Research

Cite this article: Wang J, Guan J, Hawkins N, Vollrath F. 2018 Analysing the structure and glass transition behaviour of silks for archaeology and conservation. *J. R. Soc. Interface* **15**: 20170883.
<http://dx.doi.org/10.1098/rsif.2017.0883>

Received: 26 November 2017
Accepted: 18 January 2018

Subject Category:

Life Sciences—Physics interface

Subject Areas:

biomaterials, biophysics, biomechanics

Keywords:

biopolymer, ageing, amorphous structure, UV irradiation, thermal properties, mechanical properties

Authors for correspondence:

Juan Guan
e-mail: juan.guan@buaa.edu.cn
Fritz Vollrath
e-mail: fritz.vollrath@zoo.ox.ac.uk

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3985317>.

Analysing the structure and glass transition behaviour of silks for archaeology and conservation

Jianlan Wang¹, Juan Guan², Nicholas Hawkins³ and Fritz Vollrath³

¹Shanghai Institute of Visual Arts, Shanghai, China

²Beijing Advanced Innovation Center for Biomedical Engineering, School of Materials Science and Engineering, Beihang University, Beijing, China

³Oxford Silk Group, Department of Zoology, University of Oxford, Oxford, UK

JG, 0000-0002-9784-8125

Silk is an iconic material in many cultures. Silk archaeology and conservation is affected by silk production technology as well as subsequent environmental effects such as humidity, temperature, UV radiation and ageing. The complex interactions and various effects on silk materials affect the practical use of silk, for example, in the conservation of ancient manuscripts. This study examines the various influences of silk provenance and processing, adhesive coatings and chemical treatments as well as natural and artificial ageing of the silk material. We use infrared spectroscopy (FTIR) and dynamic mechanical thermal analysis to investigate the glass transition behaviours in a range of archaeological and control silk samples. This allows us to establish structural differences in century-old museum silks and predict the effects of silk ageing and degradation.

1. Introduction

Natural commercial silks have many desirable properties. Silks have a history of use going back 5000 years when sericulture emerged in China and the Indus valley. Consequently, silks and silk products have been stored and preserved in palaces, homes and museums around the world as well as buried in sites underground, all reflecting the unique artisan crafts and cultural values of the time with considerable historical information. Museum specimens document the development of textile and fashion industries as well as informing on spinning and weaving technologies. Today silks are used not only in luxury textiles but also in cultural activities such as silk painting. Important for our study is the continued use of silk to mount paper-based artefacts in conservation techniques that have a history going back to ancient China [1].

This so-called ‘silk-facing’ or ‘silking’ technique relates to covering ancient manuscripts with translucent silk materials in order to protect the fragile manuscript papers, which often have experienced long periods of some kind of degradation. This technology is based on the concept that disintegrating, paper, typically hand crafted, could be supported and strengthened by silk facings and that this could be done without obscuring the text, as would happen with modern tissue paper (B. Borghese 2010, personal communication). However, such silk facing and the materials used (both silk and glue) sometimes failed the original purpose of preservation. Indeed, as time goes by silk facing often loses its inherent, original mechanical properties and fails to support the manuscripts due to changes in its own elasticity and extensibility.

The Stein Collection in the British Library is famous for its religious, historical and literary texts in Chinese, Tibetan and many other languages dating from the fifth to eleventh centuries. For example, the library includes the world’s earliest printed book, the Diamond Sutra, as well as many manuscripts and documents from the golden age of China (Tang dynasty, 618–907 AD) [2,3],

Figure 1. Optical photo images of the silk fabrics investigated in this work. The fabrics were placed on the same black background and the above images were acquired from the same camera setting under the same light exposure.

found in a hidden cave in the Mogao caves (莫高窟) near the Silk Road town of Dunhuang (敦煌) [4,5].

Historic silk objects have experienced complex ageing conditions that can be mimicked (to some degree), and thus studied through artificial ageing using UV irradiation or thermal treatments. For example, UV irradiation simulates the intensive energy exposures that textiles experienced during their lifetime, which enables reproduction of patterns comparable (and observable on short-term timescales) to that of textiles naturally aged over timescales of thousands of years [6–8]. Colorimetric analysis and tensile strength analysis [8–11] have previously been used to inform curators and conservators of the condition of degraded silk samples. However, silk textiles and costumes that have been dyed or are generally too fragile and/or valuable might fail to be investigated by such methods. Infrared or Raman spectroscopy, which provides non-destructive analysis, is highly relevant in scientific conservation efforts. For example: Koperska *et al.* [12] reported degradation markers of fibroin in silk using infrared spectroscopy and Kojthung *et al.* [13] reported the effects of gamma radiation on the biodegradation of *Bombyx mori* silk fibroin, while Prasong *et al.* [14] discussed how different treatments of silk (with or without sericin) may be reflected in IR spectra. However, the IR and Raman spectra of complex proteins are themselves rather complex, showing many overlapping bands of similar frequencies. Although several approaches can be used to further resolve such spectra, including signal manipulation by Fourier deconvolution [15], such complex analyses may not be available to conservators or indeed impossible to perform on spectroscopic data collected at low intensity. For example, the degree of ageing of silk from the recent 1000 years cannot be distinguished from analysing the IR spectroscopic features as reported in by Royall *et al.* [16].

Dynamic mechanical thermal analysis (DMTA), combining thermal analysis and dynamic mechanical analysis, could offer a powerful analytical tool complementary to spectroscopic analysis. Experimentally, DMTA measures the stiffness and damping properties of materials as a function of temperature, mechanical stress or other environmental conditions, e.g. humidity. All structural states/phases in materials display structural transitions, e.g. glass transition from glassy state to rubbery state, and DMTA can detect the associated mechanical property changes (storage modulus and damping parameters) caused during the structural transition. Hence, DMTA is highly sensitive to structural changes. This would be particularly interesting to textile studies as relative humidity (RH) and temperature have

always been the key concerns to the conservation objective of textile-related materials.

DMTA might also detect structural relaxations on different length and timescales. Under low temperature or high frequencies, only the smallest structural units such as the local groups can be allowed to relax; at high temperatures and low frequencies, the longer chain segments will become more relaxed and the correlated motions are activated. Therefore, through varying temperature or frequency scans on silk samples DMTA could be useful for detecting structural degradation due to ageing. Despite its popularity and importance in conventional polymer industries such as adhesives and composites, DMTA has not been deployed extensively in the field of pharmacology [16], biomaterials [17,18] and conservation [19–21]. One interesting and relevant example used DMTA to assess the crystalline structure and the influence of deterioration conditions in new and historical parchments and leathers [19].

Here, we set out to better understand the silk facing materials used in the twentieth century's conservation for protecting the ancient manuscripts, and the mechanisms causing the ineffectiveness of the original protection role of silk facings. FTIR (ATR) and DMTA were combined to investigate a variety of silk materials from the past and the present to compare silk facing materials. The following major factors were considered: (1) raw silk production origins; (2) weaving and processing methods; (3) additive coatings and (4) ageing conditions. In order to elucidate on the structural change and the interactive mechanisms between silk and other supplements, we present comparisons and analysis, and reasonable suggestions regarding the role of archaeological silk materials in the conservation.

2. Experimental

2.1. Materials

The optical photo images of the studied silk materials in this work are shown in figure 1.

2.1.1. British Library silk

Fine and gauze silks were the two main forms of facing materials studied, having been manually removed by experts at the British Library from some of its ancient manuscripts under International Dunhuang Project/IDP (accessible by library staff), with an estimated age of *ca* 60–80 years of the silk facings and the still attached adhesives. Fine silk facing is tightly woven, comparable to modern commercial

Table 1. TGA results for silk samples and other supplements found in silk samples (the definition of each result can be referred to figure 1).

silk material	moisture content (%)	weight loss at $T < 400^{\circ}\text{C}$ (%)	onset temperature for thermal degradation ($^{\circ}\text{C}$)	temperature for fastest thermal degradation ($^{\circ}\text{C}$)
Guangxi raw silk	6.02	53.86	199.9	315.3
Zhejiang raw silk	6.47	54.79	197.4	320.3
commercial loose weave	5.22	58.42	204.2	321.5
commercial fine weave	4.90	56.63	206.0	317.7
library gauze silk	6.51	62.65	179.9	310.4
library fine silk facing	6.12	56.81	185.7	318.2

fine weave silk fabric. Gauze silk facing has a looser, wavy morphology with very little compaction and is similar to commercial loose-weave silk fabric.

Our control silk materials were, firstly, modern (2014) raw silks without any industrial processing, produced in two regions in China, Guangxi Province and Zhejiang Province and provided, respectively, by Guangxi Silk Group Co. Ltd and Zhe Jiang Silk Holding Co. Ltd, who collected silks from local growers. Secondly, for the comparison of silk material after different post-processing conditions, we used commercial modern silk fabrics in two forms, loose and fine weaves purchased from Pongees Ltd (London, UK) and Amazon Minerva Fabrics Ltd, respectively. Commercial fine silk fabric had an even weave with a flat surface in contrast to creased and loose silk fabric. Moreover, fine weave fabric will have experienced steps of thermal treatments and mechanical stretching in order to stabilize the weave morphology.

In addition to the silks themselves, we had to consider the adhesive coating materials. As controls we examined commonly used animal glue (pearl glue) and rice starch comparable to adhesives traditionally used in the silk-facing process by conservators on ancient manuscripts. Pearl glue was purchased from Liberon Limited (Kent, UK). Rice starch (product number S7260) was purchased from Sigma Aldrich. Coated silks as comparisons to pure silk were prepared by immersing commercial silk fabrics in the solutions of coating materials: (1) silk coated with starch; (2) silk coated with pearl glue; and (3) silk coated with mixture of starch and pearl glue.

In order to study ageing, we used a range of techniques. Coated silk was heated in a laboratory oven at 80°C and 80% RH for eight weeks for conditioning. UV radiation and thermal ageing were applied separately to simulate natural ageing. For UV ageing, commercial fine silk samples were irradiated from 10 min to 51 days under a portable TLC plate reading UV lamp (254 nm, 120 W). For thermal ageing, commercial fine and loose silk samples were treated in an oven (Davidson & Hardy Ltd) at 125°C for 11 days (264 h). The artificial ageing parameters were chosen based on previous studies [22,23], for example, Vilaplana *et al.* used 254 nm UV for light ageing and Zhang used 125°C for dry thermal ageing.

2.2. Methods

2.2.1. Thermal gravimetric analysis (TGA)

The silk and fabric samples were cut into small pieces, and a weight of 2–4 mg sample was placed into ceramic pans

before loading onto the TGA instrument Q500. Two adhesives, rice starch and pearl glue, were tested too. The temperature range was from 20°C to 800°C at $10^{\circ}\text{C min}^{-1}$ with N_2 as the purge gas.

2.2.2. FTIR (ATR) analysis

Silk samples studied in the spectroscopic experiment were as follows: fine silk facing removed from the British Library; new silk fabrics purchased from commercial suppliers; new raw silks from two main provinces in China, (table 1). The spectra were recorded using a PerkinElmer Spectrometer 100 FT-IR instrument with the attenuated total reflection (ATR) mode. Thirty-two scans were accumulated for each collection at a resolution of 4 cm^{-1} in the range of $4000\text{--}600\text{ cm}^{-1}$.

2.2.3. DMTA

all DMTA experiment was done on a dynamic mechanical analyser Q800 (TA Instruments, Waters Ltd). Tension films clamps were used and the Liquid Nitrogen Cooling Accessory (GCA) was attached to enable subambient temperatures. The samples included: bundled raw silks produced in Guangxi and Zhejiang Province; commercial loose and fine weave silk fabrics; gauze and fine silk facing materials from the British Library. For bundled raw fibres, sections were cut approximately 30 mm in length for testing. For commercial loose and fine weave silk fabrics, strips were cut approximately 5 mm in width and 30 mm in length. For the thinner gauze, a wide sample of approximately 3 cm in width and 30 mm in length was cut. These strips were then 'rolled up' before being loaded in the DMA clamps. The following experimental conditions were used: temperature ramp from -125°C to $+250^{\circ}\text{C}$; heating rate $5^{\circ}\text{C min}^{-1}$; frequency 1 Hz; dynamic strain between 0.5% and 1.0%; force track 120%; clamping pressure 8 PSI. It is noted that the recorded data of absolute storage modulus could not be used for quantitative analysis as the samples were not in simple geometry and the size information could not be accurately considered.

2.3. Data representativeness and statistical significance

The fabrics chosen for TGA and DMTA measurement were at least $1\text{ cm} \times 1\text{ cm}$ square-sized, to contain hundreds of silk fibres and a considerable amount of adhesives for the analyses. For raw silks, a similar quantity of silk fibres was examined for comparison. This gives enough sample for TGA and DMTA to be representative for a discussion of treatment effect on the molecular structures on single silk fibres.

Figure 2. (a) TGA profiles from room temperature to 800°C for the subject silk materials: Zhejiang raw silk, Guangxi raw silk, commercial fine weave, commercial loose weave, the British Library gauze and fine silk facing. (b) The derivative of percentage mass as a function of temperature showing the onset temperature of thermal degradation and the temperature for fastest thermal degradation. (c) TGA profiles of mass loss and derivative mass loss from room temperature to 800°C for unaged silk (commercial fine silk), UV-aged silk and thermal-aged silk.

The ATR-FTIR experiment used a 4 mm diameter beam spot to collect the spectra for each sample, which is expected to contain structural information from more than a hundred fibres within the area. However, the experiment was only repeated once for each silk sample, thus there might be a hidden experimental error due to operation, equipment and sample variation.

3. Results and discussion

3.1. Silk moisture content and thermal stability

TGA was used to determine the moisture content and the thermal stability of the silk materials. Figure 2a shows the remaining mass percentage as a function of temperature

Figure 3. TGA profiles of animal glue (blue lines) and starch (red lines). The solid lines correspond to the left axis of the mass in percentage and the dashed lines correspond to the right axis of the derivative of mass over temperature to indicate the fastest thermal degradation temperature.

from room temperature to 800°C and figure 2b shows the derivative of the mass as a function of temperature in the same range. To compare the thermal stability of different silk materials, the following results are defined and determined (as shown in figure 2 and table 1): the moisture content below 120°C, weight loss below 400°C, and two thermal degradation temperatures, the onset degradation temperature and the fastest-degradation temperature. We observed that the commercial silks, especially the commercial fine weave silk, had lower moisture content and a higher onset temperature for thermal degradation compared to the raw silks. On the other hand, the library gauze silk possessed the highest moisture content, highest weight loss below 400°C and the lowest onset temperature for thermal degradation. The results suggest that silk processing from raw silk to commercial fabric textile could reduce the moisture content and enhanced the thermal stability of silk; whereas the ageing process of the library silk reduced the thermal stability of the silk.

Because rice starch and pearl glue are commonly used to stick silk facings to paper manuscripts, TGA was also conducted on the two adhesives. As shown in figure 3, rice starch as a carbohydrate possesses a much simpler TGA profile, which in our cases only showed two accelerated thermal degradation events at temperatures of approximately 65°C and 323°C, corresponding to mass loss of approximately 11% and 80%. At 500°C, rice starch was completely degraded and/or carbonized. By contrast, the pearl glue as a protein glue had four accelerated thermal degradation events at temperatures approximately 173°C, 343°C, 517°C and 584°C. The first characteristic temperature of thermal stability at 173°C for pearl glue was very close to silk's onset degradation temperature ranging from 180°C to 206°C. For the library gauze silk facings, the highest weight loss at 400°C may support starch as the adhesive for the library silk facing. However, because pearl glue has very similar thermal stability profiles, the presence of pearl glue cannot be excluded.

3.2. Structural analysis from FTIR

FTIR is often used to investigate molecular structural changes in archaeological objects. To examine age effects on library silk facings, we performed FTIR on the following silk control materials: raw silks from two different regions of China and different types of textile fabrics (commercial fine weave and commercial loose weave). Raw *B. mori* silk is mainly

Figure 4. (a) Comparing the fingerprint regions of the IR spectra of *B. mori* raw silks from two regions Guangxi (i) and Zhejiang (ii) in China; and two types of textile silk fabrics: commercial fine weave silk (iii) and commercial loose weave silk (iv). (b) Comparing commercial loose weave silk (iv) and the library gauze silk (v), and adhesive-rice starch (vi). (c) Comparing unaged commercial fine weave silk (iii) with 8 h UV-aged silk (vii) and 51 days UV-aged silk (viii). (d) Comparing unaged commercial loose silk (iv) with 264 h thermally aged silk (ix). (e) Free carbonyl region and (f) tyrosine doublet region of silks. (g) Peak deconvolution results for the spectra of typical silk materials listed above (data shown in table 2).

comprised of silk fibroin (ca 75%) as well as containing sericin (ca 25%). The raw silks are unravelled from cocoons in a reeling process that applies hot water with alkali to dissolve the sericin gum. Some of the sericin remains on the silk fibres even after intensive degumming. Therefore, in order to

evaluate the effect of the remaining sericin, the spectrum of sericin is also discussed [24]. The weaving industry uses the degummed silks to produce textile fabrics which after weaving are typically cleaned further of sericin and processing substances.

Table 2. Conformation content in the subject silk materials from FTIR peak deconvolution analysis "(amide I region)".

	Guangxi raw silk	Zhejiang raw silk	commercial fine silk	library silk facing	8 h-UV aged	51d-UV aged	thermal aged
β -sheet	57.10%	58.80%	65.40%	54.10%	42.10%	42%	65.40%
helix or coil	34.40%	33.50%	31%	35.80%	47.30%	47.60%	32.50%
β -turns	6.40%	6.20%	2.60%	6.70%	10.40%	10.30%	2%
fitting coefficient r^2	0.89	0.96	0.93	0.58	0.73	0.73	0.70

Figure 4a shows four spectra of raw silks from Guangxi and Zhejiang Provinces in China, and commercial silk fabrics of fine and loose weave forms. The raw silks from two production regions in China displayed very similar IR spectroscopic signatures (figure 4a(i)(ii)), and the effect of provenance could not be resolved by qualitative IR analysis. Also, the effects of commercial processing and weave-form were not obvious (figure 4a(iii)(iv)).

The FTIR spectra of library loose/gauze silk and commercial loose silk fabric are presented in figure 4b(iv)(v). The library silk shows obvious differences in the following regions: amide III and $1100\text{--}900\text{ cm}^{-1}$. Less intense amide III shows that the library silk may have less β -sheet structure as the $1260\text{--}1280\text{ cm}^{-1}$ peak associated with β -sheet conformation is absent. The spectrum of rice starch is shown in figure 4b(vi). A group of much stronger absorbance bands centred at 1035 cm^{-1} can be seen, which are the structural fingerprints of starch corresponding to its skeletal mode vibrations of α -1,4 glycosidic linkage (C–O–C). This absorbance region coincides with the more pronounced spectroscopic signals from 1100 to 900 cm^{-1} for the library silk facings. Therefore, it may further prove that the British Library's silk-facings were originally coated by starch or glycol-containing substances.

Apart from the complexity from the coating, silk facing materials applied to the ancient documents in the early twentieth century obviously went through a process of natural ageing, which can be investigated through simulated artificial ageing. The ageing mechanisms for silk include oxidation, hydrolysis, chain scission and chain rearrangement (also known as physical ageing) as summarized by Vilaplana *et al.* [22]. However, these changes often take place simultaneously in a complicated way and any single mechanism can be difficult to prove on its own.

The first simulated ageing was UV irradiation on commercial silk fabrics. Figure 4c compares spectra of unaged commercial fine silk fabric with UV-irradiated ones, for 8 h (8 h-UV) and 51 days (51d-UV). The representative spectra (8 h-UV and 51d-UV) were selected from a range of samples exposed to different lengths of UV irradiation from 10 min to 51 days. The 8 h-UV sample was selected because this was the point when the colour of silk started changing yellowish. The 51d-UV sample not only turned distinctly yellowish but also became remarkably stiff and brittle by simple inspections. It is believed that the tyrosine amino acid residues in silk are the most susceptible to degradations upon the exposure of UV [25]. The characteristic absorption peak around 1014 cm^{-1} for tyrosine [26] appears diminishing for the aged silks.

It is acknowledged that natural ageing in museum and library environments is far more complex than merely UV irradiation. Therefore, a second ageing process of thermal ageing was also considered. Commercial loose weave silks were thermally aged at 125°C for 264 h and the FTIR spectra

were compared with the original unaged sample as shown in figure 4d. Again, the changes in colour and hand-feel indicated that structural changes should have happened to the silk fabric; however, qualitative observation does not reveal these structural changes.

The carbonyl region ($1775\text{--}1700\text{ cm}^{-1}$) and tyrosine region ($850\text{--}835\text{ cm}^{-1}$) are important indicators of the degradation of silk, as introduced in [22]. As shown in figure 4e,f, the two FTIR regions were compared for the old library silk and the artificially aged silks. It was found that the UV-aged and thermal-aged silks showed much greater absorbance in the carbonyl region, which means higher oxidation for the artificially aged silks. This observation agrees with that in [22]. For the tyrosine doublet peak region, the UV-aged and thermal-aged silks showed the lowest absorbance, which meant greater oxidation of tyrosine by either UV light or oxidants and heat resulted in more reduction in tyrosine residues. The two regions for our library silk, especially the carbonyl index, are between artificially aged silk and the commercial new silks. This suggests the library silk in this study is less degraded than the three artificially aged silks.

The three amide regions, amide I ($1700\text{--}1600\text{ cm}^{-1}$), amide II ($1600\text{--}1480\text{ cm}^{-1}$) and amide III ($1300\text{--}1200\text{ cm}^{-1}$), are the important regions for analysing the secondary structures of proteins [27]. Peak deconvolution as a quantitative comparison method is often resorted to. We also attempted to perform automatic peak deconvolution for the amide I region using a standard procedure in PeakFit similar to that used in [28]. One example of our peak deconvolution results is shown in electronic supplementary material, figure S1. It is noted that some of the fitting results have poor fitting coefficient r^2 (refer to table 2 and figure 4g). Bearing in mind the quality of the data, the composition of the conformations in different silk could still be compared. The results presented in table 2 and figure 4g suggest: (1) the two raw silk samples from Guangxi and Zhejiang turn out to have very similar secondary structures (57.1% β -sheet, 34.3% coil or helical structure and 6.4% β -turns for Guangxi raw silk; and 58.8% β -sheet, 33.5% coil or helical structure and 6.2% β -turns for Zhejiang raw silk). This information agrees with the thermal stability data. (2) The commercial fine weave silk turned out to possess a notably higher content of β -sheet (65.8%), whereas the UV-aged silk was shown to possess a lower content of β -sheet (42.1%). (3) The secondary structure composition of the thermally aged sample was similar to that of commercial fine silk, without obvious structural deterioration as contrasted to the UV-irradiated silks. It may be further implied that the thermal ageing conditions could induce structural transitions from helix or coil to β -sheet, which could in fact prove that industrial processing may anneal helix or coil structures to more stable β -sheet. To summarize, FTIR measurements can provide valuable structural

information on β -sheet crystalline content through quantitative peak deconvolution. UV-ageing and thermal processing appeared to have opposite effect on silk structure, i.e. destructing or stabilizing the crystalline structure of silk.

3.3. Glass transitions in silks using dynamic mechanical thermal analysis

As revealed by the FTIR results, the library silk facings bear differences from recent silk fabrics due to two major factors, adhesive coating and ageing. Quantitative FTIR analysis often requires high quality IR spectra, which is difficult to achieve for archaeological samples under ATR mode. For example, Meng [29] reported that ancient Chinese silks from the Han Dynasty (206 BC–220 AD) display only small differences in their IR spectra while those from the Ming (1368–1644 AD) and Qing (1644–1912 AD) dynasties of China showed no significant difference in their IR spectra. Therefore, DMTA combining thermal and mechanical analysis as a complementary analytical tool has become our focal method in understanding the effect in library silk facing materials. The $\tan\delta$ profile reveals the structural relaxations or energy dissipation events (e.g. glass transition) in silk; while the storage modulus reveals the mechanical stiffness of silk at different temperatures.

3.3.1. The effect of provenance of silk

The two bundled silk samples from two different provinces, Zhejiang and Guangxi Provinces, denoted as Zhejiang raw silk and Guangxi raw silk, respectively, were compared using DMTA. Zhejiang Province has been a historical silk production area, and Guangxi Province has become the newly developed silk production area (2000 km away from Zhejiang) as China politically shifts the agricultural economy to the western region of the country. As shown in figure 5a, the $\tan\delta$ profile of Zhejiang silk shows the same typical structural features as that reported for *B. mori* single fibre [30], which was also from the same province. According to [30], the first three $\tan\delta$ peaks are associated with the following characteristic structural relaxations for silk polymers: (1) silk protein–water complex glass transition at around -60°C ; (2) denaturation and water evaporation from 25°C to 120°C with features of storage modulus and $\tan\delta$ both increasing; (3) silk protein glass transition at above 150°C . Interestingly, the Guangxi raw silk in figure 5a shows one extra structural relaxation event that finishes at temperatures around 0°C . The obvious modulus increase in the two silk samples between 25°C and 100°C occurs complementarily with a small $\tan\delta$ peak, which suggests chain or segmental movements. As we have seen from the TGA results, the silk materials often lose 4–6% water in the same temperature region. The modulus change occurs hand in hand with the water loss. Therefore, one might hypothesize that, as water molecules leave silk, the hydrogen bonds between water and silk are replaced by those between silk chains, as explained in more detail in [30].

Because event (4) coincides with the melting of ice, this $\tan\delta$ peak for Guangxi silk could be attributed to the softening of the ice of bulk water in sericin or the amorphous silk fibroin structures. This suggests that larger fractions of sericin or/and amorphous silk fibroin may be present in Guangxi raw silk. Meanwhile, the slightly higher peak value of $\tan\delta$

Figure 5. (a) Loss tangent $\tan\delta$ profiles of the raw silks from Zhejiang and Guangxi Provinces. Dashed lines are results of automatic peak deconvolutions after baseline removal. Four constitutive peaks are distinguished to correlate with characteristic structural relaxations: (1) silk fibroin–water complex glass transition; (2) dehydration and denaturation; (3) silk fibroin glass transition; (4) bulk water glass transition in hydrated disordered silk structure or sericin structure. (b) Storage modulus profiles of the raw silks from Zhejiang and Guangxi Provinces. Note that the storage modulus is normalized by its value at -100°C .

at the major glass transition event (3) for Guangxi silk means more amorphous structures in this silk, which could also validate the above hypothesis that Guangxi raw silk may possess more disordered structures. From figure 5b, it can be also concluded that Guangxi silk experiences more modulus loss than Zhejiang silk upon raised temperatures.

3.3.2. The effect of processing conditions

Figure 6 shows the DMTA profiles of the commercial loose and fine weave silk fabrics. The loose weave silk fabric shows characteristics similar to the Guangxi silk fibre, despite the smaller changes with increasing temperature. It is also seen that the relaxation event (2) is indicated by a slight increase in storage modulus during 25 – 100°C . More importantly, the main relaxation event (3) of the silk glass transition could not be completed, and the relaxation event (4) occurred at around 5°C . Interestingly, for the commercial fine weave silk, the storage modulus did not show any increase that may be associated with the previous relaxation events (2) and (4). A previous study showed that thermal annealing treatment up to about 120°C effectively removes the lower structural relaxations due to the removal of water

Figure 6. DMTA $\tan\delta$ (a) and storage modulus (b) profiles for two types of new commercial fabrics: loose weave in blue lines and fine weave in red lines.

[30]. Therefore, one explanation could be that the fine weave silk had been processed through high temperature and high mechanical stress which effectively removes water out of silk and therefore all the water-related structural relaxation events (2) and (4) no longer exist.

Moreover, the major glass transition of the commercial fine weave silk occurs at a much lower temperature of just above 100°C. This observation seems to contradict the TGA observation of the highest onset temperature of thermal degradation for commercial fine weave silk. Although the fine weave silk is more thermally stable, under the coupled effects of mechanical stress and thermal ‘stress’, this type of silk appears to be much weaker in the DMTA. We suspect that not only the amorphous structure but also the crystalline structure in commercial fine weave silk may be disrupted through the processing.

3.3.3. Analysing the old library silk facings

Figure 7 compares both $\tan\delta$ and storage modulus profiles of two old library silk facing materials. Both profiles possess features of the three typical relaxation events in silk facings similar to those found in new raw silks. The $\tan\delta$ profile of the gauze silk facing possesses an overall higher value compared to the library fine facing silk. We have repeated the experiment and it proved that $\tan\delta$ indeed had a higher base level. The raised baseline for the gauze silk may be associated with the presence of small molecules such as starch, whose molecular relaxations can be activated at a much lower temperature and contribute as a constant $\tan\delta$ throughout the temperature range. Most interestingly, both

Figure 7. DMTA $\tan\delta$ profiles (a) and storage modulus profiles (b) of old library silk facing materials: gauze silk facing in red lines and fine silk facing in blue lines.

profiles of storage modulus showed a dramatic increase from 50°C to 180°C, where the major glass transition of silk occurs. The storage modulus change indicates the silk at 180°C was twice as stiff as the original silk at -100°C. This phenomenon has not been observed for other silk materials and the magnitude of the storage modulus increase is unusual for a pure silk, which cannot be simply attributed to the water evaporation and protein denaturation. It is therefore proposed that the stiffening effect was caused by the non-silk adhesive substance in the old silk facings. One proposal could be starch, given its crystallization effect at temperatures above its T_g [31,32]. We will further discuss this possible adhesive effect in the next section.

3.3.4. The effect of artificial ageing and protective coating

As shown above, the old silk facings possess features of crystallization in the DMTA storage modulus profiles, which are absent in all the new silk materials including raw silk and commercial silk fabrics. The obvious differences are proposed to come from the adhesive coating used to attach the silk facing to the manuscript or ageing. In order to separate these effects, two common adhesives (rice starch and pearl glue) were first applied to the commercial loose weave silk, and the coated silks were put in the oven to stabilize before further treatments. Secondly, artificial ageing of both UV-radiation and thermal ageing as introduced in §2.1 were applied to age pure and coated silks.

Figure 8. DMTA profiles of storage modulus (solid lines) and $\tan\delta$ (dashed lines) illustrating the effects of coating and ageing on silk samples (a) aged and unaged pure silks without coating; (b) aged and unaged silks with starch coating; (c) aged and unaged silks with pearl glue coating and (d) aged and unaged silks with mixture glue coating of starch and pearl glue.

Figure 8a compares the storage modulus and $\tan\delta$ profiles of silk fabric before and after artificial UV-ageing. The $\tan\delta$ of aged silk fabric shifted to a higher base level (indicator of greater local molecular mobility), and for the main glass transition at approximately 220°C, the peak $\tan\delta$ increased significantly from 0.15 to 0.22, clearly indicating an increase in the glass transition. The amount of amorphous/disordered structures in silk is proportional to the area under the main T_g $\tan\delta$ peak [33]. The larger $\tan\delta$ peak suggests a greater amount of amorphous/disordered structures in the aged silk. It may suggest that UV-ageing caused degradation of β -sheet crystalline structure. The decrease in crystallinity from the FTIR deconvolutional analysis also agrees well with the increase in glass transition of the amorphous structure after UV-ageing. Moreover, the less increase in the storage modulus of aged silk from 50°C to 120°C suggests that a water-evaporation-induced denaturation event (2) is marginal. This could mean that the aged silk already lost its free water during ageing and that there may be less water in aged silk.

Figure 8b shows the differences caused by ageing for the rice starch-coated silk fabrics. The profiles of storage modulus and $\tan\delta$ before and after ageing are similar, but both diverged from those of uncoated silk fabric. The $\tan\delta$ profiles of the coated silk fabric possess a higher baseline, which is indicative of the wide spread of molecular motions from amorphous starch. Rice starch is also reported to possess small molecules such as lipids [34], whose molecular motions can be activated at a much lower temperature than the melting temperature ($T_g \sim -40^\circ\text{C}$ and $T_m \sim 60^\circ\text{C}$ [35]). On the other hand, the storage modulus increased from 50°C to 180°C, which is similar to the library silk and more dramatic than that of uncoated silk fabric. The storage modulus of the

starch-coated fabric became 1.3 times greater at above 180°C than the modulus at -100°C . Clearly, dehydration and crystallization of starch contribute to this storage modulus increment. This observation adds evidence to the above hypothesis that the old library silk facings were initially coated with starch or similar adhesives.

Compared to figure 8a, the pearl glue-coated silk fabrics before and after ageing in figure 8c showed similar structural relaxations to that of pure silk: (1) water–protein glass transition, (2) water evaporation and denaturation, and (3) glass transition of silk fibroin. It is noted that $\tan\delta$ for the -60°C water–protein glass transition was smaller for pearl glue-coated silk fabric, which may suggest that pearl glue suppresses the interaction between water and silk fibroin. Moreover, the main T_g peak of $\tan\delta$ of the aged coated silk only increased marginally, denoting a possible protection role of pearl glue on silk from ageing.

Figure 8d is important as it shows the coupled effect of mixture coating and ageing. It has been shown that ageing can induce the formation of disorder in silk fabric as seen in figure 8a. Although coatings can protect silk from direct contact with the ageing mediums, it may introduce other effects such as a dramatic increase in the stiffness/modulus of the silk, as happened with the rice starch-coated silk. We observed that the mixture coating of rice starch and pearl glue appears to have avoided the negative effects of undesirable stiffening and to have protected the core silk from structural deterioration. This may point to a new method for effectively protecting silk or other protein materials in the conservation. Our assessments on ageing and coating of silk materials suggest that DMTA can be deployed to analyse archaeological silks and to investigate routes for protecting precious silk relics.

Figure 9. Comparing glass transition temperatures T_g (defined by the peak temperature of $\tan\delta$) and the peak value of $\tan\delta$, for the various types of silks. (Online version in colour.)

3.4. General discussion

To further compare the glass transition behaviours of the different types of silks, the glass transition temperature T_g defined by the peak temperature of $\tan\delta$, and the peak value of $\tan\delta$, are extracted from the DMTA profiles and plotted in figure 9. As mentioned above, T_g is a measure of the molecular interactions in the amorphous region of silk, and $\tan\delta$ can indicate the quantity of the amorphous structure in silk. Figure 9 shows clearly that both library silks have lower T_g compared with untreated new silk (unaged silk); and all the UV-ageing processes resulted in higher T_g . We suggest here simulated UV-ageing causes an increase in T_g by molecular cross-linking, as proved by other studies; and the natural ageing process in low-light and low-UV environment does not cause an increase in T_g . Simultaneously, UV-ageing also induced increased amount of amorphous structure in silk if the silk was not protected by any coating material (comparing unaged and UV-aged). Here the FTIR results suggested that the crystalline content was also reduced by UV-ageing. However, the natural ageing process did not seem to induce much increase in the peak $\tan\delta$. As we also show in the electronic supplementary material, figure S2, the XRD patterns of the library silk closely resembled the unaged silk but not the UV-aged silk. This suggests that UV-ageing cannot simulate the ageing mechanisms in natural ageing in a hundred years' scale. Our results show that natural ageing mainly weakens the molecular interactions in the amorphous silk, but does not appear to induce more amorphous structure (i.e. turning β -crystalline regions to amorphous).

4. Summary

This work was initiated to evaluate the structural changes in the silk facing materials from twentieth century in collections from the British Library. In order to understand the relevant effects in the conservation of silks, we selected a series of

silks, and used three analytical methods, including TGA, Fourier-transformed infrared in attenuated reflection mode (FTIR-ATR) and DMTA. TGA results showed that ageing can reduce the thermal stability of silk materials; whereas industrial processing methods (e.g. those to produce commercial fine silk fabrics) can reduce the water content and increase the thermal stability of silk materials. Qualitative and quantitative FTIR-ATR analyses on the content of β -sheet crystalline conformation suggested that UV ageing can induce reduction in crystalline order, and that the structural degradation of library silk facing including loss of ordered crystalline structure can be attributed to similar ageing mechanisms.

We developed DMTA analysis methods to help with this conservation study on archaeological silks, and we uncovered subtle structural differences in museum silk materials. Different effects (including provenance, processing methods, ageing and coating effects) can be distinguished through analysing the thermo-mechanical properties and thermally induced structural transitions, especially glass transition. Taken together, the measurements and analysis allow us to identify the adhesive coating as starch in the British Library's silk facing materials with the ageing process weakening the amorphous structure without increasing the amorphous structure content compared to recent silks. This structural information is useful not only for conservators but also for materials scientists. Last but not the least, our work also provides a thermo-mechanical property reference for a range of silk materials, thus adding to our knowledge on understanding invaluable textiles in the museum collections and the long-term conservation strategies for cultural heritage.

Data accessibility. All essential data have been included in the main manuscript. Supporting information is available containing two figures: electronic supplementary material, figure S1 of XRD results; electronic supplementary material, figure S2 of FTIR peak deconvolution example. Other data will be available upon request.

Authors' contributions. J.W. and F.V. conceived the research; J.W. collected and prepared all the silk fabric samples; J.G., J.W. and N.H.

conducted all the experiments and analysed the data; J.G., J.W. and F.V. wrote and revised the manuscript.

Competing interests. We declare we have no competing interests.

Funding. J.G. thanks the National Natural Science Foundation of China (51503009) and the 'Zhuoyue' Associate Professor Program of Beihang

University for financial support. F.V. and N.H. thank the AFOSR (FA9550-15-1-0264) for generous funding.

Acknowledgements. J.W. thanks specially the Queen's University Belfast for sponsoring the research and the British Library for kindly offering the conservation materials.

References

- Feng Z. 1993 *中国书画装裱* Zhongguo shu Huang zhuangbiao. Shandong Science and Technology Publishing House.
- Wood FBM. 2010 *The diamond sutra: the story of the world's earliest dated printed book*. London, UK: British Library.
- Hopkirk P. 1984 *Foreign devils on the silk road: the search for the lost cities and treasures of Chinese Central Asia*. Amherst, MA: University of Massachusetts Press.
- Cohen M, Brandt A. 1998 *Conservation des manuscrits de Dunhuang et d'Asie Centrale*. Paris, France: Bibliothèque nationale de France.
- Whitfield S. 2002 *Dunhuang manuscript forgeries*. London, UK: British Library Document Supply Centre.
- Feller RL. 1995 *Accelerated aging: photochemical and thermal aspects*. Los Angeles, CA: Getty Conservation Institute.
- Appel WD, Jessup DA. 1935 Accelerated aging test for weighted silk. *J. Res. Natl. Bureau Stand.* **15**, 601–608. (doi:10.6028/jres.015.046)
- Hersh SP, Tucker PA, Becker MA. 1989 Characterization of historical and artificially aged silk fabrics. *Adv. Chem. Ser.* **1989**, 429–449. (doi:10.1021/ba-1988-0220.ch025)
- Rogério-Candelera A. 2014 *Science, technology and cultural heritage*. Oxford, UK: Taylor & Francis.
- Solazzo C, Dyer JM, Deb-Choudhury S, Clerens S, Wyeth P. 2012 Proteomic profiling of the photo-oxidation of silk fibroin: implications for historic tin-weighted silk. *Photochem. Photobiol.* **88**, 1217–1226. (doi:10.1111/j.1751-1097.2012.01167.x)
- Garside P, Wyeth P, Zhang X. 2010 Understanding the ageing behaviour of nineteenth and twentieth century tin-weighted silks. *J. Inst. Conserv.* **33**, 179–193. (doi:10.1080/19455224.2010.501293)
- Koperska MA, Pawcenis D, Bagniak J, Zaitz MM, Lojewski T, Lojesska J. 2014 Degradation markers of fibroin in silk through infrared spectroscopy. *Polymer Degrad. Stab.* **105**, 185–196. (doi:10.1016/j.polymdegradstab.2014.04.008)
- Kojthung A, Meesilpa P, Sudatis B, Treeratanapiboon L, Udomsangpetch R, Oonkhanond B. 2008 Effects of gamma radiation on biodegradation of *Bombyx mori* silk fibroin. *Int. Biodeterior. Biodegrad.* **62**, 487–490. (doi:10.1016/j.ibiod.2007.12.012)
- Prasong S, Yaowalak S, Wilaiwan S. 2009 Characteristics of silk fiber with and without sericin component: a comparison between *Bombyx mori* and *Philosamia ricini* silks. *Pakistan J. Biol. Sci.* **12**, 872–876. (doi:10.3923/pjbs.2009.872.876)
- Surewicz WK, Mantsch HH, Chapman D. 1993 Determination of protein secondary structure by Fourier transform infrared spectroscopy: a critical assessment. *Biochemistry* **32**, 389–394. (doi:10.1021/bi00053a001)
- Royall PG, Huang C-Y, Tang S-WJ, Duncan J, Van-de-Velde G, Brown MB. 2005 The development of DMA for the detection of amorphous content in pharmaceutical powdered materials. *Int. J. Pharm.* **301**, 181–191. (doi:10.1016/j.ijpharm.2005.05.015)
- Gearing J, Malik KP, Matejtschuk P. 2010 Use of dynamic mechanical analysis (DMA) to determine critical transition temperatures in frozen biomaterials intended for lyophilization. *Cryobiology* **61**, 27–32. (doi:10.1016/j.cryobiol.2010.04.002)
- Castillo FY, Socher R, Krause B, Headrick R, Grady BP, Prada-Silvy R, Pötschke P. 2011 Electrical, mechanical, and glass transition behavior of polycarbonate-based nanocomposites with different multi-walled carbon nanotubes. *Polymer* **52**, 3835–3845. (doi:10.1016/j.polymer.2011.06.018)
- Cucos A, Miu BL, Mitrea S, Sbarcea G. 2011 Dynamic mechanical analysis (DMA) of new and historical parchments and leathers: correlations with DSC and XRD. *Thermochim. Acta* **516**, 19–28. (doi:10.1016/j.tca.2011.01.006)
- Budrugaec P, Carsote C, Miu L. 2016 Application of thermal analysis methods for damage assessment of leather in an old military coat belonging to the history museum of Braşov—Romania. *J. Therm. Anal. Calorim.* **24**, 1255–1266.
- Budrugaec P, Cucos A, Miu L. 2011 The use of thermal analysis methods for authentication and conservation state determination of historical and/or cultural objects manufactured from leather. *J. Therm. Anal. Calorim.* **104**, 439–450. (doi:10.1007/s10973-010-1183-0)
- Vilaplana F, Nilsson J, Sommer DVP, Karlsson S. 2015 Analytical markers for silk degradation: comparing historic silk and silk artificially aged in different environments. *Anal. Bioanal. Chem.* **407**, 1433–1449. (doi:10.1007/s00216-014-8361-z)
- Zhang XM, Berghe IV, Wyeth P. 2011 Heat and moisture promoted deterioration of raw silk estimated by amino acid analysis. *J. Cult. Herit.* **12**, 408–411. (doi:10.1016/j.culher.2011.03.002)
- Teramoto H, Miyazawa M. 2005 Molecular orientation behavior of silk sericin film as revealed by ATR infrared spectroscopy. *Biomacromolecules* **6**, 2049–2057. (doi:10.1021/bm0500547)
- McLaren AD et al. 2014 *Photochemistry of proteins and nucleic acids: international series of monographs on pure and applied biology: modern trends in physiological sciences*. Oxford, UK: Elsevier Science.
- Taddei P, Monti P. 2005 Vibrational infrared conformational studies of model peptides representing the semicrystalline domains of *Bombyx mori* silk fibroin. *Biopolymers* **78**, 249–258. (doi:10.1002/bip.20275)
- Miyazawa T, Shimanouchi T, Mizushima S-I. 1958 Normal vibrations of N-methylacetamide. *J. Chem. Phys.* **29**, 611–616. (doi:10.1063/1.1744547)
- Hu X, Kaplan D, Cebe P. 2006 β -sheet crystallinity in fibrous proteins by thermal analysis and infrared spectroscopy. *Macromolecules* **39**, 6161–6170. (doi:10.1021/ma0610109)
- Meng Y. 2013 *Research on degradation characteristics of the silk in Xi Han dynasties*. Suzhou, China: Soochow University.
- Guan J, Porter D, Vollrath F. 2013 Thermally induced changes in dynamic mechanical properties of native silks. *Biomacromolecules* **14**, 930–937. (doi:10.1021/bm400012k)
- Jouppila K, Roos YH. 1997 The physical state of amorphous corn starch and its impact on crystallization. *Carbohydr. Polym.* **32**, 95–104. (doi:10.1016/S0144-8617(96)00175-0)
- Zavareze EDR, Dias ARG. 2011 Impact of heat-moisture treatment and annealing in starches: a review. *Carbohydr. Polym.* **83**, 317–328. (doi:10.1016/j.carbpol.2010.08.064)
- Guan J, Wang Y, Mortimer B, Holland C, Shao Z, Porter D, Vollrath F. 2016 Glass transitions in native silk fibres studied by dynamic mechanical thermal analysis. *Soft Matter* **12**, 5926–5936. (doi:10.1039/C6SM00019C)
- Ashogbon AO, Akintayo TE. 2012 Isolation, composition, morphological and pasting properties of starches from rice cultivars grown in Nigeria. *Starch-Starke* **64**, 181–187. (doi:10.1002/star.201100044)
- Shalaeve EY, Steponkus PL. 2003 Glass transition of a synthetic phospholipid in the lamellar phase. *J. Phys. Chem. B* **107**, 8734–8737. (doi:10.1021/jp0303265)