



Biosynthesis of Silver Nanoparticles by *Escherichia coli*

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Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in nanotechnology. One of the options to achieve this objective is to use natural factories such as biological systems. In this study, we have investigated the synthesis of nanoparticles of silver by reduction of aqueous Ag^+ ions with the culture supernatant of *Escherichia coli* (DH5a). UV-visible spectrum of the aqueous medium containing silver ion showed a peak at 415 nm corresponding to the plasmon absorbance of silver nanoparticles. The silver nanoparticles were 10-100 nm in dimensions as measured by TEM images. The process of reduction is extracellular, which makes it an easier method for the synthesis of silver nanoparticles.

Key Words: *Escherichia coli*, Biosynthesis, Silver nanoparticle, Bacteria.

INTRODUCTION

Nanotechnology play an increasing role in many key technologies of the new millennium¹. Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts². Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity². Silver nanoparticles have many important applications that include: selective coating for solar energy absorption and intercalation material for electrical batteries, as optical receptors, polarizing filters, catalysts in chemical reaction, biolabelling and as antimicrobial agents³. Living organisms have huge potential for the production of nanoparticles/nano devices of wide applications. However, the elucidation of exact mechanism of nanoparticles production using living organisms needs more studies⁴. The microorganisms have been explored as potential biofactory for synthesis of metallic nanoparticles such as cadmium sulfide, gold and silver³. Researchers have turned to biological synthesis because through this biological synthesis obtaining particles with good control on the size distribution than the other methods⁵ and also in this method doesn't exist chemical agents associated with environmental toxicity.

A novel biological method for the synthesis of silver nanoparticles using the fungus *Verticillium* was reported. Exposure of the fungal biomass to aqueous Ag^+ ions resulted in the intracellular reduction of the metal ions and formation of silver nanoparticles of dimensions $25 \pm 12 \text{ nm}^6$. Ahmad

*et al.*⁷ have observed that aqueous silver ions when exposed to the fungus *Fusarium oxysporum* are reduced in solution, thereby leading to the formation of an extremely stable silver hydrosol. The silver nanoparticles are in the range of 5-15 nm in dimensions and are stabilized in solution by proteins secreted by the fungus. It is believed that the reduction of the metal ions occurs by an enzymatic process⁷.

Balaji *et al.*⁸ reported the extracellular biosynthesis of silver nanoparticles (AgNP) employing the fungus *Cladosporium cladosporioides*. The silver nanoparticles were 10-100 nm in dimensions as measured by TEM images⁸.

The first of synthesis of silver nanoparticles by bacteria has been reported in 2000. Joerger *et al.* used from *P. stutzeri* AG259 to synthesize silver nanoparticles with size less than 200 nm. Bacteria were grown on Lennox L (LB) agar substrate, containing 50 mM AgNO_3 , at 30 °C for 48 h in the dark⁹. In 2008, biosynthesis of silver nanocrystals by *Bacillus licheniformis* have been researched. Aqueous silver ions were reduced to silver nanoparticles when added to the biomass of *B. licheniformis*. This was indicated by the change in colour from whitish-yellow to brown. The probable mechanism for the formation of silver nanoparticles involves the enzyme nitrate reductase¹⁰.

Silver nanoparticles were successfully synthesized from AgNO_3 through a simple green route using the latex of *Jatropha curcas* as reducing as well as capping agent. Crude latex was obtained by cutting the green stems of *Jatropha curcas* plants. Mixture was heated at 85 °C with constant stirring for 4 h in oil bath and silver nanoparticles were obtained gradually¹¹.

As mentioned above, living organisms such as bacteria, fungi and plants have huge potential for the production of metal nanoparticles. In this study, we have made an attempt to corroborate the reduction of water soluble Ag^+ to Ag^0 using *E. coli*.

EXPERIMENTAL

The culture, *Escherichia coli* was obtained from Microbiology Laboratory, Tehran University, Tehran, Iran. Muller-Hinton broth (MHB) was prepared, sterilized and inoculated with a fresh growth *Escherichia coli*. The culture was centrifuged at 5000 rpm for 15 min and the supernatant was used for the synthesis of silver nanoparticles. Distilled water was used as solvent in the synthesis of silver nanoparticles. The supernatant was added separately to the reaction vessel containing AgNO_3 at a concentration of 0.001 M (1 % v/v). The reaction between this supernatant and Ag^+ ions were carried out in bright conditions for 10 min. The silver nanoparticles were characterized by UV-visible spectroscopy (6505 UV-VIS spectrophotometer). In addition, the silver nanoparticles were analyzed by transmission electron microscopy.

RESULTS AND DISCUSSION

The aqueous Ag^+ ions were reduced during exposure to the culture supernatant of *Escherichia coli*. The colour of the reaction solution turned from yellow to brown (Fig. 1), which indicated the formation of silver nanoparticles extracellularly.

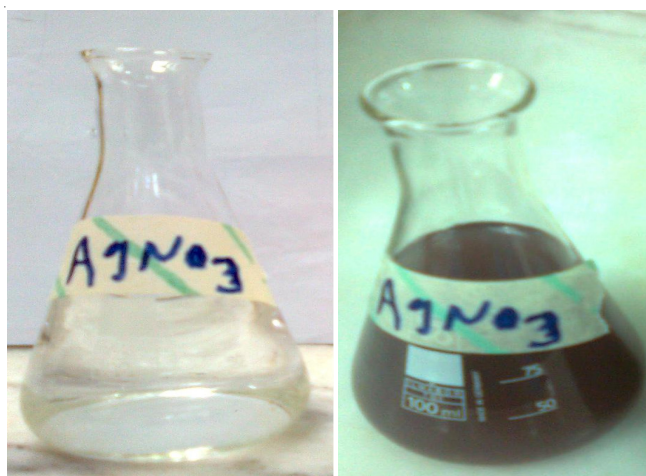


Fig. 1. Colour of the reaction solution turned from yellow to brown

The reaction was completed after 10 min of mixing that indicated it was a rapid process. The colour of the reaction solution remained brown without any changes only for three day. After three day, silver nanoparticles precipitated in the bottom of vessel. The silver nanoparticles analyzed by UV-VIS spectra and TEM. For comparison, vessels containing only the culture supernatant without silver nitrate solution and only silver nitrate (without culture supernatant) were incubated under similar experimental conditions. Upon visual observation, the culture supernatant incubated in the presence of silver nitrate showed a colour change from yellow to brown whereas no colour change could be observed in culture supernatant without silver nitrate and silver nitrate solution without the culture.

Fig. 2 shows the UV-visible absorption spectra recorded from the silver nanoparticles solution after formation. The results indicate that the reaction solution has an absorption maximum at about 415 nm attributed to the surface plasmon resonance band of the silver nanoparticles. Transmission electron microscopy shows the various shapes and sizes of silver nanoparticles. As shown in Fig. 3, the silver nanoparticles are mainly in the size range 10-100 nm.

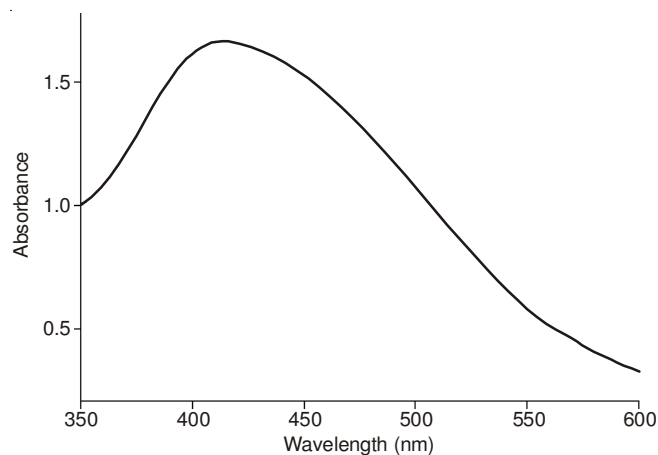


Fig. 2. UV-visible spectrum of aqueous medium containing supernatant and silver ion (1 mM)

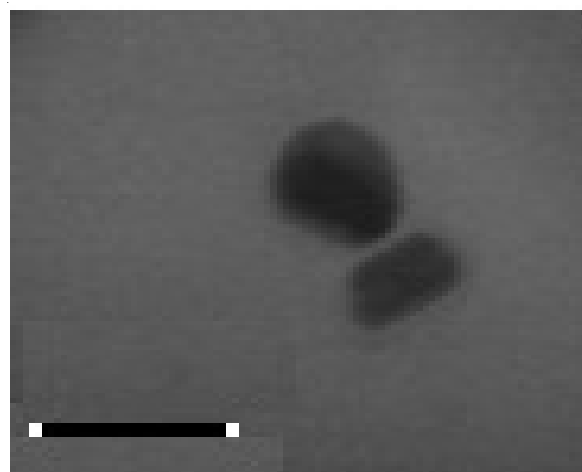


Fig. 3. TEM of silver nanoparticles produced by *Escherichia coli*

Conclusion

Silver nanoparticles in the range of 10-100 nm are synthesized by the supernatant of *Escherichia coli* (DH5a) when silver nitrate is added to it. The silver nanoparticles synthesized are unstable and separated to easy. This methodology could be used for synthesizing a number of metallic nanoparticles involving other metals with good size and shape morphology. This study would therefore lead to an easy procedure for producing silver nanoparticles with the added advantage of biosafety.

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