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Preparation of Ag nanoparticles by biological method

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Abstract

Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of application of 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 Klebsiella pneumonia. UV–visible spectrum of the aqueous medium containing silver ion showed a peak at 425 nm corresponding to the plasmon absorbance of silver nanoparticles. The DLS analysis showed silver nanoparticles with size of 85±25 nm. In addition, it was used from Scanning electron microscopy (SEM).The process of reduction is extracellular, which makes it an easier method for the synthesis of silver nanoparticles.

Key words: *Klebsiella pneumoniae*, biological method, Ag nanoparticles.

Nanotechnology will play an increasingly crucial role in many key technologies of the new millennium[6]. Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts[11]. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity[10]. Silver nanoparticles have many important applications that include: spectrally 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 agents[5]. Living organisms have huge potential for the production of nanoparticles/ nanodevices of wide applications. However, the elucidation of exact mechanism of nanoparticles production using living organisms needs much more experimentations[7]. Recently found that microorganisms have been explored as potential biofactory for synthesis of metallic nanoparticles such as cadmium sulfide, gold and silver[5]. Researchers have turned to biological synthesis because through this biological

synthesis obtaining particles with good control on the size distribution than the other methods[9] and also in this method doesn't exist chemical agents associated with environmental toxicity.

In 2001, 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±12 nm [8]. In another investigation, Ahmad *et al*.[1], 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 process[1].

Balaji *et al*.,[2] reported the extracellular biosynthesis of silver nanoparticles (AgNP) employing the fungus Cladosporiumcladosporioides. The AgNP were 10–100nm in dimensions as measured by TEM images[2]. The first of synthesis of Ag nanoparticles by bacteria has been reported in 2000. Joerger *et al*.[3], used *P. stutzeri* AG259 to synthesize Ag nanoparticles with size less than 200 nm. Bacteria were grown on Lennox L (LB) agar substrate, containing 50 mM $AgNO_3$, at 30 ºC for 48 h in the dark[3]. 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 reductase[4]. In 2009, 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 85ºC with constant stirring for 4 hour in oil bath and silver nanoparticles were obtained gradually[10].

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 Ago using a bacteria (*Klebsiella pneumoniae*).

The Bacteria culture of *Klebsiella pneumoniae* was obtained from Microbiology Laboratory,Tehran University, Tehran, Iran. Muller-Hinton broth (MHB) was prepared, sterilized, and inoculated with fresh growth of *Klebsiella pneumoniae*. The culture was centrifuged at 5000 rpm for 15 minutes and the supernatant was used for the synthesis of Silver nanoparticles. Distilled water was used as solvent in the synthesis of silver nano particles. 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 minutes. The Ag-NPs were characterized by UV-visible spectroscopy (6505 UV-Vis. Spectrophotometer). In addition, The Ag-NPs were analyzed by Scanning electron microscopy (SEM). For measuring of nanoparticles size, dynamic light scattering (DLS) analysis was

used.

The aqueous Ag+ ions were reduced during exposure to the culture supernatant of *Klebsiella pneumoniae*. The color of the reaction solution turned from yellow to brown, which indicated the formation of silver nanoparticles extracellularly.

The reaction was completed after 10 min of mixing that indicated it was a rapid process. The color of the reaction solution remained brown without any changes only for three days. After three days, Ag nanoparticles percipitated 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

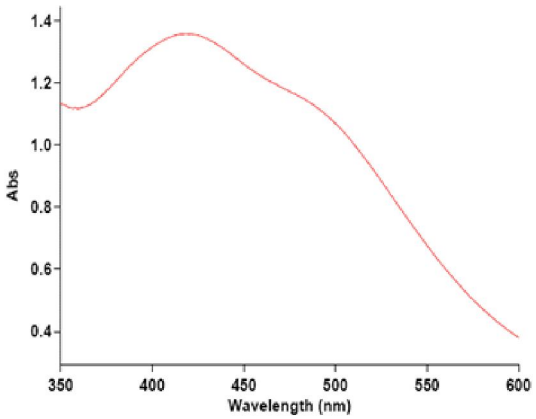

Fig. 1. UV–visible spectrum of aqueous medium containing supernatant and silver ion (1 mM)

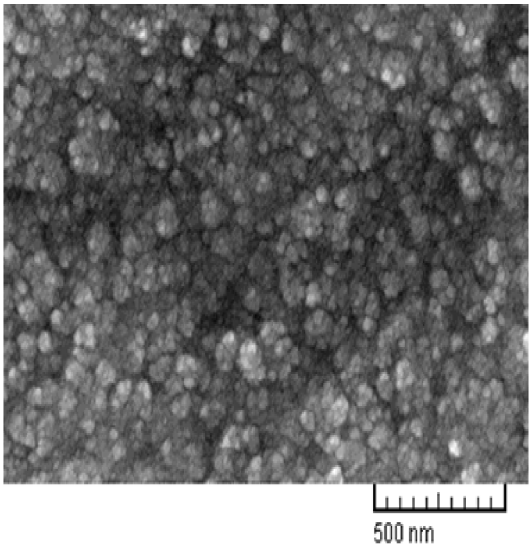

Fig. 2. SEM of silver nanoparticles produced by *Klebsiella pneumoniae*

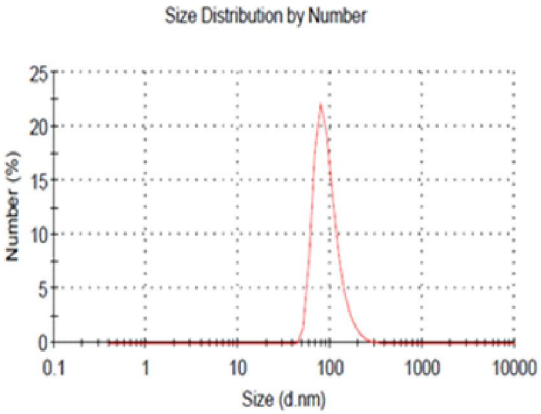

Fig. 3. The curve of size distribution by number

could be observed in culture supernatant without silver nitrate and silver nitrate solution without the culture.

Fig. 1 shows the UV–Vis absorption spectra recorded from the silver nanoparticles

solution after formation. The results indicate that the reaction solution has an absorption maximum at about 425 nm attributed to the surface plasmon resonance band (SPR) of the silver nanoparticles.

Scanning electron microscopy (SEM) measurements show the various shapes of silver nanoparticles. SEM images of the produced nanoparticles are shown in figure 2. The size distribution of the synthesized nanoparticles was shown in Fig. 3. As shown in figure, the silver nanoparticles are mainly in the size range 60–110 nm (85±25 nm).

Silver nanoparticles in the range of 60-110 nm (85±25 nm) are synthesized by the supernatant of *Klebsiella pneumoniae* 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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