Digest Journal of Nanomaterials and Biostructures Vol. 5, No 2, April-June 2010, p. 427 – 432

BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES BY USING ONION (ALLIUM CEPA) EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

ANTARIKSH SAXENA, R.M. TRIPATHI*, R. P. SINGH
Amity Institute of Nanotechnology, Amity University, Noida – 201301, U.P, India

The synthesis of metal and semiconductor nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. We have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of onion (*Allium cepa)* extract. The characterization of nanoparticles was done by using UV- Vis Spectrophotometer and Dynamic Light Scattering (DLS). The morphology of silver nanoparticles was confirmed by Transmission Electron microscopy (TEM). The antibacterial activity of these nanoparticles was studied against E.coli and Salmonella typhimurium. The bactericidal property of nanoparticles was analyzed by measuring the growth curve of bacteria and 50µg/ml concentration of silver nanoparticles was found to be effective antibacterial.

(Received April 3, 2010; accepted May 3, 2010)

Keywords: Silver nanoparticles, Biological Synthesis, Onion Extract, TEM, DLS, Antibacterial activity, Salmonella typhimurium, E.coli and Bacterial growth curve.

1. Introduction

Application of Nano scale material and structures are usually ranging from 1-100nm and is emerging area of nanoscience and nanotechnology. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms; have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties [1-4]. Synthesis of noble nanoparticles for the applications such as catalysis, electronics, environmental and biotechnology is an area of constant interest [5-6]. Generally, metal nanoparticles are synthesized and stabilized by using chemical methods such as chemical reduction [7-8], electrochemical techniques [9], photochemical reactions in reverse micelles [10] and now days via green chemistry route [11]. Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective and environment friendly easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Now days we are using bacteria, fungi for the synthesis of nanoparticles [12-18] but use of leaf extract [19-20] reduce the cost as well as we do not require any special culture preparation and isolation techniques.

Here we report synthesis of silver nanoparticles, reducing Ag^+ ions present in the aqueous solution of silver nitrate by the help of onion extract. Through elaborate screening process involving number of plants, we observed that onion (*Allium cepa)* was potential candidate for synthesis of silver nanoparticles. We also study the antibacterial property of silver nanoparticles toward E.coli and Salmonella typhimurium. Although, several previous reporter have studied the

*Corresponding author: ravi_gene@rediffmail.com, ravigene81@gmail.com

antibacterial activity of chemically synthesised silver nanoparticles [8, 21-26] but here we study the biologically (using onion extract) synthesised silver nanoparticles.

2. Experimental details

2.1Materials

For the synthesis of silver nanoparticles, we used onion (*Allium cepa)* as a extract for reducing and capping agent which is purchased from market and silver nitrate ($AgNO_3$) from Merck limited, India. Lyophilized culture of E.coli, Salmonella typhimurium was procured from the Microbial Type Culture Collection Center (MTCC) located at the Institute of Microbial Technology (IMTECH) Chandigarh, India. Luria bertani, Nutrient and Macconky media were used here and supplied by Hi-Media Laboratories.

2. 2 Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, silver nitrate ($AgNO_3$) and onion extract are taken. Onion extract was prepared by taking 25g of thoroughly washed and finely crushed onion mixed with 100ml deionized water in 500 ml of Erlenmeyer flask and then boiling the mixture for 10 min before finally decanting it. For the reduction of Ag^+ ions, 5ml of onion extract was mixed to 50 ml of 0.1mM aqueous of $AgNO_3$ solution drop wise with constant stirring at 50^0-60^0C and observe the colour change. The reduction of Ag^+ was observed by measuring the UV–Vis spectra of the solution.

2.3 Characterization

2.3.1 UV-Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-1601 pc shimadzu spectrophotometer) or ultraviolet-Visible spectrophotometry (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.

2.3.2 Dynamic light Scattering (DLS)

This is one of the most popular technique which is used to determine the size of particles. Shining a monochromatic light beam, such as a laser, onto a solution with spherical particles in Brownian motion causes a Doppler Shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. From DLS (Zetasizer, Malvern) it is possible to compute the sphere size distribution and give a description of the particle's motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function.

2.3.3 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) (Philips CM-10) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device.

2.4 Analysis of interaction of silver nanoparticles with Bacteria

2.4.1 Bacterial Growth Curve

The antibacterial activity of silver nanoparticles against E.coli and Salmonella typhimurium was analyzed by their growth curve. We have inoculated fresh colonies from agar media into 10ml of broth (Luria Bertani) media. The media is supplemented with 10-50µg/ml silver nanoparticles and bacterial cultures were incubated at 37^0C with continuous shaking at 150rpm. The growth of E.coli and Salmonella typhimurium in broth media was indexed by measuring the optical density (at λ=600nm) at regular intervals using UV-Vis spectrometer. Whereas control does not contain any exposure of silver nanoparticles synthesized from the onion extract.

3. Results and discussion

3.1 UV-Vis Spectrophotometry

The Formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of onion (*Allium cepa)* extract may be easily followed by UV–Vis spectroscopy (UV-1601 pc shimadzu spectrophotometer). UV-Vis absorption spectrum of silver nanoparticles in the presence of onion extract is shown in figure 1. The Surface Plasmon band in the silver nanoparticles solution remains close to 413nm throughout the reaction period, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum.

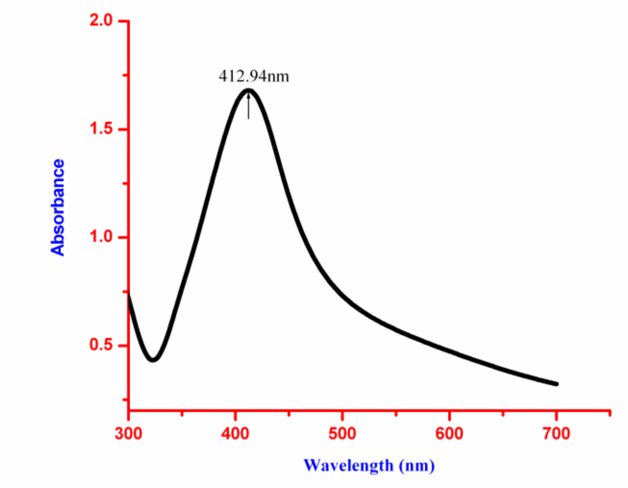

Fig.1. UV-Vis absorption spectra of Silver nanoparticles synthesized by exposure of onion broth with 0.1mM silver nitrate.

3.2 Dynamic Light Scattering (DLS)

Dynamic light scattering (also known as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) is a technique in physics which can be used to determine the size distribution profile of small particles in suspension (chemistry) or polymers in solution. Light scattering (Zetasizer, Malvern) technique was used to determine the size distribution profile of nanoparticles in suspension. The average mean size of silver nanoparticles comes out to 33.6 nm as shown in figure 2.

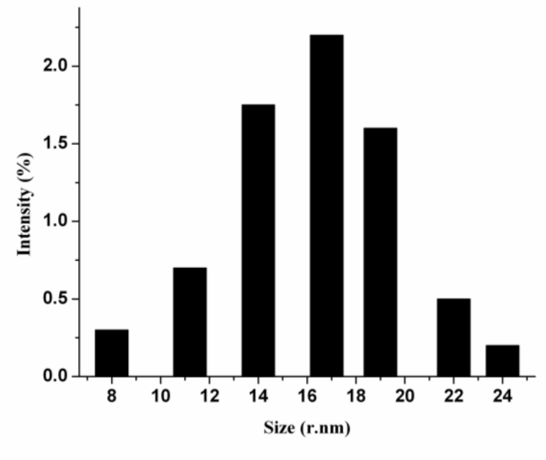

Fig. 2. DLS showing mean average size of silver nanoparticles.

3.3 TEM analysis of Silver nanoparticles

The silver nanoparticles synthesized by the help of onion extract were scanned using TEM (Philips CM-10) from which we can conclude that the average mean size of silver nanoparticles was 33.67 nm and seems to be spherical in morphology as shown in figure 3.

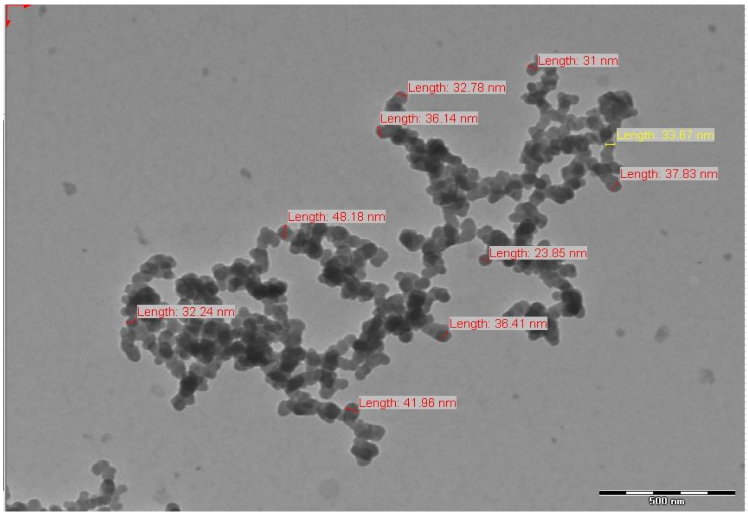

Fig. 3. TEM micrographs of silver nanoparticles synthesised from onion extract.

3.4 Analysis of Growth Curve

It was well known that silver nanoparticles exhibits strong antibacterial activity due to their well-developed surface which provides maximum contact with the environment. Here, antibacterial effect of silver nanoparticles were studied by using optical intensity as function of time for 25 hours with varying concentration of silver nanoparticles . From figure 4 and 5, we can conclude that in the absence of silver nanoparticles there is increase in optical density showing bacterial growth but as the concentration of silver nanoparticles increases, there is reduction in the bacterial growth of E.coli and Salmonella typhimurium.

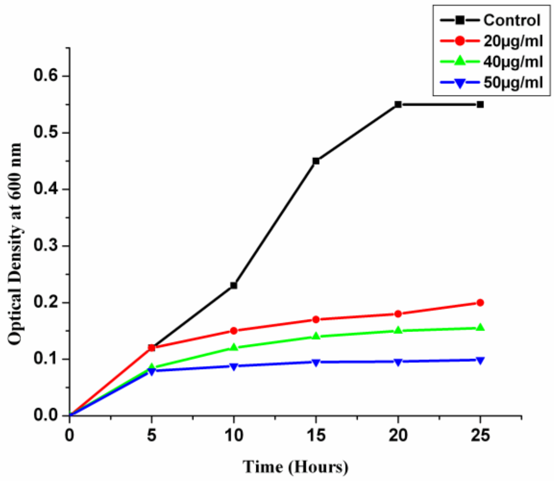

Fig .4. Effect of silver nanoparticles on E.coli growth rate.

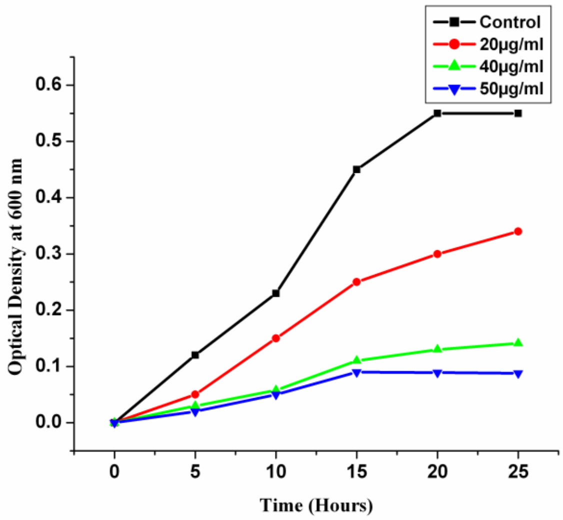

Fig. 5. Effect of silver nanoparticles on Salmonella typhimurium.

4. Conclusions

We used onion (*Allium cepa*) extract as a reducing and capping agent to minimize the serious environmental pollution problems. Silver nanoparticles were synthesised by this method having 33.6 nm average mean size. The preparation of nanoparticles by using onion extract has desired quality with low cost and convenient methods. These nanoparticles at concentration 50µg/ml were showed complete antibacterial activity against E.coli and Salmonella typhimurium.

Acknowledgement

Authors are thankful to Dr. Ashok K. Chauhan for his encouragement and providing excellent facilities for the above work.

References

[1] Catauro M, Raucci MG, De Gaaetano FD, Marotta A, J.Mater Sci Mater Med. **15**(7), (2004).
[2] Crabtree JH, Brruchette RJ, Siddiqi Ra,Huen IT, Handott LL, Fishman A, Perit Dial Int. **23**(4), (2003).
[3] Krolikowska A, Kudelski A, Michota A, Bukowska, J.Surf Sci **532**, (2003).
[4] Zhao G, Stevens, J. Se. Biometals. **11**, (1998).
[5] Hussain I, Brust M, Papworth A J, Cooper AI, Langmuir **19**, (2003).
[6] Sharma Virender. K, Yngard Ria A., Lin Yekaterina, Colloid and Interface Science **145**, (2009).
[7] Balantrapu Krishna; Goia Dan V, Journal of materials research **24**, (2009).
[8] R.M.Tripathi, Antariksh Saxena, Nidhi Gupta, Harsh Kapoor, R.P.Singh, Digest journal of nanomaterials and Biostrucutres **5(2),** (2010).
[9] Rodriguez-Sanchez.L, Blanco.M.C, Lopez-Quintela.M.A, J. Phys. Chem. **104,** (2000).
[10] Taleb. A, Petit.C, Pileni .M.P, Chemistry of Materials **9**, (1997).
[11] Begum Naznin Ara, Mondal Samiran, Basu Saswati, Laskar Rajibul A., MandDebabrata, Colloids and Surfaces B: Biointerfaces. **71(1)**, (2009).
[12] Lengke Maggy, Southam Gordon, Acta **70 (14),** (2006).
[13] Binoj Nair and T. Pradeep, Crystal Growth & Design, **2**, (2000).
[14] Shiying He, Zhirui Guo, Yu Zhang, Song Zhang, Jing Wang and Ning Gu, Materials Letters **61**, (2007).
[15] Holmes J.D., Smith P.R, Evans-Gowing R., Richardson D.J., Russel D.A., Sodeau J.R, Arch. Microbiol.**163**, (1995).
[16] Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan M.I, Kumar R, Sastry M, J. Nanotechnology **5**, (2005).
[17] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan M. I,Kumar R and Sastry M, Colloids and Surfaces B: Biointerfaces **28**, (2003).
[18] Kuber C. Bhainsa and S.F. D'Souza, Colloids and Surfaces B: Biointerfaces **47**, (2006).
[19] Shankar S. Shiv, Rai Akhilesh, Ahmad Absar and Sastry Murali, Colloid and interface science **275**, (2004).
[20] Gardea-Torresdey J.L, Parsons J.G, Gomez E, Peralta-Videa J, Troiani H.E, Santiago P and Jose Yacaman M, Nano Letters **2**, (2002).
[21] Raffi, M. Hussain, F. Bhatti, T.M. Akhter, J .I.,Hameed, A. Hasan, Journal of Material Sciences and Technology **24 (2)**, (2000).
[22] Monica Heger, A Silver Coating in the Fight Against Microbes, Silver nanoparticles could be the next step forward in antibacterial products, New York University's Science, Health andEnvironmental Reporting Program, (2008).
[23] S. Pal, Y. T. Tak, J. M. Song,Does, Applied and environmental Microbiology, **73 (6),** (2007).
[24] M. Singh, S. Singh, S. Prasad, I. S. Gambhir, Digest Journal of Nanomaterials and Biostructures, **3(3),** (2008).
[25] R. E. K.León, Study of Silver Nanoparticles Biocidal Impact on Escherichia coli Using Optical and Atomic Force Microscopy, Research Accomplishments, (2007).
[26] R. G. Cutler, E. J. Evans, Journal of Bacteriology, **9(2)**, (1996).