The Use of Fibroin-Based Biopolymers in the Medical Field and the Analysis of Fibroin Content in Silk Fiber Based on Chromato-Mass Spectrometry

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ABSTRACT

Fibroin was isolated from cocoons and chemical analysis was performed were analyzed by gas liquid chromatography and chromatography-mass spectrometry (GC-MS) to identify bioactive compounds in methanol, ethanol and acetone solution extracts. The composition and amount of amino acids in fibroin was studied. The presence of a number of biologically and chemically active substances in fibroin was checked and the validity of their use for the treatment of various wounds and silk fabrics and fibroin films for various diseases was determined.

Keywords: Silkworm, Fibroin, gas liquid chromatography, chromato-mass spectrometry

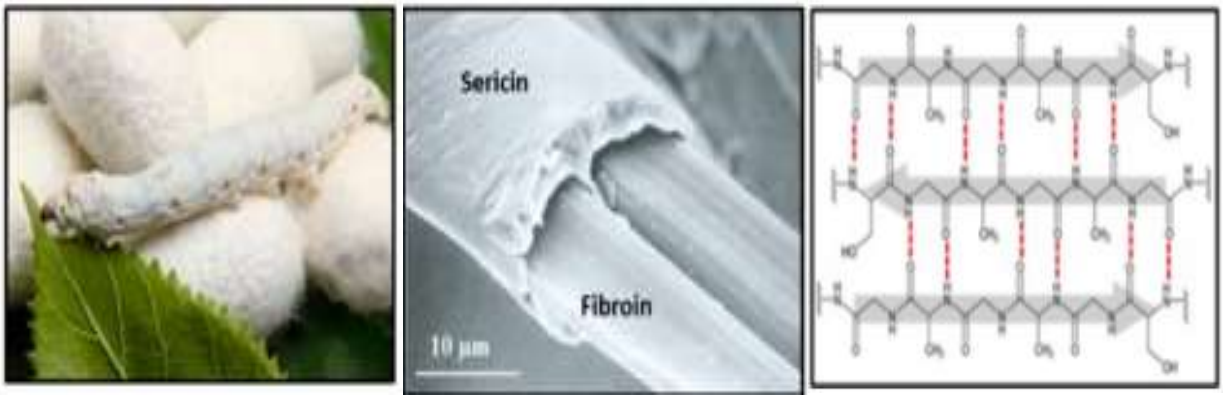
It is known that today, on a global scale, more and more attention is paid to the problem of creating biological polymer materials in pharmaceutical enterprises, waste reduction, and in the medical field. The development and study of such materials is one of the priority directions of the development of science. Today, there are several scientific directions in the field of creating biological polymer materials, which are engaged in the study of biopolymers and composites based on natural and synthetic polymers, as well as modification of synthetic polymer compositions to accelerate the destruction of polymer matrices.

The range of polymers produced by industry is very limited, and the needs of society are very large. Therefore, the main task of macromolecular chemistry is the creation of polymeric materials with the widest range of chemical and physical-mechanical properties. Among the methods of changing the properties of basic polymers, the most important is polymer modification. The modification of polymers should be understood as a purposeful

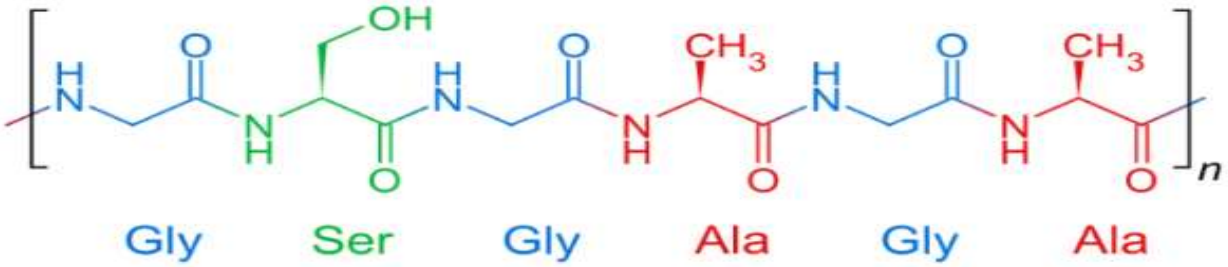
change of their properties by carrying out chemical reactions to the functional groups present in the polymer or by changing its supramolecular structure. Such a definition limits the modification of polymers by the processes of changing the structure of macromolecules in the polymer block and their phase state. The second component of the definition is very important, since the physical heterogeneity of macromolecular compounds affects their properties.

As we know, in addition to cellulose, chitosan and collagen, silk is also one of the most widespread natural polymers. In addition, silk has been widely recognized as a raw material in the textile industry for thousands of years. Silk was discovered for the first time in 2500 BC. Silk has historically been recognized as the queen of textiles due to its unique luster, tactile properties, durability, mechanical strength, flexibility, breathability, and comfort it provides in hot or cold weather.

The external coating of silk with sericin can cause hypersensitivity reactions. In addition, sericin can have various effects, enhance allergic reactions and immunity, and can be at the same level as fibroin in terms of effects. Regardless of the presence of sericin, other soluble compounds may be the main cause of silk-induced effects. However, the process of separating sericin from fibroin remains an important step. Because if we pay attention to the chemical composition of silk, it consists of fibroin (72-81%), sericin (19-28%). Fibroin serves as the inner core and provides mechanical strength, while sericin is the coating, like an outer glue. Fibroin is currently being researched for the treatment of various types of wounds.



Silk is a biomaterial that is "clinically proven" for human life. Fibroin is a heterodimer, consisting of two protein chains, which are interconnected by disulfide bonds. Fibroin-L and fibroin-H and they are glycine-43%, alanine-30%, serine-12%, small amount of thiosine-5%, valine-2% in terms of quality composition. It is important to separate fibroin from sericin in order to use fibroin for medical purposes and to obtain polymeric products that are used for various types of wounds by connecting fibroin with dextran.



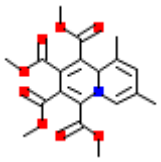
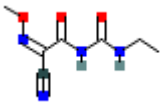
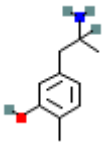
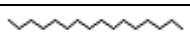
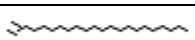
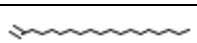
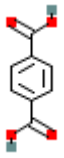
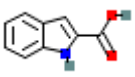
Silk fiber materials can be produced using a variety of methods, depending on the desired materials. However, it was necessary to remove sericin, regardless of the method of use in what fields. In order to remove the sericin, first, the raw silk nut must be cleaned. To carry out this

process, the raw silk cocoon is boiled in a 50% dilute solution of sodium carbonate (Na_2CO_3), then washed with clean water and the washed cocoon is dried overnight (24 hours) to remove the fibroin threads. During boiling, the concentration of Na_2CO_3 should be carefully controlled to avoid adverse effects that may occur as a result of the process. Long-term boiling of Na_2CO_3 at a concentration higher than 50% leads to the breaking of disulfide bonds between H- and L-fibroin and the breakdown of amorphous chains to the formation of fibroin with a polydispersity molecular weight.

In order to clean and use silk-based materials, the most widely used method is to dissolve fibroin and transfer it to various materials. Various solutions are used for this purpose. For example, (lithium bromide aqueous solution), AJIZAV reagent (calcium chloride: ethanol: water 1: 8: 2: water solution), calcium nitrate aqueous solution, is used for dissolving fibroin.

Each melt system offers a different melt strength and therefore requires different melt times and temperatures. After that, the electrolytes were removed by dialysis against pure water, and aqueous solutions of fibroin were obtained. At this stage, an aqueous solution of polyethylene glycol (PEG) 20% is used instead of pure water. It is used to obtain a concentrated solution of fibroin. Dialysis repeated several times depending on its concentration.

The fibroin extracted from these processes is used to obtain modified polymers based on dextran. Fibroin-based materials are currently used in a variety of fields, in plastic surgery, as well as in human clinical trials, for the preparation of wound healing or membrane repair films, by separating fibroin from sericin. Separated fibroin was obtained by different physical research methods (spectra based on Chromato-Mass spectrometry) and the analysis results were analyzed in the following table.

№	Minute	Percentage of fibroin	Substance name	The formule of the Substance	Molecular formule
1	3.272	6.98	Decane		$C_{10}H_{22}$
2	3.43	4.87	3.6-Dimethylpiperazine-2		$C_6H_{14}N_2$
3	4.917	8.04	Dodecane		$C_{12}H_{26}$
4	4.963	11.77	Dodecane, Hexadecane, 7,9-dimethyl-		$C_{19}H_{21}NO_8$
5	5.56	5.13	Piperazine		$C_4H_{10}N_2$
6	5.288	6.12	2-cyano-N-[9ethylamino0 carbonyl] – 2 – (methoxyimino)		$C_7H_{10}N_4O_3$
7	6.330	17.66	Tetradecane		$C_{14}H_{30}$
8	6.956	18.21	5-(2-Aminopropyl) - 2 methylphenol		$C_{10}H_{15}NO$
9	7.720	5.22	Methylpent-4-enylamine		$C_6H_{13}N$
10	15.104	0.03	Cetene		$C_{16}H_{32}$
11	15.238	0.01	Hexadecane		$C_{16}H_{34}$
12	18.759	0.03	E-15-Heptadecenal		$C_{17}H_{32}O$
13	18.874	0.01	Octadecane		$C_{18}H_{38}$
14	22.095	0.02	3-Eicosene		$C_{20}H_{40}$
15	25.150	0.02	1-Docosene		$C_{22}H_{44}$
16	27.955	0.01	1-Nonadecene		$C_{19}H_{38}$
17	31.015	0.01	Hexamethyl-tris		$C_6H_{18}N_3Si$
18	32.470	0.01	Terephalic-acid		$C_8H_6O_4$
19	33.898	0.01	1H-Indole-2-carboxylic acid		$C_9H_7NO_2$

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