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A multi-analytical investigation of the materials and painting technique of a wall painting from the church of Copacabana de Andamarca (Bolivia)

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ABSTRACT

The Andean church of Our Lady of Copacabana de Andamarca in Bolivia was built in 1723. Its walls are made of adobe bricks and are decorated with paintings dated from mid 18th century. Before a restoration process involving the governments of Bolivia and the Federal Republic of Germany, seven microsamples were extracted from representative colors of one of the wall paintings inside the church. The aim of our research was to characterize the chromatic palette and investigate the painting technique of this Andean colonial wall painting. To approach these goals, an integrated investigation comprising Raman and FTIR-ATR spectroscopy, optical microscopy (OM), scanning electron microscopy with X-ray microanalysis (SEM–EDS), gas chromatography (GC), and gas chromatography mass spectrometry (GC–MS) was carried out.

The results indicated the use of a mixture of two basic copper sulfates of mineral origin, brochantite and antlerite, as the green pigment, adding relevant information to the palette of green pigments in colonial art. Yellow, orange and red ochre, abundant pigments in the Andean region, were also characterized. Observation by optical microscopy of cross-sections of the embedded microsamples and analysis of organic binders by GC and GC–MS pointed to *a secco* technique and the use of a mixture of egg and vegetable oil ("tempera grassa") as the pigment binder. In conclusion, our results allowed to establish the pigment palette and offered new insights into the painting technique of Andean wall paintings.

1. Introduction

The church of Our Lady of Copacabana de Andamarca (Fig 1a), placed at 3800 masl in the Bolivian department of Oruro, is one of various churches located near the so-called Silver Route that connected the Imperial city of Potosí (Bolivia) with the port city of San Marcos de Arica in Chile. In this vast territory, intense exchange processes have taken place since prehispanic times. During the colonial period, the silver ore, among other commodities, was transported on the backs of mules along this commercial route from the Cerro Rico from Potosí to the Pacific Ocean [1].

The Andean church of Copacabana de Andamarca is a building (about 390 square meters) with stone foundations and adobe brick walls constructed with mud mortar and plaster. It was built in 1723 and the walls inside the church were decorated with paintings dated from mid 18th century. These paintings depict several themes related to escathological discourses, including the Leviathan devouring souls, Saint Michael archangel, the Virgin of Carmen rescuing souls in Purgatory, among others. Still in the 18th century, these scenes were vital for the Catholic evangelization in that region, since they had as purpose to recall the believer the finitude of life and the eternal bliss that awaited those who pursued God's path and the endless punishments reserved for sinners. This is particularly clear in the Andean context, where the paintings of the Four Last Things – that can be found in temples like Carabuco, Huaro and Caquiaviri among others – were considered essential for the evangelization of natives during the Spanish domain [2]. The walls of Copacabana de Andamarca portray Saint Michel with the balance and the opened mouth of Leviathan where reprobates fall and road to Heaven (Fig. 1b and c), all recurrent topics of this iconography, as well as decorations with vases of flowers, trees, and birds (Fig. 1d).

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Fig. 1. (a) Image of the church of Copacabana de Andamarca, Bolivia; (b) detail of a wall painting from the church; (c) detail of the figure of Leviathan in (b) and indication of samples AND1, AND2, AND3, AND4, and AND5 locations; (d) detail of the floral decoration in (a) and indication of samples AND6 and AND7 locations.

In the early 20th century, the paintings were covered with a white plaster due to deterioration processes, such as flaking and detachment of the painted areas from the wall surface. Between 2005 and 2009, the church became the object of an important restoration process involving the governments of the Federal Republic of Germany and Bolivia. In order to gather information about pigment and plaster materials as well as the pictorial technique, the restorers collected microfragments of pigment samples from various locations of the wall paintings after a cleaning treatment, previous to the restoration.

Preliminary analysis of the black sample AND3 (Table 1, Fig. 1c) by Raman spectroscopy indicated the use of a carbon-based pigment. This result prompted us to develop analytical methodologies that allowed discrimination between the manufacture and origin of these pigments [3,4]. Based on this approach, the black pigment in sample AND3 was identified unambiguously as wood charcoal. The developed methodology was further applied to the characterization of carbon-based black pigments in South American polychrome wooden sculptures by Raman spectroscopy [5]. The study revealed the use of Vandyke or Cassel earth, lampblack and wood charcoal as carbon-based pigments in four Jesuit wooden sculptures. On the other hand, a rapid analysis of the blue sample AND6 (Table 1, Fig. 1d) by direct-insertion mass spectrometry (DI-MS) of the solid microsample revealed the presence of indigo as the blue organic pigment and gave preliminary information on the organic binder [6].

The aim of the present study is the characterization of yellow, red, orange, and green pigments of the wall painting palette, the identification of the ground layer, and the technique of execution. In order to achieve these goals, a multi-analytical approach based on the use of complementary analytical techniques is applied.

Table 1
List of samples, color and location.

Sample	Color	Location
AND1	Yellow	Flame
AND2	Green	Demon's head
AND3	Black	Leviathan's head
AND4	Red	Flame
AND5	Green	Demon's leg
AND6	Blue	Handle of flower vase
AND7	Orange	Flower decoration

2. Experimental

2.1. Painting samples

Before restoration, seven microsamples were extracted with a scalpel from colored areas of a wall painting from the church of Copacabana de Andamarca in Bolivia (Fig. 1c and d). The description of their color and location is given in Table 1. Polished cross-sections were prepared embedding the fragments in an acrylic transparent resin (Subiton, Laboratories S.A., Argentina) according to traditional techniques.

2.2. Model samples

Model samples of the ground layer were prepared by applying a mixture of gypsum plaster (1 g) in water (0.6 ml) on glass slides. After drying to constant weight the gypsum layer was separated from the glass slide and cut in three pieces of 2.5 cm × 2.5 cm and a thickness of 0.5 cm. Then a thin layer of binder (about 0.15 mm) was applied with a paint brush on the gypsum layer. Three different types of binder were used: linseed oil, animal glue, and whole egg. The animal glue was prepared according to Doerner [7]. The samples were naturally aged at room temperature for 1 year. Gypsum plaster, linseed oil, animal glue and egg were purchased from local suppliers.

2.3. Scanning electron microscopy

Morphological and stratigraphic investigations were carried out by using optical microscopy (OM) and scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS).

OM images were taken with a Leica MZ6 stereomicroscope and a Leica DM 750 microscope equipped with a Cannon Power shot S50 digital camera.

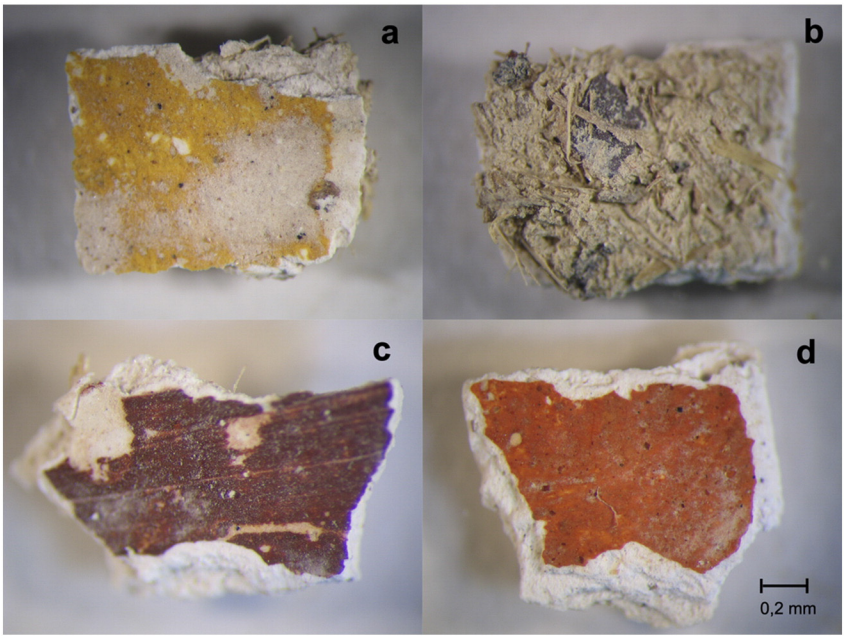

Fig. 2. Optical images of (a) the surface of sample AND1; (b) the back side of sample AND1; (c) the surface of sample AND4; (d) the surface of sample AND7, reflected light.

SEM analysis by secondary electrons (SE) and backscattered electrons (BSE) were obtained by using a field environmental scanning electron microscope (FE-SEM) with a Zeiss:Supra 40 coupled with an energy-dispersive X-ray spectrometer (SEM-EDS) INCA X Sight (Oxford Instruments). The samples were coated by sputtering with a thin (less than 80 Å) layer of gold. Several measurements were performed on selected areas or certain grains of each layer of the samples.

2.4. Micro-Raman spectroscopy

The Raman spectra were obtained with a Renishaw Raman microscope (System 3000), fitted with a Peltier cooled (−70 °C) CCD detector (Wright, 600 × 400 pixels) and with an Olympus metallurgical microscope (BH2-UMA). The spectra were excited at 632.8 nm (He-Ne laser, Spectra-Physics mod. 127) and a ×80 ultra long working distance objective (Olympus) was used to focus the laser beam on the samples and to collect the scattered light; laser power was always kept below 0.5 mW at the sample (power density $5 \cdot 10^3$ W cm^{-2}) to avoid degradation. The samples were studied as received without any type of manipulation and the spectra were analyzed using the Grams/AI package (Thermo Inc.).

2.5. Infrared spectroscopy

Fourier-transformed infrared spectroscopy (FTIR) spectra were obtained on a Nicolet iS50 FTIR spectrometer with a diamond single-bounce ATR accessory. For each sample, 64 scans were recorded in the 4000–400 cm^{-1} spectral range in the reflectance mode with a 4 cm^{-1} resolution. Spectral data were collected with the Omnic v9.2 (Thermo Electron Corp.) software without post-run processing. The spectrum of air was used as background.

2.6. Gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC–MS)

GC–MS and GC analyses were performed on three samples (AND3, AND6, and AND7) in order to detect the presence of lipids and proteins used as binders.

Samples were subjected to a previously reported extraction procedure [8] for the separation of lipids and proteins in the same small sample. Amino acid derivatives for GC analysis were prepared following the procedure of Bersani et al. [9]. Fatty acids were derivatised as their methyl esters [10] and sterols as their TMS derivatives [11].

Fatty acid methyl esters (FAME) were analyzed using a Shimadzu GCMS-QP5050/GC17A (Shimadzu Corporation, Kyoto, Japan) instrument. A ZB-5 (Phenomenex, USA) column (30 m × 0.25 mm i.d., 0.50 μm thickness) was used. The carrier gas was helium. Column temperature was programmed to increase from 100 to 180 °C at a rate of 10 °C/min followed by an isothermal period of 30 min. Injector temperature was 250 °C (splitless injection) and ion source temperature was 280 °C.

TMS sterol derivatives were analyzed using a Shimadzu GCMS-QP5050/GC17A (Shimadzu Corporation, Kyoto, Japan) instrument. An Ultra 2 (Agilent, USA) column (50 m × 0.20 mm i.d., 0.11 μm thickness) was used. Column temperature program: 1 min isothermal at 100 °C, then increase at a rate of 10 °C/min up to 240 °C followed by an increase at a rate of 4 °C/min up to 280 °C and an isothermal period of 30 min at this temperature. Injector temperature was 250 °C (splitless injection) and ion source temperature was 280 °C.

The mass spectrometer was operated in the electron impact (EI) positive mode (70 eV). Mass spectra were measured in the total ion monitoring (TIC) mode and the peak area data were used for quantitative evaluation. Individual components were identified using mass spectral data and by comparing retention time data with those obtained by authentic laboratory standards (Sigma-Aldrich Co.).

Amino acid derivatives were analyzed by gas chromatography using a Thermo Focus GC chromatograph equipped with a flame ionization detector and a VF-5ms (Varian, USA) column (25 m × 0.32 mm i.d., 0.52 μm thickness). Nitrogen was the carrier gas. Both injector and detector temperatures were set at 280 °C. Column temperature program: 3 min isothermal at 60 °C, then increase at a rate of 25 °C/min up to 260 °C followed by an isothermal period of 6 min. Individual components were identified by comparing retention time data with those obtained by amino acid standard (Sigma-Aldrich Co.) derivatives prepared in the same conditions as the samples.

3. Results and discussion

Microsamples were examined directly by OM in order to get morphological information of the surface, the ground layer and their backing. All the samples, as for example AND1, AND4 and AND7 (Fig. 2a, c and d), showed remains of the white plaster used to cover

Table 2
Results of SEM–EDS analysis on cross-sections of the wall painting samples. Major elements are marked in bold style.

Sample	Color	Pigment layer	Ground layer I	Ground layer II	Adobe layer
AND1	Yellow	**Ca, S, Si, Al,** Fe, **Na**	**Ca, S,** Si	**Ca, S,** Al, Si	**Si, Ca, Al, S,** Mg, K, Fe
AND2	Green	**Si, Cu, S, Ca,** K, Al	**Ca, S,** Al, Si	**Ca, S**, Al, Si	**Ca, Si, Al,** S, Mg, Fe, Na, K
AND3	Black	**Ca, S,** Si, Al, K	**Ca, S,** Si	**Ca, S,** Al, Si	–
AND4	Red	**Si, Fe, Ca, S, Al, K**, Mg	**S, Ca**, Al, Si	**S ,Ca**, Si	–
AND5	Green	**S, Ca, Cu,** Si, Al, K	**S, Ca**	**Ca, S,** Si, Al	–
AND6	Blue	**Ca, S**	**S, Ca**, Al, Si	**Ca, S,** Al, Si, K	**Ca, Si, S, Al,** Mg, K, Fe
AND7	Orange	**Si, S, Ca, Al,** Fe, K	**S ,Ca**, Si	**S ,Ca**, Si	–

the deteriorated paintings and that had previously been identified as gypsum [3]. The white ground layer was clearly observed in many of the microsamples together with remains of adobe from the wall of the church on their backing (Fig. 2b). Adobe was traditionally prepared as a mixture of earth, water, stone and *paja brava* straw [1]. Its elemental composition, as determined by SEM-EDS analysis of the adobe layer in the cross-sections of samples AND1, AND2, and AND6, is mainly constituted by silicon, aluminum, and calcium (Table 2), characteristic of a clayish soil. Its heterogeneous structure, including fragments of diatoms rich in silica, is clearly observed in Fig. 3d. Observation of the cross-sections by OM revealed a very simple painting technique consisting of the application of a fine pigment layer on a white ground layer, which was directly applied above the adobe church wall (Fig. 3e). The clear differentiation between the pigment layer and the ground layer pointed to *a secco* technique.

3.1. Ground layer

Detailed OM examination of the ground layer in the cross-sections revealed the superposition of two layers: I (beneath the pigment layer) and II (on the adobe wall). This is clearly appreciated in the BSE image of the cross-section of sample AND1 (Fig. 3a). Both layers show amorphous structures and differ in particle size, as observed in the SEM micrographs (Fig. 3b and c). SEM–EDS analysis (Table 2) revealed that they have very similar compositions with high amounts of calcium and sulfur and minor amounts of silicon and aluminum. This composition is indicative of calcium sulfate.

The Raman analysis performed on the white ground layer of the cross-sections of the seven samples showed a broad band at 1007–1014 cm^{-1} (Fig. 4a), characteristic of the calcium sulfate system with different levels of hydration: gypsum ($CaSO_4 \cdot 2H_2O$), bassanite ($CaSO_4 \cdot 0.5H_2O$), and anhydrite ($CaSO_4$) [12]. The intense luminescence of the spectra covered any other signals characteristic of the phases of the $CaSO_4$–H_2O system.

Analysis by FTIR-ATR of the ground layer of sample AND7 (Fig. 4b) confirmed the presence of calcium sulfate with SO_4^{2-} stretching bands at 1110, 1087, and 1008 cm^{-1} together with sharp bending bands at 595 and 660 cm^{-1}, characteristic of the $CaSO_4$–H_2O system [13]. OH stretching bands at 3604, 3536, 3402, and 3246 cm^{-1} and bands at 1619 and 1683 cm^{-1}, typical of water in gypsum and bassanite, were also observed [13]. The weak bands between 1400 and 1600 cm^{-1} are indicative of organic compounds probably used as pigment binders.

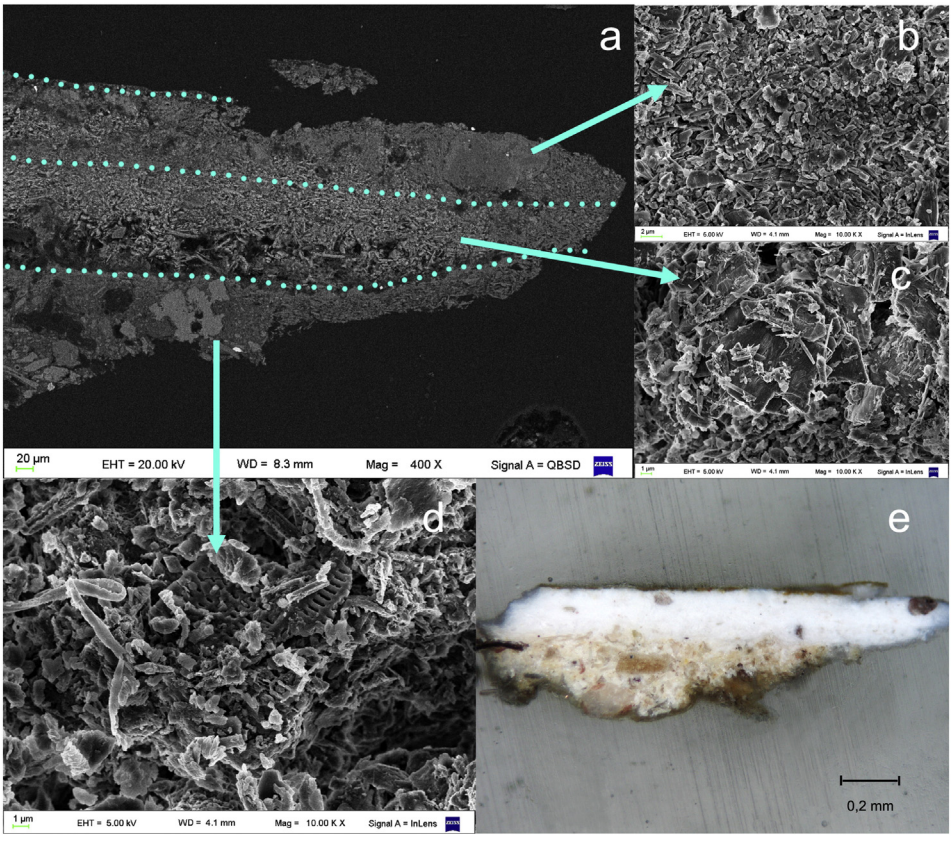

Fig. 3. Sample AND1: (a) BSE micrography (400×) of cross-section; (b) SEM image of ground layer I (10,000×); (c) SEM image of ground layer II (10,000×); (d) SEM image of adobe layer (10,000×); (e) cross-section, reflected light.

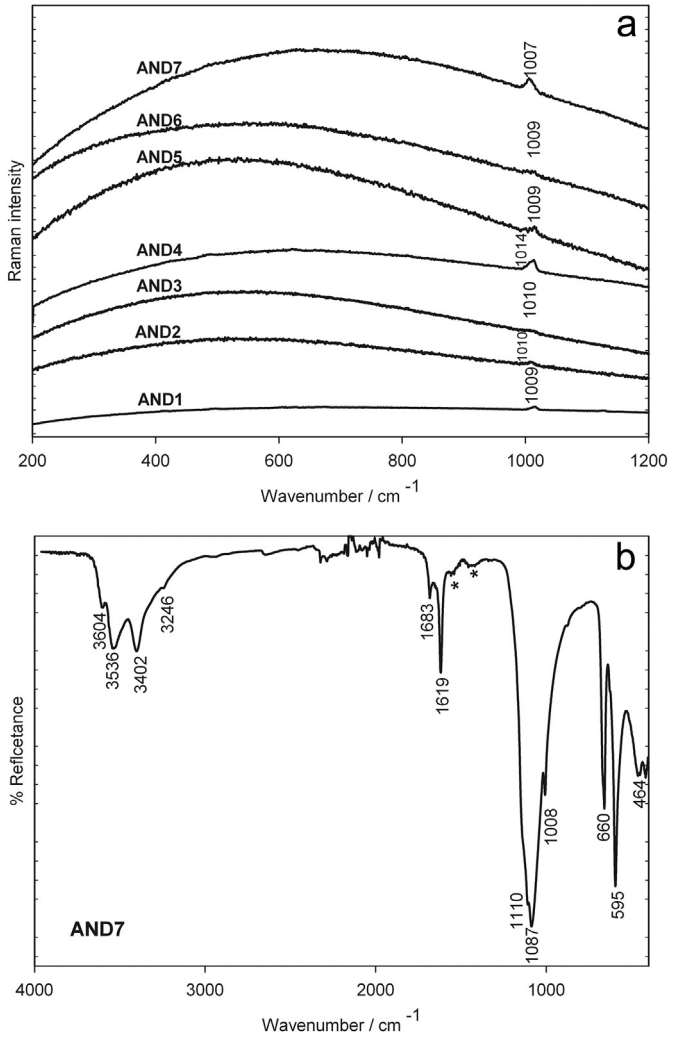

Fig. 4. (a) Raman spectra of the ground layer of samples AND1–AND7; (b) FTIR-ATR spectrum of the ground layer of sample AND7.

Gypsum in a protein medium was used as the ground layer on maguey wood in the manufacture of the late XVI century polychrome sculpture from Our Lady of Copacabana within the Viceroyalty of Peru [14]. This practice was characteristic of sculptures produced in Italy

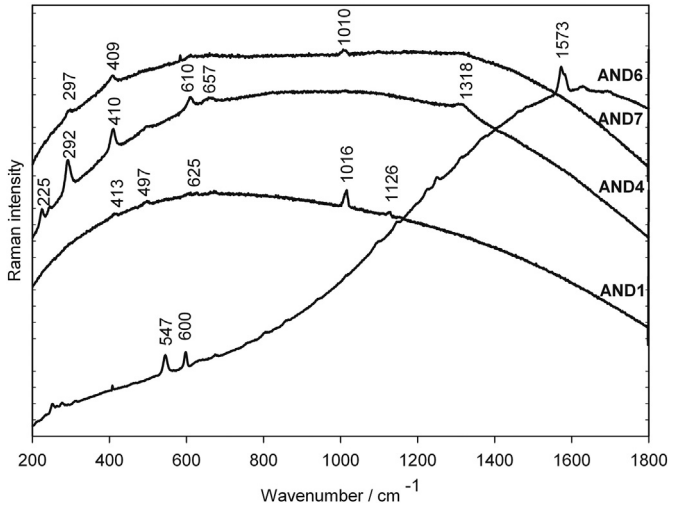

Fig. 5. Raman spectra of pigment layers of samples AND1, AND4, AND6, and AND7.

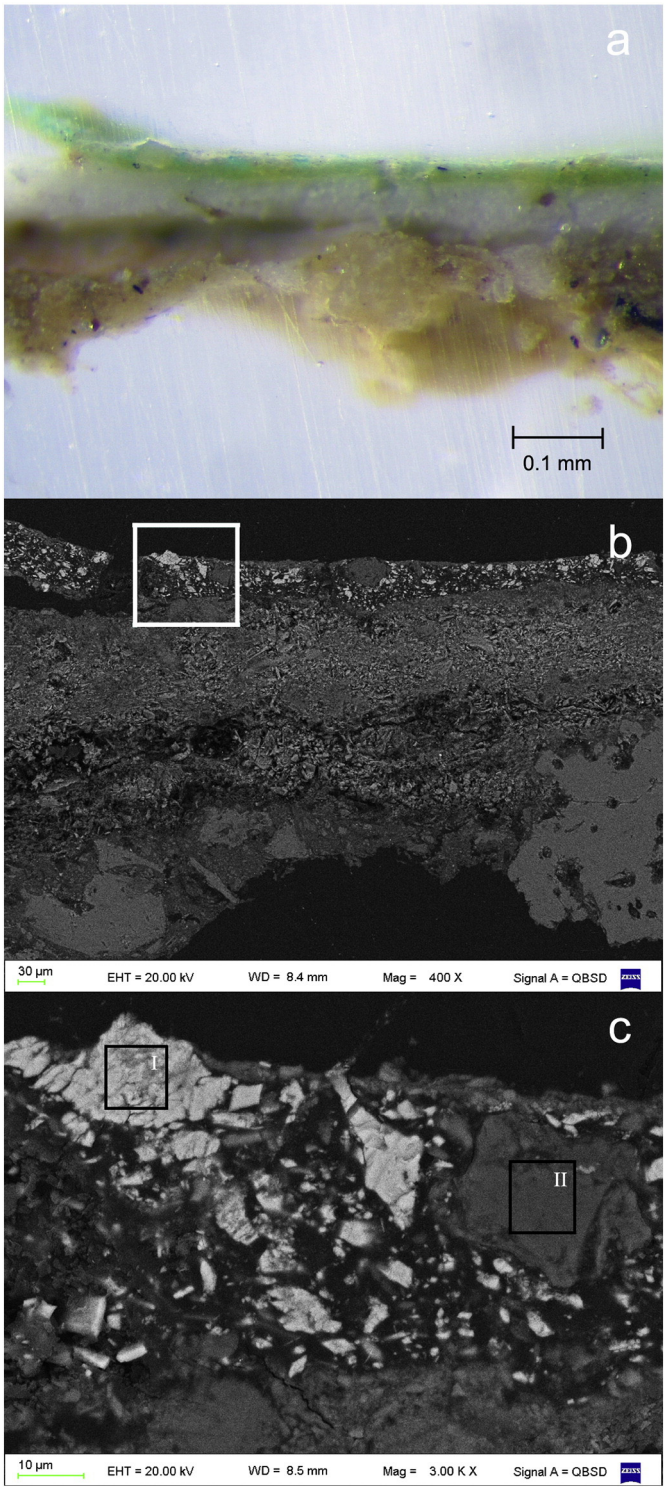

Fig. 6. Cross-section of sample AND2: (a) Optical image (50×); (b) BSE micrography (400×); (c) BSE image of the area indicated in (a) (400×); (c) BSE image of the area indicated in (b) (3000×), SEM–EDS results of zone I: Cu and S (major), Ca (minor). SEM–EDS results of zone II: Si (major), Al and K (minor).

and Spain. Nevertheless, the use of a preparation layer of gypsum in mural painting is uncommon, being lime the preferred plaster. Some authors have reported the identification of gypsum mixed with clays and calcium carbonate in the ground layer of wall paintings of churches in Sudan [15] and Spain [16]. Recently, we have identified gypsum as the preparation layer in the wall paintings of the church of San Pedro

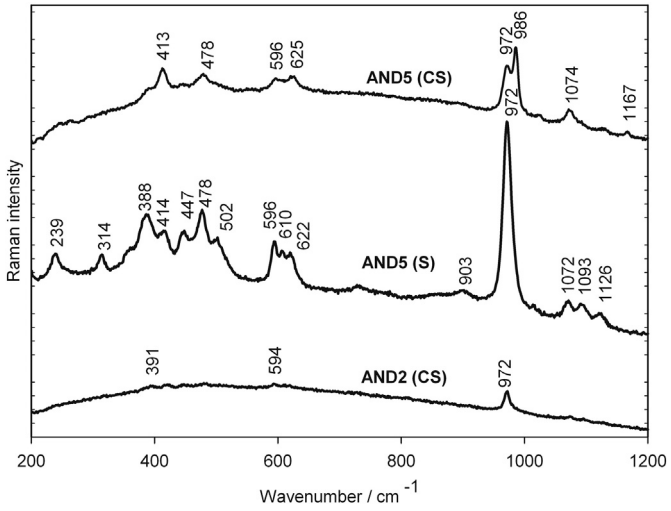

Fig. 7. Raman spectra of the green pigment layer in the cross-section of sample AND2 (CS), the surface of sample AND5 (S) and the cross-section of sample AND5 (CS).

de Pachama in Chile, a little town also located on the Silver Route and not very far away from Copacabana de Andamarca in Bolivia [17].

3.2. Pigments

3.2.1. Yellow, orange and red

Analysis by SEM-EDS of the cross-section of sample AND1 revealed that the yellow layer was constituted mainly by Ca, S, Si, Al, and Na, with minor amounts of Fe (Table 2). The Raman spectrum performed on the surface of the sample without embedding it in resin showed bands at 1126, 1016, 625, 497, and 413 cm^{-1} (Fig. 5), presumably from the gypsum that covered the paintings, but did not allow for the identification of the pigment because of a very intense luminescence which covered any other signal. Nevertheless, the elemental composition obtained by SEM–EDS permits us to hypothesize the use of a yellow ochre as pigment, most probably containing hydrated iron oxide, goethite (α-FeOOH), and clay minerals [18].

Micro-Raman analyses of the surface of microsample AND4 showed the characteristic bands of hematite (α-Fe_2O_3) at 225, 246, 292, 410, 610, and 1318 cm^{-1} together with a weak band at 657 cm^{-1} that has been reported for disordered structures in natural hematite (Fig. 5) [19,20]. Sample AND7 showed weak bands at 297 and 409 cm^{-1} ascribed to hematite. SEM-EDS analyses of both samples (Table 2) indicated, in addition to iron, the presence of silicon, aluminum, calcium, sulfur, potassium, and magnesium, suggesting the clayish origin of the hematite. The presence of clay minerals as well as the relative content of iron may be the reason of the different colors displayed by samples AND4 and AND7.

Light and dark yellow or red earths were widely used as pigments in the Andean region, as in the case of hematite or *almagra* [21]. Red earth containing hematite has been identified as a pigment in colonial paintings [22,23] and as the mordant (commonly known as bole or "Armenian bole") for the gilding of the polychrome sculpture of Our Lady of Copacabana [14].

Table 3
P/S and A/P ratios of different wall painting and model samples based on GC–MS analysis of FAME and TMS derivatives of sterols.

Sample	P/S	A/P	ΣD (%)	TMS sterol derivatives
Wall painting samples				
AND3	1.3	–	–	–
AND6	1.3	–	–	Cholesterol, cholesta-4, 6-dien-3-one, ergosta-7-en-3β-ol
AND7	1.4	0.1	1.9	Cholesterol
Model samples				
Linseed oil on gypsum	1.6	0.1	4.7	–
Whole egg on gypsum	2.8	–	–	Cholesterol

3.2.2. Blue

Analysis by SEM–EDS of the blue layer of the cross-section of sample AND6 indicated that calcium and sulfur were the main elements (Table 2). The absence of copper and iron, discarded the use of azurite (a basic copper carbonate) or Prussian blue ($Fe_4[Fe(CN)_6]_3 \times H_2O$) as pigments and suggested the presence of indigo. The Raman spectrum of the surface of the microsample indicated characteristic bands of indigo at 547, 600, and 1573 cm^{-1} (Fig. 5) [24]. This result confirms our previous identification of indigo by direct-insertion mass spectrometry and HPLC–DAD in sample AND6 [6]. Indigo, a blue dye obtained from plants of the families Papinolaceae, Brassicaceae, and Polygonaceae [25], has been one of the blue pigments most employed in the workshops of Cuzco in the 17th and 18th centuries [26,27].

3.2.3. Green

Analysis by OM of the cross-section of sample AND2 showed a thin green layer applied onto the calcium sulfate ground layer (Fig. 6a). The BSE images (Fig. 6b and c) highlight the heterogeneity of the green layer, revealing particles of different size and morphology, suggesting a natural mineral origin of the green pigment. SEM–EDS analysis of the green layer of sample AND2 indicated the presence of high amounts of silicon, copper, sulfur, and calcium together with minor amounts of aluminum and potassium (Table 2). This elemental composition is in accordance with a copper based pigment. SEM–EDS analysis of two grains of the green layer indicated in Fig. 6c revealed the presence of particles rich in copper and sulfur (indicative of a green basic copper sulfate) mixed with particles constituted mainly by silicon attributed to silicon dioxide. This heterogeneity in composition reinforces the hypothesis of the use of a green mineral pigment. Raman analysis performed on the surface of sample AND2 (Fig. 7) revealed weak bands at 972, 594, and 391 cm^{-1} characteristic of brochantite, a basic copper sulfate [$Cu_4SO_4(OH)_6$] [28,29]. On the other hand, SEM–EDS analysis of sample AND5 extracted from another region of the green figure (Fig. 1c) revealed a similar elemental composition as AND2 (Table 2). Analysis of the Raman spectrum of the surface of sample AND5 (Fig. 7) showed an intense band centered at 972 cm^{-1} and weaker bands at 1126, 1093, 903, 622, 610, 596, 388, 314, and 239 cm^{-1} that match reported assignments for brochantite [28,29]. Weak bands at 1072, 502, 478, and 447 cm^{-1} are common to both basic copper sulfates, brochantite and antlerite ($Cu_3SO_4(OH)_4$), while band at 414 cm^{-1} and the broadening of the strong band at 972 cm^{-1}, presumably due to the overlapping of the intense band at 986 cm^{-1} of antlerite, could be assigned to this basic copper sulfate. The presence of both copper based pigments is clearly evident in the Raman spectrum of the green layer in the cross-section of sample AND5 (Fig. 7). In this spectrum, strong bands at 972 and 986 cm^{-1} are consistent with a contribution of brochantite and antlerite together with weak bands of brochantite at 625 and 596 cm^{-1}, bands of antlerite at 1167 and 413 cm^{-1} as well as bands at 1074 and 478 cm^{-1} common to both basic sulfate hydroxides.

It has been reported that malachite ($Cu_2CO_3(OH)_2$) may suffer a transformation into basic copper sulfates (brochantite, antlerite, and posnjakite) in the presence of calcium sulfate [16]. Lichens and other microorganisms that excrete oxalic acid might be responsible for this degradation process. Indeed, Castro et al. [30] have suggested that malachite may decay to moolooite (copper oxalate) as result of an excess of biological activity. These authors have identified the degradation products of malachite together with weddellite (calcium oxalate dihydrate, $CaC_2O_4 \cdot H_2O$) in a wall painting of the 18th century [30]. In our study of the green samples from the wall painting of Copacabana de Andamarca, brochantite and antlerite are likely to have been used as the green pigment since no malachite or weddellite have been

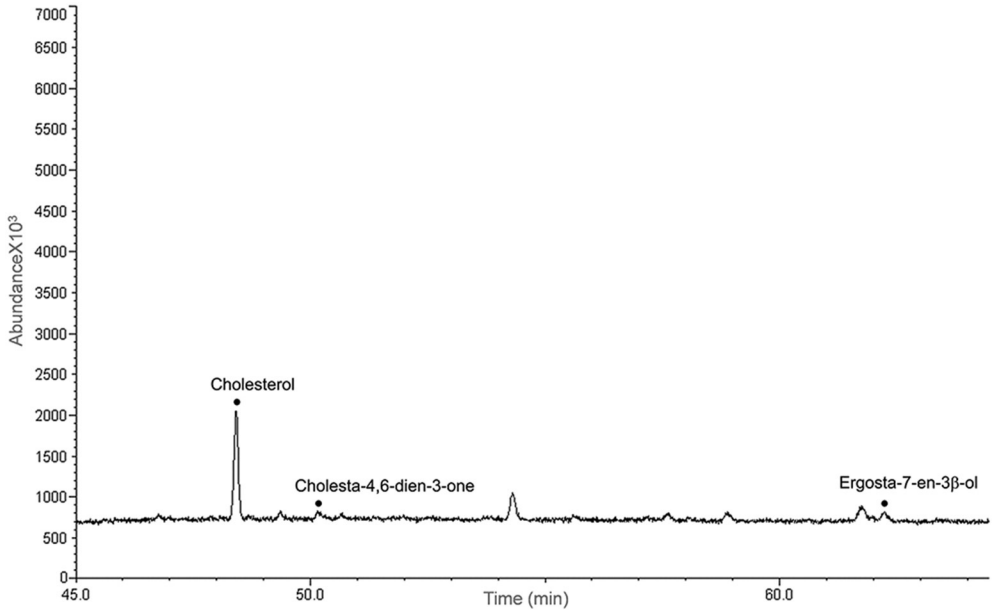

Fig. 8. Total ion chromatogram of the lipid neutral fraction of sample AND6; 1 = cholesterol, 2 = cholesta-4,6-diene-3-one, 3 = ergosta-7-en-3β-ol.

detected in the Raman spectra. Moreover, these sulfated copper hydroxides are available as minerals in the Atacama Desert in the north of Chile, which is rich in copper mines that have been exploited since prehispanic times [31]. Moreover, copper-ore based pigments, such as atacamite, brochantite and antlerite, have been identified as green paints on masks made of animal hides [32]. Recently, we have identified antlerite as the green pigment in the wall paintings of the Andean church of San Pedro de Pachama in Chile [17]. These results suggest the development of technological practices regarding the production of pigments from copper based minerals in the Andean region that extended from prehispanic times to the colonial period [21].

3.3. Organic materials

Three samples (AND3, AND6, and AND7) were investigated by GC and GC–MS in order to assess the presence of an organic binder and investigate the painting technique. Sample AND3 has been chosen as representative of the use of wood charcoal as the black pigment and to contrast our previous hypothesis on the use of water as the vehicle for the pigment [3]. Sample AND6 has been selected on the basis of preliminary information obtained by DI-MS that indicated the use of an egg tempera binding [6]. Sample AND7 has been chosen for its content of hematite, a pigment very much used in the Andean region [21].

The samples were subjected to an extraction with ammonia in order to separate lipids from proteins, as described in [8]. Saponification of the lipid fraction, separation of neutral components, and methylation of the acid fraction rendered a FAME mixture, which was further analyzed by GC–MS. The three samples revealed the presence of miristic (C14:0), palmitic (C16:0), oleic (C18:1), and stearic acids (C18:0). The parameters A/P (ratio azelaic acid (nonanedioic acid)/palmitic acid), P/S (ratio palmitic acid/stearic acid) and ΣD (sum of dicarboxylic acids) are reported in the literature as indicative of the source of the lipid material [33]. The characteristic parameters calculated for samples AND3, AND6, and AND7 were compared with the average values calculated for naturally aged reference samples of linseed oil and egg applied on gypsum (Table 3). Linseed oil, a siccative oil with a very high content of C-18 unsaturated fatty acids, has been traditionally used as a pigment binder. Whole egg or egg yolk also have been used as binders, particularly for blue colors that might turn green due to the yellowing of aged linseed oil [34]. Gypsum has been chosen as the ground layer in the model samples in order to mimic the wall painting samples. Naturally aged lindseed oil on gypsum evidenced the formation of azelaic and sebacic acids (decanedioic acid) as degradation products (ΣD = 4.7%) and a P/S ratio of 1.6. On the contrary, the reference sample of naturally aged whole egg on gypsum showed a higher P/S ratio (2.8), characteristic of egg, and no degradation products, which is in accordance with a lower content of more reactive C18 polyunsaturated acids with respect to drying oils [33]. Sample AND7 showed azelaic acid as degradation product (ΣD = 1.9%) and a P/S ratio of 1.4 close to that of the reference sample of linseed oil on gypsum (1.6) and samples AND3 and AND6 (1.3) (Table 3). In addition, analysis of the neutral fractions by GC–MS revealed the presence of cholesterol in samples AND6 and AND7, indicative of egg. Sample AND6 also showed minor peaks of cholesta-4,6-dien-3-one, the oxidation product of cholesterol, and ergosta-7-en-3β-ol, which could be ascribed to microbial contamination (Fig. 8). These results and the P/S ratio value in samples AND6 and AND7 (1.3/1.4) point to a mixture of linseed oil and egg, characteristic of a "tempera grassa" binder, in both samples.

In addition to the GC–MS analysis of the lipid and neutral fractions of the three samples, further investigation of the proteinaceous fractions

Table 4
Relative amino acid composition of wall painting and model samples.

Sample	Ala	Gly	Val	Leu	Ile	Pro	Asp	Glu	Ser	Hyp	Tyr	Trp	Phe
Wall painting samples													
AND3	10.1	22.4	4.1	7.0	3.0	16.6	10.9	15.4	7.7	–	2.8	–	–
AND6	18.8	20.7	4.8	8.0	2.0	15.7	6.8	9.9	8.9	–	4.4	–	–
AND7	17.2	21.0	7.2	4.9	2.5	10.1	8.5	14.5	7.9	–	3.4	0.6	2.2
Model samples													
Animal glue on gypsum	6.8	23.8	1.2	4.6	0.9	23.5	7.3	12.7	–	10.6	4.0	0.8	3.8
Whole egg on gypsum	11.7	6.1	6.0	13.1	4.9	8.1	13.9	15.1	5.8	–	6.0	1.0	8.3

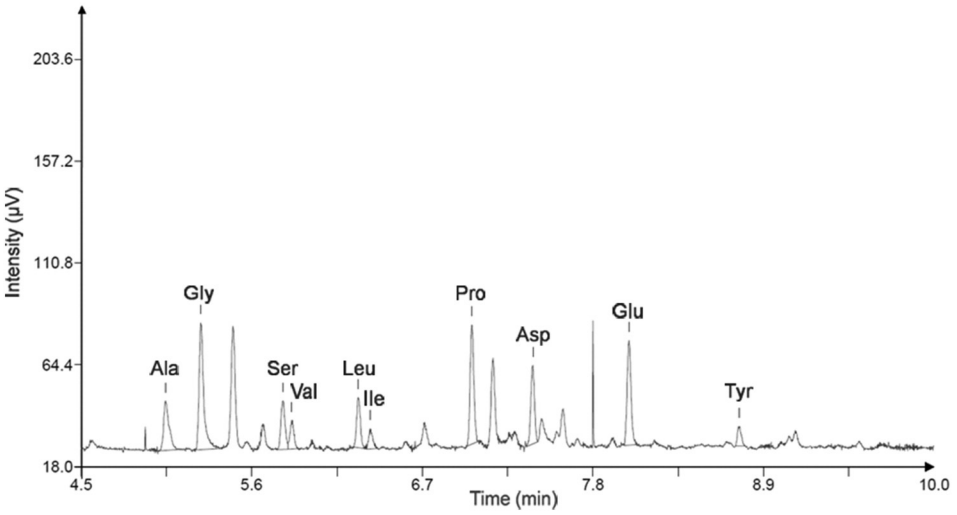

Fig. 9. Chromatogram of the amino acid fraction of sample AND3.

by GC was accomplished. Amino acids have been identified in the three samples and their relative compositions are listed in Table 4. A representative chromatogram of the amino acid profile for sample AND3 is shown in Fig. 9. Comparison of the relative amino acid compositions with those of reference naturally aged samples of whole egg and animal glue applied on gypsum (Table 4) suggested the presence of both proteinaceous media in the three samples. The high levels of glycine (Gly) and proline (Pro) in the wall painting samples as well as in the model sample of animal glue on gypsum suggest the presence of animal glue in the three investigated samples. On the other hand, the high concentrations of alanine (Ala), valine (Val), and serine (Ser) point to the presence of egg in the wall painting samples. Although cholesterol has not been detected in sample AND3, the amino acid composition supports the hypothesis of the use of egg as a component of the binder in this sample.

Regarding the wall painting technique, most probably, animal glue was applied as a primer onto the gypsum layer and then pigments mixed with egg and oil were used to color the different motifs. This is in accordance with *a secco* technique [34] as revealed by OM observation of the cross-sections of the samples in which a notable differentiation between pigment and ground layers was observed.

4. Conclusions

We have investigated the pigments and organic binders in microsamples from a wall painting of the 18th century church of Copacabana de Andamarca in Bolivia. Brochantite and antlerite, two basic copper sulfates, were identified as components of the green pigment in two of the samples. The local availability of both sulfated copper hydroxides and their heterogeneous composition in the wall painting samples point to a mineral origin of the green pigment. This is the first report on the identification of brochantite in colonial art. This finding expands our knowledge on the palette of green pigments that we have already identified in colonial easel paintings and polychrome sculptures, such as *cardenillo* (verdigris), malachite, green earth, and atacamite, as well as mixed greens of orpiment and indigo or Prussian blue [14,22,23,27]. Raman spectroscopy allowed the identification of hematite in the orange and red samples, while a yellow iron-containing ochre was identified by SEM–EDS analysis. Gypsum was identified as the ground layer on which the pigments were applied using *a secco* technique and a "tempera grassa" as the vehicle for the pigments, as determined by GC and GC–MS analysis of the binders.

The application of our multi-analytical approach permitted us to establish the pigment palette and obtain for the first time accurate and detailed information on the painting technique in an Andean wall painting. These results provide new insights into the decorative technique of these paintings and motivate us to continue investigating the pigments and organic binders in wall paintings from other churches of the Silver Route.

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