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Formation of Cellulose/Silk-Fibroin Blended Fibres

Abstract

This article presents research results concerning the formation of cellulose/silk-fibroin blended fibres. Bio-modified cellulose and silk-fibroin isolated from cocoons of the *Bombyx mori* silkworm were used for our investigations. In order to find advantageous fibre-formation conditions, the influence of bath composition and formation (spinning) speed on the silk-fibroin content in the fibres was tested. An aqueous solution of sulphuric acid with the addition of ammonium sulphate (VI) and sodium sulphate (VI) was used as the coagulation bath, and the fibre spinning was carried out at speed of 15 m/min. The fibres obtained, with a content of silk-fibroin within the range of 2% to 13.5%, were tested in order to assess their mechanical properties, and their surface and cross-sections state was evaluated by the SEM method.

Key words: cellulose, bio-modified cellulose, silk-fibroin, cellulose/silk-fibroin fibres.

Introduction

An increasing demand for dressings has been observed for a long time, although notwithstanding the great progress which has recently been made that in the field of material engineering, it must be admitted that no ideal multifunctional dressing has yet been devised. Conventional dressings, which in the majority are manufactured from cellulose, are not able to meet the requirements formulated for new-generation materials. Modern dressings should be bio-compatible, accelerate wound-healing processes, protect against secondary infection, not damage the freshly restored tissues, be characterised by appropriate mechanical properties and exhibit bio-degradability caused by bodily fluids [1 - 3]. This is why the search for new biomaterials which could meet the above-mentioned demands is constant. The Institute of Biomaterials and Chemical Fibres (IBWCh, formerly the Institute of Chemical Fibres) in cooperation with the National Institute of Agrobiological Science, Japan, has also begun scientific research into obtaining new cellulose/silk-fibroin bio-materials in the form of fibres intended for manufacturing dressing materials.

Silk-fibroin is a fibrillar protein created by the larva of the *Bombyx mori* silkworm in the shape of a continuous filament well-known as natural silk, which is the basic structural material of the cocoons. Independent of natural silk's application as a high-quality fibre, it has for centuries been used as a raw material for manufacturing surgical threads [4, 5]. Silk-fibroin macromolecules are composed mainly from the three following amino acids: glycine (Gly), alanine (Ala), and serine (Ser) in the ratio of

3:2:1. This structure of the macromolecule enables high orientation of the macromolecules as well as their high packing in the form of characteristic β -sheet structures, which is responsible for natural silk's unique properties, its excellent mechanical properties and its resistance to the majority of solvents among others. Products obtained from natural silk are characterised by good oxygen and water vapour permeability, as well as high biocompatibility with living organisms, which assures the high comfort of use of silk products [4 - 8]. Furthermore, research conducted over recent years has revealed new valuable features of silk-fibroin, such as the positive influence on fibroblasts and osteoblasts, which causes cell adhesion to improve, as well as its vitality, and growth, which in turn influences the process of wound healing thanks to the acceleration of the proliferation phase manifested by granulation. A positive influence was also attested during the third phase of wound healing, i.e. epithelisation, which takes effect in the formation of smaller scars [9 - 12]. The above-mentioned features mean that silk-fibroin is useful as a biopolymer for manufacturing dressing materials. Unfortunately, forms obtained from pure silk-fibroin, i.e. films and sponges, are too fragile and hard to be used as dressing material. Therefore compositions with different polymers have been tested, namely cellulose [13 - 15], chitosan [16 - 18], and polyvinyl alcohol [19 - 20].

Cellulose is the most widespread and at the same time the cheapest fibre-grade natural polymer, and as of now is the basic polymer used in the mass production of conventional dressing materials, such as bandages, gauzes, and cellucotton (cotton-wool). Modifying the cellulose

by forming a composition with silk-fibroin might result in obtaining cellulose/silk-fibroin biomaterials, thus joining the properties of cellulose with those of silk-fibroin, which would be especially useful as dressing materials.

The aim of our research was to develop a method of manufacturing cellulose/silk-fibroin biomaterials on laboratory scale in the form of fibres which could be used for obtaining dressing materials.

Materials and reagents

A commercial cellulose fir pulp characterised by a polymerisation degree of $DP_v = 670$ was used for our investigations.

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

The Ekonaza enzyme in the commercial form of an AB Enzymes OY (Finland) product was used for the enzymatic treatment of the cellulose pulp.

Methodology

Obtaining bio-modified cellulose pulp

The cellulose pulp was swelled in demineralised water, and next mechanically ground with the use of a tearing machine. This initially prepared cellulose pulp was bio-modified by a process consisting in treating the pulp with a solution of a cellulose enzyme complex according to the patented procedure [21].

Preparing the alkali cellulose solution

The wet, bio-modified cellulose pulp was left in an aqueous NaOH solution of

10% concentration, in order to obtain a solution of 6.5% cellulose concentration. The dissolving process was carried out in a mixer equipped with a cooling jacket and a high-speed stirrer, at an initial temperature of 0 °C. The dissolving process was performed over 60 minutes, and the temperature at the end of the process was 13 °C. The alkali cellulose solution was then filtered and degasified analogically as when using the typical viscose process.

Isolating silk-fibroin from the cocoons

In order to remove sericin and degrease the cocoons, 100g of cut cocoons were washed. The washing process consisted in heating the cocoons in an aqueous solution of a 2% concentration of soap, containing 0.5% of sodium carbonate (module 1:50), at a 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 a mixture of chloroform and methanol in the ratio of 2:1v/v, all the time shaken over 72 hours, and finally squeezed and dried at room temperature. 65% of pure silk-fibroin was obtained as the result of this process.

Preparing the alkali silk-fibroin solution

The isolated silk-fibroin was dissolved in a saturated LiBr solution, followed by dialysis against water over 72 hours with the use of dialysis sacks with a cut-off of 12,400 Da. The aqueous silk-fibroin solution was concentrated to a concentration of about 13%; next a NaOH solution of 18% concentration was added, and the whole solution was filtered using a glass filter with 1 µm pores. The final result was a 10% silk-fibroin solution in a 5% NaOH solution.

Preparing spinning solutions

The alkali solutions of cellulose and silk-fibroin were mixed at different ratios, and spinning solutions of the following differentiated cellulose to silk-fibroin weight ratios were obtained: 98:2, 95:5, 90:10, and 85:15 (all values re-calculated on the dry mass of both polymers, and related to the total polymer content of 6.63%, 6.83%, 7.15%, and 7.48% respectively). The spinning solutions obtained were used after de-aeration for spinning cellulose/silk-fibroin fibres.

Spinning cellulose/silk-fibroin fibres

An experimental spinning machine constructed in the IBWCh was used for spin-

ning the cellulose/silk-fibroin fibres. The spinning solution was fed into a coagulation bath at a temperature of 15 °C, with the use of a gear pump with an efficiency of 6 cm³/revolution through a 300-holes spinneret, each hole having a diameter of 0.08 mm. An aqueous solution of H₂SO₄ and a concentration of 100 g/dm³ with an addition of ammonium sulphate (VI) and sodium sulphate (VI) was used as the coagulation bath. The spinning process was conducted at a speed within the range of 10 m/min to 30 m/min. The fibres formed were drawn in air at a drawing ratio of R = 30%, taken up with the use of a godet, and cut into segments of about 30 cm in length. The fibres were stored in the coagulation bath at room temperature over 15 hours. Next, in order to completely remove all the components of the coagulation bath, the fibres were washed several times, firstly with water, next with an aqueous solution of C₂H₅OH, and finally with 96% C₂H₅OH, followed by drying the fibres in a free state at room temperature.

Analytical methods

The viscometric average degree of polymerisation of cellulose was determined by the viscometric method, using an iron (III) chloride-sodium-tartrate complex solution (commonly referred to by the German abbreviation EWNN [Eisen (III) Weinsäure Natrium-Komplex freier Natriumlauge]) as a solvent [2].

The dynamic viscosity of the spinning solutions was assessed with the use of a LVF-type Brookfield viscometer in accordance with the GLP procedure (certificate of Good Laboratory Practice).

The silk-fibroin content in the cellulose/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 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 cellulose/silk-fibroin fibres

The spinning solutions obtained as the result of mixing the alkali solutions of cellulose and silk-fibroin were homogeneous and stable. Some properties of the solutions depended on the silk-fibroin content; the most essential dependence was observed for dynamic viscosity. The increase in the silk-fibroin content caused a decrease in the dynamic viscosity, from a value of 9.950 cP for the cellulose solution down to the value of 8.200 cP for the mixed solution with a 15% silk-fibroin content (see Figure 1).

The first spinning tests of the cellulose/silk-fibroin 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 cellulose/silk-fibroin fibres from the point of view of minimising the silk-fibroin losses, a series of tests were carried out which

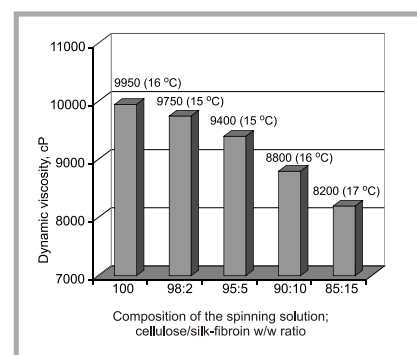


Figure 1. Changes in the dynamic viscosity of the spinning solutions in dependence on the addition of an alkali silk-fibroin solution; the temperatures of viscosity measurements are presented in brackets next to the viscosity values; the total content of polymers in the spinning solution was 6.5%, 6.63%, 6.83%, 7.15%, and 7.48% respectively.

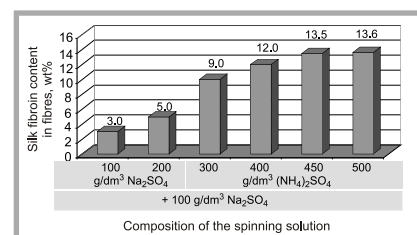


Figure 2. Influence of the coagulation bath's composition on the silk-fibroin content in cellulose/silk-fibroin fibres.

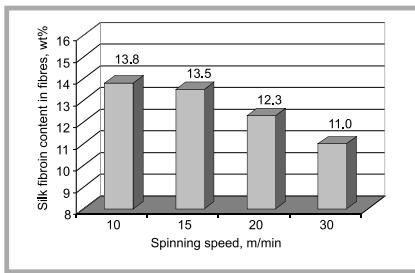


Figure 3. Influence of the spinning speed on the silk-fibroin content in cellulose/silk-fibroin fibres.

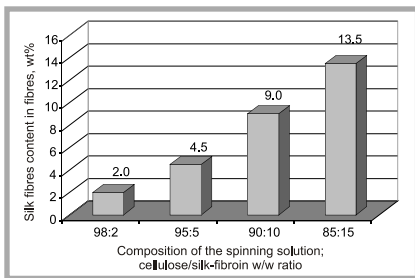


Figure 4. Silk-fibroin content in the fibres in dependence on the coagulation bath composition used for spinning.

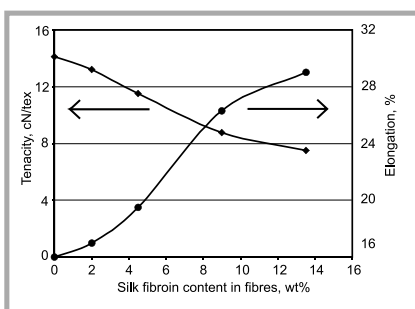


Figure 5. Influence of the silk-fibroin content in cellulose/silk-fibroin fibres on their mechanical properties: tenacity and elongation at break. For comparison, we also show tenacity and elongation at break of cellulose fibres obtained under the same conditions, but which are not typical of cellulose fibres. The process of spinning typical cellulose fibres with good mechanical properties obtained from bio-modified cellulose is described in [23].

allowed us to determine the influence of the coagulation bath's composition and the spinning speed on the silk-fibroin content in the fibres.

Figure 2 presents the research results of the coagulation bath composition's influence on the silk-fibroin content in the fibre. Two kinds of sulphuric baths with a concentration of 100 g/dm³ H₂SO₄ were tested: the first bath had sodium sulphate (VI) added to it in a concentration within the range of 100 g/dm³ to 200 g/dm³, and the second bath had

ammonium sulphate (VI) with a concentration within the range of 300 g/dm³ to 500 g/dm³. The spinning tests were carried out with the spinning solution of maximum silk-fibroin content, that is, with a cellulose to silk-fibroin ratio of 85:15, and at a spinning speed of 15 m/min. The results obtained indicated that in the case of the coagulation bath with sodium sulphate (VI), only a small part of the silk-fibroin coagulated; this means that sodium sulphate (VI), which is a typical and suitable coagulation agent in the case of cellulose [23], is significantly less effective when used for silk-fibroin. Fundamentally better results, i.e. displaying a smaller loss of silk-fibroin during the spinning process, were obtained when the coagulation bath with ammonium sulphate (VI), which allowed the highest silk-fibroin content in the fibre (13.6%) to be obtained at the highest concentration of ammonium sulphate (50%).

Figure 3 presents the research results of the spinning speed's influence on the silk-fibroin content in the fibre. Spinning speeds within the range of 10 m/min to 30 m/min were used. The spinning tests were carried out with a spinning solution with a cellulose to silk-fibroin ratio of 85:15, and in a coagulation bath with ammonium sulphate (VI). The highest silk-fibroin content in the fibre (13.5%) was obtained at the slowest speed of 10m/min.

Finally, considering the results obtained, as well as the economy factor, we accepted the following spinning conditions for cellulose/silk-fibroin fibres as the best:

- a coagulation bath with the composition of 100 g/dm³ H₂SO₄ with the addition of 450 g/dm³ (NH₄)₂ SO₄,
- a fibre spinning speed of 15 m/min.

Using the accepted spinning conditions, cellulose/silk-fibroin fibres were spun

from spinning solutions with a cellulose to silk-fibroin ratio ranging from 98:2 to 85:15. The cellulose/silk-fibroin fibres obtained, designated as I, II, III, and IV, were characterised by silk-fibroin contents from 2% to 13.5% respectively, as shown in Figure 4. Analysing the results obtained, we can state that a loss of about 10% of silk-fibroin occurs during the spinning process; this might have resulted from the presence of a peptide fraction in the solution, which was created thanks to a partial degradation of the silk-fibroin while removing sericine from the cocoons, during dissolving and dialysis.

Selected properties of the cellulose-silk-fibroin fibres

The cellulose/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 cellulose/silk-fibroin fibres and cellulose fibres obtained under analogous conditions. The mechanical properties of the cellulose fibres are not the best, because of the coagulation bath which was used. The spinning process of typical fibres from bio-modified cellulose was described in [23]. The results obtained in this work indicate that with the increase in the silk-fibroin content, the fibres were characterised by an increase in linear density, a decrease in tenacity, and an increase in the elongation at break. To better visualise the cellulose/silk-fibroin fibres' properties, the tenacity and elongation at break are also presented in Figure 5 as dependencies of the silk-fibroin content. The cellulose/silk-fibroin fibres with the highest (13.5%) silk-fibroin content are characterised by relatively low tenacity and high elongation at break. Considering application, the most interesting fibres are those with

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

Fibre	Silk-fibroin content in the fibre, %	Linear density, dtex	Tenacity in conditioned state, cN/tex	Elongation at break in conditioned state, %
Cellulose*	-	2.26	14.1	16
cellulose/silk-fibroin I	2.0	2.42	13.2	17
cellulose/silk-fibroin II	4.5	2.70	11.5	19
cellulose/silk-fibroin III	9.0	2.95	8.7	26
cellulose/silk-fibroin IV	13.5	3.14	7.5	29

* The mechanical properties of cellulose fibres obtained under analogous conditions, but which are not typical of cellulose fibres, are also presented for comparison. The spinning process of typical cellulose fibres with good mechanical properties obtained from bio-modified cellulose is described in [23].

