

Non-destructive spectroscopic investigation on historic Yemenite scriptorial fragments: evidence of different degradation and recipes for iron tannic inks

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Abstract Six fragments of different Yemenite manuscripts (three on parchment and three on paper) were analyzed by means of attenuated total reflectance Fourier transform infrared, micro-Raman and X-ray fluorescence spectroscopies. The combination of molecular and elemental techniques allowed the characterization of the conservation state of all the fragments, the identification of sizing agents and salts on the supports and the identification of the composition all red and black writing media. In particular, analysis of black inks provided interesting insights: independent of the substrate, all inks seemed to have identical composition, corresponding to rather well preserved high-quality iron tannic inks to which carbon black had not been added. However, in some samples, the most intense Raman peak of the ink was clearly shifted with respect to the typical bands of iron gall ink. Starting from the hypothesis that the shift could have been produced by the use of sources of tannin other than

gallnuts, research was undertaken by preparing and characterizing seven different tannic inks. The experimental results confirmed the hypothesis of different tannin sources.

Keywords Micro-Raman · Attenuated total reflectance Fourier transform infrared · X-ray fluorescence · Tannic inks

Introduction

In early 2007, during the restoration of the ligneous ceilings in the Great Mosque (al-Jami al-Kabir) of Sana'a performed by Italian staff, a large amount of hidden fragments of ancient parchments and papers was found. The fragments had been concealed in two hollows on the west side of the mosque, adjacent to the minaret's wall.

Archaeologists and historians hypothesized the findings to be fragments and parts of volumes, likely from the seventh and eighth centuries AD, containing mainly historical and religious texts. This discovery is quite relevant for Arab culture. This is the first important unearthing of manuscripts since the 1973 discovery of ancient texts in the same mosque.

The fragments appear to be in a bad conservation state, as proven by two successive surveys executed by the staff of the Istituto Centrale Restauro e Conservazione Patrimonio Archivistico e Librario (ICRCPAL), first in December 2007 and subsequently in October 2008. The items showed traces of attack by rodents, the presence of stains, carbonized parts, holes and tears, folds and undulations.

In the framework of a collaboration with the Yemenite institutions, the ICRCPAL Laboratory of Chemistry was charged with the study of six fragments belonging to different manuscripts. The fragments were analyzed by means of attenuated total reflectance (ATR) Fourier transform infrared

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(FTIR), micro-Raman and X-ray fluorescence (XRF) spectroscopies for the characterization of substrates, inks and pigments.

Preliminary analyses showed the presence of inks similar but not quite identical to iron gall inks. To elucidate the spectral differences recorded, research was conducted using different tannin sources.

Materials and methods

Instrumentation

Measurements were performed by means of a Renishaw inVia Reflex Raman microscope equipped with a Renishaw diode LASER emitting at 785 nm (output power 300 mW). Neutral filters were used, when necessary, to prevent sample degradation. The backscattered light was dispersed by a 1,200 line per millimetre grating and the Raman signal was detected by a Peltier-cooled (-70 °C) deep depletion charge-coupled device (RD-VIU, 578 × 384 pixels) optimized for near-infrared and ultraviolet radiation. The nominal spectral resolution obtained for the measurements was about 3 cm⁻¹. The system, equipped with a Leica DMLM microscope to focus the laser on the sample and a colour video camera, allows the positioning of the sample and the selection of a specific region for investigation. Spectral acquisitions (five to ten accumulations, 50 s each) were performed with a ×50 objective (numerical aperture 0.75). Under these conditions, the laser spot measures about 20 μm².

No absolute a priori power “on the sample” could be chosen, since each sample is unique, depending on its composition, conservation state and history. For each sample, trial measurements were attempted, starting from about 0.003 mW at the surface, and gradually increasing the intensity until an acceptable signal-to-noise ratio was obtained, without either sample degradation or modification of the spectral features (width and position of the peaks). The final experimental power ranged between 1.0 and 5 mW.

ATR measurements were performed using a Nexus Nicolet interferometer, equipped with a KBr beam splitter and extended with a ZnSe cell and a liquid nitrogen cooled MCT/A detector. Measurements were performed in the 4,000–650-cm⁻¹ range at a resolution of 8 cm⁻¹, with an average of 300 acquisitions per sample.

FTIR transmission measurements were performed, exclusively on laboratory samples, by means of a Nexus Nicolet interferometer, equipped with a KBr beam splitter and a deuterated triglycine sulphate KBr detector. The system was operated under a flux of dry air (absolute humidity less than 0.06 gm⁻³), with a resolution of 4 cm⁻¹. Samples were dispersed in KBr pellets (0.2 mg of sample in 200 mg KBr) and measurements were performed in the 4,000–400-cm⁻¹ range, with an average of 200 acquisitions per sample.

XRF spectra were recorded by means of an Assing Lithos 3000 portable spectrometer, equipped with a molybdenum X-ray tube. With such an instrument, the radiation can be collimated with different beam diameters (from 0.5 to 3 mm), depending on the area of interest. In this experiment, a 2-mm collimator was used together with a zirconium filter. A red laser (695 nm) and a camera (both integrated into the system and controlled by the instrument software) were used to choose the area to be sampled. Measurements were performed with the tube operating at 25 kV, 0.300 mA, in the 0–25-keV range, and with a resolution of 160 eV at 5.9 keV, lasting 1,800 s for each acquisition.

Samples

- Six fragments (seventh century to eighth century AD) from the Great Mosque of Sana'a in Yemen; three of them made of paper, and the others made of parchment (Fig. 1).
- Four iron tannic inks prepared using commercial extracts of hydrolysable tannins from chestnut (*Castanea sativa*), sumach (*Rhus coriaria*), myrobalan (*Prunus cerasifera*) and valonia (*Quercus macrolepis*). As a source of condensed tannins, one extract of mangrove (*Rhizophora mangle*) was used. The inks were prepared by dissolving 0.78 g of each extract in 5.0 ml of deionized water and then adding 0.31 g of gum arabic. Finally 0.47 g of iron(II) sulphate was dissolved in each solution. After 12 h of the decanting phase, the inks were ready for use.
- Two iron gall inks obtained from Aleppo and Italian (Umbria region) oak-gall nuts. The inks were prepared by dissolving 0.78 g of powdered oak-gall nuts in 5.0 ml of deionized water for 24 h. After filtration, the volume was set to 5.0 ml; gum arabic and iron(II) sulphate were then added in the same way and amount used in the preparation of the other tannic inks.

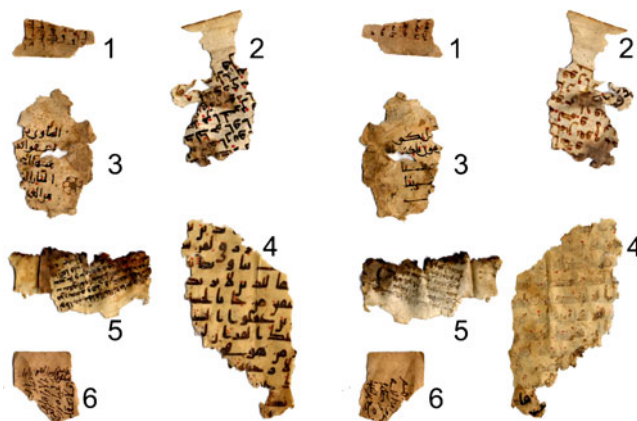


Fig. 1 Original Yemenite samples: recto (left) and verso (right). The writing support of samples 1, 3 and 6 is paper and that of samples 2, 4 and 5 is parchment

The recipe for manufacturing all the inks was developed around 20 years ago in the ICRCPAL Laboratory of Chemistry and allows one to obtain a stable non-corrosive ink. A good ink may not blur, fade, form halos, penetrate into the support passing from the verso to the recto side or corrode the support either by a hydrolysis mechanism and by oxidative action and it must be stable in light.

Results and discussion

In the following, the experimental results are discussed in two sections, one concerning the characterization of the substrates and the other concerning the studies conducted on original and laboratory inks.

Characterization of the substrates

Samples 1, 3 and 6 (Fig. 1) analyzed under the optical microscope showed that all papers were manufactured using hemp fibres, whereas samples 2, 4 and 5 are parchment from sheep.

XRF measurements, both on paper and parchment, showed the presence of many elements (Table 1). In addition to calcium, which is always used in paper sizing and parchment manufacturing, bromine and chlorine could be related to the water or the salts used for the preparation of the supports, whereas the other elements appear to be impurities related to preparation, use and conservation of the materials. For example, lead could derive from inkwells or water pipes, titanium could arise from the ceiling of the mosque, and mercury and arsenic are a contamination from diacritical marks and yellow decorations (see “[Characterization of the inks](#)”).

Fragments 2, 3, 4 and 5 presented lighter and darker areas that evidenced, within the same fragment, identical elemental composition except for iron: XRF analyses revealed a

larger amount of iron in the darker areas than in the lighter ones. The increase in iron content is probably due to migration of the graphic medium caused by localized humidity.

ATR-FTIR measurements on the cellulosic samples showed that all the papers were sized with gelatine. In particular, the gelatine layer present in sample 3 was extremely thick, and the gelatine signals covered the absorption of the cellulose underneath. Moreover calcium carbonate, always added in paper manufacturing, was degraded to calcium oxalate in fragment 3, thus indicating a possible fungal attack. Fungi are able to metabolize calcium carbonate, producing calcium or mixed oxalates [1, 2] depending on the presence of different cations on the surface. Some spectra collected from fragment 3 are reported in Fig. 2, where a mixture of calcium, iron and copper oxalates is well visible, as are some typical features of gelatine-sized papers with peaks at 2,924 and 2,853 cm^{-1} , corresponding to CH asymmetrical and symmetrical stretching, respectively, in amide [3].

The great amount of sizing materials present in all cellulosic samples affected the Raman spectra by showing a very intense fluorescence band. For this reason it was impossible to collect Raman spectra from the cellulosic substrate.

Among the three parchment samples (2, 4 and 5 in Fig. 1) we were able to collect ATR-FTIR and Raman spectra only from sample 2. The roughness of fragments 4 and 5 made it impossible to obtain good contact between the surface and the ZnSe crystal, whereas the Raman signal was masked by an intense fluorescence background.

Results of the analyses conducted on sample 2 indicated “western” manufacturing of the parchment with calcium carbonate: no presence of tannins, used for example in Jewish parchment manufacturing [4], was detected.

Moreover, the ATR-FTIR investigation revealed (Fig. 3) that the parchment support was gelatinized, as confirmed by the presence of an intense band at 1,030 cm^{-1} due to C–O–C

Table 1 Elemental composition of Yemenite black inks from X-ray fluorescence measurements

Sample	Elements in the support	Elements in the ink
Fragment 1	K, Ca, Fe, Hg, Pb, Br	K, Ca, Fe, Hg, Pb, Br
	Traces of Cl, Mn	Traces of Cl, Ti, Mn, Cu, Zn, Sr
Fragment 2	K, Ca, Fe, Br	K, Ca, Fe, Hg, Pb, Br
	Traces of Cl	Traces of P, Cl, Ti, Mn, Zn, Sr
Fragment 3	K, Ca, Fe, Hg, Pb, Br	K, Ca, Fe, Cu, Hg, Pb, Br
	Traces of Cl, Mn, Cu	Traces of Cl, Ti, Mn, Zn, Sr
Fragment 4	K, Ca, Fe, Br	K, Ca, Fe, Br
	Traces of Cl, Mn, Zn, Hg	Traces of Cl, Ti, Mn, Zn, Hg, As, Sr
Fragment 5	K, Ca, Ti, Mn, Fe, Br	K, Ca, Fe, Cu, Hg, Pb, Br
	Traces of Cl, Zn, Cu, Pb	Traces of Cl, Ti, Mn, Zn, Sr
Fragment 6	K, Ca, Fe, Br	K, Ca, Fe, Br
	Traces of Cl, Ti, Mn, Cu, Zn, Sr	Traces of Cl, Mn, Cu, Zn, Sr

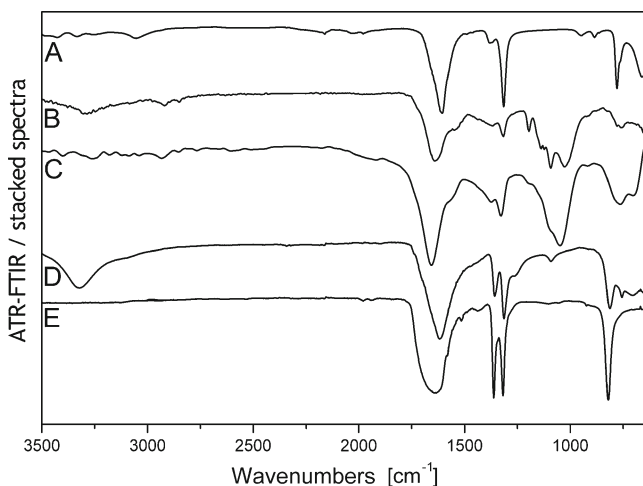


Fig. 2 Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectra recorded from two areas of sample 3 showing the presence of oxalates, compared with standard compounds: *A* Calcium oxalate standard; *B* fragment 3 calcium and iron oxalate; *C* fragment 3 calcium and unidentified oxalates; *D* aged iron(III) oxalate standard; *E* copper oxalate

stretching of the saccharide structure of the gelatine [5–7], whereas the Raman measurements showed a severe degradation of the parchment support, evidenced by the collapse of the bands around $1,650\text{ cm}^{-1}$ (Fig. 4) ascribable to the stretching of C=O amide I α -helix and β -sheet [8].

Both ATR-FTIR and Raman analyses showed the presence of a large amount of mineral salts, mostly impurities from the walls and ceiling where manuscripts had been hidden: in particular, in some areas, silicates and gypsum were found by ATR-FTIR investigation. By means of Raman spectroscopy, it was possible to perform a scan along fragment 2, where two different coloured areas were evident: one lighter and one darker. In the light area, Raman measurements showed the presence of a large amount of

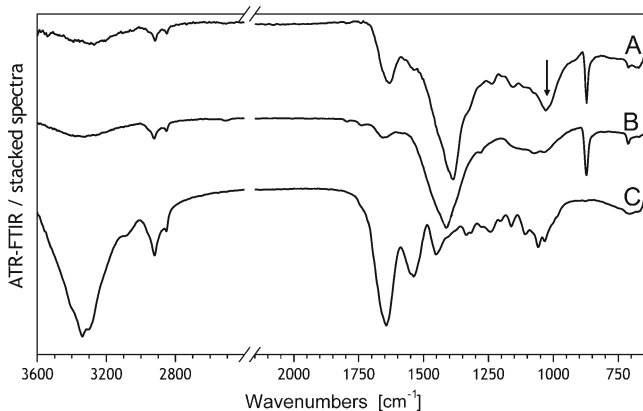


Fig. 3 ATR-FTIR spectrum recorded from sample 2 (*A*) compared with the spectra of standard parchment containing calcite (*B*) and gelatine standard (*C*). The peak at $1,030\text{ cm}^{-1}$, marked with an arrow, is attributable to the gelatinization of the support (C–O–C stretching of the saccharide structure of the gelatine)

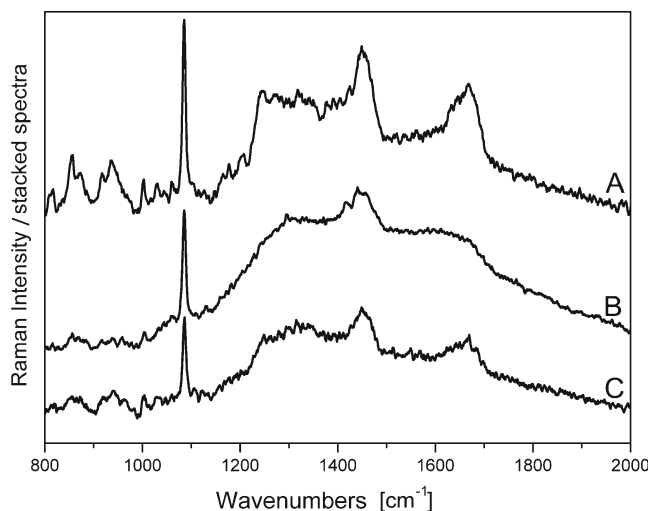


Fig. 4 Raman spectrum of the parchment support of sample 2 (*B*) compared with a standard parchment in good condition (*A*) and artificially degraded (*C*). The power on the samples was 3 mW. The characteristic bands in the region $1,200\text{--}1,800\text{ cm}^{-1}$, in particular the band around $1,650\text{ cm}^{-1}$, have “collapsed” in *B*, showing severe degradation of the support

different salts (Fig. 5): calcite (CaCO_3 , band at $1,088\text{ cm}^{-1}$), gypsum (CaSO_4 , band at $1,006\text{ cm}^{-1}$) and different iron sulphates, in particular $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ (band at $1,025\text{ cm}^{-1}$) and $(\text{Fe}^{2+}/\text{Fe}^{3+})_n(\text{SO}_4)_m$ (band at 980 cm^{-1}) [9].

On the border between lighter and darker areas, calcite and a mixture of differently hydrated calcium and mixed oxalates, in particular whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$; characteristic Raman peaks at $1,605, 1,493, 900, 596$ and 505 cm^{-1}) and weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$; characteristic Raman peaks at $1,628, 1,475, 909, 868, 596$ and 505 cm^{-1}), were detected, whereas in the dark area only weddellite was revealed (Fig. 6) [10].

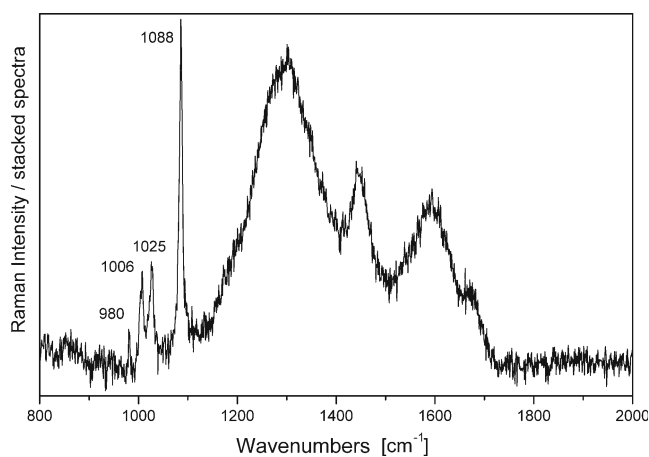


Fig. 5 Raman spectrum of the lighter area of sample 2. The power on the samples was 2.5 mW. Contamination of the substrate with calcite ($1,088\text{ cm}^{-1}$), gypsum ($1,006\text{ cm}^{-1}$) and different iron sulphates [$\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$, $1,025\text{ cm}^{-1}$, and $(\text{Fe}^{2+}/\text{Fe}^{3+})_n(\text{SO}_4)_m$, 980 cm^{-1}] is clearly visible

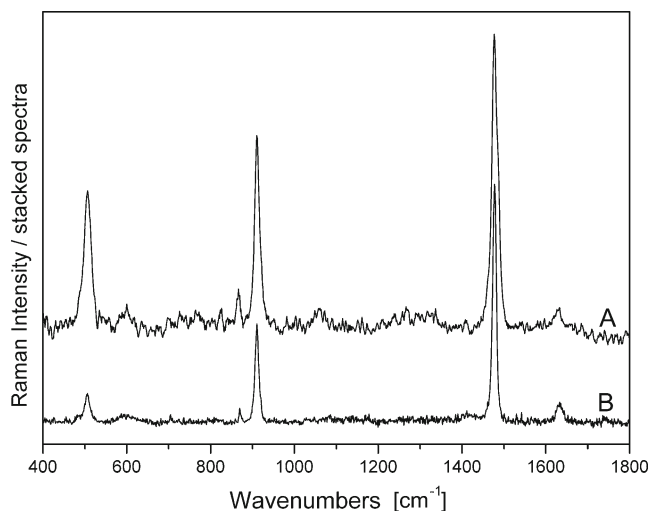


Fig. 6 Comparison between a spectrum acquired on the darker area of sample 2 (A, power on the sample 2.5 mW) and the spectrum of weddellite standard (B, power on the sample 3 mW)

This different distribution of salts, related to the different colour of the parchment, suggested a biological attack, as previously reported for the paper support, since fungi can reprecipitate secondary carbonates and biomineralize hyphae with both calcite and whewellite [11, 12].

The presence of fungi on the samples with lighter and darker areas was verified by the ICRCPAL Laboratory of Biology [13].

Characterization of the inks

The combination of elemental and molecular techniques allowed the characterization of the composition of all red, yellow and black writing media. Differently from the investigation on the substrates, it was possible to collect good-quality spectra from all samples.

Red inks used for diacritical marks were unambiguously identified as cinnabar (HgS) by means of Raman spectroscopy (bands at 253, 285 and 342 cm⁻¹).

XRF analyses of the black inks (Table 1) did not reveal significant differences in the elemental composition among inks and substrates except for a larger amount of potassium and iron in the inks that could suggest the use of iron gall inks, but such a hypothesis had to be confirmed by molecular techniques.

The Raman analysis of the black inks provided interesting insights. Black inks on fragments 1, 2 and 4 correspond to rather well preserved high-quality iron gall inks to which carbon black had not been added [14, 15]. In contrast, the Raman peak centred at 1,478 cm⁻¹, characteristic of iron gall inks, in fragments 3, 5 and 6 was clearly shifted between 1,485 and 1,490 cm⁻¹, whereas the other spectral features were well matched (Fig. 7).

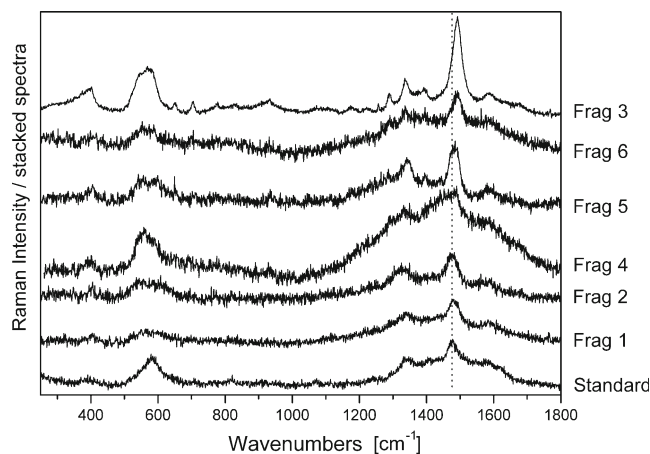


Fig. 7 Raman spectra collected from the original inks (power on the samples 1 mW), compared and iron gall ink standard (power on the samples 5 mW). The main peak of samples 1, 2 and 4 is centred at 1,478 cm⁻¹, whereas for samples 3, 5 and 6 the peak is shifted between 1,485 and 1,491 cm⁻¹

The same shifts were less recognizable in the ATR-FTIR spectra owing to the superposition between ink features and peaks belonging to the supports. Moreover, in the specific case of iron sulphate based inks, their application on parchment or paper can cause a displacement of Ca²⁺ ions from CaCO₃, resulting in the formation of CaSO₄, and the salt features in the infrared spectra are dominant over the organic matter ones.

To explain the spectral modifications recorded with Raman spectroscopy, we hypothesized that the ink could have been manufactured by extracting tannin from plant materials other than gallnuts.

To investigate this hypothesis, we decided to prepare laboratory sample inks composed of iron sulphate and tannins

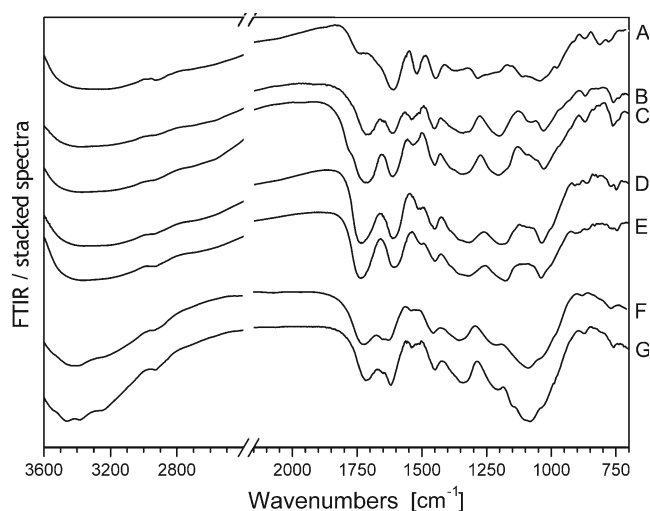


Fig. 8 FTIR spectra collected from the laboratory sample inks dispersed in KBr pellets: A mangrove, B sumach, C myrobalan, D chestnut, E valonia, F oak gall from Italy, G oak gall from Aleppo

Table 2 Fourier transform infrared (FTIR) peaks measured on the ink samples and related assignment according to the literature

Chestnut ink	Valonia ink	Aleppo oak-gall ink	Italian oak-gall ink	Myrobalan ink	Sumach ink	Approximate assignments [18, 19, 23]
1,726	1,730	1,718	1,718	1,718	1,718	ν antisymmetric C=O carboxylic acids
1,612	1,604	1,620	1,621	1,606	1,611	ν C=C chain, ν C=C aromatic, ν C–O, β C–H, β ring
1,511	1,506	1,532	1,532	1,534	1,535	
1,460	1,457	1,451	1,457	1,459	1,457	ν C–C, β C–H, β OH
1,332	1,305 1,378	1,351	1,352	1,352	1,353	ν C–OH, β C–H, β OH
		1,210	1,226			Ring breathing
1,182	1,188	1,154			1,198	ν C–O–C
1,105	1,111 (sh)	1,098	1,089	1,085 (sh) 1,066 (sh)	1,100	ν C–C, β C–H, ν =C–C (esters)
1,031	1,034	1,035	1,018	1,035	1,030	ν C–C, ν C–O carboxylic (present in gallic acid)
881	888	870	874 989	866	894	In-phase out-of-plane OH \cdots O bending, γ C–H (aromatic)
761	757	706 614	753 620	755 604	755 678	ν C–C, ν C–O carboxylic β ring, β C–O to OH
560	539	530	544		565	ν ring C–C, β ring

ν stretching, β in-plane bending, γ out-of plane bending

extracted from different plants that are fairly common in the Mediterranean and Middle Eastern areas (see “Samples”) and that are characterized by their content of hydrolysable or condensed tannins.

The main difference between the two “families” of tannins is due to the dominating presence of gallic and ellagic acid and pyrogallol in the hydrolysable tannins, whereas the condensed tannins contain polymeric flavonoids, mostly epicatechin and catechin linked via a C–C bond between the C8 terminal unit and C4 of the other molecule, with the possibility of further polymerization [16]. In particular, mangrove contains epicatechin, epigallocatechin and epicatechin gallate [17].

For comparison with the most diffused inks, two iron gall inks were also prepared.

We decided to use oak-gall nuts from different geographical provenances (Aleppo, Syria, and Italy, Umbria region) in order to investigate whether or not the different origin of the raw materials might affect their spectra.

Both Raman spectroscopy and ATR-FTIR spectroscopy showed that the typical peaks of iron gall inks were centred around the same wavenumber, irrespective of the geographical provenance of the galls, even if the relative intensity of the main peaks was different. As regards the other tannic inks, the results clearly demonstrated that different species produced different features in both vibrational techniques.

FTIR spectra (Fig. 8) recorded on the laboratory samples dispersed in KBr pellets were in very good agreement with the scientific literature on pure tannins, on single molecules mostly contained in the hydrolysable and condensed tannins and on pure gallic and tannic acids [18–23].

Table 3 FTIR peaks measured on mangrove ink samples (condensed tannins) from KBr pellets and related assignment according to the literature

Mangrove wavenumber (cm $^{-1}$)	Approximate assignments [18, 21, 22]
1,751	ν antisymmetric C=O carboxylic acids
1,641	ν symmetric C=O carboxylic acids
1,520, 1,604	ν C=C aromatic (present in catechin)
1,448	In-plane ν C–OH carboxylic, ν ring, β CH
1,371	ν ring C–C, β C–H, β OH alcoholic (present in catechin)
1,120	ν C–O carboxylic acids, β OH carboxylic, ν C–O–C in cyclic ether (present in catechin)
1,044	ν C–OH alcoholic, non-aromatic (present in catechin)
1,014	ν ring C–C, ν C–O carboxylic
970	In-phase out-of-plane OH \cdots O wagging for carboxylic acids (present in catechin)
911	ν ring C–C, ν C–O carboxylic, β ring
871	γ C–H (present in catechin)
814	γ aromatic C–H in disubstituted ring (present in catechin)
775	ν ring C–C, ν C–O carboxylic, β ring
724	ρ CH $_2$
635, 678	ν ring C–C, β and γ C–O carboxylic
586	β ring, β C=O carboxylic
540	ν ring C–C, ν C–O in C–OH, β ring
418, 446	τ ring

ν stretching, β in-plane bending, γ out-of plane bending, τ torsion, ρ rocking

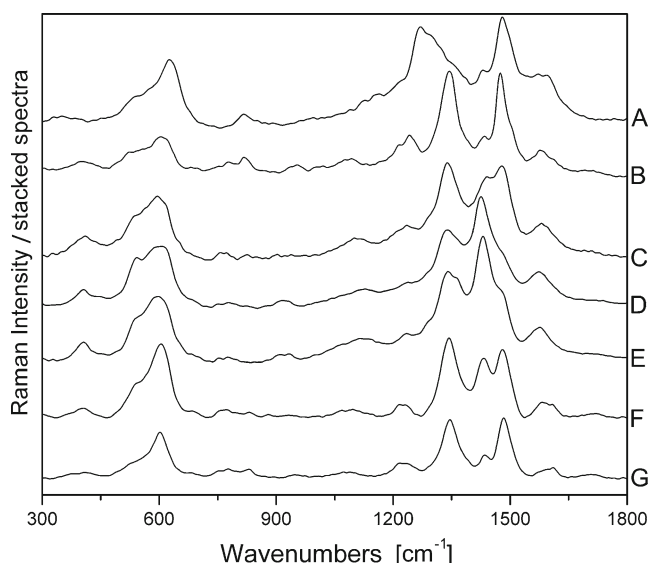


Fig. 9 Raman spectra collected from the laboratory sample inks: *A* mangrove, *B* sumach, *C* myrobalan, *D* chestnut, *E* valonia, *F* oak gall from Italy, *G* oak gall from Aleppo. The power on the samples was 5 mW

The most characteristic peaks and their attribution for hydrolysable tannins are presented in Table 2 and for condensed tannins (mangrove) in Table 3. Raman spectra are reported in Fig. 9 and the most interesting wavenumbers are reported in Table 4. The positions of the peaks presented in Tables 2, 3 and 4 were obtained by fitting the spectra with multipeak Gaussian functions in the range 1,900–700 cm^{-1} for infrared features and 900–1,750 cm^{-1} for Raman features and by minimizing the χ^2 of the fit.

It is interesting to note that in the FTIR spectra of the two oak-gall inks and the sumach and myrobalan inks there is a peak in the 1,708–1,718- cm^{-1} region, attributable to the carboxylic C=O stretching of a gallic acid residue [18, 19], whereas the spectra of chestnut and valonia inks exhibit a peak centred around 1,730 cm^{-1} , attributable to the carboxylic C=O stretching of a tannic acid residue [20], and the spectra collected from mangrove show some absorptions (at

814, 871, 970, 1,044, 1,120, 1,371, 1,520 and 1,604 cm^{-1}) related to the presence of catechin.

Moreover, as can be seen from Fig. 8 and the data presented in Table 2:

- Chestnut and valonia, which belong to the same botanical family (order Fagales, family Fagaceae), have the same absorptions at the same wavenumbers.
- The two oak-gall spectra are quite similar, as are the positions of almost all peaks.
- Mangrove has spectral features quite different from those of the other plants.

By observing all collected Raman spectra and taking into account only the mean wavenumber of the most characteristic bands (1,350–1,720- cm^{-1} region), one can recognize four “families”:

1. Chestnut and valonia, belonging to the same botanical family, with peaks centred around 1,350, 1,428, 1,476 and 1,578 cm^{-1}
2. Myrobalan and sumach, with peaks centred around 1,342, 1,445, 1,486 and 1586 cm^{-1}
3. Aleppo and Italian galls, with peaks centred around 1,346, 1,430, 1,484, 1,590 and 1,710 cm^{-1}
4. Mangrove, the spectral features of which are completely different from those of the other plants, with peaks centred around 1,480, 1,573 and 1,598 cm^{-1} .

Moreover, only in the inks obtained from oak galls is the peak due to the antisymmetric stretching of the carboxylic C=O of gallic acid (around 1,710 cm^{-1}) clearly visible.

The fine analysis of the wavenumbers obtained by fitting procedures is more complicated, because tannins are branched molecules, bonded together, sometimes polymerized, and in the ‘fingerprint region’ a large number of vibrations can occur, each vibration participating in each part of the spectrum to a different extent, as shown in [19] for pyrogallol and gallic acid.

Table 4 Raman shift of the laboratory-prepared tannic inks

Chestnut ink	Valonia ink	Aleppo oak-gall ink	Italian oak-gall ink	Myrobalan ink	Sumach ink	Mangrove ink	Approximate description [24, 29]
		952				990	w(CH ₂)
1,102	1,099	1,077	1,097	1,099	1,091	1,169	v(C–O) (alcoholic)
1,245	1,240	1,230	1,234	1,253	1,236	1,274	β(C–H); v ring; v(C–O) (ester)
1,347	1,351	1,348	1,345	1,341	1,343	1,323	v(C–O) (carboxylic)
1,425	1,430	1,431	1,428	1,448	1,433	1,429	Symmetric v(COO ⁻); β(C–H); β(O–H)
1,475	1,477	1,486	1,483	1,486	1,474 1,499	1,482	Scissor (CH ₂); v(C=C) ring (semicircle stretching); β(C–H)
1,575	1,575	1,596 1,707	1,584 1,714	1,586	1,586	1,568	v(C=C) ring (quadrant stretching) v(C=O)

For all these reasons the fine analysis did not allow us either in the infrared spectra or in the Raman spectra to recognize and point out singular or multiple bands that could be unambiguously related to a specific plant used for the preparation of a single ink. Even though the Raman spectra display differences in the Raman shift of some bands, none of these seems to be diagnostic. This probably arises from the fact that the main components of the samples analyzed (aromatic rings, carbonyls, carboxyls, carbon–carbon double bonds, etc.) are common to all tannic sources.

Tannins are, in fact, polyphenolic compounds; therefore, the most typical spectral region 1,300–1,600 cm^{-1} should involve the aromatic ring vibrations; the 1,500–1,600- cm^{-1} range involves C–C ring vibrations in which two opposite quadrants of the benzene ring stretch, whereas the other quadrants contract and the CH in plane bends [24]. In the 1,400–1,500- cm^{-1} region, the C–C ring vibration results from the stretching of one semicircle of the ring, whereas the other contracts; at same time CH bends [24]. The band around 1,340 cm^{-1} could be attributable to CH in-plane deformation.

The measured shifts in the analyzed samples depend on the different lengths and chemical compositions of the polyphenolic units of the raw materials [25].

Since no bands could be considered as a marker of the plant from which the ink originated, we decided to investigate whether the relative intensities of the characteristic bands could be regarded as diagnostic. We therefore studied the relative intensities and the related areas of the characteristic peaks for the different inks and, for completeness, we remeasured the laboratory inks after 3 years of natural ageing in order to check if the relative intensity of the main peaks could have been modified after ageing. The same

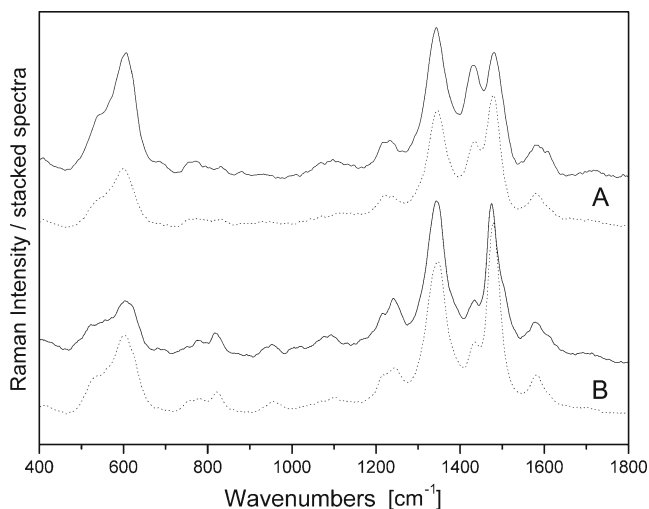


Fig. 10 Raman spectra collected from freshly prepared laboratory ink samples (2009, *straight line*) and after 3 years of natural ageing (2012, *dotted line*): *A* oak gall from Italy, *B* sumach. The power on the samples was 5 mW

samples after the natural ageing showed some variations in the relative intensity of the characteristic bands, which hence cannot be regarded as diagnostic. Two examples of the modification of the Raman spectrum as a function of ageing are shown in Fig. 10.

Conclusions

The elemental and molecular techniques used had proven to be extremely useful for the characterization of the conservation state of the original Yemenite manuscripts that were demonstrated to be in a bad condition, showing rodent and fungal attack, the presence of stains, carbonized parts, holes and tears, folds and undulations, severe degradation of the parchment and the presence of a great amount of salts, which had contaminated the surface of the documents.

The presence of many salts and impurities was detected, and in particular the recorded modification from calcium carbonate to different oxalates evidenced the effects of fungal attack on almost all samples and supports.

The analyses conducted on the original inks in the Yemenite manuscript showed the evidence of the possible use of different tannin sources [26, 27] and pointed out the need for better research on the methods used to manufacture the iron inks.

Spectroscopic research on tannic inks, other than oak-gall inks, confirmed that different tannins cause modification of the spectral features of the inks, even though it is presently impossible to distinguish the raw material directly from the spectra in a non-destructive way because the main components of the raw materials are common to all tannin sources.

From the experimental data it can be inferred that tannins from the same family (i.e. chestnut and valonia, family Fagaceae) are quite similar, with the main peaks centred around the same wavenumbers and that the geographical origin of oak galls does not produce a shift in the main peaks of the ink. Moreover, the possible use of condensed tannins is easily recognizable.

Despite the fact that the main peak (1,480–1,491- cm^{-1} region) of the Yemenite tannic inks analyzed does not match any of the samples investigated, the approach seems to be correct for characterizing iron tannic inks. Indeed, it explains the differences in wavenumbers often reported in the literature [28] and obtained from many original samples.

Even though deeper studies on the chemical and spectroscopic characterization of tannins are still in progress, our research has demonstrated, in a direct and non-destructive way, the possibility of identifying different sources of the tannins used to produce original inks.

In the case of library and archival supports, the Italian Cultural Heritage Ministry forbids sampling. For this reason we tried to characterize the inks and their manufacturing in a

non-destructive way, focusing our attention on the most intense and representative bands, which are always detectable in original samples, even very aged ones.

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