Materials Science and Engineering C 40 (2014) 85–91

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# Silk fibroin and sodium alginate blend: Miscibility and physical characteristics

Mariana Agostini de Moraes, Mariana Ferreira Silva, Raquel Farias Weska, Marisa Masumi Beppu *

*School of Chemical Engineering, University of Campinas, UNICAMP, 13083-852 Campinas, SP, Brazil*

ARTICLE INFO

*Article history:*
Received 4 November 2013
Received in revised form 6 March 2014
Accepted 18 March 2014
Available online 26 March 2014

*Keywords:*
Biopolymers
Films
Phase separation
Biomaterials

ABSTRACT

Films of silk fibroin (SF) and sodium alginate (SA) blends were prepared by solution casting technique. The miscibility of SF and SA in those blends was evaluated and scanning electron microscopy (SEM) revealed that SF/SA 25/75 wt.% blends underwent microscopic phase separation, resulting in globular structures composed mainly of SF. X-ray diffraction indicated the amorphous nature of these blends, even after a treatment with ethanol that turned them insoluble in water. Thermal analyses of blends showed the peaks of degradation of pristine SF and SA shifted to intermediate temperatures. Water vapor permeability, swelling capacity and tensile strength of SF films could be enhanced by blending with SA. Cell viability remained between 90 and 100%, as indicated by *in vitro* cytotoxicity test. The SF/SA blend with self-assembled SF globules can be used to modulate structural and mechanical properties of the final material and may be used in designing high performance wound dressing.
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## 1. Introduction

Polymer blending is one of the most versatile and economical methods to produce new multiphase polymeric materials that are able to satisfy complex demands for performance. The final properties of blends are generally governed by the miscibility between the polymers and the phase equilibrium behavior has been one of the most important research subjects to produce polymer blends. It is known that the mixing composition, the component concentration, their relative proportions and the used solvent are parameters that strongly influence the phase separation in polymer blends, resulting in different morphologies [1].

Natural polymer blends have been studied in the last years because of their importance in several areas, from food and packing industry to the biomedical field. Natural polymers are, in general, biodegradable, biocompatible and can be obtained from a renewable source with low costs. However, in some cases, natural polymers present undesired properties, as high degradation rate or unsatisfactory mechanical properties. A way to improve the properties of these materials and to produce micro-structured materials with tunable properties is blending them with other biopolymers. The behavior of blends of proteins and polysaccharides is difficult to predict and can rarely result in a single phase (miscible system). In most cases, phase separation (immiscible system) occurs, resulting in one phase rich in one of the polymers while the other phase is poor in this same polymer [2].

Phase separation can be used to produce micro-structured materials, with unique structural and mechanical properties, that can be fine-tuned depending on polymer concentration and blending ratio. Additionally, specific compounds, such as drugs and nanoparticles, can be incorporated in the microdomains obtained from phase separation resulting in a material with specific target functions and modulated properties.

Silk fibroin (SF) is a natural fiber extracted from cocoons of *Bombyx mori* silkworm that exhibit properties suitable for biomedical applications [3,4]. However, SF films cast from aqueous solution are soluble in water due to the dominating random coil structures, also called silk I. To induce conformational transitions from random coil to β-sheets (silk II) some methods are proposed, such as the use of organic solvents [5], high temperature [6] or shear stress [7]. The application of these methods turns SF films very brittle in dry state. To overcome this problem, blending SF with other polymers is a suitable alternative to improve its mechanical and physical properties. Sodium alginate (SA) appears as a promising second component to be used in blends with SF. SA is a polysaccharide of linear chain extracted from brown algae, composed by β-D-mannuronic and α-L-guluronic acids [11]. There are just a few papers in the literature regarding SF/SA blend films [8–10] and none of them have made a deep study on their miscibility.

SF has been studied in recent years for application in wound healing and tissue engineering due to its affinity with several cell types [3,4]. Its films usually present good water vapor and oxygen permeability, and blood compatibility and they are claimed to improve collagen formation and fibroblast proliferation [12]. SA is hemostatic and keeps an adequate humidity for healing of wounds and burnings [9]. It is already found in the market as dressing for wounds, under commercial names such as

* Corresponding author.
*E-mail address:* beppu@feq.unicamp.br (M.M. Beppu).

http://dx.doi.org/10.1016/j.msec.2014.03.047
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AlgiDERM® and Sorbsan® [13]. Blending SF and SA is a promising process for producing wound healing materials [9] and the control of the microstructure of the final blend is important to determine the properties and functionalities of the final material.

We investigated the blending of SF with SA within the scope of miscibility and physical properties. The miscibility between the two natural polymers was studied in terms of morphological, structural and thermal properties, and the whole system was evaluated regarding thermodynamic aspects. In addition, the physical properties and the biocompatibility of the blend films were analyzed by water vapor permeability, swelling capacity, and mechanical and cytotoxicity tests.

## 2. Material and methods

### 2.1. Blend preparation

Silk cocoons of *B. mori* silkworm (Bratac-Brazil) were degummed three times by soaking the cocoons in 1 g $L^{-1}$ of $Na_2CO_3$ solution at 85 °C for 30 min, in order to remove the sericin of the cocoons, and then rinsing in distilled water. SF fibers were dried at room temperature and dissolved in a solution of $CaCl_2$:$CH_3CH_2OH$:$H_2O$ (1:2:8 mole ratio) at 85 °C to a concentration of 5 wt.%. The SF salt solution was dialyzed in distilled water for three days at *ca.* 10 °C, to remove the salts of the solution. The final concentration of SF aqueous solution was 2.5 wt.%, and it was finally diluted in distilled water to 2 wt.%.

Sodium alginate (Vetec-Brazil) with high mannuronic acid content, extracted from *Macrocystis pyrifera* seaweed, was dissolved in 0.1 M NaOH solution to a concentration of 2 wt.% SA. Glycerin was added in SA solution to act as plasticizer.

The preparation of the SF/SA blend is briefly described below. SF and SA were blended at ratios of SF/SA 100/0, 75/25, 50/50, 25/75 and 0/100 wt.%. The blend solution was stirred for 15 min, cast in polystyrene dishes and dried for solvent evaporation. The whole process was performed at room temperature. Then, the blend films were immersed in a 0.1 M $H_2SO_4$ solution in 50 vol.% ethanol for 24 h, to stabilize the functional groups of SA and SF and turn the films insoluble in water.

### 2.2. Characterization

#### 2.2.1. Miscibility evaluation

The morphology of the blend films was observed by scanning electron microscopy (SEM). The samples were frozen in liquid nitrogen, freeze-fractured and then freeze-dried (Liobras, L101, Brazil) for 24 h. The samples were then coated with a gold layer and SEM observations were performed using a LEO 440i (Leica), with accelerating voltage of 10 kV.

Further investigation of SF/SA blend miscibility was done by observing film morphology after SF extraction from the blend films. The SF was extracted from the blend films using the same protocol used to dissolve SF fibers when preparing SF solution. For that, pieces of 1 × 1 $cm^2$ of the blend films were immersed in 50 mL of the solution $CaCl_2$:$CH_3CH_2OH$:$H_2O$, 1:2:8 mole ratio, at 85 °C, for 1 h, 1.5 h and 2 h. Time was varied in order to verify its influence in the final SF globule morphology. Subsequently, the blend films were rinsed with distilled water, frozen in liquid nitrogen, freeze-fractured and freeze-dried for 24 h prior to SEM observations.

X-ray diffraction (XRD) was performed using a X'PERT PW3050 Philips equipment, with monochromatic Cu-Kα radiation, wavelength of 1.54 Å, in the 2θ range of 10° to 35° and scanning rate of 0.6° $min^{-1}$, to evaluate the crystallinity of the blend films.

The chemical interaction between SA and SF and the molecular conformation of SF in the films were verified by Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), using a Bomem MB 102, equipped with a ZnSe cell crystal, in the range of 650–2000 $cm^{-1}$. Each spectrum was acquired in transmittance mode through the accumulation of 256 scans.

Thermogravimetric analysis (TGA) was performed in TGA-50 (Shimadzu) in a temperature range of 25–500 °C with a ramp rate of 10 °C $min^{-1}$ and a $N_2$ flow of 50 mL $min^{-1}$. The data were normalized as a function of the initial mass of the sample.

#### 2.2.2. Physical properties

The thickness of the films was measured 10 times in the dry state and also in the wet state (after swelling test) for each sample using a digital micrometer (MDC-25S, Mitutoyo).

Swelling degree of films was determined gravimetrically. Pieces of 2.5 cm in diameter of the films were weighed in the dry state ($w_i$), after reaching the equilibrium at 50% of relative humidity (48 h). Subsequently, the samples were immersed in 100 mL of distilled water (swelling medium) and were then weighed until reaching constant weight ($w_f$). The swelling degree (%) was calculated as $[(w_f - w_i)/w_i] \cdot 100$ %.

The water vapor permeability (WVP) was determined according to ASTM E 96M (2005). The samples were placed in a recipient with permeation area of 15.2 $cm^2$ containing anhydrous calcium chloride as desiccant and this recipient was then placed in a desiccator containing saturated aqueous NaCl solution, maintaining the ambient at 75% of relative humidity. The WVP through the films was determined gravimetrically by weighing the recipient every 12 h, for a period of 5 days. The rate of water vapor permeability was determined from the slope of the curve of mass change *versus* time. WVP was calculated as $WVP = [(G/t) \cdot e]/(A \cdot \Delta p)$, where G/t is the mass variation rate (slope of the straight line), in $g \cdot day^{-1}$; e is the film thickness, in mm; A is the test area, in $m^2$; and Δp is the vapor pressure difference, in kPa.

Tensile tests were performed according to ASTM D882 (2002) using a TA.XT2 texture analyzer (Stable Microsystems SMD). The films (7 × 2.5 cm) were stored under standard conditions (25 °C, RH 50%) for 48 h before the tests. For tensile test, an initial grip separation of 50 mm and crosshead speed of 10 mm $s^{-1}$ were used. The average values of tensile strength were obtained from 8 specimens.

#### 2.2.3. Cytotoxicity test

*In vitro* biocompatibility was performed according to ISO 10993-5 (2009) using Chinese hamster ovary cell line (CHO-k1). The cells were maintained in RPMI culture medium supplemented with antibiotics and antimicotic (100 units $mL^{-1}$ of penicillin, 100 μg $mL^{-1}$ of streptomycin and 0.025 μg $mL^{-1}$ of amphotericin), 2 mM glutamine, and 10% calf serum, at 37 °C in a 5% $CO_2$ humidified atmosphere until they reached confluence. For subculturing and for experiments, cells were harvested using 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline solution at pH 7.4. The films were sterilized by using UV radiation for 30 min on each side of the film. The films were immersed in RPMI culture medium in a proportion of 1 $cm^2$ $mL^{-1}$, at 37 °C for 48 h. The extracts of the films were then diluted from 100% (original extract) to 6.25 vol.% in RPMI 1640. Cytotoxicity test was performed in a 96 well microplate containing 3000 cells per well and extract of films. The microplates were incubated for 72 h at 37 °C in a 5% $CO_2$ humidified atmosphere. The cell viability was measured by adding MTS (supravital dye tetrazolium compound)/PMS (electron coupling agent) (20/1) solution and incubating for 2 h. The microplates were analyzed in a spectrophotometer ELISA at 490 nm. The test was compared with a negative control of high density polyethylene (HDPE) and a positive control of phenol 0.5 vol.% in culture medium.

## 3. Results

In the present study, tests of SF/SA blend preparation were performed, in order to obtain a homogeneously mixed solution and a film without macroscopic phase separation. Initially, we used SA aqueous

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