Synthesis of silver nanoparticles using *Bombyx mori* silk fibroin and antibacterial activity

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Abstract. In the present work, the stable silver nanoparticles (AgNPs) were produced *in situ* in the presence of white light, using aqueous silk fibroin (SF) acquired from *Bombyx mori* silk. The UV-Visible spectral study explained the production of AgNPs by displaying a distinctive surface plasmon resonance band (SPR) at the wavelength 424 nm. The crystalline nature of the produced AgNPs have been identified using XRD study. Nanocrystalline phase of silver (Ag) with face centered cubic (FCC) structure was observed by XRD. The shape evolution and size of the formed nanoparticles was studied using transmission electron microscope. The captured images shown the formed particle were spherical in shape in morphology, and diameter in the range 35 to 40 nm. In the application part, an attempt was made to evaluate the potential antibacterial activity of the biogenic silver nanoparticles against pathogenic bacteria's such as *Escherichia coli* and *Staphylococcus aureus*.

Keywords: AgNO₃, Silk fibroin, UV-Visible spectra, XRD, TEM and Antibacterial-Activity.

1. Introduction

Nanotechnology is one of the most popular areas of current research and development in all disciplines of science. It has revolutionized in the field of materials science by creating the materials of unusual properties and range of applications. Nanotechnology mainly focuses on the synthesis, characterization and modification of particles size less than 100 nm. In the last two decades the synthesis of noble metal nanoparticles received greater importance because of the wide utility in many fields of science. For the synthesis of metal nanoparticles many physical and chemical methods are available in literature [1-2]. But due to environmental issues and biological applications several authors searching alternative procedures for the synthesis of metal nanoparticles. Recently, environmentally friendly or bio-based methods leading to the evolution of nanoparticles getting more importance [3-4]. Bio based synthesis methods generally depends on the selection of solvent medium, unhazardous, reducing and stabilizing agents [5]. It is very important to mention here that synthesis of metal nanoparticles using biological/natural materials make nanoparticles more biocompatible, and environmentally friendly. In the current work, we have presented a simple, effective and
environmentally friendly method for \textit{in situ} synthesis of silver nanoparticles using SF as a reducing and stabilizing agent at room temperature in the presence of white light condition. SF is a useful biomaterial due to its biocompatible, biodegradability, renewability and unhazardous properties. The produced AgNPs were characterized by using UV-Visible, XRD and TEM techniques. The antibacterial activity of the biogenic nanoparticles were carried out with selected bacteria.

2. Material and experimental methods

2.1 Materials
Chemicals like sodium carbonate (Na$_2$CO$_3$), silver nitrate (AgNO$_3$) (>99%) and lithium bromide (LiBr) were procured from sigma Aldrich, used without any additional purification. 	extit{Bombyx mori} silk cocoons (Central Sericulture Research 4) were collected from the Department of Sericulture, University of Mysore and the aqueous solutions were prepared using double distilled water.

2.2 Preparation of aqueous silk fibroin solution
Detailed preparation method of silk fibroin solution is described in our recent work [6]. In a brief way, 	extit{Bombyx mori} silk cocoons were cut into tiny pieces and treated two times with boiling aqueous solution of 0.02 M sodium carbonate (Na$_2$CO$_3$, MW: 105.99 g/mole) for 30 min to eliminate the sticky materials. Then the fibroin mass rinsed using deionised water, and it was dried in air at normal temperature and pressure. The degummed SF fiber mass was melted in 9.3 M lithium bromide (LiBr, MW: 86.84 g/mole) salt solution at 70 °C for 3 ~ 4 h. The obtained SF and LiBr mixture solution was dialyzed using dialysis cassette (MWCO: 3,500) against double distilled water for 72 h to remove ions present in the solution. Lastly, the attained optically transparent clear solution was centrifuged at 4000 rpm for nearly 15 min to get rid of any silk aggregation during the process. The transparent SF solution was kept at 4 °C for additional use. The initial concentration of SF was about 5 wt% and was diluted to 1 wt% by adding distilled water and used for the preparation of the nanoparticles.

2.3 Preparation of colloidal silk fibroin-silver nanoparticles solution
For the preparation of colloidal solution, ten to hundred milligrams of AgNO$_3$ salt was added into 10 mL of 1 wt% SF solution. The final concentration of AgNO$_3$ in the solution was 1 and 5 mg/mL. The prepared SF-AgNO$_3$ mixture solution was irradiated using the white light (60 W, from Philips) at normal temperature for 24 h to produce AgNPs in SF. The obtained SF-AgNPs colloids were characterized using different analytical techniques.

3. Characterization of SF-AgNPs

3.1 UV-Visible spectroscopy
The UV-Visible absorption spectra of the SF and SF-AgNPs samples were recorded by using UV-visible spectrophotometer (UV-1800-Shimadzu, Japan), in the wavelength 200 to 800 nm at room temperature.

3.2 XRD measurement study
The nanostructural aspects of the silk fibroin-silver nanoparticles were characterized by using X-ray diffractometer (Rigaku Miniflex-II) equipped with Ni filter, CuK$\alpha$ radiation. The sample was scanned in the 2θ range of 10-80° with scanning rate of 5°/ min.

3.3 Transmission electron microscope images
The size and shape of the silver nanoparticles were obtained by using transmission electron microscopy (JEOL-JEM 2100 LaB6) operated at 200 keV.
3.4 Antimicrobial activity study
Antibacterial properties of the AgNPs was evaluated using disc diffusion method against two bacteria namely, S. aureus and E. coli. The tested bacteria samples were acquired from Microbial Type Culture Collection (MTCC), Chandigarh-160036, India.

4. Results and discussion

4.1 UV-Vis study
The production of AgNPs in the SF solution by the reduction of AgNO$_3$ was evident from the colour change of the reaction mixture after exposed to white light. Originally, the reaction mixture of SF-AgNO$_3$ solution was colourless, but subsequently exposed to white-light, the reaction mixture colour turns to yellow then dark brown within few minutes (figure 1).

This variation of the colour was attributed to the shape and size dependence of the AgNPs formed in the reaction mixture. The change in colour indicated the conversion of Ag ions to Ag$^0$ atoms in in the SF solution. It is well know that 18 amino acids were present in the Bombyx mori silk fibroin which are mainly Gly (43.68 mol %), Ala (29.34 mol %), Ser (11.48 mol %), Tyr (5.30 mol %), Val (2.23 mol %), Glu (1.37 mol %), and Thr (0.96 mol %) [7]. Out of these amino acid residues, tyrosine (Tyr) exhibits strong electron donating property, and which can reduce Ag$^+$ to Ag$^0$ [8-9]. In fact, the functional groups present in tyrosine have proved the reduction of AgNO$_3$ to AgNPs and the possible scheme is shown in figure 2 [10].

The UV-Vis Absorption spectra of pure SF and SF-AgNPs are presented in figure 3. From the spectra it is clear that, pure SF showed a band centered at $\lambda=275$ nm which coincides with the Tyr absorption band due to the excitation of $\pi\rightarrow\pi^*$ transition in the SF. On the other hand, SF-AgNPs, exhibited a
single absorption peak in the wavelength range $\lambda = 424-426$ nm advocated that formed nanoparticles are in spherical shape in accordance with the Mie theory \[11\]. This is also confirmed by the TEM study. We have also evaluated the effect of different concentrations of $\text{AgNO}_3$ on the synthesis of AgNPs. As the concentration of $\text{AgNO}_3$ was increased the corresponding peak intensity was also increased. The increasing trend in the intensity of the UV-abs spectra denote the increase in the number of AgNPs formed in the SF solution.

![Figure 3. UV-Visible absorption spectra of SF and SF-AgNPs.](image)

4.2 XRD study

The crystalline features of the AgNPs formed in this work was recorded and the XRD scans are illustrated in figure 4. From the figure 4, the diffraction peaks observed at $2\theta = 19.76^\circ$ and $29.34^\circ$ are corresponding to crystalline region of the pure SF \[12\]. The intense diffraction peaks riding over a SF domain with an diffraction angles $(2\theta)$ about $38.02^\circ$, $44.35^\circ$, $64.53^\circ$, and $76.34^\circ$ corresponding to reflections $(111)$, $(200)$, $(220)$, and $(311)$ indicating crystal planes of the metallic silver. These planes confirm the synthesized AgNPs represent a face centred cubic (FCC) structure compared to the pure silver is Joint Committee on Powder Diffraction Standards, silver file No.00-004-0783 \[13\]. The average crystallite size of the nanoparticles was calculated by using Debye-Scherer’s equation \[14\]. The average crystallite size of the AgNPs was found to be 35.23 nm.

![Figure 4. X-ray diffraction pattern of SF and SF-AgNPs.](image)
4.3 TEM analysis
The high resolution TEM images (figure 5 a, b) illustrate the nature of the silver nanoparticles formed in the silk fibroin solution. Almost all the nanoparticles are formed with the concentration of 5 mg/mL of AgNO$_3$ are exhibits exactly the spherical in size and shape with smooth faces. The diameter of the nanoparticles was around 35 to 40 nm.

![TEM images of AgNPs](image)

Figure 5. TEM images of AgNPs.

4.4 Antimicrobial activity
The antibacterial activity of the formed AgNPs was evaluated using Gram-positive and Gram-negative pathogens. The disc diffusion method was used in this study.

4.4.1 Disc diffusion method
Antibacterial study of the biogenic synthesized AgNPs was screened against Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) bacteria. The zone of inhibition (mm) of AgNPs was measured and is tabulated in Table 1. The results indicate that pure SF is not showed activity against any of the tested bacterial strains. But, the prepared SF-AgNPs colloidal solutions 1 and 5 mg/mL exhibits growth inhibitory effect on bacterial stains used in the study. However, the prepared samples exhibits bacterial inhibition, and is not uniform for all the tested bacteria.

| Samples | Inhibition Zone (mm) of Bacterial Strains in diameter |
|---------|-----------------------------------------------------|
| Staphylococcus aureus (Gram+ve) | Escherichia coli (Gram–ve) |

Table 1. Zone of inhibition
Sample (1 mg/mL, AgNO₃) showed diameter of inhibition zone is 12 ± 0.1 mm for *S. aureus* (Gram positive) and 12.5 ± 1.2 mm for *E. coli* (Gram negative). Sample (5 mg/mL, AgNO₃) showed enhanced zone of inhibition for all tested pathogens. AgNPs prompted antibacterial activity against bacteria was reported in earlier work [15]. In brief, the AgNPs interrupt the cell membrane integrity of the bacterial cells and upon entering into cytosol induces oxidative stress leading to the inhibition of cell advancement, which in turn, leads to cell death. The decomposition of the cell layer and the consequent decomposition of the cell membrane of the bacteria exposed to the AgNPs in the colloidal solution leads to the outflow of cytosolic contents, causing cell collapse [16].

5. Conclusion

Finally our study, spherical silver nanoparticles (AgNPs) were strongly synthesized by using SF as bio-template under UV-B light at normal pressure and temperature condition. In this study, spherical silver nanoparticles were produced by using SF as bio-template at room temperature condition. UV-Visible study established the production of silver nanoparticles by showing surface plasmon resonance band in the wavelength range 424-426 nm. The synthesized AgNPs were highly crystalline nature with average crystallite size was about 35.23 nm. The synthesized silver nanoparticle showed a broad spectrum of antibacterial activity against selected human pathogens.

6. Reference

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