

CHARACTERISATION OF CHITOSAN/HYALURONIC ACID BLEND FILMS MODIFIED BY COLLAGEN

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Abstract

In the present study, thin films based on the blends of chitosan (Ch) and hyaluronic acid (HA) with and without collagen (Coll) were characterised using tensile tests, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and thermogravimetric analysis (TGA). Ch/HA and Ch/HA/Coll polymer blends were prepared using the solvent evaporation technique. The interactions between polymer components in the binary and ternary blends were studied by FTIR analysis. Mechanical properties were studied and compared with those of simple polymer films. These results show that the addition of collagen into Ch/HA blends led to the increase of tensile strength and Young modulus. Moreover, it was found that the thermal stability of the Ch/HA binary blend significantly increased upon the addition of collagen.

Key words: *chitosan, hyaluronic acid, collagen, surface properties, polymer blends*

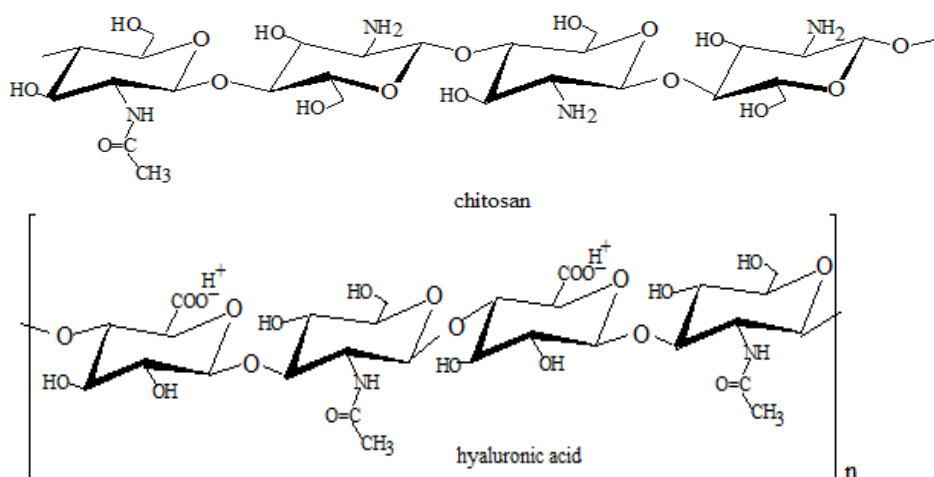
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1. Introduction

Biopolymers are useful in the biomedical field because of their structure and unique properties such as bioactivity, biodegradability, non-toxicity for humans and the ability to form complexes [1–5]. Their synthesis generally involves an enzyme-catalysed, chain growth polymerisation reaction of active monomers, which are typically formed within cells by complex metabolic processes [6]. Many properties of natural polymers can also be improved through blending and composite formation [5, 7–10]. An advantage of polymer blends is that the materials can be tailored by combining component polymers and altering the blend composition. Two or more natural or synthetic polymers can be mixed together, and such blends can lead to new materials. The number of new polymer blends and composites based on known biopolymers has increased rapidly in recent years [5, 10–15]. Thus, the main focus of this study was the preparation and evaluation of the physical properties of new blends of polysaccharides and collagen (Coll). The miscibility of collagen with chitosan (Ch) has been previously studied [16]. Collagen exists in several organs acting as the bonding material of the cell. It maintains the structural integrity of tissues and provides the hardness, stiffness and elasticity of tissues [17–20]. This protein is an interesting materials for use either in biomedical or cosmetic fields due to its favourable properties. Namely, it has excellent biocompatibility, biodegradability, good homeostatic properties and biofunctionalities and it is not immunogenic. Owing to these properties, materials based on collagen are very important for tissue engineering [18].

Chitosan and hyaluronic acid (HA) are compounds that belong to a group of hydrophilic polysaccharides (Scheme 1). These biopolymers are biocompatible, biodegradable, biofunctional and non-toxic for living organisms. Chitosan and hyaluronic acid, due to their interesting properties, are used in several fields, e.g. in medicine, pharmacy, engineering and the cosmetics industry [2, 5, 21–24].



Scheme 1. The chemical structure of chitosan and hyaluronic acid.

In the literature, there are only a few reports on the physico-chemical properties of the films and sponges based on chitosan, hyaluronic acid and/or collagen [25–28]. From these, the blends have been considered mainly as biomaterials for applications in wound

healing and different fields of tissue engineering scaffolding [26–28]. Thus, the physical properties of chitosan blends with hyaluronic acid and different additions of collagen (0–90%) are the main aim of this paper. Our previous studies of polymer blends containing collagen, hyaluronic acid and chitosan showed that the degree of miscibility between components in the Ch/HA, Coll/HA binary blends and the Ch/HA/Coll, Coll/HA/Ch ternary blends mainly depends on the blend composition and on the ionic strength of the solution [29–31]. The purpose of the present work was to prepare Ch/HA and Ch/HA/Coll blend films in different weight ratios by a solvent evaporation technique. The obtained materials were characterised using tensile tests, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and thermogravimetric analysis (TGA).

2. Materials and Methods

2.1 Materials preparation

Chitosan (Ch) and hyaluronic acid (HA) are commercial polymers and were obtained from the Aldrich company (Poland) and had a viscosity average molecular weight of 0.59×10^6 and 1.8×10^6 , respectively. The degree of deacetylation was 78% for Ch. Collagen (Coll) was prepared in our laboratory from tail tendons of young rats by means of the original method described in the literature [32]. Chitosan, hyaluronic acid and collagen were separately solubilised in aqueous acid solutions ($0.1 \text{ mol} \cdot \text{dm}^{-3}$ HCl for HA and $0.1 \text{ mol} \cdot \text{dm}^{-3}$ CH_3COOH for Coll and Ch) and then mixed at different ratios. The composition of Ch/HA was 80/20, 50/50 and 20/80. The collagen solution was added in the different ratios (10–90%) based on the Ch/HA blend in the 50/50 weight ratio. Polymer films were obtained by casting solutions onto glass plates covered with polyethylene film and evaporated at room temperature. All films had a similar thickness of about 30 μm .

2.2 TG analysis (TGA)

Thermogravimetric analysis (TGA) was carried out using a Thermal Analysis SDT 2960 Simultaneous TGA-DTA analyzer from TA Instruments in the temperature range of 20°C to 650°C at a heating rate of 20°C/min in nitrogen. From the thermogravimetric curves, we obtained the characteristic temperatures of decomposition: temperature of initial decomposition (T_{di}) and temperature at maximum decomposition rate (T_{max}).

2.3 Infrared spectroscopy

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

2.4 Mechanical properties

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 [33]. Samples were cut into dog-bone shapes of initial dimensions of 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 film samples were cut using the same shaper. For each type of film, a minimum of five samples were tested.

3. Results and discussion

3.1 Mechanical properties

Figure 1 shows the relationship between the mechanical properties (tensile strength and Young's modulus) and the chitosan content of blend films. It can be noted that the chitosan film exhibited lower tensile strength (15 MPa) than that measured for the hyaluronic acid film (20 MPa). In the Ch/HA blend with 50% chitosan content, a positive deviation from linear behaviour is observed between mechanical properties of the pure polymer films. This could be attributed to the existence of interactions between the functional groups, (e.g. $-\text{OH}$, $-\text{NH}_2$ or $-\text{COOH}$) of chitosan and HA, which could lead to the formation of a matrix due to physical crosslinking. This result is in agreement with previously published values of viscosity interaction parameters for Ch/HA blends [30]. In our previous study, we found that chitosan/hyaluronic acid blends were miscible at the weight fraction of chitosan $w_{\text{Ch}} \geq 0.5$. However, the lowest tensile strength value is observed in the blend with 80% chitosan content. This behaviour may indicate a poorer miscibility of the components. Figure 1 also shows the dependence of the Young's modulus on the chitosan content for pure polymers and blend films. The chitosan blend films with $w_{\text{Ch}} \leq 0.5$ showed higher values of Young's modulus compared to chitosan. Thus, Young's moduli of chitosan blend films increase after mixing with hyaluronic acid.

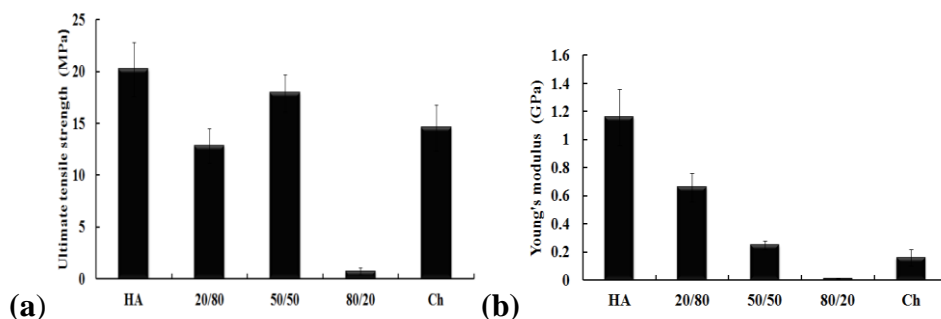


Figure 1. Effect of chitosan content (w_{Ch}) in Ch/HA blends on (a) ultimate tensile strength and (b) Young's modulus.

In both previous and present studies, we have shown that if the weight ratio of Ch/HA is equal to 1, the miscibility and mechanical properties are better than those of other weight ratios. Thus, the weight ratio of Ch/HA was kept to 1; then the binary blend was blended with different additions of collagen in this study. Figure 2 shows the dependence of ultimate tensile strength and Young's modulus on the collagen content for the ternary blend films. As can be seen, the tensile strength and Young's modulus of the binary Ch/HA blend film significantly increases after mixing the binary blend with collagen. The maximum value of ultimate tensile strength and Young's modulus (106 MPa and 3.2 GPa, respectively) is achieved for the Ch/HA/Coll blend with 70% collagen. This suggests that during mixing of the Ch/HA blend with collagen, the new crosslinking reactions in the ternary blend film responsible for the increase of mechanical properties occur.

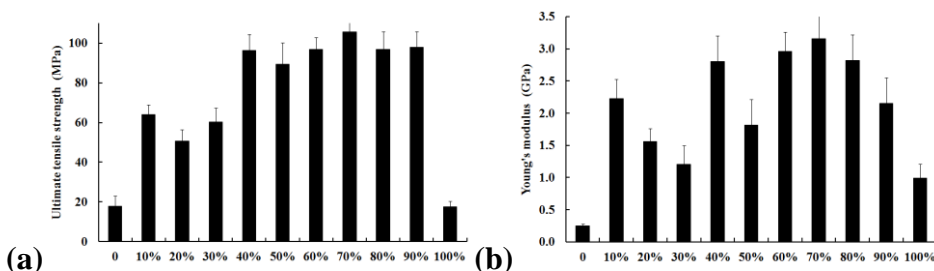


Figure 2. Effect of the addition of collagen to Ch/HA (50/50) blend on (a) tensile strength and (b) Young's modulus.

3.2 Infrared spectroscopy

It is well known that the FTIR spectroscopy has been widely used for identification of intermolecular interactions in the polymer blends [1, 5]. The properties of polymer blends are the result of interactions by hydrogen bonds and/or electrostatic interactions between the functional groups of the various polymers. In this work, on the basis of the OH, amide A, amide B, amide I (C=O) and amide II (NH) band positions and their relative intensities, the interactions between chitosan, hyaluronic acid and collagen were deduced. Thus, the FTIR spectra of blend films, in the amide I and amide II bands of Ch and HA near 1560–1650 cm^{-1} , the hydroxyl stretching band of Ch near 3300–3600 cm^{-1} , the amide A and amide B of collagen near 3300–3000 cm^{-1} have been analyzed.

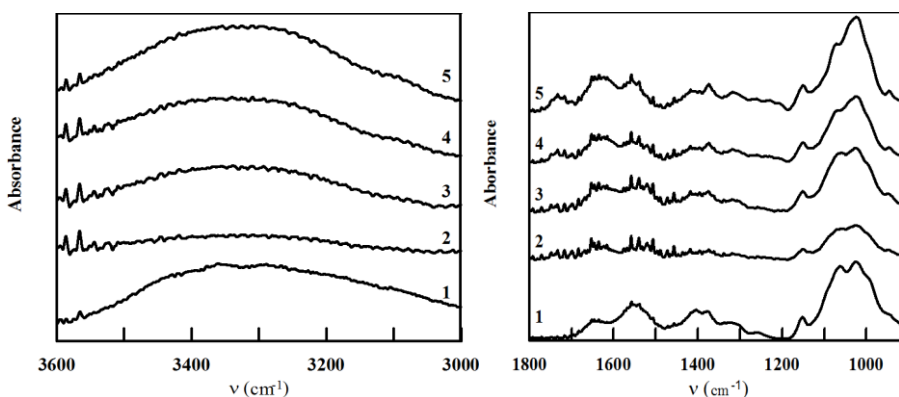


Figure 3. ATR-FTIR spectra of chitosan (Ch), hyaluronic acid (HA) and their blends with different Ch/HA ratios. 1 – Ch, 2 – 80/20 Ch/HA, 3 – 50/50 Ch/HA, 4 – 20/80 Ch/HA, 5 – HA.

Figure 3 presents the ATR-FTIR spectra of pure chitosan, hyaluronic acid and their blends. Chitosan and hyaluronic acid are the hydrophilic polysaccharides, so both give similar spectra in the ATR-FTIR method. In the case of biopolymer blends with similar structures, this method is not powerful for studying interactions. The spectrum of hyaluronic acid shows a characteristic peak of the carbonyl band at 1730 cm^{-1} , which is due to the presence of carboxylic groups in hyaluronic acid. This indicates that the ionisation of the carboxylic acid groups of HA are suppressed due to the low pH of the used solvent (0.1 $\text{mol}\cdot\text{dm}^{-3}$ HCl solution). Thus, the carboxylic group is protonated.

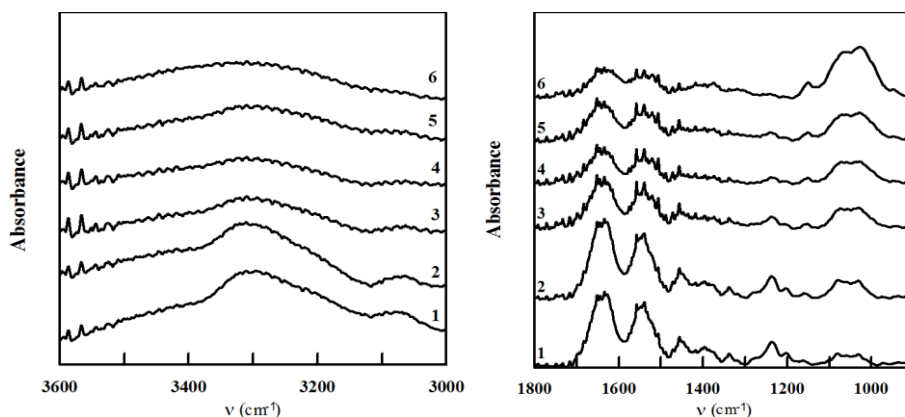


Figure 4. ATR-FTIR spectra of films made of Ch/HA (50/50) blend containing different additions of collagen: 1 – 100% of collagen, 2 – 90% of collagen, 3 – 70% of collagen, 4 – 50% of collagen, 5 – 30% of collagen, 6 – 10% of collagen.

After the addition of chitosan to hyaluronic acid, we observed a lack of a carbonyl band peak at 1730 cm^{-1} (Figure 3b). This may indicate the shift of the carbonyl band towards a lower frequency to overlap with amide bands. For the $-\text{OH}$ and $-\text{NH}_2$ bands near $3400\text{--}3300\text{ cm}^{-1}$, shifts towards lower frequencies and changes in shape were observed (Figure 3a). These observations of frequency shifts for hydroxyl, amine and carbonyl signals may be interpreted as being due to the formation of hydrogen bonds between $-\text{OH}$ and $-\text{NH}_2$ groups of chitosan and $-\text{COOH}$ and $-\text{OH}$ groups of hyaluronic acid. In the case of ternary blends (Figure 4), the ATR-FTIR spectra showed a noticeable change in the intensity and shape of amide A, amide B, amide I and amide II bands, which were observed as a broadening in the absorption peaks of the blends. The small shifts of the maximum characteristic bands in the blend spectra were observed. This may indicate a hydrogen bond of a new type between the chains of the biopolymers in the ternary blend. The formation of hydrogen bonds between different macromolecules competes with the formation of hydrogen bonds between molecules of the same polymer. Furthermore, the spectra of blends are not a simple superposition of spectra of separated components. The presence of intermolecular interactions has been confirmed by our previous studies by the viscometric method [30].

3.3 TG analysis (TGA)

Thermogravimetric analysis (TGA) has been used extensively in the study of thermal stability of polymer blends. The TGA thermograms of native biopolymers and their binary and ternary blends are shown in Figures 5 and 6.

For the hyaluronic acid film and the Ch/HA blend with 20% of chitosan, three peaks were observed on the DTG curves, while the chitosan and other binary blend curves showed two peaks (Figure 5). The first weight loss for all investigated sample (native polymers and their blends) films occurred in the temperature range of 40°C to 140°C and amounted to an approximate 12% weight loss. This stage was related to the loss of water and residual acid by evaporation or to a reduction in moisture content. The next stages at $200\text{--}380^\circ\text{C}$ were attributed to the disassociation of intermolecular side chains and the degradation of samples. It could be noticed that the chitosan sample was more thermally stable compared to hyaluronic acid, as indicated by the appearance of a higher

temperature peak for chitosan. The addition of chitosan into hyaluronic acid blends decreased the weight loss and provided a thermal barrier.

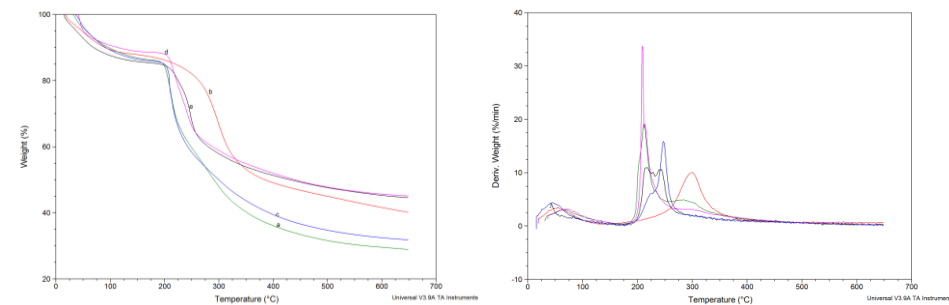


Figure 5. TGA thermograms of hyaluronic acid, chitosan and their blends: a) – hyaluronic acid (green line), b) – chitosan (red line), c) – 20/80 Ch/HA (blue line), d) – 50/50 Ch/HA (fuchsia line), e) – 80/20 Ch/HA (black line).

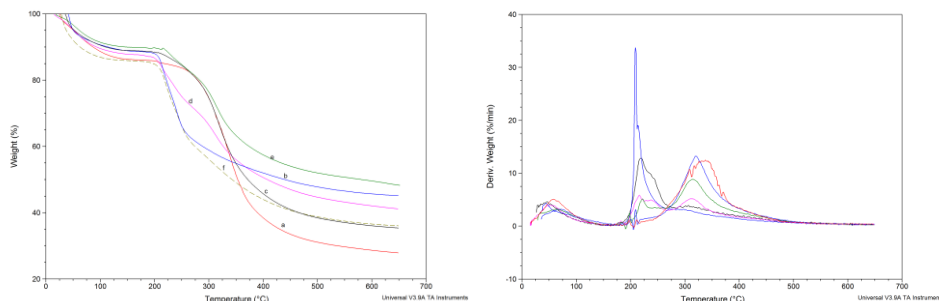


Figure 6. TGA thermograms of the Ch/HA (50/50) blend containing different additions of collagen: a) – 100% of collagen (red line), b) – 0% of collagen, Coll/HA (50/50) blend (blue line), c) – 80% of collagen (black line), d) – 50% of collagen (fuchsia line), e) – 30% of collagen (green line), f) – 10% of collagen (olive line).

For the second stage of the Ch/HA blend, the maximum temperature of decomposition increased, but it was lower than those for the chitosan sample. In the case of the collagen sample and most ternary blends, two peaks were present in the TGA curves (Figure 6). After the addition of collagen to the binary blend film, the maximum temperature of decomposition increased, especially for the ternary blends with a large content of collagen in the blend ($w_{\text{Coll}} \geq 0.7$). The weight losses of ternary blends were lower than for pure collagen. This indicates that the samples were more resistant to thermal decomposition. The addition of collagen improved the thermal stability of binary blends due to intermolecular interactions between components. This is in accordance with previously reported data for the collagen blends [5, 16, 26, 27, 31].

4. Conclusions

In this paper, the physical properties of Ch/HA and Ch/HA/Coll blend films were investigated. ATR-FTIR, thermal and mechanical results clearly highlighted intermolecular interactions between chitosan, hyaluronic acid and collagen. These interactions are related to the presence of amine, hydroxyl and/or carboxylic groups in the selected polysaccharides (chitosan and hyaluronic acid) and collagen. The ATR-FTIR analysis, considering the relative changes in –OH, –COOH and –NH₂ intensities allows the conclusion that a hydrogen bond of a new type between compounds appears. Accordingly, the improvement of mechanical and thermal properties of biopolymer blend films can be a result of hydrogen bonds between the reactive groups of components, especially after the addition of collagen into the Ch/HA binary blend.

From these results, it can be concluded that the Ch/HA and Ch/HA/Coll blends may be interesting materials for biomedical and cosmetic applications, such as for applications in wound healing or fields of tissue engineering scaffolding. Film-forming properties of the blend could also be used in hair care cosmetics.

5. Acknowledgements

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