Synthesis of Silver Nanoparticles Using *Bombyxmori* Silk Fibroin and Their Antibacterial Activity

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Abstract. Present work describes the synthesis of colloidal silver nanoparticles using *Bombyx mori* silk fibroin under white light environment at room temperature. The bio reduction of silver ions showed the unique surface plasmon resonance (SPR) band at 420 nm which was confirmed by UV-visible spectroscopy. Transmission electron microscopy (TEM) showed the synthesized AgNPs are spherical in shape with the average particle size of 35-40 nm. X-ray diffraction (XRD) pattren evidenced the crystalline nature of the AgNPs with FCC structure. The biosynthesized AgNPs showed effective antibacterial activity against bacterial stains *Bacillus subtilis,* and *Salmonella typhi.*

1. Introduction

The biosynthesis of silver nanoparticles is focussed area of research in the field of biomedical applications in recent years [1]. The make use of biological organisms in the synthesis of metallic nanoparticles developed as a novel method [2]. The advantage of risk-free chemicals, eco-friendly and viable materials are the important key role for the consideration in a biosynthesis approach [3, 4]. Recently many researchers showed that several biological organisms [5], proteins [6], peptides [7, 8] become best candidates for the synthesis of AgNPs. Recently few research groups have studied the silk protein macromolecule as a bio template for the synthesis of metallic nanoparticles [9, 10]. In the present study, we report an attempt to use Mysore silk (*Bombyxmori*) a multivoltine silk fibroin (SF) as a reducing agent for the *in situ* synthesis of AgNPs at normal atmosphere under incandescent light exposure. The synthesized AgNPs were characterized by UV-visible spectroscopy, TEM and XRD. We also carried out antibacterial activity of the colloidal silver nanoparticles against *Bacillus subtilis* (*B. subtilis*) and *Salmonella typhi* (*S. typhi*) bacteria. This study may open the new applications of SF-AgNPs in biomedical and bionanotextile industry.

2. Experimental

2.1. Materials

Sodium carbonate (Na_2CO_3), lithium bromide (LiBr) and silver nitrate ($AgNO_3$) (>99%) were procured from Sigma Aldrich. All the chemicals were used without further purification. Pure Mysore silk (PMS) – (*Bombyxmori*), multivoltine cocoons were collected from the Sericulture Department, University of Mysore, Mysore, India.

2.2. Extraction of aqueous silk fibroin (SF) solution

Extraction of aqueous SF solution was reported in our earlier work [11]. The cleaned cocoons were boiled twice with 0.02M sodium carbonate aqueous solution for an hour to remove sticky sericin material coated over the fibroin surface. The degummed SF were then rinsed twice with distilled water and dried in air at normal atmosphere. Then it is dipped into 9.3M of LiBr solution and heated at 60 ºC for 3 to 4 h until it get completely dissolved. The obtained SF-LiBr salt solution was dialyzed against distilled water for 72 hours, using dialysis cassettes (MWCO:3500) to remove salt impurities. The optically clear solution of SF obtained were maintained at 4 ºC for the further investigations.

2.3. Preparation of colloidal SF-AgNPs solution

A known quantity of $AgNO_3$ was mixed to 5 mL of SF solution (1 wt%), so that $AgNO_3$ concentration was varied from 1-15 mg/mL. This mixture solution is kept under white light (Incandescent bulb - 60W, Philips) at normal atmosphere for a day. Then the optically clear mixture solution was turned into yellow colour confirming the formation of AgNPs.

3. Characterization techniques

3.1. UV-visible spectral analysis

The optical absorption spectra of the samples was recorded using UV-visible spectrophotometer, (Shimadzu UV-1800, Japan) in the wavelength range 200-800 nm.

3.2. FT-IR spectral analysis

FT-IR scan of the sample was recorded using IR-Prestige 21 with spectral range of 4000-500 cm^{-1} having a resolution of 4 cm^{-1}.

3.3. X-ray diffraction measurements

The X-ray diffractograms of synthesized AgNPs samples were recorded using X-ray diffractometer (Rigaku Miniflex-II) with Ni-filtered CuKα radiation (λ = 1.5406 Å). The samples were mounted on sample holder and diffractograms were obtained from X-ray source for the 2θ range of 10-80º with scanning speed of 5°/min.

3.4. TEM analysis

The surface morphology, diffraction rings, size, shape and dispersion of AgNPs in the SF solution was characterized by using transmission electron microscope (TEM), under accelerating voltage of 200 keV using JEOL - JEM 1010 LaB_6.

3.5. Antimicrobial assay

The microorganisms used in antibacterial activity were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. Gram-positive bacteria namely *Bacillus subtilis* (MTCC 121) and Gram-negative bacteria *Salmonella typhi* (MTCC 733) were tested. The bacterial stains were maintained in nutrient agar (NA) slants at 4 °C in the refrigerator.

3.6. Disc diffusion method

The antibiotic activity of synthesized samples was determined using agar disc diffusion method. Briefly, the sterile paper discs were impregnated with synthesized SF-AgNPs and placed aseptically on the surface of the agar plates and incubated at 37 ºC for 24 hours. The diameter of inhibition zone formed around paper discs after 24 hours and it is measured in millimetres (mm), which shows the effectiveness of the sample against the bacterial activity. Gentamicin was served as positive reference standard [22].

4. Results and discussion

4.1. UV-visible spectral analysis

Fig. 1 shows the optical absorption spectra of pure SF and SF-AgNPs colloidal solution. The pure SF shows the absorption at λ=275 nm (not shown), which is mainly due to presence of amino acid residues such as Tyr, Phe and Try in the SF chain [12]. Initially, SF-$AgNO_3$ solution was colourless, after exposure to incandescent bulb the colour of the solution changes from colourless to yellow and then dark brown colour as shown in Fig. 2. The change of colour is evidenced that the localized surface plasmon resonance (SPR) band at 420 nm of AgNPs produced in the SF solution. As the concentration of $AgNO_3$ increased up to 15 mg/mL the corresponding peak intensity increased. The peak shift towards higher wavelength side may be particle size increased [13]. The synthesized AgNPs are spherical in shape which is further confirmed by TEM.

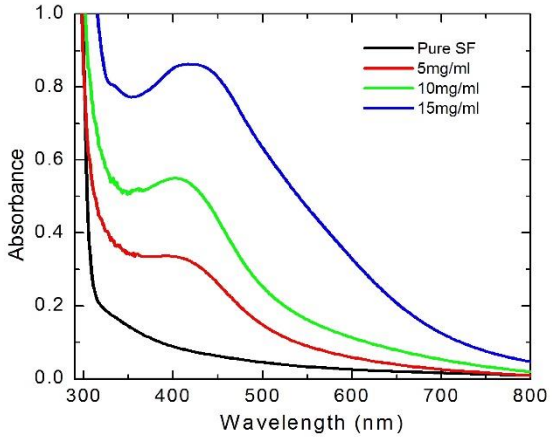

Fig. 1. Absorption spectra of pure SF and SF-AgNPs.

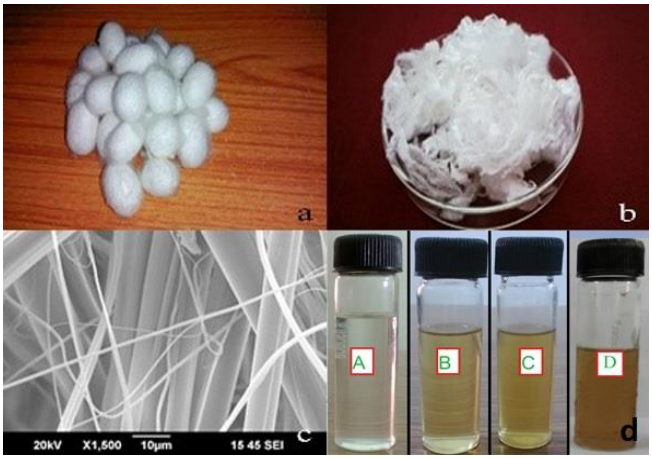

Fig. 2. Formation of AgNPs a) Cocoons b) Silk fibroin fibers c) SEM image of silk fibroin fibers d) Colloidal SF-AgNPs.

4.2. FT-IR study

The FT-IR analysis were carried out to identify the presence of biomolecules, which could be accountable for the bioreduction of silver ions (Ag^{+}) to silver neutral atom of zero valence (Ag^{0}). Fig. 3 shows the FT-IR spectra of the SF-AgNPs sample, the peak observed at 1621 cm^{-1} and 1521 cm^{-1} are corresponds to amide - I and amide - II of silk II structural conformation (β-sheet). Other absorption band observed at 1226 cm^{-1} corresponding to amide - III which is typically silk I conformation (α-helix or random coil structure) [14-15]. The peak observed at 1443 cm^{-1} due to symmetric stretching vibrations of carboxylate group and 1372 cm^{-1} corresponding to presence of methylic groups of alanine in the silk chain. The peak at 1623 cm^{-1} associated with the carbonyl (C=O) stretching vibration. The 3280 cm^{-1} is corresponding to the hydroxyl (-OH) stretching vibration from the phenolic acids of Tyr molecule of SF chain. The new peak in SF-AgNPs sample at 1725 cm^{-1} shows presence carbonyl (ketone) group and confirms that phenolic –OH group was converted into C=O group by oxidation [16].

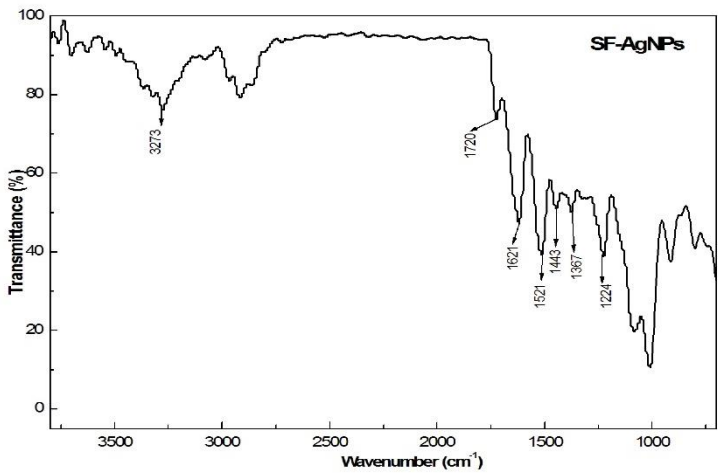

Fig. 3. FT-IR scans of SF-AgNPs.

4.3. X-ray diffraction analysis

Fig. 4 shows the X-ray pattern of powder sample obtained by SF-AgNPs colloidal solution (10 mg/mL). The prominent diffraction peaks observed at 2θ=20.28º, 38.02º, 44.35º and 77.34º confirms the presence of FCC crystalline structure of AgNPs, with (111), (200), (220) and (311) crystalline planes [17, 18]. The broad peak at 19.28º confirmed the silk fibroin present in the colloidal sample.

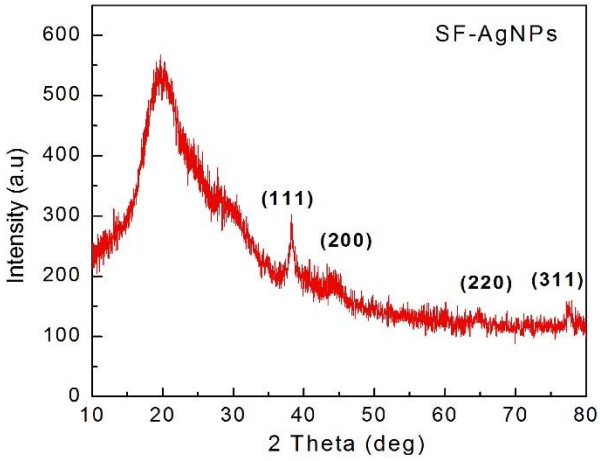

Fig. 4. XRD scan of silk fibroin-silver nanoparticles.

4.4. TEM study:

Fig. 5 shows the TEM images of the AgNPs synthesized using SF. Fig. 5 elucidates the uniform dispersion of AgNPs in the SF solution. An individual particles show the spherical shape and small size distribution evidenced the SF acts as a very good stabilizer and reducing agent. The TEM images further illustrated the nature of silver nanoparticles. The average particle size of the synthesized AgNPs was found to be 35-40 nm.

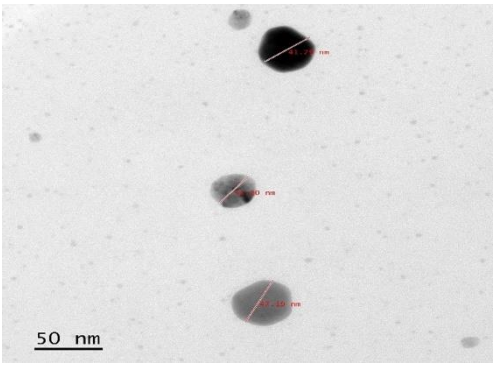

Fig. 5. TEM image of SF-AgNPs.

4.5. Antimicrobial assay

4.5.1. Disc diffusion assay

Silver is well known for its antibacterial activity. The evaluation of antibacterial activity of synthesized AgNPs were carried out for selected microorganisms. In order to study their effectiveness, MHA diffusion test were carried out using *Bacillus subtilis* (*B. subtilis*) and *Salmonella typhi* (*S. typhi*) bacterial stains.

Table 1, contains the diameter of inhibition zone values of the biosynthesized AgNPs against the tested pathogens. Pure SF is inactive against tested bacteria and hence confirms its insignificant effect on bacterial strains. Whereas 5 mg/mL, 10 mg/mL and 15 mg/mL SF-AgNPs colloidal solutions showed significant antibacterial activity shown in Fig. 6. Even though all the tested colloidal samples showed the activity against both *B. subtilis* and *S. typhi*, but they are not equally sensitive to the bacterial strains. The activity of AgNPs against these bacteria's was explained by the several authors. According to Aziz et al AgNPs increases proton motive force on the surface of the bacteria by forming ionic bond when comes in contact [17]. NPs can physically interacts with bacterial cell surface and kills the bacteria [18-20]. Briefly, the (111) planes of spherical AgNPs having high atomic density facet which is directly contact with cell membrane of the bacteria and stop the replication of DNA of the bacterial stains [21]. The AgNPs could be higher surface to volume ratio due to this reason it exhibit effective antibacterial activity against the bacterial strains.

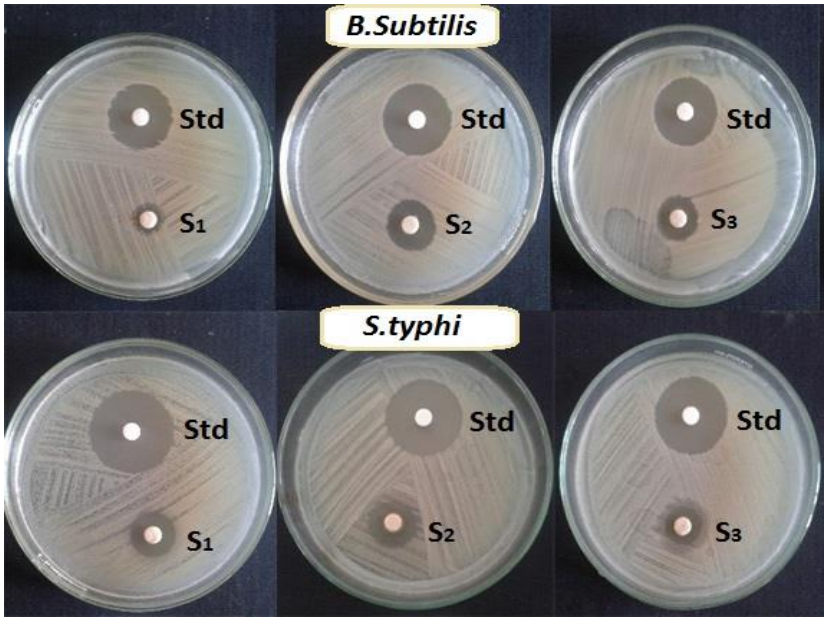

Fig. 6. Zone of inhibition, SF-AgNPs S_1 = 5 mg/mL, S_2 = 10 mg/mL, S_3 = 15mg/mL.

Table 1 Antimicrobial activity of SF-AgNPs with different bacterial species

$AgNO_3$ concentration in SF solution	Zone of inhibition (mm)	
	Bacterial strains	
	Gram-positive	Gram-negative
	Bacillus subtilis	*Salmonella typhi*
S_1	13.0	13.0
S_2	15.0	15.5
S_3	16.0	15.0
Pure	--	--

Positive control: Gentamicin

Conclusions

In this work, colloidal AgNPs were synthesized by bio based material silk fibroin as biotemplate under white light at room temperature. Silk fibroin acts as reducing and stabilizing agent in the reduction of silver ions into silver atom. The formed AgNPs were confirmed by change in colloidal solution color and the peak at 420 nm in UV-visible spectra. The synthesized AgNPs were spherical in shape and is confirmed by TEM images. The FCC structure of synthesized AgNPs were confirmed by XRD study. The synthesized AgNPs were showed enhanced antimicrobial activity against tested human pathogens.

Acknowledgements

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