Comparative Biocompatibilities of Various Sizes of AgCIT and AgPVP with their Protein Coronas Nanoparticles

[2]Abdul Hameed*, [1]Komal Saba, [1]Raheela Taj, [3]Andaleeb Azam, [3]Rohullah and [1]Aamina Paracha
[1]*Institute of Chemical Sciences, University of Peshawar, Pakistan.*
[2]*Associated Industries Limited, Amangargh Nowshera, Pakistan.*
[3]*Higher Education Department archives and library, Peshawar, KP, Pakistan.*
ham.chemist@gmail.com*

(Received on 17th February 2021, accepted in revised form 9th June 2021)

Summary: Biocompatibilities of nanoparticles are crucial for biomedical applications. Diverse silver nanoparticles (5 nm, 10 nm, 20 nm, 40 nm and 80 nm) caped with citrate and polyvinylpyrrolidone (PVP) were synthesized and primed their protein coronas. Nanoparticles were characterized with UV-visible spectroscope, Dynamic light scattering (DLS) and Transmission Electron Microscope (TEM). Comparative biocompatibilities were verified and recorded using MTS techniques. Human hepatoma carcinoma HepG2) cell line was used for measuring cytotoxic effect by MTS assays. Deleterious and comparative behaviors of citrate and PVP supported nanoparticles with varied dimensions were investigated and concluded; that citrate caped nanoparticles are comparatively less toxic and independent of size than PVP supported nanoparticles, having increased cytotoxicity with increasing size. The cytotoxic effect of citrate caped and its protein coronas nanoparticles was insignificant, while the boosted concentration of PVP supported nanoparticles enhanced the toxic effect, which endorsed enlarged size and amount of PVP supported nanoparticles. As medicinal precursors, the overwhelming use of PVP nanoparticles should be avoided, and a unique protocol must be designed if its use is crucial and unavoidable.

Keywords: Silver nanoparticles, Citrate caped, PVP caped and Protein coronas silver nanoparticles, Cytotoxic effect.

Introduction

Nanomaterials have myriad exploitation in preparation of industrial goods and medical tools followed by their consumption in industrial, medical and biological systems. Nanoparticles surfaces interact and ally with biological molecules composed of peptides, lipids and proteins, forming an adsorbed layer around nanoparticles. Such adsorbed layers of proteins around nanoparticles are called protein corona Nanoparticles (NpPC) [1, 2]. The unique physicochemical properties of protein corona nanoparticles are attributed to an alteration in shapes, charge density and hydrodynamic sizes, which modify bio-distribution, activity and toxicity [3-5]. Modifications in nanoparticles' surfaces have an impact on biodistribution and uptake of nanoparticles [6-9]. In addition, loading of nanoparticle surfaces with HAS and transferrin declines the uptake of citrate-supported silver nanoparticles in the range of 20 and 110 nm by the HEK cells, while IgG with silica-supported silver nanoparticles (AgNPs) boosts consumption [10]. Enhanced cellular ingestion of nanoparticles has been reported if fibrinogen, C3 and other proteins are individually encumbered [11, 12]. Broad studies advocate that protein corona nanoparticles have formidable effects on bio distribution and cellular intake and tend to alter the therapeutic efficacy and inadvertent noxious activities of nanoparticles. Gold nanoparticles (AuNPs) possess promising photo-thermal treatment and bio-imaging potentials, but prior to their consumption in drug delivery and the therapeutic application, their cytotoxicity and histo-compatibilities tend to be verified.

However, plasma proteins readily associate with nanoparticles that disturb the alliance between gold nanoparticles and cells, modifies biological molecules through phase transformation, reshaping, restructuring and influencing resizing and absorption on nanoparticles [13]. Studies revealed some amendments and coating to expose gold nanoparticles for therapeutic application [14, 15]. It has been understood that nanoparticle capping has a vital role in their biocompatibility[16]. It's doubtful that nanoparticles synthesized for the industrial application have the same effects if accidentally exposed as nanoparticles consumed for medical purposes. Presently AuNPs are used on a large scale for medical purposes [17-21]. The uses and synthesis of gold nanoparticles are enhanced and expedited for medical reasons due to increased ingestion, skin contact, bio-imaging and cancer therapy. Thus it's essential to evaluate the noxious properties of silver and gold nanoparticles before administration for medical purposes.

The hypothesis for current research was based on the phenomenon to synthesize and evaluate the cytotoxic effects of varied sizes (i.e. 5, 10, 20, 40, and

*To whom all correspondence should be addressed.

80 nm) citrate and Polyvinylpyrrolidone (PVP) supported silver nanoparticles. Bovine serum albumin (BSA) was used as a template for the synthesis of protein coronas nanoparticles and their relative cytotoxicity was calculated with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay using HepG2 cells.

Experiment

Analytical grade chemical reagents; Tetracholoroauric acid trihydrated ($HAuCl_4.3H_2O$), silver nitrate ($AgNO_3$), trisodium citrate, polyvinylpyrrolidone (PVP), tannic acid, sodium borohydride (NaBH4), triethylamine, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer "purchased from sigma Aldrich" are consumed for the synthesis of synergistically supported silver nanoparticles with citrate, PVP and protein corona. De-ionized water was used throughout experimental protocols. Nanoparticles were characterized with UV-Visible Spectrophotometer, Zeta Nano and Transmission Electron Microscope. HepG2 cell line, Dulbecco's Modified Eagle's Medium (DMEM), Phosphate buffer saline (PBS) and trypsin were bought from Cellgro. Fetal bovine serum albumin (BSA) from Hyclone, Trypsin from Gibco and CellTitre 96 ® Aqueous was purchased from Promega.

Cell Culture

Human hepatoma carcinoma (HepG2) cell line was obtained from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. HepG2 cells were cultured in the complete growth medium DMEM (10% FBS) at 37°C in a 5% CO_2 humidified incubator. HepG2 cells were detached with 0.25% trypsin-EDTA solution during sub-culturing.

Synthesis of AgNPs capped with Citrate

AgNPs were synthesized using general protocol; Trisodium citrate (1%, 1.5mL,), $AgNO_3$ solution (0.5%, 1 mL) and $HAuCl_4$ (0.1%, 42 µL) solution was mixed in a tube and shacked for 5 min to observed a change in color. Then the mixture was transferred to 50 mL boiling water in a round bottom flask connected to a condenser for 10 minutes. Hereafter the reaction mixture was brought to room temperature in 30 mins and then subsequently stirred for 2 hours. Initially, newly grown nanoparticles were characterized with UV-Vis spectra and further verified with Transmission electron microscopy (TEM) and Dynamic light scattering (DLS). Nanoparticles separation was achieved with a centrifuge at specified rpm (depending on the size of nanoparticles) in 20 minutes. The pellets were washed with distilled water and the process was repeated three times to remove un-reacted reagents.

Synthesis of PVP capped AgNPs

AgPVP nanoparticles were grown by mixing 15 mL (6%) of PVP with 1 mL (0.5M) HCl for 10 mins followed by the addition of $AgNO_3$ (10 mL, 2.5 mM) and stirred for 10 mins. After that, 1 mL of 0.5M NaOH was added to neutralize the free acid contents. Then, 1 mL of 20 mM NaBH4 was added and stirred for 2 hours to sufficiently reduce the salt. Thus, the synthesized AgPVP nanoparticles were initially characterized with UV-Vis spectrometer, subsequently verified with DLS and TEM. Nanoparticles separation was achieved with a centrifuge at specified rpm (depending on the size of nanoparticles) in 20 minutes. The pellets were washed with distilled water, and the process was repeated three times to remove un-reacted reagents.

Synthesis of protein corona supported nanoparticles (PcNPs)

After centrifugation, silver nanoparticles were suspended in the same amount of distilled water (as before centrifugation) and divided into two equal portions. One portion was used as reference AgNPs and the other portion (50 mL) was added into a reaction flask followed by the addition of 1 mL HEPES buffer and stirred for 5 minutes. To ensure complete ligand exchange, 5 mL of BSA (0.15 mg/5 mL) aqueous solution was added to the NP solutions and stirred overnight at 40°C. Centrifugation technique was followed to separate freshly synthesized protein coronas (PcAgCIT) and immersed in 50 mL distilled water similar in concentration to the reference NPs solutions and afterward characterized with UV-Vis spectrometer, DLS and TEM.

Cell viability assay (MTS assay)

MTS assay was executed with Cell Titer 96® Aqueous One Solution Reagent (Promega, USA). For cell viability assay to evaluate the cytotoxic effects of AgCIT, AgPVP, and protein coronas supported nanoparticles PcAgNPs with MTS assay. Simply, 100 µL $1x10^4$ HepG2 cells were seeded per well in a 96-well plate and allowed to grow in humidified incubator having 5% CO_2 at 37°C for 24 hours. Control wells were filled with 100 µL complete DMEM culture medium. The previous medium was removed after incubation for 24 hours, and 50, 100, and 150 µg of NP samples were added to the appropriate wells. The plate

was further kept in a humidified incubator at 37°C for 24 hours. 20 µL Cell Titer 96®Aqueous Solution Reagent was added into each well and was kept at 37°C for 2-hour in humidified incubator. Absorbance was measured at 490 nm with a micro-plate reader (infinite 200, Austria). The experiment was repeated at least three times to minimize the error. The relative cell viability (%) was calculated by [A]sample/ [A]control x 100%. [A] is the absorbance subtracted from the corresponding background in the cell-free condition.

Results and Discussion

Various sizes of AgCIT, AgPVP and PcAgCIT nanoparticles were prepared in early mono dispersed form. The same procedures were followed for synthesizing different sizes of nanoparticles by varying ratios of reactants.

UV-Vis Characterization

In particular, the UV-Vis region of optical density is very significant as a primary confirmation for the synthesis of AgNP [22, 23]. Citrate, PVP and proteins coronas supported nanoparticles with slight variation in colors were initially determined by measuring their absorption bands in a range of 393 to 450 nm. Initially, citrate capped silver nanoparticles were grown of varied sizes and then a ligand exchange process employed for the synthesis of protein coronas supported nanoparticles. Triumphant growth of AgCIT and PcAgNPs confirmed from surface plasmon resonance (SPR) bands (Fig-1). Color variation was observed from yellow to brownish with a minor redshift in UV-Vis spectra for all PcAgNPs (Fig-1). Moreover, a slight redshift in the LSPR peak shows the successful attachment of BSA with a possible increase in the particle sizes [24]. The SPR bands in a particular region from 395 to 450 nm were confirmed by PVP-supported varied silver nanoparticles (Fig-2).

DLS and TEM Characterization

Particle sizes and morphologies were determined with dynamic light scattering (DLS) and transmission electron microscopic (TEM) techniques. TEM images revealed monodispersed nanoparticles without aggregation and almost spherical shape nanoparticles (Fig-3). The sizes and distribution of particles were also determined by DLS techniques. The hydrodynamic diameter was slightly higher than the size of the respective nanoparticles. A slight difference of sizes in two different techniques (TEM and DLS) is attributed to the attachment of water molecules, which strongly augment nanoparticles via the salvation process.

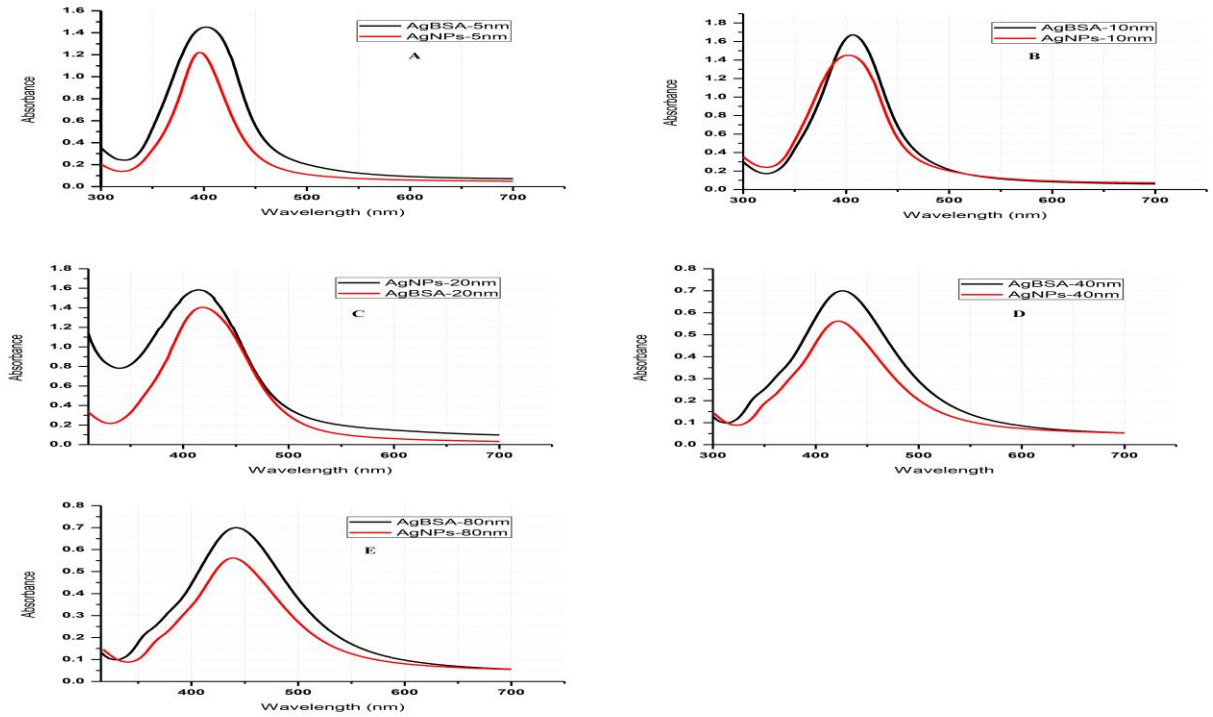

Fig. 1: SPR peaks of AgCIT and PcAgNPs of 5(A), 10(B), 20(C), 40(D), 80(E) nm sizes.

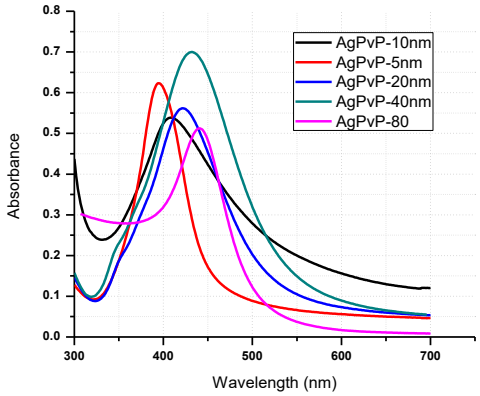

Fig. 2: Surface Plasmon Resonance Band of AgPVP nanoparticles of 5, 10, 20, 40, and 80 nm.

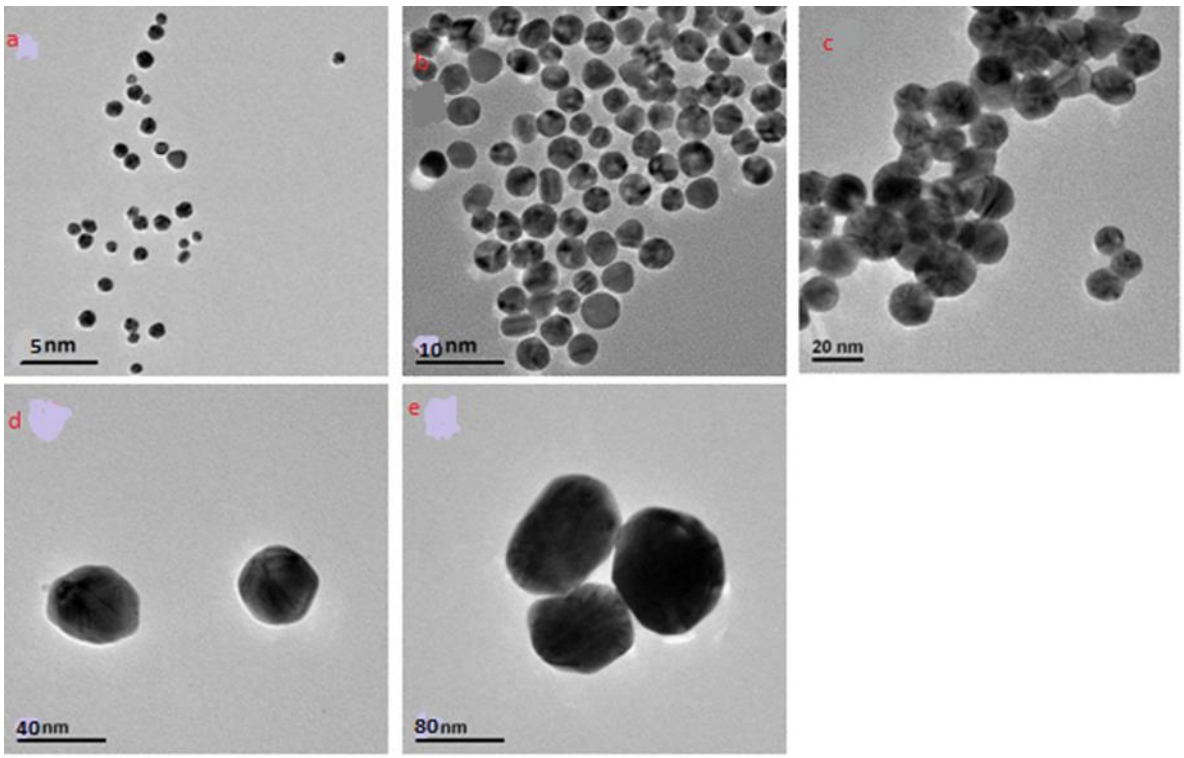

Fig. 3: TEM images of Silver nanoparticles caped with citrate (5nm(a), 10nm(b), 20nm(c), 40nm(d) and 80nm (e) in sizes).

The cytotoxicity of nanoparticles

The biocompatibility of nanoparticles was measured against the HepG2 cell line by MTS assay. HepG2 cells were exposed to the escalating concentrations of AgCIT, AgPVP, and PcAgNPs (50, 100 and 150 μg/mL) for 24 hours. AgNPs (5, 10, 40 nm) demonstrated some adverse effects on cells (Fig. 4 and 5) compared to same-sized protein coronas supported nanoparticles. This variation in adverse effect attributes to protein corona, which covered the outer surfaces of nanoparticles while AgNPs directly interact upon exposure to biological molecules. Noxious effect of nanoparticles was found independent of size up to 80 nm. Contrary to earlier mentioned, AgPVP of varied sizes has incompatibility to cells and more toxic potential compared to AgCIT. Although inside biological systems, they interact with proteins of

biological molecules, and toxicity was measured as size-dependent (Fig. 6). Percent of cells viabilities against varied sizes AgPVP nanoparticles, each of 150 ug dose, 54.54% for 5 nm, 12.14% for 10 nm, 22.84% for 20 nm, 29.33% for 40 nm, 3.8% for 80 nm. PVP itself has noxious potential and can be seen from the controlled PVP toxic effect. Enhance in toxicity was observed with increasing sizes of nanoparticles which might be due to the increased amount of PVP surrounding the enlarged outer surfaces of nanoparticles. Consequentially PVP supported nanoparticles have a more toxic effect and biologically incompatible than AgCIT, which might be due to high production of cytokines by PVP coated silver nanoparticles in the test cells. The current result suggested that extra care will be needed when using such nanoparticles as a drug for human beings.

It is reported that gold and silver nanoparticles have nontoxic to other cell lines. mPEG-coated AuNPs of 1.9 nm and 15 nm have good compatibilities with mice systems [25, 26]. AuNPs coated with HDL [27], PEI caped of 15 nm AuNPs [28], silica-coated AuNPs [29], and 153 nm gold nano-shells coated with PEG [30] have cytocompatibility under various conditions with a range of cell lines. Still, gold nanoparticles cytotoxicity also has been reported under certain conditions [14, 31, 32].

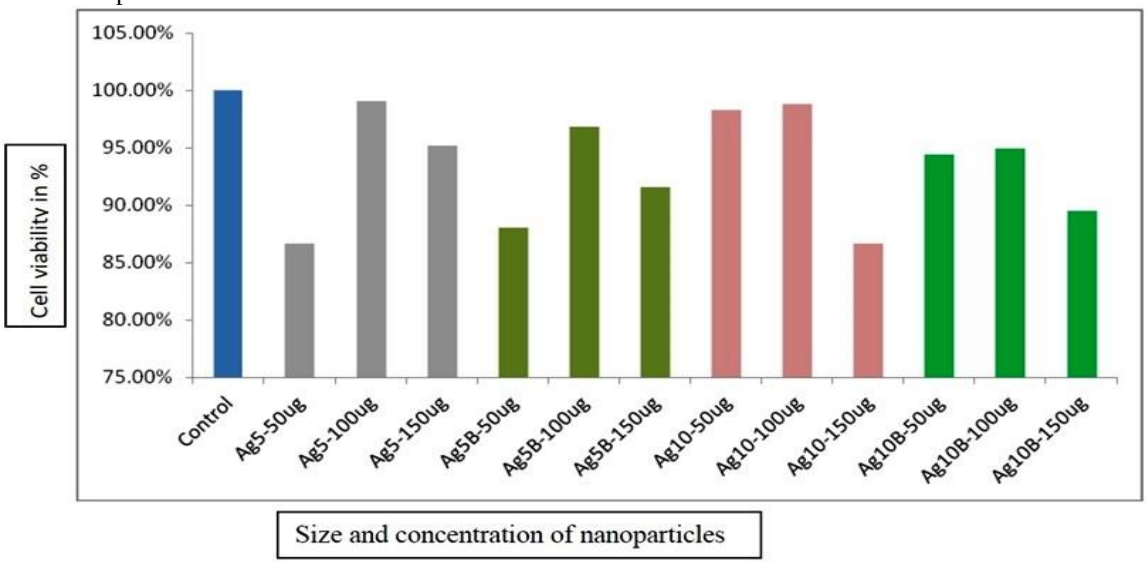

Fig 4: MTS assay results for 5 and 10 nm AgCIT and PcAgNPs (B).

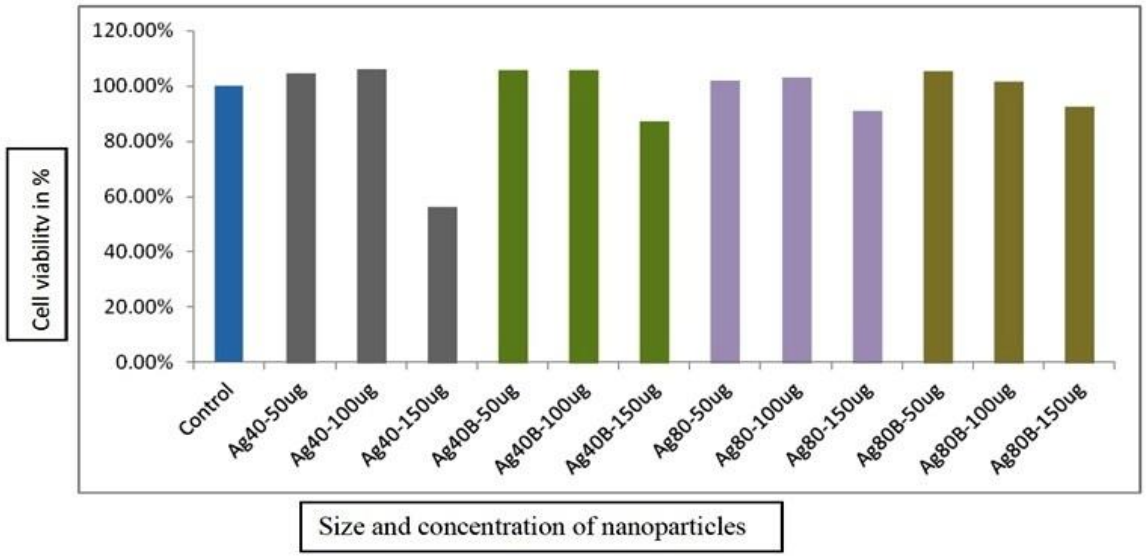

Fig 5: MTS assay results for 40 and 80 nm AgCIT and PcAgNPs (B).

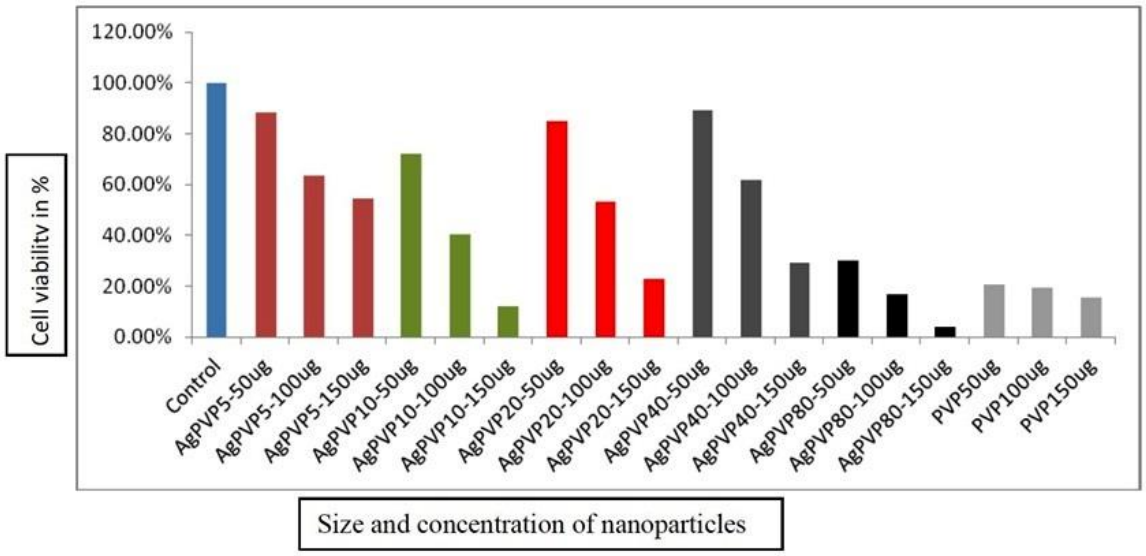

Fig 6: MTS assay results for 5, 10, 20, 40 and 80 nm AgPVP nanoparticles.

Conclusion

It is concluded that the PVP-coated silver nanoparticles prepared and studied in this project were found to be toxic for living cells. Their cytotoxicity generally increases with size up to a specific limit. PVP-coated silver nanoparticles were more deleterious as compared to AgCIT. The AgCIT conjugated with BSA was found to be biocompatible. Extreme care should be taken while using silver nanoparticles coated with PVP for therapeutic purposes. It is also essential to study the interaction of silver and other noble metal nanoparticles with different legends to make their use harmless for living organisms and the environment.

References

1. T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K. A. Dawson and S. Linse, Understanding the Nanoparticle–Protein Corona Using Methods to Quantify Exchange Rates and Affinities of Proteins for Nanoparticles, *Proc Natl. Acad. Sci.*, **104,** 2050 (2007).
2. M. P. Monopoli, C. Aberg, A. Salvati and K. A. Dawson, Biomolecular Coronas Provide the Biological Identity of Nanosized Materials, *Nat. Nanotechnol.*, **7,** 779 (2012).
3. N. A. M. Riviere, A. O. Inman and L. W. Zhang, Limitations and Relative Utility of Screening Assays to Assess Engineered Nanoparticle Toxicity in a Human Cell Line, *Toxicol. Appl. Pharmacol*., **234,** 222 (2009).
4. C. D. Walkey, J. B. Olsen, H. Guo, Emili and A. Chan, Nanoparticle Size and Surface Chemistry Determine Serum Protein Adsorption and Macrophage Uptake. *J. AM. Chem. Soc*., **134,** 2139 (2012).
5. C. D. Walkey, and W. C. W. Chan, Understanding and Controlling the Interaction of Nanomaterials with Proteins in a Physiological Environment, *Chem. Soc. Rev.*, **41,** 2780 (2012).
6. B. D. Chithrani, and W. C. W. Chan, Elucidating the Mechanism of CellularUptake and Removal of Protein Coated Gold Nanoparticles of Different Sizes and Shapes. *Nano Lett.*, **7.** 1542 (2007).
7. A. Lesniak, F. Fenaroli, M. P. Monopoli, C. Aberg and K. A. Dawson, Effects of the Presence or Absence of a Protein Corona on Silica Nanoparticle Uptake and Impact on Cells, *ACS. Nano.*, **6,** 5845 (2012)..
8. C. Lunov, T. Syrovets, O. Lunov, J. Beil and M. Delacher, Differential Uptake of Functionalized Polystyrene Nanoparticles by Human Macrophages and a Monocytic Cell Line. *ACS. Nano.*, **5,** 1657 (2011).
9. M. Safi, J. Courtois, M. Seigneuret, H. Conjeaud and J. F. Berret , The Effects of Aggregation and Protein Corona on the Cellular Internalization of Iron Oxide Nanoparticles, *Biomaterials.*, **32,** 9353 (2011).
10. N. A. Monteiro-Riviere, M. E. Samberg, S. J. Oldenburg and J. E. Riviere, Protein Binding Modulates the Cellular Uptake of Silver Nanoparticles into Human Cells: Implications for In Vitro to In Vivo Extrapolations?, *Toxicol. Lett.*, **220,** 286 (2013).

11. M. J. Clift, S. Bhattacharjee, D. M. Brown and V. Stone , The Effects of Serum on the Toxicity of Manufactured Nanoparticles, *Toxicol. Lett.,*. **198,** 358 (2010).
12. S. M. Moghimi, A. C. Hunter and J. C. Murray, Long-Circulating and Target-Specific Nanoparticles: Theory to Practice, *Pharmacol. Rev.*, **53,** 283 (2001).
13. A. E. Nel, L. Madler, D. Velegol, T. Xia and M. V. Hoek, Understanding Biophysicochemical Interactions at the Nano-Bio Interface. *Nat. Mater.*, **8,** 543 (2009).
14. A. M. Alkilany, P. Nagaria, C. R. Hexel, T. J. Shaw and C. J. Murphy , Cellular Uptake and Cytotoxicity of Gold Nanorods: Molecular Origin of Cytotoxicity and Surface Effects, *Small.,* **5,** 701 (2009).
15. H. J. Yen, , S. H. Hsu and C. L. Tsai, Cytotoxicity and Immunological Response of Gold and Silver Nanoparticles of Different Sizes, *Small.*, **5,** 1553 (2009).
16. M. M. V. Schooneveld, R. Koole, E. Vucic, Y. Zhou and J. Stocks, Improved Biocompatibility and Pharmacokinetics of Silica Nanoparticles by Means of a Lipid Coating: a Multimodality Investigation, *Nano. lett.*, **8,** 2517 (2008).
17. A. S. Thakor, J. V. Jokerst, C. Zavaleta, T. F. Massoud and S. Gambhir, Gold Nanoparticles: a Revival in Precious Metal Administration to Patients, *Nano. lett.*, **11,** 4029 (2011).
18. A. J. Mieszawska, J. M. Mulder, Z. A. Fayad and D. P. Cormode, Multifunctional Gold Nanoparticles for Diagnosis and Therapy of Disease, *Mol. Pharm.*, **10,** 831 (2013).
19. F. Hallouard, N. Anton, P. Choquet, A. Constantinesco and T. Vandamme, Iodinated Blood Pool Contrast Media for Preclinical X-ray Imaging Applications–a Review, *Biomaterials.*, **31,** 6249 (2010).
20. S. K. Libutti, G. F. Paciotti, W. E. Gannon, A. Byrnes and H. R. Alexander, Phase I and Pharmacokinetic Studies of CYT-6091, a Novel PEGylated Colloidal Gold-rhTNF Nanomedicine, *Clin. Cancer Res.*, **16,** 6139 (2010).
21. A. Bajaj, O. R. Miranda, R. L. Philips, D. J. Jerry and B. Kim, Detection and Differentiation of Normal, Cancerous and Metastatic Cells Using Nanoparticle Polymer Sensor Arrays. *Proc. Natl. Acad. Sci.*, **106,** 10912 (2009).
22. P. Vasileva, B. Donkova, I. Karadjova and C. Dushkin, Synthesis of Starch-Stabilized Silver Nanoparticles and Their Application as a Surface Plasmon Resonance-Based Sensor of Hydrogen Peroxide. *Colloids Surf. A: Physicochem. Eng. Asp.*, **382,** 203 (2011).
23. V. Amendola, Ph.D. Thesis, *Synthesis of Gold and Silver Nanoparticles for Photonic Applications*, University of Padova, Italy, (2008).
24. M. Arakha, S. Jha, S. Mohammed and B. C. Mallick, The Effects of Interfacial Potential on Antimicrobial Propensity of ZnO Nanoparticle, *Sci. Rep.*, **5,** 1038 (2015).
25. C. A. Simpson, K. J. Salleng, D. E. Cliffel and D. L. Feldheim, In Vivo Toxicity, Biodistribution and Clearance of Glutathione-Coated Gold Nanoparticles, *Nanomedicine: N. B. M.*,**9,** 257 (2013).
26. Q. Y. Cai, S. H. Kim, K. S. Choi, S. Y. Kim and S. J. Byun, Colloidal Gold Nanoparticles as a Blood-Pool Contrast Agent for X-Ray Computed Tomography in Mice, *Invest. Radiol.*, **42,** 797-806 (2007).
27. K. M. McMahon, R. K. Mutharasan, S. Tripathy, D. Veliceasa and M. Bobeica, Biomimetic High Density Lipoprotein Nanoparticles for Nucleic Acid Delivery, *Nano. Lett.*, **11,** 1208 (2011).
28. W. J. Song, J. Z. Ju, T. M. Sun, P. Z. Zhnang and J. Wang, Gold Nanoparticles Capped with Polyethyleneimine for Enhanced siRNA Delivery, *Small.*, **6,** 239 (2010).
29. A. S. Thakor, R. Luong, R. Paulmurugan, F. I. Lin and P. Kempen, The Fate and Toxicity of Raman Active Silica-Gold Nanoparticles in Mice. *Sci. Transl. Med*, **3,** 7933 (2011).
30. H. Liu, T. Liu, L. Tan and H. Wang, Impact of PEGylation on the Biological Effects and Light Heat Conversion Efficiency of Gold Nano-Shells on Silica Nanorattles. *Biomaterials.,*. **34,** 6967 (2013).
31. S. P. Boulos, T. A. Davis, J. A. Yang, S. E. Lohse and A. M. Alkilany, Nanoparticle –Protein Interactions: a Thermodynamic and Kinetic Study of the Adsorption of Bovine Serum Albumin to Gold Nanoparticle Surfaces, *Langmuir.,*. **29,** 14984 (2013).
32. B. D. Chithrani, A. A. Ghazani and W. C. Chan, Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. *Nano. Letters.*, **6,** 662 (2006).
33. K. Rao, M. Imran, T. Jabri, S. Perveen, S. Ahmed and M. R. Shah, Gum Tragacanth Stabilized Green Gold Nanoparticles as Cargos for Naringin Loading: A Morphological Investigation through AFM, *Carbohydr. Polym.*, **174,** 243 **(**2017).