Elucidating the Antibacterial Potential of PEG Modified Silver Nanoparticles

Jitendra Kumar[1], Shakeel Ahmed Ansari[2] and Rukhsana Satar[3,*]

[1]*Department of Biotechnology, Chaudhary Charan Singh University, Uttar Pradesh 200005, India*

[2]*Center of Excellence in Genomic and Medicine Research, King Abdulaziz University, Jeddah-21589, Kingdom of Saudi Arabia*

[3]*Department of Biochemistry, Ibn Sina National College for Medical Studies, Jeddah-21418, Kingdom of Saudi Arabia*

Abstract: The present study provides an insight of antibacterial potential of PEG modified AgNPs. AgNPs were found to be ~26 nm by XRD analysis apart from exhibiting its ultravoilet spectra peak at 250 nm. The Fourier transform-infrared spectroscopy further confirmed the observance of peaks between 650 to 500 cm^{-1} in the synthesized AgNPs. Antimicrobial characterization was monitored by colony forming units (CFU) on agar plates. AgNPs showed $20x10^9$/ml CFU after 24 hrs of incubation at 20 mM AgNPs concentration as against $29x10^9$/ml CFU under similar incubation conditions in the modified AgNPs. Moreover, zone of inhibition (ZOI) appeared as 3.1 and 3.8 cm at 0.2 and 0.4 mM at AgNPs, respectively in *E.coli* in contrast to 3.5 and 4.5 cm, respectively for modified AgNPs under similar conditions. ZOI for *S. aureus* showed 3.2 and 3.8 cm, respectively at 0.2 and 0.4 mM concentration for AgNPs while modified AgNPs exhibited ZOI at 3.8 and 4.4 cm, respectively under similar incubation conditions. Their toxic effect was demonstrated by MTT assay to point out their relevance as antimicrobial agent.

Keywords: Antibacterial activity, Food industries, Modified silver nanoparticles.

INTRODUCTION

It has widely been accepted that nanoparticles-based platforms can be successfully exploited in the field of biotechnology, immunosensing, biomedical sciences and of course in food packaging industries [1-3]. The outstanding features of nanoscaled structures such as their ability to reduce gaseous diffusion, particle mobility and increase in the functional surface area to volume ratio make them excellent nanomaterials to be incorporated with traditionally used polymer based food packaging to obtain an improved packaging material [4, 5]. A good packaging material is one which not only protects food from dirt, microorganisms and other harmful environmental substances but is also inert, low on production cost and easy to dispose without any significant environmental hazard [6].

In recent years, use of nanomaterials in packaging materials has gained considerable attention in food packaging industry. The use of nanomaterials in food packaging materials mainly aims to provide a stronger barrier to gaseous diffusion, better resistance to humidity and temperature, and finally better protection against microorganisms since many nanomaterials demonstrate significant antimicrobial activity [7]. These nanomaterials can also be exploited to design smart food packaging that automatically alerts the consumer about the hygiene of the packed food product. Therefore, the nanomaterials are increasingly being considered in the food packaging industry to increase shelf-life as well as hygiene of the packed food due to their peculiar characteristics that help in improving durability, flexibility, temperature tolerance, barrier properties and recycling properties of the packaging materials [8, 9]. The nanomaterials are usually incorporated in packaging materials as antimicrobials and barrier to gaseous diffusion into the food. For this purpose, nanoparticles are usually integrated into a polymer matrix which significantly slows down gaseous diffusion. Silvestre *et al*. [10] through several elegant studies have demonstrated that nanomaterials integrated into the polymer matrix, not only improve the gas barrier properties of the food packaging but also impart resistance to temperature and humidity. Since silver has significant antimicrobial properties, therefore, the use of silver nanoparticles and silver coatings with other materials such as gold, zinc oxide, silica, titanium dioxide, alumina and iron oxides into polymeric packaging materials such as polyamides, nylons, polyolefins, ethylene-vinylacetate copolymer, polystyrene, epoxy resins, polyurethane, polyvinyl chloride and polyethylene terephthalate has been extensively evaluated [11]. Efficacy of AgNP against various organisms resistant to potent chemical antimicrobials

*Address correspondence to this author at the Department of Biochemistry, Ibn Sina National College for Medical Studies, Jeddah-21418, Kingdom of Saudi Arabia; Tel: (0) 02-96356555 (Ext-349); Fax: (0) 02-626288;
E-mail: rukhsanabiochem@gmail.com and saansari@kau.edu.sa

E-ISSN: 2408-9834/16 © 2016 Cosmos Scholars Publishing House

has been evaluated by Duncan [12]. These nanoparticles have demonstrated significant antimicrobial activity against several important microorganisms such as fungi, yeast, algae, phytoplankton and few viruses [13, 14]. Moreover, the positive influence of PEG coating on reducing the toxicity of AgNP has been demonstrated earlier against human keratinocyte cell line [15] and in other bioanalytical applications [16-18].

The current study was undertaken to synthesize, surface modify AgNPs to evaluate their size-dependent physical and chemical properties for establishing their possible utility in food packaging materials. The antimicrobial and toxic potential of mAgNPs was also ascertained to highlight their suitability as a component of improved nanoparticle based food packaging material.

MATERIALS AND METHODS

The procedure covered a wide range of assays consisting of synthesis and characterization of nanoparticles, their assessment for antibacterial potential and measurement of colony forming units, and effects in cell viability assay employing standard statistical methods. Each is described as under:

Synthesis, Characterization and Modification of AgNPs

A modified method of Kim *et al*. [19] was utilized for the synthesis of silver nanoparticles (AgNPs). Briefly, 100 ml of 10 mM solution of $AgNO_3$ was mixed with sodium borohydride (250 mL, 20 M) solution. The mixture was stirred continuously for 5 hrs at 4 °C to form monodispersed nanoparticles which appeared as yellow transparent sol in aqueous medium. Then it was kept in a water bath at 4 °C for 1 hr to precipitate followed by centrifugation mediated filtration. The filtrate was subjected for crystallization to obtain its powdered form at room temperature. The nanoparticle crystals were then analyzed by XRD method employing a X-ray diffractometer (Rigaku Miniflex) with Cu-K α radiation (λ = 1.54060A°) in 2θ ranging from 20° to 80°. The Scherer formula (from 211 line width of the XRD peak) was used for the determination of average crystal size (D). The optical absorption was measured by Perkin Elmer Spectrophotometer while FTIR spectra of AgNPs were determined using a FT-IR instrument (INTERSPEC 2020 model FT-IR instrument, USA). The surface of AgNPs (1 mg) was modified by suspending them in 2 ml polyethylene glycol salt (PEG-600). The suspension was kept in a shaker and spun at 250 rpm for 4 hr. PEG modified AgNPs were centrifuged and washed thoroughly with deionized water, and stored at room temperature till further analysis.

Antibacterial Assay

Antibacterial assay was carried out by utilizing standard agar diffusion assay [20]. The test organisms were grown in sterile nutrient broth overnight at 37 °C. Agar diffusion assay was performed by pouring autoclaved nutrient agar in petri plates and allowing it to solidify. Then 100 µl of each bacterial culture was spread onto agar surface of two different petri plates. Sterile paper discs (5 mm diameter) containing AgNPs (50 mg/l) were placed onto each plates. The plates were then incubated at 37 °C for 24 hrs. Next day, the plates were observed for appearance of zone of inhibition.

CFU Measurement

The effect of AgNPs and mAgNPs on bacterial growth was determined by enumerating CFU on agar plates. *E.coli* (ATCC 25922) strain was used in the study. Overnight grown culture of *E. coli* was diluted 10^9 folds in normal saline and 50 µl of highest dilution was spread onto the agar plates containing different concentrations of AgNPs and mAgNPs with the help of a sterile glass spreader. The plates were incubated at 37 °C for 24 hrs and numbers of CFU were counted with the help of a colony counter.

Cell Viability Assay

Effect of AgNPs on A431 cell viability was evaluated by MTT reduction assay [21]. It is based on the principle of reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple formazan by succinate-tetrazolium reductase (located in mitochondrial respiratory chain which is active in viable cells only). To assess the viability, the carcinoma cells ($1x10^4$) were seeded into 100 µl medium in each well of 96-well culture plate for 24 hrs at 37 °C in 5 % CO_2 atmosphere. To begin the process, a stock AgNPs solution was prepared in double distilled water, diluted to desired concentrations (10, 50 and 100 µg/ml) and added later as per experimental design. After 21 hrs, 10 µl MTT (5 mg/mL) solutions was added in each well and further incubated for 3 hrs at 37 °C until formazan blue crystals were developed. Supernatant was discarded and dimethyl sulfoxide (100 µl) was added and kept for 10 min at 37 °C to solubilize formazan crystals. The absorbance was recorded at 540 nm with

microplate reader. The percent viability of cells was calculated by equation:

% cell viability = [(OD of control - OD of treated) / (OD of control)] x 100

Statistical Evaluation

All results were expressed as mean value ± standard error of the mean of growth inhibition zones diameters. P values lower than 0.05 were considered significant.

RESULTS AND DISCUSSION

The main purpose of a suitable food packaging material is to provide a long shelf-life to food products by protecting them against microorganisms and environmental gases. Silver nanoparticles possess distinctive optical, electrical and thermal properties, and have extensively been used as antimicrobial coatings, wound dressings and biomedical devices to provide consistent protection against bacteria [11]. Therefore, current study was undertaken to evaluate potential application of AgNPs in food packaging industry. Figure **1** shows the XRD spectrum of AgNPs. The lattice constants of AgNPs were found to be as (a) 5.758 Å and (c) 9.443 Å. XRD pattern of AgNPs showed the most intense peak at 2θ=36.30° corresponding to 211 plane of AgNPs. Average crystallite size (*D*) of AgNPs was ~26 nm which was calculated by Scherer's formula. The optical absorption of AgNPs observed in the wavelength range 250-800 nm at room temperature exhibited its highest peak at 250 nm (Figure **2**). The FTIR spectrum showed two different absorption peaks in the region from 650 to 500 cm^{-1}. The peak observed at 424.70 cm^{-1} suggests the band-stretching mode of the octahedral sites in the region from 500 to 400 cm^{-1} (Figure **3**). These results are in agreement with the findings observed by previous researchers [22-24].

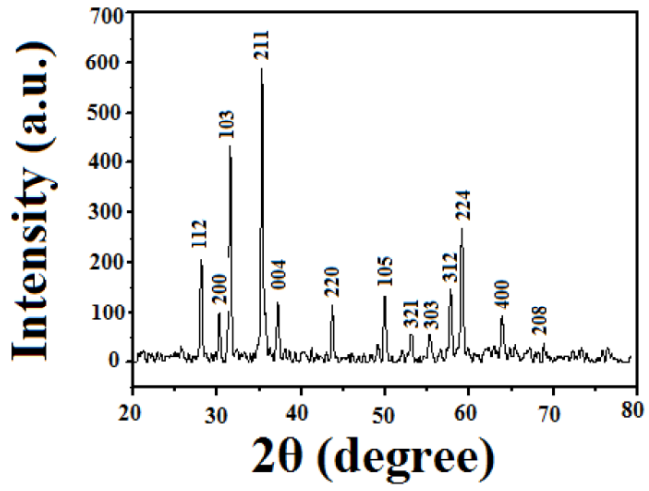

Figure 1: XRD analysis of AgNPs.
XRD was recorded at room temperature using Rigaku Miniflex X-ray diffractometer with Cu-K α radiation (*λ* = 1.54060 Å) in 2θ ranging from 20° to 80°. The particle size of the sample was found to be ~ 26 nm which was estimated from the 211 line width of the XRD peak.

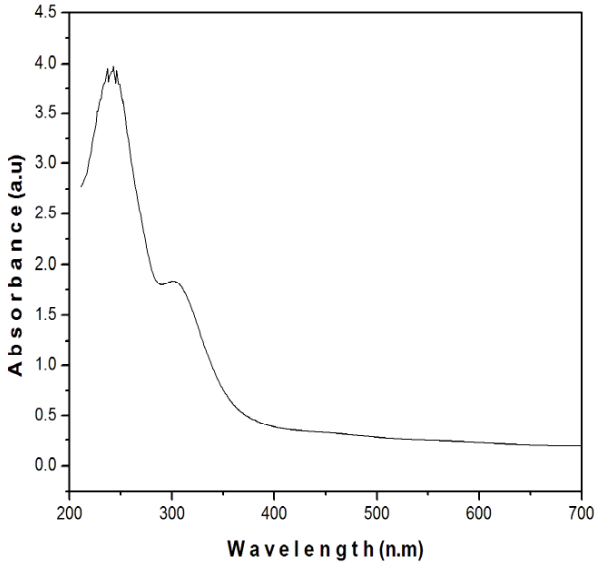

Figure 2: UV spectra of AgNPs.
UV spectra of AgNPs exhibiting its peak at 250 nm.

Polyethylene glycol (PEG) has been used earlier for functionalization and bioconjugation of colloidal inorganic nanoparticles for biomedical applications [25-27]. In this study, PEG was covalently linked to AgNP to modify the surface of AgNPs using PEG-silane coupling technique. The modified AgNPs demonstrated significant antibacterial activity against a gram-negative (*E.coli*) and gram-positive (*Staphylococcus aureus*) bacteria. AgNPs have been reported to alter the bacterial cell membrane permeability resulting in cell death because AgNPs have high chemical reactivity obtained as a result of enhanced surface area to volume ratio [28]. The plot of number of bacterial colonies grown on nutrient agar plates as a function of concentration of mAgNPs and control (AgNPs) has been illustrated in Figure **4**. It was observed that AgNPs showed $20x10^9$/ml CFU after 24 hrs of incubation at 20 mM AgNPs concentration while PEG modified AgNPs exhibited $29x10^9$/ml CFU under similar incubation conditions. Zone of inhibition (ZOI) was detected as 3.1 and 3.8 cm, respectively for *E. coli* by AgNPs at 0.2 and 0.4 mM while it was 3.5 and 4.5 cm respectively by modified AgNPs in similar situation. Similarly, ZOI for *S. aureus* by AgNPs at 0.2 and 0.4 mM was observed at 3.2 and 3.8 cm, respectively, and

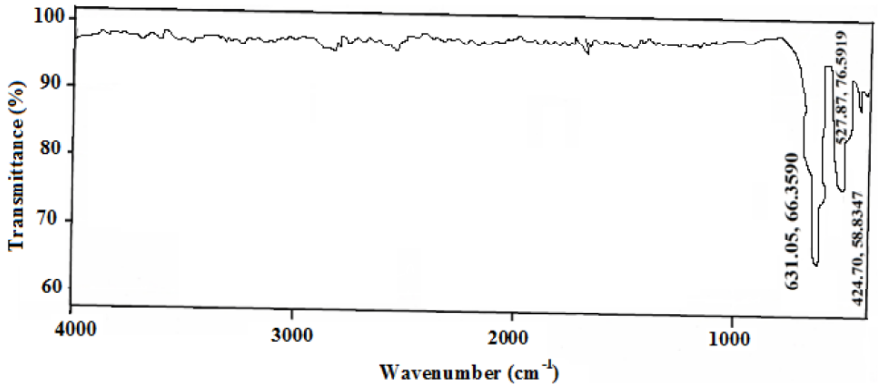

Figure 3: FT-IR spectra of AgNPs.

FT-IR spectra was monitored with INTERSPEC 2020 model FT-IR instrument, USA. The calibration was done by polystyrene film. The syringe was first washed with acetone followed by distilled water. The samples were injected by Hamiet 100 µL syringe in ATR box.

these values were 3.8 and 4.4 cm, respectively for modified AgNPs in comparison for similar incubation conditions (Figure **5**). Consequently, the antibacterial activity of both AgNPs and modified AgNPs were increased with increase in their respective concentrations. Nonetheless, the activity was greater for modified AgNPs viz-z-viz naive ANPs. It appears that the modified surfaces being hydrophilic showed resistance to the non-specific adsorption of several proteins and bacteria. Besides, MTT result indicated AgNPs induced dose-dependent toxicity in concentrations ranging from10-100 µg/mL. Looking closely at Figure **6**, we find that the percentage cell viability observed after 10, 50 and 100 µg/ml concentration of AgNPs was 65.97 %, 40.39 % and 29.68 %, respectively. The result suggested that AgNPs decreased cell viability in a dose dependent manner with the increasing concentration of AgNPs.

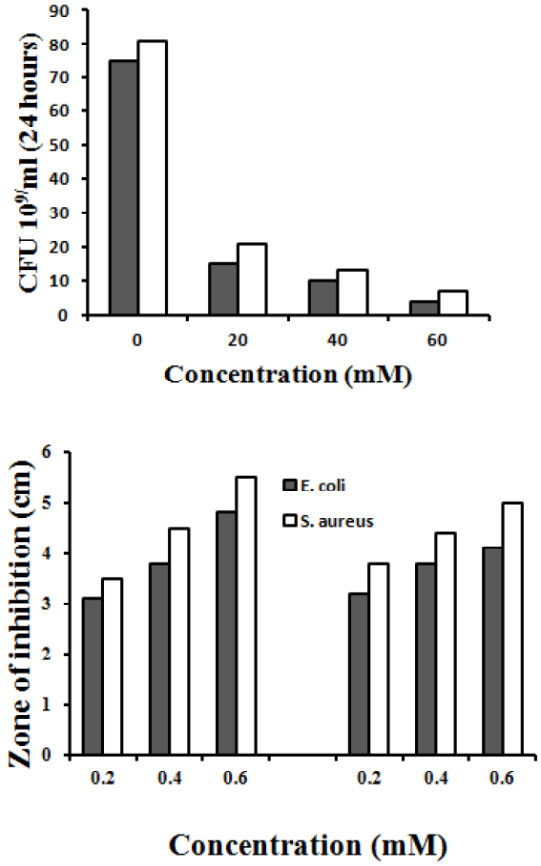

Figure 4: Antimicrobial characterization by CFU as a function of naïve and mAgNPs concentration on agar plates and zone of inhibition measurement.

Bacterial colonies were grown on nutrient agar plates as a function of concentration of naïve and mAgNPs. The numbers of CFU have been observed to reduce significantly with the increasing concentration of nanoparticles. Moreover, zone diameter was measured using an antibiotic zone measuring scale (HIMEDIA).

Hence, the self-assembled PEG-AgNPs films readily increase the hydrophilic nature of the channel surfaces, thereby retarding the non-specific adsorption of proteins and cells. This method can be readily extended to other materials relevant for food packaging applications.

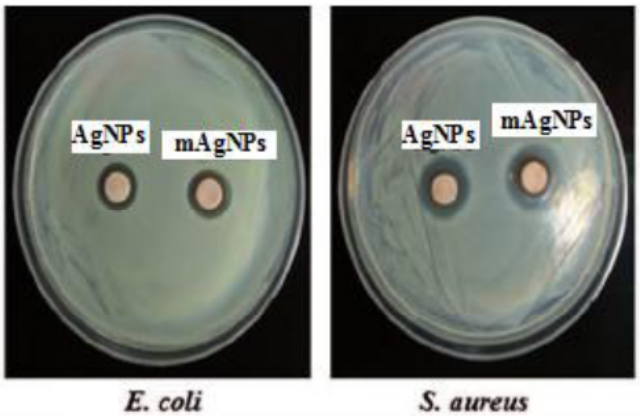

Figure 5: Zone of inhibition of antibacterial test of AgNPs and PEG coated AgNPs.

Zone diameter was measured using an antibiotic zone measuring scale (HIMEDIA).

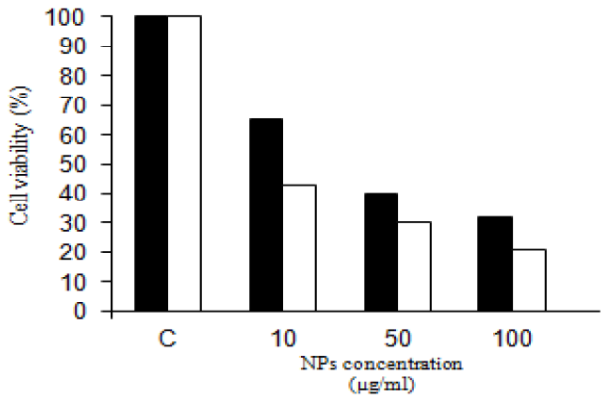

Figure 6: Viability of A431 cells after 24 hour exposure to AgNPs and PEG coated AgNPs.

Viability of A431 epidermoid carcinoma cells after 24-h exposure to AgNPs (□) and PEG modified AgNPs (■) evaluated by MTT assay at indicated concentrations.

CONCLUSION

Herein, PEG modified silver nanoparticles were successfully developed (owing to its strong toxicity to a wide range of microorganisms as ionic silver) to improve their flexibility and gas barrier properties for food packaging apart from enhanced antimicrobial/ antioxidant potential. These findings may improve the understanding and confirm a possible direction for the rational design of effective packaging by modified nanomaterial in food industries.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Microbiology, CCS University for providing the necessary infrastructure.

ABBREVIATIONS

- **AgNPs** silver nanoparticles
- **CFU** colony forming units
- **PEG** polyethylene glycol
- **mAgNPs** PEG modified silver nanoparticles
- **ZOI** zone of inhibition

REFERENCES

[1] Bergamaschi E, Poland C, Canu IG, and Prina-Mello A. The role of biological monitoring in nano-safety. Nanotoday 2015; 10: 274-277. http://dx.doi.org/10.1016/j.nantod.2015.02.001

[2] Escosura-Muniz A, Espinoza-Castaneda M, Hasegawa M, Philippe L and Merkoc A. Nanoparticles-based nanochannels assembled on a plastic flexible substrate for label-free immunosensing. Nano Res. 2015; 4: 1180-1188. http://dx.doi.org/10.1007/s12274-014-0598-5

[3] Krokowicz L, Tomczak H, Bobkiewicz A, Mackiewicz J, Marciniak R, Drews M and Banasiewicz T. In vitro studies of antibacterial and antifungal wound dressings comprising H2TiO3 and SiO2 nanoparticles. Pol. J Microbiol 2015; 64: 137-142.

[4] Ansari SA, and Q. Husain. Potential application of enzymes immobilized on/in nano materials: A review. Biotechnol Adv 2012; 16: 512-523. http://dx.doi.org/10.1016/j.biotechadv.2011.09.005

[5] Lu Y, and S. Ozcan. Green nanomaterials: On track for a sustainable future. Nanotoday 2015; 10: 417-420. http://dx.doi.org/10.1016/j.nantod.2015.04.010

[6] Barish JA, and JM. Goddard. Anti-fouling surface modified stainless steel for food processing. Food Bioprod Bioproc 2013; 91: 352-361. http://dx.doi.org/10.1016/j.fbp.2013.01.003

[7] Echegoyen Y, and C. Nerin. Nanoparticle release from nano-silver antimicrobial food containers. Food Chem Toxicol. 2013; 62: 16-22. http://dx.doi.org/10.1016/j.fct.2013.08.014

[8] Cushen M, Kerry J, Morris M, Cruz-Romero M and Cummins E. Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food and an associated exposure assessment. J Agric Food Chem 2014; 62: 1403-1411. http://dx.doi.org/10.1021/jf404038y

[9] Maisanaba S, Pichardo S, Jorda-Beneyto M, Aucejo S, Camean AM and A. Jos. Cytotoxicity and mutagenicity studies on migration extracts from nanocomposites with potential use in food packaging. Food Chem Toxicol 2014; 66: 366-372. http://dx.doi.org/10.1016/j.fct.2014.02.011

[10] Silvestre C, Duraccio D and C. Sossio. Food packaging based on polymer nanomaterials. Prog Polym Sci 2011; 36: 1766-1782. http://dx.doi.org/10.1016/j.progpolymsci.2011.02.003

[11] Ouay BL, and F. Stellacci. Antibacterial activity of silver nanoparticles: A surface science insight. Nanotoday 2015; 10: 339-354. http://dx.doi.org/10.1016/j.nantod.2015.04.002

[12] Duncan TV. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. J Coll Inter Sci 2011; 363: 1-24. http://dx.doi.org/10.1016/j.jcis.2011.07.017

[13] Kholoud MM, El-Nour A, Eftaiha A, Al-Warthan A and. Ammar RAA. Synthesis and applications of silver nanoparticles. Arab J Chem 2010; 3: 135-140. http://dx.doi.org/10.1016/j.arabjc.2010.04.008

[14] Tran QH, and Anh-Tuan L. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Adv. Nat. Sci: Nanosci Nanotechnol 2013; 4: 3001-3023. http://dx.doi.org/10.1088/2043-6262/4/3/033001

[15] Bastos V, Ferreira de Oliveira JM, Brown D, Jonhston H, Malheiro E, Daniel-da-Silva AL et al. The influence of citrate or PEG coating on silver nanoparticle toxicity to a human keratinocyte cell line. Toxicol Lett 2016; 249: 29-41. http://dx.doi.org/10.1016/j.toxlet.2016.03.005

[16] Stoiber T, Croteau MN, Romer I, Tejamaya M, Lead JR, Luoma SN. Influence of hardness on the bioavailability of silver to a freshwater snail after waterborne exposure to silver nitrate and silver nanoparticles. Nanotoxicology 2015; 9: 918-927. http://dx.doi.org/10.3109/17435390.2014.991772

[17] Simakova P, Gautier J, Prochazka M, Herve-Aubert K, Chourpa I. Polyethylene-glycol-stabilized Ag nanoparticles for Surface-Enhanced Raman Scattering spectroscopy: Ag surface accessibility studied using metalation of free-base

porphyrins. J Phys Chem C 2014; 118: 7690-7697. http://dx.doi.org/10.1021/jp5005709

[18] Shameli K, Ahmad MB, Jazayeri SD, Sedaghat S, Shabanzadeh P, Jahangirian H et al. Synthesis and characterization of polyethylene glycol mediated silver nanoparticles by the green method. Int. J. Mol. Sci. (2012) 13: 6639-6650. http://dx.doi.org/10.3390/ijms13066639

[19] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ et al. Hwang. Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol Med 2007; 3: 95-101. http://dx.doi.org/10.1016/j.nano.2006.12.001

[20] Driscoll, AJ, Bhat N, Karron RA, O'Brien KL and Murdoch DR. Disk diffusion bioassays for the detection of antibiotic activity in body fluids: applications for the pneumonia etiology research for child health project. Clin Infect Dis 2012; 54: 159-164. http://dx.doi.org/10.1093/cid/cir1061

[21] Lupu AR, and T. Popescu. The non-cellular reduction of MTT tetrazolium salt by TiO2 nanoparticles and its implications for cytotoxicity assays Toxicol Vitro 2013; 27: 1445-1450. http://dx.doi.org/10.1016/j.tiv.2013.03.006

[22] Solomon SD, Bahadory M, Jeyarajasingam AV, Rutkowsky SA and Boritz C. Synthesis and study of silver nanoparticles. J Chem Ed 2007; 84: 322-325. http://dx.doi.org/10.1021/ed084p322

[23] Bhui DK, Bar H, Sarkar P, Sahoo GP, De SP, and Misra A. Synthesis and UV–vis spectroscopic study of silver nanoparticles in aqueous SDS solution. J Mol Liq 2009; 145: 33-37. http://dx.doi.org/10.1016/j.molliq.2008.11.014

[24] Devaraj P, Kumari P, Aarti C and A. Renganathan. Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against MCF-7 cell line. J Nanotechnol 2013; Article ID 598328, 5 pages. 27Sperling RA, and WJ. Parak. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. Philos Trans A 2010; 368: 16-29.

[25] Zhang Y, Kohler N and M. Zhang. Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake. Biomaterials 2002; 23: 1553-1561. http://dx.doi.org/10.1016/S0142-9612(01)00267-8

[26] Cu Y and WM. Saltzman. Controlled surface modification with poly(ethylene)glycol enhances diffusion of PLGA nanoparticles in human cervical mucus. Mol Pharm 2009; 6: 173-181. http://dx.doi.org/10.1021/mp8001254

[27] Prabhu S, and EK. Poulose. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications and toxicity effects. Int Nano Lett 2012; 2: 32-41. http://dx.doi.org/10.1186/2228-5326-2-32

Received on 21-08-2016 Accepted on 18-10-2016 Published on 27-10-2016

http://dx.doi.org/10.15379/2408-9834.2016.03.02.04

© 2016 Kumar *et al.*; Licensee Cosmos Scholars Publishing House.
This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.