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Mechanical properties with respect to water content of gelatin films in glassy state

Iryna Yakimets [a,*], Nikolaus Wellner [b], Andrew C. Smith [b], Reginald H. Wilson [b], Imad Farhat [a], John Mitchell [a]

[a] Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
[b] Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK

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Abstract

Changes on the structural and molecular level of gelatin films induced by hydration below 25±3% water content (glass–rubbery transition at ambient temperature) were identified with DSC and FTIR spectroscopy. Three main stages of hydration were distinguished: (I) water bound by high-energy sorption centres; (II) structural water; (III) polymolecular layer water. The mechanical behaviour of gelatin films at each stage of hydration was characterized. Relaxation of the films during hydration was taken into account in the analysis of results. Hydrated gelatin films were characterized as brittle below the glass–rubbery transition at ambient temperature, however some improvement of mechanical properties related to a higher renaturation level was showed between 7 and 14% of water content in stage II (structural water).

Keywords: Gelatin film; Hydration; Mechanical behaviour

1. Introduction

The wide interest in protein films is mainly due to their biodegradability. The frequent use of gelatin films in food, pharmaceutical and photographic industries made it the most popular biodegradable protein film. Dry protein products such as packaging, capsules are easily damaged during processing, transport and storage. This damage, which often manifested itself in brittle fracture, is conditioned by a decrease of resistance to external mechanical forces and governed by the physico-chemical structure of protein films.

Gelatin is prepared from animal collagen by heating and hydrolysing with acid (type A) or bases (type B). The material properties vary, depending on source and preparation, and the physicochemical characterization of collagen and gelatin has been extensively reported previously [1–13]. The characteristic triple-helical structure of collagen is lost during the gelatin preparation, but can be reformed below the gel melting temperature. The stiffness of gelatin gels (expressed as the Bloom index) and the mechanical properties of drawn films are closely related to the renaturation level (i.e. the triple-helix content) [12]. The renaturation can be determined by different methods such as DSC, X-ray, polarimetry and others. It was shown by Achet and He that calorimetry is most suitable to monitor the real fraction of gelatin chains in a collagen-like structure and it is mainly upon the value of this fraction that the mechanical properties of gelatin films depend [13].

The molecular structures of gelatin, including its renaturation level, depend on the interactions between water and protein molecules. Consequently, water content has a great influence on the mechanical properties of protein films. An analysis of the literature data on hydration of gelatin makes it possible to subdivide the sorbed water according to its state into four types [14,15]:

1. *Water bound by high-energy sorption centres* (from 0 to 0.055 g/g (0– 10% r.h.)). This water sits at charged residues and inside the triple helix and plays a major role in its stabilization through intramolecular hydrogen bonds.
2. *Structural water* (from 0.055 to 0.14 g/g (10– 40% r.h.)). This is the water directly bound to the protein (both inside and outside helical fragments).
3. *Polymolecular layer* (from 0.14 to 0.37 g/g (40– 90% r.h.)). In excess of bound water concentration of 0.14 g/g, the monomolecular layer transforms into a polymolecular layer, which covers the triple helix structure.

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* Corresponding author. Tel.: +44 115 9516198; fax: +44 115 9516142.
E-mail address: iryna.yakimets@nottingham.ac.uk (I. Yakimets).

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4. *Free water* (above 0.37 g/g (90% r.h.)). Above 0.37 g/g free non-bound water is observed.

In order to highlight the interaction of proteins and water molecules, the transformations of gelatin induced by hydration at the molecular level should be considered. FTIR is often used in the studies of polymer modifications at the molecular level. Several infrared spectroscopy studies of collagen and gelatin have been published [16–19] but most were concerned with the secondary structures in the solution or gel state and little is known about film hydration.

It was also shown previously that at 25 ± 3% water content (dry basis), gelatin films undergo transition from the glassy to the rubbery state at ambient temperature, which changes drastically their mechanical behaviour [20]. This transition manifests itself most clearly when gelatin is subjected to impact: the resistance to impact drastically increases when the water content increases above 25 ± 3% (dry basis) [14].

Another very important point to take into account for study of gelatin is its sensitivity to physical aging. As with any glassy material, gelatin is not thermodynamically stable and during storage below T_g, its structure tends to relax towards the liquid line [21]. The physical aging is typically characterized by enthalpy relaxation and densification. It was shown that physical aging leads to an increase of the modulus, brittleness and stress at break of materials [22,23].

The aim of this study was to correlate the modifications on structural and molecular level induced by hydration below the glass-rubbery transition at ambient temperature with the mechanical properties of gelatin films in glassy state.

2. Experimental

2.1. Material

Gelatin with a relatively high bloom index, which shows a high level of renaturation, was chosen for this study. Type B gelatin from bovine skin, bloom 225 (Sigma-Aldrich Ltd, Poole, UK, G9382) was dispersed in distilled water and heated slowly to 70 °C and subsequently cooled slowly to room temperature (22 °C). This solution was used to prepare thick films by casting and thin films by spin-coating.

2.2. Films and conditions of hydration

2.2.1. Thin films

‘Thin’ films were prepared using a spin-coater (SCS-G3P8, SCS Inc, Indianapolis, PH, USA) with 5 cm diameter polystyrene Petri dishes as substrates. Spinning a 4% aqueous solution at a rate of 200–250 rpm yielded films with a thickness 10–15 μm. The films were then dried at room temperature (22 °C) and room humidity (40–50% r.h.) for 24 h on a level surface. The films were carefully peeled off the substrate and stored in large petri dishes at room temperature. The thickness of the films was measured using a micrometer and/or determined from the interference fringes in the IR.

Table 1
Relative humidities given by saturated salt solutions and corresponding water content of gelatin films at ambient temperature (22 °C)

Salt solution	Relative humidity r.h. (%)	Water content of gelatin films, dry basis (%)
P_2O_5 (powder)	0	0.8
LiCi	11.3	5.2
CH_3COOK	21.6	8.3
$MgCl_2$	32.8	11.2
K_2CO_3	44	13.6
$Mg(NO_3)_2$	52.8	15
NaBr	57.5	17.4
KI	68.9	20.8

The film sheets were hydrated dynamically in situ through the circulation of a controlled r.h. air. The controlled relative humidity was achieved by mixing dry air (0% r.h.) from a Balston air drier (Parker Hannifin, Maidstone, Kent, UK) and H_2O vapour saturated air (100% r.h.). The flow rates were adjusted with mechanical flow valves and the humidity of the combined air stream was measured with an impedance probe (Lee-Integer DHL49, UK).

2.2.2. Thick films

‘Thick’ films (thickness = 100 ± 6 μm) were prepared by casting a 4% aqueous gelatin solution into polystyrene Petri dishes and evaporating water at room humidity and temperature (40–50% r.h., 22 °C). Shortly after drying films were cut into strips for samples for tensile mechanical tests (Section 2.4). In order to erase the thermal history, these samples were reheated above the glass transition temperature and annealed at 80 °C for 1 h. Immediately after annealing, the samples were statically hydrated in a controlled r.h. chamber with saturated salt solution for 1 week. Seven saturated salt solutions and powder of P_2O_5 were used to maintain specified relative humidities in closed chambers. The saturated salt solutions used in this study and corresponding water content (dry basis)[1] of gelatin films obtained after one week of static hydration are summarised in Table 1.

2.3. Physicochemical analysis

2.3.1. X-ray

X-ray diffractograms were recorded on both thin and thick films for 2θ between 4 and 50 at 0.1° intervals (scanning rate 6 s^{-1}) using a Bruker D5005 (Bruker AXS, UK) diffractometer equipped with a copper tube operating at 40 kV and 40 mA producing Cu Kα radiation of 1.54 Å wavelength.

2.3.2. DVS

To establish the relationship between the relative humidities and water content of gelatin cast films in the case of dynamic hydration, Dynamic Vapour Sorption analyser DVS-1 (Surface Measurement Systems Ltd, London, UK) equipped with

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[1] The water content of gelatin films was determined by drying of control-samples in vacuum oven for 24 h and compared to the sorption diagram.

a Cahn D200 microbalance was used (ambient temperature, 22 °C and relative humidity range from 0 to 95% r.h.). The initial wet mass of used sample-films was 2–2.5 mg with the thickness between 7 and 20 μm. To get the dry mass, the films were dried by exposure to 0% r.h. for 260 min before start of the hydration in the DVS analyser.

2.3.3. DSC

A Perkin–Elmer Instruments DSC 7 differential scanning calorimeter was used in this study. The instrument was calibrated with pure indium ($\Delta H_m = 28.4$ J/g and $T_m = 156.6$ °C). Samples (5 and 18 mg) with different water contents were sealed in high pressure stainless steel pans and heated at 10 °C/min[2]. An empty pan of the same type as the sample pan was used as a reference. The measurements were performed in the temperature range between −20 and 200 °C. After the first heating at 10 °C/min, the samples were rapidly cooled down (50 °C/min) and reheated at the same rate of 10 °C/min. Four thermal characteristics were measured:

- Glass transition temperature (T_g), reported as the midpoint of the ΔC_p changes at the glass transition measured in the second heating scan.
- Melting temperature (T_m) reported as the maximum of melting peak in the first heating scan.
- Enthalpy of melting (ΔH_m) reported as the area of the melting peak in the first heating scan.
- Relaxation enthalpy associated with physical aging[3] (ΔH_{ag}) calculated as difference between the first and second heating scan around T_g.

2.3.4. FTIR

Fourier-transform infrared spectroscopy was used to study the effects of hydration on gelatin in term of molecular and supermolecular structure and organization. The thin film samples, prepared by spin-coating, were mounted freestanding in a hydration chamber between two BaF_2 windows. The chamber was a variation of a system described previously [24]. The system was placed into the sample compartment of a BioRad FTS175c Fourier transform infrared spectrometer (DigiLab, now Varian, USA) equipped with a narrow-band MCT detector. The tests were performed at ambient temperature. The films were dried overnight with dry air, and then the air humidity was increased stepwise from 0% r.h. to 80% r.h. in steps of 5% r.h. At each step, a single beam spectrum was acquired (256 scans, 2 cm^{-1} resolution) after equilibrium was reached[4]. For difference spectra, the measurements were ratioed against the spectrum of the dry film. Absorbance spectra were obtained with a spectrum of the empty, dry sample compartment as background. Water vapour bands due to the atmosphere in the sample compartment were removed by digitally subtracting a water vapour spectrum. Data were exported as ASCII files and the spectra analysed using MATLAB (MathWorks Inc, Natick, MA, USA).

2.4. Mechanical tests

The uniaxial tension tests were performed in order to characterize the mechanical behaviour with respect to water content. Tensile strength and percentage elongation were measured on sheet-specimens cut from the thick films (thickness = 0.1 ± 0.06 mm, width = 10 mm and gauge length = 40 mm) after their equilibration at various relative humidities, using a Texture Analyser TA.XT plus (30 kgf maximum load). A slow extension rate of 0.033 mm/s was adopted in order to consider the elongation regime as isothermal. Sheet-specimens were coated in silicon oil to alleviate the problem of water loss. The tensile tests (until break of specimens) were performed at ambient temperature using the tensile grips (A/TG).

To study the main mechanical characteristics of films with respect to water content, at least 10 samples with the same water content were stretched. The recorded tensile test data were converted to the conventional stress-strain ($\sigma_{con} = f(\varepsilon_{con})$) diagrams:

$$\sigma_{con} = F/A_0 \text{ and } \varepsilon_{con} = \delta/L_0$$

with, F, applied force; A_0, original cross-section; δ, change in gauge length; L_0, original gauge length.

The following mechanical characteristics were calculated:

- ✓ Elasticity modulus, E (slope of linear part of stress – strain diagram).
- ✓ Proportional limit (in term of strain, ε_{pl}, and stress, σ_{pl}), or elastic limit.
- ✓ Yielding strain, ε_y, and yielding stress σ_y (in this case, could be associated to the viscoelastic limit).
- ✓ Stress and strain at break, σ_b and ε_b, respectively.

To identify these characteristics, the method shown in Fig. 1 was used.

3. Results

3.1. Sorption behaviour

The DVS results were used in this study to convert the relative humidifies to the water content of thin gelatin film hydrated in situ. The obtained isothermal sorption–desorption diagram of gelatin film is shown in Fig. 2. Only the sorption part of the diagram was considered in this study.

3.2. Comparaison of thin and thick films

X-ray diffraction analysis was performed to quantify the triple-helical structure of the cast and spin-coated films as well

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[2] To measure the glass transition and melting temperature, medium heat rate (10 °C/min) was chosen to increase the sensitivity and to keep a better resolution in separating transitions occurring at close temperatures.

[3] Enthalpy of endothermic peak that appeared near the glass transition temperature during the first heating scan correlated with the extent of physical aging.

[4] According to the sorption diagram for thin film, the time necessary to reach the equilibrium for each relative humidity was identified.

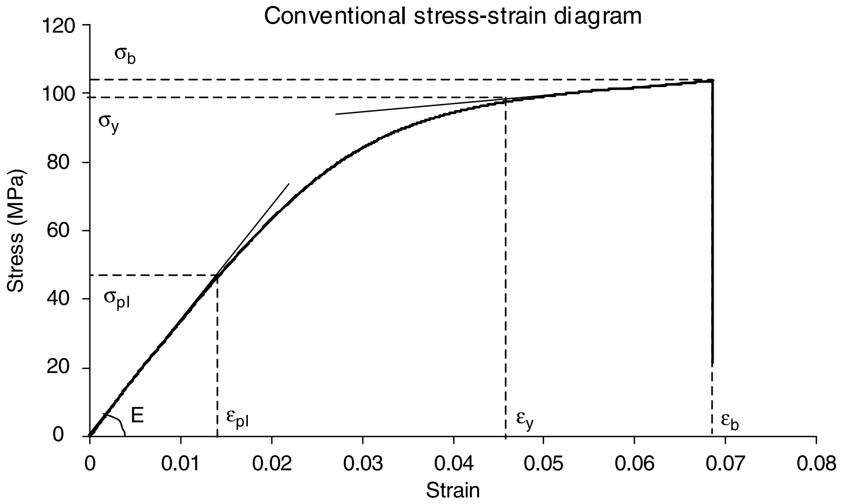

Fig. 1. Method to identify the mechanical characteristics.

as the modifications induced at the microphase structure level by annealing of the cast films. The diffractograms acquired on the gelatin films produced by casting and spin-coating were typical of a partially crystalline gelatin with the peak at $2\theta = 7°$ (Fig. 3). This characteristic peak is usually assigned to the triple-helical crystalline structure in collagen and renatured gelatin [25]. Fig. 3 shows that, shortly after drying, the cast films had a relatively high level of renaturation compared to the spin-coated film (13 and ~4%, respectively). In the thick films the slow drying resulted in the formation of the ordered triple-helical structure. On the other hand, the high drying speed and low thickness of the spin-coated films resulted in low molecular order. Annealing of the thick films made them comparable to the spin-coated film in term of molecular order, as the triple-helix content was reduced from 13 to ~3%.

3.3. Physicochemical characterization of films with respect to water content

To study the changes induced by hydration on structural and molecular level, the DSC and FTIR methods were employed, respectively.

3.3.1. Structural level

In general, the results of the DSC analyses showed the well-known pronounced plasticizing effect of water on gelatin: depression of T_m and T_g (Figs. 4 and 5, respectively). Furthermore, the renaturation level of the gelatin films, as determined from the melting enthalpy (Fig. 4), was maximal for the water content ranges between 7.6 and 14%. According to the literature, this range of water content in gelatin corresponds to the water sorbed by polar groups of gelatin or structural water (II stage), which is bound with the proteins by H-bond (both inside and outside helical fragments) [14]. For the water content below 7.6%, the quantity of water molecules is no longer sufficient to contribute to the stabilisation of high-ordered structure (I stage, Fig. 4). In contrast, for the water content above 14%, an excess of water molecules contributes to the reorganization of the molecular order and a decrease in triple-helix content (III stage, Fig. 4).

Furthermore, the enthalpy relaxation around the glass transition temperature (recorded by DSC during the first heating), observed for the cast films equilibrated at different relative humidities during one week, indicated the existence of the physical aging. Fig. 5 shows that the maximum of physical

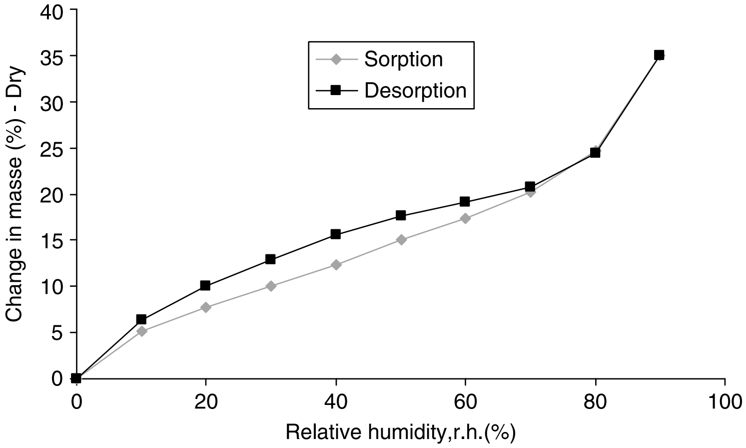

Fig. 2. DVS isotherm plot for the gelatin film (thickness = 7 μm).

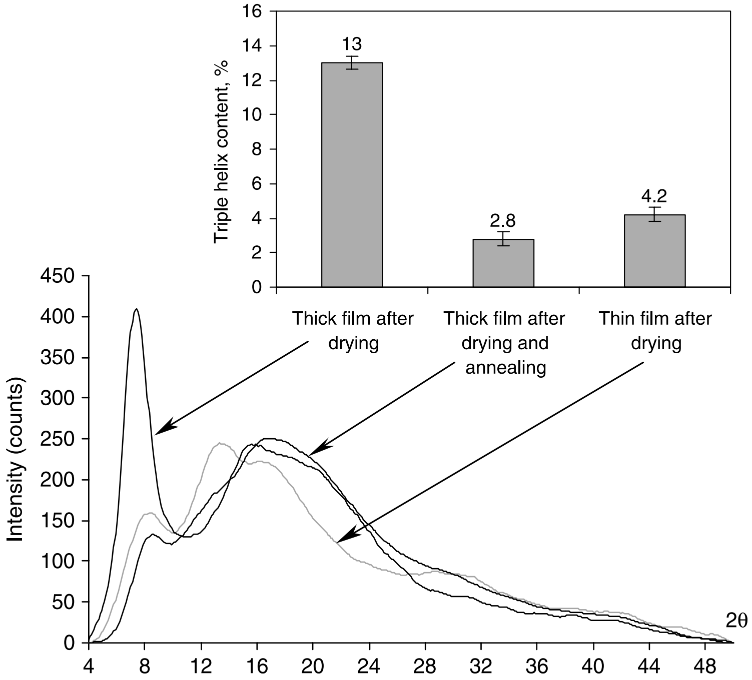

Fig. 3. X-ray patterns and quantity of helical structure of the thick film after drying, thick film after drying and annealing and thin film after drying.

aging occurred at 11.8% of water content. A similar maximum of relaxation enthalpy was already observed on normal and waxy rice starches [26]. Firstly, the ΔH_{ag} increased (0–11.8% water content) due to the increasing of the molecular mobility, which facilitates gelatin densification (this aging process being under kinetic control). Afterwards, the ΔH_{ag} started to decrease due to the approaching of the aging temperature, T_a, to the glass transition temperature, T_g, (11.8–22% water content). This contributed only to a limited extent of sample densification (this aging process now being under a thermodynamic control).

3.3.2. Molecular level

FTIR spectroscopy was used to reveal the changes on the molecular level caused by hydration of the gelatin films. Analyses of the amide I band of collagen and gelatin have shown three main components at 1660, 1643 and 1633 cm^{-1}, with the 1660 cm^{-1} peak being characteristic for the triple-helical structure of collagen and the 1633 cm^{-1} peak characteristic for the coil structure of gelatin [17]. In the freshly made film the maximum of the amide I band (νC=O) was found at 1658 cm^{-1} in the dry state, and shifted to 1649 cm^{-1} when hydrated. This position was consistent with the predominantly unordered structure observed by X-ray diffraction, as positions of 1656 and 1651 cm^{-1} have been observed in denatured rat collagen [19]. Furthermore, a band position around 1643 cm^{-1} had been reported for unordered gelatin in solution [17]. In films with higher helix content the maximum was found at 1661 cm^{-1}.

Fig. 6 shows the changes in the FTIR spectra caused by hydration of gelatin films. The most noticeable effect was an increased absorption in the 3600–3000 cm^{-1} region, with the two main peaks in the difference spectrum at 3475 and

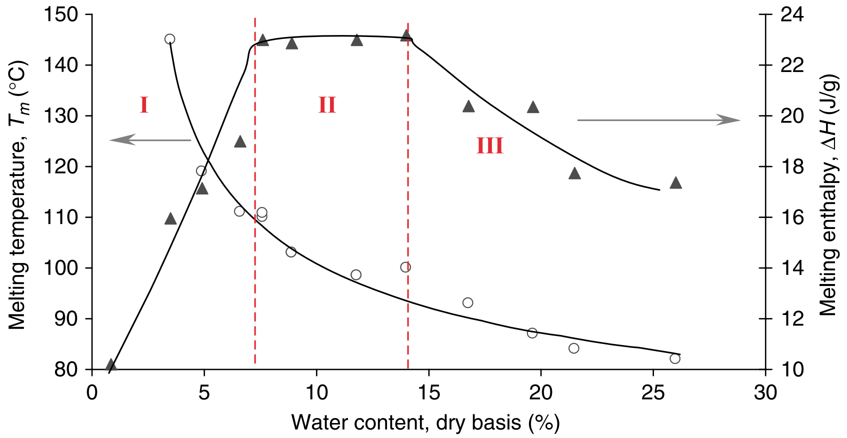

Fig. 4. Melting temperature, T_m, and melting enthalpy, ΔH, respect to water content.

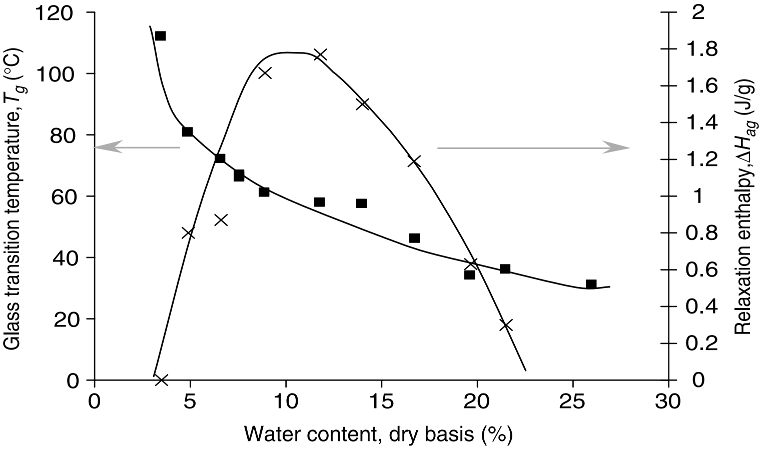

Fig. 5. Glass transition temperature, T_g, and enthalpy of physical aging, ΔH_{ag}, versus water content.

3240 cm^{-1}, due to the ν_{as} and ν_s OH stretching bands of adsorbed water molecules. The exact shape of the difference bands may be influenced by changes in the amide A band of the protein, whose maximum was found at 3340 cm^{-1} in the dry protein.

In the amide I band the hydration caused a marked loss of intensity at around 1695 cm^{-1} linked to the loss of aggregated helices [19] and increase at 1630 cm^{-1} due to the formation of hydrated chains of predominantly coil structure gelatin, which has shown a pronounced band at 1633 cm^{-1} in water [17]. In addition, some of the increase at 1630 cm^{-1} may be assigned to the deformation band of adsorbed water molecules.

In the amide II region an intensity increase at 1560 cm^{-1} and a decrease at 1500 cm^{-1} were observed, which resulted in a shift in the maximum of the amide II band 1536–1554 cm^{-1}. The amide II band is less sensitive to secondary structure than the amide I, but because of its nature (combined CN stretching CNH deformation) it is very much influenced by hydration, and gelatin/collagen studies have shown an up-shift from 1534 to 1565 cm^{-1}. Consequently, the observed band shift indicated increasing levels of protein backbone hydration. A similar argument may be used to explain the changes seen in the amide III band region, where increasing hydration caused a frequency shift from 1210 to 1240 cm^{-1} combined with an intensity loss at the lower of these two frequencies.

Plotting the rate of the spectral changes in the protein as a function of water content revealed several regions, which could be associated with the different types of hydration (Fig. 7). The FTIR spectroscopy showed that at molecular level for water content up to 5%, the protein bound the water molecules with a high energy. This high-energy sorption effect could be observed by the high initial rates of changes in the infrared

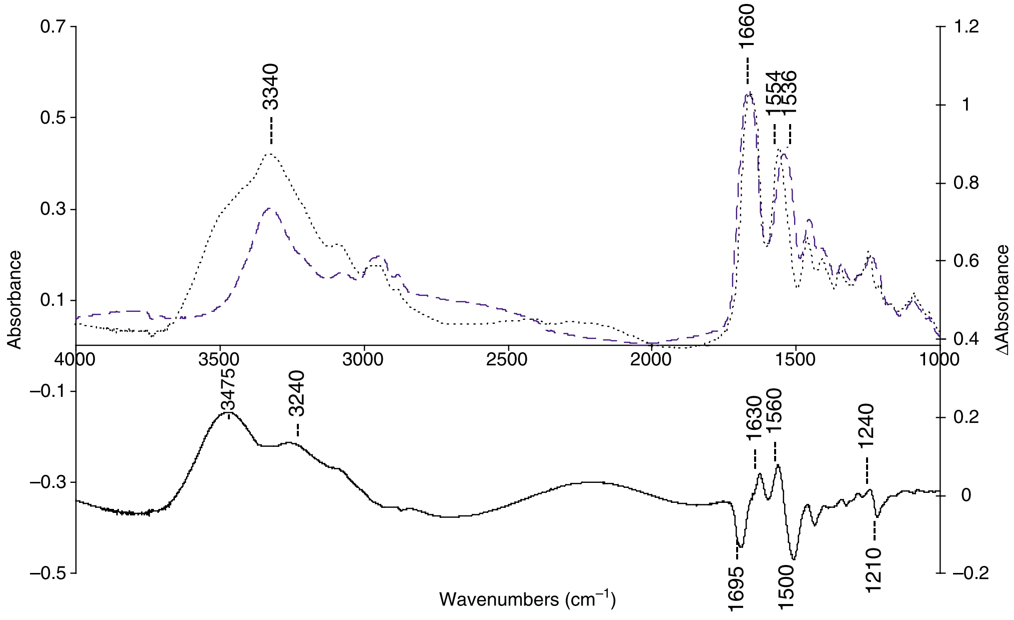

Fig. 6. FTIR spectra of the thin gelatin films: (– – –) dry, (···) hydrated to 22% water (dry basis), and (—) difference spectrum (hydrated minus dry, offset on secondary axis for clarity).

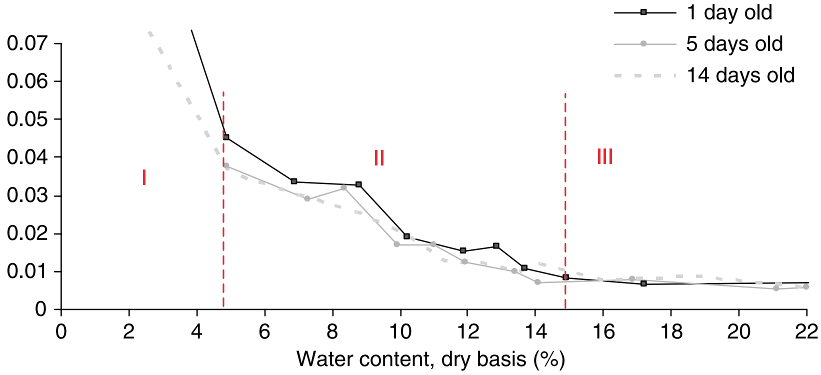

Fig. 7. Water band ratio changes as a function of hydration.

spectra for the OH band of water and the amide bands of the protein (stage I, Fig. 7). Between 5 and 15% of water content, spectroscopy showed a steady medium rate increase in hydrogen bonding of water to protein, which corresponded to the medium water activity (stage II, Fig. 7). Above this point the hydration rate slowed down markedly, as well as the spectral changes in the protein. Between 15 and 22% the amide bands continued to change slowly as polymolecularlayer water was added, and above 22% the spectra remained constant, indicating that the protein was fully hydrated and did not sense any additional water (stage III, Fig. 7). The comparison of the increase of water bands for films aged at ambient conditions (22 °C, 40–50% r.h.) for 1, 5 and 14 days showed that aging did not have a pronounced effect on hydration behaviour.

3.4. Mechanical properties of gelatin films

To link the structural and molecular modifications induced by hydration to the macroproperties of gelatin films in glass state, the elasticity modulus and other mechanical characteristics in term of strain and stress were plotted, respectively, in Figs. 8–10 as a function of water content. Fig. 8 demonstrates that at a water content above 14%, the elasticity modulus of gelatin films shows a significant decrease. This effect could be explained by the appearance of polymolecular water. The strain diagram illustrates that for water content up to 7% (stage I, Fig. 9) and from 14 to 22% (stage III, Fig. 9) the glassy gelatin films exhibited a typical brittle behaviour: the fracture occurred in the area of viscoelastic deformation (the average values of strain at break, ε_b, are lower than the yielding strain, ε_y). It means that for these water ranges, the gelatin films did not undergo any plastic deformation during tensile loading. However, for films that contained between 7 and 14% of water (stage II, Fig. 9) the fracture came slightly above the viscoelastic limit (with big dispersion of ε_b results), and it would signify the presence of some permanent plastic deformation, which preceded the fracture. On the other hand, the stress-diagram (Fig. 10) shows that for this range of water the maximum stress never exceeded the yielding stress, which means that the plastic deformation was weak and did not lead to a decrease of cross-section area. According these results, the mechanical behaviour of the gelatin films with respect to water content could be divided into three stages:

- Brittle behaviour without any plastic deformation (0– 7% water).
- Brittle behaviour with weak plastic deformation without decrease of cross-section area, which precedes the fracture (7– 14%).
- Brittle behaviour without any plastic deformation (14– 22% water).

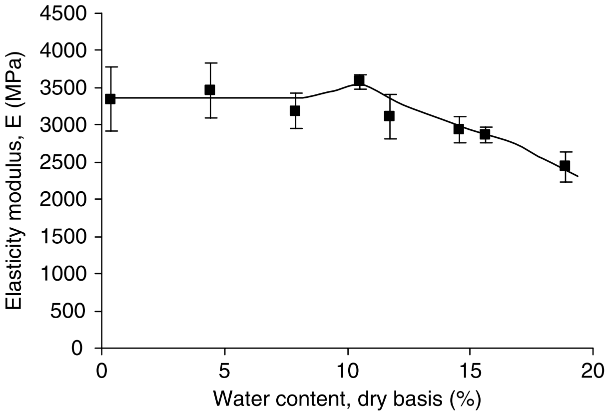

Fig. 8. Elasticity modulus versus water content.

4. Discussion and conclusion

According to literature the hydration of gelatin films proceeded through three main stages before reaching the glass-rubbery transition at room temperature: (I) water bound by high-energy sorption centres stage; (II) structural water and (III) polymolecular layer water [14]. The experiments showed that these stages of hydration can be distinguished at the microphase structure level according the renaturation level with respect to water content determined with DSC method (Fig. 4) and at the molecular level according to the water band ratio changes as a function of hydration determined with FTIR method (Fig. 7).

The aim of this study was to follow the changes of mechanical properties of gelatin films in the glass state through

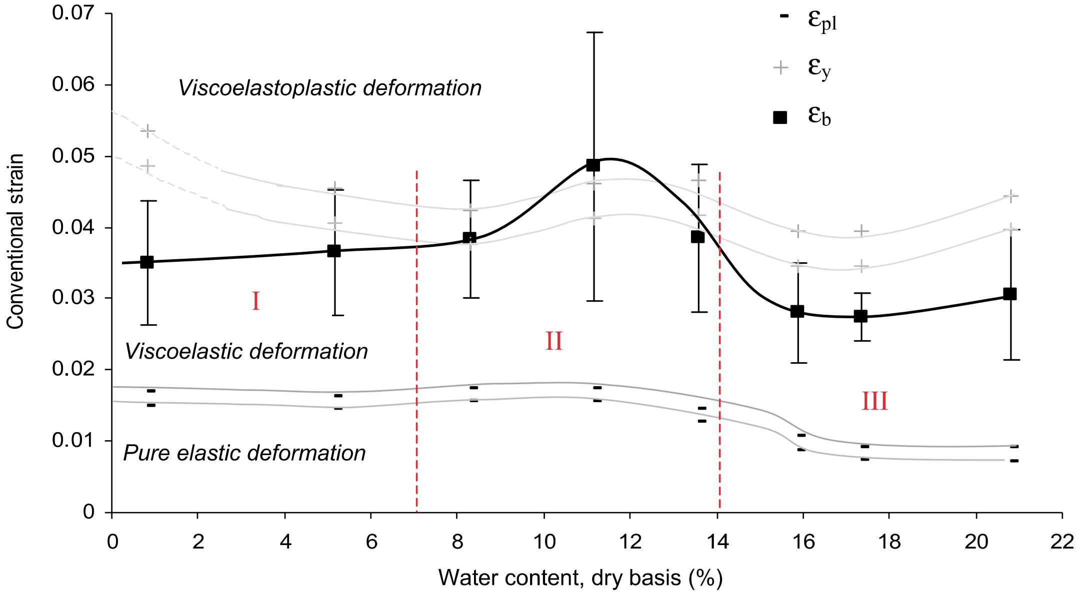

Fig. 9. Strain diagram (versus water content).

these three stages of hydration. The results showed that at first stage of hydration (water bound by high-energy sorption centres, up to 5% (FTIR) 7.6% (DSC) of water content) and at the third stage (polymolecular layer water, from 15% (FTIR) 14% (DSC) to 22%) films show brittle behaviour at fracture, which characterized by absence of plastic deformation preceding the break. We would expect that the mechanical characteristics, particularly the strain at break, will increase at high water contents (14–22%), but, in contrast, it dropped to the same values, which are shown by the brittle dehydrated coil-structure gelatin films. These two stages of hydration, characterized by high water bound energy (stage I) consumed for stabilisation of triple-helical structure and insignificant water activity due to the cover of the triple helical structure by polymolecular layer (stage III), give high content of coil structure. This fact leads to increase of brittleness of gelatin fims.

In contrast, for the second stage of hydration (structural water), the films showed an improved fracture behaviour, which manifested itself by the ε_b peak. This improvement of fracture behaviour was related to the high renaturation level of gelatin films shown by the DSC method (ε_b peak coincided with the maximum of the triple-helix content) (Fig. 4). The dispersion of strain at break values for the same water content could be explained by different orientation of triple-helical structure in the plane of fracture, which improved more or less

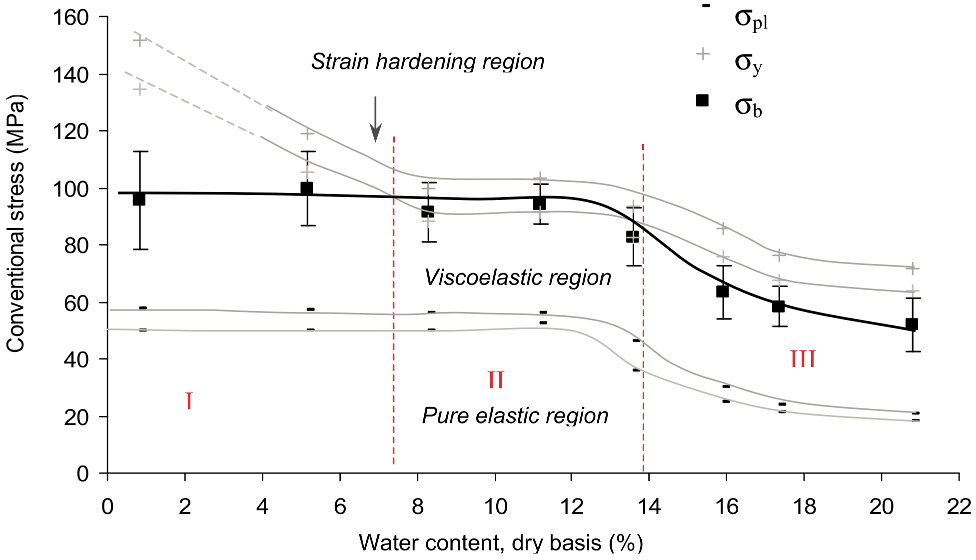

Fig. 10. Stress diagram (versus water content).

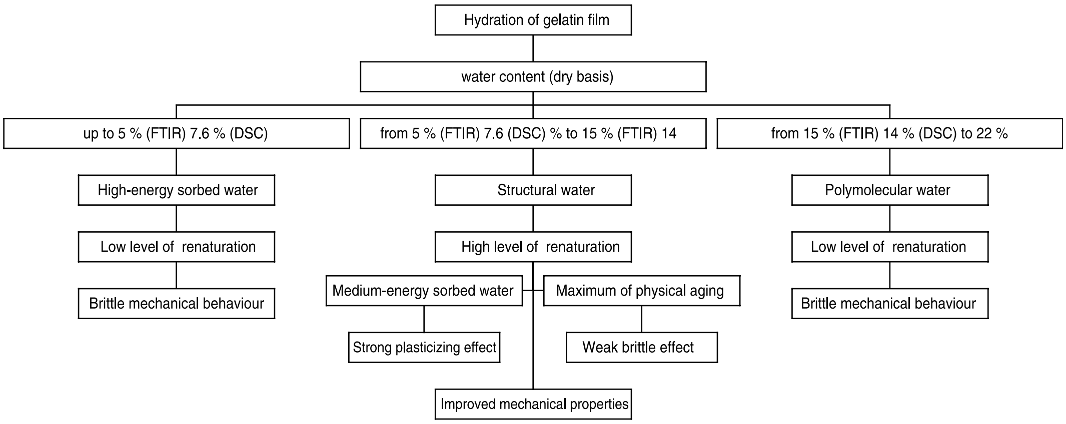

Fig. 11. Summary of results.

the fracture behaviour. On the other hand, the DSC analysis showed that, for this range of water content, the gelatin films are vulnerable to physical aging. In general, physical aging of the polymer would be expected to result in enhanced hardness and brittleness. In our study, we found that the strain at break increased for the films with water contents ranging between 7 and 14%. This phenomenon was not associated with the maximum of aging found in this range but with the maximal triple-helix content. The aging effect could be considered as unimportant compared to the influence of the renaturation level on the mechanical behaviour of films.

A short summary of the obtained results is shown in Fig. 11.

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