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Berbers, S.V.J.; Tamburini, D.; van Bommel, M.R.; Dyers, J.

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Historical formulations of lake pigments and dyes derived from lac: A study of compositional variability

Sanne V.J. Berbers[a,b], Diego Tamburini[c], Maarten R. van Bommel[a,b], Joanne Dyer[c,*]

[a] University of Amsterdam, van 't Hoff Institute for Molecular Sciences, Analytical-Chemistry Group, Science Park 904, 1098, XH, Amsterdam, the Netherlands
[b] University of Amsterdam, Faculty of Humanities, Conservation and Restoration of Cultural Heritage, Johannes Vermeerplein 1, 1071, DV, Amsterdam, the Netherlands
[c] The British Museum, Department of Scientific Research, Great Russell Street, WC1B 3DG, London, United Kingdom

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ABSTRACT

"Lac" is a term referring to a pink-red-purple organic colourant derived from an insect and used as a lake pigment or a dye. Although historical sources indicate extensive usage, findings in historical samples remain scarce and are based on the detection of laccaic acids A and B, which are the main colourant molecules in lac.

In this work various lake pigment and dye production methods were investigated, with the aim to explore the compositional variability derived from changing fundamental parameters, such as temperature and pH. Exhaustive research focusing on historical recipes was fundamental in order to create a series of 16 mock-up samples. Preliminary investigations were carried out using colorimetry and photo-induced luminescence imaging to evaluate the differences in colour and luminescence behaviour. Visible-induced visible luminescence imaging (VIVL) in the range c. 540–700 nm was particularly efficient at detecting the presence of included shellac in lac lake pigments. High performance liquid chromatography coupled with an online diode array detector followed by electrospray ionisation and quadrupole time-of-flight detection (HPLC-DAD-ESI-Q-ToF) was ultimately applied to assess the chemical composition of the samples and principle component analysis (PCA) was used to establish correlations between the chemical composition and the production methods.

The results demonstrated that, regardless of the pigment/dye production method, a transfer of shellac components always occurs from the sticklac raw material into the lake pigment or dye. More specifically, although an increase in temperature and alkaline pH during the extraction promote the inclusion of high molecular weight shellac components, a variety of shellac components are also found when mild conditions, such as room temperature and neutral pH, are used. The results obtained for artificially aged lac-dyed textile samples showed that lac colourants are more prone to degradation than shellac components. The use of mass spectrometric detection for the identification of lac in historical objects is therefore recommended, as this technique is able to detect non-colourant molecules, which may be present in the pigment or dye as a result of the production methods.

1. Introduction

Lac is a pink-red-purple organic colourant obtained from sticklac, which is the resinous secretion of some insects belonging to the *Kerriidae* family, most commonly of the genera *Kerria* and *Paratachardina,* which are indigenous to India, Thailand and Cambodia. Sticklac is also the same material used to produce the shellac resin, which is mainly used as a wood finish and varnish. Separation of shellac from the lac dye is based on the different water-solubility of the chemical constituents. Lac dye is composed of a series of water-soluble anthraquinone molecules, of which laccaic acids A and B are present in major quantities, whereas laccaic acids C, D and E are minor components [1,2]. Erythrolaccin and deoxyerythrolaccin are also colourant molecules present in sticklac but, due to their reduced solubility in water, are generally associated with shellac and are responsible for its orange colour [3,4]. Fig. 1 shows the molecular structures of these anthraquinone colourant molecules.

Lac has been used as a dye since the Vedic period (1500–500 BCE) in India [5–7]. References to its use as a textile dye can also be found in classical texts [8–13] with early finds of lac-dyed textiles from 3rd century AD Palmyra, attesting to its use in the ancient Mediterranean region [6,14,15]. However, despite mention of «λακχα» (lakcha, lac) in these ancient texts [13,16], until recently, no instances of its use as a pigment in this period had been found [17]. From the 11th century lac

* Corresponding author.
E-mail address: JDyer@britishmuseum.org (J. Dyer).

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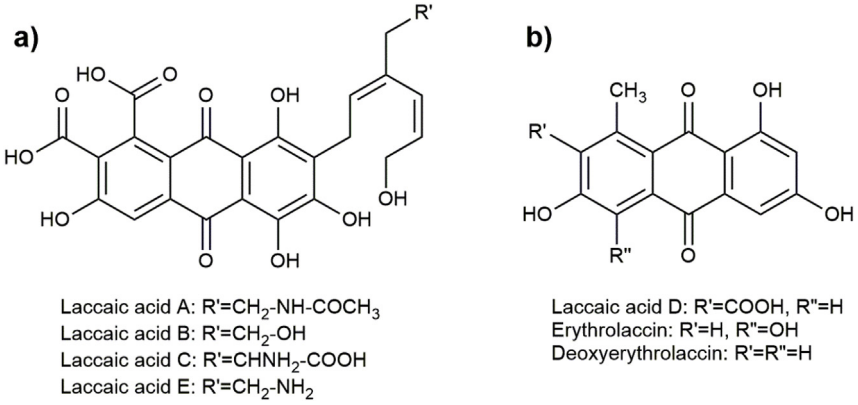

Fig. 1. The molecular structures of the colourant molecules present in sticklac, a) laccaic acid A, B, C and E, b) laccaic acid D, erythrolaccin and deoxyerythrolaccin.

lake pigments were also used for manuscript illumination [4], with recent findings also describing its use in a 12th century Orthodox icon from Cyprus [18]. Yet it was not until the 14th century that lac lakes became commonly used as part of easel painting tradition in Europe, remaining popular until the 17th century [19,20]. However, it is important to bear in mind that data on the historical use of lac lake pigments and lac dye may be skewed by the difficulties encountered in the scientific identification of the colourant in historical objects [21,22].

Shellac is a complex mixture of polyesters of hydroxyl aliphatic and sesquiterpenoid acids [23] and is considered to be insoluble in water in standard conditions (25 °C, pH 7) [3,4]. However, even with modern methods, the complete separation of shellac components from lac dye is not an easy task [2]. Therefore, when cruder historical production methods are considered, it is plausible that shellac components could have been transferred into the colourant matrix when attempting the separation. Historical recipes indicate that the inclusion of resinous components was a known and unwelcome phenomenon in dyeing practices [24], whereas it might have been a desirable feature in pigments for manuscript illumination [20], as this might have afforded improved binding properties, especially before the use of oil-based media was common practice. The possible presence of shellac in pigments has been noted in several studies on organic lake pigments [19,25]. Recent publications have also discussed this possibility in the context of both pigments and dyed textiles [26–28], and shellac components have been proposed as possible markers of lac dye in historical samples [17,19,20,27].

From an analytical point of view, the study of organic colourants can be approached in different ways, mainly depending on whether it is possible and/or permissible to sample an object. Non-invasive techniques, such as fibre optic reflectance spectroscopy (FORS) and broad band multispectral imaging have recently shown their potential for the preliminary identification of dyes [29–34]. However, if chemical information at a molecular level is required, high performance liquid chromatography (HPLC) remains the technique of choice [35–38]. The amount of sample generally needed is very small (2–3 mm of a thread or 100–150 μg of paint). Many organic colourants can be identified using HPLC coupled to a diode array detector (DAD). Despite good sensitivity and reproducibility of results, the main limitation of this method is that it is incapable of detecting colourless molecules. For this reason, the addition of mass spectrometry (MS) as a further detection method is fundamental, not only because it allows for the identification of materials other than dyes (resinous compounds, triglycerides, proteins, etc.), but also because of the ability to detect degradation products of dyes, which can be colourless. HPLC-DAD-MS is therefore extensively applied for the identification of organic colourants in cultural heritage samples [39,40].

In this work HPLC-DAD-MS, supported by colorimetry and observations of luminescence behaviour, was applied to a series of mock-up samples obtained by following different methods of lac dye and pigment production, with the aim of exploring the differences in their chemical composition and to assess the potential of using molecules other than laccaic acids for the identification of lac in historical objects.

2. Historical recipes and applications

The first recipe for lac is found in the *Mappae Clavicula*, a manuscript of which the earliest surviving copies are thought to originate from the 9th century AD, although the text is possibly of Roman origin [41]. It reports a recipe for a lac lake pigment precipitated on alum, and several other recipes describe lac for use in aqueous solution as an unmordanted colourant. However, the first complete recipe for dyeing textiles with lac from European contexts originates from 1548 and was written by Plichto [42]. This may suggest that, until that period, the import of oriental lac-dyed fabrics into Europe was predominant to local dyeing practices using the raw material. This can be corroborated by some of the contemporaneous textile finds [43–48]. Some other historical recipes are available from the 15th to the 18th century, some details of which are summarised in Table 1.

The production of lake pigments from textile extracts is also known to have been a common practice in Europe between the 14th and the 17th centuries, and seemed to have been used primarily for kermes, cochineal and madder. It is thought that lac and brazilwood lakes were more often directly made from the raw dyestuff source [25]. This is supported by the fact that none of the recipes for making lake pigments from textile clippings specifically mention lac-dyed textiles, although some sources indicate that this practice was known [49,50]. However, in his treatise on the arts, thought to be written around the beginning of the 15th century, Cennino Cennini advises against the use of lac lake made from textile clippings, indicating that this method was in use at the time but considered bad practice [51].

The overview given by this collection of recipes highlights four main variables in the different recipes, *i.e.* pH (from neutral to alkaline), temperature (from room temperature to boiling), time of extraction (from minutes to days) and additives (for example urine or ley -potassium carbonate-to adjust the pH). In order to determine the effects of some of these production parameters (in particular pH and temperature) on the final pigment/dye composition, the recipes were translated and adapted into a procedure where a standardised protocol and laboratory grade chemicals are used. This enabled variable parameters to be controlled so that their influence could be selectively studied, and the nature and extent of the sticklac components transferred into the final sample, as a consequence of the production process, could be assessed. The results of these investigations may in turn contribute to more rigorous methods for the identification of lac in historical objects.

Table 1
A chronological overview of various historical recipes for lac dyes and pigments. The main ingredients and relevant observations are reported as recorded in the recipes.

Recipe source	Date (AD)	Folio	Type	Urine*	Ley**	Colourant extraction	Precipitation***	Notes and added ingredients
Mappae Clavicula [41]	9th or 12th century	§253	Lake pigment	Urine boiled to reduce with 2/3	–	Boiled	Alum	
Ibn Badis [52]	c. 1025	–	Unmordanted dye	–	Borax and sodium carbonate	–	–	
Jehan le Begue [49]	1431	§11	Lake from clippings	–	Ley from ashes	Boil ley with clippings	More ley, with alum, add liquid	Not specifically for lac, but kermes or madder dyed cloth
		§12	Lake pigment	Boiled	Urine or ley	Boiled	Alum and sea salt with ley, boiling	After precipitation 15 days in urine/ley
		§13	Lake from clippings	Urine – not specified		Clippings in urine for some days		No alum, not specifically for lac, but kermes or madder dyed cloth
		§36	Unmordanted dye	Very old, then boiled	–	Cold	Alum	Shellac residue not removed, precipitation directly during extraction
		§37	Unmordanted dye	3 days old	–	Keep lac in urine for 3 days then boil	Roman vitriol (sulphuric acid)	
Nuremburg Kunstbuch [25,53]	15th century		Lake from clippings	–	Ley from ashes, boiling	Boiling ley with clippings	Grind with alum, then boil	Paris red from red clippings
Bolognese [49]	15th century	§110	Lake from clippings	–	Strong ley of ashes	Boil clippings with ley	Adding alum until precipitated, stirring until cool	Not specifically for lac, but kermes dyed cloth
		§111	Lake from clippings	–	Ley from ashes and quicklime (CaO)	Boil clippings with ley	Add alum and boil	Not specified what kind of cloth to be used
		§129	Lake pigment	Week old then boiled	Create ley from that urine	Warm	Alum, cold	
		§130	Lake pigment	20 days old then boiled	–	In boiling old urine with alum alum	–	Precipitation directly during extraction
Plichto [42]	1548	§118	Dyeing silk	–	–	Warm water, extract 2 times	Tarter, alum mordanted silk, boil for 1 h	Boil silk with black soap and alum for 2 days
Ziegler [54]	1677		Dyed with brazilwood	–	Tartar and alum	Boiled		Yarn in dye bath for 30 min when still hot
Hellot [24]	1789		Dyeing wool	–	–	Sticklac in a linen sack, boil	Boiling	Mentions that this recipe causes resin to adhere to the dyed fabric
			Dyeing wool	–	–	Boil, cool. Let particles sink to bottom	After concentration, dye with it, boiling with alum and tartar	
			Dyeing wool	–	–	–	–	Several methods to incorporate less resin with the dyed fabric, which use other types of ingredients
Cardon [6]	Modern India		Dyeing silk	–	–	Overnight cold water	Alum dyeing, cold/hot unspecified	
Kirby, van Bommel and Verhecken [25]	Modern adaptation		Lake pigment	–	Potassium carbonate solution	Simmering	Potash, 50 °C	Ley is replaced by potassium carbonate

*Urine has been used as a source of aqueous alkaline solutions since ancient times, described by Pliny in the first century AD to be used for dyeing [55]. Alkalinity increases with time and temperature in urine solutions, as ammonia forms [56].
**Ley (potassium carbonate) produces a strong alkaline solution (pH 9–13.5) and it is prepared by running most commonly water over wood ash [57].
**Precipitation of lac to form a lake pigment is usually done on potassium alum ($KAl(SO_4)_2{\cdot}12H_2O$), which forms an insoluble metal-dye complex. Also in the case of textiles, the mordant mentioned is potassium alum. The alum forms a chelate between the fibres and the dye resulting in a more lightfast and colour-fast textile.

3. Material and methods

3.1. Reference and mock-up samples

All the production methods described in the following sections used sticklac (*Tachcerdia lacca*) as the starting material, which was obtained from Kremer Pigmente (Aichstetten, Germany). The sticklac was approximately one year old.

After removal of macroscopic plant residues, the sticklac was ground and used to produce two sets of mock-up samples: lake pigments and dyed textiles. For both sets, the procedure as described by Kirby et al. [25] was adopted, in order to selectively control pH and temperature. A third set of mock-up samples was obtained by re-extracting the lac dye from the dyed textiles and processing it into a lake pigment, in agreement with historical descriptions of the re-use of textile clippings to obtain colourants [50,51].

3.1.1. Lac lake pigments made directly from sticklac (DP)

Sticklac (6.0 g) was finely ground using a coffee grinder and placed in a polyester netting bag. A solution (150 mL deionised water) at pH 11 (potassium carbonate 0.1 M) or at pH 7 (urea 0.1 M) was heated to 50 °C and poured onto the sticklac. These solutions were then heated to 100 °C for 10 min or kept at room temperature for 1 h. After removal of the sticklac residue, which remained contained within the netting bag, the temperature of the colourant solution was raised to 50 °C and 80 mL of warm alum solution (0.26 M) was added under stirring. The resulting lake pigment was filtered off and washed with deionised water. This process resulted in four pigments, referred to as direct pigments (DP). The extractions performed at room temperature and 100 °C are referred to as cold (C) and hot (H), respectively. The extractions performed at pH 7 and pH 11 are referred as neutral (N) and alkaline (A), respectively. As an example, the lake pigment obtained by extracting sticklac at 100 °C and pH 11 is referred to as DP_H_A. All the mock-up samples produced and investigated are described in Table 2.

3.1.2. Silk textiles dyed with sticklac (T)

Prior to dyeing, silk was mordanted using an alum solution (35% w/w, 2 L deionised water for 44 g silk) for 1 h at 70 °C, then left overnight at room temperature. Sticklac (16.5 g) was finely ground using a coffee grinder and placed in a polyester netting bag. The colourant molecules were extracted in water (500 mL, deionised) overnight at room temperature (cold - C) or for 1 h at 100 °C (hot - H). Alum-mordanted silk (5.5 g) was added to the two differently extracted colourant solutions at 70 °C for 1 h, thus obtaining two lac-dyed silk samples, referred to as textiles (T). Both of the differently extracted colourant solutions were also divided into three equal amounts, and 5.5 g of alum-mordanted silk was dyed three times (70 °C for 1 h each time), with mordanting (alum solution 15% w/w for 1 h at 70 °C) between each dye bath. To distinguish the textiles dyed once from the textiles dyed three times, a number is used in the name of these samples. As an example, the textile obtained by extracting sticklac at 100 °C and then dyeing three times, is referred to as T_H3 (Table 2). Changing the pH was not considered for dyeing, as it was not reported in any of the recipes found. Moreover, very alkaline pH values are known to damage the silk [58].

3.1.3. Lac lake pigments made from lac-dyed silk textile clippings (TP)

Silk (2.5 g) dyed with lac as described in section 2.1.2 was cut into small clippings and put into polyester netting. A potassium carbonate solution (0.1 M, 100 mL, pH 11) was added and the system was heated for 15 min at 100 °C (H) or left at room temperature (C) overnight. After removal of the silk residue contained in the netting, the colourant solution was brought to 50 °C and 25 mL of warm alum solution (0.42 M) was added under stirring. The resulting lake pigment was filtered and washed. These eight mock-up samples are referred to as textile-extracted pigments (TP). Since they were produced starting from the four textiles, the names of these samples contain the conditions of the dyeing process, as explained in section 2.1.2, and the conditions of the re-extraction (H or C). As an example, the textile pigment obtained from T_H3 by re-extracting the colourants at 100 °C is referred to as TP_H3_H (Table 2).

3.2. Photo-induced luminescence (PIL) imaging and colorimetry

All images were taken using a modified Nikon D7000 camera body with a maximum resolution of 4928 × 3264 (16.2 megapixels). The modification consists of the removal of the inbuilt UV-IR blocking filter, to exploit the full sensitivity of the CMOS sensor (c. 300–1000 nm). The lens used is a Canon EF 50 mm f/1.8II. The camera is operated in fully manual mode. An X-rite (or "Macbeth") Colorchecker® and reference grey scale, comprising a set of Lambertian black, grey and white tiles, are placed in the same plane as the object under investigation. These Spectralon® references have uniform reflectance properties across the ultraviolet, visible and infrared spectral ranges under investigation and show no luminescence properties [59].

In each case the object is illuminated by two radiation sources symmetrically positioned at approximately 45° with respect to the focal axis of the camera and at about the same height. A filter, or combination of filters, is placed in front of the camera lens in order to select the wavelength range of interest.

The combinations of radiation sources and filter(s) used for each

Table 2
A description of the reference and mock-up samples and the labels used to refer to them in the text.

Label	Description
P	Reference sample of lac dye purchased from Kremer pigmente (Lac Dye, #36020 description: Indian Lake)
T	Reference sample of undyed and unmordanted silk
DP_C_N	Direct pigment obtained by extracting sticklac at room temperature and neutral pH
DP_H_N	Direct pigment obtained by extracting sticklac at high temperature (100 °C) and neutral pH
DP_C_A	Direct pigment obtained by extracting sticklac at room temperature and pH 11
DP_H_A	Direct pigment obtained by extracting sticklac at high temperature (100 °C) and pH 11
T_C1	Dyed silk obtained by extracting sticklac at room temperature and using one dye bath
T_C3	Dyed silk obtained by extracting sticklac at room temperature and using three consecutive dye baths with mordanting in between
T_H1	Dyed silk obtained by extracting sticklac at high temperature and using one dye bath
T_H3	Dyed silk obtained by extracting sticklac at high temperature and using three consecutive dye baths with mordanting in between
TP_C1_C	Pigment made from T_C1 by re-extracting the colourants at room temperature and pH 11
TP_C1_H	Pigment made from T_C1 by re-extracting the colourants at high temperature and pH 11
TP_C3_C	Pigment made from T_C3 by re-extracting the colourants at room temperature and pH 11
TP_C3_H	Pigment made from T_C3 by re-extracting the colourants at high temperature and pH 11
TP_H1_C	Pigment made from T_H1 by re-extracting the colourants at room temperature and pH 11
TP_H1_H	Pigment made from T_H1 by re-extracting the colourants at high temperature and pH 11
TP_H3_C	Pigment made from T_H3 by re-extracting the colourants at room temperature and pH 11
TP_H3_H	Pigment made from T_H3 by re-extracting the colourants at high temperature and pH 11

Table 3
Summary of the combination of radiation sources and filter(s) used for each of the imaging techniques considered.

Imaging Technique	Radiation Sources	Filter(s) in front of camera	Range investigated
Visible-reflected imaging (VIS)	2 x Classic Elinchrom 500 Xenon flashlights, each equipped with a softbox (diffuser)	XRite CC1 UV-IR blocking bandpass filter (c. 325–645 nm, 50%)	c.350–700 nm
Ultraviolet-induced visible luminescence imaging (UVL)	2 x Wood's radiation sources (365 nm) filtered with a Schott DUG11 interference bandpass filter (280–400 nm)	Schott KV418 cut-on filter (50% transmission at c. 418 nm) + XRite CC1 UV-IR blocking bandpass filter (c. 325–645 nm, 50%)	c. 420–700 nm
Visible-induced visible luminescence imaging (VIVL)	2 x high power LED (red, green and blue) light sources (Eurolite LED PAR56 RGB spots 20 W, 151 LEDs, beam angle 21°). Blue LEDs (λ_{max} = 465 nm)	XRite CC1 UV-IR blocking bandpass filter (c. 325–645 nm, 50%) + Tiffen Orange 21 filter (50% transmission at 550 nm)	c. 540–700 nm

photo-induced luminescence imaging technique and visible-reflected imaging, which corresponds to regular photography in the visible used as the reference image, are summarised in Table 3.

All images are acquired as RAW images and transformed into 4928 × 3264 pixel resolution images in 16-bit TIF (tagged image file) format, and by turning off all enhancements (e.g. recovery, fill light, blacks, contrast, brightness, clarity, vibrance, saturation, as well as setting the tone-curve to linear). This procedure can be carried out using the camera software or external programs such as Adobe Photoshop. For further details on the conversion of images from RAW, see the manual on multispectral imaging techniques [60].

Post-processing procedures for the standardisation and calibration of the VIS and UVL are then carried out using "BM_workspace", a plug-in for Nip2, the open-source graphical user-interface of VIPS, a free image processing software [61]. For details on how to download BM_workspace and the Nip2 software, as well as descriptions, workflows and data requirements for the post-processing of these images, see the manual on multispectral imaging techniques [60].

Post-processing procedures for the VIVL images are not currently supported by the BM_workspace. However a post-processing protocol for VIVL images was described in Dyer et al. [62].

Colorimetric information on the samples was obtained by transforming the colour-calibrated visible images into CIE L*a*b* colour space in Photoshop and taking an average colour measurement (51 × 51 pixels) from each sample area.

3.3. High-pressure liquid chromatography – diode array detection – electrospray ionisation – quadrupole – time of flight (HPLC-DAD-ESI-Q-ToF)

A combined mild extraction procedure was adopted for the textile and lake pigment samples to extract both colourants and resin components. Approximately 0.6 mg of pigment sample or 20 mg of textile sample were admixed with 200 µL DMSO and heated at 80 °C for 10 min. After centrifugation, the supernatant was transferred into another vial. The residue was admixed with 200 µL of a solution of methanol/acetone/water/0.5 M oxalic acid 30:30:40:1 (v/v/v/v) and kept at 80 °C for 15 min. The solution was evaporated under N_2 and reconstituted using 200 µL of 1:1 MeOH/H_2O (v/v). The DMSO and oxalic acid extracts were combined, and the solution was centrifuged for 5 min. The supernatant was transferred into a fresh 250 µL insert and 20 µL of the solution was injected into the HPLC system. If necessary, some of the extracts were diluted using 1:1 MeOH/H_2O (v/v). The extraction procedure was optimised in the framework of the CHARISMA project (2009–2014) funded by the European Union FP 7 Research Infrastructures programme (CHARISMA Grant Agreement no. 228330) and has proven its suitability for the analysis of organic colourants in both dyes [28,29,63] and pigments [17,64] formulations.

Analysis were carried out using a 1260 infinity HPLC (Agilent Technologies), coupled in series with a DAD detector (cell volume 50 µL) and with a Quadrupole-Time of Flight tandem mass spectrometer 6530 Infinity Q-ToF detector (Agilent Technologies) by a Jet Stream ESI interface (Agilent Technologies). The HPLC conditions were: Zorbax Extend-C18 column (2.1 mm × 50 mm, 1.8 µm particle size); 0.4 mL/min flow rate; 40 °C column temperature. Separation was achieved using a gradient of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The elution gradient was programmed as follows: initial conditions 95% A, followed by a linear gradient to 100% B in 10 min, held for 2 min. Re-equilibration time for each analysis was 10 min. The ESI operating conditions were: drying gas (N_2, purity > 98%): 350 °C and 10 L/min; capillary voltage 4.0 KV; nebulizer gas 40 psig; sheath gas (N_2, purity > 98%): 375 C and 11 L/min. High resolution MS spectra were acquired in negative mode in the range 100–1700 *m/z*. The fragmentor was kept at 150 V, nozzle voltage 1000 V, skimmer 65 V, octapole RF 750 V.

The identification of the molecules was based on the comparison of retention times and mass spectra of the molecules detected in the samples with those present in an in-house database of reference compounds and in the literature [2,17,23].

Each sample was analysed in triplicate and chromatographic areas were integrated using MassHunter Agilent software.

For DAD data, the chromatographic areas of laccaic acids A and B obtained at 491 nm were considered. These data were used to obtain a semi-quantitative estimate of the amount of colourant present in each sample, as explained in more detail in section 3.2. In order for these areas to be comparable for all the samples investigated, each of the triplicate datasets were averaged and normalised according to the initial weight of the samples and dilution prior to HPLC-DAD-MS analysis.

For the MS data, the chromatographic areas obtained from the extract ion chromatograms (EIC) were considered. Extract ion chromatograms were obtained using the EIC function of the MassHunter Agilent software by extracting the calculated accurate masses of the molecules of interest ± 0.001 *m/z*. The instrumental accuracy always produced a difference between the experimental and calculated masses below 2 ppm, which allows for the third decimal digit to be reliable for identification. The molecules considered were laccaic acids A and B, deoxyerythrolaccin, erythrolaccin, butolic acid (but), laccijalaric acid (lac), jalaric acid (jal), aleuritic acid (al), al-lac ester, al-jal ester, jal-al-but diester, jal-al-jal diester, jal-al-al-jal triester, jal-al-but-al-jal tetraester and jal-jal-jal-al-al-al pentaester. These were chosen as they are reported as the most abundant components of lac dye and shellac [23]. The MS data were used to estimate the relative amounts of colourant molecules and shellac components in each sample. In order to simplify this estimate, the chromatographic areas of each compound were expressed as percentages referred to the sum of the chromatographic areas of all the compounds under consideration. The percentage areas were then averaged for the triplicates.

3.4. Data treatment (Principal component analysis - PCA)

Principal component analysis (PCA) was used as a pattern recognition technique to highlight differences and similarities between samples and correlations between variables. The percentage chromatographic areas were used as dataset and calculations were performed by the XLSTAT 2018.1.49386 software using the correlation matrix of centred data.

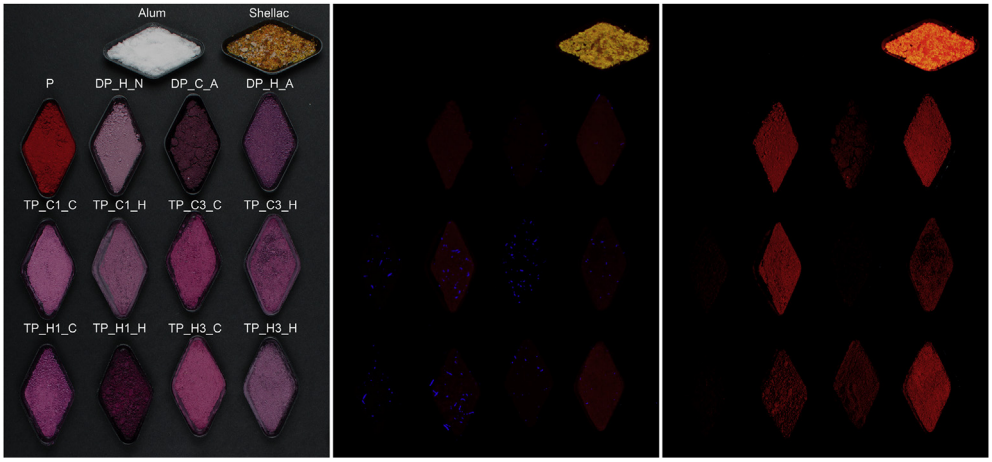

Fig. 2. VIS (left), UVL (middle) and VIVL images (right) of the pigments produced. Refer to Table 2 for sample labelling.

3.5. Ageing textile samples

Textile samples, as described in section 2.1.2, were cut into strips of 4 by 6 cm and attached to acid free cardboard with stitching. The samples were aged in an Atlas SolarClimatic SC 340 MH climatic chamber equipped with a metal halide lamp simulating solar radiation. A UV filter (5 mm LEXAN filter with a Sun-X MT20 film) was used to cut the radiation below 400 nm and reduce the total illuminance to 0.027 Mlux. Temperature (T) was set at 40 °C (higher then room temperature to accelerate the ageing) and relative humidity (RH) was set to 50%. The experiment lasted 28 days (672 h). As the visible light intensity is not completely homogeneous in the chamber, the card was rotated by 90° after 2 weeks. All the samples were therefore exposed to ca. 18.15 Mlux during ageing.

4. Results

4.1. Qualitative observations

Images of the pigments and dyed textiles are reported in Figs. 2 and 3 respectively, which show a comparison between VIS, UVL and VIVL images (the latter is not shown for the dyed textiles). References of alum and shellac are present in Fig. 2 to help with the interpretation of the luminescence images. The pigments were not ground to uniform particle size and some colour variation in the images may be due to the particle morphology.

The pigment DP_C_N, obtained at room temperature and neutral pH, is not shown in Fig. 2. This is because it was not possible under these conditions to precipitate the colourants to form a lake pigment and explains why this method was not found in the historical recipes.

None of the pigments or textiles present a pure red colouration, as exhibited by the reference sample purchased from Kremer pigmente (P). This is due to the chemical nature of the laccaic acids, which

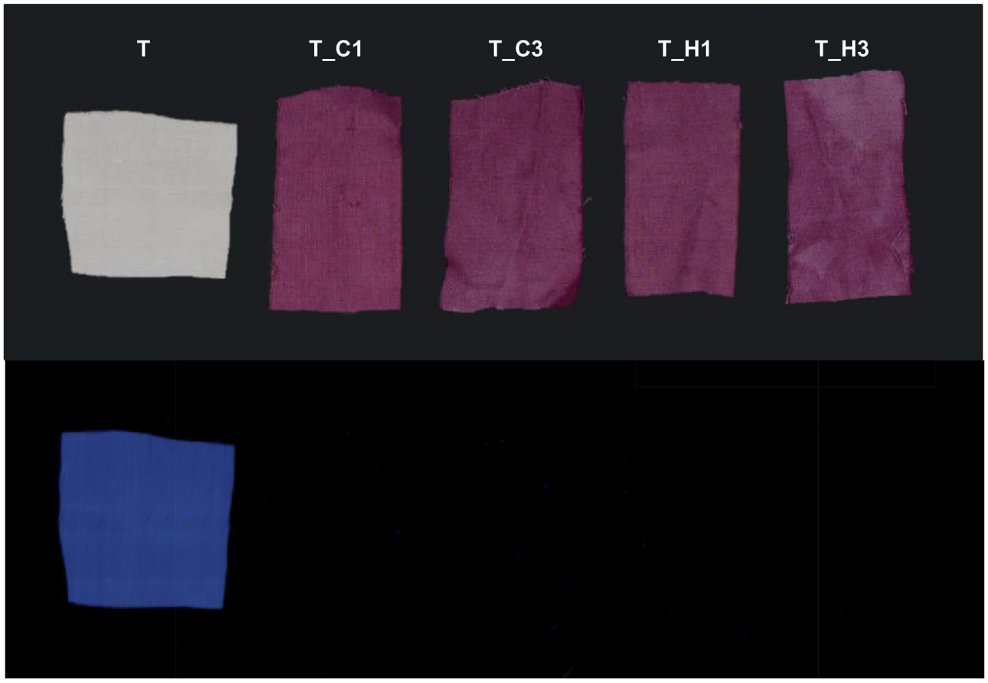

Fig. 3. VIS (top) and UVL (bottom) images of the dyed textiles produced. Refer to Table 2 for sample labelling. The textile on the left is undyed and unmordanted silk for reference.

change colour according to the pH of the solution, with relation to their three pK_a values (pH 5.6, 7.0 and 9.8) [20]. The red colour, often associated with lac, can only be obtained through an acidic pH below 5.6. However, none of the recipes considered describes the use of an acidic environment in any of the production steps. The neutral and alkaline environments adopted in this work thus led to pink-purple colourations. Sample P also turned purple when dissolved in water at neutral pH, thus the red colouration is likely indicative of its acidic preparation conditions, presumably made by evaporating the extraction solvent. This is corroborated by the absence of an inorganic substrate, as revealed by FTIR spectroscopy (Figure S-1 – Supplementary Information).[1]

Considering the three direct pigments (DP), the colour variation observed was significant, as highlighted by the colorimetry measurements. As expected, the L* component, or lightness value, was lowest for DP_C_A (the darkest sample), had an intermediate value for DP_H_A and was highest for DP_H_N (the lightest sample). The a* values, related to the green–red colour components, were relatively similar, with DP_H_A showing a slightly higher value compared to the other two, indicative of a redder colour. The b* values, related to blue–yellow colour components, revealed that all the samples tend to the blue side of the scale with DP_C_A having the highest yellow component. The colorimetry data for all samples are presented in Tables S–1 (Supplementary Information).

A difference was also noticed in the amount of pigment produced under the three different conditions. The highest yield was obtained for pigment DP_H_A, followed by DP_C_A and DP_H_N. Furthermore, the amount of residue present in the polyester netting bag after extraction varied greatly depending on the extraction conditions: high temperature and high pH (DP_H_A) resulted in the complete dissolution of the shellac, leaving only a small residue of bark pieces behind in the netting. When only heat was used (DP_H_N), the resinous shellac appeared to have only partially melted and transferred into the solution, the netting being completely covered with melted resinous components. At high pH and low temperature (DP_C_A), the dissolution of shellac was less evident, as there was no clear visual change in the appearance of the sticklac. These observations were in agreement with the fact that shellac begins to melt at 60 °C and begins to dissolve in water at pH values of 8 or higher [15,16]. The alkalinity therefore aids in the extraction of colourants from the macromolecular network of shellac by disrupting this network, however the resin is more prone to dissolve in alkaline aqueous solution, and heating appears to further enhance the process.

An interesting correspondence was also obtained considering the photo-induced luminescence imaging data (Fig. 2). The characteristic luminescence of shellac when irradiated with UV light is well attested [65]. Conversely, pure lac colourants (as represented by sample P) and alum do not give any notable luminescence under UV light (Fig. 2). As samples DP_H_A and DP_H_N show a weak but noticeable luminescence under UV, and considering the observations about the shellac residue after extraction, it is reasonable to ascribe this luminescence as deriving from the shellac included in the pigment formulation. This is also supported by considering the VIVL image (Fig. 2), which targets the wavelength range c. 540–700 nm, where shellac is known to emit (λ_{max} ~ 600 nm) [65]. The luminescence from samples DP_H_A and DP_H_N is even more evident in this image, whereas only very weak emission is observed from sample DP_C_A. The possibility of the observed luminescence as being due to complexation between Al and laccaic acids was excluded by preparing a lake pigment from sample P. No appreciable luminescence was observed.[1]

[1] The reference lac dye purchased from Kremer pigmente was also made into a lake pigment by adopting the procedure described in 2.1.1. The resulting pigment was a dark purple pigment, with no appreciable luminescence in UVL and VIVL (not shown). The FTIR spectrum is shown in Figure S-1 – Supplementary Information.

The combination of these qualitative observations suggests that the dark colour of DP_C_A is caused by a higher concentration of colourant molecules, due to an effective extraction procedure under alkaline conditions, which partially suppresses the dissolution of shellac by using room temperature. Among the colourant molecules, erythrolaccin is also extracted more effectively (see also Table S2-Supplementary Information), thus justifying the higher b* value (yellow colouration) of this sample. A less effective extraction process in terms of colourant molecules results in the lighter colours of samples DP_H_A and DP_H_N. Nevertheless, shellac, which is extracted more effectively under high temperature conditions, contributes to the more intense UV-luminescence of these samples.

The dyed textiles (Fig. 3) did not show any significant variations in colour and the colorimetric values obtained (Tables S–1 – Supplementary Information) did not vary substantially. This might be attributed to the complete colour saturation of the textile fibres, which may have been achieved after only one dye bath. However, in this case the photo-induced luminescence imaging (Fig. 3) results were not helpful. The untreated silk (T - Fig. 3) showed the characteristic luminescence of proteinaceous fibres under UV light [66], but this was completely quenched by the presence of lac colourants on the dyed textiles, and no luminescence from possible shellac components was observed in either the UVL or VIVL images (the latter not shown).

By contrast, the textile-extracted pigments (Fig. 2) produced quite variable results. Equal amounts of very similarly coloured textiles resulted in eight pigments, which were very different in appearance. This not only highlighted the effects of the two different re-extraction methods (room temperature and 100 °C), but also indicated that some differences in the textiles were actually present, although not directly related to the colour. Boiling the textile in an alkaline solution removed all traces of colourants from the textile, whereas this was not the case for extraction at room temperature. However, the colour of the pigments obtained after extraction at room temperature (C) appeared slightly more saturated compared to those obtained after extraction at 100 °C (H). The colorimetry data (Tables S–1 – Supplementary Information) did not show large differences between values of L* for the samples obtained after extraction at 100 °C (H) and the samples obtained after extraction at room temperature (C), with the exception of sample TP_H1_H, which was darker than all the other re-extracted samples. There is no simple explanation for the anomalous behaviour of this sample, however the particle size of the lake pigment and the significant inclusion of silk fibres appear to have played a role in the final colour of this sample. More positive a* values for the pigments obtained after extraction at room temperature were observed, indicating a redder component in these samples. In addition, the UVL and VIVL images (Fig. 2) showed that the pigments obtained after extraction at 100 °C (H) exhibited more intense luminescence than their equivalents extracted at room temperature (C), most likely due to a more significant transfer of shellac components. The blue-luminescing areas in the UVL image are instead attributable to some silk fibres transferred into the pigment from the textile.

4.2. Semi-quantitative determination of molecular variability

In order to translate these qualitative observations into semi-quantitative results, HPLC-DAD-ESI-Q-ToF was used to determine the variability of the molecular components present in the mock-up samples. It is important to underline that, as no calibration curve was performed in this work, none of the results presented can be considered quantitative. The main reason for not performing a quantitative analysis is that, when dealing with real samples, there are too many variables that prevent the concentration of a molecule in an extract from being correlated with the concentration of that molecule in a lake or a textile. Nevertheless, chromatographic peak areas can be exploited to obtain semi-quantitative indications of the relative amount of a molecule. The numbers obtained are valid not as absolute values, but

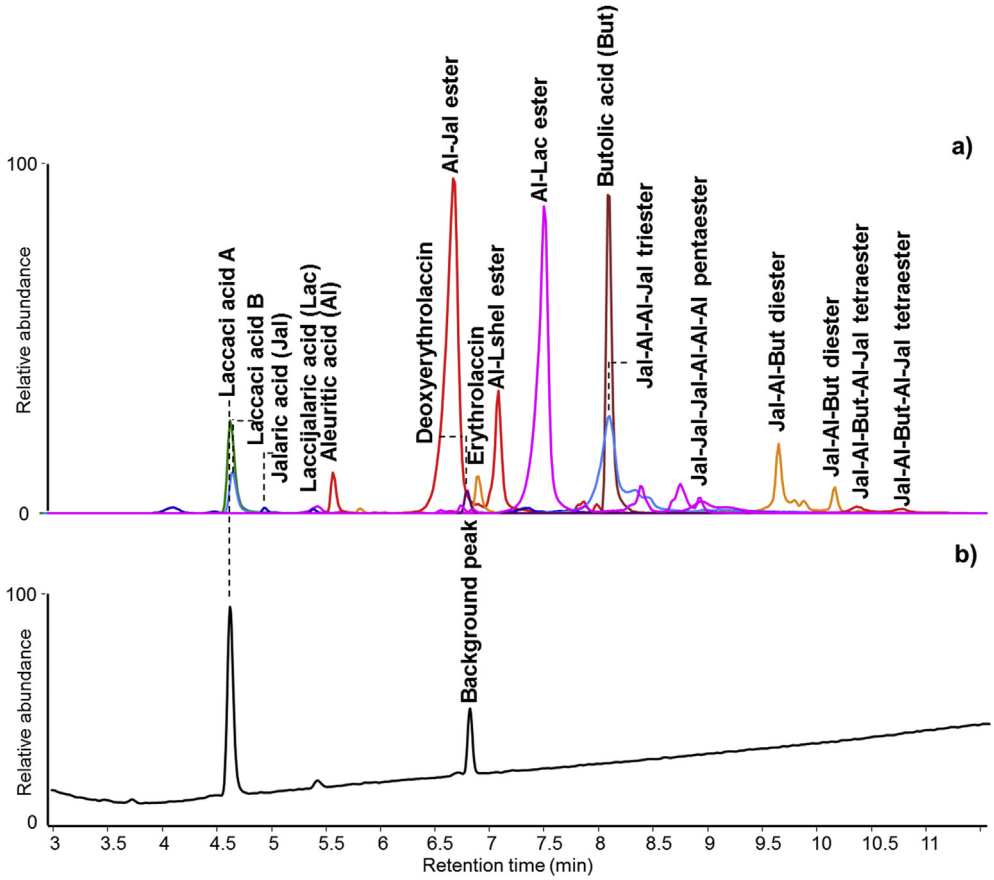

Fig. 4. a) Chromatographic profile of sample DP_C_A obtained by HPLC-DAD at 491 nm; b) extracted-ion chromatograms (EICs) obtained by HPLC-ESI-Q-ToF of the same sample, showing the lac dye and shellac compounds considered for interpretation.

acceptable if compared among samples that have undergone exactly the same analytical preparation and data treatment.

The DAD data were therefore used to compare the samples in terms of relative abundance of colourant molecules. Shellac gives a broad peak with an UV maximum at 223 nm [67], but it is unknown which components in shellac contribute to the UV absorption, therefore laccaic acids A and B were considered. These compounds are not fully chromatographically separated under these conditions, thus the chromatographic peak at 4.6 min represents the sum of both compounds. The areas were integrated and corrected for the initial weight of the sample and dilution prior to HPLC analysis, as described in section 2.3. As the samples contained different amounts of shellac, the correction for the initial weight of the samples does not make the data completely independent from this parameter. Therefore, the results have to be considered as indicative and integrated with the results obtained by MS, as described later in this section. Fig. 4a shows a DAD chromatogram for sample DP_C_A registered at 491 nm, where the peak attributed to the sum of laccaic acids A and B is indicated. Fig. 5 reports the chromatographic area of this peak for each of the samples.

The results showed that the pigment DP_C_A contains the highest relative amount of laccaic acids, confirming that the alkaline environment enhances the extraction of the colourants. Temperature appeared to play a secondary role, as sample DP_H_A showed a lower relative amount of laccaic acids. These results were in agreement with the appearance of the pigments and the colorimetry measurements, as well as their luminescence behaviour, as discussed in section 3.1.

The role of the temperature was more evident when considering the textile samples. Those dyed after extraction of the colourants at high temperature contained a higher relative amount of laccaic acids than those dyed after extraction at room temperature. In addition, the number of dye baths did not seem to be relevant in the case of the room temperature extraction, whereas three dye baths resulted in a higher concentration of colourants on the textile in the case of extraction at 100 °C. These results showed that, despite the similar appearance of these textiles, chemical differences were indeed present.

The pigments obtained after re-extracting the colourants from the textiles generally showed a higher concentration of laccaic acids when the extraction was performed at room temperature rather than at 100 °C, in agreement with what was obtained for the direct pigments and the observations described in section 3.1. It is important to underline that a direct comparison between the relative amounts of colourants in the textiles and the pigments is not possible based on these results. In fact, these data are normalised for the weight of the samples, which intrinsically includes the weight of the fibres in the case of the textiles. A comparison between the direct pigments and the re-extracted pigments should in principle be more accurate. Nevertheless, the results obtained for DP_C_A and TP_H1_H highlight some difficulties. In fact, despite similar colourimetry data obtained for these pigments, the estimated concentration of colourant molecules was very different. In addition to the inconsistencies already mentioned about TP_H1_H, the multiple extraction steps required in the production of the re-extracted pigments and the weight contributed by the silk fibres in the final composition might play a significant role in the chromatographic data obtained, making the comparison between direct and re-extracted pigments not always straightforward.

In order to then evaluate the transfer of shellac components into the final composition of the pigments and dyed textiles, the MS data were used. As aforementioned for the DAD data, these are not quantitative results and are mostly valuable for comparison within the three datasets. Due to the large variety of molecular species present in shellac, a selection was made to represent the various shellac components, namely; free acids - butolic acid (but), laccijalaric acid (lac), jalaric acid

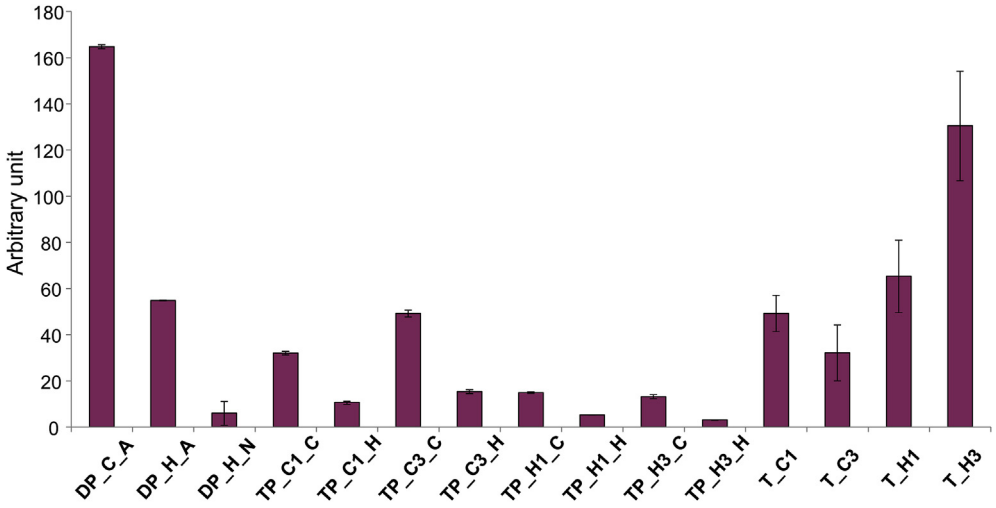

Fig. 5. Plotted values of the sum of the chromatographic areas of laccaic acids A and B for each sample, as obtained from the HPLC-DAD chromatograms at 491 nm. Refer to Table 2 for sample labelling.

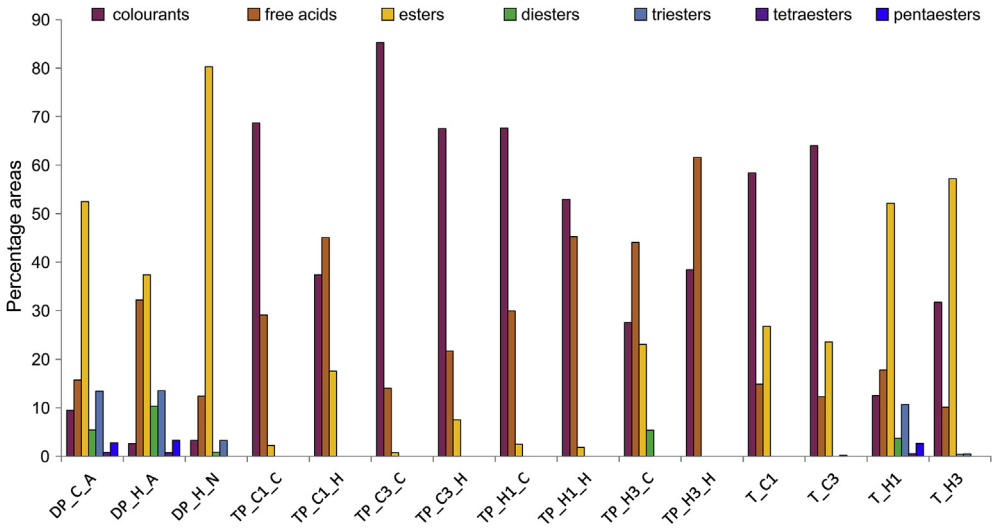

Fig. 6. Plotted values of the sum of the chromatographic percentage areas of the lac dye and shellac compounds as obtained from the HPLC-ESI-Q-ToF data. Details are presented in Tables S–2 – Supplementary Information.

(jal), aleuritic acid (al) -, esters - al-lac and al-jal -, diesters - jal-al-but and jal-al-jal -, triesters - jal-al-al-jal -, tetraesters - jal-al-but-al-jal - and pentaesters - jal-jal-jal-al-al-al. The selection was based on previous work showing that these compounds produce the most intense chromatographic peaks when shellac is analysed by HPLC-ESI-Q-ToF [23]. Erythrolaccin and deoxyerythrolaccin were also selected to represent the colourants present in shellac, and laccaic acids A and B were again included to provide an estimate for the relative abundance of shellac and lac-dye components in the samples. The chromatographic profile (EICs of the selected compounds) of sample DP_C_A is shown in Fig. 4b and the percentage areas of the compounds, calculated as described in section 2.3, are presented as a histogram in Fig. 6. The accurate masses used to obtain the EICs and the percentage areas of the compounds are reported in Tables S–2 – Supplementary Information.

Shellac components were present in all the samples, including the pigments obtained by re-extracting the textiles, which highlighted the ease of solubilising these compounds in water, despite their partial hydrophobicity. Samples DP_C_A and DP_H_A contained all of the selected molecular species, and sample DP_H_N showed the presence of free acids and esters up to the triesters, indicating that heat under neutral conditions is sufficient to extract high molecular weight components from the resinous matrix. Alkaline conditions, as evidenced by sample DP_H_A, further enhance the process. However, the relative amount of laccaic acids A and B in the pigment extracted under neutral conditions was significantly lower than the other two direct pigments, as observed from the DAD data. This could be related to a lower concentration of colourants extracted from the sticklac or to the difficulty in precipitating lac onto the alum under neutral conditions, as lake pigments are precipitated by neutralisation [25]. Both these possibilities highlight the importance of alkalinity in the production of lac lake pigments.

A clear difference was observed in the textiles dyed with colourants extracted through heating or at room temperature. T_H1 and T_H3 contained all the shellac components considered, whereas T_C1 and T_C3 only contained some of the free acids and esters, but no components of higher molecular weight, thus highlighting the role of temperature in the extraction and solubilisation of these components.

Regarding the textile-extracted pigments, the relative amount of shellac components was generally lower than in all the other samples and only free acids and esters could be detected, regardless of the original textile or extraction method. However, also for these samples the relative amount of shellac components was higher when heat was used to re-extract the colourants from the textiles.

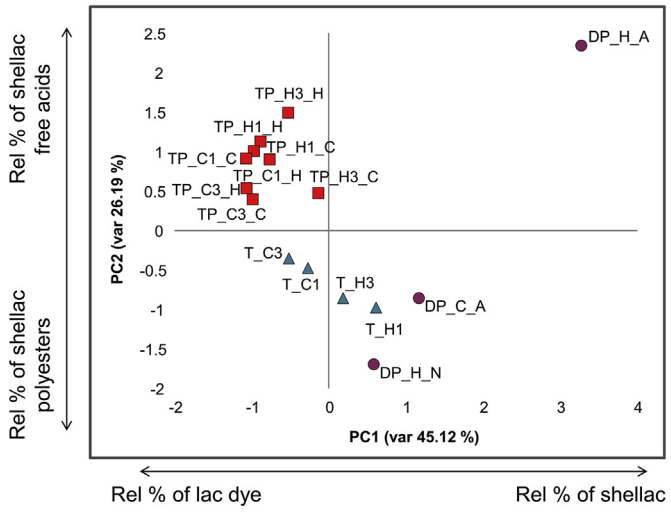

Fig. 7. PCA score plot of the chromatographic percentage areas representative of the composition of the samples analysed. The sample labels are explained in Table 2.

4.3. Principle component analysis (PCA)

To further investigate the data and visualise the correlations between the different production methods and the molecular composition of the samples, PCA analysis was applied to the dataset of chromatographic percentage areas obtained from the HPLC-ESI-Q-ToF analysis (Tables S–2 – Supplementary Information). To simplify the interpretation, diesters, triesters, tetraesters and pentaesters were considered together and referred to as polyesters.

The two main principle components (PC) described a cumulative explained variance of 71.3% of the total variance of the dataset, with PC1 representing 45.1% and PC2 representing 26.2%. This resulted in a good separation of the three types of samples - direct pigments (DP), dyed textiles (T) and textile-extracted pigments (TP) - in the PC1/PC2 score plot, as shown in Fig. 7.

The direct pigments did not completely cluster on the score plot, with DP_H_N and DP_C_A showing positive scores on PC1, but negative scores on PC2, whereas DP_H_A showed the highest positive score on PC2. In order to make sense of these differences the loading values for the two PCs were considered. These are shown in Fig. 8. As is evident, the highest loadings on PC2 are represented by the free acids from shellac and sample DP_H_A showed the highest relative amounts of these compounds. Considering that DP_H_A was obtained using alkaline and high temperature conditions, it is reasonable to hypothesise that shellac esters underwent the highest extent of hydrolysis under these conditions, therefore producing higher relative amounts of free acids, which were transferred to the pigment.

The textiles clustered approximately in the same part of the score plot, but T_H1 and T_H3 had positive scores on PC1, whereas T_C1 and T_C3 had negative scores. Considering the loadings, positive values of PC1 are related to a higher relative abundance of shellac components, whereas negative values are related to a higher relative abundance of lac colourants. Therefore, as the two groups of textiles differ for the temperature of the colourant extraction, the PCA confirmed the higher level of transfer of shellac components into the dye bath when using high temperature.

The textile-extracted pigments clustered together at negative values of PC2 and positive values of PC1. They did not really exhibit a clear trend but their positioning in the score plot was in agreement with the fact that these pigments had the highest relative amount of lac-dye colourants (negative loading on PC1) when compared to shellac components, as a result of the double-extraction step used to produce them. In addition, almost no shellac polyesters were present in these

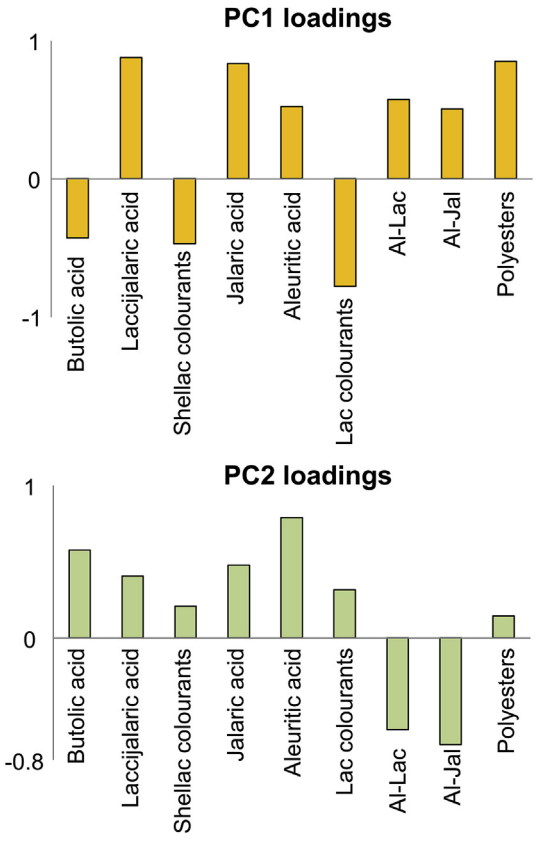

Fig. 8. Loading values for PC1 and PC2, showing the influence of each compound or category of compounds to the positioning of the samples on the score plot.

pigments, but mainly free acids, which contributed positively to PC2.

In general, PCA was able to separate the samples in terms of an indication of the relative amount of lac-dye colourants versus shellac components on PC1, and in terms of the molecular weights of the shellac components on PC2. This interpretation does not exactly justify the position of all samples, as negative and positive loadings can contribute differently based on internal compositional differences. Nevertheless, the analysis confirms that shellac was transferred less efficiently in the textile-extracted pigments and in the textiles dyed with colourants extracted at room temperature, and also adds further insights into the extent of hydrolysis of the shellac components when alkaline and high temperature conditions are adopted.

5. Discussion

Analysis of the mock-up samples by HPLC-DAD-ESI-Q-ToF showed that shellac components were present in all the samples. The degree to which high molecular weight shellac components were transferred into the dye/pigment composition was dependent on the extraction conditions. High pH and high temperatures promote the inclusion of these components, but mild conditions, such as neutral pH and room temperature, are also sufficient for a range of shellac compounds to be included. The mock-up samples displayed a range of colours, from light pink to deep purple. For the pigments some indication of the presence of shellac could be determined by UVL and VIVL imaging. However, the effects of the different production parameters on the molecular composition could only be assessed by using HPLC-MS, due to its ability to detect colourless shellac components. Despite a general agreement between observations, colorimetry and chromatographic data, some inconsistencies were noted, especially regarding the colour of certain pigments and some shellac-containing extracted pigments not

exhibiting luminescence. Further investigations on the influence of particle size on colour, as well as which molecular components are responsible for the shellac luminescence, are likely to shed additional light on these topics.

Although the data presented in this study were mainly obtained from freshly made mock-up samples, some considerations about the relevance of these results to historical samples are presented. First, a pilot study was carried out to investigate the effects of ageing on the lac dye and shellac components. The textiles were considered for this preliminary study, mainly because they represent a simpler system compared to the pigments, which would have required being made into paints with various binding media and then aged. Questions related to binding medium-pigment interactions would have to have then been considered, which are deserving of a larger dedicated study, currently ongoing.

The four textiles were aged using the conditions presented in section 2.5. Colorimetry and HPLC-DAD-ESI-Q-ToF analyses were performed on the aged textile samples and the results (Tables S–4, Tables S–5 and Figure S-2 – Supplementary Information) indicated slight fading and a relative decrease of the lac colourant molecules compared to the shellac components. These effects occurred to a greater extent for the textile samples that had undergone multiple dye baths (T_C3 and T_H3). This could indicate that in each successive dye bath the colourants added were more weakly bound and thus more easily removed. Furthermore, a slight relative decrease in shellac polyesters was noted for the textile samples that initially contained them. This could be related to the partial hydrolysis of the ester bonds induced by the ageing conditions [23]. These preliminary results generally indicate that degradation affects the colourant molecules much more rapidly than the shellac components. This is in agreement with previous research on the lightfastness of natural organic colourants [68,69]. We can assume that this is generally valid for pigment formulations as well, although the extent of degradation might vary significantly depending on other factors, including binding medium-pigment interactions. In any case, it appears that it may be more likely to find shellac components than lac colourant molecules in heavily aged historic samples.

Previous studies have indeed shown that shellac components are detected in historical objects and, when the use of shellac as a conservation treatment can be ruled out, shellac markers can suggest the presence of a lac dye/pigment. Dyer et al. identified shellac components in several paint samples from a Hellenistic terracotta oinochoe (3rd century BC). However, laccaic acids, which undoubtedly proved the presence of lac dye, were detected in only one of these samples. The shellac markers were therefore used to deduce the use of a lac pigment in all the other samples [17]. The presence of shellac components on lac-dyed textiles was shown by Tamburini et al. on Chinese textiles from Dunhuang (7th-10th century AD) [28]. Also in this case, the laccaic acids were not always detectable and the shellac markers were the only surviving indication of the use of lac. Kirby et al. also identified lac as a lake pigment containing shellac components on two European paintings from the 13th and 17th century [19].

6. Conclusions

Studying lac pigment production through a combined historical and analytical approach has resulted in an improved insight into both the variety of colours and the molecular composition of lac lake pigments and lac-dyed textiles. The experimental parameters that vary the most according to historical recipes, such as temperature and pH, were particularly considered.

The results highlighted that shellac components are transferred into the pigments or dyed textiles under all conditions adopted, even those not promoting the solubility of these components in water, i.e. room temperature and neutral pH and even when lac is re-extracted from textiles and made into a pigment. Shellac was found to be responsible for the luminescence exhibited by most pigments and the intensity of the luminescence appeared to be directly related to the relative amount of shellac, apart from a few samples. In addition, it was found that VIVL imaging in the range c. 540–700 nm was particularly efficient at detecting the presence of included shellac in lac lake pigments. This may provide an initial key indication of the presence of these components, and hence also of the presence of lac lake pigments when inspecting painted surfaces. The type and relative amounts of shellac components were dependent on the production method and varied greatly according to the experimental parameters. Generally, alkaline conditions and high temperatures favoured the solubilisation of shellac, whereas alkaline conditions and room temperature resulted in the highest relative extraction yield of lac colourants.

It was therefore concluded that, until the industrial revolution, when more refined production methods came into practice, shellac components can be expected to be found together with lac colourants in historical objects. From a preliminary study of artificially aged lac-dyed textiles, it was shown that lac colourants are more prone to degradation than shellac components. The endurance of the latter may mean that these are more reliable markers for the presence of lac. However, the identification of these colourless molecules was only possible as a result of the sensitivity afforded by mass spectrometric detection methods, and the application of this technique is thus recommended.

Not all the variations observed in the samples could be explained by the analytical results and the effect of the precipitation method on the resulting pigment morphology and on the final colour has not yet been studied in detail for lake pigments. In addition, future studies could assess the effects of using more moderate alkaline pH values and temperatures during the extraction of colourants and the contribution of the various shellac components to the luminescence properties observed. The affinity of shellac components for wool compared to silk is another interesting aspect for investigation. The study of the degradation of lac lake pigments in different binding media would also provide interesting insights into both the working properties of lac pigments (direct and extracted from textiles) and the possible difference in degradation between the colourant and the shellac components.

These conclusions represent an improved and valuable insight into both the variety of colours and the molecular composition of lac lake pigments and lac-dyed textiles and is here presented for onward technical and art historical evaluation of its significance by scientists, conservators and art historians with the intention that this knowledge may aid the future detection of lac in historical objects.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2019.107579.

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