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THERMAL AND RHEOLOGICAL BEHAVIOR OF COLLAGEN
Chitosan blends

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Abstract

Collagen:chitosan blends in 1:1 ratio were prepared and characterized by Fourier transform infrared spectroscopy, thermal (DSC, TG) and rheological studies. Apparently each material maintains its behavior and addition of chitosan does not denature collagen fibers. The rheological behavior showed that adding chitosan to collagen causes a decrease of storage modulus (G'), viscous loss modulus (G") and apparent viscosity when measured as a function of frequency. Both anionic and native collagen presented more solid-like behavior than fluid-like viscoelastic behavior. Collagen:chitosan blends exhibits a more fluid-like viscoelastic behavior.

Keywords: blends, chitosan, collagen, rheology

Introduction

Chitin is the second most abundant naturally occurring biopolymer (after cellulose) and is found in the exoskeleton of crustaceous, in fungal cell walls, and in other biological materials. It is mainly poly β-(1-4)-2-acetamide-*D*-glicose, which is structurally identical to cellulose except that a secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by acetamide group. Chitosan is a cationic biopolymer obtained by N-deacetylation of chitin. Its name does not refer to a unique defined compound but rather to a family of copolymers with various fractions of acetylated units (usually <50%). The following major characteristics of chitosan make this polymer advantageous for numerous applications: 1. it can be chemically and enzimatically modified; 2. it is biodegradable and biocompatible with many organs and cells; 3. it can be processed into several products including flakes, fine powders, beads, fibers, gels [1, 2].

Collagen exists as a triple helix, comprising three discrete chains (three dimensional structure), which leaves space for inter chain hydrogen bonding. The three α-chains of collagen are not identical but have slight variations in their amino acid

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content. Type I collagen molecules (300 nm long × 1.5 nm diameter rigid rod, 300 kD protein) [3] form fibers that possesses a regular stagger of approximately 1/4 of a rod length between each molecule and its axially aligned neighbor. This molecular architecture has been studied by electron microscopy [4]. The effect of molecular packing on the rigidity of collagen fibers can be described by treating the collagen as a liquid-crystalline phase of ordered rigid rod collagen molecules [5]. Its gel behavior results from the intrinsic properties of the interaction between two components phases: a network of collagen fibers and interstitial solution.

In the present study blends consisting of chitosan and collagen (native and anionic) in 1:1 ratio (mass/mass), to be applied in medical fields such as wound dressing or a matrix for the controlled delivery of sustained drugs, were prepared and characterized by Fourier transform infrared spectroscopy, thermal (DSC,TG) and rheological studies.

Materials and methods

Preparation of collagen and chitosan

Chitosan (QUI) used in this work is a commercial product from Fluka with a medium molar mass ($4 \cdot 10^5$ g mol^{-1}) and degree of deacetylation of 75%, determined by NMR [6]. It was purified before use, dissolving it in acetic acid solution (3%) under slight shaking at least for 24 h, and filtered at positive pressure through a 5.0 μm pore size membrane. NH_4OH was added to precipitate chitosan and it was filtered and washed with water until neutral. Purified chitosan was resolved in acetic acid solution on a concentration of 0.7% (mass/mass) and pH corrected to pH 3.5 with acetic acid.

Native collagen (NC) was obtained from purified porcine serosa and treated with 6% aqueous dimethylsulfoxide for 24 h followed by extraction in a pH 3.5 acetic acid solution. Anionic collagen (AC) was prepared by the treatment (72 h) of porcine serosa with an alkaline solution, 6% in dimethylsulfoxide, containing sulphate and chloride salts of sodium, potassium and calcium, for complete hydrolysis of amide groups from asparagine and glutamine [7]. After complete removal of excess salt, collagen was extracted in acetic acid solution, pH 3.5. Molar mass evaluation was performed by polyacrylamide sodium dodecil sulphate (SDS) gel electrophoresis [8] using a 7% stacking gel and 10% separating gel. The α_1/α_2 ration determined by densitometry was 1.9 compared to a value of 2.0, expected for type I collagen.

Preparation of collagen:chitosan blends

Collagen gels were prepared in a concentration of 0.7% (mass/mass). Blended solutions consisting of collagen anionic and chitosan (ACQ) or native collagen and chitosan (NCQ) in a 1:1 ratio (mass/mass) were prepared in aqueous acetic acid solution (pH 3.5). Membranes for FTIR and thermal analysis were cast in acrylic moulds. Chitosan, collagen and blended membranes were found to be transparent and flexible. No change in appearance were found upon storage in the dry state.

Experimental apparatus

Infrared Spectrum were recorded in a Bomem Fourier transform infrared spectrometer model MB 120. All spectra were recorded from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

For thermal analysis, TA Instruments 2010 DSC and 2050 TGA apparatus were used. Samples (10 mg) were heated at 10°C min^{-1} in a dynamic N_2 atmosphere.

Rheological measurements were performed in a Rheometer of controlled stress (AR-1000N, TA Instruments) using parallel plate geometry with 40 mm diameter, with a gap spacing of 1.5 mm at 25°C, and with a cap to avoid vaporization. Frequency response was measured in the linear viscoelastic spectra over 1–60 rad s^{-1} in a limit strain of 1–6% (i.e., G' and G" 2 independent of strain). Spectra of G' and G" *vs.* strain were recorded at the frequency of 10 rad s^{-1}. The viscosity studies were performed over four decades of shear rate (0.1–1000 s^{-1}). The viscosity $\eta_{app}(\dot{\gamma})$ is in general, a non-linear function of $\dot{\gamma}$. In the limiting case when $\dot{\gamma}$ approaches to zero, η_{app} approaches to the zero-shear rates viscosity, η_0.

Results and discussion

FTIR spectra of individual polymers and blends showed the characteristic peaks of collagen [9] and chitosan [10]: 3317 cm^{-1} (N–H stretching), 1658 cm^{-1} (amide I C=O stretching), 1552 cm^{-1} (amide II N–H bending and C–N stretching), between 1143 and 1301 cm^{-1} (amide III C–N stretching and N–H bending) and a strong absorption band between 852 and 1213 cm^{-1}, assigned to the polysaccharide structure. These absorption bands that are characteristics of collagen revealed that the structure of collagen is intact in the prepared membranes. IR absorption ratio A_{1234}/A_{1454} was near 1.0

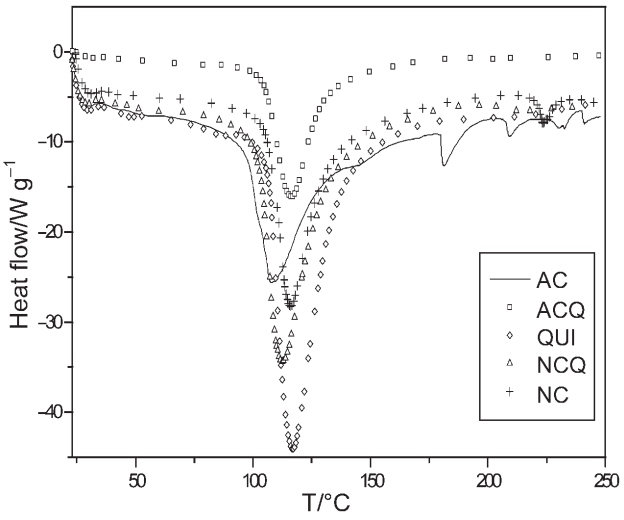

Fig. 1 DSC curves of individual biopolymers and blends

in all samples indicating that triple helix of collagen structure was preserved as compared with gelatine membranes, which have a 0.59 ratio in the same conditions [9].

Thermodynamic properties of collagen, chitosan and their blends were studied using the techniques of differential scanning calorimentry (DSC) and thermogravimetry (TG). DSC curves of chitosan, collagen and blends recorded in nitrogen, from ambient temperature to 250°C, are shown in Fig. 1. The basic DSC curves of all samples (Fig. 1) show a characteristic endothermic peak at about 110°C associated to the loss of bound water. Anionic and native collagen exhibit additional endothermic peaks at 170 and 230°C, attributed to degradation of polymer chains. The temperatures for various thermal effects are given in Table 1.

Table 1 Peak temperature and mass loss in DSC and TG during the thermal degradation of chitosan, collagen and blends

Sample	First range (25–200°C)			Second range (200–400°C)		Residue/% (600°C)
	DTG_{max}/°C	Mass loss/%	DSC/°C	DTG_{max}/°C	Mass loss/%	
AC	54.2	12.8	50.2/117.7	325.2	58.0	20.2
ACQ	49.4	16.0	47.5/116.2	303.5	46.2	28.1
QUI	51.1	18.8	–/116.8	286.2	42.0	32.5
NCQ	45.4	16.0	47.9/112.4	305.4	44.3	30.2
NC	57.5	14.8	52.9/115.9	317.0	55.7	20.2
Interpretation	Water loss	Denaturation/water loss		Decomposition		

In conventional characterization of polymer blends, measurements of glass transition temperature (T_g) by differential scanning calorimetry and dynamic mechanical analysis (DMA) have been used for estimating their miscibility. However, in this work this was not possible because some biomaterials such as cellulose and chitin or chitosan have no clear glass transition temperature. And the decrease in crystallinity of chitosan network cannot be determined from DSC analysis. Although chitosan has crystalline regions, the crystalline melting temperature is not found because of rigid-rod polymer backbone having strong inter- and/or intra-molecular hydrogen bonding. This behavior is frequently detected in polysaccharides such as chitin derivatives [11].

Denaturation of collagen is an endothermic reaction, which occurs at a very slow rate in equilibrium conditions, corresponding to the breakdown of triple collagen helix, yielding a random structure (gelatine). DSC studies of both collagen (native and anionic) and respective blends confirmed that collagen triple helix was preserved, because denaturated collagen do not have any thermal transition in the region of 47–51°C (shown with arrow in Fig. 2) which is the denaturation region. The results confirm IR analyses.

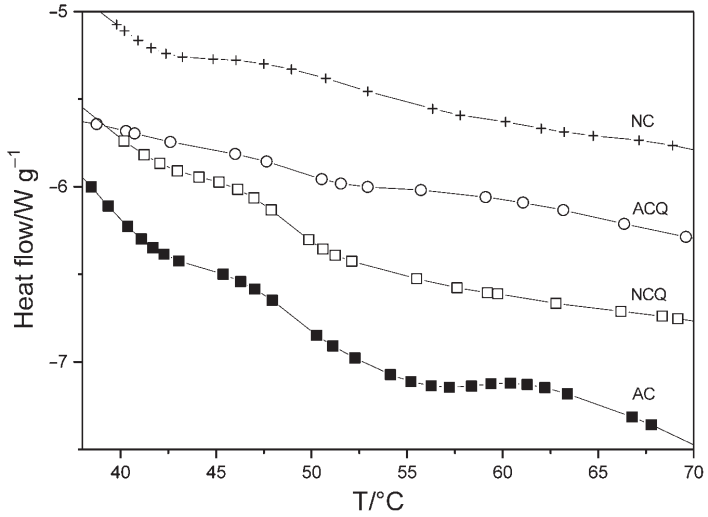

Fig 2 Thermal denaturation profile of collagen and blends

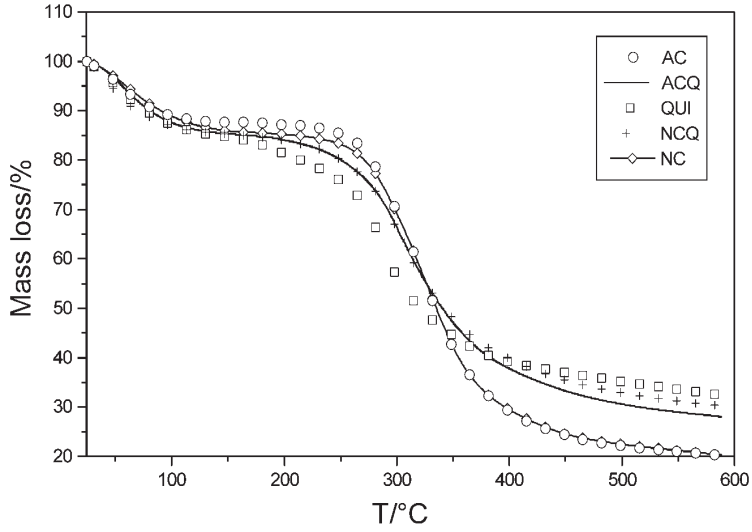

Fig. 3 Thermogravimetric curves of chitosan, collagen and blends

The heat capacity step change at the denaturation transition yields three temperature values: onset, midpoint and endset. The midpoint value, calculated as the midpoint of the extrapolated heat capacities before and after the denaturation transition, was used as the denaturation temperature values (Table 1).

TG curves of chitosan, collagen and blends recorded in nitrogen from ambient temperature to 600°C are shown in Fig. 3. The mass loss (%) and DTG_{max} are also given in Table 1. Chitosan degrades in two stages. The first one begins at 25°C with

17% mass loss assigned to the loss of water. The second stage starts at 170°C and reaches a maximum at 387°C with 43% mass loss, similarly to the behavior previously reported [12]. TG profiles for collagen and blends also show two degradation stages. When compared to its blends chitosan has higher water loss and lower thermal degradation temperature. Among the samples investigated, chitosan has the lowest thermal stability and anionic collagen has the highest one.

Rheology

A strain sweep test at frequency of 10 rad s^{-1} was performed to determine the linear viscoelastic region, from which an appropriate strain was selected (not shown). Storage modulus (G') and the viscous loss modulus (G") were measured from a constant strain frequency sweep over a frequency range of 1–60 rad s^{-1}.

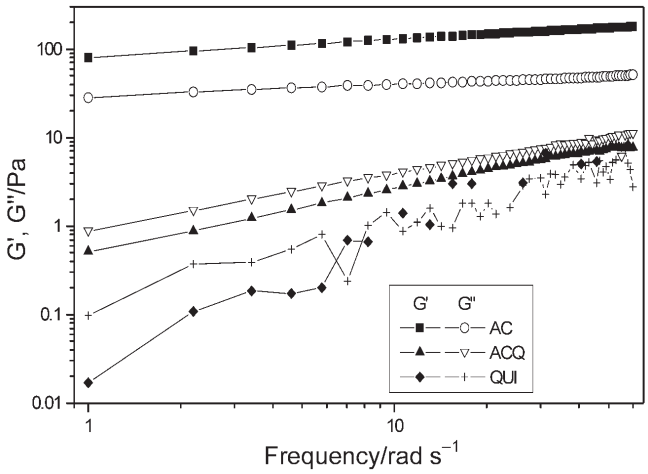

Fig. 4 Viscoelastic properties of anionic collagen, chitosan and blend ACQ (25°C)

Figures 4 and 5 show the frequency dependence of G' and G", at 25°C for chitosan, collagen and blends. As seen from Figs 4 and 5 anionic collagen has higher values of G' and G" than native collagen. Anionic collagen, blend ACQ and native collagen has a gel-like behavior, with storage and loss modulus *vs.* frequency curves being approximately parallel [13]. For anionic collagen, native collagen, blend ACQ and chitosan the frequency (ω) dependence of G' and G", in the range of 1–60 rad s^{-1}, increase with ω, reflecting finite viscoelastic relaxation mechanisms with almost constant reciprocal time [14]. Chitosan has the lowest G' and G" and the anionic collagen has the higher G' and G", and blend ACQ has G' and G" somewhere between the two individual polymers. Native collagen has higher G' and G" than the respective blend with chitosan. Both anionic and native collagen presented more solid-like than fluid-like viscoelastic behavior. Only for ACQ blend G" is higher than G' exhibiting a more fluid-like viscoelastic behavior.

Over a frequency of 15 rad s^{-1} G' of the NCQ blend starts to decrease rapidly (Fig. 5), and before this frequency G' was higher than G" and shows a more solid-like viscoelastic behavior.

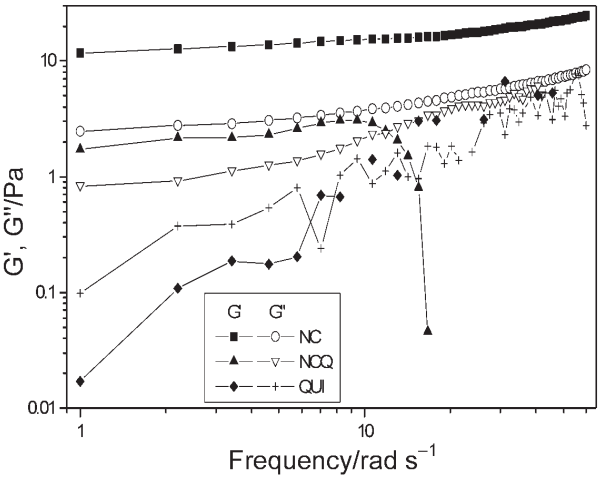

Fig. 5 G' and G" *vs.* frequency for native collagen, chitosan and blend NCQ

Almost all liquids of technical and practical importance show non-Newtonian behavior. Their shear viscosity in general will not be a constant material property but rather will depend upon the state of stress the solution is subjected. Chitosan solutions generally behave as typical non-Newtonian shear thinning fluid [15]. The orientation of the dispersed asymmetric molecules starts to occur in shear stress experiments. With an increasing shear rate this effect will become more pronounced and

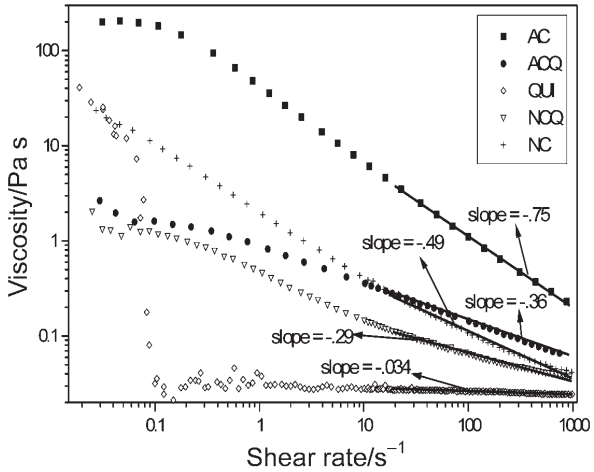

Fig. 6 Apparent viscosity behavior of collagen, chitosan and blends *vs.* shear rate

will cause a decrease of the internal friction because of a smaller effective interaction between the molecules.

Apparent viscosity dependence on shear rate is presented in Fig. 6, and steady-state viscosity plots show that there is an initial Newtonian regime, where with the increase of shear rate viscosity starts to decrease. That is, all materials have a shear thinning behavior. NCQ blend shows the smallest shear thinning (slope = –0.29) and followed by the blend ACQ (slope = –0.36), then native collagen (slope = –0.49) and anionic collagen (slope = –0.75). If consider chitosan apparent viscosity before it came to the second Newtonian regime (shear rate $<0.1\ s^{-1}$) it is the more shear thinning material (slope = –12.77). In the second Newtonian regime, chitosan has slope = –0.034.

Conclusions

There are differences between anionic collagen and native collagen, as a result of the alkaline treatment of porcine serosa during the preparation. It probably allows the entrance of more water groups, swollen the fibre with new hydrogen bonds. It can probably be associated with the increase of G' (or G") *vs*. frequency and apparent viscosity *vs.* shear rate, there in an increase of fibre rigid the rod-like collagen molecules, explained by the liquid crystal phase. FTIR and DSC studies of both collagen (native and anionic) and respective blends confirmed that collagen triple helix was preserved.

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References

1 G. A. F. Roberts, Chitin chemistry, MacMillion, London 1992.
2 W. Paul and C. P. Sharma, STP Pharma Sci, 10 (2000) 5.
3 J. B. Park, Biomaterials Science and Engineering, Third Edition, Plenum Press, New York 1984.
4 P. P. Fietzek and K. Kühn, Molec. Cell. Biochemistry, 8 (1975) 141.
5 D. G. Wallace and A. Thompson, Biopolymers, 22 (1983) 1793.
6 A. Hirai, H. Odani and A. Nakajima, Polymer Bull, 26 (1991) 87.
7 G. Goissis, A. M. G. Plepis and J. L. Rocha,. Patent (1996) BR PI 9.405.043-0.
8 U. K. Laemmli, Nature, 227 (1970) 680.
9 A. George and A. Veis, Biochemistry, 30 (1991) 2372.
10 Y. Shigemasa, H. Matsuura, H. Sashiwa and H. Saimoto, Int. J. Biol. Macromol., 18 (1996) 237.
11 S. J. Lee, S. S. Kim and Y. M. Lee, Carbohydrate Polymers, 41 (2000) 197.
12 F. A. A. Tirkistani, Polymer Degrad. Stability, 60 (1998) 67.
13 H. Jiang, W. Su, P. T. Mather and T. J. Bunning, Polymer, 40 (1999) 4593.
14 D. M. Knapp, V. B. Barocas and A. G. Moon, J. Reol., 41 (1997) 971.
15 M. Mucha, Macroml. Chem Phys., 198 (1997) 971.