

## Synthesis of silver nanoparticles using microorganisms

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Biosynthesis of silver nanoparticles using *Penicillium* fungi has been reported. The extracellular mechanism of silver nanoparticles creation was investigated by UV-Vis spectroscopy, electron microscopy and laser diffraction. The zeta potential of silver nanoparticles has also been determined.

Key words: *silver nanoparticles; Penicillium fungi; extracellular synthesis; zeta potential*

### 1. Introduction

The study of biosynthesis of nanomaterials offers a valuable contribution into materials chemistry. The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticles should be employed in the search for new materials [1]. Biosynthetic methods have been investigated as an alternative to chemical and physical ones. These methods can be divided into two categories depending on the place where the nanoparticles or nanostructures are created as many microorganisms can provide inorganic materials either intra- or extracellularly [2]. For example, bacteria *Pseudomonas strutzeri* isolated from silver mine materials is able to reduce  $\text{Ag}^+$  ions and accumulates silver nanoparticles, the size of such nanoparticles being in the range 16–40 nm, with the average diameter of 27 nm [3]. The examples also include magnetotactic bacteria which produce magnetite ( $\text{Fe}_3\text{O}_4$ ) or greigite ( $\text{Fe}_3\text{S}_4$ ) and diatoms which produce siliceous material [4]. The intracellular methods need a special ion transportation system into the microbial cell. Formation of magnetite particles proceeds through a sequence of events: reduction of Fe(III) to Fe(II), precipitation of amorphous oxide and subsequent transformation to magnetite [4].

Gold nanoparticles have also been synthesized in human cells, both in cancer and non-cancer ones [5]; the scanning microscopic images confirmed that their morpholo-

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gies differed significantly. This behaviour can have an implication to cancer diagnostics. In contrast, extracellular synthesis of nanoparticles occurs in alkalothermophilic actinomycete, *Thermomonospora* sp., which reduces gold ions.

The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for such processes. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp. [1] or *Aspergillus fumigatus* has been reported [6]. A novel biological method for synthesis of silver nanoparticles using *Vericillum* was proposed by Mukherjee et al. [7, 8]; a two-step mechanism was suggested. The first step involves trapping of  $\text{Ag}^+$  ions at the surface of the fungal cells. In the second step, enzymes present in the cell reduce silver ions.

The extracellular production of metal nanoparticles by several strains of the fungus *Fusarium oxysporum* has been described by Duran et al. [9]. The presence of hydrogenase in the *F. oxysporium* broth was demonstrated. This extracellular enzyme shows excellent redox properties and it can act as an electron shuttle in metal reduction. It was evident that electron shuttles or other reducing agents (e.g., hydroquinones) released by microorganisms are capable of reducing ions to nanoparticles.

The Neem (*Azadirachta indica*) leaf broth and aqueous solution of silver nitrate or chloroauric acid were used for the extracellular synthesis of pure metallic silver and gold particles [10]. The time required for  $\text{Ag}^+$  and  $\text{Au}^{3+}$  ions to reduce was 4 h and 2 h, respectively, being extremely short compared to both bacteria and fungi (24 h and 120 h). Surface active constituents of the leaf broth stabilize nanoparticle suspensions – an aqueous suspension showed stability even after 4 weeks.

Our aim in the present contribution was to synthesize and characterize silver nanoparticles obtained by use of *Penicillium* fungi isolated from the soil. To our knowledge, extracellular synthesis of Ag particles by these fungi has not been reported so far.

## 2. Experimental

*Synthesis of silver nanoparticles.* *Penicillium* strain isolated from soil was studied. Inoculated fungi were prepared in Petri dishes at room temperature using 2% malt extract with 0.5% yeast extract. Fungal biomass used for biosynthetic experiments was grown aerobically in liquid medium containing [g/l]:  $\text{KH}_2\text{PO}_4$  7.0,  $\text{K}_2\text{HPO}_4$  2.0,  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$  0.1,  $(\text{NH}_4)_2\text{SO}_4$  1.0, yeast extract 0.6, glucose 10.0. Erlenmeyer flasks were inoculated with spores and incubated at 25 °C with shaking (150 rpm) for 72 h. After the incubation, the biomass was filtered (Whatman filter paper No. 1) and then extensively washed with distilled water to remove any medium component. Fresh and clean biomass was taken into Erlenmeyer flasks containing 100 cm<sup>3</sup> of Milli-Q deionised water. The flasks were agitated at the same conditions as described above, then the biomass was filtered again (Whatman filter paper No. 1) and cell-free filtrate was used in experiments.  $\text{AgNO}_3$  (1 mM of final concentration) was mixed with cell-free

filtrate in an Erlenmeyer flask and agitated at 25°C in dark. Control (without silver ions) was also run along with the experimental flasks. Samples of 1 cm<sup>3</sup> were withdrawn at various times and the absorbance was measured at the resolution of 1 nm using a UV-visible spectrophotometer (HELIOS  $\lambda$ , ThermoElectron Corp.).

*Particle sizing measurements.* Particle sizing experiments were carried out by means of laser diffractometry, using an Mastersizer 2000 instrument (Malvern), equipped with HydroMu dispersing unit (Malvern). Measurements were taken in the range between 0.1 and 1000  $\mu\text{m}$ , under the following conditions: particle refractive index 0.54, particle absorption coefficient 4, water refractive index 1.33, and general calculation model for irregular particles. Ten measurement cycles of 10 s each were taken, and the data obtained were averaged by a software (Mastersizer 2000, ver. 5.20 from Malvern).

*Electron microscopy.* Scanning electron micrographs were taken using a DSM 982 GEMINI instrument (Zeiss, Germany). Samples were filtered and dried before measurements.

*The zeta potential measurement.* Measurements were carried out using a Zetasizer Nano ZS (Malvern) and a titrator MPT-2. An aqueous suspension of silver nanoparticles was filtered through a 0.45  $\mu\text{m}$  PTFE membrane before measurement. The zeta potential was calculated using Henry's equation.

### 3. Results and discussion

Upon addition of Ag<sup>+</sup> ions into the filtered cell-free culture in the dark, samples changed in colour from almost colourless to brown, with intensity increasing during the period of incubation. Control (without silver ions) showed no change in colour of the cell filtrates when incubated in the same conditions (data not shown). Formation of colloidal silver particles can be easily followed by changes of UV-Vis absorption (Fig. 1). Shankar et al. suggested that the shoulder at 370 nm corresponded to the transverse plasmon vibration in silver nanoparticles, whereas the peak at 440 nm due to excitation of longitudinal plasmon vibrations [11]. After 72 h the process was stopped and the particles were further analyzed by laser diffraction, scanning electron microscopy and zeta potential measurements.

Laser diffraction revealed that particles obtained are polydisperse mixture (uniformity 6.97), with the size ranging between hundreds on nanometers and micrometers (Fig. 2). The values of D10, D50 and D90 are 0.69, 4.87 and 13.31  $\mu\text{m}$ , respectively. There is still work to do on the control of particle sizes and uniformity. Probably changes in medium composition and *Penicillium* strain can substantially improve these parameters.

Scanning electron micrograph (Fig. 3) confirms data obtained from the laser diffraction, albeit preparation of samples (including drying) can affect their size and shape. It was observed that the nanoparticles are partially aggregated due to the drying process.

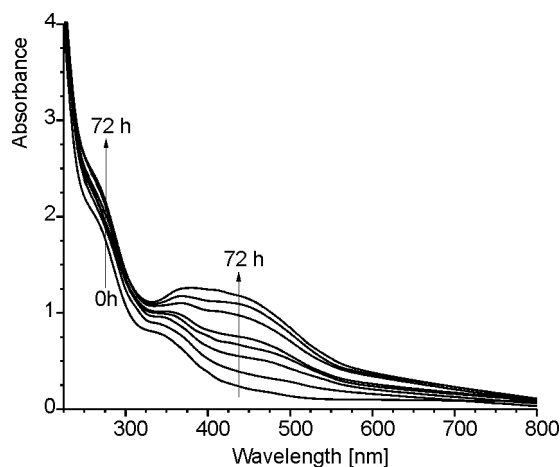


Fig. 1. Temporal evolution of UV-Vis absorption spectra during the formation of silver nanoparticles

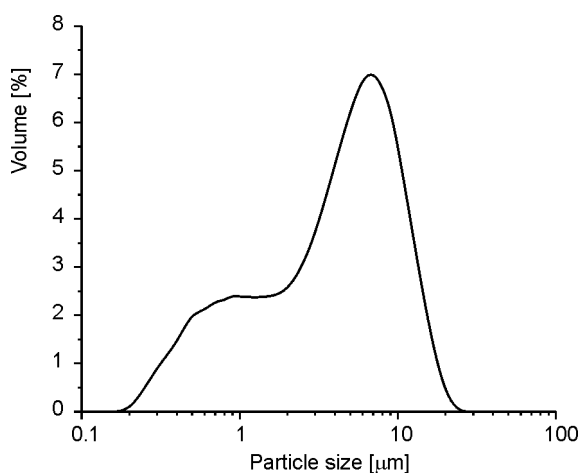


Fig. 2. Particle size distribution for silver nanoparticles

The effect of pH on the zeta potential of silver nanoparticles was also investigated (Fig. 4). At natural conditions (pH close to 8), the zeta potential was equal to  $-26.3 \pm 0.2$  mV. We can conclude that the silver nanoparticles got a negative zeta potential and the isoelectric point is below pH = 2; at pH > 8, particles are fairly stable due to the electrostatic repulsion. On the other hand, in acidic solutions low negative values of  $\zeta$ -potential clearly indicate instability of the aggregates.

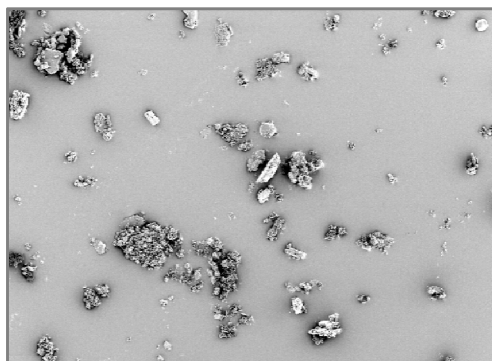


Fig. 3. Scanning electron micrograph of silver nanoparticles. Magnification 5000 $\times$

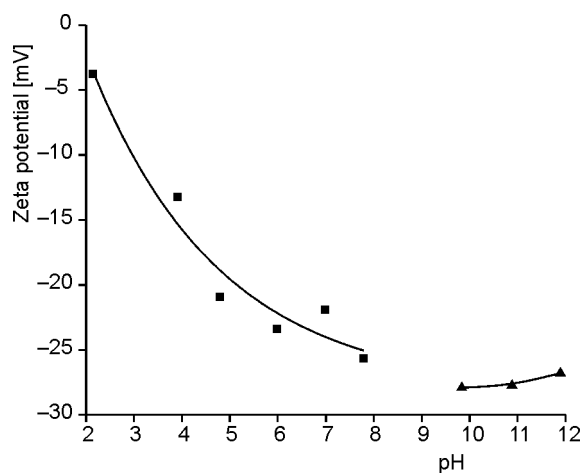


Fig. 4. Zeta potential of silver nanoparticles in function of pH

Fungi of *Penicillium* genus seem to be extremely good candidates for the synthesis of silver nanoparticles. Their formation proceeds via an extracellular mechanism but there are still some question regarding the details of the process. Nanoparticles obtained possess negative zeta potential and are fairly stable at pH value above 8 due to the electrostatic repulsion. The most important feature of *Penicillium* fungi is the fact they are widespread present in the waste biomass from pharmaceutical industry. Such cheap source of material gives an opportunity to cost-effective preparation of various silver-based nanostructures. Further research on the antimicrobial activity of produced nanoparticles will be carried out in this laboratory.

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