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AN ECO-FRIENDLY ENZYMATIC APPROACH TO SILVER NANOPARTICLE SYNTHESIS

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ABSTRACT

The synthesis of metallic nanoparticles (NPs) using eco-friendly green synthesis techniques is a promising alternative over chemical synthesis. The present study aims to develop sustainable and environment friendly approach for the synthesis of silver nanoparticle using extracellular lignin peroxidase enzyme from *Purpureocillium lilacinum (LP8i)*. It has been proposed that a variety of proteins and enzymes are important for the creation of silver nanoparticles (Ag-NPs) in fungi and bacteria. Partially purified lignin peroxidase from *Purpureocillium lilacinum (LPi)* released enzyme extract. The specific activity of the partly purified fraction is 126.61U/mg. XRD revealed face catered cubic (FCC) AgNPs with an average size of 84 nm after partially purified enzyme was exposed to silver nitrate solution. Synthesized Ag nanoparticles were characterized by UV-DRS, FTIR, FESEM- EDS, and XRD. This report describes the reduction of extracellular silver ions by fungal lignin peroxidase purified from *Purpureocillium lilacinum* (LPi).

Introduction:

Lignin peroxidase (LiP) is hydrogen-peroxide dependent heme-containing enzyme. Originally it was characterized for its ability to cleave the complex aromatic polymer lignin. In lignin peroxidase catalytic cycle H_2O_2 oxidizes the ferric heme to higher oxidation states that generate short- live radical species capable of one-electron oxidation of non-phenolic aromatic substances, this high redox potential and radical chemistry have been reused in nano-material synthesis to reduce metal ions (Anil Kumar Singh et.al 2023).

In recent studies researchers have demonstrated that the enzyme mediated formation of metallic nanoparticles; notably silver and copper. Enzymatic synthesis of metallic nanomaterial has several advantages over classical chemical methods. Enzymes operate at ambient temperature and acidic to neutral pH, avoid harsh use of reducing agent and stabilizers. Enzymes can impart biological capping, and enzyme catalyzes radical or electron transfer step to give control over nucleation and growth leading to narrow size and distinct morphology (Richa sing et. al. 2017). Singh et.al reported that extracellular LiP from *Acinetobacter sp.* Catalyzing reduction of Ag^+ to Ag^0 nanoparticles. This research provides one of the earliest experimental proofs that LiP can directly mediate metallic nanoparticle formation under mild conditions.

Sefa Nur et.al. (2025) prepare silver nanoparticle using Lignin peroxidase from *Caldibacillus thermoamylavoran,* which were cultivated on waste walnut shell, which are rich in lignin. *Caldibacillus thermoamylavoran,* is a thermophilic bacterium showing highest lignin peroxidase activity. *Caldibacillus thermoamylavoran,* produces extracellular lignin peroxidase, supernatant obtain after growth and centrifugation was used for synthesis of Ah nanoparticles using $AgNO_3$ solution. Synthesized nanoparticles were characterized by UV-DRS, FTIR, SEM-EDS, XRD. Lignin peroxidase mediated nanoparticle has broad spectrum antimicrobial activity.

Materials and Methods:

Materials

1. Partially purified lignin peroxidase obtained from *Purpureocillium lilacinum.*
2. Silver nitrate
3. Cetyltrimethyl ammonium bromide
4. Hexane

Methods

Silver nanoparticle synthesis using partially purified lignin peroxidase:

The synthesis of silver nanoparticle was carried out by challenging 0.2ml partially purified lignin peroxidase enzyme with 3ml – 1mM solution of silver nitrate. Reaction was carried out pH-3 and 20^0C temperature. After visible colour change, the spectrum was measured over the wavelength range 200-800 nm after regular interval od 24 hours. This initial small-scale synthesis was carried out to confirmed synthesis of silver nanoparticle but monitoring their characteristic surface plasmon response band (SPR) (using instrument UV- VIS Spectrophotometer by Shimadzu), which appears as an absorbance peak at specific wavelength (Richa singh et. al 2017).

After confirmation of silver nanoparticle using lignin peroxidase same experiment was carried out for large amount of silver nanoparticle synthesis. The synthesized silver nanoparticles were harvested using CTAB (0.2 mM) and hexane (35%) in 1:2 proportion. Resulting pellet was collected wash with distilled water to remove impurities from powdered particles and dried by heating at 60°C to obtain powder.

AgNP nanoparticle powder was used for nanoparticle characterization using Ultra Violet Diffuse- Reflectance spectroscopy (UV-DRS), Fourier-Transform Infrared spectroscopy (FTIR, Field Emission Scanning Electron Microscopy (FESEM), Energy – Dispersive X-ray spectroscopy (EDS), X-Ray Diffraction (XRD).

Results and Discussion:

Visible Observation: From visible observation of color change, by comparing with the control; indicates that nanoparticle formation has occurred. Control tube only contains $AgNO_3$, so there is no color change over time of periods of four days.

After 4 hours of incubation the brown coloration suggests the initial formation of silver nanoparticle (AgNPs). Silver ions (Ag+) are being reduced to metallic silver (Ag^0). The brown color due to surface plasmon response (SPR) of AgNPs collective oscillation of conduction of electrons when interacts with light.

Over the time of incubation of four days color changes from brown to gray with sedimentation at bottom. It indicates maximum Ag^+ ions were reduced to Ag^0; particle size may increase, nanoparticle agglomerate, causes sedimentation and formation of large metallic clusters. (Figure 1)

Figure 1: Visible colr change in synthesis of silver nanoparticle

UV-Visible spectroscopy:

After 4-5 hours of incubation color change was observed and sample was used for UV-Visible spectroscopy. UV-Visible spectroscopy was done using UV- VIS Spectrophotometer by Shimadzu. The optical property of synthesized silver shows that the absorption peak was observed at 318nm. The band gap was calculated and found to be 3.89ev. it indicates that synthesized nanoparticles may possess good photocatalytic activity (Figure :2)

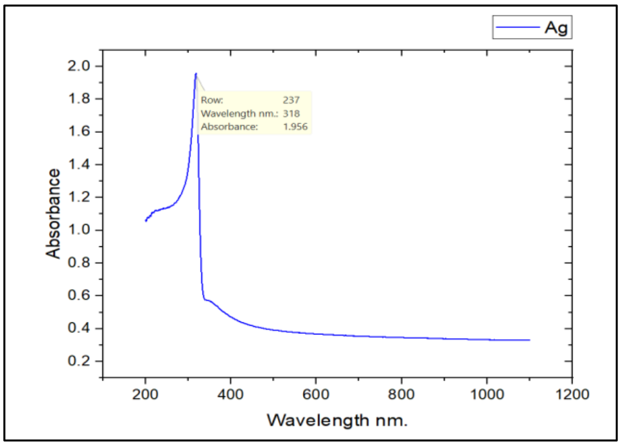


Figure :2 UV- Visible spectra of Ag Nanoparticle

Ultra Violet Diffuse- Reflectance spectroscopy (UV-DRS)

At 318 nm a dip surface plasmon response appears because a collective oscillation of electron conduction in metallic silver nanoparticle when sample was excited by light. AgNPs surface plasmon response typically occurs between 300 nm to 450 nm. Position of SPR peak depends upon particle size, as small size particle SPR occurs at short wavelength, it also depends upon shape, aggregation, and dielectric environment i.e. capping agent present in surrounding. In present study SPR occurs at 318nm, reflectance decreases sharply and increase gradually indicates minimal absorption and typical Ag^0 nanoparticle synthesis (Figure 3).

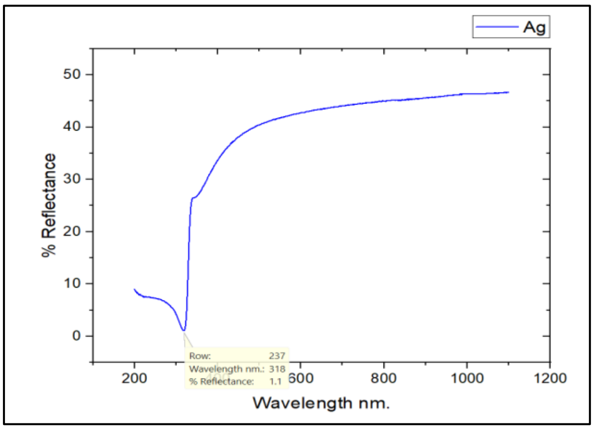


Figure 3. Ultra Violet Diffuse- Reflectance spectroscopy (UV-DRS) of AgNPs

Fourier-Transform Infrared spectroscopy (FTIR): Elemental silver (Ag) is a metal and does not exhibit significant infrared absorption bands in the typical FTIR range (400-4000 cm^{-1}). Nanoparticle material shows absorption band above 500nm, Indicating interactions with visible or UV light and biomolecules. So, for confirmation of synthesis of Ag nanoparticle using lignin peroxidase shows FTIR peaks at 3300, peak around 1630 -1650 per cm, peak near 3500-1400 and 1000 – 1100 per cm as well as low wave number peak is preset in 500-600 per cm.

Broad peak at 3300 per cm indicated there is presence of O-H /N-H stretching of Proteins. It indicates silver nanoparticles are capped by biomolecules. Peak around 1630-1650 per cm indicates presence of C=O from lignin peroxidase enzyme. It is evidence for lignin peroxidase binds to nanoparticle surface. Peaks at 1350-1400 per cm and 1000-1100 per cm shows the presence of C-N, C-O or aromatic C-C vibrations, it indicates that organic stabilizers are attached with nanoparticle. Peak at low wavenumber i.e. 500-600 per cm indicates lignin peroxidase enzyme is interacting with silver.

In conclusion, we can say a broad band peak at 3331 per cm, Strong amide or aromatic signals at 1638,1356,1097 per cm and band near 513 and 476 per cm, these are fully consistent with AgNPs capped by lignin peroxidase enzyme (Figure 4).

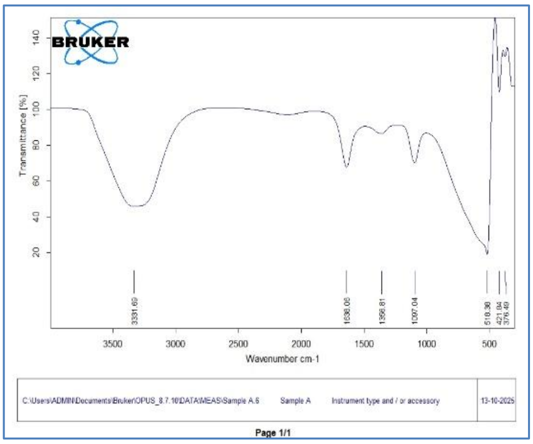


Figure 4: FTIR of silver nanoparticles.

Field Emission Scanning Electron Microscopy (FESEM): The surface structure and shape of the Ag NPs sample were studied using FESEM. Figure shows SEM images of the samples at different magnifications. the material appears as clumped or agglomerated structures. The images showed that most of the particles are spherical and evenly distributed, which is typical for silver nanoparticles prepared through both chemical and biological methods (P, J., V, G., et. al. 2024). Although some particles tended to stick together, the majority remained well-separated, indicating that the synthesis and stabilization process was effective. A few clusters were seen, which is expected because metallic nanoparticles naturally tend to clump together due to van der Waals forces. However, the overall size distribution was narrow, reflecting the good quality of the synthesis (Rana N et. al 2024) (Figure 5.)

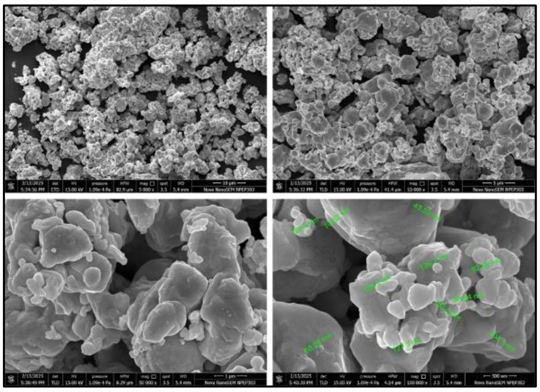
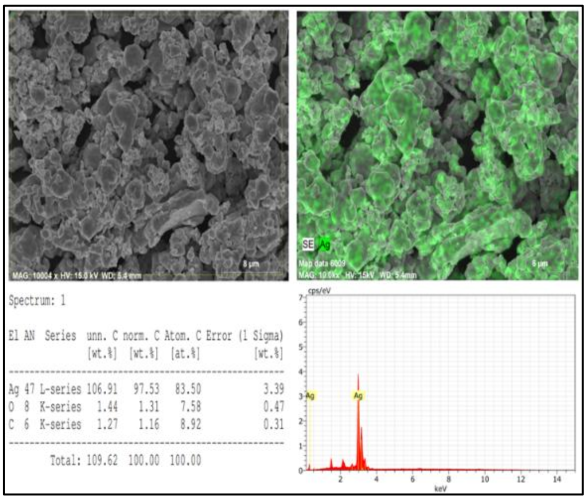


Figure 5: FE-SEM of silver nanoparticles.

Energy-Dispersive X-ray spectroscopy (EDS): The elemental composition of the silver nanoparticles was further confirmed using Energy Dispersive Spectroscopy (EDS) coupled with FESEM. The spectrum showed a strong peak between 2–4 keV, which corresponds to silver, verifying that the nanoparticles are pure and free from major contaminants. No significant peaks from other elements were detected, confirming their high purity. (D. Kumar 2022). Minor signals of carbon were sometimes observed, but these are commonly due to the carbon-coated sample grids support used during analysis, rather than being true impurities in the nanoparticles. (M. Puchalski et. al 2007). The strong silver signal further confirms the successful synthesis of high-purity silver nanoparticles, in agreement with findings reported in recent literature (Figure 6)



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Spectrum: 1

El AN  Series   unn. C norm. C Atom. C Error (1 Sigma)
                [wt.%]  [wt.%]  [at.%]          [wt.%]
------------------------------------------------------
Ag 47 L-series 106.91   97.53   83.50            3.39
O   8 K-series   1.44    1.31    7.58            0.47
C   6 K-series   1.27    1.16    8.92            0.31
------------------------------------------------------
        Total: 109.62  100.00  100.00
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Figure 6: Energy – Dispersive X-ray spectroscopy (EDS) of silver nanoparticles.

X-Ray Diffraction (XRD): The crystal structure and phase purity of the synthesized silver nanoparticles (Ag NPs) were examined using X-ray diffraction (XRD). The diffraction pattern, as shown in Figure, displays characteristic peaks at 2θ values of 38.51°, 44.69°, 64.83°, 77.76°, and 81.90°, which correspond to the (111), (200), (220), (311), and (222) crystallographic planes, respectively. These peak positions and their relative intensities closely match the standard data for face-centered cubic (FCC) silver, as given in the *JCPDS card no. 04-0783*. The intense (111) reflection indicates a preferred orientation along this plane; a feature commonly observed in Ag nanoparticle systems. (Ali MH et. al 2023). No additional peaks were detected, confirming the absence of secondary phases or oxide impurities in the sample. The sharp and intense nature of the peaks reflects the high crystallinity of the synthesized nanoparticles.

The average crystallite size of the Ag NPs was calculated using the Debye–Scherrer equation applied to the main diffraction peaks. The resulting average size was found to be approximately 22.22 nm, which is consistent with nanocrystalline Ag reported in recent studies. The agreement between observed and standard JCPDS patterns, as well as the calculated crystallite size, further supports the successful formation of pure, crystalline silver nanoparticles. (Ali, E.M.et al 2025).

Instrument parameters followed standard protocols: XRD measurements were performed using Cu Kα radiation (λ = 1.5406 Å), with a scan range from 10° to 90° 2θ, and a scan speed of 2°/min. The obtained data provide strong evidence for the successful synthesis of FCC-structured, phase-pure Ag nanoparticles with an average crystallite size in the nanometre regime. (T. M. Gesing et. al

2024).

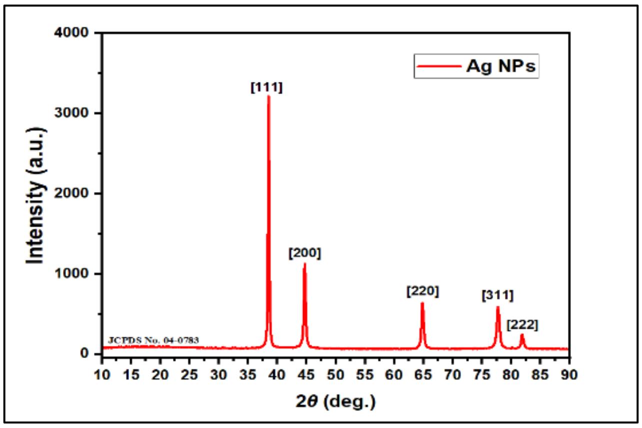

Figure 7: X-Ray Diffraction (XRD) of silver nanoparticle.

Conclusions

UV-Visible spectroscopy confirms successful synthesis of stable silver nanoparticle with band gap 3.89 eV.The UV-DRS spectrum confirms successful synthesis of metallic silver nanoparticle 3.89 eV. SPR at 318 indicates small particle size of nanoparticle with good metallic properties. SEM analysis report surface morphology of synthesized nanoparticle. Surface morphology shows cubic nature of silver nanoparticle. Average particle size from SEM image was found to be **84 nm**. The spectrum shows that the nanoparticles are pure and free from major contaminants. The XRD analysis reports that peak positions and their relative intensities closely match the standard data for face-centred cubic (FCC) silver, as given in the *JCPDS card no. 04-0783*. The sharp and intense nature of the peaks reflects the high crystallinity of the synthesized nanoparticles. Using the Debye–Scherrer equation average size was found to be approximately 22.22 nm

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