RESEARCH ARTICLE

Comparison of the antibacterial activity against Escherichia coli of silver nanoparticle produced by chemical synthesis with biosynthesis

Nguyen Phuc Quan [1],[2], Tran Quoc Vinh [1], Kieu Thi My Yen [1], Le Vu Khanh Trang [2], Nguyen Minh Ly [2], Tran Cong Khanh [1]

[1] University of Science, Vietnam National University Ho Chi Minh City

[2] University of Education –University of Da Nang

Abstract: The synthesis of silver nanoparticles (Ag NPs) has been carried out using different methods, mainly by biological and chemical methods; however, comparing antibacterial activity of Ag NPs synthesized by these methods has not been conducted before. In this study, silver nanoparticles (Ag NPs) were synthesized by methods using reducing agent NaBH4/carboxymethyl cellulose (CMC) and fungal strain Trichoderma asperellum (T.asperellum). The formation of silver nanoparticles was observed visually by color change and identified by Ultraviolet-visible (UV – vis) spectroscopy. The transmission electron microscopy (TEM) image illustrated almost nanoparticles with spherical shape and average diameter of 4.1 ± 0.2 nm and 2.1 ± 0.2 nm of samples produced from chemical reduction and biosynthesis respectively. Both samples after 180 days storing have been separated lightly, but the agglomeration and absorbance peak shifting were not observed which proved the high stability of synthesized Ag NPs. Antimicrobial activity against human bacterial pathogen Escherichia coli (E. coli) showed that the inhibition zone produced by "biosynthesis" and "chemical reduction" Ag NPs were 3.17 cm and 2.42 cm respectively. With nanoparticles size smaller than 2 mm, antibacterial activity of "biosynthesis" Ag NPs against E. coli was 31 % higher than "chemical reduction" Ag NPs, although the concentration of Ag NPs produced by biosynthesis was about 10-fold less.

Keywords: Silver nanoparticles; Trichoderma asperellum; Carboxymethyl cellulose; Antibacterial activity; Plasmon surface

1. Introduction

Silver nanoparticles (Ag NPs) have been known to be used for abundant electronic, medical, food, environmental, agricultural applications based on special physical, chemical properties including potential electrical conductivity, thermal conductivity and high bioactivity[1, 2]. In particular, synthesis of Ag NPs with excellent antibacterial properties, especially against strong pathogenic bacteria [3], and low toxicity to human and animals [4] has been a great interest in the improvement of agriculture and environment. The antimicrobial activity of Ag NPs depends on the shape, size and distribution of particle size. Therefore, it is necessary to control the properties of Ag NPs by selecting the appropriate synthetic method [5].

Ag NPs are synthesized by different methods including chemical synthesis (reduction of silver salts with reducing agents such as $NaBH_4$, N_2H_4, ethylene glycol, glucose…) [6, 7], physical method (evaporation – condensation, thermal decomposition…) [8, 9], photochemical method (irradiation by UV, visible light… of AgNO3/carboxymethylated chi-tosan…) [10, 11] and biological methods (reduction of silver salts by bacteria, fungi [12, 13], as well as plant extracts [14, 15]). In recent years, eco-friendly biosynthesis Ag NPs has been received much attention as resulted from very small nanoparticle products (2 - 5 nm) with low cost [16] and chemical methods is widely used based on simple process, controlled nanoparticles size and easy implementing on industrial scale. However, each method has its own limitations, in which the comparison of antibacterial property of Ag NPs

doi: 10.18063/msmr.v2i2.679

produced from biosynthesis and chemical method has not been conducted yet. Also, application of Ag NPs has been limited currently due to their low stability and easy deposition.

Therefore, with the aim of applying Ag NPs as antibacterial agents, this study focused on evaluating antibacterial ability against E. coli of Ag NPs produced by biosynthesis using fungal strain T.asperellum in comparison with chemical reduction using $NaBH_4$/CMC as well as evaluating the stability of synthesized Ag NPs samples following storing time.

2. Materials and methods

2.1 Materials and chemicals

Silver nitrate ($AgNO_3$, 99,0 %, Merck, Germany), carboxymethyl cellulose (CMC, Mw 90,00, Sigma-Aldrich), sodium borohydride ($NaBH_4$, 98.0 %, Scharlau, Spain), agar (99.0 %, HiMedia, India) and triple – distilled water with conductivity smaller than 1 S/cm were used.

2.2 Synthesis of silver nanoparticles

"Chemical reduction" Ag NPs were synthesized using $NaBH_4$ as a primary reductant and CMC as secondary reductant and stabilizer on the basic of the Pedroza – Toscano method [17]. The reduction process was carried out at 0 ºC. A volume of 23 mL of 20 mM $AgNO_3$ aqueous solution was loaded into the reactor. Then 68 mL of 0.12 % w/v CMC solution was added to the $AgNO_3$ solution about 30 minutes at room temperature with vigorous stirring to ensure a homogenous solution. The reaction was cooled down to 0 ± 2 ºC by an ice bath. Since temperature stability, a volume of 9 mL of 57 mM $NaBH_4$ solution was added dropwise into the reaction during vigorous stirring for 90 minutes until collecting homogenous brown yellow colloidal solution. Finally, the reaction was allowed to continue stirring at room temperature for 30 minutes.

"Biosynthesis" Ag NPs were synthesized employing fungal strain T.asperellum. T.asperellum was isolated and identified at Lab of Microbiology, Faculty of Biology-Environmental Science, The University of Education, Danang University, Vietnam [18]. T.asperellum was cultured in Czapek Dox liquid medium at temperature 28 ± 1 ºC in a rotary shaking operated at 140 rpm for 120 hours. Biomass of T.asperellum was harvested by filter paper Whatman 10, then clean the biomass by distilled water for 3 – 4 times. Add 10 g of T.asperellum biomass into an Erlenmeyer flask containing 100 mL of distilled water and inoculate for 24 hours. Then collect the supernatant by filter paper Whatman 10. Finally, add 100 mL of 1mM $AgNO_3$ in 100 mL of the supernatant and inoculate in the dark at temperature 28 ± 2 ºC.

2.3 Characterization of Ag NPs

The formation of Ag NPs was observed by UV–vis absorption spectra (Jasco V-670, Japan). A volume of 1mL of Ag NP solution was taken and diluted to 100 mL by distilled water; a 6 mL aliquot was used for the measurement. UV-Vis absorbance spectrum also was used to evaluate the stability of Ag NPs after 180 days of storing at room temperature. Ag NPs solution was separated into small layers from top to down.

The morphology and particle size were observed by transmission electron microscopy (TEM, JEOL JEM-1400) operated at an accelerating voltage of 100 kV. The samples of TEM characterization were prepared by placing a drop of the Ag NP solution onto a formvar–coated copper grid, which was then evaporated at room temperature. TEM images of the nanoparticles were used for the size distribution measurements. Over five hundred particles were measured to obtain the average particle size and size distribution.

2.4 Antibacterial activity of Ag NPs

The antibacterial activity of Ag NPs was tested by the standard zone of inhibition (ZOI) method on LB agar (Luria – Bertani) [19]. LB agar plates were inoculated with Escherichia coli (E. coli) under aseptic conditions and wells (diameter = 1 cm) were filled with 3 mL of the test samples and incubated at 30 ± 1 ºC for 24 hours. The test

sample included Ag NPs samples, 1 mM $AgNO_3$, distilled water and the supernatant of T.asperellum culture medium. After the incubation period, the diameter of the growth inhibition zones was measured.

3. Results and discussion

3.1 Synthesis of Ag NPs

3.1.1 Chemical reduction of Ag NPs by $NaBH_4$/CMC

CMC has been used for chemical synthesis of Ag NPs as the stable chemical/reducing agent stability. Adding silver nitrate into a reaction flask, CMC with COO- groups created complex bonds with Ag+[20]; Ag+ was reduced by the hydroxide groups (-OH or -CH2-OH) to generate Ag atoms which acted as nucleation centers where other Ag+ ions adhered to[21]. As shown in Figure 1a, the transition from transparent to light pink mixture of silver nitrate and CMC indicated that CMC acted as reducing Ag+ to Ag nanoparticles. A color change to russet occurred for adding NaBH4 as a reducing agent that proved that Ag+ ions were reduced and obtained a uniform colloidal solution.

(a) (b)

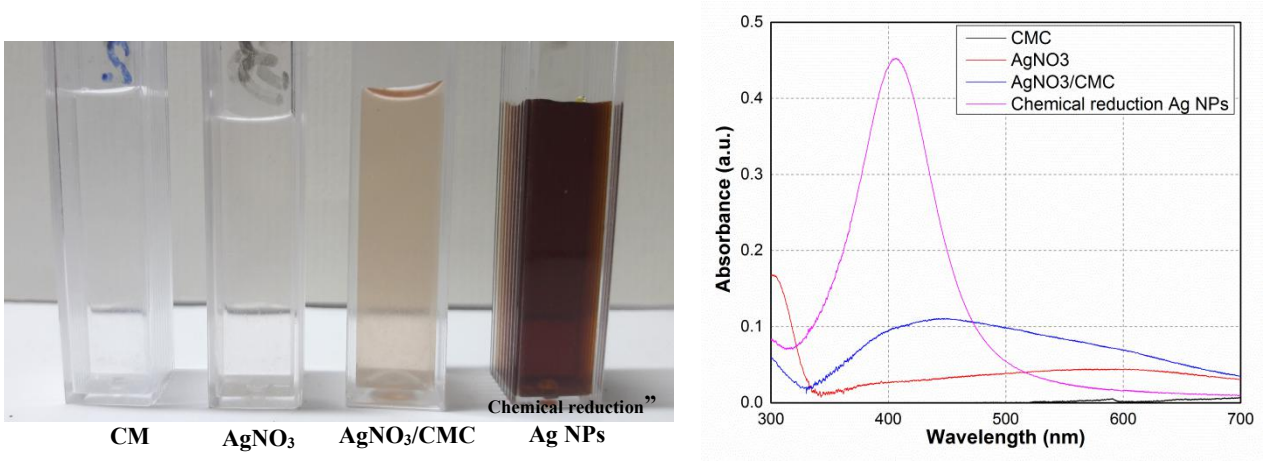


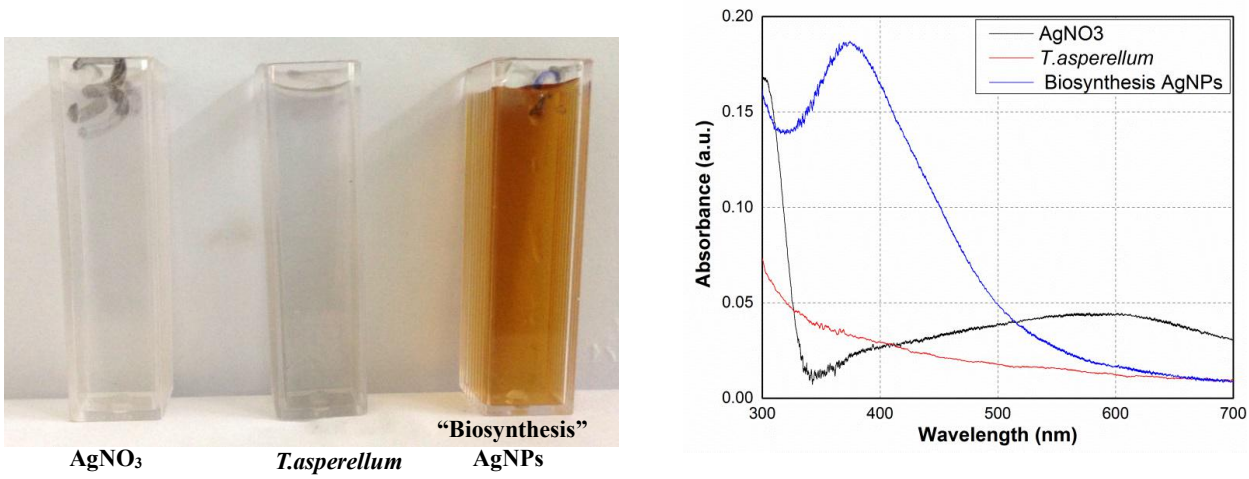
Figure 1; Colour change (a) and UV-Vis absorption spectrum of the reaction solution (b) indicating the formation "chemical reduction" Ag NPs**.**

Figure 1b showed UV-Vis spectrum of the reaction solution during formation of "chemical reduction" Ag NPs. The reducing ability of CMC was verified by the presence of absorption bands. In UV-Vis spectrum as-prepared Ag NPs colloidal solution was characterized by an intensive surface plasmon resonance of Ag NPs with a peak at 404 nm.

3.1.2 Biosynthesis of Ag NPs by T.asperellum

Ag NPs were synthesized by denitrification of nicotinamide adenine dinucleotide (NADH) of the supernatant of T.asperellum. Converting from NADH to NAD+ was released electrons which combined with Ag+ to form Ag atoms, while NO3- was reduced to N2[22, 23].

(a) (b)



$AgNO_3$ *T.asperellum*

Figure 2; Colour change (a) and UV-Vis absorption spectrum of the reaction solution (b) during formation of "biosynthesis" Ag NPs.

Figure 2 showed UV-Vis spectra of solution during synthesis procedure of "biosynthesis" Ag NPs. The change of the reaction solution color from transparent to yellow and an absorption peak at 373 nm of synthesized solution which clearly indicated the formation of Ag NPs [24].

3.2 Characterization of Ag NPs

3.2.1 Morphology of Ag NPs

The size and morphology of Ag NPs were observed using TEM shown in Figure 3. The TEM images indicated the presence of spherical and monodispersed particles of the "chemical reduction" Ag NPs with an average diameter of 4.1 ± 0.2 nm, the "biosynthesis" Ag NPs with an average diameter of 2.1 ± 0.2 nm.

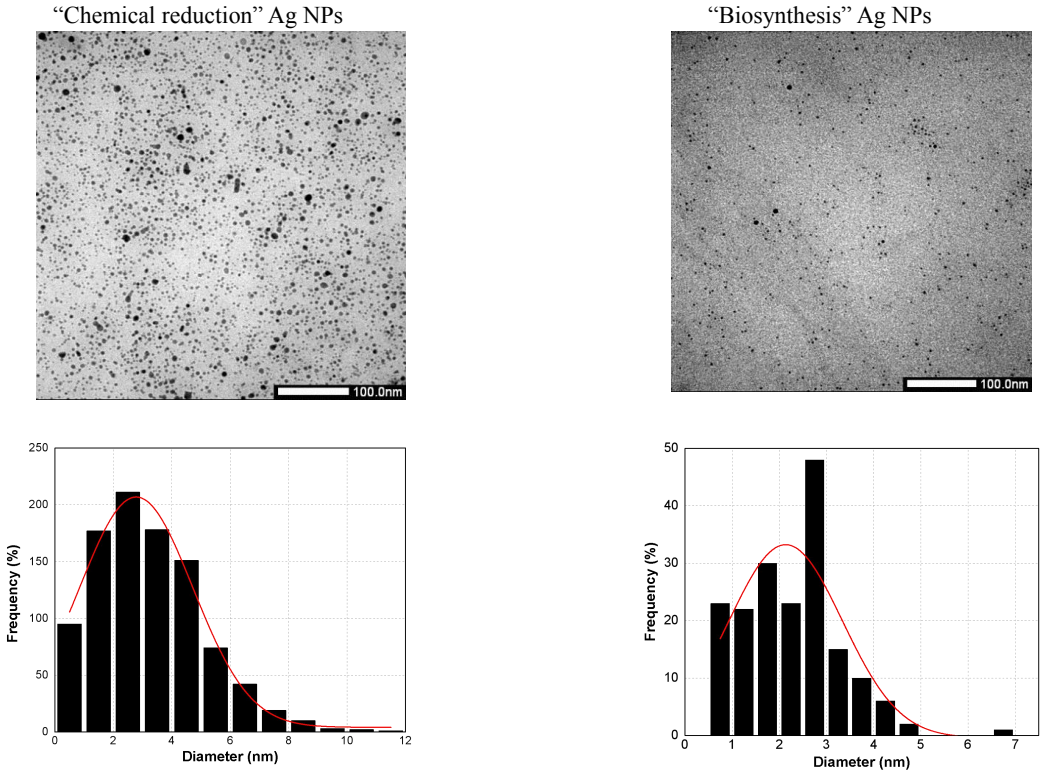


Figure 3;TEM images and graph of particle size distribution of Ag NPs**.**

The effect of nanoparticle size on the properties of Ag NPs has been studied carefully by different research groups [25, 26, 27]. Decreasing Ag NPs size from 100 nm to 5 nm resulted in the absorbance peak of Ag NPs shifting from 462 nm to shorter wavelength 393 nm. However, the effect of Ag NPs size smaller than 5 nm on their properties has not been carried out. In this study, the absorption maxima of Ag NPs shifted from 404 nm to shorter wavelength 373 nm with Ag NPs size decreased from 4.1 ± 0.2 nm to 2.1 ± 0.2 nm.

3.2.2 The stability of Ag NPs

The stability of Ag NPs by the time was evaluated by comparing the intensity and the shift in wavelength of absorbance peak in UV-Vis absorbance spectra of small solution separated from layers of "chemical reduction" and "biosynthesis" Ag NPs after 180 days storing (**Figure 4**). For "chemical reduction" Ag NPs, the intensity of absorbance (I) of separated layers was 0.80, 0.83 and 0.94 from top to down respectively, while the absorbance peak was still observed at 404 nm. Comparing to the intensity of absorbance of the as-synthesized Ag NPs solution (I^o = 0.87) revealed an insignificant change of the absorbance intensity and non-move of absorbance peak of the samples after 180 days storing. These indicated that the layers separation of "chemical reduction" Ag NP solution were insignificant after 180 days storing and the nanoparticles were not conglomerated which proved the high stability [28].

The intensity of absorbance (I) of layers of "biosynthesis" Ag NPs solution increased gradually from top to down after 180 days storing as 0.165, 0.182 and 0.186 respectively, specifically, the intensity of absorbance of bottom layer was higher than the intensity of absorbance of surface layer about 11,3 %. Therefore, "biosynthesis" Ag NPs solution was separated slightly to layers during storage. However, the conglomeration of "biosynthesis" Ag NP solution was not observed visually.

(a) "Chemical reduction" Ag NPs (b) "Biosynthesis" Ag NPs

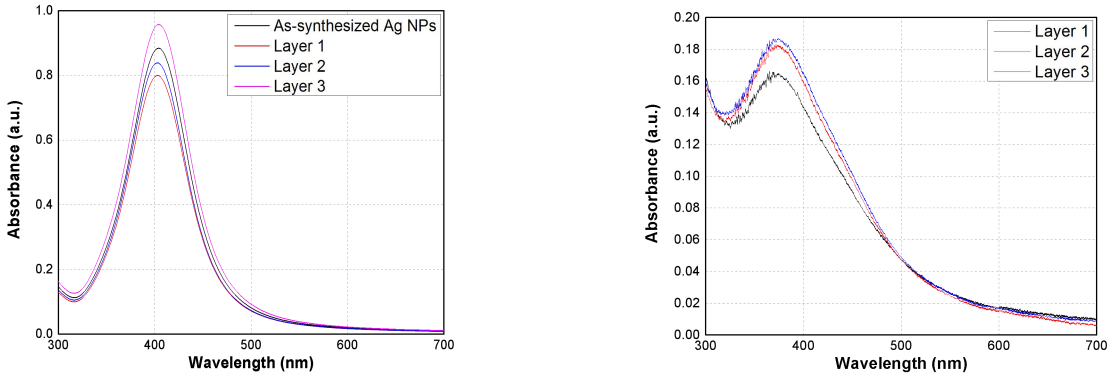


Figure 4; UV-Vis absorption spectrum of Ag NP solutions after180 days storing with separated layers from top to down respectively.

3.3 Antibacterial activity of Ag NPs

The antibacterial activity against E. coli of samples was conducted by a well diffusion method which could be observed in **Figure 5**. The supernatant of T.asperellum culture medium and $AgNO_3$ solution could not inhibit the growth of E. coli. Clearly, the inhibition was recorded by chemical reduction and biosynthesis of Ag NPs.

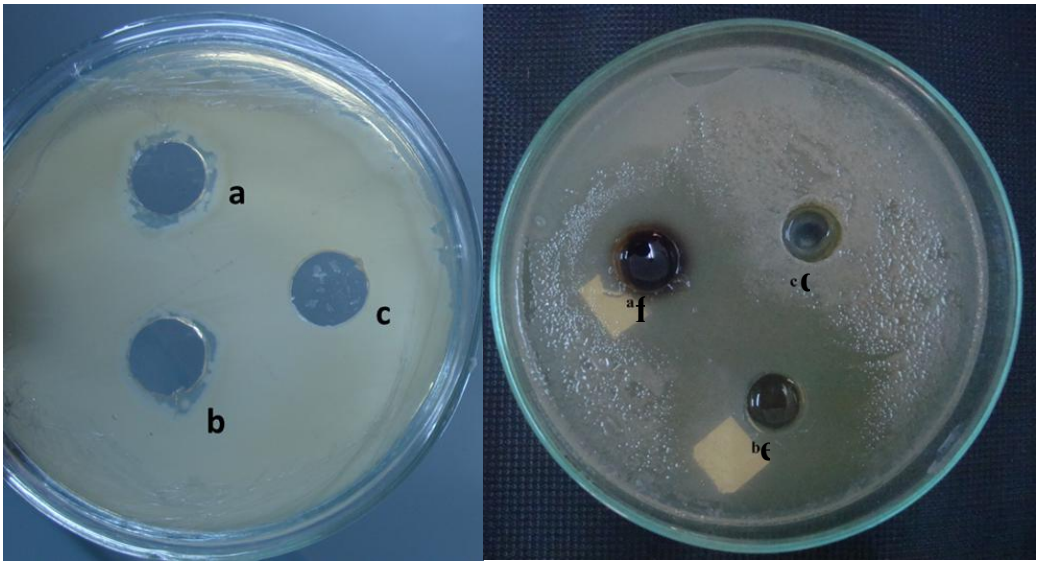


Figure 5; Inhibition zone of samples against E. coli: a) $AgNO_3$ 1mM; b) T.asperellum; c, d) distilled water; e) "biosynthesis" Ag NPs; f) "chemical reduction" Ag NPs**.**

The result of Table 1 showed that the antibacterial ability against E. coli of "biosynthesis" AgNPs was 31% higher than "chemical reduction" Ag NPs.

Microorganism	Zone of inhibition*, cm				
	Control samples			Ag NPs samples	
	Distilled water	$AgNO_3$ solution	Supernatant of T.asperellum culture medium	"biosynthesis" Ag NPs	"chemical reduction" Ag NPs
E.coli	1.0	1.2	1.3	3.17 ± 0.26	2.42 ± 0.18

* Diameter of well 1.0 cm

Table 1. Inhibition zone diameters produced by Ag NPs

"Chemical reduction" Ag NPs was synthesized based on the method of Pedroza–Toscano resulted in Ag concentration of 500 ppm with the yield 90%. By contrast, "biosynthesis" Ag NPs had Ag concentration of 54 ppm which was about 10-fold less than "chemical reduction" Ag NPs. However, "biosynthesis" Ag NPs with nanoparticle size smaller 2 nm represented more effective inhibition of E. coli. Thus, Ag NPs smaller than 5 nm resulted in excellent antibacterial property and depending on size effect.

4. Conclusion

Ag NPs produced by chemical synthesis using NaBH4/carboxymethyl cellulose and biosynthesis using T.asperellum were also spherical particles an average diameter 2 ÷ 6 nm. Ag NP solutions represented high stability after 180 days storing. The antibacterial activity of "biosynthesis" Ag NPs against E. coli was higher than "chemical reduction" Ag NPs with the inhibition zones of 3.17 cm and 2.42 cm respectively. Simple, rapid synthesis with very small particle size of 2 ÷ 6 nm are advantages of above methods, in particular, the friendly biosynthesis using T.asperellum not requiring chemical reducing will bring promising approach in high-tech agriculture.

References

1. Quang Huy Tran, Van Huy Nguyen and Anh Tuan Le, Silver nanoparticles : synthesis, properties, toxicology, applications and perspectives, Advances in Natural Sciences: Nanoscience and Nanotechnology, Vol. 4, №. 3, 2013.
2. Maqusood Ahamed, Mohamad S. Alsalhi and M.K.J. Siddiqui, Silver nanoparticle applications and human health, Clinica chimica Acta, Vol. 411, №. 23- 24, pp. 1841-1848, 2010.

3. Catalina Marambio- Jones, Eric M. V. Hoek, Areview of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment, Vol. 12, №. 5, pp. 1531-1551, 2010.
4. Shivendu Ranjan, Nandita Dasgupta, Eric Lichtfouse, Nanoscience in Food and Agriculture 5, Sustainable Agriculture Reviews, SARV, Vol. 26, №. 5, pp. 2210-4429, 2016.
5. Kholoud M.M. Abou El-Nour, Ala'a Eftaiha, Abdulrhman Al-Warthan, and Reda A.A. Ammar, Synthesis and applications of silver nanoparticles, Arabian Journal of Chemistry, Vol. 3, №. 3, pp. 135–140, 2010.
6. Shao- Feng Chen and Hongyin Zhang, Aggregation kinetics of nanosilver in different water conditions, Advances in Natural Sciences: Nanoscience and Nanotechnology, Vol. 3, №. 3, 2012.
7. Thi My Dung Dang Dang, Thi Thu Tuyet Le, Eric Fribourg-Blanc and Mau Chien Dang, Influence of surfactant on the preparation of silver nanoparticles by polyol method, Advances in Natural Sciences: Nanoscience and Nanotechnology, Vol. 3, №. 3, 2012.
8. Don Keun Lee and Young Soo Kang, Synthesis of Silver Nanocrystallites by a New Thermal Decomposition Method and Their Characterization, Vol. 26, №. 3, 2004.
9. Der-Chi Tien, Kuo-Hsiung Tseng, Chih-Yu Liao, Jen-Chuen Huang, Tsing-Tshih Tsung, Discovery of ionic silver in silver nanoparticle suspension fabricated by arc discharge method, Journal of Alloys and Compounds, Vol. 463, №. 1-2, pp. 408-411, 2008.
10. Roberto Sato-Berrú, Rocío Redón, América Vázquez-Olmos and José M. Saniger, Silver nanoparticles synthesized by direct photoreduction of metal salts. Application in surface-enhanced Raman spectroscopy, Journal of Raman Spectroscopy, Vol. 40, №. 4, pp. 376-380, 2009.
11. Ling Huang, Maolin L.Zhai, Dewu W.Long, Jing Peng, Ling Xu, Guozhong Z Wu, Jiuqiang Q. Li, Genshuan S. Wei, UV-induced synthesis, characterization and formation mechanism of silver nanoparticles in alkalic carboxymethylated chitosan solution, Journal of Nanoparticle Research, Vol. 10, №. 7, pp. 1193-1202, 2008.
12. Amanulla Mohammed Fayaz, Kulandaivelu Balaji, Morukattu Girilal, Ruchi Yadav, Pudupalayam Thangavelu Kalaichelvan, Ramasamy Venketesan, Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria, Nanomedicine: Nanotechnology, Biology, and Medicien, Vol. 6, №. 1, pp. 103-109, 2010.
13. Nalenthiran Pugazhenthiran, Sambandam Anandan, Govindarajan Kathiravan, Nyayiru Kannaian Udaya Prakash, Simon Crawford and Muthupandian Ashokkumar, Microbial synthesis of silver nanoparticles by Bacillus sp, Journal of Nanoparticle Research, Vol. 11, pp. 1811-1815, 2009.
14. T Peter Amaladhas, S Sivagami, T Akkini Devi, N Ananthi and Spriya Velammal, Biogenic synthesis of silver nanoparticles by leaf extract of Cassia angustifolia, Advances in Natural Sciences: Nanoscience and Nanotechnology, Vol. 3, №. 4, 2012.
15. M Umadevi, S Shalini and M R Bindhu, Synthesis of silver nanoparticle using D. carota extract, Advances in Natural Sciences: Nanoscience and Nanotechnology, Vol. 3, №. 2, 2012.
16. Subin Poulose, Tapobrata Panda, Praseetha P. Nair and Thomas Théodore, Biosynthesis of silver nanoparticle, Journal of nanoscience and nanotechnology, Vol. 14, №. 2, pp. 2038-2049, 2014.
17. M. A. Pedroza-Toscano, S. López-Cuenca, M. Rabelero-Velasco, E. D. Moreno-Medrano, A. P. Mendizabal-Ruiz and R. Salazar-Peña, silver nanoparticles obtained by semicontinuous chemical reduction using carboxymethyl cellulose as a stabilizing agent and its antibacterial capacity, Journal of Nanomaterials, Vol. 2017, Article ID 1390180, 7 pages, 2017, DOI: 10.1155/2017/1390180.
18. Thi Van Doan, Chau Tuan Vo, Vu Khanh Trang Le, Le Nguyen Vo, Thi Tu Uyen Pham. Study on microbial preparations production against fungi Fusarium sp. affecting the sprout of coffe seed in Kon Ray district, Kon Tum province. Vietnam Conference on Plant Pathology 2017. Vol. 16, pp. 258-266, 2017. Vietnamese.
19. Dabhi BK, Jambukiya J and RV Vyas, Anti-bacterial action of silver nanoparticles, International Journal of Chemical Studies, Vol. 5, №. 4, pp. 1740-1743, 2017.
20. M. A. Garza-Navarro, J. A. Aguirre-Rosales, E. E. Llanas-Vázquez, I. E. Moreno-Cortez, A. Torres-Castro, and V. González-González, Totally ecofriendly synthesis of silver nanoparticles ´from aqueous dissolutions of polysaccharides, International Journal of Polymer Science, Vol. 2013, Article ID 436021, 8 pages, 2013.
21. A. A. Hebeish, M. H. El-Rafe, F. A. Abdel-Mohdy, E. S. Abdel Halim, and H. E. Emam, Carboxymethyl cellulose for green synthesis and stabilization of silver nanoparticles, Carbohydrate Polymers, Vol. 82, №. 3, pp. 933–941, 2010.
22. L. Karthik, Gaurav Kumar, A. Vishnu Kirthi, A.A. Rahuman, K.V. Bhaskara Rao, Streptomyces sp. LK3 mediated synthesis of silver nanoparticles and its biomedical application, Bioprocess and Biosystems Engineering, Vol. 37, №.2, pp. 261-267, 2014.
23. Guangquan Li, Dan He, Yongquing Qian, Buyuan Guan, Song Gao, Yan Cui, Koji Yokoyama and Li Wang, Fungus-mediated green synthesis of silver nanoparticles using Aspergillus terreus, International Journal of Molecular Sciences, Vol. 13, №. 1, pp. 466-476, 2012.
24. T prameela Devi, S Kulanthaivel, Deeba Kamil, Jyoti Lekha Borah, N Prabhakaran and N Srinivasa, Biosynthesis of silver nanoparticles from Trichoderma species, Indian Journal of Experimental Biology, Vol. 51, pp. 543-547,

2013.
25. Muhammad Akram Raza, Zakia Kanwal, Anum Rauf, Anjum Nasim Sabri, Saira Riaz and Shahzad Naseem, Size- and Shape-Dependent Antibacterial Studies of Silver Nanoparticles Synthesized by Wet Chemical Routes, Nanomaterials, Vol. 6, №. 4, pp. 74, 2016.
26. Shekhar Agnihotri, Soumyo Mukherji and Suparna Mukherji, Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy, RSC Adv., Vol. 4, pp. 3974-3983, 2014.
27. J. Helmlinger, C. Sengstock, C. Groß-Heitfeld, C. Mayer, T. A. Schildhauer, M. Köller and M. Epple, Silver nanoparticles with different size and shape: equal cytotoxicity, but different antibacterial effects, Royal Society of chemistry advances, Vol. 6, pp. 18490-18501, 2016.
28. Amro M. El Badawy, Todd P. Luxton, Rendahandi G. Silva, Kirk G. Scheckel, Makram T. Suidan, Thabet M. Tolaymat, Impact of Environmental Conditions (pH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions, Environmental Science and Technology, Vol. 44, pp. 1260-1266, 2010.