Research Article

Synthesis of Silver Nanoparticles by 'Electrochemical Route' through pure metallic Silver electrodes, and evaluation of their Antimicrobial Activities

Sumit Sharma*, Kapil Choudhary, Ishu Singhal, Rishabh Saini
Research and Development Department, Nanobeach, Delhi, India.
***Corresponding author's E-mail:** sumitcctuniraj@gmail.com

Accepted on: 10-08-2014; **Finalized on:** 30-09-2014.

ABSTRACT

The present study describes a low cost, easy procedural and easy to control technique for synthesis of highly pure silver nanoparticles (AgNPs) by 'Electrochemical Route' through pure metallic silver and evaluation of their antimicrobial activities. The electrochemical setup is developed indigenously to facilitate the DC voltage operation. The synthesis process exploits the advantages such as one-step synthesis low-cost procedure, at room temperature without using any harmful chemicals. The setup brings up the oxidation of the anode and reduction of the cathode. The nanoparticles synthesized can be kept in the aqueous solution or in powder form Nanoparticles. For sample obtained, the optical characteristic is analyzed by UV-Visible spectrophotometry; morphology was studied using Scanning electron microscopy (SEM), Transmission electron microscopy (TEM). Characterization of AgNPs showed the average particle size of below 50 nm as well as revealed their spherical shapes. The antibacterial activity of silver nanoparticles against *Escherichia coli*, and *Enterobactor aurogenes* was studied. Antifungal activity was also investigated against *Candida albicanse* and *Aspergillus niger*. The results showed that AgNPs are quite pure and stable without any capping agent and displayed a significant antimicrobial activity.

Keywords: Silver nanoparticles, electrochemical reaction, antimicrobial activity.

INTRODUCTION

Currently, there is a growing need to using environmentally friendly nanoparticles synthesis route that do not produce toxic wastes in their process. To achieve this, we will use electrochemical synthesis route without using harmful chemicals. The Synthesis of AgNPs by is easy procedural, easily controlled, economic viability, and no need to use high pressure, high temperature and toxic chemicals in synthesis protocol. This approach gives a reliable process for the synthesis of nanomaterial's chemical purity because there is no other chemical used in synthesis process except silver metal in pure form and double distilled water as medium.

In the recent studies, so much efforts have been put up to synthesize Silver nanoparticles. But no studies are available for synthesis of Silver nanoparticles by electrochemical route from pure silver electrodes without using any capping agent and toxic chemicals.

The antibacterial effects of Silver (Ag) salts have been noticed since antiquity[1], and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds[2,3]. In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects. Silver nanoparticles have found tremendous applications in the field of high sensitivity bio molecular detection and diagnostics, antimicrobials and therapeutics, Catalysis and micro-electronics[4-8].

Silver has been known to possess strong active properties in nanoparticle forms. It has been found variety of application of AgNPs in different fields as medical and industrial process[9-10]. In daily life also, consumers may have nano silver containing room sprays, laundry detergents, air and water purification and wall paint[11-14]. In medical arena, nano Silver based topical dressing has been widely used as a treatment for infections in burns, open wounds and chronic ulcers, contraceptive devices, surgical instruments, catheters and bone prostheses[15,16]. AgNPs can also act as a catalyst; and disables the enzyme needed for oxygen metabolism by single-celled bacteria, viruses and fungi, so they suffocate without causing harm to human enzymes or parts of the human body chemistry[17,18].

Here in, we report the synthesis of silver nanoparticles by electrochemical reaction through pure silver metal, and these electrochemically synthesized nanoparticles were found highly toxic against different human pathogens.

MATERIALS AND METHODS

Materials

The components of the culture mediums used in growing, maintaining and experimentation of microbial cultures were supplied by Hi media laboratories.

Double distilled water (by Millipore) and silver electrodes were purchased from a local metal dealer and later molded to form electrode with a dimension (Width .5cm, Thickness 2mm). Susceptibility test conducted on E. coli, Aspergillus nigar and Candida albicance strain, enterobactor *aerugenes* strain. TDS meter is used for concentration determination which was integrated in water analyzer 370.

Synthesis of Silver Nanoparticles

Both electrodes were initially polished with zero number sandpaper and further cleaned and sonicated in ethanol for 5 minutes. After drying of the electrodes, they were spaced 3 cm apart and fixed using the electrodes clips dipped into double distilled water which was being slowly stirred as shown in the **Figure 1**.

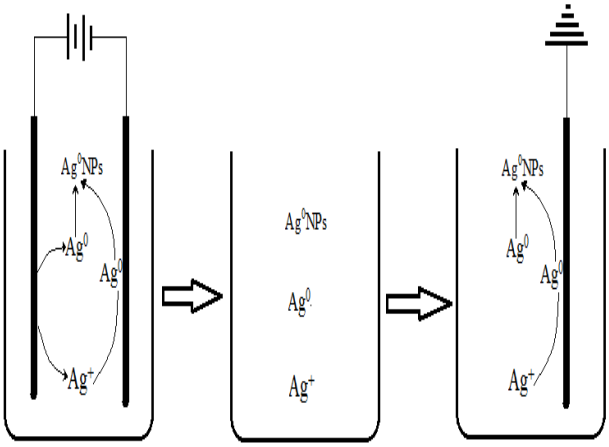

Figure 1: Block diagram of electrochemical reactions and synthesis of AgNPs.

Anode was 10 cm long spiral formed and straight cathode was of 5 cm of length. As such no capping agent was used and 200 ml of double distilled de-ionized water was taken as the medium for electrolyte cell to operate. A DC current source of 60 volts and maximum 5 mA was used. When current source was switched on and applied to silver electrode the final current was 3.2mA. DC current was applied for 2 hours with rubbed cleaning of electrodes repeatedly after each quarter of hour by doubled distilled water and filter paper. The temperature was 35° C during all of experimentation.

RESULTS

Visual inspection

The reduction of silver ions was routinely monitored by visual inspection of the solution. The solution gives brownish yellow color darkened with time and concentration. As we used only the pure metallic silver with double distilled water only for electrochemical reaction, there is no possibility of any other impurity except silver in aqueous medium. The pictures of beaker with the changes in color of initially taken water by synthesis of nanoparticles with time are shown in the Figure 2.

The appearance of brownish yellow color clearly indicates the formation of silver nanoparticles in the electrochemical reaction[19-21]. The characteristics brown color of colloidal silver solution is due to the excitation of surface Plasmon vibrations in the nanoparticle and provides a convenient spectroscopic signature of their formation[22,23].

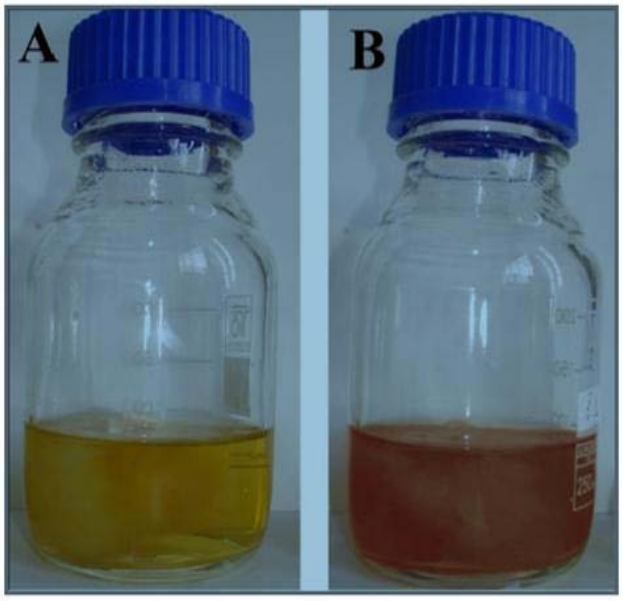

Figure 2: Pictures of beaker with time interval of 2 hours after starting the reaction (A) at starting, (B) after 1 hour, and (C) after 2 hours

UV-Vis Spectra analysis

The reduction of pure silver ions was measured by the UV-Visible spectra of the solution by periodic sampling of aliquots (2 mL) of the aqueous component. The UV-Vis spectroscopy measurements were recorded on a Shimadzu dual beam spectrophotometer (model UV-2450) operated at a resolution of 1 nm. It is well known that colloidal dispersions of metallic nanoparticles exhibit absorption bands in the UV-vis region, due to collective excitations of the free electrons (surface plasmon band)[24]. The formation of Ag nanoparticles can be analyzed easily by the UV-spectra[25]. **Figure 3(A)** shows the UV-Vis spectra recorded from the final solution after 8 hours.

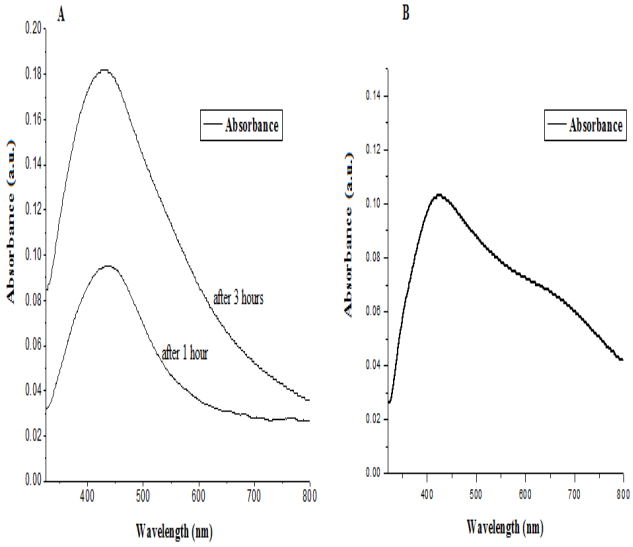

Figure 3: UV-Vis spectra of colloidal solution of AgNPs (A) after 1 AND 3 hours of starting of electrochemical reaction. (B) After 3 months

The Peak is seen at around 430 nm, suggesting that the particles are spherical shaped and dispersed in the aqueous solution with no evidence for aggregation[21]. Broadening of peak indicated fairness in the mono dispersion of particles[21]. It was observed that the silver nanoparticles of silver was stable for 3 months and onward with no aggregation even there is no any capping agent present in solution. **Figure 3 (B)** shows the UV-Vis spectra recorded from the final solution after 3 month.

SEM analysis of silver nanoparticles

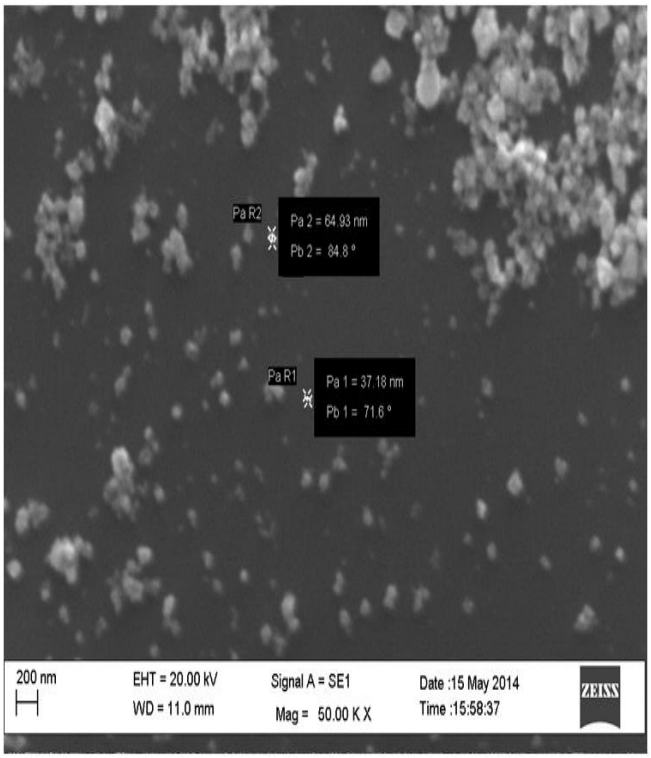

Figure 4: SEM Image of Silver Nanoparticles on silicon substrate

A drop of Solution containing Silver Nanoparticles was added to a silicon substrate. Then the substrate was heated for 15 min to obtain a film on the silicon substrate. This prepared sample was used to run the SEM characterization. As shown in **Figure 4** SEM image of silver nanoparticles synthesized from 'electrochemical reaction' Shows the silver nanoparticles are spherical in structure. Most of the nanoparticles are well separated and few agglomerations were noticed. The particle size of silver nanoparticles has been obtained as 30-70 nm From the SEM images.

TEM analysis of silver nanoparticles

Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper then the film on the TEM grid were allowed to dry by putting under mercury lamp for 5 min and used for TEM characterization. As shown in **Figure 5** TEM image of silver nanoparticles synthesized from 'electrochemical route' shows the particle size of few nanoparticles are less than 20 nm and spherical in shape without agglomerations.

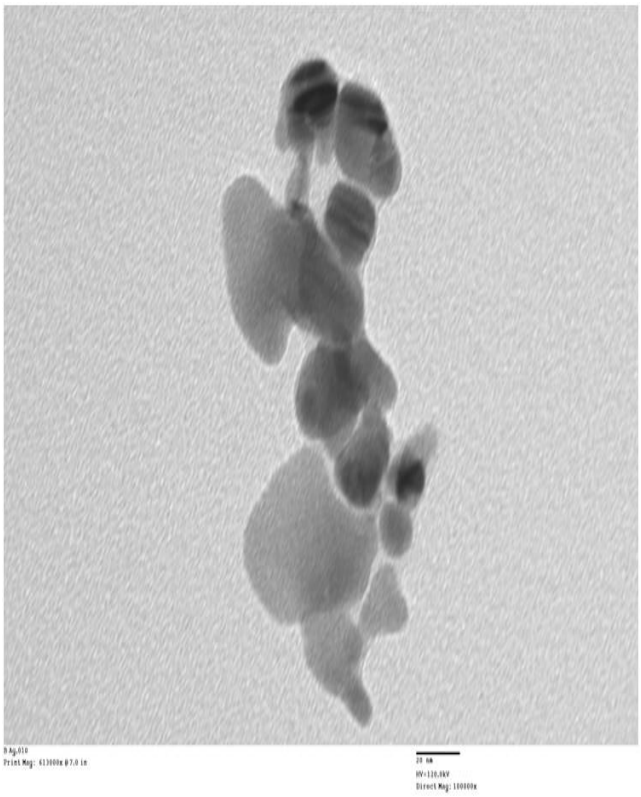

Figure 5: TEM Image of Silver nanoparticles

Antimicrobial activity

The antibacterial assays were done on human pathogenic Escherichia coli, enterobactor aeruginous, Aspergillus nigar and Candida albicance by standard disc diffusion method. Appropriate agar medium for culture of microbe was prepared and sterilized in a conical flask at a pressure of 20 lbs. for 20 min. After pouring and solidification of the media, microbe culture was spread on the solid surface of the media. Sterile paper discs of 5mm diameter (dipped in 7ppm silver nanoparticle solution for 30 hours) were placed in each inoculated petri dish.

S.NO.	Test organism	Zone of inhibition (mm)	Concentration (PPM)	Agar medium used
1	*E. coli*	12	8	L.B.
2	*E.aerugines*	12	8	MacConkey
3	*C. albicanse*	14	8	Y.P.D.
4	*A. niger*	3	8	Dextrose

Table 1: parameters and their values for antimicrobial activity

The zone of inhibition (mm) around disk, concentration (PPM) of nanoparticles used for preparation of disk, and medium used for culture are presented in the **Table 1,** while **Figure 6 (a-d)** shows the images of disks with zone of inhibition. Nanoparticles syntheses by electrochemical

reaction through pure metallic silver are found highly toxic against human pathogenic bacteria and fungi.

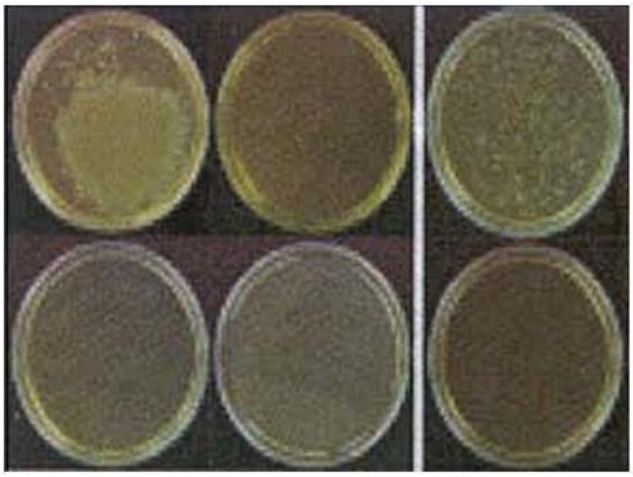

Figure 6: microbes grown on agar plates showing the zone of inhibition after treatment of the silver nanoparticle (8 PPM) integrated paper dick. Upper left, E. coli; Upper right, E.*aerugines*; bottom left, C. albicance, and bottom right, A. niger

In the results silver nanoparticles promised for highly antimicrobial activity against *E. coli, E. aerogenes* and *C. albicans* while mild antimicrobial activity was found against *A. niger*.

Overall synthesis of silver nanoparticles from 'electrochemical reaction' through pure metallic silver was investigated. Characterizations have confirmed the presence of Silver nanoparticles in the range of 50 nm and they promised for highly antimicrobial activity as non-selectable biocide against many human pathogenic microbes.

DISCUSSION

Metallic nanoparticles are traditionally synthesized by wet chemical techniques[26-29], where the chemicals used are quite often toxic or flammable. These chemicals can be also harmful to our environment also. In recent, biosynthesis of nanoparticles has received increasing attention because of use of natural products in synthesis of nanoparticles[20,30-32]. But it is not easy to obtain the concerned bio product or extract in a low cost with as huge quantity as required for large scale production of nanoparticles. The high cost, and required long time for growth and maintenance of bio resources for supply of bio product used in biosynthesis of nanoparticles makes this method worst. Additionally, Hazardous chemicals used in chemical technique and undetermined toxic biochemical in biosynthesis of nanoparticles can be absorbed on the surface of nanoparticles that may have adverse effect in the medical applications.

Electrochemical method seems as a better way to synthesis of metallic nanoparticles with trust for purity when there is a requirement of economic viable, ease procedural and easily controllable technique with which the process can be scaled up but no need to use high pressure, energy, temperature and toxic chemicals in synthesis protocol.

In the chemical and biological synthesis of nanoparticle, metallic ions obtained from there salt are reduced by a reducing agent in the presence of stabilizer or capping agent. In electrochemical procedure described in early studies to obtain particles, a metal salt is reduced at the cathode, giving rise to metallic particles stabilized by stabilizers or capping agents [33-36]. In all these strategies, metallic ions go to nanoparticles with 'bottom-up' approach of particle growth. The present study reports for the first time synthesis of silver nanoparticles synthesized by electrochemical reaction with 'top-down' approach of particle growth. In this method, metallic nanoparticles have been produced directly from pure form of bulk material. Same as other strategies, chemical reaction takes place by electrochemical route but Ag^+ ions were obtained from pure metallic anode because Ag was anodically dissolved and gives intermediate Ag^+ ions which got reduced by negative cathode of same metal and colloidal solution of silver nanoparticles was obtained which was stable by Zeta-potential. There was no need for stabilizers or capping agents for regulation of particle size in the present technique of Ag nanoparticle synthesis. Because, Particle formation was carried on only at the metallic cathode not in whole volume of solution and nano sized particles used to leave the metallic cathode immediately because of mechanical stirring after these assemblies from reduction and agglomeration of Ag^+ ions to AgNPs stabilized by negative Zeta-potential. It seems from previous and present studies that in the electrochemical reaction if once particles were formed and dispersed in a medium, they were not further agglomerate immediately but took a significant time in months. The negative Zeta potential present on electrochemically formed AgNPs (sol) causes for mutual repulsion of the similar charges then stabilizes the colloidal sol by keeping apart the particles and prevents agglomeration[37]. So there is no need of any type of capping agent but ionized water also responsible for stability of AgNPs in colloidal solution. In the results of this study AgNPs were observed as stable at room temperature for three months and onward successfully. It was also seemed that if the colloidal solution of nanoparticles was kept froze at -2° C, it was stable for six months and onward probably because of two reasons, first is the absence of motion and contact with each other of nanoparticles in solid form of medium and second is insufficient action potential at low temperature for metallic bond formation in agglomeration. Ag nanoparticles were also stable for same long time, if a stabilizer was applied just after completing the electrochemical reaction.

There was an indication got from experimental work for a precise particle-size control achieved by adjusting the current density and voltage. On the low current density and voltage the out flux of Ag^+ ions from anode and influx on cathode is comparatively slow there for assembly of

atoms to form nanoparticles on anode was also slow and small particles were obtained. Vice-versa is also true.

The best advantage of this method is the high level of purity of the particles because there is no other chemicals were used in the synthesis protocol except pure metallic silver and double distilled water.

The primary aim of this work is to adjust the general scheme to the synthesis of silver nanoparticles free from any kind of impurities. For the optimization, it is necessary to take into account the following parameters: (1) current density which mainly controls the electrochemical actions and regulation of size of nanoparticles (2) operating temperature which is considered for Brownian motion and action potential (3) time of reaction which also controls the particle size and concentration (4) Size and shape of metal electrodes for consideration of surface area as work spot which also controls the electrochemical actions. (5) Purity of metal electrodes and medium.

The Ag nanoparticles were tested against *E. coli, E. aerogenes and* C. albicans they effectively inhibited the microbial growth that was similar to that found by Sondi and Salopek-Sondi[18]. In contrast, the inhibitory effect of Ag nanoparticles was mild in *A. niger* as compared with other microorganisms; these results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain microbial species. We think that the lower efficacy of the Ag nanoparticles against *A. niger* may derive from the difference as a point of membrane structure that was similar to results and discussion of J.S. Kim, E. Kuk and colleagues[37].

In conclusion, Ag nanoparticles prepared by the 'Electrochemical route' described here have great promise as antimicrobial agents. This cost-effective and easy to control technique, can be a suitable way for the formulation of new method of nanoparticle synthesis for industrial production of highly pure metallic nanoparticle. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

REFERENCES

1. Jones SA, Bowler PG, Walker M, and Parsons D, "Controlling wound bio burden with a novel silver-containing Hydro fiber dressing.," Wound Repair Regen., vol. 12, no. 3, 2004, pp. 288–294.
2. Silver S and Phung LT, "Bacterial heavy metal resistance: new surprises.," Annu. Rev. Microbiol., vol. 50, 1996, pp. 753–789.
3. Catauro M, Raucci MG, De Gaetano F, and Marotta A, "Antibacterial and bioactive silver-containing Na2O x CaO x 2SiO2 glass prepared by sol-gel method.," J. Mater. Sci. Mater. Med., vol. 15, no. 7, 2004, pp. 831–837.
4. Schultz S, Smith DR, Mock JJ, and Schultz DA, "Single-target molecule detection with non bleaching multi color optical immune labels.," Proc. Natl. Acad. Sci. U. S. A., vol. 97, no. 3, 2000, pp. 996–1001.
5. Rai M, Yadav A, and Gade A, "Silver nanoparticles as a new generation of antimicrobials.," Biotechnol. Adv., vol. 27, no. 1, 2009, pp. 76–83.
6. Elechiguerra JL, Burt JL, Morones JR, Bragado AC, Gao X, Lara HH, Yacaman MJ, Interaction of silver nanoparticles with HIV-1, Journal of Nanobiotechnology, 29, 2005, pp. 3-6.
7. Crooks RM, "Patterning of hyper branched polymer films.," Chemphyschem, vol. 2, no. 11, 2001, pp. 644–654.
8. Gittins DI, Bethell D, Schiffrin DJ, and Nichols RJ, "A nanometre-scale electronic switch consisting of a metal cluster and redox-addressable groups.," Nature, vol. 408, no. 6808, 2000, pp. 67–69.
9. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, and Yacaman MJ, "The bactericidal effect of silver nanoparticles.," Nanotechnology, vol. 16, no. 10, 2005, pp. 2346–2353.
10. Lok C, Ho C, Chen R, He Q, Yu W, Sun H, Tam PK, Chiu J, Che C, "Silver nanoparticles: partial oxidation and antibacterial activities.," J. Biol. Inorg. Chem., vol. 12, no. 4, 2004, pp. 527–534.
11. Chou WL, Yu DG, and Yang MC, "The preparation and characterization of silver-loading cellulose acetate hollow fiber membrane for water treatment," Polym. Adv. Technol., vol. 16, no. 8 SRC – Google Scholar FG - 0, 2005.
12. Jin M, Zhang X, Nishimoto S, Liu Z, Tryk DA, Emeline AV, Murakami T, and Fujishima A, "Light-Stimulated Composition Conversion in TiO2-Based Nanofibers," J. Phys. Chem. C, vol. 111, no. 2, Dec. 2006, pp. 658–665.
13. Chen Q, Yue L, Xie F, Zhou M, Fu Y, Zhang Y, and Weng J, "Preferential Facet of Nanocrystalline Silver Embedded in Polyethylene Oxide Nanocomposite and Its Antibiotic Behaviors," J. Phys. Chem. C, vol. 112, no. 27, 2008, pp. 10004–10007.
14. Sun Y, Xia Y, "Shape-Controlled Synthesis of Gold and Silver Nanoparticles," Science, vol. 298, no. 5601, 2002, pp. 2176–2179.
15. Muangman P, Chuntrasakul C, Silthram S, Suvanchote S, Benjathanung R, Kittidacha S, and Rueksomtawin S, "Comparison of efficacy of 1% silver sulfadiazine and Acticoat for treatment of partial-thickness burn wounds.," J. Med. Assoc. Thai., vol. 89, no. 7, 2006, pp. 953–958.
16. Lansdown ABG, "Silver in health care: antimicrobial effects and safety in use.," Curr. Probl. Dermatol., vol. 33, 2006, pp. 17–34.
17. Kvítek L, Panáček A, Soukupová J, Kolář M, Večeřová R, Prucek R, Holecová M, and Zbořil R, "Effect of Surfactants and Polymers on Stability and Antibacterial Activity of Silver Nanoparticles (NPs)," J. Phys. Chem. C, vol. 112, no. 15, 2008, pp. 5825–5834.
18. Sondi I & Sondi B S, "Silver nanoparticles as antimicrobial agent : a case study on E. Coli as a model for Gram-negative bacteria," J. Colloid Interface Sci., vol. 275, 2004, pp. 177–182.

19. Durán N, Marcato PD, Alves OL, De Souza GIH, and Esposito E, "Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusariumoxysporum strains.," J. Nanobiotechnology, vol. 3, 2005, p. 8.

20. Saifuddin N, Wong CW, NurYasumira AA, "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation, E-Journal of Chemistry," vol. 6, no. 1 SRC - Google Scholar FG - 0, 2009, pp. 61–70.

21. Jain D, Daima HK, Kachhwaha S, Kothari SL, Synthesis of plant-mediated Silver nanoparticles using Papaya fruit extract and evaluation of their antimicrobial properties, Digest Journal of Nanomaterials and Biostructures, 4(3), 2009, pp. 723 – 727.

22. Mulvaney P, "Surface Plasmon Spectroscopy of Nanosized Metal Particles," Langmuir, vol. 12, no. 3, Jan. 1996, pp. 788–800.

23. Shankar SS, Rai A, Ahmad A, and Sastry M, "Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachtaindica) leaf broth.," J. Colloid Interface Sci., vol. 275, no. 2, 2004, pp. 496–502.

24. Wilcoxon JP, Williamson RL, and Baughman R, "Optical properties of gold colloids formed in inverse micelles," J. Chem. Phys., vol. 98, no. 12, 1993.

25. Wiley BJ, Im SH, Li ZY, McLellan J, Siekkinen A, Xia Y, "Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis.," J. Phys. Chem. B, vol. 110, no. 32, 2006, pp. 15666–15675.

26. Sun Y, Xia Y, Shape-controlled synthesis of gold and silver nanoparticles, Science, vol. 298 (5601), 2002, pp. 2176-9.

27. Jana NR, Gear heart L, and Murphy CJ, "Seeding Growth for Size Control of 5–40 nm Diameter Gold Nanoparticles," Langmuir, vol. 17, no. 22, 2001, pp. 6782–6786.

28. Schmid G, "Large clusters and colloids. Metals in the embryonic state," Chem. Rev., vol. 92, no. 8, Dec. 1992, pp. 1709–1727.

29. Goia DV and Matijević E, Preparation of mono dispersed metal particles, New Journal of Chemistry, vol. 22, 1998, pp. 1203-1215

30. Parashar V, Parashar R, Sharma B, Pandey AC, Parthenium Leaf Extract Mediated Synthesis of Silver Nanoparticles: A Novel Approach Towards Weed Utilization, Digest Journal of Nanomaterials and Biostructures, vol.4 (1), 2009, pp. 45 – 50

31. Bhainsa KC and D'Souza SF, "Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillusfumigatus.," Colloids Surf. B. Biointerfaces, vol. 47, no. 2, 2006, pp. 160–164.

32. Willner I, Basnar B, and Willner B, "Nanoparticle-enzyme hybrid systems for nanobiotechnology.," FEBS J., vol. 274, no. 2, 2007, pp. 302–309.

33. Reetz MT, Winter M, and Tesche B, "Self-assembly of tetra alkylammonium salt-stabilized giant palladium clusters on surfaces," Chem. Commun., no. 2, 1997, pp. 147–148.

34. Rodriguez-Sanchez L, Blanco MC and Lopez - Quintela MA, "Electrochemical Synthesis of Silver Nanoparticles," Journal of Physical Chemistry B, Vol. 104, No. 41, 2000, pp. 9683-9688.

35. Yin B, H. Ma, S. Wang and S. Chen, "Electrochemical Synthesis of Silver Nanoparticles under Protection of Poly (N-vinylpyrrolidone)," J. Phys. Chem. B, vol. 107, no. 34, 2003, pp. 8898–8904.

36. Zhu J, Liu S, Palchik O, Koltypin Y, and Gedanken A, Shape-Controlled Synthesis of Silver Nanoparticles by Pulse Sono electrochemical Methods, Langmuir, vol.16(16), 2000, pp 6396–6399

37. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, and Cho MH, "Antimicrobial effects of silver nanoparticles.," Nanomedicine, vol. 3, no. 1, 2007, pp. 95–101.

Source of Support: Nil, **Conflict of Interest:** None.