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Chemical and spectroscopic investigation of the Raphael's cartoon of the School of Athens from the Pinacoteca Ambrosiana

Marcella Ioele[1] · Armida Sodo[2] · Annalaura Casanova Municchia[2] · Maria Antonietta Ricci[2] · Alfonso Pio Russo[2]

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Abstract The cartoon of "The School of Athens", realized by the famous artist Raffaello Sanzio, is a masterpiece of Italian Renaissance. It is a full-scale (804 × 285 cm^2) preparatory cartoon, stored at the Pinacoteca Ambrosiana (Milan, Italy). In order to characterize the cartoon and the drawing and to investigate its conservation state, several scientific analyses have been performed, both in situ and on sampled fragments. This multi-analytical approach has identified most of the materials used in the original drawing and in restored areas and provided a map of previous restoration works. Here we report the results obtained by Raman and FTIR spectroscopy, scanning electron microscopy equipped with X-ray micro-analysis (SEM–EDS), pH measurements and micro-chemical tests. pH measurements proved the absence of an acidic decay of the paper. The original paper from linen and hemp fibres is well preserved. It is filled with calcium carbonate and has received a light sizing with protein glue, now almost completely transformed into oxalates. 1797 French intervention paper is of lower quality and has been covered with a patina of lead white in Arabic gum, most likely in an attempt to match the colour to the tone of the original. Both papers are stuck to a support paper with flour glue (containing starch and gluten) and glued with the same adhesive to a canvas lining. In situ Raman spectroscopy has clearly shown that the original drawing was in charcoal and lead white, while the restored areas have been drawn using charcoal and graphite.

✉ Armida Sodo
sodo@fis.uniroma3.it

[1] Laboratorio di Chimica, Istituto Superiore per la Conservazione e il Restauro, via di San Michele 23, 00153 Rome, Italy

[2] Dipartimento di Scienze, Università Roma Tre, Via della Vasca Navale 84, 00146 Rome, Italy

1 Introduction

"The School of Athens" is one of the most beautiful Italian Renaissance frescoes realized by the famous painter and architect Raffaello Sanzio (1483–1520). It was painted between 1509 and 1511 as a part of Raffaello's commission to decorate the rooms now known as the *Stanze di Raffaello*, in the Apostolic Palace in the Vatican [1]. According to the protocol of the time, the artist would draw a full-scale preparatory cartoon, before realizing the fresco, in order to show the project to the commissioner, Pope Giulio II, and to define the details of light and shade. After approval, the drawing was transferred onto a so-called sacrifice cartoon, used to report the key outlines of the project on the fresco wall, as demonstrated by the still visible pin-prick holes. The original cartoon of "The School of Athens", closely corresponding to the Vatican fresco, is the object of this investigation. Among the Renaissance cartoons, this is the only one almost entirely preserved and can be considered the world most important, as it gives a unique insight into one of Raffaello's most famous works [2]. The cartoon dates back to about 1508 and shows past great philosophers, scientists, mathematicians, astronomers and architects (often represented with the aspect of famous artists of the time), surrounding the two central figures of Plato and Aristotle (see Fig. 1). The architectural structures visible in the upper part of the Vatican fresco and the figure of Heraclitus are missing in the cartoon. It is believed that Heraclitus, with the

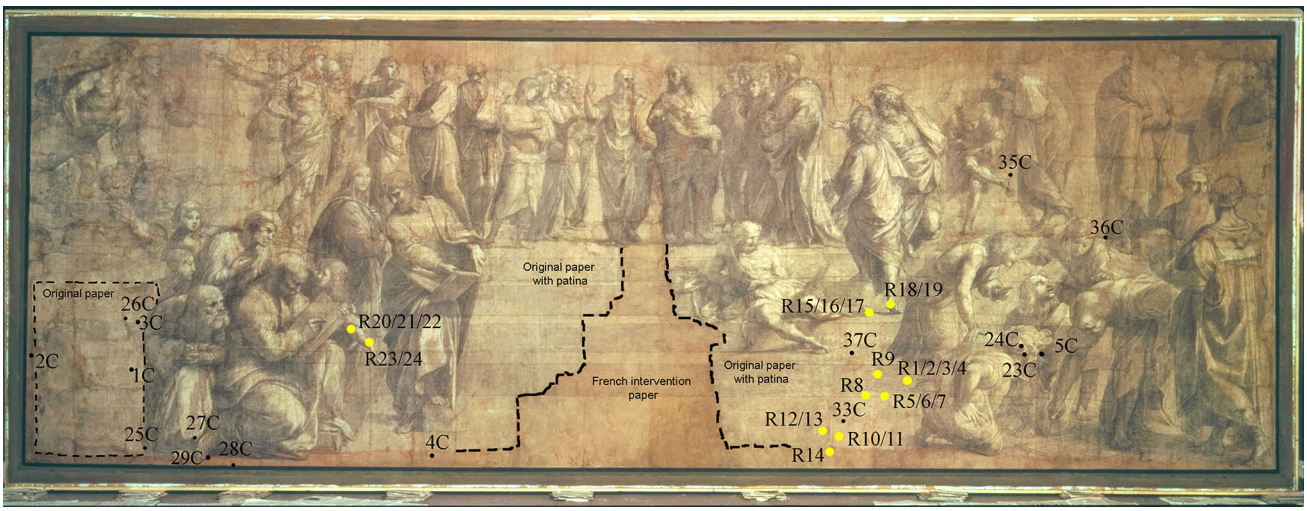

Fig. 1 Preparatory cartoon of "The School of Athens", Biblioteca Ambrosiana, Milan, Italy. In the figure, in situ Raman point of analysis (in *yellow*) and sampling areas (*black*) are reported. *Dash lines* evidence the three different typologies of the investigated paper

features of Michelangelo Buonarroti, was a late addition to the scene, as a tribute to the great rival colleague who had just accomplished his paintings in the Sistine Chapel.

This masterpiece has had a troubled storey [2, 3]. In 1796, it was transported to France by Napoleon, and in 1797 it underwent a significant restoration intervention before being exhibited at the Louvre Museum. The cartoon was re-assembled, lined with a supporting paper and a linen canvas. The largest missing parts of the cartoon, in particular in the central and border areas, were filled with restoration paper (hereafter called French intervention paper), whose colour was matched to the original by applying a slightly coloured glaze. Pictorial integrations of the missing drawing were performed directly on the restoration paper by two French painters, Jean Michel Moreau and Pierre Bouillon (the latter did the largest integrations). These restored areas are now clearly identified by the yellow–brown tone due to the alteration of the glaze.

The cartoon was returned to Italy in 1816 [4], and since then it has been stored and exhibited at the Pinacoteca Ambrosiana, where in 1967 it underwent a restoration intervention by Giuseppe Arrigoni. The cartoon was tensioned on a wooden frame, through the application of perimetral canvas strips and put into a big showcase specifically built for this item.

In July 2014, the cartoon was removed from the showcase to examine its conservation state, investigate the constitutive materials and evaluate the need for a modern restoration intervention and the possible best practice.

Preliminary diagnostic investigations, performed from July to December 2014, before the restoration intervention, involved several professional figures from different institutions, called by a Planning Group[1] to give their contribution to the study and knowledge of such an important artefact, among those, technical and scientific staff from ISCR, CCR, Roma TRE University, La Sapienza University, Politecnico di Milano, and Malcangi photographic laboratory. Experts from these institutions have provided photographic documentation, a survey on the conservative history of the cartoon,[2] along with a wealth of scientific data, by applying multi-spectral investigation (IR reflectography, trans-illumination, UV photography),[3] XRF analysis,[4] Raman spectroscopy,[5] pH measurements, FTIR and electron microscope analyses.[6] These studies have been complemented by mechanical tests[7] and biological analyses,[8] to evaluate the strength of the support and

[1] A Planning Group was established, followed by the creation of a Scientific Committee and a Technical Group, composed by representative of the Veneranda Biblioteca Ambrosiana; Soprintendenza BSAE of Milan; Curia Arcivescovile of Milan; Istituto Superiore per la Conservazione e Restauro (ISCR) of Rome; Fondazione Centro Conservazione e Restauro (CCR) La Venaria Reale of Turin; the Fondazione Cardinale Federico Borromeo; the Vatican Museums, the University La Sapienza of Roma, the restorers Pinin Brambilla Barcilon and Maurizio Michelozzi. Mons. Franco Buzzi, Veneranda Biblioteca Ambrosiana Prefect, is the President of Planning Group and Scientific Committee.

[2] Stefania de Blasi, CCR.

[3] CCR, Malcangi Laboratory.

[4] CCR.

[5] Armida Sodo, Alfonso Russo, Annalaura Casanova Municchia, Università di Roma TRE, Dipartimento di Scienze.

[6] Marcella Ioele, Chemistry laboratory ISCR.

[7] Mauro Torre, ISCR, Physics laboratory, ISCR, G. B. Broggiato, L. Cortese, University la Sapienza, Dipartimento di Ingegneria Aerospaziale; A. Cigada, E.Zappa, G. Brusca Politecnico of Milan.

[8] Gian Franco Priori, Maria Rita Giuliani, Biology Laboratory ISCR.

establish biological attack on the paper support and the lining paper and canvas.

Sharing this information has allowed for many issues of the cartoon executive technique, past conservation interventions and its actual conservation state to be clarified. Investigation will continue during the restoration intervention, and a complete report will be eventually published, reporting contributions from all institutions and professionals participating to the project.

In this paper, we report only the main results of the chemical and physical analyses performed by ISCR laboratory of Chemistry and Roma TRE Department of Science, using Raman and FTIR spectroscopy, scanning electron microscopy equipped with X-ray micro-analysis (SEM–EDS), pH measurements, digital microscopy and micro-chemical tests. The aim of this work is to characterize the paper support, to investigate and distinguish the material used in the original drawing and in the restored areas, and to investigate the cartoon conservation state. Analyses were performed both in situ and on sampled fragments. This multi-analytical approach has allowed identification of the majority of materials used in the original drawing and in the restored areas and has allowed a map of previous restoration works to be generated.

2 Experimental

2.1 Sample description and experimental procedure

As reported in the introduction, the cartoon of "The School of Athens" is a full-scale (804 × 285 cm^2) preparatory cartoon, stored at the Pinacoteca Ambrosiana (Milan, Italy). It is comprised of 210 sheets of paper linked together and glued to a canvas support. The alphanumeric labels in Fig. 1 mark the points where measurements have been made: whenever possible, these measurements were in situ. Raman investigations, pH measurements and digital microscopy observations were carried out during a short and intense in situ experimental campaign. A total of 37 micro-samples were collected from the front and back side of the cartoon for other investigations. Part of the data shown in this paper refers to 15 samples (from the front side) out of the 37. Samples not discussed in this paper were sampled from the back and predominately used for biological tests. A total of 24 points (represented in yellow in Fig. 1) were investigated by in situ Raman spectroscopy. Black points in Fig. 1 label the areas that were sampled. Hereafter, all samples will be referred to by using the labels reported in Fig. 1. As evident in Fig. 1, the distribution of sampling points is quite heterogeneous, since sampling was allowed only at sheet junctions avoiding all the drawn figures. In the first two columns of Tables 1 and 2, the name, the position and the description of the investigated samples are reported.

2.2 Experimental procedures

In the first part of the survey, the cartoon was observed under visible and UV light, by means of USB video microscopy to evidence details of the surface and inhomogeneities. Acidity of the different papers and of canvas lining was checked with a flat electrode for superficial pH measurements. Superficial dust, fragment of fabric from the lining canvas, cotton swabs for solvent extraction, sterile pads for microbiological tests,[9] fragments of superficial restoration materials, fragments of the original support, French intervention and lining paper taken from edge areas were sampled.

SEM–EDS, micro-chemical tests, micro-FTIR and micro-Raman spectroscopy were used to characterize the sampled fragments in order to investigate the conservation state and to characterize the original and restoration materials. Finally, in situ Raman investigation has been performed, to clarify some aspects, especially regarding the writing media.

2.3 Instrumentation

2.3.1 Raman spectroscopy

Raman measurements were performed using a Renishaw InVia Reflex Raman microscope equipped with a laser source at 785.5 nm (nominal output power 300 mW). In order to avoid sample damage, trial measurements were undertaken starting with a ~0.1 mW laser power gradually increasing the laser power until a spectrum with an acceptable signal-to-noise ratio was obtained. The final laser power on the sample was 5 mW. The backscattered light was dispersed by a 1200 line/mm grating. The Raman signal was detected by a Peltier-cooled (–70 °C) deep depletion charge-coupled device (CCD RD-VIU, 578 × 384 pixel, with spectral response in the range 200–1025 nm). Nominal spectral resolution was about 3 cm^{-1}. During in situ investigations, the laser beam was focused on the sample by a Renishaw probe, equipped with an edge filter and 20× microscope objective.

To analyse the material that was sampled from the cartoon, Raman spectra were collected using a Leica DMLM microscope to select a specific area of interest. One of the advantages of this instrumental set-up is the possibility of analysing different points of the same sampled fragments with a spatial resolution depending on the choice of the microscope objectives. Spectral acquisition (three

[9] Microbiological tests evidenced the absence of living microorganism either on the paper support, the lining canvas and the aspired dust (M.R. Giuliani, Gian Franco Priori, ISCR laboratory of Biology).

Table 1 Samples investigated on the front of the cartoon, techniques of analysis and main results

No.	Type of sample/*sampling area*	Analyses	Results
1C	Fragment of original paper from an overlying edge/*bottom area, left*	OM, FTIR, micro-chemical test, SEM–EDS	Paper containing calcium carbonate, oxalates, protein (traces). On the back: significant presence of fungal and spores, not vital[a]
2C	Fragment of French intervention paper/*central area, left edge near the green frame*	OM, FTIR, micro-chemical test, SEM–EDS, micro-Raman	Paper with a superficial patina containing lead white and gypsum in Arabic gum and protein. Presence of starch and gluten glue on both sides. On the back: presence of fungal spores, not vital
3C	Fragment of a drop of adhesive on the paper surface/*left, central area*	OM, FTIR, micro-chemical test	Starch and gluten (flour glue)
4C	Fragment of French intervention paper/*bottom, central left area*	OM, FTIR, micro-chemical test, SEM–EDS	Paper with a superficial patina containing lead white and gypsum in Arabic gum and protein. Presence of starch and gluten (flour glue) on both sides.
5C	Powder scraped with a scalpel from a white lighting with a pinkish UV fluorescence/*central right bottom, area*	OM, FTIR	Cellulose, oxalates, lead white, polysaccharide material
23C	Powder scraped with a scalpel from a White lighting with a pinkish UV fluorescence on a hair curl/*central right bottom, area*	OM, FTIR	Lead white, oxalates, polysaccharide material, fatty acid soap (traces)
24C	Cotton swab with de-ionized water from white lighting with a pinkish UV fluorescence on a hair curl/*central right bottom area*	OM, FTIR on the water extract	Lead white, polysaccharide (probably Tragacanth gum)
26C	Fragment of original paper without lining paper, taken from a junction between foils/*central right area*	OM, FTIR, micro-chemical test, SEM–EDS, micro-Raman	Paper containing calcium carbonate. Oxalates and traces of protein. On the back: isolated fungal spores, not vital
27C	Fragment of original paper with black drawing (probably not original) and without lining paper. Raised edge from an area of junction between foils/*bottom left area*	OM, FTIR, micro-chemical test, SEM–EDS, micro-Raman	Carbon black on the black drawing. Paper containing calcium carbonate
28C	Fragment of French intervention paper glued over original paper and without lining paper/*bottom left area under big toe of a character*	OM, micro-chemical test, SEM–EDS	Superficial patina containing lead white in Arabic gum and protein medium. Between French and original and on the back: starch and gluten (flour glue)
29C	Cotton swab with de-ionized water on the patina of French intervention paper/*bottom left area*	FTIR on the water extract	Arabic gum, gypsum, lead white
33C	Original paper with a grey patina. Cotton swab with de-ionized water, partially removing the grey layer. Paper is highly water absorbing/*central lower area of the staircase*	FTIR on the water extract	Protein, gypsum, calcium carbonate, polysaccharide material (probably Arabic gum)
35C	Fragment of original paper with grey patina without lining paper from a raised edge/*top right area*	OM, FTIR, micro-chemical test, SEM–EDS	Protein and traces of gypsum, polysaccharide material (probably Arabic gum), lead white, calcium carbonate
36C	Fragment of original paper with black drawing without lining paper from raised edge/*central right area*	OM, FTIR, micro-chemical test, micro-Raman	Carbon black on the black drawing. Polysaccharide material (probably Arabic gum), calcium carbonate and traces of gypsum. On the back: starch and gluten (flour glue)
37C	Micro-fragment of black pigment French intervention (redrawing)—on original paper/*central staircase, bottom right area*	Micro-Raman	graphite

OM = optical microscopy; FTIR = micro-infrared analysis; SEM–EDS = electron microscope analysis with an X-ray probe, micro-chemical test = Lugol test for starch detection

[a] Coltural analyses performed by ISCR laboratory of biology demonstrated the absence of fungal growth

accumulations, 10 s each, in the range 200–3200 cm^{-1}) was performed with 50× and 100× objectives, depending on the response and structure of the individual samples. WiRE software has been used for spectra acquisition and Origin software for the spectra elaboration.

2.3.2 FTIR spectroscopy

Infrared analyses were carried out using a Thermo Scientific Nicolet iN10 FTIR microscope. Spectra were carried out by transmission using a diamond cell. Spectra were

Table 2 Samples investigated by in situ Raman investigation

No.	Type of sample and position	Results
R1; R2; R3; R4	Original black ink, student dress/*bottom right area*	Carbon black; degraded animal glue
R5; R6; R7; R8	French black ink, staircase/*bottom right area*	Synthetic adhesive (nylon)
R9	French black ink, staircase/*bottom right area*	Synthetic adhesive (nylon)
R10; R11	Original 1500 paper/*bottom right area*	Cellulose (good state of conservation)
R12; R13	Original 1500 paper with greyish patina/*bottom right area*	No Raman signals; fluorescence background
R14	French intervention paper/*bottom right area*	Cellulose (good state of conservation)
R15; R16; R17	Restored white zone of Diogenes's foot/*bottom right area*	Synthetic adhesive (nylon)
R18; R19	Original white area of the heel of an unidentified backwards character/*bottom right area*	Synthetic adhesive (nylon)
R20; R21; R22	Black ink, arm of Pythagoras's assistant/*bottom left area*	Cellulose (good state of conservation); carbon black; synthetic adhesive (nylon)
R23; R 24	Restored black ink, hand of Pythagoras's assistant/*bottom left area*	Cellulose (good state of conservation); synthetic adhesive (nylon)

collected in the 4000–650/cm-1 spectral range, using a liquid nitrogen-cooled MCT detector. Cotton swabs for solvent extraction were re-extracted, and the residue was positioned on a diamond cell and analysed by micro-FTIR spectroscopy.

2.3.3 Digital microscope analyses

The paper surface was investigated in situ using a Dino-Lite AM4113, USB digital microscope, equipped with reflected white light and UV LED light. Images were taken at 60× and 220× magnification.

2.3.4 Scanning electronic microscopy coupled to energy-dispersive spectrometer (SEM–EDS)

Samples of the paper support were analysed by SEM–EDS. The instrument used was a Zeiss EVO60 electron microscope equipped with an Oxford INCA Pentaflex energy-dispersive spectrometer. Paper samples placed directly on the stub were not metal-coated and were analysed at variable pressure.

2.3.5 Determination of pH

pH was measured using a portable Hanna pH meter with a flat electrode for surface measurement. Several points for analysis were selected on the original paper, French intervention paper, lining paper, lining canvas and green paper of the frame. The pH meter was calibrated with standard solutions at pH 4 and 7 before measurements were collected. Measurements were performed by placing a drop of distilled water on the paper surface, then the electrode was positioned on this same spot, and measurements were repeated in duplicate for each point.

2.3.6 Microscopic analysis

Paper fragments were analysed under reflected white light and UV light with a Leica DM RB optical microscope at 50×, 100× and 200× magnification.

2.3.7 Micro-chemical tests

The iodine test with Lugol reagent was used to test for the presence of starch. A solution of iodine (2.5%) and potassium iodide (5%) in water was prepared. A few drops of the reagent were added to a tiny fragment of paper under microscopic observation. In the presence of starch, a dark-blue colour is observed, due to the formation of a tri-iodide anion (I_3^-) and starch complex.

3 Results and discussion

3.1 Characterization of the paper substrate

As reported in the introduction, not all of the 210 paper sheets forming the cartoon are original; in the central part and in the borders, French restorers inserted new sheets (French intervention paper) that were treated to match the original paper.

Preliminary, several areas of the cartoon surface were observed directly in situ by USB microscope under white and UV light in order to have an overview of the state of the paper and to detect differences. Optical microscopy

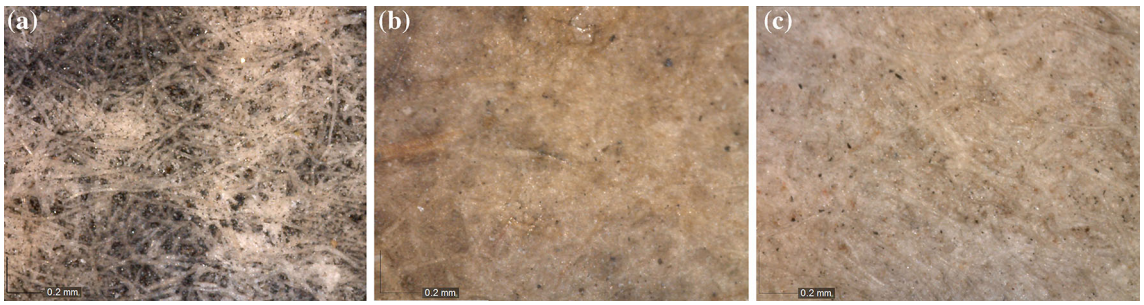

Fig. 2 USB microscope images (×220) of: **a** original paper from the *left* portion of the cartoon; **b** French intervention paper; **c** original paper with patina from *central* portion of the cartoon (*staircase* area)

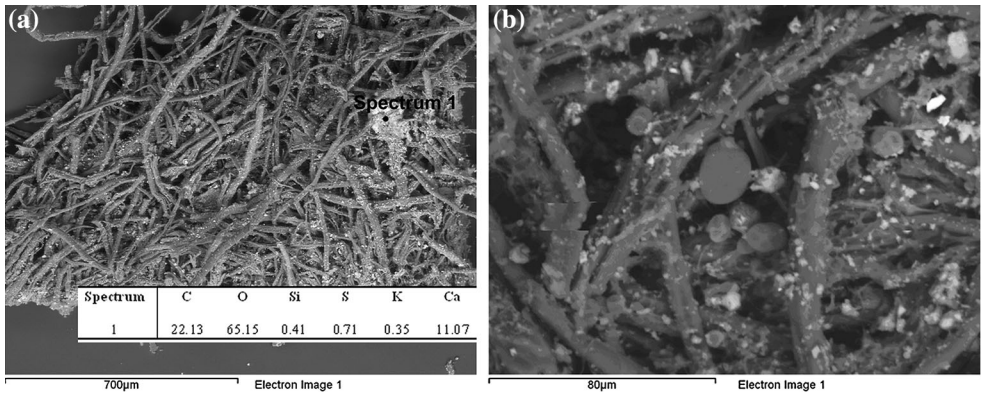

Fig. 3 Sample 26C, original paper: **a** *front side,* SEM image (BSD electrons) and EDS analysis of a mineral containing calcium; **b** *back side*, SEM image (BSD electrons) showing the presence of fungal spores

revealed that in the left portion of the cartoon (original paper) the paper fibres were clearly visible (Fig. 2a), while in the French intervention paper the cellulose fibres were covered by a patina (Fig. 2b). Interestingly, in the central bottom and right part of the cartoon, also a portion of original paper appeared to be covered by a thinner patina, probably not original (Fig. 2c). In Fig. 1, a dashed line points out the three investigated typologies of paper.

A summary of all the investigated samples and the obtained results is reported in Tables 1 and 2.

3.1.1 Original paper

The original paper was very good-quality rags paper. In XVI century, high-quality paper was produced from pieces of old clothes (rags) that were reduced to pulp and often sized with animal glue. The pH values of the investigated in situ areas were found to be between 6 and 7.

In order to determine the composition and conservation state of the original paper, six fragments (namely samples 1C, 26C, 27C, 33C, 35C and 36C) were sampled and analysed. Moreover, two points (R10 and R11) were investigated in situ by Raman spectroscopy.

SEM–EDS results indicated the presence of calcium-based fillers between fibres (Fig. 3a) [5]. Traces of potassium, silicon and some sulphur were also found. These elements can be attributed to dust sediment [6–8]. The SEM images collected from the backs of the original paper samples show the presence of fungal spores (Fig. 3b) that are non-active as evidenced by microbiological investigations carried out at ISCR laboratory of Biology.[10]

In Fig. 4c, one of the Raman spectra collected from sample 26C (representative of all other samples of original paper) is reported and compared with the spectrum of a standard pure cellulose paper in good state of conservation (Fig. 4a). This comparison shows that the paper is in good conservation state, as demonstrated by the absence of the typical cellulose oxidation bands located at about 1700–1850 cm^{-1} and attributable to carbonyl and carboxyl functions [9–13]. The FTIR spectrum from the same sample (front surface) is reported in Fig. 5a: strong oxalate bands at 1640 and 1319 cm^{-1} are observed together with the protein amide II band at 1550 cm^{-1}, whereas amide I band is superimposed to oxalate signal [14]. Bands in the 1000–1100 cm^{-1} spectral region are assigned to the presence of cellulose fibres [9]. The presence of calcium oxalate, in particular in the mineralogical form of weddellite ($Ca(C_2O_4)\cdot 2(H_2O)$) [15], is confirmed by the Raman

[10] G. Priori, INTERNAL REPORT, Biology laboratory ISCR.

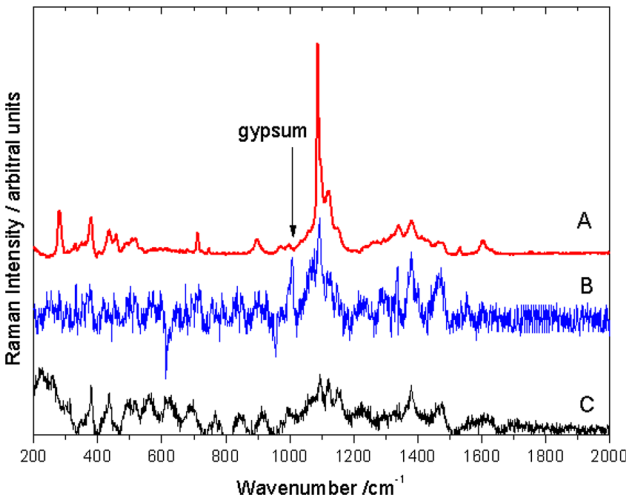

Fig. 4 Comparison of Raman spectra of (*A*) standard pure cellulose paper; (*B*) French intervention paper from sample 2C, the *arrow* show the typical band of gypsum at 1006 cm^{-1}; (*C*) original paper from sample 26C. Spectra were arbitrary stacked for a better visualization

spectrum (Fig. 6a) acquired from a white grain in sample 26C. Several studies attribute the presence of calcium oxalate to the oxidative degradation process of organic components (in particular proteinaceous materials) [16, 17] and references herein]. Consequently, we suggest that the original paper was sized with a proteinaceous material, likely animal skin glue [18].

FTIR investigation also evidenced the presence of calcium carbonate (see, for example, the spectrum from sample 1C water extract reported in Fig. 5d). The presence of calcium carbonate is most likely to be responsible for the neutral pH and is due to the paper production technique. Calcium compounds such as lime, in fact, were used in early paper manufacture to expedite swelling of cellulose and fibres shortening [19].

3.1.2 Original paper with patina

Part of the original 1500 paper in the central and right portions of the cartoon is covered by a greyish patina. The greyish patina is present also over the lining paper, visible in a lacuna area (Fig. 7a, sampling area 35C, top right portion of the cartoon): this observation suggests that the patina on the original paper is due to the French restoration intervention in 1799.

Samples of this typology of paper are labelled 33C, 35C (Fig. 7b). *In situ* Raman spectroscopy at points R12 and R13 gave no useful Raman bands due to an intense fluorescence background.

SEM–EDS investigations of these samples revealed the presence of calcium, lead and traces of potassium, silicon, iron, aluminium and sulphur (Fig. 7c). These elements can be related to an extremely thin layer of diluted gouache probably containing lead white, gypsum and/or calcium carbonate.

The presence of iron could in principle suggest the use of earths that are usually easily identified by Raman or FTIR investigations. Since both techniques have not evidenced the characteristic bands of any iron-based earth, the presence of iron may be ascribed to the papermaking process [20, 21], to dust impurities covering the paper surface or to earths concentrations below the sensitivity threshold of the experimental techniques.

The state of conservation seems to be good as demonstrated by a neutral pH. FTIR spectra collected from two water extracts from sample 33C (Fig. 8a, b) reveal the presence of calcium carbonate along with the presence of a protein (animal glue), gypsum and a polysaccharide, possibly Arabic gum. Protein is characterized by the amide I, II and III bands at 1650, 1550 and 1450 cm^{-1}, respectively, whereas the presence of a vegetal gum can be deducted from the typical polysaccharides bands in the 1000–1100 cm^{-1} region (see reference spectrum of Arabic gum in Fig. 8c) [22]. The presence of gypsum is evidenced by the band at 1158 cm^{-1} (see reference spectrum in Fig. 8d). Animal glue and Arabic gum were most likely used as patina binders. Unambiguous identification of organic materials, in particular polysaccharides, was very difficult in this artefact, due to the presence of cellulose from paper and starch contained in the adhesive used to stick the sheets of paper (see next section). As already said, samples were collected in proximity of the junctions between sheets; thus, they always contain adhesives. A Lugol test, sensitive to the presence of starch, was used to determine whether the polysaccharide bands observed in the FTIR spectra originated from starch or gum. On the front side of both investigated samples starch was not evidenced, supporting our suggestion regarding the presence of Arabic gum on the paper surface.

3.1.3 French intervention paper

French intervention paper in the cartoon is characterized by a pronounced brown orange tone (Fig. 9a). A number of fragments (namely samples 2C, 4C, 28C and 29C) of this typology of paper have been investigated by FTIR and Raman spectroscopies. Moreover, in situ Raman measurements were performed on point R14. French intervention paper is a good-quality rags paper, although formed by shorter fibres compared to the original 1500 paper.[11] No cellulose oxidation bands were observed in Raman spectra as reported in Fig. 4b, where, by the way, the band at 1006 cm^{-1} attributable to gypsum was detected. The pH of the French intervention paper was

[11] G. Priori, INTERNAL REPORT, Biology laboratory ISCR.

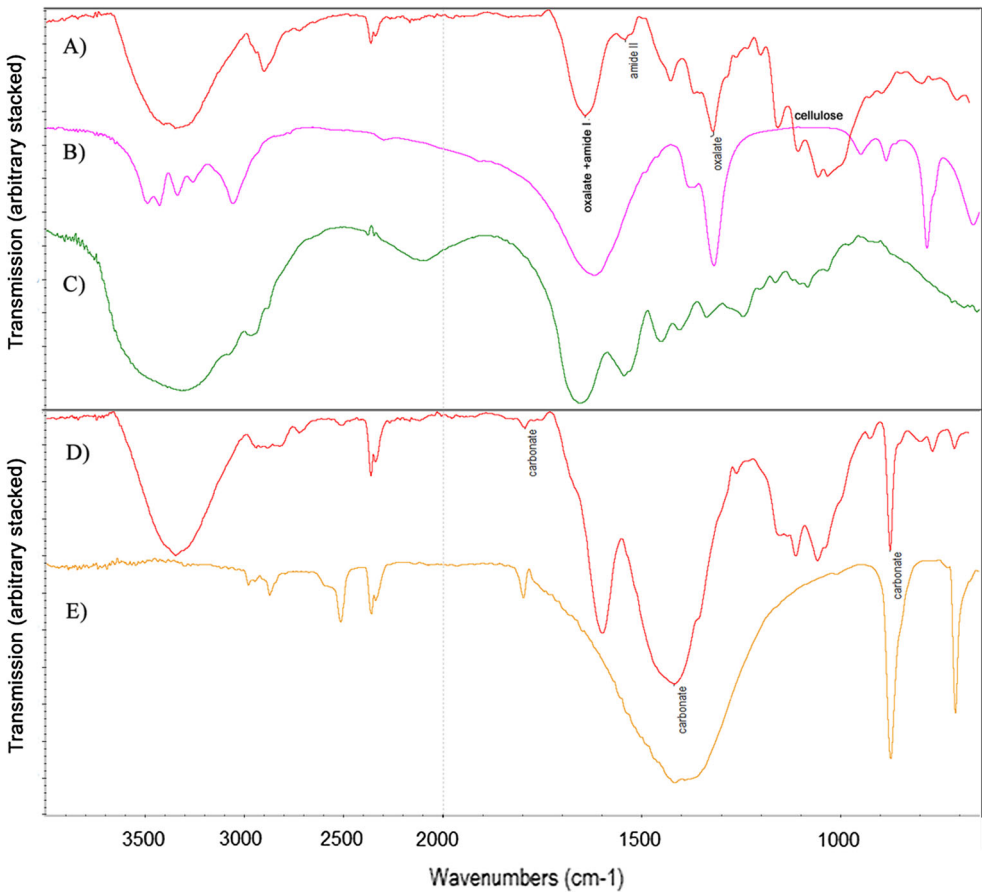

Fig. 5 FTIR spectra of: (*A*) original paper, sample 26C, front side; (*B*) reference spectrum of calcium oxalate; (*C*) reference spectrum of animal skin glue; (*D*) original paper, water extract, sample 1C; (*E*) reference spectrum of calcium carbonate. Spectra were arbitrary stacked for a better visualization

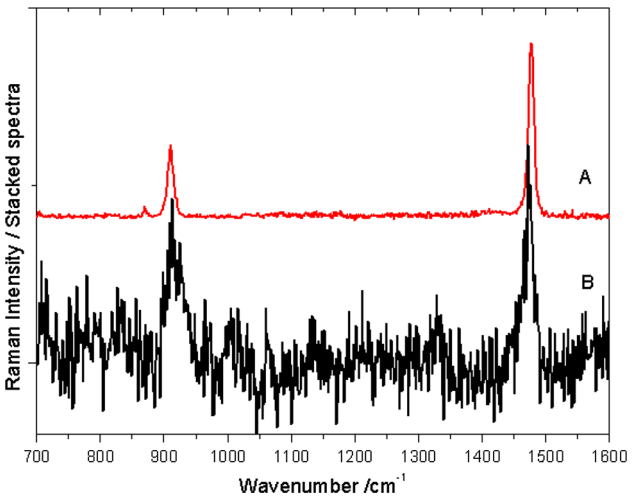

Fig. 6 Comparison between the Raman spectrum collected from sample 26C (*B*) and the Raman spectrum of standard weddellite (*A*). Spectra were arbitrary stacked for a better visualization

found to be neutral as was the original paper, while a slightly higher hydrophobicity of the patinated surface was observed.

In situ and on fragments microscopic analyses showed the presence of a quite thick brownish patina covering the paper fibres. In some areas, the patina shows craquelure and abrasions (see, for example, Figs. 2b, 9b, c). SEM–EDS investigations of these samples revealed the presence of lead, calcium, silicon, sulphur (Fig. 9c) and a small amount of iron [23]. Particularly abundant is lead, likely due to the use of lead white in the coloured glaze. The brown colour may in principle be due to organic binder alteration or to the presence of a very diluted iron-based earth pigment, used to accord the tone of the integration paper to the original.

FTIR analyses of the glaze showed the presence of a polysaccharide, protein, oxalates, lead white and a relevant amount of gypsum. In the FTIR spectrum from a portion of the patina of sample 2C (Fig. 10a), bands attributed to gypsum are observed at 595, 676 and 1158 cm^{-1}, the protein amide II band is observed at 1550 cm^{-1}, whereas the band at 1650 cm^{-1} is a complex combination of bands arising from protein (amide I), oxalate and Arabic gum. The bands in the 1000–1150 cm^{-1} region pertain to polysaccharide materials and are also observed in the FTIR spectrum of the water extract of sample 29C (not shown). The negative Lugol test in those areas proved the presence of a gum, likely Arabic gum as in the case of original paper with patina.

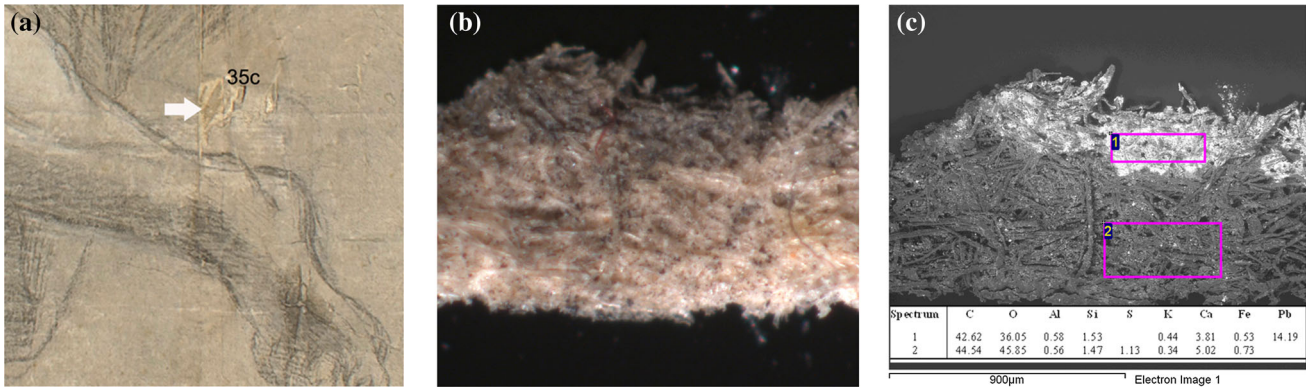

Fig. 7 Original paper with patina: **a** patina over lacuna, near area of sampling 35C; **b** sample 35C, microscope image; **c** SEM–EDS image (BSD electron) and EDS analysis of sample 35C

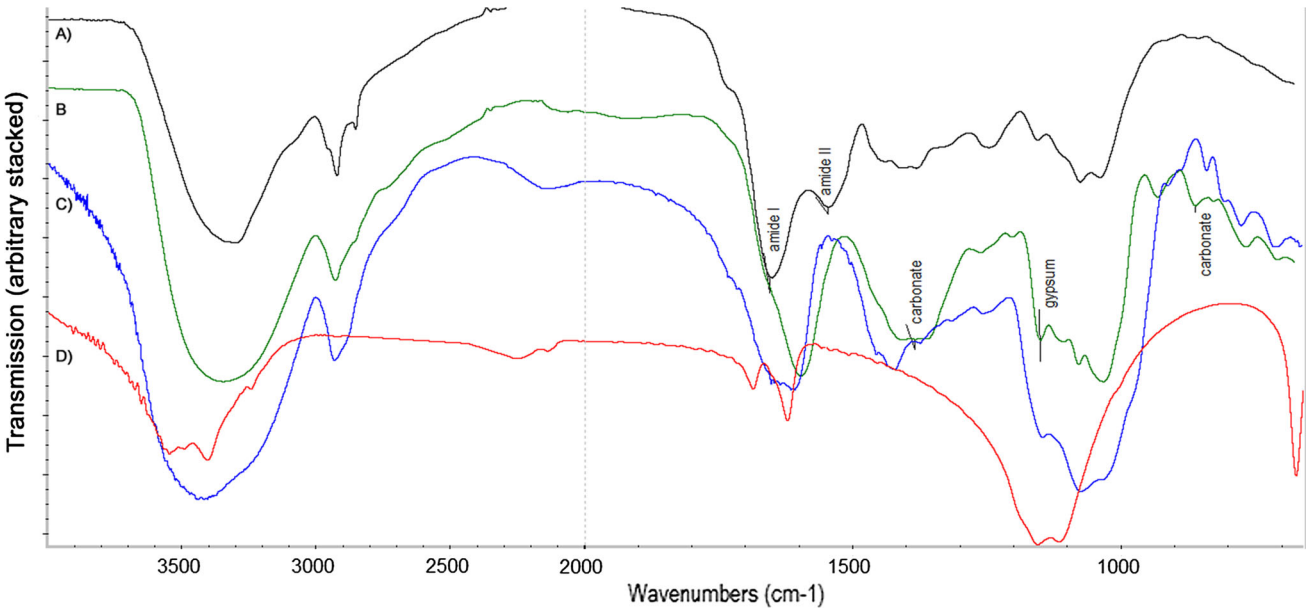

Fig. 8 FTIR spectra of: (*A*, *B*) surface of original paper with patina (portions of sample 33C water extract); (*C*) reference spectrum of a polysaccharide (Arabic gum); (*D*) reference spectrum of gypsum. Spectra were arbitrary stacked for better visualization

Raman investigation confirmed the presence of gypsum (band at 1006 cm^{-1}), see Fig. 4b; calcite (band at 1086 cm^{-1}), and lead white (band at 1050 cm^{-1}) as shown in Fig. 11 [24].

3.2 Identification of drawing materials and lighting (original vs. restoration)

Due to the preciousness of the drawing, sampling of pigments was not allowed. For this reason, in situ Raman spectroscopy was mainly used to identify and compare the composition of both the original and restoration pigments. Accidentally, samples 27C (original paper), 36C (original paper) and 37C (original paper with redrawing) contain black traces and have been investigated to identify the pigment composition.

3.2.1 *Particular cases*

Sample 27C is a fragment of original paper with an ink trace of doubtful origin (original or restoration), while 37C (again original paper) contains surely a restoration drawing. In Fig. 12, Raman spectra collected from both samples are reported. The Raman spectrum of sample 27C shows the presence of carbon black (bands at 1325 and 1580 cm^{-1}) while graphite bands at 1314 and 1571 cm^{-1} have been identified in fragment 37C [30].

3.2.2 *Original drawing*

The investigated points are reported in Fig. 1 and labelled as R1, R2, R3, R4, R20, R21, R22. Accidentally, sample 36C contains traces of the original black drawing and has

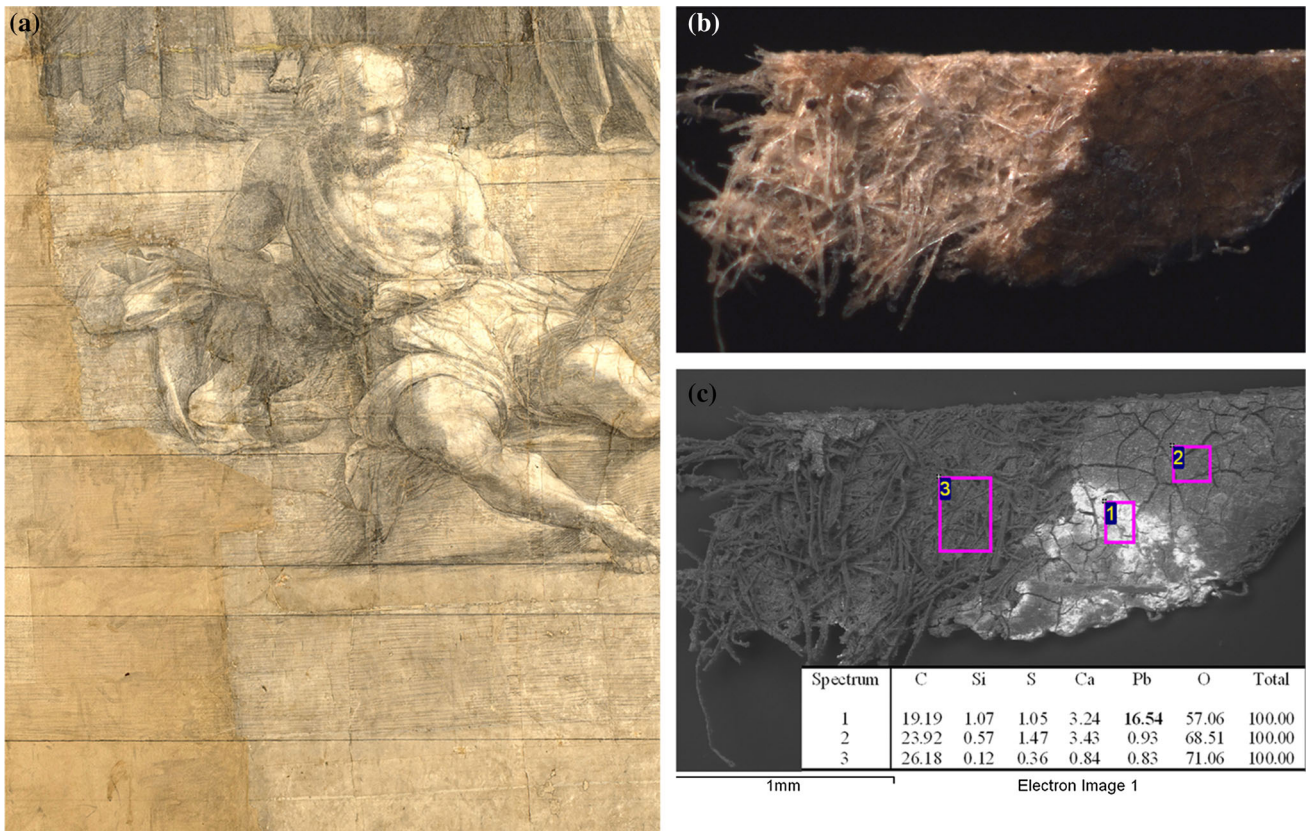

Fig. 9 **a** Central portion of the cartoon: French intervention paper is recognizable by the *brown–orange* tone; **b** microscope image of sample 2C, French intervention paper with patina, in an abraded portion; **c** sample 2C SEM–EDS image (BSD electron) and EDS analysis

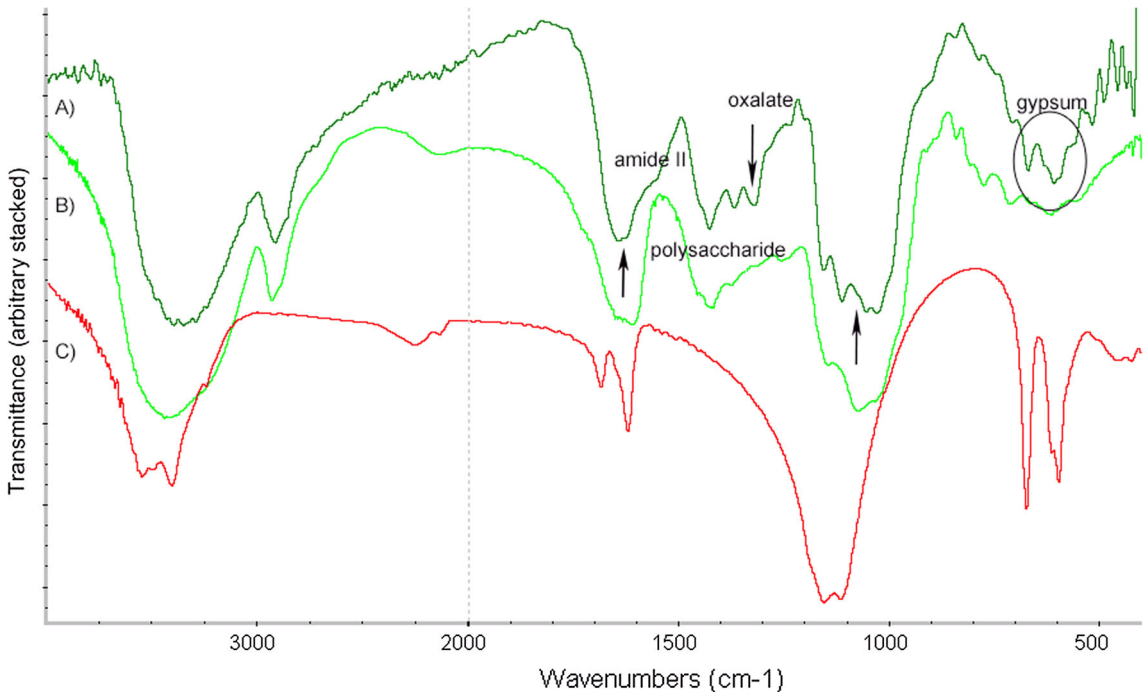

Fig. 10 FTIR spectrum of: (*A*) the brownish patina of sample 2C; (*B*) reference spectra of Arabic gum; (*C*) reference spectrum of gypsum. Spectra were arbitrary stacked for a better visualization

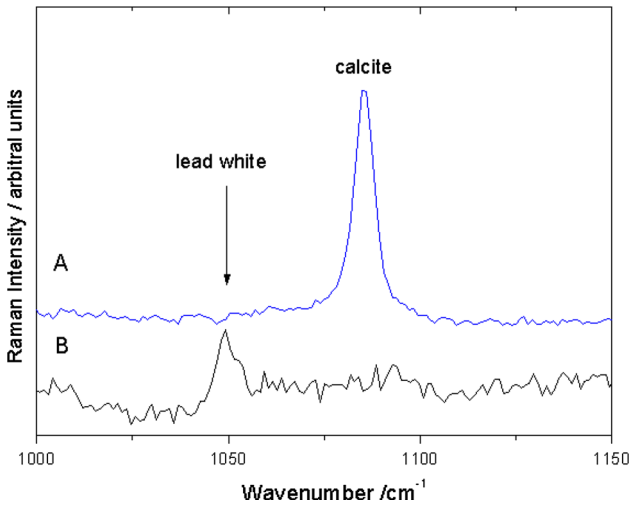

Fig. 11 Raman spectra of different white areas of sample 2C. It was possible to identify the presence of (*A*) calcite band at 1086 cm^{-1} and (*B*) lead white, band at 1050 cm^{-1}. Spectra were arbitrary stacked for a better visualization

been used to investigate the nature of the original black pigment.

All the collected Raman spectra (both in situ and from sample 36C) showed the presence of carbon black bands, centred at 1325 and 1580 cm^{-1}. In some areas (namely samples R1, R2, R3 and R4), bands around 1320, 1457 and 1591 cm^{-1} were detected (see Fig. 13a). These are attributed to amide III, C–H bending and amide I bands of proteinaceous material, respectively, and could be ascribed to a collagen-based glue. The broadness and low intensity of these bands are signatures of degradation of such glue [25–27].

Raman spectra (Fig. 13b) collected at points R20, R21 and R22 show bands at 1639, 1434 1301 and 1237 cm^{-1} that could be assigned to a modern synthetic polymer, probably used by Arrigoni during his conservation treatment (1967). [28] Observed Raman bands are compatible with a polyamide polymer, such as nylon (see Fig. 13c), generally used as restoration adhesive [29].

3.2.3 *French intervention drawing*

The in situ Raman spectra of the black pigments from the restored areas (R5, R6, R7, R8, R9, R23 and R24) did not have any characteristic bands useful for identification of their composition; the only bands present were attributable to the presence of nylon, as previously discussed. Similarly in the spectra from points R15, R16, R17, R18 and R19, only the presence of nylon has been detected. Unfortunately, the absence of signature of pigments in these samples does not allow to unambiguously assign the presence of graphite found in sample 37C to the French intervention.

3.2.4 *Lighting*

Raffaello gave volume to the figures by applying white lighting. These areas (namely samples 5C, 23C, 24C) were investigated by FTIR spectroscopy which found the presence of lead white (681, 1045, 1406 and 3536 cm^{-1}), oxalates (876, 1321 and 1625 cm^{-1}) and polysaccharides as evidenced by the typical signals in the 1000–1100 cm^{-1} region, by the broad signal centred at 1620 cm^{-1} and by the C–H bands at 2910 cm^{-1}. Examination of the FTIR spectrum (Fig. 14) of the water extract from sample 24C led us to the conclusion that the polysaccharide could be identified as Tragacanth gum. This is evidenced by the match of the bands observed in our sample 24C (in particular, the broad peak at around 1740 cm^{-1} as shown in Fig. 14a) with the Tragacanth gum reference spectrum in Fig. 14b. Tragacanth gum is a natural gum derived from a plant (*Astragalus* sp.), and it was traditionally used as binder in the making of artists pastels. [31, 32].

Moreover, the presence of a side band at around 1530 cm^{-1} could indicate the presence of traces of fatty acid soaps [33]. Thus, we hypothesize that the white drawing was executed with a white pastel of lead white mixed with a gum (Tragacanth gum) and likely fixed with traces of fatty material (e.g. wax), which underwent a saponification reaction with lead.

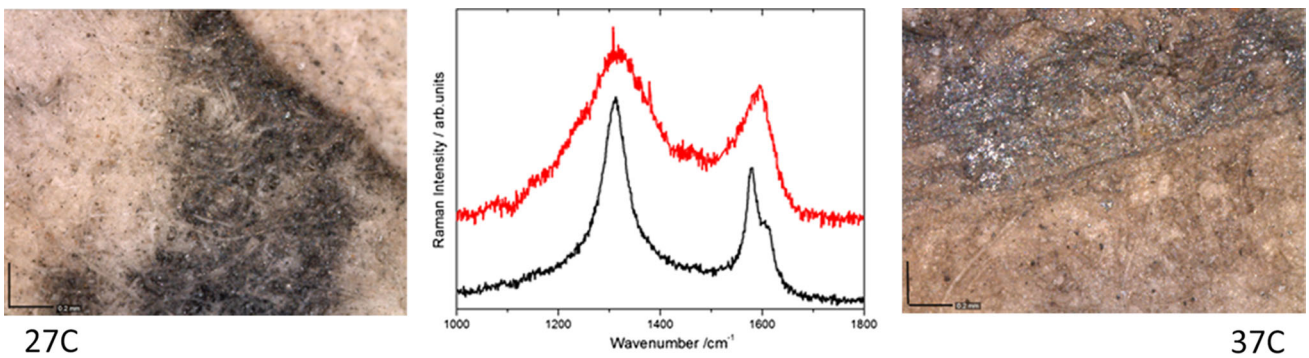

Fig. 12 Raman spectra collected from sample 27C (*red line*) and 37C (*black line*). Microscope images of the investigated samples are reported

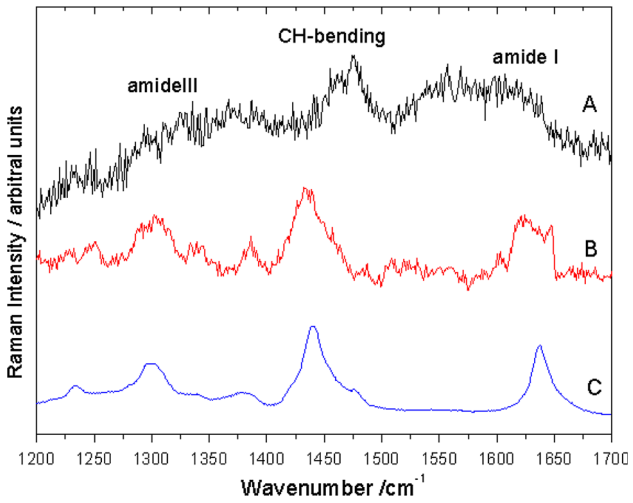

Fig. 13 Raman spectra of (*A*) sample R4 where bands attributed to a collagen-based glue are evidenced, (*B*) sample 21 where bands ascribed to a modern synthetic polymer were detected, (*C*) standard nylon

3.3 Identification of adhesives

Adhesives employed for joining paper sheets and gluing them to the lining paper and the lining canvas were investigated by micro-FTIR spectroscopy (namely on samples 2C, 3C, 4C, 26C, 27C, 28C, 35C and 36C). A subset of fragments from the samples were cut and analysed to detect the presence of starch using the Lugol reagent on both sides of the samples. Moreover, we have investigated the composition of some drops of glue visible in a few areas on the surface of original paper (Fig. 15a). These were probably dripped over the 1500 paper during the French interventions.

FTIR analyses showed that under the French intervention paper and in the junctions between the French and original paper sheets there was an adhesive containing proteinaceous material, polysaccharides and oxalates. In Fig. 15b, the FTIR spectra collected from the drop of glue and the back of sample 4C are compared to standard spectra of starch.

Since several polysaccharide substances were evidenced on the cartoon paper (cellulose, Arabic and Tragacanth gums), the Lugol test was employed to establish and confirm the presence of starch on paper fragments and to distinguish it from the Arabic gum, present in the composition of the patina and in the pigment binder. Lugol test confirmed the presence of starch on the drop glue and on the back of most samples (Fig. 16c, d). The presence of starch and proteinaceous materials confirms the use of flour glue as adhesive. Flour glue, containing starch and gluten, was widely used for paper objects during the centuries, and its adhesive properties are stronger than pure starch glue.

Flour glue was present also under the lining paper with the function of attaching the paper to the canvas. This latter adhesive has lost most of its adhesive properties, as results

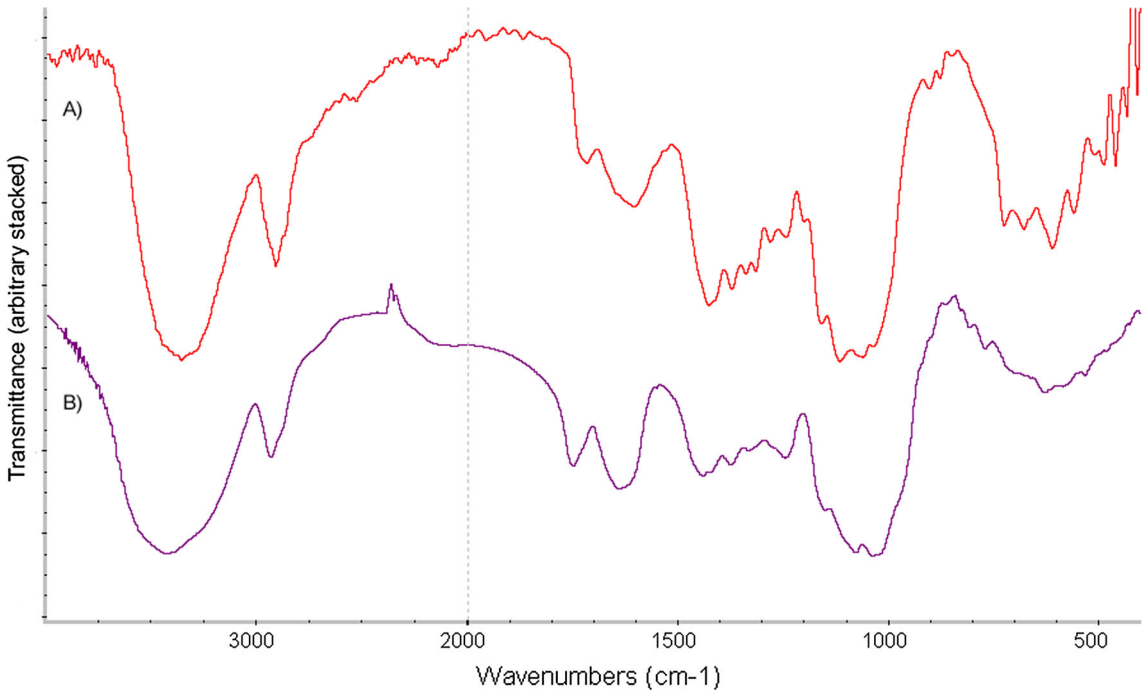

Fig. 14 FTIR spectra of: (*A*) water extract of sample 24C; (*B*) reference spectrum of Tragacanth gum. Spectra were arbitrary stacked for a better visualization

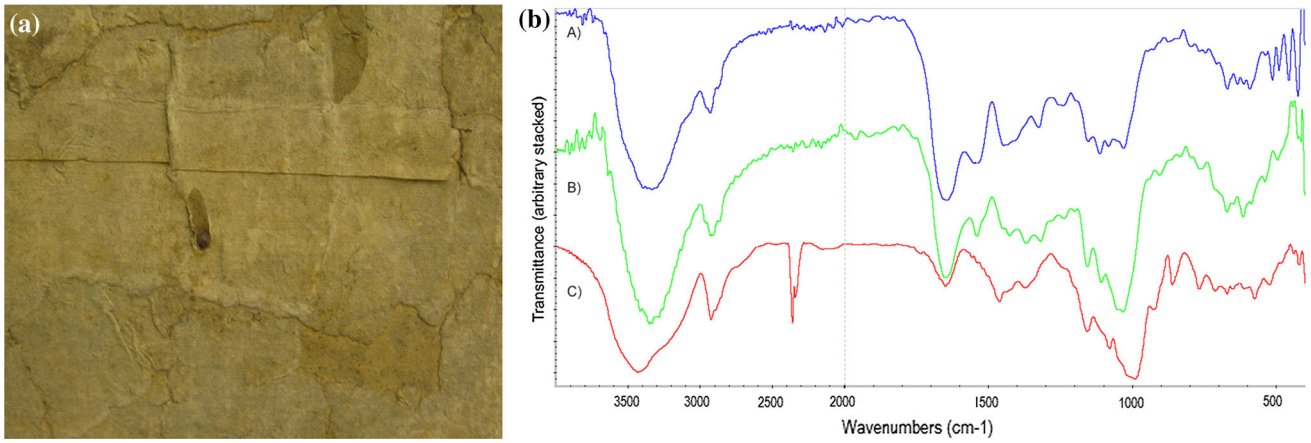

Fig. 15 **a** Drop of restoration adhesive on the surface of original paper, **b** FTIR spectra of: (*A*) a drop of adhesive over the paper surface (front of sample 3C); (*B*) adhesive under French intervention paper (back of sample 4C); (*C*) reference spectrum of starch. Spectra were arbitrary stacked for a better visualization

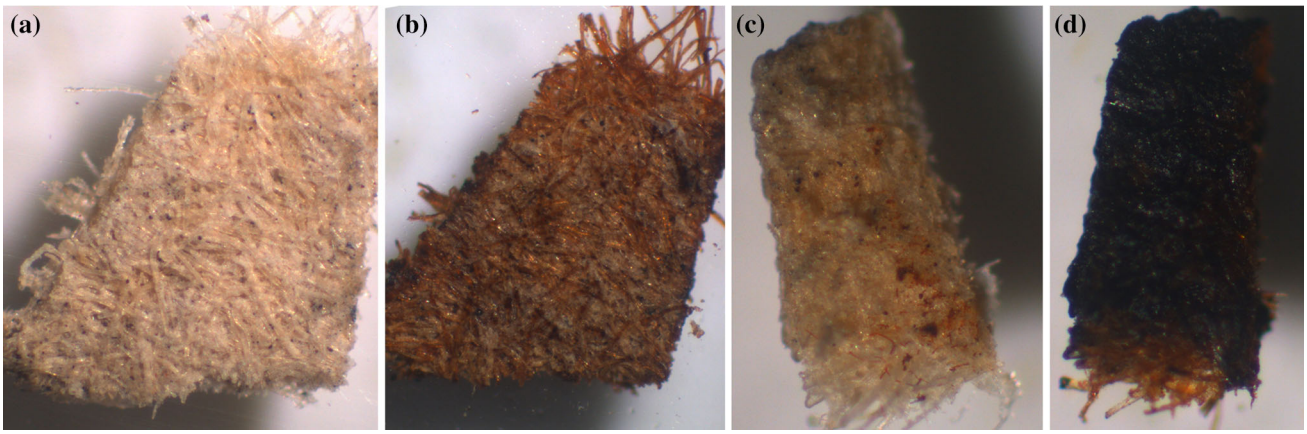

Fig. 16 Fragments of *back* portions of paper fragments in areas without lining paper: *back* of the original paper (sample 1C) before (**a**) and after (**b**) the Lugol test, negative for the presence of starch. Back of sample 4C, French paper, before (**c**) and after (**d**) the starch test, positive for the presence of starch

from the lack of contact between canvas and paper in some areas of the cartoon.

Flour glue adhesive is present in the back portion of the French paper samples, as well as under the lining paper, and is absent on the front of the samples, with the exception of some samples taken from foil junctions (2C, 4C), where the adhesive is also present on the paper surface. The Lugol test was negative for the original paper fragments (26C and 35C) collected from the areas where the lining paper was absent (Fig. 16a, b). In those samples, FTIR evidenced only traces of protein almost completely transformed into oxalates [16, 17]. The presence of protein can also be attributed to sizing of the original paper with animal glue. As far as the absence of starch on the back of original paper samples, we can hypothesize that an animal glue adhesive was employed to stick together original paper foils or that original adhesive is not longer present.

4 Conclusion

Data reported in this paper are part of a much wider study performed by several different institutions aimed at the definition of the conservation state of the preparatory cartoon of "The School of Athens" in order to identify the best conservation/restoration process.

In particular, chemical and spectroscopic investigations reported in this paper (FTIR and Raman spectroscopy, SEM–EDS analysis, pH measurements, microscopic investigation and micro-chemical tests) had the aim of characterizing constitutive materials and their state of conservation before a modern day restoration took place.

Two types of paper were investigated: the original paper dated to 1500 and the restoration paper, added during the 1799 French restoration treatment to fill a large missing part in the centre and the border of the cartoon.

Analyses revealed that both the original and French intervention paper have pH values close to the neutrality and do not need any de-acidification treatment. On the back of both paper types, fungal spores were identified; culture analyses confirmed the absence of vital colony, indicating that a curing intervention is not necessary.

The original paper is a good-quality rags paper and contains calcium carbonate, probably added as a filler. The paper surface contains traces of protein (animal skin glue) mostly transformed into oxalates, showing that the paper has undergone a mild sizing with animal glue. In the areas investigated in the left portion of the cartoon, the paper fibres appear free and not covered by organic material; on the contrary, the areas in the centre of the cartoon appeared to be covered by a light patina containing protein (animal glue), gypsum and probably polysaccharide material. This patina does not appear to be original and was possibly added during the French conservation intervention.

French intervention paper is a discrete quality rags paper; the cellulose fibres are covered by a fairly thick patina composed of a polysaccharide (Arabic gum), protein, gypsum and lead white. The patina has been added most likely to match the tone of the filling paper to the original. The brown colour of the paper is mostly due to binder alteration, even though traces of iron have been identified by SEM–EDS which could suggest the use of earth pigments, that, however, has not been confirmed by Raman and FTIR investigations. The patina makes the paper surface hydrophobic.

The original black drawings were made with charcoal pencil, Raman spectroscopic studies found the presence of carbon black, and two types of restoration black drawings were evidenced, drawing with graphite pencil and brush strokes with carbon black ink. White lighting was performed with lead white, mixed with a polysaccharide (likely Tragacanth gum) binder.

Raman investigations also found the presence of a modern synthetic polymer, likely nylon or modified nylon, that was probably used as restoration adhesive in a not documented intervention. Adhesive employed during French intervention was flour glue, containing starch and gluten. This adhesive was used to stick together French paper foils, to stick French paper to original paper and to stick the paper to the lining paper. Drops of flour glue from French intervention are visible in some areas of the cartoon. Flour glue is present also under the lining paper with the function of attaching the paper to the canvas. This latter adhesive has lost most of its adhesive properties, as results from the lack of contact between canvas and paper in some areas of the cartoon. Adhesive used to stick together original paper foils, instead, seems to be animal skin glue.

All the analyses performed allowed to better understand the composition of this extraordinary work of art and gave useful information on Raffaello technique in realizing the cartoon. In particular, analyses helped in distinguishing original materials employed by Raffaello from materials coming from following restoration intervention. The results obtained, combined with the experience of restorers, curators, art historians and all different professional figures involved in the project, allowed to establish the restoration intervention to be performed. The restoration work is actually started, additional analyses have been performed, and additional results will be reported in further publications.

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