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DEVELOPMENT OF SAMPLE PRETREATMENT OF SILK FOR RADIOCARBON DATING

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ABSTRACT. We have developed sample pretreatments for silk for radiocarbon dating. Characteristics of silk under different types of pretreatment were investigated, as well as the behavior of dye and possible contaminants. We found that dye could be removed completely, together with all other foreign materials bigger than 1.2 μm, using a glass microfiber filter after decomposition with 6N HCl. The decomposed proteins were concentrated using Centriprep® ultrafiltration concentrators with 3 different molecular weight cut-offs. By taking a molecular weight fraction—which selects for secondary structures of silk protein—^{14}C dating of silk samples can be made more reliable. This study confirms that uniformly fractured polypeptide chains of silk provide an appropriate fraction for ^{14}C age dating to select silk protein against dye particles and undecomposed foreign contaminants.

INTRODUCTION

Silk was discovered more than 4000 yr ago; Chinese legends say that silk spinning began around 2640 BC. Caterpillars produce silk from a pair of labial glands, each of which consist of silk-secreting posterior and middle regions and an outlet. The posterior region produces the fibrous silk core, whereas the middle region provides a sticky coating for the fiber and adds several low molecular components with presumably protective functions to the silk. Raw silk consists of the proteins fibroin (70~76%) and sericin (15~25%), plus wax (2%) and salt (1%). In order to make silk fabric soft and glassy, a degumming or stripping process is required. During this process, sericin is removed, and only fibroin is present in commercial silk.

Silk fibroin consists of extended protein chains of various molecular weights formed predominately from the amino acids glycine (31.6%), alanine (23.8%), and serine (18.1%), plus other residues with long hydrophobic side chains (16%) (Žurovec and Sehnal 2002). The silk core is typically composed of 3 types of proteins: heavy-chain fibroin (H-fibroin), light-chain fibroin (L-fibroin), and P25 glycoprotein. For the silkworm *Bombyx mori*, it was shown that the H-fibroin (~390 kD) and L-fibroin (~30 kD) molecules are linked by a disulfide bond, and 6 such heterodimers are assembled with a single P25 module into a complex called an elementary fibroin unit (Žurovec and Sehnal 2002). Fibroin is typified by long regions of anti-parallel beta sheets running parallel to the fiber axis. The alternation of serine with either alanine or glycine residues allows the beta sheets to pack together.

Silk can be preserved for a long time without seriously degrading, but old silk from archaeological excavations may be brittle and may contain contaminants such as mold, fungus, dirt, or other carbon-containing materials. One of the major problems in dating any textile is removing all unknown carbon-containing foreign materials (Burleigh and Baynes-Cope 1983). In particular, the measured radiocarbon age of silk may be seriously affected by microorganisms growing on the surface of silk during the entire time of preservation. Also, unless the dye used to color the silk is the same age as the silk itself, separating dye from the silk is important in order to obtain true ^{14}C ages.

Mold is very tough cellulose that can grow on the surface of silk and is very hard to remove. Although a small amount of contaminant might have only a small effect on the $^{14}C/^{12}C$ ratio of the

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silk, it is important to decontaminate the fabric as much as possible because the $^{14}C/^{12}C$ ratios of contaminant and silk may be very different. For mold on silk, conventional pretreatment methods (solvent treatment followed by acid-base-acid treatment) may not be adequate for removing all contaminants (See Figure 1). Therefore, this study focused on removing all the carbon-containing materials from silk and then separately estimating the age of the silk itself and of contaminants including dye. Figure 2 shows an image of SEM of incubated silk after contamination with soil.

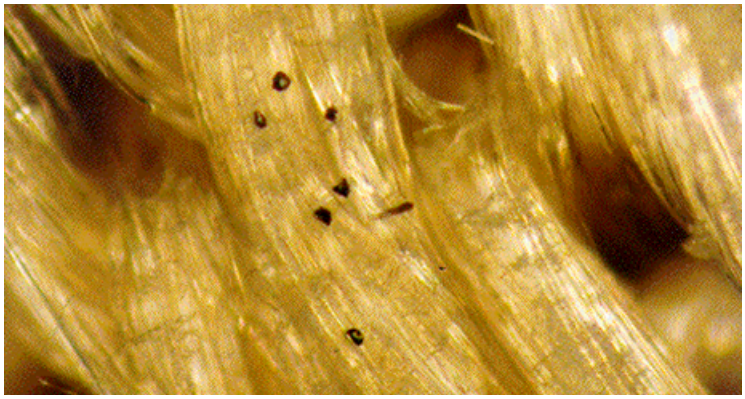

Figure 1 The surface of silk after pretreatment. Two types of mold on the surface of silk were not removed by the existing pretreatment methods.

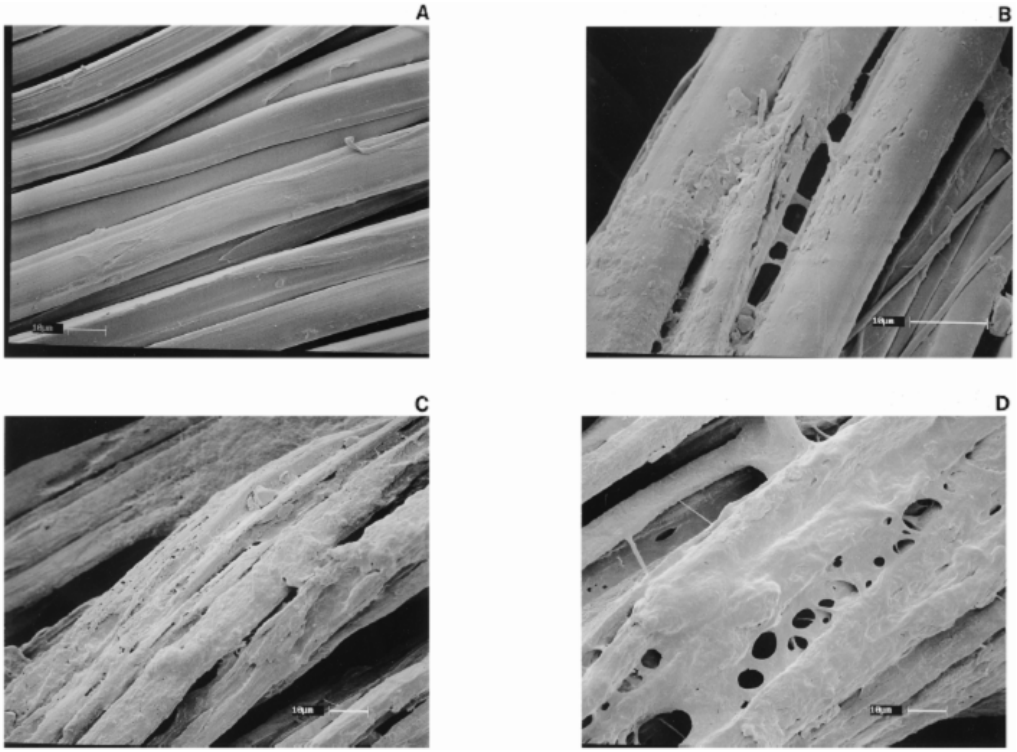

Figure 2 SEM micrographs of degummed silk fabric buried in soil initially (a), at 30 days of incubation (b), and at 60 days of incubation (c, d), modified after Seves et al. (1998).

EXPERIMENTAL

For this study, we investigated several silk samples obtained from neckties. The neckties were made in the 1970s and contain high levels of bomb carbon (~145 pMC) compared to recent biological materials. One of these, designated as S72, was separated into 3 subsamples. S72-1 was measured to determine the overall ^{14}C content of the silk sample, S72-2 was used to check pMC values from the dye and the silk fiber itself, and S72-3 was used to test the effects of deliberate contamination. The S72-3 sample was mixed with fine soil and with orange pulp in a large beaker and left for a month to absorb contaminants and grow biological materials on the silk, and was then dried for this study.

Most visible contaminants on silk can be removed by an initial wash with water in an ultrasonic bath, but a silk sample containing fine materials such as soil, fungus, or glue may not be completely cleaned with existing pretreatments because these foreign materials may be stuck on the textile between tightly woven threads. However, these can be removed by unravelling the woven silk or by decomposing the silk fibers.

In this study, NaOH and HCl treatments were used because these chemicals can decompose or hydrolyze silk protein. For example, NaOH treatments initially break silk fibers into fragments (causing woven silk to unravel), and subsequently decompose fibroin. The minimum NaOH concentration necessary to attain fibroin dissolution is around 0.5~0.6N (Freddi et al. 1995), and the greater the concentration of NaOH, the higher the rate of dissolution—but at the cost of a pronounced decrease in average molecular weight of the fibroin fragments. It is known that silk can also be hydrolyzed by heating at 110 °C for 20 hr in 6N HCl under vacuum (Tsukada et al. 1992, 1996) and that silk is very chemically reactive compared to cellulose. These characteristics of silk were utilized in this study in the development of an effective pretreatment for ^{14}C dating.

Our experiments showed that pretreatment with NaOH successfully decomposed silk, but these treatments were sometimes unnecessarily harsh. They also led to salt buildup in the final product, which in turn caused frequent breakage of combustion tubes when the samples were burned for ^{14}C analysis. Therefore, the NaOH treatments developed here are documented below for completeness, but we concluded that HCl treatments were preferable and used them in the remainder of this study.

RESULTS AND DISCUSSION

NaOH Treatment

The characteristics of silk under various temperatures and concentrations of NaOH were studied. Any woven silk can be unraveled by heating with NaOH; the time required depends on the concentration of NaOH and temperature. The times required to unravel the woven silk and for complete decomposition of the fibers were investigated (Table 1).

Table 1 The characteristics of silk under various temperatures and concentrations of NaOH.

Concentration (N)	Temperature (°C)	Unravelling time (min)	Total decomposition
0.05	105	30	—
0.05	150	10	105 °C, 1 hr
0.1	—	—	70 °C, break down only
0.5	105	15	—
1	150	7	70 °C, 3 hr

An optimum procedure for reducing woven silk to individual threads is as follows. Silk was cleaned in deionized water in an ultrasonic bath, which removed most visible contaminants except some fine materials stuck between the threads. The silk was then heated to 80 °C for 4.5 hr in 0.1N NaOH. When the sheets within the silk fiber unraveled and remained as single threads, the sample was washed with water and filtered using a 355-µm coarse filter, which retained the silk but allowed most soil particles, mold, etc. to pass through. The long silk threads on the filter were dried and examined under a microscope (40×). Most were very smooth and clean, but some did retain mold particles on the surface. The characteristics of silk under various temperatures and concentrations of NaOH are shown in Table 1. The minimum concentration and temperature of NaOH solution required to break down silk fibroin into small pieces (1 mm) are 0.1N NaOH and 70 °C, respectively. At this stage, the characteristics of the silk fibers remain unaltered, and some dye is dissolved. However, filtering small fractured silk particles can be a problem when the size of contaminants such as mold is the same as the size of the silk fragments.

Alternatively, we found that silk could be rapidly decomposed using 1N NaOH solution. The solution was then neutralized with HCl and filtered, but it was then necessary to remove the residual salt. (When a solution containing large quantities of salt is dried and combusted in sealed quartz tubes, the combustion tubes are often perforated and the CO_2 gas is lost.) In addition, the NaOH treatment can be used for only young silk whose threads are strong enough to undergo unfolding without total decomposition. In this case, the threads of silk after unfolding can be coarse-filtered and washed to remove salt. This procedure will clean the surface of silk and remove foreign materials. At least some dyes are dissolved and removed after the coarse filtration using this procedure.

HCl Treatment

Silk was successfully decomposed by treatment with 5 mL of 6N HCl at 110 °C for 20 hr in air. Since no silk fragments were visible to the naked eye, we assume that the silk was completely dissolved. The silk protein containing solution was filtered with glass 1.2-µm microfiber filters, which removed both the dye and undecomposed residue. Figure 3 shows the filtered residue on a glass microfiber filter, and illustrates that in most cases, the dye particles are bigger than 1.2 µm. Using this method, dye and any fine foreign particles bigger than 1.2 µm can be removed completely.

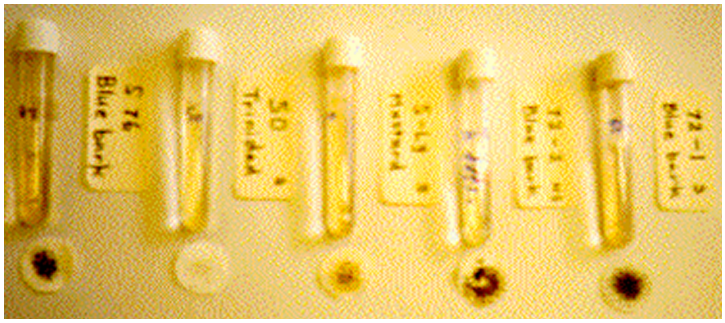

Figure 3 Dye was filtered on a glass microfiber filter from 5 different solutions of silk

The percentage of dye in various silk samples was determined by weighing the filtration residues (Table 2). We found that the dye content of 10 different samples ranged from 0.6% to a surprising 17.6%, though most were less than 3%. ^{14}C ages for silk samples with large dye contents might be seriously biased if the dye contained old or ^{14}C-dead carbon (Burleigh and Baynes-Cope 1983). In Korea, natural dyes were made from plant materials such as plant stems, leaves, seeds, and roots, but

minerals and charcoal or burnt wood were used to make dark colors. Therefore, the possibility of finding old carbon in archaeological silk samples is feasible in some cases.

Table 2 Content of dye in various textiles.

Sample ID	Sample weight (mg)	Percent of dye (%)	Color
S72-1	24.71	2.27	Blue bark
S73	11.93	17.6	Green, brown, ivory
S76-1	13.36	3.14	Blue bark
S-BMX	25.25	1.39	Brown, mustard, tan
S-DG	23.79	0.63	Blue
S-LG	9.78	1.28	Mustard

Figure 4 shows the calculated overall ^{14}C content of a silk sample for various dye contents, based on the measured ^{14}C concentrations in the dye and silk protein from sample 72-1 in this study. The data indicate that ^{14}C could be shifted significantly in some instances, though the dye used in this 1970s sample probably contained fossil-fuel-based carbon and likely represents a worst-case scenario.

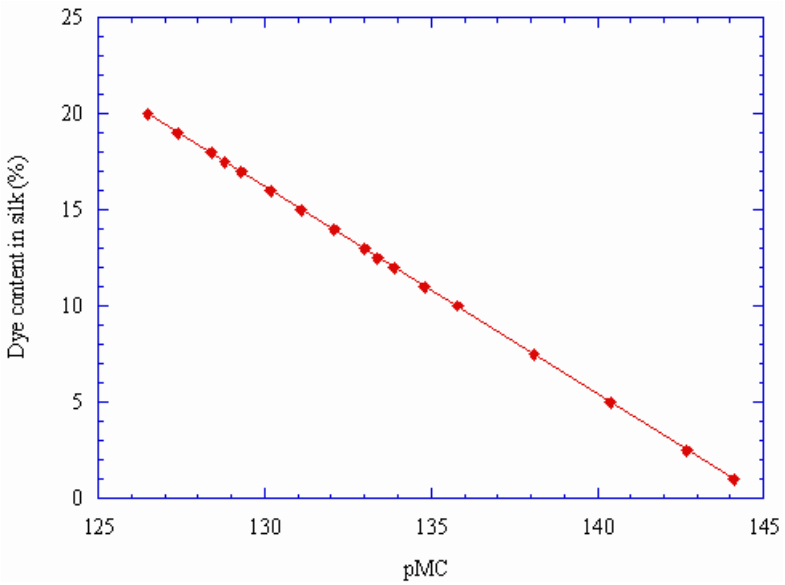

Figure 4 The calculated effect of dye content on the overall percent modern carbon (pMC) for a silk sample is shown. The measured pMC values for the dye and for silk sample 72-1 containing 2.3% dye are 52 and 143.3 pMC, respectively, and the calculated end-point value for the pure silk protein is 145 pMC.

Cellulose powder was found to be insoluble in 6N HCl when treated for over 20 hr at 110 °C. This means that any cellulose-type of contaminants such as mold will not be dissolved during the silk decomposition process in 6N HCl, and can therefore be removed using the 1.2-µm filter.

As a final step, the solution containing the proteins was separated into 3 fractions of different molecular weight ($x < 3000$; $3000 < x < 10{,}000$; and $10{,}000 < x$) using Amicon Centriprep® ultrafiltration concentrators. We assume that the first fraction might have more contaminants than the other 2

because it likely contains any large (sub-micron size) contaminants, which pass through the microfiber filter. Also, the fraction smaller than 3000 might contain very fine particles of contaminants. The chosen HCl treatment was intended to partially decompose the silk protein, breaking some hydrogen bonds in the beta-sheets but avoiding complete hydrolysis of the silk protein to amino acids. However, some hydrolyzed amino acids might be present in the solution. We assume that any low molecular weight materials such as amino acids, additives, dye, and microorganisms might be present in the third fraction. Therefore, by taking only the medium molecular weight (MW) fraction of the decomposed silk fibroin (Fraction III), relatively uncontaminated uniformly fractionated silk fibroin can be obtained and ^{14}C dated. Figure 5 shows the flow chart of the experimental method of the physical and chemical pretreatment of silk.

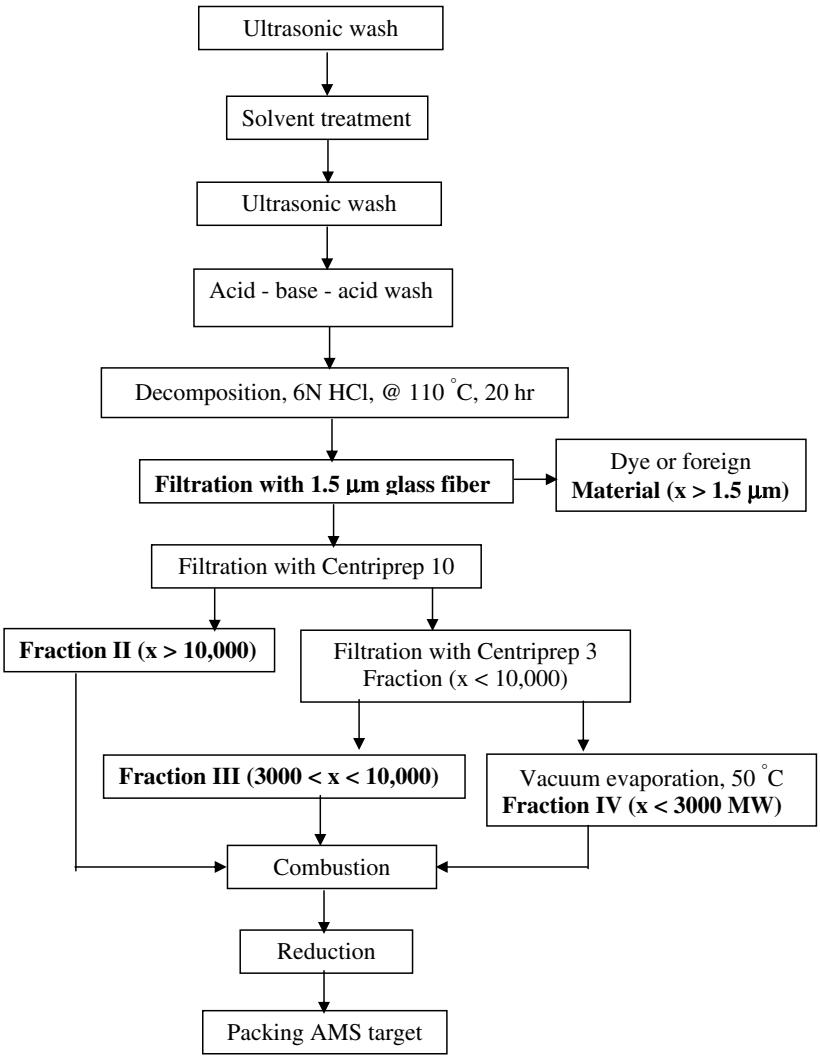

Figure 5 Flow chart of the experimental method for the physical and chemical pretreatment of silk is shown.

Most visible contaminants from the deliberately contaminated sample S72-3 were removed by the initial pretreatment cleaning steps. However, some mold and fine thread-like contaminants remained (Figure 1). The ^{14}C results of 4 fractions from this sample (the residue on the glass microfiber filter and the 3 fractions from the Centriprep concentrators) are shown in Table 3. The ^{14}C content of silk Fraction III was 140 pMC, whereas the dye (72-2) was just 52 pMC. This implies that the silk was made around 1972, but the associated dye contains old biological material or dead carbon. The ^{14}C ratio of the >1.2-µm mixture of dye and mold mixture (90 pMC) was significantly different from the ink itself (52 pMC). The ^{14}C concentration of Fraction III (MW between 3000 and 10,000) was slightly closer to that of untreated uncontaminated silk than fractions II and IV (MW >10,000 and <3000, respectively). However, this value was still slightly below the 145 pMC calculated for uncontaminated silk protein. This suggests that small amounts of contaminated 21st century biological material or soil carbon were still present, i.e. that the sample preparation procedures were not 100% effective. However, we emphasize that this extremely (and deliberately) contaminated sample represents a worst-case scenario.

Table 3 The ^{14}C contents in each fraction of a deliberately contaminated silk (72-3) and uncontaminated untreated silk are shown.[a]

Sample description (72-3)	Weight (%)	Percent modern carbon (pMC)
Ink with contaminants (Fraction I)	4.5	90 ± 6.4
x > 10,000 MWCO (Fraction II)	18	137.04 ± 0.96
3000 > x > 10,000 (Fraction III)	62.6	140.05 ± 0.99, 139.47 ± 1.06
3000 > x (Fraction IV)	19	137.47 ± 0.97
Untreated silk with ink (72-1)	100	143.27 ± 1.21, 143.38 ± 1.1
Ink only (72-2)	2.3	52.49 ± 0.38
Silk protein only (72-2)	97.7	145.38 (calculated)

[a]Note: Vacuum-dried samples may have contained small amounts of residual moisture.

Table 3 shows that the silk sample 72-3, contaminated with mold, leaves twice as much filtered material on the microfiber glass membrane as the ink from the uncontaminated silk sample 72-1 (4.5% vs. 2.3%). The residue on the glass fiber was found to be a mixture of dye, mold, and silk fragments. The ^{14}C content of dye and mold was measured as 52 and 110 pMC, respectively, and the value for silk protein was calculated as 145 pMC (see Figure 4). The ^{14}C content of the residue on the glass fiber filter was measured as 90 pMC. The ^{14}C contribution of 52 pMC for the dye was subtracted from the value for this residue using a mass balance approach, and the ^{14}C content of the mixture of mold and silk protein was determined as 128.2 pMC. From this value, it was concluded (again using a mass balance calculation) that just 1% of the silk protein was present in the residue. This shows that cellulosic contaminants and dye were separated efficiently from the majority of the protein (the filtrate), in the sense that very little protein was lost.

CONCLUSIONS

^{14}C dating textiles is important in archaeological and cultural studies. Silk can be preserved many years without degrading, but the age of dye or ink might be different from the silk, and there might be unknown microorganisms, soil, or other contaminants present on the surface of the textile. Uniformly fractured polypeptide chains of silk provide an appropriate fraction for ^{14}C age dating since this fraction selects against dye particles and undecomposed foreign contaminants. The pretreatment regime developed in this study thereby solves many of the common problems encountered in the

pretreatment laboratory, such as treating silk contaminated with soil, mold, or other organic materials and the handling of brittle samples of very old and degraded silk.

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