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Use of sericin of *Bombyx mori* in the synthesis of silver nanoparticles, their characterization and application

Prakash N Bhat, Nivedita S & Subrata Roy[a]
Central Silk Technological Research Institute, BTM Layout, Madiwala, Bangalore 560 068, India

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Silver nanoparticles have been synthesized by the reduction of silver nitrate with sodium borohydride in an aqueous medium. The silk protein sericin, extracted from the cocoons of *Bombyx mori* silkworms, has been used as an effective capping agent. The stability of the colloidal suspension is confirmed by zeta potential measurement. The nanoparticles have been characterized by UV-vis spectroscopy, SEM and TEM analyses. They are found to be fairly spherical particles of average size 15nm. These particles also exhibit antimicrobial properties when applied onto silk fabric without significantly changing the colour of the fabric.

Keywords: Antimicrobial properties, Sericin, Silk, Silver nanoparticles, UV-vis spectra

1 Introduction

Silver nanoparticles (AgNP) can be synthesized in an aqueous medium by the reduction of silver nitrate. These tiny particles possessing high surface energy are inherently unstable. Therefore, they must be captured in the early stage of the reaction by surfactants (capping or stabilizing agents), which can keep them floated in the solvent by controlling their growth and agglomeration. The PVP[1], geletin[2], polyglutamic acid[3], sophorolipids[4], mixtures of different agents[5] and PVA[6] have been used as capping agents. The silk protein fibroin has earlier been used in the preparation of AgNP as reducing and capping agent[7].

Fibroin and sericin are the two proteins in raw silk spun from the silk glands of mature caterpillars belonging to the species *Bombyx mori*. Fibroin, the silk filament is surrounded by the gummy substance sericin, which constitutes about 25% of the raw silk. Usually sericin is removed as waste during silk refinement. However, in recent times sericin has been the topic of interest for research as it is found to have useful properties and applications in varied areas[8,9].

Fibroin is insoluble in water and preparation of its solution is a tedious process involving the use of chemicals, whereas sericin which is readily soluble in water can be extracted by a simple process, i.e. by boiling[10]. The sericin has earlier been used as a reducing and capping agent in the green synthesis of AgNP of average size 25nm (ref. 11).

In the present study, a method of preparation of even smaller sized (15nm) silver nanoparticles using sericin as the capping agent has been proposed. Further, the antimicrobial efficacy of these particles when applied onto silk fabrics has also been confirmed. Although the solution itself is yellow in colour, the treated silk fabric showed no significant change in colour, which is an improvement over the previously reported method[12].

2 Materials and Methods

2.1 Chemicals used for Synthesis

Silver nitrate (pure), the precursor for silver and sodium borohydride, and the reducing agent were purchased from Merck and used without further purification. De-ionized water was used for the preparation of silver nitrate and borohydride solutions. Bivoltine cocoons of CSR2 × CSR4 race were collected after hot air stifling. A 0.29 mM solution of silver nitrate was prepared and sonicated for 20 min at room temperature. Also, 10 mL of 0.01 M sodium borohydride was prepared and aged for 3h.

2.2 Equipment used for Characterization and Application

UV-vis measurements were made on Varian Cary 50 Bio UV-visible-NIR spectrophotometer in a standard optical cell of 10mm path-length. Zeta potential was measured on the Malvern Instrument's

[a] To whom all the correspondence should be addressed.
E-mail : cstriban@csb.gov.in

Zetasier using zeta dip cell. Scanning electron microscope (SEM) photographs were taken on Nova NanoSEM 600 (FEI Company) and transmission electron microscope (TEM) photographs were taken on JEOL JEM-3010 electron microscope. The change in colour of the treated fabric was measured on the Data Colour International's computer colour matching system DC 650.

2.3 Preparation of Sericin Colloid

The cocoons were deflossed, cleaned and cut into pieces of about 5 × 5 mm^2. A 0.2 % solution of sericin was prepared by boiling 2g of the cocoon in 200mL of purified (millipore) water for 20 min (ref. 10). After cooling, the solution was filtered using 5A grade paper, which retains particulates and gelatinous precipitates (>10 µm). This was sonicated at room temperature for 20 min. This solution of sericin colloid showed an absorption peak at 276 nm (Fig. 1a).

2.4 Preparation of AgNP

The 0.5 mL of sodium borohydride solution was added to 100 mL of the silver nitrate solution drop-wise under constant stirring. The transparent solution turned yellow instantaneously, indicating the formation of silver nanoparticles[13]. The UV-vis analysis was performed immediately at this stage and an absorption peak at 389 nm was obtained (Fig 1b), thus confirming the presence of silver nanoparticles before capping. If this solution is left to stand, it turns gray within a very short span of time, presumably due to agglomeration of AgNP, and the peak disappears. The chemical reaction[14] for the reduction of silver nitrate by sodium borohydride is shown below:

$$AgNO_3 + NaBH_4 \rightarrow Ag + ½ H_2 + ½ B_2H_6 + NaNO_3$$

2.5 Stabilization of AgNP with Sericin

To the AgNP containing solution, 3% by volume sericin solution was added and mixed thoroughly. The UV-vis spectrum of the stabilized solution showed a peak at 399 nm (Fig. 1c), which confirms the presence of AgNP after capping. The zeta potential of this solution was also determined.

3 Results and Discussion

3.1 Characterization

It can be seen from the TEM photograph (Fig.2) that the shape of the particles is spherical and their average diameter is about 15 nm. The particles are of fairly uniform size, as can be observed from the SEM photograph. (Fig. 3). The peak width at half

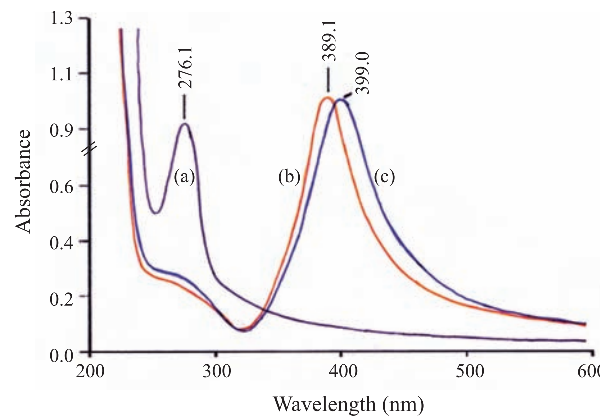

Fig. 1— UV-vis spectra of (a) sericin, (b) AgNP before capping and (c) sericin capped AgNP

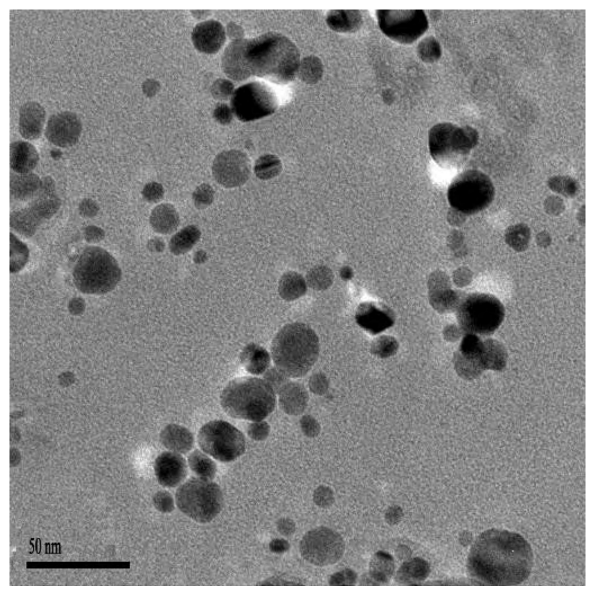

Fig. 2 — TEM photograph of silver nanoparticles

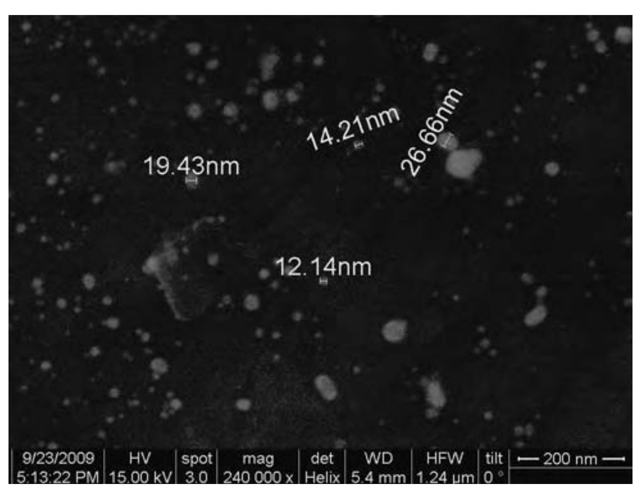

Fig 3— SEM photograph of silver nanoparticles

maximum (PWHM) calculated from the spectrum shown in Fig. 1c is about 60 nm, which also implies a fairly uniform size distribution. The zeta potential was found to be –34.6mV, which corresponds to moderate stability.

The particle size dependence on the concentrations of metallic salts has been reported[13]. In our earlier work, we have used a higher concentration (1 mM) solution of silver nitrate[11] as compared to 0.29 mM solution used in the present study. Presumably, the finer particle size has resulted due to the lower concentration of $AgNO_3$ solution. There is a shift in the UV-vis absorption peaks from 415nm in the earlier method to 398 nm in the present method, which also indicates reduction in particle size.

3.2 Role of Sericin as a Capping Agent

The Ag^+ ions resulting from the reduction of $AgNO_3$ by sodium borohydride are further reduced to Ag^0 atoms by accepting an electron. A group of nearby atoms aggregates to form AgNP, which are capped by the sericin micellae, and form colloidal particles which make the solution to appear yellow in colour.

It is well known that sericin micellae possess a negative charge[10]. The AgNP capped by sericin would therefore possess a negative charge and so they would remain suspended in water due to repulsive forces. Repulsive forces arising from the positive charges of the nuclei also contribute to electrostatic repulsion. Thus, it can be inferred that the stability is due to electrostatic means.

Stabilization may also be effected by steric means. Steric stabilization is a mechanism by which certain additives, such as hydrophilic polymers, inhibit coagulation of suspensions. Such a polymer being hydrophilic, would stay associated with water, rather than interact with any other surface, except that they are attached at one end to a particle. In particular, hydrophilic repulsion occurs in aqueous solutions between polar molecules to maximize the formation of hydrogen bonds with water. The amino acids of sericin have a high content (80%) of hydrophilic lateral groups, while also being attached to AgNP.

Thus, the sericin-capped AgNP form a moderately stable colloidal suspension in the aqueous medium which could be due to combined effects of electrostatic repulsion and steric stabilization.

3.3 Application of AgNP as Antimicrobial Agent

As reported earlier[12], AgNP when applied to silk fabric by exhaust method possess antimicrobial properties, but the treatment permanently stained the fabric with yellowish green colour. In the present study, AgNP when applied to the silk fabric also exhibits antimicrobial properties but the colour of the fabric is not affected significantly.

A plain woven mulberry silk fabric of the mass $35 gm^{-2}$ was used for the study. This degummed and undyed fabric (untreated) shows the whiteness index of 29.91. The sample was then treated with sericin-capped AgNP solution at boil for 5 min and dried in air. This treatment was repeated to impart a second coating. After drying, the fabric was cured in an oven at 100°C for 15 min. The fabric was thoroughly rinsed with water 3 times and then dried at room

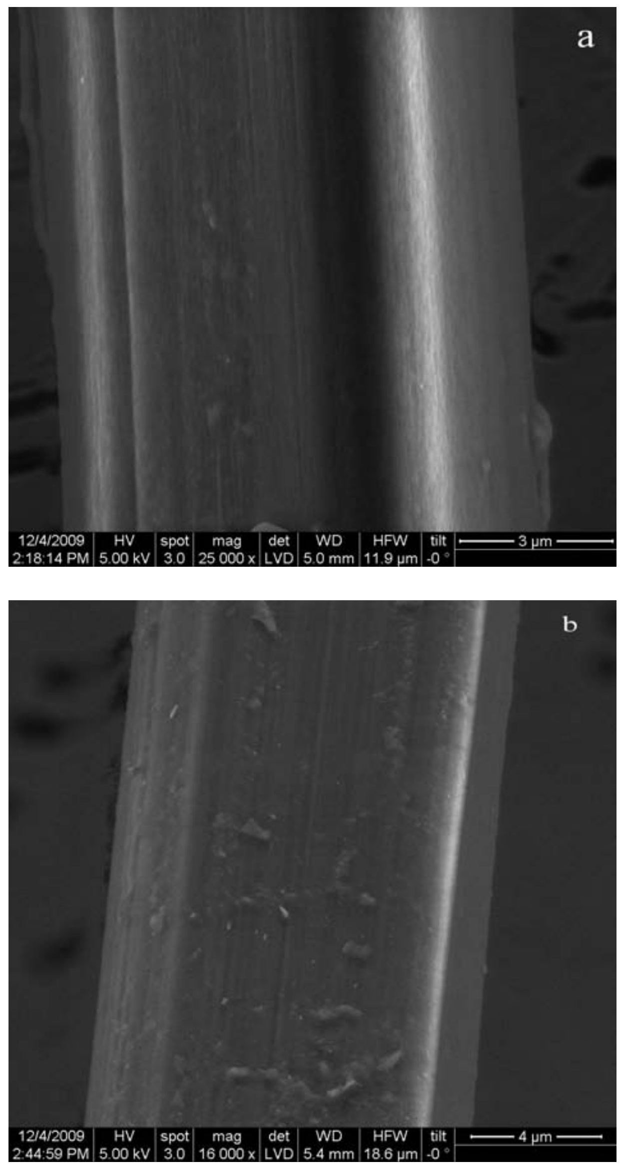

Fig. 4 — SEM image of (a) untreated and (b) treated silk filaments

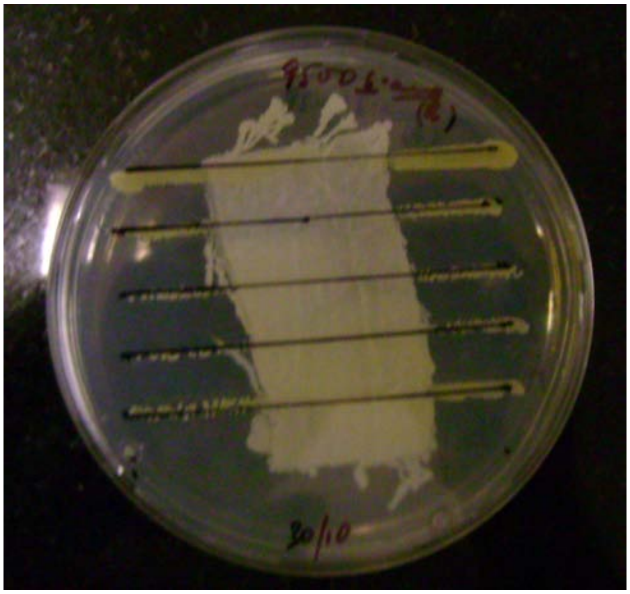

Fig. 5 — Antimicrobial test sample without zone of inhibition

temperature. This sample (treated) shows the whiteness index of 29.62. The change in colour in terms of ΔE (CMC) as compared to the control (untreated) was also evaluated. The ΔE (CMC) is found to be 0.51, which is well below the ΔE tolerance limit of 0.70.

From the SEM images of filaments removed from one of the silk yarns unraveled from the untreated and treated fabrics (Fig. 4), it can be seen that the AgNP are present on the treated filaments but not on the untreated filament.

This treated fabric was subjected to antibacterial activity assessment along with the untreated silk fabric as per the standard method (AATCC 147-2004). This method demonstrates bacteriostatic activity and provides evidence of antibacterial activity against both Gram positive and Gram negative bacteria, viz. *Staphylococcous aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). In the untreated sample, growth was observed, whereas it was not observed beneath the treated sample. Further, zone of inhibition was absent, which implies that the AgNP do not leach out of the fabric but effectively inhibit bacteria which come in contact with the fabric (Fig. 5).

The nanoparticles prepared in the present study are found to be as small as 15 nm and perhaps too small to impart any colour to the fibre and fabric significantly. However, being small, they are highly effective against the microbes as they have a high surface to volume ratio and it is the surface atoms that involve in reactions.

4 Conclusion

Sericin extracted by a simple procedure from the cocoons of *Bombyx mori* silkworms has been used effectively in the preparation of silver nanoparticles as a capping agent. Sericin effectively prevents coagulation of the AgNP and keeps them in a moderately stabilized condition in an aqueous medium. The spherical particles of average size 15nm are fairly monodispersed. Further, the sericin-capped silver nanoparticles when applied to silk fabric impart antimicrobial properties to it.

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