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Organic red colorants in Islamic manuscripts (12th-15th c.) produced in al-Andalus, part 1

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A R T I C L E I N F O

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A B S T R A C T

The conservation of Islamic manuscripts in the *Fondo Ka'ti* created the opportunity to study the organic red colourants applied in five manuscripts, which include a Koran (1198), a theology treatise (14th c.), a book of poems from Al-Sarishi (15th c.), a biography of the Prophet (1468) and manuscript 19 (1485). These dark red colours were characterized using fibre optic reflectance spectroscopy (FORS-VIS), microspectrofluorimetry and infrared spectroscopy (microFTIR). Microspectrofluorimetry detected the presence of a lac dye chromophore in all the manuscripts studied and ascribed it to specific medieval recipes for three of the manuscripts. This was based on the very good matches obtained with our database of paint reconstructions that were prepared according to medieval technical sources; the dark reds found in the Koran compared very well with the recipe 'to make red ruby from lukk' from Ibn Bādīs text (11th c.); the brighter reds applied in the book of poems and in the biography of the Prophet, with recipe 113 from the Paduan manuscript (16th c.). MicroFTIR completed the characterization of the paint formulation, identifying the proteinaceous nature of the binding media as well as the fillers. It also showed the presence of oxalate compounds, possibly, resulting from the binding media degradation, a mark of the recent and dramatic history of these books. Finally, these red dyes were successfully compared to lac dye colours previously characterized in 12th-13th c. Portuguese manuscript illuminations. From Mali to Iberia, tracing the rich diversity of a precious heritage legated by medieval Arabic culture.

1. Introduction

The rescue and conservation of a group of Islamic manuscripts, from Timbuktu, Mali, created the opportunity to study the materials and techniques used to illuminate these medieval books [1–4]. In this work, we focused on the molecular characterization and degradation assessment of the red dyes. The organic purples applied in the *Theology treatise* and all the inorganic pigments will be discussed in another publication as part 2 of this work.

These manuscripts are now preserved at the Timbuktu Andalusian Library (*Biblioteca Andalusi de Tombuctu*, Fondo Ka'ti), and its president, Ismael Diadié Haïdara, dated them between the 12th c. and the 15th c and made a first classification as follows; a Koran (1198), a theology treatise (14th c.), a book of poems from Al-Sarishi (15th c.), a biography of the Prophet (1468) and manuscript 19 (1485). Thereafter referred to as *Koran*, *Mss19*, *Al-Sarishi poems*, *Prophet biography* and *Theology treatise*. Except for the *Koran*, applied on parchment and paper over parchment, all other manuscripts are written and illuminated on paper support.

This research will increase our knowledge on the materials and techniques of manuscripts produced in al-Andalus, particularly in the use of historical dyes.

1.1. The manuscripts of Fondo Ka'ti

The five manuscripts were produced either in Andalusian territory or under the Arab diaspora. Until 2012, the manuscripts were stored in Timbuktu's libraries [5–9], when their integrity was at risk due to the

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imposition of the Sharia law by a faction of the Tuareg tribe. It was possible to rescue more than 370,000 manuscripts, in a plan co-ordinated by Abdel Kader Haïdara, and for which many people risked their lives for the safeguard of their cultural heritage [6–8]. To avoid their destruction, these works were hidden and transported in dramatic conditions [9].

1.2. State of art on the characterization of organic red colorants in Islamic manuscripts

Dye characterization in medieval illuminations is a challenging endeavour [2–4,10,11], as we need to address the fundamental chromophore as well as the full "formulation" to get in-depth knowledge about the culture that produced these precious colours - which are centuries or even millennia - old [12,13]. High performance liquid chromatography with diode array detection and mass spectrometry (HPLC-DAD-MS) combines high separation power with a complete dye characterization, but only in exceptional cases it will be possible to have a micro-sample from an illumination with enough material to be extracted for HPLC. For this reason, for dye identification, our group devised other approaches that combine high spatial resolution, with the highest sensitivity (microfluorimetry) and a molecular fingerprint (surface-enhanced Raman spectroscopy -SERS) [2,3]. To gain an insight into the paint formulations, we included fibre optic reflectance spectroscopy in the visible (FORS) and Fourier-transform infrared spectroscopy (microFTIR). *In situ* techniques, such as microfluorimetry and FORS, can only be used for an in-depth analysis if supported by a made-to-measure reference database such as the one we have been building, over the past 15 years, based on studying and reconstructing the medieval processes for making pigments and paints that were used to create medieval manuscript illuminations [14–16].

To the best of our knowledge, in only three publications organic colourants were unequivocally identified in Islamic manuscripts; indigo was detected by Raman microscopy and HPLC-DAD by Lucia Burgio (and co-workers) [17] and Teresa Espejo Arias (and co-workers) [18], respectively; carminic acid was characterised by SERS, by A. El Bakkali and co-workers [19]. The other publications rely essentially on energy dispersive X-ray fluorescence (EDXRF) and FORS that will give an indication of the possible colourants present; in the majority of the cases, authors have proposed the use of "cochineal" [20–23], and in one publication of "kermes" [21], Table 1. With FORS it is possible to discriminate between families of chromophores such as flavonoids and anthraquinones; for the latter, it is also possible do distinguish between anthraquinone rings either bearing or not bearing a carboxylate group. This is relevant because it allows to differentiate between anthraquinone chromophores produced by parasitic insects (e.g., carminic, kermesic and laccaic acids) from anthraquinones of vegetal origin, Figs. 1 and 2. The main results and analytical techniques for the

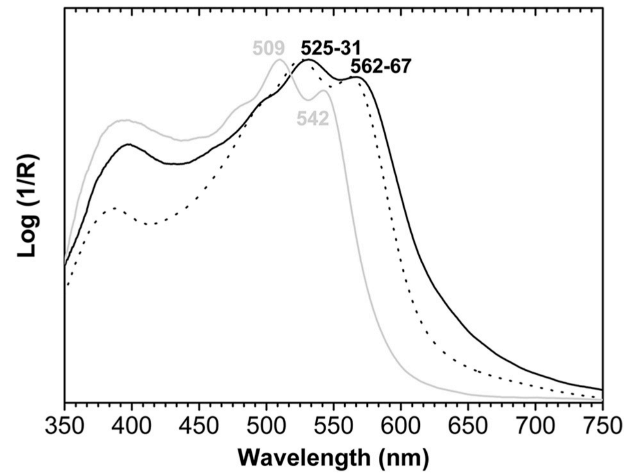

Fig. 1. Apparent absorbance spectra for anthraquinone lake pigments obtained from (*grey*) *Rubia tinctorum* roots, (*black*) *Kerria lacca,* (dashed) *Dactylopius* coccus, applied as paints; the first and latter with arabic gum in paper, and the *Kerria lacca* with egg white in parchment.

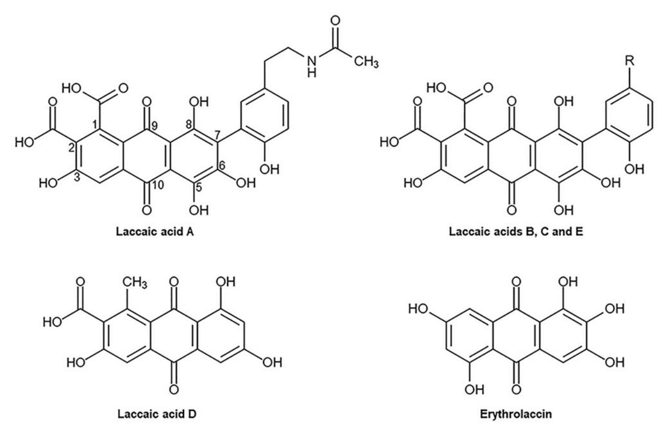

Fig. 2. Chemical structures of laccaic acids A to D (B, R = CH_2CH_2OH; C, R = CH_2CHNH_2COOH; E, R = $CH_2CH_2NH_2$) and erythrolaccin. Laccaic acid A is the main chromophore extracted from sticklac and displays a red to carmine colour; erythrolaccin is yellow and is usually found in the resin.

Table 1
Organic dyes identified in Arabic illuminated manuscript, with information on the date of the manuscript, analytical technique used for the dye identification and spectral data published.

Dyes[a]	Manuscript date	Analytical technique	Spectral data	References
Cochineal red	12^{th} - 15^{th} c.	µ-XRF; DRS	n.a.	Deroche F. et al., 2005 [20]
Cochineal red Kermes red	9^{th} - 11^{th} c.	DRS; µ-XRF; MO	n.a.	Roger-Puyo P. et al., 2015 [21]
Not found	10^{th} - 11^{th} c.	µ-XRF; µ-Raman	n.a.	Hamdan N. M. et al., 2012 [22]
Cochineal red	10^{th} - 19^{th} c.	DRS; MO; µ-XRF	λ_{abs}: 500,525,561 nm[b]	Roger P. et al., 2004 [23]
Not found	14^{th} c.	µ-XRF; µ-Raman	n.a.	Duran A. et al., 2011 [24]
Not found	14^{th} - 16^{th} c.	n.a.	n.a.	Beny A. et al., 2015 [25]
Organic red	16^{th} - 18^{th} c.	µ-Raman; SEM-EDX	SEM-EDX: Ca, Al, K, S	Tanevska V. et al., 2014 [26]
Not found	n.a.	MO; µ-XRF; Chromatography	n.a.	Arias T. 1997 [27]
Not found	n.a.	MO; µ-XRF; µ-Raman	n.a.	Biddle M. 2011 [28]
Cochineal	n.a.	µ-Raman; SERS; DRS	λ_{abs}: 500,528,570 nm SERS: 1582,1444,1330,1223 e 482 cm^{-1},[b]	El Bakkali A. et al., 2012 [19]
Indigo	16^{th} - 18^{th} c.	µ-XRF; µ-Raman	µ-Raman: 546,599, 1575, 1584 cm^{-1},[b]	Burgio L. et al., 2008 [17]
Indigo	11^{th} - 17^{th} c.	DRX; SEM/EDX; µ- FTIR; HPLC;	HPLC: indigotin	Arias T. et al., 2008 [18]

[a] Names given by the authors.
[b] Data collected from the spectra displayed in the publications (not described in the text).

study of organic colorants in medieval Islamic manuscripts are summarized in Table 1 [17–28].

Another source of information on the dyes used are medieval technical texts and textile studies in the Islamic world, which show that the main colorants used to produce reds are anthraquinone and brazilwood based [29,30].

2. Materials and methods

2.1. The manuscripts and overall modus operandi

This project encompasses the study of five manuscripts, specifically a *Koran*, *Mss 19*, *Al-Sarishi poems*, *Prophet biography* and *Theology treatise*. The dating and typology of the manuscripts were defined by the president of the Fondo Ka'ti, Ismael Diadié Haïdara. Details on the manuscripts can be found on Table S1.

The first screening of the manuscripts was carried out by FORS and microEDXRF. For each of the five manuscripts 3 folios were analysed, except for the *Koran* and *Mss*19, with 4 and 6 folios analysed respectively, and for each colour data was acquired in three areas, in a total of three points per area per folio: *Koran* (fols 1, 30, 47 and 70), *Mss 19* (fols 18, 19, 109, 110, 217 and 218), *Prophet biography* (fols 1, 4 and 107), *Al-Sarishi poems* (fols 1, 4 and 10) and *Theology treatise* (fols 17, 43 and 70). This allowed to select areas to be analysed by microRaman and sampled for microFTIR allowing us to characterize pigments, binders and gain insight into the full paint formulation. One or two areas per colour per manuscript were selected for microsampling, except for the *Al-Sarishi poems* were only vermilion red was sampled, due to the fragile condition of the support. MicroRaman was used *in situ*, with 3 points per colour per folio, or in microsamples. Microspectrofluorimetry was then used for both *in situ* and microsample analysis to identify the colorants present. The criteria for the selection of the folios for analysis was based on ensuring that all the colours present in the manuscripts were part of the selected folios and that they encompassed as much variability as possible.

2.2. Historically accurate reconstructions

The historically accurate reconstructions of lac dye have been prepared within the scope of a doctoral thesis, and more details can be found in previous publications in which these reproductions have been described [31–38]. For lac dye, fourteen recipes were selected from eight treatises/recipe books: Ibn Bādīs manuscript (c. 1025) [33], Mappae clavicula (9th–12th c.) [34], *Livro de como se fazem as cores* (‘book of all colour paints’, 15th c.) [35], the Le Begue manuscript (1431) [36], the Bolognese manuscript (15th c.) [36], the Strasbourg manuscript (15th c.) [37], the Montpellier manuscript (15th c.) [38] and the Paduan manuscript (late 16th to 17th c.) [36]. The main steps for the reproduction of lac dye lake pigments are common to all recipes. The resin, sticklac, is ground and a basic solution is added and then is filtered to remove impurities of the lac and its residual resin. Some recipes are complete at this point and do not add a complexing agent as a forth step. In other recipes, Al^{3+} in the form of alum is added, to precipitate the colorant. In this work, three recipes of lac dye will be used for comparison with the Islamic organic red colorants: chapter 6 (‘red ruby from the *lukk*’) from the Ibn Bādīs manuscript, recipe 113 from the Paduan manuscript and chapter 13 (‘para fazeres nobre karmen’) of the *Livro de como se fazem as cores*. For all three recipes the lac is added to water and no alum is present. For the first, the recipe for red ruby of Ibn Bādīs, borax and sodium carbonate from ashes, are also added; for recipe 113 of the Paduan manuscript, tartar is added at the beginning; and for chapter 13 of the *Livro de como se fazem as cores*, the extraction is carried out with urine and quicklime and ashes are then added. For a detailed description please see Ref. [31] and Table S2, for the transcription of the recipes.

2.3. Equipment

2.3.1. Micro-sampling

Micro-sampling of the manuscripts was performed with a micro-chisel from Ted Pella microtools under a Leica KL 1500 LCD microscope, (7.1x to 115x objective) and a Leica Digilux digital camera, with external illumination via optical fibers. Micro-samples were taken under a microscope, typically of 20–50 μm in diameter and as such invisible to the naked eye; as we have not yet obtained their weight, even though micro-scales have been used, we can use its detection limit to conclude that they weigh less than 0.1 μg.

2.3.2. Energy dispersive X-ray fluorescence (microEDXRF)

MicroEDXRF results were obtained using an ArtTAX spectrometer of Intax GmbH, with a low-power molybdenum (Mo) X-Ray tube attaining a microspot with a spatial resolution of circa 70 μm, an X-flash detector refrigerated by the Peltier effect (Sidrift), sustained by a mobile arm (providing a major freedom in choosing the spot of analysis). The accuracy of the incident beam position on the sample is achieved through three beams crossing diodes controlled by an integrated CCD camera; the characteristic X-rays emitted by the sample (at 40°) are detected by a silicon drift electro-thermally cooled detector with a resolution of 160 eV at Mn-Kα. This apparatus allows for a simultaneous multi-element analysis in the element range from Mg (magnesium, atomic number 12) to U (uranium, atomic number 92). The experimental parameters used were: 40 kV of voltage, 300 μA of intensity, for 120s, under Helium gas flux. Si, Mn, Cu and Pb standards were used as calibration standards in the beginning and at the end of each day of data acquisition.

2.3.3. Fibre optic reflectance spectroscopy

The reflectance spectra were obtained with a reflectance spectrophotometer Ocean Optics, MAYA 2000 Pro, with single beam optical fibres, equipped with a linear silicon CCD detector Hamamatsu, with a spectral range of 200–1060 nm. The light source is a halogen lamp Ocean Optics HL-2000-HP, 20 W output, with a spectral range of 360–2400 nm. The analyses were obtained with 8 ms integration time, 15 scans, 8 box width, and acquired at 45°/45° (light source/acquisition), with a spatial resolution of 2 mm. To calibrate the equipment a white reference was used, Spectralon® standard. The spectra were acquired in reflectance mode and presented as apparent absorbance A’ = log10 (1/R).

2.3.4. Microspectrofluorimetry

Fluorescence excitation and emission spectra were recorded with a Jobin–Yvon/Horiba SPEX Fluorog 3–2.2 spectrofluorometer hyphenated to an Olympus BX51 M confocal microscope, with spatial resolution controlled by a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot, with 50 × objective. Beam-splitting is obtained with standard dichroic filters mounted at 45°; they are located in a two-place filter holder. For a dichroic filter of 525 nm, excitation may be carried out until about 510–515 nm and emission collected after about 530–535 nm (“excite bellow, collect above” principle). The optimization of the signal was performed daily for all pinhole apertures through mirror alignment, following the manufacturer's instructions, using a rhodamine standard (or other adequate reference). Standard dichroic filters of 525 and 600 nm were used where the emission spectra were acquired exciting at 515 nm and excitation spectra were recorded collecting the signal at 610 nm. This enables both the emission and excitation spectra to be collected with the same filter holder. A continuous 450 W xenon lamp is directed into a double-grating monochromator, and spectra are collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). The pinhole aperture that controls the area of analysis is selected based on the signal-to-noise ratio. Usually, for weak to medium emitters, it is set to 8 μm (pinhole 5); in this work, for very weak signals

30 µm spot was also used (pinhole 8) with the following slits set, emission slits = 3/3/3 mm and excitation slits = 5/3/0.8 mm. Emission and excitation spectra were acquired on the same spot whenever possible. For more information please see Refs. [10,11].

2.3.5. Fourier transform infrared microspectroscopy (microFTIR)

Infrared analyses were performed using a Nicolet Nexus spectrophotometer coupled to a Continuµm microscope (15x objective) with a MCT-A detector cooled by liquid nitrogen. The spectra were collected in transmission mode, in 50 µm areas, resolution setting 4 or 8 cm^{-1} and 128 scans, using a Thermo diamond anvil compression cell. For some infrared spectra the system was purged with nitrogen prior to the data acquisition; for all infrared spectra the CO_2 absorption at circa 2400-2300 cm^{-1} was removed from the acquired spectra (4000- 650 cm^{-1}). To improve result robustness, more than one spectrum was acquired from different sample spots.

2.4. Chemometric analysis based on excitation spectra

The data from the case studies was compared with databases for red lake pigments resourcing to a chemometric analysis method [4]. The first database is built up from historically accurate reproductions composed of brazilwood, cochineal, lac dye, and kermes. For the second approach, the database is built up of data from artworks from medieval manuscripts to textiles (11th – 15th c.) where lac dye, cochineal and brazilwood were identified. The chemometric analysis was based on Hierarchical Cluster Analysis (HCA) resourcing to the Ward's algorithm and the Mahalanobis distance. For the first database, the HCA method was fed with scores resulting from a principal component analysis of the dataset. For the second, the HCA algorithm was fed with spectral data (not requiring a previous PCA step). In both cases, excitation spectra were preprocessed by applying the 1st derivative (2nd order) followed by the Haar transform. Normalization by area (unit area) is also typically used for the analysis of fluorescence data and was also considered and applied subsequently to the first two methods.

3. Results and discussion

In a first observation, the manuscripts' support displayed a reduced pliability and low mechanical strength. No extensive paint loss was observed, except for the *Koran*, where we found only traces of the original colours, Fig. S1. In the red colours that will be discussed, EDXRF analysis detected only the chemical elements of the paper support, indicating the presence of organic chromophores. The organic red colorants in all 5 manuscripts studied are perceived as dark reds, Fig. 3. When used for writing and drawing, in each manuscript, no distinction was found.

3.1. Characterization of the main chromophores

3.1.1. Microspectrofluorimetry in the visible

The emission and excitation spectra for the dark red inks/paints are plotted in Fig. 4, together with the best matches we obtained with historical medieval paint reproductions. In all cases the best match was obtained with a lac dye based colour; for *Al-Sarishi poems*, *Prophet biography* and *Koran* the match with the database is very good, enabling a safe assignment of the presence of a lac dye red.

In the *Koran*, generally, we obtained spectra with very low intensity, and it is important to remember that in this document we do not find intact paints, but traces, as seen in Fig. S1. In all excitation spectra, the presence of the shellac resin was detected through erythrolaccin (max at 474 nm) [3], and a good match with Ibn Bādīs red was obtained [31,32,38], Fig. 4. For the emission spectra, we found both the spectral envelope depicted in Fig. 4 as well as another emission signature with maxima at 560 nm and a shoulder at 585 nm, Fig. S2. In both cases, the match of Ibn Bādīs red colorants' with the emission spectrum representative of the red chromophore present in the *Koran* is not satisfactory.

Characterized by a maximum at 592 nm and a shoulder at 607 nm, the emission spectrum of *Mss 19* compares well with what is described for the red colorants in the *Koran*, and therefore the closest match is again with the reproduction from the treatise of Ibn Bādīs.

The reds used in *Al-Sarishi poems* and *Prophet biography* display similar emission and excitation spectra and for that reason, in Fig. 4, we only represent the spectra for *Al-Sarishi poems* (emission maxima found at 582 and 604 nm; and excitation at 517 and 551 nm). The spectra obtained agree very well with the reconstruction described in Paduan manuscript as 113 [31–33], characterized by a L* = 53, a* = 24 and b* = 7; i.e., a red colour with a small component in the yellow. These are perceived almost as carmines and are lighter than the dark reds from the other manuscripts that will be next described, Fig. 3.

The colorant found in the *Theology treatise* is characterized by an emission and excitation spectra shifted to lower wavelengths (emission and excitation maxima at 583 nm and 532 nm, respectively) when compared to *Al-Sarishi poems*. In this case, it was not possible to find a reference that could match closely with both spectra; one of the best matches obtained was with the carmine colour produced following the recipe 113 from Paduan manuscript.

The low intensities obtained in the excitation spectra of the dark red colours indicate that these colours are not based in Al^{3+} complexes (when this is the case we observe a tenfold increase in the signal intensity), or, if present, they are not the major chromophore. Overall, the

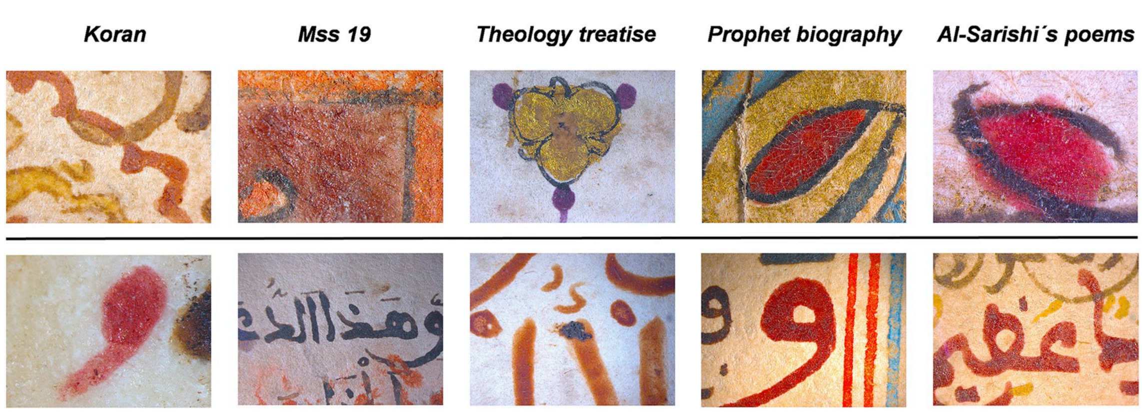

Fig. 3. Details of the red colorants used in the drawings (*top*) and writing inks (*bottom*), in the illuminated manuscripts studied. Usually based on lac dye, except for the following case studies; vermilion and an iron-gall based chromophore were detected in the writing ink used in *Mss 19* (2nd detail, bottom); in the *Theology treatise* an organic purple was used in the drawing ink (3rd detail, top). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

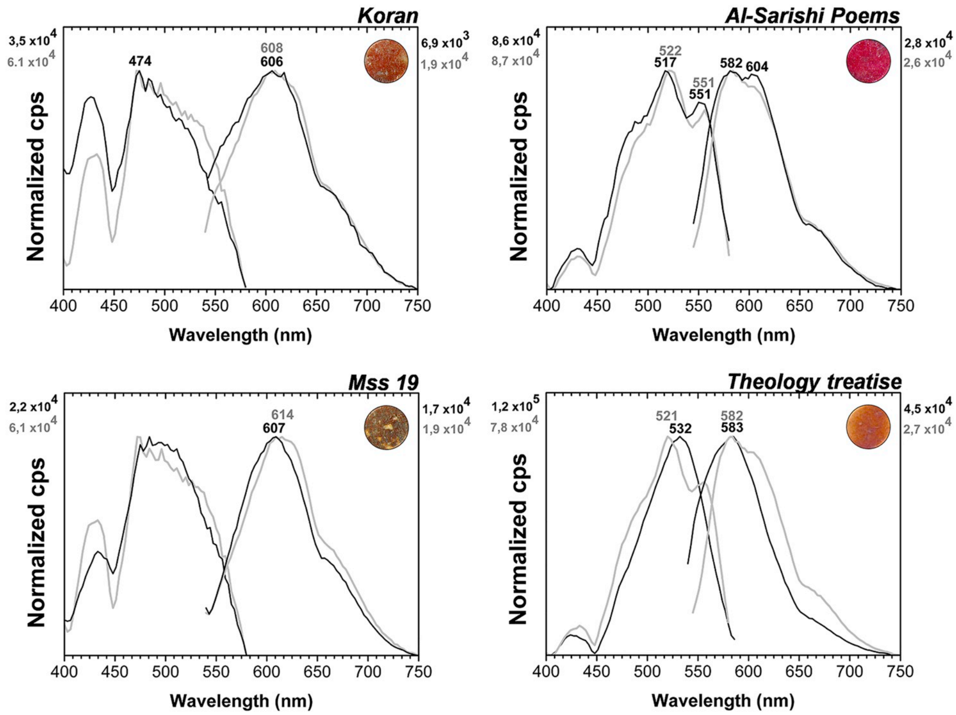

Fig. 4. Excitation and emission spectra for the organic red colorants in Islamic manuscripts (*black*), compared with historically accurate reconstructions of lac dye paints (*grey*). For the *Koran* and *Mss 19* with recipe for red ruby of Ibn Bādīs; for *Al-Sarishi poems* and the *Theology treatise* (as well as for *Prophet biography,* not shown) with recipe 113 of Paduan manuscript. For more details please see text. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

matches with Ibn Bādīs *red ruby* and Paduan 113 confirm these conclusions as these colours were prepared without the addition of alum.

3.1.2. FORS in the visible

Generally, the reflectance spectra acquired match the same paint reproductions as the fluorescence spectra, as follows: the spectrum of the red colorants of *Koran* and *Mss 19* display a satisfactory correlation with recipe for Ibn Bādīs' *red ruby; Al-Sarishi poems* and *Prophet Biography* with recipe 113 from the Paduan manuscript, Fig. 5. On the other hand, the *Theology treatise* displays a better match with chapter 13 of the 'book of all colour paints' and not with 113 from Paduan manuscript as was the case with emission and excitation spectra [31–34]. In these spectra, the first band is attributed to the reflectance from the support, at 400 nm for paper and 375 nm for parchment; possibly due to the lower thickness of the original colours when compared with our reproductions (reflectance spectra for parchment and

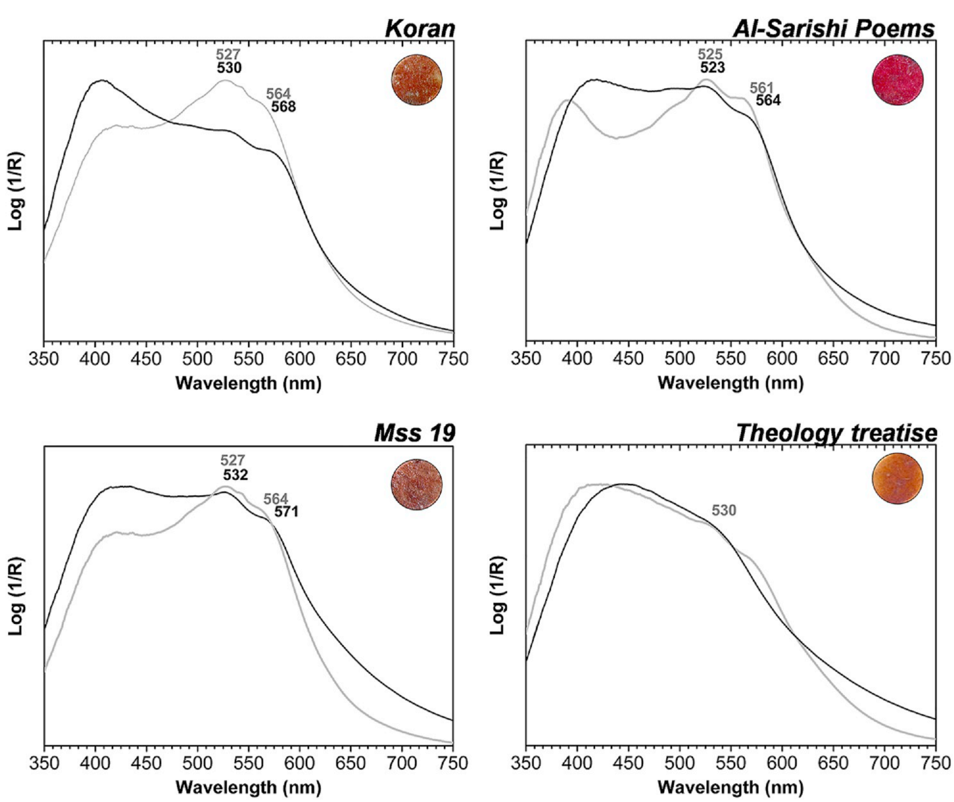

Fig. 5. Apparent absorption spectra for the organic red colorants found in Islamic manuscripts (*black*), compared with historically accurate reconstructions of lac dye (*grey*). For the *Koran* and *Mss* 19 with an Ibn Bādīs paint for *red ruby*; for *Al-Sarishi poems* with recipe 113 of the Paduan manuscript; and for the *Theology treatise* with chapter 13 of the 'book of all colour paints'. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

paper are found in Fig. S3). Also, the chromophore is not as well defined as in the reproductions, Fig. 5.

3.1.3. Chemometrics based on microspectrofluorimetry data

A chemometric model was applied to verify if additional information could be extracted from the fluorescence spectra. The analysed samples were tested against two databases of spectra from *(i)* historically accurate reconstructions of lac dye, brazilwood, cochineal and kermes and from *(ii)* historical samples dated from 11th-15th c [4]. An unsupervised modelling was chosen, hierarchical cluster analysis (HCA), Fig. S4. For the comparison with samples from the first database, the HCA method was fed with five principal components. These principal components were generated from a previous PCA model. In agreement with the microspectrofluorimetry analysis, generally, all the organic red colorants were clustered as lac dye, Fig. S4. A more detailed analysis shows that the *Prophet biography*, *Al-Sarishi poems* and *Mss 19* are also close to the kermes cluster. The *Theology treatise*, however, was more difficult to predict, and although it falls within the lac dye cluster, it alters the model greatly. These results show that for the *Theology treatise* the model could not add any relevant information.

Another approach was attempted, now comparing the testing samples against a database of historical samples, artworks from medieval manuscripts to textiles (11th – 15th c.) where lac dye, cochineal or brazilwood were present as the main chromophores. With this approach the results were substantially improved. With this database it was found that better results arose without the use of PCA, so the HCA algorithm was fed directly with pre-processed spectra. Considering the excitation spectra dataset alone, the HCA method revealed a successful clustering of the colorants, as seen in Fig. 6. The model considers the data from the *Theology treatise*, the *Al-Sarishi poems* and the *Prophet biography* within the lac dye cluster, similar to the lac dye reds used in the *Book of Birds* (1183–4) produced in the scriptoria of the monastery of Lorvão (Lorvão 5). The dark reds in this manuscript are characterized by an excitation maximum at 525 nm with shoulders at 473 nm and 553 nm, and emission maximum at 587 nm; which compares well with the excitation maximum at 517 nm with a shoulder at 551 nm, and emission maximum at 582 nm for the red colours in the *Al-Sarishi poems* and the *Prophet biography*, see Fig. 4. Possibly due to the presence of shellac resin found in the *Theology treatise*, the model predicts it similar to the *Book of Birds*.

The *Koran* colorants' are also found in the lac dye cluster, sharing similarities with the manuscript Lorvão 13 (13th c.), with excitation maxima at 472 nm and emission at 591 nm.

For the *Mss19*, however, it appears that the model locates this sample between the lac dye and the cochineal clusters, unable to place it with certainty within a colorant class.

As an overview, in Fig. 7, we represent the spectra for the organic red colorants found in Islamic manuscripts compared with real case studies of medieval Portuguese manuscripts. As expected by the fluorimetry discussion, where the low intensities obtained in the excitation spectra indicate that these colours do not present in Al^{3+} complexes as the main chromophores, also in this model all the samples were predicted within a cluster of lac dye reds in which Al^{3+} is absent or present in very low amounts.

At this point, these results reveal that other molecular fingerprint techniques, such as surface-enhanced Raman spectroscopy, should be used to confirm the identification of the red colorants present in the *Theology treatise*.

3.2. Characterization of the paints

3.2.1. Binding media characterization by infrared spectroscopy

The discussion of the binders used is more complex and will be carried out by comparison with original samples from Portuguese monastic collections (12th-13th c.), because we studied it in-depth and the number of spectra acquired makes it a robust dataset [4]. In the *scriptoria* of these three important Portuguese monasteries, a variety of lac dye red paints were prepared; e.g. fillers could be or not present (calcium carbonate or/and gypsum), different proportions of shellac resin and proteinaceous binder have been found [1,3,31].

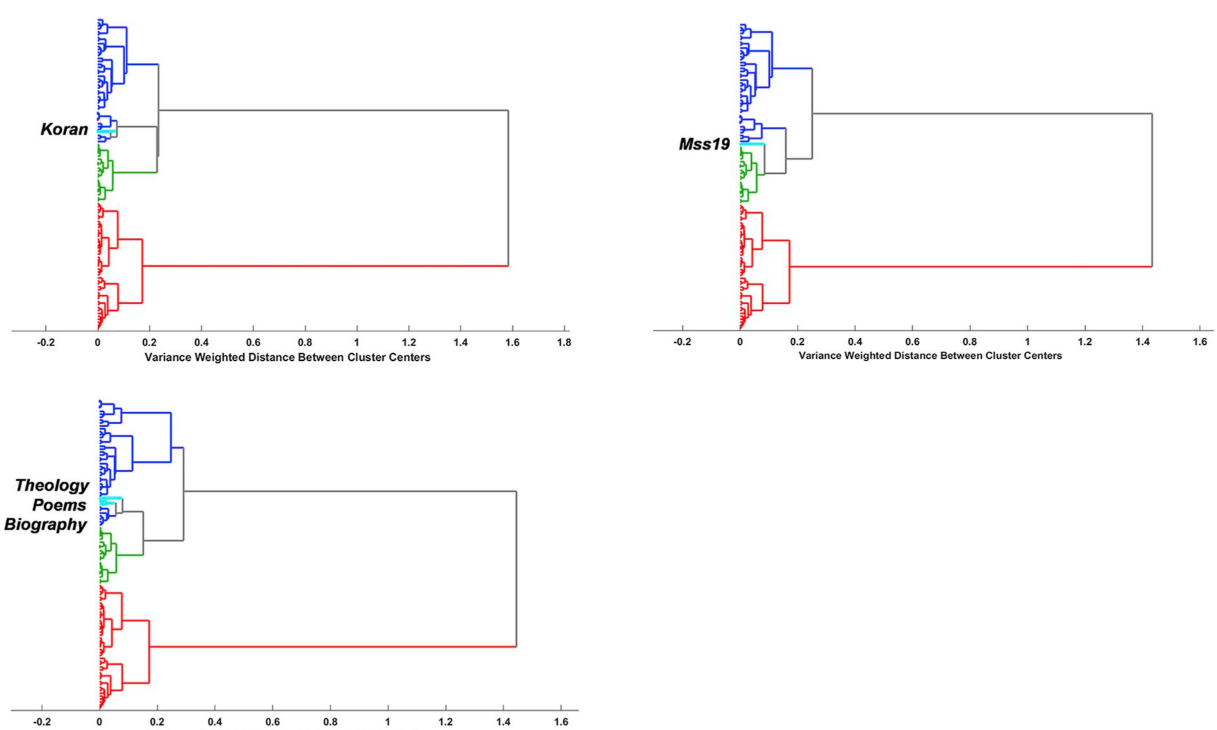

Fig. 6. Dendrogram generated by HCA applied to excitation spectra using a model composed of data acquired from artworks (lac dye (*blue*), cochineal (*green*) and brazilwood (*red*)). The data from the Islamic manuscripts was predicted within the model (*light blue*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

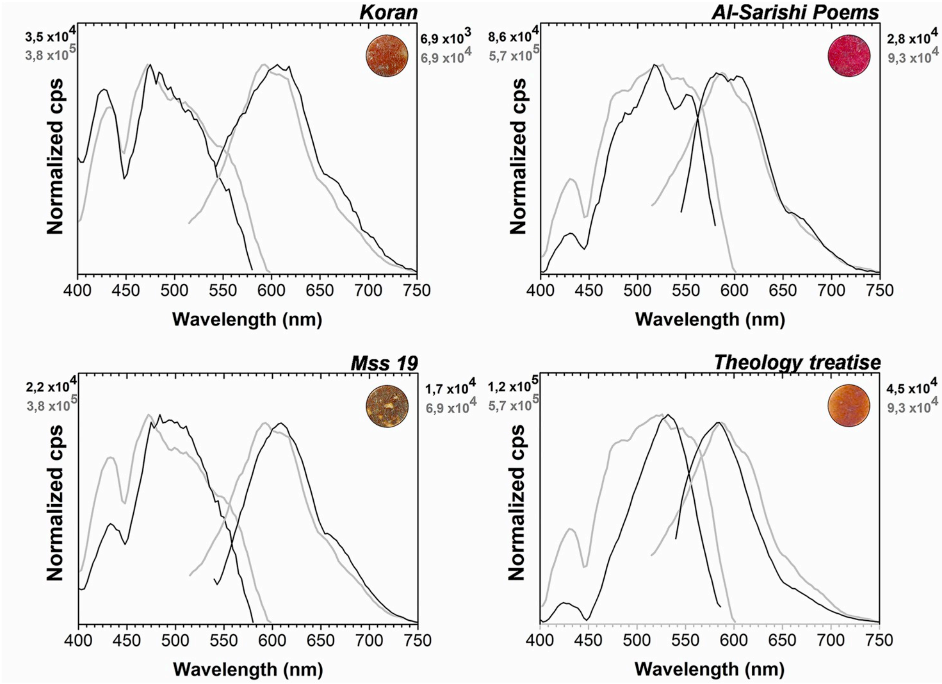

Fig. 7. Excitation and emission spectra for the organic red colorants found in Islamic manuscripts (*black*), compared with case studies of medieval Portuguese manuscripts, from monastery of Lorvão, (*grey*). For the *Koran* and *Mss 19* with the *Lectionarium Temporale*, Lorvão 13; for *Al-Sarishi poems* and the *Theology treatise* the comparison is made with the *Book of birds*, Lorvão 5. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

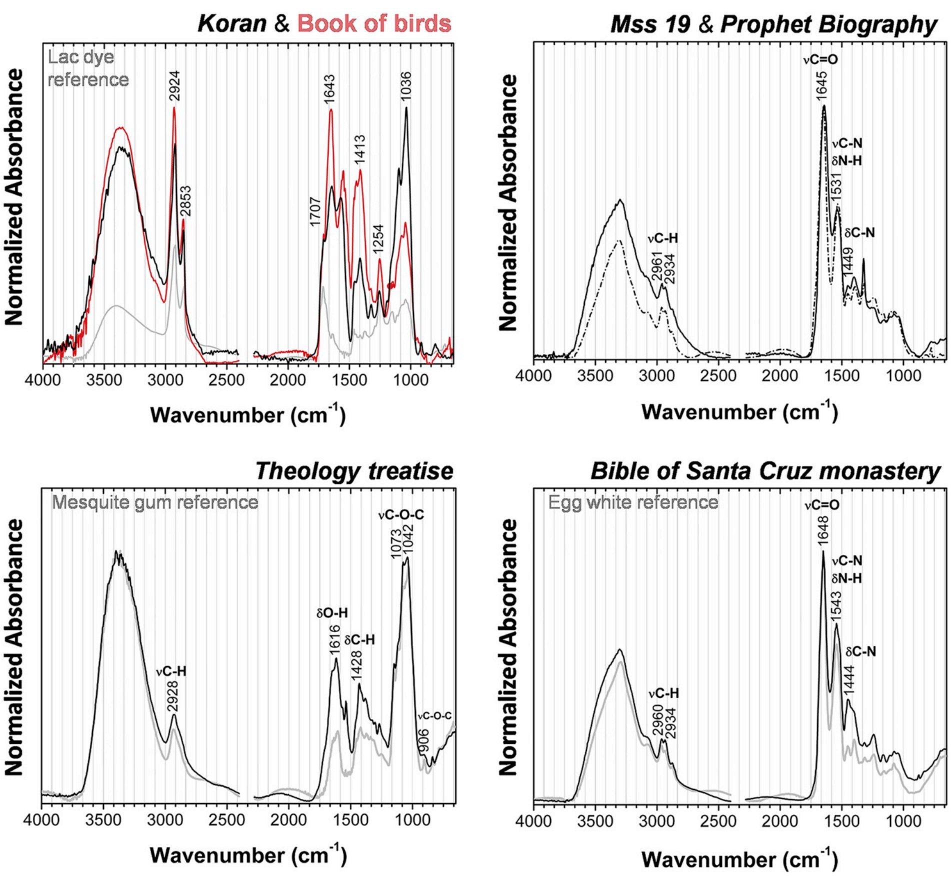

Fig. 8. Infrared spectra for the organic red colorants found in Islamic manuscripts: *from up to bottom, left to right,* Koran (*black*) with the *Book of birds* from the monastery of São Mamede of Lorvão (*red*) and lac dye reference (*grey*); comparison between the *Mss 19* (*black*) and the *Prophet Biography* (dashed); *Theology treatise* (*black*) with mesquite gum reference (*grey*); and Bible of the monastery of Santa Cruz of Coimbra (*black*) with egg white reference (*grey*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The infrared spectra of red colorants in *Koran*, *Theology treatise*, *Prophet biography* and *Mss 19* are represented in Fig. 8. The presence of the C-H stretching at 2924 and 2853 cm^{-1} is very clear in all *Koran* infrared spectra, confirming unequivocally the presence of shellac resin as anticipated by the fluorescence spectrum. A very good match was found with a lac dye red used to paint the *Dove diagram* in Lorvão *Book of birds*, Fig. 8. The shellac resin spectral envelop in the fingerprint region also compare well with shellac. Overall, the two medieval paint spectra match well, and the main differences observed are in the amount of proteinaceous binder, higher in the Portuguese colour.

A clear proteinaceous fingerprint is observed in the spectra from red paints in *Prophet biography* and *Mss 19*, but the presence of shellac resin could not be detected, possibly because if falls below the detection limits of this technique (< 10%). For these samples a very good match was found with the carmine colour used to paint the initial in fol. 1v of the monumental Bible of the Holly Cross monastery, Santa Cruz 1 (except for the presence of calcium carbonate, present in low amounts in these Portuguese paints, but not detected in the Islamic manuscripts), Fig. 8.

On the other hand, in the *Theology treatise*, a perfect match was found with a polysaccharide such as mesquite gum, Fig. 8.

3.2.2. Assessing degradation by infrared spectroscopy

Calcium oxalate was detected in most of the samples (through the sharp bands at 1625 and 1323 cm^{-1}). It was present in higher amounts in *Koran* and *Mss 19* dark reds. Nati Salvadó and co-workers, proposed that the presence of calcium oxalate, as weddellite (dihydrate form) and/or whewellite (monohydrate) in Cataluña medieval paints, may be ascribed to the binding media degradation [39]. Considering the small number of microsamples that we studied in this work, this cannot be used to conclude that in these manuscripts red colours display a higher level of degradation. However, we think it is possible to conclude that overall, they display more binder degradation than what was found in 12^{th}-13^{th} c. Portuguese carmine paints.

In f. 30r of the *Koran*, in the dark red colour, we detected for the first time the presence of a soap, which we assign to calcium palmitate by comparison with its C-H stretching at 2916 and 2850 cm^{-1} as well as carboxylate bands at 1574 and 1540 cm^{-1}, Fig. 9 [40].

4. Conclusions

For the first time the use of lac dye in Islamic manuscripts was unequivocally proved. For the red inks used in the *Koran*, *Al-Sarishi poems* and *Prophet biography* it was possible to further ascribe a specific recipe to produce the colorant and to disclose the paint formulation. In the *Koran,* lac dye cannot be present as a Al^{3+} complex, and in *Al-Sarishi* poems and *Prophet biography* our data indicate that possibly Al^{3+} complex cannot represent the main chromophore. Additives and binding medium further disclose the richness and specificities of these formulations: all lac dye based, but all different. Chemometric analysis confirmed these assignments and further matched the colourants with data from Portuguese manuscript illuminations, the *Book of Birds* and *Lectionarium Temporale* from the monastery of Lorvão, Lorvão 5 and Lorvão 13, respectively.

In previous studies, for organic red colorants only "cochineal" or "kermes" have been identified. Importantly, with one exception [19],

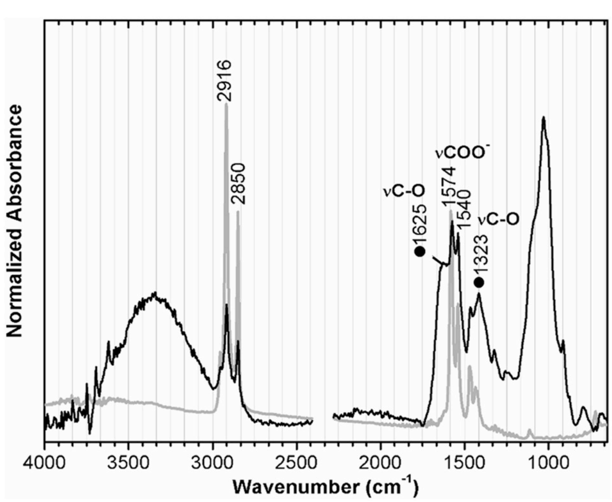

Fig. 9. Infrared spectra for the organic red colorant found in the Koran, f. 30r (*black*), with reference of calcium palmitate (*grey*) and fingerprint of calcium oxalate (●).

the identifications have been based on FORS-VIS data. For this reason, they must be considered with caution, as although this technique is able to distinguish between traditional anthraquinone dyes of animal or plant origin, Fig. 1, and to indicate the main chromophore present, it cannot assess its precise nature (kermesic, carminic or laccaic acids), in ancient manuscripts.

On the other hand, for the dark reds applied in the *Theology treatise*, *in situ* methods supported by a robust data base of medieval reconstructions could not provide an unequivocal molecular characterization of the main chromophore.

This study shows, for the first time, the importance of lac dye in al-Andalus manuscripts, highlighting the richness and diversity of the paint formulations used. The very good spectral matches with both fluorescence emission and excitation spectra as well as with infrared data enable us to conclude that the chromophore is well preserved, but the binding media show signs of severe degradation. The presence of lac dye in these manuscripts agrees with the historical sources and the use of lac dye in the Arabic world [30].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2019.03.061.

Conflicts of interest

The author declares that she has no competing interests.

Availability of data and materials

Most of the data on which the conclusions of the manuscript rely is published in this paper, and the full data is available for consultation on request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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