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Original article

Gelatine adhesives from mammalian and fish origins for historical art objects conservation: How do microstructural features determine physical and mechanical properties?

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ABSTRACT

Gelatine adhesives aka 'animal glues' are water-soluble biopolymers used in historic objects such as wooden cabinets and panel paintings since ancient times. This paper investigates the correlations between microstructural features, namely triple helices, and macroscopic properties of four different types of gelatine adhesives, prevalently used in conservation practices, irrespective of the animal origin. These adhesives include bovine bone, bovine skin, rabbit skin, and fish glues. Thin adhesive films were produced via solution casting methods in controlled climate conditions and their thermal and mechanical properties, and moisture sensitivity were investigated. XRD as a non-destructive characterisation method demonstrated good agreement with DSC in the quantification of gelatine adhesive (animal glue) triple helix content irrespective of the animal origin. Linear correlations between triple helices and gel (Bloom) strength and tensile strain energy to failure (toughness) were found for all adhesive types. Dynamic vapour sorption experiments demonstrated that lower triple helix content is correlated with higher moisture sensitivity of the adhesives. Moreover, the effect of environmental RH on the thermal behaviour of adhesives was investigated by DSC. The results demonstrated that the increase in environmental RH causes a reduction in the adhesives' glass transition and denaturation temperatures whilst triple helix content did not alter. Bovine bone glue with the lowest triple helix content showed the least toughness and highest moisture sensitivity, whilst fish glue with the highest triple helix content was identified as the most flexible glue.

1. Introduction

Animal glues have been used by artists and craftsmen as adhesives, binding media, and consolidants in historic and artistic objects such as decorative woodworking, paintings, bookbinding, and papermaking, for centuries and even since antiquity. In modern times, animal glues are widely used in the conservation and restoration of artifacts and historical objects.

Animal glues are impure form of gelatines. Gelatines are collagen-based biopolymers derived from animal products. Collagen, in which the term (kólla) is derived from the Greek word for glue, is a fibrous structural protein found in the extracellular matrix of connective tissues (e.g. skin, bone, and cartilage) of the mammalian and fish species. It provides the tissues with structural integrity and support [1–3]. Each collagen molecule essentially consists of three polypeptide chains in which the amino acids are arrayed in a specific sequence of Gly-X-Y, with Glycine repeating at every third residue, and where X is prevalently proline and Y hydroxyproline [4,5]. The origin and source of collagen also affect its amino acid profile. Collagen fibers have a hierarchical structure (see Fig. 1); they comprise of collagen microfibrils, and each microfibril is comprised of several collagen molecules. Each collagen molecule, called tropocollagen, is composed of three α-

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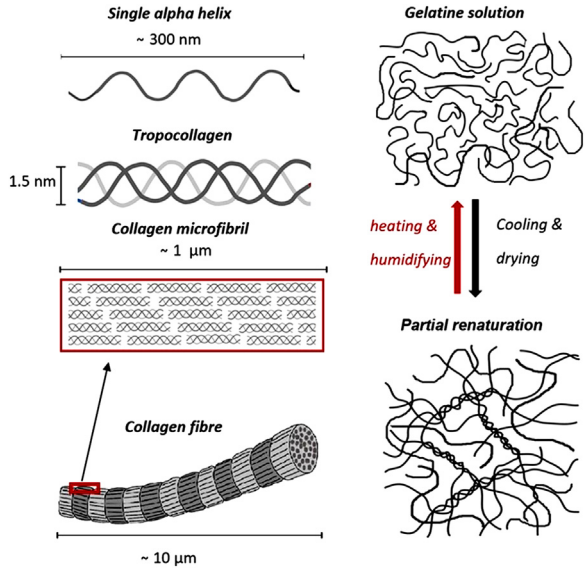

Fig. 1. (Left) Hierarchical structure of collagen; from single alpha helix to tropocollagen triple helix, to supramolecular assembly into the collagen microfibril, and to collagen fibre. (Right) Amorphous conformation of gelatine chains when dissolved in water, and partial renaturation and formation of triple helices in gelatine structure upon cooling and drying.

helix chains wound together in a right-handed triple helix structure, stabilized by intermolecular hydrogen bonds and chemical cross-links, thus forming a subunit of the larger collagen fibril aggregates [3,6].

The triple helix, a ternary structure, is the most important structural feature of the collagen molecule and it is stabilized by intra-chain hydrogen bonds between water molecules and main chain N–H and C = O groups [1,2,7].

Collagen is insoluble in cold water and can be transformed into water-soluble gelatine through a process called denaturation by the application of heat (50–80 °C) and acidic or alkali treatment followed by water extraction [3,8,9,10]. During denaturation, collagen triple helices are hydrolysed and the covalent bonds connecting the polypeptide chains are broken which leads to gelatine with a random coil structure. Upon cooling and drying gelatine chains undergo a conformational rearrangement called renaturation during which random coils partially rearrange themselves into collagen-like triple helices within or between the gelatine chains. These partially renatured helical structures only occur in sections of the chains and act as physical nodes or cross-links and form a 3D network structure [11–14]. The physical and mechanical properties of gelatines are highly influenced by the triple helix content [15–18]. The extent of renaturation in animal glues can vary due to many parameters such as the animal origin [19], glue concentration [20], preparation and casting conditions [21], molecular weight distribution [22,23], and moisture content influenced by environmental relative humidity [24,25].

Animal glues have versatile functionality in the conservation and restoration of historic objects. A comparative and systematic study on the thermal and mechanical performance of prevalently used adhesives (from different animal origins) in conservation practice is still limited and therefore it is necessary to provide practitioners with quantitative data on these materials [11,26].

This paper presents a comparative study of the thermal behaviour and mechanical performance of four animal glues commonly used in the conservation of wooden artefacts as part of Dutch Heritage collections. The adhesives investigated in this study are bovine bone, bovine skin, rabbit skin, and fish glue. These adhesives have been used by furniture conservators in the Rijksmuseum (Amsterdam, the Netherlands) in their restoration and repair practices during the last decades.

In this study, a correlation between the microstructure of these adhesives and their macroscopic mechanical behaviour is established. For this purpose, thin films of animal adhesives were prepared using the solution casting method. The physical and mechanical behaviour of the adhesives was characterized using Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), Bloom strength measurement, and uniaxial tensile tests. Thermal characterisation methods such as DSC were employed to measure glass transition and enthalpy of denaturation of the adhesives.

2. Research aim

The current study was undertaken on four types of animal glues commonly used in art conservation. The aim of this study is to develop a further understanding of the properties of gelatine adhesive films aka animal glues, irrespective of their animal origin, and to detect the effect of changing environmental conditions (relative humidity) on thermal behaviour and moisture sensitivity. This understanding will also help the conservators in their decision of the type of adhesive they use for a particular application as well as in deciding on relaxation measures regarding climate control of the museum where they keep art objects containing animal glues. Moreover, this study is a prelude to a follow-up study regarding the ageing of these adhesives in wooden cultural heritage and how the microstructural features in these adhesives can determine their rate of ageing and degradation.

3. Materials and methods

3.1. Animal adhesives

Four types of animal adhesives from different animal origins, regularly used in conservation practices, were chosen for this study in close consultation with furniture conservators of the Rijksmuseum. For the sake of consistency, the adhesives were sourced from the same supplier that delivers the adhesives to the Rijksmuseum. These adhesives include bovine bone glue, bovine skin glue, rabbit skin glue, and fish glue. Bovine bone glue (article no. O6300 and CAS no 9000–70–8), bovine skin glue (article no. O6300), and rabbit skin glue (article no. O6302), in granular form, were sourced from Labshop (Twello) B.V. (Apeldoorn, the Netherlands). Fish glue (series no. 63,080 and CAS no. 9000–70–8), in powder form, was purchased from Kremer Pigmente, Germany. MERGAL KM90 pesticide was added to the aqueous solutions of the adhesives to prevent bio-deterioration by microorganisms.

3.2. Sample preparation

Thin adhesive films with a thickness of 0.24±0.02 mm were prepared using a solution casting method. 40 gs of dry adhesive powder was dissolved in 200 ml of demineralised water (20 wt.% of glue solution) along with 0.1 ml of Mergal KM90. This mixture was then stirred using a magnetic stirrer in a 60 °C water bath for one hour. Then the mixture was homogenously and gently injected into a 10×10 cm Teflon mould using a syringe and spread evenly by circular motions. Subsequently, the samples were let to cool and dry at room temperature and a controlled environmental relative humidity of about 50%.

3.3. Physical tests

3.3.1. Differential scanning calorimetry (DSC)

To obtain the glass transition temperature (T_g), denaturation temperature (T_d), and denaturation enthalpy (ΔH_d) of the different

gelatine adhesive films, a TA Instrument DSC 250 differential scanning calorimeter was utilised. The adhesive films were conditioned at 30%, 50%, and 80% of relative humidity at room temperature in a controlled climate chamber for 72 h before testing. Subsequently, the test samples, each weighing about 8 mg, were within a minute transferred and hermetically sealed using Tzero aluminium pans to be able to measure the effect of relative humidity without the escape of water from the DSC pan during the thermal cycling. An empty pan was used as a reference. For the measurements, the samples were heated and cooled. In the heating step, the samples were heated from 10 °C to 150 °C at 10 °C/min, maintained at 150 °C for 5 min, and then cooled from 150 °C to room temperature at 10 °C/min. All the measurements were performed in triplicate. The glass transition temperature (T_g) is measured as the midpoint of the heat flow change at the glass transition in the first heating scan. The denaturation temperature (T_d) is identified as the minimum point of the endothermic denaturation peak in the first heating scan. The enthalpy of denaturation (ΔH_d) is calculated as the area of the endothermic denaturation peak in the heating scan.

3.3.2. X-Ray diffraction (XRD)

X-ray diffractograms were recorded on the adhesive films for scanning angle 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 CuKα radiation source was used (I = 15 mA, U = 40 kV). For each adhesive type, measurements were performed on three different samples, and an average representative curve was chosen for comparative reporting. It was ensured that the sample was placed at the same height and level as the rim of the sample holder and the same distance to the detector for each consecutive measurement.

3.3.3. Dynamic vapour sorption

To investigate the moisture sensitivities of the different adhesives, moisture sorption experiments were performed using an automated gravimetric Dynamic Vapour Sorption (DVS) analyser, TA Q5000 SA at an isothermal temperature of 34 °C . The uptake of the water vapour was determined gravimetrically using a high-precision balance with a mass resolution of ±0.1 µg. In the first step, the adhesive films were equilibrated at 0% relative humidity (RH) to determine a dry reference mass. After drying, the adhesive films in the DVS were exposed to a stepwise increase of%RH (0%; 20%; 40%; 60%; 80%; 90%). The same%RH profile in reverse order was employed for desorption. At each stage, the equilibrium moisture content was determined when the mass variation versus time was 0.002 mg/min for at least 10 min. The relative humidity around the sample was controlled by mixing saturated and dry carrier gas streams using mass flow controllers. Adhesive films were cut into disks and placed in flat aluminium pans with an internal diameter of 7 mm. Hence, one side of the adhesive film was exposed to the surrounding environment of the DVS chamber.

3.4. Mechanical tests

3.4.1. Bloom strength measurements

For measurement of Bloom strength of the adhesives, a 12.5 wt.% solution of the adhesive sample was prepared at 60 °C, cooled to 10 °C, and kept for 17 h at this temperature to mature according to the GME method [26]. The resulting Bloom strength was measured using a Brookfield CT3 texture analyser equipped with an AOAC plunger (with 12.7 mm diameter, plane surface, and sharp edge).

3.4.2. Uniaxial tensile measurements

Uniaxial tensile tests were performed to measure the following mechanical properties of different adhesive films namely Young's modulus, tensile strength, strain to failure, and energy to failure. For the measurements, a standard tensile INSTRON machine (model 3365) equipped with a video extensometer and a 1 kN load cell was used.

For these tests, the ISO 527–2 standard was used. The tensile test samples were cut into dogbone shapes, with dimensions of 4 mm (gauge width) × 15 mm (gauge length), and 0.2 ± 0.02 mm (thickness), using a cutting die and a stamper. The strain rate was set to 1 mm/min. The samples were conditioned for at least 48 h at a temperature of 23 °C and a RH of 50% before testing. At least 10 samples were tested for each adhesive type to give a good statistical overview of the properties. Young's modulus (E) was calculated as the linear part of the stress-strain tensile curve. The tensile strength (σ_{max}) was measured as the maximum stress, which for these materials is also stress at break. Strain to failure (ε_{max}) is reported as the strain at maximum stress. Strain energy density to failure (J/m^3) was calculated as the area under the stress-strain curve up to failure.

Table 1
From DSC experiments, values of the glass transition temperature, T_g, denaturation temperature, T_d, and denaturation enthalpy, ΔH_d, of different adhesive films preconditioned at 23 °C and 50% RH. Also, from XRD experiments, the ratio (A_c/A_a) between integrated areas of diffraction peaks at crystalline (2θ∼8°), and amorphous (2θ∼20°) regions for different adhesives.

Adhesive type	T_g (°C)	T_d (°C)	ΔH_d (J/g)	A_c/A_a
Bovine bone	52±1.5	81±1	18.5 ± 0.5	0.33±0.02
Rabbit skin	54±1	84±0.6	23±1	0.39±0.06
Bovine skin	55±1	84±1	25.5 ± 3	0.52±0.06
Fish	55±1	84±1.5	42±2	0.73±0.01

3.4.3. Dynamic mechanical analysis (DMA)

DMA measurements were performed in tensile mode using a DMA Q800 (TA Instruments, USA). Samples with a thickness of 0.2 ± 0.02 mm were cut into strips of 5 mm in width and 20 mm in length. Temperature sweep measurements were performed by heating the samples from 10 °C to 220 °C and applying an oscillation strain amplitude of 0.01, at a frequency of 1 Hz, along with a pre-tension force of 0.1 N to straighten the films. The storage and loss moduli were recorded.

4. Results and discussions

4.1. Microstructural characterisation using physical methods: measurement of triple helix content by DSC and XRD

The structural characterization of gelatine-based animal adhesives in the form of physical gels is of primary importance for understanding their physical and mechanical performance. Animal glues are thermoplastic polymers in which some segments of the chains are partially renatured in the shape of triple helices at contact points between three different strands and stabilised by hydrogen bonding. The triple helices are randomly distributed in space in a matrix of amorphous polypeptide chains.

DSC is one of the physical characterisation methods that can be employed to quantify the triple helix content of the adhesives [27,16]. For this, four types of animal glue films were acclimatised at room temperature and 50% RH before testing. Fig. 2a illustrates the thermograms of the first heating scan of different animal glue films. The stepwise change in the heat flow curve at around 55 °C is related to the glass transition and the values for the different adhesives are tabulated in Table 1. The glass transition is related to the onset of the movements of the amorphous parts of the chains and is the temperature at which the animal glue films transit from a glassy and stiff to a more soft and rubbery state. Hence, the glass

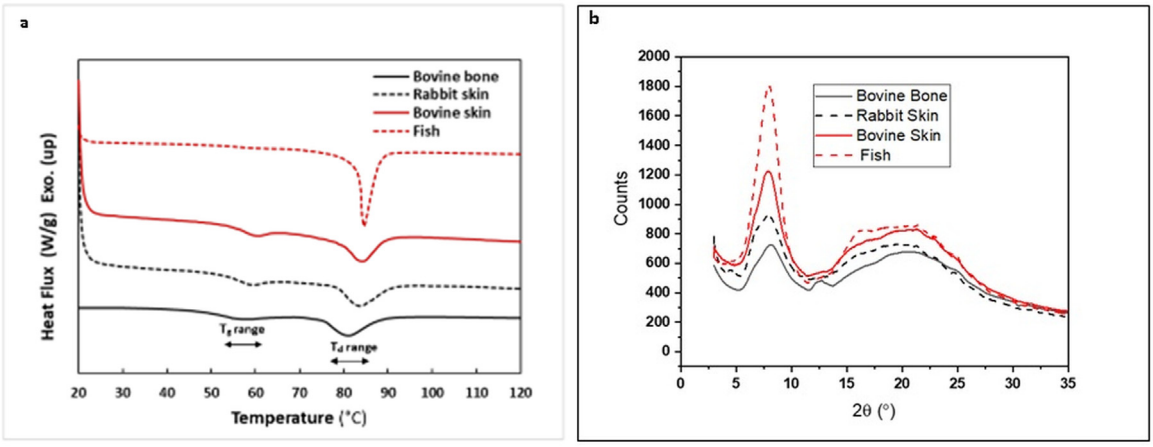

Fig. 2. (a) DSC thermograms for different animal glues from the first heating scan tested at 23 °C and 50% RH; (b) Comparative XRD diffractogram patterns of different adhesive films conditioned at 23 °C and 50% RH.

Table 2
The Measured Bloom strength of 12.5 wt% solutions of the adhesives at 10° C; values for Young's modulus, maximum stress, maximum strain to failure, and strain energy to failure for different adhesive films obtained from tensile experiments at 23 °C and 50% RH.

Adhesive type	Bloom strength (g)	σ_{max} (MPa)	ε_{max} (%)	E (GPa)	Strain Energy (MPa)
Bovine bone	169±2.5	78.0 ± 10	4.5 ± 1	2.7 ± 0.2	1.7 ± 0.8
Fish	786±12	82.0 ± 5	10.0 ± 2	2.6 ± 0.2	6.3 ± 1.2
Rabbit skin	261±4	81.0 ± 3	6.5 ± 1	2.4 ± 0.1	3.2 ± 0.8
Bovine skin	306±4.5	92±8	6.0 ± 1	2.8 ± 0.7	4.0 ± 0.9

transition is an important thermodynamic parameter which determines the change in the mechanical performance of the adhesives e.g. the creep behaviour. Mosleh et al. [16] also found a slight correlation between triple helix content in gelatine films and their glass transition in porcine gelatines with different Bloom numbers. Another aspect that affects the glass transition is the presence of water or smaller molecules (e.g. fats, sugars) that act as plasticisers and cause reduction of glass transition temperatures in animal glues, [11]. As observed in Table 1, bone glue demonstrates a slightly lower glass transition temperature compared to the other adhesives. This cannot be solely attributed to the triple helix content but also to the additives in the adhesive formulation (e.g. fat or sugar) and the moisture content of each adhesive. Nevertheless, the values of T_g of all the adhesives are well above room temperature, indicating that these adhesives are in the glassy state and hence, likely to demonstrate brittle behaviour. Following the glass transition temperature, an endothermic peak appears at a higher temperature of around 84 °C associated with the denaturation of the collagen triple-helix structures to coil structure. The denaturation temperature (T_d) is a measure of the thermal stability of the animal glues. The denaturation enthalpy (ΔH_d), which is the area associated with this endothermic peak, is believed to be related to the triple-helix content in the protein chains [27]. The values for T_d and ΔH_d are given in Table 1. As observed, the bone glue shows the lowest enthalpy of denaturation whilst the highest denaturation enthalpy is related to fish glue. This can be also demonstrated in the lower value of Bloom strength for bone glue and the higher Bloom value of fish glue reported later in Table 2. For further scrutiny of the triple-helix content, XRD measurements were performed on the adhesive films with similar thicknesses.

Representative X-ray diffraction patterns of different glues are illustrated in Fig. 2b. As observed, two characteristic diffraction peaks at angles of $2\theta\sim8°$, and $2\theta\sim20°$ are found in all different animal adhesives films. The first diffraction peak at 8° is attributed to the ordered structure of the triple-helix from a partially renatured collagen-like structure, which can also be designated as a crystalline structure, while the second peak at 20° relates to the amorphous phase with free single-helix chains [28]. A parameter *d* related to the inter-planar spacing is calculated from the Bragg equation with values of ~1.1 nm and ~2.9 nm, which can be attributed to the peaks at $2\theta\sim8°$ and $2\theta\sim20°$, respectively. These two values are attributed to the internal diameter of the triple helix structure in the 'crystalline' region and the single helix in the amorphous regions, respectively [15,28].

Table 1 summarises the ratio of peak areas to compare the content of the 'crystalline' and amorphous structure (A_c/A_a) in each adhesive. This ratio is the lowest for bone glue and highest for fish glue. This trend is in agreement with the triple helix content attributed to the enthalpy of denaturation values obtained from DSC measurements (Table 1). The lowest content of triple helix structure in bone glue can be attributed to a broader molecular weight distribution and lower average molecular weight (indicated by the steeper slope of decline in storage modulus measured by DMA that will be shown in a later section), which can potentially hinder molecular arrangements. Moreover, the results showcase that two fundamentally different physical characterisation methods, namely XRD and DSC, are in agreement in identifying the molecular structure content in gelatine-based adhesives.

In Fig. 3, the ratio of A_c/A_a (the ratio of the integrated crystalline and amorphous peaks) derived from XRD measurements is plotted against the denaturation enthalpy obtained by performing DSC on four different adhesive film types.

Both parameters give a quantitative measure of the triple helix content as explained earlier. As illustrated, a linear relationship describes the correlation between A_c/A_a and ΔH_d. It should be noted that the curve is assumed to pass through the origin, meaning that the absence of denaturation enthalpy relates to the lack of triple-helix structure and hence no diffraction peak at $2\theta\sim8°$ would occur.

The existence of a linear correlation between the XRD and DSC output was previously demonstrated for gelatine adhesives derived from one animal species (porcine skin with different bloom

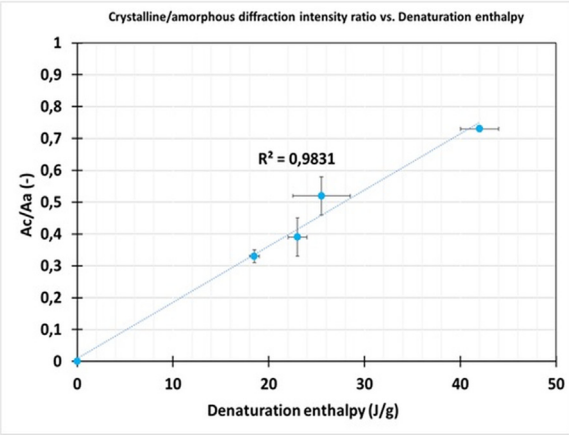

Fig. 3. Correlation between the measured ratio between integrated areas of diffraction peaks at crystalline ($2\theta \sim 8°$), and amorphous ($2\theta \sim 20°$) regions and denaturation enthalpy, both associated with the triple helix content obtained by XRD and DSC techniques, respectively.

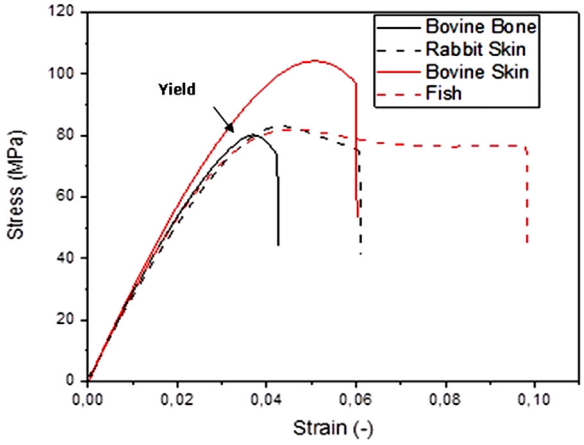

Fig. 4. Uniaxial tensile stress–strain curves of the different adhesives, at standard environmental conditions (room temperature, 50% RH).

strengths) [16]. Here, a similar linear correlation (with different slope) also for gelatine adhesives sourced from different animal species (bovine, rabbit, and fish) is yet again observed. Note should be taken that this conclusion is limited to the climate conditions that measurements have been carried out in (23 °C and 50%RH).

4.2. Macroscopic mechanical behaviour of the adhesive films

4.2.1. Bloom strength of the adhesives

Bloom strength is one of the most important physical characteristics of animal adhesives. The Bloom strength is measured as the weight (force in grams) required to depress the surface of the adhesive gel 4 mm using a 12.7 mm diameter flat-bottomed cylindrical plunger, [3].

Bloom strength is essentially a measure of gel-forming ability, cohesive strength, and stiffness of the gelatinous adhesives thus the structure formed by intermolecular hydrogen bonds. The number of nodes or junctions that are formed by hydrogen bonds within and between the molecules determines the rigidity and elasticity of the glue matrix and its Bloom strength [29]. Bloom strength generally relates to the average molecular weight of the polypeptide chains, the level of physical and chemical cross-links present in the micro-structure of these adhesives, and also to the degree of renaturation during gelation [11,15].

Table 2 summarises the measured Bloom strength values of the four different adhesives according to the procedure described in 3.2.1. As observed, bovine bone adhesive demonstrates the lowest Bloom strength, whilst the highest Bloom strength is found for fish glue. The Bloom strength is believed to be related to the degree of renaturation of microstructural features, mainly triple helices, of polypeptide chains in the adhesives [11,15,16]. This indeed proved to be the case (see Fig. 7).

4.2.2. Uniaxial-tensile measurements

Fig. 4 illustrates the comparative stress-strain curves of the different adhesives. As observed, the gelatine adhesive films demonstrate a typical elastic-plastic behaviour described by a yield point as the point at which a shift from elastic to plastic behaviour occurs. The extent of plastic deformation is the lowest for bone glue with strain to failure around 4%. The highest plasticity is demonstrated by fish glue rendering a strain to failure up to 10%.

The tensile experiments were performed at standard conditions of 23 °C and 50% RH, 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 Young's modulus (E) is almost constant for different adhesives at room temperature. This indicates that the different triple helix content of the adhesives hardly affects the elastic modulus at the conditions at which the glue films were tested (glassy state, 50% RH, and 23 °C) [16]. Though triple helices act as physical cross-links which can increase stiffness, it must be noted that these adhesives are tested in standard conditions (room temperature, 50% RH) and well below their glass transition temperature at which the amorphous phase is in its rigid state, hence the cross-link content or crystalline content hardly affects the elastic stiffness. The tensile strength, defined as the maximum stress value (σ_{max}), is the highest for bovine skin glue (around 90 MPa) whilst the other adhesives show similar strength values at around 80 MPa. The strain at break value (maximum strain, ε_{max}) which is related to the flexibility of the glue, is the highest for fish glue, whilst bone glue seems to be the less flexible adhesive. To be able to show a measure for the toughness of the different glues, the strain energy density to failure was calculated as the area under the tensile stress-strain curve of the adhesives. As indicated in Table 2, the strain energy value is the highest for fish glue, and the lowest for bovine bone glue. The observed trend in the toughness of all four adhesives can be associated with the amount of triple helix content (see also Fig. 6). Similarly, it can be observed that the value of strain to failure increases with increasing triple helix content in the adhesive films. This is in line with previous findings of research indicating a strong correlation between triple helix content and tensile strain to failure in porcine gelatine films [15,16].

To describe this phenomenon one can consider the self-organised triple helices acting as mobile physical cross-links or some type of inter/ intra chain entanglements which form a three-dimensional network within the polymer matrix, improving its elasticity and extensibility. Also, the improved failure strain energy can be because the triple helices act as denser and more compact regions around which the propagating crack path deflects leading to a larger absorbing energy.

4.2.3. Dynamic mechanical analysis

DMA experiments were performed to assess the viscoelastic properties of the four adhesives as a function of temperature. Fig. 5 (left) shows the storage modulus (E') of the different adhesive films. A steady reduction of the storage modulus can be observed as a function of temperature for all the adhesives. A change in the slope of the storage modulus curve starting around 50 °C can be observed, which is in correspondence with an increase in tanδ values in Fig. 6 (right). This softening of the adhesive is fol-

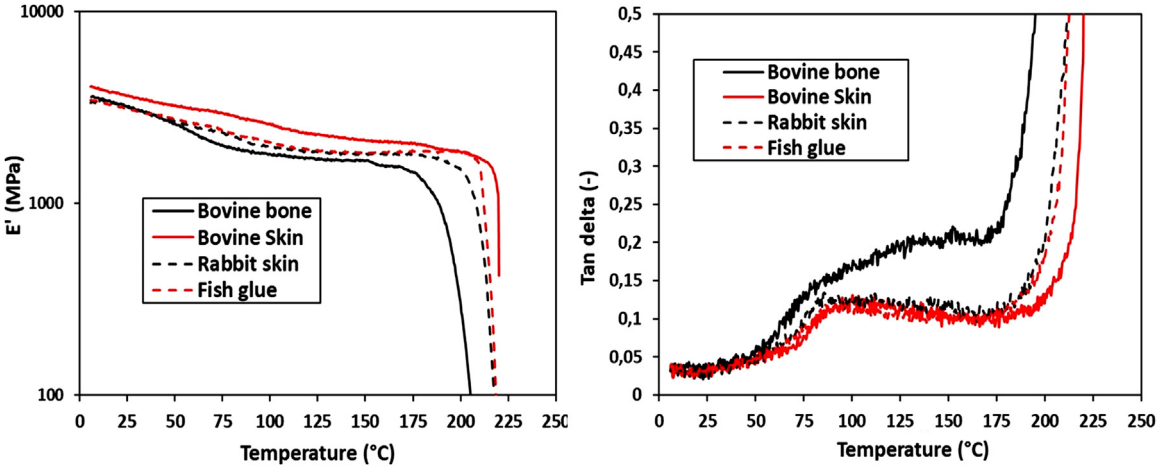

Fig. 5. Storage modulus (left) and tan delta (right) versus temperature curves of the different adhesive films obtained from DMA.

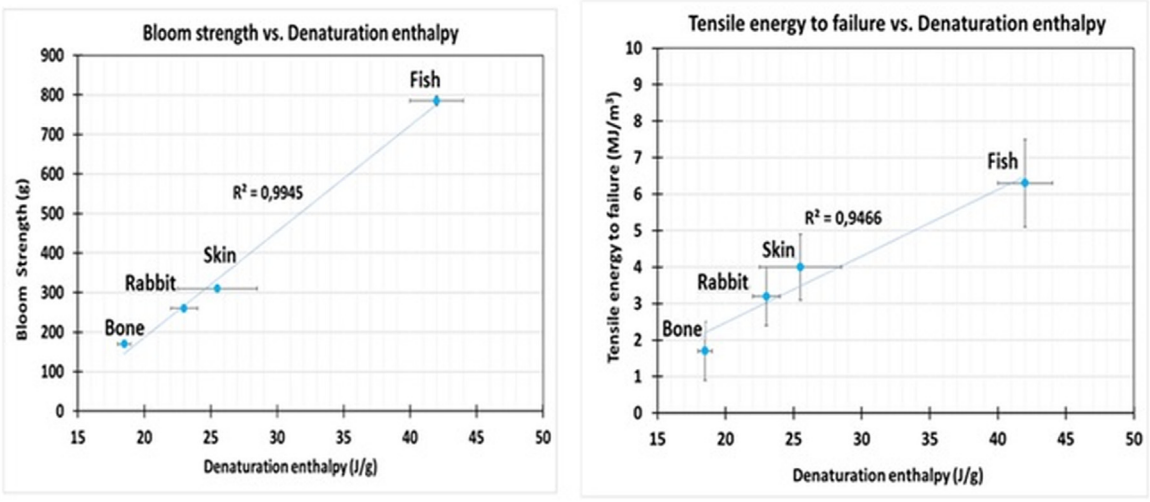

Fig. 6. (left) Bloom strength, (right) energy to failure, plotted versus denaturation enthalpy with a linear least squares regression.

lowed by a peak of tanδ around 80 °C. This gradual softening of the adhesives with increasing temperature can be attributed to transitions in the microstructure; such as the glass transition and followed by the denaturation of the triple helix to coil structure. The slope of change in both storage modulus and tanδ curves after 50 °C is comparatively more prominent for the bone glue, which has the lowest triple helix content. This is logical, since T_g is a property of the amorphous phase and bone glue has the highest amorphous content.

The major drop in storage modulus around 175 °C for bone glue, and above 200 °C for other glues, is related to the discoloration and degradation of the adhesive films which was directly observed after opening the DMA chamber at the end of the experiment. The lowest triple helix content leads to the lowest degradation temperature possibly attributed to lower energy required to break bonds for glue with the lowest triple helix content.

4.3. Effect of microstructure on macroscopic mechanical behaviour

The correlation between the microstructure of the adhesives and their mechanical properties at a macroscopic level is summarised in Fig. 6. In Fig. 6 (left), the Bloom strength of the adhesives is plotted against their denaturation enthalpy. A direct correlation between triple-helix content represented by denaturation enthalpy and the Bloom strength can be observed. Interestingly, a similar linear relation between the denaturation enthalpy of these adhesives and their strain energy to failure (toughness) is also present as illustrated by Fig. 6 (right). Previous research also demonstrated that Bloom strength, in both wet and dry gelatine films derived from porcine skin, is linearly correlated with triple-helix content. Moreover, an increase in tensile strength and strain to failure was also observed in prior research, [15,16].

4.4. Moisture sensitivity of the adhesives

4.4.1. Dynamic vapour sorption analysis: moisture uptake

Animal adhesives are hygroscopic materials and may undergo structural changes when subjected to different environmental conditions. Different environmental relative humidity can affect physical and mechanical properties such as thermal transitions, and cohesive and adhesive strengths of gelatine and collagen-based materials [11,30,31]. Hence, understanding the extent of moisture sensitivity and the parameters affecting it is necessary to establish the appropriate environmental conditions for their storage, and also for conservation practices that they are utilised in. To compare the extent of moisture uptake of the four adhesives DVS experiments were performed. As observed in Fig. 7, the bone glue demonstrates the highest level of moisture uptake whilst the fish glue absorbs

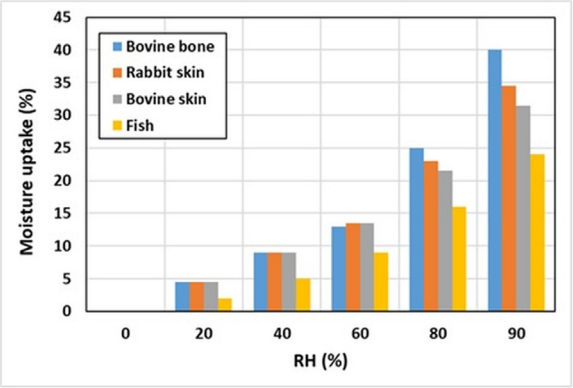

Fig. 7. Moisture uptake after reaching equilibrium mass when adhesive films are subjected to different relative humidities inside the DVS chamber.

the lowest amount of environmental moisture especially when subjected to higher RHs of 80 and 90%. The trend of the moisture absorption of these adhesives is in agreement with their triple helix content as measured by DSC and XRD techniques. Hence, a correlation between the microstructural order and the (dis)ability of water absorption can be considered. This is because the ordered domains in the form of triple helices are more densely packed thus hindering penetration of water molecules whilst amorphous regions contain more free volume allowing more absorption of water. It can be concluded that the higher amount of structural order in the form of physical or chemical cross-links can in principle reduce the moisture sensitivity of these adhesives.

4.4.2. Effect of environmental relative humidity on the thermal behaviour of the adhesives

Calorimetry is one of the most important techniques to measure temperature or moisture-induced changes in the structure of proteins. Moisture effects on the thermal properties of animal adhesives have not thoroughly been studied. DSC analysis was carried out to identify the glass transition temperature (T_g), the helix-coil denaturation temperature (T_d), and the denaturation enthalpy (ΔH_d) of the adhesives after exposure to three different relative humidities of 30, 50, and 80%.

In their study on the effect of DSC pan integrity, Mukherjee and Rosolen [32] showed that the seal integrity of the DSC pans can remarkably affect the detected thermal transitions of bovine gelatines, and they attribute this to the moisture escape during testing when the pan is not hermetically well sealed. Hence, to study the effect of moisture, hermetically sealed Al pans were used to be able to detect the changes in the thermal transitions without the effect of moisture loss.

Table 3 summarises the glass transition temperature (T_g), the denaturation temperature (T_d), and the denaturation enthalpy (ΔH_d) of the four adhesive films after exposure to three different relative humidities of 30, 50, and 80%. The glass transition is identified as the first step-wise transition which is related to the glassy to rubbery transition in the amorphous region of the protein chains. In the case of bovine bone glue at 80% RH, such step-wise transition is not discernible. This is because the T_g transition is somewhat coinciding with the denaturation event and it is hidden in the endothermic denaturation peak. Both the T_g and T_d (peak temperature of the endothermic peak) remarkably decrease with increasing relative humidities for all the adhesives. This shift in the thermal events is due to the increase in water content of the adhesives at higher relative humidities as demonstrated by DVS experiments (Fig. 7). Free water molecules act as plasticiser and facilitate the movements in the amorphous regions of polypeptide chains [24]. The reduction in the glass transition temperature of these adhesives in humid environments has repercussions for conservation practices. For instance, to avoid adhesive softening in humid environments, climate control inside museums and storage sites of art objects would become important. Also, reduced glass transition and denaturation temperatures of animal glues as a result of the increase in environmental relative humidity can lead to an increased tendency to creep at room temperature conditions.

Table 3
Values of the glass transition temperature, T_g, denaturation temperature, T_d, and denaturation enthalpy, ΔH_d, of different adhesive films when subjected to three different environmental relative humidities of 30%, 50%, and 80%. N.I. stands for not identified.

Parameter	Adhesive type	Relative humidity		
		30%	50%	80%
T_g (°C)	Bovine bone	70±0	52±1.5	N.I.
	Fish	60±1	55±1	38±4
	Rabbit skin	65±0.5	54±1	30±1
	Bovine skin	77±1	55±1	N. I.
T_d (°C)	Bovine bone	92±0.5	81±1	56±2
	Fish	100±1	84±1.5	68±1.5
	Rabbit skin	97±0	84±0.6	63±3
	Bovine skin	95±0.5	84±1	66±3.5
ΔH_d (J/g)	Bovine bone	19±2	18.5 ± 0.5	19±1
	Fish	44 ± 1	42 ± 2	42±3
	Rabbit	24±0	23±1	23±1
	Bovine skin	24.5 ± 3	25.5 ± 3	24.7 ± 1.5

However, the denaturation enthalpy seems to hardly change with respect to moisture content and environmental RH (Table 3). This as such indicates that water molecules may be absorbed mainly into the amorphous regions, and did not highly intervene with the amount of triple helices within the adhesive. But because the denaturation temperature (T_d) decreases with moisture content it can be hypothesized that there is an effect on the arrangement and the size of the domains of triple helices. Lower denaturation temperature at higher RH may indicate more dissociated and smaller crystalline domains of triple helices. This is in analogy to crystalline regions in polymers where smaller crystals have lower melting points.

5. Conclusions

This study investigated the correlation between microstructure and macro-properties in four types of animal glue commonly used in the conservation of art objects. The animal glues investigated in this study are bovine bone, bovine skin, rabbit skin, and fish glue. Thin adhesive films were produced via solution casting methods in controlled standard climate conditions (23 °C and 50% RH).

- Physical characterisation techniques such as XRD and DSC were employed to measure the triple helix content in the adhesive films which was associated with the enthalpy of the endothermic denaturation peak and the integrated intensity of the diffraction peak at around $2\theta \sim 8°$, respectively. Both techniques showed good agreement with each other in identifying the triple helix content and it was seen that bovine bone glue and fish glue contain the lowest and highest triple helix content, respectively.
- Bloom strength measurements demonstrated a linear correlation with the renaturation levels of the triple helices. Bovine bone glue showed the lowest and fish glue the highest Bloom strength. Uniaxial tensile tests on the adhesive films showed a linear correlation between the triple helix content and the strain energy to failure (toughness) of the adhesives. Particularly, the fish glue demonstrated higher flexibility and toughness, which makes it an interesting candidate for adhesively bonding parts that can be subjected to impacts or vibrations.

Bovine bone glue was identified as the most brittle and least flexible glue amongst the four types of glue evaluated.

- The current paper investigated the effect of animal origin (bovine, rabbit, fish). It was demonstrated that the linear correlation between triple helix content and mechanical properties of adhesive films previously found for gelatine adhesives from porcine skin [16], can be translated to gelatine-based adhesives derived from different animal origins (bovine, rabbit, and fish). Though this observation is valid solely for the particular standard climate conditions (23 °C and 50% RH) that tests have been performed in.
- Dynamic vapour sorption experiments showed a negative correlation between the triple helix content and the water uptake, particularly at high RH of 80 and 90%. Bone glue absorbed the highest amount of moisture whilst fish glue absorbed the least. High triple helix content means a lower amount of amorphous phase, the latter of which is expected to absorb more water.
- The effect of environmental RH on the thermal behaviour of animal glues such as the glass transition and denaturation temperatures was investigated by DSC, when the adhesive films were pre-conditioned at three different RHs of 30, 50, and 80%. The enthalpy of denaturation hardly changes concerning moisture content and environmental RHs. The fact that the denaturation temperature decreased at high humidity can be explained by the occurrence of smaller triple helix domain sizes.

The results of this study show that adhesive type as well as Bloom number, and the environment that the adhesives used in art objects are subjected to, can have repercussions for conservation practices. Hence, these results can assist conservators to make a more informed selection of the type of adhesive they use for their repair practices. For instance, the results indicate that changes in environmental relative humidity can significantly affect the thermal properties of the adhesives, causing them to soften prematurely in humid environments due to lowered glass transitions. This will increase the tendency for creep in the adhesive layer if the art objects would be preserved in humid environments without proper climate control. Furthermore, the results of this study demonstrate the importance of the use of adhesive with high bloom strength and higher strain to failure such as fish glue, for potentially creating a tougher adhesive bond that can better withstand stresses as well as possible impact loads, due to the higher toughness values of these adhesive films.

Note must be taken that apart from physical and mechanical properties, there are other factors that conservators regard when using a specific glue in their conservation practices e.g. where the glue is used, in a wooden joint or for veneering, or how much open time is required to complete the gluing procedure. Moreover, in art conservation practices, conservators at times use mixtures of different glues to further optimise the properties based on their practical experience.

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