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Sericin – A Unique Biomaterial

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Abstract: *Silk is made up of two types of proteins- silk fibroin and sericin. Silk sericin is the main residue (≈20-30%) in raw silk fiber. It is composed of 18 amino acids. Solubility, molecular weight, and gelling properties of sericin depends on the methods of isolation i.e. degumming. Sericin has proved itself as a highly potential biomaterial by wide variety of applications in medicine, pharmaceuticals, cosmetics, biosorbent and other areas. Sericin has a good capacity for use in biomedical and pharmaceutical areas. Sericin coated fabric showed a high degree of bacteriacidal activity. Sericin might be a valuable ingredient for development of antimicrobial textiles. Various groups are still working to explore some untouched areas of application of sericin.*
Keywords *: sericin, adsorption, biosorption, degumming, silk fibroin etc.*

I. Introduction

India is the second largest producer of silk in the world and has the distinction of producing all the four varieties of silk. Presently, India produces nearly16700 mt silk /y reeled silk prices are in the range of Rs 900-1300/kg, the pierced cocoons and waste silk generated at the rearing are sold at Rs 80-100/kg. This waste contributes nearly 30% of total cocoon production.[1, 2]

Silk fiber is made of two types of proteins—silk fibroin and sericin. Sericin contributes about 20-30 percent of total cocoon weight. It is characterized by its high content of serine and 18 aminoacids, including essential amino acids. There are different methods of isolation of sericin from silk filament. Solubility, molecular weight and gelling properties of sericin depend on the method of isolation.[3]

Silk is a continuous strand of two filaments cemented together forming the cocoon of silk worm, Bombyx mori. Silk filament, a double strand of fibroin, is held together by a gummy substance called silk sericin or silk gum. Silk fibroin is the protein that forms the silk filament and gives its unique physical and chemical properties.[4] Silk adapts various secondary structures, including α-helix, β-sheet and crossed β-sheet.[5]

Silk sericin, a natural protein obtained from silk-worm cocoon has a combination of many unique properties such as biodegradability, nontoxicity, oxidation resistance, antimicrobial activity, UV resistance, and absorbs moisture.[6]

It is estimated that out of about 1 million tons (fresh weight) of cocoons produced worldwide approximately 400000 tons of dry cocoons are generated, that have 50000 tons of recoverable sericin. Indian production of 1600 tons of silk can be source of about 250-300 tons of sericin per year [7]. If this sericin protein is recovered and recycled, it would be a significant economic and social benefit.

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Silk Sericin is a highly hydrophilic macromolecular protein comprising of 18 amino acids. Molecular wt of sericin protein ranges from 24 to 400 kDa with predominant amino acid group's -serine (40%), glycine (16%), glutamic acid, aspartic acid, threonine, tyrosine. Thus

it consists of polar side chain made of hydroxyl, carboxyl and amino groups that enable easy cross-linking, copolymerization and blending with other polymers to form improved biodegradable materials.[8]

Various scientists have classified sericin of cocoon shell into two classes: α-sericin and β-sericin. The outer coccon shell is made of α-sericin while inner layer of β-sericin. The α-sericin contains less C and H and more N and O than the β-sericin.[9] Solubility of α-sericin is higher than β-sericin in hot water[10].

II. Structure

Both fibroin and sericin contain similar types of amino acids[11]. The composition of C, H, N and O varies a little between fibroin and sericin as shown in Table 1.

Table 1: composition of fiber and sericin

	Fibers %	Sericin %
C	47.6	46.5
H	6.4	6.0
N	18.3	16.5
O	27.7	31.0

The fibroin is made up of a series of different amino acids as shown in Table 2. The structure is generally a polypeptide structure. The approximate compositions of different amino acids in fibroin from mulberry silks are as follows-

Table 2: Different amino acids present in fibroin (mulberry silk)

Glycine	36.8 %	Valine	2.7 %
Alanine	22.2 %	Phenylalanine	1.5 %
Serine	11.4%	Histidine	0.1 %
Tyrosine	11.1 %	Lysine	0.2 %
Leucine	1.5 %	Threonine	1.3 %
Proline	1%.0	Arginine	0.9 %

Sericin also contains various amino acids like fibroin. The amount of different amino acids is not the same as that of fibroin as shown in Table 3. Total no. of amino acids are constant in both sericin and fibroin. The different amino acids present in sericin are as follows.

Teble 3: Different amino acids present in sericin

	Mulberry silk	Wild silk
Lysine	20-30 %	20-30 %
Serine	16-20 %	7-16 %
Glycine	8-10 %	10-20 %
Aspartic acid	8-12 %	7-10 %
Glutamic acid	-	4-6 %
Arginine	-	5-15 %
Threonine	4-6 %	3-10 %
Histidine	4-6 %	3-6 %
Tyrosine	-	4-6 %

Gulrajani reported that Sericin is chemically a non-filamentous protein. Besides sericin, raw silk also contain other natural impurities namely, fat and waxes, inorganic salts and colouring matter (fibroin-70-80%, sericin-20-30%, wax matter-0.4-0.8%, carbohydrate-1.2-1.6%, inorganic matter-0.7%, pigment-0.2%).[12] Rui also studied the outer layer of the silk fiber and revealed that the sericin content is more in outer layer, where fibroin content is less[13].

Sericin may also be devided into three classes like sericin A, sericin B, and sericin C. Sericin A is outermost layer in silk and it is insoluble in water. It consists of about 17.2 % of nitrogen and amino acids like- serine, threonine, glycine, and aspartic acid. Sericin B is the middle layer and it contains 16.8% nitrogen. On acid hydrolysis this layer yields amino acids of sericin A in addition to tryptophan. Sericin C is the innermost layer and is insoluble in hot water. This layer is adjacent to fibroin and can be removed from fibroin by treatment with hot dilute acid or alkali. It contains sulphur and 16.6 % of nitrogen. On acid hydrolysis this layer yields proline in addition to amino acids of sericin B[14, 15].

III. Isolation Of Sericin From Silk Fiber

The degumming of silk is usually carried out by using chemical or biochemical systems or with water alone under pressure. The processing of raw silk produces about 50,000 tons of sericin, worldwide each year. Major part of it is discarded into the waste water stream, which leads to high chemical oxygen demand (COD) and biological oxygen demand (BOD) level [16]. Therefore, the waste water released by silk industry leads to contamination of water and environment. Various methods have been developed and patented to recover this protein material from degumming liquor. Removal of sericin gum from crude silk is based entirely upon its solubility in water. It may be extracted by dilute solution of sodium carbonate or by hot water extraction of raw silk and followed by evaporation to obtain powder. Soap plus alkali method is another way to extract sericin. Many times sericin is removed by autoclaving for one and half hour under pressure of 600-700 mm Hg (14 lb). A satisfactory yield is obtained by autoclaving at 105^0C for 30 min with good gelling property. These methods are based on adsorption, precipitation, coagulation, evaporation, chromatography and ultra filtration. The recovery of this protein substantially reduces the pollution load in the waste water[17-19]. Silk sericin is recovered by three different processes- high temperature high pressure (HTHP), alkaline and soap plus alkali (SPA), using membrane filtration technology. It has been found that sericin recovered from different methods has different- color, ash content, protein content and different molecular wt. range. The secondary structure of sericin recovered from HTHP degumming has random coil conformation with some β-sheet structure. The sericin recovered from alkaline and SPA degumming liquors has denatured secondary structure with some random coil, β-sheet, and α-helix conformation[20].

IV. Properties Of Sericin

Sericin is a unique protein due to its non-conventional properties which are summarized as follows;

4.1 Molecular Weight

Molecular weight of sericin depends on the method of extraction[28]as shown in Table 4. When sericin is extracted with 1% sodium deoxycholate solution, $M_{w\ is}$ 17100-18460. When it is extracted by hot water it shows molecular wt. of 24000 by gel electrophoresis. When it is extracted by enzyme action mol. wt. is 3000-10000 and when it is extracted with aqueous urea mol.wt. is around 50000.

Table 4 Sericin Molecular Weight by different techniques

S.No	Method of extraction	Molecular Weight
1	1% sodium deoxycholate solution	17,100-18,460
2	gel electrophoresis	24,000
3	Enzyme extraction	3000-10,000
4	aqueous urea[29]	50,000

4.2 Property of gelling-

It consists of random coil and β-sheet structure. Random coil structure is soluble in hot water and as the temperature lowers, the random coil structure convert to β-sheet structure, this results in gel formation[21, 22]. Sericin has sol-gel property as it easily dissolves into water at 50-60^{0}C and again retuns to gel on cooling[23].

4.3 Isoelectric Ph:

In sericin[24] there are more acidic than basic amino acid residues, hence the isoelectric point of sericin is around 4.0

4.4 Solubility:

Solubility of sericin in water decreases when the sericin molecules are transformed from random coil into β-sheet structure. Solubility of sericin may be increased by addition of poly
Sodium acrylate and it may be decreased by the addition of formaldehyde, polyacrylamide or resin based finishes [25-27].

V. Applications

Applications of sericin are very versatile and not yet fully known. These may be categorized as follows-

5.1 As a general adsorbent/biosorbent:

Although ion exchange resins and activated carbons have long been recognized as effective commercial adsorbents for treating industrial wastewaters containing adsorptive pollutants, their high cost and low efficiency have limited their commercial use in actual industrial scenarios. Considering their cost and efficiency, biomass-based adsorbents or biosorbents are more attractive alternatives than ion exchange resins and activated carbons[30].

Silk sericin derived from waste biomass is low cost and effective for removal of acidic dyes and other anionic dyes from water. Sericin is a complex biosorbent rich in amide groups that could be further altered to achieve different adsorption behavior and selectivity for targeted remediation of polluted water. Sericin biosorbent could selectively adsorb precious metals like gold, palladium etc. from solution containing other impurities[30A].

The use of biosorbents for the removal of toxic pollutants or for the recovery of valuable resources from aqueous wastewaters, is one of the most recent developments in environmental engineering[31]. The major advantages of this technology over conventional ones include not only its low cost, but also its high efficiency%, the minimization of chemical or biological sludges, the ability to regenerate biosorbents, and the possibility of metal recovery following adsorption [32].

Adsorptive pollutants like metals and dyes can be removed by living microorganisms, but can also be removed by dead biological material[33]. Feasibility studies for large scale applications have demonstrated that biosorptive processes using non living biomass are in fact more applicable than the bioaccumulative processes that use living microorganisms, since the later require a nutrient supply and complicated bioreactor systems[34]. In addition maintenance of a healthy microbial population is difficult due to toxicity of the pollutants being extracted and other unsuitable environmental factors like temperature and pH of the solution being treated. Recovery of valuable metals is also limited in living cells, since these may be bound intracellularly. For these reasons attention has been focused on the use of non living biomass as biosorbents[35, 36].

5.2 As a adsorbent for removal of trivalent chromium: Amphiphilic core-shell PMA-SS (poly methyl acrylate-silk sericin) nanoshperes were prepared by graft copolymerisation of methyl acrylate and silk sericin using tert-butyl hydroperoxide as initiator. The diameter of MA-SS nanospheres ranged from 100 to 150 nm, and their average size was 115 nm with narrow distribution. The PMA-SS nanospheres were found to be effective in the adsorption of trivalent chromium from aqueous solutions, and the maximum adsorption observed was 4.876 mg Cr^{3+}/g of adsorbent. The adsorption equilibrium can be reached after about 3 h. With the increase in pH values, the adsorption increased obviously. The addition of KCl had a little effect on the adsorption equilibrium. Furthermore, the values of adsorption obtained with using PMA-SS nanospheres were significantly higher that with SS powder use. The PMA-SS nanospheres are considered to have potential applications in wastewater treatment for the removal of heavy metal ions such as trivalent chromium species[37].

5.3 As a wound dressing/wound care material: Sericin has been found to posses wound healing property and can be used as wound healing covering material in the form of film[38].

Sericin, a silk protein, has high potential for use in biomedical applications. Akturk et al (2011) prepared wound dressing membranes of Sericin (S) and Collagen (C) with glutaraldehyde cross-linking at S/C; 2:1, 1:1, 1:2, and 0:1 weight ratios. They were stable in water for 4 weeks. However, increasing the proportion of sericin had decreasing effect on the membrane stability. Water swelling property of membranes was enhanced with sericin.[31] Wound dressing materials have evolved significantly in the past quarter century. An ideal wound healing material should be biocompatible, protective from secondary infections and should prevent water loss while controlling water vapor and oxygen permeabilities. In addition to these, wound dressing should have mechanical properties compatible with the skin and improve the healing process by actively attracting the cells to the wound area [40-42]. Overall results suggest that sericin/collagen membranes would be favorable as wound dressing material when sericin ratio is less than or equal to the collagen component[39].

Fibroin and sericin when sulphonated show antithrombic effect [43]. Silk sericin membranes are good bandage materials and the film has adequate flexibility and tensile strength. Sericin is a novel wound coagulant material because of its biocompatible and infection resistant nature. Its flexibility and water absorption properties promote smooth cure for defects in the skin and do not cause any peeling of the skin under regeneration when detached from the skin[44].

5.4 As a raw material for making contact lenses:

Silk sericin has the potential to find application in the development of contact lenses. The graft polymers are prepared with methyl methacrylate or styrene and are also biocompatible[45, 46]. Oxygen permeable membranes are made up of fibroin and sericin with 10-16 percent water and are used for contact lenses and as artificial skin[51].

5.5 As a medicine for improving digestion and curing digestive system:

Intake of sericin containing food relieves constipation, suppresses development of bowel cancer and accelerates the absorption of minerals. In rats consumption of sericin elevates the apparent absorption of zinc,iron, magnesium, and calcium by 41,41,21, and 17 % respectively[47].

Sericin when taken orally causes a dose dependent decrease in the development of colonic aberrant crypt foci. The incidence and the number of colon tumers are suppressed by consumption of sericin. Sericin have antitumour activity[48-50].

5.6 Improvements in properties of synthetic fabrics:

P.N.Bhat et al has conducted a study on cross linking of sericin on polyster fabric in 2010 and concluded that· High pressure, High temperature extraction technique is the best method of extraction of sericin. It also provides the purest form of sericin. The sericin stored in the dry form (powder) is also an convenient storage method and does not involve any preservative. Sericin can be fixed by both Formaldehyde and glutaraldehyde fixatives. The change in the concentration of the cross linking agents change the properties of the treated fabrics. Higher concentrations of Formaldehyde and glutaraldehyde fixatives are not necessary for the optimum cross linking. Higher concentrations of cross linking agents not only change the properties of the treated fabrics but also deteriorate the structure of fabric[52].

Functional properties of some synthetic fibers can be improved by coating with silk sericin protein. Sericin modified polyster has been reported by Yamada and Matsunaga and Wakawayasi and Sugioka[53]. Sericin modified polyster is five times more hygroscopic than untreated polyester. Although servicing application on textiles for antibacterial property enhancement has not been reported as yet, it has been found that sericin (4% w/v) treated PET fabric shows 51% reduction of P.Vulgaris and 38% reduction of S.aureous[40] and has a potential for such an application[54].

5.7 Improvements in properties of woolen fabrics:

The results of a study showed that sericin has an affinity for wool, whereas it does not have any affinity for cotton. Sericin was fixed on wool fiber under defined conditions with an exhaustion rate of about 48% for a concentration of 2.5% (w/w) (compared to the mass of sample). Concerning the effect on wool-treated fabrics; a percentage of sericin 5% (w/w) improved the touch of wool fabrics samples until a score of 4 points, as well as the absorption of water with a profit of 0.75%. The samples also showed an improved antibacterial activity. These analyses reveal the multifunctionality of sericin as a finishing agent, it improve both fabrics absorption and hand with an acceptable clear brown shade. In industry, these finishing effects are typically obtained by the use of toxic chemicals. Thus, sericin could be the investigative focus of interest to be used as a biodegradable product with significant finishing effects, because of its available properties and reactivities. Yet, it is interesting to improve the sericin exhaustion rate by using more sophisticated treatments such as grafting or cross-linking and to apply it on synthetic fibers such as polyamide and polyster[55].

5.8 Use of sericin as a finishing agent A new approach to use a natural material, sericin or adhesive silk protein to provide healthy environment is a promising future. Silk sericin could be coated onto nylon and polyster fibers and has a strong potential to be used for indoor air filters to reduce the amount of toxic free radicals, fungi and micrococcus type of bacteria. By using a simple coating technique, the sericin waste can increase the value of air filter[56].

5.9 Cosmetic application: Hence recovery of silk sericin from degumming liquor or waste cocoons not only helps to reduce the environmental pollution but also is highly desirable as the recovered sericin has a lot of commercial value finding application in creams and shampoos as a moisturizing agent and also an important biomaterial for several applications including textiles[57].

Sericin alone or in combination with silk fibroin has been used in skin, hair and nail cosmetics. Sericin when used in the form of lotion, cream and ointment shows increased skin elasticity, antiwrinkle and antiageing effects[58-60].

5.10 Antimicrobial use: Rajendran et al[61] developed a simple and effective method for extracting sericin from the cocoons of B. mori silkworm using chilled ethanol precipitation method. They focused on studying the antimicrobial property of cotton fabric coated with sericin obtained by this method. The sericin-coated fabric showed a high degree of bactericidal activity against test organisms E. coli and S. aureus used in this study. In the FTIR spectra, it showed distinct amide peaks[61].

VI. Conclusion

A large amount of silk sericin goes into waste from silk industry and increases pollution load in industrial effluent. But it may be recovered and reused. Sericin may be isolated from silk in various ways. Its mol. wt. and other properties depend on the method of extraction. Silk sericin, not recognized till now, is a highly valuable biomaterial with infinite application potentials. It may be used as a good adsorbent/biosorbent for the removal of dyes, other toxic pollutants and heavy metals like trivalent chromium from industrial effluent. Sericin has found its way in medical and health sector both in vivo and in vitro. It may be used as a wound dressing material, making contact lenses and improving or curing digestive system. It may be used to improve the properties of synthetic fabrics and woolen fabrics. It has antimicrobial property and it can be used as a finishing material for textile fabrics. It has many cosmetic applications also, such as- in formation of various creams, lotion, shampoos and ointment etc.

All above applications proved that sericin, a bi-product of silk industry can play a key role as an effective biomaterial of future.

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