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Gamma radiation assisted biosynthesis of silver nanoparticles and their characterization

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ABSTRACT

Silver nanoparticles (AgNPs) were synthesized *in situ* under gamma radiation environment at room temperature using aqueous silk fibroin (SF) solution obtained from *Bombyx mori* silk. The formation of the Ag NPs was confirmed by its characteristic surface plasmon resonance (SPR) band at around 424 nm in UV-visible spectra. The strength of the photoluminescence (PL) spectra decreases with increasing dosage reveals the optimum dose required for the synthesis of silver nanoparticles. Dynamic light scattering (DLS) measurement indicated the dose dependent size of the Ag NPs formed in the solution. The transmission electron microscopy (TEM) images showed that the formed nanoparticles are roughly spherical in shape. Further the X-ray diffraction (XRD) analysis confirms the nanocrystalline phase of silver with FCC crystal structure. From this study, it was found that the increasing the radiation dose increases the rate of reduction and decreases the particle size. The size of the Ag NPs can be tuned by controlling the radiation dose. Copyright © 2015 VBRI Press.

Keywords: Silk fibroin; gamma radiation; silver nanoparticles; UV-visible; XRD; TEM.

Introduction

In recent years noble metal nanoparticles synthesis has attracted much attention of many researchers. The development of clean synthetic procedures for the synthesis of metal nanoparticles have been the area of focused researches due to their interesting catalytic, optical, electrical, magnetic and particularly antimicrobial properties **[1]**. The metal nanoparticles, therefore, have potential uses in technological applications. Silver is a nontoxic inorganic material well known for possessing an inhibiting effect towards 650 types of microbe's growth **[2]**. Silver exhibits the highest electrical and thermal conductivities among all the metals **[3]**. A quite large number of methods are available for synthesis of silver nanoparticles for example, chemical reduction **[4]**, sol gel **[5]**, hydrothermal **[6]**, electrochemical synthesis **[7]**, thermal decomposition **[8]**, sonochemical **[9]**, photo reduction in reverse micelles **[10]**, bacterial synthesis **[11]** and microwave irradiation method **[12]**. All the above listed methods used for the synthesis of nanoparticles, involve the usage of environmentally toxic or biologically hazards chemicals as reducing agent. Therefore, for an environmental sustenance, the development of clean, eco-friendly, green route approach for synthesis of silver nanoparticles is very much needed. More recently many researchers were well documented/demonstrated the bio or green route (Irradiation with high energy radiations) synthesis of silver nanoparticles **[13-23]**. In the present work we introduced a simple, effective and environmentally friendly method for *in situ* preparation of silver nanoparticles using silk fibroin as a reducing agent at room temperature under gamma radiation environment. Since the silk fibroin extracted from *Bombyx mori* silk, a protein polymer, which is naturally abundant, nontoxic and biocompatible material, are introduced for AgNPs synthesis. The synthesized silver nanoparticles were characterized by UV-vis spectroscopy, PL, FT-IR, XRD, DLS and TEM.

Experimental

Preparation of silk fibroin (SF) solution

Pure Mysore silk (PMS) cocoons were cut into small pieces and then treated twice with boiling aqueous solution of 0.02 M Na_2CO_3, for 30 min to remove the glue like sericin protein and washed with distilled water, and allowed to dry in air at room temperature **[24-28]**. The degummed silk were dissolved in 9.3 M LiBr salt solution at 70 °C for 3~4 h, and then silk fibroin LiBr solution was dialyzed against deionized water for 72 h at room temperature using a dialysis cassette (MWCO:3500) in order to remove salt. Finally, the obtained optically clear solution was centrifuged at 4000 r/min for about 15 min to remove small

amount of silk aggregates formed during the process. Then, the clear solution of SF was stored at 4 °C for further use. The concentration of SF was about 5 wt % and was diluted to 1 wt % by adding deionized water.

Preparation of SF-AgNPs solution and gamma irradiation

Ten milligrams of $AgNO_3$ powders were added into 10 mL of 1 wt % SF solution (6 bottles) to form a transparent SF-$AgNO_3$ mixture solution. And then SF-$AgNO_3$ mixture solution was exposed to gamma radiation. The gamma irradiation of these SF-$AgNO_3$ samples was carried out on Co-60 irradiator (Energy 1.25 MeV) with a dose rate of approximately 7.065 kGy/h and source strength of 13.455 kCi. The samples were irradiated in the dose ranging from 0-80 kGy in a step of 10 kGy at CARRT centre, Mangalore University, India. The synthesized silver nanoparticles were confirmed by UV-vis absorption, photoluminescence, FT-IR, XRD and TEM analysis.

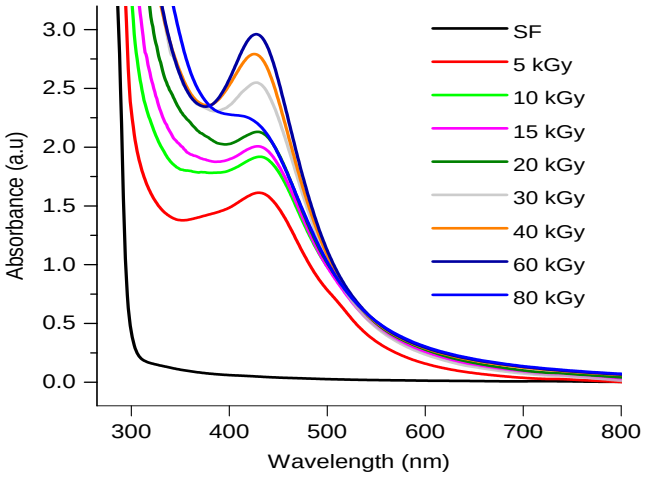

Fig. 1. UV-vis absorption spectra of silk fibroin and silk fibroin-silver nanoparticles.

UV-vis spectroscopy

UV-visible (UV-Vis) spectra were measured using a spectrophotometer (Shimadzu UV-1800) in the wavelength range 190-800 nm.

Photoluminescence (PL) study

The fluorescence spectra of the aqueous silk fibroin solution and SF-AgNPs samples were recorded using RF - 5301 fluorescence spectrophotometer equipped with a 150W Xenon lamp as the excitation source.

Fourier transform infrared spectra (FT-IR)

The FT-IR scans were used to identify the potential biomolecules in SF, which is responsible for reducing Ag^+ to Ag^0. FT-IR spectra of the SF and SF-AgNPs were measured by Perkin Elmer 2674 spectrometer having a resolution 4 cm^{-1} in the wavenumber range 500-4000 cm^{-1}.

X-ray diffraction (XRD)

Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Rigaku Miniflex-II) operating at a voltage of 40 kV and a current of 40 mA with CuKα radiation (λ = 1.5406 Å). The samples were scanned in the 2θ range 10-80° with a scanning speed and step size of 5°/min and 0.02°.

Transmission electron microscope (TEM)

The morphology of the silver nanoparticles was studied using transmission electron microscope (TEM), which was performed at 200 keV using JEOL JEM2010.

Dynamic light scattering (DLS)

The average particle size and size distribution of the silver nanoparticles formed in the silk fibroin solution was measured using the TRI-BLUE particle size analyzer, Microtrac, instrument.

Fig. 2. (a) Photograph of silk cocoons, and b) Silk fibroin solutions: A – unirradiated, B – 10 kGy, C - 20 kGy, D – 40 kGy, E – 60 kGy, F – 80 kGy.

Results and discussion

UV-vis study

UV-visible spectroscopy is one of the commonly used tools for the analysis of silver nanoparticles. The UV–visible absorption spectra of SF and SF-AgNPs samples are presented in **Fig. 1**. The recorded spectra of SF sample display absorption at λ=275 nm (not shown), which was attributed to the presence of the Tyr, Phe and Try residues in the silk fibroin (SF) chain **[29]**.

The formation of the silver nanoparticles in the aqueous RSF solution was evidenced with the change in colour. The initial SF-$AgNO_3$ solution was colourless, but after the exposure to the gamma radiation the colour turned to yellowish, then to the dark brown colour **(Fig. 2)**. Such a change was attributed to the size and shape dependent surface plasmon resonance (SPR) of AgNPs formed in the solution in the visible region **(Fig. 1)**. The surface Plasmon absorption band with a maximum of 424 nm indicates the presence of spherical or nearly spherical silver nanoparticles and this was also confirmed by TEM results **(Fig. 8)**.

Observation of the Plasmon peak is a well-known phenomenon in the case of various metal nanoparticles **[30]**. Metal nanoparticles strongly interact with the electromagnetic radiation and the interaction was discussed by several researchers **[31, 32]**. The incident light creates oscillations in the conduction electrons on the surface of the nanoparticles and the electromagnetic radiation is absorbed. From the **Fig. 1**, it is very clear that, as the gamma irradiation dose increases the corresponding peak intensity increases up to 60 kGy. The increase in intensity of UV absorption spectra indicates an increase in number of silver nanoparticles with increasing dose. At the highest dose (80 kGy) a decrease is observed in the intensity of the UV spectra may be due to the decrease in number of silver nanoparticles. It is generally accepted that the wavelength of the Plasmon absorption peak could be used to estimate the nanoparticle size and shape in the aqueous solution **[33]**. The estimated size is 35-40 nm and this value is in good agreement with the earlier reported research work **[34]**.

Photoluminescence study

Fluorescence study is considered the most effective method to study the silk fibroin as a reducing agent for the synthesis of silver nanoparticles **[35]**. Fluorescence spectra of the samples are presented in **Fig. 3**. Silk fibroin (SF) obtained from *Bombyx mori* silkworm, with the protein primarily composed of repetitive sequences of amino acid residues: glycine (42.9 mol %), alanine (30.0 mol %), serine (12.2 mol %) and tyrosine (4.8 mol %) **[36]**. Among these residues tyrosine (Tyr) is having electron donor property **[37]**.

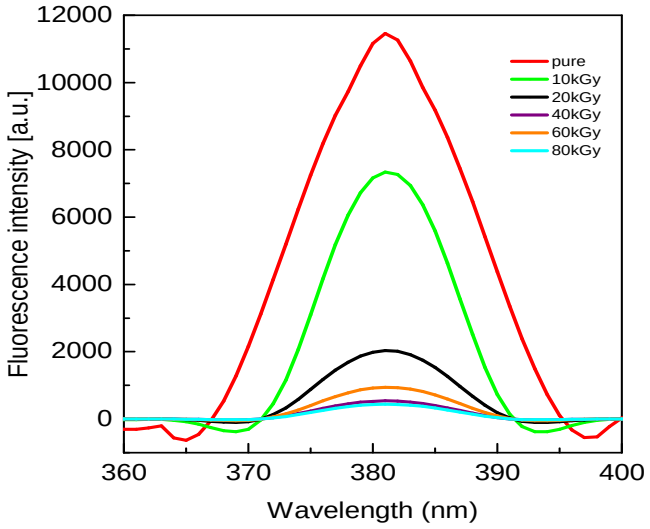

Fig. 3. Photoluminescence spectra of silk fibroin and silk fibroin-silver nitrate reaction systems with different irradiation doses (from 0 to 80 kGy) at room temperature (silk fibroin =1wt %).

In fact, tyrosine containing molecules have been proved as excellent reducing agents for synthesis of metal nanoparticles **[38, 39]**. In the present study, we have prepared 5 mg/mL, $AgNO_3$ solution, then exposed to gamma irradiation with different doses and checked the suitable dose for the reduction of $AgNO_3$. Fluorescence spectra **(Fig. 3)** reveal that the native SF shows maximum intensity and after irradiation it rapidly decreases. The highest intensity in native SF solution may be due to the tyrosine residues in the protein molecule. As the gamma irradiation dose increased the reduction in fluorescence intensity was observed. The loss of intensity indicates oxidation of Tyr in SF molecule and a possible scheme is shown in **Fig. 4 [40]**. **Fig. 3** indicates that the fluorescence intensity does not change when the irradiation dose is larger than 60 kGy. This means that almost all the Tyr residues in silk fibroin have been used. The loss of fluorescence from Tyr residues reflected the oxidation degree of the phenolic groups in them **[41]**. This was also supported by FT-IR study. The more the Ag^+ ions were reduced, the more the Tyr residues were oxidized, and less the fluorescence intensity was observed **[35]**.

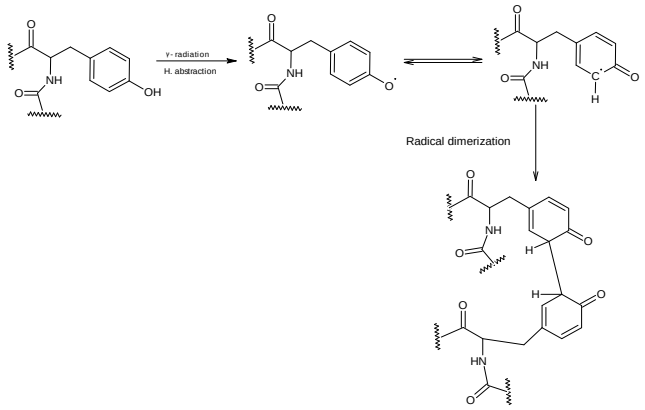

Fig. 4. Possible schematic representation of tyrosine oxidation mechanism.

Infrared spectra analysis

To further explore the *in situ* formation of silver nanoparticles FT-IR measurements were carried out to identify the biochemical radicles among biomolecules, which could be responsible for the reduction of Ag^+ to Ag^0. The FT-IR spectra of the SF, SF-AgNPs are given in **Fig. 5**. The native SF exhibited absorption bands at 1631 cm^{-1} (amide I), 1522 cm^{-1} (amide II) which corresponds with β-sheet conformation, and another band observed at 1236 cm^{-1} (amide III) was assigned to random coil conformation **[42-44]**. This shows that SF was simultaneously composed of β-sheet and random coil structure. The peaks observed at 1443 cm^{-1} and 1372 cm^{-1} could be assigned to symmetric stretching vibrations of carboxylate groups of amino acid residues with free carboxylate groups and methylic groups of alanine in the silk fibroin proteins. The peaks observed at 1623 cm^{-1} and 1160 cm^{-1} are due to the carbonyl stretching vibration from the carboxylate ions and the hydroxyl stretching vibration at 3286 cm^{-1} from the phenolic ions of Tyr molecule of native SF. Spectra B and C in **Fig. 5** show the carbonyl stretching peak at 1745 cm^{-1} which could be assigned to the formation of a quinone structure due to oxidation of the phenolic group in Tyr. The previous reports revealed that proteins can bind to metal nanoparticles through either free amine groups or cysteine residues in the proteins. In these circumstances, oxidized tyrosine molecules bind to Ag crystallites through amine groups so that Ag nanoparticles could be easily stabilized in polymer **[45]**.

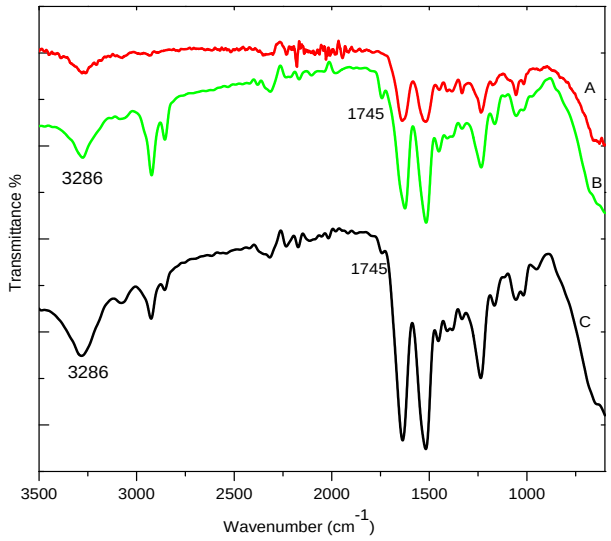

Fig. 5. FTIR scans (A) SF, (B) 10 kGy, and (C) 80 kGy irradiated samples.

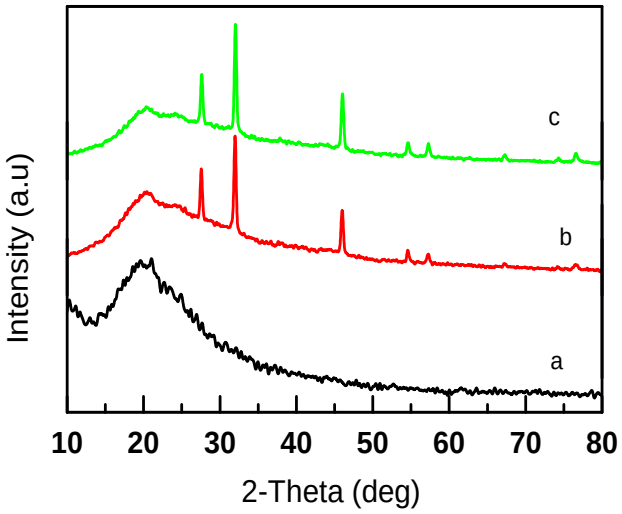

Fig. 6. XRD scans of (a) silk fibroin, and (b) and (c) SF-AgNPs.

X-ray diffraction study

The crystalline nature of the silver nanoparticles formed in this approach has been investigated with X-ray diffraction methods. The X-ray diffraction scans of SF and SF-AgNPs are presented in **Fig. 6**. From **Fig. 6(a)**, it can be seen that pure SF shows the broad peak at 2θ = 20.24°, confirming crystalline domains in silk **[46]**. Whereas the X-ray diffraction **(Fig. 6, (b)** and **(c))** spectrum of powder product obtained by drying the colloidal dispersion made from 1mg/mL SF solution irradiated with 60 kGy dose exhibits a number of peaks. The prominent diffraction peaks observed at 2θ = 27.28°, 32.02°, 46.35°, 54.53°, 57.45°, 66.32° and 76.34° **[47]** in the whole spectrum of 2θ value with the range between 10° to 80°. These peaks could be indexed as the face centred cubic (FCC) crystalline phase of the silver nanoparticles with the corresponding Bragg's reflections (111), (200), (220), (311), (222), (400) and (420) **[48, 49]**.

The average size of the crystallites was calculated from the Debye-Scherrer's equation **[50]** with the method based on the width of the diffraction patterns obtained in the X-ray reflected crystalline region: $L = (k\lambda)/(\beta\cos\theta)$, where k is Scherrer constant, λ is X-ray wavelength, β is the full width at half maximum (FWHM) of the measured reflection and θ the angle of diffraction. From the above equation the average size of the AgNPs synthesized by gamma assisted SF was 30.23 nm. This value is greater than the average particle size shown by the DLS study, which is 21.4 nm.

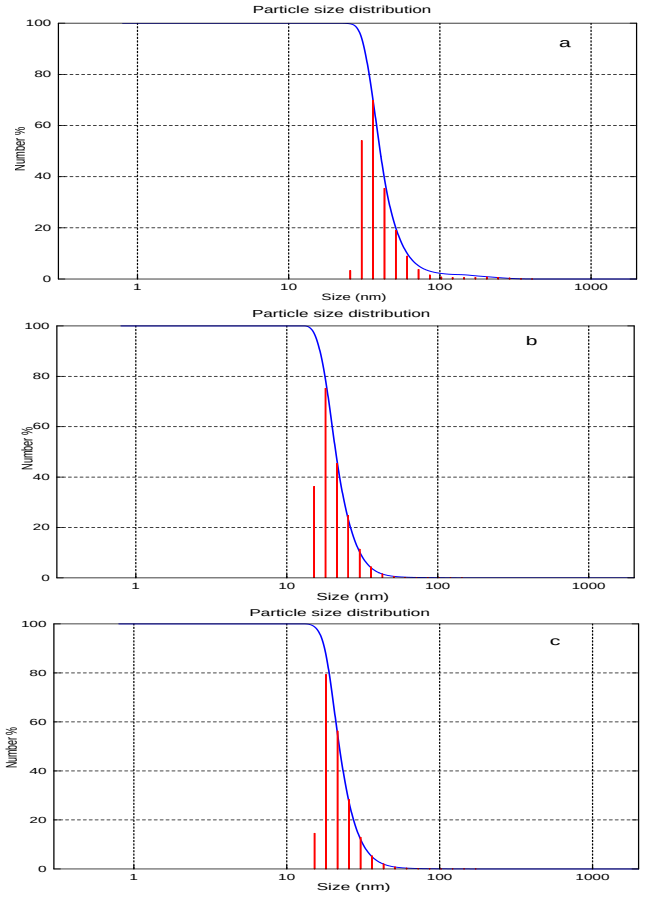

Fig. 7. DLS profile of size distribution of silver nanoparticles (a) 40 kGy (b) 60 kGy and (c) 80 kGy

Dynamic light scattering study

To examine the average size of the silver nanoparticles formed in the silk fibroin solution dynamic light scattering (DLS) experiment was performed. The DLS measurements of the selected samples are illustrated in **Fig. 7**. From **Fig. 7 (a)** it is observed that at lower doses (20 kGy) bigger particles (~40 nm) are formed. As the gamma irradiation dose increased the size of the particles decreased to ~20 nm at 60 kGy. Thus from this study it is evident that the average size of the nanoparticle decreased as the irradiation dose increased. The decreasing trend of the nanoparticle size can be explained as follows. It is well known that the ionizing radiations can cause structural changes in the biopolymer especially when there is a possibility of inter- and intra-molecular crosslinking within the SF molecules, so at higher doses, the polymer becomes a more complex matrix. Thus it prevents the aggregation of silver nanoparticles resulting in the formation of smaller nanoparticles **[51]**.

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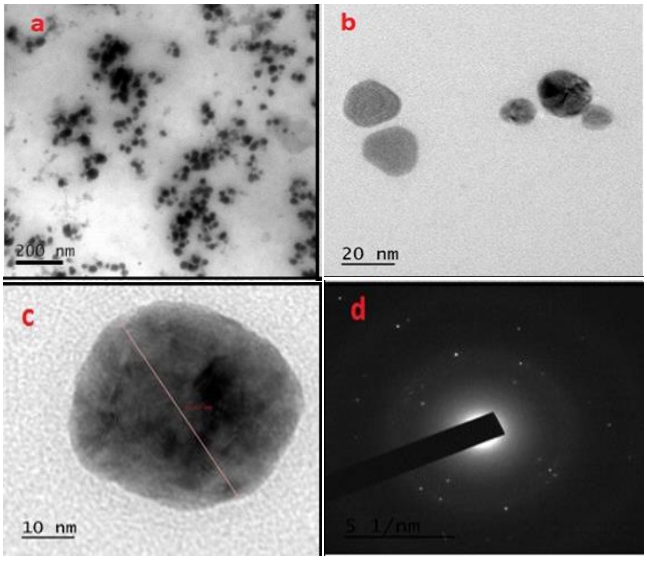

Fig. 8. (a) TEM images of AgNPs biosynthesized using the Silk fibroin, showing that particles are well dispersed (scale bar: 200 nm), (b) Typical high resolution TEM image of as biosynthesized AgNPs (scale bar: 20 nm), (c) High resolution TEM image of a single AgNP (scale bar: 10 nm) and (d) Typical selected area electron diffraction of AgNPs biosynthesized using Silk fibroin (scale bar: 5 1/nm).

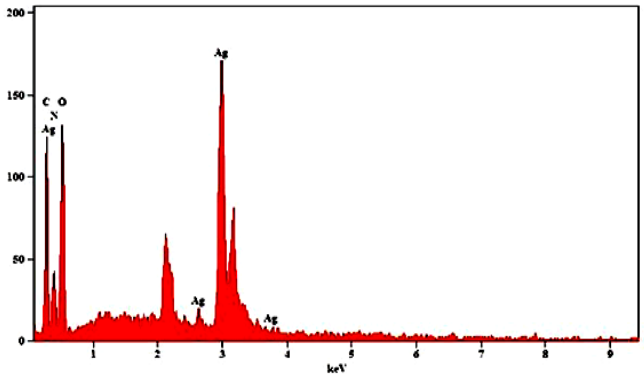

Fig. 9. SEM EDX spectra of AgNPs.

TEM analysis

Fig. 8 shows the TEM images of silver nanoparticles formed in the SF solution. Most of the silver nanoparticles were roughly spherical in shape with smooth edges. The structures were identical to those of the silver nanoparticles produced from the green route method **[52]**. The HRTEM images **(Fig 8b, c)** further illustrated the nature of silver nanoparticles. The overall morphology of the silver nanoparticles produced by reduction of Ag^{+} to Ag^{0} with 0.1mg/mL, 60 kGy irradiated sample is composed of almost uniform nanoparticles. The local elemental composition was confirmed as Ag by EDX SEM analysis and was given in **Fig. 9**.

Conclusion

In this study, silver nanoparticles were synthesized by an eco-friendly bio based green route method. *Bombyx mori* silk fibroin has acted as a reducing and stabilizing agent for the synthesis of silver nitrate into silver nanoparticles under gamma radiation environment. Synthesized silver nanoparticles were confirmed by colour change which was observed by UV-visible spectra at 424 nm. Photoluminescence analysis showed that, the intensity loss indicates the oxidation of tyrosine in silk fibroin. The synthesized silver nanoparticles were spherical in shape and their morphology was confirmed by TEM images. Further the X-ray diffraction (XRD) study reveals the nanocrystalline phase of silver with FCC crystal structure. From this investigation, it was found that the increasing the radiation dose increases the rate of reduction and decreases the particle size, and thus the size of the silver nanoparticles can be tuned by controlling the radiation dose.

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