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Short communication

Combined use of vibrational spectroscopy and GC–MS methods in the characterization of archaeological pastes from Patagonia

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Abstract

Two samples of ochre painting residues (crayons) obtained from the archaeological site Cave Loncomán (Río Negro, Argentina) were analysed by ATR-IR and FT-Raman spectroscopy revealing the presence of haematite as the red pigment. Further analysis by FTIR of the chloroform/methanol extracts from both archaeological pastes showed the presence of carboxylic acids, indicative of hydrolysis of triacylglycerols in the pastes. Analysis by GC and GC/MS indicated that the main organic constituents of both pastes were saturated ($C_{16:0}$ and $C_{18:0}$) and unsaturated ($C_{16:1}$ and $C_{18:1}$) fatty acids. Our results show that the combined FTIR and Raman spectroscopic evaluation of archaeological pastes and their organic extracts provides a very useful and simple methodology to characterize the inorganic pigment and the presence of organic binders in an archaeological sample.

Keywords: Archaeological pastes; Lipids; Haematite; Infrared spectroscopy; FT-Raman

1. Introduction

Studies on Patagonian rock art have had an outstanding role in the development of Argentine archaeology, but only a few attempts have been made to study the chemical composition of the paints used. The scarce literature on this subject is limited to inorganic component analysis by X-ray diffraction, scanning electron microscopy and FTIR [1–4]. Recently, we have performed the first chemical analysis of organic and inorganic components of rock art samples and painting residues (pastes) from archaeological excavations in Argentinian Septentrional Patagonia. Our results showed that they were composed of inorganic pigments mixed with lipids of vegetable or animal origin [5]. In continuation of our studies on Patagonian archaeological pastes, we have obtained two samples of ochre painting residues (pastes **1** and **2**) that were in stratigraphic position in the archaeological site Cave Loncomán, in Río Negro Province, Argentina. The two samples came from the top of the early occupations, dated by ^{14}C in 1960 ± 40 BP, and belong to hunter-gatherers societies that established in the region 2800 years ago, at the beginning of the stage of exploration of new territories. The pastes might have been used in the preparation of colours for painting over the engravings of the Cave (representations of human footprints and local fauna) and leathers used as shelters or for facial and corporal paintings. Preliminary results obtained by application of FTIR spectroscopy on chloroform:methanol (2:1) extracts of both pastes showed the presence of carboxylic acids as the main components [6].

Recent research on organic residues associated to archaeological fats has proved that lipids are preserved under favourable conditions [7,8]. Analysis of lipids found in vessels or buried in soil is attracting considerable attention because of their potential to yield archaeological information related to human activities, diet and resource utilization [9]. The use of complementary analytical methods, such as GC–MS, FTIR and more recently non-destructive FT-Raman spectroscopy can maximize the amount of information provided by a sample, allowing a better understanding of its degradation process. Archaeological samples are very complex in composition, since

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their preparation generally involves a mixture of materials submitted to deterioration factors that are largely dependent on the environmental conditions. Furthermore, in the case of artefacts, the technique employed in their manufacture and the use they are designed for also increases the number of variables to be considered concerning the sample heterogeneity. In the case of the archaeological pastes there is, in general, an inorganic and an organic phase present, thus adding up to the system complexity. Clearly only a set of analytical tools can characterize such samples properly. The purpose of this work is to enlarge the understanding of the artistic practices and technological knowledge of the primitive inhabitants of the Argentinian Patagonia, through the analysis of the pastes composition by a combination of spectroscopic and chromatographic methods. Modern animal fats from an American ostrich (*Pterocnemia pennata*) and from a guanaco (*Lama guanicoe*) provided comparative compositional data.

2. Experimental

2.1. Samples

Pastes **1** and **2** were collected at Cave Loncomán, Río Negro Province, Argentina at a depth of 1.20 m from the soil surface and at 0.10–0.15 m from the rock. Animal fat and marrow from an American ostrich (*P. pennata*) and a guanaco (*L. guanicoe*) were provided by local inhabitants of Estancia Loma Blanca and Potrero Consumo, Río Negro Province. The reference red mineral was obtained from Sitio Florentino, Estancia Loma Blanca, Río Negro Province.

2.2. Solvent extraction of the archaeological pastes

Pastes **1** and **2** were crushed to a powder and extracted using chloroform/methanol (2:1 (v/v), 15 ml, 15 min sonication) at room temperature. The total lipid extracts were then centrifuged (15 min, 3200 rpm), decanted and filtered through celite under vacuum. The filtrates were then dried under a stream of nitrogen and stored at −25 °C until required for derivatisation and analysis. The extractable lipid concentrations were 1120 and 160 μg g^{-1} for pastes **1** and **2**, respectively. *P. pennata* fat and *P. pennata* and *L. guanicoe* marrows where extracted under the same conditions.

2.3. Derivatisation

Fatty acid methyl esters (FAMEs) were prepared by treating the extracts with HCl 2% in methanol (0.5 ml) at 60 °C for 2 h. After cooling, water (0.5 ml) was added. The mixture was extracted with chloroform (3 × 0.5 ml) and the solvent evaporated under nitrogen.

2.4. Preparation of the mixture of fatty acids from the fat of P. pennata

P. pennata fat (3 g) was hydrolysed with 4% KOH in MeOH solution (20 ml, 70 °C, 1 h), cooled and acidified to pH 3 with 3 M HCl. The mixture of fatty acids was extracted with ethyl ether. The organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated to dryness under a stream of nitrogen. The residue was purified by vacuum-dry column chromatography on silica gel using cyclohexane-EtOAc mixtures as eluents to afford 71.9 mg of the mixture of free fatty acids.

2.5. Preparation of the potassium salt of the mixture of fatty acids from the fat of P. pennata

The mixture of fatty acids from *P. pennata* was treated with KOH in EtOH for 20 min at 70 °C, following a procedure reported previously [10]. Centrifugation and further filtration afforded the potassium salt of the mixture of fatty acids.

2.6. Instrumental

Raman spectra were obtained from a Bruker RFS 100/S, fitted with a liquid N_2 cooled Ge detector. Sample excitation was performed with the 1064 nm line of a Nd^{3+}/YAG laser. Single Bounce ATR data were obtained in a Bomem MB-100 Arid Zone FTIR spectrometer using a Silver Gate ATR accessory (Specac). FTIR spectra from the extracts were collected using a Nicolet Magna 550 spectrometer. The samples were dispersed in KBr disks. Spectra of 4 cm^{-1} resolution were accquired by coaddition of 32 scans from 4000 to 400 cm^{-1}. GC was performed on a Hewlett-Packard 5890A chromatograph equipped with a flame ionization detector and an ULTRA 2 column (30 m × 0.25 mm i.d.). Temperature program: 1 min of isothermal at 100 °C and then 100–290 °C at 10 °C min^{-1}, followed by a 10 min hold at 290 °C. GC–MS was performed on a TRIO-2 VG mass spectrometer coupled to a Hewlett-Packard 5890 chromatograph. XRD analysis was carried out on a Philips diffractometer PW 1050 using copper radiation and nickel anticathode. SEM-EDX analysis was performed on a Philips XL 30 ESEM scanning electron microscope. ^{14}C dating was performed at the Laboratorio de Tritio y Radiocarbono, Universidad Nacional de La Plata (CONICET).

3. Results and discussion

Fragments of pastes **1** (red-orange) and **2** (yellow-orange) were ground using a mortar and pestle. FT-Raman spectra of both pastes were recorded in the 100–3500 cm^{-1} region and are similar; Fig. 1 shows the Raman spectrum of paste **2**. They present a band at 1335 cm^{-1} and a broader and much weaker feature at ca. 1575 cm^{-1} overlapping an emission background (Fig. 1). These bands are typical of degraded organic materials, corresponding basically to C–C and C=C stretching vibrations. The source of the emission background can be either the emission observed for clays [11] or the degraded organic phase and will be considered later. A careful analysis in the low frequency region (200–500 cm^{-1}) reveals very weak peaks at ca. 295, 410 and 609 cm^{-1} that are ascribed to haematite vibrations [12]. In Fig. 1 the FT-Raman spectrum of paste **2** is compared with that of an inorganic pigment (reference pigment) from the same region where the samples were found.

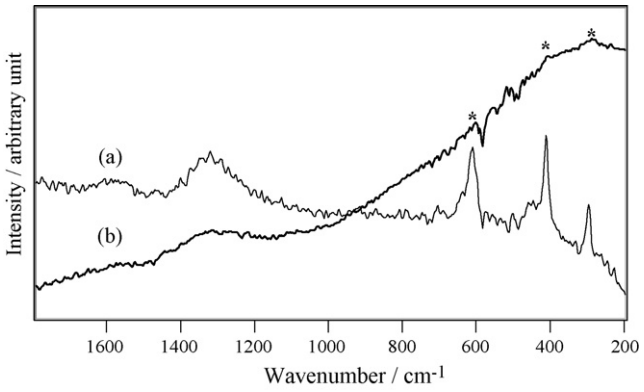

Fig. 1. FT-Raman spectra obtained from the reference inorganic pigment (a) and from paste **2** (b); the asterisk indicates the haematite peak positions.

Despite the poor data quality of the archaeological sample spectrum due to the high background there is a clear coincidence of the above cited bands and the haematite peaks.

XRD was also used in order to identify the crystalline components present in the pastes **1** and **2** and in the reference pigment. The diffraction pattern (not shown) indicated that tridymite (a silicon oxyde) and feldspar were the main components in the archaeological samples whereas quartz, kaolinite, illite and haematite were found as the major constituents of the reference pigment. Curiously haematite was not detected in the pastes in spite of the very suggestive sample colours (red-orange and yellow-orange), however, if iron compounds are present at low concentrations it is possible that XRD is not sensitive enough as to detect them.

Scanning electron microscopy coupled with an energy dispersive electron microprobe (SEM–EDX) was then used on the samples and allowed the identification of iron, oxygen, aluminum, silicon, potassium and calcium as the major elements. Iron compounds (probably oxides or oxihydroxides) can then be assigned as the chromophoric species present in the archaeological samples.

FTIR data were also recorded directly from the pastes, using a single bounce ATR accessory and Fig. 2 shows the paste **1** ATR spectrum. There is no clear sign of organic compounds in the spectrum, which is dominated by the inorganic phase

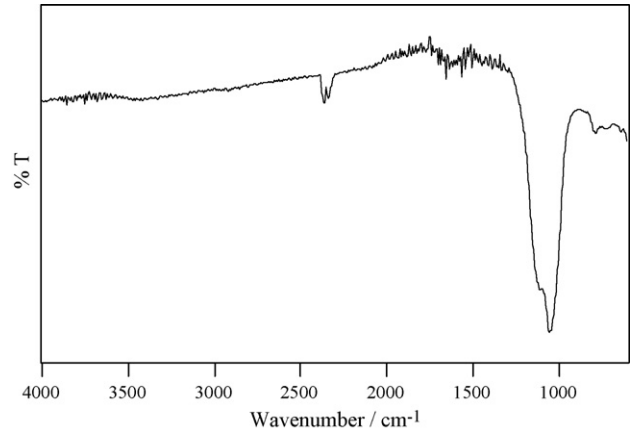

Fig. 2. Single bounce ATR FTIR spectrum of paste **1**.

components. Strong peaks at 1110 and 1048 cm^{-1} due to Si–O stretchings [13] confirm the results previously obtained by XRD.

Aiming at the detection of any residual organic compound, the samples were extracted with a chloroform:methanol (2:1) mixture and the extracts were analysed by FTIR (Fig. 3), GC and GC–MS (Table 1). In the case of FTIR the samples were prepared as KBr pellets without any previous manipulation whereas for GC and GC–MS the methyl ester derivatives were prepared. In the FTIR spectra of both archaeological samples the most important feature is the presence of a band at 1730 cm^{-1} (νCOO stretching) together with bands attributable to CH_3 and CH_2 groups at 2970–2870 and 1450–1380 cm^{-1}. The band at 1730 cm^{-1} could arise from fatty acids or from triacylglycerol groups. In order to clarify this point, the FTIR spectra were compared to that of a mixture of an animal fat of an American ostrich (*P. pennata*) and a local mineral [6]. The observation of a sharp carbonyl band at 1764 cm^{-1} in the FTIR of the reference mixture suggested the presence of free fatty acids in both archaeological pastes. Further comparison of the FTIR spectra of the organic extracts of **1** and **2** with the mixture of fatty acids obtained by saponification of the reference fat and the corresponding potassium salt of the mixture of fatty acids (Fig. 3) clearly indicated that the lipids in the archaeological samples correspond to a mixture of free fatty acids and their salts. Broad absorptions in the spectrum of the reference carboxylic salt around 3000–3700 and 1650 cm^{-1} indicate the presence of OH groups, possibly from adsorbed water in the sample [14].

Analysis by GC and GC/MS of the carboxylic acid methyl esters prepared from pastes **1** and **2** extracts, indicated that the main organic constituents of both pastes were saturated ($C_{16:0}$ and $C_{18:0}$) and unsaturated ($C_{16:1}$ and $C_{18:1}$) fatty acids, as established by GC/MS and comparison with retention times of authentic compounds. The chemical composition initially confirmes that the binders have an animal and not a vegetal origin, otherwise a much higher palmitic acid ($C_{16:0}$) concentration with respect to that of stearic acid ($C_{18:0}$) would be expected [15].

Reports from foreign travelers in the Patagonian region during the XVIII and XIX centuries give account of the use by the local inhabitants of animal fats (ostrich and guanaco) mixed with red clays for corporal and facial painting as well as for the decoration of their shelters [16]. Comparison of the fatty acid abundances of the archaeological pastes and those from fat and marrow lipids of a local ostrich (*P. pennata*) and guanaco (*L. guanicoe*) (Table 1) points to similar compositions, confirming the use of an animal source in the manufacture of the archaeological pastes. The very high abundance of the monounsaturated palmitoleic ($C_{16:1}$) and oleic ($C_{18:1}$) acids in the samples suggests that oxidative degradation of the lipids within the mineral matrix was minimal.

Considering that organic substances were only detected in the extracts, it can be concluded that they are minor components in the archaeological samples and, taking into account their age and the environmental conditions the samples were submitted to (buried, dry and cold weather) it is very likely that they degraded to a large extension. This was confirmed by

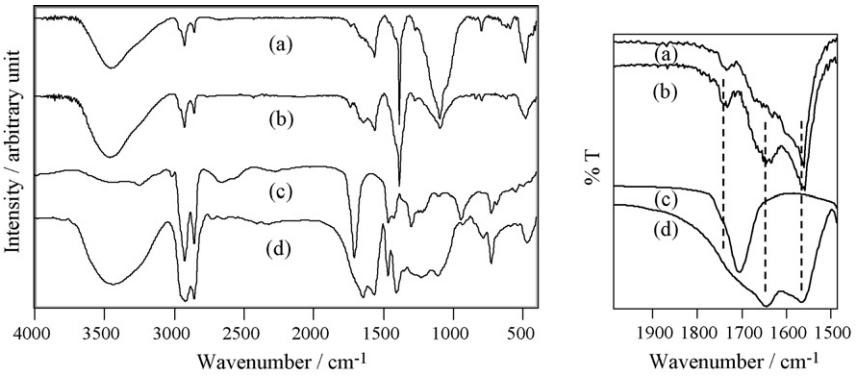

Fig. 3. FTIR spectra from paste **1** (a) and paste **2** (b) extracts, from the hydrolysis product of the reference fat (c) and from the potassium salt (d).

elemental analysis which showed a very low carbon content (below 0.4% for both samples).

The extracts thus provided important information concerning the origin of the luminescent background in the FT-Raman spectra, suggesting at first sight that it might be due to the inorganic compounds as the contribution of the organic phase is negligible. However, it has to be emphasized that fluorescence cross section is much higher than the Raman scattering one and even very small amounts of organic compounds could originate the observed background.

Another point worth mentioning is the fact that the reference pigment itself has some organic compounds in its composition as can be inferred by the broad band at ca 1570 cm^{-1} in the FT-Raman spectrum; the 1335 cm^{-1} band is swamped by a haematite mode at 1320 cm^{-1}. For this reason the pigment was extracted with the same solvent mixture used with the pastes and the FTIR spectrum was obtained as KBr pellets. In Fig. 4 the FTIR spectra of the pastes and pigment extracts are compared and clearly shows that the spectra of pastes **1** and **2** extracts have a different profile in the 1750–1200 cm^{-1} region in comparison with the pigment extract, thus reinforcing that in the archaeological samples the organic compounds detected are not from contaminants in the soil.

Further analysis by GC–MS of the organic extract of the reference pigment in the same conditions as for pastes **1** and **2** did not show the presence of carboxylic acids in agreement with the results obtained by FTIR.

Table 1
Fatty acid composition of archaeological pastes **1** and **2**[a]

Fatty acid	Paste **1**	Paste **2**	*P. pennata* fat	*P. pennata* marrow	*L. guanicoe* marrow
C_{14}	1.5	–	Traces	5.2	1.6
C_{15}	1.0	Traces	Traces	1.5	0.3
$C_{16:1}$	4.7	8.7	5.0	6.8	8.8
C_{16}	28.5	29.8	34.3	29.5	26.9
C_{17}	0.53	–	Traces	1.2	0.4
$C_{18:1}$	20.6	16.1	27.0	26.9	29.8
C_{18}	8.3	13.2	5.2	12.2	3.8

[a] Percentage determined by GC. Identification of fatty acids by GC–MS.

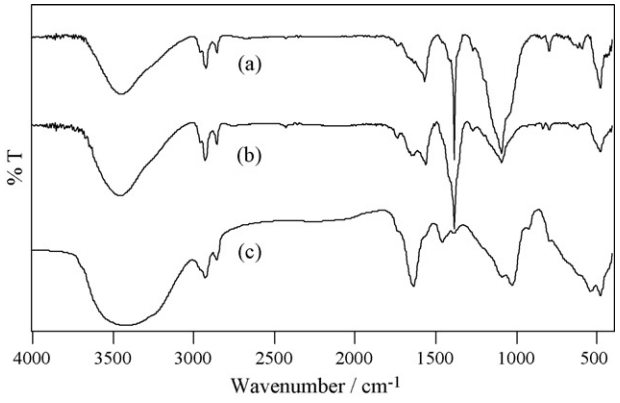

Fig. 4. FTIR spectra (KBr) from paste **1** (a), paste **2** (b) and pigment (c) extracts.

4. Conclusions

Archaeological investigation demands the use of several complementary techniques due to complex nature of the samples, arising from their chemical composition, degradation processes and contamination from the environment.

The combination of vibrational spectroscopy and GC–MS revealed that the pastes analysed in this work used an animal fat as binder, in spite of the negligible amount of organic compounds found by elemental analysis. The components of the residual organic phase are mainly free fatty acids and their salts indicating that the triacylglycerides originally present in the fat underwent hydrolysis. Recently, we have performed artificial ageing tests on a mixture of the reference mineral and the fat from *P. pennata* by heating under atmospheric conditions. The resulting product presented a quite different composition of fatty acids to those of the archaeological pastes, with the formation of short-chain linear dicarboxylic acids [17]. Considering the dry conditions of the region, this is a clear indication that the deterioration mechanisms operative in the accelerated decay assay and in the archaeological samples are not the same. It is well known that degradation of the organic phase in archaeological objects can occur through the environment chemical or microbial action. The data here reported point out to the microbial process as the most important one.

Spectroscopic and chromatographic methods were successfully used to confirm anthropological reports about the use of animal fat and inorganic clays to produce paints in the Argentine Patagonian region.

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