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Surface-enhanced Raman spectroscopy (SERS) on silver colloids for the identification of ancient textile dyes: Tyrian purple and madder

Silvia Bruni,* Vittoria Guglielmi and Federica Pozzi

Surface-enhanced Raman spectroscopy (SERS) was used for the identification of natural organic dyes belonging to indigoid and anthraquinone classes in archeological samples, and good agreement with the corresponding reference commercial materials was found. Special attention was paid to the well-known problem of anomalous bands that arise sometimes in the SERS spectra on colloids: as suggested in the literature, this problem could be reduced by the use of poly-L-lysine and ascorbic acid as aggregating agents, but we observed that also the addition first of the analyte and subsequently of suitable electrolytes to the colloid in an inverted order compared to the most widely used method can be of help in limiting the intensity of such spurious bands. This procedure allowed us to obtain, for the first time, the SERS spectra of both modern and ancient Tyrian purple and to solve a specific problem observed in the analysis of archeological wool samples dyed with madder lake, i.e. the competition in the SERS response between the dye and other compounds possibly deriving from the degradation of the peptide chain during the hydrolysis treatment during the extraction of the dye from the wool fiber.

Keywords: surface-enhanced Raman spectroscopy; Ag colloids; Tyrian purple; madder; archeology

Introduction

It is well known that, prior to the introduction of synthetic dyes in the second half of the 19th century, all colorants used in textile dyeing, an activity which started more than 4000 years ago, were derived from a variety of natural sources. Dyes coming from plants and also from other organisms like lichens, insects and shellfish were mainly blue, red and yellow, while other hues could be obtained by the combined use of different dyestuffs.

The identification of archeological textile dyes is of great interest as it can provide information regarding the technical knowledge reached by a certain population in a given historical age, the provenance of a textile material and the commercial transactions possibly allowing the usage of a certain dye far from its geographical source.

As in the case of other organic substances of archeological interest, the analysis of natural dyes from ancient textiles also was significantly improved thanks to the introduction of instrumental analytical tools, both of chromatographic and spectroscopic types.

Besides those techniques that are most frequently employed for the identification of organic dyes historically used for textiles, i.e. UV-visible spectroscopy and high-performance liquid chromatography (HPLC), in recent years the potential of surface-enhanced Raman spectroscopy (SERS) has been appreciated. Indeed, the enhancement of the Raman scattering intensity obtained for substances absorbed on metallic surfaces showing nanoscale roughness allows the analysis of samples that give a strong fluorescence background and that are available in very limited amounts. In particular, sample weights even an order of magnitude lower than those necessary for HPLC analysis are sufficient to obtain good-quality SERS spectra.

Among the possible substrates, silver colloids have been employed in many studies with satisfactory results, mostly by using potassium nitrate as the aggregating agent.[1–7]

In the present work, Ag colloids prepared according to the Lee–Meisel procedure were used; the analytical methods developed for solving specific problems occurring in SERS identification of natural organic dyes belonging to indigoid and anthraquinone classes, respectively Tyrian purple and madder, are reported. Both reference commercial materials and archeological samples were analyzed: in particular, we examined a purplish bone fragment (Fig. 1) found in the tomb of the martyrs Gervase and Protase in the Basilica of Sant'Ambrogio, Milano (4th century A.D.),[8] in which the dye is probably the only residue of a cloth that wrapped the corpses of the martyrs, and a red-dyed wool thread (Fig. 2) from the Royal Tumulus of In Aghelachem (300 B.C.–350 A.D.) in the Libyan Sahara (Italo-Libyan Joint Mission in the Tadrart Acacus and Messak, CIRSA).[9]

Tyrian purple, also known as royal purple, shellfish purple, imperial purple or purple of the ancients, is an organic dye that has been used since antiquity and has an important historical value. It can be obtained from various species of molluscs, such as *Murex brandaris, M. trunculus, Purpura haemastoma* and *P. lapillus*; the dye is not present in the live mollusc, as it is generated by enzymatic hydrolysis of precursors found in the animals'

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Figure 1. Purplish bone fragment from the tomb of the martyrs Gervase and Protase in the Basilica of Sant'Ambrogio, Milano.

Figure 2. Red-dyed wool thread from the Royal Tumulus of In Aghelachem, Libya.

hypobranchial glands, followed by photochemical conversion to the purple pigment. Only very small amount of Tyrian purple, often less than 1 mg, can be extracted from each mollusc, making this dye rare and costly. The main chromophore of Tyrian purple is 6,6′-dibromoindigotin, but the different types of molluscs provide dyes of varying composition whose components can also include 6-monobromoindigotin and indigotin, as well as smaller amounts of indirubins.[10]

Extracted from dried roots of *Rubia tinctorum*, madder lake is one of the oldest and most popular red dyestuffs; it is originated in India but it was widely cultivated in Europe and the Middle East. Belonging to the group of mordant dyes, it requires a pretreatment of textile fibers with a solution of mordant; the metal salt most frequently used as mordant was alum, often together with acid potassium tartrate. The principal coloring matters of madder are alizarin and purpurin, but other anthraquinone species such as pseudopurpurin, xanthopurpurin, rubiadin and munjistin could also be present, the relative amounts of which vary with the age of the plant.[10]

Experimental

Materials

Tyrian purple and madder lake were purchased from Zecchi (Florence, Italy); alizarin, purpurin, silver nitrate (purity ≥99.5%), hexane (assay ≥99.0%) and trifluoroacetic acid (assay ≥99.5%) were from Fluka; while trisodium citrate dihydrate (assay 100.2%) and poly-L-lysine hydrobromide were from Sigma. *N*,*N*-Dimethylformamide, ascorbic acid (assay ≥99.7%) and hydrochloric acid (assay ≥99.9%) were obtained from Riedel-de Haën, methanol (assay ≥99.9%) and acetonitrile (assay ≥99.9%) from Sigma-Aldrich, and sodium chloride (assay ≥99.5%) from Carlo Erba.

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

Extraction of madder from its inorganic support

In order to isolate the organic chromophores from the aluminated inorganic support of the lake, we added to few milligrams of the dye 2 ml of a 10% aqueous solution of hydrochloric acid and heated at 70 °C for 5 min. The mixture was extracted by 2 ml of hexane which was then drawn off from the aqueous phase with a Pasteur pipette and dried under a gentle N_2 stream.[11]

Extraction of dyes from archeological samples

In order to extract the dye from the archeological purplish relics, a small fragment of bone (about 1 × 1 mm) was suspended in 1 ml of *N*,*N*-dimethylformamide and heated at 70 °C for 5 min, before being filtered through a 0.45-μm GHP Acrodisc membrane filter.[12]

The red dye was extracted from the wool thread (a portion less than 0.5 mg) by treating the sample with 6 ml of MeOH and 200 μl of HCl 37% at 65 °C for 60 min, filtering the obtained solution through a 0.45-μm GHP Acrodisc membrane filter and drying it under a gentle N_2 stream.[13,14]

Colloid synthesis

Silver colloids were prepared according to the Lee–Meisel procedure,[15] by reduction of silver nitrate with trisodium citrate dihydrate. All glassware was washed with HNO_3 65% and in deionized and ultrapure MilliQ water in an ultrasonic bath and accurately dried. Eighteen milligrams of silver nitrate was then suspended in 100 ml of deionized water 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 boiling point for 60 min with continuous stirring. The resulting colloid could be kept in the refrigerator in the dark, by wrapping the flask in an aluminum foil, and was characterized by determining the wavelength of the absorption maximum in the visible region on a Jasco UV/VIS/NIR V-570 spectrophotometer; all the Lee–Meisel colloids we prepared had an absorption maximum between 425 and 435 nm (Fig. 3).

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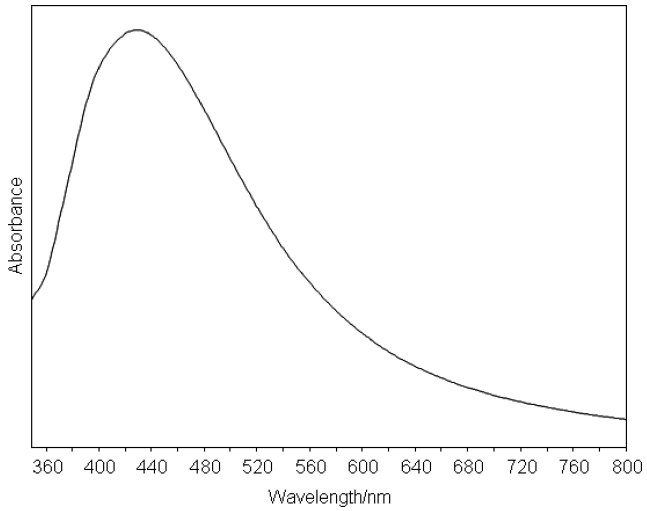

Figure 3. UV–vis spectrum of a silver colloid prepared according to the Lee–Meisel procedure.

SERS sample preparation

Solutions of commercial dyes for SERS were prepared daily at a concentration of 10^{-4} M.

Tyrian purple, which is insoluble in water and most organic solvents, was dissolved in *N*,*N*-dimethylformamide by heating at 70 °C for 5 min, while madder, alizarin and purpurin solutions were prepared in methanol. Whenever necessary, solutions were filtered through a 0.45-μM GHP Acrodisc membrane filter.

Concerning the archeological samples, the purplish dye from the relics of the martyrs was directly analyzed as a solution in *N*,*N*-dimethylformamide, while the red dye from the Libyan wool thread was dissolved in few drops of methanol.

The metallic colloid was activated by inducing a partial aggregation of the nanoparticles. To accomplish this, 35 μl of NaCl 1 M was added to 1 ml of the silver colloid, before or after the addition of analyte according to the different cases, as discussed in the following; alternatively, 150 μl of a 0.01% aqueous solution of poly-L-lysine was added prior to the analyte, the addition of which was followed by 35 μl of ascorbic acid 1 M.

Instrumentation

SERS spectra were collected with a micro-Raman instrument, equipped with a 1800 lines/mm 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 an output laser power of about 100 mW. All SERS spectra were recorded between 2000 and 200 cm^{-1} by collecting 30 scans with an exposure time of 4 s. 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.

Micro-FTIR spectra were obtained between 4000 and 600 cm^{-1} in a diamond compressing cell with an IRT-3000 Jasco spectrometer with a 4 cm^{-1} resolution, as the average of 256 accumulations.

Scanning electron microscopy-energy dispersive X-ray (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. The samples were covered with graphite in order to make them conductive for the observation and microanalysis.

Chromatographic analyses were performed with a HPLC PU-1580 Jasco pump, equipped with LG-1580-02 Jasco gradient valve and GASTORR GT-103 solvent degasser, by using a MD 1510 Jasco photodiode-array (PDA) detector in order to obtain spectral information between 200 and 600 nm. The analyses were executed using a 25-μl injection volume, on a Supelco Discovery C18 column (25 cm × 4.66 mm, 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, with a flow of 1 ml/min. Parameters for gradient elution are reported in Table 1.[16]

Table 1. Parameters for HPLC gradient elution

Time (min)	%A	%B
0	95	5
25	70	30
30	40	60
33	40	60
38	5	95
65	95	5

Results and Discussion

In previous studies on SERS spectroscopy of textile dyes, silver colloids have been usually employed with satisfactory results, mostly by using potassium nitrate in order to induce the partial aggregation of the metal nanoparticles. In the present work we found that, among the tested electrolytes, NaCl 1 M gave the best results in most cases. First of all, we tried to prepare samples according to the most common method reported in the literature, consisting in the addition to the colloid first of the chosen electrolyte and subsequently of the analyte; however, this procedure had to be modified in order to obtain the best results in the two cases described in the following.

Case study 1 - identification of Tyrian purple on archeological bones

In a previous work,[8] micro-FTIR and X-ray fluorescence analysis on the purplish bone fragment found in the tomb of the martyrs Gervase and Protase in the Basilica of Sant'Ambrogio (Fig. 1) allowed us to identify the dye as Tyrian purple. Micro-Raman spectroscopy was also applied in a nondestructive manner on the same sample, but just weak signals on a fluorescence background only could be obtained in resonance conditions using the 457.9 nm line as excitation wavelength, while the use of the 532 nm excitation line, even though closer to the absorption maximum, yielded only the fluorescence band.

At first, the SERS spectrum of the reference commercial dye showed a very weak response for its chromophore 6,6′-dibromoindigotin, especially when compared with the spectrum obtained for unsubstituted indigo (Fig. 4(c)) under the same experimental conditions. This is probably due to the steric hindrance associated with the two Br atoms, which makes the interaction of –NH groups with the metal surface more difficult; because of this fact, we could also observe in the recorded

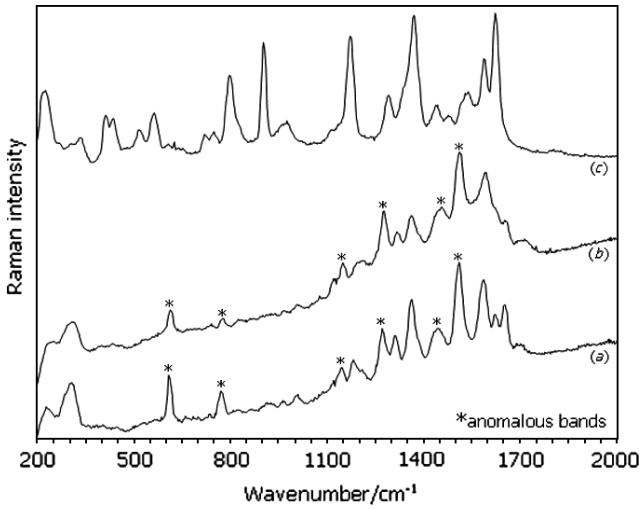

Figure 4. SERS spectra (λ_{exc} = 532 nm) of: (a) 150 µl of DMF extract of a purplish bone fragment from the tomb of the martyrs Gervase and Protase in the Basilica of Sant'Ambrogio, Milano; (b) 150 µl of a DMF solution of commercial Tyrian purple, both added to 1 ml of Ag colloid prepared according to the Lee–Meisel procedure, with subsequent addition of 35 µl of NaCl 1 M; and (c) 150 µl of a MeOH solution of commercial indigo, added to 1 ml of Ag colloid prepared according to the Lee–Meisel procedure, after the addition of 35 µl of NaCl 1 M.

spectrum of Tyrian purple many anomalous bands probably due to the preferential absorption of citrate oxidation products, as these were noticed also in the absence of the analyte. As suggested in the literature,[17] this problem can be reduced by the use of poly-L-lysine and ascorbic acid as aggregating agents, but we noticed that also the addition first of the analyte and subsequently of NaCl 1 M to the colloid, in an inverted order when compared to the most widely used procedure, can be of help in limiting the intensity of such spurious bands. The chromophore signals are thus observed at 1651, 1620, 1586, 1360, 1311, 1211, 1180 cm^{-1} (Fig. 4(b)). These wavenumbers correspond to those reported in the literature for the normal Raman spectrum of 6,6′-dibromoindigotin,[18] even though obviously the relative intensities of the bands are different in the SERS spectrum. The real effectiveness of the SERS method was confirmed by the identification of Tyrian purple on the purplish bone fragment found in the tomb of the martyrs Gervase and Protase (Fig. 4(a)), which was in good agreement with the reference commercial material.

The HPLC-UV-PDA analysis (Fig. 5) showed a different composition for the ancient and modern pigments, suggesting that they derive from two different species of shellfish: Tyrian purple from the purplish archeological bone, containing a larger amount of 6-monobromoindigotin, should derive from *M. trunculus*, which was indeed the main source of the dye in ancient times according to the literature, while the reference commercial pigment, the primary component of which is 6,6′-dibromoindigotin, should be an extract from *Nucella lapillus*.[19] Of course, we cannot exclude the fact that the relative amounts of the two bromoindigotins changed as a consequence of the dyeing technology upon exposure of the vat dye bath to sunlight as suggested in the literature.[10,20] The significant amount of 6-monobromoindigotin in the ancient dye could also support the interpretation of SERS spectra (Fig. 4), as the bands at 1620, 1360 and 1180 cm^{-1} of the archeological sample are more intense than those of the modern dye, suggesting that the interaction of –NH groups with the metal surface is in the first case easier because of the presence of a single Br atom. It is interesting to note that these are also the most prominent bands in the SERS spectrum of unsubstituted indigo. The spectrum of archeological Tyrian purple also shows a higher relative intensity of the band at 1651 cm^{-1} when compared with the modern dye, probably due to a slightly different orientation of the molecular plane with respect to the metal surface.

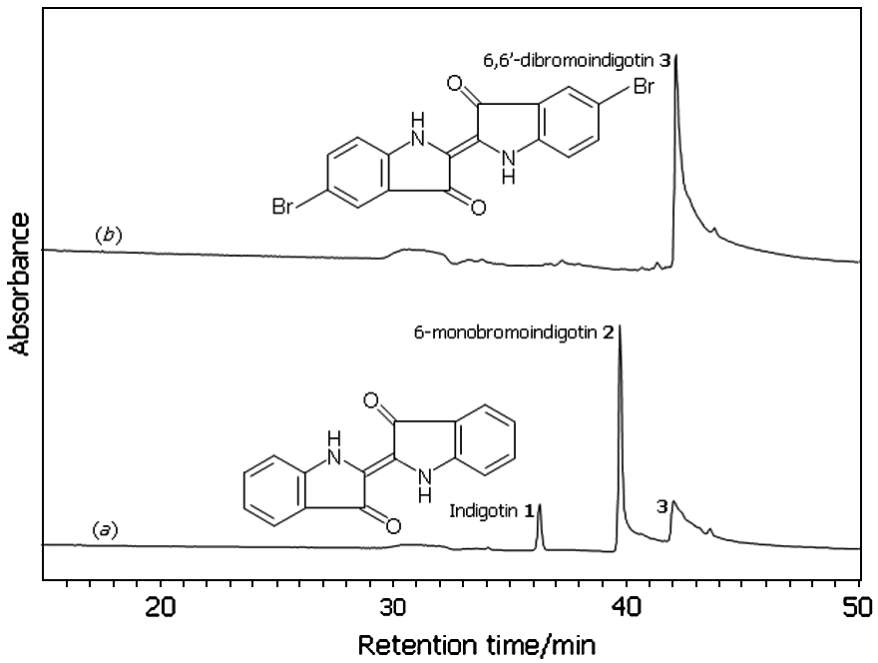

Figure 5. HPLC-UV-PDA chromatograms of: (a) 25 µl of DMF extract of a purplish bone fragment from the tomb of the martyrs Gervase and Protase in the Basilica of Sant'Ambrogio, Milano; (b) 25 µl of a DMF solution of commercial Tyrian purple. The identified compounds are (1) indigo, (2) 6-monobromoindigo and (3) 6,6′-dibromoindigo.

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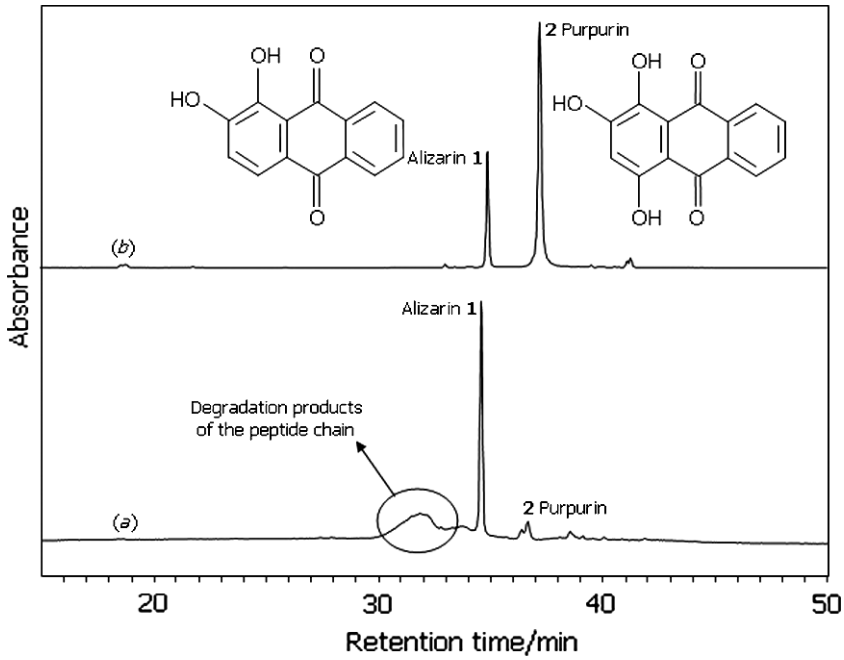

Figure 6. HPLC-UV-PDA chromatograms of: (a) 25 µl of MeOH solution of commercial madder; and (b) 25 µl of MeOH solution of the extract obtained from a red-dyed wool thread found in the Royal Tumulus of In Aghelachem, Libya. The identified compounds are (1) alizarin and (2) purpurin.

Case study 2 – identification of madder in ancient red wool threads

The HPLC-UV-PDA chromatogram (Fig. 6) of the dye extracted according to the traditional MeOH/HCl procedure from the red wool thread found in the Royal Tumulus of In Aghelachem (Fig. 2) showed the presence of alizarin and purpurin, clearly suggesting that the sample was dyed with madder.

The SERS analysis, on the contrary, did not show for such extract any signal in the experiments done with the same procedure used for the reference commercial substances (35 µl of NaCl 1 M and subsequently 150 µl of the analyte solution added to 1 µl of silver colloid). This kind of problem, observed also in other studies,[1] has been attributed to the aggressive method of extraction, which is supposed to partially hydrolyze the proteinaceous wool substrate, leading to the formation of products that inhibit the interaction between the dye and the metal nanoparticles. This hypothesis seems to be correct also in the present case, as the micro-FTIR spectrum (Fig. 7) of the same extract essentially corresponds to that of the thread not subjected to extraction, showing the typical bands of the peptide chain; similar spectra were obtained for anthraquinone dyes used in some paintings, leading to the assumption that the dye had been extracted from a textile.[21] Also, the presence of a large peak in the HPLC-UV-PDA chromatogram at a retention time around 30 min (Fig. 6) can be related to the presence of such degradation products of the peptide chain. A milder extraction method based on the use of HF was suggested[1,22] in order to obtain an SERS spectrum of the dye, but in the present case it was sufficient to apply one of the alternative methods tested in recording the spectrum of Tyrian purple, i.e. the use of 150 µl of a 0.01% aqueous solution of poly-L-lysine and 35 µl of ascorbic acid 1 M as aggregating agents, to observe for the archeological sample the typical bands of madder (Fig. 8d). The main signals are located at 1637, 1553, 1478, 1411, 1323, 1162, 656 cm^{-1}, in agreement with the spectra of reference

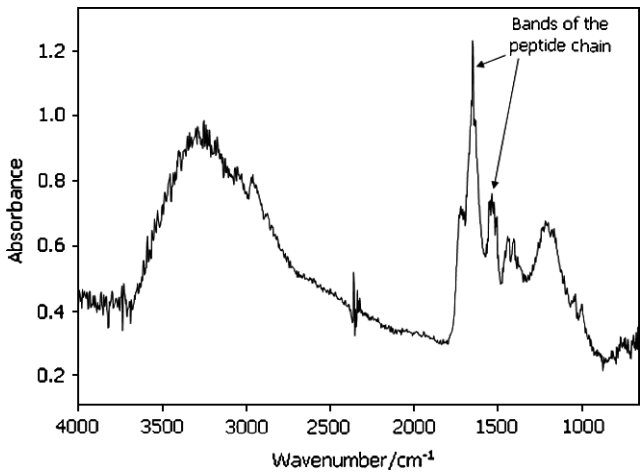

Figure 7. Micro-FTIR spectrum of the extract obtained from a red-dyed wool thread found in the Royal Tumulus of In Aghelachem, Libya.

commercial substances (Fig. 8a–c) and with the spectral patterns reported in the literature.[23–27]

The SEM-EDX analysis (Fig. 9) of the wool thread showed, besides the presence of sulfur due to amino acids, traces of metals such as Al, Mg, Fe and Cu possibly related to the mordant used to fix the dye on the fibers.[28]

Conclusions

We successfully applied SERS spectroscopy on silver colloids to the identification of two natural organic dyes, Tyrian purple and madder, as reference commercial materials and verified the real effectiveness of the method when applied to the detection of dyes extracted from ancient samples. In particular, we verified the good performance of NaCl 1 M as an alternative aggregating

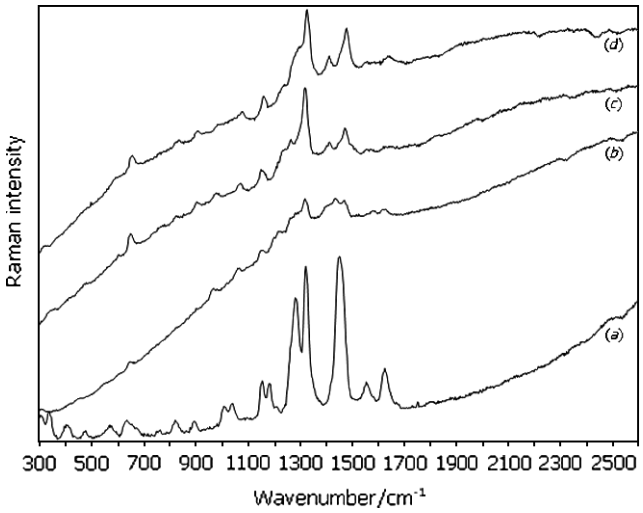

Figure 8. SERS spectra (λ_{exc} = 532 nm) of: (a) 150 µl of MeOH solution of commercial alizarin (10^{-4} M); (b) 150 µl of MeOH solution of commercial purpurin (10^{-4} M), both dyeing principles of madder; (c) 150 µl of MeOH solution of madder isolated from the commercial mordanted extract of *Rubia tinctorum* root; and (d) 150 µl of MeOH solution of the acid extract obtained from a red-dyed wool thread found in the Royal Tumulus of In Aghelachem, Libya, added to 1 ml of Ag colloid prepared according to the Lee–Meisel procedure. Spectra (a), (b) and (c) were obtained using for aggregation 35 µl of NaCl 1 M, while 150 µl of 0.01% poly-L-lysine and 35 µl of ascorbic acid 1 M had to be used as aggregating agents in order to obtain spectrum (d).

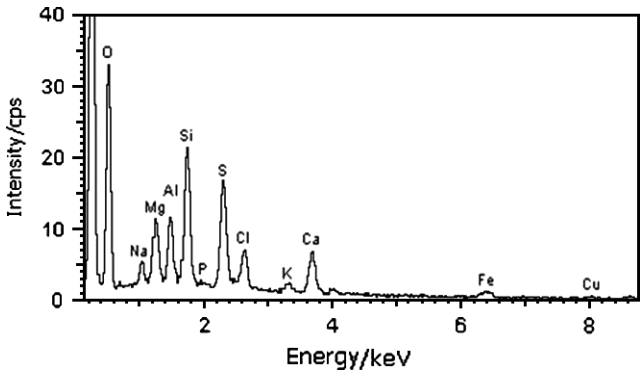

Figure 9. SEM-EDX analysis of the red-dyed wool thread found in the Royal Tumulus of In Aghelachem, Libya.

agent in comparison to those described in previous works. The aggregation of the Ag nanoparticles by poly-L-lysine and ascorbic acid, already suggested in the literature, but also a different order of addition of the analyte and the electrolyte to the colloid, was exploited to optimize the application of the SERS method to this kind of analyses. These procedures were especially useful when the interaction between the analyte and the metal particles was difficult due to the steric hindrance of the molecule, as in the case of Tyrian purple, or the competition with other substances in the solution, such as the hydrolysis products of proteins, as in the case of madder extracted from the wool thread.

Moreover, the present work allowed us to obtain, for the first time, the SERS spectrum of Tyrian purple and also to highlight, on the basis of this spectrum, how the observed variations of relative intensities of the bands can be related to two different shellfish sources for the ancient and modern dyes, in good agreement with available historical information.

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