Surface-enhanced Raman spectroscopy (SERS) on silver colloids for the identification of ancient textile dyes. Part II: Pomegranate and sumac

ARTICLE *in* JOURNAL OF RAMAN SPECTROSCOPY · MARCH 2011

Impact Factor: 2.67 · DOI: 10.1002/jrs.2736

Research Article

Journal of RAMAN SPECTROSCOPY

Received: 1 April 2010 Accepted: 25 May 2010 Published online in Wiley Online Library: 7 July 2010

(wileyonlinelibrary.com) DOI 10.1002/jrs.2736

Surface-enhanced Raman spectroscopy (SERS) on silver colloids for the identification of ancient textile dyes. Part II: pomegranate and sumac

Silvia Bruni,[a]* Vittoria Guglielmi,[a] Federica Pozzi[a] and Anna Maria Mercuri[b]

The effectiveness of surface-enhanced Raman spectroscopy (SERS) spectrocsopy on Ag colloids has been successfully demonstrated for the identification of a yellow dye in two ancient wool threads found in the Royal Tumulus of In Aghelachem, Libyan Sahara, belonging to the Garamantian period (2nd–3rd century A.D.). High-performance liquid chromatography (HPLC) highlighted the presence of ellagic acid in the extracts from the threads, excluding other chromophores. This result, together with the abundance of malic acid detected by gas chromatography-mass spectrometry (GC-MS), suggested the possible use of pomegranate rind or sumac berries as source of the yellow dye, both plants being documented in the Fezzan area during the Garamantian period. HPLC analyses and SERS spectra acquired on the extracts of the ancient threads were therefore compared with those obtained from pomegranate and sumac extracts of the corresponding fruits and reference dyed wool samples, allowing us to identify the yellow dye as deriving from pomegranate (*Punica granatum* L.). SERS spectra of ellagic acid and dyes extracted from pomegranate rind and sumac berries are reported here for the first time. A methodological improvement is also presented, based on the use of $NaClO_4$ as aggregating agent, that leads to a significant increase of the signal-to-noise ratio in the SERS spectra.

Supporting information may be found in the online version of this article.

Keywords: SERS; chromatography; ancient dyes; pomegranate; sumac

Introduction

Dyeing technology has been in existence for at least the past 4000 years. Prior to the introduction of synthetic dyes in the second half of the 19th century, all colorants used in textile dyeing were derived from a variety of natural sources, such as plants, and also from other organisms like lichens, insects and shellfish.

The identification of archaeological textile dyes can provide information regarding advances in technical knowledge, provenance of textile materials and commercial routes.

In recent years, the potential of surface-enhanced Raman spectroscopy (SERS) in this field of research has been widely demonstrated,[1,2] thus giving it a meaningful role beside the techniques that have been traditionally employed for the identification of historical textile dyes, such as ultraviolet–visible (UV–vis)[3] spectroscopy and high-performance liquid chromatography (HPLC).[4,5]

In the present study, SERS on Ag colloids and HPLC were employed to analyse two yellow-dyed wool threads (Fig. 1) from the Libyan Sahara, belonging to the Garamantian period (2nd–3rd century A.D.). Besides these techniques, also colour analysis, scanning electron microscopy combined with energy-dispersive X-ray analysis (SEM-EDX) and gas chromatography-mass spectrometry (GC-MS) were used to gain preliminary information on the samples.

In a previous paper,[6] we employed SERS on Ag colloids, with NaCl or poly-L-lysine and ascorbic acid as aggregating agents, to identify Tyrian purple on a purplish bone fragment and madder on a red wool thread, the latter found at the same excavation site in the Libyan Sahara to which the samples here investigated belong. In the present paper, the use of a different electrolyte, namely $NaClO_4$, was evaluated, giving rise to significantly improved spectral quality compared to the previously used aggregating agents.

Among the various classes of natural dyes, yellows are often difficult to identify, as their plant sources are widely distributed in the vegetable world. In most cases, flavonoids are their main colouring matter, but a number of other brownish-yellow dyes are obtained from species containing different chromophores, such as naphthoquinonoids, carotenoids, curcuminoids and tannins.[7] Because of the great variety of plants producing yellow shades, the choice of the dye source often depends on the species that are locally available.[8]

Archaeobotanical remains from archaeological sites of the Fezzan area have demonstrated that an extended plant cover was present in the area, where a large number of species existed and were used by local populations since the first millennia of the

* *Correspondence to: Silvia Bruni, Dipartimento di Chimica Inorganica, Metallorganica e Analitica "Lamberto Malatesta", Università degli Studi di Milano, via G. Venezian 21, 20133 Milano, Italy. E-mail: silvia.bruni@unimi.it*

a *Dipartimento di Chimica Inorganica, Metallorganica e Analitica 'Lamberto Malatesta', Università degli Studi di Milano, 20133 Milan, Italy*

b *Laboratorio di Palinologia e Paleobotanica, Dipartimento di Biologia, Università di Modena e Reggio Emilia, 41121 Modena, Italy*

Figure 1. Yellow-dyed wool thread (thread 2) from the Royal Tumulus of In Aghelachem, Libya. The fibre has an approximate length of 5 mm.

Holocene.[9] During Garamantian times, a number of seeds and fruits were found that could have been used as sources for yellow dyes and tanning agents[10] (Table 1). Among them, based on the results of chromatographic analyses performed on the extracts obtained from the ancient threads, in this paper we focused on *Rhus* (sumac) and *Punica granatum* L. (pomegranate), which were considered the most probable sources of the yellow dye colouring the wool discovered at In Aghelachem.

Dyes were therefore extracted from the fruits of pomegranate and sumac, which were also used to dye wool samples in our laboratory, according to ancient recipes, in order to provide a reference for chemical analysis to be compared with the results obtained for the archaeological samples.

Before extraction, the ancient threads were analysed by visible reflectance spectroscopy for colour measurements as well as by SEM-EDX. Subsequently, the extracts obtained from the two samples, as well as those of pomegranate and sumac fruits and reference dyed wool, were studied by GC-MS, HPLC and SERS.

Rhus and *P. Granatum*: Long-History Dye Plants

Rhus or sumac (*Anacardiaceae*)

This genus comprises about 200 species from the temperate and tropical/subtropical regions. The leaves, bark, wood and fruits of many species are widely used in dyeing and leather tanning. Phylogenetic studies and research on sumac extracts have shown that plants of different species have similar chemistry.

The Sicilian sumac *Rhus coriaria* L. has stems and bark rich in tannins. Leaves contain 20–35% tannin and yield a yellow dye; also, leaves can be collected as they fall in autumn and used as a brown dye or as a mordant. Its fruit and bark are also used[17,18]: stem bark yields yellow, and root bark yields brown dyes.[22] *Rhus coriaria* L. and *Cotinus coggygria* Scop. have been known and used widely since Greek times, and sumac was among the genera described by Theophrastus (4th–3rd century B.C.). *Rhus tripartita*

Table 1. Dye plants found in Garamantian archaeological records from which yellow dyes can be obtained

Wadi	Wadi Al-Ajal			Wadi Tanezzuft		
Site	Zinkekra	Tinda	Jarma	Fewet	Aghram Nadharif	
Chronology	900–400 B.C.	370–110 B.C.	400 BC–750 A.D.	200–0 B.C.	50 B.C.–1200 A.D.	
Garamantian phase	Formation phase	Late phase	Mature to late phase	Mature phase	Classical phase to medieval	Parts used to produce dyes (chromophores)
Acacia sp.				+	+	Yellow dye from flowers of *A. dealbata* Link and pods of *A. nilotica* Del. (flavonoids)
Amaranthus sp.					+	Yellow dye from whole plant of several species (betalains)
Carthamus tinctorius cf.			+			Yellow dye from flower heads (flavonoids)
Chenopodium sp.					+	Gold/green dye from whole plant of *Ch. bonus-henricus* L., *Ch. ficifolium* Sm. and some other species (flavonoids)
Foeniculum vulgare cf.	+				+	Yellow dye shoots, flowers and leaves combined (flavonoids)
Gossypium sp.			+			Orange/yellow dye from flowers of *Gossypium hopi* (flavonoids)
Medicago sp.					+	Yellow dye from seeds of alfalfa, i.e. *Medicago sativa* L. (flavonoids)
Olea europaea			+			Yellow/green dye from the leaves (flavonoids)
Punica granatum			+	+		Dark gold dye from fruit rinds (tannins)
Rhus tripartita	+					A yellow dye is obtained from leaves and fruits of *Rhus coriaria* (tannins, anthocyanins)
Vitis vinifera	+	+	+		+	Bright yellow to olive green dyes from leaves (tannins, flavonoids, anthocyanins)
References	[11,12]	[12,13]	[10]	[14]	[15–21]	

can still be found in the Central Sahara, where the wild plant has a long and important history of local exploitation. Moreover, it was found as plant macro-remains in the archaeobotanical record from the Formation phase of Garamantian times (Table 1) and also as pollen from earlier sites of the area.[23]

Punica granatum L. or pomegranate (*Punicaceae*)

This species is native to Western Asia, most likely from Iran, Northeastern Turkey and the region of the South Caspian sea, and early became a subspontaneous shrub in the Eastern Mediterranean regions.[24]

Pomegranate has been cultivated from early antiquity for its valuable fruit throughout the Mediterranean and North African regions, including Central Saharan oases. Considering the locations and context of pomegranate representations and archaeobotanical evidence, this fruit has continued to maintain a long-time tradition of a luxury food.[25] In Roman times, at the Villa Rustica in Oplontis, over a tonne of carbonized pomegranates was discovered.[26] Columella gave instructions on how to preserve pomegranate for over a year,[27] a knowledge that allowed its transport as goods.

Several parts of the plant were used as both a tanning agent and dye. In particular, the dried fruit rind yields a yellow dye which was employed for dyeing clothes and for making a hair dye, or sometimes also as a mordant.[28]

The archaeobotanical record testifies that pomegranate was present in the Fezzan area at the Mature phase of Garamantian times (Table 1). In the Fewet citadel, two seeds of *P. granatum* were found uncharred, suggesting a different depositional history compared with that of other seeds and fruits which were found in a charred state in the same context.[10]

Experimental

Archaeological samples

The archaeological threads (Fig. 1), called 'thread 1' and 'thread 2', were found in the Royal Tumulus of In Aghelachem in the Libyan Sahara. The site excavations were carried out within the Italian–Libyan Archaeological Mission in the Acacus and Messak (Central Sahara), Sapienza, University of Rome and Department of Archaeology of Tripoli (presently directed by S. di Lernia). The In Aghelachem area proved to be particularly rich in megalithic structures, mainly belonging to the Garamantian period. The Garamantes inhabited the Fezzan during the period from *ca* 500 B.C. to *ca* 400 A.D. and developed a network of sites controlling the Saharan caravan routes. Initially a sort of large tribal federation, the Garamantes had a true kingdom in the period between the last three centuries B.C. and the mid-fourth century A.D. The Royal Tumulus of In Aghelachem, dating to the 2nd–3rd century A.D., was the tomb of a person of relevant social status, as demostrated by the remains of an originally rich votive deposition, including a bronze bracelet and a vessel. Moreover, a few fragments of dyed textiles were found, made with coarse and highly twisted threads, which can be cautiously interpreted as a kind of rigid small container.[29]

Materials

Dried pomegranate rind was purchased from the herbalist's shop 'Kallidendria' (Milan, Italy), while sumac berries were harvested in Palermo (Italy). Weld and dyer's broom vegetable cut pieces were purchased from Zecchi (Florence, Italy) and Kremer (Aichstetten, Germany), respectively. Acid potassium tartrate was obtained from the pharmacy Dott. Ambreck (Milan, Italy). Ellagic acid (assay ≥96.0%), silver nitrate (purity ≥99.5%), sodium perchlorate monohydrate (assay ≥99.0%), trifluoroacetic acid (assay ≥99.5%) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane were purchased from Fluka. Methanol (assay ≥99.9%), trisodium citrate dihydrate (assay 100.2%), ethyl acetate (assay ≥99.9%) and acetonitrile (assay ≥99.9%) were obtained from Sigma-Aldrich. Hydrochloric acid (assay ≥99.9%) was purchased from Riedel-de Haën and sodium chloride (assay ≥99.5%) from Carlo Erba, while Na_2CO_3 and $KAl(SO_4)_2$ were obtained from Baslini.

All the aqueous solutions were prepared using ultrapure water (Millipore MilliQ).

Process for dyeing wool

Washing

First of all, wool samples had to be washed in order to remove any lipidic residue which would make the interaction between the dye and the fabric rather difficult. For this purpose, 15 g of raw wool was immersed in a 10% aqueous solution of Na_2CO_3, according to a partially modified procedure of the published protocol.[30] The washing bath was slowly heated up to 40 °C and then kept at this temperature for about 10 min and, after this, the wool was rinsed in distilled water and dried.[31]

Mordanting

Alum, $KAl(SO_4)_2$, reported in the literature as the most common mordant used in ancient times,[32] was employed to set the dyes on fabrics and increase their lightfastness together with acid potassium tartrate, which was added to optimize the pH value. Fifteen grams of washed wool was immersed in a solution obtained by mixing 3.6 g of $KAl(SO_4)_2$ in 150 ml of distilled water and 0.9 g of acid potassium tartrate in 720 ml of distilled water. After heating the resulting bath to 90 °C in about 30 min and then cooling it to room temperature, the wool was rinsed in distilled water and dried.[31]

Dyeing

Pomegranate and sumac dyes were extracted from 30 g of fruits in 150 ml of distilled water. The wool was then immersed in the resulting dyebath, which was heated at 90 °C for 30 min. After cooling to room temperature, the wool was rinsed in distilled water and finally dried in the dark.[31]

Wool samples were also dyed according to the above-reported procedure but without mordanting, for comparison purposes. Indeed, the capability of tannins themselves to act as organic mordants also for other dyes is well known.[33]

Extraction of dyes from archaeological and reference dyed wool samples

About 0.5 mg of each wool thread was suspended in 200 µl of HF 4 M and placed in a polyethylene test tube and was kept at room temperature under magnetic stirring for 30 min. The resulting extracts were thus loaded onto a Discovery Supelco C18 SPE cartridge previously pre-conditioned by 5 ml

of MeOH/acetonitrile 1 : 1 and 5 ml of MilliQ water. Fluorides were washed from the cartridge with 5 ml of MilliQ water with 0.01% trifluoroacetic acid, and the dyestuffs were then eluted using 3 ml of MeOH/acetonitrile 1 : 1 acidified with 0.01% trifluoroacetic acid. The obtained solutions were finally evaporated under a N_2 gentle stream.[34,35]

Extraction of dyes from plant sources

Pomegranate and sumac fruits were suspended in 50 ml of deionized H_2O at room temperature overnight; the obtained solution was then filtered and evaporated in a drying oven at 60 °C.[36,37]

Weld and dyer's broom vegetable cut pieces were treated with 6 ml of MeOH and 200 µl of HCl 37% at 65 °C for 60 min; the obtained solution was filtered and dried under a N_2 gentle stream.[31]

Analytical methods

Colour measurements

The colour of the archaeological threads was analysed by visible reflectance spectra using a Jasco UV–vis–NIR V-570 spectrophotometer equipped with an integrating sphere. A suitable software allowed obtaining from the spectra the CIELab coordinates.

SEM-EDX

SEM-EDX analyses were recorded with a Stereoscan Cambridge 360 scanning electron microscope equipped with an Oxford energy-dispersive electronic microprobe with LaB_6 filament; data acquisition was performed with 25 mm working distance and 20 kV accelerating voltage. Samples were covered with graphite in order to make them conductive for the observation and the microanalysis.

HPLC

HPLC analyses were performed with an HPLC PU-1580 Jasco pump, equipped with an LG-1580-02 Jasco gradient valve and a GASTORR GT-103 solvent degasser, by using an MD 1510 Jasco diode array detector in order to obtain spectral information between 200 and 600 nm. The analyses were done by injecting 25 µl of a methanolic solution of the sample on a Supelco Discovery C18 column (25 cm × 4.66 mm, particle diameter 5 µm) by using (A) H_2O with 0.1% of trifluoroacetic acid and (B) acetonitrile with 0.1% of trifluoroacetic acid as solvents, at a flow of 1 ml min^{-1}. The solvent gradient was as follows: 95–70% A in 0–25 min, 70–40% A in 25–30 min, 40–5% A in 30–38 min and 5–95% A in 38–65 min.

GC-MS

GC-MS analyses were carried out with a Shimadzu GC-MS QP 5050 gas chromatograph coupled with a quadrupole mass spectrometer using electron impact ionization (acceleration voltage 1.5 kV). Chromatographic separation was performed on an Equity-5 Supelco column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm) using poly(5% diphenyl/95% dimethyl)siloxane as a stationary phase and He as carrier gas (flow rate 0.7 ml min^{-1}, purge pressure 27.7 kPa). The ion source and interface temperature was 280 °C, while the scan range *m/z* 40–800. The chromatographic heating gradient was as follows: initial temperature 57 °C, 2 min isothermal, then ramped at 10 °C min^{-1} up to 200 °C, 3 min isothermal, then ramped at 20 °C min^{-1} up to 300 °C and then isothermal for 20 min. A total of 230 µg of each sample was submitted to derivatization in 20 µl of *N,O*-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane and 50 µl of ethyl acetate, heating at 70 °C for 30 min; the injection volume was 1 µl.

SERS

SERS spectra were collected with a micro-Raman portable instrument, equipped with an 1800 lines mm^{-1} grating, a notch filter, an Olympus 50× microscope objective and a Peltier-cooled CCD detector, by using a backscattering geometry; a Nd : YAG laser provided the exciting radiation at 532 nm, with a laser power at the sample of about 1.5 mW. All SERS spectra were recorded between 2000 and 200 cm^{-1} by collecting 30 scans with an exposure time of 4 s. A resolution of around 8 cm^{-1} is estimated in the examined spectral range. Ag colloids were used as the substrate, upon activation by inducing a partial aggregation of the nanoparticles. To accomplish this, 125 µl of $NaClO_4$ 1.8 M was added to 3 ml of the silver colloid under stirring, after the addition of 300 µl of the analyte methanolic solution. Solutions of pomegranate and sumac extracts obtained from fruits and reference dyed wool were prepared daily at a concentration of 10^{-4} M, while the yellow dye from the Libyan wool threads was dissolved in few drops of the solvent. The SERS measurements were performed by focusing the laser beam on a drop of the dye–nanoparticle system deposited on the surface of a glass slide.

Colloid synthesis for SERS

Silver colloids were prepared according to the Lee–Meisel procedure,[38] by reduction of silver nitrate with trisodium citrate dihydrate. All glassware was washed with diluted HNO_3 and deionized and ultrapure MilliQ water in an ultrasonic bath, and carefully dried. Eighteen milligrams of silver nitrate was then suspended in 100 ml of deionized water, which was previously degassed under a gentle N_2 stream, and heated to boiling before 2 ml of a 1% solution of trisodium citrate was slowly dropped under vigorous stirring; the solution was held at the boiling point for 60 min with continuous stirring. The resulting colloid could be kept under refrigeration in the dark (by wrapping the flask in an aluminium foil) and was characterized by determining the wavelength of the absorption maximum in the visible region; all the Lee–Meisel colloids prepared had an absorption maximum between 425 and 435 nm.[6]

Results and Discussion

Non-destructive analyses

Before extraction, the ancient wool threads were examined both by colour analysis and SEM-EDX.

The colorimetric coordinates ($L^* = 52.97, a^* = 7.93, b^* = 14.56$ for thread 1; $L^* = 66.37$, $a^* = 8.27$, $b^* = 24.60$ for thread 2) showed that the colour of the threads can be more precisely defined as reddish-yellow, as also apparent from Fig. 1.

The SEM-EDX analysis of the wool threads showed, besides the presence of sulfur due to amino acids, traces of metals such as Al, Fe and Cu, which could obviously be due to a contamination from

Journal of RAMAN SPECTROSCOPY

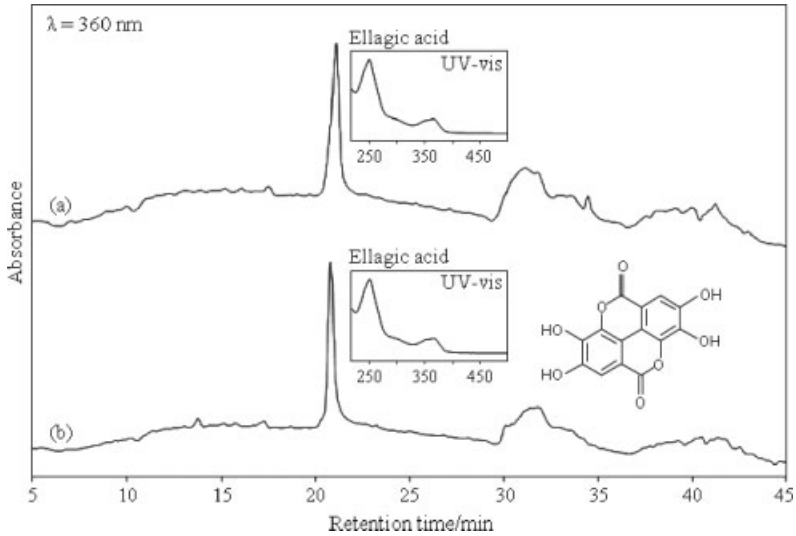

Figure 2. HPLC-UV-PDA chromatograms of (a) thread 1 and (b) thread 2 HF extracts. The only detected compound is ellagic acid.

the burial environment, even though a possible correlation with the mordant used in the dyeing process cannot be excluded.[39] A similar result was obtained for the red-dyed thread investigated by the authors in their previous paper.[6]

Chromatographic analyses

The application of a mild extraction method based on the use of HF at room temperature was essential in order to detect, in the HPLC chromatograms of the extracts obtained from the threads (Fig. 2), a relevant amount of ellagic acid (about 0.5 μg in the extracts obtained from 10 mg of each thread), allowing us to rule out the presence of other chromophores such as flavonoids from weld or dyer's broom, reported in the literature as widely used in antiquity for dyeing textiles.[40] In previous studies, the detection of ellagic acid on ancient textiles which were supposed to be dyed with natural substances was not taken into account as a crucial indication of the employed dye, as ellagitannins could also originate from the decomposition of plant fragments present in the environment, such as dead leaves and bark.[41] In the present case, the possibility that ellagic acid could derive from a contamination is excluded, as previous analyses of a red-dyed thread from the Royal Tumulus showed the exclusive presence of madder without any trace of ellagic acid.[6]

At the same time, in the extracts from the threads the presence of significant amounts of malic acid (around micrograms from 10 mg of each thread) and a slightly smaller quantity of citric acid was highlighted by means of GC-MS (Fig. S1, Supporting Information). Even though these substances are often detected in natural materials, their relative quantities in the samples investigated here are relevant and, moreover, they were not found in the red-dyed thread from the same excavation, thus suggesting that their presence is related to the dyeing process.

The results of chromatographic analyses hinted that, among the natural sources in the Fezzan area during the Garamantian period for reddish-yellow dyes,[42] the most probable candidates were the fruits of pomegranate and sumac (Table 1), i.e. plant parts that, besides containing tannins, could also be rich in malic acid (hydroxybutanedioic acid).[7,43–45]

Extracts from pomegranate rind and sumac berries, as well as from wool dyed with such colorants, were thus studied for comparison. Relevant amounts of ellagic and malic acids were found in both pomegranate and sumac extracts, ellagic acid being especially abundant in the pomegranate extract (about 60 μg mg^{-1} of extract compared to *ca* 10 μg mg^{-1} in the case of sumac) and malic acid in sumac extract (about 50 μg mg^{-1} of extract compared to *ca* 1 μg mg^{-1} in the case of pomegranate). In the extracts from the freshly dyed wool threads, comparable amounts of ellagic, malic and citric acids with those detected for archaeological samples could be determined.

Hence, the above-discussed results of HPLC and GC-MS analyses supported the possible use of pomegranate or sumac dyes for the archaeological threads, but they were not decisive in order to point out which one of the two was really employed. However, HPLC analysis was useful, as it allowed us to detect in both cases other substances besides ellagic acid, which can be considered the real chromophores because of their absorption at higher wavelengths. Indeed, sumac was found to contain also anthocyanins, which could be suggested as responsible for the intense red colour of the extract, while high quantities of ellagitannins such as punicalagin were identified in the pomegranate extract and the reference dyed wool (Fig. 3), which are supposed to give rise to the yellow colour thanks to their absorption at 380 nm. This latter observation, together with the detection of ellagic acid only in the ancient samples, allowed us to hypothesize that the yellow dye of the threads was from pomegranate. Indeed, ellagitannins were not found in the HPLC analysis of the archaeological samples, but this fact can be attributed to their complete hydrolysis to ellagic acid, which could have occurred over the centuries, and to an oxidative polymerization process which results in the formation of insoluble species.[46]

SERS analyses

SERS has proven to be an effective technique in validating the hypothesis put forward for the identification of the yellow dye on the basis of chromatographic analyses.

Our first attempt led to the collection of the SERS spectra of the archaeological samples extracts by using NaCl 1 M or poly-L-lysine together with ascorbic acid as aggregating agents (Fig. 4(a) and (b)), as this procedure easily allowed the identification of the dye in the red-dyed wool thread from In Aghelachem.[6] However, in the present case this method did not result in

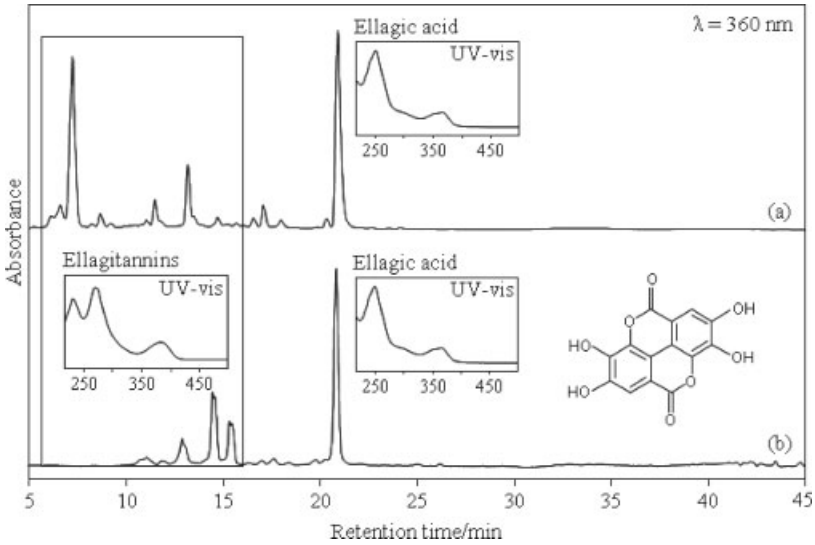

Figure 3. HPLC-UV-PDA chromatograms of (a) pomegranate extract obtained from dyed wool and (b) pomegranate extract obtained from the fruit. The detected compounds are ellagic acid and ellagitannins.

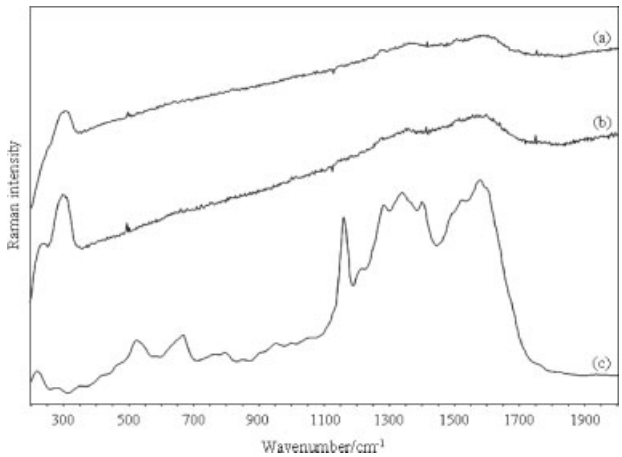

Figure 4. SERS spectra (λ_{exc} = 532 nm) of extracts from (a) thread 1 on Ag Lee–Meisel colloid aggregated by adding NaCl 1 M, (b) thread 1 on Ag Lee–Meisel colloid aggregated by the use of poly-L-lysine and ascorbic acid, (c) thread 1 on Ag Lee–Meisel colloid aggregated by adding $NaClO_4$ 1.8 M. Similar results were obtained for thread 2.

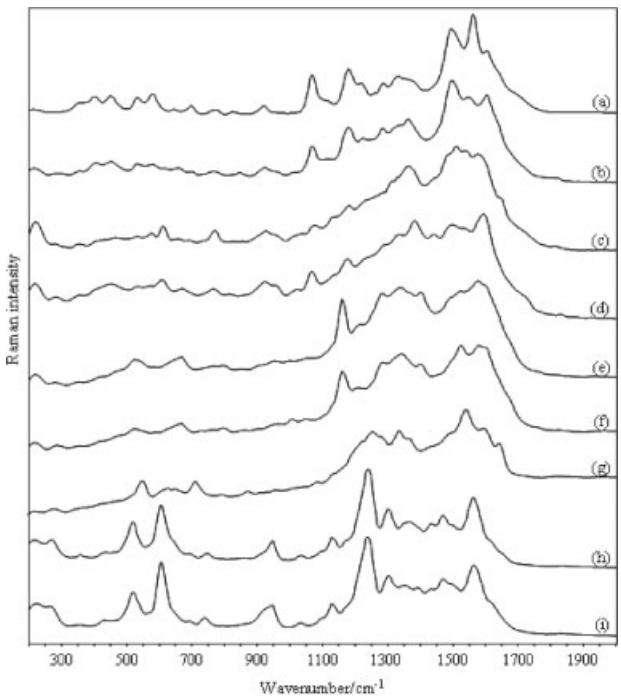

Figure 5. SERS spectra (λ_{exc} = 532 nm) of (a) ellagic acid, and extracts from (b) pomegranate rind, (c) mordanted and (d) unmordanted wool dyed with pomegranate, (e) and (f) threads 1 and 2, (g) sumac berries, (h) weld and (i) dyer's broom vegetable cut pieces. All the spectra were obtained in MeOH solutions on Ag colloid prepared according to the Lee–Meisel procedure and aggregated by adding $NaClO_4$ 1.8 M after the analyte.

high-quality SERS spectra from the extracts of the two yellow-dyed threads. The use of $NaClO_4$ 1.8 M as aggregating agent was thus evaluated and found to be crucial to obtain from the samples good-quality SERS spectra, especially when added to the colloid *after* the analyte, i.e. in an inverted order when compared to the most widely used procedure reported in the literature (Fig. 4(c)).

In accordance with the results acquired by HPLC, the SERS spectra of the extracts from the archaeological samples so obtained were found to be clearly different from those of flavonoid dyes such as weld and dyer's broom (Fig. 5, Table 2), allowing us to exclude the possible use of this latter kind of substances as colouring matter for the ancient threads.

Comparing the results obtained from the SERS analysis of pomegranate and sumac reference dyes (Fig. 5, Table 2), a greater similarity was observed between the spectra of the archaeological samples and those of the pomegranate dye.

The SERS spectrum of the sumac extract shows a very good correspondence with the Raman and resonance Raman spectra of the hydroxyflavylium structure of anthocyanins reported in the literature. In particular, the bands at 1637, 1585, 1534, 1485, 1430 and 1357 cm^{-1} are assigned to ring stretching vibrational modes, while the band at 1328 cm^{-1} is attributed to the δ(CH) modes; the band at 1247 cm^{-1} is due to the C–O stretching mode and the medium

Table 2. Wavenumbers of SERS spectra reported in Fig. 5 (cm^{-1}) with relative intensities

Ellagic acid spectrum (a)	Pomegranate rind spectrum (b)	Pomegranate-dyed mordanted wool spectrum (c)	Pomegranate-dyed unmordanted wool spectrum (d)	Thread 1 spectrum (e)	Thread 2 spectrum (f)	Sumac berries spectrum (g)	Weld spectrum (h)	Dyer's broom spectrum (i)
						1637 (s)		
1603 (m)	1603 (m)	1597 (s)	1584 (s)	1603 (sh)	1603 (sh)			
1560 (vs)	1548 (m)	1544 (s)	1544 (m)	1578 (vs)	1578 (vs)	1585 (s)	1562 (s)	1562 (s)
						1534 (vs)		
1492 (s)	1492 (s)	1507 (s)	1492 (m)	1522 (s)	1522 (vs)		1495 (sh)	1495 (sh)
						1485 (sh)		
							1467 (s)	1467 (s)
						1430 (sh)	1430 (m)	1430 (m)
1376 (sh)	1359 (m)	1359 (m)	1381 (m)	1401 (s)	1401 (s)		1390 (sh)	1390 (m)
						1357 (sh)	1367 (m)	1351 (m)
1333 (m)	1333 (sh)	1333 (sh)	1333 (m)	1335 (vs)	1335 (vs)	1328 (s)		
							1300 (s)	1300 (s)
1285 (m)	1285 (m)	1285 (sh)	1285 (sh)	1285 (s)	1285 (s)			
						1275 (sh)		
						1247 (s)		
							1239 (vs)	1239 (vs)
1220 (m)	1220 (m)	1220 (sh)	1220 (sh)	1215 (m)	1215 (m)			
1178 (m)	1178 (m)	1178 (w)	1178 (w)	1159 (s)	1159 (s)		1173 (sh)	1173 (sh)
							1128 (w)	1128 (w)
							1094 (sh)	1094 (sh)
						1085 (vw)		
1064 (m)	1064 (m)	1064 (w)	1064 (w)	1003 (vw)	1003 (vw)			
	1014 (vw)	1014 (vw)	1014 (vw)				1029 (vw)	1029 (vw)
958 (vw)	958 (sh)	958 (sh)	958 (sh)	954 (vw)	956 (vw)	960 (vw)	944 (w)	944 (w)
921 (w)	921 (w)	921 (w)	921 (w)	904 (vw)	904 (vw)		923 (sh)	923 (sh)
						873 (vw)		
827 (vw)	845 (vw)	827 (vw)	819 (vw)	802 (vw)	802 (vw)	794 (vw)		
771 (vw)	771 (vw)	771 (w)	771 (w)	766 (vw)	766 (vw)			
							745 (vw)	737 (vw)
699 (vw)	699 (vw)	699 (vw)	699 (sh)	725 (vw)	725 (vw)	709 (m)	692 (vw)	692 (vw)
649 (vw)	657 (vw)	657 (vw)	668 (vw)	668 (w)	668 (w)	650 (w)	645 (sh)	645 (sh)
						627 (w)		
	609 (vw)	609 (w)	609 (w)	618 (sh)	618 (sh)		605 (s)	605 (s)
580 (w)	580 (w)	580 (vw)	580 (vw)	582 (vw)	582 (vw)			
						548 (m)		
531 (w)	531 (w)	531 (vw)	531 (vw)	529 (w)	529 (w)			
							518 (m)	518 (m)
450 (w)	450 (w)	450 (vw)	450 (w)	470 (vw)	470 (vw)			
							429 (vw)	429 (vw)
405 (w)	405 (w)	407 (vw)	407 (vw)	412 (vw)	412 (vw)			
355 (vw)	352 (vw)	352 (vw)	352 (vw)	352 (vw)	352 (vw)		359 (vw)	359 (vw)
266 (vw)	283 (vw)	283 (vw)	283 (vw)	283 (vw)	283 (vw)	283 (vw)	266 (w)	266 (w)
222 (vw)	222 (vw)	222 (w)	222 (w)	222 (w)	222 (w)	222 (vw)	220 (w)	220 (w)

intensity bands at 709 and 548 cm^{-1} are assigned, respectively, to the δ(CC) modes of the phenyl and benzopyrylium rings.[47]

On the contrary, the SERS spectrum of the aqueous extract of pomegranate rind shows a similar, even if not identical, pattern to that of ellagic acid, indicating that the interaction between the Ag surface and the molecules of ellagitannins takes place through the polyphenolic moieties of the latter. The main difference between the spectrum of ellagic acid and that of pomegranate extract lies in the relative intensities and exact wavenumbers of the bands assigned to stretching vibrations of the aromatic rings. Indeed, such signals are observed at 1603, 1560, 1492 and 1376 cm^{-1} for ellagic acid and at 1603, 1548, 1492 and 1359 cm^{-1} for ellagitannins from pomegranate. As reported in Table 2, the remaining bands are located at the same wavenumbers in both spectra. The most prominent ones have been assigned to the combination of C–O stretching and O–H deformation vibrations (1333 and 1178 cm^{-1})[48] and to the breathing of the lacton and aryl rings (1064 cm^{-1}).[49] For comparison, Fig. 5 also reports the SERS spectra obtained from the acid extracts of mordanted and unmordanted wool dyed with pomegranate rind. Both spectra show a remarkable similarity with the pattern observed for the fruit extract, as also evidenced by the wavenumbers listed in Table 2.

As already stated, the spectra obtained for the extracts from the archaeological threads have a pattern similar to that of pomegranate dye, even though some differences can be observed. The most evident change is associated with the disappearance of the band at 1064 cm^{-1}, previously assigned to the lacton rings. Indeed, the opening of such rings is evident in oligomeric ellagitannins.[50] Moreover, the intensity of the bands attributed to the combination of C–O stretching and O–H deformation modes, located at 1335 and 1159 cm^{-1} (shifted in comparison with those observed for the pomegranate extract), is significantly increased. At the same time, in the region where the ring stretching vibrations are observed, the strongest band appears at 1578 cm^{-1} and can be considered characteristic of highly substituted phenolic structures.[51] The global enhancement of bands involving phenol groups supports again the hypothesis of the formation of polymeric ellagitannins due to oxidation upon ageing of the pomegranate dye.[46]

Therefore, the results reported above support again the use of pomegranate for dyeing the archaeological samples.

Conclusion

The analytical results here reported allowed the characterization of two natural sources of yellow dye, i.e. sumac berries and pomegranate rind, which have not been examined in detail so far from the point view of their dyeing properties for historical textiles. In the context of a multi-technique approach for the identification of such dyes, special attention was paid to SERS: the good performance of $NaClO_4$ 1.8 M as aggregating agent was highlighted in comparison with that of other electrolytes employed in previous studies on different dyes, especially when added to the silver colloid after the analyte, in an inverted order when compared to the most widely used procedure. By using this method, good-quality SERS spectra of ellagic acid and dyes extracted from pomegranate and sumac fruits were obtained, which are reported here for the first time.

From the archaeological point of view, the present work sheds new light on the dyeing technology in the Libyan Sahara during the Garamantian period and, more generally, on the use of pomegranate extract in antiquity to obtain yellow-dyed textiles. In particular, even though *P. granatum* was part of the archaeobotanical record of Garamantian sites, its seeds/fruits or pollen were not discovered in such great amounts to demonstrate its use as a dyeing plant, which instead could be ascertained in the present work. As a consequence, it is probable that the pomegranate remains discovered in the area were part of the new Mediterranean/Near Eastern luxury goods exchanged by the Garamantian merchants.

Acknowledgements

We would like to thank Dr Eleonora De Luca (Università degli Studi di Milano) for dyeing the reference wool samples, Dr Tommaso La Mantia (Università di Palermo) for collecting the sumac fruits and giving us useful information regarding this plant and Dr Lucia Mori for providing us with the archaeological samples.

Supporting information

Supporting information may be found in the online version of this article.

References

[1] K. L. Wustholz, C. L. Brosseau, F. Casadio, R. P. Van Duyne, *Phys. Chem. Chem. Phys.* **2009**, *11*, 7350.
[2] K. Chen, M. Leona, T. Vo-Dinh, *Sens. Rev.* **2007**, *27*, 109.
[3] G. W. Taylor, *Stud. Conserv.* **1983**, *28*, 153.
[4] S. M. Halpine, *Stud. Conserv.* **1996**, *41*, 76.
[5] J. Wouters, *Stud. Conserv.* **1985**, *30*, 119.
[6] S. Bruni, V. Guglielmi, F. Pozzi, *J. Raman Spectrosc.* **2010**, *41*, 175.
[7] Z. C. Koren, in *Archaeological Chemistry*, ACS Symposium Series 625 (Ed.: M. V. Orna), American Chemical Society: Washington, DC, **1996**, p 269.
[8] J. S. Mills, R. White, *The Organic Chemistry of Museum Objects*, Butterworths: London, **1994**, p 121.
[9] A. M. Mercuri, *J. Archaeol. Sci.* **2008**, *35*, 1619.
[10] A. M. Mercuri, G. Bosi, L. Olmi, L. Mori, E. Gianassi, A. Florenzano, *Bocconea* **2009**, *23*, 379.
[11] C. Ward, *World Archaeol.* **2003**, *34*, 529.
[12] R. Pelling, *J. N. Afr. Stud.* **2005**, *10*, 397.
[13] R. Pelling, in *Food, Fuel and Fields: progress in african archaeobotany, Monographs on African Archaeology and Environment*, vol. 15 (Eds: K. Neumann, A. Butler, S. Kahlheber), Heinrich-Barth Institut: Köln, **2003**, p 129.
[14] A. M. Mercuri, G. Trevisan Grandi, G. Bosi, L. Forlani, F. Buldrini, in *Aghram Nadharif. The Barkat Oasis (Sha'abiya of Ghat, Libyan Sahara) in Garamantian Time. AZA Monographs*, vol. 5 (Ed.: M. Liverani), **2005**, p 335.
[15] J. Cannon, M. Cannon, *Dye Plants and Dyeing*, Timber Press: Portland, **2003**, p 128.
[16] R. Corti, *Flora e vegetazione del Fezzan e della Regione di Gat*, Reale Società Geografica Italiana: Firenze, **1942**, p 505.
[17] K. Fern, *Plants For A Future: Edible & Useful Plants For A Healthier World*, Permanent Publications: Clanfield, **2007**, p 300.
[18] M. Grieve, *A Modern Herbal*, Harcourt Brace: New York, **1931**, p 919.
[19] I. Grae, *Nature's Colors – Dyes from Plants*, MacMillan Publishing Co.: New York, **1974**, p 229.
[20] P. C. M. Jansen, D. Cardon (Eds), *Plant Resources of Tropical Africa 3. Dyes and Tannins*, PROTA Foundation, Backhuys Publishers: Leiden, **2005**, p 216.
[21] J. C. Th. Uphof, *Dictionary of Economic Plants*, Hafner Publishing: New York, **1959**, p 400.
[22] P. M. Guarrera, *J. Ethnobiol. Ethnomed.* **2006**, *2*, 9.
[23] A. M. Mercuri, *J. Arid Environ.* **2008**, *72*, 1950.
[24] D. Zohary, M. Hopf, *Domestication of Plants in the Old World* (2nd edn), Clarendon Press: Oxford, **1994**, p 279.
[25] G. Bosi, A. M. Mercuri, C. Guarnieri, M. Bandini Mazzanti, *Veg. Hist. Archaeobot.* **2009**, *18*, 389.
[26] W. F. Jashemski, F. Meyer, *The Natural History of Pompeii*, Cambridge University Press: New York, **2002**, p 528.
[27] L. J. M. Columella, *De Re Rustica, Book 12* (trans. R. Calzecchi-Onesti), Ramo Editoriale degli Agricoltori: Roma, **1947–1948**.
[28] J. F. Dastur, *Useful Plants of India and Pakistan*, D.B. Taraporewala & Co. Pvt Ltd: Bombay, **1964**, p 260.
[29] S. Di Lernia, G. Manzi (Eds), *Sand, Stones and Bones*, AZA Monographs, vol. 3, Firenze, **2002**, pp 102 160, 165.
[30] J. H. Park, B. M. Gatewood, G. N. Ramaswamy, *J. Appl. Polym. Sci.* **2005**, *98*, 322.
[31] M. P. Colombini, A. Andreotti, C. Baraldi, I. Degano, J. J. Lucejko, *Microch. J.* **2007**, *85*, 174.
[32] F. M. Helmi, O. M. El-Feky, E. H. Salib, *Egypt. J. Anal. Chem.* **2008**, *17*, 53.
[33] W. Novik, S. Desrosiers, I. Surowiec, M. Trojanowicz, *Archaeometry* **2005**, *47*, 835.
[34] J. Sanyova, *Microchim. Acta* **2008**, *162*, 361.
[35] M. Leona, J. Stenger, E. Ferloni, *J. Raman Spectrosc.* **2006**, *37*, 981.
[36] M. A. Khan, M. Khan, P. K. Srivastava, F. Mohammad, *Colourage* **2005**, *52*, 53.
[37] H. Bozkurt, *J. Sci. Food Agric.* **2006**, *86*, 849.
[38] P. C. Lee, D. J. Meisel, *J. Phys. Chem.* **1982**, *84*, 3391.
[39] M. Müller, B. Murphy, M. Burghammer, I. Snigireva, C. Riekel, J. Gunneweg, E. Pantos, *Appl. Phys. A* **2006**, *83*, 183.
[40] E. S. B. Ferreira, A. N. Hulme, H. McNab, A. Quye, *Chem. Soc. Rev.* **2004**, *33*, 329.
[41] W. Nowik, S. Desrosiers, I. Surowiec, M. Trojanowicz, *Archaeometry* **2005**, *47*, 835.
[42] A. J. Thompson, K. A. Jakes, *Southeast. Archaeol.* **2002**, *21*, 252.

[43] S. Kurucu, M. Koyuncu, A. Güvenç, *J. Essent. Oil Res.* **1993**, *5*, 481.
[44] P. Legua, P. Melgarejo, M. Martínez, F. Hernández, *Options Méditerr.* **2000**, *42*, 99.
[45] S. M. Mavlyanov, S. Y. Islmbekov, A. K. Karimdzhanov, A. I. Ismailov, *Chem. Nat. Comp.* **1997**, *33*, 279.
[46] N. Vivas, M. F. Nonier, N. Vivas de Gaulejac, I. Pianet de Boissel, *C. R. Chim.* **2004**, *7*, 945.
[47] J. C. Merlin, J. P. Cornard, *Spectrochim. Acta A* **1994**, *50*, 703.
[48] A. Edelmann, B. Lendl, *J. Am. Chem. Soc.* **2002**, *124*, 14741.
[49] E. Vogel, W. Kiefer, *Fresenius J. Anal. Chem.* **1998**, *361*, 628.
[50] U. Vrhovsek, A. Palchetti, F. Reniero, C. Guillou, D. Masuero, F. Mattivi, *J. Agric. Food Chem.* **2006**, *54*, 4469.
[51] R. A. Alvarez-Puebla, J. J. Garrido, R. F. Aroca, *Anal. Chem.* **2004**, *76*, 7118.