Biosynthesis of spherical gold nanoparticles and their characterization

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Abstract. Colloidal gold nanoparticles (AuNPs) were synthesized by the reduction of chloroauric acid (HAuCl₄·H₂O) using silk sericin (SS) extracted from Bombyx mori silk as a biotemplate. Synthesized AuNPs were characterized by using the various analytical techniques. UV-visible (UV-vis) study confirms the formation of AuNPs in aqueous SS solution showing the surface plasmon resonance (SPR) band at λ = 530 nm. The X-ray diffraction (XRD) study revealed the crystalline phase of gold nanoparticles with face centered cubic (FCC) structure. Transmission electron microscopy (TEM) images showed the formed AuNPs are spherical in shape with diameter around 8 nm. Dynamic light scattering (DLS) experiment also confirmed the formation of AuNPs in SS solution with average size 10 nm.

Keywords: Silk sericin, XRD, TEM, DLS.

1. Introduction
Now a day’s nanotechnology is considered as one of the most important and attractive field of the modern research. Nanoscience and nanotechnology focuses on preparation, manipulation of size of the particles in the range 1 – 100 nm and characterization. [1, 2]. Production of metal nanoparticles from bulk to nanometric scale mainly gold and silver attracted greater curiosity due to their unique properties and usefulness in many field like life science [3] and materials science [4]. Different useful methods are available in literature for the production of the metal nanoparticles. Mainly chemical and physical approaches are using world-wide. In most of the chemical and physical methods starting materials are toxic and hazards in nature [7]. Therefore, for the environmental sustain researchers have been adopting/using bio-based or green route methods for fabrication of metal nanoparticles [8]. In this perspective, it is vital to mention that production of gold nanoparticles using biological systems makes nanoparticles more biocompatible and ecologically friendly [9, 10]. In the current work, we have considered Bombyx mori SS as a reducing and subsequently stabilizing agent for the production polymer consists of 18 amino acids especially rich in Serine (32%), Aspartic acid (19%) residues [11].
Source of SS is *Bombyx mori* silk and it is biodegradable, biocompatible, environmentally friendly and safe material. The synthesized gold nanoparticles were characterized by using UV-Vis, XRD, TEM and DLS experiments.

2. Experimental

2.1. Materials
Silk sericin powder and HAuCl₄·H₂O (>99%) were obtained from Sigma Aldrich and used in the work as such without auxiliary purification.

2.2. Preparation of aqueous silk sericin solution
The SS solution is prepared by dissolving known amount of sericin powder in double distilled water with constant stirring about 15 min. Finally, we achieved pure solution and was centrifuged at 3800 rpm for about 20 min to elimination of insignificant amount of silk aggregates may be present in the process. Then, the transparent solution of SS was stored at 4 °C for additional use. The initial SS concentration was about 5 wt% and it was diluted to 1 wt% by totalling deionised water.

2.3. Preparation of colloidal SS-AuNPs solution
Powder of HAuCl₄ was added into 10 mL of 1 wt% SS solution and then we got a transparent SS-HAuCl₄ mixture. The SS-HAuCl₄ mixture solution was irradiated with UV-B light for about 5 h. The colour of mixture sample changed colourless to pink. This shows that formation of gold nanoparticles in the prepared solution. The formed gold nanoparticles were established by using different characterization.

3. Characterization of SS-AuNPs

3.1. UV-Visible spectra
The absorption spectra of silk sericin and silk sericin-gold nanoparticle were recorded using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) instrument in the wavelength range 190-800 nm.

3.2. X-ray diffraction measurement study
The crystalline nature of SS-AuNPs sample was carried out on Rigaku Miniflex-II, X-ray diffractometer with Ni filtered, Cukα radiation of wavelength λ = 1.5406 Å.

3.3. Transmission electron microscope
The evolution of the nanoparticles were probed using high-resolution transmission electron microscope (HR-TEM). The study was carried out using typical JOEL-JEM 2100 instrument.

3.4. Dynamic light scattering experiment
The average size and distribution of nanoparticles was determined using the dynamic light scattering (DLS) experiment. The study was carried out using the TRI-BLUE particle size analyzer (Microtrac).

4. Results and discussion

4.1. UV-Visible spectroscopy
The gold nanoparticles formation in the course of reduction of Au³⁺ ions into Au⁰ atom was visually confirmed by the variation in colour of the reaction mixture from pale pink to dark pink as presented in figure 1.
The pure SS is solution is basically colourless, the presence of AuNPs in SS solution provides a vibrant colour to it. This is because of the collective oscillation of the electrons in the conduction band of the gold nanoparticles as a consequence of localized surface plasmon resonance with the electromagnetic radiation of incident light. The UV-vis absorption spectra (figure 2) of SS-AuNPs showed a strong single peak at $\lambda = 530$ nm approves the formation of nanoparticles in SS solution [12]. This evidenced the components of SS is able to reduce $\text{Au}^{3+}$ ions into $\text{Au}^{0}$ atoms and also acted as stabilizing agent.

![Figure 1](image1.png)

**Figure 1.** Photograph of pure SS and SS-AuNPs.

![Figure 2](image2.png)

**Figure 2.** UV-Visible absorption spectra of SS and AuNPs.

4.2. *X-ray diffraction measurement study*

The crystalline aspects of the formed AuNPs were probed using XRD. The XRD pattern of the SS-AuNPs composite film obtained from SS-AuNPs colloidal solution (2.0 mM $\text{HAuCl}_4\cdot\text{H}_2\text{O}$ in SS) and is shown in figure 3. From the XRD spectra broad peak observed at $2\theta = 22.37^\circ$ reveals the crystalline domain present in the SS [13]. The most intense characteristic peaks detected at scattering angle $2\theta$ of about 38.5°, 44.0°, 64.33°, 77.35° corresponding lattice planes, which may be indexed to (111), (200), (220), (311) Bragg’s reflection of crystalline gold (JCPDS No. 04-0784) respectively [14]. Thus the XRD pattern illustrate that formed gold nanoparticles are crystalline nature with FCC structure. The calculation of average crystalline size of the SS-AuNPs was done by using Debye-Scherrer’s formula [15]. In the current work, the width of the most prominent peak at $2\theta = 38.5^\circ$, corresponding to (111)
Bragg’s reflection was used and size of the AuNPs was found to be 7.7 nm which is close to average particles size of 6.3 nm obtained from TEM analysis.

Figure 3. XRD spectra of SS-AuNPs.

4.3. Transmission electron microscope study
The surface morphology study with size of the formed gold nanoparticles were examined using the high resolution transmission electron microscope (HR-TEM). HR-TEM image of the AuNPs are given in figure 4 (a) which approves the produced AuNPs were spherical in nature with smooth edges. The diameter of the nanoparticles was found to be around 6.3 nm. This protein capped AuNPs were stable and there is no aggregation of particles [16]. The selected area of the electron diffraction (SAED) pattern of the AuNPs are shown in figure 4 (b). The SAED pattern shows the intense circular spots corresponding to (111), (200), (220), (311) planes of AuNPs respectively [17].

Figure 4. (a) TEM image of SS-AuNPs, (b) SAED pattern of SS-AuNPs.

4.4. Dynamic light scattering experiment
The dynamic light scattering (DLS) experiment was explored to study the average particles size and size distribution of the formed nanoparticles. The average size distribution of AuNPs in the SS solution was confirmed by figure 5 and the size distribution of the AuNPs in SS solution is about 7.6
nm. This is in fact good agreement with other results. The zeta potential measurement of the colloid SS-AuNPs showed about -29.8 mV indicates the stability of the formed AuNPs in the SS solution.

![Figure 5. DLS size distribution of synthesized SS-AuNPs.](image)

5. Conclusions
In the current work, we synthesized well dispersed, stable AuNPs using silk sericin as reducing and stabilizing agent. UV-visible study established the formation of gold nanoparticles in silk sericin solution by showing absorption peak at $\lambda = 530$ nm. Further, crystalline nature with FCC structure of the formed gold nanoparticles were confirmed by X-ray diffraction measurement study. From the TEM analysis, we conclude the formed AuNPs are spherical in morphology and well dispersed in the SS solution. From the DLS experiment hydrodynamic average size and zeta potential of the nanoparticles were examined.

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6. References
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