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Original article

Validating chemical and structural changes in painting materials by principal component analysis of spectroscopic data using internal mineral standards

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ABSTRACT

This work shows the capability of principal component analysis (PCA) to detect molecular, chemical and mineralogical changes in historic painting materials subjected to a thermal ageing test (<250 °C). To simulate the heat-induced alterations an ageing accelerated process was performed on two sets of samples containing two mineral phases (hydroxyapatite and quartz) and two organic compounds (collagen and albumin). The chosen minerals behaved as internal standards during the tests since they are stable and chemically inert at the tested temperatures. Raman microscopy (RM) was applied to characterise one set of samples made of bone, containing ca. 70% hydroxyapatite and 30% collagen. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to study the other set of samples made of four different quartz/albumin mixtures with quartz contents of 30%, 50%, 70% and 90% (w/w). The aim was to identify the ideal proportion of internal standard to be validated by ATR-FTIR and PCA, determined to be 70%. PCA analyses detected changes in the molecular structures of the organic components while the internal mineral standard remained stable. Moreover, the internal standard IR/Raman bands were constant during the tests and confirmed that the results of PCA analyses were independent of instrumental and technical factors, as well as sample collecting and handling. This demonstrates the potential benefits of our approach to study historical painting materials, which have suffered any type of heat-induced alteration.

1. Research aims

This work describes a methodology based on the use of internal mineral standards to validate possible molecular changes in two sets of painting samples revealed by PCA. To this end, changes have been studied during an accelerated thermal aging test performed on albumin/quartz (SiO_2) and collagen/chicken bone [composed mainly of hydroxyapatite, HAp, $Ca_{10}(PO_4)_6(OH)_2$ samples]. Vibrational spectrocopic techniques were used to characterise these samples. Subsequently, the spectral data were analysed by PCA. In both cases, quartz and hydroxyapatite were selected as internal standard minerals since they are stable through the thermal tests and their corresponding spectral bands are invariable and non-overlapping with the bands of albumin and collagen. The ultimate goal was to identify the capability of PCA to track the ageing process of the studied samples due to harmful temperatures (<250 °C) such as those caused by exposure to candle burning.

2. Introduction

The study of alteration processes of painting materials in artefacts is challenging due to the complexity of their composition and variety of alteration mechanisms, as well as interactions among them. Historical paintings contain pigments, mainly mineral phases (e.g. azurite, cinnabar, minium), and specific binding media such as calcium carbonate, protein binders or drying oils [1–5]. Painting materials are sensitive to the influence of atmospheric pollution, humidity, temperature and varying environmental conditions associated with climate change or catastrophes [6–14]. In fact, exposure to extreme temperatures caused by fire resulting from candle burning, heating or eruptions of volcanos could involve serious damage, for example, in the inner pictorial decorations of monuments [7,14]. In this regard, other studies describe how organic components are more easily degraded than pigments due to oxidation or denaturation processes [8,9]. Knowing the stability and/or chemical modification of painting materials is critical to designing the right strategy to conserve or restore painting artworks [4–8,13,15]. In any case, to fully characterise organic and mineral painting materials and their alteration products, a combination of advanced analytical techniques is necessary [2,9,15].

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Regarding advanced analytical techniques, Raman and Infrared spectroscopies provide a wealth of information regarding chemical composition and structural features of inorganic and biomolecular compounds [16–18]. However, the complexity of analytical data from painting materials often makes necessary the use of sophisticated statistical and mathematical methods for data analysis. Therefore, there is an increasing interest in applying chemometric tools to spectral data to help characterise Cultural Heritage materials with complex composition [19–24]. Principal component analysis (PCA) is one of the most commonly used of these methods. In fact, PCA greatly facilitates analytical data interpretation and helps to classify or group samples according to similarities in composition and/or molecular conformation, e.g., which are indicative of alteration processes to which these materials have been subjected [25–27].

Nevertheless, spectral data are highly dependent on multiple factors such as instrument settings, sample preparation and handling or acquisition time. Additionally, instrumental miscalibration or measurement errors can substantially modify spectral data, altering peak intensities or shapes, or even the appearance of spurious peaks. Thus, these changes in spectral data can induce the misclassification of samples with similar characteristics in different groups when using PCA methods. This in turn may induce erroneous conclusions about alteration processes or mechanisms to which they have been subjected. Also, sensitive components can be altered during analysis, for example by laser exposition during Raman analysis [22].

In order to detect and correct these systematic and other instrumental errors it is necessary to evaluate data quality by measuring substances with well-known and accurate spectral data [28]. The spectra generated by these standard substances can be compared with the tabulated spectral data to check for any change arising from differences in sample handling, instrument miscalibration, or other problems, which may arise during sample measurement. The standard substances can be a natural component of the studied sample or can be intentionally added. Ideally, standards must be chemically stable in the face of the treatment to which the samples are subjected, and should not react with any sample components, so that their contribution to the spectra is constant throughout the test and measurement period.

Considering the above, this paper focuses on the denaturation (not destruction) temperatures of the proteinaceous binders (i.e. albumin and collagen) present in tempera model samples (i.e., binders are mixed with mineral components, namely quartz and hydroxyapatite) due to thermal alteration. To better track the alteration process of the proteinaceous binders, the selected mineral components (used as mineral standards) should be stable throughout the thermal test and their Raman/FTIR spectral bands invariable and not overlapping the organic bands of the proteins. Thus, under these conditions we were able to observe slight molecular changes in the proteinaceous binders.

3. Materials and methods

3.1. Reagents and samples

To track possible heat-induced transformation/alteration processes in historic painting materials, we have prepared two sets of model painting samples. In one set of samples the standard mineral is a natural component of the material under investigation, which is a natural composite, since fresh chicken bones (femur) composed of hydroxyapatite (70%) and collagen (30%) were chosen. The bones were ground using a cryomill (CertiPrep 6750, Freezer/Mill, SPEX). Powdered burned bones have been used as white or black pigments [29–32]. Collagen extracted from animal skins, bones or fishes has been used as binders in historical paintings and patinas since Ancient Egypt [33,34]. The other set of samples was made by mixing albumin and quartz (SiO_2), so quartz was intentionally added as mineral standard to the sample mixture. For this set of experiments, pure quartz was mixed with albumin (Kremer Pigments GmbH & Co.KG; CI: PB 63250) to prepare four different quartz/albumin mixtures with quartz contents of 30%, 50%, 70% and 90% (w/w). The use of quartz as an extender is very frequent in historical paintings [4,5,29]. In addition, the use of albumin as organic binder is common, for instance, it is present in egg which is a traditional media used in the tempera grassa painting technique [29]. In addition, non-aged (untreated) model samples were used as blanks and analysed for both studies.

3.2. Thermal aging processes

According to Rossi and Schiraldi, denaturation of the main egg proteins takes place within the temperature range 60–100 °C, while the destruction of collagen and the rest of the organic materials present in bones occurs at temperatures between 350 °C and 650 °C [35,36]. Therefore we have analysed aged model samples heated after their collagen denaturation (at T > 100 °C), but below the temperature of protein destruction. As a consequence, the maximum selected temperature for our tests was 250 °C.

The powdered bone sample was divided into four fractions. One fraction was used as a blank (control) sample and the other three were heated to different temperatures (150 °C, 200 °C and 250 °C) using a muffle Thermo Scientific Thermolyne, mod. F4791026 (USA) during 15 minutes. Subsequently, the resultant samples were analysed by RM. Similarly, the quartz/albumin mixtures were divided into four fractions. One fraction was used as control sample and the other three were heated in the same oven to 100 °C, 150 °C and 250 °C. Afterwards, such samples were analysed by ATR-FTIR.

3.3. Raman microscopy (RM)

A Renishaw Invia Raman microscope system fitted with a Peltier-cooled CCD detector and a Leica DMLM microscope was used to analyse the inorganic and organic compounds present in the bone samples. Samples were excited with a 785 nm diode laser over the range of 400–3200 cm^{-1} with an average spectral resolution of approximately 1 cm^{-1}. To improve signal-to-noise ratios, 10 spectra collected consecutively during 15 s each were averaged. Spectra were taken by placing the samples on the microscope stage and focusing on them with a 50 × objective. The video camera attached to the microscope enabled both selection of the sample area to be analysed and laser focus. Precautions were taken not to cause any damage to the samples (i.e. laser-induced degradation of painting materials). This was done by reducing the laser intensity and visually confirming the absence of damage in the sampling area with the help of the camera. Consequently, 50% laser power (150 mW) was employed. For statistical analysis, each sample treated at different temperatures was consecutively analysed 10 times from the same location on the model sample in order to avoid spatial variation.

3.4. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

A JASCOFTIR6200 spectrometer (JASCO, Tokyo, Japan) equipped with an ATR diamond crystal plate (MIRacleTM ATR accessory, PIKE Technology) was used to analyse the quartz/albumin mixtures samples treated at different temperatures. For each measurement, 200 scans were made with a resolution of 2 cm^{-1} in the region of 650 cm^{-1} to 4000 cm^{-1}. Smoothing and baseline fitting were performed using the JASCO software Spectra Manager v.2. As with the RM analysis, every sample was characterised by 10 spectra obtained

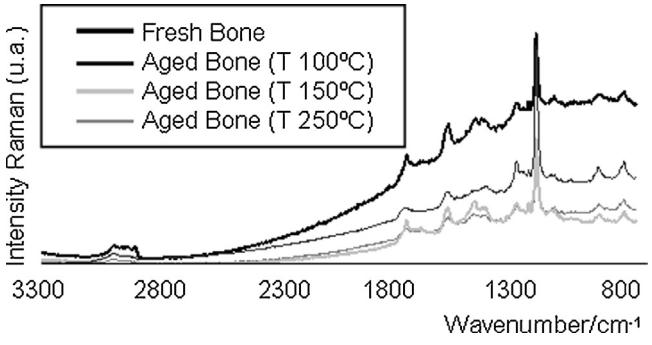

Fig. 1. Thermal ageing of bone samples. Raman microscopy spectra of fresh and aged bone samples at 100 °C, 150 °C and 250 °C from 400 to 3200 cm^{-1}.

from the same location on the model sample to avoid spatial variation.

3.5. Statistical analyses

The spectra acquired from the studied samples subjected to different thermal treatments were combined on an Excel spreadsheet and the resulting data matrices were used as input data for the PCA analyses. PCA analyses were performed using the Statistical Product and Service Solutions program (SPSS, for Windows v.15, USA). Specifically, in the case of the quartz/albumin mixtures, four different data matrices were prepared containing spectra data from each mixture prepared at different ratios, which included 10 spectra of the quartz/albumin samples at each temperature studied (fresh and aged samples). In this way, each matrix was initially formed by 40 spectra. The principal components (PCs) were obtained using both the covariance data matrices (scaling by mean-centered data) and the correlation data matrices (scaling by unit variance). PCA results from correlation data matrices were better, as showed in previous works [21,22]. Thus, the results shown and discussed here correspond to autoscaled data. In this work only regions containing relevant bands were selected to apply PCA. Similarly, for the bone samples a correlation data matrix was prepared containing the information from 40 spectra.

4. Results and discussion

Fig. 1 shows the Raman spectra of the bone samples treated at different temperatures in the region between 400 and 3200 cm^{-1} where thermal degradation is evident. The PCA analysis of the spectral data focused on the region from 650 to 1730 cm^{-1} which included the main characteristic Raman bands of the principal chemical component of bones, i.e. the phosphate band of the HAp mineral and the amide or C-H groups of proteins and lipids [37]. The two first principal components explained 99.97% of the total variance of the data (Fig. 2). Scores for PC1 and PC2 showed that data from bone samples treated at the same temperature were grouped in the same cluster, and the clusters of samples treated at different temperatures separated well. In particular, PC1 (accounting for 94.6% of total variance) distinguishes fresh bone samples with positive scores from aged bone samples heated at 100 °C, 150 °C and 250 °C, which showed negative scores (Fig. 2a). On the other hand, the information contained in PC2 (accounting for 5.1% of total variance) was associated with the thermal degradation of samples up to 250 °C (with negative scores), separating aged samples from the rest of the bone samples studied, which showed positive scores (Fig. 2a).

In addition, the analysis of the corresponding values of loadings plots of PC1 and PC2 revealed strongly negative values for the wave numbers associated with the main phosphate band of HAp (around

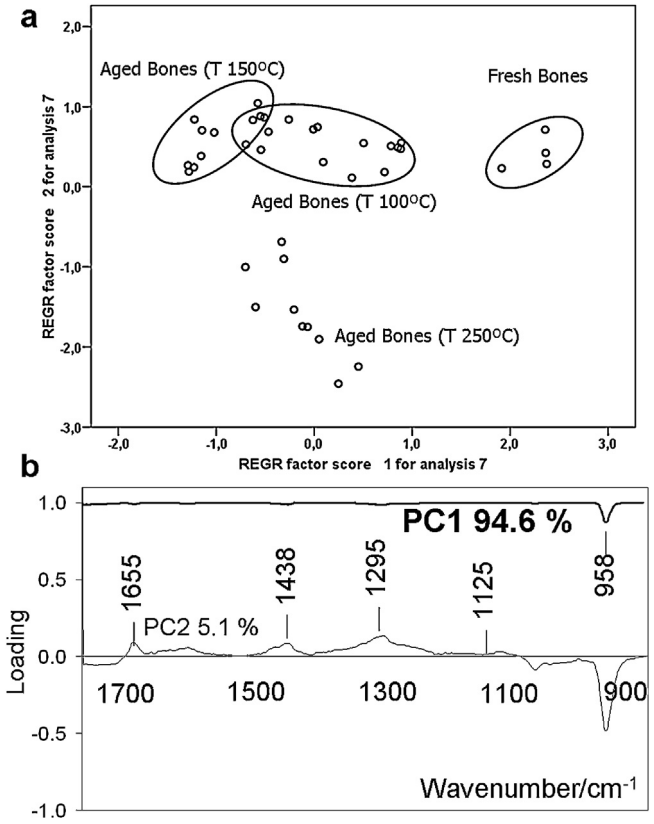

Fig. 2. Principal component analysis (PCA) in the Raman microscopy region between 650 and 1730 cm^{-1}; a: Scores plots; b: loading plots for PC1 and PC2.

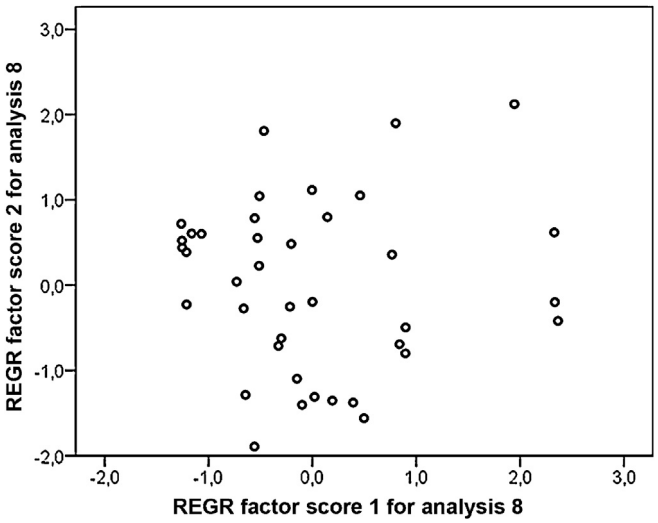

Fig. 3. Score plot of the two principal components for the hydroxyapatite Raman region between 833 and 912 cm^{-1}, PC1 and PC2 of bones samples. Figure includes ageing at different temperature (Fresh; 150 °C; 200 °C and 250 °C).

958 cm^{-1}; Fig. 2b). This result indicates that the spectral bands of the HAp mineral remain unchanged during the heating test. This is further confirmed with the PCA for the Raman region between 833 and 912 cm^{-1}, where the main Raman band of phosphate groups appears (958 cm^{-1}). The first PC, accounting for 99.97% of total variance, did not discriminate samples related to the thermal ageing. A unique cluster was identified for fresh and aged bones samples (Fig. 3). This result was expected since HAp is stable over the temperature range selected in this work, and more importantly, it

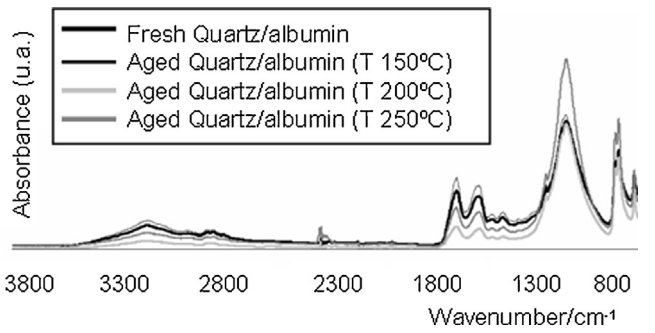

Fig. 4. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of 70% quartz/albumin sample from 650 to 4000 cm^{-1} fresh and treated at 150 °C, 200 °C and 250 °C.

confirms the quality of the results of the PCA analyses and that the data acquired are correct. By contrast, bands associated with proteins and lipids showed positive weight of the loadings plots of PC2 (Fig. 2b), indicating that these components are strongly affected by the thermal test. In particular, the PC2 loading plot clearly showed that these bands have an important contribution and are more strongly affected in samples heated to 250 °C. In this case, the most notable changes – to the amide I band at about 1640–1670 cm^{-1}, i.e. ν C=O (α helix) and for ν ($C=C_{trans}$) at 1125 cm^{-1} of phospholipids and proteins – could be indicative of a conformational change of protein secondary structure [38] (Fig. 2). The bands at 1295 cm^{-1} and 1438 cm^{-1} associated with lipids were also notably affected by the test temperatures in agreement with results from other authors [37,38].

In the case of the quartz/albumin mixtures (quartz contents of 30%, 50%, 70% and 90%), the spectral region from 600 to 1770 cm^{-1} containing the characteristic bands of quartz (779, 796, 1060 and 1089 cm^{-1}) and the albumin (1156, 1440, 1524 and 1660 cm^{-1}) were analysed by PCA [38,39]. In this work, the optimum amount of quartz contained in the composite samples has to be established to determine the minimum detectable quartz signal without interfering with the albumin bands. Four PCA analyses were carried out, one for each quartz/albumin mixture. Table 1 summarises the explained variance for the PCA analyses on each group of samples. The best results were clearly obtained when the proportion of quartz in the quartz/albumin mixture samples was 70% (Fig. 4).

The PCA results for samples containing 30% and 50% of quartz showed a greater variability in their weights (Figs. 5 and 6). In these multivariate analyses, the first calculated PC consistently accounted for more than 70% of the variance present in the spectra (Table 1), specifically 73.3% in the case of the 30% quartz/albumin samples, 77.4% in the case of the 50% quartz/albumin samples, and 95.1% in the case of the 70% quartz/albumin samples. This indicates mainly one source of variability in the spectra being observed in 70% albumin/quartz, which was related to the presence of quartz.

In particular, score plots for 30% quartz/albumin samples showed separation of samples in groups according to the treatment temperature, i.e. four groups were clearly discriminated when the first two PCs were plotted against each other (Fig. 5a). These two first PCs account for 96.9% of the total variance (Table 1). The loading plot for PC1 showed a high weight for the whole band between 1200 and 1700 cm^{-1} where some characteristic Raman bands of albumin appear. Additionally, the corresponding plots of PC2 loadings showed the highest weight at 1690 cm^{-1} revealing a shift in the position of the amide I band from 1636 to 1690 cm^{-1} (Fig. 5b). This result indicates an alteration of the conformational structure of the albumin due to its thermal degradation, in agreement with Furlan et al. [38].

On the contrary, only three clusters were identified for 50% quartz/albumin mixture samples when plotting PC1 against PC2

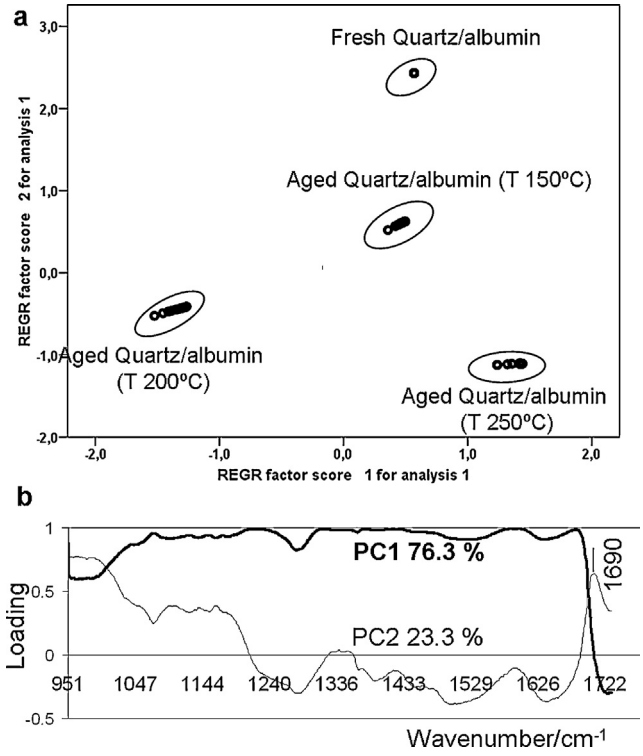

Fig. 5. Principal component analysis (PCA) of the thermal ageing of the 30% quartz/albumin sample in the attenuated total reflectance-Fourier transform infrared (ATR-FTIR) region between 760 and 1700 cm^{-1}: a: Scores plots; b: loading plots for PC1 and PC2. Figure includes ageing at different temperature (Fresh; 150 °C; 200 °C and 250 °C).

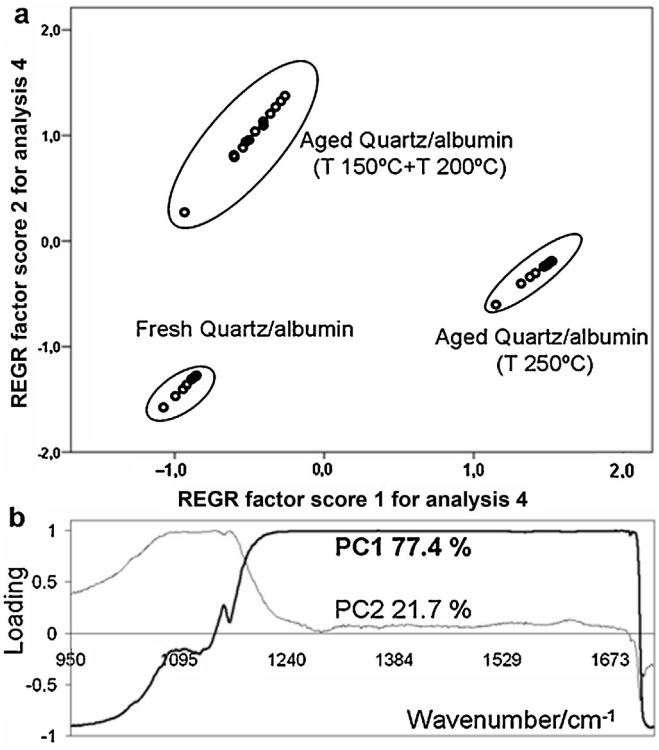

Fig. 6. Principal component analysis (PCA) of the thermal ageing of the 50% quartz/albumin sample in the attenuated total reflectance-Fourier transform infrared (ATR-FTIR) region between 950 and 1730 cm^{-1}: a: Scores plots; b: loading plots for PC1 and PC2. Figure includes ageing at different temperature (Fresh; 150 °C; 200 °C and 250 °C).

Table 1
Principal component analysis (PCA) results in the quartz/albumin mixture samples.

	ATR-FTIR region (cm^{-1})	PC	Variance explained (%)	Accumulated variance explained (%)
30% quartz/albumin	760–1700	PC1	73.6	73.6
		PC2	23.3	96.9
50% quartz/albumin	950–1730	PC1	77.4	77.4
		PC2	21.7	99.1
70% quartz/albumin	650–1730	PC1	95.1	95.1
		PC2	3.7	98.8

ATR-FTIR: attenuated total reflectance-Fourier transform infrared.

(these two PCs accounted for 99.1% of the total variance). Specifically, the untreated (fresh) samples had negative scores for PC1; while the samples treated to 150 °C and 200 °C were grouped in one cluster around zero scores for both PCs (Fig. 6a). Another group with positive score for PC1 was identified, which included data from samples heated to 250 °C. In these samples, the corresponding loading plot was less informative than for the 30% mixture samples. A broad region of high weight resulted and no relevant information was obtained (Fig. 6b).

In the case of the quartz/albumin mixtures containing 70% quartz, the score plot of PC1 (95.1%) versus PC2 (3.7%) accounted for 98.8% of the total variance, and the samples clustered well and separated according to temperature, i.e. four groups were identified in the first two component projection (Fig. 7a). The information contained in the loading plot of this PC when plotted versus the wavenumber values analysed showed that the values with the lowest weight were those at 779 cm^{-1}, 1060 cm^{-1} and 1089 cm^{-1}, which correspond to the characteristic bands of quartz (Fig. 7b). This is due to the fact that quartz is stable over the temperature range of the test and thus its spectral bands are unaffected by the thermal treatments, as expected. On the contrary, PC2 added no relevant information to the first PC, accounting for less than 5% of the variance. On the other hand, in the case of the 90% quartz/albumin mixture sample, the PCA results were not interpretable since the quartz bands masked the characteristic spectral bands of albumin (not shown). Thus, in this later case, the excessive amount of quartz in the mixture prevented the gathering of high quality data for the albumin, and it was not possible to distinguish how albumin was altered by the thermal test.

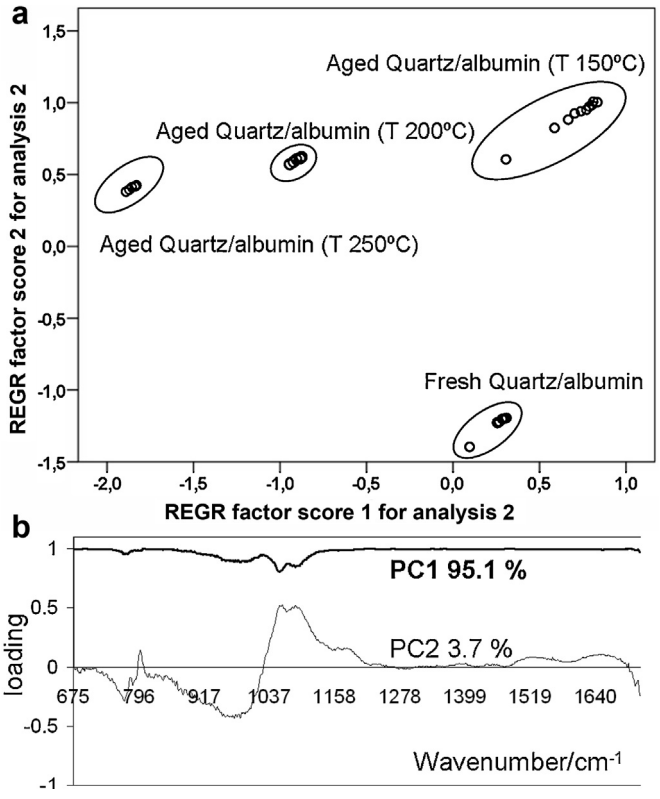

Fig. 7. Principal component analysis (PCA) of the thermal ageing of the 70% quartz/albumin samples in the attenuated total reflectance-Fourier transform infrared (ATR-FTIR) region between 650 and 1730 cm^{-1}: a: Scores plots; b: loading plots for PC1 and PC2. Figure includes ageing at different temperature (Fresh; 150; 200 and 250 °C).

5. Conclusions

This work has demonstrated the capability of PCA to distinguish slight changes in the molecular structures of the organic components present in the complex samples containing both mineral phases and organic compounds, due to an accelerated thermal aging test. The quality of the data and the validity of the PCA results were assessed by the use of quartz or hydroxyapatite as internal mineral standards in two different composite samples (i.e. artificial and natural) containing either albumin or collagen. These minerals remained unaltered in the studied samples during the thermal tests, contributing constantly to the spectra. In addition, this validation method made possible identification of the suitable proportion of internal standard to be added to a sample, in such a way that it can be used to validate results of PCA analyses. Basically, the amount of the mineral used for internal standard has to be enough to produce a relatively high intensity peak so that it can contribute significantly to the spectra. For instance, in this study the concentration of the mineral used as internal standard should be around 70% (by weight) in the mixture (inorganic-organic) sample to obtain a good spectral signal from both types of compounds.

Regarding the spectrometric techniques used to characterise the composite samples, both ATR-FTIR and RM showed similar capability to validate the quality of the PCA results. However, RM provided clearer spectral bands and thus better discrimination of samples by PCA analyses, and this evaluation was not influenced by any instrumental or technical factor.

Based on the results of this study, we propose the following recommendations in order to validate results of spectral data analysed by PCA obtained from complex samples. The substance used as an internal standard should be stable and chemically inert for the test conditions, i.e. stable against temperature or other parameters or agents such as acids or UV radiation. Regarding thermal aging processes, we propose the use of mineral phases such as quartz, apatite or hydroxyapatite, which are stable over a large temperature range. The material of choice to be used as a standard also should ideally have a simple spectrum, with a single or few characteristic spectral bands, which in addition should not mask the key bands of the organic or inorganic compounds present in the analysed sample.

The validation methodology of PCA described here, based on the use of a stable mineral standard, might be also valid for many mineralogical studies, such as archaeological investigations involving transformation or reaction processes of minerals. For instance, it can be applied to better understand the heat-induced alteration of

mineral pigments (clay minerals, realgar, minium, etc.) commonly found in wall paintings, as well as archaeological metals or potteries. Additionally, it can be useful to determine the compatibility or reaction of materials introduced during restoration interventions, with the original materials.

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References

[1] P. Vandenabeele, B. Wehling, L.H. Moens, M. Edwards, G. De Reu, Van Hooydonk, Analysis with micro-Raman spectroscopy of natural organic binding media and varnishes used in art, Anal. Chim. Acta 407 (2000) 261–274.
[2] G. Burrafato, M. Calabrese, A. Cosentino, A.M. Gueli, S.O. Troja, A. Zuccarello, ColoRaman project: Raman and fluorescence spectroscopy of oil, tempera and fresco paint pigments, J. Raman Spectrosc. 35 (2004) 879–886.
[3] D. Hradil, T. Grygar, J. Hradilová, P. Bezdička, Clay and iron oxide pigments in the history of painting, Appl. Clay Sci. 22 (2003) 223–236.
[4] C. Cardell-Fernández, C. Navarrete-Aguilera, Pigment and plasterwork analyses of nasrid polychromed lacework stucco in the Alhambra (Granada, Spain), Stud. Conserv. 51 (2006) 161–176.
[5] C. Cardell, L. Rodríguez-Simón, I. Guerra, A. Sánchez-Navas, Analysis of nasrid polychrome carpentry at the hall of the Mexuar palace, Alhambra complex (Granada, Spain), combining microscopic, chromatographic and spectroscopic methods, Archaeometry 51 (2009) 637–657.
[6] F. Becherini, A. Bernardi, E. Frassoldati, Microclimate inside a semi-confined environment: valuation of suitability for the conservation of heritage materials, J. Cult. Herit. 11 (2010) 471–476.
[7] D. Ponziani, E. Ferrero, L. Appolonia, S. Migliorini, Effects of temperature and humidity excursions and wind exposure on the arch of Augustus in Aosta, J. Cult. Herit. (2012), In Press.
[8] Ł. Bratasz, I. Harris, Ł. Lasyk, M. Łukomski, R. Kozłowski, Future climate-induced pressures on painted wood, J. Cult. Herit. 13 (2012) 365–370.
[9] Z.E. Papliaka, K.S. Andrikopoulos, E.A. Varella, Study of the stability of a series of synthetic colorants applied with styrene-acrylic copolymer, widely used in contemporary paintings, concerning the effects of accelerated ageing, J. Cult. Herit. 11 (2010) 381–391.
[10] E. Mattei, G. de Vivo, A. de Santis, C. Gaetani, C. Pelosi, U. Santamaria, Raman spectroscopic analysis of azurite blackening, J. Raman Spectrosc. 39 (2008) 302–306.
[11] E. Manzano, J. Romero-Pastor, N. Navas, L.R. Rodríguez-Simón, C. Cardell, A study of the interaction between rabbit glue binder and blue copper pigment under UV radiation: a spectroscopic and PCA approach, Vib. Spectrosc. 53 (2010) 260–268.
[12] M. Aceto, A. Agostino, E. Boccaleri, F. Crivello, A.C. Garlanda, Evidence for the degradation of an alloy pigment on an ancient Italian manuscript, J. Raman Spectrosc. 37 (2006) 1160–1170.
[13] M. Cotte, J. Susini, N. Metrich, A. Moscato, C. Gratziu, A. Bertagnini, M. Pagano, Blackening of Pompeian cinnabar paintings: X-ray microspectroscopy analysis, Anal. Chem. 78 (2006) 7484–7492.
[14] S. Rickerby, Heat alterations to pigments painted in the fresco technique, The Conservator 15 (1991) 39–44.
[15] L. Monico, G. van der Snickt, K. Janssens, W. de Nolf, C. Miliani, J. Dik, M. Radepont, E. Hendriks, M. Geldof, M. Cotte, Degradation process of lead chromate in paintings by Vincent van Gogh Studied by means of synchrotron X-ray spectromicroscopy and related methods, 2. Original paint layer samples, Anal. Chem. 83 (2011) 1224–1231.
[16] A. Nevin, J.L. Melia, I. Osticioli, G. Gautier, M.P. Colombini, The identification of copper oxalates in a 16th century Cypriot exterior wall painting using micro FTIR, micro-Raman spectroscopy and gas chromatography-mass spectrometry, J. Cult. Herit. 9 (2008) 154–161.
[17] K. Možina, K. Možina, S. Bračko, Non-invasive methods for characterisation of printed cultural heritage, J. Cult. Herit. (2012), In Press.
[18] C. Cardell, I. Guerra, J. Romero-Pastor, G. Cultrone, A. Rodríguez-Navarro, Innovative analytical methodology combining micro-X-ray diffraction, scanning electron microscopy-based mineral maps, and diffuse reflectance infrared fourier transform spectroscopy to characterize archeological artifacts, Anal. Chem. 81 (2009) 604–611.
[19] G.E. De Benedetto, B. Fabbri, S. Gualtieri, L. Sabbatini, P.G. Zambonin, FTIR-chemometric tools as aids for data reduction and classification of pre-Roman ceramics, J. Cult. Herit. 6 (2005) 205–211.
[20] J.J. Łucejko, F. Modugno, M.P. Colombini, M. Zborowska, Archaeological wood from the Wieliczka salt mine museum, Poland – Chemical analysis of wood degradation by Py (HMDS) – GC/MS, J. Cult. Herit. 13 (2012) S50–S65.
[21] N. Navas, J. Romero-Pastor, E. Manzano, C. Cardell, Benefits of applying combined diffuse reflectance FTIR spectroscopy and principal component analysis for the study of blue tempera historical painting, Anal. Chim. Acta 630 (2008) 141–149.
[22] N. Navas, J. Romero-Pastor, E. Manzano, C. Cardell, Raman spectroscopic discrimination of pigments and tempera paintmodel samples by principal component analysis on first-derivative spectra, J. Raman Spectros. 41 (2010) 1196–1203.
[23] F. Colao, R. Fantoni, P. Ortiz, M.A. Vázquez, J.M. Martín, R. Ortiz, N. Idris, Quarry identification of historical building materials by means of laser induced breakdown spectroscopy, X-ray fluorescence and chemometric analysis, Spectrochim. Acta B 65 (2010) 688–694.
[24] F. Rosi, C. Miliani, C. Clementi, K. Kahrim, F. Presciutti, M. Vagnini, V. Manuali, A. Daveri, L. Cartechini, B.G. Brunetti, A. Sgamellotti, An integrated spectroscopic approach for the non-invasive study of modern art materials and techniques, Appl. Phys. A 100 (2010) 613–624.
[25] H. Wilcken, H.R. Schulten, Quality control of paints: pyrolysis-mass spectrosmetry and chemometric, Anal. Chim. Acta 336 (1996) 201–208.
[26] M. Dyrby, D. Baunsgaard, R. Bro, S.B. Engelsen, Multiway chemometric analysis of the metabolic response to toxins monitored by NMR, Chemometr. Intel. l Lab. 76 (2005) 79–89.
[27] F.M. Verbi-Pereira, M.I.M. Silveira-Bueno, Evaluation of varnish and paint films using digital image processing, energy dispersive X-ray fluorescence spectrometry and chemometric tools, J. Coat. Technol. Res. 6 (2009) 445–455.
[28] P.M. Ramos, I. Ruisánchez, Noise and background removal in Raman spectra of ancient pigments using wavelet transform, J. Raman Spectrosc. 36 (2005) 848–856.
[29] R. Mayer, Materiales y Técnicas del Arte, Ed. Hermann Blume, Madrid, 1988.
[30] N. Eastaugh, V. Walsh, T. Chaplin, R. Siddall, Pigment compendium, a dictionary of historical pigments, Ed. Elsevier Butterworth-Heinemann, Oxford, 2004.
[31] A. Van Loon, J.J. Boon, Characterization of the deterioration of bone black in the 17th century Oranjezaal paintings using electron-microscopic and microspectroscopic imaging techniques, Spectrochim. Acta B 59 (2004) 1601–1609.
[32] A.M. Correia, R.J.H. Clark, M.I.M. Ribeiro, M.L.T.S. Duarte, Pigment study by Raman microscopy of 23 paintings by the Portuguese artist Henrique Pousao (1859–1884), J. Raman Spectrosc. 38 (2007) 1390–1405.
[33] G. Chiavari, D. Fabbri, G.C. Galletti, R. Mazzeo, Use of analytical pyrolysis to characterize Egyptian painting layers, Chromatographia 40 (1995) 594–600.
[34] J. Martín-Gil, F.J. Martín-Gil, M.C. Ramos-Sánchez, P. Martín-Ramos, The orange-brown patina of Salisbury Cathedral (West Porch) SURFACES: evidence of its man-made origin, Environ. Sci. Pollut. R. 12 (2005) 285–289.
[35] M. Rossi, A. Schiraldi, Thermal denaturation and aggregation of egg proteins, Thermochim. Acta 199 (1992) 115–123.
[36] C.M.A. Pijoan, J. Mansilla, I. Leboreiro, V.H. Lara, P. Bosch, Thermal alterations in archaeological bones, Archaeometry 49 (2007) 713–727.
[37] S. Le Blond, E. Guilminot, G. Lemoine, N. Huet, J.Y. Mevellec, FT-Raman spectroscopy: a positive means of evaluating the impact of whale bone preservation treatment, Vib. Spectrosc. 51 (2009) 156–161.
[38] P.Y. Furlan, S.A. Scott, M.H. Peaslee, FTIR-ATR study of pH effects on egg albumin secondary structure, Spectrosc. Lett. 40 (2007) 475–482.
[39] F. Bosch-Reig, J.V. Gimeno-Adelantado, M.C.M. Moya-Moreno, FTIR quantitative analysis of calcium carbonate (calcite) and silica (quartz) mixtures using the constant ratio method, Appl. Geol. Samples, Talanta 58 (2002) 811–821.