Structural and Thermal Properties of Silver Nanoparticles Embedded on Silk Fibroin Films

A Project Thesis Submitted to the Faculty of Science

Bengaluru City University

For the partial fulfilment of the degree of

MASTER OF SCIENCE

IN

PHYSICS

By

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September 2021

Bengaluru City University

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CERTIFICATE FROM THE HEAD OF THE DEPARTMENT

This is to certify that the project entitled **"Structural and Thermal Properties of Electrical Properties Silver Nanoparticles Embedded on Silk Fibroin films"** is carried out by **MANASA J (PH190205), POORNIMA K (PH190206), PRAMOD S D (PH190207) and RENUKA N ELIJER (PH190208)** students of M.Sc. in Physics for the Partial fulfilment of the degree of ***Master of Science in Physics***, prescribed by the Bengaluru City University during the academic year 2020-21.

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CERTIFICATE FROM THE PROJECT GUIDE

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CERTIFICATE FROM THE HEAD OF THE INSTITUTION

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Principal

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Place:

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DECLARATION BY CANDIDATES

We hereby declare that, the matter embodied in the project report entitled **"Structural and Thermal Properties of Electrical Properties Silver Nanoparticles Embedded on Silk Fibroin films"** is carried out by us under the guidance of Dr. Shivananda C S , Assistant Professor & PG Co-ordinator, Post Graduate Department of Physics, KLE Society's S Nijalingappa College Bengaluru. We are submitting this project report for the partial fulfilment of the requirements for the award of **Master of Science** degree in Physics by the **Bengaluru City University** during the year 2020-2021. We further declare that this dissertation or any part of it has not been submitted elsewhere, for any other degree or diploma to any other University or Institute.

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Contents

Preface

The Bionanocomposites have been extensively studied during the last decade. The main interest in these composites is to tailor the structural and thermal properties. The silk fibroin is functional biomaterial the incorporation of silver nanoparticles on silk fibroin matrix which changes the structural changes and enhancing the thermal stability of the composite materials. The prepared composite materials promising for thermo electric switching devices and implantable biomedical device application.

In this project work, the structural and thermal properties of silk fibroin - silver nanoparticles composite films have been investigated.

This project thesis contains four chapters:

Chapter 1:

This chapter is general introduction to the silk biomaterial, with reference to the *Bombyxmori* silk fibers.

Chapter 2:

The methods of preparation and characterization of silk fibroin - silver nanoparticles have been discussed. Further, the details of the analytical techniques undertaken such as XRD and Thermo gravimetric analysis have been described.

Chapter 3:

In this chapter, the XRD studies and Thermal studies have been discussed.

Chapter 4:

The significant results obtained in the present project work have been summarized in this chapter

References:

The references are listed at the end of the project thesis

Chapter 1
Introduction and Literature Review

1.1. General Introduction of Silk

Silk is a fibrous protein-based biomaterial derived from many insects and arachnids. The *Bombyxmori* silkworm is class of lepidopteron; it is an important productive insect that is emerging as a molecular genetics resource it resolves wide range of distinctive biological problems. Traditionally, it has been associated with man lifestyle, particular occasions like marriages. Hence, recent days it is named as 'queen of textiles' because of its special properties like smooth, soft texture, thin and non-slippery compared to other synthetic fibers. Generally, massive silk proteins are produced from the *Bombyxmori* silkworm it is having specialized silk gland cells, the biosynthesis process takes place within the epithelial cells. These silk proteins stored middle of the silk glands and expelled anterior duct and spinning through the spinneret opening, afterwards it can be converted into fibers [1]. The silk cocoons were fabricated by the *Bombyxmori* silkworm, it is a constituent of protein material such as collagen, elastin, keratin, fibroin and sponging [2].

1.2. *Bombyxmori* Silk Fibers

The *Bombyxmori* silk fiber is one of the most important useful protein polymer and it is environmentally preferable fiber because it consists of huge amount of amino acid groups. Silk fibers naturally expelled from *Bombyxmori* silkworm composed of both sericin and fibroin filaments held together during ejection. The silk fiber shows the core-shell structural fibroin protein material having a triangular cross-section with curved shape, typical diameter of 5-10 μm (Figure 1) [3]. The sericin protein material encapsulated over the fibroin protein and it acts like binder. The diameter of the silk fibers varies depending upon the type of species. In comparison with other natural and synthetic fibers, silk fiber occupies leading position for its characteristic properties known as chemical composition, elasticity, flexibility, heat conductivity, biodegradability, water absorption, luster, handling, washability, brilliant colour shades obtained by dyeing and also an excellent mechanical properties which gives hardness and strength [4]. Silk is commonly named as textile fiber because of its excellent mechanical properties and it exhibits tensile modulus on the order of 5Gpa and strength of 400MPa.

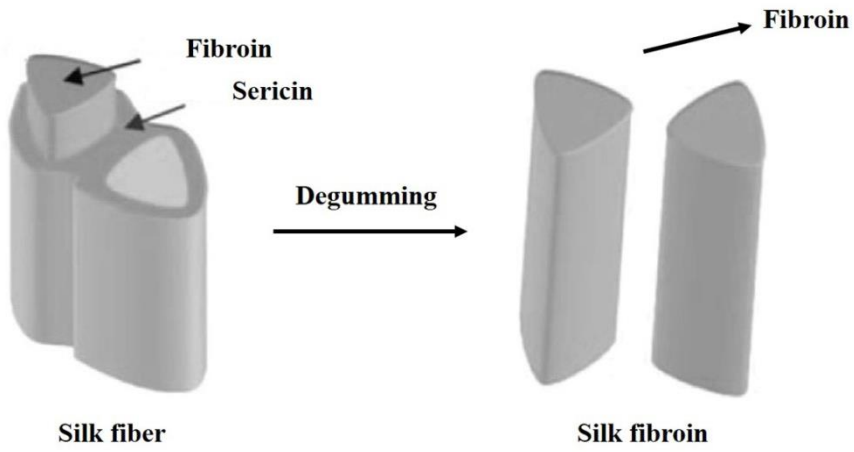

Figure 1.5. Cross-sectional view of core-shell structure of the silk fibre.

1.3. Silk Fibroin

Fibroin is a major component of the silk fiber which is in the form of thread like protein material. After removal of sericin protein from the fibroin surface it looks shining feel soft to touch. It consists of heavy (~ 390 kDa) and a light (~26 kDa) polypeptide chain present in a 1:1 ratio and it is covalently linked by a single disulphide bridge at the amino-terminus and carboxyl-terminus of the two subunits [5]. The micellar unit is formed due to the bonding between the H-L complex and a single glycoprotein P25 in the ratio of 6:1 through hydrophobic interactions. This micellar unit helps transportation of huge amount of fibroin through silk glands [6]. The silk fibroin is composed of discrete β-sheet crystal domain and amorphous domain. The H-chain of β-sheet crystal domain plays a major role in the mechanical properties of the silk fibroin, whereas the L-chain plays minor role because of its smaller size compared to H-chain. The β-sheet crystal domain are more ordered hydrophobic blocks with repetitive amino acid sequence are linked with amorphous domain, these are less ordered hydrophilic non-repetitive sequence carry moisture content and its plays a negligible role on the silk fibroin. The crystal domain is a repetitive sequence mainly composed of amino acids usually made by polypeptide chains. The silk fibroin consisting of amino acid residues known as glycine (~43-46%), alanine (~25-30%) and serine (~12%) in the ratio of 3:2:1, whereas tyrosine is a polar side chain found to ~5%. The remaining residues are valine (~2%) and it is followed by aspartic acid, phenylalanine, glutamic acid, threonine, isoleucine, leucine, arginine, lysine and histidine found to be small percentages [7]. The amino acid residues known as glycine, alanine, serine are higher proportion, whereas tyrosine, valine, threonine are found to lesser proportion combine together to form crystalline domain through which intra or intermolecular force of attraction including hydrogen bonds, van der Waals

forces, and hydrophobic interactions which helps to formation of highly ordered crystalline domain in silk fibroin. In spite of, the unrepeated sections of acidic amino acid residues known as glutamic acid, aspartic acid, arginine and lysine, which are not found in the crystallite domain but which helps to formation of semi-amorphous region in silk fibroin [8]. The H-chain of the silk fibroin was arranged in a regular pattern, it consists of 12 hydrophobic domains linked with 11 hydrophilic domains as shown in Figure 1.6.

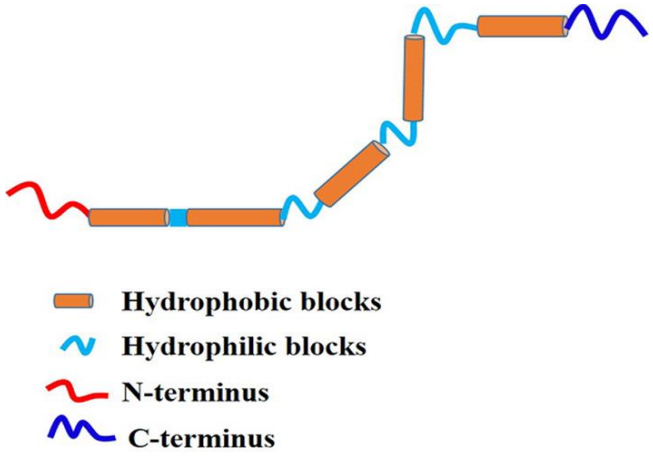

Figure 1.6. Hydrophilic and hydrophobic sequence of silk fibroin.

The main structure of the silk fibroin was dominated by repetitive sequence of [Gly-Ala-Gly-Ala-Gly-Ser]$_n$ with corresponding to the side groups known as H, CH_3, H, CH_3, H, CH_2OH as shown in figure [9]. The repetitive amino acid sequence which is responsible for the formation of antiparallel β-sheet conformation named as silk II structure and crystalline form named as silk I structure have been identified for silk fibroin [10].

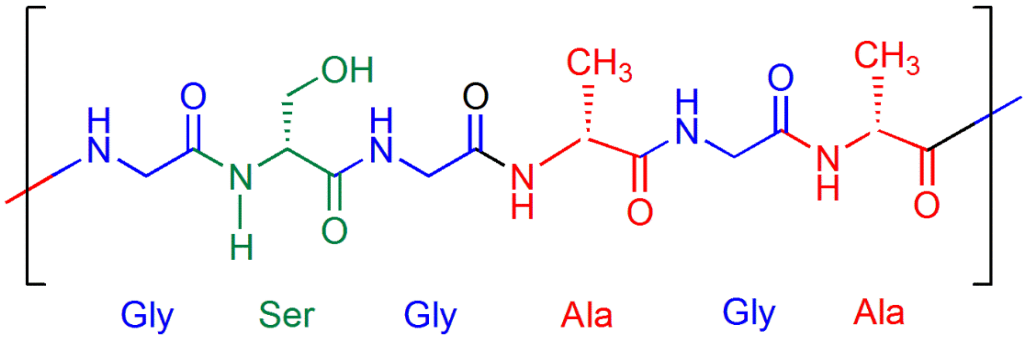

Figure 1.7. Primary structure of amino acid sequence of silk fibroin.

The main contribution of silk fibroin in the bioengineering field is mainly due to the characteristic features such as the protein arrangement, molecular weight, the ratio of hydrophilic and hydrophobic blocks [11]. These characteristic properties may cause the silk-cell and silk-drug interaction. In the biomedical field silk plays major role in the wound

dressing, surgical sutures, substrate for cell culture, drug delivery, bone regeneration because it is less toxic, non-immunogenic response, easy of sterilization, surface energy, chemical modification, non-irritable, biocompatibility and biodegradability. It can be easily fabricated into different forms, such as fibers, films, gels, powders, membranes, and three-dimensional scaffolds [12-15]. The main benefits of silk fibroin in the field of textile industry due to characteristic physical properties such as, biocompatible, biodegradable and high tensile strength, elastic modulus, stiffness, elongation to break and toughness. The earlier research reported the make of silk fibroin biomaterial as a microfluidic device in the field of drug delivery and tissue engineering [16]. The silk fibroin thin, very soft to touch, light weight, it is having well water absorbing capacity, dyeing affinity, thermos tolerance, insulation and enamelling properties.

1.4. Sericin

Sericin is a another type of silk protein material in sticky in nature coating outer layer of the fibroin filament with the molecular range ~20 to 310 kDa and it helps to cocoons formation [17]. The sericin contributes the 20 to 30% of cocoons formation of its overall weight. Sericin is an amorphous in nature which acts as an adhesive binder encapsulated over the fibroin [18]. It is a macromolecular protein which is insoluble in normal water but it is easily dissolved in hot water by breakdown of group of glycoproteins chain into smaller fractions. The sericin protein material is used in medical and pharmaceutical application due to remarkable properties i.e., resistant to UV light, resists oxidation, antibacterial properties, wound healing properties[19], cyto-compatibility [20], blood glucose, lowering the cholesterol and it easily absorbs and release moisture content [21, 22]. In order to remove the sericin component from the fibroin surface it becomes silk lustrous, soft, shining during silk fibroin extraction process. The removal of sericin gumming material considered as anunusable products. But in recent days, seri -waste products and their by-products are used in the value added products. Sericin is also used in cosmetic applications including sunscreen lotion, cream, shampoo, ointment and nail polishes [23].

1.5. General Introduction of Nanotechnology

Nanotechnology is an important and promising subject in the field of applied science, concerned with the synthesis, characterization, and determination of structures with dimensions 1-100 nm. In 1959 a famous physicist Richard Feynman gave a statement “There is a plenty of rooms at the bottom’’ at an annual meeting of the american physical society, is widely accepted and spark that initiated the nanotechnology. The nanotechnology can be difficult to determine and define, as its definition is often engineered to suit the researcher

and their field; this resulted in a need of a general working definition. In fact committee was established National Nanotechnology Initiative (NNI) and it conducts number of workshops and seminars to define the importance in the nanotechnology and estimate the following features [24].

- It is mainly dedicated to materials having dimensions in the order of 1 to 100 nm.
- It is ability to control or manipulate at nanoscale or molecular scale.
- Due small size it exhibits novel properties and different structures.

The first two aspects are easily understood and the third aspect are excited term "novel properties'' when we go from macro to nanoscale the interactions and physics between the atoms shows interesting properties because of the venture into the world of quantum mechanics rules. In the macro level the bulk materials emerges from the collective behaviour of trillionth of atoms and provides the nano world (Figure 1). Recent days a revolution in material science and engineering taking place a wide variety of research activities has been done around the world to characterize the materials at nanoscale. The word 'nano' has been widely used for the development of new materials with the outstanding optical, electrical, magnetic and mechanical properties for the use in information technology, bioengineering, energy and environmental applications.

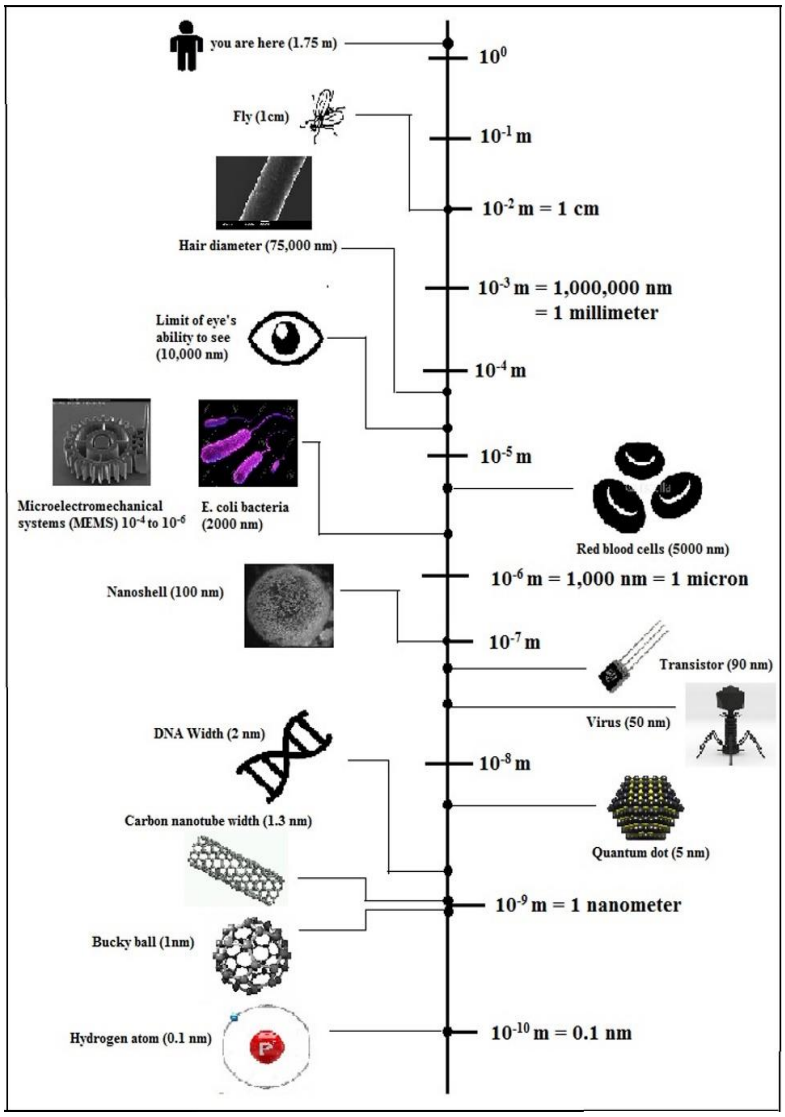

Figure 1.1. Schematic representation of size comparison scale - Macro world to nano world [25].

1.6. Importance of being nanomaterials:

Generally, the nanoscale materials with the size between the range of 1 and 100 nm. Nanomaterial structures is different from those of atoms and the bulk materials. Most of the microstructured materials behaves similar properties to the corresponding bulk materials, the material properties with nano dimensions are exhibits significant properties. In nanoscale the materials behaviour is different from those of atoms of bulk materials because of the two reasons. Firstly, the smaller particles which shows the larger surface area compared to the bulk material, due to the larger surface area materials becomes highly reactive. Materials in the larger scale it becomes inactive, whereas in the nanodimenstion it becomes highly reactive. Secondly, compared to the bulk materials nanomaterials exhibit larger surface area

to volume ratio, high surface energy, special confinement, less imperfections. Therefore the properties of the nanomaterials becomes size dependent [26]. The metallic nanoparticles which exhibit different physiochemical properties compared to bulk metals. Various research area physics, chemistry, material science and mechanical engineers are involved in the study of physical properties of metallic nanoparticles, such as linear and non-linear optical spectra, high specific surface area, mechanical strengths, and temperature dependent study of resistivity, spin resonance spectra, specific magnetization. The electronic state (energy levels) are continuous in case of bulk material except for semiconductors, whereas in nanoparticles the electronic state may be discrete because it depends on the particles size and material. These properties prove attractive in various industrial applications [27]. In day to day life the usage of nano-enabled products which plays major role for us. The nanoparticles are existed throughout the world and it can be synthesized some microorganisms and some of engineered nanoparticles which are produced by manmade. In reality, the nanomaterial based ingredients, additives and food contact materials are well established and it is expected to rapid growth in the near future [28]. Some research experts believe that the nanotechnology is rarely used in the foods, but it is very difficult to give a detail information about the presence of nanoparticles in food [29]. In some countries, nanomaterials are already used in alimentary supplements and food packaging applications.

1.7. Silver nanoparticles

Silver nanoparticles are important inorganic nanoparticles, it has been used since from ancient days with the particle size between 1 to 100 nm. It is frequently described as “silver” it is mainly composed of large amount of silver oxide due to their large surface to volume ratio to bulk silver atoms [30]. Similar to the gold nanoparticles, silver ions has a long history background which was first used by roman glassmakers and also used in jewelry, utensils, monetary currency, dental alloy, photography, explosives etc[31].The basic investigation carried out in the 1980s and1990s showed that colloidal silver nanoparticles exhibit a peculiar properties known as characteristic SPR, high thermal conductivity, electrical conductivity, SERS, chemical stability, nonlinear optical behaviour well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc[32]. Due to these valuable properties it is served as a materials in the manufacturing of new generation applications of electronic, optical and bio-sensor devices, imaging and medical purpose [33, 34]. In the present time, several researchers put an effort to incorporate silver nanoparticles into a wide range of medical equipment’s including, bone cement, surgical suture material, needles and surgical

masks etc [35]. Silver nanoparticles loaded on silk fibroin as an effective agent for enhanced wound healing properties [36]. Moreover, these nanoparticles have their attractive physiochemical properties and biological activities compared with the ordinary metal so that it received a considerable attention in the field of biomedical imaging using SERS. Due to the SPR of colloidal silver nanoparticles and large effective scattering cross-section of individual silver nanoparticles are used in molecular labeling [37].The size and shape dependent properties of silver nanoparticles exhibits greater impact on the bacteria and viruses. According to Elechiguerra et al. 2005reported that silver nanoparticles experience a size and shape-dependent interaction with HIV-1, with size range of 1-10 nm attached to the virus and inhibit its ability to bind host cells *in vitro* [38]. Silver nanoparticles exhibits wide range of antibacterial activity against microbes such as Gram positive and Gram negative bacteria. Hence, it is extensively used in the commercial products used in our daily life such as soaps, pastes, metals, textiles and antimicrobial food packaging materials etc [39]. The different shapes (spherical, rod-shaped, truncated triangular nanoplates) of silver nanoparticles were exhibit potential antibacterial activity. The earlier researchers reported the antibacterial activity of the silver nanoparticles with gram negative bacteria is mainly depends on their size and shapes [40]. In *vitro* studies of silver nanoparticles exhibits toxicity of various cells with the size ranging from 1 to 100 nm. The well distribution of silver nanoparticles exhibits antibacterial activities and subsequently causes toxicity. The size, surface area and oxidation states are identified as an important properties for the toxicity of the silver nanoparticles [41, 42].Previous research work reported the concentration variation of cytotoxicity of silver nanoparticles with the size range of 1 to 2.5 nm on peripheral blood mononuclear cells (PBMCs). Kim et al investigated the in *vivo* genotoxicity of silver nanoparticles with the size of 60 nm and different dose using micronucleated assay of bone marrow of the mice [43, 44]. The detailed investigation of toxicity effect of silver nanoparticles on human T lymphocytes and Jukart T cells using oxidative stress-related endpoint [45].

1.8. Literature Survey

A detailed literature review has been carried out in the field of biosynthesis of silver nanoparticles using *Bombyxmori* silk fibroin as as biotemplate. Number of research articles reported for the bio or green synthesis of silver nanoparticles. But only countable studies published related to the synthesis of silver nanoparticles using silk fibroin. We made an attempt to collect all the reported information for the *in situ* synthesis of silver nanoparticles using silk fibroin. The silver nanoparticles embedded on the different silk matrix such as

aqueous silk fibroin solution, fibroin fibers, scaffolds, hydrogel, films also their application in the field of biomedical and textile industry has been reviewed and reported as follows.

Zhou et al (2001) studied the core-shell nanostructure of silk-gold colloid *in situ* under redox reaction at room temperature. The presence of tyrosine molecule in silk fibroin exhibits strong electron donating properties for the bio-reduction of Au^{+3} ions to Au colloids. The thiol or amine groups are responsible for the stabilization of gold colloid in SF solution and formation of manodispersed nanoparticles. The SF molecules coating over the gold colloids which shows the spherical core-shell nanostructure with the average particle size of ~ 45 nm and size of the gold nanoparticles was found to 15 nm which is confirmed by TEM images. The detailed investigation of the composites are were not discussed but only he reported the formation of SF-AuNPs colloidal suspension in his work [46].

Dong Q et al (2005) reported *in situ* bio-reduction floriated nanoclusters on the surface of silk fibroin fibers at room temperature, the obtained nanoclusters are polycrystalline grains with the mean diameter of 5 nm. The surface morphology of the floriated structure of nanoparticles was associated with the concentration of silver nitrate as well as the primary and secondary structure of the silk fibroin macromolecule. The silk proteins attached to the surface of the silver nanoparticles through which presence of cysteine residues in the protein chain [47].

Dong Q et al (2008) published the bio-inspired method for the development of well-ordered hybrid bionanocomposites. In this approach *in situ* reduction of silver nanoparticles on the surface of degummed silk fibroin fibers, the presence of silk fibroin constituents acts as a reducing and stabilizing agents. The synthesized nanoparticles shows floriated structure of polycrystalline grains with the diameter of 10-15 nm. The XRD patterns reveals that the silk fibroin exhibits peaks at 20.64^0 which is attributed to the presence of protein chains consisting lot of amines, amides and carboxylic groups. The formed floriated silver nanoclusters shows face centre cubic structure corresponding to (111), (200), (220) and (311) planes. The FESEM images shows the cross section view, protein arrays of the silk fibroin surface and also number silver nanocrystals assembled to form a floriated structure with the diameter of 30-50 nm [48].

Chang et al (2009) reported that synthesis of AgNPs on the surface of silk fibers under gamma radiation, the synthesized AgNPs are well dispersed with the particles size was found to be < 20 nm. The XRD patterns reveals that the formed nanoparticles are in FCC structure, the antibacterial activity was carried out against gram positive bacteria *Staphylococcus aureus*. The synthesized AgNPs exhibits enhanced antibacterial activity with elevation of

gamma dose. He also reported that the Ag loaded SF fibers shows excellent washing stability [49].

Bhat et al (2011) reported that the sericin gumming material used as a capping agent for the synthesis of silver nanoparticles at room temperature. The micellae structure in sericin exhibits negative charge, which helps to formation of stable colloidal silver nanoparticles without agglomeration. The average particle size was found to be 15 nm with uniform shape which is confirmed by TEM and SEM results. The synthesized silver nanoparticles decorated on the surface of the silk fabric by exhaust method. He also reported silver coated silk fabric exhibits strong antibacterial activity against *S. aureus* and *K. pneumoniae* [50].

H. Su et al (2011) reported the formation of silver chloride nanocrystals on the silk fibroin surface under room temperature. The degummed fibroin fiber were immersed in the silver nitrate and sodium chloride mixture solution. The surface of the silk fibroin acts as a negatively charged due to the presence of amino acids residues under alkaline condition, which gives locations to immobilize silver ions and finally form silver chloride seeds. Further growth of silver chloride nanocrystals with the size of 100 nm which was confirmed by using FESEM [51].

Chapter 2

Experimental Details: Theory and Specifications

2.1. Materials and methods

Na_2CO_3, LiBr and $AgNO_3$ (>99%) were procured from Merck. CSR4 – (*Bombyx mori*), multivoltine cocoons were obtained from the Central Sericulture Training Research Institute (CSTRI), Bengaluru, India.

2.2. Preparation of Silk Fibroin Solution

The silk fibroin preparation process has been reported in our earlier work [21]. Briefly silkcocoons were cut into small pieces and then boiling in an aqueous solution of 0.02M Na_2CO_3 to remove the sericin protein material coating over the fibroin. The bundle of degummed SF was washed thoroughly with deionized water to remove any remaining sericin, impurities and surfactants, then gently dried in normal atmosphere for overnight. In order to prepare the pure silk fibroin solution the dried fiber was dissolved in 9.3M of LiBr salt solution and kept in a hot air oven at 60°C for 4 hours. Then the dissolved SF-LiBr salt solution was dialysed against distilled water multiple times to remove the LiBr ions. Finally obtained clear solution of SF was centrifuged to remove the small amount of undissolved silk aggregates formed during the process, then the obtained silk fibroin solution was stored at 4 °C for further use.

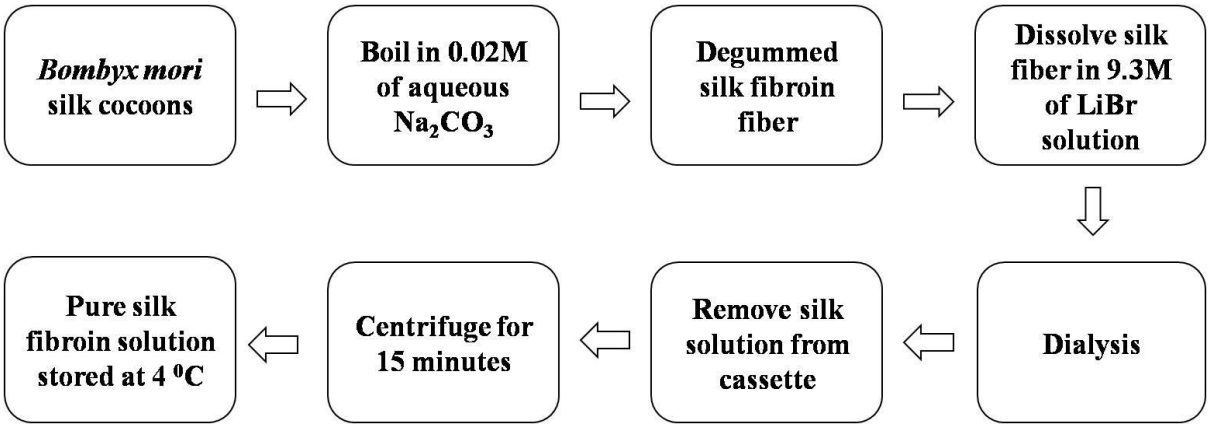

Figure 2.1. Schematic Representation of Preparation of Pure Silk Fibroin Solution

2.3. Preparation of SF-AgNPs BNCs films

Different weight (%) of $AgNO_3$ salts were added into 10 mL of 1 wt% SF solution to form a transparent SF-AgNPs mixture solution. The SF-$AgNO_3$ solution was exposed under

incandescent bulb (40 W, from Philips) for 24 h to produce SF-AgNPs colloidal solution. Preparation of SF-AgNPs composite films were reported briefly in our earlier work [21].

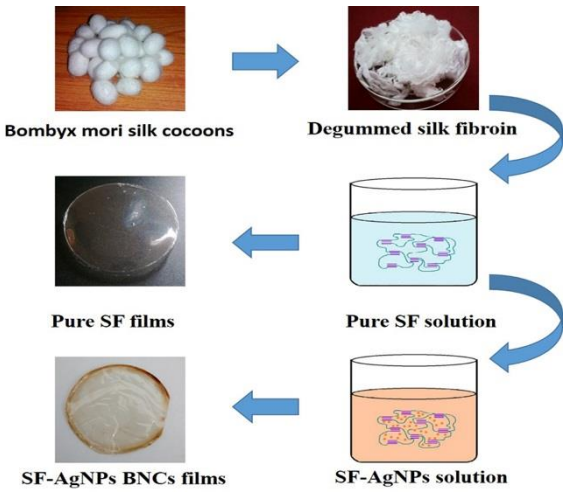

Figure 2.2. Schematic Representation of Preparation of Silk Fibroin – Silver Nanoparticles Composite Films

2.4. Characterization techniques

2.4.1. X-ray diffraction (XRD)

X-ray powder diffraction is a standard technique in many research field (mineralogist, Physicist and solid state chemists) that is capable of providing a wealth of information in areas such as phase identification of a crystalline substances, crystal phase determination, atomic spacing, particle size and strain measurements. When X-rays interacts with an atomic arrangement of the crystalline phase the diffraction pattern is observed. The peak position is obtained by a function of the radiation used. The peak intensities depends on the kinds of atoms and their arrangement in the unit cell. Therefore, the X-ray powder diffraction pattern of a phase may be used as a "fingerprint" to identify the phase. X-rays are generated when a highly focused electron beam accelerated across high voltage field bombards a stationary target material. These electron collide with atoms in the target and slows down, a continuous spectrum of X-rays are emitted. It works based on the principle of the interaction of the incident rays with the sample produces constructive interference pattern which satisfies the Bragg's Law i.e., $n\lambda=2d\sin\theta$. Where n is an integer, λ is wavelength of incident x-ray radiation, d is the interatomic spacing and θ is the diffraction angle between the incident beam and atomic planes. The average crystallite size of the silver nanoparticles was calculated from the Debye-Scherrer's formula [52].

$$L = \frac{K\lambda}{\beta cos\theta} \quad (1)$$

Where, K is Scherrer constant, λ is the X-rays wavelength (1.5406Å), β is the full width at half maximum (FWHM) value of the measured reflection and θ the angle of diffraction. The average lattice strain (ε_{av}) is the stain-induced due to the crystal disorder and lattice deformation was calculated by using the formula [53]:

$$\varepsilon_{av} = \frac{\beta}{4tan\theta} \quad (2)$$

Where, ε_{av} is the average lattice strain, β is the full width at half maximum and θ is the angle of tangent.

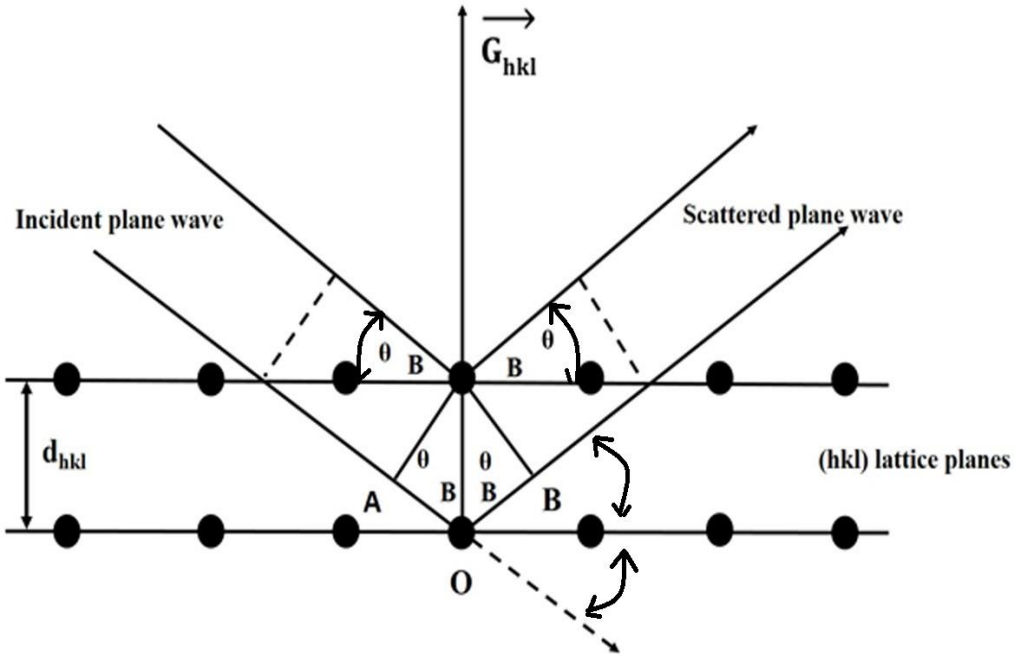

Figure 2.3. Schematic representation of incident and scattered X-ray plane waves, from the two interatomic planes. The relation by which diffraction occurs is known as Bragg's condition.

Instrument details

The X-ray diffractions of all the samples were examined by using Rigaku Miniflex-II X-ray diffractometer with an operating of voltage of 40 kV and a current of 40 mA with CuKα (λ = 1.5406 Å) radiation, Ni is used as a filter for K_β suppression, graphite used as a monochromator to obtain a single wavelength. The samples were scanned in the 2θ range 10°–80° with a scanning speed and step size of 5° min^{-1} and 0.02°, respectively.

Figure 2.7. Rigaku Miniflex-II X-ray diffractometer.

2.4.2. Thermogravimetric analysis (TGA):

The thermogravimetric analysis (TGA) is a method of measuring the amount and rate of change in the weight of a sample as a function of temperature or time in a precise atmosphere. The nitrogen gas atmosphere is needed at all time during the experiment. There is a change in temperature that can affect the sample. But not all thermal events bring a change in mass of the sample (i.e., melting, crystallization or glass transition) but some thermal events (i.e., desorption, absorption, sublimation, oxidation, decomposition, vaporization and reduction) these factors affect the drastic change in mass of the sample. TGA can be used to characterize the thermal stability and decomposition of the samples upto the temperature range of 1200º C. It can also determine the kinetics of the physiochemical process occurring within the sample. The different factors such as weight of the sample, volume, physical appearance, the shape, nature of the sample holder, nature of the pressure of the atmosphere in the sample chamber and scanning rate have most important influences on the nature of the TGA curve [54].The TGA of the prepared samples were characterized by using TA instrument SDT-Q600. Initially the selection of the pan type depends on the physical properties of sample, before loading the sample the pan should be cleaned and dried, then by taring the empty sample pan. The powder or film sample of weight around 5-7 mg loaded on the sample pan and another pan kept as empty for reference. The weight of the sample was noted on the TGA balance. The sample in the pan was covered by quartz tube in which the flow of air was maintained. The entire assembly was placed in the furnace. The experiment was started under a nitrogen atmosphere with a flow rate of 100mL/min, the sample heated from room temperature to 700^{o}C at a heating rate of 10^{o}/min. The weight loss observed as a function of temperature. The experiment was stopped at 700^{o}C, there is no further weight loss takes place in the sample [55].

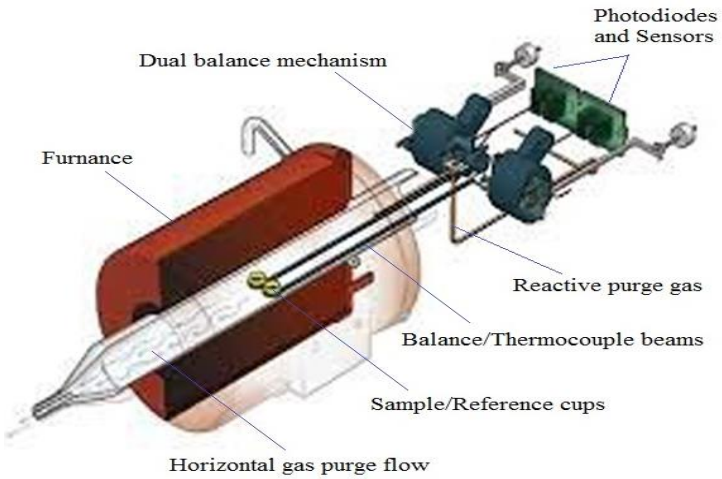

Figure 2.26. Schematic diagram of TGA.

Instrument details:

The TGA of prepared samples were carried out by using SDT – Q600 TA instruments. The entire system was designed horizontal balance & furnace. The Q600 features a highly reliable horizontal dual balance mechanism that supports precise TGA and DSC measurements. The sample capacity upto 200 mg (350 mg including sample holder), the sensitivity of the balance is 0.1 μg and bifilar wound furnace are used. The temperature ranges from ambient to 1200ºC with the heating rate of 0.1 to 100ºC/min. The Platinum/Platinum-Rhodium (Type R) thermocouples are used in this instruments. Ceramic, Platinum and alumina sample pans are used in Q600 depends on the temperature. The ceramic pan is recommended for operation to 1500ºC, platinum for operation to 1000ºC and alumina pan was used in the temperature less than 1000ºC. The measurements carried out under nitrogen atmosphere with the gas purge flow rate of 100 ml/min. Figure 2.8. Shows the typical SDT-Q600 TA instruments. The experiment was carried out DST-PURSE lab, Mangalore University.

Figure 2.8.TGA SDT- Q600 model, TA instruments.

Chapter-3

Structural and Thermal Properties of Silver Nanoparticles Embedded on Silk Fibroin films

3.1. X-Ray Diffraction study

The XRD patterns of the Pure SF and SF-AgNPs BNCs films are shown in figure 3.1. The Pure SF shows the two prominent broad diffractive peaks due to presence of plenty of amines, amides, carboxylic functional groups and few smaller peaks riding over a broad hump. The broad peaks observed at 19.63^0 and 27.56^0 which is corresponding to the crystalline nature of the silk I structure with the *d*-spacing 0.45 nm and 0.361 nm respectively. The diffraction peak at 9.1°, 18.9° and 20.07° which is corresponding to the silk II structure with the d-spacing 0.97, 0.46 and 0.43 nm respectively [52, 53].As increasing the concentration of silver nanoparticles on the silk fibroin matrix there is a decreasing the amorphous nature of the silk fibroin. The sharp peak is observed at the different angles 2θ = 32.62^0, 46.35^0, 67.29^0 and 76.27^0corresponding reflection planes are (111), (200), (220) and (311) respectively. The XRD patterns reveals that the silver nanoparticle shows the face centered cubic (fcc) structure. The table shows the structural parameters of pure SF and SF-AgNPs BNCs films.

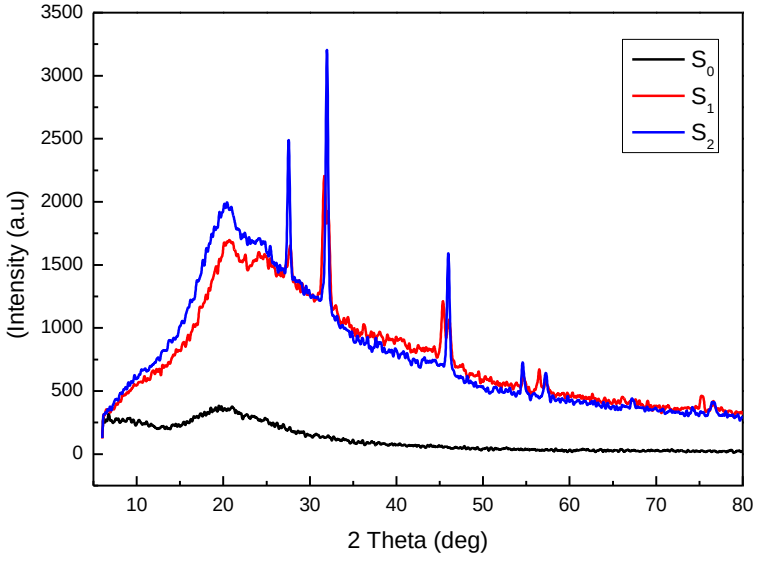

Fig. 3.1. XRD patterns of pure SF and SF-AgNPs BNCs films

Table 1 Structural parameters of SF-AgNPs BNCs films.

Sample	Peak position	P_{XRD} (nm)	Lattice Strain (%)
S_0	19.63^0	0.62	0.3402
S_1	31.50^0, 45.48^0, 56.54^0, 75.26^0 -----	-------	
S_2	32.03^0, 45.85^0, 54.58^0, 76.52^0	16.93, 14.54	0.0078, 0.0064

3.2. TGA analysis of pure SF and SF-AgNPs composite films

Figure 3.2. Shows the TGA plots of pure SF and SF-AgNPs composite films with the temperature ranging from room temperature to 700 °C. There are three distinct region of weight loss (%) was observed during heating, the first region of weight loss takes place from room temperature to 170.1 °C, it is mainly due to the elimination of adsorbed water molecules from the samples. In this region, we calculated the percentage of weight loss for pure SF and SF-AgNPs samples and are tabulated in the Table 2. The weight change was not significant and the sample was thermally stable. In the second region the weight loss takes place from the temperature range of 184.3 to 461.1 °C with the maximum weight loss observed around 37.19 %. The weight loss of SF in second region is associated with breakdown of side chain groups present in the SF, because it is composed of polypeptide chains with the amino- terminus and carboxy-terminus of the two subunits.

In the third region of weight loss takes place from the temperature ranging from 473.8 to 652.4 °C. This region attributed to the decomposition of main chain groups of SF with the maximum weight loss was around 35.52 %. Similarly, three distinct region of weight loss observed in the SF-AgNPs composites but there is a slight variation in the weight loss (%). Compared with the pure SF, the AgNPs immobilized samples exhibits greater weight loss and increases the thermal stability because nanoparticles may change the surface hydrophilicity of SF. In SF-AgNPs composites the second stage weight loss reduced due to the AgNPs bind together with the carboxylic groups of side chains of SF protein. These binding sites of AgNPs which helps to protect the thermal degradation of side chains [56].

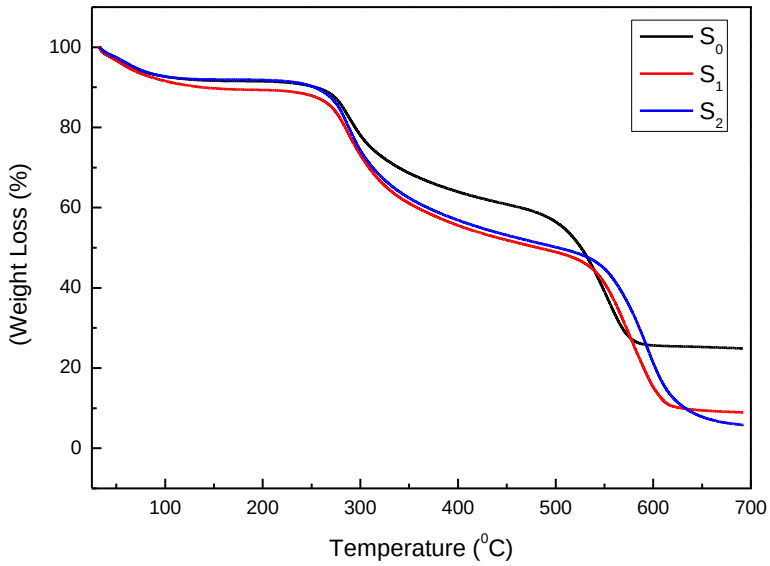

Figure 3.2. TGA plots of pure SF and SF-AgNPs composite films.

Table 2. Weight loss (%) of pure SF and SF-AgNPs composite films.

Sample	Weight loss (%) at different temperature (°C)		
	Ist Stage (RT to 170.1)	IInd Stage (184.3 to 461.1)	IIIrd Stage (473.8 to 652.4)
S_0	7.62	37.19	35.52
S_1	8.92	38.93	41.16
S_2	6.91	41.3	43.94

Chapter 4
Conclusions

The preparation of silk fibroin - silver nanoparticles a simple solution casting method under ordinary light exposure. The prepared composite films characterized using different analytical techniques and the properties studied in detail. SF is an excellent biomaterial used in biomedical field and optoelectronic filed. The research work presented in this dissertation covers the detailed investigation of SF–AgNPs BNCs. The AgNPs were synthesized by a bio-based approach. The SF acts a good stabilizing and reducing agent for the AgNPs. The XRD pattern confirmed the highly crystalline nature of the formed AgNPs with FCC structure; the thermal stability of the composites increases with increasing AgNPs in the SF matrix. The AgNPs loaded silk films are flexible and shows the significant changes in the thermal properties and it is very good applications in the field of implantable thermoelectric wireless switching devices.

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