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Synthesis and Charactrisation of Silver Nanoparticles using Fungi and its Anti-Microbial Activity

D. R. Patil

Department of Physics, R.C. Patel Arts, Commerce & Science College, Shirpur, India
dr.drpatil@gmail.com

Abstract: *In present work attempt has been made to synthesized silver nanoparticles by biosynthesis technique using different species of fungi. This is an easy, cost efficient and ecofriendly and rapid method for synthesis of silver nanoparticles and evaluates its microbial activity. Biosynthesized silver nanoparticles were characterized by using UV–Visible spectroscopy, XRD, FTIR particle size analyzer etc. And its stability is studied. silver nanoparticles exhibited maximum absorbance at 420 nm in UV–visible spectroscopy which is attributed to the Plasmon resonance of silver nanoparticles. These nanoparticles are found to stable for about 3 month. Antimicrobial activity was also studied which indicates that silver nanoparticles shows good antimicrobial activity.*

Keywords: *Bio- synthesis, silver nanoparticles, Antimicrobial activity*

1. INTRODUCTION

Nano-silver is relatively new and a different type of silver with different chemical and physical properties. Nano-silver part methodology, low cost production and the minimum time required. There for it is prepared over the chemical and physical method.The fungi produce higher amounts of protein as compared to bacteria, which result in higher production of nanoparticles [5-7]. Because of their properties such as tolerance, and metal accumulation ability high binding capacity and intracellular up take compare to other microorganisms [8]. Fungi have been extensively used for the rapid and eco-friendly biosynthesis of metal nanoparticles. It has been monitored that the fun icles can be synthesized using different are methods from metallic silver because of their antibacterial activity. The synthesis of nanostructured metallic particles is an expanding research area biosynthesis has received attention due to its cost effectiveness and environment friendly technique for green synthesis of nanomaterial's[1]. The synthesis of silver nanoparticles extensively studied by using chemical and physical methods, Traditional chemical methods of synthesizing silver nanoparticles include the role of ethylene glycol [1, 2], pyridine [3], and sodium boro hydride [4].The biological synthesis process provides a wide range of environmentally acceptable gi-mediated synthesized nanoparticles have good mono-dispersed and good dimensions [6, 9]. The intracellular and extracellular methods are being used for the synthesis of nanoparticles via a biological route. The extracellular method is more advantageous than intracellular method because the intracellular method requires an additional step to obtain the purified nanoparticles [10, 11].

Nano-silver has reported several biological properties like antibacterial activity against a broad spectrum of Gram-negative and Gram-positive bacteria including antibiotic-resistant strains [12-15]. Silver nanoparticles exhibiting well antifungal properties as it is an effective and fast-acting fungicide against a broad spectrum of common fungi, including genera such as Aspergillus, Candida, and Saccharomyces [16]. Antiviral properties of silver nanoparticles with average diameter 10 nm are responsible to inhibit HIV-1 virus [17]. Silver nanoparticles have become the focal point of much research interest due to their broad assortment of applications [18]. Their power to alter the physical, optical and the electronic properties of compounds [19] have found applications in diverse areas including electronic devices [20], chemical/biological sensing [17] and surface enhanced Raman spectroscopy [21]. In contrast, a promising usage of Silver nanoparticles as an antimicrobial agent is well experienced and has already found applications in antimicrobial paint coatings [22], textiles, water treatment, medical devices [23], and HIV prevention as well as treatment [24].

In this research work, includes biological synthesis of silver nanoparticles using different isolated fungal strains and further characterization of synthesized silver nanoparticles. This research approach may be useful to green chemistry researchers those working on synthesis and application of silver nanoparticle in different field of research.

2. MATERIALS AND METHODS

2.1 Materials

There are seven different fungal strain used such as decaying fruit, affected plant, garden soil, farm soil, affected peanuts, rotten tomato, decaying food. different fungus strain were grown on Malt extract Glucose Yeast peptone media(Hi media, Mumbai) containing yeast extract-0.3%, malt extract-0.3%, glucose-1%, peptone-0.5%. AgNO3 (1mM).

2.2 Experimental Work

In this study, seven different fungal strains obtained from sources of decaying fruit, affected plant, Garden soil, farm soil, affected peanuts, rotten tomato, and decaying food were used for experimentation. Isolated fungi were inoculated in Malt Glucose Yeast Peptone (MGYP) broth (20) at 30oC, in shaking condition (200 RPM) in the dark. After 7 days, the incubated biomass was filtered and then extensively washed with distilled water to remove any medium component. An obtained biomass was taken into sterilized flasks containing 100 ml DI water. The flasks were incubated at the same conditions. The biomass was filtered with whatman filters paper, after the incubation period of 72 h, the fungal filtrate solution was used further.

In a typically reaction procedure 4ml of fungal filtrate was added to 100ml of 1mM silver nitrate solution with stirring magnetically at room temperature . The yellow color of the mixture of extract and silver nitrate start changing this indicate bio synthesis of silver nanoparticles .the flask were incubated with shaking condition. After 96 h of incubation Farm Soil fungal strain show result from yellowish to brown, hence further work was carried out with only this fungus. Further the reduction of silver ions was observed by UV-vis spectral analysis in the range of 300-600nm.

To investigate the effect of extract concentration for synthesis of silver nanoparticles, the mixture with the ratio of 1:25,1:50,1:75,1:100,1:125 extract to 1mM silver nitrate were prepared. The reduction mechanism of silver ion into Nano silver particle was investigated with particlesizer.

The solution was kept at room temperature over a period of three month. During this period, UV-vis spectrum of solution was recorded after every ten days. The color and pH of solution were regularly noted and it does not show any change. Antimicrobial activity against E-coli was done by disc diffusion method.

The suspension of silver nanoparticles was allowed to settled, excess liquid was removed, then rinse to remove organic residue and responded in 90% ethanol for further characterization.

2.3 Characterization

Particle size analysis was performed using particle size analyzer (Zetasizer Malvern). The UV-vis spectra of silver nanoparticles were recorded as a function of wavelength using UV-visible spectrometer (Shimadzu -UV2450). X-ray diffraction was used to confirm the crystal structure of silver nanoparticles using X-ray diffractometer (Philips pw1710) on copper target using Kα Radiation having $\lambda=1.54A^0$ in the 2θ range of $20^0$and 80^0. Fourier transform infrared (FT-IR) spectral measurements were carried out by drying the suspension in lyophilizer, diluted with KBr in ratio of 1:100 and analyzed by Shimadzu IRAffinity-1S spectrophotometer with diffused reflectance mode in the range of 1000-4000 cm^{-1}.FTIR measurements are carried out to identify the possible interaction between silver and bioactive molecules which may possible by for synthesis and stabilization of silver nanoparticles.

3. RESULTS AND DISCUSSION

3.1. Characterization

The addition of biomass extract to silver nitrate solution resulted in color change of solution from yellowish to brown due to production silver nanoparticles. This distinctive to brownish red color appearance is mainly due to excitation of surface plasmon resonance with the silver nanoparticles, which is consider to be primary signature of the formation of nanoparticles [25]. An observed peak at 420 nm (Figure 1) is assigned to surface plasmon resonance band of silver nanoparticles it indicates the complete of the silver nanoparticles.

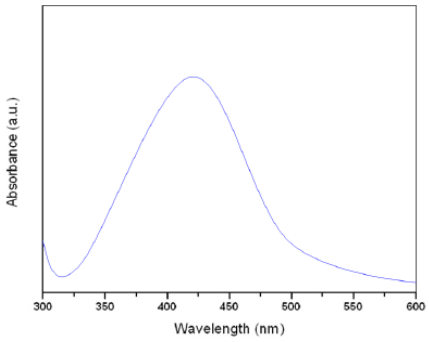

Figure 1. *Absorbance maxima at 420nm of silver nanoparticles obtained by soil fungi*

The crystalline nature of silver nanoparticles was confined by XRD. The XRD pattern of the synthesized nanoparticles is shown in Figure 2. The indexing process of a powder diffraction pattern is done and Miller indices (h,k,l) to each peak is assigned. Four prominent peaks corresponds to (111),(200),(220) and (311) reflection planes of a face center cubic (FCC) structure of silver by comparing with JCPD data , silver file No. 04-0783. Additional unassigned peak is observed at 55.30 which may be due to bio-organic compounds or metalloproteinase which are responsible for production and stabilization of resultant nanoparticles. The average grain size of the silver nanoparticles formed in the bio reduction process is determined by using Debye - Scherer equation [26, 27].

$$D = \frac{k\lambda}{B\cos\theta}$$

The average grain size estimated to be 12.5 nm which are in good agreement with previously reported result [28].

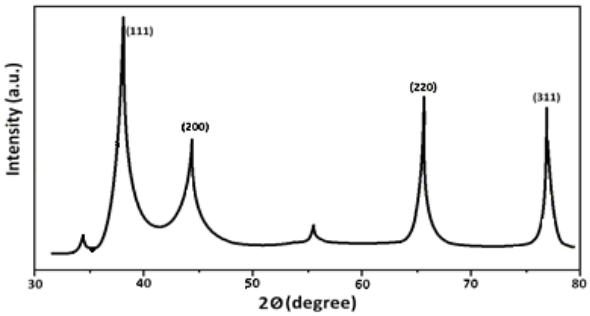

Figure 2. *XRD Pattern of silver nanoparticles synthesized by extract*

The result of FTIR study reveals that absorption peaks appear at 3410, 2934, 1748, 2624, 1371and 1163 cm^{-1} as shown in figure 3. The band at 3410 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2934 cm^{-1} corresponds to O-H stretch carboxylic acids. The peak at 1748 cm^{-1} O=H of carboxylic acid or ester. The high intense peak at 1624 cm^{-1} corresponds to the bending vibration of N-H bending vibrations of amines. The peak at 1378 cm^{-1} corresponds to C-N stretching vibrations of aromatic amine group, while the band observed at 1163cm^{-1}corresponds to C-N stretching alcohol. This indicates the presence and binding of proteins with silver nanoparticles which can lead to their possible stabilization.

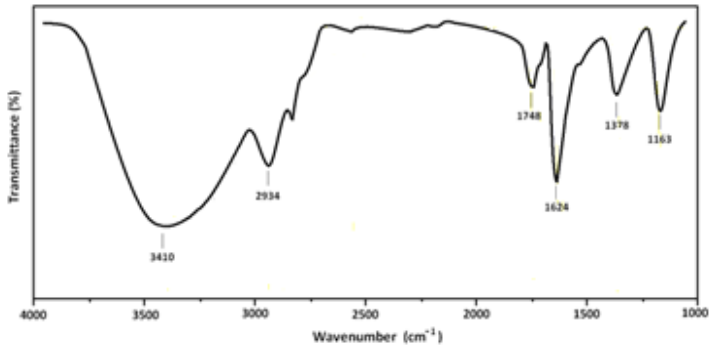

Figure 3. *FT-IR spectrum of synthesized silver nanoparticles*

3.2. Parameters affecting the formation of silver nanoparticles

The main parameters affecting the formation of nanoparticles include pH of extract, effect of incubation temperature and extract concentration.

3.2.1. Effect of pH

The effect of extract pH on the synthesis of silver nanoparticle in the range of 2 to 6 with interval of 0.5 was investigated by UV-vis spectrometer, which show that the silver nanoparticle synthesis increases at pH 4.5. The highest color intensity is observed at this pH. This difference in colour observed over the range of pH can be attributed to the dissociation constant of functional groups on biomass used [29]. These results are in good agreement with previously reported studies [30]. This may be because various biomolecules are involved in biological synthesis of silver nanoparticles. These molecules are likely to be inactive under extremely acidic conditions.

3.2.2. Effect of incubation temperature

The incubation temperature also controls the rate of reaction .When the reaction mixture is incubated at different temperatures ranging between 30^0C-70^0C. It is observed that at lower temperatures up to 50^0C, light brown colour was seen, while at 60^0C and70^0 C well developed dark radish brown color was developed. It took 24 hrs. to develop color at room temperature while dark radish brown color was developed in 96 hrs. Development of red colour is indication of completion of reaction thus by reduction of silver. At higher incubation temperature, reduction process was faster. Figure 4 show UV spectra of the samples incubated at different temperatures. Increase in incubation temperature shows development of a sharp peak which is shifting towards lower wavelength region, shifting of a peak toward lower wavelength indicates smaller size of nanoparticles. This is in good agreement with the result obtained by other researchers [31]. This may attributed to the well-known fact that at higher temperature, the reactants are rapidly consumed and leading to formation of smaller size nanoparticles. Similar results are obtained for other fungus used to synthesis silver nanoparticles [32].

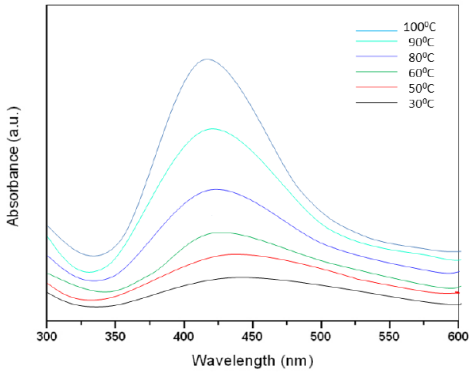

Figure 4. *UV spectra of the samples incubated at different temperatures*

3.2.3. Effect of incubation time

The intensity of the radish brown colour is proportional to reaction mixture incubation time. Increase in the absorbance with different incubation time is plotted in figure 5. Increase in the absorbance with incubation time can be ascribed to increase in the number of silver nanoparticles formation with time [33]. Here after 4 days, the color did not change any more with reaction time. The characteristic radish brown color of silver solution provided a convenient spectroscopic signature to indicate silver nanoparticles formation.

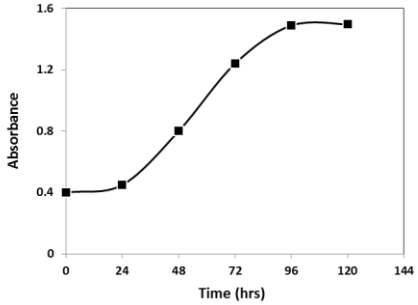

Figure 5. *Absorbance as a function incubation time*

3.2.4. Effect of extract concentration

The graph shown in figure 6 indicates that the average particle size of silver nanoparticles is influenced by biomass extract concentrations. The smaller particle size of 65 nm was obtained for extract to silver nitrates ratio of 1:125. This may attributed to the fact that increase in the concentration of filtrate increases the ratio of reduction and reduces the particle size. The reduction of the silver ions to the silver nanoparticles was found to be optimized at the ratio of 1:25 of extract to 1mM silver nitrate solution.

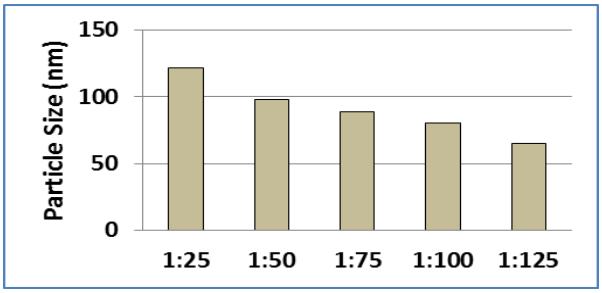

Figure 6. *Particle size of silver nanoparticles at different extract concentration*

3.3. Stability testing:

Further the reduction of the silver ions was monitored over time by UV visible spectral analysis in of the range of 300-600 nm and a quartz cuvette with water as a reference. The solution was kept at room temperature over period of 3 months. During this period, UV- vis spectrum of solution was recorded after every ten day. The color and the pH of solution were regularly noted and it doesn't show any change. Zeta potential is an important parameter to understand the state of the nanoparticle surface and predicting the long term stability of the dispersion. The zeta potential value of dispersed silver nanoparticles in distilled water was -25.7 mV recorded indicates long term stability. The suspension of silver nanoparticles was allowed to settled, excess liquid was removed then rinse to remove organic residue and suspended in 90% ethanol for further characterizations. Out of these different fungal strains obtained a soil fungus only shows a result from yellowish to brown hence further work was carried out with only this fungus.

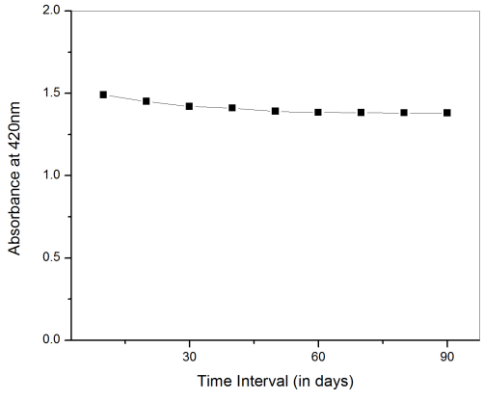

Figure 4. *Stability testing over a period of 90 days*

3.4. Antimicrobial activity

The antimicrobial activity of silver nanoparticle was evaluated using the disc diffusion method and growth inhabitation test. Pure slant culture of E-coli was used on agar plate. Two standard antibiotic disc, gentamycin and ciprofloxacin were also placed in each plate to be used as controlled antibiotics. The culture agar plates were incubated at 37^0C for 24 hours, after which zones of inhabitations were observed. These results indicate that silver nanoparticles are having stronger antimicrobial activity than standard antibiotic. Inhabitation of bacterial growth by silver nanoparticles can be elucidated by following possible mechanism. In general positively charged silver ion from nano particles are believed to become attach to the negatively charged bacterial cell wall resulting in its rapture, leading to denaturation of protein and finally cell death [34]. It is also possible that silver nanoparticles not only interact with the surface of membrane, but also penetrate inside the bacteria. Silver ions also interact with the DNA of bacteria, preventing cell reproduction.

4. CONCLUSION

Silver nanoparticles were successfully synthesized using fungi at room temperature. Synthesis of nanoparticles using this method was completed within 96 hrs. The color change occurs due to surface plasmon resonance during the reaction which is confirmed by XRD, FT-IR, UV-vis spectroscopy and zeta sizer. XRD confirms FCC crystal structure and FT-IR study ensures that amino acid residues have strong binding ability with silver silver nanoparticles. Antimicrobial study shows that silver has strong antimicrobial activity against E-coli pathogen. Furthermore, biosynthesized silver nanoparticles show good long term stability. The various physical parameters such as extract concentration, pH of extract, incubation period and incubation temperature plays an important role in synthesis of silver nanoparticles.

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