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Bio-conjugated silver nano-materials and shape-directing role of cetyltrimethylammonium bromide

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Abstract

Conventional UV-visible spectrophotometric and transmission electron microscopic technique were used to determine the morphology of silver nanoplates (AgNP) using Alstonia scholaris aqueous leaves extract for the first time. The visible spectra showed the presence of three well defined surface plasmon absorption (SRP) bands at 500, 550 and 675 nm which attributed to the anisotropic growth of Ag-nanoplates. Transmission electron microscopic (TEM) analysis of AgNP showed formation of truncated triangular, polyhedral with some irregular shapes nanoplates in the size range 7-20 nm. Cetyltrimethylammonium bromide (CTAB) has no significant effect on the shape of the spectra, position of SRP bands, size and the size distribution of AgNP. Effects of various [CTAB] were also discussed in the green extra-cellular synthesis of AgNP using Alstonia scholaris leaves extract.

Key words: Nanostructures, chemical synthesis, electron microscopy, surface properties

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1. Introduction

Alstonia scholaris is a big tree with medicinal properties belonging to the family Apocynaceae family, its leaves, flowers, roots and stem bark has long being utilized as a traditional medicine to cure various human decease (arthritis, asthma, cancer, debility, diarrhea , dog bite , fever , hepatitis, impotence, jaundice, leucorrhoea , malaria, tumour, skin diseases, wounds, etc.) [1]. Silver and its compounds have been studied for many years, not only for their antibacterial activity and antiviral activity, but also for their low toxicity [2]. Antibacterial activity of the silver-containing materials can be used, for example, in medicine to prevent bacteria colonization on catheters , cancer, dental materials , prostheses , stainless steel materials , and vascular grafts [3]. Synthesis of advanced nanomaterials of silver and gold using various biological, chemical, and physical methods has been the subject of large number of investigations made by many authors over last two decades [4]. Generally, a stabilizer (co-polymers, dendrimers, lipids, polymers, surfactants, and starch) is essential to obtain fine and stable noble metal particles obtained by these methods [5]. In addition, bio-inspired approaches (designing safer chemicals, safer solvents and auxiliaries for green chemistry [6]) were explored in the synthesis of AgNP using leaf extracts of different plants such as, Crossandra infundibuliformis, Acalypha indica, Mentha piperita , Azadirachta indica , Stevia rebaudiana, Chenopodium album , Cinnamomum camphora leaf extract , Dioscorea bulbifera tuber extract, and Padina Tetrastromatica to avoid the use of hazardous toxic chemicals [7].

Surfactants are amphiphilic in nature, which have two ends with different polarities, and serve as a protecting, capping and/or stabilizing agent for nanoparticles against external forces [8]. The use of surfactants in the synthesis of advanced AgNP based on the chemical reduction method has been the subject of several investigators owing to a broad spectrum of applications of Ag and AgNP [9] . The roles of surfactants in the green extra-cellular biosynthesis of silver nanoparticles are limited [8c]. Solution-based synthesis of advanced nanomaterials, require special mention due to their low cost, convenience and use without additional templates and apparatus. Solutions of nanometer large particles are transparent and the scattering of light can be neglected [10]. The water soluble nano-materials have advantageous over the water-insoluble forms because UV-vis spectroscopy can be used to monitor the optical changes that accompany the surface on the materials and size, the size distribution, and the aggregation state of nanoparticles would be established with the help of absorbance spectra. To the best of our knowledge, there are no reports to the synthesis of anisotropic bio-conjugated water soluble silver nano-materials using Alastonia Scholaris leaves extract. It was, therefore, thought to be of interest to prepare the water soluble silver nano-materials investigate the title reaction having an insight into the role of Alastonia Scholaris leaves constituents. In the present study, spectra of resulting colored silver sols showed different peaks in the visible region for the first time. Additionally, the approach to biosynthesis of different sized bio-conjugated nano-materials may be more advantageous than conventional synthetic chemical reduction methodologies as the methods used in the present studies are green. We therefore anticipate that bio-conjugated materials may find applications in pharmaceutical and biomedical processing technologies.

2.2. Materials and Methods

2.1 . Materials and preparation of leaf extract

Alastonia Scholaris (Devil's tree) leaves were obtained from campus of Jamia Millia Islamia (Central University), New Delhi. The 10 g leaves were washed with cooled water and chopped into fine pieces then soaked in 250 ml double distilled water , heated for 20 min on water bath at 60 ^{0}C. The extract was filtered with Whatman paper No. 1 and kept under continuous dark conditions to avoid the intervention of photochemical reactions. The AR grade silver nitrate ($AgNO_3$) and cetyltrimethylammonium bromide purchased from Merck chemicals Ltd. (Mumbai) and used as received.

2.2. Morphology determination

On treatment of aqueous solutions of $AgNO_3$ with Alastonia Scholaris leaves extract, stable yellow to dark brownish color developed as the reaction proceeds. A control setup was also prepared without leaf extract which showed no change of color. The morphology was determined by sampling the reaction mixture at definite time intervals and the absorption maxima was scanned by UV–vis spectra, operated at a resolution of 1 nm between 325 and 700 nm ranges in a Perkin Elmer UV–vis spectrometer (Lambda EZ 150) in a 1 cm quartz cuvettes. For electron microscopic studies, the images of nanoparticles were studied using high resolution TEM (Technai G2, Nether land, 200kV, 300 grid). TEM samples were prepared by placing a drop of the colored silver sol on carbon-coated copper grids and dried in air before measurements.

3. Results and discussion

3.1. Morphology of AgNP in absence of stabilizer

Henglein [10] suggested that UV-visible spectroscopy can be used to determine and easily analyzed the optical properties of colloidal metal nanp-materials. Therefore, the optical adsorption UV-vis spectra of AgNP prepared by different amounts (5, 10, 15 cm^3) of aqueous leaf extract of Alastonia Scholaris and [Ag^+] (10.0 , 20.0, 30.0 × 10^{-4} mol dm^{-3}). A visual observation (color of the solution changing from color less to dark brown) indicates the formation of AgNP due to the SPR after the addition of leaf extract to the aqueous $AgNO_3$ solution. A fig 1 and 2 shows (supplementary Figs S1 and S2) the production of AgNP at time intervals over 30 to 120 min for the variation of leaves extract and Ag^+ ions, respectively. Fig. 1 clearly shows the strong SRP at around 500 nm along with a weak shoulder at 675 nm in 5.0 cm^{3}of leaves extract for 30 min reaction time. Interestingly, 3 SRP bands at 500, 550 and 675 nm were observed for the entire range of [leaves extract] and [Ag^+] used in the present studies. The second adsorption peak at 550 nm was not clear in the

5.0 cm^3 leaves extract (Fig. 1) up to 30 min, but this peak becomes observable after 60 min and peak resolution was also very clear. At higher amounts of extract, all three SRP bands observed from the beginning of the reaction (Supplementary information; Figs S1 A and B). The absorbance of the product, silver sol, starts at zero and rises rapidly at the beginning of the reaction (Figs 1 and S1). The spectra show, however, that the absorbance change more slowly as the reaction goes on. The rates of the most chemical reactions depend upon the concentrations of the reactants. As these substances are used up, the reactions slow down. It was also observed that rates of the AgNP formation were found to be constant with [Ag^+] and has no significant effect on the morphology. Different types of natural products (alkaloids, iridoids, coumarins, flavonoids, leucoanthocyanins, reducing sugars, simple phenolics, steroids, saponins, triterpenoids, and tannins [11]) have been detected and identified from the Alastonia Scholaris plants. Out of these, alkaloids are one of the major constituents might be serve as both a reducing-and stabilizing- agents.

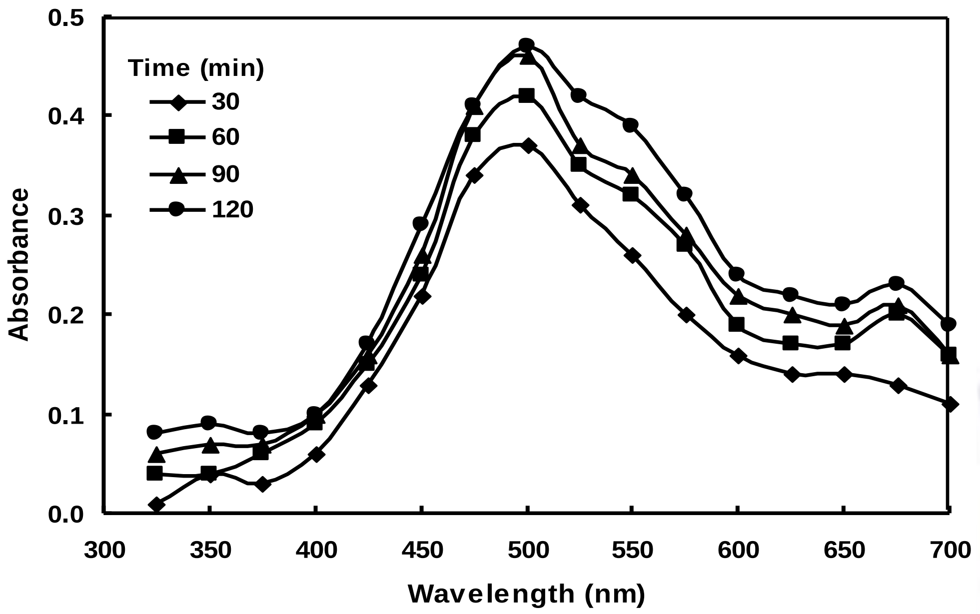


Fig. 1. UV-visible spectra of silver nanoparticles. Reaction conditions: $AgNO_3$ =10.0×10^{-4} mol dm^{-3} with variation of Alstonia Scholaris leaf extract = 5.0 cm^3 , Temp. = 30°C.

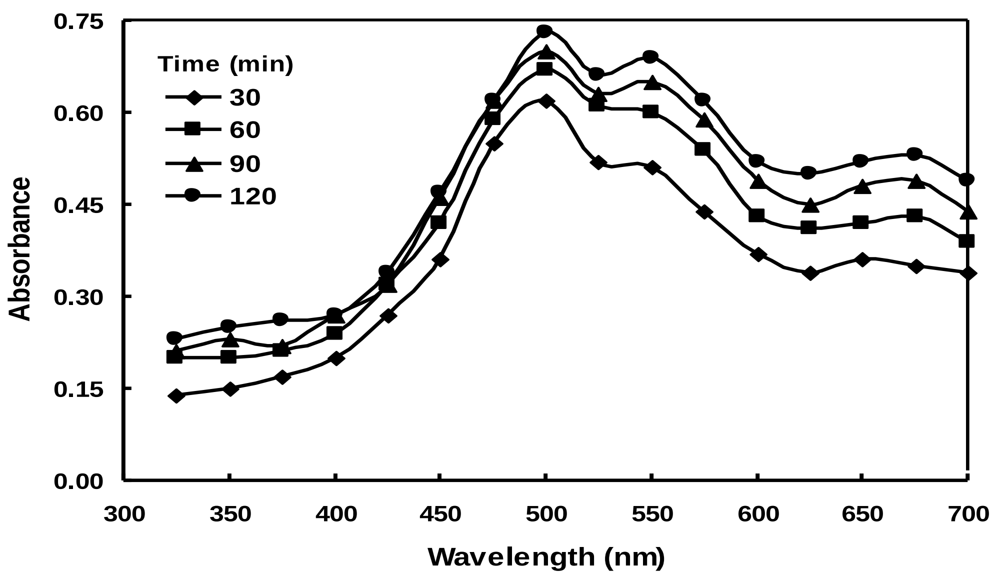
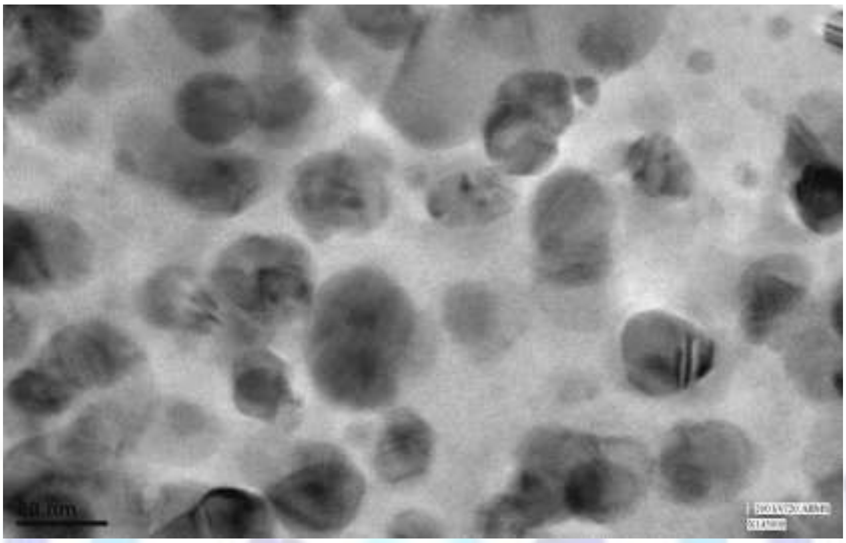


Fig. 2. UV-visible spectra of silver nanoparticles. Reaction conditions: Alstonia Scholaris leaf extract = 10.0 cm^3 with variation of $AgNO_3$ =10.0 × 10^{-4} mol dm^{-3} , Temp. = 30°C.

Figure 3 displays the TEM images of typical nanoparticles, produced by 5.0 cm^3 of leaves extract with Ag^+ ions (10.0 × 10^{-4} mol dm^{-3}) recorded at different magnifications. The resulting nanoparticles are well poly-dispersed. A large number of truncated triangular nanoplates and/or nanoprism with an irregular edge can occur at various diameters from 7 to 12 nm. It is well known that appearance of multiple-SRP bands in the spectra of silver sols due to the anisotropic growth and excitation of quadrupole and higher multipode plasmon resonance of Ag-nanoplates [12]. All 3 SRP bands at 500, 550 and 675 nm attributed to the in-plane dipole and multipole plasmon of Ag-nanoprisms and/or nanodisks which might be formed by the dissolution of the corner atoms of truncated triangular nanoplates [4b] . The SRP spectra also show the possibility of the presence of silver nanostructure of various sizes and shapes.

A



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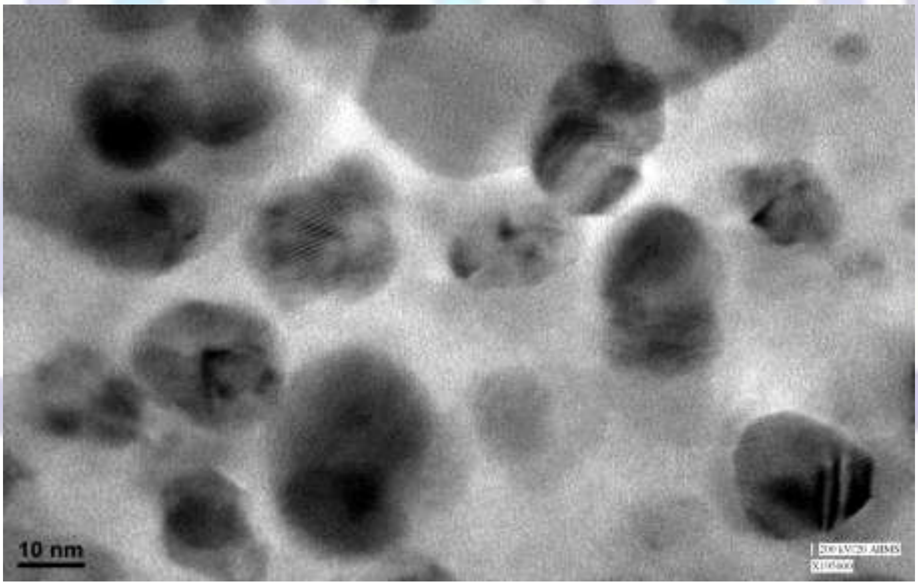

Fig. 3. TEM images of silver nanoparticles. Reaction conditions: $AgNO_3$ =10.0 × 10^{-4} mol dm^{-3} , Alstonia Scholaris leaf extract = 5.0 cm^3.

3.2. Morphology of AgNP in presence of stabilizer

The syntheses and characterization of noble metal nanoparticles by chemical reduction methods have been performed in the presence of suitable stabilizers in order to prevent unwanted agglomeration of the colloids [8]. Although a number of stabilizers (ligands, polymers, solid matrix and surfactants) were used for the stabilization of nanosize particles in solution, these are associated with some demerits [13]. In this content, surfactant aggregates, especially micelles, reverse micelles and macroemulsions, will get an edge over other stabilizers [14]. In order to see the shape-directing role of CTAB (sub-micellar, post-micellar and dilution effect) surfactant, three different [CTAB] (2.0 × 10^{-4} , 8.0 and 14.0 × 10^{-4} mol dm^{-3}) were used at constant other parameters and U.V.-vis spectra of AgNP recorded at different time intervals using Alastonia Scholaris leaves extract (Fig. 4). These observations and supplementary information (Fig.S3) also suggested that the

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paths involved in the formation of perfect transparent silver sol remains the same in the absence (Figs. 1 and 2) and presence of [CTAB] (Fig 4 and Fig S3). Figure 5 show TEM images of silver nanoparticles prepared by the leaves extract reduction of Ag^+ in presence of CTAB, also indicates the presence of truncated triangular nanoplates with some spherical-shaped particles. These results are in agreement with spectra of silver sols recorded at different time intervals. Thus, we may safely conclude that CTAB has no significant effect on the morphology of AgNP in the present system.

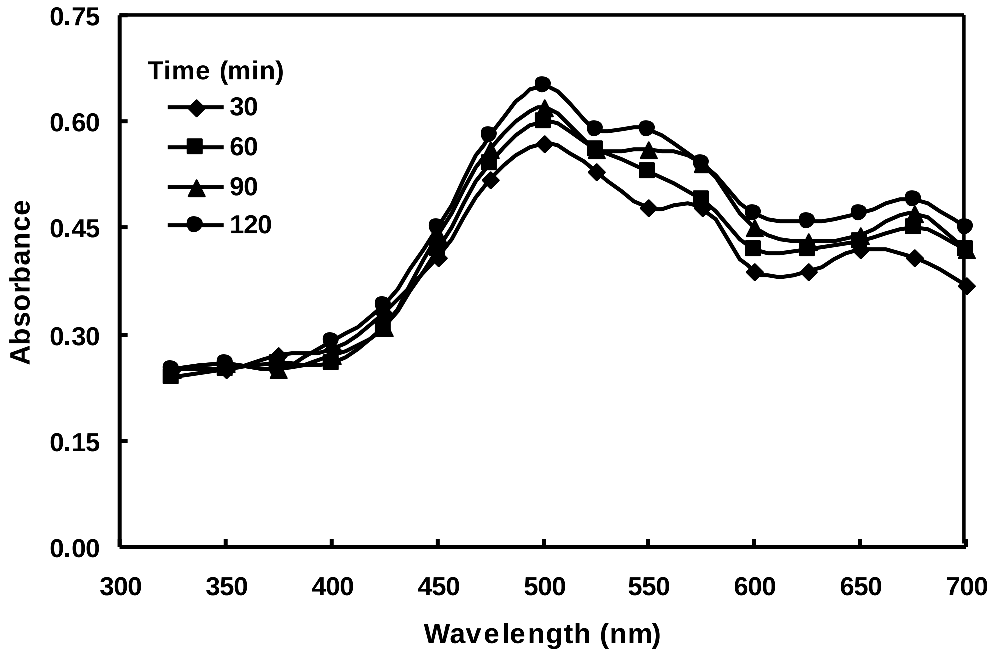
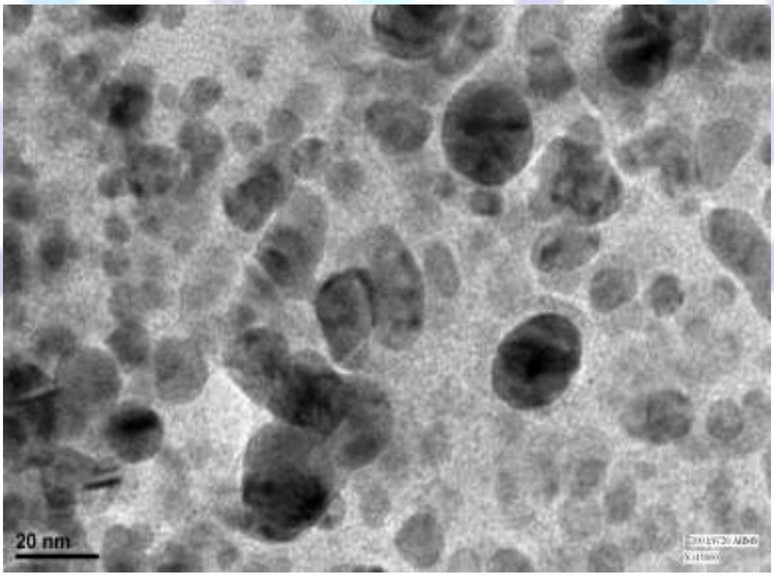


Fig. 4. UV-visible spectra of silver nanoparticles. Reaction conditions: $AgNO_3$ =10.0 × 10^{-4} mol dm^{-3}, Alstonia Scholaris leaf extract = 5.0 cm^3 with variation of CTAB = 2.0 × 10^{-4} mol dm^{-3} , Temp. = 30°C.

A



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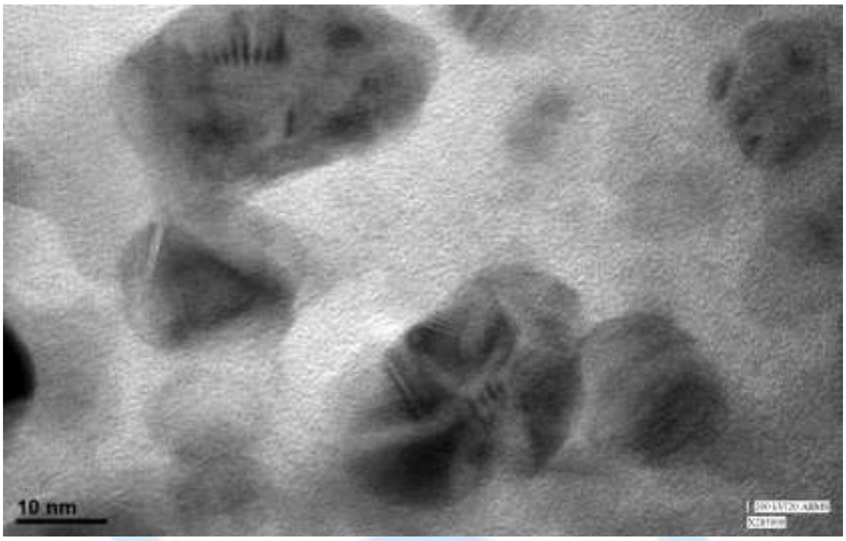

Fig. 5. TEM images of CTAB-stabilized silver nanoparticles. Reaction conditions: $AgNO_3$ =10.0 × 10^{-4} mol dm^{-3}), Alstonia Scholaris leaf extract = 5.0 cm^3 , CTAB = 2.0×10^{-4} mol dm^{-3} .

3.3. Visual observations on the morphology of AgNP

When Ag^+ ions were added, the color of solutions changed in a few minutes, which indicated the change of size and shape of particles. The color changes are observed from reddish yellow to deep red. The characteristic brown color of silver solutions provided a convenient spectroscopic signature to indicate their formation. AgNP were observed to be stable in solution and show very little aggregation (Fig.6).

A B C D E

Fig. 6. Optical photograph, captured by Nicon COOLPIX L26 , 16.1 Mega pixels digital camera, of the colloidal solution of AgNP at different time intervals. Reaction conditions: $AgNO_3$ = 10.0 × 10^{-4} mol dm^{-3}, Alstonia Scholaris leaf extract = 5.0 cm^3 , Time = just after mixing (A), 30 (B), 60 (C), 90 (D) and 120 min (E).

The origin of color is attributed to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field. With the concentration of Ag^+ and leaves extract increasing, the color of solutions after interaction was from colorless, pale yellow, orange, to red or brown. A set of color changes not typical for the preparation of spherical particles was observed during the course of the reaction [4b]. A unique property of spherical AgNP is that the SPR peak wavelength can be tuned from 400 nm (adsorb violet light and consequently appear yellow) to 530 nm (adsorb green light and appear brown-purple) due to the longitudinal plasmon mode by changing the particle size and the local refractive index near the particle surface [15]. Even larger shifts of the SPR peak wavelength out into the infrared region of the electromagnetic spectrum can be achieved by producing AgNP with rod or plate shapes.

4. Conclusions

Alastonia Scholaris leaves extract was successfully used for the synthesis of bio-conjugated silver nano-materials without adding any stabilizers for the first time. This spanking new and simple method for biosynthesis of silver nanoparticles offers a valuable contribution in the area of green synthesis and nanotechnology. Interestingly, sub-micellar, post-micellar and dilution effect of CTAB was not observed in the present system because constituents of Alastonia Scholaris leaves extract are better capping agents. Carefully monitoring the absorbance-time functions is sensitive techniques which allow an easy overview in determining if any nanoparticles aggregation has occurred on to the surface of nano-materials.

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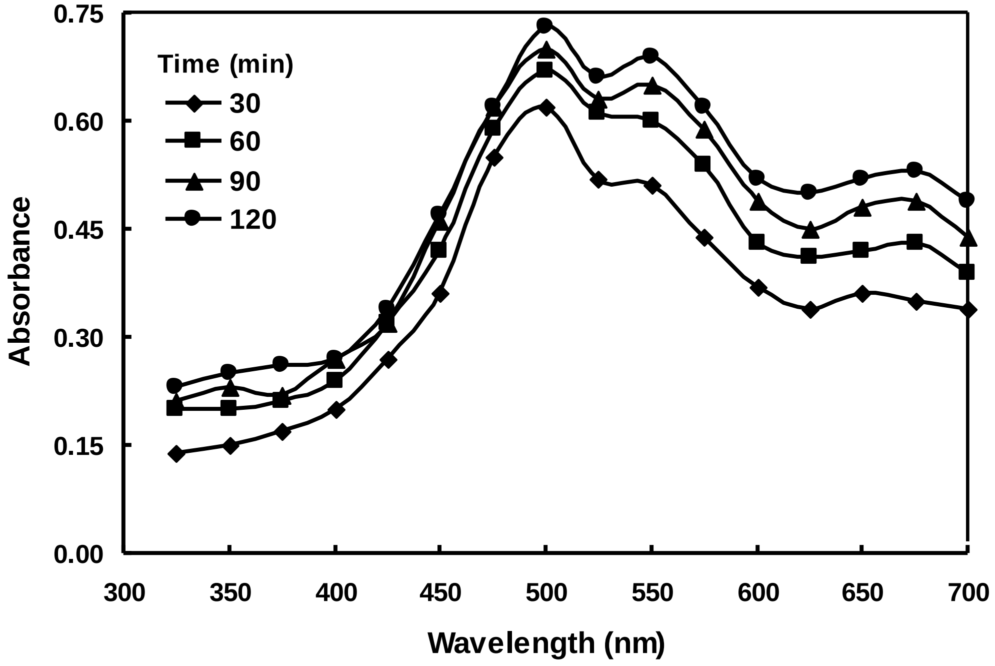
Supplementary materials

A



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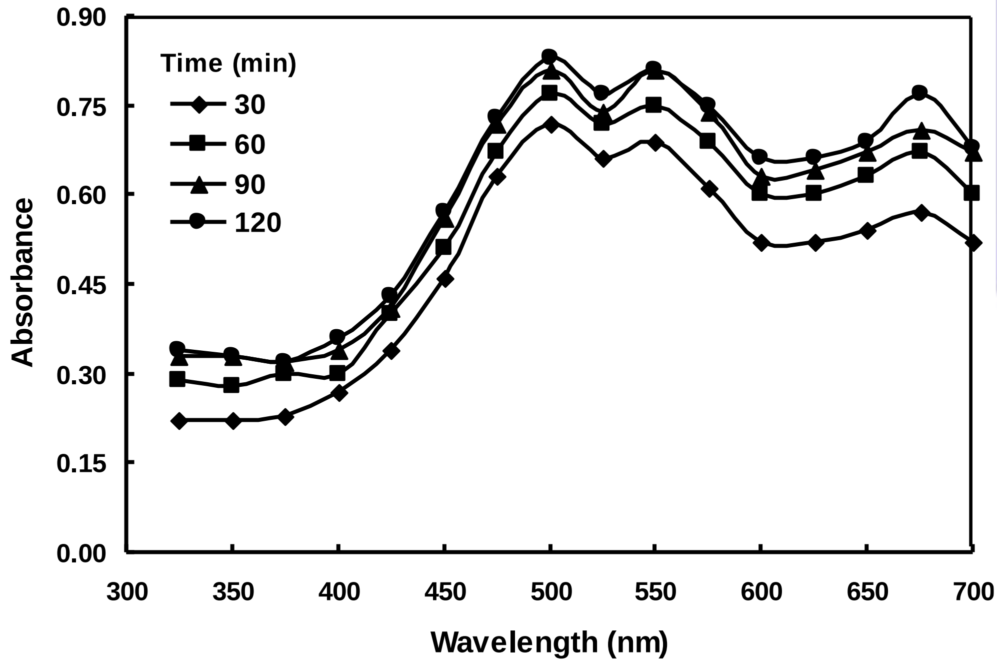
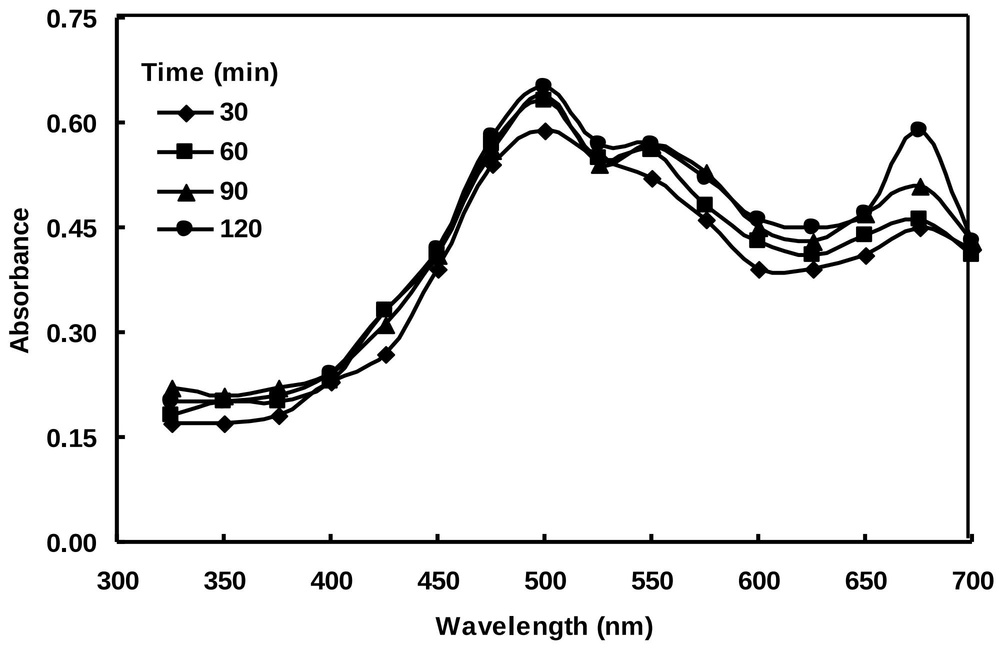


Fig. S1. UV-visible spectra of silver nanoparticles. Reaction conditions: $AgNO_3$ =10.0×10^{-4} mol dm^{-3} with variation of Alstonia Scholaris leaf extract = 10.0 (A) and 15.0 cm^3 (B), Temp. = 30^{o}C.

A



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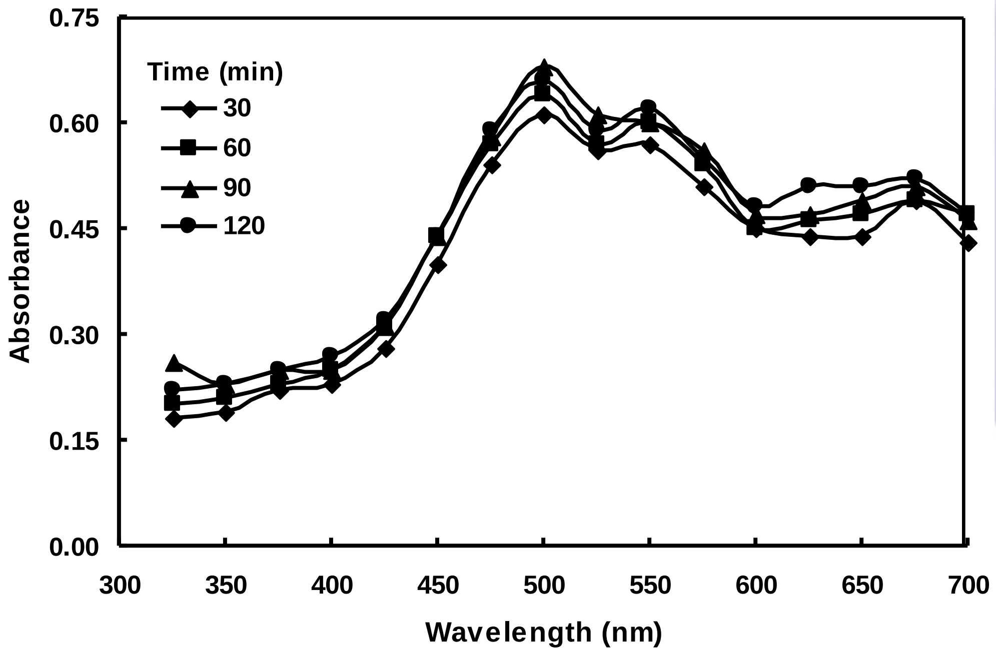

Fig. S2. UV-visible spectra of silver nanoparticles. Reaction conditions: Alstonia Scholaris) leaf extract = 10.0 cm^3 with variation of $AgNO_3$ = 20.0 (A) and 30.0 × 10^{-4} mol dm^{-3} (B), Temp. = 30^{o}C.

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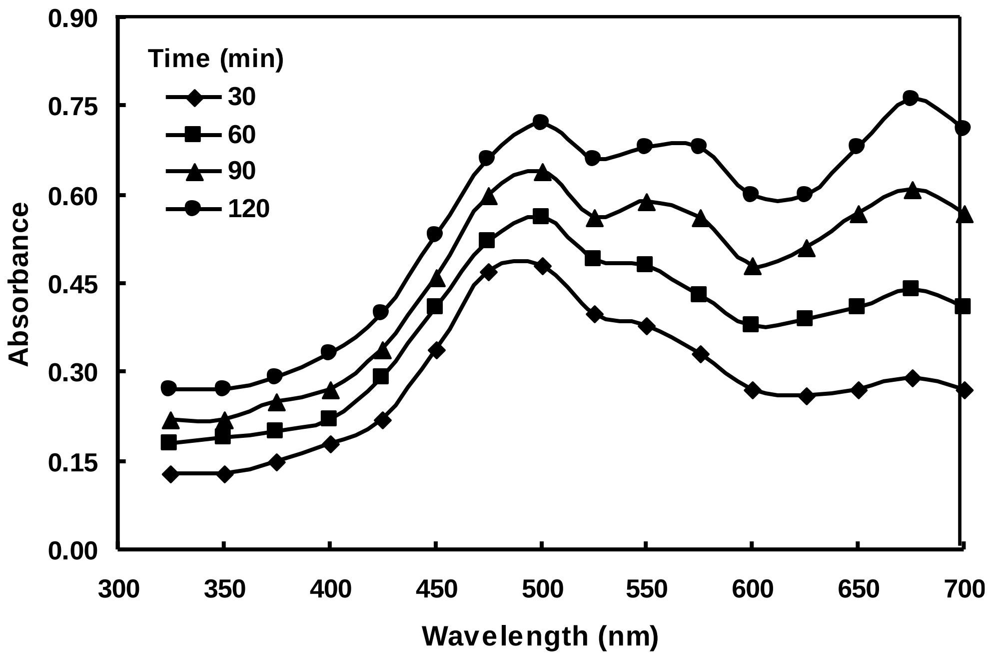
A



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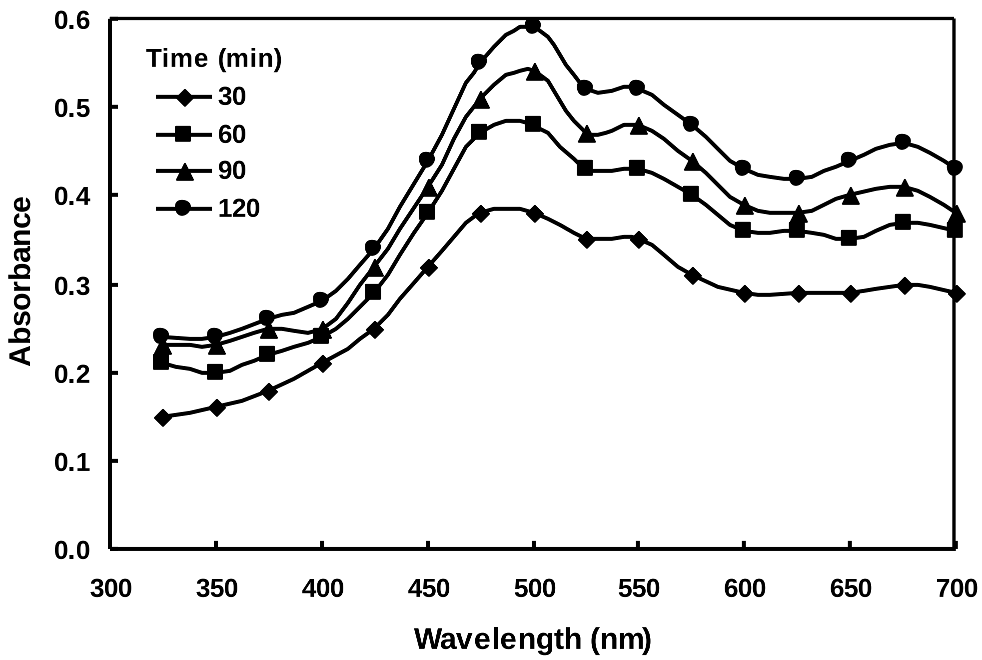

Fig. S3. UV-visible spectra of silver nanoparticles. Reaction conditions: $AgNO_3$ =10.0 × 10^{-4} mol dm^{-3}, Alstonia Scholaris leaf extract = 5.0 cm^3 with variation of CTAB = 8.0 (A) and 14.0× 10^{-4} mol dm^{-3} (B) , Temp. = 30^{o}C.