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Identification of iron gall inks, logwood inks, and their mixtures using Raman spectroscopy, supplemented by reflection and transmission infrared spectroscopy

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Abstract

Raman spectral references for various formulations of iron gall inks, logwood inks and mixtures of both types were acquired during this study. The samples, either liquid or dry inks and applied on paper, were analysed using a portable Raman spectrometer, both with 852 and 785 nm excitation lines, and a confocal dispersive Raman microscope with two different excitations, namely 785 and 532 nm. It was found that longer wavelengths are more suitable for the analysis of iron gall inks and that the spectral response of logwood inks is relatively more dependent on the excitation line. For the mixtures, it is suggested analyses be performed with both longer and shorter wavelengths, since with a 785 nm line mainly the characteristic features of the metal-polyphenol complex in iron gall inks are detected, while the spectra collected with a 532 nm excitation contain information on the presence of logwood inks. The study was complemented by non-invasive reflectance infrared spectroscopy, which enabled the detection of tannic acid in iron gall inks and in the mixtures, and by micro-Fourier transform infrared (FTIR) spectroscopy to complement the reference set. The study aims to contribute to the development of non-invasive protocols for identification of inks in historic and artistic works on paper, such as manuscripts and drawings.

Keywords Ink, Iron gall, Logwood, Mixtures, Raman spectroscopy, Infrared spectroscopy

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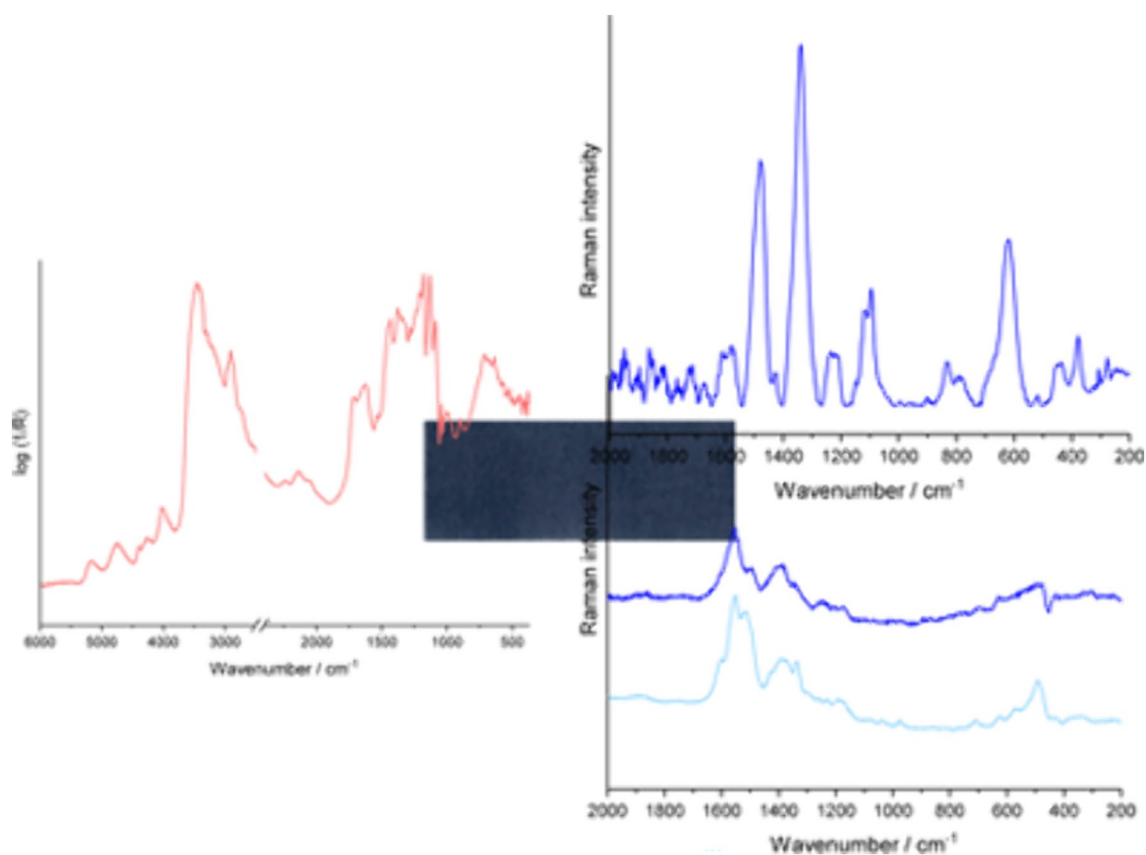
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Graphical Abstract



Introduction

The characterisation of cultural heritage is often a very challenging task due to the complexity and heterogeneity of the samples. Particularly in the case of inks, the different formulations that vary over the centuries or in relation to different regions present a further challenge that can further complicate the analysis due to the variable composition.

Logwood was often used to produce dye for colouring silk, cotton and linen [1, 2]. It was also used to make inks. The latter are made from logwood (either as an extract or in the form of chips) and combined with various metal salts and a binding agent, usually gum arabic [3]. However, there are also formulations that do not require a binder, such as inks made with chrome and logwood [3]. Logwood originates from Central America. The export of wood to Europe increased at the end of the nineteenth century, with France exporting most of the logwood from Haiti to France [4], although imports had already taken place before this, for example the first English trade in logwood began in the second half of the seventeenth

century [5]. This is probably also the reason why a large proportion of postcards in France during this period were written with logwood ink (chrome), in addition to many personal letters, as analysed by Neevel et al. [4]. In the nineteenth century, logwood with different salts (chrome (Cr), copper (Cu), iron (Fe)) were used, often also with the combination of different salts [6]. The colour or hue of the ink depends on the choice of metal salt, for example, it can take on a purple hue when using aluminium salt and be black when using iron salt. The presence of Fe-logwood has already been proven in a manuscript from the eighteenth century [7]. But even if an ink could be made with iron, it was often used as an additive to iron gall inks that were not black enough, and many recipes mention the use of both galls and logwood to make ink, also for production of copying inks [3, 8]. Logwood inks are usually described as metal complexes with hematein or its degradation products, which are formed during ink production [9]. Vibrational (Raman) spectroscopy proved to be successful for the detection of logwood, as it provided characteristics of Al [10], Cr [11] and Fe or Cu/Fe

[9] -logwood inks or it provided spectral data on their components [12–14]. Other analytical techniques such as chromatography and mass spectrometry [15, 16] were also used to analyse logwood, but mainly to characterise it as a colourant used for paintings or textiles. Logwood was identified in real cultural heritage objects such as historical documents, manuscripts and drawings [7, 11, 17, 18].

Iron gall inks were a common choice for ink for many centuries and were still used in the 19th and even the twentieth century. Over time, it has been shown that their corrosive properties attack the metal nibs on the one hand and also attack the carrier through catalytic degradation on the other. The degradation of the iron gall ink containing paper [19, 20] (e.g. discolouration, embrittlement) has often been studied and the main reasons are attributed to acid-hydrolysis and metal-catalysed oxidation of the cellulose or other components of the paper [21]. To prevent corrosion of metal nibs, Cr-logwood, for example, has also been introduced as an alternative [4]. In order to improve the colouring properties of iron gall inks, other dyes such as madder and indigo (e.g. the alizarin ink patented by Leonhardi), but also others such as aniline dyes and logwoods were often added [6]. Chemically, iron gall ink is a complex of metals with polyphenols found in galls (gallotannins). Several structural models have been proposed in the literature, the most common being the gallate structure [22, 23], which was further demonstrated by Ponce et al. [24] that the main colourant is an amorphous form of an octahedral Fe(III) gallate metal organic framework structure. Many different analytical methods have been used to analyse iron gall inks, e.g. laser-induced breakdown spectroscopy, XRF, Mössbauer and vibrational spectroscopy, chromatographic techniques [25–29]. Although some techniques can provide meaningful results about the composition of inks, they often require destructive or micro-destructive procedures (such as chromatographic techniques). However, the molecular composition can be determined non-invasively by vibrational spectroscopy. Raman spectroscopy has been used with iron gall inks to fill in the gaps in their characterisation, as it can provide information about the bonding or orientation in these metal complexes depending on the position and separation of the bands [24]. Recent studies [30] have incorporated theoretical calculations that provide detailed assignments of vibrational modes, serving as a basis for further in-depth analysis of ink structures. Additionally, these studies have demonstrated that Raman bands are influenced by factors such as pH, polyphenol structure, and metal concentration. In addition, FTIR spectroscopy can provide information about the molecular composition of the binding medium and the metal salts used in ink preparation [9].

The combination of both mentioned vibrational spectroscopic techniques, especially as they can be applied non-invasively and with portable equipment, makes them highly suitable for analysing historical works on paper in archives, libraries and galleries. This work includes vibrational spectroscopic research on iron gall inks, logwood inks and mixtures of both types. In general, different inks share many material components (e.g. similar polyphenols in galls and logwood, metal salts for ink formation, etc.), resulting in similar material properties when analysed. In addition, the presence of multiple colourants in the inks can present a difficult analytical task, requiring the use of different methods for their analysis, which also depend on the sensitivity of the technique and are often applied destructively. Therefore, the aim of this study was to create a vibrational spectral knowledge base for the evaluation of different inks of iron gall ink and logwood ink and their mixtures using non-invasive methods. Successful identification and characterisation of inks is the starting point for their further conservation and designing the appropriate preventive conservation plans. For this reason, Raman spectroscopy was used with a portable handheld Raman spectrometer, which enables on-site characterisation, as well as Raman microscopy with different excitation lines, which also enables non-invasive analysis but is limited due to the size of the cultural material. The aim of this study was also to complement the Raman spectral data with non-invasive FTIR (Fourier-transform infrared) reflectance spectroscopy to investigate its performance for analysis of inks and further also with micro-FTIR transmission spectroscopy.

Methods/experimental

Sample preparation

The materials/chemicals used in this study include tannic acid (Sigma-Aldrich 403040), iron (II) sulphate heptahydrate (Sigma-Aldrich 215422), copper (II) sulphate pentahydrate (Sigma-Aldrich 209198), oak apples, gallnuts (Kremer 37400), gum arabic from acacia tree (Sigma-Aldrich G9752), natural logwood extract (Kremer 36110), logwood cut pieces (Kremer 36100), aluminium potassium sulphate dodecahydrate (Honeywell 31242), hematein (Sigma-Aldrich 51230), hematoxylin (Sigma-Aldrich H3136), and potassium dichromate (Sigma-Aldrich P5271). Solutions were prepared with ultrapure Mili-Q water.

The ink samples were prepared following historical recipes [3] and recipes reported by other authors [9, 11, 31]. The inks were applied to aluminium foil to dry (referred to in the text as dry inks). In addition, the inks were applied to Whatman™ paper No. 1 with a brush (labelled with prefix P1_), usually with one stroke. Liquid inks were stored in a refrigerator at 4°C.

Iron gall inks (tannic acid-based inks)

As historical iron gall inks often contain other transition metal ions [21], such as copper, in addition to iron, we prepared inks with different ratios of copper to iron based on literature recipes [31]. All these inks consisted of 31.4 gL⁻¹ gum arabic, 49.2 gL⁻¹ tannic acid, 0.15 molL⁻¹ of copper (II) sulphate and iron (II) sulphate. Copper and iron sulphates were added in different proportions to achieve two different copper to iron molar ratios (Cu (II): Fe (II)), namely 0.35 and 0.7. To achieve a 0 ratio, only iron sulphate was added (7.5 mmol, in 50 mL). The inks are labelled according to their copper to iron molar ratios, as follows: Cu/Fe-TAN0, Cu/Fe-TAN0.35 (1.95 mmol copper, 5.55 mmol iron), and Cu/Fe-TAN0.7 (3.1 mmol copper, 4.4 mmol iron). As a reference, an ink labelled as Cu/Fe-GAL0 was also prepared according to the same recipe as for Cu/Fe-TAN0, but using gallic acid instead of tannic acid (the same mass).

Logwood inks

Logwood inks were made from logwood chips or logwood extract [3, 9, 11]. If the recipe called for chips but an extract was used, the quantity of the extract was one quarter of the amount of chips recommended, as suggested by Lehner [3]. The iron logwood (Fe-logwood) ink was prepared as follows: 0.75 g of logwood extract were boiled in 50 mL of ultrapure water. After cooling, 60 mL of a solution containing 3 g of ferrous sulphate and gum arabic in 8% acetic acid were added. To prepare chrome logwood (Cr-logwood) ink, 0.005 g of potassium chromate were dissolved in 50 mL of ultrapure water, 1 g of logwood extract was added, and the solution was heated for about 30 min. The aluminium logwood (Al-logwood) ink was prepared as follows: 10 g of logwood chips were boiled in 45 mL of ultrapure water, and 1 g of gum arabic and 0.4 g of alum dissolved in 5 mL of hot water were added.

Mixture of iron gall and logwood inks

A mixture of iron gall and logwood inks (Cu/Fe-GALLS/LOG) was prepared by using a slight modification of the recipe recommended for the Ribancourt's Campeachy ink [3] as follows: 2 g of logwood chips in 25 mL of water were boiled in a closed beaker for 1–2 h. The decoction was filtered, slightly cooled, and then 4 g of crushed galls, 2 g of ferrous sulphate, 0.5 g of copper sulphate, 1.5 g of gum arabic, and 0.5 g of sugar were added. For comparison, reference inks with a smaller number of components were made, by using just some of the components listed above and keeping their ratios. Fe-LOG and Cu-LOG were prepared as follows: 0.5 g of logwood extract were boiled in 50 mL of water until half of the liquid

evaporated. After cooling, 1.5 g of gum arabic and 2 g of ferrous sulphate or 0.5 g of copper sulphate were added. Fe-hem and Cu-hem were prepared as follows: 0.5 g hematein were boiled in 25 mL of water. After cooling, 1.5 g of gum arabic and 2 g of ferrous sulphate or 0.5 g of copper sulphate were added. Fe-TAN and Cu-TAN were prepared as follows: 4 g of tannic acid were dissolved in water. Then 1.5 g of gum arabic and 2 g of ferrous sulphate or 0.5 of copper sulphate were added.

Methods

Raman spectroscopy with a portable spectrometer

A BRAVO handheld Raman spectrometer (Bruker Optics GmbH, Germany) equipped with Duo LASERTM excitation (785 and 852 nm) and SSETM (Sequentially Shifted Excitation) patented fluorescence mitigation was used. The experimental parameters and the calibration were automatically adjusted; the spectrometer also has an automatic baseline correction. Band positions (in the region 1800–200 cm⁻¹) were defined in OPUS 7.0 software. The spectra of the liquid samples were collected in a glass vial. For the spectra of the dry samples, inks were applied to the aluminium (Al) foil to dry.

Raman microscopy

A Bruker SENTERA II dispersive Raman microscope with 785 and 532 nm laser excitation lines and a multi-channel thermo-electrically cooled CCD detector was used. The spectra were recorded using a 50x (long working distance) or 100x objective lens, and a 400 grooves/mm grating which gave a spectral resolution of approximately 4 cm⁻¹. Experimental parameters were adjusted for each sample. Typically, for the 785 nm line, a power of ~1–25 mW (1–25%, output laser power) was used, and integration times were set between 10 and 200 s, with 2 or 4 accumulations. For the 532 nm excitation, a power of ~0.25–6.25 mW (1–25%, output laser power) was used, with integration times between 1 and 100 s and 2 or 4 accumulations. For the spectra of the dry samples, inks were applied to the aluminium (Al) foil to dry. Band positions (in the region 1800–200 cm⁻¹) were defined in OPUS 7.0 software. Spectra are displayed without manipulation (e.g., smoothing or baseline correction), only shifted on the y-axis or scaled for the sake of comparison.

FTIR spectroscopy with a portable spectrometer

Reflection FTIR analyses were carried out using a portable ALPHA-R spectrometer (Bruker Optics, Germany/USA-MA) equipped with a dedicated external reflection module, which allows contactless and non-destructive FTIR analysis with a room-temperature deuterated l-alanine-doped triglycine sulphate (DLαTGS) detector and a 25/25° optical layout. Samples of inks applied on paper

were placed in front of the instrument approximately at a 1 cm distance. An integrated video camera provided a view of the approximately 28 mm² sampling area. Pseudo-absorbance spectra [$\log(1/R)$; R =reflectance] spectra were collected in the 8000–380 cm⁻¹ range with 160 scans, and a 4 cm⁻¹ resolution. The background was acquired using a gold flat mirror.

FTIR microscopy

FTIR analyses of dry inks were performed using Bruker's TENSOR II FTIR spectrometer coupled to a Hyperion 3000 FTIR microscope with a liquid nitrogen cooled MCT detector. Small amounts of dry inks (a small amount of the dry samples prepared on aluminium foil) were placed between the windows of a diamond anvil cell, examined under the microscope, and transmission spectra recorded using a 15× objective. Absorbance spectra in the 4000–600 cm⁻¹ range were acquired with 64 scans and a 4 cm⁻¹ resolution. The system was operated with the proprietary 32-bit software OPUS 8.1 (Bruker Optik GmbH) for measurements and analyses. For analyses in the transmission mode, three spectra were collected on

each sample, normalised, baseline corrected and averaged using the OPUS 7.0 software.

Results and discussion

Iron gall inks

The spectra of the iron gall inks collected with a portable Raman spectrometer are shown in Fig. 1, and the main Raman bands are summarised in Table 1. Iron gall inks can be distinguished by three strong features reported at ca. 1475 (ν_1), 1343 (ν_2) and 603 cm⁻¹ (ν_3), characteristic of the metal complex (metal polyphenol complex) [30].

For the three inks prepared with different Cu/Fe molar ratios: 0 (iron only, Cu/Fe-TAN0), 0.35 (Cu:Fe=0.35, Cu/Fe-TAN0.35) and 0.7 (Cu:Fe=0.7, Cu/Fe-TAN0.7), no significant differences in the positions and relative intensities of these three main bands were observed in the spectra collected with the portable Raman spectrometer (Table 1, Fig. 1). A difference is in the lower wavenumber region, where for the dry Cu/Fe-TAN0.7 ink, a relatively high intensity band is observed at ca. 298 cm⁻¹ that could be assigned to a $\nu(\text{Cu}-\text{O})$ mode, [30] a feature also reported for CuO [32]. Bands ν_1 and ν_2 are attributed

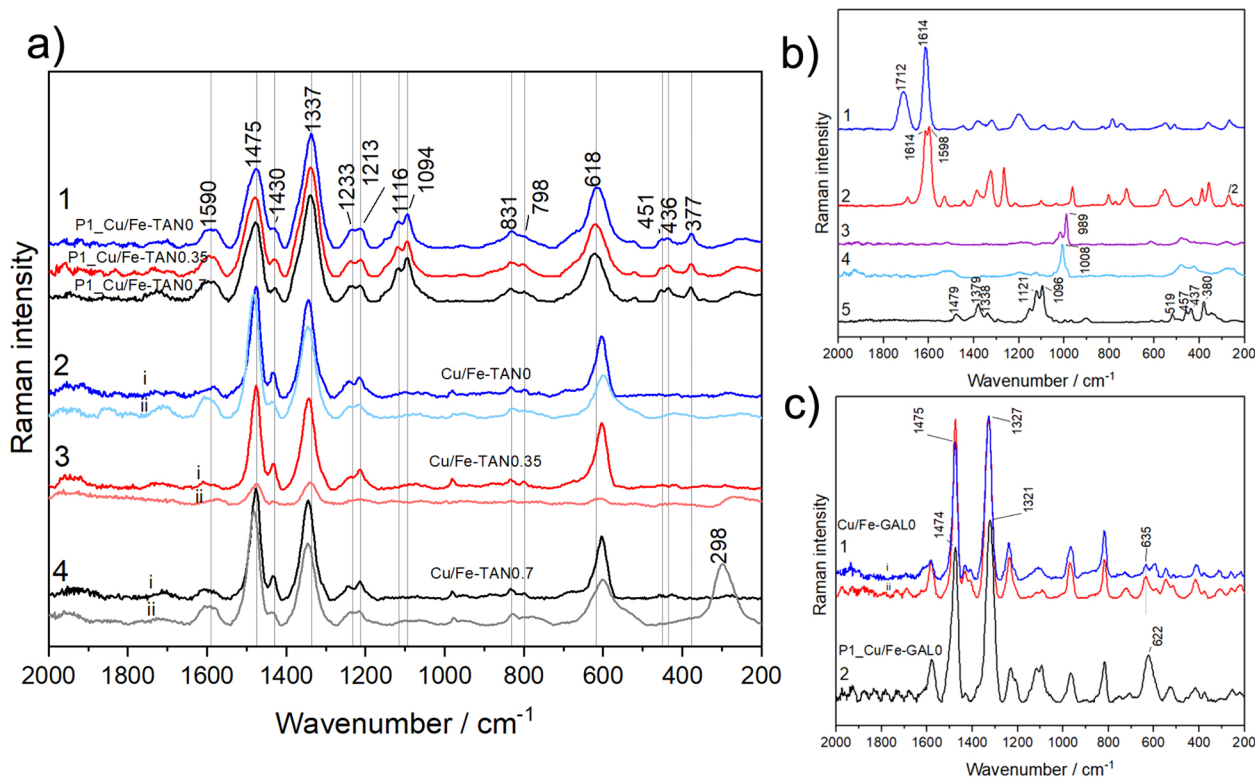


Fig. 1 **a** Raman spectra of various iron gall inks (Cu/Fe-TAN0, Cu/Fe-TAN0.35, Cu/Fe-TAN0.7) on (1) paper (P1_) and 2, 3, 4) in i-liquid and ii-dry form. **b** Raman spectra of reference materials: (1) tannic acid, 2) gallic acid, (3) ferrous sulphate, (4) copper sulphate, (5) Whatman paper. **c** Raman spectra of (1) ink prepared from gallic acid (Cu/Fe-GAL0) in i-liquid and ii-dry form and (2) ink Cu/Fe-GAL0 on paper (P1_Cu/Fe-GAL0). The spectra were recorded with a portable Raman spectrometer (785 and 852 nm excitations). The spectra are shifted on the y-axis for the sake of clarity and scaled where necessary

Table 1 Raman bands (cm^{-1}) for iron gall inks prepared with different copper to iron molar ratios, measured with a portable Raman spectrometer using 852 and 785 nm excitations, on the liquid and dry inks; and by Raman microscopy, acquired with 785 and 532 nm lines, on the dry inks

Instrument sample excitation	Raman microscopy*		
	Portable Raman*	Dry ink 852 and 785 nm	Dry ink 532 nm
Cu/Fe-TAN0	1582w, 1475vs, 1435m, 1343vs, 1240m, 1215m, 1102w, 1067w, 1045w, 980w, 832w, 799w, 793w, 694vw, 663vw, 603s, 415vw, 279vw(br)	1712w(br), 1606m(br), 1483vs, 1436m, 1345vs, 1238w, 1218m, 1062vw(br), 977vw, 950vw, 825w, 779vw, 600s, 532sh, 420vw (br), 254vw(br)	1708w(br), 1599sh, 1576m, 1475vs, 1435m, 1344vs, 1242m, 1218m, 1081w(br), 995vw, 953w(br), 828m, 799m, 777m, 702vw, 599vs, 564sh, 381w(br), 236w(br)
	1710 w(br), 1609w, 1580 (sh), 1476vs, 1432m, 1343vs, 1236w, 1213m, 1071 (br), 981w, 880vw, 833w, 800w, 674vw, 653 (sh), 604s, 450vw, 414vw, 292 vw	1581m(br), 1478vs, 1435w, 1340vs, 1242sh, 1218m, 1134w, 1059w, 996vw, 960vw, 942vw, 826vw, 760vw, 682vw, 616sh, 605m, 514vw(br), 422w(br), 271m(br)	1606w, 1580w(br), 1479s, 1431w, 1341s, 1232w, 1212w, 1095vw(br), 1057vw(br), 1038vw(br), 956vw(br), 824m, 799m, 754m, 596vs, 420vw, 222vw(br)
	1714 vw(br), 1607w (br), 1476vs, 1433m, 1344vs, 1241w, 1214m, 980w, 954vw, 886vw, 872vw, 833w, 800vw, 680sh, 603s, 426vw, 288 vw(br)	1722vw(br), 1606m, 1592m, 1578m, 1482vs, 1438w, 1346vs, 1238m, 1215m, 1103vw(br), 1063vw(br), 977w, 959vw, 798vw, 778vw, 760sh, 936vw, 829w, 778vw, 601s, 542sh, 298s	1713w(br), 1613m, 1571sh, 1501sh, 1483w, 1444vw, 1353m, 993w, 603vw(br)
Cu/Fe-TAN0.35			1713w(br), 1613m, 1487m, 1353m, 1203vw(br), 1095vw(br), 787vw(br), 596w, 553sh
Cu/Fe-TAN0.7			1710m(br), 1609s, 1585sh, 1482s, 1442vw, 1352s, 1246w, 1220w, 1098vw(br), 1012vw, 992w, 793w(br), 599m(br), 550sh

* vw very weak, w weak, m medium, s strong, vs very strong, sh shoulder, br broad

to benzene vibrations/ring stretching coupled with C-O stretching and C-H bending, while ν_3 is due to Fe-O stretching [30]. The band ν_3 is broader in the spectra of the dry inks, while ν_1 shifts slightly to higher wavenumbers for Cu/Fe-TAN0 (from 1475 to 1483 cm^{-1}) and for Cu/Fe-TAN0.7 (from 1476 to 1482 cm^{-1}). A band at ca. 980 cm^{-1} , assigned to a stretching of the sulphate group [33] in ferrous sulphate, is present in the spectra of the liquid inks. In all samples, the concentration of ferrous sulphate was higher than that of copper; the band characteristic of copper sulphate [34] is at 1008 cm^{-1} and is not present in the spectra of the inks (comparison to Fig. 1a, b, spectra 3,4). In addition, weak bands may appear at ca. 1712, 1606 cm^{-1} indicating tannic acid [28, 30] (Fig. 1b, spectrum 1), probably in excess, although the detection may also depend on the location of the measurement as the samples are not homogenous. The spectra of the inks on paper (Fig. 1a) show characteristic bands at ca. 1590, 1475, 1430, 1337, 1223, 1213, 831, 798, 618 cm^{-1} characteristic of metal complex characteristic of iron gall inks, in addition to the bands due to cellulose at ca. 1116, 1094, 451, 436, 377 cm^{-1} . In Fig. 1b, a spectrum of Whatman paper with features at ca. 1479, 1379, 1338, 1121, 1096, 519, 457, 437, 380 cm^{-1} characteristic of cellulose [35, 36] is presented. The position of bands ν_1 and ν_2 recorded on inks on paper are comparable to the spectra of liquid inks, but the Fe-O band (ν_3) shifts to higher wavenumbers, from ca. 603 to 618 cm^{-1} . This band is sensitive to different parameters such as binding and pH [30]. A wider range for the position of this band was reported by other authors [37, 38]. Furthermore, ageing was observed to influence the position, shifting it for as much as $\sim 30 \text{ cm}^{-1}$ (results not included in this study).

Although, the structure of iron gall inks has not been fully determined due to the complex (macromolecular) characteristics of natural tannins, the colouring effect is attributed to a metal-polyphenol complex [30]. The inks in this study were made from tannic acid, a compound found in galls; this commercial material is a mixture of gallotannins and galloylglucoses, which according to some authors may be even more complex than the gall extracts themselves [39]. The three main characteristic Raman bands of the metal-polyphenol complex are sensitive not only to the chemical environment (such as pH) but also to the type of polyphenol [30]. Espina et al. [30] analysed the marker Raman bands of iron complexes with tannic acid, gallic acid, pyrogallol and syringic acid. According to their results and further Raman measurements (Fig. 1c) on an ink prepared according to the same recipe as Cu/Fe-TAN0, but with the tannic acid replaced by gallic acid (labelled Cu/Fe-GAL0), it can be assumed that for all three iron gall inks (in liquid/dry form and on paper) the dominant signal in the spectra (Fig. 1a)

belongs to the complex formed between the metal and tannic acid. The Raman bands in the spectrum (Fig. 1c) of the Cu/Fe-GAL0 ink, in liquid and dry forms and on paper, are attributed to the complex formed between iron and gallic acid with the bands at ca. 1474, 1327 and 622 cm^{-1} when applied on paper and with bands at ca. 1475, 1327, 635 cm^{-1} in the spectra of liquid and dry inks (not applied on paper). The interaction and appearance of bands suggesting the interaction of metal with gallic acid could be confirmed also by the comparison to the spectrum of gallic acid (Fig. 1b, strong bands at 1614, 1598 cm^{-1}), and also aligned with the literature results [30]. The main difference between the spectra prepared from tannic acid or gallic acid lies in the position of the ν_2 band, which shifts to lower wavenumbers (to ca. 1321 cm^{-1}) in the case of ink prepared from gallic acid and ferrous sulphate (Fe-gallic acid complex). The difference between ν_1 and ν_2 therefore increases from 132 to 153 cm^{-1} . Moreover, the Fe-O (ν_3) band appears in the spectra of Cu/Fe-GAL0 ink at higher wavenumbers than in the spectra of Cu/Fe-TAN0 (Fig. 1c). This differs from the literature [30, 39], but this band could most likely also be influenced by the wavelength of excitation of Raman signals. However, the position of the mentioned band is comparable in the spectra on inks Cu/Fe-GAL0 or Cu/Fe-TAN0 applied to paper.

The spectra of the iron gall inks collected with a Raman microscope are shown in Fig. 2, and the main Raman bands are summarised in Table 1. The Raman spectra (Fig. 2a, b) of the inks acquired by Raman microscopy were comparable to those recorded with the portable instrument (Fig. 1), showing bands at ca. 1483–1475, 1352–1341, 603–596 cm^{-1} characteristic of the Fe-polyphenol complex [30]. In this case, only the spectra of the dry inks (dried on aluminium foil) are shown, as the signals from the glass (background) often interfered when the liquid inks were analysed in glass vials. A small difference was observed in the spectra of the inks (dry, not applied to paper) recorded with a 532 nm excitation: the band at ca. 1612 cm^{-1} , due to tannic acid [30], has a higher relative intensity. When applied on paper, the mentioned inks could be detected using a 785 nm excitation; in this case the characteristic bands appear at ca. 1476, 1336, 600 cm^{-1} (Fig. 2a). It was observed that, when the results are compared to those obtained with a portable Raman spectrometer, the $\nu(\text{Fe-O})$ band in the spectra of dry inks and of inks on paper does not shift significantly. The signals in the spectra recorded with a 532 nm excitation for the inks on paper were obscured by fluorescence from the paper (results not shown). It should be noted that the inks were applied to Whatman paper, which has no sizing or impregnation and can therefore absorb the ink well. This could explain the

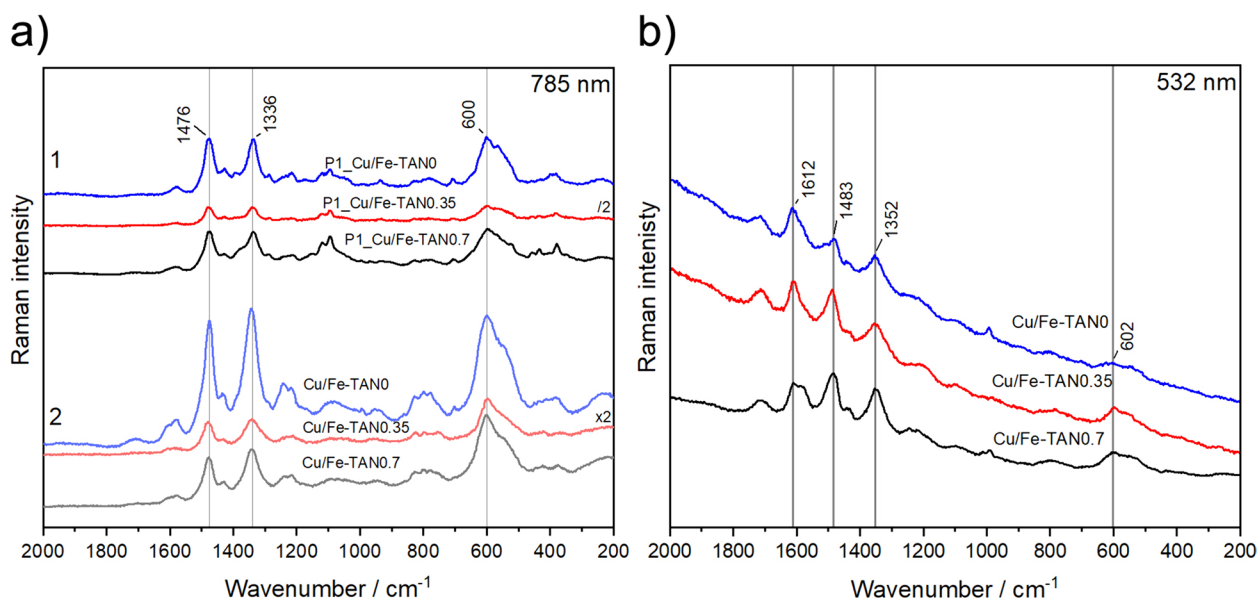


Fig. 2 **a** Raman spectra of various iron gall inks (Cu/Fe-TAN0, Cu/Fe-TAN0.35, Cu/Fe-TAN0.7), applied to (1) paper (P1) and (2) in dry form, recorded with 785 excitation wavelength. **b** Raman spectra of various iron gall inks (Cu/Fe-TAN0, Cu/Fe-TAN0.35, Cu/Fe-TAN0.7) in dry form, recorded with 532 excitation wavelength (the spectra are shifted on the y-axis for the sake of clarity and scaled where necessary)

possibility of lower concentrations of ink on the surface, which could complicate the Raman analysis. It can be seen that the Raman analysis with the 785 nm excitation line provides a better signal-to-noise ratio for analysing iron gall inks in general.

Reflection FTIR spectra of the paper substrate P1 and of the iron gall inks Cu/Fe-TAN0, Cu/Fe-TAN0.35, and Cu/Fe-TAN0.7 applied to paper P1 are illustrated in Fig. 3a. No significant differences among the spectra recorded on the three inks with different copper to iron molar ratios are observed. In all the FTIR spectra collected in the reflection mode for the iron gall inks, only two characteristic bands assigned to residual tannic acid are visible at ca. 1714 cm^{-1} (stretching vibrations of carbonyl group) and 1535 cm^{-1} (in plane deformation (C-H) of benzene ring) [40, 41]. The rest of the bands are hindered by cellulose signals from the paper. The absence of other characteristic bands of inks is most likely due to the physical properties of Whatman paper, as mentioned in the previous Raman paragraph.

Transmission FTIR spectra of the dry iron gall inks Cu/Fe-TAN0, Cu/Fe-TAN0.35, Cu/Fe-TAN0.7 are presented in Fig. 3b. All these spectra show distinctive signals of tannic acid at ca. 3394, 1712, 1615, 1536, 1447, 1325, 1203, 1088, 1035, 872 and 759 cm^{-1} [40, 41]. All the inks investigated contain components whose signals overlap in the mid-IR range. The two strong bands in all three FTIR spectra of dry inks at ca. 1615 and 1088 cm^{-1} could be present due to tannic acid and/or gum arabic binder

and/or Fe- and Cu-sulphates [40–43]. Both tannic acid and ferrous sulphate have a band at ca. 1035 cm^{-1} which can also overlap. This band has the highest intensity in the spectrum collected on Cu/Fe-TAN0. This spectrum also shows a weak band at ca. 667 cm^{-1} that could be assigned to ferrous sulphate [43]. Moreover, the strong absorbance at 1088 cm^{-1} and weak signal at 977 cm^{-1} in the transmission FTIR spectra of Cu/Fe-TAN0.35 and Cu/Fe-TAN0.7 could be associated with vibrations of copper sulphate, whereas a shoulder at ca. 1646 cm^{-1} in Cu/Fe-TAN0.35 and Cu/Fe-TAN0.7 spectra could be assigned to a ferrous sulphate vibration [43]. The features in the FTIR spectra acquired in the transmission mode on dry iron gall inks depend on the ratio between iron and copper salts used to prepare them.

Logwood inks

The spectra obtained on the logwood inks applied on paper using a portable Raman spectrometer were compared with the spectra of the inks in the liquid and dry forms. The bands observed in the liquid and dry inks are listed in Table 2 and the spectra are shown in Fig. 4. The Fe-logwood ink can be detected on paper by the strong characteristic bands at ca. 1491, 1346 and 581 cm^{-1} , in addition to the bands due to cellulose (Fig. 4, spectra 1, 2). These ink bands may be assigned to the metal complex formed between iron and hematein. A similar spectral profile was observed by other authors, such as Bicchieri et al.[7], who analysed

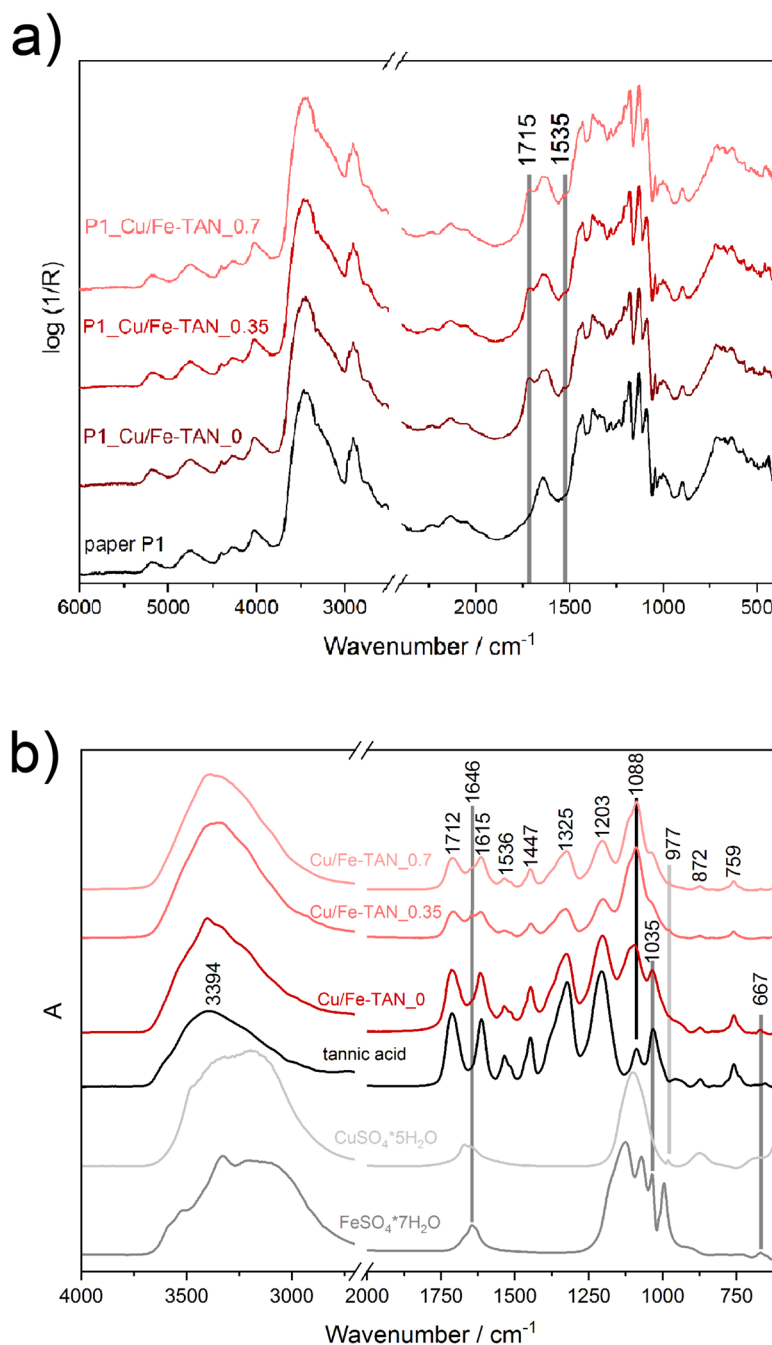


Fig. 3 **a** Reflection FTIR spectra of substrate (paper P1) and iron gall inks (P1_Cu/Fe-TAN0.7, P1_Cu/Fe-TAN0.35 and P1_Cu/Fe-TAN0) applied on paper P1. **b** Transmission FTIR spectra of dry iron gall inks (Cu/Fe-TAN0.7, Cu/Fe-TAN0.35 and Cu/Fe-TAN0) and reference materials (tannic acid, ferrous sulphate and copper sulphate)

Fe-logwood by Raman spectroscopy with a 785 nm excitation. The spectrum of hematein recorded with a portable Raman spectrometer shows bands at ca. 1575, 1515, 1435, 1364, 1338, 1220, 1194, 1166, 1138, 699, 660, 623, 547, 453 cm^{-1} (Fig. 4b, spectrum 1) which are not visible in the spectra of the ink and suggest the

formation of a complex with iron and/or the presence of hematein degradation (breakdown) products formed during ink production [9]. However, the spectrum could to some extent be confused with the spectrum of iron gall ink (Fig. 1). Bronzato et al. [9] analysed Fe-logwood inks by mass spectrometry with an electrospray

Table 2 Raman band positions (cm^{-1}) for logwood inks prepared with different metals, Fe-logwood, Cr-logwood, Al-logwood, measured with a portable Raman spectrometer, using 852 and 785 nm excitation lines, on the liquid and dry forms; and by Raman microscopy on the dry inks using 785 and 532 nm excitations

Instrument sample excitation	Portable Raman*		Raman microscopy*	
	Liquid ink 852 and 785 nm	Dry ink 852 and 785 nm	Dry ink 785 nm	Dry ink 532 nm
Fe-logwood	1606w, 1499vs, 1452sh, 1437sh, 1386sh, 1353m, 1297w(br), 1255vw, 1236w, 1200w, 1172sh, 1155sh, 1078vw(br), 1043w, 979vw, 972 sh, 892w, 861sh, 813vw(br), 736w, 712w, 628m, 578s, 495w, 447vw, 385sh, 363w(br), 266vw(br)	1606w, 1499vs, 1433vw, 1388sh, 1353m, 1301w(br), 1253w, 1234w, 1201w, 1177sh, 1084vw, 1041w, 975w, 889vw, 865vw, 815vw, 738w, 713w, 629m, 579s, 497w, 451vw, 422vw, 366w(br), 256vw(br)	1602vw, 1501s, 1434vw, 1393sh, 1350m(br), 1290vw, 1255vw, 1232w, 1200w, 1192vw, 1172vw, 1081vw(br), 1040w, 976w, 859w, 815w(br), 773vw, 735m, 711m, 681vw, 628m, 577vs, 534sh, 495m, 447w, 4258vw, 363w(br), 258w(br),	1599sh, 1554vs, 1524sh, 1391m (br), 1339w, 1260vw, 1234vw, 1197vw, 1174vw, 765vw, 713w (br), 628w (br), 569sh, 495m, 436w, 332vw(br),
Cr-logwood	1560w, 1531w, 1381w, 1336m, 1289m, 1155m(br), 1082sh, 1040sh, 977w, 713vw, 633w, 580vw, 508m, 460w, 341w(br), 323sh, 235vw	1522w, 1380w, 1340m, 1293vw, 1149w, 1095vw(br), 1001w, 889vw, 856, 808vw, 772w, 760vw, 739vw, 634w, 584w, 508m, 457w, 428sh, 271w(br)	1522vw(br), 1379vw, 1335w, 1151vw(br), 784w, 735vw, 713vw, 630vw, 522m, 505sh, 460w, 430w, 375vw(br)	1605w, 1558s, 1540sh, 1408sh, 1389m, 1242w(br), 1171vw, 766vw, 696w, 629w, 539w, 510vw, 472w, 436vw, 405vw(br), 302w
Al-logwood	1610vw(br), 1566m, 1509m, 1443vw, 1386w(br), 1341m, 1301w, 1261vw, 1240vw, 1179w, 1152w, 1086w, 1057w, 1039w, 978m, 762vw(br), 540w(br), 327vw(br)	1626vw(br), 1564m, 1509w, 1433w, 1340w, 1297vw, 1200w, 1181sh, 1097w(br), 1054vw(br), 979vw(br), 645w, 553w(br), 429w	/	1578w, 1550m, 1542sh, 1381vw, 1257vw, 516vw(br)

* vw very weak, w weak, m medium, s strong, vs very strong, sh shoulder, br broad

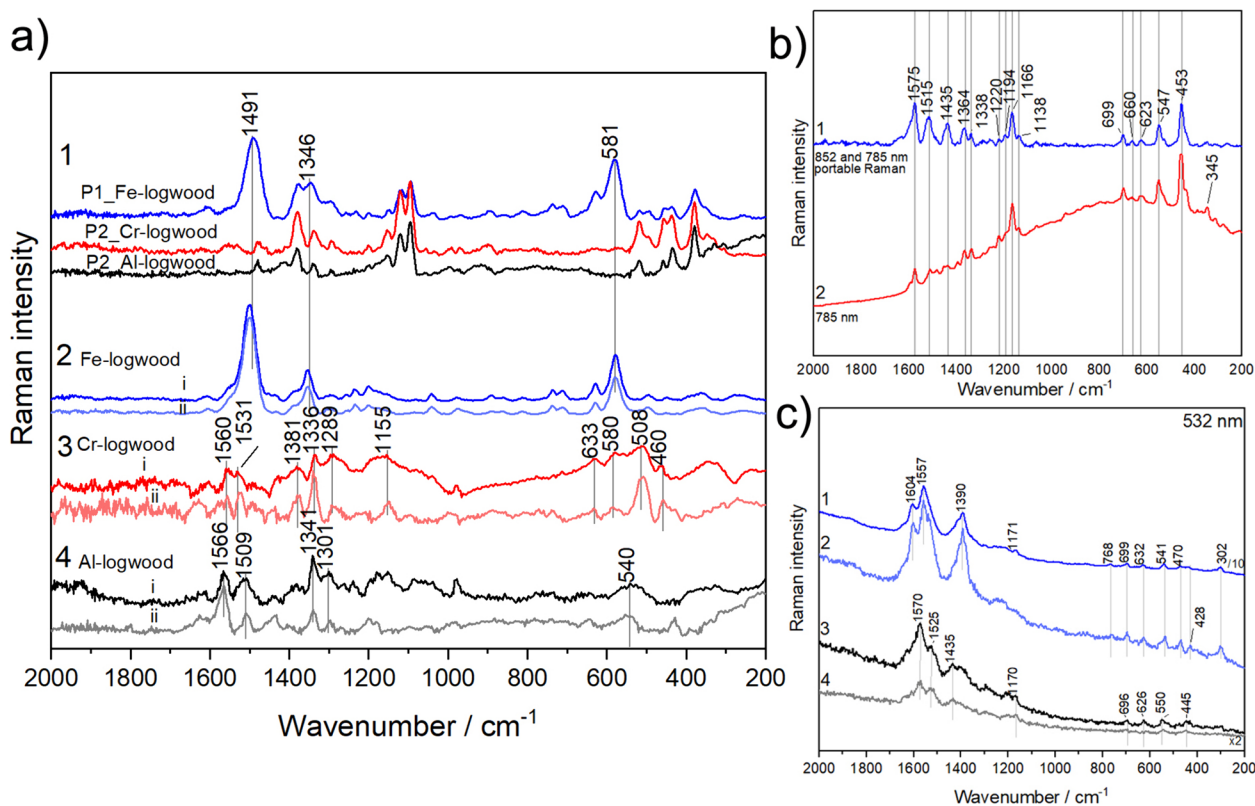


Fig. 4 **a** Raman spectra of various logwood inks (Fe-logwood, Cr-logwood, Al-logwood), applied to (1) paper (P1_) and (2, 3, 4) in i-liquid and ii-dry form, recorded with a portable Raman spectrometer (785 and 852 nm excitations). **b** Raman spectra of hematein, recorded with a portable Raman spectrometer (1) or Raman microscope at 785 nm (2). **c** Raman spectra of hematein recorded with a Raman microscope at 532 using different parameters (all 1% output laser (~0.25 mW) power)—1: 10 s with 2 accumulations, 2: 1 s with 2 accumulations, 3: 2 s with 2 accumulations, 4: 1 s with 2 accumulations. (the spectra are shifted on the y-axis for the sake of clarity and scaled where necessary)

ionization source (ESI-MS) and detected hematein degradation products, such as catechols and bicyclic compounds, which are similar to those reported for iron gall inks. The formation of metal complexes with degradation products could explain the similarities with the spectra of iron gall inks. The main difference with the Raman spectra of the iron gall inks (Fig. 1) acquired with a portable spectrometer is that the position of the most intense band for Fe-logwood applied on paper is at a higher wavenumber (ca. 1491 cm⁻¹); this band is at ca. 1499 cm⁻¹ in the spectrum of the pure ink (liquid and dry), so this could be an indication of the presence of an Fe-logwood ink, which has also been suggested by other authors [7]. Another difference is the position of the Fe–O stretching mode [30], which in the case of Fe-logwood is at lower wavenumber (ca. 581 cm⁻¹), but the position of this band may be affected by ageing, pH, and the ink preparation recipe, as in the case of the iron gall inks. The Cr-logwood and Al-logwood inks applied on paper could not be detected with a portable Raman spectrometer (Fig. 4), as the

signals from cellulose dominated the spectra obtained. In Fig. 4a (spectra 3,4), the spectra of these inks in the liquid and dry states are presented and, even though the signal–noise ratio is not optimal, bands for Cr-logwood are visible at ca. 1560 and 1531, 1381, 1336, 1289, 1155, 633, 580, 508 and 460 cm⁻¹. In the spectra of the Al-logwood ink (in liquid and dry form), bands are observed at ca. 1566 and 1509, 1341, 1301, 540 cm⁻¹. Therefore, these inks can be characterised by features at 1560 and 1531 cm⁻¹ for Cr-logwood and 1556 and 1509 cm⁻¹ for Al-logwood. These bands are shifted in the inks when compared to the pure hematein bands at ca. 1574 and 1514 cm⁻¹ (Fig. 4b), indicating the complexation with the metal. Furthermore, in the spectrum of Al-logwood, no band at ca. 453 cm⁻¹ (an aromatic ring deformation [10] of hematein (Fig. 4b), is detected. In the spectrum of the Cr-logwood ink, there is a band at ca. 460 cm⁻¹, which possibly indicates the presence of free hematein, as relatively small amounts of chromate were used in the production of this ink. In the lower wavenumber range, a broad band around 540 cm⁻¹ is

visible in the spectrum of the Al-logwood and, and in the spectrum of Cr-logwood, weak bands at ca. 633, 580, 508 cm^{-1} are detected (Fig. 4a).

Further investigation with the Raman microscope revealed that the spectra of logwood inks are influenced by the choice of the excitation wavelength (Fig. 5). This is also the case for pure haematein, for which a wavelength dependence of the spectral response is also observed (Fig. 4b, c). The band positions in the spectra of haematein recorded with a portable Raman spectrometer and a Raman microscope with a 785 nm line (Fig. 4b) are comparable; the main difference is a weak band ca. 345 cm^{-1} in the spectrum acquired with the Raman microscope. A similar spectral profile, with bands at ca. 1570, 1525, 1435, 1170, 696, 626, 550, 445 cm^{-1} , was obtained with a 532 nm line (Fig. 4c, spectra 3, 4), but at very low laser power and short exposure times (1 or 2 s at 1% power (~ 0.25 mW) output laser power). A different spectral profile was also obtained with a 532 nm excitation (Fig. 4, spectra 1,2), typically measured at slightly higher laser powers and/or exposure time, in which bands at ca. 1604, 1557, 1390, 1171, 768, 699, 632, 541, 470, 302 cm^{-1} were detected. This spectrum is in agreement with previously published haematein spectra recorded with a 488 nm line [10]. The main difference between the spectra of haematein (Fig. 4b, c) recorded with different spectrometers or with different experimental parameters is in the position of the bands in the spectrum obtained with a portable Raman (852 and 785 nm excitations) or with the Raman microscope (785 nm excitation): at ca.

1574, 1515, 1435 cm^{-1} and a strong band at 453 cm^{-1} compared to the spectrum typically obtained with 532 nm with the band positions at 1604, 1557, 1390, and 470 and 428 cm^{-1} in the lower wavenumber region. The reason for this may be the degradation of haematein under the influence of a higher power laser, the oxidation of haematein, or the possible presence of two different types of haematein (e.g. unoxidised and oxidised), whereas at 532 nm a component in lower concentration is possibly detected but resonantly enhanced. Hulme et al. [44] suggested that the hydroxyl group is the ionisation site for the provision of the deprotonated oxidised neoflavonoid species. In order to clarify this, further work is required by application of DFT studies for example. Furthermore, Al-logwood was not detected by Raman microscopy with a 785 nm line, either as dry ink or applied on paper. The signal was saturated or the interference of the cellulose dominated. Fe-logwood applied on paper, on the other hand, was easily detected (Fig. 5) and the presence of Fe-logwood can be determined from the bands at ca. 1499, 1338 and 578 cm^{-1} . The band at ca. 1490 cm^{-1} or above indicates the presence of Fe-logwood, similar to the spectra obtained with a portable Raman spectrometer when we compare the spectra with iron gall inks. The analysis of the Cr-logwood ink applied on paper by Raman microscopy with a 785 nm excitation line exhibited a low signal to noise ratio (Fig. 5); weak bands at ca. 1517, 1371, 1338, 1151 cm^{-1} , and a feature at ca. 522 cm^{-1} are present which could indicate the presence of a Cr-logwood ink (Table 2). Comparison of the spectrum

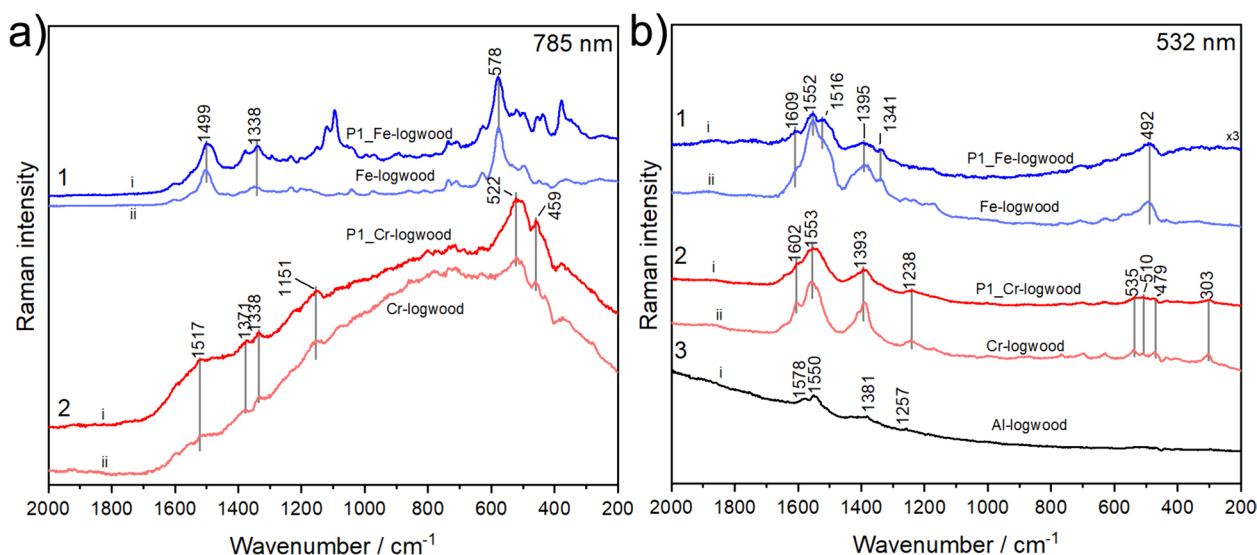


Fig. 5 **a** Raman spectra of various logwood inks: (1) Fe-logwood on i-paper (P1) and ii-as dry ink and (2) Cr-logwood on i-paper (P1) and ii-as dry ink, recorded with 785 nm excitation wavelength. **b** Raman spectra of various logwood inks: (1) Fe-logwood on i-paper (P1) and ii-as dry ink, (2) Cr-logwood on i-paper (P1) and ii-as dry ink, (3) Al-logwood as i-dry ink recorded with 532 nm excitation wavelength. (the spectra are shifted on the y-axis for the sake of clarity and scaled where necessary)

of Cr-logwood inks obtained with a portable spectrometer and a Raman microscope using a 785 nm excitation shows that the band around 522 cm^{-1} is broader and shifts to higher wavenumbers in the spectrum recorded with Raman microscope.

It was not possible to characterise the Al-logwood ink applied on paper with a 532 nm excitation line (Fig. 5). In the spectra of the dry ink, weak Raman bands are observed at ca. 1578, 1550, 1381, and 1257 cm^{-1} , which are associated with the interaction of hematein with aluminium and agree with the published spectrum recorded with a 488 nm line [10]. The spectra of the Fe-logwood ink obtained with a 532 nm excitation (Fig. 5b) shows a different profile than that obtained with a portable spectrometer and with the Raman microscope with a 785 nm excitation (Fig. 4a, Fig. 5a). The spectrum of the ink applied on paper, with characteristic bands at ca. 1609, 1552, 1516, 1395, 1341, 492 cm^{-1} , or the dry ink show similarities with the spectrum of pure hematein (Fig. 4c). However, the bands that could indicate the interaction of iron with hematein are at ca. 1552 and 1516 cm^{-1} , along with a medium strong broad band at ca. 492 cm^{-1} . Different spectral profiles collected with the Raman microscope and 785 or 532 nm lines could indicate the presence of different metal complexes in the ink, which could be a result of hematein conversion during ink production. [9] The spectrum of the Cr-logwood ink applied on paper with a 532 nm excitation (Fig. 5b) shows bands at ca. 1602, 1553, 1393, 1238, 535, 510, 479, 303 cm^{-1} and agrees with the spectrum of the dry ink (Table 2). Compared to the spectrum obtained with a 785 nm line, the bands at ca. 1553 and 1393 cm^{-1} measured with the 532 nm line are intense (Fig. 5) and, in the lower wavenumber range, three bands at ca. 535, 510, 479 cm^{-1} are present. The band ca. 510 cm^{-1} is not present in the spectrum of the pure hematein (Fig. 4c), this band is observed also in the spectra of the ink obtained with a portable Raman spectrometer (Fig. 4a), suggesting an interaction between Cr and hematein. The differences in the spectra collected with different excitation lines may reflect the fact that chromate possibly oxidises the hematein and different species are present, or it may be a consequence of the temperature effect during ink production, as even small temperature variations can cause chemical changes [11]. Comparing the Raman results obtained on the logwood-based inks, it can be concluded that if there is a doubt as to whether the inks are iron logwood or iron gall inks, especially if the spectra were recorded with longer excitation wavelengths, an additional analysis with a shorter excitation wavelengths is recommended, as the analysis with a 532 nm line clearly showed the presence of hematein-related features that are absent in the spectra of iron gall inks (Fig. 2b). On the other hand, the analysis of

different logwood inks gave to some extent similar spectral profiles when a 532 nm excitation was used (Fig. 5b), so an additional elemental analysis to identify the metal is recommended for unknown samples.

The FTIR spectra collected in the reflection mode on the three logwood inks applied to paper P1 showed no spectral features that could be attributed to these inks. In all these spectra, only bands due to the cellulose in the paper substrate are visible (spectra not shown). The transmission FTIR spectra of the logwood and logwood extract, together with those of the dry Fe-logwood, Cr-logwood and Al-logwood ink powders, are shown in Fig. 6a. The spectra of the dry Fe- and Al-logwood inks are dominated by the strongest signals of the binding medium (i.e. gum arabic) at ~ 1611 and $\sim 1071\text{ cm}^{-1}$. [42] All three FTIR spectra collected on the dry logwood inks show distinctive bands of logwood and/or logwood extract at ca. 1738, 1611, 1506, 1293, 1159, 1111, 875, 859 and 779 cm^{-1} [11]. Some of these signals overlap of Fe- and Al-logwood inks with the bands of the binder. The Cr-logwood ink does not contain an organic binder, therefore the strong band at ca. 1140 cm^{-1} could be assigned to the residual of potassium chromate [45]. Transmission FTIR spectra of reference components for logwood inks are presented in Fig. 6b. The spectrum of logwood contains characteristic signals for the basic wood components, such as cellulose (ca. 3384, 2929, 1461, 1425, 1373, 1320, 1158, 1111, 1056 and 897 cm^{-1}) and lignin (ca. 1608, 1506, 1461, 1373, 1320, 1260, 1158, 1111 cm^{-1}) [46]. Additionally, this transmission spectrum has a band at ca. 1738 cm^{-1} . Furthermore, two slightly different transmission spectra, similarly to what was observed by Raman spectroscopy, Fig. 4, were collected on the pure hematein reference powder and are labelled as hematein 1 and hematein 2 (Fig. 6b). The FTIR spectrum of hematein 2 shows characteristic hematein IR bands at ca. 3357, 1608, 1514, 1440, 1377, 1296, 1204, 1068, 1033, 859, 795, 766, 661 and 623 cm^{-1} , while the spectrum of hematein 1 shows some additional and/or shifted bands at ca. 3589, 3507, 1732, 1594, 1464 and 1270 cm^{-1} [9]. The signal at ca. 1738 cm^{-1} (C=O stretch) could indicate the presence of the oxidized form of hematein. Hematein, which is produced by the oxidation of haematoxylin, is not a stable molecule and tends to oxidise further. [47] In addition, probably the signal of oxidised hematein is visible also in the spectrum of logwood (Fig. 6b). Furthermore, the spectrum of the powder logwood extract contains pronounced signals of wood components, at ca. 1506, 1158, 1053 cm^{-1} , hematein at ca. 1608, 1293 cm^{-1} , and of haematoxylin, at ca. 1473, 1087, 1053, 1033, 956, 942, 875, 859, 779, 700 and 674 cm^{-1} . [11] The proportions of haematoxylin

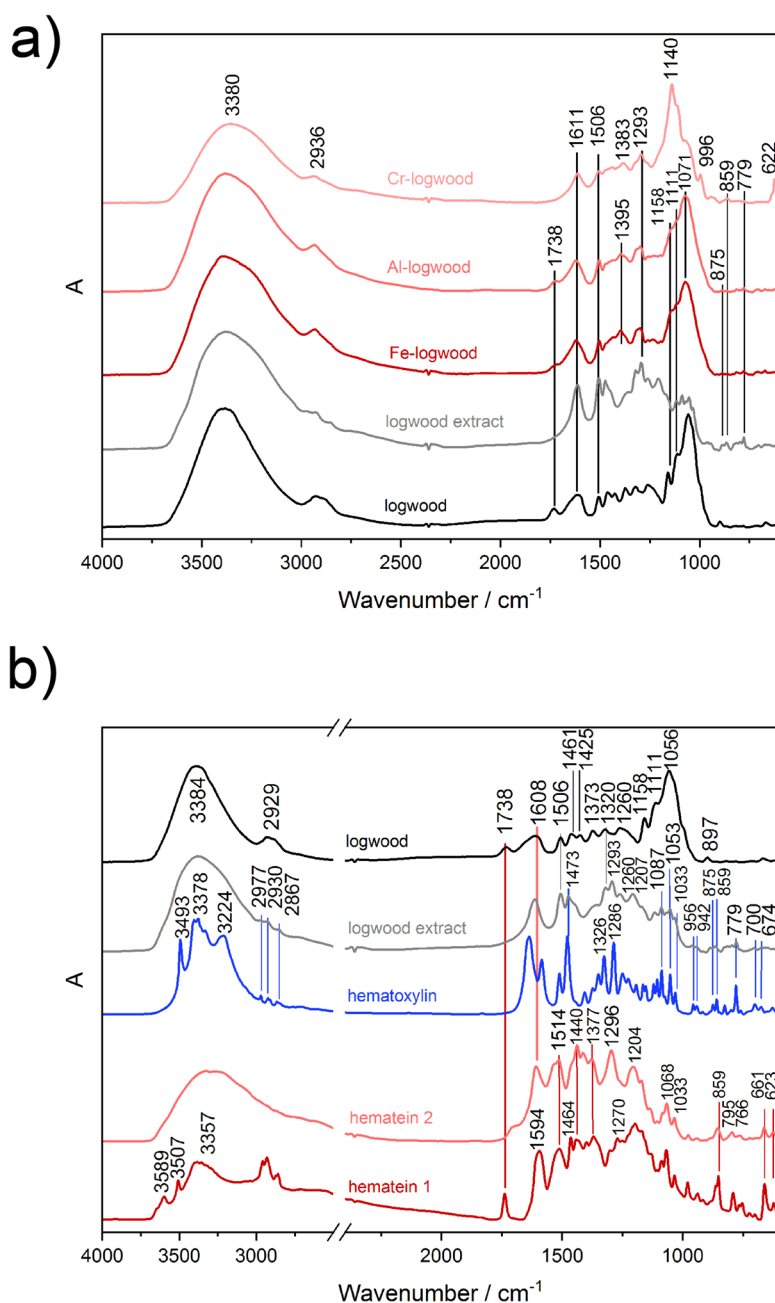


Fig. 6 **a** Transmission FTIR spectra of dry logwood inks (Cr-logwood, Al-logwood and Fe-logwood) and reference materials (logwood and logwood extract). **b** Transmission FTIR spectra of reference materials (logwood, logwood extract, hematoxylin, and hematein)

and hematein probably vary from one batch to another, depending on differences in production. The signal of hematoxylin is probably more visible in the spectrum of logwood extract, especially in the fingerprint region, due to the absence of cellulose signals. In addition, logwood contains also various amounts of other colorants, such as a derivative of brazilein and tannins, as well as other unidentified components. [1]

Mixture of iron gall and logwood inks

The spectra of an ink prepared by mixing iron(III) sulphate and copper sulphate with galls and logwood (labelled as Cu/Fe-GALLS/LOG) in the dry and liquid forms, recorded with a portable Raman spectrometer, are shown in Fig. 7a, and the bands are summarised in Table 3. The most intense bands are at ca. 1479, 1346 and 602 cm^{-1} . The spectrum of the ink on paper

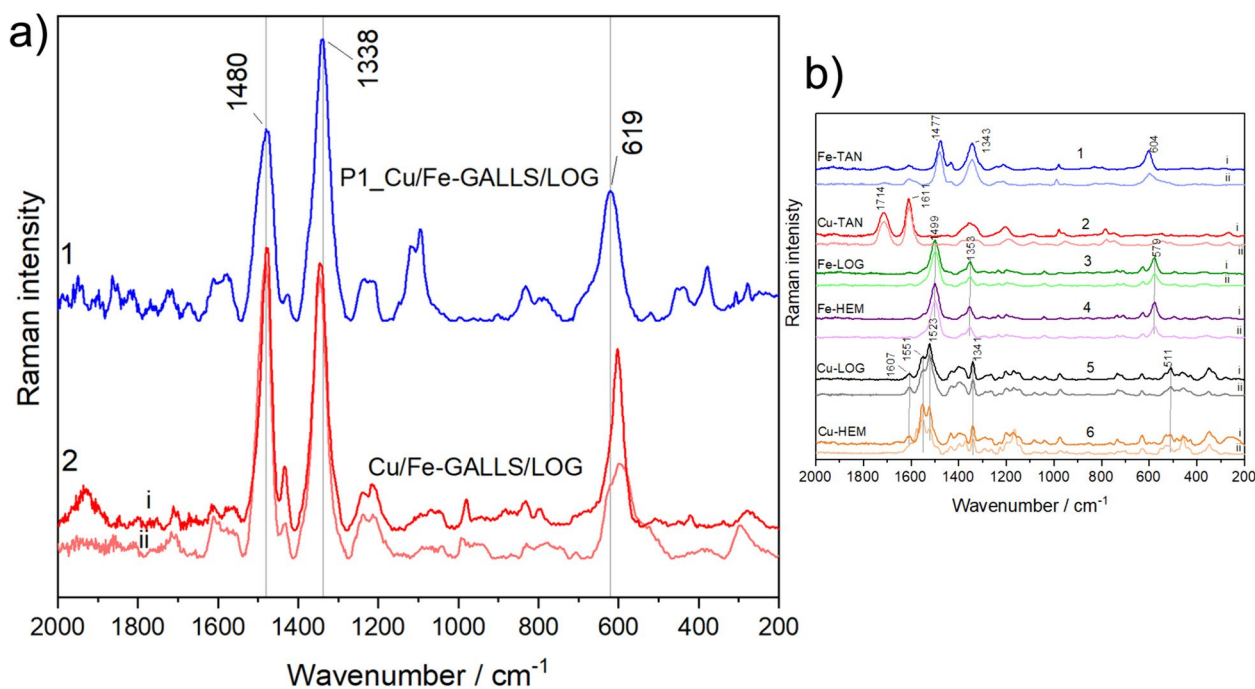


Fig. 7 **a** Raman spectra of the mixture of iron gall and logwood ink Cu/Fe-GALLS/LOG on (1) paper (P1) and as (2) ink in i-liquid and ii-dry form. **b** Raman spectra of reference samples in i-liquid and i-dry form of (1) Fe-TAN (ferrous sulphate and tannic acid), (2) Cu-TAN (copper sulphate and tannic acid), (3) Fe-LOG (ferrous sulphate and logwood extract), (4) Fe-HEM (ferrous sulphate and hematein), (5) Cu-LOG (copper sulphate and logwood extract), (6) Cu-HEM (copper sulphate and hematein). The spectra were recorded with a portable Raman spectrometer (785 and 852 nm excitations). The spectra are shifted on the y-axis for the sake of clarity and scaled where necessary

(Fig. 7a) is comparable with the spectra of the pure (liquid, dry) ink, with the band characteristic of $\nu(\text{Fe}-\text{O})$ shifted to higher wavenumbers (from 602 to 619 cm^{-1}), and 1346 to 1338 cm^{-1} . However, the signal is characteristic of the metal (iron)-polyphenol (tannic acid) complex, which was also confirmed by comparing it with the spectrum of the Fe-TAN (Fig. 7b, spectra 1) and with the spectra of iron gall inks (Fig. 1a). This Fe-TAN ink was prepared from ferrous sulphate, tannic acid and gum arabic, keeping the same mass fractions as in the original formulation of Cu/Fe-GALLS/LOG, but replacing the galls with tannic acid. The spectrum of the ink mixture (Cu/Fe-GALLS/LOG) was also compared with the spectra of reference compounds that could contribute to the overall signal (Fig. 7b). Such compounds could, for example, result from interactions between Cu and tannic acid (sample Cu-TAN), Fe and logwood/ hematein (samples Fe-LOG, Fe-HEM), Cu and logwood/hematein (samples Cu-LOG, Cu-HEM). The comparison with the spectra of the reference set (Fig. 7b) shows that the main signal in the spectrum of Cu/Fe-GALLS/LOG belongs to the signal of Fe-polyphenol complex, most probably to the Fe-tannic acid complex (spectrum Fe-TAN, Fig. 7b), characteristic for iron gall ink. The sample prepared from copper

sulphate and tannic acid (Cu-TAN) only shows signals (Fig. 7b, spectra 2) from tannic acid (1714, 1610 cm^{-1} , Fig. 1b). It is known that the interaction of polyphenols with Cu^{2+} can cause similar spectral changes as with iron, but the Raman spectra of the copper complexes are much weaker than those of the iron ions [30]. Moreover, in this sample (Cu-TAN) the copper concentrations are lower compared to the iron concentrations, which could be another reason for dominating of the signal of tannic acid. The spectra of ferrous sulphate and logwood extract (Fe-LOG) or hematein (Fe-HEM) are comparable and characterised by bands at ca.1499, 1353, 579 cm^{-1} (Fig. 7b, spectra 3,4). The spectra of the samples prepared from copper sulphate and logwood extract and/or hematein (Cu-LOG and Cu-HEM) are also comparable and bands at ca.1607, 1551, 1523 and 1341, 511 cm^{-1} are visible (Fig. 7b, spectra 5,6). These bands are different from the bands of hematein (Fig. 4b) and can therefore be attributed to the interaction and possibly the formation of a complex between copper and hematein. The comparison with the reference set shows that the dominant signal in the spectra of Cu/Fe-GALLS/LOG obtained with the portable Raman spectrometer belongs to the signal of the Fe-polyphenol complex characteristic of iron gall inks.

Table 3 Raman band positions (cm^{-1}) for a mixture of iron gall and logwood inks, measured with a portable Raman spectrometer using 852 and 785 nm excitations, on the liquid and dry inks; and with a Raman microscope on the dry inks with 785 and 532 nm excitations

Instrument	Portable Raman*		Raman microscopy*	
	Liquid ink 852 and 785 nm	Dry ink 852 and 785 nm	Dry ink 785 nm	Dry ink 532 nm
Cu/Fe-GALLS/LOG	1712vw, 1616vw, 1567vw(br), 1479vs, 1433m, 1346vs, 1239m, 1216m, 1094w, 1066vw, 1046vw, 979w, 882vw, 831w, 801w, 602s, 508vw, 421vw, 338vw, 279vw	1716vw, 1610w(br), 1578sh, 1481vs, 1432w, 1346vs, 1238w, 1214w, 1090vw(br), 1039vw, 992w, 950w(br), 829vw, 776vw, 746vw, 706vw, 599m, 523sh, 390vw(br), 295w	1545vw, 1476m, 1431vw, 1347m, 1234vw(br), 779vw(br), 581m(br), 533sh, 426vw, 372w	1599sh, 1552s, 1513s, 1427sh, 1390m, 1370sh, 1337m, 1256vw, 1228vw, 1190w, 1080vw, 1039vw, 976w, 710w, 626w, 572vw, 491m, 341w(br),

* vw very weak, w weak, m medium, s strong, vs very strong, sh shoulder, br broad

Further analyses of the ink mixture (Fig. 8a, Table 3) Cu/Fe-GALLS/LOG with Raman microscopy at 785 nm (characteristic bands of ink on paper at 1476, 1336, 594 cm^{-1} and characteristic bands of ink at 1476, 1347, 582 cm^{-1} (shift of $\nu(\text{Fe}-\text{O})$ compared to ink on paper) also showed the strongest contributing signal of the iron-tannic acid complex, as the spectrum matched the Fe-TAN spectrum (Fig. 8a, spectrum 3, characteristic bands at 1483, 1345, 596 cm^{-1}), confirming the presence of the iron-polyphenol complex [30] characteristic of iron gall ink in the mixture.

On the other hand, analysis of the same ink mixture Cu/Fe-GALLS/LOG on paper (bands at ca. 1553, 1494, 1386 cm^{-1}) and of dry ink with excitation line 532 nm (Fig. 8b, Table 3) showed the presence of logwood. The spectrum of the dry ink recorded with 532 nm show similarities with the spectrum of the dry ink prepared from ferrous sulphate and hematein (Fe-HEM, Fig. 8b, spectrum 3, bands at 1554, 1522, 1421, 1390, 1339, 1262, 1234, 1192, 1177, 1038, 710, 631, 575, 497 cm^{-1}) and indicate the presence of logwood (hematein) and/or interaction of Fe with hematein. However, it is very likely that the signal of the compounds formed by the interaction of Cu and hematein/hematein degradation products also contributes to the overall signal [9] as the spectrum of the sample prepared from copper sulphate and hematein also shows similar spectral features (bands at ca. 1546, 1380, 1257, 711, 626, 508 cm^{-1}) in the spectrum recorded at 532 nm (Fig. 8). It could be concluded

that analysing the mixture ink composed of galls and logwood only with a portable Raman or Raman microscope with 785 nm could lead to the false assumption that only gall ink is present in the ink. On the other hand, although hematein-like features can be seen in the spectra of the mixture ink on paper (1553, 1386 cm^{-1}), these are largely consistent with the spectra of Cr-, Fe- and Al-logwood ink measured at 532 nm (Fig. 5b). The recipes [3] mainly mention the use of ferrous sulphate or a mixture of ferrous and copper sulphate for the preparation of mixtures, but for an unknown sample an additional analysis is suggested to determine the elemental composition.

The reflection FTIR spectra collected on mixture of iron gall and logwood ink applied on Whatman paper substrate shows only two spectral features of iron gall part of ink (tannic acid) placed at 1715 and 1535 cm^{-1} (Fig. 9a, spectrum Fe-TAN) and no signals of logwood based ink (Fig. 9a). The same results show also FTIR analyses in transmission mode performed on dry mixture of iron gall and logwood ink, where bands of tannic acid are disclosed (3345, 1709, 1614, 1536, 1446, 1329, 1205, 872 and 761 cm^{-1}). Strong absorption at 1081 cm^{-1} could be assigned to the binding medium and/or residual of ferrous and/or copper sulphates (Fig. 9b) [43, 48].

Conclusion

Often, the analysis of works on paper by Raman spectroscopy with short excitation wavelengths is challenging as fluorescence emission may obscure the Raman spectra.

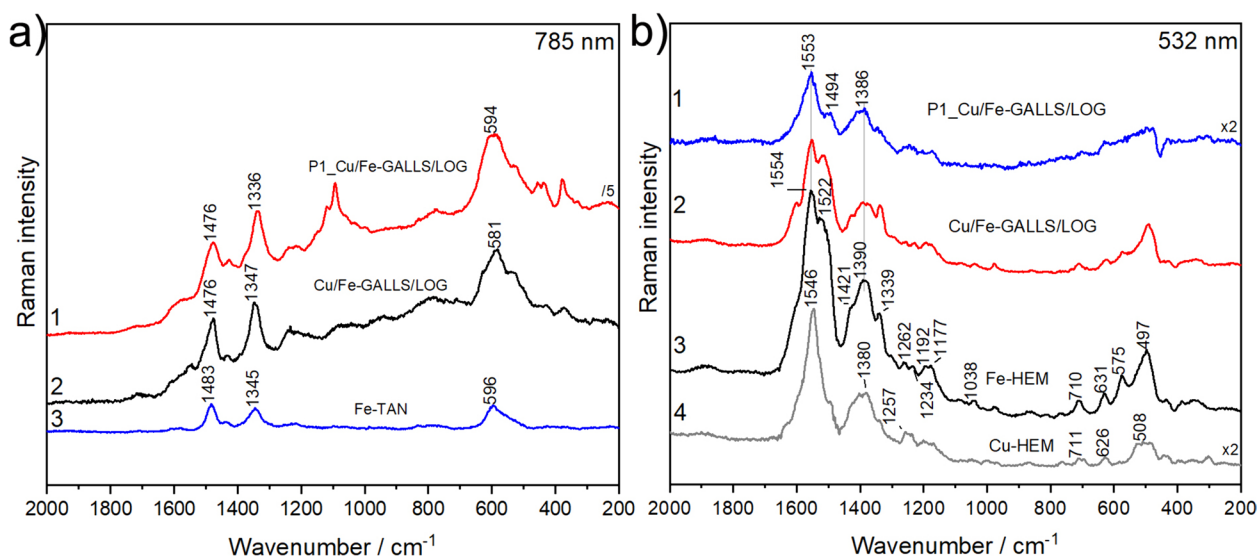


Fig. 8 **a** Raman spectra of the mixture of iron gall and logwood ink Cu/Fe-GALLS/LOG on (1) paper (P1) and as (2) dry ink, and (3) the reference sample Fe-TAN (iron sulphate and tannic acid), recorded with 785 excitation wavelength. **b** Raman spectra of the mixture of iron gall and logwood ink Cu/Fe-GALLS/LOG on (1) paper (P1) and as (2) dry ink and (3) the reference samples Fe-HEM (iron sulphate and hematein) and (4) Cu-HEM (copper sulphate and hematein) recorded with 532 nm excitation wavelength. (the spectra are shifted on the y-axis for the sake of clarity and scaled where necessary)

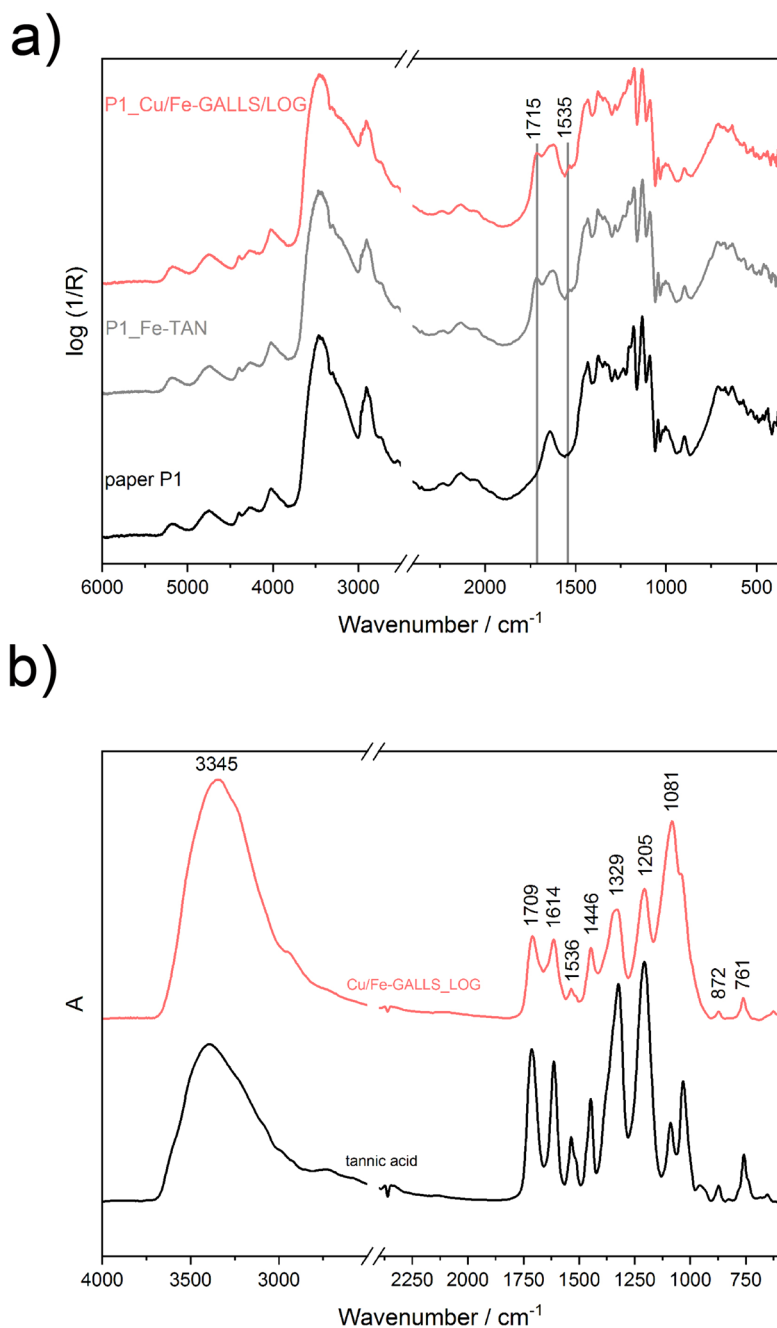


Fig. 9 **a** Reflection FTIR spectra of substrate (paper P1), mixture of iron gall and logwood ink (Cu/Fe-GALLS/LOG) and tannic acid-based ink Fe-TAN applied on paper P1. **b** Transmission FTIR spectra of dry mixture of iron gall and logwood ink (Cu/Fe-GALLS/LOG) and reference material (tannic acid)

This study confirms that iron gall inks can be analysed by Raman spectroscopy with long excitation wavelengths (either 852 and 785 nm with a portable Raman spectrometer or 785 nm in a Raman microscope) using the three characteristic bands around 1475, 1340, 600 cm^{-1} as markers. The spectral data obtained was independent of the formulation used, which varied depending on

the molar content of copper and iron. Logwood inks are more difficult to analyse with Raman spectroscopy, especially when applied on paper. Fe-logwood was the only ink that could be analysed on paper with longer excitation lines. Its spectrum at 852 and 785 nm or 785 nm is very similar to the spectrum of iron gall ink, but the most intense band is shifted to higher wavenumbers (up

to about 1490 cm^{-1}). However, the spectrum of Fe-logwood recorded at 532 nm shows hematein features and could be used to further distinguish between iron gall ink and Fe-logwood ink. All investigated logwood inks (Fe-, Cr-, Al-) provide spectral information when analysed at 532 nm, therefore Raman spectroscopy at shorter wavelengths is suggested for their investigation. The spectra show some differences in the lower wavenumber range, but are of low intensity, so that an additional analysis of the elemental composition is recommended, when analysing real works of art. Furthermore, analysis of the mixture of iron gall ink and logwood ink showed that Raman spectroscopy at 785 nm was able to detect the iron gall part of the ink, while further analysis at 532 nm also showed the presence of logwood in the inks. Therefore, analysing the inks, especially if iron gall ink is present as a component, only using Raman spectroscopy with excitation lines of longer wavelengths could lead to the false conclusion that only iron gall ink is present.

The results of the non-invasive FTIR analysis of inks and their mixtures applied to paper (Whatman) showed the dominant signals of cellulose. It did not provide additional spectral information on logwood inks, making the technique less powerful for their characterization compared to Raman spectroscopy. However, the reflection spectra of the iron gall inks and the mixtures of iron gall ink and logwood inks also showed the signals of tannic acid, which complemented Raman spectroscopy results on metal complexes present in inks. Furthermore, analyses of the dried inks using FTIR spectroscopy in transmission mode revealed signals from other ink components such as binders and/or various metal salts, in addition to the signals from logwood in logwood inks and from tannic acid in iron gall inks and in mixtures of iron gall inks and logwood inks.

Abbreviations

FT	Fourier-transform
FTIR	Fourier-transform infrared
IR	Infrared
SSE™	Sequentially shifted excitation

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Author contributions

K.R. developed the concept of the research. K.R., L.L. drafted the manuscript. K.R. and L.L. carried out Raman and FTIR analysis. J.K., P.R. have reviewed the manuscript. All authors contributed to data interpretation and writing. All authors have read, approved and agreed to the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

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References

- Cardon D. Natural dyes: sources, tradition, technology and science. London: Archetype Publications; 2007.
- Kirby J, van Bommel M, Verhecken A. Natural colorants for dyeing and lake pigments: practical recipes and their historical sources. London: Archetype Publications; 2014.
- Lehner S. Ink manufacture: including writing, copying, lithographic, marking, stamping, and laundry inks. London: Scott, Greenwood & Co.; 1902.
- Neevel H. Logwood writing inks: history, production, forensics, and use. *Restaurator Int J Preserv Lib Arch Mater*. 2021. <https://doi.org/10.1515/res-2021-0015/html>.
- Howes FN. Logwood and writing ink. *Kew Bull*. 1948;3:248–51. <https://doi.org/10.2307/4119768>.
- Reissland B, Pronao Gaibor AN, Ligterink F, Neevel JG. Exploring the late 19th-century landscape of ink manufacturing via a collection of 90 bottles. In: ICOM-CC 18th Triennial Conference Preprints, Copenhagen, 4–8 September 2017, ed. J. Bridgland, art. 0105. Paris: International Council of Museums.
- Bicchieri M, Monti M, Piantanida G, Sodo A. All that is iron-ink is not always iron-gall! *J Raman Spectrosc*. 2008;39:1074–8. <https://doi.org/10.1002/jrs.1995>
- Mitchell CA. Inks: Their Composition and Manufacture; Including Methods of Examination and a Full List of English Patents. 4th ed. London: Charles Griffin & Co.; Griffin, Limited; 1937.
- Bronzato M, Zoleo A, Biondi B, Centeno SA. An insight into the metal coordination and spectroscopic properties of artistic Fe and Fe/Cu logwood inks. *Spectrochim Acta - Part A: Mol Biomol Spectrosc*. 2016;153:522–9. <https://doi.org/10.1016/j.saa.2015.08.042>.
- Centeno SA, Ropret P, del Federico E, Shamir J, Itin B, Jerschow A. Characterization of Al(III) complexes with hematein in artistic alum logwood inks. *J Raman Spectrosc*. 2010;41:445–51. <https://doi.org/10.1002/jrs.2455>.
- Centeno SA, Bronzato M, Ropret P, Zoleo A, Venzo A, Bogialli S, et al. Composition and spectroscopic properties of historic Cr logwood inks. *J Raman Spectrosc*. 2016;47:1422–8. <https://doi.org/10.1002/jrs.4938>.
- Svobodová E, Bosáková Z, Ohlídalová M, Novotná M, Němec I. The use of infrared and Raman microspectroscopy for identification of selected red organic dyes in model colour layers of works of art. *Vib Spectrosc*. 2012;63:380–9. <https://doi.org/10.1016/j.vibspec.2012.09.003>.
- Bruni S, Guglielmi V, Pozzi F. Historical organic dyes: a surface-enhanced Raman scattering (SERS) spectral database on Ag Lee-Meisel colloids aggregated by NaClO₄. *J Raman Spectrosc*. 2011;42:1267–81. <https://doi.org/10.1002/jrs.2872>.
- Leona M, Stenger J, Ferloni E. Application of surface-enhanced Raman scattering techniques to the ultrasensitive identification of natural dyes in works of art. *J Raman Spectrosc*. 2006;37:981–92. <https://doi.org/10.1002/jrs.1582>.
- Pawlak K, Puchalska M, Miszczyk A, Rosloniec E, Jarosz M. Blue natural organic dyestuffs—front textile dyeing to mural painting. Separation and characterization of coloring matters present in elderberry, logwood and

- indigo. *J Mass Spectrom.* 2006;41:613–22. <https://doi.org/10.1002/jms.1018>.
16. Mantzouris D, Karapanagiotis I, Valianou L, Panayiotou C. HPLC-DAD-MS analysis of dyes identified in textiles from Mount Athos. *Anal Bioanal Chem.* 2011;399:3065–79. <https://doi.org/10.1007/s00216-011-4665-4>.
 17. Pagès-Camagna S, Duval A, Guicharnaud H. Study of Gustave Moreau's black drawings: identification of the graphic materials by Raman microspectrometry and PIXE. *J Raman Spectrosc.* 2004;35:628–32. <https://doi.org/10.1002/jrs.1215>.
 18. Cieřla R. Forensic examination of inks used as inscription on historical documents. *Int J Conserv Sci.* 2023;14:481–96. <https://doi.org/10.36868/ijcs.2023.02.07>.
 19. Liu Y, Fearn T, Strlić M. Dose-response Modelling for the Discolouration of Iron Gall Ink on Paper. In: *Transcending Boundaries: Integrated Approaches to Conservation. ICOM-CC 19th Triennial Conference Preprints, Beijing, 17–21 May 2021*, ed. J. Bridgland. Paris: International Council of Museums.
 20. Liu Y, Kralj Cigić I, Strlić M. Kinetics of accelerated degradation of historic iron gall ink-containing paper. *Polym Degrad Stab.* 2017;142:255–62. <https://doi.org/10.1016/j.polymdegradstab.2017.07.010>.
 21. Kolar J, Strlic M. Iron gall inks: on manufacture, characterisation, degradation and stabilisation. Ljubljana: National and University Library of Slovenia; 2006.
 22. Wunderlich C-H, Weber R, Bergerhoff G. Über eisengalltinte. *ZAAC - J Inorgan Gen Chem.* 1991;598:371–6. <https://doi.org/10.1002/zaac.19915980134>.
 23. Feller RK, Cheetham AK. Fe(III), Mn(II), Co(II), and Ni(II) 3,4,5-trihydroxybenzoate (gallate) dihydrates; a new family of hybrid framework materials. *Solid State Sci.* 2006;8:1121–5. <https://doi.org/10.1016/j.solidstatescienc.2006.04.013>.
 24. Ponce A, Brostoff LB, Gibbons SK, Zavalij P, Viragh C, Hooper J, et al. Elucidation of the Fe(III) gallate structure in historical iron gall ink. *Anal Chem.* 2016;88:5152–8. <https://doi.org/10.1021/acs.analchem.6b00088>.
 25. Caterino S, Pajer N, Crestini C. Iron-galls inks: preparation, structure and characterisation. *Microchem J.* 2023; 185. <https://doi.org/10.1016/j.microc.2022.108258>.
 26. Wagner B, Bulska E, Stahl B, Heck M, Ortner HM. Analysis of Fe valence states in iron-gall inks from XVIth century manuscripts by 57Fe Mössbauer spectroscopy. *Anal Chim Acta.* 2004;527:195–202. <https://doi.org/10.1016/j.aca.2004.04.011>.
 27. Lerf A, Wagner FE, Dreher M, Espejo T, Pérez-Rodríguez JL. Mössbauer study of iron gall inks on historical documents. *Herit Sci.* 2021;9. <https://doi.org/10.1186/s40494-021-00522-3>.
 28. Espina A, Sanchez-Cortes S, Jurařekova Z. Vibrational study (Raman, SERS, and IR) of plant gallnut polyphenols related to the fabrication of iron gall inks. *Molecules.* 2022;27(1). <https://doi.org/10.3390/molecules27010279>.
 29. Ferretti A, Sabatini F, Degano I. A model iron gall ink: an in-depth study of ageing processes involving gallic acid. *Molecules.* 2022. <https://doi.org/10.3390/molecules27238603>.
 30. Espina A, Cañamares MV, Jurařeková Z, Sanchez-Cortes S. Analysis of iron complexes of tannic acid and other related polyphenols as revealed by spectroscopic techniques: implications in the identification and characterization of iron gall inks in historical manuscripts. *ACS Omega.* 2022;7:27937–49. <https://doi.org/10.1021/acsomega.2c01679>.
 31. Malešič J, Sala M, Šelih VS, Kočar D. Evaluation of a method for treatment of iron gall ink corrosion on paper. *Cellulose.* 2014;21:2925–36. <https://doi.org/10.1007/s10570-014-0311-6>.
 32. Deng Y, Handoko AD, Du Y, Xi S, Yeo BS. In situ Raman spectroscopy of copper and copper oxide surfaces during electrochemical oxygen evolution reaction: identification of CuII oxides as catalytically active species. *ACS Catal.* 2016;6:2473–81. <https://doi.org/10.1021/acscatal.6b00205>.
 33. Chio CH, Sharma SK, Muenow DW. The hydrates and deuterates of ferrous sulfate (FeSO₄): a Raman spectroscopic study. *J Raman Spectrosc.* 2006;38:87–99. <https://doi.org/10.1002/jrs.1623>.
 34. Fu X, Yang G, Sun J, Zhou J. Vibrational spectra of copper sulfate hydrates investigated with low-temperature Raman spectroscopy and terahertz time domain spectroscopy. *J Phys Chem A.* 2012;116:7314–8. <https://doi.org/10.1021/jp302997h>.
 35. Agarwal UP. Analysis of cellulose and lignocellulose materials by Raman spectroscopy: a review of the current status. *Molecules.* 2019;24 (9). <https://doi.org/10.3390/molecules24091659>.
 36. Edwards HGM, Farwell DW, Webster D. FT Raman microscopy of untreated natural plant fibres. *Spectrochim Acta - Part A: Mol Biomol Spectrosc.* 1997;53:2383–92. [https://doi.org/10.1016/S1386-1425\(97\)00178-9](https://doi.org/10.1016/S1386-1425(97)00178-9).
 37. Lee AS, Otieno-Alego V, Creagh DC. Identification of iron-gall inks with near-infrared Raman microspectroscopy. *J Raman Spectrosc.* 2008;39:1079–84. <https://doi.org/10.1002/jrs.1989>.
 38. Piantanida G, Menart E, Bicchieri M, Strlić M. Classification of iron-based inks by means of micro-Raman spectroscopy and multivariate data analysis. *J Raman Spectrosc.* 2013;44:1299–305. <https://doi.org/10.1002/jrs.4351>.
 39. Díaz Hidalgo RJ, Córdoba R, Nabais P, Silva V, Melo MJ, Pina F, et al. New insights into iron-gall inks through the use of historically accurate reconstructions. *Herit Sci.* 2018;6. <https://doi.org/10.1186/s40494-018-0228-8>.
 40. do Menezes LLRM, da Pires RN, da Cunha PLR, de Freitas RM, de Souza BWS, de Feitosa JPA, et al. Effect of tannic acid as crosslinking agent on fish skin gelatin-silver nanocomposite film. *Food Pack Shelf Life.* 2019;19:7–15. <https://doi.org/10.1016/j.fpsl.2018.11.005>.
 41. Çakar S, Özacar M. Fe-tannic acid complex dye as photo sensitizer for different morphological ZnO based DSSCs. *Spectrochim Acta - Part A: Mol Biomol Spectrosc.* 2016;163:79–88. <https://doi.org/10.1016/j.saa.2016.03.031>.
 42. Daher C, Paris C, Le Hô A-S, Bellot-Gurlet L, Échard J-P. A joint use of Raman and infrared spectroscopies for the identification of natural organic media used in ancient varnishes. *J Raman Spectrosc.* 2010;41:1494–9. <https://doi.org/10.1002/jrs.2693>.
 43. Boyatzis SC, Velivasaki G, Malea E. A study of the deterioration of aged parchment marked with laboratory iron gall inks using FTIR-ATR spectroscopy and micro hot table. *Herit Sci.* 2016;4. <https://doi.org/10.1186/s40494-016-0083-4>.
 44. Hulme AN, McNab H, Pegg DA, Quye A. Negative ion electrospray mass spectrometry of neoflavonoids. *Phytochemistry.* 2005;66:2766–70. <https://doi.org/10.1016/j.phytochem.2005.09.001>.
 45. Vats V, Melton G, Islam M, Krishnan VV. FTIR spectroscopy as a convenient tool for detection and identification of airborne Cr(VI) compounds arising from arc welding fumes. *J Hazard Mater.* 2023;448:130862. <https://doi.org/10.1016/j.jhazmat.2023.130862>.
 46. Jacquemain A, Retko K, Legan L, Ropret P, Waentig F, Cattersel V. Exploratory investigation of historical decorative laminates by means of vibrational spectroscopic techniques. *Herit Sci.* 2023;11. <https://doi.org/10.1186/s40494-023-00941-4>.
 47. Llewellyn BD. Hematoxylin Formulae. The Internet Resource For Histo-technologists. 2013. <https://www.stainsfile.com/wp-content/uploads/2022/09/hematoxylin-formulae.pdf>. Accessed 4 Dec 2023
 48. White RL. Variable temperature infrared study of copper sulfate pentahydrate dehydration. *Thermochim Acta.* 2012;528:58–62. <https://doi.org/10.1016/j.tca.2011.11.013>.

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