Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Issue 4, 2013

ISSN - 0974-2441

Research Article

MORINDA TINCTORIA FRUIT ASSISTED BIOSYNTHESIS OF SILVER NANOPARTICLES

R.KIRUBHA* AND G.ALAGUMUTHU

P.G & Research centre, Department of chemistry, Sri Paramakalyani College, Manonmaniam Sundaranar University, Alwarkurichi-India.
E.mail: alagupathi@yahoo.co.in, rosekirubha@gmail.com

Received: 2 August 2013, Revised and Accepted: 27 August 2013

ABSTRACT

Objective: To develop a reliable, eco-friendly and easy process for the synthesis of silver nanoparticles using fruit extracts of medicinal plant '*Morinda Tinctoria*' and evaluate its anti-microbial properties.
Methods: The synthesis and characterization of silver nanoparticles was confirmed by UV–Visible, FR-IR, SEM, TEM, CV techniques and Disc diffusion assay method was used to confirm the antibacterial activity of silver nanoparticles.
Results: UV-visible spectrum of the aqueous medium containing silver nanoparticles showed a peak around 420.25nm. FTIR analysis confirmed reduction of Ag^+ ions to Ag^0 ions in synthesized silver nanoparticles.The SEM and TEM analysis showed the particle size between 17-25nm, and spherical in structure.The silver nanoparticles have shown bactericidal effect against *E.Coli, Staphylocococus aureus, Bascillus cereus, and Pseudomonas aeruginosa.*
Conclusions: The fruit extract of *M.Tinctoria* quickly reduces Ag^+ to Ag^0 and enhances synthesis of silver nanoparticles with anti-microbial activity.

Keywords: Nanoparticles; Morinda Tinctoria; TEM; Silver nanoparticle, Antibacterial activity;

INTRODUCTION

Nanomaterials have received much attention because of their structure and properties differ significantly from those of atoms, molecules, and bulk materials [1]. The synthesis of metal nanoparticles has been widely discussed in the literature due to their small sizes, large surface area and unique physical and chemical properties, which have many potential applications [2-4]. The reducing agent, reaction medium, and stabilizer are the three key factors in the synthesis and stabilization of metallic nanoparticles[5]. The nanomaterials can be synthesized by physical, chemical and biological methods. Although the physical and chemical methods produce pure, well defined particles, these methods are not cost effective and ecofriendly. This drawback can be exhausted by biological method where the microorganism or plant extract or plant biomass is used as reducing agent[6-10].Now-a-days biological synthesis of metallic nanoparticles is gaining importance as it is reliable and ecofriendly. The formation of silver nanoparticles via green route is also studied by using *Gelidiella acerosa* crude extracts[11], *Camellia sinensis*[12], *coriander leaves*[13], *Ficus benghalensis*[14], *Crossandrain fundibuliformis*[15], *neem leaf*[16] , *Cissus Quadrangularis*[17] ,*Morinda Pubescens*[18] and *Portulaca Oleracea (l.)*[19].

In our present study, we have demonstrated a suitable green method for the synthesis of silver nanoparticles using *Morinda Tinctoria* fruit extract as reducing agent. The antibacterial activity of silver nanoparticles has been tested against various pathogens.

MATERIALS AND METHODS:

Materials

Fresh fruits of *Morinda Tinctoria* were identified and collected from Tamilnadu Agricultural University, Tirunelveli, and Tamilnadu, India and the taxonomic identification was made by Botanical Survey of India, Coimbatore. Silver nitrate was obtained from the precision scientific co, Coimbatore, India.

Synthesis of silver nanoparticles

The fresh fruits of *M.Tinctoria* broth solution was prepared by taking 100g of thoroughly washed and finely cut fruits in a 500 mL Erlenmeyer flask along with 200mL of sterilized double distilled water and then boiling the mixture for 15 min before finally decanting it. The extract was filtered through Whatman filter paper no 1 and stored at -15⁰C and could be used within 1 week. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature. As a result, a brown-yellow solution was formed; indicating the formation of silver nanoparticles and it was further confirmed by UV-Vis spectrum analysis [20]. It showed that aqueous silver ions could be reduced by aqueous extract of plant parts to generate extremely stable silver nano particles in water (Figure 1).

Figure1: Photographs showing A) pure $AgNO_3$ solution B) pure *M.Tinctoria* fruit Extract C) Colour changes after adding fruit Extract with $AgNO_3$ solution.

Characterization of the synthesized silver nanoparticles using UV-spectra:

Synthesis of silver nanoparticles solution with fruits extract may be easily observed by ultraviolet-visible (UV-Vis) spectroscopy. The bio-reduction of the Ag^+ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component and measuring the UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on a Vasco 1301 spectrophotometer in 400-600 nm range operated at a resolution of 1 nm.

FT-IR Spectroscopy

FT-IR measurements is undertaken in order to confirm the formation of crystalline nanocrystals and identify adsorbed species onto the crystal surface. Generally, FT-IR is recorded using Nicolet

FT-IR spectrometer mode impact 400. The spectra were recorded at wave number in the range of 400 and 4000cm^{-1}.

Scanning electron microscopy (SEM)

The electronic images were made on Hitachi S-4500 SEM Analyzer.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) (HITACHI, H-7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. Ag nanoparticle image was formed from the interaction of the electrons transmitted through the specimen; the image of Ag nanoparticles was magnified and focused onto an imaging device.

Cyclic Voltammetry analysis

Analysis through cyclic voltammetry(CV) confirmed the presence of elemental silver signal of silver nanoparticles .The change in the oxidation state of the metal ion was studied by CV technique, using platinum electrode with fresh surface at the rate of 25mVs^{-1} in the potential range between -1.0 and 1.0V.

Antimicrobial activity study

Antimicrobial activities of the synthesized Ag nanoparticles were determined by using the agar disc diffusion assay method. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized petri plates. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h.100 mL of broth culture of each test organism (1105 cfu/mL) was used to prepare lawns. Agar of 5 mm diameter was prepared with the help of a sterilized stainless steel cork borer. Five plates were prepared in the agar plates. Ciprofloxacin was used as standard and positive controls. The plates containing the test organism and Ag nanoparticles were incubated at 37 ^{o}C for 24 - 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the plates. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

RESULTS

UV-VIS spectra analysis

Reduction of Ag ion into silver nanoparticles during exposure to the plant extracts could be followed by color change. Silver nanoparticle exhibit dark yellowish - brown color in aqueous solution due to the surface plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential plants for synthesizing the Ag nanoparticles. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min has absorbance peak at 420.25 nm, broadening of peak indicated that the particles are polydispersed (Figure 2).

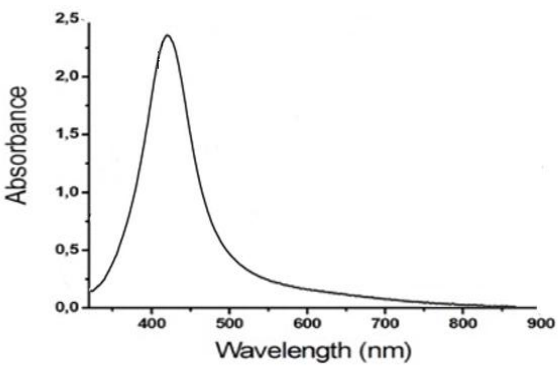
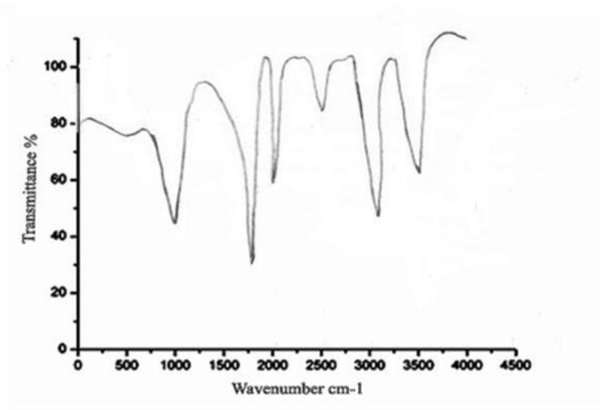


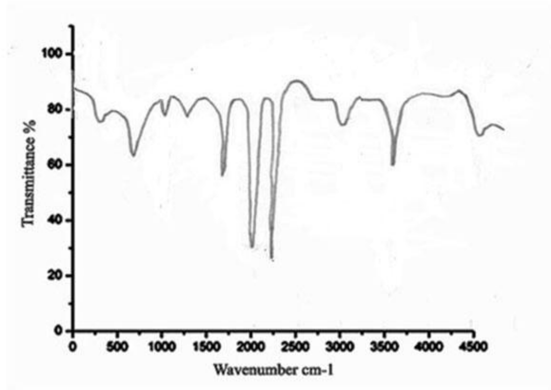
Figure 2: UV Spectra of silver nanoparticles

FT-IR studies

FTIR analysis was carried out to identify the possible interaction between the biomolecule and Ag^{+} during the biogenric reduction reactions. Figure 3(a) and 3(b) shows, the FTIR data for *Morinda Tinctoria* fruit extract and the biosynthesized Silver nanoparticles. The band at 3524cm^{-1} is assigned for O-H stretching vibration of alcohol and phenol compounds and band observed at 1627.3 cm^{-1} corresponds to N─H groups of primary amines. The band at 1034.2 cm^{-1} shows that the C─O stretching vibrations of alcohols and carboxylic groups. Absorption peaks located at about 3382.24 cm^{-1}, 2915.81 cm^{-1}, 1638.02 cm^{-1}, 1381.96 cm^{-1}, 1033.34 cm^{-1}, and 479.82 cm^{-1} in the region of 4000 cm^{-1} to 500 cm^{-1} indicates the presence different functional groups like secondary Alcohol (O-H stretch, H-bonded), Alkanes (-C-H- stretching), Alkene (C=C- stretching), Alkane (-C-H bending), Ether (C-O stretching) and Alkene (=C-H bending). Proteins present in the extract can bind to AgNPs through either free amino/carboxylic group in the proteins. The FTIR values showed reduction and capping of silver ion which may be due to the presence of alkaloids. Particularly, the peak at 1034.2cm^{-1} of the extract changed is to 1037cm^{-1} after synthesis confirming the reduction of silver ion to silver nanoparticles.



3(a)



3(b)

Figure 3: FT-IR spectra of 3(a) *Morinda Tinctoria* fruit extractand 3(b) biosynthesized Silver nanoparticles.

SEM Analysis of Silver nanoparticle

SEM images provided information about the morphology and size of the biosynthesized Silver nanoparticles. The Silver nanoparticles were found to be cubic in shape. The diameter of synthesized nanoparticle was identified as 18.2 nm and shown in figure 4. SEM image also showed the high density Silver nanoparticles synthesized by the *Morinda Tinctoria* plant extract. This confirms the development of Silver nanostructures by the plant extract.

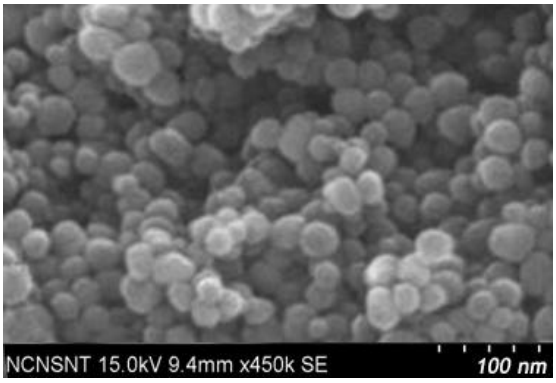


Figure 4: SEM analysis of Silver nanoparticles

TEM analysis of Ag nanoparticles

Based on the TEM analysis, which we conclude that the average mean size of Ag nanoparticles was *18.2* nm and seems to be spherical in morphology as shown in Figure. 5.

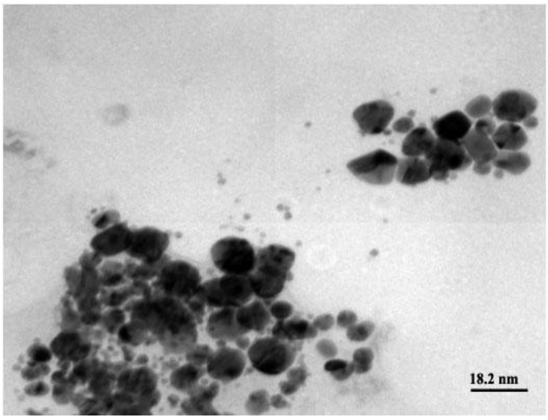


Figure 5: HR-TEM analysis of silver nanoparticles

Cyclic Voltametry analysis

In cyclic voltammetric analysis the *Morinda Tinctoria* fruit extract free solution makes all the metal ions are reduced to lower oxidation state, since there is no possibility for the formation of NPs. On addition of *Morinda Tinctoria* extract in the reaction medium, the cathodic peak shifted towards the negative potential direction, implying that the reduced silver NPs are stabilized by *Morinda Tinctoria* extract (Fig. 6). The extent of decrease in anodic peak current is greater than that of the cathodic peak current due to the fact that the rate of reduction of silver ion may be greater than its oxidation. This might be because of the electron donating methoxy, hydroxyl and amine groups present in *Morinda Tinctoria* extract can provide a suitable environment for the formation of nanoparticles. The cyclic voltammogram of AgNPs shows the peaks observed at -0.43 and 0.052V.

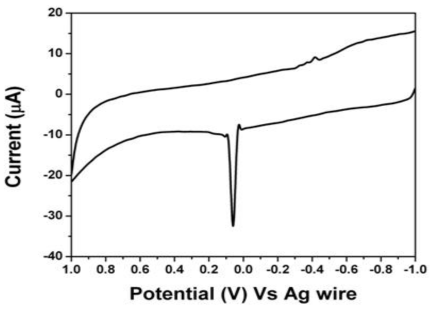


Figure 6: Cyclic voltammograms of silver nanoparticles.

Antibacterial Activity study

The antibacterial activity of silver nanoparticle was tested against the following microorganism, viz; *E.Coli, Staphylococus aureus, Bascillus cereus, and Pseudomonas aeruginosa* by disc diffusion method. The silver nanoparticle has shown the antibacterial activity against all tested microorganism and maximum zone of inhibition was found against *Staphylococus aureus* (figure 7).

Figure 7: Antibacterial activity of Silver Nanoparticles using *M.Tinctoria* fruit extract.

DISCUSSION

Antibiotic resistance by the pathogenic bacteria has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents. In the present scenario, Ag nanoparticles as antimicrobial agents have come up as a promising candidate in the medical field [21]. The extremely small size of nanoparticles exhibits enhanced or different properties when compared with the bulk material. There are different physical and chemical methods for the synthesis of nanoparticles, but there is always a need for the development of eco-friendly route for the synthesis process [22, 23]. Hence, our current study proves to be an important step in this direction. Formation and stability of Ag nanoparticles in aqueous colloidal solution are confirmed using UV-Vis spectral analysis. UV–Vis spectra of aqueous component as a function of time variation of fruit broth with 1mM aqueous $AgNO_3$ solution. Metal nanoparticles have free electrons, which give surface plasmon resonance absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver colloids were observed at *420.25* nm. The intensity of absorption band increases with increasing time period of aqueous component and consequent color changes were observed from colorless to reddish brown. These characteristic color variations is due to the excitation of the of the surface plasmon resonance in the metal nanoparticles.

From the analysis of FTIR studies, we revealed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of *Silver* nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of Silver nanoparticles in the aqueous medium. These results imply that proteins, sugars and amino acid present in *Morinda Tinctoria* plant extract are play a major role on reduction of Ag^+.

Transmission electron microscope image of silver nanoparticles obtained from *Morinda Tinctoria* extract. The morphology of the nanoparticles was spherical in nature. The obtained nanoparticles are in the range of sizes 17-25 nm and few particles are agglomerated. The mean size of the silver nanoparticle was obtained as *18.2*nm from HR-TEM is a good agreement with the size obtained in the SEM measurements.

Fig. 6 illustrates the response of a reversible redox couple during a single potential cycle. It is assumed that only the oxidized form Ag^+ is present initially. Thus, a negative-going potential scan is chosen for the first halfcycle, starting from a value where no reduction occurred. As the applied potential approaches the characteristic *Eo* for the redox process, a cathodic current begins to increase, until a peak is reached. The sweep is reversed after traversing the potential region where the reduction process takes place. During the reverse scan, Ag^0 molecules are reoxidized back to Ag^+ and it result in an anodic peak.

It is well known that Ag ions and Ag-based compounds have strong antimicrobial effects [24], and many investigators are interested in using other inorganic nanoparticles as antibacterial agents[25-27]. These inorganic nanoparticles have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. Thus far, these antimicrobial agents based on chemicals have been effective for therapy; however, they have been limited to use for medical devices and in prophylaxis in antimicrobial facilities. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed desperately, especially in medical devices, etc. Ag ions and Ag salts have been used for decades as antimicrobial agents in various fields because of their growth-inhibitory capacity against microorganisms. Also, many other researchers have tried to measure the activity of metal ions against microorganisms [28, 29]. However, Ag^+ ions or salts has only limited usefulness as an antimicrobial agent for several reasons, including the interfering effects of salts and the antimicrobial mechanism of the continuous release of enough concentration of Ag^+ ion from the metalform. In contrast, these kinds of limitations can be overcome by the use of Ag nanoparticles.

CONCLUSION

In this investigation, the bio-reduction of aqueous Ag^+ ions by the fruit extract of the plant *Morinda Tinctoria* was studied and characterized by UV-Vis, FT-IR, SEM, CV and HR-TEM analysis. The potential antimicrobial activity of silver nanoparticles was performed and the maximum antibacterial activity was observed against *Staphylococus aureus.* Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials. Toxicity studies of silver nanoparticles on human pathogen open a door for a new range of antibacterial agents and anticancer agents.

REFERENCES

1. Rosei F. Nanostructured surfaces: challenges and frontiers in nanotechnology. J Phys Condens Matter 2004; 16:S1373–S1436.
2. 2. Papp S, Patakfalvi R, Dekany I. Metal nanoparticle formation on layer silicate lamellae. Colloid Polym Sci 2008; 286:3–14.
3. Chen D, Qiao X, Qiu X, Chen J. Synthesis and electrical properties of uniform silver nanoparticles for electronic applications. J Mater Sci 2009; 44:1076–1081.
4. Foglia S, Suber L, Righini M, Size tailoring of CdS nanoparticles by different colloidal chemical techniques,Colloids Surf. A 2001; 177(1):3-12.
5. Liu J, He F, Gunn TM, Zhao D, Roberts CB. Precise seed-mediated growth and size-controlled synthesis of palladium nanoparticles using a green chemistry approach. Langmuir 2009;25:7116–7128.
6. Mohanpuria P, Rana Nisha K and Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res 2008; 10:507-517.
7. Li L, Liu C, Liu Z, Tsao R, Liu S. Identification of phenylethanoid glycosides in plant extract of *Plantago asiatica* L. by liquid chromatography-electrospray ionization mass spectrometry. Chinese J. Chem 2009; 27:541-545.
8. Padmadhas R, Ragunathan R. Effect of lead nano particle present in the leaf of *Calotrphis Gigantea* which result in the loss of the painted grass hoppe of the Western ghats species in India. Int, J. Nanotechnol. Appl 2009; 3: 89-96.
9. Geoprincy G, Vidhya srri BN, Poonguzhali U, Nagendra Gandhi N, Renganathan S. A Review on Green Synthesis of Silver nanoparticles. *Asian* J Pharm Clin Res 2013;6(1): 8-12.
10. Meena N , Jeya M, Aroumugame S, Arumugam P and Sagadevan E. Green synthesis of silver nanoparticles using leaves of *lepisanthes tetraphylla* and evaluation of their antibacterial activity against drug resistant clinical isolates. Int J Pharm Bio Sci 2012; 3(2): 592-601.
11. Vivek M, Senthil Kumar P, Steffi S, Sudha S. Biogenic silver nanoparticles by *Gelidiella acerosa* extract and their antifungal effects .Avicenna. J.Med.Biotech 2011; 3(3): 143-148.
12. 12. Vilchis-Nestor A, Sa´nchez-Mendieta V, Camacho-Lo´ pez M, Go´ mez-Espinosa R, Camacho-Lo´ pez M, Arenas-Alatorre. Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. J. Mater. Lett 2008; 62: 3103.
13. Narayanan KB, Sakthivel NJ. Coriander leaf mediated biosynthesis of gold nanoparticles. Mater. Lett 2008; 68: 4588-4590.
14. Saxena A, Tripathi RM, Zafar F, Singh P. Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antibacterial activity. Mater Lett 2012; 67: 91–94.
15. Kaviya S, Santhanalakshmi J, Viswanathan B. Biosynthesis of silver nano-flakes by *Crossandra infundibuliformis* leaf extract. Mater Lett 2012; 67: 64–66.
16. Asmita J Gavhane, Padmanabhan P, Suresh P Kamble , Suresh N Jangle. Synthesis of silver nanoparticles using extract of *neem leaf* and *triphala* and evaluation of their antimicrobial activities. Int J Pharm Bio Sci 2012; 3(3): P 88 – 100.
17. Alagumuthu G, Kirubha R.Green synthesis of silver nanoparticles using *Cissus quadrangularis* plant extract and their antibacterial activity. Int. J. of Nanomater. Bio2012; 2(3) :30-33.
18. Jancy Mary E , Inbathamizh L. Green synthesis and characterization of nano silver using leaf extract of *Morinda Pubescens*. *Asian* J Pharm Clin Res 2012;5(1):159-162.
19. Firdhouse M, Jannathul, Lalitha P. Green Synthesis of Silver nanoparticles using the aqueous extract of *Portulaca Oleracea (l.). Asian* J Pharm Clin Res2013;6(1):92.
20. Isse JJ, Falciola L, Mussiri PR, Gennaro A, Chem.Common. 2006; 344-346.
21. Duran N, Alves OL, De Souza GIH, Esposito E Marcato PD. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J Biomed Nanotechnol 2007; 3: 203-208.
22. Chen JC, Lin ZH, Ma XX. Evidence of the production of silver nanoparticles via pretreatment of Phoma sp. 3.2883 with silver nitrate. Lett Appl Microbiol 2003; 37: 105-108.
23. Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M. Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. Curr Nanosci 2008; 4: 141-144.
24. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM, et al. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. J Antimicrob Chemother 2008; 54:1019- 24.

25. Abuskhuna S, Briody J, McCann M, Devereux M, Kavanagh K, Fontecha JB, et al. Synthesis, structure and anti-fungal activity of dimeric Ag(I) complexes containing bis-imidasole ligands. Polyhedron 2004; 23:1249- 5.
26. Hamouda T, Myc A, Donovan B, Shih A, Reuter JD, Baker Jr JR. A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. Microbiol Res 2000; 156:1 -7.
27. Crabtree JH, Burchette RJ, Siddiqi RA, Huen IT, Handott LL, Fishman A. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. Perit Dial Int 2003; 23(4): 368- 74.
28. Russel AD, Hugo WB. Antimicrobial activity and action of silver. Prog Med Chem1994; 31:351- 70.
29. Marsh PD. Microbiological aspects of the chemical control of plaque and gingivitis. J Dent Res 1992; 71:1431- 8.