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Original article

Identification of red colorants in van Gogh paintings and ancient Andean textiles by microspectrofluorimetry

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Abstract

Red lake pigments and dyes used in works of art were characterized by microspectrofluorimetry, a new tool in the field of cultural heritage. Emission and excitation spectra were obtained with high spatial resolution (8–30 μm) in cross-sections from paintings by Vincent van Gogh and Lucien Pissarro and from millenary Andean textiles. The fluorophores were identified by comparing their spectra with those from historic reconstructions assembled in a database. In the paints, purpurin and eosin lakes were detected. In the Paracas and Nasca textiles, dated from 200 B.C. to A.D.1476, purpurin and pseudopurpurin were the red dyes used. Carminic acid was detected in textiles dated close to the Inca Empire, A.D. 1000–1476. The results obtained with this new technique were confirmed and are in agreement with those obtained with conventional methods, requiring microsampling, such as HPLC-DAD-MS and SEM-EDX.

Keywords: Microfluorescence spectroscopy; Red lakes; Andean textiles; Vincent van Gogh; Impressionism

1. Research aims

New non-invasive and sensitive analytical approaches are necessary for the study of organic dyes and lake pigments in the field of cultural heritage. The development of microspectrofluorimetry techniques proved to be useful for in situ characterization of chromophores in different materials, such as textiles and cross-sections.

2. Introduction

Organic dyes and their complexes have been used for textiles, manuscript illuminations, paintings and other historic works of art. The colorants reflect important artistic and historic values and may provide clues to the understanding of ancient cultures. Identification of these materials in art objects and characterization of their deterioration may be important to historians and conservators. However, the fugacity of these materials and the difficulty in obtaining samples for analysis may explain why they have received relatively little attention in the literature [1,2]. Anthraquinones and their hydroxy derivatives have been used as red dyes and pigment lakes[1] since prehistoric times [3,4], while eosin was synthesized in the 19th century and is a xanthen derivative (Fig. 1). The lake pigments based on these colorants were very popular among impressionist painters, including Vincent van Gogh. Eosin lakes are particularly unstable and fade on exposure to light sometimes within a few years [5]. The importance of impressionists and Van Gogh to the European culture is well known [6,7], and while pre-Columbian textiles are arguably less famous, they are unique as a cultural

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[1] A "lake" pigment is obtained by precipitating the chromophore in solution with metal salts, such as alum.

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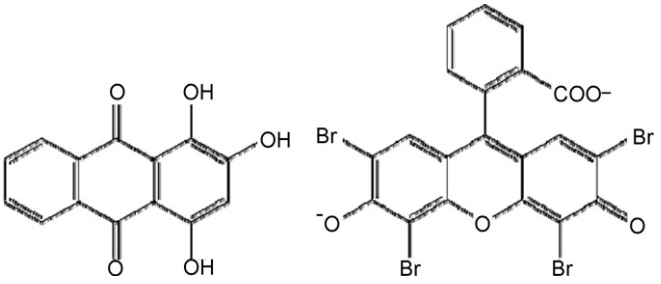

Fig. 1. Purpurin (1,2,4 trihydroxy anthraquinone) and eosin Y.

and historic record, representing the longest continuous textile record in world history [8,9]. Fortunately, in extremely arid archeological sites, the cultural heritage of different Andean cultures such as Paracas, Nasca, Chancay, Lambayeque, has been preserved. The reds used to dye at Peru before the Inca Empire (14–15th century), were based on purpurin chromophore (Fig. 1) obtained from *Relbunium sp.* [4,10,11]. The dye was bound to the textile fiber through a metal ion, known as mordant, such as Al^{3+}. These dated works of art, with difference in centuries and continent origin, have in common the presence of the red anthraquinone chromophores, expected to be found in the form of a coordination complex with a metal ion, i.e., a red lake.

Recently we have explored the potential of confocal microfluorescence spectroscopy for the non-invasive analysis of model paints of selected red lake pigments based on alizarin, purpurin and eosin (weak, medium and strong emitters) [12]. The information acquired was used to build up a consistent database. In the present study, the technique is applied to samples from works of art, ranging from textiles to cross-sections. Samples from paintings by Lucien Pissarro (Courtauld Institute of Art) and Vincent van Gogh (Van Gogh Museum), and pre-Columbian Andean textiles (Museum of Fine Arts, Boston) are examined. In all the samples, the red chromophores can be expected to occur in the form of a coordination complex with a metal ion. Selected samples were also analyzed by other techniques requiring microsampling such as HPLC-DAD and SEM/EDS, and the results compared. Another issue explored in this work is the possibility of acquiring information on the environment using the chromophore emission as a probe. The fact that the media can strongly influence the fluorescence emission is well known and this is the reason why fluorescent molecules are currently used as probes for the investigation of physicochemical, biochemical and biological systems [13–15].

3. Methodologies

3.1. Samples

3.1.1. Andean textiles

From the MFA-Boston collection 76 reds from Andean textiles, were sampled (yarns usually weighed 0.1–0.4 mg). These textiles, dated from 200 B.C. to A.D. 1476, were created by cultures of Paracas (Early Horizon Period to Early Intermediate Period), Nasca (Early Intermediate Period), Wari Huari (Middle Horizon), Chancay (Late Intermediate Period) and Lambayeque (Late Intermediate Period). All the samples studied in this work were camelid fibers. Longitudinal sections of fibers were examined under 200 × magnification using an Olympus polarizing light microscope. Visual identification was made using physical features of the camelid fibers, such as the uniform diameter, smooth scale profiles, and appearance of the medulla.

3.1.2. Paint cross-sections

Paint samples prepared as cross-sections embedded in polyester resin from oil paintings by the impressionist and post-impressionist painters, Lucien Pissarro (one sample) and Vincent van Gogh (cinq samples), were studied. From Lucien Pissarro, Old mark's field 1932 (ref 249); from Vincent van Gogh: *Head of an old woman* 1885 (ref. F174), *Montmartre: Quarry, the Mills* 1886 (ref. F229), *Allotments on Montmartre* 1887 (ref. F316), *Two white butterflies* 1889 (ref. F402) and *Wheat Field Under Clouded Sky* 1890 (ref. F778).

3.2. Fluorescence emission measurements

3.2.1. Apparatus

Fluorescence spectra were acquired by a Jovin-Yvon Spex Fluorog 3-2.2 spectrofluorometer. The measurements were obtained with the microSPEX (Spex® FluoroMap with Manual Microscope Stage) using a set-up described elsewhere [16], where the Spex Fluorog 3-2.2 is connected to a Olympus BX51 M confocal microscope, with spatial resolution controlled with a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot with a 50 × objective. For steady-state fluorescence spectra, a continuous 450 W Xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator. The incident excitation beam is directed onto the sample and its fluorescence is directed back up into the microscope. To view the sample's fluorescence directly, a binocular eyepiece and a digital camera are available. Beam-splitting is obtained with standard dichroic filters of 500 nm (Glen Spectra) and 570 nm, 25 mm diameter, used at 45°. Daily, was carried out the optimization of the signal, through mirror alignment in the optic pathway of the microscope, following the manufacturer instructions.

3.2.2. Spectra acquisition

In model and historical reconstructions, samples spectra were acquired after focusing for the maximum signal. The cross section samples have to be analyzed using a black cover avoiding reflection from the resin. Andean textile fibers were put in concavity glass slides. Fluorescence spectra were not corrected for the wavelength response of the system. If not otherwise stated, all spectra were acquired in a 30 μm spot, using pinhole 8. Emission spectra were aquired with 2 nm spectral resolution and excitation spectra with 1 nm. Following the manufacture recommendations, for pinhole 8, the entrance of the emission slits was made equal to 2 mm and the exit of the excitation slits was equal to 0,7 mm. For pinhole 5, the entrance of the emission slits was made equal to 2 mm and the exit of the excitation slits was equal to 0,7 mm. As we are operating with double monochromators the complete slits set was the following:

- pinhole 8 (30 μm), emission slits 2/3/3 mm; excitation slits 5/3/0.7 mm;
- pinhole 5 (8 μm): emission slits 0.8/3/3 mm; excitation slits 5/3/0.2 mm.

3.2.2.1. Andean fibers. Emission spectra were acquired using a 500 nm dichroic filter, exciting at 490 nm. Excitation spectra were performed with a 570 nm dichroic filter, collecting the signal at 590 nm [12]. For all the samples, a minimum of three emission spectra was acquired together with the excitation spectra, which closely matches the dye absorption.

3.2.2.2. Cross sections. As for the Andean textiles, emission spectra were acquired with a 500 nm dichroic filter and excited at 490 nm. Excitation spectra were collected at 590 nm using the 570 nm dichroic filter. A minimum of seven emission spectra, together with excitation spectra, was acquired for each sample.

3.2.2.3. Model reconstructions. For each painted eosin lake, the emission spectra were acquired in seven different points, with very good reproducibility. The emission and excitation spectra for the eosin lakes were obtained using 500 nm dichroic filter, exciting at 490 nm and 570 nm dichroic filter, collected at 590 nm, respectively.

3.3. Scanning electron microscopy

Textile fibers were mounted on double-sided carbon tape attached to a graphite disc. Analyses were carried out in a JEOL JSM-6460LV scanning electron microscope with an Oxford Instruments "INCAx-sight" energy-dispersive X-spectrometer (SEM/EDS). SEM was operated in low-vacuum mode with a chamber pressure of 35 Pa.

3.4. Extraction of dyed textiles

Extractions were only performed in some of the studied samples using the oxalic acid method developed by Claude Andary and Pauline Guinot [17], but without acetone in the mixture. Threads with 0.3 mg of weight were placed in a vial with 400 μl of methanol/water/oxalic acid (80:20:1, v/v) and extracted in a water bath for 30 minutes at 60 °C. Once the dyes were extracted, the fibers were removed after centrifugation; then, the extracts were dried under vacuum over NaOH pellets. The upper 30 μl of solution was removed with a pipette for HPLC-DAD analysis, in which 25 μl was injected.

3.5. Analysis of extracts

Samples were analyzed by high performance liquid chromatography with diode array detector (HPLC-DAD) with a ThermoFinnigan, Surveyor PDA 5, a RP-18 column (Nucleosil 250 × 4.6 mm, 30 nm–5 μm) and a pre-column, with a water (pH 1.5)/methanol gradient [18].

Dye analyses was also performed on a LC-MS instrument consisted of a ProStar 410 autosampler, two 212-LC chromatography pumps, a ProStar 335 diode array detector and a 500-MS ion trap mass spectrometer equipped with an ES ion source (Varian, Inc., Palo Alto, CA, USA). In this case the HPLC separations were carried out using a Polaris C18-A, with 5 μm of particle size (150 × 2 mm). The isocratic mobile phase consisted of a mixture of acetonitrile (75%) with 0.08% (v/v) formic acid (aq.) (25%). The operating parameters were optimized for the standard purpurin and pseudopurpurin and were as follows: the spray needle voltage was set at –5.1 kV (negative mode); nitrogen was used both as nebulising and drying gas (35 and 15 psi, respectively), drying gas temperature 350 °C, capillary voltage 55 V for negative ions, and RF loading tuned for 70 V.

3.6. Preparation of the model samples

For the preparation of the model samples distilled water, reagent grade chemicals and eosin Y (Sigma-Aldrich) were used.

Samples with eosin Y and different coordinating metal ions were prepared with aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), alum ($KAl(SO_4)_2 \cdot 12H_2O$) and lead(II) acetate trihydrate ($Pb(CH_3CO_2)_2 \cdot 3H_2O$). In 50 ml of NaOH solution (pH = 12), 0,1 g eosin Y was dissolved and then the different complexing metal agents were slowly added till a bright pink precipitate has formed (pH ≈ 6). Precipitates were left to settle, filtered, washed and dried at room temperature. The lakes were applied as paint layers with the aid of a brush, after thorough grinding in a mortar, first only with the powder, and then with the binding media: linseed oil (Talens®), similar to paints that were found in paintings by van Gogh [5].

4. Results and discussion

4.1. Andean textiles

Microspectrofluorimetry was used to analyze the 76 microsamples taken from different Andean textiles dated from 200 B.C.–A.D. 1476. The majority of the samples present a red colour, but fibers with pink and purple colour were also analyzed.

The SEM-EDX screening enabled to confirm the use of aluminum ion as a mordant, i.e., the metal ion that was used to bind the dye to the fiber; and also, to conclude that all the red samples studied were made of camelid fibers. This was also confirmed by optical microscopy.

The spectra obtained, in a 30 μm spot, can be classified in two groups, a major one in which good excitation and emission spectra were obtained, displaying good spectral resolution, and a second group in which only weak excitation spectra could be collected. In Fig. 2 are shown some representative spectra for the first group, three of the red samples and a purplish one. Both shape and maxima (emission and excitation) closely match what would be expected for a purpurin lake (Table 1), being very similar to those obtained for an aluminum lake in solution [12]. These spectra (Fig. 2) show a good mirror-image relationship between the excitation and emission spectra for the purpurin complex. The small Stokes' shift indicates a similar geometry for the complex in the ground and first excited state. It is also worth noting that the excitation spectrum is similar to the

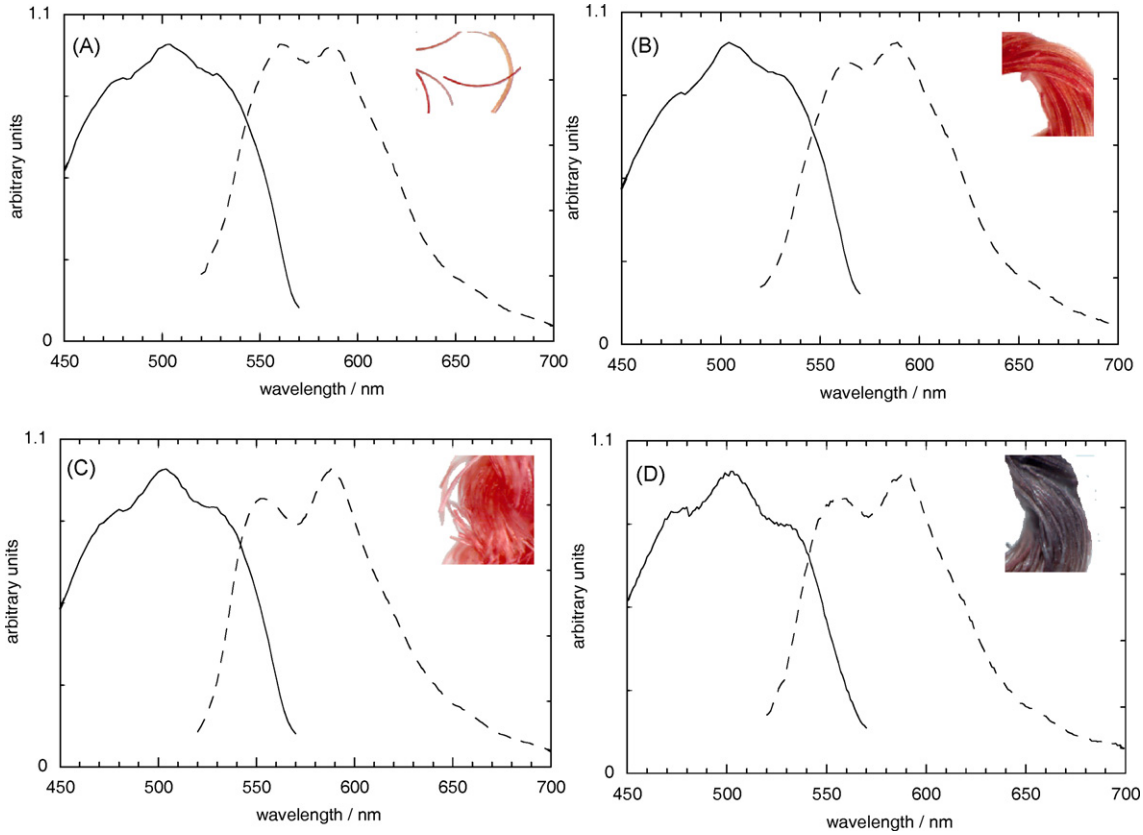

Fig. 2. Emission and excitation spectra, acquired in a 30 μm spot, for: A: Andean Paracas man's poncho (mfa31.496), 100 B.C.–0; B: ornamental braid (mfa21.2557), 0-A.D. 200; C, D: Nasca fragment of a tabled fringed border (mfa31.500), A.D. 300. λ_{exc} = 490 nm, 500 nm and λ_{em} = 590 nm.

Table 1
Emission and excitation maxima of the analyzed samples and the purpurin and pseudopurpurin aluminum lakes used as references[a].

	31.496	21.2557	31.500 red	31.500 purple	Purpurin-Al^{3+}[a]	Pseudo-purp: Al^{3+}[a]
Excitation λ_{max}/nm	480, 504, 532	482, 504, 534	484,504, 534	480,502, 536	502, 531	493, 520
Emission λ_{max}/nm	560, 587	564, 591	552, 587	556, 589	551, 595	542, 583

[a] Anthraquinone: Al^{3+} 1:1000, pH = 3.7 in H_2O:MeOH (25%:75%, v/v).

absorption spectrum obtained on full chelation with aluminum in solution[2], which exhibits vibronic maxima about 480, 504 and 534 nm, attesting the validity of the method to fingerprint the presence of red lakes. The emission spectra contain specific fluorescence bands whose maxima emission wavelengths are at circa 555 nm and 588 nm. It should be stressed that, in the samples studied, the relative intensity of these two bands varies, and the relative intensities range from 0.8 to 1.16; also, the second maximum is usually maintained but the first one can shift from 550 to 560 nm. The above-mentioned variations in the emission spectra of the Andean fibers could be explained by a different chromophore environment or to the presence of other emitters. We had already observed, for the emission of the purpurin aluminum complex in solution, that, depending on the pH, these bands could display a small shift. On the other hand, the presence of a second fluorophore was indeed confirmed by HPLC-DAD-MS as being pseudopurpurin; in all the samples analyzed by HPLC-DAD the major chromophore was purpurin, but pseudopurpurin in a proportion ranging from 30 to 45% was always detected. The emission of the pseudopurpurin fluorophore present in the Andean textiles was obtained after extraction followed by HPLC separation and finally complexation with Al^{3+} in MeOH:H_2O (75%:25%, v/v) solution (Fig. 3). Two bands, whose maxima excitation wavelengths are found at 493 and 520 nm, characterize its excitation spectrum; the emission spectrum has also two maxima emission wavelengths at 542 and 583 nm (Fig. 3). When compared to the purpurin aluminum lake emission, the values for pseudopurpurin emission are shifted to lower wavelengths, both in the emission and excitation spectra; also, in the emission spectra, the relative intensities of the two vibronic peaks are inverted. Consequently, the shifts and shapes in the spectra of Andean reds could be explained by the presence of pseudopurpurin and purpurin in variable amounts as follows: in the excitation spectra, the vibronic observed at 480 nm reflects the presence of pseudopurpurin; in the emission spectra, the higher the relative concentration of pseudopurpurin the higher

[2] At pH = 3.4 to 4; pH was not increased above 4.5, to prevent $Al(OH)_3$ precipitation.

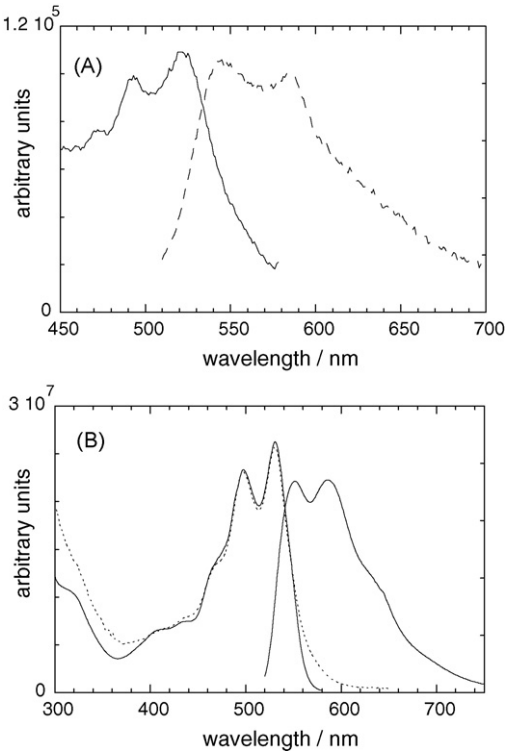

Fig. 3. Emission (λ_{ex} = 490 nm) and excitation (λ_{em} = 590 nm) spectra, in homogeneous media, for pseudopurpurin lake from an Andean textile (A) and purpurin lake in MeOH:H_2O (75%:25% v/v) (B).

will be the ratio between the two emission bands, at circa 555 and 588 nm. It was possible to confirm that the highest ratios were observed in samples with the highest relative concentration of pseudopurpurin (determined by HPLC-DAD). The relative amount of purpurin and pseudopurpurin could provide further clues for madder's source and methods used to dye. But, observing Fig. 2 and Table 1, a direct connection between the places of origin, such as Paracas or Nasca, and the relative amount of each chromophore, could not be made.

In the second group, with samples from Chancay and Lambayeque cultures dated from AD 1000–1476, carminic acid was found to be present. In this case, the quality of the fluorescence signals obtained did not enable a conclusive characterization, which was carried out by HPLC-DAD. The emission spectra are of very low intensity and it was not possible to obtain well-resolved excitation spectra. Nevertheless, with these fibers we obtained far better signals than with model painted samples of carminic acid.

In this study, emission and excitation spectra were obtained directly from fiber (microsamples) set in the microscope stage, but analysis could have been performed on the entire textiles, as no preparation for samples is required.

4.2. Paintings

Microsamples from paintings by Vincent van Gogh (five) and Lucien Pissarro (one) (Fig. 4) were analyzed in cross-sections.

4.2.1. Allotments on Montmartre (F316), Montmartre: Quarry, the Mills (F229) and Head of an old woman (F174) by Vincent van Gogh

The red glaze layer of the F316 cross-section has been previously characterized by HPLC-DAD [19] and found to contain purpurin and two other red dyes. Moreover, aluminum was detected by SEM-EDX, indicating the presence of a purpurin-aluminum complex on an alumina substrate. Representative excitation and emission spectra are depicted in Fig. 5, and both match the spectra obtained for Kopp's purpurin in oil binding media [12], prepared as an historic reconstruction [19]. The two vibronic bands present in the excitation spectra, that reflects the absorption spectra of the chromophore, are the

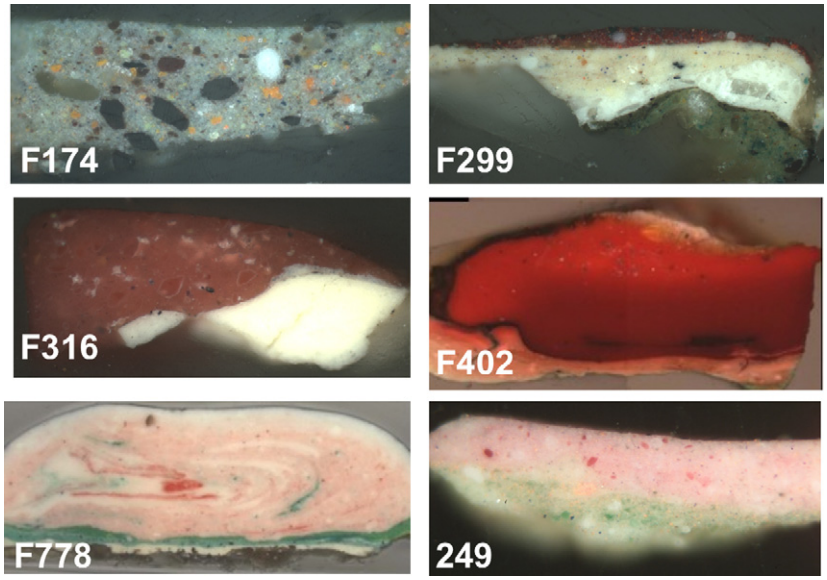

Fig. 4. Cross sections of five different paintings by Vincent van Gogh: *Head of an old woman* (F174); *Montmartre: Quarry, the Mills* (F229); *Allotments on Montmartre* (F316); *Two white butterflies* (F402); *Wheat Field Under Clouded Sky* (F778). and *Old Mark's field* by Lucien Pissarro (249).

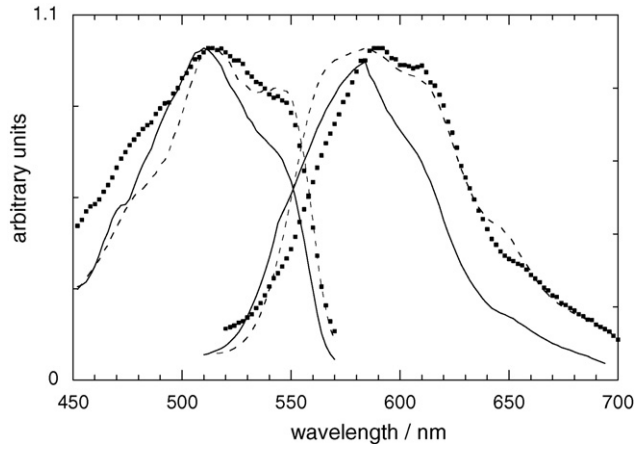

Fig. 5. Emission and excitation spectra, acquired in a 30 μm spot, for the following cross sections: Vincent van Gogh F316 (—), Lucien Pissarro (■) and Kopps purpurin (—),(λ_{exc} = 490 nm, and λ_{em} = 590 nm).

same as those obtained with Kopp's purpurin (513 and 546 nm), even if the second one (546 nm) appears broader. The emission wavelength maxima (λ_{max} = 580 nm) in the historical reconstruction is slightly different to that found with Kopp's purpurin, λ_{max} = 586 nm. However this difference is not significant and in practical terms it falls within the same range of the Kopp's purpurin or other purpurin lake prepared in the laboratory. These differences could be due to different ligands, different complex geometry or other environmental factors. These in turn, can be derived from ageing or to a different manufacturing process of making the purpurin lakes.

Although, in both samples, F174 and F229, purpurin was detected by HPLC-fluorescence [20] through microspectrofluorimetry, the results were not completely conclusive, as the emission spectra were similiar to the purpurin lake but the excitation spectra could not be obtained.

4.2.2. Old Mark's field by Lucien Pissarro

The organic red colour (Fig. 4) was present in the uppermost layer that contained large red particles in a pink matrix. The emission and excitation spectra obtained in this cross-section indicate the presence of a purpurin complex as the spectra envelop reproduces the purpurin lakes' spectra. Moreover the excitation spectrum makes a almost perfect match with the absorption spectra of the purpurin lake samples (Fig. 5). The similarity between the Lucien Pissarro's and Kopp's purpurin emission and excitation spectra is reflected in the two identical vibronic bands in the excitation maxima (λ_{max} = 513 and 546 nm), clearly reproducing the chromophore's absorption spectra. With regard to the emission spectra, the two vibronic maxima, λ_{max} = 590 and 610 nm, are in very good agreement with those found for Kopp's purpurin, λ_{max} = 586 and 610 nm.

4.2.3. Two white butterflies (F402) and Wheat Field Under Clouded Sky (F778) by Vincent van Gogh

The analyses performed with HPLC-DAD and Fluorescence [20] of the *Two white butterflies* (F402) and *Wheat Field Under Clouded Sky* (F778) revealed that both cross sections are composed by a red layer of eosin lake. The substrate present contains lead in F402 and aluminum in F778, as detected by SEM-EDX [20]. This last sample also contains iodine-substituted lakes related to eosin, possibly erythrosine, and red lead [20]. On the *Two white butterflies*, it was detected the presence of only two other components related to eosin, and aluminum silicates [20].

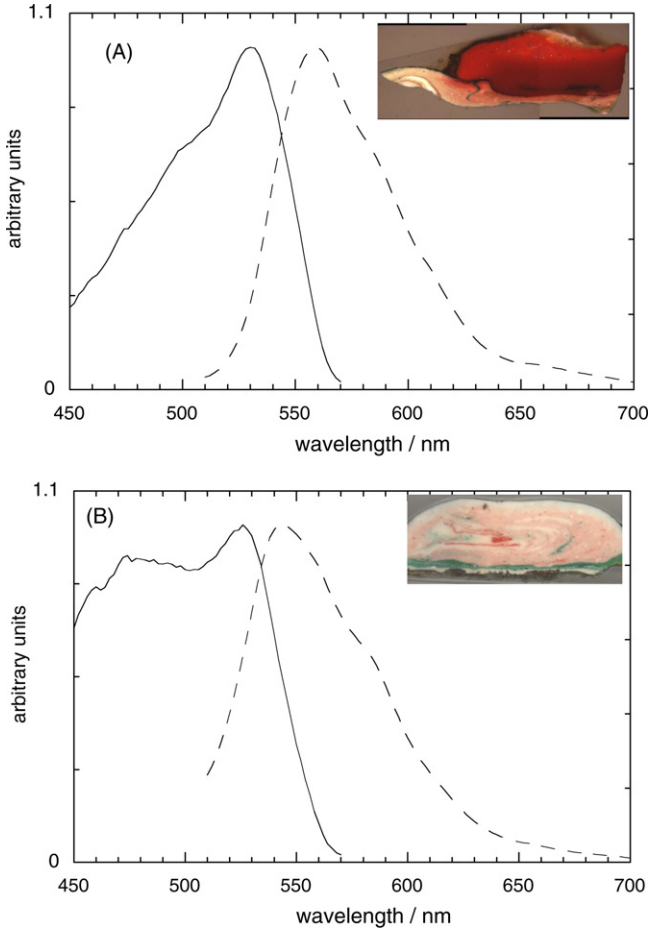

Fig. 6. Emission and excitation spectra, acquired in a 30 μm spot, for the cross sections F402 (A) and F778 (B). λ_{em} = 490 nm and λ_{em} = 590 nm.

The emission and excitation spectra of these two red layers on samples taken from paintings by van Gogh are represented in Fig. 6. Similar emission and excitation maxima were obtained for both samples, revealing the emission of an eosin lake (Table 2). The excitation spectra of the F778 is not so well resolved as it was expected because there is an interference near to the maximum excitation that could be related to the heterogeneity of the layer, eosin lake is mixed with erythrosine, green and blue pigments.

Evaluating these results with those obtained with the created eosin lake model samples (see next section) it is clear that the F778 has a behavior similar to the eosin lake prepared with aluminum chloride hexahydrate, corroborating the previous results obtained with invasive analytical techniques (SEM-EDX). The emission and excitation wavelength maxima obtained in the F402 sample were similar to the values obtained when the eosin lake was prepared with alum. Even if these wavelength maxima

Table 2
Emission and excitation maxima of cross-sections (F402 and F778) and painted model samples of eosin lake.

Sample	Excitation spectra λ_{max}/nm	Emission spectra λ_{max}/nm
F402	530	558
F778	526	542
Eosin lake prepared with $AlCl_3$ $6H_2O$	524	544
Eosin lake prepared with $KAl(SO_4)_2$ $12H_2O$	528	558
Eosin lake prepared with $Pb(CH_3CO_2)_2$ $3H_2O$	528	546

are similar to the ones of alum eosin lake, the intensity of these two spectra is not very high, as what expected for that lake – in fact the intensity is comparable to the Pb eosin based lake –, promoting a quenching of the fluorescence, thus inhibiting the intensity of the emission and excitation spectra. This likely results from a more efficient spin-orbit coupling induced by the heavy atom effect of the lead atom(s) promoting a quenching of the fluorescence emission. In fact, the classical spin-orbital operator for a single electron in a central potential field is given by $H_{SO} = \kappa\zeta\ (\vec{L}.\vec{S})$, where ζ is a term that depends on the field of the nucleous, κ is a constant which depends on the molecule and L and S are respectively the electron orbital and spin angular-momentum operators [21]. In the case of the lead atom the value of ζ is 7294 cm^{-1} versus 28 cm^{-1} for carbon [21], clearly showing that the spin-orbit coupling is more effective when the electron is close to atoms of high atomic number.

4.2.4. Probing the environment

4.2.4.1. Eosin lake pigments. It was observed that depending on the complexing agent the intensity of the spectrum increases, being the lower with $Pb(CH_3CO_2)_2{\cdot}3H_2O$, going to $KAl(SO_4)_2{\cdot}12H_2O$ and the higher with $AlCl_3{\cdot}6H_2O$ (Table 2); in all the samples the metal to ligand ratio as well as the sample thickness were maintained constant. In which concerns the spectra shape, even if the maxima excitation wavelength are similar $\lambda_{max} = 528$ nm and $\lambda_{max} = 524$ nm, the emission maxima displays different values being, those of the eosin lakes prepared with $AlCl_3{\cdot}6H_2O$ and $Pb(CH_3CO_2)_2{\cdot}3H_2O$, the lowest values, $\lambda_{max} = 544$ nm and $\lambda_{max} = 546$ nm respectively, and the one prepared with alum the higher ($\lambda_{max} = 558$ nm). The differences found for the aluminum eosin lakes could be explained by a different lake structure induced by the nature of the counter-ions. For alizarin aluminum complexes for example, it has been described that depending on the nature of counter-ions the final lake structure could be different, even if the ligand to metal ratio was preserved [22]. Soubaryol et al. showed that, if Na^+ and Ca^{2+} were present, a closed structure is obtained, on the other hand for K^+ and Ba^{2+} an open one was the final result [22]. By itself, a different structure with different ligand geometry can induce profound differences in the chromophores fluorescence emission; also, a more closed structure could possibly protect the lake from quenching by other ions present in the paint.

These results reveal that the chromophore environment, depending on the complexing agent, can originate small but relevant differences that allow the interpretation of the real samples with a non-invasive analytical tool.

5. Conclusions

Fluorescence spectra obtained from the analysis of paint cross-sections from post-impressionist paintings and ancient textiles demonstrates the potential of this analytical tool to characterize red purpurin and eosin based colours in works of art. The possibility of in situ analysis without sampling or alternatively on paint cross-sections offers a new spatially resolved analytical technique for red dyestuffs. The spatial resolution achieved of 30 μm, is appropriate for the analysis of individual pigments or aggregates in a paint film, enabling selective excitation of these compounds. This study has shown that dyes can be characterized in different environments in samples from paintings and ancient textiles. All the results obtained were in agreement with what known by other currently used techniques, such as HPLC-DAD-MS (dye characterization) and SEM-EDX (metal ions).

The acquisition of the spectra is rapid. Besides the full emission spectra, it was possible to acquire well-resolved excitation spectra, which is a great advantage when studying complex aged samples, where different kinds of dyes' mixtures or species can be present. The homogeneity of model samples enables an analyzing area of 8 μm (pinhole 5) but in real samples, in the presence of additives and ageing, in order to have a good signal-to-noise ratio the 30 μm (pinhole 8) resulted to be a good compromise; this spatial resolution still allows to selectively excite pigment aggregates in a paint film. In the future, combined with other analytical techniques such as HPLC-DAD or HPLC-DAD-MS that enable an unequivocal assignment of the dye present, it will be possible to gather accurate data on the environment of the chromophore and its degradation state. In the case of the red and pink colours investigated, resulting from metal complexes of organic fluorophores, an accurate characterization of the environment will enable to obtain data concerning the metal ion involved, the ligands present in the complex and the media surrounding the dye. Work is currently in progress to maximize the information present in fluorescence emission and excitation spectra acquired. The possibility of obtaining simultaneously the chromophores lifetime will be also explored [23].

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