NEW INSIGHTS INTO MOLECULAR EVOLUTION OF OIL/COLOPHONY VARNISHES: TOWARDS PYROLYSIS-GAS CHROMATOGRAPHY/MASS SPECTROMETRY-BASED QUANTITATION

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Abstract

Mixtures of a drying oil with a Pinaceae resin are among the most widespread natural varnish formulations encountered in coating of artifacts. GC/MS and Py-GC/MS are to date the choice analytical methods in the cultural heritage field for their qualitative molecular identification.

This research was carried out on reference varnish films made with various proportions of linseed oil and colophony aged naturally in the same conditions for one year. It aimed at (1) better understanding the influence of the various proportions of oil and resin on the molecular composition of the reference films, and (2) suggesting a Py-GC/MS semi-quantitative method applicable to micro-samples. Qualitative analysis of these reference varnishes and of a historical film is first presented. Then, semi-quantitative analysis of the reference films suggested that the reaction mechanisms involved in their ageing processes are highly related to each ingredient’s proportions. Linseed oil may have an activating role in oxidation reactions occurring on colophony compounds, and the conservation state of varnishes could thus be closely linked to the resin’s proportions. Such results provide new insights into molecular evolution of linseed oil/colophony varnishes, and the semi-quantitation seems useful to further explore mechanisms occurring between diterpenoids and fatty acids.

1 Introduction

Mixtures of a drying oil with a Pinaceae resin are among the most widespread varnish formulations encountered on musical instruments until the 18th century1. For instance, they have been identified in 16th century Bolognese and Paduan lutes2,3 and in an early 18th century violin made by Jacques Boquay in Paris4. Five instruments made by Antonio Stradivari in Cremona, Italy, between c.1692 and 1724 are also coated with an oil/Pinaceae resin film1,3. Besides, such coatings were frequently used for furniture, easel paintings and in decorative arts5,6. In particular, pine resin is reported to be quite frequently detected together with oil in the binding medium of red and green glazes on 16th and 17th century Italian easel paintings7.

Gas chromatography coupled with mass spectrometry (GC/MS) is to date the choice method for the molecular characterisation of historical varnish samples weighing as little as a few micrograms. Very efficient multi-steps protocols have been developed for the detection of various types of chemical compounds, but requiring larger sample quantities8-10. When very little material is available for analysis, one-shot protocols allowing the simultaneous analysis of compounds of oils and natural resins have been developed, extensively used and optimised in the past decades. Most of those are based on an apolar column and a derivatization reaction ahead of the chromatography step. The first aim of the derivatization procedure is to reduce the polarity of certain molecules or moieties, in particular the carboxyl and hydroxyl groups, by an esterification, methylation or silylation procedure. Derivatization also improves peak shapes and intensities. Transesterification
The present research was carried out on a set of model varnish films made with various proportions of linseed oil and colophony. They are all aged under the same natural conditions for one year. Other parameters, such as the nature of the drying oil, the varnish preparation process (e.g. time, temperature), and the ageing conditions (e.g. photo ageing, thermo ageing, presence of oxygen, duration) are not considered.

The aims of this research are:
- to better understand the influence of the various proportions of oil and resin on the molecular composition of the reference varnish films.
- to develop and assess the reliability of a Py-GC/MS method.

In this paper, after a brief summary on the main well-established mechanisms occurring upon ageing of films made with a mixture of oil and Pinaceae resin, results from the qualitative analysis of the reference one year old varnish films and of a historical varnish film are first presented. The method developed for the semi-quantitative data analysis of the reference one year old varnish films is described and evaluated. Lastly, the molecular evolution of the films considering possible phenomena and mechanisms specific to the mixture of linseed oil with colophony, as well as the input of the method developed to further perform Py-GC/MS are discussed.

2 Ageing of linseed oil and Pinaceae resin: a brief summary

Curing and ageing mechanisms of films made of linseed oil have been extensively studied, in multiple ageing conditions, with addition of pigments, on model and paint samples from artworks. The predominance of particular reactions over others depends on the ageing conditions, but the main steps of the curing and ageing of linseed oil are well described in the literature. The mechanism governing the curing of linseed oil-based films is generally referred to as auto-oxidation, which describes the radical chain reactions involving spontaneous addition of oxygen on the triacylglycerol’s chains to form hydroperoxides.

The first step of curing involves the formation of radicals on (poly)unsaturated alkyl chains by thermal, photochemical or catalytic processes. The aliphatic double bonds isomerize, oxygen reacts with the radical to form peroxide radicals, leading to hydroperoxides. Then several processes may overlap in the curing phase: (I) oxidation within fatty acids chain forming cyclic peroxides, di-hydroperoxides, epoxides; (II) polymerization reactions leading to formation of peroxy, carbon and ether bonds between fatty acid chains and, (III) formation of low molecular weight volatile compounds (aldehydes, ketones, carboxylic acids, small hydrocarbons) mainly formed by β-scission of hydroperoxides.

Less information is available on long-term degradation of linseed oil. Oxidation goes on, forming more oxidized compounds like carboxylic acids, peresters, lactones, polymerization stabilizes and chain scission...
are favoured in carbons position 7 and 15. To date, the most oxidized compound identified is 15-OH-7-oxo-dehydroabietic acid. Regarding the ageing reactions of the pimaric fraction of colophony less is known, but these compounds may polymerize via the extra-cyclic double bond as suggested by Scalarone.

Though a lot is known about the ageing of linseed oil and colophony, there are, to our knowledge, only two studies dealing with the ageing behavior of oil and colophony mixtures. It was suggested that the presence of colophony may promote oil hydrolysis and that molecules from linseed oil and colophony may react with each other. Daher recently observed that oxidation of colophony was favoured by the presence of linseed oil. Such studies suggest a possible reactivity between oil and resin compounds and enlighten the need for additional information on oil/resin mixtures.

3 Experimental

3.1 Preparation of reference varnish films

Reference liquid varnishes with five different proportions of linseed oil and colophony (80/20, 66/33, 50/50, 40/60, 25/75, wt%) were manufactured according to historical recipes dating from the 14th to the 18th century which include ingredients used for preparing varnishes for musical instruments or other objects.

Reference linseed oil (10 g) namely clarified linseed oil (Laverdure, ref. #181070) was heated slowly to 170 °C in a beaker. The heating plate was then stopped and various quantities (2.5, 5, 10, 15 and 30 g) of the solid reference colophony (Laverdure, ref. #341865) were added to obtain five varnishes. Before being added to the hot linseed oil, the colophony had been crushed in an agate mortar. The dissolution of the colophony was achieved under magnetic stirring in about one hour. In a general way, the viscosity increases with the proportion of colophony, and similarly, the color of the varnishes becomes darker with increase in the proportion of colophony.

Different solid varnish films were obtained by carefully spreading the liquid varnish on a clean microscope glass slide. Thicknesses of the varnish films were not perfectly homogeneous, and were measured from 10 µm to 150 µm.

Films were then aged one year upon indoor storage in room conditions (i.e. with indirect natural sunlight, values for relative humidity and for temperature are 34% and 24 °C, respectively).
3.2 Historical varnish

The varnish coating the soundbox of a theorbo was micro-sampled. This instrument belonging to the lute family, was made in the late 16th or early 17th century in Venice by Magno Dieffopruchar (a.k.a. Tieffenbrucker) and is now in the collection of the Musée de la musique in Paris (inv. E.1778)\textsuperscript{37}. Its dark red-brown coating is considered by lute historians as quite typical of those encountered on Venetian lute-type instruments in this period. Special care was taken in the selection of the sampling area, in order to avoid any detectable touching up or non-original material.

3.3 Pyrolysis (TMAH)-GC/MS analytical conditions

Reference varnish samples were analyzed using a single-step TMAH-Assisted Py-GC/MS procedure with thermally assisted methylation.

3.3.1 Sample preparation

For each run, a few milligrams (not weighted; estimated about 20 µg) of each reference varnish film were collected by scraping the surface of the microscope glasses with a sterile scalpel blade under a binocular microscope (Microvision Zeiss Discovery V20; magnification 7.5). Each sample (reference and historical varnish) almost invisible by the naked eye was directly placed into a 50 µl stainless steel Eco-cup (Frontier Laboratories). A new Eco-cup is used for each run. With the aim to check the run and to further obtain the relative amount of some diterpenoids and carboxylic acids in each sample (i.e. semi-quantitative analysis), 4 µl of nonadecanoic acid, nC19:0 (Sigma-Aldrich), dissolved in dichloromethane (Sigma Aldrich, ACS reagent, > 99.5%) with a concentration of 8.2. 10\textsuperscript{-4} mol/L was also introduced into the Eco-cup using a micro-syringe. Nonadecanoic acid was chosen as internal standard because it is absent from both colophony and linseed oil molecular compositions, and because its chemical structure is very close to colophony and linseed oil molecular compositions, consistent with the literature. In particular, expected methylated forms of carboxylic acids (from linseed oil) and diterpenoids (from colophony) are detected.

The molecular assignment was done either by direct analysis of the mass spectrum and/or comparison with a reference mass spectral library (US National Institute of Standards and Technology, NIST 02 version 2.0 a, 2002) and/or comparison to pyrograms and mass spectra of published literature.

Pyrograms from all reference varnishes have profiles consistent with the literature. In particular, expected methylated forms of carboxylic acids (from linseed oil) and diterpenoids (from colophony) are detected. Fig. 2 presents the pyrogram from the reference varnish film with a 50/50 (wt%) linseed oil/colophony proportion. Peak assignments of fatty acid and diterpenic compounds are provided in Tab. 1 and Tab. 2, respectively.
Differences in relative peak intensities are observed between the reference varnishes. Chromatographic peaks assigned to linseed oil are methyl esters of palmitic (nC16:0, P), stearic (nC18:0, S), oleic (nC18:1, O) and linoleic (nC18:2, L) acids, which are present in the varnish as free acids or bound in triglycerides. Linoleic (nC18:2, L) and linolenic (nC18:3, Ln) acids are the major fatty acids in fresh linseed oil, but as may be expected from their high reactivity in the oxidation and polymerization processes of the oil, linolenic is not detected and linoleic is detected in very small amounts. Interestingly, oleic acid is still detected in reference samples after one year of natural ageing whereas it was not detectable anymore after fifteen hours of accelerated photoageing equivalent to five months of natural ageing\(^{26}\). Two dicarboxylic acids, suberic (di-nC8, Su) and azelaic (di-nC9, Az) acids, formed by homolytic cleavage during radical oxidation processes of unsaturated fatty acids, are also detected.

As for colophony, methylated forms of five diterpenic acids are detected. In particular, qualitative identification of sandaracopimaric acid (Sand), abietic acid (Ab), dehydroabietic acid (DHA), 7-oxo-dehydroabietic acid (7-oxo-DHA) and 15-OH-7-oxo-DHA are consistent with compounds reported in the literature using a similar analytical method\(^{13,20}\). A previous article pointed out that thermally assisted methylation can lead to the formation of multiple derivatives from a single compound and induce isomerizations\(^{15,20}\). Within this work, to avoid any misinterpretation, for each of the five diterpenic compounds, we took into account the highest-intensity chromatographic peak which corresponds to the major compound with both the hydroxyl and carboxylic methylated functional groups\(^{15}\).

On the basis of previous articles\(^{3,4,19,28,38}\), the five above-mentioned compounds were selected and considered as markers for evaluating the amounts of diterpenoids and fatty acids as a function of the proportion of linseed oil and colophony.

### Table 1. Fatty acid compounds identified in the reference varnishes with five different oil/colophony proportions, and in the historical varnish. Peaks are numbered according to their elution time. Check mark indicates detected compound (above the detection limit).

<table>
<thead>
<tr>
<th>Peak number/abbreviation</th>
<th>Compound</th>
<th>Mw (methylated)</th>
<th>Tr (min)</th>
<th>Linseed oil/colophony proportion</th>
<th>Historical varnish</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Octenoic acid</td>
<td>156</td>
<td>9.10</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>Octanoic acid</td>
<td>158</td>
<td>9.61</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>Nonanoic acid</td>
<td>172</td>
<td>11.13</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>4/Su</td>
<td>Octanedioic acid</td>
<td>202</td>
<td>14.22</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>5/Az</td>
<td>Nonanedioic acid</td>
<td>216</td>
<td>15.52</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>6</td>
<td>Decanedioic acid</td>
<td>230</td>
<td>16.90</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>7</td>
<td>Tetradecanoic acid</td>
<td>242</td>
<td>18.08</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>8</td>
<td>Pentadecanoic acid</td>
<td>256</td>
<td>19.63</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>9</td>
<td>Hexadecanoic acid or isomer</td>
<td>268</td>
<td>20.83</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>10/P</td>
<td>Hexadecanoic acid or isomer</td>
<td>270</td>
<td>21.55</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>11</td>
<td>Heptadecanoic acid</td>
<td>284</td>
<td>23.46</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>12/O</td>
<td>Octadecanoic acid or isomer</td>
<td>296</td>
<td>24.92</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>13</td>
<td>Octadecanoic acid or isomer</td>
<td>296</td>
<td>25.05</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>14/S</td>
<td>Octadecanoic acid</td>
<td>298</td>
<td>25.44</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>15/L</td>
<td>Octadecadienoic acid</td>
<td>298</td>
<td>26.70</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>20</td>
<td>Eicosanoic acid</td>
<td>326</td>
<td>29.44</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>28</td>
<td>Docosanoic acid</td>
<td>354</td>
<td>30.44</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>31</td>
<td>Tetracosanoic acid</td>
<td>382</td>
<td>31.44</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

![Figure 2](https://example.com/figure2.png)  
Figure 2. Pyrograms obtained from (a) the reference varnish film with a 50/50 (wt%) linseed oil/colophony proportion and (b) the historical varnish. Numbered peaks are listed in Tab. 1 and Tab. 2. (NI): compounds of which chemical structures were not identified.
Table 2. Diterpenoids identified in the reference varnishes with five different oil/copal proportion, and in the historical varnish. Peaks are numbered according to their elution time. Check mark indicates detected compound (above the detection limit).

<table>
<thead>
<tr>
<th>Peak number/ abbreviation</th>
<th>Compound</th>
<th>Max. (methylated)</th>
<th>Ti (min)</th>
<th>Linseed oil/ copal proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>9,10-seco-dehydroabietic acid or isomer</td>
<td>316</td>
<td>26.21</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>16</td>
<td>9,10-seco-dehydroabietic acid or isomer</td>
<td>316</td>
<td>26.61</td>
<td>X</td>
</tr>
<tr>
<td>18/Sand</td>
<td>Sandaracopimaric acid</td>
<td>316</td>
<td>28.37</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>19/IsoP</td>
<td>Isopimaric acid</td>
<td>316</td>
<td>28.95</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>21</td>
<td>Methyl 6-dehydrodehydroabietic acid or isomer</td>
<td>312</td>
<td>29.57</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>22/DHA</td>
<td>Dehydroabietic acid</td>
<td>314</td>
<td>29.64</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>23</td>
<td>7-Methoxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10 a-octahydrophenanthrene-1-carboxylic acid</td>
<td>302</td>
<td>30.25</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>24/Ab</td>
<td>Abietic acid</td>
<td>316</td>
<td>30.62</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>25</td>
<td>Methyl 6-dehydrodehydroabietic acid or isomer</td>
<td>312</td>
<td>31.29</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>26</td>
<td>Methyl 6-dehydrodehydroabietic acid or isomer</td>
<td>312</td>
<td>31.50</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>27/15-OH-DHA</td>
<td>15-methoxy-dehydroabietic acid</td>
<td>344</td>
<td>32.40</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>29/7-oxo-DHA</td>
<td>7-oxo-dehydroabietic acid</td>
<td>328</td>
<td>34.17</td>
<td>XX XX XX XX XX</td>
</tr>
<tr>
<td>30/15-OH-7-oxo-DHA</td>
<td>15-hydroxy-7-oxo-dehydroabietic acid</td>
<td>372</td>
<td>34.47</td>
<td>XX XX XX XX XX</td>
</tr>
</tbody>
</table>

4.2 Qualitative analysis of a 16th century varnish film

The pyrogram of the historical varnish film is characteristic of a drying oil/Pinaceae resin system (Fig. 2b). As for fatty acids, we identified methyl esters of palmitic, azelaic and suberic acids.

The palmitic/stearic ratio was calculated to 1.43 which strongly corresponds to values obtained for linseed oil with similar analytical method14. The azelaic/palmitic and suberic/palmitic ratios were calculated to 0.66 and 0.30, respectively. Typical markers of Pinaceae resin, sandaracopimaric, isopimaric, dehydroabietic and 7-oxo-dehydroabietic acids were also detected. Peak assignments lead to the identification of a drying oil admixed to a diterpenic resin, probably a Pinaceae one.

As expected in such centuries-old sample, linolenic, linoleic, oleic as well as abietic acid are not detected, because of their well-known reactivity in the ageing of drying oils and colophony respectively. Interestingly, the 15-OH-7-oxo-dehydroabietic acid is not detected in this four hundred years-old varnish, whereas previous works mentioned that it is a highly oxidized product of colophony ageing. Two hypotheses can be suggested: this compound is present in a lower quantity which cannot be detected with our method or it is only an intermediate compound towards still undescribed further ageing mechanisms.

4.3 Semi-quantitative analysis of the reference one year old varnish films

Until now, interpretation of chromatographic analyses of diterpenoids in Pinaceae resins mostly relied on the detection or not of molecules usually associated to various states of oxidation, after various artificial ageing protocols, and in historical (several centuries old) samples. More recently, the GC/MS analysis of a collection of materials from an early 18th century chemist’s cabinet illustrated that conservation conditions and physical form of samples could have a strong influence on the relative intensities of ageing markers39. Given the chemical state of the resin material, markers corresponding to the different oxidations states could either be detected as major components, minor components or not detected. Van den Berg selected four markers (Ab, DHA, 7-oxo-DHA and 15-OH-7-oxo-DHA), each corresponding to an oxidation state of the material, and normalized the sum of their peak areas to 100%. This allowed to assess the oxidation state of the material from a sequence of four numbers19 and, later, from an “Index for the Degree of Oxidation” called IDOX, a linear combination of these four normalized peak areas20.

Our research is an adaptation of this method and aims to suggest a semi-quantitative approach. With this aim, we selected ten compounds as markers: five for colophony (sandaracopimaric acid, abietic acid, dehydroabietic acid, 7-oxo-dehydroabietic acid and 15-OH-7-oxo-dehydroabietic acid) and five for linseed oil (oleic acid, stearic acid, palmitic acid, azelaic acid and suberic acid. For each sample, the area of each compound was divided by that of the internal standard and the value obtained was then adjusted by a factor corresponding to the relative content of oil (in the case of the fatty acids and dicarboxylic acids), or the relative content of resin (in the case of terpenoid compounds), and finally divided by the sample weight. Each semi-quantitative value likely obtained is represented as a function of the proportion of colophony (Fig. 3).

The relative standard deviation (RSD, based on four injections) of the internal standard area is calculated to 10.2%. This low value indicates that the Py(TMAH)-GC/MS developed method which required sample of a minimal quantity of matter from a varnish on an artifact, under a stereomicroscope, has a good reproducibility.
of natural ageing. In general, these higher RSD may be explained by a "matrix effect".

For these ten markers, with an exception for oxidised colophony compounds, 7-oxo-DHA and 15-OH-7-oxo-DHA, standard deviations are acceptable, considering the high areas variations caused by the pyrolysis injection (see 7-oxo-DHA and 15-OH-7-oxo-DHA in Fig. 3). The higher values obtained for 7-oxo-DHA and 15-OH-7-oxo-DHA account for the heterogeneity within each varnish film and could be enhanced by the low amounts of these diterpenoids after only one year of natural ageing. As for linseed oil markers, peak areas of palmitic and stearic acids (see P and S in Fig. 3) follow the same variation from one reference varnish to another and are only representative of the inherent variability of injections. The palmitic/stearic ratio is constant at 1.58 ± 0.15 independently of the proportions of linseed oil and colophony (see P/S in Fig. 3). This result reinforces
that P/S ratio is still relevant to determine the vegetal origin of the drying oil even in such mixtures, where these molecules are weakly and/or similarly reactive.

Oleic acid is still detectable in reference varnishes after one year of natural ageing, and its signal increases with the proportion of colophony (see O and O/P in Fig. 3). The higher amount of oleic acid in varnishes containing high proportions of colophony (60% and 75%) suggests that the autoxidation of unsaturated fatty acids occurring within the linseed oil is slowed down by colophony compounds.

Regarding the suberic (see Su and Su/P in Fig. 3) and azelaic (see Az and Az/P in Fig. 3) acids, the results, despite the quite high relative standard deviations, suggest that they are formed in lesser amounts for the highest colophony proportion (75%) which would reinforce the previous suggestion. Such a correlation has already been observed on photo-aged oil samples. The Az/P values are slightly superior to those obtained for three month old-linseed oil films.

As for colophony compounds, it is highlighted that the more colophony in the mixture, the higher the amount of abietic acid, sandaracopimaric acid and dehydroabietic acid (see Ab, Sand and DHA in Fig. 3). As previously recalled, abietane-type diterpenoids such as abietic and dehydroabietic acids are supposed to oxidise over time leading to dehydroabietic acid and subsequently to 7-oxo-dehydroabietic acid. It is then likely that these compounds are less present in mixtures with high content of oil because they reacted to form oxidised compounds.

Contrary to abietane-type diterpenoids, pimarane compounds such as sandaracopimaric acid initially present in fresh colophony are not expected to form oxidised compounds. Though they should be less prone to polymerization than abietane-type diterpenoids, cross-linked oligomeric structures could be formed from the reaction of their separated double bonds. This can be a possible explanation for the low amount of sandaracopimaric in low-colophony varnishes.

As a consequence of the oxidation of abietic and dehydroabietic acids, we would have expected to see the amount of 7-oxo-dehydroabietic acid and 15-0H-7-oxo-dehydroabietic acid to decrease with the proportion of colophony in the mixture (see 7-oxo-DHA and 15-0H-7-oxo-DHA in Fig. 3). However, it is very difficult to discuss these evolutions because of the high relative standard deviations. The small amount of fresh diterpenic compound (abietic and sandaracopimaric acids) and first-stage oxidation compound (dehydroabieticacid) in mixtures with a high proportion of oil raises questions about the sole use of these markers to assess the degree of oxidation/ageing of resinous fraction in oil varnishes. It also suggests that linseed oil has an activating role in the oxidation reactions occurring on colophony compounds.

This study shows that the amount of the less oxidised compounds (abietic, sandaracopimaric, dehydroabietic and oleic acids) in oil/colophony mixtures increases with the colophony proportion. It might be worth noting that colophony admixed to linseed oil in a film has an influence on the curing and degradation of the oil, as pigments could have in an oil-based paint layer. Conversely, linseed oil has an influence on the ageing of colophony. We calculated the IDOX from our data set as defined by van den Berg (see IDOX in Fig. 3). This indicates that for identical ageing time and conditions, the oxidation state of the resin compounds in varnishes can be very different, depending on the oil proportion.

**4.4 Possible phenomena and mechanisms specific to linseed oil/colophony mixtures**

Our results indicate that compounds of linseed oil and colophony evolve differently in the binary system oil/colophony than separately. The modification of the molecular compositions of each of those is influenced by the presence of the other and as chemical phenomena may be considered as possible factors explaining it, even if, obviously, further specific studies should be necessary to evidence them.

One of the two materials may act as a photo-absorbing agent, which would then minimize photo-initiated mechanisms involved in the evolution of the other material. Absorptions of abietane-type diterpenoids present maxima up to 280 nm whereas fatty acids absorb photons up to 250 nm and fresh drying oil films up to 300 nm.

One hypothesis could be that the conjugated double bonds of the diterpenoids would react preferentially to those of the oleic acid if colophony reaches a given amount, and/or that colophony plays an antioxidant role in linseed oil. This hypothesis is consistent with the lower calculated amounts of azelaic and suberic acids at high colophony proportion. These acids arise from the unsaturated fatty acids in linseed oil, among which the oleic acid. So, the higher the amount of oleic acid, the lower the expected amount of azelaic and suberic acids. Likewise, the relative amount of DHA could be linked to the medium reactivity, and thus to the respective proportions of linseed oil and colophony in the binary system.

This would suggest that a competition among reaction mechanisms or among specific compounds could play a role depending on the composition stoichiometry of the reference varnishes.

These results question the methods usually used for characterising oil/colophony varnishes that are based on a qualitative search for specific degradation markers. Similarly, the influence of the Az/P ratio may modify the criteria that have been used so far for the attribution of the vegetal origin of oil and its discrimination from egg yolk.

**4.5 Towards Py(TMAH)-GC/MS quantitation**

The Py(TMAH)-GC/MS semi-quantitative method developed and applied to micro-samples from the reference varnishes allows to go beyond qualitative method applied up to now on such mixtures. Data obtained suggest that the reaction mechanisms involved in the process of drying, oxidation and degradation are highly related to the proportions of linseed oil and colophony within the varnishes. This result provides new insights into molecular evolution of linseed oil/colophony. To our knowledge, this aspect has never been published so far. To go further in the
understanding of the chemical mechanisms involved in the evolution of films containing drying oils and Pinaceae resin and thus to better assess the reactivity between carboxylic acids and diterpenoids, it seems necessary, in next steps, to track the quantities of the various compounds which appear or disappear during the film drying and ageing by performing molecular quantitation using a Py(TMAH)-GC/MS method we aim to develop.

5 Conclusion

This paper presents the first stage of results obtained in the process of optimising a single-step TMAH-Assisted Py-GC/MS method, in an approach going towards the qualitative analysis of markers in mixture of drying oil with a Pinaceae resin that are frequently encountered in coating of musical instruments, furniture, various decorative art objects as well as in easel paintings.

This research performed with the aim to better understand the molecular ageing of coating based on oil/resin highlights the variability of the semi-quantitative composition of samples of the same age and kept under the same conditions. Results obtained suggest that linseed oil has an activating role in the oxidation reactions occurring on colophony compounds, and that conservation state of varnishes could be closely linked to the proportion of resin in the mixture. Questions of the relevance of previously frequently used markers to assess the state ageing of such varnishes and of the need to reconsider conservation strategies are thus raised.

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