were checked and was found to contain a great deal of Ag, using EDX analysis in Fig. 5.

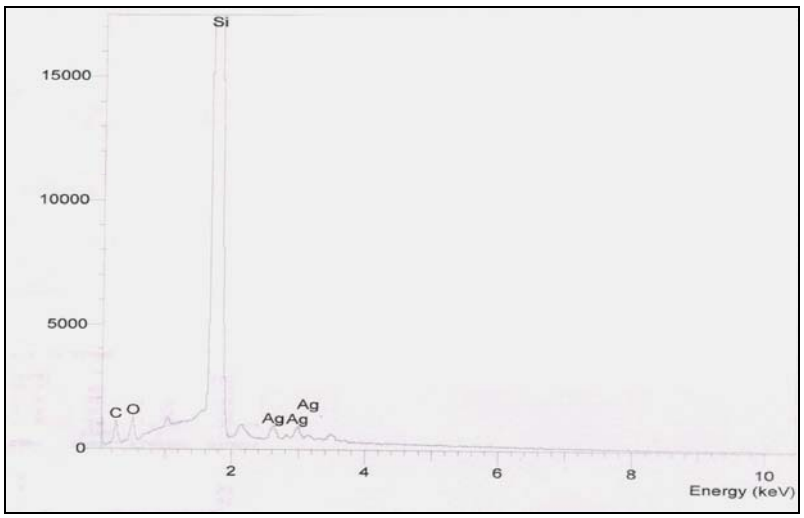


*Fig. 5. EDAX spectrum recorded from drop-coated films of silver nanoparticles synthesized using Hormoconis on glass substrates. Different X-ray emission peaks are labelled.*

Figure 5 shows the EDAX (energy dispersive analysis of X-rays) spectrum recorded in the spot-profile mode from one of the densely populated silver nanoparticle regions on the surface of film. Strong signals from the silver atoms in the nanoparticles are observed, while weaker signals from C, O, S, P, Na, Mg and Ca atoms were also recorded. The C, O, S, P, Na, Mg and Na signals are likely to be due to X-ray emission from proteins/enzymes present in the cell wall of the biomass.

## 4. Conclusions

In conclusion, it has been demonstrated that the *Hormoconis resinae* is capable of producing silver nanoparticles extracellularly and the silver nanoparticles are quite stable in solution. And this is an efficient, eco-friendly and simple process. We are focusing to prepare monodispersed nanoparticles by controlling several parameters as concentration of $Ag^+$ and/or the content of biomass as formed nanoparticles are of different shapes and sizes. This report will also lead to the development of a rational biosynthetic procedure for other metal nanomaterials with the *Hormoconis resinae*.

### Acknowledgements

The authors gratefully acknowledge the financial support (SR/S5/NM- 22/2006) given to this research from the Department of Science & Technology, New Delhi under the Nano Science Technology Initiative Scheme. We are also grateful to Dr. Renu Pasricha, NPL, New Delhi for TEM analysis and Dr. N.K.Saini, Dr. N.K.Juyal & Dr. Samay Singh from WIHG, Dehradun for XRD & EDX measurements.

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