Microchemical Journal 124 (2015) 31–35

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Fourier transform infrared spectroscopic study of rabbit glue/inorganic pigments mixtures in fresh and aged reference paint reconstructions

Davide Pellegrini [a], Celia Duce [b], Ilaria Bonaduce [b], Simona Biagi [a], Lisa Ghezzi [b], Maria Perla Colombini [b], Maria Rosaria Tinè [b], Emilia Bramanti [a,*]

[a] National Research Council of Italy, C.N.R., Istituto di Chimica dei Composti Organo Metallici-ICCOM-UOS Pisa, Area di Ricerca, Via G. Moruzzi 1, 56124 Pisa, Italy
[b] Dipartimento di Chimica e Chimica Industriale, Via Risorgimento 35, 56127 Pisa, Italy

ARTICLE INFO

Article history:
Received 23 July 2015
Accepted 26 July 2015
Available online 31 July 2015

Keywords:
Collagen
Inorganic pigments
Fourier transform infrared spectroscopy (FTIR)

ABSTRACT

We studied the interactions of rabbit glue, a collagen-based proteinaceous binder, with azurite ($Cu_3(CO_3)_2(OH)_2$), calcium carbonate ($CaCO_3$), hematite ($Fe_2O_3 \cdot nH_2O$), red lead (Pb_3O_4) and cinnabar (HgS) by Fourier transform infrared spectroscopy (FT-IR). The research was carried out on a set of paint reconstructions, which were analysed before and after artificial light ageing. A deconvolution of the amide I FT-IR absorption peak was performed with a written-in-house *LabVIEW* program to study the secondary structure of the glue.
The changes in the glue conformation highlighted that all the inorganic pigments interact with the proteinaceous binder. The conformational changes were correlated with a loss of stability of the collagen structure, especially after ageing, likely due to the interlayer coordination of metals salts and oxide with protein functional groups. These results were correlated with the lower thermal stability of the glue/pigment mixtures with respect to the pure glue, evidenced by thermogravimetry (TG) and differential scanning calorimetry (DSC) analyses performed in a previous step of this work.

1. Introduction

Fourier-transform infrared spectroscopy (FT-IR) is a well-established technique to characterize organic and inorganic painting materials present in artworks [1], being non destructive, fast and low cost technique, and requiring minimum sample preparation [2]. Moreover, the use of different FT-IR configurations (e.g. ATR, DRIFT, PAS spectroscopies, FT-IR microspectroscopy, etc.) ensures high versatility in investigating both organic and inorganic materials in Cultural Heritage, for several purposes, such as diagnostic [1–6] and localization of materials in paint cross sections [7–12]. Infrared spectroscopy is also a fundamental tool to investigate the degradation and ageing of organic materials in Cultural Heritage. Among these, proteinaceous paint binders are particularly complex, given their composition and structure [1,13–15].

FT-IR can be used to study proteins to gain molecular and conformational information. The spectral data are, indeed, interpreted in terms of the vibrations of the structural amide bond repeated unit, which give rise to nine characteristic IR absorption bands, namely amide A, B and I–VII [16–19]. Among these, amide I and amide II bands are the two most prominent vibrational bands of the protein backbone, with amide I (1700–1600 cm^{-1}) being the most sensitive one in terms of conformational information [20]. The amide I band is due almost entirely to the C═O stretching vibrations of the peptide bonds and the frequencies arising from each component of this absorption are found to be closely correlated to the protein secondary structure [21]. The peak fitting procedure applied to the amide I absorption band provides, indeed, information about the various secondary motifs (*e.g.* helix, β-sheets, turns, random coils, etc.) and the contribution of each component to the secondary structure of the protein [22–25]. This approach was used to study proteinaceous paint binders, in order to investigate the interaction occurring between selected pigments and ovalbumin and casein and their changes with ageing [26].

Animal glues are the other commonly used proteinaceous binders in paintings. Animal glues are obtained by extraction and partial hydrolysis of collagen from animal hides and bones. Collagen in its natural state is water insoluble, while animal glues are soluble in hot water [27], although the solubility of animal glue decreases with ageing [28,29].

In this work we study the interaction of pigments with animal glue. In particular we present the results of the study of the interaction of five pigments (azurite ($Cu_3(CO_3)_2(OH)_2$), calcium carbonate ($CaCO_3$), hematite ($Fe_2O_3 \cdot nH_2O$), red lead (Pb_3O_4) and cinnabar (HgS)) with rabbit glue binder and their chemical modification with ageing at molecular level by FT-IR spectroscopy. Analyses were carried out on model painting samples before and after artificial ageing under indoor light ageing. The results are discussed in terms of protein conformation changes due to the interaction with pigments and ageing. This study completes the previous investigation of proteinaceous binders and pigments [27,30].

* Corresponding author at: National Research Council of Italy, C.N.R., Istituto di Chimica dei Composti Organo Metallici-ICCOM-UOS Pisa, Italy.
E-mail address: bramanti@pi.iccom.cnr.it (E. Bramanti).

http://dx.doi.org/10.1016/j.microc.2015.07.018
0026-265X/

2. Experimental section

2.1. Chemicals and samples

Paint reconstructions were prepared using rabbit glue (53921) purchased from Bresciani srl (Milan, Italy) in mixtures or alone, with azurite ($Cu_3(CO_3)_2(OH)_2$), calcium carbonate ($CaCO_3$), hematite (Fe_2O_3), red lead (Pb_3O_4) and cinnabar (HgS). The glue was dissolved in water and heated in a bain-marie until a clear/fluid solution was obtained. The pigment was mixed with the fluid binder in proportions that produced a paintable paste. The paint was then applied with a brush on glass slides for the microscope. A set made up of each typology of pigment/protein replica was analyzed before and after artificial indoor ageing in the Solarbox (see Apparatus and methods) and then stored at room temperature in the laboratory.

2.2. Equipments

2.2.1. FT-IR spectroscopy

Infrared spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrophotometer, equipped with a universal attenuated total reflectance (ATR) accessory and a triglycine sulfate TGS detector. After recording the background spectrum, for each sample 128 interferograms were recorded, averaged and Fourier-transformed to produce a spectrum with a nominal resolution of 4 cm^{-1}. The spectra were run and processed by means of Perkin-Elmer spectrum software and a written-in house LabVIEW program for peak fitting, respectively. Analyses were performed on the model samples before and after the artificial ageing.

Prior to curve processing, a straight baseline passing through the ordinates at 1800 and 1480 cm^{-1} was subtracted and spectra were normalized in the 1700–1600 cm^{-1} region. This approach was taken in order to avoid artefacts in absorptions near the limits of the region examined (1700–1600 cm^{-1}). Then, the second derivatives of the amide I band of the spectra examined (1700–1600 cm^{-1} region) were analysed in order to determine the starting data (number and position of Gaussian components) required for the deconvolution procedure. The choice of the amide I band for structural analysis is due to the very low contribution of the amino acid side chain absorptions present in this region [31], and to its higher intensity with respect to other amide modes. On the basis of the infrared assignment of amide components, assuming that the extinction coefficient is the same for all the secondary structures, the secondary structure composition can be obtained from the FTIR spectra. The values for the percentages of the different secondary structures were estimated by expressing the amplitude value of the bands assigned to each of these structures as a fraction of the total sum of the amplitudes of the amide I components. While the general validity of the assumption above made about the extinction coefficients remains to be tested, the good correlation found between the secondary structure results obtained by FTIR approaches and x-ray crystallography indicated that this is a reasonable assumption [32].

2.2.2. Solarbox

The Solarbox (1500e RH), purchased from Erichsen (Germany), was used for artificially ageing the paint replicas. The exposure conditions were 720 h at 25 °C, 50% relative humidity (RH) and irradiance 550 W/m^2. A soda-lime glass UV filter was used to simulate indoor exposure. Irradiation uniformity was guaranteed by a parabolic reflector chamber with the xenon lamp in the focus.

3. Results and discussion

3.1. FT-IR amide I peak fitting

Fig. 1 shows the comparison of the FT-IR spectra of glue, aged glue, glue/pigment and aged glue/pigment paint replicas in the 1750–850 cm^{-1} region.

A careful investigation of specific absorption bands is fundamental to obtain useful information about the interaction of pigments with collagen hydrolysate. The strong absorption bands in the range of 1628–1636 and 1528–1539 cm^{-1} were attributed to amide I (C═O stretching) and amide II (CN stretching and NH bending) of the glue. Absorption bands in the fingerprint region from 1450 to 1000 cm^{-1} were attributed to CH_2 wagging, CH_3 deformation, C—N stretching and C—OH stretching of the proteinaceous binder [33].

The FT-IR spectra of glue and aged glue paint replicas do not show significant differences, suggesting that the pure glue is stable during ageing. The addition of pigments to the pure glue induces a modification of the absorption bands shapes in the fingerprint region. Particularly, the FT-IR spectra of glue/$CaCO_3$ exhibit the characteristic absorption peak at 870 cm^{-1} (C—O stretching of CO_3^{2-}) and the other absorption peak features of carbonate in the fingerprint region (1500–1400 cm^{-1}), which cover all protein characteristic absorptions except for the amide I band [26,34].

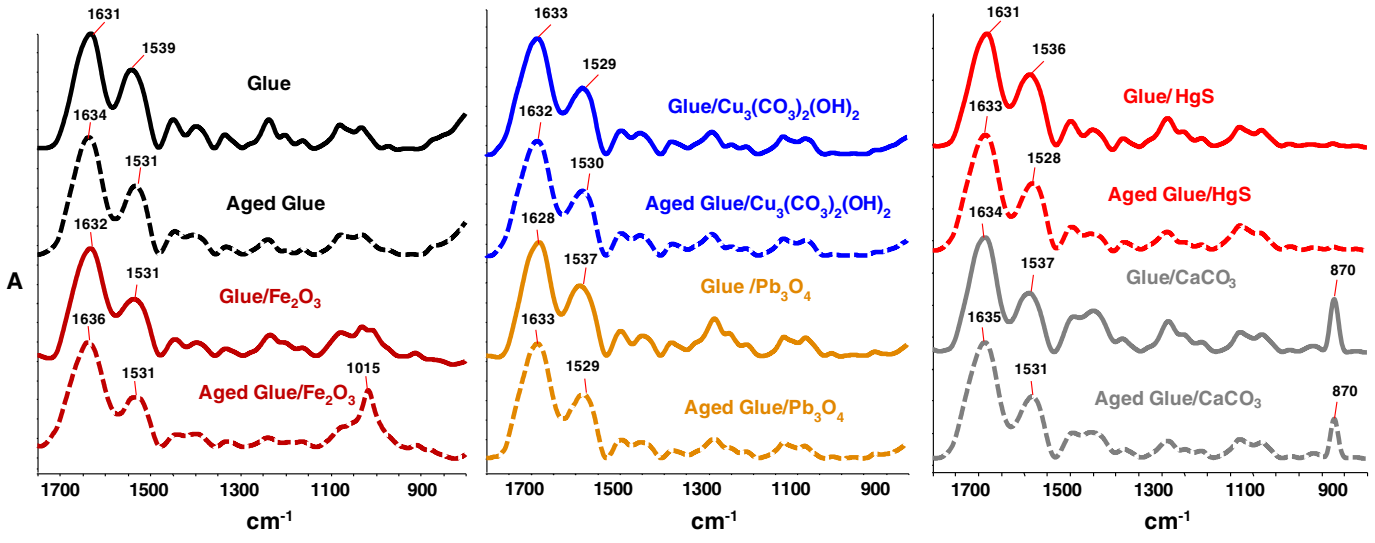

Fig. 1. Comparison of the FT-IR spectra of glue, aged glue, glue/pigment and aged glue/pigment paint replicas in the 1750–850 cm^{-1} region.