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LEDμSF: a new tool for the study of UV fl uorescence in cultural heritage materials

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Introduction

Photoluminescence is a phenomenon of the emission of photons by an atom or a molecule in an electronic state excited by the absorption of photons. Fluorescence emission is one of the radiative de-excitation processes of photoluminescence, with lifetimes of 10^{-10} - 10^{-7} s [1]. Fluorescence, known since the XVI e century, is theorized by Stockes in the mid-nineteenth e century [2].

In the field of the examination of cultural objects, methods which rely on the emission of fl uorescence were developed after the invention of Wood's lamp (1913), in particular for the examination of the surface of old paintings: observation under UV lighting can, for example, highlight any repainting or recent restorations. Based on this fl uorescence emission from certain materials, spectro fl uorimetry is a very sensitive analytical technique used mainly for the study of organic compounds. From the end of the 1970s, the work of René de La Rie [3] made it possible to establish the fl uorescence properties of certain pigments, binders and varnishes.

This chapter aims to recall the principles of spectro fl uorimetry before developing the technical characteristics of new portable instruments. The data processing and interpretation methods are explained and illustrated by the presentation of spectra of pure fresh and degraded products (pigments, dyes,

binders and supports). The di culty of studying mixtures of compounds and degraded products by spectro fl uorimetry is presented on the basis of examples.

1 Principle of spectro fl uorimetry

1.1 Reminder of physical principles

We call *fl uorimetry* and *spectro fl uorimetry* analytical techniques which consist in analyzing the phenomenon of fl uorescence of molecules or atoms. Fluorescence is a phenomenon of emission of electromagnetic radiation (or photons) by a molecule (or an atom) following the excitation of the latter (or of the latter) by absorption of electromagnetic radiation of higher energy. In the case of molecular fluorescence, in order to excite the molecules, monochromatic ultraviolet or visible radiation is used. This is referred to as molecular fluorescence or UV fluorescence. Any molecule capable of absorbing in the UV-visible is called a chromophore. Groups of atoms capable of re-emitting absorbed energy in the form of electromagnetic radiation are called fluorophores. There are two distinct phenomena of light emission (photoluminescence) for molecules excited by absorption of photons: phosphorescence and fl uorescence. The di ff erence between the two consists of di ff erences in electronic transitions, and results in a slower phenomenon in the case of phosphorescence (Figure 1) [2].

The excitation of molecules occurs by transition of electrons constituting the bond between two atoms of a molecule. The energy transported by UV-visible photons allows these electrons to pass from a fundamental level, of low energy, to an excited level, of higher energy. The energy of the molecules also increases, we say that they are excited. This phenomenon is called *absorption.* Secondly, the molecule loses its excess energy. This loss of energy can be accompanied by the emission of photons, we then speak of *radiative transitions,* and the emitted photons are at the origin of the phenomenon of fl uorescence [2].

1.2 "Classic" laboratory spectro fl uorimeter

There are two general types of instruments: filter fl uorimeters and lattice monochromator spectro fl uorimeters. In both cases, the exciting light passes through the filter or the monochromator, then through the sample which can absorb some of the radiation, inducing fluorescence of some molecules in the sample. Some of the fluorescent light is then focused on a filter (or monochromator), which is sometimes placed at an angle of 90 ° to the excitation light which is often the case for solid samples. The light emitted by the sample is then picked up by a detector.

Various light sources can be used as excitation sources: lasers (Laser Induced Fluorimetry or LiF), photodiodes and lamps such as xenon arcs and mercury vapor lamps.

In the laboratory, “classic” spectro fl uorimeters are imposing systems, made up of a xenon lamp and a double monochromator going from UV to IR. As an example, the SPEX Fluorolog spectrofluorimeter, model 212 (Horiba

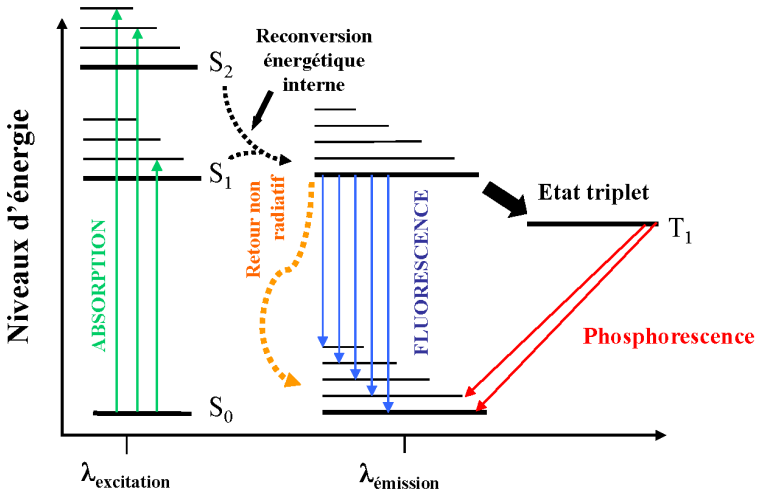

Figure 1 - Perrin-Jablonski diagram and illustration of the relative positions of the absorption, fluorescence and phosphorescence bands.

Jobin-Yvon) used for our research, consists of a 450 W xenon arc source, giving continuous polychromatic radiation, a double monochromator made up of flat arrays for excitation, a door compartment - sample, of a second double monochromator with gratings for the emission and of a photomultiplier.

The devices record either the excitation spectrum or the fl uorescence emission spectrum [1], or both simultaneously which makes it possible to obtain excitation / emission maps. Fluorescence emission spectra are generally collected at 90 °.

The devices which record the excitation spectra have the advantage of complementing the characterization of the fl uorophores responsible for the fl uorescence emission. However, the intensity of the beam delivered by the lamp and monochromator assembly varies as a function of the excitation wavelength. These fl uctuations therefore disturb the measurement of an excitation spectrum. The corrected spectrum will therefore be equal to the ratio between the measured spectrum and a correction function which can be determined using the photon counting device. The device consists of a triangular tank containing a very concentrated reference solution (generally rhodamine) whose intensity of the emission spectrum does not depend on the excitation wavelength.

This system has a relatively small analysis chamber allowing work on quartz slides or cells with an analysis area of 1 cm x 5 mm. It is therefore, with this system, impossible to analyze objects in a non-invasive manner. However, the movement of works from their place of storage (museum, reserves, etc.) to

1. Illustration of a fluorescence emission spectrum and an excitation spectrum in Figure 4.

laboratories often remain limited, even exceptional. The impossibility of removing or moving these works made it impossible for them to be analyzed by these classical systems; however, it is possible to equip the instrument (for example in our laboratory: iHR Fluorolog 320 [Horiba Jobin-Yvon]) with an optical fiber and a microscope in order to be able to study objects which can be moved in the laboratory.

1.3 Fluorescence spectra

Due to the existence of vibrational relaxation phenomena, the position of the fluorescence emission maximum of a molecule is at an energy lower than its absorption maximum, ie at a longer wavelength. The emission band is generally in the UV-visible-IR range of the electromagnetic spectrum. Several parameters are important in the study of a spectrum, in particular, the shape of the spectrum, the luminescence intensity, the wavelength of the maximum intensity, the width at half height of the bands, etc. Under certain conditions (low concentrations, pure compounds...) There exists, as for absorption spectrometry, a relation of proportionality between the intensity of fl uorescence and the concentration of the species considered.

The shape of the fluorescence spectrum for a molecule does not depend on the excitatory wavelength. However, in the case of a mixture of compounds, not all of the compounds are excited in the same way depending on the selected exciting wavelength. The fluorescence spectrum of a mixture of compounds therefore varies as a function of the excitatory wavelength [2].

2 Portable spectro fl uorimeters

2.1 Overview of existing systems

The portable spectro fl uorimeter makes it possible to abolish the size limit of the analyzed objects and to move the instrument near the object. The general principle is to miniaturize the detector, to choose LED excitation sources with optical fibers to transmit the light signal of excitation and fluorescence emission.

B. Brunetti and C. Miliani, for example, carried out a development within the framework of the European project CHARISMA [2] in order to be able, with a single system, to record the reflectance, fl uorescence and fl uorescence lifetime spectra of the compounds [4]. Their apparatus includes laser diode sources (LEDs at 375, 455 and 650 nm) for fluorescence measurement, a halogen-deuterium lamp for absorption measurements and pulsed laser diode sources for measuring lifetimes. Three detectors are needed to collect the signal (CCD sensors). The analysis area is approximately 5 mm^2. Although the components were the smallest available on the market in 2011, the system is quite large as a whole and is more transportable than portable since it requires the use of a cart to place the various components. The Italian teams have shown the interest of the

2. Cultural Heritage Advanced Research Infrastructures: Synergy for a Multidisciplinary Approach to Conservation / Restoration, FP7 Capacities - speci fi c Program Integrated Activities for Research Infrastructure, n ° 228330.

UV fluorescence, especially for the identi fi cation of indigo [5]. They have integrated mathematical treatments to their data, such as the Kubelka-Munk formula in order to eliminate the self-absorption phenomena allowing to visualize only the emitted fluorescence [6].

Other systems are available on the market such as the one offered by Ocean Optics (Jazz system). It requires speci fi c modules for the di ff erent measurement con fi gurations and their handling is not always easy, which has led us to develop our own system according to the constraints specified above. The system had to be light, portable, allowing very short analysis times while controlling the power of the LEDs and the analysis time in order to adapt the operating conditions to any type of material.

2.2 Development of an original system: the LEDµSF

We present here the development phases of the portable LED micro-spectro fl uorimeter (LEDµSF) [3] (Figure 2a, b, c, d).

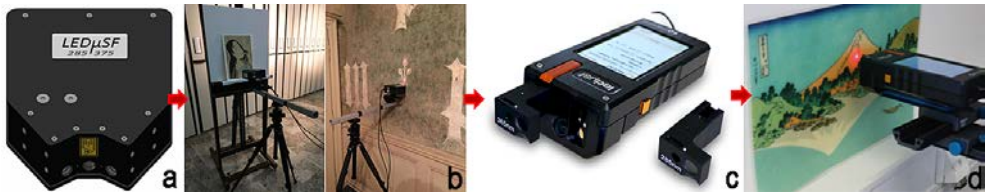

Figure 2 - (a) First prototype produced by a local company, Erma Electronique with two exciting LEDs; (b) LEDµSF v1 in front of a Japanese print from the Zaragoza museum (Spain) and in front of a wall painting from the castle of Germolles in Burgundy (France); (c) LEDµSF v2 augmented with LED + filter modules offering a wide range of exciters from UV to IR and a white LED to record both fluorescence and reflectance emission spectra. The device is marketed by Freiberg Instruments (Germany); (d) LEDµSF v2 in front of a stamp, the focus is adjusted using two red lasers.

The Thorlabs spectrometer, CCS200 / M which equipped the 1st version (Figure 2a and b) has been replaced by a Qmini Wide VIS spectrometer (Pembroke Instruments) for version 2 (Freiberg Instruments, Figure 2c and d). The spectral range of the latter extends from 200 to 1000 nm. The signal enters the spectrometer through an optical fiber (Ø 400 µm).

For the excitation of materials, the UV sources are in version 1, low power LEDs with di ff erent wavelengths: 285 nm (300 µW) and 375 nm

3. The LEDµSF system was designed and developed in partnership between, on the one hand, the Research Center in Physics Applied to Archeology, one of the components of the Institute for Research on Archaeomaterials (IRAMAT-CRPAA) of the University of Bordeaux Montaigne and, on the other hand, the Institute of Molecular Sciences (ISM) of the University of Bordeaux. Thanks to the support of Aquitaine Science Transfert, the device was the subject of a patent filing with the National Institute of Intellectual Protection (INPI) on December 24, 2014 (France n ° 14 63318). The geographical extension of patent protection was made on December 23, 2015 by filing an international application under the Patent Cooperation Treaty (PCT / FR2015 / 053744). SATT Aquitaine conducted the marketing study and fi nanced the creation of an industrial prototype. A local company, Erma Electronique (Charbonnier Group,

(5 mW) with associated high-pass filters (respectively 320 nm for the first and 455 nm for the second). Version 2 o ers a larger number of interchangeable modules (LEDs + fi lters). Those we currently have emit at the following wavelengths: 255, 285, 310 and 365 nm for UV, 457, 525, 590 and 625 nm for VIS. A white LED also makes it possible to record the reflectance spectra on the same analysis point.

The spectrometer's range (200 to 1000 nm) is wider than that of laboratory devices (200-720 nm).

The working distance, adjusted using two red lasers, is 4 cm. The diameter of the analysis spot on the sample is approximately 2 mm. The fluorescence-emitting spot of light is imaged on the entrance of the analysis fiber through a short focal length lens.

For fragile objects or when the signal is too strong, the power of the LEDs can be changed to decrease the intensity of the sources.

A module (Arduino [4] in version 1) allows the activation and deactivation of laser pointers to obtain the correct working distance, activation / deactivation of UV sources for sample excitation, integration time and start analysis. The software allows you to select the integration time from 10 µs to 60 s and to display the programmed exposure time. The analysis time depends on the intensity of the emission of the materials studied. In the case of weak signals, a smoothing of the spectra may be necessary. The very short analysis time allows the method to be applied to sensitive works and in particular for the study of paintings.

The LEDµSF v1 weighs less than 1.5 kg for a measuring head dimension of 15 x 15 x 8 cm while version 2 weighs 0.8 kg for a size of 17 x 8 x 5 cm. If the 1 er system was powered and controlled by USB connection via a laptop and by means of a graphical interface realized with "Windows Forms [5] », The commercial version has an electric power supply (or possibly a battery) and a touch screen for adjusting operating conditions and recording data. In order to finely adjust the working distance, the device is fixed on a millimeter plate. The horizontal or vertical positioning is also manually adjustable. The whole is attached to a photo stand, infinitely adjustable. It has a central column that can be reversed with a ball joint that allows an adjustable 90 ° pivot to adjust the tilt (Figure 2b and d). The device (Figure 2c) can also be hand-held when measuring in hard-to-reach places.

While the LEDµSF is globally less sensitive than conventional laboratory devices, the signal-to-noise ratio is largely sufficient for our applications. The LEDµSF only records the emission spectra (and not the excitation spectra), which does not always allow a fi nite characterization of the fl uorophores.

4. Cards (materially free) on which there is a microcontroller that can be programmed for the analysis and production of electrical signals.

5. Interface realized with the Arduino IDE and MS Visual Studio Express C ++.

Fluorescence corrections can be envisaged, in particular thanks to the addition of the white LED which allows the recording of the reflectance spectrum. [6] in the visible in the same analysis area.

The main advantages of the LEDμSF are portability, weight and ease of use and set-up. Its speed of analysis is also a real asset. A few seconds are sufficient to collect a fluorescence emission spectrum, 1 to 2 seconds for an organic compound and 10 to 20 seconds for an inorganic pigment (about 5 minutes with a fixed laboratory apparatus). The very short analysis time and the quality of the signal make it possible to work on fragile works and to study the dyes and binders in paintings, textiles, prints and more generally works of cultural heritage which are sometimes little studied because of the the inability to sample or move them to laboratories.

3 Approach for the interpretation of spectra fluorescence emission

Interpretation of fluorescence emission spectra is facilitated by the prior study of pure materials with laboratory instruments. These make it possible to record the excitation spectra in order to characterize the fl uorophores at the origin of the fl uorescence emission. The excitation maxima specific to each compound notably guide the choice of the most suitable excitation LED to reveal the fluorescence of the material.

3.1 Maximum fluorescence emission wavelength

Certain materials have fluorescence properties which can make it possible, under certain conditions, to identify them, in particular by their maximum fluorescence wavelength.

However, in the case of portable instruments, these fluorescence emission maxima may exhibit band shifts which can be explained by experimental parameters (analysis time, choice of source) and by the reproducibility of the measurements [7].

The fl uorescence spectra resulting from the experiment are complex to interpret due to the composition of the materials and their state: the support, the binder, the mixtures of pigments, their preparation, the thickness of the layers and the alterations can modify the material. spectrum and shift the emission maxima measured on pure materials. Self-absorption and absorption phenomena occur, especially in the case of mixtures (pigments + binders) [6, 8, 9].

Treatments, including those based on Kubelka-Munk (KM) theories, can be applied to minimize the e ff ects of these phenomena [8, 9].

This KM treatment is generally applied to spectra of mixtures of the pigment + binder type, which makes it possible to eliminate the absorption by the pigment and to allow only the intrinsic fluorescence of the binder to appear. However, in the case of dyes in

6. Fraction of the re-emitted flux in all directions for each wavelength.

non-opaque and transparent layers, the conditions for applying KM theory are not met. This correction must therefore be adapted to the case of mixtures of various fluorophores for which characteristic band shifts have been observed [6].

In addition to these spectral data processing, it is possible to interpret the fluorescence spectra by comparison with those of a database of intrinsic fluorescence emission spectra of constituents alone or of mixtures of compounds.

3.2 Creation of a database of reference compound spectra

The essential work of constituting benchmarks is a long-term investment in the sense that any material can be studied alone or combined, mixed or superimposed, on a fl uorescent support or not, aged according to di ff erent modes (UV, relative humidity or the conjunction of two...) and that the applications go far beyond the field of cultural heritage materials. Each team establishes its own reference samples according to its object of study and records its own reference spectra. Most of the published articles present the study of a particular case of pigment (or color), dye or lacquer [9, 10, 11]. In 2005, Claudia Daffara's Italian team with Anna Pelagotti published a database on the study of UV fl uorescence of paint materials with fl uorescence emission spectra and reflectance [12]. In 2017, Derrick and his colleagues published an article dealing with dyes used in Japanese prints and a database is presented [13]. We therefore have a certain amount of data on which we can rely to con fi rm our results even if, most of the time, these published data have been obtained with devices of more traditional con fi guration.

We have built our own database of reference spectra with the portable system. The results obtained were validated with a conventional spectro-fluorimeter. As regards the latter, the acquisition parameters chosen are as follows: angle of 22.5 ° between the incident beam and the detector; 366 nm exciter, constant exciter flux mode. The slit width is 2.2 mm, which corresponds to a pass band of 4 nm, for a step of 0.5 nm and an integration time of 0.5 seconds. Our database currently has nearly 200 reference spectra. It was mainly established from two color charts produced using old techniques. The first was done on parchment, the second on pure cellulose paper. The pigments or lacquers have been mixed with a binder (tempera,

We present some reference spectra in part 4 of this chapter.

3.3 Complementarity with other analytical techniques

Portable fl uorimetry is complementary to “traditional” analytical techniques (X-ray Fluorescence Spectroscopy [XRF], Raman or Fiber Optic Reflectance [FORS] spectrometry and Hyperspectral Imaging [HSI]) which help interpret the results. obtained with the LEDμSF. The techniques currently used (XRF, Raman...) Make it possible to respond essentially to the identification.

inorganic pigments by providing information on the basic composition or molecular structure of the minerals, respectively. That of organic materials remains problematic but possible, in particular thanks to LEDμSF.

For example, the combination of X-ray fluorescence, reflectance and fluorescence spectra has solved many questions about the identi fi cation of the lacquers employed in Japanese prints. Elemental analysis (XRF) makes it possible to identify the mordant (alum, calcium...) Used to precipitate the dye and to confirm the hypothesis of the presence of a lacquer. The reflectance spectrum makes it possible, for example, to define the absorption bands and thus to better choose the exciting LED for the characterization of the fluorophore (s) present. The integration of this new LEDμSF system into the range of portable devices available opens up new avenues and objects of study [14].

Hyperspectral imaging under UV can benefit from a database of fluorescence emission spectra of pigments, dyes and reference binders such as the one we have developed (Table 1): it is thus possible to map the Fluorescence of materials by choosing observation wavelengths *ad hoc.*

For example, one can locate the presence of cinnabar which emits a fluorescence at λ_{max} 610 nm, thanks to the imaging obtained by selecting the length of interest. This complementarity of techniques was the subject of a publication dedicated to the study of medieval miniatures by Mounier *et al. [*15].

Table 1 - Maximum characteristic fluorescence emission of pigments mixed with gum arabic analyzed with the 375 nm LED (L375) or the 285 nm LED (L285) as well as mixtures of pigments.

Pigments	LEDμSF (λ_{max})		Pigment mixtures	LEDμSF (λ_{max})
Cinnabar	610 nm (L375)		Red ocher + indigo	592, 720 nm (L375)
Minimum	590 nm (L375)		Red ocher + azurite	605 nm (L375) 430 nm (L285)
Red ocher	605 nm (L375)		Cinnabar + indigo	590, 725 nm (L375)
Cochineal	640 nm (L375)		Cinnabar + indigo + organic red	619, 720 nm (L375)
Brazil wood	630 nm (L375)		Cinnabar + minium	620 nm (L375)
Azurite	440 nm (L285)		Cinnabar + minium + organic red	615 nm (L375)
Lapis-Lazuli	440, 710 nm (L285)		Indigo + yellow ocher	560, 615, 730 nm (L375)
Egyptian blue	440, 560, 710 & 890 nm (L285)		Indigo + orpiment	560, 720 nm (L375)
Indigo	730 nm (L375)		Verdigris + indigo	560, 710 nm (L375)
Yellow ochre	575 nm ((L375)		Malachite + azurite	440, 695 nm (L285)
Lead yellow and tin	545 nm (L285)			
Orpiment	525 nm (L285)			
Bourdaine	520 nm (L285)			
Buckthorn	570 nm (L285)			
Stil-de-grain	550 nm (L285)			
Malachite	430 nm (L285) 550 nm (L375)			

4 Applications

4.1 Pure fresh products

4.1.1 Coloring matters

We present here some reference spectra obtained on a color chart of 150 pigments produced by the Marlier workshop. [7] according to the recipes described in ancient treatises (Cennino Cennini, Monk Théophile...). Pigments and binders come from various suppliers (Kremer, Okhra, Marlier, prepared in the laboratory). The pigments were mixed with the binders most used in medieval illuminations, such as gum arabic, rabbit skin glue or egg white, deposited on slides or on parchment.

The coloring materials used are inorganic or organic in nature. In medieval illuminations, for example, organic dyes are most often used as highlights on inorganic pigments (cinnabar, lapis lazuli, minium, malachite, ochres) [8].

Red pigments

Five red pigments, fixed on an inorganic support, conventionally used in medieval illuminations were studied by fluorimetry. Each of them (minium, cinnabar, red ocher, Brazil wood and cochineal in the form of lacquers) was bound with gum arabic and deposited on a quartz slide. Laboratory studies have shown that the excitation wavelength at 375 nm is well suited to excite the fl uorophores present [7]. The fluorescence emission spectra were recorded with the LEDµSF V1 while exciting at 375 nm with the 455 nm filter, for an analysis time of 30 s. Figure 3 shows the fluorescence emission spectra of these five red pigments. Cinnabar gives a maximum emission wavelength around 610 nm, minium emits around 590 nm, cochineal at 640 nm and Brazil wood at 630 nm. The red ocher gives no characteristic band even if a shoulder is visible around 600 nm. Thus, in this example, these five red pigments under the most favorable conditions can be differentiated by their fluorescence emission spectra.

The appearance of the spectra and the fluorescence emission wavelength values were con fi rmed by the laboratory apparatus on these same samples for longer analysis times (approximately 5 minutes per spectrum).

The maximum excitation wavelength (that which excites the fluorophore most effectively) may be characteristic of dyes and their study by conventional spectro-fluorimetry equipment may be essential. Thus, for example in the case of cochineal lake, the excitation maxima at 540 and 580 nm correspond to the carminic acid complex (FIG. 4). In the Brazilian wood,

7. http: //www.enluminure-peinture.fr/. Renaud Marlier is a specialist in medieval illumination. he manufactures its own materials (pigments, binders,...) and proceeds to the production of *facsimile.*

8. Projects coordinated by Floréal Daniel and funded by the Regional Council of Aquitaine (ENLUMI-NURES) and the LaScArBx labex (HYPERSPEC).

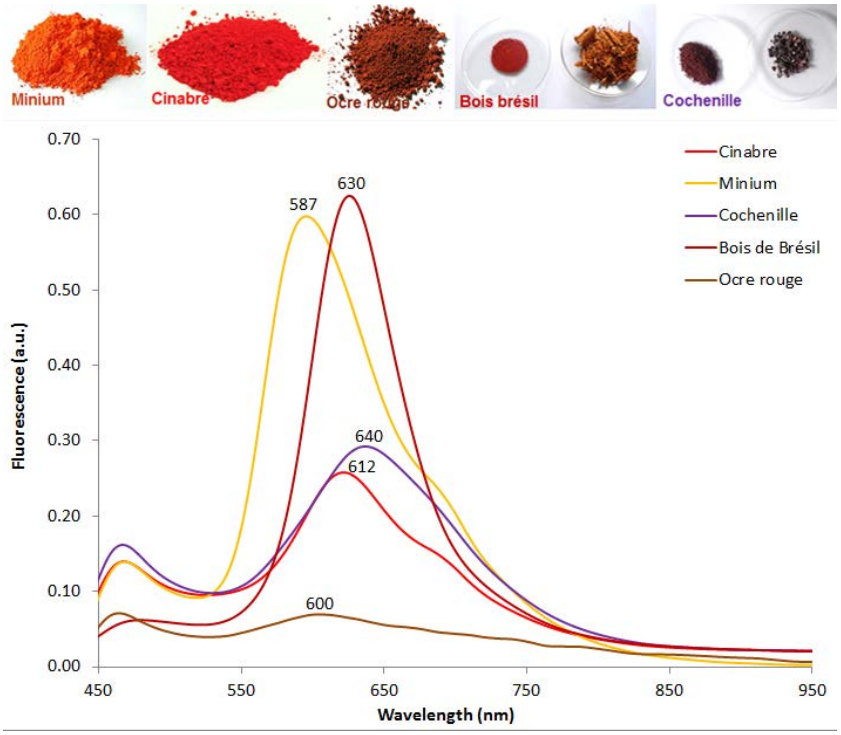

Figure 3 - Fluorescence emission spectra of 5 red pigments mixed with gum arabic, deposited on a quartz slide, excited at 375 nm (filter 455 nm), for an analysis time of 30 s with the LEDµSF. Each pigment has a specific spectrum and maximum emission, which makes it possible to distinguish it.

brazilein is the only fluorophore with an excitation maximum centered at 510 nm [7]. These results are con fi rmed by the literature [16-20].

Reproducibility

Regarding the response between two calibrated laboratory devices whose curves are corrected, a shift of a few nm is noted for each red pigment studied. The LEDµSF gives *maxima* higher for all red pigments and the shift is even greater for organic pigments [15].

Blue pigments

The same methodology was applied to blue pigments (azurite, lapis lazuli, Egyptian blue) mixed with gum arabic with excitation at 285 nm. All the spectra show a common band centered around 440 nm [7]. Lapis lazuli has an additional band at 730 nm and Egyptian blue at 890 nm. Cu ion $^{2+}$, which gives the color to Egyptian blue, absorbs around 620 nm and is known to fluoresce around 900 nm [21].

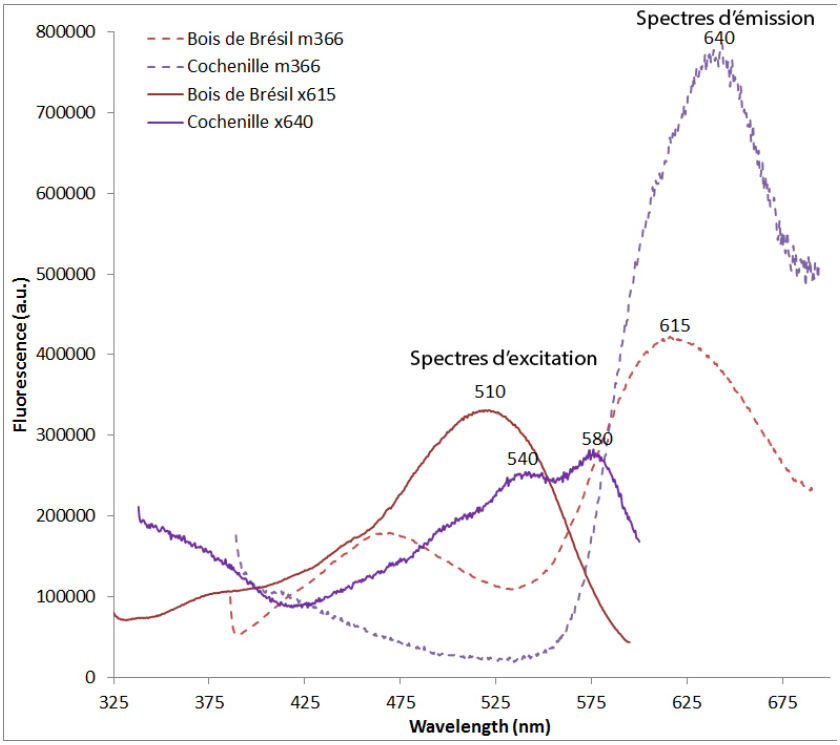

Figure 4 - Fluorescence emission spectra of Brazilwood (emission at 615 nm) and of cochineal (max at 640 nm) by exciting at 366 nm with the laboratory system; the excitation spectra inducing emissions at 615 and 640 nm, reveal the fl uorophores at the origin of the fl uorescence (510 nm for brazilin and 540 and 580 for anthraquinones).

Yellow pigments

Five yellow pigments are commonly used in medieval manuscripts: gold, lead and tin yellow, buckthorn, buckthorn, and stil-de-grain. The last three are made from the berries or bark of shrubs of the rhamnaceae family and, chemically, the colored compounds are fl avonoids (green fruits), anthraquinones (red fruits) and anthocyanins (black fruits).

The five yellow pigments were excited at 285 nm with the filter at 320 nm for an analysis time of 50 s (Figure 5). The lead and tin yellow gives a maximum fluorescence around 545 nm and the orpiment at 525 nm.

With regard to the three organic dyes from the same plant, the ripeness of the berries could be the cause of variations in the maximum wavelength (up to 50 nm) observed in the fluorescence spectra. The more mature the array, the more the maximum emission wavelength shifts towards long wavelengths.

In fact, buckthorn has a λ_{max} at 570 nm, grain stil at 550 nm and buckthorn at 520 nm. To make buckthorn, we use a green berry, a berry
more mature for stil-de-grain and a red berry, very ripe for buckthorn [7].

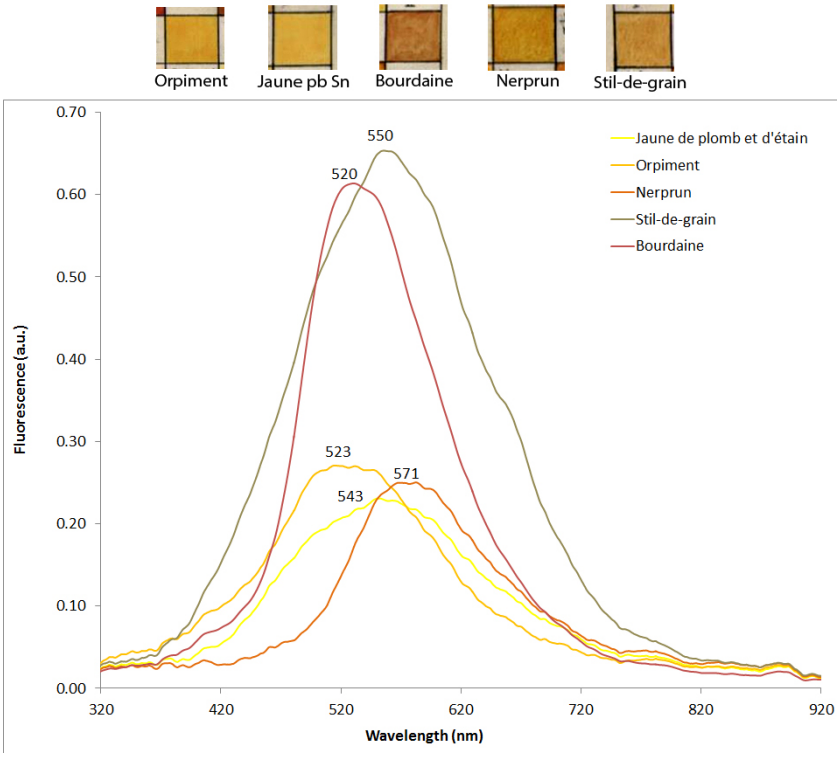

Figure 5 - Fluorescence emission spectra obtained with the LEDµSF on five yellow pigments by exciting at 285 nm (filter at 320 nm) for an analysis time of 50 s. The 5 spectra are di ff erent and in this example, each pigment gives a maximum emission and its own spectrum appearance.

The LEDµSF system made it possible to distinguish them and to record the characteristic bands for this specific case. If this technique alone only gives clues for this type of compound, it should be noted that no other non-invasive technique is capable of giving an identification.

The intensity of fluorescence, in some special cases, can help to differentiate organic compounds from inorganic ones. For example, the intensity of fl uorescence, for the same analysis time, is three times greater for buckthorn than for orpiment. In all cases, the analysis by XRF or by UV-Vis reflectance spectroscopy makes it possible to remove the uncertainties.

4.1.2 Binders

While pigments and dyes may have intrinsic fluorescence emission bands, they are usually mixed with an organic binder which, too, fluoresces and contributes to the overall spectrum recorded. In the above examples, gum arabic and tempera have little impact because the exciting LED is chosen

depending on the pigment to be analyzed (eg 375 nm for red pigments) and to minimize fluorescence of the binder [22].

The fluorescence of binders (glues, oils...) Has been extensively studied with laboratory spectrofluorimeters and their fl uorophores have been identified [3, 10, 22, 23]. Gum arabic and fish glue give maximum fluorescence emission at 440 nm. Fresh linseed oil emits at 430 nm and aged linseed oil exhibits a maximum wavelength around 560 nm. This bathochrome e [9] is explained by the crosslinking of the oil during its drying and the formation of the solid, continuous, hard and resistant film, causing its yellowing [3, 23, 24]. Egg yolk exhibits maximum fluorescence emission at 520 nm. The presence of a binder and its degradation over time can therefore have an influence on the apparent fluorescence of the pigment or dye measured in the mixture comprising the pictorial layer.

The laboratory study by spectro fl uorimetry of the binders used in paints has made it possible to show that the protein binders (rabbit skin, bone, nerve glues) or carbohydrate (gum arabic) fl uorescent in the blue, while the lipid binders (linseed oil, walnut oil...) rather give a yellow fluorescence.

Accelerated photochemical aging carried out on these binders has shown that the maximum emission wavelength shifts towards long wavelengths (yellowing) and that the addition of a drier (lead pigment) increases the intensity. fluorescence [22, 23].

4.1.3 Supports

We cannot over-emphasize the influence of the medium on the emission spectra and in particular on the apparent displacement of the maximum emission wavelengths, criterion which we have chosen for the identification.

The support of the paintings (parchment, preparatory layer, paper...) Can indeed emit a fluorescence and thus contribute to the total fluorescence recorded. This is why the coloring matters studied were studied on a *facsimile* [10] illumination of the XIV e century produced by the Marlier workshop [7]. The parchment emits a very intense fluorescence and a very broad band regardless of the excitation wavelength. However, in the various measurements carried out on the *facsimile,* the fluorescence of the parchment has little impact on that of the pigments because the pigmented layer is thick and opaque.

The variability of the spectra depends on the process for preparing the pigment and on its application to the support (thickness, concentration of the layers, type of binder, etc.). On the other hand, for a low thickness of the dye, the measurement is hampered by the superposition of the spectra due to the pigment and to the support.

9. Modi fi cation of the position of the spectral band towards the longest wavelengths (or frequencies weaker).

10. Reproduction of a Dominican antiphonary, 14th century. kept at the municipal library of Colmar (cod. 311, fol. 123), initial letter N with the representation of the Nativity, parchment by Ets Lieutard. Pigments and mixtures of pigments or binders are known.

4.2 Mixtures of dyes

In the simple case of acquiring intrinsic fluorescence or when alum is used as a mordant to fix the dye, identification appears to pose less difficulty. On the other hand, in the case of mixtures or fixation of the dye on a calcium-based filler, cochineal and Brazilwood can for example be confused [7]. Only the excitation spectra can dispel the ambiguity. It is therefore advantageous to use the same type of device to produce the database and to guarantee the reproducibility of the measurements.

The identification of materials in the case of mixtures is difficult if one considers only the fluorescence emission spectra. However, hypotheses can be made and the comparison of the results with those obtained by other techniques (hyperspectral imaging, FORS, XRF, etc.) can help in the interpretation of the bands and in the identification of the pigments. In the absence of spectral data processing, it is sometimes difficult, for complex systems such as paints, to distinguish certain pigments.

If the pigment is often combined with an organic binder, the pigments and dyes can be mixed or superimposed. For example, in medieval illuminations the dyes are usually highlighted on a layer of pigment, while in Japanese prints the mixture of pigments / dyes is usual. The engravings are colored by superimposing layers or mixtures of pigments in order to vary the tones. In addition, organic dyes are widely used and make identification more complex as many fluorophores may be present. These dyes are also known to be unstable.

We produced in the laboratory the speci fi c dyes for 18th century Japanese engravings. e to XX e century and made pseudo-prints [11]. In addition to the traditional pigments used in Western paints (vermilion, minium, ocher, lead white, malachite, etc.), the use of speci fi c and / or local dyes enrich the palette (St. John's Wort, cochineal, madder, safflower. , indigo, gamboge...). These dyes are of animal or vegetable origin. This material thus makes it possible to extend our database of reference fluorescence emission spectra. About ten mixtures of pigments (50/50 by volume percentage) were made to study their e ff ect on the fluorescence spectra measured.

For example, violet is a very present color in prints, several combinations of mixtures are possible to obtain it. Likewise, it is not uncommon to find a mixture of vermilion, minium and an organic red to subtly vary the shades of reds [26].

Mixtures were recreated in the laboratory to obtain a violet tone (vermilion + indigo or vermilion + indigo + cochineal) (Figure 6) and deposited on quartz slides and on Whatman non-fluorescent paper under our operating conditions (time and lengths of excitation wave). The reference spectra of vermilion and indigo

11. INDIGO project led by Floréal Daniel (CRP2A) including a thesis in progress led by Carole Biron, directed by Rémy Chapoulie (IRAMAT-CRP2A) and Laurent Servant (ISM) on the applications of infrared spectroscopy.

appear in Figure 6 for a direct comparison of the bands (LED 375 nm, filter 455 nm, analysis time 30 s). The fluorescence emission maxima of the studied mixtures are given in Table 1. In the first mixture, there appears an apparent shift of the maximum wavelength due to the vermilion towards the shorter wavelengths (612 nm. 590 nm), the same for indigo (730,725 nm). In the second case, three pigments are mixed and analyzed with the same experimental parameters. We note the presence of a wide maximum band centered at 620 nm with a slight shoulder around 720 nm. The main band is between that of the vermilion (612 nm) and that of the cochineal (640 nm). The indigo band (reference at 730 nm) is present but shifted towards 720 nm. Vermilion and indigo have

λ_{max} fluorescence of 610 nm and 730 nm, respectively. In mixture, the λ_{max} of the two components shifts to the shorter wavelengths, 20 nm for the
vermilion and 5 nm for indigo. In general, additional close emissions widen the bands and the appearance of new bands is possible, possibly due to the excitation of new fluorophores formed during the interaction between the mixed pigments / lakes. It is also possible that these are apparent emission maxima generated by the absorption properties of the overall mixture. The reasons for these trips have been discussed above. Pigments are capable of absorbing and / or dispersing the radiation emitted by surrounding materials. Self-absorption phenomena can occur between molecules, which shifts the characteristic bands.

By way of illustration, here is an example of the attribution of the bands on a Japanese ukiyo-e print (ref. 49868, Figure 7) which shows all the difficulty of interpreting the fluorescence spectra in the case of mixtures (pigments, dyes , binder) on a support which is itself fl uorescent. Indeed, the spectrum of the violet dye shows a number of bands at 502, 566, 610 and 690 nm. Those with wavelengths less than 550 nm correspond to the fluorescence of the paper. The 610 nm band seems to resemble that of vermilion but other hypotheses can be put forward:

- Displacement of the maximum apparent emission wavelength of the pigment due to the high fluorescence intensity of the paper. A treatment like that proposed by Pottier *et al. [* 25] would distinguish the fluorescence of coloring matter from that of paper filtered by the paint layer.

- Presence of cochineal or a mixture of red and blue pigments. It cannot be indigo which gives a characteristic band much higher at around 730-40 nm, absent on the spectrum. However, another blue dye (day ower or aobana) extracted from the petals of common commeline, very frequent in prints for its blue-violet color, could be used. We do not have a reference for this dye in our database. However, the characteristic absorption bands of this pigment at 594 and 645 nm, described in the literature [14, 27], have been measured by reflectance spectrometry. The di use re fl ectance spectrometry made it possible to identify the dye.

  aobana in this print.

Depending on the complexity of the objects studied and the di ff erent parameters involved (multilayer object, heterogeneity of materials, organic and inorganic,

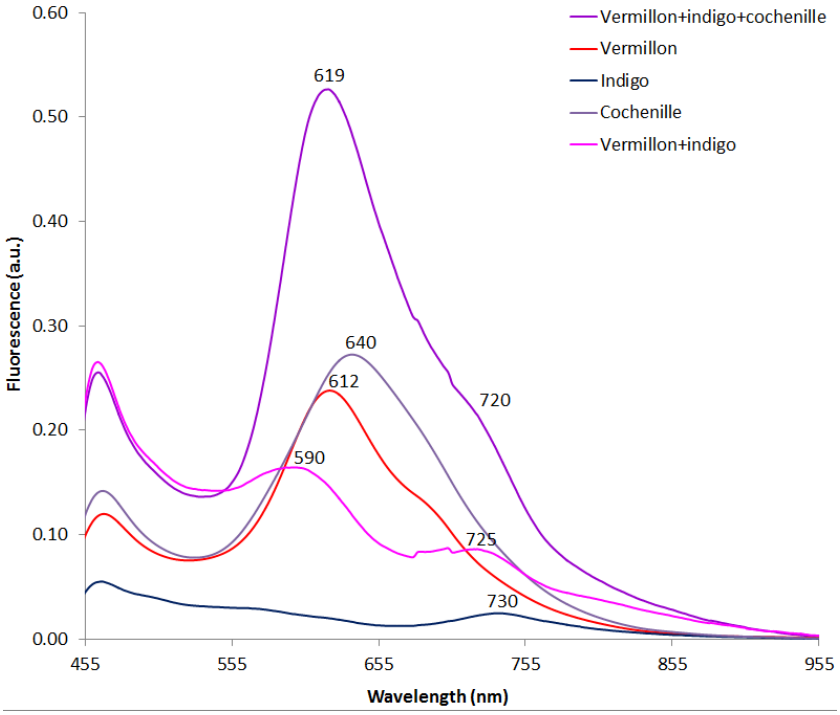

Figure 6 - Fluorescence emission spectra of mixtures of pigments to obtain violet: vermilion + indigo and vermilion + indigo + cochineal. Reference spectra of the pigments alone have also been added to facilitate comparison of the bands. In the case of mixtures, the characteristic bands of the pigments alone are staggered or else overlap, making it difficult to identify the pigments; corrective treatments (Kubelka-Munk, deconvolution...) could help this dissociation.

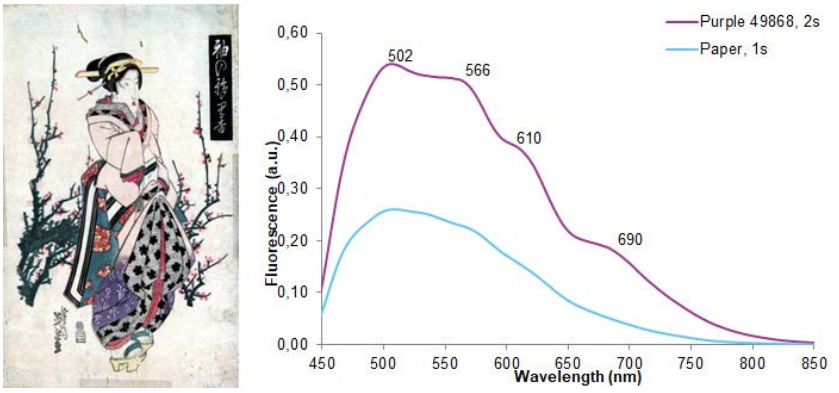

Figure 7 - Japanese print from the Torralba collection (Zaragoza museum) referenced 49868 (artist Eisen, XIX e s.). Fluorescence emission spectra measured in violet and in paper. The latter exhibits a very important fluorescence. The bands between 500 and 550 nm can therefore come from the support and not from the pigments.

supports, binders, pigments. . .) corrective treatments (Kubelka-Munk, deconvolution...) could help to dissociate the signal due to the di ff erent constituents and in particular characterize the intrinsic fluorescence of the pigment by eliminating, in particular, the self-absorption phenomena .

These methodological aspects still require the development of models. Recently, standard correction procedures (Kubelka-Munk) have been applied to experimental fl uorescence data collected from the polychromatic surface of an Aztec manuscript of the XVI. e century [25]. These results are compared to a new method based on the hypothesis of non-emitting transparent paint layers. This method makes it possible to establish the origin of the emission detected and to distinguish the fluorescence of the substrate transmitted by the pictorial layer from the real intrinsic emission generated by the coloring matters studied.

4.3 Degraded coloring matter

The study of sensitive materials and in particular organic dyes leads to consider the alteration of these materials and more mainly the phenomenon of discoloration [3].

Spectro fl uorimetry is not, from a certain level of degradation of the colorant, suitable for the identification of the latter, nor are the excitation spectra because it is probable that certain fl uorophores are degraded. during aging.

The same is true for binders alone, such as for example linseed oil, the emission wavelength of which goes from 440 nm beyond 500 nm during photochemical aging [23].

To verify the evolution of the fluorescence spectra of the coloring matters as a function of the degradation, we subjected samples to the flux of an LED (275 nm,
1.6 mW) and followed the discoloration of pigments (Figure 8) under power conditions 5 times greater than that of LEDµSF under analysis conditions and irradiation times of up to 12 hours, corresponding to a energy exposure of 5,870 mJ / cm 2. Fluorescence spectra were recorded regularly over time.

The first tests were carried out on indigo and cochineal mixed with gum arabic, a fragile and very brittle binder. The degradation of dyestuffs depends on the concentration and nature of the binder, the mode of preparation of the dye and the filler to which it is attached. Indeed, according to Saunders and Kirby [28], the higher the binder concentration, the less the fluorescence spectrum of the pigment changes. On the other hand, cochineal discolors more quickly when mixed with gum arabic rather than oil.

Figure 8 shows the fluorescence emission spectra of indigo and cochinillus alone before irradiation, and after 12 h of irradiation at 275 nm. The characteristic indigo band at 730-740 nm remains stable. This result is consistent with previous published studies [11]. The spectrum of the cochineal, before irradiation, shows a band at 640 nm and a small shoulder at 575 nm. The fl uorescence of the cochineal is due

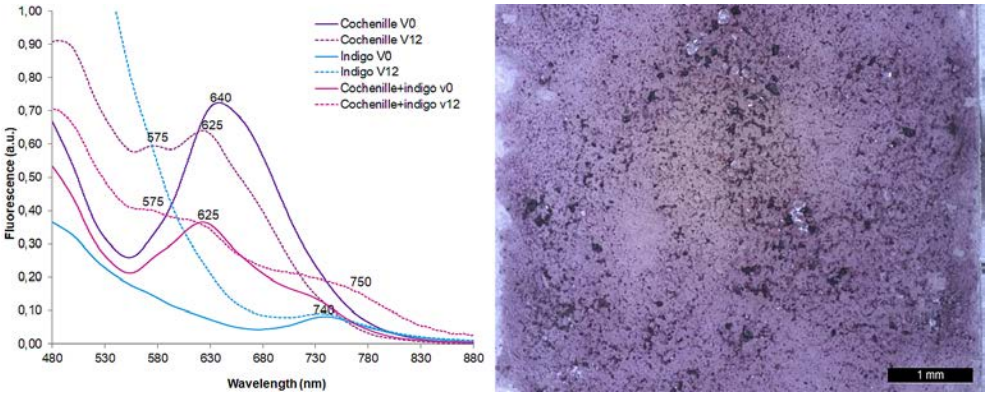

Figure 8 - Fluorescence emission spectra obtained at 375 nm on cochineal, indigo and the mixture of the two pigments before irradiation (V0) and after 12 hours of irradiation (V12, i.e. an energy exposure of 5870 mJ / cm 2) with an LED at 275 nm. Indigo is little affected by prolonged UV irradiation, unlike cochineal which degrades and becomes discolored. The main 640nm band shifts to 625nm and a 575nm band grows under irradiation. In the case of the cochineal + indigo mixture, the same phenomenon is observed. After 12 hours of UV irradiation, an orange spot appears, which clearly shows the discoloration / deterioration of the pigment.

to the anthraquinones which compose it: carminic acid (red compound, band at 640 nm) and laccaic acid (yellow compound, band around 575 nm). After 12 hours of irradiation at 275 nm, a molecular change is noted. The main band shifts towards the shortest wavelengths (640 to 625 nm) and there is a strong predominance of the 575 nm band. According to Saunders and Kirby, red compounds are destroyed by UV exposure [28]. This discoloration, measurable by reflectance spectrometry (decrease in the intensity of the reflection factor), even begins to be visible to the naked eye from 10 hours of irradiation. Under a microscope, a yellow-orange spot clearly appears in the irradiated area (Figure 8).

In the case of the indigo + cochineal mixture, before irradiation, two bands are noted at 625 and 730-740 nm with a slight shoulder towards 575 nm. After 12 hours of irradiation, the intensity drops due to the destruction of the red compounds of the cochineal to give way to the yellow compounds. The indigo band is shifted towards the long wavelengths (750 nm) (Figure 8). The presence of indigo seems to accelerate the degradation of the cochineal since the contribution of yellow compounds appears from 3 hours of irradiation. In addition, there is an inversion of the bands, that of yellow compounds is more important than that of reds. The influence of indigo on the cochineal in its alteration process is not established but some hypotheses can be made on the factors in play such as the modification of the pH,

Therefore, the fluorescence spectra evolve with the disappearance or appearance of the fluorophores modifying the absorption properties during degradation. The interpretation of the data is therefore more delicate, in particular for

sensitive organic dyes. The combination of the results with those obtained by reflectance spectrometry makes it possible to resolve the ambiguities [14].

Conclusion

The applications of LEDμSF for the study of paints and in particular for the identification of pigments and dyes are of definite interest in the simplest cases (monolayers of pigment, little binder). They require the establishment of a database of fluorescence spectra of reference compounds. In more complex cases (mixtures of materials), corrective treatments can be applied to the data, although the protocols in this field are still poorly defined for our applications. The development of fl uorescence simulations of pure fresh, mixed or degraded products would make it possible to better understand the spectra.

However, the uncertainties can be resolved by complementary approaches: either by modifying the wavelengths of the LED sources to selectively excite the fl uorophores of the species to be identified, or by obtaining the excitation spectra using conventional laboratory fl uorimeters. , or by using elementary and molecular analysis techniques. Indeed, experience shows that portable spectro fl uorimetry must be integrated into a non-invasive analytical methodology in order to compare the results and enrich them with information on the organic materials or chemical families of organic compounds used. This is why LEDμSF is a complementary technique to FORS and to X and Raman fluorescence spectrometry.

The results obtained with the first version of the portable system presented here are consistent with those obtained with laboratory devices. Our collaboration with Freiberg Instruments made it possible to materialize the improvements that we had tested in the laboratory, in order to increase the possibilities, the fields of application and a better choice in the excitation wavelength. The device produced and now marketed by this German company offers several excitation sources and a white LED allowing both fluorescence and re fl uence to be measured.

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The LEDμSF project was selected by the CNRS to be exhibited at the Salon Innovatives SHS 2015, which gave rise to numerous press releases. A 3-minute film produced by the LaScArBx labex is available on the web [12].

12. http: //www.iramat-crp2a.cnrs.fr/spip/ and https: //www.youtube.com/watch? V = YdXTKyvNTxg.

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