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# Green synthesis of biogenic silver nanoparticles using *Solanum tuberosum* extract and their interaction with human serum albumin: Evidence of "corona" formation through a multi-spectroscopic and molecular docking analysis

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ABSTRACT

Biogenic silver nanoparticles (AgNPs) have been synthesized by using *Solanum tuberosum* (potato) extract (PE) as a reducing as well as stabilizing agent which is reasonably cheaper, non-toxic and easily available material. The green synthesis of silver nanoparticles has been carried out by very simple method and the nanoparticles were characterized by surface plasmon band as well as TEM measurements. The PE-AgNPs were highly dispersed in the solution and found to be spherical with around 10 nm in size. Interaction of these nanoparticles was studied with plasma protein HSA by means of various spectroscopies, such as, UV–visible, fluorescence, DLS, CD and FTIR spectroscopies. The HSA was found to form the protein "corona" around the starch-capped PE-AgNPs. Absorption spectroscopy revealed that the interaction between HSA and PE-AgNPs resulted in the ground state complex formation. Due to the strong absorption of PE-AgNPs, the inner filter effect was corrected for the fluorescence data. PE-AgNPs were found to quench the fluorescence of HSA with a small blue shift attributed to the increase in the hydrophobicity near tryptophan residue due to the presence of amylopectin and amylose units in the starch. The value of n, Hill's constant, was found to be > 1 which determines the existence of a cooperative binding between nanoparticle and albumin. Several parameters such as Stern-Volmer and binding constants in addition to the thermodynamic parameters have been analyzed and discussed which established that the complex formation has taken place via static quenching mechanism and the corona formation between albumin and PE-AgNPs was entropy driven process. Binding of biogenic PE-AgNPs to the HSA slightly affected the secondary structure of latter with a small decrease in α-helical contents resulting in the partial unfolding of the protein, though the structural motif remained the same. Molecular docking simulations revealed various possible binding modes between PE-AgNPs and albumin.

## 1. Introduction

Noble metal nanoparticles have provoked ample attention because their structures display considerably unique and better physical, chemical, and biological properties, and functionalities due to their ultra small sizes. Amongst the numerous noble metal nanoparticles, silver nanoparticles have drawn the much more attention owing to their applications in many fields including, medical, pharmaceutical, cosmetics and agriculture, etc. AgNPs have also provided a diversity of superior properties, for instance, improved antimicrobial and antifungal activities, enhanced catalytic and optical properties [1–3]. Apart from these accomplishments, AgNPs can be used in bio-labelling, sensors, drug delivery system, and filters [4,5]. The activities of nanoparticles vary on their way of synthesis, for example, their size, morphology, toxicity, stability and mode of action can be different on the basis of their method of synthesis [6]. A lot of chemical and physical methods are there to synthesize the nanoparticles but all these required either high temperature and high pressure conditions or a lot of organic solvents which are very toxic in nature [7]. Therefore, it is needed to replace these classical, non-biocompatible and expansive methods by simple, economic and biocompatible methods. To overcome these situations green syntheses have got the attentions of researchers which is easier and harmless as compared to the physical and chemical methods. These are environment-friendly and require non-hazardous solvents which are, generally, extracts obtained from the plants or other living materials. These types of green syntheses have been carried out

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by using algae [8], fungi [9,10], bacteria [11] and other plant materials [12–17]. Reports have shown that gold [18] and zinc oxide [19] nanoparticles have been prepared by using potato extract with promising potential for biological and biomedical applications. The syntheses were simple and contained environmentally benign and renewable material. Cysteine-functionalized AgNPs have also been synthesized by using *Solanum tuberosum* (potato) extract which were used for the biological function [20].

The size of these nanoparticles is trivial enough to move in nearly all parts of the body, even in cells and organelles, leading potentially to a new approach to medicine or even a source of biological hazard. Despite the remarkable speed of development of nanoscience, relatively very few reports are available in the literature about the interaction of nanoparticles with biological systems, and this is now a serious bottleneck in the whole nanomedicine and nanotoxicology enterprise. In our previous work, we have synthesized polyvinyl thiol coated AgNPs and studied their interaction with several important proteins such as lysozyme [21], human serum albumin [22] and bovine serum albumin [23]. In the line of these studies we have designed the present study. In this report PE-AgNPs have been synthesized by using only potato extract via one pot synthesis and characterized by means of surface plasmon band as well as TEM. Interaction of these nanoparticles was seen with HSA (an important and most abundant protein of human plasma). Serum albumin is a vital globular protein found in the circulatory system and has a tendency to bind various endogenous and exogenous substances, such as, fatty acids, bilirubin and drugs. It is responsible for the transport of these substances throughout the body due to its binding capacity. Binding of the drug as well as delivery agent to the serum albumin plays important roles in the pharmacokinetics. Weak binding of the pharmaceutical substance with albumin leads to the short lifetime or poor distribution whereas strong binding can decrease the amount of free substance in the plasma. It is reported that interaction of nanoparticles with bio-fluids may lead to the “corona” formation, which may result in the particle with different properties when mixed with serum albumin, therefore, the protein-nanoparticle interaction is a topic of particular interest [24–26]. Interaction of HSA with PE-AgNPs obtained by green synthesis using potato extract has been seen by using a variety of techniques, such as, UV–visible, fluorescence, CD, DLS and FTIR spectroscopies. Additionally, we have used molecular docking for in silico analysis of the PE-AgNPs-HSA interaction which is, relatively, a less studied topic.

## 2. Experimental

### 2.1. Materials

HSA (lyophilized powder, Fatty acid free, Globulin free, ≥ 99%, Sigma) and silver nitrate (ACS reagent, ≥ 98.0%, Sigma) were used without further purification. Fresh potatoes were purchased from the local market. Rest of the chemicals/reagents used in the study were of analytical grade and purchased from Sigma, USA and used as received.

### 2.2. Methods

Perkin-Elmer Lambda 650 Spectrophotometer was used for measuring UV–Visible spectra of PE-AgNPs and HSA-PE-AgNPs interaction. The surface plasmon resonance for PE-AgNPs formation the spectrum was collected from 300 to 650 nm while for the interaction studies the spectra were recorded in the range of 240–320 nm using quartz cuvettes of 1 cm. TEM measurements were carried out on Transmission Electron Microscope (TEM, JEOL JEM-2100 F) electron microscope. DLS experiments were carried out on Nano Plus zeta/nano particle analyzer using quarts cuvette of 1 cm. Fluorescence measurements were performed on Hitachi spectrofluorometer (Model F 7000). Intrinsic fluorescence was measured by exciting HSA at 295 nm. Synchronous fluorescence spectra were collected at $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm. 3d–Fluorescence spectroscopy has been carried out between 200 and 500 nm at an interval of 5 nm for excitation wavelengths. CD was carried out with JASCO J-815 spectropolarimeter. FTIR spectra were recorded on a SHIMADZU IRPrestige-21/IRAffinity-1/FTIR-8400S spectrophotometer. Unless stated otherwise, most of the experiments were performed at 25 °C.

The PDB format of HSA (1AO6) was taken from protein databank (http://www.rcsb.org./pdb). Hetero atoms and side chain B were deleted from the protein before the docking using discovery studio visualizer (DSV) [27]. As the size of protein is significantly smaller than that of nanoparticles the latter can be considered as a large crystalline surface [28]. For that purpose, two layered silver sheet comprising 200 atoms each was built from the Hyperchem 8.0 software and the charge on the nanoparticles was kept zero [29]. Amylose and amylopectin units were made by ChemDaw Ultra 8.0 and their geometries were optimized using Hyperchem software [29]. After geometry optimization, capping agents (amylose and amylopectin units) were merged with nanoparticle sheet. The resultant capped structure of PE-AgNPs was then saved in PDB format for using in molecular docking simulations. Molecular docking was carried out using Hex online server (http://hexserver.loria.fr/) [30]. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of protein. Hex performs protein docking using Spherical Polar Fourier Correlations [31]. The parameters used for docking include: correlation type – shape only, FFT mode – 3D, grid dimension – 0.6, receptor range – 180, ligand range – 180, twist range – 360, distance range – 40. The docked structures were then visualized by using DSV.

### 2.3. Synthesis of PE-AgNPs Using Potato Extract

For the synthesis of Ag nanoparticles, we used potato (*Solanum tuberosum*) extract as the unique reducing and stabilizing agent, silver nitrate as the metal precursor, and deionized (DI) water as dispersing medium. Fresh potatoes were collected from nearest market for preparation of potato extract. 50 g potatoes were cleaned, peeled and cut into small pieces and boiled in 250 ml double distilled water at 90 °C for 15 min. After cooling to room temperature, the mixture was filtered using Whatman filter paper 1. To prepare 10 mM stock solution of silver nitrate (purchased from Merck India Ltd.), 0.17 g of $AgNO_3$ was dissolved in 100 ml deionized water. For preparing biogenic PE-AgNPs, 5 ml potato extract was added dropwise to 10 ml silver nitrate solution (conc. 10 mM) and was added 5 ml of deionized water so that the final concentration of the mixture remained 5 mM. The mixture was kept at room temperature and its color began to change within a few minutes of addition of potato infusion was collected for further study. The PE-AgNPs were washed three times with double-distilled water by centrifugation to remove any impurities. It was then lyophilized and stored in air tight vials under ambient conditions. The FTIR studies were performed to ascertain the involvement of the potato starch which seems to be accountable for the capping of PE-AgNPs. For that purpose, we have performed the FTIR measurements for both PE and the PE-AgNPs in order to establish whether the capping agent is potato extract. The FTIR spectrum of starch capped nanoparticles is similar to the spectrum of potato starch (Fig. 1(A)) which confirms that the potato starch is serving as functionalization agent.

### 2.4. Characterization of PE-AgNPs Synthesized from Potato Extract

The PE-AgNPs were characterized by TEM (Fig. 1(B)) and surface plasmon band in UV- absorption spectrum which is given in Fig. 2(A) (the spectrum of pure PE-AgNPs). UV–visible spectroscopy is an important technique for the characterization of nanoparticles attributable to the presence of surface plasmon band which shifts to longer wavelengths with increase in the size of nanoparticles [32]. Absorption of PE-AgNPs is also dependent on the morphology [33]. In present case peak at 419 nm is corresponds to the surface plasmon resonance and