Red Lakes from Leonardo's Last Supper and other Old Master Paintings: micro-Raman spectroscopy of anthraquinone pigments in paint cross-sections

Iacopo Osticioli[1], Marco Pagliai[2], Daniela Comelli[3], Vincenzo Schettino[2], Austin Nevin[4,5]

[1] Institute of Applied Physics "Nello Carrara" National Research Council (IFAC-CNR) Via Madonna del Piano, 10, 50019 Sesto Fiorentino, Firenze, Italy

[2] Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy

[3] Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, Milano 20133, Italy

[4] Istituto di Nanotecnologie e Fotonica National Research Council (IFN-CNR), Piazza Leonardo da Vinci 32, Milano 20133, Italy

[5] Department of Conservation, University of Gothenburg, Box 130, 40530, Gothenburg, Sweden

Abstract

The analysis of red particles in paint cross-sections from Leonardo da Vinci's *Last Supper*, Masolino da Panicale's wall painting *Beheading of St. John the Baptist* in Castiglione Olona, Tintoretto's *The Discovery of the Body of Saint Mark* and Paolo Veronese's *Supper in the House of Simon* has been carried out with micro-Raman measurements. Subtracted shifted Raman spectroscopy methods have been employed to resolve the signals in the presence of fluorescence. Taking advantage of the vibrational assignments based on recent *ab initio* calculations of aluminum-complexes of anthraquinones, the approach allowed the discriminate between anthraquinone dyes and lakes based on Kermesic and Carminic acids present in the studied samples for the first time without heavy sample treatment.

Keywords: Raman microscopy, organic pigments, cochineal, kermes, carmine

1. INTRODUCTION

Red lakes precipitated from dyes, have been used for millennia as pigments in works of art due to their unique color arising from extensive conjugated bonds in aromatic rings[1]. Natural red lake pigments originate from dyes extracted from plants or insects. Despite their optical similarity, lakes may differ in chemical composition, reactivity and ease of photodegradation depending on their

origin and the materials used in their preparation. Madder[2,3], from *rubia tinctorum,* or other kinds of *rubia*, is a mixture of purpurin and alizarin[4], while Brazilein[5], from *Brazilwood*, is particularly light sensitive. Laccaic acids[6] account for the dye produced by the lac beetle "Kerria lacca Kerr". The extracted dyes yield a solid pigment when precipitated with a mordant. Carminic, kermesic and flavokermesic acids can be extracted from insects: Mexican Cochineal (*Dactylopius coccus*) chiefly contains carminic acid,, while extracts from Armenian (*Porphyrophora hamelii*) and Polish Cochineal (*Porphyrophora polonica*) contain larger concentrations of kermesic and flavokermesic acids[7, 8].

The main structural difference between the many anthraquinone-based red lakes is the nature of the side chains on the anthraquinone backbone[9,10]. Carminic acid contains a glycosidic ring, while flavokermesic and kermesic acids contain both a methyl group and three or four hydroxyl groups, respectively. Alizarin and purpurin, by contrast, contain only two or three hydroxyl groups[11, 12], respectively. Historic recipes for the preparation of red lake pigments vary in the methods of extraction of the dyes, the pH of the dye solution and the mordants used for precipitation of the complexes[13]. For example, lake pigments can be prepared by extracting the dyes directly from the insects or from shearings of dyed textiles, with common mordants including Alum ($KAl(SO_4)_2 \cdot 12H_2O$), calcium carbonate ($CaCO_3$) or starch. Other metal ions were commonly used to create darkly coloured textiles with the same dyes. Red lakes are widely documented in paintings including works by Leonardo, Tintoretto and Veronese[1, 14, 15].

While the analysis of many inorganic pigments may be straightforward and can be achieved optically or non-destructively with well-established techniques[16], organic lakes are notoriously difficult to identify or discriminate. Their identification is mainly carried out through the detection of different markers or acids by liquid chromatography (LC) which stands as the standard method for the analysis of lakes due its specificity and sensitivity even though it involves extraction and destruction of the sample[17-20]. Extensive research with LC at the National Gallery London revealed a range of red lake pigments made from madder and brazilwood in paints by Old Masters showing that the use of Kermes-based red lakes was followed by the adoption of Carminic-acid based lakes, for example by Veronese, extracted from Mexican Cochineal[14].

In much published research lake pigments are simply recognized on the basis of their fluorescence, but cannot be easily discriminated[21]. Raman spectroscopy is routinely used for the non-destructive analysis of pigments and numerous case studies demonstrate its strength for the determination of both inorganic and organic materials in paint samples[22]. Often the direct Raman analysis of red lakes in paint samples or in paintings is compromised by the strong fluorescence of the pigment which masks Raman signal[23-26].

For these reasons Surface Enhanced Raman Spectroscopy (SERS)[27-33] has gained significant popularity as an alternative method for the analysis of red lake pigments. SERS, like LC, is based on the extraction of dyes from micro-samples and on subsequent interaction of the extracted molecules with nano-structured substrates for the detection of an enhanced Raman signal[34-36]. SERS spectra can be directly measured on cross-sections following sample treatment[37] or laser ablation micro-sampling[38]. A general problem with the extraction of dyes from pigments - whether for SERS or for LC - is that information regarding the coordination of the dye molecules is lost[39].

The interpretation of the Raman spectra of red lakes and their parent dyes in terms of normal modes is a major challenge because of the large number of active modes and the vanishing intensity of some of them. For instance, in the case of kermesic acid there are 96 Raman active modes, with 53 of them occurring between 1800-600 cm^{-1}. For this reason, most of the Raman studies of red lakes rely on standard Raman bands taken as markers of specific red lakes or dyes. Although this is a very simple, practical and economic approach for the identification of the lake it does not offer much information on the actual normal modes at work, apart from correlations with traditional group frequencies.

In this work we apply a different approach relying on our recent work[40] where *ab initio* Density function theory (DFT) calculation of the vibrational frequencies and infrared and Raman intensities has been carried out for the anthraquinone family dyes, including anthraquinone, alizarin, purpurin, carminic and kermesic acid and their Al-complexes. The computational results have been compared with all the available infrared and Raman spectra leading to a vibrational assignment of unprecedented accuracy for this category of chemical compounds.

The samples presented in this work were examined with normal Raman spectroscopy. The comparison between the experimental Raman spectra with available experimental data and with DFT calculated spectra allows the clear identification of red lakes, and the normal modes characterizing the observed Raman profile can be resolved. It will be shown that the present results do not contradict the results of SERS investigations, whenever available, but provide superior spectroscopic and structural information while, at the same time, an alteration of the sample is avoided.

With the outlined procedure we report the analysis of the composition of organic red lake pigments in samples from Leonardo da Vinci's wall painting *The Last Supper*, from Masolino da Panicale's wall painting depicting *The Beheading of St. John the Baptist in the Baptistery* (Castiglione Olona, Italy), from two canvasses: Tintoretto's monumental *The Discovery of the Body of Saint Mark* and Veronese's canvas *Supper at the House of Simon*, both on display in the Pinacoteca of Brera in Milan.

2. EXPERIMENTAL AND METHODOLOGY

Raman analyses were carried out directly on cross-sections. Cross-sections from the paintings were taken during conservation work and are now stored in the Archivio Gallone[1]. All samples were prepared in cross-section using a polyester- based resin and hand polished.. A Renishaw 2000 micro-Raman spectrometer using excitation either with the out of resonance 785 nm line of a diode laser and with the near resonant 514 nm line of an Ar^+ laser was employed. The power at the surface of the sample was 6 mW. The laser was focused on the sample using a 50× objective (NA: 0.75), and an area of approximately 2-4 μm in diameter was analyzed at a spectral resolution of 4 cm^{-1} with typical integration times of 100 seconds per acquisition. The luminescence background in the experimental Raman spectra was eliminated by subtracted shifted Raman spectroscopy (SSRS) which is described in detail elsewhere[41-45].

3. RESULTS AND DISCUSSION

3.1 Leonardo da Vinci: *The Last Supper*

The wall painting of *The Last Supper* was executed by Leonardo for the Refectory of Santa Maria delle Grazie in Milan between 1494 and 1498. A sample from the blue drapery of Saint Bartholomew's cloak has been analyzed. The paint layers contain the precious blue ultramarine ($Na_{8-10}Al_6Si_6O_{24}S_{2-4}$) as the main pigment mixed with azurite ($Cu_3(CO_3)_2(OH)_2$). A layer of finely ground pure pigment is on top of a light blue layer containing fine particles of lead white and large inclusions of a dark red lake pigment. A thin layer of ground lead white covers the surface of the wall and the plaster. Deep red particles are dispersed in a layer of brilliant ultramarine (Figure 1a) in one of the many chromatic variations and pigment mixtures adopted by Leonardo for his masterpiece; other samples from blue hues in the painting contain mixtures of azurite and ultramarine particles over black or grey (for example in the the tablecloth) or green (the mountains), with no red lake particles observable. We hypothesise that Leonardo added red particles to the blue layer here to slightly modify the hue of the paint which may have appeared more purple after its execution than the drapery of Saint Bartholomew does today, as the red pigment is known to fade. It is also noted here that some samples of the wall painting contain continuous red lake layers, as has also been reported in contemporary icons, a more common practice for the application of red lakes [46]. On the basis the fluorescence of the dark red particles in the sample in Figure 1a, Antonietta Gallone identified madder lake (or *Lacca di Garanza*) in samples from Leonardo's *Last Supper* [1].

Figure 1. *(a) Photomicrograph of a sample from the figure of Saint Bartholomew from Leonardo da Vinci's Last Supper. Dark red particles are distributed in an ultramarine blue layer mixed with azurite [3] over a white lead groud [2] and the plaster wall [3].*

(b) Photomicrographs of the cross-section from Masolino da Panicale's wall painting "The beheading of St. John the Baptist"from the Baptistry, Castiglione Olona. The red layer [1] is covered by a lead white based adhesive [2] on which a layer of tin [3] has been applied.

The Raman spectra of the deep red particles in the cross-section from *The Last Supper* taken with the 785 and 514 nm exciting lines are shown in Figure 2. The intensity profile of the spectrum changes appreciably approaching the resonance conditions. As seen in Figure 2, the experimental Raman spectrum compares favorably with the calculated spectrum of the Al complexed kermesic acid (hereafter kermes), when allowance is made for the broadening of the bands in the experimental spectrum and small differences between calculated and observed frequencies[40]. The identification of the red particles as kermes is confirmed through a comparison with available experimental data.

The Raman spectrum of kermes has been reported by various authors. In the report by Burgio and Clark[47] only two peaks are barely discernible at 1603 and 1451 cm^{-1} on the fluorescence background, showing the significant improvement of the approach of the present paper. The Raman spectrum of Fig. 2 is identical to the spectrum of kermes reported by Papaevangeliou et al.[48]. Instead, in the normal Raman spectrum of kermes reported by Whitney et al.[31] no peaks can be detected. Comparison with SERS spectra of kermes reported by Whitney et al.[31] and by Leona et al.[30] is not straightforward primarily because, as far as one can argue from the data reported in the two papers, the experimental spectra are not identical with significant differences in both the frequencies and the intensity profile. This variation can be ascribed to the different treatment of the samples and the possible chemical effects associated with the absorption of the samples on the surface. Indeed the large variability of the SERS spectra of lakes and dyes depending on the experimental conditions has been pointed out by Pozzi et al.[35] and by Idone at al.[37], this latter with particular reference to kermes.

Relying on the reported Raman spectra and on the comparison with calculations, we identify the red particles in the Leonardo's painting as kermes and attempt a vibrational assignment of the observed bands as reported in Table 1. It can be noted that the spectrum does not show the typical bands associated with madder at 1325 and 1481 cm^{-1} arising from δ (OH) and δ (CH) vibrational modes of the alizarin component. Non-complexed kermesic acid is responsible for the bands observed at 964 and 1644 cm^{-1}. Traces of basic lead carbonate ($2PbCO_3 \cdot Pb(OH)_2$) account for the band at 1049 cm^{-1}. Resonance bands are observed with 514.5 nm excitation in spectral regions around 1150, 1220, 1480 and 1640 cm^{-1} and are ascribed to OH and CH bending (1150, 1220 cm^{-1}), to bending modes

involving the methyl group (1480 cm^{-1}) and the C=O stretching mode (1640 cm^{-1}) of kermes and kermesic acid, respectively.

Figure 2. *Raman spectra of the red particles in the cross-section from the Last Supper (see Fig. 1a) with excitation at (a) 514.5 nm and (b) 785 nm. Ab initio DFT calculated Raman spectra of (c) Al-kermes complex and (d) kermesic acid from Ref. 40. Calculated spectra are shown with FWHM=40cm^{-1} for a close comparison with experimental spectra.*

3.2 Masolino da Panicale: *Beheading of St. John the Baptist* (Baptistery of Castiglione Olona)

The second sample presented in this work is from the *Beheading of St. John the Baptist* by Masolino da Panicale, a wall painting in the baptistery of Castiglione Olona dated to 1435. The available cross-section is from the red dress of Salome and, as seen in Figure 1b, two red colored layers, under a white layer (lead white) and a dark grey layer (Sn), are observed. SEM-EDS analyses of the two red-colored layers revealed the presence of aluminum (Al), potassium (K) and lead (Pb) as main components[49]. The presence of Al and K is a clear indication that the red lake was obtained by precipitation of an organic dye with potash alum.

The Raman spectra of the red pigment particles in the Masolino's painting are compared with the spectrum of the particles in the Leonardo's *Last Supper* in Figure 3. The spectra are practically identical with both excitation lines, apart from differences in intensities, particularly for excitation at 514 nm that are ascribed to resonance effects. The experimental data clearly demonstrate that the same pigment, kermes, has been used by the two artists.

Figure 3. *A comparison of Raman spectra of the red particles in the cross-section from Leonardo's Last Supper (black) and the wall painting by Masolino da Panicale (red) with excitation at 785 nm(a,b) and at 514.5 nm (c,d).* The spectra are y-axis normalized on the most intense band at around 1300 cm^{-1}.

3.3 Tintoretto: *The Finding of the Body of Saint Mark*

The third sample considered in thiswork is a cross-section from the blue and red garments worn by Saint Mark in the oil painting on canvas entitled *The Finding of the Body of Saint Mark* by Jacopo Robusti, known as Tintoretto, painted in 1562 and conserved at the Pinacoteca di Brera in Milan (http://pinacotecabrera.org/en/collezione-online/opere/the-finding-ofthe-body-of-saint-mark/). The

cross-section reveals Tintoretto's typically complex painting technique with multiple layers of paint (see Figure 4a).

Figure 4. *(a) Photomicrograph of a cross-section from a sample from Tintorett's The Discovery of the Body of Saint Mark, Pinacoteca di Brera, Milan. From top to bottom: a varnish layer [7], a blue layer containing ultramarine and smalt [6], a layer containing lead white and ochre pigments [5], a 40 μm thick pink layer containing red lake particles (smaller than 5 μm in diameter) mixed with lead white [4], a thin layer containing lead white and ochre particles[3], and finally a layer containing red lead [2] over a ground [1].*
(b) Cross-section from a sample of Veronese's Supper in the House of Simon, Pinacoteca di Brera, Milan,Stratigraphy from top to bottom: a thin layer of varnish [4]; a 40-100 μm thick pink-paint layer with particles of red lake mixed with white pigments bound in a drying oil (with the original red-particles faded at the surface)[3]; intensely colored homogenous red particles with length from 1 to approximately 20 μm mixed with lead white[2] over a lead-based ground [1]. Larger particles of red pigments which can be up to 100 μm in length are found in the dark red layer and were analyzed with micro Raman spectroscopy.

The Raman spectra of the red lake particles in Tintoretto's painting are shown in Figure 5. With the 785 nm excitation line a sharp intense peak is observed at 1050 cm^{-1} which, as in the sample from Leonardo's painting discussed above, must be assigned as the C-O stretching mode of lead carbonate. Changes in the intensity profile of the spectrum are observed also in this case with excitation at 514 nm. It is remarkable that the appropriately corrected normal Raman spectra appear of suitable quality for the identification of red lakes. The spectra in Fig. 5 significantly differ from those of the samples analyzed in the previous sections and must be associated with a different red lake which can be identified as Al-carmine complex (hereafter carmine). The Raman spectra of a reference cochineal and various other cochineals have been reported by Pozzi et al.[36] and, when allowance is made for some variability, the spectra appear coincident with the spectra of the Tintoretto's red particles. The Raman spectrum of a reference carmine sample has been measured in the present work and has indeed been found identical with the spectrum reported by Pozzi et al.[36]
It is nonetheless noted that the Raman spectra and the SERS spectra of cochineal reported by Pozzi et al.[36] differ significantly. This certainly implies that the simple Raman spectrum as used in the present work constitutes an alternative or useful tool for the recognition of red lakes. However the Raman spectrum at 785 nm of the present work compares favorably with the SERS spectrum of cochineal reported by Whitney et al.[31], which however is affected by a significant background.

Finally, the variability already noted of SERS of dyes depends on sample treatment is well documented by the specific work of Canamares et al.[50] on carminc acid.

The spectrum of the red lakes in Tintoretto's painting is compared with DFT calculated spectra of carminic acid and its Al complex in Figure 5, and a tentative assignment of the observed bands is attempted in Table 2. (See Supplementary information for a comparison between DFT calculated and experimentally measured reference samples.) The limited resolution of the experimental spectrum does not allow for a clear cut identification of free carminic acid in the lake. However, by comparing with the DFT spectrum of carminic acid, it is observed that the peak just below 1500 cm^{-1} that becomes more intense with 514 nm excitation, is a possible marker for the acid.

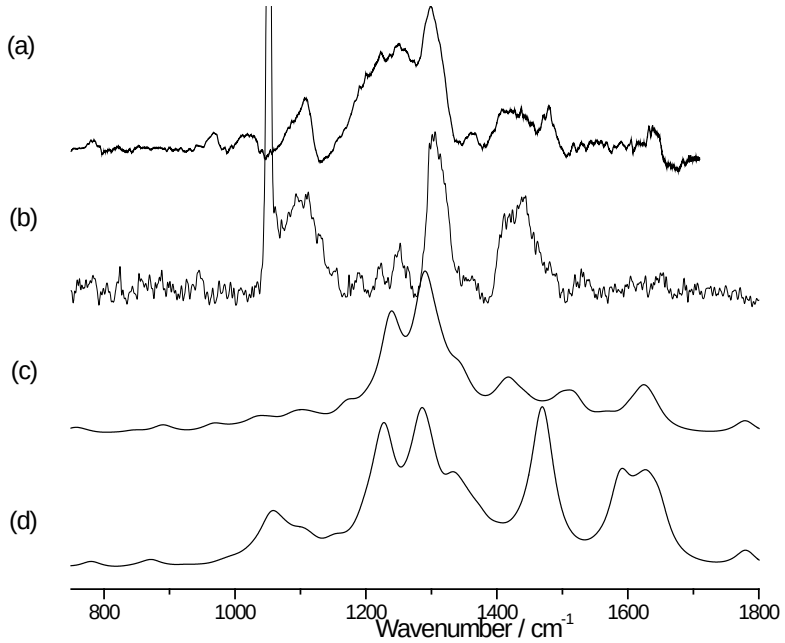

Figure 5. *Raman spectra acquired from the red particles in the cross-section from Tintoretto's painting The Finding of the Body of Saint Mark (see Fig. 4a) following excitation at (a) 514.5 nm and (b) 785 nm. Ab initio calculated Raman spectra of (c) Al-carmine complex and (d) carminic acid [40]. Calculated spectra are shown with FWHM=40cm^{-1} for easy comparison with experimental spectra.*

3.4 Veronese: *Supper in the House of Simon*

Finally, we present the analysis of a sample from the *Supper in the House of Simon*, by Veronese. The sample was taken from the red dress of the man with the glass, in the center-left of the painting and the cross-section is shown in Figure 4b with a description of the stratigraphy. Two layers of red paint can be seen and the analysis was carried out on darker large grains of a deep red color which

are covered by a lighter layer containing particles of smaller size with a fiery red color, alternating with particles of white.

The comparison of the results for the cross-sections from paintings by Tintoretto and Veronese (Fig. 6) shows that the Raman spectra are essentially identical apart from the different broadening of the peaks. We therefore conclude that the same red pigments (carmine) were used by the two artists.

Figure 6. *A comparison of Raman spectra acquired from the dark red particles in the cross-sections from Tintoretto's Finding of the Body of Saint Mark (black) and Veronese's Supper in the House of Simon (red), with excitation at 785 nm (a,b) and at 514.5 nm (c,d).* Spectra were y-axis normalized on the most intense cochineal band at around 1300 cm^{-1}.

4. CONCLUSIONS

The results obtained in this work clearly show that normal micro-Raman spectroscopy, when appropriately improved with Subtracted Shift Raman spectroscopy methods is a practical approach for accurate studies of Old Master paintings to discriminate red lake particles of varying sizes (as small as 5 μm) and obtain information on their chemical composition. Strong similarities have been found between Raman spectra from cross-sections of wall paintings by Leonardo and Masolino, and from samples from paintings by Veronese and Tintoretto. From the experimental data and from comparison with available experimental data and DFT calculations it has been established that similar kermes-based lakes were used in paintings by Leonardo and Masolino and similar carmine-based lakes in paintings by Veronese and Tintoretto. To our knowledge, this is the first time that micro-Raman spectroscopy has been used for discriminating between lake pigments in painting cross-sections without further sample preparation. This approach could complement traditional methodologies for the analysis of lake pigments with the remarkable advantage that further sample preparation from existing cross-sections is not required.

An explanation of the use of kermes-based lakes by Masolino and Leonardo and carmine-based cochineals by Tintoretto and Veronese can be found by considering the different historical periods in which these artists lived and is supported by technical evidence from the analysis of other paintings[14]. Red dyes used by Leonardo and Masolino were probably European kermes-based including Polish Cochineal[51], whereas Tintoretto and Veronese lived in Venice in a time when the city was at the center of commercial and cultural exchanges, and analysis from other paintings by Veronese at the National Gallery London has reported his use of cochineal lakes. Similar trends have been observed in the production of icons where kermes was replaced with cochineal [51]. It is

known that the *Dactylopius coccus Costa* species of cochineal from Mexico replaced, by the year 1523, the Polish dye due to its higher carmine-based colorant content (10–12 times more than kermes)[52].

Acknowledgements

The authors would like to dedicate this work to the memory of Antonietta Gallone Gallassi

REFERENCES

1. Gallone, A. Analisi Stratigrafica di campioni di colore dell'Ultima Cena, in Leonardo l'Ultima Cena, Indagine, Ricerche, Restauro (Eds. G. Basile, M. Marabelli) Nardini Editore, Firenze, 2007, 145-54.

2. G. Cuoco, C. Mathe, P. Archier, C. Vieillescazes, J. Cult. Herit. 2011, 12, 98.

3. S. Bruni, V. Guglielmi, F. Pozzi, J. Raman Spectrosc. 2010, 41, 175.

4. C. Grazia, C. Clementi, C. Miliani, A. Romani, Photochem. Photobiol. Sci. 2011, 10, 1249.

5. M. Harris, B. K. Stein, J. H. P. Tyman, C. M. Williams, J. Chem. Res. 2009, 407.

6. J. Echard, L. Bertrand, A. Von Bohlen, A. Le Hô, C. Paris, L. Bellot-gurlet, B. Soulier, A. Lattuati-derieux, S. Thao, L. Robinet, B. Lavedrine, Stephane Vaiedelich, Angew. Chem. Int. Ed. 2010, 49, 197.

7. L. F. C. de Oliveira, H. G. M. Edwards, E. S. Velozo, M. Nesbitt, Vib. Spectrosc. 2002, 28, 243.

8. A. Cheili, J. Sanyova, B. Doherty, B. Brunetti, C. Milani, Spectrochim. Acta. A 2016, 162, 86.

9. M. V. Canamares, M. Leona, J. Raman Spectrosc. 2007, 35, 1259.

10. M. V. Canamares, J. V. Garcia-Ramos, C. Domingo, S. Sanchez-Cortes, J. Raman Spectrosc. 2004, 35, 921.

11. C. Lofrumento, E. Platania, M. Ricci, C. Mulana, M. Becucci, E.M. Castellucci, J. Molec. Struct. 2015, 1090, 98.

12. L. Legan, K. Retko, P. Ropret, Microchem J. 2016, 127, 36.

13. J. Wisniak, Indian J. Hist. Sci. 2004, 39 (1), 75

14. J. Kirby, R. White in National Gallery Technical Bulletin, (Ed. A. Roy), National Gallery Publications, London, 1996, 17, 56.

15. S. Bruni, V. Gugglielmi, F. Pozzi, J. Raman Spectrosc. 2011, 42, 1267.

16. I. Petroviciu, I. Cretu, I. V. Berghe, J. Wouters, A. Medvedovici, F. Albu, D. Creanga, e-PS, 2012, 9, 90

17. J. Sanyova, Microchim. Acta 2008, 162, 361.

18. J. Wouters, Stud. Conserv. 1985, 30, 119.

19. G. Balakina, V. Vasiliev, E. Karpova, V. Mamatyuk, Dye. Pigment. 2006, 71, 54.

20. D. Fabbri, G. Chiavari, H. Ling, J. Anal. Appl. Pyrolysis 2000, 56, 167.

21. J. Seixas. de Melo, A. P. Moura, M. J. Melo, J. Phys. Chem A, 2004, 108, 6975

22. I. Marcaida, M. Maguregui, H. Morillas, C. García-Florentino, V. Pintus, T Aguayo, M. Campos-Vallette, J. M. Madariaga Anal Bioanal Chem. 2017, 409, 2221.

23. I. Osticioli, A. Nevin, D. Anglos, A. Burnstock, S. Cather, M. Becucci, C. Fotakis, E. Castellucci, J. Raman Spectrosc. 2008, 39, 307.

24. M. L. Franquelo, A. Duran, L. K. Herrera, M. C. J. De Haro, J. L. Perez-rodriguez, J. Mol. Struct. 2009, 924-926, 404.

25. N. Marchettini, A. Atrei, F. Benetti, N. Proietti, V. Di Tullio, M. Mascalchi, I. Osticioli, S. Siano, I. T. Memmi, Surf. Eng. 2012, 29, 153.

26. C. M. Schmidt, K. A. Trentelman, e-PS Sci. 2009, 6, 10.

27. F. Pozzi, M. Leona, J. Raman Spectrosc. 2015, 47, 67.

28. F. Casadio, M. Leona, J. R. Lombardi, R. Van Duyne, Acc Chem Res, 2010, 43(6), 782.

29. E.Casanova-González, A.García-Bucio, J. L. Ruvalcaba-Sil, V. Santos-Vasquez, B. Esquivel, T. Falcón, E. Arroyo, S. Zetina, M. L. Roldán, C. Domingo, J. Raman Spectrosc. 2012, 43, 1551.

30. M. Leona, J. Stenger, E. Ferloni, J. Raman SLeopectrosc 2006, 37, 981.

31. A. V Whitney, R. P. Van Duyne, F. Casadio, J. Raman Spectrosc. 2006, 37, 993.

32. C. L. Brosseau, K. S. Rayner, F. Casadio, C. M. Grzywacz, R. P. Van Duyne, Anal. Chem. 2009, 81, 7443.

33. M. Ricci, E. Trombetta, E. Castellucci, M. Becucci, J. Raman Spectrosc. 2018, 49, 997

34. C. Lofrumento, M. Ricci, E. Platania, M. Becucci, E. Castellucci, J. Raman Spectrosc. 2013, 44, 47.

35. F. Pozzi, K. J. van den Berg, I. Fiedler, F. Casadio, J. Raman Spectrosc. 2014, 45, 1119

36. F. Pozzi, J. R. Lombardi, M. Leona, Herit. Sci. 2013, 1, 23.

37. A. Idone, M. Aceto, E. Diana, L. Appolonia, M. Gulmini, J. Raman Spectrosc. 2014, 45, 1127.

38. A.Cesaratto, M. Leona, J. R. Lombardi, D. Comelli, A. Nevin, P. Londero, 2014, Angew. Chem. Int. Ed. 2014, 53, 14373.

39. J. Sanyova, J. Reisse, J. Cult. Heritage, 2006, 7, 4, 229-235

40. M. Pagliai, I. Osticioli, A. Nevin, S. Siano, G. Cardini, V. Schettino, J. Raman Spectrosc. 2018, 49, 668.

41. I. Osticioli, A. Zoppi, E. M. Castellucci, Appl. Spectrosc. 2007, 61, 839.

42. F. Rosi, M. Paolantoni, C. Clementi, B. Doherty, C. Miliani, G. Brunetti, A. Sgamellotti, J. Raman Spectrosc. 2010, 41, 452.

43. H. Macandrew , D. Howard, J. Dick, J. Plesters, The Burlington Magazine 1985, 127, 501.

44. P.A. Mosier-Boss, S. H. Lieberman, R. Newbery, Appl. Spectrosc. 1995, 49. 630.

45. J. U. N. Zhao, M. I. K. E. M. Carrabba, F. S. Allen, Appl. Spectrosc. 2002, 56. 834.

46. I.Karapanagiotis, D. Lampakis, A. Konstanta, H. Farmakalidis,J Arch Sci 2013, 40, 3,1471

47. L.Burgio and R.J.H. Clark, Spectrochim. Acta A 2001, 57, 1491

48. P. Papaevangeliou, A. Zoppi, C. Lofrumento, E. Castellucci, G. Lanterna, M. Galeotti, C. Lalli, La Spettroscopia Raman per la differenziazione e lo studio di lacche rosse, in Atti del IV Congresso Nazionale di Archeometria, Pisa, 2006

49. D. Comelli, A. Nevin, G. Valentini, I. Osticioli, E. M. Castellucci, L. Toniolo, D. Gulotta, R. Cubeddu, J. Cult. Herit. 2011, 12, 11

50. M.V. Canamares, J.V. Garcia-Ramos, C. Domingo, S. Sanchez-Cortes, Vib. Spectrosc. 2006, 40, 161.

51. L. Valianoua, S.Wei, M.S. Mubarak**,** H.Farmakalidis,E.Rosenberg, S. Stassinopoulos, **J Arch Sci**, 2011, 38, 2, 246.

52. I. Serafini, L. Lombardi, G. Vannutelli, C. Montesano, F. Sciubba, M. Guiso, R. Curini, A. Bianco, Microchem. J., 2017, 134, 237.

Table 1. *Raman spectrum (cm^{-1}) of the sample from Last Supper with excitation wavelength at 514.5 nm and 785 nm and vibrational assignment.*

Exp (this work)		**Calculated (Ref. 40)**		**Assignment [b]**
514.5 nm [a]	**785 nm [a]**	**Kermes**	**Kermesic acid**	
904(w)		898, 899	873	$\delta_{ring} + \delta_{OH}$
964(w)		968		γ_{CH}
995(w)	1002(w)	978, 1021		γ_{CH}
1135-1170(m,br)	1150(w)	1137	1130, 1174 1150	$\delta_{OH} + \delta_{CH}$ δ_{CH}
1210-1260(m,br)	1200-1250(m,sh)	1197, 1205, 1241,1270	1184, 1229, 1241	$\delta_{OH} + \delta_{CH}$
	1302(s)	1287, 1296	1286,1297	δ_{OH}
1318(s)	1316(s)	1313		$\delta_{OH} + \delta_{CH}$
1360-1370(m,sh)		1347,1357	1349,1368	$\delta_{OH} + \delta_{CH}$
1432(w)	1430(w,br)	1419,1424,1435,1445	1448,1455	$\delta_{CH(m)} + \delta_{OH}$
1484(m)		1474, 1478	1470	$\delta_{CH(m)}$
1502(sh)		1541		$\delta_{OH} + \delta_{CH}$
	1575-1620(m,br)	1553,1587 1624,1628	1587 1611	$\nu_{ring} + \delta_{OH}$ $\nu_{ring} + \delta_{OH} + \delta_{CH}$
1644(m)			1653	$\delta_{OH} + \nu_{C=O}$

[a] weak (w), strong (s), medium (m), shoulder (sh), broad (br)

[b] stretch (ν), bend in plane (δ), deformation (γ), methyl (m).

Table 2. *Raman spectrum (cm^{-1}) of the cross-section from The Discovery of the Body of Saint Mark with the 514.5 nm and 785 nm excitation wavelengths.*

Experimental (this work)		**Calculated (Ref. 40)**		**Assignment**
514.5 nm	785 nm	Carmine	Carminic acid	
968(w)		967	976	γ_{CH}
1000-1040(w**)**		980, 996, 1003, 1017, 1032	980, 994 1022, 1030, 1038	Gly $\delta_{OH} + \delta_{CH}$
1085-1110(m)	1085-1110(m)	1087, 1130 1101, 1113	1100, 1120 1095, 1107, 1117	$\delta_{OH} + \delta_{CH}$ Gly
1195, 1200	1190 (w**)**	1202	1198 1215	$\delta_{OH} + \delta_{CH}$ Gly
1225	1223(w)	 1235, 1238	1227 1221, 1231	Gly $\delta_{OH} + \delta_{CH}$
1240, 1255	1252(w)	1244, 1250	1263	Gly
1305(s)	1305(s)	1291, 1311 1296	1294 1299, 1307	Gly δ_{OH}
1318(s,sh)	1320(s,sh)	1322	1333, 1341 1327	$\delta_{OH} + \delta_{CH} + \delta_{ring}$ Gly
1360(w)	1355(w,sh)	1349,1353, 1359	1357 1368, 1376	Gly
1410-1450(m,br)	1410-1450(m,br)	1417, 1418, 1426, 1434, 1442, 1444	1407, 1413, 1420, 1433 1400, 1426, 1448	Gly $\delta_{CH(m)} + \delta_{OH}$
1480 (m)	1481(w,sh)	1476, 1498	1469, 1485 1471 1478	δ_{CH} Gly $\delta_{CH(m}$
	1528(w)	1554	1556	$\delta_{OH} + \delta_{CH}$
1640(m)	1650(w)	1623, 1640	1613, 1628 1647	$\nu_{ring} + \delta_{OH} + \delta_{CH}$ $\delta_{OH} + \nu_{C=O}$

[a] weak (w), strong (s), medium (m), shoulder (sh), broad (br) [b] stretch (ν), bend in plane (δ), deformation (γ), methyl (m), normal modes localized on glycosidic moiety (gly)