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Review article

Evaluation of the effective factors on size and anti-bacterial properties of biosynthesized Silver nanoparticles

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ABSTRACT

Developing reliable and eco-friendly processes for the synthesis of metallic nanoparticles is an essential step in the field of application of nanotechnology .Using natural factories such as biological systems is a way of developing such processes. Nowadays, the synthesis of silver nanoparticles is very common due to its numerous applications in different fields. The synthesis of these nanoparticles is carried out through physical, chemical and biological methods. However, due to its inexpensive and environmentally friendly features, the biological method is preferred to the other two methods. In the present article, the factors influencing the size of synthesized silver nanoparticles using biological methods (Which consists ofliving organisms such as: Plants, Fungi, Bacteria and Yeast) and the anti-bacterial properties of silver nanoparticles were investigated.

Keywords: *Silver nanoparticles; Biosynthesis; Effective factors; Silver precursor; Antibacterial.*

INTRODUCTION

Since nanoparticles are a bridge between voluminous and atomic or molecular state of materials, they have always been of considerable interest [1]. It is predicted that in the 21st century, nanotechnology will significantly influence science, economy and daily life and will become one of the driving forces of the next industrial revolution. Different aspects of this new technology include production, determination of properties and manipulation of structures in the nanoscale. The use of nanoparticles has recently received much attention due to its well-defined chemical and mechanical properties [2, 3]. Among different nanoparticles, metallic nanoparticles are the most promising due to anti-bacterial properties, which is a result of the high volume of these particles. This has been considered by many researchers because of the resistance of microbial growth in the presence of metal ions, antibiotics and development of resistance strains.

The change in size or composition level can influence the physical and chemical properties of nanoparticles [4, 5]. In recent decades, the use of metallic nanoparticles has become very common due to its widespread applications in different industries [6]. Nanoparticles have unique electrical, physical, chemical and optical properties [7].

When the dimensions of metallic particles reach this size range (1-100 nm), their physical, chemical and electric properties change. These properties depend on the size of particles; and by changing the size and shape of silver nanoparticles,and also some properties including melting temperature, magnetic behavior, redox potential and their colors can be controlled [8].

Today, silver nanoparticles have been considered and used due to their anti-microbial effect and their wide range of applications in different sciences and industries. For example, they are used in medicine for treating HIV virus, in food industries as anti-bacterial factors, in packaging food products, as catalyses in chemical reactions [9] and in many other fields due to their unique catalytic [10], electronical, optical [11, 12] and anti-bacterial [13] properties.

Extraordinary optical properties of silver nanoparticles were discovered and used at the time of the Roman emperors. Basic studies on silver nanoparticles in 1980s indicated that silver nanoparticles have valuable composites, unique optical properties, developed levels and catalytic activity [14]. Optical properties of silver nanoparticles have been investigated in different papers concluding that the optical properties of these nanoparticles highly depend on the unique properties including size, shape, composite, presence of absorbing layers and their structures [15, 16]. Furthermore, it is realized that the anti-microbial properties of silver depend on the amount of consumption and release of silver. Silver is stable in metal form, however, it reacts with the moisture in the environment or on body skin and ionizes [17]. Low concentration of silver is not dangerous for human cells; however, it is fatal for most bacteria and viruses. So, it is widely used in medicine, food industries and many other fields [18]. It seems impossible for microorganisms being able to resist against silver through genetic mutation. Silver ions attack most of proteins in a cell and this valuable characteristic has been increasingly considered due to the increase of bacteria-induced varieties which are resistant against a wide spectrum of antibiotics [19].

Nowadays, the researchers found that living organisms like bacteria, fungus, plants, etc have great ability in synthesizing metal nanoparticles. Furthermore, microorganisms have been explored as potential biofactories for synthesizing metallic nanoparticles like cadmium sulfide, silver and gold, Moreover, they prefer biological synthesis because of better control over size distribution of nanoparticles [20, 21] and the environmental toxicity, which along with chemical methods is not present in this method [22].

Evaluation of the Effective Factors on synthesized silver nanoparticles through biological methods

The investigations have shown that size, morphology, stability and (physical, chemical) properties of metal nanoparticles are strongly influenced by reaction conditions, interaction synthesis of metal ions with reducing factors and the absorption process of stabilizer with metal ions [9].

It could be said that the size of synthesized nanoparticles depends on the materials used in producingthe nanoparticles. The ability of size-controlled synthesis of nanoparticles is important due to the fact that some properties like solubility, transparency, color, absorbance and emission wavelengths, conductance coefficient (index), melting point, and catalytic behavior just change by the size of different particles [23].

- **The effect of temperature on the size of silver nanoparticles**

Gurunathan et al. indicated that by controlling environmental conditions of nanoparticle synthesis, it is possible to produce silver nanoparticles in different sizes and shapes. They investigated this proposition by changing temperature, pH, and supernatant of *E. coli* .They conducted their experiments by adding supernatant to 1 mM $AgNO_3$ solution and its incubation in different temperatures and pH. Silver nanoparticles sizing 50 nm were synthesized at room temperature, while at 60° C, the particles sizing 15 nm were synthesized. As it can be seen, the results of investigations show that the size of silver nanoparticle reduces when the temperature increases [24, 8].

In another article presented by Ghorbani et al. the size of producing nanoparticle was investigated in 5, 25, 40 and 90°C and the optimum temperature was reported as being 5°C. The results of this study show that at 90°C, no nanoparticle is synthesized, which can be justified by denaturation of proteins and disappearance of Nitrate Reductase enzyme in the culture medium. Furthermore, it becomes clear that by increasing temperature, the size of particles increases [25]. Fayaz et al. carried out the biosynthesis of silver nanoparticles using fungus varieties and investigated the effect of temperature on the size of silver nanoparticles. The results of this investigation showed that temperature is one of the factors, bywhich, we would be able to synthesize size-controlled silver nanoparticles,for the temperature increase leads to size reduction in silver nanoparticle and increase of monodispersity in them [26].

In their article, Iravani et al. (2013) synthesized 10-40 nm silver nanoparticles using the *Pinuseldarica Bark Extract*. This research group investigated the effect of different temperatures (25, 50, 100 and 150°C) on the size of produced silver nanoparticles and observed that increase of reaction temperature resultsincrease, which can be due to increase in keloid silver nanoparticles' production and revival rate. Furthermore, when the reaction temperature increases from 25°C to 150 °C, the size of produced nanoparticles decreases [27]. ManjuBala et al. (2013) performed the biosynthesis of silver particles using aqueous extract endophytic fungus *aspergillusfumigatus* and investigated the effect of temperature (0, 37 and 100°C) on the size of synthesized nanoparticles and observed that when the temperature increases from 0 to 100, the size of produced silver nanoparticles increases [28]. Goldie Oza et al. (2012) studied the extracellular synthesis of silver nanoparticles by using *Pseudomonas aeruginosa*. The size of produced nanoparticles in this method was reported to be in the range of 20-50 nm. These researchers also investigated the effect of two different temperatures (30 and 100°C) on the size of silver nanoparticles and observed that the optimum temperature of this process was 100°C [29].

- **The effect of pH on the size of silver nanoparticles**

Solution pH is one of the factors affecting the produced silver nanoparticles. The research results indicate,the size of nanoparticles in acidic pH as 45 nm, while the size of synthesized nanoparticles was 15 nm in base pH (pH= 10) [8, 30]. Ghorbani et al. (2011) studied the effect of the pH of 5.5, 7.5 and 9 on the size of synthesized silver nanoparticles and observed that the size of produced nanoparticles increases as the pH increases from 5.5 to 9 [25]. Iravani et al. investigated the effect of different levels of pH (3, 5, 9, 7 and 11) on the size of produced silver nanoparticles by *Pinuseldarica Bark Extract*. The results indicated that a rise in the pH of the reaction mixture increases the absorbance which can be due to the increase in the production of keloid silver nanoparticles and revival rate. It seems that pH is effective on the number of produced nanoparticles and their stability. Thus, pH impactsthe revival rate of the reaction. In their study, it becomes clear that in lower pH (acidic), bigger silver nanoparticles are formed while in base pH, smaller nanoparticles are synthesized [27]. Goldie Oza et al. (2012) investigated the effect of different levels of pH (9, 8, 6, 2 and 10) on the size of silver nanoparticles with the aim of finding optimum pH in the process. Finally, reviewing the resultsdemonstrated that, pH=10 was the optimum condition [29]. Generally, the results obtained from different studies [30-33] also confirm this fact that the nanoparticles, formed in lower PH, are bigger than the ones formed in higher pH. A schematic imaginary diagram demonstrating the reasons for size control over the synthesis of silver nanoparticles has been shown in Figure1.

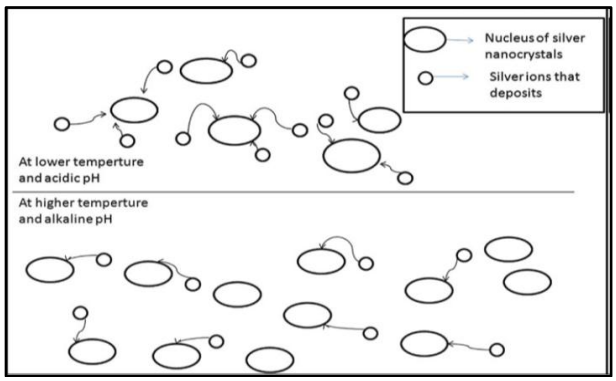

Fig.1. At lower temperature and pH, less nucleation occur thereby forming larger particles whereas at higher pH and temperature more nucleation may occur thus forming smaller particles [40]

The effect of microorganism type

- **Bacteria**

The first synthesis of the silver nanoparticles was conducted by *p. stutzeri AG259* bacteria in 2000, the size of produced nanoparticles was reported smaller than 20 nm. The used silver source was 50 mM silver nitrate with LB agar as medium culture [34]. In 2008, Kalimuthu et al. could synthesize nanoparticles using *Bacillus licheniformis* [35]. The mechanism for synthesis of silver nanoparticles by *Bacillus licheniformis* has been shown in Figure2.

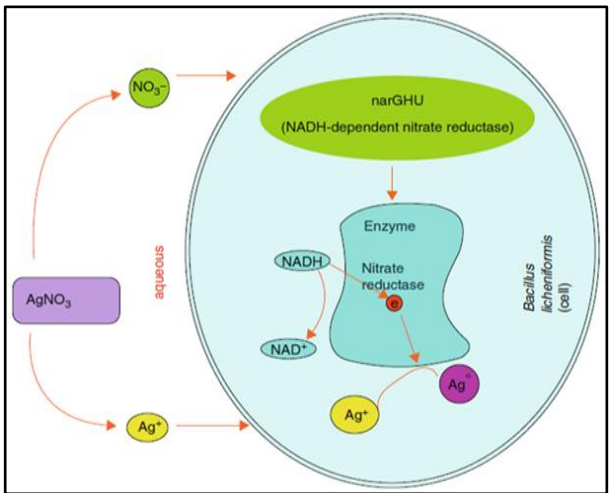

Fig.2. Likely mechanism for synthesis of silver nanoparticles by *Bacillus licheniformis* including Nitrate Reductase enzyme depending on NADH which might transform Ag+ to Ag° [35]

To decrease the synthesis time of silver nanoparticle, different varieties of bacteria were studied. The results of these investigations showed that supernatant of the culture medium of *Klebsiella pneumonia, Escherichia coli* [36, 37] and *Enterobacter cloacae* bacteria have the ability of synthesizing silver nanoparticles in a shorter time (5 min) [38].

In biological methods, revival of Ag^+ silver ions in biomolecules is performed by enzymes/proteins, amino acids, poly-saccharides and the available vitamins, which are all compatible with the environment. However, the mechanism, which is usually accepted for biosynthesis of silver nanoparticles, is the presence of Nitrate Reductase enzyme [39].

The presence of α-NADPH-dependent nitrate reductase is required in laboratory synthesis of silver nanoparticles to prevent downstream processes. During the reaction, nitrate is converted into nitrite and free electrons are transferred to silver entering ions [37, 40]. Ghorbani (2013) synthesized silver nanoparticles using *Salmonella typhirium* extract. The bacteria extract was separated by 0.22 mm (PVDF) Durapore membrane filter, and then added to the reaction vessel including, silver sulfate of 0.0005 M concentration. Using dynamic light scattering analysis the size of produced silver nanoparticles was reported as 50-150 nm with an average size 129 nm [48].

Here, we summarize some bacterial species used in the biosynthesis of Ag nanoparticles (Table 1).

- **Fungi**

In recent years, fungi have been added to the list of microorganismsused for the production of Nano-powders. The interest in the use of fungi in production of nanoparticles is due to the significant amount of special enzymes in these microorganisms and also their easy application in the laboratory [55, 56]. Anil Kumar et al. (2007) used purified Nitrate Reductase enzyme obtained from *Fusariumoxysporum* for the synthesis of silver nanoparticles. The reaction mixture includes Nitrate Reductase, silver nitrate and NADPH. The color of reaction mixture turns to brown which is the first sign of Nitrate Reductase enzyme involved in the synthesis of silver nanoparticles [39]. The proteins separated from fungi culture are able to synthesize metal nanoparticles. Gaikwad Sagar et al. (2012) used *Aspergillusniger* fungi for the synthesis of silver nanoparticles. 50 ml cell filtrate was mixed with 10 ml of 10 mM $AgNO_3$. For purification purposes, synthesized silver nanoparticles were two times placed in centrifuge at 10000 rpm for 10 minutes. The size range of synthesized silver nanoparticles was 1-20 nm and their shape was spherical [76]. Table 2 shows Synthesis of metallic nanoparticles by different kinds of Fungi.

Table 1. Synthesis of Ag nanoparticles using different kinds of Bacteria

Organism	Size range(nm)	Ref./year
Pseudomonas stutzeri AG259	200	(34)/ 2000
Bacillus megaterium	46.9	(41)/1999
Klebsiella pneumonia (culture supernatant)	(52.5) 28.2-122	(38)/2007
Bacillus licheniformis	50	(42)/2008
Bacillus licheniformis	40-50	(35)/2008
Corynebacterium sp.	10-15	(43)/2005
Bacillus subtilis (culture supernatant)	5-60	(44)/2009
Morganella sp.	20 ± 5	(45)/2008
Proteus mirabilis	10-20	(46)/2009
Staphylococcus aureus	1-100	(47)/ 2009
Salmonella typhirium	50-150	(48)/2013
Lactobacillus Sps.	2-20	(49)/2012
Idiomarina sp. PR58-8	25-26	(50)/2012
Corynebacterium sp.	24.46-32.05	(51)/2012
Marine Cyanobacterium, OSCILLATORIA WILLEI NTDM01	100-200	(52)/2010
Pseudomonas aeruginosa.	20-50	(29)/(2012)
Spirulinaplatensis	average size=12	(53)/2012
Bacillus strain CS 11	42-92	(54)/2013

Table 2. Synthesis of Ag nanoparticles by different kinds of Fungi

Organism	Size range(nm)	Ref./year
Fusariumoxysporum	20-50	(57)/2005
Fusariumoxysporum	2-5	(58)/2007
Aspergillusfumigatus	5-25	(59)/2006
Verticillium	25±12	(60)/2001
Phanerochaetechrysosporium	100	(61)/2006
Aspergillusflavus	8.92± 1.61	(62)/2007
Cladosporiumcladosporioides	10-100	(63)/2009
Fusariumsemitectum	10-69	(64)/2008
Trichodermaviride	5-40	(65)/2010
Penicilliumfellutanum	1-100	(66)/2009
Fusariumsolani	5-35	(67)/2009
Fusariumacuminatum	5-40	(68)/2008
Aspergillusclavatus	10-25	(69)/2010
Penicilliumcitrinum (MTCC9999)	20-30	(70)/2013
TrichodermaHarzianum	30-50	(71)/2011
TrichodermaReesei	5-50	(72)/2011
Sphaerulinaalbispiculata	6-12	(73)/2012
Penicillium sp.	58.35 ± 17.88	(74)/2010
Aspergillusniger	3-30	(75)/2010
Aspergillusniger	1-20	(76)/2012

- **Yeast**

There are a few reports about the synthesis of metal nanoparticles with yeast; among which the extracellular synthesis of silver nanoparticles by yeast resistance to silver *MKY3* can be mentioned. In this report, the size of produced silver nanoparticles ranged from 2 to 5 nm and the concentration of silver solution was 1 mM [77].

- **Plants**

The advantage of using plants in the synthesis of silver nanoparticles is that they are easily accessible and include a wide spectrum of metabolites that help the revival of metal nanoparticles Table 3.

In 2003, a natural resource, i.e. *Alfalfa Sprouts*, was used for the synthesis of silver nanoparticles. The particles are usually found in certain areas of the plants, i.e. the inner anatomy of *Alfalfa stem*. Plants absorb silver atoms in certain channels, therefore, silver cores or a collection of particles are absorbed inside these channels. However, the particles could also be found outside channels. This observation is either due to the decomposition of plant structure which caused the silver nanoparticles to move out of them or the penetration of silver nanoparticles into different places [78]. Using *Cycas leaf extract*, Jha et al. (2009) biosynthesized the silver nanoparticles. 20 ml of 0.25 M $AgNO_3$ was added to the obtained extract and then it was placed in the water bath for 20 minutes (until the color of solution turned into brown). Finally, the size of produced silver nanoparticles was reported to be 2-6 nm [79].

Table 3. Synthesis of Ag nanoparticles using plants

Organism	Size range(nm)	Reff./year
Alfalfa Sprouts	2-20	(78)/2003
Pelargonium graveolens	16-40	(80)/2003
Azadirachtaindica (Neem)	5-35	(81)/2004
Emblicaofficinalis	10-25	(82)/2005
Aloe vera	15.2±4.2	(83)/2006
Cinnamomumcamphora	55-80	(84)/2007
Cinnamomumcamphora Leaf	5-40	(85)/2008
Carica papaya	60-80	(86)/2009
Gliricidiasepium	10-50	(87)/2009
Jatrophacurcas	10-20	(88)/2009
Apin (from henna leaves)	21-39	(89)/2009
Ocimum	3-20	(90)/2011
Cassia auriculata	20-40	(91)/2011
Eucalyptus chapmaniana leaves extract	60	(92)/2013
Mangosteen leaf extract	35	(93)/2010
OleaeuropaeaLeaves Extract	10±1	(94)/2012
MORINGA OLEIFERA L. LEAF	40-50	(95)/2013
Callicarpamaingayi Stem Bark Extraction	12.4 ± 3.27	(5)/2012
Leaves Of TECOMA STANS (L.) KUNTH	average particle size=15	(96)/2013
CynodonDactylon Leaf Extract	30-50	(97)/2013
Leaves OfMurrayaPaniculata (L.) JACK	20-50	(98)/2013
Olive leaf extract	20-25	(99)/2013
carob leaf	5-40	(100)/2013

- **The effect of the concentration of silver precursor**

It has been confirmed that the concentration of silver precursor directly affects the size of synthesized silver nanoparticles. Using chemical methods, Ajitha et al. (2013) investigated the effect of silver precursor concentration on the size of produced silver nanoparticles. In their study,they used 3 different concentrations of silver nitrate (0.01, 0.02 and 0.03 M). The results of the experiments and tests showed that the average size of particles increased from 23 to 44 nm by an increase of silver precursor from 0.01 to 0.03M [101]. Similar investigations have been conducted in the synthesis of silver nanoparticles through biologic methods which confirm the results of Ajitha et al. research.

Using *Rumexhymenosepalus extracts*, Rodríguez-León et al. could synthesize 2-40 nm silver nanoparticles. These researchers investigated the effect of different concentrations (2.5, 5, 7.5, 10 and 15 mM) of $AgNO_3$ on the synthesis of silver nanoparticles and observed that a rise in silver nitrate concentration, increasesthe peak of UV-Vis apparatus,which is indicative of the increase of produced silver nanoparticles' size [102].

In another article, Ghorbani et al. used 0.001, 0.005 and 0.01 M of silver nitrate for investigating this effect and observed that when the concentration increases from 0.001 to 0.005, the absorbent peak (λmax) increases from 422 to 436 nm. Furthermore, the concentration of 0.01M was studied using UV-Vis spectroscopy and no peak was observed at this concentration, which indicatesthat the morphology of the particles in micrometer [25]. The results of all investigations seem to confirm the fact that there is a direct relationship between the silver precursor concentration (in these investigations, silver nitrate) and the size of produced silver nanoparticles.

- **Studying the stabilizer amount**

The stabilizers are often used in synthesis processes of silver particles to prevent particle accumulation. At their presence, the probability of collision and coalescence of nanoparticles reduces due to the reactions of stabilizer function groups and silver nanoparticles. Thus, they prevent the connection of silver particles and sediment formation [103- 107]. Various polymers such as: Poly Vinyl Pyrrolidone (PVP) [108], Poly Vinyl Alcohol (PVA) [109], polyethylene glycol [110], Poly (Methyl Methacrylate) [111], Polyaniline [112] and Polyacrylonitrile [113] have been used as stabilizers in the synthesis of silver nanoparticles. Among all stabilizer polymers of silver nanoparticles, PVP has the best function due to its unique structure [103, 114, 115]. Dagmara Malina et al. investigated the effect of different concentrations of PVP (0.1, 0.2, 0.5, 0.7, 1, 1.5, 3, 5, 10 and 15%) on the synthesized silver nanoparticles through chemical method. To this end, they investigated the effect of different concentrations of PVP (stabilizer) on the size of silver nanoparticles. The results indicated that size difference, size distribution and capacity depend on the PVP concentration for accumulation. Furthermore, in this study, it becomes clear that when the stabilizer concentration (PVP) is 1-10% , it leads to synthesis of nanoparticles in nano- size and Z- Average is between 20 to 30 ± 0.43-0.21 [116].

In some methods of biologic synthesis of silver nanoparticles, PVP has been used to provide stability and prevent the connection of synthesized silver ions, which results in sediment-like formation [25]. In most studies, the PVP concentration used is 1%, and therefore, this effect is not investigable.

The relationship between the size and shape of silver nanoparticles with their anti-bacterial properties

Amany A. El-Kheshen and Sanaa F. Gad El-Rab studied the anti-bacterial activity of silver nanoparticles on the gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria, *E. Coli* and concluded that the antibacterial activity of silver nanoparticles increases with increase of their area to the volume. Furthermore, they observed that the control area of silver nanoparticles was more for *Staphylococcus Aureus* (a gram positive bacterium) than *Escherichia coli* (gram negative bacteria) [18]. Hang Lee-U et al. (2010) studied three different sizes of silver nanoparticles: (1) small, 1.16±3.08 nm, (2) average, 1.12±5.75 nm and (3) large, 6.06±24.85 nm to investigate the anti-bacterial effect and their poisonous effect. In this study, it becomes clear that smaller silver nanoparticles have better antibacterial effects and higher cellular poisonous effect. Furthermore, it can be demonstrated that by the increase of the size of silver nanoparticles, their

antibacterial activity reduces and this confirms the previous findings indicating that in smaller silver nanoparticles, the profanity of interaction with bacteria increases due to the higher ratio of area to volume which results in aggregation of antibacterial effect [19].

Tyler S. Radniecki et al. (2011) concluded that silver nanoparticles of, 20 nm have a higher poisonous effect than silver nanoparticlesof 80 nm, and this can be attributed to the higher ratio of area to volume in small particles. Furthermore, it is noticeable that the preventive effect of silver nanoparticles on bacteria depends on the concentration of nanoparticles and the initial number of the bacteria, so that the growth of bacteria cells resumes with the decrease in concentration of nanoparticles [117].

Sukdeb Pal et al. (2007) proved that there is a good agreement between the shape of silver nanoparticles and their antibacterial properties. They investigated three different shapes (spherical, triangulate and rod) of silver nanoparticles with the aim of comparing their antibacterial effect. They observed that triangular nanoparticles have the most antibacterial effect in comparison with spherical and rod shapes [118]. Ahmed et al. (2010) showed that the poisonous property of coated silver nanoparticles is more than the uncoated ones, which is due to their accumulation [119]. Furthermore, in a similar study by Aranout et al. (2012), the effects of three different coatings (PVP, gum arabicum (GA) and acid citric) on silver nanoparticles were investigated. They observed that the citrate and gum arabicum result in the increase of the poisonous effect of silver nanoparticles on *Nitrosomonaseuropaea* bacteria [120].

CONCLUSIONS

As mentioned before, the antibacterial effect of silver nanoparticles depends on their shapes [118], coatings [119,120] and size [19,117]. So, the size of synthesized silver nanoparticlesis one of the reasons for the significance of production of silver nanoparticles with controlled size. In this investigation, the effect of the factors, including temperature, pH, organism type, silver precursor concentration and the amount of stabilizer on the size of synthesized silver nanoparticles was investigated using a biological method.

As the results indicate, in higher temperature and pH, the synthesis of silver nanoparticles is observable with smaller sizes [27, 29], which can attributed to the increase of silver ions' dynamism and shaping of nucleation areas due to accessibility of –OH ions and temperature increase [40]. The investigation of silver precursor concentration indicates a direct relationship between the size of synthesized silver nanoparticles and concentration of silver precursor**.**

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