e-PRESERVATIONScience

e-PS, 2014, **11**, 64-75
ISSN: 1581-9280 web edition
ISSN: 1854-3928 print edition

e-Preservation Science (e-PS)

is published by Morana RTD d.o.o.
www.Morana-rtd.com

PRELIMINARY INVESTIGATION OF THE CHEMICAL COMPOSITION OF EUROPEAN LACQUERS USING PYROLYSIS GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Steven Saverwyns[1], Marc Vermeulen[1], Emile van Binnebeke[2]

SCIENTIFIC PAPER

This paper is based on a presentation at the sixth international meeting of the Users'Group for Mass Spectrometry and Chromatography (MASC) in Pisa, Italy, 5th - 6th June 2013.

Guest editors:
Klaas Jan van den Berg, Ilaria Bonaduce, Ester Ferreira, Ken Sutherland, David Peggie and Chris Maines

1. Royal Institute for Cultural Heritage (KIK/IRPA), Laboratory Department, Jubelpark 1, 1000 Brussels, Belgium

2. Royal Museums of Art and History (KMKG/MRAH), European Sculpture and Furniture, Jubelpark 10, 1000 Brussels, Belgium

corresponding author:
steven.saverwyns@kikirpa.be

received: 15/12/13
accepted: 30/01/2014

key words:
European lacquer, pyrolysis gas chromatography-mass spectrometry (Py-GC/MS), tetramethylammonium hydroxide (TMAH), natural resins, marker compounds, cross-sections

Abstract

The organic constituents of two pieces of furniture with European lacquer from the collection of the Royal Museums of Art and History (Brussels, Belgium) were examined with pyrolysis gas chromatography-mass spectrometry in combination with derivatisation with tetramethylammonium hydroxide (TMAH-Py-GC/MS). Using marker compounds, complex mixtures of oils and (hard) resins were identified. Cross-sections permitted to study the stratigraphy of the lacquer system, a prerequisite for sampling individual lacquer layers. Because of the limited thickness of some of the layers and their visual resemblance, individual sampling of layers was not always possible, and this complicated the interpretation of results. Selected results of this study, that forms the pilot study of an interdisciplinary research project focusing on the technological evolution of European lacquers, are presented together with the advantages and disadvantages of the analysis protocol applied.

1 Introduction

Lacquerware is created by applying lacquer - the sap from several species of trees within the Anacardiaceae family, genus *Rhus* - to wooden objects to give it a fine finish and lustre. It has been used to protect and decorate a wide variety of objects: furniture, household items of all kinds, musical instruments, weapons, and even buildings.[1]

It is generally acknowledged that the techniques involved were developed in China and Japan over 4000 years ago. The use of European lacquers, however, can be proved to be developed only fairly recently. The first oriental lacquered objects were dispersed into Europe via the famous Silk route, but in very limited amounts and their existence was not widely spread known. Extensive trade with China via shipping routes only started as early as the 16th century. With well-established ports in Bruges, Antwerp and Ghent, Flanders became in the 16th century an important transit region.[2] Quickly the demand for these luxury Oriental Lacquers exceeded supply. As the export of the raw Oriental lacquer material - the lacquer sap - was forbidden and initially the technique of coating was not understood, local craftsmen applied their own skills and techniques to produce lacquers using familiar materials, i.e. Western resin-based varnishes. Since the 17th century many pieces of European furniture and other products were "japanned" or "lacquered" using Western resins and techniques.[1,3,4] These pieces are valued in their own right, but are intrinsically different from Oriental Lacquer, and labelled *European lacquers*. Although the basic raw materials used differ strongly - lacquer in Asia and natural resins in Europe - the final aspect of the European lacquers resembles often closely that of their Oriental equivalent. The European lacquers remained popular until the end of the 19th century, after which they slowly went out of fashion, and were gradually replaced by synthetic polymers in the 20th century. A selection of japanned ware has in time entered numerous museum collections, and can, amongst others, be found in the collections of the Royal Museums of Art and History (RMAH) in Belgium.

The quest to imitate Oriental lacquer has resulted in an immense collection of recipes, describing not only the materials used, but also the practical aspects of producing European lacquer. In spite of, or maybe just because of this vast number of recipes and hence variations in composition and technology, often little is known about the technical and chemical aspect of japanned ware in (Belgian) museum collections.

From a chemical point of view, European lacquers are superimposed layers of varnishes with complex composition based on a mixture of natural resins, oils, solvents, additives, pigments and/or fillers.[1] Their identification forms a major analytical challenge. Not only the simultaneous presence of several (hard-drying) resins impedes the analyses, but also chemical degradation as a consequence of varnish preparation and of natural ageing adds to the complexity of analysis. Different chemical processes may occur at the same time, making the prediction of the deterioration of European lacquer much more difficult than for Oriental lacquer. In order to preserve japanned objects in museum collections, to understand the materials used in the lacquer preparations and to study their degradation, it is necessary to obtain data on the chemical composition of the lacquers.

Main ingredients of European lacquer are drying oils, essential oils or alcohols and mixtures of vegetal resins such as sandarac, copal resins, amber, mastic, benzoin, and others.[1] One resin exuded by an insect - shellac - is mentioned also in some recipes. Most natural resins are complex mixtures of neutral and acidic mono-, sesqui-, di- or triterpenoid components.[5] The distinction between different resins is usually made based on the presence of some molecules that are considered to be chemical markers for natural resins. Especially markers present in aged resins play an important role in their identification, as drying and ageing through mainly oxidation processes alter the original composition of the resins.[6] In some cases markers are fairly unique and permit the straightforward identification of the resin, in other cases only variations in relative quantities of certain compounds are observed. Especially in aged resin mixtures the identification of the individual resins present can be complicated.

Resins and varnishes have been studied by different analytical techniques including infrared and Raman spectroscopy.[7-12] More detailed information is obtained by hyphenated techniques in which often gas chromatography (GC) is coupled to mass spectrometry (MS) in order to combine the high separation power of GC with the high sensitivity and specificity - through detailed information on the molecular composition of the resins - provided by the MS.[13-16] Besides the classic sample introduction as liquids, pyrolysis (Py) forms an interesting alternative to introduce samples to the GC. Solid samples can be analyzed directly, without bringing them into solution, by breaking them down in smaller molecules through flash heating of the sample.[17-21] Especially hard resins difficult to dissolve, such as amber or copal, may profit from a solid introduction of the sample in the analysis system. The volatility of the compounds can be improved by simultaneously carried out derivatization reactions. Thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) is in that context one of the more popular protocols for the analysis of natural resins with Py-GC/MS.[17,22-26]

Although natural resins and varnishes related to cultural heritage objects have been studied by Py-GC/MS, European lacquers have been largely neglected. The aim of this work is investigating the chemical composition of some European lacquers from the collection of the RMAH, mainly with TMAH-Py-GC/MS as analysis technique. The focus is placed on two japanned object from the collection of the RMAH: a writing desk (no inventory number) and a writing desk with drawers (inventory number V.0015). The art-historical information on the two objects is rather limited. The construction of the writing desk and the materials used, such as the hinges and screws, seem to point to a construction date somewhere around the third quarter of the 19th century. Based on the style of the writing desk with drawers, it should date from the second half of the 19th century. Besides Py-GC/MS analyses, samples of the japanned objects are also studied in their stratigraphic context, a prerequisite in order to carry out layer-by-layer sampling, necessary for accurate TMAH-Py-GC/MS analysis of the lacquers. Results are completed by inorganic analyses with scanning electron microscopy in combination with energy dispersive X-ray detection (SEM-EDX) and micro-Raman spectroscopy (MRS), while synthetic colorants were identified with high-performance liquid chromatography in combination with diode array detection (HPLC-DAD). Results from this pilot study are presented, and used to evaluate TMAH-Py-GC/MS for the future study of European lacquers in an interdisciplinary context.

2 Experimental

2.1 Instrumentation and methodology

The main analytical technique used during this study is pyrolysis gas chromatography-mass spectrometry (Py-GC/MS). A Curie-point pyrolysis unit (Pyromat, GCGMess- und Analysengeräte) was directly mounted on the split/splitless injector of a TraceGC gas chromatograph (Thermo), in turn hyphenated with a PolarisQ Ion Trap mass spectrometer (Thermo). Pyrolysis is carried out at 625 °C during 10 s in a helium atmosphere. The injector of the chromatographic system was kept at 250 °C. Separations were accomplished on a SLB-5ms capillary column (Supelco, 20 m x 0.18 mm i.d. x 0.18 µm film thickness) applying following temperature program: initially the oven temperature was maintained at 50 °C for 1 min during split injection (split ratio 8). Next, a 50 °C min^{-1} gradient was applied until 100 °C, followed by a 5 °C min^{-1} gradient until 180 °C; finally the column was heated to a temperature of 320 °C at a rate of 10 °C min^{-1}; this temperature was maintained during 5 min. Carrier gas was helium at a constant flow of 1.3 mL min^{-1}. Ionization was carried out in the ion volume of the ion trap mass spectrometer under the standard EI positive mode at 70 eV. The mass spectrometer was scanned in the 35-500 amu range, with a cycle time of 0.5 s.

Cross-sections were prepared by mounting the sample in between two poly(methyl methacrylate) (PMMA) cubes: the sample is placed on one cube and covered with freshly prepared and still liquid PMMA resin, followed by placing the second cube on the sample covered with liquid PMMA, and gently pushing. After curing of the resin, which takes about 15 minutes, the embedded sample is polished using Micro-Mesh papers up to 12000 grit. The cross-sections were

observed with an optical microscope (Zeiss Axioplan), using polarized white light and UV-light (excitation bandpass filter from 390 to 420 nm, beamsplitter at 425 nm and emission lowpass filter from 450 nm).

The inorganic composition of the European lacquers was determined by analyzing the cross-sections with a scanning electron microscope coupled to an energy-dispersive X-ray detector (SEM-EDX, Jeol 6300 and Si[Li]) detector Pentafet, Oxford Instruments). As with SEM-EDX only information on the elemental composition of the pigments is obtained, additional measurements were carried out by a dispersive micro-Raman instrument (Renishaw inVia) providing molecular information on the pigments. A diode laser at 785 nm (Innovative Photonic Solutions) in combination with a 1200 l mm^{-1} grating was used for all measurements. The Raman signal was registered by a Peltier cooled (203 K) NIR enhanced deep depletion CCD detector (576 x 384 pixels). Cross-sections were analysed with the aid of the Leica DMLM microscope at a magnification of 500 or 1000x. Laser power was reduced to values between 0.1 and 1 mW to avoid damage to the samples. Identification of the Raman spectra was done by comparison with in-house compiled reference libraries.

The identification of synthetic organic colorants present in some lacquers was carried out by high-performance liquid chromatography (HPLC) in combination with diode array detection (DAD). The sample was dissolved in a mixture of HCl/H_2O/MeOH 2/1/1 (v/v/v) and 10 min heated at 105 °C. After a purification step, and re-dissolution in $MeOH/H_2O$ 1/1 (v/v) 20 µL of this solution was injected onto the temperature controlled column (end capped LiChrosorb RP-18, 125 mm x 4 mm i.d., 5 µm particle size, 100 Å pore diameter, VWR-Merck). Applied solvents are pure methanol as solvent A (grade: for HPLC > 99.8%, from Acros Organics) while solvent B is made of methanol and MilliQ water (ASTM Type I, resistivity: 18 MΩ.cm and TOC < 5ppb, Waters) in the volumetric ratio of 1/9. Solvent C is prepared by the use of 55 mL of phosphoric acid (85 wt% pro analisi, Acros Organics) to which 945 mL Milli-Q water is added. The HPLC analysis is performed with the use of the following gradient: 0-3 minutes: isocratic 23A/67B/10C, 3-29 minutes: linear gradient to 90A/0B/10C, 30-35 minutes: isocratic 23A/67B/10C; elution programme at a constant flow rate of 1.2 ml min^{-1}; creating a system back-pressure of 1600 psi. An Alliance HPLC instrument with automatic injection is applied from Waters Chromatography BV. The mobile phase is degassed by an online vacuum degasser. The PDA detector (PDA model 996, Waters, USA) uses 512 diodes, scanning the absorbance within the wavelength range between 200 and 800 nm, with a resolution of 1.2 nm with 1 scan s^{-1}. The applied software system for data treatment of the chromatographic analyses is Empower 2 (Waters, USA). Interpretation of the results is carried out using reference spectral libraries.

Finally protein analyses were carried out according to a procedure described by Schilling *et al.* [27], based on the hydrolysis of proteins to amino acids with 6M HCl (Sigma-Aldrich) during 24h at 110 °C and the subsequent derivatisation of the amino acids with ethyl chloroformate (98,0% GC, Fluka) to obtain compounds volatile enough to be analysed with GC/MS. The same chromatographic system as earlier described was used, but with 1 µL liquid injections, and an adjusted temperature program: the column is heated from 70 °C until 180 °C at a speed of 10 °C min^{-1} and then to 280 °C at 40 °C min^{-1}. The final temperature of 280 °C is maintained for 1 min. A mass spectrometer (PolarisQ, Thermo) detects the amino acids (scanning of the masses between 50 and 500 amu). Proteins are identified by interpretation of the amino acid composition data.

2.2 Samples

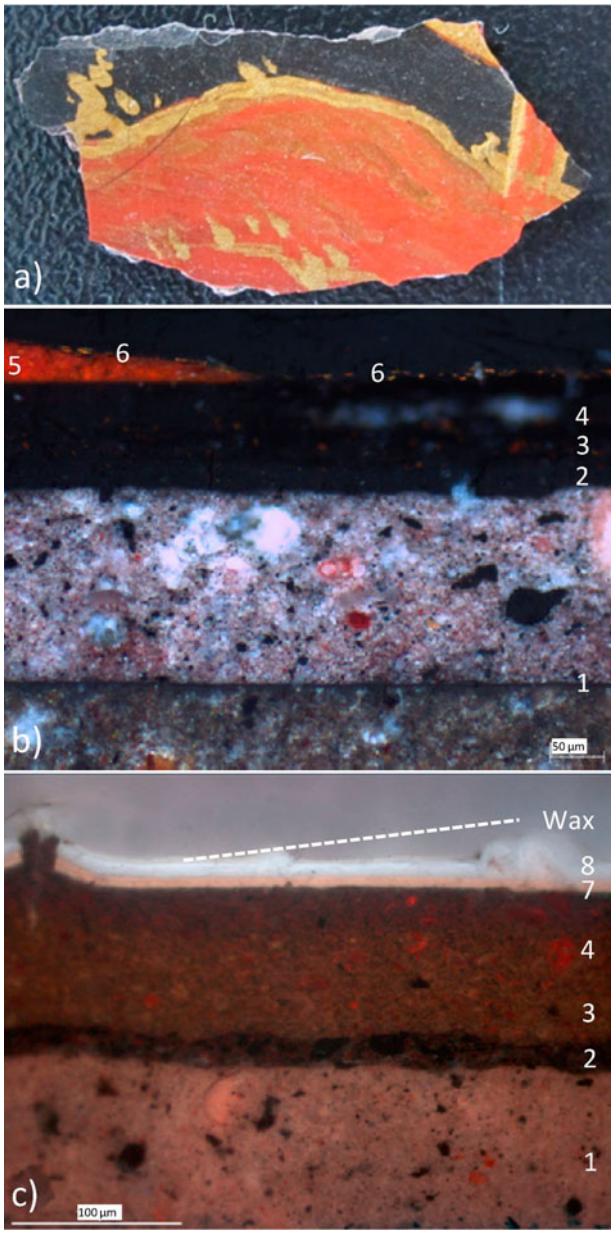
Two pieces of furniture from the collection of the RMAH - a writing desk (no inv. nr.) and a writing desk with drawers (inv.nr. V.0015) - were chosen as pilot objects to be studied into detail (figure 1), with the emphasis on the identification of the composition of the individual lacquer layers superimposed to create the illusion of an Oriental lacquer. Only limited information is available on the objects, but stylistically both

Figure 1: The two pieces of furniture with European lacquer that form the subject of this study: a) writing desk and b) writing desk with drawers.

were dated in the second half of the 19th century. Both the pieces of furniture were at the moment of sampling kept in the reserve of the RMAH. Because of space restrictions in the reserve, the in-situ study of the lacquered layers and sampling them individually was not possible. The objects however were quite damaged, and flakes of samples were collected permitting the close observation of the layer built-up and subsequent sampling under an optical microscope or binocular. The exact location of the samples was hence not known, but based on observations of the samples and the pieces of furniture, attribution to a possible location became possible. For each piece of furniture only one sample was available big enough to permit sampling of the individual lacquer layers. Other smaller samples could be used for cross-sections giving additional information on the layer structure. These cross-sections were also used for identification of inorganic compounds, both with SEM-EDX and micro-Raman spectroscopy.

2.3. Experimental procedure

Once the samples collected, they were taken to the laboratory. In a first stage they were carefully observed under the optical microscope to check if they were complete; the most representative ones were chosen for further analysis. A small selection was used to prepare cross-sections in order to have an idea of the general layer built up of the pieces of furniture. From two bigger samples, one from each piece of furniture, a small chunk was cut off with a scalpel and transformed into a cross-section. This cross-section aided in identification of the different layers that were as much as possible sampled layer by layer by scraping with a scalpel with rounded blade from the remainder of the sample. All scrapings were carried out under a stereomicroscope using both visible and UV light. Because of the limited thickness of some varnish layers (< 5 µm) and/or their visually similar aspect, individual sampling of all layers was not feasible. The scrapings were transferred to glass vials with conical shape, to which 10 µL of 2,5% tetramethylammonium hydroxide (TMAH) in methanol (prepared from 25% wt in methanol; Sigma-Aldrich) was added. The small samples were gently crunched with a glass rod; 2 µL of this



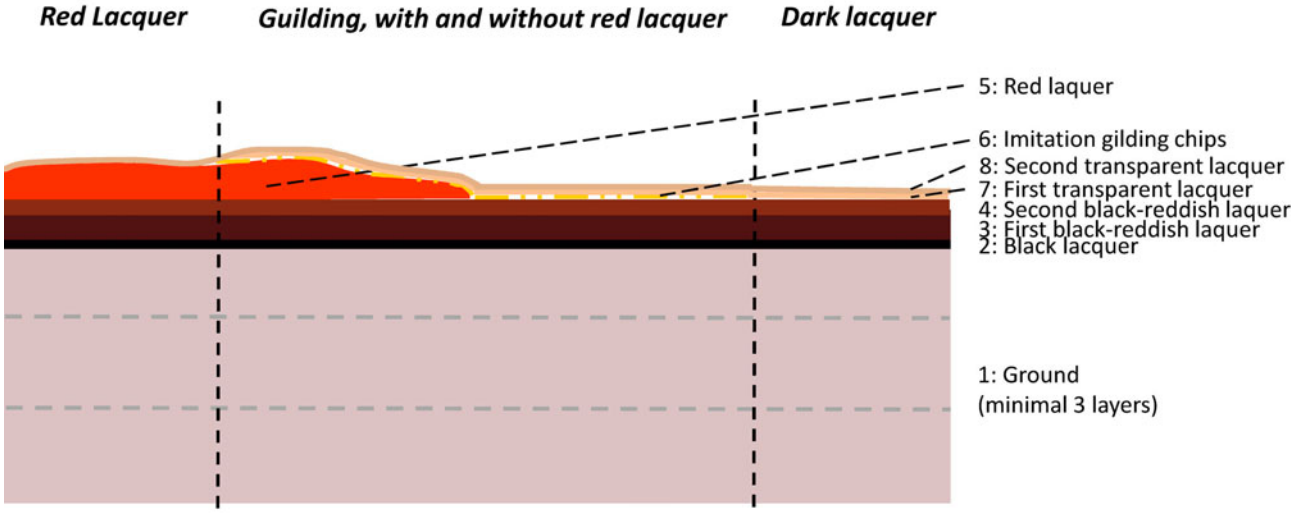


Figure 2: a) A piece of lacquer (approx. 9 mm x 5 mm) from the writing desk, of which a small part at the intersection between the red and black lacquer was used to prepare the cross-section b) Detail of the cross-section showing the transition between the red and black lacquer under white light (100x magnification) c) Other detail of the cross section showing the stratigraphy of the black lacquer under UV-light (200x magnification) d) Schematic representation of the layer structure.

solution or suspension was brought on the pyrolysis wire and after drying at room temperature inserted in the pyrolysis oven, after which the samples were analysed under instrumental conditions earlier described. The GC/MS results obtained on the lacquers were interpreted using NIST and previously in-house compiled mass spectra databases, and other published mass spectra results, as well as fingerprinting with reference materials and (unidentified) mass spectra.

3 Results and discussion

3.1 Writing desk

3.1.1 Stratigraphy and inorganic composition

Different cross-sections prepared from samples of the writing desk allow a clear understanding of the layer structure. A typical flake of sample collected can be seen in figure 2a. The sample is composed of black lacquer, which is decorated with red lacquer. Both lacquer layers are partially finished with gold paint. A smaller part of the flake, at the intersection between the black and red lacquer, was used to prepare a cross-section; the transition between the red and black layer can clearly be seen on the images of the cross-section (figures 2b and 2c). A schematic representation to ease reading of the cross-section is made combining observations on different cross-sections from the same object (figure 2d).

As the wooden support is not present in the cross-sections, only estimates can be made about the thickness of the ground layers. The ground layer was applied in three thick layers (only the upper layers are visible in the cross-sections), with a total minimal thickness of 750 µm. For the identification of the inorganic compounds throughout this study SEM-EDX was used, complemented with additional micro-Raman measurements in case the elemental information obtained by SEM-EDX did not allow the accurate identification of the pigment. Analyses of the ground layers point to the use of chalk mixed with earth pigments (red, yellow and black earths), a carbon-based black pigment and a small amount of zinc white or zinc sulphide (under the conditions used, MRS could not give further information on which of the two zinc compounds is present). These layers are followed by a relatively thin (15-20 µm) black lacquer layer, rich in an iron containing pigment, probably a black earth, next to a lower amount of red earth. This thin black layer is followed by two layers with a very dark aspect under white light, but with a reddish tonality under UV-light, and practically not distinguishable from each other. SEM-EDX in combination with MRS analyses prove the presence of chalk and barium sulphate, zinc white or zinc sulphide and some earth pigments in low concentration. Possibly also some carbon based black pigment is present. The presence of white pigments in such dark layers is striking. Chalk and barium sulphate can however also be used as substrate for synthetic organic dyes or pigments. Indeed, during the preparation of the cross-sections a reddish colorant seemed partially to dissolve in the liquid embedding resin (PMMA), something which only rarely occurs, and points to the use of synthetic dyes. In the description of the organic part of the composition of these layers more informa-

Writing desk			
Layer number	**Layer description**	**Layer composition (pigments, gilding and dyes)**	**Lacquer composition (organic materials)**
8	Transparent lacquer*	-	Colophony, drying oil(?), benzoin(?)
7	Transparent lacquer*	-	Shellac, drying oil(?), benzoin(?)
6	Imitation gilding	Brass (Cu, Zn)	-
5	Red lacquer	Red earth (Fe), chrome yellow (Cr), zinc white or zinc sulphide (Zn), barium sulphate (Ba, S),	Colophony, heated (tung) oil, African/Congo copal, dammar
4	Black-reddish lacquer+	Chalk (Ca), barium sulphate (Ba, S), zinc white or zinc sulphide (Zn), earth pigments (Fe), carbon-based black pigment (pitch/tar?), acid red 23, acid orange 7, basic violet 3	Colophony, heated (tung) oil, African/Congo copal, dammar
3	Black-reddish lacquer+	Chalk (Ca), barium sulphate (Ba, S), zinc white or zinc sulphide (Zn), earth pigments (Fe), carbon-based black pigment (pitch/tar?), acid red 23, acid orange 7, basic violet 3	
2	Black lacquer+	black earth (?; Fe), red earth (Fe),	
1	Ground	chalk (Ca), earth pigments (Fe), zinc white or zinc sulphide (Zn), carbon-based black pigment,	Heated (linseed) oil, African/Congo copal, colophony, benzoin(?), dammar(?)
Writing desk with drawers			
Layer number	**Layer description**	**Layer composition (pigments and gilding)**	**Lacquer composition (organic materials)**
9	Transparent lacquer	-	Heated linseed oil, Asian copal, colophony, benzoin, dammar
8	Gold foil	Alloy of gold (Au), silver (Ag) and copper (Cu)	-
7	Yellow mordant	Lead white (Pb), chrome yellow (Cr, Pb)	-
6	Mass	Lead white (Pb), chalk (Ca), earth pigments/synthetic Fe_2O_3 (Fe)	Heated linseed oil, Asian copal, colophony, benzoin, dammar
5	Transparent lacquer	Lead (as siccative) (Pb)	Heated linseed oil, Asian copal, colophony, benzoin, dammar
4	Dark lacquer	-	Heated linseed oil, Asian copal, colophony, benzoin, dammar
3	Red lacquer°	Red earth (Fe), vermillion (Hg, S)	
2	Red-orange lacquer°	Red earth (Fe), vermillion (Hg, S), chrome yellow (Cr, Pb), chrome orange (Cr, Pb), traces of bone black (Ca, P) and lead white (Pb)	
1	Ground	Chalk (Ca)	Walnut (?) oil and semi-drying oil, colophony, beeswax and a hydrocarbon wax, benzoin, animal glue (collagen)

Table 1. Summary of the composition of the different layers of the two objects studied. In between brackets the elements detected by SEM-EDX are given; results of the pigment analyses were further refined by MRS analyses. Dyes were analysed by HPLC-DAD; the composition of the lacquers finally was determined by Py-GC/MS. The symbol (*,+,°) next to some layers, indicate which layers were analysed together (according to symbol) towards lacquer composition.

tion is given on the dyes as identified by HPLC. The red lacquer layer is applied directly on the top dark layer, without an intermediate transparent lacquer. Different pigments were identified including red earth, chrome yellow, zinc white or -sulphide and barium sulphate. The possible presence of organic dyes in the red lacquer could not be confirmed due to lack of sufficient sample to carry out an HPLC analysis. The red lacquer is in parts decorated with a paint imitating gold, composed of brass (copper-zinc alloy) flakes. These flakes are enclosed by a transparent lacquer. On top of this lacquer, a second transparent lacquer, with orange appearance under UV-light, has been applied. This lacquer seems to be only present on the imitation gold decoration, and directly on the black lacquer; on the red lacquer itself it seems to be absent. Finally a transparent lacquer is applied on the complete surface. These last transparent lacquers have each a thickness in the order of 5 to 10 µm. An overview of the composition of each layer analysed is given in table 1.

3.1.2 Organic composition

The dark layers (black-reddish under UV-light) could not be separated from each other as visibly almost no difference was observed between them. Because of the bleeding of a red colorant into the liquid PMMA resin when preparing the cross-sections, it was assumed the reddish-black layers contained synthetic organic dyes, which were analysed using HPLC-DAD. At least three different synthetic dyes could be identified: acid red 23 (monoazo dye, colour index (CI) 16130), acid orange 7 (monoazo dye, CI 15510) and basic violet 3 (triarylmethane dye, CI 42555). Since these dyes are synthetic, their dates of discovery are well known and give information of an earliest possible creation date of the writing desk. The different dyes identified were discovered respectively in 1873, 1876 and 1883, in agreement with a creation date of the second half of the 20th century as was presumed from stylistic observations.

The emphasis of the research was however placed on the resinous composition of the lacquer layers. Due to the limited thickness of some layers, or because of their too similar aspect, not all layers could be sampled individually. This forms a restriction in the current research as composition might differ from one lacquer layer to another. Four different samples could be taken by scraping: the transparent top lacquers, the red lacquer, the black lacquers (composed of the two dark black-reddish layers containing the synthetic dyes and the thinner black layer on top of the ground layers) and the lower ground layer.

The total ion chromatogram (TIC) of the transparent lacquers is reported in figure 3. Table 2 lists the marker compounds, indicated with numbers in the chromatogram, used to identify the resins present in the varnish samples. The chromatogram is dominated by two peaks, unidentified, but characteristic for aged shellac[22] as also shown by the analysis of naturally aged shellac samples from the KIK/IRPA reference collection. The latter part of the chromatogram shows the typical pattern of beeswax: a series of saturated fatty acids with an even number of carbon atoms, maximizing around FA-C24:0, alternating with odd linear hydrocarbons, in combination with a relatively high FA-C16:0 (palmitic acid) concentration. The presence of even linear hydrocarbons suggests also the presence of a hydrocarbon wax. It is believed that these waxes are not original but originate from later polishing of the writing desk with wax containing compounds to increase the gloss of the objects. Methyl esters of fatty acids of siccative oils and their characteristic degradation products, fatty diacids and other degradation products, govern the first part

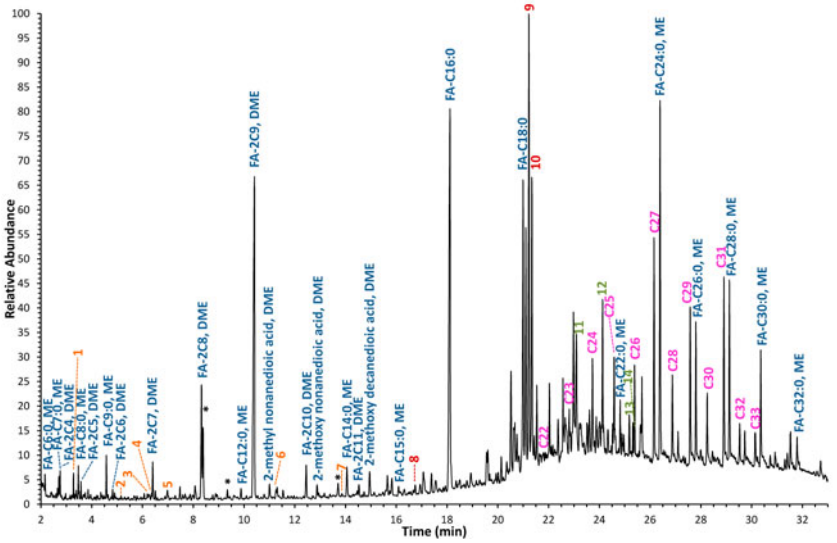


Figure 3. TIC of the transparent lacquers. Fatty acids, fatty diacids and hydrocarbons identified are indicated in the chromatogram; numbers point to resin markers summarised in table 2; * indicates contamination with modern phthalates. FA-Cx:y indicates a fatty acid with x the number of carbon atoms and y the number of unsaturated bonds. FA-2Cx points to fatty diacids with x the number of carbon atoms. Cx finally stands for linear hydrocarbons with x number of carbon atoms. ME stands for 'methyl ester', DME for 'dimethyl ester'.

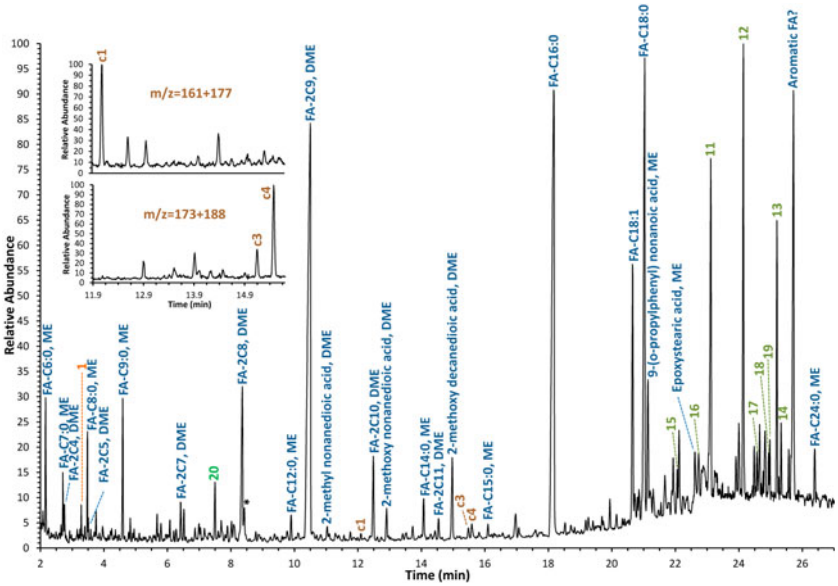


Figure 4. TIC of the red lacquer from the writing desk, with the inset showing markers for African/Congo copal (selective ion chromatogram, plotted masses mentioned).

Marker compounds	Peak number	Retention time (min)	Characteristic ions (m/z)*
Benzoin[22]			
Benzoic acid, ME	1	3.28	51, 77, 105, **136**
4-methoxy-benzaldehyde	2	5.09	63, 77, 92, 135, **136**
Cinnamic acid, ME	3	6.23	51, 77, 103, 116, 131, **162**
3-methoxy-benzoic acid, ME	4	6.35	63, 77, 92, 107, 135, **166**
4-methoxy-benzoic acid, ME	5	6.98	63, 77, 92, 107, 135, **166**
3,4-dimethoxy-benzoic acid, ME	6	11.25	65, 79, 94, 107, 125, 137, 153, 165, **196**
3,4,5-trimethoxy-benzoic acid, ME	7	13.86	124, 155, 195, 211, **226**
Shellac[22]			
Butolic acid, methyl ether, mehyl ester	8	16.74	67, 83, 95, 127, 159, **272**
Unidentified marker	9	21.22	77, 91, 131, 159, 187, 229, 247
Unidentified marker	10	21.34	77, 91, 131, 159, 187, 215, 244, 247
Colophony[6]			
Methyl dehydroabietate (DHA ME)	11	23.09	197, 239, 299, **314**
7-oxo-dehydroabietate (dimethylated derivative)	12	24.12	95, 187, 209, 227, 267, 283, 327, **342**
7-oxo-dehydroabietate, ME	13	25.18	187, 211, 253, 268, 296, **328**
15-hydroxy-7-oxo-dehydroabietate (trimethylated derivative)	14	25.32	73, 141, 225, 265, 281, 297, 313, 325, 340, 357, **372**
Sandaracopimaric acid, ME	15	22.05	79, 91, 105, 121, 180, 241, 258, 290, 301, **316**
Isopimaric acid, ME	16	22.71	105, 121, 241, 256, 301, **316**
15-hydroxy-dehydroabietate, methyl ether, methyl ester	17	24.57	203, 273, 269, 313, 329, **344**
15-hydroxy-7-oxo-dehydroabietate (dimethylated derivative)	18	24.93	225, 265, 281, 325, 340, **372**
7-oxo-dehydroabietate (trimethylated derivative)	19	24.98	201, 230, 241, 281, 341, **356**
Dammar[35]			
Unidentified marker	20	7.50	39, 55, 67, **83**, 96, 114, 139, 154, 182, 199, 215
Africa/Congo copal[24]			
Unidentified marker	c1	12.10	67, 79, 91, 105, 161, 177, **236**
Unidentified marker	c2	14.00	91, 105, 119, 133, 145, 161, 173, 189, **248**
Unidentified marker	c3	15.15	91, 105, 119, 175, 191, 235, **250**
Unidentified marker	c4	15.47	91, 105, 119, 133, 145, 173, 188, 248
Eperuic acid, ME	-	21.92	41, 81, 95, 177, 191, 305, **320**
Asian copal24			
Unidentified marker	b1	11.33	79, 91, 105, 119, 161, 176, 204, 221, **236**
Unidentified marker	b2	13.20	91, 133, 145, 159, 173, 189, **248**
Unidentified marker	b3	14.31	119, 175, 191, 235, **250**
Unidentified marker	b4	14.48	91, 119, 133, 173, 188, **248**

* The use of an ion trap mass spectrometer as detector can result in the formation of slightly different ion fragments, with difference in relative intensity, in comparison to the more standardly used quadrupole mass spectrometer.

Table 2. List of TMAH-Py-GC/MS marker compounds used for the identification of different natural resins found in the lacquers analysed. References in the table point to publications where the marker compounds (and their mass spectra) are further discussed.

of the chromatogram. Those oils can in general be identified based on the FA-C16:0/FA-C18:0 (palmitic/stearic acid; P/S) ratio, but in the presence of beeswax this is no longer feasible. The report of the di-acids azeleic to suberic acid (FA-2C9/FA-2C8 = 4.0) provides information of the preheating of the sample. The relatively low value obtained suggests that the oil has been heated.[24,28] In the beeswax region also compounds formed upon oxidation of abietic acids are detected: methyl esters of dehydroabietic acid and 7-oxo-dehydroabietic acid, as well as the methyl ester, methyl ether form of 7-oxo-dehydroabietic acid. These compounds are markers for aged diterpenic Pinaceae resins, such as colophony. In the first part of the chromatogram also some compounds in relatively small concentration are detected that are considered to be markers for benzoin: methyl esters of benzoic acid, cinnamic acid, 3-methoxy benzoic acid, 4-methoxybenzoic acid and 3,4-dimethoxy benzoic acid.[22] Summarised, these different top transparent layers contain following compounds: a drying oil, shellac, colophony (markers for other Pinaceae resins such as Venice turpentine were not detected), benzoin, beeswax and a hydrocarbon wax. Close observation of a cross-section from a black part of the writing desk suggests the presence of three thin finishing layers (figure 2c), and permits to make some assumptions on the composition of the individual layers. The lowest layer of the varnishes under consideration, applied on top of the black-reddish lacquer layers, produces an orange fluoresce when observed under UV-light. This is a typical fluorescence behaviour of shellac, implying that the shellac compound found in the TMAH-Py-GC/MS analysis is situated in this lower layer. As mentioned before, waxes are not listed in the old recipes as ingredients of lacquers. Therefore the assumption is made that the waxes found are not original, but come from a later intervention, and are most logically found on top of the object (not represented in the schematic drawing, as considered as being non-original). In between the shellac layer and these wax-containing layers probably the colophony is situated. From the Py-GC/MS results and the observation of the cross-section, no information could be deduced which layer, or maybe both lower layers, contain drying oil or benzoin. Only sampling of individual layers, or additional measurements directly on the cross-section, with e.g. attenuated total reflection - Fourier Transform Infrared spectroscopy (ATR-FTIR) might give more detailed information on the composition of the individual layers, but this falls outside the scope of the actual pilot study. Shellac has mistakenly often been mentioned as a key ingredient in European lacquers, because its hardness with a degree of elasticity, high gloss, and some abrasive resistance makes it an ideal lacquer resin.[29] It produces however no clear transparent lacquer, and hence it was not used on white, green or blue japanning.[1] Here it seems to be only present on the imitation gilding and the black lacquer, two zones where the darker colour of shellac is

of no or lesser importance.

The second sample of the writing desk analysed towards organic composition, is the red lacquer, of which the TIC is given in figure 4. A large number of peaks, especially in the diterpenes area, can be distinguished. Oxidised abietic acid compounds are identified (see also table 2), indicating the presence of colophony. Again fatty acids and fatty diacids are found, next to some other degradation compounds of drying oils (such as hydroxyl dicarboxylic acids). An aromatic fatty acid was identified as the methyl ester of 9-(o-propylphenyl) nonanoic acid. This compound can be formed upon heating of a triply unsaturated conjugated system, such as eleostearic acid.[30,31] Tung oil, or Chinese wood oil, is composed of about 80% of eleosteraic acid, and because of its good properties as drying oil it has been used for centuries as principal component of finishes for wooden objects.[32] The presence of 9-(o-propylphenyl) nonanoic acid in relatively high concentration could hence point to the use of heated tung oil. Epoxystearic acid is an oxidised intermediate in drying oils, but can also indicate heating of an unsaturated oil.[33,34] The oil can have been heated to pre-polymerise it, which leads to much faster drying times, or it was necessary to heat the oil to dissolve hard resins in it. Many lacquer recipes mention the use of hard resins, such as copal, sandarac or amber, as these are required to obtain a hard lacquer that can be polished in order to obtain a smooth and high gloss surface. The preparation of such lacquers requires harsh heating treatments. The hard resins are heated until they melt, and then slowly added to oil heated to temperatures above 300 °C. Carefully searching the chromatogram lead to the identification of compounds considered to be markers for African/Congo copal (see inset figure 4 and table 2). Compounds initially present such as eperuic acid, copalic acid and ozic acid react to a large extent away during the preparation and ageing of the African/Congo copal lacquer, especially when the resin has been subjected to severe heat treatment in combination with drying oil.[28] Only smaller dicyclic reaction products of labdanes related to ozic acid can still be found in low concentration, making use of selective ion chromatograms as shown in the inset of figure 4. These molecules, labelled c1 to c4 in accordance with the publication of van den Berg and co-workers[24], can survive to some extent the harsh manufacturing, oxidative drying and ageing processes, and are considered markers for copal resins. A trace of eperuic acid (t_R= 21.92) can be found as well. Other compounds usually found in fresh copal can no longer be detected.[26] Although the evidence for heating of the oil is quite clear, and the presence of copal explains the reason for this, the FA-2C9/FA-2C8 ratio is rather high (5.5), and also the unsaturated fatty acid FA-C16:1 (oleic acid) is still present in high concentration. This implies that probably the oil/copal resin mixture has been diluted with non- or slightly heat-bodied oil. Finally a marker compound for aged dammar was identified.[35] As only one marker for benzoin was found no conclusions could be drawn on its presence in the sample.

The third sample from this lacquered object concerns the dark black-reddish layers, together with the thin black layer. The composition of these layer(s) is very similar to the one of the red lacquer, although relative concentrations vary slightly. The concentration of oleic acid (in comparison with stearic acid) seems to be lower, and also the value of FA-2C9/FA-2C8 = 3.5 indicates that, besides the presence of a heat-bodied oil, a smaller amount of (or even no) non- or slightly heat-bodied oil is added. A marker for retene has been observed, but only in trace concentrations. This points to the presence of tar or pitch[36], and might form an explication for the dark colour of these layers besides the previously identified mixture of synthetic organic dyes.

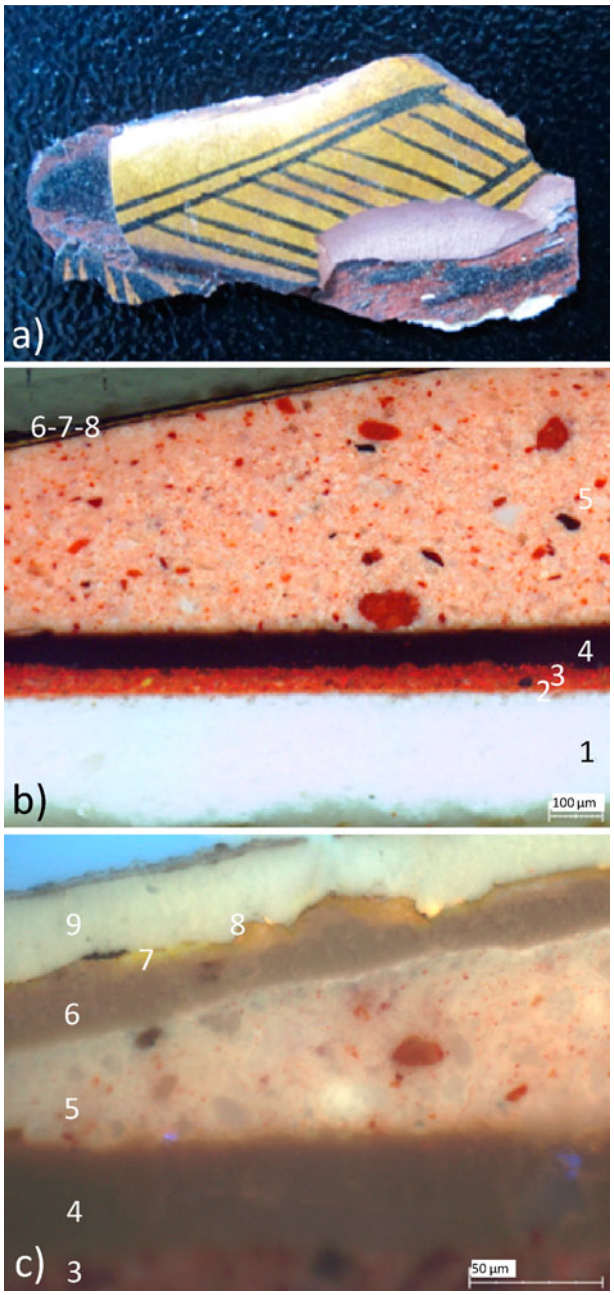
The lower ground layer is the last of these successive layers analysed. The chromatogram is dominated by fatty acids and fatty diacids, as well as diterpenic compounds. The FA-C16/FA-C18 ratio of 0.8, as well as the low FA-2C9/FA-2C8 ratio of 2.8, together with an important concentration of epoxystearic acid clearly indicates a heat-bodied oil, probably linseed oil. Markers for African/Congo copal are noted, giving a possible explanation for the heating of the oil. Diterpenes identified all originate from abietic acids, and are formed during the preparation and ageing of colophony varnish. Finally a trace amount of benzoin and some dammar might be present. In this layer no tung oil (or another oil rich in triply unsaturated conjugated fatty acids) could be detected.

From these analyses it can be concluded that the organic composition of the pigmented lacquer layers is quite similar for all layers. All lacquer layers contain colophony and African/Congo copal, mixed with a heat-bodied oil (likely tung oil), further adulterated with non- or slightly heat-bodied oil. Also the ground layer(s) show a similar composition as the coloured lacquers, with the exception that heated linseed oil was used (without adulterating the oil). Likely some dammar and benzoin are added to modify the lacquer characteristics.

3.2 Writing desk with drawers

3.2.1 Stratigraphy and inorganic composition

The sample from the writing desk with drawers discussed, originates from a raised decoration. On the finished black lacquer, filled lacquer is added to create a three-dimensional structure, illustrated by the cross-section in figures 5a en 5b. At least three ground layers, with a total thickness between 150 and 200 µm form the basis of the lacquerware. All three are composed of chalk, but in the last two layers the chalk is more finely grinded than in the first lower layer. On top of the ground layers, two red lacquer layers are applied, very similar in colour. The lower of the two layers contains besides red earth and vermillion also chrome yellow and chrome orange, and some traces of bone black and lead white. The upper red layer is mainly composed of red earth and vermillion alone. These layers are followed by a relatively thick (ca. 75 µm) black organic layer, the basic black lacquer layer of the japanned writing desk with drawers. It is not clear if this layer was applied as such, or is composed of many thinner layers with similar composition. No black pigments could be identified in this layer with SEM-EDX or micro-Raman spectroscopy. A pink mass is applied on this dark layer, with a thickness between 0.5 and 1 mm (in the part sampled), and coloured with lead white, chalk and earth pigments or synthetic iron oxide. Both the pink mass and the black basic layer are

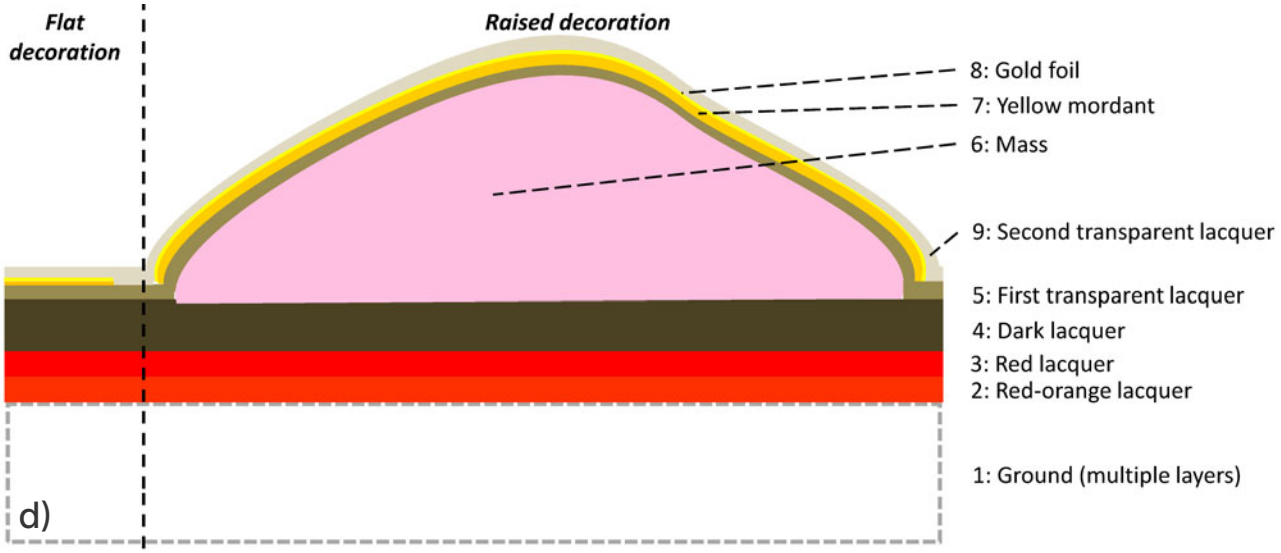


covered with an organic layer, with some lead as siccative. In cases gilding is applied, both for flat or raised areas, a yellow layer, mainly composed of lead white and chrome yellow, follows this rather transparent organic layer. It serves as mordant for the gold gilding (alloy of gold, silver and copper). A second transparent organic lacquer covers this gilding as well as the first transparent layer for parts where no gilding is applied. A final layer, likely dirt disposal, can be seen in the cross-section. A schematic overview of the layer built-up is given in figure 5d (not showing the last dirt layer). The presence of synthetic chrome based pigments points to a 19th century (or later) creation date, which agrees with a creation date of the second half of the 19th century, as was presumed on observations of the style.

3.2.2 Organic composition

Again it was not feasible to sample all layers individually. Six different samples were taken for the identification of the organic compounds: the transparent lacquer on the gilding, the transparent lacquer covering the mass, the mass itself, the dark lacquer layer, both red lacquer layers and the ground layers. The composition of all layers, with the exception of the ground layers, was very similar. The sample available was very limited, especially for the transparent lacquer layers. The chromatogram of the pink mass is given as being representative for all lacquer layers (figure 6). Fatty acids and fatty diacids indicate the presence of a drying oil. Based on the ratio of FA-C16:0/FA-C18:0 (1.3) and FA-2C9/FA-2C8 (2.7) the oil was identified as heated linseed oil. Markers for copal resins are present and become clear when plotting characteristic marker ions (selective ion chromatogram; inset in figure 6). In this case the dicyclic reaction products, labelled b1-b4 according to the publication by van den Berg *et al.* [24], can be related to communic acid, and hence to Asian copal. The presence of sandarac was ruled out as typical markers for this resin - hydroxy- and acetoxy sandaracopimaric acids - could not be found. Asian copal

Figure 5: a) A piece of lacquer (approx. 8 mm x 4 mm) from the writing desk with drawers, of which a small part was used to prepare the cross-section b) Detail of the cross-section under white light (100x magnification) c) Other detail of the cross section under UV-light (500x magnification) d) Schematic representation of the layer structure.



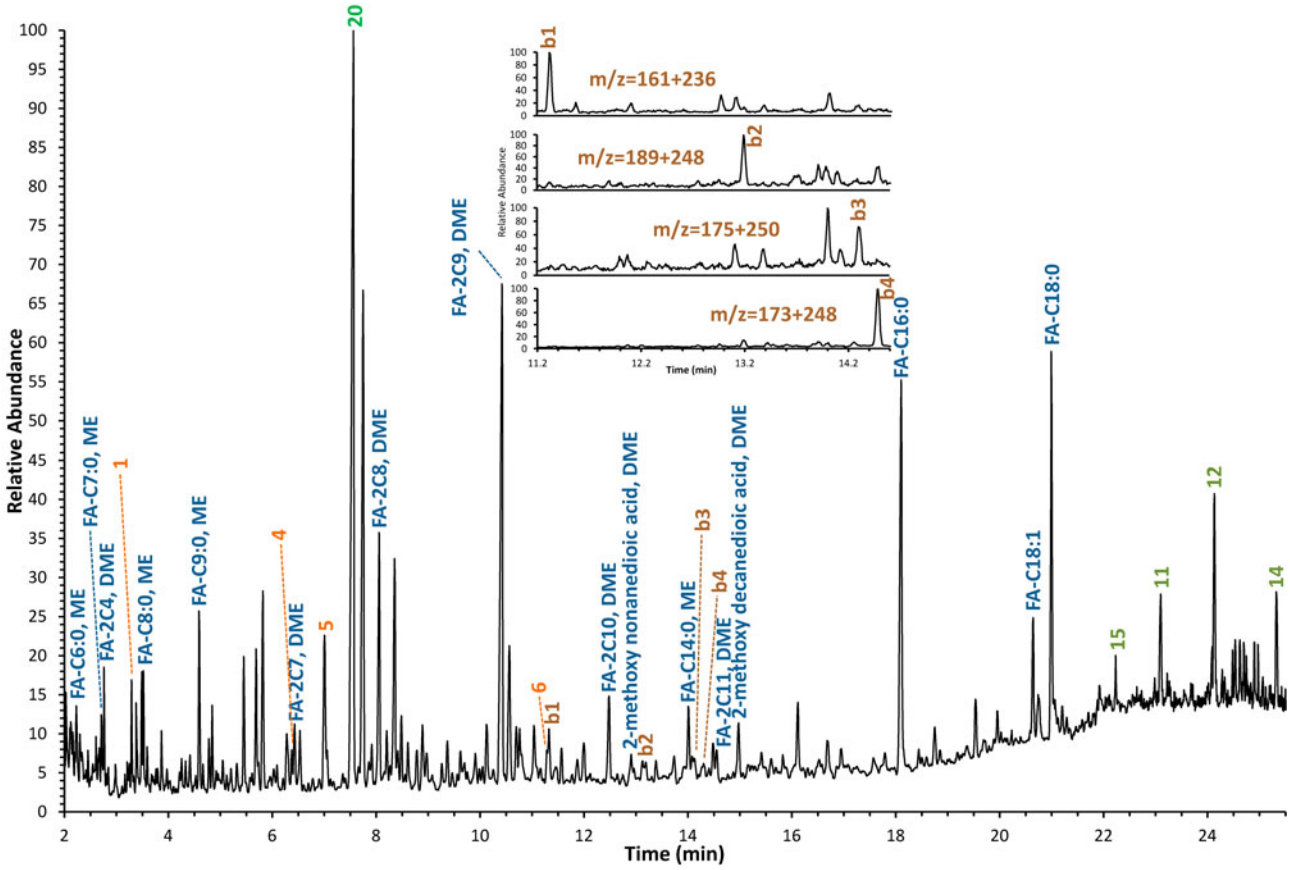


Figure 6. TIC of the pink mass from the writing desk with drawers, with the inset showing markers for Asian copal (selective ion chromatogram).

seems to be present in all layers sampled, but because of the often very limited sample size, not all dicyclic markers were found; often only the b1 and b4 markers were identified. Methyl esters of dehydroabietic acid and 7-oxo-dehydroabietic acid, and other markers mentioned in table 2, point to the presence of a Pinaceae resin, typically colophony. Some markers for benzoin in relatively high concentration are also present (table 2). Around 7.5 minutes some compounds are detected that could be related to aged dammar. As no triterpenes are detected further analysis of aged dammar reference samples is required to confirm this.

The results of the analysis of the ground layers are deviating from the ones of the other layers. Again fatty acids and fatty diacids indicate the presence of oil, but the high FA-C16:0 and FA-C18:0 levels are striking. The FA-2C9/FA-C16 ratio (0.3) is too low to indicate a pure drying oil, for which this ratio is usually higher than 1. The FA-C16:0/FA-C18:0 ratio (2.4) on the other hand can point to walnut oil, although other oils cannot be excluded. In the higher temperature region of the chromatogram longer chain fatty acids can be detected, in a low concentration, maximising around FA-C24:0, and alternating with odd linear hydrocarbon chains, a typical pattern for beeswax. Beeswax is known to contain high levels of FA-C16:0 (and sometimes FA-C18:0)[37], but the low concentration in which it seems to be present cannot alone account for the high levels of FA-C16:0 and FA-C18:0, so the presence of an as yet not identified (semi)drying oil is assumed. The chromatogram shows also some even hydrocarbons indicating the presence of a second wax in low concentration, besides characteristic markers for benzoin and colophony. Finally markers for proteins are detected in the chromatogram. The presence of proteins was confirmed by amino acid analysis using GC/MS as described in the experimental section. The protein source was identified from amino acid molar percentages by correlation of the so-called seven stable amino acids to published amino acid composition data as described by Schilling *et al.*[38] The amino acid molar percentages calculated (alanine: 12.1; valine: 2.1; isoleucine: 1.4; leucine: 2.9; glycine: 57.6; proline:12.3, hydroxyproline: 11.5; all values expressed as mole%) showed a good correlation (correlation coefficient 0,99) with collagen (animal glue). No copal was detected in these layers. As the ground is composed of three layers that could not be sampled separately, because of the too similar aspect of the individual layers, it is not clear if all layers have the same composition as elucidated here, or that differences between the ground layers occur. As waxes are not mentioned in the historic literature as being an essential part of European lacquers, they more than likely originate from later restoration actions, which contaminated the ground layers (taken into consideration that the writing desk with drawers is heavily damaged). Quite some peaks in the chromatogram with some important ones around the aged dammar marker, could not be identified. Detailed analyses with TMAH-Py-GC/MS of aged reference samples are required to allow identification of the source of these (pyrolysis) compounds, foreseen in the near future.

4 Conclusion

The study of cross-sections from European lacquerware, in combination with the organic analysis of (indi-

vidual) lacquer layers with TMAH-Py-GC/MS permitted to give a detailed image of the construction of two japanned objects from the collection of the Royal Museums of Art and History, Brussels, Belgium. This study formed the pilot study of a larger research project and permitted to pin point weaknesses and strengths in the applied analysis protocol. The TMAH-Py-GC/MS method is capable of analysing a whole range of compounds at once with detailed information on resins, waxes and oils, and indications on the presence of proteins, even when mixed together as often the case with European lacquers. Retrieving all information from one single chromatogram is however often labour intensive and time-consuming. The use of markers characteristic for aged resins (and other compounds) plays a crucial role in the final elucidation of the lacquer composition. In the upcoming project efforts will be undertaken to have the chromatogram screened automatically on most of the compounds mentioned in traditional recipes, making use of specific markers. The possibilities of double shot pyrolysis will be evaluated, in order to separate more volatile compounds from less volatile or polymeric ones, which might ease the interpretation. In double shot pyrolysis the sample is first heated to a lower temperature than the actual pyrolysis temperature. Upon heating of the sample the more volatile compounds are thermally desorbed, and analysed separately from the less volatile or polymeric ones. In a second step (and after the first chromatographic run of the desorbed compounds) the same sample is undergoing flash pyrolysis. As the analysis of the copal containing lacquers have shown, the way the lacquer is prepared has a tremendous influence on the (marker) compounds finally present in the lacquer. Lacquer systems will be prepared based on old recipes and artificially aged in order to be able to follow degradations that occur when preparing the lacquers and upon artificial ageing. The appearance of specific markers might lead to more information on the production process of European lacquers. As performing TMAH-Py-GC/MS may be, difficulties in sampling thin individual layers, with often visually similar aspect, hamper the interpretation of results, as often contamination with adjacent layers occurs. Use of a new generation of digital microscopes with long working distance, might permit sampling under high magnification, reducing the risk of sampling several layers at once. Finally TMAH-Py-GC/MS results can be completed by additional binding medium analysis on cross-sections using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR), giving indications of (organic) composition of the lacquer system in a stratigraphic context.

The analytical study of the lacquers can also give a new impetus to art-historical research. Very limited historical information was available on the two studied pieces of furniture. The fact that in both cases oil varnishes were used allowed classifying them more than likely as post 18th century, as these kinds of lacquers were only produced by then.[1] Pigment analysis permitted to further refine results as synthetic inorganic and organic compounds were found that were not in use until the last quarter of the 19th century. These results are in agreement with a creation date of the second half of the 19th century, as assumed on stylistically observations.

It is our hope that the thorough research on European lacquers, on an art-historical, technological and chemical level, of which some of the results of a pilot study are discussed in this paper, will deepen our insight in the technological history of European lacquers. The emphasis of the future research will be placed on objects made in Belgium (and surrounding areas) between the 18th and 20th century.

5 Acknowledgements

The authors would like to thank Marie-Christine Macquoi for the analysis of the synthetic dyes and the proteins, and Louise Decq for the preparation of the cross-sections (both from the Laboratories Department of the Royal Institute for Cultural Heritage). Our gratitude also goes to Henk van Keulen from the Cultural Heritage Agency of the Netherlands (RCE) for kindly sharing his list of reference markers on natural resins and a wide range of other organic materials, and the fruitful discussions on the chromatographic results. Finally the Belgian Science Policy is greatly acknowledged for partially funding this research through the brain.be project "European lacquer in Context: art-historical, technological and chemical characterisation of European lacquer in Federal collections".

6 References

1. M. Webb, *Lacquer: Technology and Conservation: a Comprehensive Guide to the Technology and Conservation of Asian and European Lacquer*, Butterworth-Heinemann, Oxford, 2000.

2. W. De Kesel, G. Dhont, *Flemish 17th century lacquer cabinets*, Stichting Kunstboek, Oostkamp, 2012.

3. T. Wolvesperges, *Le meuble français en laque au XVIIIe siècle*, Editions Racine, Brussels, 2000.

4. M. Kühlenthal, Ed., *East Asian and European Lacquer Techniques*, Bayerisches Landesamt für Denkmalpflege, Munich, 2000.

5. J.S. Mills, R. White, *The organic chemistry of museum objects*, Butterworth-Heinemann, Oxford, 2nd ed., 1994.

6. K.J. van den Berg, J.J. Boon, I. Pastorova, L.F.M. Spetter, *Mass spectrometric methodology for the analysis of highly oxidized diterpenoid acids in Old Master paintings*, J. Mass Spectrom., 2000, **35**, 512-533.

7. H. Khanjian, M. Schilling, J. Maish, *FTIR and Py-GC/MS investigation of archaeological amber objects from the J. Paul Getty museum*, e-Preserv. Sci., 2013, **10**, 66-70.

8. R.H. Brody, H.G.M. Edwards, A.M. Pollard, *Molecular and Biomolecular Spectroscopy: A study of amber and copal samples using FT-Raman spectroscopy*, Spectrochim. Acta Part A, 2001, **57**, 1325-1338.

9. D. Lau, M. Livett, S. Prawer, *Application of surface enhanced Raman spectroscopy (SERS) to the analysis of natural resins in artworks*, J. Raman Spectrosc., 2008, **39**, 545-552.

10. P. Vandenabeele, H.G.M. Edwards, L. Moens, *A Decade of Raman Spectroscopy in Art and Archaeology*, Chem. Rev., 2007, **107**, 675-686.

11. S. Prati, G. Sciutto, R. Mazzeo, C. Torri, D. Fabbri, *Application of ATR-far-infrared spectroscopy to the analysis of natural resins*, Anal. Bioanal. Chem., 2010, **399**, 3081-3091.

12. M.T. Doménech-Carbó, *Novel analytical methods for characterising binding media and protective coatings in artworks*, Anal. Chim. Acta, 2008, **621**, 109-139.

13. U. Baumer, P. Dietemann, *Identification of resinous materials on 16th and 17th century reverse-glass objects by gas chromatography/mass spectrometry*, Int. J. Mass Spectrom., 2009, **284**, 131-141.

14. A. Lluveras, I. Bonaduce, A. Andreotti, M. Colombini, *GC/MS Analytical Procedure for the Characterization of Glycerolipids, Natural Waxes, Terpenoid Resins, Proteinaceous and Polysaccharide Materials in the Same Paint Microsample Avoiding Interferences from Inorganic Media*, Anal. Chem., 2010, **82**, 376-386.

15. J.P. Echard, C. Benoit, J. Peris-Vicente, V. Malecki, J.V. Gimeno-Adelantado, S. Vaiedelich, *Gas chromatography/mass spectrometry characterization of historical varnishes of ancient Italian lutes and violin*, Anal. Chim. Acta, 2007, **584**, 172-180.

16. F. Modugno, E. Ribechini, M. Colombini, *Chemical study of triterpenoid resinous materials in archaeological findings by means of direct exposure electron ionisation mass spectrometry and gas chromatography/mass spectrometry*, Rapid Commun. Mass Spectrom., 2006, **20**, 1787-1800.

17. J.M. Challinor, *Review: the development and applications of thermally assisted hydrolysis and methylation reactions*, J. Anal. Appl. Pyrolysis, 2001, **61**, 3-34.

18. G. Chiavari, S. Prati, *Analytical Pyrolysis as Diagnostic Tool in the Investigation of Works of Art*, Chromatographia, 2003, **58**, 543-554.

19. M.T. Doménech-Carbó, A. Doménech-Carbó, L. Osete-Cortina, J. de la Cruz-Canizares, *Characterisation of organic materials in archaeometry and art conservation*, Technol. Artis, 2006, 39-59.

20. D. Scalarone, O. Chiantore, *Py-GC/MS of Natural and Synthetic Resins*, in: M.P. Colombini, F. Modugno, Eds., *Organic Mass Spectrometry in Art and Archaeology*, John Wiley and Sons, Chichester, 2009, 327-361.

21. D. Scalarone, M. Lazzari, O. Chiantore, *Ageing behaviour and pyrolytic characterisation of diterpenic resins used as art materials: colophony and Venice turpentine*, J. Anal. Appl. Pyrolysis, 2002, **64**, 345-361.

22. A. Heginbotham, H. Khanjian, R. Rivenc, M. Schilling, *A procedure for the efficient and simultaneous analysis of Asian and European lacquers in furniture of mixed origin*, in: *15th Triennial Conference New Delhi, 22-26 September 2008: Preprints, ICOM Committee for Conservation*, Allied Publishers, New Delhi, 2008, 608-616.

23. F. Shadkami, R. Helleur, *Recent applications in analytical thermochemolysis*, J. Anal. Appl. Pyrolysis, 2010, **89**, 2-16.

24. K.J. van den Berg, J. Ossebaar, H. van Keulen, *Analysis of copal resins in 19th century oil paints and resin/oil varnishes*, in: R. Van Grieken, K. Janssens, L. Van't Dack, G. Meersman, Eds., *Proceedings of Art2002, 7th International Conference on Non-destructive Testing and Microanalysis for the Diagnostics and Conservation of the Cultural and Environmental Heritage, 2-6 June 2002*, Antwerp, Belgium, 2002.

25. I. Pastorova, K.J. van den Berg, J.J. Boon, J.W. Verhoeven, *Analysis of oxidised diterpenoid acids using thermally assisted methylation with TMAH*, J. Anal. Appl. Pyrolysis, 1997, **43**, 41-57.

26. G. Chiavari, S. Montalbani, V. Otero, *Characterisation of varnishes used in violins by pyrolysis-gas chromatography/mass spectrometry*, Rapid Commun. Mass Spectrom., 2008, **22**, 3711-3718.

27. M.R. Schilling, H.P. Khanjian, L.A.C. Souza, *Gas chromatographic analysis of amino acids as ethyl chloroformate derivatives. Part 1, Composition of proteins associated with art objects and monuments*, J. Am. Inst. Conserv., 1996, **35**, 45-59.

28. K.J. van den Berg, J. van der Horst, J.J. Boon, *Recognition of copals in aged resin/ oil paints and varnishes*, in: *Preprints ICOM Committee ICOM for Conservation 12th Triennial Meeting, Lyon, France, 29 Aug-3 September 1999*, James & James, London, Lyon, 1999, **3**, 855-861.

29. K. Walch, *Baroque and Rococo Transparent Gloss Lacquers. I. The Replication of 'White Lacquers' on the Basis of Historic Sources and Scientific Investigation*, in: K. Walch, J. Koller, Eds., *Lacke des Barock und Rokoko / Baroque and Rococo Lacquers*, Bayerisches Landesamt fur Denkmalpflege, Munich, 1997, 21-52.

30. J.D.J. van den Berg, J.J. Boon, K.J. van den Berg, I. Fiedler, M.A. Miller, *Identification of an Original Non-Terpenoid Varnish from the Early 20th Century Oil Painting 'The White Horse' (1929), by H. Menzel*, Anal. Chem., 1998, **70**, 1823-1830.

31. R.P. Evershed, M.S. Copley, L. Dickson, F.A. Hansel, *Experimental Evidence for the Processing of Marine Animal Products and Other Commodities Containing Polyunsaturated Fatty Acids in Pottery Vessels*, Archaeometry, 2008, **50**, 101-113.

32. F.C. Izzo, *20th Century Artists' Oil Paints: a Chemical-Physical Survey*, PhD thesis, Università Ca' Foscari Venezia, 2010.

33. J. Velasco, O. Berdeaux, G. Márquez-Ruiz, M. C. Dobarganes, *Sensitive and accurate quantitation of monoepoxy fatty acids in thermoxidized oils by gas-liquid chromatography,* J. Chromatogr., 2002, **982**, 145-152.

34. J. Velasco, S. Marmesat, O. Bordeaux, G. Márquez-Ruiz, C. Dobarganes, *Formation and evolution of monoepoxy fatty acids in thermoxidized olive and sunflower oils and quantitation in used frying oils from restaurants and fried-food outlets*, J. Agric. Food Chem., 2004, **52**, 4438-43.

35. H. van Keulen, Cultural Heritage Agency of the Netherlands, oral communication.

36. S. Wei, X. Fang, J. Yang, X. Cao, V. Pintus, M. Schreiner, G. Song, *Identification of the materials used in an Eastern Jin Chinese ink stick*, J. Cult. Herit., 2012, **13**, 446-452.

37. I. Bonaduce, M.P. Colombini, *Characterisation of beeswax in works of art by gas chromatography-mass spectrometry and pyrolysis-gas chromatography-mass spectrometry procedures*, J. Chromatogr. A, 2004, **1028**, 297-306.

38. M.R. Schilling, H.P. Khanjian, *Gas chromatographic analysis of amino acids as ethyl chloroformate derivatives. Part 2, Effects of pigments and accelerated aging on the identification of proteinaceous binding media,* J. Am. Inst. Conserv., 1996, **35**, 123-144.