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The Structure–Property Correlations in Dry Gelatin Adhesive Films

Yasmine Mosleh,* Willem de Zeeuw, Marlies Nijemesland, Johan C. Bijleveld, Paul van Duin, and Johannes A. Poullis

Gelatins are proteinaceous natural materials that are widely used in areas such as conservation and restoration of artifacts as adhesives and consolidants, in pharmaceuticals as drug delivery carriers, and in the food industry as structurants. Herein, type A porcine gelatin adhesive films are prepared via solution casting method and their physical and mechanical properties are investigated using X-ray diffraction (XRD), differential scanning calorimetry, contact angle measurement, dynamic mechanical analysis, and uniaxial tensile tests. The results demonstrate a linear correlation between microstructure of gelatin films in terms of their triple-helix content and their macroscopic mechanical properties such as tensile strength and gel (Bloom) strength. Moreover, the findings of this study can help the scientists, in, e.g., art conservation and restoration, to predict the mechanical performance of these adhesives by performing a less material demanding and nondestructive physical measurement such as XRD.

1. Introduction

Gelatins are collagen-based biomaterials that are widely used as adhesives, more widely known in this field as animal glues, for preservation and restoration of artifacts and objects of cultural heritage such as panel paintings, decorated furniture, and books. They have been also used as consolidants, paint binder, and canvas lining over the last centuries.^[1–3] For many centuries, they were used for the production of objects of cultural heritage,

such as furniture, panel paintings, books, hats, and many other objects.

Moreover, gelatins are also used in the pharmaceutical and food industry.^[4,5] Particularly, fish gelatin is considered as a candidate for food packaging due to its flexibility, transparency, and superior barrier properties against oxygen and UV light.^[6]

Collagen is a primary structural protein in skin, bone, and connective tissues of animals. Collagen molecules are composed of three polypeptide chains, wound together in a right-handed triple helix, ≈ 280 nm long, stabilized by intermolecular hydrogen bonds and chemical cross-links.^[7] Collagen is water insoluble and can be turned into water-soluble gelatin through the denaturation process during which the triple-helix

structure in collagen unwinds and turns into random coil polymer chains giving gelatin. The process of gelatin extraction involves the use of acid (type A) or base (type B) for collagen hydrolysis at a temperature range of 50–80 °C, factors which can subsequently affect the average molecular weight of the obtained gelatin.^[8,9]

During the gelling and subsequent drying of gelatin, the random protein coils undergo partial renaturation back into triple helices.^[3,10,11] These triple helices in gelatin act as physical cross-links leading to a continuous 3D network structure.^[12] The triple-helix content which is linked to the degree of renaturation is an important physical characteristic of the gelatin adhesive films dominating their thermal and mechanical performance.^[10,13] The triple-helix content in gelatins can be influenced by many factors such as animal origin defining amino acid profile of polypeptide chains (e.g., hydroxy proline content),^[14] gelatin concentration,^[15] preparation and casting conditions,^[16] molecular weight distribution,^[8,17,18] and moisture content.^[19,20]

Another important parameter when choosing gelatin for a certain application is its Bloom value (strength). The Bloom value is the force in grams required to press a specific plunger into a gelatin gel of a defined concentration.^[21,22] The degree of renaturation is believed to also be related to the Bloom value (strength).^[13]

To measure the triple-helix content, methods such as X-ray diffraction (XRD), differential scanning calorimetry (DSC), and polarimetry have been utilized in previous studies.^[10,13,19]

Due to extensive variety in the functionality of gelatins in conservation and restoration of historic objects such as furniture

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and panel paintings as adhesives, binding medium, and consolidants, developing comprehensive knowledge on the thermal and mechanical behavior of these adhesive systems is crucial to give people in the field better knowledge about the behavior of historic glue films and to choose the right material with the right properties for conservation purposes.

In this article, films of type A porcine skin gelatin with four different Bloom values were prepared using the solution casting method. The materials were then conditioned at room temperature and 50% environmental relative humidity. Physical and mechanical behavior of the gelatin adhesives was characterized using DSC, XRD, contact angle measurement, uniaxial tensile testing, and dynamic mechanical analysis (DMA).

This article investigates the existence of clear structure–property relations in dry gelatin adhesive films. It is evaluated if nondestructive and less material demanding physical measurement techniques such as XRD can predict the mechanical performance such as tensile and gel strength in gelatin films. This will help scientists in different research fields, using gelatins, to be able to limit the type and number of characterization methods on these materials.

2. Materials

Four different type A porcine gelatin powders were sourced from Trobas Gelatin B.V., based in Dongen, the Netherlands. Two of these were food grade type with Bloom strengths of 240 and 180 g, referred to in this article as PG240 and PG180, respectively. The other two types of gelatin were technical gelatins with Bloom strengths of 110 and 60 g, respectively. Therefore, the technical gelatins are referred to as TG110 and TG60. To prevent bio deterioration, MERGAL KM90 pesticide was added to the gelatin aqueous solutions as pesticide.

2.1. Sample Preparation

Gelatin adhesive films were produced using a solution casting method. For this, a 30 g of gelatin powder was added to 300 mL of demineralized water and subsequently heated to 60 °C for an hour. To avoid the biological decay by microorganisms, 0.1 v/v% of MERGAL KM90 pesticide was added to the gelatin aqueous solution. After an hour of heating, the gelatin solution was cast on a square Teflon mold. The gelatin film was left to dry for 48 h at a controlled temperature of 22 °C and a relative humidity of 50%.

3. Experimental Section

3.1. Physical Tests

3.1.1. Differential Scanning Calorimetry

To obtain the glass transition temperature (T_g), denaturation temperature (T_d), and the denaturation enthalpy (ΔH_d) of different gelatin adhesive films, a TA Instrument DSC 250 differential scanning calorimeter was utilized. The gelatin films were conditioned at 50% relative humidity at 22 °C in a controlled climate chamber for 72 h before being tested. Subsequently,

the test samples, each weighing about 5 mg, were hermetically sealed using Tzero aluminum pans, whereas an empty pan was used as reference. For the measurements, the samples were heated and cooled and again heated in three subsequent temperature sweeps. In the first heating step, the samples were heated from 10 to 150 °C at 10 °C min⁻¹, maintained at 150 °C for 5 min, cooled from 150 to 10 °C at 10 °C min⁻¹, maintained for 5 min at 10 °C, and finally heated again from 10 to 150 °C at 10 °C min⁻¹. All the measurements were performed in triplicate. The apparent glass transition temperature (T_g) was measured as the midpoint of the heat flow change at the glass transition in the first heating scan. The denaturation temperature (T_d) was reported as the minimum point of the endothermic denaturation peak in the first heating scan. The enthalpy of denaturation (ΔH_d) was calculated as the area of the endothermic denaturation peak in the first heating scan.

3.1.2. X-Ray Diffraction

X-ray diffractograms were recorded on the gelatin films for 2θ between 3° and 60° at 0.1° intervals and a speed of 1.0°/min using a Rigaku MiniFlex 600 with a NaI scintillator detector. A Cu K α radiation source was used ($I = 15$ mA and $U = 40$ kV). For each gelatin type, at least three samples were tested. All the films had a thickness of 0.13 mm. Moreover, it was ensured that the sample was placed at the same height and level with the rim of the sample holder and the same distance to the detector for each consecutive measurement.

3.1.3. Scanning Electron Microscopy

The morphology of the adhesive films' surface and cross section was monitored using scanning electron microscopy (SEM), with a JEOL JSM-7500F instrument.

3.1.4. Contact Angle Measurements

Static contact angles were measured utilizing a KSV Instrument CAM 200 system (Biolin Scientific, Helsinki, Finland) based on the sessile drop method. For each gelatin type, measurements were taken at room temperature using different samples and locations on the films to yield representative results. Two test fluids, namely, water and fully dispersive diiodomethane, were used.

3.2. Mechanical Tests

3.2.1. Uniaxial Tensile Measurements

Uniaxial tensile tests were performed to measure the mechanical properties such as Young's modulus and tensile strength of different gelatin films as a function of the Bloom value. For these tests, ASTM D882-12, the standard for tensile testing of thin plastic materials, was used. The tensile test samples were cut into strips with dimensions of 20 mm (width) \times 175 mm (length), and 0.13 ± 0.01 mm (thickness). The tensile tests were performed using a Zwick 20 kN general purpose mechanical testing device with a 1 kN load cell, and a long-stroke extensometer with

5 μm accuracy set at a gauge length of 100 mm. The strain rate was set to 1 min^{-1} . Before testing, the samples were conditioned for at least 48 h at a temperature of 23°C and a relative humidity of 50%. At least 10 samples were tested for each gelatin type. Young's modulus (E) was calculated as the linear part of the stress–strain tensile curve. Tensile strength (σ_{max}) was taken as the maximum stress, which for these materials was identical to stress at break. Strain to failure (ϵ_{max}) was reported as strain at maximum stress.

3.2.2. Dynamic Mechanical Analysis

DMA measurements were performed in tensile mode using a RSA-G2 solids analyzer (TA Instruments, USA). Samples were cut into strips of 5 mm width and 20 mm length, and the thickness was $0.2 \pm 0.02 \text{ mm}$. Temperature sweep measurements were performed by heating the samples from 10 to 220°C and applying an oscillation strain amplitude of 0.01%, at a frequency of 1 Hz, along with a pretension force of 0.1 N to straighten the films. The storage and loss moduli were recorded.

4. Results and Discussion

4.1. Thermal Analysis using DSC

Gelatin adhesive films (conditioned at 50% environmental relative humidity) can be regarded as a semicrystalline polymer in which so-called crystalline domains are corresponding to the partially renatured triple-helix structures similar to collagen^[23] embedded in a matrix of random coil material. During drying and cooling of gelatin, triple-helix structures are formed which act as physical cross-links at microscale and the amount of these structures can affect the macroscale behavior of gelatins, which will be discussed later in this article.

Thermograms of the first heating scan of gelatin adhesive films with different Bloom values are shown in **Figure 1**. The first stepwise change in the heat flow versus temperature curve

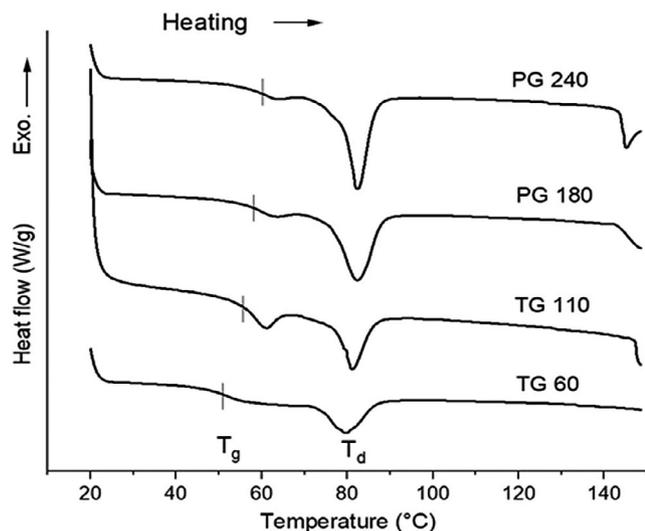


Figure 1. First heating thermograms of gelatin films obtained by DSC.

is related to the glass transition and is indicated by a small vertical line on the thermograms. Glass transition is a second-order time–temperature-dependent transition which is due to the onset of movements inside amorphous regions comprising of random coil chain segments. In gelatins, the glass transition is dependent on parameters such as thermal history (e.g., drying time and temperature) and moisture content.^[19,23] In this article, different gelatin films were prepared with a similar procedure and were all conditioned at 50% environmental relative humidity to ensure similar thermal history and environmental conditioning.

In addition, an endothermic peak appears at higher temperatures related to the denaturation of collagen triple-helix structures. The denaturation enthalpy (ΔH_d), which is the area associated with the endothermic peak, is directly related to the triple-helix structure content in the gelatin polymer^[24] and is caused by the disruption of hydrogen bonds between the chain segments and unfolding of the protein structure.^[25] The denaturation temperature (T_d) is a measure of the thermal stability of the gelatins.

The values for glass transition temperature (T_g), denaturation temperature (T_d), and denaturation enthalpy (ΔH_d) for all tested gelatin films are shown in **Table 1**. As shown in **Figure 1** and **Table 1**, the glass transition temperature is slightly increased in gelatins with higher Bloom values. This can be due to the fact that the triple helices act as physical cross-links; in general, increase in the number of cross-link points in polymer networks results in more hindered chain movements and therefore in an increase in glass transition temperature.^[26] However, the denaturation temperature (T_d) seems to be unaffected by Bloom value, and suggests that the triple-helix domain size is rather constant. As indicated by the enthalpy values in **Table 1**, gelatins with higher Bloom strength indeed contain more crystalline/ordered domains in the form of triple helices. For further confirmation of the possible relationship between Bloom strength and triple-helix content, XRD measurements were performed and will be discussed in the next section.

4.2. X-Ray Diffraction

For further confirmation of the possible relationship between Bloom strength and triple-helix content in gelatin films, XRD measurements were performed on different gelatin films of the same thickness.

The results of the XRD analyses are shown in **Figure 2a,b**. **Figure 2a** compares the X-ray patterns over a wide angle range for the gelatins of highest and lowest Bloom strength. As observed, the gelatins show two characteristic peaks at $2\theta \approx 8^\circ$ and 20° which correlate to periodicity distances of 1.1 and 0.29 nm attributed to triple-helix diameter and the distance

Table 1. Glass transition temperature, T_g , denaturation temperature, T_d , and denaturation enthalpy, ΔH_d , of gelatin films at different Bloom values.

Gelatin type	T_g [$^\circ\text{C}$]	Onset of T_g [$^\circ\text{C}$]	T_d [$^\circ\text{C}$]	ΔH_d [J/g]
PG 240	59 ± 0.5	50 ± 0	82.5 ± 0.5	39 ± 2.3
PG 180	57 ± 1	50 ± 1	82.5 ± 0.5	36.5 ± 3
TG 110	55 ± 1.5	49 ± 2	81 ± 1	23.5 ± 2
TG 60	51 ± 1	42 ± 2	80 ± 1	19.5 ± 1.4

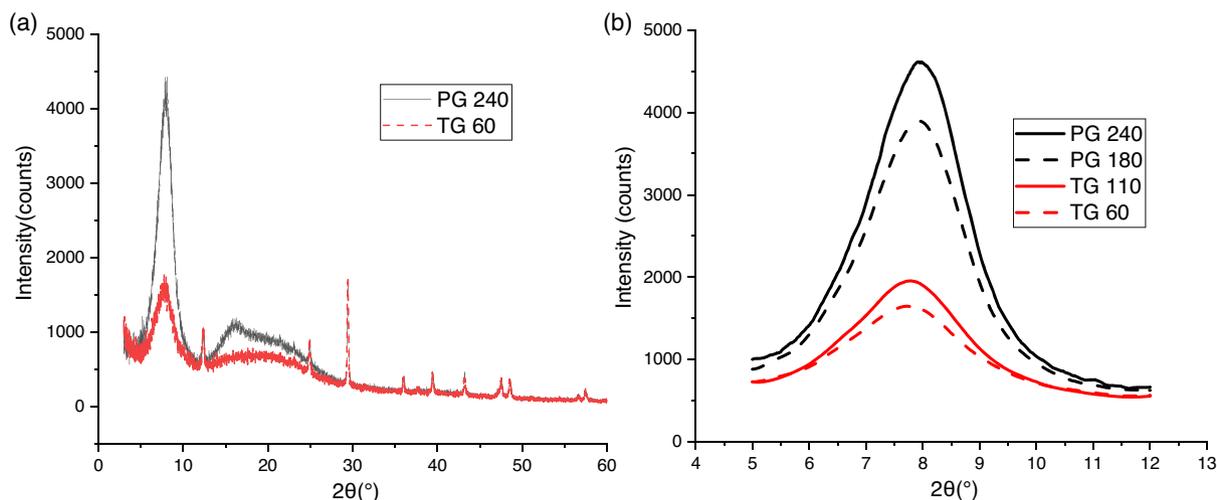


Figure 2. a) Comparative XRD patterns of PG 240 and TG 60 over a wide range of 2θ (3° – 60°); b) comparative XRD patterns of gelatins at $2\theta \approx 8^\circ$.

between amino acid residues along the helix, respectively.^[13] Diffraction peak intensity at $2\theta \approx 8^\circ$ is the strongest peak which is correlated with the relative triple-helix content in gelatin films. Figure 2b clearly shows a significant reduction of peak intensity at 8° with decreasing Bloom value indicating a decrease in triple-helix content. This is in line with the denaturation enthalpy results obtained from DSC which showed that pure gelatin of high Bloom value demonstrates higher denaturation enthalpy associated with higher triple-helix content.

4.3. Uniaxial Tensile Testing

Comparative stress–strain curves of gelatin adhesives are shown in Figure 3. As observed, the gelatin adhesive films demonstrate a typical brittle behavior without undergoing much plastic deformation and a limited strain to failure up to 2.6%.

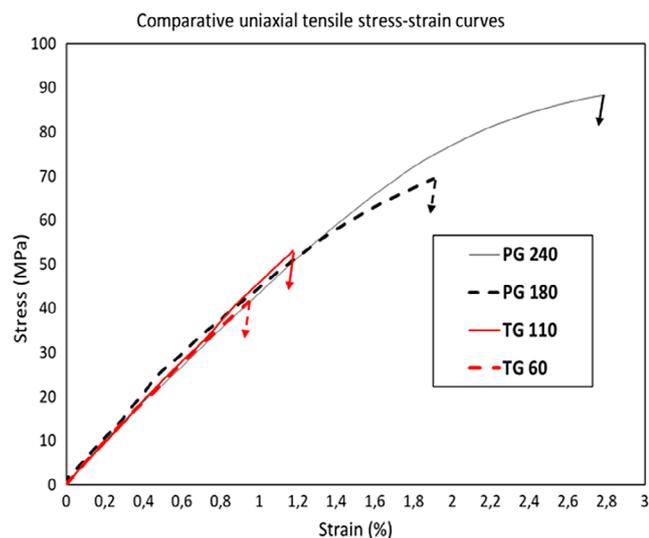


Figure 3. Uniaxial tensile stress–strain curves of gelatin films with different Bloom values, at standard environmental conditions.

Care should be taken that the tensile experiments were performed at standard conditions of 23°C and 50% relative humidity, meaning that the adhesive films were below their glass transition temperature (as indicated by DSC experiments) and in their glassy state. The results of the tensile tests per type of glue are shown in Table 2. The first interesting observation is that the Young's modulus (E) is almost constant with an increase in Bloom value indicating that the triple-helix content, where the triple helices act as physical cross-links, hardly affects the elastic modulus. This can be related to the fact that these adhesive films are, as mentioned, below their glass transition temperature, where also the amorphous phase is in its rigid state. The second result is that the strength (which is defined here as maximum stress value, σ_{max}) increases with an increase in Bloom value. This can be explained by the increase in triple-helix content as demonstrated by DSC and XRD measurements discussed earlier.

As shown in Figure 3, the strain at break value (maximum strain, ϵ_{max}) increases with Bloom value resulting in an improved fracture energy. This improvement can also be attributed to the higher triple-helix content.

4.4. Dynamic Mechanical Analysis

Figure 4 shows the storage modulus (E') of different gelatin films obtained from DMA. A stepwise change in storage modulus starting around 50°C is indicative of a glass transition

Table 2. Values for Young's modulus, maximum stress, and maximum strain for gelatin films with different Bloom values obtained from tensile experiments at 23°C and 50% RH..

Gelatin type	E [GPa]	σ_{max} [MPa]	ϵ_{max} [%]
PG 240	4.7 ± 0.3	83 ± 10	2.7 ± 0.9
PG 180	4.5 ± 0.4	71 ± 15	1.9 ± 0.7
TG 110	4.6 ± 0.4	53 ± 11	1.2 ± 0.3
TG 60	4.8 ± 0.6	45 ± 15	1.0 ± 0.3

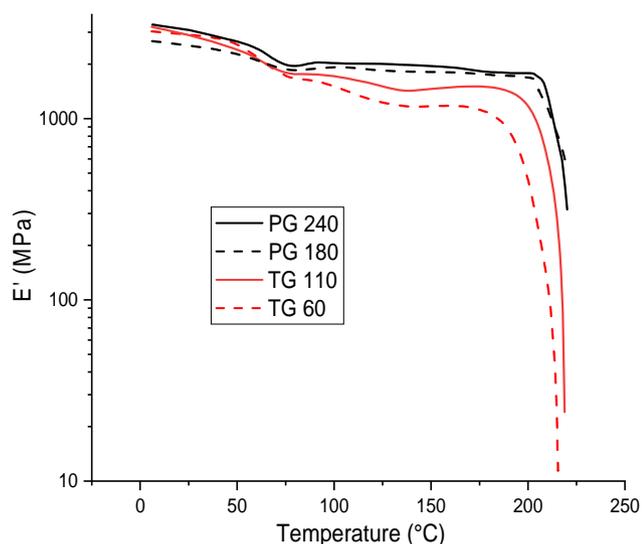


Figure 4. Storage modulus–temperature curves of different gelatin films obtained from DMA.

temperature. The onset temperature of the glass transition is shown in **Table 3**. The storage modulus of different gelatins, obtained from DMA below T_g , shows similar values for gelatins with different Bloom values. This is in line with the values of Young's modulus at room temperature obtained from tensile tests indicating that the triple-helix content merely affects the stiffness of gelatin films above T_g . This is because below glass transition, both amorphous and crystalline domains (helical structures in gelatins) are in their rigid state and therefore an increase in triple-helical structure does not influence the molecular mobility and therefore stiffness. The values of the storage modulus at 25 °C (below glass transition) and 100 °C (above glass transition and denaturation temperature) are shown in **Table 3**.

A second stepwise transition around 80 °C and consequently a continuous decrease in storage modulus is observed in both technical gelatins (TG 110 and TG 60). This transition is attributed to the denaturation of some triple helix to coil structure. As shown in **Figure 3**, after glass transition, the drop in elastic modulus is more prominent for gelatins with lower Bloom value while pure gelatins with high Bloom values keep higher storage modulus values. This must be due to the higher triple-helix content in the pure gelatins (as shown by DSC and XRD measurements) and suggests that at the denaturation temperature of around 80 °C, sufficient structuring material is left in these materials to maintain a high modulus value.

Table 3. Values of storage modulus of different gelatin adhesive films at 25 and 100 °C, and onset of glass transition obtained from DMA..

Gelatin type	E' at 25 °C [GPa]	E' at 100 °C [GPa]	Onset of T_g [°C]
PG 240	3.0 ± 0.3	2.0 ± 0.1	55 ± 2
PG 180	2.6 ± 0.4	1.9 ± 0.5	55 ± 1
TG 110	3.0 ± 0.1	1.8 ± 0.1	53 ± 1
TG 60	3.0 ± 0.1	1.5 ± 0.1	49 ± 2

The technical gelatins typically contain more impurities compared to pure gelatins in the form of oligomers, fat, and ash which can partially hinder triple-helix structure formation during drying of the film. Moreover, the technical gelatins with lower Bloom values have a possibly lower average molecular weight which can result in less chain entanglements and less engagement of the polypeptide chains in forming helical structure.^[8,17]

Another major drop in storage modulus occurs around 170, 200, 220, and 220 °C for TG 60, TG 110, PG 180, and PG 240, respectively. This transition is related to discolorification and degradation of gelatin films. As observed, the pure gelatins of higher Bloom number degrade at higher temperatures (around 220 °C) in comparison to technical gelatins, indicative of a higher molecular weight.

4.5. Microstructural Evaluation Using SEM

Samples were cut into standard environmental conditions (room temperature and 50% RH) with a scissors to study the cross-sectional morphology of the different films by SEM. Also, SEM images were taken of the film surfaces. As shown in **Figure 5**, a rough fracture surface resembling a layered structure is discernible in the cut cross section of all the different films. This is possibly due to plastic deformation of the gelatin films while undergoing shear loading during cutting. As observed, the extent of plastic deformation at the cut surface seems most pronounced for technical gelatins with lower Bloom strength, although also a more irregular failure is visible. This is believed to be due to higher impurity and higher inhomogeneity of the technical gelatins leading to a more irregular fracture surface. The observations of the microstructure are in line with tensile testing results which show lower elongation to failure at lower Bloom strength; this points to premature failure at irregularities when loaded in tension, although all gelatins seem inherently ductile from the sheared fracture surfaces. The SEM micrographs of the surfaces indicate a smooth surface of the gelatins although small scratches or impurities can be observed on the surfaces of the technical gelatins.

4.6. Contact Angle Measurements on Gelatin Film Surface

Table 4 shows the contact angle values for water and diiodomethane on the different gelatin films. High contact angles for water suggest that gelatins have a hydrophobic nature. Although much lower contact angles can be observed when diiodomethane is used, confirming the hydrophobicity of the gelatin film surface. Differences between the different gelatins are small; if anything, the suggestion is that the TG 60 is the most hydrophobic, whereas the PG 180 is the least hydrophobic. Despite the generally hydrophilic character of gelatin chains, the contact angle measurements suggest that their film surface is relatively hydrophobic.

Previously, some researchers have observed the relative hydrophobicity of gelatin film surfaces.^[27–29] They explained the gelatin surface hydrophobicity to a preferred orientation of hydrophobic moieties at the gelatin–air interface. This orientation of the hydrophobic groups toward the surrounding air would

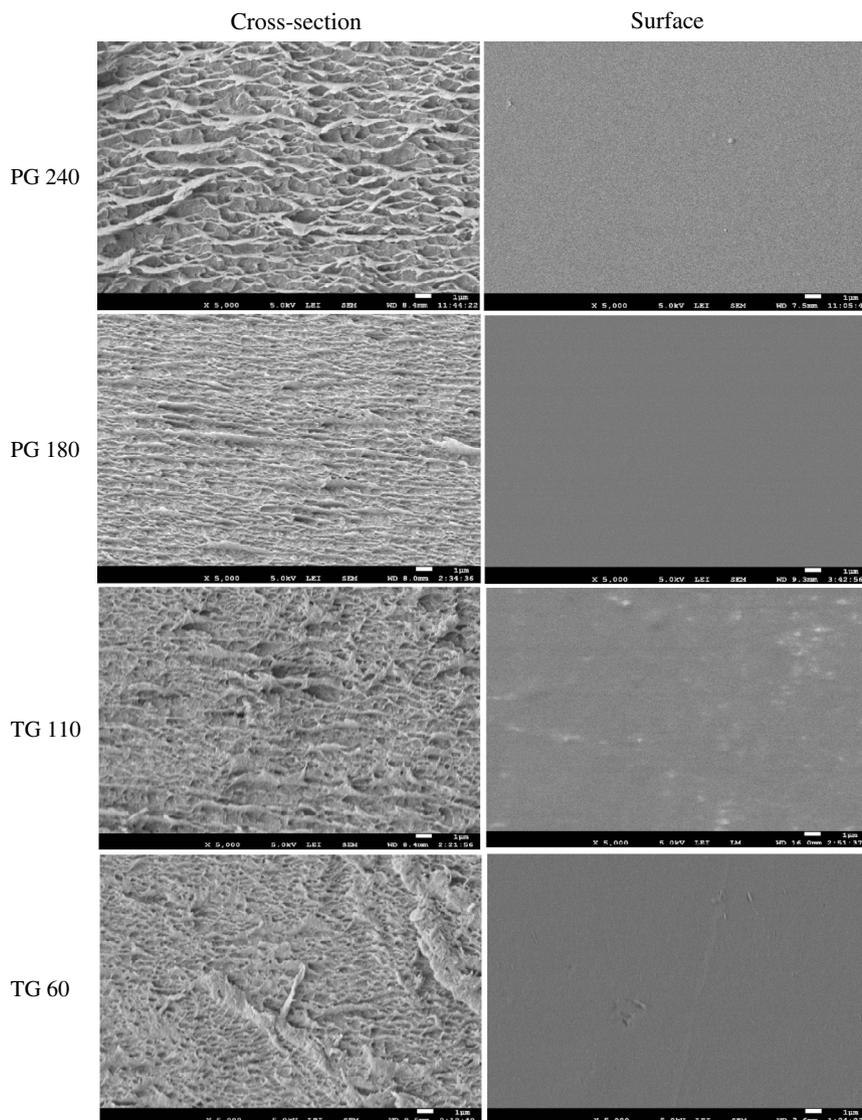


Figure 5. SEM micrographs of cut cross sections and surfaces of different gelatin films.

Table 4. Contact angle values of water and diiodomethane with gelatin films.

Gelatin type	PG 240	PG 180	TG 110	TG 60
Gelatin/water contact angle [θ]	$75^\circ \pm 4$	$70^\circ \pm 4$	$71^\circ \pm 5.5$	$79^\circ \pm 5.5$
Gelatin/diiodomethane contact angle [θ]	$34^\circ \pm 2.5$	$41^\circ \pm 4$	$37^\circ \pm 4$	$34^\circ \pm 3.5$

happen during gelification, when most hydrophilic groups such as amino groups and carboxyl groups aggregate inwards and form hydrogen bonds with internal hydrophilic groups, whereas hydrophobic groups, such as aliphatic chains and aryl groups, tend to arrange themselves on the surface of the film.^[29]

Bialopiotrowicz and Jańczuk^[28] even showed that the surface hydrophobicity is strongly dependent on the aqueous gelatin concentration used to prepare the gelatin films.

4.7. Correlation between Molecular Level Microstructure and Macroscopic Mechanical Behavior

Figure 6 shows how the two different methods used for the measurement of triple-helix content in gelatin films, namely, DSC and XRD correlate with each other. For this, the enthalpy of denaturation obtained from DSC experiments is plotted against the integrated intensity which is the area underneath the peak corresponding to the inner diameter of the triple helix, obtained from XRD measurements. As observed, a power-law relationship seems to describe this correlation. It should be noted that the curve is assumed to pass through the origin, and this is because lack of renaturation enthalpy is associated with the absence of triple-helix structure and therefore the absence of any X-ray pattern at around $2\theta \approx 8^\circ$.

Figure 7a,b show the existence of a correlation between the structure of the gelatin films at molecular level and mechanical

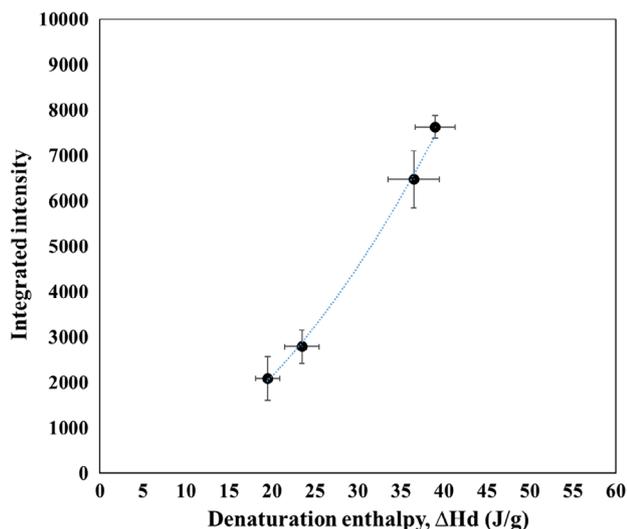


Figure 6. Correlation between integrated intensity of XRD peak at $2\theta \approx 8^\circ$ and enthalpy of denaturation of triple-helix coil obtained from DSC.

properties at macroscopic level. As shown in Figure 7a,b, there is a direct association between triple-helix content represented by the XRD integrated peak intensity and the mechanical strength (both Bloom and tensile strength, σ_{\max}). It seems, based on the four investigated materials, that a linear equation can predict the Bloom and tensile strength values based on the triple-helix content (as quantified by XRD).

For the sake of completeness, a similar correlation as shown in Figure 7 is found if the strength values are plotted as a function of the triple-helix content obtained from the DSC denaturation enthalpy.

Previous research by Bigi et al.^[13] also demonstrated that Bloom strength, in porcine skin wet gelatin films, is linearly correlated with triple-helix content. Moreover, similar to the findings of this article, they also reported an increase in both

tensile strength and strain at break with increase in Bloom strength of gelatin. In another study, Bigi et al.^[30] found a linear correlation between the degree of renaturation obtained by DSC measurements with tensile strength of air dried drawn cross-linked gelatin films. Achet and He^[10] showed that renaturation enthalpy obtained by DSC method is a suitable measure to monitor the real fraction of gelatin chains in a collagen-like structure, which is related to the mechanical properties of gelatin films.

It should be noted that the found correlations between the physical properties of gelatins at microlevel (triple-helix content) and their macroscopic mechanical performance is suggested in the first instance for the here tested type A porcine skin gelatin adhesive films preconditioned at room temperature and 50% relative humidity. σ_{\max} , e.g., not only depends on the material properties of the gelatin but also on the amount of defects in the casted tensile test samples. However, as the XRD measurements only measure the triple-helix content, regardless of the origin of the glue, it is expected that this principle can be extended to other animal glues.

5. Conclusion

This study clearly demonstrates the structure–property relations in (porcine) gelatin adhesive films in a comprehensive manner. More specifically, the relationship between triple-helix content, associated with the renaturation level of gelatin films, with thermal and mechanical performance is demonstrated. For this, films of type A pig skin gelatin with four different Bloom values were prepared and their physical and mechanical properties were characterized. The physical characterization of gelatin films demonstrated a clear correspondence between enthalpy of renaturation obtained by DSC and the integrated intensity of the X-ray pattern at around $2\theta \approx 8^\circ$ (attributed to triple-helix internal diameter). Moreover, DSC measurements showed that the increase in triple-helix content (associated to denaturation enthalpy) in gelatin films of higher Bloom value caused an

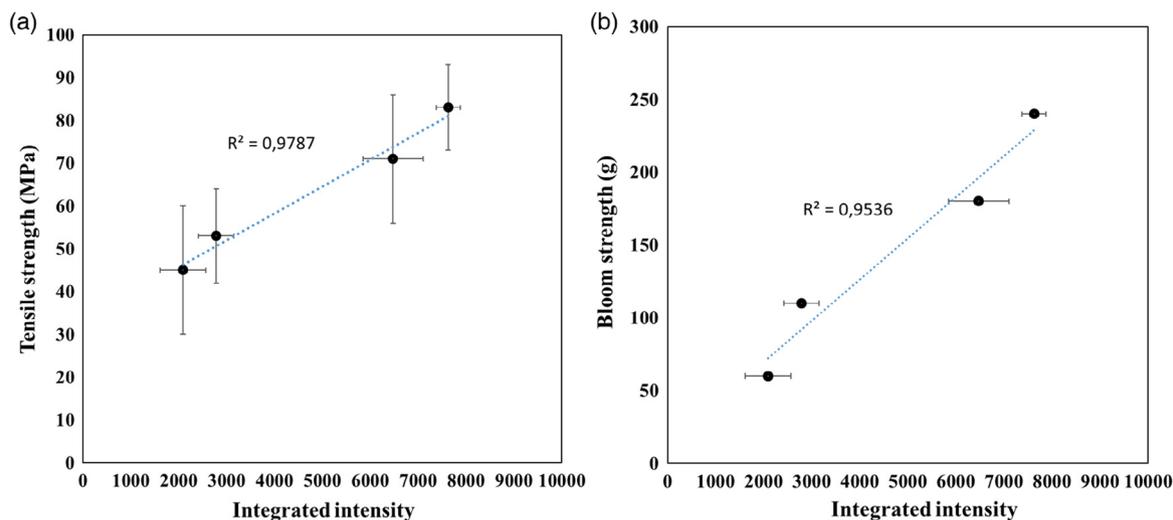


Figure 7. a) Tensile strength plotted versus integrated intensity of the XRD patterns at $2\theta \approx 8^\circ$ with a linear least squares regression; b) Bloom strength plotted versus integrated intensity with a linear least squares regression.

increase in glass transition temperature of the gelatin films; however, the denaturation peak temperature remained unaffected, indicating that triple-helix domain size stayed constant. Static uniaxial tensile experiments and DMA (in tension mode) both showed that the stiffness of gelatin films is independent of their triple-helix content, which fits with T_g values well above room temperature. On the contrary, the tensile strength and strain to failure improve with increasing triple-helix content for higher Bloom strength gelatin films.

Moreover, a linear correlation was found between the triple-helix content derived from XRD and DSC measurements with mechanical properties such as gel (Bloom) strength and tensile strength.

Contact angle measurement on different gelatin surfaces using water and diiodomethane fluids showed that all gelatin films have a hydrophobic surface nature which is believed to be due to the tendency of the hydrophobic parts of gelatin chains to orient themselves at the surface of the film during gelification. The SEM micrographs of cut cross sections of gelatin films demonstrated a ductile fracture surface while undergoing shear deformation during cutting. Technical gelatins showed more irregularities at the fracture surface possibly due to their higher level of impurities, which would lead to local strain magnification and can explain their lower tensile strain to failure.

The results of this article, strictly for the given gelatin type and environmental preconditioning (room temperature and 50% relative humidity), demonstrate how the molecular level structure dictate macroscopic mechanical behavior of dry gelatin films. In addition, physical techniques such as XRD and DSC can be used to predict the macroscopic mechanical properties of gelatin films.

This article is a showcase of how nondestructive and less material demanding physical characterization methods such as XRD can assist scientists in various fields, e.g., art conservation, to predict the mechanical behavior of gelatin adhesives. Structural features at molecular level characterized by a nondestructive physical characterization method can be predictive of macroscopic mechanical response of the material.

Moreover, from an art conservation point of view, the results of this article are an important step toward choosing an appropriate animal glue for specific conservation purposes. It also provides essential input for future experiments to assess how ageing influences the macroscopic mechanical properties of gelatin films, which is important to understand the behavior of historic animal glues.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

adhesives, bloom strength, gelatins, renaturation, triple helix

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- [1] S. Hackney, *On Canvas: Preserving the Structure of Painting*, The Getty Conservation Institute **2020**.
- [2] A. Pataki-Hundt, E. Hummert, *Int. J. Preserv. Lib. Arch. Mater.* **2016**, *37*, 137.
- [3] N. C. Schellmann, *Stud. Conserv.* **2007**, *52*, 55.
- [4] J. Ahmed, H. S. Ramaswamy, M. O. Ngadi, *Int. J. Food Prop.* **2005**, *8*, 179.
- [5] R. Zamani Alavijeh, P. Shokrollahi, J. J. Barzin, *J. Mater. Chem. B* **2017**, *5*, 2302.
- [6] S. F. Hosseini, M. C. Gomez-Guilen, *Trends Food Sci. Technol.* **2018**, *79*, 125.
- [7] M. M. Schmidt, R. C. P. Dornelles, R. O. Mello, E. H. Kubota, M. A. Mazutti, A. P. Kempka, I. M. Demiate, *Int. Food Res. J.* **2016**, *23*, 913.
- [8] P. Díaz-Calderon, E. Flores, A. Gonzalez-Munoz, M. Pepczynska, F. Quero, J. Enrione, *Food Hydrocolloids* **2017**, *71*, 118.
- [9] A. A. Karim, R. Bhat, *Food Hydrocolloids* **2009**, *23*, 563.
- [10] D. Achet, X. W. He, *Polymer* **1995**, *36*, 787.
- [11] M. Djabourov, *Contemp. Phys.* **1988**, *29*, 273.
- [12] S. B. Ross-Murphy, *Polymer* **1992**, *33*, 2622.
- [13] A. Bigi, S. Panzavolta, K. Rubini, *Biomaterials* **2004**, *25*, 5675.
- [14] G. Aguirre-Alvarez, T. Foster, S. E. E. Hill, *J. Food* **2012**, *10*, 306.
- [15] J. L. Gornall, E. M. Terentjev, *Phys. Rev. E* **2008**, *77*, 031908.
- [16] M. Usta, D. L. Piech, R. K. MacCrone, W. B. Hillig, *Biomaterials* **2003**, *24*, 165.
- [17] J. Eysturskard, I. J. Haug, A. S. Ulset, K. I. Draget, *Food Hydrocolloids* **2009**, *23*, 2315.
- [18] J. Eysturskard, I. J. Haug, A. S. Ulset, H. Joensen, K. I. Draget, *Food Biophys.* **2010**, *5*, 9.
- [19] I. Yakimets, N. Wellner, A. C. Smith, R. H. Wilson, I. Farhat, J. Mitchell, *Polymer* **2005**, *46*, 12577.
- [20] F. W. Kotch, I. A. Guzei, R. T. Raines, *J. Am. Chem. Soc.* **2008**, *130*, 2952.
- [21] F. A. Osorio, A. Bilbao, R. Bustos, F. Alvarez, *Int. J. Food Prop.* **2007**, *10*, 841.
- [22] R. Schrieber, H. Gareis, *Gelatine Handbook: Theory and Industrial Practice*, Wiley-VCH, Weinheim **2007**.
- [23] Y. Kato, T. Hagiawara, T. Suzuki, R. Takai, *Res. Soc. Jpn.* **2001**, *26*, 659.
- [24] A. Bigi, G. Cojazzi, S. Panzavolta, N. Roveri, K. Rubini, *Biomaterials* **2001**, *22*, 763.
- [25] M. Shafiqur Rahmana, G. Al-Saidia, N. Guizania, A. Abdullah, *Thermochim. Acta.* **2010**, *509*, 111.
- [26] J. Shen, X. Lin, J. Liu, X. Li, *Macromolecules* **2019**, *52*, 121.
- [27] T. Yasuda, T. Okuno, H. Yasuda, *Langmuir* **1994**, *10*, 2435.
- [28] T. Białopiotrowicz, B. Jańczuk, *Langmuir* **2002**, *18*, 9462.
- [29] J. Zhao, F. Pan, P. Li, C. Zhao, Z. Jiang, P. Zhang, X. Cao, *ACS Appl. Mater. Interfaces* **2013**, *5*, 13275.
- [30] A. Bigi, M. Borghi, G. Cojazzi, A. M. Fichera, S. Panzavolta, N. Roveri, *J. Therm. Anal. Calorim.* **2000**, *61*, 451.