Anal Bioanal Chem (2015) 407:6505–6514
DOI 10.1007/s00216-015-8816-x

RESEARCH PAPER

Tailored micro-extraction method for Raman/SERS detection of indigoids in ancient textiles

Elena Platania[1] · Cristiana Lofrumento[1] · Elisabetta Lottini[2] · Elena Azzaro[1] · Marilena Ricci[1] · Maurizio Becucci[1,3]

Received: 5 March 2015 / Revised: 27 May 2015 / Accepted: 1 June 2015 / Published online: 17 June 2015

Abstract Indigoid dyes are well known as vat dyes. In their oxidized dichetonic form they are stable and insoluble in water, whereas in their reduced form, commonly known as *leuco*, they are soluble in water and able to be attached to fabric for dyeing purposes. These blue dyes are usually easily detectable in art objects by means of Raman spectroscopy by adopting for analyses a laser line at a high wavelength, such as a 785 nm diode laser. Unfortunately, in ancient artworks, that are often highly degraded, it is not always possible to collect high quality Raman spectra, which makes the analysis and identification of these compounds particularly challenging. In this work, we present a tailor-made methodology for the extraction and the recognition of indigoid dyes in works of art, which exploits the solubility of these compounds in their reduced form. Excellent Raman and surface enhanced Raman spectroscopy (SERS) spectra of indigo were acquired after micro-extraction on ancient and reference textiles, confirming the reliability of the presented procedure. Moreover, the methodology has been applied also for the extraction of the indigoid dye Tyrian purple on a reference textile, showing excellent results. This analytical method has been found to be extremely safe both for the reference textiles and the investigated ancient textiles, thus being a promising procedure for the selective analysis and detection of indigoid compounds in objects of artistic relevance.

Keywords Raman · SERS · Indigo · Tyrian purple · Agar gel · Ag nanoparticles

Electronic supplementary material The online version of this article (doi:10.1007/s00216-015-8816-x) contains supplementary material, which is available to authorized users.

✉ Marilena Ricci
marilena.ricci@unifi.it

[1] Department of Chemistry "U. Schiff", University of Florence, 50019 Sesto Fiorentino, Firenze, Italy

[2] Department of Chemistry "U. Schiff" and INSTM Research Unit, University of Florence, 50019 Sesto Fiorentino, Firenze, Italy

[3] European Laboratory for Nonlinear Spectroscopy (LENS), 50019 Sesto Fiorentino, Firenze, Italy

Introduction

Natural indigoids, such as indigo and Tyrian purple, were employed since ancient times both as pigments and dyes. Indigo, whose main chromophore is the molecule indigotin, is one of the most stable natural organic dyes because of its high light-fastness. It was extracted by fermentation of the leaves of the plant species *Isatis tinctoria* (woad), cultivated in Europe and extensively adopted from the 12th to the 17th century, and of the Asian species *Indigofera tinctoria*. The quality of indigo extracted from the Asian species was much brighter because of the higher concentration of indican (indoxyl-β-D-glucoside) precursor [1, 2]. Tyrian purple, also known as "Royal purple," was the most precious and expensive indigoid dye, known since pre-Roman times by the ancient Phoenicians. Its main chromophore, 6,6'-dibromoindigo, was extracted from the hypobranchial gland of sea snails of the family *Muricidae* in the Mediterranean Sea, and one shellfish was able to provide only few drops of the valuable secretion [3]. Indigoids belong to the class of vat dyes. As a matter of fact, since their dichetonic form is insoluble in water, they must be reduced to a water-soluble form, called *leuco*, in order to be attached to a fabric for dyeing purposes. In particular, the *leuco* species exists in three forms, identified respectively as vat acid (neutral form), mono-ionic, and di-ionic. The mono-ionic form, which exists at 10-11 pH

values, is the one presenting the strongest affinity for the fabric fiber [4, 5]. Indigo dye is usually easily detectable in dispersive conditions by means of Raman spectroscopy by adopting for analyses laser lines at high wavelength [6], which are sufficient to quench the fluorescence background and to detect the molecule. Unfortunately, the recognition of this compound may be quite difficult when analyzing ancient artworks, as for instance textiles, where the dye is present at very low concentrations, or paint layers, where the colorant is generally incorporated within complex pigment mixtures [7–9]. Moreover, degradation processes or bad state of conservation of the art object may further affect the analyses of the blue dye. In the last decade, the successful application of the high sensitive surface enhanced (resonance) Raman spectroscopy [SE(R)RS] for the detection of organic dyes in works of art has strongly improved the possibility of identifying fluorescent organic dyes according to minimal invasive [10–14] and microdestructive approaches [15–17]. Up to the present, the majority of the developed SERS procedures dealt mainly with the detection of red and yellow dyes [15–26], whereas only a few works have investigated the characterization of indigoids by means of SERS/SERRS. One of the main problems is that indigo is insoluble in the aqueous silver colloid dispersion and it must be treated by specific solvents prior to SERS analyses. The first semiquantitative study of indigo using SERS/SERRS was reported by Shadi et al. [27], who investigated methanolic solutions of the blue dye combined with silver colloidal dispersions aggregated by means of HCl. In the specific case of cultural heritage applications, SERS-based detection of indigo has been reported for the analysis of paintings [28], textiles [8, 29, 30], and archaeological objects [31]. Recently, a pioneering application of tip enhanced Raman spectroscopy (TERS) has also been presented for the in sit*u* identification of indigo in paper artworks [32].

In this work, we present a new methodology concerning the minimally invasive extraction of insoluble indigoid dyes in textiles and textile fibers of artistic relevance. In particular, the extraction process is achieved by means of an agar hydrogel cube of a few millimeters size, which is loaded with a drop of solution of *leuco*-reagents ($NaOH/Na_2S_2O_4$) that works as a vehicle for the dye extraction. The use of *leuco*-reagents is able to trigger the inverse dyeing process of the indigoids. In this manner, controlling the reagents concentration and contact time, a few molecules are converted into their reduced water-soluble form (*leuco*), and are easily absorbed by the gel matrix during the extraction procedure. The extracted dye is subsequently revealed by means of Raman or, in order to obtain higher quality spectra, by means of SERS, by the introduction of silver nanoparticles (AgNPs) in the gel's structure, which are able to enhance the Raman scattering. The high compatibility of agar hydrogel with the artwork's surface is well documented in the field of conservation, where it was widely and successfully applied for cleaning purposes [33]. This work demonstrates also that the structure and morphology of agar gel is not affected by the presence of the AgNPs within its structure. For this purpose, the nanocomposite matrix was observed for the first time by a helium ion microscope (HIM), in order to obtain an accurate evaluation of the pores' structure of the gel, and the size, aggregation, and spatial distribution of the AgNPs. In order to further improve sensitivity of the applied methodology, we adopted for the collection of the Raman spectra the excitation laser line at 514 nm. This wavelength is well above the first electronic transition of indigoids, allowing to work under resonance Raman conditions and near the plasmonic absorption band of the AgNPs. The described approach is a tailored procedure specific for this class of dyes and has a high potential as a valid tool for the extraction and recognition of indigoids in mixtures. The safety of the methodology was assessed by means of X-Ray fluorescence (XRF) and colorimetric analyses that confirmed the suitability of the technique for the identification of indigoids in textiles. The developed protocol is extremely fast and easy to be applied, and the extraction process has the advantage of being performed in situ. Moreover, using a gel as a medium for the solvent mixture allows for a more localized and controlled micro-extraction essay, which involves the analysis of extremely small areas of the object under study. The procedure was tested first on reference laboratory textiles dyed with indigo and, subsequently, once the safety of the procedure was assessed, it was applied on ancient textile fibers of artistic relevance. The methodology was also applied on a reference textile dyed with Tyrian purple showing excellent results.

Materials and methods

High purity natural indigo and Tyrian purple were purchased from Zecchi, Florence, Italy (Fig. 1). Tyrian purple was obtained from the hypobranchial gland of the mollusk *Murex trunculus* harvested in the area of Dubrovnik, Croatia. Sodium hydroxide (NaOH) and sodium dithionite ($Na_2S_2O_4$) were provided by Merck (Darmstadt, Germany). $AgNO_3$ (99.9 %), trisodium citrate, and tridistilled water (HPLC gradient grade) were purchased from Sigma-Adrich (St. Louis, Missouri, USA). Common grade agar-agar in flakes was used for the synthesis of the hydrogel.

Three textiles of artistic relevance were investigated. A precolombian piece of cotton textile (private collection), a fragment of a XV^{th} century cotton tablecloth of Italian manufacture (Museo del Tessuto, Prato, Italy), and a blue thread of a Medici's tapestry in wool, silk, silver, and gilded silver of the XVI^{th} century (cartoon of Bronzino) depicting Joseph escaping from Potiphar's wife (Palazzo Vecchio, Sala dei Duecento, Florence, Italy restored by Opificio delle Pietre Dure, Florence).

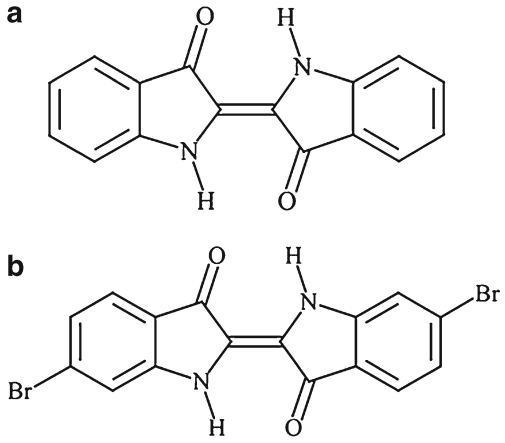

Fig. 1 Indigoids's molecular structures: **(a)** indigo; **(b)** 6,6′ dibromoindigo

Indigo

Pieces of cotton were dyed according to the recipe reported by Schweppe [34]. The dyeing liquor was prepared by stirring 15 g of indigo powder with 75 mL of warm water in a beaker glass until it formed a paste. In a second vessel, 30 g of sodium hydroxide, NaOH, were dissolved in 120 mL of warm water; 60–70 mL of this solution was poured over the indigo paste, stirring vigorously. Then, 30 g of sodium dithionite, $Na_2S_2O_4$, was added with continuous stirring, and 1 L of warm water was added stirring carefully. This mixture was heated to 55 °C. Undyed pieces of cotton were immersed in warm water until the fabric was thoroughly wet. The fabric was then put inside the dyeing liquor and kept in the dye-bath for a few minutes. The pieces of cotton were then taken out of the vat, squeezing the liquor out thoroughly. When the fabric comes out of the vat, it has a typical green-yellow color, which turns blue when exposed to air.

Tyrian purple

The dyeing procedure adopted was similar to the previous one used for indigo, using a colorant/NaOH/$Na_2S_2O_4$ ratio equal to 1:2:4. Since Tyrian purple is a mixture of brominated indigoids, 6-bromoindigo (MBI) and 6,6′ dibromoindigo (DBI), it is subject to debromination because of the action of UV-light. For this reason, when the reagents were heated to 55 °C, the light was turned off and the flask was wrapped in aluminum foil. A yellow-greenish homogeneous solution was obtained, and the wet fabric was put into the dye-bath for 15 min. Then it was removed from the flask and exposed to air, while still protected from light. After 1 h of air exposure, the fabric was rinsed in aqueous solution and allowed to dry.

Agar and Ag-agar gel synthesis

Glassware was cleaned with HNO_3. Agar gel was synthesized according to the procedure previously published [10]. In brief, 0.2 g of agar-agar in flakes was mixed in a beaker with 10 mL of water or with silver colloidal water dispersion to obtain the agar gel or the Ag-agar gel, respectively. The mixture was then heated in a common microwave oven for a few seconds at the lowest heating power and then poured into a Petri dish. Once cooled, the Petri dish containing the gel was stored in the dark inside a sealed glass container. In these storage conditions, the gel was found to be stable for up to 6 mo. The colloidal solution used for the synthesis of Ag-agar gel was prepared according to the Lee and Meisel procedure [35].

Ag-agar gel preparation for HIM imaging

Ag-agar gel was sliced into a pellet of $5 \times 5 \times 5$ mm^3 in a way to expose the inner structure. In order to minimize sample perturbation and maximize the preservation of the gel structure, the section of Ag-agar gel was dehydrated in a graded ethanol series from water through 50 %-75 %-95 %-100 %-100 % ethanol. Subsequently, the sample was dried by liquid CO_2 replacement of ethanol in a K850 (Quorum Technologies, Laughton, East Sussex, UK) critical point drying apparatus.

Micro-extraction procedure by means of *leuco*-reagents

In order to accomplish the micro-extraction (Fig. 2), a small cube of about $4 \times 4 \times 4$ mm^3, easy to handle, was cut off from the agar hydrogel by means of a spatula. A smaller agar cube can also be used for the micro-extraction, being the microRaman analysis area about 2 μm. A drop of 20–30 μL of *leuco* solution (NaOH/ $Na_2S_2O_4$ 1:2 w/w) was then added on the top of the cube and, after a couple of seconds, placed on the point of interest of the artwork surface. During the dye's extraction, the gel cube was covered with a container in order to avoid dehydration. After the time required for extraction, the gel cube was removed from the artwork surface, placed bottom-side-up on a microscope glass slide, and allowed to dry. The drying process usually takes place in about 30 min, and a water loss of 92 % is measured. The resulting sample becomes very thin with an area of about 3×3 mm^2 still easy to manipulate. SERS measurements were taken on the dried Ag-agar gel. It is crucial in terms of SERS signal enhancement that measurements are taken on the dried cube, since by shrinking it increases the AgNPs and the analyte's concentration [9]. Also, this process creates a high-density distribution of NPs within the dried nanocomposite matrix that possibly improves the substrate's enhancement efficiency because of the strong electromagnetic local field produced.

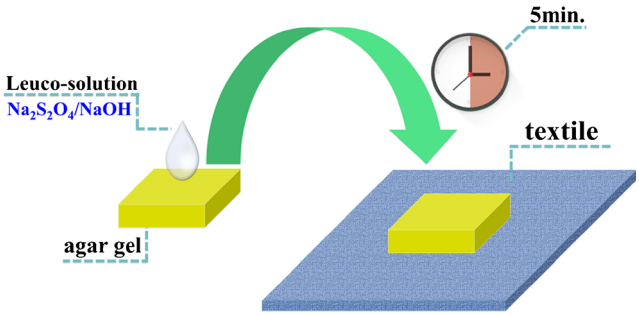

Fig. 2 Extraction procedure by means of *leuco*-reagents on Ag-agar gel

Instruments

The Raman/SERS spectra were recorded with a micro-Raman Renishaw RM2000 spectrometer using a 514.5 nm excitation laser line. A 50× objective of a Leica (Milano,Italy) microscope was used for both excitation and signal collection (180° scattering geometry). Laser power at the surface of the agar gel's cube was estimated to be ~20 μW. The time constant for signal accumulation was typically 10 s and the laser spot diameter was about 2 μm.

High resolution morphological characterization of the Ag-agar gel was achieved by using a helium ion microscope (HIM, Orion Plus; Carl Zeiss located at Consorzio GRINT) equipped with an Everhart-Thornley detector. Images were acquired in secondary electron mode with an acceleration voltage of 25 kV with a probe current ranging from 0.2 to 0.8 pA. Neutrality of the samples was maintained through the use of a low energy electron flood gun properly synchronized with the imaging beam.

Results and discussion

Ag-agar gel imaging

The high resolution characterization of the morphology of the Ag-agar gel structure was performed by means of the HIM setup described above. Using a HIM system avoids any preliminary sample metallization; moreover, it provides the imaging of insulating samples without producing any change on the surface. This aspect offers remarkable advantages to thoroughly understand the properties of the employed Ag-agar matrix used as SERS substrate. It is well known that the agar gel is characterized by a porous structure with pore size of about 100–200 nm [36, 37]. Our measurements show that the size of the pores ranges between 50 and 250 nm whereas the pore wall thickness is about 30 nm, in good agreement with the previous reports (Fig. 3). HIM images also showed how both the single and the aggregated AgNPs are homogeneously distributed within the gel structure. AgNPs sizes, ranging between 50 and 450 nm, are in accordance with the UV-Vis absorption spectrum, which has a maximum at 418 nm and a full width at half maximum of about 100 nm.

The HIM images of Ag-agar gel matrix suggest that the gel shrinkage upon drying process reduces the distance between the AgNPs, almost isolated in the hydrogel, favoring the generation of high plasmonic electromagnetic fields.

Leuco-extraction of indigo on reference sample

The use of the *leuco*-reagents, sodium hydroxide and sodium dithionite, triggers an inverse dyeing process when the gel is put in contact with the textile: the dichetonic molecules of indigo are converted into their reduced form, well known as *leuco*-indigo. The latter is water-soluble and less stable than indigo in its oxidized form. Following the reduction process, molecules of *leuco*-indigo are free to move to the gel cube which, working as a sponge, absorbs them. After the extraction, when the gel starts to dry, molecules of *leuco*-indigo are converted again into their oxidized form by atmospheric oxygen, and subsequently revealed by Raman measurements. The Raman spectra have been further improved by using the Ag-agar gel cube, in order to collect SERS spectra of the extracted indigoid dye. The minimum extraction time required for the detection of indigo in the gel cube was 5 min. The *leuco*-extraction procedure was at first applied on a cotton reference textile dyed with indigo in order to evaluate the efficiency and the safety of the proposed methodology. Figure 4 reports a comparison among the SERS and Raman spectra recorded after the *leuco*-extraction on the agar gel cube with or without the presence of AgNPs (Fig. 4a and b, respectively) and the Raman spectrum collected on the reference cotton textile dyed with indigo (Fig. 4c). These spectra clearly show all the characteristic bands associated to the molecule of indigotin, the Raman spectrum of which is available in the Electronic Supplementary Material (ESM, Fig. S1). Table 1 reports the assignment of fundamental Raman bands in the analyzed spectral region.

Moreover, as no unassigned bands are present in the Raman/SERS spectrum of extract, we do not have evidence for presence of by-products attributable to the action of the *leuco*-reagents on the sample.

In order to assess the safety of the method, X-ray fluorescence (XRF) and colorimetric analyses were performed in correspondence of the analyzed points of the textile. XRF was chosen in order to reveal transfer of AgNPs and sulphur from the extraction gel to the textile. No evidence for Ag presence was detected whereas the presence of sulphur, attributable to the sodium dithionite present in the *leuco*-solution, was noticed only for extraction times longer than 20 min (see ESM, Fig. S2). Colorimetric measurements did not reveal any appreciable fading of the investigated area, further corroborating the safety of the proposed method. (Table S1 in the ESM)

The spectrum reported in Fig. 5, obtained immediately after a 5′ extraction on agar gel cube, is the reduced form of indigo,

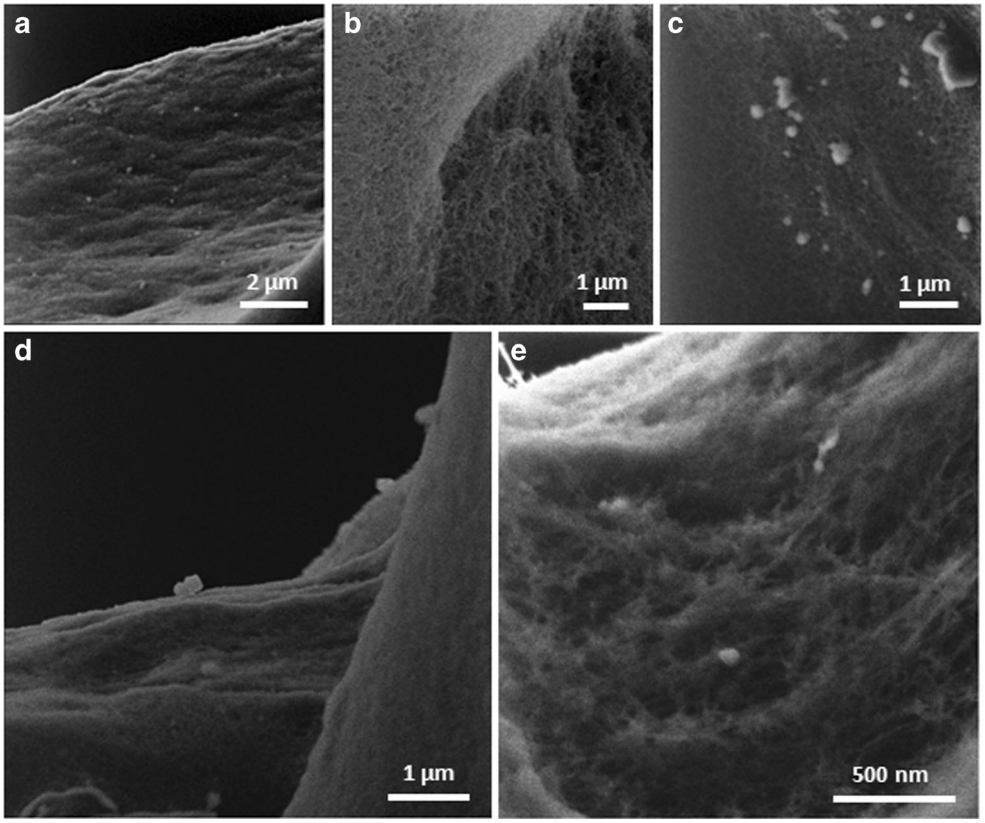

Fig. 3 HIM images of a section of Ag-agar gel at different magnifications

leuco-indigo. The sample, after 15′, in the presence of oxygen, turns from yellowish back to blue, and on repeated measurements, the observed spectrum is again the one of indigo. *Leuco*-indigo is a high conjugated system with a higher delocalization of π electrons that explains the shifting of the Raman bands to lower wavenumbers. Besides the disappearance of the carbonyl stretching vibration ν(C=O) present in indigo around 1699 cm^{-1}, among the most important spectral changes with respect to indigo worth mentioning are the shifts of ν(C–C) and δ(C–H) bands of the benzene ring centered, respectively, at 1577, 1484, and 1450 cm^{-1} [40]. The stretching vibrations C–O bonds explain the bands around 1120 and 1100 cm^{-1}.

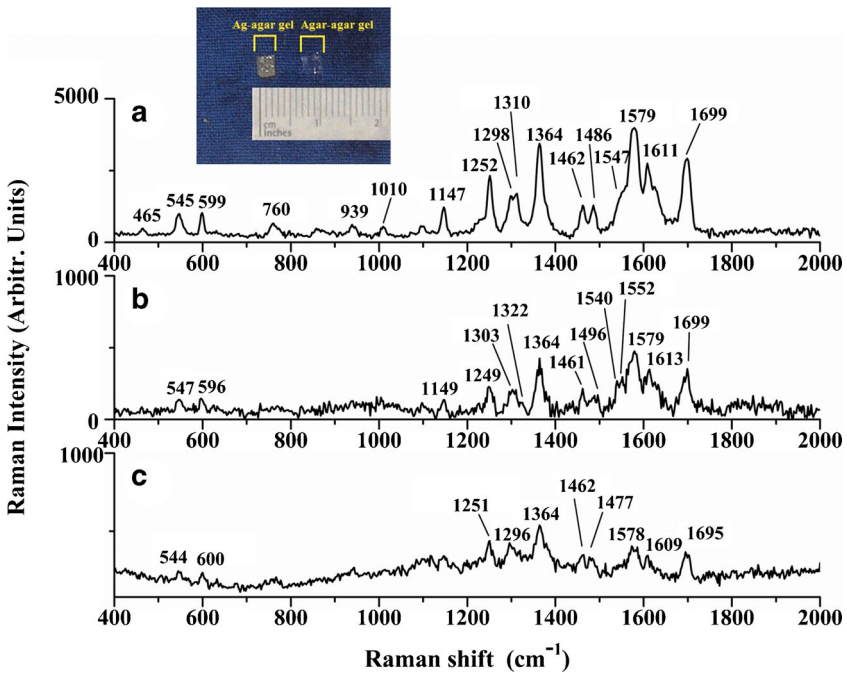

Fig. 4 **(a)** SERS spectrum collected on Ag-agar gel following *leuco*-extraction on the reference textile dyed with indigo; **(b)** Raman spectrum of indigo collected on the agar gel cube following *leuco*-extraction; **(c)** Raman spectrum of the reference textile dyed with indigo

Table 1 Main experimental (Raman and SERS) wavenumbers (obtained by 514.5 nm exCitation) of indigo spectra and relative assignments based on literature [38, 39]

Raman wavenumbers (cm^{-1})[a]		Band assignments[b]
Raman (powder)	SERS on Ag-agar gel	
1704 s	1699 s	νC=C, νC=O (δN-H)
1633 s	1628 sh	δC-C_{ring}, νC-C, δN-H
	1611 s	
1580 vs	1579 vs	νC=C, (νC=O δN-H)
1489 m	1486 m	δC-H, δN-H, δC-C_{ring}
1462 m	1462 m	δC-H, δC-C_{ring} (δN-H)
1364 s	1364 s	δN-H, δC-H
1315 m	1310 m	δC-H (δC=C)
1253 s	1252 s	δC-H, δC=C, δN-H
1222 w	1225 w	δN-H, δC-H
1147 w	1147 m/w	δC-C, δC-H
1100 w		γC-H
1013 w	1010 w	γC-H (γC-C_{ring})
940 w	938 w	γC-H, γC=C (γC-C)
860 vw	860 vw	δC-C, δC-N
761 w	760 w	δC=C, δC-N (γC-C_{ring})
675 w		δC-C_{ring}, δN-H, δC=C, δC=O
602 m	599 w	δC-C_{ring}, δC-N
547 m	545 w	δC=C-CO-C, δC-N
465 vw	465 w	γC=C, γC-H, γC=O
252 w		γC=C

[a] The experimental wavenumbers are marked as s (strong), m (medium), w (weak), sh (shoulder), and v means 'very'

[b] The vibrational modes are reported as ν (stretching), δ (in-plane bending), γ (out-of-plane bending)

The dyeing process of the fabrics goes into different interaction mechanisms between the dye and the fiber. Indigoids become water soluble after the reaction with a reducing agent (*leuco* form), and then, once attached to the fabric, the *leuco* form is oxidized into the blue insoluble and colored form. A further dyeing example regards the case of anthraquinonic mordant dyes, which are (partially) water soluble, and are attached to the fiber attributable to the presence of metal ions. We have already reported in a previous work that it is possible to extract anthraquinonic dyes by adopting as a solvent for the extraction, an aqueous solution of a chelant reagent [11].

In order to confirm the specificity of the extraction mechanism proposed, we tried to use the same extraction protocol on a cotton reference textile dyed with anthraquinonic mordant dye, alizarin. As expected, alizarin being a mordant dye, we were not able to extract any dye molecule from the fabric. The experimental result is shown in Fig. S3 in the ESM.

Leuco-extraction of indigo on real samples from artworks

Once the suitability of the *leuco*-methodology for indigoids in textiles fibers was assessed, it was applied to three different samples of artistic relevance: a precolombian textile, a cotton tablecloth of the XVth century, and a Medici's tapestry in silk, wool, and silver of the XVIth century. The Raman spectrum collected on the blue area of the pre-Columbian piece of textile, and the Raman and the SERS spectra acquired on the dry gel cube following extraction on the same sample by means of *leuco*-reagents are shown in Fig. 6.

The Raman spectra collected on the cotton fiber (Fig. 6c) and on the agar gel cube following extraction on the textile (Fig. 6b) present a strong fluorescence background that covers any possible Raman spectrum. Conversely, an excellent SERS

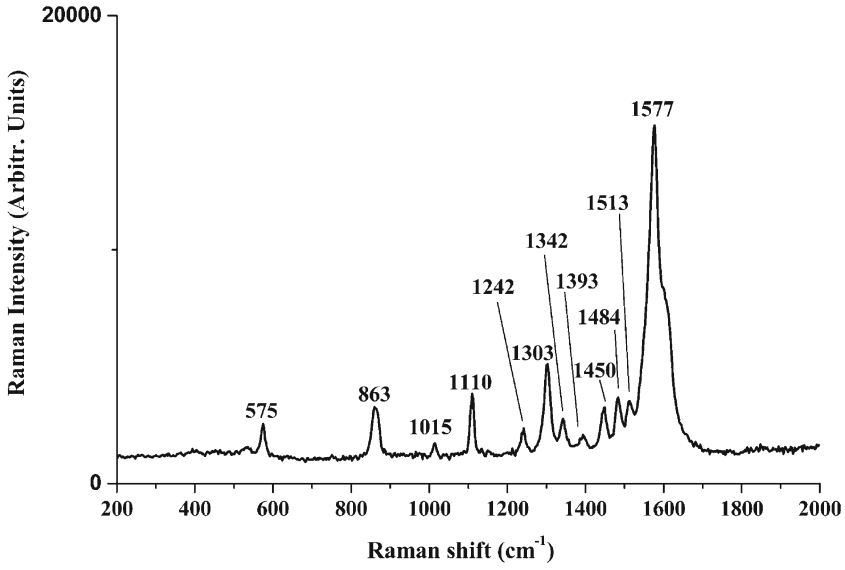

Fig. 5 Raman spectrum of *leuco*-indigo collected on the agar gel cube following extraction by means of *leuco*-reagents

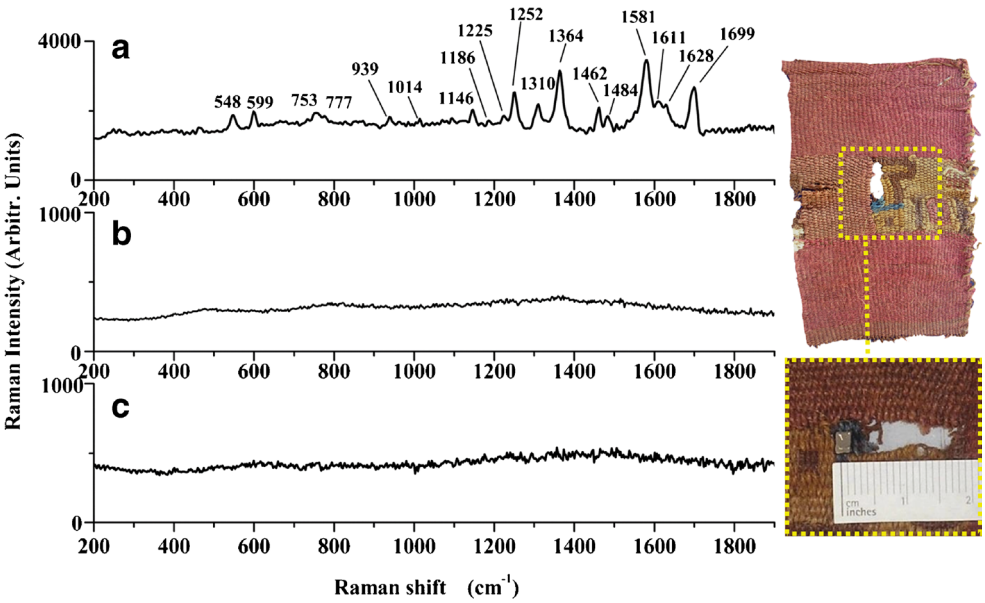

Fig. 6 **(a)** SERS spectrum collected on Ag-agar gel following *leuco*-extraction on the blue area of the precolombian textile; **(b)** Raman spectrum of indigo collected on the agar gel cube following *leuco*-extraction; **(c)** Raman spectrum acquired on the blue area of the precolombian textile

spectrum (Fig. 6a) has been collected, which reports the characteristic bands of indigo.

Excellent SERS spectra of indigo were also observed after the analysis of the nanocomposite gel cube following extraction on the XVIth century tablecloth fragment (Fig. 7), provided by the Museo del Tessuto in Prato, Italy.

The methodology was confirmed to be extremely valuable also for the identification of the blue dye on a thread of a Medici's XVIth century tapestry designed by Bronzino, depicting Joseph escaping from Potiphar's wife (Palazzo Vecchio, Sala dei Duecento, Florence, Italy, restored by Opificio delle Pietre Dure, Florence). As illustrated in Fig. 8, although the Raman spectrum collected on the fiber (Fig. 8a) did not allow for the immediate detection of the dye, the SERS spectrum acquired on the Ag-agar gel following extraction by means of *leuco*-reagents allowed for the detection of indigo, showing the typical frequencies of the blue dye. The relative intensity fluctuation can be explained by the presence of a different chemical environment or by the effect of aging processes concerning the ancient textile.

Leuco-extraction of natural Tyrian purple on reference sample

In order to assess the suitability of the *leuco*-extraction for the detection of other indigoid dyes, the methodology was also applied on a reference textile dyed with Tyrian Purple, which is a mixture of brominated indigoids, such as 6-bromoindigo

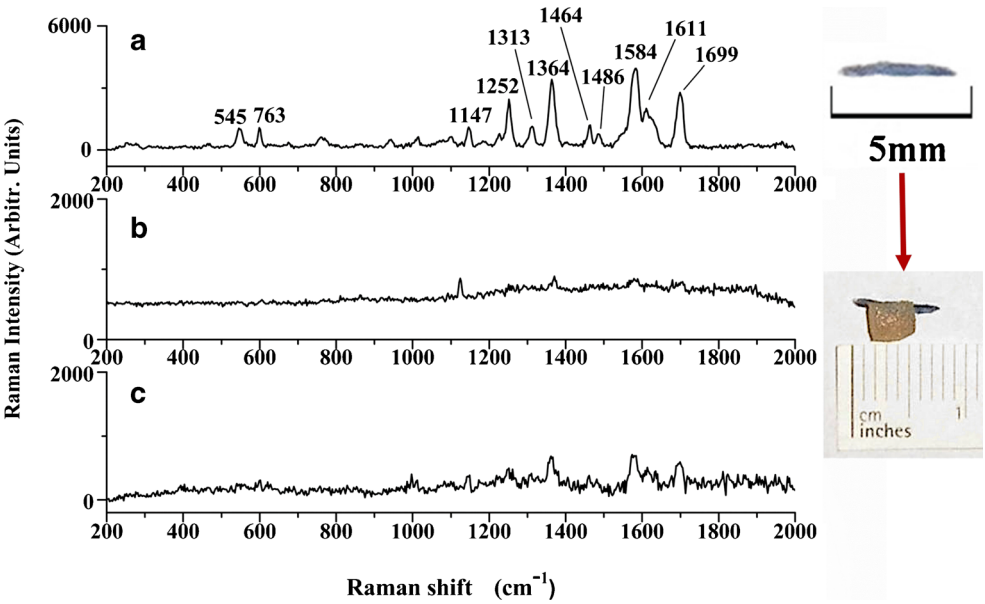

Fig. 7 **(a)** SERS spectrum collected on Ag-agar gel following *leuco*-extraction on the thread of a XVth century tablecloth; **(b)** Raman spectrum of indigo collected on the agar gel cube following *leuco*-extraction; **(c)** Raman spectrum acquired on the blue area of the fragment of textile of the tablecloth

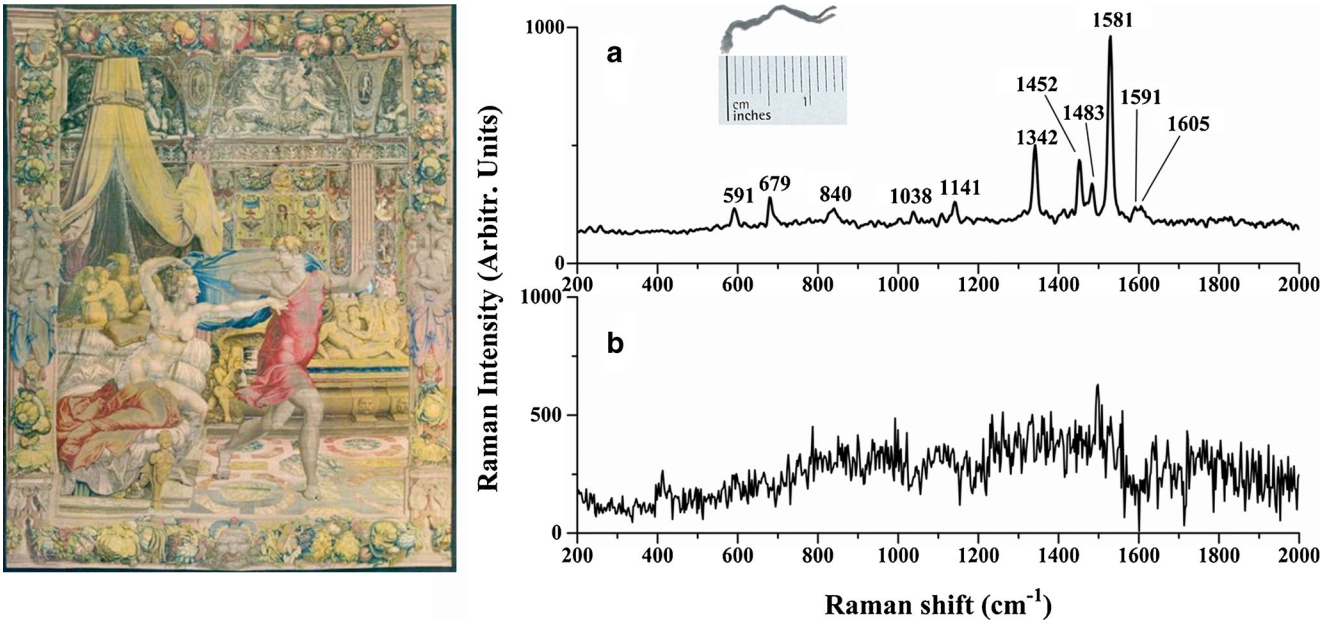

Fig. 8 **(a)** SERS spectrum of indigo collected on Ag-agar gel cube following extraction by means of *leuco*-reagents on the blue thread of the XVIth century Medici's tapestry; **(b)** Raman spectrum collected directly on the blue tapestry's thread

(MBI) and 6,6′ dibromoindigo (DBI). Different types of mollusks provide dyes of different composition. Excellent Raman and SERS spectra were acquired following *leuco*-extraction of the reference textile (Fig. 9). In order to get comparable quality, the reported Raman spectrum is the average of 20 acquisitions.

The spectral region between 1580 and 1700 cm^{-1} is characteristic of ν(C=C), ν(C=CH), and ν(C=O) stretching motions and is typical of indigoid molecules even if significant changes are induced by the halogen substituents in the ring quadrant modes. The vibration attributed to a ν(C=C) in conjugation with the two carbonyl group, each of which is itself further conjugated with an aromatic ring, is at 1625 cm^{-1}, whereas this band is weaker in the spectrum of indigo where it appears as a doublet at 1628/1611 cm^{-1}.

The 308 cm^{-1} band is attributed to the C-Br stretching vibration, fingerprint frequency of compounds with bromine atom [9]. The band assignments confirm the presence of 6,6′-dibromoindigotin as the principal component of purple. In particular, as evidenced in the reported spectrum, debromination processes of the molecule were not observed, confirming the safety of the *leuco*-solution for extraction purposes of the insoluble dye.

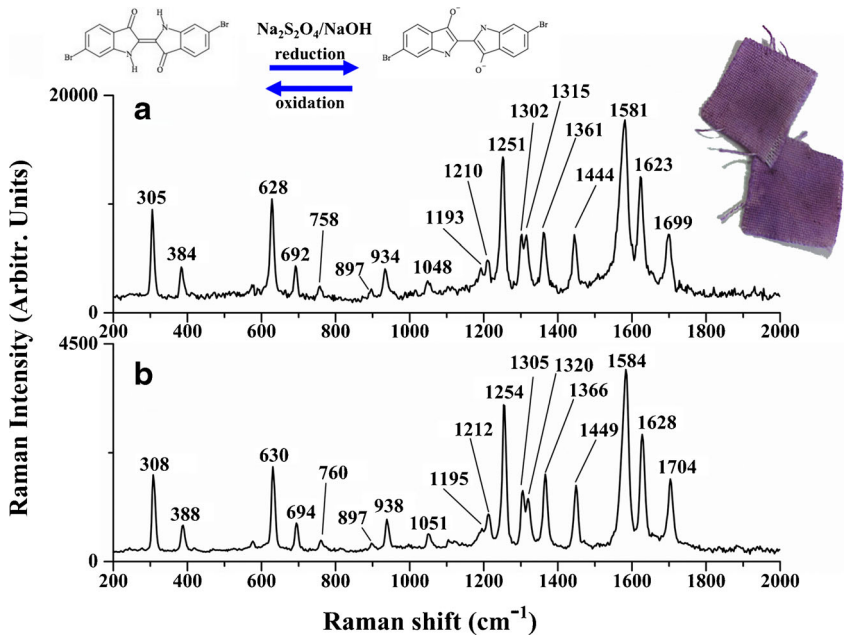

Fig. 9 **(a)** SERS and **(b)** Raman spectra of 6,6′-dibromoindigotin acquired on agar gel cube following *leuco*-extraction on a reference textile dyed with high purity Tyrian purple. SERS and Raman spectra are acquired at the same experimental conditions. In order to get comparable quality, the reported Raman spectrum is the average of 20 acquisitions

Conclusions

To date, the reliable and unambiguous detection of indigoids in art objects has proven to be challenging. In this work, we focused on the development of a new methodology for the selective extraction and identification of indigoids in the typical concentration used in textiles by means of an ecocompatible homogeneous nanostructured matrix.

The extraction system was modulated according to the chemical properties of the target analyte by choosing appropriate reagents for the extraction and optimizing the extraction time. The adoption of *leuco*-reagents (sodium hydroxide/sodium dithionite), which helps the conversion of the water-insoluble dichetonic form of indigo into its reduced (water soluble) form, called *leuco*-indigo, has allowed the trapping of indigo molecules into the agar-gel and Ag-agar gel. Excellent indigoid Raman and SERS spectra of indigoid dyes were obtained after micro-extraction by means of 514.5 nm excitation wavelength, which is intermediate between those of the dye's first electronic transition and of AgNPs' visible plasmonic band. The presence of AgNPs offers the advantage of gathering excellent SERS spectra of dyes by enhancing the Raman signal while quenching fluorescence, thus removing the fluorescence background problem usually occurring in resonance Raman spectra.

The effectiveness of the method is further confirmed by the fact that the Raman and SERS spectra recorded on the gel following extraction by means of *leuco*-reagents are quite similar: as a matter of fact, no significant changes in the band positions and their relative intensities were observed. On the contrary, an electromagnetic contribution to the enhancement factors explains the higher intensity of the SERS features. A more detailed evaluation about the SERS enhancement mechanisms will be carried out in a new study.

Moreover, no chemical transformation (e.g., debromination processes) of the Tyrian purple indigoid dye was noticed, confirming the safety of the developed extraction method for labile vat dyes.

The technique has been found to be extremely suitable and valuable for the minimal invasive detection and the recognition of indigoid dyes. In particular, the efficacy of methodology has been widely demonstrated by its application on several textiles of artistic relevance, validating the suitability of the proposed method on the investigated samples.

The high efficiency of this methodology confirms that the tailored gel extraction is a promising nondestructive technique for SERS identification of dyes.

Acknowledgments This work was supported by the Italian MIUR (grant no. 20100329WPF_007), the European Union (LASERLAB-Europe contract no.284464), and the Ente Cassa di Risparmio di Firenze (grant no. 2012-0849). Consorzio GRINT (http://www.consorziogrint.it) is gratefully acknowledged for providing the authors access to HIM.

References

1. Clark RJH, Cooksey CJ, Daniels MAM, Withnall R (1993) Endeavour 7:191–1999
2. Bechtold T, Mussak R (2009) Handbook of Natural Colorants. John Wiley & Sons, New York
3. Cooksey CJ (2001) Molecules 6:736–769
4. Etters JN (1993) J Soc Dyers Color 109:251–255
5. Etters JN (1995) Textile Chem Color 27:17–22
6. Smith G, Clark RJH (2001) Rev Conserv 2:96–106
7. Miliani C, Romani A, Favaro G (1998) Spectrochim Acta A 54: 581–588
8. Oakley LH, Fabian DM, Mayhew HE, Svoboda SA, Wustholz KL (2012) Anal Chem 84:8006–8012
9. Devièse T, Ribechini E, Baraldi P, Farago-Szekeres B, Duday H, Regert M, Colombini MP (2011) Anal Bioanal Chem 401:1739–1748
10. Lofrumento C, Ricci M, Platania E, Becucci M, Castellucci E (2013) J Raman Spectrosc 44:47–54
11. Platania E, Lombardi JR, Leona M, Shibayama N, Lofrumento C, Ricci M, Becucci M, Castellucci E (2014) J Raman Spectrosc 45: 1133–1139
12. Leona M, Decuzzi P, Kubic TA, Gates G, Lombardi JR (2011) Anal Chem 83:3990–3993
13. Doherty B, Brunetti BG, Sgamellotti A, Miliani C (2011) J Raman Spectrosc 42:1932–1938
14. Doherty B, Presciutti F, Sgamellotti A, Brunetti B, Miliani C (2014) J Raman Spectrosc 45:1153–1159
15. Leona M, Stenger J, Ferloni E (2006) J Raman Spectrosc 37:981–992
16. Pozzi F, Lombardi JR, Bruni S, Leona M (2012) Anal Chem 84: 3751–3577
17. Pozzi F, Lombardi JR, Leona M (2013) Heritage Sci. doi:10.1186/2050-7445-1-23
18. Chen K, Leona M, Vo-Dinh T (2007) Sensor Rev 27:109–120
19. Leona M (2009) Proc Natl Acad Sci USA 106:14757–14762
20. Cañamares MV, Garcia-Ramos JV, Domingo C, Sanchez-Cortes S (2006) Vibr Spectrosc 40:161–167
21. Cañamares MV, Garcia-Ramos JV, Domingo C, Sanchez-Cortes S (2004) J Raman Spectrosc 35:921–927
22. Jurasekova Z, Domingo C, Garcia-Ramos JV, Sanchez-Cortes S (2012) J Raman Spectrosc 43:1913–1919
23. Bruni S, Guglielmi V, Pozzi F (2011) J Raman Spectrosc 42:1267–1281
24. Whitney AV, Van Duyne RP, Casadio F (2006) J Raman Spectrosc 37:993–1002
25. Pozzi F, Shibayama N, Leona M, Lombardi JR (2013) J Raman Spectrosc 44:102–107
26. Corredor C, Teslova T, Canamares MV, Chen Z, Zhang J, Lombardi JR, Leona M (2009) Vibr Spectrosc 49:190–195
27. Shadi IT, Chowdhry BZ, Snowden MJ, Withnall R (2003) Spectrochim Acta A 59:2213–2220
28. Jurasekova Z, del Puerto E, Bruno J, Garcìa-Ramos JV, Sanchez-Cortes R, Domingo C (2010) J Raman Spectrosc 41:1455–1461
29. Pozzi F, Poldi G, Bruni S, De Luca E, Guglielmi V (2012) Archaeol Anthropol Sci 4:185–197
30. Bruni S, Guglielmi V, Pozzi F (2010) J Raman Spectrosc 41:175–180
31. Proietti N, Presciutti F, Di Tullio V, Doherty B, Marinelli AM, Provinciali B, Macchioni N, Capitani D, Miliani C (2011) Anal Bioanal Chem 399:3117–3131
32. Kurouski D, Zaleski S, Casadio F, Van Duyne RP, Shah NC (2014) JACS 136:8677–8684

33. Anzani M, Berzioli M, Cagna M, Campani E, Casoli A, Cremonesi P, Fratelli M, Rabbolini A, Riggiardi D (2008) Quaderno n.6/ CESMAR7. Il Prato, Padova
34. Schweppe H (1997) In: FitzHugh EW (ed) Artists' pigments: a handbook of their history and characteristics. National Gallery of Art, Washington, DC
35. Lee PC, Meisel DJ (1982) J Phys Chem 86:3391–3395
36. Deguchi S, Tsudome M, Shen Y, Konishi S, Tsujii K, Ito S, Horikoshi K (2007) Soft Matter 3:1170–1175
37. Chui MM, Phillips RJ, McCarthy MJ (1995) J Colloid Interphase Sci 174:336–344
38. Sánchez del Río M, Picquart M, Haro-Poniatowski E, van Elslande E, Hugo Uc V (2006) J Raman Spectrosc 37:1046–1053
39. Tatsch E, Schrader B (1995) J Raman Spectrosc 26:467–473
40. Fiedler A, Baranska M, Schulz H (2011) J Raman Spectrosc 42: 551–557