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# Crystallinity and degradation of silk: correlations between analytical signatures and physical condition on ageing

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**ABSTRACT** Adequately characterising the physical condition of historic textiles, and understanding the microstructural changes that occur in these materials, is essential when considering appropriate conservation, display and storage strategies. Our work has concentrated on developing non-destructive or micro-destructive methodologies that will permit this for one of the most important historic fibres, silk. We have been able to demonstrate that correlations can be drawn between the physical deterioration of silk samples and certain measurable spectroscopic, chromatographic and chemical signatures. Understanding the way in which these signatures arise then allows the microstructural changes within the crystalline and amorphous content of the fibres to be investigated and more fully interpreted. The techniques developed by our group and by other researchers in the field include polarised FTIR-ATR (Pol-ATR) and near infrared (NIR) spectroscopy, HPLC microsampling analyses and pH measurements. The results of these analyses correlate to measurable mechanical properties and thus suggest that the physical state of historic silk fabrics might be adequately characterised for conservation purposes by such indirect micromethodology.

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## 1 Introduction

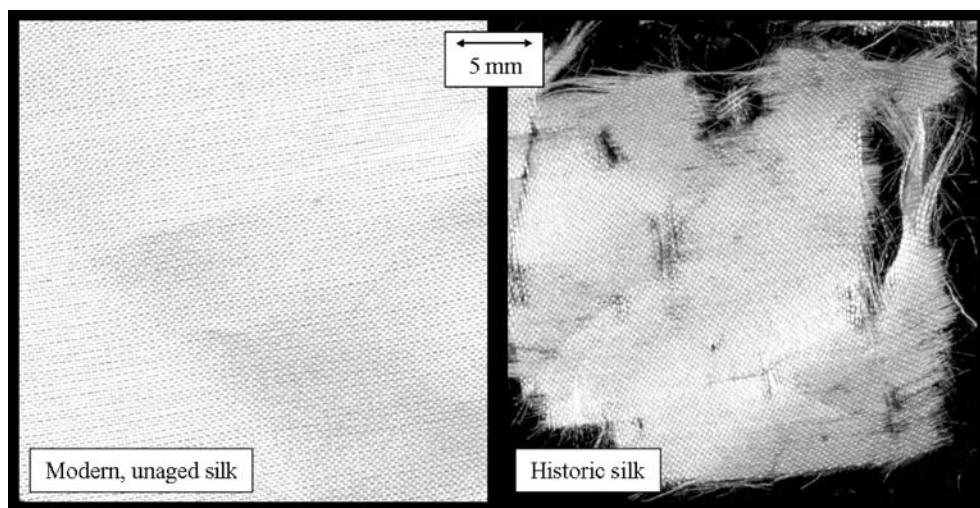
Silk fibres are found in many important historic textiles and artefacts. In order to determine suitable approaches to the treatment, display and storage of these items, it is important to be able to characterise the physical and chemical state of the fibres. Simple assessments of a textile, such as microscopic analysis, chemical stain tests or determination of mechanical properties, have a variety of disadvantages: they either provide only limited, qualitative information or they require large samples, which is ethically unacceptable. Ideally the condition of the silk might be quantified indirectly, by techniques which yield signatures that correlate with mechanical properties. The aim of our research is to develop either a micro-sampling or, preferably, completely non-invasive methodology that would yield the necessary information on

the mechanical and structural condition of the fibres. This, in turn, would enable us to gain a greater understanding of the microstructural changes that occur within the crystalline and amorphous regions of the polymer as the material deteriorates over time.

Silk is a proteinaceous fibre, produced by the larvae of the domestic silk worm, *Bombyx mori*, to form a cocoon during pupation. In its natural state, the fibre consists of two filaments of the protein fibroin, which are highly crystalline and roughly triangular in cross-section, bound together by a second, principally amorphous protein, sericin; in commercial use, the sericin is normally removed (via 'degumming'), leaving the fibroin filaments. Fibroin is largely formed from a hexapeptide repeat motif (-Gly-Ala-Gly-Ala-Gly-Ser-), folded into anti-parallel  $\beta$ -sheets, which aggregate into crystallites (of the order of 10 nm in diameter). These  $\beta$ -crystallites are strongly aligned with the axis of the fibre, an orientation that is imposed during the extrusion of the filaments, and are embedded in an amorphous matrix formed from the remainder of the protein, which is rich in residues with bulky, polar side-chains. The extensive crystallinity of silk dictates its physical and chemical properties, such as high strength, high resistance to chemicals and micro-organisms, and low elasticity and extensibility. When the fibres undergo degradation, these reactions normally proceed from the readily accessible, poorly ordered amorphous regions, which are rich in reactive residues; this continues until the matrix deteriorates to the point that it no longer consolidates the crystallites, which consequently begin to lose their exquisite alignment, and the material fails. Figure\*Fig. 1 shows typical examples of modern, pristine silk and historical 'shattered' silk). Only in the final stages of degradation does damage proceed to the crystallites themselves. Therefore, an understanding of the current degree of crystallinity and the crystalline alignment of a silk sample will provide a valuable insight into its condition and its likely future behaviour.

There are a variety of ways in which this may be achieved. The ideal method of probing the crystallinity of a fibre may be via X-ray diffraction experiments, as these allow a direct observation of the extent and orientation of the long-range ordering of the polymer. However, this approach has the disadvantages that the results are not necessarily easy to interpret and access to the requisite equipment is likely to be limited; therefore, a more readily available and useable technique may

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**FIGURE 1** Examples of (a) modern, unaged silk and (b) historic silk, showing characteristic signs of 'shattering'

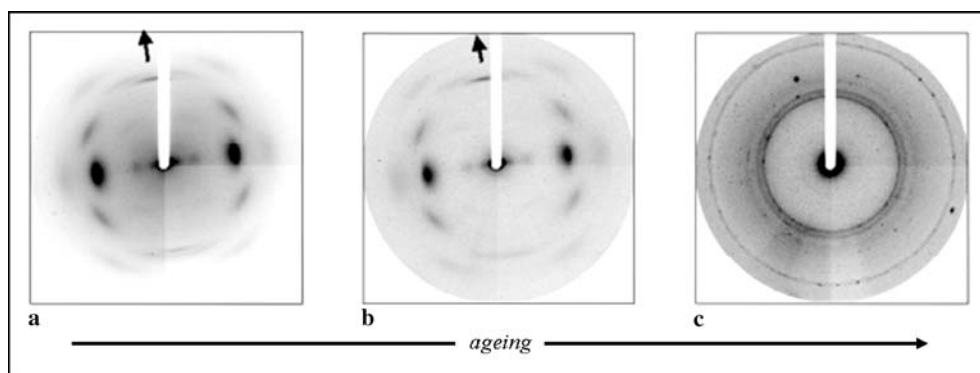
be appropriate for everyday use within the field of conservation. Similar results to the X-ray experiments can be achieved with polarised infrared spectroscopy, particularly using the attenuated total reflectance (ATR) sampling technique, which allows the degree and alignment of crystalline components within the material to be assessed. Other methods can be used to complement this type of analysis: liquid chromatography using size-exclusion techniques, as developed by the 'Monitoring of Damage to Historic Tapestries' project [1–4], can be employed to give an indication of the range of lengths of polymer chain in the sample, and thus the manner in which the degree of polymerisation changes with deterioration; by examining the distribution of the chain fragments. It is also possible to identify the presence of stable crystallites and susceptible amorphous regions. Recent research has also shown that simple pH determination gives an indication of the state of the fibres, with preferential degradation in the amorphous component perhaps allowing the acidic side-chains found in this region to become more accessible.

The validity of all of these approaches to measure the extent of crystallinity, as a marker for the state of silk fibres, is supported by demonstrating that each of these methods provides a good correlation to mechanical properties such as tensile strength. This suggests that the condition of historic silk fabrics might be adequately characterised for conservation purposes by such micromethodologies.

## 2 Methodology

### 2.1 X-ray diffraction

X-ray diffraction studies of modern and archaeological silk samples highlight microstructural changes occurring within the fibres as the materials degrade [5, 6]. Silk samples from Tang dynasty silk artefacts, from the Famen Temple (near Xian, China) were assessed by Greiff et al. [5]; these materials covered a range of states of deterioration, representing a progressive loss in structural integrity. Synchrotron radiation microdiffraction experiments carried out at the European Synchrotron Radiation Facility on both single fibres and on bulk fabric samples yielded a set of diffraction patterns that correlated well with the observed state of the materials (see Fig. 2 for the patterns for the fabric samples). These patterns indicate the ordering within a material, with the intensity, spacing and orientation of diffraction spots signalling the extent and arrangement of crystalline components; amorphous regions produce no signal. The data for the pristine, modern material reveals a sharp, strong pattern, arising from the 210 reflection, corresponding to the known structure of the silk fibres, with extensive  $\beta$ -crystallites held in strong alignment with the fibre axis. As the material deteriorates, the initial changes in the diffraction pattern are subtle, as exemplified by the differences between Fig. 2a and b, with the major reflections broadening slightly, but generally retaining their position and orientation, reflecting a slight loss in orientation



**FIGURE 2** X-ray diffraction patterns derived from a succession of historically aged fibres, demonstrating the manner in which these patterns change with progressively more degraded materials (for (c), while there is still significant crystallinity, the crystallites have become disordered, producing the ring patterns); reproduced from [5]

of the crystallites but no dramatic alteration in microstructure. The patterns will become progressively less well defined as more degraded materials are considered, until, with the most heavily deteriorated sample (Fig. 2c), the signal spreads out into a continuous, poorly defined, hazy contour, indicating the continued presence of the crystalline domains (bands are observed at the same distance from the center as before), but their almost complete loss of ordering (orientational information has been lost, with sharply defined points broadening to uniform rings). A greater subtlety of information can be achieved using wide angle X-ray scattering (WAXS) imaging [6], which can monitor the deterioration within a fabric as a measure of the orientation distribution of the fibroin crystallites within silk fibrils and the macroscopic orientation of the fibres, which themselves may break upon embrittlement.

This data suggests that the crystalline regions in the silk protein polymer remain largely intact, even if degradation processes have already deprived the material of its physical integrity, with damage and chain scission occurring preferentially in the amorphous component. It is only during the ultimate stages of deterioration that the crystallites are also affected.

## 2.2 Polarised ATR spectroscopy

It is possible to monitor the way in which the arrangement of the crystalline and amorphous components change with deterioration using polarised ATR (Pol-ATR) spectroscopy. Polarised infra-red techniques have been shown to be well suited to the investigation of long-range ordering in polymers, and the ATR technique requires just a few fibres or a short section of yarn for analysis. Building on earlier work carried out using Pol-ATR, in which the use of the technique in investigating the crystallinity and microstructure of cellulosic fibres has been shown [7, 8], a method was developed to assess the degree of orientation of crystalline fibroin in silk fibres [9]. By recording pairs of spectra with the fibre aligned parallel with and then perpendicular to the direction of polarisation of the incident radiation, it is possible to monitor how the orientation of the crystallites changes over time. It can be shown that as the fibres deteriorate, these crystalline regions lose their strong alignment with the fibre axis and become increasingly more disordered, due to the degradation of the amorphous component that constrains them; however, the crystals themselves do not appear to exhibit deterioration to any great extent.

Spectra of silk samples were initially recorded without a polariser; pairs of spectra were then recorded with an infra-red polariser placed in the beam path before the specimen (aligned such that the electric vector of the radiation was perpendicular to the beam path), as noted above. The samples themselves were prepared using a range of historically accurate methods, and subjected to artificial ageing by means of either elevated temperature or intense illumination with simulated sunlight, to yield a range of specimens reflecting the variety of conditions encountered by historic textiles.

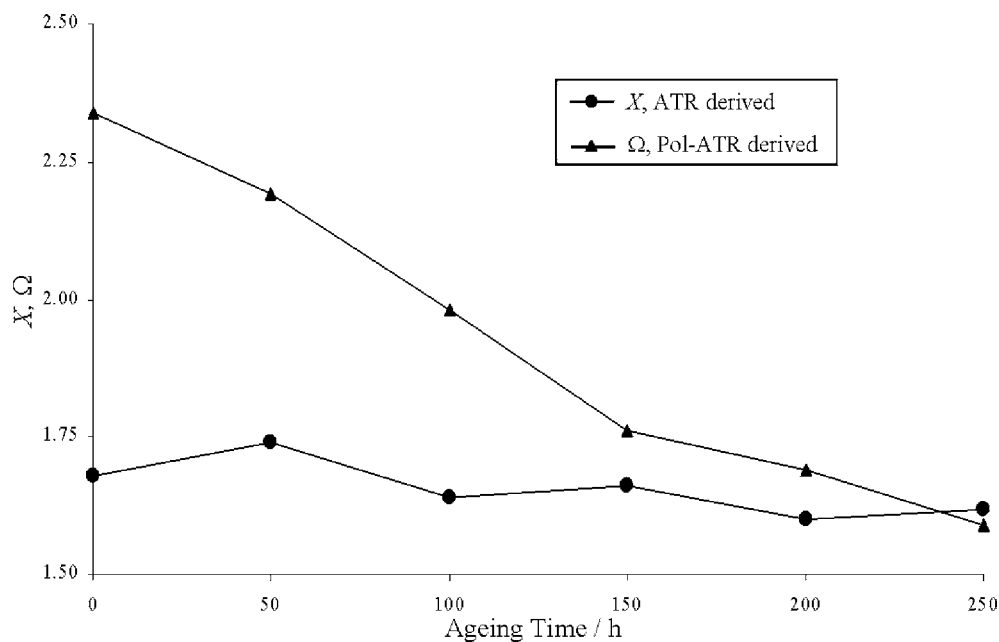
A crystallinity index,  $X$ , can be defined as the ratio of directly measured intensities within the amide I band, at points corresponding to the theoretical maxima for the  $\beta$ -sheet ( $1615\text{ cm}^{-1}$ ) and  $\alpha$ -helix/random coil ( $1655\text{ cm}^{-1}$ ) motifs,

above a baseline drawn from  $1730$  to  $865\text{ cm}^{-1}$ , giving the intensities  $I_\beta$  and  $I_\alpha$  respectively:  $X = I_\beta/I_\alpha$ . These values can then be derived from both conventional ATR spectra, and from pairs of polarised spectra, recorded with an infra-red polariser placed in the beam path before the specimen (aligned such that the electric vector of the radiation was perpendicular to the beam path), as noted above. The data from the pairs of polarised spectra are then used to provide an orientational order parameter,  $\Omega$ , defined as the ratio of the spectral crystallinity indices for the fibres aligned perpendicular ( $X_{90^\circ}$ ) and parallel ( $X_{0^\circ}$ ) to the incident electric vector:  $\Omega = X_{90^\circ}/X_{0^\circ}$ . This value reflects how well the  $\beta$ -crystallites of fibroin are aligned to the fibre axis, with higher values reflecting a greater degree of alignment. Calculated values for  $X$  (derived from the conventional ATR spectra) and  $\Omega$  (from the Pol-ATR spectra) for a typical sample, show that whilst  $X$  varies little over the course of the ageing process,  $\Omega$  diminishes as shown in Fig. 3 (for a complete loss of crystallite order,  $\Omega = 1$ ). This suggests that the overall ratio of crystalline to amorphous material does not change greatly as the fibres deteriorate, but instead the orientation constraint imposed on the embedded  $\beta$ -crystallites is relaxed and the crystallites can 'drift' away from the initially strong alignment with the fibre axis. This probably results from the loss of integrity of the amorphous matrix which would normally constrain such movement.

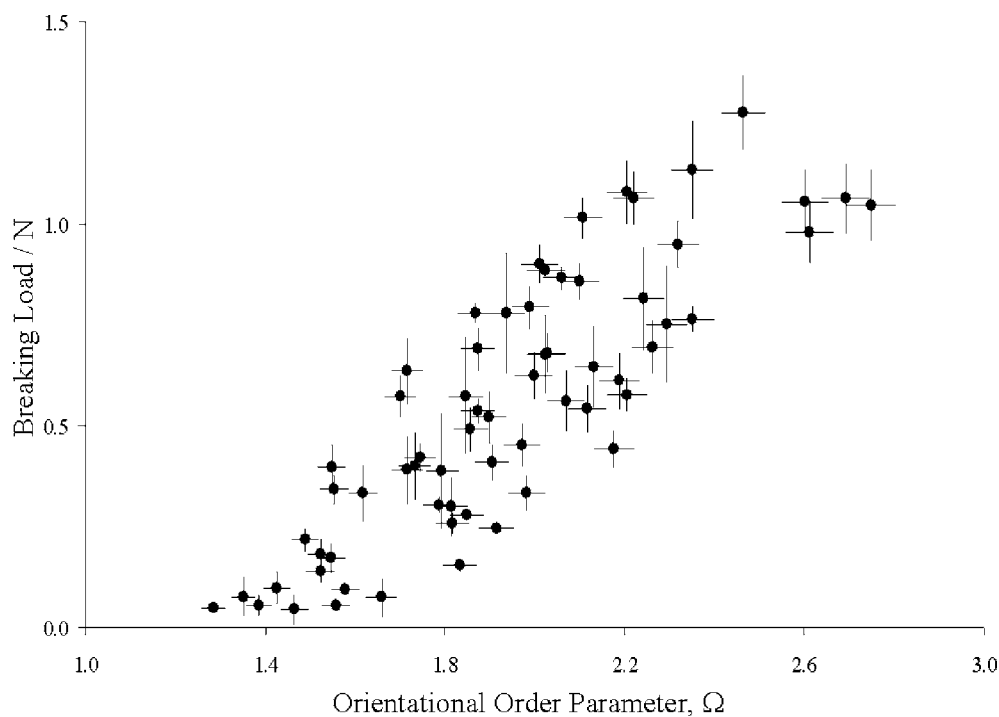
To complement these values, the breaking strengths of yarns from these samples were also measured. When the orientational order parameter,  $\Omega$ , was plotted against breaking strength (Fig. 4), it became apparent that there is a good correlation, with  $\Omega$  decreasing as the fibres age and become physically weakened. The progressive disorientation of the crystalline domains with ageing has been established through X-ray analysis; the Pol-ATR experiment confirms this, but is of particular use due to the ease with which the technique may be used to rapidly assess a large set of samples, thus allowing a more comprehensive analysis of the data, such as the demonstration of a direct relationship between the loss of crystallite ordering and the loss of the mechanical integrity of the fibre.

## 2.3 Liquid chromatography

The development of liquid chromatographic techniques for assessing the state of silk fibres was a fundamental aspect of the 'Monitoring of Damage to Historic Tapestries' (MODHT) project, based at the Textile Conservation Studio at Hampton Court Palace [1–4]. This approach employs size exclusion chromatography (SEC), a well-established technique for investigating the extent of polymer degradation; samples are characterised on the basis of the size (hydrodynamic volume) of the polymer chain fragments, a property which is related to their molecular weight. More massive molecules are retained less efficiently on the chromatographic column, and are thus eluted relatively rapidly, with a low retention time ( $t_R$ ); smaller molecules however, remain trapped on the column for longer, and so elute at higher retention times. Thus, when suitably calibrated, the technique can provide a molecular weight distribution, which is of value as the average molecular weight decreases as the polymer breaks down. In order to successfully use this method, samples must first be taken into solution, which is potentially problematic for



**FIGURE 3** Calculated values for the apparent crystallinity,  $X$  (derived from the conventional ATR spectra), and the orientational order parameter,  $\Omega$  (from the Pol-ATR spectra), for a typical silk sample, demonstrating that whilst  $X$  varies little over the course of the ageing process,  $\Omega$  diminishes



**FIGURE 4** The correlation between mechanical strength (breaking load) and the spectroscopically derived orientational order parameter,  $\Omega$

silk; it can, however, be achieved using a lithium thiocyanate solution, which acts as a hydrogen bond disruptor, breaking down the secondary structure of the protein but, importantly, not interfering with the primary structure or causing chain scission. In general the more degraded a sample is, the more readily it will go into solution. A Tris-HCl/urea pH 7 solvent system acts as a suitable mobile phase; a detection wavelength of 280 nm is used. Calibration with molecular weight standards (6500 to 2 000 000 Daltons) enables molecular weight averages to be calculated. As the material degrades, the retention time distribution broadens and becomes higher, reflecting a wider but lower polymer size range. This can be successfully correlated with physical prop-

erties such as extension, demonstrating that the technique can be used to monitor the bulk deterioration of silk fabrics. Furthermore, although this approach does require sampling, it has been refined to use as little as 0.2 mg of silk, enabling small specimens to be taken from loose threads or hidden areas, minimising the ethical difficulties associated with sampling.

More recent developments of the technique suggest that relatively stable domains in the molecular weight distribution may be related to the presence of stable crystallites, thus potentially enabling more detailed information on the microstructure of the protein, and the role it plays in the overall integrity of the material, to be determined.

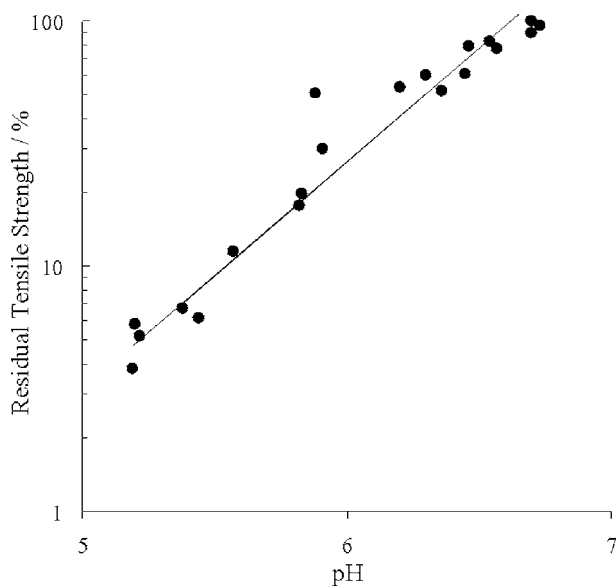


FIGURE 5 Correlation plot of pH of silk extracts versus % residual tensile strength; error bars are omitted for clarity

## 2.4 Acidity measurements

Acidity measurements also give an indication of the way in which the microstructure of the material influences its degradative behaviour [10]. These measurements may be taken by immersing a small sample of the silk in 0.1 M NaCl; the pH of this extract is then measured using a glass electrode. The extraction is carried out in saline solution to allow sodium ions to displace protons in the Donnan equilibrium [11, 12], effectively allowing the equilibration of immobile acid within the solution, and thus permitting a truer measurement of the sample's acidity. If the same quantity of silk was used for each extraction, the pH values may be taken as indicative of the relative acidity of the samples.

When data for a variety of artificially aged silks are compared with the tensile strength of the fabrics, a first order dependence between the two (Fig. 5) is found. Silk is a highly crystalline fibre, and it is the amorphous matrix, rich in the bulkier and acidic and basic amino acids, in which these crystallites are embedded that is particularly susceptible to degradation. Fibroin is relatively rich in acidic residues and has an isoelectric point at pH 2–3 [13]; in its native state these acidic groups may be involved in secondary bonding (hydrogen bonding and salt linkages), supporting the secondary structure of the polymer. However, as the fibres deteriorate, the polymer in this region is broken down, enhancing access and thus allowing a truer measure of the acidity of the protein to be measured than can be achieved with the pristine material.

## 3 Conclusions

The techniques described highlight a variety of ways in which the microstructure and crystallinity of silk fibres may be probed to allow a better understanding of the processes of fibre degradation.

The ATR and Pol-ATR studies demonstrate that the overall crystallinity of the material (as measured by the ratio of the  $\beta$ -sheet component to the randomly oriented component of the

polymer) does not vary greatly as ageing occurs, but that instead the crystallites progressively lose their strong alignment with the fibre axis. Chromatography shows a gradual reduction in the degree of polymerisation over the same timescale, as the polymer chains begin to fragment, but also demonstrate that there are particular components of the chain which are relatively stable and do not suffer scission in the same way; this may be linked to the breakdown of the amorphous component of the fibroin, leaving the crystallites intact. The acidity measurements show that the fibres appear to become steadily more acidic as they deteriorate, which is again associated with the degradation of the amorphous regions exposure of acidic residues. Taken as complementary studies, these results can be used to construct a picture of the way in which silk fibres degrade, with damage and chain scission occurring predominantly in the amorphous component, leaving the  $\beta$ -crystallites relatively unaffected, but compromising the integrity of the fibre as a whole, and causing the highly ordered nature of pristine silk, with its precise ordering of the crystalline regions, to be lost. These results are confirmed by the more involved X-ray diffraction analyses, which indicate that initial crystallite disordering is even effected by the original processing of the silks.

Therefore there exists a range of relatively simple analytical techniques which may be readily employed to gain a greater understanding of the state of silk fibres within an artefact, and to determine how the material is likely to behave in future; this information is of particular value to conservators and curators when considering the most suitable conservation, display, handling and storage strategies.

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