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UV-Excited Fluorescence as a Basis for the In-Situ Identification of Natural Binders in Historical Painting: A Critical Study on Model Samples

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Abstract: The fluorescence emission by aged organic binders used in painting is a well-known phenomenon. Several literature studies were devoted to its investigation, both on pure binders and on their mixtures with some pigments. Nevertheless, a systematic study about the real possibility of exploiting such a phenomenon for the non-invasive identification of binders in ancient paintings is still lacking. In the present work, a prototype portable fluorimeter was used to analyze a significant number of model painting samples containing different binders (drying oils, egg yolk, milk, animal glue, and gum Arabic) mixed with various pigments having different hues. The model samples were naturally aged in a period ranging from fifteen to one year. The effects on the spectral pattern due to the different binders, the recipes used to prepare them, and the pigments mixed with them were examined. The fluorescence spectra were corrected for the absorption of the emitted radiation due to the pigments. Finally, the corrected spectra were treated by principal component analysis to determine if the possibility of distinguishing at least the most fluorescent and common binders, i.e., drying oils and egg, existed. It was shown that, even if the technique cannot be effectively applied in the case of mixed or superimposed binders, it allows to put forward at least a preliminary hypothesis when pure binders are used.

Keywords: spectrofluorimetry; paintings; organic binders; ageing; self-absorption correction; principal component analysis

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1. Introduction

Most of the investigations on materials in pictorial works are dedicated to identifying the substances responsible for color, i.e., pigments, as color is obviously one of the most impactful aspects of these works. However, the binders, i.e., the means by which the pigments are dispersed to be applied in the pictorial layers, are also of considerable importance not only from the point of view of the visual effect but also, and above all, from that of the conservation of the artifacts and of the knowledge of the artists' technique.

Binders are mainly organic substances, which until the beginning of the 20th century and the advent of synthetic resins were of natural origin [1]. They include materials of a lipid or protein nature or even waxes or carbohydrates. In particular, in paintings on panels, the use of egg tempera was predominant until the 14th century, while, starting from the 15th century, the use of drying oils in paintings on panels and then on canvas was progressively affirmed. This evolution took place through an intermediate phase in which both binders were used, sometimes also in a mixture or in different layers.

From the point of view of the analytical methods aimed at identifying binders, they are largely dominated by micro-invasive techniques, as the non-invasive ones commonly used for the recognition of pigments, such as X-ray fluorescence or visible reflectance spectroscopy, are not applicable. Indeed, X-ray fluorescence cannot be applied to most organic substances as it is not useful for molecular analysis and, as for elements, it allows

to identify just those having a medium-high atomic number [2]. At the same time, visible reflectance cannot be used for the identification of binders whose characteristic absorptions, being colorless, reside in the UV region. Therefore, chromatographic techniques prevail, in particular gas chromatography-mass spectrometry [3–7]. Micro Fourier-transform infrared (micro-FTIR) spectroscopy is instead applied to the study of binders in the different layers of a painting through the analysis of cross-sections of micro-samples from the painting itself [8]. Both methods, however, require the taking of a sample and are, in fact, micro-destructive.

The use of non-invasive spectroscopic techniques for the identification of binders has also been proposed, based in particular on the reflectance in the near IR (NIR) region [9,10]. NIR reflectance spectroscopy allows for the recognition of the use of a drying oil rather than an egg tempera or even mixed binders such as *tempera grassa* [10]. However, in the literature, the measurement of the characteristic fluorescence emission of some binders, especially if aged, has also been suggested as a non-invasive method for their investigation [11,12]. This emission has been associated in particular, at least experimentally, both with fluorophores already present in the fresh binder and, above all, with degradation products that are formed as a result of its aging [11]. In the case of drying oils, the ester linkages and unreacted double bonds that remain in the dried oil matrix are reactive sites for degradation phenomena. The latter are mainly based on oxidation reactions, which lead, among other things, to the yellowing of the dried oil, with condensation of fatty acids oxidized at more than one position to give quinone-type structures, or possibly condensation with nitrogen-containing compounds, such as, for example, atmospheric ammonia or proteins belonging to the oil itself [13]. It has been shown that there is a correlation between yellowing and fluorescence of these binders [14–16]. Indeed, quinone-like structures can act as fluorophores with emission maxima between 380 and 520 nm [17], and fluorescence properties have been suggested for conjugated imines [11]. For proteinaceous binders, amino acid oxidation and the Maillard reaction between amino acids and sugars have been proposed as sources of fluorescent products [10,18], although egg white and yolk have been observed to contain higher levels of easily oxidizable aminoacids than animal glue [19]. In the case of egg yolk, the lipid component also contributes to the observed fluorescence in a similar way to that described for drying oils [11].

These previous studies were, in most cases, aimed at pure binders, and the data reported on painting models are not always comparable to one another. For this reason, in the present work, spectrofluorimetry was systematically applied to a wide number of model painting layers, obtained with different pigments in multiple binders, prepared over a period of time between 15 and 1 year from now, and then naturally aged.

In particular, the spectroscopic behavior of the various binders and the effect of pigments on their emission spectra were considered, adopting appropriate procedures for the correction of these spectra as well as principal component analysis (PCA) of the data to compare them and thus obtain a better evaluation of the actual efficiency of the method.

A portable spectrometer was used to prospectively evaluate the effective possibility of using spectrofluorimetry for the in-situ recognition of binders in real paintings. The instrument adopted is very compact, being based on a rather simple optical setup (as described below) and using a small battery-powered UV LED as the source of the exciting radiation. Combined with a halogen source, it also has the advantage of allowing the acquisition of visible reflectance spectra, which are useful for correcting the emission spectra on the same measurement areas in which these spectra are recorded.

2. Materials and Methods

2.1. Materials

Siccative oils, animal glue, and gum Arabic were purchased from local paint shops in Milano (Italy). Lead white, azurite, yellow ochre, burnt Sienna, cadmium red, ultramarine blue, and madder lake were purchased from Zecchi (Firenze, Italy). Red lead and hematite were bought from Sigma Aldrich.

2.2. Reference Samples

The mock-up samples analyzed in the present work, simulating the layered structure of real paintings, were prepared from 1 to 15 years ago using a wood panel or a canvas as support. In general, the wood panel was covered with a ground layer of a white mineral material, namely gypsum (this is the case of the 1- and 15-year-old samples) or calcite (5- and 2-year-old samples) mixed with animal glue. On the other hand, a commercial canvas was already prepared with kaolinite and a synthetic binder (3- and 6-year-old samples). Then, a layer of color was applied by mixing the pure binder (linseed, poppy or walnut oil, whole egg or yolk, animal glue, and milk) with the ground colorants in a 1 to 1 ratio. The coloring substances were the inorganic pigments lead white, red lead, ultramarine blue, azurite, yellow ochre, hematite, burnt Sienna, and cadmium red. In addition, madder lake was also used. Referring to the coexistence of different binding media, *tempera grassa* was obtained by mixing yolk and a drying oil in the same proportion. Finally, to prepare multi-layer samples, a layer of oil was spread on a layer of tempera or vice versa.

The described procedures were mostly followed for the samples, but in some cases, more complex recipes were adopted. In particular, in the case of most of the binders based on whole egg or yolk, a small amount of vinegar, about one-tenth of the volume of egg, was also added to prevent fermentation, as reported in ancient treatises [20,21] (Table 1). In addition, the so-called Doerner's tempera was also prepared based on a mixture of equal volumes of whole egg and linseed oil and a double volume of water [21].

Table 1. List of the pigments and binders in each of the model painting samples examined. The age of the sample in years (y) is indicated in brackets next to the identification number.

Binder	Pigment	Number [a]
Egg yolk	Lead white	5 (1y), 11 (2y), 17 (6y) *, 21 (3y) *, 24 (15y) *
	Ultramarine blue	28 (15y) *, 33 (5y)
	Azurite	36 (2y)
	Cadmium red	43 (5y) *
	Madder lake	47 (3y)
	Burnt Sienna	53 (5y) *
	Red lead	54 (15y)
Whole egg	Lead white	1 (15y)
	Ultramarine blue	27 (15y)
Linseed oil	Lead white	8 (1y), 14 (2y), 16 (2y)
	Ultramarine blue	31 (5y)
	Azurite	39 (2y), 40 (2y)
	Cadmium red	41 (5y)
	Hematite	45 (1y)
	Burnt Sienna	51 (5y)
Walnut oil	Lead white	4 (1y), 15 (2y)
Tempera grassa (egg yolk + linseed oil)	Lead white	6 (1y), 12 (2y), 18 (6y), 20 (3y), 25 (15y)
	Ultramarine blue	29 (15y), 30 (15y), 32 (5y)
	Azurite	37 (2y)
	Cadmium red	42 (5y)
	Madder lake	48 (3y)
	Hematite	49 (1y)
	Burnt Sienna	52 (5y)

Table 1. *Cont.*

Binder	Pigment	Number [a]
Tempera grassa (egg yolk + walnut oil)	Lead white	7 (1y), 13 (2y)
	Azurite	38 (2y)
	Hematite	46 (1y)
Tempera grassa (egg yolk + poppy seed oil)	Lead white	19 (3y)
Doerner's tempera (whole egg + linseed oil)	Lead white	23 (15y)
	Ultramarine blue	26 (15y)
Tempera on linseed oil	Lead white	3 (1y)
	Hematite	44 (1y)
Tempera on walnut oil	Lead white	22 (1y)
Linseed oil on tempera	Lead white	2 (1y), 9 (2y)
	Azurite	34 (2y)
	Hematite	50 (1y)
Walnut oil on tempera	Lead white	10 (2y)
	Azurite	35 (2y)
Animal glue	Lead white	55 (15y)
Milk	Lead white	56 (15y)

[a] All samples containing egg yolk or whole egg as a binder were prepared by adding a small amount of vinegar as preservative, except those marked with an asterisk.

All of the samples were kept for most of the time in the dark and in ambient conditions of humidity and temperature (the first ranging from 40 to 60% and the second from 20 to 25 °C approximately).

Table 1 shows a list of the samples, indicating the binder and the pigment used, as well as their age.

2.3. Instrumentation

Spectrofluorimetric analyses were performed using a portable microprobe (Figure S1), suitable for both visible reflectance and fluorescence measurements. The microprobe, equipped with an Olympus 20× objective, is connected by optical fibers to a Lot Oriel MS125 spectrometer (grid 400 lines/mm) provided with an Andor CCD detector (1024 × 128 pixel) cooled by means of a Peltier device. The wavelength calibration was based on the emission spectrum of a neon lamp.

For fluorescence analyses, an LED source emitting at 365 nm with a power of 5.5 mW was used as the exciting source. The radiation from the source was sent to the measurement area with an incidence angle of 45°, and the emitted radiation was collected through the objective of the above-described microprobe. Fluorescence spectra were collected as a sum of 30 scans with an exposure time of 2 s, and the analyses were preceded by the acquisition of a background spectrum in the absence of the incident radiation.

For visible reflectance analyses, a beam splitter 30/70 in the spectral range of 400–700 nm was assembled into the probe. A halogen source (maximum power 150 W) was connected to the probe by optical fibers, and the radiation was sent in a direction perpendicular to the microscope objective. Reflectance spectra were acquired as a sum of 30 scans with an exposure time of 0.01 s, and the analyses were preceded by the acquisition of background and reference spectra. A metal target coated with barium sulphate was used as a reference.

2.4. Multivariate Analysis of Data

The emission spectra were processed by principal component analysis (PCA), performed by the statistical package MINITAB 14. The spectra were first normalized between

zero and one to avoid the possible variability due to the emission intensity and then cut between 420 and 680 nm. To perform PCA, the covariance matrix was selected to reduce the baseline contribution.

2.5. Kubelka–Munk Correction for Self-Absorption of Fluorescence Emission

To take into account fluorescence self-absorption and reemission in the solid-state, a model based on the Kubelka–Munk theory of diffuse reflectance was applied [22,23]. According to it, it is possible to obtain true emission spectra dividing the experimental data by a function $\gamma(\lambda, \lambda_0)$, defined by Equation (1):

$$\gamma(\lambda,\lambda_0) = \left(1/\left(1+\sqrt{Rem[R(\lambda)]/(Rem[R(\lambda)]+2)}\right)\right) x (1/(1+\sqrt{Rem[R(\lambda)]\{Rem[R(\lambda)]+2\}/Rem[R(\lambda_0)]\{Rem[R(\lambda_0)]+2\}})) \quad (1)$$

where λ and λ_0 are the emission and excitation wavelength and $Rem[R(\lambda)]$ is the total remission function defined by the following Equation (2):

$$Rem[R(\lambda)] = k(\lambda)/s(\lambda) = [1-R(\lambda)]^2/2R(\lambda) \quad (2)$$

where $s(\lambda)$ and $k(\lambda)$ are the scattering and the absorption coefficient, respectively, and $R(\lambda)$ is the diffuse reflectance at the corresponding wavelength λ.

The self-absorption correction was performed by means of the GRAMS/AI software.

3. Results and Discussion

In the following sections, the fluorescence emission of several model samples will be discussed, taking into account three different aspects: the effect of the binders, the effect of the recipe, and the effect of fluorescent/non-fluorescent colorants. The emission spectra of the pure binders were also acquired for comparison and are shown in Figure S2 (Supporting Information). In this regard, it should be noted that the yellowing due to the aging of films of pure binders can cause a shift of the fluorescence band by self-absorption, as highlighted in Figure S3 (Supporting Information), especially for egg yolk. For this reason, in addition to the greater resemblance to real paintings, it was decided to consider only the spectra of model pictorial layers in the following.

3.1. The Effect of the Binder

As widely discussed in the literature (see in particular [11]), different binders have fluorescence emissions with different characteristics, even as a result of aging. Figure 1 shows the emission spectra obtained from mock-up samples of lead white spread with linseed or walnut oil, egg yolk, milk, and animal glue and with different degrees of aging. Lead white was chosen as it has been historically the most important of all white pigments and the only white pigment used in European painting until the 19th century [24].

With regard to this pigment, it has been reported that it influences the emission of linseed oil, both making it evident after a short time from its drying (a process which is accelerated by the pigment) [25] and causing it to shift at wavelengths greater than that of the fresh binder [11]. At least the first aspect is undoubtedly evident in Figure 1a, which shows how the fluorescence intensity of linseed oil is particularly intense after only 2 years of aging in a pictorial layer containing lead white. In this regard, it should be considered that the mock-up samples analyzed have been mainly kept away from light, a condition that seems to favor the accumulation of the species responsible for the emission [14]. This effect is much less evident in the case of walnut oil, which also generally appears less fluorescent than linseed oil (Figure 1e). In fact, it is reported that the first oil yellows to a lesser extent than the second [13,26], and it can therefore be assumed that to the same degree, it gives rise to the formation of a smaller number of degradation products responsible for the emission of fluorescence.

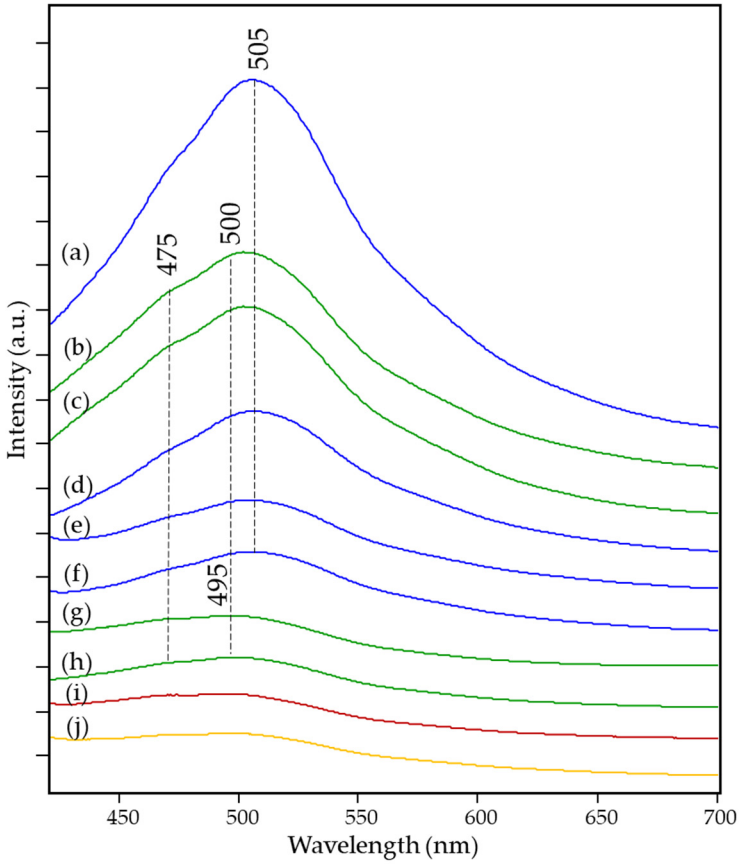

Figure 1. Emission spectra (λ_{exc} = 365 nm) of pictorial layers of lead white mixed with different binders: (**a**) linseed oil 2y, (**b**) egg yolk 15y, (**c**) egg yolk 3y, (**d**) linseed oil 1y, (**e**) walnut oil 2y, (**f**) walnut oil 1y, (**g**) egg yolk 2y, (**h**) egg yolk 1y, (**i**) milk 15y, (**j**) animal glue 15y. Color legend: blue = drying oil; green = egg yolk; red and yellow = other proteinaceous binders.

Egg yolk (Figure 1b,c,g,h) mixed with lead white also shows an increase in emission intensity with aging, although even over the longest time-lapse among those considered in this study (approximately 15 years), the intensity achieved is in any case lower than that found for linseed oil. A decidedly weaker emission was instead found for the pictorial layers spread with milk (Figure 1i) or animal glue (Figure 1j).

As a general rule, regarding the use of the emission intensity as a discriminating parameter, in any case, it must obviously be remembered that since the fluorescence intensity is proportional to the concentration of the species that are responsible for it [27], differences related to the proportion of binder present in each mock-up sample can also be detected, a situation which is, moreover, completely realistic when we consider old paintings rather than models prepared in the laboratory.

Concerning the wavelength of the emission maxima of the two binders that have the most appreciable fluorescence, the egg yolk maximum is at a wavelength of about 500 nm or a few nanometers below. Instead, for drying oils, the maximum is slightly above this value. Furthermore, again for egg yolk, a shoulder around 475 nm is more evident in comparison with linseed and walnut oils. However, as will be said in the next section, this trend can undergo variations, and, moreover, the differences discussed above appear negligible if compared with the typical width of the fluorescence bands.

3.2. The Effect of the Recipe

Another important aspect to take into account is that, in ancient paintings, the individual binders were often components of more complex mixtures, which could also include other ingredients intended to improve the properties of the binding medium. Therefore,

the effect that these substances might have on the behavior of the prevailing binder cannot be ignored. In the present work, this phenomenon is particularly evident for the tempera mock-up layers.

Figure 2 shows how the presence or absence of vinegar in the formulation used for egg tempera causes a different trend in the observed emission spectrum, even for the same pigment and regardless of the degree of aging. In particular, in the mock-up samples of lead white tempera prepared according to recipes involving the use of vinegar (Figure 2a,b,f), an emission maximum is observed at about 496 nm accompanied by an evident shoulder at about 473 nm, while in the absence of this component, the maximum shifts to about 500 nm and the shoulder becomes less pronounced (Figure 2c–e). Therefore, in the second case, it can be said that the overall pattern of the fluorescence band is more similar to that found for drying oils, in particular linseed oil.

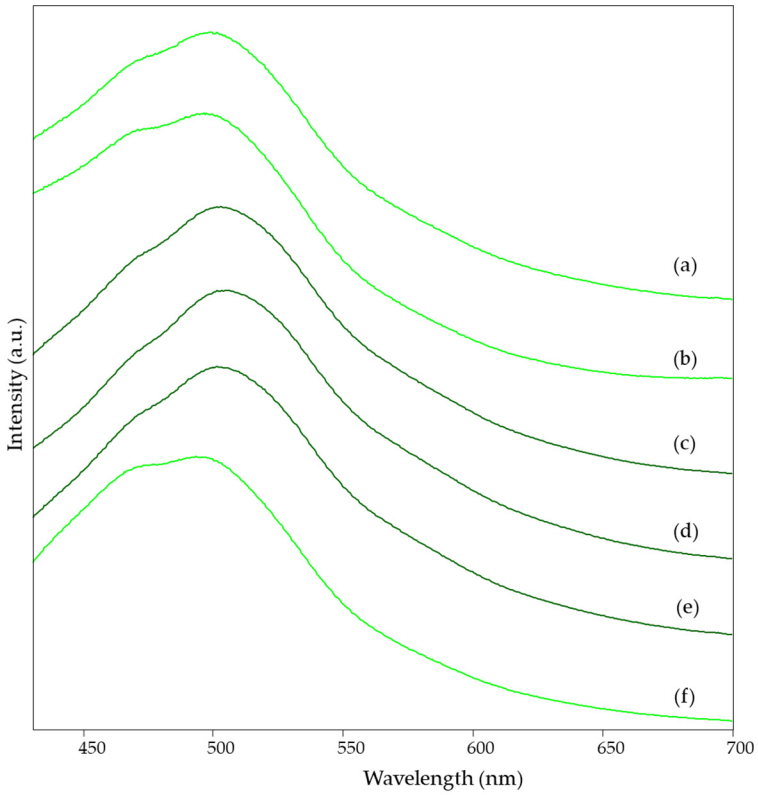

Figure 2. Emission spectra (λ_{exc} = 365 nm) of pictorial layers of lead white mixed with egg yolk or whole egg according to different recipes and with different degrees of aging: (**a**) egg tempera + vinegar 1y; (**b**) egg tempera + vinegar 2y; (**c**) yolk + egg white + water 2:1:1 3y; (**d**) egg tempera 6y; (**e**) yolk + water 15y; (**f**) whole egg tempera + vinegar 15y. Color legend: light green = egg tempera with added vinegar; dark green = egg tempera without vinegar.

Although the use of vinegar as a preservative has been documented for Renaissance painting [20], it could have negative effects on some pigments (lead white and ultramarine blue) and thus was not always added [21]. It is, therefore, a variable to be taken into consideration.

3.3. The Effect of the Pigment

3.3.1. The Self-Absorption Phenomenon

Figure 3 (left) well represents the effect associated with different pigments dispersed in a selected binder. In fact, being compounds capable of absorbing in the visible region, the coloring materials can also reabsorb part of the radiation emitted as fluorescence by the binding medium itself [11]. This self-absorption phenomenon causes an evident shift in the

emission maximum or even a variation in the spectral pattern depending on the color. This effect has already been described in the literature for painting layers that have different concentrations of the colorant. Regardless, so far, a correction procedure has been carried out only in a limited number of cases [22,23]. Hence, in this work, the Kubelka–Munk theory (see Section 2.4) was applied to correct emission spectra for self-absorption. The obtained results were satisfying from the point of view of the correspondence of spectral pattern and emission maximum for pictorial layers with different pigments, as shown in Figure 3, where the same spectra are displayed before (left box) and after (right box) this correction.

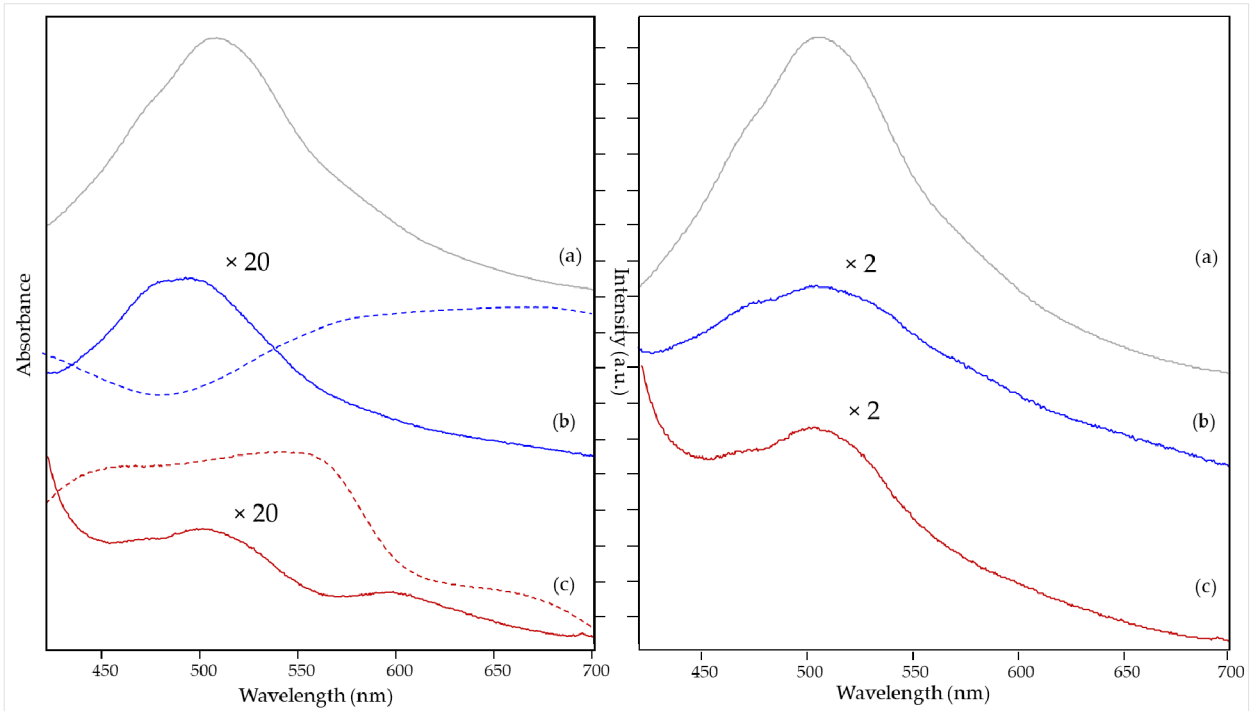

Figure 3. (**Left**): emission (λ_{exc} = 365 nm) (solid lines) and absorption (dashed lines) spectra of mock-up samples prepared with different pigments in linseed oil (1- or 2-years old). (**Right**): emission spectra (λ_{exc} = 365 nm) of the same pictorial layers after self-absorption correction. Spectra (**a**) refer to lead white (grey lines), (**b**) to azurite (blue lines) and (**c**) to hematite (red lines).

3.3.2. The Effect of Fluorescent Coloring Substances

Some pigments can themselves be fluorescent, so the corresponding emissions are added to those of the binder, introducing a further element of complexity, as already reported [11]. This is exemplified in Figure 4 for madder lake and red lead (Pb_3O_4). Emissions around 600 nm, typical of the lake derived from madder, one of the most widespread natural dyes in ancient times, are well documented in the literature [28,29]. On the other hand, an intense fluorescence emission has also been documented for the red-orange pigment minium, particularly when spread with egg yolk as a binder [30]. A weak emission of around 600 nm has been reported for the more recent pigment cadmium red, a cadmium selenide [31]. The fluorescence spectra of the pure pigments are reported in Figure S4 and show their emission maxima at wavelengths higher than the corresponding painting layers due to the effect of self-absorption, which is obviously more relevant for the pure pigments themselves.

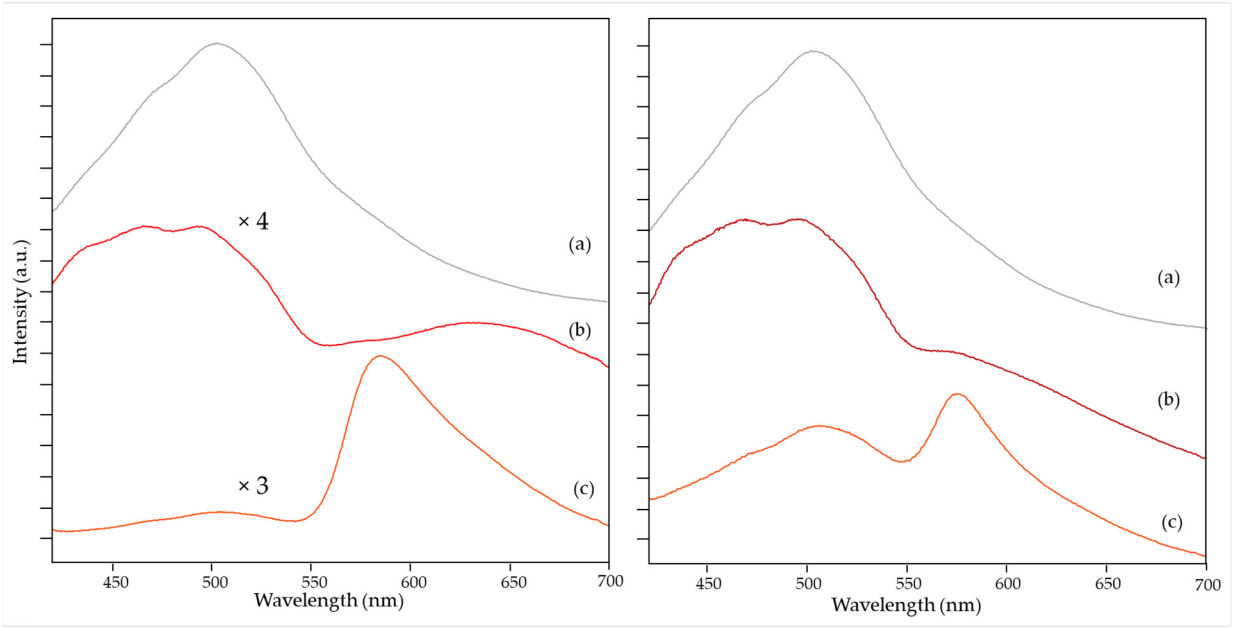

Figure 4. Emission spectra (λ_{exc} = 365 nm) of: (**a**) a pictorial layer of lead white (grey lines) compared with those of the two fluorescent colorants (**b**) madder lake (red lines) and (**c**) red lead (orange lines), spread with egg yolk or whole egg (15- or 3-years old respectively). (**Left**) before and (**right**) after the self-absorption correction.

As can be seen for madder lake and red lead (Figure 4, right box), the correction of the fluorescence spectra of the model samples for self-absorption also allows, in this case, to obtain an undistorted emission band for the binder, but obviously it does not eliminate the contribution of the fluorescence emission due to the coloring substances.

3.4. Principal Component Analysis Applied to Emission Spectra

As demonstrated in the previous section, the correction of the spectra for the absorption due to the pigments allows us to obtain comparable trends for the same binder. However, it must be considered that the low specificity of the electronic spectra due to their broad bands and the proximity of the emission maxima observed for different binders (already discussed in Section 3.1 and shown in Figures S5–S12 and Table S1, Supporting Information) can make fluorescence data difficult to interpret for identification purposes.

In order to investigate this aspect, PCA was applied to the emission spectra. In particular, the aim was to evaluate the possibility of distinguishing the two most used binding media in ancient paintings on wooden panels and on canvas, namely drying oil and egg tempera. Figure 5 shows that, although it is possible to identify approximately two groupings corresponding to model samples prepared with each of the two binders, there is considerable overlap between them. At least in the case of the tempera models, a possible reason for the dispersion of the corresponding points in the score plot seems to be, as already discussed in Section 3.2, the presence or absence of vinegar used as a preservative in the formulation of the binder. This component, in fact, was not added to those samples that are located in the left part of the egg group. Since the fluorescence emission of these materials is linked to alteration products of their components, it is plausible that the addition of a preservative influences the formation of these products or a part of them, thus modifying the emission spectrum. On the other hand, no significant age dependence is observed in the score plot, as expected because the spectra were normalized prior to PCA to take into account only their pattern and not the intensity of the emission bands. Finally, the spectra of the pictorial models that included fluorescent pigments such as madder lake or cadmium red obviously do not group with those of the corresponding binder.

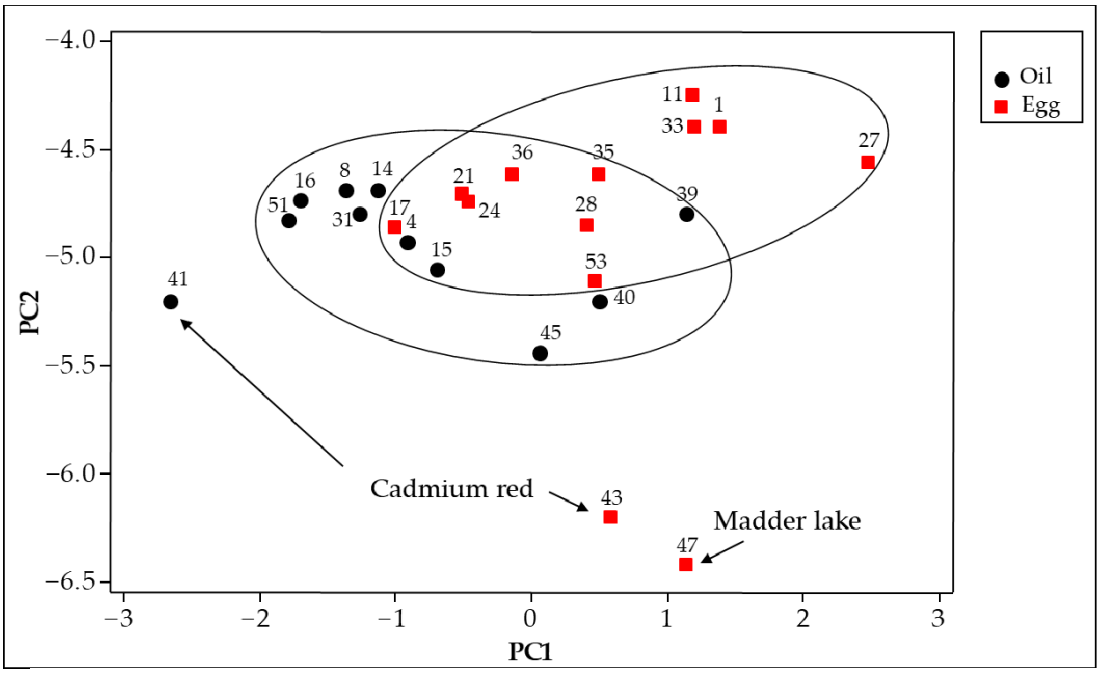

Figure 5. Score plot of the first two principal components of the emission spectra (λ_{exc} = 365 nm) of pictorial layers with egg tempera and oil as binders, mixed with different pigments (explained variance 89.3%). For the numbering of the samples, see Table 1.

PCA was then applied to a greater number of model samples, also including *tempera grassa*, in which tempera and oil coexist, and the superimposed layers, each containing a different binder. The score plot obtained (Figure 6) shows that it is not possible to distinguish these "mixed" cases from those in which a single binder was used. In fact, the numerous examples of *tempera grassa* are scattered throughout the two large groups of drying oil and egg tempera. Even for models in which layers have been superimposed with each of the two binders, a reliable prediction cannot be made regarding the binder of the top layer. Only in the case of an oil layer on egg tempera can it be observed that those models whose spectra have a better correspondence with egg tempera rather than oil presented a thinner top oil layer compared to those that group best with drying oils. In particular, on the basis of the observation of cross-sections under an optical microscope (as described in ref. [9]), for samples 9 and 10, an overall thickness of the two color layers of 40 µm was estimated, compared, for example, to sample 2 for which the two layers had a total thickness of about 100 µm. The spectrum of model 34, composed of two layers of azurite, of which the upper part with linseed oil and the lower part with egg tempera, does not group with similar samples, probably due to an overestimation of the correction for the absorption due to the pigment.

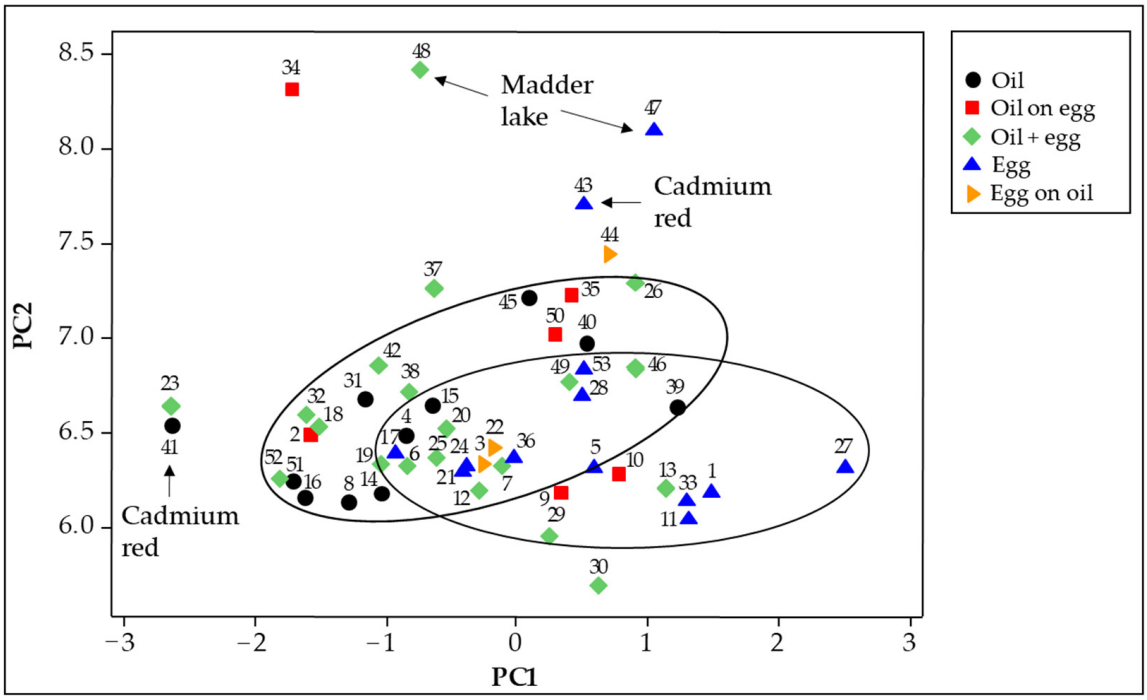

Figure 6. Score plot of the first two principal components of the emission spectra (λ_{exc} = 365 nm) of pictorial layers with egg tempera and oil as binders, pure or in mixture and super-imposed layers (explained variance 90.2%). The models containing just a drying oil or egg are highlighted by the two ellipses. For the numbering of the samples, see Table 1.

4. Conclusions

As already known, the present paper highlights that the binders of natural origin used in painting for many centuries can have significant fluorescence emission. This is especially true of drying oils and egg yolk (or whole egg). Furthermore, it was also confirmed that the intensity of this emission increases as the binder ages, especially for linseed oil. However, with the same binder considered, various factors can influence the trend of the emission spectrum, which is, moreover, quite similar for the two types of binders examined. Among these factors are:

- The presence of other components in the preparation of a specific binder, as exemplified in particular by the differences between the spectra obtained for tempera prepared with or without the addition of vinegar as a preservative;
- The partial reabsorption of the radiation emitted by the pigments mixed with the binder, which can cause a shift in the emission maximum or make it difficult to observe the fluorescence band; this effect can be at least partially corrected with an appropriate mathematical procedure;
- The overlapping of the emission due to the binder and that of the pigment/lake dispersed in it.

The processing of the spectra by PCA showed that two groups could be identified for binders based on drying oil and egg tempera, respectively. However, these groups are quite large and partly overlapping. Consequently, in the case of mixed binders, the distribution of the spectroscopic data does not allow for the formulation of hypotheses and the recognition of the use of a mixture with respect to that of a single substance. It can therefore be concluded, at least with reference to model samples, that the method based on spectrofluorimetry can allow a first hypothesis on the nature of the binders used in a painting but must necessarily be accompanied by more specific analytical techniques, even non-invasive, such as vibrational spectroscopies. As previously mentioned, NIR reflectance spectroscopy allows an in-situ analysis [9,10], with the possibility of distinguishing mixed

binders or overlapping layers containing different binders, or even obtaining information on the binder when a surface layer of varnish is present on the painting, as demonstrated in references [10,32], on a part of the model samples examined in the present work and on ancient paintings. The greater specificity of the vibrational spectra is due to the fact that they show a greater number of narrower signals for a given substance in comparison with electronic spectra, both of absorption and emission, that have just a few broad bands. However, the spectrofluorimetric measurements are, in principle, faster and, therefore, could be used for a broader screening of the artwork to select those areas where the most specific NIR investigation should be performed. To deepen this conclusion, however, an extension of the present study from models to real paintings will be necessary.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors10070256/s1, Figure S1: Schematic diagram of the portable spectrometer used for UV-excited fluorescence and visible reflectance measurements; Figure S2: Emission spectra of pure binders; Figure S3: Comparison between the emission and the reflectance spectra of pure egg yolk and an egg tempera layer with lead white; Figure S4: Emission spectra of fluorescent pigments; Figure S5: Normalized and corrected emission spectra of model samples of tempera painting with egg yolk; Figure S6: Normalized and corrected emission spectra of model samples of tempera painting with whole egg; Figure S7: Normalized and corrected emission spectra of model painting samples with linseed oil as binder; Figure S8: Normalized emission spectra of model painting samples of lead white with walnut oil as binder; Figure S9: Normalized and corrected emission spectra of model samples of *tempera grassa* painting with linseed oil; Figure S10: Normalized and corrected emission spectra of model samples of *tempera grassa* painting with walnut and poppy seed oil and of Doerner's tempera painting; Figure S11: Normalized and corrected emission spectra of model samples of overlapped paint layers each containing egg or oil binder; Figure S12: Normalized emission spectra of model painting samples of lead white with animal glue or milk as binder. Table S1: Wavelengths of the emission maxima of the examined model pictorial layers (λ_{exc} 365 nm).

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