Yeast Mediated Synthesis of Silver Nanoparticles

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(Received; 30 Apr 2008 and Accepted; 29 Sep 2008)

Abstract
A green low-cost and reproducible yeast mediated synthesis of silver nanoparticles is reported. The synthesis is performed at room temperature. X-ray and transmission electron microscopy analyses are performed to ascertain the formation of Ag nanoparticles. Nanoparticles almost spherical in shape having a size of 6-20 nm are found.
Keywords: *Nano silver; Nanoparticles; Saccharomyces sp.; Eco-friendly.*

1. INTRODUCTION

Nature by dint of its diversity provides exponential possibilities in the form of 'mini' nano-factories and microorganisms are one of them. Both bacteria and fungi make such an exciting category of microorganisms having naturally bestowed property of reducing metal ions into metallic nanoparticles. Use of fungal members for the purpose of synthesizing metallic nanoparticles has been a fairly recent development, due the fact that they are eukaryotic organisms having well understood metabolism at the cellular level. So far, Fusarium [1], Verticellium [2] and Aspergillus [3] have been found quite promising. Extra-cellular synthesis of silver nanoparticles taking use of Ag-tolerant strain of yeast MKY3 has also been reported [4]. However, it is suggested to be advantageous if a fungus is used for developing a process keeping in mind handling of the biomass and downstream processing of the nanoparticles [5]. Further, a procedure of nano-fabrication is much soughted which does not cause undesired lattice strain in them during synthesis. Biological system ensures such an advantage compared to the other mechanical or physico-chemical fabrication protocols.

Yeast being a member of the class Ascomycetes (also called sac fungi) in kingdom fungi, has been taken into regular use as media supplement in different culture procedures and this organism itself has been a very good source of different enzymes and vitamins. Besides, legislation on waste electrical/electronic equipment (WEEE) and restriction of hazardous substances (RoHS) has been issued recently by the European Union. To meet the requirements and growing technological

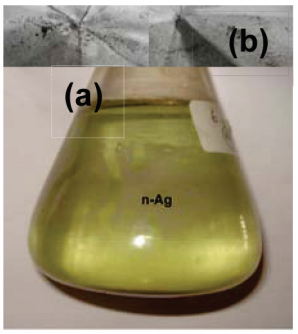

Figure 1: Photograph showing (a) deposition of n-Ag with medium and (b) deposition of n-Ag on whatman filter paper.

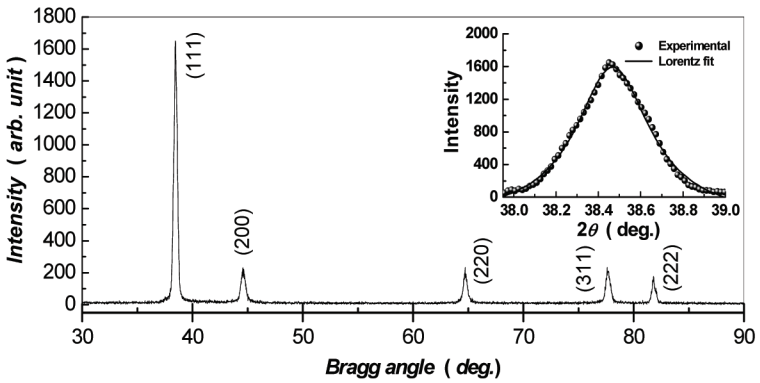

Figure 2: X-ray diffraction pattern of n-Ag at room temperature. Inset: Enlarged view of (111) peak with Lorentzian fit.

demand, there is a need to develop an eco-friendly approach for nanomaterials synthesis that should not use toxic chemicals in the synthesis protocol. In the present effort, the baker's yeast (Saccharomyces cervasae) has been taken in order to assess its potential as putative candidate fungal genera for the transformation of silver nanoparticles (abbreviated hereafter n-Ag). Tolerance of the organism towards silver ion has also been assessed. Furthermore, we have tried to explore a cost effective, eco-friendly and reproducible approach for the purpose of scaling up and subsequent downstream processing.

2. EXPERIMENTAL

Nanoparticles of Ag were prepared by using baker's yeast. Yeast cells were allowed to grow as suspension culture in presence of suitable carbon and nitrogen source for 36 hours. This was treated as source culture. A small portion of it (25 ml) was filtered and diluted four times by adding 30% Et OH containing nutrients. This diluted culture was again allowed to grow for another 24 h until it attains a light straw colour. Now, 20 ml of 0.025(M) $AgNO_3$ solution was added to the culture solution and the culture was allowed to incubate. The colour of the culture solution changes immediately into silver gray after the addition of $AgNO_3$ solution. It turns yellowish brown within ten minutes. Such a rapid extracellular synthesis was also reported from Aspergillus biomass filtrate [3]. Transformation was observed to take place within 10 min. after the addition of Ag+ ions into the culture solution which continues up to four days until complete precipitation of n-Ag takes place. After three to four days, the culture solution was observed to

have distinctly markable deposits at the bottom as well as on the wall of the conical flask (Fig.1a). A remarkable change in pH was observed at this stage. The possible synthetic mechanism could be as follows:

$$Ag^{+} + e \Leftrightarrow Ag^{0} \downarrow$$

The sooty gray nanoparticles of silver were filtered under the laminar flow through whatman filter paper (Fig.1b), allowed to dry under blow of hot air after which they were used for X-ray and TEM characterizations. The formation of single-phase compound was checked by X-ray diffraction (XRD) technique. The XRD spectra were taken with a X-ray diffractometer (XPERT-PRO, PW3050/60) at room temperature, using CuKα radiation λ = 1.5406 Å over a wide range of Bragg angles ($10° \leq 2\theta \leq 90°$). TEM micrograph and selected area electron diffraction (SAED) pattern of n-Ag were obtained at 88.0 K using Philips CM200 transmission electron microscope.

3. RESULTS

Fig.2 shows the X-ray diffraction profile of n-Ag. The XRD confirms the crystalline nature of the particles. The peaks of the XRD-pattern were indexed and cell parameters were determined using experimental 2θ-values of peaks on different crystal systems. The five peaks of XRD were assigned to the diffraction {111}, {200}, {220}, {311} and {222} planes of face-centered cubic (FCC) silver respectively. The cell parameter and unit cell volume respectively estimated to be 4.067 Å and 67.27 Å^3 with the space group of *Fm3m* which is in agreement with the literature report (PCPDF No. #03-0931). It is important to note that the ratio $I_{\{111\}}/I_{\{200\}}$ comes out to be 9 which is much higher than the conventional value '4'. This indicates that the nanoparticles are abundant in {111} planes. Thus, diffraction intensity of {111} plane should be greatly enhanced in comparison to that of other planes. This result is in consistent with the earlier report [6]. The inset Fig.2 shows the (111) Bragg reflection of silver along with a Lorentzian fit to the reflection.

$$I = I_o + (2A/\pi)\ [\ w/\{\ 4(\theta - \theta_c)^2 + w^2\}\ ]$$

where A, w and θc are respectively the area, width and centre of the curve. The fitting parameters as obtained are I_o = -258.94, A = 1243.77, w = 0.427 and θc = 38.47. The value of regression coefficient (R^2) was found to be 0.9943. An estimate of the size of the nanoparticles was made from the line broadening of the (111) reflection using the Debye-Scherrer formula [7]:

$$P_{hkl} = 0.89\lambda / \text{FWHM} \cos\theta$$

where FWHM = full width at half maximum. The average particle size was estimated to be of the order of 18 nm.

Fig.3 illustrates the TEM micrograph at 100 nm of the n-Ag being formed using Saccharomyces strain. The micrograph clearly shows individual nanoparticles with variable shapes, most of them present in spherical in nature. The measurement of size was performed along the largest diameter of the particles. The particles are found to have a size of the order of 11 nm. It is found that the estimate of the size of the nanoparticles was made from line broadening of (111) reflection using Scherrer's equation to be in fairly good agreement with the nanoparticle size estimated by the TEM analysis. Inset Fig.3 shows the selected area electron diffraction (SAED) pattern obtained from n-Ag shown in Fig.3 The Scherrer ring characteristic of fcc silver is clearly observed, showing that the structure sheen in the TEM image are nanocrystalline in nature. Also, the structure of n-Ag as observed in SAED pattern resembles with XRD result. The particle size histogram of n-Ag (Fig.4) shows a broad distribution of particle sizes which follows a Lorentzian model:

$$N = N_o + (2A/\pi)[w/\{4(d - d_c)^2 + w^2\}]$$

where A, w and dc are respectively the area, width and centre of the curve. The fitting parameters as obtained are N_o = 4.013, A = 83.591, w = 1.664 and d_c = 10.831. The value of regression coefficient (R^2) and Chi^2/DoF were found respectively to be 0.984 and 3.166. The size of particles ranged from 6-20 nm. Majority of the n-Ag were scattered with only a very few of them showing aggregates of varying sizes as observed under TEM. The difference in size is possibly be due to the fact that the nanoparticles are being formed at different times, which may

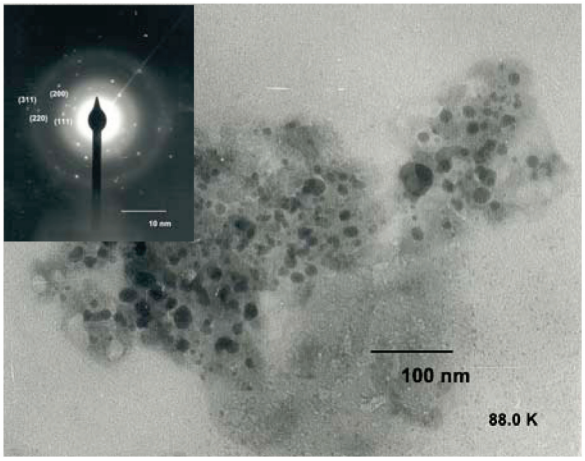

Figure 3: TEM photograph of n-Ag at 88.0 K. Inset: Selected area electron diffraction pattern of n-Ag.

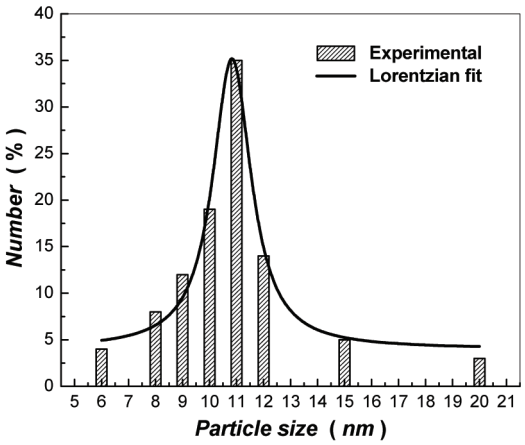

Figure 4: Bar chart showing particle size distribution (%) of n-Ag.

limit the nanoparticle size due to constraints related to the particles nucleating inside the organisms. The results presented in this paper are at single pH value and is a part of our systematic work which is still in progress.

4. DISCUSSION

Generation of n-Ag by the assistance of different fungal members has been a comparatively new approach [8]. The synthesis could either be intra- or extra-cellular depending upon the species being taken into use. Earlier workers have observed appearance of brown colour on addition of the silver ion, into the medium which indicated the excitation of surface plasmon vibrations, typical of silver nanoparticles [1]. During the present work, the fungal biomass after thirty six hours of incubation with nutrients, and subsequent dilution in 30% Et-OH and further incubation for 24 h, was

challenged with $AgNO_3$ 0.025(M) solution. The visible changes in culture solution which ranges from silver gray to yellowish brown, and finally to sooty gray indicated extra-cellular synthesis of n-Ag. Although, such a synthesis was also reported in Fusarium [1], Aspergillus [3] and in MKY3 yeast strain [4], but in the present case we have tried to generate n-Ag using commonly available baker's yeast which generate nanoparticles at a much faster pace, is hazard free (because Aspergillus being a toxin producing fungi) and may reduce the cost of production appreciably.

Further, in yeast cells the reduction of metal ions in extra-cellular manner could be due to one of the following mechanisms: (i) participation of a sulphate or nitrate reductase system capable of working in the presence of ATP and NADH or anthraquinone [9]. (ii) activation of a specific polypeptide chain involving a repeating sequence of general formula (γ-Glue-Cys)nGly with n values commonly ranging from 2-6 (as found in case of Cd). They might bind to the cationic species immediately forming a cationic-PC complexes that are transported into the vacuole, where this complex is degraded and the nano particles are formed [10,11]. This was found in the case of Cd while challenging Candida glabrata cells. (iii) Ag nanoparticles are formed on the surface of fungal cells which might have happened due to an electrostatic interaction between the Ag+ and the negatively charged carboxylate groups present either on specific enzymes, protein or even polypeptides present on the cell wall. Bioreduction and stabilization takes place in situ probably due to capping of Ag nanoparticles by some specifically secreted proteins, like cysteine [12]. As reported in case of Aspergillus fumigatus [3] a much faster rate of transformation compared to Fusarium may be accounted to the physiological or metabolic advantage of being a member of a higher class of fungi (Ascomycetes) like yeast, having a well defined lifecycle [13].

5. CONCLUSION

In conclusion, the present biotechnological method is capable of producing Ag nanoparticles. Also, it is a green high yield, fast and low cost approach.

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