Miscibility and physical properties of chitosan and silk fibroin mixtures

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ABSTRACT

Miscibility investigations of chitosan (Ch) with silk fibroin (SF) mixtures with different weight percentage compositions (100/0, 80/20, 50/50, 20/80, 0/100) were studied using viscometric method. The viscosity interaction parameter of each polymer in 0.1 mol·dm^{-3} solution as well as the ternary systems (polymer A, polymer B/solvent) has been determined. By comparing the experimental and ideal viscosity data it was clearly seen that the investigated system are miscible at the weight fraction of chitosan in the mixtures $w_{Ch} \leq 0.5$. Ch, SF and their mixture films were prepared by casting technique. The effect of mixture composition on mechanical and thermal properties was also investigated in this study. The mechanical properties such as ultimate tensile strength, Young modulus and tensile strain at break depend on the weight fraction of chitosan and were changed irregularly. The thermal properties of the film made of Ch/SF mixtures were similar to those of chitosan film.

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1. Introduction

Natural polymers play an important role in medicine, pharmacy and tissue engineering, because of their valuable properties, such as bioactivity, biocompatibility and biodegradability [1–3]. However, the increase in practical applications for the biopolymers is related to the modification of their properties through suitable changes of molecular and chemical structure [4–6]. One way is a simple blending with other natural or synthetic polymers. In this study, a natural polysaccharide, chitosan, has been blended with silk fibroin. It is well known that, chitosan (Ch) a deacetylated product of chitin is a high molecular weight polysaccharide with composed mainly of β-(1 → 4)-2-amino-2-deoxy-D-glucopyranose units [1–4]. Due to its good biocompatibility, biodegradation and bioactivity, chitosan has been frequently applied in medicine, pharmacy and in cosmetic industry. Silk fibroin (SF) is a fibrous protein produced by different types of silkworms, spiders, mites and flies. Specific properties of silk fibroin such as good mechanical properties, remarkable biological compatibility, non-toxicity and biodegradability have resulted in an increasing interest of its investigation and application e.g. tissue engineering, medicine and pharmacy [6–11]. This biopolymer is often combined with other polymers, such as cellulose [7–10], sodium alginate [12–14], carboxymethyl chitosan [15] and poly(vinyl alcohol) [13,16,17]. The present work is a continuation of our previous studies on the physico-chemical properties of chitosan with silk fibroin [18–20]. In our earlier measurements, the surface properties of thin films based on the mixture of chitosan and silk fibroin have shown that the wettability and the surface roughness of chitosan and its blend films have been altered by UV-irradiation.

Moreover, the molecular interaction between the polymeric components in the mixture was observed by FTIR spectroscopy. The purpose of the present work was to determine the miscibility and physical properties of chitosan/silk fibroin mixtures, using viscosity measurements of dilute solution, thermogravimetric analysis (TGA) and tensile tests. Characterization of films made of chitosan with silk fibroin has been reported [21–27]. It was found that the mechanical properties were greatly enhanced with increasing chitosan content and showed a maximum value at the composition of 30% chitosan [21]. FTIR study and thermal analysis showed the intermolecular interactions between chitosan and silk fibroin [19,23–26]. However, the swelling property, mechanical properties and degradation of Ch/SF mixture films were affected by proportion of chitosan and silk fibroin [25]. To the best of our knowledge, the miscibility of chitosan with silk fibroin in diluted solution by viscometric method has not been studied before.

2. Materials and methods

Chitosan powder (Ch) (degree of deacetylation DD = 80% $\overline{M}_v$ = 1.9×10^5 g/mol) was obtained from Aldrich and used without further purification. The viscosity average molecular weight $\overline{M}_v$ of chitosan was measured with the Ubbelohde viscometer using 0.2 M sodium acetate and 2% acetic acid as a solvent [27] and calculated from the viscosity of solutions according to the Mark–Houwink–Sakurada equation [28]. The degree of deacetylation (DD) of chitosan was estimated by the conductometric titration method [29]. Silk fibroin (SF) was prepared in our laboratory from *Bombyx mori* cocoons (Jedwab Polski Sp. z o.o. company). Cocoons were boiled for 1 h in aqueous solution of 0.5% Na_2CO_3 according to the procedure found in literature [30] and then rinsed thoroughly with distilled water to extract the sericin proteins. The subsequent procedure was the

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same as described earlier [18,19]. The SF solution with a concentration of 5% was obtained.

The solutions of chitosan and silk fibroin were solubilised, separately, in aqueous 0.1 mol·dm^{-3} CH$_3$COOH and then mixed at different proportions. The polymer films were obtained by solution casting method. The films were dried in vacuum at room temperature.

Viscosity measurements of dilute polymer solution were carried out in a controlled thermostatic bath at 25 ± 0.1 °C using the Ubbelohde capillary viscometer. The flow times were recorded with an accuracy ± 0.01 s. Before measurements the solutions were filtered through G1 sintered glass filters. The intrinsic viscosity and the interaction parameter and Huggins coefficient values were determined according to Huggins equation [31] using solution of 5 concentrations. The values of experimental interaction parameter are determined from the plot of η_{sp}/c vs c (Eq. (1)) for solutions containing both polymers.

$$\frac{\left(\eta_{sp}\right)_m}{c_m} = [\eta]_m^{exp} + b_m^{exp} c_m \tag{1}$$

where:

b_m^{exp} the experimental, viscosity interaction parameter of polymer mixture;

$[\eta]_m^{exp}$ the experimental, intrinsic viscosity of the polymer blends;

c_m total concentration of solution.

The ideal value of the interaction parameter can be defined by different equations [32–38]. In this study, we considered two different criteria to estimate the polymer–polymer miscibility in dilute solution such as: the Krigbaum and Wall criterion [32] and the Garcia et al. criterion [36]. Krigbaum and Wall [32] have defined the ideal value of the interaction parameter b_m^{id} by the expression:

$$b_m^{id*} = b_A w_A^2 + b_B w_B^2 + 2b_{AB}^{id} w_A w_B \tag{2}$$

$$b_{AB}^{id} = b_A^{1/2} b_B^{1/2} \tag{3}$$

where:

b_A, b_B, b_{AB} interaction parameters of like (AA, BB) and unlike (AB) polymer molecules, respectively;

w_A, w_B weight fractions of polymers A and B, respectively.

Whereas, Garcia et al. [36] defined the ideal value of the interaction parameter, b_m^{id}, as:

$$b_m^{id**} = b_A w_A^2 + b_B w_B^2. \tag{4}$$

The polymer mixture is miscible if $\Delta b_m = b_m^{exp} - b_m^{id} > 0$ and immiscible if $\Delta b_m = b_m^{exp} - b_m^{id} < 0$.

Thermogravimetric analysis (TGA) was carried out using Thermal Analysis SDT 2960 Simultaneous TGA-DTA analyser of TA Instruments in the temperature range of 20 °C–600 °C at heating rate 10 °C/min in nitrogen. From thermogravimetric curves the characteristic temperatures of decomposition: temperature of initial decomposition (T_{di}) and temperature at maximum decomposition rate (T_{max}) of the investigated samples were determined.

Mechanical properties of the materials were measured at room temperature using a tensile tests Zwick&Roell at a crosshead speed of 5 mm/min in accordance with the Polish Norm PN-81/C-89034 (ISO 527-1 i 527-2) standard procedure. Samples were cut with a shaper into dog-bone shapes of initial dimensions 50 mm length with a

Table 1
The values of interaction parameters for Ch/SF mixtures.

w_{Ch}	b_m^{exp} (dL/g)^2	b_m^{id*} (dL/g)^2	Δb_m	b_m^{id**} (dL/g)^2	Δb_m	Miscibility
0.2	36 ± 2	10 ± 1	26	9 ± 0.5	27	Miscible
0.5	58 ± 3	58 ± 3	0	57 ± 3	1	Miscible
0.8	115 ± 6	147 ± 7	-32	147 ± 7	-32	Immiscible

b_m^{id*} — determined according to Krigbaum and Wall [32], b_m^{id**} — determined according to Garcia et al. [36].
w_{Ch} — the weight fraction of chitosan in the mixtures.

4.1 mm width and 30 μm thickness. The thickness of the samples was determined using an ultrameter type A-91 (producer: Manufacture of Electronic Devices, Warsaw, Poland). All the film samples were cut using the same shaper. For each kind of film, at least five samples were tested.

3. Results and discussion

According to the Huggins Eq. (1) from the slope of the plots η_{sp}/c versus c, b_m^{exp} is obtained. The values of b_m^{id*} and b_m^{id**} are obtained by substituting all terms in Eqs. (2) and (4), respectively. Table 1 gives viscometric data for mixtures containing chitosan and silk fibroin. As it can be observed, the Δb_m values are positive, exceeding the range of the experimental error for $w_{Ch} \leq 0.5$ and are negative for the weight fraction of chitosan (w_{Ch}) above 0.5. Thus, chitosan and silk fibroin are miscible with the exception of the blend for high content of chitosan ($w_{Ch} \geq 0.8$), according to the used criteria of polymer–polymer miscibility. This conclusion confirmed earlier studies in the solid state [19, 21] where the interactions between chitosan and SF were observed. The miscibility is a result of specific interaction between two polymeric components.

The results of thermogravimetric analysis (TGA) of unmodified chitosan, silk fibroin and their mixture films are shown in Table 2. The three weight-loss steps of the investigated samples were found. In the case of the chitosan and mixture films, the first and second steps were attributed to the loss of adsorbed and bound water and the residue of acetic acid. The third step of weight loss was believed to be caused due to chemical reaction, such as main chain disassociation or rearrangement. Decomposition of silk fibroin also proceeded in three stages. The first weight loss at 90–170 °C was due to the moisture vaporization [23]. The second stage which begins at about 200 °C, mainly involves the breakdown of side chain groups of amino acid residues and dehydration accompanied by the formation of some volatile products. In the third stage, the residues are further degraded at 399 °C to yield carbon and hydrocarbons. Decomposition of mixture film showed the greatest weight loss in the range 200–380 °C which are believed to be due to the disintegration of intermolecular structure and breaking of the molecular structure. For the first and second stages, we observed a shift to higher temperature which can indicate some interactions between the polymer components in the mixture.

The characteristic stress–strain curves of chitosan films and films made of Ch/SF mixtures were obtained by Zwick&Roell 0.5 testing machine. Addition of silk fibroin to chitosan is shown here to have an

Table 2
The weight loss (Δm) and temperature at maximum decomposition rate (T_{max}) of Ch/SF mixtures.

w_{Ch}	Δm (%)			T_{max} (°C)		
	I	II	III	I	II	III
0.0	6	39	12	143	281	399
0.5	6	5	47	123	188	260
1.0	6	8	47	97	168	265

w_{Ch} — the weight fraction of chitosan in the mixtures.