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The Determination of the Optimum Parameters for the Formation of Silver Nanoparticles by Aspergillus Niger BDU-A4

Eynulla Musayev
Department of Microbiology, Faculty of Biology,
Baku State University, Baku, Azerbaijan
musayev.eynulla@mail.ru

Abstract

In this work, the formation of silver nanoparticles was investigated by *Aspergillus niger* BDU-A4 at different stages/phases/culture media: a) pH: 4.82,5.79,7.42,8.88, 9.86;b) indicators of temperature (20°C, 25°C, 30°C, 37°C və 45°C) and c) indicators of different concentration of the salt of $AgNO_3$. As a result of UV-V, absorbance spectrum is defined as the optimal conditions for the formation of maximum amount of Ag nanoparticles from $AgNO_3$ - substrate by *Aspergillus niger* BDU-A4. This condition determined pH = 7.4 at 37 °C,with 10 grams of biomass within 1mm $AgNO_3$ solution in 100 ml suspension during 6 hours incubation.

Keywords: *Aspergillus niger*, Ag nanoparticles, pH, biomass, temperature, incubation period

Introduction

The size, shape, method of synthesis and other major properties of nanoparticles play an important role in the determination of practical importance (Mansoori *et al.,* 2007; Taylor *et al.,* 2013; Pundir, 2015).

The synthesis of metal nanoparticles as well as copper, zinc, titanium, magnesium, gold, cadmium and silver has particular importance among the synthesized nanoparticles. So they are widely used in the medical fields, manufacturing optical and electronic equipment, chemical industries, also agriculture in the form of elements and compounds (Mukherjee *et al.,* 2001a, 2001b; Bell *et al.,* 2003; Gilbert *et al.,* 2003; Chovanec *et al.,* 2005; Begum *et al.,* 2009; Rai & İngle, 2012). The environmental risks of nanoparticles were investigated also by researchers as well as their synthesis and application (Fabrega *et al.,* 2014).

The optimal conditions were determined by different researchers for the synthesis of individual metal nanoparticles in different metal oxides and salts. Also the influence spectrum of various physical and chemical parameters was well investigated that affects the process of the synthesis of nanoparticles (Ottow & Von Klopotek, 1969; Sastry *et al.,* 2003; Vasilenko *et al.,* 2010; Desai *et al.,* 2011; Singh & Balaji Raja, 2011; Wu *et al.,* 2012; Li *et al.,* 2013; Ali *et al.,* 2014; Das *et al.,* 2014; Parmar *et al.,* 2014; Kameyama & Senna, 2014; Selishchev & Pavlishchuk, 2014; Yongju *et al.,* 2014; Aliakbari *et al.,* 2015; Makhdoomi *et al.,* 2015).

The antibacterial and inhibitor effects of silver along with its metal properties make it important to perform research on more effective ways to synthesize the silver (Jaidev & Narasimha, 2010; Fayaz *et al.,* 2012). Although the synthesis of silver nanoparticles is available with biological and other methods, "green" synthesis is more effective both financially and environmentally. The main cost - effective method in synthesis of nanoparticles is using microorganisms at "Green nanotechnology". Different species of bacteria and fungi that are products of nanoparticles haven't been investigated yet. Fungi synthesize silver nanoparticles from silver ions of their salts through intracellular and extracellular enzymes (Mukherjee *et al.,* 2001a; Ingle *et al.,* 2008; Castro-Longoria *et al.,* 2011; Jain *et al.,* 2011; Vahabi *et al.,* 2011; Abd El-Aziz *et al.,* 2012; Li *et al.,* 2012).

To increase the synthesis of silver nanoparticles with minimum investment by microorganisms, it is required to select the optimum culture conditions and suitable physical parameters for their products. Each of the selected active strains is able to be synthesized in different amount and form of silver nanoparticles/ synthesize different amount and form of silver nanoparticles, depending on the factors as follows: definition of biomass, pH, temperature, light intensity, nutritious environment, concentration, incubation period and so on. (Safekordi *et al.,* 2011; Yin *et al.,* 2012; Chan & Don, 2013; Sonal *et al.,* 2013).

It was determined that smaller - sized and spherical - shaped nanoparticles are synthesized at higher pH, while studying the correlation between the characteristics of silver nanoparticles and the environmental acidity. While at lower pH, relatively large, rod- or triangle-shaped nanoparticles are formed (Sonal *et al.,* 2013; Alqadi *et al.,* 2014). It was determined/found/revealed through the investigation of relation between the size of silver nanoparticles synthesized by fungi and temperature that the increasing of the reaction temperature leads to the reduction of nanoparticle size and their monodispersity (Fayaz *et al.,* 2009; Chan & Don, 2013; Sonal *et al.,* 2013).

The optimal concentrations of biomass and substrate were studied by researchers to increase the production of silver nanoparticles on an industrial scale by fungi and bacteria (Korbekandi *et al.,* 2012; Chan & Don, 2013; Korbekandi *et al.,* 2013; Sonal *et al.,* 2013). The optimal physio-cultural conditions affecting the synthesis of silver nanoparticles was determined/measured/studied/found by Sonal and his collaborators. They have studied the effect of temperature and pH, biomass and substrate concentrations, as well as the effects of nutrient - rich environment, filtration level of suspension and intensity of light for optimal synthesis of nanoparticles by *Fusarium oxysporum* (Sonal *et al.,* 2013).

Plant extracts have been investigated as nanoparticle product and their optimal synthesis conditions was observed/recorded during the study of rapid synthesis of silver nanoparticles (Geetha *et al.,* 2013; Krishnaraj *et al.,* 2013; Thamer & Almashhedy, 2014; Christopher *et al.,* 2015; Pourmortazavi *et al.,* 2015).

At previous studies, the strains of mold fungus were separated from soil samples and different rotten plant residues to identify and test their ability to generate silver nanoparticles. It was studied that *Aspergillus niger* BDU-A4 active strain has the ability to form silver nanoparticles intensively (Ganbarov *et al.,* 2013; Ganbarov *et al.,* 2014a, 2014b). We also studied the relation of silver nanoparticles synthesis by Aspergillus niger BDU-A4 with the incubation period and the amount of biomass (Musayev *et al.,* 2013). The main purpose of the present study is to test other factors which can affect the formation of silver nanoparticles by *Aspergillus niger* BDU-A4 and identify the optimal performance of these factors for the rapid synthesis of silver nanoparticles.

Materials and Methods

Fungal culture: Weekly cultures of *Aspergillus niger* BDU-A4 taken from the cultures collection of Baku State University.

Reagents: For the synthesis of silver nanoparticles as the primary source, we used/applied1mM $AgNO_3$ solution, also the solution of 1 N HCl and the solution of 1 M NaOH for preparation of solutions at relevant pH, and distilled water for preparation of solutions. The composition of the nutrient medium (g / l) was: - Sucrose-20; $NaNO_3$-3; K_2HPO_4-1; $MgSO_4 \cdot 7H_2O$ - 0.5; KCl- 0.5; $FeSO_4 \cdot 7H_2O$-0.01.

UV-Vis spectral analysis of silver nanoparticles: For UV(UV-Vis) spectrophotometer, "Analytic Jena" spectrophotometer was used/applied (model: Specord - 250 pulse, German). UV spectrophotometer (UV-vis) is a valuable tool for determination, characterization and study of nanomaterials. UV-vis is the equipment which calculates the dispersed or absorbed light of a given sample at the solution. The suspension of silver nanoparticles has dark golden-yellow color through the surface plasmatic resonance. These are the motions of electrical conductance as a response to electromagnetic waves. Silver nanoparticles have a spectrum absorption characteristic at the "vision" circle of UV-vis. Spectral characterization of silver nanoparticles strongly depends on their size, shape, inter-particular gaps and surroundings (Rao *et al.,* 2002). The dependency of quantum size affects the passage of red or blue spectra of surface plasmatic resonance. Therefore, the absorption peaks can be used as a means in determining particles' size and stability. Nano-scaled silver nanoparticles form peak at the wavelength of 400 - 420 nm. However, when the size of particle increases, its absorption spectrum goes beyond nanoscale.

Synthesis of silver nanoparticles: The fungal culture was incubated in 250 ml Erlenmeyer flasks that contain 100 ml nutrient medium, at 28°C, at 120 rpm, within 120 hours. Received biomass washed by distilled water, it was incubated again within 24 hours with 100 ml of distilled water at 28 °C, to clean from nutrient medium completely. Then the suspension is drained through Watman filter paper (№1) and the gained/collected wet biomass is weighted. For the synthesis of silver nanoparticles, 100 ml of suspension was prepared that consisted of 10 grams of biomass, 1 ml of distilled water and 1 mM $AgNO_3$in 250 ml Erlenmeyer flasks. Suspensions were incubated at different pH and temperature in dark conditions. Silver nanoparticles were synthesized as a result of reduction of silver ions to metallic silver at the optimal indicator of each factor after incubation.

Selection of the optimal conditions for the synthesis of Ag nanoparticles:

In order to ensure fast and stable synthesis of silver nanoparticles by *Aspergillus niger* BDU-A4, we studied the effects of following factors: medium acidity (pH), temperature, incubation period, the amount of biomass and concentration of silver nitrate. The experiments were carried out twice throughout the study of each physical and cultural indicator.

a) Effect of pH on the formation of nanoparticles

The biomass was incubated in solutions with a pH of 4.8, 5.8, 7.4, 8.9 and 9.9 to study the effect of pH on the formation of nanoparticles.

b) Effect of temperature on the formation of nanoparticles:

The suspension was incubated in a thermostat at stable temperatures of 20 °C, 25 °C, 30 °C, 37 °C and 45 °C to study the effect of temperature on the formation of nanoparticles (**experimental procedure is shown above).**

c) Effect of concentration of $AgNO_3$ salt on the synthesis of nanoparticles:

10 grams of biomass was incubated in $AgNO_3$ salt solutions in different concentrations of 0.5 mM, 1 mM, 2 mM, 3 mM and 5 mM to study the effect of concentration of $AgNO_3$ salt on the synthesis of silver nanoparticles.

Results and Discussion

a) Effect of pH on the formation of nanoparticles

The formation of silver nanoparticles by *Aspergillus niger* BDU-A4 was investigated at different pH values (4.8, 5.8, 7.4, 8.9, 9.9) in UV-vis spectrophotometer and the results obtained are shown in Figure 1. According to the results of spectrum, the synthesis of silver nanoparticles has been observed at all tested pH values.

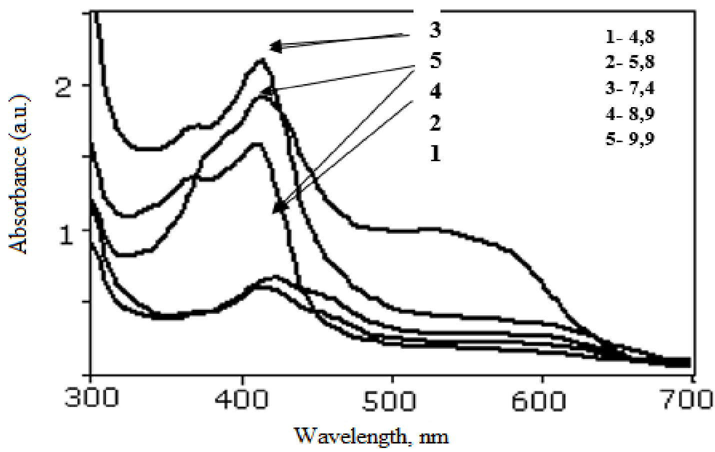

Figure 1. The effect of pH on the formation of silver nanoparticles by *Aspergillus niger* BDU-A4

But it is known that the maximum synthesis of silver nanoparticles occurs in an alkaline environment (8.9 and 9.9pH values). According to reduction of pH value from pH 7.4 toward acidic environment, the amount of synthesized nanoparticles is decreased, and when the pH level reaches 4.8, it results in aggregation of particles. After incubation, the flocculation of particles was not observed in none of the solutions with different pH values. However, after one week the aggregation of particles occurred in a solution of 4.8 pH. But in solutions of alkaline, stable diffusion of the nanoparticles was observed.

Stable diffusion or aggregation of silver nanoparticles in solutions may depend on zeta potential of particles and the amount of OH^- anions in solution. Thus, the zeta potential of particles is high in alkaline environment as a result of adsorption of OH^- anions by the silver nanoparticles. Thereby, the electrostatic repulsion force formed in particles provides their sustainability in solution. Lack of anions OH^- weakens zeta potential of particles in the acidic environment that makes their aggregation inevitable (Sonal *et al.,* 2013).

b) Effect of temperature on the formation of nanoparticles:

The formation of silver nanoparticles by *Aspergillus niger* BDU-A4 was studied at different temperatures of 20 °C, 25 °C, 30 °C, 37 °C, 45 °C in UV-vis spectrophotometer and the results are shown in Figure 2. According to analysis of spectra, the synthesis percentage and surface plasmatic absorption of silver nanoparticles increase gradually as the temperature increases.

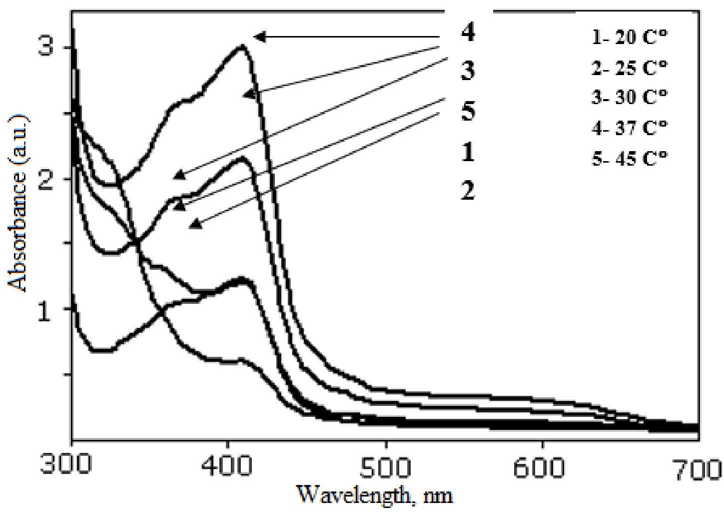

Figure 2. The effect of temperature on the formation of silver nanoparticles by *Aspergillus niger* BDU-A4

The highest density of nanoparticles is observed at 37 °C but reduced at 45 °C respectively. Such a change in absorption and the increase in surface plasmatic resonance report an increase in the number and size of silver nanoparticles in the suspension of flasks and directly cause the solution to get dark silver color.

c) Effect of concentration of $AgNO_3$ salt on the synthesis of nanoparticles:

10 grams of biomass was incubated in $AgNO_3$ salt solutions in different concentrations of 0.5 mM, 1 mM, 2 mM, 3 mM and 5 mM to study the effect of concentration of $AgNO_3$ salt on the synthesis of silver nanoparticles and the formation of nanoparticles was tested in UV-vis spectrophotometer. The obtained results are shown in Figure 3.

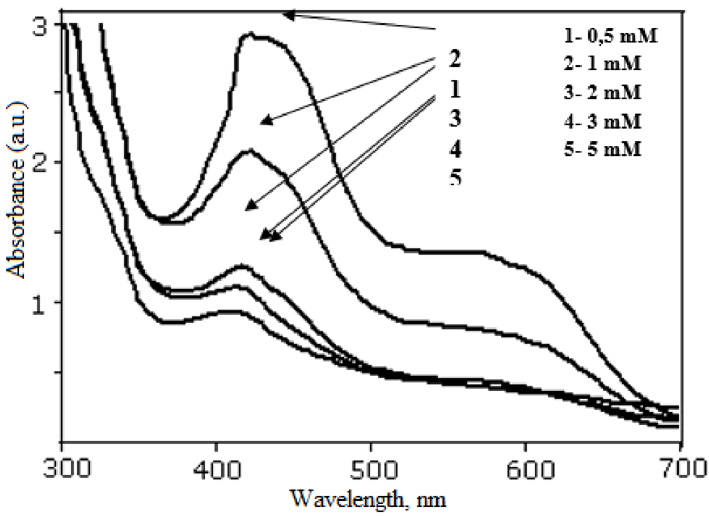

Figure 3. The effect of different concentrations of $AgNO_3$ salt on the formation of silver nanoparticles by *Aspergillus niger* BDU-A4

According to analyses of spectra, the surface plasmatic adsorption was also increased as the concentration of silver-nitrate increased. But the highest density of silver nanoparticles was observed in 1 mM concentration of silver nitrate.

Conclusion

In this research, we have studied the effect of certain parameters of physical conditions in synthesis of silver nanoparticles: medium acidity (pH), the effect of temperature and the concentration of $AgNO_3$ salt by active strains of *Aspergillus niger* BDU-A4. The effect of different pH values was analyzed in UV-Vis spectroscopy and it was revealed that the optimum pH value for the highest synthesis of silver nanoparticles is 7.4 at 28 °C in suspension of 1mm $AgNO_3$ and 10 grams of biomass. According to UV-Vis analysis of temperature effects, the optimum temperature is 37 °C for getting the highest density of silver nanoparticles in the suspension of 1mm $AgNO_3$ and 10 grams of biomass. Also, it was determined/observed/revealed that more synthesis of Ag nanoparticles is observed in the solution of 1mm concentration of $AgNO_3$salt.

Finally, optimization of the medium acidity (pH), effect of temperature and concentration of salt $AgNO_3$, which affect the synthesis of silver nanoparticles by *Aspergillus niger* BDU-A4, can be used in the synthesis of silver nanoparticles on an industrial scale and in the production of substances with antimicrobial properties.

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