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Synthesis and Characterization of Silver Nanoparticles using Bread Mold Extract

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ABSTRACT

Silver nanoparticles were synthesized through green methods using 0.05M $AgNO_3$ as precursor salt and black bread mold extract as reductant. X-ray diffraction pattern confirms the formation of nanoparticles with indexed Bragg reflection peaks in accordance with the JCPDS data corresponding to face centered cubic lattice of silver. The average crystallite size was estimated using Debye-Scherer equation and the obtained crystallite size was estimated to be 37 nm. The UV-visible spectrum, FTIR and antibacterial analysis were also performed on the sample. The silver nanoparticles so formed exhibited moderate antibacterial activity as evidenced from the growth inhibition ring (8 mm dia) observed for both *E coli* and *S aureus*.

Keywords: Silver nanoparticles, Bread mold, E coli, S aureus etc.

I. INTRODUCTION

Green methods have now become the routine tool for nanoparticle synthesis [1]. Zero valent metal nanoparticles such as silver, gold, iron etc. have been widely synthesized using green techniques [2]. Among them, synthesis of silver nanoparticles is particularly important because of their affluent impact on antimicrobial utilities [3]. Extracts of various plant parts and fungi have been extensively used as reductant for such synthesis. Among them, Black Bread Mold comes under kingdom fungi in the phylum zygomycota and class zygomycetes. The order and family is mucoraceae. In the rhizopus genus, it comes under the species rhizopus stolonifer, and hence the scientific name is *Rhizopus stolonifer*. It is a threadlike mold and a heterotrophic species which consumes sugar or starch as source of carbon substances for food. As the mold matures it begins to turn black, which breaks down organic matter through decomposition. When kept in a moist environment, such as a piece of bread, the parasite can quickly spread within a few days. Its spores are commonly found in the air. The spores grow most rapidly at temperatures between 15°C and 30°C and germinate to their full potential. Black Bread Molds are commercially used for manufacturing alcohol and organic acids. Figure 1 shows the collection of black bread mold plucked carefully from a 10 days old bread surface.

Figure 1: Black bread mold collected from a 10 days old bread surface

In this paper, the detailed discussion of characteristic results obtained for the silver nanoparticles synthesized using 0.05M $AgNO_3$ as precursor salt with Bread Mold extract as reductant is scripted.

II. EXPERIMENTAL

The black bread molds were plucked carefully from 10 days old bread surface during May 2017. Around 6 g of molds were weighed and washed properly first with normal water and then using de-ionized water. 100 mL of de-ionized water was then taken in a beaker and the molds were added to it and stirred well with heating at 60 ^oC for almost one hour. The solution was left over night and filtered twice using ordinary filter paper and then by whatmann filters paper. The filtrate was measured and kept for further process.

(a) (b) (c) (d)

Figure 2: The different stages in sample synthesis (a) 0.05 M $AgNO_3$ solution (b) the bread mold extract (c) formation of silver nanoparticles and (d) the crushed fine powder of nanoparticles

For the preparation of precursor salt solution, 0.05 M $AgNO_3$ solution was prepared in dark, covered with aluminium foil, and stirred well in magnetic stirrer. Then the filtered extract solution covered with aluminium foil was placed over the magnetic stirrer and the precursor salt solution was added drop wise in 3:2 ratio and stirred well for almost 3hrs at a temperature of 60 ^oC. The pH and colour of solution were also noted frequently. Initially pH was below 4. Then 80% saturated ammonium sulphate ($(NH_4)_2SO_4$) solution was added drop wise to sustain the pH level. The solution was gradually turned brown which confirmed the formation of Silver nanoparticles. The gel like accumulation of the mixture was then kept for 3hrs with constant heating & stirring. It was necessary to keep pH between 2 to 6 in order to obtain better precipitation of silver nanoparticles. The

solution in the magnetic stirrer was fully covered with aluminium foil and the xero-gel obtained was kept overnight to settle down. The residue obtained was dried and further annealed at about ~130 ^{o}C for proper aging of crystals. The crushed fine powders of silver nanoparticles were then kept in air tight packet (Figure 2) after proper labeling for further characteristic studies and measurements. The samples were hereafter referred to as Ag-NP-BM.

III. RESULTS AND DISCUSSION

Figure 3 shows theX-ray diffraction pattern recorded for Ag-NP-BM sample, using XPERT-PRO diffractometer system having type 0000000083005153 with continuous scan mode of step size 0.0170 in the gonio axis in the 2θ range 10° to 89.9°, installed at National Centre for Earth Science Studies (NCESS), Thiruvananthapuram.

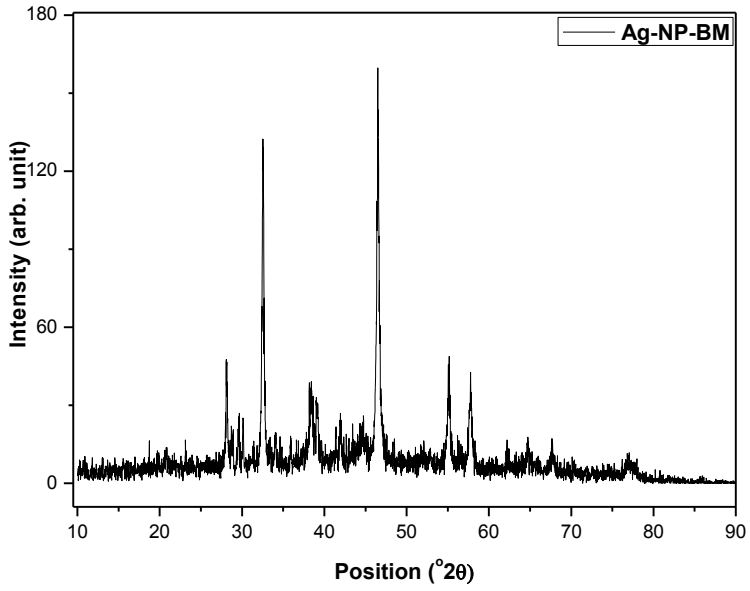


Figure 3: X-Ray diffraction pattern recorded for Ag-NP-BM

X-rays from the Cu anode material including K-aplha1, K-alpha 2 and K-beta radiations with respective wavelengths, 1.54060 Å, 1.54443 Å, and 1.39225 Å with K-alpha 2 to K-alpha1 ratio equal to 0.50000 were allowed to impinge on the samples. The 240 mm goniometer radius having 100 mm dist. focus-diverge-slit was used with generator settings 30 mA, 40kV. The indexed Bragg reflection peaks with respective d-values were found to be in accordance with JCPDS: ICDD PCPDF WIN #PDF 893722, which is corresponding to the face centered cubic lattice of Silver. Also, traces of silver oxide phases are evident from XRD patter due to the presence of an intense peak with matching d value at 32.5^{o}.

Considerably broadened x-ray reflection peaks from various crystallographic plane surfaces indicate the distribution of crystallite size in nanometric regime. The average crystallite size was estimated using Debye-Scherer equation [4]:

$$D = \frac{k\lambda}{\beta \cos\theta_B}$$

where D is the thickness (diameter) of the particle, λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) of the main peak under consideration, k is the shape factor and θ_B is the Bragg angle of reflection. The main intense peak at 46.5370° was taken for analysis to obtain the size estimation using Scherer formula. Accordingly, the estimated crystallite size is 37 nm.

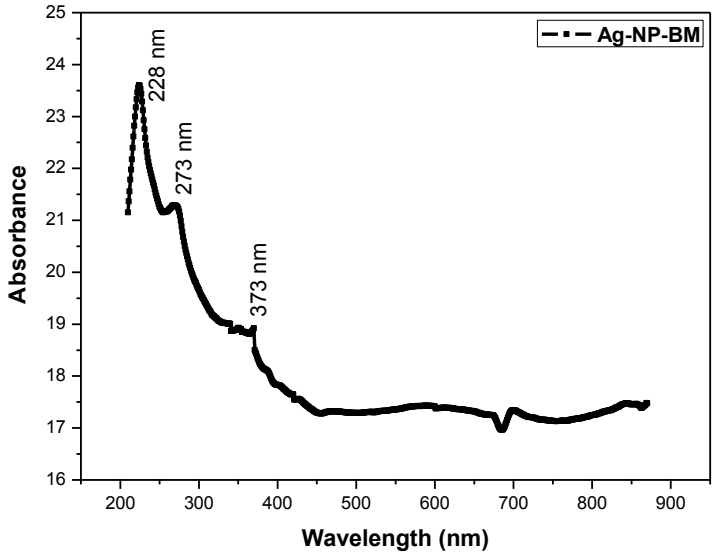


Figure 4: UV-Visible Spectrum recorded for Ag-NP-BM

UV-Visible absorption spectrum of the sample was recorded using JASCO V-650 B118561150 spectrometer installed at Department of Physics, Sree Narayana College, Kollam. The data were obtained in absorbance photometric mode using a bandwidth of 5nm in 1 nm interval at a san speed of 400 nm/min. Figure 4 represents the spectrum obtained for the prepared Ag-NP-BM sample. The peaks obtained at wavelength 228 nm, 273 nm and 373 nm are corresponding to the elemental absorption of Ag in Ag-NP-BM sample.

FTIR spectral measurement was performed on Perkin Elmer Spectrum Version 10.5.2 installed at Department of Chemistry, Christian College, Chengannur. Figure 5 shows the room temperature FTIR spectrum recorded in the wavelength range 4000 cm^{-1} -400 cm^{-1} for the sample, Ag-NP-BM. Five bands centered at 3206.68 cm^{-1}, 1645.26 cm^{-1}, 1403.00 cm^{-1}, 1047.00 cm^{-1} and 607.33 cm^{-1} were observed. The band at 607.33 cm^{-1} corresponds to the stretching vibrations at the metallic site of silver in the crystal. The intense broad band at 3206.68 cm^{-1} is due to the asymmetric and/or symmetric stretching modes of water molecules (H-O-H bonding) in the crystal. The band at 1645.26 cm^{-1} is attributed to the C=C ring stretching in flavonoids (organic capping) [5, 6]. The band at 1403.00 cm^{-1}corresponds to the in-plane bending vibrations of –OH group in flavonoid [5,6] and that at 1047.00 cm^{-1} relates to the stretching vibration of C-O-C. The weak absorptions at the inorganic band positions against the comparatively larger absorptions at the capped polymer rings again infer that samples formed are of much reduced crystallites, which agrees well with the XRD result.

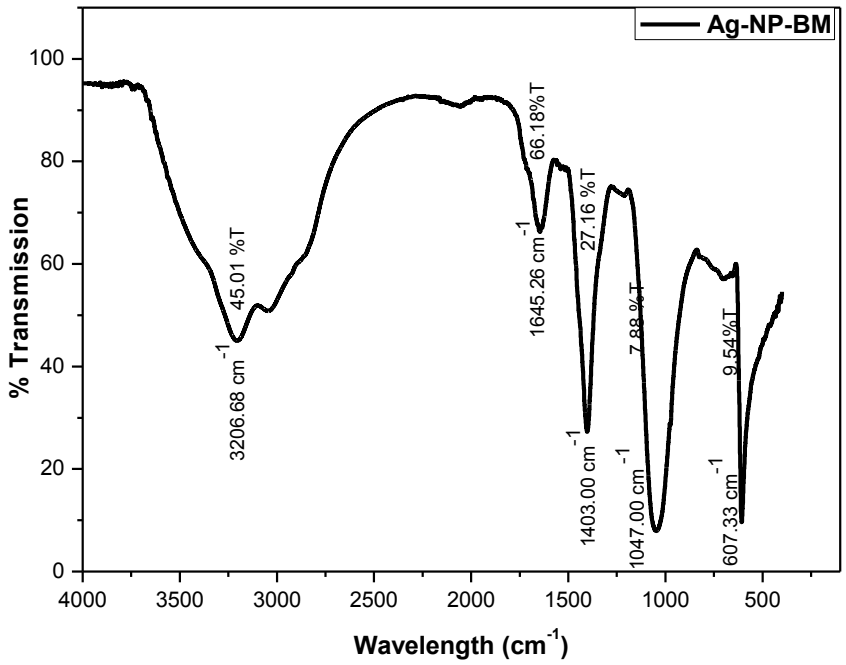
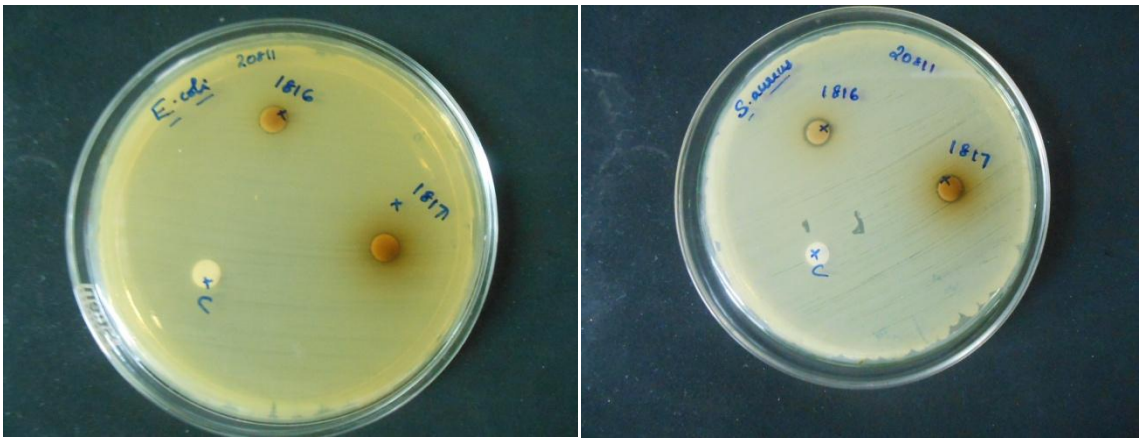


Figure 5: FTIR spectrum recorded for Ag-NP-BM



(a) (b)

Figure 6: Antibacterial activity of Ag-NP-BM solution against (a): E. Coli (1817) (b): S. aureus (1817)

Figure 6 shows the antibacterial activity of Ag-NP-BM for *Escherichia coli (E coli)*, Gram negative and *Staphylococcus aureus (S aureus)*, Gram positive, measured by CLSI Standard M02-A10 method. The Ag-NP-BM (1817) shows moderate antibacterial activity as evidenced from the growth inhibition ring (8 mm dia) depicted in figure 3.4(a) and (b) for both *E coli* and *S aureus*).

The mechanism for antibacterial action of silver nanoparticles is bacterial membrane disruption by the ions silver released from the solution [3]. The Ag ions form insoluble compounds with sulphydryl groups in the cellular wall of the microorganism that are responsible for the inhibition halo in the seeded culture media as observed in figure 3.4(a) and (b). The diminution in nanocrystallite site has also a great impact in antibacterial

activities. All antibacterial activity tests were performed in triplicate and were done at least two different times to ensure reproducibility.

IV. CONCLUSION

Nanoparticles of silver could be successfully synthesized using the extract of black bread mold. The face centered cubic lattice phase of silver and the average crystallite size were estimated from x-ray diffraction pattern. From the UV-visible spectrum and FTIR, the existence of zero valent silver could be confirmed. The anti bacterial impact was found to be moderate against the media of E. Coli and S. aureus respectively.

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