

Synthesis and Characterization of Silver Nanoparticles by Escherichia Coli

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ABSTRACT

This work investigates the environmentally friendly biosynthesis of AgNPs utilizing Escherichia coli. The bacterial strain was grown and then subjected to silver nitrate (AgNO₃) for nanoparticle production in both the supernatant and pellet. As a result of surface plasmon resonance, the hue changed from light yellow to brown, confirming that AgNPs had been formed. The nanoparticles underwent X-ray diffraction and scanning electron microscopy characterization after being centrifuged to separate them. SEM showed spherical particles 5–25 nm in size, while XRD verified a crystalline structure with an average size of 118 nm. The work highlights the potential nanotechnology uses of proteins by emphasizing their function in stabilizing AgNPs.

Keywords: Silver nanoparaticles, Escherichia coli, Synthesis, Pellet, Supernatant

1.INTRODUCTION

The creation and use of nanoparticles have propelled nanotechnology to the forefront of scientific inquiry as a whole in the last few years. Silver nanoparticles (AgNPs) stand out among the others because of their exceptional physicochemical features, wide-ranging antibacterial action, and many potential uses in fields as diverse as medicine, biology, ecology, and electronics. Avoiding the environmental concerns linked with traditional chemical and physical procedures has made the development of sustainable and environmentally friendly ways for synthesizing AgNPs all the more crucial. An efficient, green, and cost-effective alternative to conventional nanoparticle manufacturing is the biosynthesis of silver nanoparticles via the use of various biological systems, such as microbes and plants. In this regard, the Gram-negative bacteria Escherichia coli (E. coli) stands out as a perfect model organism for the synthesis of nanoparticles because of its adaptability, simplicity of genetic engineering, and capacity for extracellular nanoparticle production.

Multiple metabolic processes are involved in the complicated production of silver nanoparticles by E. coli. The process of reducing silver ions to metallic silver is mostly controlled by the bacterium's own enzymes and proteins. Enzymes like nitrate reductase are essential for the reduction process because they help turn silver ions into silver nanoparticles by donating electrons to them. Bacterial metabolites and proteins may also function as capping agents, keeping the nanoparticles from clumping together and stabilizing them. As an additional tool for nanoparticle stabilization,

Escherichia coli's extracellular polysaccharides serve as a matrix for the synthesis and distribution of AgNPs in the ambient medium.

Environmental factors, including temperature, pH, and concentration of silver ions, also have a significant role in determining the biogenesis of silver nanoparticles. The nanoparticles' size, shape, and dispersion may be drastically altered by adjusting these factors. A few examples include how changes in temperature may hasten the reduction process and produce smaller nanoparticles and how changes in medium pH can affect the stability and surface charge of the nanoparticles. Scientists may create nanoparticles with precisely the right characteristics for their uses by fine-tuning the synthesis process under these circumstances.

To comprehend their characteristics and behavior in various settings, it is essential to characterize the E. coli-synthesised silver nanoparticles. Dimensions, form, surface morphology, and crystallinity of the produced nanoparticles are ascertained by means of a number of analytical procedures. Nanoparticles may be seen at the nanoscale using imaging techniques like scanning electron microscopy and transmission electron microscopy, and their hydrodynamic size and distribution in suspension can be measured using dynamic light scattering. The crystalline nature of the nanoparticles can be confirmed through X-ray diffraction analysis, and the optical properties of the AgNPs, including their characteristic surface plasmon resonance peak, which usually occurs between 400 and 450 nm, can be monitored through UV-visible spectroscopy.

Another essential approach for identifying the functional groups on the surface of the nanoparticles and the

biomolecules involved in stabilizing and capping them is Fourier-transform infrared spectroscopy. Further analysis of the nanoparticles' elemental composition and confirmation of silver presence may be achieved by combining scanning electron microscopy or transmission electron microscopy with energy-dispersive X-ray spectroscopy. When taken as a whole, these characterisation methods give light on the structural and functional characteristics of *E. coli*-synthesised silver nanoparticles, illumination that is crucial for assessing their prospective uses.

Due to their exceptional antibacterial characteristics, the biosynthesized silver nanoparticles from *E. coli* have shown tremendous promise in several domains. Because of their extensive research on antimicrobial, antifungal, and antiviral properties, AgNPs are a good choice for use in wound dressings, medical devices, and coatings for surfaces that are susceptible to contamination by microbes. Furthermore, the use of silver nanoparticles in cancer treatment, medication delivery systems, and biosensing has been investigated. When it comes to healthcare applications, biosynthesized AgNPs are very desirable due to their low toxicity and biocompatibility.

Additionally, silver nanoparticles may be used for purposes other than medicine. Their antimicrobial characteristics aid in the disinfection of polluted water and the elimination of dangerous contaminants, making them useful in environmental remediation activities including water treatment and pollution control. Another example of AgNPs' adaptability and economic importance is their use in the textile, electronics, and catalysis industries.

II. REVIEW OF LITERATURE

Kareem, Ekhlas et al., (2023) Because it can reduce metals to the nanoscale, altering their physical, chemical, and biological properties in comparison to their bulk counterparts, nanotechnology has attracted a lot of attention this century. Physical, chemical, and biological methods are used to produce nanoparticles. There has been a rise in interest in biological synthesis by plants as a solution to the problems with physical and chemical synthesis methods, such as the production of harmful by-products and the high energy consumption caused by the use of high temperatures, pressures, powers, and dangerous compounds. A new field known as "green nanotechnology" has emerged as a result of scientists combining nanotechnology with the concept of sustainability.

Hussain, Zawar et al., (2023) The antibacterial activity of silver nanoparticles (AgNPs) created biologically was a

major factor in the medicinal application of nanotechnology. Using the antibacterial characteristics and harvesting *Mentha piperita* leaves, scientists looked into an efficient and eco-friendly method of making silver nanoparticles. We used XRD, SEM, and UV Visible Spectroscopy for characterization after producing AgNPs from mint plant extract using a green synthesis approach.

Egodawaththa, Nishal M et al., (2022) As an antimicrobial, silver has been around for a long time. Conjugating chemical ligands with silver nanoparticles allows for the synthesis of antimicrobial nanoparticles with improved pharmacodynamic properties and reduced toxicity. Based on dynamic light scattering (DLS), the typical diameters of the nanoparticles were determined to be 25-278 nm. They looked at spherical and nanotube-like shapes using scanning and transmission electron microscopy.

Xuan Hoa, Vu et al., (2018) In order to create the colloidal silver solution, sodium borohydride was used to reduce silver nitrate, and starch was added as a stabilizing ingredient. Using ultraviolet-visible (UV-Vis), Fourier transform-infrared (FTIR), and transmission electron microscopy (TEM) spectroscopy, the optical characteristics and size of the produced AgNPs were thoroughly examined. Additionally, the impact of various factors on AgNPs was examined. The findings demonstrated that the produced spherical silver nanoparticles were of a certain size and could dissolve in water.

Gurunathan, Sangiliyandi et al., (2009) Nanoscience and nanotechnology are seeing rapid growth in the field of applications involving materials and structures on the nanoscale, defined here as 1–100 nm. Solar energy conversion, catalysis, medicine, and water treatment are just a few areas where nanomaterials show promise in addressing technical and environmental issues. A significant problem now is the creation of methods for the controlled synthesis of nanoparticles with well specified size, shape, and composition for applications in biomedicine and other fields like optics and electronics. In order to advance the area of nanotechnology applications, it is crucial to create safe and environmentally acceptable methods for synthesizing metallic nanoparticles. Using "natural factories," like biological systems, is one way to accomplish this goal. This paper details the best conditions for maximizing the production of AgNPs by reducing Ag(+) ions in the *Escherichia coli* culture supernatant.

III.MATERIALS AND METHODS

Production of biomass

The culture library of the School of Biosciences was used to get *Escherichia coli* (*E. coli*). To cultivate biomass, the bacterial strain was grown in nutrient broth. At 100 rpm, the culture was placed on a rotary shaker for incubation. Centrifugation at 12,000 rpm for 10 minutes was used to extract the biomass after 24 hours of growth. Silver nanoparticle production required the collection of both the supernatant and the pellet.

Synthesis of silver nanoparticles

To the reaction flask, which already contained silver nitrate (AgNO_3) at a concentration of 10-3 M (1% v/v), the collected supernatant was added independently. In addition, AgNO_3 was thoroughly combined with the pellet. Under intense lighting, the supernatant and pellet reacted with Ag^+ ions over 24 hours. After 24 hours, the color changed from yellowish white to brown, indicating that silver nanoparticles had been synthesized.

Separation of silver nanoparticles

Centrifugation at 12,000 rpm for 10 minutes separated the synthesized silver nanoparticles. We kept the pellet we made at -40 degrees Celsius.

Techniques for Characterization of silver nanoparticles using *E. coli*

To comprehend and manage nanoparticle production and uses, characterization of nanoparticles is crucial. Many methods, including powder X-ray diffractometry and scanning electron microscopy are used for characterization.

IV.RESULTS AND DISCUSSIONS

Within 24 hours of inoculation, the reaction mixture's color changes from light yellow to brown, indicating the production of silver nanoparticles by pellet and supernatant cultures. It is well-known that when metal nanoparticles are excited to vibrate their surface plasmons, they take on a yellowish-brown hue in water. The distinctive stimulation of surface plasmon vibrations in silver nanoparticles is responsible for the color shift. The dipole oscillation that results from coupling a visible-range electromagnetic field to the collective oscillations of conduction electrons is the source of surface plasmon vibrations.

Figure 1 displays the outcome. There is no visible change in color when the control without silver nitrate is incubated under the same conditions. We still don't know much about

how to reduce silver ions and make silver nanoparticles. In the creation of silver nanoparticles, protein molecules and enzymes like nitrate reductase serve as effective regulators.

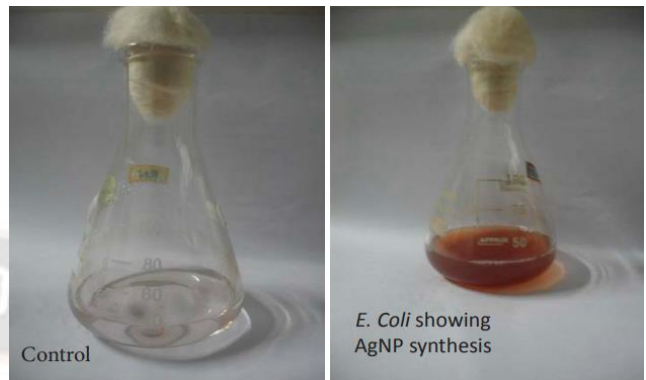


Figure 1: Synthesis of nanoparticle using *E. coli*

Characterization of Silver Nanoparticles using *E. coli*

XRD pattern

Figure 2 displays the XRD pattern of silver nanoparticles synthesized from *E. coli* supernatant. At $2\theta=32.18$, there is a distinct and strong peak that corresponds to the diffraction plane of silver with the fcc lattice. The Scherer formula tells us that the silver nanoparticles' average crystal size (t) may be calculated using the $\text{Cu K}\alpha$ radiation's X-ray wavelength ($\lambda = 1.54\text{\AA}$), Bragg's angle θ , and the maximum radian width of the peak at half height (β). The synthesized nanoparticles' predicted particle sizes are listed in Table 1.

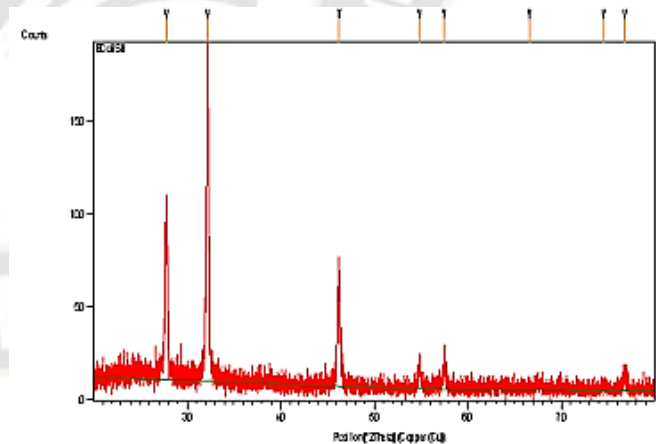


Figure 2: Use of XRD pattern for characterization of silver nanoparticles synthesized from *E. coli* supernatant

Table 1. Debye Scherrer's equation for crystalline size determination of silver nanoparticles

2	θ	θ	FWHM	$\beta = \lambda^* \frac{\text{FWHM}}{180}$	$t = 0.9 \frac{1}{\beta \cos \theta}$
(Degrees)				(radians)	(nm)

32.18	16.12	0.51	0.0082	118
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Scanning electron micrograph

Figure 3 shows a scanning electron micrograph of silver nanoparticles that were synthesized from *E. coli* supernatant. Spherical form was shown by the synthesized silver nanoparticles.

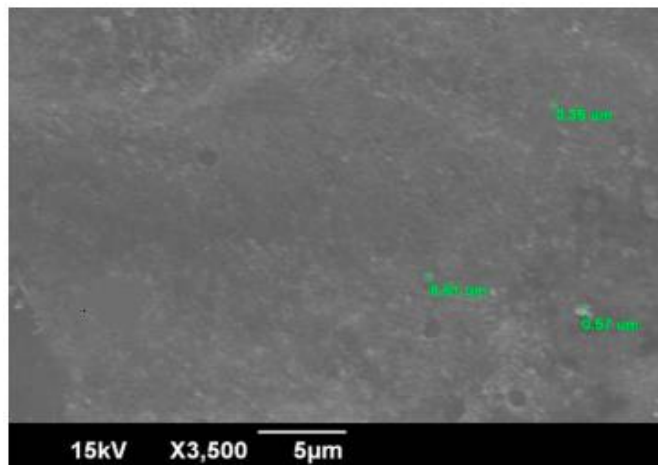


Figure 3: Synthesis of silver nanoparticles from *E. coli* supernatant and their characterization by scanning electron microscopy

The silver nanoparticles seen in the scanning electron micrograph seemed to be quite uniformly distributed and had a spherical shape. The diameters of the silver particles ranged from 5 to 25 nanometers. Silver nanoparticle biosynthesis using the culture supernatant of recently identified *Bacillus* sp. extrinsically, silver nanoparticles with a size distribution ranging from 50 to 120 nm were synthesized. Nanoparticles produced by biological systems often exhibit such morphological and dimensional heterogeneity. To make silver nanoparticles a practical substitute for the present chemical approach, however, tighter regulation of particle size and polydispersity is necessary.

Protein nanoparticles may bind to one another by cysteine residues or free amine groups, or through the electrostatic attraction of enzymes' negatively charged carboxylate groups, as is well known. The aromatic amines' band at 1381 cm⁻¹ and the aliphatic amines' band at 1032 cm⁻¹ are both caused by the stretching vibrations of carbon and nitrogen, respectively. These findings point to the existence of proteins and their binding to silver nanoparticles, which may cause the proteins to become more stable.

CONCLUSION

This work confirms the possibility of using *E. coli* for the biosynthesis of silver nanoparticles (AgNPs), an

environmentally benign and sustainable technique. Surface plasmon resonance, a property of silver nanoparticles, caused a color shift from light yellow to brown, indicating the synthesis. The presence of nanoparticles in both the supernatant and pellet of *E. coli* cultures indicates that bacterial biomolecules play an active role in the reduction of silver ions. Based on the results of the XRD and SEM analyses, the produced AgNPs were found to have a face-centered cubic (fcc) crystalline structure, a strong diffraction peak at $2\theta=32.18^\circ$, and an average particle size of about 118 nm, as determined by Debye-Scherrer's equation. The scanning electron microscopy examination showed that the nanoparticles, which were 5–25 nm in size, were spherical and mostly uniformly distributed. Although biosynthesis offers a promising substitute for chemical approaches, it still has to be optimized further to achieve the desired level of control over nanoparticle size and polydispersity, which are crucial for industrial-scale uses. In sum, this method provides a green way to make silver nanoparticles, which might have uses in nanomedicine, catalysis, and antibacterial agents.

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