

ANALYTICAL STUDY OF THE WALL PAINTINGS IN THE BORGHERINI CHAPEL IN THE SANT PIETRO IN MONTORIO CHURCH (ROME, ITALY) BY SEBASTIANO DEL PIOMBO

Laura Osete Cortina, María Luisa Vazquez de Agredos Pascual¹, María Teresa Doménech Carbó and Antonio Doménech Carbó²

Instituto Universitario de Restauración del Patrimonio de la Universitat Politècnica de València

¹Departamento de Historia del Arte de la Universitat de València,

²Departamento de Química Analítica, Universitat de València

CORRESPONDING AUTHOR: María Teresa Doménech-Carbó, tdomenec@crbc.upv.es.

ABSTRACT: *The wall paintings “La Trasfigurazione” and “La Flagellazione” studied in the present work are located in the Sant Pietro in Montoro Church, which is an outstanding monument in Italian Renaissance style. Borgherini family commissioned the decoration of one of the Chapels in this church to Sebastiano del Piombo, one of the most relevant Italian painters at that time. The color palette identified in these paintings is in correspondence with the artistic technique used by the artist in each one of the paintings. Thus, earth pigments, green earth and smalt compose the color palette in “La Trasfigurazione”, together with gilded decoration in punctual areas. In contrast, a richer palette has been characterized in the oil-based “La Flagellazione”, including a number of copper pigments, lead white, tin yellow, vermillion, among other pigments characteristics of this technique. Interestingly, different artistic techniques have been identified in the paintings analyzed. Whereas fresco technique was used in “La Trasfigurazione”, a drying oil has been identified in “La Flagellazione” suggesting the use of an oil technique here. Conventional analytical techniques such as Scanning Electron Microscopy-X-Ray Microanalysis, FTIR Spectroscopy, Gas Chromatography-Mass Spectrometry, Pyrolysis-Gas Chromatography-Mass Spectrometry have been combined with the voltammetry of microparticles.*

KEYWORDS: *piombo*, Renaissance, SEM-EDX, voltammetry of microparticles, FTIR spectroscopy, GC-MS, Py-GC-MS

1. INTRODUCTION

Shortly after King Ferdinand II of Aragon had ordered the reconstruction of the church of San Pietro in Montorio between 1481 and 1500, the trader Pierfrancesco Borgherini commissioned Sebastiano del Piombo with the mural decoration of the church chapel, commonly known as the Borgherini Chapel, where one may still contemplate today Sebastiano's scenes of the Flagellation and Transfiguration adorning the wall and vault of the chapel. The records show that work was started on the paintings in 1521 exactly one decade after Sebastiano del Piombo had arrived in Rome from his native Venice in 1511 (Strinati, 2009). Over these ten years his painting had undergone a gradual and evident transformation from the rich combination of colours of the Venetian school, influenced by the masters Giovanni Bellini and Giorgione, towards the monumental forms and volume instilled by Michelangelo throughout these Roman years. The close friendship that had bonded both painters over this time and the high esteem in which Sebastiano del Piombo held Michelangelo as a painter, explain why he sought his assistance shortly after being commissioned to paint the Borgherini Chapel, as is made evident by the letter sent to Michelangelo with this purpose in mind on 6 September 1521. Michelangelo answered by sending a sketch of the scene of the Flagellation which Sebastiano del Piombo reproduced with great fidelity in 1524 and this explains why the sculptoric depiction of the figure of Christ bound to a column frequently evokes the work of Michelangelo (Stone, 2004)

The importance of the mural paintings of the Flagellation and Transfiguration is not only to be found in the close relation between two of the most emblematic painters of the Roman Cinquecento, as already narrated by the painter and historian Giorgio Vasari (Vasari, 2005) but also, and more particularly, because these two painters personified the leading debate of the day concerning the technique to be employed in mural painting: oil or fresco? In

technical terms, the convenience of the latter of these two left no room for doubt. But how was it possible to take up the innovative technique that Antonello da Mesina had brought to Naples from the workshop of Jean Van Eyck just one century earlier and which had extended to Florence shortly afterwards by the hand of Friar Filippo Lippi? (Radke and Paoletti, 2002) Why could one not experiment with the freedom, and ensuing naturalism of colour offered up by drying oil binders?

The work described afterwards concentrates on the chemical characterization of the wall paintings in the Borgherini Chapel of the church of San Pietro in Montorio by Sebastiano del Piombo.

A wide variety of analytical methodologies have been applied in recently reported studies that deal with the characterization of wall paintings. Most of them are based on the application of complementary instrumental techniques such as Light Microscopy (LM), Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX), Raman Spectroscopy, X-ray diffraction (XRD), X-ray Fluorescence (XRF) and Chromatographic techniques, among others (Ospitali et al., 2007; Herrera et al. 2009; Bianchin, 20097; Katsibiri and Howe, 2010; Bianchin et al., 2009; Daniilia et al., 2007). More recently, an increasing number of studies are based on the simultaneous application of less-destructive techniques such as Laser Induction Breakdown Spectroscopy (LIBS), that does not require sample preparation and Proton-Induced X-Ray Emission Spectroscopy (PIXE), as a powerful multi-elemental technique due to its high sensitivity and its potential for performing spatially resolved analysis and the favorable excitation of light elements and the simultaneous combination of infrared reflectography, microscopic examination of surfaces, X-ray fluorescence (EDXRF) and radiographic techniques (El Aal et al., 2009; Serifaki et al. 2009). However, in most cases, the application of conventional destructive techniques such as SEM/EDX or chromatographic techniques are required in order

to achieve a more complete understanding of the inorganic and organic materials present in the paintings. The development of solid state electrochemistry is an interesting alternative which provides useful analytical information about the composition, speciation and coordination environment of the electroactive centers present in the sample. In particular, voltammetry of microparticles (VMP) has been successfully applied for the identification of pigments in the wall paintings of the rock-hewn churches in Lalibela (Ethiopia), the wall paintings from San Juan del Hospital church in Valencia, and in the frescoes painted by Antonio Acisclo Palomino y Velasco (1698) in the vault of the Sant Joan del Mercat church in València (Spain) (Doménech et al., 2000; Doménech et al., 2007).

In this paper, a multi-method approach to the study the wall paintings in the Borgherini Chapel in the Sant Pietro in Montorio Church by Sebastiano del Piombo is proposed, that includes analysis of inorganic and organic materials present in the works of art. The use of Light Microscopy (LM), Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX), Voltammetry of Microparticles (VMP) in combination with Gas Chromatography (GC-MS) and Pyrolysis-Gas Chromatography-mass Spectrometry (Py-GC-MS) and FTIR Spectroscopy, allowed the scientific investigation of the employed materials and the painting technique. Combination of those instrumental techniques has provided essential analytical information about the inorganic and organic materials present in the paint samples with high sensitivity. Additionally, a new micro- and nanosampling method has been used in order to reduce the amount of sample used for the pigment analysis.

2. EXPERIMENTAL

2.1 Materials

Reagents: The following reagents were used to treat the samples: hexamethyldisilazane (HMDS) and methyl chloroformate (ECF) (purity 99%) (Sigma-Aldrich, Steinheim, Germany). Absolute pyridine and chloroform at 98% for GC (Acros, Cambridge, MA, USA). The absolute ethanol used for analysis was from Carlo Erba, Rodano, Italy. The sodium hydrogencarbonate used for analysis was from Panreac, Barcelona.

Micro and nanosampling: Microsamples for microscopy, spectroscopy and chromatography analysis were collected by excising them with the help of a scalpel from different areas of the paintings with different tones in order to investigate the stratigraphic structure and characterize the materials employed by the artist.

Micro and nanosamples for VMP analyses were obtained by abrasive fixing of a few micro and nanoparticles from the surface of the painting on the basis of a sampler consisting of a thin cylinder of graphite ($d=0.5-1$ mm) inserted into a handler ($d=1$ cm, 10 cm length) for facilitating handling. This graphite cylinder is directly put in the electrochemical cell acting as work modified electrode.

Table 1 shows the reference, location and description of samples studied. In Figure 1, a photograph of "The Transfiguration" can be observed.

2.2 Instrumentation

Optical microscopy. Microsamples were prepared as cross sections by embedding them in polyester resin (Seryfix, Struers) and then mechanically polished to a 4000 silicon carbide grit finish in a polishing machine (Knuth-rotor 2, Struers, Erkrath, Germany) for examination under a Leica DMR microscope using polarized reflected light at X25-X400 magnification.

Scanning electron microscopy-energy dispersive x-ray microanalysis. Samples prepared as cross-sections were studied by SEM-EDS using

a Jeol JSM 6300 scanning electron microscope operating with a Link-Oxford-Isis X-ray microanalysis system. The analytical conditions were: 20 kV accelerating voltage, 2×10^{-9} A beam current and 15 mm as working distance. Samples were carbon coated to eliminate charging effects. Elemental analysis was performed in parallel to the morphological examination of the microsamples. Qualitative analysis was performed by point and area acquisitions. Quantitative microanalysis was carried out using the ZAF method that is based on the correction of the matrix of multi-elemental effect that takes place in the simultaneous determination of the concentration of each element present in a multielemental material. This theoretical method provides a correction of the x-ray intensity of each element present in the material by means of the application of a series of correction factors for the atomic number effect (which describes the depth of electron penetration and the fraction of electrons that backscatter from the sample), the absorption correction (which describes the absorption of x-rays in the matrix as function of the composition and depth of electron penetration) and the fluorescence correction (which describes the secondary fluorescence of one element by the others present). In the present study, the counting time was 100 s for major and minor elements. Concentrations were calculated by stoichiometry from element percentages generated by ZAF software on the Oxford-Link-Isis EDX instrument.

Voltammetry of microparticles. Voltammetric measurements were performed at sample-modified paraffin-impregnated graphite electrodes immersed into deaerated 0.50 M sodium acetate buffer at pH 4.85, using a CH I420 equipment. A conventional three-electrode arrangement was used, with a Pt-wire auxiliary electrode and an AgCl (3M NaCl)/Ag reference one. Samples were pressed on a freshly polished graphite electrode and subjected to voltammetry analysis (see 2.1 section).

Fourier Transform Infrared Spectroscopy. IR spectra of the samples were obtained using a Vertex 70 Fourier-transform infrared spectrometer with a FR-DTGS (fast recovery deuterated triglycine sulphate) temperature-stabilized coated detector. Number of co-added scans: 32, obtained with a spectral resolution of 4 cm^{-1} .

Gas Chromatography-Mass Spectrometry. A small quantity (0.5 mg) of sample was grinded and placed in a 0.3 mL minivial (Supelco Bellefonte, PA, USA) and hydrolyzed with 100 μL of 6 M HCl in an Ar atmosphere for 24 h at 110°C . The resulting solution was evaporated to dryness under infrared radiation lamp (230 V, 250 W, Osram Siccatherm, Germany) and then 100 μL of water and 100 μL of CHCl_3 were added, shaking vigorously to facilitate the extraction of fatty acids in the chloroformic phase. The aqueous and chloroformic phases were separated and treated independently as described below. Proteinaceous components in the samples are best separated in the aqueous phase. 50 μL of ethanol/pyridine 4:1 were added to 50 μL of aqueous phase. This solution was treated with 8 μL of ECF, shaking for 10 s after the addition to help the reaction. The reaction mixture was then extracted with 50 μL of 1% CHCl_3 in ECF and, finally, 50 μL of a saturated solution of NaHCO_3 was added and shaken vigorously. Two phases (aqueous and chloroformic) were obtained and 1.5 μL of the organic phase was injected into the chromatograph. The same process was followed for the chloroformic phase, which was first evaporated to dryness and the residue re-dissolved in 50 μL of a water/ethanol/pyridine mixture (5:4:1). The chromatographic conditions used for the analysis of the chloroformic phase were: initial temperature of the gas chromatograph 50°C , a gradient of $40^\circ\text{C}/\text{min}$ up to 295°C , which was held for 12 minutes. The carrier gas was He with an inlet pressure of 72.5 kPa and a split ratio of 1:20. For the aqueous layer initial temperature of the gas chromatograph was 100°C , a gradient of $5^\circ\text{C}/\text{min}$ up to 155°C , ramping up at $15^\circ\text{C}/\text{min}$ up to 295°C , which was held for 5 minutes.

Pyrolysis-Gas Chromatography-Mass Spectrometry. Pyrolysis-Gas Chromatography-Mass Spectrometry for the characterisation of the binding media was carried out with an integrated system composed



Figure 1. Photograph of the wall painting by Sebastiano del Piombo in the Borgherini Chapel in the Sant Pietro in Montorio Church (Rome, Italy)

of a CDS Pyroprobe 1000 heated filament pyrolyser (Analytical Inc., NY, USA), and a Gas Chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, Ca, USA) equipped with an on-column injection system and coupled with an Agilent 5973N mass spectrometer (Agilent Technologies) and equipped with a pyrolysis injection system. A capillary column HP-5MS (5% phenyl-95% methylpolysiloxane, 30 m x 0.25 mm I.D., 0.25 mm film thickness, Agilent Technologies) was used in order to provide an adequate separation of components for analysis. Pyrolysis of samples was

performed at 600°C for 10 s using a pre-calibrated Pt coil type pyrolyser (CDS pyroprobe). The pyrolyser interface and the inlet were set at 250°C. Samples were injected in split mode (split ratio 1:40). The chromatographic conditions were: initial temperature 100°C, increased at 5°C/min up to 295°C held for 25 min. Helium glass flow in constant mode was set at 1.2 mL.min⁻¹. Ions were generated by electron ionisation (70 eV) in the ionisation chamber of the mass spectrometer. The mass spectrometer was scanned from m/z 20 to m/z 800, with a cycle time of one second. Tuning of the mass spectrometer was checked using perfluoro-tributylamine. Agilent ChemStation software G1701CA MSD was used for GC-MS control, peak integration and mass spectral evaluation. EI mass spectra were acquired in the total ion monitoring mode and peak area data from total ion current (TIC) chromatograms were used for quantitative analysis. The temperatures of the interface and the source were 280 and 150°C, respectively. The Wiley and NIST Libraries of Mass Spectra were used for identifying the compounds.

Off-line derivatisation. Samples were dissolved in 50 µl ethanol-pyridine (4:1, v/v). Then 10 µl ECF were added. The reaction mixture was vortexed for about 10 s and the derivatives were extracted in 50 µl chloroform containing 1% ECF. After that, 50 µl of a saturated solution of NaHCO₃ was added and the mixture was shaken carefully. A 1.5 µl aliquot of the organic phase was injected into the GC-MS system.

In-situ thermally assisted pyrolysis and silylation of samples. Samples were taken from the works of art by scraping with the help of a scalpel, and were placed in a microquartz pyrolysis tube. Then two portions of quartz wool were introduced into both sides of the quartz tubes to avoid undesirable displacements of the sample. Afterwards, 5 µL of HDMS was added.

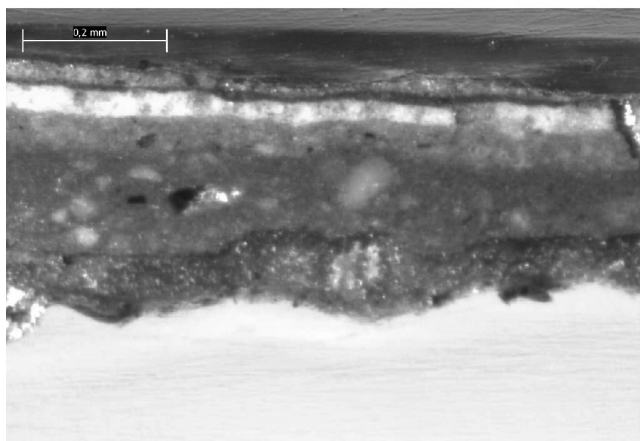
3. RESULTS AND DISCUSSION

3.1 Characterization of pigments and grounds

3.1.1 "The Flagellation"

For the determination of the stratigraphic structure of the paintings and the characterization of the pigments and grounds used by the artist, samples from both works of art were prepared in cross-sections and subsequently examined by light microscopy and analyzed by using Scanning Electron Microscopy Energy-Dispersive X-ray Microanalysis.

a)



b)

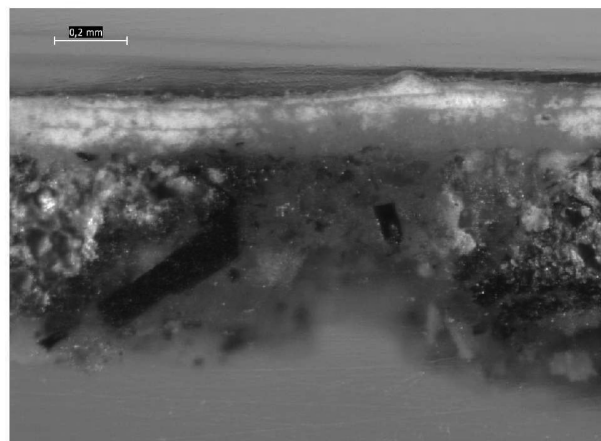


Figure 2. Microphotographs of samples F12 (2a) and T10 (2b) in cross-section

Reference	Location	Color
The Flagellation		
F1	Mantle of the personage at the back of the main scene	Green
F2	Wrap of Jesus Christ	White
F3	Flesh color. Right heel of the flagellator on the right.	Dark ochre
F4	Wrap of the flagellator on the left	Grey
F5	Flesh color. Jesus Christ's right leg	Light ochre
F6	Vest of the flagellator on the left	Green-Blue
F7	Mantle of the personage at the bottom on the left	Red-ochre
F8	Floor	Yellow-grey
F9	Geometric bands on the floor	Blue-grey
F10	Personage at the bottom on the left of the main scene	Dark ochre
F11	Clothes of the personage at second plane on the right of the main scene	Red-blue
F12	Background on the top at the right	Yellow
F13	Pilaster at the bottom on the right	Ochre
F14	Book covers	Red
F15	Saint Peter's sleeve	Light blue
F16	Saint Peter's mantle	Orange-Yellow
F17	Saint Peter's tunic	Violet
F18	Bottom of the painting	Black
The Transfiguration		
T1	Entablature (transition between "The Flagellation" and "The Transfiguration")	Black
T2	Entablature	Blue-green
T3	Entablature	Dark orange
T4	Entablature	Ochre
T5	Mantle of the personage on the right	Red-purple
T6	Mantle of the personage on the right	Green
T7	Outer ornamental band	Yellow-ochre
T8	Outer ornamental band	Red-ochre
T9	Sleeve of the personage on the right of the vault	Light-ochre
T10	Tunic of the personage on the left	Orange-yellow
T11	Tunic of the personage in the foreground on the left	White
T12	Tunic of the personage on the left	Red
T13	Tunic of the personage in the foreground on the left	Dark green

Table 1. References, description and location of the samples studied

Observation of the polished-cross-sections by means of light microscopy revealed two ground layers in most part of the samples studied. Gypsum as main compound and clayish minerals in small proportion were identified by using Scanning Electron Microscopy Energy-Dispersive X-ray Microanalysis in the inner light-ochre ground with a porous appearance. The analysis by using FTIR spectroscopy corroborated the presence of gypsum, showing the stretching absorptions of SO_4^{2-} groups at 1102 cm^{-1} , and the stretching and bending IR absorptions of the water of hydration ν O-H at 3520 , 3400 cm^{-1} and the δ O-H at 1683 , 1622 cm^{-1} . On the other hand, a heterogeneous distribution of rounded white grains of lead-white pigment and some gypsum aggregates make up the thinner and greyish ground bellow the paint layers (average thickness ranging

from $20\text{--}150\text{ }\mu\text{m}$). This layer was probably applied by the artist as an intermediate stratum between the inner ground and the paint layers to enhance the hue of the pigments and it is supposed that small particles of carbon black were diffused by the artist in order to get the grey colour. It is noteworthy, that an intense diffusion of this layer into the inner ground is observed in some of the samples studied, which could indicate its application on the not totally dry plaster. Microphotograph of sample F12 is shown in Figure 2a.

It is noticeable that in the grey areas of this painting, such as in sample F2 from the Jesus Christ's wrap on the floor, the outer greyish ground layer acts as a pictorial stratum as well. Another interesting feature is the presence of carbon black particles between the paint

layer and the ground in sample F2, which could correspond to the preliminary drawing.

In most samples, only one paint layer is identified with an average thickness between 8 to 100 μm , except in samples F12 and F17, in which two and four strata are superimposed.

Red earth (Fe_2O_3 , and clayish minerals), lead white (2PbCO_3 , $\text{Pb}(\text{OH})_2$), ochre ($\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$ and clayish minerals), vermilion (HgS), azurite ($2\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$), malaquite ($\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$), tin-lead yellow (Pb_2SnO_4) and a red organic pigment, were identified in the paint layers by elemental analysis using SEM/EDX. It is interesting to note, that this colour palette is more representative of an oil-based technique due to the wide variety of colours and the use of pigments such as lead white, which can act, additionally, as siccative.

A number of samples from "The Flagellation" painting, namely F1, F6, F16, F20 and F22, were analysed by VMP using a micro/nanosampling method by potential scanning from +0.45 V vs. AgCl/Ag in the negative direction and using acetate buffer. In all cases, overlapping cathodic peaks at -0.05 and -0.15 V were found, that are ascribed to azurite. Sample F6, additionally, exhibited an intense reduction peak at -0.56 V that is associated to lead white. Despite the sampling procedure used provides samples in the range of a few micro- to nanograms, the peaks ascribed to the different electroactive species present in the samples were well resolved in the voltammograms so that the identification of pigments was satisfactory.

3.1.2 "The Transfiguration"

Microphotographs of the samples from "The Transfiguration" prepared in cross-section showed the common structure of a wall painting and a different layer distribution than the one for "The Flagellation". The ground observed in these samples is a lime plaster integrated by a heterometric distribution of translucent, ochre and black silicate grains, and a microcrystalline whitish matrix followed by the paint layers with an average thickness ranging from 70 to 230 μm .

Detailed examination of the samples studied showed that in most parts of painting, the paint stratum was applied in a single layer and appeared gently mixed with lime, according to the fresco technique, but in a few cases, the pigments were spread in several layers in which the absence of diffusion between the plaster and the paint stratum could indicate little adjustments or retouchings made by *secco* technique (samples T9, T10, T13). This particular paint layer distribution can be seen in Figure 2b, where microphotograph of the sample T10 is shown.

SEM/EDX analysis of the paint layers proved a limited number of pigments, mainly yellow ochre, red, green earths and smalt, all of them suitable for painting on wet plaster. On the other hand, in some samples, copper pigments which are not preferred for *fresco* technique have been identified, maybe employed by the artist for obtaining a wider range of blue tones. In these cases, the pigments could have been applied on dry plaster with the help of a binding medium to ensure their fixation to the ground.

Samples from the entablature, which is the transition area between both paintings "The Flagellation" and "The Transfiguration" (samples T1, T2, T3 and T4) present a stratigraphic distribution which differs from the rest of the samples. Two ground layers were identified, the inner one similar to the ground layer observed in the samples from "The Transfiguration" and a second one thinner in orange colour. X-ray spectra obtained from the point analysis carried out on this outer ground revealed a mixture of lead-white and earth pigments. Another difference to highlight is the higher number of superimposed paint strata recognized in these samples, and the presence of remains of a gold sheet corresponding to a gilded

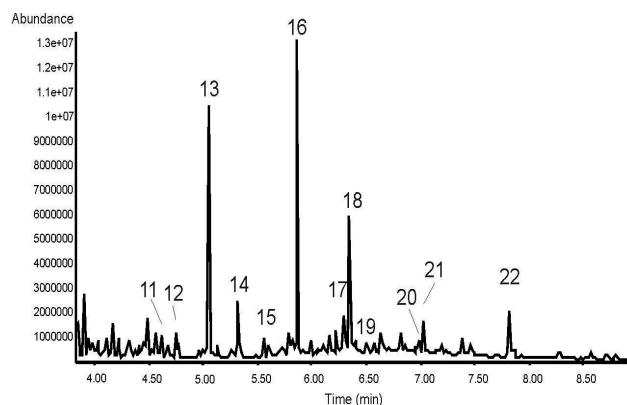


Figure 3. Gas Chromatogram of sample F3. Compounds identified are listed in Table 2

decoration over the ground layer in sample T2. As for the pigments employed by the artist in this area, elemental analysis performed by SEM/EDX revealed minium, lead-white, red earth, smalt and green earth, which indicates a richer colour palette than the one identified in "The Transfiguration".

3.3 The painting technique

3.3.1 "The Flagellation"

For the identification of binders, FTIR Spectroscopy, GC-MS and Py-GC-MS were performed. FTIR spectroscopy in ATR mode was carried out in order to obtain analytical information about the nature of the binding materials employed. These results confirmed that an oil-based technique was employed in "The Flagellation". IR spectra collected in the outer layer of the samples F1 and F12 from "The Flagellation" showed prominent absorption bands at 2919 and 2850 cm^{-1} ascribed to stretching C-H vibrations, that together with the carbonyl stretching band at 1706 cm^{-1} are indicative of the presence of a siccative oil highly deteriorated due to hydrolysis of fatty esters to free fatty acids (Erhardt et al. 2005). Besides, the identification of absorption bands at 1612, 1319 and 820 cm^{-1} characteristic for calcium oxalates (wheddellite) and bands at 1438, 1365 and 782 cm^{-1} attributed to copper oxalates (moooloite) in sample F1 (Salvadó et al. 2005) are in agreement with the significant

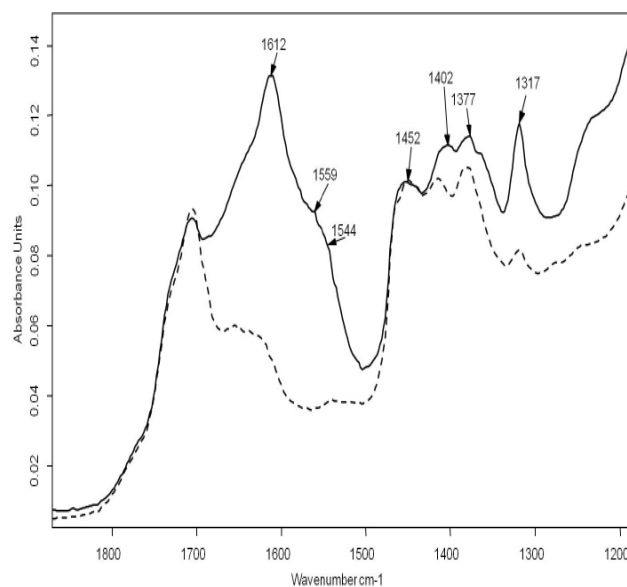


Figure 4. Details of infrared spectra of samples F1 (solid line) and F12 (dashed line)

Peak number	t _R	Compound	m/z
The Transfiguration			
1	5,74	Alanine, N-ethoxycarbonyl-, ethyl ester	<u>116</u> , 100, 88, 72, 44
2	5,87	Glycine, N-ethoxycarbonyl-, ethyl ester	<u>102</u> , 74, 56, 30
3	8,08	Valine, N-ethoxycarbonyl-, ethyl ester	<u>144</u> , 116, 72, 55
4	9,58	Leucine, N-ethoxycarbonyl-, ethyl ester	<u>158</u> , 102
5	9,94	Isoleucine, N-ethoxycarbonyl-, ethyl ester	<u>158</u> , 129, 102
6	10,43	Proline, N-ethoxycarbonyl-, ethyl ester	<u>142</u> , 114, 98, 70
7	13,93	Metionine, N-ethoxycarbonyl-, ethyl ester	<u>175</u> , 129, 101, 61
8	14,45	Glutamic acid, N-ethoxycarbonyl-, ethyl ester	230, <u>202</u> , 156, 128, 84
9	15,08	Phenilalanine, N-ethoxycarbonyl-, ethyl ester	192, <u>176</u> , 148, 120, 91
10	17,76	Lysine, N-ethoxycarbonyl-, ethyl ester	<u>156</u> , 128, 102, 84
The Flagellation			
11	4,74	Octadienoic acid diethyl ester (Suberic acid ethyl ester)	185, <u>143</u> , 97, 69, 55
12	4,78	Decanoic acid 9-oxo ethyl ester	169, <u>157</u> , 111, 83, 43
13	5,05	Nonanodioic acid diethyl ester (Azelaic acid diethyl ester)	<u>199</u> , <u>152</u> , 125, 111, 83, 55
14	5,32	Decanedioic acid diethyl ester	<u>213</u> , 171, 125, 98, 55
15	5,60	Tetradecanoic acid ethyl ester	<u>227</u> , 185, 149, <u>98</u>
16	5,87	Hexadecanoic acid ethyl ester	284, 241, 157, 101, <u>88</u>
17	6,29	Octadecenoic acid ethyl ester (Oleic acid ethylester)	310, 264, 222, 180, 83, <u>55</u>
18	6,34	Octadecanoic acid ethyl ester (Stearic acid ethyl ester)	312, 269, 157, 101, <u>88</u>
19	6,40	Octadecadienoic acid ethyl ester (Linoleic acid ethyl ester)	308, 263, 135, 95, <u>67</u>
20	6,99	Di-dehydroabietic acid	326, 266, <u>237</u> , 197, 165
21	7,03	Dehydroabietic acid ethyl ester	328, <u>239</u> , 197, 173, 129
22	7,81	7-oxo-dehydroabietic acid ethyl ester	342, <u>253</u> , 213, 187

Table 2. Retention time, peak number, m/z and compounds identified by using GC-MS
 state of deterioration of the painting medium present

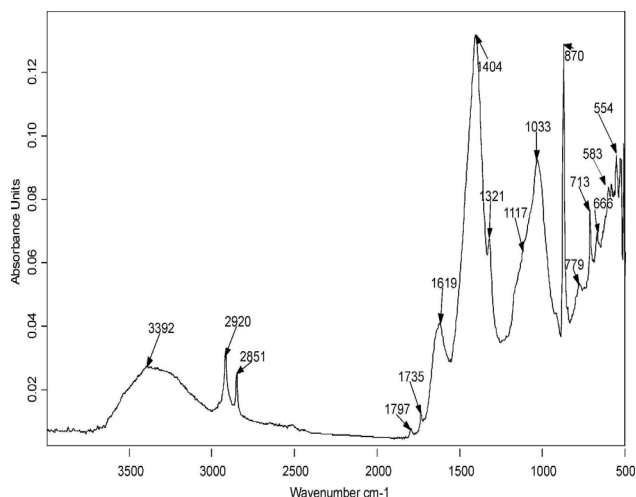


Figure 5. Infrared spectrum of sample T10

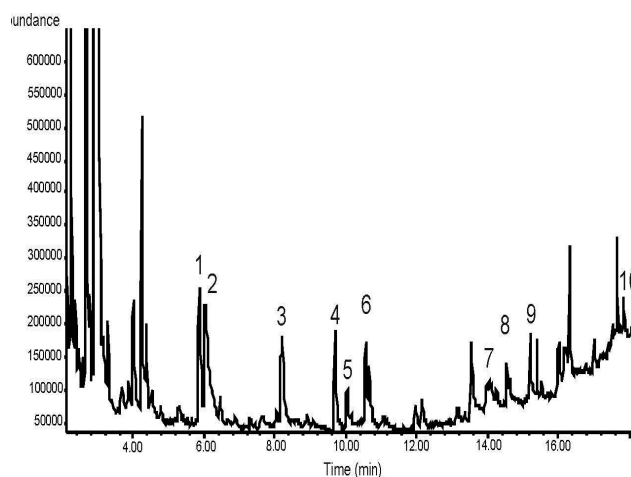


Figure 6. Gas Chromatogram of sample T10

Representative samples from “The Flagellation” were analyzed by GC-MS and Py-GC-MS with the aim of corroborating the results obtain by using FTIR Spectroscopy and to achieve a deeper knowledge about the nature of the organic materials present in these works of art.

A methodology developed by the authors based on the simultaneous characterization of lipids, proteins and diterpenoids by GC/MS using Ethyl Chloroformate (ECF) as derivatization reagent was applied for the identification of the binding media in these wall paintings. This methodology has been successfully applied to the analysis of lipids and proteins used as binding media (Doménech-Carbó et al. 2005).

Figure 3 shows the chromatogram obtained in sample F3. In parallel, peak numbers, retention times and m/z values for the compounds identified in this sample have been listed in Table 2. This chromatogram revealed the presence of: hexadecanoic (palmitic) and octadecanoic (stearic) and nonanodioic (azelaic) acids, corresponding to the most prominent peaks and, in a less amount, tetradecanoic acid and some oxidation products of the unsaturated fatty acids, such as octanedioic (suberic), decanedioic and decanoic acid, 9-oxo. It is noticeable the small amount of linoleic acid identified suggesting that the oil oxidation has not reached a significant extent.

According to the literature (Mills and White, 1994) A/P (azelaic/palmitic) and P/S (palmitic/stearic) ratios can be used for the identification of an oil binder. In the samples studied P/S ratios range between 2.10-2.60 which could be related to walnut oil. On the other hand, A/P ratios obtained present a significant variation margin (from 0.15 to 1.13) which could be ascribed to different degrees of oxidation of the oil and the formation of complexes between the metal ions of the pigments and fatty acids. The occurrence of metallic soaps (azelates, palmitates and stearates) is confirmed by the presence in the IR spectra of vibrational bands ascribed to them, at 1559, 1544 and 1452 cm^{-1} , as can be seen for sample F1. In contrast, in a number of samples, the relative intensity of these IR bands is much lower, for example, in sample F12. The comparison between both spectra is shown in Figure 4.

Interestingly, the chromatograms obtained contain several peaks relative to oxidation products of abietadiene acids, such as, dehydrodehydroabietic acid, dehydroabietic acid and 7-oxo-dehydroabietic acid. Thus, the presence of a *Pinaceae* resin together with the drying oil was corroborated.

A second series of analysis were performed by means of Py-GC/MS attempting to complement the information found previously

shown. This technique was selected due to its minimum sample size and pre-treatment requirements. This methodology has been successfully applied to the analysis of lipids used as binding media and protective coatings in works of art and in archaeological finds (Doménech-Carbó, 2008).

In-situ thermally assisted pyrolysis and silylation with HMDS has been carried out for the analysis of the organic materials occurring in the samples studied, since this technique has demonstrated its effectiveness in obtaining adequate derivatives with high amount of reaction yields and low amount of secondary products in comparison with thermally-assisted pyrolysis and methylation with TMAH. The pyrograms obtained showed the presence of fatty acids and, in a very low proportion, some diterpenoid oxidation products, confirming the results obtained by GC/MS previously mentioned.

3.3.2 “The Transfiguration”

FTIR spectroscopic analysis enabled the identification of small amounts of fatty materials in a number of samples, as indicated by the vibrational band at 1736 cm^{-1} ascribed to the stretching vibration of C-O bond in ester groups. The low intensity of this band could suggest the presence of unburnt residues of candles smoke condensed on the surface of the paintings. The characterization of the deposits of dirt scraped from different areas of the painting corroborated this hypothesis as waxy materials were also identified in some extent by FTIR spectroscopy.

Interestingly, IR spectra collected in the outer layer of those samples in which the painting was applied in several layers (samples T9, T10, T13) evidenced the presence of a proteinaceous material, as indicated by the band at 1644 cm^{-1} ascribed to the stretching vibration of C=O groups associated to amide I functionalities of proteinaceous materials. These results suggest that *secco* technique was used in these areas of the painting that could be associated to retouchings made by the artist. Significant IR bands ascribed to calcium oxalates that appear at 1620, 1317 and 780 cm^{-1} are also found in this series of samples (Figure 5). FTIR spectroscopic analysis of the blue-green paint layer of sample T13 indicated the formation of copper oxalates as well, and it is an indication of the transformation of the constituent materials in degradation products. Besides, it is interesting to note that gypsum was identified in some of the samples studied, indicating a significant salt induced decay processes.

The analytical characterization by means of FTIR spectroscopy of samples from the entablature, located between both paintings, showed characteristic absorption patterns attributed to a siccative

oil. Spectra collected from the outer layers of these samples showed significant absorption bands at 1733 and 1700 cm^{-1} related to the stretching vibration of carbonyl bond from an ester group and a carboxyl group, respectively, indicating the partial degradation of the oil owing to the hydrolysis of fatty esters.

Gas Chromatographic analysis of samples from "The Transfiguration" confirmed the presence of fatty acids at trace level ascribed to residues from the combustion of candles. On the other hand, alanine, glycine, valine, proline, isoleucine, methionine, glutamic acid, phenylalanine and lysine amino acids were found, in a low proportion, in samples T9, T10 and T13, corroborating that these samples correspond to a retouching. For illustrating this finding the chromatogram of sample T10 is shown in Figure 6. However, the low intensity of them and the significant degree of degradation, evidenced for the high content of oxalates identified by FTIR (see Figure 6), does not enable to do a right assignment of the kind of protein present.

3. CONCLUSIONS

The analytical results presented here confirm that Sebastiano del Piombo chose to use an oil technique to paint the scene of the Flagellation that decorates the wall above the altar in the Borgherini Chapel, while resorting to the *fresco* for the episode of the Transfiguration, painted on the chapel's vault. In this way the two mural paintings of the Borgherini Chapel represent the technical dilemma faced by many of the painters of the Italian Renaissance during the 15th and 16th centuries. This being the very same dilemma that years earlier had led Leonardo da Vinci to chose oil as the technique for the preparation of The Last Supper (1495-1497), and which in 1534 led to the confrontation and ensuing coolness between Michelangelo and Sebastiano del Piombo following their disagreement over the technique that Michelangelo should use to paint the Last Judgement in the Sistine Chapel.

The different techniques employed in the Flagellation and Transfiguration clearly explain the differences in the color palettes used in these painting, which were identified by the scientific study of the works carried out by means of microscopy, spectroscopic, electrochemical and chromatographic techniques. In the Flagellation we may find red earth, lead white, ochre yellow, vermilion, azurite, malaquite, tin-lead yellow and red dye, which remit to the rich colour range of the Venetian school created by the combination of many pigments in one single paint layer. A color palette based on iron oxide based pigments, and smalt, among others, with traditional dry finishing touches, were employed in this case by Sebastiano del Piombo, and demonstrate a perfect knowledge of the use of the colour palette to suit the main conditioning factor: the pictorial technique.

ACKNOWLEDGEMENTS

Financial support is thanked to the Spanish (MICINN) R+D Project CTQ2011-28079-C03-01 and 02 also supported with ERDF funds. Research was conducted within the "Grupo de análisis científico de bienes culturales y patrimoniales y estudios de ciencia de la conservación" microcluster of the University of Valencia Excellence Campus (Ref. 1362). The authors would like to thank Dr. Rosario Otegui, Director of the Real Academia de España in Rome, who enabled the study of the paintings. The authors wish also to thank Mr. Manuel Planes i Insausti and Dr. José Luis Moya López, the technical supervisors responsible for the Electron Microscopy Service of the Universitat Politècnica de València, technical supervisor of Scientific.

REFERENCES

Bianchin, S., Casellato, U., Favaro, M., Vigato, P.A. (2007): "Painting technique and state of conservation of wall paintings at Qusayr Amra, Amman - Jord" *Journal of Cultural Heritage* **8**, 289-293.
Bianchin, S., Favaro, M., Vigato, P.A., Botticelli, G., Germani, G., Botticelli

S., (2009): "The scientific approach to the restoration and monitoring of mural paintings at S. Girolamo Chapel - SS. Annunziata Church in Florence" *Journal of Cultural Heritage* **10** 379-387.

Daniilia, S., Tsakalof, A., Bairachtari, K., Chryssoulakis, Y. (2007): "The Byzantine wall paintings from the Protaton Church on Mount Athos, Greece: tradition and science" *Journal of Archaeological Science*, **34** 1971-1984.

Doménech-Carbó, M.T., (2008): "Novel analytical methods for characterization of binding media and protective coatings in artworks" *Analytica Chimica Acta*, **621** 109-139 and references therein.

Doménech, A., Doménech-Carbó, M.T., Mas-Barberá, X. (2007): "Identification of lead pigments in nanosamples from ancient paintings and polychromed sculptures using voltammetry of nanoparticles/atomic force microscopy" *Talanta* **71** 1569-1579.

Doménech-Carbó, M.T., Casas-Catalán, M.J., Doménech-Carbó, A., Mateo-Castro, R., Gimeno-Adelantado, J.V., Bosch-Reig, F., (2001): "Analytical study of canvas painting collection from the Basilica de la Virgen de los Desamparados using SEM/EDX, FT-IR, GC and electrochemical techniques", *Fresenius' Journal of Analytical Chemistry* **369** 571-575.

Doménech, A., Doménech-Carbó, M.T., Moya-Moreno, M., Gimeno-Adelantado, J.V., Bosch-Reig, F., (2000): "Identification of inorganic pigments from paintings and polychromed sculptures immobilized into polymer film electrodes by stripping differential pulse voltammetry" *Analytica Chimica Acta* **407** 275-289.

El Aal, S.A., Korman, A., Stonert, A., Munnik, F., Turos, A., (2009): "Ion beam analysis of ancient Egyptian wall paintings" *Vacuum* **83** 54-58.

Erhardt, D., Tumosa, C.S., Mecklenburg, M.F., (2005): "Long-Term Chemical and Physical Processes in Oil Paint Films" *Studies in Conservation* **50** 143-150.

Herrera, L.K., Montalbani, S., Chiavari, G., Cotte, M., Solé, V.A., Bueno, J., Durán, A., Justo, A., Rodriguez, J.L., (2009): "Advanced combined application of μ -X-ray diffraction/ μ -X-ray fluorescence with conventional techniques for the identification of pictorial materials from Baroque Andalusia painting" *Talanta* **80** 71-83.

Katsibiri, O., Howe, R.F., (2010): "Characterisation of the transparent surface coatings on post-Byzantine icons using microscopic, mass spectrometric and spectroscopic techniques" *Microchemical Journal* **94** 83-89.

Mateo-Castro, R., Doménech-Carbó, M.T., Peris-Vicente, V., Gimeno-Adelantado, J.V., Bosch-Reig, F. (1997): "Study of binding media in works of art by gas chromatographic analysis of amino acids and fatty acids derivatized with ethyl chloroformate" *Journal of Chromatography A*, **778** 373-381.

Mills J, White R (1994): *The Organic Chemistry of Museum Objects*, Butterworth, London.

Nevin, A., Melia, J.L., Osticioli, I., Gautier, G., Colombini, M.P. (2008): "The identification of copper oxalates in a 16th century Cypriot exterior wall painting using micro FTIR, micro Raman spectroscopy and Gas Chromatography-Mass Spectrometry" *Journal of Cultural Heritage* **9** 154-161.

Ospitali, F., Rattazzi, A., Colombini, M.P., Andreotti, A., di Lonardo, G., (2007): "XVI century wall paintings in th" *Cultural Heritage* **8** 323-327.

Salvadó, N., Buti, S., Tobin, M.J. Pantos, E., John A., Prag, N.W., Pradell, T., (2005): "Advantages of the Use of SR-FT-IR Microspectroscopy: Applications to Cultural Heritage" *Analytical Chemistry* **77** 3444-3451.

Serifaki, K., Böke, H., Yalcin, S., Ipekoglu, B. (2009): "Characterization of materials used in the execution of historic oil paintings by XRD, SEM-EDS, TGA and LIBS analysis" *Materials Characterization* **60** 303-311.

Stone, I (2004): *Michelangelo: La biografía novelada del genio del Renacimiento*, Planeta, Barcelona.

Strinati, C., (2009): *Sebastiano del Piombo, 1485 - 1547*, Federico Motta Editore, Italia.

Vasari, G (2005): *La vida de los más excelentes arquitectos, pintores y escultores italianos desde Cimabue a otros tiempos*, Cátedra, Madrid.

Radke, G M & Paoletti J (2002) *El arte en la Italia del Renacimiento*, Akal, Madrid.