Journal of Molecular Liquids 220 (2016) 726–730

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

The miscibility of collagen/hyaluronic acid/chitosan blends investigated in dilute solutions and solids

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ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 2 May 2016
Accepted 4 May 2016
Available online xxxx

Keywords:
Collagen
Hyaluronic acid
Chitosan
Polymer blends
Miscibility

ABSTRACT

In the present study, the results from viscometric measurements, tensile tests and Fourier transform infrared spectroscopy (FTIR) of polymer blends containing collagen (Coll) and hyaluronic acid (HA) with and without chitosan (Ch) are presented.
The viscosity interaction parameter of each polymer in 0.1 mol·dm^{-3} CH_3COOH/0.3 mol·dm^{-3} NaCl solution for the collagen and 0.3 mol·dm^{-3} NaCl or 0.1 mol·dm^{-3} HCl solution for the hyaluronic acid and 0.1 mol·dm^{-3} CH_3COOH/0.3 mol·dm^{-3} NaCl solution for the chitosan as well as the two-component blend systems and the ternary blend systems have been determined. These studies indicated that collagen/hyaluronic acid blends were miscible at any composition (Coll/HA: 80/20, 50/50, 20/80) in 0.1 mol·dm^{-3} CH_3COOH/0.3 mol·dm^{-3} NaCl and 0.1 mol·dm^{-3} HCl at 25 °C. In the case of Coll/HA/Ch ternary blends, the polymeric components were partially miscible. The mechanical properties of films such as tensile strength and Young's modulus depend on the blend composition. Coll/HA blends possessed a tensile strength and Young's modulus of 16.3–57.6 MPa and 1.0–1.9 GPa, respectively. The addition of chitosan to the blend led to an increase in tensile strength by approximately 50%. The results of FTIR analysis suggested that there was the existence of intermolecular interactions between functional groups of polymers.

1. Introduction

Polymer/polymer blending is an important method to improve the original properties of the components [1–8]. This method is usually cheaper and less time-consuming for the creation of materials with new properties than the development of new monomers and/or new polymerization routes. An additional advantage of polymer blends and composites is that the materials can be tailored by combining component polymers and changing the blend composition. Currently, there is a tendency toward a greater use of natural polymers that are obtained from renewable resources, especially those that are sourced from food industry waste and the agricultural and pulp and paper industries [8–14]. The purpose of this study was the preparation and evaluation of the miscibility and surface properties of new blends of polysaccharides and collagen in solutions and in solids. Collagen is the most abundant natural polymer in animals. Collagen a major structural protein of extracellular matrix, supports the growth of a wide variety of tissues while its structure imparts favourable properties such as mechanical strength [2,15–17]. Collagen is very important biopolymer and its blends can be of significant practical application [15]. Natural polysaccharides are widely used in the cosmetic industry as raw materials and hold promise for biomedical applications because of their film-forming ability, controlled bioactivity, biocompatibility and biodegradability. Chitosan and hyaluronic acid are the hydrophilic polysaccharides, showing unique properties such as solubility in an aqueous solution, ability to form complexes and non-toxicity to humans which have resulted in increased interest in investigation and application, e.g., in medicine, pharmacy and cosmetics [18–21]. Characterization of films and sponges made of collagen with hyaluronic acid and chitosan have been reported [22–23]. Cai et al. [22] have described the coagulation property of hyaluronic acid-collagen/chitosan complex film. These results indicated that the hyaluronic acid/collagen type I/chitosan (HA-Col-I/Ch) film possessed promising coagulation property, cell compatibility and anti-bacteria property, and the potential in future clinical application such as wound healing and bandage. In the case of collagen/hyaluronan/chitosan composite sponges [23], the results showed that the 9:1:1 mixing ratio of collagen, hyaluronan and chitosan to be the optimal ratio for the manufacture of complex scaffolding in different fields of tissue engineering based on its properties, cell biocompatibility and low cost.

To the best of our knowledge, the miscibility of collagen with hyaluronic acid in different solvent and collagen blends with hyaluronic acid and chitosan in diluted solution by viscometric method has not been studied before. The present paper is a continuation of our previous studies on the miscibility and surface properties of chitosan blends with hyaluronic acid and collagen [24]. A previous study has shown that the degree of miscibility between the polymer components in the Ch/HA

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http://dx.doi.org/10.1016/j.molliq.2016.05.009
0167-7322/

binary blends and the Ch/HA/Coll ternary blends mainly depends on the blend composition and on the thermodynamic goodness of the solvent. In this study, methods such as viscometric method, tensile tests and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) were used. The blending of collagen with hyaluronic acid and chitosan makes it possible to obtain new materials, in which intermolecular interactions between the various components occur mainly due to hydrogen bonds and electrostatic interactions.

2. Materials and methods

Collagen (Coll) was extracted in our laboratory from rat tail tendon. Tendons were washed and dissolved in 0.1 M acetic acid. The obtained solution was then spun at 10,000 rpm in a Sorvall centrifuge and the soluble fractions were decanted and lyophilized [25]. Hyaluronic acid (HA) is a commercial polymer from Aldrich Company with a viscosity average molecular weight of 1.8×10^6. Chitosan powder (a degree of deacetylation of 78% and a viscosity average molecular weight of 540,000) was supplied by Aldrich, Poland. The polymeric samples were solubilized separately in the solvent. Collagen and chitosan were solubilized in aqueous 0.1 mol·dm^{-3} CH_3COOH/0.3 mol·dm^{-3} NaCl. For the hyaluronic acid, we used different solvents such as 0.3 mol·dm^{-3} NaCl, 0.1 mol·dm^{-3} CH_3COOH/0.3 mol·dm^{-3} NaCl and 0.1 mol·dm^{-3} HCl. Coll/HA blends were prepared from mixed polymer solutions. The composition of Coll/HA was 80/20, 50/50 and 20/80. The chitosan solution was added in the different ratios (10–90%) based on the Coll/HA blend in 80/20 weight ratio. These solutions were cast to prepare the polymer films. Polymer films were obtained by casting solution onto glass plate at room temperature.

Viscosity measurements of dilute polymer solution ($c = 0.1\%$) 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 values were determined according to Huggins equation [26] using solution of 5 concentrations. The miscibility is estimated by comparison of the experimental and ideal values of b_m. The values of interaction parameters were obtained using the same methods as shown in previous papers [24,27–29].

ATR-FTIR spectra of the polymer samples and their blends were recorded on Genesis II FTIR spectrophotometer Mattson (USA) equipped an ATR device (MIRacleTM PIKE Technologies) with zinc selenide (ZnSe) crystal, in the wavelength range between 4000 and 600 cm^{-1}, resolution of 2 cm^{-1} and 64 – times scanning. The samples were prepared from 1% solutions. All spectra were obtained for films of the similar thickness.

The mechanical properties of the materials were measured at room temperature using tensile tests, Zwick Roell (Germany), at a crosshead speed of 50 mm/min in accordance with the standard procedure [30]. Samples were cut into dog-bone shapes of initial dimensions of a 50-mm length, 4.2-mm width and 30 μm thickness. Sample thickness was determined using an ultrameter type A-91 (Manufacture of Electronic Devices, Warsaw, Poland). All of the film samples were cut using the same shaper. For each type of film, a minimum of five samples were tested.

3. Results and discussion

An important aspect of properties of polymer blends is the miscibility of its components. The degree of miscibility of the components depends on the interactions between them. In recent years, viscometry has been widely used to study the polymer–polymer interaction and miscibility because this method is a simple, quick and an inexpensive [24,27–29,31]. Many researchers proposed criteria to determine polymer-polymer miscibility by the viscometric technique [32–36]. As published by Krigbaum and Wall [32] and Garcia et al. [33], the Δb_m parameter calculated by the equation: $\Delta b_m = b_m^{exp} - b_m^{id}$, can be utilized to evaluate the presence of interactions. If $\Delta b_m > 0$, attractive forces are dominant which proves the miscibility of the components. If $\Delta b_m < 0$, repulsive forces prevail and immiscibility is expected.

All the Huggins plots (the reduced viscosity (η_{sp}/c) versus polymer concentration (c), curves not shown) show linear behaviour in the range of concentration studied. Collagen, hyaluronic acid and chitosan are polyelectrolytes and, in aqueous solution, in limit of low ionic strength the electrostatic interactions are weakly screened. In this study the ionic strength in the investigated systems was high enough to prevent the polymer chains showing a polyelectrolytes effect. According the Huggins equation [26] from the slope of η_{sp}/c versus c, b_m^{exp} is obtained. The values of b_m^{id} were determined from the equation proposed by Garcia et al. [33]. The parameters of the miscibility criterion and comparisons between the different solvent used to dissolve the HA are tabulated in Table 1. It is clearly seen that the differences of Δb_m values in the three respective systems are significant. The Δb_m are positive for all the ratios in 0.1 mol·dm^{-3} HCl while $\Delta b_m < 0$ for the other two solvents. In the case of the solution in which HA was dissolved in 0.3 mol·dm^{-3} NaCl, the large addition of collagen to the blend causes a strong interaction between the polymeric components and phase separation in the selected blend systems ($wt_{Coll} \geq 0.5$). These reasons could probably be responsible for the negative value of the interaction parameter. However, the addition of NaCl to the collagen solution decreases the negative values of the interaction parameters. Such differences in the values of interaction parameters are caused by the rise of ionic strength in the polymer solution which influences the decrease of electrostatic interactions between polymer chains in the used solvent. Therefore, it has been proved that collagen and hyaluronic acid are miscible in 0.1 mol·dm^{-3} HCl solution.

These viscometric results illustrate if the weight ratio of Coll/HA binary blend is higher than or equal to 0.5, the miscibility of polymeric components is better than those of other weight ratios. Therefore, the weight ratio of Coll/HA was kept to 80/20 then the binary blend was blended with different amounts of chitosan solution in this study.

Table 2 gives viscometric data for the Coll/HA (80/20) blend containing the different addition of chitosan. As it can be observed, the Δb_m values show a nonmonotonic change with the change in blend composition. Thus collagen blends with hyaluronic acid and chitosan are partially miscible.

Mechanical properties such as the ultimate tensile strength and Young's modulus of films have been determined and compared. The influence of collagen content in the Coll/HA blend on the mechanical properties was evaluated by a Zwick & Roell testing machine in room temperature and is shown in Fig. 1. With the miscible polymer blends, the mechanical compatibility is assured and a property compromise

Table 1
A comparison of the experimental and ideal viscometric parameters b_m of Coll/HA blends using the criterion proposed by Garcia et al. [33].

wt_{Coll}	b_m^{exp} [dL/g]^2	b_m^{id} [dL/g]^2	Δb_m
Coll/HA solvent of HA: 0.3 mol·dm^{-3} NaCl; solvent of Coll: 0.1 mol·dm^{-3} CH_3COOH			
0.2	53.0 ± 4.8	48.6 ± 4.0	4.4
0.5	**2.7 ± 0.2**	**45.1 ± 3.7**	**−42.4**
Coll/HA solvent of HA: 0.3 mol·dm^{-3} NaCl; solvent of Coll: 0.1 mol·dm^{-3} CH_3COOH + 0.3 mol·dm^{-3} NaCl			
0.2	47.6 ± 4.0	45.9 ± 3.7	1.9
0.5	**4.7 ± 0.3**	**27.9 ± 1.2**	**−23.2**
0.8	31.9 ± 2.3	30.0 ± 1.5	1.9
Coll/HA solvent of HA: 0.1 mol·dm^{-3} HCl; solvent of Coll: 0.1 mol·dm^{-3} CH_3COOH + 0.3 mol·dm^{-3} NaCl			
0.2	19.7 ± 1.1	12.0 ± 0.8	7.7
0.5	47.5 ± 4.0	22.3 ± 1.2	25.2
0.8	73.0 ± 6.2	48.7 ± 4.0	24.3

wt_{Coll} – weight fraction of collagen,
Immiscible system is marked in bold.