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AN INTERDISCIPLINARY APPROACH TO THE STUDY OF COLOUR IN PORTUGUESE MANUSCRIPT ILLUMINATIONS

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Resumo

Nesta tese de doutoramento são descobertos os materiais e técnicas que os iluminadores medievais utilizaram na produção de iluminuras portuguesas surpreendentes, datadas dos séculos XII e XIII. Novas técnicas e aplicações foram também testadas para melhor caracterizar a composição das tintas.

Na primeira parte desta tese, foi desenvolvida uma metodologia para estudar e caracterizar a paleta da iluminura medieval do *scriptorium* do Lorvão, para tal foram seleccionados nove manuscritos iluminados do *scriptorium* do Lorvão, por serem representativos e por poderem sujeitar-se a um estudo analítico. Foram realizados estudos mais detalhados em dois manuscritos datados, muito importantes: o *Apocalipse* e o *Livro das Aves*. Por comparação com outras cópias portuguesas ou europeias concluiu-se que o *Apocalipse* do Lorvão é bastante diferente dos outros comentários de Beatus, seus contemporâneos, tanto pela excepcional escolha de cores utilizadas – laranja, vermelho e amarelo – como pelas escolhas iconográficas; no *Livro das Aves*, descobriram-se muitas semelhanças com a cópia produzida no mosteiro de Santa Cruz. Foi criada uma base de dados de tintas baseadas nos tratados e receituários medievais e posteriormente caracterizada por HPLC-DAD, UV-VIS, µ-FTIR, µ-EDXRF, µ-Raman e µ-espectrofluorimetria. O que permitiu uma caracterização mais eficaz dos componentes das tintas, através de técnicas não invasivas: ligantes, corantes e pigmentos orgânicos e inorgânicos. Nestes últimos, foi possível verificar que tanto eram aplicados por si só, como em mistura de dois ou três pigmentos, como no caso da tinta laranja do *Apocalipse* ou da tinta azul do *Livro das Aves*. Quando as misturas foram detectadas, utilizou-se a análise com EDXRF para quantificar os componentes inorgânicos das tintas. Foi também identificado, com a técnica de µ-Raman e XRD, a galena como produto de degradação do mínio, presente na maioria da tinta laranja do *Apocalipse*.

A segunda parte desta tese resulta do estudo sobre corantes orgânicos vermelhos, que poderão ser encontrados na iluminura de manuscritos medievais: garança, goma-laca e cochinilha. Os seus cromóforos principais: alizarina, purpurina, ácido lacaico A e ácido carmínico, foram caracterizados fotofísica e fotoquimicamente. Foram realizados estudos de fotodegradação em meio homogéneo (alizarina, purpurina e ácido carmínico) e em meio heterogéneo. Com base neste estudo, a µ-espectrofluorimetria foi utilizada como uma técnica inovadora para identificar corantes vermelhos em reconstruções, no *Livro das Aves*, nos têxteis Andinos milenares (uma colaboração com Museum of Fine Arts de Boston) e nas estratigrafias de pinturas de impressionistas, como Vincent van Gogh ou Pissarro (uma colaboração com o projecto internacional *Red lakes*). O estudo mostrou que é possível caracterizar lacas vermelhas e tintas de alizarina, purpurina e eosina (emissores fraco, médio e forte) e que a µ-espectrofluorimetria também pode ser usada como um método

semi-quantitativo em lacas de garança. Permitindo determinar a razão de laca de purpurina na mistura de lacas de purpurina e alizarina.

Abstract

This dissertation explores the materials used and practice undertaken by medievalist illuminators to produce some remarkable Portuguese illuminations from the 12th and 13th centuries. New techniques and applications were also explored to better characterize the paints' composition.

In the first part of this dissertation a methodology to study the illuminations of nine Lorvão *scriptorium* manuscripts, selected as representative and for being suitable for an analytical study, was developed and used to characterize the Lorvão's *scriptorium* medieval palette. Further studies were performed in two very important dated manuscripts: *Apocalypse* and *Book of Birds*. Comparing them with other Portuguese or European copies it was concluded that the *Lorvão Apocalypse* differs from other contemporaneous Beatus *Commentaries*, either on the uniqueness of the colours used –orange, red and yellow- or on iconographic choices; and the *Book of Birds* was found to have many similarities with the copy made on Santa Cruz monastery. Based on medieval written sources, a database of paint reconstructions was built and characterized by HPLC-DAD, UV-VIS, µ-FTIR, µ-EDXRF, µ-Raman and µ-spectrofluorimetry. It allowed an easier description of the manuscripts' paints components by non-invasive techniques: binders, organic and inorganic pigments. The last ones were found singly applied or in a mixture of two or three pigments, such as the orange paint of the *Apocalypse* or the blue paint of the *Book of Birds*. When mixtures were found, EDXRF analysis was used to quantify the inorganic paint components. The red lead degradation product in most of orange paint applied on *Lorvão Apocalypse*'s folia was recognized as galena, using µ-Raman and XRD techniques.

The second part of this dissertation outcomes from the study of red organic colorants that could be expected in medieval illumination manuscripts, based on madder, lac dye and cochineal. Their main chromophores: alizarin, purpurin, acid laccaic A and carminic acid, were photochemically and photophysically characterized. Photodegradation studies were performed in homogeneous (alizarin, purpurin and carminic acid) and heterogeneous media. Based on this study, the use of microspectrofluorimetry as a new technique to identify red colorants was applied on red lake paint reconstructions, on the *Book of Birds*, on millenary Andean textiles (a collaboration with Museum of Fine Arts, Boston) and applied on impressionists' (Vincent van Gogh or Pissarro) paintings' cross-sections (a collaboration with *Red Lakes* international project). It revealed that it is possible to characterize red lake pigments and paints based on alizarin, purpurin and eosin (weak, medium and strong emitters) and that can also be used as a semi-quantitative method for madder lake pigments, enabling the determination of purpurin lake ratio in a mixture of purpurin and alizarin.

Symbols and Notations

ε	Molar absorption coefficient
Φ_f	Quantum yield of fluorescence
Φ_R	Quantum yield of reaction
λ_abs	Absorption wavelength
λ_fluo	Fluorescence wavelength
λ_max	Maximum wavelength
λ_em	Emission wavelength
λ_ex	Excitation wavelength
µ-EDXRF	Micro- energy dispersive X-ray fluorescence spectroscopy
µ -FTIR	Micro- Fourier transform infrared spectroscopy
µ -Raman	Micro- Raman spectroscopy
µ -SPEX	Micro-spectrofluorimetry
µ -XRD	Micro- X-ray diffraction spectroscopy
τ_f	Fluorescence lifetime
A	Absorbance
A	Neutral form species
A^-	Mono-anion form species
A^{2-}	Di-anion form species
A^{3-}	Tri-anion form species
A^{4-}	Tetra-anion form species
ALC	Alcobaça collection
ATR	Attenuated Total Reflectance
DMSO	Dimethyl sulfoxide
DGARQ-ANTT	Diracção Geral de Arquivos- Arquivo Nacional Torre do Tombo
ESPT	Excited state proton transfer
ESIPT	Excited state intra - and intermolecular proton transfer
f.	Folio (manuscript page)
ff.	More than one folio (folia)
H_2O	Purified water with millipore
HPLC-DAD	High performance liquid chromatography – diode array detector
$h\nu$	Light
I_0	Intensity of the incident light
I_abs	Total absorbed light
Kα, Kβ, Lα, Lβ, Mα,	Spectral peaks due to different energy x-rays

K_{a1}	Equilibrium constant of (A) species
K_{a2}	Equilibrium constant of (A^{-}) species
K_{a3}	Equilibrium constant of (A^{2-}) species
K_{a4}	Equilibrium constant of (A^{3-}) species
K_f	Rate constant for fluorescence (radiative) deactivation
K_{nr}	Rate constant for nonradiative deactivation
k_{ic}	Rate constant for internal conversion deactivation
k_{isc}	Rate constant for intersystem crossing deactivation
k_f	Rate constant for fluorescence deactivation
k_p	Rate constant for phosphorescence deactivation
LUMO	Lowest-energy unoccupied molecular orbital
MeOH	Methanol
MFA	Museum of Fine Arts (Boston)
Ms	Manuscript
NMR	Nuclear Magnetic resonance
$\mathrm{p}K\mathrm{a}$	Acid-base equilibrium constant
PMMA	Poly(methyl methacrylate)
PIXE	Particle induced X-ray emission spectroscopy
PVA	Polyvinyl acetate
S_0	Singlet state
S_1	First excited singlet state
SEM-EDX	Scanning electron microscopy and energy-dispersive X-ray spectrometry
sp.	A single species
spp.	More than one species
T_1	First triplet state
t_r	Retention time
UV	Ultraviolet light
UV-VIS	Ultraviolet-visible spectroscopy
v	Verso (of the folio)
v/v	Volume ratio
$\mathrm{V}_{\mathrm{sol}}$	Solution volume
wt	Weight

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A. Colours on Portuguese Medieval manuscripts illuminations

> "Entering into the seductive world of illuminations is an act full of fascinations and challenges. Coming into this world is also returning to a time when the word, writing, reading and its fruition were very different than today and even more than they will be tomorrow (…). We can understand that illumination was much more than a technique and nothing less than an art form."
>
> Carlos Reis, in *A iluminura em Portugal*

1. Introduction

1.1. Preamble

Illuminated manuscripts are a crucial piece of evidence to better understand the medieval art landscape. Both art and science are consistently represented by this sort of craftsmanship. Being one of the most important colour legacies in the Portuguese medieval art, manuscripts illuminations of this period are an issue that demands further studies besides the global knowledge about production, genealogy and circulation of manuscripts, developed in the last century [1-4], such as the technological art research of the materials used to paint the illuminations.

Being attentive to the uniqueness and the importance of two dated manuscripts, *Apocalypse* (1189) and *Book of Birds* (1183/84), from the Lorvão collection, in a context of Romanesque illumination, and despite the various studies carried out by art historians [3-11], there were enduring doubts regarding their genealogy, the existence of a *scriptorium* so skilled as to produce them, the composition and quality of the palette and even the illuminators aesthetics options. All these facts made clear the need for a more profound study, a study on pictorial material and techniques applied, which created an interdisciplinary pioneering work, involving Art History and Conservation Sciences, in the project *An interdisciplinary approach to the study of colour in Portuguese manuscript illuminations*.

Based on a chronological period, second half of 12th century and first quarter of 13th century, seven other manuscripts representative of Romanesque illumination, of considerable artistic value and a wide-ranging colour palette were selected, to enrich the colour study of this *scriptorium*.

Although the written sources can provide essential and wide evidence of medieval materials and techniques, the remaining doubts associated with geographic or temporal aspects that could emerge from a material study on the illumination manuscripts, can only be addressed with the analysis of their own materials. The multiplicity of analytical techniques used in this work enabled a larger set of data to be collected, allowing a full characterization of materials used by illuminators, crosschecking and understanding some conservation problems of the paints.

> “Andalus…might be here or there, or anywhere…a meeting
> place of strangers in the project of building human culture…
> It is not only that there was a Jewish-Muslim coexistence,
> but that the fates of the two peoples were similar…
> Al-Andalus for me is the realization of
> the dream of the poem.”
> Mahmoud Darwish

1.2. Illumination

Illumination, a word derived from the Latin *illuminare*, which means to enlighten or illuminate [12], was first mentioned in the Lucca manuscript or *Compositiones variae* as *alluminatura* or *illuminatura* [13], a manuscript dated between the 8th and 9th centuries [14-16]. Medieval authors used this expression to name any illustration or ornamentation in manuscripts, where the splendour of the colours or the application of metal (gold or silver) leaves enable some parts of the text to be highlighted. This term can also derive from the word *alluminare*, which according to Franco Brunello has a technical nature. The word could mean highlight, as referred before, or paint with lakes (*lacche alluminate* or *illuminati*), produced by precipitating the extracted organic dyes with the alum (in medieval treatises: *lume*, but most probably should be the verb *alumen*, as *Al-lu-ha-rum* was used in ancient texts from Mesopotamia to refer to alum substances) because they are often mentioned in medieval written sources [13].

Illuminations are also named “miniatures”, a name from the Latin verb *miniare*, which means “to colour with red”, as the medieval red was frequently named *minium* [17,18].

Some manuscripts just have red colour on the titles, chapter headings, or colophon, called *rubricae* because, traditionally, they were written in red ink [19]. But many initials are decorated according to their level of significance, they are used to indicate the importance of a section of the text or to highlight and differentiate textual divisions [12,17]. Initials can be painted in more than one colour, providing beautiful colour combinations or they can be decorated with different types of motives, such as anthropomorphic, zoomorphic, the combination of these two (Figure 1.1) or historiated [20-22] (Figure 1.3).

Figure 1.1. Examples of initials in Portuguese manuscripts with two different motives: zoomorphic in the *Book of Birds* (*f.* 4) and zoo-anthropomorphic in *Lorvão 16* (*f.* 71v).

During the 12th century in Western Europe men started to appreciate the world in a different way, by its colour and “this was a germinal time, pregnant with a thousand possibilities” [23]. According to Umberto Eco “the pleasure in colour and light is given from a spontaneous reaction, which only then is articulated with the scientific interest and organized in metaphysical speculations” [24]. For this author, the beauty of colour is simple and immediately achieved. The selection of the colours used in illumination depends on the illuminator’s penchant and on the rigorous knowledge of the text that needs to be articulated with the “iconic element” [25], which means that the symbolic meaning is not only defined by a single colour or even a range of colours, but also depends on the context.

Besides the initials, the illuminations could be painted over the whole folio (although, until the 12th century, the entire folio illuminations are usually only present in the Commentaries on Apocalypse) or just on part of it [17], but always related to the text. They occasionally have a narrative feature, meaning that the story could be told through the images.

Although there was an original manuscript to be copied, through the Middle Ages scribes and illuminators revealed enormous creativity in inserting the images at the folia and in the codex context. In the margins of some manuscripts instructions indicating the letter and the colour to use [21, 26], and also some small drawings (*marginalia*[1]), like in the *Lorvão Apocalypse* (Figure 1.2.) are still visible.

Figure 1.2. *Marginalia* in *Lorvão Apocalypse* manuscript (*f.* 115 – first two, and *f.* 65).

In the 12th century, the manuscripts were produced in monasteries, at the *scriptoria*. According to Aires de Nascimento this word only got the meaning of the place where the manuscripts were made in the 10th century; before that time it could be associated to writing instruments or just a place where "reading and meditation could be made in silence" [25]. The structure was very well defined: the Abbot should choose the books to copy and the *armarius* (who commanded the *scriptorium* activities), who should select the people and the materials to work with.

"The writer's job is to revive the reader. The former becomes
physically debilitated and the latter is mentally strengthened.
Therefore, strive to remember the hard-working laborer,
whoever you are that benefits from this toil..."
Beatus de Silos (colophon), 12th century

1.3. How medieval monks made their own colours

Just as illuminations give light significance and brightness to the codices, they also offer the possibility of learning and serve as intermediaries in the attempt to reach the sacred: "what the spirit of the humble hardly could reach with the eyes of knowledge, at least it can understand with those of the body; and sight will comprehend what the ear would learn with difficulty" [11].

Recognizing the skills of the medieval monks regarding the colours that we can see nowadays, perfectly applied on manuscripts, leads to a marvellous investigation into the medieval *scriptorium*. That is only possible by a deep look at the manuscripts themselves, by comparing one with the others; by searching in written sources: treatises, which contain not only recipes but also practical technical instructions, or recipe books. In the

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[1] *Marginalia* is a Latin word that means "things in the margin".

absence of these sources, by searching in texts describing artefact production or related uses of colouring materials (dying or painting) and by examining all the contemporary texts available “regardless of their subject matter, and searching for incidental clues” [27].

Figure 1.3. *Sta Cruz 20* (*f.* 139).

With all this knowledge it is possible to go further in order to realize the sense or nonsense of the medieval technical instructions, since in many aspects, the information described is ambiguous, incomplete, obscure or contradictory. "Fortunately, there is an alternative record of medieval technology: the medieval objects themselves. More can be learned from a detailed laboratory examination of medieval illuminations than could have been told by the makers themselves." [15]. Therefore "it has become common to proceed by reviewing contemporary written technical sources, or "recipe books", followed by preparing reconstructed reference samples based on these sources, and then comparing analyses of these standards with analyses of artefacts." [28]. These reconstructions would also enable to better understand the complexity of the materials and of their aging process, to test the limits and potentialities of the analytical methodologies that are currently been used, as well as to test and/or develop new ones and finally to establish or improve the characterization *modus operandi*.

1.3.1. Written sources

Citing Voltaire: “History is a bag of tricks which the dead have played upon historians”, Lynn White Jr. comments that “the most remarkable of these illusions is the belief that the surviving written records provide us with a reasonably accurate facsimile of past human activity” [29]. This is why research in written sources must be developed wisely, rigorously and critically.

The written sources, contemporary to the manuscripts, are important documents concerning the technique and materials used, and at present some of these treatises and recipe books are accessible in excellent editions and translations [13, 15, 30-43]. Although there are just a few from the 12th and 13th centuries, posterior sources should reflect what happened in these centuries or even before them.

From the group of written sources analysed, *De Clarea*[2] [35], dated from the 12th century, is the only one well-known truly contemporary to the illuminated manuscripts

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[2] *De Clarea* is also referred to as: Berne Burgerbibliothek, ms. A 91.17.

under study. At the same time, it is a manuscript only dedicated to illumination techniques [14, 16, 37], referring the white egg as the binding medium to use.

Medieval written sources dated before the 12th century are extremely rare, an example of that being the *Compositiones variae*, also named the Lucca manuscript (dated from the between 8th and 9th centuries), with its origin in older manuscripts such as the *Naturalis Historia* (Pliny), *De Architectura* (Vitruvius) or *De materia medica* (Dioscorides), and including text fragments of Leyden and Stockolm papyrii [42]. Another text is the *Mappae Clavicula*, dated from the 9th or 10th centuries, but with additions from the 11th-12th centuries, found in many manuscripts including the Lucca *Compositiones variae*, Phillipps Ms. 3715, Sélestat Ms 17 and Bibliothèque Nationale de Paris Ms. 6514 [14-16]. All the manuscripts have recipes to produce colours that can be applied in illuminations. Finally, the *Schedula diversarum artium* or *De diversis artibus*, has some references to illumination techniques in the first book, even though the main subject of this treatise is metal craft [32].

The remaining analysed written sources are all dated later than the Lorvão manuscripts studied, mainly from the 14th century, and they usually refer the preparation and application of pigments not only in illumination, but also in painting technique. Examples of that are: *De coloribus naturalia exscripta et collecta*, also known as ms. Amplonius Quarto 189 [38, 43], *Liber de coloribus iluminatorum sive pictorum* [31], and the Montpellier manuscript (Ms. 277) has its first book, of four, dedicated to illuminations, explaining colour preparation, the leaf and the metal inks [14, 38]. In *Il libro dell' arte* some pages are dedicated to illumination instructions but, more than illuminations and painting techniques, this treatise embraces all techniques where colour is present, which made it a reference for several years.

The later 13th century written sources, completely dedicated to the illumination technique are *De arte illuminandi* [13], dated from the 14th century, and the *Göttingen Model Book* [37], dated from the 15th century. The former teaches in significant detail how to prepare paints and binders[3]; the latter one goes further using models to explain and illustrate the different steps to follow in the illumination technique, from the initial drawing to the final shadows and highlights, saying that anyone who follows those steps can do graceful illuminations.

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[3] Paint is constituted essentially by a colorant, such as a pigment, a dye or a lake, and a binder. The binder is responsible for the formation of the pictorial layer as well as for its adhesion to the support. Additives may be added to improve the performance of the paint, for instance, fillers (or extenders) may be used to enhance the mechanic properties as well as the film opacity.

The manuscript *O livro de como se fazem as cores* was believed to have been written by a Portuguese Jew, after the 13th century [16, 33]. A recent publication demonstrated that it should be dated from the 15th century [44]. This manuscript is a Portuguese text written in Hebrew characters, and was first published in 1928 by Blondheim [33]. It is an important record, produced by a practitioner, written in a synthetic and clear language, with careful and minute descriptions of the most important steps of the described procedures. In this treatise one can learn how to make one's own paints as well as how to apply them on parchment, and how to produce the final colour effects. In the workshop colorants had to be ground, mixed with the binding medium and other additives, such as plasticizers and fillers; even if the leading role was ascribed to the colorants, the other constituents, binding medium and additives, also played a crucial role in the final visual effect. The way colour was constructed, whether by layers or mixture, was also determinative.

Reading all these written sources carefully one can verify that the range of materials cited is massive and includes minerals, earths and metals, animals (parts like skin, bones, tendons, teeth, blood, or products such as urine or dung), plants (parts as roots, stems, leaves, berries, flowers, resins, or products like wine and vinegar). The cited names are not always familiar to us and some remain mysterious until the recipes are tried (by reasonable guessing) and tested. There are a lot of identical recipes, denoting the copies made on earlier manuscripts.

Probably from lack of knowledge, due to calligraphic problems or even on purpose (with a view to keeping some secrets or because some things were so obvious to the monks that it would be unnecessary to write them down), some of the recipes do not make sense at all. An instance of that is the vast number of recipes for the production of vermillion[4] mixing mercury and sulphur, some better than others that were tried in the laboratory and did not work out.

This proves that further studies are needed to better understand medieval paint composition, including the comparison with other medieval manuscripts.

1.3.2. Uncovering the colour materials

Colour production in medieval illuminations was a complex process, and the study of the materials and technology underlying it can be tested as a tool to probe the

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[4] The vermillion synthesis using the dry process, which according to Gettens was the first method used to produce this red pigment [45].

provenances of the manuscripts. In this area, as reported by Mark Clarke, "Considering the large amount written about medieval manuscript decoration there have been surprisingly few analyses published. The study of the materials used for making manuscripts is, compared to the study of paintings on other supports, in its infancy." [28]. Even if some pioneering complete studies have been accomplished [46-52], most of the results published on medieval illumination deal with pigment analysis [53-61], and the main conclusions point to a common palette for the main inorganic pigments. Therefore, their use as markers could be limited. More complex information will be related with the way colour was constructed, as well as by the binder formulation.

1.3.2.1. Analytical techniques

The most proper techniques to characterize colorants in medieval manuscripts have been reviewed, and their strengths and weaknesses assessed [28]. In the same work, a compilation of the published analyses results is presented, "showing those pigments that have been positively identified by reliable techniques, for medieval European manuscripts" [28]. Concerning colorant identification, powerful techniques are the µ-Raman, µ-FTIR, µ-XRD (molecular techniques) and µ-EDXRF (elemental technique). These techniques can be used *in situ* or with previous collected micro-samples, the decision being based on the accessibility of samples, the type of equipment available and the quality or quantity of information that can be gained [48, 62].

In situ techniques, using soft radiation sources, have the advantage of enabling the acquisition of a higher amount of data, allowing statistical analysis to be carried out [63]. On the other hand, micro-sampling performed under the microscope, if well planned, can give extensive and more detailed information. Also, micro-sampling does not require removal of the manuscripts from their location[5] and involves much less handling than *in situ* methods [48]. Micro-sampling should indeed be used when the analytical technique involves high-energy radiation sources, to ensure that only the samples, and not the entire manuscript, are subjected to irradiation. These sources can produce a very high amount of radicals in organic as well as inorganic compounds that will sooner or later start to disintegrate the paint layer, or even the support.

µ-Raman is very efficient in characterizing inorganic pigments since each compound has its own fingerprint [58]. It has proved to be invaluable for *in situ* analysis of artefacts in a non-destructive way and is now often used to identify pigments and its degradation products in a wide range of artworks [64, 65]. The data provided are essentially immune to

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[5] Which not only is better to preserve the manuscripts in their best-controlled conditions but is also cheaper, as the insurance of certain manuscripts is extremely expensive.

interference from either binders or adjacent pigments, because of high spatial (< 2 µm) and high spectral (< 1 cm^{-1}) resolution of the instrumentation. However the µ-Raman does not allow determine the percentage of different compounds of each colour, which makes the µ-EDXRF or/and µ-FTIR indispensables for this purpose.

µ-EDXRF is one of the most used analytical tools to investigate inorganic materials such as pigments [66] and nowadays it is also used to quantify inorganic mixtures. Being an elemental technique, the results are not so directly interpreted, as for the other mentioned techniques, although is the technique that enable to easily obtain a better colour representativeness. Using µ-FTIR, the identification of compounds is made by analyzing the fingerprints that resulted of inter-atomic vibrations caused by the absorption of infrared radiation [67, 68]. This technique[6] allows obtain crucial information, regarding the paints composition, being the only of these techniques that can be used to calculate the relative proportions of pigments, fillers, binders and dyes.

HPLC-DAD is another available molecular technique used to identify organic compounds. However the amount of sample required and the impossibility of recovering it once analysed, made it a scarcely used technique in illumination characterization. The necessity of micro-samples and the uncertain characterization of some colouring organic materials analyses, namely the lakes, demonstrate the required development of new analytical techniques. In part B of this thesis a new developed technique, µ-spectrofluorimetry, to analyse these chromophores is presented and discussed. This is another non-transportable technique, since it is adapted to a microscope, but it offers the possibility to acquire complete steady-state fluorescence spectra *in situ*, from samples placed directly on a programmable x-y-z microscope stage. The fluorescence from the sample is directed back up into the microscope and it is also possible to view the fluorescence directly, through a binocular eyepiece and a digital camera. Beam splitting, that enables the separation of emission and excitation light, is achieved using dichroic filters [69]. The potential of this technique is very promising due to its high sensitivity, good spectral (< 0.5nm) and spatial resolution (2µm-60µm), and data reliable and reproducible [70].

1.3.2.2. Experimental design

To study the medieval colour palette, a visual comparison of the diverse paints applied in the manuscripts with paints made according to the recipes from some of the

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[6] µ-FTIR can be used with ATR accessory, avoiding the need to take micro-samples, although it is always necessary to contact with the colour material, which in an certain way turns it in an invasive technique. Nevertheless, the binders are barely characterized with this *in situ* accessory, when mixed in a paint.

medieval painters' manuals should be the first approach.

The reconstructions are also very useful to improve the performance of the experimental design for data collection and analyses. They serve as models to what would be performed in the real manuscripts. That allowed us to optimize the micro-sample selection, to control the timings to select, to collect, to keep and cataloguing the required micro samples; to verify the best analytical approach, that is to select the sequence in which each technique should be used; to realize which should be the better conditions to perform the analyses, which materials were not dispensable and what could be accidentally disregarded.

Some difficulties would emerge regarding, for example, the large number of pigments described in medieval references, the possible use of paints or paint components not referred to in any of the consulted books and the assumption that the medieval paints underwent little or no change throughout the centuries.

The following approach involved using non-invasive techniques. In all the selected[7] folia, the inorganic colour material characterization was made with μ-EDXRF, μ-Raman and μ-FTIR. Using first the μ-EDXRF, *in situ*, will optimize the information complementariness provided by these techniques, because it offer a global approach of the paints composition, revealing also some possible areas to be analyzed by μ-Raman and μ-FTIR.

To perform the study of organic colour materials, μ-FTIR, μ-spectrofluorimetry, μ-Raman and HPLC-DAD were used. The first three mentioned techniques can be used *in situ*, but in order to have a better paint characterization, micro-sampling was performed to analyze by μ-FTIR. HPLC-DAD needs mandatory micro-sampling, however the micro-samples were just used when really necessary, meaning that HPLC-DAD was used when the other techniques were not enough to characterize the molecules in doubt or to corroborate any dubious result.

In a previous study on *Apocalypse* [71, 72] where, for the first time, the colour materials of Portuguese medieval illuminations were characterized, one micro-sample of each red, orange and yellow colours were analysed by μ-FTIR and μ-EDXRF. The results revealed the presence of parchment glue as a binder and lead to the hypotheses of pigments mixture in orange and red colours. In this study, several folia were analysed in

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[7] A selection of some representative illuminated folia was made. By representative it is understood the group of folia that compose the entire Lorvão palette, for each manuscript when possible and enough folia that can provide consistent and statistically relevant results.

order to do the quantification of orange (*Apocalypse*), red (Lorvão collection) and also blue (*Book of Birds*) paints.

1.3.3. Published case studies of the Medieval palette

Although the majority of published case studies [46, 53-61, 73] are only about pigment identification, it could be important to establish comparisons with the Lorvão palette. The techniques used to identify the pigments were mostly Raman spectroscopy, X-ray Fluorescence and less X-ray diffraction, FTIR spectroscopy and PIXE spectroscopy.

According to published studies (Appendix II, section II.1.), there is no doubt that the most used red pigment in 13th-14th centuries illuminated manuscripts was pure vermillion [28, 29, 47, 48, 74, 75]. Orange pigments identified in different medieval illuminations revealed the use of red lead [60].

The blue paint could be made with lapis lazuli [48, 54, 58, 76], with azurite [49, 60, 84] or a mixture of both [48, 77] which would be clever if the illuminator intended a cheaper shade of lapis lazuli, since lapis lazuli (Figure 1.4.) was the most sought-after pigment in the Middle Ages, being more expensive than gold [93]. Indigo was also used to paint deeper blues, some of them almost bluish grey [54].

Figure 1.4. Lapis lazuli

Greens are the most problematic pigments to identify because they could be created by mixing other pigments, such as orpiment with ultramarine [54, 48] or azurite with lead-tin yellow [60] and possibly by developing new, unique recipes. These new recipes and mixtures could be very localised and distinctive, and this is the reason why several authors [78, 79, 83] have pointed out that greens are a useful indicator for specific artists, workshops, periods or countries. There are many kinds of copper green pigments: malachite [80, 81], a copper carbonate $Cu_2CO_3(OH)_2$, v*erdigris*[8], a neutral copper acetate $[Cu_2(CH_3COO)_4.2H_2O]$ [54, 76], copper sulphates [82]. Copper proteinates have been also identified, namely a deep green pigment in a 15th century German illumination [83], and some still remain unidentified [58].

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[8] Verdigris was considered the main artificial green pigment on medieval illuminations, but recent studies have questioned this fact [83].

The use of white lead in mixtures and to highlight is common, but as white colour by itself is not so common until the 13th century, at least in Anglo-Saxon manuscripts, where calcium carbonate [28, 50] or even calcium sulphate or calcined bone [48, 84] were used.

Black paint was found to be mainly carbon black [54, 55, 76, 81, 82]. The majority of references do not distinguish the type of carbon black and what is its source. V. Orna refers that the black found in the illuminations studied was from charcoal [48]. Besides the carbon black, iron gall is also detected as a black colour [54, 76].

Carmine-coloured is only referred by V. Orna, as an alizarin lake analysed by microchemical tests [48]. In other publications, there is also a reference to an organic red compound applied over the red or orange colour [80, 84]. In any other reference, this colour is not cited, this is possibly due to its organic nature, and to the difficulty in characterizing it with non-invasive techniques or with the ones that only require a minuscule amount of sample.

The colour yellow is mainly orpiment, although there are two studies that mentioned the possibility of it being an organic compound, such as saffron mixed with gypsum [55] or gamboge [48]. Yellow ochre [81] and massicot [48] are also detected in two manuscripts from the 12th-13th centuries. Some publications pointed out the presence of lead-tin yellow, but the manuscripts are dated later than the 14th century [60, 78].

The characterization of the nature and the manufacture procedure of dyes and lakes used in illuminations, could be a very important achievement to recognize the medieval palette. The nonexistence of efficient techniques able to characterize this sort of material made necessary the development of other techniques, such as fluorimetry, already used at New University of Lisbon and University of Perugia (also used by MOLAB team). New data have already been discovered, pointing to new perspectives of the medieval palette.

Brown colour is never mentioned, except in the V. Orna publication, as being an earth pigment.

The inks of all the manuscripts analysed are iron gall based inks (when referred), as was common in Western Europe [25, 47].

Regarding the nature of the binders used in medieval paints, they are usually not identified, probably due to the difficulties inherent to performing micro sampling or just because it is not such a direct analysis as for pigments characterization. The majority of the publications about binders are related to panel painting and not to illumination binders, which probably is due to the difficult access of micro sampling; bar this, there is no reasonable reason, as FTIR already has micro accessories that can be used to enable the characterization of micro samples, with dimensions of *circa* 20µm. So far, four publications mentioned the binder as a protein [59], without specifying which sort, another mentioned the use of animal glue [46, 83], and the other reports it as a polysaccharide binder; again no details about the kind of gum were offered [46, 49]. There is another study on binders, but it is about the effect of lead white on the proteinaceous binding media [85]. The other studies are related to the characterization of the most common binders used in art, by using new techniques such as FT-Raman or Raman spectroscopy [86, 87].

1.3.4. Common paint materials

1.3.4.1. Colour materials

Figure 1.5. Cinnabar

- **red:** vermillion, the synthetic HgS, was already described in the Lucca manuscript (8th-9th centuries) because alchemists showed an interest in this process. There have been many recipes of vermillion in technological written sources since then [88], most of them explaining that vermillion is obtained by only mixing sulphur and mercury in almost equimolar proportion and heating it in a closed container. Although laboratory experiences made in proved that this process is not so easy, there are some details, such as time and temperature, usually not described in the medieval texts that are decisive in the synthesis of vermillion. Cinnabar, a mineral HgS (Figure 1.5.), has been obtained since Antiquity by grinding it into powder [45]. The main source of this mineral in Europe, were the Spanish mines from Almadén [36, 89].

- **orange:** *minium* or red lead (Pb_3O_4) is a pigment used since Ancient times that could have been obtained through the mineral or by synthesis, however the synthetic pigment is more used than the mineral one considering it is uncommon to find it in nature. It is a semi-conductor lead tetroxide and a mixed-valence compound, with valence IV and II, since it has both lead atoms octahedrally coordinated to the oxygen atoms, as PbO_2 (plattnerite, lead (IV) oxide), and pyramidically coordinated, as PbO (lead (II) oxide) [90]. Vitruvius (1st century B.C.) refers that *minium* was first produced by heating white lead, accidently and that one is better than the natural one [91, 92]. This pigment seems to have been used in European illuminations since the 8th century.

Figure 1.6. Orpiment

- **yellow:** orpiment (As_2S_3) (Figure 1.6.) was a pigment found naturally in mineral form, "but artists preferred a synthetic version" [93], which Cennino considered a task best left to alchemists. It was already used by the Egyptians (17-16th centuries B.C.) as a pigment for mural painting and sarcophagus adornment [94]. Although the most important medieval yellow had been metallic gold, yellow pigments were also used. In illuminations, orpiment was especially used due to its luminosity[9]. Saffron was another source of yellow. The Arabs introduced it in the Iberian Peninsula markets in 711, and produced it since 961, using it as a dyeing agent, mainly for textiles [96]. Made from the stigmas of crocus flowers, predominantly from *Crocus sativus* L., when mixed with glair, saffron produces a strong and transparent yellow due to the presence of the chromophore crocetin and its glicosil derivatives. Usually to paint with this colour is like painting with an watercolour because the technique is to soak a tissue (the *clothlet*) as much as possible in the dye and binder and let it dry; whenever it is needed, a wet brush takes the paint off [36].

- **blue:** lapis-lazuli or ultramarine blue (thus called by the 14th century to distinguish it from azurite [30]) is a mixture of minerals, the colour blue a result of the dominant aluminosilicate lazurite $(Na,Ca)_8$ $(AlSiO_{12})(S,SO_4,Cl)$, which has the sulphur ion as chromophore, incorporated in its aluminosilicate solid matrix [93, 97]. Although Theophrastus and Pliny mention lapis lazuli in ancient treatises, there are no remarks of its use as a pigment by the Egyptians, Romans or Greeks [98]. The laborious process of extracting this pigment (different grades of pigment both in size and in quality, depend on the purification method [99]) and the fact that the main deposits were located in Afghanistan (already mentioned by Marco Polo [100]) made it very expensive [93]. Azurite, a mineral based on copper carbonate ($2CuCO_3 \bullet Cu(OH)_2$) was easily available through the Mediterranean commercial route [88] and it could be found in some mines in Alentejo. Indigo, an organic colour extracted from the fermentation of the *Indigofera* or *Isatis* species leaves [30, 101-103], has been noticed since Antiquity, for instance in a robe from Thebes

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[9] This pigment's name derives from the Latin word *auripigmentum*, already used by Pliny [95] in the 1st century. Due to its appearance, which is similar to gold, it was believed that orpiment did contain gold [93].

dated at around 3000 B.C. [93]. It was used in illuminations during the Middle Ages, but it cannot be compared to the other blues already mentioned, neither its presence in manuscripts [104] nor in medieval recipes, as it is just cited in one of the texts from Jehan Le Begue's manuscript: *Alcherius de Coloribus Diversis* [30].

- **green:** malachite (Figure 1.7.) a basic copper pigment ($CuCO_3.Cu(OH)_2$), was a mineral already used by the Egyptians during the pre-dynastic era, in the statues eyes [105, 106]. During the Antiquity the Greeks also used this pigment, but the Romans abandoned it in favour of green earth [102], although they continued to use it as medicine [102, 106]. In medieval times it was used in illuminations in parallel with verdigris. Verdigris is a neutral copper acetate ($Cu_2(CH_3COO)_4.2H_2O$), it was one of the main artificial green pigments used in medieval illuminations. It was made by exposing the strips of copper to wine or vinegar vapours, as described in ancient and medieval texts, [15, 32-34, 95]. According to Georgius Agricola, it "was first brought to Germany from Spain" [107], which could indicate the Arabic influence. It varies its colour hue easily depending on the chemical structure and binder used, from green, *bottle-green*, bluish-green to a greenish-blue. Very often this pigment was used to replace malachite [108] because it has a more intense colour; nevertheless it has a corrosive behaviour on parchment.

Figure 1.7. Malachite

- **white:** white lead ($2PbCO_3.Pb(OH)_2$) was the first white pigment synthesized; known since Antiquity, it was used in China and Japan in mural painting, paper and silk [109]. In *De Arte Illuminandi* [13] it is described as the ideal white for illumination, due to its remarkable consistency. Both Pliny and Vitruvius revealed its production, by putting lead near hot vinegar vapours [89]. In the medieval written sources, besides its production, its application is also explained in *Mappae Clavícula* [15], *On Divers Art* [32], *Il Libro dell' Arte* [36], *O livro de como se fazem as cores* [33, 34], and others. Calcium carbonate ($CaCO_3$) is a white pigment that can also works as filler (see section 1.3.4.3.).

- **black:** bone black, ivory black and vine black, all these black pigments are produced by the combustion of animal (like bones or ivory) or vegetal material (wood, vine, peach stone) and they were already used in Antiquity. Both Pliny and Vitruvius named them artificial blacks [108]. The high quality and homogeneity of the particles suggests that they should be scraped from cooking containers [94]. But, according to Thompson [110], the most important black in the Middle Ages was writing ink, based on carbon and on iron.

- **Carmine-coloured:** madder lake, alizarin lake and purpurin lake are anthraquinones that have been used as red dyes since pre-historic times [101], and there are written accounts of their use in ancient Egypt [42] to dye textiles with mordants[10], such as alum [111, 112]. These red chromophores were extracted from the roots of a variety of plant from the Rubiaceae family (madder family) [101, 113-117], alizarin and purpurin being the major occurring species in *Rubia tinctoria* L.. The red extracts were also prepared for use as pigment for painting by precipitating the extract in solution with aluminium salts, such as alum. The pigments were known as "lakes": madder lake was widely used in manuscript illuminations [13, 33, 34, 36]. Lac dye is a colorant collected from the segregation produced by a phitoparasite insect *Laccifer lacca* [101] from India and Southeast Asian. Its use is reported since the 16th century B.C., and it probably started to be used in Europe at the end of the 12th century [77]. The colour is a mixture of anthraquinones, laccaic acid A being the major component. Cochineal lake is another colorant based on the anthraquinone carminic acid, obtained from a parasite *Porphyrophora polonica* (Polish cochineal) or *hamelii* (Armenian cochineal), used in medieval Europe and from *Dactylopius coccus*, previously known as *Coccus cacti* [101, 102, 118]. Spanish people, who brought *Dactylopius coccus* from the New World [119-121], were the first to commercialize it in the 16th century, noticing that the colouring agent, carminic acid, was in a higher content it that parasite (15-20% carminic acid dye per dry insect for the *Dactylopius coccus* and circa 1% for the *Porphyrophora spp.*) [101]. Dragon’s blood, a natural resin extracted mostly from *Dracaenaceae*, *Palmae* and *Euphorbiaceae* [122, 123] owes its deep red colour to the presence of flavylium compounds. It was reported as a source of red, in *Historia Naturalis* it is described as “the blood of dragons and of elephants” [95]. According to Merrifield this resin has been used “from a very early period in miniature painting, but is not considered a durable colour” [30].

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[10] Mordant examples used in the past are metalic ions of Al, Fe, Cu and Sn [101].

1.3.4.2. Binders

The binder is one of the paint components necessary to make the adhesion between the paint and the parchment surface, and to maintain the cohesion of the paint. It also protects the chromatic layers and produces optical effects depending on the binder's nature and/or quantity in the paint mixture. However, there are some pigments more susceptible than others concerning their final colour or their stability.

The research on written sources and on case studies was not very conclusive regarding on the nature of the most common binder used during the 12th and 13th centuries. In the written sources, all the varieties of binders are mentioned: animal and parchment glue [30, 32-34, 36, 124], egg white [13, 30, 35] and yolk[11] [13, 30, 32, 35], fruit tree gums [13, 31, 32], the gum arabic (exuded from *Acacia Senegal*, easily imported from Africa to Europe [125]) being the one most referred, and also the mixture of two different types of binders [125]. In the case studies, the binders were not studied.

<u>Gum arabic</u> was one of the gums more cited in the texts and moreover Portugal had an intense Muslim influence from the recent past. Gum arabic consists of several polysaccharides and their salts, which on hydrolysis yield L-arabinose (Figure 1.8.), D-galactose, L-rhamnose and glucoronic acid [126].

<u>Glair</u> (or egg white) is almost composed by water (~89%) and also by proteins (10%), mainly ovalbumin (~55%), ovotransferrin (~12%) and ovomucoid (~11%) [127]. It was mostly used with organic components, to intensify some colours like reds, or to shade colours like orange.

<u>Parchment glue</u> was also used. Monks would probably took advantage of the scraps made when the bifolia were cut[12]. It is mainly composed by collagen, which is a natural polymer made of linear sequences of 20 different amino acids [128]. It has around 30% of glycine and 15 to 30% of proline and hydroxyproline (Figure 1.8.). The spatial form of these two last amino acids origins a helical structure; collagen is composed by three of this structures linked [129] (Figure 1.8.).

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[11] The egg yolk is referred in *De Coloribus et Artibus Romanorum* as a binder; in the other references it is cited as being used in very specific situations, depending on the pigments; for instance Theophilus says to used it with azurite to intensify its colour.

[12] Considering that the monasteries of new cities in a new country, should be a cautious monastery, a place where no waste of available resources would be condoned.

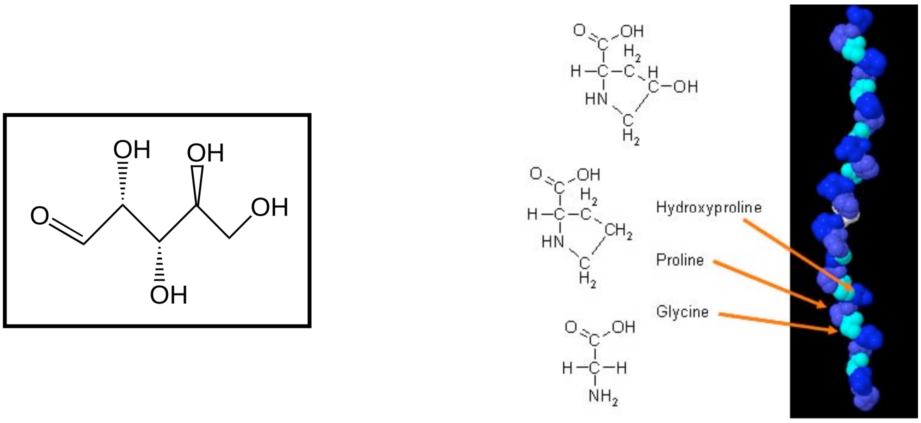

Figure 1.8. L-Arabinose structure (left) and principal amino acids structures of collagen: glycine, proline and hydroxyproline, and the collagen structure (right), i*n* [130].

The use of plasticizers, like honey [13, 124] or sugar [13], knowing that it was introduced by the Arabs in Iberian Peninsula at 711 [96], could be a technique used to improve the paint properties.

1.3.4.3. Fillers

Calcium carbonate ($CaCO_3$) is a filler, which intensifies colour to enhance opacity, without changing the colour, and it also improves the mechanical resistance. It could be obtained from eggshells or from a very white and soft sedimentary rock, chalk. Used since Ancient times, it has been detected in illuminations; its application is described by Cennino [36].

Another filler used was calcium sulphate, which can be hemihydrate ($CaSO_4 \bullet 1/2H_2O$), known as plaster of Paris, or dihydrate ($CaSO_4 \bullet 2H_2O$) namely gypsum, or anhydrous ($CaSO_4$).

> “Nearby, I saw a rubricator, Magnus of Iona, who had finished scraping his vellum
> with pumice stone (…). Another, next to him, Rabano of Toledo, had fixed the parchment
> to the desk, (…) with a metal stylus, he was now drawing very fine horizontal lines.
> Soon the two pages would be filled with colours and shapes (…).
> Those two brothers, I said to myself, are living their hours of paradise on earth.”
> Umberto Eco, in *The name of the rose*

1.4. Lorvão *scriptorium*

Al-Andalus, the Muslim-ruled part of Iberia by its Arabic name, once a prosperous region of the Roman Empire, which suffered economic and cultural transformation under the Visigoths, would only see its economy and cultural life revived under the Muslim rule [131].

The foundation of the Lorvão monastery [132] probably dates back to the 9th century, when the Reconquest of Coimbra occurred (878) and when there was a requirement to repopulate the land previously occupied, and to increase the “celebration of life”, which is attested by the various chapels, churches and monasteries built. From the 11th century onwards, the monks who lived there, would have resisted the introduction of the Order of St. Benedict, which emerges in monastic Galiza by the 10th century [133], keeping the Hispanic liturgy that also motivated the reading of the Commentary as a way of cultivating and developing their spirituality[13].

In the beginning of the 13th century the monks’ withdrawal from the Lorvão monastery was led by the bishop of Coimbra, D. Pedro Soares [132]. It ended with their expulsion and the entrance of the first Cistercian women in the monastery in 1206[14], converting it into a feminine Cistercian monastery [132]. How this happened leaves some questions open, namely the possibility that the monks had brought some of the existent manuscripts with them and also the inherent motives that made them give up some liturgical books that were fundamental to their order, among them the *De avibus* (or *Book of Birds*) attributed to Hugh of Fouilloy[15] and the *Commentary on Apocalypse* attributed to Beato of Liebana[16].

During the 12th century, the monastery would receive several donations that highlighted its importance in the context of the political strategy of the Reconquest, assuming the function of maintaining the peace and the articulation of the social order. In

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[13] Per research conducted by Prof. Aires de Nascimento and still in progress.

[14] The first reference made on the presence of women at the monastery dated from 1206, although just in 1211 after the interference of Pope Innocent III himself, the lawsuit between D. Teresa and the monastery finished, enabling the queen and forty religious women settled in the Lorvão monastery as a women Cistercian order [134].

[15] DGARQ – Torre do Tombo, Lorvão 5 (C.F. 90).

[16] DGARQ – Torre do Tombo, Lorvão 43 (C.F. 160).

1106, Count Henry and Countess Theresa[17] made an important donation to the monastery. A document from 1138 tells us about an annual payment to be made by someone named Nuno Mendes to the monastery, consisting of a "lamb skin", an essential material in a *scriptorium*. Throughout the 12th century, the first king of Portugal, Afonso Henriques, granted several donations to the monastery, which attested its growing importance[18].

It is during the government of Abbot João (1162-1192), considered to be a period of prosperity and activity of the “scriptorium laurbanense”, according to Nelson Correia Borges [132], that three of the manuscripts for which the colophon informs their dates will be made: Saint Augustine’s *Enarrationes in Psalmos* (1183), Hugo de Folieto’s *De avibus* (1183/1184), and the *Apocalypse Commentary* (1189) [135] (Figures 1.9. and 1.10.).

Figure 1.9. *Enarrationes in Psalmos*, *f.* 64v (left) and *Apocalypse*, *f.* 90. Photo: DGARQ-ANTT (right).

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[17] Parents of the first King of Portugal, Afonso Henriques.

[18] We know of an important donation, made in 1199, from Queen Theresa, the daughter of King Sancho I and granddaughter of King Afonso Henriques.

Figure 1.10. *Book of Birds*, *ff.* 4 and 6.

1.4.1. Lorvão collection

The manuscripts from the Lorvão monastery, preserved at the DGARQ (National Portuguese Archives) were all collected after 1834, when the prohibition to admit novices was decreed and therefore the extinction of religious Orders began to be promoted [136]. Deprived of any subsistence system, the monastery became extremely poor and the last nun died in the year of 1887. In 1853 Alexandre Herculano, the director of the National Archives, at that time known as Torre do Tombo, received from the nuns, at least nine manuscripts of these monastery *scriptorium*, such as *Apocalypse*, which was marked by an inscription made by his own hand.

This part of the thesis is mainly focused on two manuscripts, *Apocalypse* and *Book of Birds* but seven other manuscripts kept at DGARQ belonging to the Lorvão collection and attributed to the 12th and 13th centuries, with Romanesque esthetical characteristics, were also analysed: *Enarrationes in Psalmos of Saint Augustine* (Lorvão 50) dated from 1183, *Psalterium* (Lorvão 3) dated from the 12th century, Martyrology, (Lorvão 16) dated 1176-1200, Saints lectionary[19] (Lorvão12), Temporal lectionary (Lorvão 13) both dated

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[19] Lectionary is a book of lessons for the Divine Office [137].

from the 13th century, Gradual[20] (Lorvão 15) dated from the first quarter of 12th century and the Book of Calends[21] (Lorvão 17) , attributed to the 13th century.

Notwithstanding the diversity of the Lorvão collection, the existence of colophon[22], referring the date and location of the books' manufacture, in two of the manuscripts, *Apocalypse* and *Book of Birds*, make them particularly important [77].

"…the key of things past, the knowledge of things to come;
the opening of what is sealed, the uncovering of what is hidden"
Joachim of Fiore, late 12th century

1.5. Commentary on Apocalypse

The Apocalypse[23] (which in Greek means *Revelation*) is a biblical text attributed to the Apostle John [139], who seems to have been a late first-century itinerant Christian prophet active in Asia Minor, the location of the seven churches to whom the introductory letters are directed [140]. The majority of modern scholars, in accordance with some ancient sources, date the text to the last years of the first century (*ca.* A.D. 95), during the reign of the emperor Domitian, traditionally seen as a persecutor of Christians. Others still argue that it was written almost thirty years earlier, at the end of the reign of Nero (d. 68) [141, 142].

According to McGinn [140], the Apocalypse is not only a book containing a secret message but also a manuscript full of book imagery, especially the closed book with the seven seals (Rev. 5:1-7) and the open one that the "strong angel" commands John to eat (Rev. 10:2-11)[24].

It is important to note that the symbolic mode of communication of the Apocalypse makes it very powerful in the medieval culture. The special characteristic of its symbolism is the way in which it portrays the absoluteness of the final struggle between good and evil through a wide range of opposites, for instance the opposed feminine figures: the *Great*

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[20] Gradual is a book containing words and music for liturgical chants [137].

[21] Calends means the first day of the month [137].

[22] In the *Book of Birds* colophon "Ad honorem dei et sancti Mametis in monasterio laurbanense est scribtus [sic] liberiste IN Dlebus Iohanis abbatis FINITO LIBRO DONA DENT~ LARGIORA m a g i s tro (date between the letters of m,a,g,i,s, de magistro)" [138] ; In the Lorvão Apocalypse Commentary colophon: "Iam liber est scriptus / qui scripsit sit benedictus / qua … … / ERA MCCXIIa [1189] / Ego egeas qui hunc librum scribsi si in aliquibus / a recto tramite exivi, delinquenti indulgeat / karitas que omnia superant." [7].

[23] The moral message of apocalypticism is a call to the elect to reaffirm their commitment to God [140].

[24] Rev. 10:9 - "So I went to the angel and asked him to give me the small scroll. He said to me, "Take it and eat it. It will turn bitter in your stomach, but it will be as sweet as honey in your mouth."

Whore riding the seven-headed Beast versus the *Woman on the sun and the dragon*. (Figure 1.11.)

Figure 1.11. The *Great Whore riding on the seven-headed Beast* (*f.* 186v) and the *Woman on the sun and the dragon* (*f.* 153v). Photos: DGARQ-ANTT.

The first known Latin interpretation of the Apocalypse was the commentary written by Victorinus of Pettau, around 300. In North Africa and after 80 years, Tyconius wrote a second version. In the sixth century four more commentaries were written, one of them by Apringius of Beja (ca. 550) in Iberia, under the Visigoth[25] domain. His interpretation seems to have originally been a set of homilies intended for the liturgical time between Easter and Pentecost, as used in the Mozarabic rite [144, 145]. Another commentary was written by Primasius (ca. 540), which influenced all latter commentaries, including the one written by Beatus of Liébana (ca. 780), probably the latest of the three, dated from the 8th century. The Beatus of Liébana commentary has influences from four previous commentaries (Victorinus, Tyconius - ca. 380, Primasius and Apringius), as well as from a number of other sources [144].

Beatus of Liébana was a monk who lived in Liébana at the turn of the 8th to the 9th century [146], and wrote the Commentary to the *Apocalypse of Saint John* in an environment of belief in the end of the world, accentuated by the closeness to the end of

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[25] Visigoths and Suevi converted to Arianism, a version of Christianism, originated with Arius, presbyter of Alexandria Church in the fourth century, stating that Christ was a created human being, inferior to God, although was a Great master of wisdom and awareness of all divine mysteries [145].

the millennium. It is believed that the original manuscript[26] would have been illuminated and that it would communicate the visual message of the events that occurred from the Revelation of Christ to the religious realm, through the delivery of the Book to John, up until the moment of the reconstruction of New Jerusalem.

Beatus wrote the first commentary edition in the year of 776, as a spiritual lesson for monks [147]. There is evidence [148] pointing to a second edition (784) has only some reviews, and the last one, was edited in 786, an year after he had written the controversial *Adversus Elipandum*. In this last edition he made some alterations using it against the heretics and the antichrist followers, inserting a few more illuminations, transforming a contemplative work into a controversial denunciation [5]. According to Dodds, only in the 10th century would the false prophets of the commentary be related to Muslims, "an allusion which would make its way into many Beatus's images" [148].

Through the *Apocalypse*, the monastic world reacts again to the confrontation of religions and cultures by means of artistic work in which strong and abstract images exorcize the demoniac forces and promise the salvation of the chosen ones.

The commentary of Beatus is divided into twelve books, each with a prologue; the titles are related to the sanctity of the Church and the defence of the divinity of Christ against the Adoptionist theology[27] of the followers of Bishop Elipandus of Toledo. After a brief preface and a longer *Summa dicendorum*, Beatus divided the entire Apocalypse into sixty-eight sections, or *storiae*, and each one was followed by the *explanatio*. Most of the surviving manuscripts are illustrated with pictures based on the narrative *storiae*, inserted between each *storiae* and its *explanatio* (Figure 1.12.), resulting as a double explanation: the illustrated one and the written one [147]. According to Williams the iconographic uniformity and the way they are connected to the text, confirms that the first commentary, or one of the earliest copies made in Beatus's circle, was illustrated and was used as model for all the Hispanic copies that followed [6]. This large collection of manuscripts is known as *Beatus*[28].

The ensemble of the *Beatus* is divided into two main Branches, Branch I and Branch II, which are further subdivided in other branches. This *stemma*, proposed by Peter Klein [150], based on Wilhelm Neuss, results from the analysis of the existing *Beatus* and their intrinsic relationships. Branch I is considered closer to the prototype.

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[26] Although the original copy has been lost, according to Neuss, we should look for a prototype that would date back to the time of the Beatus himself, and which will have served as a model for later copies. Klein [3] also attributes the introduction of illumination in the text to the edition of 784. The first known image is the famous figure of Silos' fragment, dated from the end of the ninth century [3].

[27] The reason for the rise of Adoptionism (an option that accepts that Christ as described in the Koran, was a prophet, an adopted son of God, not His true son, as believed by Christians) at this time was the Muslim conquest of Visigoth Spain, which impressed upon Spanish theologians the need to develop a Christological doctrine compatible with the Islamic notion of strict monotheism [149].

[28] According to Echegaray there are 34 copies [8].

Figure 1.12. Examples of pictures based on the narrative *storiae*, inserted between each *storiae* and its *explanatio* (*ff.* 14v and185v). Photos: DGARQ-ANTT.

The Lorvão *Apocalypse Commentary*, an important manuscript in the artistic Portuguese context, copied in the *scriptorium* of the Monastery of St. Mamede of Lorvão in 1189, is part of the *Beatus* [150, 151]. It appears in a period of consolidation of Portugal as a kingdom. Afonso Henriques established his political frontiers with Castile but he also enlarged his territory in the Christian expansion beyond the Tagus River (see its localization in Figure 1.13.). In this geographic space, a Christian culture, an Arabic culture and a Jewish culture coexisted[29] [153, 154] as attested by a *security letter*, dated from 1170, written by King Afonso Henriques prohibiting the maltreatment of Moors by Christians and Jews[30] [156]. The Christian culture was assured by religious and military Orders, as well as by the Crusaders that came at the request of Afonso Henriques, to help in the conquest of the city of Lisbon [157, 158]. The Arabic culture had famous writers and historians, such as al-Mutamid (governor of Silves), Ibn Abdum (a resident of Évora) or Ibn Bassan (a resident of Santarém), considered by Mattoso as the last representatives of the Western Arab culture in Portuguese territory [159]. Although there were not religious buildings with the same magnificence as the ones in al-Andalus, there are remains of mosques in Moura, Idanha-a-Velha, Elvas and Loulé, as well as Mértola, which would have been built in the seventies. (Figure 1.13.)

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[29] According to Cordeiro others authors, Beinart, H., Burgos, F. and Vallecrosa,M. argue that Jews had been in Portugal for a long time before the Moor invasion happened [152].

[30] The presence of the Jews in Portugal is described by Ferro. According to her, Santarém had a large and prosperous community during the Muslim period and after the city conquest to the Moors (1147) it became part of Portugal [155].

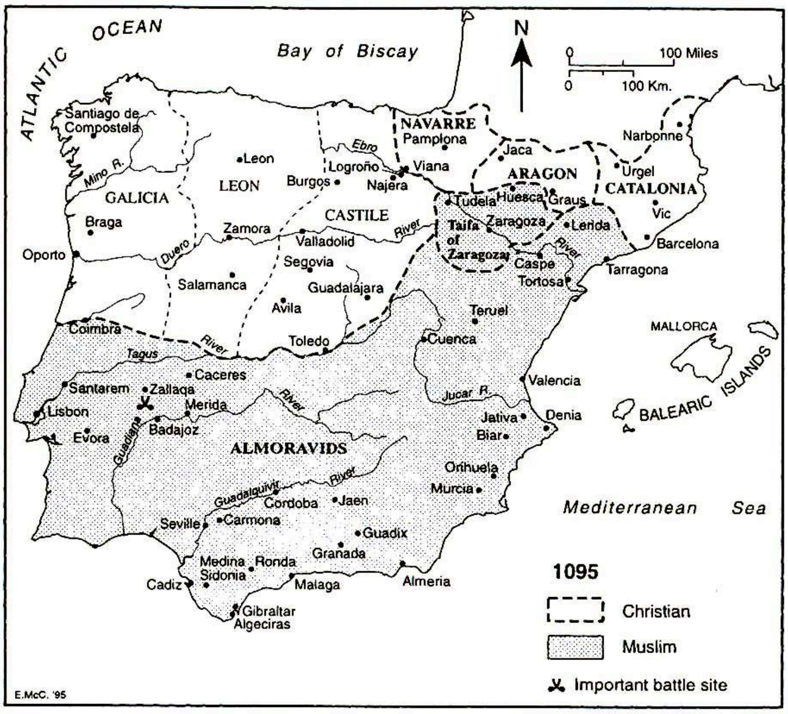

Figure 1.13. Iberia Peninsula map at 1095, showing the cultures territories (*in* [157]).

The coexistence of cultures was interrupted by the political unification and religious severity brought by the Almoravids and Almohads[31]. The year the *Lorvão Apocalypse* was made, 1189, coincides exactly with the Christian expansion, which leads to the first conquest of Silves and to the consequent counter-offense directed by the Caliph Yaqub al-Mansur[32].

The *Lorvão Apocalypse* belongs to Branch I[33], which copies the most ancient tradition of the Beatus. It is written in Latin, in primitive Gothic letter (Figure 1.14.) and has the dimensions (345x245mm) of a manuscript made to be exhibited in specific times of the year, which is consistent with the average Beatus

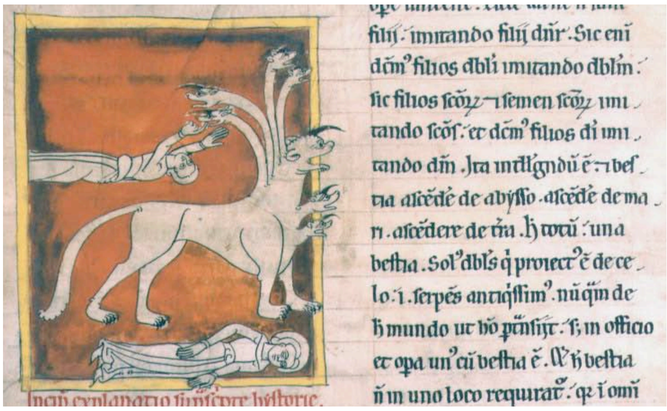

Figure 1.14. Primitive Gothic letter (*f.* 158).

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[31] The Almohads were especially militant and zealous [160].

[32] There is another contemporary Commentary on Apocalypse of Beatus (ALC. 246 beato de Liébana Commentarium in Apocalypsin, 330 X 220mm), conserved at National Library; this do not has illuminations, there is only the representation of the Antichrist tables (f. 144).

[33] The *Apocalipse do Lorvão*, the only Portuguese manuscript of the *Beatus*, apart from being the only from the twelfth century that is dated (1189), belongs to Branch I.

of the ninth to eleventh centuries. We have found that in the context of the Beatus of the 12th century[34], only Lorvão's and Navarra's follow the average dimensions of pre-Romanesque manuscripts, respecting the dimensions of the original model. The codicological analysis of this manuscript has been conducted by Horácio Peixeiro [7] and, more recently and deeply, by Peter Klein [3].

The text begins with a prologue emphasizing the end of the first millennium, and continues articulating the images between the *historia* and the *explanatio*. This organization of the Beatus makes the illuminator sometimes opt for centring the representation of the text in the *historia* or the *explanatio*. The colophon, with an appositive in folio 219v, informs us of the scribe's name as well as the date of its execution: «Iam liber est scriptus / qui scripsit sit benedictus / qua … … / ERA MCCXII^{a} [1189] / Ego egeas qui hunc librum scribsi si in aliquibus / a recto tramite exivi, delinquenti indulgeat / karitas que omnia superant.». In this case, it was implicit that *Egeas* was not the illuminator, because a narrative series of such order would assume an artistic specialization that in most cases was not the copyist's own.

The symbolic and eschatological character of the text and the image in the literary tradition of the commentary on the Apocalypse and the need to publicize a message in the political-religious context of the peninsular Christian expansion, would have lead the artist of the *Lorvão Apocalypse* to create an iconographic program which translates into 88[35] images, using contrasting and bright colours for the backgrounds[36] and transparent bodies, which appear to exalt the spirituality present in the text.

The study of these illuminated manuscripts demonstrates that the illuminator did not always follow the text, modifying elements according to his own creativity and visual tradition [135] which, at the time, raised interpretation problems to the twenty-first century historian, like Peter Klein [3], who considers all the iconographic deviation to be an error on the illuminator's part, at the same time attributing to another artist the capability to introduce new iconographic elements [161]. Some representative examples, discussed by Ana Lemos, are [135]:

1) In *The Four Knights of the Apocalypse*[37] (*f.* 108v) (Figure 1.15.), the iconographic differences in the Lorvão manuscript, assumed by some authors as "iconographic errors",

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[34] In this study, the fragments of the *Beatus* were not considered.

[35] Tables, schemes and marginal elements are included in this count.

[36] The images in the available bibliography do not agree with these chromatic characteristics.

[37] Rev 6:1-8 - "Then I saw when the Lamb broke one of the seven seals, and I heard one of the four living creatures saying as with a voice of thunder, "Come." I looked, and behold, a white horse, and he who sat on it had a bow; and a crown was given to him, and he went out conquering and to conquer. When He broke the second seal, I heard the second living creature saying, "Come.". And another, a red horse, went out; and to him who sat on it, it was granted to take peace from the earth, and that men would slay one another; and a great sword was given to him. When He broke the third seal, I heard the third living creature saying, "Come." I looked, and behold, a black horse; and he who sat on it had a pair of scales in his hand. And I heard something like a voice in the centre of the four living creatures saying, "A quart of wheat for a denarius, and three quarts of barley for a denarius; and do not damage the oil and the wine.". When the Lamb broke the fourth seal, I heard the voice of the fourth living creature saying, "Come." I looked, and behold, an ashen horse; and he who sat on it had

can be explained if we take into account the historical context and the value of the affirmation of the Christian knight compared to the Islamic one, in the process of the Reconquest, with its more powerful symbols: the sword and the cross. The replacement of the balance by the sword symbolizing justice, and of the sword of the fourth knight by the cross, accentuates the idea of salvation associated to the knight of the Christian expansion.

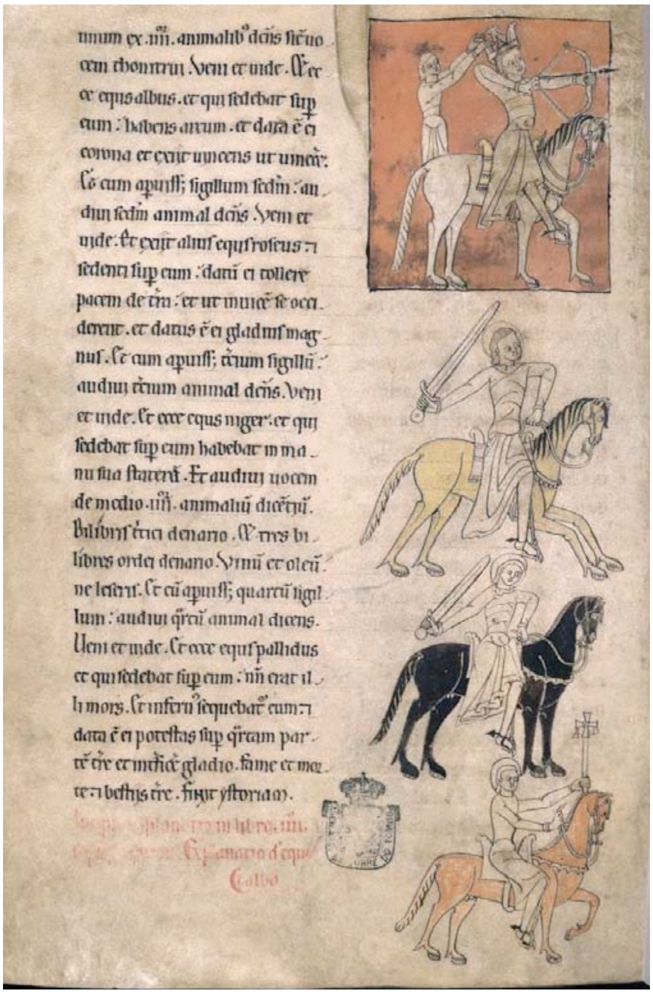

Figure 1.15. *The Four Knights of the Apocalypse* (*f.* 108v). Photo: DGARQ-ANTT.

2) The Lamb that holds the cross (the apocalyptic Lamb), is represented as an adult lamb with curved horns in the *Lorvão Apocalypse* (for example, *f.* 90);

3) The representation of a beardless Christ (*f.* 14v) again integrates this manuscript in the Lorvão environment, making it possible to compare it with the Christ who appears in the *Book of the Birds* (*f.* 25) (Figure 1.16.). This thus assumes a genealogy that dates back to the Paleochristian period and that would possibly be present in the iconographic representation of the manuscripts from this *scriptorium* [135].

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the name Death; and Hades was following with him. Authority was given to them over a fourth of the earth, to kill with sword and with famine and with pestilence and by the wild beasts of the earth.” [162].

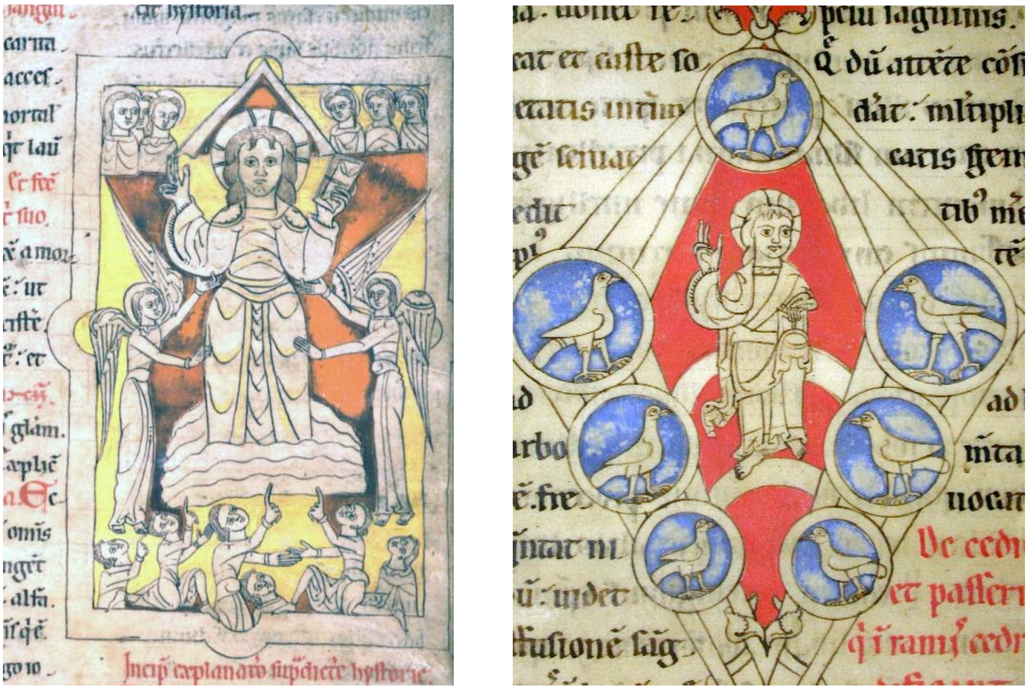

Figure 1.16. *Apocalypse* (*f.* 14v) and *Book of Birds* (*f.* 25) of the beardless Christ representation.

The artistic traditions and the historical context are clearly evident in the illuminations of this manuscript, but it becomes more difficult to rigorously establish a genealogy for the illuminated commentaries on the Apocalypse produced during the Romanesque period, because that was a period of internationalization, where multiple influences convened. The iconographic aspects discussed allow us thus to include the *Lorvão Apocalypse* in a precise historical and ideological context; some iconographic particularities imply a diversity of influences that go beyond the characteristics of Branch I.

In the Iberian context, the *Lorvão Apocalypse* presents affinities with some of the Beatus, especially the Beatus of Osma; from an iconographic point of view, innovative relations were found with the Beatus of Seu from Urgell belonging to Branch II and prior to the *Lorvão Apocalypse*, which allowed the perception of the use of certain elements, although not its justification. A remarkable example is the representation of *Heavenly Jerusalem* in the shape of a rectangle[38] (*Lorvão Apocalypse*, *f.* 209v and Beatus of Seu d'Urgell, *f.* 198v) and not as a square as was visible in the remaining Beatus (Figure 1.17.).

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[38] The representation of the City of Jerusalem in the shape of a rectangle appears equally in folio 253v of Window 14-2, also belonging to Branch II. However, in this last manuscript, other comparative analogies of the illuminated manuscripts with the ones of the Beatus of Lorvão have not been established.

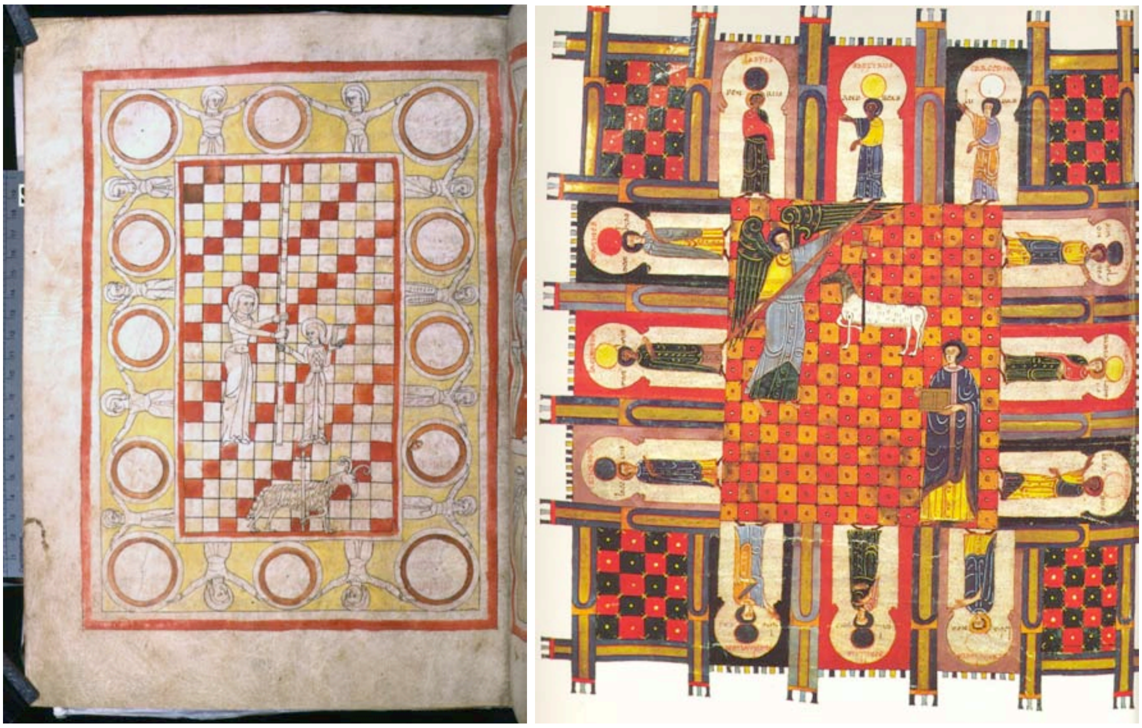

Figure 1.17. Representation of the *Heavenly Jerusalem* in the shape of a rectangle in *Lorvão Apocalypse* (*f.* 209v) (photo: DGARQ-ANTT) and in the shape of a square in *Beatus Facundus* (*f.* 253v).

In the *Lorvão Apocalypse* there is a primacy of the drawing[39] over colour [150], which is applied in certain spaces with a very precise intentionality, revealing a symbolic character but also fundamental to define the composition and to determine the spaces where the scene unfolds as we can see in the reading of our illuminations [163].

Symbolically, the three colours present in the illuminations can be explained by the persistence of a palette that privileged yellow and red, and by an aesthetic option of the illuminator, who used colours that were more adequate to the aesthetics of the light that awakened in the Western world from mid-11th century [24, 164, 165]. The application of only three colours[40] (yellow, orange and red) throughout all the illuminated manuscripts constitutes a particularity of the *Lorvão Apocalypse* within the Iberian Beatus. Even though the characteristic use of these colours may be found specifically in a manuscript in the National Library of Madrid (Ms 5780, Cassianus, Abbas Maxiliensis: Of Incarnatione libri septem-S-), from the 11th century, where the use of a reduced palette of colours, notably orange and yellow, is equally found [135].

In this context, colour is applied not in the bodies, but in the backgrounds, in order to highlight their transparency through the skin of another body, the parchment. This

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[39] According to Yarza Luaces, Lorvão Apocalypse belongs to the apocalyptic manuscripts' group where the drawing is the "main protagonist" [150]. It is in this way that a study and analysis of the body in the Apocalypse led us to highlight two basic concepts: transparency and metaphor. Lorvão's artist is the only one among all the preserved Commentaries, to represent the transparent bodies, using a drawing of considerable quality. This absence of painting can be found in the text of the commentary: "Es, pues, el resplandor de la luz interior de Cristo, que dice el Apóstol que habita en él. Pero cual es esta alma de tal calidad, a la que me refiero, o esta semejanza, sino la vida celeste espiritual, que no mancha pasión alguna, ni vicio, ni lujuria, ni corrompe engañosos tintes de colores". [8].

[40] The colors black and brown are also present, but only in a few details.

relationship between the soul and the body arises from an anti-corporeal ideology, formally expressed by the absence of volumes, a feature common to all the bodies in the Apocalypse [163]. In this way the monastery tradition and an aesthetic conception of the time that derives from the affirmation of Gregorio Magno: "the body is the abominable clothing of the soul" [166] are combined. Referring to the satanic bestiary, Michel Pastoureau writes, "Satan est souvent l'élément le plus saturé de l'image, le plus dense chromatiquement. C'est un moyen d'attirer l'attention sur lui et d'évoquer la densité suffocante de l'enfer, cette opacité des ténèbres qui s'oppose au caractère translucide de la lumière et de tout ce qui est divin" [167]. The illuminator of the *Lorvão Apocalypse* reflects this intentionality in folio 153v (Figure 1.11.) by painting the beast's body in orange, the same colour applied to the robe of the figure that represents the Devil, which is an exceptional feature in a manuscript where bodies and clothes are transparent. Also in folio 191 (Figure 1.18.), it is interesting to notice the link that the illuminator establishes between the characters with the heads covered with *pileum cornutus*[41], decorated with red strokes, the same used to fill the body of the Beast.

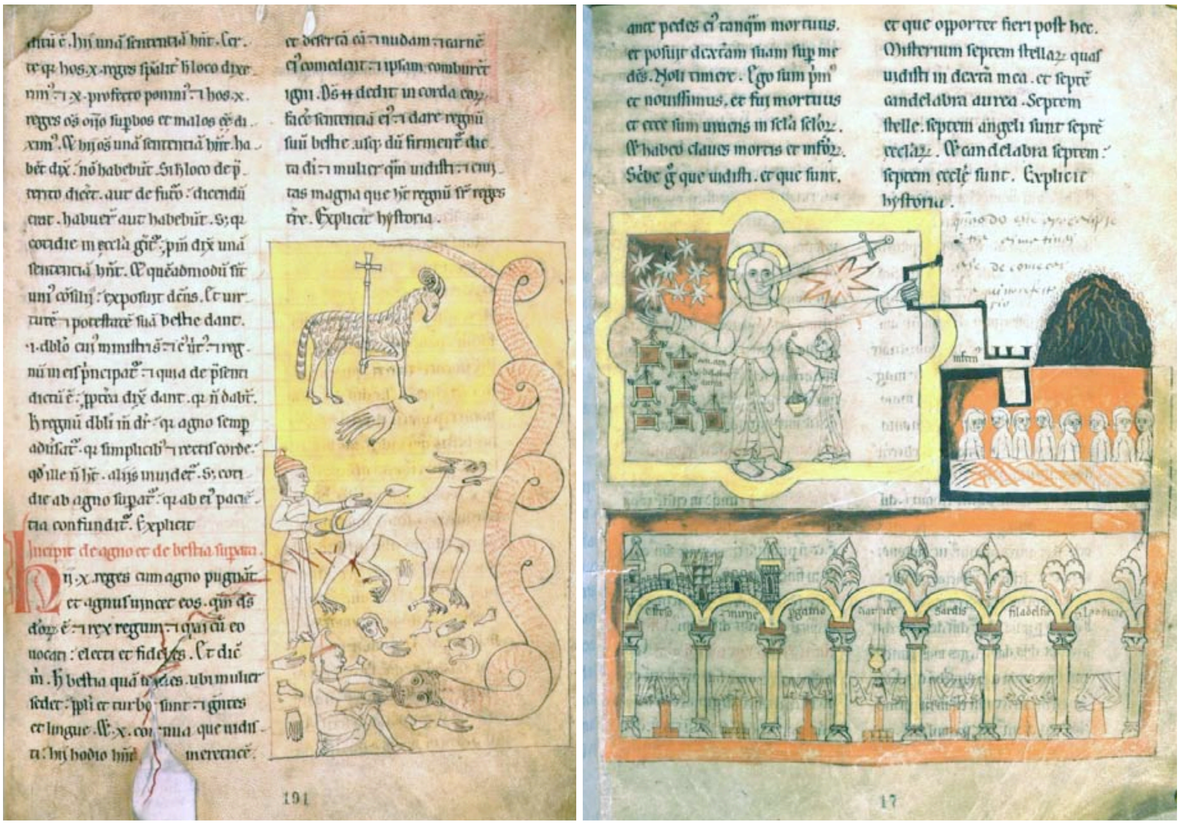

Figure 1.18. *Lorvão Apocalypse*: *The victory of the Lamb over the Beast* (*f.* 191) and *Commission to write* (*f.* 17). Photos: DGARQ-ANTT.

In the representation of "the Parusia" (*f.* 14v) (Figure 1.16.), orange brings to light the figure of Christ, thus emphasizing the text message. Also, in the representation of

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[41] Iconographic element that might allow the identification of these characters as Jewish.

Commission to write (*f.* 17) (Figure 1.18.), the illuminator divides the composition in three scenes, in which colour assumes an important role in the reading and comprehension of the space where the action unfolds and in the iconographic elements that participate in it. The illuminator makes equal use of colour in the backgrounds to highlight the characters' gestures and the relations between them. For example, in the *Message to the Church of Pergamum* (*f.* 59) (Figure 1.19.), the illuminator highlights the importance of the transmission of the message of the angel to John through the opposition of orange in the existing space between them, contrasting with the yellow of the surrounding background [135].

Figure 1.19. *Lorvão Apocalypse*: *Message to Pergamon* (*f.* 59) and *Message to the Church of Smyrna* (*f.* 54).

Also interesting is the use of orange colour in *Message to the Church of Smyrna* (*f.* 54) (Figure 1.19.), creating a sense of movement that accompanies the rising nature of the elevation of the cross. In this same illumination, the orange colour is also strategically applied between John's arms that hold the Bible/Book, emphasizing the gift of the Word [135].

“Cum scribere illitterato debeam, nom miretur, diligens lector,
si ad aedificationem illitterati de subtilibus simplicia dicam”[42]
in *De avibus*

1.6. Book of Birds

The *Book of Birds*, written by Hugh of Fouilloy, is one of the various manuscript copies spread in Europe [168, 169].

De bestiis et aliis rebus is a text written between 1130-1140 by Hugh of Fouilloy, an Augustinian prior, and dedicated to a convert named *Rainerus* [170]. It is based on illustrated traditional late Roman *Physiologus*[43], the first Christian bestiary and animal breviary and in the Middle Ages was as well-known as the Bible [171]. The authors of bestiaries aimed to educate and instruct the sinful, arguing that the ”Creator had made animals, birds and fishes, and had given them their natures or habits, so that the sinner could see the world of mankind reflected in the kingdom of nature, and learn the way to redemption by the examples of different creatures” [172]. Beyond the moral message that each creature represents, the mystical significance is also an important aspect that can cause some problems to the writers, since the same creature might represent both good and evil in different texts of the Bible. They usually solved this difficulty by attributing both meanings to the same creature, with several shades of probable meaning between the two extremes as well [172].

In this text, the birds are related to moral allegories as examples to be followed by monks and canons. In the first part, the Bible is the major source and is repeatedly cited; in the second part the author uses the bestiary from Isidore of Seville, *Etymologiae* and *De rerum naturis* (The Nature of things) from Rabanus Maurus[44], as models.

There are several copies of this book scattered throughout Europe, three of them in Portugal. From the Portuguese copies of the *Book of Birds*, Lorvão 5 (DGARQ-ANTT), ALC. 238 (National Library) and Ms. 43 Santa Cruz (Public Municipal Library from Oporto) (Figure 1.20.), belong to the group of manuscripts which follows the original manuscript from Heiligenkreuz abbey, *ms.* 226 [173], also dated from the end of the 12th century. It should be noted that the *Book of Birds* from Lorvão and Santa Cruz have some particular

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[42] Translation: “As I have to write to an illiterate, do not be surprised, zealous reader, if for enlightenment of those, I will say simple things about subtle themes” [11]

[43] The *Physiologus* is a text written in Alexandria, in Greek by an unknown author, probably dated from the 2nd century. This text compiles many traditional texts: zoological description, animal fables, evangelic word and biblical paraphrase. Each animal is described, and a tale follows, from which the moral and symbolic qualities of the animal are derived.

[44] Rabanus Maurus used mostly Isidore’s material, but had added his own moral sings and biblical quotations.

affinities between them, since all the birds are placed in a circle[45] and the drawings are similar (Figure 1.20.). In general, only the manuscripts from Lorvão and Santa Cruz have all the birds shown in a circle, while for instance, in the *ms*. 177, Troyes (*f.* 141) [174] one of the birds is inserted in a circle[46], and all the others are painted in a proper space of the folio (Figure 1.20.).

Figure 1.20. The representation of the heron in the three Portuguese *Book of Birds*: *Lorvão 5* (*f.* 59v), *ALC. 238* (*f.* 222v) and Ms. 43 Santa Cruz (*f.* 106).

The *Book of Birds* from the Lorvão collection (210 x 137 mm) has two colophons that specify the Lorvão monastery as the place of production, and provide two different dates: 1183 and 1184.

The author, Hugh of Fouilloy, theorizes on the power of the image to justify the richness and variety of images painted in these codices. The illustration does not only explain the text but it also holds the attention and stimulates the imagination of those to whom this codex is dedicated, as explained in the first prologue:

> *Cum scribere illiterato debeam, non miretur, diligens lector, si ad aedificationem illiterati de subtilibus simplicia dicam. Nec imputet laeuitate quod accipitrem uel columbam pingam, cum beatus Iob et propheta Dauid huiusmodi uolucres nobis relinquerint ad doctrinam. Quod enim doctioribus innuit Scriptura, hoc simplicibus pictura. Sicut enim sapiens delectatur subtilitate scripturae, sic simplicium animus detinetur simplicitate picturae. Ego autem plus laboro ut simplicibus placeam, quam ut doctioribus loquar et quasi uasculo pleno latices infundam. Qui enim sapientem uerbis instruit quasi uasculo pleno latices infudit.* [47]

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[45] Except the birds at the prologue.

[46] The circle is also present in the *Lorvão Apocalypse*, for example in: the “crown of life” (*f.* 54), the composition of circles of the “Vision of the Lamb and the four beings” (*f.* 90), the illustrations of “Silence of the sky” (*f.* 134) and the “Fifth angel sounded the trump and opens the abysm with the key” (*f.* 140v), the “Colours’ description of precious stones” (*ff.* 208v-209), the “New Jerusalem” (*f.* 209v). It is also represented in the folia 112,115,118,139, 142, 153v, 169, 172v, 180v, 196v, 202v, 207, 210, 217

[47] Translation based on the Portuguese version (p. 59) written by Maria Gonçalves: “As I have to write to an illiterate, do not be surprised, zealous reader, if for enlightenment of those, I will say simple things about subtle themes. And do not assign frivolously as I paint the hawk or the dove, when the reasonable Job and the prophet David left us this type of birds to instruct. In fact, what the Scripture make clear to the skilled, the image will enlighten the humble: as the skilled delights the subtlety of the prose, the spirit of the humble is fascinated by the simplicity of the image. As for me, I prefer to commit myself in please the humble than talk to the more talented, as if I put liquid in a filled container. In fact, who teach a sapient man by words is putting liquid in a filled container.” [11].

The prologue begins with the dove and the hawk framed by a horseshoe arch (Figure 1.21.), an element absent in the other *De Avibus* (for instance BNF, Manuscrit latin 2495 [169]), but also present in the *Lorvão Apocalypse*, which could be related to the contemporaneous architecture of Coimbra [132]. In folio 5v, the illuminator presents two archers with crossbows probably to mention “the nobility members”[48] as appear in the *ms.* 226 (the horse rider and the hawk). The illuminator follows the program representing the dove, with mystic (*f.* 6) and moral (*ff.* 7v and 8) meanings (Figure 1.10.). There is another representation of the hawk flapping its wings (*f.* 16) (Figure 1.21.) which, according to the text symbolizes a feather changing, symbolically associated to a military horse rider who leaves his earlier life to initiate a clerical life going back to “a much lighter flight as much younger” (*f.* 17).

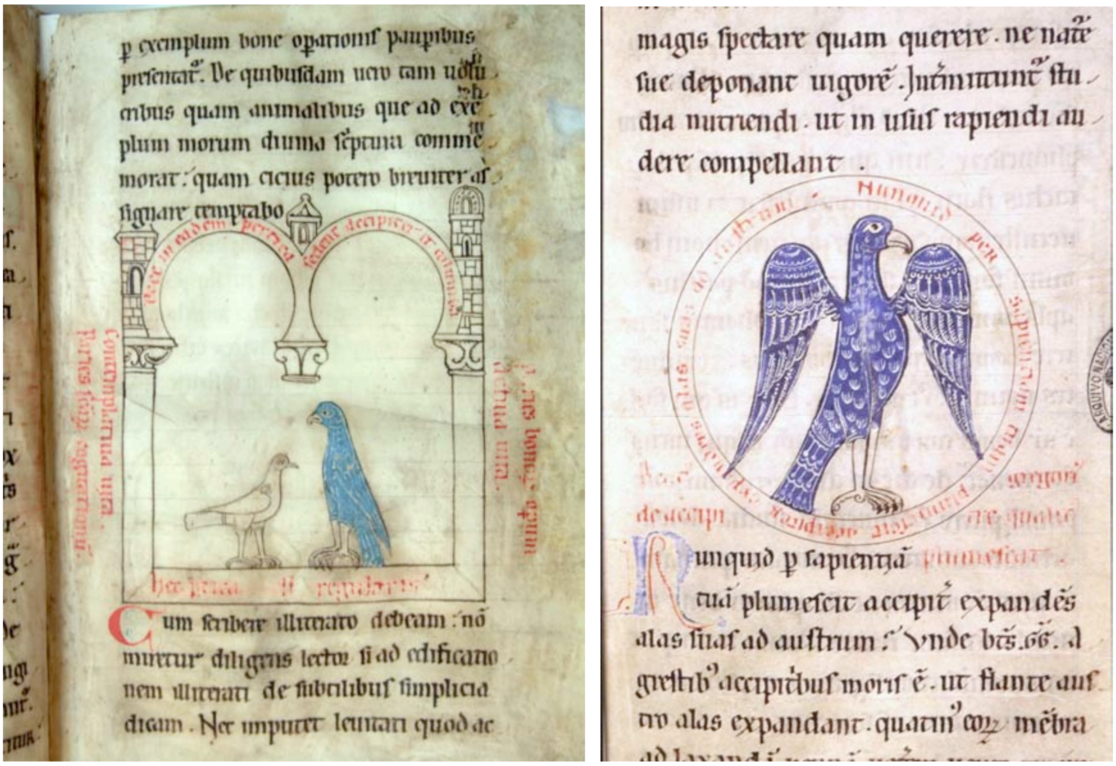

Figure 1.21. Representation of the dove and the hawk in folia 5 and 16 of the Book of Birds.

The palm tree (*f.* 20v), as well as the turtledove (*f.* 21v) (Figure 1.22.) denotes a reflection of the fair person associated to the palm tree, which in turn symbolizes Christ himself; the fruit is the result of the conversion through faith. The turtledove comes out as a contrite person.

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[48] *Book of Birds*, prologue, *f.* 5

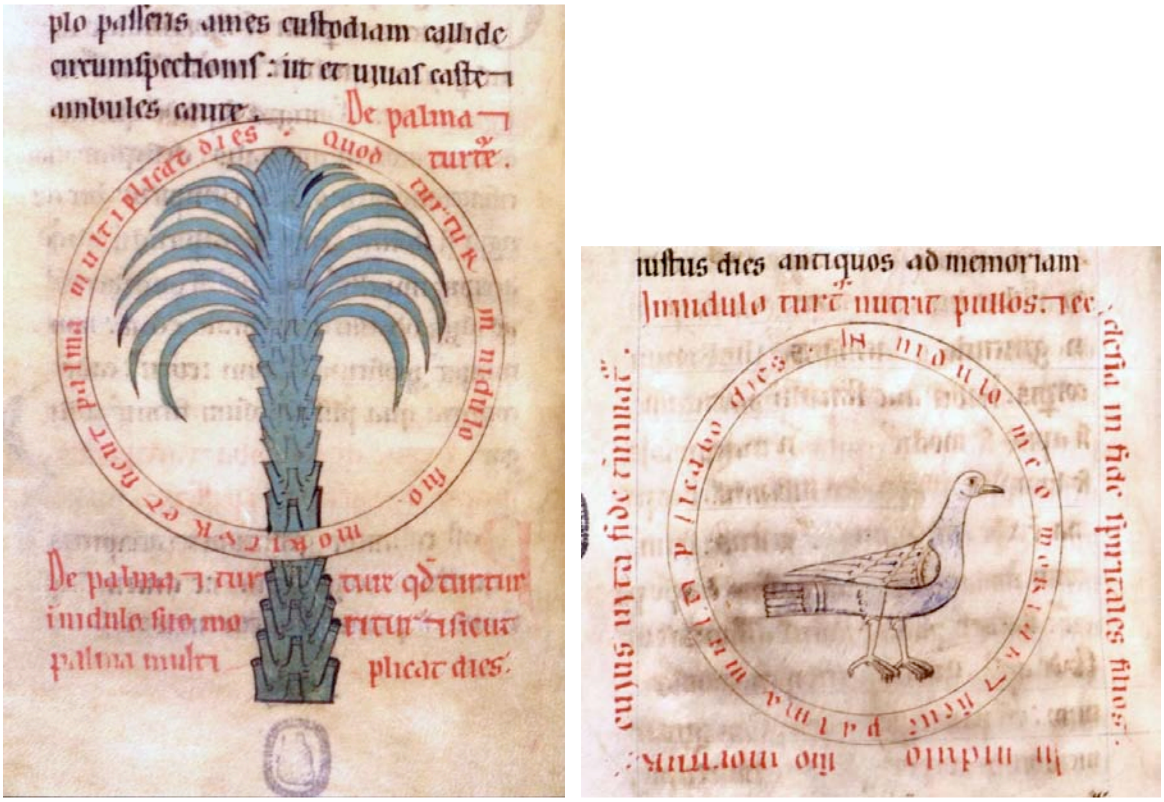

Figure 1.22. The palm tree (*f.* 20v) and the turtledove (*f.* 21v). Photos: DGARQ-ANTT.

Before the numerous birds' representation, the illuminator gives special attention to the Cedar of Lebanon (*f.* 25) (Figure 1.23.), the tree that, according to the *Song of Songs*, symbolizes Christ; the birds surrounding it are judged as the souls of the preachers. The Lorvão illuminator, as the one from Santa Cruz, represented a closer iconography to the text in which Christ appears in the centre of the tree, surrounded by seven birds, interpreted as the seven gifts of the Holy Saint (Figure 1.23.) [175]. Hugh of Fouilloy illustrates the other 22 birds[49] as moral examples through their virtues and faults (Appendix V).

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[49] *Book of Birds*: Pelican (*f.* 30v), Nighthawk (*f.* 32), Raven (*f.* 33), Cock (*f.* 36v), Ostrich (*f.* 40), Vulture (*f.* 46v), Crane (*f.* 48), Kite (*f.* 49v), Swallow (*f.* 50v), Stork (*f.* 52v), Blackbird (*f.* 54), Carrion crow (*f.* 56v), Goose (*f.* 58v), Heron (*f.* 59v), Caladrius (*f.* 60v), Phoenix (*f.* 61), Partridge (*f.* 62v), Quail (*f.* 63v), Hoopoe (*f.* 64v), Swan (*f.* 65v), Peacock (*f.* 66v), Eagle (*f.* 69).

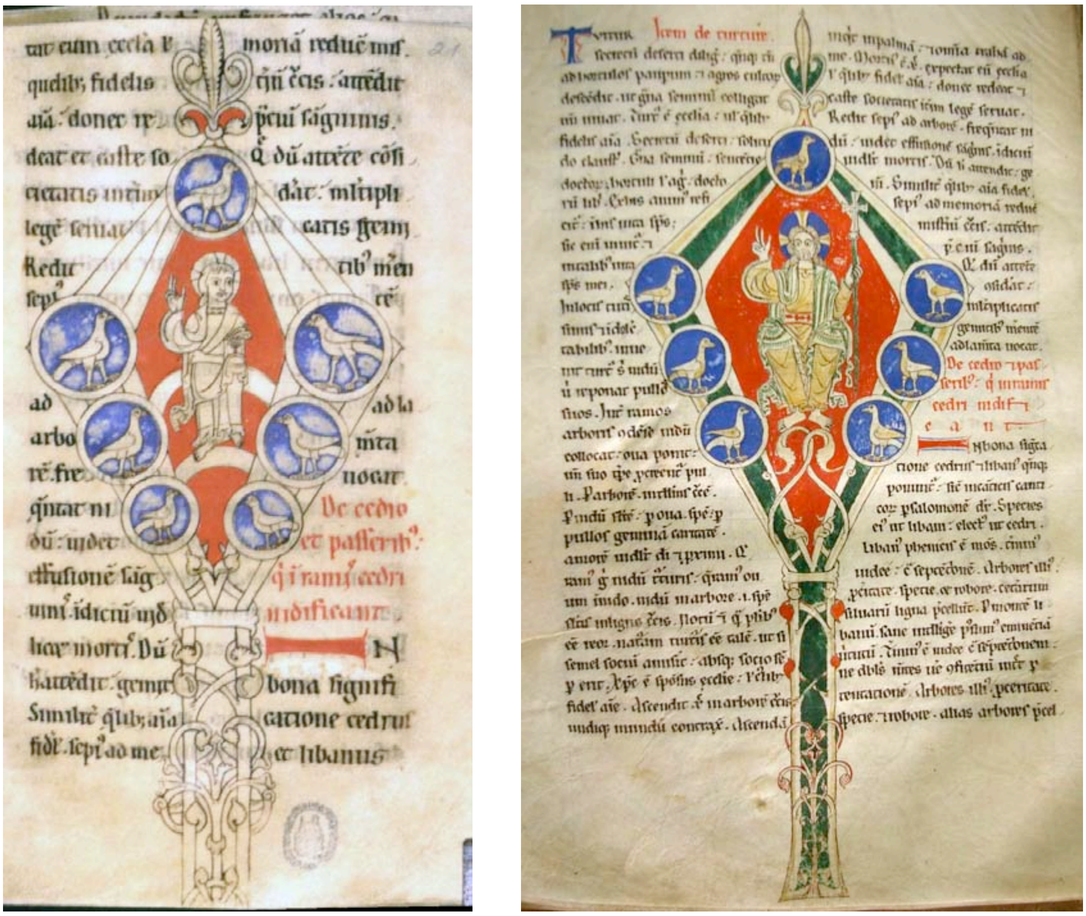

Figure 1.23. The cedar of Lebanon represented in Lorvão (*f.* 25) (Photo: DGARQ-ANTT) and Santa Cruz (*f.* 94v) manuscripts.

The illuminator individualizes the birds from a morphological point of view with very good drawings (most of the birds can be identified by the similarity to the natural world) and places them inside double circles with a red inscription. In this manuscript it is important to stress the decorative nature that some of the birds have, as well as the strength of the gesture relating the birds to the respective text (the cock is an example, *f.* 36v) (Appendix V). Some of the birds of the set can be marked by their lapis-lazuli blue colour, for instance the hawk (*ff.* 5 and 16)[50], revealing the importance given to nobility.

The *De avibus* iconographic analyses enabled new correlations to be established that suggest new hypothesis linked to the *stemma* proposed by Willene Clark [173]; perhaps the Santa Cruz manuscript is directly associated to the original lost manuscript and the one from Lorvão followed this model but adopted its own distinctive monastery style to the illuminations. The interactions between Portuguese canons with Saint Rufo of Avignon (a

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[50] Other four birds have all their body painted: the raven is painted in black (*f.* 33); the swallow (*f.* 50v) is painted in black and red, with white reliefs; the blackbird (*f.* 54) is black and has a red beak; the swan (*f.* 65v) is white and has red beak and feet. From all these birds only the swallow has a positive meaning in the Hugh of Fouilloy's text. It is "the penitent soul, which always yearns for the beginning of Spring, because in everything it keeps the measure of discretion and temperance. Behold how a simple bird teaches those that Divine Providence has made prudent from the beginning" [11].

very important Augustinian centre in the south of France, from where there is another *de Avibus* produced in the same period) are known. In this case, the transmission could have been established through the Augustinians and not the Cistercians, since the *Book of Birds* from the Alcobaça monastery had a privileged connection with Claraval [77, 176, 177].

Although the three Portuguese *Book of Birds* manuscripts are included in the same group, which uses as manuscript model the one from the Heiligenkreuz Abbey (Ms. 226), the ones from Lorvão and Santa Cruz presented some specificity between them by using a circle surrounding all the birds and also by their affinity in style of drawing.

1.7. Other Lorvão manuscripts analysed

The other seven Lorvão manuscripts were analysed and their colours compared with *Apocalypse* and the *Book of Birds*, to get more information about the Lorvão Romanesque illumination palette.

Besides the *Apocalypse* and the *Book of Birds*, the manuscript *Enarrationes in Psalmos of Saint Augustine* (*Lorvão 50*), dated from 1183, is the only one of this group that is also dated. This manuscript (365 x 242 mm) is a copy of the text written by Saint Augustine in the 4th century, revealing his knowledge of the Scripture and his theology.

Lorvão 3, the *Psalterium* (a book of psalms) from the 12th century is the smaller manuscript of the group (175 x 130 mm) and the illuminations consist of a group of 13 expressive human figures (Figure 1.24. and 1.25.), painted in some decorated initials or in the margins of the folia, and in this latter case they are always linked to the initials, by looking or pointing to them.

Figure 1.24. *Lorvão 3*: folia 107v, 108 (above) and 98v, 71v (below).

Figure 1.25. *Lorvão 3*: folia 17v, 82v, 98v, 107, 107v and 109.

In the Saints (*Lorvão 12*) and Temporal (*Lorvão 13*) lectionaries, colour is applied to paint the main initials. The lack of illuminations could be justified by the rigorous beliefs of the Cistercian Order.
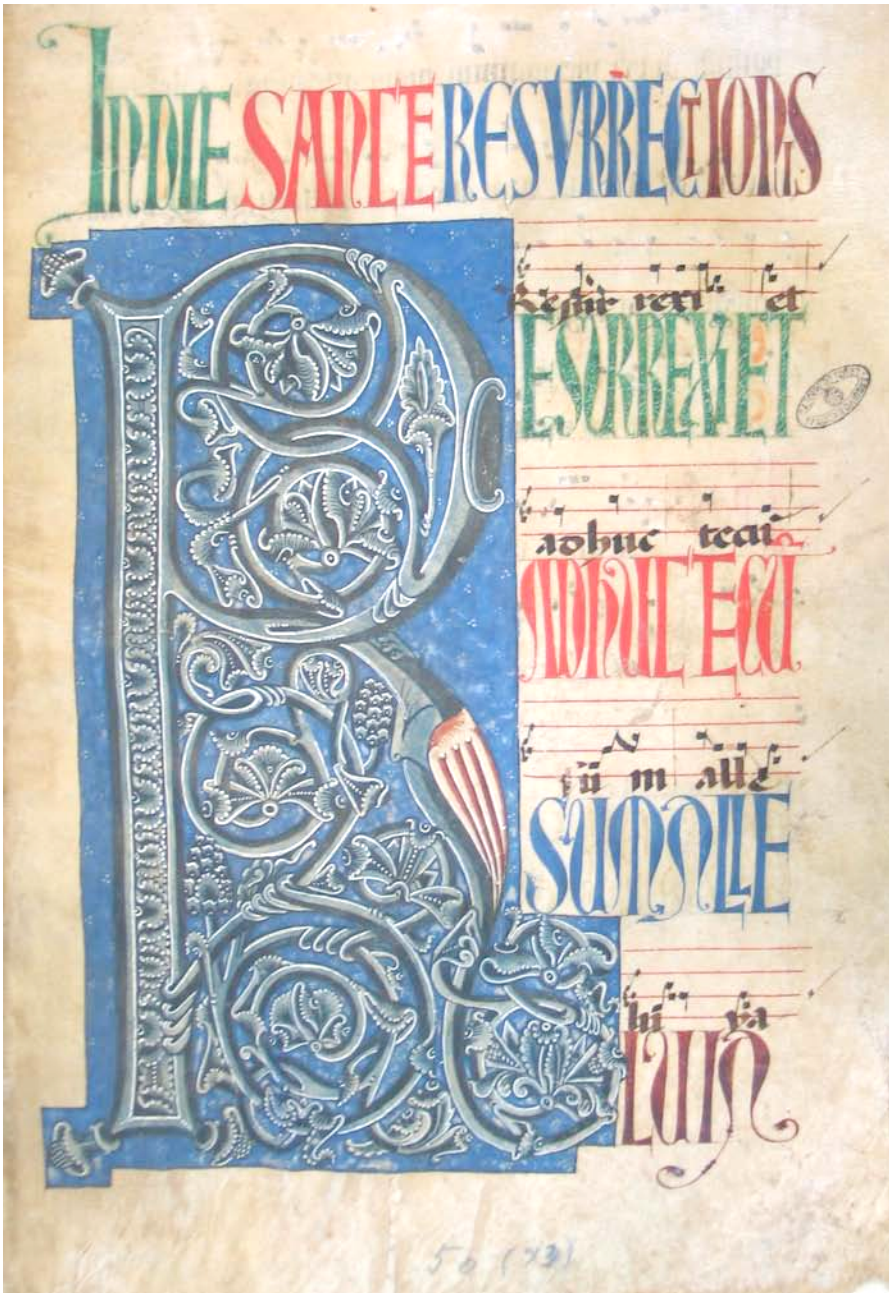
Figure 1.26. Ornamented initial with painted background in Lorvão 15 (*f.* 50).

Lorvão 15, the Gradual, is a music book of Gregorian chant[51], dated from the 13th century. Usually graduals are very large, like the Lorvão one (395 X 260mm), so that

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[51] This kind of music is called Gregorian because it was the Pope Gregory I who established rules to sing a serial of songs during the liturgical calendar, standardizing the liturgy required by the quick expansion of Christianity.

several members of the choir could gather around a single copy, and provided music for all times of the year (Figure 1.26.).

There is a Martyrology (*Lorvão 16*) in this group dated from the end of the 12th century (380 x 250 mm) with 218 folia. In this manuscript the initials have their backgrounds painted with simple and alternate colours creating effects between the parchment, which is left unpainted and the background (Figure 1.27).

Figure 1.27. Initials of Lorvão 16: *ff.* 32, 34 (left above and below, respectively) and 59v (right).

Finally, the book of Calends (*Lorvão 17*), attributed to the 13th century, is a large book (325 x 225 mm) with the main initials painted but with unpainted background. (Figure 1.28.)

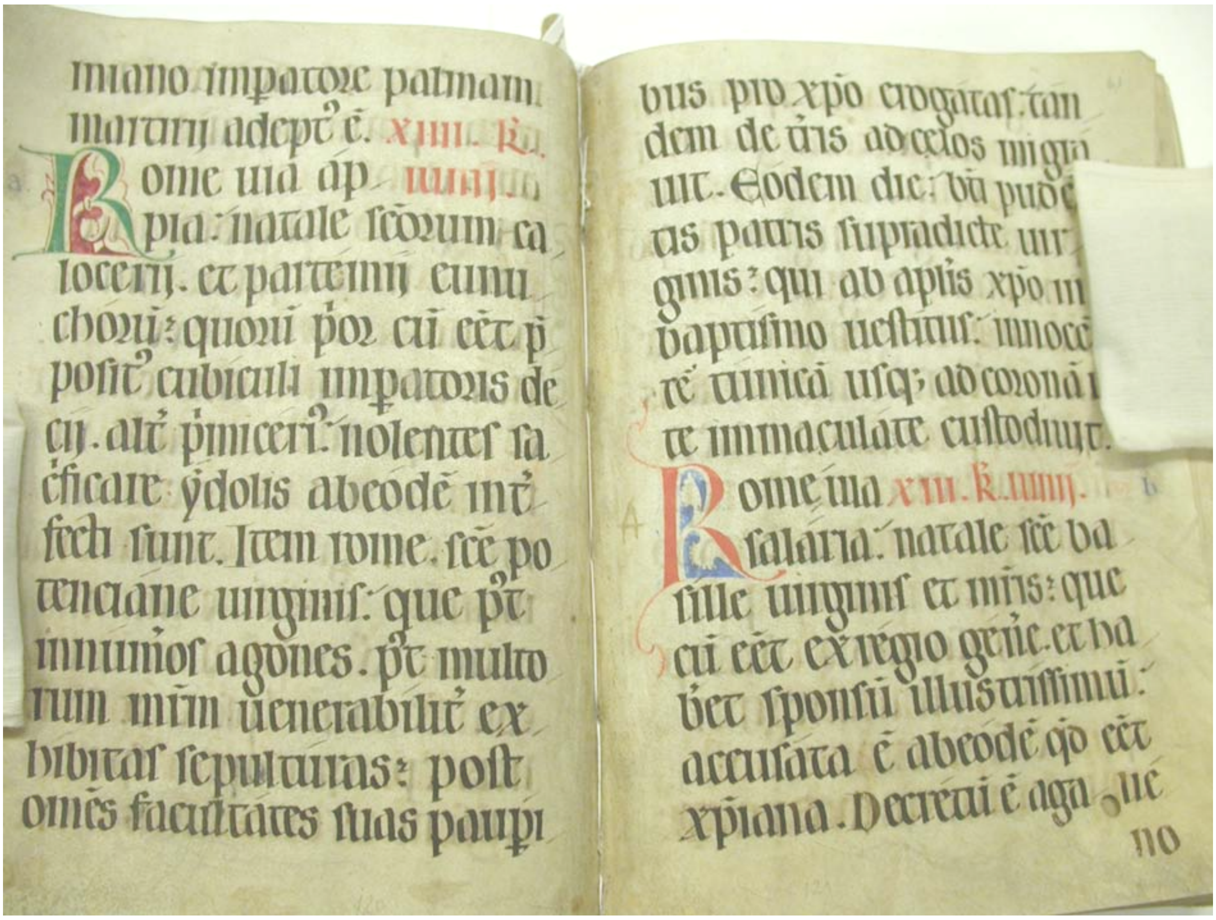

Figure 1.28. Initials present in Lorvão 17, folia 120v and 121.

Lorvão manuscripts 12, 13, 15 and 17 present a very close connection with the manuscripts from the Alcobaça monastery, in which the colours and their combination are used in a different way, which according to some art historians' hypotheses, could be related to the palette's modification or indicate the possibility of being copies made in this monastery and not in the Lorvão monastery. Lorvão 16 presents considerable similarities to Santa Cruz Homiliary (Santa Cruz 4), dated from 1136, belonging to a genealogy that lead to Peninsular Iberia illumination and to the manuscripts from the Moissac and Limoges *scriptoria*, dated from the eleventh-twelfth centuries [20].

“Painters need paint. Colour is their medium of expression and
communication, but to make their dreams visible it needs substance.”
Philip Ball, in *Bright earth*

2. Results: characterizing the colour of Lorvão manuscripts

2.1. Preamble

The study of the Lorvão colour palette started by observing the manuscripts in a macro-scale, regarding not only their colour specificities but also their conservation condition. Then, a visual comparison was performed of the diverse paints applied in the manuscripts with paints made according to the recipes from some of the main medieval painters’ manuals: *Mappae Clavicula* [15], *De Coloribus et Artibus Romanorum* [30], *Liber de Coloribus Illuminatorum Siue Pictorum* [31], *De Diversis Artibus* of Theophilus [32], *De Arte Illuminandi* [13], *O Livro de como se fazem as cores or The Book on how to make colours* [33, 34] and *De clarea* [35],.

To understand the paint composition, the manuscripts were analyzed at a micro-scale level, and the colour materials were characterized. Inorganic colour materials were analysed *in situ* by µ-EDXRF followed by µ-Raman (Appendix I, section I.3.1.1.1). Further studies were performed, in order to determine the contribution of each paint inorganic component in the final blue, orange and red colours. Organic materials were characterized by µ-FTIR and eventually by µ-Raman, and red lakes were also studied by using a new technique, µ-spectrofluorimetry (study developed in part B of this thesis).

A preliminary microscopic observation would allow a better micro-sampling of organic materials, pigments and extenders, that eventually would need EDXRF and Raman data confirmation or complementary information, by other non-transportable techniques (µ-FTIR, µ-spectrofluorimetry, HPLC-DAD…). µ-EDXRF will allow to get an idea of pictoric elemental composition in a larger area, while the µ-Raman will reveal the pigments in a smaller area, but no so representatively. Selecting in each folio, and for each colour, five representative areas and three points in each colour area (Appendix I, section I.3.1.1.1.) will enable to have relevant and representative data, allowing to have statistical value that can also be used in other analytical techniques, such as chemometrics. To quantify mixtures even more acquisitions are needed.

An estimate time to analyse a 10x10 cm illumination with the entire palette applied, reveals that five hours would be needed to assure its representativeness. For instance in a blue colour, 11 µ-EDXRF analyses, 11 µ-Raman and 1 micro-sampling would take at least 50 minutes (Appendix I, section I.3.1.1.2.). Regarding the number of illuminations by manuscript and the real time to analyse them, one must choose between a folio by folio analysis or selecting representative illuminations.

2.2. The Lorvão's palette, a macroscopic characterization

The characterization of the manuscripts started with their visual observation at Torre do Tombo. Although only nine manuscripts have been analysed and just from the end of the 12th century to the first quarter of the 13th century, they revealed that the Lorvão's palette was not only diverse but also very rich, as expensive pigments like lapis lazuli [93, 98, 99, 108] seemed to be present in almost every manuscript.

Apocalypse - As already mentioned, a distinctive feature of this manuscript was the use of a colour palette mainly restricted to yellow, orange and red, three meaningful colour of emblematic simplicity that "scholars believe was probably inherent from a lost North African tradition in Apocalypse manuscripts" [52]. The illuminator rarely uses black or brown; the former is used whenever contrast colour is necessary mainly into details (Figure 2.1.), and the brown colour was just used in three folia (*ff.* 177, 178v and 179), representing the earth[52] (Figure 2.2.).

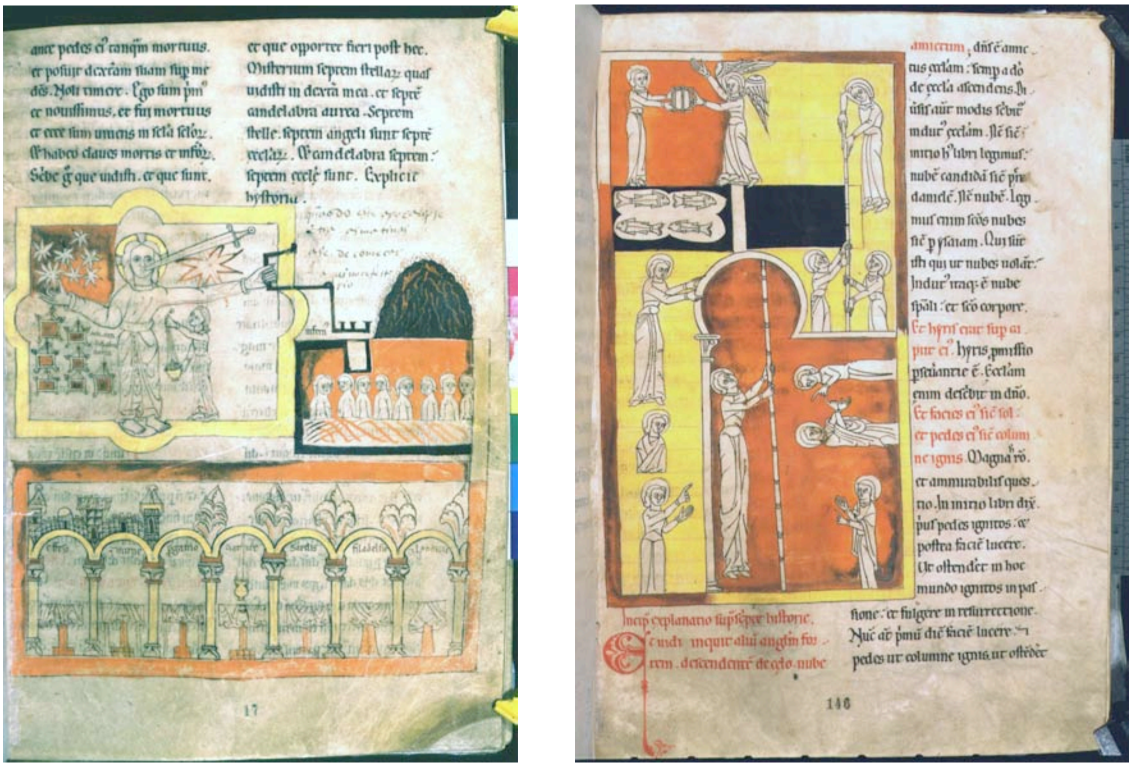

Figure 2.1. The use of black colour in *Apocalypse*, from left to right: *Mystery of the seven stars* (*f.* 17) and *Measuring the new temple* (*f.* 146). Photos: DGARQ-ANTT.

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[52] In chapter 16 of the Apocalypse is written: (16:2) "And the first (angel) went and poured out his vial upon the earth. And there fell a sore and grievous wound upon men who had the character of the beast: and upon them that adored the image thereof."; (16:3) "And the second angel poured out his vial upon the sea. And there came blood as it were of a dead man: and every living soul died in the sea. "; (16:4) "And the third (angel) poured out his vial upon the rivers and the fountains of waters. And there was made blood." As can be seen the colour brown is only applied to the earth.

Figure 2.2. The use of brown colour in *Apocalypse*, from left to right: T*he first angel poured out his vial* (*f.* 177), *The first angel poured out his vial* (*f.* 178v)[53] and *The second and the third angels poured out theirs vials* (*f.* 179). Photos: DGARQ-ANTT.

Book of Birds – In this manuscript it is possible to find what is known as the complete medieval palette: besides red and orange colours, already mentioned for *Apocalypse*, blue, green, white, black, carmine-coloured, yellow, brown and gold leaf are present.

Blue is the predominant colour. Besides being applied to the backgrounds of the ornamented initials (*ff.* 4 and 6v) and the circles with doves (*f.* 25), it can also be seen in folio 95v, in some of the close gardens that appear over the portico. This colour is also used in the bodies of the birds: to define the feathers (for example in *ff.* 40 and 66v), colouring the entire body (the hawk in *ff.* 5 and 16 and the crane *f.* 48) or the majority of it (for instance in *ff.* 30v, 59v and 63v) (Figure 2.3.). Depending on the folio this colour can have distinctive shades. (Figure 2.3.)

Figure 2.3. Different shades and applications of blue colour: to define the peacock's feathers (*f.* 66v), to colouring the entire hawk's body (*f.* 16) and the majority of quail's body (*f.* 63v) of the *Book of Birds*.

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[53] In the Lorvão *Apocalypse* there are two reresentations of the first angel pouring out his vial.

The green colour is just applied in two folia: 20v (palm tree) and 95v (Creation). The appearance of the green applied in the palm tree does not look the same as the green on all the other manuscripts from Lorvão, which was called “bottle green”. On the other hand, the one present in Christ’s mantle seems to be identical to the common Lorvão’s green.

Black, as well as the white, was used both in some details but also on the birds’ body. The black and white colours were used to decorate the hawk’s body in folio 16, to simulate the feathers, with lights and shadows (Figure 2.4.). The black colour was used on the entire body of the three naturally black birds (swallow, raven and blackbird) of the manuscript, but some differences between the paint applied to the swallow and to the other two birds can be observed (Figure 2.4.).

Figure 2.4. The black colour, applied to shade (*f.* 16, magnified: 12.5x); and to paint the bodies of swallow (*f.* 50v, magnification 16x), raven (*f.* 33, magnification 7x) and blackbird (*f.* 54, magnification 12x) (reading clockwise, from top left).

The use of white on the birds like stork, caladrius and swan (Figure 2.5) is not a common feature in the 12th century; usually whites were left unpainted taking advantage of the parchment colour [84]. White was also used as *impasto* drops to decorate the initials (Figure 2.5).

Figure 2.5. The white colour, applied to paint the bodies of caladrius (*f.* 60v) and swan (*f.* 65v). And white colour applied like drops, detail from an initial (*f.* 6v, magnification: 80x).

Carmine-coloured was used as shade on the orange colour (*f.* 4), a common feature detected in the Lorvão collection. It was also used by itself, for instance in the dove diagram (*f.* 6). Because it is transparent, the illuminator takes advantage of this to make different shades of carmine-coloured. The yellowish colour was just applied to some illuminated letters. (Figure 2.6.)

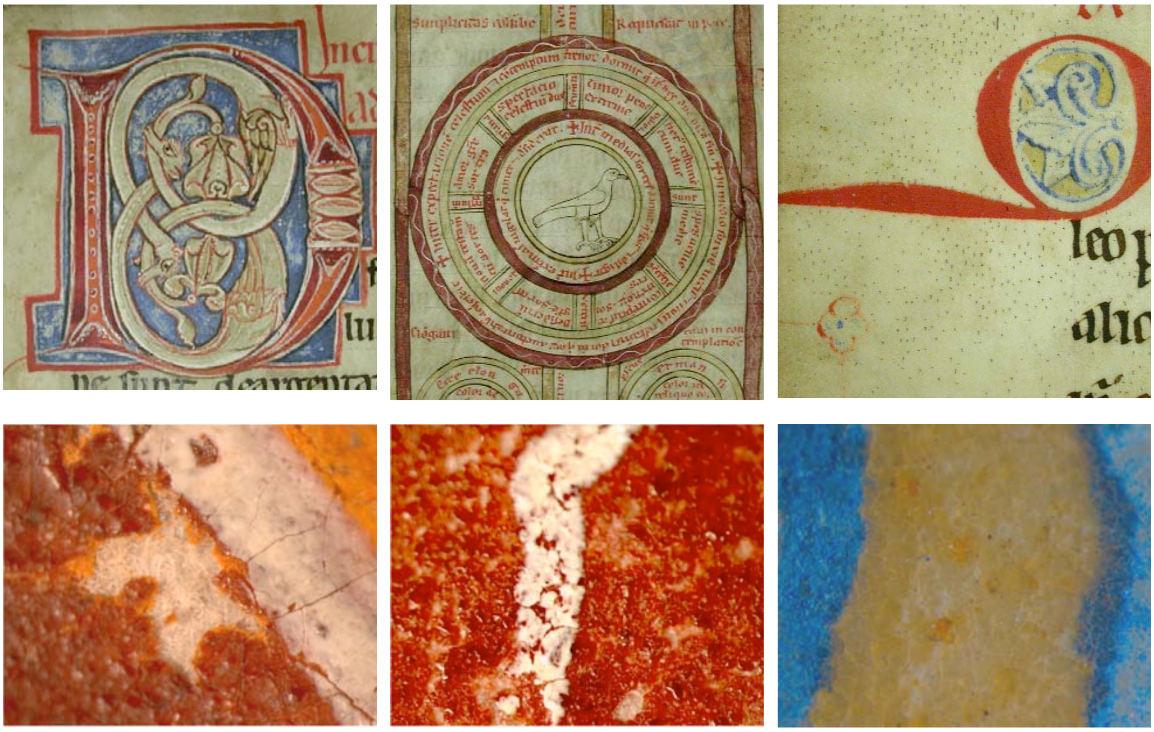

Figure 2.6. Carmine-coloured applied as shade in folio 4 (detail - magnification 80x), by itself in folio 6 (detail – magnification 80x) and yellow colour applied in folio 72v (detail - magnification 80x).

Yellow brownish was mostly used as a watercolour, for instance in the cock (*f.* 36v), it seems almost a diluted ink, applied to give more realistic visual effect. The gold leaf was only applied on the last folio (95v), to Christ's nimbus.

The other manuscripts from the 12th century – In *Enarrationes in Psalmos*, the other dated manuscript, it is possible to find the same colours present in the manuscripts already mentioned: blue, red, green, white, carmine-coloured, a yellow brownish diluted colour (as in the *Book of Birds*), and another shade of colour, the salmon pink colour. The gold leaf was also applied to some of this initials' manuscript.

Although the *Psalterium* (Lorvão 3) has some of the colours already cited, like red, blue and green, there are also hues that do not look the same: for instance, some of the blue colours are deeper (Figure 2.7.); the yellow colour is also different, it is neither a strong colour like the one used in *Apocalypse*, nor it is the vanished colour of the *Book of Birds*, but an opaque and pale colour (Figure 2.11.). The colour brown (Figure 2.18.) is very often applied, which is not common in the Lorvão collection.

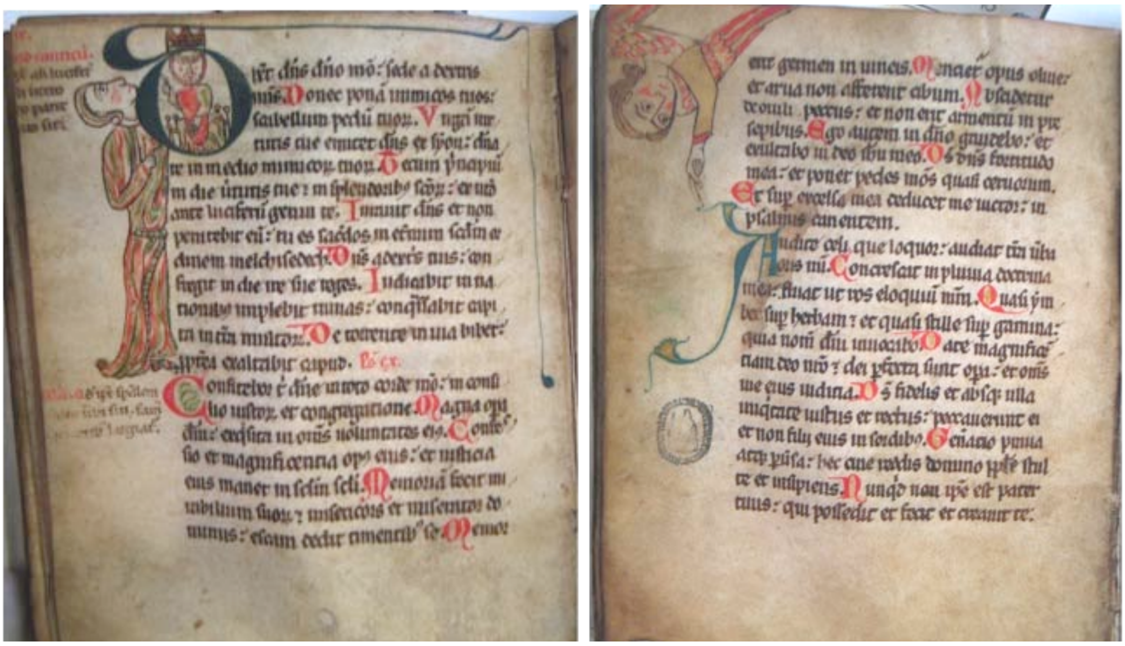

Figure 2.7. Different shades of blue in folia 82v and 110v, this one similar to the most used blue.

Finally the other 12th century manuscript, the *Martyrology* (Lorvão 16), has a very complete palette, with seven colours: blue, red, orange, green, brown, yellow and a pink colour. In this manuscript the colour is just applied to the initials. Although the colour disposition in these initials is very assorted, the pink colour was just applied once at folio 165v.

Other manuscripts from the 13th century – The *Saint* (Lorvão 12) and the *Temporal* (Lorvão 13) *Lectionaries* have almost the same palette applied to the decorated initials: red, green, black, deep carmine-coloured, a yellow colour and a brown colour. The white colour is applied in both manuscripts. The orange colour is also present but they have two different shades, the one present in *Lorvão 12* being a deeper orange shade, and the one of the *Lorvão 13* the most common orange. The blue colour is only applied in *Lorvão 13*.

The *Gradual* (Lorvão 15) is one of the manuscripts showing all the colours of the Lorvão palette. The colour in this manuscript was applied not only in minor initials but also in decorated initials that filled almost the entire page (Figure 2.8.). In this manuscript blue with different shades, red, green, orange, salmon, white, black, deep carmine-coloured, brown and grey colours were applied.

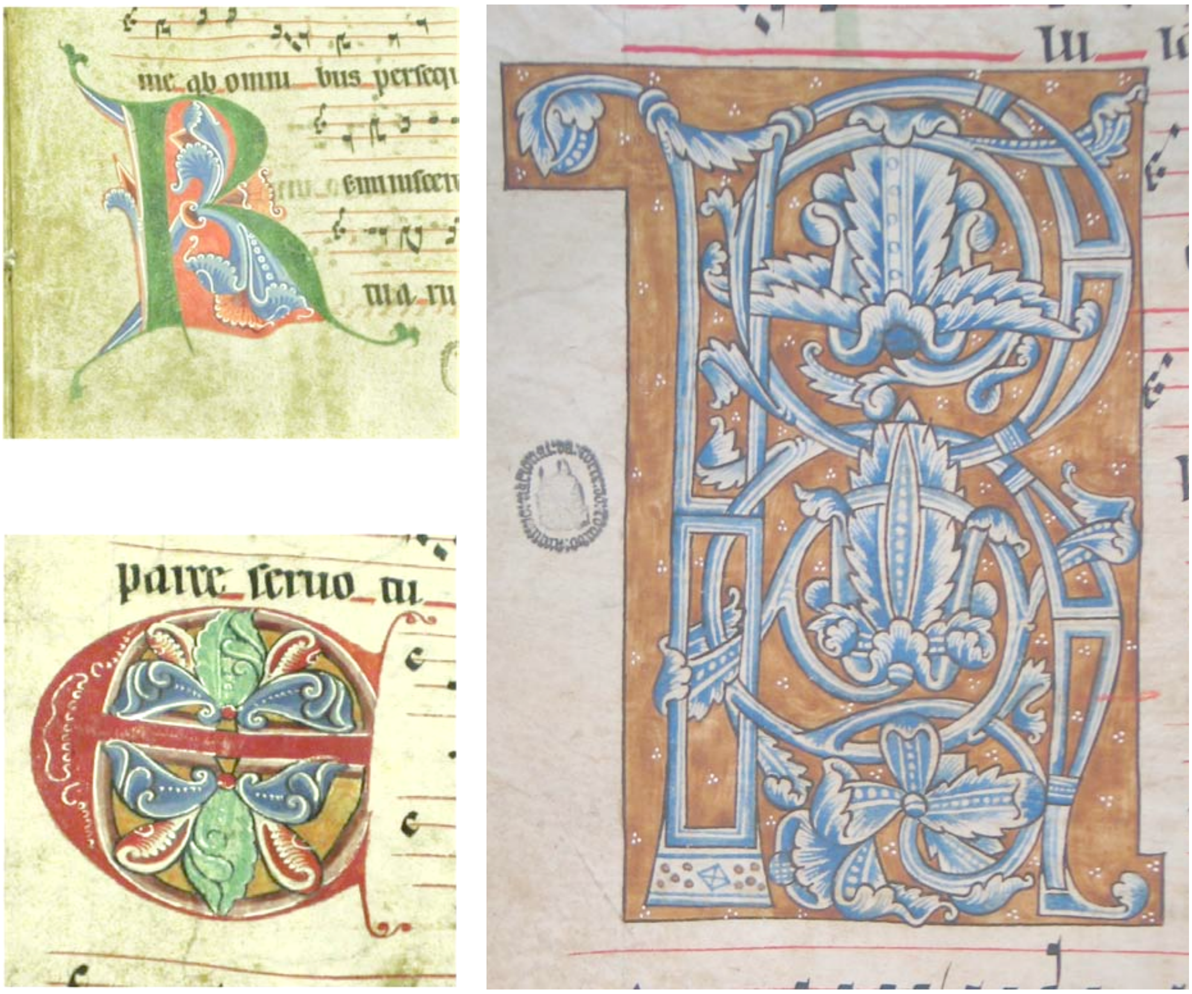

Figure 2.8. *Lorvão 15*, minor initials (*ff.* 11 and 26) and ornated initial (*f.* 125v).

The *Book of Calendas* (Lorvão 17) has only some painted initials, with the most common colours found in the Lorvão palette: blue, red, green, the characteristic “bottle green” present in all manuscripts with this colour, deep carmine-coloured and brown.

2.3. Book description and conservation condition

All manuscripts, except for the *Book of Birds*, were bound with non-original bindings, all of them dated from the 15th or 16th centuries (Figure 2.9.). The *Book of Birds* used to be bound, like the other manuscripts but a previous restoration made it impossible to handle it properly. This situation led to rebuild the binding in the manner of the original one, following the original sewing stations[54]. This, in turn, allowed us to easily observe all the illuminations of the manuscript while it was unbound.

Figure 2.9. *Apocalypse* binding (15th-16th century on wood covered with leather and brass bosses).

Regarding the conservation condition, all manuscripts are very well preserved considering their age. Although there is some degradation in the orange paint, the green paint recurrently presents a high degree of detaching from the parchment, which is also true of the blue paint but not so often, the paints are generally still in good condition. (Figure 2.10.)

Figure 2.10. Orange paint degradation in *Apocalypse*, *f.* 136 (left); Green paint detachment in *Lorvão 3*, *f.* 109v (center); Blue paint detachment in *Lorvão 16*, *f.* 32 (right).

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[54] The restoration intervention was performed at Conservation and Restoration department of ANTT.

Although the orange colour of *Apocalypse* is suffering from an extensive degradation process, where the bright orange is changing into a grey-blackish lustred colour [178], the manuscript is very well preserved, considering its 800-year existence and its exhibition presences. From the 69 illuminations, 18 are full illuminated folios and only three do not show visual orange degradation.

The *Book of Birds* is also very well preserved, although some of the blue paint has detached[55], for instance in folios 4, 25 and 63v. The yellowish paint applied in some minor initials, which would have been more intense at the time it was made, is now a trace that barely can be seen. The green paint applied to Christ's mantle, in the last folio (*f.* 95v), is detaching from the parchment, revealing some serious lacunas.

Lorvão 3 presents some corrosion problems in the parchment, implicit in the blue paint: where it was applied there is now a green section in the back. The yellow paint is cracking and coming loose, probably due to its thickness (Figure 2.11.).

Figure 2.11. Green section in the back of folio, where it was applied the blue paint, *f.* 71 (left) and yellow paint detail from *Lorvão 3*, *f.* 108 (magnification: 50x) (right).

Lorvão 12, *13*, *15*, *16* and *17* do not present problems besides the loss of some blue and green paints. In *Lorvão 16* the losses of the blue paint resulted in white sections (for example in *f.* 126), this was probably the original colour of the parchment after it had been treated with $CaCO_3$, which was usually performed before the illuminations were painted[56] [36, 43, 52] (Figure 2.12.). The loss of the blue paint in

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[55] The blue paint has been detaching, probably due to the large size of the pigment particles, which makes the cohesion action of the binder less efficient and facilitates detaching by running through.

[56] Ms. Amplonius Quarto 189 from Erfurt library refers: "…de creta, i.e., de petra alba quam spargunt scriptores super libros," [43]; Cennini advises the use of burnt-out and ground chicken bones: "Bisogna sapere che osso è buono. Togli osso delle cosce e delle alie delle galline, o di cappone; e quanto più vecchi sono, tanto sono migliori. (…) Nella pecorina tu puoi disegnare, o vero dibusciare, collo stile detto, mettendo prima del detto osso, seminato isparso e nettato con zampa di levre, per su per la carta, asciutto e spolvoreato in forma di polvere o di vernice da scribere." [36].

Lorvão 17 is evident in almost every minor initial and the green paint has been reacting with the parchment in such a way that in at least two folios (*ff.* 5v and 7) the parchment has perforations instead the green paint (Figure 2.12.).

Figure 2.12. Detail from *Lorvão 16*, *f.* 161v, where blue paint loss is patent (left); Detail from *Lorvão 17*, *f.* 5v, where corrosion occur in the parchment due to the reaction with green paint. (A recent restoration was performed to integrate the loss of parchment) (top right); Detail of gold detachment from a gilded initial of *Lorvão 50*, *f.* 64v (magnification: 25x) (down right).

Lorvão 50 is one of the best preserved manuscripts, even for those paints more susceptible to come off (green and blue). The visible lacunas are in the gilded initials, where it is possible to see some missing parts of gold (Figure 2.12.). This is probably due to the application technique or the quality of the gold leaf.

After a previous full colour observation it is necessary, as already mentioned, to go further and to analyse the paint composition at the molecular level. Compare it with other sources, in order to better understand what material colours could have been applied to the manuscripts and how medieval men have made them.

2.4. Creating a database

Creating a modern medieval palette, a method developed by Roosen-Runge back in the 60’s, seeing the difficulties (or lack thereof) of making medieval paints, can be a genuine challenge, but is truly rewarding when the colours and textures of these paints look similar to the ones in the manuscripts and even more when databases of diverse techniques can be built in order to enable a better characterization of the manuscripts’ paints. Considering previous research (see section 1.3), a range of colour paints that may be present in the Lorvão's manuscripts was created.

In this approach some aspects were considered: ***1)*** to create own samples of medieval materials, either when they are not available commercially, or to compare those made to the marketed ones; ***2)*** to understand the difficulties, or lack thereof, in producing some materials in order to appreciate their profuse or modest use in the illuminations, as well as the quality of the illuminations; ***3)*** to realize various possibilities of making the same colour paint with different sorts of pigments or binding media, different quantities of pigments when applied in mixture; ***4)*** to experiment with some paint techniques, for instance mixing the pigments in the same paint or applying different paints in layers; ***5)*** to evaluate the behaviour of the paints applied on the parchment over time; ***6)*** to develop a broad palette which would make possible a visual comparison with the illuminations of the manuscripts, either by naked eye colour comparison or by microscope surface comparison; ***7)*** to build a database for each technique that would be used in the characterization of the illuminations: μ-EDXRF, μ-Raman, μ-FTIR, HPLC-DAD,UV-Vis and μ-spectrofluorimetry.

Once a group of the more common medieval pigments, lakes and binders had been assembled, samples were painted, using different methods, onto several supports, like goat parchment, filter paper or PMMA. The last support was used whenever was necessary to obtain a signal of the paints' components without interference of it, while using μ-EDXRF technique.

2.4.1. Binders

Some medieval recipes appear too fantastic or very difficult to decipher, but the ones related to the binders were clear and quite easy to reproduce, which made possible the manufacture of all binders used in the reconstructions. The parchment glue was made following the indications of *O Livro de como se fazem as cores* [33, 34]; gum arabic was made according to *De Arte Illuminandi* [13] and glair was easier and more advantageous to made by stirring than squeezing, both suggestions being described in *De Clarea* [35].

Parchment glue and glair were used in most of the reconstructions[57]. Mixtures of glair and parchment glue were also made to reproduce the sort of varnish present for instance in the *Book of Birds*. The benefit of using honey or sugar was seen mainly in

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[57] Gum arabic was only used in the first reproduction set, before the three microsamples of *Apocalypse* were analysed.

the glair, the less used binder, because it is very brittle when dried and does not coat the pigment particles when used in the proportions recommended by the written sources [179]. The benefits of honey and sugar do not seem enough to use them continuously in the other used binders, parchment glue and gum arabic, that are more plastic by themselves.

In order to characterize the binders, the μ-FTIR technique was used. Were analysed, not only the ones manufactured to use in the paints' reconstructions, but also the binders that could possibly appear in one of the Lorvão manuscripts, like egg yolk, casein, collagen, rabbit and sturgeon glues, peach and plum tree gums (Figure 2.13.) (Appendix II, section II.2.1).

μ-FTIR proved to be a good technique to characterize both binders' families: proteinaceous or polysaccharide. Nevertheless, when it is necessary to distinguish between two proteinaceous binders, as parchment glue and glair, it may be seen that the spectra are overlaid, which makes a more detailed characterization by using just this technique difficult, without a full interpretation of the protein absorbance. Although when separately we can observe that glair CH stretching peaks are at higher wavenumber (2979 and 2943 cm^{-1}) than the ones from parchment glue (2975 and 2935 cm^{-1}). Chemometric analyses are presently being tested to better characterize the binders in illuminations[58].

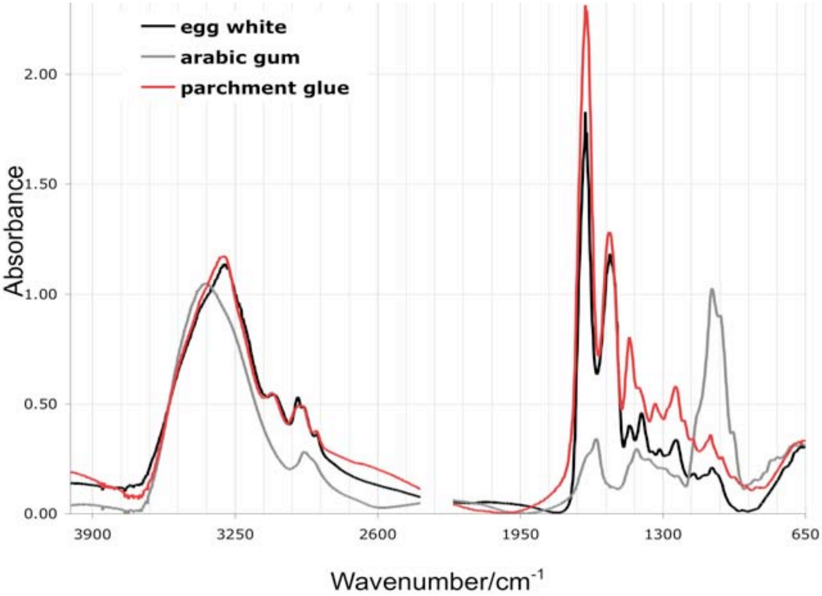

Figure 2.13. IR spectra of egg white (black), gum arabic (grey) and parchment glue (red).

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[58] A project about illuminations "The identity of Portuguese medieval manuscript illumination in the European context", project reference: PTDC/EAT/65445/2006 (2007-), in which the chemometric studies have been developed, is still ongoing.

The proteinaceous binder was detected by the presence of the polyamide pattern, namely the amide I (CO stretching, 1653 cm^{-1}), amide II (CN stretching and NH bending, 1550 cm^{-1}) and CN bending at 1450 cm^{-1}, and also by the OH and NH stretching at 3400-3000 cm^{-1} [180-186] (The assignments of the vibration bands are presented in Appendix II, section II.2.1, Table II.2.*).*

Polysaccharide binder presents an intense and broad absorption resulting from the hydroxyl groups and bound water (OH stretching, ≈3384 cm^{-1}), which overlaps in part the CH stretching; the carboxylate group is present by a strong vibration (C=O stretching asymmetric 1617 cm^{-1}) and a weak vibration (symmetric at ≈1419 cm^{-1})[187]. In the fingerprint region the C-H bending are produced by the C-H bonds (spectral envelop between 1420 cm^{-1} and 1220 cm^{-1}). Finally but essential, the region from 1200-1000 cm^{-1} contains skeletal C-O and C-C vibration bands of glycosidic bonds, and usually presents a well defined structure, which enables the characterization of the sort of gum [188-190]. The following vibrations can be assigned, at 1150 cm^{-1} COC stretching, at 1120 cm^{-1} CC (CO) stretching, 1080 cm^{-1} OH bending and CO stretching and finally at 1040 cm^{-1} CC(CO) stretching [187] (see Appendix II, section II.2.1,Table II.3.).

2.4.2. Pigments, fillers and paints

The majority of the pigments used in this study, were obtained commercially or by grinding the minerals, as in Medieval period. Following recipes, mainly from *O Livro de como se fazem as cores* [33, 34] pigment syntheses were prepared, some more successfully than others.

Based on the already mentioned research, the colorants use to produce the paints were vermillion, *minium*, orpiment, saffron, lapis-lazuli, azurite, indigo, malachite, verdigris, white lead, calcium carbonate, calcium sulfate, bone black, ivory black, vine black, madder lake, alizarin lake, purpurin lake, lac dye, cochineal lake and dragon's blood (Appendix I, section I.3.1.2.).

Small samples were painted with all these colorants and they were also painted as mixtures occasionally cited in the medieval texts (Figure 2.14.). The mixtures made were: for <u>red</u>: vermillion with *minium*, vermillion with white lead and vermillion with calcium carbonate; for orange: *minium* with vermillion[59], *minium* with orpiment, *minium* with vermillion and orpiment, *minium* with fillers such as calcium carbonate or white lead; for blue: lapis lazuli with azurite, lapis lazuli with white lead, and lapis lazuli with white lead and with azurite were also made. The proportion of each component in the mixture varied according to the final colour required, for instance when white lead is added, or according to the use given to the paint, meaning that a red paint could be used to write (ink) or to paint.

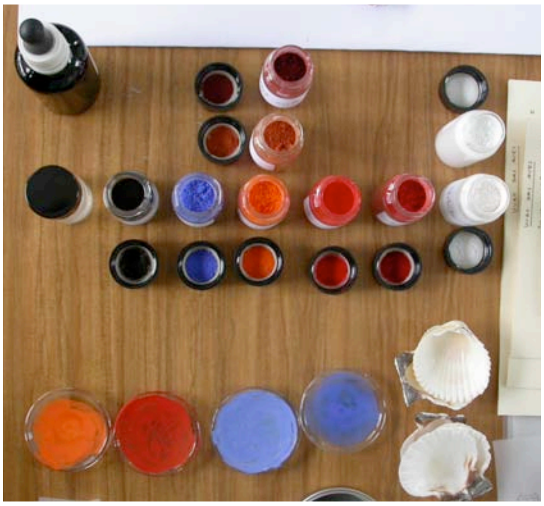

Figure 2.14. Some examples of paints made with *minium*, vermillion, lapis lazuli and white lead in different proportions, using parchment glue as a binder.

When producing the coloured paints, some of the aspects described in the medieval written sources were observed, for instance the care when lapis lazuli needed to be ground as reported in the *Göttingen Model Book*, “Blue you should not grind, unless it is rather coarse” [37]. This is also true for all the pigments based on transition metals, like malachite, azurite and verdigris. In all these pigments the element responsible for the colour is the copper ion that has forbidden d-d transitions [191]. Therefore the molar extinction coefficient (ε) will be low [192], which means that these pigments may not be ground into a fine powder because scattering will dominate over absorption, increasing the colour loss. On the other side are the pigments based on semiconductors[60], like white lead, *minium* (Pb) or vermillion (Hg). The colour of these pigments arises through a “very long-ranged rearrangement of electrons: absorption of light liberates electrons entirely from their orbits around particular ions and sets them free to wander through the solid” [93], making the material more

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[59] There is a warning in the Jehan Le Begue manuscrit, regarding the use of *minium*: “if anyone wishes to illuminate a manuscript he must not do that with *minium* only, because, although the letters may be well formed yet they would not be beautiful, for they would be too pale; he must therefore mix *minium* with vermillion, so that the colour may be brighter. (…) If the vermillion is very good and new, I put two parts of it, and scarcely the third part of *minium*. But if the *minium* is dusky and very old, put a half or a third part of vermillion, and make the remainder of *minium*” [30].

[60] Semiconductors are solid substances that have electrical conductivity [191], they need only little energy to boost electrons into a mobile state [93].

electrically conducting. Therefore, what Cennino wrote “if it is ground every day, for 20 days, the colour will be more beautiful” [36], makes perfectly sense.

The design of this database enabled us to better understand the challenge involved in paint medieval illuminations, to learn some tricks and to recognize advantages and/or disadvantages in different paint techniques tested. It also permitted us to figure out the difference between badly or well done paints, not only by seeing the common low cohesion and adhesion to the parchment, but also by observing its appearance microscopically. It allowed us to characterize the look of the pigments, lakes or dyes applied as paints to the parchment or other supports, the nature of a pure paint or a paint made with a mixture of two or more components.

The colours in the manuscript could then be checked against those "known" under each of the non-destructive techniques. However it is necessary to be cautious when comparing colour because it is quite impossible to know if the ones in the manuscripts have altered from their original structure. To avoid misinterpretations, mainly to organic compounds, since they degrade easier than inorganic ones, photodegradation and degradation studies are crucial to know which products could be found (Part B of this thesis).

It is also problematic to assume that the "re-created" lakes, inks and pigments are appropriate without being completely certain of all of the materials and techniques in the treatises. Once the information obtained from all the non-invasive techniques had been correlated, the manuscripts could be studied in a more efficient way. The suggested reconstructed paints could now be used to confirm, to clarify or disprove the medieval colour composition from the Lorvão *scriptorium*.

2.5. Inorganic material analysis

2.5.1. Characterization of the main paint materials

The characterization of inorganic colour material from the *Apocalypse* was performed in 24 folios (Appendix I, section I.3.1.1.1.), a selection of the most representative, meaning the ones where the three key colours (red, orange and yellow) were used simultaneously in the same folio (for instance *ff.* 43, 49 and 217) or separately used, like in the folios where the stones of Heavenly Jerusalem are described and each circle was painted regarding the stone colour [61] (*ff.* 208v and 209),

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[61] In Lorvão Apocalypse, only the circles representing the red (amethyst, although this stone is purple it is also represented with red colour in other Beatus [193], sardonyx and chysoprase, this one is green but according to Isidore of Seville is red at night [193]) or yellow (sapphire, chrysolite and hyacinth) stones were painted, the others circles representing jasper, topaz and emerald (green), chalcedony and beryl (in both cases, one of their natural colours is white) were left unpainted.

or a group of folios with a heterogeneous orange degradation (like in *ff.* 49, 115, 118) and even the three folios where no degradation of orange seems to be occurring (*ff.* 43, 185v and 186v) (Figure 2.15.) (Table 2.1.).

Figure 2.15. Three of the *Apocalypse* folios selected, from left to right: The woman over the beast (*f.* 43), Of stones and colours (*f.* 208v), Message to Efeso (*f.* 49). Photos: DGARQ-ANTT.

From the *Book of Birds* 19 folios (Appendix I, section I.3.1.1.1.) were selected to study one of the most complete Lorvão inorganic palettes. That allowed all the colours present in the manuscript to be covered. When the folios selection was made, particular attention was given to the folios with the blue colour, in order to understand what originates the difference shades of the blue paints (for instance between the folios 5 and 16). In the same manuscript, the characterization of the black paint used in three birds (*ff.* 33, 50v and 54) (Figure 2.4.) was also an objective to accomplish. Lastly, understand the association pigments/techniques used to paint some peculiar details on the initials (*ff.* 4 and 6v) and on the birds (*ff.* 5, 16 and 50v) was also one of the aims. Since this manuscript has some illuminations with less paint applied or with very little details, the balance between the representativeness and the quality of the information acquired in such folios was also a very important criteria to consider, which favours the selection of the folios with more paint, making it more suitable for analyses with all the non-invasive techniques, mainly the µ-EDXRF which from all the techniques is the one that has the major area of analysis (70µm) (Table 2.1.)

The selection of the other manuscripts' folios followed similar criteria, giving special attention to red, blue and green colours, in order to realize if there was a pattern or a correlation between all the contemporary Lorvão manuscripts. All the folios studied (not only with inorganic but organic materials too) are in Table 2.1. (See also Appendix I, section I.3.1.1.1.). In the *Lorvão 3* a higher number of folios was analyzed because the sort of colours in this manuscript considerably differs from the other ones (Figure 2.16.). So, in order to have a consistent characterization, more folios needed to be examined. From the nine Lorvão manuscripts, 154 folios were analyzed.

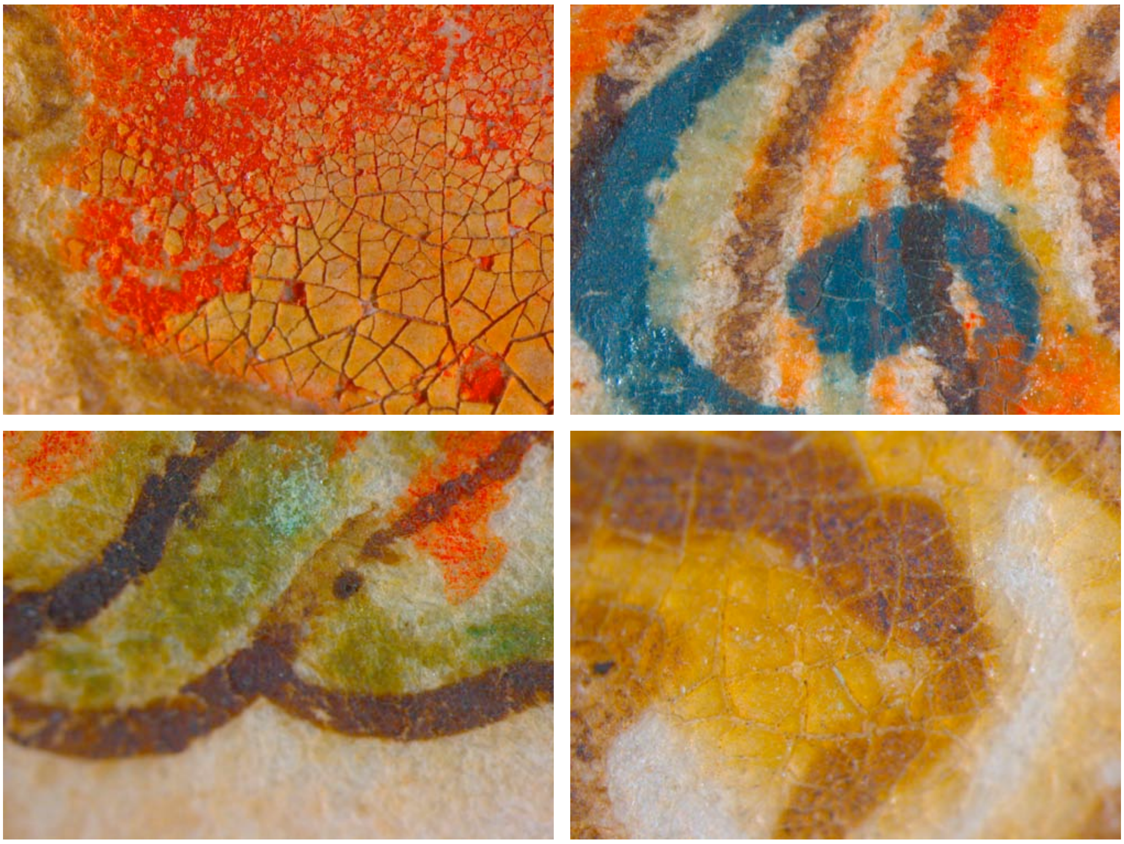

Figure 2.16. Details from the colours of *Lorvão 3*: red (*f.* 98v, magnification 25x); blue, orange and red (*f.* 109v, magnification 63x); brown (*f.* 107v, magnification 40x); green (*f.* 82v, magnification 32x) (reading clockwise, from top left).

Table 2.1. Folios selected to be analysed with different analytical techniques.

Manuscript	***Analyzed folios (μ-sampling, μ-EDXRF, μ-Raman)***	***Total folios***
Apocalypse	1*, 43, 49, 79, 115, 118, 122v, 136, 138v, 158, 172v, 177, 178v, 179, 185v, 186v, 193, 196v, 200, 208v, 209, 217, 217v, 218v	24
Book of Birds	4, 5, 5v, 6, 6v, 7v, 16, 20v, 25, 33, 36v, 48, 49v, 50v, 54, 56v 59v, 72v, 95v	19
Lorvão 3	1, 8v, 9v, 14v, 15, 16, 17, 17v, 19, 19v, 23, 27, 35v, 36v, 43, 46, 46v, 51v, 54v, 59v, 71, 71v, 82v, 87v, 98v, 101, 107, 107v, 108, 109v, 110v, 113, 139v, 146v, 149	35
Lorvão 12	6v, 7v, 8, 11, 17, 23v, 30, 38v, 39, 50, 53v, 64, 94	13
Lorvão 13	1v, 6v, 19v, 21v, 30, 35v, 39v, 44v, 63v, 73, 80v, 85, 92v, 114	14
Lorvão 15	5v, 6, 11, 16, 26, 36v, 38v, 50, 125v, 154v, 167v	11
Lorvão 16	2v, 7v, 8, 11, 24v, 25, 32, 34, 35v, 59v, 71v, 73, 126, 155, 161v, 165, 165v, 188	18
Lorvão 17	7v, 9, 9v, 34, 35, 74v, 127, 162v, 169v, 230	10
Lorvão 50	1v, 17v, 34v, 37v, 64v, 173, 173v, 196v, 197v, 248v	10

*This folio was analysed, although it does not belong to the original *Apocalypse*. Therefore its results are not presented.

In *Apocalypse* the 24 folios were analysed by µ-EDXRF, a total of 271 points (five points per area); ten folios were studied by µ-Raman and it were collected 35 micro-samples from seven folios. In *The Book of Birds*, 17 of the 19 folios were analyzed by µ-EDXRF, about 205 points (three points per area) (Figure 2.17.). This characterization was complemented with the information obtained from the nine folios analyzed by µ-Raman and the 46 micro-samples, performed in 14 folios, interpreted by µ-FTIR. The study of these two manuscripts enabled to create the Lorvão palette, which was then compared to the other Lorvão manuscripts, not so exhaustively studied (Table 2.1.).

Figure 2.17. Book of Birds (*f.* 95v) analysed by µ-EDXRF.

The results obtained using these three techniques enable the characterization of the inorganic paint components for:

Oranges: The orange colours analyzed are based on *minium*. The main element observed by µ-EDXRF, in all manuscripts was lead (Pb)[62] and *minium* was identified by µ-Raman (characteristic bands at 548, 390, 223, 149 and 122 cm^{-1}) [195] (Appendix I, section I.3.1.2.). The orange of *Lorvão 13* and *Lorvão 15* is made only by *minium*. Some of the oranges have other compounds besides the *minium*, like in the *Apocalypse*, vermillion and orpiment were also detected in minor or just residuals quantities (Figure 2.18.). *Lorvão 12* and *Lorvão 16* revealed a different composition, being vermillion the main element detected in the mixture of it and *minium* (representative spectra of all manuscripts can be see in Appendix II, section II.3.1.)

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[62] Absorptions coefficients and the quantum yields of emission (Mo anode: 17,44 keV) of all detected elements [194] are given in Appendix II, section II.4.2.1.

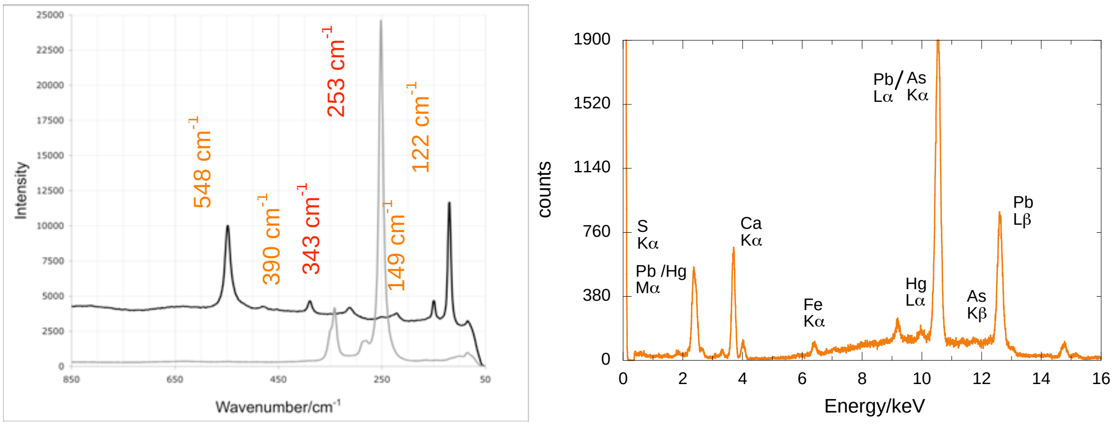


Figure 2.18. Raman spectra of orange colour, folio 179 (black line) and red colour, folio 49 (grey line) from *Apocalypse* (left). EDXRF spectrum of orange color of folio 185 from *Apocalypse* (right).

Lorvão 3 and *Lorvão 50* do not have orange but a salmon colour, in the first manuscript, analyzed by µ-EDXRF, the Pb and Hg were found together, unfortunately this colour was not analyzed by µ-Raman to better understand if it results from a mixture of vermillion, and lead white, or one of these two pigments with *minium*. In *Lorvão 50* only Pb was detected by µ-EDXRF, but a mixture of *minium* and white lead was detected by µ-Raman (the spectra are in Appendix II, section II.3.1.).

When analysed by µ-EDXRF, the difference between the degraded and non-degraded oranges, in the majority of folios, revealed a higher ratio of Pb and S for degraded orange. This observation is inconclusive but a recently study [178] revealed through the analyses performed on the *Apocalypse* by µ-Raman and using samples aged in laboratory, that the main lead degradation product formed when red lead and orpiment are reacting is galena, and arsenic trioxide seems to be forming.

<u>Reds</u>: the analyses performed on the Lorvão manuscripts revealed that are two sorts of reds, the ones used on the illuminations and the others applied in *rubricae* (Figure 2.19.). Usually in the illuminations it was detected by µ-Raman, vermillion (characteristic bands at 343, 285 and 253 cm^{-1}) (Figure 2.20.) [195] (Appendix I, section I.3.1.2.).; *minium*, white lead and calcium carbonate (the last two also confirmed by µ-FTIR) (Figure 2.21.), are in minor quantities. An extensive study was performed mainly by µ-EDXRF (200 points in 94 different folios), which makes the characterization of the lead pigment dubious, as it could be white or red lead (Figure 2.22.), but according to medieval texts the use of *minium* and vermillion mixtures was common [30]. In *rubricae* vermillion was detected alone (representative spectra are in Appendix II, section II.3.1.). The possibility of cinnabar could have been used was rejected because by µ-EDXRF, there were no traces of other elements usually

associated in natural minerals, such as magnesium (Mg) or chlorine (Cl) and the synthesis of vermillion was already developed [15].

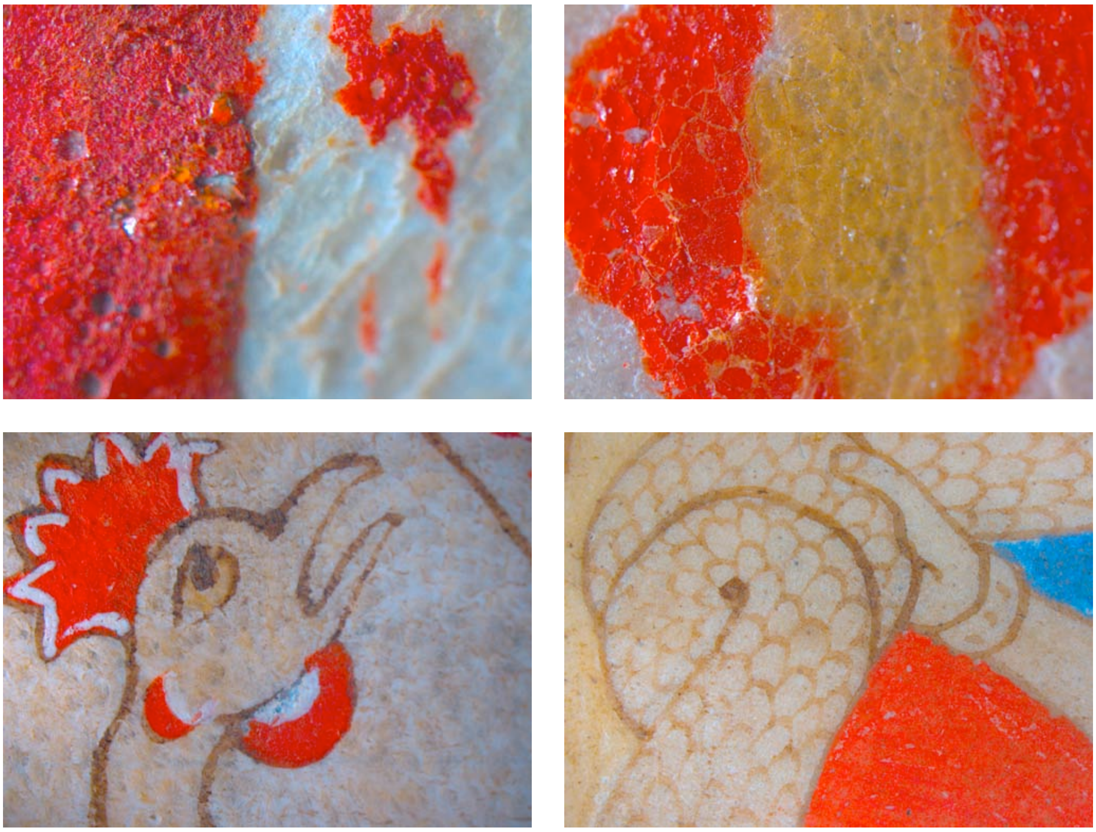


Figure 2.19. Details of different sorts of red used, from left to right: *Apocalypse* (*f.* 118, magnification 63x) and *Lorvão 3* (*f.* 17v, magnification 40x) are *rubricae*; *Lorvão 16* (*f.* 171v, magnification 7.1x) and *Book of Birds* (*f.* 36v, magnification 10x) are from illuminations (reading clockwise, from top left).

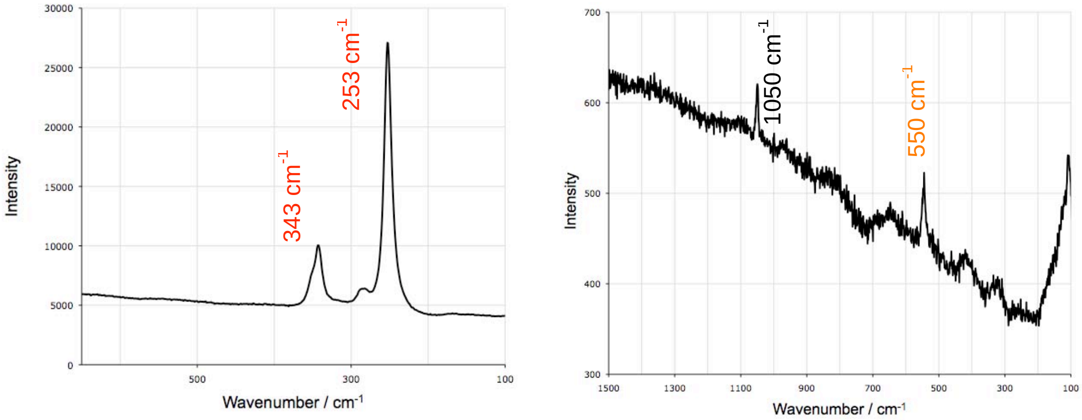


Figure 2.20. Raman spectra showing the presence of vermillion (left) and the red and white lead (right), of the red used to illuminate folio 92v from *Lorvão 13*.

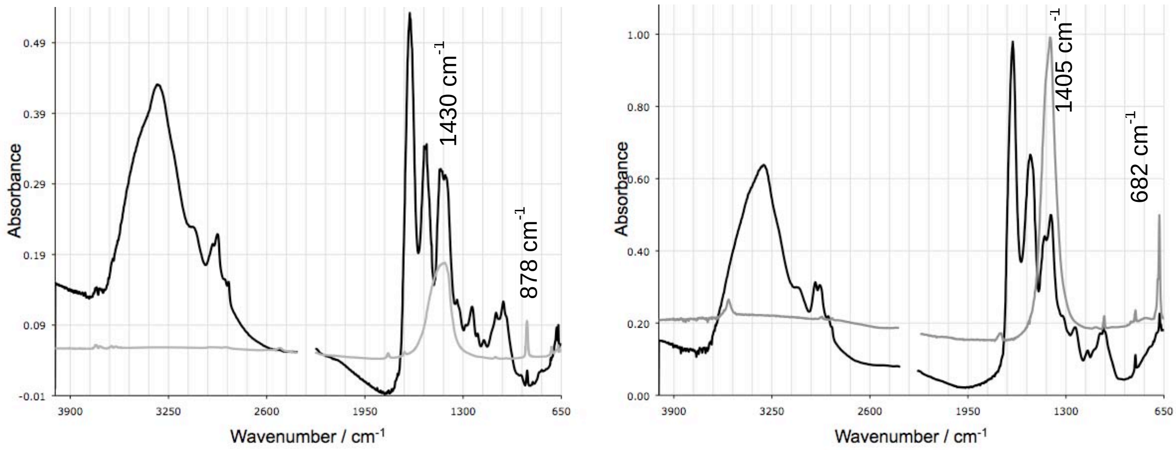
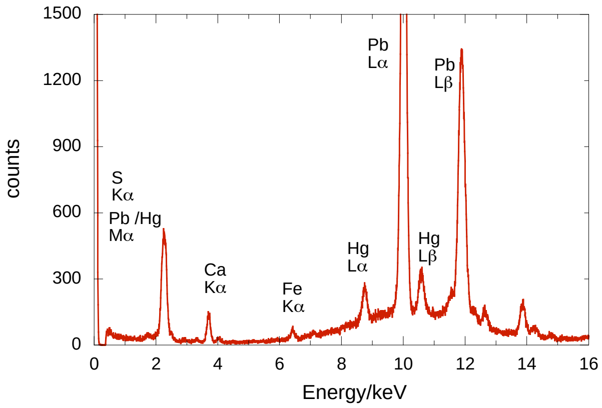


Figure 2.21. FTIR spectra of the red colour used to illuminate folio 200 from *Apocalypse* (black line) and calcium carbonate (grey line) (left); and folio 17v from *Lorvão 3* (black line) and white lead (grey line).



Detail from Lorvão 13 , *f.* 6v (magnification: 7x)

Figure 2.22. EDXRF spectrum of the red used to illuminate folio 92v from *Lorvão 13*.

Yellows: In Lorvão *scriptorium*, the only inorganic yellow found was orpiment, but only in the *Apocalypse*, being identified by µ-Raman the characteristics bands of this pigment at 353, 309, 292, 154 cm^{-1} [195] (Figure 2.23.)(Appendix I, section I.3.1.2.). The other yellowish colours of the remaining manuscripts are of organic nature, because there were no elements detected, although the ones of *Lorvão 3, 12* and *13* could have been mixed with a filler based on $CaSO_4$, detected by µ-FTIR and µ-EDXRF since in these situations Ca was detected in higher quantities than the ones obtained just from the parchment and S was also identified (Figure 2.24.) (representative spectra are in Appendix II, section II.3.1.). In one of the folios (*Lorvão 3*, *f.* 108) analyzed by µ-Raman the presence of gypsum was detected [196] (Figure 2.11.). The µ-FTIR could have clarified what kinds of organic yellows were used, but the spectra obtained revealed only the binder that is probably in more quantity than the dye (if the watercolour technique was applied), making presently impossible the characterization of the organic yellow.

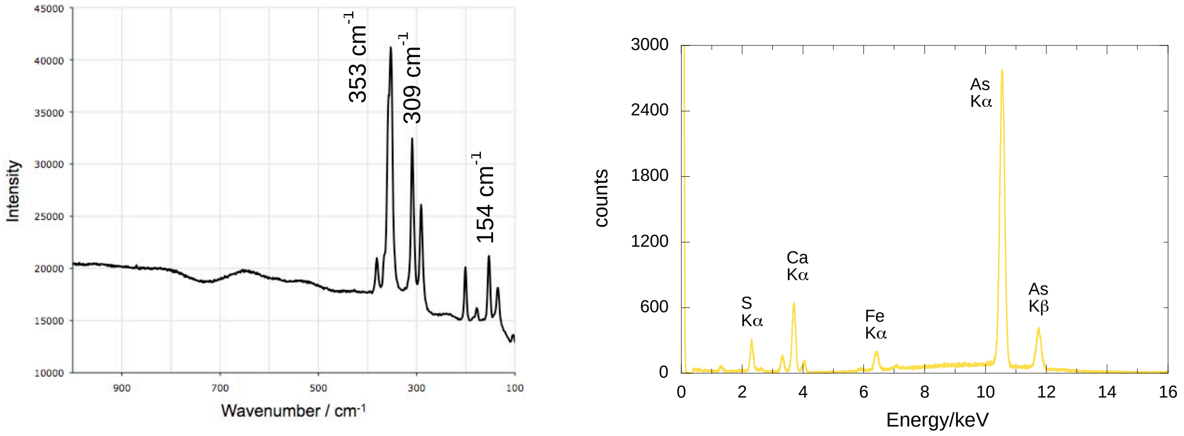


Figure 2.23. Raman (left) and EDXRF (right) spectra of the yellow used in folio 43 from *Apocalypse*.

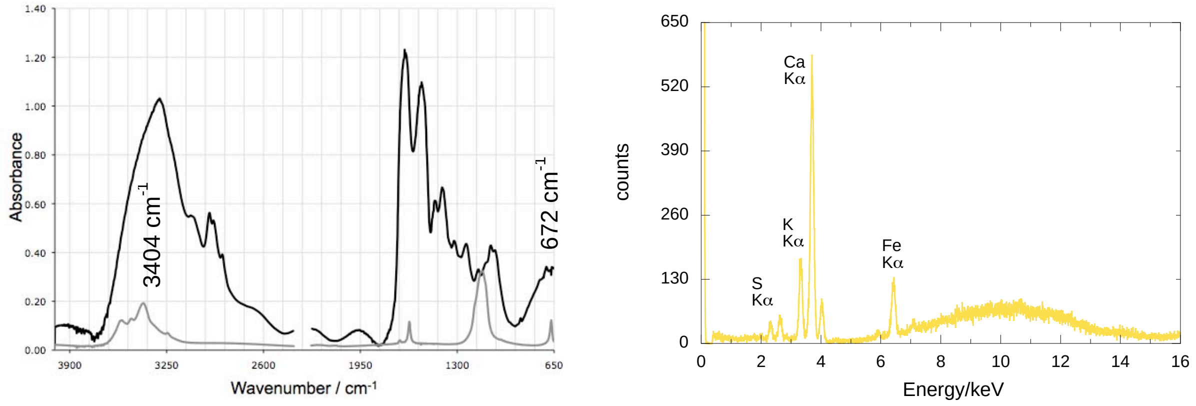


Figure 2.24. FTIR spectra (left) of the yellow (black line) used in folio 108 from *Lorvão 3*, and of the calcium sulphate dihydrate (grey line); and EDXRF spectra of the yellow used in folio 51v from *Lorvão 3* (right).

Blues: this colour is mainly composed of lapis-lazuli, identified by all the techniques used: μ-EDXRF detected the elements that compose lazurite[63]: Al, Si, K and Ca and Fe from pyrite; μ-Raman: revealed its bands at S_3^-: 255 and 285 (bending), 544 stretching, S_2^- 580 cm^{-1} [197-199] and with μ-FTIR it was observed the typical Si-O stretching region of aluminosilicate matrix at 1050 cm^{-1}, which has also a recognizable asymmetric shape from a shoulder near 1200 cm^{-1} [66]; the Al-O vibrations below 750 cm-1 [200] (Appendix I, section I.3.1.2.). However it is important to realize that when pure lapis-lazuli [201] was applied in the illuminations, in the same folios azurite is also found [202] in the minor initials. Its low concentration does not enable us to conclude if it was a impurity or if it was added with a purpose, which could be identical to the one

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[63] Lapis lazuli main component is lazurite $(Na,Ca)_8$ $(AlSiO_{12})(S,SO_4,Cl)$, and usually, it also has pyrite (FeS_2) associated.

used in red, when vermillion is mixed with *minium*[64] (Figure 2.25). This will be further discussed in section 2.5.2.1.. The presence of azurite identified by the presence of the element copper with µ-EDXRF and with µ-Raman (the two most intense bands: 401 and 294 cm^{-1} corresponding to Cu-O bond stretching and O-Cu-OH bending vibration, respectively [60]), was confirmed by µ-FTIR, through the presence of an intense broad band of COO stretching and a sharp peak of the bending, also by the characteristic asymmetric and/or symmetric stretching at 1504 and 1417cm^{-1}, and bending at 837cm^{-1}, due to carbonate group [184]. Furthermore, the expected absorption due to the OH stretching is observed at 3430cm^{-1} (Appendix I, section I.3.1.2.).

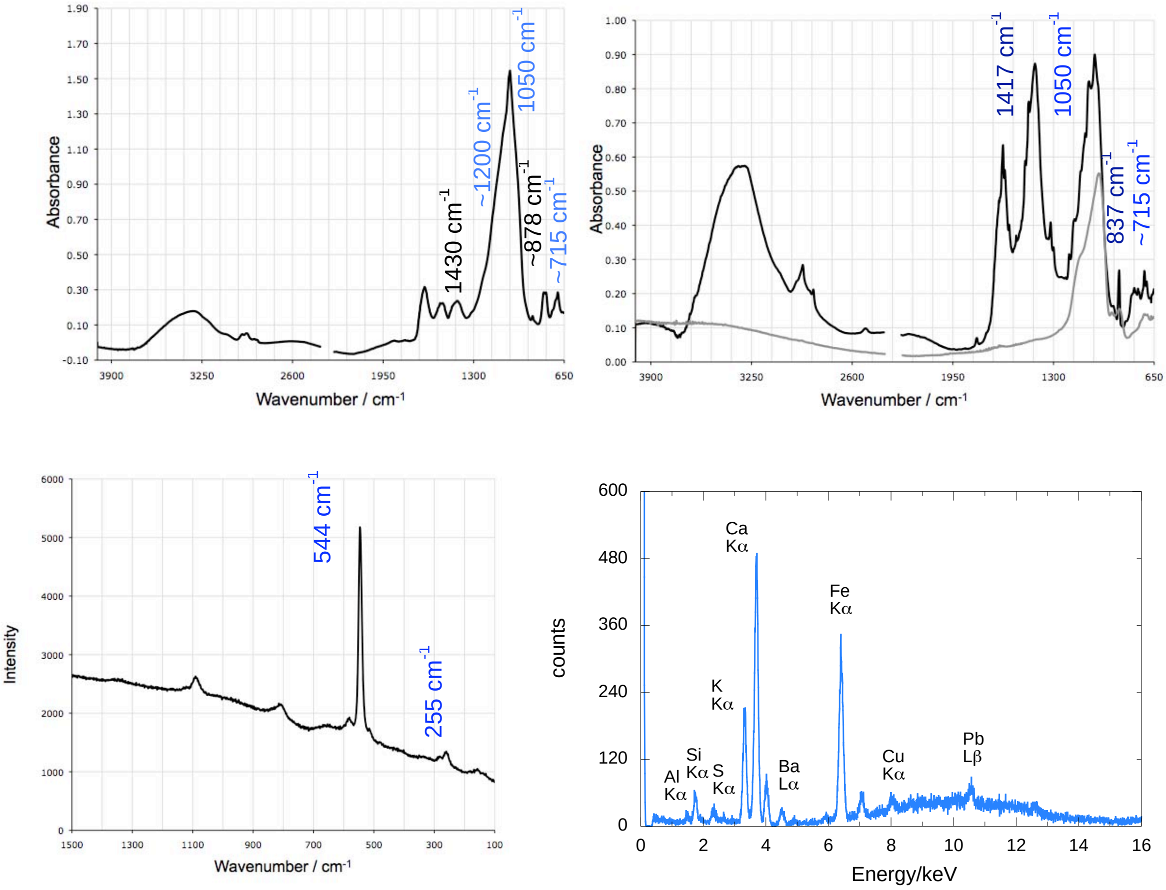

Figure 2.25. Blue colours present in the same folio 50 from *Lorvão 15*: FTIR spectra (above) where the pigment detected was only lapis lazuli mixed with the filler calcium carbonate (left) and other where a lapis lazuli is mixed with azurite (right); Raman spectrum showing the presence of lapis lazuli (left below) and EDXRF spectrum denoting elements that could belong to lapis lazuli (Al, Si, S, Ca, Ba and Fe), azurite (Cu) and white lead (Pb) (right below).

The *Book of Birds* has some blues highlighted with white lead (see section 2.5.2.1)

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[64] By mixing lapis lazuli with azurite the cost of the paint would decrease, without changing the look of the final colour. In the same way that vermillion a pigment more difficult to obtain than *minium*, would reduce the costs associated to the red colour.

Lorvão 3 presents two sorts of blues: 1) a grey-bluish hue achieved by mixing lapis-lazuli, azurite and indigo, this one was observed with µ-Raman (presents two of its characteristic bands assigned to bending vibrations involving the central C=C (549 cm^{-1} and 599 cm^{-1}) [199, 203]) and with µ-FTIR (N-H stretching at 3268 cm^{-1}, C=O stretching at 1628 cm^{-1}, six-member ring C-C at 1585, 1483, 1461, 1317 cm^{-1} and N-H in plane bending at 1392 cm^{-1} [184, 204]) (Figure 2.26); 2) and other with a larger quantity of azurite (Figure 2.27.), when compared with the other blues that can justify the green degradation present in the verse of the folios. This sort of degradation is common in manuscripts where copper based pigments were applied, but usually is associated to copper green pigments [205, 206].

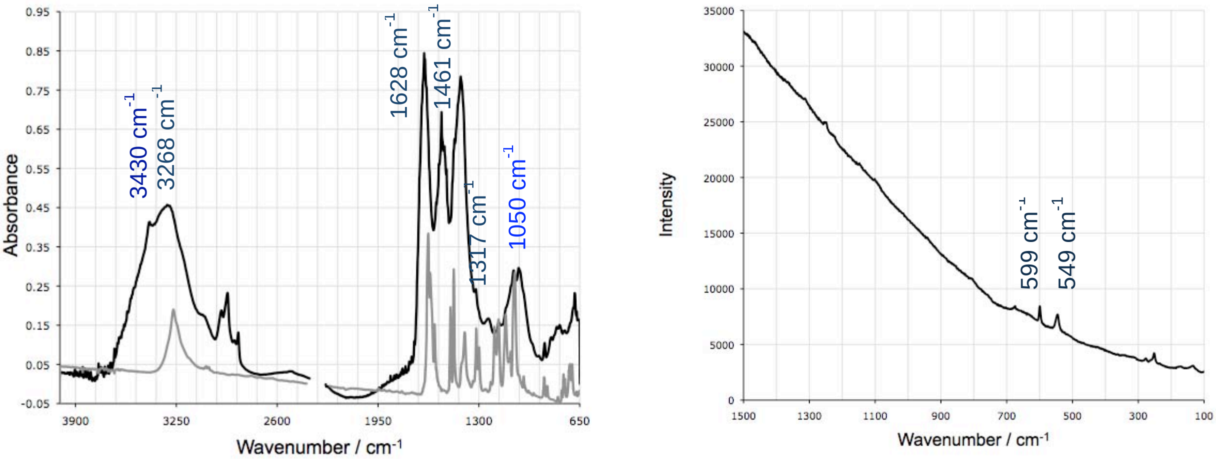

Figure 2.26. FTIR spectra (left) of the blue (black line), a mixture of indigo, azurite and lapis lazuli, used in *Lorvão 3,* folio 46v, and indigo (grey line). Raman spectrum of blue applied in *Lorvão 3*, folio 107v.

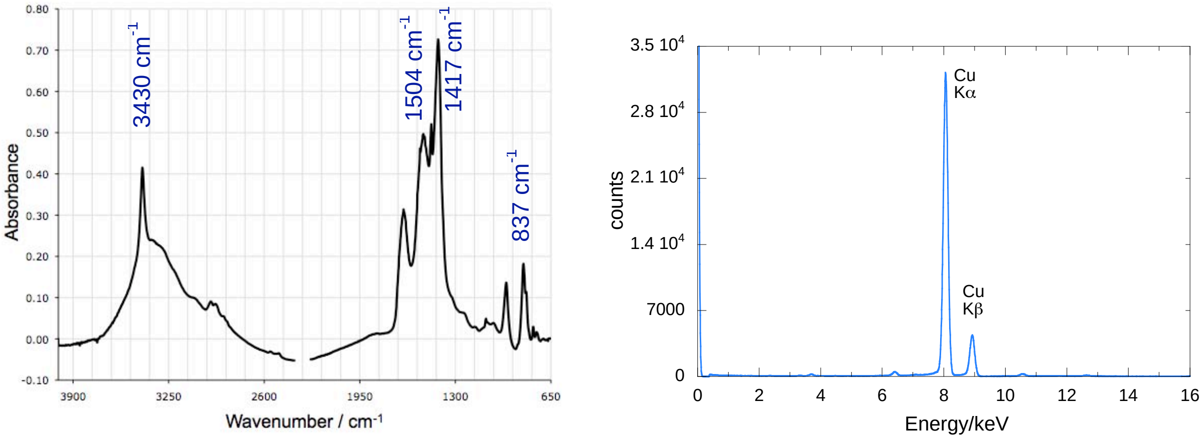

Figure 2.27. FTIR (left) and EDXRF (right) spectra of the blue used in folio 23 from *Lorvão 3*, which is producing the green corrosion on the parchment.

Lorvão 15, *16* and *17* also have blues highlighted with white lead and the grey-bluish hue, which is a mixture of lapis-lazuli, azurite and indigo. All three manuscripts have just one folio (*ff.* 50, 73 and 230, respectively) (Figure 2.28.) where indigo and carbon black are mixed in order to obtain a deeper blue, in *Lorvão 16* and *Lorvão 17*, and an almost a grey shade, in *Lorvão 15*, also explained by the addiction of white lead, and the filler $CaCO_3$ added in all the blue paints of this manuscript (Figure 2.25.) (representative spectra are in Appendix II, section II.3.1.).

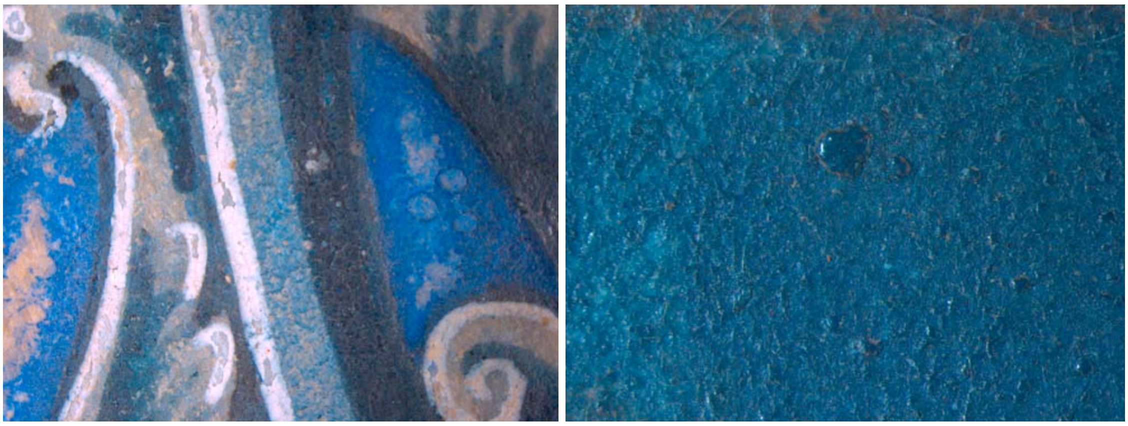

Figure 2.28. Details from *Lorvão 15*, *f.* 50 (left, magnification: 10x) and *Lorvão 16*, *f.* 73 (right, magnification: 50x).

Greens: There were identified two kinds of green colours. One was just observed in two folios of different manuscripts (*Book of Birds – f.* 20v and *Lorvão 17 – f.* 169v) as being probably malachite, identified by µ-Raman[65] (the characteristics bands are at 143, 432, 754, 1001, 1052 and 1094 cm^{-1} [207]) (Figure 2.29.) (Appendix I, section I.3.1.2.). This was not confirmed by µ-FTIR, but if it were malachite then it should have been easy to identify (carbonates stretching COO at 1500 cm^{-1} and a sharp peak of the bending at 1095 cm^{-1}, double peak due to OH stretching at 3410 and 3330 cm^{-1}, and a very intense doublet of the OH bending at 1098 and 1047 cm^{-1}) [67]. This could mean that this pigment may be similar to malachite or eventually has been reacting and changing to some similar compound. µ-EDXRF identified copper, zinc and lead as the main elements present in all the green colours (Figure 2.29.), pointing out to the possible use of brass, a copper zinc alloy, as starting material for pigment green synthesis (Table 2.2.). Other elements, such as chlorine, potassium and calcium were also detected, but they are also present in the parchment (representative spectra are in Appendix II, section II.3.1.).

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[65] The results obtained by µ-Raman were not very clear since the laser used (632 nm) is not the best for analysing green pigments. Better would be a 514.5 nm laser.

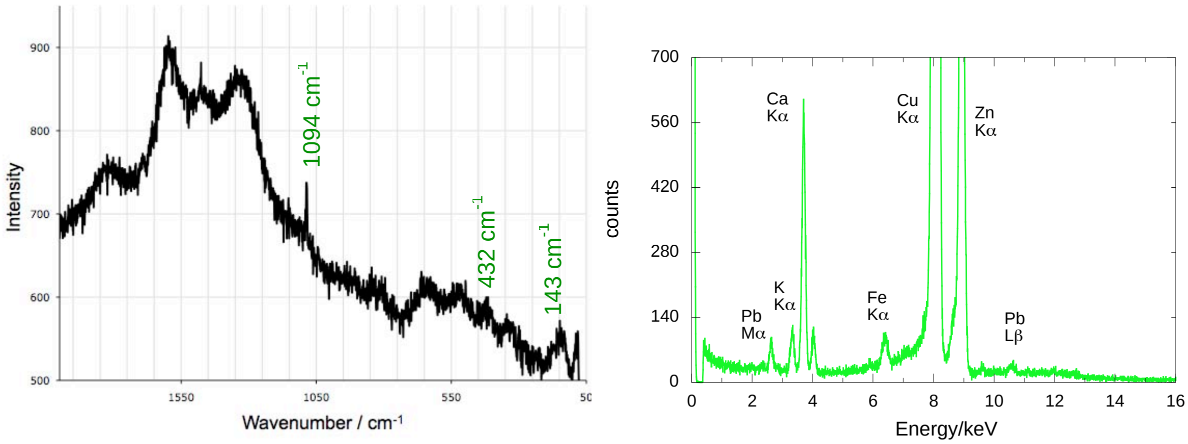

Figure 2.29. Raman (left) and EDXRF (right) spectra of the green used in Lorvão 17, folio 169v.

Table 2.2. Elemental intensity ratio of Cu/Zn and Cu/Pb of green paints from Lorvão manuscripts (see spectra in Appendix II, section II.3.2.).

Folio	*Cu/Zn*	*Cu/Pb*
Lorvão 12**, **f.** **8	37	-
Lorvão 12**, **f.** **50	36	-
Lorvão 13**, **f.** **44v	103	347
Lorvão 13**, **f.** **63v	86	279
Lorvão 15**, **f.** **6	132	75
Lorvão 15**, **f.** **50	134	108
Lorvão 16**, **f.** **7v	17	-
Lorvão 16**, **f.** **188	18	-

The second kind of green colours (Figure 2.30.), have µ-FTIR similar spectra to neutral copper acetate[66], in which the acetate asymmetric and symmetric absorptions appear at *circa* 1635 and 1375 cm^{-1}, respectively; the OH stretching absorptions are at 3400-3100 cm^{-1} region although the binder does not allowed to see them separately. In the Lorvão greens, namely in the more intense greens, the amide I, II from the binder (see section 2.4.1.) and the acetate absorption bands seem to have deformed into a single very broad band, (Figure 2.31.). The OH and NH stretching also display a different absorption pattern, where the NH absorption is not so evident. These differences could reflect a chemical reaction, namely the formation of a different copper complex with the protein, a copper proteinate [208]. This research is currently in progress with the historic reconstructions[67].

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[66] Neutral copper acetate is a binuclear complex with four carboxylate bridges and two water end groups [209].

[67] This is a work in progress in the scope of the project “The identity of Portuguese medieval manuscript illumination in the European context” (PTDC/EAT/65445/2006), 2007-

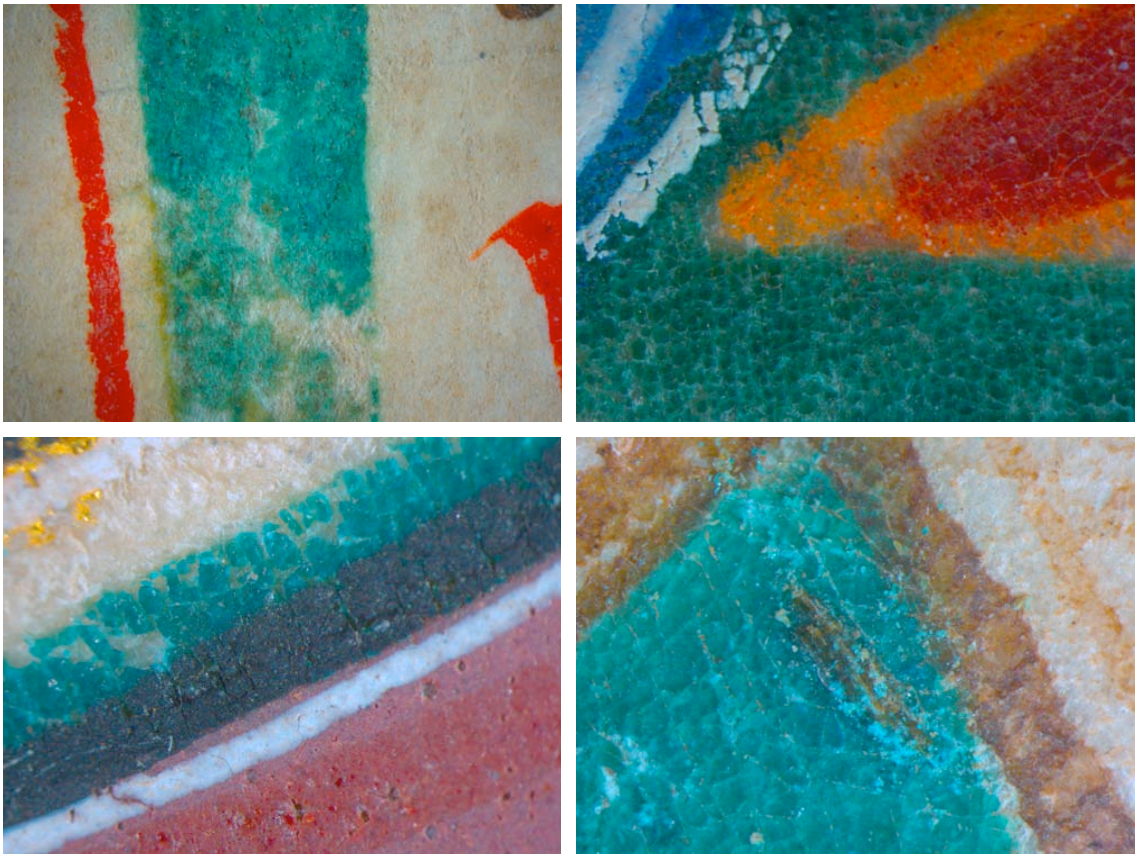

Figure 2.30. Details of different sorts of green used, from left to right: *Lorvão 12* (*f.* 17, magnification 12x), *Lorvão 15* (*f.* 11, magnification 32x), *Lorvão 16* (*f.* 59v, magnification 50x) and *Lorvão 50* (*f.* 64v, magnification 40x) (reading clockwise, from top left).

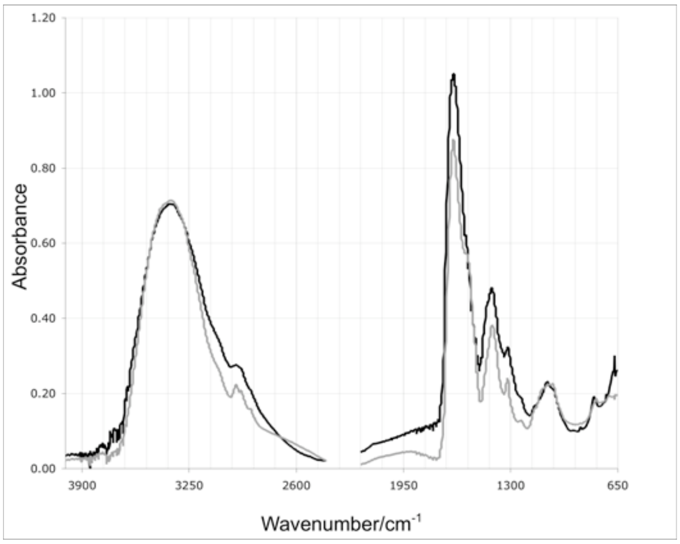

Figure 2.31. FTIR spectra of green colour from *Lorvão 13*, *f.* 44v (black) and *Lorvão 16*, *f.* 59v (grey).

With µ-Raman it was not possible to characterize the pigment responsible for the green colour. Further analyses with a more appropriate laser (a green laser, λ=514.5 nm) for green pigments or other structural characterization approach, shall be performed to accomplish more conclusive results.

Whites: the white colour is always identified as white lead, by the presence of lead in μ-EDXRF and by the characteristics absorption bands detected with μ-FTIR: the strongest absorption in the spectra of the white colour is due to the CO_3^{2-} stretching at 1405 cm^{-1} (Appendix I, section I.3.1.2.). This absorption together with the sharp peak of carbonate group bending at 682 cm^{-1} indicates the presence of basic lead carbonate [109] and the expected broad absorption due to OH stretching appears at ≈3404 cm^{-1}. The μ-Raman spectra is not so easy to obtain, although it presents the typical spectra with the υ_1 symmetric stretching mode of CO_3^{2-} anion, at 1055 cm^{-1}, instead the theoretical doublet with bands at 1048 and 1053 cm^{-1}, but so far all the publish spectra showed only a single band, probably due to a lower spectral resolution [210]) (Figure 2.32.) (representative white colour from different manuscripts are in Appendix II, section II.3.1.).

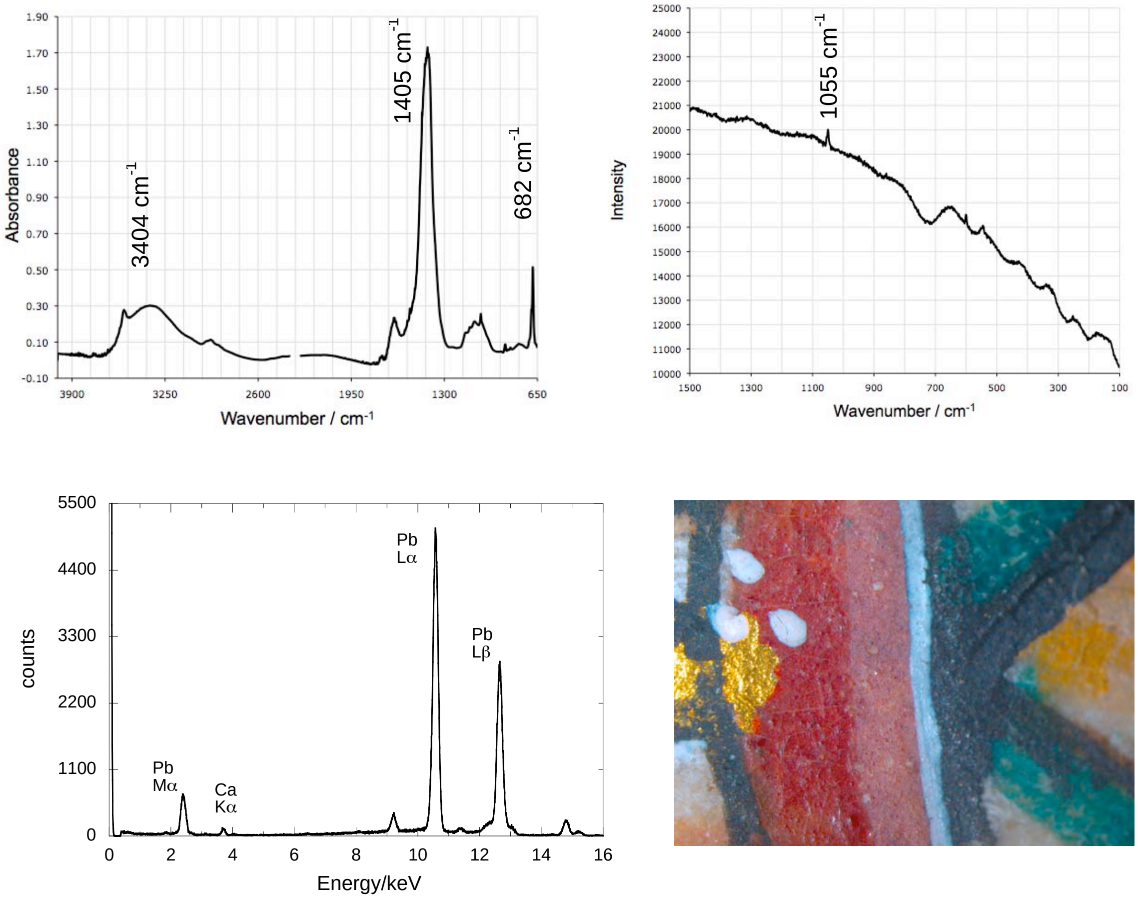

Figure 2.32. FTIR (left above), Raman (right above) and EDXRF (left below) spectra of white colour and a detail (magnification: x25) from *Lorvão 50*, *f.* 64v.

Browns: the brown colour from the Lorvão *scriptorium* is the most heterogeneous colour, it never looks the same in each manuscript, however there is always iron in its composition (representative spectra in Appendix II, section II.3.1.). *Apocalypse* presents a different kind of brown colour, being a mixture of vermillion with *minium* and a not identified element with iron (EDXRF). The possibility of being an iron oxide was considered, however it was neither identified by µ-Raman nor by µ-FTIR. The other inorganic browns present on *Lorvão 3*, *12* and *13* were not fully characterize but the presence of iron, copper and sulphur detected by µ-EDXRF, could indicate the use of an iron and copper sulphate based ink; or a mixture of it with fillers, such as white calcium carbonate (*Lorvão 12* and *Lorvão 13*), since Ca peak appears with high intensity in this colour (Figure 2.33.).

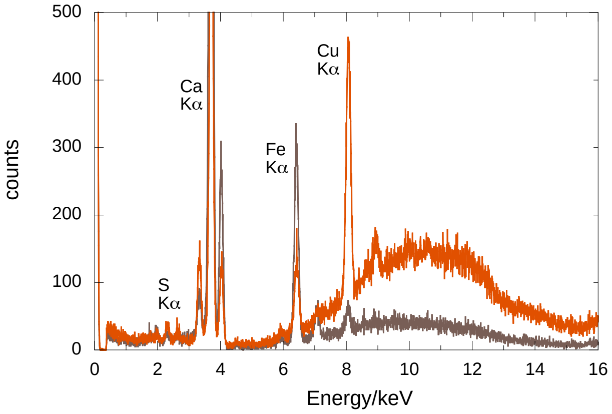

Figure 2.33. EDXRF spectra of brown applied on *Lorvão 13*, *ff.* 30 (brown line) and 92v (orange line).

Gold: the presence of gold was immediately identified by µ-EDXRF (Figure 2.34.). This technique indicates also the presence of iron, detected due to the presence of *bolus* applied below the gold leaf, used to enrich the tonality of the gold and to make the areas to which the ground had been applied more visible [12].

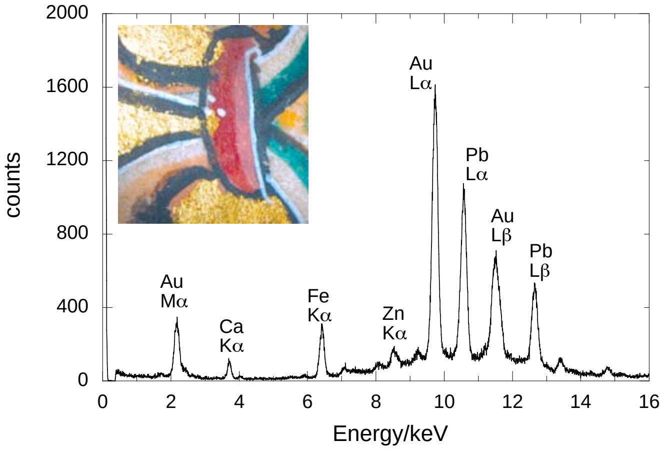

Figure 2.34. EDXRF spectrum of the gold applied on *Lorvão 50*, folio 64v. And inset there is a detail of it (magnification: 10x).

Fillers: calcium based fillers were not easily identified neither by µ-EDXRF because the calcium detected could be due to residual lime from the processing of the skin and/or to a surface coating like chalk (once the paint layers are thin) or to the fillers, nor by µ-Raman as the spectra were not clear. White lead can also be considered a filler, since the final colour is not modified, but it was not clearly visible in mixtures, by µ-Raman. Taking advantage of the fact that *minium* and vermilion do not absorb in the wavenumber range (4000-600 cm^{-1}) available in µ-FTIR used, it was possible to observe, in both colours, the presence of fillers, such as:

calcium carbonate: These extender is characterized by the carbonate group which have a broad and intense band of COO stretching at 1430 cm^{-1} and a weaker and straight band of COO bending at 878 cm^{-1} [183] (Appendix I, section I.3.1.2.); these absorptions were easily observed in the red and orange colours (Appendix II, section II.3.1.).

basic lead carbonate: the strongest absorption in the spectra of the white colour, is due to the CO_3^{2-} stretching at 1405 cm^{-1}. This absorption together with the sharp peak of carbonate group bending at 682 cm^{-1} indicates the presence of basic lead carbonate [109] and the expected broad absorption due to OH stretching appears at ≈3404cm^{-1} (Appendix I, section I.3.1.2. and Appendix II, section II.3.1.).

The calcium carbonate has also been detected in the blues (Figure 2.25.), blacks, browns and organic yellows of *Lorvão 12* (Appendix II, section II.3.1.). The calcium sulfate dihydrate was only identified mixed with the organic yellow of the *Lorvão 3* (Figure 2.24.), through the SO_4^{2-} doublet stretching bands at 1140 and 1119 cm^{-1}, the two stretching modes of the water groups found at 3548 and 3404 cm^{-1} and its bending band at 672cm^{-1} characterize gypsum, a dihydrate calcium sulfate [211] (Appendix I, section I.3.1.2.).

In all the manuscripts analyzed, calcium (Ca), iron (Fe) and potassium (K) from the parchment were always detected by µ-EDXRF, revealing as expected, a relative thin paint layer (Appendix II, II.3.1.).

In order to find out more about some of these colours, and regarding the equipment potentialities, a new approach was carried on, when semi-quantification of mixtures in paints with µ-EDXRF and characterization of degradation products with µ-Raman were performed.

2.5.2. Paint composition: a quantitative study of blues, oranges and reds

Multi-elemental quantitative analyses have been largely applied in other fields like metals, glass, ceramics and paintings [212-214], but not on illuminated manuscripts. Besides the previous characterization of inorganic colour materials, the use of μ-EDXRF was tested to perform semi-quantitative analyses when mixtures of pigments composed the paint: in orange and red paints from the *Apocalypse* and blue paint from the *Book of Birds*. In all the analyzed folios, at least 3 spectra were collected from each analyzed area and, when possible, three different areas of the same colour were examined. The standards used to perform this semi-quantification were based in the manuscripts' colours (for more details see Appendix I, section I.3.1.3.).

2.5.2.1. Quantification of the blue paint of the *Book of Birds*

From the 34 illuminations present in this manuscript, 26 have the blue paint applied. However, from these folios only six were selected since the analyzing areas must be higher than 70 µm in diameter. The illuminations with blue paint, in which over layers like vermillion or red lead could be present, were also excluded (Figure 2.35.).

Figure 2.35. The folios analysed: *f.* 5 – *The dove and the falcon* (A), *f.* 6v - *Inhabited initial* (B), *f.* 7v –*Initial* (C), *f.* 16 – *Falcon* (D), *f.* 25 - *Lebanons' Cedar* (E) and *f.* 48 -*Common crane* (F).

The illuminations were analysed in several blue areas, above 11 for each folio, to ensure a representative database, which allowed quantify the components present in each folio paint layer. All spectra obtained have the same elements although the proportions are highlight different depending on the colour shades, mainly in folio 5, the lighter one, and in folio 7v, the deeper one (Figure 2.36. and Table 2.3.).

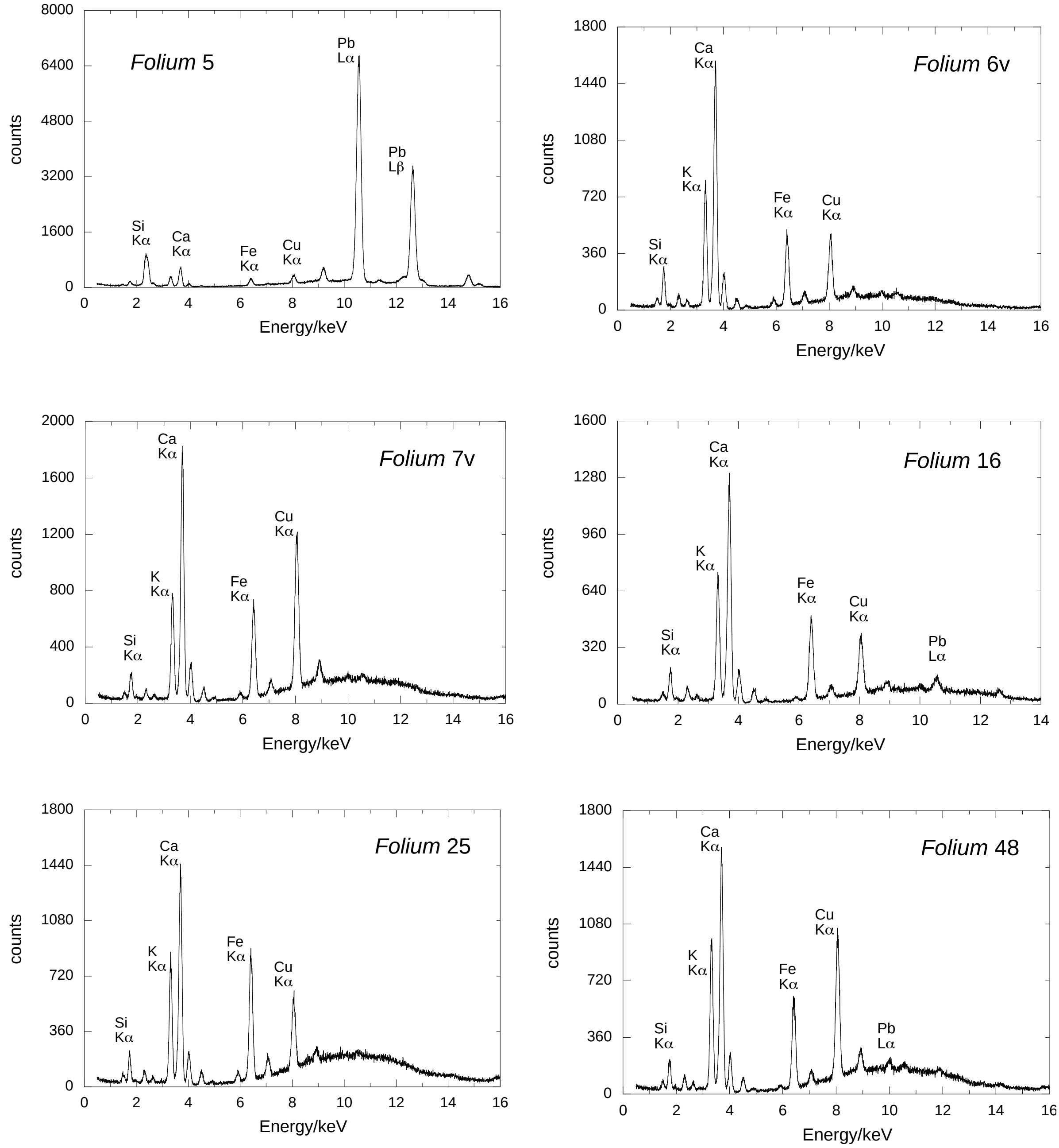

Figure 2.36. Representative µ-EDXRF spectra of the analysed folios: *f.* 5 – *The dove and the falcon* (A), *f.* 6v - inhabited initial (B), *f.* 7v –minor initial (C), *f.* 16 – *falcon* (D), *f.* 25 - *Lebanons' Cedar* (E) and *f.* 48 - *common crane* (F), acquisition time 300 seconds.

Table 2.3. Peak area for the blue colours' main elements (using ArtTAX software) of *ff.* 5, 6v, 7v, 16, 25 and 48.

Folio	Al	Si	S	Cu	Pb
5	528	2329	7219	6761	174852
6v	784	3342	1060	26172	6269
7v	715	3280	1936	32791	5213
16	747	3075	2028	13374	4432
25	864	3333	1278	20840	7756
48	678	2615	1044	23006	4549

Standards were made with two sorts of lapis lazuli (from Afghanistan) and one of lazurite from Afghanistan previous characterized by µ-EDXRF and PIXE, which indicated that all raw material has an analogous major elemental composition (Appendix II, section II.4.1.), although with different elemental contents, being the main difference in the potassium contents, about one order of magnitude lower in lazurite.

Pigments were ground in a mortar, then mixed with parchment glue, and were applied as paint layers with a brush over a PMMA support.

Different composition paints of lazurite or lapis lazuli, lead white and azurite were then prepared to: ***1)*** test the influence of different amounts of each pigment in the paint; ***2)*** to find out which way of applying the paint simulates better the manuscript blue paints (using brush or film applicator) (Figure 2.37.); ***3)*** to define how the thickness can induce the quantification results (ranging from 100 µm to 200 µm) (for more details about the standards, see Appendix II, section II.4.2.).

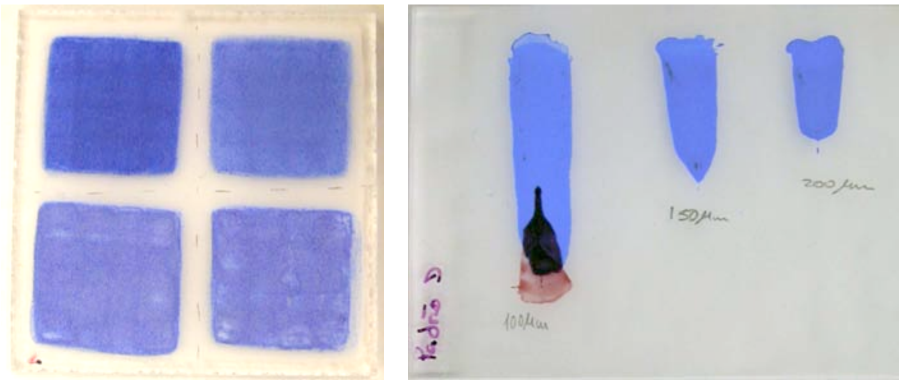

Figure 2.37. Standards of blue paint made using brush (left) or film applicator (right).

Using the analytical program WinAxil to simulate the semi-quantification of the standards with themselves as references, the results showed that the semi-quantification is better obtained when selecting the Lα peak of Pb to white lead, Ca[68]

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[68] Calcium was the element selected because the characterization of lapis lazuli and lazurite revealed that together with aluminum, these were the components with the same percentage in the three different minerals. Ca (20) was chosen rather than Al (13) considering the high atomic number of the other two representative elements (the only X-ray

Kα peak to lapis-lazuli, instead selecting all the lapis-lazuli components (Al, Si, S, Ca and K), and azurite is better quantified if it is calculated by difference than by selecting the Cu Kα peak to azurite (Appendix II, section II.4.2.2.).

The results indicated that the standards must have a composition similar to what it is going to be quantified (which can be roughly done considering the results obtained with the EDXRF software) with a 10% maximum margin of error, otherwise the results would not be reliable.

The standards that most close, matched the already made reconstructions were the ones made with a brush, and not with the film applicator. This is probably due to the affinity to the original paint technique (Appendix II, section II.4.2.4.) applied in the manuscripts.

Regarding the thickness of the paint layers, between 100 and 200 µm, it is possible to realize that the thicker the paint layer was, more inaccurate the results were, mainly in the calculation of lead white followed by azurite, since the mass attenuation coefficients vary widely with X-ray energy and material composition. Usually, X-rays of low energy do not penetrate very deep in a matrix of heavy material, whereas high-energy X-rays in a light material have high penetration, which affect the quantification of elements' concentration [215]. The mass absorption coefficient of the main elements of lead white (Pb: 3839 cm^{-1}) and of azurite (Cu: 3995 cm^{-1}) are higher than the others main elements from lapis lazuli (Mg: 673 cm^{-1}, Al: 1254 cm^{-1}, Si: 1350 cm^{-1} and K: 959 cm^{-1}). It was also possible to realize that the intensity of the characteristic X-rays from Pb or Cu increase linearly with the thickness, the thinner the standard was, the lower the intensities were (Appendix II, section II.4.2.3), which characterizes these paint model samples, has thin samples [215].

The thickness of the manuscripts' paints was estimated to be lower than 100 µm, since the only cross-section performed (from *Apocalypse*) has 72 µm thickness. This means that they could be also considered as thin films that allow quantifying all elements of the paint.

The *velatura* (Figure 2.37), the first application on the picture from the right), usually a thin layer of red organic material, does not influence the quantification of the blue paint layer, as its thickness is small and its attenuation effect on the quantification is only due to the presence of non emitting X-ray organic material.

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emitters) of the paint composition: LαPb and KαCu and because of the mass absorption coefficient of Ca: 165 cm^2g^{-1} to lazurite and 239 cm^2g^{-1} to lapis lazuli, and of Al: 380 cm^2g^{-1} to lazurite and 411 cm^2g^{-1} to lapis lazuli.

To quantify the paint components of each folio, three painted standards (all with 100 µm thick, determine by sample cross-sections) were selected (wt%): **(A_1)** 97,5:1:1.5 (lapis lazuli : lead white: azurite), **(B_1)** 97,5:1:1.5 (lazurite : lead white : azurite) and **(B_3)** 87,5:11.5:1 (lapis lazuli : lead white : azurite). The results obtained reveal a very good reproducibility (Appendix II, section II.4.2.5).

The colour blue used in the *Book of Birds* is rich as its composition is mainly of lapis lazuli. The lead white was used to obtain a lighter colour and the azurite quantity is so low that it is difficult to conclude if it is an impurity or if it was intentionally added (Appendix II, section II.4.2.5). Therefore, the lighter colours reveal a composition with a high percentage of lead white (around 24%), such as at folio 5, and the deep colours have a minor amount of white pigment (around 1%), like folio 16. These results are also in agreement with what we can see by µ-FTIR (Figure 2.38.), since even folios 7, which is one of the paints with less than 1% of white lead quantified, presents the characteristic carbonate group bending at 682 cm^{-1} of white lead.

The small amount of white lead in the blue of folios 6v, 7v, 25 ad 48 (below 1%) could be considered as an impurity, but in folios 16 it could also be associated to a intended added filler.

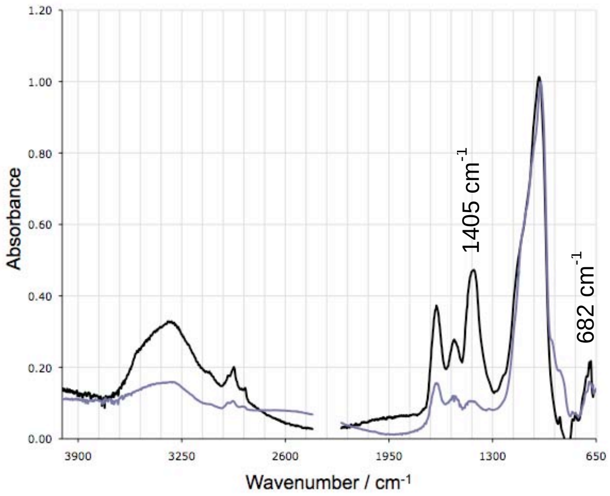

Figure 2.38. µ-FTIR spectra of the blue paint of *Book of Birds*, *f.* 5 (black line) and *f.* 7v (grey line).

2.5.2.2. Quantification of orange and red paints of the *Apocalypse*

The *Apocalypse* has 69 illuminations and between them a total of 16 folios, with red or orange colours, were quantified (*ff.* 43, 49, 115, 118, 136, 138v, 158, 172v, 178v, 179, 185v, 186v, 193, 196v, 217 and 217v). Usually, in each folio each colour was analysed 2 to 3 times in different areas. A total number of 107 spectra for the orange, from degraded and non-degraded areas and 19 for the red were collected.

Based on the spectra obtained in the selected manuscript folios, standards for μ-EDXRF semi-quantitative analyses of different mixtures of cinnabar (HgS), red lead (Pb_3O_4) and orpiment (As_2S_3), were prepared in the same way has the previous blue paint (standards composition in Appendix I, section I.3.1.3.). The thickness of the paints was determined for each standard, in cross-sections, as *ca.* 100μm.

Using these standards, the semi-quantification of the orange paint in degraded and non-degraded areas was carried on with the analytical program WinAxil, using the same approach as for the blue colour. Meaning that was selected the Pb Lα peak as representing *minium*, Hg Lα peak as vermillion and As Kα as orpiment.

In all the 16 folios analysed (Table 2.4.), as already described, the orange paint is a mixture of red lead and orpiment, the latter ranging from *ca.* 1.2% in folio 138v (one of the two illuminations with vermillion) to *ca.* 4.4% in folio 49 (a degraded orange paint folio). The presence of vermillion is not always detected in the orange paint, but when it is, it is lower than *ca.* 1.5%. Despite the seeming-relation between the amount of arsenic in the orange paint and its degradation (in general non-degraded folios contain less amount of orpiment in the orange paint composition when compared with the degraded ones), it was not possible to establish a direct connection between the degradation extent and the arsenic amount on the orange paint.

Table 2.4. Orange paint composition in *Lorvão Apocalypse* determined by micro-EDXRF (% wt).

Folio	Pb_3O_4	HgS	As_2S_3
43*	96.5	-	3.5
49	95.6	-	4.4
115	94.5	1.5	4.0
118	96.6	-	3.4
136	98.5	-	1.5
138v	97.5	1.3	1.2
158	96.8	-	3.2
172v	96.5	-	3.5
178v	96.4	-	3.6
179	98.0	-	2.0
185v*	97.8	-	2.2
186v*	96.9	-	3.1
193	98.0	-	2.0
196v	97.6	-	2.4
217	96.6	-	3.4
217v	97.0	-	3.0

* these folios did not present signals of degradation

For the reds, vermillion was mixed with red lead, in such proportions that the final red colour is not changed, either with the addition of red lead or with the addition of a filler as calcium carbonate ($CaCO_3$). The vermillion amount in these mixtures, determined by μ-EDXRF, range between 70 - 95% (wt %), (Table 2.5.). The use of these colour formulations enhances the red, allowing the artist to save one of most valuable pigments in the medieval palette, vermillion (HgS).

Table 2.5. Red paint composition in *Lorvão Apocalypse* determined by μ-EDXRF (% wt).

Folio	HgS	Pb_3O_4
49	91	9
115	91.4	8.6
177	76	24
200	70	30
209	95	5
217v	95	5

The semi-quantification of reds was also performed for all the others manuscripts using the WinAxil software and selecting the Pb Lα peak for *minium* and calculating the vermillion by default. Except for *Lorvão 16*, all the manuscripts have reds as a mixture of vermillion with *minium*. The range of *minium* could differ from 0% (in the minor initials) to 40% in *Lorvão 3* (*f.* 17), 15% in *Lorvão 12* (*f.* 7v), 14% in the *Book of Birds* (*f.* 6v), 12% in both *Lorvão 13* (*f.* 63v) and *Lorvão 50* (*f.* 34v), 7% in *Lorvão 15* (*f.* 154v) and finally 3% in *Lorvão 17* (*f.* 7v) (for ore details see Appendix II, section II.5.).

2.6. Organic colour materials

From all the studied manuscripts, only the *Apocalypse* does not present any organic colour. All the others manuscripts have, at least, one paint made of organic materials (table 2.6.). In the Book of Birds the yellowish colour is always applied in the minor initials (Figure 2.4.).

Table 2.6. Organic material colours used in Lorvão manuscripts

	Carmine	Yellowish	Blue	Black	Brown
Book of Birds	4, 6	40, 59v, 72v		33, 50v, 54	
Lorvão 3	-	51v, 59v, 108	82v, 107v, 139v	98v	107, 107v
Lorvão 12	6v, 11, 30, 53v	6v, 11, 39, 50, 64, 94	-	6v, 11, 30	6v
Lorvão 13	1v, 6v, 30, 80v	30, 44v, 63v, 80v, 85	-	30	30, 92v
Lorvão 15	5v, 11, 26, 38v, 50, 125v, 167v	-	50	26, 127v	125v
Lorvão 16	165v	7v, 24v, 71v, 155, 165v	73	30, 73	126,155,161v
Lorvão 17	74v, 169v	-	230	-	230
Lorvão 50	1v, 64v, 173v	-	-	1v,64v,173v	-

109 microsamples were collected using appropriate microtools, under microscope observation. To characterize the organic colour material, μ-FTIR and μ-Raman (for indigo) were the first techniques to be used: μ-Raman because as been already used to characterize inorganic colour materials and was taken to DGARQ-TT, and μ-FTIR, for being a wide-ranging and relatively fast technique.

The results obtained using these techniques enabled, in some cases, the characterization of the organic paint components for:

<u>Carmine-coloured</u>: this colour was identified by μ-FTIR as lac dye in the *Book of Birds, Lorvão 12*, *13* and *17* (representative spectra in Appendix II, section II.3.1.). Its characterization was more difficult than what previously expected because the binder used, the glair mixed with resin (shellac), has almost the same characteristic vibrations of the colour material (OH and NH stretching at ~3410 cm^{-1}, characteristic amide bending at 1591 cm^{-1} and CN bending at 1450 cm^{-1}, stretching aromatic ring C=C at 1473 cm^{-1}) (Figure 2.39.), and this colour application was not described in medieval treatises. The reconstructions created to simulate this colour with different shades, as appeared in the *Book of Birds*, revealed that it is necessary to join in different proportions the two components of lac dye: the resin and the dye, in order to obtain a more or less intense bright red. μ-spectrofluorimetry was used to obtain the emission spectra of the colour together with the emission of the parchment, by exciting at 500nm (to obtain a spectra with the higher intensity) and 550nm, since at this last excitation wavelength the emission from the parchment is almost negligible [49]. In this case, μ-spectrofluorimetry was not very useful because the emission spectra were of very low intensity and it was not possible to obtain well-resolved excitation spectra, not enabling a conclusive characterization. HPLC-DAD would be the last step if the extraction of the red chromophore had been successfully achieved, but after trying soft extraction methods, the microsample was submersed in the most strong acids (H_2SO_4, $HClO_4$, HCl and BF_3 in methanol) and was still insoluble!

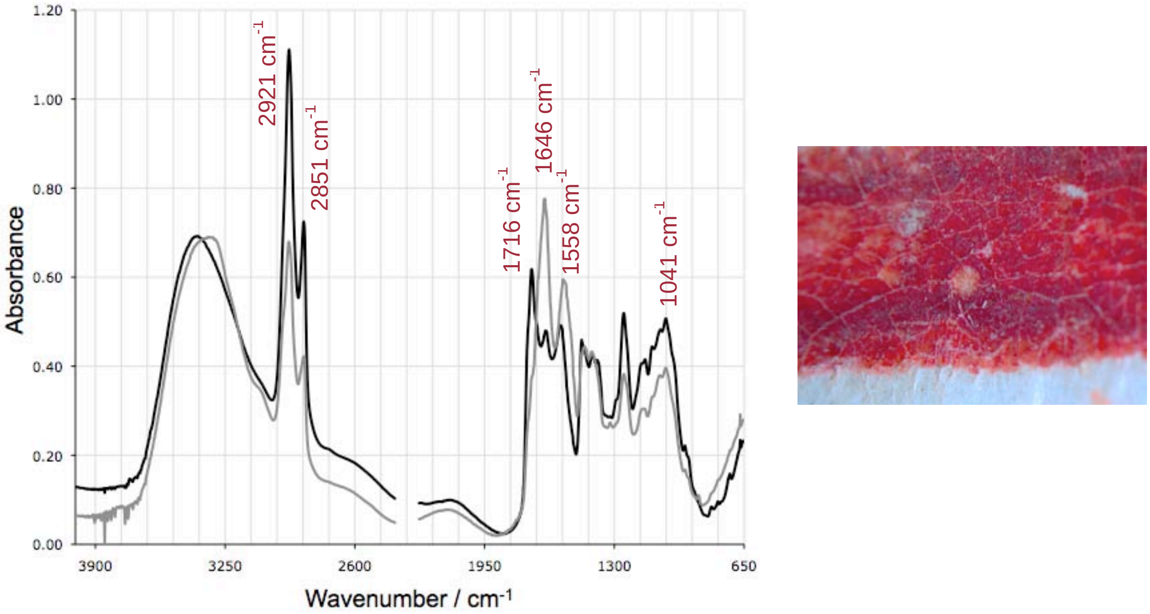

Figure 2.39. FTIR spectra of carmine-coloured from the *Book of Birds*, folio 6, more (black line) and less (grey line) intense colour; and a carmine-coloured detail (magnification 40x).

The characterization of the carmine-coloured colours, that seemed to have a different nature from the previous ones (*Lorvão 15*, *16* and *50*), was performed with µ-EDXRF, in order to find the metal element used to complex the chromophore, if a lake was applied. In *Lorvão 15* was detected Pb, in *Lorvão 16* the presence of Cu and *Lorvão 50* has Pb and Cu (Appendix II, section II.3.1.), this one in a large quantity (Figure 2.40.). These elements can justify the shade differences, if the same dye was applied in all manuscripts. µ-FTIR characterization was performed in *Lorvão 15*, which revealed a proteinaceous matrix mixed with an non identified organic dye and white lead (*f.* 26) or calcium carbonate (*f.* 50), and in *Lorvão 50*, disclosing the use of glair (*f.* 173v) but not the dye.

This sort of material is not easily characterized by µ-Raman due to the intrinsic materials fluorescence. Although SERRS technique has been developed to minimise this effect when analyse red colorants [216, 217] however this identification requires micro-samping.

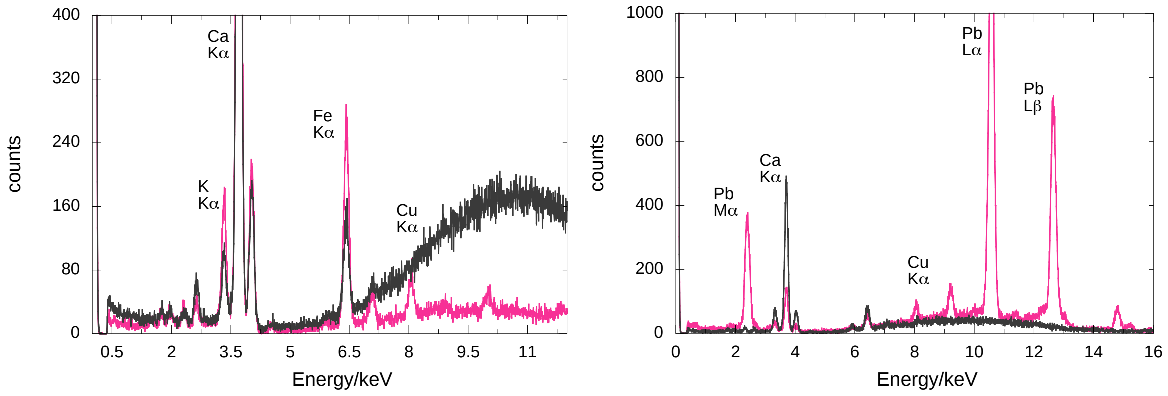

Figure 2.40. EDXRF spectra of carmine-coloured (pink line) from the *Lorvão 16*, folio 165v and Lorvão 50, folio 64v (grey line – parchment).

Yellowish: all the analysed samples revealed only the proteinaceous binder. Even if there is saffron present in this colour, for instance, possibly the bands of the dye can be masked as a result of a low ratio dye:binder. The minimal amount of the dye, if even present in the collected micro-samples did not enable to perform other kind of characterization (representative spectra in Appendix II, section II.3.1.).

Blue: micro-sampling the blue colour enabled to verify the presence of indigo, which was straightforwardly identified by μ-FTIR: N-H stretching at 3268 cm^{-1}, C=O stretching at 1628 cm^{-1}, six-member ring C-C at 1585, 1483, 1461, 1317 cm^{-1} and N-H in plane bending at 1392 cm^{-1} [184, 204] (Figure 2.41.).

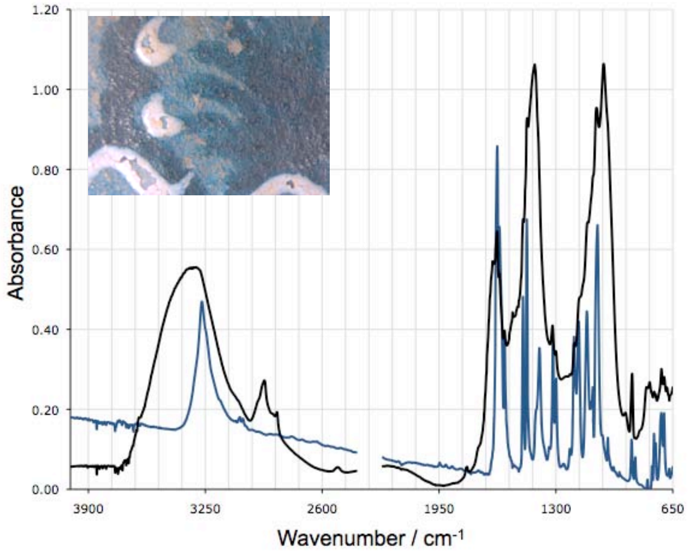

Figure 2.41. FTIR spectra of blue colour from *Lorvão 15*, folio 50 (black line) and indigo (blue line); inset a detail (magnification 25x).

When analysed by µ-Raman looked like to be there but not very clear, because indigo was found combined with lapis lazuli or azurite, never detected alone as a blue paint, for instance in *Lorvão 15* (*f.* 50), *Lorvão 16* (*f.* 73) and *Lorvão 17* (*f.* 230) (representative spectra in Appendix II, section II.3.1.).

Black: although in *Apocalypse* the black colour is probably a mixture of iron gall ink (detected by µ-EDXRF a high amount of Fe and detected by µ-Raman by comparing with a reference sample) and carbon black (detected by µ-Raman: at 1590 and 1324 cm^{-1}, usually named "G band" arise from stretching vibrations of the well ordered polyaromatic rings in the planar graphite structure [218]), all the other manuscripts have only carbon black based paint (representative spectra in Appendix II, section II.3.1.). By µ-Raman it was possible to distinguish two sorts of carbon black: animal carbon black (the intensity of the peak at 1324 cm^{-1} is higher than the one at 1590 cm^{-1} [218]), in *Lorvão 3* and *Lorvão 15* and vegetal carbon black (where the intensity of the characteristic peaks is reversed higher for 1590 cm^{-1} and the shape is broader and also by the presence of the phosphate vibrations at ~961 cm^{-1} [218]) used in a drawing ink in the Book of Birds and in *Lorvão 3* (*f.* 98v) (Figure 2.42.).

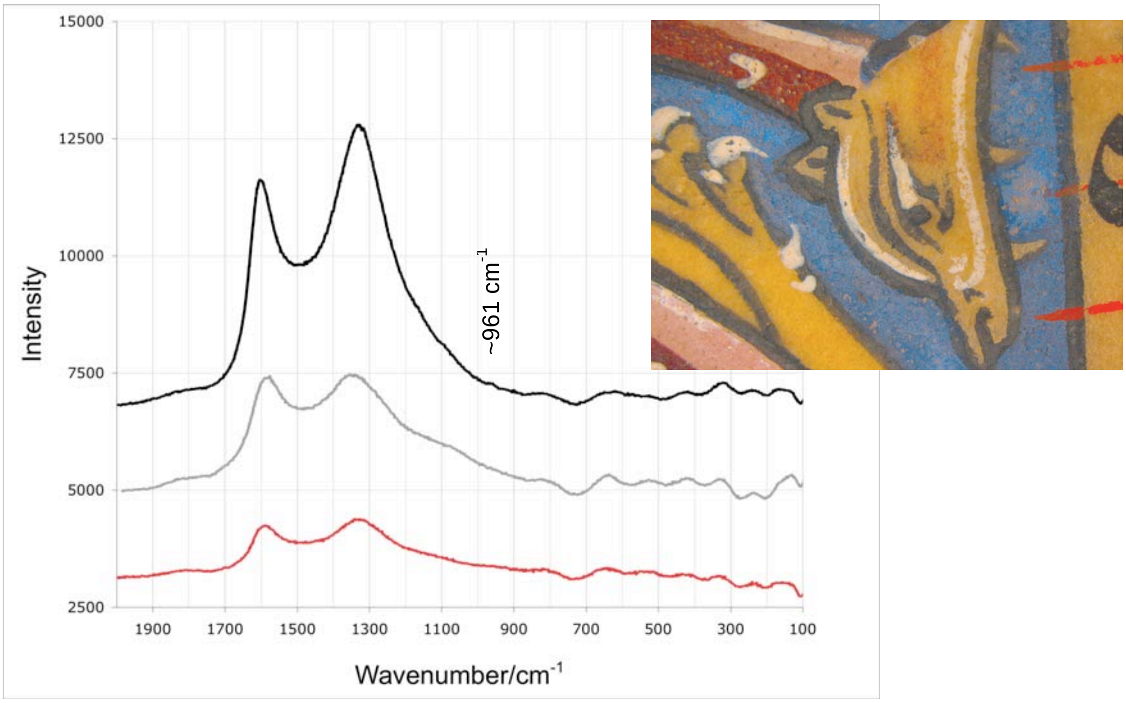

Figure 2.42. Raman spectra of two carbon black sorts: ivory black (black line) and vine black (grey line), the colour black present in Lorvão 15, folio 167v (red line); inset a detail (magnification: 7.1x).

Brown: This colour was only analysed in *Lorvão 13*, revealing a proteinaceous matrix of glair mixed with the filler calcium carbonate. This could mean that the brown colour of this and probably of the other manuscripts too, is based in iron gall ink (in µ-

EDXRF was detected mainly Fe) diluted in a high amount of binder (representative spectra in Appendix II, section II.3.1.).

These results reinforce the need of creating a consistent and proper database to enable the characterization of organic materials in illuminations, regarding all practicable and possible alternatives.

2.6.1. Red lakes: µ-FTIR studies

In order to better recognize the spectra differences between the red colours applied in manuscripts illuminations purpurin, alizarin and their respective lakes made with $AlCl_3{\bullet}6H_2O$, madder (from Kremer), dragons' blood, carminic acid and its lake (from Aldrich), laccaic acid A, lac dye and cochineal lake were analysed.

Lac dye and laccaic acid A (its major component) can be distinguish from other organic reds since they are the only amides of the group, with the characteristic NH stretching at 3400-3000 cm^{-1}, and the amide I (CO stretching, 1653 cm^{-1}), amide II (CN stretching and NH bending, 1550 cm^{-1}) and CN bending at 1450 cm^{-1}, and also by the OH and NH stretching (Figure 2.43.). This advantage, when the dyes are analysed in powder, could be in fact a disadvantage when it is necessary to characterize a paint with a proteinaceous binder, as this has also an amide group on its structure that may mask the organic red amide.

Dragon's blood has also a typical spectra, as it is constituted by natural flavylium compounds [219], therefore the characteristic C=O stretching of an ether, at ~1700 cm^{-1}, is expected to appear in its spectra (Figure 2.43.).

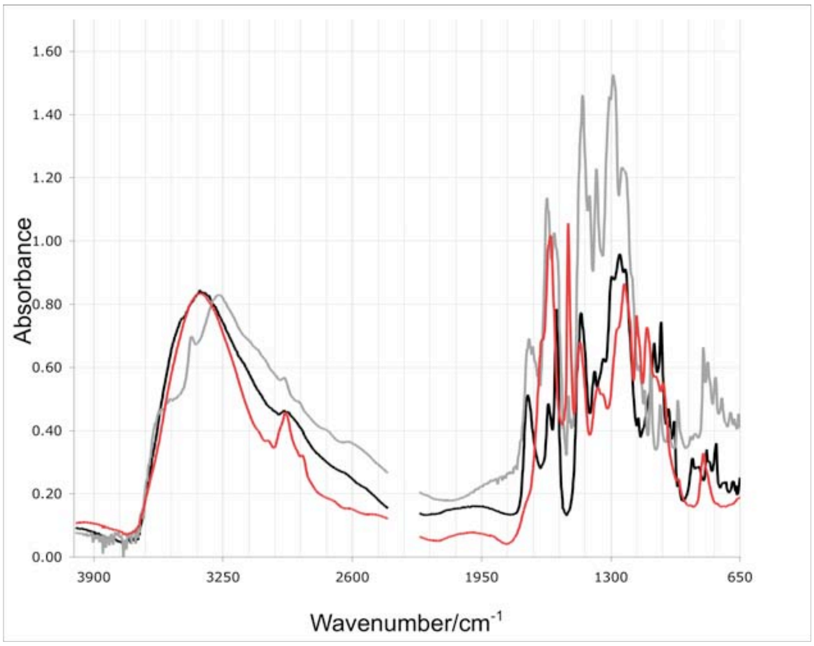

Figure 2.43. FTIR spectra of carminic acid (black line), laccaic acid A (grey line) and dragon's blood (red line).

Alizarin (1,2-dihydroxy anthraquinone) and purpurin (1,2,4-trihydroxy anthraquinone) have almost the same structure, but their infrared spectra differs in the C=O stretching. While alizarin has two characteristic absorption bands at 1662 cm^{-1} and 1633 cm^{-1}, purpurin should have just one at 1627 cm^{-1}, due to the formation of intramolecular hydrogen bonding between oxygen of C=O and the hydrogen coming from OH groups at C1 and C4 positions (Figures 2.44. and 2.45.) [220, 221]. Although, by looking to the spectra it is possible to verify that pupurin presents two absorption bands at 1669 cm^{-1} and 1614 cm^{-1}, instead of one, this could be explained by the influence that hydroxyl group in C2 position.

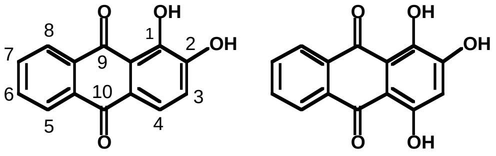

Figure 2.44. Alizarin (1,2 dihydroxy anthraquinone) and purpurin (1,2, 4 tri-hydroxy anthraquinone). Both, alizarin and purpurin are 9,10 quinones.

Besides these two peaks, alizarin can be identified by other peaks: the ring stretching at 1587 cm^{-1} and 1286 cm^{-1}, ring-H bending at 1047 cm^{-1} and stretching the ring-H common of 1,2-di substituted aromatic group at 713 cm^{-1} [184]. Purpurin has almost the same peaks but shifted: 1581, 1066, 719 and 709 cm^{-1}.

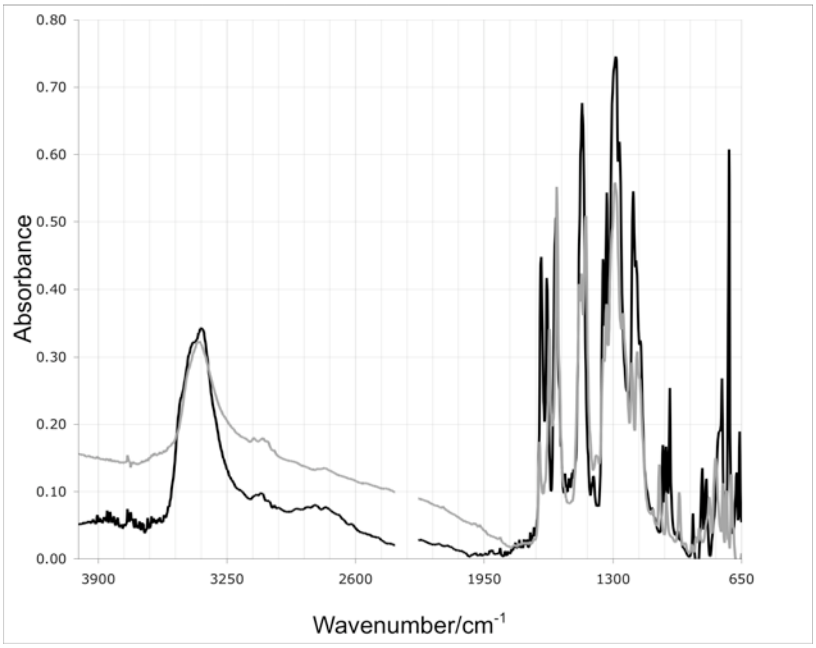

Figure 2.45. FTIR spectra of alizarin (black) and purpurin (grey).

Carminic acid can be identified by its anthraquinone structure linked to a carboxylic acid and to a gluclosidic unit. Despite the characteristic bands of sugars, there is also the representative C=O stretching at 1716 cm^{-1}.

Distinguish between red lakes is not a so easy task since the fingerprint region of the aromatic rings is masked by the Al-O vibrations. Although it was possible to verify that alizarin, purpurin and carminic acid lakes continue to present some of their characteristic bands with a minor deviation: alizarin lake (1591, 1286, 1043 and 719 cm^{-1}), purpurin lake (1581, 1066, 719 and 702 cm^{-1}) and carminic acid lake (1612, 1571, 1384, 1080, 1047, 981, 900 and 858 cm^{-1}) (Figure 2.46.).

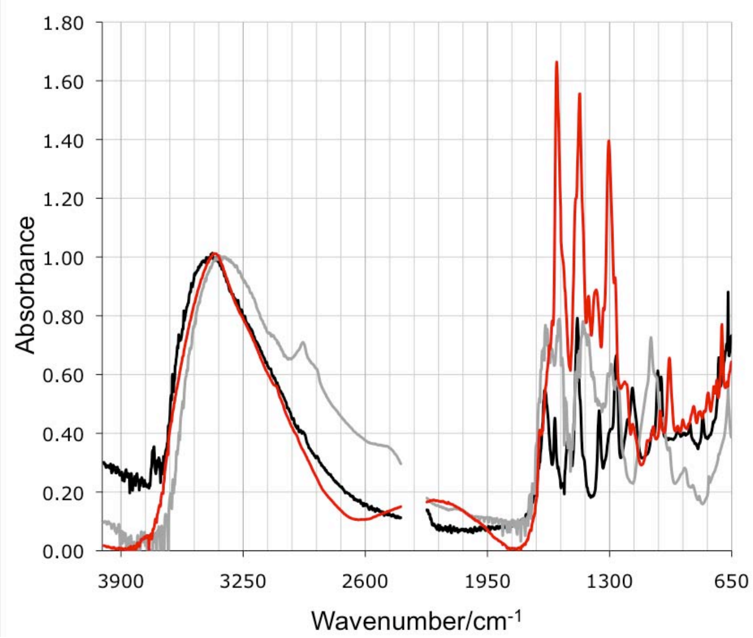

Figure 2.46. FTIR spectra of alizarin lakes (black line), purpurin lake (red line) and carminic acid lake (grey line).

Madder lake is very difficult to identify by µ-FTIR. From two samples analysed, the one from Kremer Pigmente® revealed one band, also presented in alizarin at 848 cm^{-1}, and one from the purpurin at 1066 cm-1, again with a small shift to 852 and 1070 cm^{-1}, respectively. In the other sample, recreated at the laboratory following medieval recipes, did not show any evidence of been composed by alzarin and purpurin, because the inorganic contribution was more concentrated than the dye.

2.6.2. Red lakes: µ-Spex studies – a new approach

Lac dye is not an emitter or is a very weak one, since the emission spectra was not resolved and clear. On the other hand, this technique proved to be very useful to characterize two sort of paints, the ones based on anthraquinones, such as pupurin or alizarin with maxima emission wavelengths *circa* 585nm, and the other composed by cochineal and pernambuco lakes, as well as dragon's blood resin with a maximum emission wavelength of 620nm.

The fluorescence emission could have provide useful information for the characterization of the colorant used in the *Book of Birds*, although in this case the quality of the fluorescence signals obtained did not enable a conclusive characterization. The emission spectra are of very low intensity and it was not possible to obtain well-resolved excitation spectra, reinforcing the possibility of being lac dye, which was verified by µ-FTIR analyses (further studies will be present in part B of this thesis).

2.7. Organic binder materials

The binder used in the Lorvão *scriptorium*, analysed by µ-FTIR, is proteinaceous. It was detected the presence of the polyamide pattern, namely the amide I (CO stretching, 1653 cm^{-1}), amide II (CN stretching and NH bending, 1550 cm^{-1}) and CN bending at 1450 cm^{-1}, and also by the OH and NH stretching at 3400-3000 cm^{-1} [180-183] (the binder can be easily observed in colours red and orange in Appendix II, section II.3.1.).

The most probable binders to find in these manuscripts are parchment glue mainly composed by collagen, and glair by albumin, as already mentioned, but the FTIR spectra differences between the first two binders are not very clear. Amide I and II maxima can be used to distinguish pure binders, by small differences, but the amide I maxima for the mixtures shift toward higher wavenumbers, while the amide II maxima shift toward lower wavenumbers with respect to the values observed for the pure binder [85]. Another approach used to differentiate the parchment glue and glair is through the CH stretching, also with minor but significant differences. Applying the spectral deconvolution to CH symmetric (2979 cm^{-1} for glair and 2975 cm^{-1} for parchment glue) and asymmetric (2943 cm^{-1} and 2935 cm^{-1} for glair and parchment glue, respectively) distension vibrations, it was possible to verify a short difference in the areas obtained, being slightly greater to parchment glue than to glair. It was also seen that glair presented a better resolution for both distension peaks. These enabled to distinguish the use of glair when organic dyes were applied, and the probably use of parchment glue to the other colorants. Attributing these small differences to the age of the binders under study (*circa* 800 years old), the precise characterization of the binder it is even more complicated.

Several micro-samples from the *Apocalypse* illuminations, of degraded and non-degraded orange areas, were analysed, evidencing the use of a proteinaceous binder with the presence of lead white, sometimes mixed with chalk, as extenders. In Figure 2.47., the infrared spectra for a degraded and non-degraded orange were compared. The fingerprint for the protein is clearly observed in the non degraded orange, on the contrary, in the degraded orange the amide I and II absorption bands appear like broad unresolved peaks that have collapsed into a single band (Figure 2.47. A). A similar phenomenon has been observed when proteinates are formed by complexation with metal ions [208]. The differences observed are the result of a chemical reaction, possibly involving the metal ions from the degraded pigments or other reactive intermediates. The OH and NH stretching also display a different absorption pattern, where the NH absorption is not so evident [178].

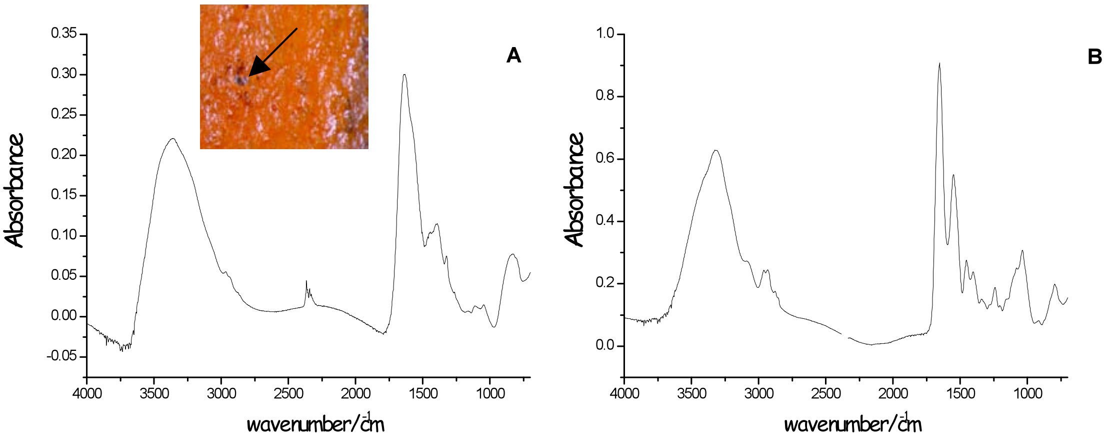

Figure 2.47. µ-FTIR spectra from the orange colour applied in folio 138: (A) degraded blackish area, and (B) orange "non-degraded" area, displaying a clear fingerprint for the proteinaceous binding media. In the inset, a detail of the orange paint, (magnification: 80x) indicating the point where the sample was collected.

2.8. Other materials

The remaining materials not yet focused on are the parchment, the ruling and the ink.

Parchment

The parchment of all manuscripts was analysed under microscope and by µ-EDXRF. In *Apocalypse*, besides the analyses performed in the illuminated folios, like was done for entire manuscripts group, the flyleaf and endpaper, as well as five folios (*ff.* 1, 79, 79v, 122v, 218v) with no illuminations, were also analysed, avoiding to have other materials from the illuminations or from written text that could mislead the results' interpretation.

The type of skin used as the support for the Lorvão manuscripts is undetermined but the colour and the follicle pattern suggests sheepskin or goatskin[69].

The results obtained by µ-EDXRF, revealed a more accentuated presence of the elements Ca, K, Fe and not so intense the elements Mn, Cl, S e As. Being the average ration Ca/Fe equal to 5 (representative spectra in Appendix II, section II.3.1.).

Analysed by µ-FTIR, it was possible to identified the fingerprint of the collagen by its characteristic polyamide absorption pattern, namely the amide I (CO stretching, 1653 cm^{-1}), amide II (CN stretching and NH bending, 1550 cm^{-1}) and CN bending at 1450 cm^{-1}, and also by the OH and NH stretching at 3400-3000 cm^{-1} [222].

Ruling

Under magnification the appearance of the ruling in the manuscripts were like silvery or graphite. The analyses performed in these lines to all manuscripts, using µ-EDXRF, revealed the presence of lead (Figure 2.48.) (representative spectra in Appendix II, section II.3.1.). According to many authors a metal point of lead or an alloy of lead (¾) and bronze (¼) [12, 223, 224] was used[70], since the 12th century, to mark the lines.

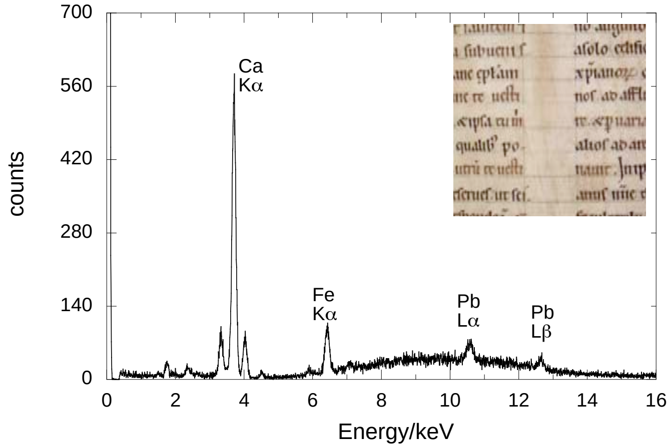

Figure 2.48. Common EDXRF spectrum of the lines present in the folios, this one is from *Lorvão 50*, *f.* 64v.

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[69] According to DGARQ-IANTT technicians of parchment manuscripts.

[70] To J. Alexander [225] these lines were made with graphite, used as a pencil, but according to Michelle Brown graphite was not generally used before the 17th century [12].

The ruling of *Lorvão 3* did not present lead, although it is marked, which can indicate another instrument based on bones or a metal harder than lead. Before the late eleventh century, ruling was generally executed with a hard point [223] that leaves a ridge-and-furrow effect on the writing surface rather than a graphic mark. This evidence could bring some new data, regarding the manuscript date or question if it really belongs to the same group of the other studied Lorvão manuscripts, since besides this difference, this manuscript has also a different drawing style and colours shade applied, as already mentioned.

In all the folios of the *Book of Birds*, where circles are drawn, there is a hole from a compass point at the center of each of the circles[71].

Inks

The basic text inks of the manuscripts are rich browns. There is evidence of the ink's having eaten through or into the parchment, as usually occurs with iron gall ink. And according to the analyses performed with µ-EDXRF it is possible to say that all the texts were written with iron based ink (Figure 2.49.). By µ-Raman it was possible to identify the peaks at 1480, 1338 and 595 cm^{-1}, which according to A.S Lee et al. [226] can be used to perform a positive identification of iron gall ink in historical samples. Although further analysis should be performed to identify the tannins.

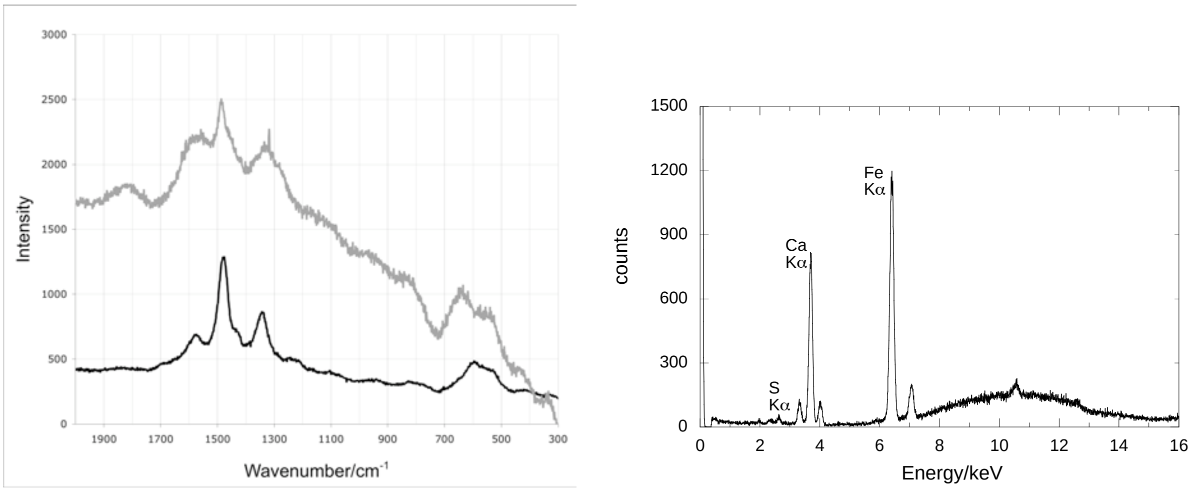

Figure 2.49. Raman spectra of the ink used to write in *Lorvão 12 f.* 6v (grey line), and iron gall ink made in the laboratory as reference (black line) (left); EDXRF spectrum of the ink used to write in Lorvão 12, *f.* 7v (right).

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[71] References to the use of the compass are found [21, 32], for example in Theophilus, chapter 17 on the laying out of windows:" Take the measurements, namely, the length and breadth of one section in a window, and draw it on the board with a rule and compasses with [a point made of] lead or tin.".

2.9. Dissemination

The results achieved with this interdisciplinary study were publicized in Portuguese through a step-by-step web book, “À descoberta da Iluminura Medieval”[72] [51] made for a public ranging from 4 to 99 years old (Figure 2.50.). This book is composed by a short introduction on Lorvão *scriptorium*, the materials used to paint medieval illuminations, an “activity book” and an “exercise book” that can be use in schools, school outings and in workshops developed in cultural institutions, as already happened in the faculty and Oporto Library. The activity book is composed of different activity sheets (see an example in Appendix II, section II.6.), explaining how illuminations from *Apocalypse* or from *Book of Birds* can be made, step-by-step. The “exercise book” is composed of questions and ideas to discuss, which would be a starting point for further projects related to medieval illuminations.

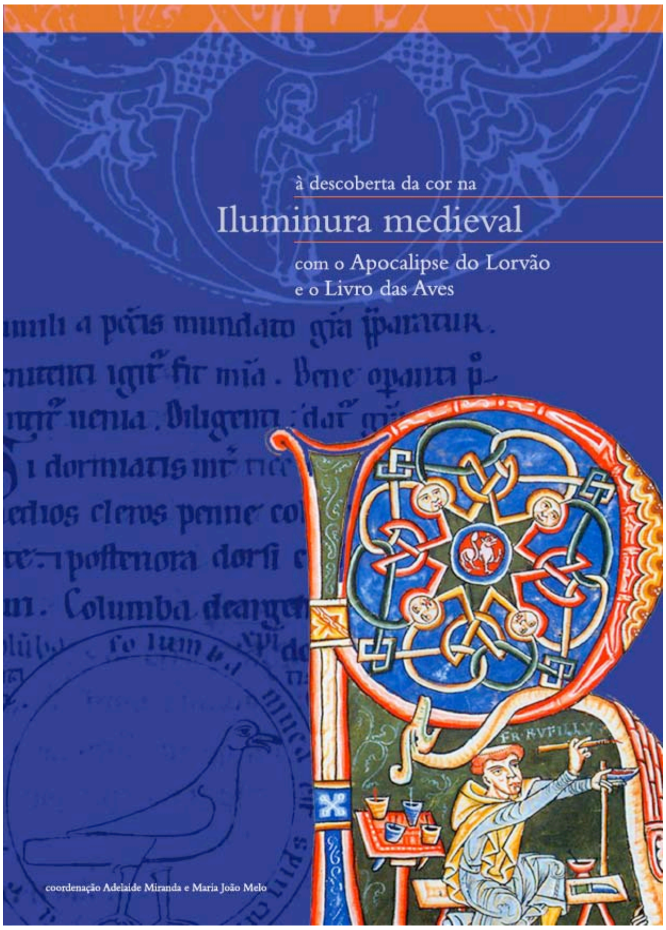

Figure 2.50. *À descoberta da Iluminura Medieval* web book, front cover.

Internationally a workshop was organized in one of the most attended medieval international congress, in Leeds (Figure 2.51.). It was the first time the congress held a hands-on workshop, which was supported by the activity sheets from *À descoberta da*

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[72] “Discovering the Medieval Illuminations”.

Iluminura Medieval. They were successfully used and Portuguese illuminations from the *Book of Birds* were reproduced and spread to different countries.

Figure 2.51. The workshop organized for 15 people (left) and one of the participants reproducing the hawk (*f.* 16) from the *Book of Birds* (right).

3. Conclusions

This study enabled to conclude that although there were some studies concerning the colour on Lorvão *Apocalypse* [3, 7], a full characterization of colour, which was never done on medieval Portuguese manuscripts, can provide different information than the one obtained just by analysing the text and iconographic aspects. This characterization enriched the knowledge about the history technology of art and can be one of the keys to better understand the history of Portugal.

Being the first approach done in this area and regarding the few international publications available, it is difficult to compare the results obtained with others, to established any correlation with other *scriptoria* or countries and to perceive if there is a characteristic palette or technique for region, period or *scriptoria*. When further studies would be carried on, this small step may reveal important aspects as geographical, temporal or cultural influences.

The multi and interdisciplinary work that was carried out, involving the knowledge from different fields of Conservation, Art History and Chemistry, has enabled to solve, for the most part, the initial questions. Those concerning the place where these manuscripts were produced, the specificity of minor components of the materials that may be use as a signature, illuminators' aesthetic and symbolic options, as well as the paints composition of one of the 12th century Portuguese *scriptorium*.

It was possible to verify that more important than *in situ* or micro-sampling is what sort of information was relevant to acquire in order to answer the most pertinent queries. Once again, the importance of a multi and interdisciplinary team is directly related with the best possible questions and the choice of the best possible methods of analysis and problem solving. Knowing that, there was always a compromise between what one would like to know and the sampling or handling it will require. On the other hand, more information is obtained if several complementary analytical techniques are used.

The correlation established between the *Apocalypse* and the *Book of Birds*, regarding the drawing (the architectonic structures, the use of the circle to inset the birds and human figures, the beardless Christ following the Early Christian representation) and the data provided by the manuscripts themselves, enable to consider their production in the same *scriptorium*. The illuminator's intentionality in painting the *Apocalypse* with only three colours was proved by: 1) the use of orpiment (single application in all Lorvão's collection); 2) the fact that there were other colours available in the *scriptorium* as they were used, for instance, in *Lorvão 16* dated between 1176 and 1200, which is during the *Apocalypse* production (1189); 3) because the applied colours are associated to the twelfth century aesthetic of light. In the *Apocalypse*, the illuminator's creativity is expressed by the choice of strong colours in the backgrounds. It, together with the unpainted human and animal elements represented without the notion of volume, originates symbolical contrasts and dynamic compositions, establishing a unique relation even among the Romanesque Beatus close to the first illuminated Commentaries. The *Lorvão Apocalypse* is thus a complete work, in which figures were purposefully left without colour.

Lorvão 3 was the only manuscript analysed that probably do not belong to the studied group, since the drawing and the colours applied are very different from the rest of the collection. Also, unlike the others, the ruling was not made by a lead point, but with a hard point. This could mean that this manuscript was made in a different *scriptorium* or by other person. In this way, the analysis provided new data for art historians, until now imperceptible.

The materials analyses revealed that the binders used are parchment glue or a mixture of it with glair. Except lapis lazuli, azurite and orpiment, all the other pigments used in the Lorvão manuscripts were obtain by synthesis: vermillion, *minium*, white lead, carbon black and copper green pigment. Like in the rest of Europe, lapis lazuli was used for the blue colour, vermillion as the main pigment of reds and *minium* was used in the orange colour. The use of indigo is always in a mixture with lapis lazuli, detected in four manuscripts: *Lorvão 3*, *15*, *16* and *17*. Lac dye was profusely used directly on the parchment, over *minium* to darken it or even mixed with lead white to shade and create the scale from dark to bright. The use of lac dye can be a signature of Portuguese medieval illuminations. Also characteristic is the application of white lead as a white colour, scarce in 12th century illuminations where $CaCO_3$ is used instead. In Lorvão collection we can also find a characteristic use of carmine-coloured and "bottle green" appearing together in several initials. In Figure 2.52. we can see what is the characteristic medieval Portuguese palette, so far studied, and in Table 2.7. the colorants found per manuscript and century.

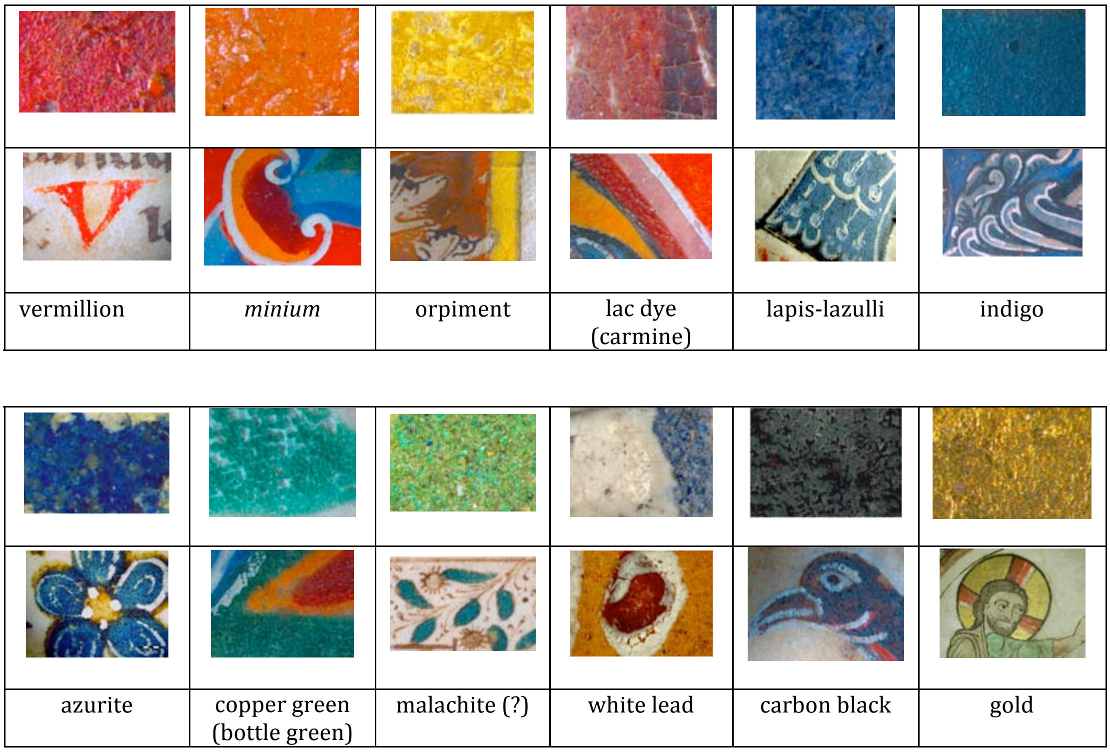

Figure 2.52. Palette of Medieval Portuguese illuminations, created with medieval illuminated details from Lorvão collection (except the ones from a *Charter of Vila Flor* (1512), azurite and malachite). In the top we can see microscopic photographs (magnification between 63 x and 80 x), followed by photographs with none magnification to 32 x; and in the bottom the major pigments characterized.

Table 2.7. Colorants identified per Lorvão's manuscript and per century.

Ms	Date	Colorants										
		vermillion	minium	orpiment	lac dye	lapis lazuli	indigo	azurite	copper green	malachite	white lead	carbon black
Apocalypse	12th	x	x	x								
Book of Birds	12th	x	x		x	x		x		?	x	x
Lorvão 3	12th	x	x			x	x	x	x		x	x
Lorvão 12	13th	x	x		x				x			x
Lorvão 13	13th	x	x		x	x			x		x	x
Lorvão 15	12th	x	x			x	x	x	x		x	x
Lorvão 16	12th	x	x			x	x	x	x			x
Lorvão 17	13th				x	x	x	x	x		x	
Lorvão 50	12th	x	x			x			x		x	x

For the first time, a quantification of mixtures of pigments in paints of illuminations was accomplished, using µ-EDXRF technique. It revealed the capacity to quantify the pigments, when adequate standards were used. It was discovered that the blue paint, in the *Book of Birds* is a mixture of lapis lazuli and white lead which varies between 24% (weight) in the lighter paints (*f.* 5) and 0% for the dark paints (*f.* 7v), no more than 1.7% of azurite was also quantified. In *Apocalypse,* the orange paints are diverse mixtures of *minium,* orpiment (varying between 1 and 4% in weight) and vermilion (less than 2%). More interesting, red paints were found as a mixture of vermillion and *minium* being the amount of HgS between 70% (in the larger areas) and 95%; $CaCO_3$ was also frequently mixed. This can be understood as a creative way of saving high cost materials, since vermillion was a pigment more expensive than *minium* and the final colour in both situations is the same. The addition of $CaCO_3$ improves the opacity of the colour, once again without changing it.

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B. Characterization of red lakes

> "Almost everywhere, red as the color of fire, the sun, and blood
> (and thereby life itself) has unusual significance, symbolizing magnanimity
> and fortitude, majesty and power, both temporal and supernatural"
> R. A. Donkin

1. Introduction

1.1. Preamble

The identification and detailed characterization of organic red colorants used in the past, as dyes or lakes, stills a challenge in the area of Conservation of Cultural Heritage. However the common analytical techniques are not enough to characterize the main chromophores present in illuminations, HPLC-DAD being an exception, although for illumination the micro-sample needed is not so small as it should be. And surprisingly, a systematic approach to the study of the photophysics and photochemistry of these colorants is not easily found in the literature [1,2]. In those, emphasis has been put on the molecular characterization (lifetimes and fluorescence emission) rather than on a full understanding of the photodegradation mechanisms.

Some of the expected organic colorants to be found in this sort of cultural heritage are madder, lac dye and cochineal, all composed by anthraquinones hydroxy derivatives: alizarin, purpurin, laccaic acid A and carminic acid. In the second part on this dissertation the characterization of these chromophores photochemical and photophysical was achieved. Photodegradation studies of these molecules were also performed, since they are coloured molecules they will absorb in the UV-VIS region and therefore photooxidation may be the main degradation mechanism. Knowing the main degradation products would enable to characterize what one can find in aged samples, where a mixture of products can already occur, making harder a proper analysis. The photochemical characterization was first made in homogeneous and then in heterogeneous media, to be able to compare with the real samples.

As the main reason for the lack of information and characterization on organic red colours applied in manuscript illuminations is their limited access to perform micro-sampling, that could be filled by non-invasive and powerful techniques, such as microspectrofluorimetry. The potential of this technique to characterize red organic dyes was explored, not only the ones applied in medieval manuscripts but also others used in textiles and Impressionist paintings. Although, the use of this technique may be only possible after a full understanding of the excited state nature of these historically important molecules.

“Life is pretty simple: You do some stuff. Most fails. Some works.
You do more of what works. If it works big, others quickly copy it.
Then you do something else. The trick is the doing something else.”
Leonardo da Vinci

1.2. Molecular and photochemical studies on historical dyes

1.2.1. Equilibria solution

To recognize and characterize the chromophores used to paint the illuminated manuscripts, usually not identified, could be very important or even determinant in the study of medieval illuminations, revealing signs until now ignored. Therefore, is necessary to understand their molecular behaviour and how they photochemically change through time, which can be achieved through a photochemical characterization, joined to a photophysical study necessary to characterize the excited state.

All these anthraquinones undergo, in aqueous MeOH solution, various structural transformations (multistate systems) driven by pH changes with weak organic acids. Characterizing their acid-base equilibria in aqueous solution, as well as understanding their response in the excited state and determining the irreversible chemical conversions are important data to achieve. These chromophores are also able to form complexes with metal cations, which are very important to characterize since these chromophores were used in the same way to dye textiles and to paint in the form of lakes.

The photochemical characterization and photodegradation study of the main chromophores of each selected dye was first performed in homogeneous media, as there are analytical approaches already developed that could be followed. Also in these homogeneous media, their complexes were characterized. It is also important to notice that these chromophores are medium emitters when complexed with aluminium ion, which enables to characterize their fluorescence emission. This first approach makes easier the characterization of the solid state, a more complex and real environment. The emission together with the excitation spectra was characterized in this thesis

1.2.1.1. Photophysical and photochemical characterization

Physical and chemical processes induced by the absorption of light can occur in organic dyes, creating excited new molecules. As Turro mentioned "electronically excited states of molecules are the heart of all *photoprocesses*" [3], because once excited, the molecule is energetically unstable, compared to the ground state. The molecule can react until reaching again the lost stability, in two distinct ways: chemically if the excited molecule reacts structurally, reaching a different irreversible constitution or configuration, or physically if the involving bond breaking and formation are reversible, for instance ESPT [4-8], or if it loses the excess of energy by interconverting to other excited states or by returning to the ground state [3, 9-11].

When the excited molecule returns to the ground state, with a lifetime[1] usually in the order of nanoseconds, this can occur by radiative or radiationless processes. Radiative processes are characterized by the "allowed" emission, also be called fluorescence ($S_1 \rightarrow S_0 + h\upsilon$) or by phosphorescence ($T_1 \rightarrow S_0 + h\upsilon$), a "forbidden" emission. (Figure 1.1.). Radiationless decay occurs by internal conversion (direct return to the ground state) or intersystem crossing (transition between two isoenergetic vibrational levels belonging to electronic states of different multiplicity [9]), possibly followed by emission of phosphorescence or delayed fluorescence, as schematically described in Figure 1.1. by the traditional Jablonski diagram.

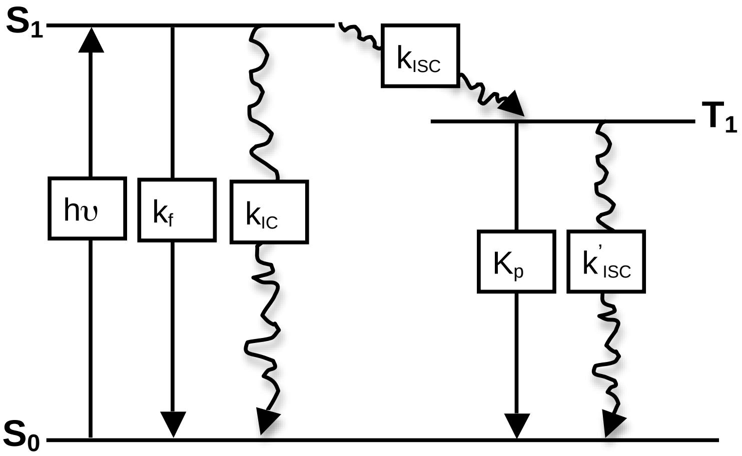

Figure 1.1. Adapted Jablonski diagram [9], where S_0 and S_1 are the singlet and excited singlet state respectively and T_1 is the first triplet state; k_f and k_p are the radiative rate constants for fluorescence and phosphorescence; the non-radiative rate constants for internal conversion, k_{ic} and intersystem crossing k_{isc} ($S_1 \rightarrow T_1$) and k'_{isc} ($T_1 \rightarrow S_0$).

A molecule can be photophysically characterized by the absorption, fluorescence and phosphorescence spectra and by determining the triplet-triplet absorption, fluorescence lifetimes, quantum yields and calculating the rate constants responsible

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[1] Lifetime is the average time during which the molecules stay in the excited state [9]

for the excited state deactivation and the fluorescence quantum yield, which takes in account the fluorescence rate coefficient (k_f) and all the efficiency of the occurring processes while the excited molecule stabilizes (Σk_i) [9, 10]:

$$\Phi_f = \frac{k_f}{k_f + \Sigma k_i}$$

To characterize a photochemical reaction, the quantum yield of reaction can be defined as [12]:

$$\Phi = \frac{amount\ of\ consumed\ reactant\ or\ formed\ product}{amount\ of\ absorbed\ photons}$$

Alizarin, purpurin and carminic acid chromophores have already been studied in relation to some of their excited state properties [1, 2, 13], but a full photochemical characterization is still to be made. Photodegratation studies are very important to understand the molecular mechanisms of degradation in molecules used since Antiquity, in order to be able to plan strategies to prevent their fading and improve their durability.

> “A color (…) scale insect does not announce, through its (…) glands, the presence
> of any dyestuff. Only the dyer knows that, through his chemical manipulations,
> brilliant and hardy colors can be derived from the often unattractive natural materials.”
>
> Gösta Sandberg, in *The red dyes: cochineal, madder*
> *and murex purple, a world tour of textile techniques*

1.3. Madder

1.3.1. Natural sources and main chromophores

Madder is the common name of the plant genus *Rubia* and family *Rubiaceae*, it can be found wild or cultivated. Most growers prefer the cultivated *Rubia tinctorum* L. (Figure 1.2.), known as the dyers’ madder, which yields more powerful, colour powder [14]. According to Brian Murphy, wild madder (*Rubia peregrina* L. or *Rubia sylvestris* L.) is now more of a novelty or vestige of the plant’s glory days that ended more than a century ago [15].

Figure 1.2. *Rubia tinctorum* L. (image by Thomas Schoepke)

It has been used since pre-historic times [16, 17], there are written accounts of its use to dye, in ancient Egypt [18-20]. The ancient Greeks and Romans also used it as a medicinal plant but its medicinal properties are not as significant as its use as a dye plant. Madder was mentioned by Dioscorides in his *Materia Medica*, where he distinguished between wild and cultivated varieties [21] and by Pliny (*Rubia passiva*), who believed the most bountiful madder flourished in gardens near Rome, although “almost all provinces grow this in abundance” [22].

Even being the most popular and dominant red dyestuff in European markets [21, 23], dyeing with madder requires knowledge and great precision because madder roots vary greatly in quality [24].

Recent identification of madder dye in more than thousand years old archaeological textiles [25-28] revealed the presence of purpurin and alizarin (among the organic dyes most resistant to light-induced fading [29]), the main madder chromophores in certain species. The red extracts were also prepared to use as a pigment for painting, by precipitating the extract in solution with aluminium salts, such as alum. The pigments were known as "lakes". Madder lake, a mixture of alizarin and purpurin lakes, was first described by Heraclius in the 10th century [30], it was used in manuscript illuminations [31-33] and paintings [34,35], and is still in use as artists' material.

Figure 1.3. Madder dried roots

The part of the plant used for the dye is the root (Figure 1.3.), which according to Cardon contains 28 anthraquinonic derivatives [16]. Alizarin, which is the main colorant (for the majority of madder species), pseudo-purpurin, ruberythric acid[2], munjistin, christophin and a lucidin heteroside have all been detected in fresh roots. In dried roots purpurin, xanthopurpurin, nordamnacanthal, quinizarin and two of its derivatives, anthragallol and one of its derivatives, alizarin 1-methylether and rubiadin 1-methylether can also be found [16, 25]. But, according to Rafaëlly *et al.*, depending on the analytical technique (HPLC-UV at 250nm [38], HPLC-MS and HPLC-DAD [39]) used to identify the madder constituents, the anthraquinones detected are not the same and are far from a complete detection [40].

Alizarin and purpurin were studied in this thesis because are the main chromophores in *Rubia tinctorum* L., the most important species of the family Rubiacea, probably one of the most used in Portugal.

1.3.2. Alizarin, purpurin and their lakes

Alizarin (1,2-dihydroxy anthraquinone) can be found in the roots of *Rubia* spp. or other species like *Galium* L., or be synthesised (Figure 1.4.). Purpurin (1,2,4-trihydroxy anthraquinone) (Figure 1.4) can be found in the roots of madder or other species like *Relbunium* L..

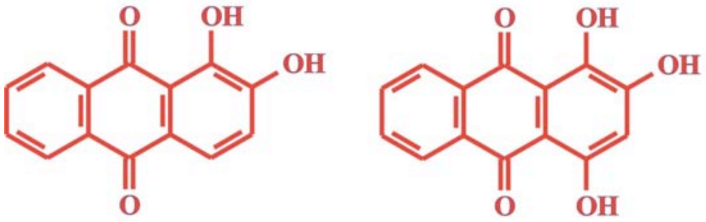

Figure 1.4. Alizarin (left) and purpurin (right) structures

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[2] Rubyrethyric acid is how alizarin can be found in the madder fresh roots. Schunck was responsible for identifying the colour precursor rubyrethyric acid isolation, during a major study of madder that started in 1846 and occupied Schunck for one decade [36, 37].

Synthetic alizarin was the first of the new generation of synthetic reds. Created, not accidently, since its chemical structure was discovered by Graebe and Liebermann at 1868, it was the first ”copy” of a natural dye. The discovery, made simultaneously by Perkin and by chemists working for the German firm BASF in 1869 [41], took a few years (1874) to reach the market, but when it did it was a tremendous success. Purer, brighter, and less costly than natural madder, not only did it completely destroy the market for madder[3], but it made inroads on the cochineal market, too [24].

Alizarin red S (Figure 1.5.) is an industrially important anthraquinone dye coming from alizarin sulfonation. Is commonly used for being water-soluble, although it is a very polluting dye [43, 44].

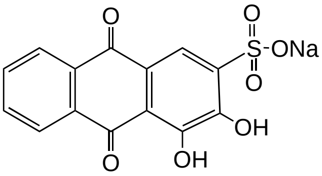

Figure 1.5. Alizarin Red S (3,4-Dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt) structure.

More recently alizarin, as chromophore, has found new and high-tech applications, namely in Solar Energy conversion, development of new imaging materials and in environmental strategies [45-49].

The most common metal salt used to form an insoluble complex with alizarin is potassium aluminum sulfate or alum ($KAl(SO_4)_2 \cdot 12H_2O$), creating a bright red. Other salts were introduced in the 16th century to dye textiles and in the end of 18th century on paints [50]. For instance copper sulfate, potassium dichromate, ferrous sulfate, tin, barium, lead, magnesium and calcium salts [51] originating colours such as red-violet (Sn), purple (Ca, Ba and Pb), black-violet (Fe), violet (Mg), yellow (Cu) or brown-violet or red-brown (Cr) [25, 52] were used.

1.3.2.1. Ground and excited state properties

In the last years there has been a comprehensive body of published work on the physical-chemistry and chemical properties of hydroxy, amino and acylamino derivatives [53-59]. The acidochromic effects in the excited state properties, for alizarin and purpurin, have been recently studied by Favaro and Milliani [1]. From these studies it has been concluded that the substitution pattern of hydroxy anthraquinones is

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[3] According to Finlay, in 1868 the price of madder in London was 30 shillings a hundred weight, but in 1869 it was just eight [42].

determinant in which concerns their excited state properties, where efficient radiationless processes, such as excited state proton transfer (ESPT) and excited state intra- and intermolecular proton transfer (ESIPT) play a determinant role in the overall stability of the molecules. It was shown that the excited-state surface can be sensitively controlled by substituents, allowing the formation of a 1, 10 keto tautomer, T*, which ratio (compared to the excited 9,10) can be varied at least two orders of magnitude (Figure 1.6.).

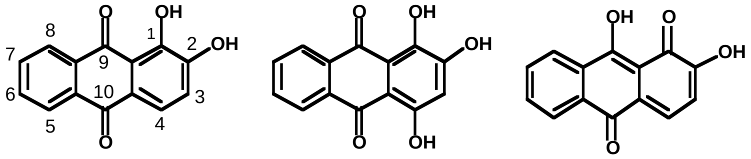
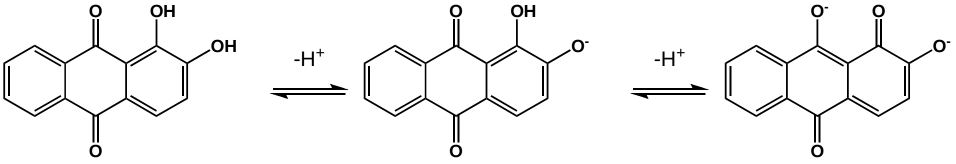


Figure 1.6. Alizarin (1,2-dihydroxy anthraquinone), purpurin (1,2,4-tri-hydroxy anthraquinone) and the 1, 10 keto tautomer of alizarin, T^*. Both, alizarin and purpurin are 9,10 quinones.

The ESIPT process can be extremely rapid (<300 fs) and was believed to involve proton tunneling [55]. ESPT and ESIPT with excited state tautomer formation can be considered to induce a photoprotective mechanism for the molecule, enabling a very fast and effective dissipation of the excess energy of the excited state. Light is absorbed, an excited state tautomer is formed (involving an adiabatic proton transfer), and will decay with recovery of the initial ground state molecule.

Moreover, the hydroxy derivatives are multi acid-base equilibria systems, *interconvertible* by pH inputs (Figure 1.7.) [1]. Therefore, in the ground state the system is easily characterized through the acid-base equilibrium constants, pK_a, but in the excited state the acid-base equilibria are found to compete with intra- and intermolecular proton transfer [1, 54-57].



A **A**$^{-}$ **A**$^{2-}$

Figure 1.7. Acid-base equilibrium system of alizarin

The formation of an excited 1,10 keto tautomer, T*, (Figure 1.6.) was observed in α-hydroxyanthraquinones which lack the 1,4-substitution pattern, and the dyes are all weak emitters [54-57]. On the other hand, in the presence of a 1,4-substitution pattern, the fluorescence quantum yield was found to be moderate to high [54]. The above results are consistent with purpurin acting as a moderate emitter (no ESIPT) and alizarin a very weak one (ESIPT present) [1, 54-57]. The fact that ESIPT is suppressed in the case of the 1,4-hydroxy substitution is an intriguing result, and a possible explanation was already proposed by Gillispie in 1984, based on the existence of six-membered *pseudo*-ring created by the intramolecular hydrogen bonds: "In the 1,4-substituted compounds there are two such rings which can interact conjugatively through their common outer ring of the anthraquinone skeleton. Probably this leads to a stabilization of the 9,10-keto form in the S_1 sufficiently strong to make ESPT energetically unfavourable." [54] (Figure 1.8.).

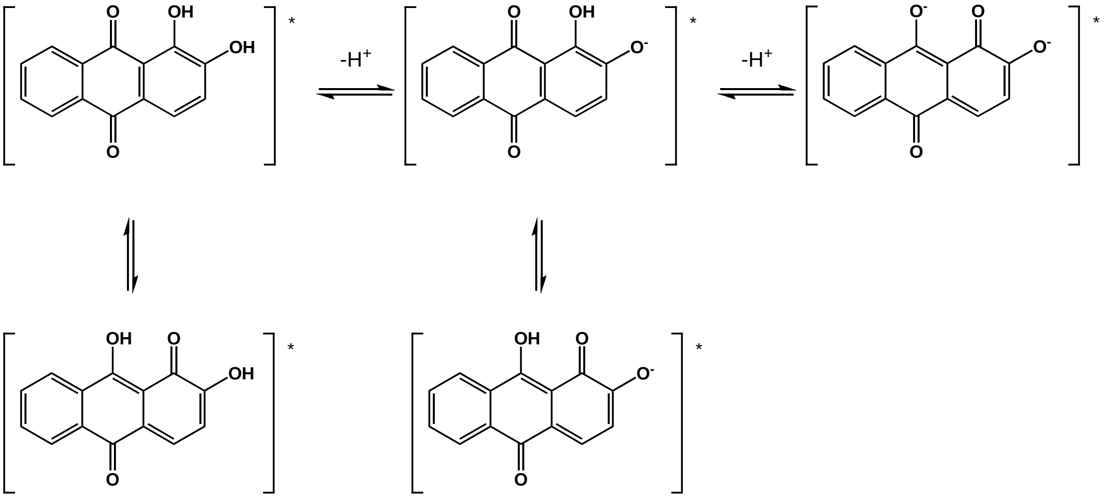


Figure 1.8. Acid-base equilibrium system of alizarin and its tautomer forms in the excited state.

This molecule is good electron acceptor since the hydroxyl substituents are electronegative, thus increase the molecular conjugation, a great number of $\pi \rightarrow \pi^*$ transitions are then possible [53]. Alizarin is used as dye because of the weak energy gap between its frontier orbitals [60].

While there is significant data from studies of anthraquinone derivatives in solution, less is known about the excited state properties of red lakes [2, 61, 62]. Many structures of alizarin lakes have been proposed since 1893, by Liberman who was the first to synthesise alizarin crimson [52]. Other structures of the calcium-aluminum lake of alizarin were also proposed by Kiel and Heertjes, based on model deduction [63, 64] (Figure 1.9.). The alizarin molecules are capable of forming six-member chelate rings with aluminum ions [51].

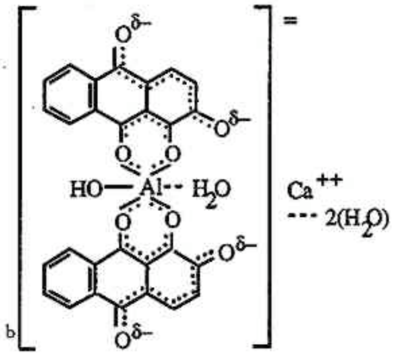


Figure 1.9. Alizarin lake structure proposed by Kiel and Heertjes.

Solid state NMR studies of alizarin lake made with aluminium and using a large scale production technique suggested that if Na^+ or Ca^{2+} are the counterions used, the lake formed should have a closed structure, although if K^+ or Ba^{2+} are used, an open structure is formed due to their higher ion volume as depicted in Figure 1.10. [65]. Sanyova [52] has also studied this lake, reviewing all the structures described until then, but neither a structure was accepted nor proposes were suggested.

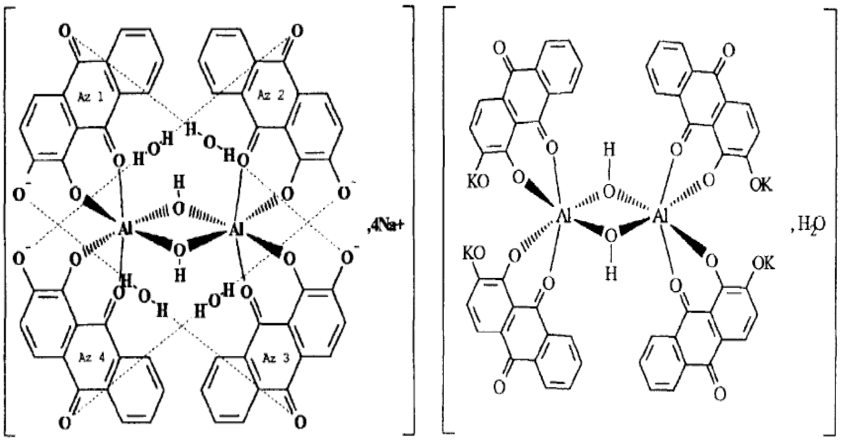


Figure 1.10. Closed and open structures purposed by Soubayrol et al.

1.4. Lac dye

1.4.1. Natural sources and main chromophores

Lac dye is other red dye used since Antiquity. Dioscorides and Bolus Democritus may allude to lac, and there are unequivocal references in Hebrew and early Arabic sources [23]. Native to India and Southeast Asia [21, 23, 66-69], lac was made from insect *Kerria lacca*, from the *Kerriidae* family, also known by numerous synonyms such as *Laccifer lacca*, *Carteria lacca*, *Tachardia lacca* and *Lakshadia lacca* [16] (Figure 1.11.). It is a source of both lac and shellac.

Figure 1.11. Lac dye and the products extracted [66] (left) and phytoparasite insects and a stick covered with the resin (right).

The female insect produces a sticky resin on tree twigs, which envelopes their bodies and is collected with the bugs still inside it. Ber trees are considered amongst the best for rearing lac insects, although when used by them, its use as trees for fruits is not viable, since the insect devitalises the tree [70].

The colour stuff is extracted from the water-soluble resin fraction, and after the extraction, it is evaporated until cakes are formed. The water-insoluble part, the shellac, is then extracted with alcohol, usually ethanol, or other organic solvents, to be commercialized as a varnish.

The colour was used to dye textiles, although European dyers found it expensive, and the gummy substance hard to work with and used it primarily for dyeing leather [24]. Different shades could be obtained using alum (carmine hue), potassium dichromate, stannous chloride (scarlet hue), copper sulfate and iron salts (purple hue) which have also the function of improving colour fastness [71, 72].

1.4.2. Laccaic acid A

The colour of lac dye is due to the presence of different laccaic acids: A, B, C, D and E[4], being the laccaic acid A (3,5,6,8 – tetrahydroxy -7(3-(2-hidroxyethilphenyl)-9,10-dioxo-9,10-dihydroanthracene-1,2-dicarboxilic acid) the main chromophore (Figure 1.12.).

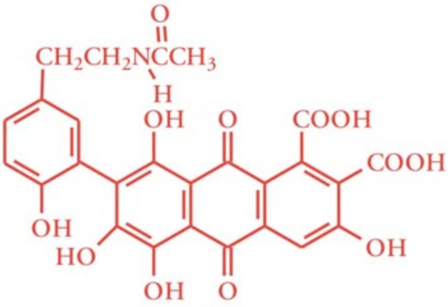


Figura 1.12. Laccaic acid A structure.

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[4] The amount of each laccaic acid A depends on the locality and the season [73, 74]

1.4.2.1. Ground state properties

There are studies performed by Indian Lac Research Institute, but the results are not easily accessed to general public. This chromophore still one of the most neglected, concerning its characterization, usually it is only identified with chromatographic techniques [73, 75, 76], and the absorption maxima in different solvents (ethanol, water and acetone) were also published [71]

1.5. Cochineal

1.5.1. Natural sources and main chromophores

Cochineal insects belong to the family Coccidae and to two distinct genus: the *Porphyrophora polonica* (Polish cochineal) or *hamelii* (Armenian cochineal), used in medieval Europe and the genus *Dactylopius* (Costa), the former are parasites hosted in diverse plant's roots, which grow on the sandy soils of Central Europe [21]; and the later are parasites of cacti belonging to the genera *Opuntia* and *Nopalea* (Figure 1.13.) [21, 77].

Figure 1.13. Cochineal on Opuntia cactus (photo by Mark Dimmitt, 2001) and dry cochineal.

To some authors the name cochineal is due to the similar appearance with woodlice (Oniscidea suborder), in Spanish "cochonilla de la humedad" [78].

Cochineal[5] (*Dactylopius coccus*) has a long history covered "in political intrigue and embellished by folklore" [79]. It was used since Aztec, Maya and Incan peoples. The Spanish people, who first though that was the kermes of their native Spain, were immediately impressed with its utility, starting a monopoly trade in the 16th century [80].

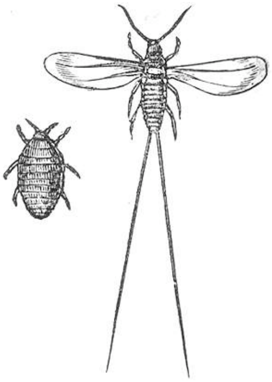

Figure 1.14. Female and male cochineal

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[5] Domesticated insects are usually a single species, *Dactylopius coccus* (which is double the size of other species and takes twice as long to complete its life cycle), the wild or partly domesticated insects to several species.

Collecting the mature cochineal (also called *grana*) was a patient process. Depending on the drying process, it could be necessary *circa* 70000 insects for a pound [81]. To increase the number of cochineals, Mexicans started to cultivate them (Figure 1.14.) [72]. Domestic cochineal creation was reported by Gonzalo Gómez de Cervantes (*Vida económica y social de Nueva España*, *ca.* 1599*)* and José Antonio de Alzate y Ramírez (*Memoria en que trata del insecto grana ò cochinilla*, 1794-1795) [21]. Uncultivated cochineal, called by the Spanish *grana Silvestre*, was only half as large as the cultivated, and it yielded an inferior dye [81].

The red dye is obtained from the eggs of the female, from the wild ones it might be collected throughout the year. From the domesticated species there are harvest seasons, usually twice or even thrice in the course of a year. The females were cropped before depositing the eggs, leaving only sufficient to ensure the production. [16, 21]

Cochineal substituted the kermes, the most used red dye in Europe until then, which could be justified by the higher amount of carminic acid present in cochineal [82].

1.5.2. Carminic acid and its lake

Carminic acid is the main chromophore of cochineal. It could be extracted by different ways: handmade, by grinding the dry cochineal and boiling it in water, easily dissolved due to the presence of many free polar groups in the anthraquinone and glucose parts of the dye molecule [83]; or industrially where cochineal is transformed in a fine powder with intense purplish red colour [84]. The adult females of cochineal (*Dactylopius coccus* Costa) contain 19 to 24% carminic acid (in dry weight) [82].

Pigments made with cochineal (usually named carmine) were, according to Donkin, available in Mexico in the sixteenth century and probably long before [21]. The *Codex Florentino* (*ca*. 1570) describes the preparation of colours and shows an illuminator at work with several cakes of *nocheztli* [6] (Figure 1.15.). There are records (Sahagún, 1540 and Father Cobo, 1653) that alum was commonly used to dye with cochineal and to make the cakes [21].

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[6] *Nocheztli*, was the name given by Aztecs that in their language means “nopal blood” [78]

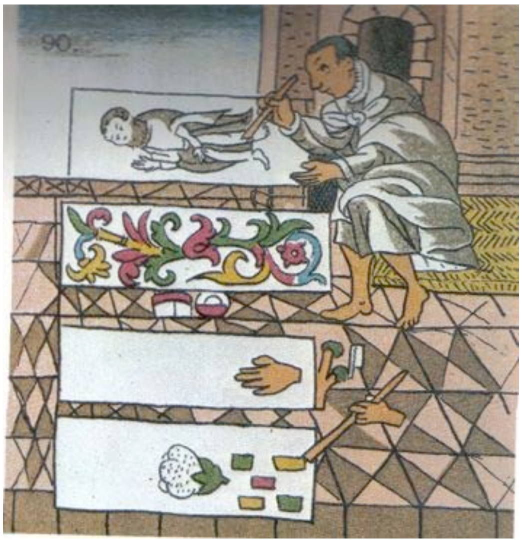

Figure 1.15. Indian learning how to paint in the European method at a church school, Codex Florentino, 1540.

Carminic acid has been the goal of many studies, mainly focused in its identification applying chromatographic techniques [75, 76]. In the last years the cochineal chromatographic analyses has enabled to discover some "markers" associated to carminic acid that allows the determination and characterization of the *Dactylopius* species [16, 85, 86]. Although regarding photochemical characterization, there are just some photophysical characterization, the transient fluorescence and reorientation responses of carminic acid in water and a series of primary aliphatic alcohols [87]. Its excited state properties in DMSO (Dimethyl sulfoxide) were also studied [2], but a full photochemical characterization is still to be made.

1.5.2.1. **Ground state properties**

Carminic acid (7- β-D-glucopyranosyl-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid). It has an anthraquinone nucleus with carboxylic acid and hexose sugar attached (Figure 1.16.). The oxygen atoms and hydroxyl groups, create an ample opportunity for hydrogen bonding.

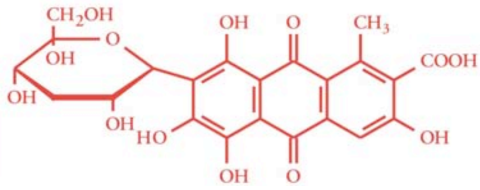

Figure 1.16. Carminic acid structure.

According to Golikov, carminic acid has two positions where chelate complexes with metal cations can be formed [79, 83]. Based on comparable structures of alizarin, previous referred by Kiel [63], Meloan et al. proposed the structure based on Stuart-Briegleb model, represented in Figure 1.17 [88]. Nevertheless, Dapson argue that this proposed structure is unfavoured, since the two dye molecules are in the same plane, and bond angles for aluminium do not permit a single plane configuration. According to him, the carminic acid groups must lie crosswise to one another at an angle about 120° and the two carminic acid groups are in the *trans*, not *cis*, position [79].

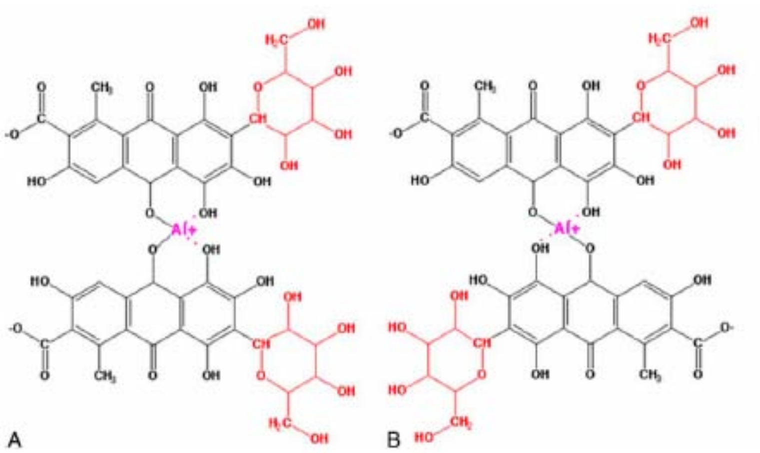

Figure 1.17. Structure of carmine. A) Two-dimensional model proposed by Meloan et al. [88] that had both carminic acid in the same plane as hexose units (red) on the same ends. B) Structure as verified by molecular modeling software in which the hexose units are on opposite ends [79].

1.6. Photodegradation studies

Photodegradation studies have been performed, commonly on wool textiles dyed with madder, revealing that madder has fair light fastness [89], stronger when subject to UV radiation than to visible light [90]. Recently Clementi *et al* studies on dyed wool with alizarin and purpurin [91] concluded that "degradation results in a decrease of absorption and emission intensities of the dye and a blue shift of the dye fluorescence maximum.", also that degradation is generally higher for purpurin than for alizarin and the mordant has a protecting action on degradation. Fading experiments with dyes on wool have traditionally been reported in terms of colorimetric measurements only [52, 92], whereas the literature dealing with the effects of light exposure on the spectral properties of natural dyes is scarce [91].

Until now photodegradation studies enabled to conclude that alizarin reacts but no reaction product was proposed. Photodegradation studies are scarcer for purpurin than for alizarin. The results published are from Clementi *et al.* [91], stated that degradation is generally higher for purpurin than for alizarin. No published studies were found about lac dye, laccaic acid, cochineal or carminic acid photodegradation or any reference to their stability.

In this thesis, both monochromatic and polychromatic irradiations were used to follow the photodegradation reaction. In order to determine effectively the quantum yields of reaction of alizarin and purpurin, to better characterize them, monochromatic irradiation was used. To simulate the real conditions, meaning the solar spectrum, that chromophores could be subject, polychromatic irradiation was used for lac dye, the one found in Lorvão collection.

1.7. Fluorescence emission spectroscopy as a non-invasive technique

Analytical techniques based on fluorescence detection were selected to take advantage of the opportunity for high sensitivity and selectivity, combined with good spatial resolution [9, 93-95]. Using fluorescence microscopy in confocal configuration (Figure 1.18.) an excitation area of 2 µm can be defined for visible radiation with the advantage of similar resolution in three-dimensions [9].

Fluorescent molecules can also be used to probe and characterize their environment, which is one of the reasons for their popularity in biochemical and pharmaceutical applications and in medicine, for monitoring of species of clinical relevance. Although analytical techniques based on fluorescence detection are widely used, it is the first time that potential for emission fluorescence microspectroscopy is investigated for the study of red lakes used in cultural heritage, with the possibility of acquisition of emission and excitation spectra [7].

Figure 1.18. Confocal microscope objective and a 18th century manuscript book.

The potential of this technique was used in reconstructions based on historical written sources [30-33, 92, 96], in order to characterize and create a database of red organic colorants applied in cultural heritage, and then was also used to identify their application on works of art like illuminated manuscripts, textiles and paintings.

2. Results

2.1. Photochemical characterization

2.1.1. Ground state: acid –base equilibrium in solution

2.1.1.1. Alizarin

Alizarin characterization was performed in a mixture of methanol and water (75%:25%, v/v), since its solubility is very limited in water, but not in methanol, with no O_2 , in order to avoid the degradation of deprotonated alizarin. At a very acidic pH values, the absorption band neutral alizarin (**A**) is yellow (λ_{max}=430nm). Increasing the pH, a new maximum at 530 nm is reached, due to the red mono-anion alizarin (**A**$^-$) formed, pK_{a1}=7.5. Increasing even more the pH, the characteristic band of the purple di-anion alizarin (**A**$^{2-}$) is formed with its maximum at 570 nm, pK_{a2}=13.5 (Figures 2.1. and 2.2.).

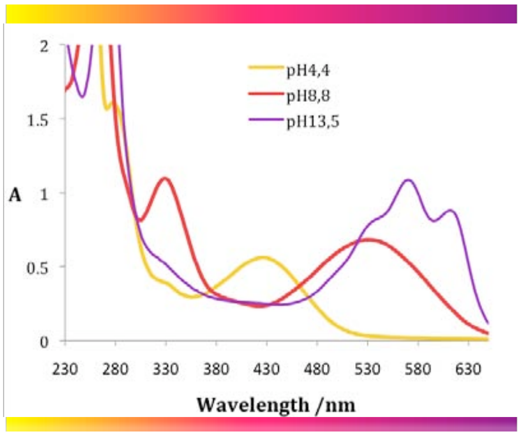

Figure 2.1. UV-VIS spectra of alizarin (8 x10^{-5} M) in neutral (yellow line), mono-anion (red line) and di-anion (purple line) forms.

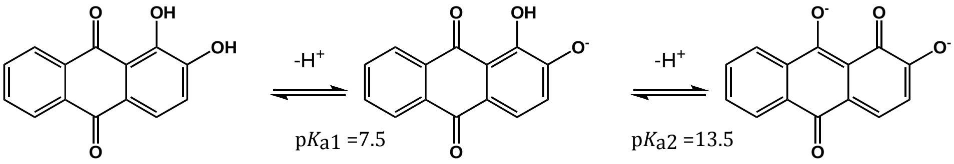

Figure 2.2. Acid-base equilibrium system of alizarin.

A second study was also performed on alizarin red S, since it would be used in the solid state photodegradation. Although this molecule is soluble in water, the same conditions (MeOH:H_2O, 75%:25%) were used to enable any comparisons. As expected the pK observed are different due to the influence of the sulfonate group: pK_{a1}=6.5 and pK_{a2}=11.8 (Figure 2.3.) [97].

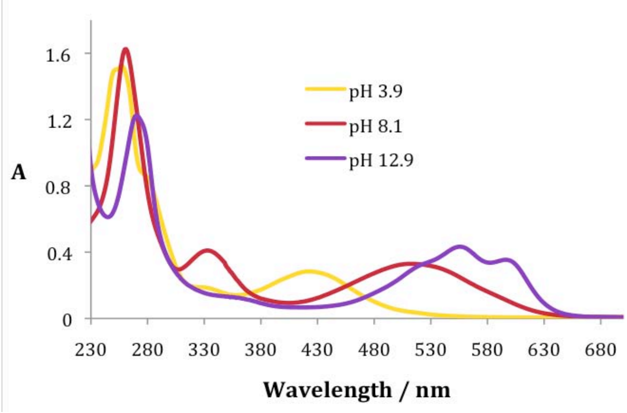

Figure 2.3. UV-VIS spectra of alizarin red S (8 x10^{-5} M) in neutral (yellow line), mono-anion (red line) and di-anion (purple line) forms.

As there are no comparable published results from studies of the photophysics of the aluminum complexes in solution, a brief study was carried out for alizarin in the presence of aluminum ion, Al^{3+}, after a previous characterization of the chromophore in the same conditions.

The method of continuous variations (or Job's method) and the mole-ratio method [98, 99] were used to determine the stoichiometry of the alizarin and aluminium complex (Appendix III, section III.1.1.). The results were not conclusive since it appears that two complexes can be formed, one in which the Al atom co-ordinates two dye molecules through the 1-hydroxy oxygen and the adjacent carbonyl oxygen (Figure 2.4.) [7, 100], and other where Al atom co-ordinates one dye molecule.

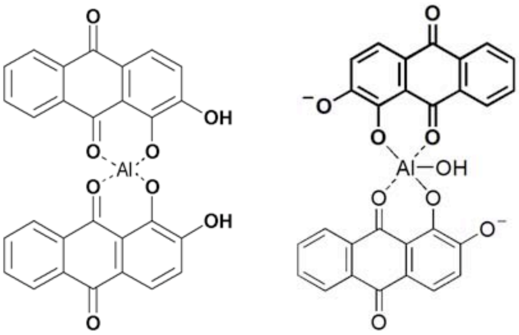

Figure 2.4. Two molecular structures proposed for an Al^{3+}:alizarin 1:2 complex.

Analyses performed with NMR [101], and currently in progress, pointed out that a tautomer could be formed and react in two diferent modes when Al^{3+} is added. Further studies are still been developed using liquid and solid NMR technique [101] in order to better understand how this complex is forming.

2.1.1.2. Purpurin

Purpurin characterization was performed in a mixture of methanol and water (75%:25%), due to its limited solubility in water, but not in methanol; and in absence of O_2, by the same reason presented to alizarin. In acidic solutions (pH ≤5.8) the absorption spectrum of neutral purpurin (**A**) showed a maximum at 480nm. With decreasing acidity, the absorption shifted to longer wavelengths and the solution turned from yellowish orange to red, with a maximum wavelength at 510nm. An isosbestic point was observed at 430nm. The increase of the pH shows that maximum wavelength does not change although the spectrum presents variations in its shape and there is an enhancement of absorbance. A further pH increase caused a shift of the spectrum and red turned into a purple colour. At pH ≥12.8, the spectral shape and intensity remained constant. This spectrum, which is assigned to the tri-anion is vibronically structured and shows maxima located at 545 nm (Figures 2.5 and 2.6.).

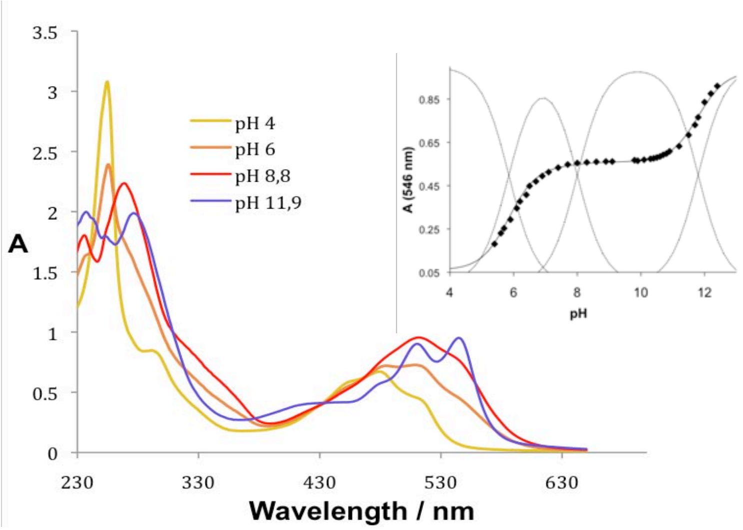

Figure 2.5. UV-VIS spectra of alizarin (8 x10^{-5} M) in neutral (yellow line), mono-anion (orange line), di-anion (red line) and tri-anion (purple line) forms. In the inset are the different forms according to the pH.

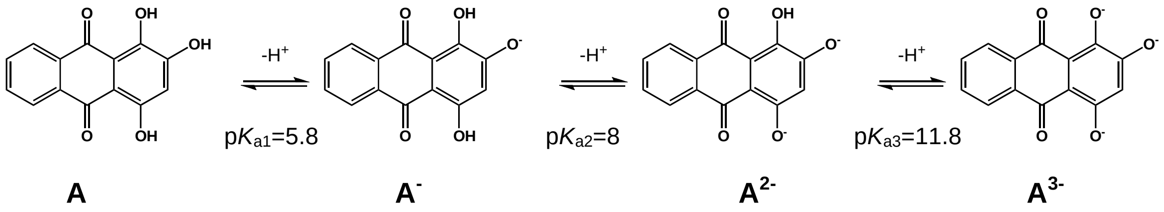

Figure 2.6. Acid-base equilibrium system of purpurin.

A study was carried out for purpurin in the presence of Al^{3+}, since purpurin, like alizarin, has no comparable published results from studies of the photophysics of the aluminum complexes in solution. Similarly to alizarin, the Job's method was also applied to purpurin, in order to determine the complex stoichiometry. The results point to 1:1 Al^{3+}: purpurin complex, although the mole-ratio method was inconclusive (Appendix III, section III. 1.2.). Nevertheless it is easier to accept that this complex's structure is 1:1 since purpurin is larger than alizarin, due to one more hydroxyl group present and the molecular rearrangement is more limited. Further studies need to be done.

2.1.1.3. Laccaic acid A

Laccaic acid A characterization was performed in a mixture of methanol and water (75%:25%, v/v), to maintain the same conditions used to the alizarin and purpurin. At low pH the absorption spectrum of neutral laccaic acid A (**A**) has a maximum wavelength at 488nm. Increasing the pH, the orange solution turns into red and a new maximum at 525 nm is reached, pK_{a1}=5.6. Continuing to increase the pH the di-anion (**A^{2-}**) is formed with the same maximum (525nm) as the mono-anion (**A^{-}**), but with a more intense and different absorption spectrum shape, pK_{a2}=7. Increasing even more the pH, the characteristic absorption spectrum of the purple tri-anion (**A^{3-}**) with vibronic structure is formed. The maximum wavelength is at 564 nm, pK_{a3}=9.8, (Figures 2.7. and 2.8.). Net isosbestic point at 430 nm for the laccaic acid A forms and an isosbestic point at 486 for mono-anion, di-anion and tri-anion forms, were observed.

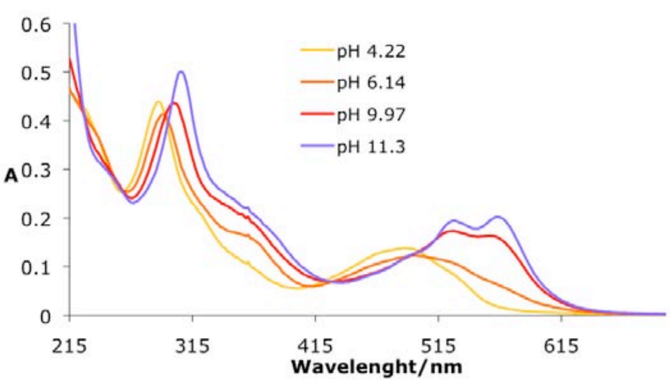

Figure 2.7. UV-VIS spectra of laccaic acid A (8.4 x10^{-5} M) in neutral (yellow line), mono-anion (orange line), di-anion (red line) and tri-anion (purple line) forms.

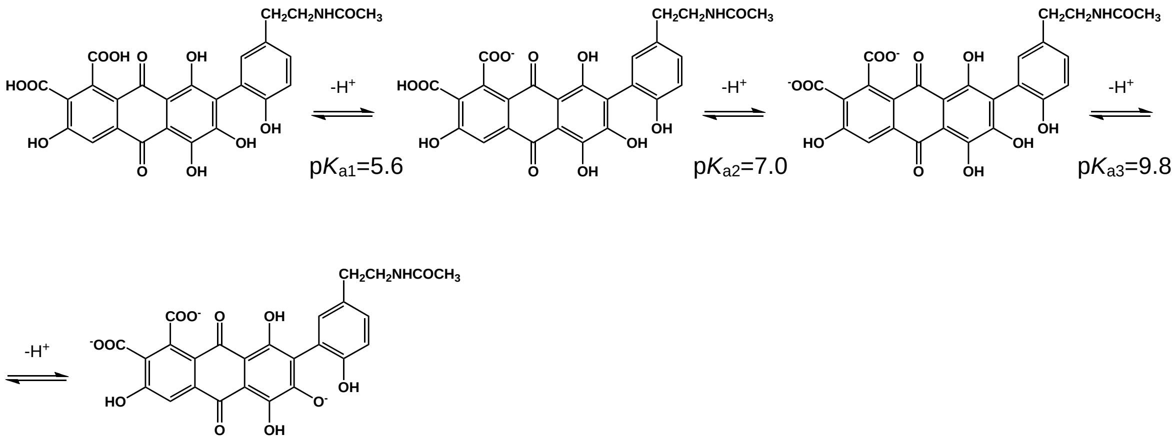

Figure 2.8. One possible acid-base equilibrium system of laccaic acid A.

2.1.1.4. Carminic acid

Carminic acid was characterized in a mixture of methanol and water (75%:25%, v/v), to maintain the same conditions used to the previous studies. It was possible to define four p*K*s: $pK_{a1}=2.9$; $pK_{a2}=6.6$; $pK_{a3}=10.3$ and $pK_{a4}=13.4$. The neutral (**A**) and mono-anion forms (**A**$^-$) have their maxima wavelength absorption at 492 nm and 496nm, respectively, di-anion form (**A**$^{2-}$) has its maximum at 528 nm and tri (**A**$^{3-}$) and tetra-anion forms (**A**$^{4-}$) have the same maximum at 564 nm. With increased pH, the solution changes from orange (pH ≤2.9) to red (pH≈4-10) to purple (pH ≥10.3). A net isosbestic point was observed at 430 nm. It was also possible to verify an intensity change in the spectrum of tetra-anion form and, like to the other chromophores, a vibronic structure. (Figures 2.9. and 2.10.).

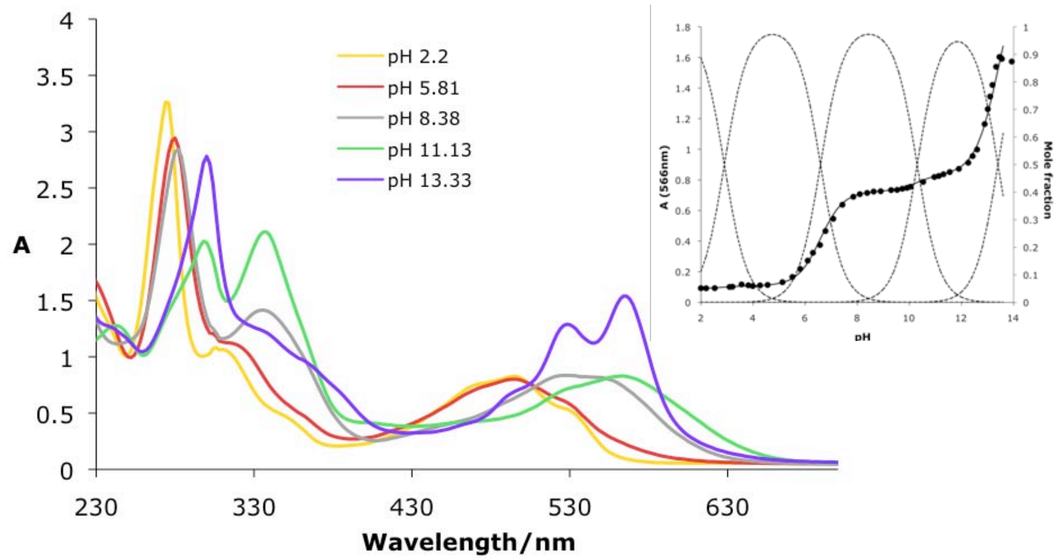

Figure 2.9. UV-VIS spectra of carminic acid (8.6 x10^{-5} M) in neutral (yellow line), mono-anion (red line), di-anion (grey line), tri-anion (green line) and tetra-anion (purple line) forms. In the inset are the different forms according to the pH.

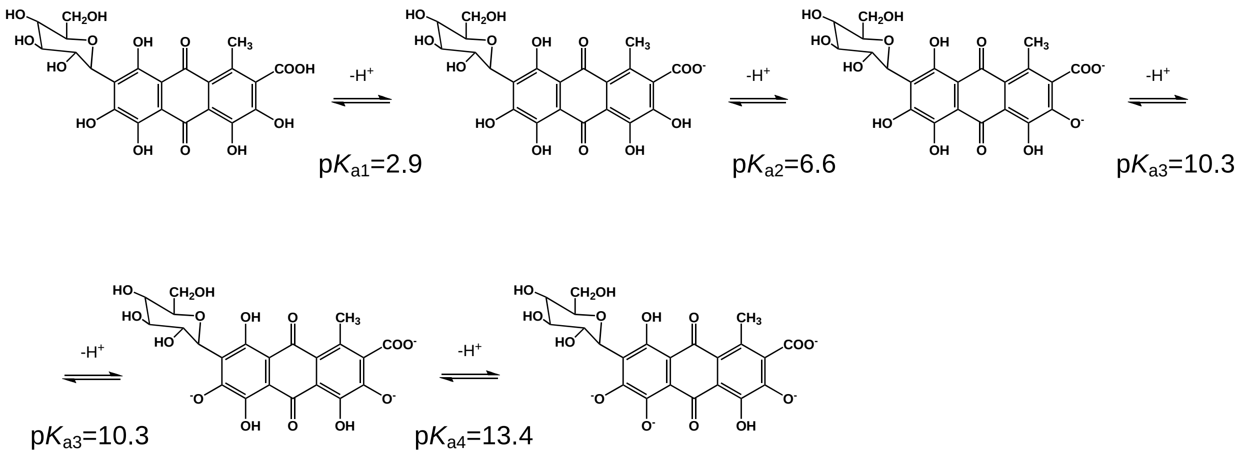

Figure 2.10. One possible acid-base equilibrium system of carminic acid.

2.1.2. Excitated state characterization

Excitation and emission spectra were obtained to be used as reference guides to the understanding of the solid state behaviour of the paint models (Figure 2.11.). Fluorescence quantum yields, for the Al^{3+} chelates, were also obtained and are reported in Table 2.2, where they can be compared to fluorescence quantum yields of the neutral forms of the respective free chromophores in solution (Table 2.1.). As expected for fluorogenic ligands where the metal chelates are much more fluorescent than the free ligands themselves [102], the aluminum complexation enables in the majority of the chromophores, more than a ten fold increase in the ϕ_f values.

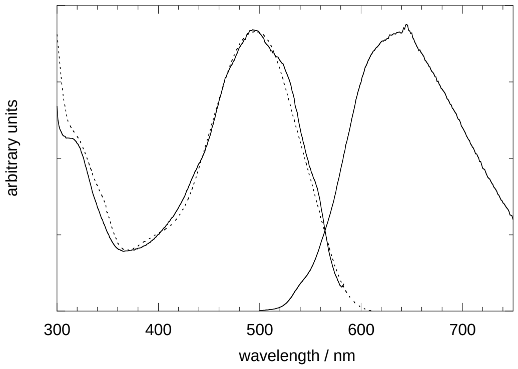

Figure 2.11. Corrected emission and excitation spectra (λ_{em}= 590nm and λ_{exc} = 503nm; abs at the λ_{exc} ≈0.018), full line, for alizarin aluminium complex, in the presence of a 1:100 excess of Al^{3+}, in a methanol/water solution (75:25%) at pH= 3.4. Dotted line, absorption spectra.

Table 2.1. Spectral and photophysical data obtained in solution, MeOH:H_2O (75:25 /v:v) @ 293K, for the neutral forms of alizarin, purpurin, laccaic acid A and carminic acid.

Compound	λ_{abs}(max)/nm	ε_{max}/ $M^{-1}cm^{-1}$	λ_{fluo}(max)/nm	ϕ_f	τ_f/ns	k_f (ns^{-1})*	k_{nr} (ns^{-1})**
alizarin	430	5830	578	5×10^{-4}	0.03^a	1.7×10^{-2}	33.3
purpurin	480	7770	582	4.0×10^{-3}	0.29^a	1.3×10^{-2}	3.4
laccaic acid A	488	9750	582	3.0×10^{-3}	0.03^a	1.0×10^{-1}	33.2
carminic acid	492	7910	590	6×10^{-3}	0.29^a	2.0×10^{-2}	3.4

* $k_F= \phi_F/\tau_F$; ** $k_{NR}= (1-\phi_F)/ \tau_F$; [a]This is the major component of the biexponential decay.

Table 2.2. Spectral and photophysical data obtained in solution, MeOH:H_2O (75:25 /v:v) @ 293K, for 1:100 ratio aluminum complexe (pH=3.4) of alizarin, purpurin, laccaic acid A and carminic acid.

Compound	λ_{abs}(max)/nm	ε_{max}/ $M^{-1}cm^{-1}$	λ_{fluo}(max)/nm	ϕ_f	τ_f/ns	k_f (ns^{-1})*	k_{nr} (ns^{-1})**
alizarin lake	500	7440	640	6×10^{-3}	0.26^a	2.3×10^{-2}	3.80
purpurin lake	531	9540	595	2.9×10^{-1}	5.60	5.2×10^{-2}	0.13
laccaic acid A lake	515	9270	623	1.5×10^{-2}	0.94^a	1.7×10^{-2}	1.10
carminic acid lake	517	8700	618	4×10^{-2}	2.20^a	1.8×10^{-2}	0.44

* $k_F= \phi_F/\tau_F$; ** $k_{NR}= (1-\phi_F)/ \tau_F$; [a]This is the major component of the biexponential decay.

In purpurin, the aluminum complexation enables almost more than a hundred fold increase in the ϕ_f values; the ϕ_f of the alizarin aluminum complex compares with the emission quantum yield of neutral form of purpurin; and the purpurin lake displays the strongest emission, and may be considered a moderate to strong emitter. Moreover, the spectra represented in Figure 2.12. show a reasonably good mirror-image relationship between the excitation and emission spectra for the purpurin complex, with small Stokes' shift, which can reflect the absence of ESIPT processes.

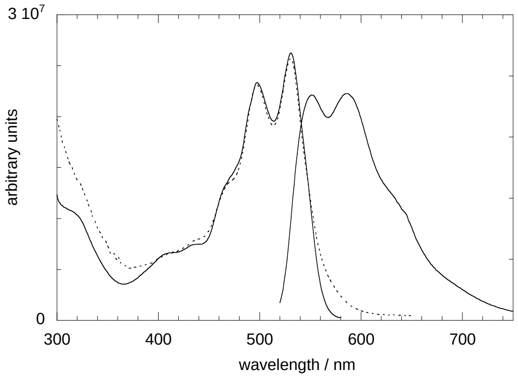

Figure 2.12. Corrected emission and excitation spectra (λ_{em}= 590nm and λ_{exc} = 503nm; abs at the λ_{exc} ≈0.018), full line, for purpurin aluminum complex, in the presence of a 1:100 excess of Al^{3+}, in a methanol/water solution (75:25%) at pH= 3.4. Dotted line, absorption spectra.

The aluminum complexation of laccaic acid A and carminic acid A enables a ten fold increase in the ϕ_f values compared with the chromophores, which enable their characterization as a moderate and weak emitters, respectively. The spectra of the laccaic acid A and carminic acid, represented in Figure 2.13. show a well resolved excitation spectrum, and the characteristic broad emission spectrum, that could be related to the amide (laccaic acid A) and carboxylic groups (laccaic acid A and carminic acid) [9].

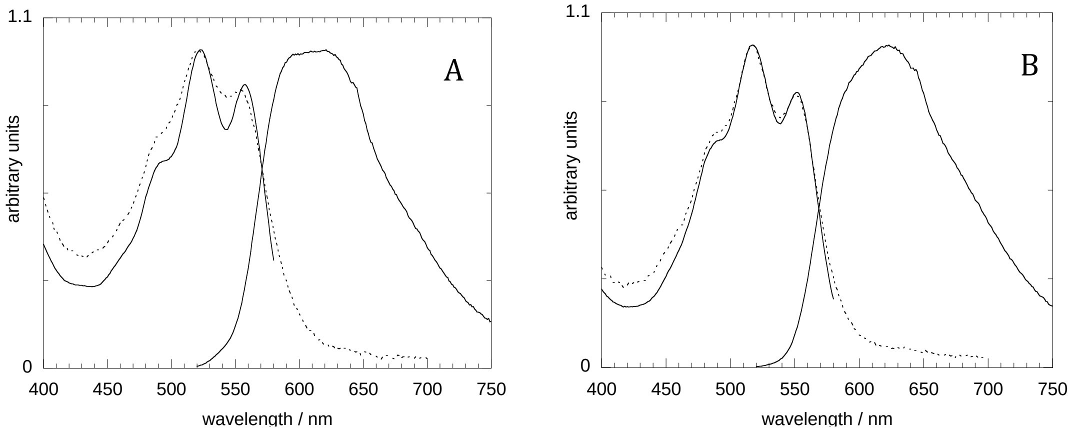

Figure 2.13. Corrected emission and excitation spectra, full line, for laccaic acid A aluminium complex (A) and carminic acid (B), in the presence of a 1:100 excess of Al^{3+}, in a methanol/water solution (75:25%) at pH= 3.4. Dotted line, absorption spectra.

Comparing the photophysical data obtained we can realize that the quantum yield of fluorescence (ϕ_f) of neutral forms of laccaic acid A and carminic acid can be compared to purpurin, while alizarin presents a ϕ_f of an order of magnitude lower which could be explained by the existence of ESIPT. Regarding the fluorescence lifetimes laccaic acid A can be compared to alizarin with a very short lifetime (0.03 ns), and carminic acid with purpurin (0.29 ns). The short lifetime of the laccaic acid A could be explained by the proximity of the amide that can reduce the excited state by resonance energy transfer. When radioative (k_f) and nonradiative (k_{nr}) constants are compared one can observe that carminic acid stills having the same behaviour as the one of purpurin but, despite the k_{nr} of alizarin and laccaic acid A are similar, as expected, the k_f differ in one order of magnitude, which could be explained by the different molar absorption coefficients (ε), which can also explain the difference of their ϕ_f. Meaning that laccaic acid A could eventually have ESIPT as alizarin, noticed by the k_{nr}, but uncovered in its k_f, due to its high ε.

Analysing the photophysical results of the lakes it is possible to identify again a similar behaviour between purpurin and carminic acid lakes, and one closer to be similar of alizarin and laccaic acid A lakes. In this group the purpurin lake has a ϕ_f higher than any of the other lakes, being a hundred fold higher than alizarin lake.

Regarding the lifetime decays once again purpurin lake is different from the other lakes, being the only one that presents a monoexponetial decay, with a long lifetime (5.6 ns). Both alizarin and laccaic acid A lakes have short lifetimes, smaller than 1 ns, carminic acid lake has middle lifetime (2.2 ns). As for purpurin and carminic acid, their lakes have similar low k_{nr} and laccaic acid A and alizarin lakes have higher k_{nr}, being the one for alizarin lake the highest (3.8 ns). When k_f is compared we can verify that purpurin lake present the highest k_f. The other lakes have a similar behaviour. The difference of the ε is not so intense has for the non complexated neutral forms and alizarin presents a higher ϕ_f when compared to carminic acid and laccaic acid A lakes, which can justify a similar k_f.

With this data it is possible to identify a similar behaviour between the two groups, the one made by purpurin and carminic acid, the other composed by alizarin and laccaic acid A. Which indicates that carminic acid and laccaic acid A having a similar anthraquinone structure to purpurin, could have a very different behaviours, according to the substituents, when excited.

2.1.3. Photodegradation study

In this work, quantum yields of reaction[7] were obtained at 430 and 480 nm irradiation wavelengths in homogenous organic and aqueous solutions for alizarin and purpurin, by this order. In proteinaceous transparent gels, alizarin and purpurin were irradiated at 430 and 480 nm, respectively. This design aimed to reproduce the dye environment in paints, and monochromatic irradiation wavelengths were used in order to determine effectively the quantum yields of reaction. UV-visible absorption was used to monitor the reaction over time, whereas HPLC-DAD was applied to determine relative compositional changes that occurred after exposure (final time exposure for monochromatic irradiation and each time exposure in polychromatic study). A Xenon polychromatic source, with a spectral distribution close to the solar spectrum, was used to irradiate laccaic acid A and lac dye in the solid state, in order to better understand the analysed carmine-coloured of Lorvão manuscripts.

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[7] The quantum yields of reaction were determined using the process described by Montalti et al. using $K_3[Fe(C_2O_4)_3 \cdot 3H_2O]$ as actinomer [11].

2.1.3.1. Monochromatic irradiation in homogeneous media

Alizarin, alizarin red S and their lakes were irradiated at or near the maximum absorption wavelength, 430 nm (both alizarins) and 490 nm (alizarin lakes)(tables 2.1. and 2.2.), in MeOH:H_2O (90%:10%, v/v) solution. Irradiations below wavelengths 280 nm were avoided, since this type of irradiation is rarely, if ever, encountered under glass indoors or outdoors and can give rise to different reactions than those induced by the near ultraviolet and visible [103].

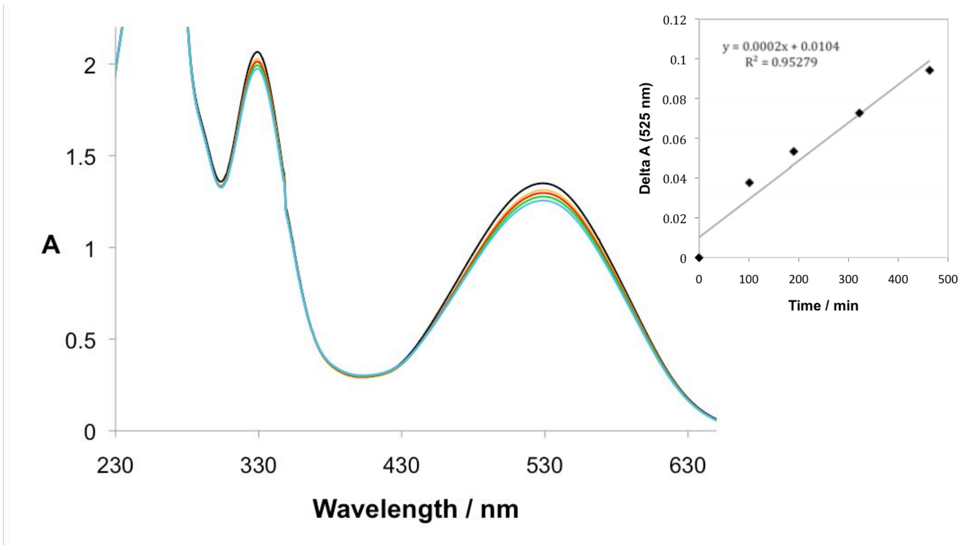
The quantum yields of reaction, Φ_R, obtained at room temperature, were very low (Table 2.3.)(Figure 2.14.).

Table 2.3. Quantum yields of reaction, for alizarin, alizarin red S, purpurin and their respective lakes, in MeOH:H_2O (90%:10% / v:v) at T=293 K.

	alizarin	alizarin lake	alizarin red S	alizarin red S lake	purpurin	purpurin lake
Φ_R homo	$6\text{x}10^{-5}$	$3\text{x}10^{-5}$	$4.6\text{x}10^{-5}$	$4\text{x}10^{-5}$	$6\text{x}10^{-5}$	$8\text{x}10^{-4}$
Φ_R hetero	$8\text{x}10^{-5}$	*	$2.8\text{x}10^{-4}$	*	$2\text{x}10^{-4}$	*

*The experiences were performed but due to reaction of the lakes with the gelatine, the transparency of the gel was lost and the results adulterated.

Comparing the UV-VIS spectra obtained to each alizarin, the variation is minor (Figure 2.14.), even when irradiation time has reached 14 hours, due to the low value of Φ_R.



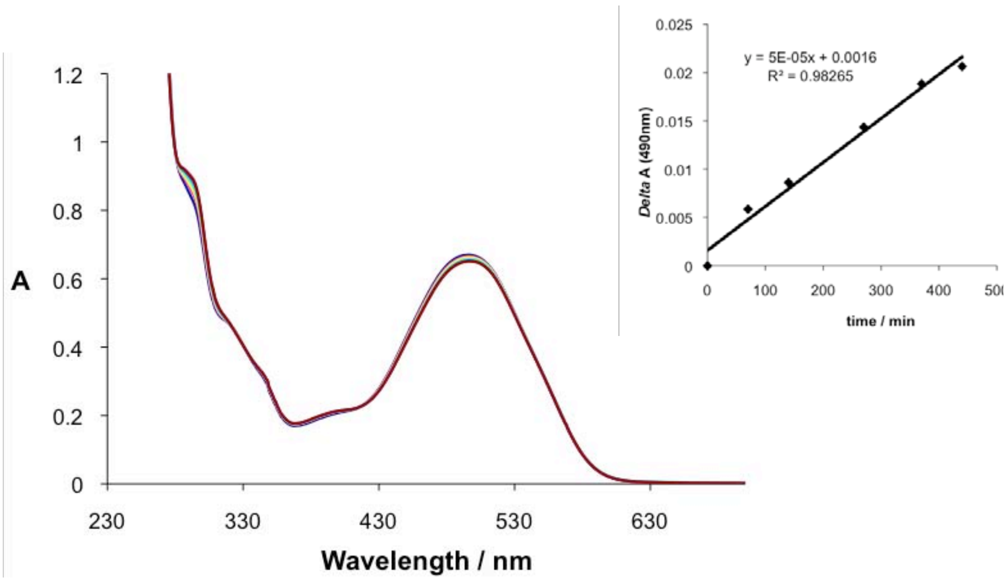


Figure 2.14. Photodegradation of alizarin at pH=8.8 (above) and alizarin lake (below) in MeOH:H_2O (90%:10%, v/v) irradiated at 430 and 490 nm, respectively. In the inset the absorbance variation followed at 525 and 490nm, respectively, is plotted over time, giving the *m* parameter of the Φ_R equation (Appendix III, section III.2.1.).

The analyses performed by HPLC-DAD (Appendix III, section III.2.1., Table III.2.) revealed that alizarin changes the relative area of the main peak (t_r=23.2 min) in 17% after irradiated, and have an increase on the t_r=21.9 min peak in 16.5% (Figure 2.15.). Alizarin red S presents almost no difference between irradiated and non irradiated samples.

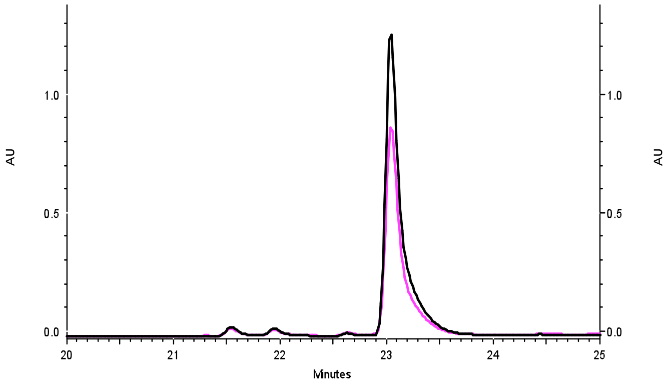


Figure 2.15. Chromatogram, λ=280 nm, for alizarin non irradiated (black) and irradiated by 15 hours (purple).

It was possible to verify the decreasing of alizarin lake peaks' areas as expected, but very small differences were observed during irradiation time. If photodegradation products of alizarin red S lake have been formed they can be associated to t_r= 18.2 min, only visible at UV wavelengths. This compound could be due to a double bond cleavage of carbonyl group, forming a more polar molecule.

Purpurin and its lake were irradiated at the maximum absorption wavelength, 480 nm and 500 nm, respectively (Tables 2.1. and 2.2.), in MeOH:H_2O (90%:10%, v/v) solution. The quantum yield of reaction, Φ_R, of purpurin was very low (Table 2.3.), comparable to the ones obtained for alizarin and alizarin lake. Although the one obtained for purpurin lake is one order of magnitude higher. Comparing the UV-VIS spectra obtained both to purpurin and its lake, the variation is very small (Figure 2.16.).

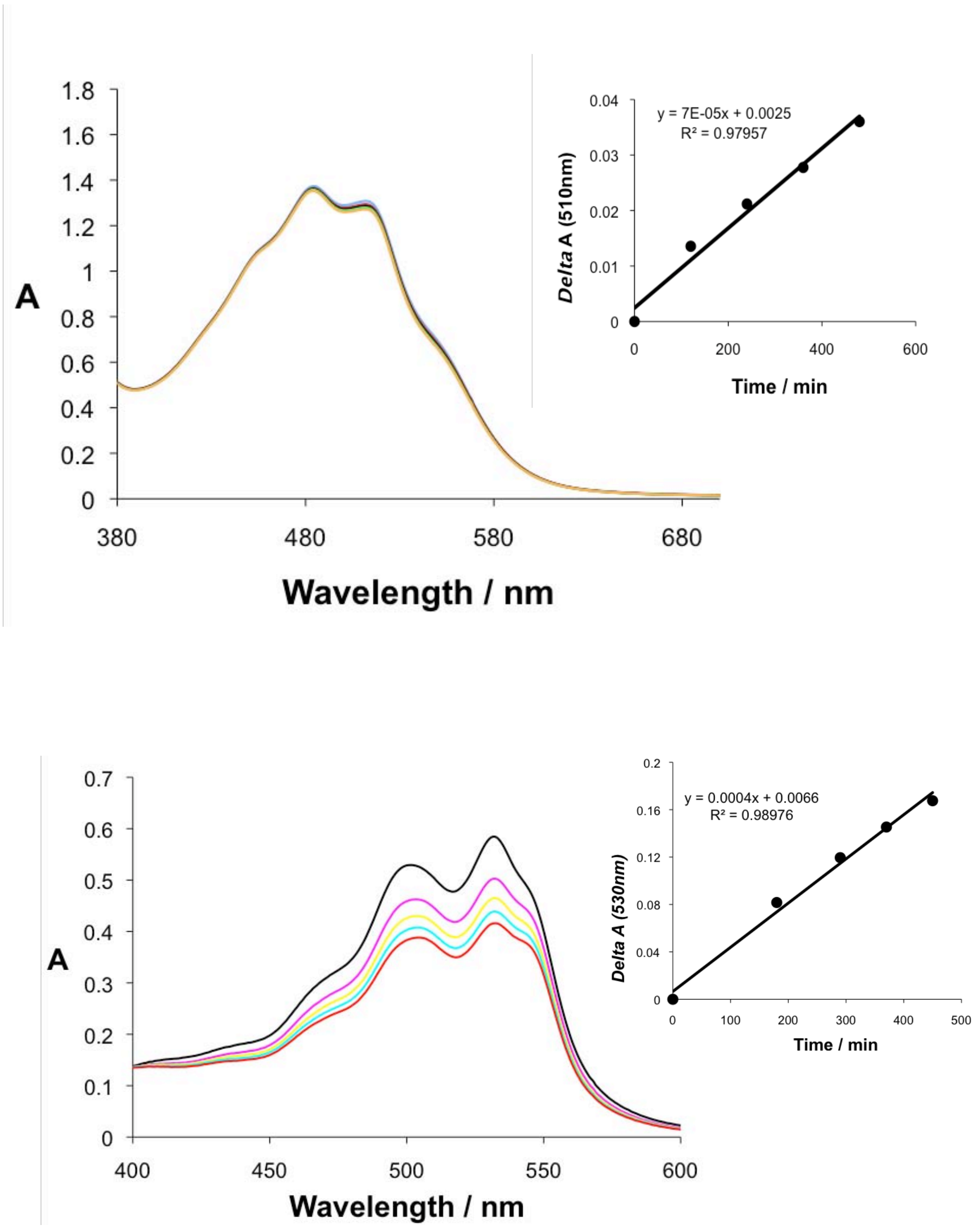


Figure 2.16. Photodegradation of purpurin at pH=6.3 (above) and purpurin lake (below) in MeOH:H_2O (90%:10%, v/v) irradiated at 480 and 500 nm, respectively, and in the inset a detail (left); the absorbance variation followed at 510 and 530 nm, respectively, is plotted over time, giving the *m* parameter of the Φ_R equation. (Appendix III, section III.2.1.).

Purpurin did not present any variation in HPLC-DAD chromatogram, but purpurin lake, as expected, due to its higher Φ_R, revealed a 3% decrease of the main peak relative area (t_r=25.1 min), and the increase of two other peaks at t_r=20.7 min and t_r= 22.8 min (Appendix III, section III.2.1., Table III.3.). These peaks may be related to the carbonyl group changing, since the polarity is higher.

2.1.3.2. Monochromatic irradiation in heterogeneous media

Recent studies had shown that it is possible to determine Φ_R in transparent gel solutions, using the same equations and methodology developed for solution, if the active volume of irradiation is considered and the incident light is distributed uniformly over the irradiated surface [104]. Providing that irradiated volume is defined by the product of the area of irradiated face and optical pathway, the Lambert-Beer's law can be applied when the concentration is uniform, like in stirred solution or in transparent gel.

Commercial gelatine, mainly composed by collagen, was used to simulate the protein based binders used in illuminations manuscripts. This gelatine has been used in research studies as it has good purity [105, 106] and transparency in the visible region [107]. This study was only performed to alizarin, purpurin (dissolved in ethylene glycol) and alizarin red S (in water).

The quantum yields of reaction obtained for alizarin (Φ_R, = 8 x10^{-5}), alizarin red S (Φ_R, = 2.8 x10^{-4}) and purpurin (Φ_R= 2x 10^{-4}) were like in the homogeneous media very low (Table 2.3.), that it is why looking to Figure 2.17., is not possible to see any difference between irradiated and non irradiated areas. Comparing to the ones obtained for homogeneous media, it can be see the all chromophores have a higher quantum yield of reaction in heterogeneous media, circa one order of magnitude, except to alizarin, which is of the same magnitude.

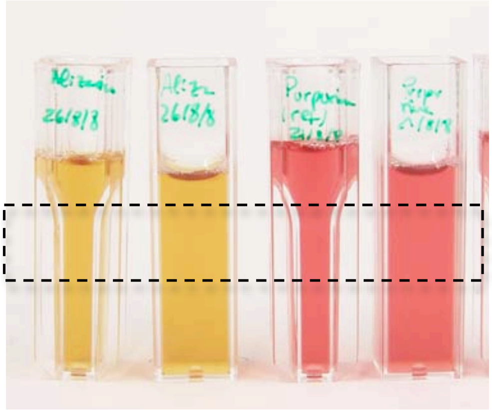


Figure 2.17. Alizarin and purpurin references and after irradiation, the irradiated area is market with the dotted line.

2.3.1.3. Polychromatic irradiation in heterogeneous media

Frosted glasses were used to deposit a homogeneous coat of laccaic acid and acid lac dye[8]. The red colorants were irradiated with a Xenon lamp with a cut-off filter till 300 nm, simulating the sunlight exposure. The reaction was followed measuring colour changes with a colorimeter[9] and the photodegradation was characterized by µ-FTIR and as previously by HPLC-DAD, after removing the dyes with MeOH.

After 4250 hours of irradiation, which corresponds to 203 years in the museum display (Appendix III, section III.3.) laccaic acid A and lac dye did not faded totally (Figure 2.18.)

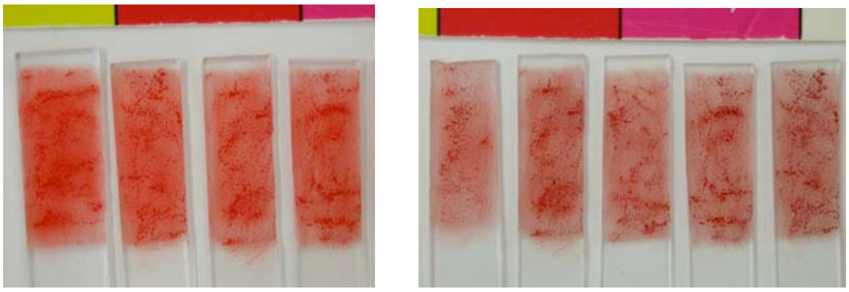


Figura 2.18. Laccaic acid A before and after irradiation, during 108 h, 203 h, 366 h, 500 h and 4250 h, from left to right.

HPLC-DAD analyses performed on laccaic acid A, allowed to see 30% decrease of the main peak (t_r=19.8 min), while new others were appearing as the irradiation time increased (Table 2.4.). Lac dye has a similar behaviour since the two main peaks of laccaic acid A and laccaic acid B (t_r= 19.5 min) decrease 30% and 20%, respectively; and new peaks appeared at t_r= 18.8 min and t_r= 15.4 min.

Table 2.4. Compounds detected by HPLC-DAD after photodegradation of laccaic acid A, and lac dye.

Compound		Laccaic acid A (%)	Δ	Lac dye (%)	Δ
t_r=8.8 min* λ_{max}=292nm	t_0 t_{irrad}	0.0 2.2	+2.2	0.0 2.4	+2.4
t_r=10.5 min* λ_{max}=287nm	t_0 t_{irrad}	0.0 1.6	+1.6	0.0 2.1	+2.1
t_r=15.4 min* λ_{max}=284/**489** nm	t_0 t_{irrad}	0.0 2.0	+2.0	0.5 8.3	+7.8
t_r=18.8 min* λ_{max}=282/ **492** nm	t_0 t_{irrad}	0.0 14.4	+14.4	0.2 31.2	+31.0
t_r=19.5 min* λ_{max}=284/**490** nm	t_0 t_{irrad}			22.5 2.2	-20.3
t_r=19.8 min* λ_{max}=284/**490** nm	t_0 t_{irrad}	99.3 69.1	-30.2	53.2 23.5	-29.7

*Relative area %, calculated at 280 nm

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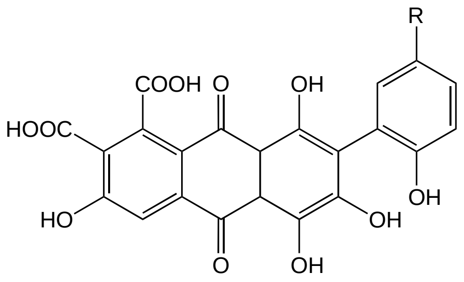
[8] The lac dye used is from Fluka, By HPLC-DAD, it were detected the two main laccaic acids present in lac dye: A and B.

[9] The system CIELab, was developed to characterize the colour, which is than defined by the coordinates L* (measures the luminosity, varies between 0 (black) and 100 (white), a* and b* measure the hue (-60 (green) < a* < 60 (red) and -60 (blue) < a* < 60 (yellow)) [108].

The FTIR spectra revealed that the NH distension attributed to the secondary amide present in the laccaic acid A (Figure 2.19.), has almost disappeared, being the 3415 cm^{-1} peak after the irradiation just a shoulder (Figure 2.20.) and the second peak at 3075 cm^{-1} more intense. Probably a conversion of secondary amide into a primary amine could occur, since a new band at 3245cm^{-1}, characteristic of primary amines [109], is now more evident, which could mean the loss of the group $C=O-CH_3$; and the ratio between the C=O amide I asymmetric distension (1620 cm^{-1}) and amide II NH bending and CO distension (1570 cm^{-1}), decreases after irradiation. Those could explain the transformation of the secondary amide into primary amine, which presents only the NH_2 bending vibration at ~1600 cm^{-1}, in fact there is a shoulder appearing at that wavenumber, in the irradiated dye. In the fingerprint region it is possible to note at 1407 cm^{-1} (C=O distension of aromatic ring) the decreasing intensity, being after irradiated, a shoulder.



Laccaic acid A	R= $CH_2CH_2NHCOCH_3$
Laccaic acid B	R= CH_2CH_2OH
Laccaic acid C	R= $CH_2\underset{\overset{\vert}{NH_2}}{C}HCOOH$
Laccaic acid E	R= $CH_2CH_2NH_2$

Figure 2.19. Structure of laccaic acids A, B, C and E.

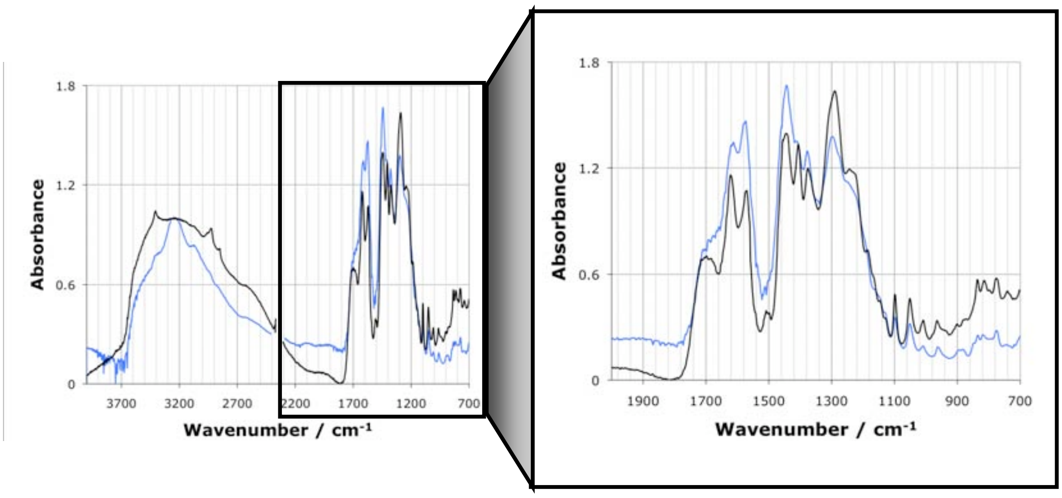


Figure 2.20. FTIR spectra from laccaic acid A before (black line) and after irradiation for 4250 hours (blue line).

2.1.4. Conclusions

Photophysical data obtained for the analysed chromophores, revealed the possibility of two kinds of substituents, sugar in carminic acid and amide in laccaic acid A, strongly modify in distinct ways, the chromophores excited behaviour. Laccaic acid A and carminic acid, even having a similar structure to the one of purpurin, predicting an absence of ESIPT, showed that in the excited state laccaic acid A is almost similar to alizarin and carminic acid is similar to purpurin. The differences obtained in the lakes should be related to the structures of the complexes formed with aluminium ion, since previous studies have already shown that carminic acid is not a planar molecule [79], neither laccaic acid A due to CH-CH linkage between the anthraquinone and the amide, although alizarin and purpurin are planar, which could influence the excited state properties.

Stoichiometry of Al^{3+}:alizarin complex seems to be a mixture of two complexes, a 1:2 and 1:1 complex Al^{3+}:alizarin, which could be explained by the existence of two tautomers identified by liquid and solid state NMR [101], reacting simultaneously. Stoichiometry of purpurin: Al^{3+} complex seems to be 1:1, which regarding the molecule structure is suitable, as there are two hydroxyl groups near the carbonyl groups that could react with Al^{3+}. Even though further studies using more and new techniques available should corroborate this study.

Important evidences about apparently similar red chromophores were discovered with this work. A small structural difference can produce a broad of different ground but mainly excited state behaviours, which does not allow creating one single paradigm of these ancient molecules, but a set of them.

This work confirmed the fading resistance of all the chromopheres studied, in homogeneous and heterogeneous media. The difference between the Φ_R obtained in monochromatic irradiation in homogeneous and heterogeneous media is higher to alizarin red S (ten folder) than alizarin (same magnitude order), which could be explained by the sulfonate group interaction with gelatine.

It was confirmed that the photodegradation of alizarin and purpurin are not so intense as for their lakes. Alizarin lake irradiated allowed to notice the formation of something more polar, which could be due to the presence of free radicals that would attack the double bonds of the carbonyl groups. According to these results, in heterogeneous media purpurin is in fact more photoreactive than alizarin. In homogeneous, it was concluded that the mordant has not a protecting action on their photodegradation, like it has on wool [91]. Despite of the results for purpurin lake in proteinaceous gel were not conclusive, the absorbance intensity decreasing in the first hours, while the gel was transparent, was faster than in homogeneous media, revealing its higher reactivity.

Laccaic acid A revealed to be a vey resistant dye to photodegradation, since after been subject to a polychromatic irradiation of 12455 MJ/m^2 , the colour did not totally fade. HPLC-DAD enabled identify new compounds formed as the irradiation was carried on. The most expressive peaks have a retention time at 18.8 min and 15.4 min. Knowing that laccaic acid B is eluted at 19.5 min, and laccaic acid C and E are co-eluted ate 15.9 min (Figure 2.19.), probably the compounds eluted at 18.8 min is an amine with the methyl group changed or with some of the carboxylic groups deprotonated or could be due to the loss of carbonyl. The one eluted at 15.4 min, should have a structure similar to laccaic acid C or E. These hypotheses were also in agreement with what observe in the FTIR spectra.

> “The real magic lies not in seeing new
> landscapes, but in having new eyes”
> Marcel Proust

2.2. µ-spectrofluorimetry: a new non-invasive technique to analyze red lakes in cultural heritage

2.2.1. Reconstructions

Reconstructions of artists' paints made with red lake pigments, based on medieval and 19th century recipes were examined. Additional test paints were made using the same lake pigments bound in a range of binding media including gum arabic, collagen, PVA (a vinyl emulsion) and linseed oil. Samples were prepared on different supports, including paper and melinex, glass slides and sapphire cells (Appendix I, section I.3.2.1.)

2.2.1.1. Alizarin and purpurin lakes

Alizarin and purpurin lake model samples were used to test the reproducibility of the microfluorescence data. The model samples used were the 1 cm square paintings of purpurin and alizarin lakes and their mixtures, in a PVA emulsion, applied in a Whatman filter paper, with four replicates for every composition (Figure 2.21.). For each of the prepared paintings 6-9 emission and excitation spectra were acquired, in different days, in different points, and the data was shown to be completely reproducible. Other supports, including melinex sheets, glass slides and sapphire cells, were also used and do not influence the shape of the emission/excitation spectra obtained.

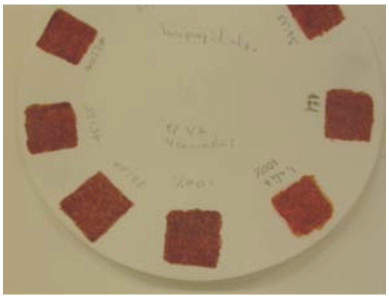


Figure 2.21. Alizarin and purpurin model samples.

To test the semi-quantitative potential of the technique, mixtures of purpurin and alizarin lakes of known concentration (wt %) were prepared and the respective spectra acquired[10] (Figure 2.22.). Taking into account the intrinsic heterogeneity of the solid media, the correlation coefficient of the curve representing the concentration of purpurin in the mixture as a function of the emission intensity can be considered very good, R^2= 0.96 (inset Figure 2.22.). The emission spectra for the solid state are satisfactory resolved, comparing well with the spectra acquired in solution (section 2.1.). It was observed that the spectra decrease in intensity with the increase of alizarin proportion in the mixture, which was an expected behaviour due to the relative fluorescence yields of the two compounds (section 2.1.).

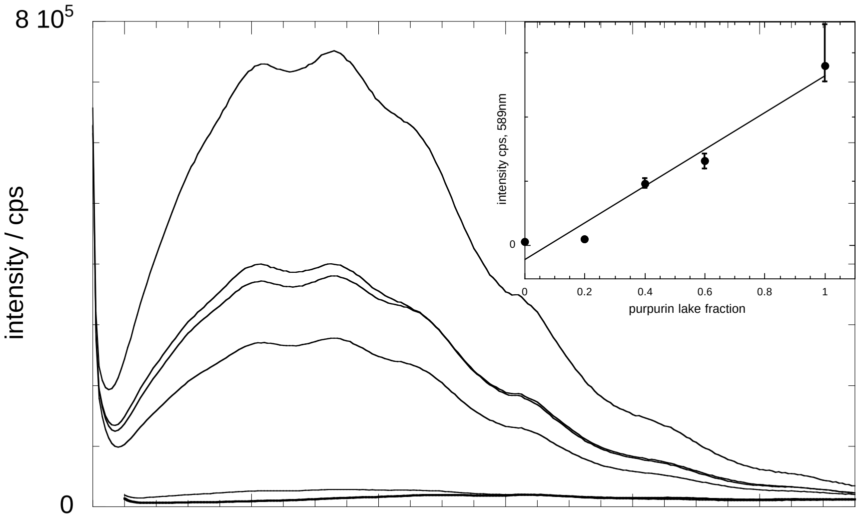


Figure 2.22. Microemission spectra (not corrected) obtained for mixtures of purpurin and alizarin lakes in a PVA medium, applied in Whatman filter paper; mixtures, weight:weight, of purpurin:alizarin, 0:100, 20:80, 40:60, 60:40 and 100:0 with λ_{exc}= 490 nm and dichroic filter of 510 nm. In the inset, the resulting calibration curve with R^2=0.98 is shown (y = 8212.5x - 61557); the calculated uncertainties correspond to a confidence level of 95%.

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[10] Each concentration point results from a mean value of 36 spectra, obtained in four different experiments (9x4).

Even if it is difficult to see the emission spectra of the alizarin lake with the scale axes of figure 2.22, there is also a blue-shift of the overall spectra since the maximum emission of alizarin is also blue-shifted relative to purpurin. Due to this shift and to the difference between the fluorescence quantum yields of the two lakes, it was possible to collect the purpurin lake emission directly from the raw data, without any further spectral deconvolution. The emission spectra acquired for both lakes using other binders such as parchment glue and gum arabic compares well to what obtained using PVA as binder.

The emission from the model samples was also collected with a fibre optic adaptor (without microscope). In this case, for each sample, only one emission spectrum was acquired as the area covered by the fibre optic, 0.5 cm, takes into account the heterogeneity of the sample. Again, a similar trend in the emission spectra was observed (Figure 2.23.). The linear fitting for the concentration of purpurin in the mixture as a function of the emission intensity was very good, R^2= 0.98 (inset figure 2.23.), enabling a semi-quantitative analysis to be carried out.

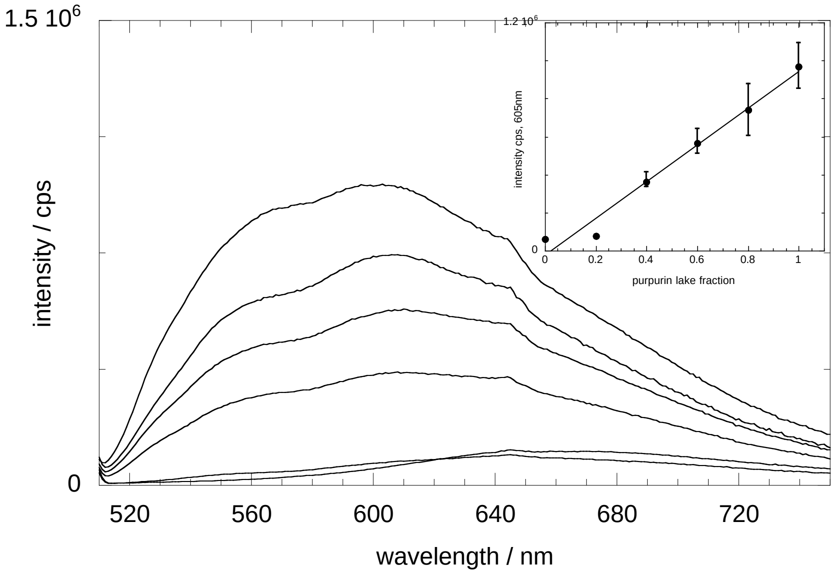

Figure 2.23. Emission spectra (not corrected) obtained for mixtures of purpurin and alizarin lakes in a PVA medium, applied in Whatman filter paper, through fibre optic; mixtures, weight: weight, of purpurin:alizarin, 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0 with λ_{exc}= 490 nm and dichroic filter of 510 nm. In the inset, the resulting calibration curve with R^2=0.98 is shown (y = 9634x – 19537); the calculated uncertainties correspond to a confidence level of 95%.

The detection limits found are ≈11% in both cases, μ-SPEX and fibre optic (calculated as three times the standard deviation of the sample where the amount of purpurin was 0%-blank signal [110]). This reveals that below this percentage of purpurin in the alizarin:purpurin mixture there is a significant degree of uncertainty in the measurements.

The spectra envelope was less resolved than that obtained with the µ-SPEX. This could be explained by the relatively higher light intensity output of the fibre optic compared with the microscope, and also that the light reflected from the sample is more efficiently collected. Therefore it is possible that interference due to reflection affected the emission spectra obtained with fibre optic system. Another disadvantage of using fibre optic is that it is not possible to acquire excitation spectra of the samples, as it is with the µ-SPEX.

Kopp's purpurin lake

Kopp’s purpurin (Appendix I, section I.3.2.1.) was widely used in the latter part of the 19th century for making madder lakes [34]. It was prepared by extracting madder root with sulfurous and sulfuric acids and contains largely pseudopurpurin and purpurin [35]. Aluminum lakes of dye extracted from the madder root or from a madder derivative were popular artists' pigments in the 19th century[11] [111].

Historic reconstructions with this lake were made and emission and excitation spectra for *Kopp's Purpurin* lake, where white lead was applied as an underlayer and the lake was applied in oil media are presented in Figure 2.24.. In this lake reconstruction, different dihroic filters (510 nm and 570 nm) were used to obtain the emission spectra, which allowed to conclude that the 570 nm cannot be used for this red lakes as part of the emission is cut-off, with consequent loss of information that can induce to a misinterpretation of the experimental results. On the other hand, it was possible to obtain good excitation spectra with the 570 nm dichroic filter. This excitation spectrum reflects the absorption spectra of the sample, enabling a better identification of the chromophore. This is an important advantage in the field of cultural heritage, since in ancient samples, complex aged mixtures can display also a complex emission spectra, and having access to a well resolved excitation spectra will facilitate the characterization of the chromophores present.

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[11] Since impressionists paints will be analysed it was helpful having Kopp’s purpurin and eosin lakes characterized.

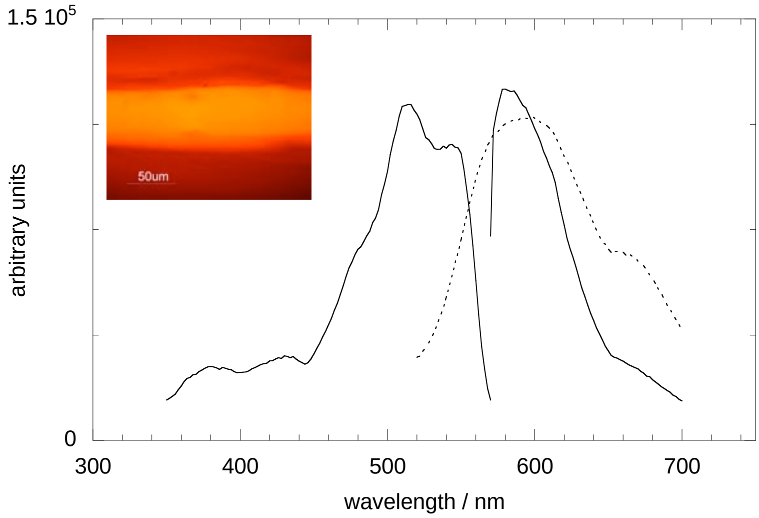

Figure 2.24. Kopp's purpurin lake (emission (570 (—) and 510 (---) nm dichroics) and excitation (570 nm dichroic) spectra (8 μm spot). Inset: cross section of Kopp’s purpurin lake model sample - Image acquired with green light (filter set 14 – BP510-560 FT 580 LP 590).

2.2.1.2. Cochineal lake

Model painted samples of carminic acid or cochineal lakes have emission spectra with very low intensity, and it was not possible to obtain well-resolved excitation spectra. Although it is possible to identify the emission λ_{max} at 620 nm, when excited at 550 nm (to avoid the binder spectrum intrusion) this technique is not the best to identify this sort of organic red colours, since this emission maximum is similar to other organic reds like brazil wood lakes and dragon´s blood resin.

2.2.1.3. Eosin Y lake

First discovered by Hofmann and marketed by BASF in 1874 [112], eosin is primarily used as a histological stain, a cosmetic colorant, and a colorant in red inks [113]. In the late 19th and early 20th century, eosin was also used as a red paint pigment. Despite the warnings, as the one of Jean-Georges Vibert to whom the aniline dyes were a “catastrophe for painting” [112], painters like Vincent van Gogh did not resist to the allure of these new colours [114, 115]. Eosin lake, one of the pigments that he regularly used, revealed latter as a disaster for some of his works, because it is particularly unstable and fade on exposure to light sometimes within a few years [116].

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 ($Pb(CH_3CO_2)_2 \cdot 3H_2O$) (Appendix I, section I.3.2.1.). The lakes were applied as paint layers with the binding media: linseed oil (Talens®), similar to paints that were found in paintings by Van Gogh [116].

The emission and excitation spectra for the eosin lakes were obtained, as for *Kopp's Purpurin* lake, from a cross section (in 8 μm and 4 μm spots) (Figure 2.25.). It was possible to acquire an excitation spectrum with the 570 nm dichroic filter. As expected, being eosin a strong fluorophore, the emission intensity is *circa* one order of magnitude higher than that obtained for *Kopp's* lake.

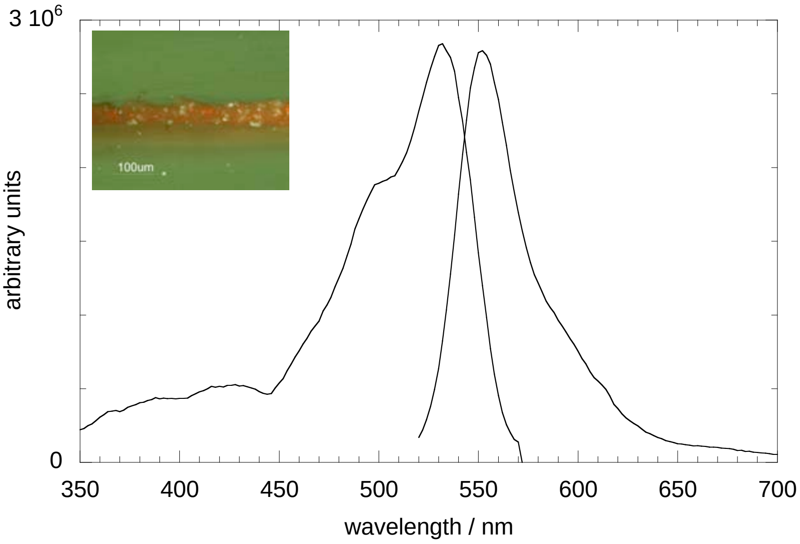

Figure 2.25. Eosin lake (with alum) emission and excitation (570 nm dichroic) spectra with an 8 μm spot. Inset: cross section of Eosin lake model sample; image acquired with UV light.

It was observed that depending on the complexing agent the intensity of the spectrum increases, being the lower with $Pb(CH_3CO_2)_2 \bullet 3H_2O$, going to $KAl(SO_4)_2 \bullet 12H_2O$ and the higher with $AlCl_3 \bullet 6H_2O$ (Figure 2.26.) (Table 2.5.).

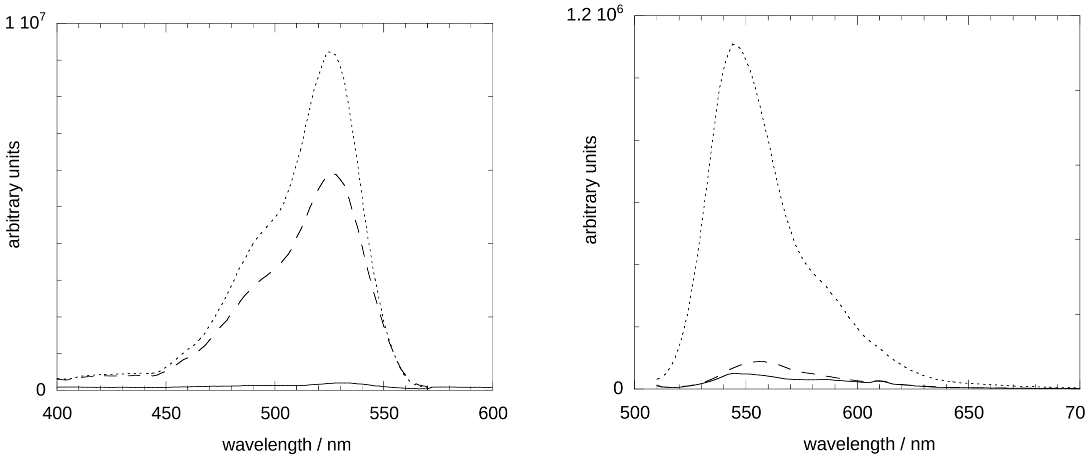

Figure 2.26. Excitation and emission spectra of eosin lakes with aluminum chloride (- - - -), alum (— — —) and lead(II) acetate trihydrate (———), showing the metal ion influence on the intensity of the spectra.

Table 2.5. Emission and excitation maxima of the three painted model samples of eosin lake.

sample	Excitation spectra, λ_{max} / nm	Emission spectra, λ_{max} / nm
Eosin lake prepared with $AlCl_3 \bullet 6H_2O$	524	544
Eosin lake prepared with $KAl(SO_4)_2 \bullet 12H_2O$	528	558
Eosin lake prepared with $Pb(CH_3CO_2)_2 \bullet 3H_2O$	528	546

Even if the maxima excitation wavelength are similar (528 nm and 524 nm), the emission maxima displays different values being, those of the eosin lakes prepared with $AlCl_3 \bullet 6H_2O$ and $Pb(CH_3CO_2)_2 \bullet 3H_2O$, the lowest values, 544 nm and 546 nm respectively, and the one prepared with alum the higher (558nm).

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.

2.2.2. Book of Birds

The folia 4, the one with the initial (Figure 2.27.) and 6 (The Diagram) where organic red colour is present. In folio 4, carmine-coloured is applied over the *minium* and in folio 6 there are no other pigments mixed. Both were analysed but the results were inconclusive, since lac dye have a very low intense emission spectra, and it was not possible to obtain well-resolved excitation spectra.

Figure 2.27. Folio 4 from the *Book of Birds*.

2.2.3. **Andean textiles**

Pre-Columbian textiles are unique as a cultural and historic record, representing the longest continuous textile record in world history [117-119]. 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 (14th-15th centuries), were based on purpurin and pseudopurpurin chromophores obtained from *Relbunium sp.* [17, 120, 121] (Figure 2.28.).

Figure 2.28. Mantle border from Paracas, early Horizon-early interm. Period, 100B.C. – A.D. 100; Man’s poncho from Paracas, early Horizon Period, pos. phase 10, 100B.C. – 0; Bag with human and vegetal motif from Nasca, early Middle Horizon, A.D. 500 – 600.

From the MFA-Boston collection 76 reds from Andean textiles, were sampled (yarns usually weighed 0.1-0.4mg). These textiles, dated from 200B.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). The SEM-EDX screening enabled to confirm the use of aluminium ion as a mordant, i.e., the metal ion that was used to bind the dye to the fibre; and also, to conclude that all the red samples studied were all made of camelid fibres. This was also confirmed by optical microscopy [8].

Microspectrofluorimetry was used to analyse all microsamples. The majority of the samples present a red colour, but fibres with pink and purple colour were also analysed (Figure 2.29.).

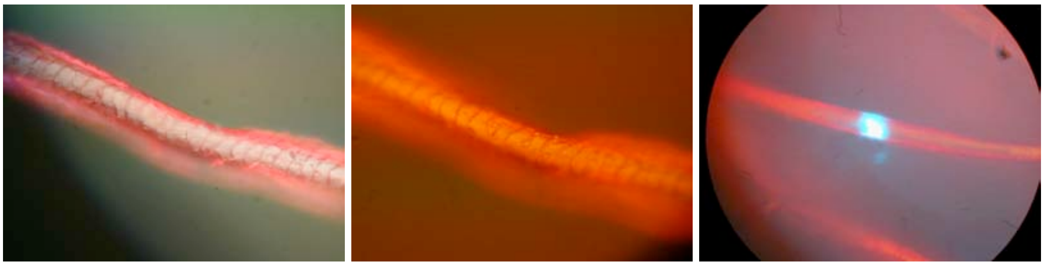

Figure 2.29. A dyed wool fiber under the microscope observation, illuminated with visible light (left), under excitation light (centre) and with the spot area analyses of 30µm visible (right).

The spectra obtained, in a 30µm spot (Figure 2.29), can be classified in two groups, a major one in which good excitation and emission spectra were obtained, with a spatial resolution, and a second group in which only weak excitation spectra could be collected.

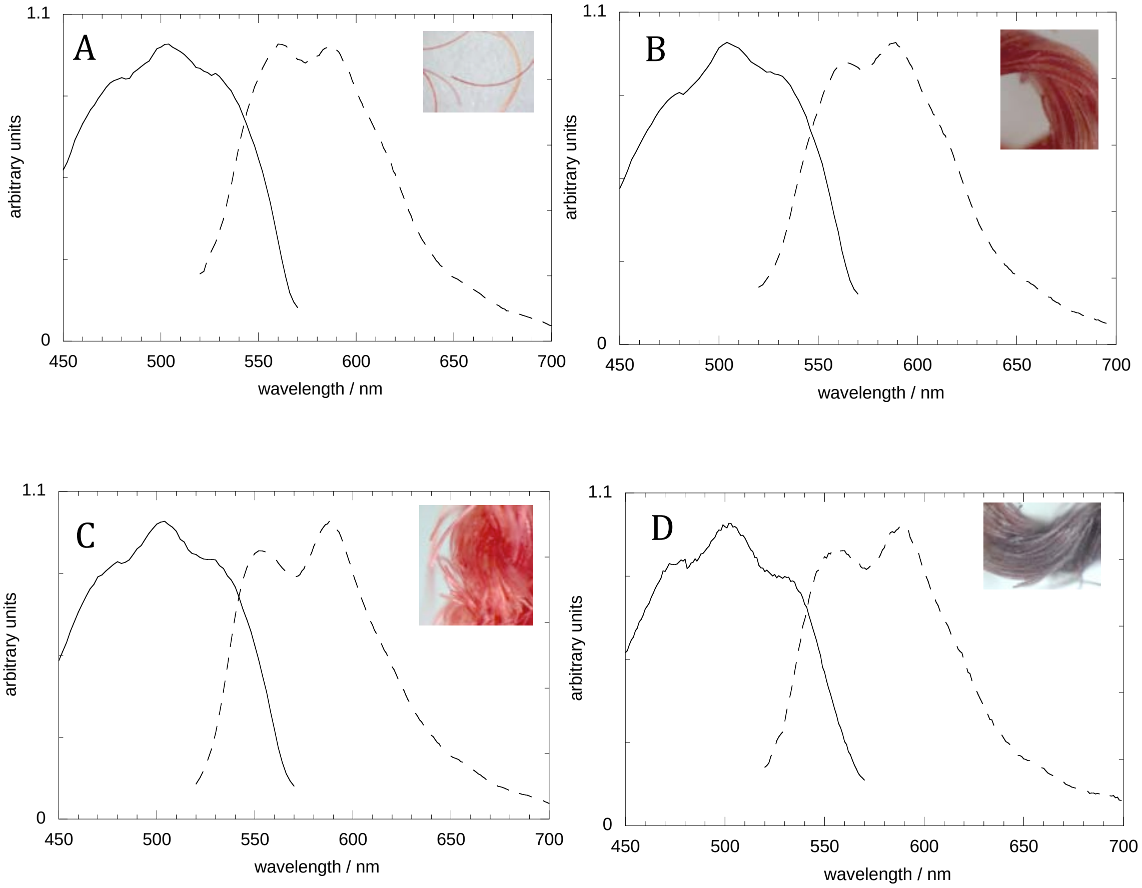

Figure 2.30. Emission and excitation spectra, acquired in a 30 µm spot, for the following red fibres: A- Andean Paracas man's poncho (mfa31.496), 100B.C.–0; B- ornamental braid (mfa21.2557), 0-A.D.200; C, D- Nasca fragment of a tabled fringed border (mfa31.500, 2 samples), A.D. 300. (λ_{exc}= 490nm, 500nm dichroic; λ_{em}= 590nm, 570nm dichroic).

In Figure 2.30. 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 2.6.), being very similar to those obtained for an aluminium lake in solution [7]. These spectra (Figure 2.30.) 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.

Table 2.6. Emission and excitation maxima of the analysed samples and the purpurin and pseudopurpurin aluminium lakes used as references (* anthraquinone: Al^{3+} 1:1000, pH=3.7 in H_2O:MeOH (25%:75%, v/v)).

	mfa 31.496	**mfa 21.2557**	**mfa 31.500** **(red sample)**	**mfa 31.500** **(purple sample)**	**purpurin-Al^{3+}***	**pseudopurp:Al^{3+}***
excitation, λ/nm	480, 504, 532	482, 504, 534	484,504, 534	480,502, 536	502, 531	493, 520
emission, λ/nm	560, 587	564, 591	552, 587	556, 589	551, 595	542, 583

It is also worth noting that the excitation spectrum is similar to the absorption spectrum obtained on full chelation with aluminium in solution[12], which exhibits vibronic maxima about 480 nm, 504 nm 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 fibres could be explained by a different chromophore environment or to the presence of other emitters. It has been already observed, for the emission of the purpurin aluminium 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; and in all the samples analysed by HPLC-DAD the major chromophore was purpurin but, pseudopurpurin in a proportion ranging from 30% to 45% was always detected (Figure 2.31.).

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[12] at pH=3.4 to 4; pH was not increased above 4.5, to avoid $Al(OH)_3$ precipitation.

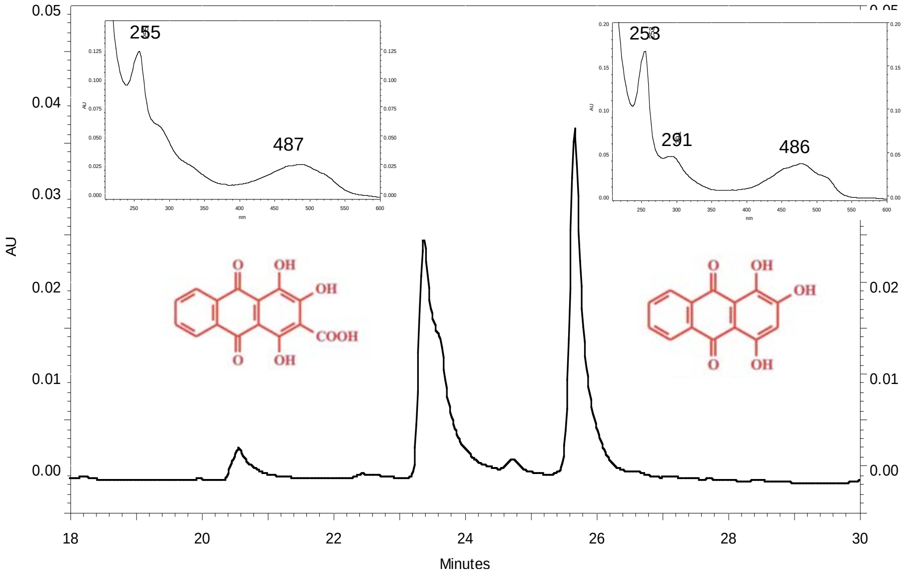

Figure 2.31. Chromatogram of a red sample (21.2581, nº3), insets with UV-VIS spectra of the eluted compounds pseudopurpurin (tr=23.4 min) and purpurin (tr= 25.6 min)

The emission of the pseudopurpurin fluorophore present in the Andean textiles was obtained after extraction (Appendix IV, section IV.2.1.) followed by HPLC separation and finally, complexation with Al^{3+} in $MeOH:H_2O$ (75%:25%, v/v) solution (Figure 2.32.).

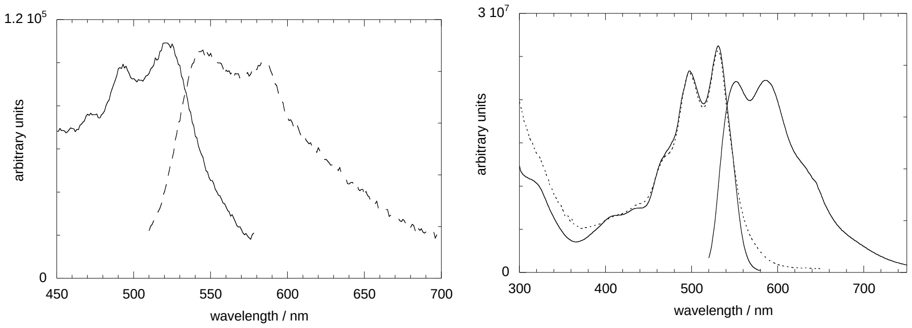

Figure 2.32. Emission (λ_{ex}= 490 nm) and excitation (λ_{em}= 590 nm) spectra, in homogeneous media, for pseudopurpurin lake from an Andean textile (left) and purpurin lake in $MeOH:H_2O$ (75%:25%, v/v).

Two bands, whose maxima excitation wavelengths are found at 493 nm and 520 nm, characterize its excitation spectrum; the emission spectrum has also two maxima emission wavelengths at 542 nm and 583 nm (Figure 2.32.). When compared to the purpurin aluminium 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 will be the ratio between the two emission bands, at circa 555 and 588 nm (Figure 2.33.). It was possible to confirm that the highest ratios were observed in samples with the highest relative concentration of pseudopurpurin (determined by HPLC-DAD)(Appendix IV, section IV.2.2., table IV.1). The relative amount of purpurin and pseudopurpurin could provide further clues for madder's source and methods used to dye. But, observing Figure 2.30. and Table 2.6., a direct connection between the places of origin, such as Paracas or Nasca, and the relative amount of each chromophore, could not be made.

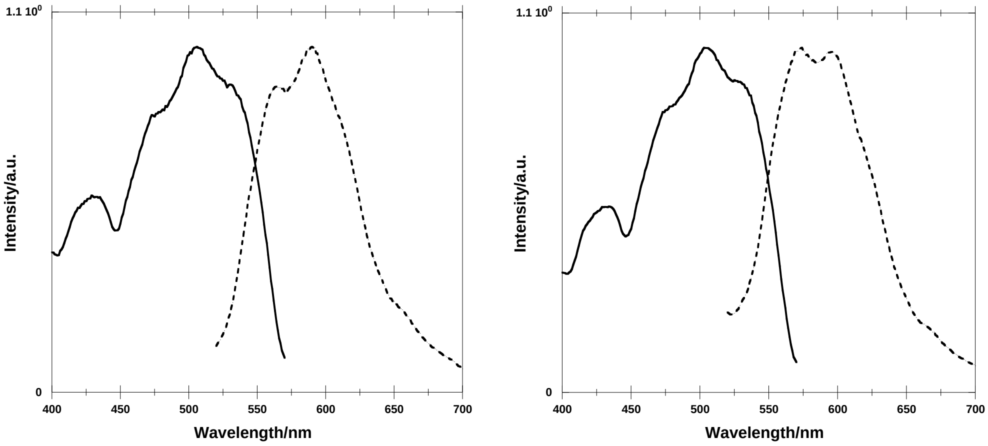

Figure 2.33. Emission and excitation spectra, acquired in a 30 µm spot, for the red fibres of (left) Andean Paracas skirt (mfa21.2581), 200B.C.–A.D. 200 (pseudo/purp=0.71); (right) Paracas man's poncho fragment (mfa31.699), 0-A.D. 100 (pseudo/purp=0.46). (λ_{exc}= 490nm, 500nm dichroic; λ_{em}= 590nm, 570nm dichroic).

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 (micro-samples) set in the microscope stage, but analysis could have been performed on the entire textiles, as no preparation for samples is required.

2.2.4. 19th century paintings

Paint samples prepared as cross-sections embedded in polyester resin from oil paintings by the Impressionist and post-Impressionist painters, Lucien Pissarro (1 sample) and Vincent van Gogh (5 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) (Figure 2.34.).

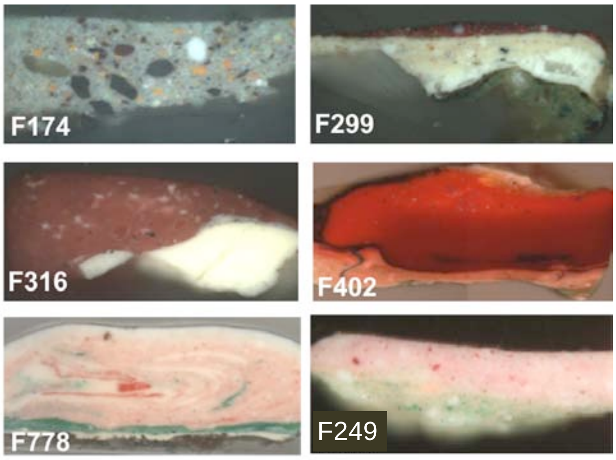

Figure 2.34. Cross sections of five different paintings by Vincent Van Gogh: 1- *Head of an old woman* (F174) (length 2mm); 2- *Montmartre: Quarry, the Mills* (F229) (length 1.4mm); 3- *Allotments on Montmartre* (F316) (length 0.41mm); 4- *Two white butterflies* (F402) (length 1.6mm); 5- *Wheat Field Under Clouded Sky* (F778) (length 3.7mm) and *Old Mark's field* by Lucien Pissarro (249) (length 0.5mm).

- *Allotments on Montmartre* (F316) (Figure 2.35.), *Montmartre: Quarry, the Mills* (F229) and *Head of an old woman* (F174) by Vincent van Gogh

Figure 2.35. *Allotments on Montmartre* by Vincent van Gogh

The red glaze layer of the F316 cross-section has been previously characterized by HPLC-DAD [35] and found to contain purpurin and two other red dyes. Moreover aluminium was detected by SEM-EDX, indicating the presence of a purpurin-aluminium complex on an alumina substrate. Representative excitation and emission spectra are depicted in Figure 2.36., and both match the spectra obtained for Kopp's purpurin in oil binding media [7], prepared as an historic reconstruction [35]. The two vibronic bands present in the excitation spectra, that reflects the absorption spectra of the chromophore, are the same as those obtained with Kopp's purpurin (513 nm 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 [122] 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.

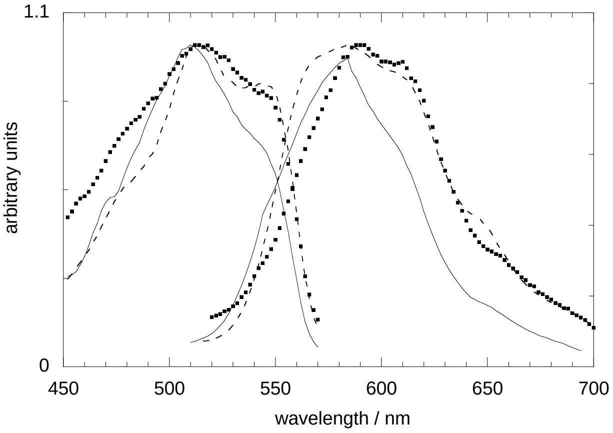

Figure 2.36. Emission and excitation spectra, acquired in a 30 µm spot, for the following cross sections: Vincent van Gogh F316 (—), Lucien Pissarro (■) and Kopps purpurin (---), (λ_{exc}=490nm, 500nm dichroic filter; λ_{em}=590nm, 570nm dichroic filter).

- *<u>Old Mark's field</u>* <u>by</u> Lucien Pissarro

The organic red colour (Figure 2.34.) 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 an almost perfect match with the absorption spectra of the purpurin lake samples (Figure 2.16.). 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 nm and 546 nm), clearly reproducing the chromophore's absorption spectra. With regard to the emission spectra, the two vibronic maxima, λ_{max}=590 nm and 610 nm, are in very good agreement with those found for Kopp's purpurin, λ_{max}=586 nm and 610 nm.

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

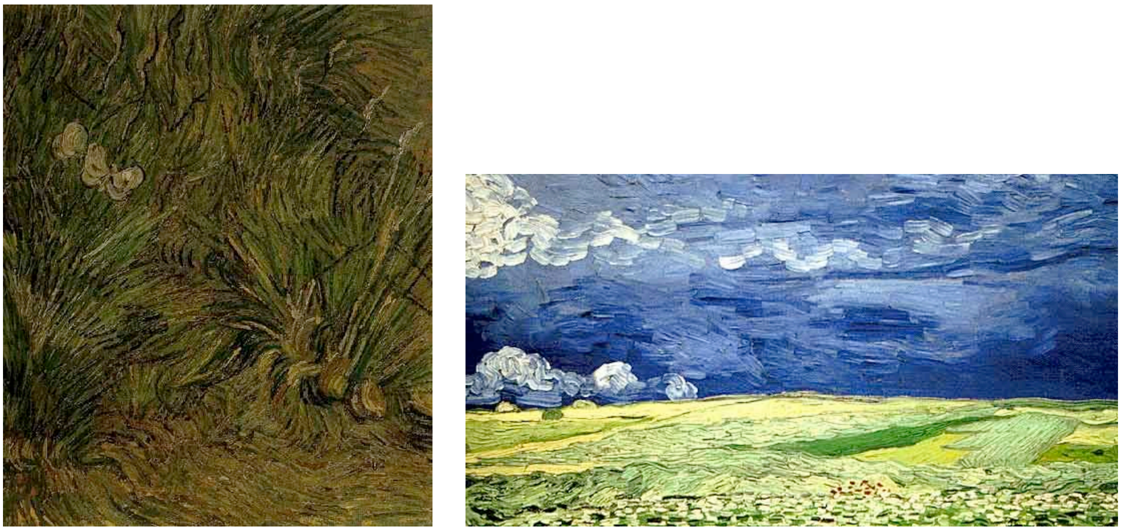

Figure 2.37. *Two white butterflies* (left) and *Wheat Field Under Clouded Sky* (right) by Vincent van Gogh.

The analyses performed with HPLC-DAD and fluorescence [122] of the *Two white butterflies* (F402) and *Wheat Field Under Clouded Sky* (F778) (Figure 2.37.) revealed that both cross sections are composed by a red layer of eosin lake. The substrate present contains lead in F402 and aluminium in F778. This last sample also contains iodine-substituted lakes related to eosin, possibly erythrosine, and red lead [122]. On the *Two white butterflies*, it was detected the presence of only two other components related to eosin, and aluminium silicates [122].

The emission and excitation spectra of these two red layers on samples taken from paintings by Van Gogh are represented in Figure 2.38.. Similar emission and excitation maxima were obtained for both samples, revealing the emission of an eosin lake (Table 2.5.). 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.

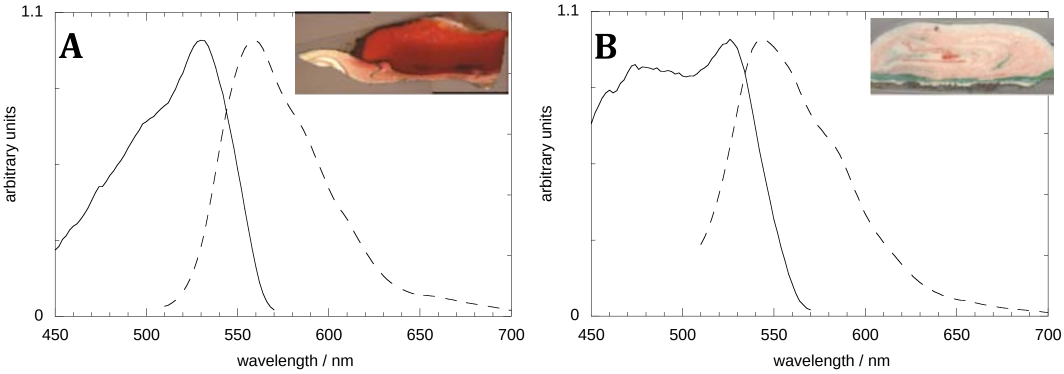

Figure 2.38. Emission and excitation spectra, acquired in a 30 µm spot, for the cross sections of Vincent van Gogh F402 (A) and F778 (B). λ_{em}= 490nm, 500nm dichroic filter; λ_{em}= 590nm, 570nm dichroic filter).

Evaluating these results with those obtained with the created eosin lake model samples (see section 3.1.1.3.) it is clear that the F778 has a behaviour similar to the eosin lake prepared with aluminium 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 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 $H_{SO} = \kappa\xi\,(\vec{L}.\vec{S})$, where ξ is a term that depends on the field of the nucleous, κ is a constant which depends on the molecule and $\vec{L}$ and $\vec{S}$ are respectively the electron orbital and spin angular-momentum operators [123]. In the case of the lead atom the value of ξ is 7294cm^{-1} vs. 28cm^{-1} for carbon [123], clearly showing that the spin-orbit coupling is more effective when the electron is close to atoms of high atomic number. This suggests that the eosin lake from F402 sample, could have been made using Pb ion but has the influence of the impurities of aluminium silicates.

2.2.5. Conclusions

Micro-emission molecular fluorescence proved to be a promising analytical tool to identify the presence of selected red lake pigments combined with a range of binding media. The radiation used is of low intensity and non-destructive to the molecules. Analysis may be carried out *in situ*, without sampling, or on prepared paint cross-sections that offers the opportunity to look at materials in discrete layers of a painting. The spectra acquisition is fast, with high spatial resolution and the data reliable and reproducible.

The analytical method developed for the identification of red lakes prepared as aluminium complexes with purpurin and eosin defines the optimal conditions for the acquisition of the steady-state emission fluorescence spectra using a 510 nm dichroic filter, with excitation at 490 nm for the red anthraquinone lakes. For eosin type lakes the 570 nm dichroic filter was used, and excitation was performed at the absorption maxima. All steady-state excitation spectra were acquired with the 570 nm dichroic. Spatial resolution of 8 μm showed to be appropriate for the analysis of individual pigments particles or aggregates in a paint film. When spatial resolution was not an important requirement 30 μm of area analysis is preferred.

This work suggests that micro-emission fluorescence can also be used as a semi-quantitative method for madder lakes, enabling the determination of purpurin lake fraction in a mixture of purpurin and alizarin [8], and to identify the chromophore eosin environment, depending on the complexing agent.

In which concerns the comparison of the method with the currently available transportable fibre-optic spectrofluorimeters, the advantages of micro-emission molecular fluorescence are the unique possibility to acquire both emission and excitation spectra, not only with high spatial resolution but also with the possibility of in-depth profiling. Moreover, the quality of the spectral information, namely the spectral resolution and signal to noise ratio, is very high when compared to what obtained with the fibre-optic spectrofluorimeters. It is important to stress that, an excitation spectrum corresponds to the chromophore absorption spectrum, a relevant parameter for the characterization of a dye or pigment. The simultaneous acquisition of emission and excitation (absorption) spectra together with the high spectral resolution will contribute for an accurate identification of dye molecules and lakes by using micro-emission molecular fluorescence.

Although the molecular structures of lakes are difficult to determine by fluorescence emission alone, combined with data from other analytical techniques, information about the environment [124-127] of the colorant and its state of degradation can be obtained.

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General conclusion

Illuminated manuscripts proved to be one of the most precious testimonies of medieval colours. Kept in privileged conditions, the colours are rarely exhibited (mainly nowadays) or restored, enabling to discover what were the real colouring materials used by Medieval monks.

One aim of this work was to characterize the Lorvão palette, which not only permitted to have, for the first time, the perception of what material colours were used in a medieval Portuguese monastery, but also to perceive the historical and geographical influences, the particularities of its *scriptorium* and the challenge of identifying some materials, mainly the organic ones. Until now, red lakes remained without a proper identification, essentially due to limited access to them and to the required analytical techniques to characterize them *in situ*. To fill this gap was the other aim of this work, which was achieved by the development of the microspectrofluorimetry to this field. Its use was improved with a previous study of the main red chromophores, in order to understand their photophysical and photochemical properties in homogeneous media, not so difficult to characterize as the solid state; and to identify their photoproducts which, together with the chromophores, provide a different information than what one is expecting, but that could be found in 800-years illuminations.

In part A, the characterization of Lorvão palette, based on nine manuscripts, allowed to create an essential interdisciplinary team to perform this work. The knowledge of different fields enabled to make pertinent questions, to rationalize the ideas with different points of view and the improvement of strategies to be followed. An experimental design was achieved, combining the different analytical techniques used and regarding the limited time to analyse the manuscripts. This revealed to be a crucial step to get both the maximum and the representative information from each illumination. The characterization of Lorvão palette allowed

1) to establish a correlation between almost all manuscripts studied, except for Lorvão 3, by the selection of characteristic combination of colours.

2) to perceive the richness of the *scriptorium*, which had all the medieval typical colouring materials applied in the manuscripts;

3) to realize some methodologies of mixing pigments in order to economize;

4) and to conclude the illuminator’s intentionality on painting the Apocalypse with only three significant colours.

The reconstruction choices made during this work, concerning the limitation to gain access to some written sources, and the few analytical techniques available, attested that even more questions could be made and that a lot of issues (un)known are still to be explored.

In part B, it was possible to conclude that red chromophores based in similar anthraquinones could have very different photophysical properties. Although laccaic acid A and carminic acid have a closer structure to purpurin than to alizarin, the former revealed a resemblance to alizarin, which could be justified y the presence of the linked amide. Photodegradation studies revealed that alizarin and purpurin are very stable, displaying very low quantum yields of reaction in homogeneous and heterogeneous media, as well as their lakes in homogeneous media. Microspectrofluorimetry proved to be a powerful analytical tool *in situ* to characterize emitter chromophores in very small samples (≥ 2 μm). It can be used not only in illuminations but also in other works of art like textiles and paintings, in the last one, when micro-samples are analysed one can selected the colorant emitter individual particles or aggregates in different paint layers. It also proved to enabled the characterization of madder lakes, by semi-quantifying the purpurin lake fraction in a mixture of purpurin and alizarin lake; and to differentiate the chromophore environment, depending on the complexing agent of eosin lakes.

Appendix I - Experimental section

I.1. General

All reagents and solvents used were of analytical grade. The water used was of Millipore grade.

For medieval paint reconstructions and standards were used pigments and binders from Sigma-Aldrich: vermillion, *minium*, lead white, verdigris, alizarin, purpurin, carminic acid, lac dye (Fluka) and alum carmine; from Panreac: calcium carbonate and calcium sulphate; Kremer Pigmente: orpiment, azurite, indigo, malachite, ivory black, madder roots and oak galls; from Zecchi: saffron, vide black, madder roots, cochineal, Pernambuco, gum arabic and goat parchment; from Wako: laccaic acid A; from Musée du Parchemin: parchment.

For modern red lakes reconstructions purpurin and eosin Y from Sigma-Aldrich, madder ground root from Kremer and Eosin, Meister Lucius & Brüning, ICN-reference collection, inv. nr. 4328 were used. Linseed oil was obtained from organic linseeds, and the vinyl media was obtained from a commercially available Portuguese artist brand, white glue V7®.

I.2. Instrumentation

I.2.1. µ-FTIR

µ-FTIR analyses were performed on a Nicolet Nexus spectrophotometer interfaced with a Continuµm microscope with a MCT-A detector cooled by liquid nitrogen. The spectra were collected in transmission mode, with a spatial resolution of 50-100 µm, an optical resolution of 4 cm^{-1} and 128 or 256 scans, by using a Thermo diamond anvil compression cell. When necessary, the system was purged with nitrogen prior to the data acquisition. The spectra are shown as acquired, without corrections or any further manipulations, except for the occasional removal of the CO_2 absorption at approx. 2300-2400 cm^{-1}.

I.2.2. µ-EDXRF

µ-EDXRF results were obtained using an ArtTAX spectrometer of Intax GmbH, with a molybdenum (Mo) anode, Xflash detector refrigerated by the Peltier effect (Sidrift), with a mobile arm. A set of polycapillary lens forms a microspot, circa 70µm, of primary X-radiation of high intensity on the sample surface [1]. The exact position of the incident beam on the sample to be analyzed is given through three beam-crossing diodes and can be controlled by an integrated CCD camera. The characteristic x-rays emitted by the sample (at 40º) are detected by a silicon drift electro-thermally cooled detector with a resolution of 160eV at Mn-Kα.

The experimental parameters used were: 40kV of voltage, 300 μA of intensity, for 200 seconds, under Helium gas flux. Si, Mn, Cu and Pb standards were used to calibrate the equipment in the beginning and at the end of the day.

I.2.3. μ-Raman

Raman Microscopy was carried out using a Labram 300 Jobin Yvon spectrometer, equipped with a HeNe laser 17 mW operating at 632.8 nm. Spectra were recorded as an extended scan. The laser beam was focused either with a 50 x or a 100 x Olympus objective lens. The laser power at the surface of the samples was varied with the aid of a set of neutral density filters (optical densities 0.3, 0.6, 1 and 2).

I.2.4. SPEX

Fluorescence spectra were acquired by a Jovin-Yvon Spex Fluorog 3-2.2 spectrofluorometer. The measurements were obtained with the μ-SPEX (Spex® **FluoroMap** with Manual Microscope Stage) using a set-up described elsewhere [2], where the Spex Fluorog 3-2.2 is connected to a Olympus BX51M confocal microscope, with spatial resolution controlled with a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot with a 50x objective. For steady-state fluorescence spectra, a continuous 450W 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 (Figure I.1.).

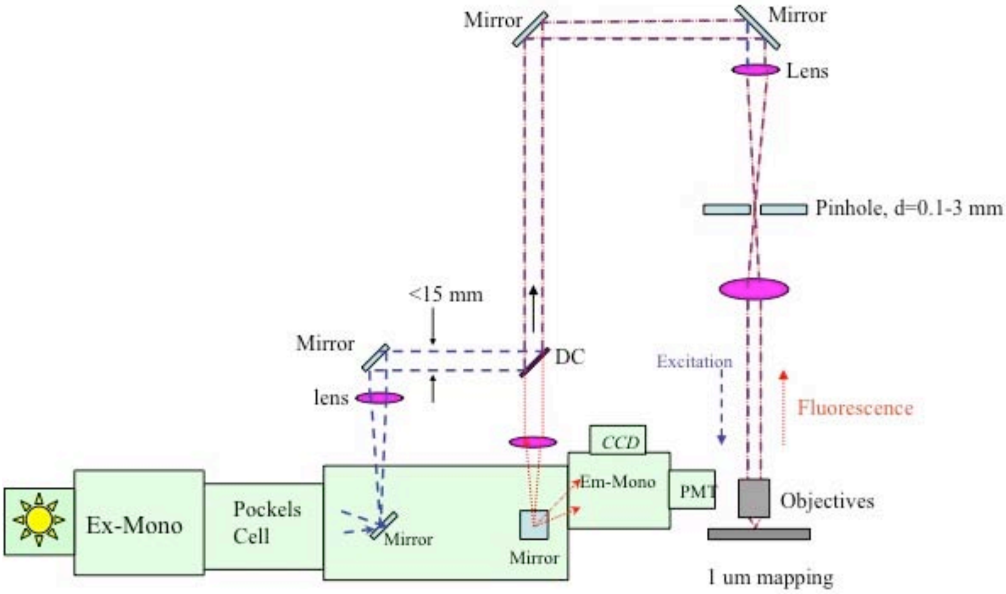


Figure I.1. Scheme of µ-SPEX apparatus.

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 45º. The optimization of the signal was carried out daily, through mirror alignment in the optic pathway of the microscope, following the manufacturer instructions.

For the measurements with the optic-fibre a SPEX Model F-3000 fibre-optic adaptor was used, and the signal optimized as recommended by the manufacturer.

I.2.5. HPLC-DAD

An analytical ThermoFinnigan Surveyor HPLC-DAD system with a PDA 5, using a RP-18 analytic column (250x4.6 Nucleosil 300-5 C18), was used to perform the dye analyses. The column was stabilised at 35 ºC. The samples were injected onto the column via a Rheodyne injector with a 25 µL *loop*. The system was re-equilibrated at the starting eluent composition for 3-5 min before next injection. The isocratic mobile phase consisted of **A**-pure methanol and a mixture of **B**-0.15% perchloric acid 70%: water (v/v), the solvent gradient was used at a flow rate of 1.7 ml/min; 0-2 min 7A:93B isocratic, 8 min 15A:85B linear, 25 min 75A:25B linear, 27 min 80A:20B linear, 29-40 min 100A isocratic [3].

I.2.6. Optical Microscopy

The optical analysis were carried out in an optical Zeiss Axioplan Z Imaging microscope with a Nikon digital camera DMX 1200F and a mercury lamp HBO100, and in a Leica MZ16 stereomicroscope with a Leica digital camera (Digilux 1) with fiberoptic light Leica system (Leica KI 1500 LCD).

I.2.7. PIXE

Elemental analysis on two ground sorts of lapis lazuli and one of lazurite were carried out by PIXE using 2.3 MeV protons generated by a Van de Graff accelerator at the Instituto Tecnológico e Nuclear (ITN). The induced X-rays were detected by a LinkTM Si(Li) detector. For all the measurements, a 350 μm thick Mylar® absorber was used. PIXE spectra were analysed with AXIL-PC [4] computer program and the concentration calculations performed with the computer code DATTPIXE [5].

I.2.8. Monochromatic irradiation

The monochromatic irradiations were performed in a 450W Xenon lamp with a Jobin Yvon Divisional Instruments SA monochromator. Entrance and exit slit widths were set at 2mm. The chromophores were irradiated using a Melles-Griot set-up.

I.2.9. Solar Box Camera

The polychromatic irradiation was performed in a 3000e irradiation camera, with a 3500W Xenon lamp; a cut-off filter was used to use only higher wavelengths than 300 nm. The irradiation intensity was controlled to be 800 W/m^2 and 50 ºC BST.

I.2.10. UV-VIS spectroscopy

UV-VIS absorption spectra were recorded on a Shimadzu UV-2501PC or Cary 100 Bio UV-VIS Varian spectrophotometer and on a Shimadzu at 21ºC.

I.2.11. Colorimeter

The reflectance spectra and the Lab* coordinates were measured with a Microflash mobile colorimeter DataColor International, which is provided with a Xenon lamp. The sampling area is of 8mm diameter. The CIELab* system was used, defining the D65 illuminant and the 10º observer; the calibration was made with white (porcelain) and black prototypes.

I.3. Methods

I.3.1. Manuscripts

I.3.1.1. Data acquisition for Lorvão manuscripts

The data acquisition of the selected set from Lorvão *scriptorium* was performed in two missions, one in February 2006, at DCR scientific laboratory, and other in November 2007, at Torre do Tombo. µ-EDXRF, µ-Raman and µ-SPEX analyses were done *in situ* while µ-FTIR analyses was performed in micro-samples collected *in situ* throughout these missions.

The strategy adopted to analyse the colours begun with µ-EDXRF analyses, since the spatial resolution is 70 µm followed by µ-Raman analyses (4 µm) in some of the previous µ-EDXRF analysed areas. At least two representative areas in each folio, for each colour were selected. In each area three local spots were analysed by µ-EDXRF and two to three by µ-Raman, to ensure statistical relevance (see section I.3.1.1.1.).

Micro-sampling performed to do µ-FTIR analyses was carried out with a micro chisel from Ted Pella micro tools under microscope (Leica). The organic material micro-sampled was usually performed independently of the µ-EDXRF and µ-Raman results. Inorganic materials were only micro-sampled if there were doubts remaining, such as the presence of fillers mixed with pigments, the nature of greens and the possibility of organic colours were mixed with pigments.

Micro-sampling was mainly performed on lacunas or detached zones, followed a strategy of minimum intervention in the manuscript, yet ensuring a representative sampling of the paints.

I.3.1.1.1. Analysed areas

The blue, red and green dots are marking the areas where µ-EDXRF, µ-Raman µ-FTIR analyses were performed, respectively. All photographs of *Apocalypse* folia are from DGARQ-ANTT.

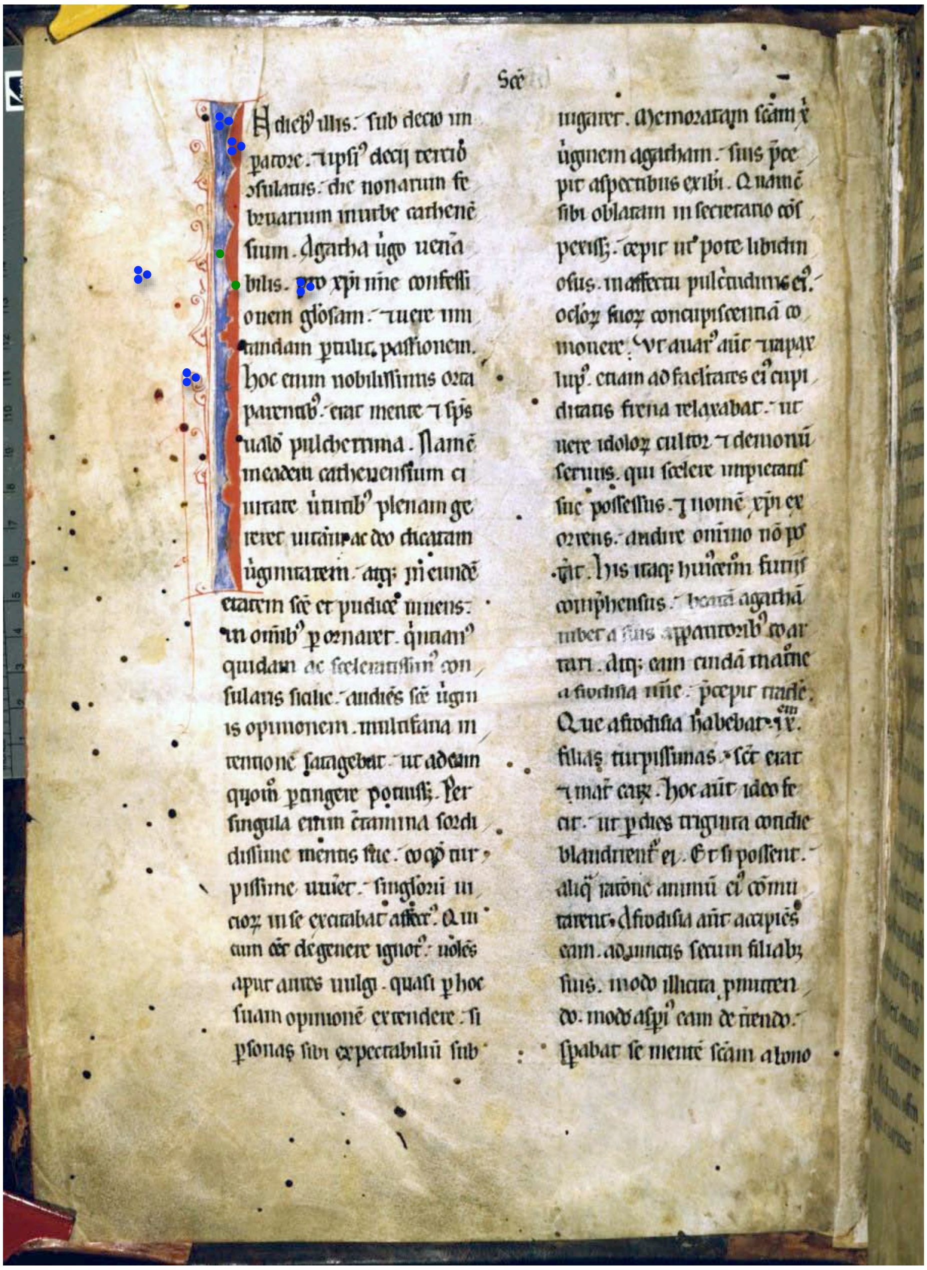


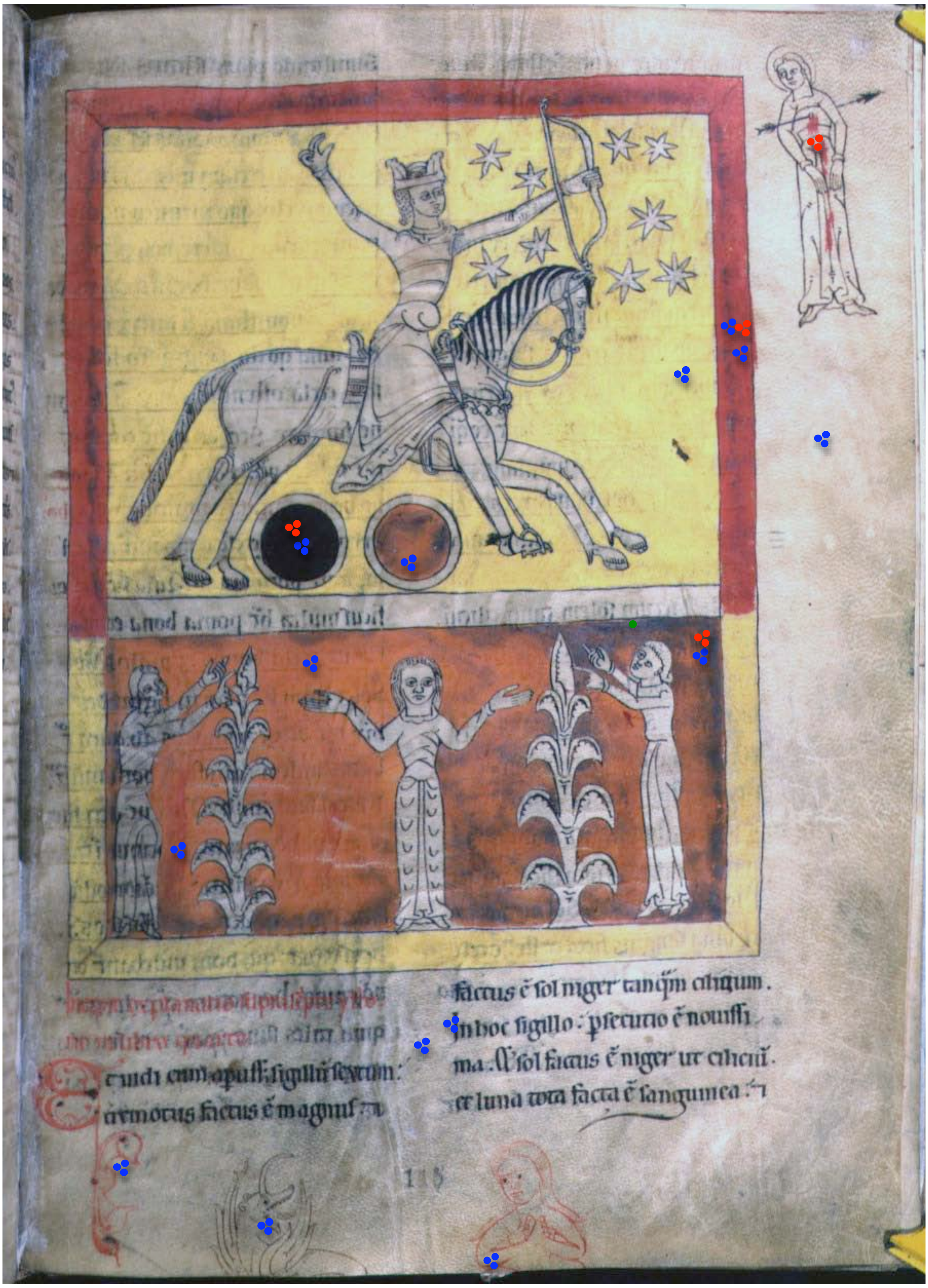
Figure I.2. *Apocalypse*, *f.* 1v

Mulier sup bestiam ē corruptela. et opa nequitie. et uoluptates. fornicatio. et inmundicia. et auaricia ⁊ zelus. detractio. et inuidia. uana gl'a. supbia. et ingluuia uētris. Qui de prosperitate mundi letant͛. qui caritatē nō habet. qui paupib' bene nō facit. qui seruos dei iniuriis et contumeliis afficit. qui ppria nō tribuit s; adhuc aliena rapit: qui ad

43

Figure I.3. *Apocalypse*, *f.* 43

Figure I.4. *Apocalypse, f.* 49



[illegible]

Et uidi cum apuisset sigillum sextum:
terremotus factus ē magnus et

factus ē sol niger tanquam cilicium.
In hoc sigillo: percussio ē nouissi
ma. Sol factus ē niger ut cilicium.
et luna tota facta ē sanguinea

115

Figure I.5. *Apocalypse*, *f.* 115

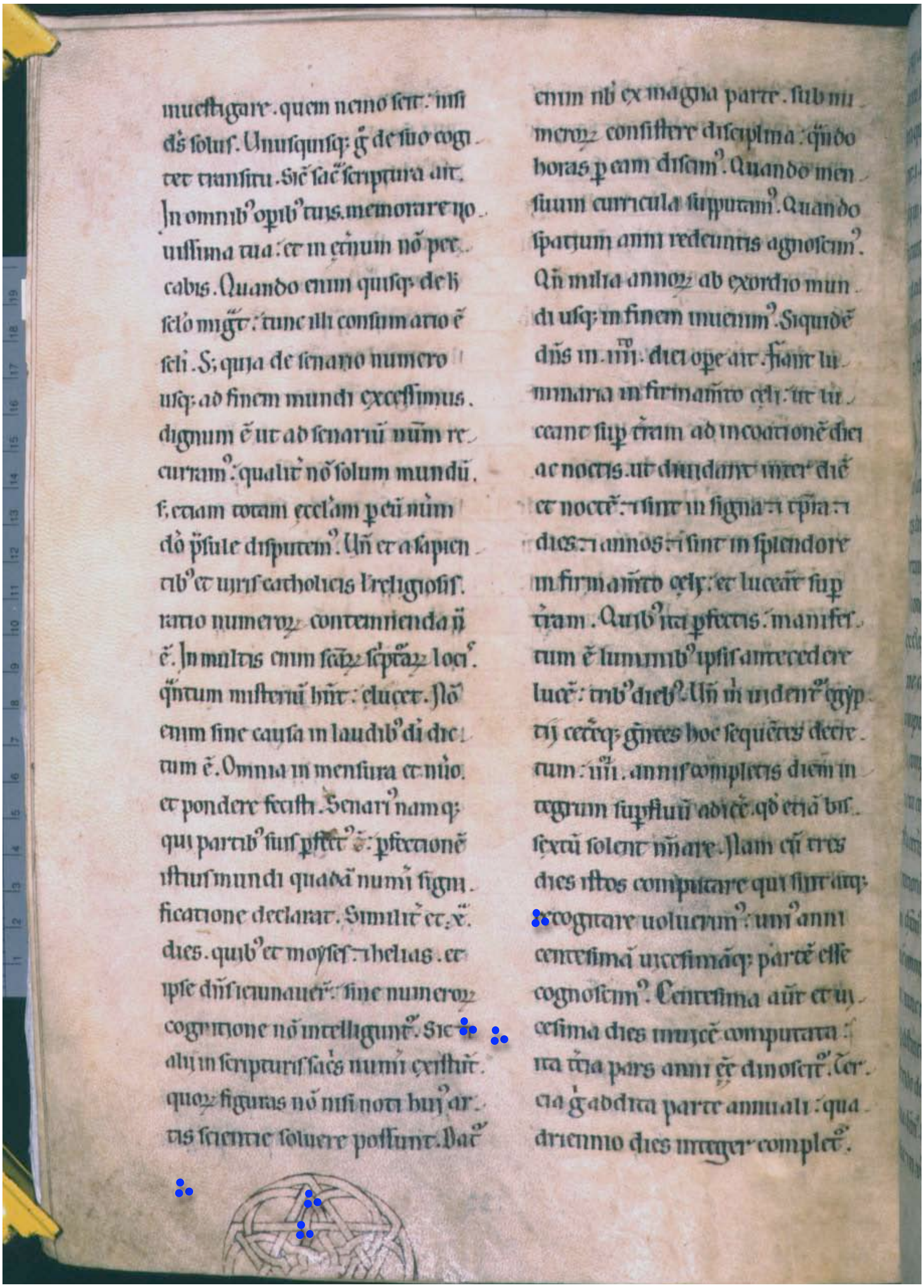
originē redit. et recapitulat eadem. breuit᷑. atq; alit᷑ dicitur. Explicit explanatio ſexti ſigilli.

Incipit hiſtoria .iiiior. angloꝝ .iiii. uentos tenentium.

Et poſt h' uidi .iiiior. anglos ſtantes in .iiii. angulos t̃re. tenētes .iiiior. uentos t̃re. ne flarent in t̃rā. neq; in mare. neq; in ullā arborē. Et uidi aliū angl'm aſcēdentē ab ortu ſolis. hntē ſignū dī uiuētis. Et clamauī uoce magna .iiiior. anglis. quib' data ē poteſtas. ledē t̃ram. et mare dicēs. Nolite ledere t̃ram. neq; mare. neq; arbores. donec ſignem' ſeruos dī nr̄i in frontib' eoꝝ. Explic' hiſtoria.

118

Figure I.6. *Apocalypse*, *f.* 118



inuestigare . quem nemo scit : nisi
ds solus . Unusquisq; g de suo cogi-
tet transitu . Sic sac scriptura ait .
In omnib' opib' tuis . memorare no-
uissima tua : et in eternum nō pec-
cabis . Quando enim quisq; de h
sclo migt : tunc illi consummatio ē
scli . S; quia de senario numero
usq; ad finem mundi excessimus .
dignum ē ut ad senariū nūm re-
curram' : qualit nō solum mundū .
s; etiam totam ecclām p eū nūm
dō psule disputem' . Un et a sapien-
tib' et uiris catholicis & religiosis .
ratio numerorum contempnenda n
ē . In multis enim scāz scptāz loci' .
qntum misterii hēt : elucet . Nō
enim sine causa in laudib' dī dic-
tum ē . Omnia in mensura et nūo .
et pondere fecisti . Senari' namq;
qui partib' suis pfect' ē : pfectionē
istius mundi quadā numi signi-
ficatione declarat . Similit et . xl .
dies . quib' et moyses & helias . et
ipse dns ieiunauer : sine numerorum
cognitione nō intelligunt . Sic
alii in scripturis sacs numi exsistūt .
quorum figuras nō nisi noti huj' ar-
tis scientie soluere possunt . Hac

enim nisi ex magna parte . sub nu-
merorum consistere disciplina : qndo
horas p eam discim' . Quando men-
sium curricula supputam' . Quando
spatium anni redeuntis agnoscim' .
Qn milia annorum ab exordio mun-
di usq; in finem inuenim' . Siquidē
dns in . iiii . diei opeatr . fiant lu-
minaria in firmamto celi : ut lu-
ceant sup trām ad incoationē diei
ac noctis . ut diuidant inter diē
et noctē : & sint in signa & tpa &
dies & annos : & sint in splendore
in firmamto celi : et luceāt sup
trām . Quib' ita pfectis : manifes-
tum ē luminib' ipsis antecedere
lucē : trib' dieb' . Un in uident egyp-
tii ceteriq; gentes hoc sequētes decre-
tum : iiii . annis completis diem in-
tegrum supfluū adicē . qd etiā bis-
sextū solent nōiare . Nam cū tres
dies istos computare qui sunt anq;
recogitare uoluerim' : uni' anni
centesimā uicesimāq; partē esse
cognoscim' . Centesima aūt et ui-
cesima dies inuicē computata :
ita t'cia pars anni ēē dinoscit' . Ter-
cia g addita parte annuali : qua-
driennio dies integer complet' .

Figure I.7. *Apocalypse*, *f.* 122v

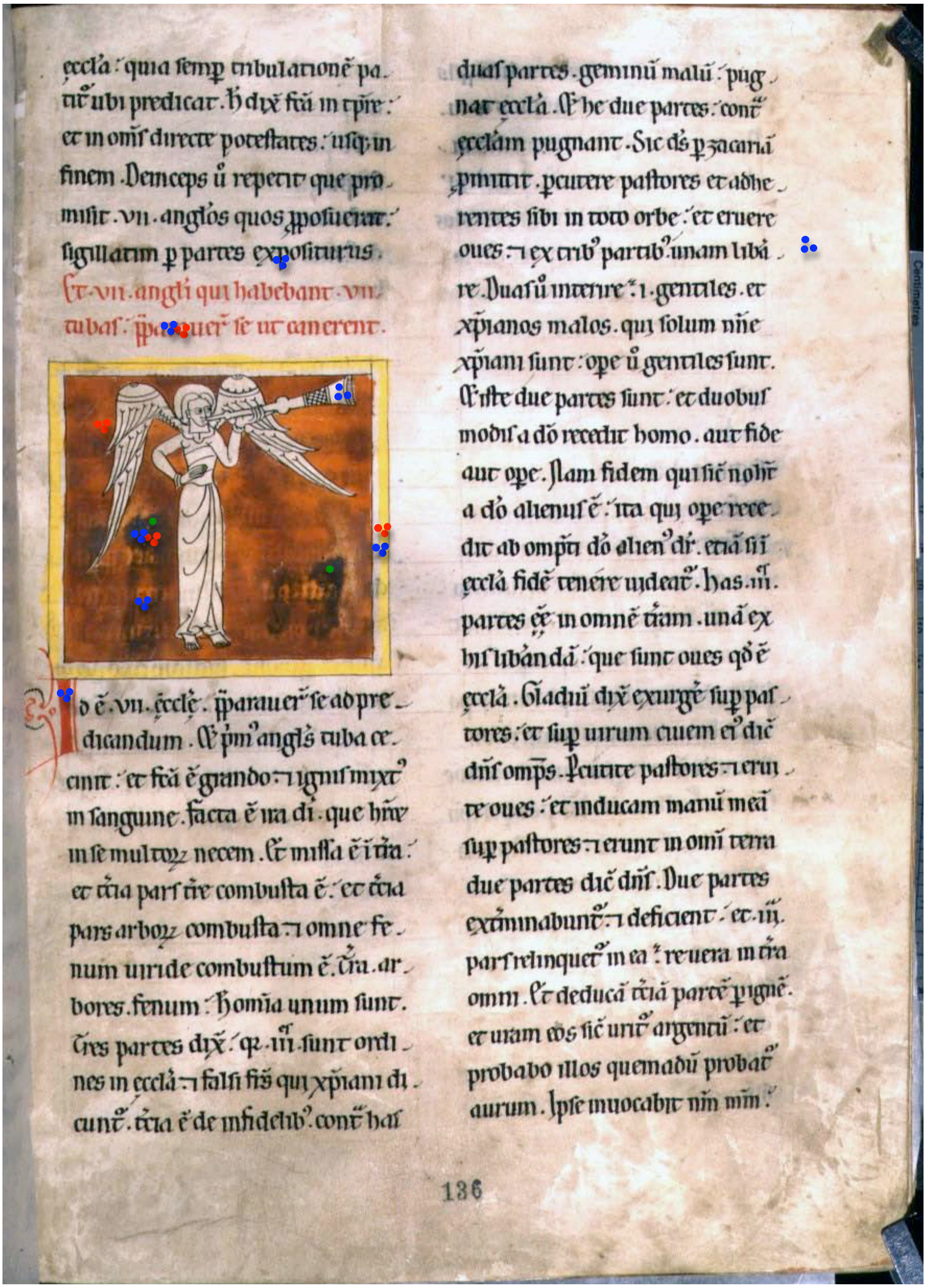


Figure I.8. *Apocalypse*, *f.* 136

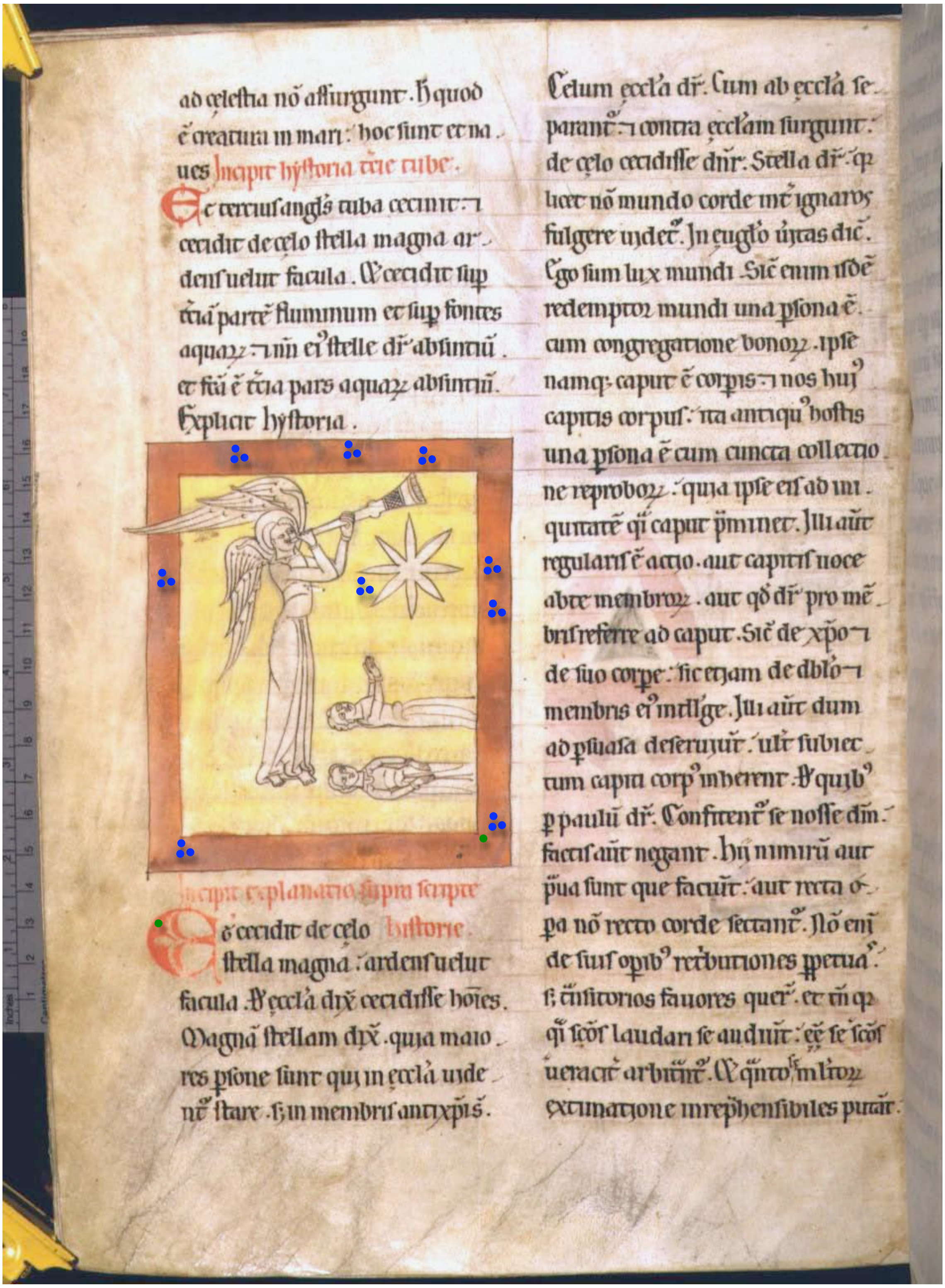


Figure I.9. *Apocalypse*, *f.* 138v

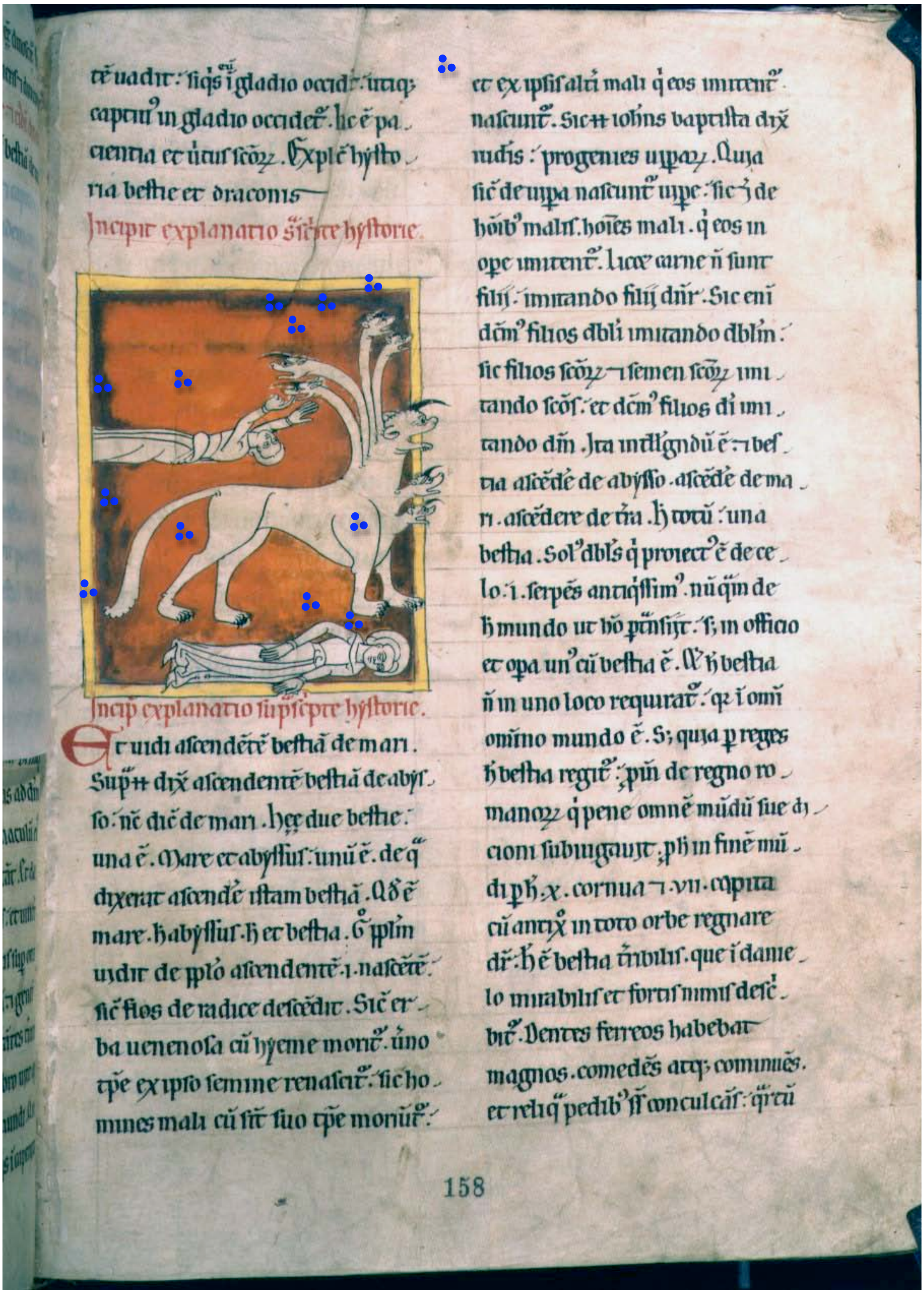
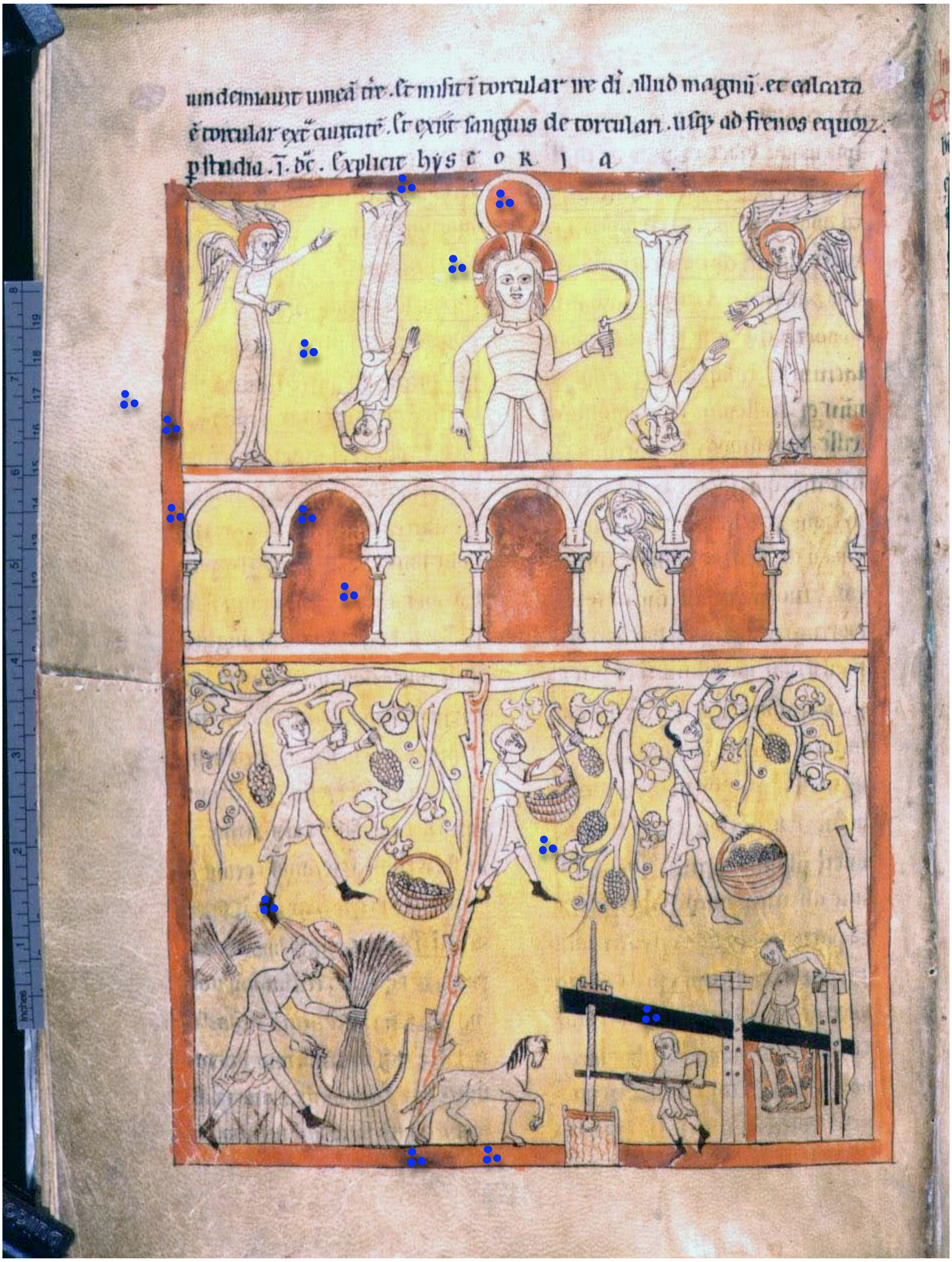


Figure I.10. *Apocalypse*, *f.* 158



uindemiauit uineā t̄re. Et misit ī torcular ire dī. illud magnū. et calcata
ē torcular ext̄ ciuitatē. Et exiit sanguis de torculari. usq; ad frenos equoꝝ.
p stadia .ī. dc̄. Explicit hys t o r i a

Figure I.11. *Apocalypse*, *f.* 172v

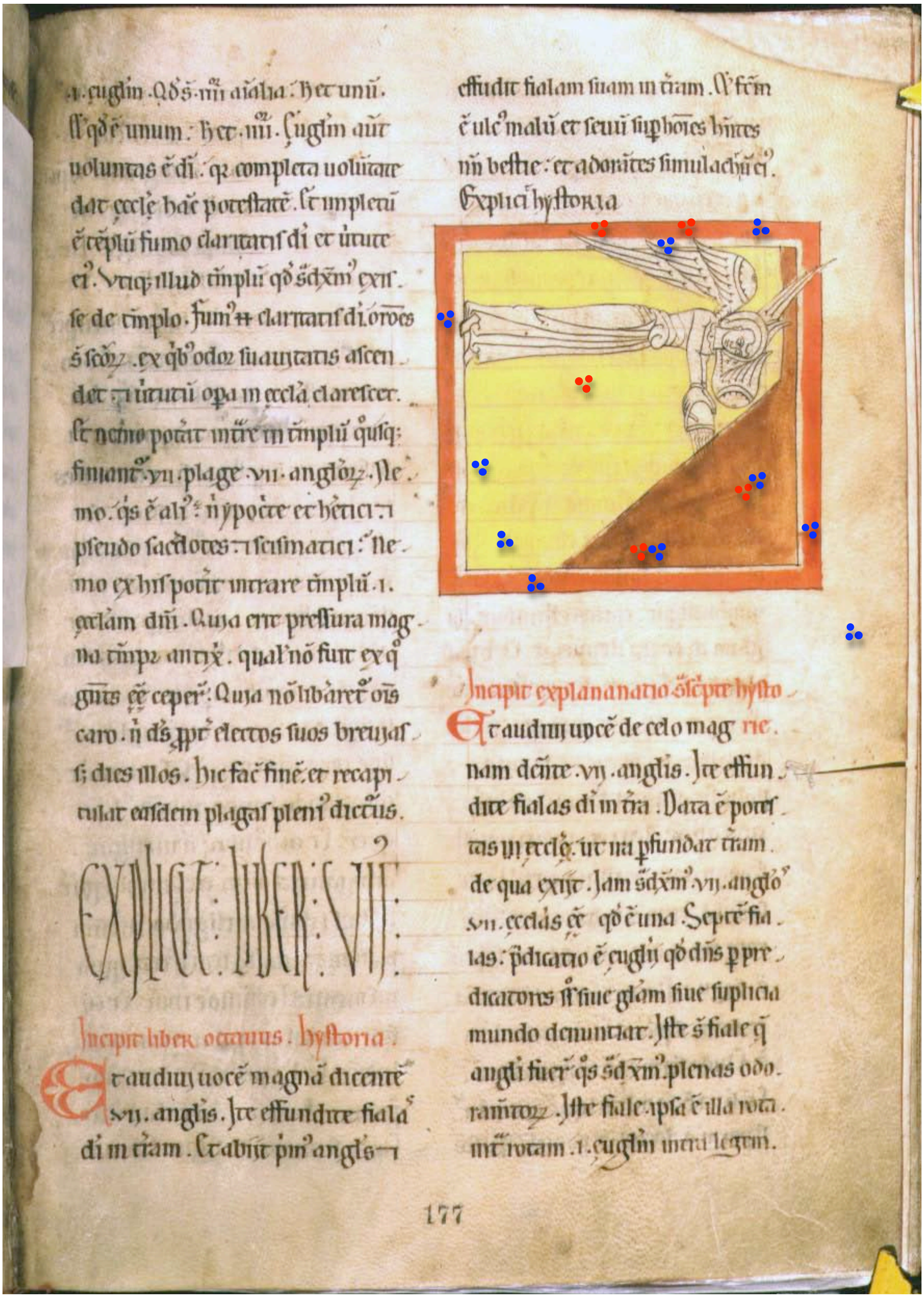


Figure I.12. *Apocalypse*, *f.* 177

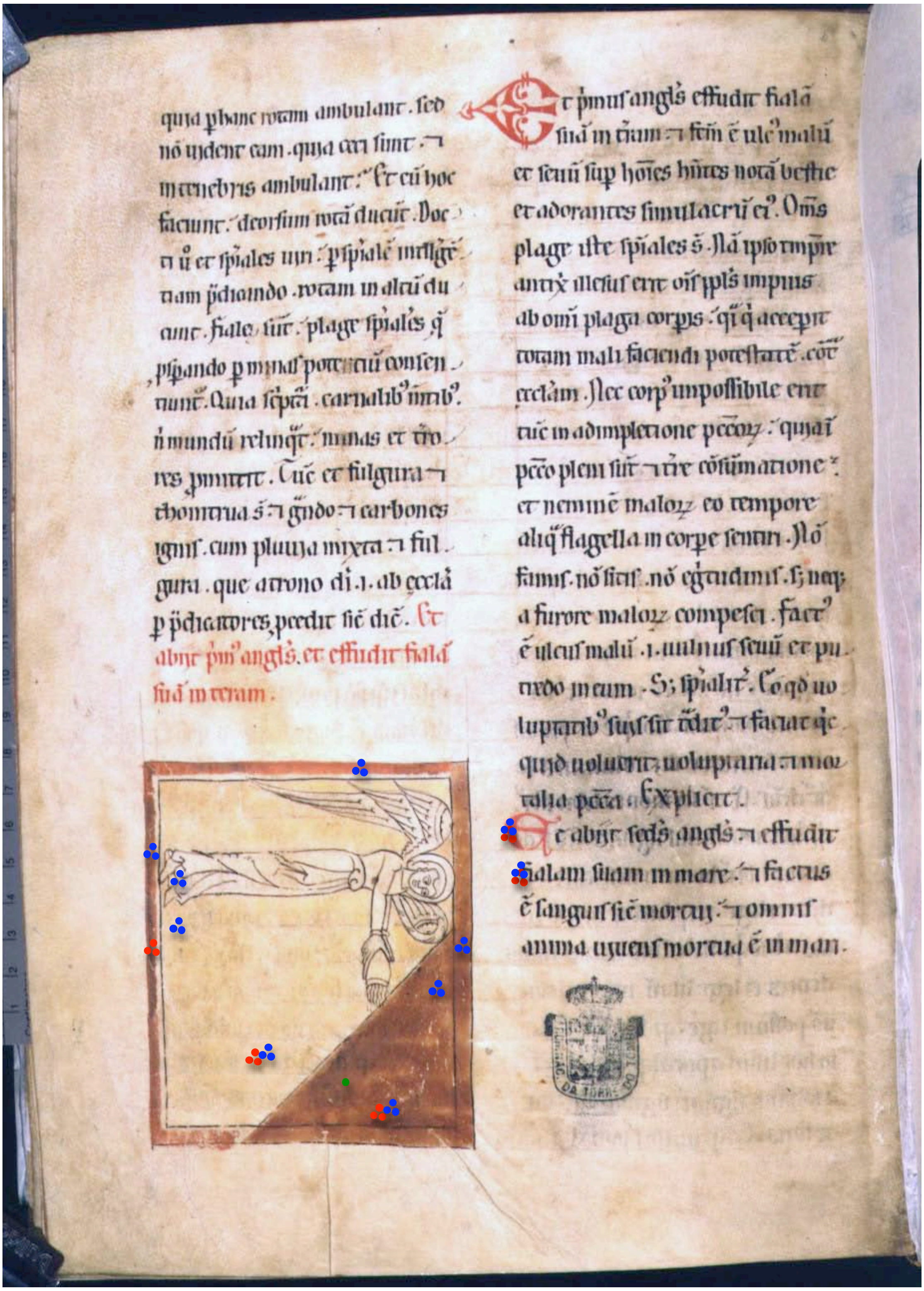


Figure I.13. *Apocalypse*, *f.* 178v

Figure I.14. *Apocalypse*, *f.* 179

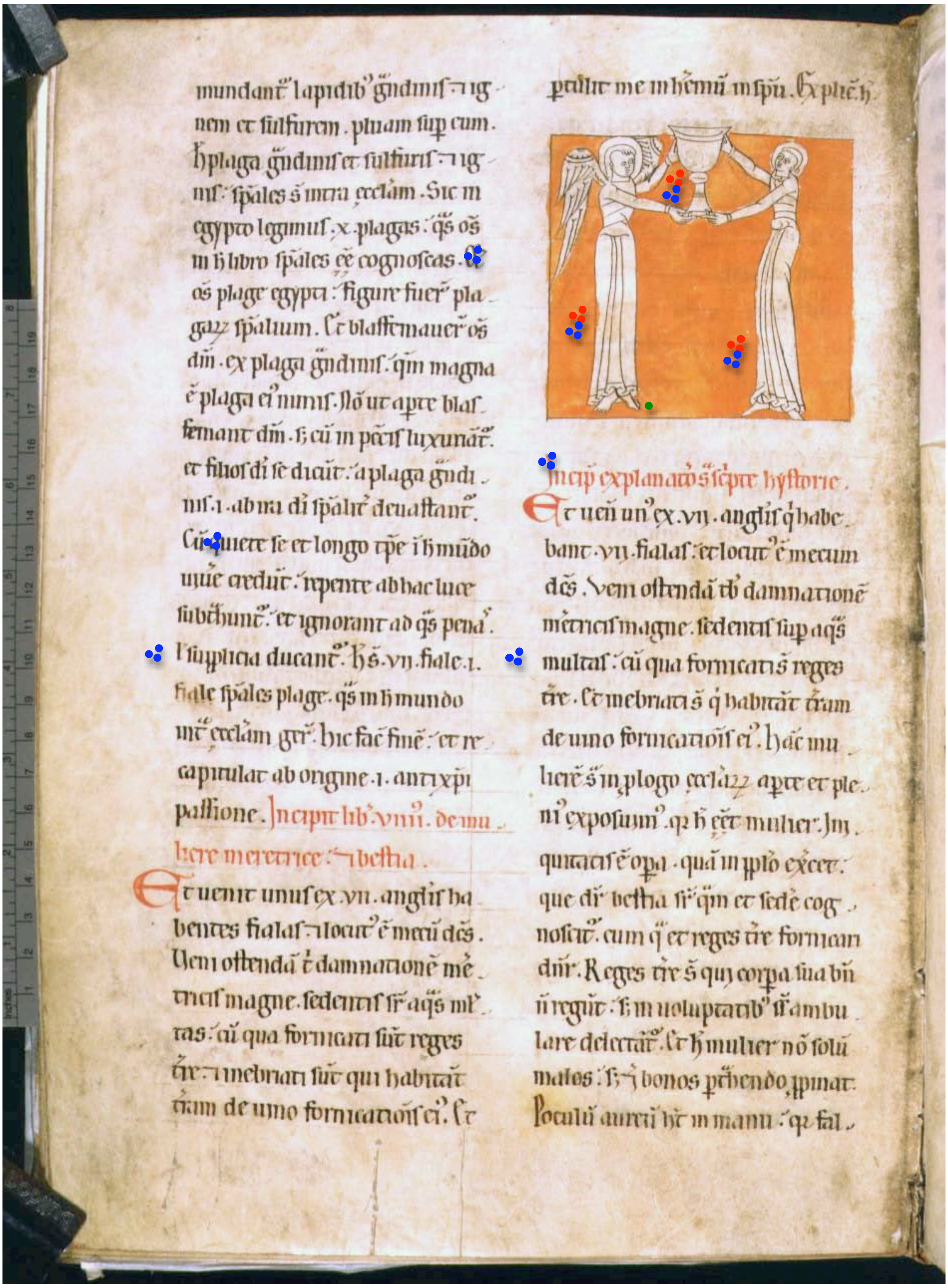


Figure I.15. *Apocalypse*, *f.* 185v

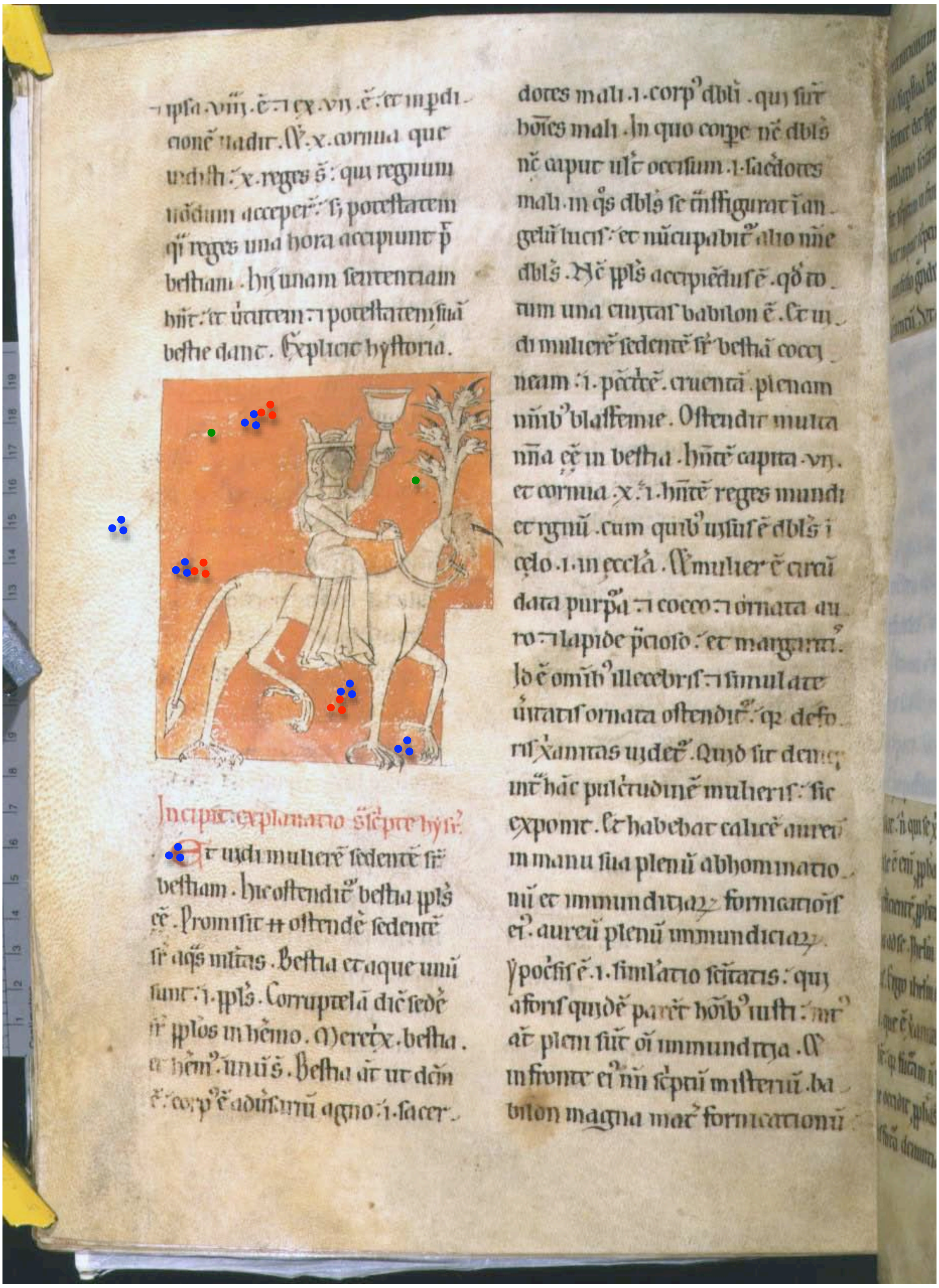


Figure I.16. *Apocalypse*, *f.* 186v

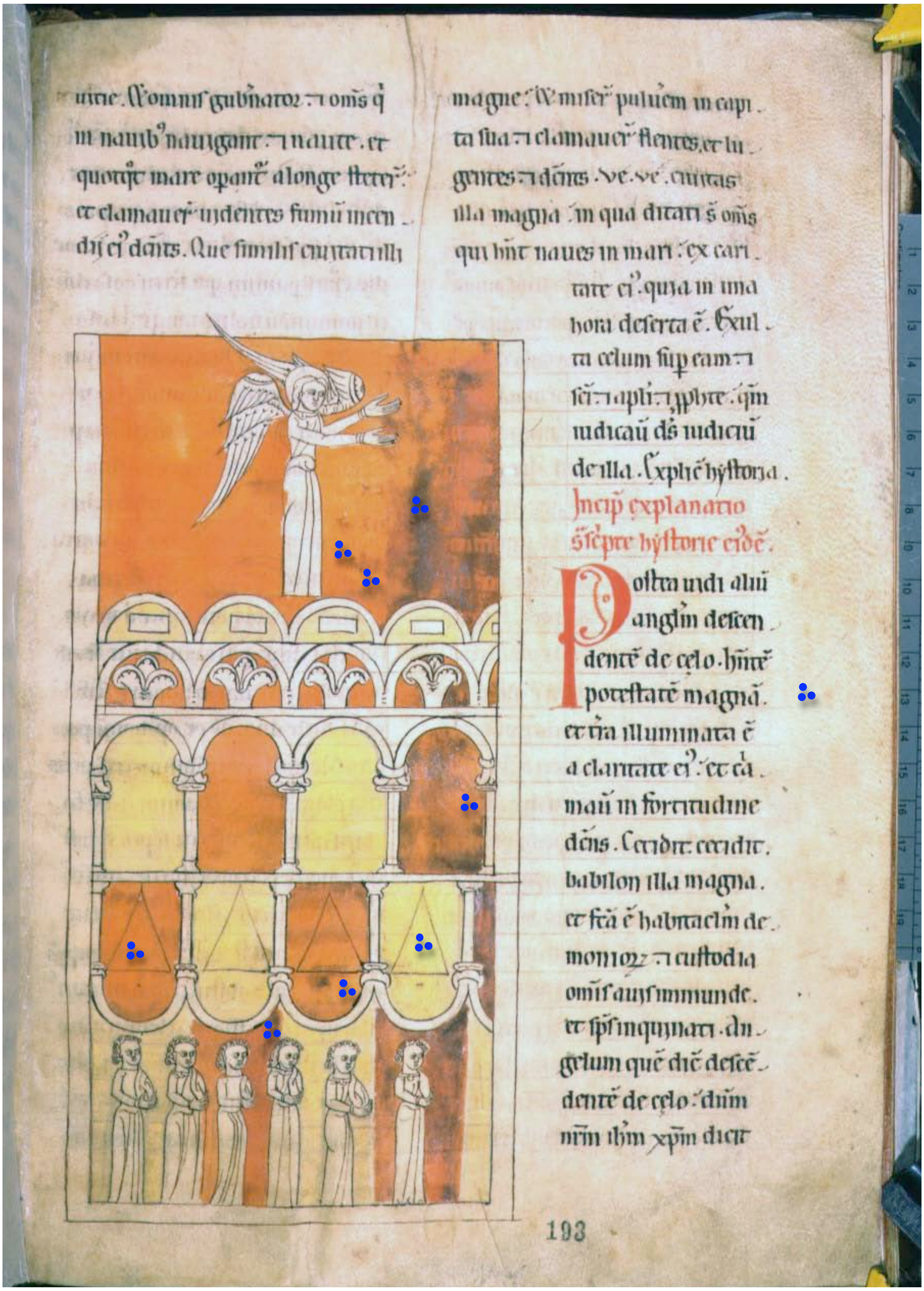


Figure I.17. *Apocalypse*, *f.* 193

Figure I.18. *Apocalypse*, *f.* 196v


que ē tīa ſanguinis: omnis omnino
mund' ē. Et ipſa ē ciuitas dbli que
uſq; in finē ſċli cruciatr ⁊ pſequit.
Et in una naſcit xpc. ⁊ in alia antix.
Ꝑptea dic gladiū dñi in iugulatio-
nē. in ydumea ⁊ boſor. i. in malos
fr̄s. qui ſe ſyon mentiunt. Sic ſcp-
tum ē: Poplm di. oſtium ſangui-
nem bibere. Explicit. Incipit hyſ-
toria de beſtia ⁊ de regib' tre.
Et uidi beſtiam ⁊ reges tre ⁊ ex-
ercitus eoꝝ congregatos: facē bellū
cum ſedente ſup equū ⁊ cum exer-
citu ei'. Et apphenſa ē beſtia ⁊ cum
illo pſeudo pphe. qui fecer ſigna
in conſpectu ei'. in quibus ſeduxit

accipientes notam beſtie: ⁊ ado-
rantes ſimulachrū ei'. Viuentes
miſſi ſunt ipſi duo in ſtagnū ignis
ardentis. in ſulfure. Reliqui u
occiſi ſunt gladio ſedentis ſup
equum: exeunte de ore ei': ⁊
omnes aues ſaturate ſunt de
carnib' eoꝝ. Explicit hyſtoria.

200

Figure I.19. *Apocalypse*, *f.* 200

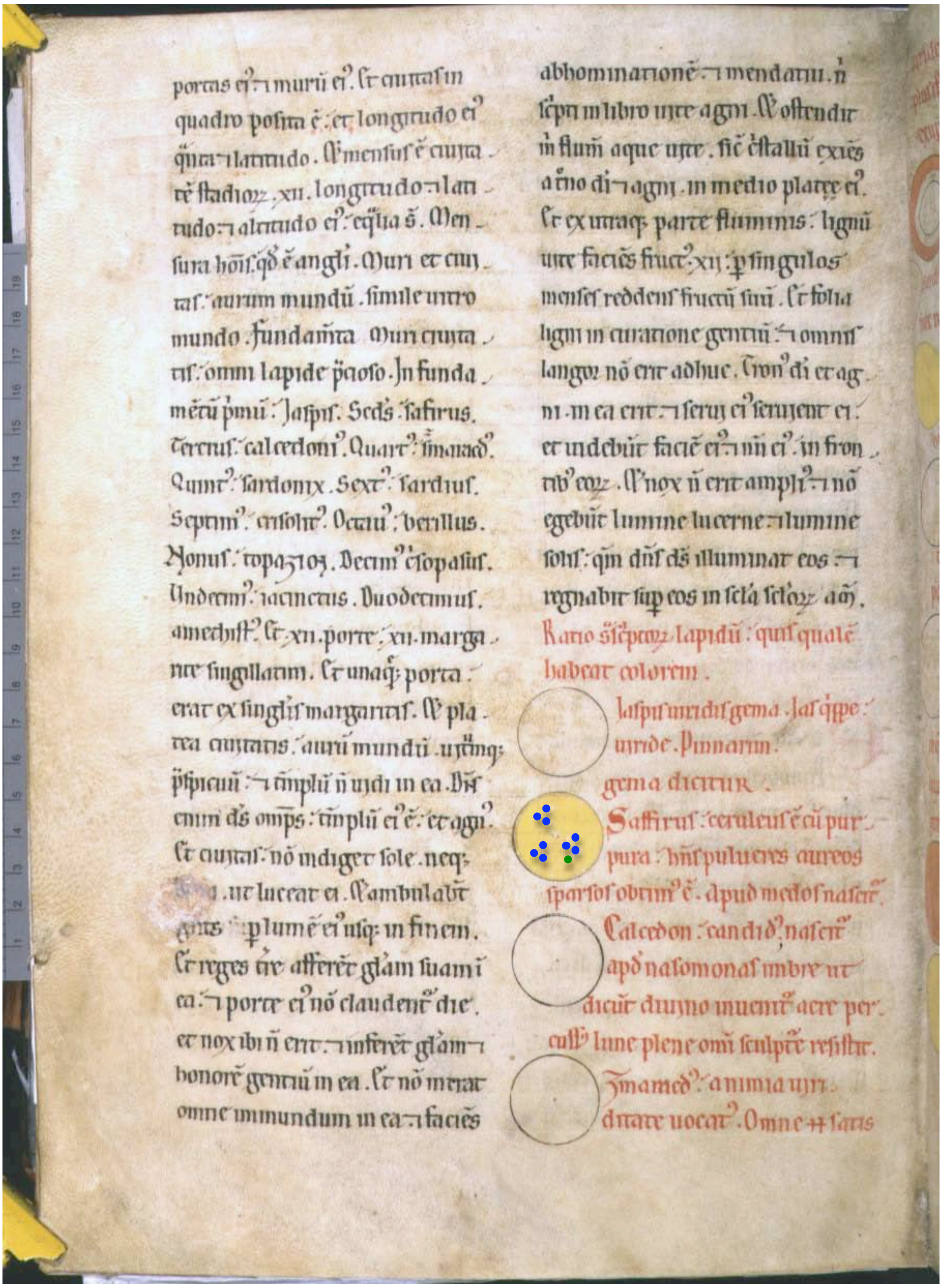


Figure I.20. *Apocalypse*, *f.* 208v

Figure I.21. *Apocalypse*, *f.* 209

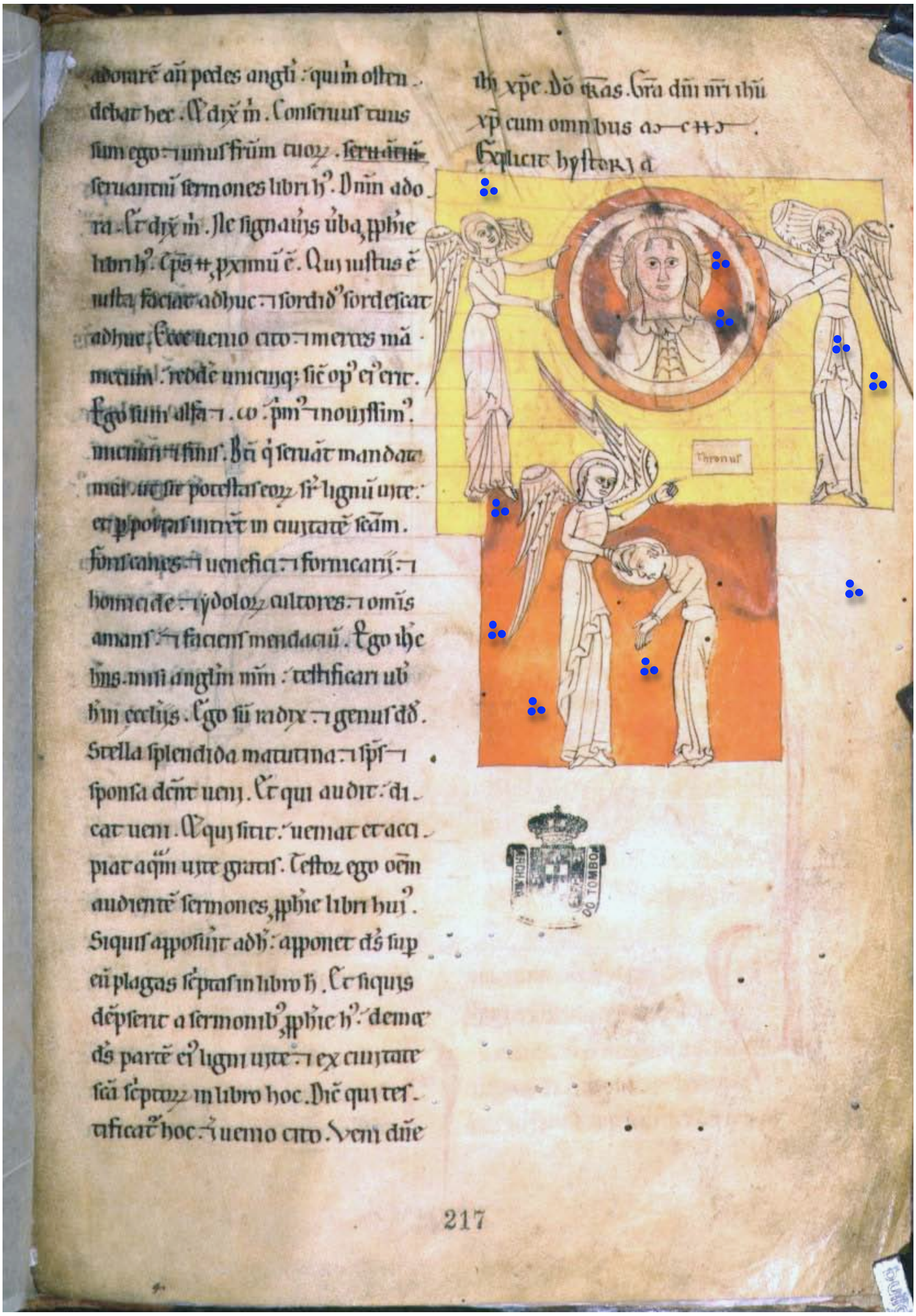


Figure I.22. *Apocalypse*, *f.* 217

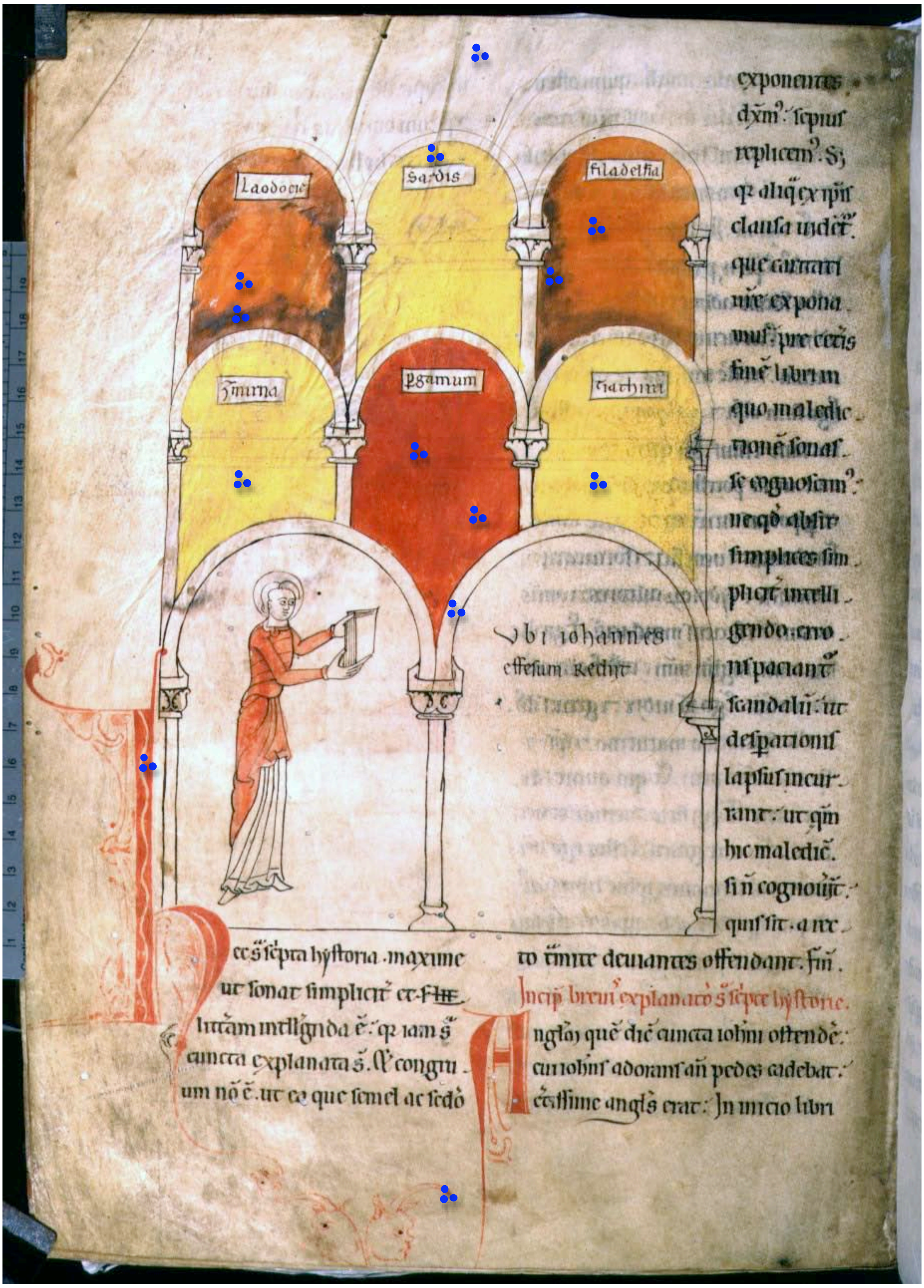


Figure I.23. *Apocalypse*, *f.* 217v

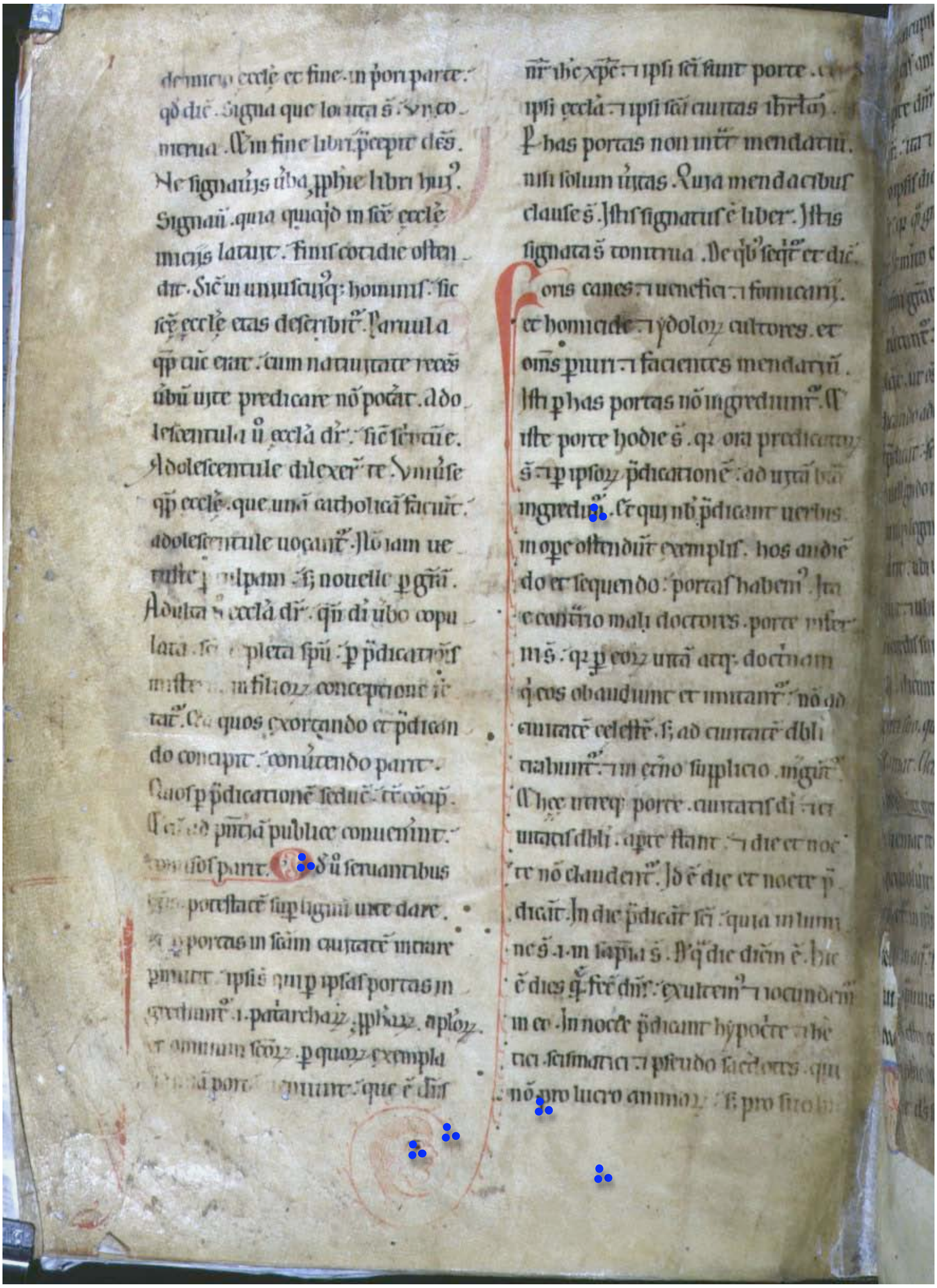


Figure I.24. *Apocalypse*, *f.* 218v

Figure I.25. *Book of Birds*, *f.* 4

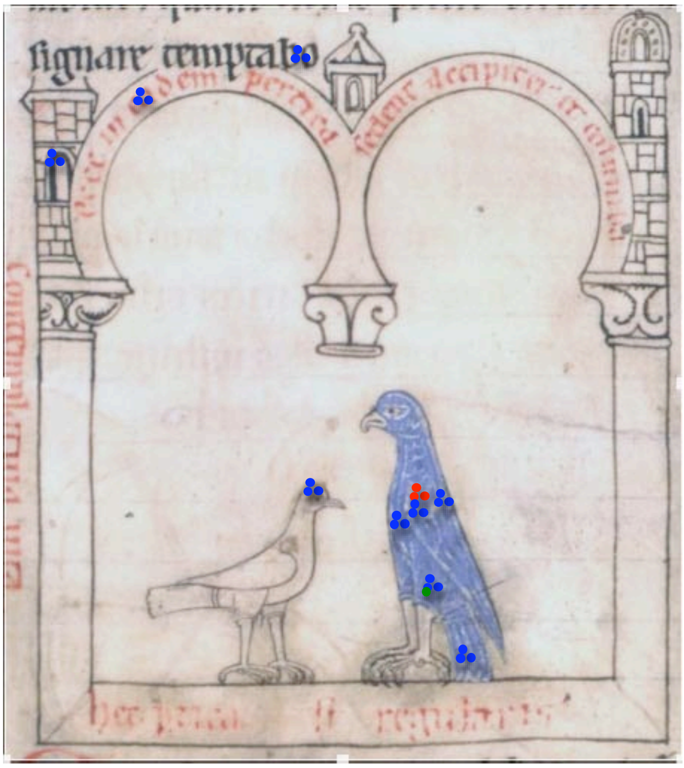
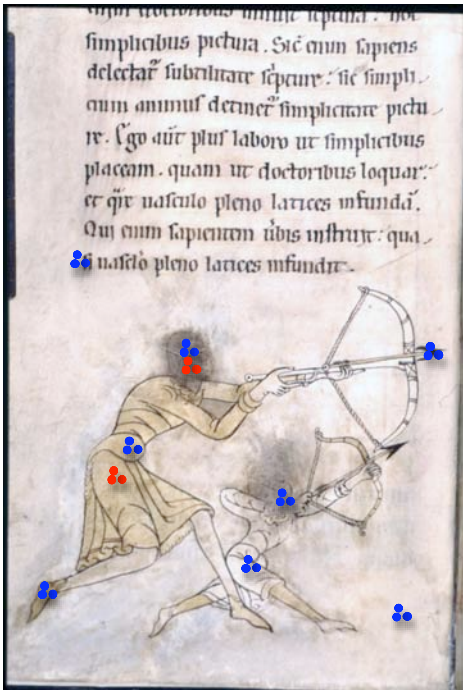


Figure I.26. *Book of Birds*, *f.* 5



ſimplicibus pictura. Sic enim ſapiens delectatur ſubtilitate ſcripture: ſic ſimplicium animuſ detinetur ſimplicitate picture. Ego autem pluſ laboro ut ſimplicibus placeam. quam ut doctoribus loquar: et quasi uaſculo pleno latices infundam. Qui enim ſapientem uerbis inſtruit: quaſi uaſculo pleno latices infundit.

Figure I.27. *Book of Birds*, *f.* 5v

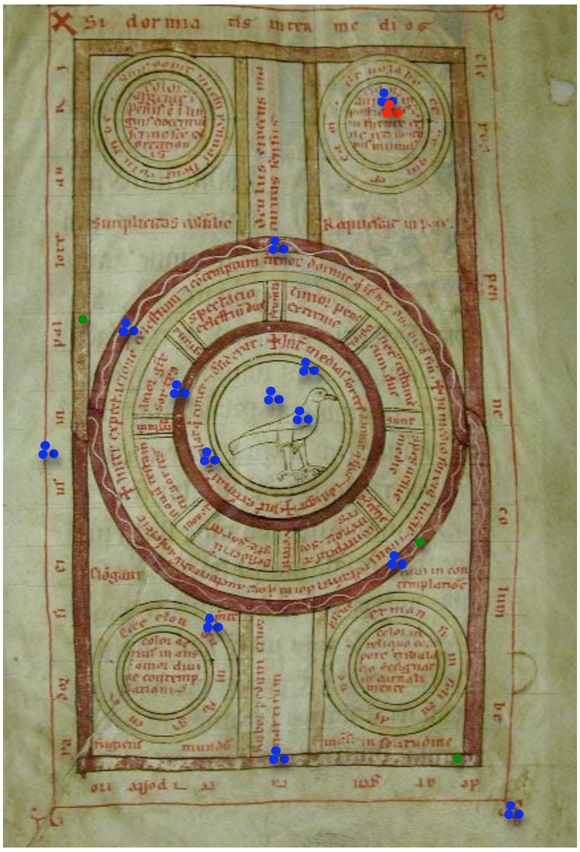


Figure I.28. *Book of Birds*, *f.* 6

Figure I.29. *Book of Birds*, *f.* 6v

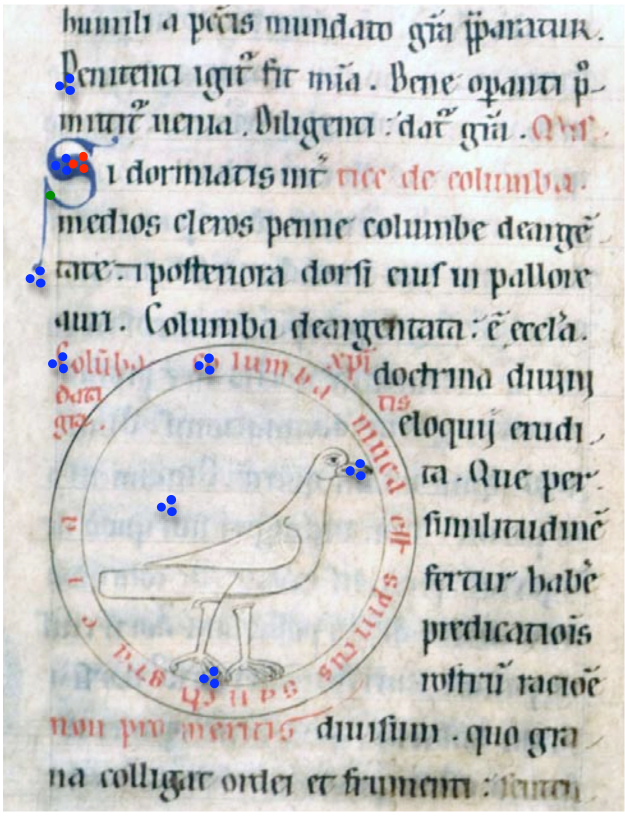


Figure I.30. *Book of Birds*, *f.* 7v

Figure I.31. *Book of Birds*, *f.* 16

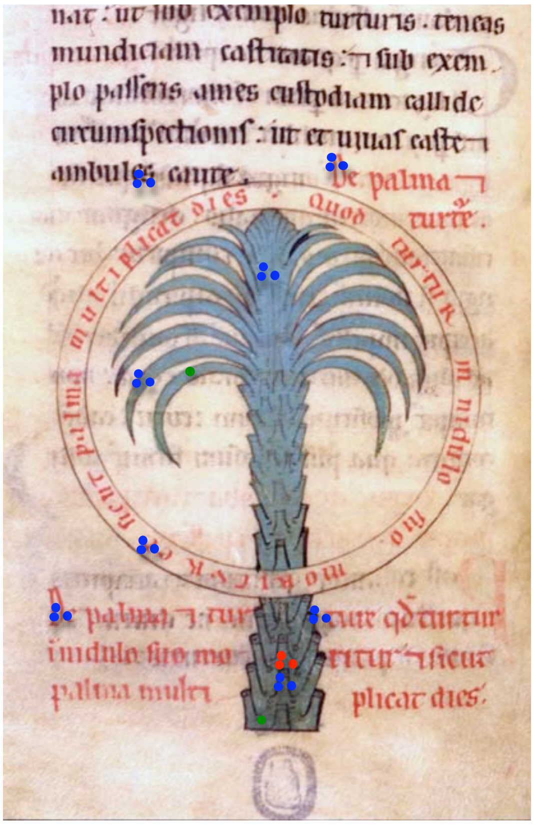


Figure I.32. *Book of Birds*, *f.* 20v

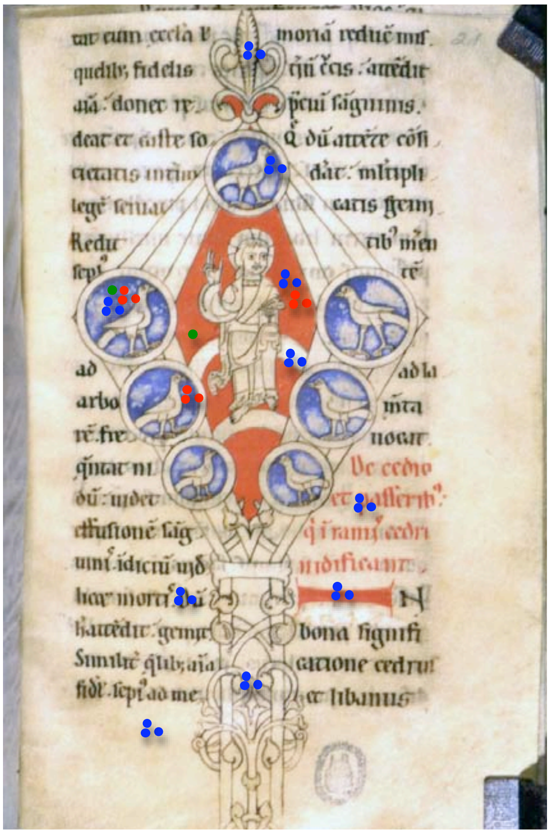


Figure I.33. *Book of Birds*, *f.* 25

Figure I.34. *Book of Birds*, *f.* 33

Figure I.35. *Book of Birds*, *f.* 36v

Figure I.36. *Book of Birds*, *f.* 48

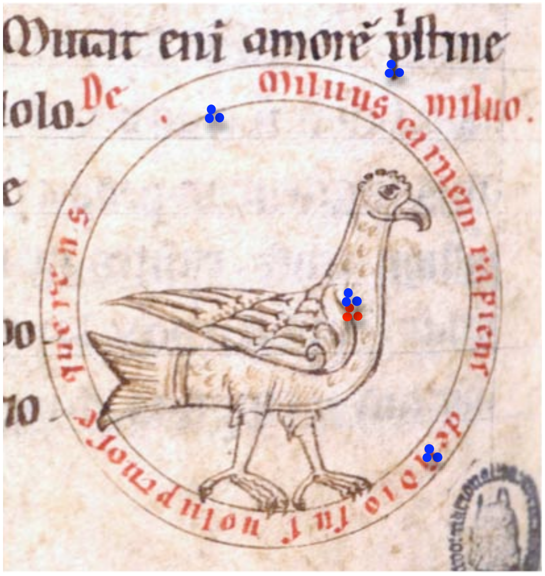


Figure I.37. *Book of Birds*, *f.* 49v

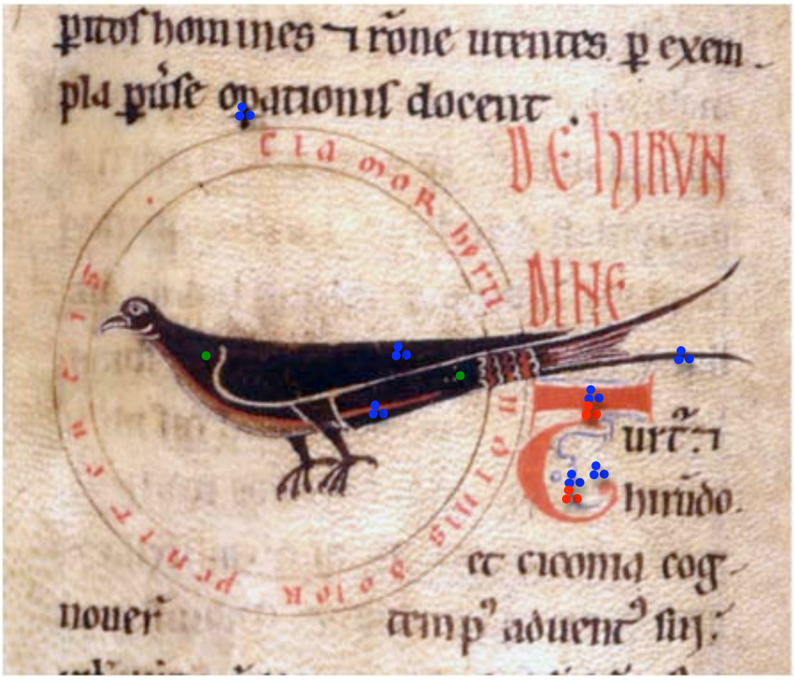


Figure I.38. *Book of Birds*, *f.* 50v

Figure I.39. *Book of Birds*, *f.* 54

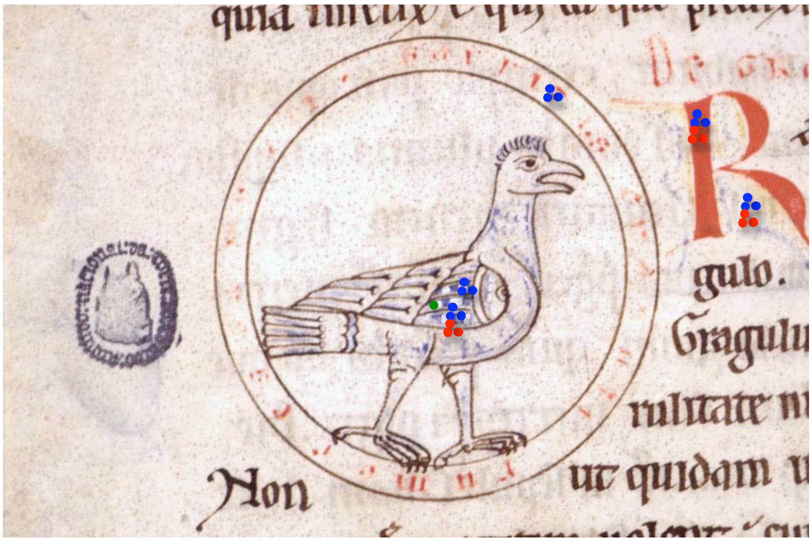


Figure I.40. *Book of Birds*, *f.* 56v

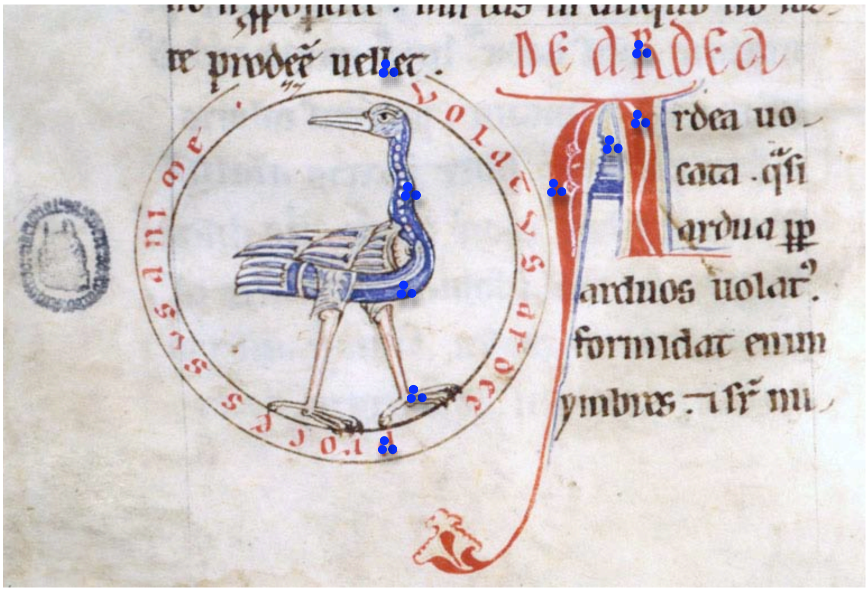


Figure I.41. *Book of Birds*, *f.* 59v

Figure I.42. *Book of Birds*, *f.* 72v

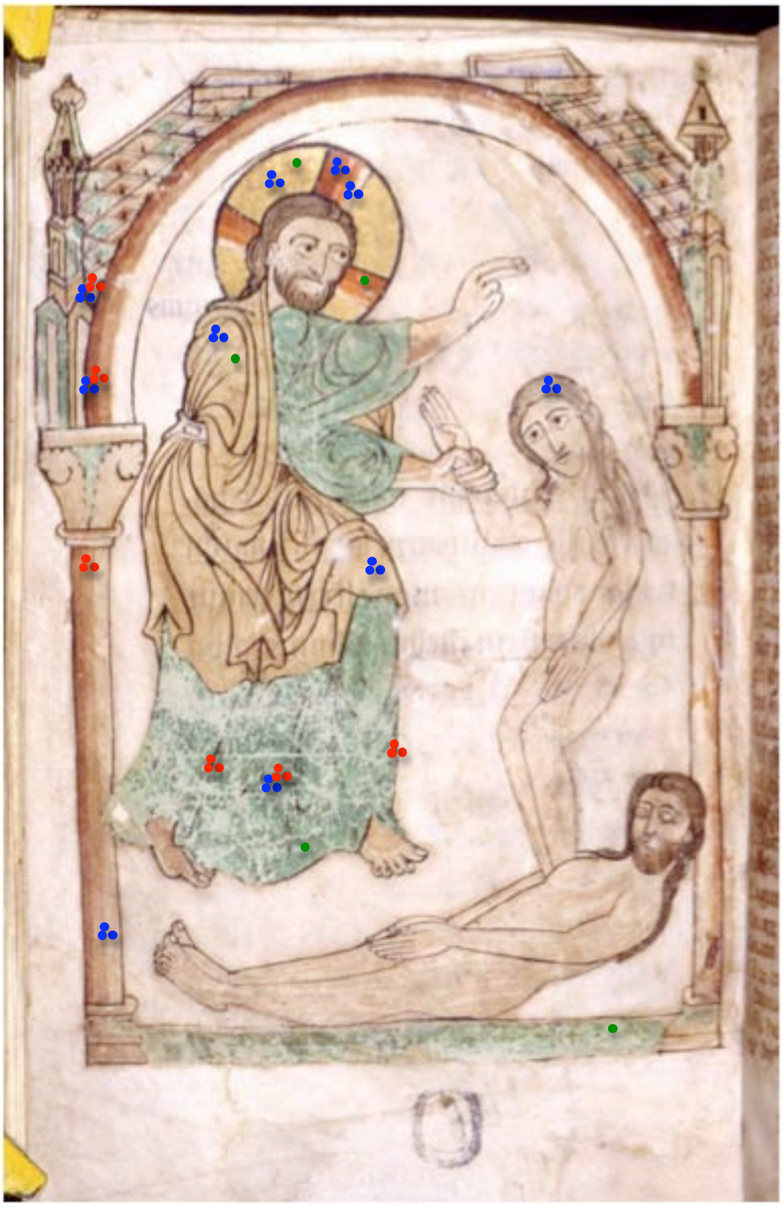


Figure I.43. *Book of Birds*, *f.* 95v

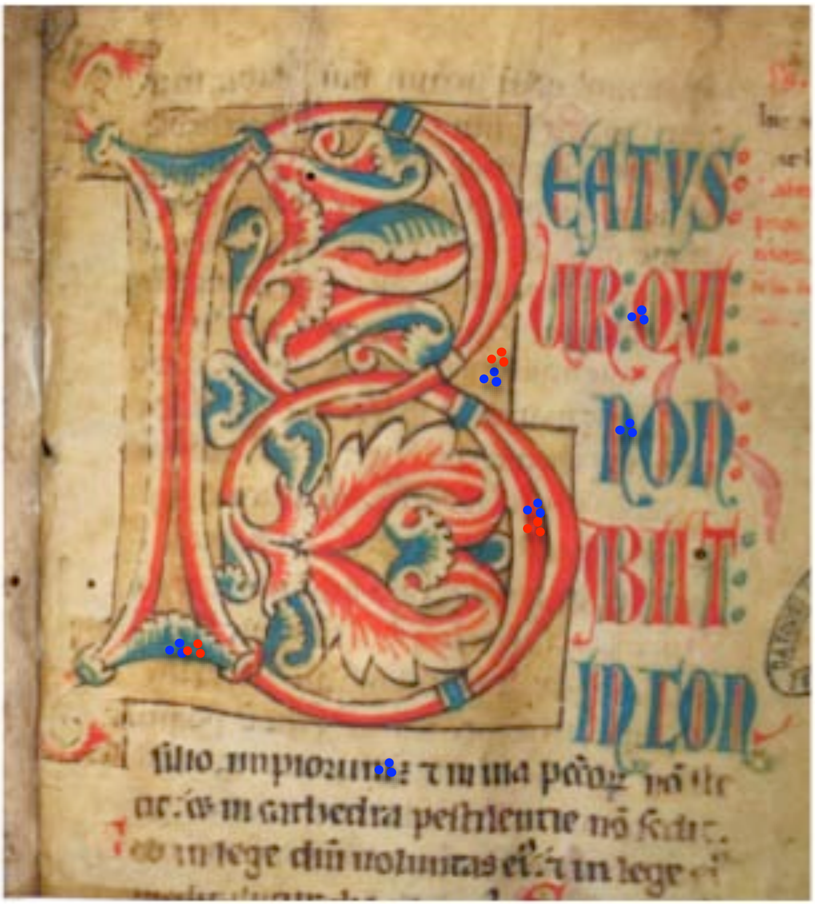


Figure I.44. *Lorvão 3, f.* 1

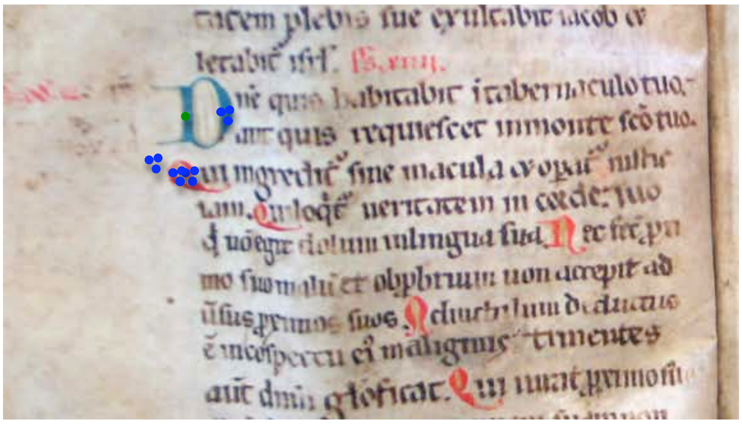


Figure I.45. *Lorvão 3, f.* 8v

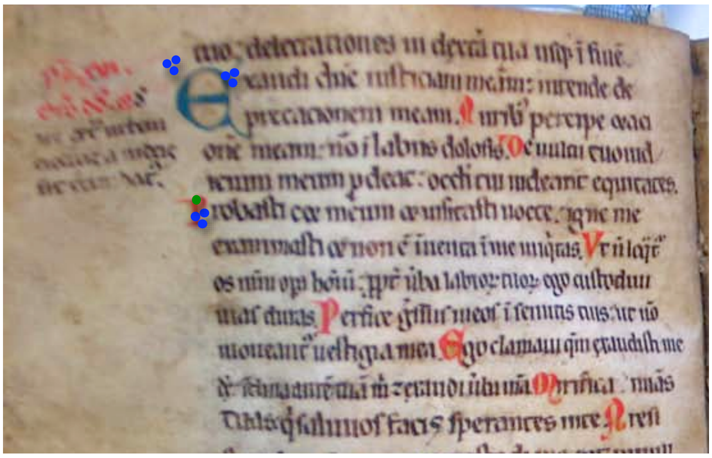


Figure I.46. *Lorvão 3, f.* 9v

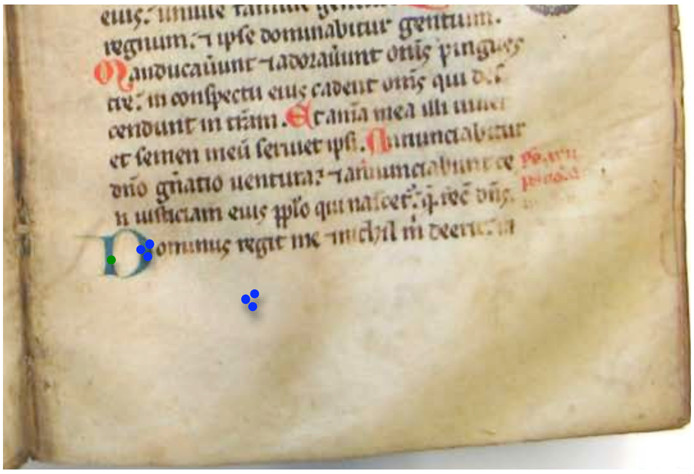


Figure I.47. *Lorvão 3, f.* 15

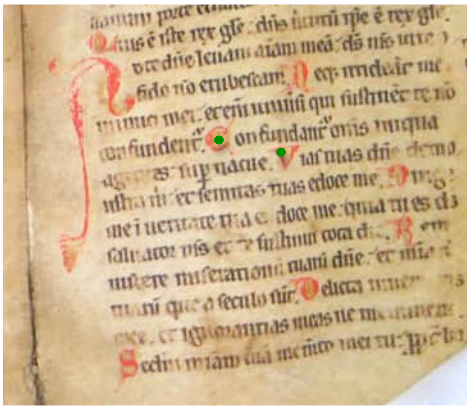


Figure I.48. *Lorvão 3, f.* 16

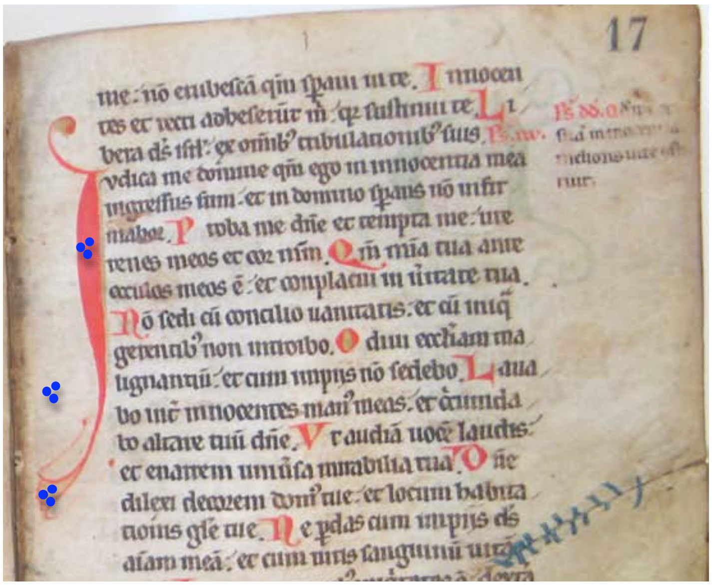


Figure I.49. *Lorvão 3, f.* 17

Figure I.50. *Lorvão 3, f.* 17v

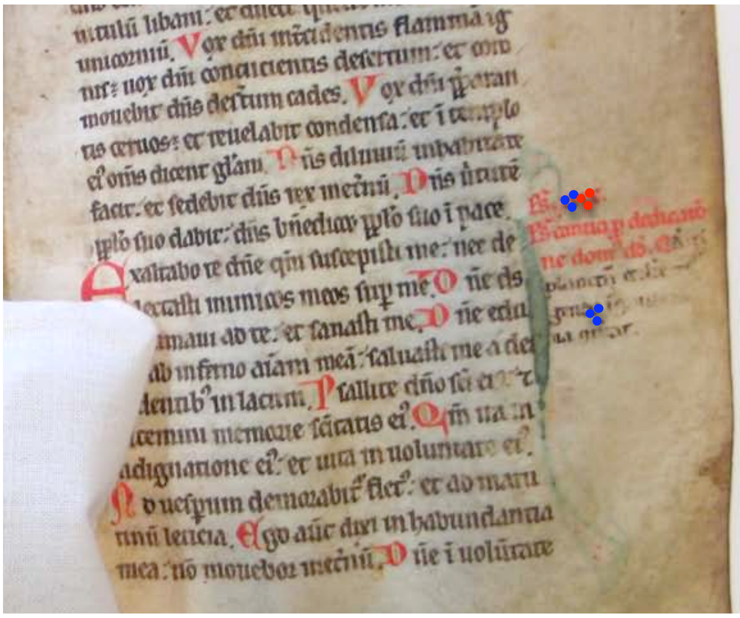


Figure I.51. *Lorvão 3, f.* 19

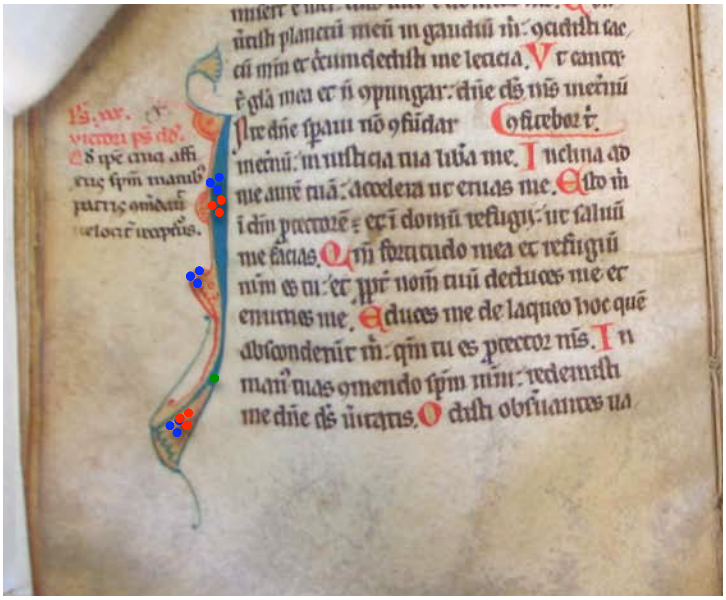


Figure I.52. *Lorvão 3, f.* 19v

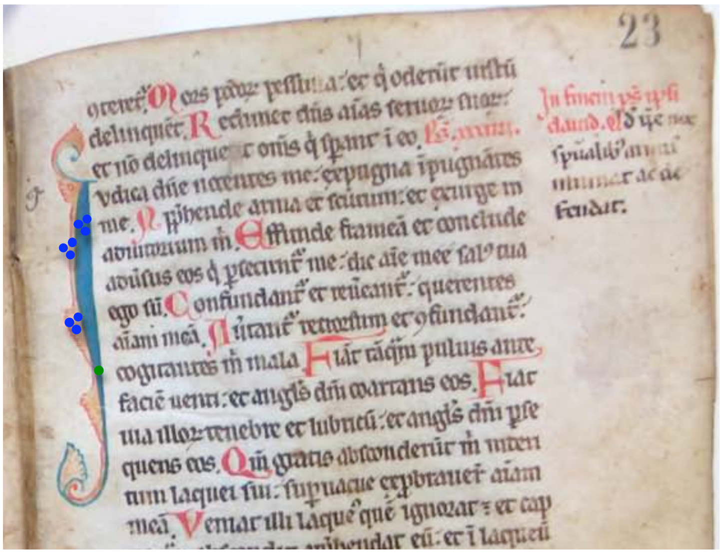


Figure I.53. *Lorvão 3, f.* 23

Figure I.54. *Lorvão 3, f.* 27

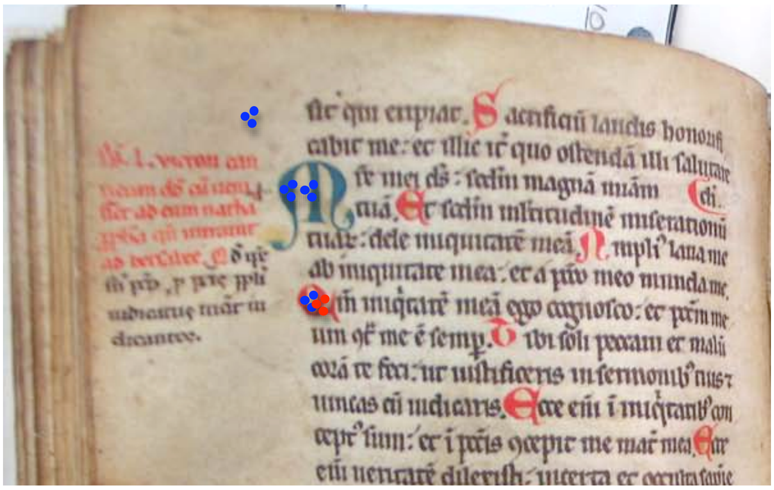


Figure I.55. *Lorvão 3, f.* 35v

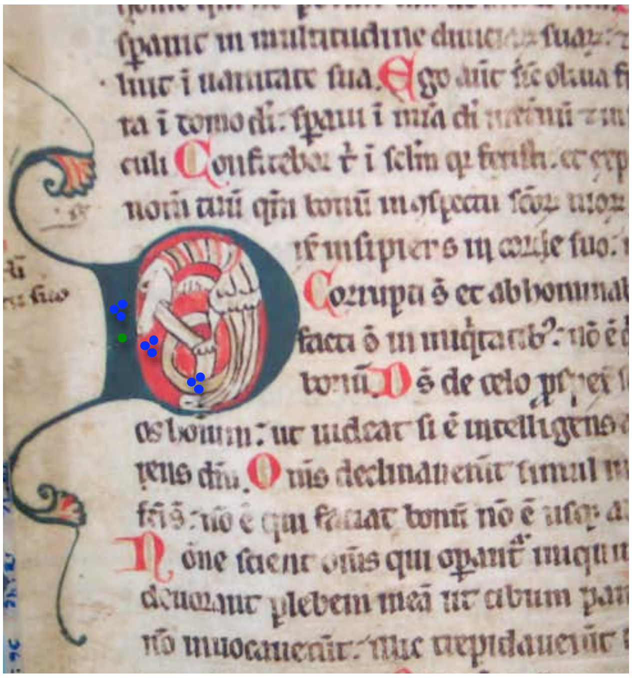


Figure I.56. *Lorvão 3, f.* 36v

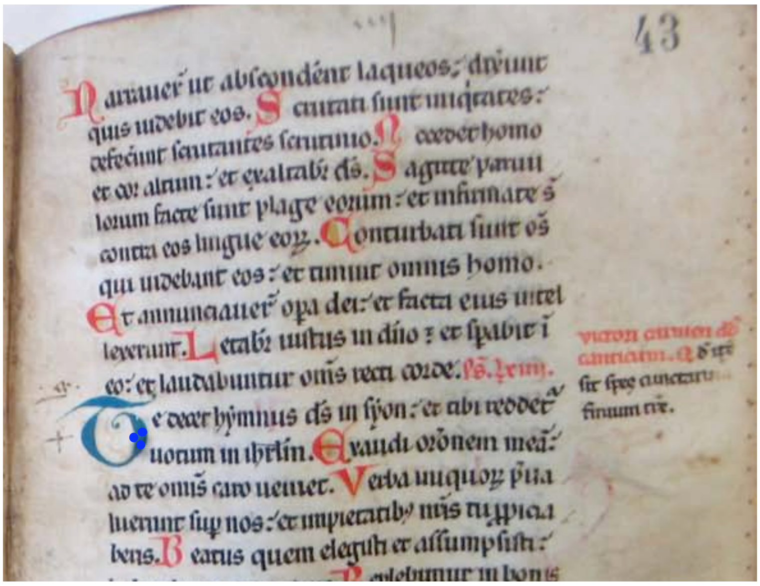


Figure I.57. *Lorvão 3, f.* 43

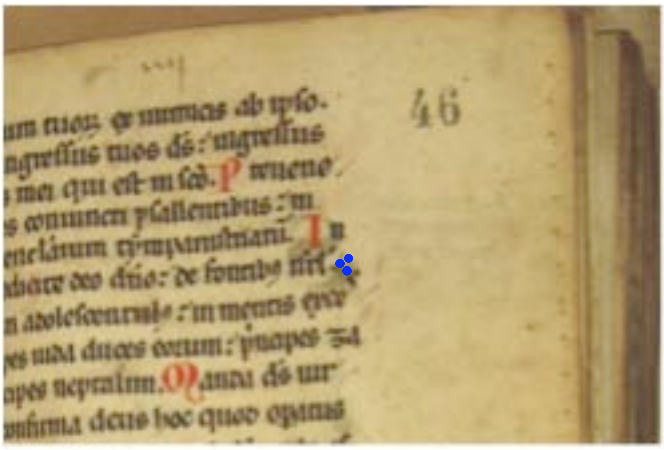


Figure I.58. *Lorvão 3, f.* 46

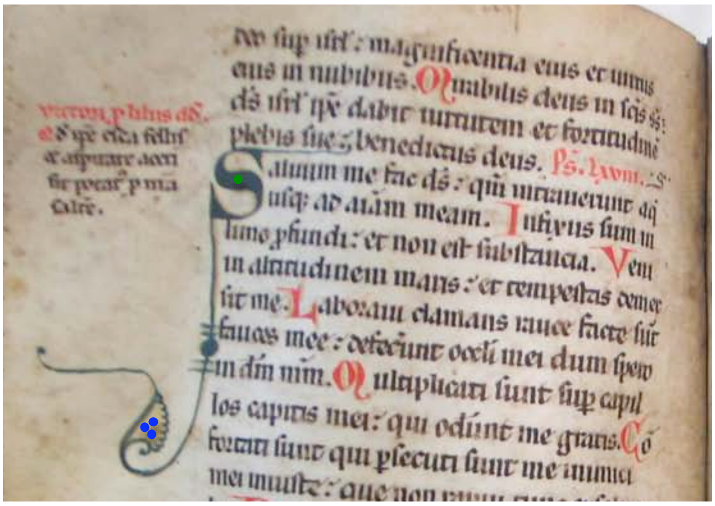


Figure I.59. *Lorvão 3, f.* 46v

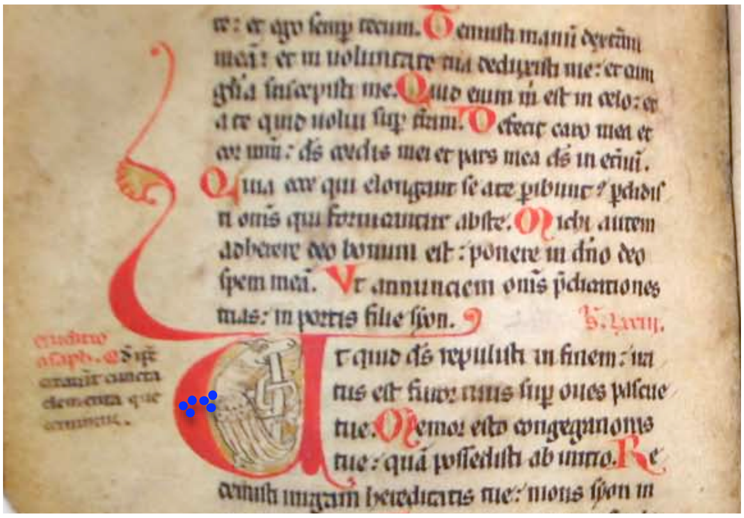


Figure I.60. *Lorvão 3, f.* 51v

Figure I.61. *Lorvão 3, f.* 54v

Figure I.62. *Lorvão 3, f.* 59v

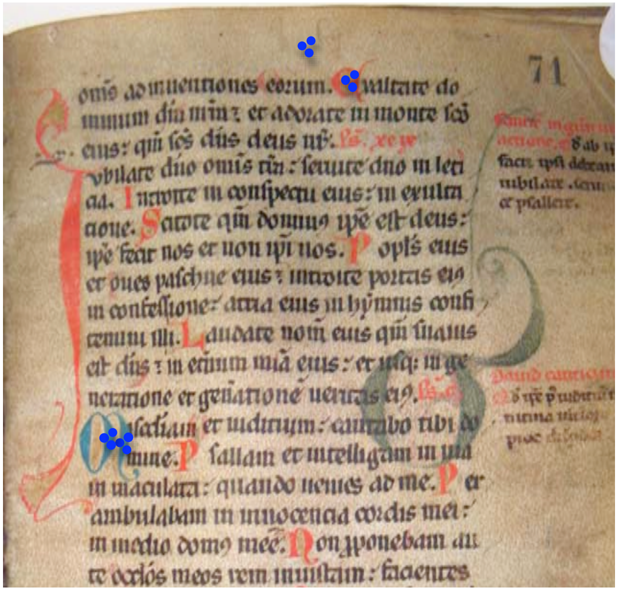


Figure I.63. *Lorvão 3, f.* 71

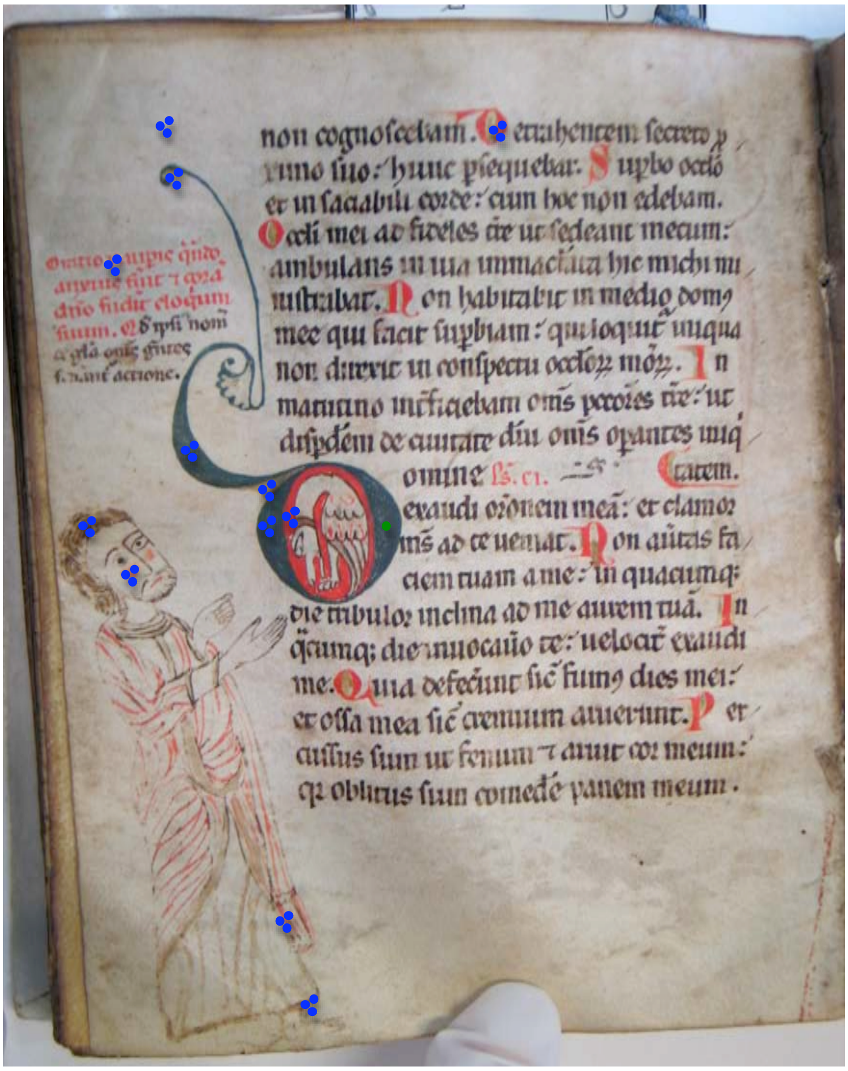


Figure I.64. *Lorvão 3, f.* 71v

Figure I.65. *Lorvão 3, f.* 82v

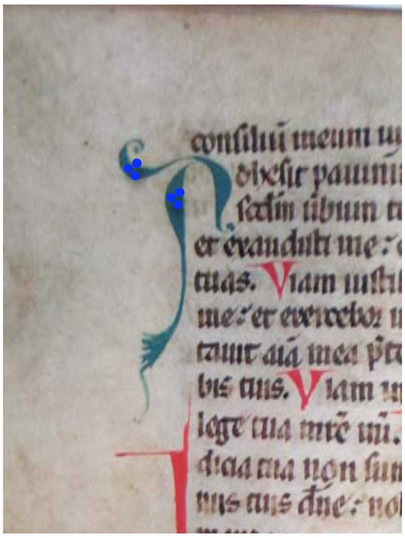


Figure I.66. *Lorvão 3, f.* 87v

Figure I.67. *Lorvão 3, f.* 98v

Figure I.68. *Lorvão 3, f.* 101v

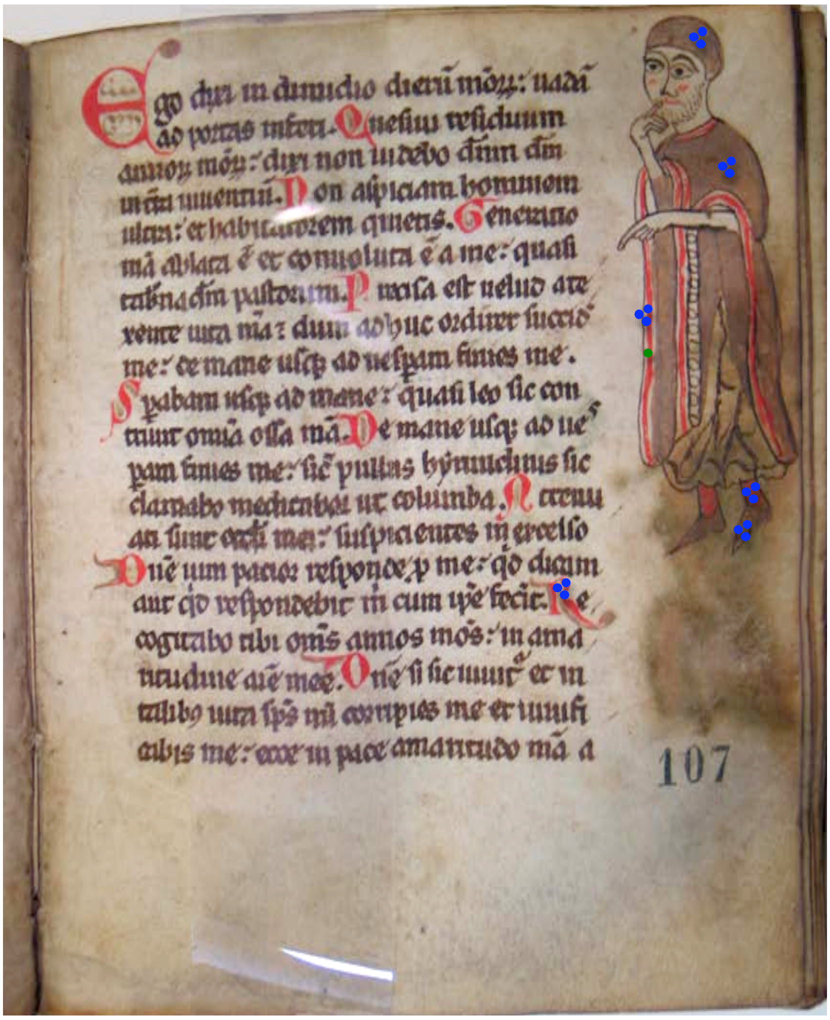


Figure I.69. *Lorvão 3, f.* 107

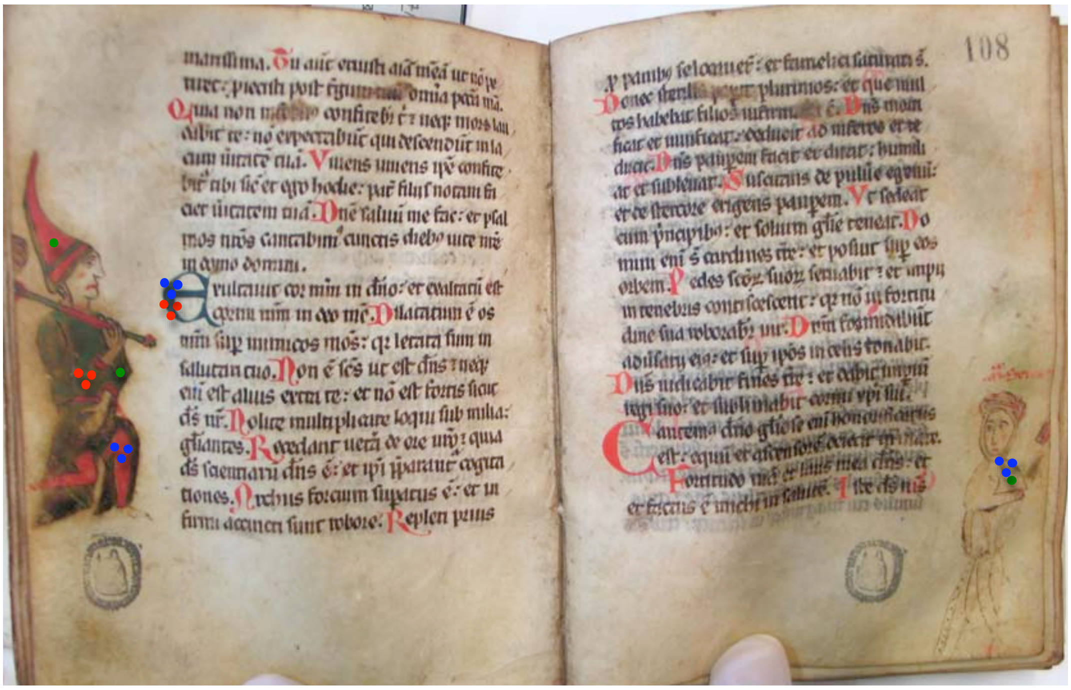


Figure I.70. *Lorvão 3, ff.* 107v-108

Figure I.71. *Lorvão 3, f.* 109v

Figure I.72. *Lorvão 3, f.* 110v

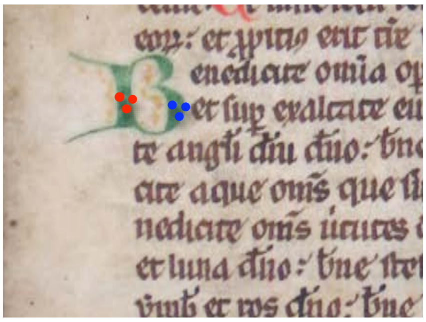


Figure I.73. *Lorvão 3, f.* 113

Figure I.74. *Lorvão 3, f.* 139v

Figure I.75. *Lorvão 3, f.* 146v

Figure I.76. *Lorvão 3, f.* 149

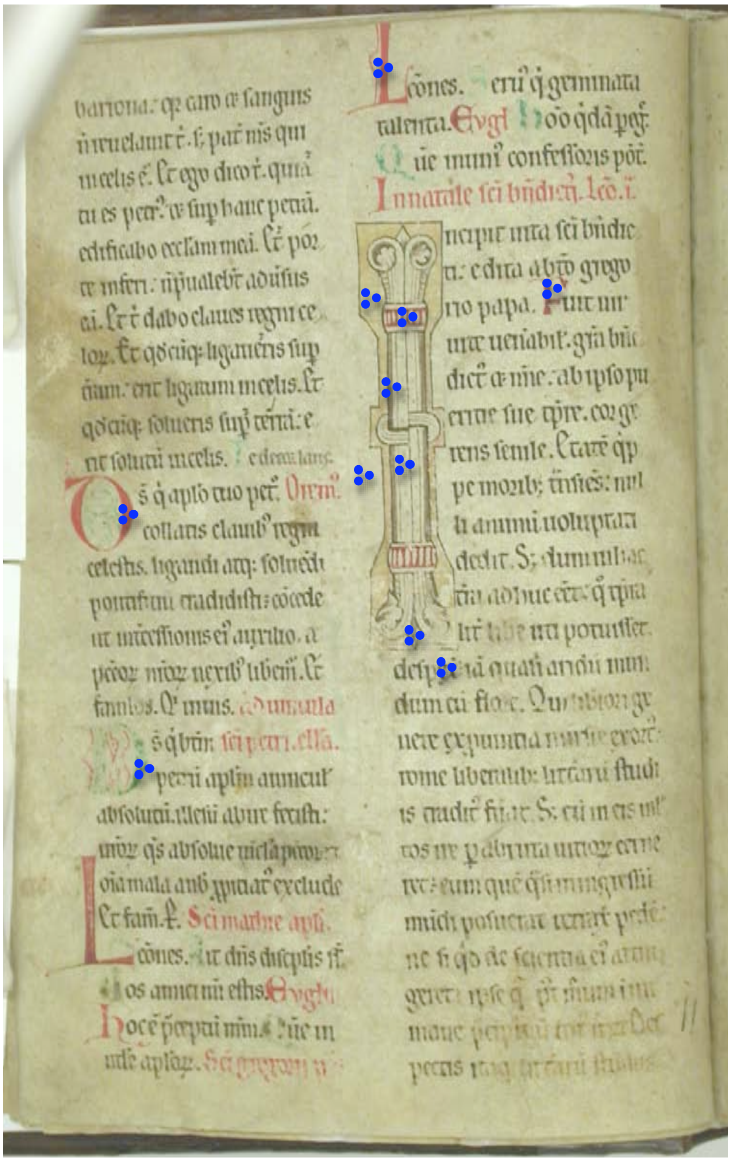


Figure I.77. *Lorvão 12*, *f.* 6v

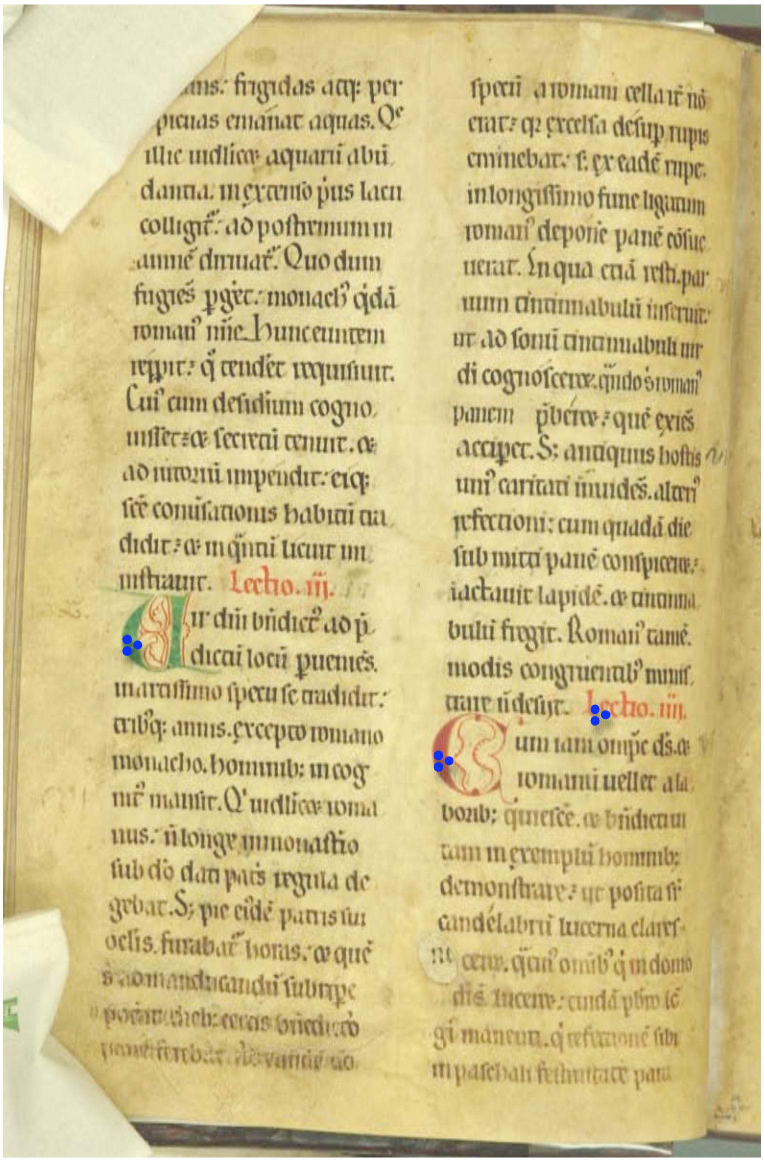


Figure I.78. *Lorvão 12*, *f.* 7v

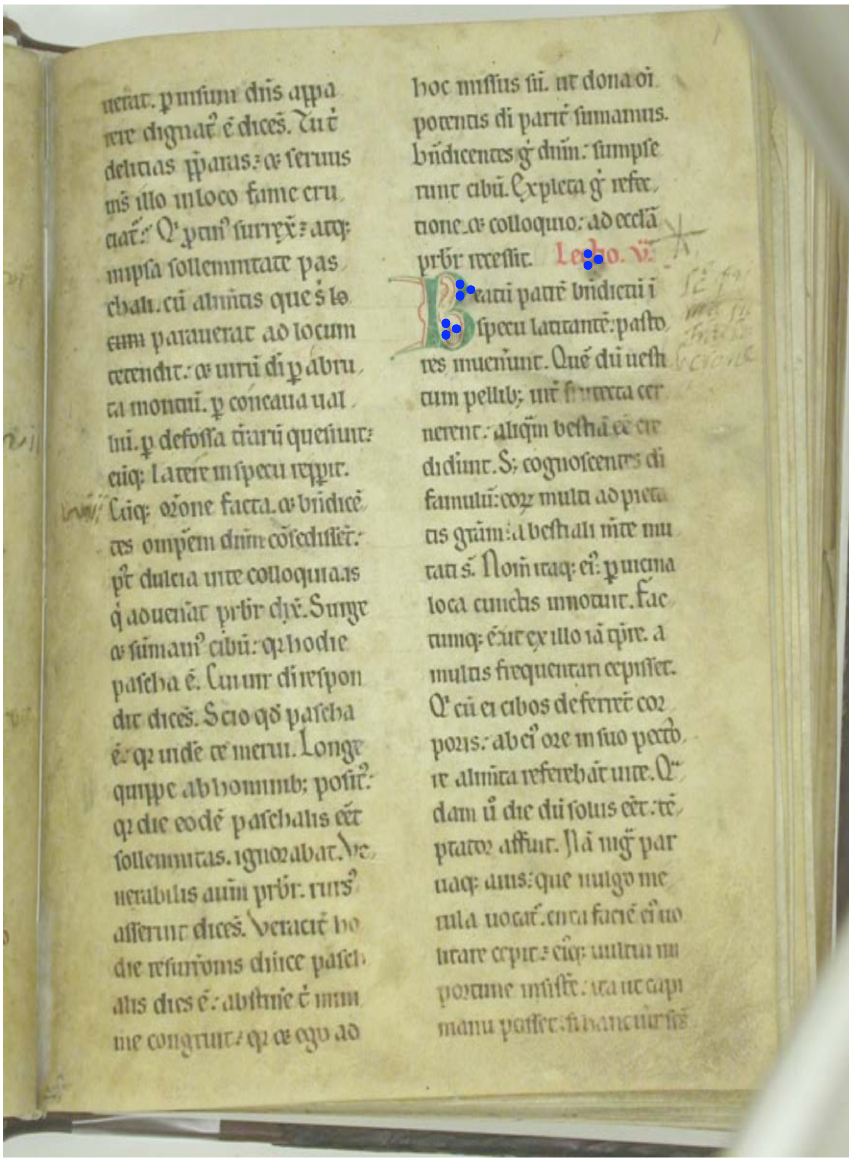


Figure I.79. *Lorvão 12*, *f.* 8

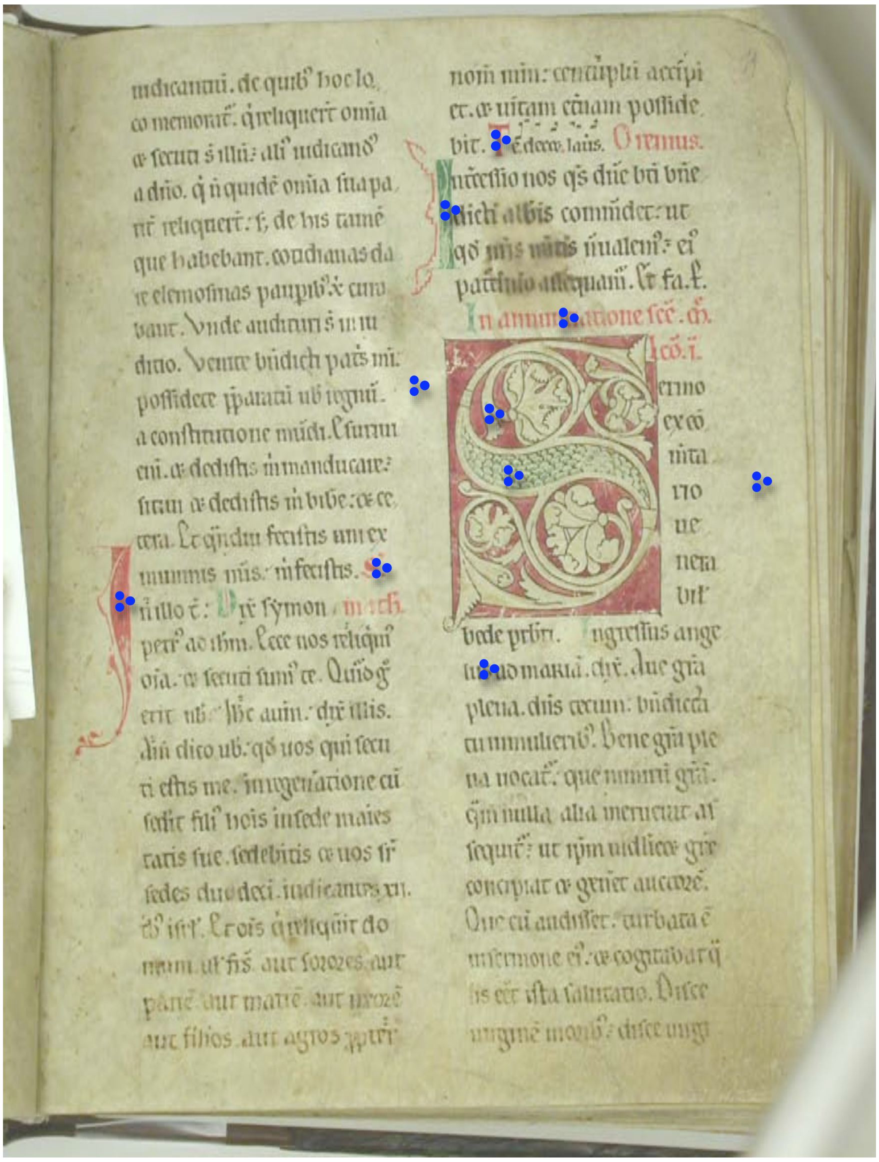


Figure I.80. *Lorvão 12*, *f.* 11

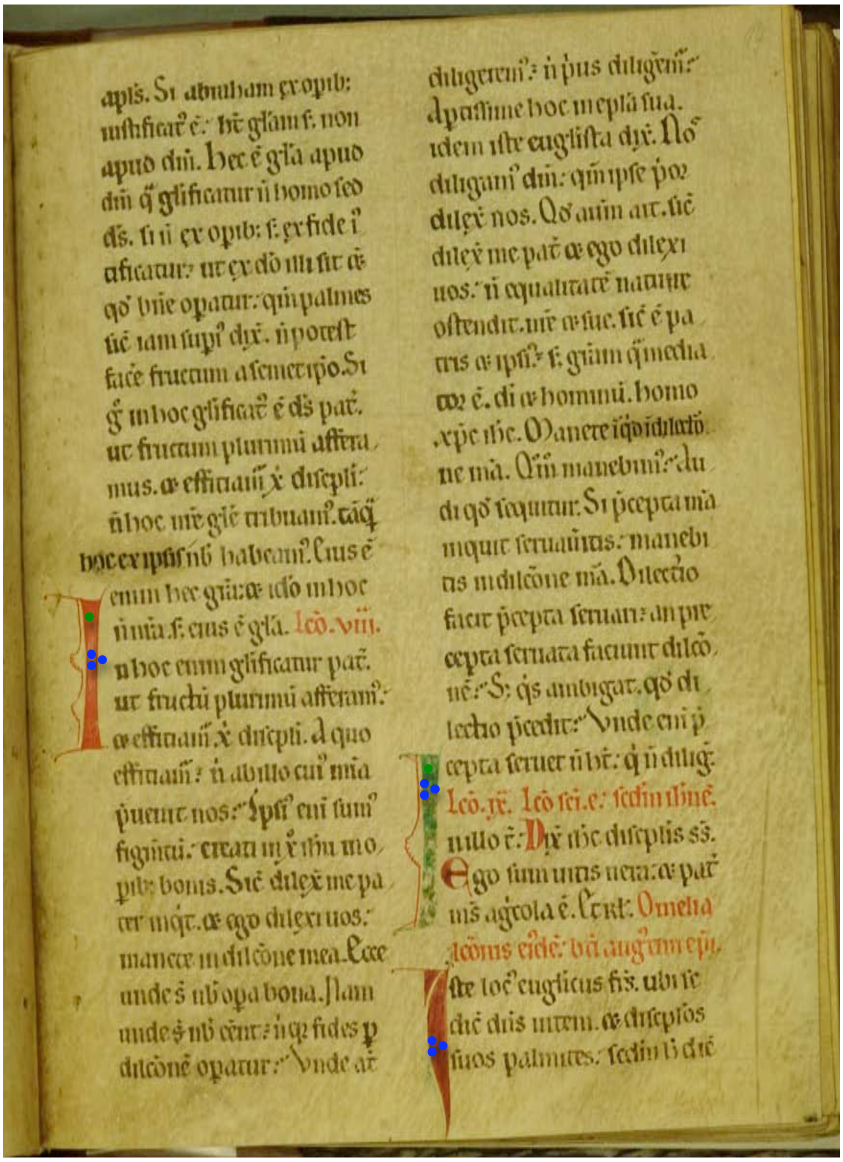


Figure I.81. *Lorvão 12*, *f.* 17

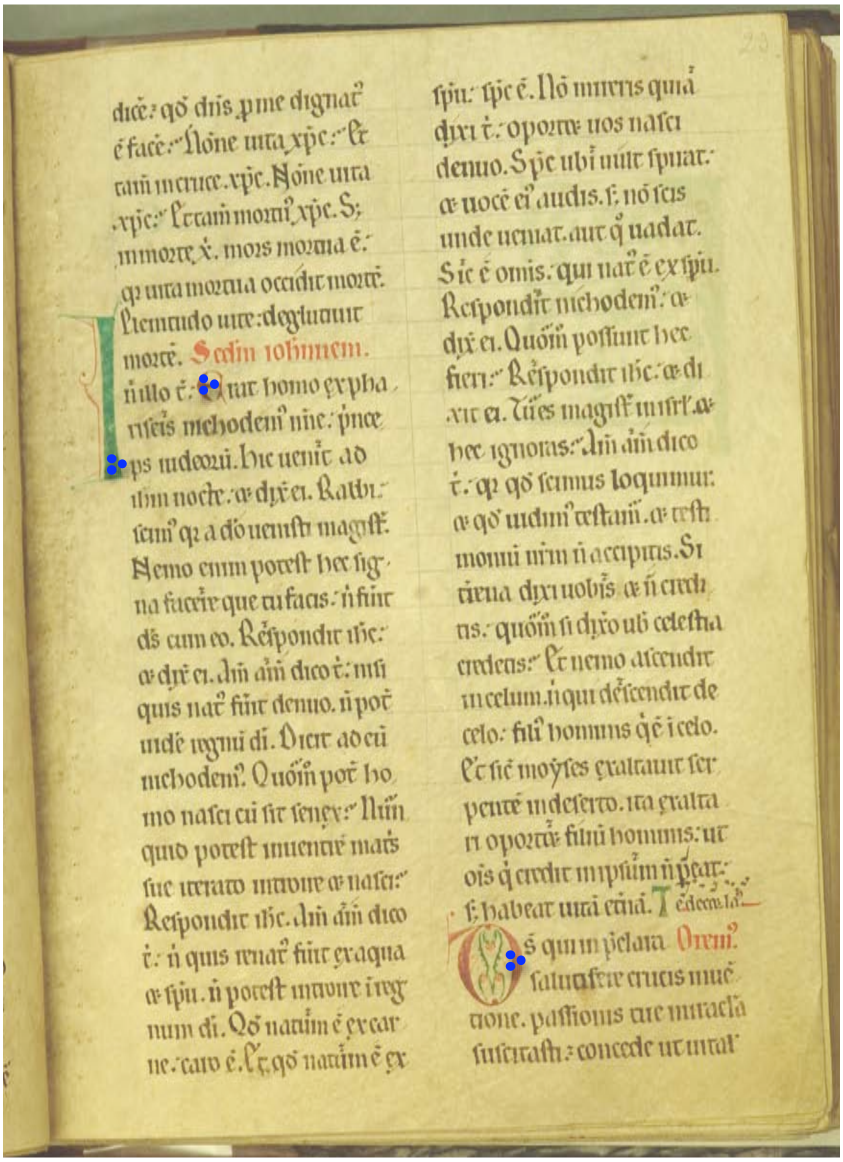


Figure I.82. *Lorvão 12*, *f.* 23v

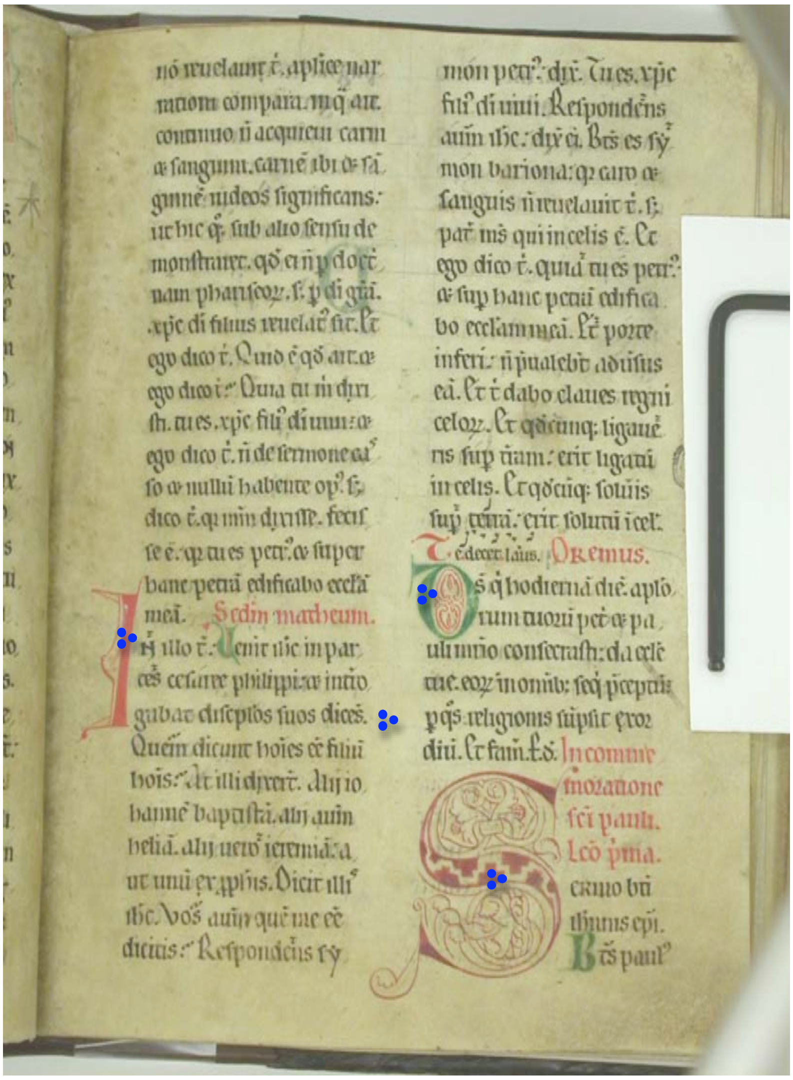


Figure I.83. *Lorvão 12*, *f.* 30

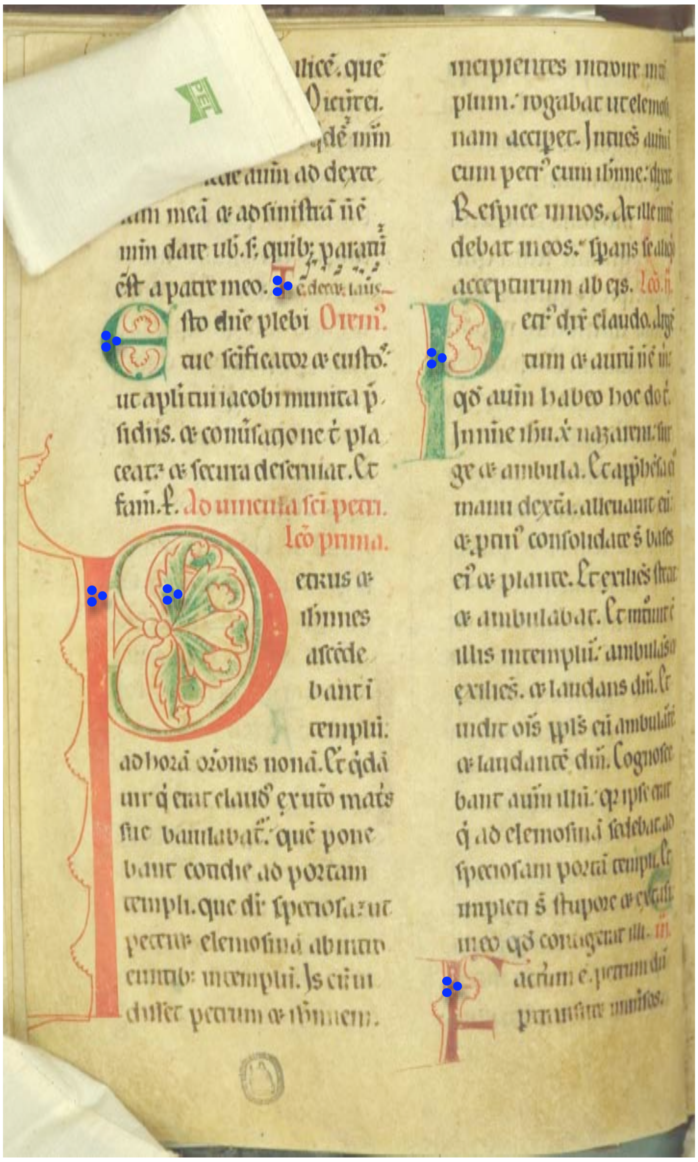


Figure I.84. *Lorvão 12*, *f.* 38v

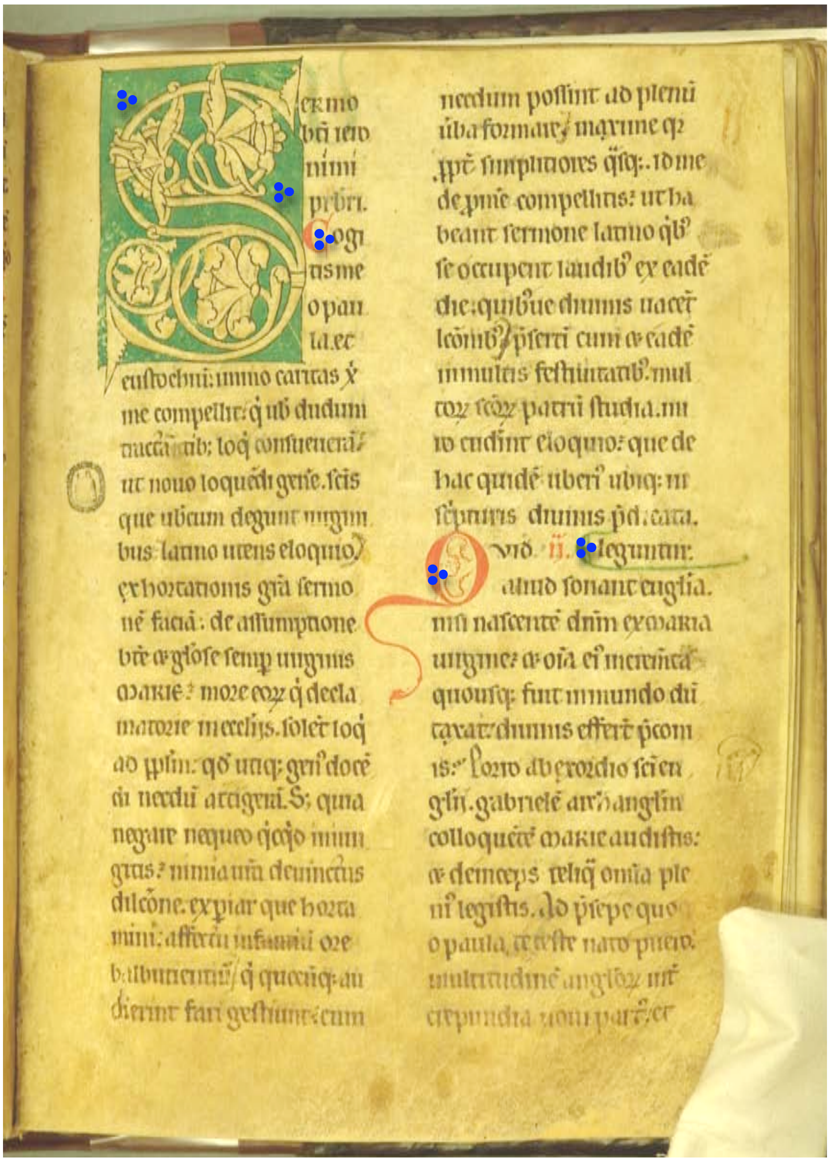


Figure I.85. *Lorvão 12*, *f.* 39

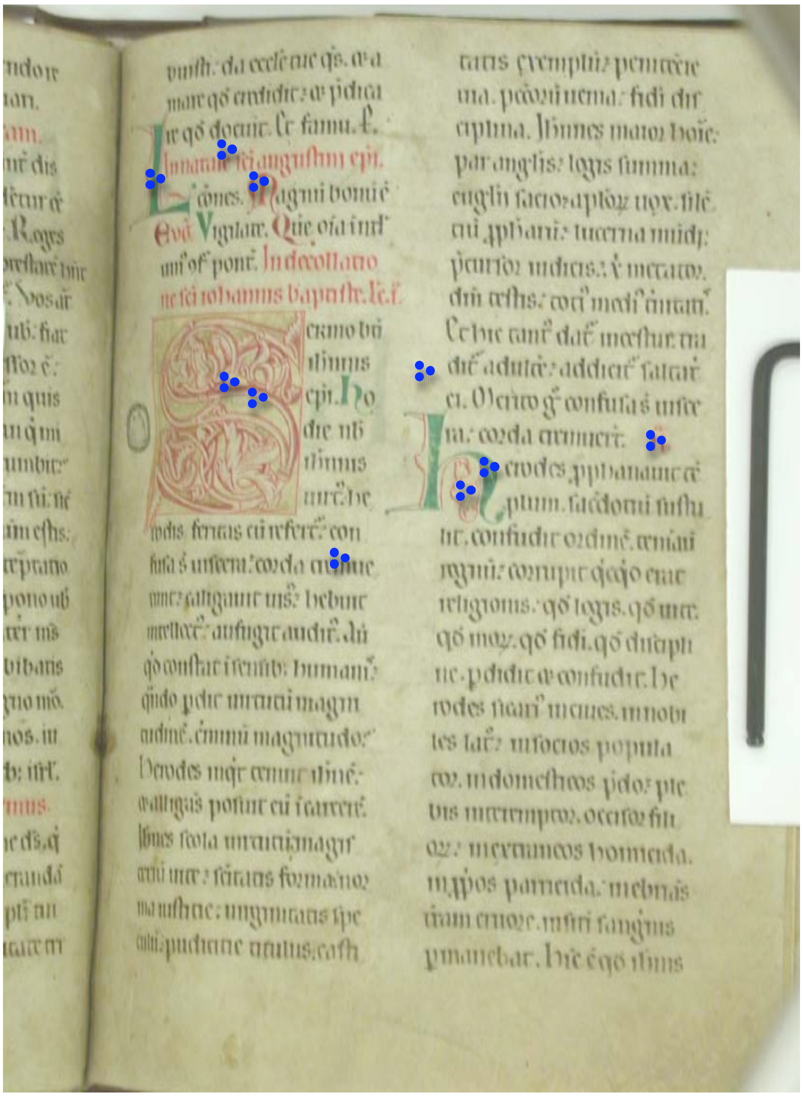


Figure I.86. *Lorvão 12*, *f.* 50

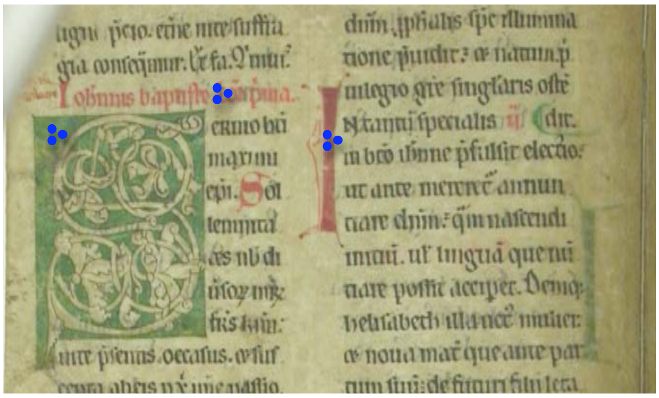


Figure I.87. *Lorvão 12*, *f.* 53v

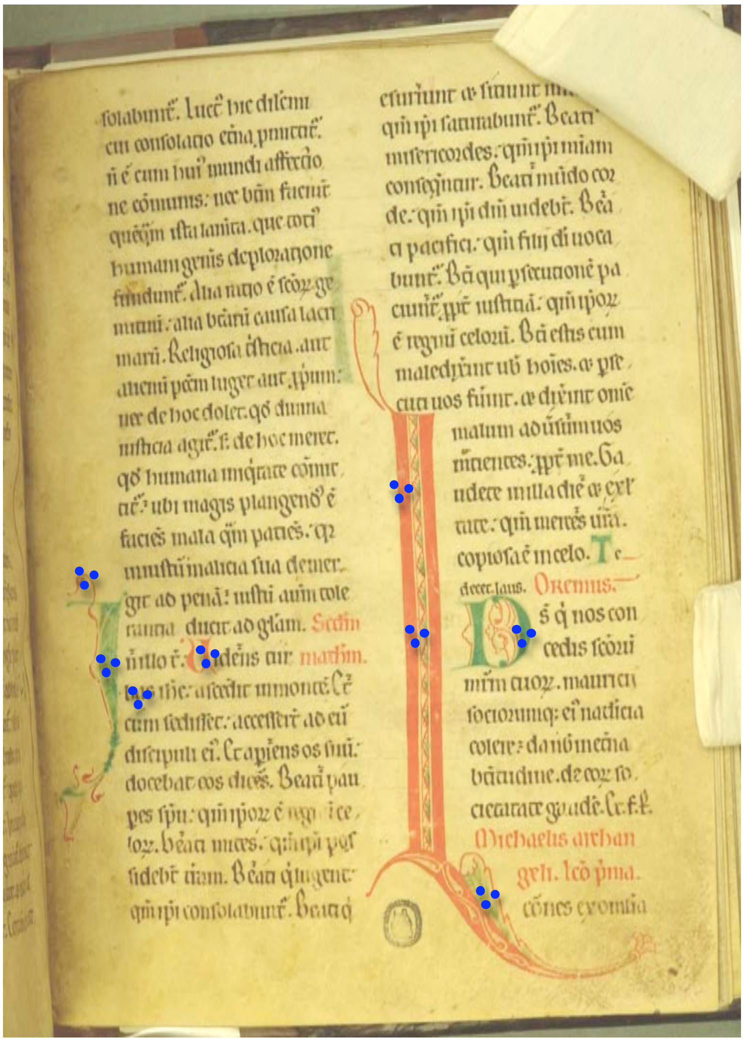


Figure I.88. *Lorvão 12*, *f.* 64

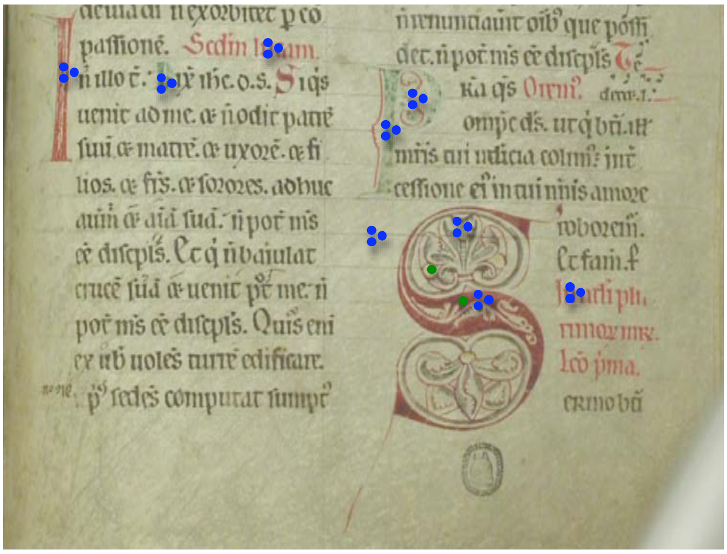


Figure I.89. *Lorvão 12*, *f.* 94

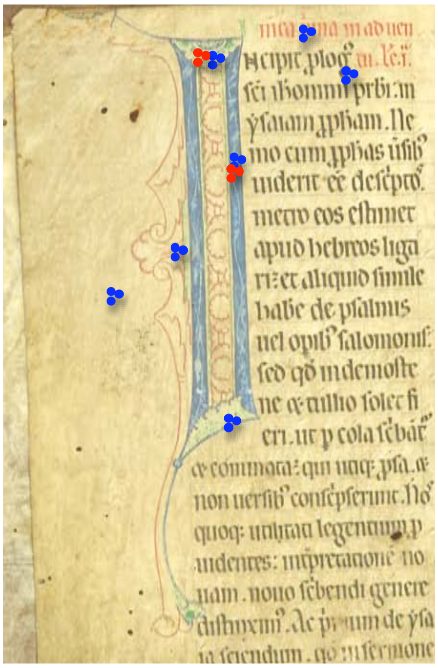


Figure I.90. *Lorvão 13*, *f.* 1v

Figure I.91. *Lorvão 13*, *f.* 6v

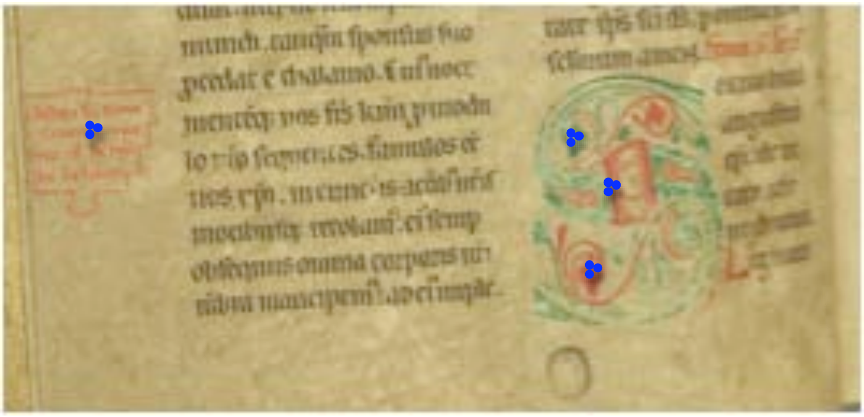


Figure I.92. *Lorvão 13*, *f.* 19v

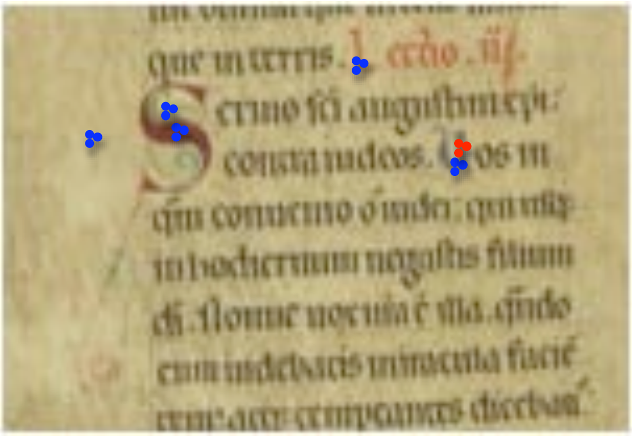


Figure I.93. *Lorvão 13*, *f.* 21v

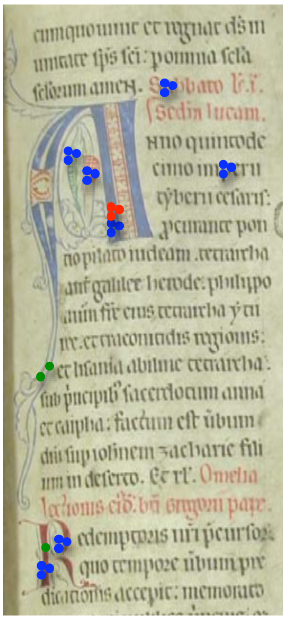


Figure I.94. *Lorvão 13*, *f.* 30

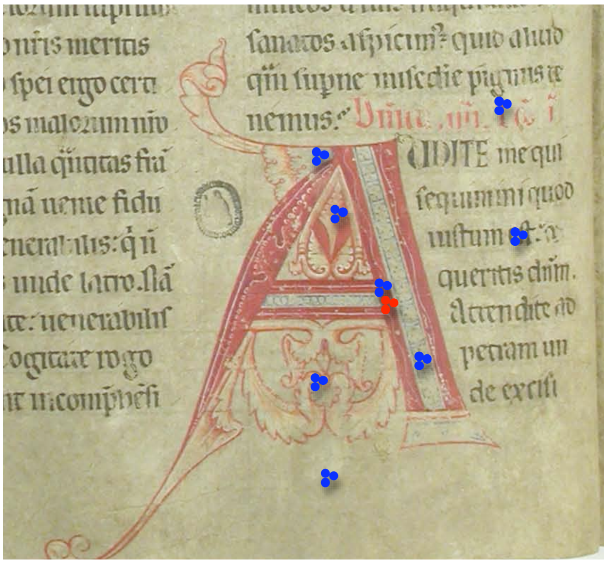


Figure I.95. *Lorvão 13*, *f.* 35v

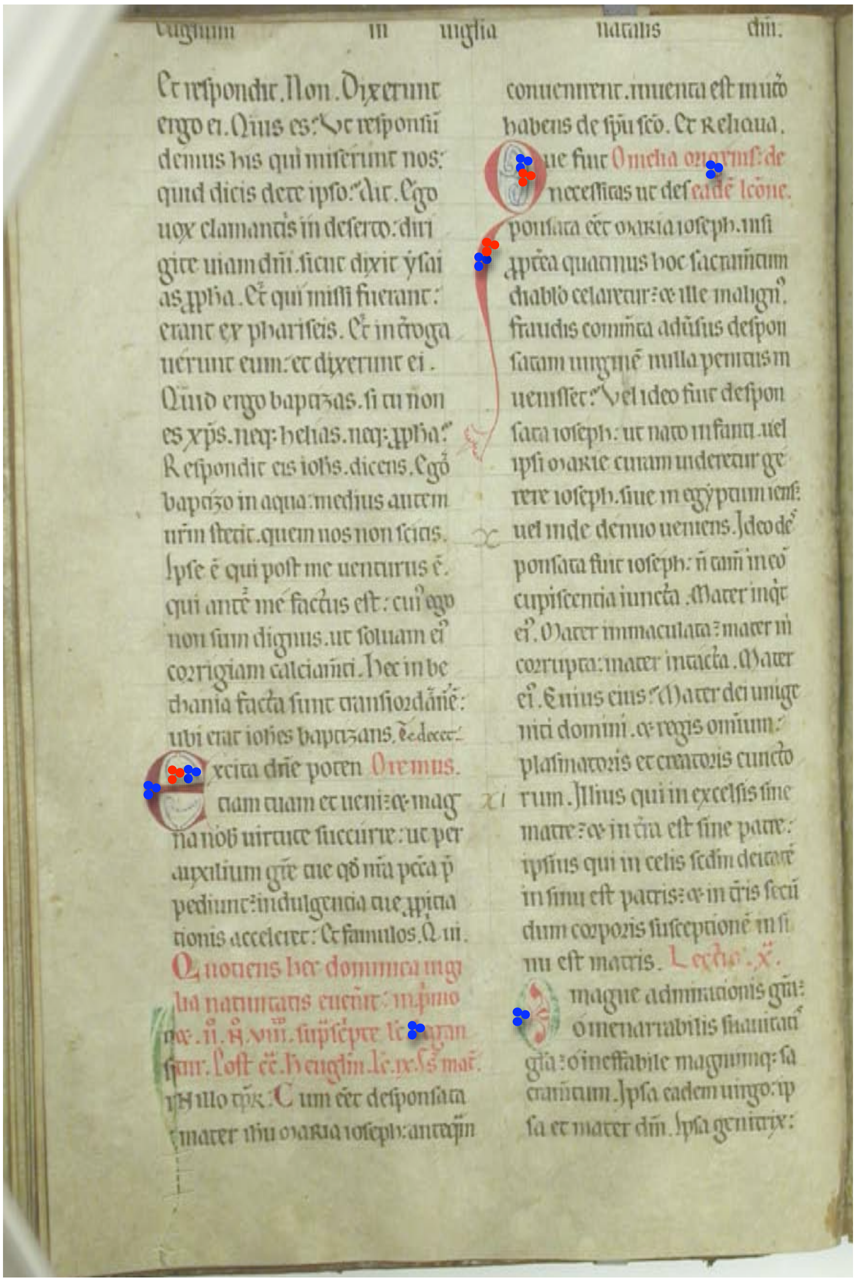


Figure I.96. *Lorvão 13*, *f.* 39v

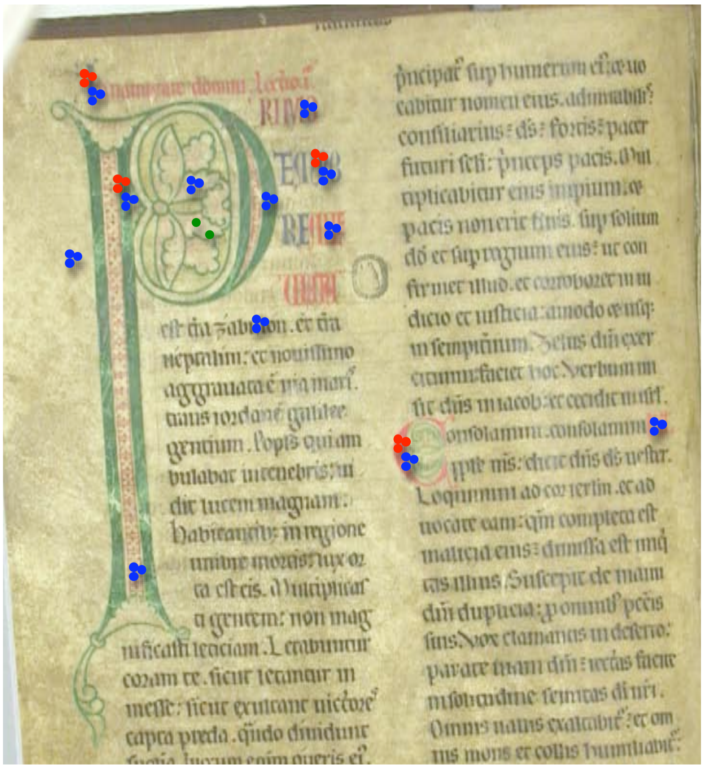


Figure I.97. *Lorvão 13*, *f.* 44v

Figure I.98. *Lorvão 13*, *f.* 63v

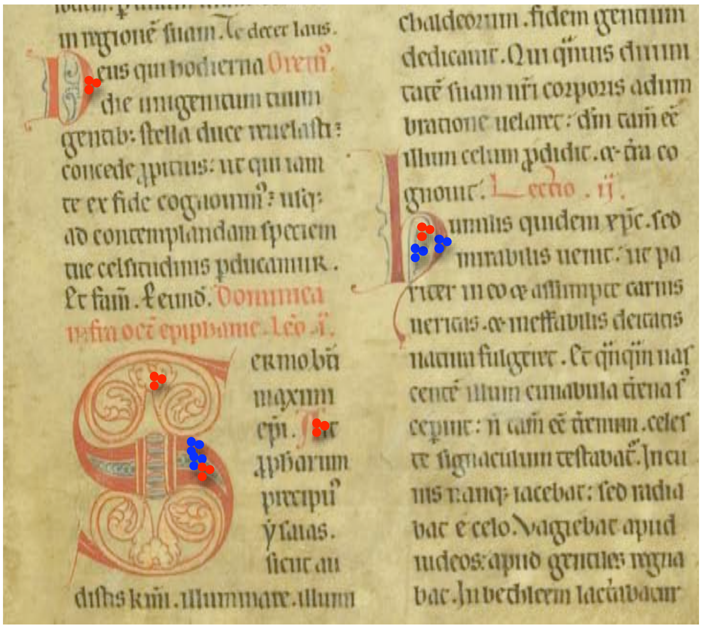


Figure I.99. *Lorvão 13*, *f.* 73

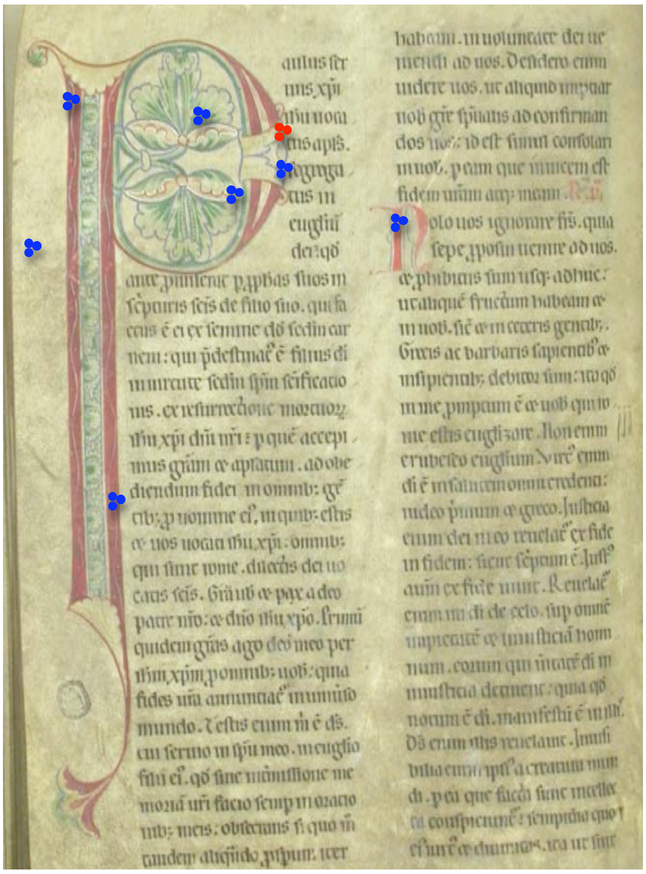


Figure I.100. *Lorvão 13*, *f.* 80v

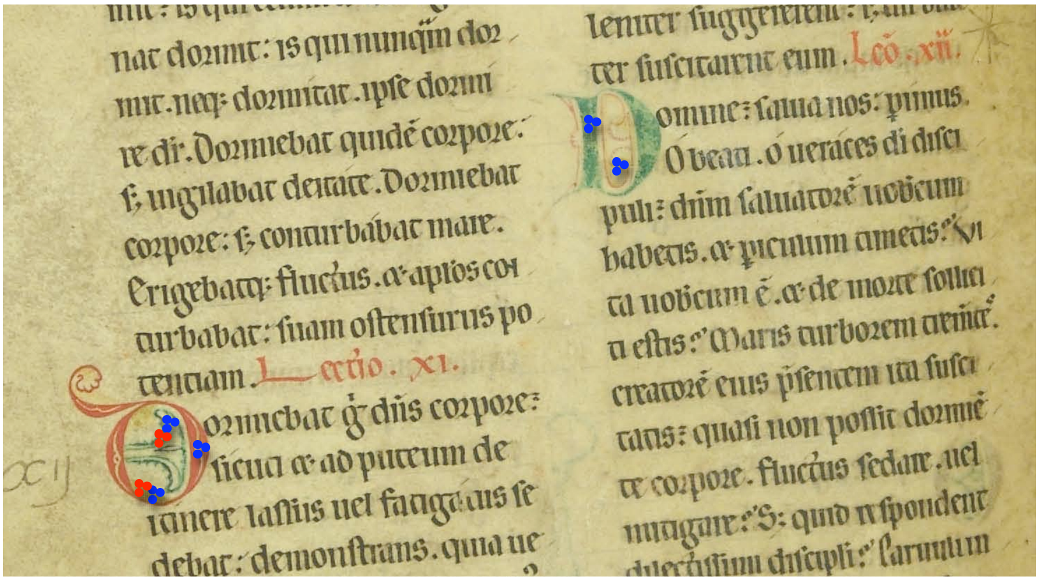


Figure I.101. *Lorvão 13*, *f.* 92v

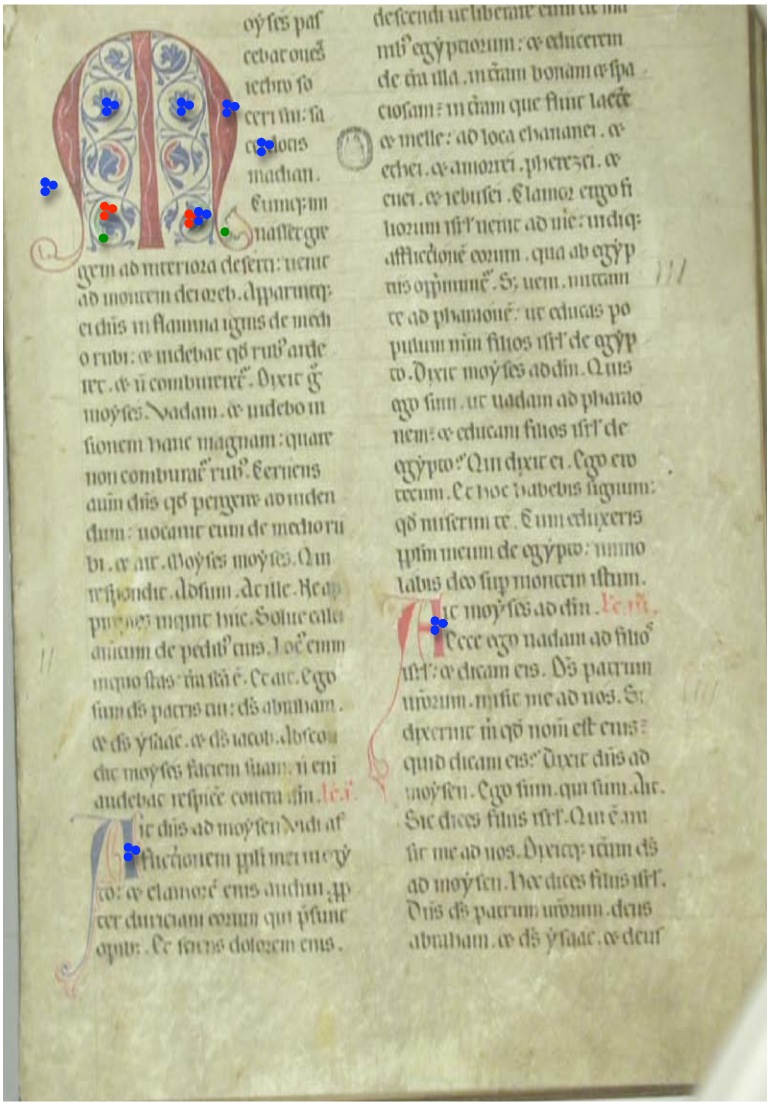


Figure I.102. *Lorvão 13*, *f.* 114

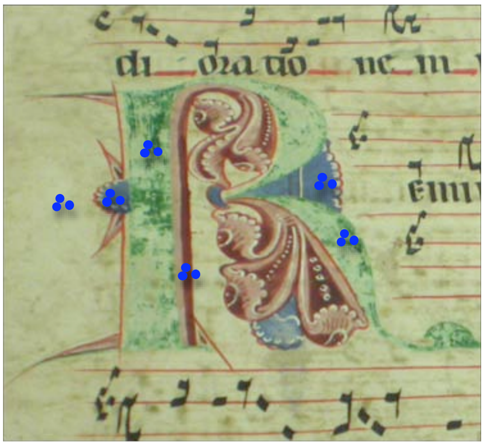



Figure I.103. *Lorvão 15*, *f.* 5v

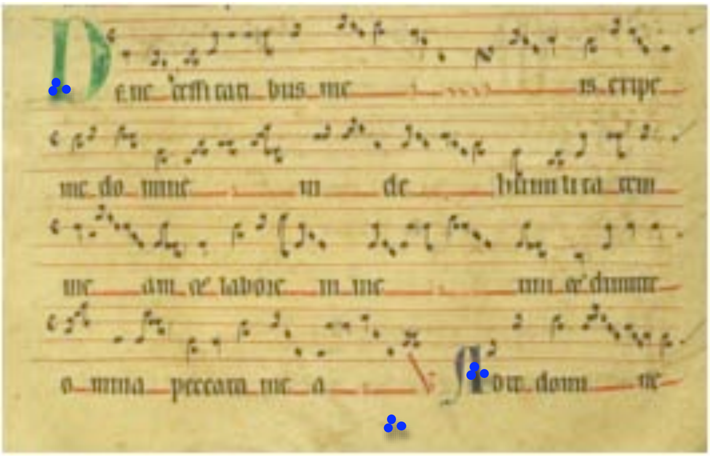



Figure I.104. *Lorvão 15*, *f.* 6

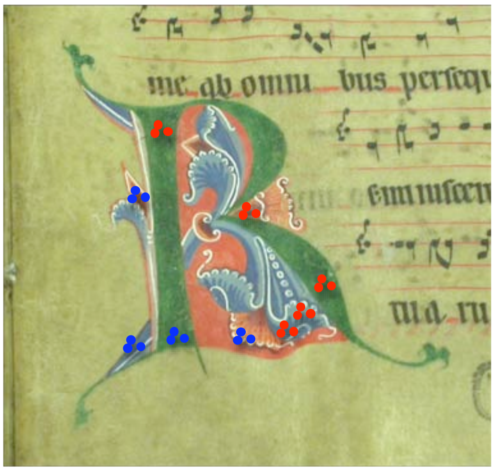



Figure I.105. *Lorvão 15*, *f.* 11

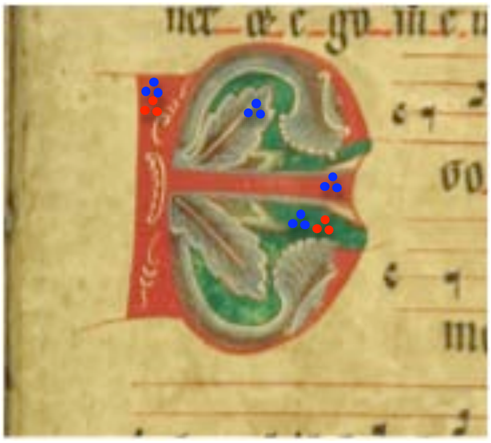



Figure I.106. *Lorvão 15*, *f.* 16

Figure I.107. *Lorvão 15*, *f.* 26

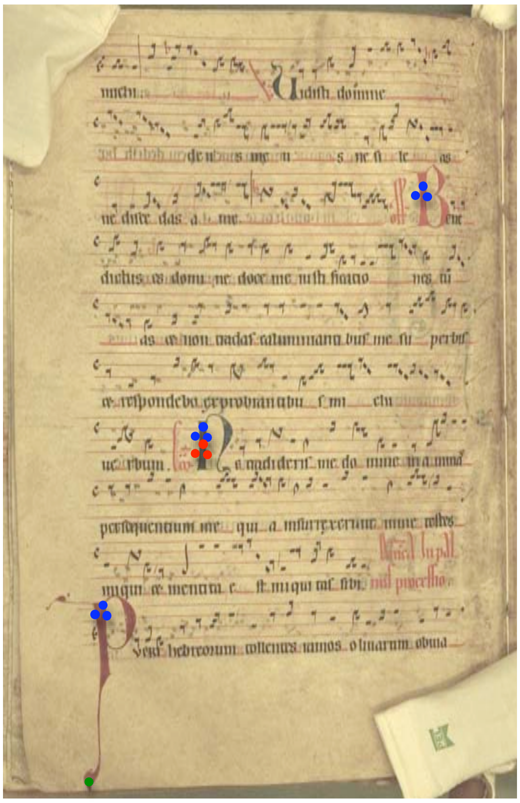



Figure I.108. *Lorvão 15*, *f.* 36v

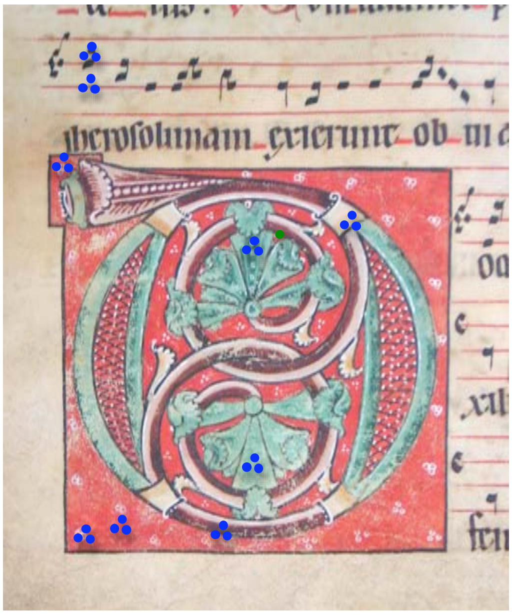



Figure I.109. *Lorvão 15*, *f.* 38v

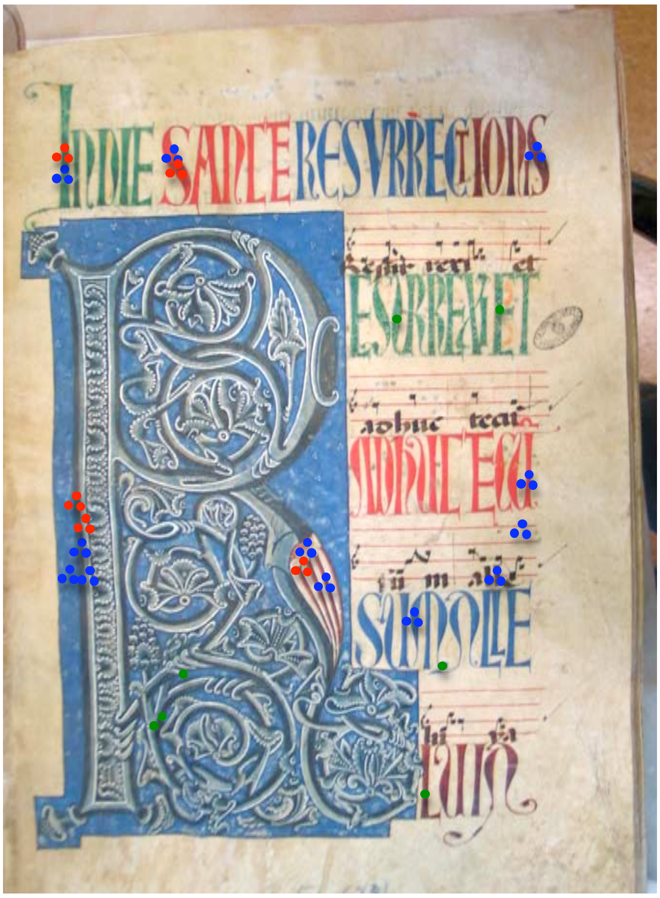



Figure I.110. *Lorvão 15*, *f.* 50

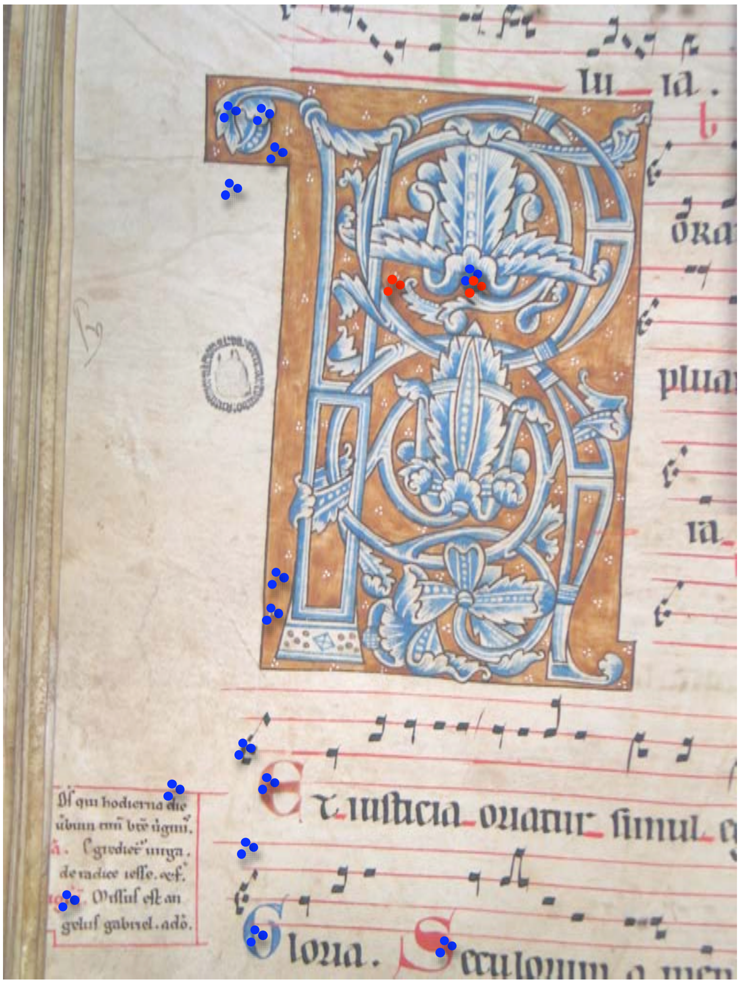



Figure I.111. *Lorvão 15*, *f.* 125v

Figure I.112. *Lorvão 15*, *f.* 154v

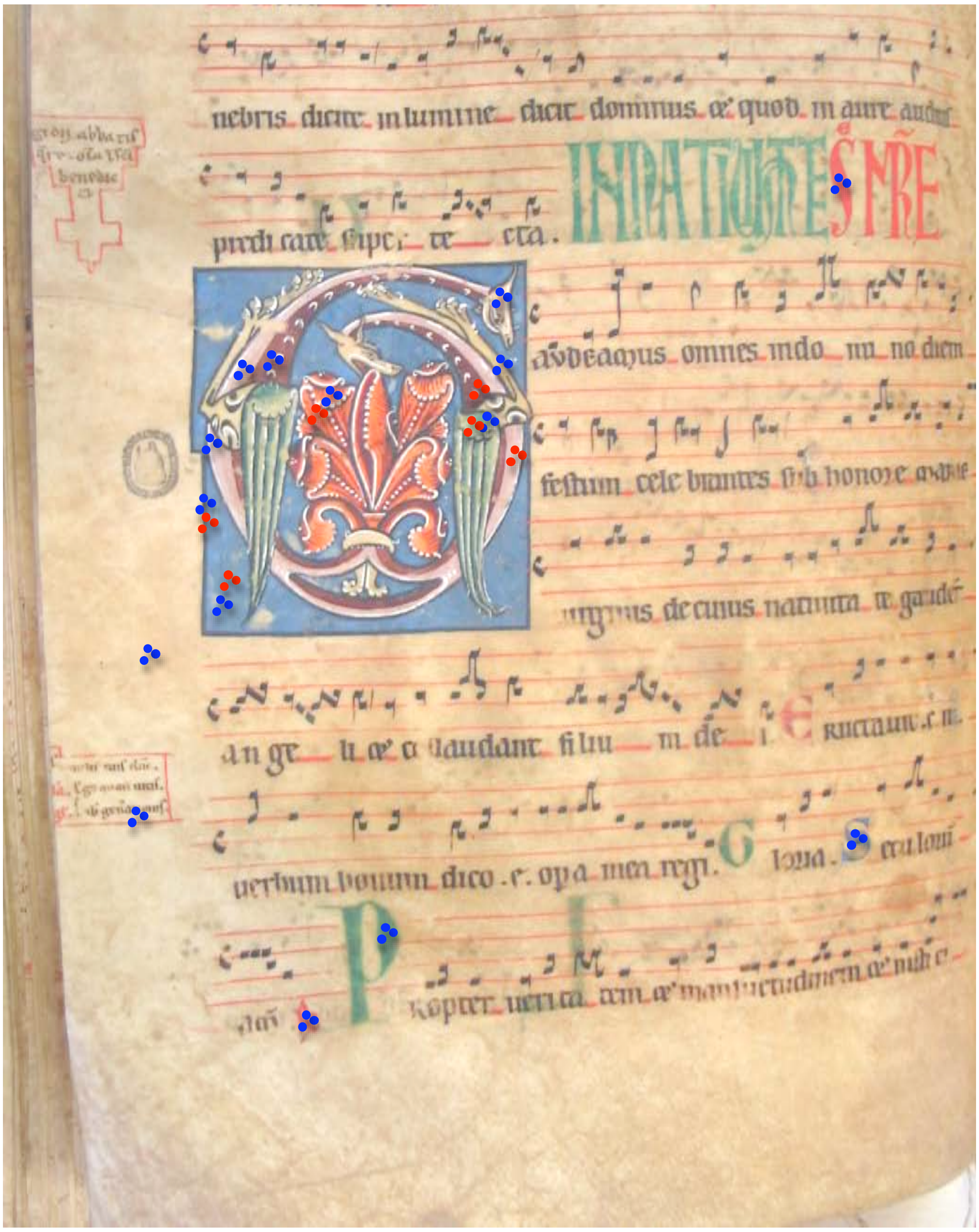



Figure I.113. *Lorvão 15*, *f.* 167v

Figure I.114 *Lorvão 16*, *f.* 2v

Figure I.115. *Lorvão 16*, *f.* 7v

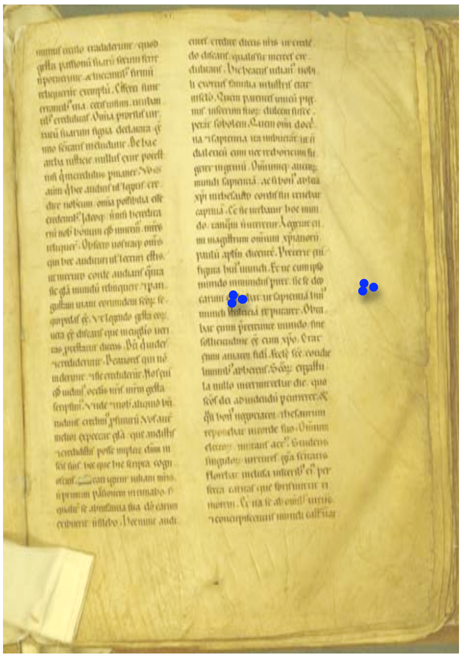


Figure I.116. *Lorvão 16*, *f.* 8

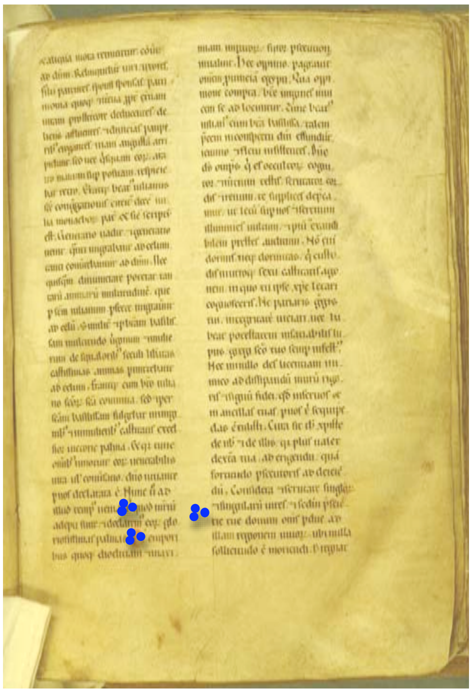


Figure I.117. *Lorvão 16*, *f.* 11

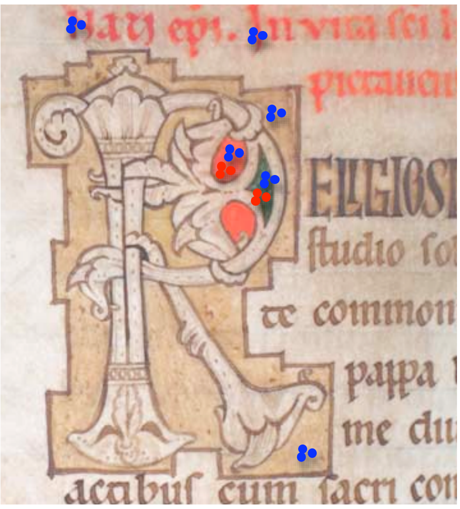



Figure I.118. *Lorvão 16*, *f.* 24v

Figure I.119. *Lorvão 16*, *f.* 32

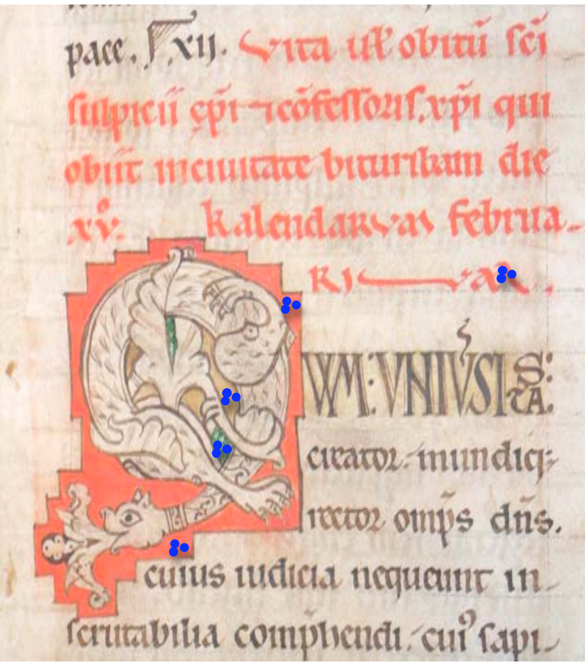


Figure I.120. *Lorvão 16*, *f.* 34

Figure I.121. *Lorvão 16*, *f.* 35v

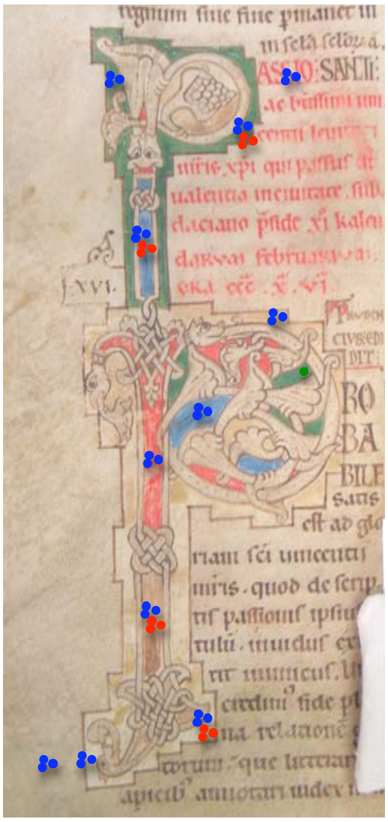


Figure I.122. *Lorvão 16*, *f.* 59v

Figure I.123. *Lorvão 16*, *f.* 71v

Figure I.124. *Lorvão 16*, *f.* 73

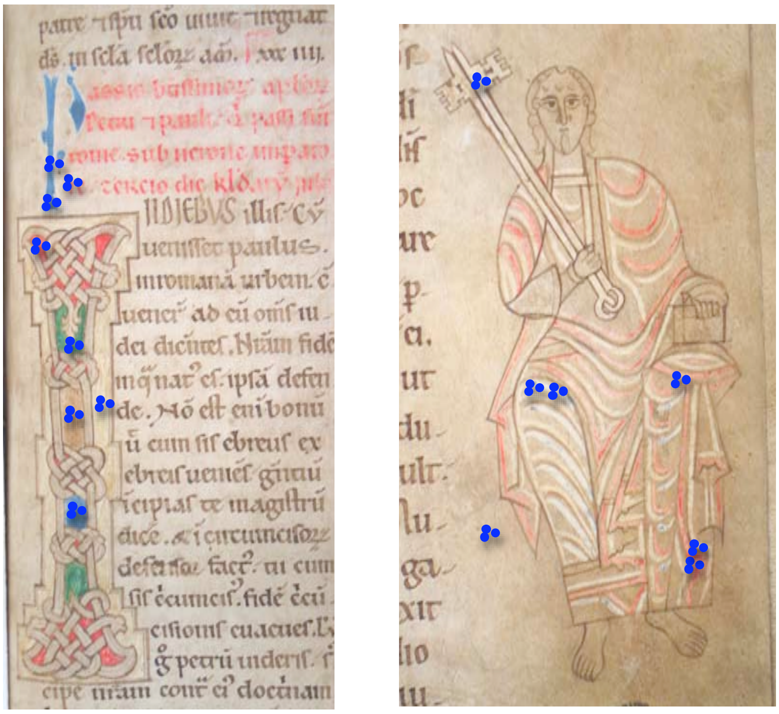


Figure I.125. *Lorvão 16*, *f.* 126

Figure I.126. *Lorvão 16*, *f.* 155

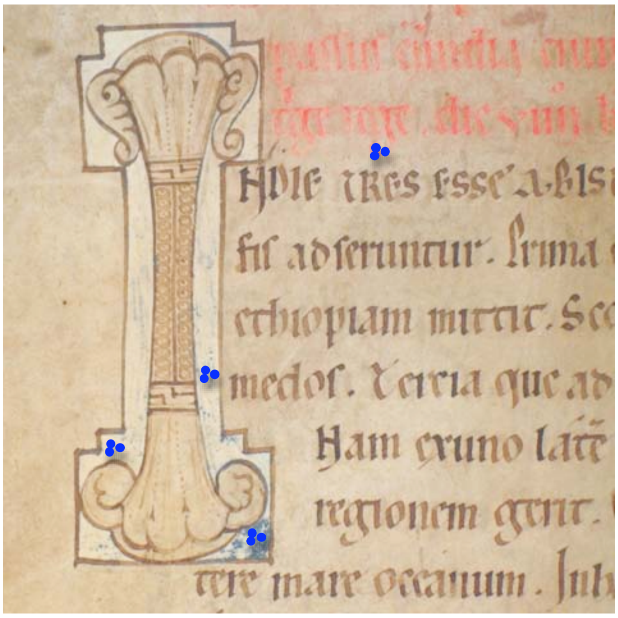



Figure I.127. *Lorvão 16*, *f.* 161v

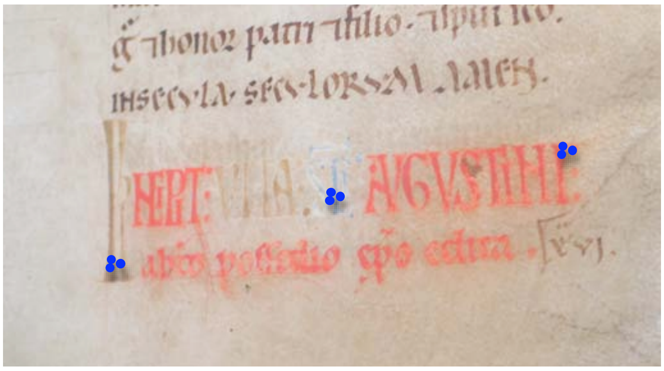


Figure I.128. *Lorvão 16*, *f.* 165

Figure I.129. *Lorvão 16*, *f.* 165v

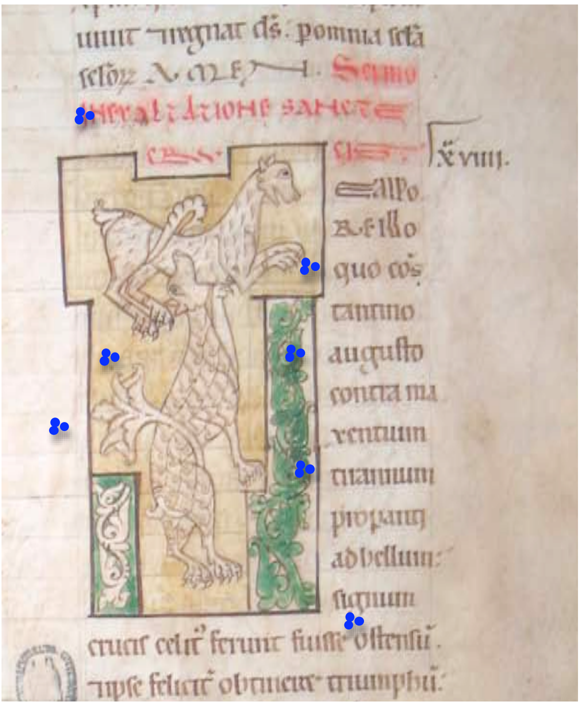


Figure I.130. *Lorvão 16*, *f.* 188

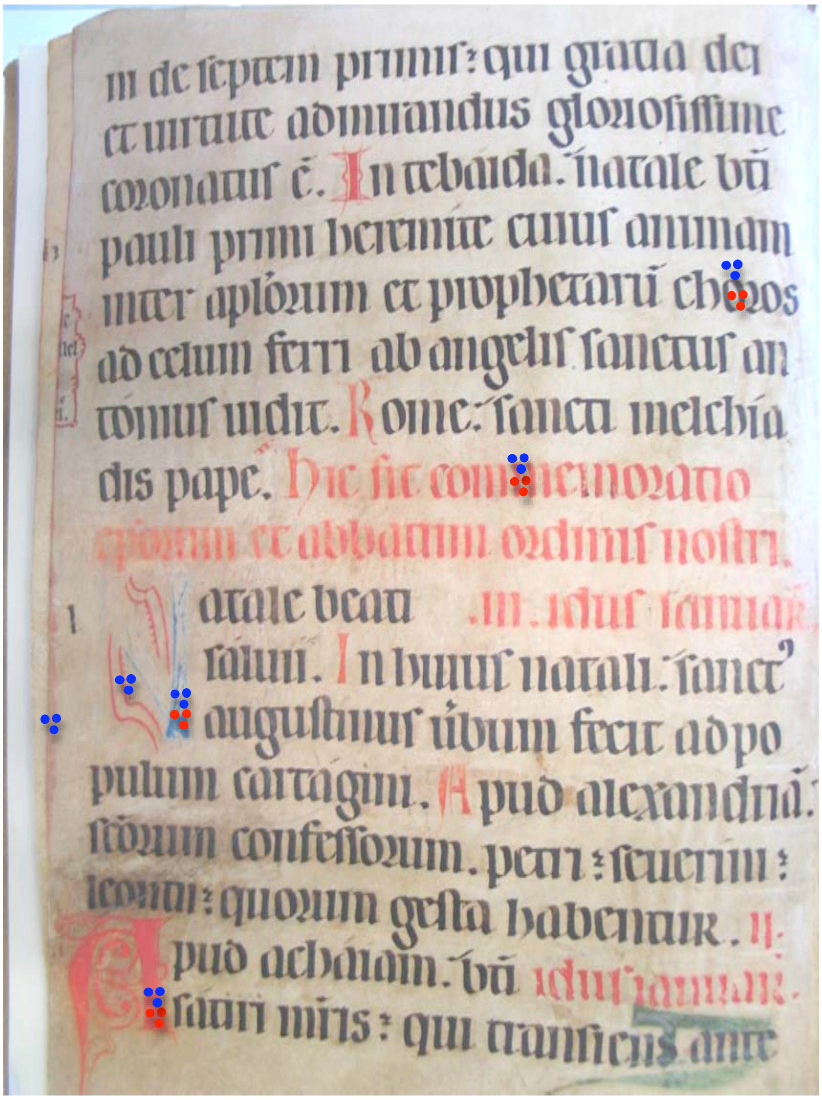

m de septem primis: qui gratia dei
et uirtute admirandus gloriosissime
coronatus ē. In tebaida. natale bti
pauli primi heremite cuius animam
inter aplōrum et prophetarū choros
ad celum ferri ab angelis sanctus an
tonius uidit. Rome: sancti melchia
dis pape. Hic fit commemoratio
epōrum et abbatum ordinis nostri.
Natale beati .III. idus ianuar
saluii. In huius natali: sanctꝰ
augustinus ūbum fecit ad po
pulum cartagini. Apud alexandriā:
scōrum confessorum. petri: seueriani:
leontii: quorum gesta habentur. II.
Apud achaiam. bti idus ianuar.
satiri miris: qui transiens ante

Figure I.131. *Lorvão 17*, *f.* 7v

Figure I.132. *Lorvão 17*, *f.* 9

Figure I.133. *Lorvão 17*, *f.* 9v

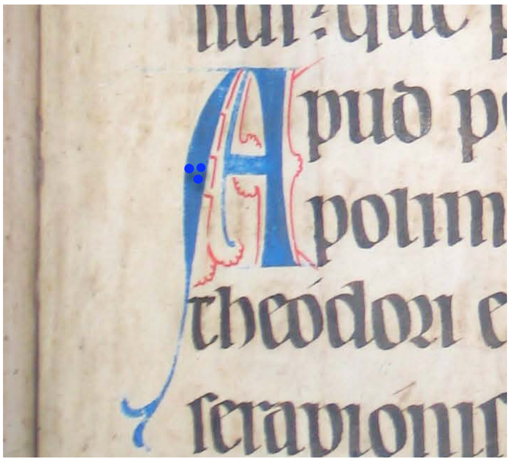


Figure I.134. *Lorvão 17*, *f.* 34

Figure I.135. *Lorvão 17*, *f.* 35

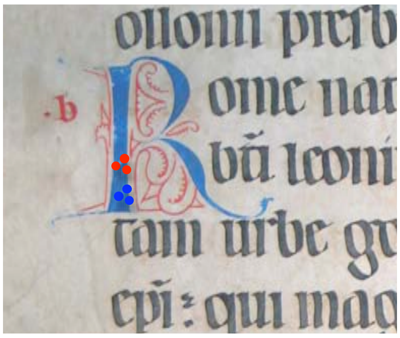


Figure I.136. *Lorvão 17*, *f.* 74v

Figure I.137. *Lorvão 17*, *f.* 127

Figure I.138. *Lorvão 17*, *f.* 162v

Figure I.139. *Lorvão 17*, *f.* 169v

Figure I.140. *Lorvão 17*, *f.* 230

Figure I.141. *Lorvão 50*, *f.* 1v

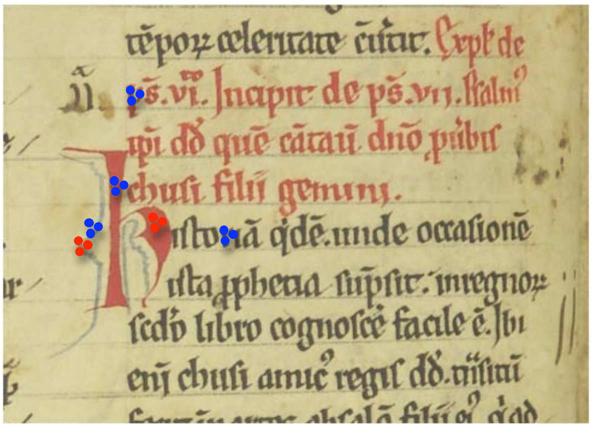


Figure I.142. *Lorvão 50*, *f.* 17v

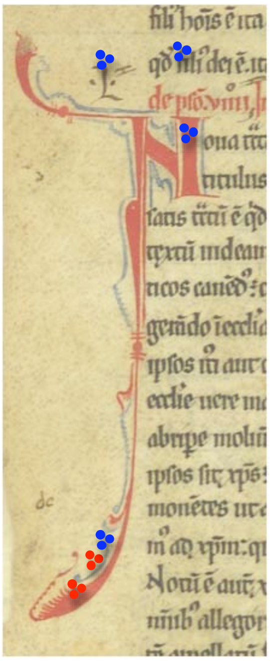


Figure I.143. *Lorvão 50*, *f.* 34v

Figure I.144. *Lorvão 50*, *f.* 64v

Figure I.145. *Lorvão 50*, *f.* 173v

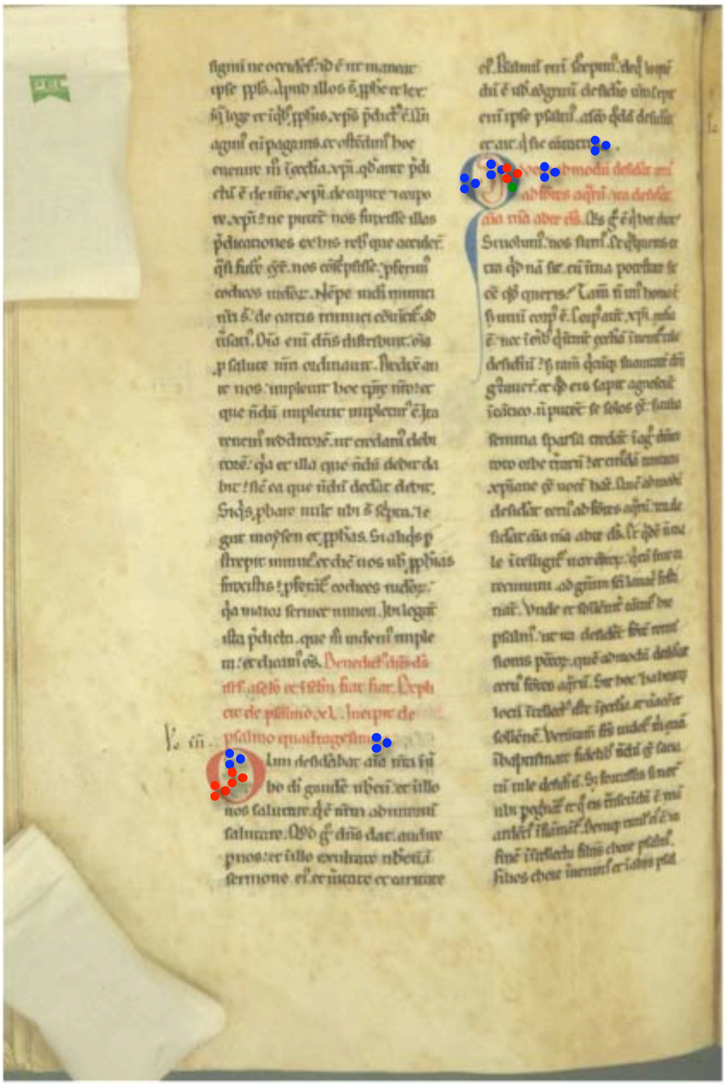


Figure I.146. *Lorvão 50*, *f.* 196v

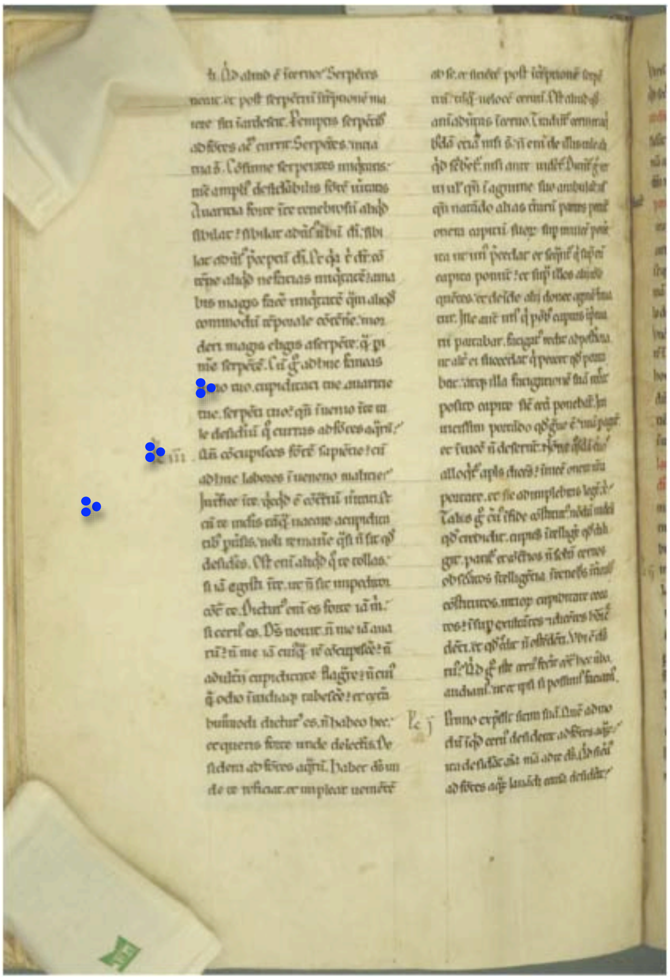


Figure I.147. *Lorvão 50*, *f.* 197v

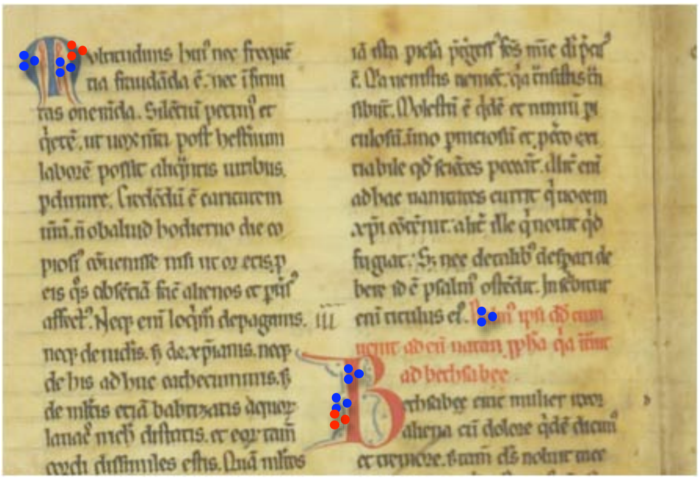
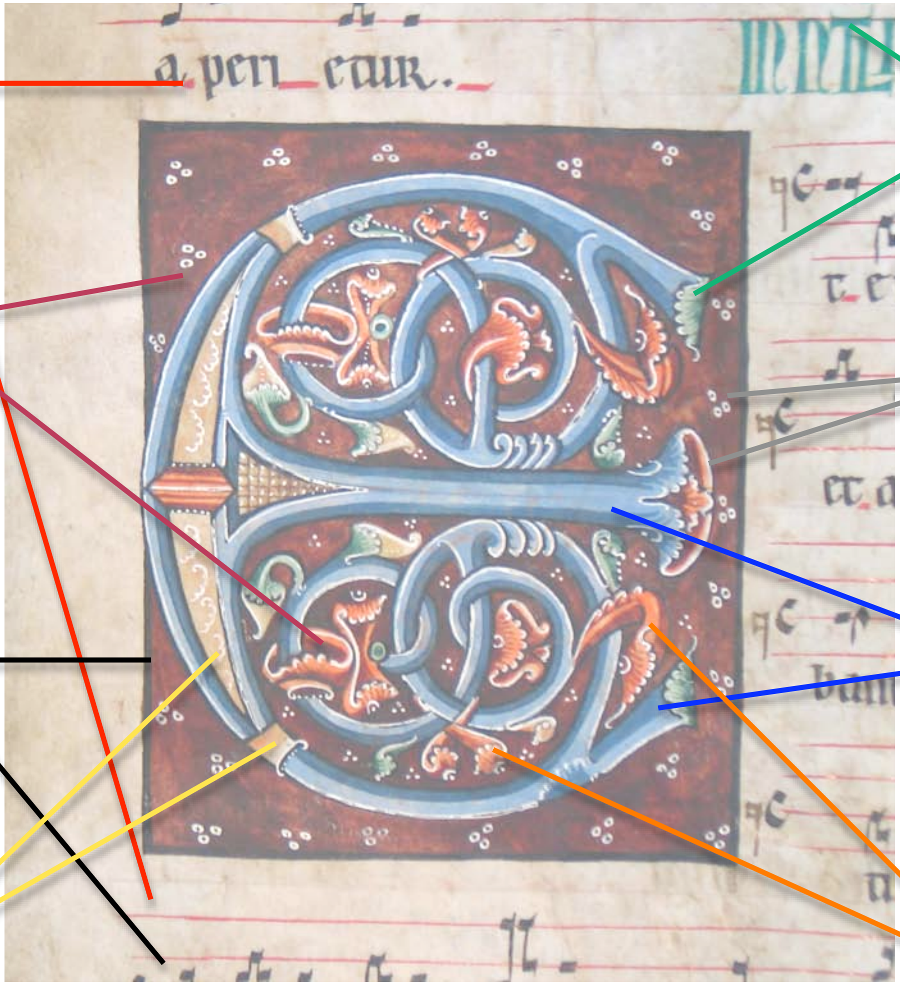


Figure I.148. *Lorvão 50*, *f.* 248v

How to do

Lorvão 12, *f.* 102 (~10 x 10 cm)



Red (~20 min)
5 µEDXRF
5 µRaman

Carmine (~30 min)
3 µEDXRF
2 µsamples

Black (~60 min)
5 µEDXRF
5 µRaman
for 3 different points

Yellow (~35 min)
3-5 µEDXRF
3-5 µRaman
for 3 different points

Green (~50 min)
11 µEDXRF
11 µRaman
1 µsampling

White (~35 min)
3-5 µEDXRF
for 3 different points

Blue (~50 min)
11 µEDXRF
11 µRaman
1 µsampling

Orange (~20 min)
5 µEDXRF
5 µRaman

Total time ~5h00

I.3.1.2. Creating a database

The yellow colorant of saffron was extracted with hot water (~60ºC) for 10 minutes. Lac dye was extracted with water, from a stick with the resin. Dragon's blood colorant was extracted with methanol, at room temperature. Madder lake was prepared by grinding the madder roots, which were left in a bath of water with yeast (Fermipan®) for three days at 40ºC, to extract the sugar compounds; after this period the roots were washed and left submersed in alum aqueous solution (5% p/v) at 40ºC for 2 days; as colorant extraction was achieved, the roots were removed, the pH of the remaining solution was 2.74. K_2CO_3 (5%) was added until a pink precipitate was formed (pH≈6). The solution was filtered and the precipitate (madder lake) was washed. Cochineal was ground in a mortar and dissolved in 0.21 M alum solution for 10 minutes, until the dye is extracted (pH~3). After filtration, K_2CO_3 is added until the precipitate is formed (pH~7), which is then washed.

To prepare the paints, pigments were ground in a mortar, then mixed with binder and applied as paint layers with a brush over the parchment or PMMA.

I.3.1.3. µ-EDXRF semi-quantification

Semi-quantification of blue, orange and red paints was carried on with the analytical program WinAxil using the fundamental parameter method, to deconvolute X-ray spectra measured by ArtTAX, according to the experimental conditions under which the spectrum was collected and comparing with previously prepared standards.

Blue standards: The blue pigments were collected directly from minerals. Lapis lazuli and lazurite $(Na,Ca)_8 (AlSiO_{12})(S,SO_4,Cl)$ were ground from three different Afghanistan minerals. Azurite $(2CuCO_3 \bullet Cu(OH)_2)$ was collected from a mineral that comes out of Portuguese mines.

Based on the results obtained by analyzing the blue paint on the manuscript, with µ-EDXRF and quantified by ArtTAX software, several model painted samples were made. Different mixtures of lapis lazuli, lead white and azurite were prepared (wt%): 97.5:1:1.5 (lapis lazuli : lead white: azurite), 97.5:1:1.5 (lazurite : lead white : azurite) and 87,5:11.5:1 (lapis lazuli : lead white : azurite). Paints were applied in two different modes: with brush, controlling the thickness through the number of layers applied and through cross-sections lately performed (~100 µm); and with a metal film applicator (Zehntner GmbH Testing Instruments), with 3 different thicknesses (100 µm, 150 µm and 200 µm) (Table I.2.). This allowed study the influence of the paint application mode and thickness in the quantification process.

Table I.2. Painted model samples to use as standards to quantification of the blue paint components from the *Book of Birds*.

painted model sample	composition	application mode	thickness µm
A_1	97,5% lazurite + 1% lead white + 1,5% azurite	brush	100
A_2	89% lapis lazuli + 10% lead white + 1% azurite	brush	100
B_1	97,5% lazurite + 1% lead white + 1,5% azurite	film applicator	100
B_2	97,5% lapis lazuli + 1% lead white + 1,5% azurite	film applicator	100
B_3	87,5% lapis lazuli + 11,5% lead white + 1% azurite	film applicator	100
B_{3a}	87,5% lapis lazuli + 11,5% lead white + 1% azurite	film applicator	150
B_{3b}	87,5% lapis lazuli + 11,5% lead white + 1% azurite	film applicator	200
B_{3d}	87,5% lapis lazuli + 11,5% lead white + 1% azurite with a *velatura*[1]* of lac dye	film applicator	100

A colorimeter was used to analyse the colour of the created model samples. Three points and three measures in each point have been analysed.

Red and orange standards: Different mixtures of vermillion, *minium* and orpiment, were prepared (wt%): 100 (*minium*), 99:1 (*minium* : vermillion), 95:5(*minium* : vermillion), 95:5(*minium* : orpiment), and 96:2:2 (*minium* : vermillion : orpiment).

To all standards, pigments were ground in a mortar, then mixed with parchment glue and applied as paint layers with a brush over a PMMA support. The thickness of the paints applied with brush was determined for each standard, in cross-sections, as *circa* 100 µm.

I.3.2. Red lakes

I.3.2.1. Reconstructions

Alizarin solution was prepared in a basic solution of NaOH (pH=9), since this anthraquinone is more soluble in basic solutions (≈10^{-5} M). To obtain the lake, a 1 M solution of aluminum chloride was added, drop-by-drop, until precipitation of the pink/red lake occured; the final pH solution was circa 5. The alizarin lake was filtered and dried at room temperature. The purpurin lake was prepared following the same procedure.

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 selected aqueous binding media. For gum arabic a 10% weight solution was used, based on a recipe

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[1] *Velatura* is a thin layer of red organic material applied over the pigments to intensify its colours, as it seems to be the case of the original illuminations.

from *De Arte Illuminandi* treatise [6]. Parchment glue, was obtained following *O Livro de como se fazem as cores* recipe [7]. Parchment pieces were boiled in a small amount of water, until the water was almost all evaporated. This procedure was repeated until the final glue, the extracted collagen. Glair was made based on *De Clarea* recipe [8]. Egg white was beaten until it is converted into "the likeness of snow" [8]. It is left over night, than the liquid part is collected to be used as binder.

For the semi-quantitative analysis of mixed alizarin and purpurin lakes, different ratios (wt:wt) of each lake: 0:100; 20:80; 40:60; 60:40; 80:20 and 100:0 (purpurin lake : alizarin lake) were prepared. The lakes were weighed, dissolved in acetonitrile and ground in a mortar. After complete evaporation of the solvent, a diluted PVA medium (white glue V7®), was added to the lakes mixture. With this paint, three layers were applied in 1cm^2 squares, made in a Whatman filter paper. For each purpurin and alizarin lakes ratio, four reconstructions were made, on individual filter papers.

Historical reconstructions

The following description of the lake pigments used in this dissertation is based on a full description published by Kirby et al [9].

- Kopp's Al^{3+} lakes

Kopp's lake was obtained with powdered madder root mixed with a 0.6% solution of sulphurous acid (H_2SO_3); left overnight; filtered. The residue was treated again with 0.4% H_2SO_3 and filtered. The filtrates put together; added 2% of total vol. conc. H_2SO_4 and heated to 45–50 ^oC for approx 20 min: brown flakes of Kopp's. The brown flakes were treated with solution of 10 g potash alum ($AlK(SO_4)_2 \cdot 12H_2O$) in 300 ml water, stirred and gently heated, keeping temperature below 70 ^oC. Filtered hot; added more portions of hot alum solution until flakes dissolved. Solution made up to 90 g potash alum in 1 l water. A rose-pink lake was precipitated with 125 ml of 31 g K_2CO_3 in 125 ml water. The precipitated lake was washed with water in a Büchner funnel and dried under a stream of dry nitrogen for several days. Washings were checked for residual sulphate ions (from K alum) with barium chloride.

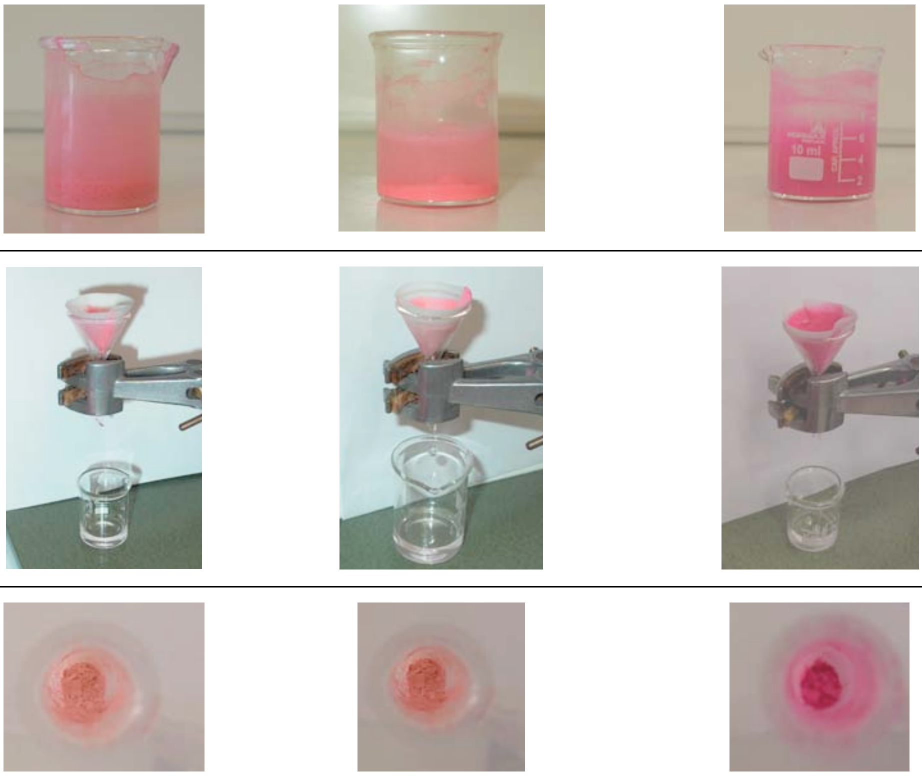
Kopp's lake was applied in water-washed linseed oil [10].

- Eosin lakes

Eosin (tetrabromofluoroscein, sodium salt) Na_2CO_3 solution (120 g in 700 ml) was slowly added to 114 g aluminium sulphate ($Al_2(SO_4)_3 \cdot 18H_2O$) in 500 ml water until pH neutral (400 ml needed). Precipitate (form of hydrated alumina) left to settle; filtered; washed. Suspension in water (approx. 1 l) heated to about 50 °C using magnetic stirrer. 1.5 g eosin in 70 ml water added to suspension; heated to 65 °C; 10 g lead (II) acetate trihydrate ($Pb(CH_3CO_2)_2 \cdot 3H_2O$) in 100 ml water was added to form the pigment.

The bright bluish pink precipitated was washed with water in a Büchner funnel and dried under a stream of dry nitrogen for several days. Eosin lake on alum with lead acetate was applied in water washed linseed oil [10].

The other samples with eosin were prepared with aluminum chloride hexahydrate ($AlCl_3 \bullet 6H_2O$), alum ($KAl(SO_4)_2 \bullet 12H_2O$) and lead (II) acetate trihydrate ($Pb(CH_3CO_2)_2 \bullet 3H_2O$). In 50ml 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 (Figure I.149.). 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 [10].



Eosin Y with $AlCl_3 \bullet 6H_2O$ | Eosin Y with $KAl(SO_4)_2 \bullet 12H_2O$ | Eosin Y with $Pb(CH_3CO_2)_2 \bullet 3H_2O$

Figure I.49. Making off of red eosin lakes with aluminium chloride hexahydrate ($AlCl_3 \bullet 6H_2O$), alum ($KAl(SO_4)_2 \bullet 12H_2O$) and lead(II) acetate trihydrate ($Pb(CH_3CO_2)_2 \bullet 3H_2O$).

I.3.2.2. Actinometry

For the monochromatic irradiation the intensity of the incident light (I_0) was calculated with the potassium ferrioxalate actinometer $K_3[Fe(C_2O_4)_3 \bullet 3H_2O]$ [11-13].

To obtain the lamp I_0 at the different wavelengths used to irradiate the different red chromophores (430 nm and 490 nm for alizarin group, 480 nm and 500nm for purpurin group and 500 nm for carminic acid), a 3 ml solution of $K_3[Fe(C_2O_4)_3 \bullet 3H_2O]$ 0.15M in water, was irradiated in a quartz cell with 1 cm optical path under constant agitation for 30, 60 and 90 seconds, corresponding each irradiation time to an individual 3 ml quartz cell. Moreover, a thermal blank was kept in the dark for comparison with the irradiated samples. Immediately after the irradiation, 500 µl of phenanthroline (phen) 0.1% (p/v) in water was added in each quartz cell, including the blank solution (this procedure was made under dark red light), and kept in the dark for 1 hour, until the complexation is done. Finally, the UV-Vis spectra of the solutions with the resulting $[Fe(phen)_3]^{2+}$ complex were traced and the formation of the product was followed at 510 nm.

The irradiation of $K_3[Fe(C_2O_4)_3]$ causes the reduction of ferric ions to ferrous ions when oxalate is present, according to equation (1) [12]:

$$2Fe^{3+} + C_2O_4^{2-} \xrightarrow{h\nu} 2Fe^{2+} + 2CO_2 \qquad (1)$$

The mechanism of this reduction, according to Hislop and Bolton [14] “includes a ligand to metal electron transfer in the ion-oxalate complex with formation of an oxalyl radical anion”, the final reaction stoichiometry is described in equation (2) [12]:

$$Fe^{III}(C_2O_4)_3^{3-} \xrightarrow{h\nu} Fe^{2+} + CO_2 + 2.5C_2O_4^{2-} \qquad (2)$$

To obtain the lamp I_0, the released Fe^{2-} is complexed with phenanthroline forming a pale orange compound, Fe(II)-1,10-phenanthroline complex [13]:

$$Fe^{2+} + phen \xrightarrow{h\nu} [Fe(phen)_3]^{2+} \qquad (3)$$

To calculate the intensity of light, the following expression was used:

$$I_0 = \frac{V_{sol} \bullet \left(\dfrac{\Delta A}{\Delta \varepsilon}\right)}{1000 \bullet \Phi_R \bullet \Delta t} \qquad (4)$$

where, V_{sol} is the volume of irradiated solution in ml (3 ml), ΔA is the change in absorbance at the monitoring wavelength (510 nm) during the irradiation time period, Δt, corrected by the light absorption of the reagent according with the irradiation wavelength used to each chromophore (λ_{irr}), $\Delta\varepsilon$ is the difference between the molar absorption coefficients of reagent (ε(R)=11100 $M^{-1}cm^{-1}$) and product[2] at the monitoring wavelength (510 nm), and Φ_R is the quantum yield of reaction (2) (Φ_R=1.11 when irradiated at 430 nm; Φ_R=1.08 when irradiated at 480 nm or 490 nm; Φ_R=0.86 when irradiated at 500 nm) [11]. The I_0 was calculated with Moggicor program.

I.3.2.3. Homogeneous media: monochromatic irradiation

The different chromophore solutions were dissolved in MeOH:H_2O (90:19 / v:v) in different concentrations (Table I.3.), in 3 ml quartz cells with 1 cm optical path and stirred . The solutions were irradiated at different wavelengths according to the chromophore (Table I.3.), in the presence of O_2 , at 21 ºC. From the final irradiated solutions, 25 µl were analysed with HPLC-DAD.

Table I.3. Concentration of neutral forms of alizarin, purpurin and alizarin red S in solution, MeOH:H2O (90:10 / v:v) and in gelly and ethylene glycol, @ 293K.

compound	**medium**	**Concentration (M)**	**λ_{irr} (nm)**	**slits**
alizarin	homogeneous	3.0 $x10^{-4}$	430	-
	heterogeneous	2.9 $x10^{-4}$	430	2mm
alizarin lake			490	
purpurin	homogeneous	2.3 $x10^{-4}$	480	-
	heterogeneous	2.7 $x10^{-4}$	490	2mm
purpurin lake			500	
alizarin Red S	homogeneous	4.8 $x10^{-4}$	430	-
	heterogeneous	7.7 $x10^{-4}$	437	2mm
alizarin Red S lake			490	
carminic acid	heterogeneous	8.7 $x10^{-5}$	500	-

I.3.2.4. Heterogeneous media: monochromatic irradiation

Gels of red chromophores in commercial gelatine (Jerónimos) were irradiated (Table I.3.). Chromophores were diluted in ethylene glycol; 0.4 g of gelatine was dissolved in 2 ml of hot water (at *circa* 90 ºC) with agitation, until a liquid gel was formed; 3 ml of chromophore solution and 2 ml of ethylene glycol were added to gelatine. Before gelatine solidification, the solution was transferred to a 3 ml plastic cell with 1 cm optical path and submitted to ultrasonic bath until the air bubbles disappeared. The same procedure was done to another cell to be kept in the dark as thermal control. When completely solid, *circa* 5 minutes at 21 ºC, the monochromatic irradiation was performed.

---

[2] The molar absorption coefficient of products was not used, since the products were not characterized and their amount in the final irradiated solution is almost insignificant. Instead the difference of molar absorption coefficients, only the one of the reagent was used.

I.3.2.5. Heterogeneous media: polychromatic irradiation

In each frosted glass (~1cm^2), 0.8 mg of each chromophore were deposited surface with a brush and irradiated in the Solar Box camera during 4250 hours. Each cell was taken at each different time of irradiation. Then the dye was extracted with MeOH to perform HPLC-DAD analysis.

I.3.2.6. Quantum yield

The quantum yield was calculated through the following equation:

$$\Phi_R = \frac{V_{sol} \bullet \left(\frac{\Delta A}{\Delta \varepsilon} \right)}{1000 \bullet I_{abs} \bullet \Delta t} \qquad (5)$$

where, I_{abs} is the total light absorbed by the solution at the irradiation wavelength; the I_{abs} is equal to I_0 x (1-10^{-Airr}) when A<2 or I_{abs}=I_0 when A>2. The other terms were already defined for equation 4.

In the calculation of quantum yield in heterogeneous media only half volume of the gel was irradiated, therefore the volume considered was 1.45 ml (calculated through the measurement of the optical path exposed to light) [11].

The program Moggiccor was also used to obtain the Φ_R.

I.3.3. µ-SPEX spectra acquisition

I.3.3.1. Homogeneous media

Fluorescence spectra were corrected for the wavelength response of the system.

The absorption and emission measurements, in homogeneous media, were performed with 5x10^{-5} M for chromophores and with 2x10^{-6} M solutions for the respective lakes. The fluorescence quantum yield for the lakes were determined using purpurin as standard (ϕ_f= 0.004 in MeOH:H$_2$O (75:25, v:v); and the fluorescence quantum yield of purpurin was obtained by comparison with a fluorescein standard solution (ϕ_f=0.79 in 0,1M NaOH) [15]. The ϕ_f value for the purpurin compares well with previously published values, using quinine sulphate as standard [16].

The pH of the solutions was measured with a Metrohm 713 pH meter or a MetLab pHM240. Calibration was performed with a set of Metrohm buffers of pH4.00 and 7.00. Adjustment of the desired pH values was accomplished by addition of $HClO_4$ or NaOH.

When studying the fluorescence emission of these chromophores, the greatest care had to be taken to avoid contamination by metal ions that are able to form fluorescent complexes with the anthraquinone hydroxy derivatives. Low concentration of metal ions, such as 10^{-6} M may induce a significant contribution in the global fluorescence emission in solution where purpurin concentration is typically ≈ 10^{-6}-10^{-7}M. Hence it is advisable to not use common paper for the cleaning of the pH electrode or other glassware as it contains a large amount of Al^{3+} ions. In alternative, it was used filter paper.

I.3.3.2. Heterogeneous media

For all the solid state measurements uncorrected spectra are presented. In heterogenous media, for the μ-SPEX, spectra were acquired after focusing, and for the optic fibre by gentle contact through a black o-ring.

The set of samples prepared to test the technique can be divided in two categories, model samples and historic reconstructions; Model samples are purpurin and alizarin lakes bound in each of the following media: gum arabic, parchment glue (collagen) and PVA emulsion. Historical reconstructions were made following 19th Century English and French recipes [9], for the red lake pigments, which were then made into linseed oil paints, similar to paints that were found in paintings by Van Gogh [10].

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 fibres were put in concave 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 mm spot, using pinhole 8.

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. For all Andean fibres samples, a minimum of three emission spectra was acquired together with the excitation spectra, which closely matches the dye absorption. For the cross sections, a minimum of seven emission spectra, together with excitation spectra, was acquired for each sample.

I.3.4. Andean fibres extraction

Extractions were only performed in some of the studied samples (see Appendix IV, section IV.2.2., table IV.1.) using the oxalic acid method developed by Claude Andary and Pauline Guinot [17], but without acetone in the mixture (for further details see *Appendix IV*, section *IV.1.1.*). 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 60ºC, in a magnetic stirrer. Once the dyes were extracted, the fibres 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.

I. 4. References

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Appendix II – Colouring materials data

II.1. Comparative case studies

A research on the publications where colour materials of medieval illuminations were identified or characterized, was done (Table II.1.). It allowed conclude that the most common pigments identified were vermillion, *minium*, azurite and ultramarine blue, verdigris, orpiment, white lead and carbon black; and mixtures were not very used

Table II.1. Summary of the colouring material identified in different dated medieval manuscripts, according to published sources.

	8th	9th	11th		12th	13th		14th		14th - 15th	15th				Late 15th	16th	
	[1]	[1]	[1]	[2]	[2]	[3]	[4]	[5]	[6]	[7]	[4]	[8]	[9]	[10]	[11]	[12]	[13]
Red																	
vermillion				x	x	x	x		x	x	x	x	x	x^{g}		x	x
minium	x^{g}	x^{i}															
ochre	x								x								
vermillion+ *minium*										x							
vermillion+ organic lake									x								
Orange																	
minium	X	X		X		X	X	X				X				X	
minium+ massicot																	X
vermillion+ white lead							X										
minium+ organic^{k}								X								X	
Blue																	
ultramarine^{i}			X	X	X	X	X				X	X	X				X
azurite						X		X	X	X	X	X	X	X	X	X	X
indigo				X			X					X					
ultramarine azurite						X											X
Green																	
malachite												X	X		X	X	X
verdigris	X	X	X				X		X			X					
copper sulphate														X			X^{d}
malachite+ copper sulphate																	X^{d}

	8th	9th	11th		12th	13th		14th		14th - 15th	15th				Late 15th	16th	
	[1]	[1]	[1]	[2]	[2]	[3]	[4]	[5]	[6]	[7]	[4]	[8]	[9]	[10]	[11]	[12]	[13]
lead-tin yellow+ copper green								X									
orpiment+ blue pigment		X									X						
Yellow																	
orpiment	X	X	X	X		X	X		X								
realgar						X											
massicot						X						X					
lead-tin yellow								X[f]				X[e]					X[e]
ochre												X					
organic						X				X[j]							
White																	
lead white	X		X	X		X	X	X			X	X			X	X	
gypsum			X			X[b]											
calcined bone						X			X								
calcite				X								X					
chalk							X										
Black																	
carbon black	X		X			x				X	X	X					
iron gall		x[h]	x[h]				x[h]										
Brown																	
iron oxide						X											X
vermillion+ black coal+ iron oxide															X		
minium		X															
Carmine-coloured																	
organic						X[c]									X	X	X
Grey																	
carbon							X										
indigo			X														
azurite+ carbon black+ white lead																	X
ultramarine +white lead																	X

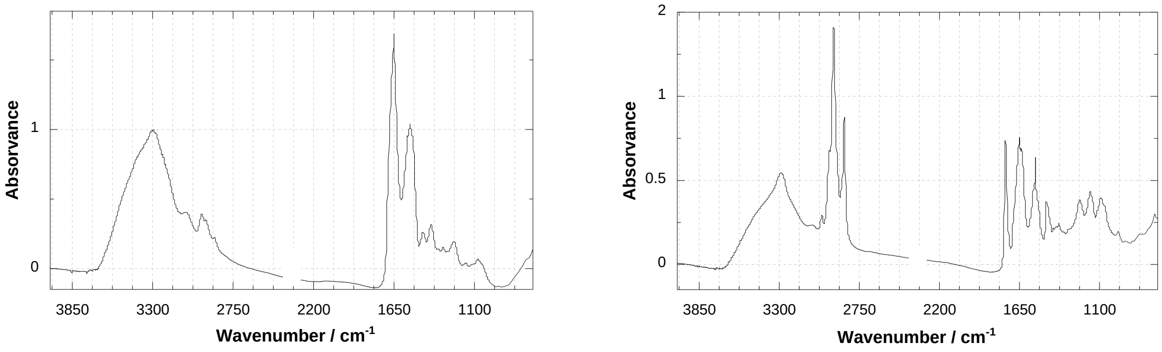
a- yellow tentatively identified as gamboge, a gum resin produced by trees of the genus *Garcinia*; ***b-*** and also anhydrous calcium sulphate; ***c-*** alizarin lake; ***d-*** basic copper sulphate; ***e-*** lead-tin yellow type I; ***f-*** lead-tin yellow type II; ***g-*** rubric letter; ***h-*** probably; ***i-*** initial; ***j-*** the best organic match is saffron and is mixed with gypsum; ***k-*** deep shade; ***l-*** ultramarine blue or lapis-lazuli

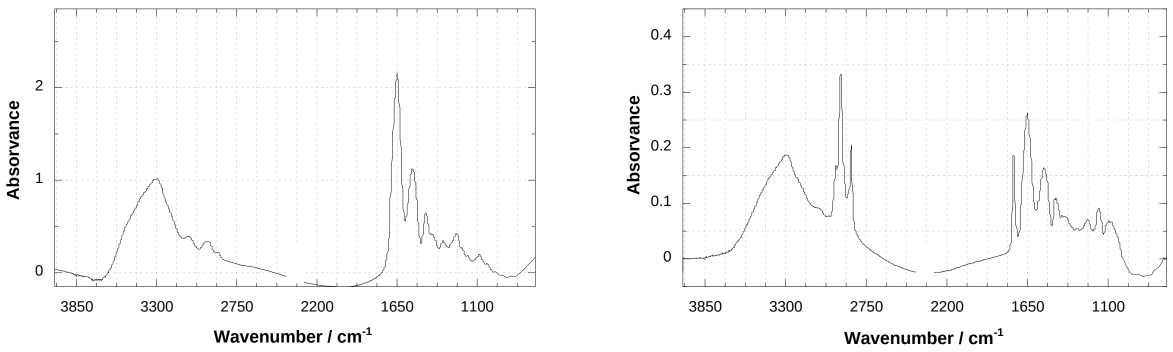
II.2. Reconstructions

II.2.1. Binders

Different sorts of binders were analysed by μ-FTIR, to build up the database, that would be used to identify the binders used to paint the illuminations from Lorvão manuscripts (Figure II.1. a-j)

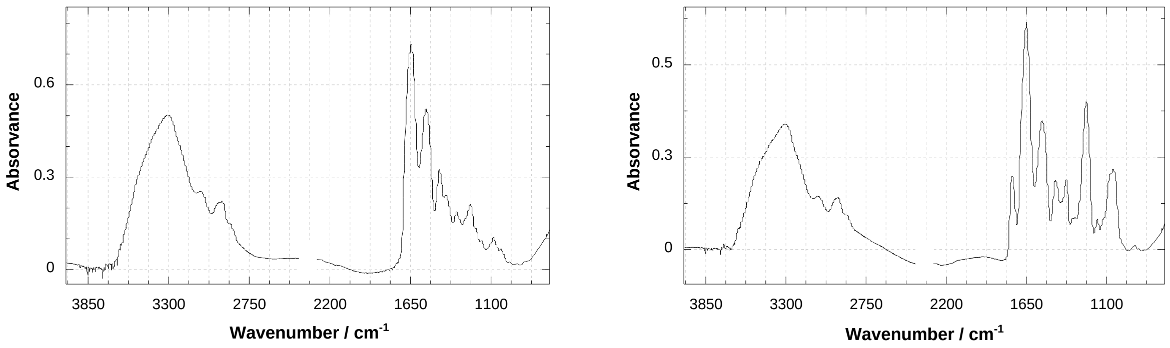
(A) egg white (B) egg yolk





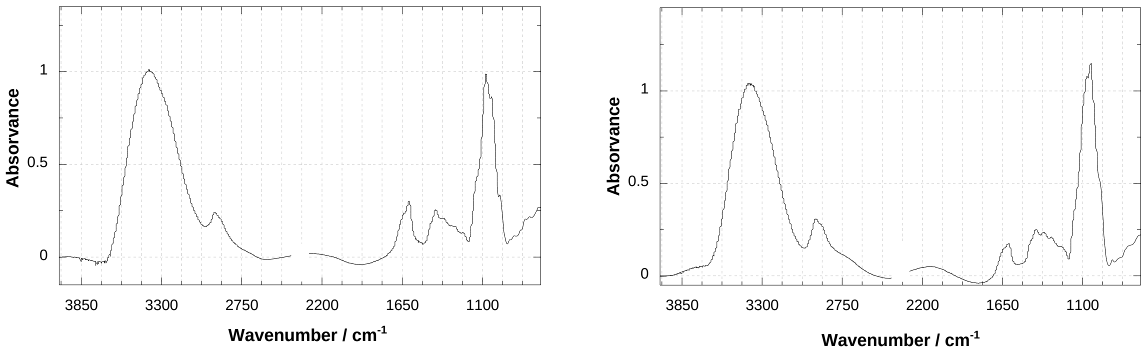
(C) parchment glue

(D) casein



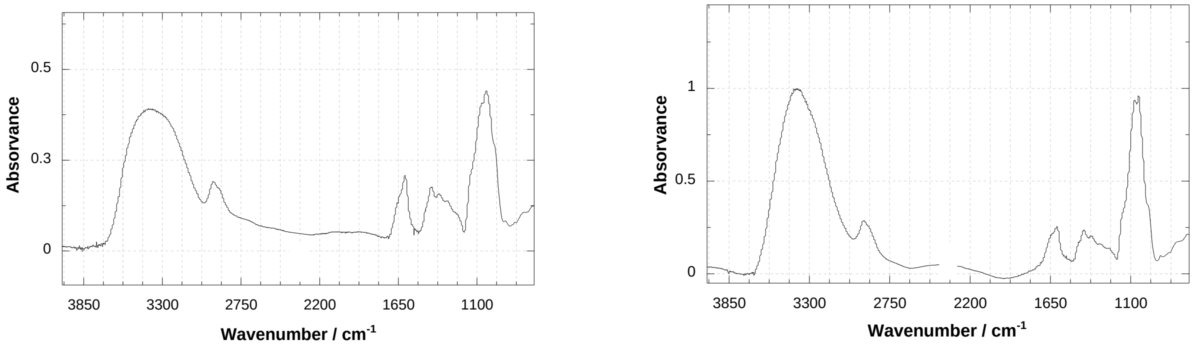
(E) rabbit glue

(F) sturgeon glue



(G) gum arabic

(H) peach tree gum



(I) plum tree gum

(J) almond tree gum

Figure II.1. FTIR spectra of different sorts of binders possible to find in medieval illuminations, from A to J: egg white, egg yolk, parchment glue, casein, rabbit glue, sturgeon glue, gum arabic, peach tree gum, plum tree gum and almond tree gum (the gaps in the spectra between ~2400 and 2300 cm^{-1}, correspond to the stretching C=O attributed to CO_2).

Table II.2. Observed infrared fundamental modes (in cm^{-1}) of the proteinaceous binder, in the wavenumber region 700-4000 cm^{-1}.

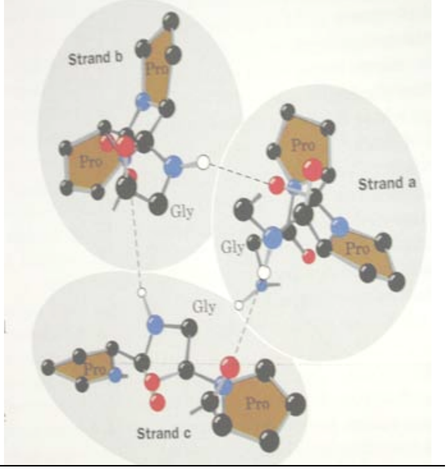
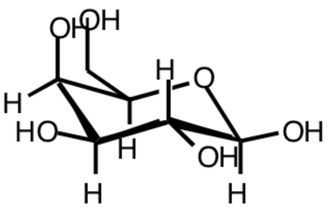
Wavenumber (cm^{-1})	**vibration**	**intensity**	**Notes**
~3300	stretching O-H	w , vb	
3350	stretching N-H	m-w , sh	
3080			
2918	stretching C-H	m-w	
2850			
1631	stretching C=O (amide I)	vs	Three characteristic bands of proteins, falling like a stair
1545	stretching C-N; bending N-H (amide II)	s	
1457	bending C-N (amide III)	m	
1430	See notes	See notes	Composite and complicated bands, corresponding to structural vibrations, bending C-O-C, C-H, etc.
1335			
1240			
1083			
1031			
s - strong; m - medium; w - weak; v - very; b – broad; sh – sharp			

Table II.3. Observed infrared fundamental modes (in cm^{-1}) of the polysaccharide binder, in the wavenumber region 700-4000 cm^{-1}.

Wavenumber (cm^{-1})	**vibration**	**intensity**
~3300	stretching O-H	w , vb
2924 & 2853	stretching C-H (sp^3)	m-w
1615	bending O-H	
1420	bending C-H	s
1324		
1155	stretching C-O-C	m-w
1123		vs
1078		
1040		s
907		
s - strong; m - medium; w - weak; v - very; b – broad; sh - sharp		



II.2.2. Colouring materials

The most common found pigments and dyes were analysed by µ-FTIR, µ-Raman, µ-EDXRF and HPLC-DAD, depending on the nature of each one, to build up a consistent database for this techniques. The spectra are presented below.

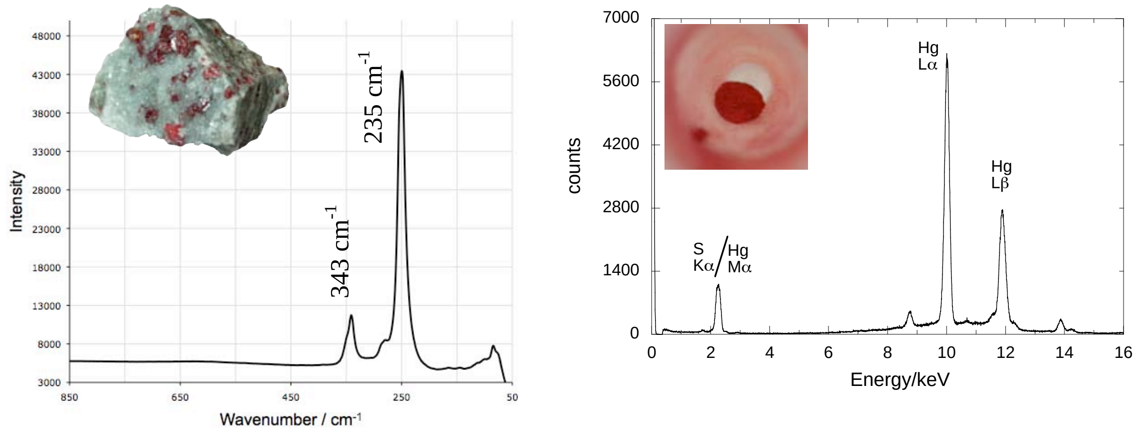


Figure II.2. Raman and EDXRF spectra of vermillion

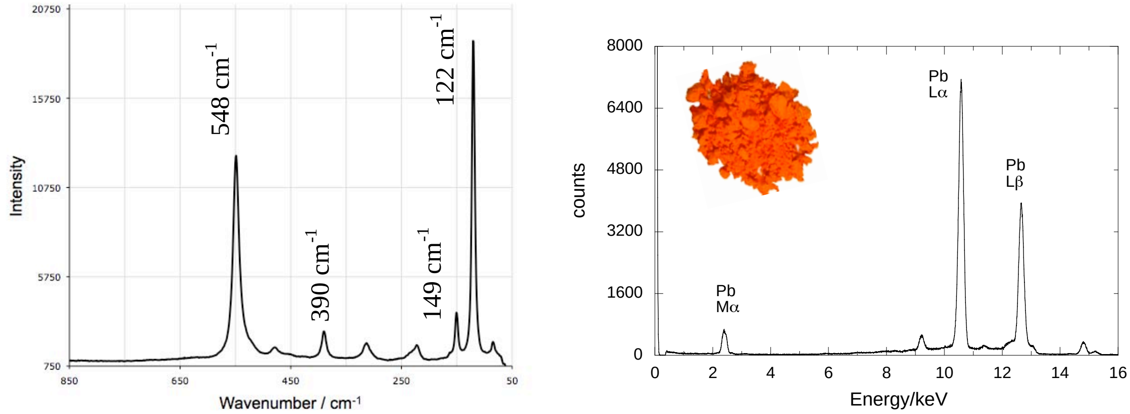
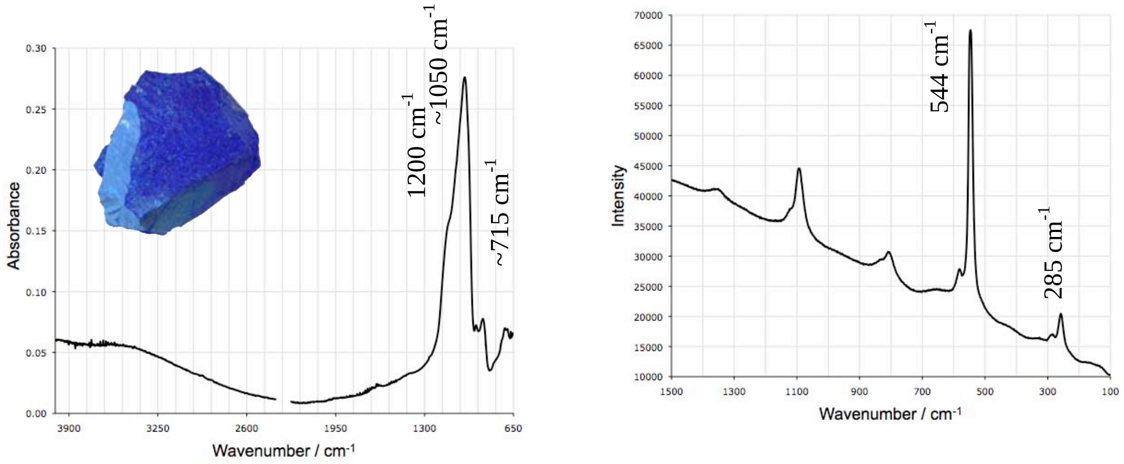


Figure II.3. Raman and EDXRF spectra of *minium* (red lead]



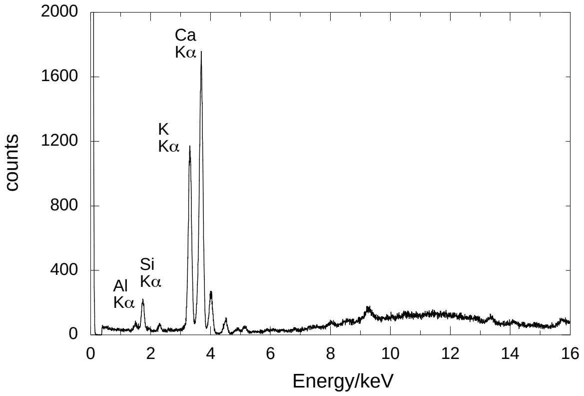


Figure II.4. FTIR, Raman and EDXRF spectra of lapis lazuli.

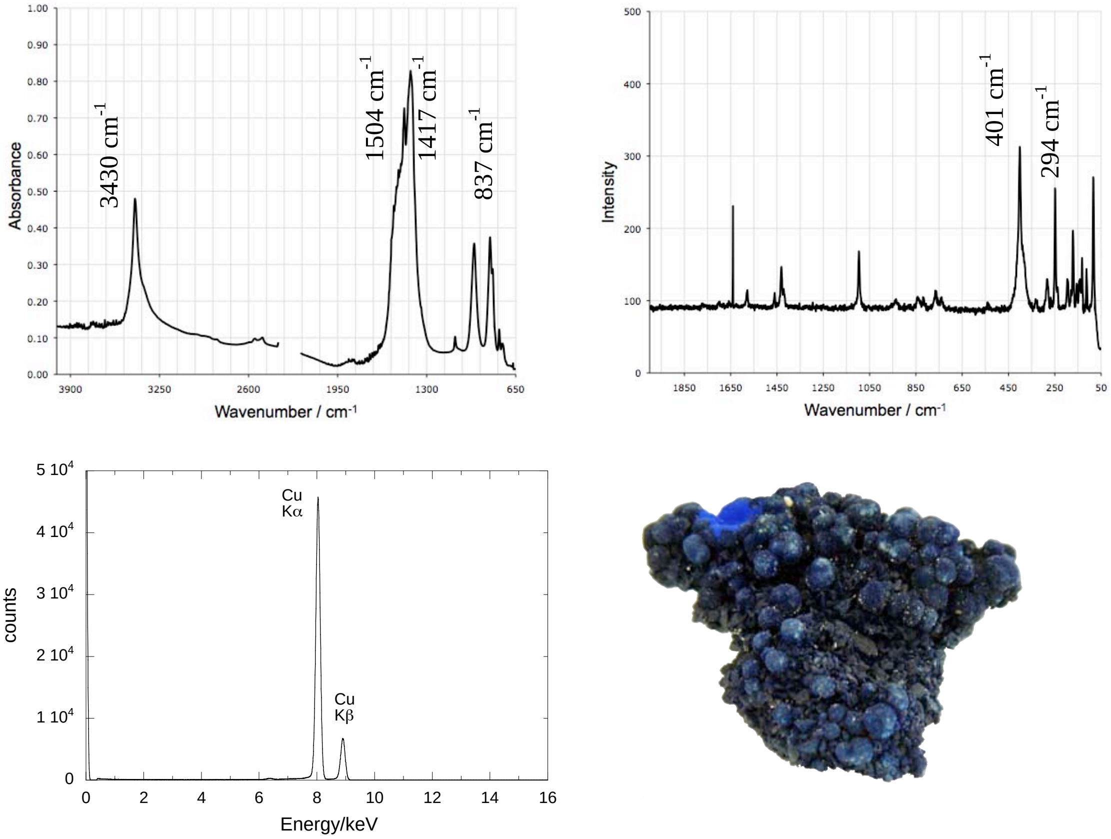


Figure II.5. FTIR, Raman and EDXRF spectra of azurite.

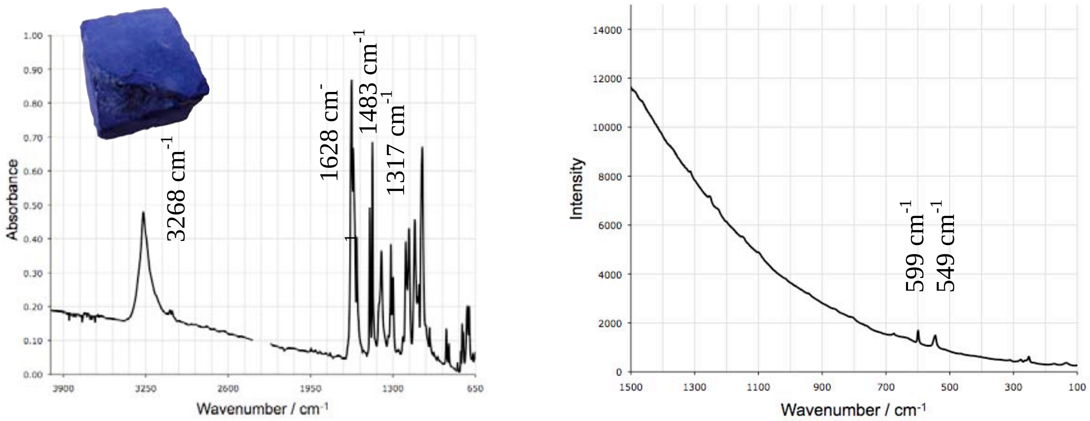


Figure II.6. FTIR and Raman spectra of indigo.

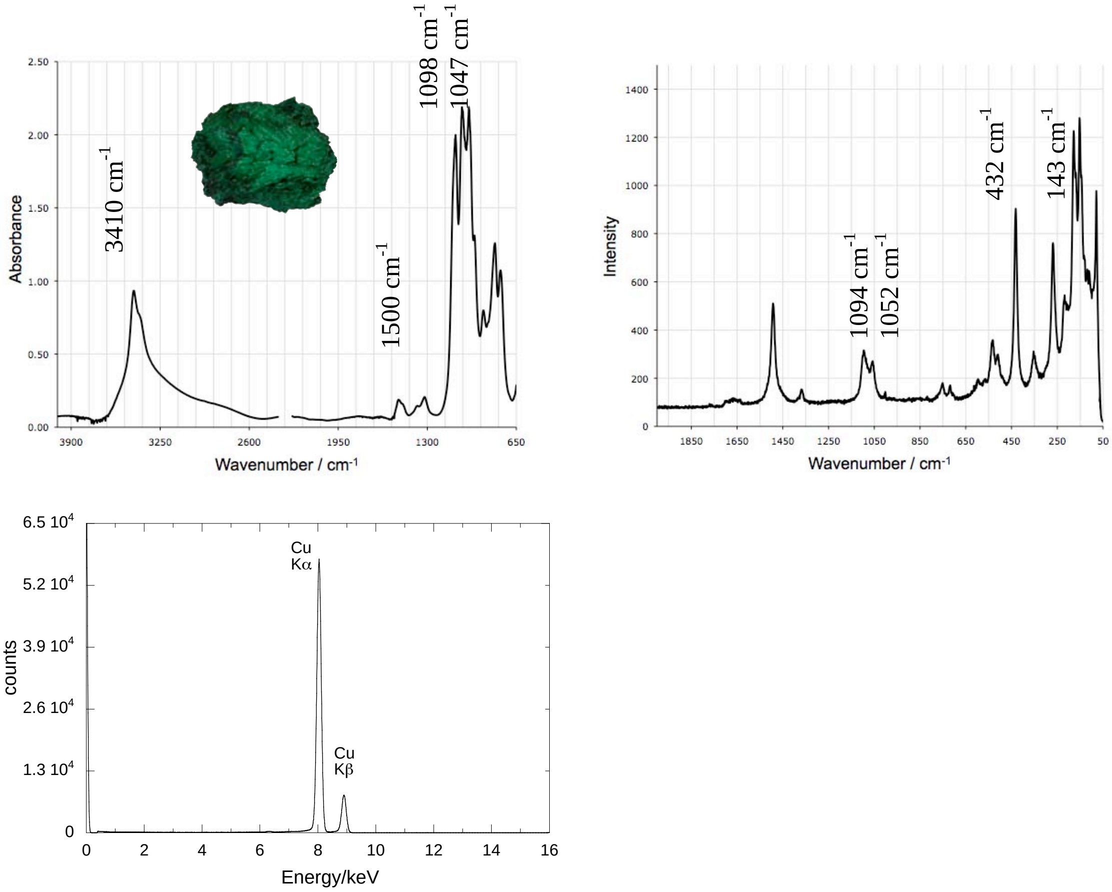


Figure II.7. FTIR, Raman and EDXRF spectra of malachite.

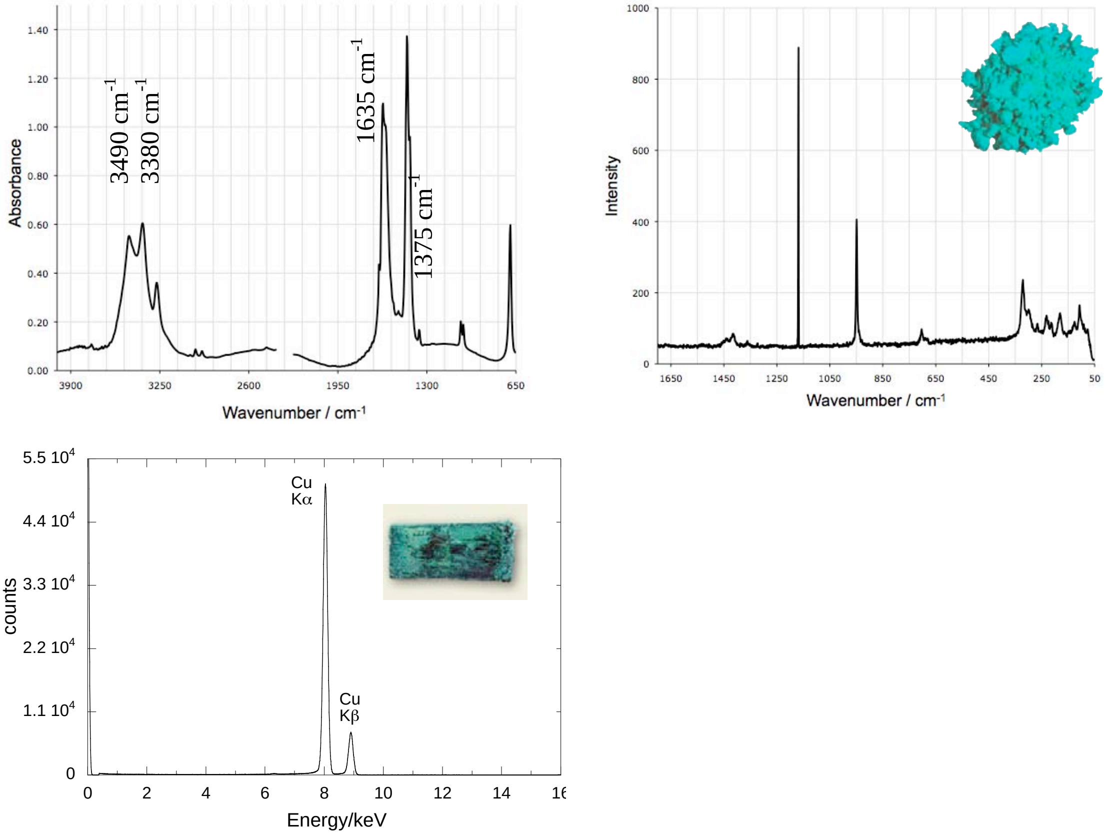


Figure II.8. FTIR, Raman and EDXRF spectra of verdigris.

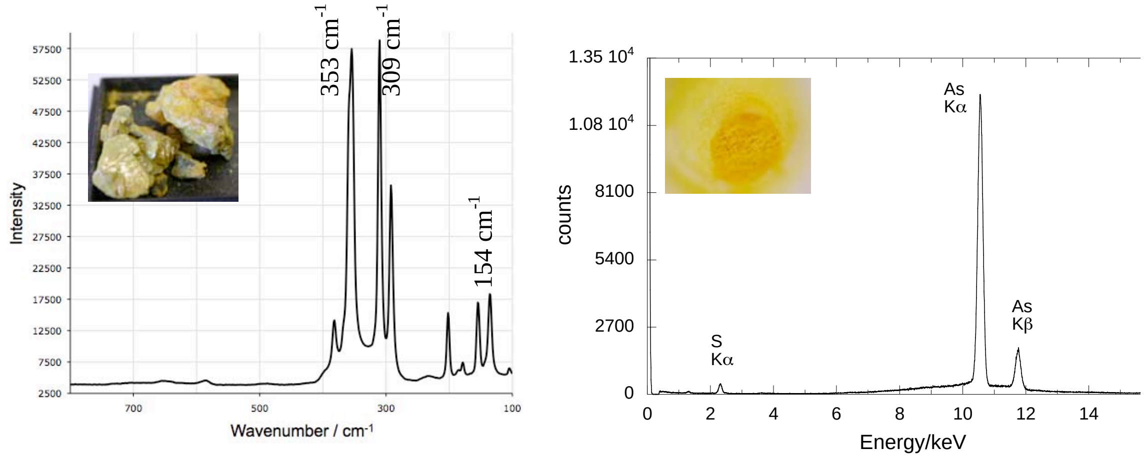


Figure II.9. Raman and EDXRF spectra of orpiment.

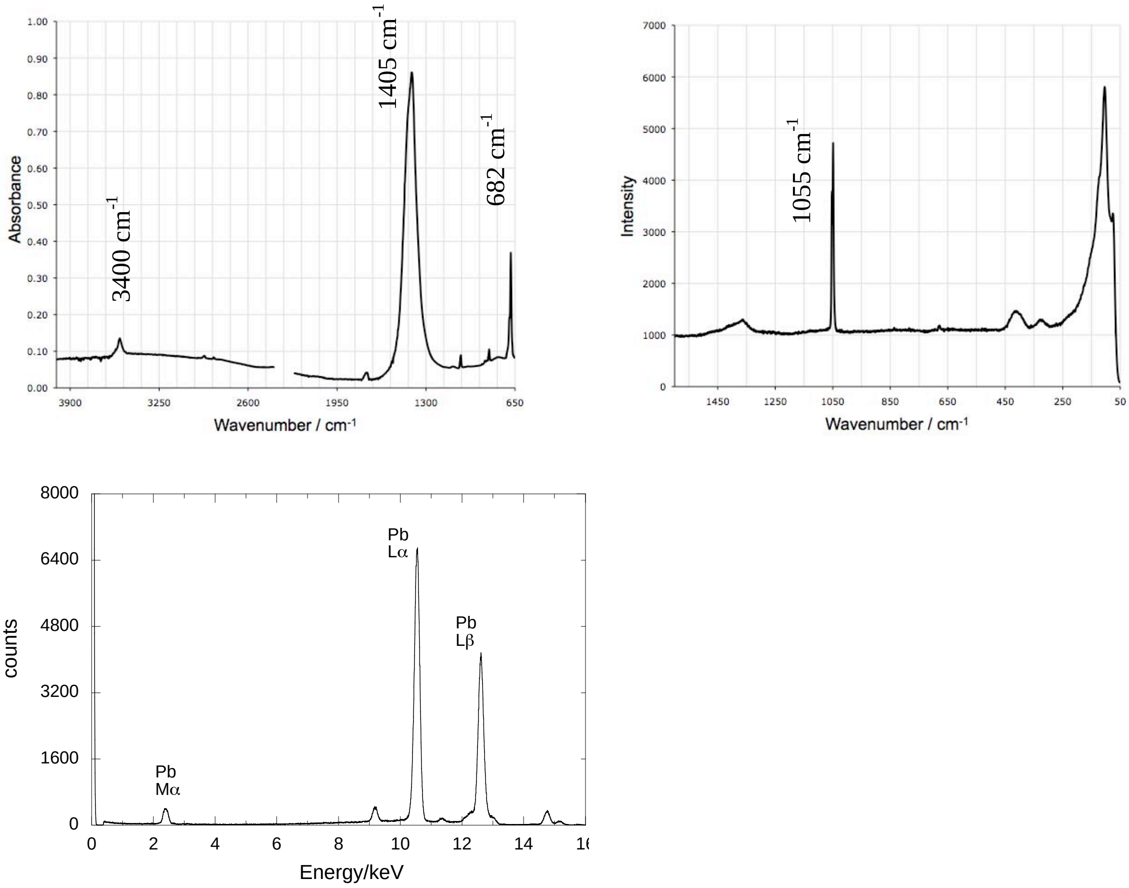


Figure II.10. FTIR, Raman and EDXRF spectra of white lead.

$1430\ cm^{-1}$

$878\ cm^{-1}$

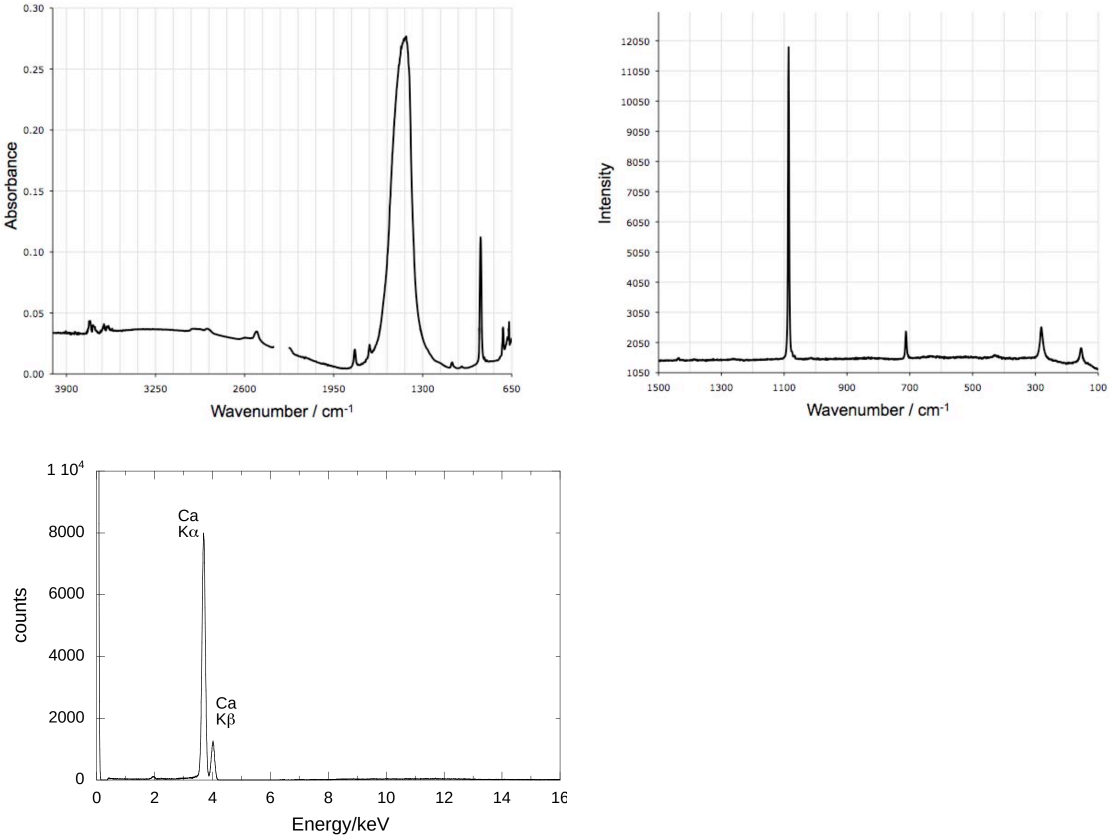


Figure II.11. FTIR, Raman and EDXRF spectra of calcium carbonate.

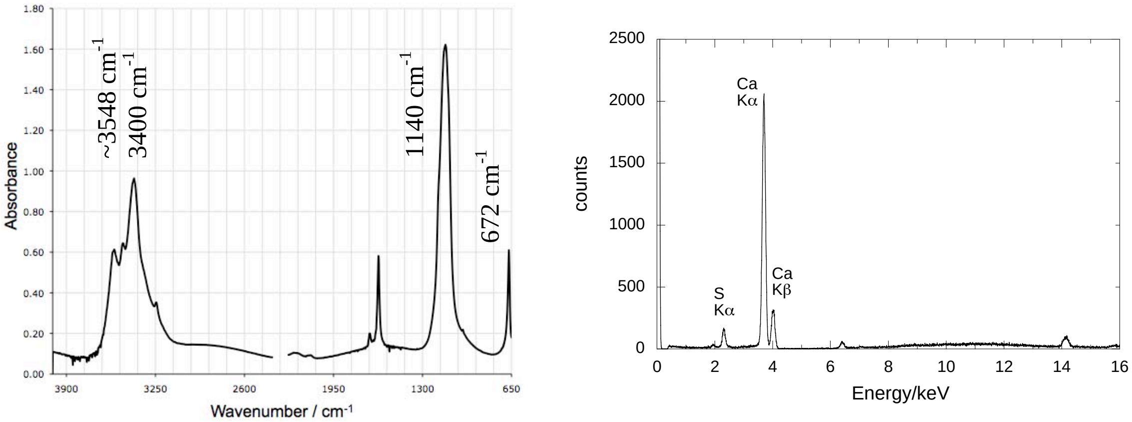
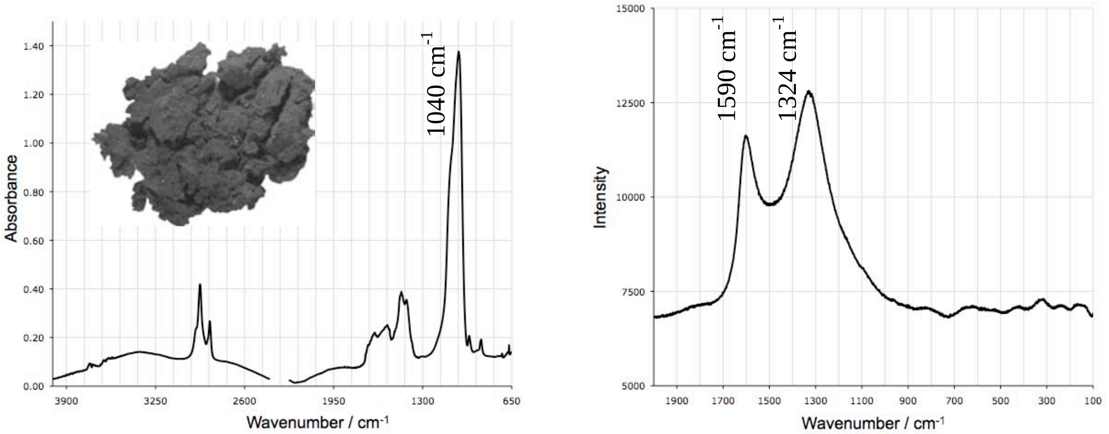


Figure II.12. FTIR and EDXRF spectra of calcium sulphate.



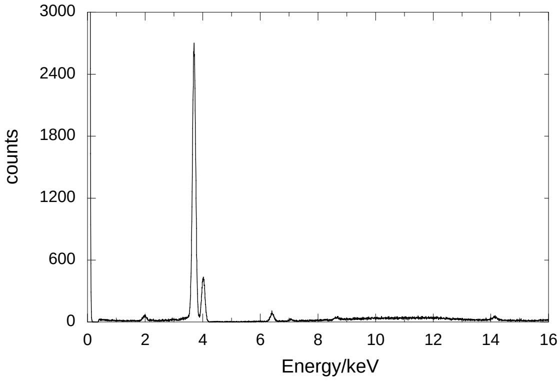


Figure II.13. FTIR, Raman and EDXRF spectra of ivory black.

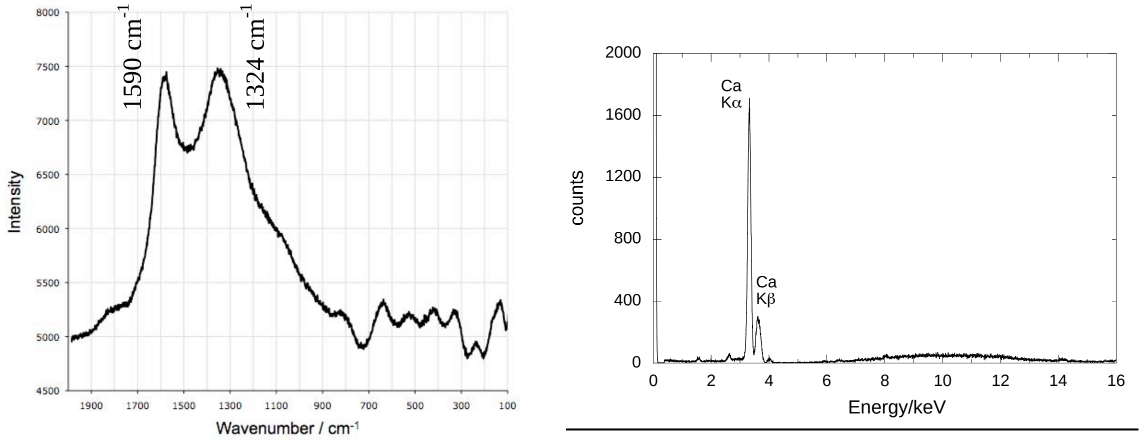
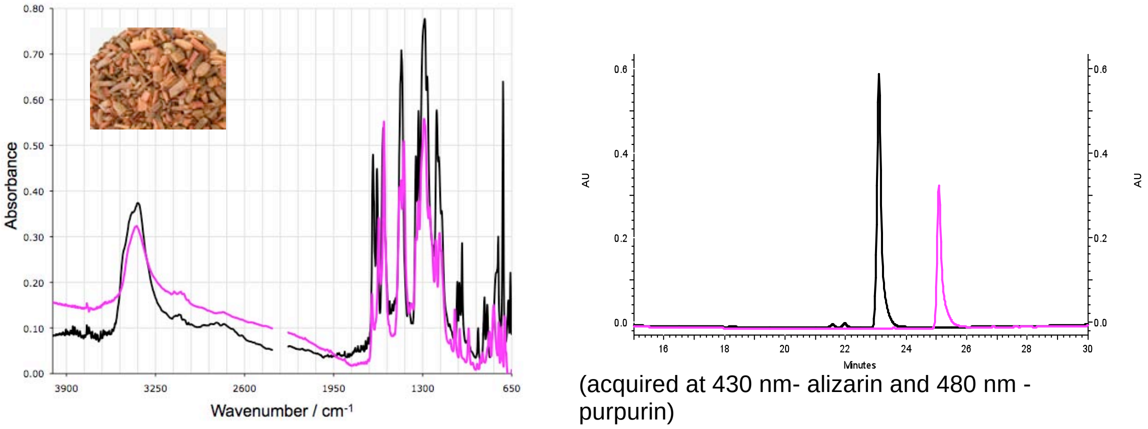
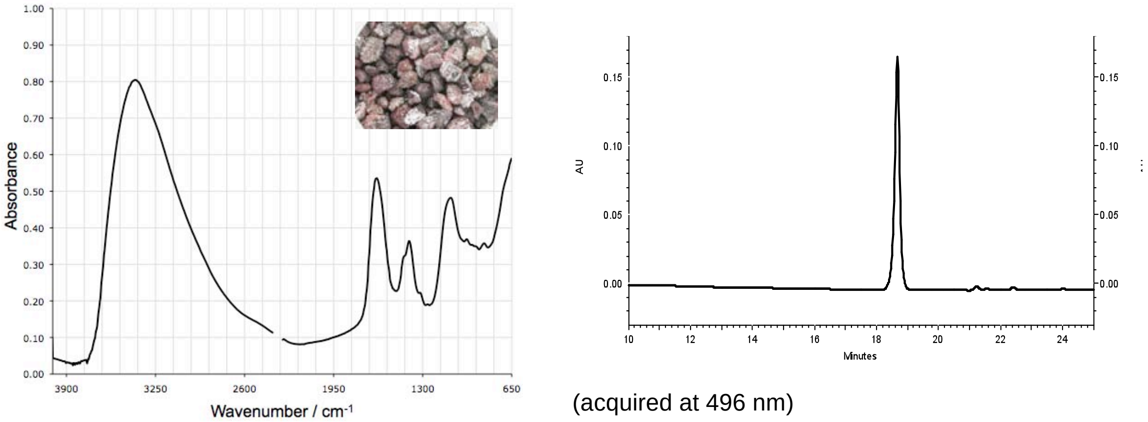


Figure II.14. Raman and EDXRF spectra of vine black.



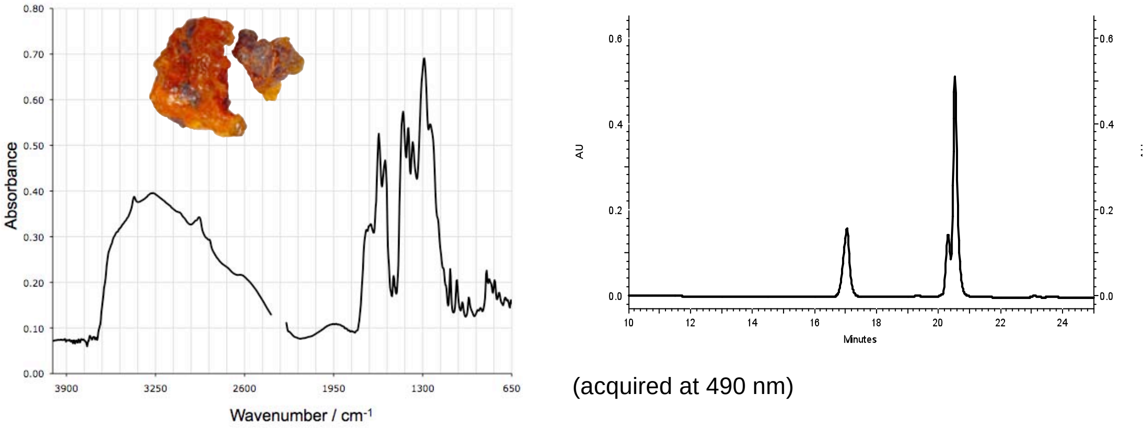
(acquired at 430 nm- alizarin and 480 nm - purpurin)

Figure II.15. FTIR spectra and chromatogram of alizarin (black line) and purpurin (purple line)



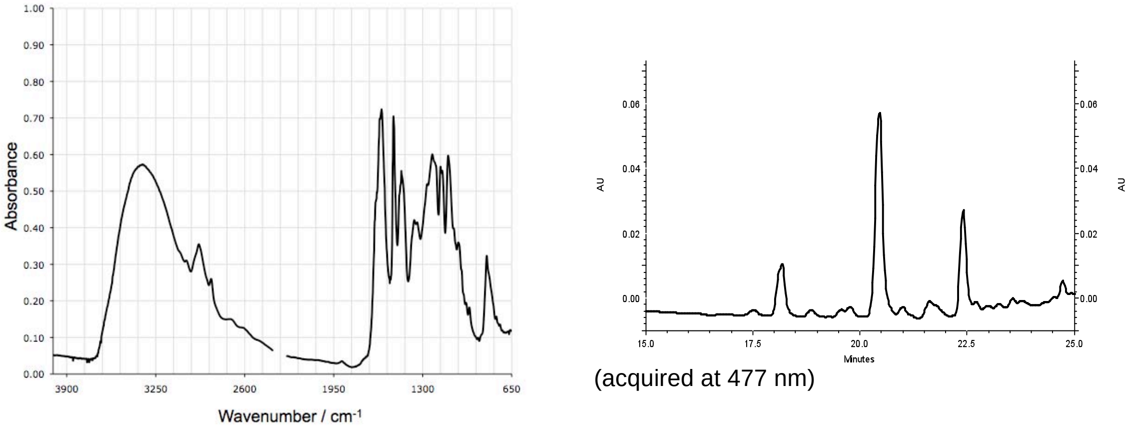
(acquired at 496 nm)

Figure II.16. FTIR spectrum and chromatogram of cochineal.



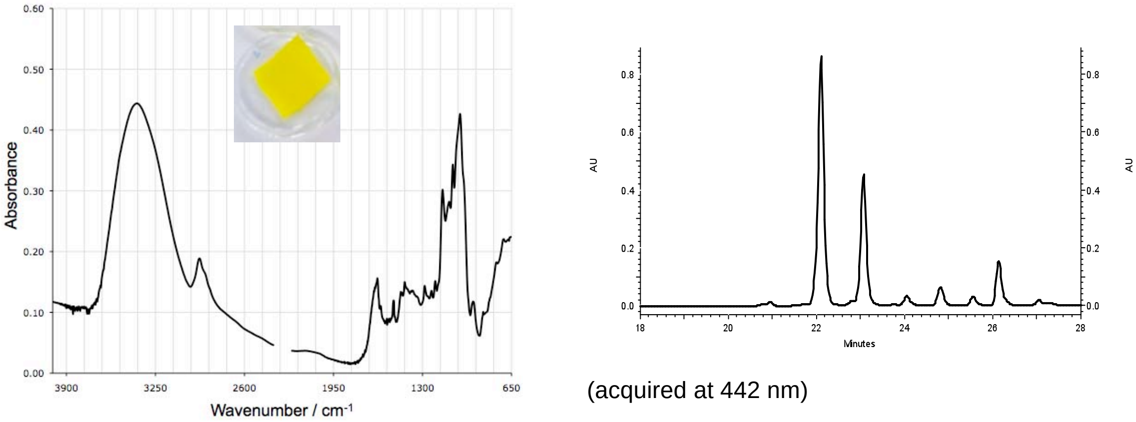
(acquired at 490 nm)

Figure II.17. FTIR spectrum and chromatogram of lac dye.



(acquired at 477 nm)

Figure II.18. FTIR spectrum and chromatogram of dragon's blood.



(acquired at 442 nm)

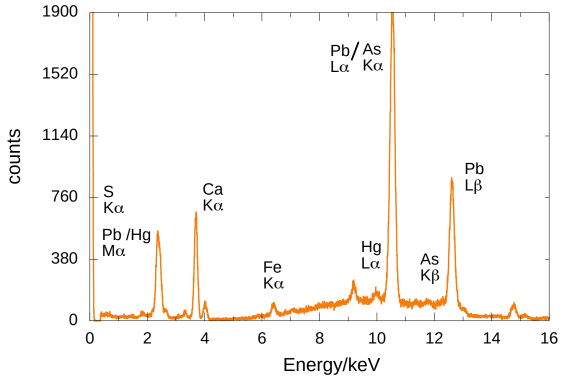
Figure II.19. FTIR spectrum and chromatogram of saffron.

II.3. Lorvão data

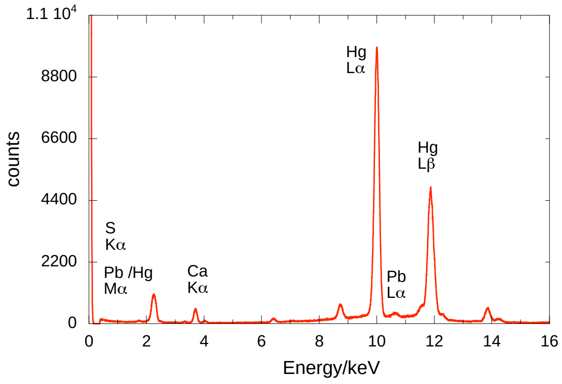
II.3.1 Representative spectra of Lorvão collection

Apocalypse

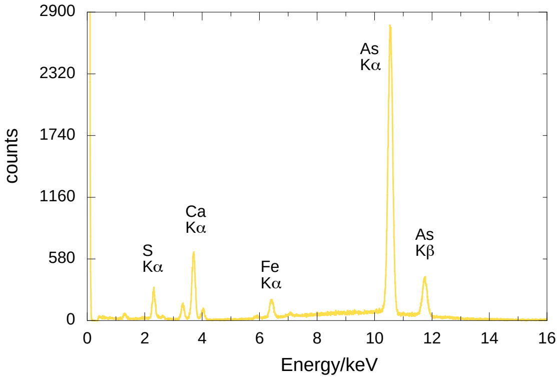
<u>μ-EDXRF</u>



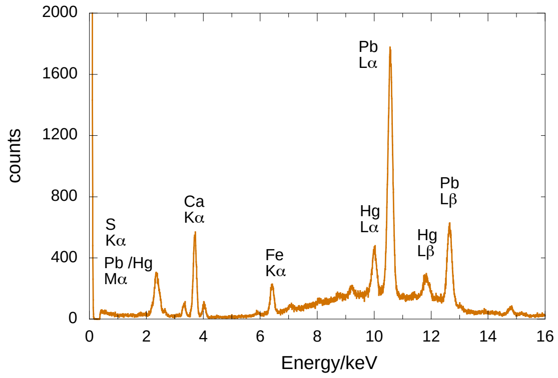
orange colour, *Apocalypse*, *f.* 185v



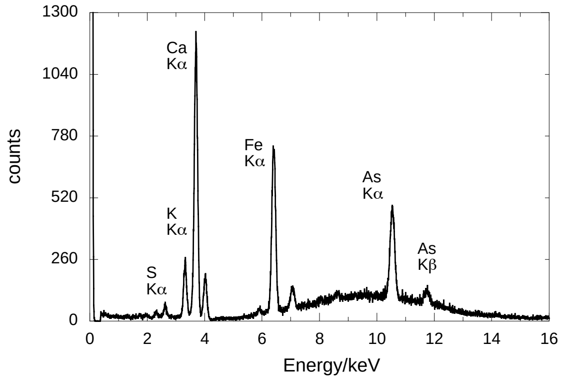
red colour, *Apocalypse*, *f.* 43



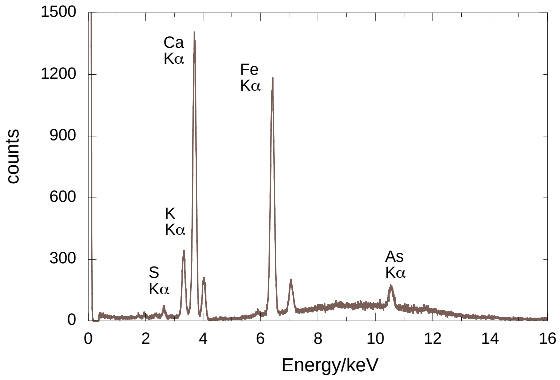
yellow colour, *Apocalypse*, *f.* 43



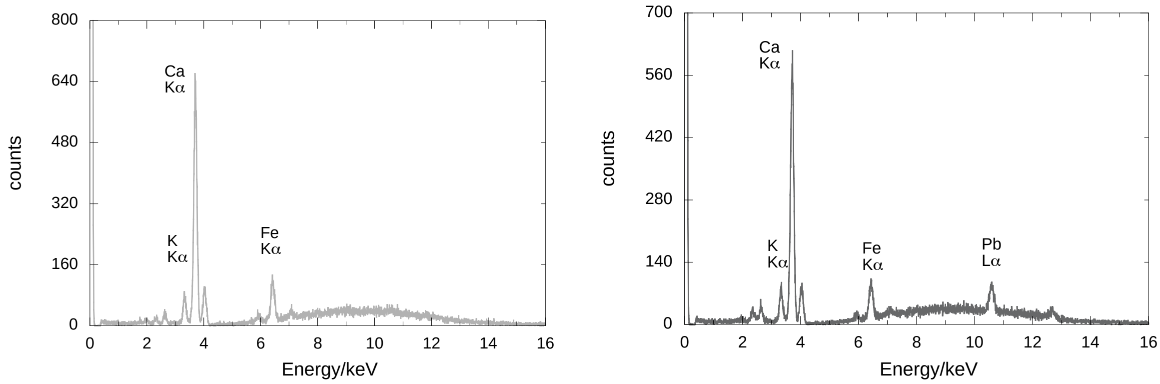
brown colour, *Apocalypse*, *f.* 177



black colour, *Apocalypse*, *f.* 172v



writing ink, *Apocalypse*, *f.* 43

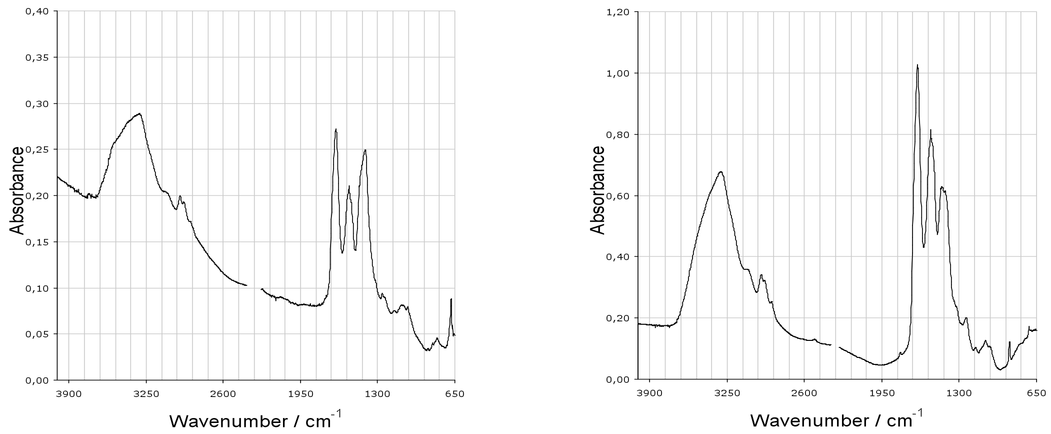


parchment, *Apocalypse*, *f.* 79

ruling, *Apocalypse*, *f.* 43.

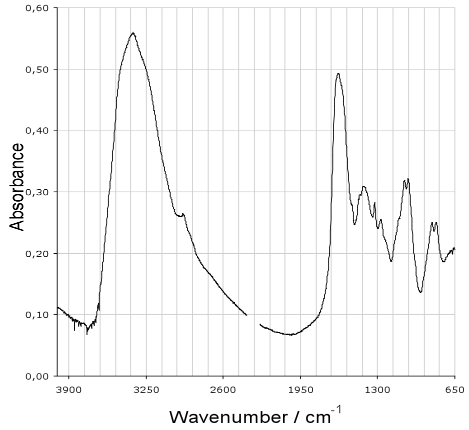
Figure II.20. Representative EDXRF spectra of the orange, red, yellow, brown and black colours, writing ink, parchment and ruling of *Apocalypse*.

µ-FTIR



orange colour (mixed with **white lead**),
Apocalypse, *f.* 200

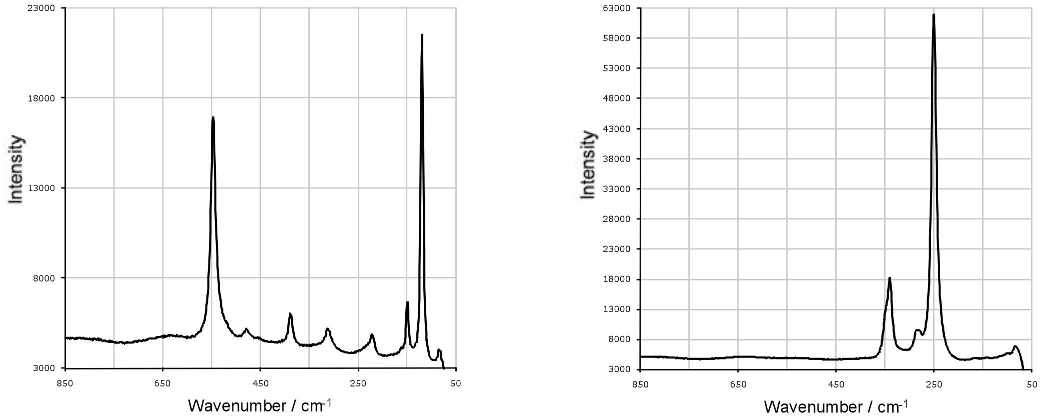
red colour (mixed with **calcium carbonate**),
Apocalypse, *f.* 185v



black colour (mixed with **calcium carbonate**),
Apocalypse, *f.* 172

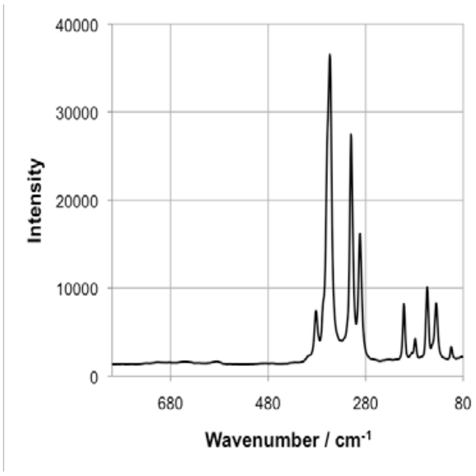
Figure II.21. Representative FTIR spectra of the orange, red and black colours of *Apocalypse*.

µ-Raman



minium in orange colour, *Apocalypse*, *f.* 185v

vermillion in red colour, *Apocalypse*, *f.* 177

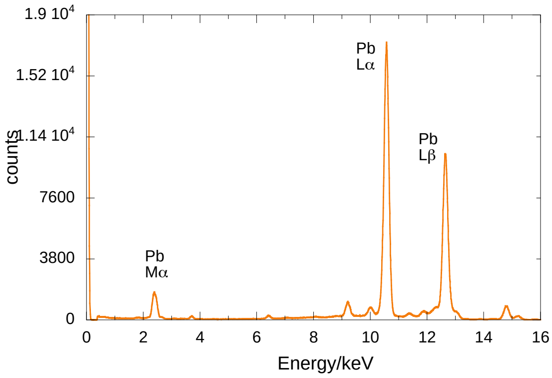


orpiment in yellow colour, *Apocalypse*, *f.* 43

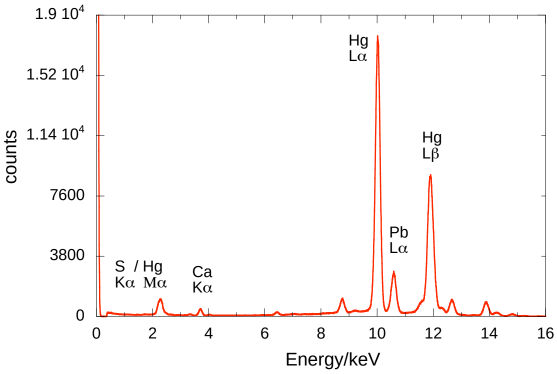
Figure II.22. Representative Raman spectra of the orange, red and yellow colours, of *Apocalypse*.

Book of Birds

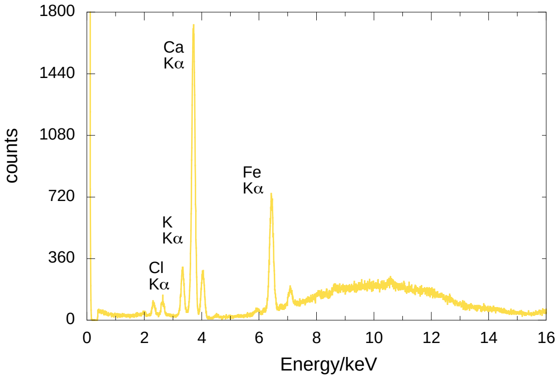
µ-EDXRF



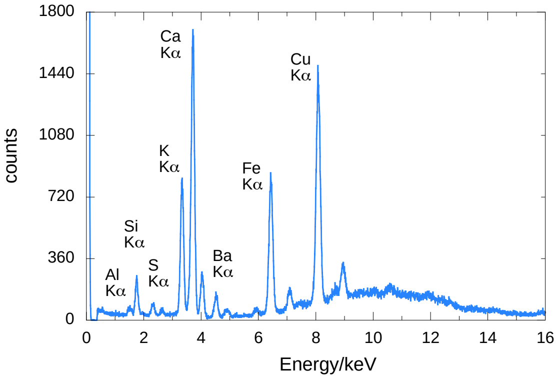
orange colour, *Book of Birds*, *f.* 4



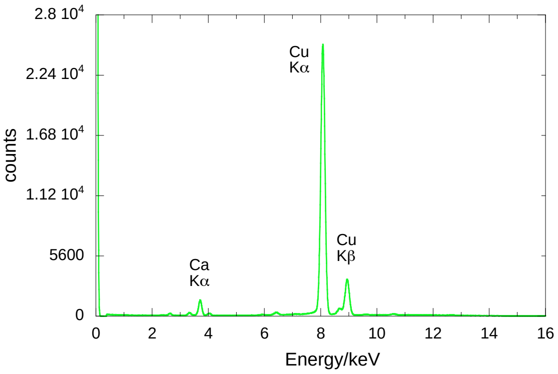
red colour, *Book of Birds*, *f.* 6



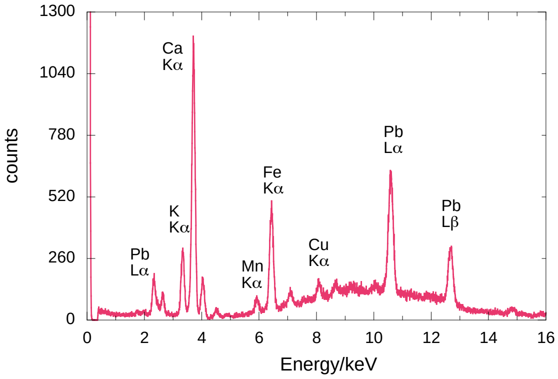
yellow colour, *Book, Birds*, *f.* 72v



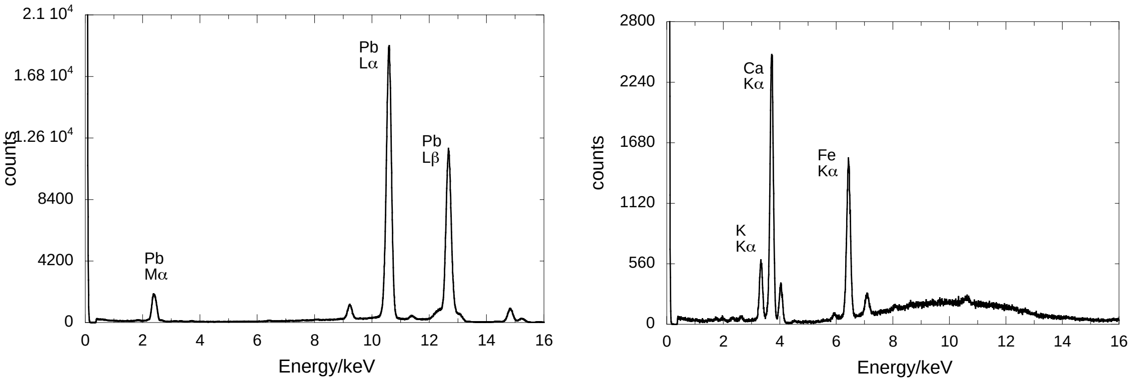
blue colour, *Book of Birds*, *f.* 7v



green colour, *Book of Birds*, *f.* 20v

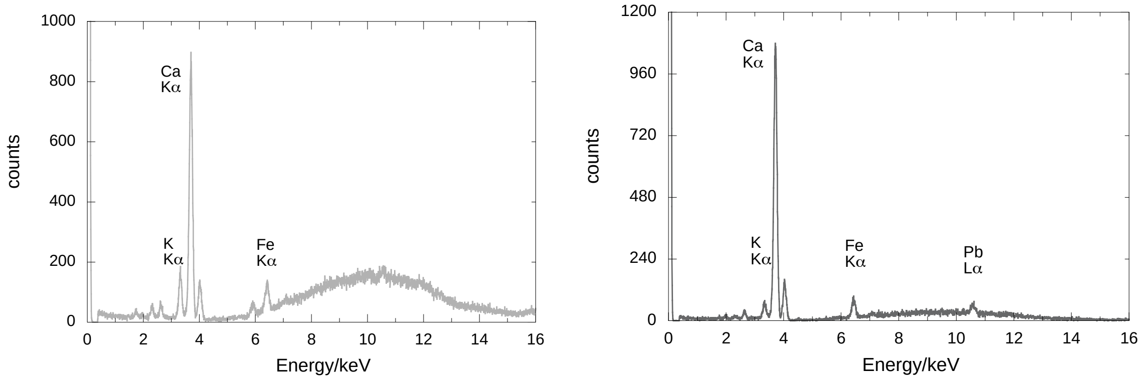


carmine-coloured, *Book of Birds*, *f.* 6



white colour, *Book of Birds*, *f.* 56v

black colour, *Book of Birds*, *f.* 50v

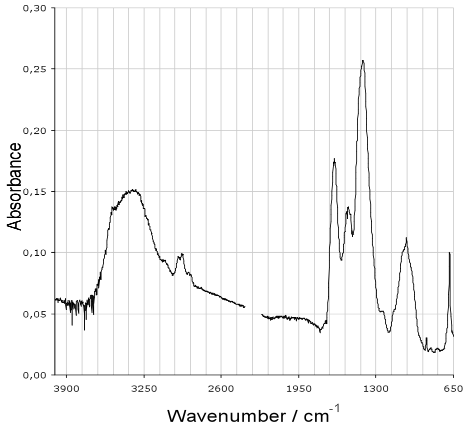


parchment, *Book of Birds*, *f.* 33

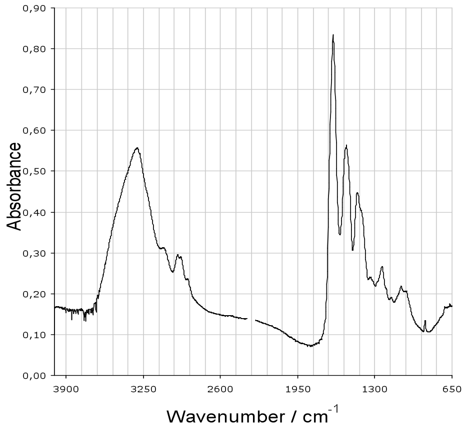
ruling, *Book of Birds*, *f.* 33

Figure II.23. Representative EDXRF spectra of the orange, red, yellow, blue, green, carmine-coloured, white and black colours, parchment and ruling of the *Book of Birds*.

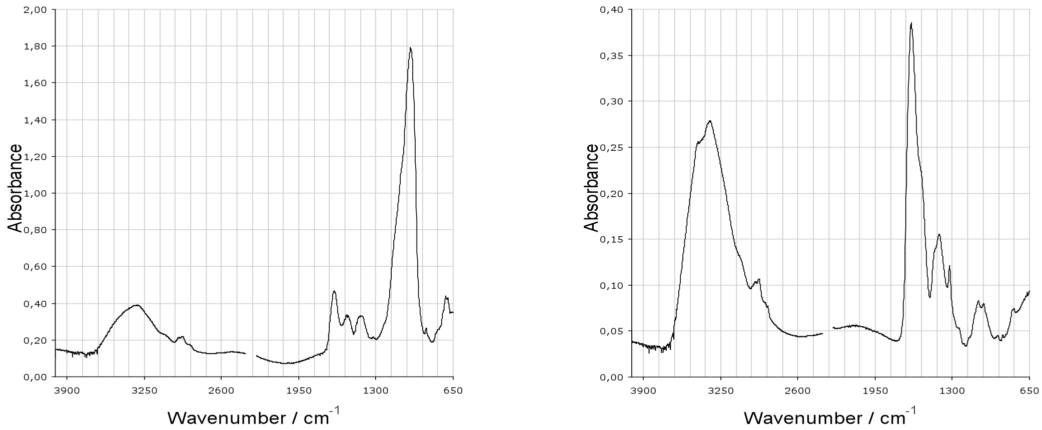
μ-FTIR



orange colour (mixed with **calcium carbonate** and **white lead**), *Book of Birds*, *f.* 4

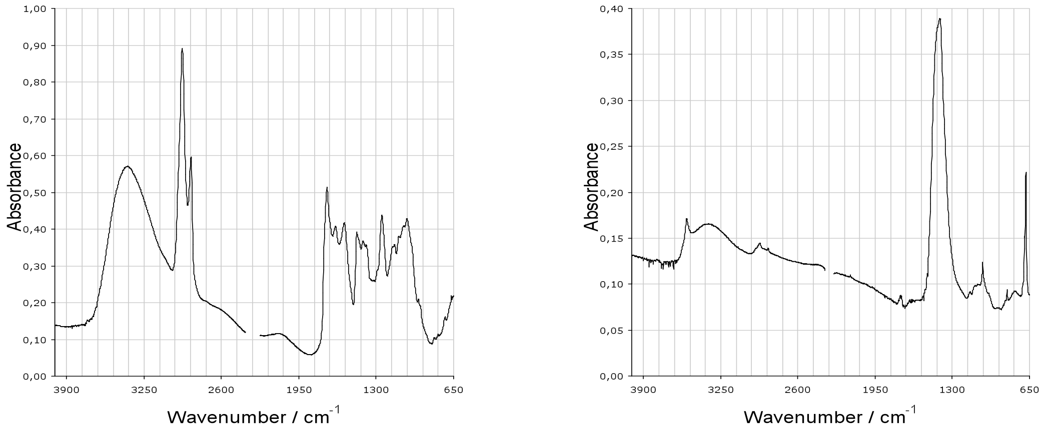


yellow colour (**calcium carbonate** identified), *Book of Birds*, *f.* 72v



blue colour (**lapis lazuli** and **calcium carbonate**), *Book of Birds*, *f.* 7v

green colour, *Book of Birds*, *f.* 20v

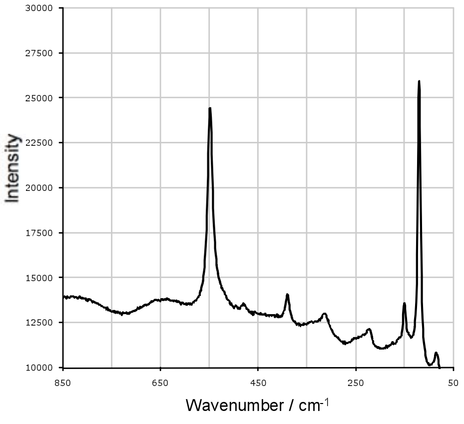


carmine-coloured, *Book of Birds*, *f.* 6

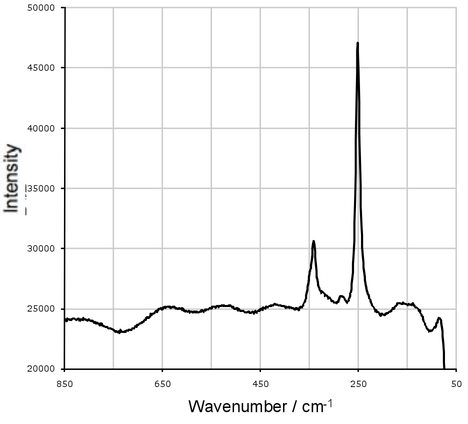
white colour (**white lead**), *Book of Birds*, *f.* 56v

Figure II.24. Representative FTIR spectra of the orange, yellow, blue, green, carmine-coloured and white colours of the *Book of Birds*.

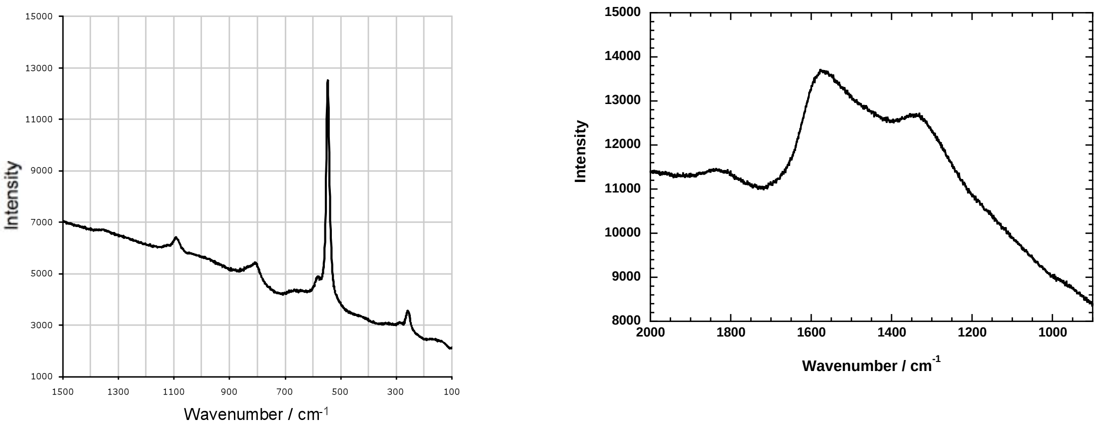
µ-Raman



minium in orange colour, *Book of Birds*, *f.* 4



vermillion in red colour, *Book of Birds*, *f.* 6v

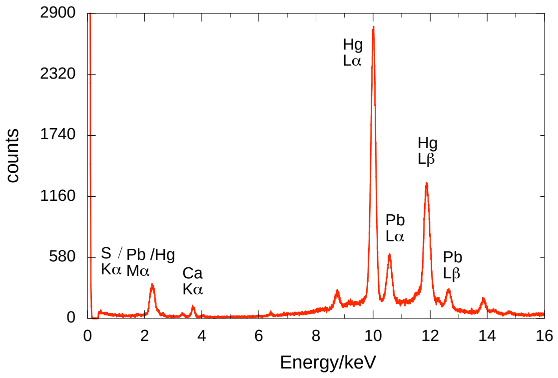


lapis lazuli in blue colour, *Book of Birds*, *f.* 4

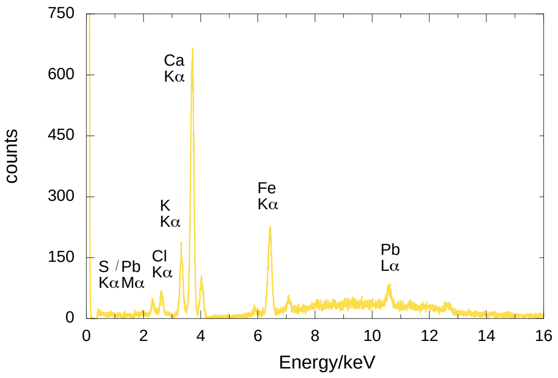
Figure II.25. Representative Raman spectra of the orange, red, blue and black colours of the *Book of Birds*.

Lorvão 3 – Salterium

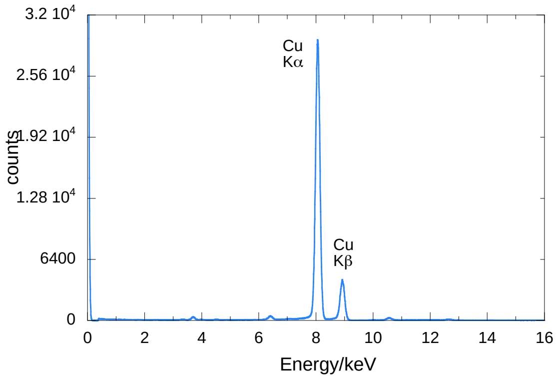
<u>µ-EDXRF</u>



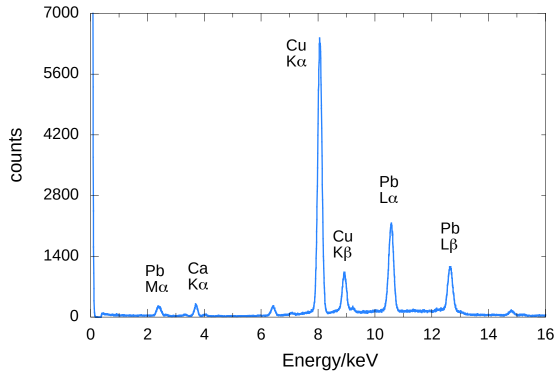
red colour,*Lorvão 3*, *f.* 8v



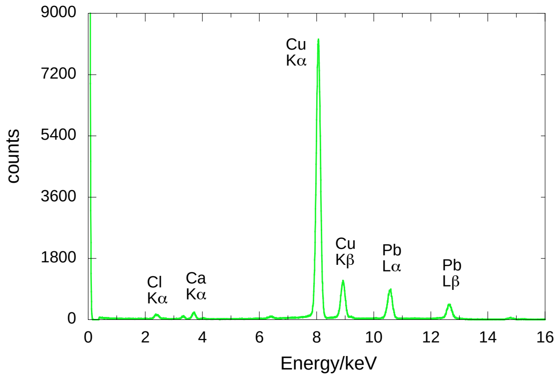
yellowish colour, *Lorvão 3*, *f.* 36v



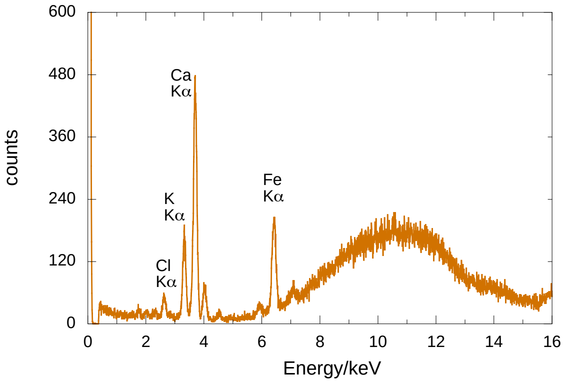
blue colour, *Lorvão 3*, *f.* 59v



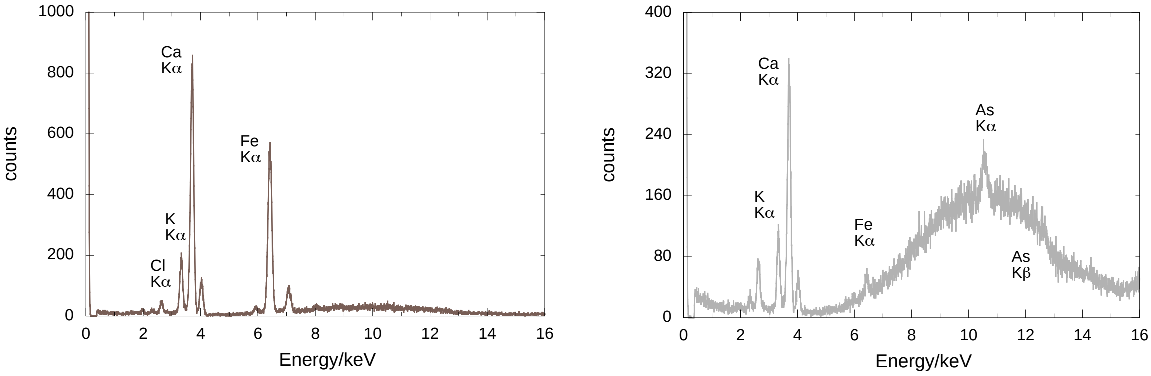
blue colour, *Lorvão 3*, *f.* 46v



green colour, *Lorvão 3*, *f.* 107v



brown colour, *Lorvão 3*, *f.* 107

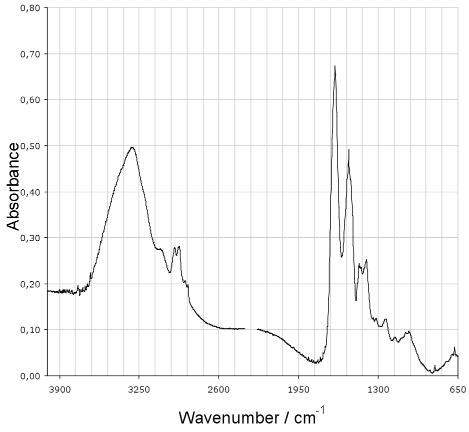


writing ink, *Lorvão 3*, *f.* 107

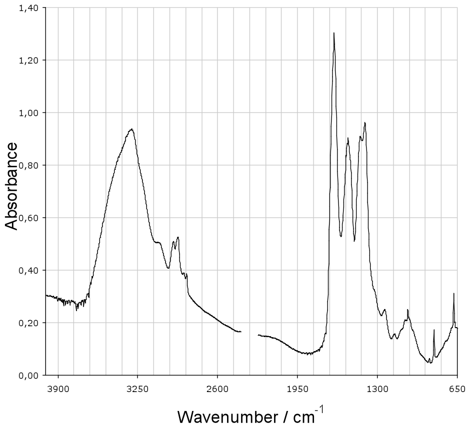
parchment, *Lorvão 3*, *f.* 8v

Figure II.26. Representative EDXRF spectra of the red, yellow, 2 different blues, green and brown colours, writing ink and parchment of *Lorvão 3*.

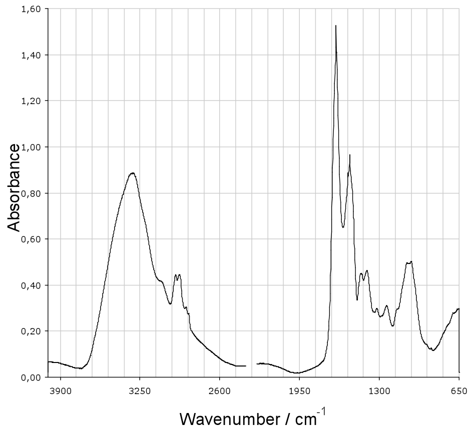
μ -FTIR



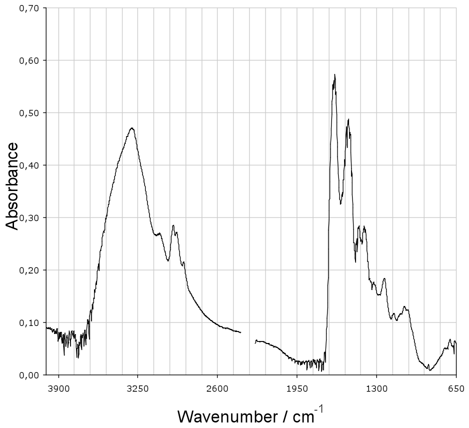
red colour with**calcium carbonate** and **white lead**, *Lorvão 3*, *f.* 109v



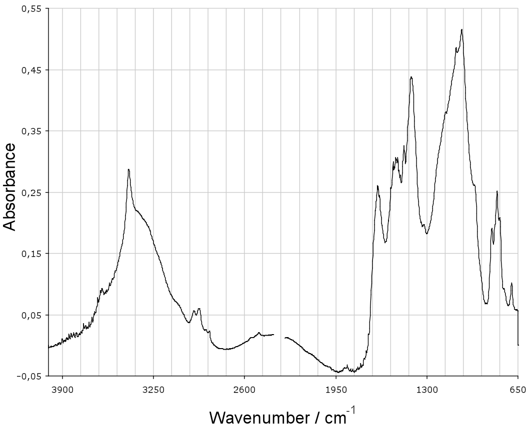
red colour with **calcium carbonate** and **white lead**, *Lorvão 3*, *f.* 139v



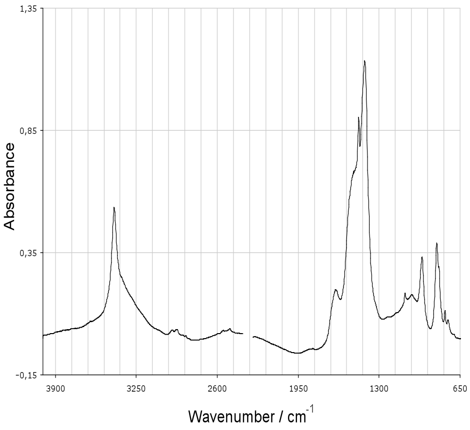
yellowish colour, revealing only the binder, *Lorvão 3*, *f.* 16



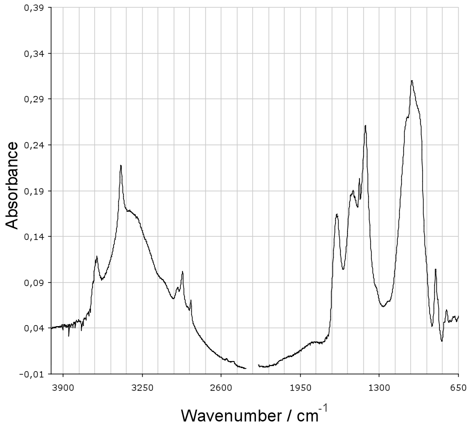
yellowish colour with **calcium sulphate**, *Lorvão 3*, *f.* 17v



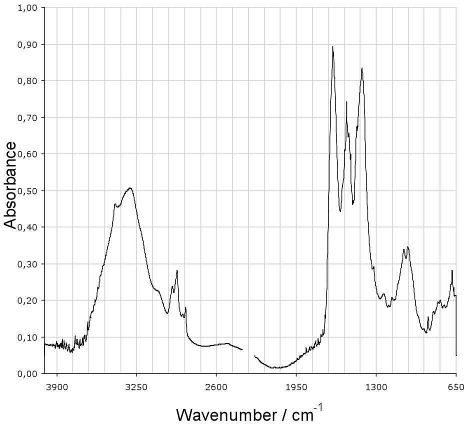
lapis lazuli, **azurite** and **calcium carbonate** in the blue colour, *Lorvão 3*, *f.* 14v



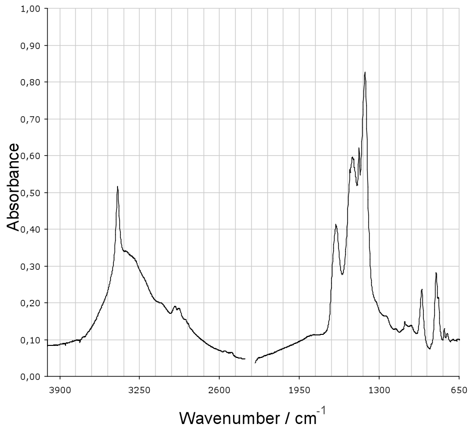
blue colour with **azurite**, *Lorvão 3*, *f.* 14v



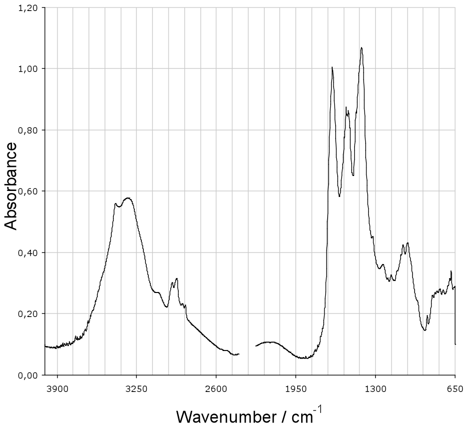
blue colour with **lapis lazuli**, **azurite**, and **calcium carbonate**, *Lorvão 3*, *f.* 59v



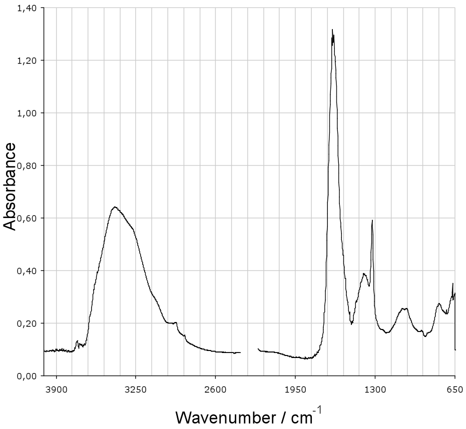
blue colour with **indigo**, **azurite** and some mixed **white lead**, *Lorvão 3*, *f.* 46v



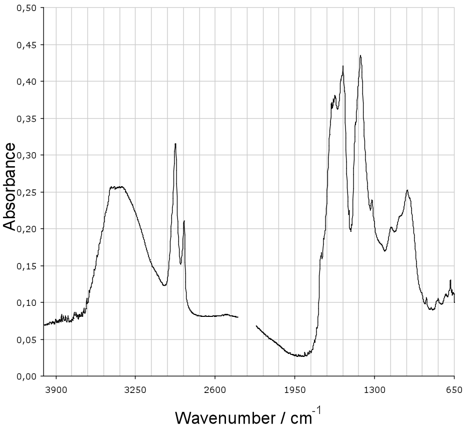
blue colour with **azurite**, *Lorvão 3*, *f.* 23



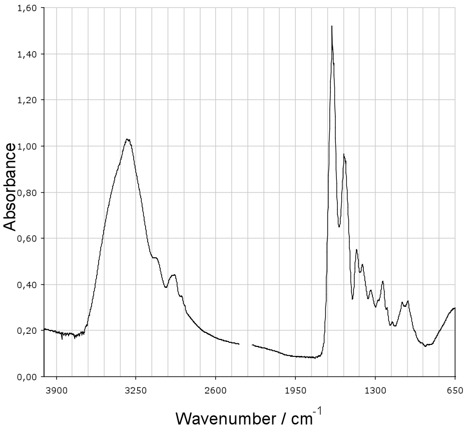
blue colour with **indigo**, **lapis lazuli** and **azurite**, *Lorvão 3*, *f.* 17v



green colour, *Lorvão 3*, *f.* 82v



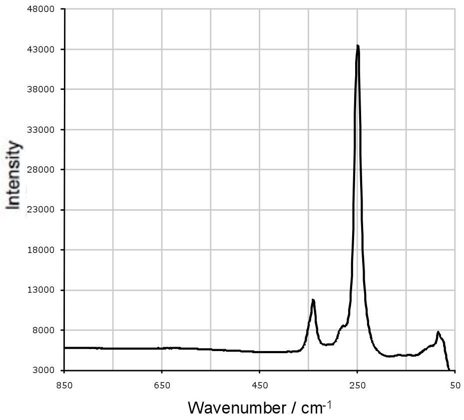
green colour, *Lorvão 3*, *f.* 107v



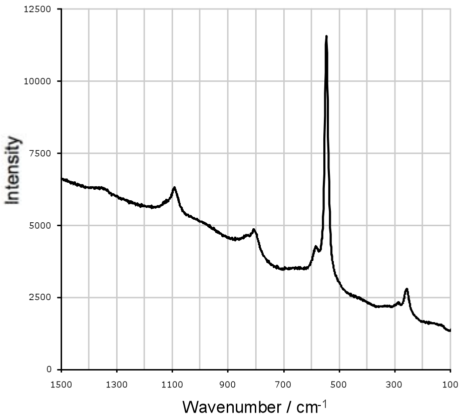
brown colour, *Lorvão 3*, *f.* 107

Figure II.27. Representative FTIR spectra of two reds, two yellows, four blues, two greens and brown colours of *Lorvão 3*.

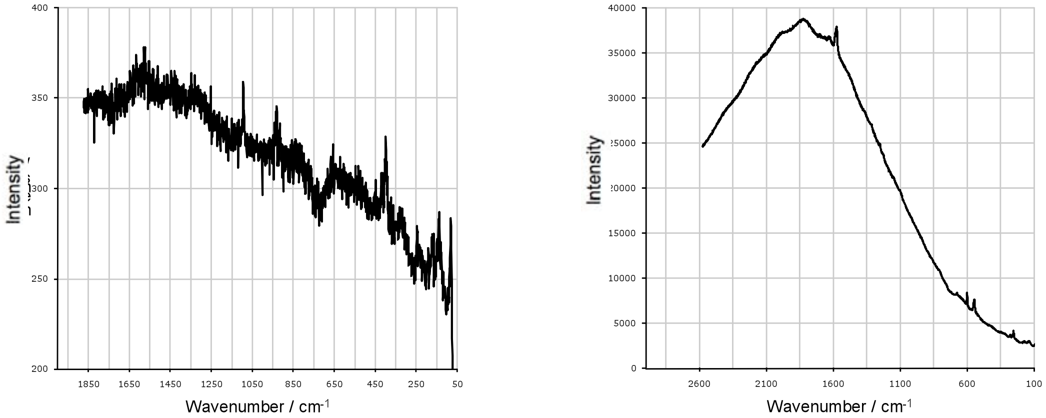
µ-Raman



vermillion in red colour, *Lorvão 3*, *f.* 107v

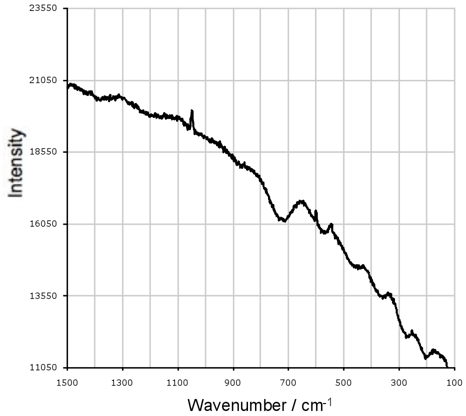


lapis lazuli in blue colour, *Lorvão 3*, *f.* 54v



azurite in blue colour, *Lorvão 3*, *f.* 54v

indigo in blue colour, *Lorvão 3*, *f.* 107v

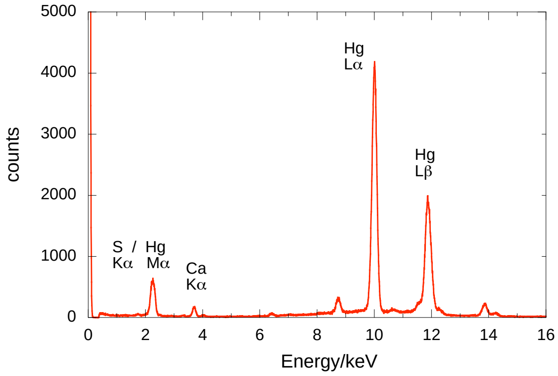


white lead in white colour, *Lorvão 3*, *f.* 146v

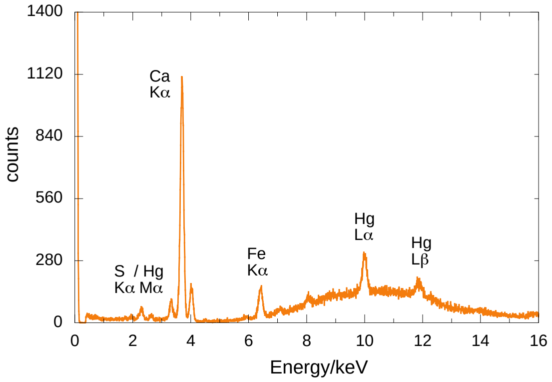
Figure II.28. Representative Raman spectra of the red, three different blues and white colours of the *Lorvão 3*.

Lorvão 12 – Saint lectionary

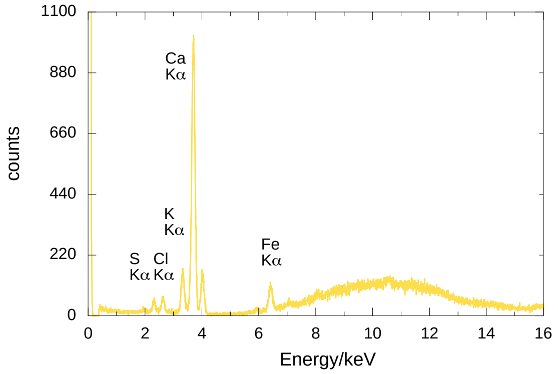
μ-EDXRF



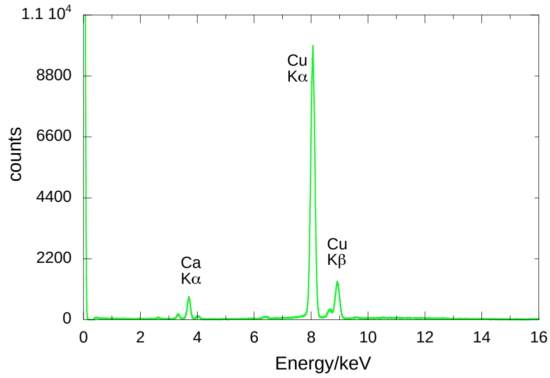
red colour, *Lorvão 12*, *f.* 17



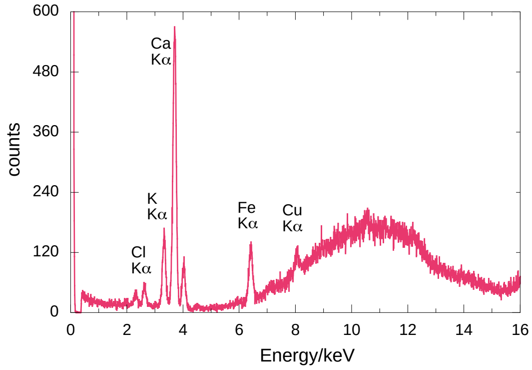
orange colour, *Lorvão 12*, *f.* 6v



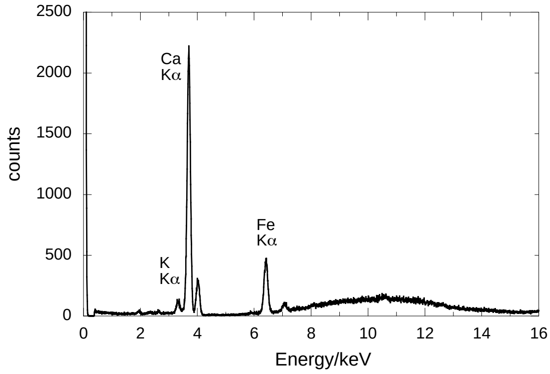
yellowish colour, *Lorvão 12*, *f.* 94



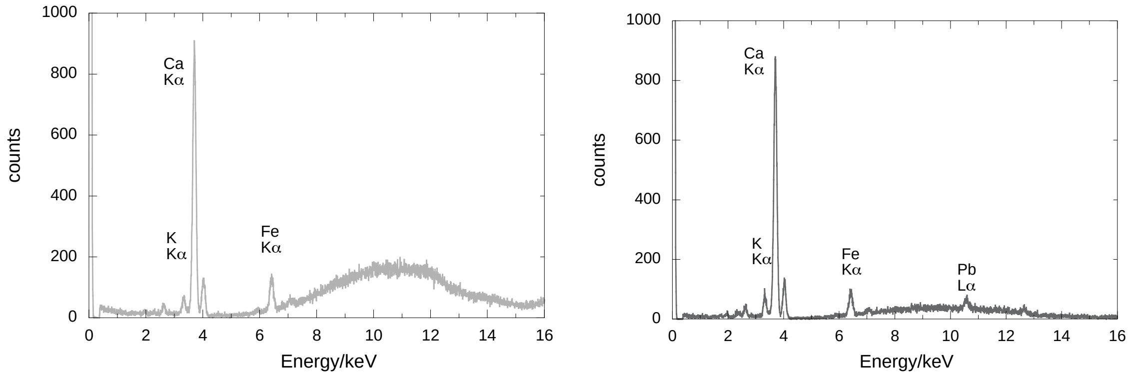
green colour, *Lorvão 12*, *f.* 17



carmine-coloured, *Lorvão 12*, *f.* 30



black colour, *Lorvão 12*, *f.* 6v

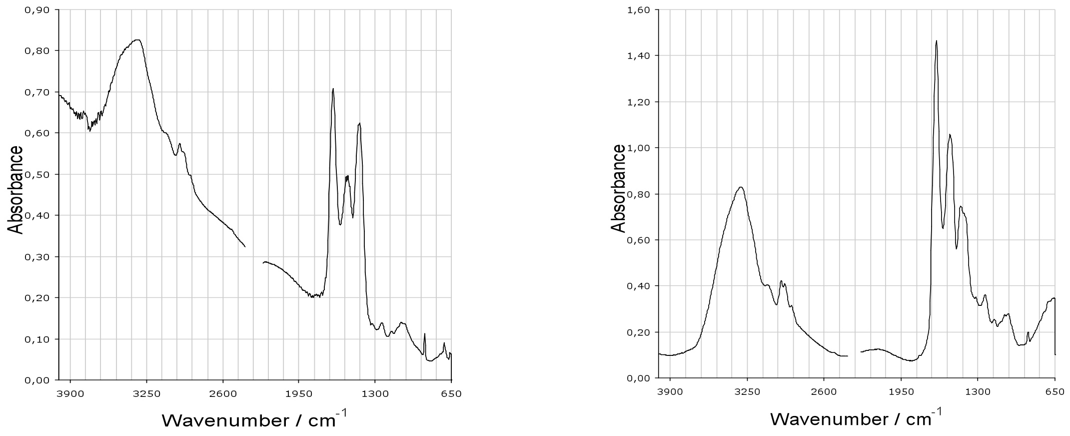


parchment, *Lorvão 12*, *f.* 11

ruling, *Lorvão 12*, *f.* 50

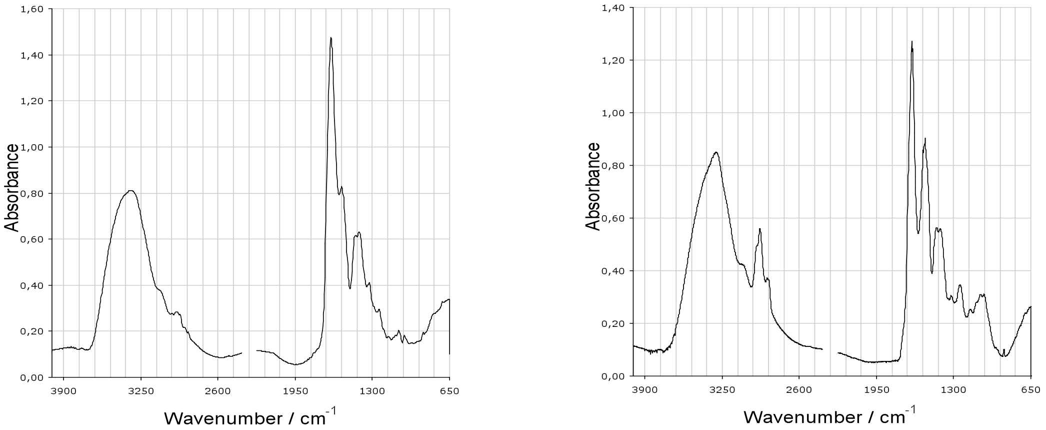
Figure II.29. Representative EDXRF spectra of the red, orange, yellow, green, carmine-coloured and black colours, parchment and ruling of *Lorvão 12*.

μ -FTIR



mixture of **calcium carbonate** in red colour,
Lorvão 12, *f.* 17

mixture of **calcium carbonate** in yellow colour,
Lorvão 12, *f.* 94

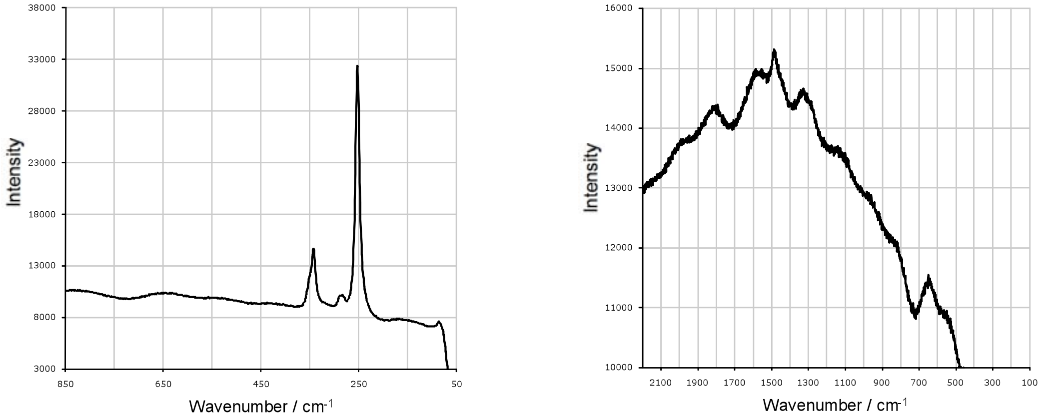


synthetic copper based green colour,
Lorvão 12, *f.* 17

lac dye with a small amount of **calcium carbonate** in
carmine-coloured, *Lorvão 12*, *f.* 17

Figure II.30. Representative FTIR spectra of the red, yellow, green and carmine-coloured colours of *Lorvão 12*.

μ-Raman



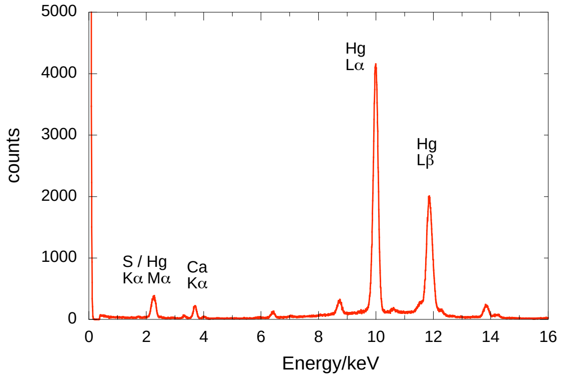
vermillion in red colour, *Lorvão 12*, *f.* 6v

carbon black in black colour, *Lorvão 12*, *f.* 6v

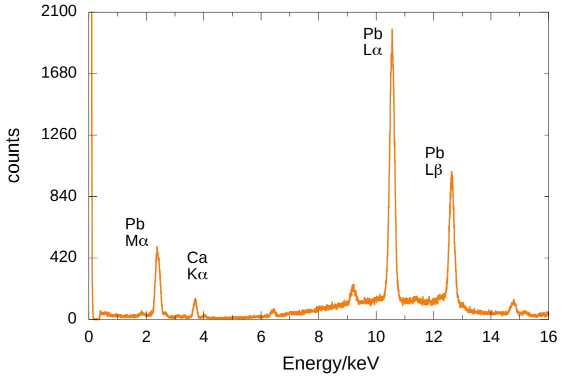
Figure II.31. Representative Raman spectra of the red and black colours of *Lorvão 12.*

Lorvão 13 – Temporal lectionary

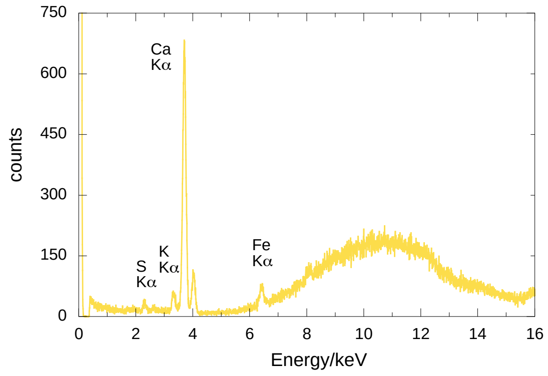
μ-EDXRF



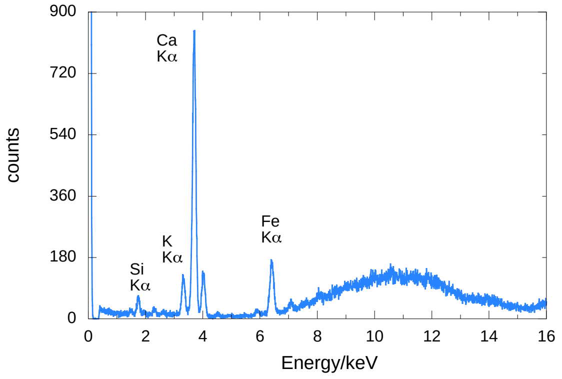
red colour, *Lorvão 13*, *f.* 6v



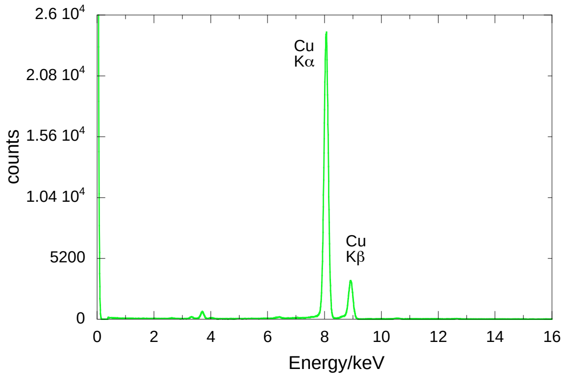
orange colour, *Lorvão 13*, *f.* 6v



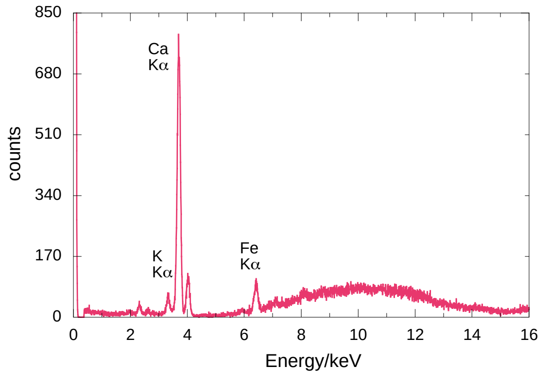
yellowish colour, *Lorvão 13*, *f.* 85



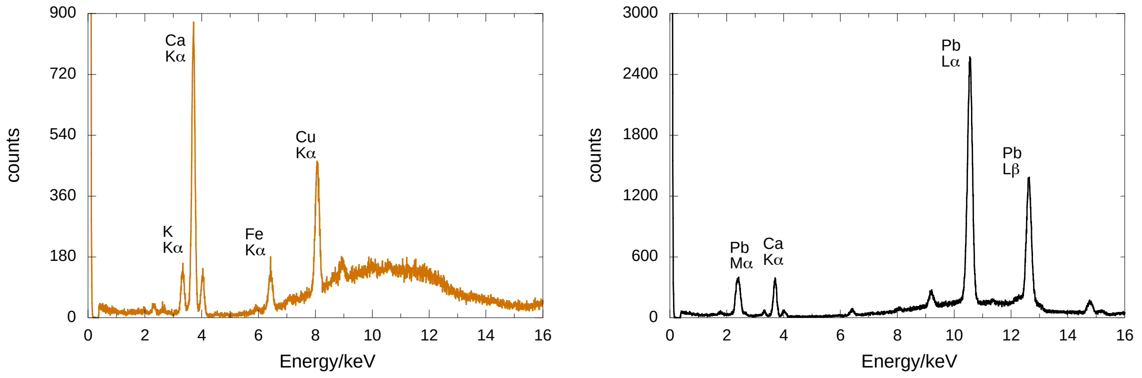
blue colour, *Lorvão 13*, *f.* 6v



green colour, *Lorvão 13*, *f.* 44v

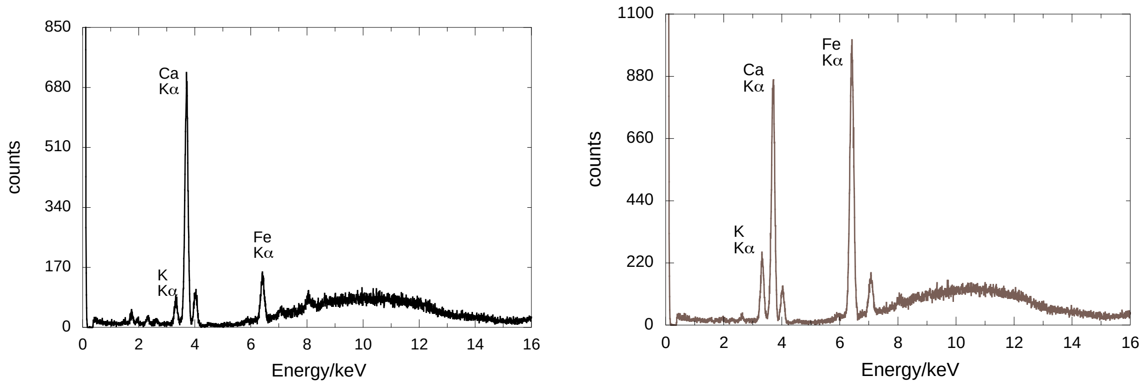


carmine-coloured, *Lorvão 13*, *f.* 30



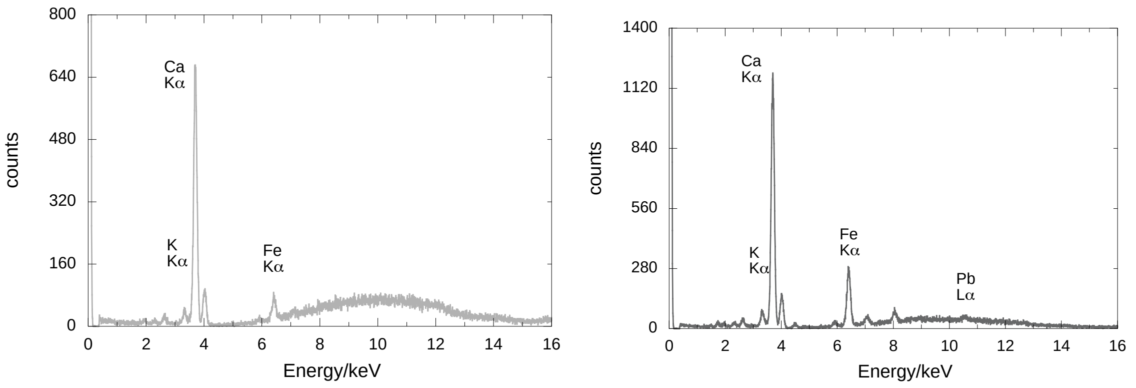
brown colour, *Lorvão 13*, *f.* 92v

white colour, *Lorvão 13*, *f.* 1v



black colour, *Lorvão 13*, *f.* 30

writing ink, *Lorvão 13*, *f.* 6v

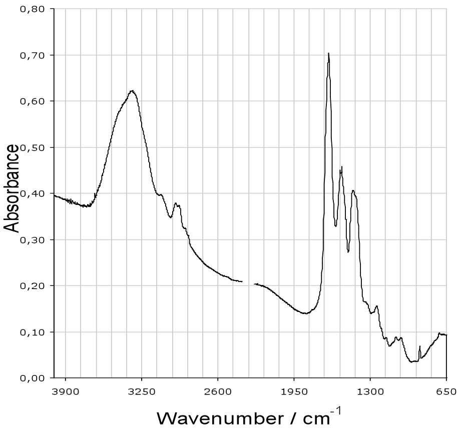


parchment *Lorvão 13*, *f.* 30

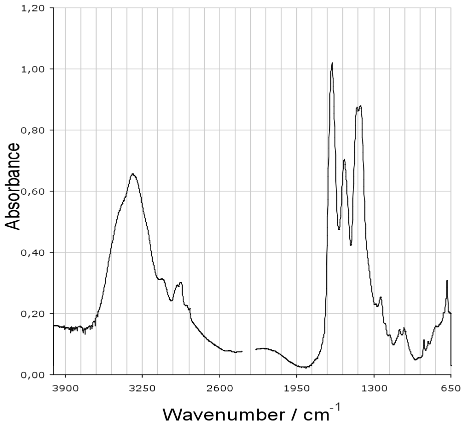
ruling, *Lorvão 13*, *f.* 1v

Figure II.32. Representative EDXRF spectra of the red, orange, yellow, blue, green, carmine-coloured, brown, white and black colours, writing ink, parchment and ruling of *Lorvão 13*.

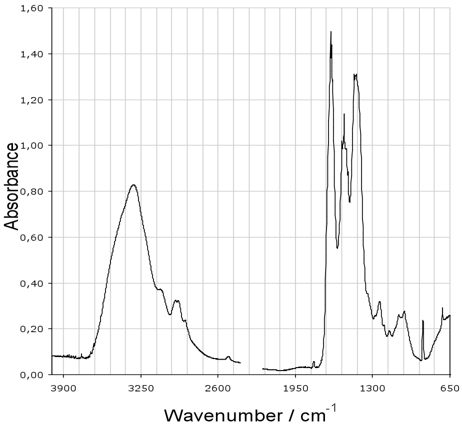
μ-FTIR



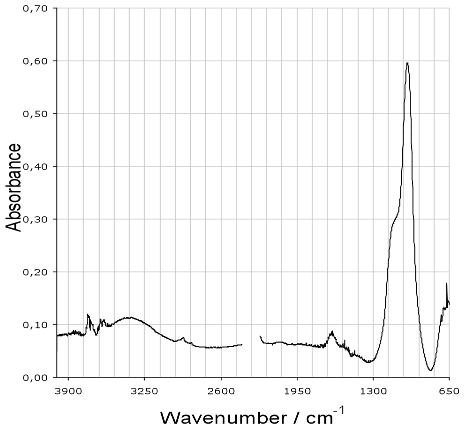
mixture of **calcium carbonate** in red colour, *Lorvão 13*, *f.* 6v



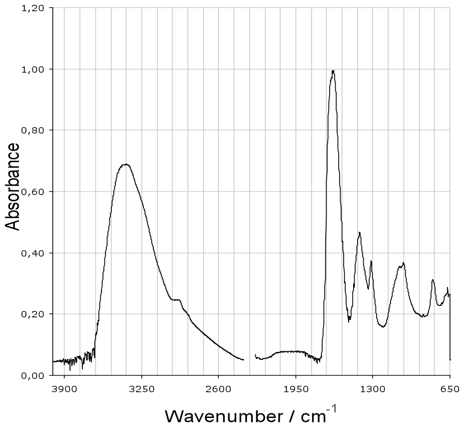
mixture of **white lead** and some **calcium carbonate** in orange colour, *Lorvão 13*, *f.* 6v



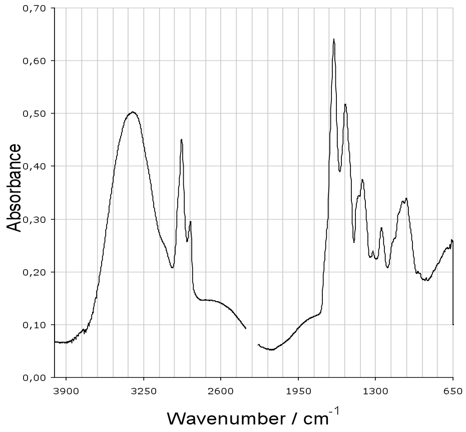
yellowish colour, *Lorvão 13*, *f.* 114



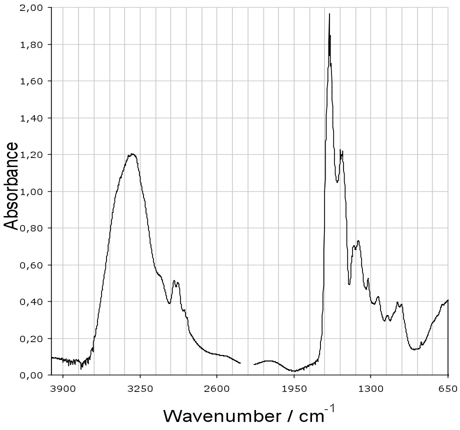
mixture of **lapis lazuli** in blue colour, *Lorvão 13*, *f.* 6v



synthetic copper based green colour, *Lorvão 13*, *f.* 44v



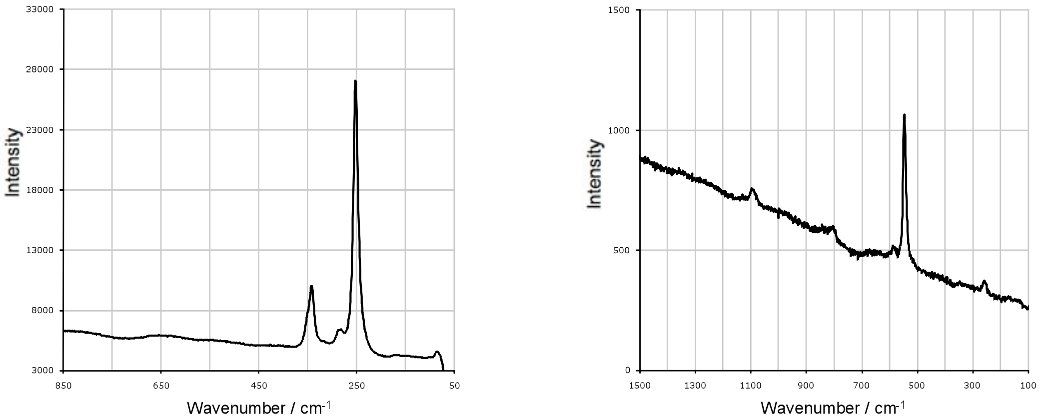
lac dye in carmine-coloured, *Lorvão 13*, *f.* 30



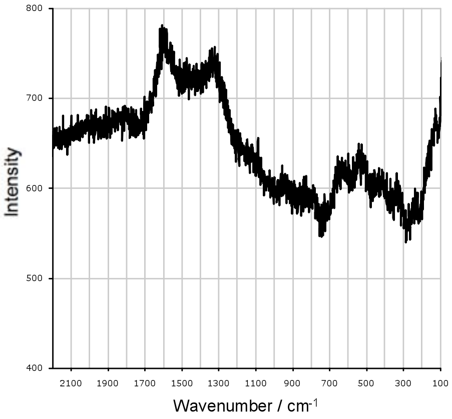
brown colour, *Lorvão 13*, *f.* 92v

Figure II.33. Representative FTIR spectra of the red, orange, yellowish, blue, green, carmine-coloured and brown colours of *Lorvão 13*.

µ -Raman



vermillion in red colour, *Lorvão 13*, *f.* 92v **lapis lazuli** in blue colour, *Lorvão 13*, *f.* 92v

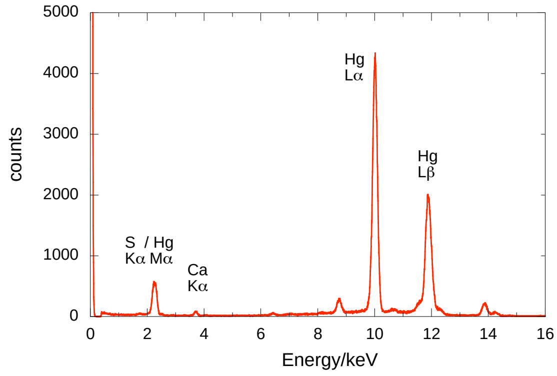


carbon black in black colour, *Lorvão 13*, *f.* 44v

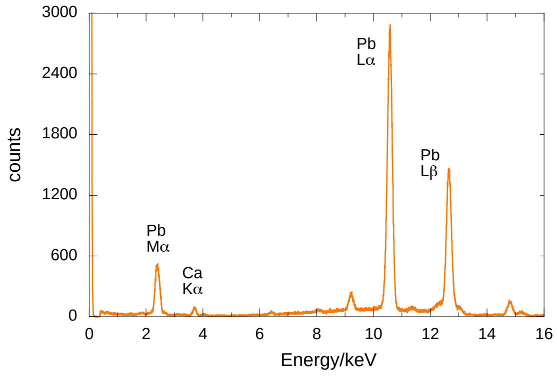
Figure II.34. Representative EDXRF spectra of the red, blue and black colours of *Lorvão 13*.

Lorvão 15 – Gradual

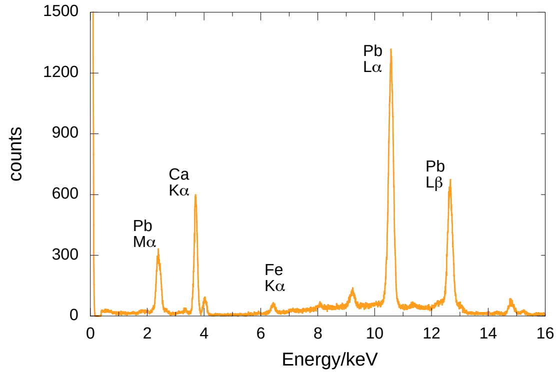
μ -EDXRF



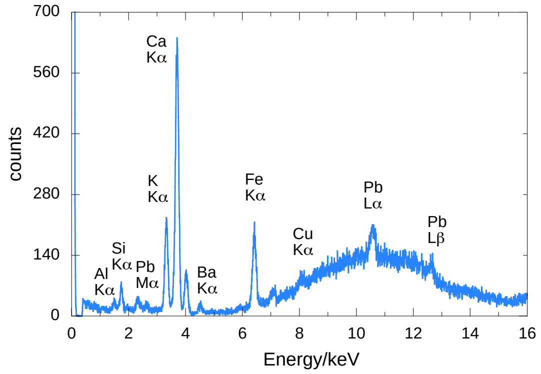
red colour, *Lorvão 15*, *f.* 26



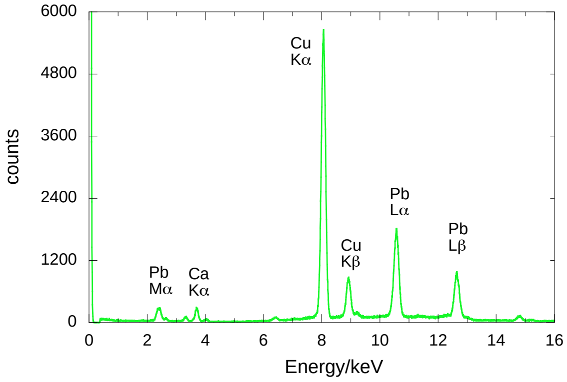
orange colour, *Lorvão 15*, *f.* 11



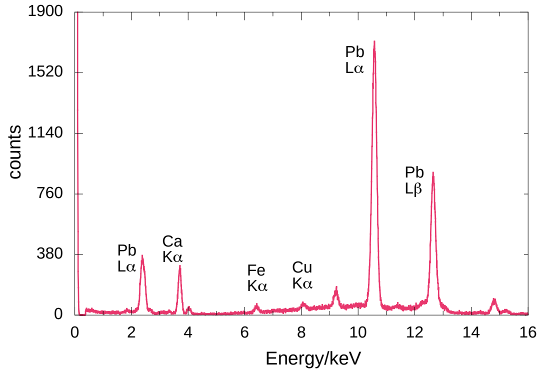
salmon colour, *Lorvão 15*, *f.* 26



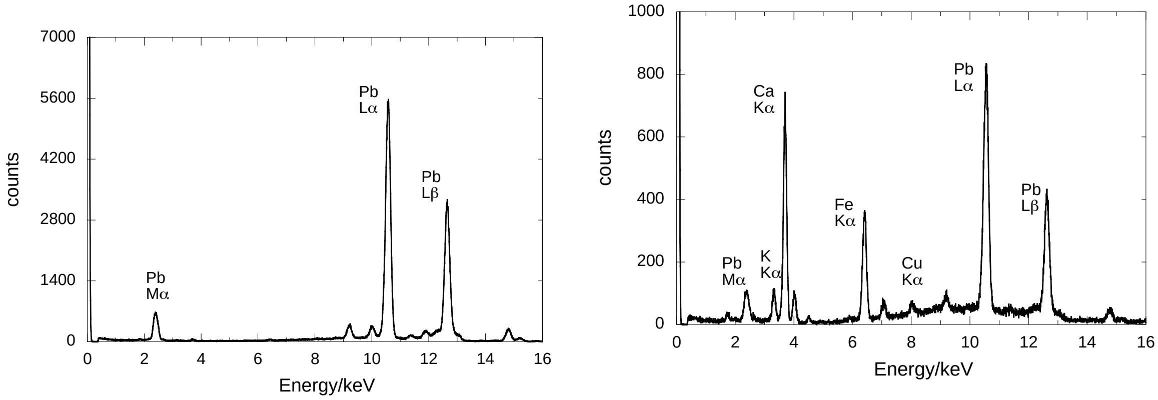
blue colour, *Lorvão 15*, *f.* 167v



green colour, *Lorvão 15*, *f.* 26

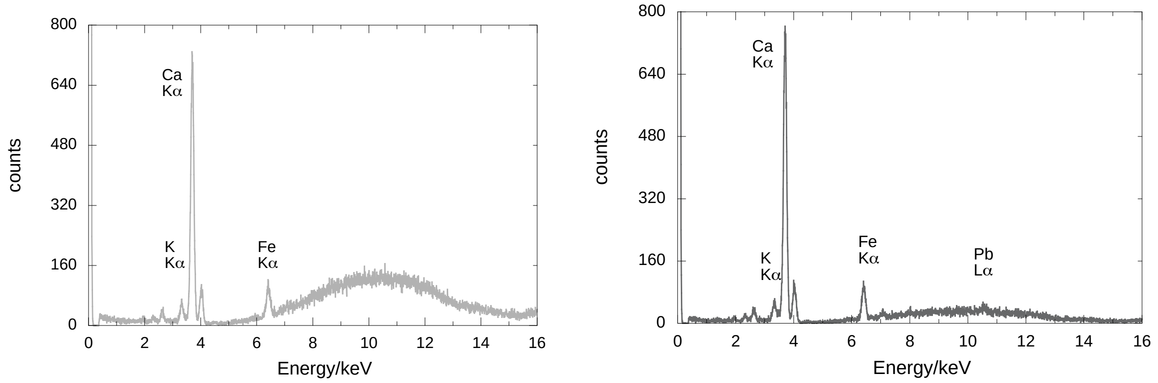


carmine-coloured, *Lorvão 15*, *f.* 26



white colour, *Lorvão 15*, *f.* 26

black colour, *Lorvão 15*, *f.* 50

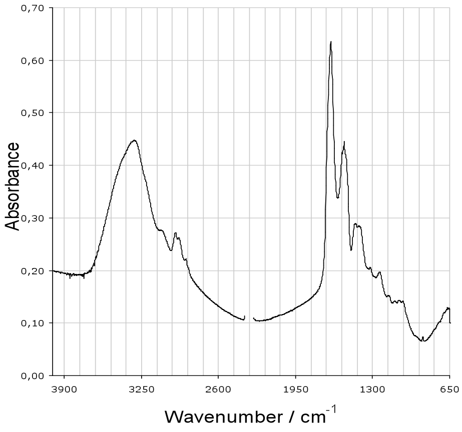


parchment, *Lorvão 15*, *f.* 125v

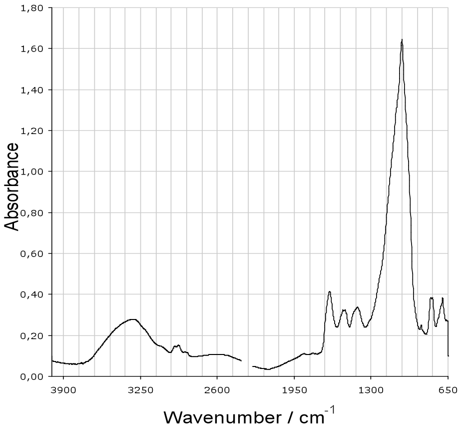
ruling, *Lorvão 15*, *f.* 125v

Figure II.35. Representative EDXRF spectra of the red, orange, salmon, blue, green, carmine-coloured, white and black colours, parchment and ruling (lines) of *Lorvão 15*.

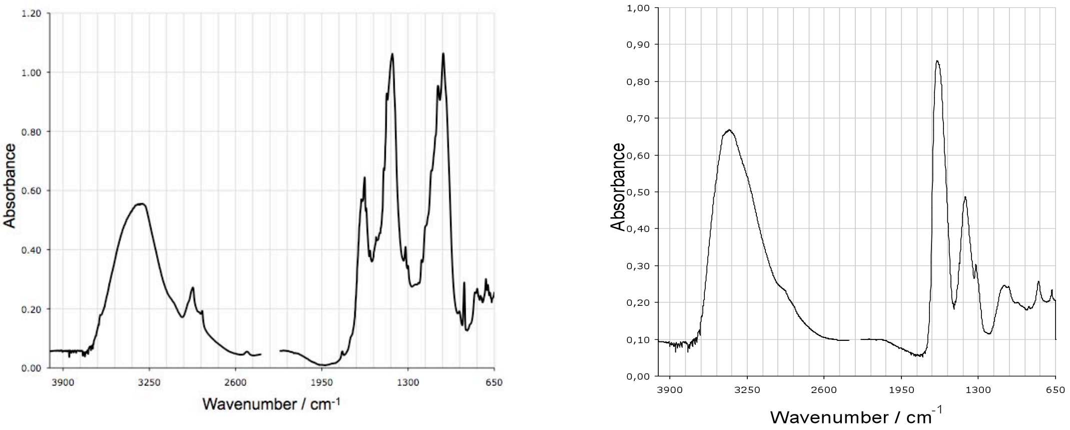
µ -FTIR



red colour mixed with a small amount of **calcium carbonate**, *Lorvão 15*, *f.* 26

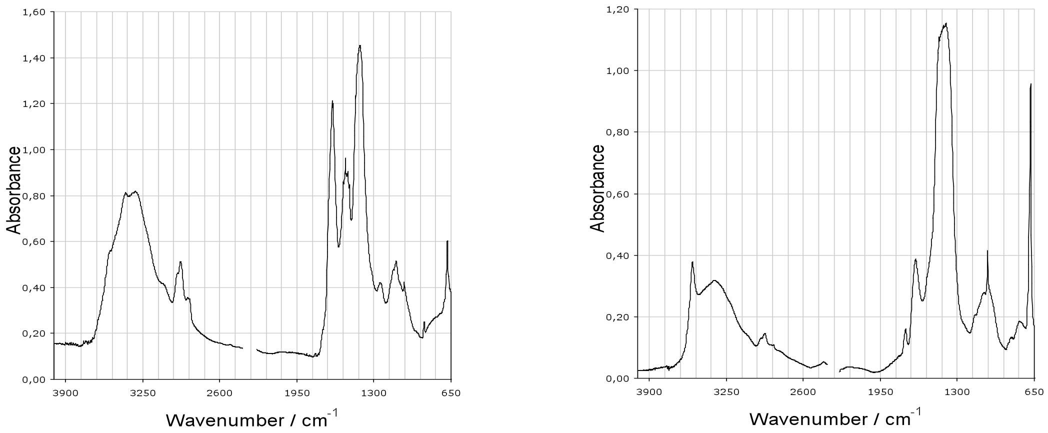


blue colour (**lapis lazuli** with some **calcium carbonate**), *Lorvão 15*, *f.* 26



blue colour (**lapis lazuli** with **indigo** and some **white lead**), *Lorvão 15*, *f.* 50

synthetic copper based green colour, *Lorvão 15*, *f.* 26

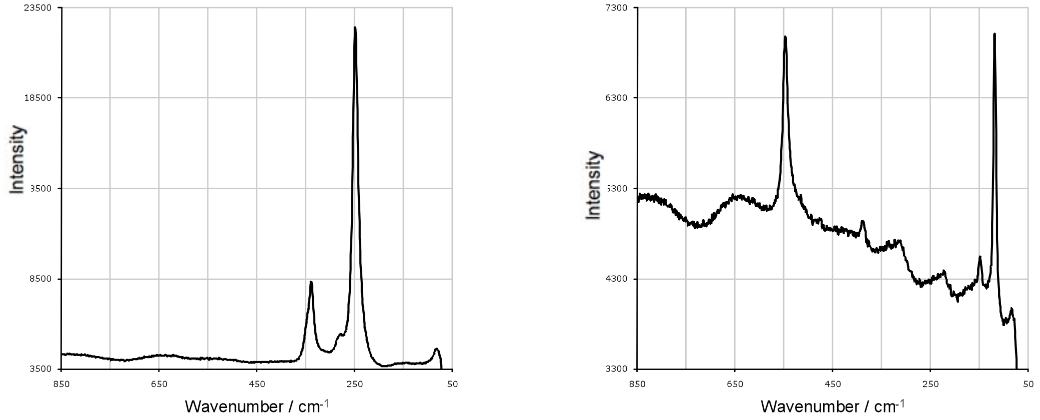


carmine-coloured mixed with a considerable amount of **white lead**, *Lorvão 15*, *f.* 26

white lead in white colour, *Lorvão 15*, *f.* 26

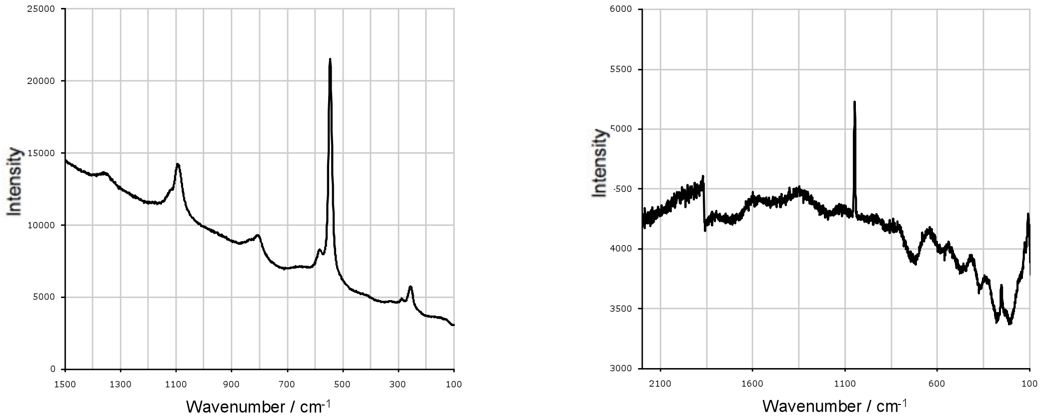
Figure II.36. Representative FTIR spectra of the red, two different blues, green, carmine-coloured and white colours of *Lorvão 15*.

µ -Raman



vermillion in red colour, *Lorvão 15*, *f.* 50

minium in orange colour, *Lorvão 15*, *f.* 167v



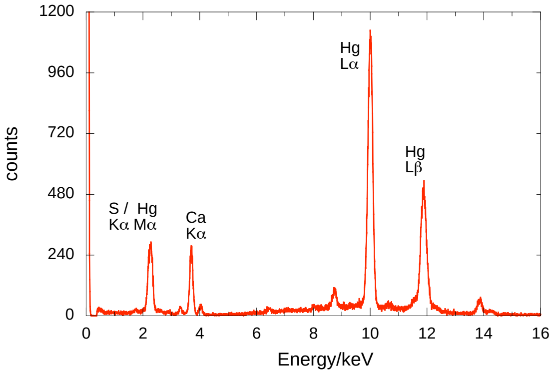
lapis lazuli in blue colour, *Lorvão 15*, *f.* 11

white lead in white colour, *Lorvão 15*, *f.* 167v

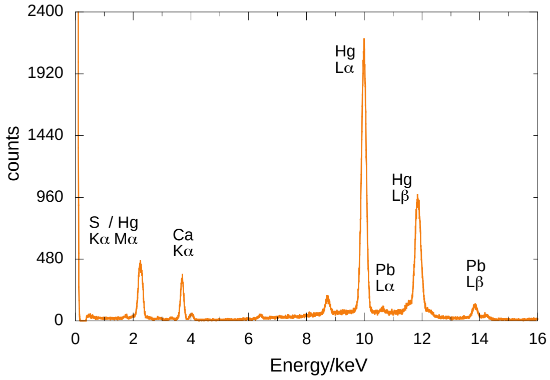
Figure II.37. Representative Raman spectra of the red, orange, blue and white colours of *Lorvão 15*.

Lorvão 16 – Martyrology

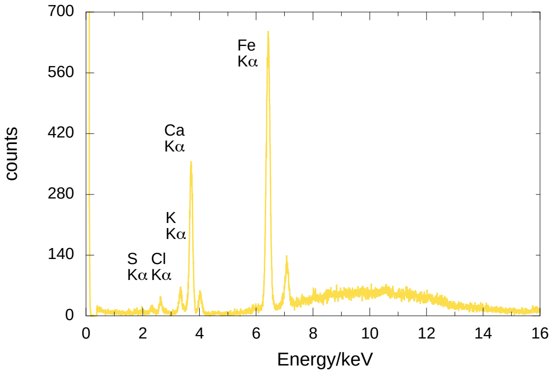
µ -EDXRF



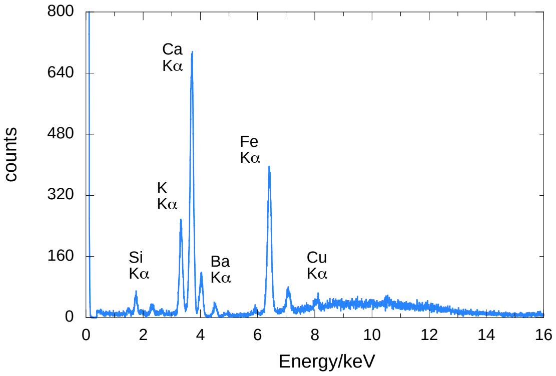
red colour, *Lorvão 16*, *f.* 126



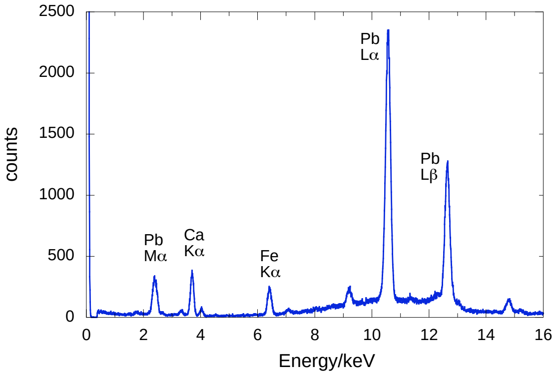
orange colour, *Lorvão 16*, *f.* 2v



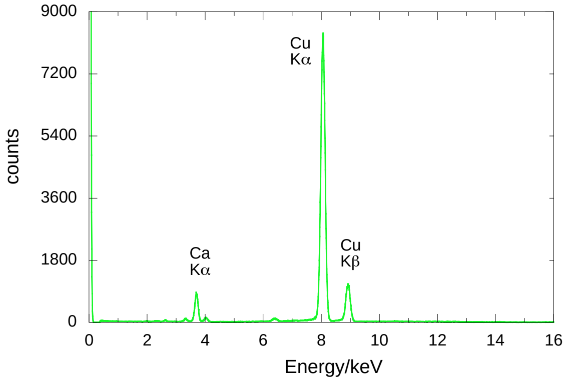
yellowish colour, *Lorvão 16*, *f.* 188



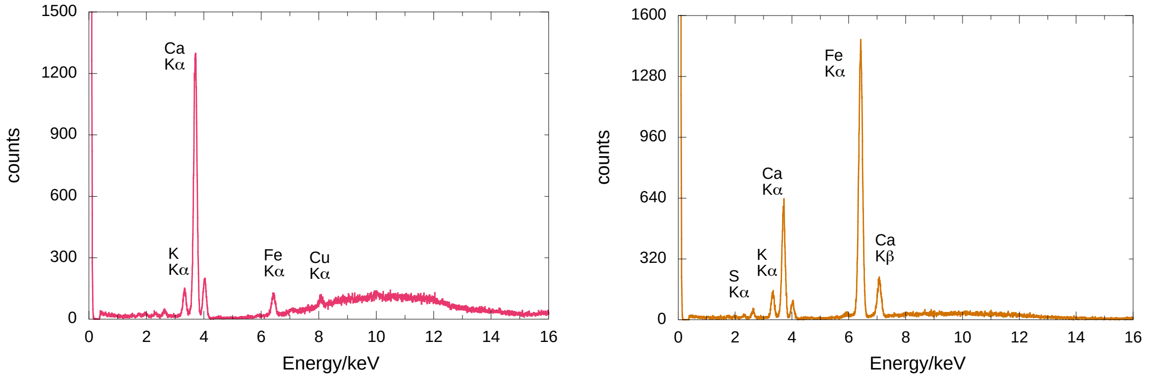
blue colour (lapis lazuli), *Lorvão 16*, *f.* 71v



deep blue colour (indigo), *Lorvão 16*, *f.* 73

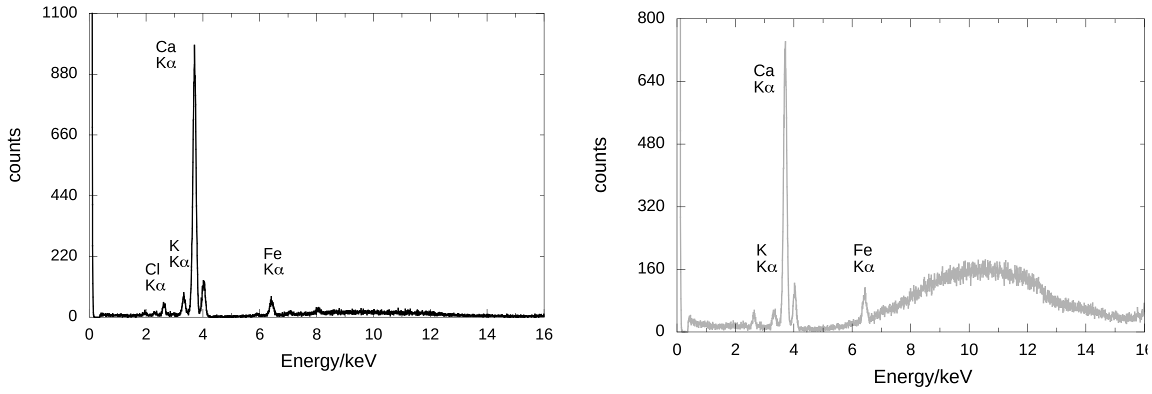


green colour, *Lorvão 16*, *f.* 59v



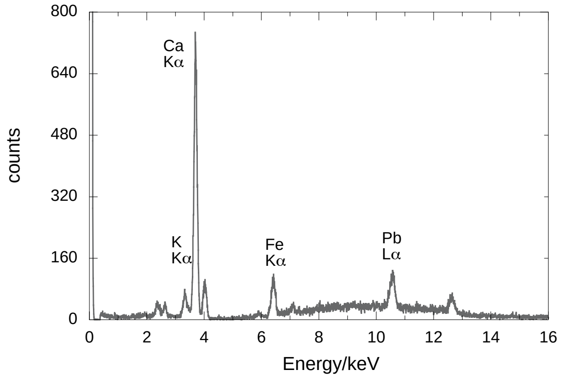
carmine-coloured, *Lorvão 16*, *f.* 165v

brownish colour, *Lorvão 16*, *f.* 155



illumination background*, *Lorvão 16*, *f.* 126

parchment, *Lorvão 16*, *f.* 188

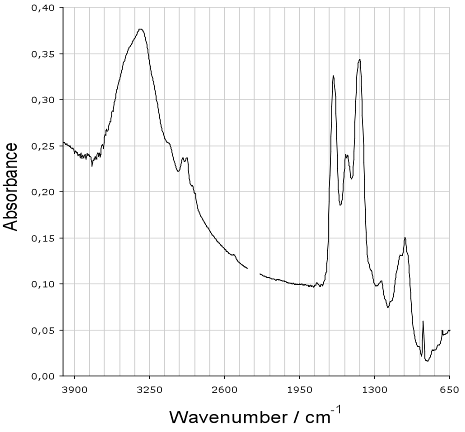


ruling, *Lorvão 16*, *f.* 188

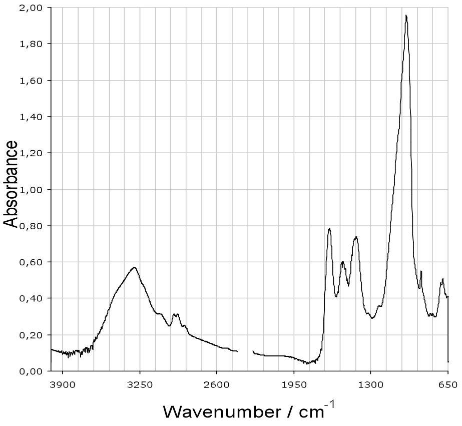
* The background colour is the white colour that is below the colouring layer. Where this layer has detached, we could be seeing the white preparation or the treatment given to the parchment before the pictorial layer is applied.

Figure II.38. Representative EDXRF spectra of the red, orange, yellow, blue, green, carmine-coloured and brownish colours, illumination backgound, parchment and ruling of *Lorvão 16*.

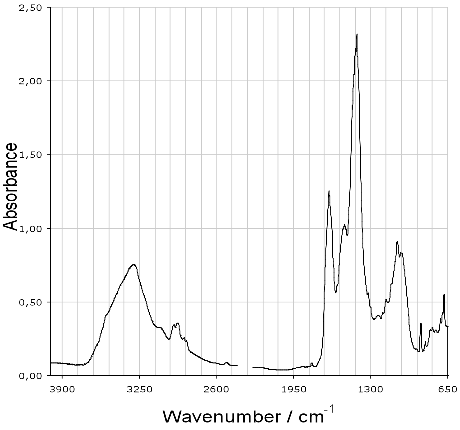
µ -FTIR



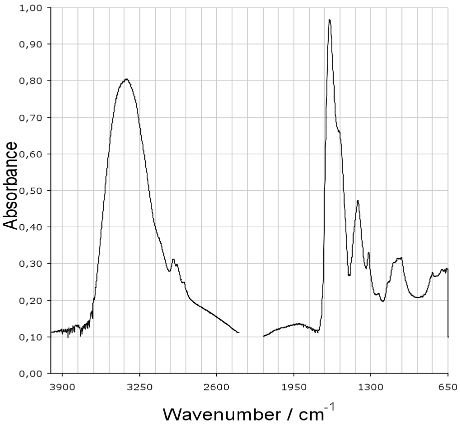
red colour with a considerable amount of **calcium carbonate**, *Lorvão 16*, *f.* 71v



blue colour with **lapis lazuli** and **calcium carbonate**, *Lorvão 16*, *f.* 71v

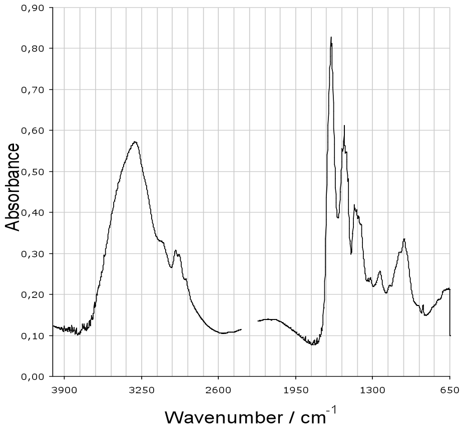


blue colour with **lapis lazuli** and **calcium**, **white carbonate lead** and **indigo**, *Lorvão 16*, *f.* 73



synthetic copper based green colour,

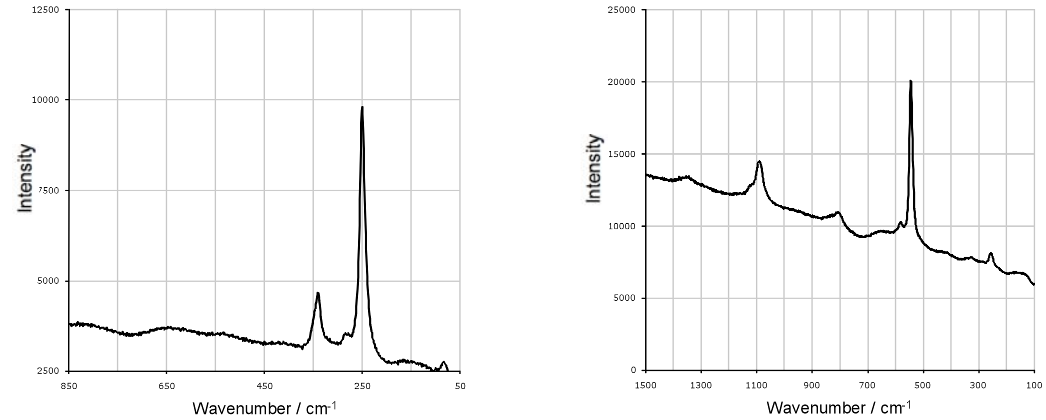
Lorvão 16, *f.* 59v



illumination *background* (proteinaceous binder and some calcium carbonate), *Lorvão 16*, *f.* 2v

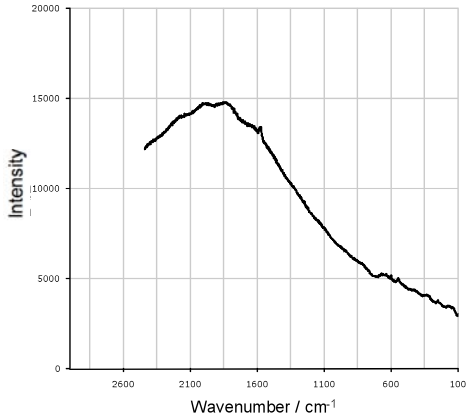
Figure II.39. Representative FTIR spectra of the red, two blues and green colours, and illumination background of *Lorvão 16*.

µ -Raman



vermillion in red colour, *Lorvão 16*, *f.* 7v

lapis lazuli in blue colour, *Lorvão 16*, *f.* 2v

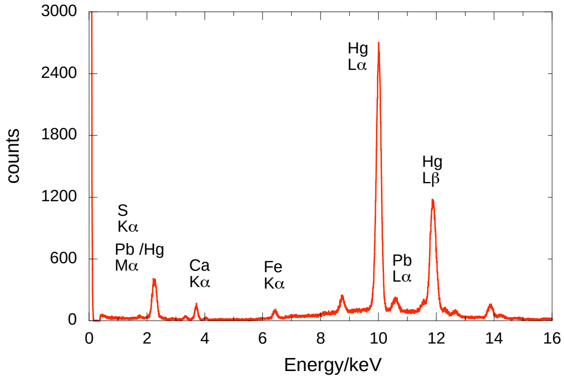


indigo in blue colour, *Lorvão 16*, *f.* 73

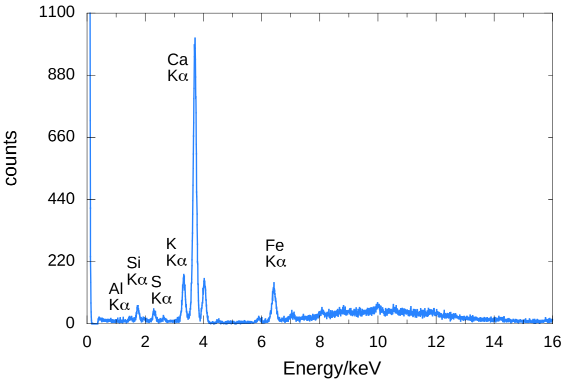
Figure II.40. Representative Raman spectra of the red and two sorts of blue colours of *Lorvão 16*.

Lorvão 17 – Book of Calendas

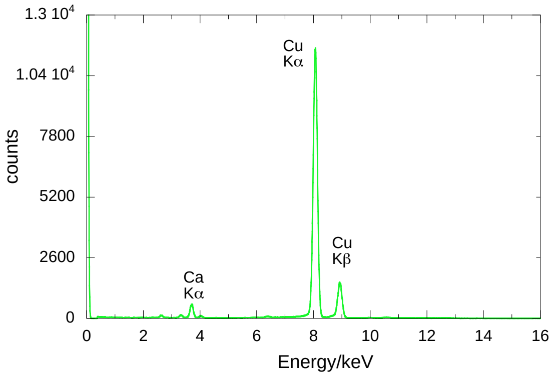
μ -EDXRF



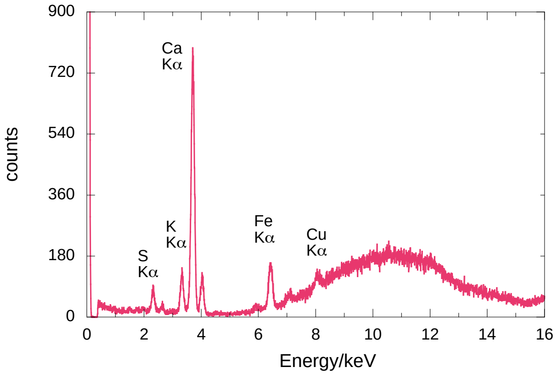
red colour, *Lorvão 17*, *f.* 169v



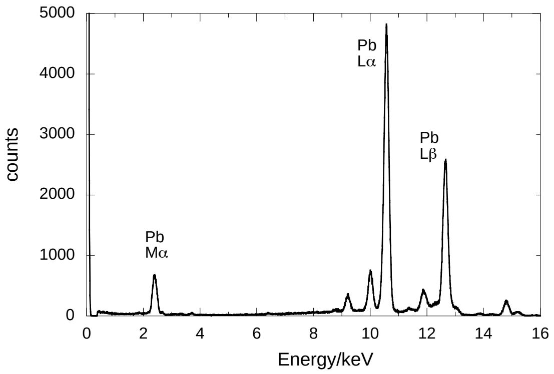
blue colour, *Lorvão 17*, *f.* 7v



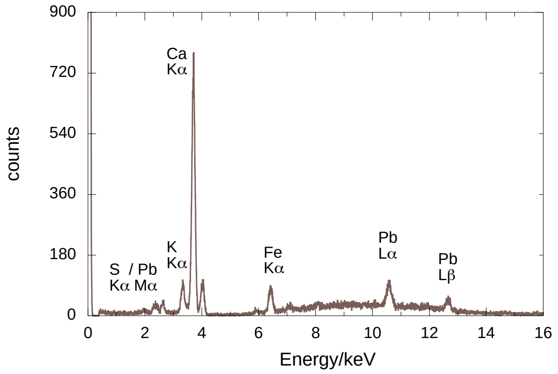
green colour, *Lorvão 17*, *f.* 9v



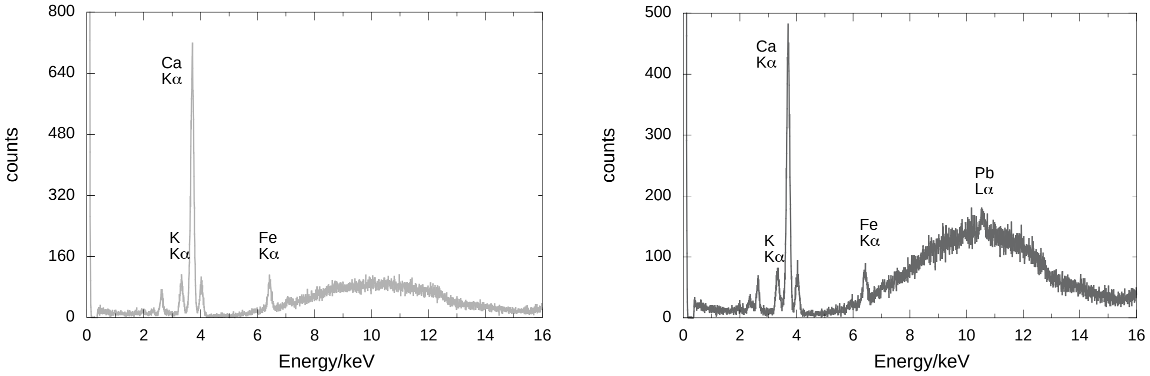
carmine-coloured, *Lorvão 17*, *f.* 169v



white colour (near of a red colour), *Lorvão 17*, *f.* 162v



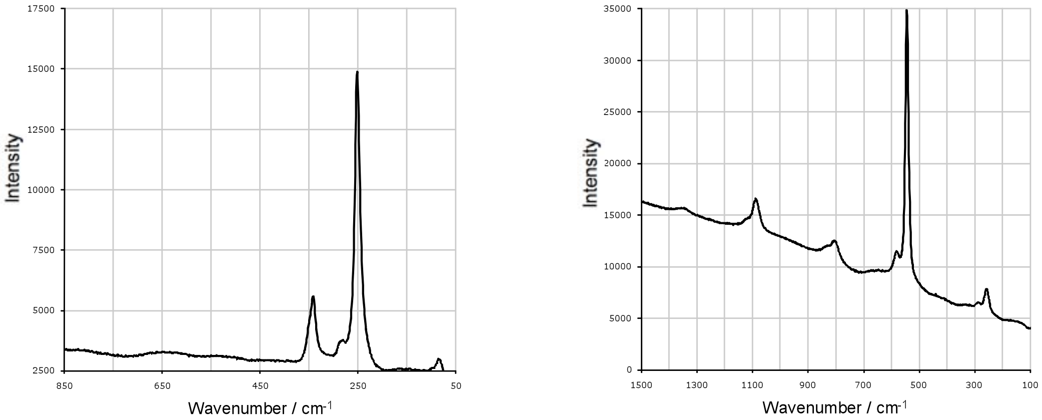
writing ink, *Lorvão 17*, *f.* 169v



parchment, *Lorvão 17*, *f.* 169v　　ruling, *Lorvão 17*, *f.* 162v

Figure II.41. Representative EDXRF spectra of the red, blue, green, carmine-coloured and white colours, writing ink, parchment and ruling of *Lorvão 17*.

<u>µ -Raman</u>

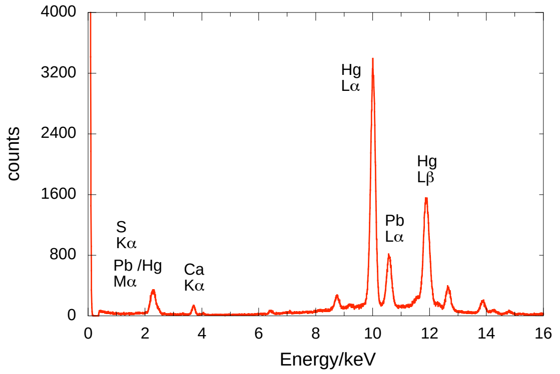


vermillion in red colour, *Lorvão 17*, *f.* 9　　**lapis lazuli** in blue colour, *Lorvão 17*, *f.* 7v

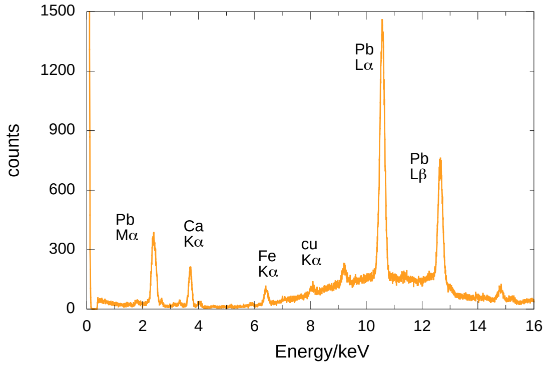
Figure II.42. Representative Raman spectra of the red and blue colours of *Lorvão 17*.

Lorvão 50 – Enarrationes in Psalmos of Saint Augustine

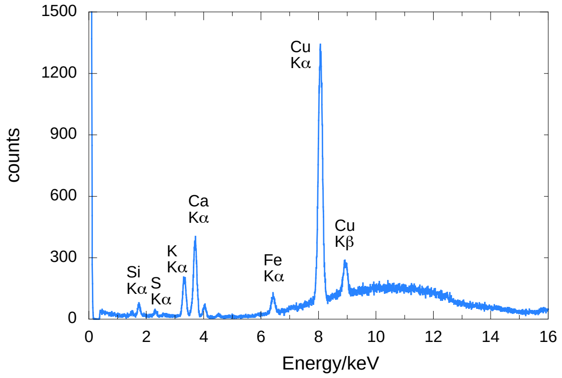
μ -EDXRF



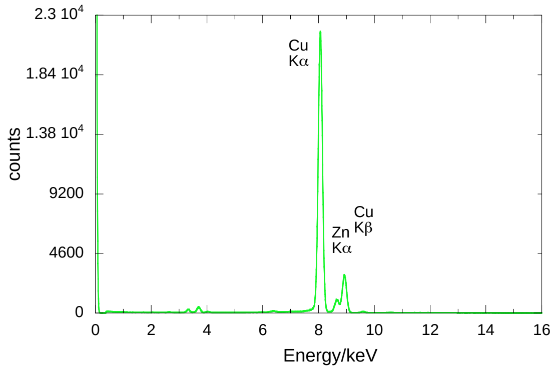
red colour, *Lorvão 50*, *f.* 34v



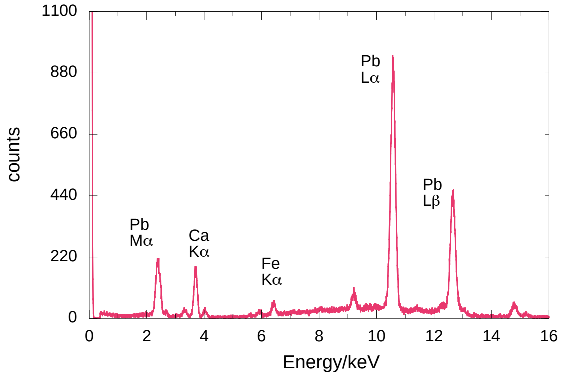
salmon colour, *Lorvão 50*, *f.* 64v



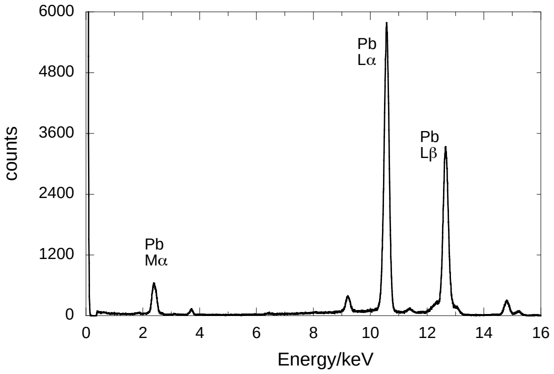
blue colour, *Lorvão 50*, *f.* 196v



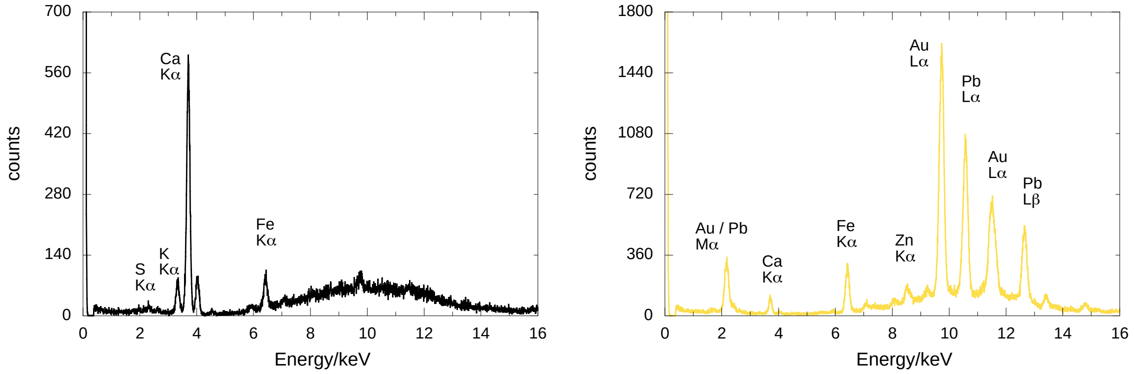
green colour, *Lorvão 15*, *f.* 64v



carmine-coloured, *Lorvão 50*, *f.* 173v

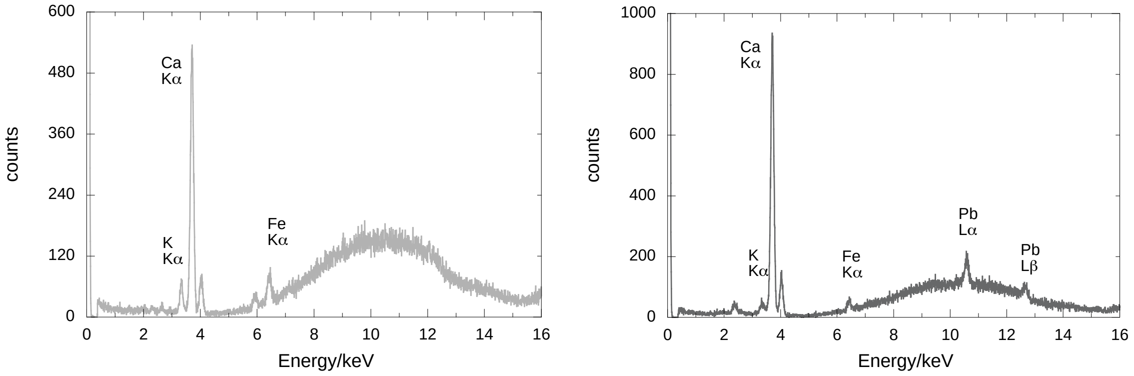


white colour, *Lorvão 15*, *f.* 173v



black colour, *Lorvão 50*, *f.* 64v

gold leaf, *Lorvão 50*, *f.* 1v

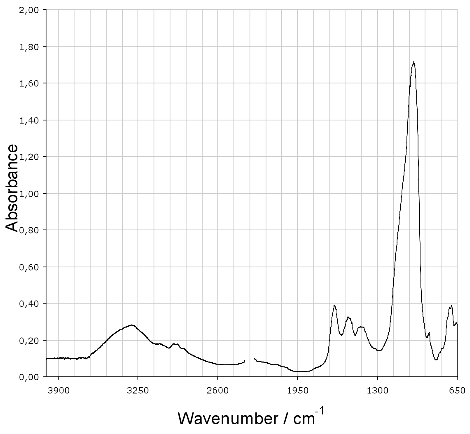


parchment, *Lorvão 50*, *f.* 173v

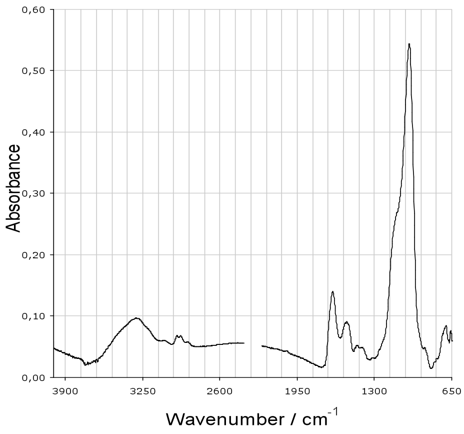
ruling, *Lorvão 50*, *f.* 248v

Figure II.43. Representative EDXRF spectra of the red, salmon, blue, green, carmine-coloured, white and black colours, gold leaf, parchment and ruling of *Lorvão 50*.

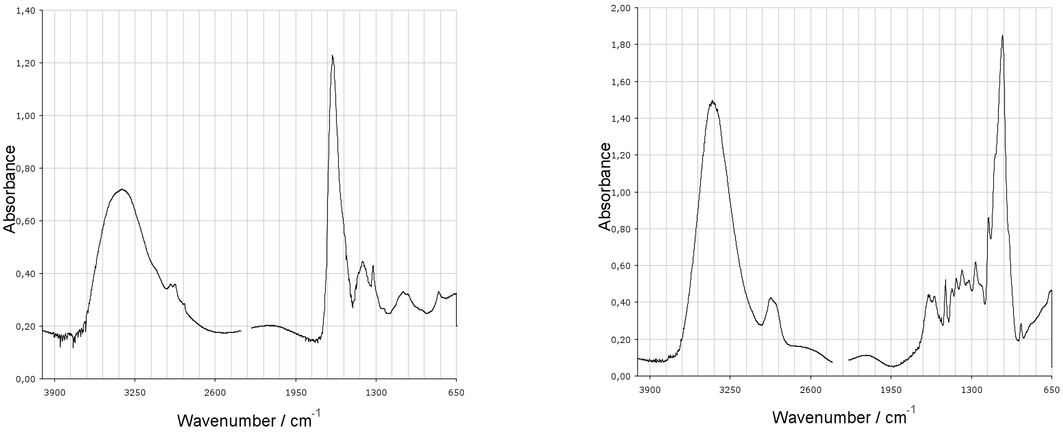
μ -FTIR



blue colour with **lapis lazuli** and **calcium carbonate**,
Lorvão 50, *f.* 1v

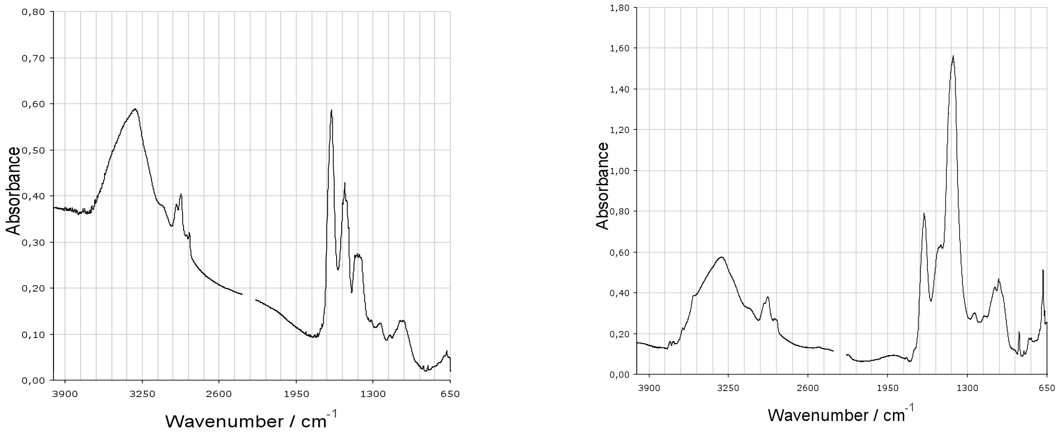


blue colour with **lapis lazuli**,
Lorvão 50, *f.* 196v



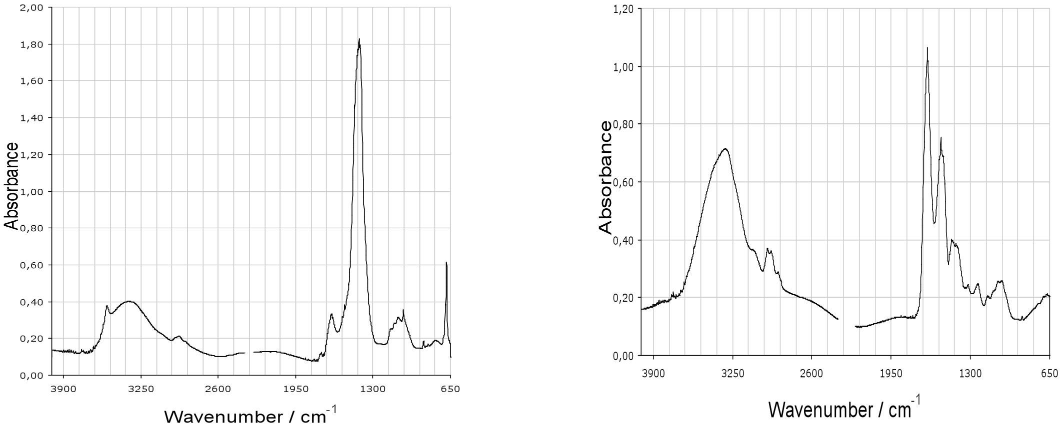
synthetic copper based green colour, *Lorvão 50*, *f.* 64v

carmine-coloured mixed with **calcium carbonate**, *Lorvão 15*, *f.* 1v



carmine-coloured, *Lorvão 50*, *f.* 173v

carmine-coloured (with **white lead** and some **calcium carbonate**), *Lorvão 50*, *f.* 64v

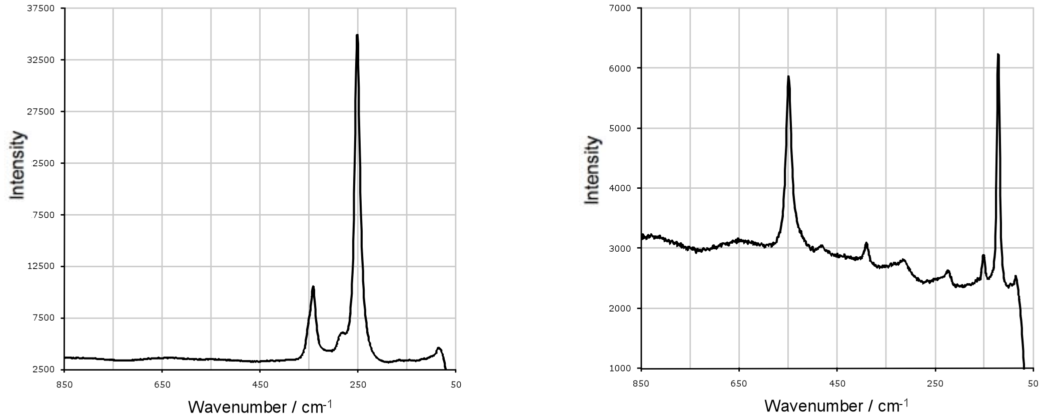


white lead of white colour, *Lorvão 15*, *f.* 64v

black colour (with a very small amount of **calcium carbonate**), *Lorvão 50*, *f.* 1v

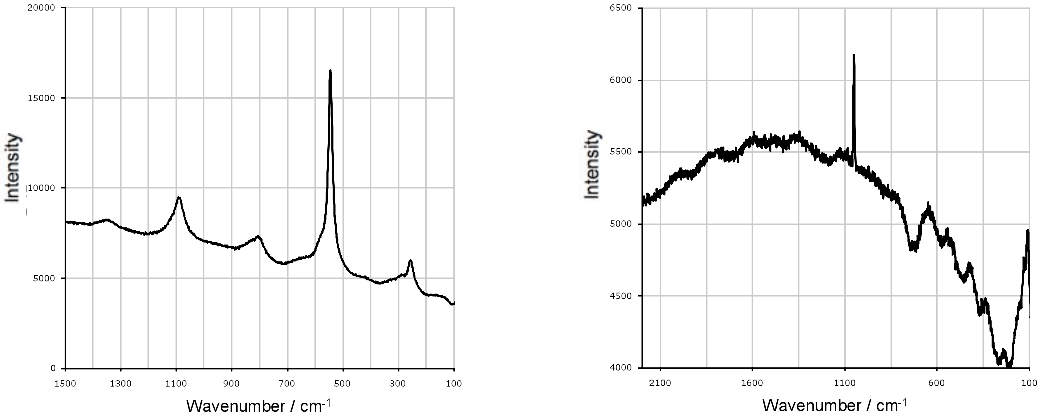
Figure II.44. Representative FTIR spectra of the two blues, green, three carmine-coloureds, white and black colours of *Lorvão 50*.

µ -Raman



vermillion in red colour, *Lorvão 50*, *f.* 1v

minium in salmon colour, *Lorvão 50*, *f.* 64v

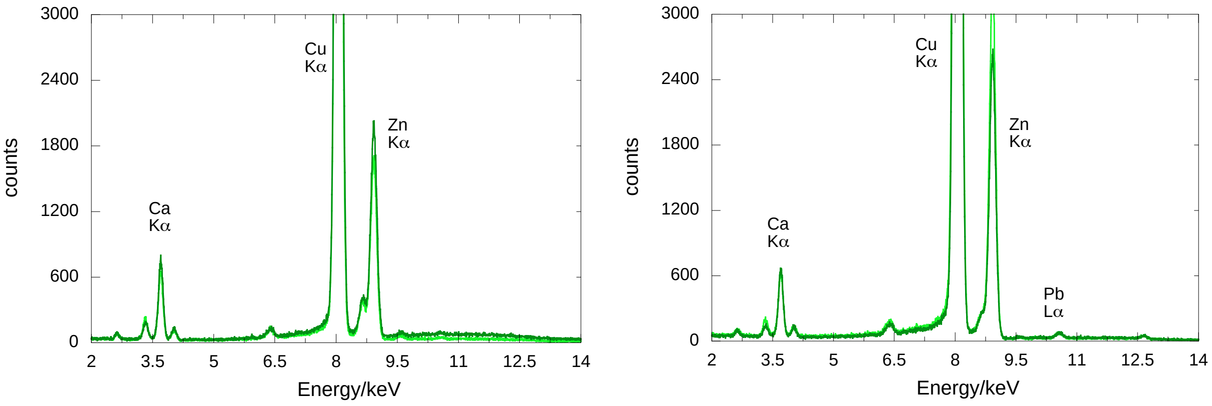


lapis lazuli in blue colour, *Lorvão 50*, *f.* 1v

white lead in white colour, *Lorvão 50*, *f.* 64v

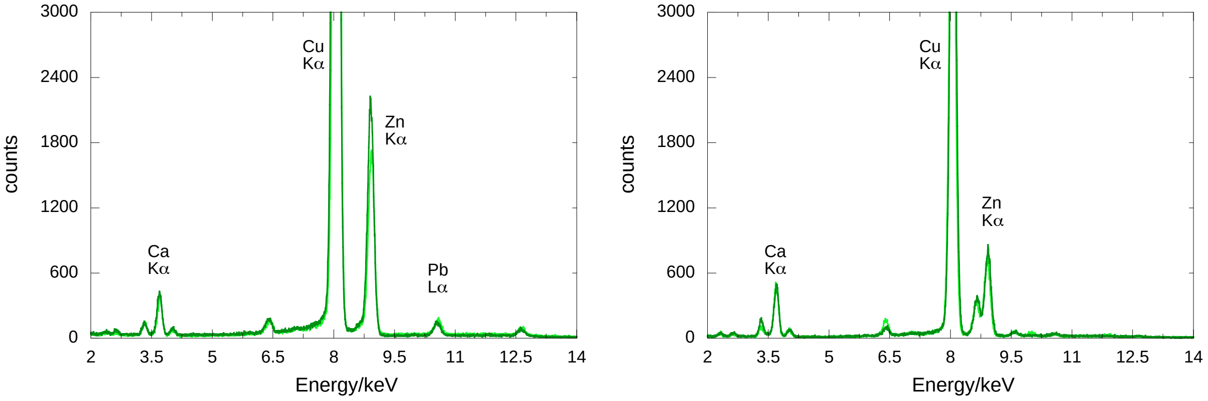
Figure II.45. Representative Raman spectra of the red, orange, blue and white colours of *Lorvão 50.*

II.3.2. Green colour



Lorvão 12, *ff.* 8 (light green) and 50 (deep green)

Lorvão 13, *ff.* 44v (light green) and 63v (deep green)



Lorvão 15, *ff.* 6 (light green) and 50 (deep green)

Lorvão 16, *ff.* 7v (light green) and 188 (deep green)

Figure II.46. EDXRF spectra of the greens analysed, in which ratio Cu/Zn and Cu/Pb were determined (part A, section 2.5.1, table 2.2.)

II.4. Blue colour from the *Book of Birds*

The EDXRF results already presented in subsection *Semi-quantification of the blue paint of the Book of Birds* were complemented with Raman analyses.

Two folia (*ff.* 6v and 16) were also analyzed by Raman spectroscopy, making easy the identification of the main component, lapis lazuli (Figure II.47.). The lead white and the azurite were not detected, the first one due the 632.8 nm excitation line, not so efficient as the 514 nm one, and the second one due to its low content in the paint which, with a high special resolution of 4µm spot, it is even more difficult to locate in the manuscript.

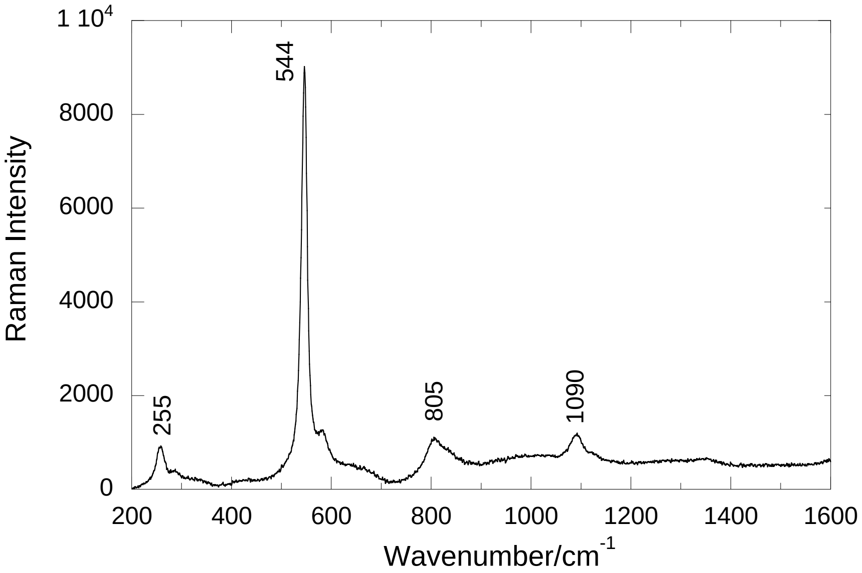


Figure II.47. Raman spectrum of folio 16. Laser excitation, 632.8 nm; objective, 50x ULWD; laser power intensity 1.7mW.

The lapis lazuli is easily observed in the Raman spectra by its bands at 255, 285, 544, 580, 805 and 1090 cm^{-1}, being the spectrum of S_3^-: 255 cm^{-1} and 285 cm^{-1} (bending) and 544cm^{-1} strectching, and the spectrum of S_2^- 580 cm^{-1} [14-16]; and the 1090cm^{-1} band an overtone [17].

II.4.1. Raw material

II.4.1.1. µ-EDXRF

Lazurite and two sorts of lapis lazuli, were analyzed by µ-EDXRF, in several different local areas of the minerals. Both kinds of lapis lazuli gave similar results and lazurite reveals an analogous major elemental composition, which is Ca, K, Si, S and Al. Comparing the spectra obtained it was also possible to detect a lower amount of potassium (K) on lapis lazuli than on lazurite (Figure II.48.).

This technique provides some more details associated to lapis lazuli, like the presence of barium (Ba), which is common to appear in its composition, as lapis lazuli is formed by limestone metamorphism by an alkaline igneous intrusion [18] and Ba is preferentially found in igneous rocks [18].

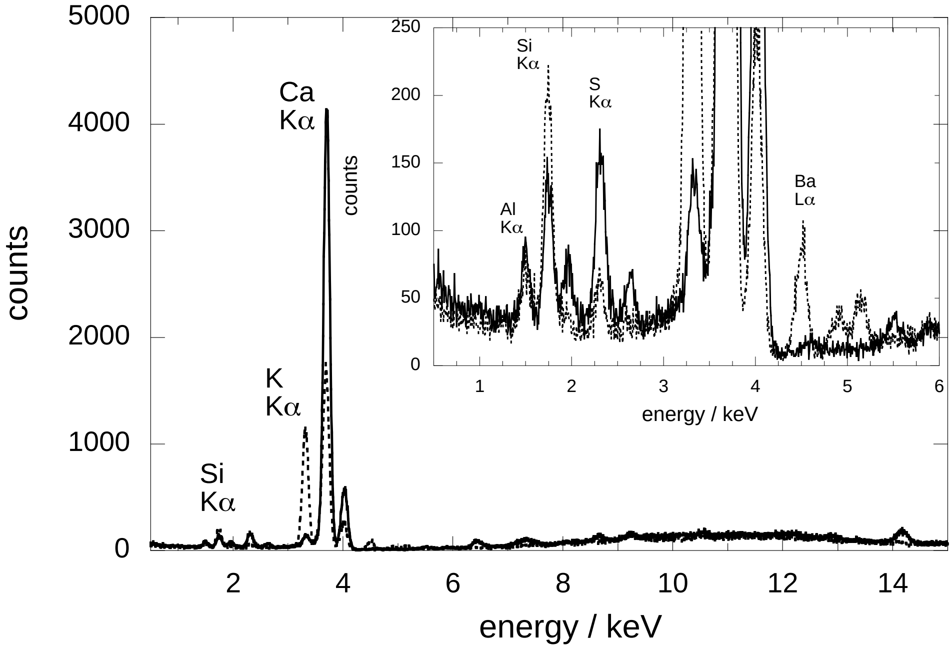


Figure II.48. μ-EDXRF spectra of the lazurite (—) and lapis lazuli (----), Using a tube voltage of 40kV of voltage, 300 μA current intensity, during 300 seconds.

II.4.1.2. PIXE

In order to get some more information to distinguish these three minerals, analyses were performed by PIXE, in powder, to quantify the main elements present in each one. These results were necessary to determine if there was a considerable difference between the mineral usually bought in medieval times, lapis lazuli, and the mineral responsible for its colour, lazurite; and also to find out if different sources, even from the same country, could enable different elemental or quantitative composition.

Both kinds of lapis lazuli gave similar results and lazurite reveals an analogous major elemental composition (Table II.4.), although with different elemental contents. The main difference, about one order magnitude, is in the K contents, much lower in lazurite.

Table II.4. Results of the elemental components of lazurite and lapis lazuli obtained using PIXE

Element	Lazurite (%)	Lapis lazuli 1 (%)	Lapis lazuli 2(%)
Mg	3.0	2.2	2.2
Al	15.0	13.0	13.0
Si	14.5	23.5	24
S	4.0	2.0	2.0
K	**0.7**	**6.5**	**6.7**
Ca	7.6	4.5	4.6
Ba	-	0.2	0.2

II.4.2. Standards

II.4.2.1. Mass absorption coefficient

Mass absorption coefficients of lapis lazuli and lazurite were calculated (Table II.5.) to evaluate how quickly the beam would lose intensity due to the absorption of the constituents of these two blue pigments.

Table II.5. Mass absorption coefficients for lapis lazuli and lazurite considering different elements' energy.

	KαCa (3.69 keV)	KαAl (1.49 keV)	KαSi (1.74 keV)	KαS (2.31 keV)
$\mu_{lapis\ lazuli}$ / cm^2•g^{-1}	292	411	611	745
$\mu_{lazurite}$ / cm^2•g^{-1}	205	380	700	610

II.4.2.2. Peak selection

Blue paint mixtures of the manuscript were quantified after model samples were quantified with WinAxil software, using the B_3 painted model sample as reference and B_1 and B_2 as hypothesis painted samples (Appendix I, section 1.3.1.3., Table I.2.). Two approaches were followed: the first one, by selecting the Kα peak of all the components detected as lapis lazuli/lazurite (Al, Si, S, Ca and K) and the second one by selecting just the Ca Kα peak as lapis lazuli/lazurite representative element. PIXE analysis reveled almost the same percentage of Ca and Al for the three different minerals, being a mass absorption coefficient of Ca: 165 cm^{2}g^{-1} to lazurite and 239 cm^{2}g^{-1} to lapis lazuli, and of Al: 380 cm^{2}g^{-1} to lazurite and 411 cm^{2}g^{-1} to lapis lazuli. Being the atomic number of Ca (20) and Al (13), the Ca Kα peak was selected as representative of lapis lazuli/lazurite. Considering the atomic number of the other two representative elements (the only X-ray emititors) of the paint composition: Lα peak of

Pb (82) to lead white ($2PbCO_3{\cdot}Pb(OH)_2$) and $K\alpha$ peak of Cu (29) to azurite ($2CuCO_3{\cdot}Cu(OH)_2$).

The quantification is better obtained by selecting the Ca $K\alpha$ peak than selecting all the components (Al, Si, S, Ca and K) (Table II.6.).

Table II.6. Quantification performed with WinAxil software of painted model samples B_1 and B_2, using as reference the painted model sample B_3.

Samples	B_1	B_2
Composition	97.5% lazurite 1.0% lead white 1.5% azurite	97.5% lapis lazuli 1.0% lead white 1.5% azurite
Ca $K\alpha$ peak	97.0% lazurite 1.5% lead white 1.5% azurite	96.8% lapis lazuli 1.4% lead white 1.8% azurite
Al, Si, S, Ca, K $K\alpha$ peaks	96.3% lazurite 2.0% lead white 1.7% azurite	95.4% lapis lazuli 3.0% lead white 1.6% azurite

II.4.2.3. Thickness

Software WinAxil was used to validate the influence of the paint layer thickness and the influence of the presence of a *velatura* (Table II.7.).

Table II.7. Calculated percentage, using the WinAxil software, of each pigment in the paint composition, varying the thickness of the paint layer (100, 150 and 200 µm) and with a *velatura* applied.

Painted model sample	lapis lazuli (%)	lead white (%)	azurite (%)
B_3 (100 µm)	87.5	11.5	1.0
B_{3a} (150 µm)	79.0	19.0	2.0
B_{3b} (200 µm)	74.5	23.0	2.5
B_{3c} (100 µm with *velatura*)	86.0	13.0	1.0

Comparing the results obtained it is possible to realize that the thicker the paint layer was, the more inaccurate the results were, mainly when lead white and azurite were present, since the mass attenuation coefficients vary widely with X-ray energy and material composition. Usually, X-rays of low energy do not penetrate very far in a matrix of heavy material, whereas high-energy X-rays in a light material have high penetration, which induce the quantification of elements' concentration [19]. The mass absorption coefficient of the main elements of lead white (Pb: 3839 cm^{-1}) and of azurite (Cu: 3995 cm^{-1}) are higher than the other main elements from lapis lazuli (Mg: 673cm^{-1}, Al: 1254 cm^{-1}, Si: 1350 cm^{-1} and K: 959 cm^{-1}). It was also possible to realize that the intensity of the characteristic X-rays from Pb or Cu increase linearly with the thickness, which characterizes these paint model samples, has thin samples [19].

The *velatura*, usually a thin layer of red organic material, does not influence the quantification of the blue paint layer, as its thickness is very reduce and its attenuation effect on the quantification is only due to the presence of non emitting X-ray organic material.

II.4.2.4. Brush *vs* film applicator

To select the best painted model sample to be use in the quantification of the manuscript blue paint, a previous quantification was made, using the painted model samples with brush and with film applicator. The painted model sample B_2 (Appendix I, section I.3.1.3., Table I.2.) was selected as reference to quantify other painted model samples: A_1, A_2 (painted with brush) and B_1 (painted with a film applicator) (Table II.8.). The results were very good for both techniques, although the quantification of azurite was not possible to do, since the error was above 20%, which can be justified by its low percentage in the paint composition.

Table II.8. Percentages of lapis lazuli and lead white obtained using the paint model sample B_2 and the Ca Kα peak selected, in WinAxil software.

Samples	Lapis lazuli (%)	Lead white (%)
B_1	98.3	1.0
A_1	98.4	1.0
A_2	90.2	9.3

II.4.2.5. Quantifying the blue paint in the Book of Birds with 3 standards

To quantify the paint components of each folio, three painted model samples were selected: A_1, B_2 and B_3 (all with thickness 100µm) with the Ca Kα peak, as lapis lazuli component, Pb Lα peak as the lead white and the azurite was quantified by difference. The results obtained reveal a very good reproducibility (Table II.9.).

Table II.9. Quantity (in percentage) of each blue paint component (lapis lazuli, white lead and azurite) applied on real illuminated folios (*ff.* 5, 6v, 7v, 16, 25 and 48), obtained using three different painted model samples as reference (A_1, B_2 and B_3).

Real illuminated folios	Painted model sample A_1			Painted model sample B_2			Painted model sample B_3		
	lapis lazuli	white lead	azurite	lapis lazuli	white lead	azurite	lapis lazuli	white lead	azurite
5	76.3	23.4	0.4	74.4	25.5	0.1	66	33.6	0.3
6v	98.5	0.7	1	99.5	0.3	0.4	98.4	0.5	0.9
7v	98.4	0.1	1.6	99.3	0.2	0.6	98.1	0.2	1.5
16	98.4	1.1	0.5	98.7	1.2	0.2	97.8	1.7	0.4
25	98.7	0.4	0.8	99.4	0.4	0.3	98.5	0.6	0.7
48	98.1	0.3	1.7	99.1	0.3	0.7	98.1	0.5	1.5

These results enabled the reconstruction of the blue palette present in these manuscript folia. Also revealed that the painted model with brush (A_1) give a better quantification when compared with the ones obtained with film applicator (B_2 and B_3), as the layer painted with brush reproduces a better model of what it is in the real illuminations.

II.4.2.6. Colorimetry

Lab* coordinates were determined (Table II.10.) and visually compared with the real blue colours, since the blue details are smaller than the analysing area. Although in the future it could appear a colorimeter with a small area analysis, able to compare to this previous results. The a* coordinate was always positive, except for folio 5, in which the blue is not so pure as it has more lead white mixed (25%). The coordinate b* revealed the hue of the colour blue, which was higher in the simulation of the folio 7 simulation, as it has practically no white lead (0.1%), and lower in the folio 5 simulation.

Table II.10. Lab* coordinates of the reconstructed blue palete of folia 5, 6v, 7v, 16, 25 and 48, based on the quantification results obtained with painted model A_1, and the real blue colours of these folia.

Folio	L*	a*	b*
5	68.73	-0.57	-37.05
6v	65.96	0.23	-42.49
7v	63.78	2.73	-44.75
16	64.26	1.88	-44.23
25	64.35	2.16	-44.3
48	64.73	1.61	-44.24

II.5. Red colour in Lorvão manuscripts

Red was analysed by μ-EDXRF and the semi-quantification was performed with WinAxil software. Pb Lα peak was selected to determine the percentage of *minium*, and vermillion was calculated by default, since this was the major pigment used in the red colour (Table II.9.). The spectra were only collected once, since the time was limited and there were several folia to analyse; and the majority of the results present in Table II.11. are from the illuminations and not from the minor initials. Lorvão 16 has only vermillion as red.

Table II.11. Red paint composition in Lorvão manuscripts determined by µ-EDXRF (% wt)

Manuscript	Folio	HgS	Pb_3O_4
Apocalypse	49	91	9
	115	91	9
	177	76	24
	200	70	30
	209	95	5
	217v	95	5
Book of Birds	4	95	5
	6	94	4
	6v	86	14
	16	92	8
	25	95	5
	48	97	3
	50v	92	8
	56v	88	12
	72v	93	7
Lorvão 3	1	96	4
	8v	92	8
	9v	94	6
	15	96	4
	17	60	40
	19v	96	4
	23	98	2
	27	99	1
	35v	90	10
	36v	75	25
	51v	92	8
	59v	99	1
	71	92	8
	71v	100	0
	82v	78	22
	98v	97	3
	107	98	2
	109v	96	4
	110v	94	6
	139v	95	5
	146v	91	9
	149	96	4
Lorvão 12	6v, 7v	100	0
	8	100	0
	11	100	0
	17	100	0
	23v	92	8
	30, 38v	100	0
	39, 50	100	0
	53v	96	4
	64, 94	100	0
Lorvão 13	1v	99	1
	6v	99	1
	30	96	4
	35v	99	1
	39v	98	2
	44	100	0
	63v	88	12
	73	99	1
	85	99	1
	92	98	2
Lorvão 15	5v	100	0
	11	97	3
	26	100	0
	38v	96	4
	50	100	0
	125	98	2
	154v	93	7
	167v	97	3
Lorvão 17	7v	97	3
	162v	98	2
	169v	98	2
Lorvão 50	1v	98	2
	34v	88	12
	37v	92	8
	64v	93	7
	173v	93	7
	196v	88	12
	248v	92	8

II.6. Dissemination*:* Activity sheets

An example of the activity sheets developed to do the step-by-step manual and distributed in the Medieval congress workshop in Leeds is presented below.

II.7. References

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Appendix III – Red chromophores data

III.1. Job's method

III.1.1. Alizarin lake

Using the Job's method (Figure III.1.), a simple and effective approach to the determination of chemical reaction stoichiometry by relating it with the maximum absorption achieved. In alizarin: Al^{3+} complex it is $X_M=0.33$[1], meaning that alizarin lake is an aluminum two-ligand complex.

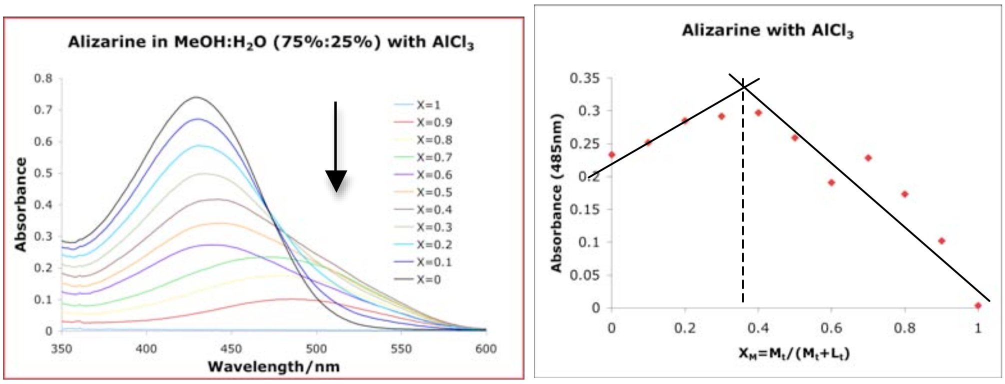

Figure III.1. Determination of alizarin complex stoichiometry using the Job's method.

III.1.2. Purpurin lake

Like to alizarin, the Job's method was also applied to purpurin, in order to determine the complex stoichiometry. The results point to 1:1 Al^{3+}: purpurin complex (Figure III.2.).

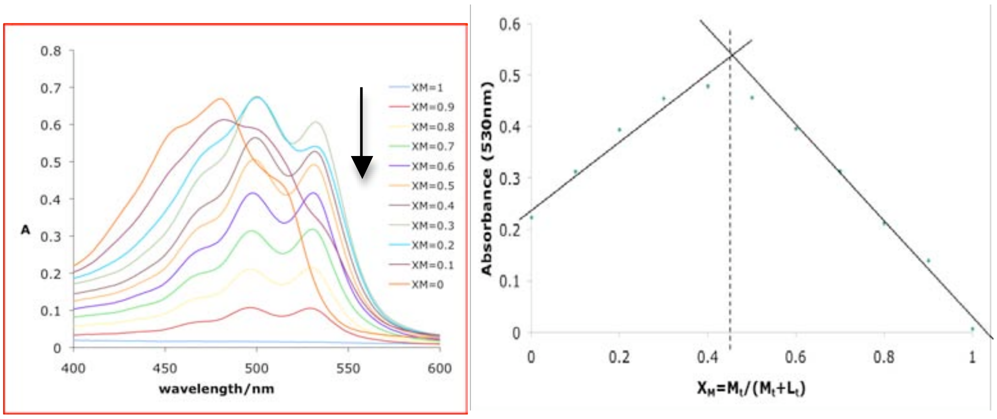

Figure III.2. Determination of purpurin complex stoichiometry using the Job's method.

III.2. Intensity of light (I_0)

I_0 were obtained for the different wavelengths of irradiation (Table III.1.), through the equation 4 (Appendix I, section 1.3.2.2).

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[1] In alizarin: Al^{3+} complex is $X_M=0.33$, (X_L = 1- X_M = 0.67 and $X_M/X_L = n_M/n_L$ = 0.33/0.67 = ½), meaning that it is a complex with stoichiometry 1:2 (ML_2).

Table III.1 – I_0 and parameters considered for the 430, 490 and 500 nm irradiations, using always 3 ml of solution.

Actinometry	I_0 (Einstein/min)	m*	slits
430 nm	1.19×10^{-6}	0.0132	-
	2.03×10^{-6}	0.0231	-
	4.50×10^{-7}	0.0046	2 mm§
490 nm	8.51×10^{-7}	0.0055	-
	8.23×10^{-7}	0.0052	-
	7.12×10^{-7}	0.0044	2 mm
	3.44×10^{-7}	0.0011	2 mm
500 nm	2.13×10^{-7}	0.0002	-
	2.71×10^{-7}	0.0006	2 mm

*m is the equation slope of ΔA over the irradiation time period, Δt for the reagent at the irradiation wavelength. § Entrance and exit slits were used.

III.2.1. HPLC-DAD data

HPLC-DAD results obtained before and after alizarin, purpurin, alizarin Red S and their lakes were irradiated with monochromatic irradiation (Tables III.2 and III.3.).

Table III.2. Compounds detected by HPLC-DAD after photodegradation of alizarin, alizarin red S and their lakes.

Compound		Alizarin (%)	Δ	Alizarin lake (%)	Δ	Alizarin red S (%)	Δ	Alizarin red S lake (%)	Δ
t_r=13.7 min* λ_{max}=259/**427** nm	t_0 t_{irrad}							4.2 5.7	+1.5
t_r=14.7 min* λ_{max}=263 nm	t_0 t_{irrad}							0.5 1.1	+0.6
t_r=18.2 min* λ_{max}=256/**422** nm	t_0 t_{irrad}					99.5 98.7	-0.8	94.3 92.1	-2.2
t_r=21.9 min* λ_{max}=271/**416** nm	t_0 t_{irrad}	1.5 18.2	+16.7	0.3 0.3	-				
t_r=23 min* λ_{max}=258 nm	t_0 t_{irrad}					0.5 1.3	+0.8		
t_r=23.2 min* λ_{max}=250/**430** nm	t_0 t_{irrad}	95.7 78.8	-16.9	97.9 95.2	-2.7				

*Relative area %, calculated at 280 nm

Table III.3. Compounds detected by HPLC-DAD after photodegradation of purpurin, and its lake.

Compound		Purpurin (%)	Δ	Purpurin lake (%)	Δ
t_r=20.7 min* λ_{max}=253/**483** nm	t_0 t_{irrad}			4.2 5.8	+1.6
t_r=22.8 min* λ_{max}=254/**481** nm	t_0 t_{irrad}	0.7 0.7	-	0.5 1.1	+0.6
t_r=25.1 min* λ_{max}=253/**480** nm	t_0 t_{irrad}	88.1 87.9	~	95.0 92.7	-2.3

*Relative area %, calculated at 280 nm

III.3. Polychromatic irradiation

Total light annual exposure found in a museum in London is, according to Feller [1] 1.55% of the exterior exposure (1100 kWh/m^2 [2]).

Red chromophores have just little fading with 12455MJ over 4250h of irradiation with a light source simulating the outdoor exposure (λ>300nm). Which means that in a museum they will be expected to fade after *circa* 200 years of continuously exhibition, which correspond to a compound class A (excellent material for conservation) [1]:

One year of light exposure in London: 1100 kWh=3960 MJ.
3960*1.55%=61.38
12455/61.38=202.91 years in a museum

III.4. - References

[1] Feller, R. *Accelerated aging – Photochemical and Thermal aspects*. The Getty Conservation Institute: United States of America, 1994.

[2] http://www.geni.org/globalenergy/library/renewable-energy-resources/world/europe/solar-europe/solar-united-kingdom.shtml, November 2008.

Appendix IV – Case studies data

IV.1. Eosin lakes reconstructions

Eosin lakes reconstructions enabled to see the excitation and emission spectra differences between the three lakes made with aluminum chloride hexahydrate ($AlCl_3 \bullet 6H_2O$), alum ($KAl(SO_4)_2 \bullet 12H_2O$) and lead(II) acetate trihydrate ($Pb(CH_3CO_2)_2 \bullet 3H_2O$) (Figure IV.1.).

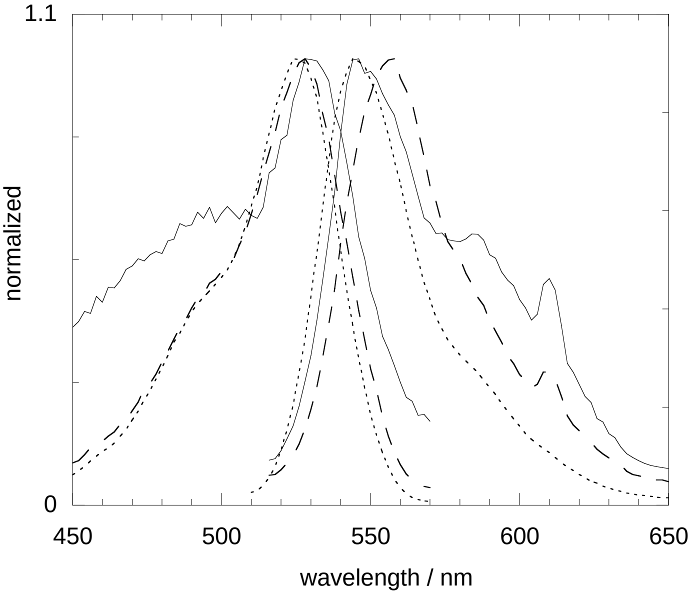


Figure IV. 1. Excitation and emission spectra of eosin lakes with aluminum chloride (- - - -), alum (— — —) and lead(II) acetate trihydrate (———), showing the difference between the maxima excitation and emission wavelengths.

IV.2. Andean textiles

IV.2.1. Extraction methods tested

Different extractions methods were tested in order to obtain as much information as possible from Andean textiles micro-samples. 11 micro-samples from the same Andean textile sample, a poncho fragment (mfa 31.699) were subject to mild extraction methods such formic acid, the one based in the oxalic acid method developed by Claude Andary and Pauline Guinot [1], in the one developed by Sanyova [2, 3] using hydrofluoric acid; it was also used a more aggressive extraction method with hydrochloric acid to compare with the others.

The results showed that when formic acid was used, there was almost no extraction of dye from the fibres. When hydrochloric acid was used pseudopurpurin decomposes into purpurin (Figure IV.2.).

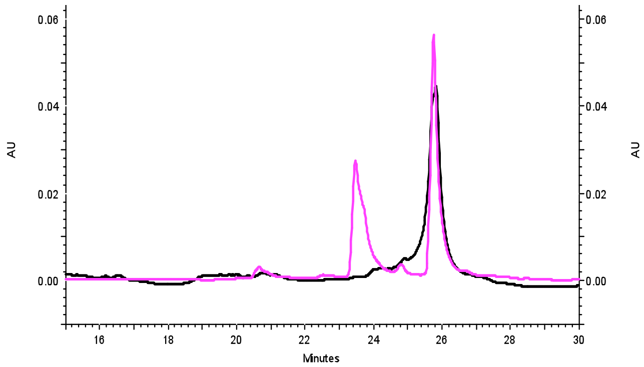


Figure IV.2. Chromatograms (acquired at 486 nm) of extracted dye from micro-samples of Andean textile (mfa 31.699), using two extraction methods: oxalic acid in MeOH:H_2O (80:20 / v:v) for 30 minutes (purple line) and MeOH:H_2O (80:20 / v:v) with HCl (black line), until a pH~1.5, for 20 minutes, both extractions were done at 60 ºC.

The hydrofluoric acid was so efficient as oxalic acid method, although the manipulation of hydrofluoric acid is more dangerous, therefore oxalic acid was preferred to hydrofluoric acid. The method was optimized to extract the maximum of dye from each fibre, keeping the concentration of oxalic acid the same and varying the ratio MeOH: H_2O. The respective areas of the compounds were calculated at their maximum wavelength (486 nm) with the chromatographic program Chromquest [4]. The results revealed that depending on the higher MeOH percentage, the amount of purpurin extracted is also higher (Figure IV.3.).

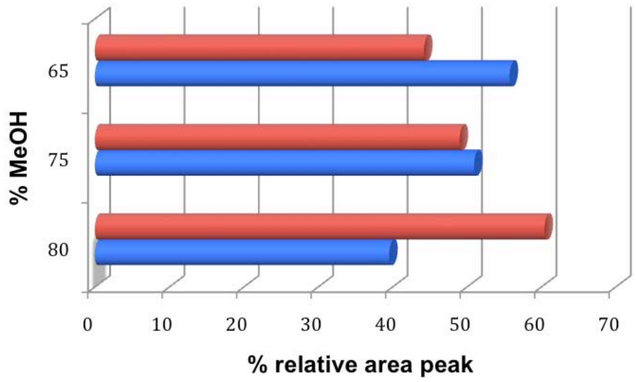


Figure IV.3. Relative area peak percentage of purpurin (red bar) and pseudopurpurin (blue bar) extracted from sample mfa 31.699, according the percentage of MeOH used in the extraction method, acquired at 486 nm.

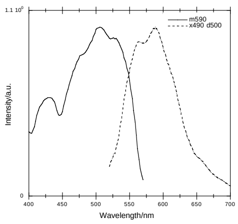
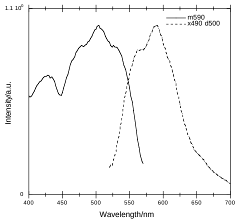
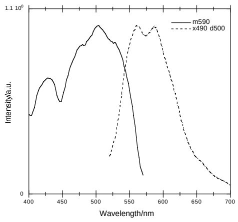
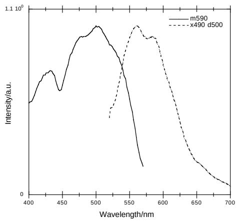
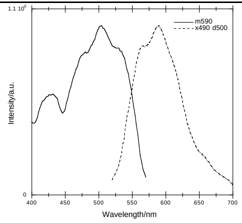
IV.2.2. μ-SPEX and HPLC-DAD data

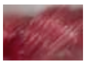
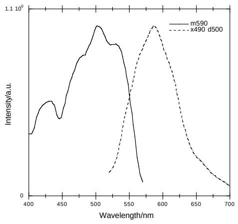
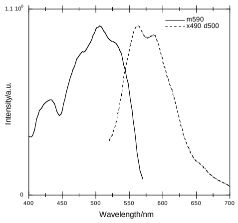
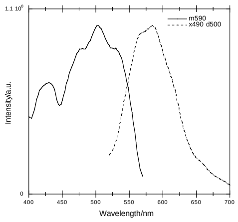
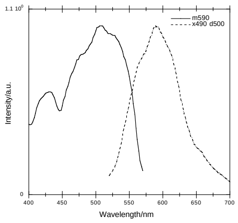
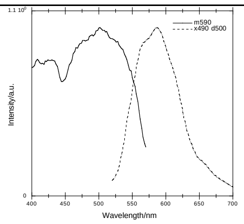
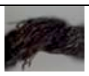
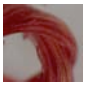
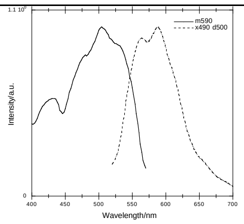
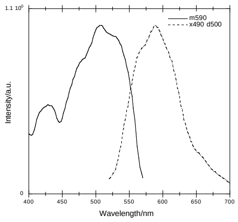
The red Andean textile samples analysed revealed two sorts of dyes, one sort based on pseudopurpurin and purpurin, founded in Paracas (200B.C.- A.D. 300) and Nasca (A.D. 0-300) textiles; and other sort based on carminic acid present in Chancay (1000-1476) textiles (Table IV.1.).

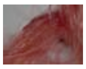
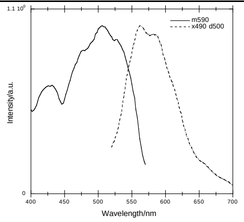
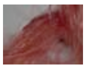
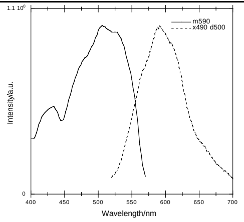
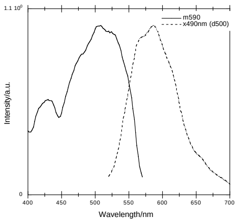
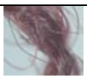
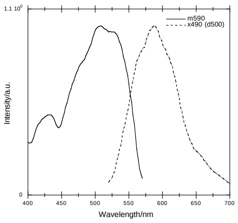
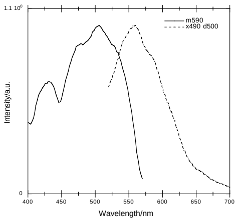
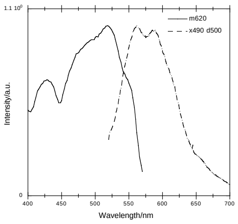
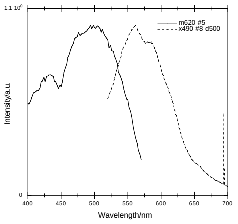
By HPLC-DAD, it was determined the ratio between pseudopurpurin and purpurin relative area peaks, acquired at 486 nm, which can explain the difference of the maxima emission wavelength for each sample. A database of dyed fibres with different kinds of pseudopurpurin and purpurin sources must be created to verify this hypothesis.

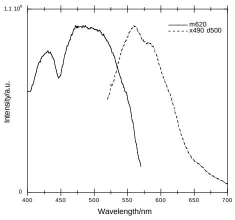
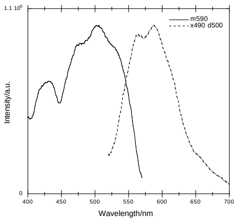
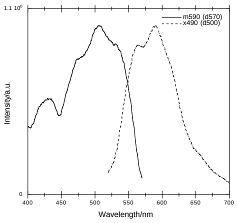
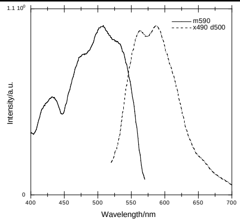
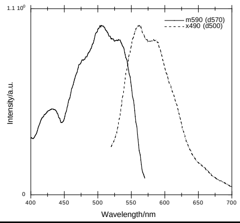
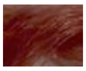
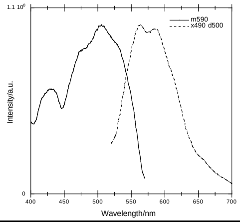
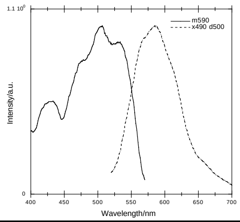
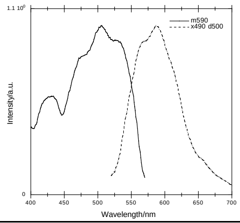
When carminic acid was detected through HPLC-DAD technique, it was possible to see the marker cd III associated (Figure IV.4.).

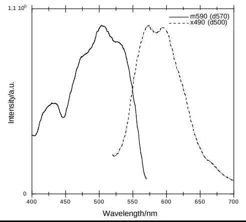
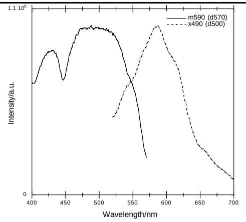
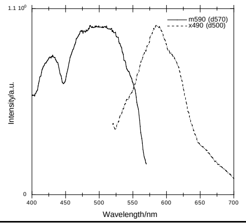
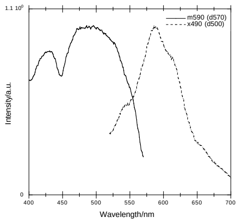
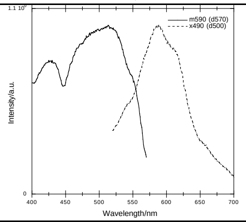
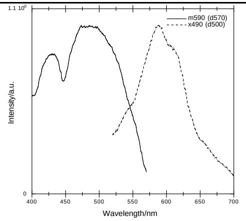
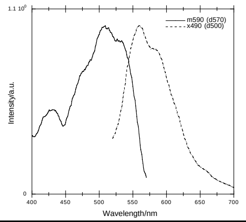
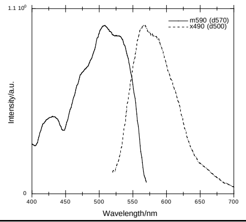
Table IV.1. Results obtained by μ-SPEX and HPLC-DAD of the red Andean textiles analysed (blank spaces were left when the spectrum has not enough resolution; ratio λ_{em} is obtained between the intensities of the two λ_{em} maxima; pseudo/purp is the ratio of the relative area peaks of pseudopurpurin and purpurin).

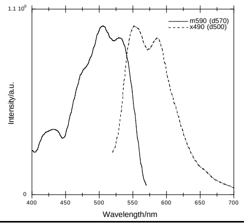
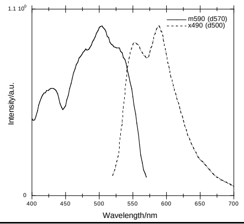
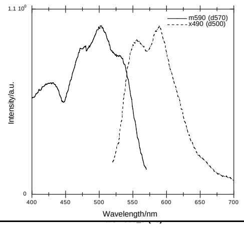
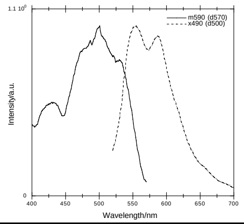
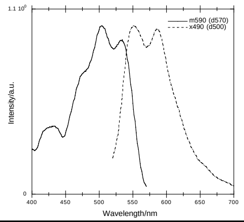
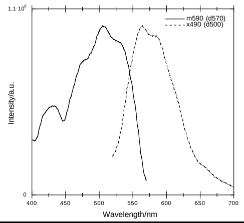
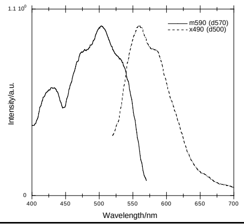
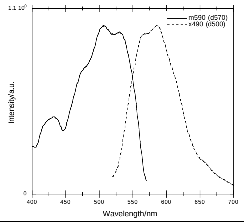
Textile	Sample		Excitation and emission spectra	λ_{max} ex (nm)	λ em (nm)	ratio λ em	Observations
Paracas, 100B.C. – 0 (Man's poncho)	31.496_3			506	564; 588	0.894	
	31.496_4			504	568; 589	0.858	
	31.496_5			504	560; 587	1.004	
	31.496_ex			504	560; 586	1.069	pseud/purp=0.4
Paracas, 100 B.C. – A.D. 100 (Mantle border)	67.85_1			504	566; 590	0.881	

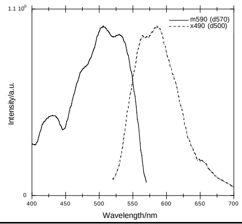
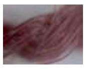
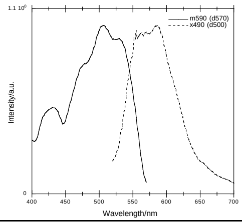
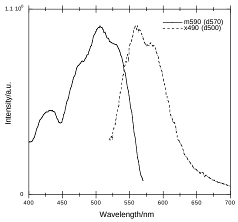
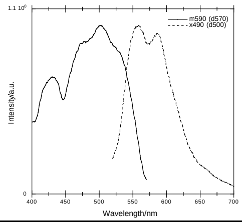
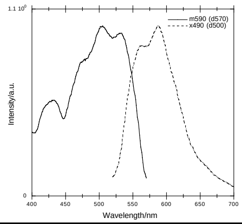
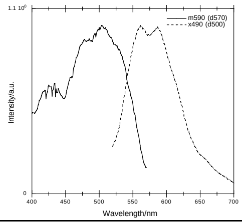
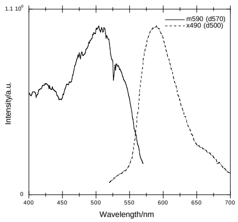
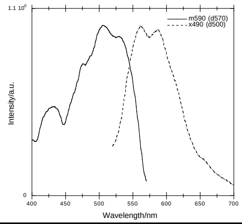
	67.85_2			502	586		
Paracas, 0 – A.D. 200 (Poncho)	31.498_2			506	564; 586	1.057	
	31.498_4			502	566; 589	0.968	
	31.498_7			506	591		
Paracas, 100 B.C. – A.D. 100 (Border fragment)	31.659_4				587		
Paracas, 0 –A.D. 200 (Ornamental braid)	21.2557_1				570		
	21.2557_7			504	564; 591	0.948	
	21.2557_8			508	589		

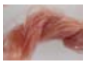
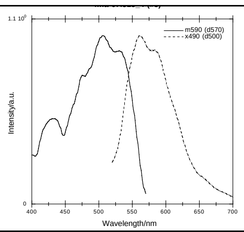
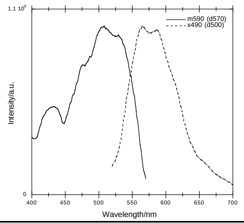
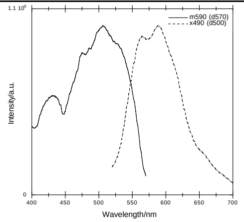
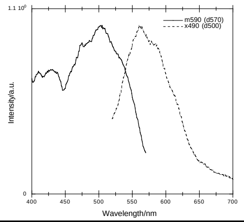
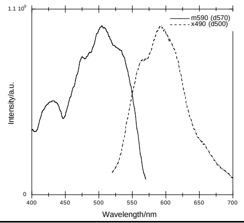
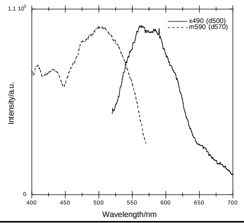
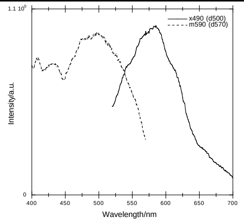
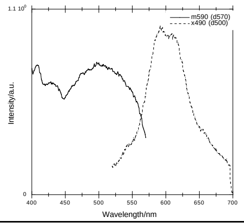
Paracas, 0 – A.D. 50 (Border fragment)	51.571_1			506	562: 590	1.086	
Paracas, 100 B.C. - 0 (Border fragment)	51.572_1			506	591		
Paracas, 100 B.C. - 0 (Border fragment)	35.1125_3				592		
Paracas, A.D. 100 – 200 (fragment-figures detached)	1977.134_1			508	568; 587	0.930	
	1977.134_4				566; 586	0.986	
	1977.134_9			508	585		
Paracas, 100B.C.- A.D. 100	31.499_1			506	560		
	31.499_2			516	562; 589	1.025	
	31.499_5			504	562; 589	1.140	

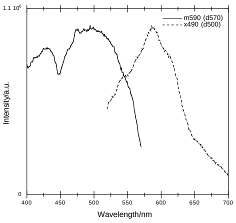
	31.499_6				559; 590	1.160	
	31.499_7			506	565; 587	0.946	
Paracas, 200 B.C.-A.D. 200 (skirt)	21.2581_3			506	564; 588	0.957	pseud/purp=0.7
	21.2581_6			508	565; 587	0.968	
Paracas, 200 B.C.- 0 (border fragment)	31.900_1			503	564; 591	0.925	
	31.900_5			506	563; 585	1.024	
	31.900_5*			505	569; 585	0.926	pseud/purp=0.4
Paracas, 0 –A.D. 100 (poncho fragment – Snails)	31.699_4			506	587		

	31.699_9			504	574; 596	1.012	pseud/purp=0.5
Chancay A.D. 100-1476 (Weaver's workbasket)	02.680_1				587		carminic acid
	02.680_5				585		carminic acid
Lambayeque A.D. 1000-1476 (tapestry strip with figures and large birds)	10.267_1				586		carminic acid
	10.267_2				588		carminic acid
	10.267_6				588		carminic acid
Paracas, 0 – A.D. 200 (Border fragment)	16.36_2			509	559		
	16.36_7			508	566		
	16.36_8				590		

Nasca, A.D. 300 (fragment of a tabled and fringed border)	31.500_1			506	554; 585	1.072	
	31.500_6			504	552; 587	0.909	
	31.500_7			502	556; 589	0.918	
	31.500_9			501	555; 587	1.064	
	31.500_ex			504	552; 585	1.020	
Early Nasca A.D. 200 (Border fragment)	16.37a_1			505	564; 590	1.100	pseud/purp=0.7
	16.37a_6			503	561; 582	1.160	
Paracas-Nasca, 0 – A.D. 200B (Border fragment)	31.696_1			506	585		

	31.696_3			506	586		
Paracas-Nasca, 0 – A.D. 200B (Fragment)	31.697_1			504	587		
	31.697_2			506	563		
	31.697_4			503	558; 587	1.047	
	31.697_7			504	560; 588	0.880	
Nasca, A.D. 500 – 600 (Bag with human and vegetal motif)	30.253_2			503	561; 587	1.010	
	30.253_4				590		
Paracas-Nasca A.D. 200 (*Fragments of a hanging*)	67.313_1				561; 587	1.026	

	67.313_4			506	561		
	67.313_7			508	564; 585	1.020	
	67.313_8			506	564; 588	0.936	
	67.313_9			503	560		
	67.313_12			504	566; 593	0.802	
Neo-Inca 1550 (*Tapestry with Tocapu motifs*)	1988.325_1			505	564; 589	1.052	
	1988.325_2				585		
	1988.325_4				592		

Chancay A.D. 1000-1476 **(***Fringed tunic with interlocked birds*)	47.1087_1				586		

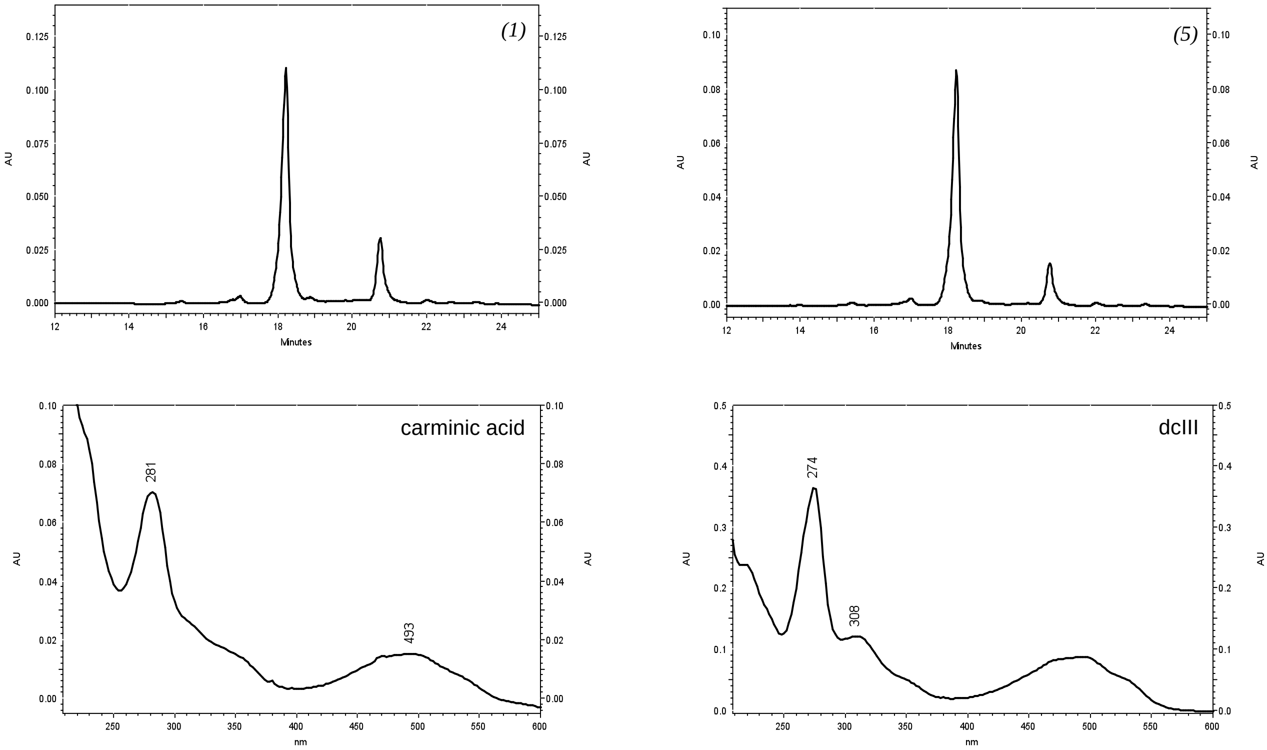


Figure IV.4 Chromatograms of the dye extracted from 2 samples (nº1 and 5) of *Weaver's workbasket (*Chancay Late Intermediate Period, A.D. 1000-1476), acquired at 490nm, and the UV-Vis spectra of carminic acid (t_r=18 min) and dc III (t_r=20.8 min) of sample nº1.

IV.3. References

[1] P. Guinot, C. Andary, Molecules involved in the dyeing process with flavonoids, proceedings of the 25th Meeting of Dyes in History and Archaeology, 2006 *in press.*

[2] Sanyova, J. *Michrochim Acta* **2008**, *162*, 361.

[3] Sanyova, J.; Reisse, J. *J. Cult. Herit* **2006**, *7*, 229.

[4] 4.1 ed., *ChromQuest 4.1*, Thermo Scientific, 2003.

Appendix V – Birds from the *Book of Birds*

Dove *f.* 7v

Hawk, *f.* 16

Turtledove, *f.* 21v

Pelican, *f.* 30v

Nighthawk, *f.* 32

Raven, *f.* 33

Cock, *f.* 36v

Ostrich, *f.* 40

Vulture, *f.* 46v

Crane, *f.* 48

Kite, *f.* 49v

Swallow, *f.* 50v

Stork, *f.* 52v

Blackbird, *f.* 54

Carrion crow, *f.* 56v

Goose, *f.* 58v

Heron, *f.* 59v

Caladrius, *f.* 60v

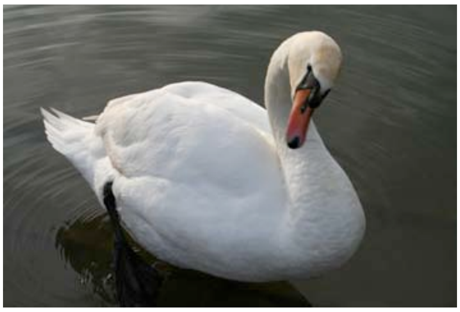
Phoenix, *f.* 61

Partridge, *f.* 62v

Quail, *f.* 63v

Hoopoe, *f.* 64v

Swan, *f.* 65v



Peacock, *f.* 66v

Eagle, *f.* 69

Book of Birds

The dove and the hawk

This illumination introduces the main characters of the *Livro das Aves* (Book of Birds). The characters were chosen because their attitude, way of living and behaviour will have a greater impact on the public. That is, they represent those persons that everybody should or would like to emulate. On one hand, we have the most learned of men, the clergy, on the other, the most famous of men, the nobility. And thus, in this face-to-face, the dove represents the clergy; the hawk, the nobility. The arches under which they stand symbolize the "cloister", a protective space associated, in the text, with a birdcage. The author introduces these main characters in a passage in which he describes the following image: "Behold, the hawk and the dove stand on the same perch, we both came to the cloister, I from the clergy, and you from the military, so that both of us will remain in the monastic life, as if we were in a birdcage". The Book is written and dedicated by someone from the clergy, the author, to someone who has been converted, Rainerius, who has abandoned the military to enter religious life. Regardless of whether or not this is a literary device, the author states that he is writing the book at the request of Rainerius in order to "enlighten the minds of ordinary people by means of painting: so that those things that the spirit of ordinary people can hardly perceive with the eyes of understanding, may, at least, be comprehended with their bodily eyes; and the sight will understand what the ear could only grasp with difficulty.".

The illumination was painted with inks obtained by mixing a proteinaceous glue, e.g. parchment glue (*Caderno de ligantes*, Binders workbook) with the mineral lapis lazuli (blue), and the synthetic pigments white lead and vermilion. The outline was done using an iron-gall ink. (*Caderno de instrumentos de escrita*, Writing instruments workbook). The pigment is previously ground, and then well mixed.

Try on

The materials 1 – parchment (10x10 cm), may be replaced by another paper with a surface similar to parchment; 2 – white paper tape to fix the parchment (e.g. *Comply^{TM} Indicator Tape*); 3 – tracing paper; 4 – pencil and nib pen (or quill pen); 5 – agate pestle; 6 - Small round glass dishes to mix the colorants (watch glasses (5.08 cm)); 7 – iron-gall ink (or another ink not water-soluble); 8 – parchment glue (a white glue can be an alternative); 9 – pigments, for the blue: ultramarine blue, $Na_8[Al_6Si_6O_{24}]S_n$; white: white lead, $2PbCO_3.Pb(OH)_2$ and red: vermillion (HgS); 10 - paintbrushes (nº2 and 3), Cups to wash the paintbrushes, Eyedroppers to dilute the inks and *Kleenex* or other absorbent tissue paper.

Step by step

1 – Fasten the parchment to a rigid support. It will tend to curl up with minor fluctuations in humidity. Therefore, it is convenient to fasten each of its four corners to a support using white paper tape.

2 – Photocopy the drawing, enlarging the original so as to obtain a copy with the size of the real image: 10 x 10 cm; make a copy of the drawing into tracing paper, and then transfer it to the parchment (a).

3 – Dipping the nib of the pen in the ink (b), mark the drawing with iron-gall ink. The line may seem light, but do not worry.

4 – Ink preparation: grind the pigment together with the parchment glue in an agate mortar with the aid of a pestle until you have obtained an uniform ink; if necessary, add a few drops of water: if you add to much water it will take longer to obtain an homogeneous ink, if you have too little water, then it is difficult to mix the components. The ink should first be air-dried, and then it can be applied to the parchment with a brush moistened with water, as if it were a watercolour or a gouache (you can prepare it a day or more in advance).

5 – Start with the blue ink, painting the hawk's body (c). Then, use the white ink to paint the details that highlight the hawk's plumage (c). Finally, use the ink made with vermilion to paint the red letters (d).

Attention! If you use white lead avoid its inhalation and swallowing. Before start the activity, please read the *Chemical Safety Card*.


a


b


c


d

Book of Birds

The dove and the hawk

Background

This "Aviary" is conserved in the Portuguese National Archives-Lisbon (DGARQ). It is an illuminated manuscript dated from 1184 and produced in Lorvão Monastery, near Coimbra. The *Livro das Aves* (Book of Birds) starts with a prologue addressed to Rainerus, the lay brother, starting with the following passage (from the Portuguese translation by Maria Isabel Rebelo Gonçalves, cited work, p.59): “Wishing to satisfy your request, I have decided to paint the dove (...) and enlighten the minds of ordinary people by means of painting: so that those things that the spirit of ordinary people can hardly perceive with the eyes of understanding, may, at least, be comprehend with their bodily eyes; and the sight will understand what the ear could only grasp with difficulty. I did not want only to paint the dove giving it a form, but I also wanted to describe it in words, to elucidate the picture through writing: so that the morality of the written text may at least please those that dislike the simplicity of painting.”

Then, in the first part of the book, the author introduces us to the main characters: the dove and the hawk. In reality, there will be three doves:

“Noah's dove, David's dove, and Jesus Christ's dove: Noah represents quietude; David, bravery; Jesus, salvation.” And therefore, by means of the doves “the minds of ordinary people may be indoctrinated to change their lifes”.

The book can be divided into two distinct parts. The first part provides the ideological framework of the Book, and it is in this section that the main characters are described. The author presents the symbols associated with the dove, the hawk, the turtledove, and the bird, as well as with the winds Auster (the south wind) and Aquilo (the north wind), the Palm-three, and the cedar of Lebanon. In the second part, a chapter is dedicated to each bird.

Notes

Safety

Some of the pigments used here are toxic. It is required to read the *Chemical Safety Cards*, where risk and safety rules are described. Ingestion and inhalation must be completely avoided.