Digest Journal of Nanomaterials and Biostructures Vol. 6, No 2, April - June 2011, p. 535-542

A GREEN BIOGENIC APPROACH FOR SYNTHESIS OF GOLD AND SILVER NANOPARTICLES USING *ZINGIBER OFFICINALE*

CHANDAN SINGH[a], VINEET SHARMA[b], PRADEEP KR NAIK[a], VIKAS KHANDELWAL[c], HARVINDER SINGH[a*]

[a]*Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan 173234, Himachal Pradesh, India.*

[b]*Department of Physics, Jaypee University of Information Technology, Waknaghat, Solan 173234, Himachal Pradesh, India.*

[c]*Department of Genetics and Plant Breeding, N.M.College of Agriculture, Nausari Agriculture University- 396 450, Gujrat, India*

We report here the biosynthesis of gold and silver nanoparticles using leaf extract of ginger (*Zingiber officinale)*. The treatment of aqueous solution of $AgNO_3$ and $HAuCl_4$ with ginger leaf extract resulted in rapid formation of stable nanoparticles for both metals. The growth of nanoparticles is monitored by UV-vis spectrophotometer and complemented with characterization using Transmission Electron Microscopy, X-ray Diffraction and Fourier Transform Infrared Spectroscopy. Plausible mechanism for the formation of nanoscale and dfference in reduction time for gold and silver nanoparticle synthesis is discussed on the basis of pH. Transmission Electron Microscopy revealed the presence of polydisperse gold and silver nanoparticles with an average size of 10nm and 30.31nm respectively. X-ray diffraction studies corroborated that the biosynthesized nanoparticles are crystalline gold and silver. Furthermore, this green biogenic approach is rapid and simple alternative to chemical synthesis.

(Received January 10, 2011; accepted March 09, 2011)

Keywords: Gold nanoparticles (GNP), Silver nanoparticle (AgNP), pH, *Zingiber officinale*

1. Introduction

Nanotechnology mainly deals with the fabrication of nanoparticles having various shapes, sizes and managing their chemical and physical parameters for further use in human benefits. Their growing applications in various fields like biosensors [1] bioremediation of radioactive wastes [2], functional electrical coating[3], synthesis of enzyme electrodes[4] and particularly in medicine such as delivery of antigen for vaccination [5], gene delivery for treatment or prevention of genetic disorder[6], inspired the scientists to develop environment friendly procedures for the synthesis of nanoparticles and to avoid use of hazardous chemicals, which are traditionally used. Synthesis methods using organisms, both unicellular and multicellular like yeast, fungi and bacteria came into the picture, which were able to synthesize inorganic materials either extracellularly [7] or intracellularly [8].When it comes to the synthesis of gold nanoparticles with bacteria, initially *Bacillus subtilis* 168 was used to reduce Au^{3+} ions and octahedral gold nanoparticles(GNP) were produced having dimensions(5-25 nm) within bacterial cells by incubation of gold chloride[9]. Reduction of Au(III) ions by Fe(III)-reducing bacteria *Shewanella* algae is also reported, which results in the formation of GNP's with 10-20 nm in diameter[10]. It is well known that silver is very toxic to the microorganisms, still a lot of silver resistant strains were reported for the synthesis of silver nanoparticles (AgNP)[11], silver resistant strain such as *Pseudomonas stutzeri* AG259 produces AgNP with dimension within 35 nm to 46 nm [12],while

*Corresponding author: harvinder_07@yahoo.com

the larger nanoparticles with size of approximately 200 nm were obtained when *Pseudomonas stutzeri* AG259 isolated from silver mine and placed in a concentrated solution of silver nitrate [13].

Fungus like *Colletotrichum sp*growing on the geranium leaves was used for the synthesis of multiple shaped gold nanoparticles[14], *Fusarium oxysporum*was used for the synthesis of GNP while *Aspergillus fumigates* [15, 16] used for the synthesis of AgNP within size range of 5-25 nm. Various strains of yeast has also been used for the synthesis of AgNP and GNP [17,18]. Magnetic nanoparticles is one of the most common product of bacterial iron reduction, Octahedral shape tiny nanoparticles with diameter 12 nm were synthesized using a thermophilic fermentative strain TOR-39 [19].

Apart from the synthesis of AgNP and GNP their application in the synthesis of semiconductor nanoparticles is also very attentive, since compared to chemicals methods which are often energy intensive, employ toxic chemicals and require high temperatures but synthesis using biological routes occurs at ambient temperature and pressure, such as synthesis of luminicent CdSe quantum dots at the room temperature by fungus *Fusarium oxysporum* [22].Use of microorganism such as bacteria[23] and yeast[24] in the remediation of toxic metals is very well known, but their use in the synthesis of metal nanoparticles is relatively new and has a potential for development.

Compared to microorganisms which suffer from various problems like availability, maintenance in cell cultures and cost effectiveness during the scale up process, various plants have been successfully used for the synthesis of gold, silver and sometimes for bimetallic silver and gold nanoparticles. A number of plants such as alfalfa (*Medicago sativa*) produced GNP of 4-10 nm [25]. Biomass of oat (*Avena sativa*) and wheat (*Triticum aestivum*) were reported for irregular and rod shaped GNP with size range spaning 10-30 nm[26], bengal gram bean(*Cicer arietinum*) was also reported for gold nanotriangles [27].Similarly for the synthesis of AgNP plants such as *Capsicum annuum* [28], Quercetin [29] were used. In addition to the independent synthesis of silver and gold nanoparticles various plants such as sun dried biomass of *Cinnamommum camphora* was used for triangular and spherical gold and silver nanoparticles with size range between 55-80 nm[30], fruit extract of Amla (*Emblica officinalis*) for silver and gold nanoparticles with dimensions spaning 10-20 nm and 15-25 nm respectively[31], leaf extract of *aloe vera* produced triangular and spherical silver and gold nanoparticles[32], and neem (*Azadirachta indica*) leaf broth was used for silver, gold and Ag core–Au shell[33].These methods are very efficient in the production of silver and gold nanoparticles because of their simplicity, accuracy and cost effectiveness, also from the green chemistry perspective and their biomedical application.

We herein repot the synthesis of silver and gold nanoparticles by the reduction of aqueous Ag^+ and $AuCl_4^-$ with the extract of ginger (*Zingiber officinale)* rhizome .Ginger is known for it's medicinal values such as ginger has been used to treat skin diseases, colorectal cancer, arthritis, heart condition and also have been reported for it's antibacterial properties [34,35,36]. In addition to these medicinal uses, ginger continues to be valued around the world as important cooking spice. The approach followed by us appears to be cost efficient alternative to conventional methods and completely biogenic method of synthesis of silver and gold nanoparticles. To the best of our knowledge this is the first report on use of ginger as biological system for the synthesis of silver and gold nanoparticles. Presented below are the details of the investigation.

2. Materials and methods

For the synthesis of gold and silver nanoparticles, Gold (III) chloride hydrate, ($HAuCl_4.3H_2O$) from Sigma Aldrich, silver nitrate ($AgNO_3$) from fisher-scientific and fresh ginger (*Zingiber officinale)* rhizome were used. The extract was prepared by taking 10 gm thoroughly washed ginger rhizome, taking out the upper part by the knife, chopped into the fine pieces and converting it into the paste using mortar-pestle followed by addition of 40 ml Millipore water, boiling for 2 min then filtering, centrifuged at 5000 rpm for 15 min and collection of suspension, which was further used as ginger rhizome broth for the experiment.

2.1 Synthesis of silver and gold nanoparticles

For reduction of Ag^+ and $AuCl_4^-$ ions 5 ml of ginger rhizome broth was added to 50 ml of 1.0×10^{-3}M $AgNO_3$ solution and 50 ml of 1.0×10^{-3} M $HAuCl_4$ solution separately at room temperature, then solutions were shaken at 120 rpm in dark at 37°C.

2.2 UV-Visible spectrum analysis

Bioreduction of Ag^+ and $AuCl_4^-$ in the aqueous solution was monitored by periodic sampling, after diluting small aliquots (0.3ml) of sample with 3 ml of Millipore water. UV-vis spectroscopy analyses of silver and gold nanoparticles synthesized were recorded as a function of time at room temperature using PERKIN-ELMER spectrophotometer at a resolution of 1 nm.

*2.3 XRD (*X-ray Diffraction*) analysis*

After the complete reduction of silver and gold nanoparticles these solutions were lyophilized with lyophilizer (MartinChrist,Germany) and the dried mixture was analyzed for the crystalline nature of Ag and Au nanoparticles by an X'Pert Pro x-ray diffractometer operated at 40 mA current and 45 kV voltage with CuKα radiation.

2.4 FTIR (Fourier Transform Infrared Spectroscopy) analysis

After the complete reduction of Ag^+ and $AuCl_4^-$ ions by the ginger rhizome extract, 10 ml solution of each gold and silver nanoparticles was centrifuged at 4000 rpm for 10 min and the resulting suspension was redispersed into 20 ml of Millipore water, the process of centrifuging and redispersing was repeated three times to make nanoparticles free from proteins or other bioorganic compounds present in the solution. There after the purified suspension was completely dried in lyophilizer and analyzed byPerkinElmer-Spectrum RX-IFTIR.

2.5 Transmission Electron Microscopy (TEM) analysis

TEM samples of the gold and silver nanoparticles synthesized using ginger rhizome extract were prepared by placing a drop of nanoparticle solutions on carbon coated copper grids and allowing water to evaporate. TEM measurements were performed on a Morgagni 268(D) (Netherlands), which was operated at accelerating voltage of 100 kV.

3. Results and discussion

Bioreduction of aqueous Ag^+ and $AuCl_4^-$ ions can easily be followed by UV-visible spectrophotometer, and one of the most important feature in optical absorbance spectra of metal nanoparticles is surface Plasmon band, which is due to collective electron oscillation around the surface mode of the particles.

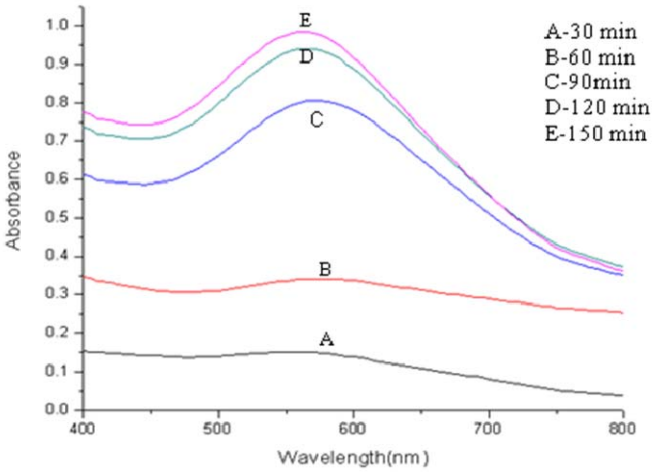


Fig. 1 UV-vis absorption spectra recorded as function of time for gold nano particles $AuCl_4^-$ ions using leaf broth of Zingiber officinale.

Previous studies have shown that gold exhibits red wine colour and silver exhibit yellowish-brown colour due to the excitations of their surface Plasmon response (SPR)[37], when dissolved in water. Figure 1 represents the UV-Vis-NIR spectrum of the $AuCl_4^-$ ions as a function of time. The colour of the gold solution changes from pale yellow to red wine within 30 minutes after the addition of ginger rhizome broth at the room temperature. The maximum absorption was observed at 560 nm, from the figure it is clear that absorption increases with time starting from the addition of the broth till the completion of the reduction i.e. 24 hours. In the case of spherical GNP the surface Plasmon resonance occurs as a band centered at about 520 nm, but when the particles deviate from the spherical geometry, then both transverse and longitudinal dipole polarizability is unable to produce equivalent resonances. As a result of that two plasma resonances appears with broadened and red shift longitudinal plasma resonance and a transverse Plasmon resonance, whose absorbance peak remains essentially unchanged from that of spherical particles. Various colloidal gold systems such as gold aggregates [38] and gold nanorods [39]supports this theory. In our case various gold aggregates were observed, but it's consequence in the UV-Vis-NIR spectrum was completely missing.

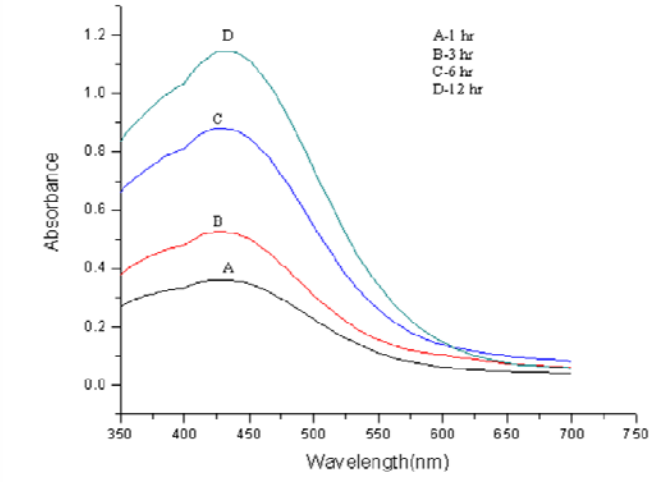
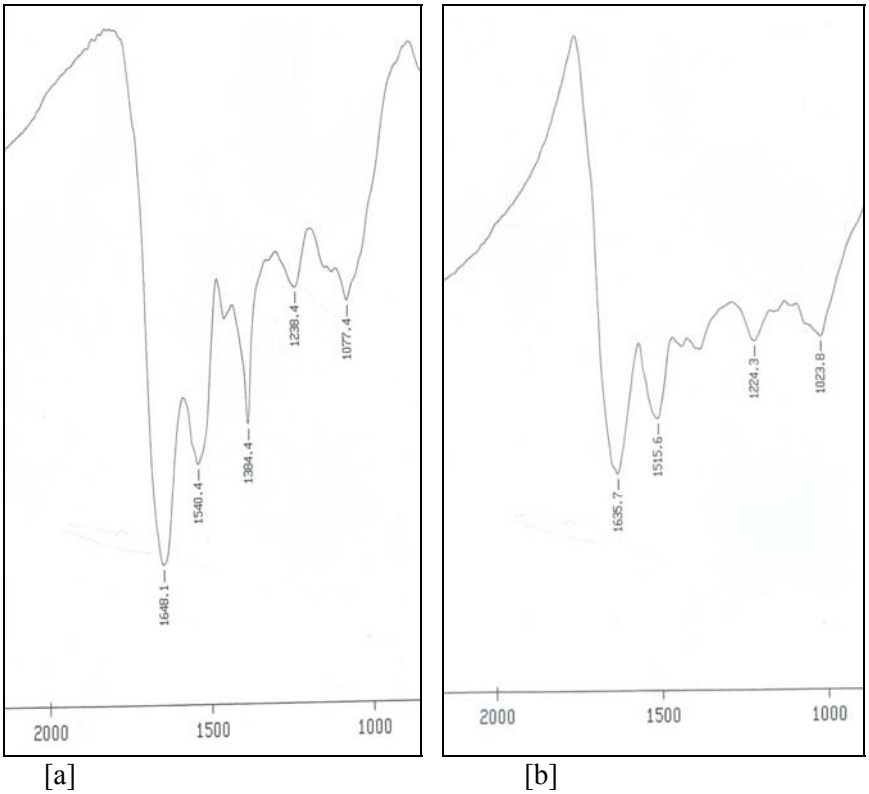


Fig. 2. UV-vis absorption spectra recorded as function of time for silver nano particles after bioreduction $AgNO_3$ using leaf broth of Zingiber officinale

Similarly figure 2 shows the UV-Vis-NIR spectrum of the reduced Ag^+ ions using ginger rhizome broth reaction medium as a function of time. The colorless solution of $AgNO_3$ gets transformed into yellowish brown colour, after the complete reduction, In the case of gold the reduction started within 5 minutes after the addition and completed in 2 hrs, but for silver the reduction time is much higher than that of gold, which is clearly visible in figure 2.The possible explanation of difference in the reduction time could be due to the difference in their reduction potential for both the metal ions. The maximum absorption in the case of silver was at 430 nm. In both the cases the spectral shift is mainly due to the dielectric constant of the medium. Here both silver and gold nanoparticles synthesized using ginger rhizomes were stable for more than one month, when stored at room temperature.

FTIR measurements were performed to identify the potential biomolecules in the ginger rhizome responsible for reduction and then providing stability to the bioreduced silver and gold nanoparticles. In the FTIR spectrum several absorption peaks were centered at 1648, 1540 1384, 1238 and 1077 cm^{-1} which was in the region range of 1000-2000 cm^{-1} (figure 3a). The most wide spectrum absorption was observed at 1648 and 1635 cm^{-1} and it can be attributed to the stretching vibrations of -C=C (alkane) [40], absorption peaks centered at 1515 and 1540 cm^{-1} can be attributed to the stretching vibration of -C=C (aromatic ring). Absorption peaks 1238 and 1224 cm^{-1} may result from the stretching vibrations of -C-O (acid) and absorption peaks at 1023 and 1077 cm^{-1} can be to attributed to the stretching vibrations – C=O (ester) [40]. Figure 3b depicts the FTIR spectra of AgNP; the peak was centered at 1384 cm^{-1} which was not observed in the FTIR spectra of GNP, which indicated presence of NO_3^- in the residual solution [41]. Now various functional groups mentioned above are mainly derived from heterocyclic compounds and these are the water soluble components of ginger rhizome. So it can be assumed that different water soluble heterocyclic compounds such as alkanoids, flavoinds etc worked as the capping ligand for the synthesis of silver and gold nanoparticles and the presence of oxygen atoms helped in the stabilization of nanoparticles by facilitating the absorption of heterocyclic compounds on nanoparticles.



[a] [b]

Fig. 3 FTIR absorption spectrum obtained from [a] silver nanoparticles biologically synthesized by reduction of Ag^+ ions and [b] gold nanoparticles biologically synthesized by reduction of $AuCl_4^-$ ions using leaf broth of Zingiber officinale

During the synthesis of silver and gold nanoparticles a huge difference in their total reduction time was observed. In the case of gold complete reduction time was 2.5 hours, but in the case of silver it was 12 hours. Here pH of the solutions 10^{-3}M $AgNO_3$ and 5.0×10^{-4} M $HAuCl_4$ are 5.0 and 3.4 respectively, according to the equation pH+pOH=14 the pOH of silver and gold solutions are 9 and 10.6, so at this stage number of hydroxyl ions present in the $AgNO_3$ are higher than that of $HAuCl_4$ solution (number of hydroxyl ions, $[OH^-] = 10^{-OH}\times$Avagrado's number) and since biomass mainly contains negatively charged groups as confirmed by FTIR analysis, so when these groups approaches silver solution for binding they are repelled due to negative charges already present there, as compared to the gold solution, which ultimately leads towards the slow reduction of silver ions. Thus the presence of high number of hydroxyl ions in $AgNO_3$ solution may be responsible for reduced the rate of reaction.

The XRD pattern of the silver and gold nanoparticles is shown in the figure 4[a and b], various Bragg reflections clearly indicated the presence of (111), (200), (220) and (311) sets of lattice planes and further on this basis of they can be indexed as face-centered –cubic structure of silver and gold. Hence from the XRD pattern this is clear that silver and gold nanoparticles formed using ginger rhizome broth were essentially crystalline in nature.

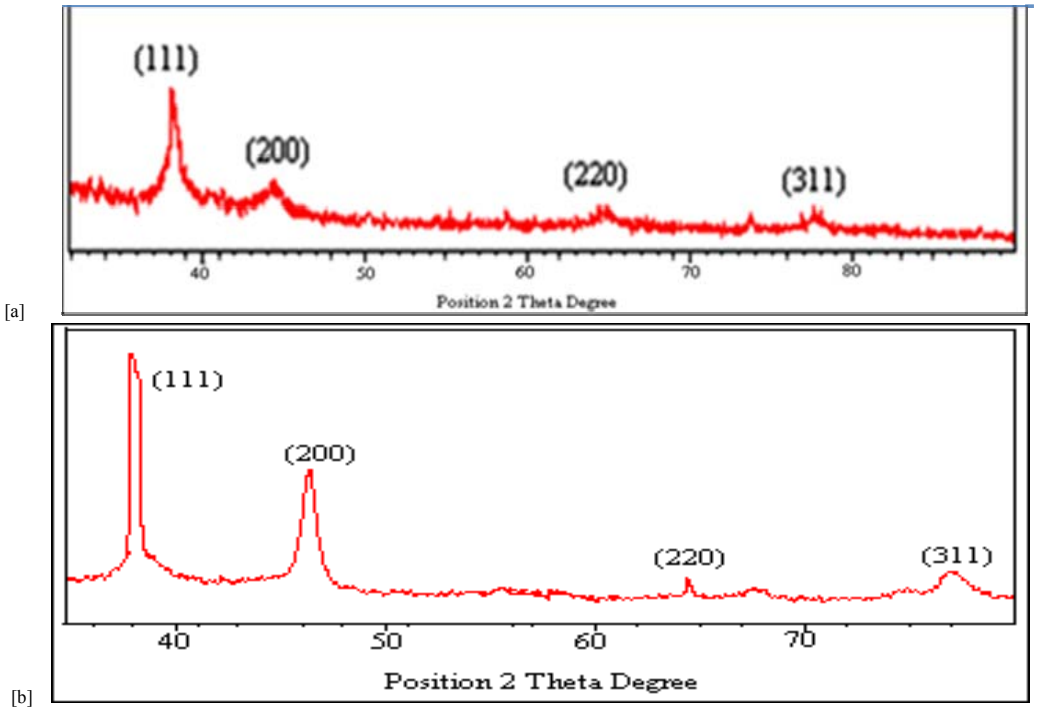


Fig. 4 X-ray diffraction Spectrum of [a] gold and [b] silver nanoparticles synthesized by reduction of $AuCl_4^-$ ions and Ag^+ ions using leaf broth of Zingiber officinale Labelled peaks correspond to characteristic diffaraction peaks of elemental Au(0) and Ag(0)

Fig. 5 represents the TEM images of biologically synthesized AgNP. From the figure it is clear that most of the AgNPs were spherical and their dimensions ranging from 10.1 to 65.91 nm with an average size of 30.31nm. Similarly figure 6 depicting the TEM images of GNP were mainly spherical in nature with few of them irregular in shape. The diameter of spherical GNP varied from 8.27 nm to 18.31 nm, with an average size 10 nm comparatively much smaller than AgNPs. The marked difference of shape control between gold (spherical to irregular) and silver (mostly spherical) may be attributed to the protective and reductive biomolecules present in the ginger biomass, shape of nanoparticles is an important criterion, since it is directly related to its optical and electrical properties [30,42]. As far as size is concerned the smaller size GNPs and AgNP are very important for effective targeted drug delivery, photo thermal therapy and in the treatment of wounds [43, 44].

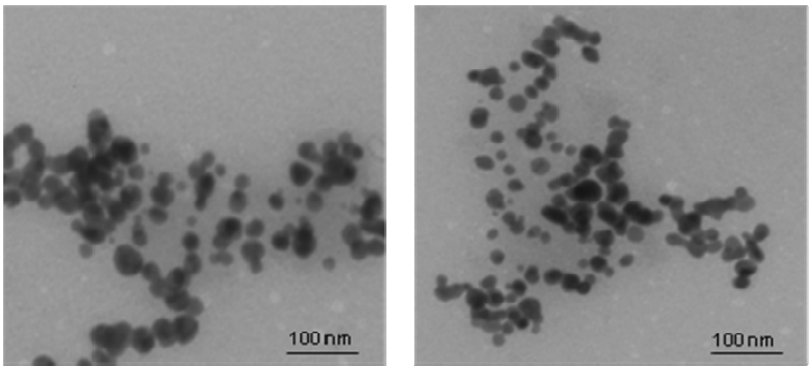


Fig. 5 Representative TEM images illustrating the formation of gold nanoparticles biologically synthesized by reduction of $AuCl_4^-$ ions using leaf broth of Zingiber officinale

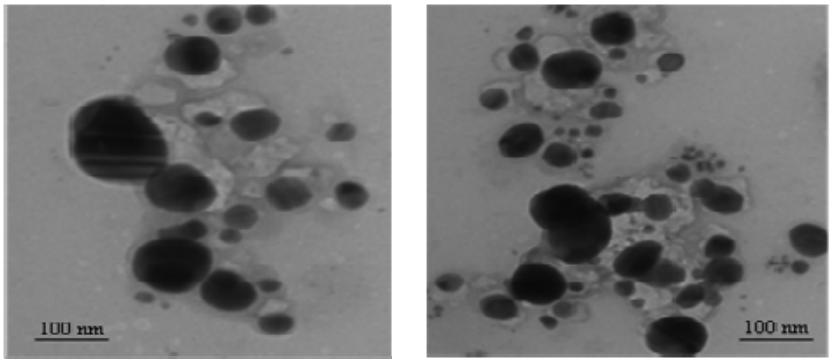


Fig. 6 Representative TEM images illustrating the formation of silver nanoparticles biologically synthesized by reduction of Ag^+ ions using leaf broth of Zingiber officinale.

4. Conclusions

In this study we present a low cost, simple and rapid approach for the bio reduction and synthesis of silver and gold nanoparticles. The most important feature of this procedure is that it is free from any chemical process, which satisfies the green biogenic approach of synthesis and gives an edge over various chemical procedures usually applied for the synthesis of gold and silver nanoparticles. Analysis of difference in the total reduction time for gold and silver nanoparticles on the basis of pH is very interesting and is important in understanding the mechanism of binding and growth of nanoparticles with respect to their pH values. Further experiments are needed in order to determine the atoms in the functional groups that are involved in the binding and stability of GNP and AgNP.

Acknowledgements

Sophisticated analytical instruments facility (SAIF) centers New Delhi and Chandigarh are gratefully acknowledged for providing the characterization facility of silver and gold nanoparticles.

References

[1] C. Jianrong, M. Yuqing, H. Nongyue, W. Xiaohua, L. Sijjiao, Nanotechnology and biosensors. Biotechnol Adv **22**,505 (2004).
[2] N. Duran, P.D. Marcato, S. De, I.H. Gabrie, O.L. Alves, E. Esposito. J Biomed Nanotechnol **3**,203 (2007).
[3] A. Singh, M. Chaudhary, M. Sastry. Nanotechnology **17**, 2399 (2006).

[4] A.L. Crumbliss, S.C. Perine, J. Stonehuerner, K.R. Tubergen, J. Zhao, R.W. Henkens, J.P. O'Daly. Biotechnol Bioeng **40**,483 (1992).
[5] B. Pulliam, J.C. Sung, D.A. Edwards, Expert Opin in Drug Delivery **4**, 651 (2007).
[6] A. Ragusa, I. Garcia, S.Penades. IEEE Trans Nanobioscience **6**,319 (2007).
[7] K. Simkiss, K.M. Wilbur, Biomoneralization,Academic Press,New York (1989).
[8] S. Mann, Biomimetic Materils Chemistry,VCH,New York (1996).
[9] T.J. Beveridge, R.G.E. Murray, J Bacteriol **141**,876 (1980).
[10] Y. Konishi, T. Nomura, T. Tsukiyama, N. Saitoh, Trans Mater Res Soc Jpn **29**, 2341 (2004).
[11] S. Silver, FEMS Microbiol Rev **27**, 341 (2003).
[12] R.M. Slawson, M.I. Van Dyke, H. Lee, J.T. Trevor, Plasmid **27**, 73 (1992).
[13] T. Klaus, R. Joerger, E. Olsson, C.G. Granqvist, Proc Natl Acad Sci USA **96**,13611(1999).
[14] S.S. Shankar, A. Ahmad, R. Pasrichaa, M. Sastry, J Mater Chem **13**,1882 (2003a).
[15] P. Mukherjee, S. Senapati, D. Mandal, A. Ahmad, M.I. Khan, R. Kumar, M. Sastry, Chem Bio Chem **3**,461 (2002).
[16] K.C. Bhainsa, S.F. D'Souza, Colloids Surf B: Biointerf **47**,160 (2006).
[17] M. Kowshik, S. Ashtaputre, S. Kharrazi, W. Vogel, J. Urban, S.K. Kulkarni, K.M. Paknikar, Nanotechnology **14**, 95 (2003).
[18] M. Gericke, A. Pinches, Hydrometallurgy **83**, 132 (2006a).
[19] C. Zhang, H. Vali, C.S. Romanek, T.J. Phelps, S.V. Liu, Am Mineral **83**,1409 (1998).
[20] A.P. Philipse, D. Maas, Langmuir **18**, 9977 (2002).
[21] H. Lee, A.M. Purdon, V. Chu, R.M. Westervelt, Nano Lett 4,995 (2004).
[22] S.A. Kumas, A.A. Ayoobul, A.Absar, M.I. Khan, J Biomed Nanotechnol 3,190(2007b).
[23] J.R. Stephen, S.J. Macnaughton **3**,230 (1999).
[24] R.K. Mehra, D.R. Winge J.Cell.Biochem **45**,30 (1991).
[25] J.L. Gardea-Torresdey, J.G. Parsons, E. Gomez, J. Peralta-Videa, H.E. Troiani, P. Santiago, M.J. Yacaman AmChemSoc **2**,397 (2002).
[26] V. Armendariz, J.L. Gardea-Torresdey, M. Jose-Yacaman, J. Gonzalez, I. Herrera, J.G. Parsons, in Proceedings –Waste Research Technology Conference at the Kansas City, Mariott-Country Club Plaza July30–Aug1 (2002)
[27] K. Ghule, A.V. Ghule, J.Y. Liu, Y.C. Ling, J Nanosci Nanotechnol **6**, 3746 (2006).
[28] S. Li, Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang, Q. Zhang, Green Chem **9**,852 (2007).
[29] E.M. Egorova, A.A. Revina, Physicochem Eng Asp **168**, 87 (2000).
[30] J. Huang, Q.Li, D. Sun, Y. Lu, X. Yang, H. Wang, Y. Wang, W. Shao, N. He, J. Hong, C. Chen, Nanotechnology **18**, 105104(11pp) (2007).
[31] B. Ankamwar, C. Damle, A. Ahmad, M. Sastry, JNanosciNanotechnol **5**,1665 (2005).
[32] S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Biotechnol Prog **22**,577 (2006)
[33] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, J Colloid Interf Sci **275**, 496 (2004).
[34] Glorious Ginger: Root out Ailments with This Ancient Spice published by thefoodpaper.com
[35] University of Maryland Medical Centre (2006). http://www.umm.edu/altmed/articles/ginger 000246.htm
[36] M. O'Hara, D. Kiefer, K. Farrell, K. Kemper, Archives of Family Medicine **7**,523 (1998).
[37] P. Mulvaney, Langmuir **12**,788(1996).
[38] A.N. Shipway, M. Lahav, R. Gabai, I. Willner, Langmuir **16**, 8789 (2000).
[39] Y. Yu, S.S. Chnag, C.L. Lee, C.R.C. Wang, J.Phys.Chem.**b101**, 6661 (1997).
[40] M. Zhu, Apparatus Analyses, Higher education press, Beijing (2000).
[41] L. Luo, S.Yu, S. Qian, T. Zhou, J Am Chem Soc. **127**, 2822 (2005).
[42] K.L. Kelly, E. Coranodo, L.L. Zhao, G.C. Schatz, J.Phys.Chem. B **107**,668 (2003).
[43] B.S. Atiyeh, M. Costagliola, S.N. Hayek, S.A. Dibo, Burns **33**,139 (2007).
[44] A.B. Lansdown, Current Problems in Dermatology **33**, 17 (2006).