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Bomyxs Mori Silk Fibroin Fiber Cleaning Methods

M. A. Sodikova
Laboratory of biologically active macromolecular systems of the Academy of Sciences of the Republic of Uzbekistan

Sh. A. Shomurotov
Doctor of Chemical Sciences, Associate Professor, Laboratory of Biologically Active Macromolecular Systems of the Academy of Sciences of the Republic of Uzbekistan

A. Karimov
Tosheknt Pharmaceutical Institute, Department of Organic Synthesis, Doctor of Chemical Sciences, Professor

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ABSTRACT

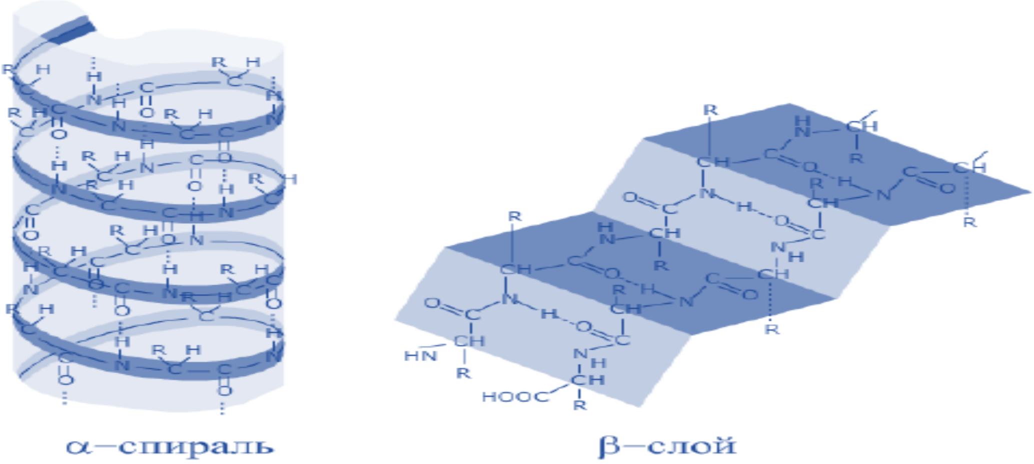
Today, there are several scientific directions in the field of creating biological polymer materials, which are engaged in the study of biopolymers and bicomposites based on natural and synthetic polymers, as well as modification of synthetic polymer compositions to accelerate the destruction of their matrices. Among the methods of changing the properties of basic polymers, the most important is polymer modification. Modification of polymers should be understood as a purposeful change of their properties by carrying out chemical reactions to the functional groups present in the polymer or by changing its supramolecular structure. This definition limits the modification of polymers by processes of changing the structure of macromolecules in the polymer block and their phase state. The second component of the definition is very important because the physical heterogeneity of macromolecular compounds affects their properties.

Virtually inexhaustible resources, unique properties and biodegradability of natural polymers cause them constant interest of scientists and technologists. From cellulose and chitin polysaccharides, which are reproduced in large quantities, polypeptides - fibroin and keratin - are undoubtedly distinguished. Cellulose, fibroin and keratin have been widely used by mankind for centuries. It can be processed into fibers and films through solutions during recycling. As we know, in addition to cellulose, chitosan and collagen, silk is the most common natural polymer. In addition, silk has been widely recognized as a raw material in the textile industry for thousands of years. Silk was first discovered in 2500 BC. Silk has historically been recognized as the queen of textiles for its unique luster, tactile properties, durability, mechanical strength, flexibility, breathability, and comfort in hot or cold weather. Fibroin is the central nucleus covered with a layer of atrophy sericin. Silk protein shares similarities with proteins such as collagen, elastin, keratin, fibroin, and sporgin and is an important component of cocoon fiber. In the last decade, the demand for biocompatible, biodegradable and bioresorbable materials has been increasing. In particular, biodegradability is an important feature of this biomaterial. Natural biodegradable polymers such as collagen, gelatin, chitosan, and silk fibroin have distinct advantages over synthetic polymers due to their distinct properties, including excellent biocompatibility, biodegradability, and bioresorption (bioabsorbability). Their physical and chemical properties can be easily modified to achieve desired mechanical and degradation properties.

Fibroin-based biomaterials have been proven to enhance cell migration, adhesion, proliferation and tissue repair in vivo. The biofilm form of silk fibroin is its important form. Its applications extend from connective tissues such as artificial skin, tendon (ligament), skin cell culture to biofilms for cell engineering, and also in drug delivery systems.

Extraction of fibroin from various natural silk raw materials and preparation of polymer compositions based on fibroin-polysaccharides, research of their composition, structure, physico-chemical and medical-biological properties is urgent.

Bombyx mori silk fibroin, which forms the central core of the silk fiber and is covered with a sericin coating. Silk fiber consists of fibroin (72-81%), sericin (19-28%) and other oils and waxes. Fibroin serves as the inner core of the silk fiber and provides mechanical strength. According to the chemical composition, Bombyx mori fibers consist of at least 16 amino acid residues, the ratio of which varies between different regions of the fibroin supramolecular structure. The total mole fraction of glycine, alanine, serine and tyrosine residues is 90%; their sequence is represented by a general formula. Figure 1 shows the projections of segments of macromolecules forming a-helix and b-sheet structures. The α-helical structure is formed by hydrogen bonds within the molecule, and the hydrophobic fragments move to the periphery. In the β-folded structure, macromolecules are arranged parallel or antiparallel, forming a sheet or layer (β-sheet).



1-rasm

Although sericin is protein in structure, unlike fibroin, it causes allergies in the body and has side effects. Therefore, fibroin requires purification from sericin before its use for medical purposes.

There are several ways to extract fibroin from silk. In the textile industry, in order to remove sericin from silk fiber, it is carried out in boiling water or using detergents [1]. In laboratory conditions, it is done using sodium carbonate solution [2]. Sericin-free fibroin fiber or thread is not considered a universal material for obtaining medical devices. It is necessary to obtain a fibroin solution when obtaining bases (frames) for various medical devices, biomaterials in the form of films, as well as when using them for other medical purposes.

Dehydrated calcium chloride or lithium bromide solutions are often used to obtain fibroin solutions [3, 4]. There are some toxicity concerns when using lithium bromide. Lithium is an essential element for the human body [5] and has been used in the treatment of some mental disorders. But due to the high side effects on the body, its use in these treatment methods has been stopped [6]. If these salts are used, after obtaining the fibroin solution, it is necessary to clean the solution from these salts. The dialysis method is used to remove salts from the fibroin solution. In this study, we studied the effects of boiling water, detergent, and sodium carbonate solution on the efficiency of fibroin extraction from silk fiber and its properties, as well as the effect of dialysis duration on the removal of fibroin solutions.

We used Bombyx mori silkworm cocoons in our research. Initially, the cocoons were crushed to increase the surface area. The ground cocoons were freed of sericin in three different ways. In all methods, the silk:solution was obtained at a modulus of 1:100 and was carried out by heating at 80 ^{0}C for 1 hour. In the first method, distilled water was used as a solvent. The second method was carried out with the addition of a neutral detergent to distilled water (3:4 ratio of cocoon fragments: detergent). The third method was carried out by adding sodium carbonate (cocoon pieces:Na_2CO_3 in a ratio of 10:1) to deionized water. After stopping the process in all three methods, the silk was washed several times with distilled water and air-dried.

The characteristics of fibroin obtained by the above three methods were studied and its structure was studied using the methods of physical and chemical analysis. As a result of research, it was shown that a small amount of sericin was preserved in the fibroin extracted by the first and second methods, i.e. with distilled water and detergent, and there was no sericin in the fibroin purified using sodium carbonate. Ajizawa reagent (calcium chloride:ethanol:water (1:8:2) molar ratio solution) was used to dissolve the isolated fibroin in our further studies. Fibroin was dissolved in ajizawa solution (fibroin:ajzawa solution in the ratio of 1:20) at a temperature of 55 ^{0}C for 1 hour under constant stirring.

The fibroin solution was dialyzed to remove salts. The dialysis process was carried out for 1-7 hours in distilled water using dialysis bags with a pore diameter of 3 kDa. Complete purification of fibroin from salt ions was determined by qualitative reaction with chloride ion $AgNO_3$, and by measuring the electrical conductivity of the solutions.

Studies have shown that the concentration of salt ions decreases as a result of constant replacement of the dialysis water of the fibroin solution, and the solution is significantly cleaned of salts within 5 days. However, for medical purposes, fibroin needs to be completely desalted. Obtaining such fibroin was achieved by carrying out the dialysis process for up to 7 days. Thus, after evaluating the three separation methods, it was found that the sodium carbonate treatment method can separate sericin and fibroin more easily and efficiently than the distilled water and detergent treatment. The effectiveness of the dialysis method in cleaning the solution of fibroin in Ajizawa reagent from salts was demonstrated. In particular, it was determined that dialysis should be performed for 5 days to significantly remove salts from the solution, and 7 days for complete cleaning. After that, the fibroin solution was centrifuged (3500 rpm 10 min) (centrifuge CenLee 20K Power supply AG220v)

After cleaning the fibroin solution from salts, it was dried in a lyophilizer in order to obtain pure fibroin proteins from the fibroin solution in powder form. (10-12 hours).

Bombyx mori silk fibroin purification process

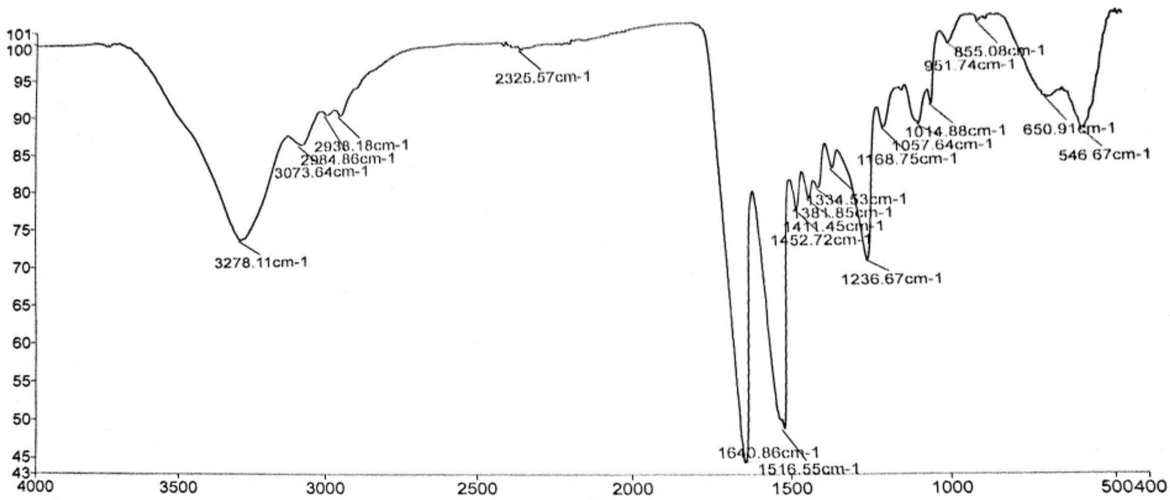
2-picture

Bombyx mori silk fibroin purification process

1-table

№	Cleaning process	Solutions used	Temperature	Time
1.	Cleaning process	0.1M li Na_2CO_3	80 ^0C	1- hour
2.	Melting process	(calcium chloride: ethanol: water (1:8:2)	55 ^0C	1- hour
3.	Dialysis process	dis-water	At room temperature	5-7 day

After that, the structure of the fibroin obtained in the powder state was studied using the IR-spectroscopy method and the following conclusions were drawn. 3278 sm^{-1} in the field –NH an 1638 – 1640 sm^{-1} in the field – COOH absorptions characteristic of vibrations of groups, 1516 sm^{-1} in the field – CO-NH- group-specific absorptions, 2938 sm^{-1} in the field – CH_2 , 2984 sm^{-1} tertiary in the field -CH- group-specific absorptions, as well 1638,1516 and 1236 in the fields Amid I, II and III specific absorptions have been determined.



Compositions can be obtained by combining fibroin protein isolated from silkworm cocoons with various polysaccharides. For example: currently, silk threads are used in surgical operations on various organs: lips, eyes, oral cavity operations, and are used in the treatment of skin wounds. As the properties of silk fibroin are being studied, it is increasingly being used in other areas of biomedicine.

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