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Chitosan Fibres Modified by Fibroin

Abstract

This article presents research results concerning the formation of chitosan fibres modified by silk-fibroin. Silk-fibroin isolated from the *Bombyx mori* silkworm cocoons and chitosan were used for our investigations. In order to find advantageous fibre-formation conditions, the influence of bath composition and spinning solution composition on the silk-fibroin content in the bath were tested. The best coagulation bath for spinning solution with 5% silk-fibroin content was the aqueous solution of NaOH at concentration of 10%. In the case of spinning solution with 10 and 15% of silk-fibroin the best coagulation bath was aqueous solution of NaOH at concentration of 10% with an ethyl alcohol in the ratio of 70:30. The chitosan/silk-fibroin fibres obtained under these conditions were characterised by a silk-fibroin content from 4,5 to 11% and by mechanical properties sufficient for their further processing into dressing materials.

Key words: chitosan, silk-fibroin, chitosan/silk-fibroin fibres.

Silk-fibroin is one of the natural polymers which has been extensively studied in recent years. It is a fibrillar protein produced by the silk-worm larvae *Bombyx mori* in form of the well known natural silk. Products obtained from natural silk are characterized by good oxygen and water vapour permeability and high biocompatibility with living organism [8-12]. Macromolecules of silk-fibroin are built up mainly of three following amino acids: glycine (Gly), alanine (Ala) and serine (Ser) amino-acids in the 3:2:1 proportion. This composition of macromolecules enables their high orientation in the form of β -sheet structures which is responsible for unique natural silk's mechanical properties and its resistance to the majority of the solvent. Furthermore, research conducted over recent years has revealed a lot of additional properties useful in the wound healing process such as the positive influence on fibroblasts and osteoblasts which accelerates the filling of skin defects with granulation tissue [13 - 16].

Chitosan is well known nature-derived biopolymer characterizing unique biological properties that are, like in fibroin, particularly useful in wound healing, such as biostimulation of wound healing, acceleration of tissue reconstruction [17 - 19].

It is expected that by combining the two biopolymers, new materials can be made with unique properties surpassing those of the single components.

The purpose of the work presented here was to recognize the possibility of preparing chitosan/silk fibroin biomaterials in form of fibres that could be suitable for a future construction of dressing materials.

Materials and reagents

Chitosan characterised by viscometric molecular weight of 700 kDa and deacetylation degree of 88% was supplied by the LA NI Co Ltd., Vietnam.

The *Bombyx mori* silkworm cocoons were received from the National Institute of Agrobiological Science, Tsukuba (Ibaraki), Japan.

Methodology

Preparing the chitosan solution

Chitosan was dissolved in aqueous acetic acid solution of 3% wt. concentration in order to obtain a solution of 5% wt. chitosan concentration. The dissolving process was carried out in a mixer equipped with high-speed stirrer and was performed over 120 minutes. The chitosan solution was filtered and degasified over 20 hours.

Preparing the silk-fibroin solution

The cut cocoons were degummed in aqueous solution of a 2% wt. concentration of soap containing 0.5% wt. of sodium carbonate (module 1:50), at the temperature of 95 °C over 30 minutes, followed by washing several times with hot water and drying at room temperature. Next the cocoons were treated by mixture of chloroform and methanol in the ratio of 2:1 v/v, all the time shaken over 72 hours, and finally squeezed and dried at room temperature. The isolated silk-fibroin was dissolved in a saturated LiBr solution, followed by dialysis against water over 72 hours with use of dialysis sacks with cut-off of 12.4 kDa. The aqueous silk-fibroin solution was concentrated to a concentration of 13% [20].

Introduction

An ideal dressing material has not been found yet, despite enormous progress in material engineering [1]. Modern dressings should be bio-compatible, protect against secondary infection, accelerate wound-healing processes, exhibit biodegradability caused by bodily fluids [2 - 4]. This is the reason why investigations in that direction are still under way. Also in the Institute of Biopolymers and Chemical Fibres research has been begun in cooperation with the Japan National Institute of Agrobiological Sciences to prepare novel biomaterials designed for dressings [5 - 7]. Amongst the most promising materials for that application are chitosan/silk-fibroin biomaterials in the form of fibres intended for manufacturing dressing materials.

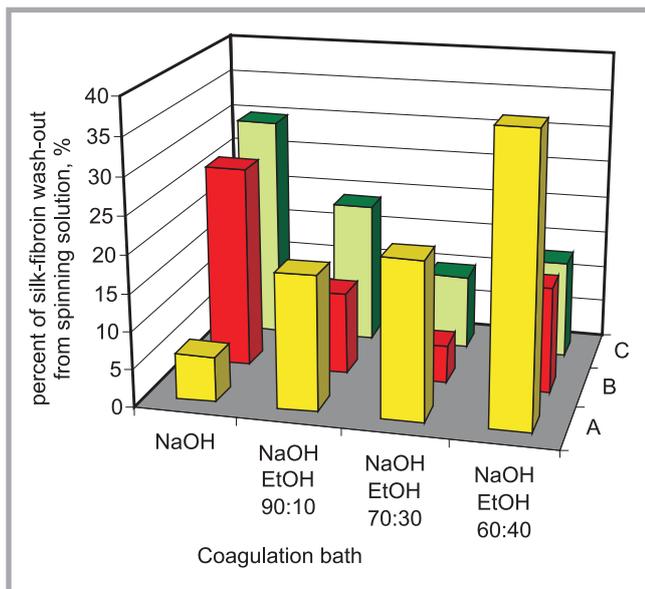


Figure 1. Influence of the coagulation bath and spinning solution composition (chitosan:silk-fibroin: A) 95:5, B) 90:10, C) 85:15) on the percent of silk-fibroin washed-out from the spinning solution on relation to the initial content.

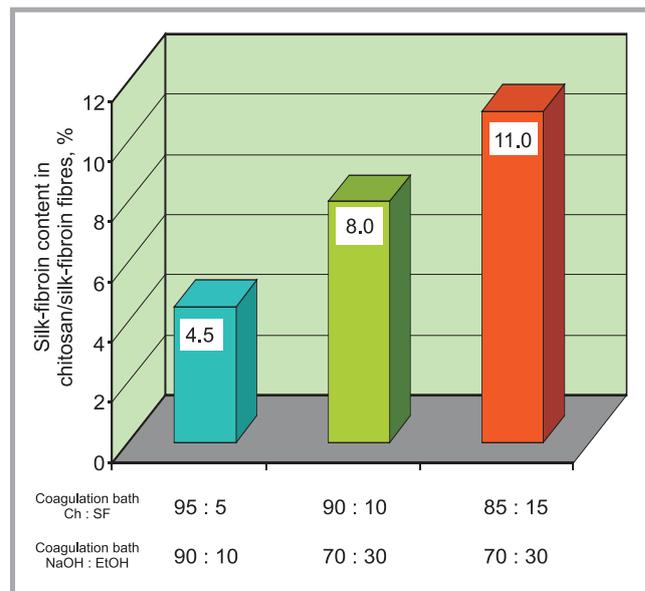


Figure 2. Silk-fibroin content in the chitosan/silk-fibroin fibres in dependence on the spinning solutions and coagulating bath composition.

Preparing spinning solution

The chitosan solution and the silk-fibroin solution were mixed at different ratios, and spinning solutions of the following chitosan to silk-fibroin weight ratios were obtained: 95:5; 90:10; 85:15 (all values re-calculated on the dry mass of both polymers content of 6.83%, 7.15% and 7.48% respectively). The spinning solutions obtained were used for spinning chitosan/silk-fibroin fibres after degastation [21].

Spinning chitosan/silk-fibroin fibres

An experimental spinning machine constructed in the IBWCh was used for spinning the chitosan/silk-fibroin fibres. The spinning solution was fed into a coagulation bath with the use of a gear pump with an efficiency of 3 cm³/revolution through a 300-holes spinneret, each hole having a diameter of 0.08 mm. Aqueous solution of NaOH and concentration of 10% wt. with an addition of ethyl alcohol in the ratio of 90:10, 70:30 and 60:40 v/v was used as the coagulation bath. The spinning process was conducted at a speed of 16.8 m/min. The fibres

formed were drawn in air at drawing ratio of R = 36%, washed with water and ethyl alcohol/water mixture, drying and winding onto spool [21].

Analytical methods

The viscometric average molecular weight of chitosan was determined by the viscometric method [22] and degree of deacetylation by potentiometric titration [23].

The silk-fibroin content in the chitosan/silk-fibroin fibres was estimated by determining the nitrogen content by the Kjeldahl method, in accordance with the GLP procedure.

The mechanical properties of the fibres were tested using textile tester Instron from (USA) in accordance with the following standards: PN-EN ISO 5079:1999 and PN-ISO 1973:1997.

The fibres' surface and cross-sections were estimated with the use of a Quanta 200 scanning electron microscope, from

FEI (USA), in accordance with the GLP procedure.

Results and discussion

Investigation into the process of spinning chitosan/silk-fibroin fibres

The spinning solutions obtained as the result of mixing solutions of chitosan and fibroin were homogenous and stable. The first test of spinning fibres indicated problems with the coagulation process efficiency of the silk-fibroin component, which resulted in a smaller content of the silk-fibroin component in the fibres than in the spinning solution. Therefore in order to determine the most advantageous spinning conditions for the silk-fibroin fibres from the point of view of minimizing the silk-fibroin losses, a series of coagulation tests were carried out which allowed us to determine the influence of the coagulation bath's composition on the silk-fibroin content in the coagulation bath after coagulation process. Figure 1 presents the research results of the coagulation bath composition's influence

Table 1. Selected mechanical properties of chitosan/silk-fibroin fibres.

Type of fibers	Silk Fibroin content in Ch/SF fibre, %	Linear density, dtex	Variation coefficient of linear density, %	Tenacity in conditioned state, cN/tex	Elongation at break in conditioned state, %	Variation coefficient of elongation at break in conditioned state, %
Chitosan	0.0	3.04 ± 0.03	0.76	17.2	9.2 ± 0.5	14.6
Ch/SF I	4.5	2.99 ± 0.05	1.55	15.9	8.5 ± 0.7	19.2
Ch/SF II	8.0	2.75 ± 0.03	3.55	15.2	8.1 ± 0.9	22.0
Ch/SF III	11.0	2.60 ± 0.02	5.46	13.4	7.8 ± 1.1	27.0

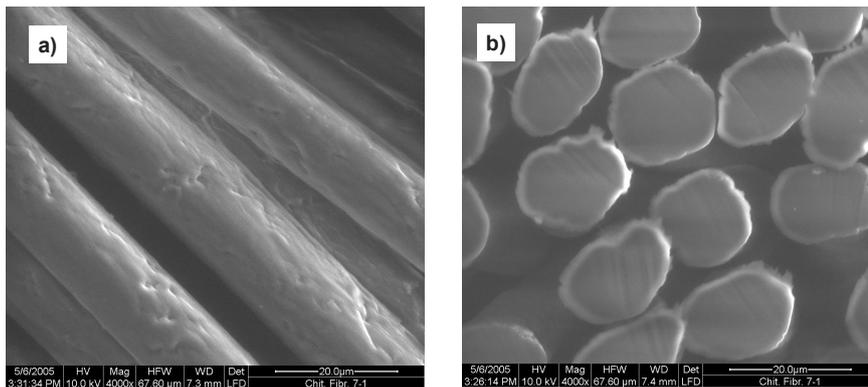


Figure 3. Photos from the scanning electron microscope (SEM) of the chitosan/silk-fibroin fibres Ch/SF III with a silk-fibroin content of 11 %; a) view of the fibres' surfaces, b) view of the fibres' cross-sections.

and the spinning solution composition on the silk-fibroin content in the coagulation bath. Two kinds of coagulation bath were tested: the first one was the aqueous solution of NaOH at concentration of 10% wt. and the second one was the aqueous solution of NaOH at concentration of 10% wt. with an addition of ethyl alcohol in the ratio of 90:10, 70:30 and 60:40 v/v. The results obtained indicated that in the case of spinning solution with a chitosan to silk-fibroin ratio of 95:5 the best coagulation bath was aqueous solution of NaOH at concentration of 10% wt. because of the smallest amount of silk-fibroin in the coagulation bath was found. In the case of spinning solutions with a chitosan to silk-fibroin ratio of 90:10 and 85:15 the best coagulation bath was aqueous solution of NaOH at concentration of 10% wt. with an ethyl alcohol in the ratio of 70:30.

Finally, considering the results obtained from coagulation tests silk-fibroin fibres were spun from spinning solutions with a chitosan to silk-fibroin ratio of 95:5, 90:10 and 85:15 to the proper coagulation bath. The silk-fibroin fibres obtained, designated as I, II and III, were characterized by silk-fibroin content of 4.5%, 11.0% and 8.0% respectively, as shown in the Figure 2. Analysing the results obtained, we can state that a loss of silk-fibroin occurring during the spinning process might have resulted from the presence of low molecular protein fraction.

Selected properties of the silk-fibroin fibres

The silk-fibroin fibres were tested in order to determine their mechanical properties, and to evaluate their surfaces and cross-sections, the latter by means of the SEM technique. Table 1 presents the test results of the mechanical properties

of silk-fibroin fibres and chitosan fibres obtained under analogous conditions as chitosan/silk-fibroin fibres I. The results obtained in this work indicate that mechanical properties of chitosan fibres are very good and that with the increase in the silk-fibroin content, the fibres were characterized by an increase in linear density, a decrease in tenacity, and an increase in the elongation at break. Considering application, the most interesting fibres are those with the highest silk-fibroin content and mechanical properties suitable for further processing, i.e. those with a silk-fibroin content of 11 %.

The view of the surfaces and cross-section of chitosan/silk-fibroin fibres with a silk-fibroin content of 11% are presented in Figures 3.a and 3.b respectively. The pictures presented indicate that the fibres are characterized by a certain deformation of the cross-section shape in relation to a cylindrical cross-section, which indicates weak coagulation properties

Summary

- Chitosan can be modified by silk-fibroin in such manner that chitosan/silk-fibroin fibres can be obtained.
- As the result of the research work we have carried out, we have determined the best process conditions (from the point of view of minimizing the silk-fibroin loss) we used to spin chitosan/silk-fibroin fibres. The best coagulation bath for the case of spinning solution with a chitosan to silk-fibroin ratio of 95:5 was aqueous solution of NaOH at concentration of 10% wt. In the case of spinning solutions with a chitosan to silk-fibroin ratio of 90:10 and 85:15 the best coagulation bath was aqueous solution of NaOH at concentration of 10% wt. with an ethyl alcohol in the ratio of 70:30.

- The chitosan/silk-fibroin fibres obtained under these conditions were characterized by a silk-fibroin content from 4.5 to 11% in dependence on the ratio of both polymers in the spinning solution. The chitosan/silk-fibroin fibres are characterized by mechanical properties sufficient for their further processing into dressing materials.

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