

CHARACTERIZATION OF BIOCHEMICALLY SYNTHESIZED SILVER NANOPARTICLES (AGNPS) USING *GANODERMA LUCIDUM* EXTRACT**Tuneer Khelkar¹, Ashish Saraf^{2*}, Kamlesh Kumar Shukla³ and Jasmeet Kaur Sohal⁴**^{1, 2, 4}School of Sciences, MATS University, Raipur, C.G, India³SoS in Biotechnology, Pt. Ravishankar Shukla University, Raipur, C.G, India**ABSTRACT**

“Green chemistry” is a simple and easily reproducible method that provides nanoparticles characterized by better stability and good dispersion in an aqueous solution. Nanoparticles can be synthesized by algae, bacteria, fungi, and plant extracts. *Ganoderma lucidum* is a commonly used medicinal mushroom with distinctive biological properties, such as antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, etc. In this study, aqueous mycelial extracts of *Ganoderma lucidum* were used to reduce AgNO_3 to form silver nanoparticles (AgNPs). The biosynthesized nanoparticles were characterized by UV-visible spectroscopy, fourier transform infrared spectroscopy, transmission electron microscopy, atomic absorption spectroscopy analysis and dynamic light scattering.

Keywords: AgNPs, characterization, *Ganoderma lucidum*, green chemistry

1. INTRODUCTION

Nanotechnology is one of the growing field because of its promising applications in the fields of information and communication technologies, biotechnology and medicine, optoelectronics and solar-cell. Nanotechnology is primarily concerned with the synthesis, characterization as well as exploration of different kinds of nanoparticles. The term nanoparticle can be used to describe a particle having size less than 100 nm. The word “nano” is derived from the Greek word which means dwarf or extremely small (Rai *et al.*, 2008). They have the tendency to react differently than their bulk counterparts due to their higher surface to volume ration, thus permitting them to be employed in novel applications (El-Nour *et al.*, 2010). The field of nanotechnology have grown rapidly especially from the last two decade, due to the ease of use of advanced characterization techniques as well as large number of synthesis methods for nanomaterials.

Silver nanoparticles have grabbed more attention, out of all the known metallic nanoparticles because of its unique physical, chemical and biological properties. One of the promising products in the nanotechnology industry are silver nanoparticles. Current research in nanotechnology focuses on the development of consistent processes for the synthesis of silver nanoparticles. Various literatures depict many ways for the synthesis of silver nanoparticles which include various types of physical, chemical, and biological methods. The physical and chemical methods used for the synthesis of nanoparticles are not only energy consuming but also non eco-friendly due to the use of toxic solvents and stringent techniques (Sohal *et al.*, 2017). Therefore in the past few years, green synthesis methods have replaced various physical and chemical methods for nanoparticle synthesis, to avoid toxicity of the process and increase quality. Biological methods used to synthesize nanoparticles comes under the principles of green chemistry, these methods does not use any harsh, toxic and expensive chemical substances. In recent years the biological methods based on microorganisms, whole plant or plant extracts have been demonstrated to be cost-effective and environmentally benign and yet produce highly stable nanoparticles.

Many fungi are reported in the synthesis of silver nanoparticles, and among these, the most studied are *Fusarium* (Husseiny *et al.*, 2015), *Penicillium* (Yassin *et al.*, 2021), endophyte *Talaromyces* sp. (Sharma *et al.*, 2022), and various *Aspergillus* species (Shahzad *et al.*, 2019; Lotfy *et al.*, 2021). Fungi are preferred for biogenic synthesis due to their high tolerance of metals, simple conditions for cultivation, good biomass production and to its ability to secrete large quantities of extracellular proteins that are involved in biosynthesis. Among species reported to be good candidates for the synthesis of nanoparticles, *Basidiomycetes* have also been mentioned, which are a group of macro fungi secreting extracellular enzymatic systems, proteins,

polysaccharides, and secondary metabolites with diverse functions. The species studied belong to *Pleurotus*, *Lentinus*, *Grifola*, and *Ganoderma* genera, with the properties of biogenic nanoparticles being closely related to the mushroom species used. (Jogaiah *et al.*, 2019; Al-Ansari *et al.*, 2020; Dat *et al.*, 2021; Chopra *et al.*, 2022; Dandapat *et al.*, 2022; Nguyen *et al.*, 2022; Sudheer *et al.*, 2022).

A major interest is to find evidence of the capacity of certain microorganisms, especially fungal strains, to synthesize nanoparticles in mild conditions with low costs. Furthermore, depending on the fungus used, specific parameters of fungal growth as well as the time required for actual biosynthesis could be decisive in the process and in further utilization of AgNPs. In this regard, our attention was focused on *Ganoderma lucidum*, a commonly used medicinal *Basidiomycete* with distinctive biological properties, such as antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, etc. In addition, the *Ganoderma* species biosynthesize silver and gold nanoparticles, an important feature for their use in medical applications where the nanoparticles must be compatible and obtained by non-toxic methods. AgNPs are synthesized by *Ganoderma* through an extra or intracellular mechanism, depending on the location where nanoparticles are synthesized. The characteristics of nanoparticles, namely size, morphological shape, and surface characteristics, influence their properties, playing a significant role in subsequent use.

In the present work, reflecting on the above-mentioned aspects, an attempt has been made to develop a low-cost, simple, and eco-friendly method for the microbial synthesis of AgNPs using *Ganoderma lucidum* extract. The nanoparticles were synthesized using fruit body extract from *Ganoderma lucidum* (GFAgNPs), and were characterized by analysis via UV-visible spectroscopy, FTIR spectra, DLS and TEM images. This study reveals that the biosynthesized silver nanoparticles from hot water extract from *G. lucidum*.

2. MATERIAL AND METHODS

Collection of Sample

Ganoderma lucidum was collected from Kanger valley National Park and regions of Bastar forest, Chhattisgarh, India. Identification of the collected sample was confirmed by Dr. Kamlesh Kumar Shukla, SoS in Biotechnology, Pt. R.S. University, Raipur, C.G.

Fungal Extract Preparation

After collection of the sample, fruiting body was washed several times with deionized water and dried at 40 °C in the oven for 3 days. The dried sample was grounded into powder form using mortar and pestle. 5 gram of powdered sample was extracted using water (200 ml) via Soxhlet extractor at 80 °C for 8 h. Thus obtained extract was filtered through Whatman No: 1 filter paper and then concentrated to 100 ml under 60 °C using rotary evaporator. The extract was stored at 4°C in the refrigerator until further use.

Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, 10 ml of mushroom extract was added into 150 ml of a conical flask containing 90 ml of 1 mM silver nitrate (AgNO₃) solution and incubated at 60 °C in dark, also the stirring of the reaction solution was done in a different time interval. The consequent reduction of silver ions (Ag⁺) was monitored periodically for 24 h. After 4 hours of incubation, the color of the reaction mixture changed from light yellow to pale yellow color, further the color was changed into dark brown indicating the formation of AgNPs (Gurunathan *et al.*, 2013).

Purification of Silver Nanoparticles

The GFAgNPs synthesized were collected by centrifugation at 10,000 rpm for 30 min at 4°C. The clear supernatant was discarded and the pellet of colloidal silver was washed three times with double distilled water to remove impurities and the unbound extract components. Finally, GFAgNPs were dried at 60°C in the hot air oven and were used for further characterization.

3. Characterization of Synthesized GFAGNPs

3.1 Ultraviolet-Visible Spectroscopic Analysis

UV-Vis spectrum analysis of the reaction medium was done to monitor the bioreduction of silver ions by aqueous extract of *Ganoderma lucidum*, which was performed by using UV-Vis spectrophotometer (Systronics, Double beam spectrophotometer, 2203) and the absorption maximum was scanned by doing spectrum scan between the wavelength of 200-500 nm. Colloidal suspension of the synthesized GFAGNPs (2 ml) was taken in the quartz cuvette in order to measure the absorption and detecting the surface Plasmon resonance.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

The extract before and after the formation of the GFAGNPs was examined for the presence of different functional groups responsible for the synthesis and stabilization of AgNPs, using Fourier Transform Infrared Spectroscopy (FTIR).

3.3 Transmission Electron Microscopy (TEM)

The studies on size and morphology of GFAGNPs were performed by transmission electron microscopy. For preparing transmission electron microscope (TEM) samples a drop of dispersed GFAGNPs solution was placed onto carboncoated copper grid. The micrographs were obtained on TECNAI G2 Spirit (FEI, Netherland) equipped with Gatan digital camera operated at an accelerating voltage at 80 kV.

3.4 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopic analysis was done to monitor the conversion of Ag^+ present in silver nitrate solution to AgNPs where aqueous extract of *Ganoderma lucidum* was the reducing agent, which was performed by using Atomic absorption spectroscope (Perkin Elmer PinAAcle 990F). The AAS analysis was done by withdrawing reaction samples at various time intervals of the reaction. The reaction samples were then centrifuged and supernatant was analyzed because Ag^+ present in silver nitrate solution are very smaller and hence cannot be separated on centrifugation, where as the AgNPs are in 0 valent state which can be separated on centrifugation around 15000 – 17000 rpm.

3.5 Dynamic Light Scattering (DLS)

The average hydrodynamic diameter and bio-molecular stability of the synthesized GFAGNPs in solution was analyzed by DLS particle size analyzer [Nano-ZSP instrument (Malvern Instruments, UK)]. Prior to DLS measurements the synthesized GFAGNPs were subjected to centrifugation to get rid of surplus reducing agents. In a quartz cuvette, 3 ml of the synthesized GFAGNPs was taken and the measurements were taken by intensity. To determine the stability surface charge of GFAGNPs was measured.

4. Result and Discussion

Central part of the India is rich in diversity and climatic conditions prevalent in the state have made it a natural habitat for large number of mushroom species. The present investigation was undertaken with the aim to synthesized silver nanoparticles using hot water extract of *Ganoderma lucidum* fruit body. To this end, extensive and regular surveys were conducted in important forests and tribal areas of Chhattisgarh during the monsoon season.

4.1. Spectroscopic Analysis of Synthesized GLAGNPs

In the present study, various extracts of *Ganoderma* sp. were prepared in different solvents. Water has a polar arrangement of oxygen and hydrogen, this property makes water capable of dissolving most of the substances as compared to other solvents. In this work also, when various extracts were mixed with different concentrations of *Ganoderma* extract and AgNO_3 solution, incubated at room temperature for 1 hour, only hot water extract from fruit body of test mushroom was able to synthesize AgNPs. It was evident from change of color from yellow to light brown and later dark brown (Fig.1), indicating the formation of AgNPs.

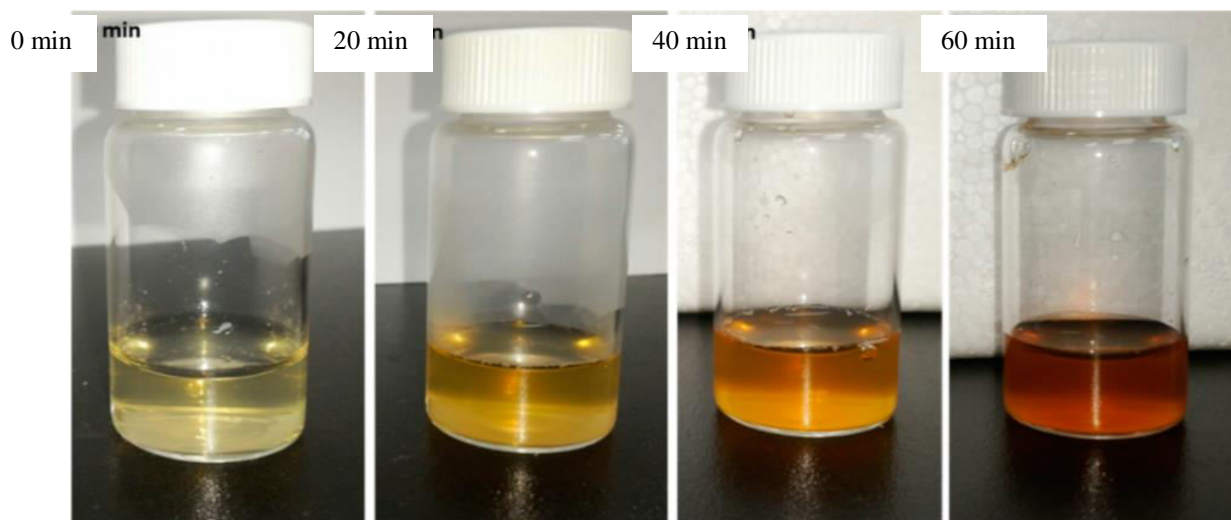


Fig. 1. Time dependent variations in the colour of colloidal solution of silver nanoparticles synthesized using aqueous extract of *Ganoderma lucidum*

Change in color was attributed to the surface Plasmon resonance (Ahmad *et al.*, 2003). The colloidal silver nanoparticles are dispersed in water and the PL emission spectra are recorded for the excitation wavelength at 430 nm (Fig.2). Whereas, control set showed no change in color and did not show any absorption at this wavelengths (430 nm). The purified silver nanoparticles were subjected to various sophisticated analysis for characterization.

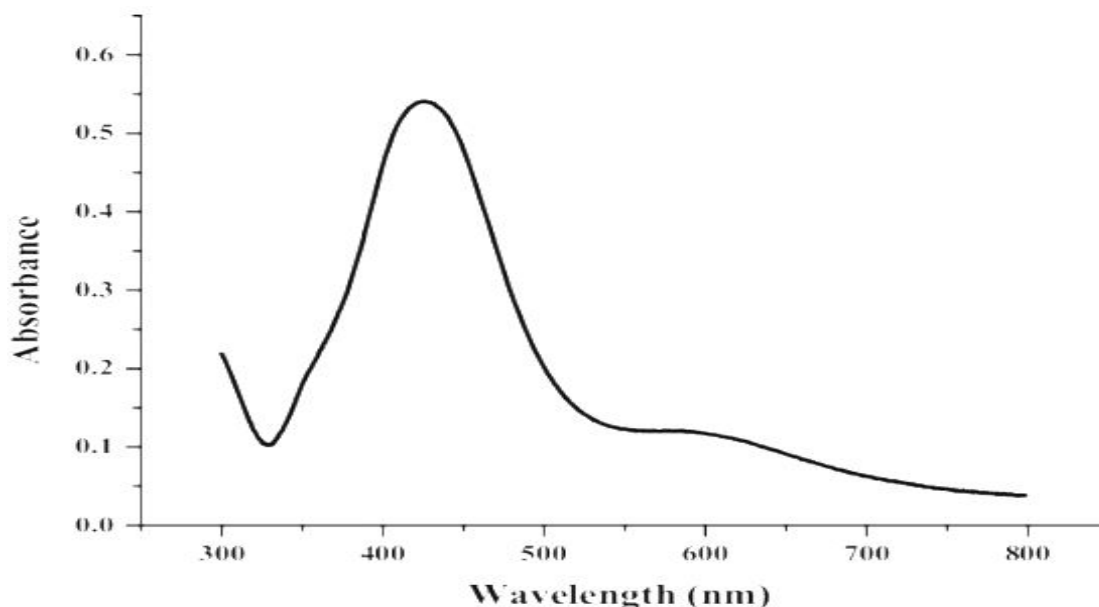


Fig. 2. Spectrophotometric determination of AgNPs synthesized from Hot Water Extract of *Ganoderma lucidum*

4.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a frequently employed technique for identifying the functional groups found in both pure substances and mixtures. The interaction between AgNPs and the functional groups of *Ganoderma lucidum* fruit body extract was investigated using FTIR characterization. The FT-IR result reveals that some functional groups are shared by both solutions, either at comparable locations or with minor alterations. These are the groups that prevent NPs from clumping and serve as a capping agent. Absorbance bands in Fig.3 are observed in the region of 450-4000 cm^{-1} are 3366, 2933, 2123, 1633, 1423, 1372, 1251 and 1026 cm^{-1} . These absorbance bands of the FTIR

spectrum are identified to be allied with the stretching vibrations for O-H (hydrogen bonded alcohols and phenols), C-H (Alkanes), C≡C (Alkynes), C=C (Alkenes), C-H (Alkanes), C-H (Alkanes), C-N (Amines, Amides) and C-F (Aliphatic fluoro compounds) respectively which proves the presence of phenolic and polyphenolic compounds such as flavonoids, terpenoids etc and proteins (Field et al., 2012) and disappearance of these band after bio-reduction (Fig. 3) gives confirmation for the participation of these phytoconstituents in the formation and stabilization of AgNPs.

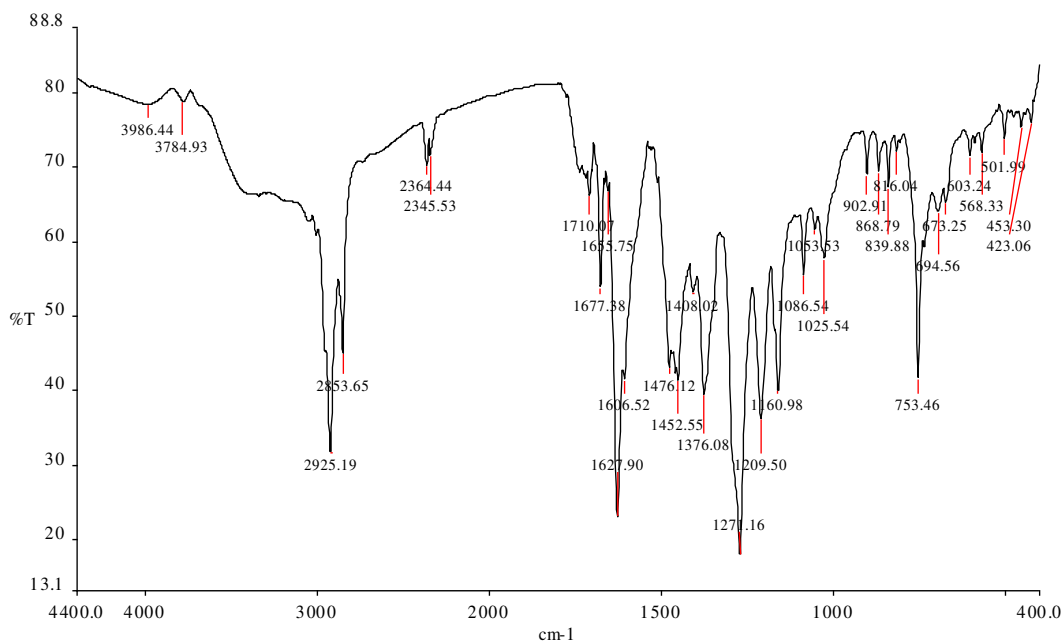


Fig. 3.: FT-IR spectra of biochemically synthesized silver nanoparticles from *Ganoderma lucidum* fruit body extract

The absorption peak at 1107.57 cm⁻¹ can be associated with Ester linkages. The peak at 1421.22 cm⁻¹ represents lignin and cellulose. The presence of amide I group is confirmed by the peak at 1637.72 cm⁻¹. Likewise, the presence of proteins, carbohydrates, flavonoids and tannins is evident from absorption peak at 3446.58 cm⁻¹. These peaks were also present with minor changes in the position in the spectrum of solution containing AuNPs which confirmed that the carbonyl group and peptides might have formed a layer on the AuNPs and might be responsible for reduction of the Au ions to atoms (Philip, 2009; Bhat *et al.*, 2013). These groups seem to have prevented agglomeration of the NPs making them stable. The results are supported by the findings of Bankura *et al.* (2012) and Dubey *et al.* (2019). In their study, some functional groups present in *A. bisporus* extracts were found to be involved in the synthesis and stabilization of silver and gold NPs.

4.3. Transmission Electron Microscopic Analysis (TEM)

TEM analysis provided details about the morphology and size of the synthesized GFAGNPs. The GFAGNPs formed was found to have an average size of 60.6 nm and spherical in shape and capped by mushroom extract constituents that prevented their aggregation. Silver nanoparticles of similar size were prepared by flower extract of *Rhododendron dauricum* (Mittal et al., 2013). Natural capping offers supplementary advantage of the stability in the synthesis through green chemistry route as shown in Fig. 4. This stability is attributed to the phytoconstituents present in the extract, these results are in consensus with the reports by Ahmad *et al.*, 2012.

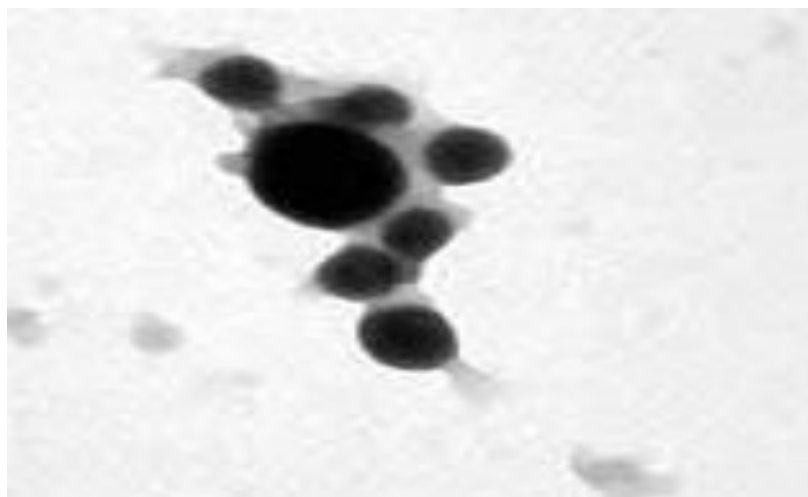


Fig.4. TEM image of biochemically synthesized silver nano particles GFAGNPs

4.4. Atomic Absorption Spectroscopic Analysis (AAS):

Silver ion concentration was analyzed by AAS which showed the conversion of Ag^+ ions into Ag^0 nanoparticles. Initially, a standard solution of 10 ppm of AgNO_3 was prepared and analyzed with AAS at 0 min. The Ag^+ ion concentration in the reaction solution, after adding *Ganoderma* fruit body extracts was monitored at different time intervals. The result showed a decrease in concentration of Ag^+ ions with reaction time, indicating the complete conversion of Ag^+ ions into Ag^0 nanoparticles at 40 mins of reaction time at 75°C

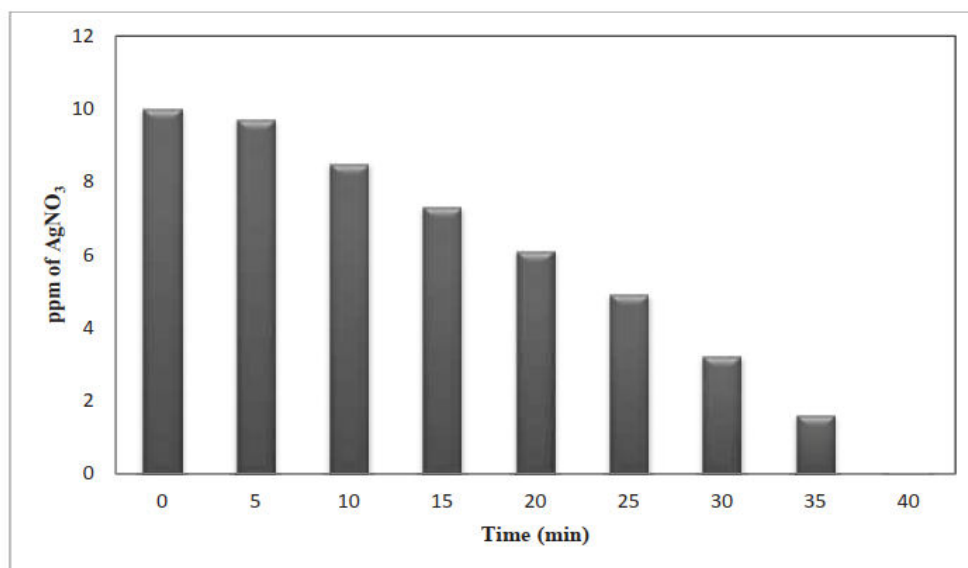
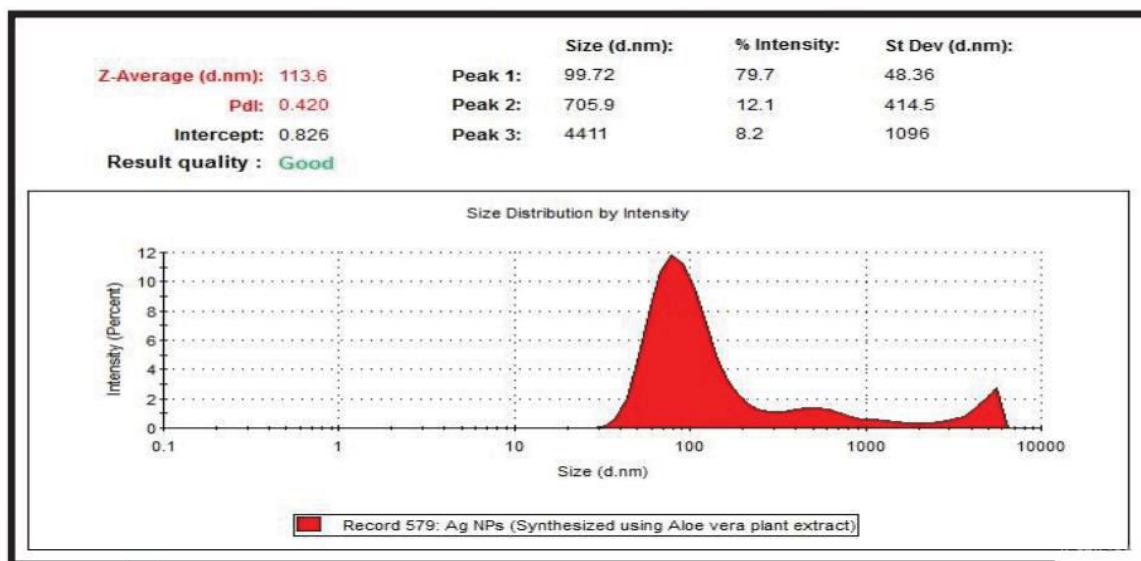


Fig. 5.: AAS analysis of silver nitrate (ppm)with reaction time (min) using *Ganoderma lucidum* fruit body extracts

4.5. Dynamic Light Scattering Analysis (DLS)

Dynamic light scattering analysis quantify the size distributions and more precise quantity of monodispersity of AgNPs in colloidal solutions. The particle size distribution (PSD) by intensity of the synthesized GFAGNPs is shown in Fig. 6. The average

hydrodynamic magnitude of biochemically synthesized AgNPs was found to be 103.2 nm and PDI was found to be 0.416 which indicates good monodispersity.



5. CONCLUSION

Study reports the single step process for the biological synthesis of silver nanoparticles using aqueous extract *Genoderma lucidum* which comes up with ecofriendly, easy and proficient method for the synthesis of innocuous nanoparticles. When the mushroom extract was mixed with AgNO_3 in the ratio 1:4 and incubated at 75°C for 40 minutes at neutral pH, its color changed to yellowish brown, indicating the formation of AgNPs, this is preliminary identification of AgNPs formation. Change in color was due to the excitation of surface plasmon vibrations in metal nanoparticles. Reaction medium containing AgNPs was subjected to UV-Vis spectral analysis where it showed sharp absorbance at 440 nm which is specific for AgNPs. The solo step process for nanoparticles synthesis is extremely appropriate for large scale production as it is very fast and eliminates the complex processes employed in protocols based on other biological systems.

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