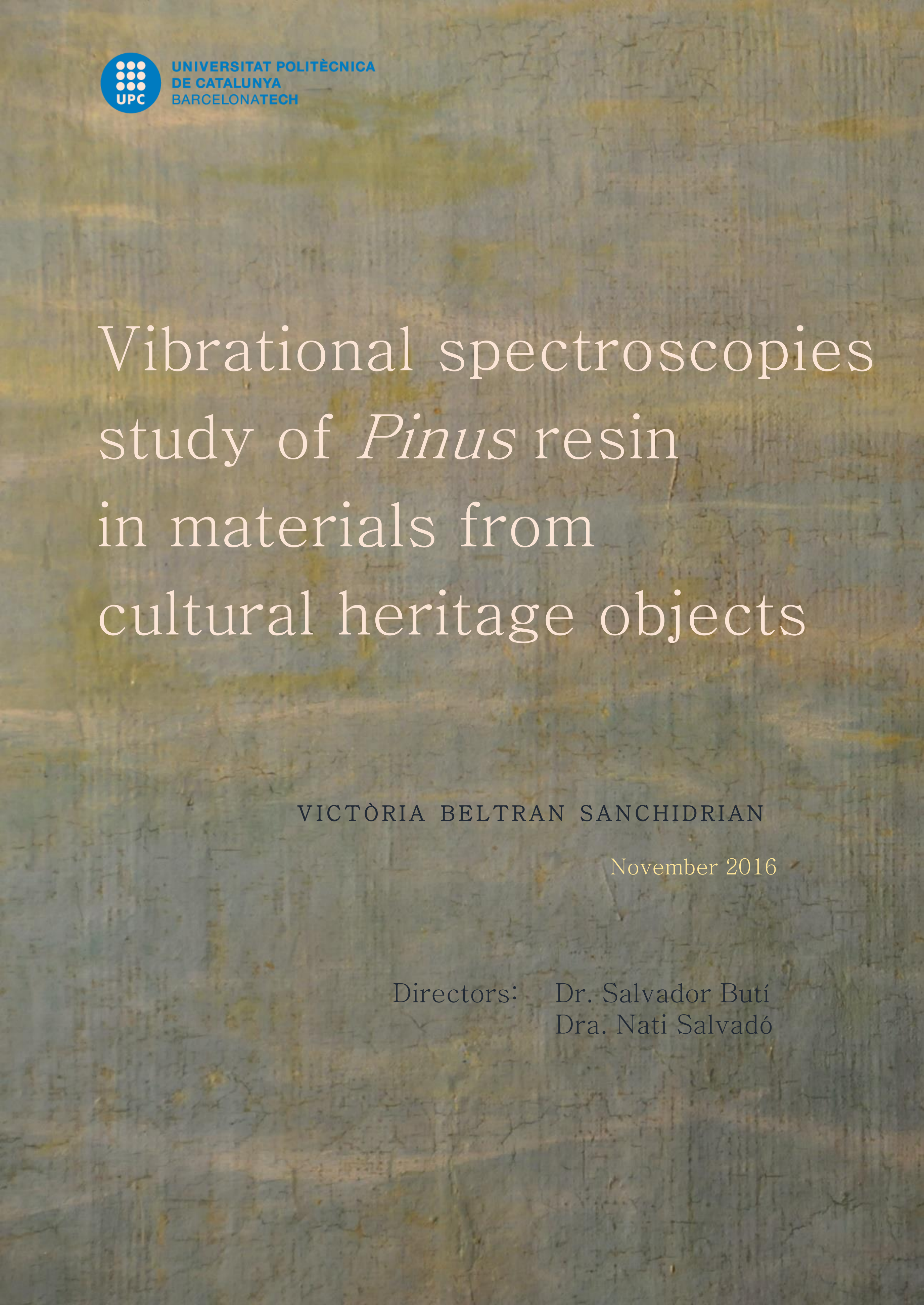

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# Vibrational spectroscopies study of *Pinus* resin in materials from cultural heritage objects

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November 2016

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# Vibrational spectroscopies study of *Pinus* resin in materials from cultural heritage objects

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A Thesis presented for the degree of
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This work has been carried out in the research group AMPC Anàlisi de Materials de Patrimoni Cultural, from the Department of Chemical Engineering of the UPC Universitat Politècnica de Catalunya BarcelonaTech. The research group is located in EPSEVG Escola Politècnica Superior d'Enginyeria de Vilanova i la Geltrú.

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# ABBREVIATIONS

| | |
|---|---|
| **BSE** | Backscattered Electrons |
| **EDX Spectroscopy** | Energy Dispersive X-ray Spectroscopy |
| **FTIR Spectroscopy** | Fourier Transform Infrared Spectroscopy |
| **μFTIR Spectroscopy** | Micro Fourier Transform Infrared Spectroscopy |
| **μSR-FTIR Spectroscopy** | Micro Fourier Transform Infrared Spectroscopy with Synchrotron Radiation |
| **GC/MS** | Gas Chromatography couple to Mass Spectrometry |
| **OM** | Optical Microscopy |
| **SEM** | Scanning Electron Microscopy |
| **XRD** | X-ray Diffraction |
| **μXRD** | Micro-X-ray Diffraction |
| **μSR-XRD** | Micro-X-ray Diffraction with Synchrotron Radiation |

The studies included in the work belong to the same research line, leading to four papers published in international journals and another one already submitted.

Beltran, V., Salvadó, N., Butí, S., & Cinque, G. (2015). Micro infrared spectroscopy discrimination capability of compounds in complex matrices of thin layers in real sample coatings from artworks. Microchemical Journal, 118, 115-123.

Beltran, V., Salvadó, N., Butí, S., Cinque, G., Wehbe, K., & Pradell, T. (2015). Optimal sample preparation for the analysis of micrometric heterogeneous samples. Analytical Chemistry, 87(13), 6500-6504.

Beltran, V., Salvadó, N., Butí, S., & Pradell, T. (2016). Ageing of resin from Pinus species assessed by infrared spectroscopy. Analytical and Bioanalytical Chemistry, 408(15), 4073-4082.

Salvadó, N., Butí, S., Pradell, T., Beltran, V., Cinque, G., Juanhuix, J., Font, L., Senserrich, R. (2016). Low molecular weight organic acid salts, markers of old fungi activity in wall paintings. Analytical Methods, 8(7), 1637-1645.

Beltran, V., Salvadó, N., Butí, S., Cinque, G., & Pradell, T. Markers, reactions and interactions during ageing of Pinus resin assessed by Raman spectroscopy *Submitted in Journal of Natural Products*

Results have also been presented in international conferences.

“Art’14 – 11th International Conference on Non-destructive Investigations and Microanalysis for the Diagnostics and Conservation of Cultural Heritage (June 11 – 13, 2014), Museo Arqueológico Nacional, Madrid (Spain)”

> Oral presentation entitled “FTIR spectroscopy of natural varnish components in cultural heritage: limitations and possibilities” (V. Beltran, N. Salvadó, S. Butí). Book of Abstracts, p. 81.

“ChemCH 2014: Third International Congress on Chemistry for Cultural Heritage (July 1 – 5, 2014) Academy of Fine Arts, Vienna (Austria)”

> Poster entitled “Metal foil coatings in altarpieces: a microanalytical study of the materials and their alterations” (N. Salvadó, S. Butí, G. Cinque, J. Juanhuix, C. Clemente, V. Beltran, T. Pradell). Book of Abstracts, p. 264.

“Synchrotron Radiation and Neutrons in Art and Archaeology (SR2A-2014) (September 9 – 12, 2014), Musée du Louvre, Paris, (France)”

> Poster entitled “Synchrotron Radiation based techniques for the study of altered metal foil coatings in Baroque altarpieces” (N. Salvadó, S. Butí, G. Cinque, J. Juanhuix, C. Clemente, V. Beltran, T. Pradell). Book of Abstracts, p. 120.

"Metal Soaps In Art (March 14 – 15, 2016), Rijksmuseum, Amsterdam, (Netherlands)"

> Poster entitled "Identification and distribution of soaps and oxalates in paint layers using synchrotron radiation techniques" (N. Salvadó, S. Butí, T. Pradell, V. Beltran, G. Cinque, J. Juanhuix). Book of Abstracts, p. 21.

"Twelfth Infrared and Raman Users Group (IRUG) Conference (May 23 – 25, 2016), Ormylia Foundation Art Diagnosis Centre (Greece)"

> Oral presentation entitled "Ageing processes in diterpenic resins used in artwork coatings" (V. Beltran, G. Cinque, N. Salvadó, S. Butí, T. Pradell). Book of Abstracts, p. 47.

CHAPTER ONE

# Introduction

## 1.1 VARNISHES AND COATINGS IN HISTORIC OBJECTS

### 1.1.1 Preliminary considerations

Historical objects are remnants of the past that have cultural heritage value. They might also have artistic value, but in any case, their study always provides information about the human history and its technology.

The surface of these historical objects is the part most exposed to the environment and, consequently, the most vulnerable and can be easily modified. Its modification is a major issue since it affects the appearance of the whole object and also because it is an extremely thin layer that can be easily damaged.

An example of the effects of the alteration of surface layers is evidenced in those coatings that have changed their physical properties, for instance when transparent varnishes become yellow affecting the perception of the chromatic layers and the appearance of the artwork (Figure 1.1).

**Figure 1.1** Detail of the canvas entitled "El port de Barcelona" (The port of Barcelona), of Eliseu Meifrén, 1887, owned by the Autoritat Portuaria de Barcelona. Archive image from AMPC research group (UPC).

The analysis of the historical objects' surface provides information about the nature of the materials; allowing to discriminate between those deposits resulting from environmental contamination from those coatings applied intentionally.

Environmental contamination includes both the environmental gases and the deposition of inorganic materials transported by the air such as dust, calcium carbonates, gypsum, silicates or calcium oxalates, but also of substances related to biodegradation processes, such as microorganism skeletons (bacteria, fungi…), as well as, the residues produced by their activity.

Coatings applied intentionally appear usually forming thin layers (less than 100 µm thick) and are made of different materials depending on their purpose (varnishes, gildings…). The materials used for coating historical objects like paintings, altarpieces, sculptures, furniture, musical instruments, cartographic documents… have changed through history depending on the technology, natural sources available or fashion. As time passes, materials are altered due to their own ageing processes but also due to the interaction with the other materials of the object. They are also affected by the environmental conditions such as moisture, temperature and presence of contamination. Besides, restoration and conservation procedures may also induce changes in the chemical composition and reactivity among the materials.

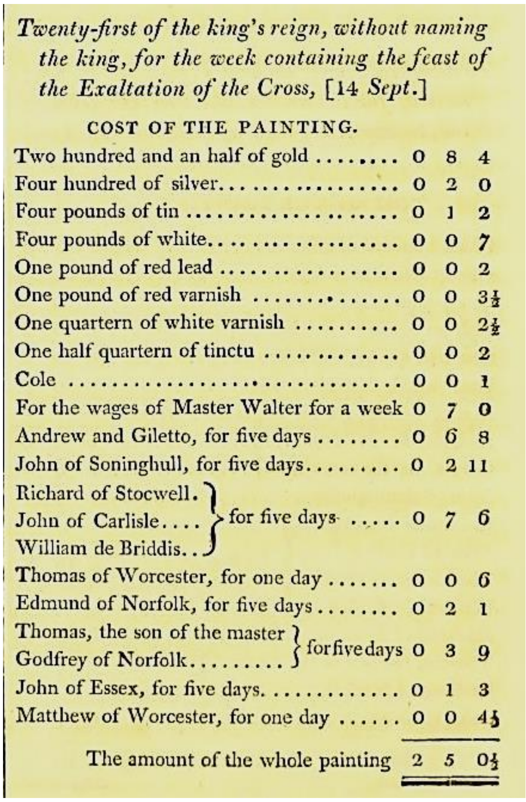
The study of the coatings of historical objects is of great interest for a number of reasons; firstly, provides information about the manufacture of the objects, both the techniques and the materials used. Secondly, identifies the materials and ageing degree, which on its turn, gives information about the optimal conservation conditions and, if necessary, the best restoration strategy.

This work attempts to deal with the analysis of the coatings applied to historical objects and the specific problems which can arise. It will be focused on the analysis of coatings made with *Pinaceae* resins, specifically in resins from *Pinus* species, since they were frequently used.

### 1.1.2 Definition of natural coatings

Varnishes and coatings are layers, normally organic, which are applied on historic and contemporary manufactures, either to protect them from the environment or to give them specific properties, or simply to modify their aspect (1,2). Natural varnishes are defined as those ones made with substances of natural origin which are unprocessed or just minimally processed.

There are references to varnishes and varnishing process in various text sources. Art treatises and manuscripts from different ages detail many varnish formulas. Also, several artworks contracts detail the varnish materials that should be used, i.e. the register of St Stephen's Chapel from the old Palace of Westminster (Figure 1.2) (3,4), and even some guild statutes deal with the varnishing process (5).



*Twenty-first of the king's reign, without naming the king, for the week containing the feast of the Exaltation of the Cross,* [14 *Sept.*]

COST OF THE PAINTING.

| | | | |
|---|---|---|---|
| Two hundred and an half of gold | 0 | 8 | 4 |
| Four hundred of silver | 0 | 2 | 0 |
| Four pounds of tin | 0 | 1 | 2 |
| Four pounds of white | 0 | 0 | 7 |
| One pound of red lead | 0 | 0 | 2 |
| One pound of red varnish | 0 | 0 | 3½ |
| One quartern of white varnish | 0 | 0 | 2½ |
| One half quartern of tinctu | 0 | 0 | 2 |
| Cole | 0 | 0 | 1 |
| For the wages of Master Walter for a week | 0 | 7 | 0 |
| Andrew and Giletto, for five days | 0 | 6 | 8 |
| John of Soninghull, for five days | 0 | 2 | 11 |
| Richard of Stocwell.<br>John of Carlisle<br>William de Briddis — for five days | 0 | 7 | 6 |
| Thomas of Worcester, for one day | 0 | 0 | 6 |
| Edmund of Norfolk, for five days | 0 | 2 | 1 |
| Thomas, the son of the master<br>Godfrey of Norfolk — for five days | 0 | 3 | 9 |
| John of Essex, for five days. | 0 | 1 | 3 |
| Matthew of Worcester, for one day | 0 | 0 | 4½ |
| The amount of the whole painting | 2 | 5 | 0½ |

**Figure 1.2** Fragment of the list of materials used in St Stephen's Chapel (old Palace of Westminster), 14th century. Lines 6 and 7 detail the cost of the varnish used (7).

These documents demonstrate that varnishes have been frequently used through history (6).

Varnishes use to be a transparent coating which modifies the colour saturation and the gloss of the artwork. Different varnishes have been applied through history depending on what the covering was intended for, the availability of the materials, the ease of application, or simply the fashion in the historic period.

According to the materials described in the treatises, early varnishes were dark yellow or dark reddish yellow. Possibly, this was intentional since there are no comments in ancient manuscripts or treatises about the importance of working with non-coloured varnishes. Moreover, some of the formulas mentioned, even those recommended for paintings, include red and yellow colorants such as minium (a red lead oxide, $Pb_3O_4$) or aloe (extract from Aloe *barbadensis* Mill. used to colour oil varnishes).

Recipes of colourless varnishes are given in later treatises, for instance the *De' veri precetti della pittura*, from Giovanni Battista Armenini (second half of 16th century) (7), which explicitly states that the good varnish is colourless. Throughout the modern era most of the varnishes detailed in the sources pretend to be colourless and give much importance to this feature.

Additionally, it is also possible that different types of varnishes were used depending on the item category or even depending on the region. Some varnish formulas are recommended for special objects, i.e. paintings, furniture or musical instruments; also some sources describe different varnishes for the dark and light areas of the same object or depending on the specific surfaces, like gildings and mecca gildings (coatings over metal foils).

Finally, we should not forget that many restoration procedures include the application of an additional varnish layer.

## 1.2 THE ANALYSIS OF SAMPLES FROM CULTURAL HERITAGE

### 1.2.1 The specific features of cultural heritage samples

The chemical study of samples from historic objects is an intricate problem and has specific features.

Samples extracted from cultural heritage objects are extremely small (submillimetric). They are usually structured forming micrometric layers, from a few to some hundreds of micrometres thickness and of heterogeneous nature.

It is very important to analyse each layer separately to understand its role in the appearance, stability and conservation of the studied object. In most cases, it is also fundamental to determine the variations in the chemical composition within each layer, as well as across the various layers, in order to identify the reactions happening among the different substances present in the layers and with the environment.

The samples composition tends to be very complex. An example of this complexity is shown in Figure 1.3. A cross-section of a sample from the painting entitled "The port of Barcelona", of Eliseu Meifrén, 1887 is shown in Figure 1.3a. Enlarged details of the cross-section are displayed in Figure 1.3b and c where the presence of a great diversity of particles can be distinguished in each layer.

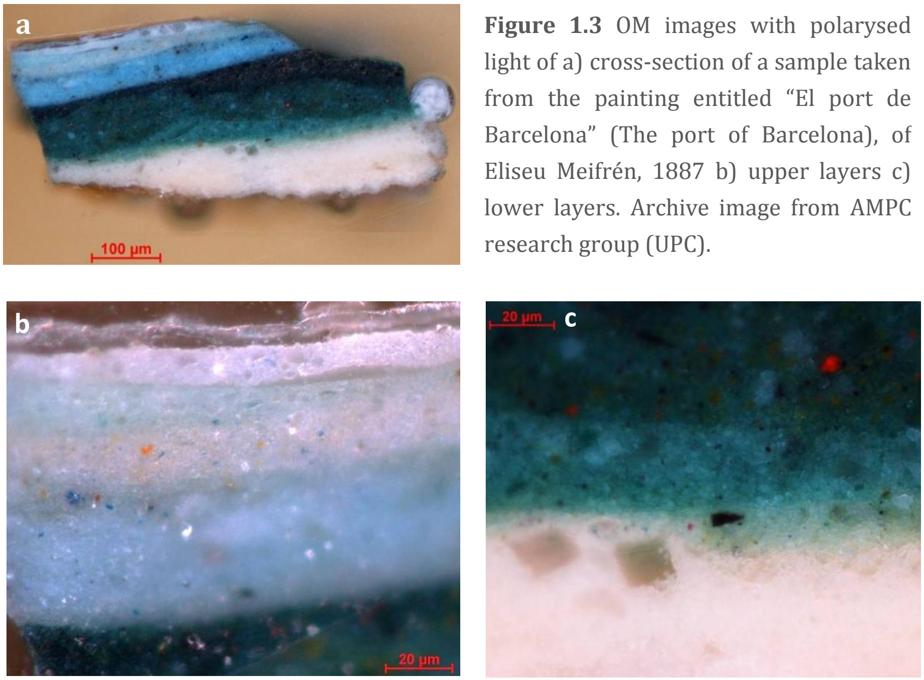


**Figure 1.3** OM images with polarysed light of a) cross-section of a sample taken from the painting entitled "El port de Barcelona" (The port of Barcelona), of Eliseu Meifrén, 1887 b) upper layers c) lower layers. Archive image from AMPC research group (UPC).

Besides the wide variety of substances used, the materials themselves were often processed in different ways changing its chemical composition and incorporating other substances. Moreover, most of the substances present in the layers appear altered due to their own ageing processes and to the interaction with other materials or with the environmental contamination. These chemical changes usually affect the physical properties of the materials (colour, fragility, adherence…).

It is worth to notice, that the chemical changes depend on the original composition of the object, but also on the materials combination and on the environmental conditions. Consequently, in most cases the nature and mixture of substances present is unique and, can hardly be compared to any reference, so each sample has to be carefully studied taking in account its distinctive features. Additionally, it is extremely important to use an optimal sample preparation method able to discriminate and identify all the substances present and the reactions existing among them.

### 1.2.2 Vibrational spectroscopies for the analysis of historical objects coatings

Vibrational spectroscopies, namely Fourier Transform Infrared (FTIR) and Raman, are among the most widely used techniques for the characterization of cultural heritage materials (8). These techniques allow the identification of a wide range of compounds, both organic and inorganic. Besides, are useful for the characterization of macromolecules and the analysis of intermolecular bonds.

The coupling of vibrational spectroscopies to a microscope (µFTIR and µRaman) extends its capabilities into the microanalytical range, providing the spatial resolution necessary for the identification of submillimetric structures/particles. It is more and more common to find infrared and Raman microscopes in laboratories dedicated to the microanalysis of cultural heritage materials.

The principal advantages of vibrational spectroscopies are related to the small quantity of sample needed, the ease of identification of very diverse materials, the speed at which results can be obtained via IR and Raman fingerprinting, the low cost of analysis and the non-destructive character of the technique. With the advantage of being non-destructive, the same sample preparation may be reused (9).

Nevertheless, the main weak point of vibrational spectroscopies is the interpretation of results, which can be very difficult especially for mixtures of different compounds. In these mixtures, the spectra of all compounds appear overlapped; this normally causes the occurrence of overlapped bands with complex shapes and peak maximum displacements hindering the identification of the bands and thus the interpretation of the spectra.

There are two different ways commonly used to overcome the limitations of vibrational spectroscopies; on the one hand, the use of spectral processing procedures and, on the other hand the use of a multitechnique analytical approach. Spectral processing procedures such as curve fitting or Fourier self-deconvolution help to resolve spectra when the bands appear largely overlapped. The multitechnique analytical approach implies the use of different analytical techniques: each one providing complementary information that helps to overcome the limitations of each single technique.

For the analysis of organic coatings of historical objects the combination of vibrational spectroscopies is very useful. Coupling Raman and FTIR spectroscopies provides complementary information of both molecular composition and intermolecular interactions as weak bands in the infrared spectrum may be strong in the Raman spectrum, or vice versa (10,11).

For instance, strong IR absorption bands occurring for the polar groups such as –OH, appear weak in the Raman spectrum. Consequently, a broad IR band resulting from a set of overlapped single IR bands can be simpler in the Raman spectra (fewer bands) facilitating their identification. Overlapped bands are more frequently observed as materials ages due to the increasing number of compounds present, consequently, the combination of Raman and IR spectroscopies might be particularly useful for the investigation of organic coatings from cultural heritage materials.

For making the most of FTIR and Raman analysis it is crucial to use an optimal sample preparation. The proper sample preparation will provide accurate information of the sample and the obtained spectra will be free from interfering substances, thus easier to interpret.

Besides, the use of other microanalytical techniques such as SEM, optical Microscopy, micro-XRD, GC/MS… will also be fundamental in the study of complex aged materials.

## 1.3 OBJECTIVES

The general objective of this work is to analyse the main compounds of varnishes and coatings used in historical objects, specifically the ones made from the *Pinaceae* species resin. Vibrational spectroscopy techniques are selected considering their non-destructive nature. Results will be applied to the study of samples from historical objects using an optimized sample preparation method.

In order to achieve the main objective, three specific tasks are addressed:

1. To study by means of FTIR and Raman spectroscopy of the reactions and interactions during ageing in pimaranes and abietanes, main compounds

of the *Pinaceae* species used in varnishes and coatings of historical objects. This will be centred in the study *Pinus* resin as its main compounds are abietanes and pimaranes, while the composition of other *Pineaceae* species used in historical objects is more complex. In order to characterize the ageing process, chemical references of the main compounds, fresh resins and aged samples will be analysed. Fresh resins will be collected directly from the trees in order to ensure their freshness and botanical origin.

2. To develop a specific sample preparation method for the analysis of historical coatings, and other layers present in the object. The method should be adequate also for the analysis of the layers by different analytical techniques while preserving their structure in order to obtain complementary information of the same sample helpful in the identification of all the compounds.

3. To study a selection of historical objects with coatings containing *Pinus* species resins based on the previous study of *Pinus* resin and using, when required, the sample preparation method developed. Use different analytical techniques for the identification of the compounds, including also synchrotron sources for the characterization of thin layers or small particles.

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## CHAPTER TWO

# Methods and materials

# 2.1 VIBRATIONAL SPECTROSCOPIES

## 2.1.1 µFTIR spectroscopy

Coupling a microscope to a FTIR spectrometer (µFTIR spectroscopy) allows the analyses of microscopic samples or microscopic areas of larger samples. This is particularly useful for the analyses of heterogeneous samples as it is possible to analyse specific areas and particles. The use of a transmission µFTIR with a globar source allows the measurement of a spot as small as 50x50 µm$^2$.

In this work, µFTIR measurements were taken using an AIM-8800 microscope with a MCT detector coupled to the Shimadzu IRAffinity$^{-1}$ FTIR spectrometer (Figure 2.1). The measurement interval was 4000–700 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ and 256 scans. All measurements were taken in transmission mode, which requires a more complex sample preparation but provides the desired sensitivity and spectral quality for the adequate identification of the substances.

**Figure 2.1** Microscope AIM-8800 with a MCT detector coupled to the Shimadzu IRAffinity$^{-1}$ FTIR spectrometer.

## 2.1.2 µRaman spectroscopy

Likewise µFTIR, the use of µRaman extends the possibilities of Raman spectroscopy. Raman measurements were taken by means of a confocal Raman-Microscope Bruker Senterra R-200L with a thermo-electrically cooled CCD detector (1024x256 pixels) and an Olympus BX51 microscope using a 50x objective lens (Figure 2. 2).

Raman requires no sample preparation, although it is necessary to know the nature of materials before performing the analysis to choose the proper analytical conditions. Wrong analytical conditions, like too long integration time, can damage the samples and give fake results.

Raman data were acquired at 9-18 cm$^{-1}$ resolution with a 50 µm pinhole aperture during 50 s integration time with 5 co-additions; the spectral footprint was about 2µm. Spectra were obtained with a 785 nm laser wavelength which gives a good signal in samples that emit fluorescence at higher excitation wavelengths. Despite some studies where the resins were analyzed with a 1064 nm laser with successful results (1–6) the use of a 785 nm laser is proposed. The 785 nm laser permits working at a lower intensity and consequently, is more suitable for the analysis of photo-sensible samples and which can be damaged if exposed to the 1064 nm laser (7).

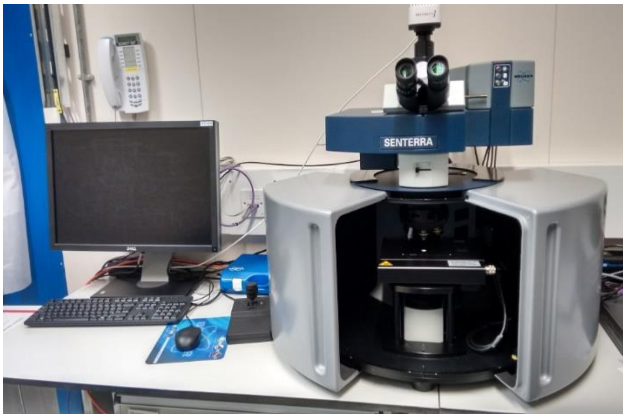

**Figure 2.2** Confocal Senterra R-200L Raman Microscope with a thermoelectrically cooled CCD detector. Beamline MIRIAM B22 of the Diamond Light Source (UK).

## 2.3 COMPLEMENTARY TECHNIQUES

The use of complementary techniques helps to overcome the limits of a single analysis. Thus, other analytical techniques were also used in some cases in order to obtain complementary data or corroborate the obtained results.

### 2.3.1 Optical Microscopy (OM)

The first step of the analysis is the microsamples observation. This has been done by means of optical microscopy.

Samples handling is performed under a stereomicroscope Nikon SMZ800 with magnification from 10x to 63x. For the observation of the samples and corresponding cross-sections, an optical microscope Nikon Eclipse LV100 with magnification from 50x to 500x was used, working under different microscopy techniques (polarized light, dark field, bright field or fluorescence) (Figure 2.3).

**Figure 2.3**
Stereomicroscope Nikon SMZ800 (left) and optical microscope Nikon Eclipse LV100 (right).

### 2.3.2 Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX)

SEM/EDX is considered a relatively rapid and non-destructive approach to surface analysis and provides information that cannot be obtained by OM techniques. The focused electron beam produces high resolution images of surface topography with excellent depth of field (secondary electrons). The backscattering images provide elemental contrast images (backscattered electrons) and the EDX (Energy Dispersive X-ray Spectroscopy) detector semi-quantitative chemical elemental analysis.

Measurements were made by means of two different equipment, a JEOL-5600 with PCXA.LINK EDS micro-analyzer and, a GEMINI SEM equipment with a Shottky-FE column at 4pA-20 nA, 0.1 to 30 kV and 1nm resolution for 20KV. Elemental analysis was obtained with an INCAR Penta FETX3 detector and a 30 $mm^2$ ATW2 window.

### 2.3.3 Gas Chromatography coupled to Mass Spectrometry (GC/MS)

GC/MS was used in order to confirm the data obtained by µFTIR and compare the results with the ones from the literature, as main studies of *Pinus* resin have been performed with GC/MS.

GC/MS analyses were performed with an Agilent Chromatograph (6850 model and HP-5MS column) coupled to an Agilent Mass Spectrometer (model 5975C) (Figure 2.4). The samples were dissolved in 20 µL of methanol, derivatized with 15 µL of MethPrepII and heated at 66.6$^0$C for 2 hours. Splitless injection was used with a non-linear heating ramp from 60 to 300$^0$C; the spectrometer temperature was fixed at 300$^0$C. Molecules were identified comparing to the NIST database.

**Figure 2.4** Chromatograph Agilent (6850 model with HP-5MS column) coupled to a Mass Spectrometer Agilent (model 5975C). Laboratory of Centre de Restauració de Béns Mobles de Catalunya CRBMC.

## 2.4 SYNCHROTRON SOURCES

Synchrotron radiation is produced when electrons accelerated to high energy, typically in the GeV range, are affected by strong magnetic fields perpendicular to the electron beam that accelerates the electrons radially. When the electron beam is bent by the magnets, the electrons emit polarised electromagnetic radiation.

Synchrotron Radiation is notable because of its high brilliance, high level of polarization and high collimation. The frequencies generated range over the entire electromagnetic spectrum, from Infrared to hard X-rays. Consequently, FTIR and XRD (X-ray Diffraction) can be performed with micrometric resolution.

The Synchrotron Radiation is particularly useful to obtain quantitative and precise data on micrometric areas (i.e. varnish layers thinner than 20 µm), and provides information about the atomic and molecular structure, composition and distribution of the various substances present. In particular, is crucial to determine the substances distributed in thin layers and especially in the areas of contact between different layers in coatings, paintings etc.

### 2.4.1 Diamond Light Source

Diamond Light Source is a 3rd generation Synchrotron Light facility located at the Harwell Science and Innovation Campus (Oxfordshire) (Figure 2.5). It is operated by the Science and Technology Facilities Council.

Synchrotron-based µFTIR (µSR-FTIR) measurements were taken at beamline MIRIAM B22 of the Diamond Light Source (Figure 2.6). Two Bruker 80 V Fourier Transform IR Interferometers are equipped with Hyperion 3000 microscopes and high sensitivity MCT detectors (broadband and narrower band).

The spectra were obtained in transmission mode using a small beamspot of 12x12 $\mu m^2$, 4 $cm^{-1}$ spectral resolution, coadding 256 scan at scanner velocity 80 kHz (35 sec). Wave number range was between 4000 to 650 $cm^{-1}$ or 500 $cm^{-1}$. IR maps of the molecular composition were obtained by scanning the sample via a micrometric resolution motorized X–Y stage.

**Figure 2.5** Diamond Light Source. Image obtained from www.diamond.ac.uk

**Figure 2.6** Beamline MIRIAM B22 from Diamond Light Source.

### 2.4.2 ALBA-CELLS

ALBA-CELLS is a 3rd generation Synchrotron Light facility located in Cerdanyola del Vallès, (Barcelona) (Figure 2.7), being the newest source in the Mediterranean area. It is managed by the Consortium for the Construction, Equipping and Exploitation of the Synchrotron Light Source (CELLS).

Synchrotron-based micro-X-ray diffraction measurements (μSR-XRD) were taken from cross-sections of samples extracted from the artwork at Beamline XALOC of the ALBA Synchrotron (Figure 2.8a).

The focused beam was 50 μm x 6 μm (fwhm) (Figure 2.8b), acquisition time was 1 s and energy was 12.6 keV. A detector Model Pilatus 6 M (Dectris) virtually noise-free with a large (424 mm × 435 mm, 6 megapixels) active area was used. The diffraction patterns were obtained by scanning the samples thin sections over ca. 150 μm with a step of 6 μm.

**Figure 2.7** ALBA-CELLS. Image obtained from www.cells.es

**Figure 2.8** a) Beamline XALOC from ALBA-CELLS, b) Beam of 6x50 μm used for μSR-XRD.

## 2.5. SAMPLE PREPARATION METHODS FOR FTIR ANALYSIS

In order to perform transmission μFTIR analysis and obtain high quality spectra it is necessary to prepare the samples with a specific thickness. Thus, two sample preparation methods have been used: a diamond compression cell and a new method developed in this work and described in detail in chapter 6.

## 2.5.1 Diamond compression cell

For this kind of analyses, small fragments of the sample are extracted and selected by means of specific tools (micro-knifes, scalpels, tungsten needles…) (Figure 2.9), under a stereo microscope and an optical microscope.

The fragment is squeezed between the diamond cells using a compression holder (Figure 2.10) in order to have the adequate thickness required for transmission analysis. The diamond cell and compression holder are from Thermo Scientific (ref. 0045-344 and 0049-434 respectively). A diamond compression cell was used for the analysis of resin samples, as they were sticky and it was not possible to prepare a KBr pellet, and for samples from historical artworks.

This sample preparation provides the adequate thickness for transmission analysis producing the high quality spectra necessary for the study of complex mixtures. Nevertheless, the exact location of the compounds identified cannot be established as the compression cell distorts the sample shape. Analysis can be obtained with a Globar source or a Synchrotron source depending on the size of the spot which is needed to discriminate the substances in the sample.

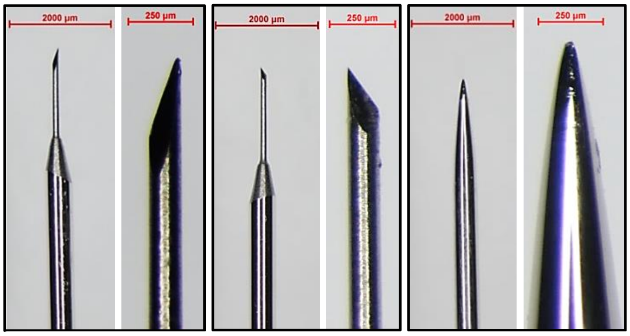

**Figure 2.9** OM image of specific tools used for handle the samples: $20^{0}$ microknife (left), $45^{0}$ microknife (center) and tungsten needle (right).

## 2.5.2 Thin sections

The preparation of thin sections is much more complex than those for the diamond cell; however it allows knowing the exact location of the analysis. This is useful for the study of the sequence of layers that constitute the coating and the reaction compounds appearing at the interfaces. This preparation method has been used for the analysis of samples of coatings and varnishes from historical objects.

This methodology has been developed in this work and is detailed in chapter 6.

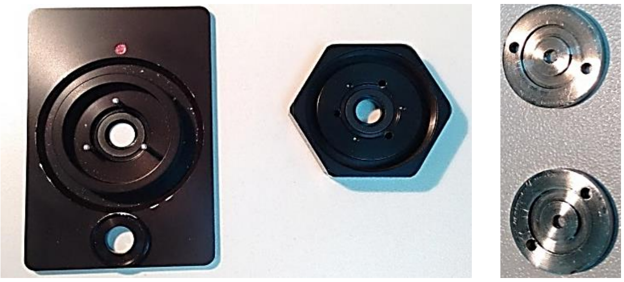

**Figure 2.10** Compression holder (left) and diamond cell (right).

## 2.6 MAIN MATERIALS USED AS NATURAL COATINGS IN EUROPE

The materials used in varnishes are very diverse in nature. Varnishes were made of natural products until the 19th century, when synthetic resins were discovered and started being used (8).

The chief materials used in varnish formulas are natural resins and drying oils. But other materials like wax and proteins are also found. Natural resins can be classified in three groups; sesquiterpenes, diterpenes and triterpenes.

The main sesquiterpenic resin used in varnishes is shellac. It is a resin extracted from the nests of the insect Laccifer Lacca Kerr, mostly composed by an oligomer made with alicyclic sesquiterpenes and molecules similar to fatty acids (Figure 2.11).

Diterpenic resins can be classified in abietane-type and labdane-type. The main compounds of abietane-type resins are diterpenes with a structure similar to abietic acid while labdane-type resins are also mainly diterpenes but with a structure similar to communic acid (Figure 2.12).

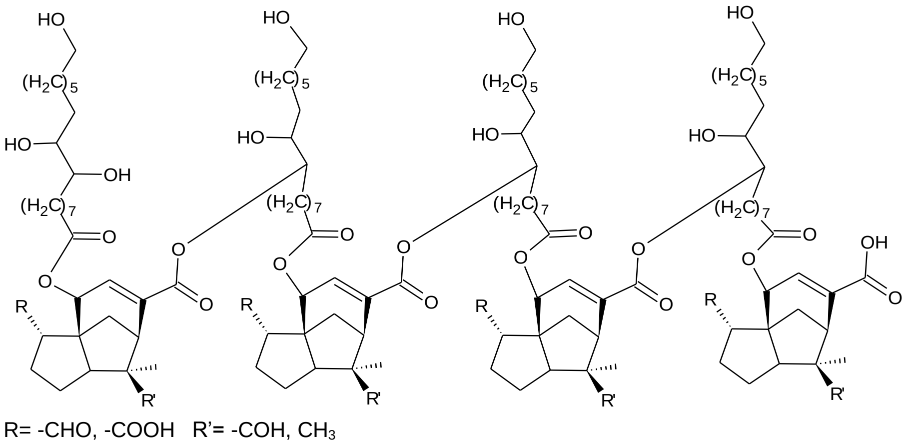

**Figure 2.11** Main oligomer of shellac (8).

The main abietane-type resins used in varnishes are obtained from the trees of *Pinaceae* family, especially from the *Pinus* species, *Abies alba* Mill. and *Larix Decidua* Mill.

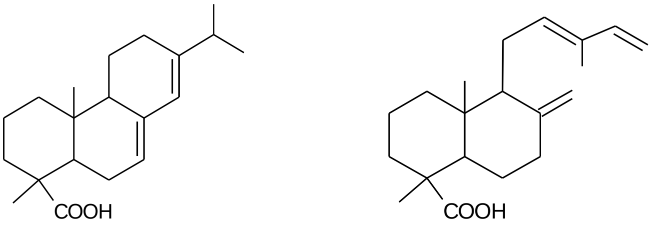

**Figure 2.12** Abietic acid (left) and communic acid (right).

The main labdane-type resins are sandarac, obtained from *Tetraclinis articulata* Vahl, and amber, a fossilized resin. Sandarac was frequently used in varnishes. Amber appears mentioned in some varnish formulas, but it is not always clear they actually refer to the fossilized resin. The fossilized resin called amber is a high cross-linked polymer, and consequently, hard to dissolve in oil or other solvents. Some accounts detail that it was an expensive material and even some art treatises supply formulas for producing fake amber (9,10). Thus, it is possible that in varnish recipes, "amber" refers to any other material, or else to a high quality sandarac more purified than the common one.

The main triterpenic resins used in varnishes are mastic, dammar and elemi. Mastic is extracted from *Pistacia lentiscus* L, which can be found along the Mediterranean basin (Figure 2.13). Dammar and elemi are obtained from tropical trees; elemi is mostly obtained from *Canarium Luzonicum* A. Gray and dammar from various trees of the *Dipterocarpaceae* family. The composition of the triterpenic resins depends on the specific substance but, generally speaking, is complex and includes many different types of molecules.

Nevertheless, we sometimes find unexpected substances in the varnishes whose presence must be accounted for. To illustrate the complexity of the substances which can be found in varnishes and coatings, a selection of case studies will be discussed.

**Figure 2.13** *Pistacia lentiscus* L. from Athens, Greece. Archive image from AMPC research group (UPC).

## 2.6.1 Case 1: guanine, unexpected coating

The first example corresponds to the coating material used in the interior of a hope chest dating from the 16th century. A hope chest is a piece of furniture traditionally used by young unmarried women in anticipation of married life. Samples were taken during the restoration process (Figure 2.14 and 2.15). The hope chest belongs to the Museu del Disseny of Barcelona (Catalonia) and was restored at Centre de Restauració de Bens Mobles de Catalunya (CRBMC)(11).

A very thin coating layer covering the inside was directly applied onto the wood; the material was fragile, translucent and colourless (Figure 2.16). The sample was mechanically extracted under a stereomicroscope to help selecting the most representative areas, which were then measured with μFTIR.

**Figure 2.14** Outside of the 16th century hope chest (image from Carles Aymerich, CRBMC.

**Figure 2.15** 16th century hope chest a) outside b) inside, where the sample was extracted (image from Carles Aymerich, CRBMC).

Two different materials were identified; anhydrous guanine (12) and a proteinaceous substance which are shown in Figure 2.17 a and b, respectively. Guanine is a substance used in pigment production to obtain a nacreous (pearlescent) effect, which was also used to make synthetic pearls and cosmetic products.

Guanine appears forming small crystallites of elongated shape, about 10–40 μm in length and 40–50 μm in thickness; it has a low density 1.6 g/cm$^3$, is stable to the light, brilliant/iridescent, and has a refraction index of 1.85 (13). It is a typical interference type pigment showing a white pearlescent effect.

Guanine is obtained from white fish scales. The extract has a composition of 75%–97% guanine and 3%–25% hypoxanthine, the exact ratio depends on the specific fish species (14,15). Guanine ($C_5H_5N_5O$) is a nucleic acid, specifically, a purine base. From a historical point of view, guanine was first reported in 1656 by François Jacquin (a rosary maker from Paris) who isolated it from a fish scale suspension (15).

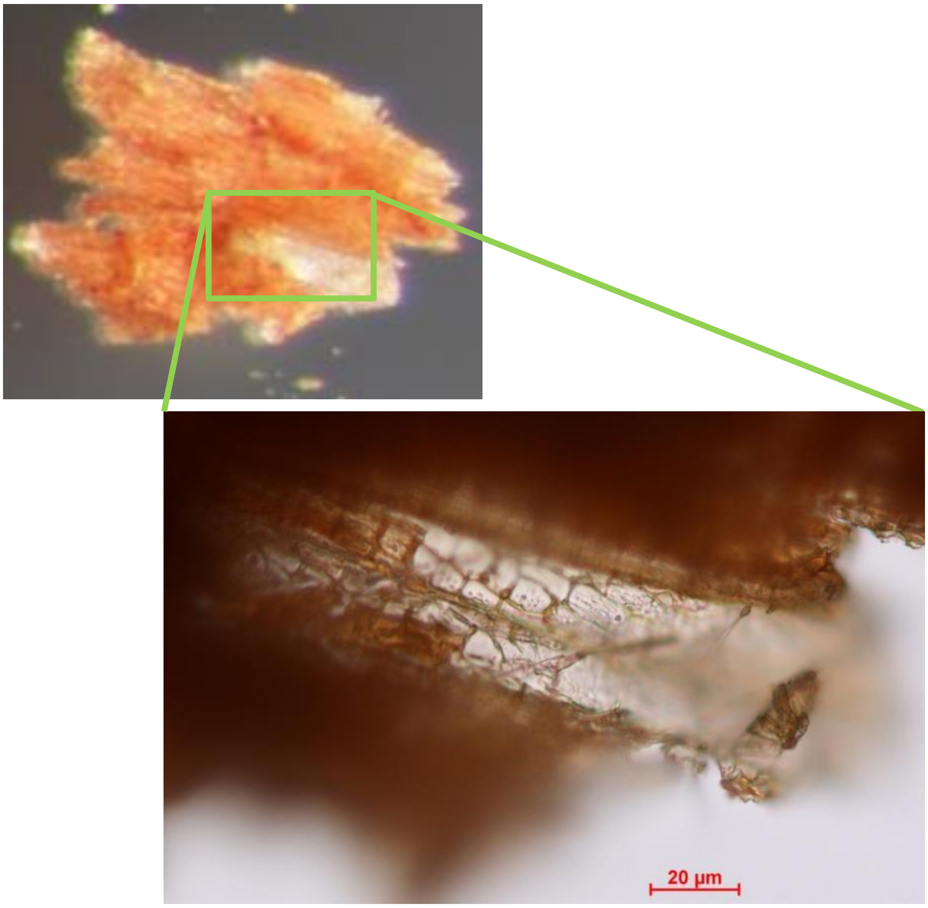

**Figure 2.16.** Sample from the interior of the hope chest. Archive image from AMPC research group (UPC).

The infrared spectrum obtained from the sample is very similar to the reference spectrum from the literature. However, assigning the absorption bands to specific vibrations is complex. Bands at 1672 and 1697 $cm^{-1}$ (Figure 2.17a) have been related to C=O coupled to the $NH_2$ scissoring and N-H in-plane bending vibrations (12).

Finally, the protein substance determined may also be related to the fish or to the glue used to stick together the pigment particles. The coating used in this case, guanine, is a substance that has never been described or found in artworks and thus it was wholly unexpected.

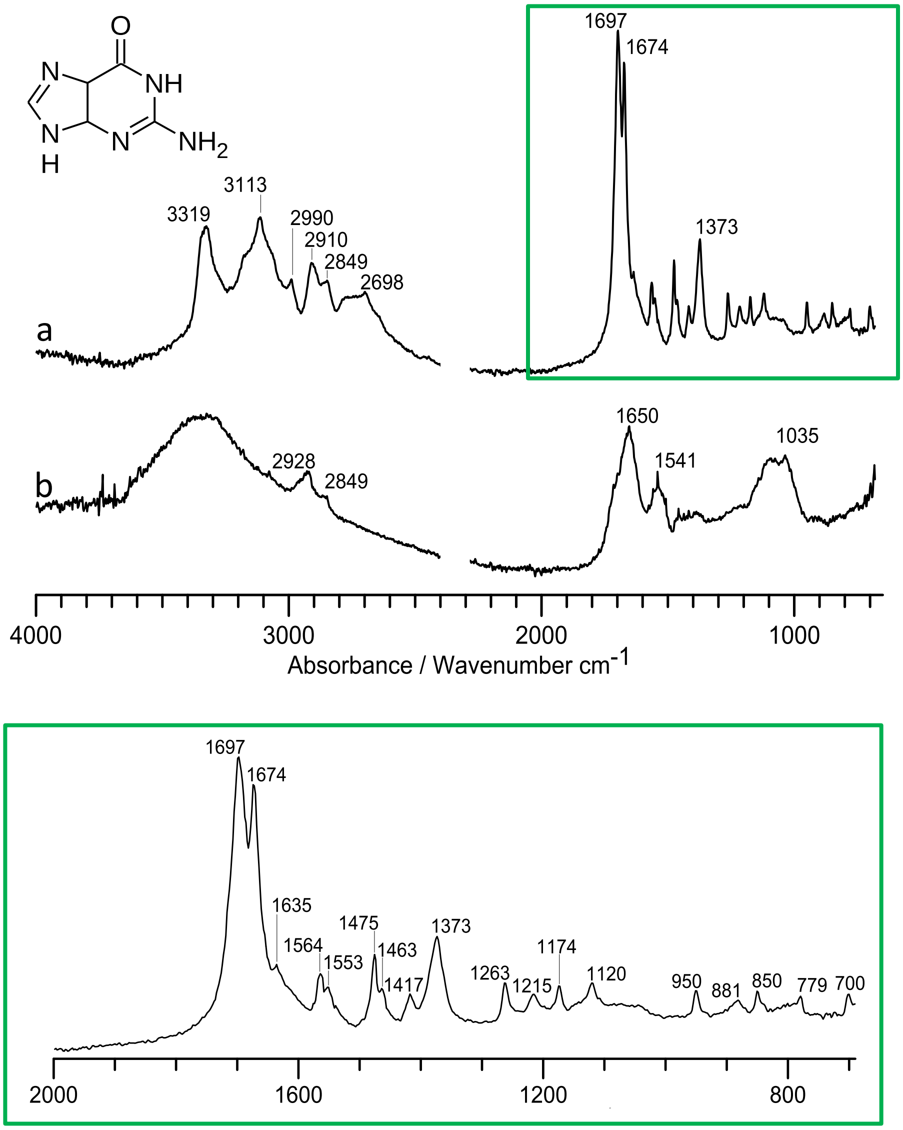

**Figure 2.17.** µFTIR spectra from sample prepared using a diamond cell (measured area, 50 µm x 50 µm), guanine identified in Spectrum a and protein identified in Spectrum b; in the bottom there is a magnification of the region between 700–2000 cm⁻¹ of spectrum a.

## 2.6.2 Case 2: reducing the brilliance of a gold foil

The second example presented corresponds to the coating applied over the gold foils from the 17th century baroque altarpiece of Santa Càndia and Santa Còrdula

(Figure 2.18) placed in the Cathedral of Santa Maria in Tortosa (Catalonia) (16). The samples were extracted during the process of restoration (11).

Altarpieces from this period were extensively gilded (17). The gold foil was applied over a bole (a red or yellow clay mixed with animal glue). The bole provides certain plasticity in the support facilitating the application of the metallic foil. Several finishing processes were given to obtain a specific colour and/or shine.

**Figure 2.18** Altarpiece of Santa Càndia and Santa Còrdula, from the Cathedral of Santa Maria de Tortosa, Catalonia. Archive image from AMPC research group (UPC).

In order to maximize brightness and to give the illusion of solid gold, the surface was polished. Sometimes, however, on certain parts of the altarpiece, a tarnish or colour finishing was desired and thus, it was necessary to apply a specific coating (10).

However, distinguishing between dirt deposits or a chromatic coating layer is often difficult but at the same time it is very important in order to ensure the adequate restoration treatment. In Santa Càndia and Santa Còrdula altarpiece a thin coating covering the whole gold surface was detected (Figure 2.19).

Three samples from different points were extracted avoiding the cracks (to prevent contamination) and the material from the inner layers.

Analysis by SEM-EDS (Figure 2.20) shows the presence of Ca, Si and S in this layer, which is extremely thin (<2 µm).

**Figure 2.19** Dark-field OM image from the surface of the sample taken from a gilded area of the altarpiece to Santa Càndia and Santa Còrdula. Archive image from AMPC research group (UPC).

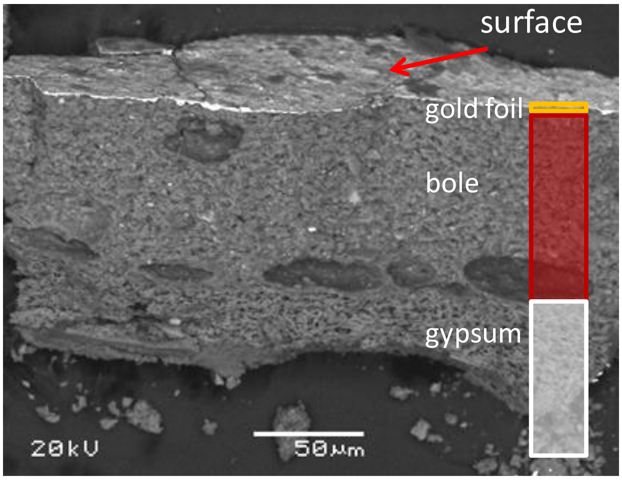

**Figure 2.20** Backscattered Electrons (BSE) image from a sample fragment placed in perspective; this image shows the surface face consisting of a layer less than 2 µm thickness. Archive image from AMPC research group (UPC).

A representative µFTIR analysis of the coating is shown in Figure 2.21.

Results show characteristic absorption bands of substances related to environmental deposition and alteration (Figure 2.21a silicates, calcium carbonates, gypsum and calcium oxalates) but also to a protein-based substance (Figure 2.21 b) with characteristic absorption bands at 3300 $cm^{-1}$ N–H stretching, ~1650 $cm^{-1}$ C=O stretching of amide I, ~1540 $cm^{-1}$ $-NH_2$ of amide II and ~1450 $cm^{-1}$ C–H deformation (18). This spectrum can be related to animal glue.

In order to confirm this, a full chromatographic analysis would be needed. The result demonstrates that the thin coating, made with protein, was intentionally applied over the gilding and it is not just dirt. This is important because during the restoration of artworks the surface dirt is removed (16).

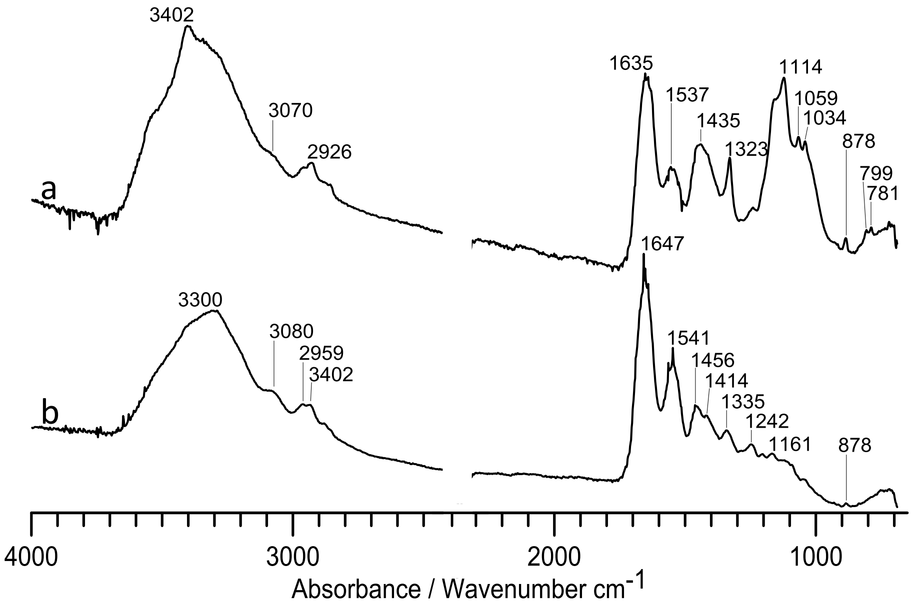

**Figure 2.21** µFTIR spectra from the superficial layer of the altarpiece a) a mixture of protein, silicates, gypsum, calcium carbonate and calcium oxalates, b) characteristic protein absorption bands.

This case demonstrates that identifying the materials to the latest extent is fundamental, as often distinguishing between the original coatings and environmental deposition is not an easy task.

### 2.6.3 Case 3: beeswax from conservation treatments and wax from natural impurities in shellac

Wax is another kind of material that is often found in artworks, either original or resulting from a restoration process as beeswax has been employed in conservation treatments to consolidate paint layers (11).

Beeswax is naturally secreted by the intraabdominal glands of bees (Apis mellifera L.). The composition of beeswax is a complex mixture which includes saturated hydrocarbons, esters and free fatty acids (19). Fatty acids from esters and free fatty acids present in beeswax are responsible for the presence of reaction compounds such as carboxylate salts, which are produced when the waxes come into contact with compounds containing metals.

Wax may also be found in other materials produced by insect secretions such as the Laccifer lacca Kerr (20). This resinous secretion, called Seed Lac once semi-refined, contains resin, dyes and 6%–7% wax (21). Seed Lac is processed by separating the wax from the principally resinous part, shellac. However, small wax impurities can still be found in shellac, which was principally used in varnishes and coatings.

A superficial treatment with beeswax from an historical restoration is found on the canvas painting entitled View of the Barcelona Port, by Francesc Soler i Rovirosa painted in 1889, located in the Museu Marítim of Barcelona (Figure 2.22). It caused a tarnishing of the paint surface, after its identification it was partly eliminated.

**Figure 2.22** Detail of the painting entitled "View of the Barcelona Port", by Francesc Soler i Rovirosa (1889). Archive image from AMPC research group (UPC).

The µFTIR spectra of this coating are displayed in Figure 2.23. Different spectra were obtained from the various samples showing that the layer was not homogeneous.

After a mechanical separation by means of specific tools, the spectrum of non-purified beeswax could be separated from the other components. This kind of wax can be differentiated from the other natural waxes traditionally employed in artworks (purified beeswax and shellac wax) by comparing to the corresponding reference spectra (22). This compound was also found in the painting layers, demonstrating its penetration.

Waxes can be recognized by the C–H stretching vibrations of $CH_2$ bonds which give strong and narrow bands at 2918 and 2850 cm$^{-1}$; a small band at 2956 cm$^{-1}$ due to the $CH_3$ group. Also sharp doublets at 1473 and 1463 cm$^{-1}$ related to $CH_2$ bending vibrations; rocking vibrations at 729 and 719 cm$^{-1}$ of the methylene groups characteristic of a compound with a long aliphatic chain; and C–H bending vibrations in the 1475–1377 cm$^{-1}$ region from aliphatic chains.

Non-purified beeswax (Figure 2.23III) shows also a C=O stretching band at 1737 cm$^{-1}$ and a C–O stretching band at 1176 cm$^{-1}$ from the ester and a band at 1712 cm$^{-1}$ from the carboxylic groups (not present in purified beeswax) and, in addition, a progression of absorption bands between 1300 and 1200 cm$^{-1}$, which are assigned to wagging and twisting vibrations of successive methylene groups coupled to a carboxyl group (not present in shellac wax) (22,23).

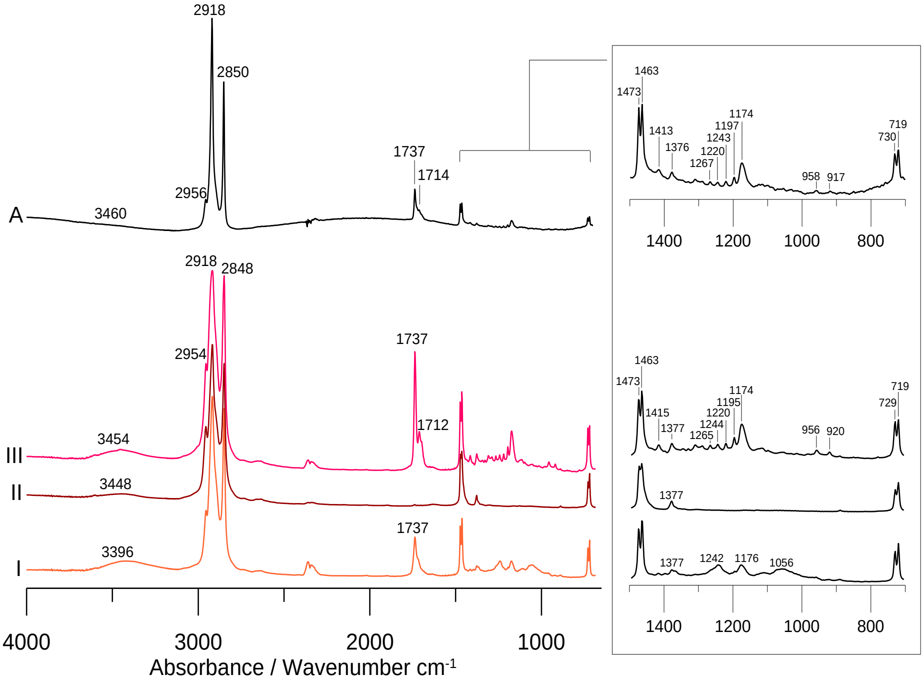

**Figure 2.23** µFTIR spectra using a diamond cell of I) shellac wax, II) microcrystalline wax and III) non-purified beeswax obtained from reference materials and A) beeswax spectrum from the superficial layer.

The presence of beeswax on the painting (Figure 2.23A) is therefore confirmed, although the 1712 cm$^{-1}$ band is unexpectedly small. This is due, in part, to the reaction of the free fatty acids from the beeswax with the metals from the pigment particles, producing carboxylates.

In fact, the presence of zinc carboxylates (bands at 1540 cm$^{-1}$, 1398 cm$^{-1}$ and 1463 cm$^{-1}$) and of copper carboxylates in smaller amounts (1586 cm$^{-1}$ band) (24,25) is confirmed, as shown in Figure 2.24. Zinc comes from the zinc pigments used as filler in the chromatic layer.

Additionally, the copper ions belong originally to the green pigment of the painting, copper acetoarsenite ($3Cu(AsO_2)_2 \cdot Cu(CH_3COO)_2$), a synthetic pigment used in the 19th century and early 20th century (26).

Finally, the presence of calcium carbonate is also determined forming a chalk layer mixed with different colour pigments. This layer was directly applied over the canvas, although not on the whole surface. This layer is porous in nature and thus, easily incorporates substances from the more superficial layers. The mixture of substances increases the complexity of the interpretation of the spectrum, and thus the best possible separation of the compounds is imperative to effectively identify the several substances present.

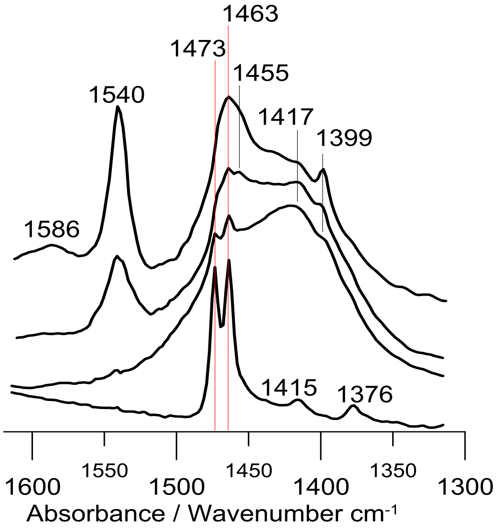

**Figure 2.24** 1300–1650 cm$^{-1}$ region of the spectra related to the compounds identified in the superficial layer.

A different story can be found in the coating applied over a silver foil on a wood moulding from the 15th century altarpiece of Our Lady by Pasqual Ortoneda, on display at the Museum Vinseum (Vilafranca del Penedès, Catalonia). The coating was applied with the double intention of protecting the silver surface from the environment and changing the colour to simulate gold (Figure 2.25) (27).

The analysis of the coating by µSR-FTIR indicates in this case the presence of shellac containing shellac wax impurities (Figure 2.26).

A noteworthy difference between the spectra of each material is the shape and position of the bands belonging to 2800–3000 cm$^{-1}$ where the $CH_3$ and $CH_2$ stretching bands appear. In the shellac wax (Figure 2.26, black line), these bands are narrower than in shellac (Figure 2.26, dashed line) due to the presence of numerous $CH_2$ groups showing long aliphatic chains from the wax compounds, and the corresponding frequencies are slightly lower owing to this. Some shellac bands hide the other shellac wax bands.

**Figure 2.25** Altarpiece of Our Lady by Pasqual Ortoneda, 15th century. Archive image from AMPC research group (UPC).

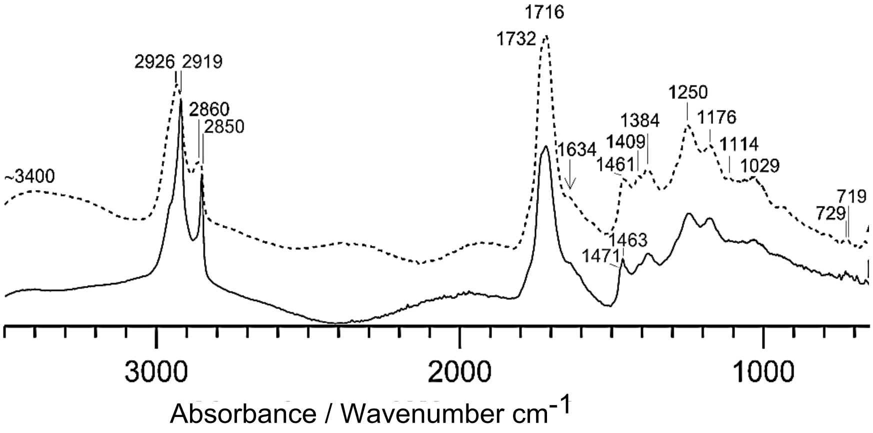

**Figure 2.26** µSR-FTIR analysis of the coating applied over a silver foil of the altarpiece of Our Lady by Pasqual Ortoneda. Dashed line corresponds to shellac and solid line to shellac with wax impurities.

The shellac wax residue in this artwork coating indicates that the shellac employed was of low purity.

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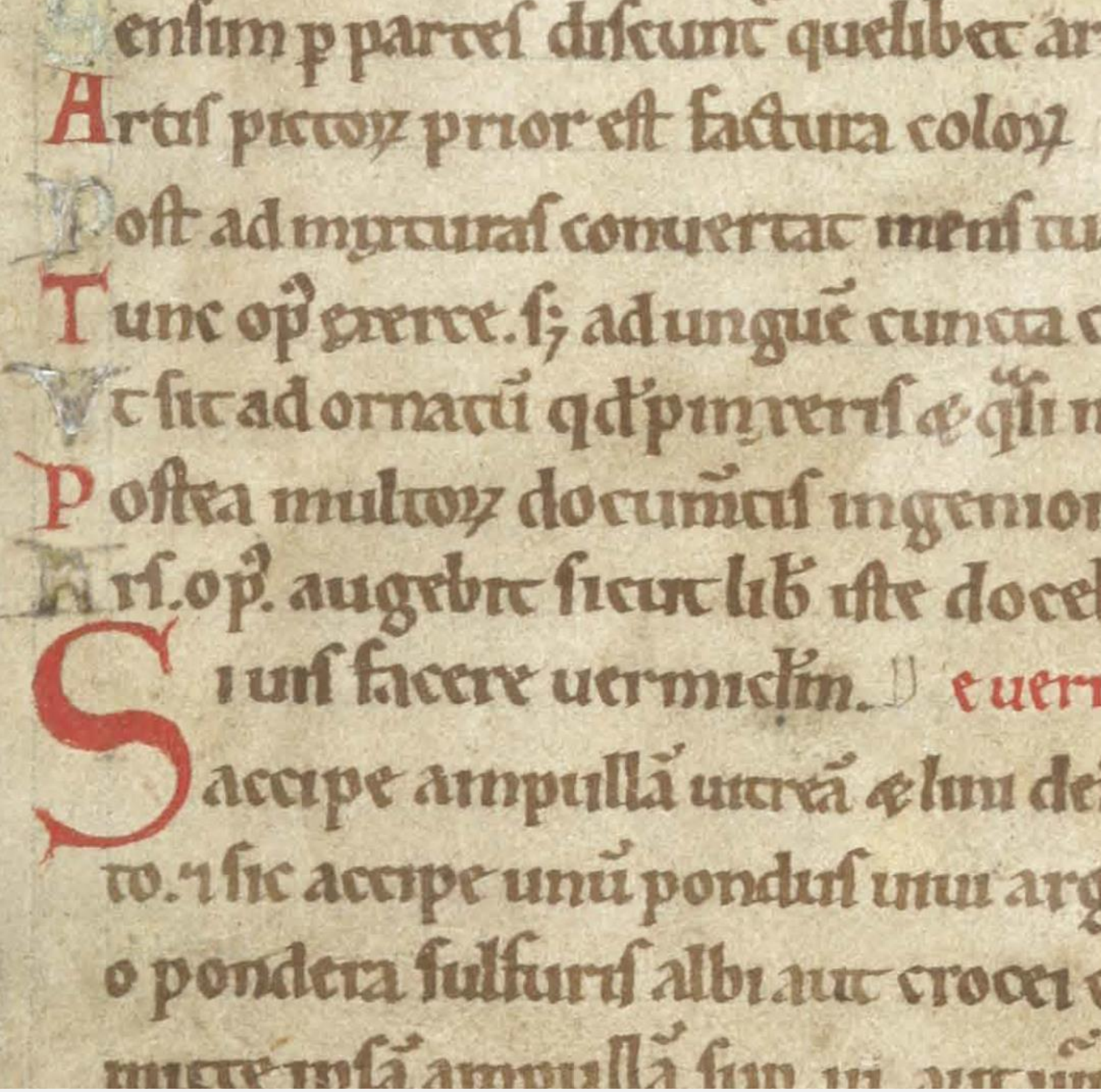
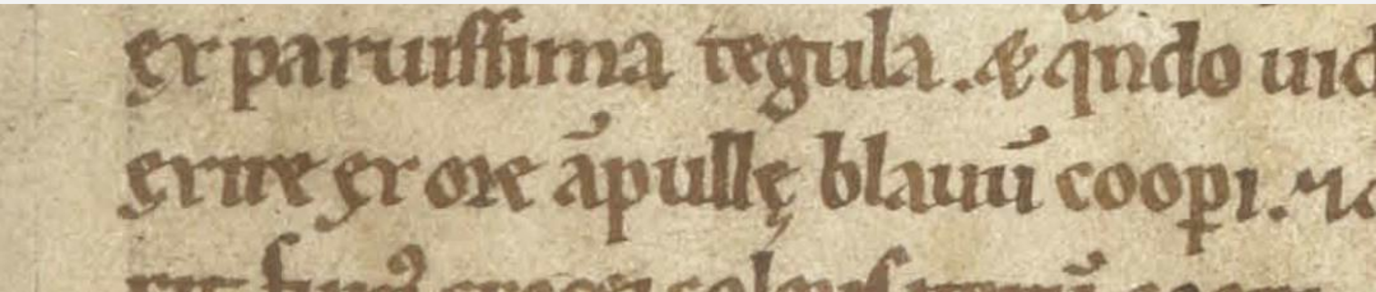


CHAPTER THREE

# Abietane-type resins in varnishes and coatings



## 3.1 NATURAL SOURCES OF ABIETANE-TYPE RESINS

Abietane-type resins obtained from *Pinaceae* genus trees have been often used in varnishes because trees which produce these resins are easily found in Europe and they exudate resin in copious amounts through the cracks of their bark (Figure 3.1).

There are three main resins used in varnishes and coatings, each one is obtained from different species: *Larix decidua* Mill., *Abies alba* Mill. and species from *Pinus* genus.

**Figure 3.1** *Pinus Pinaster* Aiton. (left) and *Pinus sylvestris* L. (right) resin exudation (Tarragona, Catalonia). Archive image from AMPC research group (UPC).

The *Pinus* genus belongs to the *Pinaceae* family and includes many species (Figure 3.2), mainly trees, found mostly in the north hemisphere (Figure 3.3) (1,2). One of the features of the *Pinus* species is that they exudate a resinous secretion known as rosin or colophony which has chemical and physical properties adequate for many uses.

*Pinus* resin is a cheap material easy to obtain so it has been widely used. Besides being used as varnishes and coatings, it has also been employed in printing inks, adhesives, soaps, food additives… (3–5).

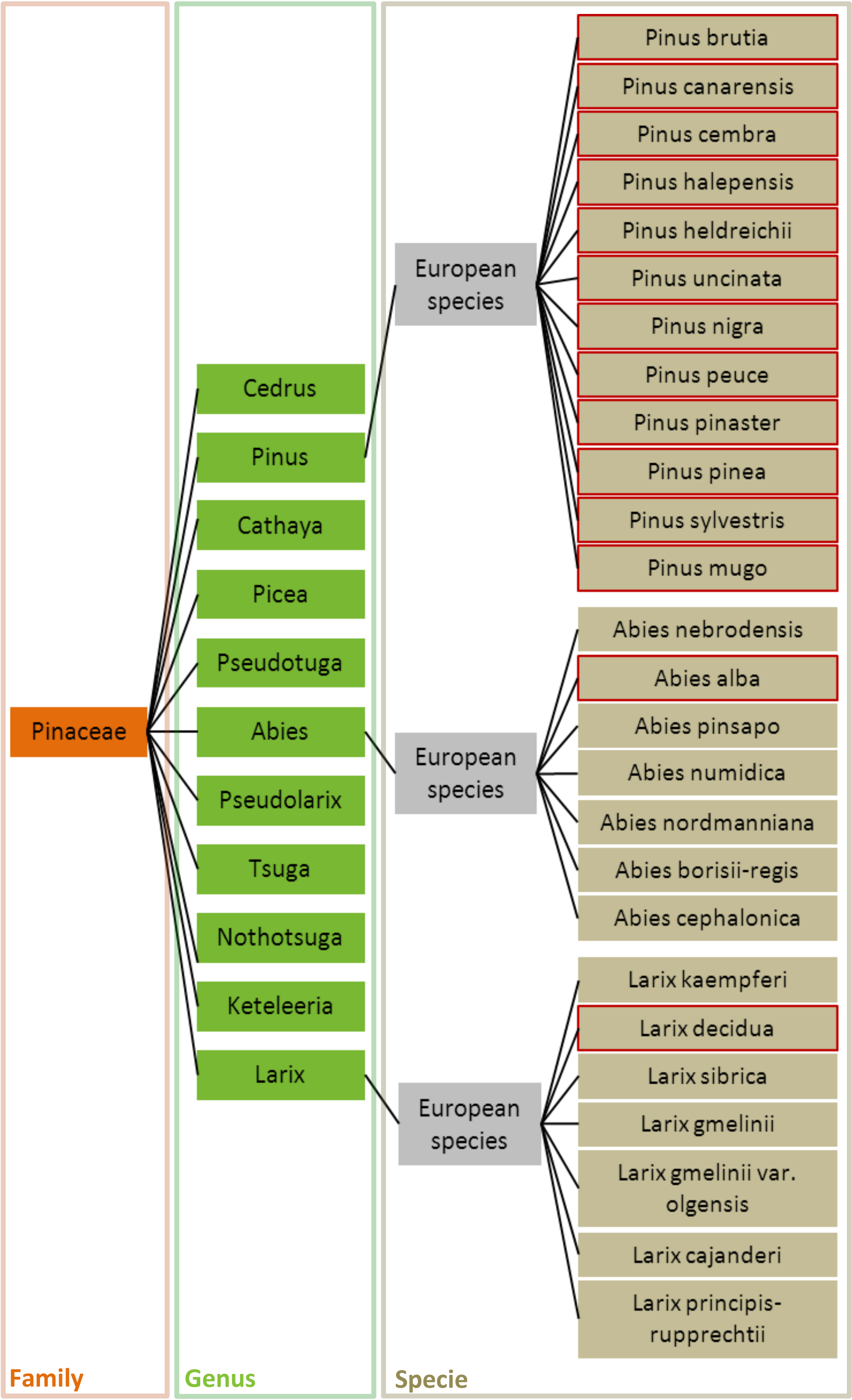


**Figure 3.2** Classification of European *Pinaceae* genus, marked in red the species used in varnishes and coatings production.

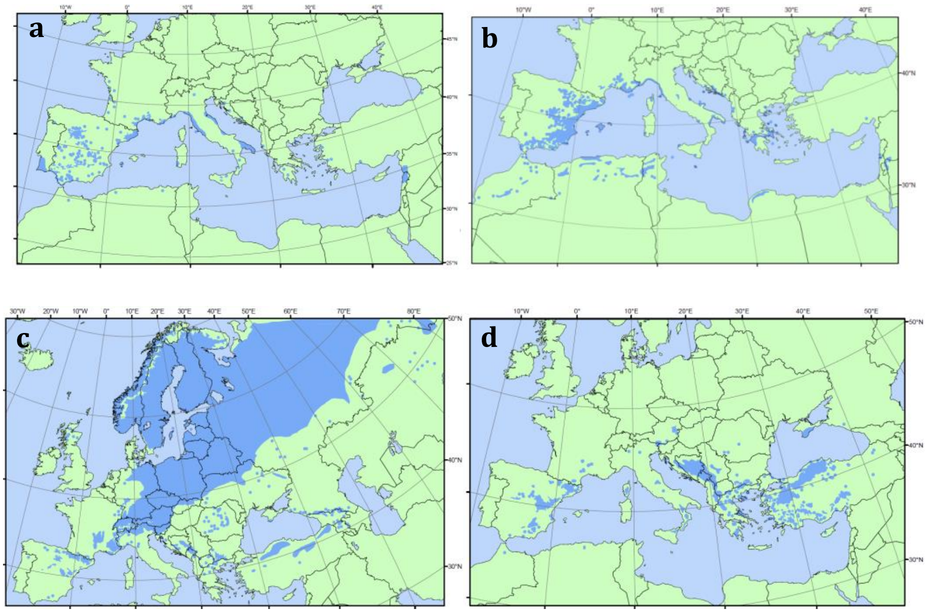


**Figure 3.3** Geographical distribution of some *Pinus* species a) *Pinus pinea* L. b) *Pinus halepensis* Mill. c) *Pinus sylvestris* L. d) *Pinus nigra* Arnold (6).

In manuscripts and art treatises, *Pinus* resin is also called pine resin, as *Pinus* trees are also commonly called pines. Besides, there are other terms which are related to the *Pinus* resin as well, such as turpentine, colophony, Greek pitch, pece greca and pine gum. Turpentine alludes to a fluid/semifluid resin, which relates to the fresh resin which still retains the monoterpenes fraction, or to the result of fresh resin distillation which contains mainly monoterpenes but also small amounts of abietanes and pimaranes. Colophony, Greek pitch and pece greca are solid, they usually refer to either the residue obtained from the distillation or to the fresh resin that has been dried or heated to eliminate the monoterpene fraction. These are not standard terms and their meanings have probably changed over the years. (7–10)

*Larix decidua* Mill. and *Abies alba* Mill. trees are less frequent than *Pinus* species. Resin from *Larix decidua* Mill. and *Abies alba* Mill. was less abundant (Figure 3.4) so it was more expensive and therefore, was considered a high quality material (7).

Manuscripts and art treatises usually refer to *Larix decidua* Mill. as Venetian turpentine, and *Abies alba* Mill. as Strasbourg turpentine of fir oil. Nevertheless, it is possible that terms like “the best turpentine” or “the lightest turpentine” were used to allude to *Larix decidua* Mill. or *Abies alba* Mill. resins as they were considered a better material than common turpentine, i. e. pine resin. Likewise, it should be taken into consideration that sometimes *Larix decidua* Mill. and *Abies alba* Mill. resins were adulterated with *Pinus* resin to reduce their price (7,9).

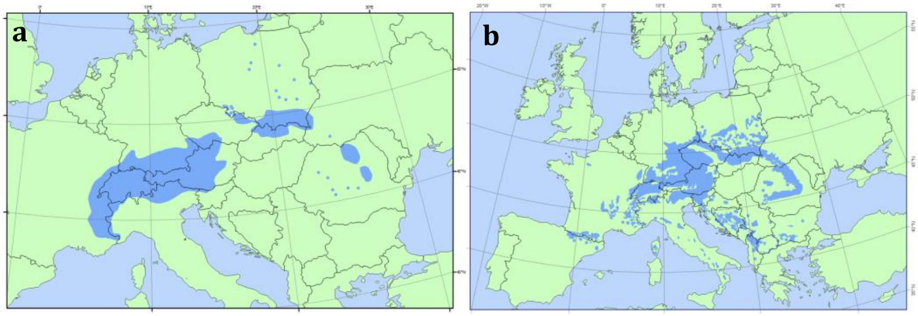


**Figure 3.4** Geographical distribution of a) *Larix decidua* Mill. and b) *Abies alba* Mill. (6).

## 3.2 HISTORICAL REFERENCES ABOUT THE USE OF DITERPERNIC RESINS IN VARNISHES

Detailed indications of the materials used in artworks and crafts can be found in art treatises and manuscripts. Some of them have survived and provide an incomparable source for the study of the ancient techniques. These texts provide information about the varnishes formulas used thorough history. (11,12)

### 3.2.1 Early varnishes

Diterpenic resins have been often mentioned in historical documents as components of varnishes. The most ancient references we have found are in the work of Pliny the Elder, *Historia Naturalis* (1st century), where he talks about *pissoceros*, a material used as a coating for ships which was made with wax and resin extracted from pines. The same mixture is also cited in the work of Pedanius Dioscorides, *De Materia Medica* (1st century), but called *zopissa* or *apochyma*. However, there are not clear references about diterpenic resin varnishes used in artworks in any of them. (13)

The first allusions to diterpenic varnishes unmistakably used for coating artworks are apparently from the early middle ages. Examples are found in the *Lucca manuscript* (late 8th or early 9th century) and in the *Mappae Clavicula* (Figure 3.5).

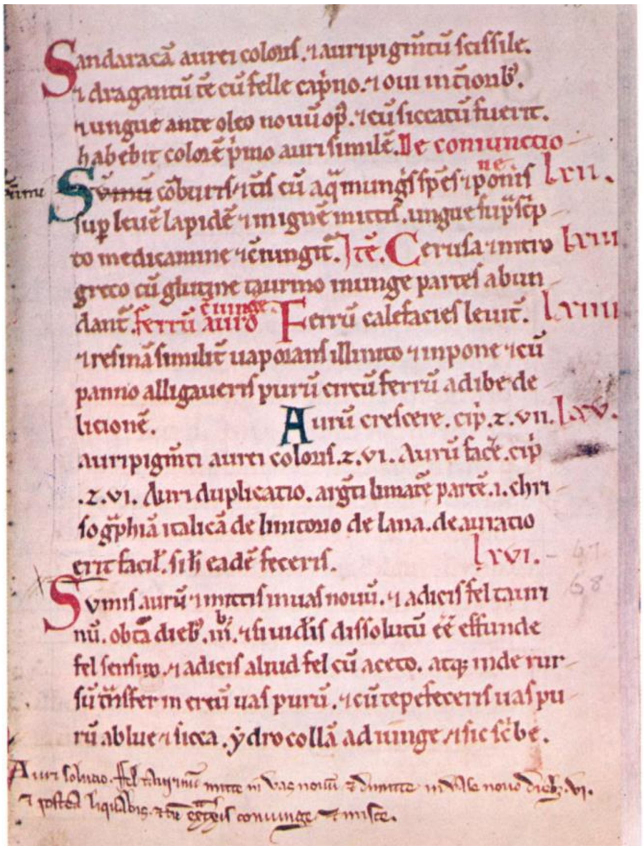


**Figure 3.5** Folio 18r of the Phillipps - Corning manuscript of the Mappae Clavicula (17).

The Lucca manuscript, also known as *Compositiones ad tingenda musiva* and *Compositiones variae* (14–16), is a profusely studied manuscript dated to late 8th or early 9th century although it is possible that some of the recipes originally belong to older manuscripts. It describes different methods and materials related to the elaboration of artworks and crafts, such as pigments, dyes, glazes… *Mappae Clavicula* is a compilation of manuscripts that contains the recipes of the *Lucca manuscript* among many others. The oldest fragment dates to the 9th century (17).

Among the recipes described in *Mappae Clavicula*,six refer to varnishes understood as artwork coatings. Three of them contain diterpenic resins among many other substances. In particular, abietane type resins like pine and larch resins are found in the first two recipes (table 3.1 and 3.2) and sandarac (in this case called *varnish*) is mentioned in all the three recipes (table 3.1, 3.2 and 3.3).

| **TABLE 3.1 Description of the varnish entitled "a recipe for a gold-coloured transparent varnish" from *mappae clavicula*** (17) |
|---|
| 5 oz. of linseed oil, 2 oz. of galbanum, 1 oz. of turpentine, 1 oz. of Spanish pitch. Melt these 3 substances (i.e., galbanum*, turpentine, Spanish pitch) into one with a little linseed oil. Now afterwards mix some linseed oil and ox glue with 2 oz. of oriental saffron, 3 oz. of frankincense, 2 oz. of myrrh, 2 oz. of mastic, 2 oz. of pine resin, 2 oz. of early-blooming poplar flower, and 2 oz. of varnish; strain into a copper bowl. When they seethe all together, mix with them 1 oz. of cherry-tree gum. When all these have been united, make them boil in 3 ounces of linseed oil by measure. After the cooking strain them through a linen cloth and mix the above-mentioned substances (i.e., galbanum, turpentine and Spanish pitch), and if later it is at all defective so that it cannot be dried, add as much mastic as you want, namely either an ounce or half an ounce, and it will be corrected.<br>* resin from *Ferula gummosa* Boiss. |

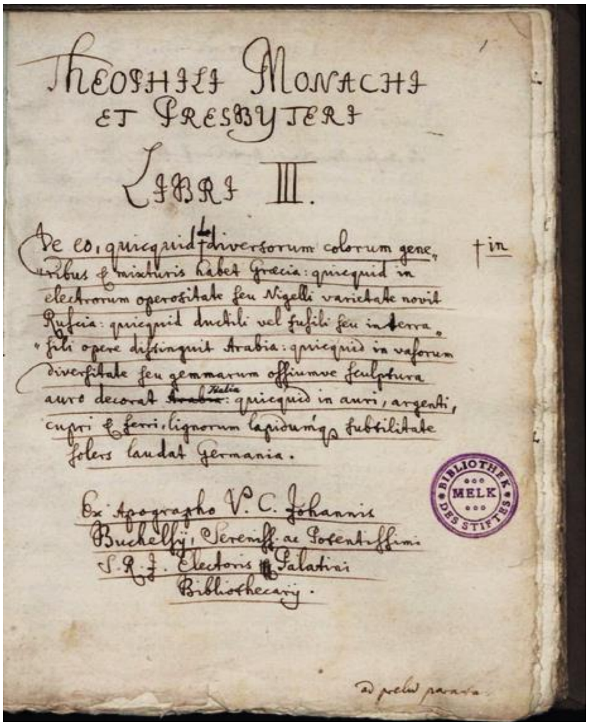
| **TABLE 3.2 Description of the varnish entitled "How transparent varnishes ought to be put over pigment" from *Mappae Clavicula*** (17) |
|---|
| 3 oz. of linseed oil, 3 oz. of turpentine, 2 oz. of galbanum, 2 oz. of larch, 3 oz. of frankincense, 3 oz. of myrrh, 3 oz. of mastic, 1 oz. of varnish, 2 oz. of cherry-gum, 2 oz. of poplar flowers, 3 oz. of almond-gum, 2 oz. of pine resin. All these should be pounded and sifted, then [put] into a brass dish with the above-mentioned linseed oil; and they should be put into a hot oven where they may be cooked without flame in such a way that they do not evaporate. And when they have been cooked, they should be strained through a clean linen cloth: and if they come out thin, cook them down until they become thick. Afterwards you will be able to varnish what- ever kind of work you want, either paintings or carved work. When they are varnished, leave them to dry |

| **TABLE 3.3 Description of the varnish entitled "Making Greek glue" from *Mappae Clavicula*** (17) |
|---|
| Make a flour out of varnish by grinding it on marble and sifting it. Put it into a rough cooking pot, thoroughly sealed with a cover, so made that there is a small hole in the middle of the cover and in this hole a pointed iron rod. Now, put it on iron sheets on a goldsmith's forge, the fire in which should have been previously started. Then put dry sticks of wood, cut up very small underneath [the pot] and [the varnish flour] melts as soon as it grows hot. Take out the iron rod and put a little drop on your fingernail; and if it seems liquid, take it off the fire and pour on 2 parts of oil squeezed from flaxseed [i.e. |

| |
|---|
| linseed oil] to 1 part of varnish, and again put wood underneath and cook a short hour, and use it. But if there is a grain of mastic [in it], it liquefies more slowly |

The methods mentioned in the texts are quite different among them: the first ones (table 3.1 and 3.2) include a wide range of materials and complex procedures while the second includes only two substances and a much simpler processing. In later treatises this kind of simple methods are more frequent and, indeed, the simplest varnish made only of a resin mixed with a solvent (like drying oil) is often mentioned.

Another important source for the study of medieval technology is the manuscript commonly called *Schedula Diversarum Artium* attributed to Theophilus Presbyter, who has been identified as Roger of Helmarshausen (18) (Figure 3.6). The text is an original work with detailed insights and practical information about the techniques used in the applied arts. Although the original manuscript did not survive there are many copies of it, the earliest from 12th century, that allowed to date the original manuscript from, at least, the beginning of 12th century.



THEOPHILI MONACHI
ET PRESBYTERI
LIBRI III.

De eo, quicquid † diverſorum colorum gene- † in
ribus & mixturis habet Græcia: quicquid in
electrorum operoſitate ſeu Nigelli varietate novit
Ruſcia: quicquid ductili vel fuſili ſeu interra-
ſili opere diſtinguit Arabia: quicquid in vaſorum
diverſitate ſeu gemmarum oſſiumve ſculptura
auro decorat ~~Arabia~~ Italia: quicquid in auri, argenti,
cupri & ferri, lignorum lapidumque ſubtilitate
ſolers laudat Germania.

Ex Apographo V. C. Johannis
Buchelfij, Sereniſſ. ac Potentiſſimi
S.R.I. Electoris Palatini
Bibliothecarij.

ad prelum parata

**Figure 3.6** *Schedula Diversarum Artium* from the Library of the Melk Abbey (Ms. 768, fol. 1r) (54).

Two simple recipes of artwork varnishes using drying oil and a resin are described in the *Schedula Diversarum Artium* (table 3.4). Many different opinions have been formulated about what exactly refers to *goma fornis*, mentioned in the text, as this term does not appear in other treatises. It is not clear if it refers to amber, to sandarac (which was also called *glassa*) (18,19) or if it corresponds to amber in the first recipe and to sandarac in the second one (13). Anyway it is another example of a varnish made of a mixture of diterpenic resin and drying oil.

**TABLE 3.4 Description of the varnish entitled "Of the varnish gluten" from *Schedula Diversarum Artium*** (18)

Put linseed oil into a small new pot, and add, very finely powdered, the gum which is called fornis, which has the appearance of the most lucid Thus, but when broken, it yields a brighter lustre. When you have placed which over the fire, cook carefully, so that it may not boil up, until a third part is consumed, and guard against the flame, because it is very dangerous and is extinguished with difficulty if it be raised. Every painting, covered over with this gluten, is made both beautiful and for ever durable.

Another method - Place together four stones which may be able to sustain the fire without flying to pieces, and place a common pot upon them, and put into it the above mentioned gum fornis, which in Romaic is called glassa, and upon the mouth of this pot place a smaller pot, which has a small hole in the bottom, and lute a paste round it, so that no vapour may come out between these pots. Then place fire carefully underneath, until this gum liquefies. You will also have a thin iron rod fitted to a handle, with which you will stir this gum, and with which you can feel when it is quite liquid. Have also a third pot nigh, placed upon the coals, in which is hot linseed oil, and when the gum is quite liquid, so that the iron being extracted a kind of thread is drawn out with it, pour the hot oil into it and stir it with the iron, and thus cook them together that they boil not violently, and at times draw out the iron and daub a little over a piece of wood or stone, to try its substance. And take care in this that in weight there are two parts of oil and the third part of gum. And when you have carefully cooked it to your wish, removing it from the fire and uncovering it, allow it to cool.

A similar varnish (table 3.5) made with drying oil and resin can be found in the treatise of Eraclius (also known as Heraclius) commonly called *De coloribus et artibus Romanorum*. This work contains detailed instructions about the materials used in artworks like pigments, gildings or binders.

The original manuscript did not survive, but fortunately it was a widespread text and many transcriptions have been preserved. The oldest copies date from the

13th and 15th centuries respectively; the latest included in the compilation of manuscripts of Jehan Le Begue, though it appears that the original manuscript should be dated before the 10th century (9).

**TABLE 3.5 Description of the varnish entitled "Of auripetrum. — Secundum Magistrum R." from *De coloribus et artibus Romanorum*** (9)

Take oil made from linseed, and put it into a new jar, and take the bark of vesprum very well dried and well ground in a mortar, and let it He for a night in the oil. The next day boil it as long as you may think proper, but not much, and then pas it through a cloth into another jar, and boil it again a little over the fire with myrrh and aloes. Strain it again, and immediately put vernix with it, and heat it upon the coals. But if you have no vernix take glassa and put it with the aloes and myrrh instead of vernix and, as I said before, strain it again. If you have not the bark of vesprum take dry incaustum or else the bark of black-thorn dried and ground, and, as I said before, boil it with the myrrh and aloes, and afterwards remove it from the fire, and when it is cold, put it away in a vase to preserve as long as you like. You must collect the bark in March or April, and dry it in May, and keep it as long as you like.

The diterpenic varnish mentioned in *De coloribus et artibus Romanorum* contains sandarac (in this case called *vernix*). It is not clear if this varnish was used only for gildings or also over paintings and artworks. Anyway, it is another evidence that sandarac was a common material from the earliest times and that the mixture of sandarac and drying oil was frequently used as a varnish. This is also confirmed with the methods and materials described in Mount Athos Manuscript (11th century) (18).

### 3.2.2 Historical sources of the late medieval period and Renaissance

More original texts are preserved from the late medieval period even though they are chiefly anonymous. In these sources, varnishes made with resin and drying oil are also described, but the use of abietane-type resins is more frequent.

Some examples of varnish formulas made with abietane-type resins and drying oil are found, for instance, in the Byzantine Manuscript where a varnish recipe made with *pegola* and a drying oil (here called *peseri*) (table 3.6) is described. In the same text, another method for preparing *pegola* by heating fir resin (table 3.7), probably to evaporate the volatile compounds, is also detailed.

The term *pegola* alludes to pine resin in other treatises, nevertheless in this manuscript refers explicitly to fir resin as it can be seen in table 3.7, demosntrating how the meaning of some terms may change depending on the historical source (13).

**TABLE 3.6 Description of the varnish from the Byzantine Manuscript** (13)

Take of peseri which has been baked in the sun four parts, of pegola three parts. Put them in a vessel on the fire to melt them together. Strain this varnish, and, in using it, expose the picture to the sun. Take care to let the first coat be as thin as possible, to avoid bubbles. If the mixture be too thick, so as to be difficult to spread, add naptha or raw peseri. If you have a good stock of mastic, take two parts of pegola and one of mastic [instead of three of pegola]. This mixture will give you a very good brilliant varnish.

**TABLE 3.7 Description of the preparation of *pegola* from the Byzantine Manuscript** (13)

Take fir resin in the quantity required; place it in a copper vessel (which it should only half fill), and set it on the fire. Take care that it does not run over; if you see it rise, remove it from the fire, and blow on it with a reed, or place the vessel in another that is full of cold water ; this instantly stops the tumefaction. Replace it on the fire, and repeat the operation several times, till the resin ceases to swell. Thus pegola is prepared. Remove it from the fire, and pour it into a copper vessel full of water, ready for the purpose. Afterwards gather up the pegola and preserve it.

Nonetheless, the most frequent varnish formulas include oil, sandarac and sometimes abietane type resins.

An example of varnish formulas including oil, sandarac and pine resin can be found in the Sloane Manuscript 2584 (13,18), dating from early 14th century. In this manuscript, a varnish (table 3.8) made of two diterpenic resins, turpentine (called here *terbentyne*, although it is not specified if the material has been processed) and sandarac (called here *frankensence*).

**TABLE 3.8 Description of the varnish from the Sloane Manuscript 2584** (18)

Take of terbentyne 1lb., of gume arabyk 1lb., of frankensence 1lb., and melt them togeder, and put there to oyle of Lynsed als a mochel as it nedes: and thus you schalt assay zif it be wele molten to geder. Take a drope or 2 of clere water and sprinkle therein, and then take a litel there of be twene your fingers and zif it is helding togeder, als wer gumed, it is goode, and zif it is nost so put yet more oyle (sic)

In the Venetian Manuscript (first half of 14th century) three recipes of varnishes which also use both sandarac and turpentine together with drying oil (table 3.9, 3.10 and 3.12) are found.  Nonetheless, there is another recipe including only sandarac and drying oil (table 3.11) (13,20).

| **TABLE 3.9 Description of the varnish from the Venetian Manuscript** (13) |
|---|
| Pulverised sandarac is added by degrees to the boiled oil; concrete turpentine (pece Greca), in the proportion of two thirds to the quantity of oil |

| **TABLE 3.10 Description of the varnish entitled "To make Vernice liquida" from the Venetian Manuscript** (13) |
|---|
| Take of sandarac not pulverised one lb., linseed oil three lb., concrete turpentine three lb.; this will be good for varnishing cross-bows. |

| **TABLE 3.11 Description of the varnish entitled "To make Painters' Varnish" from the Venetian Manuscript** (13) |
|---|
| Take of linseed oil four oz., boil it in a copper vessel, removing the scum as long as it forms any; then take an oz. and half of sandarac in grain, and put it in another vessel, with a little of the aforesaid boiled oil in the bottom. Let it boil, and continue to add the oil, little by little, till you have poured in all. Let the ingredients still boil, for the more they boil the better [the varnish will be]; and take care that the fire does not reach the oil. This is a good varnish for varnishing whatever you please." |

| **TABLE 3.12 Description of the varnish from the Venetian Manuscript** (13) |
|---|
| Take yolks of eggs and vernice liquida, equal quantities, incorporate them well, and apply the mixture, as a coating, with the brush. It is proof against water and everything else. |

It is noteworthy that one of the varnishes made with turpentine (table 3.10) is recommended for its use on crossbows. Conversely, the method described in table 3.11 is specifically recommended for paintings and, in particular, the last recipe (table 3.12) is directly related to the Italian painter Alesso Baldovinetti (1425 – 1499), who used it to protect his artworks from damp (21). All these suggest that the varnishes which also include turpentine were considered of lower quality, so more appropriate for daily objects like crossbows.

Another example of the combined use of sandarac and pine resins in varnishes are found in the formulas described in the Sloane manuscript 416 (table 3.13 and 3.14), dated from first half of 15th century (13,22).

The first recipe (table 3.13) is very similar to the one described in the Venetian Manuscript (table 3.10) where it is again recommended for crossbows. The second recipe is recommended to painters and includes pine resin (in this case called Greek pitch) only as an optional ingredient; thus, asserting the use of sandarac and drying oil mixtures for paintings varnish and the addition of pine resin for daily objects.

Besides the varnishes made with pine resin there are also formulas including only sandarac and oil, sometimes with additional materials but without pine or any other resin. This is the case of the varnishes described in the Bolognese Manuscript 165, dated from first half of 15th century (table 3.15, 3.16 and 3.17). It is remarkable that pine resin is cited in formulas for other purposes but it is not included in any varnish recipe.

**TABLE 3.13 Description of the varnish entitled "To make vernice liquida" from the Sloane Manuscript 416** (18)

| |
|---|
| Take vernix, whole, lb 1 ; linseed oil, lb 1; Greek pitch (common white pine resin from which the oil has been evaporated over hot water) 3 lb. It will be good for varnishing crossbows. |

**TABLE 3.14 Description of the varnish entitled "To make painters' varnish" from the Sloane Manuscript 416** (18)

| |
|---|
| Take the quantity you please of linseed oil, place it on the fire and boil it until upon putting a pen into the boiling oil it warps as if burnt; and when it is cooked, take it from the fire, and cast pounded and sifted vernix into the said oil, little by little, and remember not to put too much at a time, because it will rise up and swell so as to run over. And when you have put in all the vernix, replace it for a short time upon the fire until it has become slightly hot again, then strain it and it is done.<br>Note — that the best manner to make it is, by putting to the said oil, Greek pitch, two parts as much of the resin as there is oil. And a third way of making it is by putting to the same oil, when cooked, as much resin as there is oil, and this is according to Nicolao di Bertoldo. |

**TABLE 3.15 Description of the varnish entitled “To make liquid varnish in another manner” from the Bolognese Manuscript 165** (9)

Take 1 lb. of linseed oil, and put it into a new glazed jar, and then take ½ a quarter [of an ounce] of roche alum in powder, and an equal quantity of minium or vermilion ground fine, and ½ oz. of incense also ground fine. Mix all these ingredients together and put them into the oil to boil, stirring it with a stick; and when the oil is boiling, as it is likely to run over, have another glazed jar ready, and put it by that which contains the oil, so as to catch the oil that runs over, in order that it may not ran on the ground, and in this manner make it boil up 3 or 4 times, and each time pour back what has run over, on that which is boiling in the jar. Having done this set fire to the oil on the right hand side with a lighted straw, and let the oil burn on the upper part, but so that the jar may not burn on the inside, on account of the too great heat, for otherwise the oil would smell unpleasantly. When you light the oil with the straw, remove the jar from the fire, and let it bum while you can say three paternosters, then extinguish the oil with a wooden cover, putting it upon, the jar, and when it is extinguished, remove the cover in order to let the vapour escape. Then put it back over the fire; do this 3 times, and it is done.

**TABLE 3.16 Description of the varnish entitled “To make vernice liquida” from the Bolognese Manuscript 165** (9)

Take of the gum of die juniper [sandarac], two parts, and one part of linseed oil, boil them together over a slow fire, and if the varnish appears to you to be too stiff; add more of the oil and take care not to let it catch fire, because you would not be able to extinguish it, and even if you could extinguish it, the varnish would be dark and unsightly. Let it boil for half an hour, and it will be done.

**TABLE 3.17 Description of the varnish entitled “To make good vernice liquida” from the Bolognese Manuscript 165** (9)

Take 2 lbs. of common oil, and 2 lbs. of fresh linseed, and boil them together in a glazed pipkin until it is reduced one-half, and then pour it into another glazed vase, such as a pipkin, and take a tripod and place the pipkin on it and make a clear fire under it, and when the liquid begins to boil add to it 30 or 40 cloves of garlic, cleaned and scraped fine, and a little roche alum at discretion, and let it boil ; and if you wish to know when it is well done, take a hen's feather and dip it in the mixture. If the feather is burnt it is done well ; take it from the fire, and before it is cold add to it one pound of sandarac well pounded, a little at a time, and keep continually stirring it round with stick, and when it is nearly cold add six or eight whites of egg, well beaten and cleared, as they are used for vermilion, and mix the whole well, and then place the varnish for one day in the sun, stir it every hour, and keep it in a cool place, and it will be good.

Another example of a varnish made with sandarac and oil, without pine resin, can be found in the Sloane Manuscript 345 (second half of 15th century). The same formula is also in the Strasbourg Manuscript (early 15th century) (table 3.18) (13,23) and a very similar one can be found in the additional recipes of the Le Begue Manuscript (sandarac is called here *glassa*) (table 3.19) (9).

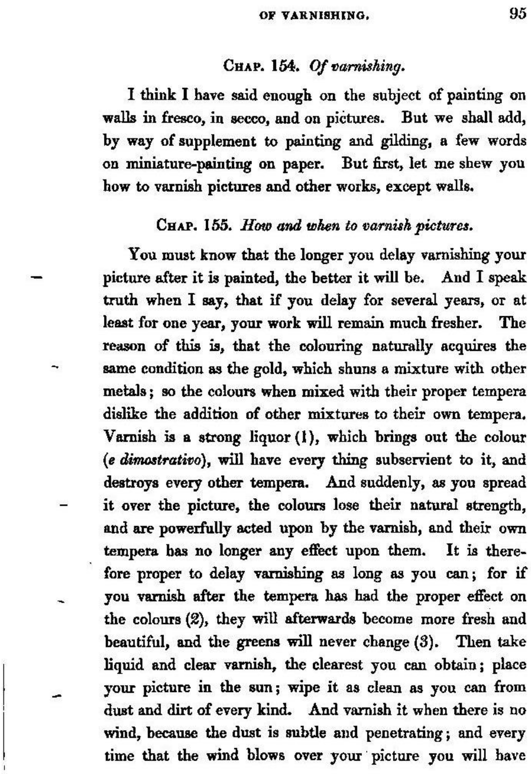
| **TABLE 3.18 Description of the varnish entitled "To make good vernice liquida" from the Bologna Manuscript 165** (13) |
|---|
| In the first place take 1lib. of sandarac or of mastic, whichever you please, and pulverise it in a clean mortar. Then take 3 lb. of linseed oil, or hempseed oil, or old nut oil, and boil this in a clean vessel skimming it and taking care, above all, that it does not run over. After it has boiled and has been skimmed throw in and stir the powdered resin little by little in the boiling oil: thus the powder dissolves in the oil. When it is quite dissolved let the varnish seethe gently with a moderate heat, stirring it continually that it may not burn; and when you find that the composition has become thick, like melted honey, take a drop of the varnish on a knife, and, after suffering it to cool a little, touch it and draw your finger slowly off; if the varnish strings it is well boiled, but if not, boil it better till it strings. Then take it from the fire and suffer it to cool; strain it through a strong. |

| **TABLE 3.19 Description of the varnish entitled "To make a good liquid varnish for painters" from the Le Begue Manuscript** (9) |
|---|
| Take glasse aromatique, which is dark or dull outside, and inside when broken is clear and shining like glass; put some of it in a new jar, which must stand on the mouth of another jar, which must be well luted to it. The upper jar must be well covered so as to be smoke-proof, and its bottom must be pierced. Then light a fire beneath it, and leave it until the glasse is melted, when you must take two parts of linseed, or hemp-seed, or nult-oil, and heat this oil slowly over a fire, not making it too hot. You most then poor it on to the said glasse, make the fire hotter, and let it boil for an hour, taking care that the flame does not touch it. Then take it off the fire and put it into a clean vessel, and when you wish to varnish any dry painting take some of this liquid and spread it over the painting with your fingers, for if you were to do it with a pencil it 'would be too thick and would not dry. You will thus have good varnish. |

The famous treatise from Cennino Cennini entitled *Il Libro dell'Arte* (early 15th century) also details varnish formulas. This widespread treatise gives many details about the painting technique and, fortunately, many transcriptions have survived. Knowing that the *Il Libro dell'Arte* treatise is recommended to painters and painter apprentices, the varnishes described should be used on paintings and not in other objects (Figure 3.7).

Cennini does not give specific recipes or formulas, but he explains how varnish should be applied and which materials should be used. Specifically, he recommends the use of *vernice liquida*, which he defines as the mixture of sandarac and linseed oil (13,24).

This agrees with the notes of contemporary painters like Leonardo da Vinci, who also recommends the use of a varnish made with sandarac and a drying oil (25); and agrees with other contemporary treatises which also recommend using varnishes made mainly by sandarac and a drying oil.



OF VARNISHING. 95

CHAP. 154. *Of varnishing.*

I think I have said enough on the subject of painting on walls in fresco, in secco, and on pictures. But we shall add, by way of supplement to painting and gilding, a few words on miniature-painting on paper. But first, let me shew you how to varnish pictures and other works, except walls.

CHAP. 155. *How and when to varnish pictures.*

You must know that the longer you delay varnishing your picture after it is painted, the better it will be. And I speak truth when I say, that if you delay for several years, or at least for one year, your work will remain much fresher. The reason of this is, that the colouring naturally acquires the same condition as the gold, which shuns a mixture with other metals; so the colours when mixed with their proper tempera dislike the addition of other mixtures to their own tempera. Varnish is a strong liquor (1), which brings out the colour (*e dimostrativo*), will have every thing subservient to it, and destroys every other tempera. And suddenly, as you spread it over the picture, the colours lose their natural strength, and are powerfully acted upon by the varnish, and their own tempera has no longer any effect upon them. It is therefore proper to delay varnishing as long as you can; for if you varnish after the tempera has had the proper effect on the colours (2), they will afterwards become more fresh and beautiful, and the greens will never change (3). Then take liquid and clear varnish, the clearest you can obtain; place your picture in the sun; wipe it as clean as you can from dust and dirt of every kind. And varnish it when there is no wind, because the dust is subtle and penetrating; and every time that the wind blows over your picture you will have

**Figure 3.7** Transcription of the beginning of the chapter about varnishes from the treatise entitled *Il Libro dell'Arte* from Cennino Cennini (55).

### 3.2.3 Historical sources of the modern era

There are many treatises and manuscripts from this period. In part due to the invention and popularization of the printing press but also because more texts from this period have survived.

During the modern era, treatises still describe the varnishes made with oil and sandarac. Nevertheless, abietane-type resins appear also in the varnish formulas. Not only pine resin, but also other substances are mentioned like *Larix Decidua* Mill. (Venetian turpentine) and *Abies alba* Mill. (Strasbourg turpentine), which were better considered than pine resin.

An example of the use of *Abies alba* Mill. is seen in the treatise of Giovanni Battista Armenini entitled *De' veri precetti della pittura* (26), were he describes its use (here called *olio di abezzo chiaro*, i. e. clear fir oil) mixed with mineral oil obtained from Sasso (here called *olio di sasso*, i. e. oil from sasso) (27) to obtain a high quality varnish (table 3.20). Similar varnishes were used by painters like Joshua Reynolds (18th century) (28).

Armenini also details two more varnishes made of sandarac and abietane-type resins (table 3.21 and 3.22), without any comment about their quality. However, as long as the manuscript is focused in painting materials they are probably recommended for paintings.

| **TABLE 3.20 Description of the varnish from the De' veri precetti della pittura treatise** (26) |
|---|
| Alcuni dunque pigliavano dell'olio di abezzo chiaro, e lo facevano disfare in un pignaltino a lento fuoco, e disfatto bene gli ponevano tant' altro olio di sasso, gettandovelo dentro subito, che essi lo levavano dal fuoco, e mesticando con la mano così caldo, lo stendevano sopra il lavoro prima posto al Sole , e alquanto caldo, sicché toccavano con quella da per tutto egualmente; e questa vernice è tenuta la più sottile e più lustra d'ogni altra, che si faccia. lo ho veduto usarla cosi per tutta la Lombardia dai più valenti: e mi fu detto che cosi era quella adoprata dal Correggio e dal Parmigiano nelle sue opere, s'egli si può credere a quelli, che gli furono discepoli.<br>Some of them took clear fir oil and melted it over slow heat with the same amount of sasso oil [mineral oil] for a short time. It was applied over the work, previously exposed to the sunlight, when it was still hot. This was considered the thinnest and shiniest varnish. I have seen it is used in this way along all Lombardy by the bravest ones and they told me that this was the varnish used by Correggio and Parmigiano in their artworks, and also their disciples. (author's translation) |

**TABLE 3.21 Description of the varnish from the De' veri precetti della pittura treatise** (26)

Ci sono alcuni, che pigliano un'oncia di sandracca, e un quarto di pece greca, e ne fanno polvere col pestarla insieme, e la fanno passare per setaccio; di poi posta in un pignattino nuovo, la cuoprono bene con acquavite di tre colle, e la fanno bollire al fuoco ben lento per fino che è ben disfatta; di poi si lascia raffreddare innanzi che si adopri, e si lieti sempre coperta, e quando si vuole adoperare si scalda a lento fuoco: questa è buona sulle tele a secco.

Some of them took one ounce of sandarac and one ounce of pece greca, and they grind it together and strain the dust. Then, they put the dust in a container and they cover it with acquavite di tre colle [ethanol solution], and it is boiled over low heat until it is melted. It is left until it is cold before use it, it should be covered; it has to be heated before using it. It is good for dry fabric. (author's translation)

**TABLE 3.22 Description of the varnish from the De' veri precetti della pittura treatise** (26)

Altri ancora pigliano tanto mastice quanta sandaracca, e ne fanno sottilissime polveri, e le cuoprono con olio di noce al fuoco nel mudo delle altre di sopra, le quali poi colate, vi aggiungono un terzo di olio di abezzo, e lo incorporano con quelle, ma vogliono bollir poco, perché la vernice verrebbe viscosa : e tutte queste predette vernici, mentre si fanno disfare al fuoco, si mesticano sempre con una picciola bacchetta, le quali poi, coperte nel loro vasetto , si conservano lungo tempo, con farsi più purgate e sottili.

Other ones take as much mastic as sandarac, and grind it to a fine powder, then it is covered with nut oil like the second recipe (table 3.21), then it is strained and a third of fir oil is added. It has to be boiled slightly because if not, it will be too thick. All these varnishes have to be stirred with a stick while they are being heated. If varnishes are covered they can be kept for a long time. (author's translation)

The use of *Abies alba* Mill. is also reported in the manuscript entitled *Raccolti di Secreti, Specifici, Remedj, ora atesso di Fra Fortunato da Rovigo, Laico Capucino, Infermiere nel convento dei Capucini di Verona* (early 17th century). In this manuscript, the use of varnishes made with sandarac, fir oil and turpentine is recommended. However, no indications about the quality of the varnishes obtained are given (9).

Nonetheless, varnish formulas containing pine resin are also frequent. Examples can be found in the Brussels Manuscript 15552 entitled *Recueuil de essaies des* *meraveilles de la peinture* written by Pierre le Brun (first half of 17th century) (table 3.23 and 3.24).

| **TABLE 3.23 Description of the varnish entitled "Fine varnish" from the Recueuil de essaies des meraveilles de la peinture** (9) |
|---|
| Is made with turpentine melted over the fire; when melted, remove it from the fire, and add oil of spike with mastic, and, if required, sandarac. |

| **TABLE 3.24 Description of the varnish entitled "Gros varnish" from the Recueuil de essaies des meraveilles de la peinture** (9) |
|---|
| Is made with turpentine, oil of turpentine, and resin, melted up together. |

Another example of a varnish formula containing pine resin is given in the manuscript entitled *Racolta di varj secreti si per far colori da miniare che di vernici d'ogni sorte et altri per varj mali, provati effica(ci)ssimi, come ancora, alcuni attenenti all'Arte degl'Orefici*, which describes recipes from the 15th and 16th century. The use of sandarac with small amounts of turpentine is detailed. Moreover, there are formulas containing light turpentine: the emphasis in the light colour possibly alludes to *Larix Decidua* Mill. or *Abies alba* Mill. resin (29) instead of regular pine resin.

The use of sandarac varnishes is described in many manuscripts and art treatises. Among them we can highlight the Marciana manuscript and the treatise entitled *El arte de la pintura*, (first half 16th century).

The Marciana manuscript is actually a compilation of manuscripts entitled *Secreti Diversi*, probably made by a monk, from early 16th century and located in the National Library of St Mark of Venice (Italy).

There is one varnish formula with sandarac (table 3.25), which is specially recommended for artworks. Additionally, there are four more methods with pine resin, one of them is recommended for paintings (table 3.26) but the other three are for daily objects such as armours and musical instruments (table 3.27 to 3.29). This again agrees with the tendency observed of sandarac being a more suitable varnish for paintings while pine resin for other objects.

Finally, a varnish formula containing only *Abies alba* Mill. (table 3.30), like the method described in *De' veri precetti della pittura* (table 3.20), is found.

**TABLE 3.25 Description of the varnish entitled "varnish tried by Master Jacopo de Monte San Savino, the Sculptor, which is proper for every kind of work and on all materials" from the Marciana manuscript** (9)

Take one ounce of sandarac, ground to a very fine powder, and 3 ounces of clear nut oil. Heat the oil in a glazed pipkin over a slow fire in the same manner as linseed oil is boiled; then add the powdered sandarac a little at a time until it is dissolved ; add to it also at the same time so much clear incense finely powdered as will impart a pleasant savour to the whole mixture, stirring it well that it may dissolve, and, if you please, you may also add a sufficient quantity of burnt and pounded roche alum to have a sensible effect on the whole composition ; and the addition of the alum will improve the varnish if you stir it until it is dissolved. It should then be strained through a linen cloth, and afterwards exposed to the sun and dew until a sediment is formed, which should be separated by pouring off the clear varnish, after which it will be ready for use.

**TABLE 3.26 Description of the varnish entitled "varnish which spreads like oil, dries quickly, and is very lustrous and beautiful, appearing like a glass mirror, and which is admirable for adhering firmly and for varnishing lutes and similar things" from the Marciana manuscript** (9)

Take one pound of linseed oil, boil it in the proper manner in a clean glazed pipkin, add to it half a pound of well pulverized clear and fine Greek pitch, and stir and incorporate the whole over a slow fire ; then add half a pound of powdered mastic, and the moment you have done so, withdraw the pipkin gradually from the fire, because it swells up, and incorporate the ingredients thoroughly; then replace the pipkin on the fire, and keep it there until everything is well dissolved and mixed, when some burnt and pounded roche alum of the size of a nut should be added and mixed, until that also is entirely dissolved and incorporated. Then take the varnish off the fire and strain it through an old linen cloth. Your varnish is then made, and it will be found to be beautiful varnish for wood, iron, paper, leather, and all kinds of painting and works, and for withstanding water. When you find it too viscous, dilute it with linseed oil in the proper manner.

**TABLE 3.27 Description of the varnish entitled "A most excellent clear and drying varnish proper for colours both in oil-painting and other kinds of painting" from the Marciana manuscript** (9)

Take 2 ounces of clear and good nut oil, one ounce of clear and good Greek pitch, and half an ounce of clear and good mastic grind the pitch and the mastic [separately] to a very fine powder, and place the oil in a clean glazed pipkin over a charcoal fire, and let it boil gently until it is done sufficiently, that is, until one-third has evaporated ; then put in the powdered pitch a little at a time, mixing and incorporating it well ; afterwards throw in the mastic in the same manner, and when it is dissolved, take the varnish of the fire and strain it through a fine and old linen cloth.

**TABLE 3.28 Description of the varnish entitled "A most excellent varnish for varnishing arquebuses crossbows and iron armor" from the Marciana manuscript** (9)

Take of linseed oil, lbs. 2 ; varnish in grains (sandarac), lb. 1 ; clear Greek pitch, oz. 2. Boil the oil, then dissolve in it the other ingredients, and strain through a much worn linen cloth, and when you wish to use the varnish, scrape and polish the work, and heat it in a hot oven, because that is the best place to heat it ; and when it is of a proper heat, that is, when the varnish adheres to it firmly and does not fry [bubble or blister from too great heat], then lay it on thinly with an instrument of wood, so that you may not bum your fingers, and it will make a beautiful changing color.

**TABLE 3.29 Description of the varnish entitled "An excellent common varnish, good for varnishing whatever you please" from the Marciana manuscript** (9)

Take 2 ounces of clear and good linseed oil, and one ounce of good and clear Greek pitch, but 2 ounces of the latter also will make the varnish thicker and give it more body ; boil the oil over a slow fire, and then put in the pounded pitch a little at a time, that it may incorporate well, and add a little roche alum previously burnt and pounded, and when it is incorporated and boiled sufficiently, that is, when you try a little of it in your fingers and find that it is done, strain it and keep it. When it is used it will be beautiful and good; if it is too tenacious you will dilute it with a little oil.

**TABLE 3.30 Description of the varnish entitled "A varnish of fir oil which dries both in the sun and in the shade" from the Marciana manuscript** (9)

Take fir oil which must be genuine and not adulterated, and if you wish to know whether it is falsified, distemper it with nut or linseed oil, or naphtha, heating both the oils and spread it on a work, when, if it is not genuine, it will not dry for a long time, and then badly, because it is adulterated with turpentine, but if it is genuine it will dry quickly and perfectly.

If you desire to varnish delicate works which will not be exposed to water, but merely to bring out the colours and show their beauty, distemper the olio di abezzo as above. But if you wish to varnish more permanently on works which are intended to resist water, do not distemper the olio di abezzo with other ingredients, but heat it in a vase, melt it, and varnish with it.

The art treatise entitled *El arte de la pintura* was written by Francisco Pacheco (first half 16th century) and it describes the preparation of the materials used for paintings. It includes some varnish formulas using sandarac (here called *grasa*, table 3.31 to 3.33) and abietane-type resins (table 3.34 and 3.35). Abietane-type resins described are *trementina de veta blanca* (i. e. white grain turpentine) and *trementina de veta de Francia* (i.e. grained tupertine of France), which probably allude to Larix Decidua Mill. or Abies alba Mill. resins (30).

**TABLE 3.31 Description of the varnish from the *El arte de la pintura* treatise** (30)

Media libra de aceite de linaza en una olla vidriada, cuando esté bien caliente se echan tres cabezas de ajos mondados y se cuecen hasta que estén dorados. Se echa una pluma de gallina para ver si está cocido: si lo está esta sale quemada. Entonces se añaden cuatro onzas de grasa molida (que es la goma que los árabes llaman sandáraca) y se cuece hasta espesar.

Put half pound of linseed oil in a glazed in a pot, when it is hot add three peeled garlic heads and cook it until they are golden. Add a hen’s feather to check if it is enough cooked: if it is the feather will be burnt. Then, add four ounces of grounded grassa (which is the gum that Arabs call sandaraca) and cook it until it thickens. (author's translation)

**TABLE 3.32 Description of the varnish from the *El arte de la pintura* treatise** (30)

Se toma una onza de trementina de veta de Francia que sea muy clara y se calienta en un vasito hasta deshacerla, se quita del fuego y se echa el aguaraz removiendo hasta mezclarlo. Se conserva hasta un mes pero es mejor hacerlo fresco para cada cuadro.

Take one ounce of grained turpentine of France, very light, and heat it until it melts. Remove it from the fire and add turpentine and stir until it mixes. You can keep it until one month but it is better to prepare it immediately before its application each painting. (author's translation)

**TABLE 3.33 Description of the varnish from the *El arte de la pintura* treatise** (30)

Dos onzas de grasa en polvo muy delgada, dos onzas de aguardiente de siete cociduras y media onza de aceite de espliego, y calentar a fuego lento hasta que se mezcle bien (recomendado para tablas)

Two ounces of fine powdered grasa and half an ounce of lavender oil, It is cooked over low heat until it is mixed (recommended for panels). (author's translation)

**TABLE 3.34 Description of the varnish from the *El arte de la pintura* treatise** (30)

Se echa la cantidad conveniente de aceite de espliego y grasa molida en polvo, con un paño atado dentro con almáciga molida, y al rescoldo, y fuego manso. Estando desecha la grasa se aparta del fuego y se saca el paño. Se echa un poco de agua de aguardiente de la más fuerte hasta el espesor deseado.

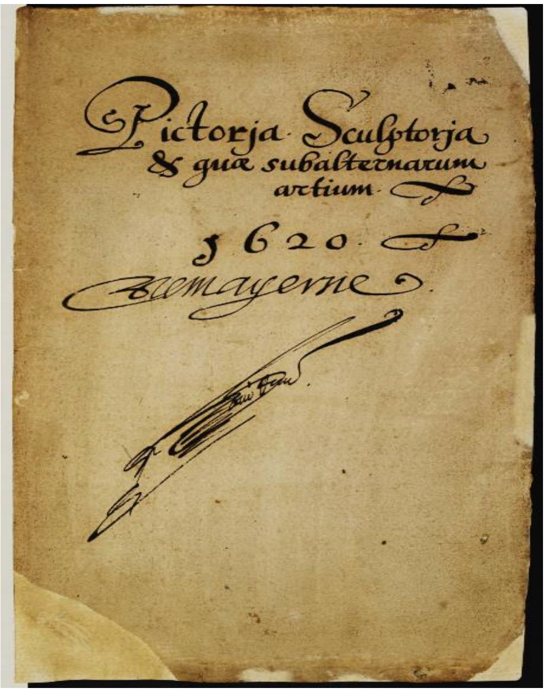
Add the appropriate amount of lavender oil and grounded grasa, and mastic inside a tied cloth, and cook over low heat. When the grasa is melted, remove it from the fire and take off the cloth. Add liquor until get the suitable thickness. (author's translation)

**TABLE 3.35 Description of the varnish from the *El arte de la pintura* treatise** (30)

Una onza de menjuí con dos onzas de aguardiente de siete coseduras y calentar a fuego lento. Estando caliente echar media onza de trementina de veta blanca.

One ounce of mastic with two ounces of refined liquor, and cook over low heat. When it is heated add half an ounce of white grain turpentine. (author's translation)

Examples of all previous varnish formulas can also be found in the manuscript entitled *Pictoria, Sculptoria, Tinctoria, et quae subalternarum Artium* wrote by Sir Theodore Turquet de Mayerne (early 17th century) (8) (Figure 3.8).



Pictorja Sculptorja
& quæ subalternarum
artium
1620
Tdemayerne

**Figure 3.8** Front page of the manuscript wrote by Sir Theodore Turquet de Mayerne (British Library, Sloane Manuscript 2052) (8).

Sir Theodore Turquet de Mayerne studied medicine and was designated main physicist of King James I of England and King Charles I of England. From this exceptional position, he studied the chemical composition of materials used in paintings. He also maintained contact with painters under the patronage of the king like Peter Paul Rubens or Anthony van Dyck. His notes provided much valuable information about the techniques and materials used in his period and confirm the use of abietane-type resins for varnishes and coatings.

### 3.2.4 Conclusions from the historical sources consulted

Formulas of diterpenic varnishes, specifically abietane-type varnishes, can be found in treatises and manuscripts from different periods.

According to the consulted sources, varnishes made with sandarac (labdane-type resin) or with *Larix Decidua* Mill. or *Abies alba* Mill. were generally considered of a higher quality than *Pinus* resin varnishes and were used mainly in higher quality works.

According to literary sources, *Pinus* resin is used over occasionally over paintings but it was mostly used for daily objects and low-quality artworks. The varnish called *vernice grossa* or *vernice commune* refers to a cheap varnish made mainly with *Pinus* resin and was recommended for furniture, amour, tools, etc. Many authors and treatises refer to this kind of low-quality varnish since the 16th century, like Leonardo Fioravanti (16th century) (31), Francesco Domenico Bisagno (17th century) (32), Theodore Turquet de Mayerne (17th century) (8) or Filippo Bonanni (18th century) (33). Additionally, analytical studies have demonstrated the presence of this kind of varnishes in a wide range of artworks (34–39) and it has also been found in artworks from the Mediterranean basin where specimens of *Pinus* are numerous.

In conclusion, *Pinus* resin was a common material in artwork manufacture, so its study can provide valuable information about this artworks background and the suitable conservation and restoration procedures.

## 3.3 PREVIOUS RESEARCH OF ABIETANE-TYPE RESINS CHEMICAL COMPOSITION

Previous analyses made by Gas Chromatography coupled to Mass spectrometry (GC/MS) of *Pinus* resins have shown that the fresh resin contains mainly two kinds of diterpenoid structures: abietanes and pimaranes (7).

Abietic, palustric, laevopimaric and neoabietic acids are some of the unoxidized abietanes present in the fresh resin. All non-oxidized abietanes are interconvertible; being abietic acid the most stable form. In the fresh *Pinus* resin monoterpenes are also present; monoterpenes are liquids at room temperature and give fluidity to fresh resin.

In atmospheric conditions, abietic acid evolves into more oxidized molecules (7,37,40–42), the most oxidized molecule determined so far being 15-hydroxy-7-oxodehydroabietic acid (43,44) (Figure 3.9).

Since functional groups like –OH and –COOH are liable to react, it is possible that some macromolecules could be formed in aged samples; nonetheless they are probably not detected by GC/MS analysis.

When *Pinus* resin loses the monoterpene fraction becomes brittle. This is because of the arrangement of the polar groups, which doesn't allow making many intermolecular bonds. Probably, this substance was used more frequently because of its low price rather than because of its physical properties.

Although pimaranes are more stable, some studies revealed also their diminution during aging (40,45). This reduction has sometimes been related to the pimaranes polymerization; however, the resulting polymer has not yet been identified, leaving the pimaranes ageing paths unsolved.

Studies made by GC/MS provided valuable information about the molecular composition. However, we have to bear in mind that GC/MS cannot detect neither macromolecules, because they are not volatile, nor interactions between molecules, since samples need to be dissolved. Moreover, the sample preparation required which usually implies dissolving and derivatization of the sample, is a long process which modifies its chemical composition, so after GC/MS the sample cannot be analysed by another technique.

This suggests that a study of *Pinus* resin using only GC/MS will not give a full characterization and the data should be completed with other analysis able to provide information about the fate of pimaranes and the reactions and interactions between molecules.

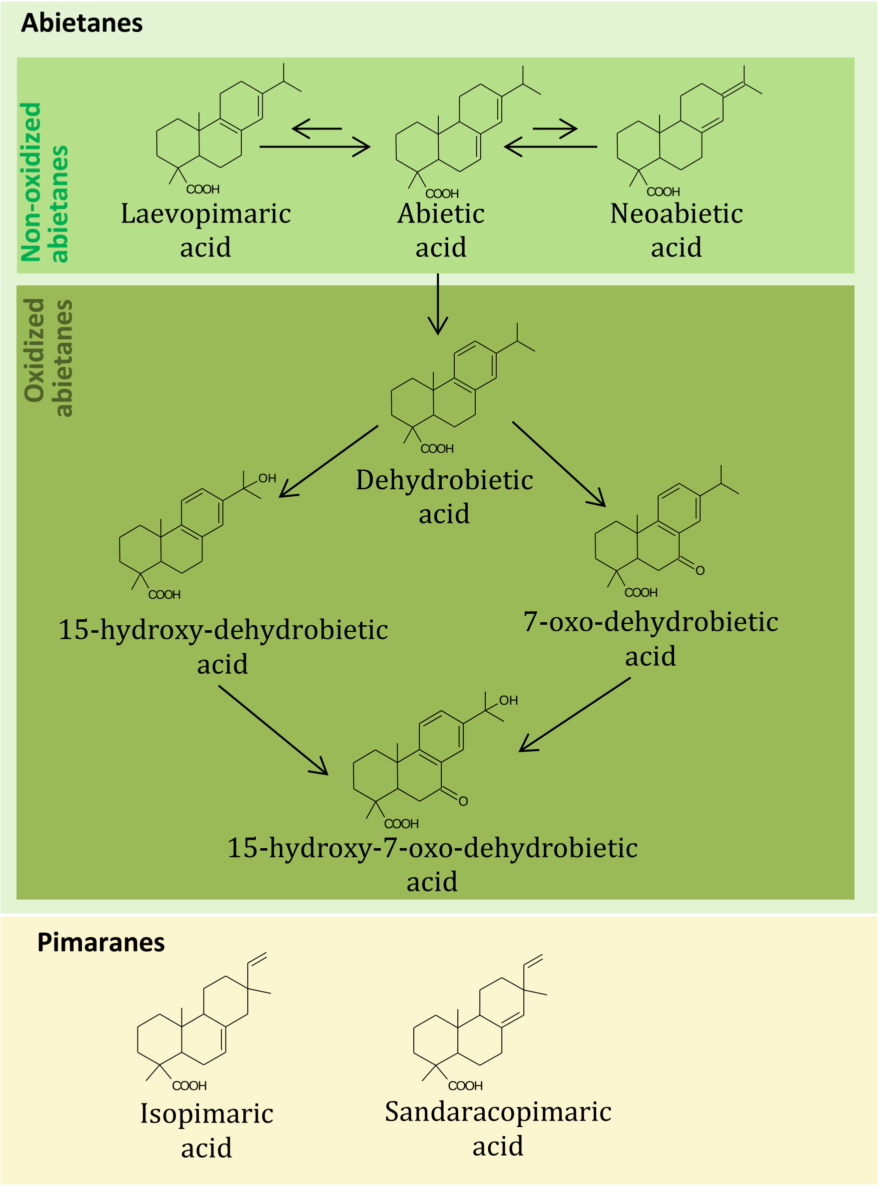


**Figure 3.9** Main abietanes (both non-oxidized and oxidized) and pimaranes of fresh and oxidized *Pinus* resins (43).

A spectroscopic dedicated study of the ageing process is expected to give very valuable additional information since, unlike GC/MS, it can detect macromolecules and it doesn't require any sample treatment such as dissolving or derivatization. Moreover, FTIR is able to identify a wide range of substances, how the molecular species are organized and which reactions or interactions exist between them. Moreover, only a small quantity of sample is needed, analyses are fast and of low cost. Additionally being a non-invasive technique, after the analysis the same fragment can be analysed again, consequently, is a good choice in multitechnical studies. There are studies of *Pinus* resin made by FTIR Spectroscopy (38,46–50) and Raman Spectroscopy (51–53), however they are mainly focused on the identification of the *Pinus* resin by determining its characteristic fingerprints.

The first molecular changes are observed already a few weeks after resin collection, consequently the identification of the oxidative pathways and how they affect the chemical composition is essential for the correct identification of the material, its degradation degree and for the determination of the long term resin degradation processes. In fact, since the recognition of resins is heavily interfered by the decay happening since their exudation, the identification of both the resins and the degradation stage is essential for their study.

Chemically, *Larix decidua* Mill. and *Abies alba* Mill. resins contain abietanes and pimaranes like *Pinus* resin, which evolve in the same way, plus other additional molecules. *Larix decidua* Mill. contains larixyl acetate, also smaller amounts of larixol and epimanool (Figure 3.10a, b and c). *Abies alba* Mill. contains abienol, which tends to polymerize (Figure 3.10d). (40)

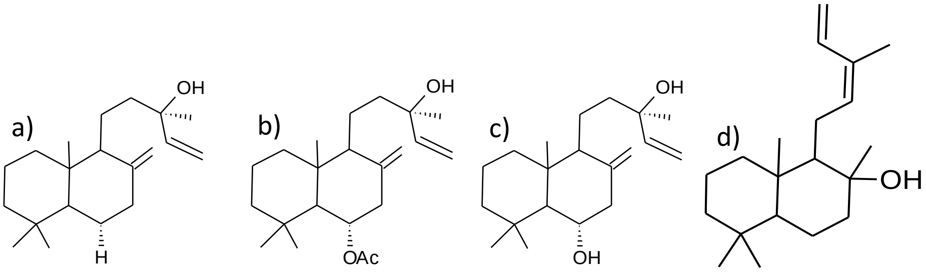


**Figure 3.10.** Main compounds of Larix decidua Mill.: a) larixyl acetate, b) epimanool, c) larixol and d) abienol.

The arrangement of the polar groups of the *Larix decidua* Mill. molecules help to the formation of more intermolecular bonds than abietanes and pimaranes. *Abies* *alba* Mill. contains polymerized abienol. The molecular composition of both *Larix decidua* Mill. and *Abies alba* Mill. makes them less brittle than *Pinus* resins.

Since the abietanes and pimaranes structures are the major compounds in all *Pinaceae* resins used in historic objects coatings, knowing their evolution during ageing provide valuable information about the ageing not only about *Pinus* resin but also about *Larix decidua* Mill. and *Abies alba* Mill. resins.

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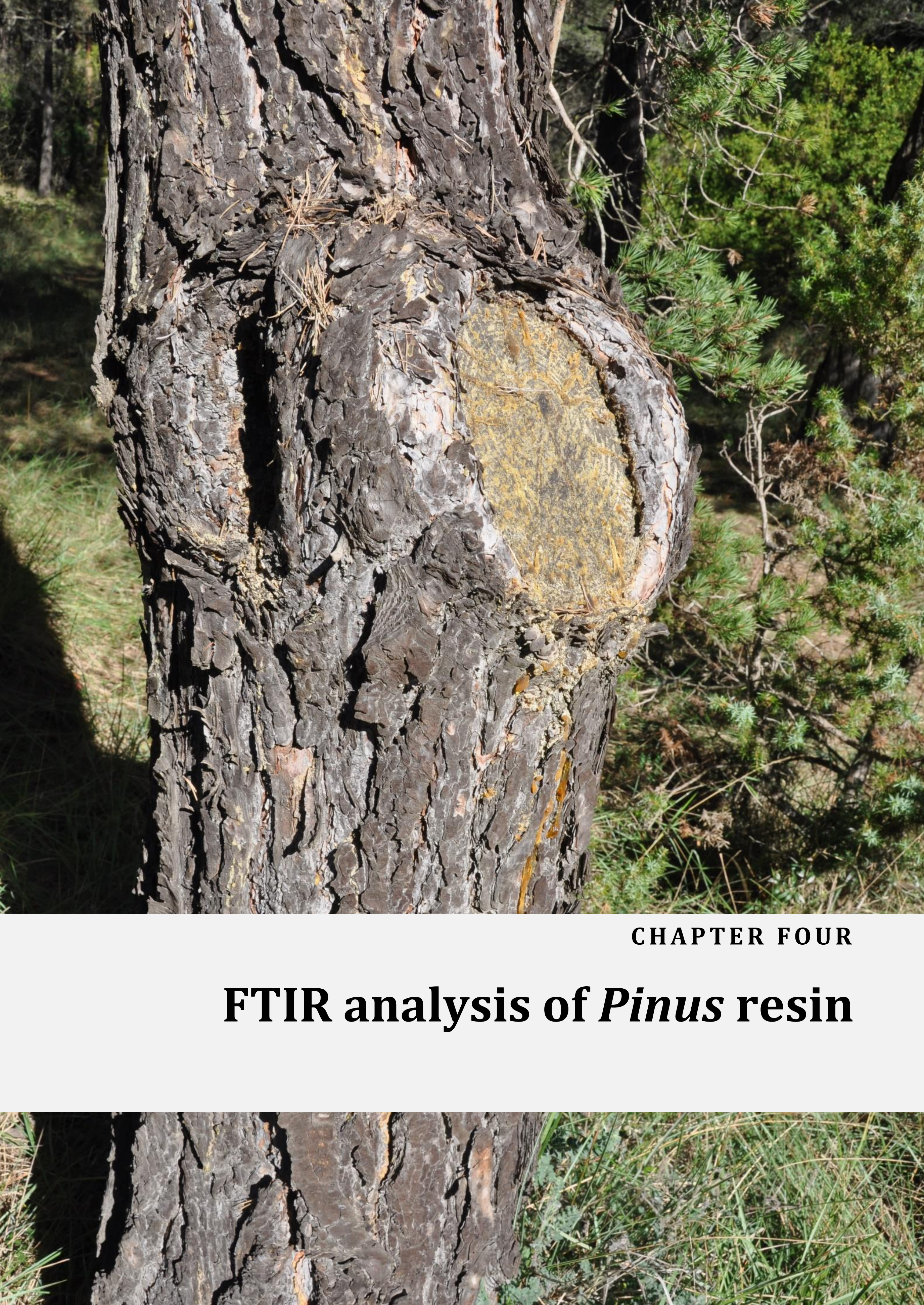


## CHAPTER FOUR

# FTIR analysis of *Pinus* resin

## 4.1 INTRODUCTION

The aim of this chapter is to monitor the compositional changes, reactions and interactions between molecules occurring during natural ageing of *Pinus* resins by FTIR spectroscopy complemented with GC/MS analysis to verify the presence of specific molecules (1).

Firstly, the main reference *Pinus* resin components are analyzed and the corresponding band assignment proposed. Secondly, the first stages of natural ageing in the fresh resin but also in abietic acid itself, which is the chief compound of *Pinus* resins, are monitored by FTIR, and the spectral features related to the process identified.

The fresh resin was collected directly from the tree in order to ensure its freshness. A wound was performed in the bark and the resin exudate was collected in glass containers to avoid contamination (Figure 4.1).

**Figure 4.1** Obtention of fresh resin from *Pinus halepensis* Mill.

Finally, long-term natural ageing studies are undertaken, and for this, well dated old resins are studied. Old *Pinus* resins are found in archaeological and artistic objects; being the resin a cheap material easy to find all over Europe (2,3), it was widely used in historical times. *Pinus* resins are found in varnishes and protective coatings (furniture, weapons…) applied over daily use objects and artworks (4,5), and also as adhesives to fill gaps and consolidate panels (6,7). Additionally, fresh and aged resins are also found in some Collections of Botanical gardens.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Chemical reagents

Abietic, dehydroabietic and isopimaric acids were purchased from Sigma Aldrich (ref. 101392305, ref. SMB00089 and MKBT3436V respectively) and their purity checked by GC/MS. 15-hydroxy-7-oxodehydroabietic acid were purchased to Chemfaces (ref. CFN97533).

Methanol from Sigma Aldrich (ref. 3460) and MethPrepII from Grace (ref. 18007) were used in the reactivity tests and chromatographic analysis and acetic acid from Panreac (ref. 131008.1611) was used for acidification.

### 4.2.2 Resin samples

Resins from different sources and ages were studied in order to account for the degradation. The genus and species from all the natural resins studied were certified but subspecies and variants were not considered. Only a selection of the most illustrative samples is shown, details are summarized in Table 4.1.

Fresh resins of *Pinus canariensis* C.Sm. ex DC., *Pinus sylvestris* L., *Pinus halepensis* Mill. and *Pinus pinea* L. were collected directly from the tree and were naturally aged both at open air exposed (although not directly) to sunlight (Figure 4.2) and in a dark closed cupboard.

**TABLE 4.1 Samples summary**

| Description | Origin | Age |
|---|---|---|
| *Pinus canariensis C.Sm. ex DC.* | Botanic garden "Jardí Botanic de Barcelona" | <1 week |
| *Pinus sylvestris L.* | | <1 week |
| *Pinus pinea* L. resin | Coast of Tarragona (Catalonia) | <1 week |
| *Pinus halepensis* Mill. resin | | <1 week |
| resin from *Pinus halepensis* Mill. sold in a market of Syria, ref. 79882 | Economic Botany Collection of the Royal Botanic Gardens, Kew | 2007 |
| *Pinus sylvestris* L. resin ref. 28417 | | probably 1850-1900 |
| *Pinus nigra* J.F.Arnold resin displayed at the Forestry Exhibition, Edinburgh (1884) ref. 28172 | | 1850-1900 |
| 16th century varnish | Canvas entitled "Comte Borrell" by Filippo Ariosto | 16th |
| Resin from a knot of the wooden panel of a ceiling | Santa Maria de Paretdelgada (Selva del Camp, Catalonia) | 14th century |

**Figure 4.2** Ageing of the samples.

Panel no. 173 from the ceiling of Santa Maria de Paretdelgada Chapel (Selva del Camp, Catalonia) dated to the 14th century (Figures 4.3) is made of wood and has a knot in the back, where resin has exuded. Samples from that part were extracted. The wood was identified as *Pinus* (Figures 4.4).

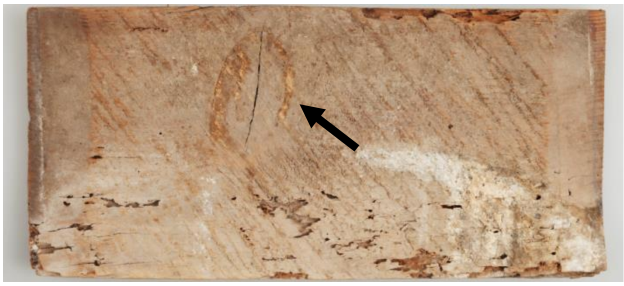


**Figure 4.3** Back of the panel 173, the knot is indicated with an arrow.

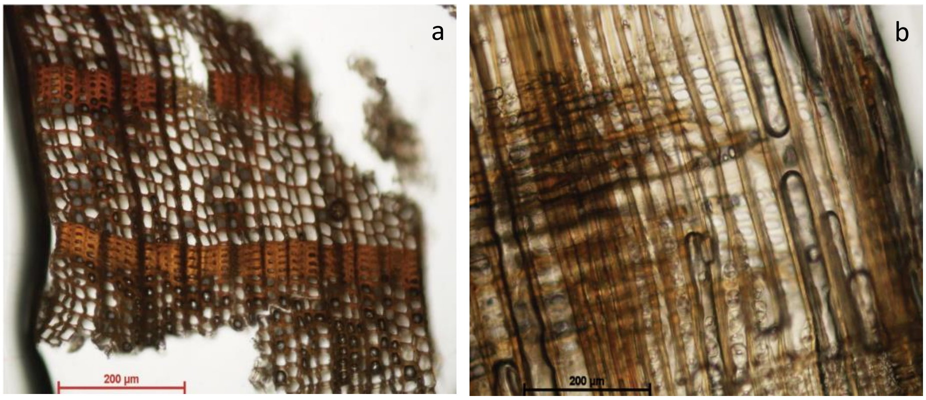


**Figure 4.4** Sections of the wood support from panel 173 wood where the *Pinus* features can be seen a) tangential section, b) transverse section.

### 4.2.4 Instrumental

GC/MS and FTIR measurements were taken following the conditions described in chapter 2.

For FTIR analysis a diamond compression cell and spot areas of 100x100 µm were used. The sticky texture of fresh resin does not allow the preparation of KBr pellets. At least three spectra were measured to ensure the reproducibility of the data. Neither baseline correction nor smooth procedures were applied to the spectra.

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Reference materials

The correct assignment of the *Pinus* resin infrared bands is fundamental for any further study. Consequently, abietic acid, dehydroabietic acid, 15-hydroxy-7-oxodehydroabietic acid and isopimaric acid were analyzed by FTIR spectroscopy and used as reference materials for the molecular structures of the main components present in the resins.

Specific markers characteristic of the molecular structures were identified and band assignment proposed (Figure 4.5).

The FTIR spectrum corresponding to the abietic acid is shown in Figure 4.5a. The bands at 3200 $cm^{-1}$, 2648 and 2534 $cm^{-1}$ correspond to the group –COOH; the former to free OH (not the main one) and the last two to bonded OH, the most common as in solid state, the carboxylic groups tend to form dimers (8). These last bands appear overlapped with overtones and combination bands of lower-frequency vibrations from the COOH group (9). The C-H stretching absorption bands show also a complex shape due to the presence of =CH, $–CH_3$, $–CH_2$ and –CH groups, where the $–CH_2$ bands appear split due to the presence of conjugated C=C bonds (10). The narrow band at 1693 $cm^{-1}$ belongs mostly to the stretching of C=O, but it also shows a shoulder at 1626 $cm^{-1}$ and an overlapped band at ~1670 $cm^{-1}$ related to the presence of conjugated C=C bonds. Finally, in the region of wavenumbers below 1500 $cm^{-1}$ a great number of bands are observed. The most intense at 1280 $cm^{-1}$ corresponds to the C-O deformation from the –COOH group and the peak at 891 $cm^{-1}$ is related to the C-H deformation out of plane of conjugated double bonds (8,10–14).

The infrared spectrum corresponding to dehydroabietic acid (Figure 4.5b) is similar to those of abietic acid although shows some minor differences. The band at about 3000 $cm^{-1}$ corresponds to C-H stretching, but contrarily to the abietic acid , the $–CH_2$ bands appear un-split because the conjugated C=C bonds are related to the aromatic group . As in abietic acid, the bands around 3200 and 2600 $cm^{-1}$ correspond to O-H stretching, overtones and combined bands. The 1695 $cm^{-1}$ band corresponds to the C=O stretching belonging to –COOH group. Below 1500 $cm^{-1}$, the band at 1498 $cm^{-1}$ corresponds to the =C-H stretching of the aromatic groups and the band at 819 $cm^{-1}$ to the out of plane deformation of the aromatic C-H group. Finally, the band at 891 $cm^{-1}$ is not present as it is related to conjugated double bonds (10,14,15).

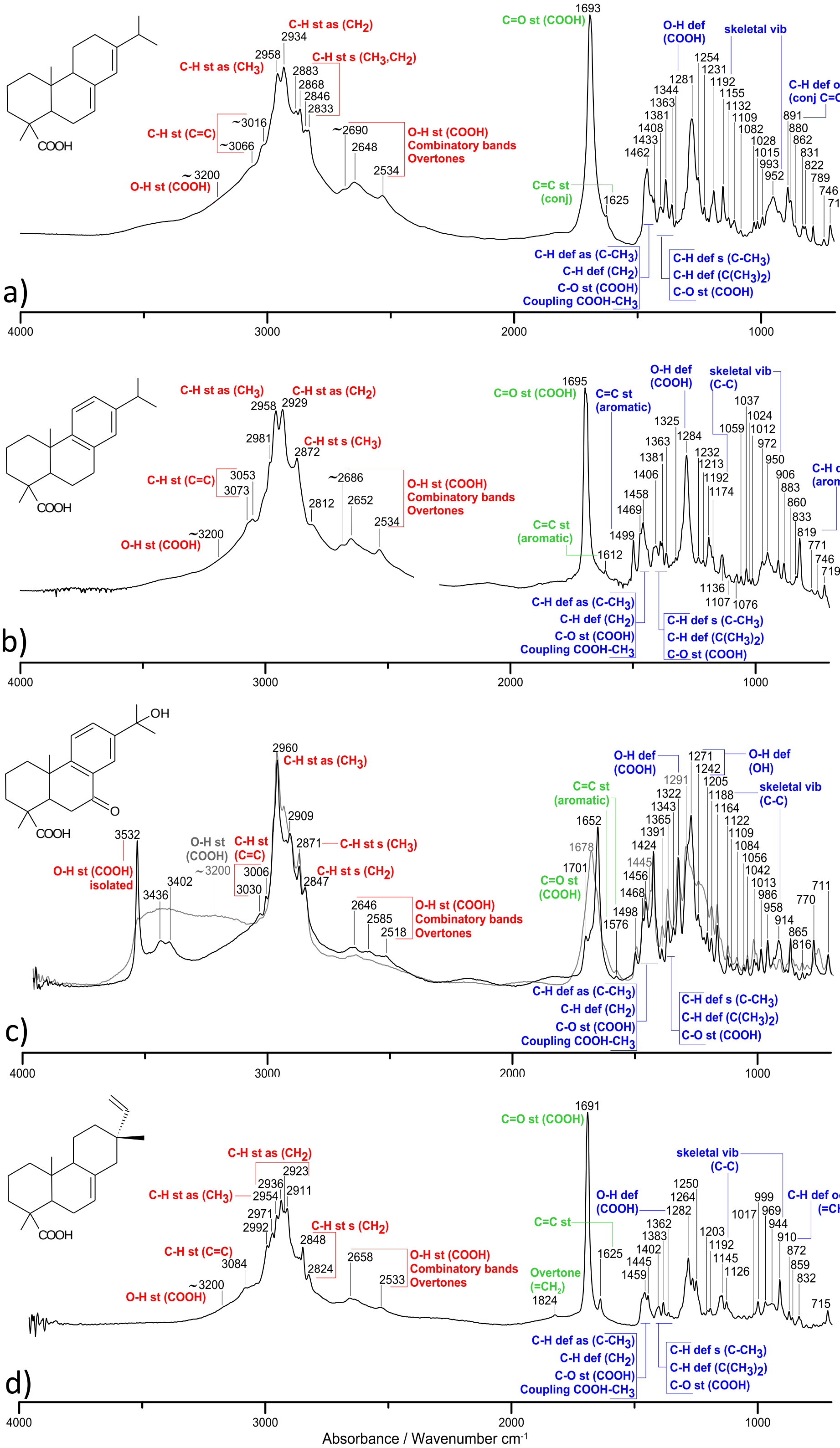

a)
COOH
C-H st as (CH$_2$)
2934
2958
C-H st as (CH$_3$)
C-H st s (CH$_3$,CH$_2$)
2883
2868
2846
2833
C-H st (C=C)
~3016
~3066
~2690
2648
2534
O-H st (COOH)
Combinatory bands
Overtones
~3200
O-H st (COOH)
1693
C=O st (COOH)
C=C st (conj)
1625
O-H def (COOH)
skeletal vib
1254
1231
1281
1192
1344
1155
1363
1132
1381
1109
1408
1082
1433
1028
1462
1015
993
952
C-H def oop (conj C=C)
891
880
862
831
822
789
746
719
C-H def as (C-CH$_3$)
C-H def (CH$_2$)
C-O st (COOH)
Coupling COOH-CH$_3$
C-H def s (C-CH$_3$)
C-H def (C(CH$_3$)$_2$)
C-O st (COOH)
4000
3000
2000
1000
b)
COOH
C-H st as (CH$_3$)
2958
2929
C-H st as (CH$_2$)
2981
C-H st s (CH$_3$)
2872
C-H st (C=C)
3053
3073
~2686
2812
2652
2534
O-H st (COOH)
Combinatory bands
Overtones
~3200
O-H st (COOH)
1695
C=O st (COOH)
C=C st (aromatic)
O-H def (COOH)
skeletal vib (C-C)
1037
1024
1325
1059
1012
1363
1284
972
1381
1232
950
1213
1406
1192
906
1458
1174
883
1469
860
833
1499
819
771
746
719
C=C st (aromatic)
1612
C-H def oop (aromatic)
1136
1107
1076
C-H def as (C-CH$_3$)
C-H def (CH$_2$)
C-O st (COOH)
Coupling COOH-CH$_3$
C-H def s (C-CH$_3$)
C-H def (C(CH$_3$)$_2$)
C-O st (COOH)
4000
3000
2000
1000
c)
OH
O
COOH
2960
C-H st as (CH$_3$)
2909
2871
C-H st s (CH$_3$)
C-H st s (CH$_2$)
2847
3532
O-H st (COOH) isolated
3436
3402
O-H st (COOH)
~3200
C-H st (C=C)
3006
3030
2646
2585
2518
O-H st (COOH)
Combinatory bands
Overtones
O-H def (COOH)
1271
1242
O-H def (OH)
1291
1205
C=C st (aromatic)
1322
1188
skeletal vib (C-C)
1343
1164
1652
1365
1122
1678
1391
1109
1424
1084
1701
1445
1056
C=O st (COOH)
1456
1042
1468
1013
986
958
1498
914
1576
865
816
770
711
C-H def as (C-CH$_3$)
C-H def (CH$_2$)
C-O st (COOH)
Coupling COOH-CH$_3$
C-H def s (C-CH$_3$)
C-H def (C(CH$_3$)$_2$)
C-O st (COOH)
4000
3000
2000
1000
d)
COOH
1691
C=O st (COOH)
C-H st as (CH$_2$)
2923
2936
2911
C-H st as (CH$_3$)
2954
2971
2992
C-H st s (CH$_2$)
2848
2824
C-H st (C=C)
3084
2658
2533
O-H st (COOH)
Combinatory bands
Overtones
~3200
O-H st (COOH)
skeletal vib (C-C)
1250
O-H def (COOH)
1264
1282
999
969
944
910
C-H def oop (=CH$_2$)
C=C st
1362
1383
1203
1192
1017
1402
1145
1445
1126
1625
1459
872
859
832
Overtone (=CH$_2$)
1824
715
C-H def as (C-CH$_3$)
C-H def (CH$_2$)
C-O st (COOH)
Coupling COOH-CH$_3$
C-H def s (C-CH$_3$)
C-H def (C(CH$_3$)$_2$)
C-O st (COOH)
4000
3000
2000
1000
Absorbance / Wavenumber cm$^{-1}$

**Figure 4.5** Infrared spectra from a) abietic acid, b) dehydroabietic acid, c) 15-hydroxy-7-oxodehydroabietic acid (black line corresponds to the pure compound, the grey line to the compound after dissolving it in methanol and evaporate the dissolvent) d) isopimaric acid. Different colors have been used in order to distinguish the proposed assignments from each region.

The 15-hydroxy-7-oxodehydroabietic acid is the most oxidized compound determined in aged resins so far. Its infrared spectrum (Figure 4.5c) shows differences depending on to what and how the –OH groups are bonded to (10,14). This is clearly seen by comparing the spectrum corresponding to the commercial compound (Figure 4.5c, black line) with the one obtained from the dried acid after dissolving it in methanol (Figure 4.5c, grey line).

The narrow band at 3532 $cm^{-1}$ corresponds to free –OH groups; this band is more intense in the commercial compound but a new broad band around 3400 $cm^{-1}$ corresponding to –OH groups with intermolecular bonds appears in the treated acid. Differences in the –OH structure also affect the 1700 $cm^{-1}$ and 1200 $cm^{-1}$ regions. The 1700 $cm^{-1}$ band appears broader in the treated compound due to the presence of hydrogen bonds between hydroxyls and carboxylic acid groups. Owing to the intermolecular bonds created in the treated acid the 1271 $cm^{-1}$ band decreases while the 1242 $cm^{-1}$ band rises (non-bonded and bonded C-O stretching).

Consequently, the detection of this molecule is problematic since different –OH bands are to be expected in its spectrum depending on the molecules to which it bonds, nevertheless, the rise of the ~3400 (O-H stretching of bonded hydroxyl group) and ~1245 $cm^{-1}$ (C-O deformation of bonded hydroxyl group) bands (Figure 4.5c) is indicative of its presence.

Finally, the spectrum corresponding to the isopimaric acid (Figure 4.5d) is similar to those of the abietic and dehydroabietic acids. A complex band at about 3000 $cm^{-1}$, consequence of the variable environment of the $–CH_3$, $–CH_2$ and –CH groups, is observed. Moreover, the characteristic O-H stretching, overtones and combination bands at 3200 and 1600 $cm^{-1}$ are also present. The functional group characteristic of the pimaranes is the $=CH_2$ with bands at 1824 $cm^{-1}$ (overtone of $=CH_2$) and 910 $cm^{-1}$ (C-H deformation out of the plane). (10,14)

### 4.3.2 Fresh materials

Fresh *Pinus* resin is made mainly of non-oxidized abietanes and a smaller portion of pimaranes the exact amount of each varying depending on the specie (16–22). This is seen in the infrared spectra displayed in (Figure 4.6): different specie resins show variations mainly in the intensities of the bands directly related to the precise amount of each compound present.

The main compound is abietic acid (Figure 4.6, red lines) in all the cases, due to the fast conversion of non-oxidized abietanes (palustric acid, neoabietic acid, laevopimaric acid…) to abietic acid in the resin.

However, still some differences between abietic acid and fresh resins spectra (Figure 4.6, black lines) may be appreciated. The C-H stretching absorption band shows the largest differences. The higher intensity of the C-H stretching absorption band at ~2930 cm$^{-1}$ in some fresh resins can be explained by the higher proportion of –$CH_2$ groups compared to those of pure abietic acid. The un-split bands corresponding to the symmetric C-H stretching is due to the lower proportion of C=C bonds (absent in the pimaranes) in the resins.

There are also some other additional bands in the fresh resins spectra due to the presence of other compounds in minor amounts. For instance the bands appearing at 1181 cm$^{-1}$ which, as we will see in the next section, disappear during ageing most probably belong to either non-oxidized abietanes or other volatile compounds, while on the contrary, the small band at 1020 cm$^{-1}$ which remain during ageing belong most probably to pimaranes.

### 4.3.3 Ageing changes

Figure 4.7 shows the changes happening in a fresh *Pinus halepensis* Mill. resin at open air and indirect daylight.

The most important and significant changes happen in the 1695 and ~1245 cm$^{-1}$ bands. The change the 1695 band is associated to the C=O stretching group. Although the peak position and intensity of the 1695 cm$^{-1}$ band is not affected by aging (both non-oxidized and oxidized -COOH species are present in the resin), it broadens due to the presence of a shoulder at 1725 cm$^{-1}$ probably related to the C=O stretching of the ketone group of the 7-oxodehydroabietic and 15-hydroxy-7-oxo-dehydroabietic acids.

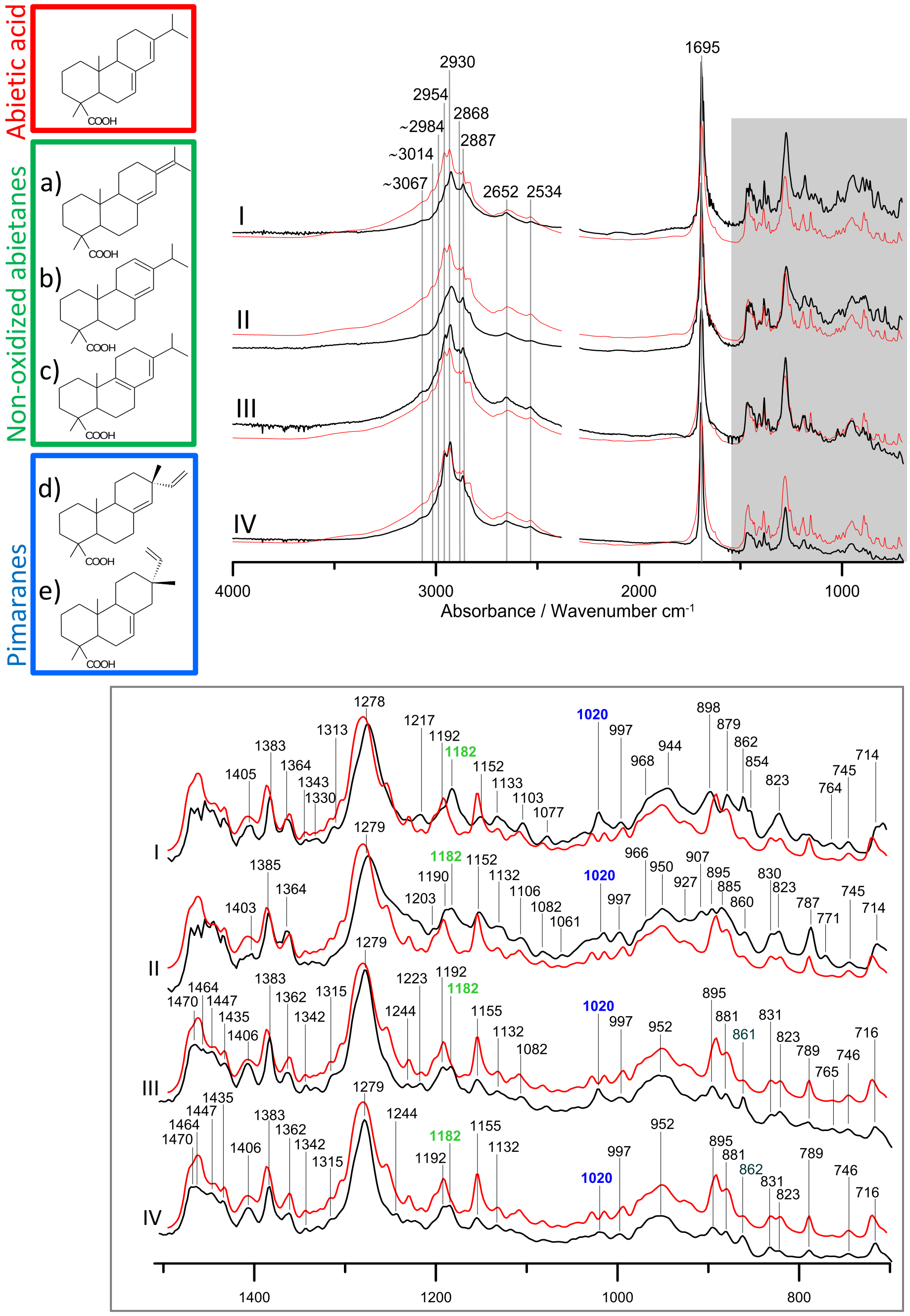
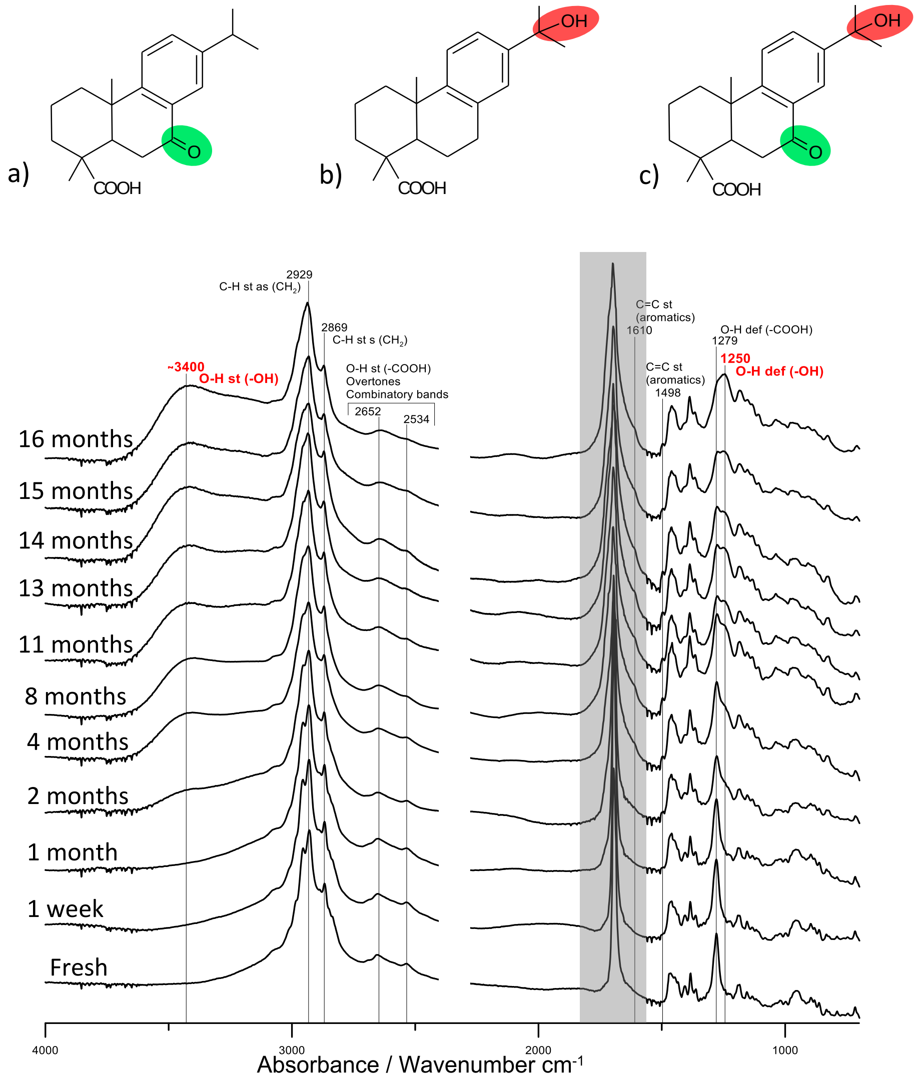


**Figure 4.6** Comparison between the infrared spectra of abietic acid (red lines) and various fresh *Pinus* resins (black lines); I: *Pinus canariensis* C.Sm. ex DC., II: *Pinus sylvestris* L., III: *Pinus pinea* L., IV: *Pinus halepensis* Mill.. An enlargement of the 1500-700 cm$^{-1}$ region is shown below. Green wavenumbers are proposed for the identification of abietanes and blue for the pimaranes. The structure of some abietanes (a: neoabietic acid, b: laevopimaric acid, c: palustric acid) and pimaranes (d: sandaracopimaric acid, e: isopimaric acid) are shown at the left.



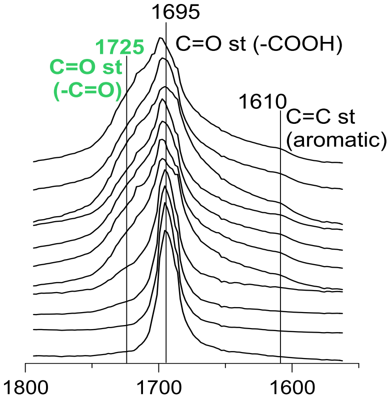


**Figure 4.7** From bottom to top infrared spectra corresponding to the first 16 months of *Pinus halepensis* Mill. resin ageing. A magnification of the carbonyl band is shown at the bottom. The main oxidized abietanes are shown at the top (a: 7-oxodehydroabietic acid, b: 15-hydroxydehydroabietic acid, c: 15-hydroxy-7-oxodehydroabietic acid). Functional groups are in the same color as their proposed specific markers.

The occurrence of those acids is also corroborated by the presence of the ~1610 and ~1500 $cm^{-1}$ bands related to the aromatic groups of the oxidized species. Additionally, the broadening of the 1696 $cm^{-1}$ band may also be related to the presence of hydrogen bonds from the –OH and –COOH groups and to a minor contribution from oxalates.

The change of the ~1245 $cm^{-1}$ band is associated to the 15-hydroxy-7-oxo-dehydroabietic acid as indicated before. The increasing of the 1245 $cm^{-1}$ band hides the 1280 $cm^{-1}$ peak, which cannot be distinguished in aged resins.

Other changes observed are the broadening of the bands around 2650 and 2530 $cm^{-1}$ which are explained by the increase in the chemical variability around the -COOH groups.

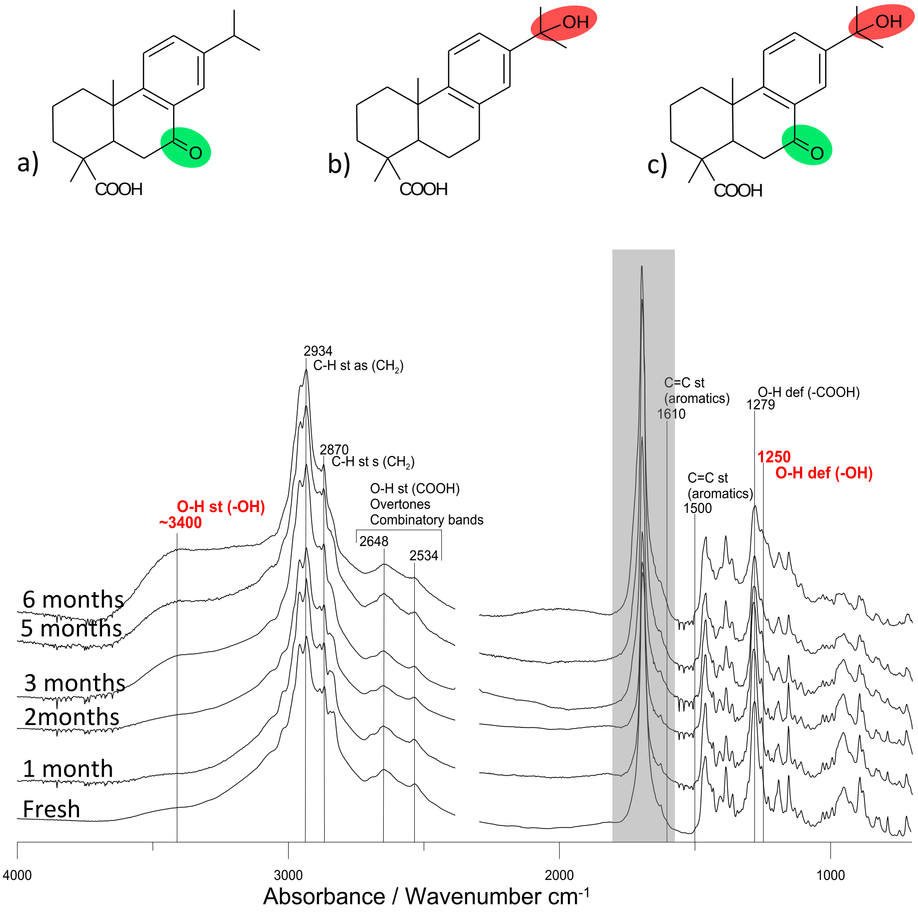
The regions 1200-1100 and 1050-800 $cm^{-1}$ where skeletal vibrations appear, show a raised absorption which confirms also the formation of more types of molecules due to aging.

Abietic acid was also aged similarly to study the oxidative process without the interference of pimaranes and the corresponding FTIR spectra are shown in Figure 4.8. The initial processes fully agree with those of *Pinus* resin and, consequently, we can conclude that the main changes observed in the *Pinus* resin spectra correspond to the oxidation of abietic acid.

As the –COOH and –OH band assignments are fundamental for this study, some chemical treatments were performed to the *Pinus pinea* L. resin in order to verify them (Figure 4.9).

The first treatment consisted in the derivatization (esterification) of the resin to transform the –COOH groups into $-COOCH_3$. The esterification has been done by adding an excess of MethPrepII and heating at 66.6$^0$C for 2 hours. The FTIR spectra corresponding to the untreated and treated resins are shown in Figure 4.9a and Figure 4.9b respectively.

The second treatment consisted in the first acidification and then derivatization with MethPrepII of the resin to convert the –COOH groups into $-COOCH_3$ and the –OH groups into $-OCH_3$. The acidification has been done by adding an excess of acetic acid and heating for 2 hours, then it has been left uncovered until acid has been evaporated (about 1 hour). The derivatization performed is the same than Figure 2.10b. The spectrum is shown in Figure 4.9c.



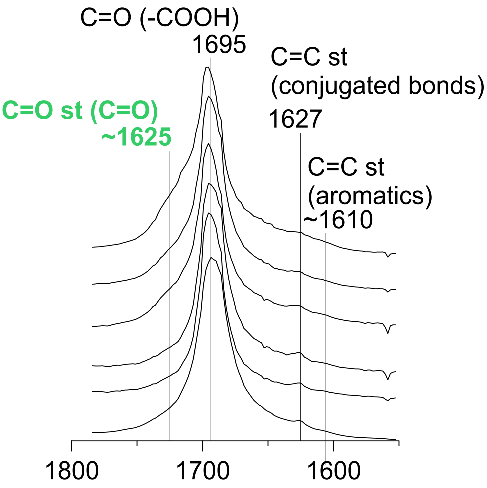


**Figure 4. 8** From bottom to top infrared spectra corresponding to the first 6 months of the ageing of the abietic acid. A magnification of the carbonyl band is shown at the bottom. The main oxidized abietanes are shown at the top (a: 7-oxodehydroabietic acid, b: 15-hydroxydehydroabietic acid and c: 15-hydroxy-7-oxodehydroabietic acid). Functional groups are in the same color as their proposed specific markers.

The bands related to the –COOH groups (O-H stretching at ~3200 and ~2600 cm$^{-1}$, C=O stretching at 1695 cm$^{-1}$ and C-O deformation at 1280 cm$^{-1}$), as well as, the –OH bands (O-H stretching at ~3400 cm$^{-1}$ and C-O deformation at 1250 cm$^{-1}$ from –OH) appear reduced in the treated resins with respect to the untreated resin confirming band assignments. Other minor bands related to –COOH and –OH, such as O-H deformation out of the plane, also change, but as they have lower intensity they are more difficult to determine.

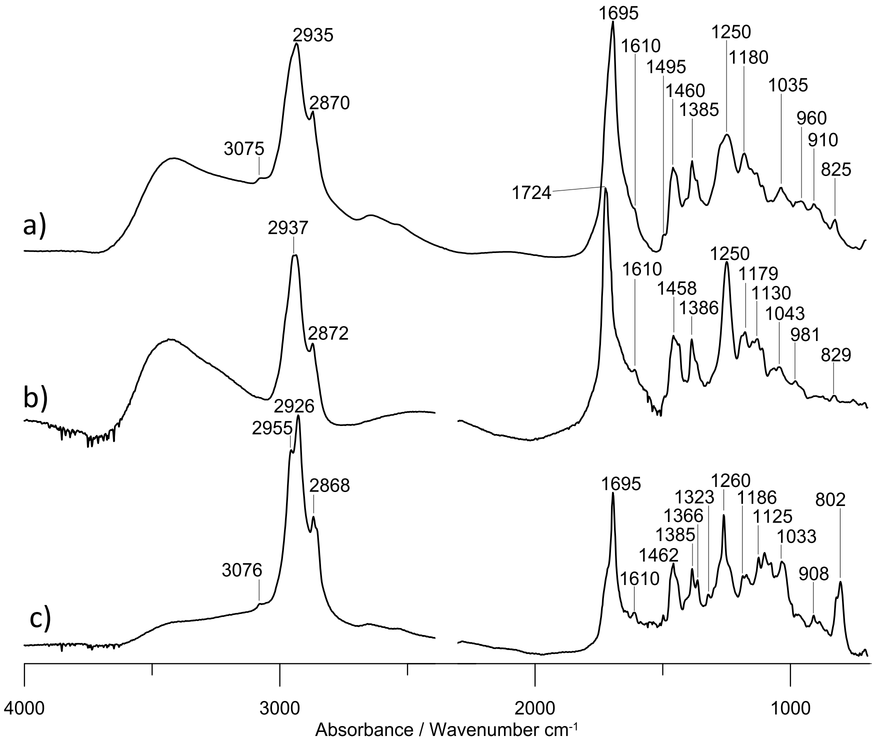


**Figure 4.9** Infrared spectra of a) untreated *Pinus Pinea* L. b) *Pinus Pinea* L. after derivatization with MethPrepII c) *Pinus Pinea* L. after acidification with acetic acid and derivatization with MethPrepII.

Figure 4.10 shows the FTIR spectra corresponding to the *Pinus pinea* L. (Figure 4.10, green lines) and *Pinus halepensis* Mill. resins (Figure 4.10, orange lines) before and after the first ageing stages, both in darkness and open-air conditions. The small differences observed among the various fresh species can hardly be appreciated in the aged resins. This was not wholly unexpected as all the abietanes evolve into the same oxidized form.

The samples aged under different conditions (darkness and open-air) show also similar spectra. However, comparing the bands corresponding to the oxidized abietanes (3400, 1725, 1606, 1500 and 1245 cm$^{-1}$) for an equivalent ageing period, the samples kept in a dark environment appear less oxidized than those aged in open-air conditions.

Consequently, open air accelerates the degradation process but the infrared spectra obtained show the same progression.

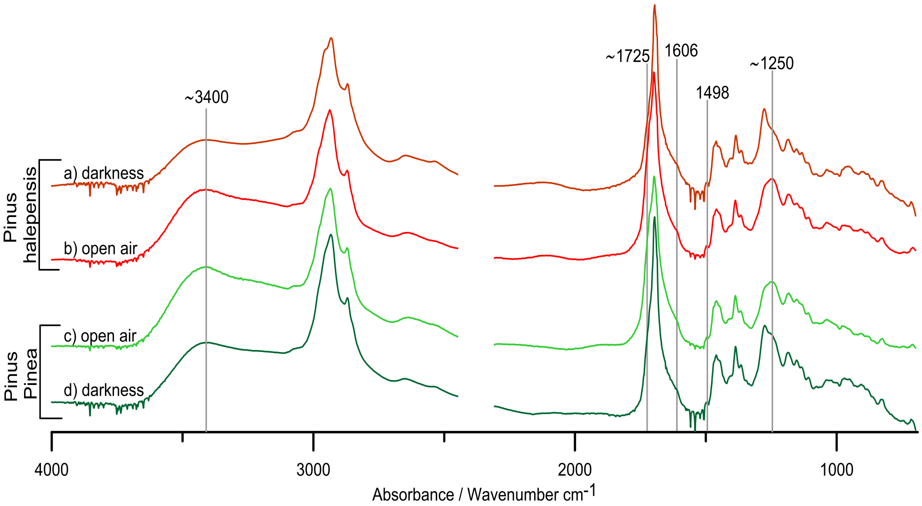


**Figure 4.10** Comparison between infrared spectra from different *Pinus* resins aged under diverse conditions. a: *Pinus halepensis* Mill. aged in darkness, b: *Pinus halepensis* Mill. aged resin in open air and indirect daylight, c: *Pinus pinea* L. aged in open air and indirect daylight,, d: *Pinus pinea* L. aged in darkness. Marked wavenumbers belong to the bands proposed for the identification of oxidized abietanes.

Additionally, it is important to notice that the changes observed during natural ageing differ from those ones observed in artificial photo ageing (23).

### 4.3.4 Ancient resins

To identify the changes at advanced degradation stages we have to rely on the study of well dated ancient resins. However, the degradation degree depends not only on age but also on the storage environmental conditions (24,25). In order to verify the chemical composition and in particular the presence of oxidized compounds, the resins were first analyzed by GC/MS (Figure 4.11).

According to GC/MS data (Figure 4.11, right), the older resins show also the higher concentration of oxidized compounds, i.e. a more advanced degradation.

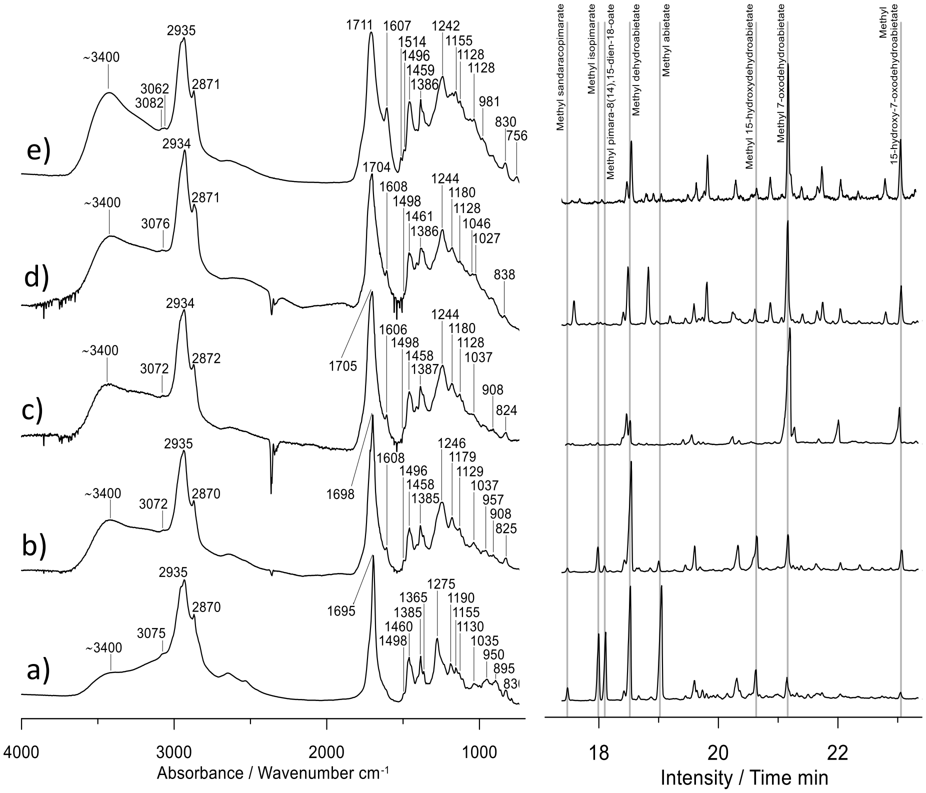


**Figure 4.11** FTIR spectra (left) and GC/MS (right) from aged natural resins: a) *Pinus halepensis* Mill. (dated in 2007) b) *Pinus sylvestris* L. (unknown date) c) *Pinus nigra* J. F. Arnold (dated in 1884). Other historical samples d) varnish from a 16th century canvas e) resin from a 14th century wooden support.

In the infrared spectra (Figure 4.11, left), the bands related to the hydroxyl, ketone and aromatics groups present only in the oxidized compounds increase. The bands associated to the hydroxyl groups appear at ~3400 cm$^{-1}$ (O-H stretching) and ~1245 cm$^{-1}$ (O-H deformation); those related to the ketones appear at ~1725 cm$^{-1}$ (C=O stretching). Finally, those related to the aromatic C=C stretching appear at 1606, 1514 and 1496 cm$^{-1}$ (the last probably belonging to dehydroabietic acid).

All those changes are also observed in other aged *Pinus* resin FTIR spectra found in the literature (26–29).

The increased absorption observed between 1740 and 1800 cm$^{-1}$ and also at about 1155 cm$^{-1}$ cannot be linked to the presence of a functional group of any related molecule (Figure 4.11e). Nevertheless, they might be associated to the formation of anhydride acids by the reaction of two carboxylic acid groups from different molecules (abietanes or pimaranes); those anhydride acids show C=O symmetric and asymmetric stretching bands between 1740 and 1800 cm$^{-1}$ and the C-O-C or C-C-O stretching band at about 1155 cm$^{-1}$.

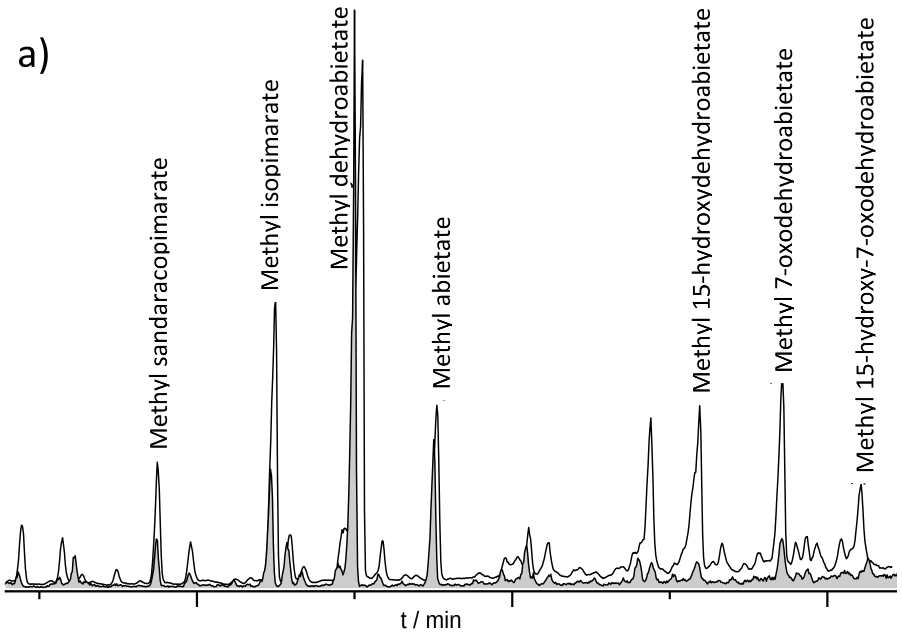
In order to confirm this, we have hydrolyzed these anhydride acids from an aged *Pinus Pinea* L. resin. These hydrolysis has been done by adding an excess of water and ethanol (50% v/v) and heating for three hours, then it has been left uncovered until water has been evaporated (about 1 hour) (30). The corresponding μFTIR spectrum and a GC/MS analysis are shown in Figure 4.12.

The GC/MS analysis (Figure 4.12a) shows a higher amount of pimaranes together with a higher concentration of other oxidized compounds demonstrating that they were originally in anhydride form and could not be detected by GC/MS. At the same time the shoulder between 1740 and 1780 cm$^{-1}$ observed in the FTIR spectrum decreases after hydrolyzation (Figure 4.12b) confirming also the assignment of this band to the C=O stretching from anhydride acid bonds. This could also explain why the presence of pimaranes was not detected in aged samples by GC/MS.

It also and agrees with the results obtained by Size Exclusion Chromatography showing the presence of high molecular weight compounds in aged resins (27).

Finally, the O-H stretching band at ~3400 cm$^{-1}$ is still present even in the most degraded materials (Figure 4.11) suggesting that esters are not formed, or are in an undetectable amount, by the reaction between the hydroxyl group from 15-hydroxy-7-oxo-dehydroabietic acid and a carboxylic acid group from other molecules as other studies have proposed (31).

A summary of the processes happening during ageing is shown in Figure 4.13 where specific FTIR markers for the identification of the various compounds produced during the degradation stages are also proposed.



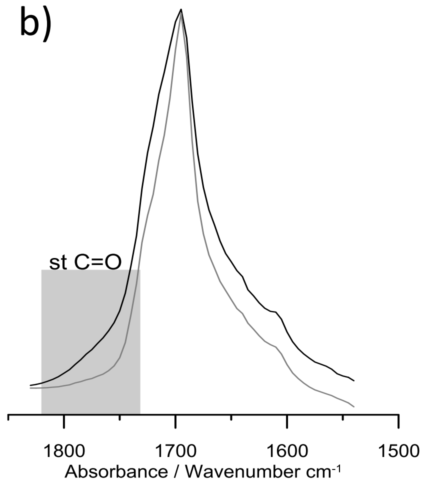


**Figure 4.12** *Pinus pinea* L. resin sample before and after the hydrolyzation. a) GC/MS analysis: original sample in grey and hydrolyzated sample in white b) FTIR spectra: black line is for original sample and grey line for hydrolyzed sample; grey area tags the region of C=O stretching from acid anhydrides.

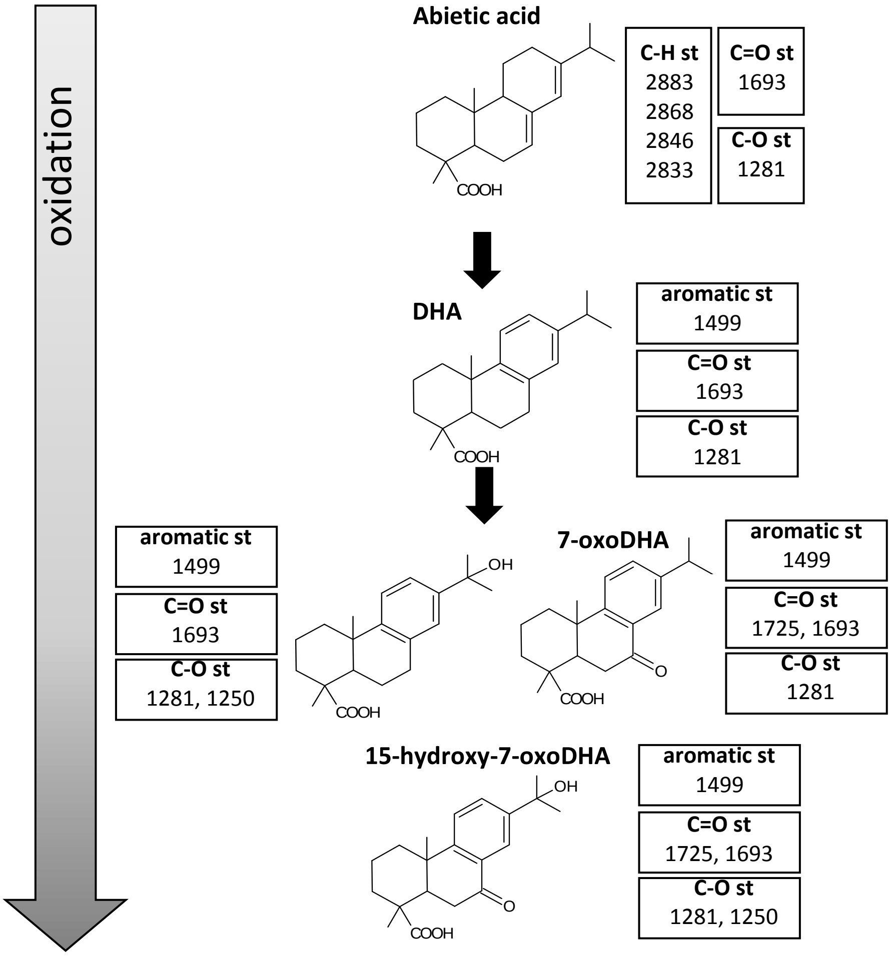


**Figure 4.13** Synopsis of the proposed specific markers in the infrared absorption spectra (numbers correspond to the wavenumbers in cm$^{-1}$).

## 4.4 CONCLUSIONS

A systematic study of the natural ageing of some *Pinus* resins and related compounds has been performed by FTIR. The oxidative processes and molecular changes happening during ageing have been identified.

The results confirm the initial transformation of the abietic acid into dehydroabietic acid and then into more oxidized species. At advanced stages of the transformation, reactions and interactions among molecules happen.

On the one hand, the hydroxyl group of the 15-hydroxy-7-oxo-dehydroabietic acid stablishes intermolecular bonds. On the other hand, the reaction between two carboxylic acid groups from different molecules (abietanes or pimaranes) gives rise to the formation of anhydride acids. This explains the decrease of the amount of pimaranes at the late stages of ageing in a more reliable way than the suggested polymerization between them. Moreover, other reactions which have also been proposed, such as the formation of esters, have not been determined.

Consequently, resins of the various species evolve into a similar molecular composition (oxidized abietanes and high molecular weight compounds).

Different ageing conditions (open air and darkness) follow the same oxidative pathways although with a different rate. Thus, it is difficult to establish a direct relationship between the age and the degradation of a sample.

Finally, both unaltered FTIR bands as well as those related to the different stages of the transformation have been identified. Since the identification of the resins is handicapped by the decay mechanisms happening since their exudation, the determination of both resins markers and degradation degree markers is essential.

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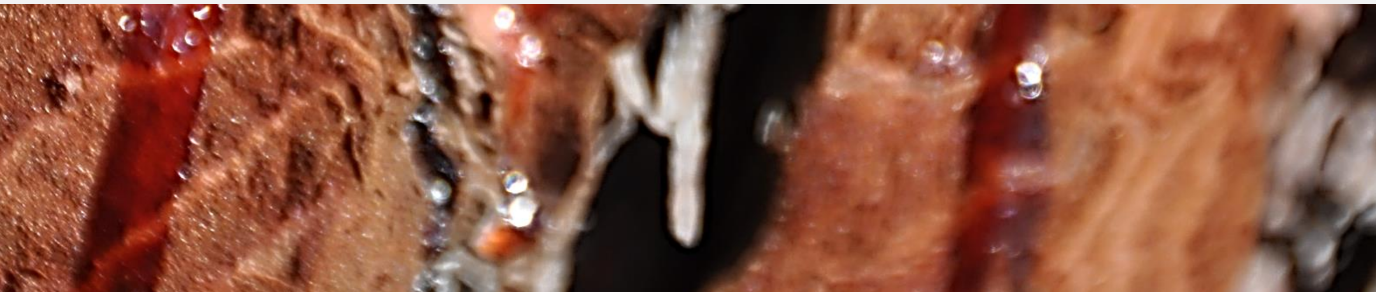
CHAPTER FIVE

# Raman analysis of *Pinus* resin



## 5.1 INTRODUCTION

The interpretation of data from vibrational spectroscopies can be very complex, especially mixtures of high weight molecules. A reliable band assignment, i. e. the correlation between the spectra bands and a specific molecular vibration, is crucial for the interpretation of the obtained results.

An accurate band assignment would not only permit a more precise identification of the material, but also could provide information about the sample chemical composition, the compounds structure as well as, of the chemical reactions and interactions among them.

Characteristic frequencies of molecular vibrations in FTIR and Raman spectroscopies are very similar, however the band intensities can be very different due to the specific excitation mechanisms and selection rules of each technique. The use of both techniques on the same material provides complementary spectra that can be used to confirm the band assignment established by each technique. (1)

Like FTIR spectroscopy, Raman has many advantages like the suitability for microanalysis, being non-invasive and a limited or null sample preparation. However, a previous knowledge of the nature of the substance is necessary to adjust the analytical conditions to avoid fluorescence, increase sensitivity and prevent damage. For instance, the sample can be burnt if the laser power is excessive. Lowering the laser power significantly reduces the risk of sample damage; nonetheless, different substances respond differently to the laser power.

The 785 nm laser wavelength provides excellent sensitivity minimizing the fluorescence of diterpenic resins. Higher energy lasers, emitting in the visible region, generate the release of significant background fluorescence.

A study of *Pinus* resins using Raman in order to complement the FTIR data (chapter 4) (2) is presented. The complementary use of both techniques for the analysis of differently aged resins corroborated and completed the initial bands assignments, aided identifying the specific Raman markers of fresh and aged compounds and gave more insightful information about the ageing processes of *Pinus* resin.

The chemical reagents of the main molecules found in fresh and aged resins were analyzed and a reliable band assignment established by comparing them to similar molecules. A broad selection of resins from different species, fresh and slightly aged as well as some severely aged resins was studied. Well dated lengthy aged samples from *Pinus* resins were obtained from archaeological objects and artworks.

Studies about historical remnants are important, not only for the information that they can provide about the long-term resin evolution, but also from the conservation and historical perspectives of the historical objects.

## 5.2 METHODS AND MATERIALS

### 5.2.1 Chemical reagents

Chemical reagents were used as references of the main resin compounds. Abietic, dehydroabietic and isopimaric acids were purchased from Sigma Aldrich (ref. 101392305, ref. SMB00089 and MKBT3436V respectively) and 15-hydroxy-7-oxodehydroabietic acid from Chemfaces (ref. CFN97533). Their purity was checked by GC/MS.

The abietic acid was aged for one year in an open air place exposed indirectly to sunlight but protected from weather inclemency (wind, rain…).

Compounds with similar functional groups to the main chemical references were also selected for comparison. Cumene (ref. C87657), 2-phenyl-2-propanol (ref. P30802), 1-methyl-1-cyclohexanecarboxylic acid (ref. 142824), α-tetralone (ref. T19003), isopropylcyclohexane (ref. I21904), vinylcyclohexane (ref. 111406) and cyclohexanone (ref. 398241) were purchased from Sigma Aldrich.

### 5.2.2 Resins

Resins from different sources and ages were studied in order to account for the degradation. The genus and species from all the natural resins studied were certified but subspecies and variants were not considered. Only a selection of the most illustrative samples is shown, details are summarized in Table 5.1.

Fresh and slightly aged resins (S1-S6) were collected directly from the tree.

**Table 5.1 Samples summary**

| Sample number | Description | | Origin | Age |
|---|---|---|---|---|
| **S1** | *Pinus pinaster* Aiton resin | | South of Coastal and Pre-Coastal Range of Catalonia | <1 week |
| **S2** | *Pinus halepensis* Mill. resin | | | <1 week |
| **S3** | *Pinus pinea* L. resin | | | <1 year |
| **S4** | *Pinus halepensis* Mill. resin | | | <1 year |
| **S5** | *Pinus pinaster* Aiton resin | | | <1 year |
| **S6** | *Pinus halepensis* Mill. resin | | | 2 years |
| **S7** | *Pinus halepensis* Mill. resin | | Botanic garden “Jardí Botanic de Barcelona” | 2 years 6 months |
| **S8** | resin from *Pinus halepensis* Mill. sold in a market of Syria, ref. 79882 | | Economic Botany Collection of the Royal Botanic Gardens, Kew | 2007 |
| **S9** | *Pinus sylvestris* L. resin ref. 28417 | | | probably 1850-1900 |
| **S10** | *Pinus nigra* J.F.Arnold resin displayed at the Forestry Exhibition, Edinburgh (1884), | ref. 28149 | | 1850-1900 |
| **S11** | | ref. 28172 | | 1850-1900 |
| **S12** | | ref. 31083 | | 1850-1900 |
| **S13** | Resin from a knot of the wooden panel of a ceiling | | Santa Maria de Paretdelgada (Selva del Camp, Catalonia) | 14th century |

## 5.2.3 Equipment

Raman and FTIR measurements were taken following the conditions described in chapter 2. The spectral range displayed from 2000 to 150 $cm^{-1}$, covers the region of interest where the specific markers are found. Three spectra were taken for each sample to assure that the data were reproducible. No smooth or baseline corrections have been applied to any spectra displayed, so the spectra show the quality of data which can be expected from the analysis.

Different absorbance scales are selected for FTIR and Raman when present in the same graph for better comparison of the data.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Reference compounds

The abietane acids are known markers of the degradation of the *Pinus* resin. Isopimaric acid may be used to determine the presence of pimaranes in the resins as all the molecules of this category contain the same functional groups. For this reason, the FTIR and Raman spectra of abietic, dehydroabietic, 15-hydroxy-7-oxodehydroabietic and isopimaric acids were collected and studied.

A comparative study based in the structural parts shared between all compounds and the ones which are exclusive of each one (Figure 5.1) is performed in order to propose the bands assignment.

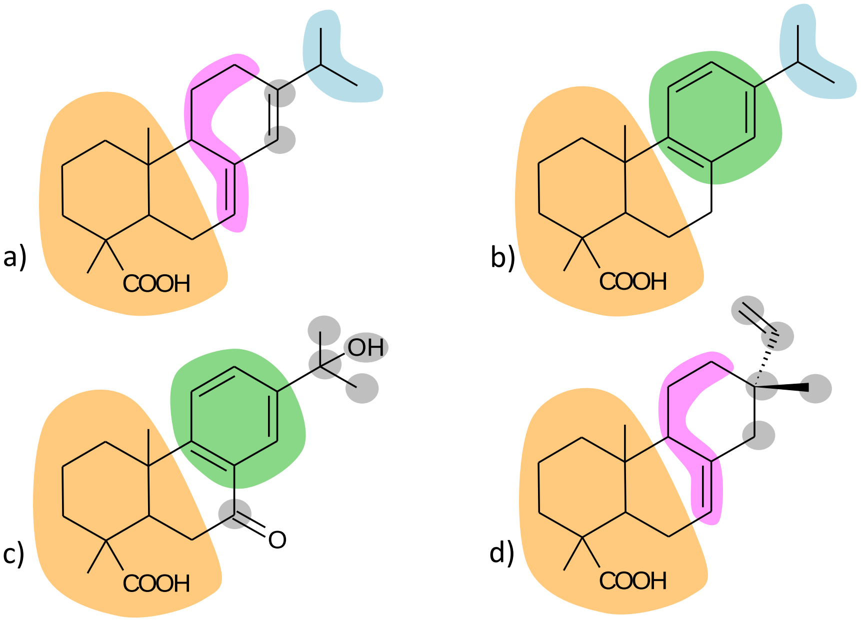


**Figure 5.1** Scheme of the main resin compounds a) abietic acid, b) dehydroabietic acid, c) 15-hydroxy-7-oxodehydroabietic acid and d) isopimaric acid showing shared functional groups with the same colour; distinctive groups are marked in grey.

The FTIR and Raman vibration bands common to all them were related to the vibration of the shared structure indicated in orange in Figure 5.1. The bands present only in the abietic and dehydroabietic acids were associated to the isopropyl group, marked in blue in Figure 5.1. Finally, the bands present only in the dehydroabietic and 15-hydroxy-7-oxodehydroabietic acids were associated to the aromatic group, marked in green in Figure 5.1. No vibrational bands in either the FTIR or Raman spectra are assigned to the shared structure of abietic and isopimaric acids, marked in pink in Figure 5.1, because no specific markers were found for them. The same comparison was also carried out with the corresponding Raman spectra.

The molecules assignment was corroborated comparing the FTIR and Raman spectra to those of molecules that contain the same structures (Figure 5.2, 5.3 and 5.4) and also with data from the literature (1,3–7). A summary is given in Tables 5.2 a-c.

The 15-hydroxy-7-oxodehydroabietic and isopimaric acids contain functional groups which are not shared with the other main resin reference materials; they are shown in grey in Figure 5.2. For this reason, those bands appearing only in their FTIR and/or Raman spectra were selected. Their spectra were also compared with those corresponding to simple molecules that contain the same structures and with data from literature (1,3). The results are displayed in Tables 5.3 a and b.

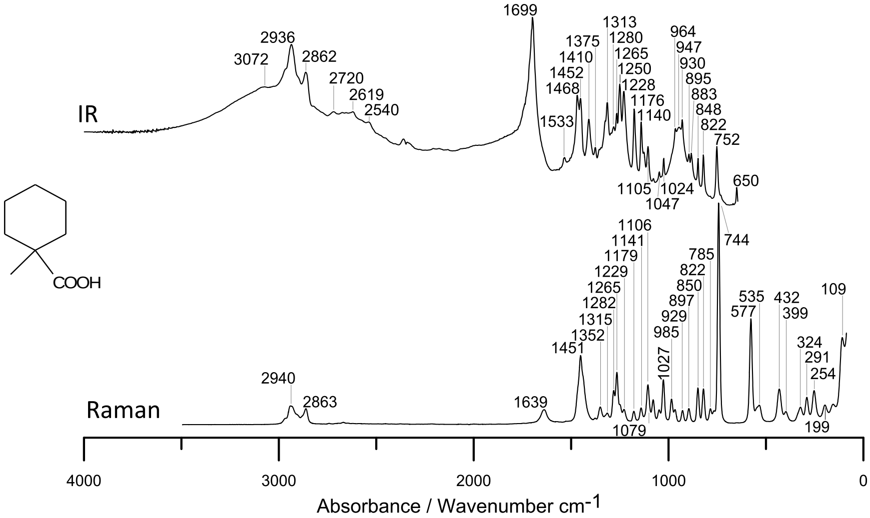


**Figure 5.2** FTIR and Raman spectra corresponding to 1-methyl-1-cyclohexanecarboxylic acid.

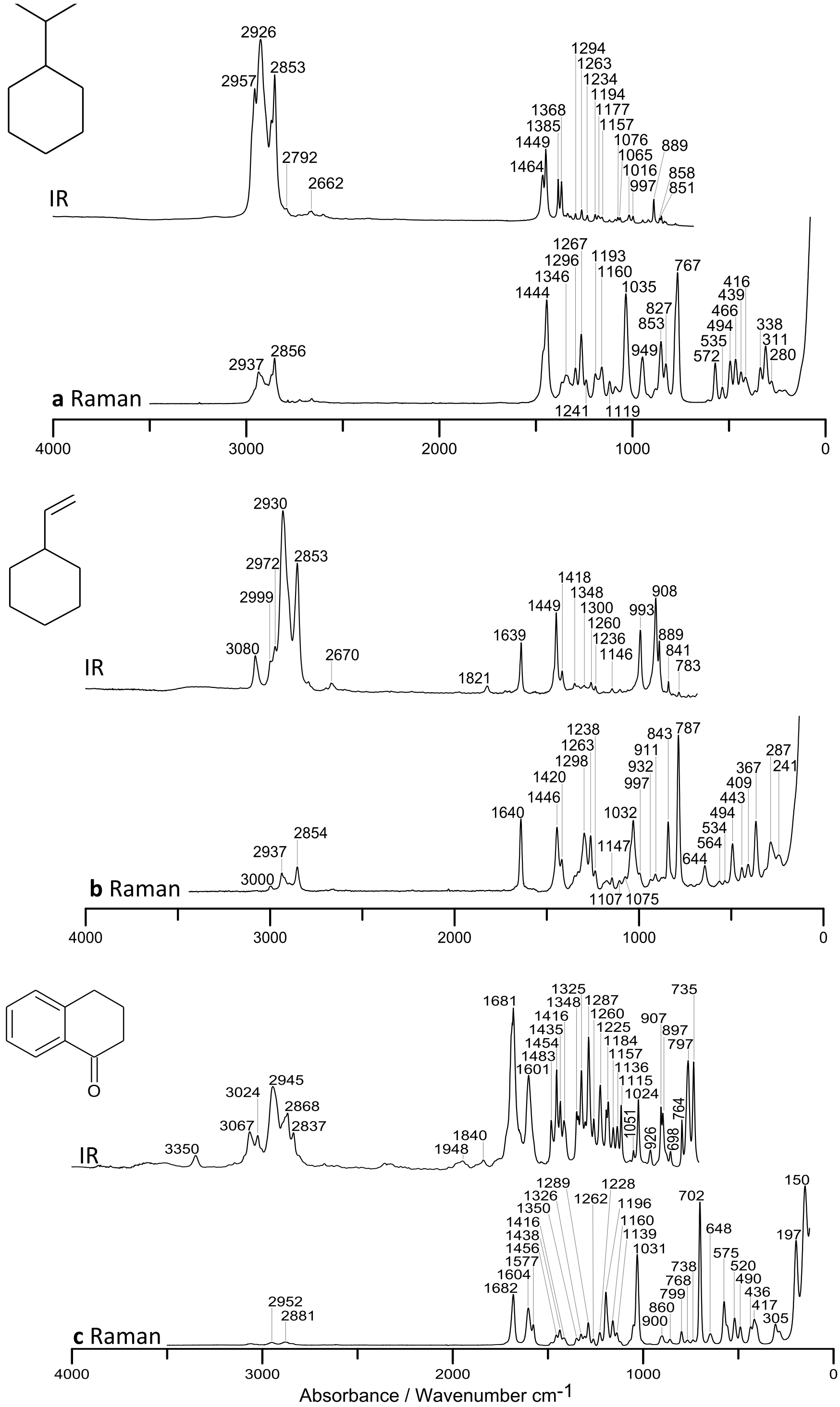


**Figure 5.3** FTIR and Raman spectra corresponding to a) isopropylcyclohexane, b) vinylcyclohexane, c) α-tetralone.

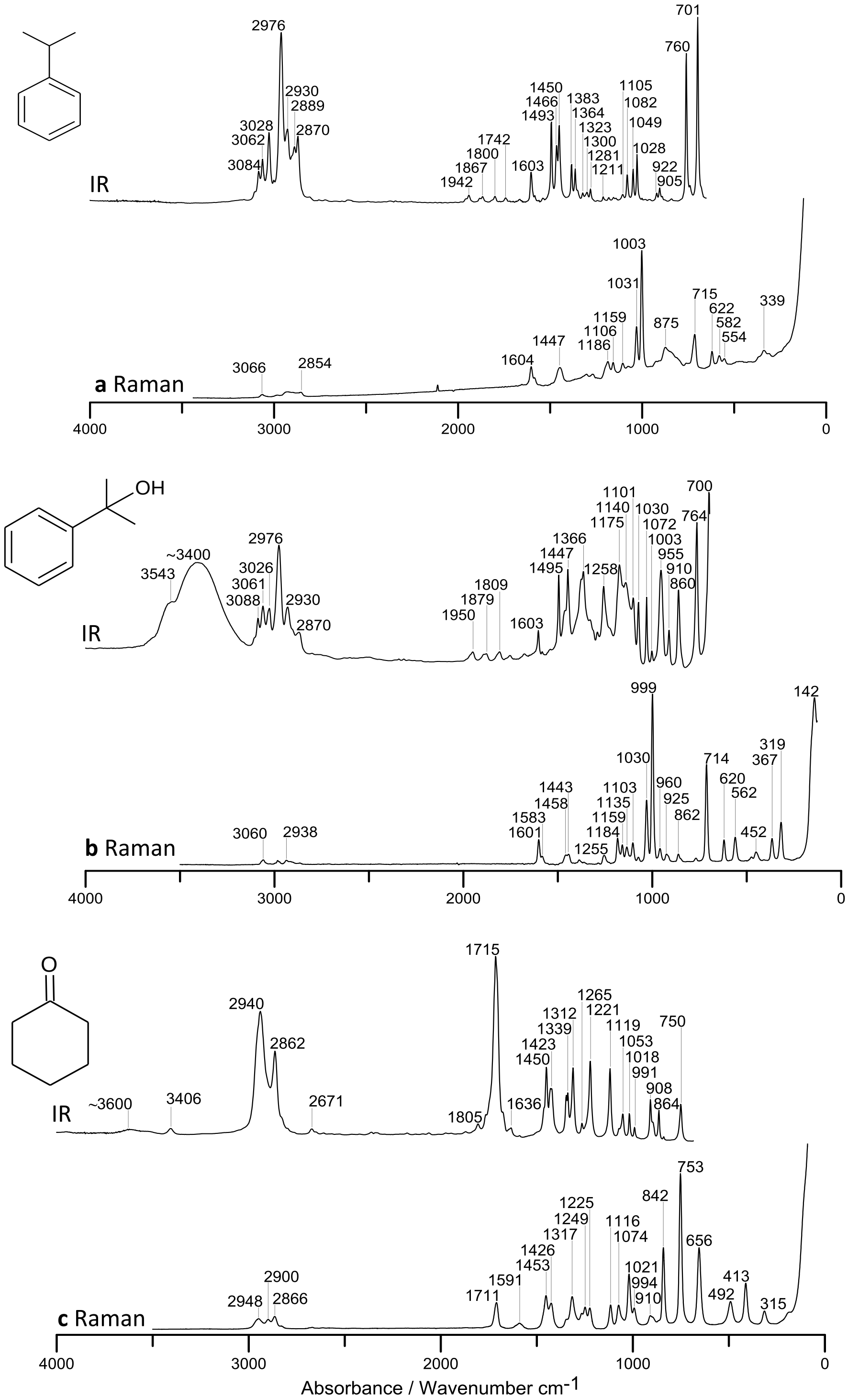
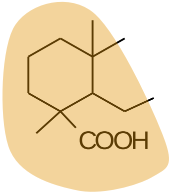
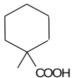


**Figure 5.4** FTIR and Raman spectra corresponding to a) cumene, b) 2-phenyl-2-propanol and c) cyclohexanone.

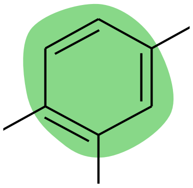
**TABLE 5.2a Characteristic bands of the common structure**

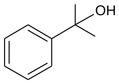
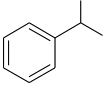


| Abietic acid | Dehydroabietic acid | 15-hydroxy-7-oxodehydroabietic acid | Isopimaric acid |  | Assignment |
|---|---|---|---|---|---|
| 1693 | 1695 | 1701 | 1691 | 1699 | C=O st |
| | | | | | |
| ~1470 | 1469 | 1468 | ~1470 | 1468 | C-H def |
| ~1470 | ~1470 | 1469 | 1473 | ~1470 | |
| 1462 | 1458 | 1456 | 1459 | 1452 | |
| ~1460 | 1463 | ~1460 | ~1460 | 1451 | |
| 1281 | 1284 | 1291 | 1282 | 1280 | Coupled C-O/O-H def |
| | | | | | |
| 952 | 950 | 958 | 944 | 947 | Skeletal vibration |
| | | | | | |
| 719 | 719 | 711 | 715 | | |
| 715 | 708 | 710 | 715 | | |

Characteristic FTIR (white background) and Raman bands (grey background) positions in cm$^{-1}$ corresponding to the shared groups among the main resin compounds. The symbol ~ indicates the approximate position of overlapped bands.

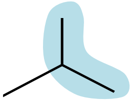
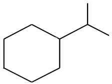
**TABLE 5.2b Characteristic bands of the aromatic ring**



| Dehydroabietic acid | 15-hydroxy-7-oxo dehydroabietic acid |  |  | Assignment |
|---|---|---|---|---|
| 1612 | ~1615 | 1603 | 1603 | C=C st (ring) |
| 1612 | 1623 | 1601 | 1604 | |
| 1499 | 1498 | 1495 | 1493 | C=C st (ring) |
| | | | | |
| 1037 | 1042 | 1030 | 1028 | C-H def (ring) |
| ~1035 | 1045 | 1030 | 1031 | |

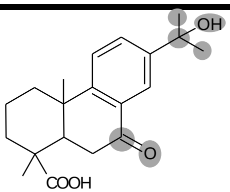
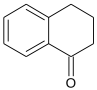
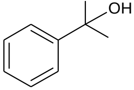
Characteristic FTIR (white background) and Raman bands (grey background) positions in cm-1 corresponding to the shared groups among the main resin compounds. The symbol ~ indicates the approximate position of overlapped bands.

**TABLE 5.2c Characteristic bands of the isopropyl group**

|  | | | |
|---|---|---|---|
| Abietic acid | Dehydroabietic acid |  | Assignment |
| 891 | 883 | 890 | Skeletal C-C st |
| | | | |

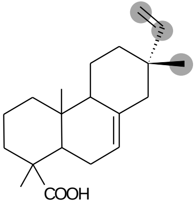
Characteristic FTIR (white background) and Raman bands (grey background) positions in cm-1 corresponding to the shared groups among the main resin compounds. The symbol ~ indicates the approximate position of overlapped bands.

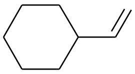
**TABLE 5.3a Characteristic bands of the 15-hydroxy-7-oxodehydroabietic acid specific groups**

|  | | | |
|---|---|---|---|
| 15-hydroxy-7-oxodehydroabietic acid |  |  | Assignment |
| | | | C=C st (ring) |
| 1579 | 1577 | 1583 | |
| 1188 | 1184 | | skeletal vibration |
| 1192 | 1196 | | |
| 1242 | | 1258 | C-C-O st (-OH) |
| | | | |
| 770 | | 764 | C-C-O st (-OH) |
| | | | |
| | | | C-C-O def (-OH) |
| 367 | | 367 | |

Characteristic FTIR (white background) and Raman bands (grey background) positions in cm-1 of 15-hydroxy-7-oxodehydroabietic acid according to the literature. The symbol ~ indicates the estimated position of overlapped bands.

**TABLE 5.3b Characteristic bands of the terminal C=C**



| Isopimaric acid |  | Assignment |
|---|---|---|
| 1641 | 1639 | C=C st |
| 1640 | 1640 | |

Characteristic FTIR (white background) and Raman bands (grey background) positions in cm$^{-1}$ of isopimaric acid according to the literature. The symbol ~ indicates the estimated position of overlapped bands.

Figure 5.5, 5.6, 5.7 and 5.8 shows the FTIR and Raman spectra obtained from the abietic, dehydroabietic, 15-hydroxy-7-oxodehydroabietic and isopimaric acids respectively. Proposed band assignments resulting from the previous comparative study are marked in green and those corresponding to data obtained from the literature are marked in blue.

Figure 5.8 shows the FTIR spectra corresponding to the 15-hydroxy-7-oxodehydroabietic acid obtained directly from the acid containing free –OH groups (Figure 5.8, red line) and after dissolving it in methanol and the solvent evaporated to enhance the formation of intermolecular bonds (Figure 5.8, black line), following the method reported in chapter 2. This comparison helps to highlight the differences among the bands related to the –OH group, heavily affected by its structure.

Among the band assignments proposed we can highlight the FTIR band around 1695 cm$^{-1}$ related to C=O stretching of carboxylic acids (Table 5.2a), although in the 15-hydroxy-7-oxodehydroabietic acid this band is overlapped with the C=O stretching from the ketone. This is confirmed comparing it with the corresponding band in the 1-methyl-1-cyclohexanecarboxylic acid (Figure 5.3b) which has the same functional group in a similar chemical environment.

Moreover, the FTIR band at 1280 cm$^{-1}$ can be related to the coupled C-O and O-H in-plane deformation modes of the –COOH group, although this is not so clearly seen in the 1-methyl-1-cyclohexanecarboxylic acid (Figure 5.3b) probably also due to the different chemical environment (4).

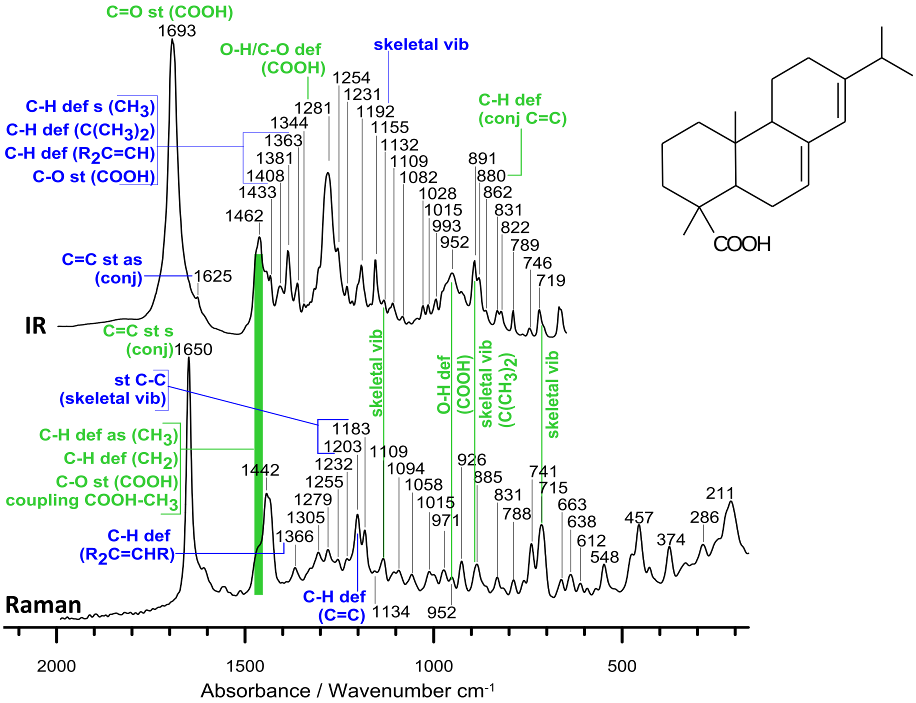


**Figure 5.5** Proposed band assignments for abietic acid. In green are the assignments resulting from the comparative study and in blue those based only in the literature.

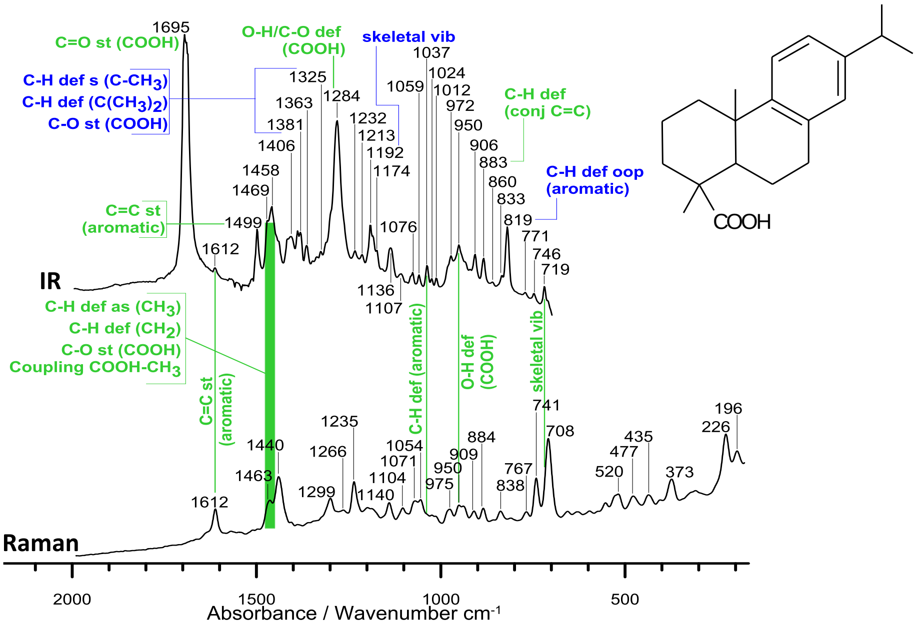


Figure 5.6 Proposed band assignments for dehydroabietic acid. In green are the assignments resulting from the comparative study and in blue those based only in the literature.

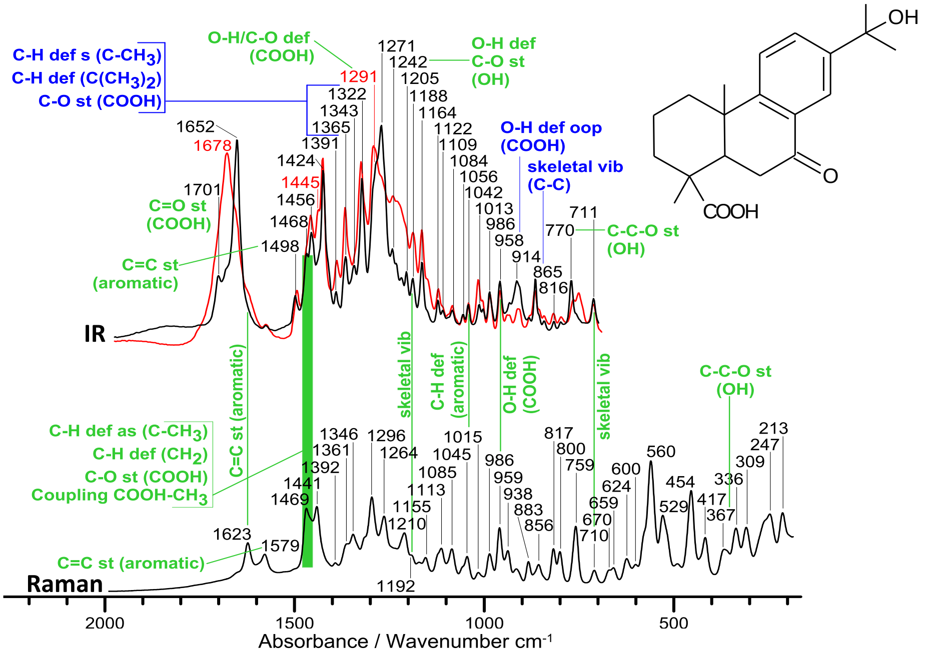


**Figure 5.7** Proposed band assignments for 15-hydroxy-7-oxodehydroabietic acid. In green are the assignments resulting from the comparative study and in blue those based only in the literature.

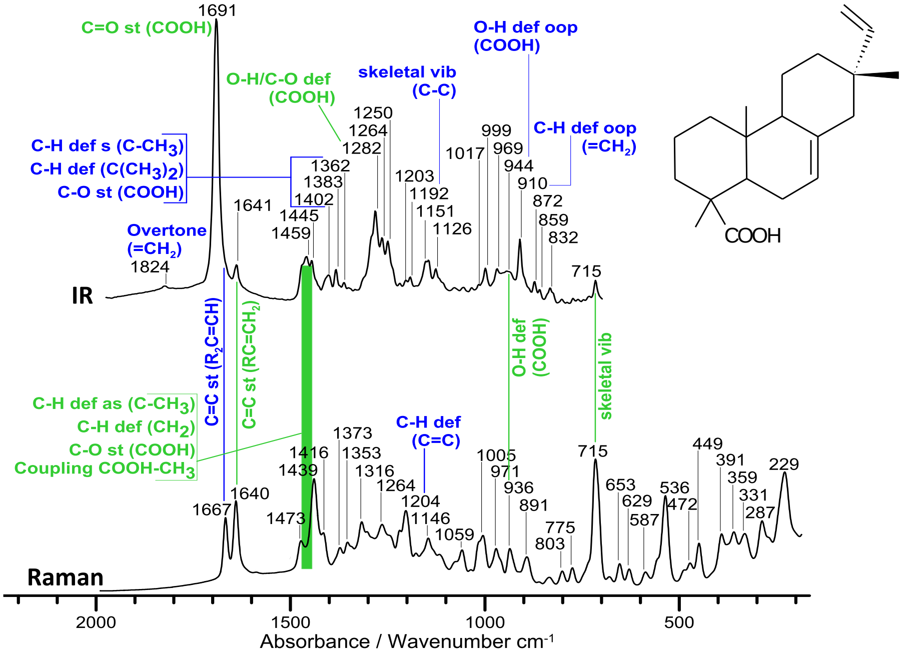


**Figure 5.8** Proposed band assignments for isopimaric acid. In green are the assignments resulting from the comparative study and in blue those based only in the literature.

The C=O group is normally Raman inactive, and this helps the analysis of the C=C stretching band. The C=C stretching band appears in the Raman spectra at 1650 $cm^{-1}$ in the abietic acid (Figure 5.5) resulting from C=C stretching of conjugated bonds, at 1612 $cm^{-1}$ in dehydroabietic acid (Figure 5.6) from vibration of the aromatic ring (Table 5.2c) and at 1623 and 1579 $cm^{-1}$ in 15-hydroxy-7-oxodehydroabietic acid (Figure 5.7) also from vibration of the aromatic ring with more polar substituents (Table 5.3a). In isopimaric acid (Figure 5.8) there are two bands related with C=C at 1640 and 1667 $cm^{-1}$, first one is related to $RC=CH_2$ (Table 5.3b) and second one probably to $R_2C=CH$.

Finally, the FTIR 950 $cm^{-1}$ band and the FTIR and Raman 715 $cm^{-1}$ band are both related to skeletal vibrations (table 5.2a).

Although the FTIR spectra normally contain more vibrational bands and, consequently, give more information about the structure, the Raman bands appear less overlapped, which greatly facilitates their identification.

### 5.3.2 Fresh resins

FTIR and Raman spectra of fresh resins, measured less than 2 weeks after collecting them from two different *Pinus* species (S1 and S2), as well as from abietic acid are shown in Figure 5.9a, 5.9c and 5.9b respectively.

The spectra corresponding to the resins show a similar spectral profile to those of the abietic acid. This is due to the fact that the abietic acid is the major constituent of the fresh *Pinus* resins, the differences are due to the presence of other minor compounds (8–14).

The differences between the FTIR spectra corresponding to the fresh resins and the abietic acid are subtle whilst are more prominent in the Raman spectra. The non-oxidized abietanes such as palustric acid or neoabietic acid and pimaranes, minor compounds of the fresh resin, can be distinguished from the abietic acid mainly by the C=C groups. Their bands are more easily identified in the Raman spectra (Figure 5.9, black lines) than in FTIR (Figure 5.9, blue lines).

For instance, in the 1650-1600 $cm^{-1}$ region of the Raman spectra the C=C stretching bands are clearly distinguishable while, on the contrary, appear overlapped with the C=O stretching bands from –COOH groups in the FTIR spectra.

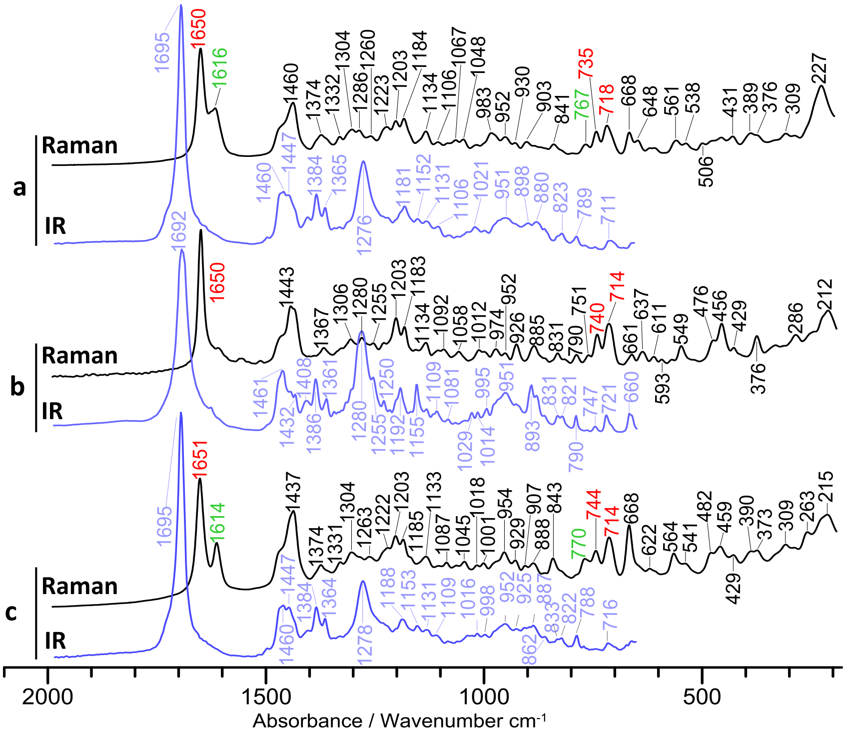


**Figure 5.9** FTIR (blue line) and Raman (black line) spectra of fresh resins extracted from two different *Pinus* species a) *Pinus pinaster* Aiton (S1) resin and c) *Pinus halepensis* Mill. (S2) resin compared to b) abietic acid. In red the wavenumbers shared by the Raman spectra of fresh *Pinus* resins and abietic acid and in green those appearing only in the fresh *Pinus* resins.

Specifically, the band at 1650 cm$^{-1}$ appearing in the abietic acid and related to conjugated C=C bonds is also observed in the fresh *Pinus* resins spectra. Moreover, the shoulder around 1615 cm$^{-1}$ more intense in the fresh resin spectra than in abietic acid, is probably related to the C=C bonds from the other minor compounds. The peak shape of the 1615 cm$^{-1}$ band in both resins (S1 and S2) appears slightly different, which could be related to the different amount of the various minor compounds present in each *Pinus* species.

Another remarkable difference between the Raman spectra of fresh *Pinus* resins and abietic acid is found in the 800 to 650 cm$^{-1}$ region and related to the skeletal vibrations. The same region of the FTIR spectra, show overlapped bands which greatly complicates their identification.

The bands at ~740 and ~715 cm$^{-1}$ observed in the Raman spectra from the fresh resins and abietic acid are related to the vibration of groups shared by abietanes and pimaranes (Figure 5.1, orange area). Contrariwise, the band at ~770 cm$^{-1}$ appears only in fresh resins spectra and is related to the presence of other minor compounds. Again, the different intensities are consequence of the variability of the exact composition of each resin.

### 5.3.3 First ageing changes

The FTIR and Raman spectra of different *Pinus* resin species (S3, S4 and S5) at the initial ageing stage, measured within one year after collecting, are compared.

Figure 5.10 shows the sequence of spectra from the freshest (top) to the most aged (bottom) resin. Samples were extracted from different *Pinus* species because, although the bands related to pimaranes are present in all of them (8–14) they can differ in intensity depending on the *Pinus* species. Thus, *Pinus* resin specie does not affect to the abietanes ageing and it only mean a difference in the proportion of non-oxidized abietanes, which disappear in aged samples due to all of them convert first to abietic acid and then to more oxidized abietanes.

The FTIR spectra in Figure 5.10 (blue lines) show an increase in the intensity of the 1715 cm$^{-1}$ shoulder (C=O stetching of ketones) and of the ~1610 cm$^{-1}$ (C=C stretching of the aromatic ring) and ~1245 cm$^{-1}$ (O-H deformation or C-O stretching of alcohols) bands with respect to the spectra corresponding to the fresh resins shown in Figure 5.10. All this data can be related to the presence of oxidized abietanes.

In Raman spectra of Figure 5.10 (black lines), the main band at 1650 cm$^{-1}$ related to the abietic acid decreases during ageing due to the oxidation processes. Other bands related to the abietic acid (Figure 5.5) (such as those at 1371 and 1204 cm$^{-1}$ also decrease.

In parallel, it is noticeable the increase of the Raman bands related to oxidized abietanes, such as, 1612 and 1569 cm$^{-1}$ related to the stretching of the aromatic ring, although the last at this early stages of aging is still small and broad.

The small shoulder about 1715 cm$^{-1}$ belongs to the C=O stretching of the ketone group from the 7-oxodehydroabietic and 15-hydroxy-7-oxodehydroabietic acids. The C=O stretching band of the ketone group, unlike the C=O stretching band from the –COOH groups, can be observed in the Raman spectra due to the resonance of the aromatic ring (1). The presence of this effect is also confirmed in the Raman spectra of α-tetralone (Figure 5.3c), where the intensity of the carbonyl C=O stretching band at 1682 $cm^{-1}$ appears also enhanced.

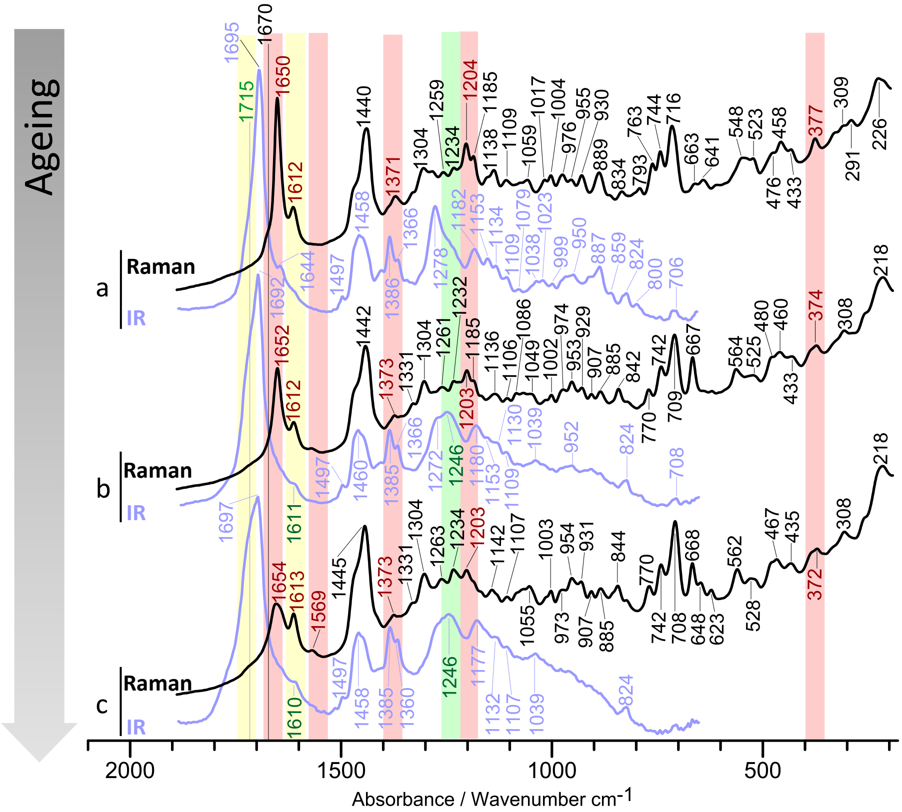


**Figure 5.10** First ageing stages (less than one year), more aged resin at the bottom. FTIR (blue line) and Raman (black line) spectra of *Pinus* resins: a) *Pinus pinea* L. (S3) b) *Pinus halepensis* Mill. (S4) c) *Pinus pinaster* Aiton (S5). The bands which change during aging in both FTIR and Raman spectra are highlighted in yellow, in red and green those that show changes only in the Raman or FTIR spectra respectively.

Other small changes observed in the Raman spectra are, for instance, the broadening of the small band at ~377 $cm^{-1}$ related to the presence of oxidized abietanes such as the dehydroabietic or the 15-hydroxy-7-oxodehydroabietic acids which have bands in this region (Figure 5.6 and 5.7).

Finally, the shoulder appearing at 1670 cm⁻¹ in the Raman spectra of the aged resins is related to the presence of isopimaric acid (C=C stretching). The Raman spectra of the aged resins confirm the presence of this double bond in all the aged resins related to the presence of pimaranes, which could hardly be noticed in the FTIR spectra.

Figure 5.11 shows the first year ageing of the abietic acid where the same changes observed in *Pinus* resin ageing are also observed. That is, as soon as the 1650 cm⁻¹ band decreases, the ~1610 and 1568 cm⁻¹ bands (C=C stretching of aromatic rings) increase and a shoulder at 1715 cm⁻¹ (C=O stretching of ketones) appears (Figure 5.11 at the bottom). Other variations occurring in the first steps of fresh resins ageing can also be detected.

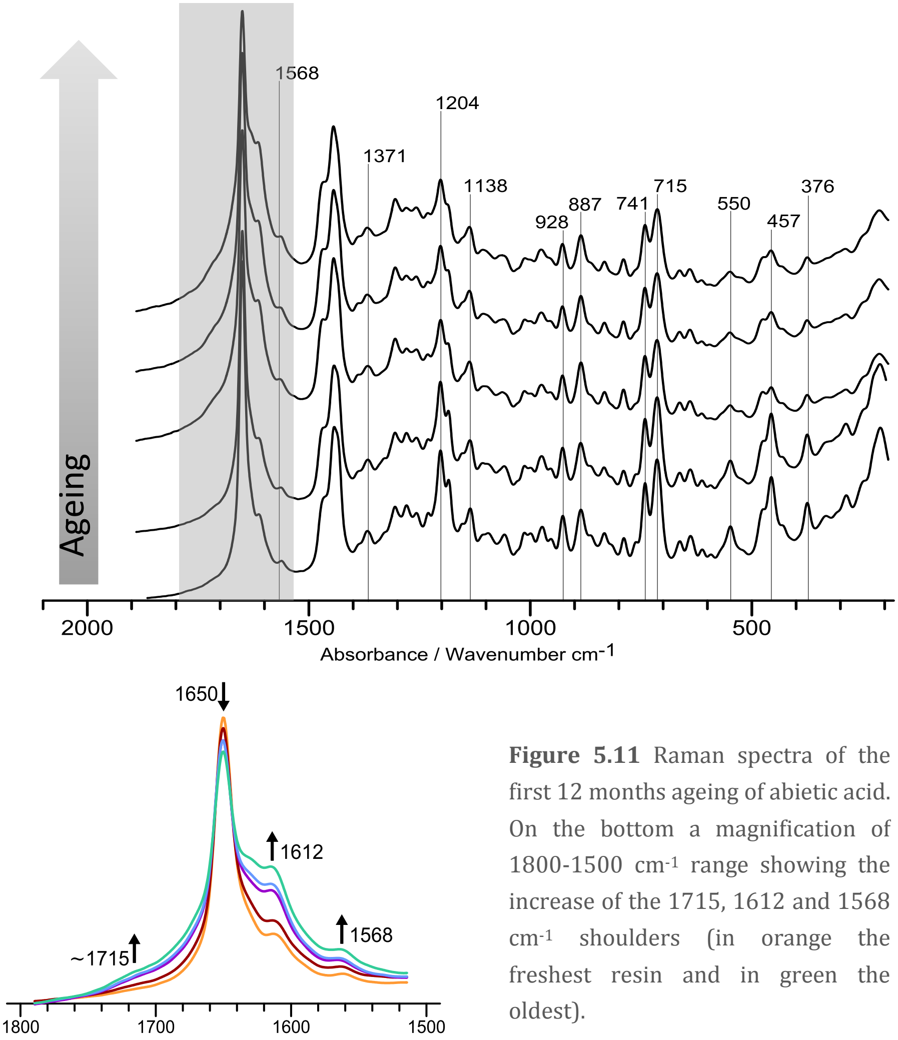


**Figure 5.11** Raman spectra of the first 12 months ageing of abietic acid. On the bottom a magnification of 1800-1500 cm⁻¹ range showing the increase of the 1715, 1612 and 1568 cm⁻¹ shoulders (in orange the freshest resin and in green the oldest).

Consequently, the main changes at the initial stage of *Pinus* resin ageing are mostly related to the changes happening in the abietic acid and in the abietanes.

### 5.3.4 Long term ageing

The Raman spectra from various *Pinus* species resin aged between 2 years and 6 centuries are shown in Figure 5.12.

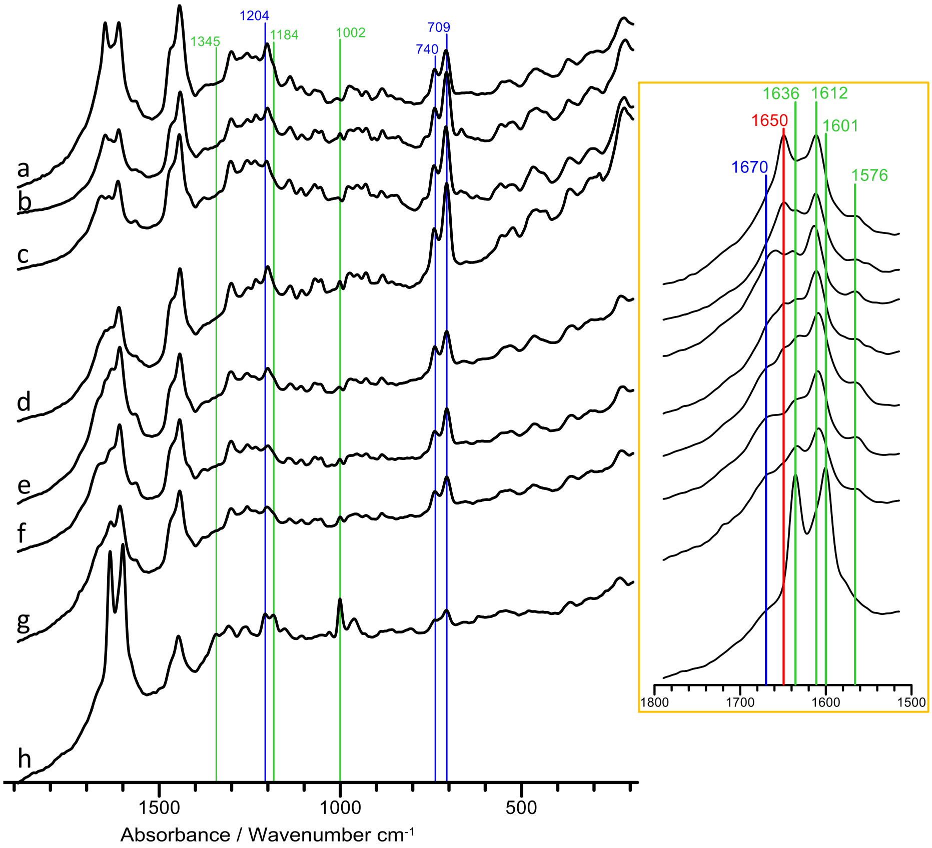


**Figure 5.12** Raman spectra of aged resins: a) two years old *Pinus halepensis* Mill. (S6), b) two years and half old *Pinus halepensis* Mill. (S7), c) nine years old *Pinus halepensis* Mill. (S8), d) *Pinus sylvestris* L. probably collected between 1850 and 1900 (S9), e-g) *Pinus nigra* J. F. Arnold collected between 1850 and 1900 from different sources (S10, S11 and S12), h) resin extracted from a knot dated from the 14th century (S13). On the right a magnification of the 1800-1500 cm$^{-1}$ region is shown. Marked in red those bands which disappear during ageing, in green those which appear during aging and in blue the bands that appear unaffected.

The spectra tend to smooth out during ageing due to the increasing presence of different molecules in the resin which diversifies the chemical environment of the characteristic functional groups. Nonetheless, some spectral features are still clearly seen.

For instance, the Raman bands at ~740 and ~709 $cm^{-1}$ remain unchanged during ageing, (Figure 5.12, blue lines) because they are related to the skeletal vibrations of that part of the abietanes and pimaranes structure which do not change.

Moreover, it is significant the presence of a band at 1204 $cm^{-1}$ even in the oldest resins. This band is found in the Raman spectra of both the abietic and isopimaric acids and is most probably related to the $RHC=CR_2$ groups. In aged resins, considering that none of the spectral markers associated to the abietic acid are observed, it should be assigned to the presence of isopimaric acid.

The main bands that increase in the Raman spectra during ageing (Figure 5.12, green lines) are related to the formation of oxidized compounds, such as the 1612 and 1576 $cm^{-1}$ bands related to aromatic groups or those at 1345 $cm^{-1}$ related to the 15-hydroxy-7-oxodehydroabietic acid. Moreover, the rise of the 1184 and 1002 $cm^{-1}$ bands can be probably related to the C-C-O and C-O-C stretching groups of acid anhydrides which have been reported before (15).

Other bands from oxidized compounds cannot be distinguished, like 1623 $cm^{-1}$ band from 15-hydroxy-7-oxodehydroabietic acid probably overlapped.

The decrease observed on the intensity of the 1650 $cm^{-1}$ Raman band corresponding to the abietic acid in the aged resins (Figure 5.13, red line) uncovers the presence of hidden bands mainly ~1670, 1636 and 1601 $cm^{-1}$. The first two may be assigned to the vibrations of the C=C groups from pimaranes and, they were probably present already in the spectra of the fresh resins but overlapped. Those two bands are also present in the Raman spectrum of isopimaric acid (Figure 5.9) and they are expected to exist also in all the other pimaranes because they are related to the presence of $RHC=H_2$ and $R_2C=CH$ groups. Pimaranes $RHC=H_2$ bands seem to be stable along ageing; this means that this bonds don’t polymerize or at least not sufficiently to be detected by Raman.

On the other hand, the band at 1601 $cm^{-1}$ cannot be related to any functional group of the oxidized abietanes; we propose to assign it to the hydrogen bonds formed between ketones, hydroxyls and carboxylic acid groups whose bands appear in this region (3). Since the resin at this stage is a solid, the reorganization of the molecules necessary to form these bonds is a slow process; consequently, it is also expected that the corresponding bands are seen only in very old resins.

To confirm this hypothesis, the oldest resin (S13) was dissolved in methanol in order to break the intermolecular hydrogen bonds. The Raman spectra before and after the treatment are displayed in Figure 5.13.

In the figure a decrease in the intensity of the bands at 1636 and 1601 cm$^{-1}$ related to hydrogen bonds is clearly seen. Isopimaric acid has also a band at 1636 cm$^{-1}$ but, as long as it does not react with methanol, the band should not be affected by the treatment. Consequently, two bands vibrate at 1636 cm$^{-1}$ one related to isopimaric acid and the other to hydrogen intermolecular bonds, last one increasing in aged samples.

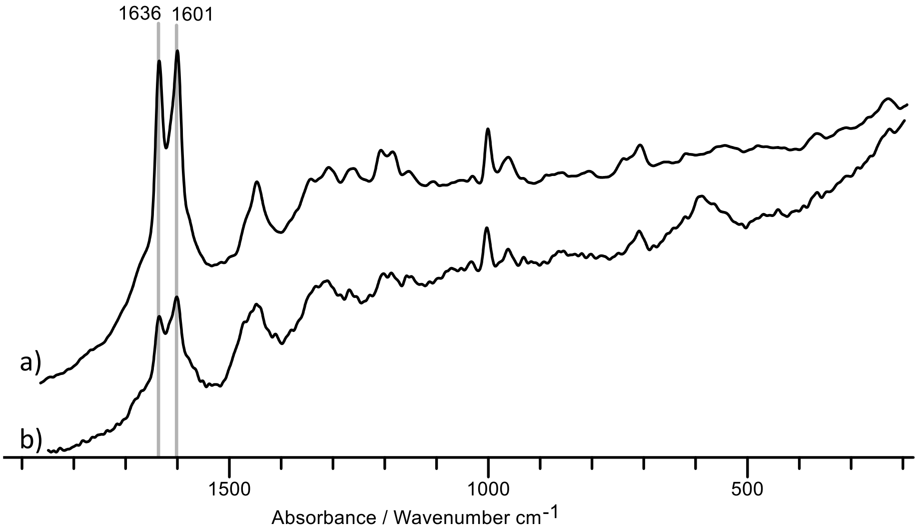


**Figure 5.13** Raman spectra of a resin dated from 14th century (S13) a) untreated and b) after dissolving it in methanol and the dissolvent evaporated at room temperature. The bands marked are those proposed for the vibration of hydrogen bonds.

Since the presence of hydrogen bonds between carboxylic acids (dimeric form) has already been reported in fresh resins and considering that hydrogen bonds between –OH from the 15-hydroxy-7-oxodehydroabietic acid are easily formed on dissolving the resin in methanol (Figure 5.7), the bands at 1636 and 1601 cm$^{-1}$ may be related to the long time molecule reorganization resulting in the development of these new kind of hydrogen bonds.

Characteristic vibrational markers of pimaranes, non-oxidized and oxidized abietanes and hydrogen bonds which appear along ageing are summarized in Figure 5.14. The small peak shifts observed are due to the overlapping among vibrational bands typical of multicomponent mixtures.

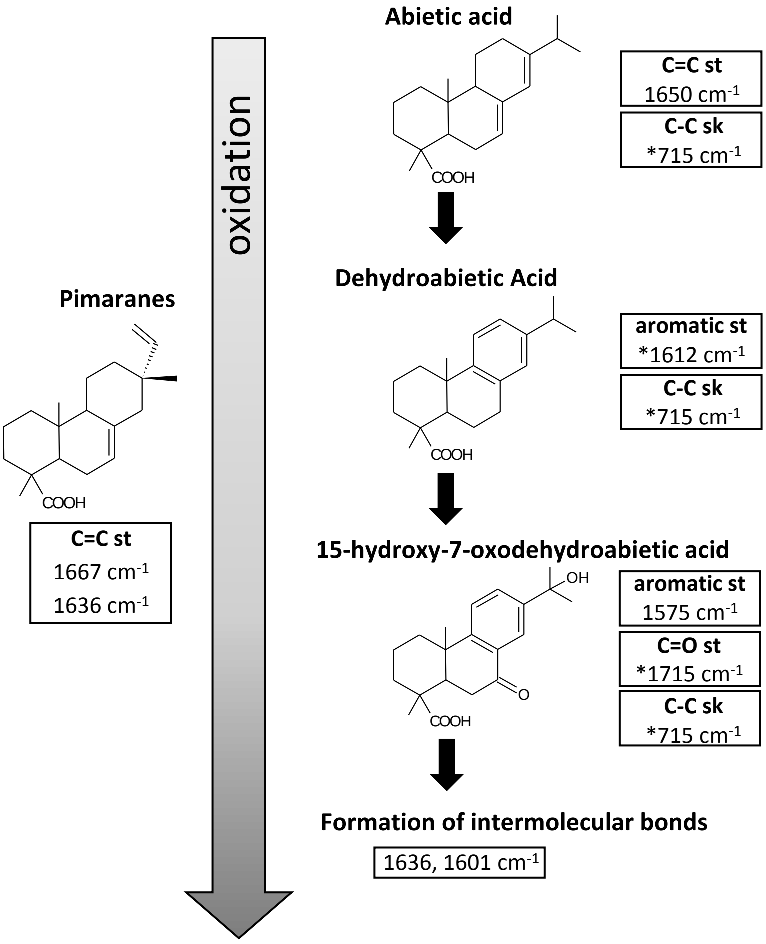


**Figure 5.14** Summary of the Raman band positions characteristic of fresh and aged compounds. (*) refers to those bands that are also present in FTIR spectra.

## 5.4 CONCLUSIONS

A systematic study of the ageing of *Pinus* resins by Raman spectroscopy has been done confirming the usefulness of this technique for this kind of material. Obtained results shed light on the molecular changes of *Pinus* resins and complement the information provided by other analytical techniques.

A study of the FTIR and Raman spectra of chemical references of the main resin compounds confirmed the formation of oxidized abietanes during ageing. At more advanced stages the interaction between these oxidized abietanes producing hydrogen bonds which show very intense bands has been demonstrated thanks to Raman spectroscopy. This cannot be clearly seen in FTIR spectra because these peaks are overlapped by the C=O stretching of ketones and carboxylic acids. Consequently, the Raman bands related with hydrogen bonds can be used to identify long-aged resins.

The experiments brought information about the pimaranes evolution during ageing. It is difficult to confirm the formation of acid anhydrides by Raman spectroscopy as their characteristic vibrations are not very intense, however bands assigned to the $RHC=H_2$ of pimaranes still appear in aged samples. This would denote that a polymerization process between pimaranes affecting these bonds has not taken place, or at least it happens in such low amounts that cannot be appreciated by Raman spectroscopy. The lack of polymer networks would explain the fragility observed in aged resins.

From the results of the comparative Raman-FTIR study, different specific Raman markers for the fresh and aged compounds are proposed which can be used in the analysis of unknown samples. This is useful in many fields, for example for the study of historical artworks coatings and varnishes. The obtained results can be also extended to the study of other abietane based resins like *Larix decidua* Mill. or *Abies alba* Mill.

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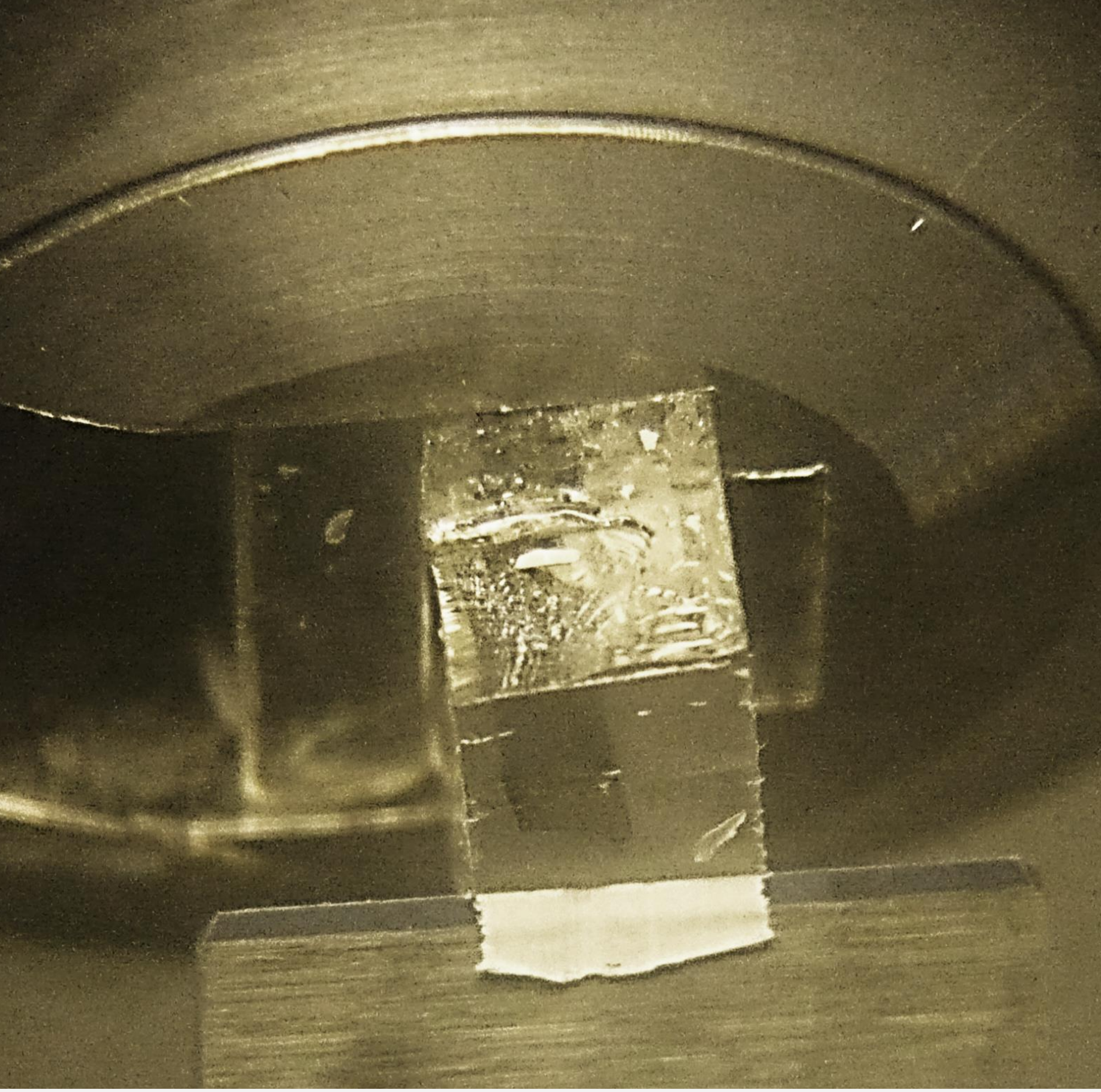
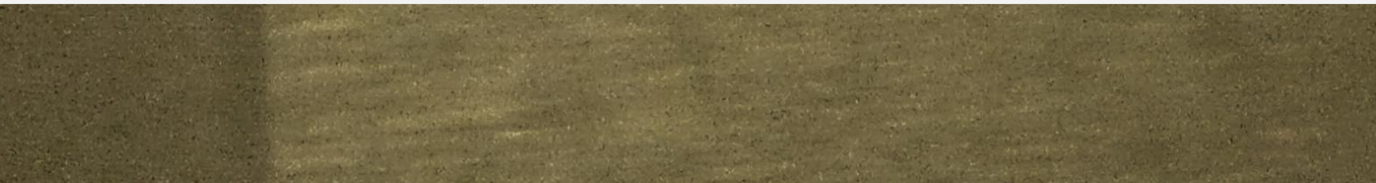


## CHAPTER SIX

# Optimization of sample preparation for the analysis of complex layered microstructure samples



## 6.1 INTRODUCTION

Precise microanalytical techniques are essential in many fields and in particular for the study of Cultural Heritage materials.

The samples often show a complex layered microstructure containing a wide range of materials of diverse nature and hardness which requires of accurate sample manipulation and preparation. Non-invasive sample manipulation and preparation is required to avoid, as far as possible, the sample contamination which may strongly limit the identification of materials (1).

Different strategies are used to assist sample manipulation and to make the most of each fragment; among them, one of the most common is to embed sample fragments in a supporting medium (2). Although the embedding process may alter the samples, on the other hand it preserves them for future examinations.

This procedure helps handling even the tiniest and most fragile samples for polishing or microtoming. Polished cross-sections and thin preparations are needed to analyse the layer microstructure by means of Optical Microscopy (OM), Scanning Electron Microscopy (SEM/EDX), Infrared Spectroscopy FTIR, Raman spectroscopy, X-Ray Fluorescent Spectroscopy (XRF) or X-Ray diffraction (XRD) (3–7).

The requirements of an optimal embedding medium for micrometric heterogeneous samples such as those found in Cultural Heritage materials (paintings, furniture, leather…) have been widely discussed elsewhere (8–10). A good embedding medium must be transparent (needed for localizing the area of interest), hard enough to be cut and polished, should not shrink during curing, cure at room temperature (the materials are easily damaged by temperature changes) and should not react or penetrate the sample. Other desirable features are low toxicity, low cost and a fast curing.

Among those requirements, we have to highlight the penetration of the embedding medium in the sample surface, as its presence may interfere with the analysis and complicates the interpretation, in particular for micrometric samples containing a great diversity of substances such as happens with Cultural Heritage materials. This is especially important for the organic samples analysed by FTIR or Raman spectroscopy as the embedding medium gives a strong signal.

Regardless the fact that a perfect embedding medium has yet to be discovered; the most commonly used materials are different types of synthetic resins, primarily epoxy and polyester. Both match the requirements quite well except for the low reactivity and penetration capability (7,11–15). Other synthetic resins which have been used, such as acrylic, cyanoacrylate or polyethylene based polymer, have neither a lower reactivity nor a lower penetration and display more limitations with the other requisites. (5)

Mathematical subtractions have been tested to remove the embedding medium contribution in the contaminated sample spectra (10); but to define a precise method has proved to be difficult and may lead to wrong conclusions.

Another strategy is the minimization of the embedding medium infiltration which may be attained by protecting the surface by a coating layer. However, this is problematic as the embedding medium acts also as a consolidator and aids cutting, polishing or microtoming fragile samples which otherwise may easily crumble.

Despite the difficulties, some smart approaches of protecting samples are giving promising results. Full substitution of the synthetic resin by an IR-transparent salt has been tested, but the block obtained is more brittle and forbid the use of water lubricated diamond saws or polishers (10).

Sample coatings, which do not interfere with sample analysis such as molten or dissolved organic media layers (wax, gels, cyclododecane among other) (5,11) and metallic coatings (12) have also been considered.

The simplest and least invasive method is the application of a gold coating (5 nm), which has already been tested with non-consistent results (5). Gold sputtered coatings offer many advantages: they are easy and fast to apply, non-toxic and they do not react or interfere with the sample; consequently, in this study we examine the possibilities of thicker gold sputtered coating layers to protect the samples from the embedding medium contamination. The only handicap of this method is that it disturbs the analysis of those samples that already contain gold; for such cases, we also test the protective capability of carbon sputtered coatings.

Most of the materials of interest in Cultural Heritage are varnished and consequently, the most external layer of the samples is very often a varnish. Subsequently, the varnish layer is likely to be in direct contact with the embedding medium and more affected by the resin penetration. For this reason, we selected those compounds constituting the most common natural varnishes to check the best sample preparation method. Although synthetic materials were also employed as a varnish, their use started in the 19th century while natural resins are found in earlier objects provided that they have not been restored.

The penetration of the embedding medium into the sample surface is evaluated by means of μFTIR spectroscopy. μFTIR is known to provide broad species identification with minimal sample area (16–18) of particular interest in the identification of organic compounds (19).

Although different setups are available for FTIR spectroscopy, only the transmission geometry gives the desired sensitivity and spectral quality for unambiguous identification of the substances present. Consequently, the embedding medium contamination is estimated by transmission μFTIR spectroscopy from coated microtomed thin sections of natural varnishes embedded in synthetic resin.

Finally, real artwork samples have also been prepared and analysed by μSR-FTIR, μSR-XRD and SEM to demonstrate the potentiality of the sample preparation method proposed.

## 6.2 METHODS AND MATERIALS

A series of experiments have been designed to test the protective capability of embedding medium infiltration either for gold and carbon sputtered coating layers; both for test materials and real artwork samples.

### 6.2.1 Test materials

Natural and 15 years aged egg white (room temperature and protected from direct sun light) were selected. Beeswax was obtained from a honeycomb (Riudoms, Catalonia) and shellac wax was purchased at Zecchi (Ref. 2750).

Among the resins, dammar was bought at CTS (Ref. 01125501) and sandarac at Zecchi (Ref. 2250); amber, shellac (gommalacca rubino) and mastic samples were obtained from particular collections. Finally, *Pinaceae* resin was extracted from a *Pinus Sylvestris* L. (Jardí Botànic de Barcelona).

### 6.2.2 Artwork samples

Samples of different artowrks were studied using the proposed method. Case I is related to the samples from one of the canvas of the Sant Francesc d'Assís life series painted by Antoni Viladomat Manalt (1678-1755) and kept in the Museu Nacional d'Art de Catalunya MNAC (Barcelona). Case II is related to the Calvari altarpiece (15th century) kept in the Museu Nacional d'Art de Catalunya MNAC (Barcelona). Case III is related to the pictorial decorations of Sant Miquel Chapel, located in the Royal Monastery of Pedralbes (Museu d'Història de Barcelona).

### 6.2.3 Embedding medium

Epoxy and polyester resins were tested.

The polyester resin, bought at CCP Composites, is a copolymer of pthalic anhydride and ethylene glycol with styrene and dicyclopentadiene for cross linking (NORSODYNE® O 12335 AL) catalysed with a Methyl Ethyl Ketone Peroxide (LUPEROX® K1 G). The unsaturated resin has a low viscosity and its composition has also other minor compounds like methyl methacrylate. It needs three days to cure.

The epoxy resin, also purchased at CCP Composites, is a copolymer of bisphenol A and epichlorohydrin which has other components such as oxirane and 2-P-tolyloxymethyl-oxirane. It is mixed with a hardener made of 5-amino-1,3,3-trimethylcyclohexanemethylamine and trimethyl-1,6-hexanediamine which gives a cross linked polymer. It needs 24 hours to cure.

To embed the samples, a resin bed of either polyester or epoxy was left to polymerize for 30 minutes and four hours respectively before the samples were placed and covered with resin.

### 6.2.4 Analytical instrumentation

#### 6.2.4.1 Turbo evaporator

Protective gold and carbon coatings were applied with a turbo evaporator Emitech K950X using a vacuum of $3x10^{-3}$ mbar (Figure 6.1).

Carbon coatings were obtained from graphite rods (Ted Pella, product 61-15, grade 1, 1/8” x12”). Two kinds of coatings were produced: thin and thick coatings obtained after 13 and 28 discharges respectively (900ms each discharge, coatings thickness are approximately from 30 to 40 nm and from 60 to 80 nm). Gold coatings were obtained from a 4cm gold strand of 0.08’’ diameter purchased at Ted Pella (Ref. 21-10) after two discharges (from 20 to 30 nm coatings thickness).

Three sets of preparations, one covered with a thin carbon layer, another covered with a thick carbon layer and a third one covered with gold were obtained. Real artwork samples were also gold-coated with four repetitions to ensure a better protection (40 to 60 nm). Pressure changes happening in the turbo evaporator are likely to eject small samples.

**Figure 6.1** Turbo evaporator Emitech K950X from Centre de Recerca en Nanoenginyeria CRnE (UPC).

Thus, an adhesive (Sylgard 184, ref. 761036-1EA from Sigma Aldrich) placed in a small box was used to hold the samples in the evaporator chamber.

Afterwards, the samples were mechanically removed from the adhesive using tweezers. The uncoated part of the sample was kept and used to check the efficiency of the coating.

#### 6.2.4.2 Microtome

The microtome is a Motorized Rotary Microtome RMC MT-990 (Figure 6.2), with a tungsten carbide blade and using the manual mode. Despite the difficulties found in some cases sections from all the materials and samples embedded were obtained. The most uniform sections were obtained using a cutting angle of 12º.

A scheme of the whole process can be seen in Figure 6.3

**Figure 6.2** Motorized Rotary Microtome RMC MT-990 from Diamond Light Source (UK).

#### 6.2.4.3 Optical Microscopy (OM)

Microsamples were manipulated under a Stereomicroscope, sections and thin slides were observed with an optical microscope. Equipment is described in chapter 2.

#### 6.2.4.4 Infrared spectroscopy

μFTIR and μSR-FTIR equipment and conditions are described in chapter 2. Analysed areas are 30 μm x 30 μm for μFTIR and 15 μm x 15 μm for μSR-FTIR.

A micro compression cell Specac GS02520 with KBr windows of 13x2mm has been used for the analysis of the test materials sections; artwork samples were pressed between two thin KBr (Scharlau PO 01680100) pellets.

All the collected infrared spectra were processed with Opus 7.2 (Bruker Optics, Inc). Spectra were taken for each test material, embedding medium and coating layer from an area immediately under the sample surface, at 10 µm and at 20 µm depth. At least 3 spectra were obtained at each depth.

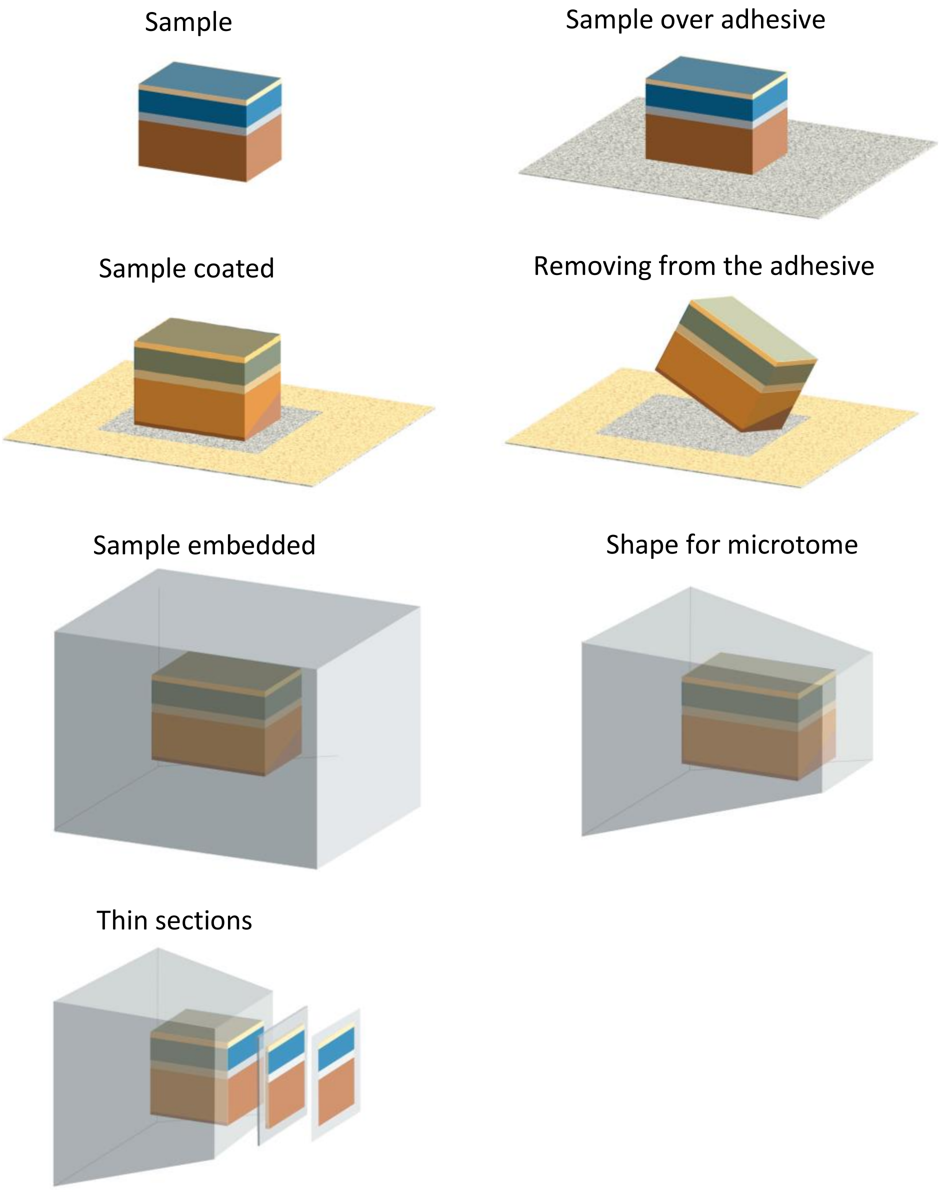


**Figure 6.3** Scheme of the proposed sample preparation method.

The region between 1700 and 900 $cm^{-1}$ was selected for data treatment: baseline correction (scattering correction with 64 baseline points) and normalization were first applied. Then, all the spectra corresponding to the same depth were averaged and the standard deviation calculated. In addition, the absorbance of the materials was measured from pellets obtained diluting the compounds with KBr.

#### 6.2.4.5 X-ray diffraction

µSR-XRD has been performed using the equipment and conditions are described in chapter 2.

#### 6.2.4.6 Scanning Electron Microscope

SEM equipment and conditions are described in chapter 2.

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 Embedding medium

The natural varnishes were embedded in epoxy and polyester resin to test the best embedding medium.

Although between 8 µm and 1 µm thick sections could be obtained from both resins, the epoxy resin is more transparent and the slices are more easily cut and more consistent. Polyester is more fragile and is often fragmented during microtoming; sections obtained are sticky, can be easily broken and it is convenient to analyse them immediately after cutting. Some examples of the thin sections obtained for shellac are shown in Figure 6.4.

### 6.3.2 Thickness optimization

8, 5, 2 and 1 µm thick sections of all the compounds were cut to determine the optimal sample thickness for µFTIR transmission analysis. As an example the µFTIR spectra corresponding to shellac are shown in Figure 6.5.

The optimal thickness is assessed from the absorption data and corresponds to those sections of thickness varying between 5 µm and 2 µm.

Spectra obtained for 8 µm thick sections appear saturated. Contrariwise, although 1 µm thick sections give good IR spectra, the sections are extremely fragile and complete sections are difficult to obtain and keep. This thickness study is in good agreement with other studies (8,12,20,21).

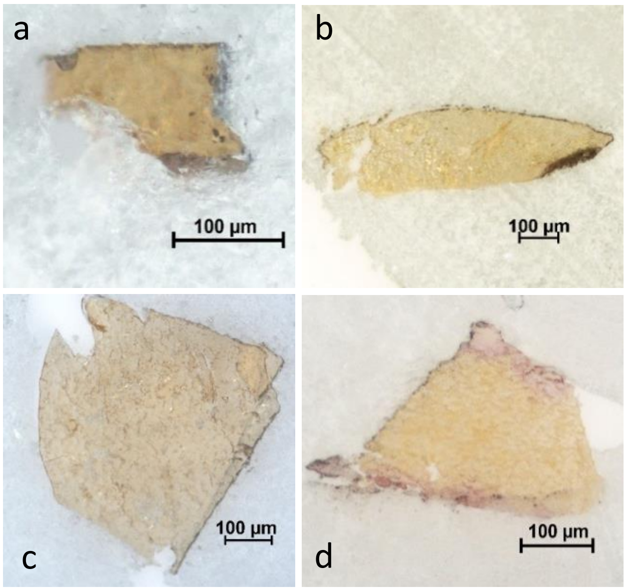


**Figure 6.4** OM images with polarized light shellac thin sections coated with a) a thin carbon coating b) a thick carbon coating and c) a gold coating embedded in epoxy resin. Comparison with d) a thin carbon coating and embedded in polyester resin.

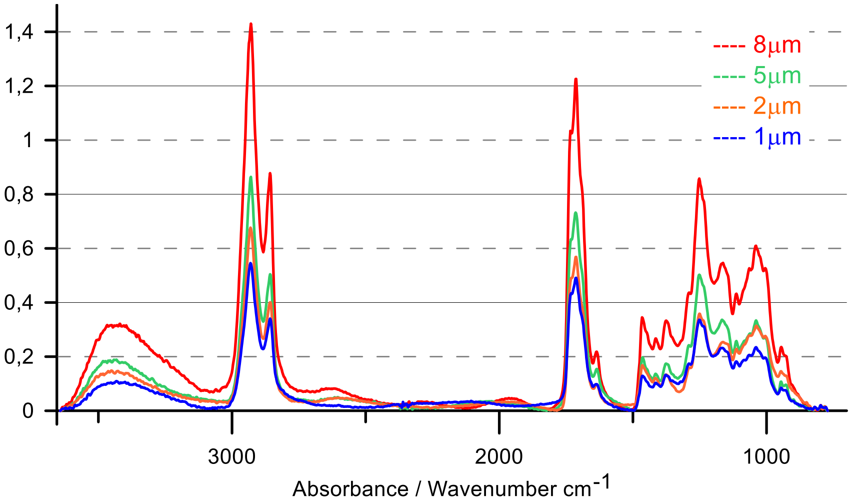


**Figure 6.5** µFTIR absorbance of shellac thin sections of various section thickness.

We have to mention that the molar absorptivity is compound dependent, for this reason the molar absorptivity of some of the test materials was also measured (Figure 6.6).

Not all the compounds were measured because some have unsuitable properties for making pellets. As shown in Figure 6.6 (black line) mastic shows the highest molar absorption, amber and dammar the lowest and sandarac shows different values depending on the wavenumber. This is taken into account in the analysis of the data.

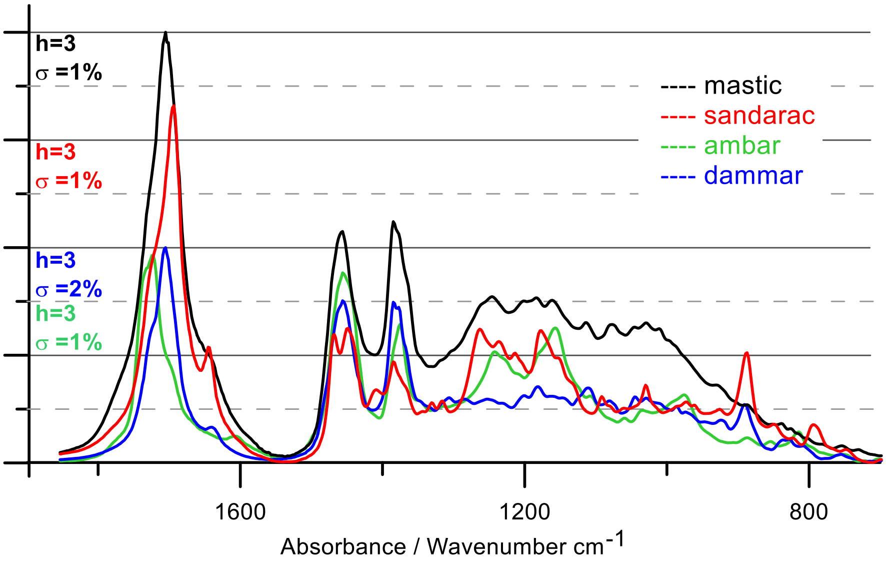


**Figure 6.6** μFTIR absorbance of a) shellac thin sections of various section thickness and b) various materials (h= number of averaged hits, σ= standard deviation).

### 6.3.3 Embedding medium infiltration

The corresponding IR spectra for the polyester and epoxy resins are shown in Figure 6.7.

Epoxy appears very suitable for embedding natural varnishes because the band with the highest absorbance appears at 1510 $cm^{-1}$ frequency for which most the materials commonly used as natural varnishes are band free. Epoxy has also a medium intensity double peak at 1608 and 1580 $cm^{-1}$ which can be used as a specific marker for detecting its presence (all the bands are associated to the stretching of the aromatic groups) (22–25).

Conversely, the IR spectrum of polyester resin has the highest absorbance at 1731 cm$^{-1}$ (associated to the stretching of the C=O bond) (25,26), overlapping with many important bands corresponding to the varnishes. A double band at 1600 and 1580 cm$^{-1}$ (associated to the stretching of the aromatic groups) (25,27) which could be used as a specific marker for detecting polyester contamination has a low intensity that makes it unsuitable for low contamination cases. This is probably one of the weakest points of polyester resin.

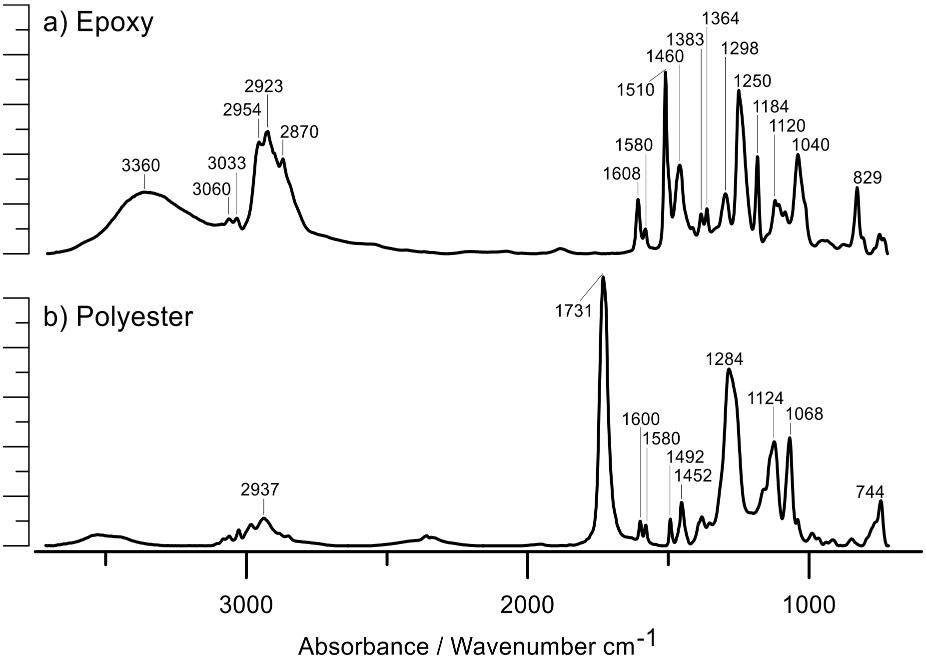


**Figure 6.7** µFTIR spectra corresponding to the embedding medium tested.

The most important factor to consider is the penetration of the embedding medium inside the coated samples. The 1510 cm$^{-1}$ and the double band at 1608 and 1580 cm$^{-1}$ are markers for the detection of epoxy resin, and the band at 1731 cm$^{-1}$ and the distortion caused by the 1285 cm$^{-1}$ band are characteristic markers of the presence of polyester resin.

The results obtained show that, generally speaking, the polyester resin penetrates deeper in the samples than the epoxy for equivalent coatings, as is shown for two cases in (Figure 6.8).

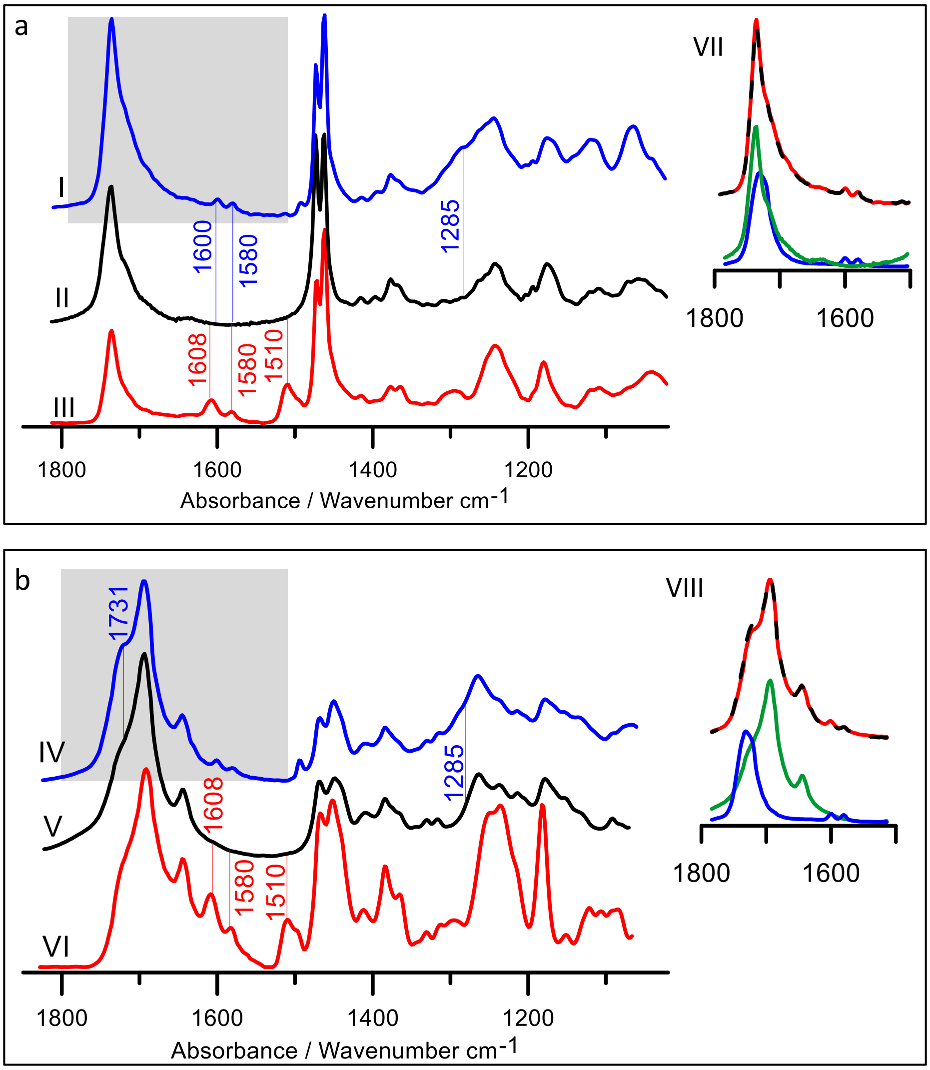


**Figure 6.8** µFTIR spectra comparison between infiltrations depending on the embedding medium a) Comparison with shellac wax samples with a thin carbon coating: I) embedded in polyester, II) pure, III) embedded in epoxy. Comparison with sandarac with a thin carbon coating: IV) embedded in polyester, V) pure, VI) embedded in epoxy. A deconvolution has been applied in band 1700 of polyester embedded sample for see the contribution of polyester: red line for calculated spectrum, black dotted line for experimental spectrum, blue line for polyester and red line for shellac wax (VII) or sandarac (VIII).

In particular Figure 6.8a shows the case of shellac wax protected with the thinnest carbon coating embedded with both epoxy and polyester resin. Measurements taken immediately below the sample surface (spectra I and III) are compared with the spectrum of shellac wax (spectrum II). As it can be seen in Figure 6.8a, the intensity of the epoxy markers is really low (the slight increase observed at the 1737 cm$^{-1}$ band is related to the decrease of shellac wax concentration because of epoxy penetration).

Contrariwise, the polyester markers show a higher intensity, the shoulder at 1285 cm$^{-1}$ and the contribution at 1731 cm$^{-1}$ which overlaps with some bands corresponding to the shellac wax: curve fitting has been applied to resolve this band from the polyester band intensity can be seen (Figure 6.8a, IV). Finally, the distortion produced by the presence of other bands related to the polyester resin is also observed.

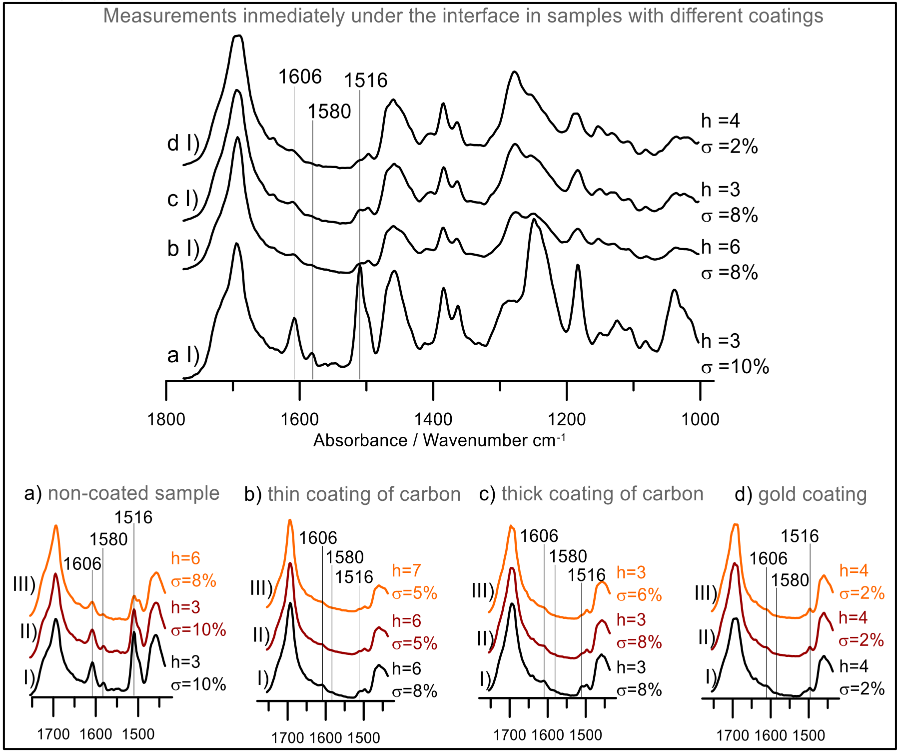
Figure 6.8b shows the same than Figure 6.8a but corresponding to sandarac (spectra V, VI and VII). The spectrum of sandarac embedded in epoxy resin shows a medium intensity band at 1510 cm$^{-1}$ which is directly related to the concentration of epoxy resin. The intense shoulder at 1730 cm$^{-1}$ overlapping the 1694 cm$^{-1}$ sandarac band is related to the presence of polyester resin; curve fitting shown in (Figure 6.8a, VIII) demonstrates the importance of the polyester band.

Therefore, we can conclude that the polyester concentration is higher than the epoxy concentration for equivalent sandarac sample preparations.

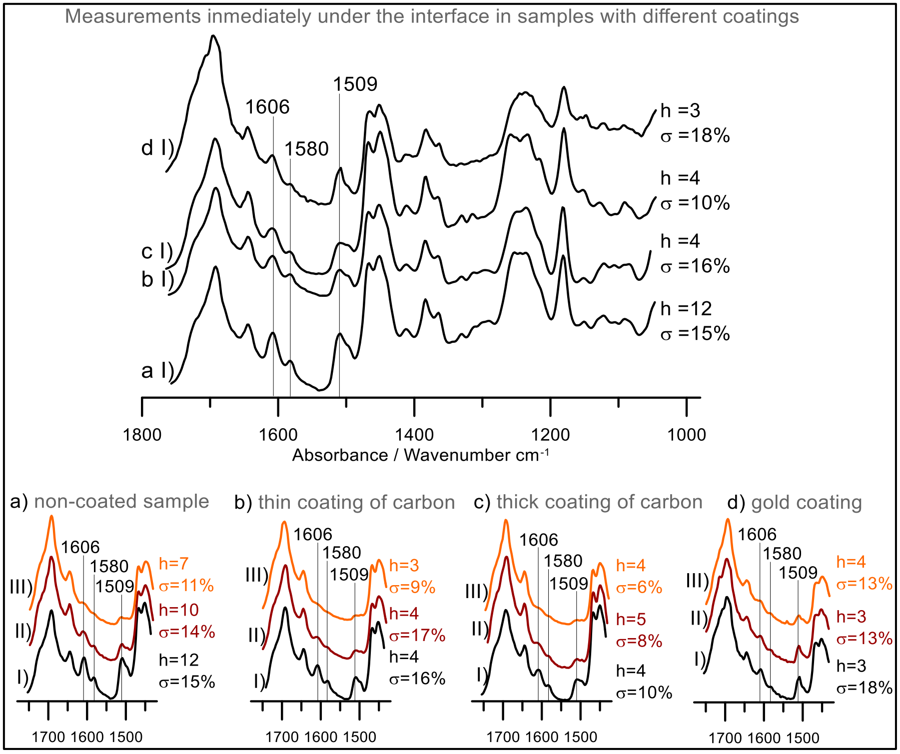
The results show that polyester penetrates more than epoxy into the test materials. This can be explained because, on the one hand, polyester needs longer time to be cured than epoxy (polyester cures in three days and epoxy in 24 hours) and on the other hand, polyester monomers are smaller than epoxy's. Epoxy resin is less invasive; consequently, the other comparative studies (infiltration of the resin depending on the test material embedded and the protective coating) were performed with epoxy resin only.

The results from the study of infiltration in embedded sample are shown in Figure 6.9. Measurements have been taken immediately under the interface between epoxy resin and each material (Figure 6.9,I), at 10 µm (Figure 6.9,II) and at 20 µm (Figure 6.9,III) below it. A scheme of the measurements is displayed in (Figure 6.9). The number of hints averaged (h) and the standard deviation (σ) are shown for each spectrum.

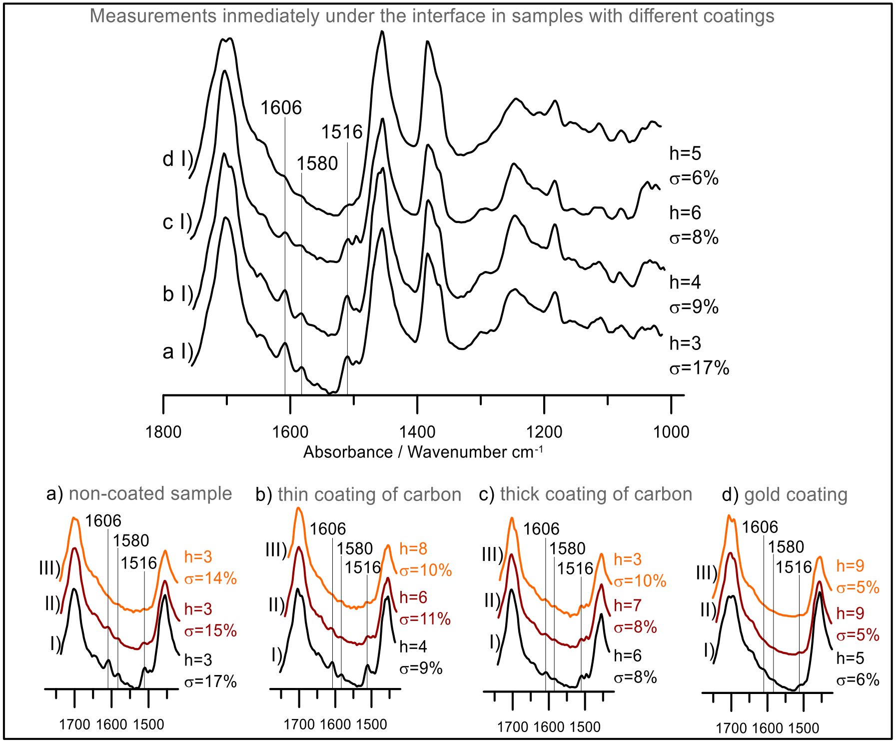
### *Pinus* resin



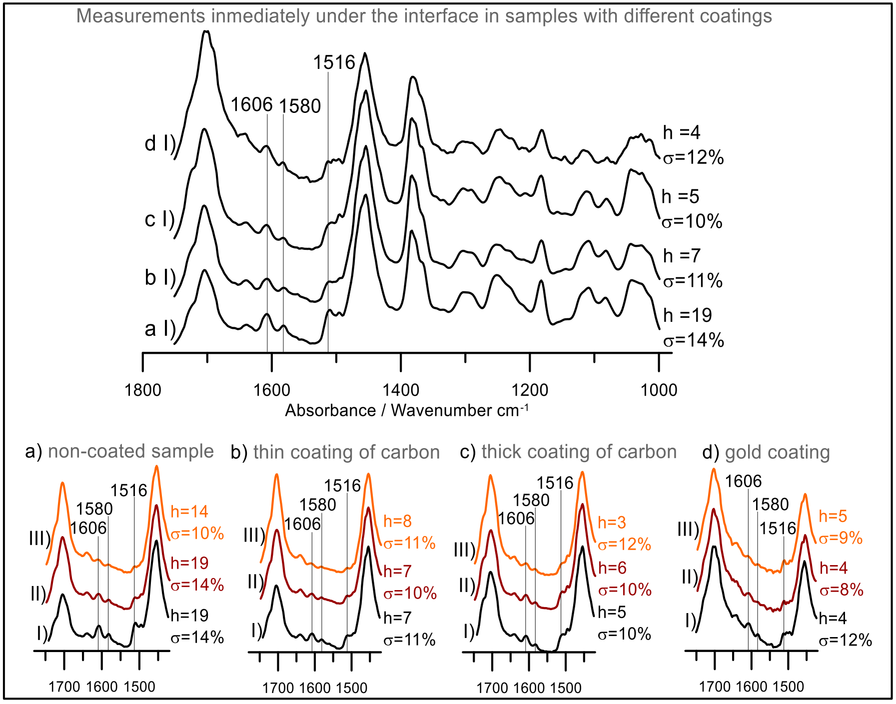
### Sandarac



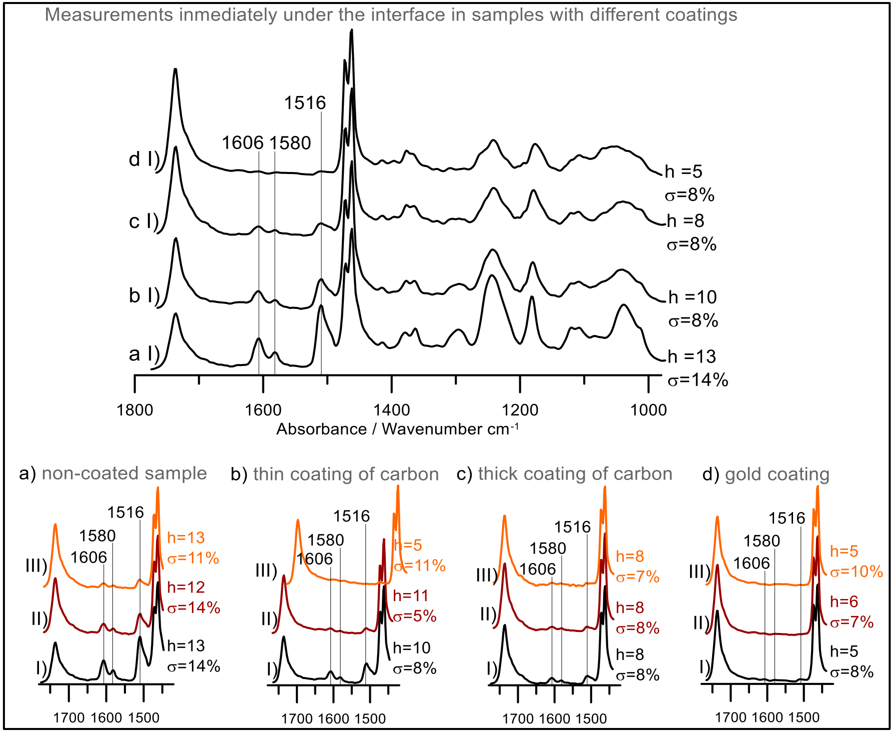
Mastic



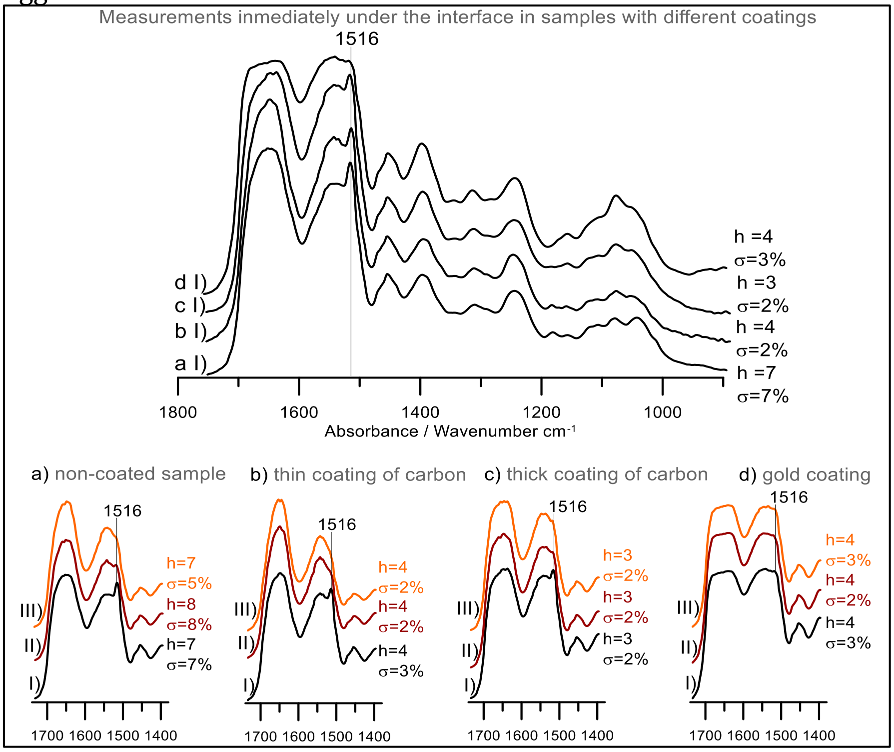
Dammar



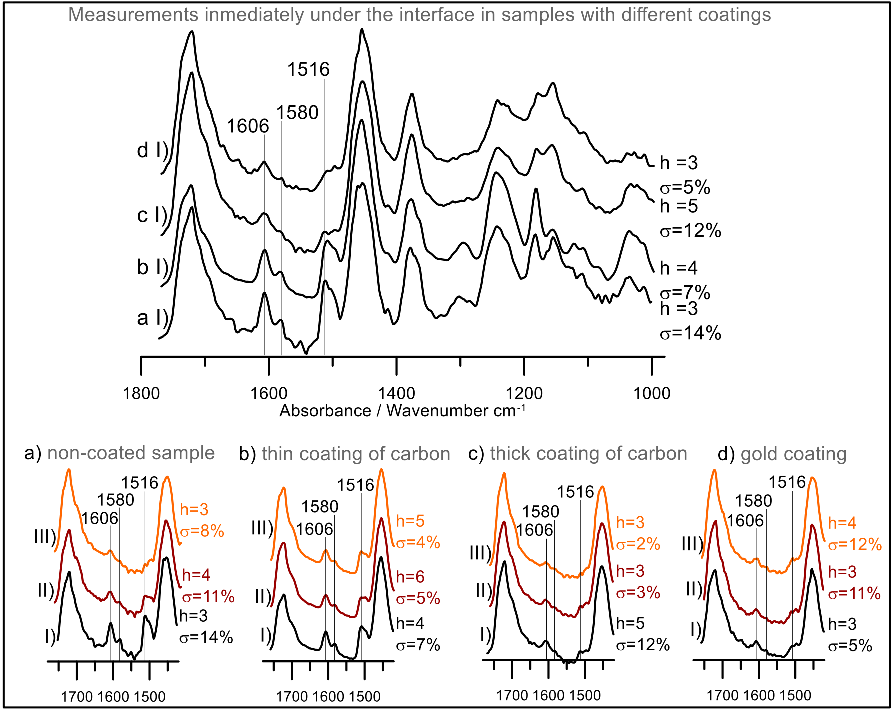
## Shellac wax



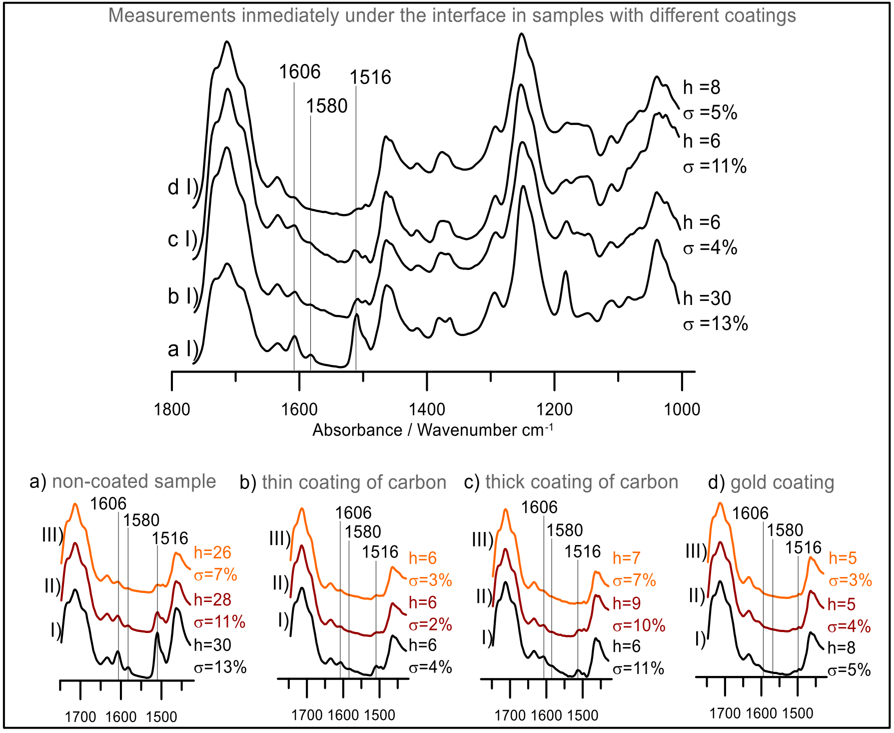
## Egg white



## Amber



## Beeswax



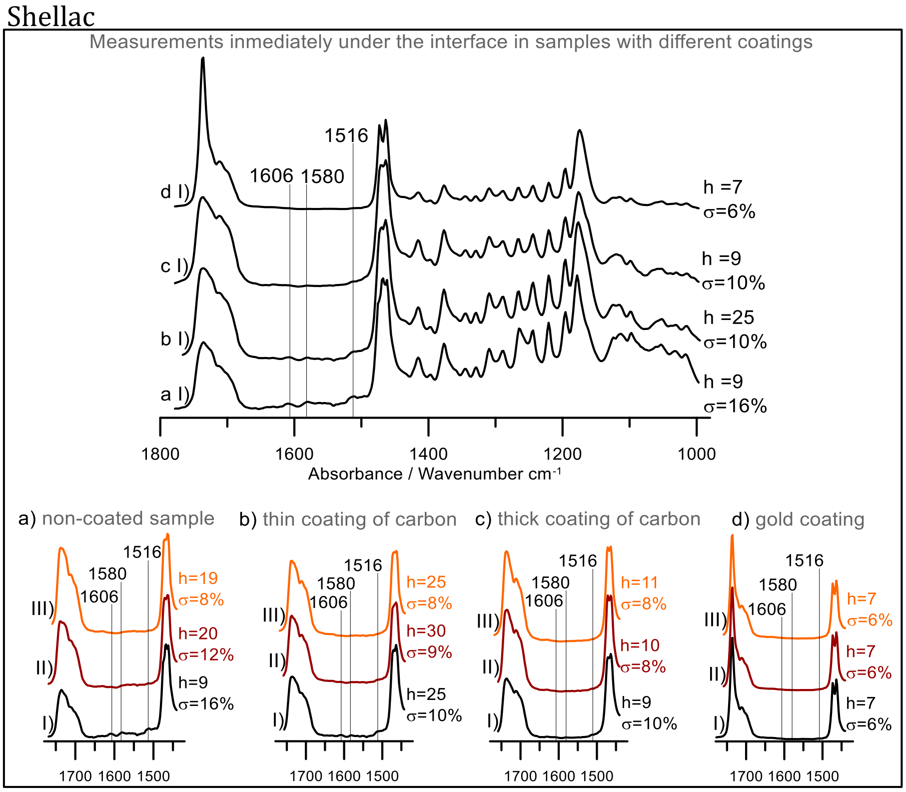


**Figure 6.9** µFTIR spectra of reference materials, h indicates the number of averaged hints and σ the standard deviation a) non-coated sample b) sample with a thin coating of sputtered carbon c) sample coated with a thick coating of sputtered carbon d) sample with a coating of sputtered gold. I) immediately under the surface, II) 10 µm under the surface, III) 20 µm under the surface.

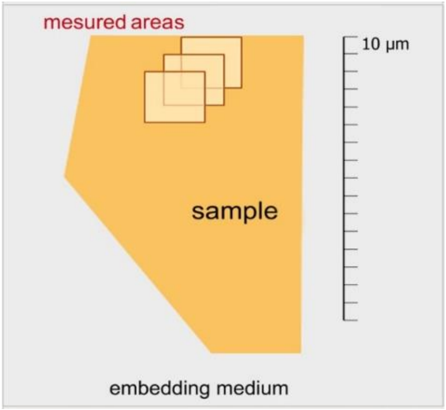


**Figure 6.10** Scheme of the performed measurements displayed in Figure 6.9. Yellow area has been used to indicate the sample, measured areas are marked with white squares.

These measurements have been repeated in unprotected samples (Figure 6.9a), samples protected with a thin coating of sputtered carbon (Figure 6.9b), samples protected with a thick coating of sputtered carbon (Figure 6.9c) and samples protected with a coating of sputtered gold (Figure 6.9d).

From them, we can state that the embedding medium penetration is a real problem that may distort the spectra of the external layers in some cases the embedding resin may penetrate as deep as 20 µm).

Although all the coatings considered reduce the embedding medium contamination (Figure 6.11), gold is the best.

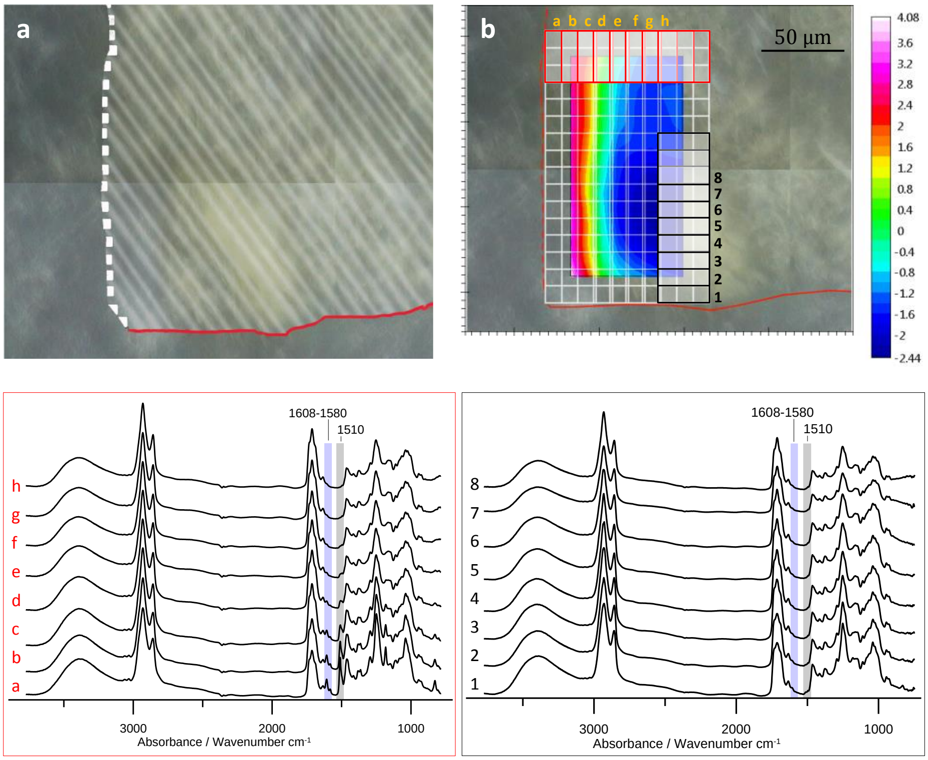


**Figure 6.11** Section of shellac (striped area) with a thin coating of carbon and embedded in epoxy resin (non-striped area) a) analysis have been made in the coated face (in red) and in the uncoated face (in a white dot line) b) analysed spots at increasing distances from the interface between the shellac and epoxy, obtained spectra are displayed below. As it can be seen, the coated face has less epoxy contamination (band at 1510 cm$^{-1}$ and double peak at 1608 and 1580 cm$^{-1}$).

Gold protects extremely well egg white, mastic, shellac and shellac wax. With other materials such as amber, sandarac and fresh *Pinus* resin, the contamination is very limited affecting very little the IR spectra. Although even gold does not reduce impressively the epoxy penetration in dammar, it protects the surface reasonably well.

Finally, despite carbon is less effective, occasionally, as happens for shellac and beeswax, it is enough to substantially avoid the embedding medium contamination. Differences between test materials results can be explained because of its different molar absorptivity.

### 6.3.4 Artwork sample analysis

Protective coatings were also tested for real artwork microsamples (<400 µm$^3$ in size).

#### 6.3.6.1 Case I: 18th century oil painting on canvas

A small fragment (125 µm x 200 µm x 400 µm) of a blue painting from a canvas of the Sant Francesc d'Assís life series from Antoni Viladomat Manalt (1678-1755) (Figure 6.12) was gold coated (from 40 to 60 nm) to improve the protection, embedded in epoxy resin from which 2 µm thick cross-sections were cut with the microtome for the FTIR analysis. (15th century)

The sample selected is particularly complex; it shows a multi-layered structure (4 layers) formed by various compounds of very different hardness. This is seen in the thin sections, which expose a polished sample surface, free of cutting and polishing contamination (diamond, alumina, silicon carbide, etc.) (Figure 6.13).

A brown preparation layer (>150 µm) containing calcium carbonate (calcite $CaCO_3$), clay minerals (illite and kaolinite), quartz, iron oxides (hematite) and calcium oxalates (weddellite) is followed by a layer of lead white (mainly cerussite $PbCO_3$ with a small amount of hydrocerussite $2PbCO_3{\cdot}Pb(OH)_2$) mixed with carbon black particles (15-20 µm); over it, a lead white paint with smalt blue particles (~30 µm) and a final diterpenic varnish (<4 µm).

**Figure 6.12** Detail of the canvas of the Sant Francesc d'Assís life series by Antoni Viladomat Manalt.

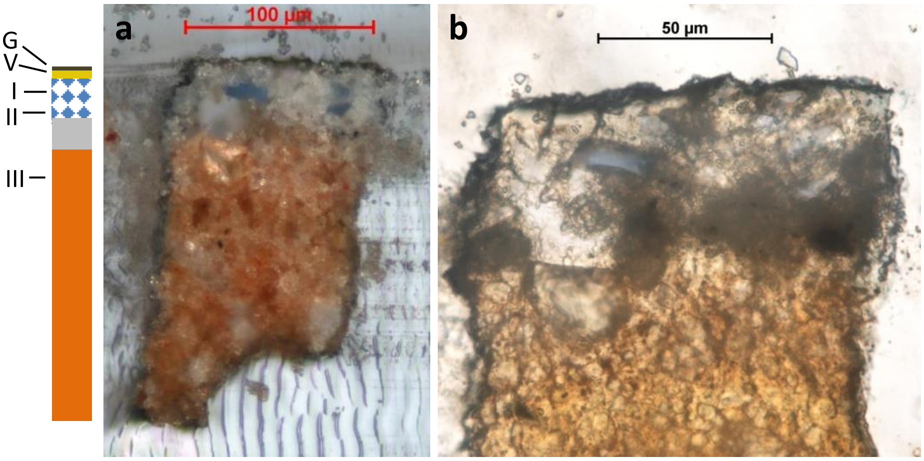


**Figure 6.13** Scheme of the layers, G: gold coating, V: varnish, I: first chromatic layer, II: second chromatic and layer III: preparation layer (left) and thin sections of the sample by OM a) polarized light b) transmission light.

The elemental composition was studied by SEM-EDX as microtome polishing is adequate for this technique (Figure 6.14) (28). The backscattering image shown in shows clearly each layer and the thin gold protective coating on top of the varnish. EDX analysis have been used to characterize the blue particles (mainly Si, K, Fe, Co and As as minor components). Acceleration high voltage (25KeV) has been used because of heavy elements like Pb found in the chromatic layer.

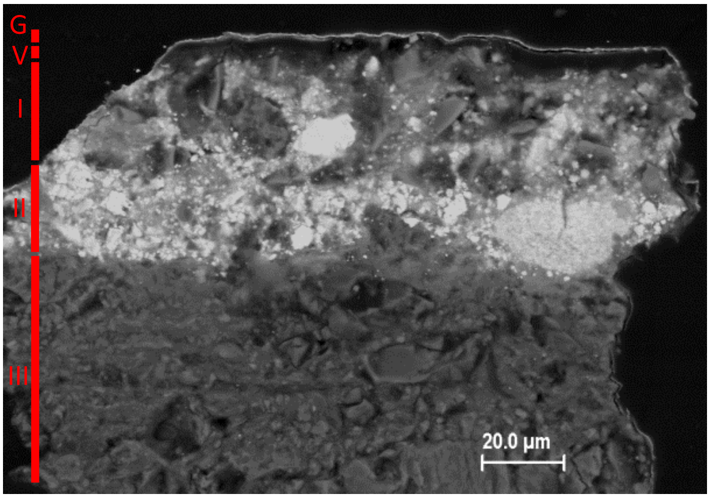


**Figure 6.14** BSE image of the bulk.

Thin cross-sections were analysed by means of µSR-FTIR to determine the embedding medium penetration. The epoxy resin presence has been measured by integrating the double peak at 1608 and 1580 cm$^{-1}$ (Figure 6.15), that shows the presence of resin at a depth of ~5 µm, affecting only the most external varnish layer. Actually, the optical diffraction limit at the wavenumbers used (1580 and 1608 cm$^{-1}$) is around 5 µm, so epoxy resin may in fact not be penetrating at all.

The thin cross-section has also been analysed by conventional µFTIR with a Globar light source on 30 µm$^2$ areas obtaining good quality spectra which permits to determine the layers composition (Figure 6.16).

The paint layers show the bands characteristic of drying oil, lead white $PbCO_3/2PbCO_3 \cdot Pb(OH)_2$ and calcium carbonate ($CaCO_3$) (Figure 6.16, I and II). The middle paint layer shows the presence of a band between 1520 and 1500 cm$^{-1}$ related to the presence of lead carboxylates (asymmetric stretching of $COO^-$) produced by the reaction between lead white and drying oil, (Figure 6.16, II). Finally the preparation layer shows the bands characteristic of silicates and calcium carbonate (Figure 6.16, III).

The thin cross-sections are also suitable for µSR-XRD but the signal obtained from 2 µm thin section is low and, despite results can still be interpreted, a better signal is obtained with 20 µm thin sections. Thin sections of 20 µm thick are cut from the same fragment and analysed using a 50 µm x 6 µm spot. Measurements were taken every 3 µm across the different layers (Figure 6.17). The crystalline compounds present in the layers are determined.

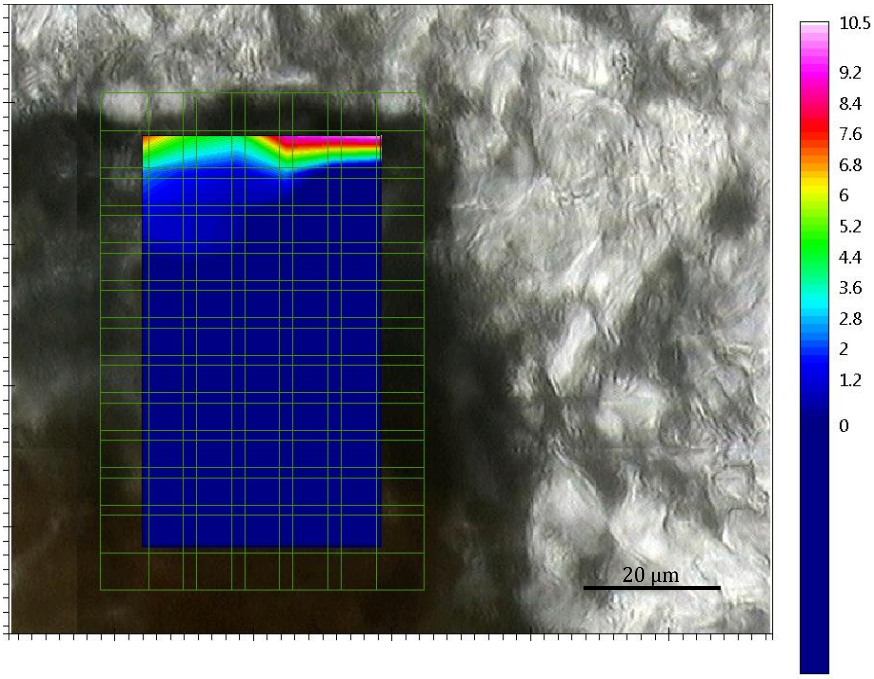


**Figure 6.15** SR-μFTIR analysis of epoxy infiltration. The integration map belongs to the double peak at 1608 and 1580 cm-1.

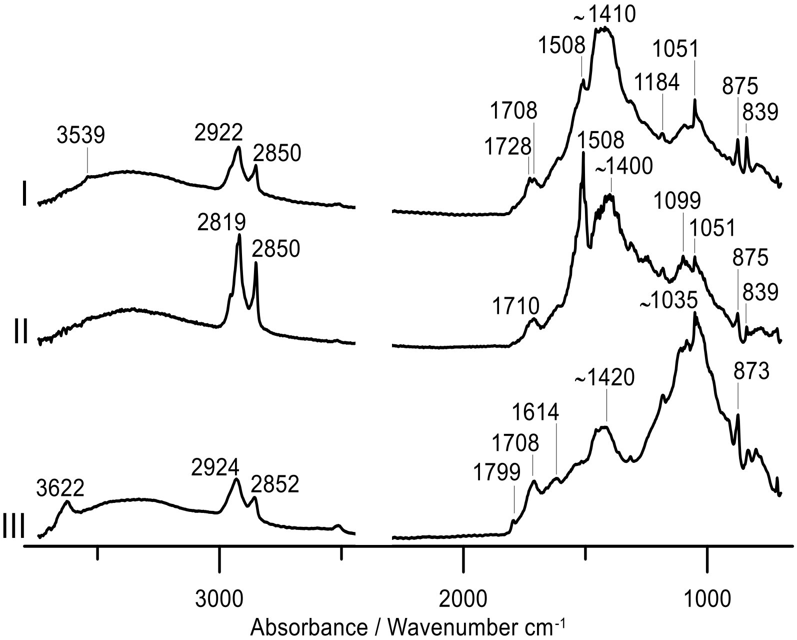


**Figure 6.16** μFTIR analysis of the thin cross-section. I: first chromatic layer, II: second chromatic and layer III: preparation layer.

Neither carbon nor smalt are identified, because the carbon main peaks overlap with those of quartz and because smalt is not crystalline. The presence of metal carboxylates of various natures is also determined at low diffraction angles in the preparation and both painting layers. The peaks corresponding to the metal carboxylates are broad and may be clearly distinguished because of the large size of those molecules. (29)

However, more work should be devoted for a full identification of the metal carboxylates and their relationship with the conservation of the paint.

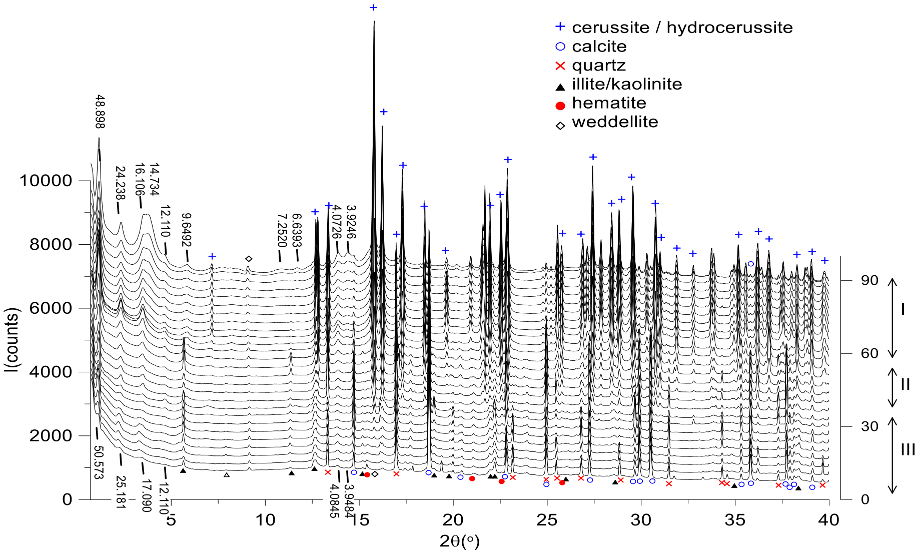


Figure 6.17 µSR-XRD diffraction from layer I, II and III.

### 6.3.6.2 Case II: 15th century egg-tempera altarpiece

A sample from a red area of the Calvari altarpiece (anonymous, 15th century) was extracted (Figure 6.18).

It was first gold coated (from 40 to 60 nm) and then embedded in epoxy resin. Sections of 2 µm thick were obtained for the FTIR analysis (Figure 6.19). This sample shows three layers: a varnish (Figure 6.19, V), a chromatic layer (Figure 6.19, I) and a preparation layer (Figure 6.19, II).

**Figure 6.18** Detail of the calvari altarpiece.

**Figure 6.19** Scheme of the layers, G: gold coating, V: varnish, I: chromatic layer, II: preparation layer (right) and thin section of the sample by OM with polarized light (left).

The embedding medium penetration has been measured by integrating the double peak at 1608 and 1580 cm$^{-1}$ (Figure 6.20). It demonstrates that the epoxy resin penetrates around ~6 µm.

The SR-µFTIR analysis of the thin section reveals the complex composition of the chromatic layer (Figure 6.21).

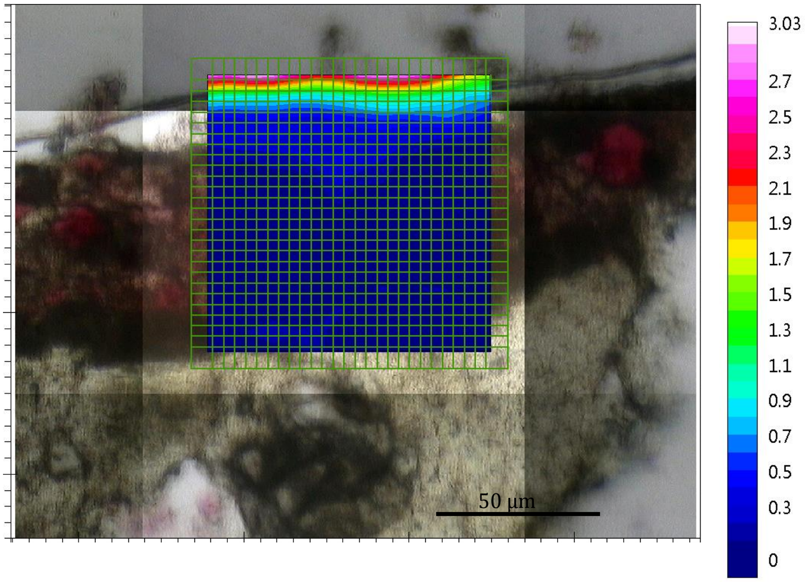


**Figure 6.20** SR-µFTIR analysis of epoxy infiltration. The integration map belongs to the double peak at 1608 and 1580 cm$^{-1}$.

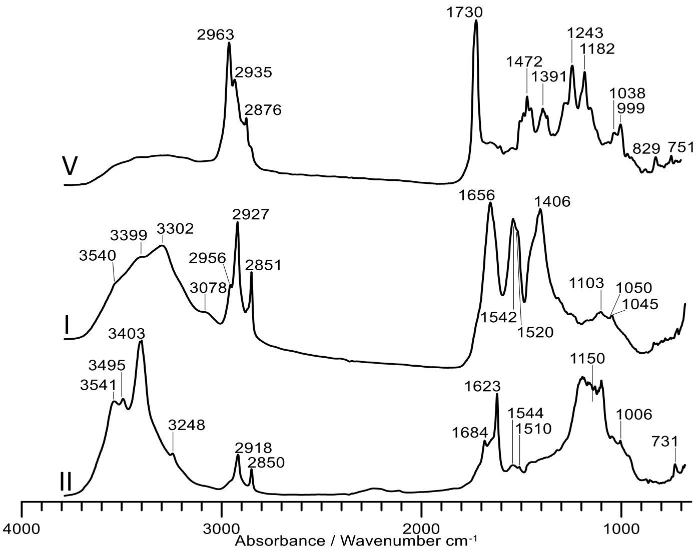


**Figure 6.21** SR-µFTIR obtained analysis of the thin section. V: varnish, I: chromatic layer, II: preparation layer.

The spectra obtained shows that varnish (Figure 6.21, V) is made with an acrylic resin, probably Paraloid@ B-67, also called Acryloid@ B-67, a colourless acrylic resin composed by an isobutyl methacrylate polymer. This is a modern material and it is related to a recent restoration process. The preparation layer (Figure 6.21, II) is made mainly of gypsum ($CaSO_4{\cdot}2H_2O$), although the main band between

1050 and 1250 cm-1 (stretching of $SO_4^{2-}$ group) is saturated due to his high absorptivity (30).

Chromatic layer (Figure 6.21, I) shows a mixture of compounds: it can be detected characteristic bands from lead white $PbCO_3/2PbCO_3{\cdot}Pb(OH)_2$ (main band centred at 1406 cm$^{-1}$), protein from the binding media and the red lake pigment (bands at 1656 and 1542 cm$^{-1}$). The shoulder at 1520 cm$^{-1}$ is related to the presence of lead carboxylates, as a result of the reaction between fatty acids and lead white. This is in good agreement with the sharp bands at 2927 and 2851 cm$^{-1}$ that correspond to lead carboxylates but also to the fatty acids. The binding media is probably egg, as it is a protein material that also contains lipids. The red lake pigment has not been identified by SR-µFTIR.

The SR-µFTIR analyses show that main compounds in the chromatic layers are distributed heterogeneously. Integration maps have been done in order to detect this distribution (Figure 6.22).

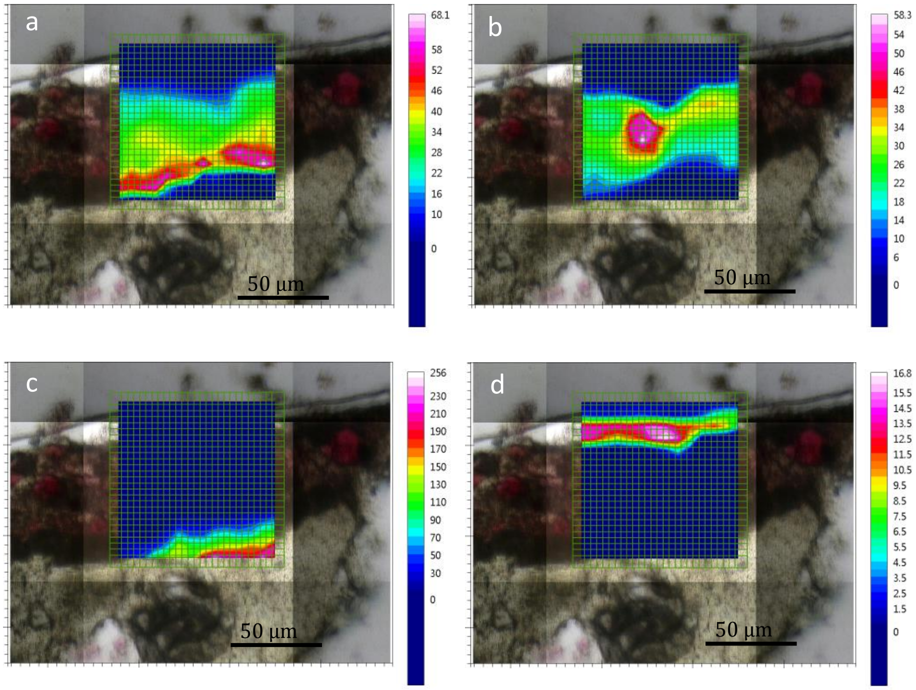


**Figure 6.22** SR-µFTIR analysis of compounds distribution. Intensity maps of a) lead white $PbCO_3/2PbCO_3{\cdot}Pb(OH)_2$ (band at 1406 cm$^{-1}$), b) protein (band at 1656 cm$^{-1}$), c) gypsum (band centred at 1151 cm$^{-1}$) and e) acrylic resin (band at 2963 cm$^{-1}$).

Knowing the distribution of the compounds allows knowing the formation of reactivity compounds between original materials, such as carboxylates.

Lead white $PbCO_3/2PbCO_3{\cdot}Pb(OH)_2$ is located predominantly in the bottom of the chromatic layer (Figure 6.22a). This explains the intermediate stratum between the preparation and the chromatic layer that can be seen in some cross-sections. The application of this intermediate stratum was a common practice among some painters in order to prepare a specific area before the application of the chromatic layer. The protein is mainly in the top of this layer (Figure 6.22b).

Additionally, a characteristic band of the acrylic resin and gypsum has been integrated (Figure 6.22d and e) showing that this two compounds are found almost exclusively in varnish and preparation layers respectively.

Additionally, a μSR-XRD has been performed in a thin section of 20 μm and crystalline compounds can have been identified (Figure 6.23). Results confirm the presence of gypsum, and lead white (cerussite and hydrocerussite). Lead carboxylates (peaks between lead palmitate- lead stearate) are also detected, that agrees with FTIR data.

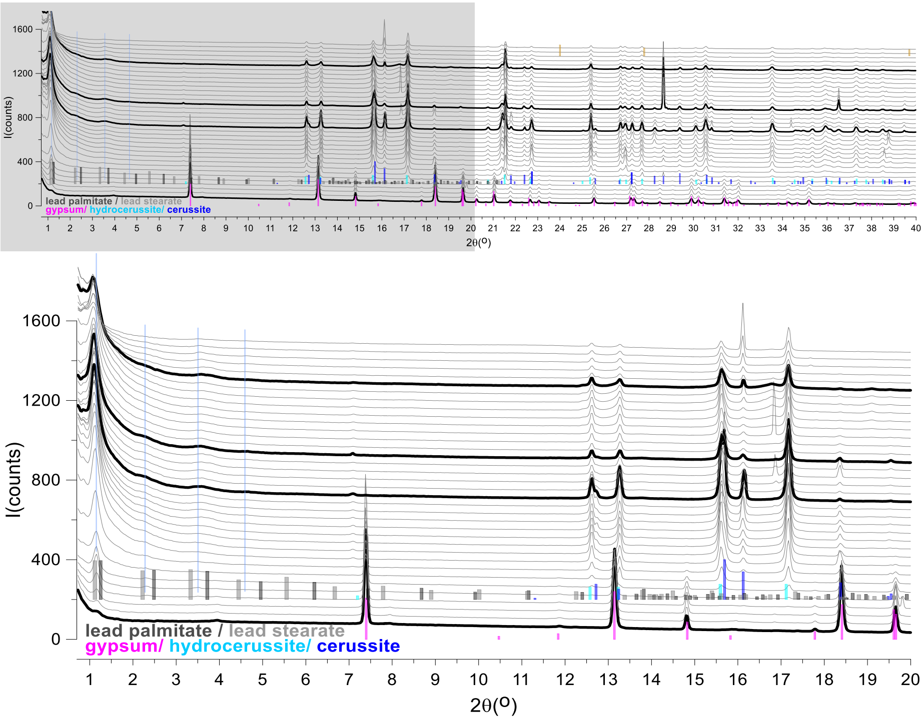


**Figure 6.23** μ-XRD diffraction (top) and magnification of the low angles region (bottom).

#### 6.3.6.3 Case III: surface remnants of old fungi activity in wall paintings

Some samples from the pictorial decorations of Saint Michael's Chapel have been studied. These decorations are situated in the Cloister of the Royal Monastery of Pedralbes in Barcelona and were carried out in 1346. They have been long attributed to the painter Ferrer Bassa.

Small dark spots (occupying a surface of few millimetres in size) randomly distributed on some areas of the wall paintings are observed. They were presumed to be the remnants of old fungi activity, as the distribution did not show any relationship with the substratum. Fungi attacks are a frequent problem in paintings and, generally speaking, are not easily diagnosed as very often fungi are not any longer active. (31–34)

Among other substances, fungi produce organic acids of low molecular weight (LMWOA) such as lactic, citric, oxalic, succinic, glutamic, fumaric, malic or acetic acids, mostly related to the reactions happening in the Krebs cycle (31,32). Some of these acids or the corresponding salts produced by their reaction with metal cations are relatively stable. Calcium oxalates, for example, are found on many surfaces, although their origin is diverse and, consequently, they cannot be considered as an indicator of fungi activity. In contrast, the presence of salts from malic, fumaric or succinic acids is directly related to the existence of old fungi activity.

A sample from a brown spot was extracted including the chromatic and the fungi deposition layer. It was first gold coated (from 40 to 60 nm) and then embedded in epoxy resin. Sections of 2 µm thick were obtained for the SR-µFTIR analysis (Figure 6.24).

The epoxy infiltration has been measured by integrating the double peak at 1510 cm$^{-1}$, as it is the one which is not overlapped (Figure 6.25). Likewise Case I and Case II, epoxy resin penetrates around ~6 µm.

The analysis of the thin section by SEM allows the observation of the superficial layer, which has a different morphology (Figure 6.26). For the analysis of this surface layer a µSR-XRD analysis was performed (Figure 6.27). Two layers are observed; an ochre paint below (Figure 6.27a) and the most external layer containing the substances related to old fungi activity on top (Figure 6.27b and c).

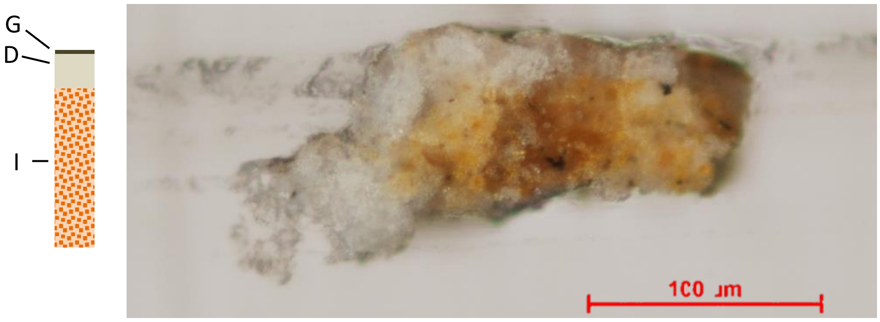


**Figure 6.24** Scheme of the layers, G: gold coating, D: deposition layer, I: chromatic layer (left) and thin section of the sample by OM with polarized light.

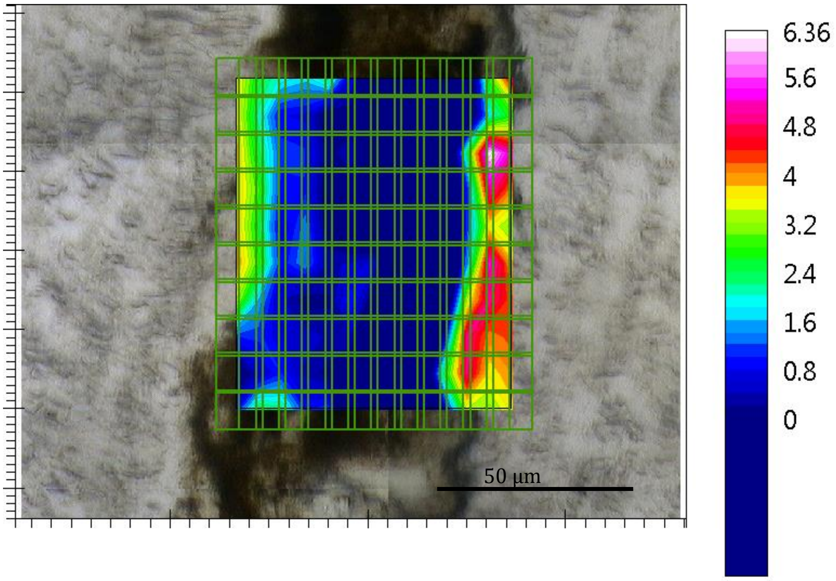


**Figure 6.25** SR-µFTIR analysis of epoxy infiltration. The integration map belongs to the peak at 1510 cm$^{-1}$.

The ochre paint is essentially a clay containing goethite ($FeO(OH)$), quartz ($SiO_2$), calcite ($CaCO_3$), weddellite (calcium oxalate dihydrate), $CaC_2O_4·2H_2O$ and bassanite (calcium sulphate hydrate, $CaSO_4·0.5H_2O$) which has broad peaks and, consequently, low crystallinity. This is in good agreement with the EDX data, namely magnesium, aluminium, silicon, sulphur, potassium, calcium and iron, which are usually found in the composition of an ochre clay.

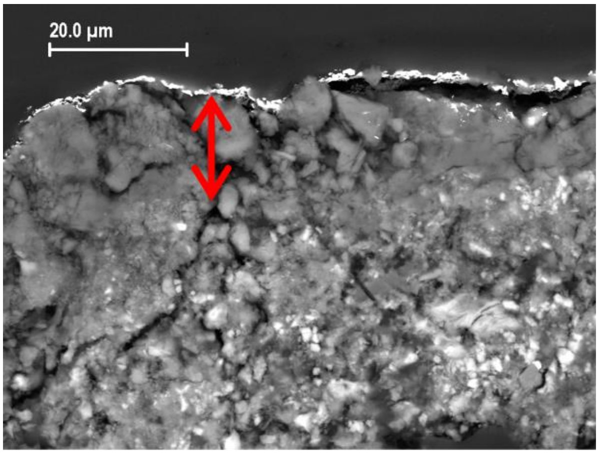


**Figure 6.26** BSE image of the thin section. Red arrow indicates the deposition layer.

The µSR-XRD pattern corresponding to the most external layer shows an unknown crystalline compound and some calcite in the surface; this is in good agreement with the fact that calcium, together with some chlorine related to atmospheric contamination, is the main compound determined by EDX analysis (Figure 6.27c, b and II).

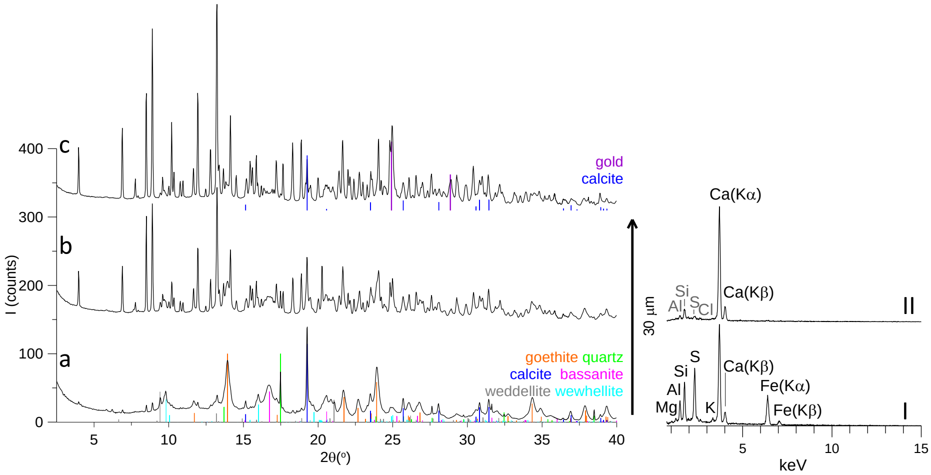


**Figure 6.27** µSR-XRD patterns (left) and EDX spectra (right) from the thin section. The components of the ochre paint (a and I) and the superficial fungi activity layer (c, b and II) are determined. The presence of gold is related to the sample preparation method used.

The small thicknesses of the microtomed cross-sections of the samples together with the small size and high brilliance of the beam have been crucial to obtain distinct µSR-XRD patterns of the crystallites related to the fungi activity considering the low intensity of the diffraction patterns of the salts and the small thickness of the layers formed.

Additionally, µSR-FTIR have been performed (Figure 6.28). Different spectra have been detected in the surface but all of them have the bands around 3580 cm$^{-1}$, that are related to calcium LMWOA salts (Figure 6.28a and b) (35). These characteristic bands have been integrated and the map is displayed in Figure 6.28d. It confirms that these compounds are only in the most external layer while in the internal layers these bands cannot be seen (Figure 6.28c)

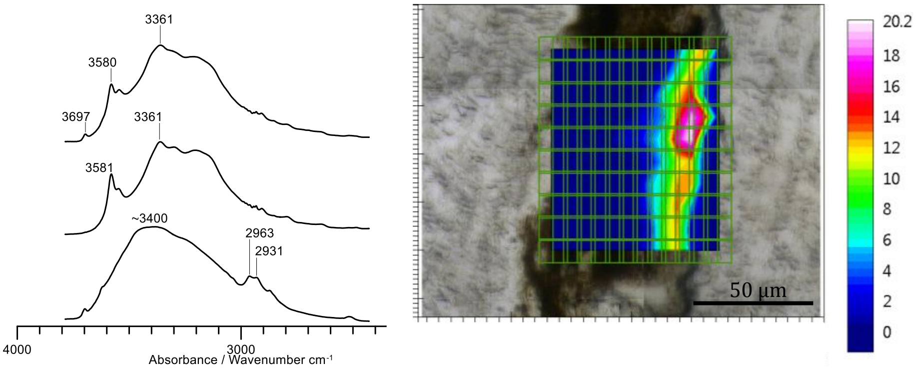


**Figure 6.28** SR-µFTIR analysis of LMWOA salts distribution. The integration map belongs to the peak at 3580 cm$^{-1}$.

As XRD and FTIR data depends on the exact substance degree of hydration and crystalline growth habits, a GC/MS was performed in order to confirm the family of substances. In particular, succinic, fumaric, malic and lactic acids were determined which are known to be secreted in variable proportions by various fungi species (36,37). Moreover, among them, glycerol was also determined; glycerol can be produced by some species of fungi when stressed by water shortage.(32)

## 6.4 CONCLUSIONS

The study has proved that the embedding resin infiltration is a real problem in thin layered microstructured samples where the resin can penetrate and unutilize the most external layers.

The sample preparation methodology proposed consisting in, first the application of a coating, then epoxy resin embedding and finally microtoming (between 20 µm to 2 µm) has proved to be very successful for the analysis of heterogeneous fragile microsamples. Gold and carbon have been tested as coating metals, and although both work, gold is more protective. It is demonstrated that the gold coating is able to minimize the epoxy resin penetration which may interfere with the analysis at the same time while the embedding resin gives to the preparation the necessary consistency for cutting.

Epoxy and polyester resins have been tested as embedding medium. Among them, epoxy has proved to give the best protection at the same time that gives adequate mechanical properties to facilitate cutting. Moreover, epoxy is also easily detected in varnished samples of maximum interest in the study of cultural heritage materials.

Finally, it is important to highlight that the same preparation may be used for µFTIR, µSR-XRD, µSR-XRF, SEM, EDX and OM. The potentiality of the methodology is demonstrated in the analysis of an artwork sample with micro layered structure including a wide range of materials, original organic and inorganic pigments and binders, and reaction, aging and deposition products.

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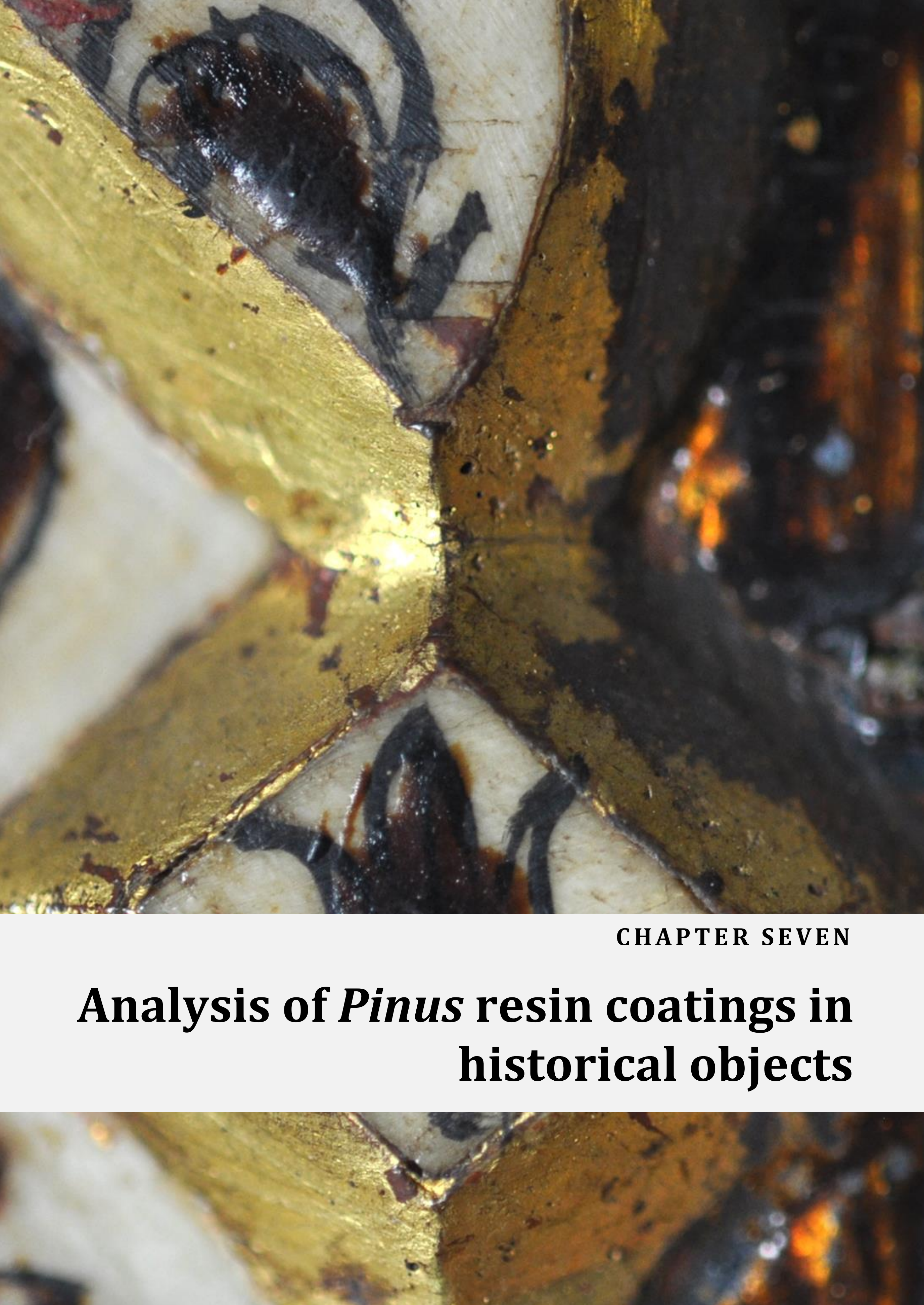


## CHAPTER SEVEN

# Analysis of *Pinus* resin coatings in historical objects

## 7.1 INTRODUCTION

The former study of *Pinus* resin ageing allows the characterization of the *Pinus* resin coatings from historical artworks. Using the specific markers of fresh and oxidized compounds some examples of these kind of coatings have been studied.

The diamond anvil cell together with a globar source was used for FTIR analysis of single layer homogeneous coatings, as it provides good quality spectra that allow the characterization of the substances. For heterogeneous coatings, the analysis of smaller areas is required and therefore, a synchrotron source was used instead of the globar source. Thin sections prepared following the method described in chapter 6 have been used in some samples, specifically for the analysis of thin layers and to know the compounds distribution and the variations of the composition along the layers sequence.

Raman analyses were performed in samples previously identified by FTIR but where the markers of fresh and oxidized compounds could not be clearly distinguished. A previous identification of the materials nature is necessary as Raman analysis require the selection of proper analytical conditions before perform the analysis. Wrong conditions can damage the sample and produce fake results.

Thin sections of 20 µm thick are the most adequate for µSR-XRD analyses, so this method has been used to obtain additional information about crystalline compounds when required. Finally, other analytical techniques including SEM-EDS and GC/MS were occasionally used to obtain information about complementary elemental or molecular composition.

## 7.2 CASE I: THE INFLUENCE OF THE AGEING CONDITIONS IN *PINUS* RESIN COATINGS

### 7.2.1 The map of Alfara de Carles (Tarragona)

The samples were extracted from the map of Alfara de Carles entitled *Dirección General del Instituto Geográfico y Estadístico – Trabajos topográficos- Provincia de Tarragona – Ayuntamiento de Alfara*, from 1916, which is owned by the Alfara de Carles Town Hall. The map, made of cellulose and drawn with ink, has a deep yellow coating (Figure 7.1 and 7.2).

Samples from different points were taken during the restoration process made by L'Escola d'Art i Disseny de la Diputació de Tarragona at Tortosa. Samples from the surface and also from the sideways where the coating is protected by a pleat of fabric were extracted (Figure 7.3).

**Figure 7.1** Map of Alfara

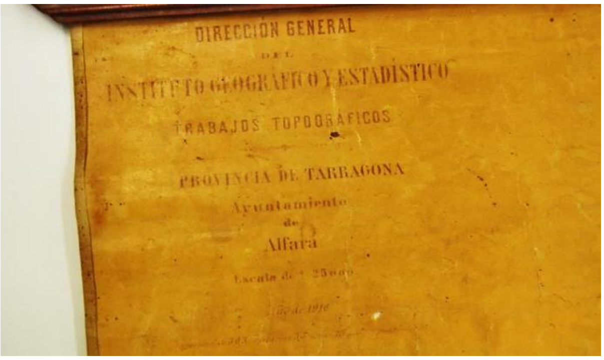


**Figure 7.2** Detail of the map. The pleat of fabric in the sideway is seen.

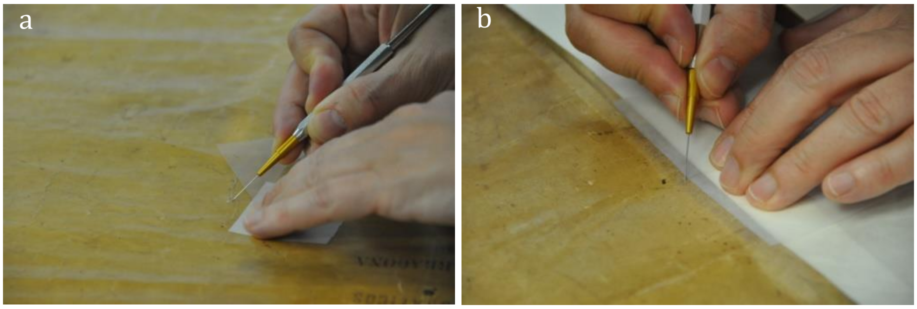


**Figure 7.3** Sample extraction of a) the surface and b) below the pleat of fabric

### 7.2.2 Sample preparation method

Some fragments of each sample were selected and analysed using a diamond compression cell with 100 x 100 µm spots. Analyses were performed in a µ-FTIR spectrometer using a globar source as the coating was a homogeneous material and there was enough amount to perform an analysis.

### 7.2.3 Results and discussion

Samples from the different points were analysed. All spectra obtained show the same spectral features showing that the main compound of the coating is unequivocally *Pinus* resin (Figure 7.4).

Nevertheless, there are subtle differences between the spectra from the resin protected by the pleat of fabric and those unprotected. In particular the position of the band centred at 1700 cm$^{-1}$ varies from 1705 cm$^{-1}$ in the unprotected area (Figure 7.4a) to 1697 cm$^{-1}$ (Figure 7.4b) in the protected area.

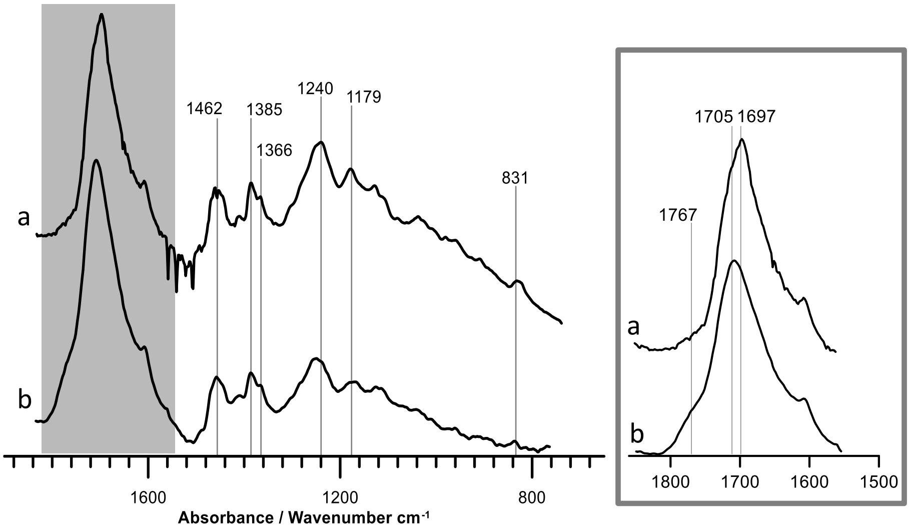


**Figure 7.4** µFTIR spectra of the map coating using a diamond cell a) sample protected by pleat of fabric and b) sample from the surface (unprotected). The shadow region is magnified at the right and compared with a sample from below the pleat of fabric of the sideway.

Specific markers corresponding to oxidized compounds are more intense in the unprotected samples than in protected ones. Band at 1697 $cm^{-1}$ is related to the C=O stretching of –COOH groups. The oxidation process produces oxidized compounds like 7-oxodehydroabietic and 15-hydroxy-7-oxodehydroabietic acids that contain a ketone group, which C=O stretching band appears at 1715 $cm^{-1}$. Consequently, in the most oxidized sample (Figure 7.4b) the maximum of the band at 1697 $cm^{-1}$ is shifted to higher wavenumbers due to the presence of a shoulder at 1715 $cm^{-1}$ while in the less oxidized sample (Figure 7.4a) the proportion of oxidized compounds with ketone groups is not enough to affect the 1697 $cm^{-1}$ band.

Furthermore, an increase in the intensity of the shoulder at 1767 $cm^{-1}$ is observed in the spectrum of the unprotected resin (Figure 7.4b), which is characteristic of oxidized resin (C=O from acid anhydrides).

These differences in the peak position and shape of the band result from the different ageing conditions of each varnish region; the pleat of fabric protects the coating from the light slowing down the ageing process, so it is less oxidized than the rest of the resin coating (lower concentration of oxidized compounds).

IN CONCLUSION. This example illustrates how different ageing conditions may slow down or accelerate the oxidation process, so differently oxidized *Pinus* resins are found in the same object, although all the coating has the same age.

## 7.3. CASE II: *PINUS* RESIN DIFFERENTLY AGED IN THE SAME ARTWORK

### 7.3.1 Varnished ceiling panel

The sample was extracted from the panel 173 from the coffered ceiling of Paretdelgada Chapel (La Selva del Camp, Catalonia) during its restoration process in the Centre de Restauració de Béns Mobles de Catalunya CRBMC. This ceiling is constituted by several painted wooden pieces of approximately 10x30 $cm^2$ and dated from the 14th century (1–3).

The panel 173 (Figure 7.5) is polychromed over a white preparation layer. The surface is covered by a thick varnish coating and (Figure 7.6). A knot with exuded resin is found in the back of the wooden panel.

**Figure 7.5** Panel 173 of the coffered ceiling from the chapel of Paretdelgada (La Selva del Camp, Catalonia).

**Figure 7.6** Varnished surface detail where the sample was taken from.

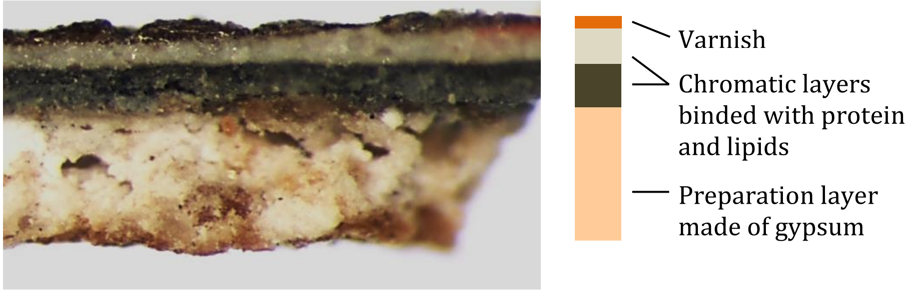


**Figure 7.7** OM with polarised light of cross-section of the sample from the panel 173 (left) and layers scheme. The main materials present were identified by FTIR.

### 7.3.2 Sample preparation method

Some fragments were carefully selected in order to avoid the environmental deposition present on the surface. Selected fragments of the varnish layer were separated and analysed using a diamond compression cell. Analyses were performed in a μ-FTIR spectrometer using a globar source and a 100 μm x 100 μm spot.

### 7.3.3 Results and discussion

The varnish is an irregular coating of thickness varying between 40 and 10 µm (Figure 7.8).

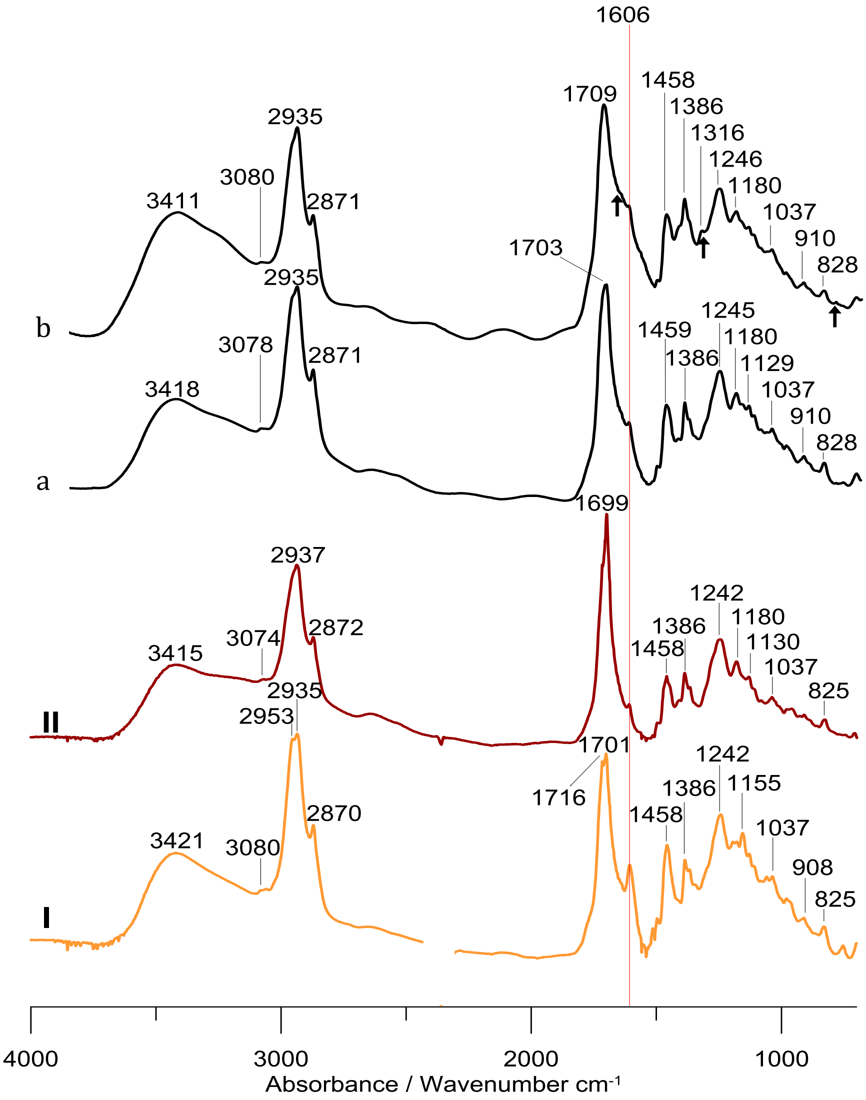


**Figure 7.8** µFTIR analysis of the varnish of panel 173 by diamond cell (100x100 µm spot) a) varnish b) varnish with environmental deposition, the arrows mark the bands related to calcium oxalates. Spectra of I) *Pinus sylvestris* L. and II) resin exudated from the knot are shownin order to compare the results.

The spectrum corresponding to the varnish (Figure 7.8a) is compared to the spectra from the resin exuded from knot of the back (Figure 7.8I) and from an aged resin from Pinus sylvestris L. kindly provided by the Economic Botanical Collection of Kew (ref. 28417) and dated between 1850 and 1900 (Figure 7.8II). The spectrum of the varnish shows the presence of Pinus resin (Figure 7.8a).

The band related to the aromatic ring of oxidized abietanes at 1606 $cm^{-1}$ is more intense in the varnish sample than in the 19th century *Pinus sylvestris* L. but less intense than the spectra from the resin of the knot. Also, the band at 1280 $cm^{-1}$ related to –COOH is hidden by the more intense 1245 $cm^{-1}$, related with –OH, which means that the resin contains a substantial amount of 15-hydroxydehydroabietic acid and 15-hydroxy-7-oxodehydroabietic acid. This comparison demonstrates that the resin of the varnish is more oxidized than the 19th centruy *Pinus sylvestris* L. but less oxidized than the resin from the knot.

Analysed fragments were methodically selected, nevertheless some spectra show the characteristic bands resulting from alterations and/or environmental deposition like calcium oxalate (1640, 1320, 790 $cm^{-1}$ indicated with an arrow in Figure 7.8b).

IN CONCLUSION. Despite belonging to the same historical object, the resin from the knot and the varnish coating have a different chemical composition, appearing the resin from the knot more oxidized than the varnish coating. This can be explained due to the resin from the knot is older than the varnish but also because the oxidation process of the varnish was slower due to specific environmental conditions.

## 7.4 CASE III: GREEN COATING MADE OF *PINUS* RESIN MIXED WITH DRYING OILS AND COPPER PIGMENT

### 7.4.1 Writing desks

Two writing desks were analysed both belonging to the Museu del Disseny de Barcelona. The first one is dated from early 20th century (MADB71747 reference number, Figure 7.9) and the second one from the last quarter of the 17th century (MADB64160 reference number, Figure 7.10) (3).

The coatings are applied over silver metal foils to highlight certain motifs of the decoration, assure the stability of these metal foils against corrosion and give them a glossy finish. Initially the coatings were green but nowadays the colour has changed to dark brown. Samples of these coatings have been extracted and studied.

**Figure 7.9** Writing desk from early 20th century (MADB71747) (left) and sample location (right).

**Figure 7.10** Writing desk from the last quarter of the 17th century (MADB64160 (left) and sample location (right).

## 7.4.2 Sample preparation method

Samples from the writing desks were analysed by means of the diamond cell using a synchrotron source, a sample from MADB71747 was also prepared in thin sections in order to compare the results obtained by each way.

### 7.4.3 Results and discussion

Both resins layers are 50 µm thick, they appear dark, with a brown shade. When observed under the optical microscope, they appear heterogeneous showing the presence of green particles in the green-coloured area, (Figure 7.11 and 7.12).

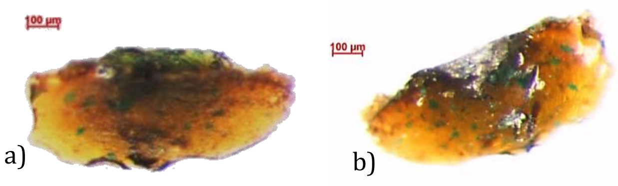


**Figure 7.11** Sample of the coating of MADB64160 (17th century) a) obverse and b) reverse,

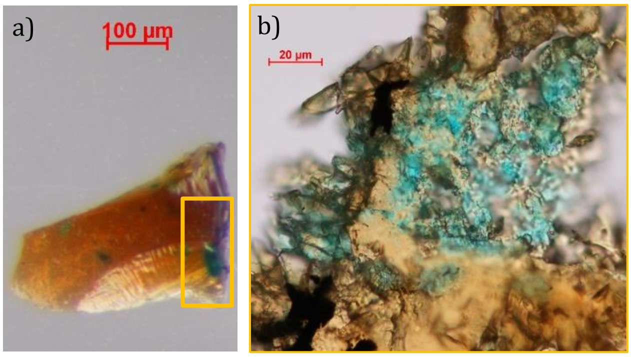


**Figure 7.12** Sample of the coating of MADB71747 (20th century), a) sample fragment and b) the same fragment smashed.

Due to the heterogeneity observed in the coatings of both desks different FTIR spectra have been obtained. They are displayed in Figure 7.13.

The spectra corresponding to the green particles observed in both coatings correspond to copper II acetate monohydrate, $Cu(CH_3COO)_2 \cdot H_2O$, commonly called *verdigris* (Figure 7.13A) while the matrix is a *Pinus* resin.

The reaction between copper pigment and the abietates of *Pinus* resin produces copper abietates. Copper abietate was synthetized following a method previously described (4) using abietic acid (Fluka Analytical ref. 101392305) and $CuSO_4 \cdot 5H_2O$ (Panreac ref. 131270.1211) in order to compare its spectra. The characteristic colour is shown in Figure 7.14 and its IR spectrum in Figure 7.13I.

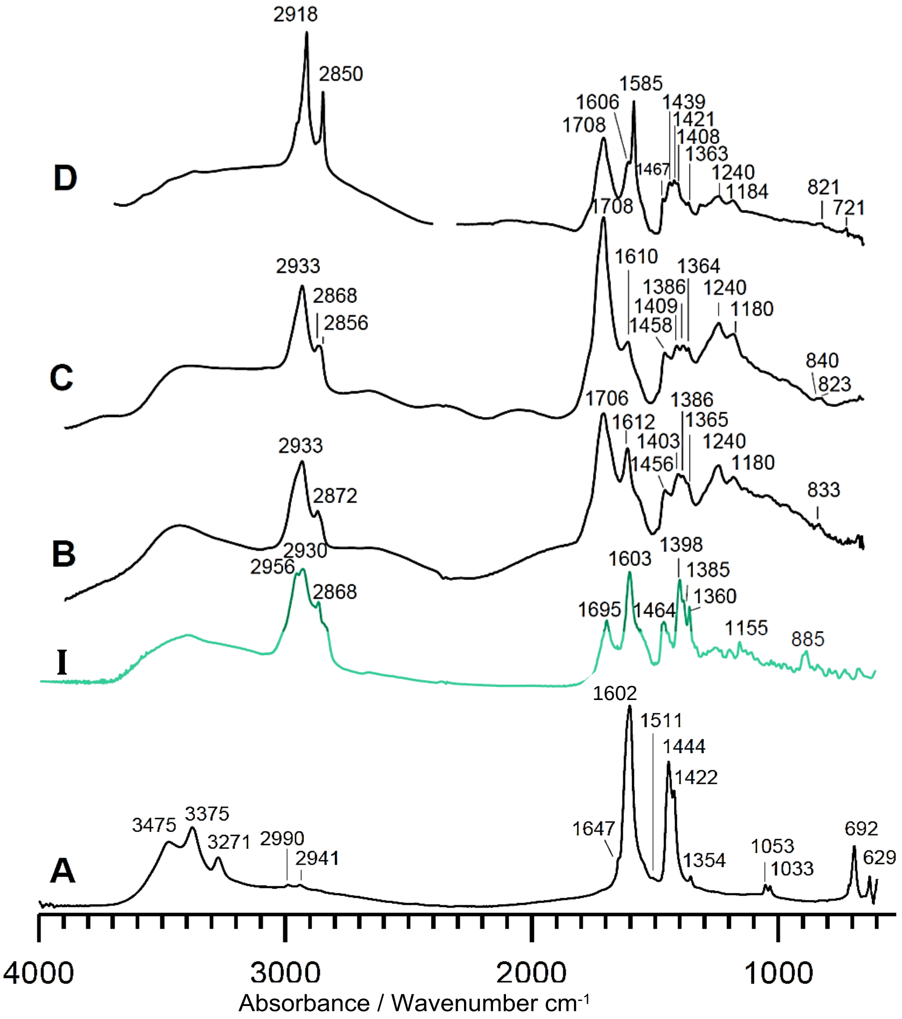
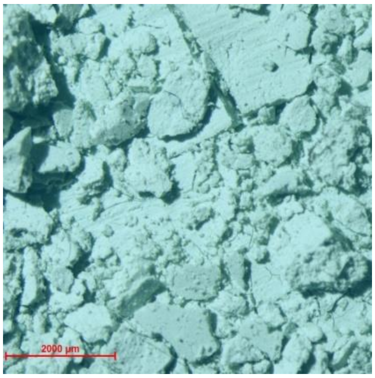


**Figure 7.13** µSR-FTIR spectra (12x12 µm spot) of A) green pigment particle of sample of 17th century desk corresponding to Cu(CH$_3$COO)$_2$·H$_2$O, B) of green coating sample of MADB64160 corresponding to *Pinus* resin and copper carboxylates of abietanes skeleton acids (abietates) C) of green coating sample of MADB71747 corresponding to *Pinus* resin and drying oil, D) of green coating sample of MADB71747 corresponding to copper carboxylates from fatty acids and from abietic acid and abietane skeleton acids and I) µFTIR (100x100 µm spot) spectra of copper abietate synthetized in the laboratory.

Copper abietate can be distinguished by an intense peak at 1603 cm$^{-1}$ associated to the antisymmetric stretching of the C=O from the COO$^-$ group (5). Thus, in the 1600 cm$^{-1}$ region, the band related to the C=O of copper abietate and the band related to C=C stretching of aged *Pinus* resin (around 1610 cm$^{-1}$) appear overlapped. In order to know the degradation degree of *Pinus* resin in samples containing copper abietate other regions of the spectrum should be studied, like the ones at 1240 or the 1500 cm$^{-1}$. In this case, the high intensity of the 1240 cm$^{-1}$ band and the low intensity of 1500 cm$^{-1}$ band show the resin has started the oxidation process.

The presence of an intense band at 1400 cm$^{-1}$, associated to symmetric stretching of $COO^{-}$ group of copper abietate (5), can help to determine its presence. However, determining the existence of copper abietate in a matrix of *Pinus* resin can be very difficult, especially when copper abietate appears in a low proportion (Figure 7.13C).



Copper abietate was prepared from abietic acid by the following general procedure.
Acid was dissolved in about 10 times its weight of denatured alcohol and nearly neutralized with a strong sodium hydroxide solution. This neutral abietate solution was then poured into a solution of a salt of the desired metal. The resulting soap was filtered on a Büchner funnel and washed thoroughly with water until free from soluble material. The thoroughly washed product was dried in small portions in a vacuum in order to avoid oxidation.

**Figure 7.14** OM with polarised light of copper abietate synthetized (left) and the general method used (right) (4).

In the 20th century varnish (MADB71747), copper carboxylates (Figure 7.13D) are also determined. These carboxylates are formed from the reaction between the saturated fatty acids of the drying oil and the copper pigment. The good quality of the spectra, i.e. low signal-to-noise ratio and straight baseline, has permitted to apply a curve fitting processing (6), evidencing the presence of oil (Figure 7.15).

The curve fitting process consists in firstly, to determine the number of overlapped bands, secondly, to fit the band parameters (intensity and width of each band) and finally, check whether the result is reasonable. Determining the number of overlapped bands is critical, it can be performed by two different methods: function derivative or Fourier Self-Deconvolution. In our case a first derivative was applied to the spectrum and the resulting number of bands compared to those determined from the second derivative. This procedure, when giving consisting data, ensures the validity of the mathematical treatment applied (7,8).

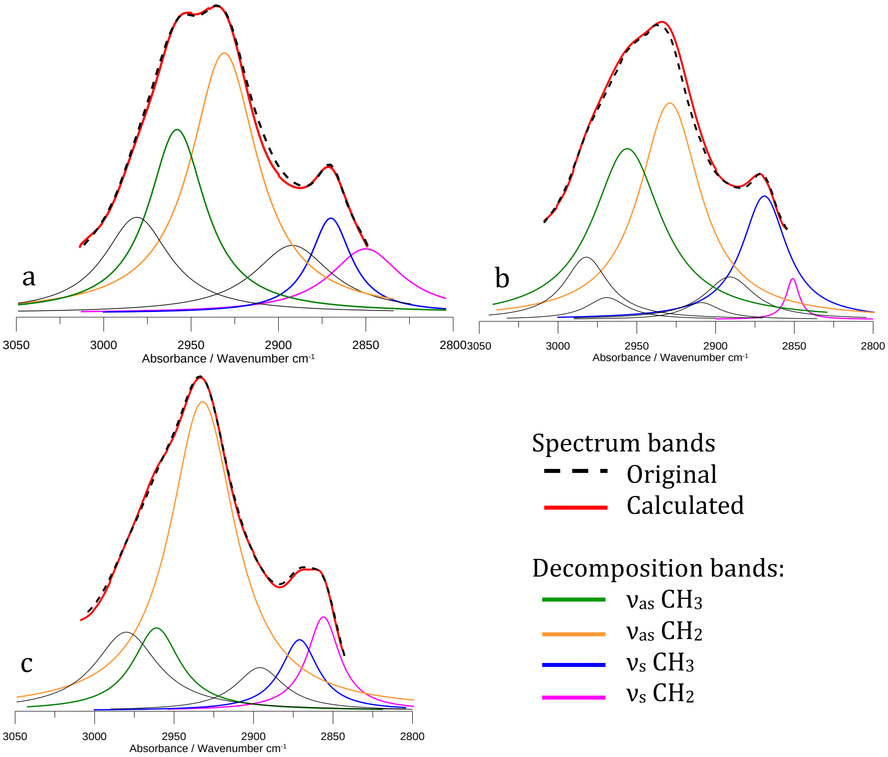
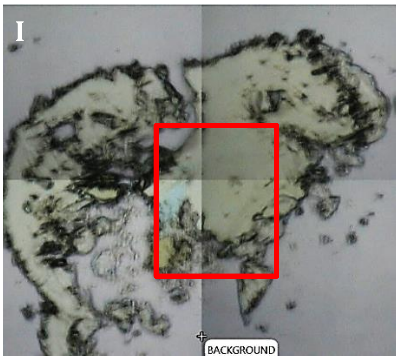


**Figure 7.15** Curve fitting of a) resin extracted from the wooden knot of the panel from chapter 5.3, b) resin from *Pinus sylvestris L.* dated between 1850 and 1900 and c) matrix from MADB71747.

Curve fitting allows seeing the intensity of the asymmetric and symmetric stretching bands of the –CH$_2$ group related to long fatty acid chains. This is possible because the distance between the maximum of these bands is larger than the shoulder limit (9–11). This band is more intense in the matrix of the 20th century varnish (MADB71747) than in any of the other sample of aged *Pinus* resin (Figure 7.15C): the resin extracted from the wooden knot of the panel from chapter 7.3 (Figure 7.15A) and the 19th century resin from *Pinus sylvestris* L. (Figure 7.15B).

Varnish samples from both desks were spread over a diamond cell and the compounds distribution determined. Figure 7.16 and 7.17 show the maps distribution of the compounds in the MADB71747 and the MADB64160 varnishes respectively.



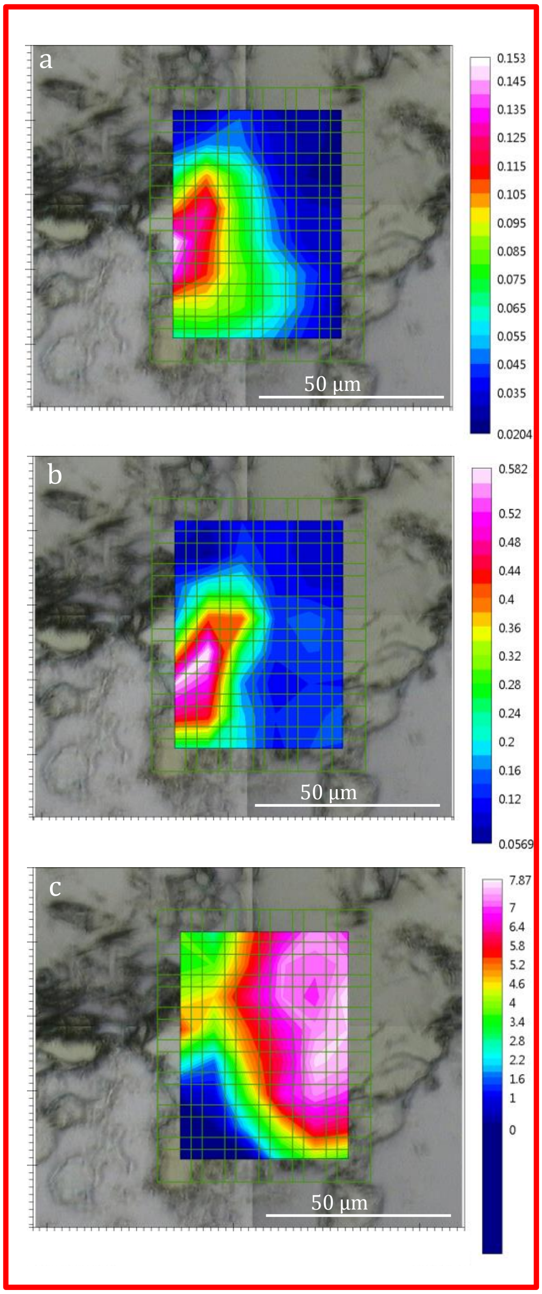


**Figure 7.16** Distribution map of MADB71747 I) optical image of the sample spread on a diamond window, squared area has been analysed. Intensity maps of: a) copper abietate, (band at 1400 cm⁻¹); b) copper carboxylates (band at 2850 cm⁻¹) c) *Pinus* resin (band at 1700 cm⁻¹).

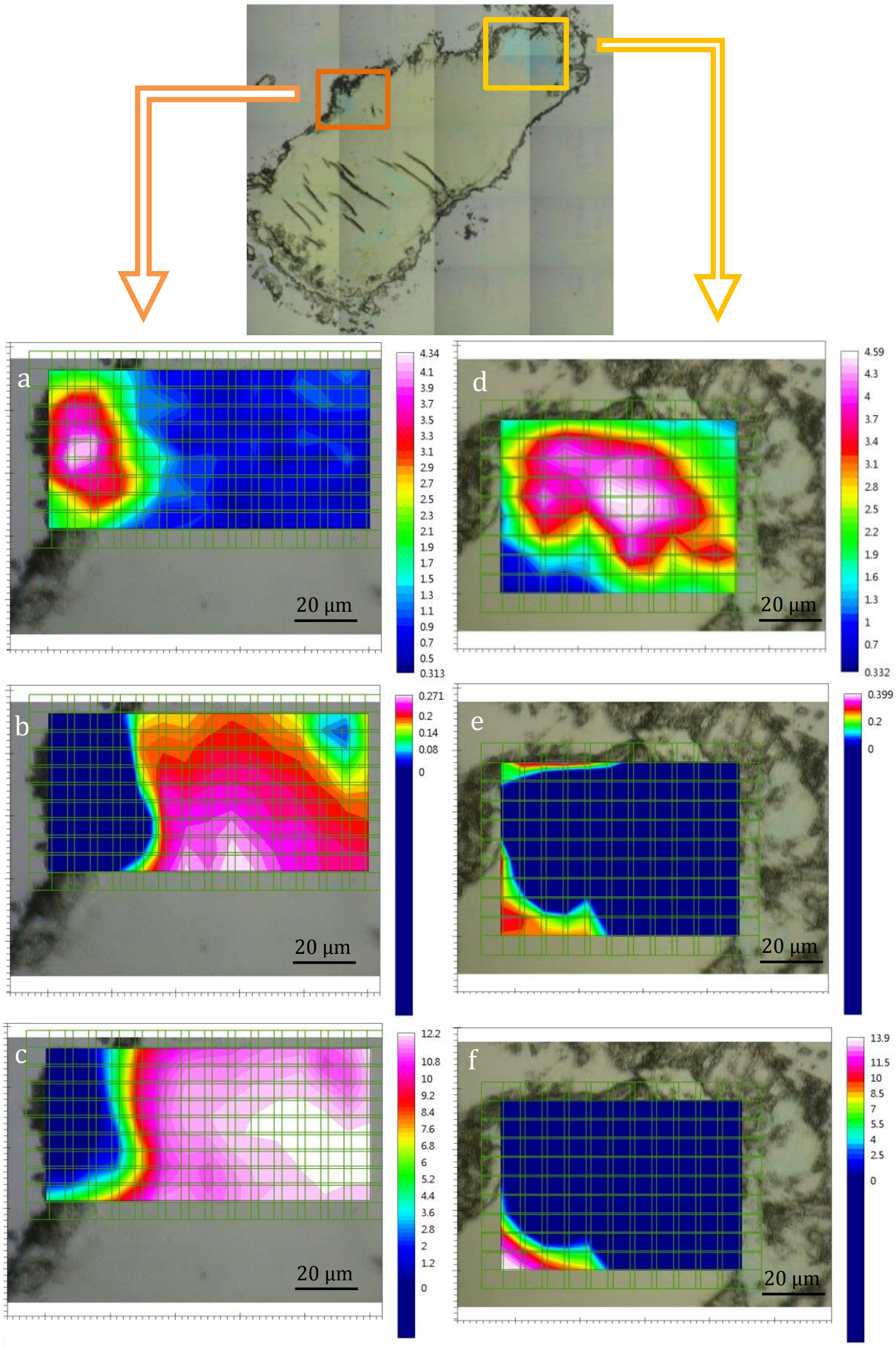


**Figure 7.17** Distribution map of MADB64160 I) optical image of the sample spread on a diamond cell, squared areas has been analysed. Intensity maps of: a and d) $Cu(CH_3COO)_2 \cdot H_2O$, (band at 690 cm$^{-1}$); b and e) copper abietate, (band at 1400 cm$^{-1}$); c and f) *Pinus* resin (band at 1700 cm$^{-1}$).

Intensity map of MADB71747 is displayed in Figure 7.16. The matrix of this coating contains *Pinus* resin and oil (Figure 7.16c) and fewer pigment relics remain. Around them, copper carboxylates are formed (Figure 7.16b) by the reaction with the fatty acids, which coexist with copper abietates (Figure 7.16a). Notably, in the areas where copper carboxylates formed from the fatty acids, copper oxalates are also found in small quantities.

Two intensity maps of MADB64160 have been made, they are displayed in Figure 7.17 and the analysed area is marked in yellow and orange. In both maps, it is noticeable that copper abietates (Figure 7.17b and f) are found in higher proportion beside the green particles (Figure 7.17a and e) and *Pinus* resin can be seen around them (Figure 7.17c and d). The identification of the reaction compounds helps to identify the original compounds present in the coating (which sometimes are no detectable by themselves).

Thin sections of another sample from MADB71747 were also prepared in order to compare the results obtained by each sample preparation method (Figure 7.18).

Thin sections show the distribution of compounds across the sample. At the surface of the section, there is epoxy resin from the embedding medium (Figure 7.18a) which has penetrated about 6 µm. Copper carboxylates are located in the middle of the sample in a small area around a pigment particle (Figure 7.18b) while copper abietate together with *Pinus* resin appear in a wider area at the inner part of the coating (Figure 7.18c).

These results are in good agreement with the previous results, which show that the layer is not homogeneous.

IN CONCLUSION. Reactions between the *Pinus* resin and the other compounds present in the coating took place. Copper abietate is detected as a result of the reaction between abietanes and copper ions from the pigment particles in the coatings of both writing desks. In MADB71747 also copper carboxylates are identified related to the reaction between fatty acids from the binding media and copper ions. These reactions changed the chemical composition of the coatings and explain the physical changes observed, i.e. darkening.

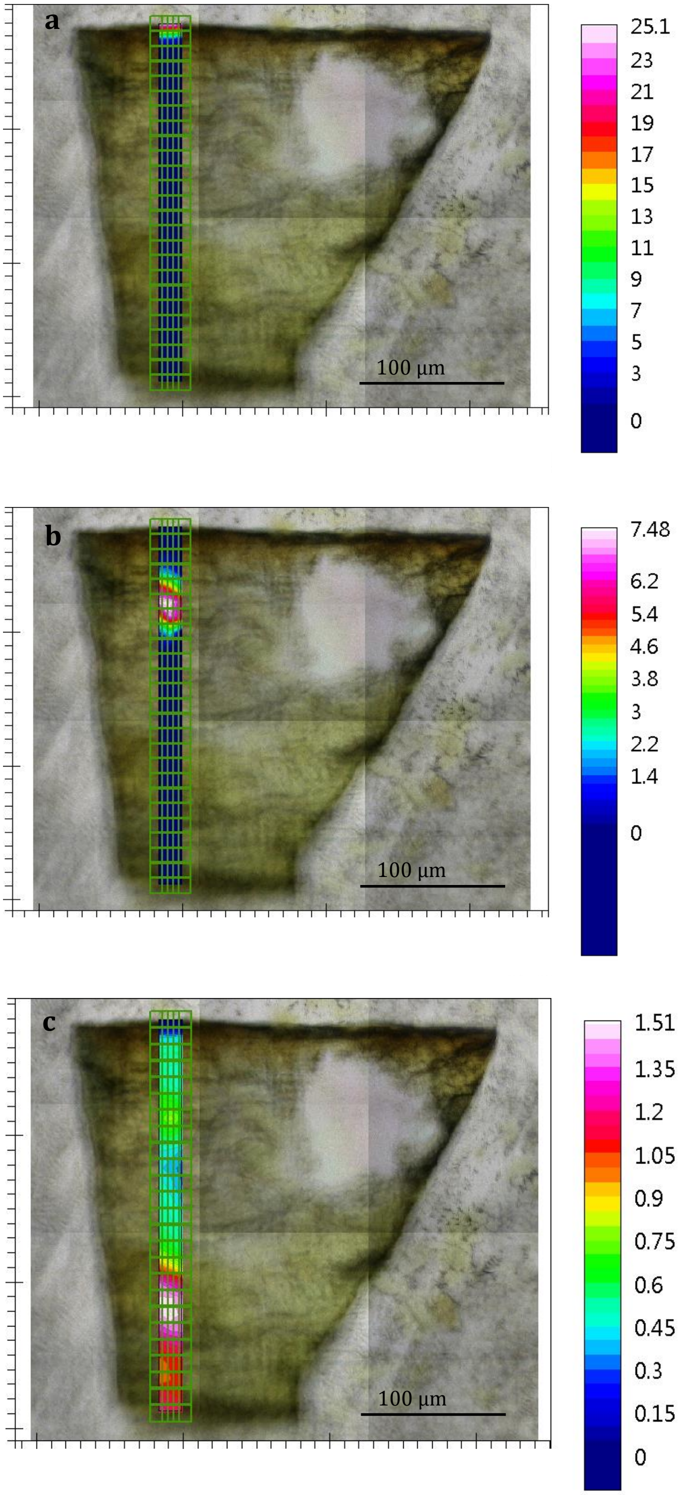


**Figure 7.18** Thin section of MADB71747. Intensity maps of: a) epoxy resin (band at 1510 cm⁻¹); b) copper carboxylate, (band at 1585 cm⁻¹); c) copper abietate, (band at 1400 cm⁻¹).

## 7.5 CASE IV: COLOURED GILDINGS

### 7.5.1 Sant Ruf altarpiece

The altarpiece dedicated to Saint Ruf is a baroque artwork from the 18th century (Figure 7.19). It is located in the Cathedral of Santa Maria de Tortosa (Catalonia).

Some parts of this altarpiece have silver foils coated with coloured gildings. The gilding is applied over the silver foils, which have a layer of bole and a ground-layer below. Samples of red and green gildings were extracted and analysed.

### 7.5.2 Sample preparation method

Samples of red and green gilding were analysed using the diamond cell with a synchrotron source and also prepared in thin sections in order to compare both results.

**Figure 7.19** Sant Ruf altarpiece from the Cathedral of Santa Maria de Tortosa (Catalonia).

## 7.5.3 Results and discussion

### 7.5.3.1 Red gilding

The red gilding layer has a deep red colour and is less than 50 μm thickness (Figure 7.20).

A sample of the gilding was prepared using a diamond cell and analysed using a synchrotron source (μSR-FTIR). Despite its homogeneous appearance, different spectra were obtained (Figure 7.21).

The spectra obtained correspond to three different compounds: *Pinus* resin matrix (Figure 7.21, orange line), starch grains typically added as an extender of the pigment (Figure 7.21, grey line) and protein related to a carmine lake pigment which was extracted either from a wool textile or wool shearing waste (Figure 7.21, red line).

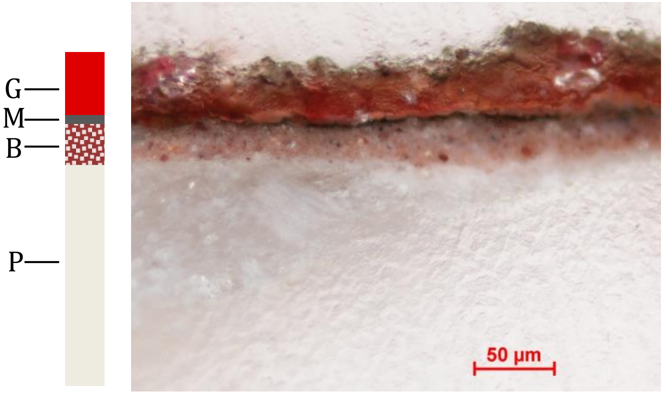


**Figure 7.20** OM with polarized light of the cross-section from the red gilding sample of Sant Ruf altarpiece (right) and layer scheme G: gilding, M: metal foil, B: bole and P: preparation layer (left).

Considering that the *Pinus* resin coatings is dated from 18th century, the markers of oxidized compounds have a lower intensity than expected. This might be explained by the dark environment of the artwork, which could slow down the oxidation process. It is also possible that the presence of other compounds in the sample can stabilize the abietanes preventing its evolution to more oxidized stages.

Samples were also prepared in thin sections and the corresponding compounds distribution maps are shown in Figure 7.22.

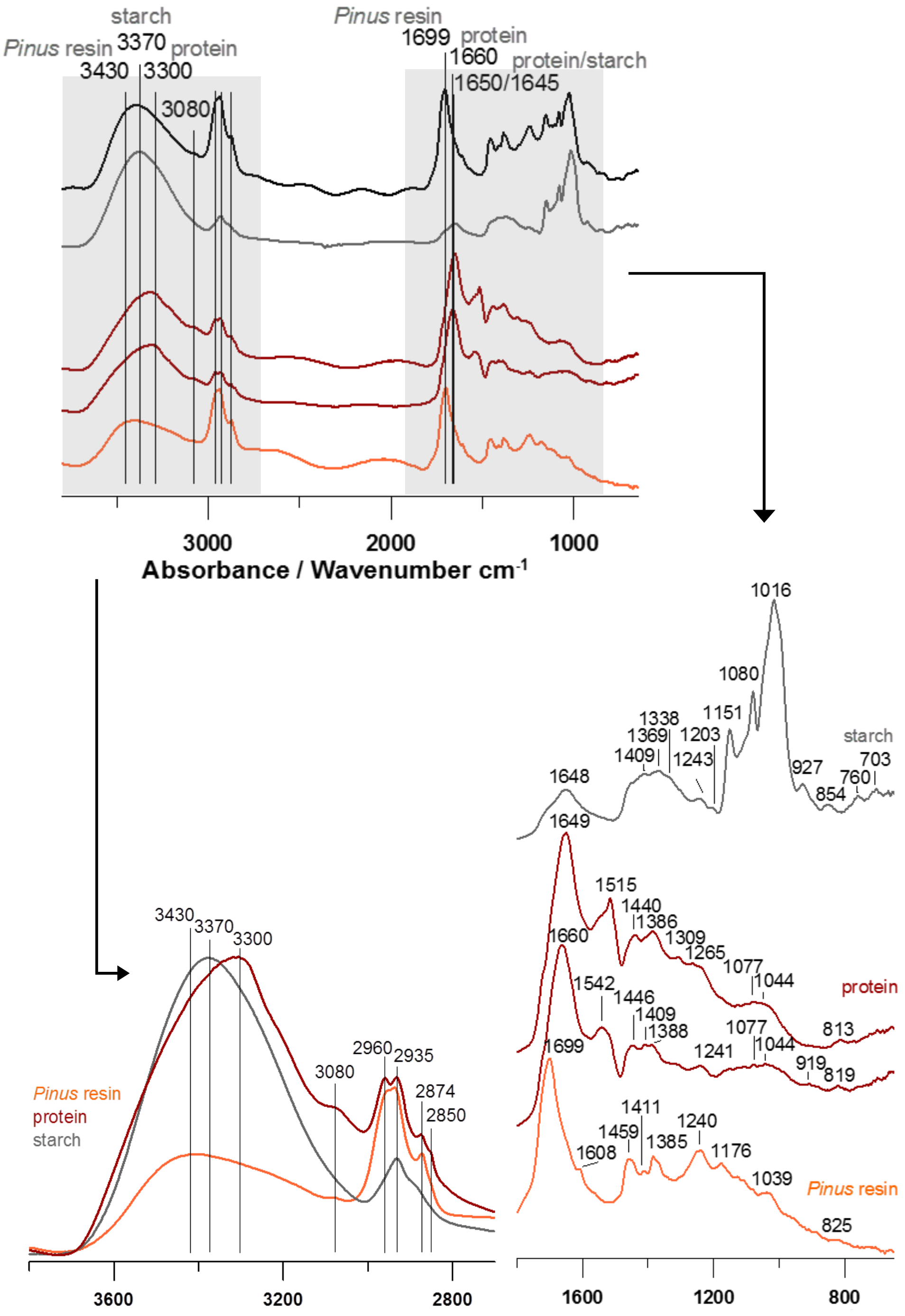


**Figure 7.21** μSR-FTIR spectra of the red gilding using a diamond cell (top). A magnification of the main regions can be seen at the bottom.

The integration of the characteristic bands shows the distribution of the epoxy embedding medium (Figure 7.22a), the protein (Figure 7.22b), the *Pinus* resin (Figure 7.22c) and the starch (Figure 7.22d). No reaction products between the compounds are detected.

The distribution of compounds demonstrates that the gilding is made with a single layer of a coloured coating.

Complementary analyses by SEM-EDX revealed the presence of aluminium and small amounts of silicon, phosphor, sulphur, chlorine, potassium and calcium in the carmine particles.

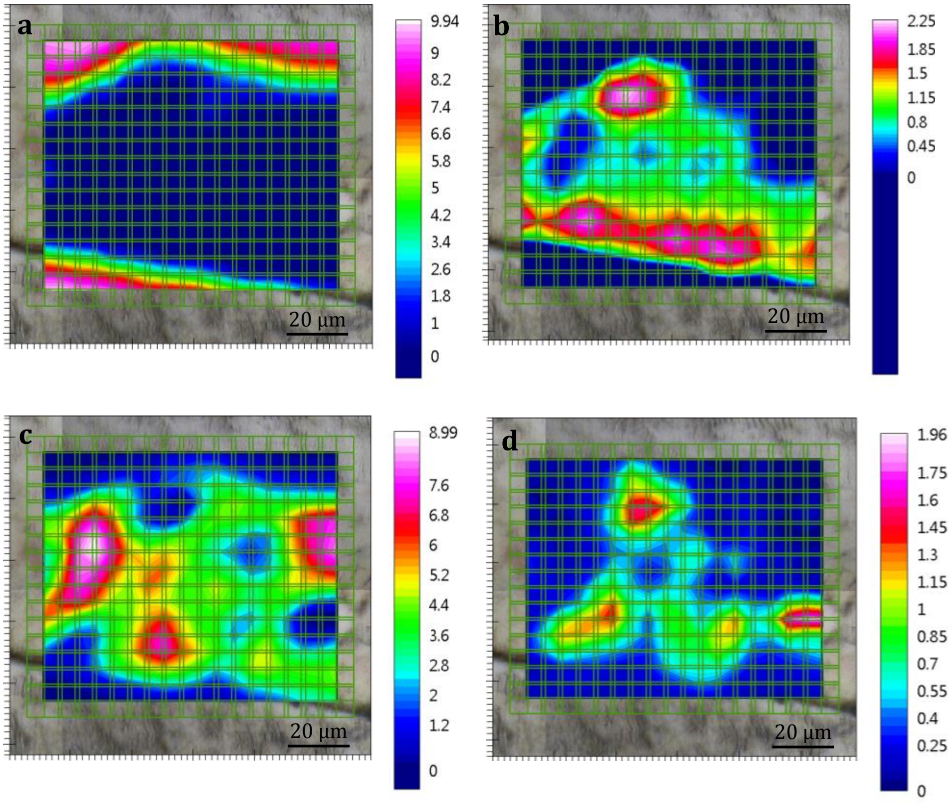


**Figure 7.22** Thin section of a red gilding sample. Intensity maps of: a) epoxy resin (band at 1510 cm$^{-1}$), b) protein (band at 1540 cm$^{-1}$), c) *Pinus* resin (band at 1699 cm$^{-1}$) and d) starch (band at 1151 cm$^{-1}$).

This result agrees with µSR-XRD data, which show the presence of minor amounts of alunite and new reprecipitated potassium and aluminium sulphates (metalunogen and arcanite). The presence of alumina and other inorganic compounds is associated to the production of the carmine lake pigment because they were normally used as precipitating agents.

#### 7.5.3.2 Green gilding

Green gilding samples has an heterogeneous colour and is around 50 µm thickness, approximately the same than red gilding samples (Figure 7.23).

A sample of the gilding was prepared using a diamond cell and analysed using a synchrotron source (µSR-FTIR) and different spectra were obtained (Figure 7.24). The spectra reveal the presence of a green copper pigment, $[Cu(CH_3COO)_2]_x[Cu(OH)_2]_y \cdot nH_2O$ and $CaCO_3$;. There are also copper and calcium abietates resulting from the reaction between the *Pinus* resin matrix and the inorganic compounds (particles of $CaCO_3$ have been detected occasionally in the surface).

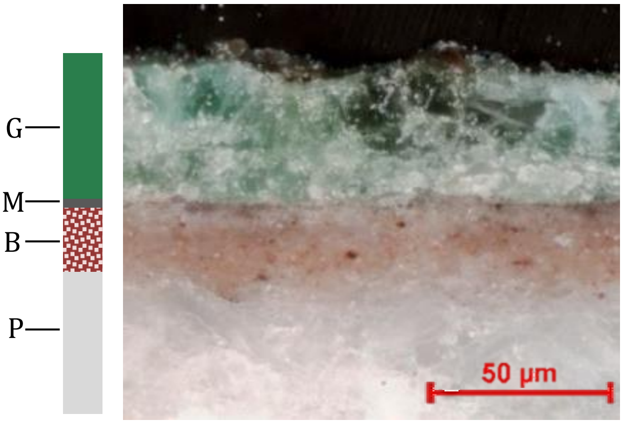


**Figure 7.23** OM with polarized light of the cross-section from the green gilding sample of Sant Ruf altarpiece (left) and layer scheme G: gilding, M: metal foil, B: bole and P: preparation layer (right).

Calcium abietates are detected by the presence of the 1542 $cm^{-1}$ symmetric stretching of $COO^-$ group band (12). Copper abietates are also be detected by their symmetric stretching of $COO^-$ group at 1608 $cm^{-1}$. However, other bands at 1708, 1672/1673 and 1648 $cm^{-1}$ are seen in their spectra which do not appear in pure abietic acid.

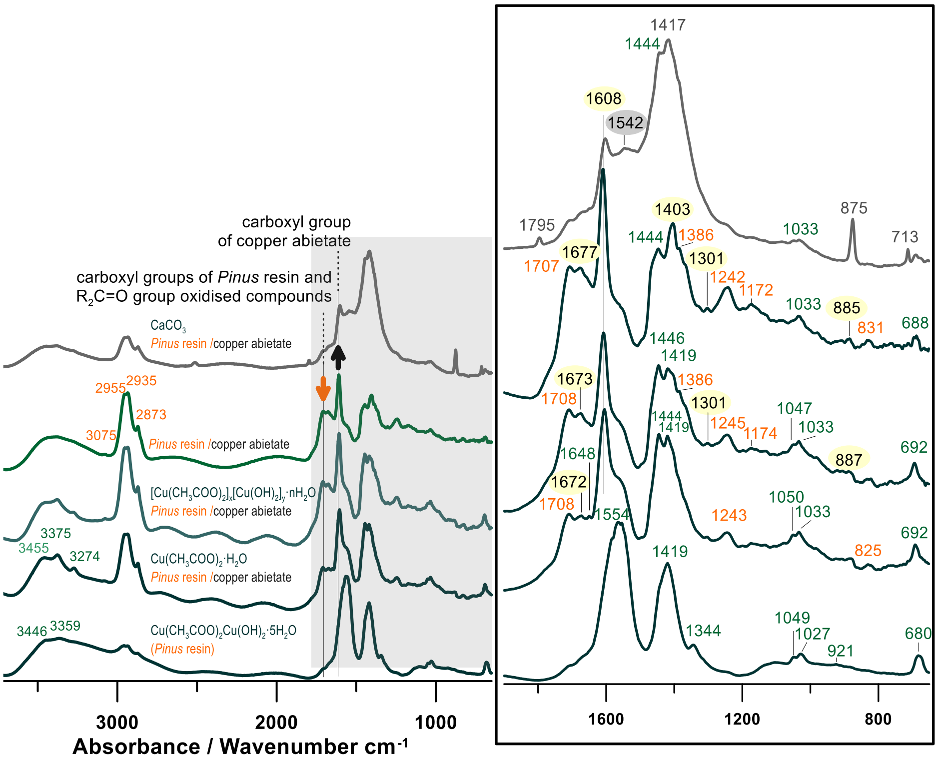


**Figure 7.24** µSR-FTIR of the green gilding prepared with a diamond cell. At the right the magnification of the 1700-700 cm-1 region is shown.

The band at 1708 cm-1 is related to the C=O stretching of aged *Pinus* resin, as in aged resin there is enough proportion of ketones to move the maximum of the –COOH band to higher wavenumbers. The 1672/1673 and 1648 cm-1 bands are related to the presence of pimaranes (C=C stretching): these bands are normally overlapped by the C=O stretching intense band and can only be seen clearly in the Raman spectra where the C=O stretching is normally not active. In this case, *Pinus* resin has reacted almost completely with copper so the band of –COOH is much less intense, allowing to see these bands.

The presence of C=C stretching bands of pimaranes in aged samples demonstrates that these compounds don't polymerize, or at least that the polymerization is not enough and C=C bands can still be seen in a FTIR spectra.

Thin sections from the green gilding were also prepared and analysed (Figure 7.25). Intensity maps show the distribution of epoxy as embedding medium

(Figure 7.25a), copper pigment (Figure 7.25b) and the mixture of *Pinus* resin and copper abietate (Figure 7.25c). Calcium abietate and $CaCO_3$ were not found, suggesting that they are present in a very low proportion.

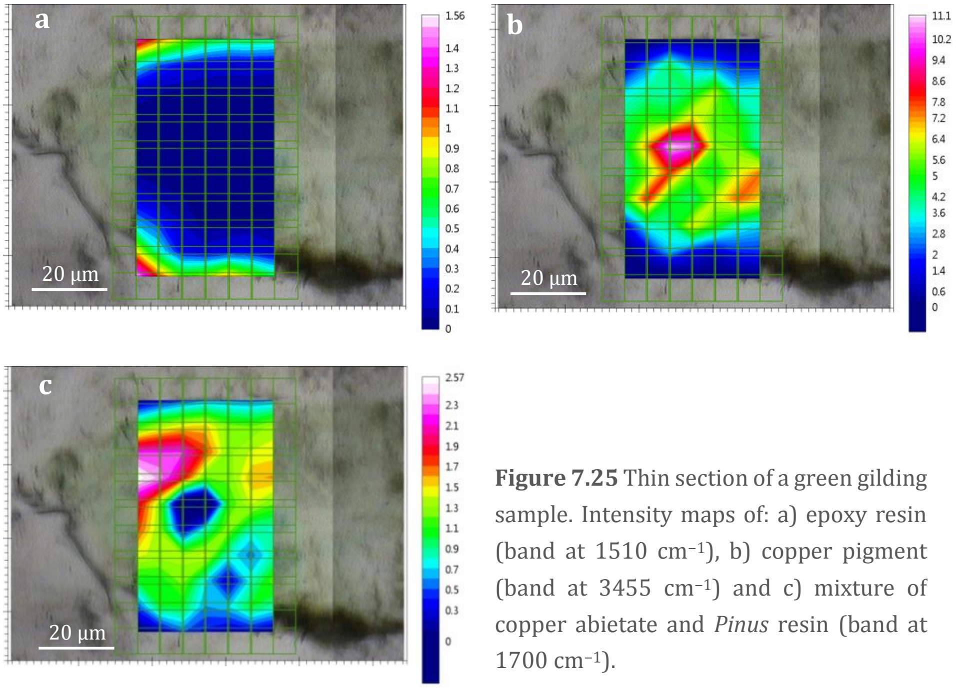


**Figure 7.25** Thin section of a green gilding sample. Intensity maps of: a) epoxy resin (band at 1510 cm⁻¹), b) copper pigment (band at 3455 cm⁻¹) and c) mixture of copper abietate and *Pinus* resin (band at 1700 cm⁻¹).

Unlike the coatings from the writing desk (section 4), *Pinus* resin is not found alone in any area of the preparations. In this case the *Pinus* resin of the gilding has reacted almost completely with the copper pigment. Likewise the red gilding sample, the distribution show that the compounds are mixed, so the gilding is made with a single layer of a coloured coting.

Thin sections were also analysed by µSR-XRD. Results show the presence of $Cu_2Cl(OH)_3$ in the green layer, probably from weathering of copper acetates and basic copper acetates. Some other crystalline copper reaction compounds are also found although the phase could not be identified.

IN CONCLUSION. These two coloured gildings contain *Pinus* resin, but the presence of other substances affected it in different ways. No reaction compounds are detected in the red gilding so the *Pinus* resin spectra is fully distinguishable. On the contrary, the copper from the green gilding has reacted with *Pinus* resin making the identification of the original materials more complex. This example illustrates the importance of the complete analysis of all substances present in the coatings by complementary techniques in order to understand its chemical composition.

## 7.6 CASE V: SINGLE-LAYERED AND MULTI-LAYERED COATINGS

### 7.6.1 Sant Joaquim Altarpiece

The altarpiece dedicated to Joaquim is a baroque artwork from the 18th century (Figure 7.26). It is located in the Cathedral of Santa Maria de Tortosa (Catalonia) like the altarpiece dedicated to Sant Ruf.

Some of the metal foils of this altarpiece have been coated. Despite the similar appearance of some of these coatings, it cannot be said that they have the same composition as and differences could be found due to different facts, like different restoration treatments.

### 7.6.2 Sample preparation method

Two kinds of samples have been studied, ones from an area with a coating made of a single layer and other ones from an area with a coating made with more than one layer (Figure 7.27).

Thin sections of the samples were prepared as some of the layers are very flimsy and have similar appearance, so it would be difficult to mechanically separate a fragment of each layer for analysis.

### 7.6.3 Results and discussion

#### 7.6.3.1 Single-layer*ed* coating

A thin section of the sample is displayed in Figure 7.28. The coating over the silver foil looks homogeneous. However, the integration of µSR-FTIR results (Figure 7.29) show a thin layer where the major compound is protein (Figure 7.29a) immediately over the metal foil, and then a coating where the major compound is *Pinus* resin (Figure 7.29b).

**Figure 7.26** Sant Joaquim altarpiece from the Cathedral of Santa Maria de Tortosa (Catalonia).

**Figure 7.27** Detail of the varnished surface where the sample was taken.

Protein and *Pinus* resin layers are no perfectly separated. Impurities of protein are seen in the spectrum of *Pinus* resin layer, like the band at 1560 cm$^{-1}$ from amide II mode (13) (Figure 7.29II) that appears at higher wavenumber than usual due to the overlapped bands. Additionally, impurities of *Pinus* resin can be seen in the protein layer, like the band at 1690 cm$^{-1}$ from –COOH of abietanes and pimaranes (Figure 7.29I).

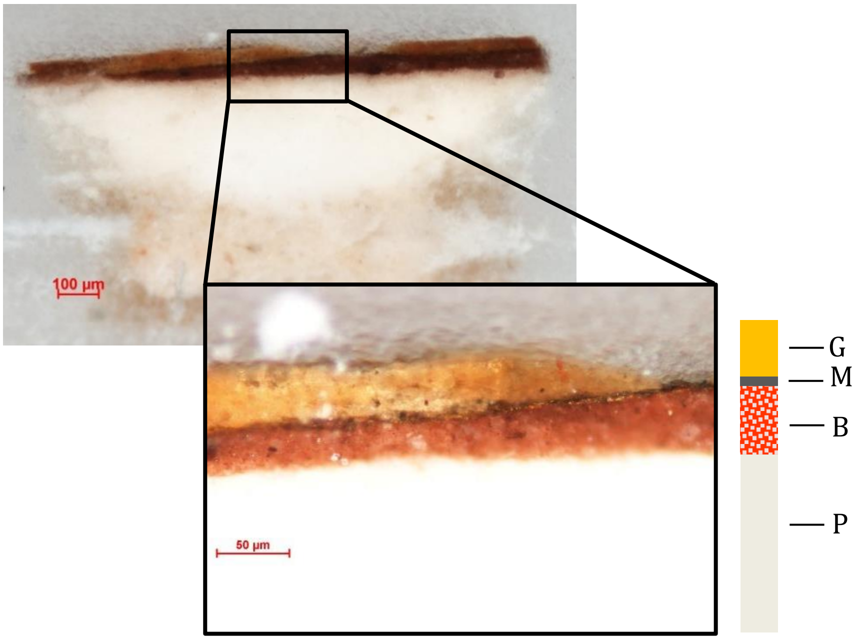


**Figure 7.28** OM with polarised light of 2 µm thin section of the sample with a single-layered coating (left) and layer scheme G: gilding, M: metal foil, B: bole and P: preparation layer (right).

Protein has bands in the same region than *Pinus* resin, consequently their specific bands cannot be clearly distinguished and it is difficult to identify the specific markers of the oxidation degree. Knowing the materials of this sample, a Raman analysis of the *Pinus* resin (Figure 7.30) using the suitable conditions was considered. In this case, the presence of protein does not interfere with the detection of *Pinus* resin bands related to the oxidation, in particular those around 1600 as protein has no bands in this region (14).

For the Raman analysis a fragment was separated and analysed without any further preparation. The equipment and analytical conditions used are described in chapter 2.

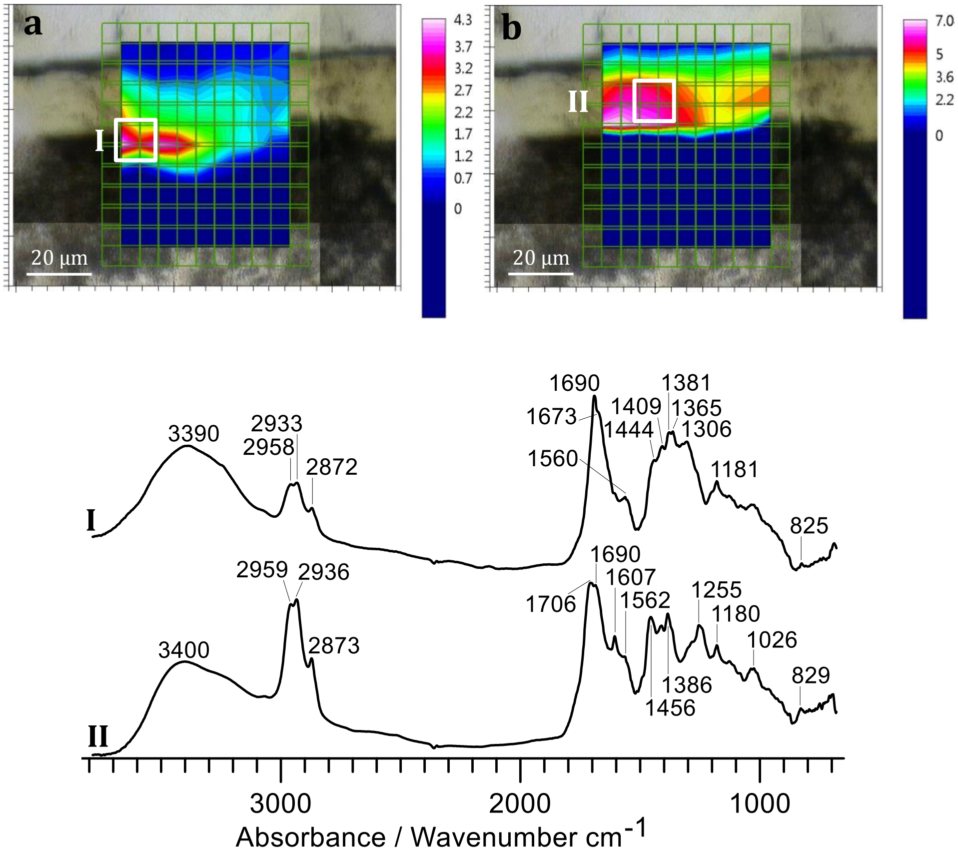


**Figure 7.29** Integration maps from the single-layered coating where the protein (band at 1560 cm-1) and the *Pinus* resin layers (band at 1710 cm-1) can be distinguished. A spectrum of the area where each material predominates is shown below I) protein as the major compound and II) *Pinus* resin as the major compound.

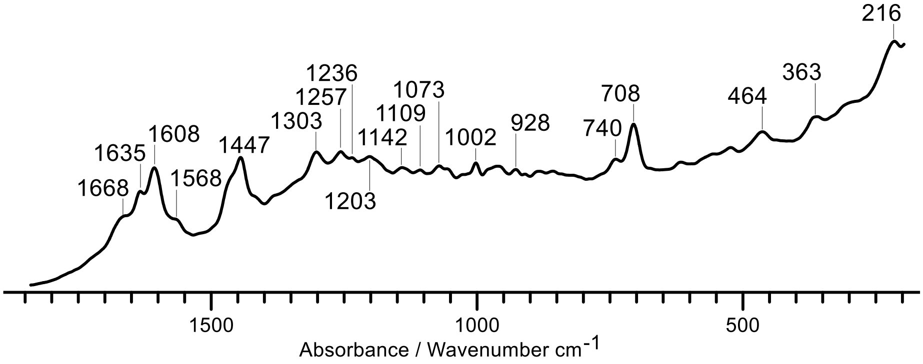


**Figure 7.30** Raman spectrum from the single-layered coating.

The Raman spectra obtained from the coating analysis shows the features of aged *Pinus* resin. Band from C=C stretching of abietic acid, that appears at 1650 $cm^{-1}$, has a low intensity and the peak can no longer be distinguished. Additionally, the band from C=C of dehydroabietic acid can be seen at 1608 $cm^{-1}$, also there is a small band at 1635 $cm^{-1}$ from the hydrogen bonds that appears in high aged resins. Results show that the resin has an advanced oxidation degree.

### 7.6.3.2 Multi-layered coating

The thin section of a sample with a multi-layered coating is shown in Figure 7.31, the scheme at the right helps to identify each layer. At the bottom there is the silver foil over the bole layer, above the silver foil three coating layers can be clearly distinguished (Figure 7.31 layers I, II and III).

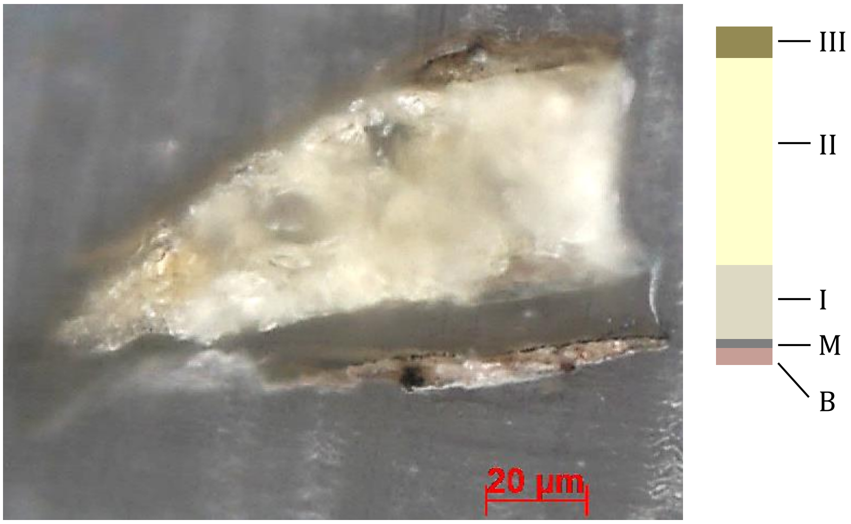


**Figure 7.31** OM with polarised light of 2 µm thin section of the multi-layered coating. At the left there is a scheme where the different coatings are numbered, B: bole, M: metal foil, I, II and III: layers of coating.

Several spectra were obtained from each layer (Figure 7.32). Epoxy resin has been used as embedding medium and can be seen around the sample (Figure 7.32a). Spectrum corresponding to layer I (Figure 7.32b) is related to protein, this agrees with the presence of protein in the sample from section 6.3.2 which belongs to the same altarpiece. Small particles are observed in this layer, SEM-EDS analysis revealed the presence of magnesium, which has been probably used as a colourant in this layer (15).

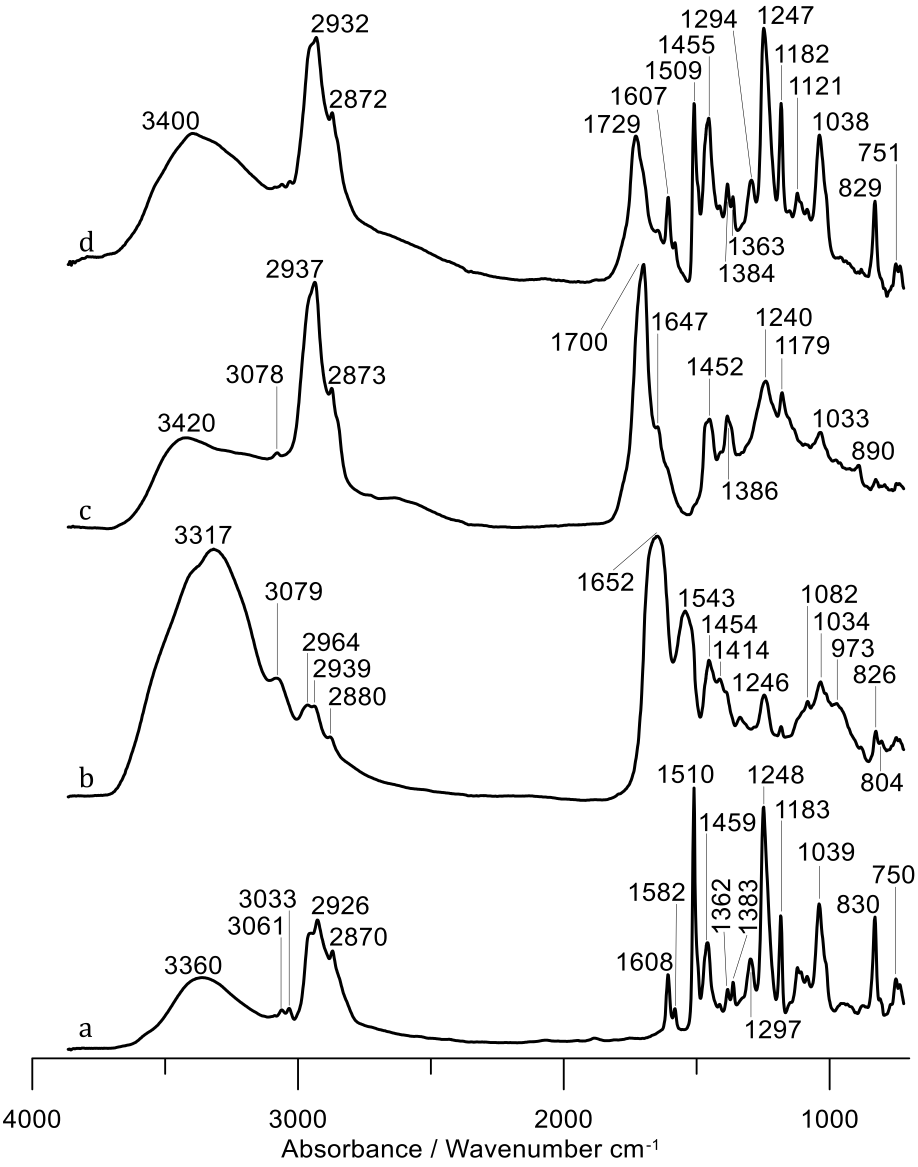


**Figure 7.32** µSR-FTIR spectra from different parts the sample a) epoxy resin from the embedding medium, b) layer I of the coating, c) layer II of the coating and d) layer III of the coating.

Spectra from layer II has a complex shape where the specific markers of *Pinus* resin can be distinguished, however other bands from the region between 1800-700 cm$^{-1}$ do not belong to these materials like the ones at 1647, 1033 and 890 cm$^{-1}$.

These bands are related to the presence of sandarac. As mentioned, sandarac is a diterpenic resin which main compound is polymerized communic acid. Communic acid structure is very similar to abietic acid and their FTIR spectra share some bands like the one around 1695 cm$^{-1}$ due to the –COOH which have almost the same chemical environment. However still some differences are appreciated like the band around 1645 cm$^{-1}$ from conjugated C=C in sandarac spectra.

The composition of sandarac also changes upon ageing due to oxidation processes. To ensure that the bands assigned to sandarac do not change during ageing it has been compared the FTIR spectra of differently aged samples (Figure 7.33).
The bands at 1647, 1033 and 890 cm$^{-1}$ are still present in aged samples, consequently, the presence of these bands in the spectrum of the layer II of the coating are safely related to sandarac.

To confirm the presence of *Pinus* resin and sandarac in the layer II of the coating, the spectrum is compared with the addition of the spectra corresponding to moderately oxidized resins aged *Pinus nigra* Arnold resin from 1884 (Economic Botany Collection of Kew, ref. 28172) and aged sandarac from 1953 (Economic Botany Collection of Kew, ref. 95431) (Figure 7.34). The comparison reveals that the sum of the two compounds is very similar to the spectra obtained experimentally. The presence of *Pinus* resin and sandarac has been also confirmed by GC/MS.

Varnishes made with sandarac and *Pinus* resin are described in historical sources. As mentioned, *Pinus* resin was cheap so it was usually utilized to adulterate more expensive materials. Consequently the mixture of *Pinus* resin and sandarac in a varnish or coating is plausible.

Spectrum from layer III (Figure 7.32d) is overlapped with the spectrum of epoxy resin, nevertheless there is an intense band at 1729 cm$^{-1}$ is not related to the embedding medium or any of the materials from the other two layers of the coating. Consequently, layer III is a different material, but, as it is very thin (less than 10 µm), it was not possible to obtain a pure spectrum of this substance. Nevertheless, as other parts of the altarpiece have been coated with a modern acrylic resin, with a typical band at 1730 cm$^{-1}$, it can be suggested that it is the substance found in layer III.

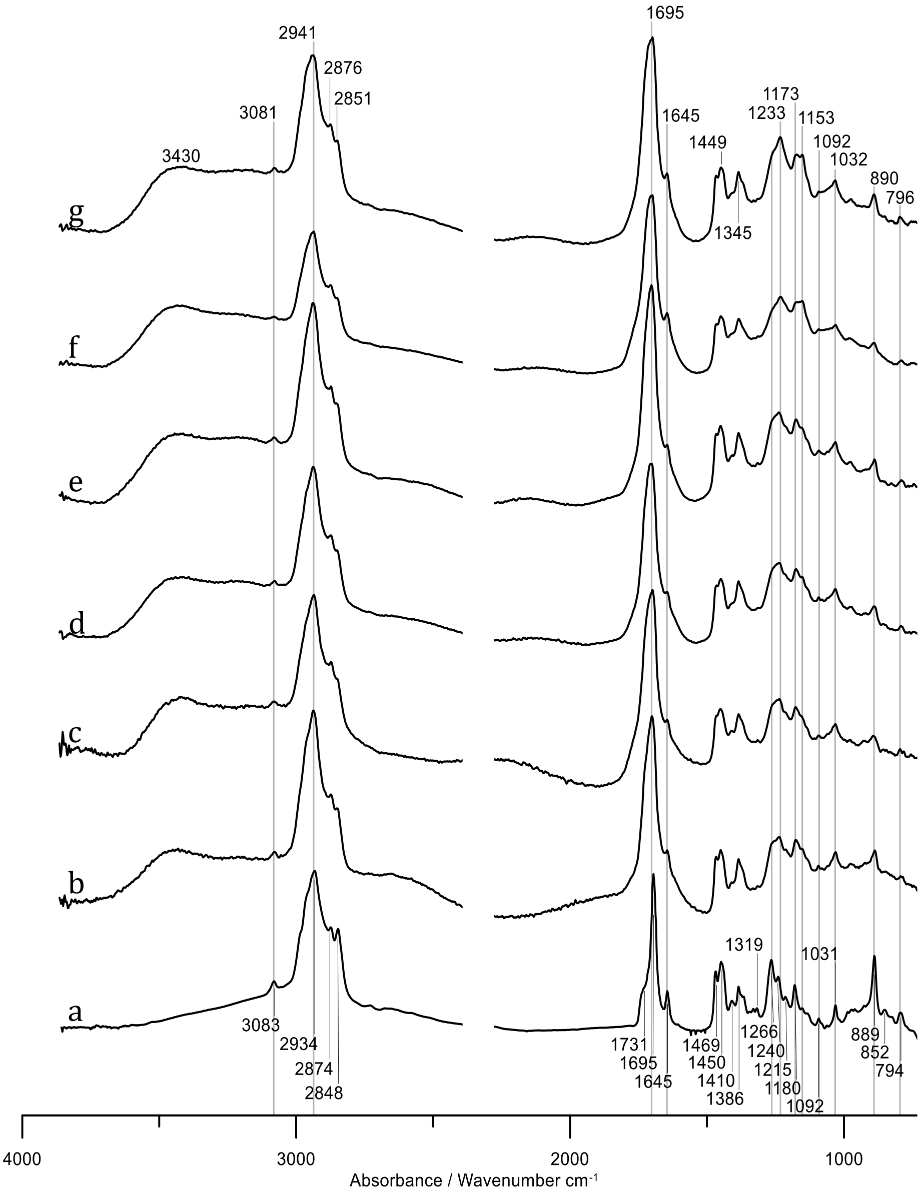


**Figure 7.33** FTIR spectra of aged sandarac a) fresh resin collected from *Tetraclinis articulate* Vahl. compared with aged resins from the Economic Botany Collection of Kew b) sample from 1961 (ref. 94133), c) sample from 1960 (ref. 94193), d) sample from 1955 (ref. 94135), e) sample from 1953 (ref. 95431), f) sample from 1886 (ref. 28830) and g) sample from 1863 (ref. 28828).

A specific band of each spectrum (coatings layers I, II and II and epoxy resin) has been integrated (Figure 7.35). Results confirm that the substances described in Figure 7.32 are not mixed but structured in a sequence of layers of different composition and thickness.

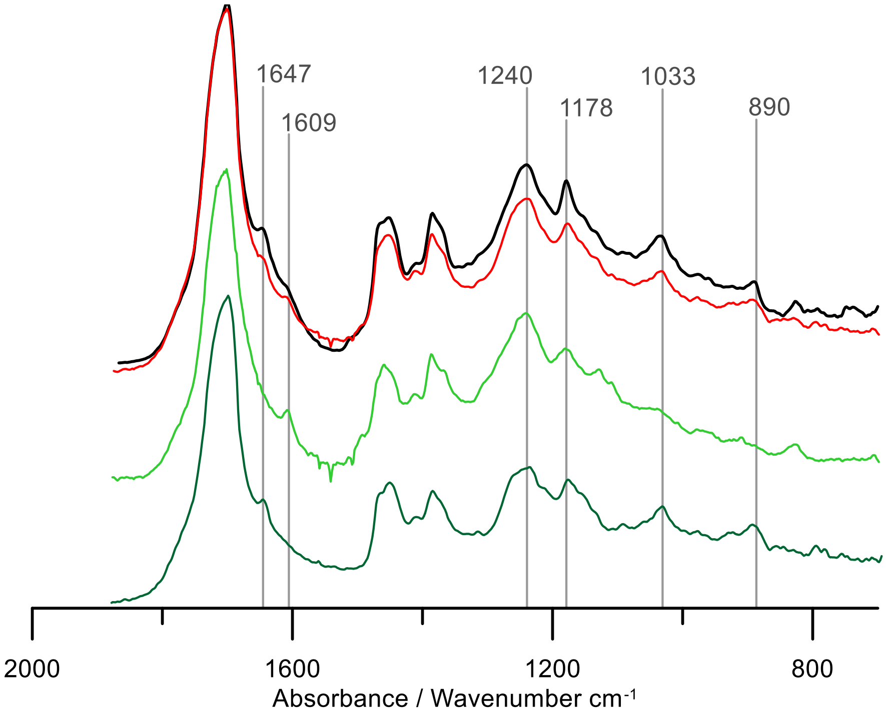


**Figure 7.34** Comparison between the spectrum of layer II of the coating (black line) and the calculated spectrum (red line) obtained from the addition of the FTIR spectra of *Pinus nigra* Arnold resin from 1884 (Economic Botany Collection of Kew, ref. 28172, light green) and sandarac from 1953 (Economic Botany Collection of Kew, ref. 95431, dark green).

IN CONCLUSION. The structure and the materials of the coating determine the best analytical strategy. The combination of different analytical techniques is useful in the analysis of coatings made of mixtures, while thin sections are crucial for the analysis of coatings made of a sequence of micrometric layers.

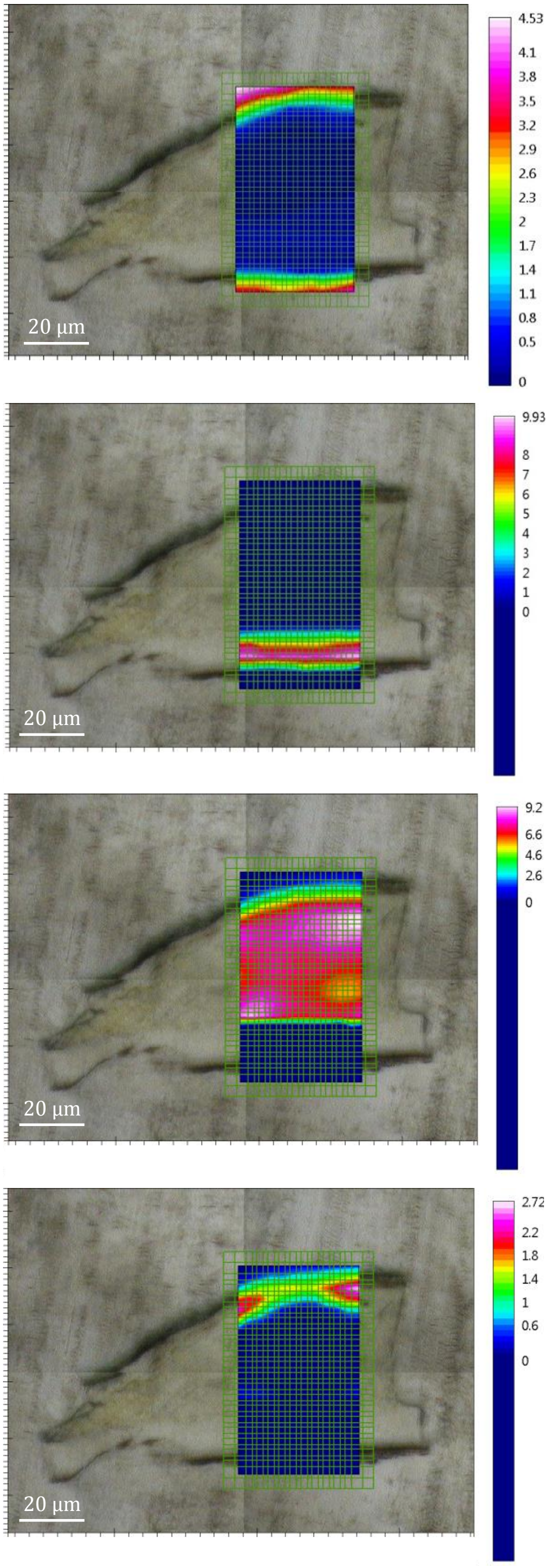


**Figure 7.35** Thin section of the multi-layered coating. Intensity maps of: a) epoxy resin (band at 1510 cm$^{-1}$), b) layer I (band at 1543 cm$^{-1}$), c) layer II (band at 1700 cm$^{-1}$) and d) layer III (band at 1729 cm$^{-1}$).

## 7.7 CONCLUSIONS

Samples from historical objects with coatings of *Pinus* resin were analyzed by FTIR spectroscopy. Different sample preparation methods were used depending on the characteristics of the coating, materials and structure. Complementary analytical techniques were applied when required to corroborate the data.

Results show that the ageing study described in chapter 4 and 5 was crucial for the identification of *Pinus* resin in coatings from artworks. Either thin homogeneous or multilayered coatings the preparation described in chapter 6 was adequate for the identification of the composition of each layer and provided high quality spectra for the materials characterization.

## 7.8 REFERENCES

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## CHAPTER EIGHT

# Conclusions

Vibrational spectroscopies are useful techniques for the study of a wide range of materials. This work has demonstrated their applicability to the study of *Pinus* resin chemical composition and their changes due to the oxidation processes.

FTIR and Raman spectroscopy have proved to be suitable techniques for the analysis of coatings of *Pinus* resin allowing to tag the specific markers of fresh and oxidized compounds in each spectrum and to shed light on the ageing mechanisms of this material. The use of combined FTIR and Raman spectroscopies facilitates the interpretation of the spectral features and provides complementary information allowing not only a better characterization of the materials, but also a more complete band assignment of each spectrum.

However, FTIR analysis of *Pinus* resin requires high quality spectra that involve specific sample preparation methods. Although Raman spectroscopy can be used without any specific sample preparation (it can be performed directly over the surface of sample fragments or cross sections), the nature of the materials should be known beforehand because wrong working conditions can damage the sample and give fake results.

A specific sample preparation method has been developed for FTIR analyses of coatings from historical objects able to analyse micrometric layers as well as the compounds distribution in each layer and across the sample. In order to obtain the information at the resolution required, a Synchrotron Source is essential, as it provides the small probe size, broadband spectrum, lateral resolution and high brilliance necessary.

By means of a study combining spectroscopic techniques and a suitable sample preparation, it is possible to characterize *Pinus* resins from historical objects. This can help to shed light in the historical background of each item and to find suitable conservation conditions and, if necessary, a proper restoration process.

Future work. It will be focused in the study of resin from other *Pinaceae* species used in varnishes and coatings, such as *Larix decidua* Mill. and *Abies alba* Mill. as they contain abietanes and pimaranes, main compounds of *Pinus* resins, but also other specific molecules. The possibility of distinguishing different Pinaceae species resin is very interesting since, according to historical sources, the resin of *Larix decidua* Mill. and *Abies alba* Mill. were more expensive, so those varnishes had a higher quality.

Additionally, the interactions among pigments and colorants and abietanes and pimaranes will also be studied; as these materials are often found in varnishes and coatings. Some preliminary results suggest that their presence can stabilize certain oxidized molecules and thus affect the resin oxidation process.