Research Article Adv. Mater. Lett. 2015, **6**(3), 260-264 ADVANCED MATERIALS Letters

www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2015.5655 Published online by the VBRI press in 2015

Green synthesis of silver nanoparticles using *Asiatic Pennywort* and *Bryophyllum* leaves extract and their antimicrobial activity

Dulen Saikia[1*], Pradip K. Gogoi[2], Pallabi Phukan[1], Nilave Bhuyan[3], Sangeeta Borchetia[4], Jibon Saikia[5]

[1]*Material Science Laboratory, Department of Physics, Sibsagar College, Joysagar 785665, Assam, India*
[2]*Centre for Nanoscience and Composite Materials, Deptt. of Chemistry, Dibrugarh University, Dibrugarh 786004, India*
[3]*Department of Zoology, J.B.College, Jorhat, Assam 785001, India*
[4]*Department of Biotechnology , J.B.College, Jorhat, Assam 785001, India*
[5]*Department of Physics, J.B.College, Jorhat, Assam 785001, India*

[*]Corresponding author. Tel: (+91) 9435055085; E-mail: dulen.s@rediffmail.com

Received: 26 September 2014, Revised: 26 November 2014 and Accepted: 12 December 2014

ABSTRACT

Silver nanoparticles of average sizes ~18-21 nm in diameter were prepared by a green approach via chemical reduction of silver nitrate ($AgNO_3$) using *Asiatic Pennywort* and *Bryophyllum* leaf extracts as reducing and capping agents. The bio-reduced silver nanoparticles were characterized by UV-Vis spectroscopic, XRD and TEM techniques. The characteristic surface plasmon band of colloidal solutions of AgNPs synthesized from *Asiatic Pennywort* and *Bryophyllum* leaf extracts were found at 445 nm and 405 nm respectively. The results of XRD and SAED pattern showed that the biosynthesized AgNPs have a crystalline structure with cubic phase (fcc). The antimicrobial activities of the as synthesized AgNPs were investigated against gram negative bacteria *Pseudomonas Fluorescens* and gram positive bacteria *Staphylococcus Epidermidis*. It was observed that silver nanoparticles obtained from *Asiatic Pennywort* was more effective on gram positive bacteria *Staphylococcus Epidermidis* while AgNPs obtained from *Bryophyllum* was more effective on gram negative bacteria *Pseudomonas Fluorescens* indicating size dependent activity of AgNPs.

Keywords: Green synthesis; silver nanoparticles; plasmon resonance band; antimicrobial activity.

Dulen Saikia obtained his Ph.D Degree from Dibrugarh University, and presently holding the post of Associate Professor in the Department of Physics, Sibsagar College, Joysagar, Assam (India). He is also visiting faculty of Apex Professional University, Arunachal Pradesh, India. His research interests are synthesis of novel nanocomposite materials, fabrication of solar cells by CBD technique and bio-synthesis of nanoparticles. He has published more than 14 papers in reputed International Journals.

Pradip K. Gogoi (b. 1954) did M.Sc, in Chemistry in 1975 from Dibrugarh University, followed by Ph.D. in Indian Institute of Technology, Bombay in 1981. For a short period he was in petrochemical industry. Since 1985 he is at Department of Chemistry, Dibrugarh University where he is a Professor since 2001. In 1992-93 he was a Marie Curie Post-doctoral Fellow at Imperial College of Science, Technology & Medicine, London. He was also a visiting Scientist at LPV Institute of Physical Chemistry, Ukrainian Academy of Sciences, Kiev during 2008. His fields of interest are co-ordination chemistry, catalysis, synthesis of novel materials, petroleum technology and coal chemistry.

Introduction

Due to multifarious applications, the synthesis of nanoscale materials has gained manifold impetus recently **[1-3]**. There is wide spectrum of areas such as medicine, electronics, biotechnology and catalysis, where the nanoparticles find significant uses **[4, 5]**. The noble and platinum group metals show promising antimicrobial activity due to their large surface to volume ratio, electronic configuration and crystallographic surface structure **[6-11]**. Among the noble metals, silver (Ag) has attracted particular interest in biology and medicine **[11]** as it has been shown to exhibit effective antimicrobial activity **[1-4]**. There are a wide variety of methods for synthesis of silver nanoparticles, such as, chemical reduction **[12, 13]**, electrochemical technique **[14]** and photochemical reduction in presence of reverse micelles **[15]**. Recently, green synthesis has become popular **[16]** due to its simplicity, eco-friendliness and low

production cost, as these techniques made use of plant extracts or biomass or microorganism **[17-19]** which are easily available in nature.

Although a number of plant extracts such as *Ocimum sanctum, Citrus limon* (lemon), *Euphorbia hirta, Acacia leucophloea, Coriandrum sativum, Trianthema decandra* **[20-26]** has been utilized for the synthesis of silver nanoparticles, the plant extracts which are edible as well as having medicinal significance are of current research interest. G. Singhal et al reported the biosynthesis of silver nanoparticles within the size of 4-30 nm using extract of *Ocimum sanctum* leaves **[20]**. T.C. Prathna et al revealed that silver nanoparticles could be rapidly synthesized at room temperature by treating silver ions with the *Citrus limon* (lemon) extract. The effect of various process parameters like the reductant concentration, mixing ratio of the reactants and the concentration of silver nitrate were also studied in detail **[21]**. K.A. Priyadarshini et al reported the synthesis of AgNPs using *Euphorbia hitra* (*E. hitra*) plant extract and investigated their activity against malarial vector *Anopheles Stephensi* **[22]**. K. Murugan et al synthesized AgNPs through a biomimetic route employing the bark extract of the readily available medicinal plant *Acacia leucophloea*, and investigated their antibacterial activity against common bacterial pathogens with the goal of biomedical application **[23]**. AgNPs of ~26 nm size were reported by Sathyavathi et al using *Coriandrum sativum* leaf extract as the reducing agent **[24]**. Aqueous leave extract of *Catharanthus roseus* was used by Mukunthan et al for the bio-production of AgNPs of size in the range of 48-67 nm **[25]**. Geethalakshmi et al. **[26]** utilised extract of *Trianthema decandra* to prepare 15 nm sized AgNPs and demonstrated their antimicrobial activity using the Kirby-Bauer method.

The synthesis of bio-reduced AgNPs using locally available medicinal plant extracts is of considerable research interest due to its potential medicinal application. In North East India, especially in Assam, *Paederia foetida, Houttuynia cordata, Bacopa monnieri, Mentha arvensis, Bryophyllum, Asiatic pennywort* etc are some of edible plants which are also used as ethno-medicines. However, only a few reports **[27-29]** are available on the synthesis and application of silver nanoparticles using medicinal plant extracts like *Bryophyllum* and *Asiatic pennywort*. In the present study we report the bio-synthesis of silver nanoparticles (AgNPs) using extracts of *Asiatic Pennywort* and *Bryophyllum* leaves as reducing and capping agents. The structural, morphological and optical properties of the as synthesized silver nanoparticles were investigated by XRD, TEM, and UV-Vis spectroscopy. It was of interest to see the effect of different plant extracts on the resultant size of AgNPs. Further, the antimicrobial activities of AgNPs as obtained were screened against gram negative bacteria, *Pseudomonas Fluorescens* and gram positive bacteria *Staphylococcus Epidermidis*.

Experimental

Materials

Silver nitrate with 99.5% purity was purchased from Merck Specialities Private Limited, Mumbai (India) and used as received without further purification. Deionised water (pH = 7 ± 0.5) purchased from Nova Biotech, Kolkata (India) was used throughout the experiments. *Asiatic Pennywort* and *Bryophyllum* leaves were collected from Sivasagar (26° 59' 0" North, 94° 38' 0" East), Assam (India) for the synthesis of AgNPs.

Method

Locally available medicinal plant leaves such as *Asiatic Pennywort* (local Name in Assam, India: Manimuni) and *Bryophyllum* (local Name in Assam, India: Dupartenga) were used for bio-synthesis of AgNPs. The plant leaf extracts were prepared by mixing 5 g of dried leaves in 100 ml of deionized water in an erlenmeyer flask, heated for 30 minutes at 80^{o}C and then filtered through a Whatman 42 no. filter paper. In a typical reaction, 9 ml of the leaf extracts was added to the 1 ml of $AgNO_3$ solution (1-5) $\times 10^{-3}$mol dm^{-3} and the reaction mixture was allowed to stay at room temperature for the reduction to AgNP to take place. The AgNPs prepared from *Asiatic Pennywort* leaf extract is labeled as AgNP1 and that obtained from *Bryophyllum* leaf extract is labeled as AgNP2. The optical properties of the as synthesized AgNPs were studied with the help of UV-Vis spectra recorded in a Hitachi (U 3900) UV-Vis spectrophotometer. The structural properties of AgNPs were investigated by X-ray diffraction (XRD) technique using an X-ray diffractometer (Make: Seifert, Model 003 T/T) with CuKα radiations operated at 40kV and 30mA. Transmission Electron Microscopic (TEM) analysis of AgNPs were done using a 200 KV JEOL JEM 2100 Transmission Electron Microscope.

Results and discussion

Fig.1 (a) and **(c)** shows the optical images of *Asiatic Pennywort* and *Bryophyllum leaves*, locally collected from the district of Sivasagar, Assam (India). When the leaf extract was mixed in the aqueous solution of the silver nitrate, it started to change colour (within 30 minutes) from brown to blackish green in case of *Asiatic Pennywort* (**Fig. 1b**) and from light brown to deep brown in case of *Bryophyllum* (**Fig. 1d**). The observed colour change might be due to excitation of surface plasmon vibrations, which is indicative of the formation of AgNPs **[30]**. The optical photograph of colour change in the colloidal solution with different concentration of $AgNO_3$ but for a fixed amount of *Asiatic Pennywort* and *Bryophyllum* leaf extracts are shown in **Fig. 1(b)** and **(d)** respectively. Usually, most of the metal nanoparticles exhibit surface plasmon resonance (SPR), which is dependent on size and morphology. Typical absorption spectra of AgNP1 and AgNP2 using different concentrations of aqueous $AgNO_3$ solution (1- 5×10^{-3}mol dm^{-3}) are shown in **Fig. 1(e)** and **(f)** respectively. The characteristic surface plasmon band for AgNP1 and AgNP2 have appeared at 445 and 405 nm respectively and it is found to be more prominent at 5mM (5×10^{-3}mol dm^{-3}) concentrations of $AgNO_3$ solution. The absorption band is found to be red shifted for AgNP1 which is an indication of the formation of larger AgNPs, with different shapes and sizes **[31]**. This is also supported by the TEM results. The shifting of the surface plasmon band may be attributed to particle size, shape, chemical surrounding, adsorbed species on the surface and dielectric constant **[30, 32]**.

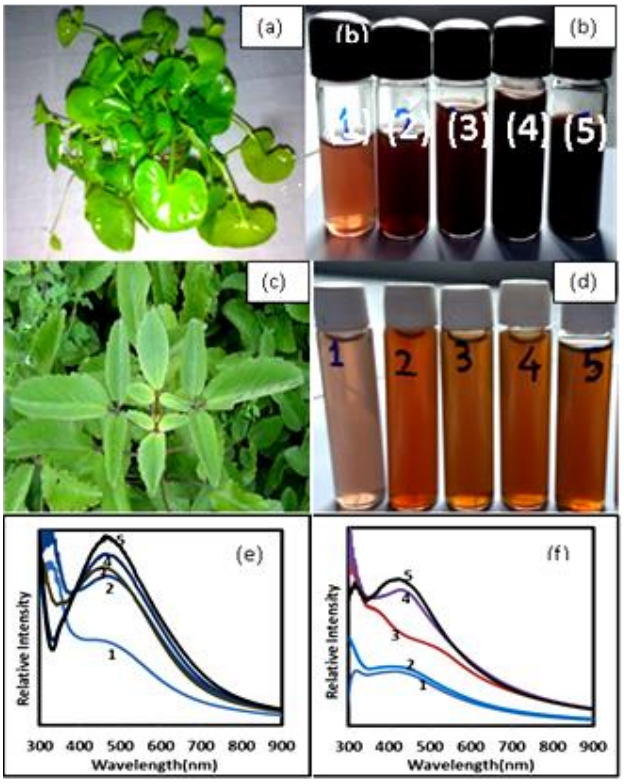

Fig. 1. (a) Image of *Asiatic Pennywort* leaf (b) Colour change of $AgNO_3$ solution with the change of concentration $AgNO_3$ solution (1mM to 5 mM) containing *Asiatic Pennywort* leaf extract (c) Image of *Bryophyllum* leaf (d) Colour change of $AgNO_3$ solution with the change of concentration of $AgNO_3$ solution (1mM to 5 mM) containing *Bryophyllum* leaf extracts; (e) UV-Vis absorption spectra of AgNP1 using different concentrations of aqueous $AgNO_3$ solution, (1) 1×10^{-3}mol dm^{-3}, (2) 2×10^{-3}mol dm^{-3} , (3) 3×10^{-3}mol dm^{-3} , (4) 4×10^{-3}mol dm^{-3} , (5) 5×10^{-3}mol dm^{-3} ; (f) UV-Vis absorption spectra of AgNP2 using different concentrations of aqueous $AgNO_3$ solution, (1) 1×10^{-3}mol dm^{-3}, (2) 2×10^{-3}mol dm^{-3} , (3) 3×10^{-3}mol dm^{-3} , (4) 4×10^{-3}mol dm^{-3} , (5) 5×10^{-3}mol dm^{-3}.

The XRD pattern of AgNP1 and AgNP2 are shown in **Fig. (2)**. Appearance of many peaks in both the samples is an indication of good crystalline nature of the AgNPs. The diffraction peaks at 2θ values of 27.2°, 32.24°, 38.04°, 44.92°, 64.48° and 76.68° for AgNP1 may be assigned to the diffraction lines produced by (226), (264), (111), (200), (220) and (311) reflection planes of the face-cantered-cubic (fcc) structure. The peaks observed at 2θ values of 27.2°, 32.04°, 38.12°, 44.8°, 64.4° and 77.04° for AgNP2 may be assigned to (226), (264), (111), (200), (220) and (311) reflection planes of the face-centered-cubic (fcc) structure.

The TEM images of AgNP1 and AgNP2 shown in **Fig. 3(a)** and **(b)** respectively reveals that the nanoparticles are almost spherical in nature. The size histogram of AgNP1 and AgNP2 depicted in **Fig. 3(e)** and **(f)** respectively shows that AgNPs of average size of ~21nm and ~18 nm were obtained from *Asiatic Pennywort* and *Bryophyllum* leaves extract respectively. The TEM images show that the silver nanoparticles are bounded by a thin layer of other materials, which may be composed of organic materials contained in the *Asiatic Pennywort* and *Bryophyllum* leaf broth. The selected area electron diffraction (SAED) pattern of the nanoparticles in **Fig. 3(c)** and **(d)** shows face centered cubic (fcc) crystalline structure of silver with indexed different diffracting planes.

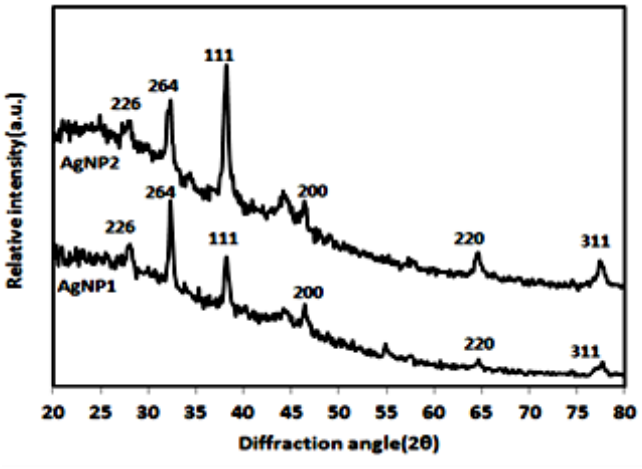

Fig. 2. XRD of AgNP1 and AgNP2.

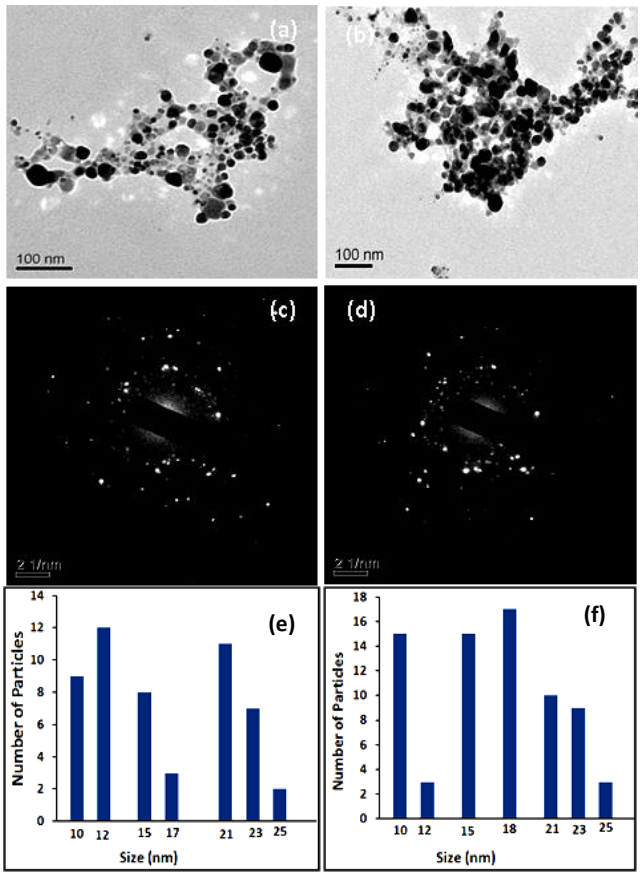

Fig. 3. (a) TEM images of AgNP1 (b) TEM images of AgNP2 (c) SAED pattern of AgNP1 (d) SAED pattern of AgNP2 (e) Size distribution of AgNP1 and (f) Size distribution of AgNP2.

Biosynthesized AgNPs were further investigated for antimicrobial activity against gram negative bacteria *P. fluorescens* and gram positive bacteria *S. epidermidis* on nutrient agar plates by disc diffusion method containing 60µL of AgNP1 and AgNP2. It was observed that, the bacterial strains were sensitive to both AgNP1 and AgNP2. The biosynthesized AgNPs showed a clear inhibition zone against *S. epidermidis* and *P. fluorescens* bacteria (**Fig. 4**)

and recorded zone of inhibitions from 15 mm to 31.5 mm. The minimum inhibitory concentrations (MIC) are listed in **Table 1**. The effect of AgNP1 and AgNP2 on the growth of *S. epidermidis* and *P. fluorescens* is shown in **Fig. 5**. It shows that both AgNP1 and AgNP2 can affect the bacterial growth of *S. Epidermidis* and *P. fluorescens*. However, AgNP1 is more effective on gram positive bacteria *S. epidermidis* while AgNP2 was more effective on gram negative bacteria *P. fluorescens* and produced maximum growth inhibition zone of 30.5±1.2 mm which could be due to similar sizes of respective bacteria and nanoparticles.

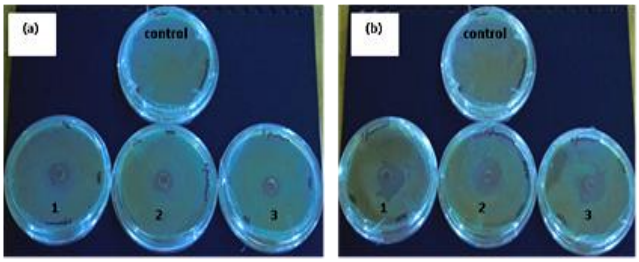

Fig. 4. (a) Antibacterial activity of AgNP1 on *S.Epidermidis* (Control and Repeat 1, 2, 3) (b) Antibacterial activity of AgNP2 on *P. Fluorescens* (Control and Repeat 1,2,3).

Table 1. The antibacterial activity of AgNP1 and AgNP2 on Gram +ve bacteria.

Expt.	Zone inhibition value (mm) of AgNP1 against		Zone inhibition value (mm) of AgNP2 against	
	S. Epidermidis	*P. Fluorescens*	*S. Epidermidis*	*P. Fluorescens*
Control	No effect	No effect	No effect	No effect
Repeat 1	20.4	19	17.1	31
Repeat 2	21.2	15.7	17.7	31.5
Repeat 3	19.4	17	17.9	29.2
Mean	20.3	17.2	17.5	30.5
SD	0.9	1.6	0.4	1.2

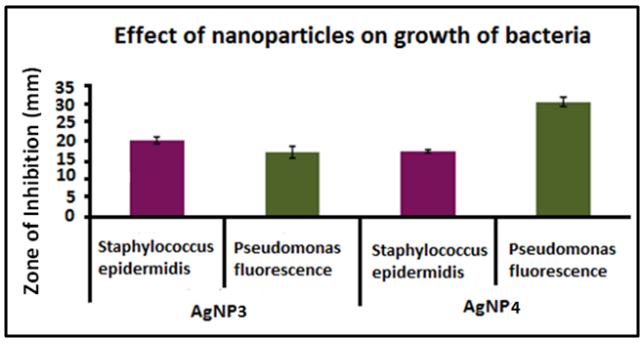

Fig. 5. Effect of AgNP1 and AgNP2 on the growth of gram negative bacteria P. fluorescens and on the growth of gram positive bacteria S. epidermidis.

AgNPs of average sizes of ~21 nm and ~18nm were obtained via bio-reduction of the silver ions by *Asiatic Pennywort* and *Bryophyllum* leaves extract and have been demonstrated through UV-Vis, XRD and TEM analysis. The TEM images show that the AgNPs are bounded by a thin layer of other materials, which may be composed of organic materials contained in the *Asiatic Pennywort* and *Bryophyllum* leaf broth. The selected area electron diffraction (SAED) pattern of the nanoparticles in **Fig. 3(c)** and **(d)** shows face centered cubic (fcc) crystalline structure of silver with indexed different diffracting planes.

Conclusion

Extracts of *Asiatic Pennywort* and *Bryophyllum* leaves were used as reducing agent for the synthesis of AgNPs. TEM studies revealed that, stable and almost spherical shaped AgNPs of average size ~18-21nm were rapidly synthesized by this biological approach. The antimicrobial activity of AgNP1 and AgNP2 was evaluated against *S. Epidermidis* and *P. Fluorescens* and found to be effective growth inhibitors against *S. Epidermidis* and *P. Fluorescens* bacteria. However, AgNP1 was more effective on gram positive bacteria *Staphylococcus Epidermidis* while AgNP2 was more effective on gram negative bacteria *Pseudomonas Fluorescens* and it could be due to size dependent interaction of AgNPs.

Acknowledgements

One of the authors (D. Saikia), acknowledges UGC, New Delhi for financial support under a minor project to Sibsagar College, Joysagar, Assam, India. Co-authors Nilave and Sangeeta Borchetia also wish to acknowledge DBT, Government of India for their generous funding through the DBT Hub programe.

Reference

1. Jahn, W.; *J. Struct. Biol.* **1999,** *127*, 106. **DOI:** 10.1006/jsbi.1999.4123
2. Naiwa, H. S.; *Ed. Hand Book of Nanostructural Materials and Nanotechnology Academic Press New York*. **2000,** 1. **DOI:** 10.1016/B978-012513920-5/50000-7
3. Murphy, C. J.; *J. Mater Chem.* **2008,** *18*, 2173. **DOI:** 10.1039/B717456J.
4. Hussain, I.; Brust, M.; Papworth, A. J.; Cooper, A. I.; *Langmuir.* **2003**, *19(11)*, 4831. **DOI:** 10.1021/la020710d
5. Sharma, K. V.; Yngard, A. R.; Lin, Y.; *Advances in Colloid and Interface Science*, 2009**,** *145(1–2)*, 83. **DOI:** 10.1016/j.cis.2008.09.002
6. Catauro, M.; Raucci, M. G.; Gaetano, F. D.; Marotta, A.; *J Mater Sci Mater Med.* **2004,** *15(7)*, 831. **DOI:** 10.1023/B:JMSM.0000032825.51052.00
7. Crabtree J. H., R. J. Brruchette, Perit, *Dial Int.* **2003,** *23(4)*, 368. **DOI:** 0896-8608/03
8. Krolikowska, A.; Kudelski, A.; Michota, A.; Bukowska, J.; *Surf. Sci.*, **2003,** *532*, 227. **DOI:** 10.1016/S0039-6028(03)00094-3.
9. Zhao, G.; Stevens, S. J.; *Biometals.* **1998,** 11(1), 27. **DOI:** 10.1023/A:1009253223055
10. Leela, A.; Vivekanandan, M.; *African Journal of Biotechnology.* **2008**, *7(17)*, 3162. **DOI:** 10.5897/AJB08.425
11. Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J. H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C. Y.; Kim, Y. K.; Lee, Y. S.; Jeong, D. H.; Cho, M. H.; *Nanomedicine: Nanotechnology, Biology and Medicine.* **2007**, *3*, 95. **DOI:** 10.1016/j.nano.2006.12.001.
12. Balantrapu, K.; Goia, D.; *J Mater Res.* **2009,** *24(9)*, 2828. **DOI:** 10.1557/jmr.2009.0336.
13. Khan, Z.; Thabaiti, S.; Ahmed, A.; Obaid, A. Y.; Youbi, A.O.; *Colloids and Surfaces B: Biointerfaces*. **2011,** *82(2,1)*, 513. **DOI:** 10.1016/j.colsurfb.2010.10.008.
14. Sanchez, L. R.; Blanco, M. C.; Lopez-Quintela, M. A.; *J. Phys. Chem.B.* **2000,** *104*, 9683. **DOI:** 10.1021/jp001761r.
15. Taleb, A.; Petit, C.; Pileni, P. M.; *Chemistry of Materials.* **1997,** *9 (4)*, 950. **DOI:** 10.1021/cm960513y

16. Begum, N. A.; Mondal, S.; Basu, S.; Laskar, R. A.; Mandal, D.; Colloids Surf B Biointerfaces, **2009,** *71(1)*, 113.
DOI: 10.1016/j.colsurfb.2009.01.012.
17. Sastry, M.; Ahmad, A.; Khan, I. M.; Kumar, R.; *Wiley-VCH, Weinheim*. **2004**, 126.
DOI: 10.1002/3527602453.ch9.
18. Bhattacharya, D.; Rajinder, G.; *Crit. Rev. Biotechnol.* **2005,** *25(4)*, 199.
DOI: 10.1080/07388550500361994.
19. Mohanpuria, P.; Rana, N. K.; Yadav, S. K.; *Journal of Nanoparticle Research*, **2008,** *10(3)*, 507.
DOI: 10.1007/s11051-007-9275-x
20. Singhal, G.; Bhavesh, R.; Kasariya, K.; Sharma, A. R.; Singh, R. P.; *Journal of Nanoparticle Research*, **2011,** *13(7)*, 2981.
DOI: 10.1007/s11051-010-0193-y.
21. Prathna, T. C.; Chandrasekaran, N.; Raichur, A. M.; Mukherjee, A.; *Colloids and Surfaces B: Biointerfaces.* **2011,** *82(1)*, 152.
DOI: 10.1016/j.colsurfb.2010.08.036.
22. Priyadarshini, K. A.; Murugan, K.; Panneerselvam, C.; Ponarulselvam, S.; Hwang, J. S.; Nicoletti, M.; *Parasitol Res.* **2012** , *111(3)*, 997.
DOI: 10.1007/s00436-012-2924-8.
23. Murugan, K.; Senthilkumar, B.; Senbagam, D.; Sohaibani, S. A.; *Int J Nanomedicine*. **2014** *9*, 2431.
DOI: 10.2147/IJN.S61779.
24. Sathyavathi, R.; Krishna M. B.; Rao, S. V.; Saritha, R.; Rao, D. N; *Advanced Science Letter*. **2010,** *3(2)*, 138.
DOI: 10.1166/asl.2010.1099.
25. Mukunthan, K. S.; Elumalai, E. K.; Patel, T. N.; Murty, V. R.; *Asian Pacific Journal of Tropical Biomedicine*. **2011,** *1(4)*, 270.
DOI: 10.1016/S2221-1691(11)60041-5.
26. Geethalakshmi, R.; Sarada, D.V.L.; *Int. J. Eng. Sci. Technol*. **2010**, *2(5)*, 970.
DOI: 10.2147/IJN.S36516.
27. Tatsimo, S. J. N.; Tamokou, J. D.; Havyarimana, L.; Csupor, D.; Forgo, P.; Hohmann, J.; Kuiate, J. R.; Tane, P.; *BMC Research Notes.* **2012,** *5*,158.
DOI: 10.1186/1756-0500-5-158.
28. Logeswari P.; Silambarasan S.; Abraham, J.; *Journal of Saudi Chemical Society*. **2012,** 16.
DOI: 10.1016/j.jscs.2012.04.007.
29. Logeswari, P.; Silambarasan, S.; Abraham, J.; *Scientia Iranica F*. **2013,** *20 (3)*, 1049.
DOI: 10.1016/j.scient.2013.05.016.
30. Mukherjee, P.; Roy, M.; Mandal, B. P.; Dey, G. K.; Mukherjee, P. K.; Ghatak, J.; Tyagi, A. K.; Kale, S. P.; *Nanotechnology.* **2008,** *19*, 075103.
DOI: 10.1088/0957-4484/19/7/075103.
31. Bar, H.; Bhui, D. K.; Sahoo, G.P.; Sarkar, P.; De, S.P.; Misra, A.; *Colloids and Surfaces A:Physicochemical and Engineering Aspects*. **2009,** *339(1–3)*, 134.
DOI: 10.1016/j.colsurfa.2009.02.008.
32. Das, M. R.; Sarma, R. K.; *Colloids and Surfaces B: Biointerfaces.* **2011,** *83(1)*, 16.
DOI: 10.1016/j.colsurfb.2010.10.0

