A SPECTROSCOPIC APPROACH TO THE STUDY OF ORGANIC PIGMENTS IN THE FIELD OF CULTURAL HERITAGE

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ABSTRACT. Characterization of pigments in archaeological finds requires an experimental approach able to avoid the destruction or perturbation of the artwork. Surface Enhanced Raman Spectroscopy technique may provide useful information in terms of chemical composition by using very small sample quantities and without samples manipulation. In this paper some pigments and an archeological find, discovered in Messina, have been analyzed with new SERS substrates prepared with Pulsed Laser Deposition (PLD) technique. In both cases we quenched the fluorescence phenomena and enhanced Raman peaks.

1. Introduction

Identification and characterization of organic pigments used in works of art and cultural heritage play a fundamental role in order to detect a precise geographic area and a specific historic moment (Claro et al. 2008; Clementi et al. 2009). Micro-Raman spectroscopy (µRS), being a non-destructiveness technique, has been largely used for the analysis of dyes of interest in the cultural heritage field. Unfortunately, pigments and dyes commonly employed in artistic production are often extremely fluorescent, making detection through Raman spectroscopy difficult or even unreliable. Conventional Raman spectra are, in fact, affected by the dyes intense fluorescence that can hinder, the much weaker Raman peaks, thus inhibiting their detection and identification (Oakley et al. 2011; Fazio et al. 2013; Pozzi et al. 2016). Surface Enhanced Raman Spectroscopy (SERS) on the contrary satisfies many of the requirements of an ideal analytical technique in detecting chemical species in artworks. In presence of a nanostructured noble-metal surface the Raman signal can be enhanced by several order of magnitude, allowing the detection of species at very low concentration, when adsorbed on or in proximity of rough metallic surfaces. In particular when dealing with organic species, characterized by the presence of delocalized electron systems, the Raman scattering signal is greatly enhanced. Moreover, the fluorescence results strongly quenched by energy transfer mechanism between molecule and metal. So that SERS has been successfully employed for the detection and the identification of organic pigments and...
modern synthetic dyes (Cañamares et al. 2006). On site measurements are often required in the cultural heritage field. Many objects cannot be sampled or moved outside museums, or are non-movable by nature because these are part of architectural monuments and, in general, it is easier to reach each object rather than to gather them all in one lab (Ziemann 2006). Mobile Raman spectrometers are deemed in these cases because are able to collect Raman spectra from the artefacts in their location. Moreover when coupled with SERS active substrates the detection of substances in the artefact at very low concentration is possible and data analysis can be performed onsite or subsequently. In this paper ad hoc SERS substrates, made by a thin layer of silver nanoparticles deposited on sandpaper sheets by means of the pulsed laser ablation technique (PLA) were prepared (D’Andrea et al. 2009; Fazio et al. 2009, 2011). The so obtained substrates can be gently swabbed, for example, on a painted surface or on small decorated fragments in order to retain small quantities of the superficial layer. Raman spectra, then, are collected on the substrate surfaces avoiding in such a way direct laser exposition of the work of art. The substrates were firstly tested on laboratory prepared layers of some organic dyes, i.e., alizarin, purpurin, curcumin and stil de grain, then were employed on an archaeological find discovered in the North necropolis of Messina, excavated from Salinas and Bears in 1913-15, under the Government House.

2. Experimental Section

The pigments were purchased from Aldrich and Zecchi and used as received. Sandpaper sheets were covered by silver NPs deposited by means of with PLD (pulsed laser deposition). Pure silver targets, placed in a vacuum chamber, were ablated by an excimer laser operating at 248 nm (COMPEX 205 Lambda Physik) in presence of controlled Argon atmosphere (pressure 70 Pa) at room temperature and at the laser fluence of 2.0 Jcm$^{-2}$. The distance

![UV-Vis spectrum of Ag-NPs.](image)

**Figure 1.** UV-Vis spectrum of Ag-NPs.
between the target and the substrates (sandpaper sheets and glass slides) was fixed to 35 mm. Under the adopted deposition conditions, NPs with size between 3 and 20 nm are obtained. The NPs size and number density on the substrate depend on the adopted Ar pressure value and on the total laser pulses fixed to $3 \times 10^4$. More details about the deposition process can be found in (D’Andrea et al. 2009). We report in Fig. 1 the UV-Vis spectrum of Ag NPs obtained by a home made UV-Vis set-up (Avantes AvaLight-DHS Deuterium-Halogen Light Source coupled with the Avaspec 2048L spectrometer). The maximum of the surface plasmon resonance peak is located at about 500 nm. The chemical structure formula of the investigated pigments are reported in Fig. 2. After preparing the substrate, it was gently rubbed on the a test pigmented sample or on the finding sample, thereafter Raman spectra were acquired on its surface. To collect spectra (Normal Raman and SERS) two different Raman instruments were used. The first is a Jobin Yvon HR 800 micro-Raman spectrometer using the 632.8 nm He-Ne laser line as exciting source and equipped with a Peltier-cooled charge-coupled device (CCD) sensor. In order to avoid samples degradation, the laser power at the sample surface was kept as low as possible using a long working distance 50 microscope objective and integration times no longer than 60 s. The second instrument (mobile-Raman) is a BRAVO Handheld Raman Spectrometer by Duo LASER (Conti et al. 2016). BRAVO uses a new patented technology (SSE: Sequentially Shifted Excitation, patent number US8570507B1) (Cooper et al. 2013) to mitigate fluorescence phenomena and is equipped with two excitation lasers with wavelengths (DuoLaser) located in the range between 700 and 1100 nm. The lasers work together to mitigate the fluorescence and it is not possible to separate them by means of the experimental setup. Unfortunately, the patent covers the additional information on the combined Duo-Laser system (for example the exact incident wavelength). Taking into account the observed SPR peak position on the glass slide, the 632.8 nm line of the He-Ne laser should be in a better resonance condition with respect to the one experienced by the Bravo exciting laser lines. Nevertheless, when considering the

![Chemical structures of pigments](image_url)
position of the SPR, it should be noted that the different surface morphologies between the sandpaper sheets and a flat surface, like the glass one, can result, in principle, to a different NPs distribution on the surface, and hence to different plasmonic properties.

3. Results and discussion

In Fig. 3 we report both the normal Raman and the SERS spectra acquired on alizarin and purpurin using the 632 nm laser excitation. Alizarin and purpurin are natural dyes and their chemical structures are anthraquinone. These pigments have been used since ancient times and were originally extracted from the roots of the common madder plant (Rubia...
A spectroscopic approach to the study of organic pigments... A5-5

Figure 4. Two pigments powder used as test for sandpaper.

tinctorium L.). These pigments are widely used in works of art (Schweppe and Winter 1997) and have been studied by several research groups. Madder has been cultivated as a dyestuff since antiquity in central Asia and Egypt, where it was grown as early as 1500 B.C. Cloth dyed with madder root pigment was found in the tomb of the Pharaoh Tutankhamun and in the ruins of Pompeii and ancient Corinth. In the middle ages Charlemagne encouraged madder cultivation. Normal Raman spectra acquired on the pigments in powder form show, as expected, a strong fluorescence background completely overwhelming the Raman signal. In the same graph, together with normal Raman spectra, we also reported the SERS spectra acquired on Ag NPs decorated sandpaper with the dyes. These are put on sandpaper by rubbing the surface directly in sample box containing the powder pigments as it is showed in Fig. 4. Both spectra types have been acquired in the same spectral region under identical experimental conditions (integration time, laser power, etc.).

4. Red and yellow pigments

In Fig. 2, as explained in the experimental section, the chemical structures of investigated dyes are showed. SERS spectra show the appearance of strong Raman peaks and no appreciable fluorescence contribution. Concerning the SERS of alizarin (see Fig. 3A) Raman peaks can be observed at 1265, 1299, 1324 and 1421 cm$^{-1}$, less intense peaks are observed at 643, 812, 905, 1052, 1159, 1191, 1210, 1506, and 1624 cm$^{-1}$. The peak at 812 cm$^{-1}$ is attributed to C-H out-of-plane bending mode, while the remaining peaks to stretching modes of aromatic C atoms. Peaks observed at 1052 and 1624 cm$^{-1}$ are attributed to ring and C-C stretching respectively. In the SERS spectrum of purpurin intense Raman peaks are clearly visible, similarly to what observed for alizarin (see Fig. 3A and Fig. 3B). The most intense peaks are located at 1154, 1213, 1286, 1325 and 1480 cm$^{-1}$ (the last one assigned to stretching mode of C-O bonds), while low intensity peaks, but still clearly visible, at 461, 611, 656, 907, 976, 1068, 1157 and 1609 cm$^{-1}$. The peak at 611 cm$^{-1}$ is assigned to the C-C-C in plane bending mode. The peaks at 1323 and 1158 cm$^{-1}$ are assigned to C-C stretching), while, the peaks at 1294 and 1210 cm$^{-1}$ are assigned to C-H bending mode. Peaks positions and relative intensities are in good agreement with the reference spectra (Van Elslande et al. 2008; Brousseau et al. 2009; Jurasekova et al. 2010).
The investigated yellow pigments were curcumin and stil de grain. Curcumin is a bright yellow chemical produced by some plants, it is the main curcuminoid of turmeric (Curcuma longa) cultivated in India, a member of the ginger family (Zingiberaceae) (Abdeldaiem 2014). It has been extensively employed as a dyestuff of textiles, furniture and lacquer. Stil de grain yellow is a pigment traditionally derived from unripe buckthorn berries. An archaic name for this colour, used in the 17th century, is pinke. It is also known as sap green, although in contemporary art the term sap green often indicates a mixture intending to resemble the traditional sap green or stil de grain yellow.

In Fig. 5 we report the analysis on the powders of curcumin and stil de grain dyes. The results are very similar to ones observed for the red pigments: specifically the conventional
Raman does not produce any significant Raman peak, being hindered by the strong fluorescence signal. On the contrary, in the SERS spectra, peaks related to the vibrational modes are evident.

For the curcumin the most intense Raman peaks are observed (see Fig. 5A) at 1156, 1193, 1260, 1609 and 1636 cm\(^{-1}\), while peaks having lower intensities are located 584, 974, 1326 and 1436 cm\(^{-1}\). The peaks at 1326 and 1156 cm\(^{-1}\) are assigned to C-C stretching, at 1193 cm\(^{-1}\) is located the O-H in-plane bending. In Fig. 5B stil de grain characteristic peaks are at 474, 880, 1136, 1255, 1280, 1306, 1357 and 1614 cm\(^{-1}\). The peak at 1255 cm\(^{-1}\) is assigned to C-O stretching, at 880 cm\(^{-1}\) is assigned to C-H out-of-plane bending and at 474 cm\(^{-1}\) to C-C skeleton out-of-plane bending. The peaks at 1306, 1614 and 1136 cm\(^{-1}\) to C-C stretching. Some of the peaks are located above a broad band in the 1000-1700 cm\(^{-1}\) region, probably due to the presence of amorphous carbon originating from the photo-degradation of the dye (Mayhew et al. 2013). The results reported above show that it is possible to obtain good SERS spectra from small quantities of pigments collected by Ag NPs covered sandpaper wiped on bulk samples. It is worth to mention that, due to the peculiar collection method, i.e., the swabbing of the SERS active substrates on the samples surface, only detection and recognition of the adsorbed substances is possible, while concentration determination is unreliable. After the promising results on test samples the same procedure was performed on a work of art to assess its reliability in a real case study.
5. A case study

The finding was discovered in a necropolis in the north part of the city of Messina (Sicily, Italy) in February 1914 during the excavations for the foundations of the new headquarters of the Prefecture of Messina, located near the church of St. John of Malta. Some objects were found together with the remains of walls and funeral newsstands. An analysis of sedimentary alluvial benches, due to secular flooding of Boccetta creek streams, allows deducing that the necropolis was buried between II and III century d.C.

Raman measurements, following the same approach used for the test samples, were performed on the surface of a fragment coming from the decoration of the wall and hereafter refereed as sample 1. Sample 1 is one of many fragments and shows a decoration in the upper part and a red-brown colour stripe in the bottom, see upper part of Fig. 6. Measurements were performed on the red-brown stripe. It was not possible to reconstruct the entire decorative element because were found only the fragments shown in Fig. 6. Firstly a normal Raman spectrum was acquired on the surface of sample 1, then an Ag
Figure 8. SERS spectra of sample 1 arrows indicate the peaks identified as alizarin (A) or purpurin (P).

NPs covered sandpaper sheet was swabbed on its surface and thereafter analysed. Normal Raman and SERS spectra were acquired with both the Raman instrument described in the experimental section, and are shown in Fig. 7. The spectrum acquired with the 632 nm (Fig. 7A) exciting laser source is dominated by the strong fluorescence signal, some weak Raman peaks are observed, but their origin most probably come from the matrix underneath. On the contrary, SERS spectra acquired from the material collected by the Ag NPs covered sandpaper, after the wiping procedure, show now intense and well defined Raman peaks. Measurement performed using the handheld spectrometer (BRAVO instrument) are shown in the right panel of Fig. 7. In this case the use of about 785 nm exciting source avoid the excitation of the fluorescence in the normal Raman spectrum and some peaks are evident. Nevertheless, these contributions were identified as due to calcite (1086 cm\(^{-1}\)) and to plaster bulk (710 cm\(^{-1}\)). So no contribution from the presence of the pigment on the surface of sample 1 is evident. The SERS spectrum, also in this case, is wealthy of Raman peaks. The contribution from the underneath matrix is now absent or hardly detectable, pointing out for the pigmented layer as the origin of the observed peaks. In the 1700-2000 cm\(^{-1}\) region the spectrum acquired with the Bravo instruments shows some Raman features absent in the spectrum acquired using the HR800 instrumentation. In particular two major peaks are evident at about 1730 and 1890 cm\(^{-1}\). Such peaks cannot be identified as belonging to alizarin or purpurin dyes, in fact they were not observed in previous works in which different excitation wavelengths where used (Whitney et al. 2006; Abdeldaiem 2014; Rambaldi et al. 2015). The origin of such peaks is unclear, also owing to patent protected acquisition procedure of the Bravo equipment, and therefore were not used for the dye identification.

In Fig. 8 we report the sample 1 SERS spectrum and the normal Raman spectra of purpurin and alizarin collected on the powders, and acquired with the handheld Raman spectrometer. The peaks of SERS spectra at about 785 nm of alizarin and purpurin are: SERS of purpurin 1610, 1560, 1468, 1462, 1392, 1356, 1288, 1204, 1154, 1096, 1028,
1002, 858, 790, 704, 640 and 526 cm\(^{-1}\); SERS of alizarin: 1614, 1554, 1390, 1320, 1290, 1202, 1186, 1094, 1040, 1010, 890, 856, 705 and 632 cm\(^{-1}\). The differences between the spectra are attributed to presence of OH in 4 position and are relevant in 1200-1600 cm\(^{-1}\) range. This structural difference effects some frequencies such as C-O stretching about 1400 cm\(^{-1}\) and C-C-C ring about 1300-1200 cm\(^{-1}\), respectively. The most intense peaks in the spectrum of sample 1 are at about 1610, 1570, 1440, 1396, 1308, 1283, 1192, 1134, 1096, 1028, 1000, 858, 786, 706, 664, 633 and 532 cm\(^{-1}\). A comparison with the spectra of alizarin and purpurin spectra allow for the identification of the pigment in sample 1 as madder lake, a mixture of the two.

6. Conclusions

Silver NPs deposited by pulsed laser ablation on sandpaper sheets proved to be suitable for the detection and identification of organic dyes by SERS. While SERS technique allows overcoming typical drawbacks of organic dyes, such as strong fluorescence and/or weak Raman activity, the proposed substrate allows for the collection of small sample quantities from the surface of a work of art and their identification by Raman spectroscopy. Moreover the use of handheld Raman spectrometer allowed the in the field use of the SERS technique.

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