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Using FTIR spectroscopy to detect sericin on historic silk

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Silks represent some of the most precious ancient and historic textile artefacts in collections worldwide. Their optimum preservation demands an appreciation of their characteristics. One important concern, especially with regard to ancient Chinese silks, is whether the fabrics have been degummed. Silks with remnant sericin gum coating the fibroin fibres would require different conservation protocol. In previous research on aged silks, the presence of sericin has been inferred from amino acid analysis of hydrolysates. In the study reported here, the potential of FTIR spectroscopy to provide a simpler and rapid method of detecting sericin on silk has been investigated. Both fibroin and sericin exhibit singular IR absorptions. Attenuated total reflectance spectroscopy was found to highlight the sericin coating more effectively than transmission and reflectance spectroscopy. Three particular peak intensity ratios were identified which might provide a quantitative estimate of the sericin content of new silk, to a sensitivity of 1%–2%. These were also shown to be valid indicators for the presence of sericin on artificially aged and archaeological silks, although quantitation was now not possible. Besides the peak intensity ratios, two signature peaks were also seen to be useful markers for silk fibroin, and their presence in a spectrum could be used to infer a degummed silk.

infrared spectroscopy, attenuated total reflectance spectroscopy, historic silk

1 Introduction

Most textile silk is derived from the domesticated silkworm, *Bombyx mori*. Prior to pupation, the caterpillars make a cocoon from silk bave. The bave consists of two brins, wedge-shaped fibroin filaments (~80%), which are extruded from glands in the head of the worm and cemented together by sericin gum (~20%). (There are also small amounts (~3% in total) of waxes, colourants, polysaccharides and inorganics).

In processing for textile use, the cocoon is softened in water, and the long silk filaments are carefully unwound and then spun lightly into threads. Either as the thread or the woven fabric, silk is usually degummed to improve its sheen, colour, texture and handle. The degumming procedure takes advantage of the distinct properties of the two proteins, i.e., the water soluble nature of sericin, compared with the insolubility of fibrous fibroin.

While most silk textiles have little residual sericin, there are some that are woven and used without degumming. The latter include Chinese Juan, for example, which was a substrate for documents before paper was invented, and is still used in calligraphy and drawing, and also for mounting pictures. Some archaeological silk fibres excavated from tombs in Mongolia [1] and South China [2] have been shown to be sericin rich. Perhaps surprisingly, even the US First Ladies' gowns in the Smithsonian Institution, Washington, appear to have a significant sericin content [3].

For curators and conservators of historic textile collections it is essential that the fabrics are characterized as fully as possible. This enables the most appropriate decisions about display and conservation to be taken, in relation to access and preservation of the artefacts. For example, while

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degummed silks may be wet cleaned to enhance their appearance and release potentially harmful soiling, such an approach would be unsuitable for a sericin-coated silk. Distinguishing untreated and degummed silk is therefore of some importance. Previous work has involved hydrolysis of small samples with subsequent aminoacid analysis by high performance liquid chromatography [1, 2].

A simpler, rapid method for determining the presence of sericin gum on silk would be invaluable. In view of the ethical constraints associated with cultural artefacts, the protocol should ideally involve minimal sampling. We have therefore been investigating the application of Fourier transform infrared spectroscopy in this context. FTIR spectra of model silk fibres with varying sericin content were recorded. Initially the optimum sampling mode was investigated, and transmission, reflectance and attenuated total reflectance were compared. The characteristic absorption bands for sericin and fibroin were confirmed, and subsequently a method for estimating the relative proportions of the two proteins was developed, based upon band intensity ratios. The methodology was refined using artificially aged silks and then tested on two archaeological specimens.

2 Experimental

2.1 Materials

2.1.1 Natural silk

New untreated (cocoon), partially-degummed and fully-degummed *Bombyx mori* silk textiles were obtained from the Suzhou Shanshui Silk Co., China. To determine the sericin contents, the fabric samples (in triplicate) were weighed before and after degumming treatments. The samples (1 g per 100 cm^3 solution) were heated in 0.6% (w/w) $NaHCO_3$ at 88 °C for 40 min, rinsed thoroughly with deionized water and air dried. The samples were conditioned at 50% relative humidity prior to weighing. Degumming was repeated to constant weight. Evaporation of a portion of a degumming solution and subsequent treatment of the residue with deionized water, to release the inorganic salt, produced a sample of sericin gum.

2.1.2 Artificially aged silk

In China, as elsewhere, archaeological textiles are most frequently recovered from very dry or very wet sites, where the conditions favour preservation. Although sericin could easily be leached in a waterlogged burial environment, it may persist under arid conditions, or where the textiles have been stored above ground at intermediate humidity.

Samples of each of the new silks were subjected to sunlight equivalent ageing for one week, to mimic daylight exposure during normal use. Further samples were thermally aged (125 °C) for two weeks to mimic long-term burial under arid conditions, and a third set of silks were subjected to high temperature and high humidity aging (100 °C, 76% RH) for a similar length of time, to reproduce the equivalent of long-term storage at intermediate humidity.

2.1.3 Archaeological silk

Fibres were obtained from two archaeological silks (H1, H2), which are around 2000 years old, excavated from a Han dynasty tomb in Fenghuangshan, Jingzhou, Hubei. It was suggested that one of these specimens was untreated and that the other had been degummed.

2.2 Microstructural and molecular characterization

Short lengths of thread were removed from each of the silk fabrics. Prior to recording transmission and reflectance spectra with an IR microscope, individual silk fibres were teased from the threads and flattened in a diamond compression cell. The fibres were either left on one of the diamond windows, for transmission, or moved to a small gold mirror, for reflection. For attenuated total reflectance a short thread was pressed to the surface of the optical element of an FTIR-ATR accessory; care was taken to apply a consistent pressure each time. A sample of the extracted sericin gum was also analyzed.

IR spectra were recorded over the range of 4000–700 cm^{-1} using a Nicolet Magna-IR 750 spectrometer, fitted with a Nic-Plan IR microscope and ATR-Omni attachment. The 45° single reflection ATR accessory has a germanium element which allows a relative shallow sampling depth (~0.7 μm at 2000 cm^{-1}).

Spectral resolution was 8 cm^{-1} and 64 scans were acquired. Thermo-Galactic Grams AI v8 software was used for spectral processing. Local baselines were taken for calculating peak ratios: 2000–885 cm^{-1} for the intensity ratios I_{1070}/I_{1165} and I_{1650}/I_{1625}, and 1476–1293 cm^{-1} for the intensity ratio I_{1400}/I_{1445}.

3 Results and discussion

The mean sericin contents (%, w/w) of the three silks were as follows: untreated (cocoon) 22 ± 2, partially-degummed 10 ± 1, fully-degummed 0 ± 0.1.

3.1 Natural silk

Transmission and reflectance spectra from a flattened untreated silk fibre and an ATR spectrum from a bundle of such fibres are shown in Figure 1. There are spectral shifts amongst them, most notably a 40 cm^{-1} shift to high wavenumber for the two peaks in the region of 1500–1700 cm^{-1} seen in the reflectance spectrum. Similar shifts have been noted by others [4], though there does not appear to be

an immediate explanation. The quality of the reflectance spectrum is relatively poor. While the transmission spectra are of good quality, the ATR spectra appear to highlight the presence of sericin much better, as illustrated in Figures 2 and 3. The ATR technique is advantageous as it probes to only a shallow depth [5] and so emphasizes any surface coatings. A minor inconvenience is the partial polarization of the infrared beam by the ATR accessory, which necessitates consistent alignment of the silk fibres on the ATR top-plate. In view of these results, subsequently only ATR spectra were recorded.

The distinct amino acid composition of fibroin and sericin together with the consequent difference in secondary structure of these proteins lead to characteristic infrared absorbances (Figure 3).

While fibroin has a high degree of crystallinity due to stacked β-sheets, sericin has a more random structure. This accounts for the differences in the main spectral features which relate to vibrations of the peptide backbone moieties. So sericin has a broad amide A band (3270 cm^{-1}), and amide I and II peaks at 1650 and 1530 cm^{-1} (as opposed to 1625 and 1520 cm^{-1} for fibroin). There is a further signature peak for sericin at 1400 cm^{-1} and a much enhanced absorbance at 1070 cm^{-1}. The prominence of these features may be ascribed to the relatively high content of carboxylic acid and alkyl hydroxyl containing amino acid side chains in sericin (Table 1). Associated water and the raised hydroxyl content together also contribute to the broad absorption at

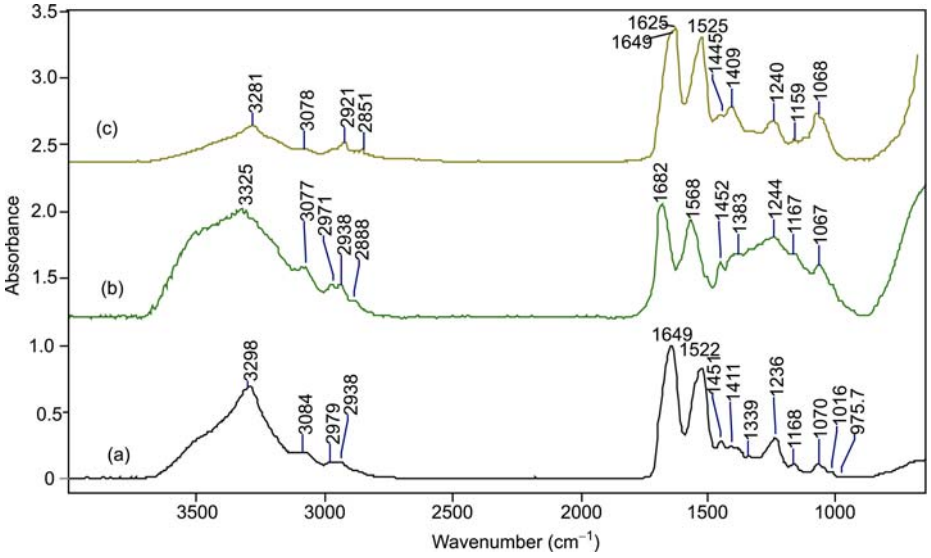

Figure 1 IR spectra of untreated (cocoon) silk: (a) transmission; (b) reflectance; (c) ATR (uncorrected).

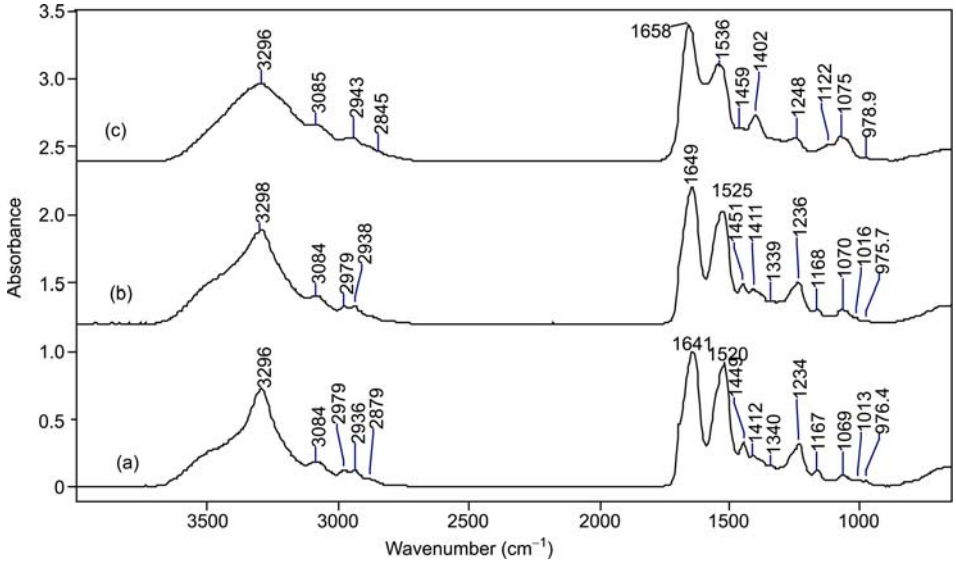

Figure 2 Transmission spectra of (a) degummed silk, (b) untreated silk and (c) sericin. For untreated silk, sericin signature peaks appear at around 1650, 1400, 1070 cm^{-1}, for example.

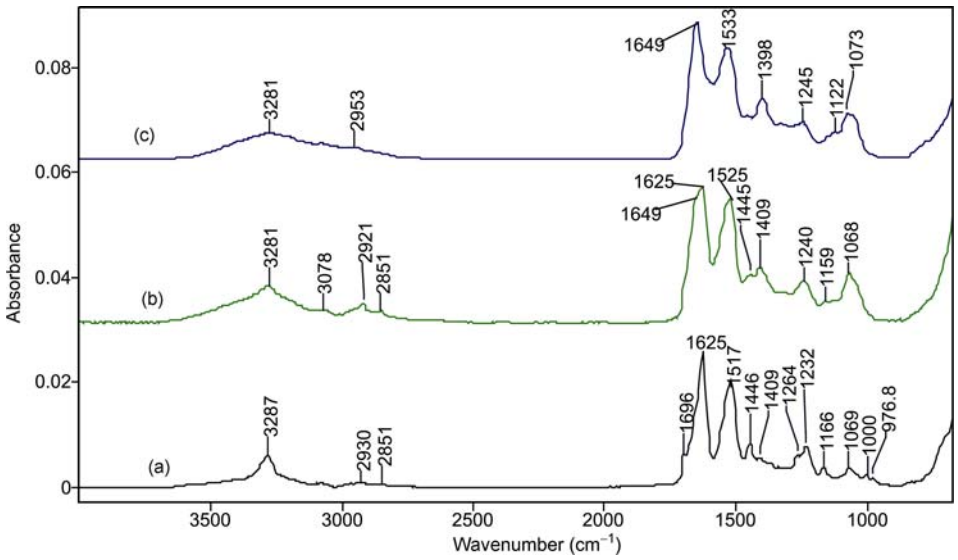

Figure 3 ATR spectra of (a) degummed silk, (b) untreated silk and (c) sericin.

Table 1 Sericin and fibroin: the content of the selected amino acids. Values are quoted as weight % to allow for an approximate content comparison in an equivalent volume [6].

	Fibroin	Sericin
Gly	42.8	8.8
Ala	32.4	4.0
Ser	14.7	30.1
Thr	1.2	8.5
Tyr	11.8	4.9
Asp	1.7	16.8
Glu	1.7	10.1
Lys	0.5	5.5

around 3400 cm^{-1}.

The presence of a sericin coating seems to mask two distinctive fibroin bands at lower wavenumber (Figure 3(a) and (b)). These bands at 1000 and 975 cm^{-1} are, respectively, characteristic of –gly–gly– and –gly–ala– peptide backbone motifs [7]. Their observation in the spectrum of an uncharacterized silk would then suggest that there is little or no sericin coating the fibroin.

In an attempt to find a means of estimating the sericin content of silk by FTIR-ATR, various peak intensity ratios were assessed. The most promising are listed in Table 2, which shows data calculated for the untreated, partially degummed and fully degummed silks. Adjacent fibroin-related peaks were selected for ratio; the relevant 1445 and 1165 cm^{-1} fibroin bands arise from the vibrations of alanine and tyrosine side-chains.

3.2 Artificially aged silk

Since the components of historic silk will undoubtedly have undergone deterioration, aged surrogates were prepared to see if the sericin content could still be assessed as above.

Table 2 Peak intensity ratios calculated from ATR spectra for untreated (cocoon), partially degummed and degummed silk. Values are the averages from 6 spectra; the standard error (se) is quoted.

	Peak intensity ratio		
Sericin (%)	$I_{1650}/I_{1625} \pm$ se	$I_{1400}/I_{1445} \pm$ se	$I_{1070}/I_{1165} \pm$ se
22 (cocoon)	0.88 ± 0.03	2.03 ± 0.26	2.37 ± 0.12
10	0.74 ± 0.01	1.23 ± 0.09	1.26 ± 0.02
0 (fibroin)	0.67 ± 0.01	0.27 ± 0.01	0.89 ± 0.01

All three peak intensity ratios noted in Table 2 seem to be useful quantitative indicators of the sericin content of silk. The experimental error places the detection limit at around 1% to 2% sericin.

Protein aging, with consequent peptide backbone cleavage, side-chain modification and conformational changes in the secondary structure, could affect the peak intensity ratios.

Aging under the variety of conditions has a marked effect on the peak intensity ratios (Table 3 and Figure 4). This seems to preclude quantitative estimation of the sericin content for such material. Nevertheless, it appears that it is still possible to determine whether sericin is present, perhaps the more readily with the last two peak ratios.

3.3 Archaeological silk

The method was used to assess the two archaeological silks (H1, H2) (Figure 5 and Table 4). FTIR-ATR spectroscopy requires compliant fibres, which might restrict its application, but both of these samples were amenable.

The resulting peak intensity ratios (Table 4), however, suggest that there is little if any sericin in either case. For specimen H2 the intensity ratio for the peaks at 1070 and

Table 3 Peak intensity ratios calculated from ATR spectra for artificially aged untreated, partially degummed and degummed silk. Aging conditions: (a) simulated sunlight; (b) 125 °C; (c) 100 °C, 76% RH

Sericin (%)	Aging conditions	Peak intensity ratio		
		I_{1650}/I_{1625}	I_{1400}/I_{1445}	I_{1070}/I_{1165}
22 (cocoon)	none	0.88	2.03	2.37
	(a)	1.24	1.21	1.40
	(b)	0.82	0.88	1.38
	(c)	0.71	1.16	1.83
10	none	0.74	1.23	1.26
	(a)	0.89	1.34	1.52
	(b)	0.87	1.19	1.38
	(c)	0.74	1.15	1.77
0 (fibroin)	none	0.67	0.27	0.89
	(a)	0.80	0.14	0.87
	(b)	0.69	0.16	0.79
	(c)	0.57	0.20	1.04

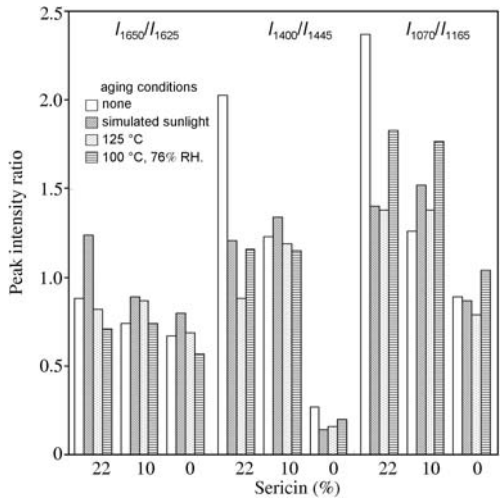

Figure 4 Bar chart presenting peak intensity ratios calculated from ATR spectra for artificially aged untreated silk (22% sericin), partially degummed (10% sericin) and degummed silk (0% sericin).

1165 cm^{-1} appears anomalous. Further detailed spectral analysis confirmed that sericin was not present, but that there were other constituents contributing underlying absorptions. A general multicomponent spectral analytical method has been suggested by Calvini *et al*. [8] which may be appropriate in such circumstances. As confirmation of the absence of a significant sericin coating, the characteristic fibroin bands at 1000 and 975 cm^{-1} were clearly seen in the spectrum of H1. For H2, these were observed as shoulders on a broad band centred at 1050 cm^{-1}.

4 Conclusions

Attenuated total reflectance appears to be the best FTIR spectroscopic method, compared with transmission and reflectance, for detecting a sericin coating on silk. The FTIR-ATR spectrum of sericin gum shows characteristic broad bands at 3400 and 3270 cm^{-1}, and further signature absorptions at 1650, 1530, 1400 and 1070 cm^{-1}, in contrast to silk fibroin which has a quite different primary and secondary structure. The peak intensity ratios I_{1650}/I_{1625}, I_{1400}/I_{1445}, and I_{1070}/I_{1165} are each useful quantitative indicators of the sericin content of processed and unprocessed new silk, permitting an estimation sensitivity of 1%–2% sericin. However, for aged silk, while the presence of sericin may still be inferred from the peak intensity ratios, the amount cannot be readily determined. The analysis of historic silks must be approached with further caution, since additional constituents may give anomalous results, and a consideration of all three intensity ratios is necessary. Besides the peak intensity ratios, the observation of bands at 1000 and 975 cm^{-1} can also offer a useful indicator of the lack of sericin gum, though again extraneous components may interfere.

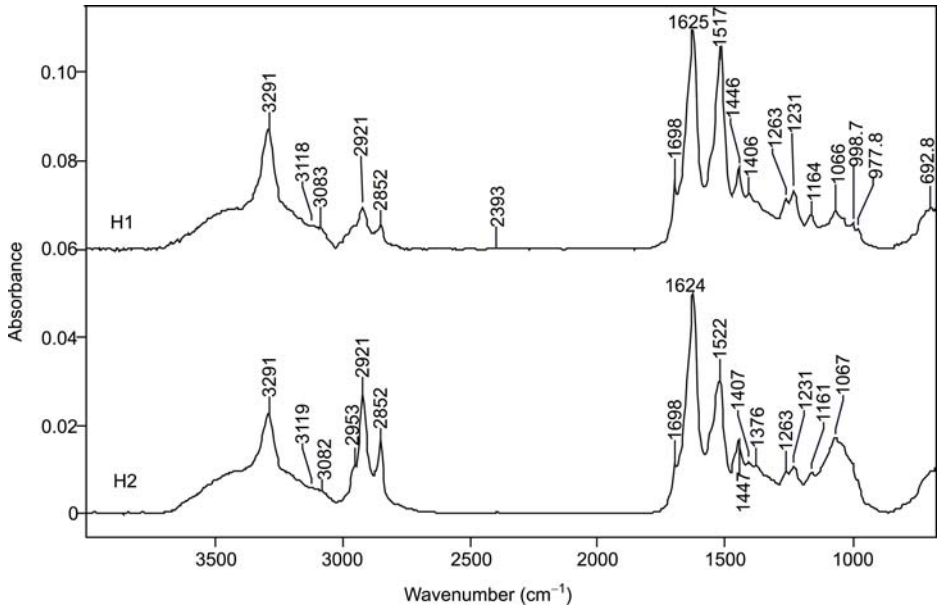

Figure 5 ATR spectra of H1 and H2.

Table 4 Peak intensity ratios calculated from ATR spectra for two historic silks

Silk specimen	Peak intensity ratio		
	I_{1650}/I_{1625}	I_{1400}/I_{1445}	I_{1070}/I_{1165}
H1	0.61	0.33	1.09
H2	0.57	0.40	1.90

FTIR-ATR spectroscopy (especially employing a high refractive index optical element which provides a shallower sampling depth) would seem to be an appropriate rapid method for distinguishing silks that still carry a thin sericin coating and those that have been degummed, in support of textile conservation. A thread just one or two millimetre in length is sufficient for the analysis.

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