RADIOCARBON, Vol 53, Nr 3, 2011, p 429–442

MONITORING THE PRESENCE OF HUMIC SUBSTANCES IN WOOL AND SILK BY THE USE OF NONDESTRUCTIVE FLUORESCENCE SPECTROSCOPY: QUALITY CONTROL FOR ^{14}C DATING OF WOOL AND SILK

Mathieu Boudin[1,2,3] • Pascal Boeckx[2] • Peter Vandenabeele[4] • Sylvia Mitschke[5] • Mark Van Strydonck[1]

ABSTRACT. Radiocarbon dating of degraded wool and silk provides ^{14}C results of questionable reliability. In most cases, degraded wool/silk contains humic substances (HSs). Thus, a nondestructive fluorescence spectroscopy method, using a fiberoptic probe, was developed to monitor the presence of HSs in degraded wool and silk. This method can provide information about the presence of HSs before and after pretreatment and about the ^{14}C age reliability. This study suggests considering with care wool/silk samples ^{14}C dating wherein HSs are detected, because the conventional solvent pretreatment method using a NaOH wash is in most cases not sufficient to remove all humic substance contaminants. As a result, unreliable ^{14}C dates can be provided.

INTRODUCTION

Wool and silk are proteinic fibers. Wool is an animal fiber that consists mainly of keratin. Raw silk is obtained from the cocoons of the larvae of the mulberry silkworm *Bombyx mori* and consists of the proteins fibroin and sericin. In order to make silk fabric soft and glassy, a degumming process is necessary to remove sericin, leaving only fibroin in silk to manufacture textiles (Sashina et al. 2006).

Wool and silk are gaining more attention as suitable radiocarbon dating material due to their short lifespan, potentially presenting the true age of an object made of these materials (Van Strydonck et al. 2004). However, wool and silk are susceptible to rapid decomposition and are rarely excavated in archaeological sites. Archaeological wool and silk fibers can be degraded by microorganisms (Janaway 1985; Gillard et al. 1994). Moreover, humidity and heat accelerate the degradation of these textiles (Sibley and Jakes 1984). Only if the growth of microorganisms is hindered in a special environment can the textile survive (Sibley and Jakes 1984; Cronyn 2001). Such special environments include peat bogs, lake bottoms, deserts, salt mines, and permafrost soils. In such cases, the activity of the microorganisms is slowed down due to anaerobic conditions, the absence of available water, and the presence of metals such as copper (Kars and Smit 2003).

Degraded wool and silk may contain contaminants such as mold, fungus, dirt, humic substances (HSs), or other carbon-containing materials (Kim et al. 2008), which may affect the ^{14}C date. Conventional pretreatment methods (solvent treatment followed by acid-base-acid treatment) for ^{14}C analyses may not be adequate for removing all contaminants (Kim et al. 2008). HS contamination of wool or silk is a major problem in obtaining reliable ^{14}C dates. Thus, HSs can be classified according to the ease in which they are soluble in alkaline or acidic solutions (Head 1987):

1. Humic acid is the fraction extracted by the alkaline solution that becomes insoluble after acidification.
2. Fulvic acids are soluble both in acid and alkaline solutions.
3. Humin is the residue that is insoluble in acid and alkaline solutions.

[1]Royal Institute for Cultural Heritage, B-1000 Brussels, Belgium.
[2]Ghent University, Faculty of Bioscience Engineering, Laboratory of Applied Physical Chemistry, Coupure Links 653, B-9000 Ghent, Belgium.
[3]Corresponding author: Mathieu.Boudin@kikirpa.be; Mathieu.boudin@ugent.be.
[4]Ghent University, Department of Archaeology, Sint-Pietersnieuwstraat 35, B-9000 Ghent, Belgium.
[5]Curt-Engelhorn Stiftung für die Reiss-Engelhorn–Museen, C5 Zeughaus, 68159 Mannheim, Germany.

Quality control of sample material (e.g. charcoal, collagen) is receiving considerable attention in order to obtain more reliable ^{14}C dates (DeNiro 1985; Alon et al. 2002; Van Strydonck et al. 2005). Therefore, a fluorescence spectroscopy method is developed in this study for screening textiles for humic substance presence in order to obtain reliable ^{14}C dates.

Fluorescence spectroscopy should be ideal for independently detecting HSs present in archaeological wool and silk samples. Fluorescence is the result of a 3-stage process (excitation, excited-state lifetime, emission) that occurs in certain molecules, generally polyaromatic hydrocarbons or heterocycles, called fluorophores (Lakowicz 1999). HSs are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, and aliphatic compounds involved in linkages between the aromatic groups (Stevenson 1982). Thus, HSs have fluorescent properties. Fluorescence spectroscopy is a common technique used in soil science by analyzing HSs in solution (Bachelier 1980–1981; Simpson et al. 1997). The new technique is applied on solid material (e.g. wool or silk fabric/fiber) using a fiberoptic probe to detect HSs.

This technique has the advantage over solution studies in avoiding the formation of any new fluorophores and/or the destruction of others during protein hydrolysis or solubilization. For example, some of the fluorophores easily detected in solid-state studies on wool keratin or silk fibroin are barely detectable after protein hydrolysis or solubilization (Millington et al. 2002). These chemical treatments are necessary in order to measure the fluorescence of a solution. This new method is favored over other techniques because it is nondestructive. The fiberoptic probe takes light to the sample via an optical light guide (excitation). The sample absorbs this light and emits light back via an optical light guide to the spectrofluorometer (fluorescence) (Figure 1).

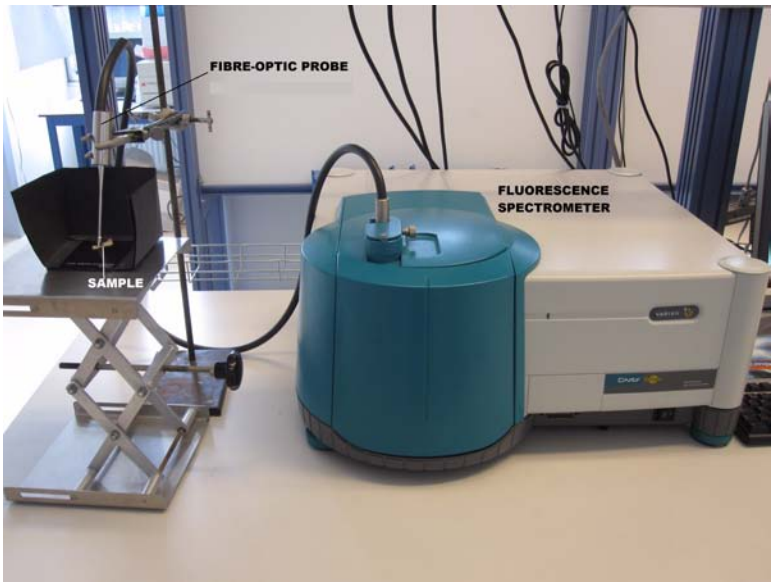

Figure 1 Varian Cary Eclipse fluorescence spectrophotometer with a fiberoptic probe

In this study, a qualitative/semiquantitative fluorescence spectroscopy technique is developed that distinguishes the (naturally dyed) textile or fiber from the HSs by choosing the appropriate excitation and emission wavelengths (Bachelier 1980–1981; Simpson et al. 1997; Clarke 2002).

MATERIALS AND METHODS

Sample Selection

Modern, undyed silk fabric was acquired from a textile shop (La Fourmi, Brussels, Belgium). The modern wool fibers were delivered by a Belgian farmer (Galle, Emelgem). The undyed silk fabric and wool were analyzed using spectrofluorescence spectroscopy as a means to obtain reference spectra, free of humic substances (HSs).

Commercially available HSs were used: humic acid depur, called "HA Roth" in this paper (Carl Roth, Karlsruhe, Germany); humic acid practical grade, called "HA MP Biomedicals" (MP Biomedicals, Brussels, Belgium); and humic acid sodium salt, "HA Sigma" (Sigma-Aldrich, Bornem, Belgium). HSs are complex aromatic macromolecules with amino acids, amino sugars, peptides, and aliphatic compounds involved in linkages between the aromatic groups (Stevenson 1982). Therefore, these commercially prepared HSs were analyzed using fluorescence spectroscopy in order to determine similarities and/or differences in their spectra.

Archaeological samples, chosen to represent various ages and preservation environments, were obtained from sites listed in Table 1, and analyzed with fluorescence spectrometry before ^{14}C pretreatment and ^{14}C dating. All the archaeological samples were naturally dyed fabrics. Some of these samples were examined with fluorescence spectroscopy before and after ^{14}C pretreatment in order to determine the extent to which HSs were removed by the pretreatment.

Table 1 Archaeological samples, chosen to represent various ages and preservation environments, were obtained from different sites.

Sample name	Archaeological site	Country	Material type	Preservation environment	Presumed historical date
Mainz 1	Mainz	Germany	Wool	Waterlogged soil	5 BC (Roman)
Mainz 2	Mainz	Germany	Wool	Waterlogged soil	5 BC (Roman)
Mainz 3	Mainz	Germany	Wool	Waterlogged soil	5 BC (Roman)
Mainz 4	Mainz	Germany	Wool	Waterlogged soil	5 BC (Roman)
Beerlegem	Beerlegem	Belgium	Wool	Presence of metal	AD 600–650 (Merovingian)
Ieper	Ieper	Belgium	Wool	Waterlogged soil	13th century AD
OS2562	Oudenburg	Belgium	Wool	Well	Late Roman
OS24909	Oudenburg	Belgium	Wool	Well	Late Roman
1924-01	Unknown	Afghanistan	Wool	Sand	AD 1000–1200
Bourelet	Unknown	Egypt	Wool	Sand	AD 200–450 (Coptic)
1923-02	Unknown	Egypt	Silk	Sand	AD 700–1000 (Coptic)
Hallstat 79429	Hallstat	Austria	Wool	Salt mine	800–400 BC

Sample Preparation

Modern silk fabric and modern wool fibers were washed separately:

1. With a 1% neutral, non-ionic soap solution (ETS-René Dejonghe, Ghent, Belgium) with a maximum temperature of 80 °C for the wool and 60 °C for the silk;
2. With Milli-Q water and;
3. Finally, dried at 60 °C.

This washing procedure has no influence on the amino acid composition of the silk and wool. This was internally tested and confirmed by the textile laboratory in the Royal Institute of Cultural Heritage (Brussels, Belgium).

About 650 mg of each HS was dissolved in 125 mL of Milli-Q™ (Millipore) water. The pH of the HS solution was ~5. To this solution, we added ~450 mg of silk and slowly agitated the mixture at room temperature for different durations (3 hr, 64 hr, and 188 hr). About 500 mg of wool was added to the HS solution and the mixture was slowly agitated for 120 hr or 200 hr at 80 °C. Wool has greater stability in an acidic environment due to its disulfide bonds. Therefore, a temperature of 80 °C was used to accelerate wool degradation.

The wool or silk was then removed from the HS solution, rinsed several times with Milli-Q water, and finally washed with Milli-Q water for 15 min in an ultrasonic bath before drying at 40 °C. Sample names consist of the material type (wool or silk), brand of HS, and agitation duration (e.g. Silk HA Roth 3 hr).

The wool and silk contaminated with HSs were analyzed with fluorescence spectroscopy in order to register reference spectra. The HS-contaminated wool (fibers) and silk (fabric and yarn) were pretreated with 1% NaOH for different durations to remove the HSs, and finally analyzed with spectrofluorescence to determine the degree of HS removal.

Fluorescence Spectroscopy

Analyses were made in natural atmosphere at room temperature using a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies). Nondestructive analysis of the textile fibers was carried out using a fiberoptic probe. Spectra were acquired in the excitation wavelength range of 340–475 or 400–475 nm and 509 nm as the emission wavelength. The following wavelengths were chosen: the main excitation bands of humic acids at 465, 480, and 490 nm (Simpson et al. 1997); the principal emission bands of humic acids in the wavelength range of 509–515 nm (Bachelier 1980–1981).

No interfering fluorescence originated from the natural organic dyes at these wavelengths (Clarke 2002). Synthetic organic dyes were introduced in the mid-19th century, with mauveïne the first synthetic organic dye produced in 1856 (Herbst et al. 1997; Holme 2006). Therefore, all archaeological dyed textiles samples manufactured before 1856 can be analyzed with our method.

Excitation was produced by a 15W xenon pulse lamp. The excitation and emission band width were set at 10 nm. Scan control (medium) consisted of 600 nm min^{-1}, average time of 0.1 s, and a data interval of 1 nm. The software automatically chose the excitation and emission filter during analysis. Fluorescence intensity was measured in arbitrary units (au). The fluorescence spectrophotometer also has room light immunity that excludes fluorescence contributed by ambient light.

^{14}C Dating

Archaeological wool and silk samples were pretreated as follows:

1. Extraction in an ultrasonic bath for 15 min with hexane (twice);
2. Rinsing with acetone;
3. Extraction in an ultrasonic bath for 15 min with acetone (twice);
4. Rinsing with acetone;
5. Extraction in an ultrasonic bath for 15 min with ethanol (twice);
6. Rinsing with Milli-Q water;

7. Extraction in an ultrasonic bath for 15 min with Milli-Q water;
8. Rinsing with Milli-Q water;
9. 15 min in cold 1% NaOH;
10. Rinsing with Milli-Q water;
11. 15 min in cold 1% HCl;
12. Rinsing with Milli-Q water;
13. Drying of sample at 40 °C.

All chemical products were purchased from Merck (Belgium).

The solvent pretreatment started with the most apolar solvent (hexane) and ended with the most polar solvent (ethanol). Rinsing with acetone is necessary after hexane and acetone extraction and with Milli-Q water after ethanol extraction in order to remove organic solvent remains. The duration of the NaOH step depends on the quality of the sample. This step was stopped when the sample started to fall apart or eventually started dissolving. The wool samples from Ieper and Beerlegem dissolved completely in the NaOH step due their degraded state. They could only be pretreated with the solvents.

^{14}C dates were measured by accelerator mass spectrometry (AMS) at the Leibniz Labor für Altersbestimmung und Isotopenforschung in Kiel, Germany (Nadeau et al. 1998). CO_2 was obtained by sample combustion in the presence of CuO and Ag. Graphitization was done with H_2 over a Fe catalyst. Targets were prepared at the Royal Institute for Cultural Heritage in Brussels, Belgium (Van Strydonck et al. 1990). ^{14}C calibrations were performed using OxCal 3 (Bronk Ramsey 1995, 2001) and the IntCal09 calibration curve data (Reimer et al. 2009).

RESULTS AND DISCUSSION

Fluorescence Spectroscopy

The uncontaminated wool in this study shows 2 excitation band centers (Figure 2a):

- at 380 nm: This fluorescence has previously been assigned to N-formyl kynurenine, which is a known oxidation product of tryptophan (Smith 1995).
- at 430 nm. Previous work has shown that oxidized wool contains β-carboline fluorophores derived from tryptophan in exactly this position (Smith et al. 1994).

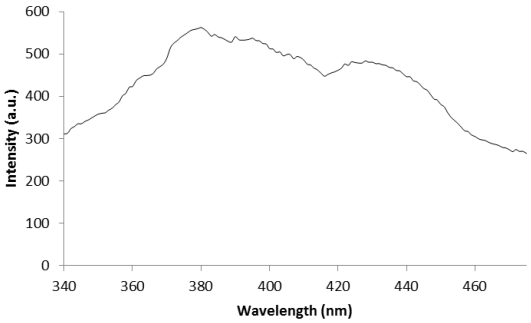

Figure 2a Excitation spectrum between 340 and 475 nm of uncontaminated wool fibers. Excitation band centers are observed at 380 and 430 nm.

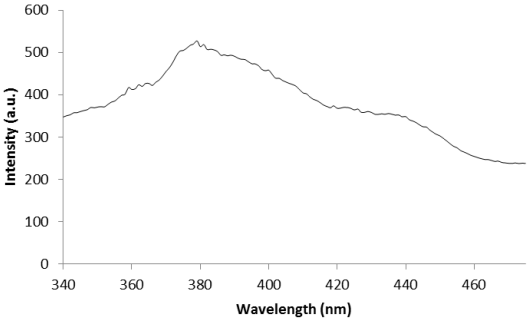

Figure 2b Excitation spectrum between 340 and 475 nm of uncontaminated silk fabric. The excitation band center is observed at 378 nm.

The uncontaminated silk fabric has an excitation band center at 378 nm (Figure 2b). This position was observed in silk fabric exposed to UV light. Untreated silk has very little visible fluorescence, but after exposure to UV light, a new feature develops, which is very similar in position and appearance to the fluorescence in reduced wool. It is likely that both features are due to protein oxidation products that are nonreducible (Millington and Kirschenbaum 2002). All analyzed HSs show a clear fluorescence increase between an excitation wavelength of 430 and 475 nm (Figure 3a–c).

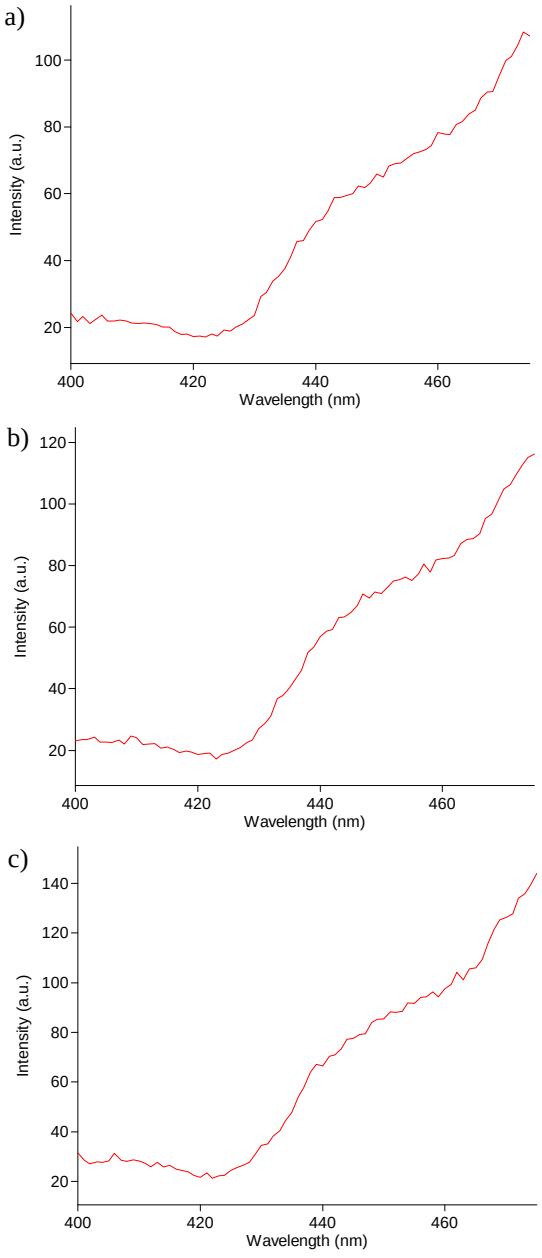

Figure 3 Excitation spectra between 400 and 475 nm of the humic substances HA Roth (a), HA Sigma (b), and HA MP Biomedicals (c).

Further analyses started at an excitation wavelength of 400 nm instead of 340 nm in order to detect mainly the HS and minimize the fluorescence interference of the textile (Table 2), and to make the analysis faster and more straightforward.

Table 2 Excitation and emission band centers (nm) of different materials (Bachelier 1980–1981; Simpson et al. 1997; Millington and Kirschenbaum 2002).

	Excitation band center (nm)	Emission band center (nm)
Wool	375	450
Oxidized wool	430	500
Silk after UV light	370	440
Humic acids	465, 480, and 490	509–515

If we compare the spectra of the HS-contaminated silk samples Silk HA Roth 64 hr (Figure 4a) and Silk HA Roth 188 hr (Figure 4b) with those of HS-contaminated (Figure 3) and uncontaminated silk (Figure 2b), we observe a linear increase in fluorescence between 460 and 475 nm in both contaminated samples. This is not the case for uncontaminated silk (Figure 2b), but it is for HA Roth (Figure 3a). This fluorescence can be explained by the presence of HSs in the fiber (Table 2). The color of the silk fabrics was a good indicator for HS contamination. The contaminated silk fabric samples were light brown, after agitation for 64 hr in HS solution, and brown, after 188 hr in HS solution, indicating that contamination with HSs occurred, because the uncontaminated silk fabric was white.

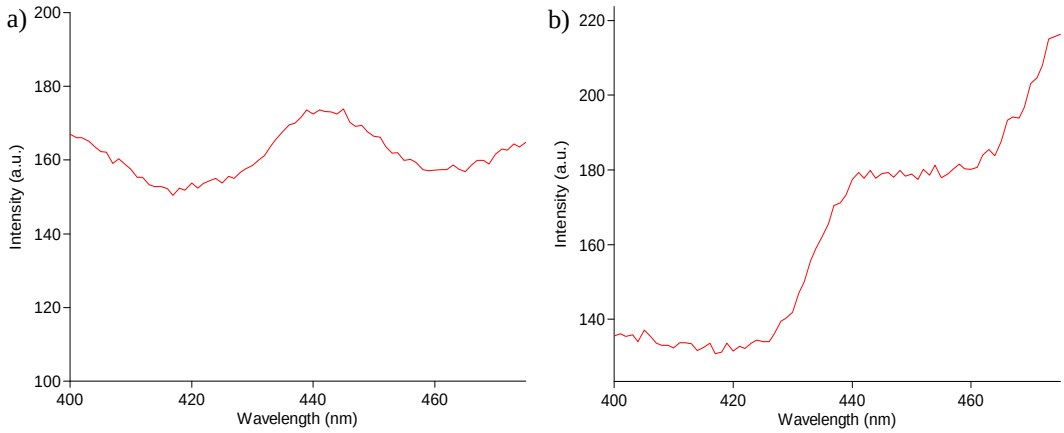

Figure 4 Excitation spectra of modern silk fabric immersed in mixture of humic substance (Roth) and Milli-Q water during 64 hr (a) and 188 hr (b).

The fluorescence intensity measured between 460 and 475 nm can be explained by the presence of HSs. Therefore, the slope was calculated using a linear fit (least squares) to the curve between 465 and 475 nm (Table 3). All analyzed humic substances (HA Roth, HA MP Biomedicals, and HA Sigma) have a positive slope. Uncontaminated wool and silk both have a negative slope, while HS-contaminated samples (Silk HA Roth 3 hr, Silk HA Roth 64 hr, Silk HA Roth 188 hr, Wool HA Roth 120 hr, and Wool HA Roth 200 hr) have a positive slope. Thus, the slope can be used as a qualitative indicator for the presence of HS: a negative slope indicating HS absence and a positive slope indicating HS presence.

Table 3 Spectrofluorescence slope values of analyzed HSs and HS-contaminated silk or wool samples, with applied PMT detector voltage. The slope was calculated using a linear fit (least squares) to the curve between 465 and 475 nm.

Sample name	Slope	PMT detector voltage (V)
HA Roth	+2.63	950
HA MP Biomedicals	+3.60	950
HA Sigma	+1.73	950
Uncontaminated silk	–0.67	650
Silk HA Roth 3 hr	+0.77	650
Silk HA Roth 64 hr	+0.76	650
Silk HA Roth 188 hr	+3.05	800
Silk HA Aldrich 64 hr	+0.01	650
Uncontaminated wool	–0.22	750
Wool HA Roth 120 hr	+0.20	750
Wool HA Roth 200 hr	+0.77	1000

The chosen photomultiplier tube (PMT) detector voltage influences the slope value. If a higher voltage was applied on the same sample, it resulted in a higher slope. This can be explained by the disproportionate fluorescence intensity increase when a higher PMT voltage is applied (Lawaetz and Stedmon 2009). A fluorescence intensity calibration is necessary to compare slope values measured with different PMT voltages. Lawaetz and Stedmon (2009) developed a fluorescence intensity calibration using the Raman scatter peak of water for liquid samples. Research is ongoing to develop a fluorescence intensity calibration method for solid samples using an fiberoptic probe.

A higher PMT voltage was necessary if the wool/silk samples were in a degraded state, but the slope value remains positive. Possible explanations are mineralization of the fibers. This can be defined as the combination and/or replacement of the organic matrix, including the fluorophores, of the fiber with an inorganic one (Gillard et al. 1994). Another possibility is protein degradation (fluorophores are part of the protein) caused by microbial attack or by microenvironmental conditions (Sibley and Jakes 1984).

If we want to compare slopes of the same sample before and after ^{14}C pretreatment, the same voltage must be applied. In this case, the slope can be used as a semiquantitative indicator for HS presence. The slope should decrease after ^{14}C pretreatment mostly due to the NaOH wash and signifies HS removal. This HS removal is shown in Table 4 where the slope value of the contaminated test samples decreases as a function of the NaOH wash duration. However, no complete HS removal was obtained.

Table 4 Spectrofluorescence slope value of Silk HA Roth 188 hr (fabric and yarn) and of Wool HA Roth 200 hr (fibers) as a function of NaOH wash duration (min). The slope was calculated using a linear fit (least squares) to the curve between 465 and 475 nm. PMT voltage was 800 V for the silk samples and 1000 V for the wool samples.

	Slope value as a function of NaOH wash duration (min)					
Sample name	0	15	30	45	60	90
Silk HA Roth 188 hr - *Fabric*	3.05	2.61	1.69	1.27	0.53	0.49
Wool HA Roth 200 hr - *Fibers*	0.77	0.67	0.05	n.a.	n.a.	n.a.

Table 5 demonstrates the dependency of the NaOH treatment duration on the sample type, e.g. HS-contaminated silk fabric can be pretreated longer than HS-contaminated silk yarns taken from fab-

ric. HS-contaminated wool fibers are especially susceptible to NaOH: ~85% of the sample is lost after 30 min of treatment. It is really exceptional that archaeological samples can be fully treated with 1% NaOH during 15 min in our laboratory.

Table 5 Weight (mg) of Silk HA Roth 188 hr (fabric and yarn) and Wool HA Roth 200 hr (fibers) as a function of NaOH wash duration (min).

	Weight (mg) as a function of NaOH wash duration (min)					
Sample name	0	15	30	45	60	90
Silk HA Roth 188 hr - *Fabric*	4.78	4.21	3.8	3.65	3.25	2.93
Silk HA Roth 188 hr - *Yarn*	5.82	4.31	1.62	n.a.	n.a.	n.a.
Wool HA Roth 200 hr - *Fibers*	41.2	29.1	6.4	n.a.	n.a.	n.a.

The slopes of different unknown samples contaminated with HSs should not be compared even if the same voltage is applied, because the chemical composition of the HSs is unknown and fluorescence depends on the amount of fluorophores (polyaromatic hydrocarbons or heterocycles) present in the HS. These assessments are supported by the analyses of 3 humic substances: HA Roth, HA MP Biomedicals, and HA Sigma. These samples were analyzed applying the same voltage (950 V). The calculated fluorescence slope is different for the 3 humic substances (Table 3).

The analyzed archaeological samples (Table 1) can therefore be divided into 2 groups:

1. The first group showing an excitation spectrum in accordance with the spectrum of sample Silk HA Roth 188 hr (Figure 4b): Mainz 1, Mainz 2, Mainz 3, Mainz 4, Beerlegem, Ieper, OS2562, and OS24909. This is an indication of HS presence.
2. The second group having an excitation spectrum matching the spectrum of the uncontaminated silk (Figure 2b): 1924-01, Bourelet, 1923-02, and Hallstat 79429. This indicates HS absence.

The slope of these archaeological samples (Table 1) was calculated (Table 6). Analytical precision for the slope was greater than 0.30 (pooled standard deviation), as determined by multiple measurements of 4 samples (Silk HA Roth 3 hr, Beerlegem, Hallstat 79429, and Mainz 2) at 750 V. Positive and negative slopes indicate the presence and absence of HSs, respectively. As such, it is suggested that archaeological silk or wool samples with a positive fluorescence slope be proposed as an indicator for less reliable ^{14}C dates unless *in situ* humification has occurred.

The samples Mainz 1, Mainz 2, Mainz 3, Mainz 4, Beerlegem, Ieper, OS2562, and OS24909 show a positive slope and consequently HS presence in the textile. The samples 1924-01, Bourelet, 1923-02, and Hallstat 79429 have a negative slope, indicating HS absence in the textile.

Four archaeological samples (Mainz 2, Mainz 4, Beerlegem, and Ieper) were analyzed before and after ^{14}C pretreatment applying the same voltage (950 V). The slope difference of the uncontaminated and pretreated textiles was calculated (Table 6). A slope decrease for the 4 samples is noted, meaning that the amount of HSs decreases after pretreatment. However, the slope for the 4 samples remains positive, indicating that there were still HSs present in the 4 samples after ^{14}C pretreatment.

The samples Mainz 2 and Mainz 4 underwent the full pretreatment including the NaOH wash. Partial dissolution of the fulvic acid likely occurred during the 15-min sample treatment with Milli-Q water in the ultrasonic bath. The NaOH step should be able to remove fulvic and humic acids because fulvic acids are soluble in acid and alkaline solutions and humic acid is the fraction extracted by alkaline solution (Head 1987). However, the NaOH wash is not sufficient to remove all HSs as proven by a positive fluorescence slope after ^{14}C pretreatment. A harsher NaOH wash results

Table 6 Spectrofluorescence slope values of analyzed archaeological samples, applied PMT detector voltage, and difference of slope values before and after ^{14}C pretreatment. The slope was calculated using a linear fit (least squares) to the curve between 465 and 475 nm.

Sample name	Slope	PMT detector voltage (V)	Slope difference between untreated and ^{14}C pretreated sample
Mainz 1 untreated	+4.37	950	
Mainz 3 untreated	+3.98	950	
Mainz 2 untreated	+5.44	950	
Mainz 2 after ^{14}C pretreatment	+2.97	950	2.47
Mainz 4 untreated	+3.73	950	
Mainz 4 after ^{14}C pretreatment	+2.45	950	1.28
Beerlegem untreated	+13.54	950	
Beerlegem after ^{14}C pretreatment	+9.89	950	3.65
Ieper untreated	+4.51	950	
Ieper after ^{14}C pretreatment	+3.00	950	1.51
OS2562 untreated	+2.15	950	
OS24909 untreated	+1.08	950	
1924-01 untreated	–1.16	650	
Bourelet untreated	–0.53	800	
1923-02 untreated	–1.03	650	
Hallstat 79429 untreated	–3.69	750	

in completely dissolving of the textile-humic mixture, more quickly than releasing HSs from the contaminated textile. Therefore, most of the protein-humic linkages are at least similarly strong as the forces that keep the α-helices (wool) or β-sheets (silk) together (and thus soluble). Van Klinken and Hedges (1995) noted this phenomenon for collagen-humic mixtures. In the case of wool, alkalis act simultaneously on 3 bond types of the keratin: the peptide bond, the S-bridges, and the salt bonds (Sibley and Jakes 1984). This causes fast decomposition of the wool. The fibroin silk fibers are also susceptible to decomposition by alkalis and acids: alkali only breaks the bond between the last amino acid and the rest of the polymer, while acids break the bonds between the amino acids on random places in the polymer (Tímar-Balázsy and Eastop 1998).

The samples Beerlegem and Ieper could not be pretreated with NaOH. However, the slope decrease can be explained by dissolution of fulvic acids during the 15-min sample treatment with Milli-Q water in the ultrasonic bath, since fulvic acids are soluble in acid and alkaline solutions (Head 1987).

AMS Dating

The ^{14}C-dated textiles can be divided in 2 groups (Table 4):

1. The first group consists of the textiles containing humic substances, proven by spectrofluorescence: Mainz 1, Mainz 2, Mainz 3, Mainz 4, Beerlegem, and Ieper;
2. The second group where no humic substances were detected: samples 1924-01, Bourelet, 1923-02, and Hallstat 79429.

The ^{14}C dates of the Mainz samples (Table 7) are older than the expected archaeological date for the textiles based on the dated coins and the typochronologically dated Italic Samian pottery found in association with the dated textiles (~5 BC) (Böhme-Schönberger and Mitschke 2005). Moreover, all ^{14}C dates for the Mainz samples are very close to each other. This would indicate a uniform HS contamination, even after pretreatment. The average ^{14}C date of the 4 Mainz samples is 2116 ± 13 BP

(χ^2 test: $df = 3$; $T = 4.2(5\%\ 7.8)$). The difference of the average ^{14}C date and the presumed archaeological (5 BC) date is 105 (68.2%) 175 BC; 50 (1.3%) 70 BC, 80 (94.1%) 200 at 95.4% probability.

Table 7 ^{14}C age and calibrated age and presumed historical data of archaeological textile samples.

Sample name	Archaeological site	Lab code	^{14}C age (BP)	Calibrated age (2 σ)	Presumed historical date
Mainz 1	Mainz	KIA-41534	2120 ± 25	340 (1.1%) 320 BC 210 (94.3%) 50 BC	5 BC
Mainz 2	Mainz	KIA-41535	2075 ± 25	180 (94.5%) 30 BC	5 BC
Mainz 3	Mainz	KIA-41536	2145 ± 25	360 (23.7%) 290 BC 230 (71.7%) 90 BC	5 BC
Mainz 4	Mainz	KIA-41537	2125 ± 25	350 (4.6%) 320 BC 210 (90.8%) 50 BC	5 BC
Beerlegem	Beerlegem	KIA-42365	1705 ± 30	AD 250 (95.4%) 410	AD 587
Ieper	Ieper	KIA-43347	750 ± 25	AD 1220 (95.4%) 1285	13th century AD
1924-01	Unknown	KIA-40874	980 ± 20	AD 1010 (54.5%) 1060 AD 1080 (40.9%) 1160	AD 1000–1200
Bourelet	Unknown	KIA-39433	1675 ± 30	AD 250 (11.8%) 300 AD 320 (83.6%) 430	AD 200–450
1923-02	Unknown	KIA-42114	1090 ± 30	AD 890 (95.4%) 1020	AD 700–1000
Hallstat 79429	Hallstat	KIA-42750	2555 ± 30	810 (53.5%) 740 BC 690 (16.4%) 660 BC 650 (25.5%) 550 BC	800–400 BC

From Table 7 above, the following conclusions can be made: 1) The difference is provoked by HS contamination of the textiles and the ^{14}C age is older than the expected archaeological date. 2) But the presence of HSs is not always an indication for ^{14}C contamination. *In situ* humification of the material is not influencing the ^{14}C date. An archaeological hypothesis is that the textile fragments were used to fortify the bog ground when the Romans build their camp. Therefore, the use of older, worn textiles is assumed and can also explain the calculated difference.

The wool of Ieper has been excavated from a watercourse. Two archaeological hypotheses can be proposed: 1) Textile manufacturing started in the mid-13th century at that site (Haneca et al. 2009). It is possible that the excavated wool was deposed in the watercourse during that period. In this case, the ^{14}C date is in agreement with this archaeological date (Table 7). However, fluorescence spectroscopy proved the presence of HSs. *In situ* humification of the material can explain the presence and the agreement between the ^{14}C and the archaeological date. 2) Nevertheless, it is also possible that the textile deposit occurred when a new port was built in AD 1290 (dendrochronological date of the felling of an oak tree used in the construction, with bark still present, Haneca et al. 2009). Then, the ^{14}C date is too old. This can be due to HS contamination and may explain the difference of the ^{14}C date and the dendrochronological date (AD 1290) that is between 5 and 70 yr (95.4% probability).

^{14}C dating of wool or silk, showing HS presence by fluorescence analysis, does not aid archaeologists in clarifying the archaeological context where different archaeological considerations (e.g. in Mainz and Ieper) can be made.

The ^{14}C date of Beerlegem (AD 250–410 with 95.4% probability) is older than the archaeological date (first half of the 7th century) (Table 7). The older ^{14}C date confirms the HS contamination of the textile analyzed by spectrofluorometry. The archaeological evidence and information suggest the unreliability of the ^{14}C date:

1. The textile was found in a burial chamber. The features of the grave goods (glass beakers, an urn, fragments of a bronze dish, beads, a golden ring, a bronze decorative disc, a silver necklace with a bone pendant) date this grave to the first half of the 7th century AD and indicate that a wealthy woman was buried in this chamber (Roosens 1959).
2. The felling date of a bottom plank of the grave was AD 587 ± 10, obtained by dendrochronology (Roosens 1977).
3. The study of textile fragments resulted in spinning and weaving techniques with a great diversity and demonstrated a high development degree of the Merovingian textile technique in Belgium (Lefève 1959).
4. The Merovingians arrived in Belgium in the second half of the 5th century (Wightman 1985).

The samples Beerlegem and Ieper could not be pretreated with NaOH to remove humic acids from the fiber. ^{14}C dating of these textiles can provide more unreliable results than textiles pretreated with a NaOH wash. The fluorescence analysis of Mainz 2 and Mainz 4 before and after ^{14}C pretreatment showed that the solvent pretreatment with NaOH and HCl wash is not sufficient to remove all the HSs in the textile sample (Table 6).

Therefore, we suggest to consider with care ^{14}C dating of wool/silk that show HS presence when undergoing fluorescence analysis. As a result, unreliable ^{14}C dates can be provided. The ^{14}C dates of the samples 1924-01, Bourelet, 1923-02, and Hallstat 79429 (Grömer 2005), wherein no HSs could be detected with fluorescence analyses, are in agreement with the determined archaeological dates (Table 7).

CONCLUSION

The fluorescence spectrometry method is a quick and nondestructive prescreening method to detect the presence of contaminant humic substances (HSs) in naturally (un)dyed wool and silk and gives information about the reliability of the ^{14}C dates. However, one must carefully consider wool/silk samples wherein HSs are detected with fluorescence analyses for ^{14}C dating, because the conventional solvent pretreatment method with NaOH wash is in most cases not sufficient to remove all HS contaminants. As a result, unreliable ^{14}C dates can be provided.

Compound-specific ^{14}C dating of individual amino acids (Van Klinken and Cook 1990; Tripp et al. 2006; McCullagh et al. 2010) of the silk/wool protein can be a potential method for dating proteinic textiles showing HS contamination. However, the conventional pretreatment method yields reliable ^{14}C dates of proteinic textiles, wherein no HSs were observed with spectrofluorescence.

ACKNOWLEDGMENTS

The authors wish to thank the European Project “Clothing and identities. New perspectives on textiles in the Roman Empire (DressID)” (Contract nr 2007–1765/001–001 CTU COOPMU) for financial support for ^{14}C dates; and Sofie Vanhoutte, Marc De Wilde, and Kristof Haneca (Flemish Heritage Institute); Johan Deschieter (Archaeological Museum of Velzeke); and Antoine De Moor for their collaboration. This research (WI/39/J05) was funded by the Belgian government.

REFERENCES

Alon D, Mintz G, Cohen I, Weiner S, Boaretto E. 2002. The use of Raman spectroscopy to monitor the removal of humic substances from charcoal: quality control for ^{14}C dating of charcoal. *Radiocarbon* 44(1): 1–11.

Bachelier G. 1980–1981. Étude spectrographique de la fluorescence des acids humiques et des acides fulviques de divers sols. *Cahiers ORSTOM, série Pédologie* 18(2):129–45.

Bohme-Schonberger A, Mitschke S. 2005. Die romis-

chen Stoffe aus Mainz. *Archaeological Textiles Newsletter* 41:22–4.

Bronk Ramsey C. 1995. Radiocarbon calibration and analysis of stratigraphy: the OxCal program. *Radiocarbon* 37(2):425–30.

Bronk Ramsey C. 2001. Development of the radiocarbon calibration program. *Radiocarbon* 43(2A):355–63.

Clarke M. 2002. Limitations of fluorescence spectroscopy as a tool for non-destructive *in situ* identification of organic pigments, dyes and inks. In: Van Grieken R, Janssens K, Van't dack L, Meersman G, editors. *Proceedings 7th International Conference on Non-Destructive Testing and Microanalysis for the Diagnostics and Conservation of the Cultural and Environmental Heritage (Art 2002)*. University of Antwerp.

Cronyn JM. 2001. The deterioration of organic materials. In: Brothwell DR, Pollard AM, editors. *Handbook of Archaeological Sciences*. New York: Wiley Interscience. p 627–36.

DeNiro MJ. 1985. Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317(6040): 806–9.

Gillard RD, Hardman SM, Thomas RG, Watkinson DE. 1994. The mineralization of fibres in burial environments. *Studies in Conservation* 39:132–40.

Grömer K. 2005. The textiles from the prehistoric saltmines at Hallstatt. In: Bichler P, Grömer K, Hofmann-de Keijzer R, Kern A, Reschreiter H, editors. *Hallstatt Textiles: Technical Analysis, Scientific Investigation and Experiment on Iron Age Textiles*. British Archaeological Reports International Series 1351. Oxford: Archaeopress. p 17–40.

Haneca K, Dewilde M, Ervynck A, Boeren I, Beeckman H, Goetghebeur P, Wyffels F. 2009. De 'houten eeuw' van een Vlaamse stad. Archeologisch en dendrochronologisch onderzoek in Ieper (prov. West-Vlaanderen). *Relicta* 4:99–133.

Head MJ. 1987. Categorisation of organic sediments from archaeological sites. In: Ambrose WR, Mummery JMJ, editors. *Archaeometry: Further Australian Studies*. Department of Prehistory, Research School of Pacific Studies, ANU, Canberra. p 143–59.

Herbst W, Hunger K. 1997. Industrial organic pigments. Production, properties, applications. *Journal of American Institute of Conservation* 45:107–25.

Holme I. 2006. Sir William Henry Perkin: a review of his life, work and legacy. *Coloration Technology* 122(5): 235–51.

Janaway RC. 1985. Dust to dust: the preservation of textile materials in metal artefact corrosion products with reference to inhumation graves. *Science and Archaeology* 27:29–34.

Kars H, Smit A. 2003. Handleiding fysiek behoud archeologisch erfgoed. Degradatiemechanismen in sporen en materialen. Monitoring van de conditie van het bodemarchief. In: Aalbersberg G, Burke E, Kars H, editors. *Geoarchaeological and Bioarchaeological Studies 1*. Amsterdam: Vrije Universiteit. p 1–109.

Kim KJ, Southon J, Imamura M, Sparks R. 2008. Development of sample pretreatment of silk for radiocarbon dating. *Radiocarbon* 50(1):131–8.

Lakowicz JR. 1999. Introduction to fluorescence. In: Lakowicz JR, editor. *Principles of Fluorescence Spectroscopy*. 2nd edition. New York: Springer. p 1–24.

Lawaetz AJ, Stedmon CA. 2009. Fluorescence intensity calibration using the Raman scatter peak of water. *Applied spectroscopy* 63(8):936–40.

Lefève R. 1959. Onderzoek van de Merovingische textielrestn uit Beerlegem. *Bulletin Koninklijk Instituut voor Kunstpatrimonium* 2:152–7.

McCullagh JSO, Marom A, Hedges REM. 2010. Radiocarbon dating of individual amino acids from archaeological bone collagen. *Radiocarbon* 52(2–3):620–34.

Millington K, Kirschenbaum L. 2002. Crosslinking and visible fluorescence in fibrous proteins. 25th Australasian Polymer Symposium, Armidale. Paper B9/1.

Nadeau M-J, Grootes PM, Schliecher M, Hasselberg P, Rieck A, Bitterling M. 1998. Sample throughput and data quality at the Leibniz-Labor AMS facility. *Radiocarbon* 40(1):239–45.

Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, Blackwell PG, Bronk Ramsey C, Buck CE, Burr GS, Edwards RL, Friedrich M, Grootes PM, Guilderson TP, Hajdas I, Heaton TJ, Hogg AG, Hughen KA, Kaiser KF, Kromer B, McCormac FG, Manning SW, Reimer RW, Richards DA, Southon JR, Talamo S, Turney CSM, van der Plicht J, Weyhenmeyer CE. 2009. IntCal09 and Marine09 radiocarbon age calibration curves, 0–50,000 years cal BP. *Radiocarbon* 51(4): 1111–50.

Roosens H. 1959. Houten VII-eeuwse grafkamer met vrouwensieraden te Beerlegem. *Bulletin Koninklijk Instituut voor Kunstpatrimonium* 2:138–51.

Roosens H. 1977. Dendrochronologie van graf 111 van Beerlegem. *Archaeologica Belgica* 196:60–2.

Sashina ES, Bochek AM, Novoselov NP, Kirichenko DA. 2006. Structure and solubility of natural silk fibroin. *Russian Journal of Applied Chemistry* 79(6): 869–76.

Sibley LR, Jakes KA. 1984. Survival of protein fibres in archaeological contexts. *Science and Archaeology* 26: 17–27.

Simpson AJ, Boersma RE, Kingery WL, Hicks RP, Hayes MHB. 1997. Applications of NMR spectroscopy for studies of the molecular compositions of humic substances. In: Hayes MHB, Simpson AJ, editors. *Humic Substances, Peats and Sludges: Health and Environmental Aspects*. Cambridge: The Royal Society of Chemistry. p 46–63.

Smith CI, Fuller BT, Choy K, Richards MP. 2009. A three-phase liquid chromatographic method for $\delta^{13}C$ analysis of amino acids from biological protein hydrolysates using liquid chromatography-isotope ratio

mass spectrometry. *Analytical Biochemistry* 390:165–72.

Smith GJ. 1995. New trends in photobiology (invited review). Photodegradation of keratin and other structural proteins. *Journal of Photochemistry and Photobiology B: Biology* 27(3):187–98.

Smith GJ, Markham KR, Melhuish WH. 1994. The presence of photoreactive β-carboline fluorophores in weathered wool keratin. *Photochemistry and Photobiology* 60(3):196–8.

Stevenson FJ. 1982. Genesis, composition, reactions. In: Stevenson FJ, editor. *Humus Chemistry.* New York: Wiley-Interscience. p 1–443.

Tímár-Balázsy A, Eastop D. 1998. Fibres. In: Tímár-Balázsy A, Eastop D, editors. *Chemical Principles of Textile Conservation.* Oxford: Butterworth-Heineman. p 1–431.

Tripp JA, McCullagh JSO, Hedges REM. 2006. Preparative separation of underivatized amino acids for compound-specific stable isotope analyses and radiocarbon dating of hydrolyzed bone collagen. *Journal of Separation Science* 29:41–8.

Van Klinken GJ, Cook WG. 1990. Preparative high-performance liquid chromatographic separation of individual amino acids derived from fossil bone collagen. *Radiocarbon* 32(2):155–64.

Van Klinken GJ, Hedges REM. 1995. Experiments on collagen-humic interactions: speed of humic uptake, and effects of diverse chemical treatments. *Journal of Archaeological Science* 22(2):263–70.

Van Strydonck M, Van der Borg K. 1990–1991. The construction of a preparation line for AMS-targets at the Royal Institute for Cultural Heritage, Brussels. *Bulletin Koninklijk Instituut voor Kunstpatrimonium* 23: 228–34.

Van Strydonck M, De Moor A, Bénazeth D. 2004. ^{14}C dating compared to art historical dating of Roman and Coptic textiles from Egypt. *Radiocarbon* 46(1):231–44.

Van Strydonck M, Boudin M, Ervynck A. 2005. Humans and Myotragus: the issue of sample integrity in radiocarbon dating. In: Alcover JA, Bover P, editors. Proceedings of the International Symposium "Insular Vertebrate Evolution: The Palaeontological Approach." Palma. *Monografies de la Societat d'Història Natural de les Balears* 12:369–76.

Wightman EM, editor. 1985. *Gallia Belgica*. Berkeley: University of California Press. p 304–5.