<u>Synthesis of Starch/PVA Bombyx mori silk fiber reinforced blends and composites</u>

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science in Chemistry

By: BHAVANA SONI

(11605130)

Under the Guidance of

Dr. HIMANSHU

Transforming Education Transforming India

SCHOOL OF PHYSICAL SCIENCES AND CHEMICAL ENGINEERING

Lovely Professional University, Punjab, India

(November, 2017)

INDIA'S LARGEST UNIVERSITY*

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education, Transforming India

DECLARATION

I hereby affirm that the dissertation entitled **"Synthesis of Starch/PVA Bombyx mori silk fiber reinforced blends and composites"** submitted for the award of Master of Science in Chemistry and submitted to the Lovely Professional University is the original and authentic study that I have carried out from August 2017 to November 2017 under the supervision of Dr. Himanshu, Assistant Professor. It does not contain any unauthorized works of other scholars, those referred are properly cited.

Date: ....

Name and Signature of the Student

Name: BHAVANA SONI

Reg. No.: 11605130

CERTIFICATE

This is to certify that Miss. Bhavana Soni has completed the dissertation report entitled**, "<u>Synthesis of Starch/PVA Bombyx mori silk fiber reinforced blends and composites</u>"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study.

Date: 30 November, 2017

Dr. HIMANSHU KAPOOR

CONTENTS

ACKNOWLEDGMENT

I acknowledge the Holy Spirit, my greatest help, coach, and strength through this study. Immense appreciation to my supervisor Dr.Himanshu, for being so gracious and helpful throughout this work. Ma'am, I appreciate your humility and modesty.

My acknowledgment also goes to my department and to my H.O.D. Dr. Ramesh Chand Thakur, thanks for providing a wonderful environment and opportunity to do this project. I appreciate affection of our teachers to clear our doubts at any time of day. Thanks to my tutor, Dr. Himanshu- thanks for believing in me and for being a part of my work, and for your positive influence for me.

Lastly, much gratitude to my father Yatendra Kumar Soni, whose valuable ideas and encouragement gives me strength and to my mother Madhu Soni, whose prayers yields great fruits in my life. My thanks and appreciation also goes to my friends who have willingly helped me out with their abilities.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND:

In recent days the environmental concern or ecological concerns have resulted in development of ecologically friendly materials (green composites) for the industrial products. This has been done as an attempt to minimize the use of petroleum based products. Petroleum based products are toxic and non-biodegradable. With the increasing environmental consciousness and ecological risks, the green composites are emerging as a realistic alternative to petroleum based products, because they are derived from renewable resources.

Green composites are also classified as bio-composites which are formed when biodegradable matrix (resins) combine with natural fibres having significantly different physical and chemical properties, which on combining produce the product with characteristics that are different from the individual components **[1].** A composite material has two components, first component is called the reinforcing phase which are strong long fibres and withstand maximum load. Second component is the matrix which holds these fibres in place and deforms to distribute the stress among the reinforcing fibres under applied force. The reinforcing phase provides mechanical support and intensifies the mechanical properties of the composite like strength and stiffness. Green composites can be classified on the basis of matrix used. When organic materials are used as matrix, the composite is termed as organic matrix composite (OMC's). The OMC's are further classified as Polymer Matrix Composite (polymer used as a matrix) and Carbon Matrix Composite generally called as Carbon-Carbon composite. Similarly, we have Metal-Metal Composites (metal is used as matrix) and Ceramic Matrix Composite (ceramic is used as matrix).

Polymer matrix supported with fibres forms an integral part of the fibre reinforced polymer composite materials. These composites are so much popular because of their easy manufacturing process, high quality, higher strength, durability and minimal efforts required in production. Every scientific discovery has some drawbacks, the downside of these FRP composites are the high coefficient of thermal and moisture expansion, low working temperature and low elastic properties. Instead of these drawbacks several FRP composites offers a blend if both quality and modulus that are either practically identical or superior to the conventional materials. The fibre generally used in FRP composites are glass or carbon although other fibres such as paper or wood is also used sometimes. The polymeric framework usually involves thermoplastic (polypropylene, nylon, polyethylene, and so on) and thermoset (epoxy, vinyl ester, polyesters and so on) polymer. The thermoplastic polymers can be remoulded into different shapes on increasing the temperature but thermosetting plastic polymers cannot be remoulded on increasing the temperature once they are moulded into some shape or in other words they are in-fusible.

Recently, research and innovations in the field of synthetic fibre composites has been shifted to natural fibre composite (NFC) area **[2]**. Interest has shifted in this area due to the advantage that they are more eco-friendly, are affordable and have high potential applications. The fibre used could be plant, animal or mineral fibre. All plant fibres contain cellulose as their major structural component, while protein is the content of animal fibre. Minerals of asbestos group are included in mineral based natural fibre composites, but now the use of these natural fibres composite has been avoided due to related health issues, thus are banned in many countries. Plant fibres provide relatively high strength and stiffness with high performance than the available animal fibres. The silk fibre emerged as an exception to this, with high strength.

1.2 POLYMERS

History has given us several evidences about the use of natural polymers from past several years. The early attempts to chemically modify the natural polymers were started in 1800s. Through vulcanisation process, Charles Goodyear in 1844 was able to transfer the latex of natural rubber into an elastomer. Till that time the word ‘polymer’ was not in use. It was coined by Berthelot in 1866, who in an article published in the Bulletin of the Chemical Society of France, noted that “*styrene, heated at 200^{0}C during a few hours, transform itself into a resinous polymer”* **[3]**.This was the first recognized synthetic polymer.

In the year 1920, *Hermann Staudinger* was the first person to put forward the idea of polymers in such a way that we use today. His idea gave rise to the modern concept of polymers. He proposed that naturally occurring polymers such as starch, cellulose, protein and rubber consist of covalently linked long chains of repeating units. The Nobel Prize was awarded to him in 1953 for his enormous contribution towards polymer chemistry and specifically for proposing the structure of rubber based on isoprene units.

Polymers are the large molecules containing large number of structural units (monomers) which are joined by the same type of linkages. These substances are often chain like in structure. Both naturally occurring and synthetic polymers are seen today. Some naturally occurring polymers are cellulose, starches, proteins and latex. Polymers are also synthesized on a large scale for commercial purpose and these synthetic polymers are associated with a wide range of enhanced properties, and have been used in myriad of applications. One of the good examples of synthetic polymer is plastic. A large number of monomers in a chemical reaction are joined sequentially to form long chain polymers. A polymer could be formed either from only one type of monomers or a combination of two or more different types of monomers. Polymers could be classified on the basis of their structure, molecular forces present in them, the sources from where they are produced or obtained and mode of polymerization.

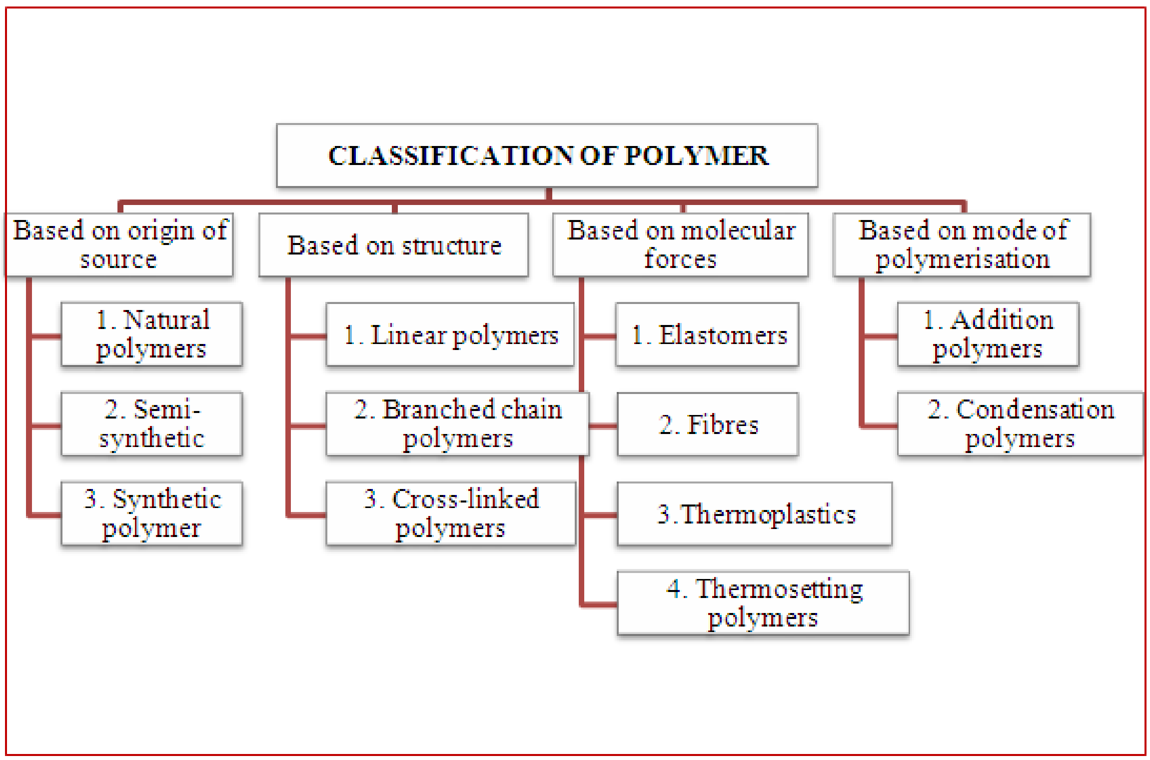


Fig 1.1 : Classification of polymers [4].

Today polymers are an essential part of everybody's life because of their extra ordinary characteristics, such as they have low cost, light weight, adaptability, hardness, durability, consumption resistance, simplicity of handling and are utilized for various applications. At first, the utilization of polymers was limited, but as the modern applications of polymers started to develop, different significant properties were fused into polymeric materials effortlessly. The polymers could be derived either from fossil fuels or from other natural resources. Those polymers which are derived from fossil fuels carry the biggest drawback that they are non-renewable and non-biodegradable. The polymeric materials derived from natural resources have become the topic of interest in recent years because of their simplicity of accessibility and most importantly they do not pose any danger for the Mother Nature.

The polymers that are found in nature incorporate polysaccharides (chains of sugar), polyamides (chains of proteins), and polynucleotides (chains of nucleotides). Polymers from natural origin are non-toxic in nature and discover enthusiasm for the fields of beautifiers and pharmaceuticals commercial ventures. Among all the natural polymers, natural fibres are most oftenly utilized as reinforcement in composites. Natural fibers are effortlessly accessible from plants and animals and their consistent use in innovative applications guarantee the best possible utility of this biomass without forcing any damage or harm to our very own blue planet.

1.3 NATURAL FIBRES

Natural fibres are the hair like raw materials which are obtainable either from an animal, vegetable, or mineral sources and are convertible into fabrics which are not woven for e.g. papers or into clothes and yarns after spinning.

Natural fibres can also be explained as an assembly of cells with diameter negligible as compared to its length **[5]**. In addition to the economic considerations, natural fibres are also having commercial importance in terms of their properties such as length, strength, pliability, elasticity, abrasion resistance, absorbency, and various surface properties. Textile fibres are having elasticity and when they are put under tension, they regain their original length either fully or partially when tension is released.

Unlike synthetic fibres, natural fibres are non-thermoplastic i.e., they do not get soft when heat is applied. In recent times, Natural fibres are used in the substitution of the traditional glass fibres as reinforcement and this is the significant step that has been taken towards the promotion of the ecological sustainability. **[6]**.

Natural fibres can be classified according to their origin.

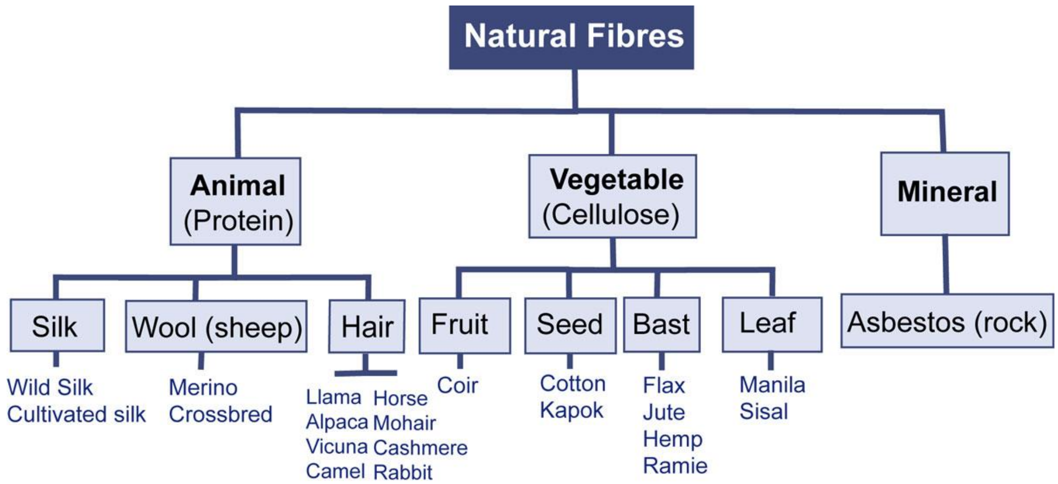


Fig 1.2 : Classification of natural fibres [7].

Chemically all the vegetable fibres are consist of cellulose with diversifying amounts of other substances such as hemicellulose, lignin, pectin and waxes which have to be removed or reduced by processing. Animal fibres extensively contain proteins and are a part of fur or hairs that serve protective epidermal covering of animals. But the exception is silk, silk fibres are secreted from the larvae of the moths and are used by them to spin their cocoons.

a. Plant Fibre

Plant fibres are obtained by breaking vines, destroying jungle plants and sometimes drop from farming other plants such as cotton. A large variety of plants like cotton, kapok, jute, flax, ramie, sisal and hemp, are used for the production of plant fibres and there are many plants that are grown as field crops to make paper, clothes and ropes. Different types of plant fibres are seed fibre, bast fibre and hard fibres.

Seed fibres: they are extracted from seeds or seed cases. e.g. cotton and kapok.

Bast fibres: They are extracted from the inner bark of the plant that surrounds the stem of the plant. This fibre has highest tensile strength and thus is most commonly used for packaging and papers. Examples are flax, jute, kenaf, hemp.

Hard fibres: they are extracted from leaves. Examples are sisal, banana and agave. Coir around the hard shell of coconut is also an example of hard fibre.

The essential constituents of plant fibre are cellulose, hemicellulose and lignin. Cellulose is hydrophilic in nature. Hemicellulose is made out of highly branched polysaccharides. Lignin is aromatic, amorphous and highly complex that gives stiffness to the cell wall and protects the cellulose.

b. Animal-Based Fibres

They are the fibres which are obtained from animals, like wool, silk, feathers and hairs, etc. are some examples of animal-based fibres.

Wool is the textile fibre which we get from sheep and other animals, including mohair, cashmere from goats, quivit from muskoxen, angora from rabbits and other types of wool from camelids. Wool is a very soft and smooth fabric also it provides warmth, because of which it is the most popular choice in winters. Wool also has elasticity and is good for wrapping. Wool comes in different colors as it can be easily dyed, and this makes it a fashionable fabric in winters. α-keratin protein constitutes the wool.

Silk is a natural fibre and is one of the oldest fibres used by humans in textile industries form past many years. It is obtained from silk worms. The most popular and commercial type of silk is obtained from the mulberry silk worm also called as Bombyxmori. Mulberry silk is the cultivated silk while apart of this we have large varieties of wild silk obtained from different silkworms China, India, Nepal and Europe are the traditional producers of good quality silk on a very large scale. Silk fibre is very soft and smooth in touch and has a unique sheen which is not found in any other fabric. All these properties of silk make it a popular choice for sarees and many royal dress materials. On the other hand silk is also used for home furnishings. Sericin and fibroin proteins are the two main proteins that constitute the silk fibre.

Hair is a protein fibre which grows from the follicles found in the dermis, or skin. It is a defining characteristic of mammals. Hair is a very important biomaterial as it provides protection and warmth and is mainly composed of keratin protein.

Feathers also grow on the epidermis and provide outer covering to the birds and serve the function of protection. They are the complex structures found in the vertebrates and covers whole body of the birds. They are grown over the skin surface from some well-defined tracts.

They are of different colors and this helps them to communicate with each other. They help birds during flight and provide them the thermal insulation.

c. Mineral Fibres

A large number of natural fibres are obtained from the mineral resources and extensively are the derivatives of silica or other metal oxides.

The fibrous crystalline forms of the natural minerals are termed as asbestos. Initially the asbestos is crushed to open up the fibre mass and then it is carded followed by spinning to yield yarn. Asbestos fibre is not affected by heat and burns, it is also resistant to acid and alkalies. Asbestos does not get deteriorated easily and also is resistant to attack of insects and microorganism. Although it has low strength but then also it is used for manufacturing of conveyor belts and fireproof clothing. They have been removed from textile markets because the inhalation of asbestos fibre causes serious health issues.

1.4 PROPERTIES OF NATURAL FIBRES

Natural fibres are important to researchers because of worthwhile qualities, for example, minimal effort; high strength to weight ratio, non-corrosive property, low density per unit volume and acceptable strength, notwithstanding their renewable and degradable attributes **[8]**. Natural fibres are a renewable resource that is found in bounty in nature and are savvy contrasted with that of synthetic fibres. In addition to the economic considerations, natural fibres are also having commercial importance in terms of their properties such as length, strength, pliability, elasticity, abrasion resistance, absorbency, and various surface properties. Textile fibres are having elasticity and when they are put under tension, they regain their original length either fully or partially when tension is released. Natural fibres seem to have little resistance towards environmental influences.

However, the use of natural fibres in composites is not free from drawbacks. The fibres generally show low capacity to hold fast to common non-polar matrix materials. Moreover, the hydrophilic nature of fibres leads to moisture absorption. Natural fibre composites tends to swell significantly with water uptake and as a result the mechanical properties, for example, firmness and quality, are adversely affected.

Fibre matrix adhesion can be enhanced using several physical and chemical treatment methods. These methods in result enhance the physical properties of the fibre composites. The effects of moisture on the composite can be reduced by changing the hydrophilic character of the natural fibres with the help of different treatment methods **[9].**

CHAPTER -2

2.1 INTRODUCTION TO SILK

Silk is a wonderful, soft, light and luxurious fiber obtained from the cocoons of the silkworm. It is a natural fibre and is one of the ancient fibres discovered by man. Out of all the fabrics available silk is regarded as the finest and the most beautiful fabric. It has an amazing sheen and can be dyed into wonderful colours.

Silk is made of proteins secreted in the liquid state as a single filament by silkworm. Silk is the only fibre which is in the form of filament. Different types of silk are produced by a wide variety of silkworms and insects instead of caterpillars, but still none of these has been used much commercially. Silk, also called as the "Leader of fibers" is known for its smoothness; fragile surface, high moistness retentiveness, fineness, extraordinary flexibility and splendor which isolate it from rest of regular and designed fibers. Silk is extremely phenomenal in its strength, light weight and insulating characteristics. Silk is strong enough to be used for surgical sutures. It is also fire and rot proof.

Silk is produced from the larva of the insects which has complete metamorphosis. Silk is produced by many insects apart of silkworm like by spiders and by mussel but most of commercial silk comes from the caterpillars of silk moths of the super family *Bombycoidea*. Several different groups of silk moth are used to produce wild silk but the cultivated silk comes from larvae of the Mulberry Silkworm moth, *Bombyxmori*, and accounts for the commercial silk production. The silk exclusively produced by the spider has very limited commercial applications, for example it is used in manufacturing weapons, telescope, and other optical instruments. **[10]** From past many years the silk form Bombyxmori silk has been used for bio-medical suture material. This fibre has proved to be the best clinical repair option for many applications because of its unique mechanical properties [**11].**

Silk fiber in view of its biodegradability, mechanical properties and characteristic soundness, has got the thought of various investigators working in various fields. Out of all the silk fibers much research is done on silk threads from tamed silkworm *Bombyxmori* and orb weaving Spiders in light of their stunning mechanical and warm properties **[12].**

2.2 HISTORY

Historical evidences showed that silk was discovered in China and the industry spread from there to other parts of the world. The Chinese are using silk since from 27th century B.C. The Chinese have domesticated silk worms and fed them with mulberry leaves. They unwounded the silkworms' cocoons to produced long strands of silk fiber which were then woven into yarns.

Silk is one of the oldest fibers known to man. Discovery of the fiber that can be woven is credited to the Lady Xi Ling Shi, the 14-year-old bride of the Emperor Huang Ti, known as the 'Yellow Emperor'. According to Confucious, one day when the princess was sitting under the mulberry tree and was having a cup to tea, suddenly a cocoon fell into her cup containing hot tea. She then noticed that the delicate fibers started to unravel in the hot liquid and thus she was credited as the first person to 'reel' or unravel a silk cocoon and use the filament to create a yarn for weaving and all this happened in 2640 B.C. For hundreds of years after that only the Chinese royal families were allowed to wear silk. Xi Ling Shi is now honored as the goddess of silk in China. May be these legends are not true, but it is certain that the earliest surviving references to silk production are from China and that for nearly three decades, the Chinese had a global monopoly on silk production.

Silk was exported along the Silk Route (the ancient trade route linking China and the Roman Empire). That trade route brought a great wealth to China but the chinese never revealed the secret of silk production. Chinese were not able to keep their monopoly forever and the secret of silk production was said to have spread to Tibet when a Chinese princess, carrying silkworm eggs and mulberry tree seeds in her headdress, married the king of Khotan in Tibet. From Tibet the industry spread slowly to India and Persia **[13].**

The earliest evidence of silk was found at the sites of Yangshao culture in Xia Country, Shanxi, where a silk cocoon was found cut into half by a sharp knife, dating back to between 4000 and 3000 B.C. that species was identified as Bombyxmori , the domesticated silkworm.

Evidences of silk weaving includes impressions found on a bronze urn dated to 1330 B.C. the provincial museum in Hangzhou displays silk threads and embroidery knots that may be 4,500 years old. In 1982 brickyard workers fell across an ancient tomb from 300 B.C with preserved silk quilts and gowns. Tombs in the Hubei province in the 4^{th} and 3^{rd} centuries BC contains amazing examples of silk work, including brocade, gauze and embroidered silk and the first complete garments.

The secret of making silk remained in china for thousands of years. No one is sure when the secret first seeped out of china, but is known to have reached Japan by way of Korea by the A.D. 4th century and said to have been brought there by four Chinese girls.

2.3 TYPES OF SILK

Silk can be produced by a variety of silkworms and sometimes no worms at all. Whatever is the source of silk, these moths and worms produce a protein called "fibroin" which is the main element in silk. Different types of silkworm produce silk with different characteristics. The known varieties are as follows:

1. MULBERRY SILK

This is the most common among many varieties of silk. 90% of the silk supply in the world is that of mulberry silk. This silk is produced by *Bombyxmori* silkworms, which are fed from the mulberry bush and thus the silk is called mulberry silk. China, Japan and Korea are the countries which have abundance of it in particular.

Fig 2.1: Bombyxmori silkworm with cocoon and moth [14].

2. TUSSAH SILK

Tussah silk is produced by tussah silkworms, Antheraea mylitta. Like other silks, this one doesn't have white color instead has a distinct light golden to dark brown colour. This color is because of the tannin rich leaves that tussah silkworm consumes. This silk is said to be one of the strongest fiber. That's why these tussah silk is an ideal material for couches, jackets and sweaters.

Fig 2.2 : (a) tasar silkworm [15]. (b) tasar cocoon [15].

3. MUGA SILK

Muga silk is similar to Tussah silk, and is produced by the semi-domesticated silkworm Antheraea assamensis. Muga originates in india and is known for its golden brown and glossy texture. It is also called as the assam silk as the fabric was exclusively used by the assam royal families for over 600 years. Muga production is considered to be eco-friendly as the silkworm doesn’t require the delicate care. This makes it an affordable silk type too. The porosity of Muga silk sometimes become its disadvantage as it limits the use of bleach (which for us is more eco-conscious but it also implies that it cannot be dyed).

Fig 2.3 : muga silkworm [16]. muga cacoon [16].

4. ERI SILK

It is produced by the domesticated silkworm *Philsamia renini*. It is almost white in color and is very fine. Though it is spun from the cocoons of domesticated silkworms, but it is a peace silk because silk caterpillar is not destroyed inside the cocoons and is allowed to emerge as a moth and live a full lifecycle. It has a matt like appearance of cotton and wool, but also has sheen and softness of the silk and is commonly cultivated in India, China and Japan. These silkworms feed on castor plant leaves.

Fig 2.4: *Eri* silkworm [17].

***Eri* cacoon [17].**

5. SPIDER SILK

It's fascinating to know that spider silk has also been used in ancient cultures. But it is very difficult to produce comparative to other silk types, as spiders cannot be domesticated like silkworms and also they cannot produce as much fibre as silkworms. Though the production of this type of silk seems to be difficult but its output is worth the efforts. It is regarded as one of the most durable silk as it is being utilized in the production of telescopes, bulletproof vests and wear resistant clothing.

Fig 2.5 : spider silk [18].

6. MUSSEL SILK

This type of silk is produced by mussels- the one found in the seabed. It is also called as a sea silk. It is surprising to know that it is being produced since from the 8th century BC. It is one of the most unusual silk types. It is very difficult to source this type of silk nowadays because its sources are being affected due to increasing pollution. Since its production is very rare, it is counted among one of the most expensive and rarest silk. The most common type of mollusk used in the production of this type silk is Byssus, and thus the fabric is called as Byssus cloth.

Fig 2.6: mussel [19].

2.4 LIFE CYCLE OF *Bombyxmori*

The silkworm, *Bombyxmori* produces the silk for commercial importance. The cocoon of Bombyxmori silkworm is used to produce silk. The insect is also called the silkworm moth. The life cycle of Bombyxmori demonstrate the most advanced form of metamorphosis. There are four distinct stages in the life cycle of the Bombyxmori egg, larvae, pupa and imago.

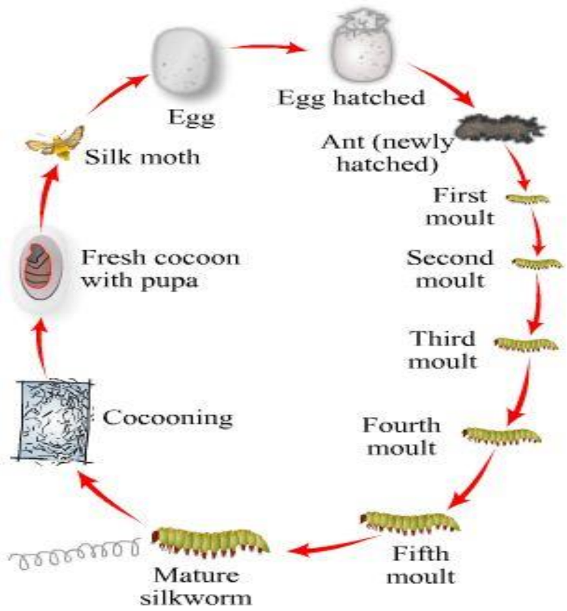


Fig 2.7: life cycle of silk worm [20].

1. EGG (OVA)

The very first stage of its life cycle is egg. The female moth lays the egg of the size about an ink dot during the summer or the early fall. Larvae does not came out of egg until spring arrives. The warmth of the spring stimulates the egg to hatch. The egg is very small and hard structure and the shell provides a protective covering to the developing embryo.

2. LARVAE

Larva is a vegetative stage, the stage at which growth takes place and is commonly called as silkworm. It is the specific host of mulberry. Young silkworms feed only on the tender leaves but as they grew they can eat big and tough mulberry leaves. This stage lasts for 27 days. During this growth period larva goes through five growth stages called as moult or instars.

3. PUPA

As the silkworm prepares to pupate, it spins protective cocoon around itself and looks like a cotton ball. The cocoon is prepared from one continuous strand of silk. The cocoon serves as the protection for pupa. The shade of cocoon could vary from white, cream and yellow depending upon the genetics of the silkworm. After final moult inside the cocoon, the larva develops into the brown, chitin covered structure called as pupa and metamorphic changes in pupa result in an emerging of moth. It takes 2-3 weeks for larva to emerge as moth.

4. IMAGO

The adult stage of Bombyxmori is call imago and is the reproductive stage where adult moth and female moth lays egg. If the moth came out the cocoon by breaking it then the silk of cocoon is not of any use for commercial purpose. So the silk is extracted before adult emerge out by boiling the cocoon in water to kill the inhabitant and to remove the glue between the silk thread that holds the cocoon.

2.5 STRUCTURE OF THE SILK FIBRE

Silk is a naturally occurring fibre which contains amino acids. Silk fibers consist of 97% of proteins called as "fibroin", approximately 75% of the filamentous protein and nearly 25% of sericin which is a non-filamentous protein. Fibroin and sericin contain same 18 amino acids such as glycine, alanine and serine in different proportion. The core fibroins are covered by a coat of sericin, which is a hydrophilic protein and holds the two fibres together **[21]**. Bombyxmori silk filaments are 10-20 µm thick. The silk strand additionally contain little amount of carbohydrates, wax and inorganic components which also act as structure elements in fiber development.

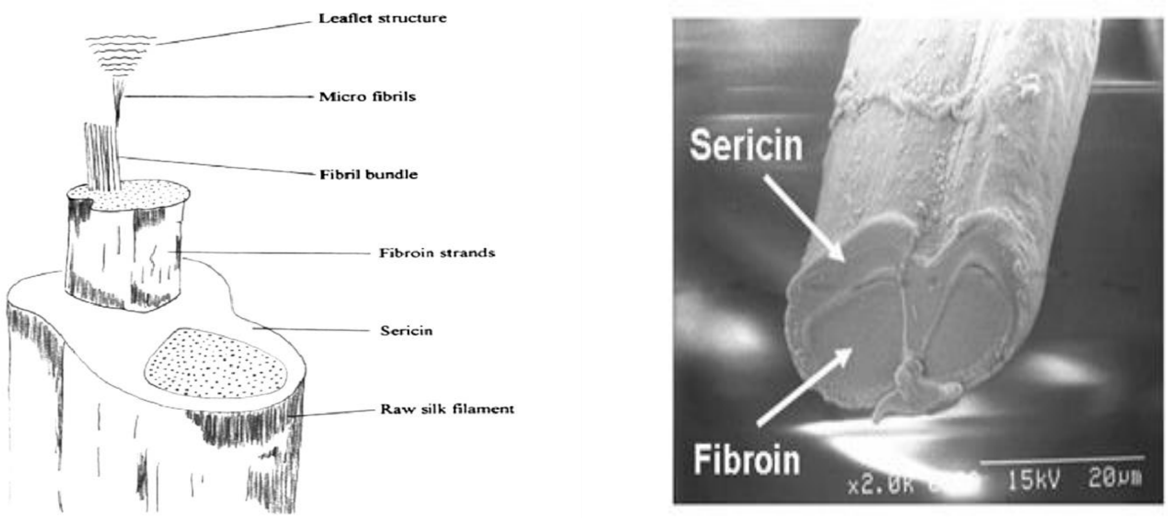


Fig 2.8: Structure of silk fiber [22-23].

Fibroins are the core fibers and are not soluble in hot water. It is having the chemical formula $C_{15}H_{26}N_5O_6$. The Sericin forms the outer layer of the silk fiber and is a form of gum with chemical formula $C_{15}H_{23}N_5O_6$. Sericin gives rough texture to the fibre and hides the gloss and whiteness of the fibre. Silk sericin can be separated from cocoon by boiling water or boiling alkaline solution. The Bombyxmori silk fibres after the removal of sericin seem to be shiny and delicate fibres. Silk doesn't have a cellular structure.

Silk fibroin is made up of amino acids Gly-Ser-Gly-Ala-Gly-Ala and form beta pleated sheets.

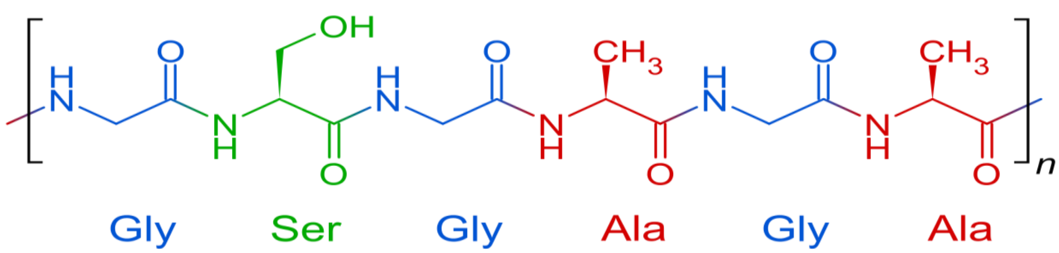


Fig 2.9 . primary structure of silk fibroin [23].

Bombyxmori silk fibroin mainly contains three α-amino acids in large proportion, these are glycine- 45%, alanine-29%, and serine-12% in approximate molar ratio 3:2:1 respectively. Tyrosine, valine, aspartic acid, glutamic acid, etc. makes up the remaining 13% of the fibre **[24].** The amino acid composition of the silk protein is given below:

Table 2.1: Components of silk protein [22].

symbol	Amino acid	Sericin	Fibroin
G	(glycine)	14	45
A	(alanine)	5	29
S	(serine)	33	12
Y	(tyrosine)	3	5
V	(valine)	3	2
D	(aspartic acid)	15	1
R	(arginine)	3	1
E	(glutamic acid)	8	1
I	(isoleucine)	1	1
L	(leucine)	1	1
F	(phenylalanine)	1	1
T	(threonine)	8	1
C	(cystine); half	0	0
H	(histidine)	1	0
K	(lysine)	4	0
M	(methionine)	0	0
P	(proline)	1	0
W	(tryptophan)	0	0

Glycine is the smallest amino acid and is present in the large proportion in the silk protein and allows tight packaging which makes the fibre strong and breaking resistant. Many hydrogen bonds are present in the protein which increases its tensile strength. But when silk is wet it loses its 20% of strength. Its elasticity ranges from moderate to poor and if elongated to a small amount it remains stretched. Silk fibre also weakens if exposed to too much sunlight [**24]**.

CHAPTER 3

LITERATURE REVIEW

3.1 LITERATURE SURVEY

Sang Muk Lee et al., have studied the mechanical and thermal properties of Bombyxmori silk fiber supported with poly butylene succinate (PBS) bio-composites. They have also studied the tensile properties and recorded the microscopic observations. Their result shows that the mechanical property of the PBS can be enhanced with the help of silk fibroins. Their work also suggested that the properties and the performances of the polymer can be enhanced if animal fibre is used as reinforcement fibre composites **[25].**

Application of natural fibres in composite plastics is gaining popularity in many industries and particularly the automotive industry. Recently there is observed shift of interest of the automotive industries towards green outlook, due to consumers demand for more environmentally friendly vehicle. Natural fibers are biodegradable and replenishable. Among the natural fibres with proven potential application as reinforcement for polymers, Bombyx mori woven silk fibre is one that recently received special attention from researchers. According to the work of *A.U. Ude and his co-workers*, it is one of the best fiber discovered in nature, providing high mechanical properties over frequently used natural fibres like sisal, jute, hemp and coir. **[26].**

Mobini S et al., have developed a novel bone tissue engineering composite from *Bombyxmori* silk fibroins. This is a 3D scaffolds which are invented when the degummed fibre is regenerated followed by freeze drying. They have studied the influence of different fibre to fibroin ratio on mechanical and biological properties. They have used SEM to prove that the interconnected porous structure is suitable for the cell penetration in scaffolds. To support the fact that fibroin scaffolds can support cell attachment they have performed an experiment on cell culture using human mesenchymal stem cells [**27**].

Raghu et al. have synthesized chemical resistant composite of silk fiber epoxy laminate. They have prepared composites of unsaturated polyester and alkali treated or untreated silk-sisal hybrid composites by using hand lay-up technique. The 2 cm long sisal fibers were treated with

2% NaOH. They have studied treated and untreated silk/sisal hybrid composites to check their chemical resistance to various acids, alkalies, and solvents [**28**].

Ismail et al. studied the effects of polyethylene -g- maleic anhydride (PE-g-MA) as a compatibilizer on morphology and tensile properties of thermoplastic sago starch (TPSS)/low density polyethylene low density polyethylene - kenaffiber composites. They observed that the Young's modulus and tensile strength of TPSS/LDPE-kenaffiber composites with PE-g-MA were greater than the PE-g-MA absent composites [**29**].

Biocomposites are derived from the natural fibers and are utilized for the biomedical applications such as drug, gene delivery, tissue engineering and orthopedics. The first essential requirement of materials to be used as biomaterial is their acceptability by human body. Biocomposites have the potential to replace or yo serve as a framework allowing the regeneration of traumatized or degenerated tissues or organs, thus improving the patients quality of life. Farideh Namvar, et al., have discussed about the utilization of plant fibers and its composites in the biomedical applications and considers potential future research directed at environmental friendly biodegradable composites for biomedical applications [**30**].

Zhi liu et al. have studied the effect of sodium carbonate concentration on degumming process. Their results have shown that the transformation of silk fibroin from nano-spheres to nano-fibrils can be determined by sodium carbonate. Further they have evaluated the effect of sodium carbonate degumming concentration on the resultant electrospun of silk fiber nano-fiber properties. They have prepared three weight ratio of sodium carbonate (0.5 wt%, 0.05 wt%, 0.005 wt%) for degumming and lithium bromide-formic acid system was prepared for the dissolution of silk fiber. The results have shown that for varying weight ratio of Na_2CO_3, silk fiber morphology changed from nano-fibers to nano-fibrils [**31**].

From more than a century the biologists, chemical scientists and material scientists are paying attention towards the animal silk because of the amazing combination of modulus, strength and extensibility. Chengji Fu et al., have discussed the formation of natural silks as well as their hierarchical organization. They have focused on the recent development in the measurements of various mechanical properties related to the structures of silk. Among all different types of silks they have exclusively focused on only two types of silk i.e., orb weaving spider silk and Bombyx mori silk [**32**].

Zuo et al., have studied the changes in the molecular weight of the silk fibroins when they are dissolved in neutral salt solution. They have also established the relationships of structural change of the regenerated SF fibers with their mechanical properties and degradability. Their results show that the biodegradability of the regenerated fibroin is enhanced while the mechanical properties have deteriorated as compared to the native silk [**33**].

Yang Cao and Bouchu Wang have discussed about various silk based biomaterials and the degradation behavior of these materials. They have focused upon silk fibroin from the silkworm, Bombyx mori.their work suggested that the silk has wonderful properties such as biocompatibility, biodegradation, non-toxicity and adsorption properties. They have also studied the degradation behavior of the silk [**34**].

Yu-Qing, et al., have discussed that the regenerated liquid silk fibroins with degraded peptide mixture can be obtained from dissolution of silk fibroins in concentrated calcium chloride solution. They suggested that with the help of water and a polar aprotic organic solvent the liquid silk can be converted into silk fibroin nano-particles. They have done the characterization of the nano-particles with the help of TEM, SEM, AFM and laser sizer. They have also discussed about the formation of β-sheet configuration and α-helix form of silk nanoparticles with the help of X-ray diffraction. They have stuied in detail by using infrared, fluorescence and Raman spectroscopy, DSC, 13C CP-MAS NMR and electron diffraction [**35**].

Sung-Won, et al., have dissolved the *Bombyx mori* silk fibroin in calcium nitrate tetrahydrate-methanol system. They have constructed a constitutional phase diagram at various concentrations of the solvent system. They have observed that the regenerated powder were resoluble in water but not in ethanol and methanol. They have also gone for Atomic absorption analysis which revealed that the calcium cations strongly interact with fibroin molecules in dialyzed fibroin solution, and may interfere with the regeneration of a strong fiber. They have also compared the thermal behavior of both original and regenerated silk with the help of DSC. They have also discussed about the surface and cross-section of the regenerated fibroins using SEM. [**36**].

Sandeep Kumar and Sandeep Kumar Singh have investigated about the effect of degumming time on the structural property of silk fiber obtained from silk cocoon of Bombyx mori and also they have prepared regenerated silk fibroin (RSF) solution which could be moulded into silk nanoparticles. They have investigated silk fibers degummed with different media at different time intervals for degumming loss and characterized them using fourier transform infrared (FTIR), differential scanning caloriemetry (DSC), XRD, and SEM. They have observed that maximum degumming occurs when fibers are treated with Na_2CO_3 for 60 min. The SEM and AFM images of RSF solution showed that the aggregation of silk globules results in the formation of solvated macrochains and giving it an appearance of island like morphology. They investigated that the prepared nanoparticles may further be explored for loading drug entities and targeting [**37**].

Lu, et al., have modified the Bombyx mori silk fiber with Nano-TiO_2 and Chitosan dispersion system by the cross-linking reactions of citric acid and maleic anhydride. The average size of the Nano-TiO_2 particles in the aqueous dispersion system was 36.7 nm. The scanning electron microscopy micro-graphs showed that the Nano-TiO_2 particles were spherical and homogeneously dispersed in the dispersion system, and the surface of B. mori silk fiber treated with the Nano-TiO_2 and chitosan dispersion system was rougher than that of the untreated one. The XRD and FT-IR Spectrometry indicated that the crystallinity of the B. mori silk fiber increased after treatment. It was also found that the Nano-TiO_2 and Chitosan contributed to significantly enhance the mechanical properties including breaking strength, breaking elongation, initial modulus, rupture work, and elastic recovery property of the *Bombyx mori* silk fiber [**38**].

Jinfa Ming, et al., has found that the *Bombyx Mori* silk fiber is soluble in formic acid/hydroxyapatite system. They have examined the structure behavior of silk fibroin films prepared by formic acid/hydroxypatite dissolution method. They observed that the secondary structures of the silk fibroin are not much affected by the hydroxypatite contents in the dissolved solution. The XRD results showed that hydroxyapatite crystalline nature exists in silk fibroin films; however, when the hydroxyapatite content was 5.0 wt. % in dissolved solution, some hydroxyapatite crystals were converted to calcium hydrogen phosphate dehydrate in the silk fibroin dissolution process. This result was also confirmed by FT-IR and DSC measurement. They have noticed that the silk fibroin films prepared by this dissolution method had higher breaking strength and extension at break and thus could be utilized for preparing advanced materials especially in the field of biomaterial applications [**39**].

Lawrence has highlighted various processing methodologies that may be utilized to create a variety of structural forms from the protein fibroin, which is derived from the Bombyx mori silkworm cocoon. He had explored various methodologies for sericin removal and fiber extraction. He had reported various scaffolding techniques which include materials such as native fibers, electrospun fibers, sponge scaffolds, hydrogels and microspheres. He had also discussed about the future trends related to fibroin protein based technologies [**40**].

Phillips, et al., has reported the suitability of the ionic liquids for the dissolution and regeneration of the *Bombyx Mori* silk and have examined the structural properties of the silk films cast from ionic liquid solutions. They have employed multiple methods for preparation of silk fibroin solutions. This involved stripping of sericin in a Na_2CO_3 wash, rinsing and drying the silk and dissolution of silk in a high-concentration, aqueous lithium salt solution or a $CaCl_2$/ethanol and water solution. These solutions were subsequently dialyzed to remove the salt. They have also suggested that the silk fibroin can be dissolved into 1,1,1,3,3,3-hexafluro-2-propanol or hexafluroacetone.[**41**].

Shah and Pramanik, have investigated the method for the preparation of silk fibroin solution for possible applications in tissue engineering. They have extracted the raw silk from *Bombyxmori* silk cocoon then degummed it using sodium carbonate solution and further dissolve it in lithium bromide solution. They have used 0.02M sodium carbonate solution, 80°C temperature and heated the raw silk for about 40 min. The proper conditions of dissolution as suggested by them was 9.3M LiBr, 70°C temperature, and three hours dissolving time. The morphology of degummed silk was investigated by SEM at different magnification. SEM revealed the absence of glue like sericine over the silk fibroin surface at optimal degumming condition. The result of their study indicates that the regenerated silk fibroins can be used for fabrication of porous silk fibroin scaffolds for applications in the field of tissue engineering [**42**].

Mihaela Ramona Tudora, et al., have synthesized and characterized the domesticated Bombyx mori silk fibroin micro- and nano-particles for their application in controlled drug delivery systems. They have prepared silk fibroin micro- and nano-particles by precipitating them in different organic solvents. They degummed the fibroin by boiling it in a solution of $NaHCO_3$ and SDS and then extracted the silk fibroin by dissolving in LiBr solution at 600 C for five hrs. They characterized the silk fibroin by FT-IR spectroscopy and SEM [**43**].

WU, et al., have prepared the silk fibroin nanofibers by dissolving the *Bombyx mori* silk in $CaCl_2$/Formic acid and calcium chloride/ethanol and water. They have studied morphology, structure and mechanical properties of nanofibers prepared by electrospinning. They have compared the nanofibril structures exhibited by silk fibroin solution in the given two different mixtures. They reported that the average diameter of nanofibers prepared by $CaCl_2$-formic acid dissolution method was 375±26nm, and also the durability, breaking strength and elongation properties have also improved in $CaCl_2$ and formic acid solution relative calcium chloride/ethanol and water [**44**].

Zhang et al., have reported that the degummed silk fibroin from *Bombyx mori* (silkworm) has potential carrier capabilities for drug delivery in humans. They treated degummed silk with four kinds of calcium-alcohol solutions, and performed secondary structure measurements and enzyme activity test to distinguish the differences between the regenerated fibroins and degummed silk fibroin. The results of their study reveals that $Ca(NO_3)_2$-methanol, $Ca(NO_3)_2$-ethanol, or $CaCl_2$ and methanol treatments produced more lower molecular weights of silk fibroin than CaCl2-ethanol. Collectively their results suggest that $CaCl_2$ and ethanol processing method produces silk fibroin with biomaterial properties that are appropriate for drug delivery [**45**].

Freng Zhang,et al., have obtained the native silk nano fibrils by dissolving the degummed silk in salt-formic acid solution such as $CaCl_2$ and formic acid. The $CaCl_2$–FA dissolves the silk by breaking hydrogen bonds in the crystalline region while preserving the nano fibril structures. They determined the dissolution behavior with the help of sodium carbonate concentration at the time of degumming which was regulated by calcium chloride concentration during the process of dissolution. They have also reported that silk fibroin nano particles can be processed into films [**46**].

Zheng et al. have prepared silk microfibers with silk solution to produce silk composite (membrane) scaffolds with the microfibers randomly oriented in the membrane. The composite membranes were studied to investigate the morphology, biodegradation, chemical structural properties, mechanical properties and toxicity. According to their study, the addition of silk micro fibres into the silk microfiber-reinforced composites enhances the tensile properties as compared to the plain silk fibre membranes. Silk membranes consisting of 1 wt% microfibers showed homogeneous distribution of microfibers in the membrane matrix and thus a smooth surface. The silk fibroin composites reinforced by silk microfibers developed in the study were not toxic [**47**].

Xu Wu et.al, have used tetrahertz spectroscopy to study the new aspects of the biomolecular structures of silk fibroins. For the first time they have used this method to study the structural changes in the in the silk fibroin in the presence of methanol and water. The results of IR spectroscopy and XRD shows that silk fibroin initially exist in a typical silk I and reassembled into a predominant silk II (antiparallel β-sheet crystal) structure after methanol treatment. The analysis of XRD and IR reveals that as the concentration of methanol increase the formation of β-sheet structure takes place.they have ysed tetrahertz method because this provides them additional information about the structural changes in natural silk [**48**].

Wang et.al., have reported the effect of degumming and fibre dissolution on the structural properties of the silk fibroins. They have reported that the thermal stability and crystallinity decreases on degumming. They did comparison between the fibres degummed with urea and sodium carbonate, and came to the conclusion that chemical properties of silk fibroin reduces when it is degummed with sodium carbonate. Their study reveals that the Sodium carbonate solution leads to breakage of peptide chain in silk fibroin. The degree of the breakage of peptide chain depends upon storage time of liquid silk fibroin. The regenerated condition of silk protein is overlooked and study shows that different silk degumming and fiber dissolution systems will have different influences. The study of the effect of the silk degumming and fiber dissolution on the molecular structure and property of silk fibroin gives idea of using silk protein as advanced functional biomaterial [**49**].

Zheng et.al., have reported a novel dialysis strategy to purify the silk fibroin from *Bombyxmori* protein in order to avoid the pre-mature formation of β-sheets and fibroin agitation. This was lithium free processing of the silk fibroin and is less expensive process. They have used Ajisawa solution to dissolve degummed silk and dialyze it against distilled water. They got the outcome nearly close to the fibroin purified by the lithium bromide [**50].**

Teuschl et.al., have reported a novel way to remove sericin from the compact and highly ordered raw silk fibre matrix. They have demonstrated that the sericin can be removed from the silk fibroin via a borate buffer based system. This borate buffer has shown non-toxic behavior and also doesn’t alter the proliferation properties of the cells. They have suggested that the sericin can be easily removed after textile engineering process and it also expands the use of silk as scaffolds in tissue engineering and regenerative medical applications. [**51].**

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 MATERIALS:

Lithium bromide (98% anhydrous) was obtained from Loba Chemie India, Lithium chloride was obtained from Pallav Chemicals and Solvents India, calcium chloride was obtained from Alpha Chemikal India, Sodium carbonate was also obtained from Loba Chemie India, and ethanol was obtained from Chong Yu Hi-Tech Chemicals.

4.2 INSTRUMENTATION:

4.2.1 Infra-red (IR) spectroscopy:

IR spectra were recorded using KBr pallets by SHIMADZU FTIR 8400S, Fourier Transformation, Infrared spectrophotometer (Department of Chemical Engineering and Physical Sciences, Lovely Professional University).

4.3 EXPERIMENTAL PART:

4.3.1 DEGUMMING OF RAW SILK

1 gram of raw silk was taken and was degummed by boiling with 0.02M, 100mL Na_2CO_3 solution at 90-100°C for about 30 minutes with stirring to remove sericin. Later the fibres were repeatedly rinsed with distilled water for 3 to 4 times to completely remove the sericin protein and then the fibroins were air dried in hot air oven at 60°C.

NOTE: the solution containing the sericin was not discarded and was stored for further usage.

4.3.2 DISSOLUTION OF SILK FIBROINS

For the dissolution of silk fibroins three different methods were used. These methods are as follows:

4.3.2 (a) Dissolution using lithium bromide

0.13 grams of degummed silk fibroins were dissolved in 50 mL aqueous solution of LiBr of different molarities (1.5M, 2.5M, 3.5M and 4.6M) and was heated for 2 to 3 hours at 60°C with stirring.

4.3.2 (b) Dissolution using lithium chloride

0.13 grams of degummed silk fibroins were dissolved in 50mL, 4.6M aqueous solution of lithium chloride and was heated at 60°C for about 3 hours.

4.3.2 (c) **Dissolution using Ajisawa Solution**

0.3 grams of degummed silk fibroins was dissolved in a mixture of calcium chloride: ethanol: water in the molar ratio 1:2:8 and heated at 80°C for 45 minutes.

Table 4.1: degumming and dissolution of silk fibre

Sr. no.	Salt	Molarity of solution	Amount of water used	Quantity of silk taken	Time for dissolution/ degumming	Temperature (°C)
1.	Na_2CO_3	0.02M	500Ml	0.368 g	2hrs	98°C
2.	LiCl	4.6 M	100mL	0.192g	3hrs	60°C
3.	LiBr	1.5M	50mL	0.135g	3hrs	60°C
4.	LiBr	2.5M	50mL	0.139g	1hr30min	60°C
5.	LiBr	3.5M	50mL	0.139g	1hr	60°C
6.	LiBr	4.6M	50mL	0.139g	45min	60°C
7.	$CaCl_2$:EtOH:H_2O	1:2:8	50mL	0.214g	45min	80°C

4.3.3 REGENERATION OF SILK FIBROINS

Regeneration of silk fibroins includes dialysis of fibroin solutions using a cellulose dialysis membrane. Different molar fibroin solutions were dialyzed against deionized distilled water using cellulose dialysis membrane. The distill water was changed after every 2 hours and was done for one week.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 DEGUMMING OF SILK FIBRE

I have used the raw silk which contains sericin and our aim was to remove that sericin to get silk fibroin. Initially the raw silk looks rough and was not having any shine. After degumming in Na_2CO_3 solution for 2hrs we got degummed silk with shiny surface and also it became soft. The sericin was removed and got dissolved in the solution making the solution light brown in color. The difference between raw silk and degummed silk could be visualized from following **figures**:

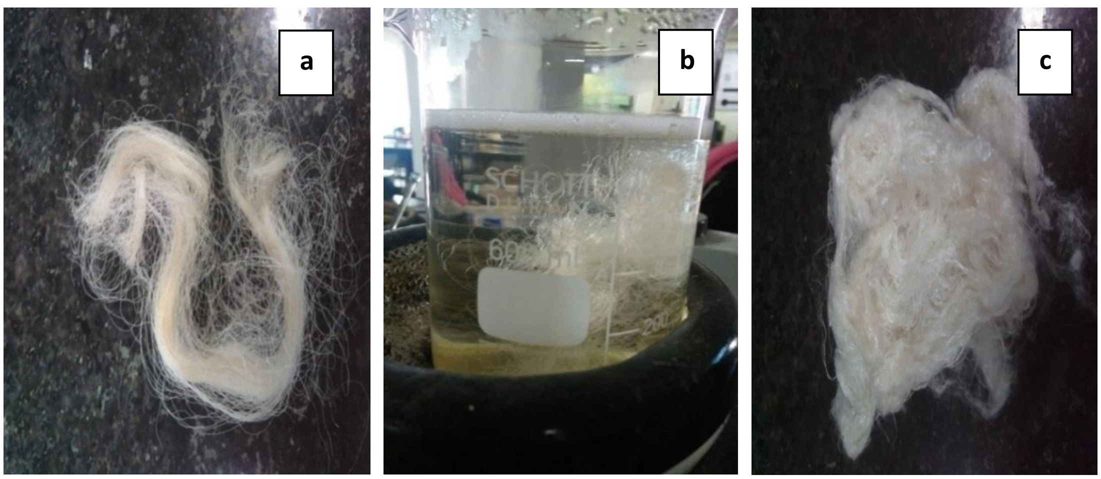


Fig 5.1: (a) Raw silk (b) degummed process (c) degummed silk fibers.

Fig 5.2 : Extraction of Sericin.

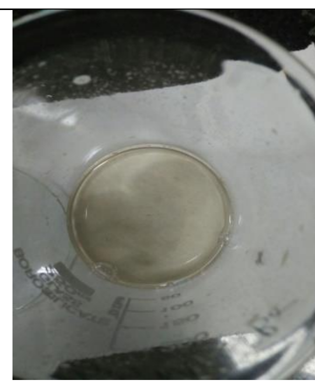
5.2 DISSOLUTION OF SILK FIBROIN

Dissolution of silk fibroin was done in different molar concentrations of different salts. We have prepared solutions of lithium chloride, lithium bromide and ajisawa solution and obtained following results for different concentration solutions:

5.2. 1 Dissolution in LiCl (4.6 M):-

In 4.6 M LiCl solution the silk fibroin was dissolved and heated for 3hrs but the dissolution has not completed and the silk fibroins got converted into small sticky balls that were brown in colour. After keeping for 2 to 3 days lithium chloride got settled at the bottom of the flask and water got separated while the fibroin balls remain scattered over the surface of the salt.

5. 2. 2 Dissolution in Lithium bromide solutions:-

1.5 M	**2.5 M**	**3.5 M**	**4.6 M**
			
Silk fibroin dissolved in 1.5M solution on heating at 60°C get converted into very fine particles	Silk fibroin dissolved in 2.5M solution on heating at 60°C get converted into very fine particles	Silk fibroin dissolved in 3.5M solution on heating at 60°C get converted into very fine particles	Silk fibroin dissolved in 4.6M solution on heating at 60°C get converted into very fine particles

5.2. 3 Dissolution in Ajisawa solution

Ajisawa solution contains calcium chloride, ethanol and water in the molar ratio 1:2:8. When silk fibroin was dissolved in this and heated at 80°C the fibroins got converted into a jelly type layer and was settled down at the bottom surface of the flask. Same is shown in the figure.

5.3 REGENERATION OF SILK FIBROIN:

Dissolve silk fibroin was regenerated by dialysis through cellulose dialysis membrane. After performing the dialysis for one week we were able to separate the lithium bromide solution from the silk particles and then the particles were collected by normal filtration using whattman filter paper. After filtration the silk particles were air dried in the hot air oven maintained at 60°C temperature. The following figures shows the dialysis process employed and the after filtration particles.

Fig 5.3 : Dialysis process.

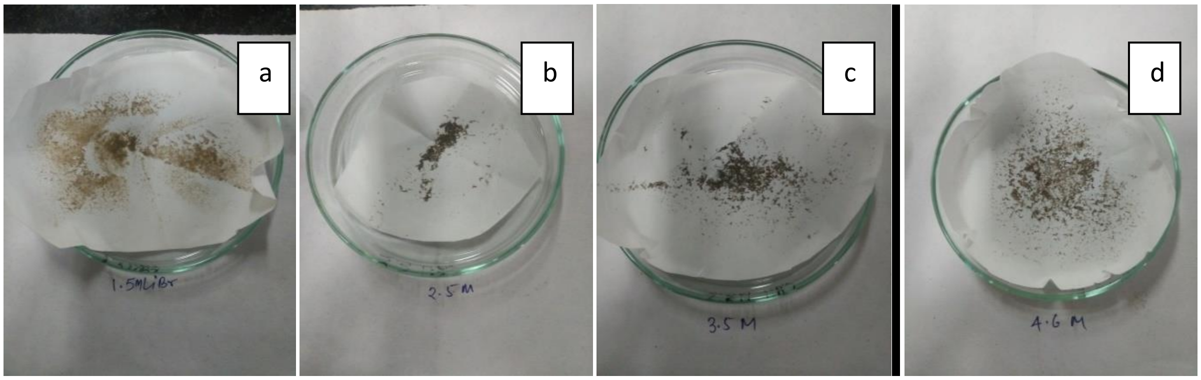
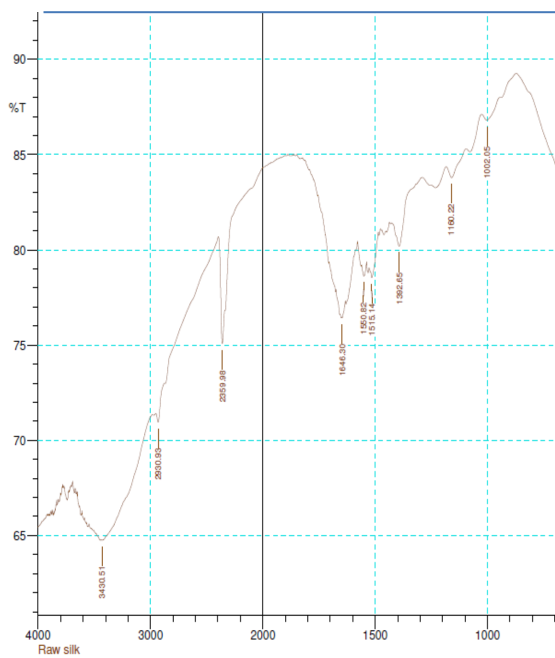


Fig 5.4 : Showing the silk fibroin obtained after dissolution with (a) 1.5M LiBr (b) 2.5MLiBr (c) 3.5M LiBr and (d) 4.6M LiBr.

5.4 CHARACTERIZATION TECHNIQUE

5.4.1 FT-IR of raw silk



3430.51 cm^{-1} – OH (H-bonding)

2930.93 cm^{-1} – sp3 stretch

1646.30 cm^{-1} – amide stretch

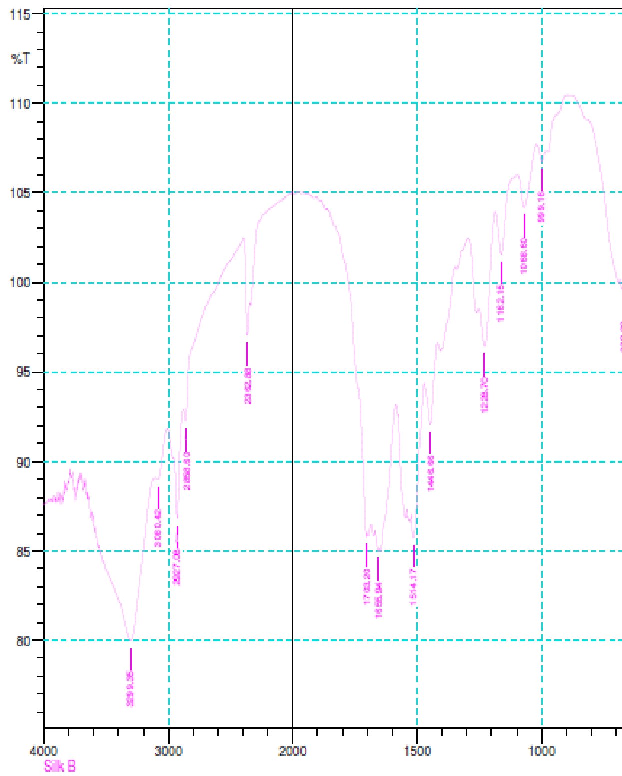
1550.82 cm^{-1} –NH bending

1392.65 cm^{-1} –CH3 bending

1160.22 cm^{-1} –C-N stretch

1002.05 cm^{-1} –C-O stretch

5.4.2 FT-IR of degummed silk



3299.35 cm^{-1} –OH & -NH (stretch) merged

2927.08 cm^{-1} –sp3 stretch

1703.20 cm^{-1} –amide stretch

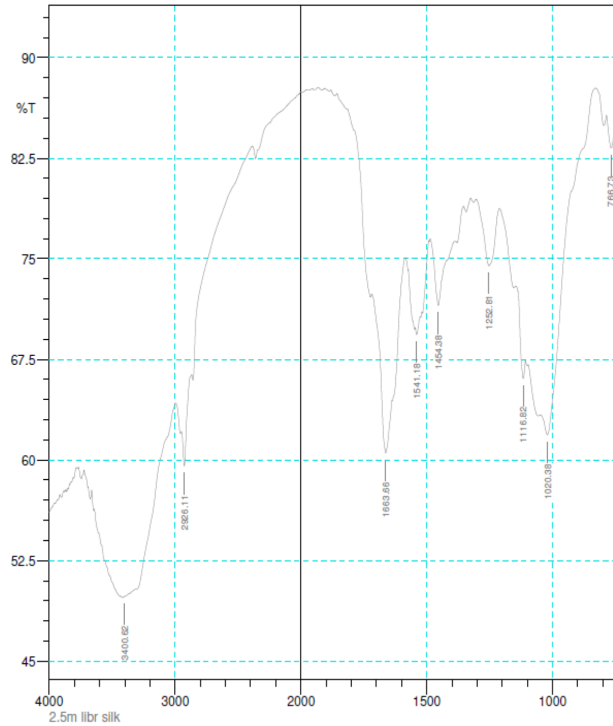
1514.17 cm^{-1} –NH bending

1228.70 cm^{-1} –C-C stretch

1162.15 cm^{-1} –C-N stretch

1068.60 cm^{-1} –C-O stretch

5.4.3 FTIR For particles obtained from 2.5M LiBr solution



3400.62 cm^{-1} –OH & -NH (stretch) merged

2926.11 cm^{-1} –sp3 stretch

1663.66 cm^{-1} –amide stretch

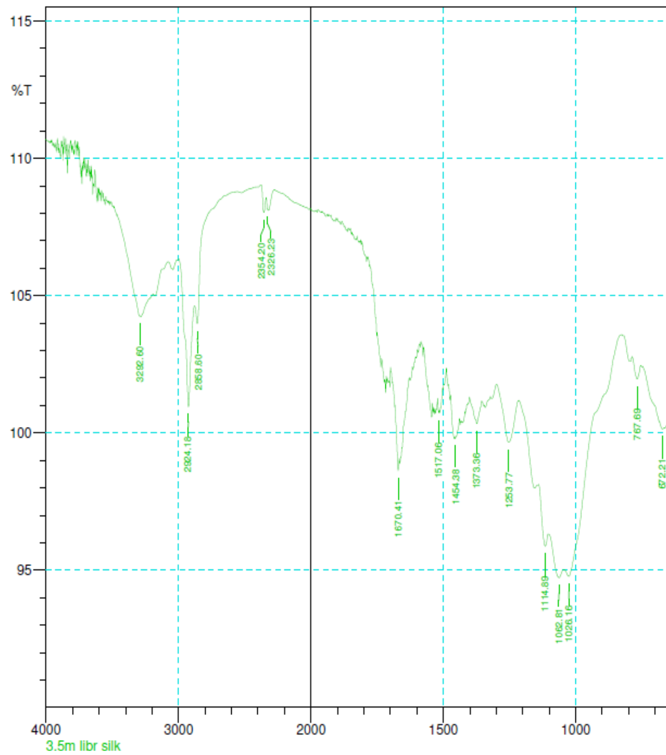
1541.18 cm^{-1} –NH bending

1252.81 cm^{-1} –C-C stretch

1116.82 cm^{-1} –C-N stretch

1020.38 cm^{-1} –C-O stretch

5.4.4 FTIR for particles obtained from 3.5 LiBr solution:



3292.60 cm^{-1} –OH & -NH (stretch) merged

2924.18 cm^{-1} –sp3 stretch

1670.41 cm^{-1} -amide stretch

1517.06 cm^{-1} –NH bending

1373.36 cm^{-1} –CH3 stretching

1253.77 cm^{-1} –C-C stretch

1114.89 cm^{-1} –C-N stretch

1062.81 cm^{-1} –C-O stretch

5.5 CALCULATIONS:

I have degummed 0.368g of raw silk in sodium carbonate solution and after degumming our silk fibroin weighs 0.304g. this means some of the weight is lost in the form of sericin and this weight loss is called degumming loss.

Formula used for finding degumming loss is :

$$\frac{\textbf{Initial weight of fibre – weight of fibroin}}{\textbf{Initial weight of fibre}} \times 100$$

Therefore,

Degumming loss = {(0.368-0.304)/0.368}x100 = **17.39%**

Upto 25% degumming loss is acceptable and if the loss is more than 25% than it indicates that the degradation of fibroin has been started during degumming. And ou degumming loss is nearly 17% so our fibroin has not been decomposed and sericin has been successfully removed.

CHAPTER 6

CONCLUSION

From our experimental wok we can conclude that Bombyxmori silk can be easily converted into fibroins with the help different concentrations of lithium chloride salt and these fibroins can be further used as the alternatives for synthesise of biodegradable composites that can be use for various biomedical applications.

REFERENCES:

1. www.bc.bangor.ac.uk/_includes/docs/pdf/biocomposites

2. http://www.sciencedirect.com/science/article/pii/S1359835X15003115

3. http://www.polymerexpert.fr/en/presentation/histoire-des-polymeres/

4. http://www.chemistry-assignment.com/wp-content/uploads/2013/04/b.png

5. https://www.britannica.com/topic/natural-fiber

6. Nguong, C. W., Lee, S. N. B. and Sujan, D., *"A Review on Natural Fibre Reinforced Polymer Composites", International Journal of Chemical, Molecular, Nuclear, Materials and Metallurgical Engineering; 7:2013.*

7. https://teachingexchange.arts.ac.uk/trog/assets/files/pages/natural.jpg

8. Namvar, F&et.al, *"Potential use of plant fibres and their composites for biomedical applications" (2014 "Cellulosics for biomed uses,"BIo Resources 9(3)*.

9. A.K Bledzki, J Gassan, *"Composites reinforced with cellulose based fibres"*doi: *10.1016/S0079-6700(98)00018-5.*

10. https://texeresilk.com/article/silk_making_how_to_make_silk

11. Altman GH1, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL., *"Silk-based biomaterials."* doi: *10.1002/adhm.201500002*.

12. Chengjie Fu, Zhengzhong Shao and Vollrath Fritz. *"Animal silks: their structures, properties and artificial production". DOI:10.1039/B911049F, Chem. Commun., 2009,0, 6515-6529.*

13. http://factsanddetails.com/world/cat54/sub349/item1224.html

14. http://ih2.redbubble.net/image.7904184.2712/flat,800x800,070,f.jpg

15. http://img02.deviantart.net/0074/i/2008/175/b/c/antheraea_mylitta_larva_1_by_jymsfr.jpg.

15.b http://cdn.c.photoshelter.com/img-get/I0000BUd2hzAAVwM/s/600/600/3035493.jpg

16. http://static.panoramio.com/photos/large/65190639.jpg
https://s-media-cache-ak0.pinimg.com/236x/1f/15/c2/1f15c266cdcf55ff85ca3f8401ebdb63.jpg

17. http://www.stickinsect.org.uk/ERISIL~1-1.JPG,
http://www.dyedinthewool.biz/wp-content/uploads/2015/01/eri.jpg

18. http://www.altawhid.org/wp-content/uploads/2014/07/06100424_400.jpg

19. http://gallery.redorbit.com/national-science-foundation-gallery/183_d9ff670007d54f9c7eadb92b76e8bb55.jpg
20. https://www.pinterest.com/pin/400538960585996541/
21. Yang Cao and Bochu Wang, *"Biodegradation of Silk Biomaterials"* Int. J. Mol. Sci. 2009, 10, 1514-1524; doi:10.3390/ijms10041514.
22. https://www.researchgate.net/profile/Hao_Wang82/publication/235666098/figure/fig4/AS:393483547365381@1470825196523/Fig-4-Structure-of-silkworm-silk-fibre-21.png
23. http://s718.photobucket.com/user/pupesosweet3/media/Deep%20review/CURMIN%20Lift/Silk.png.html
24. http://wwwchem.uwimona.edu.jm/courses/CHEM2402/Textiles/Animal_Fibres.html
25. Sang Muk Leea, Donghwan Choa, Won Ho Parkb, Seung Goo Leeb, Seong Ok Hanc, Lawrence T. Drzald, "*Novel silk/poly(butylene succinate) biocomposites: the effect of short fibre content on their mechanical and thermal properties"*.
26. A.U. Ude , R.A. Eshkoor, R. Zulkifili, A.K. Ariffin, A.W. Dzuraidah, C.H. Azhari, "*Bombyx mori silk fibre and its composite: A review of contemporary developments". DOI: 10.1016/j.matdes.2013.12.052.*
27. Sahba Mobini, Birgit Hoyer, Mehran Solati-Hashjin, Anja Lode, Nasim Nosoudi, "*Fabrication and characterization of regenerated silk scaffolds reinforced with natural silk fibers for bone tissue engineering". DOI: 10.1002/jbm.a.34537.*
28. Raghu K, Khanam PN and Naidu SV, *"Chemical resistance studies of silk/sisal fiber reinforced unsaturated polyester-based hybrid composites", J ReinfPlast Comp, 27: 2008.*
29. Majid RA, Ismail H, and Taib RM*, "Effects of polyethylene-g-maleic anhydride on properties of low density polyethylene/ thermoplastic sago starch reinforced kenaf fibre composites", Iran Polym J, 19 (7): 2010, 501-510.*
30. Farideh Namvar, Mohammad Jawaid, et al., "*Potential use of Plant Fibers and their Composites for Biomedical Applications". (2014"Cellulosics for biomed uses,"BIo Resources9(3).*
31. Zhi Liu, Yu-Qin, Wan Hao, Dou, Ji-Huan He, "*Effect of Na2CO3 Degumming Concentration on LiBr-Formic acid silk fibroin solution properties"*. THERMAL SCIENCE: Year 2016, Vol. 20, No. 3, pp. 985-991.

32. Chengjie Fu, Zhengzhong Shao and Vollrath Fritzb, "*Animal silks: their structures, properties and artificial production". DOI: 10.1039/b911049f.*

33. B. ZUO, L. DAI, Z. WU, "*Analysis of structure and properties of biodegradable regenerated silk fibroin fibers". J MATER SCI 41 (2006) 3357–3361.*

34. Yang Cao and Bochu Wang, "*Biodegradation of Silk Biomaterials*" Int. J. Mol. Sci. 2009, 10, 1514-1524; doi:10.3390/ijms10041514.

35. Yu-Qing Zhang, Wei-De Shen, Ru-Li Xiang, Lan-Jian Zhuge, Wei-Jian Gao and Wen-Bao Wang, *"Formation of silk fibroin nanoparticles in water-miscible organic solvent and their characterization". Journal of Nanoparticle Research (2007) 9:885–900, 2006 DOI 10.1007/s11051-006-9162-x.*

36. Sung-Won Ha, Young H. Park, and Samuel M. Hudson, *"Dissolution of Bombyx mori Silk Fibroin in the Calcium Nitrate Tetrahydrate-Methanol System and Aspects of Wet Spinning of Fibroin Solution." Biomacromolecules 2005, 6, 1722-1731.*

37. Sandeep Kumar & Sandeep Kumar Singh, *"Fabrication and Characterization of Fibroin Solution and Nanoparticle From Silk Fibres of Bombyx mori". DOI: 10.1080/02726351.2016.1154908.*

38. Y. H. Lu, H. Lin, Y. Y. Chen, C. Wang1, and Y. R. Hua, *"Structure and Performance of Bombyx mori Silk Modified with Nano-TiO2 and Chitosan". Fibers and Polymers 2007, Vol.8, No.1, 1-6.*

39. Jinfa Ming, Zhi Liua, Shiyu Bie , Feng Zhang , Baoqi Zuo, *"Novel silk fibroin films prepared by formic acid/hydroxyapatite dissolution method".DOI: 10.1016/j.msec.2013.12.041.*

40. B. D. LAWRENCE, *"Processing of Bombyx mori silk for biomedical applications". DOI: 10.1533/9780857097064.1.78.*

41. David M. Phillips, Lawrence F. Drummy, Deborah G. Conrady, Douglas M. Fox, Rajesh R. Naik,Morley O. Stone, Paul C. Trulove, Hugh C. De Long, and Robert A. Mantz, *"Dissolution and Regeneration of Bombyx mori Silk Fibroin Using Ionic Liquids". J. AM. CHEM. SOC. 2004, 126, 14350-14351 DOI: 10.1021/ja046079f.*

42. M. K. Sah and K. Pramanik, "*Regenerated Silk Fibroin from B. mori Silk Cocoon for Tissue Engineering Applications". International Journal of Environmental Science and Development, Vol.1, No.5, December 2010 ISSN: 2010-0264.*

43. Mihaela-Ramona TUDORA, Cătălin ZAHARIA, Izabela-Cristina STANCU, Eugeniu VASILE, Roxana TRUŞCĂ, Corneliu CINCU, "*natural silk fibroin micro- and nanoparticles with potential uses in drug delivery systems*". U.P.B. Sci. Bull., Series B, Vol. 75, Iss. 1, 2013 ISSN 1454-2331.
44. Huiying WU , Yan ZHOU , Ping YAO , Maomao DING, *"Property of electrospinning silk fibroin nanofibers prepared by different dissolved methods*". DOI: 10.1051/matecconf/20166701011.
45. Hao Zhang, Ling-ling Li, Fang-yin Dai, Hao-hao Zhang, Bing Ni, Wei Zhou1, Xia Yang and Yu-zhang Wu, *"Preparation and characterization of silk fibroin as a biomaterial with potential for drug delivery". 2012, 10:117.*
46. Feng Zhang, Qiang Lu, Jinfa Ming, Hao Dou, Zhi Liu, Baoqi Zuo, Mingde Qin, Fang Li,a David L. Kaplanc and Xueguang Zhang, *"Silk dissolution and regeneration at the nanofibril scale". DOI: 10.1039/c3tb21582b.*
47. Gang Li, Fei Li, ZhaozhuZheng, TingtingLuo, Jian Liu, Jianbing Wu, Xiaoqin Wang and David L. Kaplan, "*Silk microfiber-reinforced silk composite scaffolds: fabrication, mechanical properties and cytocompatibility",.*
48. Xu Wu[1], Xiaodong Wu[1], Bin Yang[1, 2,*], Min Shao[2, *], and Guojin Feng[3], *"Methanol–Water-Dependent Structural Changes of Regenerated Silk Fibroin Probed Using Terahertz Spectroscopy". DOI: 10.1177/0003702817706368.*
49. Hai-Yan Wang and Yu-Qing Zhang*, *"Effect of regeneration of liquid silk fibroin on its structure and characterization". DOI: 10.1039/c2sm26945g.*
50. Zhaozhu Zheng[1,*], Shaozhe Guo[1,*], Yawen Liu[1], Jianbing Wu[1], Gang Li[1], Meng Liu[1], Xiaoqin Wang[1] and David Kaplan[2], *"Lithium-free processing of silk fibroin". DOI: 10.1177/0885328216653259.*
51. Andreas Teuschl[1,2], Martijn van Griensven[1,2], Heinz Redl[1,2], *"Sericin removal from raw Bombyx mori silk scaffolds of high hierarchical order".(doi: 10.1089/ten.TEC.2013.0278).*