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Surface enhanced Raman spectroscopic investigation of orchil dyed wool from *Roccella tinctoria* and *Lasallia pustulata*

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In this work Raman spectroscopic techniques have been utilized to characterize the vibrational spectral features of orchil dyed wool samples. Specifically, it is noted by surface enhanced Raman spectroscopy that wool dyed purple with two historically used orchil species (*Roccella tinctoria* and *Lasallia pustulata*) show spectral differences possibly owing to their specific dye-precursor constituents. The additional natural dyestuff woad (*Isatis tinctoria* L.) overdyeing the *R. tinctoria* orchil dyed wool is a further challenge when distinguishing the mixed dye components given by the co-adsorption of the dyestuffs as permitted by the selection rules of surface enhanced Raman spectroscopy. Furthermore, the effects of dilution of the *L. pustulata* species in its spectral detection have been assessed along with the evaluation of subsequent lichen extract boiling before dyeing which resulted in the detection of a degraded form of the orchil dye. Proof of concept included the surface enhanced Raman spectroscopy (SERS) investigation of a purple dyed tapestry (XVI century) which permitted an aged orchil dye to be determined. This contribution utilizes SERS as a fast, reproducible and specific method for both orchil dye detection and alteration induced by degradation. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: orchil dyed wool; surface enhanced Raman spectroscopy; dye co-adsorption; lichen thermal degradation; lichen species characterization

Introduction

Considering the limited concentration of organic dyestuff which may be present in an artistic textile of cultural heritage importance together with the nowadays justified caution over sampling, less invasive identification methods are becoming increasingly preferential. A variety of non destructive analytical techniques and protocols have in the last years been successfully employed for the *in-situ* identification and conservation evaluation of textile threads, mordants and dyestuffs including, X-ray fluorescence, UV–Vis fluorimetry, infrared and Raman spectroscopy.[1–4] However, it is the more destructive/micro-destructive techniques which have been established as methods of choice for the analysis and identification of organic dyestuffs and include chromatographic techniques, mass spectrometry and thermally assisted hydrolysis and methylation.[5,6]

The investigation of natural organic colorants of cultural heritage importance by Raman spectroscopy for the elucidation of historical, artistic and technical information is noted by the vast wealth of literature in the field over the last decades coinciding with instrumental advancements.[7,8] However, the success of Raman can often be limited when working with organic materials due to the large fluorescence signals which can effectively cover the characteristic and often weak scattering. This is even more pronounced when dyestuffs have been mordanted forming complexes with metal ions[9,10] or when minute concentrations of dyestuffs are present. Surface enhanced Raman spectroscopy (SERS) instead has provided an excellent tool for the simultaneous amplification of Raman scattering and quenching of fluorescence signals by the absorption of the dyestuff/pigment under study on specifically prepared nanostructures.[11] Still a contemporary technique, SERS has been adopted widely in the cultural heritage field since the turn of the century for the study of natural (animal or plant origin) and synthetic organic colorants, dyestuffs (mordant, vat and direct dyes) and lake pigments which are present in archaeological objects, paintings and indeed historic textiles. Numerous SERS methods have been proposed and utilized ranging from the application of silver colloids (with multiple preparative methods involving wet chemistry and *in-situ* photo-reduction) to films, with no sample pre-treatments and various hydrolysis methods that are carried out directly on the sample of interest or on micro-samples.[12–16] Current research trends in SERS in the cultural heritage field are aiming towards measurements with portable instrumentations[17] and less invasive

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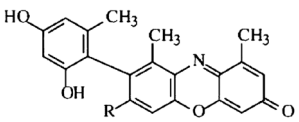
measurements with gel substrates[18–20] which may lead to future *in-situ* campaigns. However, a common agreement to all these studies is in the compilation of in-house reference databases and controlled procedures for nanoparticle production, stability and SERS execution methods which have been shown to vastly strengthen the reproducibility of measurements.

Orchil is a purple dye of lichen origin whose historical terminology is uniquely confusing derived both from country of provenance and botanical source leading to the naming variants orcein, cork, cudbear, oricello, litmus and so forth.[21] It has been known and used since antiquity for dyeing, primarily wool, often alone and in combination with other dyes in substitution of the regal Tyrian purple.[22] It is given that lichen dyes do not require a mordant as they are substantive, yet the use of mordants and various extraction methods permits colour variations to be achieved. This dye does not afford high lightfastness, and probably for such a reason its confirmation in artworks remains limited, although current instrumental advances and acute scientific interest signify forthcoming identifications.[23–26] The main precursors of the colouring matter, orcein, are esters, depsides or depsidones.[22] It is in the preparation of the lichens by methods such as immersion in ammonia-rich solutions that the depside or depsidone components are hydrolysed to orsellinic acid which in turn yields orsinol through its decarboxylation. It is through subsequent condensation reactions with nitrogen from ammonia that the main orcein components are formed.[27] Separation by chromatographic methods has been shown to lead to the elucidation of the main components: α, β and γ amino- and hydroxy-orcein as shown in Table 1.[28,29]

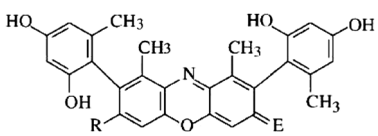
In this paper, six orcein dyed wool samples are examined and characterized by Raman/SERS spectroscopy. In particular, two orchil species from *Roccella tinctoria* and *Lasallia pustulata* are investigated to highlight any spectral differentiation. The vibrational contributions of dual components with a second dyeing on the *R. tinctoria* orchil dyed wool with woad (*Isatis tinctoria*) are examined. Furthermore, the detection limits of the *L. pustulata* species on wool are analysed on variable dyestuff dilution. Finally, the effects of subsequent heating of the same lichen extract in after-baths as a further dye preparative method will also be assessed.

Table 1. Principal components of orcein

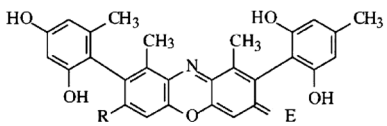
Molecular constituents of orcein



α-Amino orcein R=NH_2
α-Hydroxy orcein R=OH



β-Amino orcein R=NH_2, E=O
β-Hydroxy orcein R=OH, E=O
β-Amino orcein imine R=NH_2, E=NH



γ- Amino orcein R=NH_2, E=O
γ-Hydroxy orcein R=OH, E = O
γ-Amino orcein imine R=NH_2, E=NH

Experimental

Raman instrumentation

A Jasco Ventuno with a Peltier-cooled CCD detector and a Nd-YAG laser with excitation at 532 nm was utilized. Spectral resolution was maintained at 2 cm^{-1} over the range of 150–2000 cm^{-1}, with a laser power of 5–10 mW and acquisition times of 1–5 s and three to five accumulations. All spectra were calibrated with polystyrene.

Nanoparticle preparation and SERS analyses

Citrate-reduced silver colloids were prepared according to the Lee and Meisel procedure[30] by the reduction of silver nitrate (Aldrich 99.9%) with sodium citrate (Aldrich 99%). The prepared colloid gave an absorption maximum at 426 nm and full width at half maximum of ~110 nm, as measured with a Hewlett Packard 8453 photodiode array UV–Vis spectrometer (on 1:9 dilution with ultrapure water to observe maximum absorbance within the instrumental range). The SERS measurements were carried out by adding a 5-μl drop of colloid aggregated with magnesium sulphate[31] directly onto the wool samples, where a constant quality of spectra were obtained between 2 and 5 min permitting the colloid drop to be removed before complete evaporation. All spectra have been baseline corrected.

Dyed wool samples

The dyed wool samples were prepared by Dominique Cardon. The *R. tinctoria* used in this study was obtained by Beatriz Ballester, from the Canary Islands, expert in the natural and local history of uses of local orchil-producing lichens. *L. pustulata* was grown and collected by D. Cardon, in the mountains of Cévennes (Gard, France) on granitic rocks, at an altitude of 650 m above sea level. The recipes for the preparation of the lichens to the dyeing methods and procedures (Table 2) are as follows:

Preparation of lichens

- *R. tinctoria* from the Canary Islands was shredded and mixed in a solution of ammonia/water 1:3 for a period of 3 weeks.
- *L. pustulata* (25 g) from Cévennes, France was shredded and mixed in a solution of ammonia/water 1:3 for a period of 3 weeks. All solutions were maintained in a warm environment and regularly aired to favour the reaction with oxygen.

Preparation of overdyeing with woad

- Couched woad was powdered and soaked in stale urine for 3 days at 50 °C.

Table 2. Wool samples dyeing methods and procedures

Orchil dyed wool samples	Dyeing preparation details
S1 *Roccella tinctoria*	Washed wool was placed in a 50 °C orcein/water solution (1:2) for 15 min, removed and rinsed with cold water.
S2 *Roccella tinctoria* + woad (*Isatis tinctoria*)	Washed wool was placed in a 50 °C orcein/water solution (1:2) for 15 min. Unrinsed, the wool was placed in the woad vat solution for 15 min, removed and rinsed with cold water.
S3 *Lasallia pustulata*	Washed wool was placed in the orcein/water solution (1:2) for 15 min at 80 °C, removed and rinsed with cold water.
S4 *Lasallia pustulata* diluted	Washed wool was boiled at 80 °C in the orcein/water solution (1:4) for 15 min, removed and rinsed with cold water.
S5 *Lasallia pustulata* afterbath 1	Washed wool was brought to 80 °C in the S4 afterbath, orcein/water solution (1:4) for 15 min, then more water was added to the solution (1:6) and boiled for a further 15 min. The sample was removed and rinsed with cold water.
S6 *Lasallia pustulata*- afterbath 2	Washed wool was brought to 80 °C in the S5 afterbath, orcein/water solution (1:6) for 15 min, then more water was added to the solution (1:8) and boiled for a further 15 min. The sample was removed and rinsed with cold water.

Results and discussion

When examining historically dyed textiles with Raman spectroscopy, it is often possible to experimentally note three main origins to the bands, that is when fluorescence is not overwhelming. First, the observance of characteristic bands resulting from only the textile substrate could indicate a low dye coverage. Second, signals acquired from only the dyestuff could indicate the presence of colorant free from any form of complexation, as mordant, confirmed when the spectrum is compared to the reference colorant powder, or a complexed form of the colorant when spectral modifications are noted. Third, when dual signals derived from both the dye and the textile are simultaneously observed.[32] In our specific case, any useful conventional Raman scattering signals derived from the wool, the dyestuff and indeed the wool/dye complex for the orchil dyestuff on wool were completely covered by fluorescence. For such reason, the SERS Raman technique was utilized adopting a non hydrolysis *in-situ* application of silver colloid directly on the wool substrate of interest. As this measurement could not be carried out in a totally non destructive manner, it was feasible to apply the aggregated colloid whose amount (5 μl) permitted adequate interaction with the wool sample and allowed reproducible spectra to be recorded over a short period of time. Following such, it was advantageous to render this approach less invasive by removing the remainder colloid before total solvent (water) evaporation so that at least some of the silver could be removed and importantly no visible stain could be noticed afterwards. Ulterior working methods highlighted in literature could involve further restricted amounts of colloids for equally successful less invasive measurements.[15,33]

The SERS spectra of wool dyed with orchil from the *R. tinctoria* (S1) and the *L. pustulata* (S3) species may be observed in Fig. 1 where bands from the wool substrate are negligible and anomalous colloid bands are highlighted with an asterisk. On comparison with the SERS of the reference orcein powder (Aldrich), which coincides respectably with that noted in literature with excitation wavelengths at 785 nm[15] and more recently that acquired under FT-Raman conditions (1064 nm)[34] (Fig. 1a), the two different species observe a similar spectrum across the entire range with tentative assignments in Table 3.[35] It may be given that the interaction of the dye with the silver nanoparticles is principally through the carbonyl group as observed by the most intense peak at 1637–1647 cm^{-1}. According to SERS selection rules, the enhancement of the Raman modes indicates a preferably perpendicular orientation of the adsorbed analytes on the nanoparticles.[36] It is plausible to hypothesize that the spectral similarities between orcein powder, S1 and S3 are due to characteristic modes of vibration of the α, β and γ amino- and hydroxy-orcein components which are common to all the investigated materials. It follows then that any spectral differences should

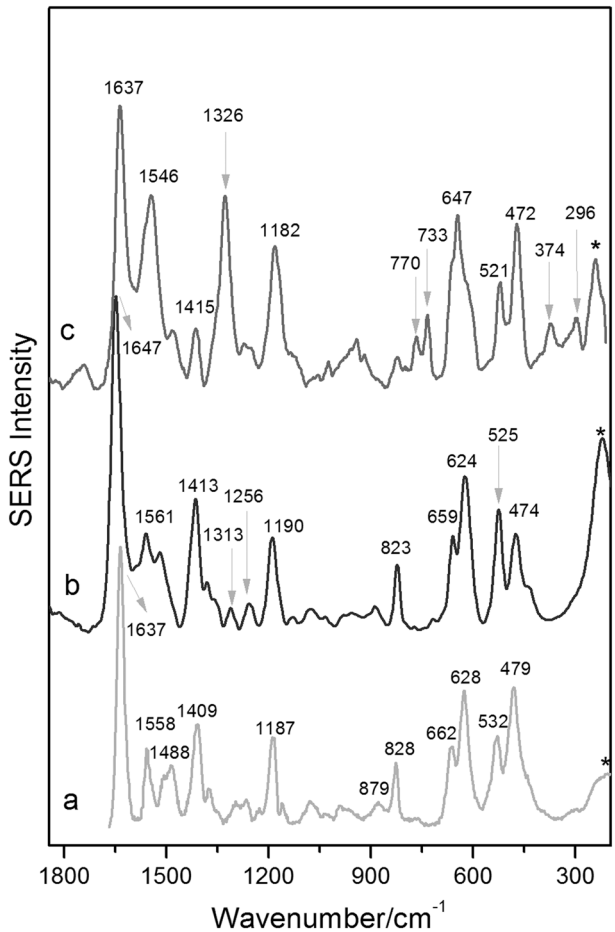


Figure 1. SERS spectra of a) orcein reference, b) S1 orchil dyed wool from *Roccella tintoria* and c) S3 orchil dyed wool from *Lasallia pustulata*.

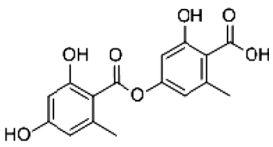
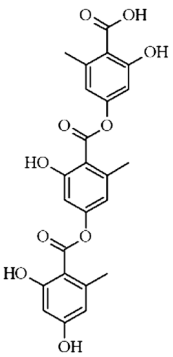
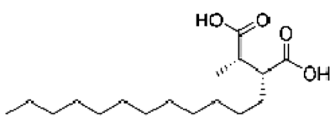
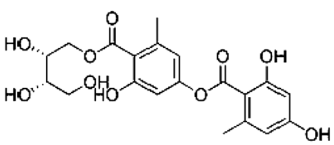
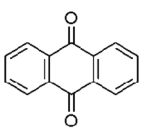
Table 3. SERS of orcein reference and orcein dyed wool

Orcein (Aldrich) SERS	Dyed wool *Roccella tinctoria* SERS	Dyed wool *Lasallia pustulata* SERS	Approximate assignments
1637 vs	1647vs	1637vs	ν(C=O, ester) δ(OH)
1558 m	1561m	1546m	ν(C=C) aromatic
1512 w	1518w		ν(C=C) aromatic
1488 m		1480sh,m	δ(NH), ν(C=C) aromatic
1409 s	1413s	1415m	$\delta(CH_2)$ $\delta(CH_3)$
1373 d, w	1379sh, w 1361sh, vw 1313m	1326vs	Ring stretches Possible anthraquinone
1300 w			Ring str.
1268 w	1256m	1251m	ν(C—O)
1187 s	1190s	1182s	ν(C—O), ν(CC), ν(CN)
1079 w	1075vw		ν(C—O), ν(CC), ν(CN)
994 w		943w	
879 w	889w		ν(CC)
828 m	823m	823m 770m 733m	ν(CC), ν(CN) δ(CCN) δ(CNC)
662 d, s	659d,s	647s	ν(CC), ν(CN) δ(CCN) δ(CNC)
628 vs	624vs	616sh	δ(CCO)
532 d, s	525d, s	521d,s	δ(COC)
479 vs	474s 435sh,w	472vs 374m 296m	ν(CC), ν(CN) δ(CCN) δ(CNC)

a vs, very strong; s, strong; m, medium; w, weak; vw, very weak, sh, shoulder. *b* ν, stretching (s, symmetric; as, asymmetric); δ, bending; γ,out-of-plane deformation; d, doublet.

Table 4. Ulterior (minor) components found in *Roccella tinctoria* and *Lasallia pustulata* species

Ulterior components beyond orcein in species examined[36,37]

Roccella tinctoria	*Lasallia pustulata*
	
Lecanoric acid	Gyrophoric acid
	
Roccellic acid	
	
Erythrin	Anthraquinone basic skeleton

depend on both the dyestuffs molecular interactions with the wool and the presence of ulterior components which are specific to each species and could conceivably be used as key markers for their respective identification.[37,38] Considering this, sample S1 (*R. tinctoria*) (Fig. 1b) observes minor differences to the orcein reference powder as noted by the additional bands at 1313 cm^{-1} and 1256 cm^{-1}. A change too in the relative intensity of the band at 525 cm^{-1} can also be appreciated. In literature, the lichen *R. tinctoria* species is reported to contain roccellic acid ($C_{19}H_{20}O_7$) as well as the para-depsidone erythrin ($C_{20}H_{22}O_{10}$), and lecanoric acid ($C_{16}H_{14}O_7$) of which the latter is given to a 15% composition[39] and where the molecular formulae may be observed in Table 4. On comparison with its Raman spectrum, a possible attribution can be made with the most intense bands of lecanoric acid at 1654 cm^{-1}, that in this work is covered by the orcein itself, yet the visible bands at 1311 cm^{-1} assigned to the $\beta(CH) + \delta(OH) + \nu(CC) + \nu((C—O)$, 1255 cm^{-1} due to the $\beta(CH)$ and 532 cm^{-1} the most intense band of a triplet given by the δ(ring) are all observed.[37] Sample S3 (*L. pustulata*) (Fig. 1c) differs notably from the aforementioned species due to the appearance of a singlet at 1544 cm^{-1}, and a strong band at 1329 cm^{-1}. Other minor doublet bands at 770, 733 and 374 and 296 cm^{-1} aid in distinguishing this species on wool. The *Lasallia* species instead is reported to contain gyrophoric acid ($C_{24}H_{20}O_{10}$) and anthraquinones ($C_{14}H_8O_2$) (Table 4) which could very well have given rise to these ulterior bands especially the anthraquinone signature (ν(CC)) at 1326 cm^{-1} which is not, however, sufficient for its confirmation.[32,38,39]

Wool dyed with *R. tinctoria* was subsequently dyed with woad (*I. tinctoria*) yielding sample S2. *I. tinctoria* contains isatan A (1H-indol-3-yl6′-O-(carboxyacetil)-betaD-ribohex-3′-ulpyranoside and isatan B (1H-indol-3-yl beta-D-ribohex-3-ulopyranoside),[40] as a major indigo precursor and indican as a minor component. The former is converted into indoxyl by enzymatic hydrolysis and then oxidized into indigotin. It is also suggested in literature that the leaves may contain flavonoid components (quercetin and kaempferol).[41,42] The conventional Raman and SERS spectra of this sample are noted in Fig. 2a i and ii, respectively, where each dyestuff has contributed its own diagnostic modes of vibration. The characteristic modes of orcein by SERS can be observed, and in particular the distinguishing bands pertaining to the *R. tinctoria* species, including the aforementioned intense band at 525 cm^{-1} can still be appreciated in this sample along with the further bands at 1313 and 1256 cm^{-1}. The only variation to the lichen spectrum on overdyeing is the disappearance of the band at 659 cm^{-1} attributed to the $\nu(CN) + \nu(CC)$. Given such, simultaneous signals of the woad dyestuff are observed, even if notably weaker in comparison to the orcein, namely the bands at 1582 cm^{-1} given by the $\nu(C{=}C)$ and $\nu(C{=}O)$ and a slightly shifted band at 1348 cm^{-1} attributed to the δ(NH), δ(CH).[32,43] This result suggests a co-absorption of both the orcein and woad on the silver nanoparticles, where the orcein appears to retain a closer vicinity, or benefits from a higher interaction with the silver given the more predominant bands. No characteristic bands relative to a flavonoid component were observed.

It is possible to observe the SERS spectra in Fig. 3i–ii, respectively, of the *L. pustulata* dyed wool (S3) that on subsequent dilution from 1:2 to 1:4 in water gave S4. No spectral modifications are noted highlighting the sensitivity of the SERS technique on dilution of this dyestuff. When instead the afterbath S4 was utilized for the preparation of S5 by further orchil dilution to 1:6 in water accompanied by a further period of heating, spectral differences may be noted in Fig. 3iii. The bands at 296, 374 and 770 cm^{-1} have completely disappeared, and a doublet at 618 and 656 cm^{-1} is

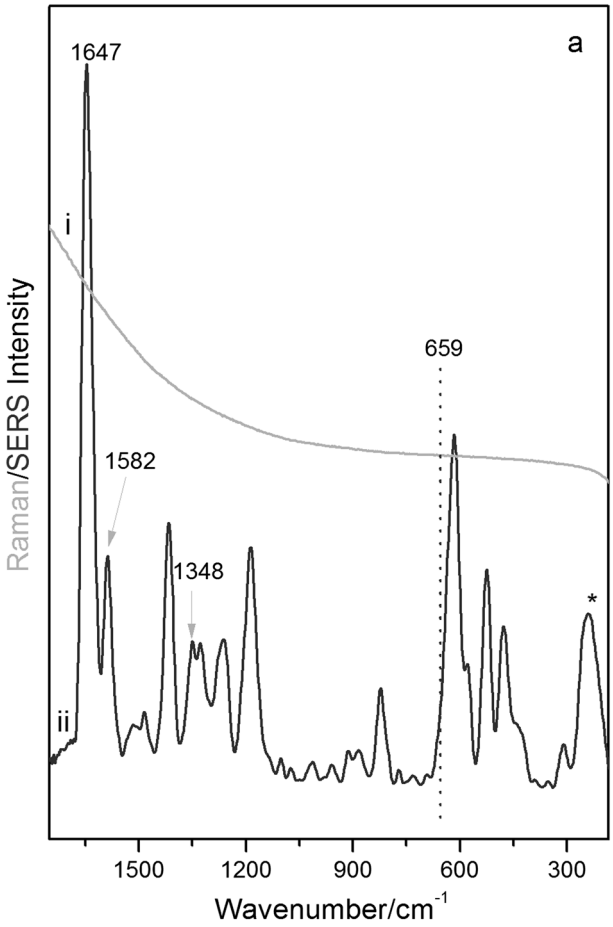


Figure 2. Orchil dyed wool Roccella tinctoria followed by a second dyeing with woad (*isatis tinctoria*) S2 i) conventional Raman spectrum and ii) SERS.

now visible along with a medium intense band at 817 cm^{-1}. It is on still further dilution (1:8 in water) with additional heating that the sample S6 is prepared giving rise to the spectrum in Fig. 3iv. The high limits of detection of orchil by SERS are evidenced, and no new bands are pronounced, although it is possible to appreciate the progressive diminishing of the bands at 472, 1182, 1326 and 1554 cm^{-1}. These generated spectral modifications, except for the band at 1329 cm^{-1} (given in this work as a possible anthraquinone component, albeit slightly shifted, of this particular orcein species), coincide with those noted by Rosi *et al.* using the subtracted shifted Raman spectroscopic technique on the artificial photoageing of orchil dyes in artworks provoking a degraded form of the orchil dye.[24]

The colloidal SERS working method was feasibly conducted on a purple region of an original artwork, a fragment of a Renaissance tapestry from Brussels (XVI century) where previous UV–Vis *in-situ* fluorimetry suggests the possible presence of orcein.[25] As observed in Fig. 4i, a conventional Raman spectrum gave rise to a predominately fluorescence background with negligible scattering, yet on application of SERS, an appreciable spectrum has been obtained (Fig. 4ii). This spectrum can be clearly identified as orcein on comparison with the principal modes of vibration of the standard references presented in Fig. 1. Furthermore, regarding the actual orchil species, it is not possible to pronounce a clear distinction as no visible precursors from this study can be highlighted; however, it is possible to observe differences in the

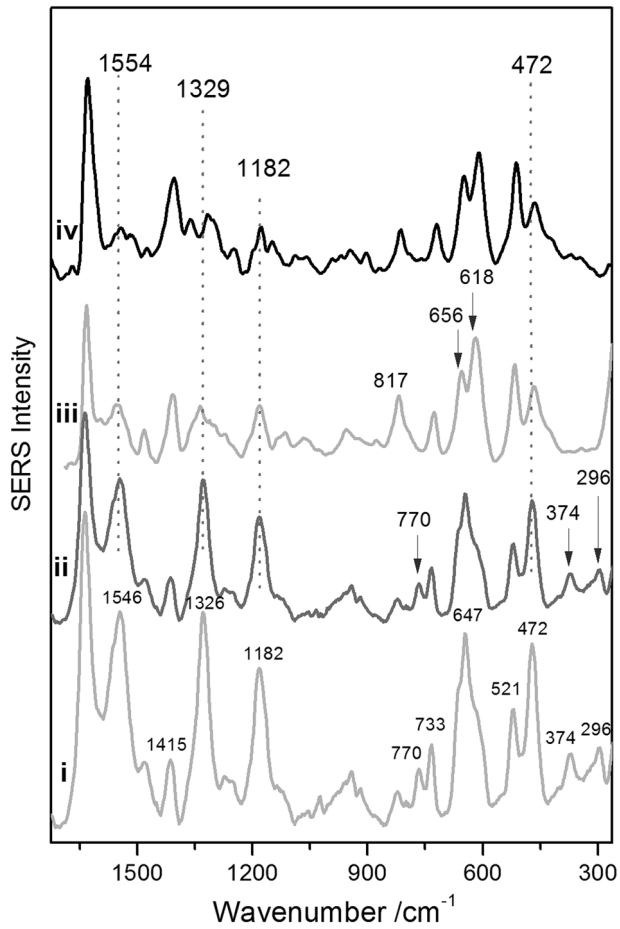


Figure 3. SERS spectra of *Lasallia pustulata* orchil dyed wool on dyeing following dilution i) 1:2 (S3), ii) 1:4 (S4), iii) 1:6 (S5) and on subsequent thermal treatment iv) S6.

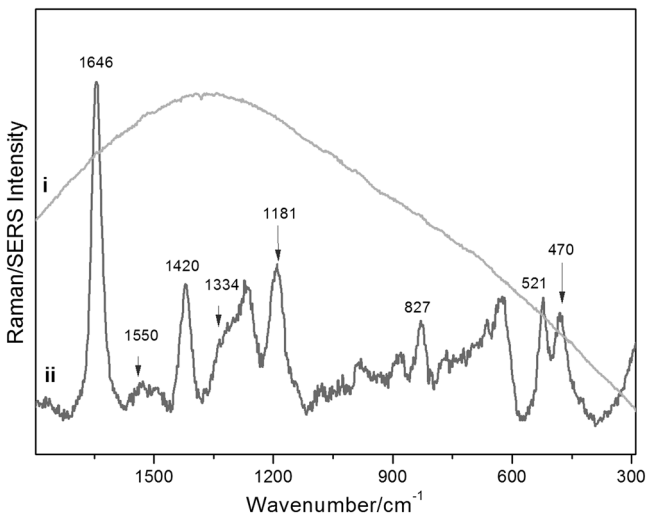


Figure 4. Investigation of a purple region of a tapestry from Brussels (XVI century) i) Raman spectrum and ii) SERS spectrum.

intensity of the bands at 470 cm^{-1}, 1181 cm^{-1}, 1334 cm^{-1} and 1550 cm^{-1}, respectively, hypothesizing a degraded form of the orchil dye utilized.

Conclusions

The exploitation of SERS as a valid micro-invasive technique for the assessment of orchil dyes on wool is shown through the reproducibility and specificity of results presented in this work. In particular, it has been attempted to distinguish the characteristic vibrational modes of two orchil species, *R. tinctoria* and *L. pustulata*, respectively, when dyed on wool where main spectral differences seemingly refer to the minor residual para-depside and anthraquinonic constituents, respectively. Interestingly, the overdyeing of the *R. tinctoria* wool with woad permitted this dyestuff to be identified together with orcein due to a co-absorption on the silver colloids. The same technique has been noted to be a feasible method for orchil dye detection even on substantial dilution of the dyestuff and furthermore on ulterior thermal ageing, where spectra gave rise to the identification of a degraded form of orchil similar to the induced by photoageing. The investigation of a purple dyed tapestry examined by SERS permitted an aged orchil dye to be determined. Overall results attained suggest that SERS could be potentially applied to a wider base of lichen species and variety within the same species for specific spectroscopic characterization and ultimately diagnostic purposes of art historic materials provenance and state of conservation.

References

[1] A. Romani, C. Clementi, C. Miliani, G. Favaro, *Acc. Chem. Res.* **2010**, *43*, 837.
[2] C. Margariti, S. Protopapas, N. Allen, V. Vishnyakov, *Dyes Pigments* **2013**, *96*, 774.
[3] M. Gulmini, A. Idone, E. Diana, D. Gastaldi, D. Vaudan, M. Aceto, *Dyes Pigments* **2013**, *98*,136.
[4] C. Clementi, B. Doherty, P. L. Gentili, C. Miliani, A. Romani, B. G. Brunetti, A. Sgamellotti, *Appl. Phys. A.* **2008**, *92*, 25.
[5] D. M. Grim, J. Allison, *Int. J. Mass Spectrom.* **2003**, *222*, 85.
[6] D. Fabbri, G. Chiavari, H. Ling, *J. Anal. Appl. Pyrol.***2000**, *56*, 167.
[7] I. M. Bell, R. J. H. Clark, P. J. Gibbs, *Spectrochim. Acta A.* **1997**, *53*, 2159.
[8] L. F. C. Oliviera, H. G. M. Edwards, E. S. Velozo, M. Nesbitt, *Vib. spectros.* **2002**, *28*, 243.
[9] G. Favaro, C. Clementi, A. Romani, V. Vickackaite, *J. Fluorescence* **2007**, *17*, 707.
[10] C. Grazia, C. Clementi, C. Miliani, A. Romani, *Photochem. Photobiol. Sci.* **2011**, *10*, 1249.
[11] F. Casadio, M. Leona, J. R. Lombardi, R. Van Duyne, *Acc. Chem. Res.* **2010**, *43*, 782.
[12] M. Leona, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 14757.
[13] C. L. Brosseau, A. Gambardella, F. Casadio, R. P. Van Duyne, C. Grzywacz, J. Wouters, *Anal. Chem.* **2009a**, *81*, 3056.
[14] Z. Jurasekova, C. Domingo, J. V. Garcia-Ramos, S. J. Sanchez-Cortes, *J. Raman Spectrosc.* **2008**, *39*, 1309.
[15] M. Leona, J. Stenger, E. J. Ferloni, *J. Raman Spectrosc.* **2006**, *37*, 981.
[16] C. L. Brosseau, K. Rayner, F. Casadio, R. P. Van Duyne, C. M. Grzywacz, *Anal. Chem.* **2009b**, *81*, 7443.
[17] A. Brambilla, A. Philippidis, A. Nevin, D. Comelli, G. Valentini, D. Anglos, *J. Mol. Struct.* **2013**, *1044*, 121.
[18] M. Leona, P. Decuzzi, T. A. Kubic, G. Gates, J. R. Lombardi, *Anal. Chem.* **2011**, *83*, 3990.
[19] B. Doherty, B. G. Brunetti, A. Sgamellotti, C. Miliani, *J. Raman Spec.* **2011**, *42*, 1932.
[20] C. Lofrumento, M. Ricci, E. Platania, M. Becucci, E. Castellucci, *J. Raman Spectrosc.* **2013**, *44*, 47.
[21] N. Eastaugh, V. Walsh, T. Chaplin, R. Siddall, in *Pigment Compendium, A Dictionary and Optical Microscopy of Historical Pigments*, Butterworth-Heinemann, Elsevier, Oxford, UK, **2008**.
[22] D. Cardon, Natural Dyes: Sources, Tradition, Technology and Science, Archetype Publications: London, **2007**.
[23] S. Bioletti, R. Leahy, J. Fields, B. Meehan, W. Blau, *J. Raman Spectrosc.* **2009**, *40*, 837.
[24] F. Rosi, C. Clementi, M. Paolantoni, A. Romani, R. Pellegrino, B. G. Brunetti, W. Nowik, C. Miliani, *J. Raman Spectrosc.* **2013**, *44*, 1451.

[25] C. Clementi, C. Miliani, A. Romani, G. Favaro, *Spectrochim. Acta A.* **2006**, *64*, 906.
[26] C. Clementi, C. Miliani, A. Romani, U. Santamaria, F. Morresi, K. Mlynarska, G. Favaro, *Spectrochim. Acta A.* **2009**, *71*, 2057.
[27] E. S. B. Ferreira, A. N. Hulme, H. McNab, A. Quye, *Chem. Soc. Rev.* **2004**, *33* 329.
[28] H. Musso, *Chem. Ber.* **1956**, *89*, 1659.
[29] H. Musso, *Planta Med.* **1960**, *8*, 431.
[30] P. C. Lee, D. Meisel *J. Phys. Chem.* **1982**, *86*, 3391.
[31] S. Bell, *J. Am. Chem. Soc.* **2006**, *128*, 15580.
[32] B. Doherty, C. Miliani, I. Vanden Berghe, A. Sgamellotti, B. G. Brunetti, *J. Raman Spectrosc.* **2008**, *39*, 638.
[33] F. Pozzi, J. R. Lombardi, S. Bruni, M. Leona, *Anal. Chem.* **2012**, *84*, 3751.
[34] C. Zaffino, S. Bruni, V. Guglielmi, E. De Luca, *J. Raman Spectrosc.* **2014**, *45*, 211.
[35] G. Socrates, Infrared and Raman Characteristic Group Frequencies Tables and Charts (3rd edn), John Wiley & Sons, Inc., New York, **2001**.
[36] M. Moskovits, J. S. Suh, *J. Phys. Chem.* **1984**, *88*, 5526.
[37] L. F. C. de Oliveira, P. C. C. Pinto, M. P. Marcelli, H. F. Dos Santos, H. G. M. Edwards, *J. Mol. Struct.* **2009**, *920*, 128.
[38] H. G. M. Edwards, E. M. Newton, D. D. Wynn-Williams, *J. Mol. Struct.* **2003**, *651*, 27.
[39] I. M. Brodo, S. D. Sharnoff, Lichens of North America, Yale University press, New Haven, **2001**.
[40] C. Oberthür, B. Schneider, H. Graf, M. Hamburger, *Chem. Biodivers.* **2004**, *1*, 174.
[41] J. H. Hofenk de Graaff, The Colourful Past, Origins, Chemistry and Identification of Natural Dyestuffs, Abegg-Stiftung and Archetype Publications, Switzerland and London, **2004**.
[42] T. Maugard, E. Enaud, P. Choisy, M. D. Legoy, *Phytochemistry* **2001**, *58*, 897.
[43] C. Coupry, G. Sagon, P. Gorguet-Ballesteros, *J. Raman Spectrosc.* **1997**, *28*, 85.