PhD thesis

Analytical techniques for the investigation of natural dyestuffs

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XXVI COURSE

Ogni erbaccia fa tinta

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PREFACE

Analytical techniques are usually classified according to their ability to preserve the investigated sample for further analyses. In particular, a technique that preserves the sample is said to be non-destructive, while one which does not allow to recover the sample after the analysis is called destructive. This classification is somewhat limited when samples from the cultural heritage are considered. In this field the main issue stands not only in the possibility of performing several analyses on the same sample, but also in preserving the object from which the sample is taken. As a matter of fact, works of art are precious and their integrity is a priority even when scientific analyses are aimed at the conservation of the artifact, as they would allow a correct restoration and storage of the object under investigation. For this reason, when dealing with analytical chemistry devoted to the cultural heritage, scientists prefer to talk about non-invasive and invasive (or micro-invasive) techniques.

Non-invasive techniques do not require to detach a sample from the artwork; they possibly work *in situ* by means of portable instrumentations, even though for small movable objects, also laboratory instrumentations with appropriate sample-holders could be employed according to a non-invasive approach. On the contrary, invasive techniques need sampling; samples could simply be deposited on the sample-holder of the instrument or they can undergo preparation and pre-treatments before the analysis. The term *micro-* often employed before *invasive* aims to focus on the reduced dimensions of the samples entailed for the analytical technique and should not be confused with the same term used to highlight the coupling of an instrumental technique with a microscope (for example micro-Raman spectroscopy). As a consequence, it is evident that the use of non-invasive techniques is highly preferred for the analysis of works of art, as they would allow to characterize their materials without compromising their integrity. For this reason, the research efforts in recent years were aimed at setting up and optimizing portable techniques for the analysis of cultural heritage materials and/or at minimizing the amount of material required for the invasive approaches. Despite this, not every analytical problem of interest for the cultural heritage can be at present faced through non-invasive techniques. This is particularly due to the lack of research strategies devoted to optimize the non-invasive approach on materials of interest for the cultural heritage field, but also to the intrinsic nature of some of the compounds of interest.

All these reasons are valid also for the analysis of natural dyes in ancient textiles and paintings, as this analytical task has been largely unexplored since recent years, leading to a lack of analytical procedures and databases; in addition, the organic nature of dyes exclude the use of elemental analytical techniques such as X-ray fluorescence spectrometry. Moreover, several dyes can be contained in a single dyestuff, thus making arduous the correct identification of the material with molecular spectroscopic techniques, in which the signals from the different matters are integrated. The most suitable technique for the analysis of natural dyes in historical and archaeological samples is at present high performance liquid chromatography. The technique is invasive (although micro-samples can be considered) and destructive. It enables the identification of major

and minor dyeing species after their separation by employing specific detectors. The identification of the principal dyeing molecules enables the identification of the raw materials (e.g. the dyestuffs) employed.

The analysis of natural dyes was also attempted through non-invasive techniques, mainly fiber optics reflectance spectrometry and portable spectrofluorimetry, although a systematic work on their applicability still lacks in the literature. As reflectance spectroscopy and molecular spectrofluorimetry do not give insights into the molecular structure of the investigated compounds, also micro-invasive approaches should be taken into account for a more detailed characterization of the dyes. In particular, surface-enhanced Raman scattering (SERS) seems nowadays the most promising technique for micro-invasive molecular analysis of dyestuffs, as proved by the steep increase in the number of papers published in the field in the last ten years.

In the course of my PhD research, I explored both non-invasive and micro-invasive techniques for the analysis of natural dyes. My research principally focused on the optimization of analytical protocols in order to develop new analytical strategies for the analysis of natural dyes that could be integrated within the analytical procedures of Laboratorio Analisi Scientifiche, from Direzione Ricerca e Progetti Cofinanziati of Regione Autonoma Valle d'Aosta, which hosted my research project. The techniques were selected as they were the most promising for the analysis of natural dyes and they would allow to minimize the sampling from the artwork. In particular, UV-Vis fiber optics reflectance spectrometry (UV-Vis FORS) was employed for the preliminary non-invasive investigation of dye-containing artworks, while surface-enhanced Raman scattering was used to acquire molecular information on the investigated dyes. In order to drastically reduce the amount of sample required and to avoid the pre-treatment of the sample, the optimization of a procedure for direct extractionless non-hydrolysis SERS was put into place. Both techniques were firstly optimized on reference samples and then applied to historical artworks from different matrices (textiles, paper, parchments, polychromies) of various provenance and dating. As for historical works of art, several complementary techniques were employed in order to confirm the results and verify the suitability of the proposed procedures, as well as to get further information on the investigated artifacts.

The work was carried out mainly in Laboratory Analisi Scientifiche (LAS) which is part of Soprintendenza per i Beni e le Attività Culturali of Regione Autonoma Valle d'Aosta and has been devoted to the investigation of art and archaeological objects through instrumental analytical techniques for about thirty years. The main goal of LAS is to help the conservation of the diverse and rich cultural heritage of Valle d'Aosta and to support the conservative treatments undergone on the artworks. The laboratory staff interacts with many institutions in Italy and abroad and is regularly involved in multi-disciplinary projects aiming at the investigation and conservation of local and external cultural heritage materials. During my PhD, I also spent three months partly at the Art Institute of Chicago (Illinois) and partly at the Northwestern University (Evanston, Illinois), working in the group of Professor Richard P. Van Duyne. My research was financially supported by the European Union, the Regione Autonoma Valle d'Aosta and the Italian Ministry of Labour and Social Policy through grants of the European Social Fund.

In this PhD thesis the considered dyestuffs are discussed from both an historical and a chemical point of view. The dissertation reviews the literature, then it describes and discusses the experimental work and the results obtained in the course of the selection and optimization of the analytical paths. Finally, it presents several case studies that were performed within the research project and that demonstrate the applicability of the proposed analytical protocols on historical or archaeological objects.

In particular, the first chapter of the thesis reviews the main features of dyes and discusses the role of natural dyestuffs through history and their uses. It also illustrates the different types of dyeing processes. The different types of classification of natural dyes are reported and a concise description of the dyestuffs employed in the experimental work is given.

The second and third chapters are related to the non-invasive techniques employed for the investigation of natural dyestuffs. The second chapter introduces the principles of UV-Vis spectroscopy. It also presents an overview of the literature about the techniques used in the field of dyes identification and debates the main strong and weak points of each technique. On the other hand, the third chapter reports the results of the experimental work concerning the optimization of Vis-FORS for the analysis of natural dyes. The methodology employed to analyze mock-up samples of dyed textiles and purple colored parchments is described. The results obtained, i.e. the selection of spectral features that are relevant for dyes identification in historical samples, are discussed and the variables affecting the identification of the considered dyes are pointed out. Finally, the applicability of portable UV-visible reflectance spectroscopy for the identification of different classes of dyes is debated under the light of the experimental evidences.

The fourth and fifth chapters of the thesis deal with micro-invasive techniques, with particular concern to surface-enhanced Raman scattering (SERS). In the fourth chapter, an overview of the principles of Raman spectroscopy, surface-enhanced Raman scattering and of the literature about the application of micro-invasive techniques to the analysis of natural dyes are presented. In particular, the Raman effect is briefly described and the main factors involved in the enhancement of Raman signals are treated. Secondly, the features of the SER substrates and of the SER probes are highlighted. Literature about the use of SERS in the field of dyes identification is thoroughly reviewed and discussed. The last part of the chapter gives an overview of the other micro-invasive techniques that are traditionally used in the field of dyes identification. The fifth chapter reports the results of the experimental work and focuses on the optimization of SERS for the identification of dyes in various art materials. In particular, mock-ups of dyed textiles and parchments were prepared. The experimental section describes the synthesis of the SERS substrates and the methodology for their characterization. It also indicates the preparation of the different samples for SERS analysis and the extraction procedures tested on parchments mock-ups. The results of substrate characterization are presented and compared to SERS results obtained with the use of diverse substrates. SERS measurements carried out on the considered samples are fully discussed in order to assess the reproducibility of the method and the information that can be obtained by means of this technique.

Several case studies are discussed in the sixth chapter. The analyzed art objects cover a wide geographical area and a large time interval. In fact they come from the former Persian Empire to

Turkey, Egypt and continental Europe, with also a particular concern to objects presently conserved in Aosta Valley but having possibly various provenance. The considered objects are dated from the first centuries AD to the beginning of the 20th century. Most of the artworks are made of textiles (e.g. fragments of clothes, carpets, vestments, tapestries, interior textiles, swatches for dresses) but also a significant number of Persian manuscripts were considered. Moreover, a fragment from the dyed parchment of a precious purple codex dated to the 6th century AD and some cross sections from wooden polychrome statues and mural paintings were investigated. For some of the considered case studies, only non-invasive measurements were authorized, while for other objects it was possible to take samples. The works of art were analysed *in situ* through portable reflectance spectroscopy as well as, in some cases, by means of portable microscopy, portable X-ray fluorescence and portable molecular fluorescence. Surface-enhanced Raman measurements were carried out on some of the available samples and the results were compared to the data obtained with high-performance liquid chromatography when the quantities of samples allowed the use of this latter technique.

At the end of the thesis, general conclusions about the entire research work are given, with considerations about the reached goals and possible future developments of the different topics touched during the PhD.

CHAPTER 1
NATURAL DYES

Natural dyes in the latest years have been the object of a renewed interest. From one side, many people are more and more receptive to the return to natural products for health and environmental reasons and thus the use of natural dyes to color textiles or as food colorants is now addressed with attention. From the other side, a growing number of researchers in the field of cultural heritage are looking at the properties and application of natural dyes for conservative purposes. In the field of archaeometric investigation, a number of publications deals with the investigation of uses, history and the production techniques of natural dyes. Among the most comprehensive publications, the books "The colourful past" by Judith H. Hofenk de Graaf (2004) and "Natural dyes" by Dominique Cardon (2007) encompass most of the present knowledge about natural dyes and represent the peak production of authors that dedicated their whole life to the subject. The intent of the present chapter is to give a general overview of natural dyes in order to integrate the following chapters of the thesis, devoted to dyes analysis in complex analytical matrices such as those found in objects from the cultural heritage. Readers interested to more specific aspects are referred to the above cited books and to the other publications cited in the following paragraphs.

In particular, this chapter will begin by discussing the proper terminology and the main features of dyes. Then, an introduction on the mechanisms of absorption of light by the dyeing molecules will be given. The main uses of dyes and the reasons for their abandon in the last centuries will be briefly debated. The second part of the chapter includes a brief overview on the information that can be obtained from ancient treatises about textile dyeing and about the use of natural dyes as painting materials; it also describes the dyeing process, the preparation of lake pigments and the main technical properties of natural dyes. Finally, the third part will introduce the various classification of natural dyes. It will then reports the main features of the natural dyestuffs considered for the analytical work of the present PhD research, which are discussed in the following sections of this thesis.

1.1 NATURAL DYES: TERMINOLOGY, FEATURES, DEVELOPMENT OF THE COLOR AND USES

The word *colorant* indicates in English the whole set of substances that can confer a color to something, especially to artworks. Colorants can be distinguished into *pigments* or *dyes*. In particular, pigments in ancient times were usually inorganic, while dyes are organic compounds; both pigments and dyes can be of natural (mineral or plant/animal, respectively) or synthetic origin. The word *dyestuff* instead can be used to indicate a substance that can be used as a dye or from which a dye can be obtained (Collins). In this dissertation, the word *dye* will be used when the focus is on the dyeing behavior of the material, while the word *dyestuff* will be used in order to indicate the raw material (of plant/animal origin) from which a dye is obtained, and thus can be applied only to natural dyestuffs.

Pigments are insoluble in water and they should be mixed with an organic binder to be spread on the substrate to be colored: they cover the substrate by an opaque film and for that reason they are said to have "body". On the other hand, dyes are soluble in water and they impart color to the substrate by chemical bonds or physical absorption on it. Another important difference is that pigments are generally chemically stable and lightfast, while the color of a significant number of dyes depends on the pH conditions and on the exposure, especially extended, to light. For these reasons, pigments and dyes are applied in art to obtain diverse effects and often they are employed on different materials. In general, pigments are widely employed on polychromies such as easel and mural paintings, sculptures, manuscripts, while the greater use of dyes is reserved to textile artworks. Dyes can be also employed on polychromies to confer particular effects to the final aspect of the work of art when they are used as glazes, i.e. thin colored transparent layers. In addition, dyes can be "converted" into pigments by precipitating them onto an inert inorganic substrate, such as gypsum or calcium carbonate, thus obtaining the so-called *lake pigments* or *lakes*. Some dyes, like indigo and Tyrian purple, are inherently insoluble in water and they can be employed in painting as pigments, and in fact they require specific chemical treatment to be employed on textile fibers as other organic dyes.

The molecular structure of dyes allows them to absorb part of the visible light and thus they appear colored to the human eyesight. The groups that allow the absorption of light in the visible range of the electromagnetic spectrum are conjugated double bonds, which can be found in all the dye molecules. Those groups are said *chromophores* and their absorption will be briefly treated in paragraph 2.1.1. In addition, most of the considered molecules present also *auxochrome* groups, which conjugate with the chromphore groups and diminish the energy of the π^* orbitals, thus increasing the absorbed radiation and causing an hyperchromic effect (intensification of the color). The auxochrome groups create also a bathochromic shift of the absorption, thus developing color in molecular structures that would otherwise have absorbed light in the UV range. The main auxochrome groups are NH, NH_2, OH, COOH, SH and SO_3H, which are often found in dyeing molecules (Table 1.1). In addition, auxochrome groups are polar or even ionizable groups that enables the solubility in water of the chemical species and are also involved in the forming chemical bonds that bond the dye to the fiber (Poldi 2010).

As previously mentioned, dyes have been widely used to color textiles, even though some of them were also employed to color the human body or hair for decorative, religious or sanitary purposes. In addition, some dyes can be used also as spices or food colorants. Differently from pigments, some of which had been already synthesized several millennia before Christ, dyes were mainly of natural origin, both from plant and animal sources. Few exceptions are known, and consists only on extraction from natural organic materials in acid or basic environment in order to convert the precursors contained in the raw material into colored molecules (Farrar 1974), and not on actual synthesis processes like that employed, as an example, to produce the pigment Egyptian blue (Pages-Camagna, Colinarte, and Coupry 1999).

The date of introduction of dyeing is not known, but it is likely that it can be situated in the area of Ancient civilizations (Mesopotamia and Egypt but also India and Pakistan) in a period near to the first manufacturing of metals, since most of the dyes require the use of metallic compounds to be steadily bound to the textile fibers, as it will be discussed in the following paragraphs. Anyway, before the discovery and exploit of metals to fix the color, barely stable dyed textiles could be produced. Dyes became to be traded also from exotic countries and the ability of Phoenicians traders allowed them to build their power on the commerce of a precious dye, known as Tyrian purple. The art of dyeing rapidly grew and it is well known that Romans had skilled dyers with a strict subdivision of the dyes employed for each workshop. This partition continued throughout the Middle Ages to the epoch of the municipalities in Italy, where the guilds of dyers became very powerful. The enlarging of the world with the discovery of new territories further widened the availability of dyestuffs, as well as the trades of dyestuffs and textiles with the Far East. In fact, the art of dyeing was well known and highly skilled both in Eastern countries (India, China, Japan) and in Central and South America. The importance of natural dyes lasted since 1856, when W. H. Perkin synthesized mauve, the first synthetic dye, followed quickly by patents for other new dyes. By the end of the 19th century, a large number of synthetic dyes was available on the market and soon replaced natural sources of dyes. In fact, the cost of synthetic products was considerably lower than that of natural dyes, they were easier to use, more stable and the final color was more reproducible. In particular, as the most precious natural dyes were imported from all over the world, their final price was strongly influenced by the costs of their transport. In addition, natural dyes are generally composed of more than one dyeing molecule, whose relative quantity is variable depending on many factors among which provenance and environmental conditions play a major role. As a consequence, different lots of the same natural dyestuff could confer different colors to textiles. On the contrary, synthetic dyes are composed of one molecule and thus the dyeing procedure is in this case highly reproducible.

1.2 DYEING PROCEDURES AND PREPARATION OF LAKE PIGMENTS

The first written work which describes the use of dyes as well as some dyeing procedures is the *Naturalis Historia* by Pliny the Elder (1952), dated to the 1st century AD. In the Late Antique and Middle Ages, several treatises containing recipes to prepare pigments and dyes were written. Most of these treatises were compiled for monks or painters, who employed dyes mainly to prepare lake pigments or to obtain particular colors on illuminations. Among them, the *Manoscritto di Lucca* (Smith and Hawthorne 1974), dated to the 8th century AD, is the older known, but also the *Mappae Clavicula* (Smith and Hawthorne 1974), the Leyden Papyrus X (Caley 1926), the Stockholm Papyrus (Caley 1927) and the 14th century *De arte illuminandi* (Brunello 1971) should be mentioned. Since the art of dyeing was reserved to the members of a guild, very few information was diffused in specific works and the first treatise about dyeing techniques was published only in 1548 by Giovan Ventura Rosetti: *Plictho de larte de tentori che insegna a tanger pani telle bambasi et sede si per larthe magiore come per la commune*. After this work, many treatises about dyeing were published in the following centuries, some mainly devoted to practical aspects of this art while others tried to explain the chemical and physical aspects of the processes involved in the various phases. Further information on dyeing textiles can be found in the previously cited books by Hofenk de Graaff (2004) and Cardon (2007), where sources are clearly detailed and debated, while a more specific discussion about historical information on the processes employed to color parchment in purple is reported in section 3.2.

Besides the previously cited chemical stability, solubility in water and lightfastness there are some other important properties of the dyes that should be taken into account when selecting them. In particular, of main interest is their affinity with the fiber, i.e. the dye must fix to it, and its solidity, that is the ability of the dye to remain fixed to the fiber for a long time and despite the external stresses. Another essential property of dyes is their resistance to washing, especially for everyday textiles like dresses or linens, while this property is less relevant for textiles originally intended as decorative or luxury objects, which were hardly ever washed. Some works related to the ascertaining of some of these properties are reported in paragraphs 2.3 and 4.6.

The dyeing process involves different phases. Firstly, the dyes should be extracted from the raw material and dissolved in water. After that, the substrate should be prepared to receive the color. Then, the substrate is kept in contact with the dye in the dye-bath and finally the dyeing molecules that did not fixed to the substrate should be removed.

The first step is the preparation of the dye-bath. The dyestuff is generally soaked in water for a certain amount of time (some hours up to several days) and then the extracting procedure is completed by heating the mixture for a limited amount of time. The solution is then filtered and the solid residue can be further extracted until exhaustion. In some cases, particular conditions are required to allow the molecules contained in the dyestuff to be converted into dyeing molecules. For this purpose, acid or basic solutions can be employed, depending on the dye. Before the advent of modern chemistry, acid baths were prepared by employing natural acids like lemon juice, while for basic solutions the use of potash or urine were diffused. The extensive use of fermented urine in the dyeing processes, and the resulting unpleasant smell, caused the dyeing workshops to be located out of the cities in the past. In the case of insoluble species (e.g. indigoid

dyes like indigo and Tyrian purple), their transformation into a soluble form, that can be put into contact with the fibers, naturally occurs after several weeks of maceration in anoxic conditions. The procedure was performed in large vats, therefore indigoid dyes are referred to as vat dyes. The obtained solution has a different color than the original molecule, as it is yellow-green both for indigo and Tyrian purple. More recently, the reduction of insoluble dyes is obtained in some minutes with the use of reducing agents such as sodium dithionite (sodium hydrosulphite) in a basic environment, orpiment (As_2S_3) or vitriol ($FeSO_4 \cdot 7H_2O$), a procedure known as chemical vat.

The amount of raw material required to dye textile fibers strongly depends on the yield of the dyestuff, and the affinity of the dyes with the fiber and varies from about 1% to 400% as respects the weight of the dry fiber to be dyed.

Textile fibers can be dyed before spinning, in yarn or even after the weaving. Dyeing of flocks (i.e. before spinning) is mainly reserved to wool fibers, while for the other natural fibers yarn or cloth dyeing are preferred. The substrate should be thoroughly washed with soap in order to remove the hydrophobic protective layers of the raw natural fibers, which can prevent the fixation of the dye. Then, depending on the dye to be fixed, the fibers might be subjected to a further treatment, called mordanting. In fact, some dyes fix directly to the fiber, while others require the use of a mordant. This is a metallic salt in which the cations interact both with the dyeing molecules and with the fiber. The most employed mordant in antiquity was alum ($KAl(SO_4)_2 \cdot 12H_2O$), often employed with cream of tartar (potassium hydrogen tartrate, $K_2C_4H_5O_6$) as an additive to give shine to the final color. Also iron(II) sulfate ($FeSO_4$), tin(II) chloride ($SnCl_2$), copper(II) sulfate ($CuSO_4$) and potassium dichromate ($K_2Cr_2O_7$) were employed, even though they modified the final color of the fiber. Mordanting can be performed before dyeing, as in the case of alum, during dyeing or after dyeing (post-mordanting) depending on the mordant employed and on the desired result. Mordants are added to warm water in quantity that varies from about 1% to 20% compared with the weight of the dry fiber. The metallic salt is allowed to dissolve and then the fibers are immersed into the solution and let in from some minutes to one hour.

Prior to be dyed, the fibers are soaked in water to swell the fibers and then they are added to a warm dye-bath which is subsequently heated at higher temperature. The quantity of water is not crucial: it must be enough to allow the fibers to move in the bath, since in any case it is the dye/fiber ratio in weight that determines the saturation of the final color, and not the concentration of the dyes within the dye-bath. The temperature employed for dyeing strongly depends on the fiber to be treated. Wool is more resistant and thus can be dyed at about 95°C, while silk suffers temperatures higher than 70°C. In addition, the temperature of a vat dye should not exceed 55°C. The fibers are kept in the dye-bath at the required temperature for some minutes up to one hour. The longer is the time, the higher is the saturation of the final color. The bath shall be frequently mixed in order to get a homogeneous dyeing and to allow the dye to penetrate the fibers.

After that , the fibers can generally be allowed to cool in the dye-bath, even though in some cases this can cause undesired effects. In the case of vat dyes, the fibers must be removed immediately and then exposed to air in order to oxidize the dye and thus allow it to precipitate on the fibers. Despite the specific case of vat dyes, when fibers should be removed from the dye-bath, they have

to be put into water at about the same temperature of the dye-bath in order to avoid matting. Fibers should then be washed in water several times until no color dissolves in the water and allowed to dry in the shadow. The final color can be obtained by several subsequent dyeing, employing the same dyestuff or by mixing different materials.

The preparation of lake pigments, similarly to dyeing, generally requires the extraction of the dyes from raw materials, although dyes ware also obtained from clippings of dressmakers and wastes of dyeing workshops. Then, an inert solid is added to the solutions. Most of the inert substrates used in the past were white substances, also employed as white pigments or preparation layers in painting. In particular, gypsum ($CaSO_4 \cdot 2H_2O$), calcium carbonate ($CaCO_3$) and lead white ($2PbCO_3 \cdot Pb(OH)_2$) were widely employed. Hereafter, alum is added to the solution causing immediate effervescence due to the release of carbon dioxide and followed by the precipitation of the lake. The solution is decanted or filtered, precipitated and the precipitate is allowed to dry.

1.3 CLASSIFICATION AND OVERVIEW ON NATURAL DYES

The classification of natural dyes can be made following different distinctions. The most immediate is certainly a classification based on the final tint of the dye: red, orange, yellow, purple, blue, green, brown dyes. In addition, they can be classified depending on the origin of the dyestuff, as dyes can be extracted from animals, plants but also from fungi and lichens. A third, more practical, classification divides the dyes according to the dyeing technique that should be used to fix them to the fibers. In this case, we can speak about direct (or substantive) dyes, mordant (or adjective) dyes and vat dyes (Hofenk de Graaff 2004; Cardon 2007), according to dyeing processes discussed in the previous paragraph 1.2. A fourth classification consider the chemical families of the different dyes: quinones (anthraquinones and naphtoquinones), flavonoids, indigoids, chalcones, anthocyans, carotenoids, curcuminoids, alkaloids, tannins and other minor groups (Cardon 2007).

In the following presentation of dyestuffs, their classification on the basis of the color will be used. The color is indeed the first evidence to identify an unknown dye and can guide the researcher in the analytical path, even though in some cases discoloring and degradation processes could alter the original color. Only dyestuffs that were considered in this research are presented; for each of them, the basic information about origin, dyeing properties and history is given. In addition, the main dyeing molecules are listed and reported in Table 1.1. Most of the information is obtained from the already cited books by Cardon (2007) and Hofenk de Graaf (2004). Green colors for the research work were all obtained by superimposing a blue dye to a yellow dye (Appendix 1), as no stable green dyes are found in nature and thus they were seldom employed in the past. In each section, the dyestuffs are listed in alphabetical order.

1.3.1 BLUE DYES

INDIGO

Indigo refers to dyes obtained from Indigofera species, among which *Indigofera tinctoria* L. is the most popular. It is a perennial herb native of India, which nowadays grows in tropical and subtropical regions from Africa to southeast Asia. Indigo is a vat dye, produced by macerating the leaves and stems for several weeks or, after the 18th century, by chemical vat, as detailed in

paragraph 1.2. The main coloring agents of indigo is indigotin (Table 1.1), an indigoid compound. The plant does not contain the dye itself but the precursor of the dye, indican, which hydrolyzes and forms indoxyl. Indigotin, forms from the combination of two indoxyl molecules through exposure to oxygen. Minor components are indirubin (2-15% depending on the species, indigoid), also formed from indoxyls, and kaempferol glycosides (flavonoids). Other *Indigofera* species are found all over the world in tropical regions. Indigo is the most used blue dye all over the world in all periods and it was known in India before the 1st millennium BC. Synthetic indigotin is still employed nowadays to dye blue jeans. In 1740 Saxon blue was invented by adding concentrated sulfuric acid to the indigo paste obtained after maceration. The disulfonic acid (disodium 5,5'-indigotin disulfonate, Table 1.1) obtained is soluble in water and thus does not require the complex procedure of vat dyeing, even though it has a poorer light and washing fastness. A high-quality Saxon blue was produced in 19th century and is known as indigo carmine.

LOGWOOD

Logwood is a mordant dye obtained from the heartwood of *Haematoxylum campechianum* L., a small tree or shrub, native of the Yucatan peninsula. It was imported into most of Central America, West Africa, India and Indian and Pacific Ocean islands. The main dyeing molecule is haematoxylin (Table 1.1), a homoisoflavanone, which can oxidizes to the more colored haematein (Table 1.1, homoisoflavonoid); minor quantities of brazilein (Table 1.1) and other homoisoflavonoids and tannins are also present. Logwood was already employed in pre-Columbian America and its use was discovered firstly by Spanish and then by English, who fought the "Logwood War" to control the Campeche Bay in central America. Blue and purple colors obtained from logwood show poor lightfastness, while logwood is the best dyestuff, if combined with a proper mordant and sometimes with other dyes, to obtain durable black.

WOAD

Woad is the indigoid dye obtained from *Isatis tinctoria* L., a biennial herb growing wild in most of Europe, in North Africa and in temperate regions of Asia. As indigo, it is a vat dye in which indigotin (Table 1.1) is the main coloring agent. The dye is obtained by maceration of the rosette leaves of the plant. Among the precursors, isatan A and isatan B, only the second one hydrolyzes to form indoxyls, which then combine to create indigotin. The indigotin yield is lower compared with indigo, but the distinction between these dyes in textiles is still arduous. Woad was used in Europe from the Neolithic to the 17th century, even though some cultivations resisted in France and Germany until the 19th century, while in Italy the last documented woad harvesting was carried out in 1902. The reason of its abandon has to be found in the increasing importation of indigo from Asia from the 12th century onwards. Most European countries regulated the use of indigo in order to protect the local economical interests, but after some centuries the superiority of indigo forced rulers to allow its use.

1.3.2 RED AND ORANGE DYES

ANNATTO

Annatto is a direct dye obtained from *Bixa orellana* L., an evergreen shrub native of tropical America which is now also cultivated in Africa, India and Sri Lanka. Annatto is obtained from the pressed seeds of the plant fermented over a week. The dyeing molecules of annatto are carotenoids, among which bixin and norbixin (Table 1.1) are the most abundant. Annatto was used in the Amazon for body-painting and by Mayas and Aztecs and imported in Europe in 16th century. It was and is still widely used as a food colorant.

ARMENIAN COCHINEAL

Armenian cochineal is a mordant dye obtained *from Porphyrophora hamelii* (Brandt, 1833), an insect of the Coccoidea family (1 cm X 7mm). These insects live on the roots and rhizomes of two grass plants, *Aeluropus littoralis* (Gouan) Paul. and *Phragmites communis* Trin., in dry steppes or semi-desert environments in central Asia. It is found also in Turkey, Iran and in the area of Mount Ararat. The dyestuff is obtained by drying the adult females and possibly also the third instars of males. The main dyeing molecule of Armenian cochineal is carminic acid (0.8% of the dried insect, about 90% of the colorants, Table 1.1). It contains also kermesic and flavokermesic acid (Table 1.1), other colorants named dcIV and dcVII plus a yellow substance named dcII. Armenian cochineal was mainly employed to dye light tones of silk, as it could not compete with the coloring power of kermes. Despite the greater amount of colorant with respects to Polish cochineal, higher quantities of dye were needed as the dried insect contains about 30% of fats which prevent the dyeing.

BLOODRED WEBCAP

Bloodred webcap indicates *Cortinarius sanguineus* (Wülf.: Fr.) S. F. Gray, a dark blood red fungus living in damp areas of Europe and North America. The whole fruiting body can be used to obtain a direct or a mordant dye. It contains anthraquinones: emodin (Table 1.1), dermocybin, dermorubin, physcion, endocrocin, dermoglaucin, dermolutein.

BRAZILWOOD

Brazilwood is a mordant dye obtained from the heartwood of *Caesalpinia echinata* Lamarck, a small-medium tree living in Brazil. The main dyeing molecule is brazilin (Table 1.1), a homoisoflavanone, which like heamatoxylin can oxides to a deeper colored molecule, brazilein (Table 1.1). In addition, other colorless or yellow flavonoids can be found. An Asian species is also known, *Caesalpinia sappan* L., from which sappanwood was obtained. Sappanwood was imported into Europe from the Early Middle Ages from Asia, where it was widely used as a dyestuff. It was called with names like brazil, brasile, brisil derived from *brasa* which means embers. After the discovery of the New World, similar trees were found and soon the Portuguese changed the name of their new territory in South America to Brazil, the name of the dyestuff. As for logwood, several wars were fought to get control of the area where brazilwood grew.

HENNA

Henna is the name of the dyestuff obtained from *Lawsonia inermis* L., a shrub or small tree native of western India which is now cultivated in most of the tropical and subtropical regions of the world. The dried leaves can be used to dye directly or with a mordant. The main dyeing molecule is lawsone (Table 1.1), a naphtoquinone, while yellow flavonoids and tannins are present as minor components. Henna has been used since ancient times both to dye textiles and to color the body and the hair. Its use as a textile dye still survives in some Islamic regions, while body decorations and hair dyeing seemed not to suffer from the advent of industrial synthetic products.

KERMES

Kermes is a mordant dye obtained from *Kermes vermilio* (Planchon, 1864), an insect of the Coccoidea family (6-8 mm in diameter) which exclusively lives on *Quercus coccifera* L., also known as kermes oak. This tree can be found in dry thickets of the Mediterranean region. Kermes is obtained from the adult females full of eggs or from the eggs themselves. The main constituent of kermes is the anthraquinone kermesic acid (Table 1.1), but also flavokermesic acid (Table 1.1) and other red and yellow colorants are present at lower levels. Kermes was one of the most prestigious dyes in antiquity, charged with symbolic values and traded all over the Old World. Mexican cochineal, imported from the New World in the 16th century, totally supplanted kermes in a short while. Kermes is now a threatened species and thus should not be employed for dyeing except in small quantities for research purposes.

LAC DYE (INDIAN LAC)

Lac dye is a mordant dye obtained from *Kerria lacca* (Kerr, 1782), an insect of the Coccoidea family (5 mm long) which lives on various species of trees and bushes from Pakistan to southern China. Each insect is surrounded by a lac or resin-like material bounded with those of the other insects of the colony, forming a red-brown continuous encrustation. The quality of the dye depends on the ratio between the insect bodies and the resin and thus insects should be preferably collected after the females die. Lac dye is composed of at least seven different colorants, among which laccaic acids A, B, C, D (flavokermesic acid, Table 1.1) and E (Table 1.1). The use of lac as a dye is attested in India from the 4th century BC and it remained the most used red dye for Chinese silks until the advent of Mexican cochineal.

MADDER

Madder is a general name employed to indicate dyes obtained from Rubiacea family. Among them, *Rubia tinctorum* L. is the most popular. It is an herbaceous plant with a perennial root system, native of the Middle East and the East of the Mediterranean. It was successfully introduced in southern and central Europe, Far East, America and Africa. The mordant dye is obtained from the dried roots of the plant, which contain a number of anthraquinone derivatives. In particular, alizarin (Table 1.1)is found, together with purpurin (Table 1.1), and a number of other compounds such as pseudopurpurin, rubiadin, munjistin, christofin, lucidin, rubianin, xanthopurpurin, nordamnacanthal, quinizarin, anthragallol and others. Madder is the most employed red dye in history and its use as a mordant dye dates to 1900 BC in Mesopotamia and at

least 1050 BC in Egypt. Madder was substituted only in 1869 by synthetic alizarin patented in that year by Heinrich Caro. Other species from the Rubiaceae family can be found all over the world, containing different relative quantities of the above mentioned anthraquinones.

MEXICAN COCHINEAL

Mexican cochineal is a mordant dye obtained from *Dactilopius coccus* (O. Costa, 1835), an insect of the Coccoidea family (about 4.5 cm long) living on cactus of various *Opuntia* species of Central and South America. It was exploited before the arrival of the Spanish in the Peruvian Andes and in Mexico. Among the various attempts of naturalization, the unique success was realized in the Canary Islands. The dyestuff is obtained from the dried females full of eggs. The main dyeing molecule of Mexican cochineal is carminic acid (about 95% of the colorants, Table 1.1), but also kermesic acid (0.1%, Table 1.1), flavokermesic acid (0.4%, Table 1.1), dcII (2.3%) and other red colorants are present (dcIV, dcVII and dcIII). Compared to Armenian cochineal, Mexican cochineal has a higher amount of dcII and a minor quantities of kermesic acid. Mexican cochineal arrived in Europe in 1523 and soon became the most requested red dyestuff. It reached also Asia thanks to the trades of Venice and in the 17th century it replaced Indian lac for silk dyeing and painting in the Far East.

POLISH COCHINEAL

Polish cochineal is a mordant dye obtained from *Porphyrophora polonica* (Linnaeus, 1758), an insect of the Coccoidea family smaller than Armenian cochineal (1.5-6.5 mm in diameter) which lives underground except in the fertilization period from continental European regions to Russian Far East. The dyestuff is obtained from the dried cysts, the third instars of males and the adult females are collected. The composition depends on the stage of development of the insects but in general Polish cochineal is mainly composed of the anthraquinone carminic acid (0.6% of the dried insect, 84% of the colorants, Table 1.1). Also kermesic and flavokermesic acid (Table 1.1) are present and other colorants named dcIV, dcVII and ppIII. The use of Polish cochineal was similar to that of Armenian cochineal, but it was cheaper than Armenian cochineal, as minor quantities of the dyestuff should be employed.

SAFFLOWER

Safflower indicates *Carthamus tinctorius* L., an annual plant originating from the area from northern India to the Near East cultivated in Mediterranean and central Europe and along the silk road to China. Nowadays it can be found also in America, Australia and Asia. The florets separated from the capitulum are washed several times until all the soluble yellow colorants are removed, then they are mixed with soda or ash in a filter and water is poured in order to obtain the red dyes in a solution. After that, lemon juice is added to precipitate the dye. The main red dyeing molecule is carthamin (Table 1.1), a quinochalcone, but a number of yellow quinochalcones are also present, some of them turning into red dyes by oxidation, as anhydrosafflor yellow B. Safflower red is a direct dye. The yellow solution obtained from the washings was employed to dye yellow in Persia, India and China, which were among the greater users of safflower red, especially for precious Chinese silk. This dye was used despite its high cost and very poor lightfastness.

SANDALWOOD

Sandalwood is a mordant dye obtained from the heartwood of *Pterocarpus santalinus* L., a medium tree which lives in hot dry climates of the Andhra Pradesh and Madras states in India. The main dyeing molecules are santalin A and B (Table 1.1), an association of isoflavonoids and flavonoids, plus santalin C and other dyes of the flavonoid family. These coloring matters are only partially soluble in water and dyers should know some guarded secret to obtain a color from sandalwood. Despite this, sandalwood had a role in European dyeing industry and was for example used for the uniforms of the French army in 19th century.

1.3.3 PURPLE DYES

ALKANET

Alkanet is a mordant dye obtained from the roots of *Alkanna tinctoria* (L.) Tausch., a perennial plant which lives in dolomitic soils all around the Mediterranean and in central Europe. The main coloring matter is alkannin (Table 1.1), a naphtoquinone, present in the form of various esters; depending on the provenance, shikonin (Table 1.1) can be found. Since most of its dyeing molecules are insoluble in water, its use was limited before 18th century. In addition, in ancient treatises very similar names were employed to indicate henna and alkanet, thus generating confusion.

FOLIUM

Folium is the name used in European Middle Ages to indicate dyes obtained from *Chrozophora tinctoria* (L.) A. Juss., a plant living in coastal areas of the Mediterranean, such as Turkey, Italy, France. Very few information is known about this dyestuff. The plant is also called turnsole and folium is the water extract obtained from its fruits, said to be used to obtain purple colors in ancient treatises. It can be used as a mordant dye. No molecular information is available about the composition of the dyes, since this aspect has not been investigated yet.

ORCHIL

Orchil is a dye obtained from a wide number of lichen species, among which *Roccella* spp., *Ochrolechia* spp., *Pertusaria dealbescens* Erichs., *Lasallia pustulata* (L.) Mérat and *Melanelia fuliginosa* (Fr. Ex Duby) Essl. The dye is obtained by reaction of orcinol, a derivative of orsellinic acid, with ammonia and oxygen in the air. The whole thallus of the lichen is employed in the process. This procedure is quite complex and requires up to twenty days. Potassium carbonate and urine were employed to obtain the suitable chemical environment. Litmus was also obtained from lichen species and it can instead be prepared with the same procedure by adding lime, soda or potash, but its use was more directed to scientific applications as it is a pH indicator. Since the possible sources of orchil are many, it is not easy to locate the provenance of the raw materials, but among the producing areas one can cite the Mediterranean coasts, the eastern Atlantic and its islands, comprised the Canary and the British islands. The main dyeing molecules of orchil are orceins (Table 1.1), while the main precursors are lecanoric acid and erythrin. Orchil was used since the era of ancient Mediterranean civilizations as it could replace or at least economize the high quantities of mollusks employed to dye purple with Tyrian purple, even though it is less

lightfast. Its fortune lasted also after the disappearance of Tyrian purple in the Mediterranean area and it is said that the Florentine family of merchants Ruccellai, who discovered the lost secrets of orchil in the Levant, owed its name to this dye.

SHIKON

Shikon is a mordant dye obtained from the roots of *Lithospermum erythrorhizon* Siebold & Zuccarini, a perennial plant from uncultivated areas of China, Korea, Russia and Japan. The roots of the plant are soaked in water and rice vinegar, as its main dyeing molecule, shikonin (Table 1.1) is only partly soluble in water at neutral pH. Also another naphtoquinone, alkannin (Table 1.1), can be found in this dye. Chinese and Japan silks were finely dyed with shikon, while its use was reserved to the royal family in the Nara period in Japan (8th century AD).

TYRIAN PURPLE

The so-called Tyrian purple is the most precious dye ever produced by the mankind. It is obtained, like indigo, through a complex process from various mollusks of the *Muricidae* family living in the Mediterranean, among which *Bolinus brandaris* (*Linnaeus*, 1758), *Hexaplex trunculus* (*Linnaeus*, 1758) and *Stramonita heamastoma* (*Linnaeus*, 1766). It is called "Tyrian" from the name of the Phoenician town where it is said to be discovered by chance. Now it is known that also the populations of British Isles, Central and South America and Japan knew how to exploit the local purple mollusks to dye textiles. The part of the mollusk containing the precursors of the dyeing molecules is the hypobranchial gland, which in the Mediterranean area was detached from the animal by killing it. On the contrary, the native American populations developed a different method to extract the dye that kept the animal alive thus allowing further extractions over a certain time lapse. The precursors contained in the mollusk are sulfate esters of indoxyls, varying from one species to another, which hydrolyze to yellow derivatives that combines to form a green photolabile compound. When exposed to light, the green compound is converted into indigotin, 6-bromoindigotin and 6,6'-dibromoindigotin (Table 1.1). In addition, indirubin and 6,6'-dibromoindirubin can be formed by another process. The relative quantity of these compounds determines the color of the dye, from purplish-red to violet-blue. As indigo, Tyrian purple is a vat dye. The first evidences of the use of purple in the Mediterranean are dated to 1800-1600 BC in Crete. Most of the monarchs of Ancient civilizations of the Mediterranean and the Middle East wore purple as a sign of power and distinction. Its use ceased in Europe with the end of the Occidental Roman Empire in 5th century AD, but in continued in smaller quantities in the Eastern Mediterranean. Byzantine emperors continued indeed to wear purple throughout the Middle Ages and to send textiles dyed with purple as precious gifts to European kings and princes. The production of purple came to a definitive end in 1453 with the Turk conquest of Constantinople, followed in 1464 by a decree by Pope Paul II who ordered its substitution with kermes.

1.3.4 YELLOW DYES

COMMON BUCKTHORN

The name indicate a mordant dye obtained from the barks and fruits of *Rhamnus cathartica* L., a shrub living in Europe, except in the extreme south. It can be found also in North Africa and in Asia. It contains anthraquinone derivatives, among which emodin (Table 1.1) glycosides, as well as flavonoids aglycones are contained in the fruits (rhamnetin, rhamnazin, rhamnocitrin, quercetin, kaempferol). The barks confer red color, while the immature fruits dye yellow and the mature fruits create a green color. The fruits are included in the sources for Avignon or Persian berries, important yellow dyes.

DYER'S BROOM

Dyer's broom is *Genista tinctoria* L., a shrub found in dry areas in most of Europe, except the extreme north and some islands. The flowering branches are employed to obtain the mordant dye. Its main dyeing molecules are luteolin (Table 1.1), a flavone, together with apigenin (Table 1.1) and genistein (Table 1.1). Dyer's broom is cited in several dyeing treatises, among which *Plichto* by Rosetti, and it is known to have been widely employed in England from the Middle Ages to the 19th century.

OLD FUSTIC

Old fustic is obtained by the heartwood of *Chlorophora tinctoria* (L.) Gaud., a large tree living in the west coasts of Central America, in the Caribbean and in tropical South America. The dye can be used both directly and with a mordant, but this second option was generally preferred in Europe. Old fustic contains morin (Table 1.1), kaempferol (Table 1.1), both flavonols and maclurin (Table 1.1), a benzophenone. Old fustic was included with other dyes in several recipes to dye black, yellow and green since its importation into Europe. It continued to be used until very recently in the US and it is still required from dyeing and tanning industries.

SAFFRON

Saffron (*Crocus sativus* L.) is a small perennial herb cultivated from Western Europe through temperate and subtropical Asia to China. A direct dye can be obtained from the dried stigmas of the flower. The main constituents are crocetin and crocin (Table 1.1), diapocarotenoids. Saffron has a high cost but also a high tinctorial strength. It was used in Europe and Asia since ancient times, as it gives a golden yellow, but it was rapidly substituted with dyes from the New World due to its elevate cost.

TURMERIC

Turmeric is the dyestuff obtained from the dryed rhizomes of *Curcuma* species, among which *Curcuma longa* L. is the most popular. It is a perennial herb original of southern Asia, which can be cultivated in most tropical and subtropical areas with adequate rainfalls or irrigation. As saffron, is a direct dye with high tinctorial strength. The rhizomes of the plant contain curcumin, demethoxycurcumin and bisdemethoxycurcumin (Table 1.1), all curcuminoid molecules. Its use is

widespread in India and neighboring areas, as well in the Pacific islands, while it was less diffused in Europe, mainly to give a golden hue to yellows or turn scarlet into orange.

WELD

Weld indicates *Reseda luteola* L., an annual or biennial herb living in sandy or rocky soils all over Europe. It can be found also in North Africa, the eastern Mediterranean, Turkey and southwest Asia. The whole plant was used to obtain a mordant dye, even though it is now ascertained that the coloring matters are contained only in the leaves, inflorescences and fruits. The main dyeing molecule is luteolin (Table 1.1), a flavone, followed by apigenin (Table 1.1). Weld has been used in Europe since prehistoric times and continued to be employed by Egyptians and Romans, during the Middle Ages and until the 19th century, when it was replaced in industry before the advent of synthetic dyes by quercitron bark. It was also employed in the Ottoman Empire.

1.3.5 BROWN DYES

GALLS

Galls are obtained from *Quercus infectoria* Oliv., a semi-evergreen shrub or small tree found in Asia Minor, eastern Mediterranean, southern Europe and Iraq up to 2000 m of altitude. The irregular spherical excrescences produced on the branches of the oak by the puncture of the female oak gall wasps, called galls or gallnuts, contain mainly gallotannins, the simplest being ester of the gallic acid (Table 1.1) and are employed to dye directly or with the addition of a mordant. The reaction with iron to obtain black colors was already known by Romans and Greeks. The use of galls to obtain black and brown colors continued until the 20th century.

QUEBRACHO

Quebracho is obtained from the heartwood of *Schinopsis quebracho-colorado* (Schltdl.) F. A. Barkley & T. Mey., a large tree living in open pampas of northern Argentina, eastern Bolivia and Paraguay. The heartwood contains gallic acid (Table 1.1), ellagic acid (Table 1.1), condensed tannins, yellow flavonols and aurones. It was used with copper and iron mordanting.

WALNUT

Walnut (*Juglans regia* L.) is a tree native of Asia Minor and now naturalized in temperate regions of Europe and China. The husks, the leaves and the barks of the branches and the roots contain dyeing molecules which can be employed directly. Walnut contains a high number of dyes belonging to different chemical classes, among which the most important is the naphtoquinone juglone (Table 1.1), but also tannins and flavonoids are found. Walnut husks were usually allowed to ferment for a long time (one year or more) to dye brown, while immature fruits gave grayish-green colors. Browns dyed with walnut were obtained in every country where species of walnut grew since the early days of dyeing in Persia. All the above cited parts of walnut were widely employed in the Middle Ages workshops all over Europe. It was also employed to produce fawn colors and *brunitures* in tapestries.

Table 1.1: Main dyeing molecules of the considered dyestuffs.
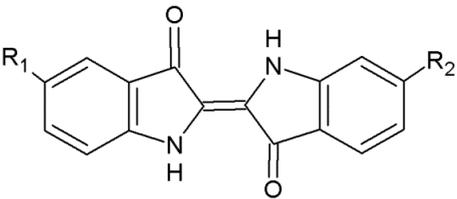
Molecule	Functional group(s)	Name of the compound
	$R_{1,2}=H$	indigotin
	$R_1=H$ $R_2=Br$	6-bromoindigotin
	$R_1=Br$ $R_2=Br$	6,6'-dibromoindigotin
	$R_1=NaO_3S$ $R_2=NaO_3S$	disodium 5,5'-indigotin disulfonate
	$R_{1,2}=H$	brazilin
	$R_1=OH$ $R_2=H$	haematoxylin
	$R=H$	Brazilein
	$R=OH$	haematein
	$R=CH_3$	Bixin
	$R=H$	Norbixin
		lawsone
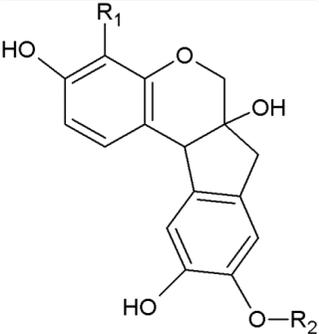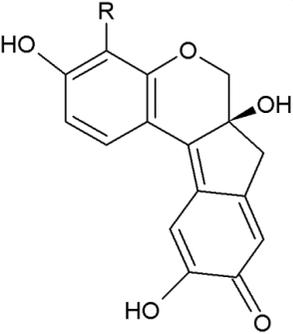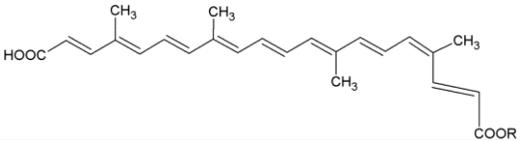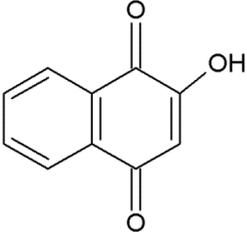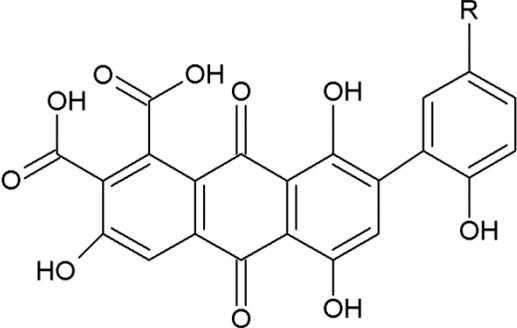
Molecule	Functional group(s)	Name of the compound
	$R=CH_2CH_2NHCOCH_3$	Laccaic acid A
	$R=CH_2CH_2OH$	Laccaic acid B
	$R=CH2CHCOOH$ NH_2	Laccaic acid C
	$R=CH_2CH_2NH_2$	Laccaic acid E
	R_1=Glu $R_{2,3,4}$=OH R_5=COOH R_6=CH$_3$	Carminic acid
	R_1=H $R_{2,3,4}$=OH R_5=COOH R_6=CH$_3$	Kermesic acid
	$R_{1,3}$=H $R_{2,4}$=OH R_5=COOH R_6=CH$_3$	Flavokermesic acid Laccaic acid D
	$R_{1,3,5}$=H R_2= CH$_3$ $R_{4,6}$=OH	emodin
	R_1=OH $R_{2,3,4,5,6}$=H	alizarin
	$R_{1,3}$=OH $R_{2,4,5,6}$=H	purpurin
		carthamin
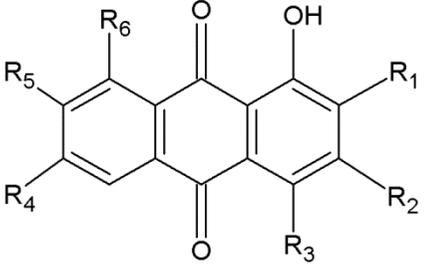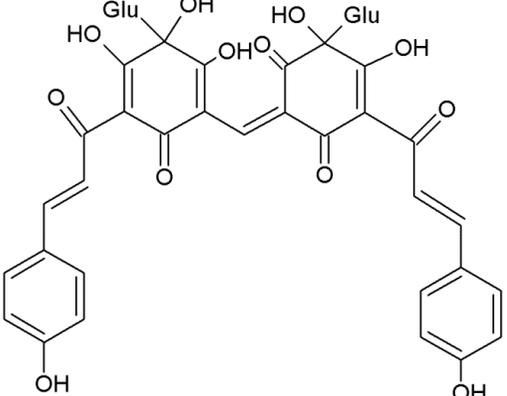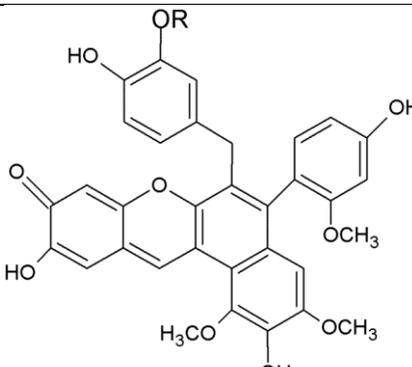
Molecule	Functional group(s)	Name of the compound
	R=H	Santalin A
	R=CH$_3$	Santalin B
		alkannin
		Shikonin
	R=NH$_2$	α-amino-orcein
	R=OH	α-hydroxyorcein
	R$_{1,2,5,6}$=OH R$_4$=H R$_3$=OCH$_3$	Rhamnetin
	R$_{1,2,6}$=OH R$_{4,5}$=H R$_3$=OCH$_3$	Rhamnocitrin
	R$_{1,2,3,5,6}$=OH R$_4$=H	Quercetin
	R$_{1,2,3,6}$=OH R$_{4,5}$=H	Kaempferol
	R$_{1,4}$=H R$_{2,3,5,6}$=OH	Luteolin
	R$_{1,4,5}$=H R$_{2,3,6}$=OH	Apigenin
	R$_{1,2,3,4,6}$=OH R$_5$=H	Morin
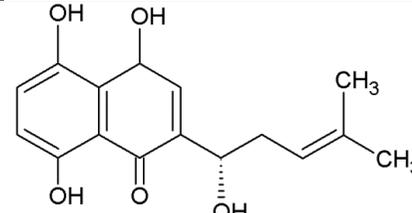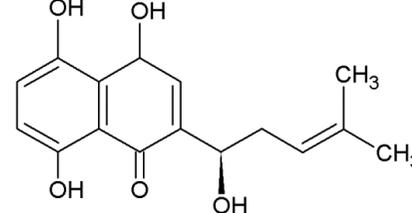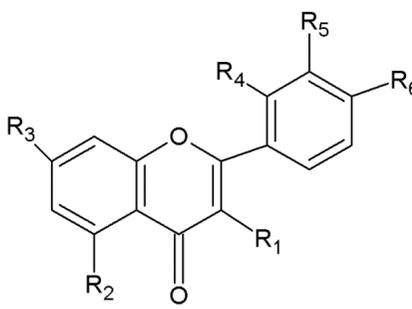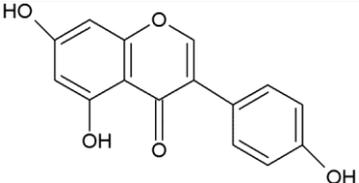
Molecule	Functional group(s)	Name of the compound
		Genistein
		Maclurin
	R=H	crocetin
	R=β-D-gentiobiosyl	Crocin
	$R_{1,2}=OCH_3$	Curcumin
	$R_1=OCH_3$ $R_2=H$	Demethoxycurcumin
	$R_{1,2}=H$	Bisdemethoxycurcumin
	R=H	Gallic acid
	R=polyol	Galloyl ester
		Ellagic acid
		juglone
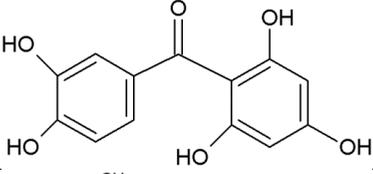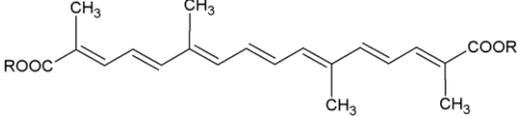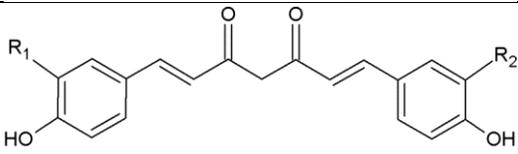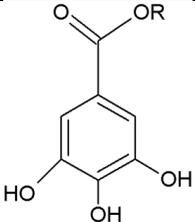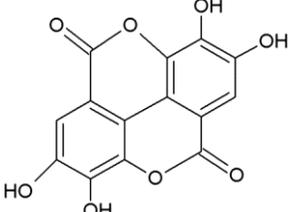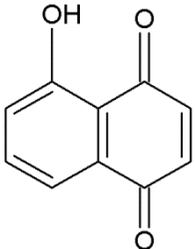
NON-INVASIVE TECHNIQUES FOR THE ANALYSIS OF NATURAL DYES

Non-invasive techniques play an important role in the analysis of cultural heritage materials, as underlined in the introduction of this work. Protocols for the investigation of dyes can be derived from more general procedures proposed for the study of historical and archaeological artworks (Aceto *et al.* 2012; Miliani *et al.* 2010), but presently no procedures are specifically intended for the investigation of natural dyes on works of art. One of the drawbacks of non-invasive techniques is that most of them do not give unambiguous molecular information of the analyte and thus the recorded data should be compared with spectra acquired on known materials to perform an attribution of the compounds. As for natural dyes, there is still a lack of published databases of reference materials for most of the employed techniques. In addition, most of the published works on dyes deal with the characterization of specific compounds or groups of compounds and thus dyes are investigated in solution. These studies are very useful to underline the spectral features of the dyes, but the experimental conditions differ by far from those that could be employed non-invasively on historical artworks. Also, the possible interference of the matrix is generally not considered.

Among the non-invasive techniques, UV-Vis diffuse reflectance spectroscopy, mainly with fiber optics probes that enable the portability of the instrument (FORS), has been employed for the analysis of dyes in different matrices (textiles, paintings, manuscripts). In some cases it was possible to identify the dyestuff or to highlight the chemical family of the dyes by comparison with spectra acquired on reference materials. Also *in situ* spectrofluorimetry was employed with similar a approach, while portable X-ray fluorescence is obviously not suitable for the analysis of organic dyes, but it can play a role in revealing mordants. After having reviewed the literature on the topic and having performed some preliminary tests, fiber optics reflectance spectrophotometry (FORS) in the UV-Visible field was selected as the most promising non-invasive technique for the analysis of natural dyes to be investigated within the present PhD research project. The NIR range is not of particular interest for the topic, since the electronic transitions of organic dyes mainly occur in the UV and visible range of the electromagnetic spectrum, as described in the following paragraph. The experimental approach employed here for the optimization of FORS procedures for the identification of natural dyes on textiles and of purple dyes on parchment will be discussed in chapter 3. The present chapter is intended to give an overview on theory and bibliography in order to introduce the following experimental chapter. In particular, this chapter will introduce the main principles that rule reflectance spectroscopy, with also some mentions to the features of portable instrumentations. In addition, a thorough overview of the published works about FORS and an overall view of the dyes analyzed with laboratory non-portable UV-Visible (-NIR) spectroscopies will be given. Finally, other non-invasive techniques employed for the characterization and identification of natural dyes will be briefly discussed.

2.1 UV-VIS ABSORPTION: PRINCIPLES AND INSTRUMENTATION

2.1.1 PRINCIPLES OF UV-VIS ABSORPTION SPECTROSCOPY

The term spectroscopy generally indicates an analytical technique which employs electromagnetic radiation to obtain qualitative information on the composition of an investigated material. Spectroscopies are usually classified on the basis of energy of the incident radiation used. The response of the analyte is highly variable depending on the selected regions of the electromagnetic spectrum, as the interaction of light of different energies promotes transitions involving different quantum levels of molecules. In particular, the absorption of the electromagnetic radiation by a material occurs when the Bohr condition is satisfied:

$$h\nu = E_f - E_i$$

that is when the energy of the incident beam equals the difference of energies between two molecular quantum levels (Bacci 2000). In particular, the regions of the electromagnetic spectrum employed in the so called UV-Vis-NIR spectroscopy may extend from the ultraviolet to the near infrared, i.e.: from 150 nm to about 3μm. The Bohr condition implies that the absorption of UV-Vis-NIR radiation enables electronic transitions of the molecules. Molecular species originate a band spectrum, as also vibrational and rotational transitions are activated by the interactions with the radiation and the fine structure of the spectrum cannot be resolved by the measuring device. Figure 2.1 reports the excitation scheme of a polyatomic system in the UV-Vis and in the IR.

The most important molecular electronic transitions that can be investigated though UV-Vis spectroscopy are those between delocalized molecular orbitals, the charge-transfer transitions, the ligand field transitions and the energy band transitions (Bacci 2000). As for dyes, they have an electronic structure that permits large electron delocalization (Bacci 2000) and enable electronic transitions between delocalized molecular orbitals. In particular, the transitions which occur by absorption of radiation in the visible or near UV range are n→π* (doublets to double bonds excited) and π→π* (double bonds to double bonds excited), as transitions σ→σ* and n→σ* occurs at higher energies which are not considered in traditional instrumentations (<200 nm) (Kellner *et al.* 2003). It should also be noted that by increasing the number of conjugated double bonds the energy of the transition is shifted towards the red region of the visible range.

The absorption of the incident radiation can be recorded in different modes according to the features of the material under analysis. In particular, it is possible to measure the light transmitted or reflected by the sample, thus working in transmission or reflection mode, respectively. The transmission mode can be considered only for transparent or translucent samples (generally solutions). When working in transmission mode the analytical signal is T (transmittance), defined as:

$$T\% = \frac{I}{I_0}$$

where I_0 is the intensity of the incident light and I is the intensity of the transmitted light. Transmittance is used to obtain absorbance, defined as:

$$Abs = \log\frac{1}{T} = \log\frac{I_0}{I}$$

The concentration of the adsorbing analyte in solution can be derived from the Lambert-Beer's law:

$$Abs = \epsilon \, b \, C$$

where ϵ is the absorption cross section [L * mol^{-1} * cm^{-1}], which is analyte- and wavelength-dependent, b is the path length (usually 1cm) and C is the molar concentration of the analyte.

However, the transmission mode cannot be employed with most of the materials of interest for the cultural heritage field, as they are usually opaque. In this case, it is possible to measure the reflected light and to record the spectral features of the sample by R (reflectance) as:

$$R_\lambda\% = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} * 100$$

where S_λ is the light intensity diffused by the sample, D_λ is the emission intensity of the black body, and R_λ the reflectance of a standard material. From this equation, it is apparent that additional measurements should be performed in order to express the measured data in terms of reflectance. In particular, the reflectance of the standard is measured on a 100% reflective material, while the emission of the black body is measured by obscuring the detector and accounts for the electronic noise of the instrument. Reflectance measurements can be performed either in specular or in diffuse mode, i.e including or excluding the specular reflection. Otherwise, both the components can be included in the measurement. In addition, diffuse reflectance can be expressed in Kubelka-Munk units:

$$F(R_\infty) = \frac{(1 - R_\infty)^2}{2R_\infty} = \frac{K(\lambda)}{S(\lambda)}$$

where R is the diffuse reflectance of a sample of infinite thickness, K(λ) is the absorption coefficient and S(λ) is the diffusion coefficient. Data provided by Kubelka-Munk function are proportional to the absorption of the material (Bacci 2000).
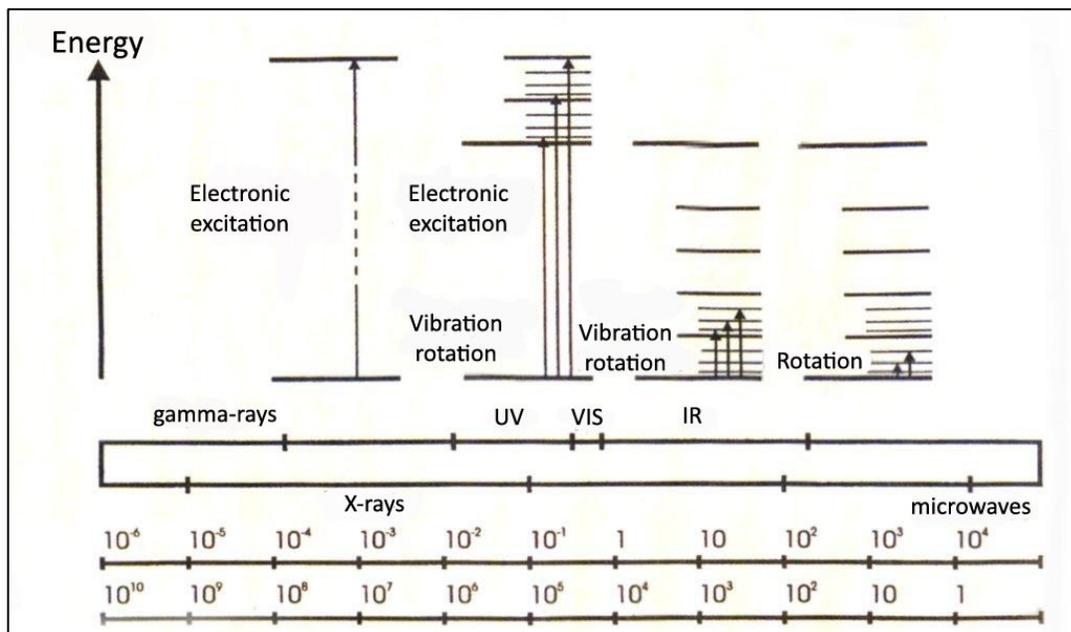
Figure 2.1: excitation scheme of a polyatomic system (adapted from Kellner *et al.* 2003).

2.1.2 INSTRUMENTATION FOR PORTABLE REFLECTANCE SPECTROSCOPY

The instrumentation for portable reflectance spectroscopy is usually composed by a light-source, a dispersing element and a detector. In addition, one or more probes which bring the incident light to the sample and collect the reflected light are required. The light-source typology depends on the spectral range to be considered. In particular, visible and near infrared radiation is emitted by incandescent or halogen lamps, while to obtain ultraviolet radiation it is necessary a gas-discharge lamp (mainly with argon, mercury, deuterium or xenon). Generally two or more light-sources are coupled to cover the entire UV-Vis-NIR spectral range. The light spectrum emitted by the source is subtracted from the spectrum of the sample by measuring the reflectance of a highly diffusing white standard, indicated in the equation of paragraph 2.1.1 with R_λ.

The dispersing element separates the different wavelengths and it is placed between the source and the sample or between the sample and the detector, depending on the instrument geometry. The dispersing element usually consists in a diffraction grating, produced through photolithographic methods, with a step comparable to the wavelength that should be separated. The diffraction follow the Bragg's law for n=1:

$$\lambda = p * \sin \theta$$

where λ is the wavelength diffracted, p is the step of the diffraction grating and θ is the diffraction angle. In a sequential equipment the dispersing element is rotated in order to focus a range of selected wavelengths on the exiting slit of the element.

The detector can be a phototube, a photodiode or a charged-coupled device (CCD). In a phototube, the incident light impacts of a cathode and extracts electrons, which are accelerated by an electric field and they hit a series of metallic electrodes, thus being multiplied by a factor of more than 10^6; the whole process is accomplished under vacuum. On the contrary, photodiodes do not operate under vacuum: the electrons pass through a semiconductor dispositive, are collected by electrodes and generate a photocurrent. In this case, the choice of the semiconductor is fundamental, as each material shows the maximum response in different regions of the electromagnetic spectrum, and thus instruments which operate in the whole UV-Vis-NIR range usually require the use of more than one detector. Both phototubes and photodiodes operate sequentially, i.e. the diffraction grating should be moved in order to enable the recording of signals within the whole considered spectral range. Therefore, long acquisition times are required. This issue can be overcome by the use of CCD, or arrays of photodiodes, which are placed on the focal plane of the dispersing element without the need of any slits. Every photodiode of the CCD reveals simultaneously a specific range of the spectrum, even though the sensitivity of the instrument may be lower with respect to sequential detectors.

The probes employed for portable reflectance spectroscopy are usually fiber optics, which permit to bring the light over long distances exploiting the principles of total reflectance. In fact, in two dielectric media of refractive indices n_1 and n_2 incident light do not enter medium 2 if the incident angle is higher than the critical angle. Fiber optics are thus constituted of two cylinders, the internal core with higher refractive index and the external cladding with lower n. The cladding is

protected by a non-optical material such as plastic or metal. The material of choice for the core depends on the spectral range of application: glass and polymeric fiber optics can operate only in the visible field, while fluorinated glass and fused silica are well-suited for NIR and the whole UV-Vis-NIR range, respectively (Bacci 2000). Instruments for reflectance spectroscopy may employ an integrating sphere, that allows the detection of more intense signals. Integrating spheres are made by a white reflecting material and are particularly suitable for color measurements. An integrating sphere can be coupled with fiber optics, although in compact instrumentations, these are replaced by a series of lens and mirrors.

2.2 PORTABLE UV-VIS REFLECTANCE AND UV-VIS SPECTROSCOPIES FOR THE ANALYSIS OF NATURAL DYES

The first application of portable reflectance to the analysis of natural dyes dates to 1987, when Miyoshi and Matsuda employed a reflectance spectrometer with an integrating sphere to investigate the light fading of silk textiles dyed with Japanese traditional techniques (Miyoshi and Matsuda 1987). In addition, Guineau (1992) reported his experiments for the identification of dyes *in situ*. In recent years, portable UV-Vis reflectance has been successful in the identification of dyes from different matrices (paintings, textiles, parchments), even though, as previously underlined, there are no methodological works that thoroughly discuss the variability of the spectral features for different dyestuffs according to the preparation procedures, the mordant typology and, for textiles, the fiber. Reflectance spectroscopy has been employed mainly with fiber optics (FORS) but also with more compact spectrophotometers, which exhibit a lower resolution. The relevance of considering a large set of spectral data collected from several samples dyed with known raw materials was aptly pointed out by Angelini *et al.* (2009) and Poldi (2011), which considered several textile reference samples for their identification of Anatolian and Caucasian rugs and Chinese carpets, respectively. Unfortunately, the variation of the spectral features were not thoroughly discussed in the first work, while in the latter the resolution of the instrument strongly limits the possibility of evaluating the spectral variations and besides the number of the considered samples and the preparation procedures are not reported. In addition, methodological works to identify the dyestuffs with principal components analysis (PCA) were carried out on wool dyed with yellow dyes with different mordants (Bacci *et al.* 2001; Cazenobe *et al.* 2002). The possibility of identifying carminic acid and an alizarin lake at different concentrations over (or mixed with) different inorganic pigments was also investigated (Bisulca *et al.* 2008). Moreover, the electronic properties of some red and yellow lakes obtained from natural dyes were discussed by Clementi *et al.* (2008).

As for the identification of dyes on historical textiles by means of FORS, besides the previously cited works by Angelini *et al.* and Poldi, it was reported for a group of kaitags from Daghestan (Bruni *et al.* 2010; Pozzi *et al.* 2012), investigated with a multi-technique approach. In addition, scale insects and indigo were identified on two Japanese paintings (Leona and Winter 2001) and a red anthraquinonic lake was found on a Renoir painting (Miliani *et al.* 2007), while several dyes were detected in various manuscripts. In particular, a red lake was found on a painted parchment by Mantegna (Bruni *et al.* 2008), whereas cochineal and yellow unidentified dyes were detected in a pre-columbian codex (Miliani *et al.* 2012) and a 6th century Mediterranean manuscript revealed

the presence of a mixture of madder and indigo (Aceto *et al.* 2012). Other applications of FORS involve the investigation of the ageing of madder (Clementi *et al.* 2007) and the investigation of modern synthetic dyes (Montagner *et al.* 2011). It should also be added that Londero *et al.* (2013a) recently developed a wavelength-tunable microscope which allows the acquisition of Raman, reflectance and fluorescence spectra by the same instrument.

Benchtop UV-Vis equipments were employed to characterize dyestuffs, dye molecules and their complexes with metal ions by considering solutions of the investigated chemical species. Many papers deal with indigoid dyes and their derivatives (Amat *et al.* 2011; Brode, Pearson, and Wyman 1954; Chen 1996; Koren 2012; Miliani, Romani, and Favaro 1998; Serrano-Andrès and Roos 1997; Seixas de Melo, Moura, and Melo 2004). Also anthraquinones (Grazia *et al.* 2011; Miliani, Romani, and Favaro 1998; Miliani, Romani, and Favaro 2000), weld (Amat *et al.* 2010), safflower (Clementi *et al.* 2014) and brazilein (Wongsooksin *et al.* 2008) were investigated. In addition, the degradation of saffron (Vickackaite *et al.* 2004) and the photo-ageing processes of carminic acid and carmine lake were examined. Regarding historical artworks, absorption spectrometry was employed by Taylor (1983) to identify the dyes extracted from Scandinavian textiles, and by Wallert and Boytner (1996) to investigate dyed textiles from Peru. Moreover, UV-Vis spectroscopy, mainly in the absorption and reflectance modes, was also employed in some cases to support fluorimetry measurements. In particular, it was used on alizarin, purpurin and eosin lakes (Claro *et al.* 2008), orcein (Clementi *et al.* 2006; Doherty *et al.* 2013) and carmine lake (Clementi, Miliani, Verri, *et al.* 2009).

2.3 OTHER NON-INVASIVE TECHNIQUES FOR THE ANALYSIS OF NATURAL DYES

The first pioneering work on fluorimetry for the cultural heritage was made by De la Rie (1982), who investigated its potentialities for the characterization of painting and varnish layers. Fluorimetry is presently a commonly employed technique for the characterization of natural dyes, both *in situ* with portable instruments and in the laboratory using benchtop equipments. Romani *et al.* (2010) recently accounted the state-of-the-art of the technique and its potentialities for the analysis of dyes in historical and archaeological materials.

In particular, *in situ* fluorimetry was employed for the characterization of orcein on mock-ups (Clementi *et al.* 2006). In addition, the application of a correction factor based on the Kubelka-Munk model was proposed by Verri *et al.* (2008) and, for the study of carmine lakes, by Clementi, Miliani *et al.* (2009). Moreover, steady-state fluorimetry coupled with luminescence lifetime measurements in a portable equipment proved to be effective in discriminating between cochineal and lac and in identifying orcein and indigo in an early medieval manuscript (Romani *et al.* 2008). On the other hand, three-dimensional fluorescence was employed for the identification of early synthetic dyes (van Bommel *et al.* 2007).

In situ fluorimetry allowed to identify the dyes in several historical artworks: orcein and indigo in Renaissance tapestries (Clementi *et al.* 2006; Clementi, Miliani, Romani, *et al.* 2009), carmine lake in a Vasari painting (Clementi, Miliani, Verri, *et al.* 2009), a red anthraquinonic lake in a Renoir painting (Miliani *et al.* 2007), lac and indigo on medieval Portuguese illuminations (Melo and Claro 2010), Gardenia augusta in Chinese wall paintings (Melo and Claro 2010), an hydroxy-

anthraquinone lake in Domus Aurea (Romani *et al.* 2010), orcein in a 9th century manuscript (Doherty *et al.* 2013).

Non-portable fluorimetry was used for the characterization of quinone, lichen and redwood dyestuffs (Wallert 1986) and also for anthraquinones and indigoid dyes (Miliani, Romani, and Favaro 1998). The use of confocal microspectrofluorimetry for the analysis of alizarin, purpurin and eosin lake pigments was proposed by Claro *et al.* (2008) and compared with *in situ* fluorimetry. This technique allowed to identify purpurin and eosin lakes on Pizarro's and Van Gogh's paintings and purpurin, pseudopurpurin and carminic acid on Andean textiles (Claro *et al.* 2010).

Other techniques were used with a non-invasive approach, even though with limited application. In particular, multispectral analyses were employed for the identification of dyes on Persian manuscripts (Isacco and Darrah 1993), while the application of colorimetry in the field of natural dyes is more related to fading studies and dyeing tests (Manhita *et al.* 2011; Saunders and Kirby 1994; Thomas *et al.* 2010; Tsatsaroni, Liakopoulou-Kyriakides, and Eleftheriadis 1998; Vankar and Shanker 2009; Yoshizumi and Crews 2003; Zarkogianni *et al.* 2010). In addition, micro-Raman spectroscopy was used in a non-invasive way on several Persian manuscripts, directly putting the selected pages or the entire book on dedicated sample holders. Indigo was thus identified on several manuscripts (Clark and Mirabaud 2006; Slogget 2007; Burgio *et al.* 2008; Muralha, Burgio, and Clark 2012), while Indian yellow was found on Indian illuminations (Ravindran *et al.* 2011; Ciomartan and Clark 1996) and carmine lake on a 17th Persian manuscript (Muralha, Burgio, and Clark 2012).

FIBER OPTICS REFLECTANCE SPECTROSCOPY (FORS)

FOR THE ANALYSIS OF NATURAL DYES

The investigation of dyestuffs in historical and archaeological materials was addressed in several works, as highlighted in chapter 2. Despite this, there is still a lack of methodological works devoted to ascertain the variability of the spectral features of the dyestuffs related to different lots. In particular, as regards textiles, the aspects concerning the internal variability of the spectral features of samples dyed with a specific dyestuff with various provenance, dyeing conditions and substrate fibers have not been discussed yet. The first part of this chapter aims at filling this gap, as it investigates the many aspects in order to optimize an analytical path for the analysis of dyed animal fibers through fiber optics reflectance spectroscopy in the visible range. In addition, an analytical procedure for the analysis of purple colored parchments by means of UV-Vis FORS was set up, and it is illustrated in the second part of the chapter. A selection of the results presented in this chapter was reported in two papers, published in the international journals Dyes and Pigments (Gulmini *et al.* 2013) and Spectrochimica Acta part A (Aceto, Idone *et al.* 2014), respectively.

3.1 OPTIMIZATION OF THE ANALYTICAL PATH FOR THE ANALYSIS OF DYED TEXTILE FIBERS THROUGH FORS

Visible fiber optics reflectance spectroscopy (FORS) was chosen as the most suitable technique for *in situ* preliminary analysis of natural dyestuffs in historical samples. The choice was performed after several tests which also involved measurements with *in situ* spectrofluorimetry and FORS extended to the UV and NIR ranges. In addition, measurements on a restricted number of reference samples were performed with two different portable instrumentations in order to determine which one had the best resolution and was therefore more suitable to build a spectral database. It was also ascertained that the spectral responses of the two instrumentations were similar enough to consider the database of general applicability as a reference. After these initial tests, a number of reference samples was obtained and an operative procedure was set up in order to record reflectance spectra and treat the reference samples according to a standardized process. The following paragraphs briefly describe the instrumentation and the set of reference samples employed. Details on the reference samples are reported in Appendix 1 and Appendix 2. The analytical procedure is illustrated here and the results are thoroughly discussed by considering the signals changes according to the considered variables. When possible, diagnostic spectral features for the considered dyestuffs are identified and pros and cons of visible FORS for the identification of dyes on textiles are discussed.

3.1.1 MATERIALS AND METHODS

REFERENCE SAMPLES

Reference samples were obtained from five different reference sets of animal fibers dyed independently by different laboratories. In some cases, only wool samples were available, while two sets include also silk fibers. The overall set encompasses dyed wool flocks, wool yarns and (in fewer cases) woven wool, while silk samples were all woven. Twenty-two natural dyestuffs were considered, both employed alone or with another dyestuff in a double dyeing. In addition, several mordants, dyeing procedures and different concentration of the dyestuff were considered. 210 reference samples were considered as a whole for this work.

Blue samples were dyed with indigo (*Indigofera tinctoria* L.) and woad (*Isatis tinctoria* L.) using a chemical vat, while logwood (*Haematoxylum campechianum L.*) samples were dyed with alum, with alum and cream of tartar, with alum, cream of tartar and iron(II) sulfate and with potassium bichromate. In addition, indigo was treated with concentrated sulfuric acid and employed to dye wool and silk mordanted with alum to obtaine the disodium 5,5'-indigotin disulphonate called "Saxon blue" (1.3.1).

The considered red samples were obtained from widely employed dyestuffs: i.e. Mexican cochineal (*Dactilopius coccus* Costa), madder (*Rubia tinctorum* L.) and brazilwood (*Caesalpinia* spp.) dyed on silk and wool with alum, with alum and cream of tartar and with alum, cream of tartar and iron(II) sulfate. In addition, some samples from less common red and orange dyestuffs were considered. These were employed on animal fibers mordanted with alum and cream of tartar. As for red dyestuffs, reference samples of bloodred webcap (*Cortinarius sanguineus* (Wulf.: Fr.) S. F. Gray) on silk and wool, as well as wool samples of sandalwood (*Pterocarpus santalinus* L.) were considered. Orange (or red-orange) dyestuffs considered here are substantive dyes such as annatto (*Bixa orellana* L.) and henna (*Lawsonia inermis* L.). The former was applied on wool and silk, whereas samples of the latter consisted of dyed wool.

A broad number of yellow dyestuffs was considered for this work, even though for some of them only a limited number of reference samples (in some instances only one or two samples) was available (Table 3.3 and Appendix 1). In particular, samples dyed with common buckthorn (*Rhamnus cathartica* L.), dyer's broom (*Genista tinctoria* L.), old fustic (*Chlorophora tinctoria* (L.) Gaud.), safflower (*Carthamus tinctorius* L.), saffron (*Crocus sativus* L.), turmeric (*Curcuma longa* L.) and weld (*Reseda luteola* L.) were investigated. Most of the dyestuffs were employed to color either wool or silk and wool mordanted with alum or with alum and cream of tartar. For saffron and turmeric, also direct dyeing and mordanting with copper(II) sulfate were taken into account, while weld was dyed using tin(II) chloride as a mordant too.

Three brown dyestuffs were analyzed, mainly dyed directly or mordanted with alum and cream of tartar only on wool or on wool and silk. In particular, gall (*Quercus infectoria* Oliv.), quebracho (*Schinopsis quebracho-colorado* (Schltdl.) F. A. Barkley & T. Mey) and walnut (*Juglans regia* L.) were considered. Wool dyed with gall was also mordanted with cream of tartar and iron(II) sulfate.

Purple samples were obtained from two purple dyestuffs, alkanet (*Alkanna tinctoria* (L.) Tausch.) and shikon (*Lythospermum erythrorhizon* Siebold & Zuccarini), and by superimposition of indigo on red fibers dyed with madder or cochineal. Alkanet and shikon were dyed directly on wool and silk, while the mordant employed on wool yarns dyed with madder and cochineal is not known; indigo was dyed in a chemical vat.

Green samples were all obtained through double dyeing with a blue dyestuff and a yellow dyestuff, as no stable green dyestuffs are present in nature (Cardon 2007). In particular, indigo obtained with chemical vat, Saxon blue and logwood mordanted with chromium were employed as blue dyestuffs, while yellow dyestuffs used were old fustic, weld, saffron, safflower and turmeric.

A complete record of the reference samples, with details about their preparation, when known, is reported in Appendix 1.

INSTRUMENTATION

Vis-FORS spectra were obtained employing a Corona45Vis spectrophotometer by Zeiss (Oberkochen, Germany). This instrument is equipped with a halogen source A10 and the detector covers the range from 380 to 1100 nm. The spectral resolution is 3.3 nm. The instrument is equipped with fiber optics which can be connected to the instrument in different ways in order to obtain various geometries. Further technical information, as well as details about the here employed geometries, can be found in Appendix 2. A first set of measurements was recorded with both the 2x45°/0° and the 0°/0° geometry in order to check for the reproducibility of the obtained spectra. Even though the intensity of the spectra with the 2x45°/0° geometry was less variable, possibly due to a better shielding system, the 0°/0° arrangement was preferred as the spot of analysis was smaller (about 3 mm) compared to 2x45°/0° geometry (about 8 mm). A smaller analytical spot would be suitable also for the analysis of tiny details of historical and archaeological objects. The spectra were referenced against a SphereOptics (Uhldingen, Germany) diffuse reflectance standard made of highly reflecting PTFE. Each spectrum was acquired as the sum of three spectra with an integration time between 118 and 150 ms. The instrument was managed by Aspect Plus dedicated software, running under Windows.

For some of the analyzed samples, color coordinates were collected by a Minolta (Tokyo, Japan) CM-700d Spectrophotometer employing the D65 standard illuminant, the CIE 1964 10° standard observer and setting the measuring aperture to 3 mm. Color coordinates in the CIE L*a*b* color space were calculated as the mean of five measurements obtained on different portions of the same sample and are reported in Table A1.1 of Appendix 1.

ANALYTICAL PROCEDURE

The investigation of textiles presents some issues that are not encountered in the analysis of other materials, as pointed out by Poldi (2011). As a matter of fact, textile surfaces are barely homogeneous and thus obtaining reproducible spectra is far more complex than on other cultural heritage materials. In addition, with the 0°/0° geometry, extreme care must be taken in order to avoid the contribution of external light. Problems may also arise from the investigation of thin textiles which allow light to pass through them. It should also be observed that, in general, exposed sides of the artifacts show a higher tendency to light-fading, as observed for the case study reported in section 6.1 of the chapter related to the cases studies. The analytical procedure was conceived in order to overcome most of these issues. In particular, the white calibration was performed for each reference sample, so as to minimize the possible source fluctuations. Four spectra were acquired from each sample, every time after having removed and repositioned the fiber optics on the sample. Each spectrum was then singularly considered and compared with the other spectra in order to reject those with anomalous bands that may arise from the contribution of external light. The spectra that passed this fist check were normalized to 100% reflectance, in order to accentuate the spectral features, and averaged in order to obtain one spectrum for each reference sample. The position of the eventual inflection points was determined upon calculation of the first derivative of the spectrum.

3.1.2 RESULTS AND DISCUSSION

3.1.2.1 BLUE REFERENCE SAMPLES

Spectra recorded on wool dyed with **indigo** and **woad** with chemical vat (Figure 3.1) revealed the presence of a strong absorption band at about 660 nm (Table 3.1), and a second band at about 350 nm, barely appreciable due to the poor sensitivity of the instrument in that spectral region. Spectra showed also the presence of a maximum of reflectance in the range 420-485 nm (Table 3.1), which is dependent on the chroma, variable from pure blue for indigo to greenish-blue or even green for woad (Table A1.1, Appendix 1). Moreover, as pointed out by Poldi (2011), lighter colors showed a bathochromic shift in the reflectance maxima, which is in agreement to what suggested by Gargano *et al.* (2006) concerning the addition of a white colorant to a colored substrate. In addition, a sharp increase towards the infrared region was observed, with an inflection point at 700-730 nm (Table 3.1); in darker samples, a second inflection point at about 395 nm was found. It should also be noted that different concentrations of the dyes did not affect the position of the absorption bands, but only their intensity (Figure 3.1). In addition, for very dark hues the identification of the position of the minimum was difficult, if even impossible (Figure 3.1, green spectrum).

The typical absorption band of indigotin in the vapor phase is the strong HOMO → LUMO transition centered at 540 nm, that undergoes a bathochromic shift in solution (600 nm in chloroform, 614 in DMSO), and the appearance of a second feature at 700 nm in the solid state: these effects have been attributed to the formation of hydrogen bonded aggregates (Monahan and Kuder 1972; Miliani, Romani, and Favaro 1998; Amat *et al.* 2011). The second absorption in the visible region is the HOMO-2 → LUMO transition, detectable at about 350 nm (Serrano-Andrès and Roos 1997). The absorption bands recorded on wool samples can therefore be attributed to solid indigotin, the main dyeing molecule of indigo and woad (1.3.1) supported on the wool fibers and represented a robust indication of the presence of indigo (or woad) in blue samples.

FORS measurements performed on silk and wool dyed with **Saxon blue** (e.g. indigo treated with sulfuric acid, Figure 3.2) presented an absorption band at about 615 nm, with a broad structured reflectance band from 350 to 550 nm, having a relative maximum at about 495 nm and an inflection point at 654-678 nm (Table 3.1). Also in this case, the position of the absorption band was not influenced by the concentration of the dye, while the use of different fibers affected the position of the maximum and the inflection point as Saxon blue proved to confer to silk more blue colors with respect to the blue-greenish hues of wool (Table A1.1, Appendix 1).

Spectra obtained on wool and silk mordanted with alum or alum and cream of tartar and dyed with **logwood** (Figure 3.3) showed an absorption band at 574-592 nm, while a weaker band was observed at about 435 nm (Table 3.1). This latter band created a broad reflectance band, whose main maximum is placed at about 455 nm (Table 3.1). In addition, the steep increase towards the infrared region determined a largely variable inflection point from about 630 to 710 nm, while a second inflection point was sometimes observed between 435 and 445 nm (Table 3.1). Similarly to what observed for indigo, both the absorption bands and the relative maximum of reflectance could not be observed in darker samples (Figure 3.3, red and black spectra). In addition, the

absorption band in the blue region and its related maximum remained undetected also in some among the lighter samples. Even though silk dyed with logwood displayed a more reddish blue than wool (Table A1.1, Appendix 1), the position of the absorption band at 435 nm was very reproducible in wool and silk samples.

The use of different mordants, such as iron(II) or chromium(VI), generated very dark colors (Table A1.1, Appendix 1), which, as observed in the darker samples obtained with alum or alum and cream of tartar, did not allow the identification of the absorption bands (Figure 3.3, cyan and magenta spectra). In addition, the increase of reflectance was shifted towards the infrared region, with the inflection point positioned at about 780 nm (Table 3.1).

The main dyeing molecule deriving from logwood is haematein (1.3.1): in neutral solution it presents a strong absorption in the visible region at 445 nm that shifts to longer wavelengths as a result of both deprotonation of hydroxyl groups and complex formation with metal ions (Shirai and Matsuoka 1996). The reflectance band in the blue region of the visible spectrum generally shows two relative maxima, due to the presence of an absorption band centered at about 430 nm, possibly related to neutral haematein. The interaction of haematein with mordanting ions, such as Al^{3+} (Bettinger and Zimmermann 1991) or Fe^{3+} (Shirai and Matsuoka 1996), investigated in aqueous solution via UV-Vis spectrophotometry is revealed by the appearance of a feature at about 550 nm (apart from the feature at 450 nm attributable to undissociated haematein). The position of the intense absorption band at about 580 nm and that of the weaker band at 435 nm, as well as the reflection maximum at about 445-455 nm, can be considered the most significant features of the reflectance spectra collected from wool or silk dyed with logwood and mordanted with alum and alum and cream of tartar, also in accordance with data indicated by Poldi (2011). In this paper, other absorption bands were also indicated, but these were not systematically detected in the reference samples considered here.

The above discussed attribution of the spectral features to one of the here considered blue dyestuffs allowed us to highlight that the position of the absorption bands is diagnostic for the identification of blue dyestuffs by means of Vis-FORS (Figure 3.4). Despite this, issues may arise when dark samples are considered, while spectra of lighter samples generally maintain their diagnostic features. For dark samples, the identification could not be performed by considering the inflection point in the red-infrared region, even if it proved to be quite reproducible in indigo, woad and Saxon blue samples, nevertheless logwood showed a very variable position of the inflection point, that varies according to the mordant employed.

Table 3.1: spectral features of blue dyestuffs on textile reference samples.

Dyestuff	Mordant	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Indigo	Chemical vat	659-669	421-429	393-397 714-727	5
Indigo	A, H_2SO_4	613-618	486-496	654-678	9
Logwood	A/A+T	431-436* 574-592*	454-459*	435-445* 633-712	10+11
Logwood	A+T+Fe			779	1
Logwood	Cr			781	1
Woad	Chemical vat	654-658	440-485	700-709	5

A=alum, T=cream of tartar, Fe=iron(II) sulfate, Cr=potassium dichromate; * feature observed only in some of the investigated samples
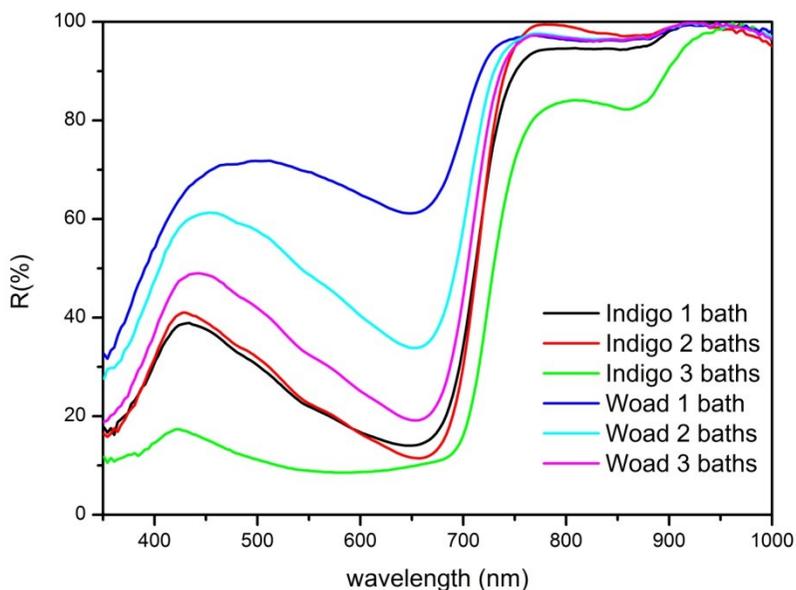
Figure 3.1: reflectance spectra recorded on blue reference samples of wool dyed with: indigo, 1 bath (black spectrum); indigo, 2 baths (red spectrum); indigo, 3 baths (green spectrum); woad, 1 bath (blue spectrum); woad, 2 baths (cyan spectrum); woad, 3 baths (magenta spectrum).
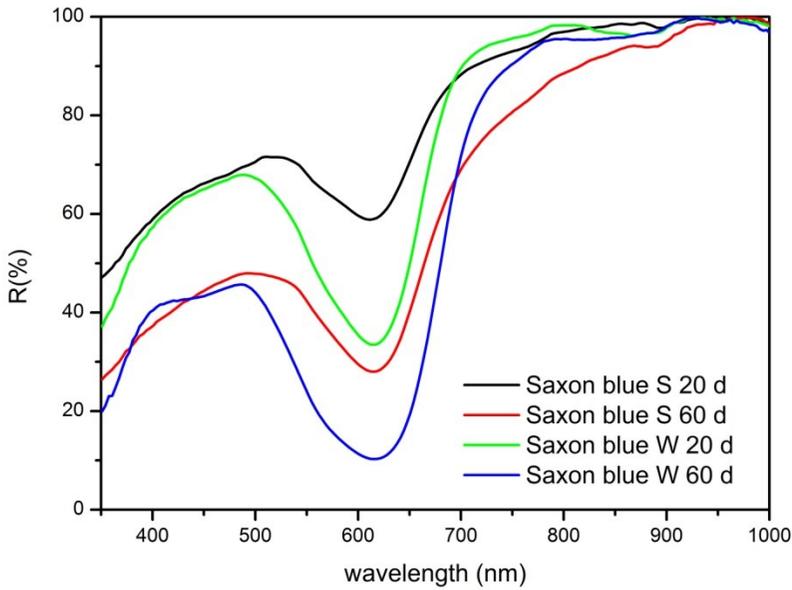
Figure 3.2: reflectance spectra recorded on blue reference samples dyed with Saxon blue: silk, 20 drops (black spectrum); silk, 60 drops (red spectrum); wool, 20 drops (green spectrum); wool, 60 drops (blue spectrum.
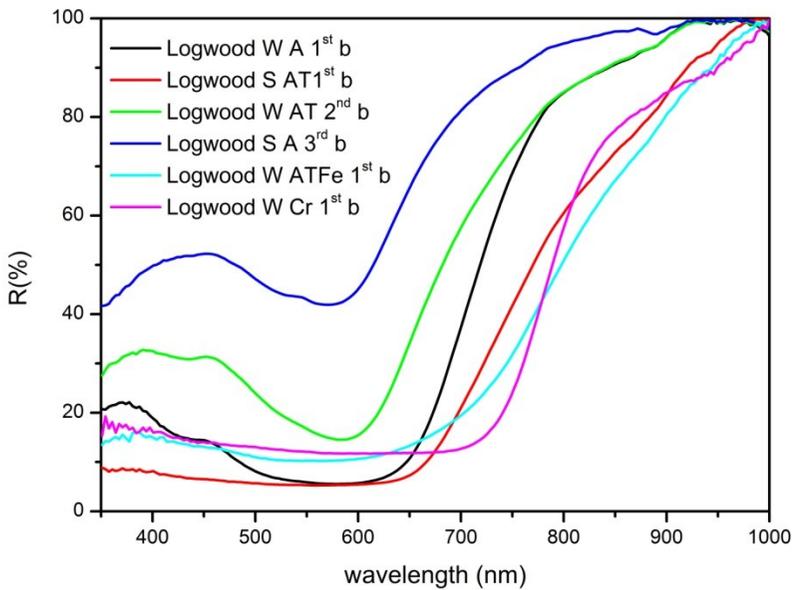
Figure 3.3: reflectance spectra recorded on blue reference samples dyed with logwood: wool, mordanted with alum, 1st bath (black spectrum); silk, mordanted with alum and cream of tartar, 1st bath (red spectrum); wool, mordanted with alum and cream of tartar, 2nd bath (green spectrum); silk, mordanted with alum, 3rd bath (blue spectrum); wool, mordanted with alum, cream of tartar and iron sulfate, 1st bath (cyan spectrum); wool, mordanted with potassium chromate, 1st bath (magenta spectrum).
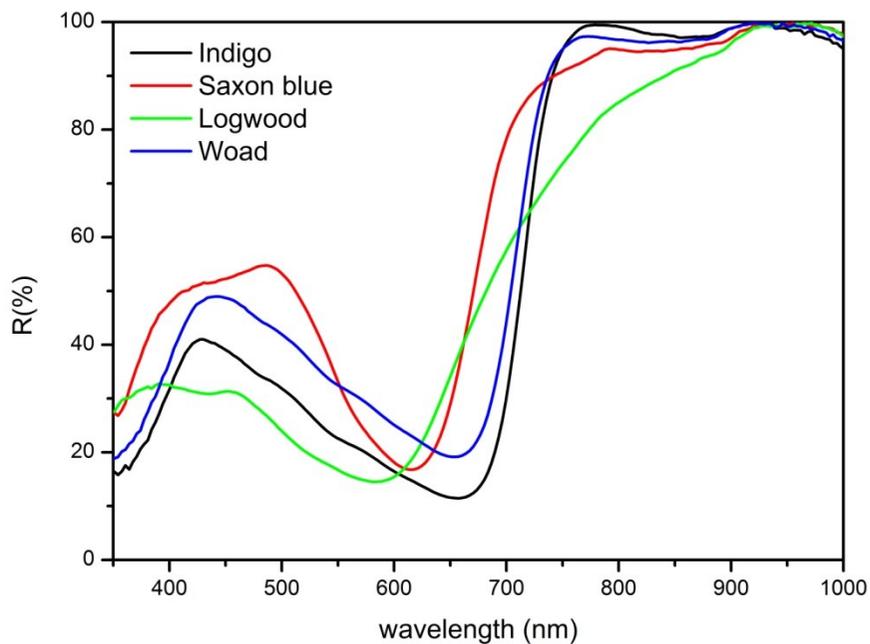
Figure 3.4: reflectance spectra recorded on blue reference samples: indigo, 2 baths (black spectrum); Saxon blue, mordanted with alum, 1st bath (red spectrum); logwood, mordanted with alum and cream of tartar, 2nd bath (green spectrum); woad, 3 baths (blue spectrum).

3.1.2.2 RED AND ORANGE REFERENCE SAMPLES

Spectra recorded on samples of wool and silk mordanted with alum or alum and cream of tartar dyed with **cochineal** (Figure 3.5) showed an absorption band structured into two sub-bands at about 530 and 560 nm (Table 3.2). In addition, a maximum of reflectance was found in the blue region, at 415-436 nm, while the increase towards the infrared region was characterized by an inflection point between 586 and 646 nm. These spectral features were observed in all the analyzed samples and the position of the absorption bands proved not to be influenced by the different fibers or by the concentration of the dye. No significant color variations were observed for the different fibers considered here (Table A1.1, Appendix 1), while lower concentrations of the dye led to more yellowish colors (Table A1.1, Appendix 1), testified by the hypsochromic shift of the inflection point in lighter samples.

FORS measurements on cochineal samples of wool mordanted with iron(II) sulfate (Figure 3.5, cyan spectrum) did not evidence significant variations in the spectral features reported for samples mordanted with alum or alum and cream of tartar (Table 3.2).

The absorption features occurring in the visible region for carminic acid, the main dyeing molecules of cochineal (1.3.2) are principally due to n → π* transitions of the carbonyl groups, as the absorption band connected to a π → π* transition is observed below 350 nm (Bisulca *et al.* 2008). As reported in chapter 2, the spectroscopic properties of carminic acid lake and cochineal lake were investigated by Bisulca *et al.* (2008) and Clementi *et al.* (2008), while Favaro *et al.* (2002) studied the photo-ageing processes of carminic acid and carminic lake. It resulted that carminic acid shows a strong dependency of the absorption maxima to the chemical neighborhood of the hydroxyl groups of the anthraquinone unit, as in neutral water solution it has a maximum in the visible region at 490 nm, that undergoes a bathochromic shift depending on the pH or the complexation with metal ions (Favaro *et al.* 2002). In particular, the absorption maximum is found at 527 nm for carminic lake in DMSO, while a second maximum appears at about 560 nm (Favaro *et al.* 2002). The identified spectral features are comparable with those observed in previous works for lake pigments (Bisulca *et al.* 2008; Clementi *et al.* 2008) and colored yarns (Poldi 2011), even though some of the signals reported in Poldi 2011 were not identified in the analyzed references, thus excluding their possible use for identification purposes.

FORS measurements performed on silk and wool samples mordanted with alum or alum and cream of tartar and dyed with **madder** (Figure 3.6) revealed the presence of a structured absorption band at about 510 and 545 nm (Table 3.2), which was often not identifiable, both in darker and lighter samples. A weak and variable maximum appeared in few cases in the blue region (Table 3.2), while the inflection point was positioned in the red region at 575-614 nm. In general, the identification of the spectral features, except for the inflection point, was difficult for darker colors, while most of the fibers dyed with less concentrated dye generally presented clear absorption bands and sometimes also the reflectance maximum. It should also be noted that silk samples obtained with less concentrated dyes showed a different trend in the reflectance spectrum, with fewer, if even none, diagnostic features compared to wool samples, according to their peculiar color, which is usually less reddish than wool samples (Table A1.1, Appendix 1).

Wool samples dyed with madder and mordanted with alum, cream of tartar and iron(II) sulfate showed similar spectral features to those recorded on the other madder samples, with also the same issues regarding darker colors (Figure 3.6, cyan spectrum).

Photophysical properties of Al(III) complexes of the main coloring matters contained in madder, e. g. alizarin and purpurin (1.3.2), were investigated both in solution (Miliani, Romani, and Favaro 2000; Grazia *et al.* 2011) and in solid state as lake pigments (Bisulca *et al.* 2008; Clementi *et al.* 2008; Grazia *et al.* 2011). Similarly to carminic acid, the chemical environments of the hydroxyl groups of the anthraquinone unit strongly influence the position of the absorption maxima. The methanol solution of alizarin, the most abundant dye component of madder, shows a strong $\pi \rightarrow \pi^*$ adsorption at 429 nm, that undergoes a bathochromic shift to 473 nm in the 1:1 Al^{3+} complex and to 490 nm in the 2:1 complex (Say-Liang-Fat and Cornard 2011). In addition, the Al(III)-alizarin chelate in DMSO shows an absorption maximum at about 500 nm, while the maximum of solid alizarin lake is found at 495 nm; a similar shift is observed between the Al(III)-purpurin chelate and purpurin lake, whose maxima are at 505 and 538 nm for the first and at 500 and 535 nm for the latter (Grazia *et al.* 2011). The absorption maxima of the di-anion of purpurin in water:dioxane (2:1, v/v) were found at 485, 513 and 547 nm (Miliani, Romani, and Favaro 2000). Also in this case, the here reported spectral features are similar to those registered in previous papers on lakes (Bisulca *et al.* 2008; Clementi *et al.* 2008) and on fibers (Poldi 2011).

FORS spectra obtained on **brazilwood** dyed on silk and wool mordanted with alum and alum and cream of tartar (Figure 3.7) highlighted the presence of a broad intense absorption band at about 520 nm (Table 3.2), whose position was not always clearly identifiable (Figure 3.7, black spectrum). A second weaker absorption band was centered at about 445 nm and was found in all the analyzed samples. This band determined a shoulder in the blue-violet region and a relative maximum at about 400 nm (Table 3.2), while the increase of reflectance towards the red was placed at 584-599 nm, being more reproducible than the previously discussed red dyes. In general, the positions of the broad absorption band and that of the maximum of reflectance were highly variable in brazilwood samples, as they proved to be influenced both by the dyeing procedure, by the concentration of the dye and by the fiber substrate. The variability of the spectral features generated also quite different colors, mainly in terms of the b* component (Table A1.1, Appendix 1).

Spectra of brazilwood recorded on wool mordanted with alum, cream of tartar and iron(II) sulfate (Figure 3.7, cyan spectrum) showed an hypsochromic shift of the band found at 520 nm (Table 3.2), which was in some samples barely identifiable. On the contrary, the position of the band in the blue region was maintained (Table 3.2), even though it was very weak. Also the position of the inflection point was comparable with the other brazilwood samples (Table 3.2), while the maximum of reflectance shifted towards the red (Table 3.2).

UV-Vis spectra of brazilein and of brazilein-Al(III), the main dyeing molecules of brazilwood (1.3.2), in solution were discussed by Wongsooksin *et al.* (2008). They highlighted the presence of a strong absorption centered at 446 nm, associated with the absorption due to the B-ring of the cinnamoyl system. When brazilein interacts with Al(III) ions, a complex is formed between the ion and the lone pair of electrons on a carbonyl group of the dye. The formation of the complex stabilizes the excited state leading to a bathochromic shift of the absorption maximum (Christie 2001; Zollinger 2003), as a new band at 509 nm appears. For the considered fibers, the weak signal at about 445

nm, which do not vary with different mordants, can be possibly attributed to brazilein, whereas the more intense absorption at 520 nm is possibly related to complexes with the mordanting cations. This absorption band was in fact also detected in reflectance spectra from red painting lakes obtained following ancient recipes for illumination (Roger, Villela-Petit, and Vandroy 2003), while it underwent a bathochromic shift (540 nm) in a sappanwood lake (Clementi *et al.* 2008). Also Poldi (2011) reported variable features for fibers dyed with brazilwood.

The spectra recorded on samples dyed with **bloodrep webcap** (Figure 3.8, red spectrum) evidenced the presence of a broad absorption band at 452-462 nm, while a structured absorption band at about 520-555 nm (Table 3.2) was found only on darker samples, which also presented a reflectance maximum at about 400 nm (Table 3.2). Inflection points were positioned at about 490, 535 and 580 nm (Table 3.2). The variation of the concentration of the dye and of the fiber did not affect the position of the absorption bands.

FORS measurements undertaken on **sandalwood** samples (Figure 3.8, blue spectrum) revealed the presence of a structured absorption band at about 480 and 505 nm, with a reflectance maximum at about 415 nm and an inflection point at 550 nm (Table 3.2). The position of the absorption band was not influenced by the variation of the concentration of the dye, even though a larger sample set should be considered in order to confirm this consideration.

Wool and silk samples dyed with **annatto** showed reflectance spectra (Figure 3.8, black spectrum) with variable features. In particular, a structured absorption band was found in some samples with minima at about 460 and 505 nm, while no reflectance maxima were identifiable (Table 3.2). In addition, an inflection point was observed at 515-533 nm. The intensity of the absorption bands was conditioned by the dyeing procedure, by the fiber and by the concentration of the dye and often led to a difficult identification of their position. The sample dyed directly, without the use of a mordant, did not show any absorption bands.

Reflectance spectra of **henna** dyed on wool did not present clear spectral features (Figure 3.8, green spectrum). The sample dyed directly showed a broad absorption band, whose position was in the 450-500 nm range, a reflectance maximum at about 420 nm and two inflection points at 387 and 540 nm. Those features were not found in the sample of wool mordanted with alum and cream of tartar and thus could not be employed for the identification of this dyestuff.

Most of the investigated red and orange dyestuffs presented a number of spectral features, among which the diagnostic ones appeared to be the absorption bands, as previously discussed for blue dyestuffs. In the case of brazilwood, also the position of the inflection point was reproducible in all the considered samples, but as its position superimposed with those of other commonly used dyestuffs (e.g. cochineal and madder) therefore it can be employed for identification purposes only in addition to other more selective spectral features. Several among the analyzed dyestuffs displayed absorption bands structured into two sub-bands which allow their discrimination. In particular, cochineal, madder and brazilwood can be easily distinguished (Figure 3.9), even though sometimes the identification of brazilwood can be difficult due to the shift of the band related to the brazilein complex with the mordant ion and to the weakness of the band of brazilein at about 445 nm. In addition, these dyestuffs can be discriminated also from some among the less common

red dyestuffs, as sandalwood (Figure 3.8, blue spectrum), as the position of the absorption bands is found at different wavelengths (Table 3.2), while bloodred webcap showed ambiguous features in the 515-560 nm region of the spectrum which were, though, always accompanied by an intense and peculiar band at about 455 nm (Figure 3.8, red spectrum). Finally, also the spectral features of two orange dyestuffs were investigated. In particular, annatto can be identified in some cases from the position of the structured absorption band, while for henna no diagnostic spectral features could be selected (Table 3.2).

Table 3.2: spectral features of red and orange dyestuffs on textile reference samples.

Dyestuff	Mordant	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Bloodred webcap	A+T	452-462 517-520* / 554-557*	396-400*	487-496 526-548 578-584	6
Brazilwood	A/A+T	443-451 (517-519)*	392-403	584-599	1+9
Brazilwood	A+T+Fe	442-444 462-493	415-426	589-591	3
Cochineal	A/A+T	522-532 / 556-569	415-436	586-646	6+12
Cochineal	A+T+Fe	523-525 / 565-566	425-430	604-609	2
Madder	A/A+T	506-514 / 543-547*	424-454*	575-614	7+11
Madder	A+T+Fe	496-510 / 548*	428-429*	575-578	3
Sandalwood	A+T	478-483 / 504-509	408-421	540-552	2
Annatto	-/A+T	458-479* / 501-505*		515-533	1+8
Henna	-/A+T	476*	419*	387* 540-543	1+1

A=alum, T=cream of tartar, Fe=iron(II) sulfate, - =direct dyeing; * feature observed only in some of the investigated samples; ( ) position of the spectral feature not clearly identifiable.
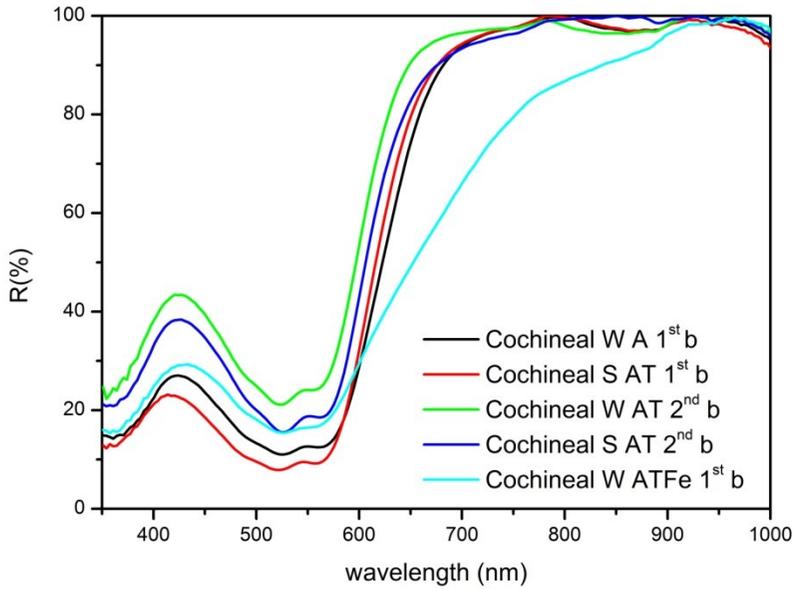
Figure 3.5: reflectance spectra recorded on red reference samples dyed with cochineal: wool, mordanted with alum, 1st bath (black spectrum); silk, mordanted with alum and cream of tartar, 1st bath (red spectrum); wool, mordanted with alum and cream of tartar, 2nd bath (green spectrum); silk, mordanted with alum and cream of tartar, 2nd bath (blue spectrum); wool, mordanted with alum, cream of tartar and iron sulfate, 1st bath (cyan spectrum).
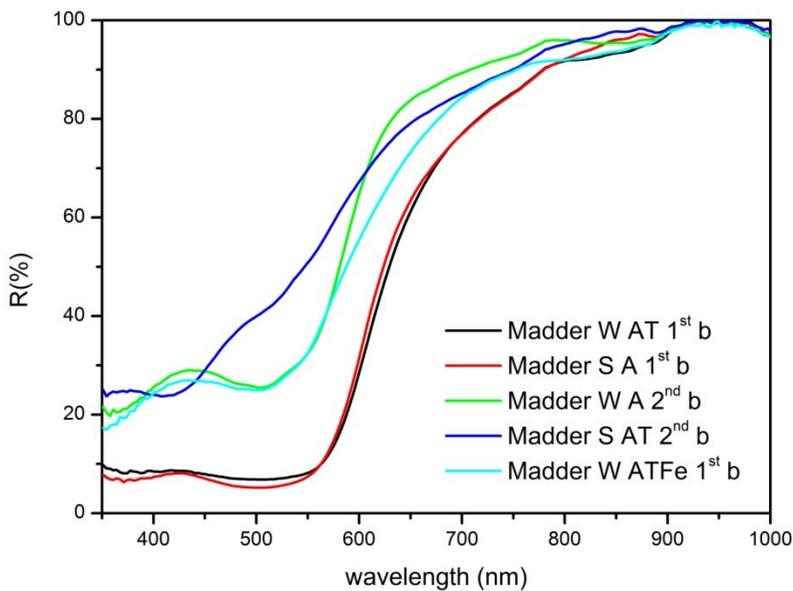
Figure 3.6: reflectance spectra recorded on red reference samples dyed with madder: wool, mordanted with alum and cream of tartar, 1st bath (black spectrum); silk, mordanted with alum, 1st bath (red spectrum); wool, mordanted with alum, 2nd bath (green spectrum); silk, mordanted with alum and cream of tartar, 2nd bath (blue spectrum); wool, mordanted with alum, cream of tartar and iron sulfate, 1st bath (cyan spectrum).
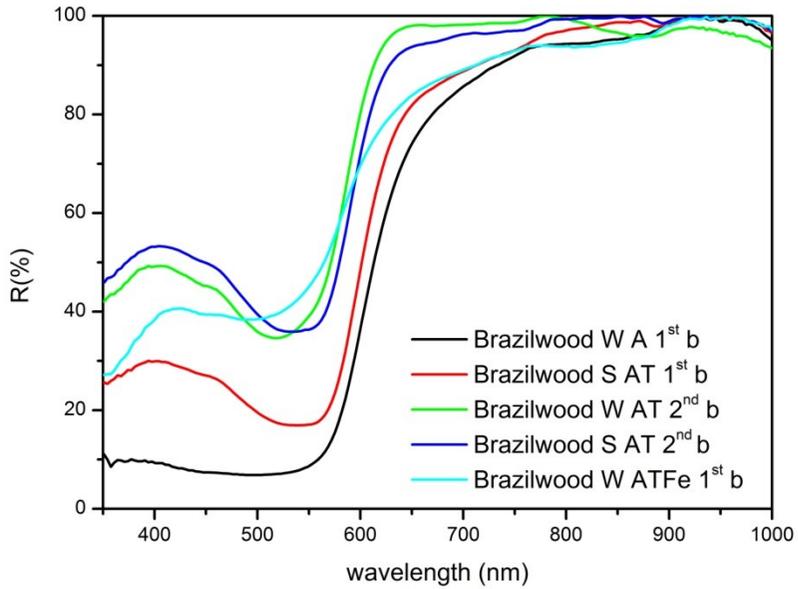
Figure 3.7: reflectance spectra recorded on red reference samples dyed with brazilwood: wool, mordanted with alum, 1st bath (black spectrum); silk, mordanted with alum and cream of tartar, 1st bath (red spectrum); wool, mordanted with alum and cream of tartar, 2nd bath (green spectrum); silk, mordanted with alum and cream of tartar, 2nd bath (blue spectrum); wool, mordanted with alum, cream of tartar and iron sulfate, 1st bath (cyan spectrum).
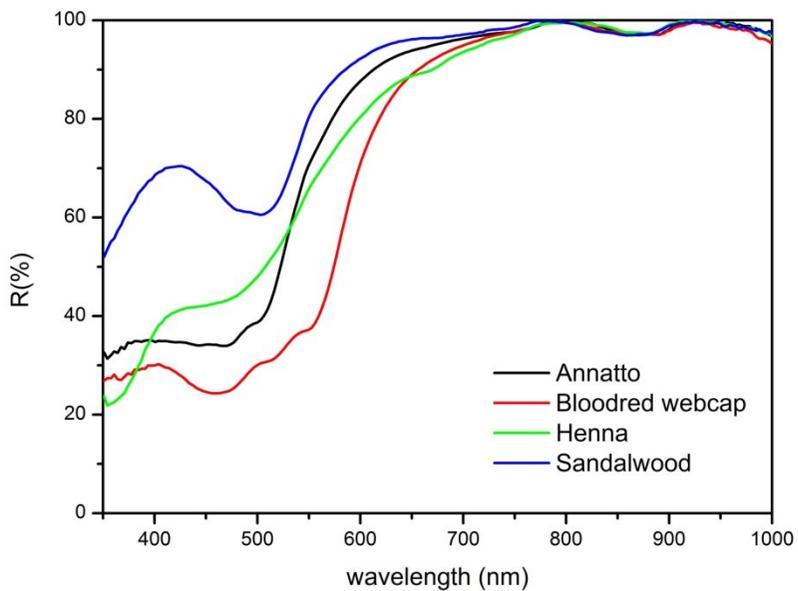
Figure 3.8: reflectance spectra recorded on red and orange reference samples of wool, 1st bath: annatto, mordanted with alum and cream of tartar (black spectrum); bloodred webcap, mordanted with alum and cream of tartar (red spectrum); henna (green spectrum); sandalwood, mordanted with alum and cream of tartar (blue spectrum).
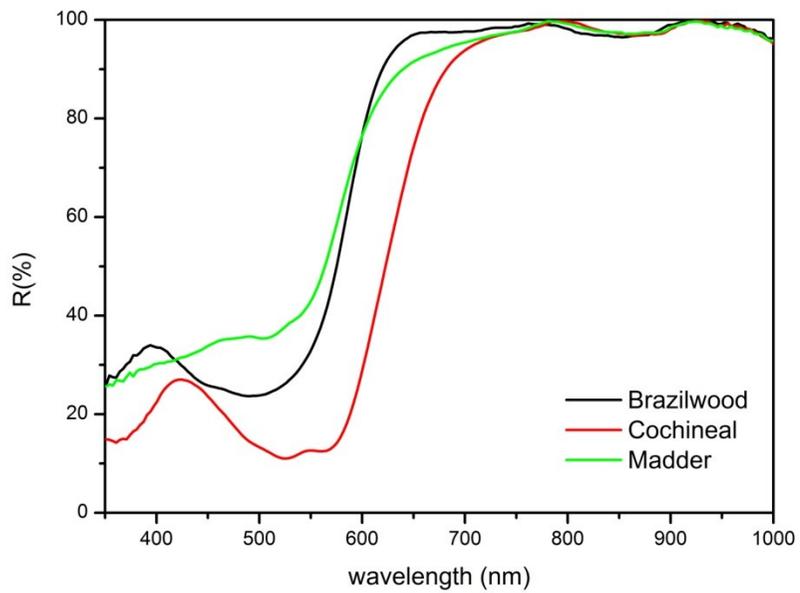
Figure 3.9: reflectance spectra recorded on red reference samples of wool: brazilwood, mordanted with alum and cream of tartar, 1ˢᵗ bath (black spectrum); cochineal, mordanted with alum, 1ˢᵗ bath (red spectrum); madder, mordanted with alum and cream of tartar, 2ⁿᵈ bath (green spectrum).

3.1.2.3 YELLOW REFERENCE SAMPLES

In general, reflectance spectra obtained on yellow samples in the visible spectral range did not show absorption maxima as those observed for blue, red and orange samples. The only spectral feature found in all the analyzed samples was the presence of a steep increase towards the red (Figure 3.10, Figure 3.11), whose position proved to be highly variable (Table 3.3) due to different dyeing procedures, to the concentration of the dye and to the substrate fiber. As for old fustic, in some samples two inflection points were observed. Nevertheless, their position was highly variable, being one centered between 461 and 494 nm and the other one between 530 and 604 nm.

Some samples presented additional features, mainly absorption bands. In particular, the unique sample of **common buckthorn** showed a band at 451 nm (Figure 3.10, black spectrum), while some samples of **old fustic** presented a band at 427-450 nm (Figure 3.10, red spectrum). The presence of this band was influenced by the dyeing procedure and by the fiber, as it was not found on silk samples (Figure 3.10, green spectrum). Also **weld** presented in some samples an absorption band around 400-450 nm, found also on samples mordanted with tin(II), but its position was not clearly identifiable (Figure 3.10, blue spectrum), while for **dyer's broom** the band was positioned around 370 nm, where the instrument has a poor sensitivity (Figure 3.10, cyan spectrum). As for **safflower** (Figure 3.10, magenta spectrum), the band was found around 350-415 nm for all the samples, but a more precise identification of its position was not possible.

It is not surprising that most of the dyestuffs which showed absorption bands in the 350-450 nm region contain flavonoids (e.g. common buckthorn, old fustic, weld and dyer's broom, 1.3.4), even though common buckthorn includes also a certain amount of anthraquinones. In fact, solutions of flavonoids generally show, in the visible region of the electromagnetic spectrum, absorptions in the 320-385 nm interval (band I) that are attributable to $\pi \rightarrow \pi^*$ transitions of the cinnamoyl part of the flavonoid unit (de Souza, Sussuchi, and De Giovanni 2003; Malesev and Kunti 2007). The interaction with metal ions determines an evident red shift to 400-450 nm, also observed in painting lakes (Clementi *et al.* 2008).

Regarding safflower, its main soluble coloring matters are quinochalcones (Cardon 2007), which were recently investigated by means of UV-Vis absorption by Clementi *et al.*(2014). Hydroxysafflor yellow A and safflor yellow A presented a maximum of absorption at 400 nm while anhydrosafflor yellow B presented an absorption band centered at 405 nm. Wool and silk dyed with safflower yellow presented absorption bands at 420 and 400 nm, respectively.

Unfortunately, the position of the above discussed absorption bands cannot be exploited for the selective identification of the considered flavonoids and quinochalcones yellow dyestuffs, as in solid samples the bands are poorly constant and, as discussed above, arduously identifiable.

The only yellow dyestuff with clear and reproducible absorption bands was **saffron**, which presented a structured band with minima at about 445 and 470 nm (Table 3.3) in all the investigated samples (Figure 3.11, black, red and green spectra). The samples of saffron and turmeric on wool mordanted with copper(II) sulfate (Figure 3.11, green and blue spectra), which are green-yellow (Table A1.1, Appendix 1), showed an absorption band at about 710 nm, barely appreciable in one of the turmeric samples. In the sample dyed with saffron and mordanted with copper(II) sulfate (Figure 3.11, green spectrum), a reflectance maximum at 566 nm was observed.

As a consequence of the above reported features, saffron can be safely identified according to the presence and the position of its absorption bands, which are attributed to $\pi \rightarrow \pi^*$ transitions of carotenoids, its main components (Vickackaite *et al.* 2004; Orfanou and Tsimidou 1996). It is known in fact that the conjugated double-bond system in carotenoids determines the presence of a band in the 400-500 nm range, structured in three sub-bands, whose position is determined by the number of conjugated double bonds present in the specific compound (Rodriguez-Amaya 2001). In polar solvents, generally only two major bands are detected (Tarantilis, Tsoupras, and Pellissiou 1995). The presence of the broad band at about 710 nm in samples mordanted with Cu(II) is attributable to the d-d electron transition of Cu(II) ion and the position of the band suggests a weak field exerted by ligands.

It should also be added that the similar features of saffron and annatto (Table 3.2, Table 3.3) are due to the related chemical composition of their coloring matters, which are mainly carotenoids (Cardon 2007), and that the bathochromic shift of the sub-bands of annatto owes a more orange color to this dyestuff, compared with the yellow to yellow-orange color of saffron.

As for above-cited **turmeric**, reflectance spectra showed s-shaped curves (Figure 3.11, blue and cyan spectra) with an inflection point at about 500 nm (Table 3.3) and no clear identification of the absorption band over a wide range (350-470 nm). The yellow dyes of turmeric are mainly curcuminoids (1.3.4). The enol form of curcumin, when dissolved in polar media, show an intense $\pi \rightarrow \pi^*$ absorption in the range 408-437 nm (Priyadarsini 2009), that is red-shifted by effect of Al^{3+} chelation to 432-465 nm (Jiang *et al.* 2011). The shape of the spectra in the considered samples did not allow us to ascertain a reliable indication on the possible presence of the dyestuff.

The position of the inflection point of the reflectance spectra of yellow dyestuffs could be possibly exploited for diagnostic purposes and deserves some comments. The number of samples for some of the dyestuffs considered here is too low in order to ascertain the variability of the position of the inflection point according to different mordants, fibers and dye concentrations. For those dyestuffs for which an adequate number of reference sample was available, it is evident that most of them present inflection points from about 460 to 520 nm (Table 3.3). On the contrary, the range of the inflection point in some samples of weld did not superimpose over the ranges covered by the inflection point other yellow dyes; the same stands for the inflection point of old fustic between 530 and 604 nm. Despite this, a tentative identification of these two dyestuffs based on the position of the inflection point is not advisable, as this spectral feature proved to be strongly dependent on many factors. Moreover, yellow dyestuffs are among the most numerous dyeing materials, as they can be found in many common plants (Cardon 2007; Hofenk de Graaff 2004), and thus their identification based on such a variable feature is hazardous.

Finally, some authors (Bacci *et al.* 2001; Cazenobe *et al.* 2002) proposed the use of chemometrics to treat reflectance data in order to identify the different yellow dyestuff employed on wool reference samples. The same approach was attempted on the large dataset obtained here, but the method was not effective in discriminating the samples dyed with different yellow dyestuffs. In particular, multivariate treatments of the data were not able to separate turmeric from saffron and, for some samples, safflower; moreover, also weld and old fustic were not discriminated despite many attempts.

Table 3.3: spectral features of yellow dyestuffs on textile reference samples.

Dyestuff	Mordant	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Common buckthorn	A	451		491	1
Dyer's broom	A	(370)		415	1
Old fustic	A/A+T	427-450*		461-494 530-604*	1+8
Safflower	A/A+T	(350-415)		473-492	1+3
Saffron	-/A/A+T	443-449 / 470-475		494-511	3+1+2
Saffron	Cu	445 / 468 711	566	494	1
Turmeric	-/A+T/?			490-516	2+3+1
Turmeric	Cu	710*		496	2
Weld	A/A+T	(380-420)*		426-475	7+4
Weld	Sn+T	(400)		445-451	2

A=alum, T=cream of tartar, Cu=copper(II) sulfate, Sn=tin(II) chloride, - =direct dyeing; * feature observed only in some of the investigated samples; ( ) position of the spectral feature not clearly identifiable.
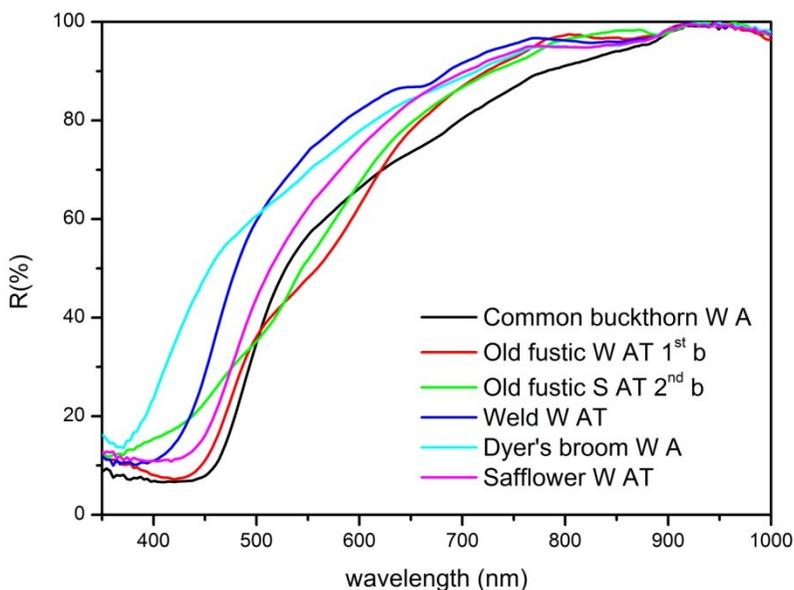
Figure 3.10: reflectance spectra recorded on yellow reference samples of wool: common buckthorn, mordanted with alum (black spectrum); old fustic, mordanted with alum and cream of tartar, 1st bath (red spectrum); old fustic, mordanted with alum and cream of tartar, 2nd bath (green spectrum); weld, mordanted with alum and cream of tartar (blue spectrum); dyer's broom, mordanted with alum (cyan spectrum); safflower, mordanted with alum and cream of tartar (magenta spectrum).
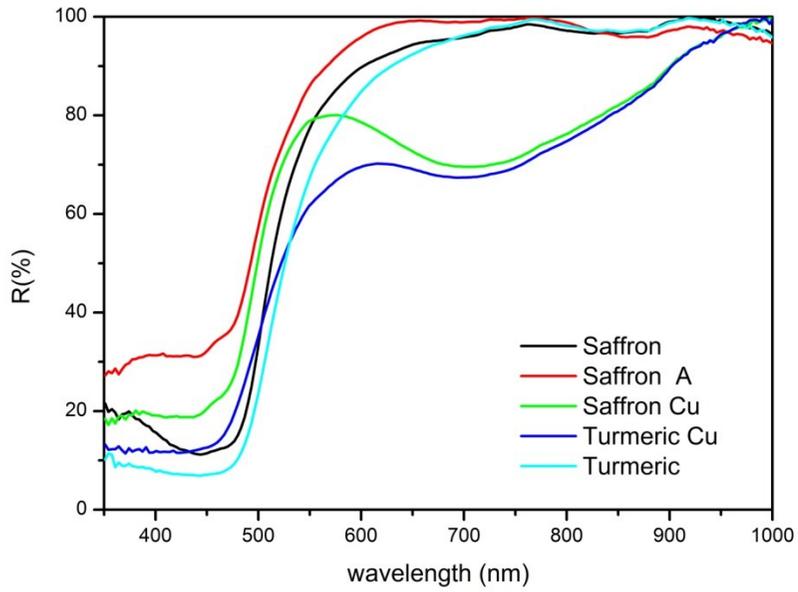
Figure 3.11: reflectance spectra recorded on yellow reference samples of wool: saffron (black spectrum); saffron, mordanted with alum (red spectrum); saffron, mordanted with copper sulfate (green spectrum); turmeric, mordanted with copper sulfate (blue spectrum); turmeric (cyan spectrum).

3.1.2.4 BROWN REFERENCE SAMPLES

Spectra recorded on brown dyestuffs (Figure 3.12) presented a gradual increase of the reflectance towards the infrared, with no significant spectral features (Table 3.4). Only in some samples of quebracho and in one sample of galls an absorption band appeared at 452-477 nm and at 414 nm, respectively (Figure 3.12, black and green spectra). Due to the lack of characteristic spectral features, the discrimination among different brown dyestuffs according to their reflectance spectra could not be assessed.

Table 3.4: spectral features of brown dyestuffs on textile reference samples.

Dyestuff	Mordant	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Galls	A+T	414*			2
Galls	Fe+T				1
Quebracho	A+T	452-477*			6
Walnut	-/A+T				1+6

A=alum, T=cream of tartar, Fe=iron(II) sulfate, - =direct dyeing; * feature observed only in some of the investigated samples.
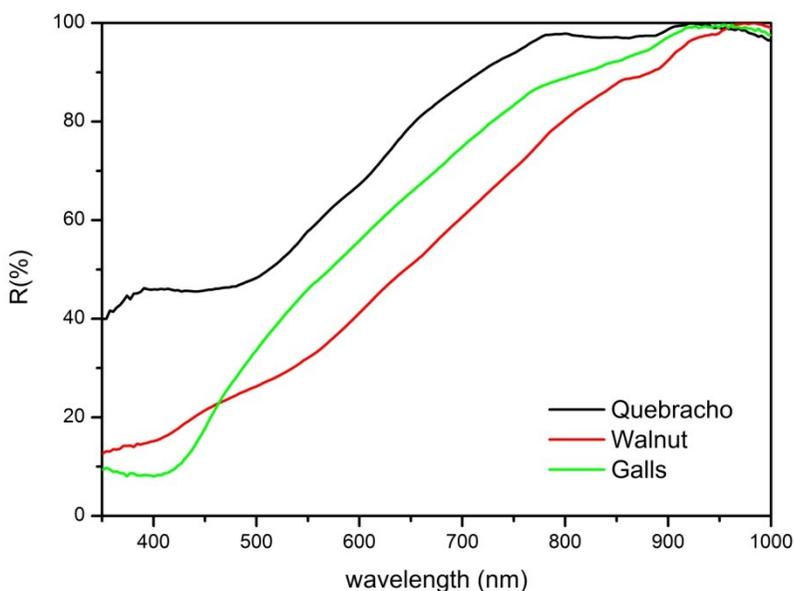
Figure 3.12: reflectance spectra recorded on brown reference samples: quebracho, wool mordanted with alum and cream of tartar (black spectrum); walnut, silk mordanted with alum and cream of tartar (red spectrum); galls, wool mordanted with alum and cream of tartar (green spectrum).

3.1.2.5 PURPLE REFERENCE SAMPLES

The spectra of samples dyed with **shikon** did not show the presence of detectable absorption bands or reflectance maxima (Figure 3.13, red spectrum), while three inflection points were observed (Table 3.5, Figure 3.14, green and blue spectra).

On the contrary, in some samples dyed with **alkanet** a structured absorption band was found (Figure 3.13, black spectrum), even though the identification of its position was difficult (Table 3.5). In addition, reflectance maxima were detected at about 460 and 775 nm (Table 3.5). Also in this case, an inflection point was located at about 620 nm (Table 3.5, Figure 3.14 black and red spectra), correspondent to the main inflection point of shikon.

In the case of double dyeing, the presence of **indigo** was always identifiable by the position of its main absorption band at about 660 nm, while the identification of the bands pertinent to the red dyestuff was strongly dependent on the concentration of the dyestuff (Table 3.5, Figure 3.13, green and blue spectra), as it was not observed in the purple sample with a higher concentration of **cochineal**. The identification of the absorption bands was rather difficult for **madder** (Figure 3.13, blue spectrum). Additional not-diagnostic spectral features related to indigo and the red dyestuff were found in all the investigated samples (Table 3.5).

The discrimination of the considered purple dyestuffs is not conclusive in the case of shikon and alkanet, which inflection points fall within similar wavelength intervals. In fact, not only their main dyeing matters belong to the class of naphtoquinones, but also alkannin, the main molecule of alkanet, might be present with shikonnin in shikon (1.3.3). However, it seems that in some cases alkanet samples presented absorption band not revealed in shikon samples, although a higher number of samples should be considered in order to ascertain this behavior. On the contrary, it is possible to distinguish between purple samples dyed with one purple dyestuff and samples obtained with double dyeing, employing a blue dyestuffs over a red dyestuff. In particular, the features of indigotin are always very clear also when other dyeing molecules are present, while the attribution of red dyestuffs can be performed only when the position of the characteristic absorption bands can be clearly identified.

Table 3.5: spectral features of purple dyestuffs on textile reference samples.

Dye 1	Dye 2	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Alkanet	-	(480-570)*	458-462* 774*	616-623	3
Shikon	-			442* 537-540 625-640	2
Cochineal	Indigo 1 bath	527* / 565-573 662-664	423-429	583-596 711	2
Madder	Indigo 1 bath	(500-550) 667	615	585 710	1

- =no second dyeing; * feature observed only in some of the investigated samples; ( ) position of the spectral feature not clearly identifiable.
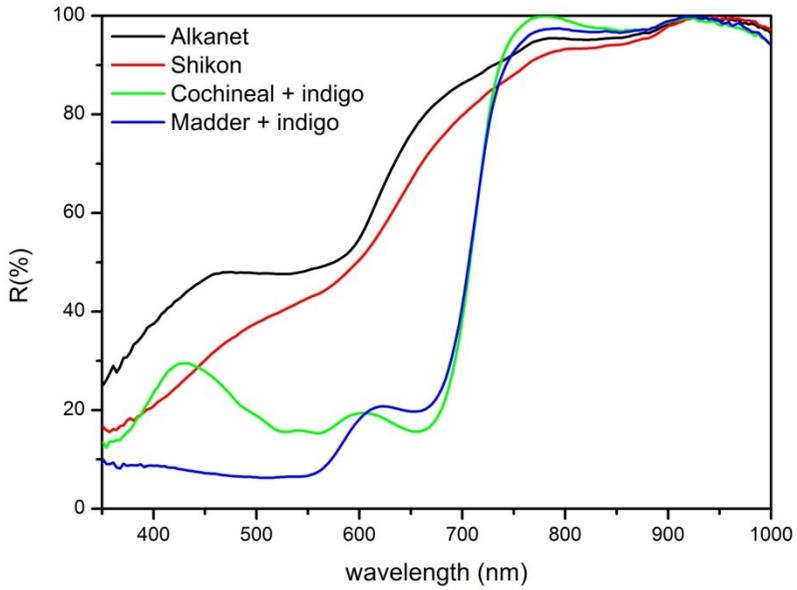
Figure 3.13: reflectance spectra recorded on purple reference samples: alkanet, wool (black spectrum); shikon, silk (red spectrum); cochineal + indigo, wool (green spectrum); madder + indigo, wool (blue spectrum).
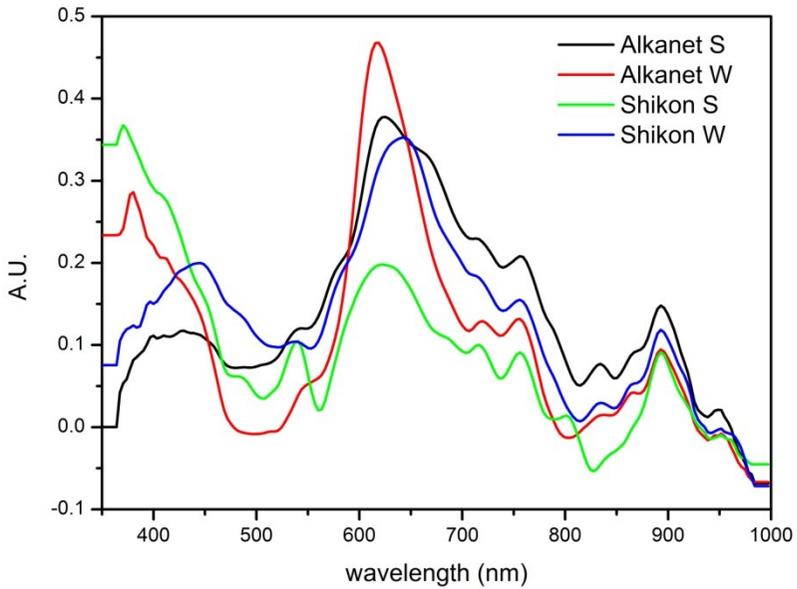
Figure 3.14: first derivative spectra calculated from the reflectance spectra recorded on purple reference samples: alkanet, silk (black spectrum); alkanet, wool (red spectrum); shikon, silk (green spectrum); shikon, wool (blue spectrum).

3.1.2.6 GREEN REFERENCE SAMPLES

The identification of **indigo** by means of its absorption bands at about 660 nm was evident in all the investigated samples (Table 3.6, Figure 3.15, red, blue and cyan spectra), as previously observed for purple samples. Similarly, **Saxon blue** was easily detected, as an absorption band at about 615-620 nm was observed in all the analyzed samples (Table 3.6, Figure 3.15, green spectrum). On the contrary, on samples of **logwood** mordanted with chromium the position of the main absorption band of logwood was not ascertained (Figure 3.15, black spectrum), mainly because the color obtained on the samples was very dark, thus making the identification of the spectral features difficult. In the sample obtained with logwood and weld, a weak absorption band at about 445 nm was observed (Figure 3.15, black spectrum), possibly related to the band observed at about 435 nm (Table 3.1) in blue samples obtained with logwood.

As for yellow dyestuffs in green samples, the diagnostic bands of **saffron** were identified in the sole reference sample available (Table 3.6, Figure 3.15, red spectrum). In addition, an absorption band at 415-427 nm was found in wool samples dyed with Saxon blue and **old fustic** (Table 3.6, Figure 3.15, green spectrum), which was not visible on silk samples and it was barely identifiable in wool samples dyed with indigo and old fustic. An absorption band was also observed in the range 350-405 nm in wool samples dyed with **weld** (Table 3.6, Figure 3.15, blue spectrum), which remained undetected in silk samples, in lighter wool samples and in the sample dyed with logwood. The bands observed in green samples dyed with old fustic and weld are possibly related to the above discussed (3.1.2.3) absorption band of yellow flavonoids. Moreover, an absorption band at about 350-390 nm was found for the green sample dyed with **safflower** (Table 3.6, Figure 3.15, magenta spectrum), similarly to what observed for yellow samples (Table 3.3). Except for those related to blue dyestuffs, no absorption bands were identified in green samples dyed with **turmeric** (Table 3.6, Figure 3.15, cyan spectrum). The position of the inflection points below 600 nm of green samples is consistent with those observed for yellow samples dyed with the same yellow dyestuffs (Table 3.3, Table 3.6). It should also be observed that the position of the maxima of reflectance in all the samples obtained with double dyeing, both purple and green, is strongly dependent on the final color of the considered sample.

As previously evidenced for purple samples obtained through double dyeing (3.1.2.5), the identification of the blue dyestuff in green samples is generally possible due to the strong absorption bands of the main blue molecules. Similarly to what observed for blue samples, some issues can derive for very dark colors obtained with logwood, as the position of the absorption band in this case is not evident. Regarding yellow dyestuffs, similar spectral features were found among green and yellow samples dyed with the same dyestuff, even though to perform a univocal attribution of the dyestuff the considerations made for yellows should be taken into account.

Table 3.6: spectral features of green dyestuffs on textile reference samples.

Dye1	Dye2	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Old fustic A+T	Indigo H$_2$SO$_4$	415-427* 616-620	518-559*	461-475 657-664	6
Old fustic A+T	Indigo chemical vat	660-663	500-584	483-491 707-711	2
Weld A	Indigo H$_2$SO$_4$	(350-410)* 616-619 875-891	498-537	452-469 652-664	9
Weld A+T	Indigo chemical vat	657-664	477-491	423-450 710-711	2
Saffron A	Indigo chemical vat	446 / 475 662	507	488 708	1
Safflower A	indigo chemical vat	(350-390) 661	518	469 708	1
Turmeric -	Indigo chemical vat	662	514	482 707	1
Logwood Cr	Old fustic			463 756	1
Logwood Cr	Weld	445		460 753	1

A=alum, T=cream of tartar, Cr=potassium bichromate, - =direct dyeing; * feature observed only in some of the investigated samples; ( ) position of the spectral feature not clearly identifiable.
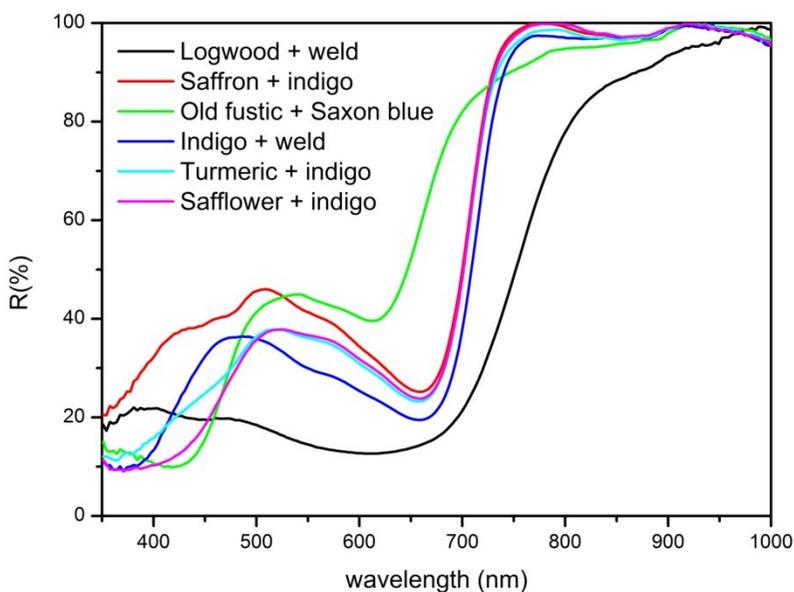

Figure 3.15: reflectance spectra recorded on green reference samples: logwood + weld (black spectrum); saffron + indigo (red spectrum); old fustic + Saxon blue (green spectrum); indigo + weld (blue spectrum); turmeric + indigo (cyan spectrum); safflower + indigo (magenta spectrum).

3.1.3 CONCLUSIONS

This work investigated by means of visible fiber optics reflectance spectroscopy a wide set of reference animal fibers (wool and silk) dyed with twenty-two dyestuffs, alone or superimposed on other dyestuffs, following different dyeing procedures. The reference samples were obtained from five different sets which considered, in some cases, various concentrations of the dyestuff. For some dyestuffs, only few samples were available, while for others a wider number could be considered. The comparison of the obtained spectra allowed to highlight the spectral features that can be confidently considered diagnostic for the analyzed dyestuffs and to ascertain their reproducibility while varying the dyeing procedure, the fiber, the mordant and the concentration of the dye. In the case of some widely used red and blue dyestuffs (e.g. madder, cochineal, brazilwood, logwood and indigo), the results substantially confirmed some previously published data (Angelini *et al.* 2009; Poldi 2011), which, conversely, did not report a systematic investigation of the spectral features for the considered dyestuffs. For other dyestuffs, there are no records in the literature of previously published reflectance spectra, even though in some cases UV-Vis absorption measurements were carried out on solid samples, mainly lake pigments (Clementi *et al.* 2008). In addition, the spectral features identified on the reference samples were also discussed according to spectroscopic studies of the considered dyestuffs or of their main dyeing molecules in solution. In general, the results obtained are consistent with previously published data and with the nature of the molecules contained in the dyestuffs, although for some dyestuffs not all the reported bands could be detected in the considered reference samples.

The identification of the dyestuff is easier when considering blue or red dyestuffs, while for yellow, brown and purple dyestuffs the spectral features are often highly variable. In particular, blue and red dyestuffs generally present strong absorption bands clearly identifiable in terms of position, which proved to be the main diagnostic features for the considered samples and are strongly related to the molecular structure of the coloring matters contained in the investigated dyestuffs. The detection of these bands is sometimes prevented in darker blue and red samples and, in these cases, other spectral features cannot be employed for an univocal identification. The position of the inflection points proved indeed to be strongly influenced by the dyeing conditions (e.g. mordant, concentration of the dye) and partially superimposed over different dyestuffs of the same color, while the reflectance maxima showed poor relationship with the molecular structure of the dye molecules. Only in the case of henna, an orange-red dyestuff, no diagnostic spectral features could be selected.

The position of the absorption bands can be seldom safely ascertained in yellow, purple and brown dyestuffs, and therefore it is not possible to distinguish these dyestuffs on the basis of these spectral features. Only saffron, a yellow carotenoid dyestuff, presents a clear and reproducible structured absorption band. In some of the other considered yellow dyestuffs (e.g. old fustic and weld), the ranges of the inflection points do not superimpose over the other dyestuffs of the same color, even though it is still very variable as observed for red and blue dyestuffs. In addition, some variously colored dyestuffs present more than one inflection point (e.g. old fustic, alkanet, shikon), thus possibly allowing their identification according to their reciprocal positions. It shall nevertheless be considered that, for some of the above cited dyestuffs, only a limited number of

reference samples was available, and therefore the data recorded are not adequate to determine the possible spectral variations within the dyestuff. Moreover, yellow dyestuffs were often obtained from local plants, and so a thorough consideration of all the possibly employed dyestuffs is arduous. However, on the light of the above discussed results, it is possible to determine the spectral features, in terms of position of the inflection points, of napthoquinones-containing purple dyestuffs (e.g. alkanet and shikon). An identification of yellow dyestuffs by means of multivariate analysis was attempted but, unlike the results obtained by Cazenobe *et al.* (2002) on a more limited set of reference samples, a discrimination among the different materials was not achieved.

Blue dyestuffs were also identified in purple and green samples obtained through double dyeing with red and yellow dyestuffs, respectively. Differently, the identification of the absorption bands of madder and cochineal in purple samples was strongly dependent on the concentration of the dye. As for yellow dyestuffs, similar spectral features to those of yellow samples were observed in green samples, but their unambiguous identification was not possible, except for saffron.

In conclusion, a thorough investigation of the reflectance spectral features of several natural dyes is reported. The work highlighted the potentiality of visible FORS for the analysis of dyestuffs but also raised some weak points of the technique, which is sometimes influenced by the concentration of the dye, by the mordant employed and by the fiber. These factors in some cases prevented the correct identification of the dyestuff, mainly when they contribute to confer dark colors to the sample. Widely used and less common blue and red dyestuffs can quite easily be identified by means of FORS, while for yellow and purple dyestuffs only discriminations among some classes of molecules are possibly achievable. Also with these limitations, FORS proved to be a valuable technique for non-invasive investigation of natural dyestuffs, as it is suitable for preliminary analysis of art objects. FORS is indeed rapid, since it allows the analyst to record a wide number of analytical spots directly on the object and then it permits to compare a large number of spectra in order to sort out areas of the object that show similar spectral features and that are therefore pertinent to a same dyestuff. Therefore, whenever sampling was allowed, the preliminary investigation by means of FORS strongly limited the number of samples required for further invasive or micro-invasive investigations.

This methodological work was complemented by a wide number of measurements performed on historical and archeological textiles, reported in chapter 6 of this thesis (paragraphs 6.1 and 6.3-6.6). In some cases, FORS analysis were followed or integrated by the use of other non-invasive or micro-invasive techniques, which allowed to compare and verify the results obtained by FORS and to further support the indication of strong and weak points of this technique for the analysis of natural dyestuffs. The technique was also applied to the analysis of dyes on paper manuscripts (paragraph 6.2) in order to ascertain whether the spectral considerations about textiles were valid also for the analysis of dyes on a different matrices.

3.2 FORS INVESTIGATION FOR THE ANALYSIS OF PURPLE-COLORED PARCHMENTS

The use of colored paper or parchment is not common in the European manuscript production, as was for Persian manuscripts (paragraph 6.2). The use of colored parchments in Europe was mainly reserved to the so called "purple codices". These are very peculiar and precious manuscripts created from the Late Antique to the Medieval ages. Purple codices were usually written with golden or silver inks on purple parchment and contained texts from the Holy Writings. The joint use valued materials charged these manuscripts with a highly symbolic value, produced to be owned by kings and emperors.

Roman written sources already mentioned the production of purple codices (Baroni 2012; Roosen-Runge 1967; Pliny the Elder 1952), together with a number of technical treatises dated to the Late Antique and Medieval Ages (Caley 1926; Caley 1927). The Leyden Papyrus X (Caley 1926), the Stockholm Papyrus (Caley 1927) and Pliny the Elder's Naturalis Historia (Pliny the Elder 1952) reported information about the dyeing of textiles with Tyrian purple or other purple-colored dyes. The use of a Tyrian purple lake in painting, called *purpurissimum* by Pliny the Elder, was mentioned as well, but information concerning the process of imparting purple color to parchment is relatively rare. Medieval treatises report generically about the use of Tyrian purple or of its cheaper substitutes. In particular, the most important treatise on miniature painting, the 14th century manuscript *De arte illuminandi* (Brunello 1971), did not even mention the use of Tyrian purple. In 7th century, Isidore of Sevilla in his Etymologies (Barney *et al.* 2006) wrote that ''[..] purple parchment is stained with purple dye [..]'' with no further indication. In the 8th century manuscript known as *Manoscritto di Lucca* or *Compositiones ad tingenda musiva* (Smith and Hawthorne 1974) three recipes are reported, and they suggest the use of different dyes to impart the purple color to writing supports: i.e kermes, madder and a purple dye from mollusks. Recently, Brun (2012) cited a small treatise on the production of purple codices called *Conchylium*, datable to 4th- 5th century AD, in which a recipe describes a dyeing procedure typical of vat dyes such as indigoid dyes; this recipe does not explicitly mention parchment, but one can reasonably think about parchment dyeing, since the following recipes in the treatise describe the preparation of gold and silver inks, which are typically used in writing on purple codices. Finally, Travaglio (2012a) cited another Early Medieval treatise, *Ut auro scribatur*, in which the use of orchil is described to impart the purple color to parchment; moreover, this book suggests that parchment was painted and not dyed. A similar procedure for coloring parchment is described in ms. Palatino 941, a 15th century manuscript held at the Biblioteca Nazionale Centrale in Firenze (2012b), in which the use of cloths, the so-called *pezzuole* or *cimature*, is described: they were soaked into extracts obtained from a natural dyestuffs and rubbed on the parchment.

The information obtained from the above mentioned ancient treatises is vague and often ambiguous. The treatises revealed that the color of parchment could not be conferred by Tyrian purple, as only *Manoscritto di Lucca* explicitly reported the use of dyes from mollusks. In addition, there is sometimes ambiguity in the use of the term ''purple'', which seems mainly related to the color rather than to a specific compound. Also the processes employed to color the parchment are unclear, as the different authors mentioned either dyeing or painting. It should also be added that ancient treatises are often compilations of older sources; in the process of transferring knowledge,

the text can be intentionally or unintentionally modified and therefore it can accumulate misunderstandings in the later versions.

According to the above described bibliographic information, several dyestuffs might be employed instead of Tyrian purple obtained from different species of mollusks (*Hexaplex trunculus* (Linnaeus, 1758), *Bolinus brandaris* (Linnaeus, 1758), *Stramonita haemastoma* (Linnaeus, 1766)). In particular, the use of folium, which is the aqueous extract from turnsole (*Chrozophora tinctoria* A. Juss., 1.3.3), or orchil, which is the alkaline extract from various species of lichens (1.3.3), can be suggested. Moreover, a purple color could be achieved also by applying a red anthraquinonic dyestuff on a surface previously dyed with indigo, a technique already known to the Romans and cited by Pliny the Elder (Pliny the Elder 1952). Among the possible anthraquinones dyestuffs, madder (*Rubia tinctorum* L.), kermes (*Kermes vermilio* (Planchon, 1864)), Armenian and Polish cochineal (*Porphyrophora hamelii* (Brandt, 1833) and *Porphyrophora polonica* (Linnaeus, 1758), respectively) should be cited, while Mexican cochineal could evidently be ruled out from the set, as it was imported from the New World (1.3.2).

The information coming from previous chemical analysis on purple codices is poor and will be discussed extensively in paragraph 6.7 of this thesis, where the identification of the dyestuffs of a 6th century purple codex is discussed on the basis of a non-invasive and micro-invasive multi-analytical approach. The aims of the methodological work discussed here are to investigate the spectral features of the above listed purple dyestuffs by means of visible or UV-visible fiber optics reflectance spectroscopy, in order to determine whether this technique could be employed for their identification on parchments. For this purpose, reference samples were prepared by painting or, when possible, dyeing the parchment with the selected dyestuffs. Additional measurements were carried out on the reference samples with other non-invasive and micro-invasive techniques in order to fully characterize the materials. The results obtained with other non-invasive techniques are reported in paragraph 6.7, while those achieved by means of surface-enhanced Raman scattering are thoroughly discussed in paragraph 5.2 of this dissertation.

3.2.1 MATERIALS AND METHODS

REFERENCE SAMPLES

Paints and dyed samples of Tyrian purple and of other purple colorants were prepared following ancient recipes and applied on parchment. Tyrian purple, madder, kermes and Armenian cochineal were obtained from commercial products (Kremer Pigmente GmbH & Co., Aichstetten, Germany; Turkish Cultural Foundation, Washington D.C., USA). Folium was obtained by extraction of the fruits of *Chrozophora tinctoria* (L.) A. Juss from Turkey. The fruits were treated with cold water at neutral pH for one hour and the extract was filtered and allowed to dry. Orchil, following the indications by Kok (1966), was obtained by extraction of scraps of *Roccella tinctoria* DC from Canary islands in 30% v/v ammonia for 3 weeks, with frequent stirring to favor the oxidation of orcin to orcein; the extract was filtered and allowed to dry. Additionally, samples of *purpurissimum* and of parchment dyed with the juice of *Hexaplex trunculus* (Linnaeus, 1758) were obtained from Rolf Haubrichs (Université de Genève). The production procedures employed for these samples could be only partially reconstructed.

Painted samples were prepared using a solution with 1 g/ml in gum Arabic and 2 g/ml in saccarose as painting medium. Different colorant/medium ratios were used in order to obtain darker and lighter samples. Tyrian purple and *purpurissimum*, being insoluble in aqueous media, were prepared as pigments, i.e. dispersed in the medium and the resulting paste was applied on parchment by means of a brush. Folium, orchil, madder, kermes and cochineal were dissolved in the medium and the solution was brushed on the parchment.

Dyed parchment samples were prepared according to the features of the colorants: folium and orchil were used as mordant dyes, i.e. soaking parchment in a solution containing dye and 30% alum (w/w with respect to the weight of parchment), while Tyrian purple was used as a vat dye, i.e. firstly reducing it in a 60% sodium dithionite and 30% sodium hydroxide solution (same as alum) at 60°C for 10 min, then soaking
parchment in the solution containing the leuco form, finally exposing the parchment sample to air in order to cause reoxidation of the leuco form to insoluble 6,6'-dibromoindigotin that precipitated on parchment imparting purple color. Also in this case, different concentrations of the dyestuff were employed to vary the saturation of the color.

INSTRUMENTATION

Fiber optics reflectance spectroscopy was performed with an Avantes (Apeldoorn, The Netherlands) AvaSpec-ULS2048XL-USB2 model spectrophotometer equipped with an AvaLight-DH-S-BAL balanced deuterium–halogen light source with a wavelength range of 190–2500 nm or with an AvaLight-HAL tungsten/halogen lamp with emission from 360 to 2500 nm. The instrumental parameters were as follows: 30 ms integration time, 50 scans for a total acquisition time of 1.5 s for each spectrum. The light source and the detector were connected with fiber optics to an Ocean Optics (Dunedin, Florida) R-400 model reflection probe. In this configuration, light was sent and retrieved with a unique fiber bundle positioned at 45° from the surface normal, in order to exclude specular reflectance. Diffuse reflectance spectra of the samples were referenced against the WS-2 reference tile provided by Avantes and guaranteed to be reflective at 98% or more in the spectral range investigated. The whole system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 7. Further technical details about the instrumentation are given in Appendix 2.
The use of AvaLight-DH-S-BAL light source allowed to extend the range into the UV, but the definition in the range 400-600 nm was lower due to the low emission – and therefore low reflectance - of the lamp in this region. For this reason, most of the samples were analyzed using both light sources. In some cases, dyed samples were analyzed in the front and the reverse side.

3.2.2 RESULTS AND DISCUSSION

The main spectral features identified in the analyzed reference samples are summarized in Table 3.7. The position of the inflection points was identified upon calculation of the first derivative of the considered spectra. R

eference samples colored with **folium** showed in their reflectance spectra the presence of a structured absorption band with minima at about 540 and 575 nm, while a maximum of reflectance was placed at about 475-500 nm (Table 3.7, Figure 3.16, black and red spectra). In addition, the increase towards the infrared region determined an inflection point at about 600-620 nm. The position of the absorption bands was similar in dyed and painted samples, while that of the reflectance maximum was not clearly identifiable in painted samples; the inflection point underwent a bathochromic shift of 10-20 nm in painted samples.

Also the reflectance spectra of **orchil** (Figure 3.16, green and blue spectra) presented a structured absorption band, whose position of the first minimum was similar to that observed in folium, while the second was slightly red-shifted at about 585-590 nm (Table 3.7). These bands were less evident in painted samples (Figure 3.16, blue spectrum). A reflectance maximum at 430 nm was observed only in the lighter sample of dyed parchment, while the inflection point was located at 609-622 nm, its position being more reproducible in painted samples.

A number of absorption bands was found in reflectance spectra recorded on samples of **Tyrian purple** and ***purpurissimum***, dyed or painted (Table 3.7, Figure 3.17). In particular, an intense absorption band centered at 524-539 nm was observed in all the analyzed samples. In addition, a broad absorption band at about 640-660 nm was distinguishable in all the sample (Figure 3.17). One parchment painted with *purpurissimum* presented also a structured band in the NIR region (Table 3.7). A variable maximum of reflectance was found between 424 and 505 nm in all the investigated samples. Variable inflection points were found at 396-416 nm and 677-709 nm; a more reproducible inflection point was identified at 547-557 nm in all the samples (Table 3.7). Some darker samples did not present the inflection point around 700 nm. The sample painted with *purpurissimum,* which showed additional bands in the NIR, presented also two inflection points in this region (Table 3.7).

Reflectance spectra of madder and Armenian cochineal painted on parchment (Figure 3.18, black and red spectra) presented similar spectral features to those observed for textile reference samples (3.1.1.2, Table 3.7), even though in that case cochineal was obtained from a different cochineal species (e.g. Mexican cochineal). In addition, reflectance spectra recorded on the parchment painted with a kermes lake (Figure 3.18, green spectrum) showed an absorption band at 490 nm, a structured absorption band at 525 and 570 nm, a maximum of reflectance at 453 nm and an inflection point at 600 nm.

It is apparent from the discussion of the spectral features that the here considered dyestuffs presented a structured absorption band in the 500-600 nm range as principal diagnostic feature, with the exception of Tyrian purple and its lake pigment *purpurissimum*, which presented also an absorption band at about 640-660 nm. This situation is illustrated in Figure 3.19 and 3.20 which

illustrate some of the reflectance spectra of the considered dyestuffs (Figure 3.19) and some examples of spectra converted into apparent absorbance (Log 1/R coordinates, Figure 3.20), which shows more clearly the absorption features of the here considered samples. The position of the sub-bands can be employed to distinguish between most of the investigated dyestuffs, as in anthraquinones from plant sources (e.g. madder) they are located at about 510 and 545 nm, while in anthraquinones from animal sources (e.g. cochineal and kermes) they are red-shifted at about 525 and 560-570. The presence of the band at 490 nm for the kermes sample might allow its discrimination with Armenian cochineal. A further bathochromic shift undergoes for folium and orchil, whose first band is positioned at about 540 nm, while the second is found at about 580-590 nm. The distinction between orchil and folium on the basis of the position of the second sub-band appears somewhat tentative, as this band proved to vary of several nanometers when analyzing different samples colored with the same dyestuff (Table 3.7). It should nevertheless be observed that the aspect of these sub-bands is qualitatively different, as in folium samples the second sub-band is substantially stronger than the band of orchil. As for dyes and lakes obtained from mollusks (e.g. Tyrian purple and *purpurissimum*), they are not distinguishable one from another as they showed common spectral features, among which the absorption band at about 520-540 nm is the most robust, while the presence of the absorption band in the 640-660 nm range of dyed samples can be attributed to the presence of the non-brominated molecule indigotin (cf. Table 3.1, 3.1.1.1).

Table 3.7: spectral features of purple dyestuffs on parchment reference samples.

Dyestuff	Application	Absorption bands	Reflectance maxima	Inflection points	N. of samples
Folium	dyed	539 572	476	598	1
Folium	painted	536-545 578-586	(475-500)	610-620	2
Orchil	dyed	539-550 589-591	430*	609-622	2
Orchil	painted	547-551 584-589		608-611	2
Tyrian purple	dyed	524-539 640-660*	445-505	319* 404-416* 547-553* 685-709*	5
Tyrian purple	painted	384 528 650	492	414 552 696	1
Purpurissimum	dyed	521-537 643-661	424-467	396-405 542-553 677-702	4
Purpurissimum	painted	529-539 644-662 800* 850*	446-469	400-408* 550-557 686-708 810* 878*	4
Madder	painted	512-513 543-547	432-435	578-593	3
Kermes	painted	490 527 560	453	600	1
Cochineal	painted	525 570	437	626	1

* feature observed only in some of the investigated samples; ( ) position of the spectral feature not clearly identifiable.
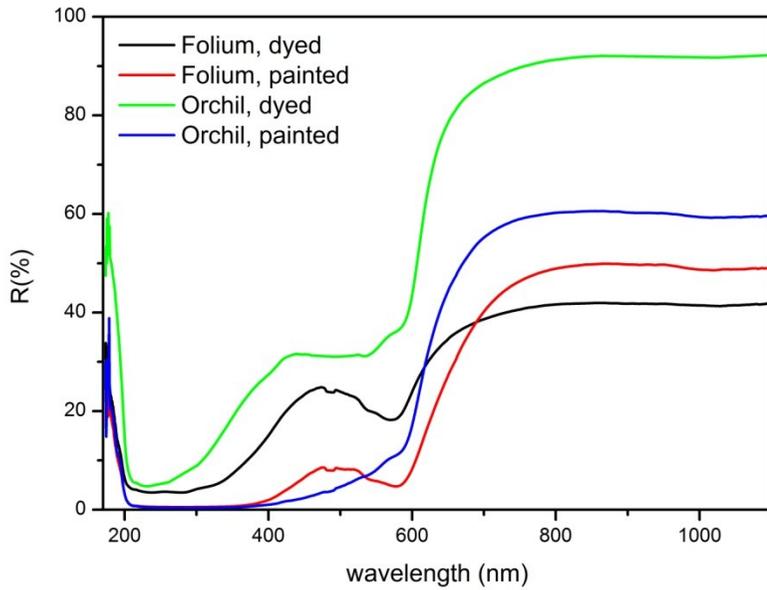
Figure 3.16: reflectance spectra recorded on parchment reference samples: folium, dyed (black spectrum); folium, painted (red spectrum); orchil, dyed (green spectrum); orchil, painted (blue spectrum).
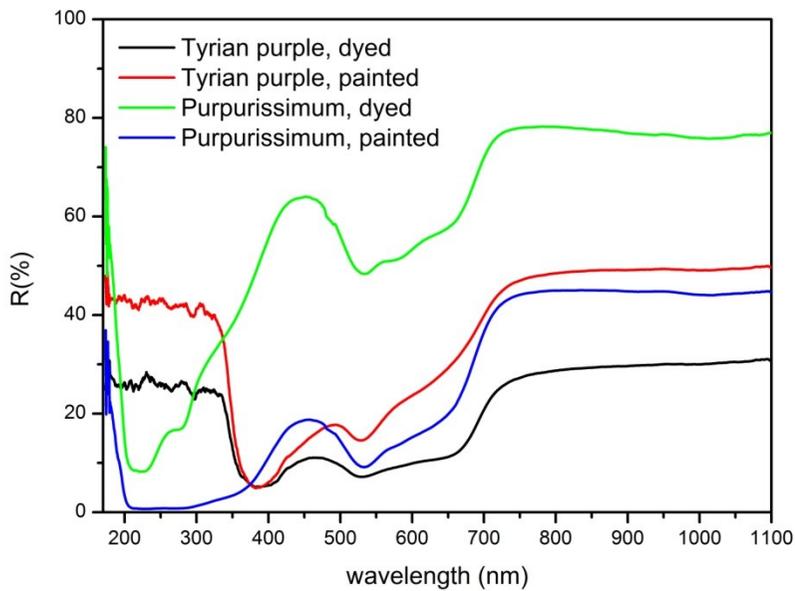
Figure 3.17: reflectance spectra recorded on parchment reference samples: Tyrian purple, dyed (black spectrum); Tyrian purple, painted (red spectrum); *purpurissimum*, dyed (green spectrum); *purpurissimum*, painted (blue spectrum).
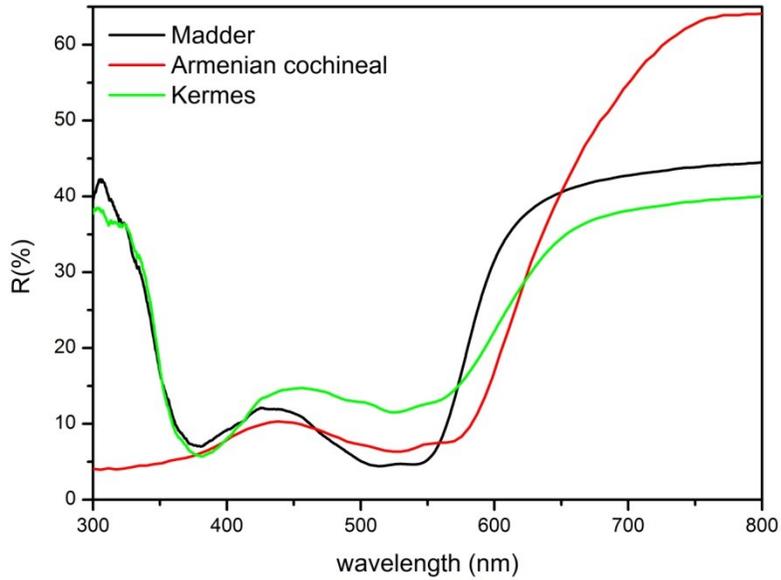
Figure 3.18: reflectance spectra recorded on parchment reference samples, painted: madder (black spectrum); Armenian cochineal (red spectrum); kermes (green spectrum).
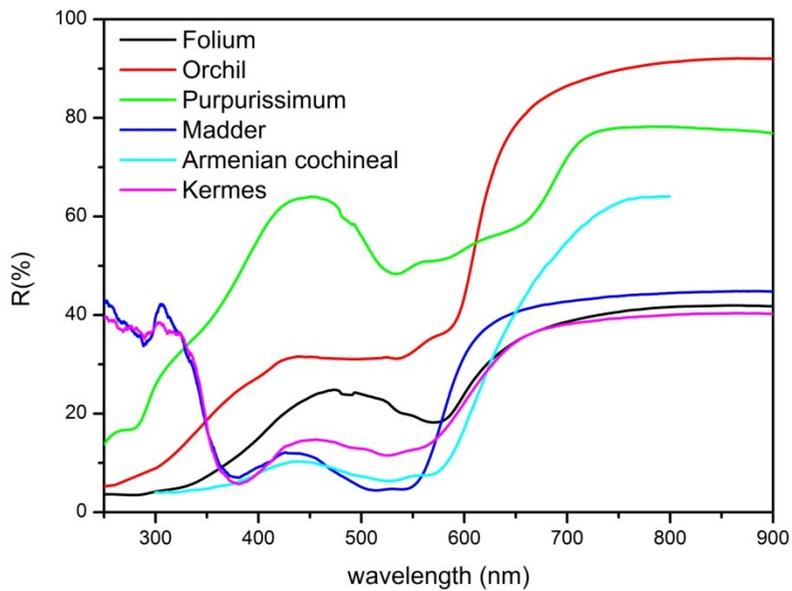
Figure 3.19: reflectance spectra recorded on parchment reference samples: folium (black spectrum); orchil (red spectrum); *purpurissimum* (green spectrum); madder (blue spectrum); Armenian cochineal (cyan spectrum); kermes (magenta spectrum).
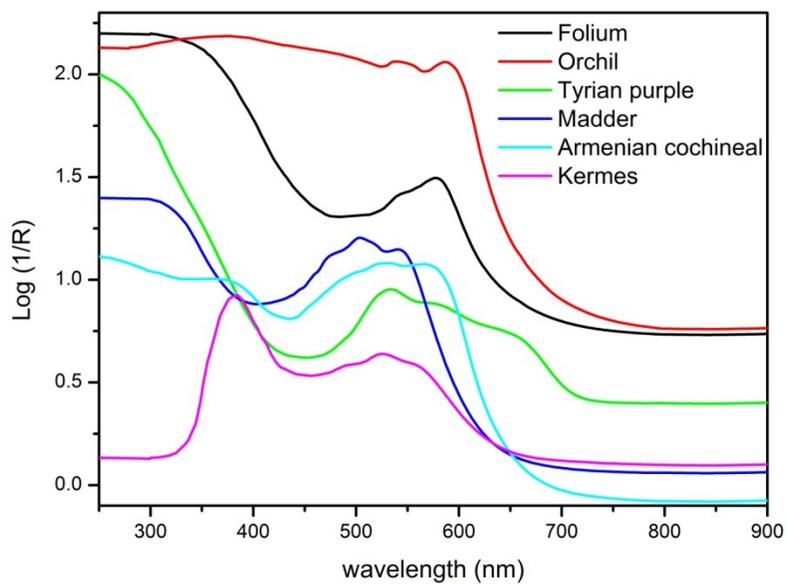
Figure 3.20: apparent absorbance spectra recorded on parchment reference samples: folium (black spectrum); orchil (red spectrum); *purpurissimum* (green spectrum); madder (blue spectrum); Armenian cochineal (cyan spectrum); kermes (magenta spectrum).

3.2.3 CONCLUSIONS

This work allowed us to ascertain the UV-Vis spectral features of purple dyestuffs possibly employed for the realization of purple codices by means of fiber optics reflectance spectroscopy. Reference samples of colored parchments were prepared in order to verify the variability of the spectral features according to the concentration of the dyestuffs and the coloring technique. In particular, dyed and painted samples were considered.

In general, the spectral features were similar in painted and dyed samples. As previously observed for dyed textiles, different concentrations of the dyestuff led to variations of some spectral features, mainly in the positions of reflectance maxima and inflection points; despite this, the identification of the diagnostic spectral features was possible both in lighter and darker samples.

The diagnostic spectral features identified for each dyestuff allowed the identification of madder and Tyrian purple/*purpurissimum* and of the groups of anthraquinone animal dyestuffs and of folium/orchil. Discrimination between kermes and Armenian cochineal on the basis of the position of the second absorption sub-band at 560-570 nm is tentative, as a higher number of samples should be considered in order to determine the internal spectral variations. On the contrary, the presence of a band at 490 nm for kermes might be diagnostic for this dyestuff. The same considerations are valid also for the distinction among folium and orchil through the precise identification of the position of the band at about 580-590 nm, even though in this case the presence of folium can be ascertained when strong absorption bands at about 590 nm are observed. It should be reported that obtaining samples of these latter four dyestuffs is very arduous nowadays and thus very little comparisons can be made with different raw materials.

In conclusion, the above reported considerations on the spectral features of several purple dyestuffs can be employed to perform a non-invasive attribution of the dyestuffs used to color the parchments of purple codices by means of UV-Vis FORS. These results were actually of help in the identification of the dyestuffs of a 6th century purple codex, as described in paragraph 6.7 of this thesis.

CHAPTER 4
MICRO-INVASIVE TECHNIQUES FOR THE ANALYSIS
OF NATURAL DYES

Non–invasive techniques are seldom conclusive in the identification of minor organic components in complex analytical matrices, as discussed in chapter 3, and thus the use of more selective techniques is required to achieve a more in-depth characterization. In particular, as for natural dyes, the possibility of enhancing the Raman signals offered by surface-enhanced Raman scattering (SERS) is of paramount interest in the field of cultural heritage, as it would quench the fluorescence emitted by most of the chemical species extracted from natural dyestuffs, with the exception of indigotin and few others, that usually hides Raman signals when a traditional analytical approach is performed. Even though the reference technique for dye analysis is still high performance liquid chromatography (HPLC), the fast development of SERS for natural dye analysis in recent years enables a comparison between the two techniques. In fact, HPLC offers the possibility of accurately identifying the major and minor dyeing components after their separation; on the other hand, SERS gives molecular information on the dyes using a very low amount of sample, much lower than that needed to perform a chromatographic separation. In addition to HPLC and SERS, other micro-invasive techniques have been employed for the analysis of natural dyestuffs in samples of historical or archaeological relevance.

As a great number of excellent books consider the topic in depth (E. Smith and G. Dent "Modern Raman Spectroscopy. A Practical Approach" (2005) and "Principles of Surface-Enhanced Raman Spectroscopy and related plasmonics effects" by E. Le Ru and P. Etchegoin (2009), this chapter will briefly outline the main principles of Raman scattering and surface-enhanced Raman scattering and will offer an overview of the main features of modern Raman instrumentations. Most of the information reported in the following paragraphs was gained from the above cited books. Moreover, the fourth part of the chapter deeply discusses the literature concerning the application of SERS to the analysis of natural dyes in samples from archaeological or historical relevance, with an insight on substrates and sample pre-treatments, as well as on the most innovative approaches reported. It also gives a thorough review on molecules and dyestuffs that were considered for SERS analysis, and on the results achieved by analyzing natural dyes in the various analytical matrices of many historical samples of different origin. In addition, a general overview on the state-of-the-art for the analysis of natural dyes by HPLC and other micro-invasive techniques is reported in the fifth and sixth parts of this chapter.

4.1 PRINCIPLES OF RAMAN SCATTERING[1]

An incident radiation can be absorbed, transmitted, reflected or diffused/scattered on the hit material. In particular, scattering processes consist on the simultaneous absorption of an incident photon and emission of another photon. Scattering can be elastic or inelastic. Elastic (or Rayleigh) scattering is the most likely process and involves the emission of a photon with the same energy of the incident photon, but with different direction and/or polarization. When nanoparticles are involved in scattering photons, the elastic scattering is called Mie scattering, since it is described by the Mie solutions to Maxwell's equations. On the contrary, when inelastic scattering occurs the scattered photon has a different energy than the incident photon. This is known as Raman effect and was discovered by C. V. Raman in 1928, who won the Nobel Prize for its discovery in 1931.

The Raman effect is correlated with the transition between two different states in the molecules of the target material and involves a small fraction of the photons that interact with the target material (about 1 in a million). In particular, the incident photon excites the molecule to an intermediate quantum state (either stationary or virtual) and the Raman effect is observed only when the molecule decays to a different vibrational state (Figure 4.1). As the number of molecules involved in this process is limited, a significant Raman effect can be observed only by using high-efficiency monochromatic sources and therefore this effect was exploited for analytical purposes only after the invention of lasers and of high-efficiency detectors. In general, the most likely situation is that the molecule decay from the intermediate state to a higher energy level (i.e. an excited vibrational state) and thus the scattered photon has a lower energy than the incident one (Stokes process, Figure 4.1). In fewer cases, the molecule is already in an excited vibrational state due to thermal excitation and the scattered photon has a higher energy than the incident one if the molecule decay to a lower vibrational level (anti-Stokes process, Figure 4.1). At room temperature, the anti-Stokes process is therefore weaker than the Stokes one and thus anti-Stokes signals are generally excluded when Raman spectroscopy is employed for analytical purposes. Nevertheless, anti- Stokes signals have the advantage of on not being affected by fluorescence, as they occur at higher energies in respect to the excitation source. Scattered photons emerge from few micrometers below the surface of the sample and thus Raman scattering gives information only about the composition of the surface of the investigated sample. Finally, when the energy of the intermediate state is that of a real electronic state, the scattering efficiency is increased of a factor from 10^2 to 10^6 and the process is named resonant Raman scattering. The terms *scattering* or *spectroscopy* associated to the Raman effect can be employed depending whether the stress is on the optical effect or on the technique and its applications, respectively.

The Raman spectrum reports the wavelength or energy dependence of the Raman scattered intensity at a given incident wavelength. The energy lost by the photons in the scattering process is called shift, defined as:

$$\Delta E = E_s - E_i$$

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[1] Most of the information reported in the paragraph was gained from the book by E. Smith and G. Dent

where E_s is the energy of the scattered photons and E_i the energy of the incident photons. The Raman shift is hence positive for Stokes and negative for anti-Stokes signals. Conventionally, the Raman intensity is plotted against the Raman shift, expressed in wavenumber ($1/\lambda$, cm^{-1}). In this way, the positions of the signals are independent on the wavelength of the source and the Rayleigh scattering (e.g. the signal generated by the scattered photons with the same energy than the incident photons) is placed at zero position in the spectrum.

Raman scattering takes place according to specific selection rules. Indeed, photons can be Raman scattered only when the vibration changes the polarizability of the electron cloud around the molecule. Differently from IR spectroscopy, where the active vibrations determine a change in the dipole moment, which occurs in asymmetric vibrations, the change in polarizability is characteristic of symmetrical molecules. For these reasons, Raman and IR spectroscopy are often complementary techniques. The number of degrees of freedom for each molecule can be calculated on the basis of the following equation:

$$D = 3N - 6$$

where N is the number of vibrations and the number 6 indicates the three transitions of the molecule in space and the three rotational movements; this number should then be changed into 5 for linear molecules, which present only two rotational movements. In the case of a diatomic molecule as O_2 the degree of freedom is only one, corresponding to a single Raman-active symmetrical stretch, while for carbon dioxide the degrees of freedom are three, although only the symmetrical stretch determines a Raman signal, while the bending and the asymmetric stretch cause little polarizability change and thus are not Raman-active. The shift of these vibrations on the Raman spectrum depends on the energy of the vibration, i.e. the stronger the bond the higher the frequency, and on the atoms involved, i.e. light atoms present vibrations at higher frequencies. For larger molecules, the positions of vibrational modes can be calculated through density functional theory (DFT).

In general, the Raman spectrum can be divided into different regions according to the vibrational modes that generates the signals:

- 4000-2500 cm^{-1} region of the single bonds (X-H)
  - 3500-3000 cm^{-1} region of the hydroxyls (OH)
  - 3100-2800 cm^{-1} region of the aliphatic and aromatic –CH bonds
- 2500-2000 cm^{-1} region of the multiple bonds (-N=C=O)
- 2000-1500 cm^{-1} region of the double bonds (-C=O, -C=N-, -C=C-)
- <1500 cm^{-1} complex patterns of C-C and C-N bonds and O=N=O bonds
- <650 cm^{-1} inorganic groups, metallorganic groups, lattice vibrations.

Further information about the interpretation of Raman spectra can be found in specific books, such as the one by Mayo, Miller and Hannah (2004).

Raman peaks are often narrower and less abundant than infrared bands, but their aspect can be broadened by the sum of several rotational or vibrational modes with similar energy. Although the

position of the Raman peaks is independent from the excitation wavelength, their intensity depends on the fourth power of the frequency of the source, and thus lasers in the UV region determine higher signals than lasers in the NIR region. In reverse, higher energies can activate unwanted electronic transitions which can generate fluorescence and also cause photodecomposition of the sample. Raman spectra generally present a background, mainly attributed to the presence of impurities or residual fluorescence. As a rule of thumbs, the above mentioned Resonant Raman scattering can be achieved by employing lasers of the complementary color of the material under analysis.

Raman spectra present a unique fingerprint of the investigated molecule as they give information about the molecular composition, the bonds, the chemical environment, the phase and the crystalline structure of the samples under analysis. In addition, the technique is not destructive and samples can be investigated in any state: gaseous, liquid, amorphous or crystalline solid. For these reasons, Raman scattering is a valuable technique for the identification of a large number of natural and artificial compounds of interests in the field of cultural heritage investigation. Despite this, this technique fails in the identification of most of the natural dyes, which often present a high background fluorescence when investigated by means of Raman spectroscopy.
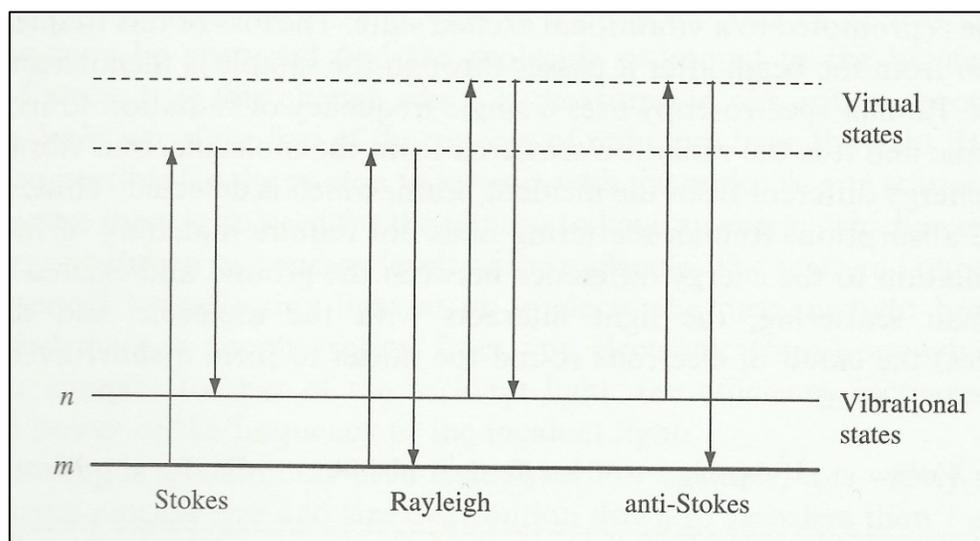

Figure 4.1: Diagram of the Rayleigh and Raman processes. The lower vibrational state is indicated with *m* (E. Smith and Dent 2005).

4.2 INSTRUMENTATION FOR RAMAN SPECTROSCOPY[2]

A suitable source for Raman spectroscopy should be monochromatic and have a high power density, as previously mentioned. These features can be found in lasers, both solid-state and gas lasers. For cultural heritage applications, lasers in the visible or near-infrared region are preferred to avoid the above discussed damaging of the sample often encountered with the more energetic UV lasers. The laser light is directed by a series of lenses and mirrors to the sample and can be attenuated by a series of neutral density filters. Raman spectrometer are often coupled with an optical microscope (micro-Raman spectroscopy), in order to visualize the investigated areas and to focus the laser beam onto the sample. The spot area can indeed be very small, as it depends on the type of laser and on the microscope magnification. Spots of few square micrometers can be obtained with a 100X objective. The incident radiation hence passes through the microscope lenses and hits the sample in the sample holder. The scattered photons are then collected from the microscope objective and proceed by an inverse path to a filter which shields the photons scattered by the Rayleigh process. The inelastically scattered photons are focused by means of a series of lenses through a slit. Dispersive Raman spectrometers present after the slit a polychromator, generally a diffraction grating, which disperses the photons to a detector, usually a CCD. On the contrary, Fourier-Transformed Raman spectrometers include a Michelson interferometer and a detector cooled with liquid nitrogen. The time of analysis varies from few seconds to some minutes. Raman spectrometers can be also provided with external probes, among which fiber optics and extended optical paths are the most diffused. These accessories can be very useful for cultural heritage analyses as totally non-invasive measurements can be carried out on movable objects with a bench instrument. Besides, external probes dissipate part of the incident radiation and also of the scattered photons along the optical path because every connection point along the probe-detector path causes a loss of signal, and thus lower quality Raman signals can be recorded.

Portable Raman instrumentations are nowadays available with a discreet assortment of excitation wavelengths. These instruments are becoming more and more compact compared with the heavy instrumentations of few years ago. Despite this, in the case of cultural heritage materials, the main issue to perform *in situ* measurements stands on the high resolution of this technique, which implies also that the analysis strongly suffers from subtle vibrations. These cannot be avoided in most of the places where immovable artworks or precious movable objects are commonly stored, like churches, museums, libraries, archives. In addition, vibrations are often a major impediment when performing Raman measurements in archaeological sites and restoration areas.

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[2] Most of the information reported in the paragraph was gained from the book by E. Smith and G. Dent "Modern Raman Spectroscopy. A Practical Approach" (2005).

4.3 PRINCIPLES OF SURFACE-ENHANCED RAMAN SCATTERING[3]

The SERS effect was observed for the first time in 1974 by Fleischmann *et al.* (1974), but it was correctly understood only three years later thanks to the independent works by Jeanmarie and Van Duyne (1977) and by Albrecht and Creighton (1977). In particular, Fleischmann *et al.* observed a very strong Raman scattering from pyridine absorbed from an aqueous solution onto an electrochemically roughened silver electrode. They interpreted the unusual intensity of the scatter phenomenon as due to the large surface area of the electrode, but it was evident that such an enhancement could not be attributed merely to that. At present, the SERS effect has not been completely understood, but scientists proved that the amplification of the signal originates mainly from the electromagnetic interaction of light with metals. This interaction creates amplifications of the laser field through excitations known as plasmon resonances. Molecules should be absorbed on a metal surface in order to profit from these excitations. The following paragraphs will briefly discuss the main features of the metal substrate and the chemical species that show enhancement, as well as their mutual interactions; then, the basics of SERS enhancement will be introduced as well as the main features of the SERS signals. Further information can be found in the already cited comprehensive book about SERS by Le Ru and Etchegoin (2009), while a more detailed discussion of the substrates employed for the characterization of dyes in cultural heritage materials will be given in paragraph 4.4.1. Furthermore, the features of the substrates selected for the experimental work of this thesis will be dealt together with the discussion of the obtained results in paragraph 5.1 of the experimental chapter about SERS.

In SERS, the molecular species that should be detected are called *probes*, while the metallic structures which enable the enhancement of inelastic scattering are known as *substrates*. Many SERS substrates have been developed since the discovery of the SERS effect, but some general considerations about the substrates can still be enunciated. Substrates are composed of metallic nano-structures with dimensions of the sub-wavelength range (<100 nm), down to less than 1 nm for rough surfaces. Flat metallic surfaces determine instead lower amplifications. The most employed metals are silver and gold; platinum and copper have been used too, even though they produce lower enhancements. Substrates can also be divided between those which provide local strong enhancement (hot-spots), suitable for single-molecule detection, and those which present a more uniform enhancement and thus allow some reproducibility. The SERS enhancement that can be obtained with a substrate is strongly dependent on the excitation wavelength. In particular, gold and silver substrates present high plasmon resonances in the visible and near-infrared region, from about 400 to 1000 nm, i.e. the operative spectroscopic region of most of the commercial lasers for Raman spectroscopy. SERS substrates can be metallic particles dispersed in a liquid medium (e.g. colloids), planar metallic structures (e.g. nanoparticles supported on a planar substrate) or rough metallic electrodes. These latter present lower enhancement compared to the other two types of substrates and thus they have been no longer employed out of specific electrochemical applications. The SER signal obtained from colloids hails from a three-dimensional volume (e.g. scattering volume) and thus is influenced by the nanoparticles concentration and their dynamics. Colloids can be dried or a attached to a proper support to produce planar metallic

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[3] Most of the information reported in the paragraph was gained from the book "Principles of Surface-Enhanced Raman Spectroscopy and related plasmonics effects" by E. Le Ru and P. Etchegoin (2009).

structures, even though recently also ordered metallic structures have been produced. In the case of two-dimensional planes, the main issue stands on transferring the probes on these planes.

The probes, as previously mentioned, should interact with the substrate, more precisely the molecules have to be absorbed by the substrate. Two main processes are involved: chemisorption and physisorption. Chemisorption implies the formation of a chemical bond between the molecule and the substrate, for example a covalent bond, while when physisorption occurs the molecules are not chemically bonded to the substrate and their interaction is based on physical forces such as electrostatic attractions. For these reasons, the substrates are not generally suitable for any probes and thus the more recent approaches to SERS analysis try to functionalize the substrate for specific probes. Besides, many factors can condition the absorption, among which the concentration of the probe, the competition from other chemical species and the method of preparation of both probes and substrates. In particular, other chemical species may affect the probe absorption by imparting surface charge to the metal substrate, or by competing with the analyte for absorption sites or even by changing the pH of the environment. One must consider that electrostatic interactions may also play a role. This is a major hindrance in the case of the absorption of negatively charged probes to most SERS colloids, typically negatively charged too.

The sensitivity of the technique is strictly related to the enhancement ability of the substrate, which is, in turn, correlated with its absorption efficiency. Despite this, also substrates with high absorption efficiency for a specific probe can be ineffective in enhancing Raman signals. As a matter of fact, the most intense SERS signal can be observed when the maximum number of molecules is absorbed on the surface layer of the substrate, but this condition can be not enough in the case of weak Raman scatterers. This problem can be overcome by employing a substrate with a larger average enhancement, by investigating a larger surface area or by increasing the laser power and thus the scattering volume. Returning to the above mentioned problem of transferring the molecules to a two-dimensional substrate, this can be achieved by following different approaches when the probe is in solution. The substrate can therefore be dipped into the solution, or a drop of the solution can be dried onto the substrate or either deposited by spin-coating. None of these procedures allows to control the number of transferred molecules. In addition, the surface density is not uniform in the first two cases while it is more uniform when employing spin-coating, even though in this latter case the transfer efficiency will be lower.

The magnitude of the enhancement is indicated by the enhancement factor (EF), which is one of the most important values for characterizing the SERS effect. This figure is nevertheless very difficult to be measured or calculated (Le Ru *et al.* 2007), also due to the lack of a unambiguous definitions. EF depends on the number of molecules producing the SER signals. High EFs (up to 10^{12}) characterize the so-called hot-spots, e.g. areas with very strong enhancement. No control can be achieved on creating hot-spots at a given position or on positioning a probe on an hot-spot, therefore high EFs generally are associated to poor reproducibility of the signal intensities. In general, maximum EF of about 10^{7}-10^{8} are sufficient to observe single-molecule signals with probes that exhibit intense scattering. On the other hand, the average EF is the enhancement expected for molecules randomly absorbed and varies between 10^{2}-10^{3} in typical SERS conditions to 10^{7}-10^{8} for very good SERS substrates.

SER signals which arise from a specific chemical species are generally similar to Raman signals in terms of frequency, even though some differences can be observed. As above mentioned, the plasmon resonances are dependent on the wavelength and therefore different regions of the spectrum are differently amplified (e.g. the relative intensities are not comparable). In addition, the intensity of each peak is influenced by the orientation of the molecules and by the formation of complexes with the substrate. The complexes formation due to chemisorption processes can generate the broadening of some peaks, slight shifts and also the quenching of other peaks in the SER spectrum as opposed to the Raman spectrum. On the other hand, inactive modes in the Raman spectrum can be activated and new modes from the complex can be observed in the SER spectrum. Moreover, other modifications may occur, like the change in the polarization properties of the SER spectrum and intensity fluctuations usually not observed in Raman spectra. In addition, a fluctuating background is observed, called *SERS continuum*, whose origins are still unclear. Finally, SERS probes can photo-bleach as in traditional Raman spectroscopy, the electromagnetic enhancements that create the SERS effect being a further cause of photo-bleaching phenomena.

4.4 SURFACE-ENHANCED RAMAN SCATTERING ANALYSIS OF NATURAL DYES

The first application of surface-enhanced Raman scattering to the cultural heritage field dates to more than a decade after its discovery. In fact, it was only in 1987 that Guineau and Guichard identified madder in an historical textile using silver electrodes as the SERS substrate (Guineau and Guichard 1987). After that, Anantha *et al.* (1996) performed a SERS characterization of saffron, widely used as food colorant, by using silver nanoparticles deposited by vacuum evaporation on glass plates. Despite the pioneering work by Guineau and Guichard, more than fifteen years passed after further works specifically aimed at the analysis of natural dyes in samples from the cultural heritage were carried out. In particular, the papers by Shadi *et al.* (2003) and Cañamares *et al.* (2004) reported the use of chemically reduced silver colloids. These were produced according to Lee and Meisel (1982) and to Leopold and Lendl (2003) and were employed to investigate indigo and alizarin with different excitation wavelengths at various pHs and concentration of the dye. In the subsequent years, the interest for the application of SERS to natural dyes rose quickly and ten years later more than fifty works on the subject have been published. Published works proposed the use of different SERS substrates, investigated the behavior of pure dyeing molecules at different conditions, explored various pre-treatments of the samples and reported database of SER spectra of pure molecules and/or natural dyestuffs. Furthermore, the results obtained on historical objects were also described. In the last years, most of the efforts in the field were aimed at a dramatic reduction of the quantity of sample required to perform analysis by SERS. In addition to experimental data, several authors reported Density Functional Theory calculations of SER peaks for the investigated molecules. In some cases, the samples were also subsequently investigated by other techniques, such as FT-Raman spectroscopy, FTIR spectroscopy, HPLC coupled with different detectors and also GC-MS.

4.4.1 SERS SUBSTRATES FOR THE ANALYSIS OF NATURAL DYES

Chemically reduced silver colloids are presently the most employed SERS substrate for the analysis of natural dyes. In particular, the colloid produced according to Lee and Meisel, which is obtained by reduction of silver nitrate with sodium citrate (Lee and Meisel 1982), has been widely used in the field of cultural heritage analysis, mainly because of its ease of preparation. Several authors suggested modifications to the original procedure for the synthesis of the colloid, or proposed the addition of aggregating agents, in order to get a better stability of the colloid and to promote a suitable coating of the sample with the aggregated silver nanoparticles. In particular, several aggregating agents were proposed, such as potassium nitrate (Cañamares, Garcia-Ramos, and Sanchez-Cortes 2006; Cañamares *et al.* 2006; Jurasekova *et al.* 2006; Leona, Stenger, and Ferloni 2006), sodium chloride (Leona and Lombardi 2007), sodium perchlorate (Bruni, Guglielmi, and Pozzi 2011a) and sodium sulfate (Heo *et al.* 2011). In addition, Leona *et al.* (2006) and Brosseau *et al.* (Brosseau, Gambardella, *et al.* 2009) achieved the aggregation of the silver nanoparticles by concentrating the Lee and Meisel colloid through centrifugation and subsequent removal of the supernatant solution. In the first case, the concentration was achieved with only one centrifugation cycle, while Brosseau *et al.* performed ten subsequent cycles in order to get a very concentrated colloid, referred to as silver colloidal paste. Another procedure that was employed to produce chemically reduced silver colloids that have been employed as SERS substrate for the analysis of natural dyes, is the above-mentioned Leopold and Lendl procedure (2003). According to Leopold and Lendl, the silver nanoparticles were obtained by reducing silver nitrate with hydroxylamine hydrochloride in an alkaline environment. The colloid was used aggregated with potassium nitrate (Cañamares *et al.* 2004). In addition, Van Elslande *et al.* (2008) and Centeno and Shamir (2008) employed the procedure suggested by Creighton *et al.* (1979), which employs sodium borohydride to reduce silver nitrate, while Leona *et al.* (2006) and Chen *et al.* (Chen, Leona, *et al.* 2006) employed Tollens mirrors as SERS substrates. Moreover, Leona (2009) presented a microwave assisted procedure for the synthesis of silver nanoparticles, which employed a solution of silver sulfate (precipitated upon addition of sulphuric acid to a solution of silver nitrate) reduced by sodium citrate and glucose. In this latter case, the partial removal of the solution though centrifugation and the addition of the same volume of water to replace the supernatant was performed in order to reduce the levels of citrate, that would compete with the analytes for adsorption sites on the silver nanoparticles.

Even though chemically reduced silver colloids represent nowadays the most used substrates for dye analysis in the field of cultural heritage, some authors used physical methods for the production of silver nanoparticles. In particular, these were obtained with silver electrodes (Teslova *et al.* 2007; Wang *et al.* 2007; Baran *et al.* 2009), by *in situ* laser photoreduction of silver nitrate (Cañamares *et al.* 2007; Jurasekova *et al.* 2008; Jurasekova *et al.* 2010) and by pulsed laser ablation (Fazio *et al.* 2013; Fazio, Trusso, and Ponterio 2013). Recently, a UV-photoreduced substrate stabilized with hydroxypropyl cellulose was also proposed (Retko, Ropret, and Cerc Korosec 2013). In addition, vacuum evaporation was employed to deposit thin layers of silver directly onto the sample (Chen, Vo-Dinh, *et al.* 2006; Whitney, Van Duyne, and Casadio 2006), onto glass microscope slides (Leona, Stenger, and Ferloni 2006) or onto expressly produced surfaces, as in the cases of Al_2O_3 substrates (Chen, Leona, *et al.* 2006) and of silver film over nanospheres

(AgFONs) (Whitney, Casadio, and Van Duyne 2007; Brosseau, Gambardella, *et al.* 2009; Greeneltch *et al.* 2012).

4.4.2 SAMPLE PREPARATION FOR SERS ANALYSIS

The pre-treatment prior to SERS analysis strongly depends on the material under investigation. As a matter of fact, most of the published works on dyes analysis via SERS deal with pure molecules, which are usually dissolved in water or methanol before being mixed with the colloid. When a solid substrate was employed, a solution of the dye was incubated on the substrate for a fixed time (some minutes up to several hours). In addition, also SER measurements on raw materials (e.g. natural dyestuffs) were generally performed on solutions obtained after having extracted the dyeing materials (plants or insects) with hot water or with the help of other solvents and acid solutions (Bruni, Guglielmi, and Pozzi 2011).

As far as dyed fibers are concerned, mock-ups or fragments sampled from historical textiles were treated according to various extraction procedures that were adapted from those employed to extract the dyes prior to HPLC separations. Most of the works carried out on extracts from mock-ups of dyed fibers focused on red dyestuffs, with the exception of the work by Leona *et al.* (2006), in which also a weld-dyed silk was considered. In particular, these latter authors extracted the samples with dimethylformamide and water (1:1 v/v) with 1% EDTA, according to Tiedemann and Yang (1995) and also employed the treatment with 3N HCl and methanol (1:1, v/v) proposed by Wouters (1985). This latter procedure was also employed by Chen *et al.* (2006). In addition, Whitney *et al.* (2006) employed a solution of HCl:MeOH:H_2O (2:1:1, v/v/v) while Brosseau *et al.* (Brosseau, Gambardella *et al.* 2009) tested several procedures and achieved the best results with methanol and formic acid (85:15, v/v). In addition, Mayhew *et al.* (2013) tested several pretreatments for the analysis of yellow dyestuffs and lake pigments and they concluded that a solution of HCl and methanol (1:3, v/v) yielded the best results among the tested procedures.

As for historical samples, the above-mentioned procedure by Wouters was employed to extract red dyestuffs from a red Lybian wool (Bruni, Guglielmi, and Pozzi 2010) and from red fibers of Kaitag textiles (Pozzi, Poldi, *et al.* 2012). The same authors of this latter work employed treated also yellow, green, brown and black fibers with hydrofluoric acid (Sanyova 2008), that was previously employed also on two wool fragments from Lybia (Bruni *et al.* 2011), while the blues were extracted with a 1:1 pyridine/water solution. In addition, a purple dye was also extracted from a bone with dimethylformamide (Bruni, Guglielmi, and Pozzi 2010).

Extraction of the dyes from the matrix aims to break the bonds between the dyes and the mordant ions ion order to release the dyeing molecules in a solution. The silver nanoparticles can then interact with the dyes thus enabling the enhancement of the signals. Conversely, approaches that perform SERS directly on the sample (i.e.:*in situ*) without hydrolysis nor extraction of the dyes were explored by several authors. In particular, silver colloidal pastes proposed by Brosseau *et al.* (Brosseau, Gambardella *et al.* 2009) were aimed specifically at *in situ* extractionless SERS analysis and they proved to be effective on several historical matrices, such as textile fibers (Brosseau, Gambardella, *et al.* 2009; Idone *et al.* 2013; Wustholz *et al.* 2009), pastels (Brosseau, Rayner, *et al.* 2009) and watercolors (Brosseau, Casadio, and Van Duyne 2011). On the other hand, Jurasekova *et*

al. (2008; 2010) employed laser photoreduction to perform *in situ* SERS on reference textile fibers and on a red Coptic textile, while Chen *et al.* (2006) directly coated mock-ups of painting fragments with a vacuum evaporator. In addition, Casanova-Gonzales *et al.* (2012) performed SERS measurements on mock-ups of wool dyed with Mexican dyestuffs employing the Lee and Meisel colloid aggregated with potassium nitrate. *In situ* SERS was also performed on a purple pigment from Santorini (1650 BC) and on a pink lake from the Greco-Roman period using the silver colloid reduced with sodium borohydride (Van Elslande, Lecomte, and Le Ho 2008) and on cross sections from two 18th century oil paintings with partially concentrated Lee and Meisel colloid (Oakley *et al.* 2011). The interest for SERS as a tool for analyze lake pigments embedded in stratigraphic cross sections of painting layers is further demonstrated by the application of a UV-photoreduced colloid to the analysis of a cross section obtained from an organic glaze from a mock-up panel (Retko, Ropret, and Cerc Korosec 2013).

A further approach considers the pre-treatment of the sample through an extractionless hydrolysis employing HF vapors. The procedure was proposed by Leona *et al.* (2006) and has been widely used to pre-treat samples prior to the application of the Lee and Meisel colloid employed with aggregating agents or the glucose-citrate reduced colloid. The procedure was tested on reference samples (Teslova *et al.* 2007; Corredor *et al.* 2009; Cañamares *et al.* 2010) and employed for the investigation of historical textiles (Leona, Stenger, and Ferloni 2006; Leona and Lombardi 2007), lake pigments and glazes from paintings and statues (Leona 2009; Pozzi, Lombardi, *et al.* 2012), dyed leather (Leona 2009b), painted cloths and musical instruments (Pozzi, Lombardi, *et al.* 2012), as well as handbooks of colors (Pozzi, Lombardi, and Leona 2013). A comparison of the results that can be obtained with and without the HF hydrolysis pre-treatment was performed by Cañamares *et al.* (2010) for Cape jasmine (*Gardenia augusta* L.) and by Pozzi *et al.* (2012) for several mock-ups of dyed textiles and historical samples of different materials. These works evidenced that the hydrolysis with HF generally improves the quality of the SER spectra, even though silk fibers were hydrolyzed by hydrofluoric acid. Also carminic acid generally shows higher signal-to-noise ratio without the HF treatment. As a conclusion, Pozzi *et al.* proposed a two-step approach: SER spectra from a same sample were obtained sequentially without and with hydrolysis by washing out the silver nanoparticles after the first analytical step.

Besides some innovative approaches, that are discussed in the next section, all the reported procedures that use chemically reduced silver colloids imply the deposition of a drop of colloid onto the sample (after pre-treatment, if that be the case). Some procedures suggest to focus the laser beam onto the drop in order to gain signals when the silver nanoparticles are still dispersed in their aqueous medium. Other procedures suggest instead to perform the analysis on the dried colloid. In this latter case, the silver nanoparticles form a more or less homogeneous coating on the sample, that enables the enhancement of the signals.

4.4.3 INNOVATIVE SERS APPROACHES

In the very last few years, some innovative approaches have been proposed to perform SERS in order to analyze dyes in samples of historical and archaeological relevance. Most of these very new approaches have been presented at the 7th International Congress on the Application of Raman in Art and Archaeology (RAA), held in September 2013 in Ljubljana (Slovenia). In particular, a first paper that reports the application of NIR-SERS to an early-synthetic dye, eosin γ, was recently published (Greeneltch *et al.* 2012). In addition, FT-SERS has been proposed for the analysis of berberine (Leona and Lombardi 2007) and textile dyes (Zaffino, Bruni, and Guglielmi 2013). Another interesting possibility is the coupling of SERS with thin layer chromatography although this procedure has been presently explored only on mock-ups (Brosseau, Gambardella, *et al.* 2009; Pozzi, Shibayama, *et al.* 2013; Cañamares, Reagan, and Leona 2013). Coupling SERS to a separative pre-treatment might allow the unhambiguous identification of major and minor compounds. Moreover, the application of chemometrics to SER spectra was also examined, in order both to develop robust procedures for library search (Pozzi, Porcinai, *et al.* 2013) and to obtain more information about the techniques employed for the production of the artwork (Castro *et al.* 2013).

Efforts were accomplished in order to employ SERS within non-invasive analytical procedures. To this aim, active hydrogels have been proposed in order to selectively remove some molecules of dyes from the artwork, performing a totally unperceivable sampling of the analytes. In particular, a gel was synthesized with an industrial co-polymer and applied to a red fiber from a 15th century Netherlandish tapestry and to a 1892 Japanese woodblock print. The gel was then coated with microwave-assisted or Lee and Meisel colloids and SER signals of alizarin and crystal violet could be collected (Leona *et al.* 2011). Moreover, the Lee and Meisel colloid was directly mixed with agar-agar to obtain a gel that was applied to cotton fibers dyed with alizarin, purpurin or carminic acid and to a pre-Columbian textile, in which madder was identified (Lofrumento *et al.* 2013). Also the use of a cellulose film embedding a glucose-reduced colloid was proposed, even though in this latter case only red lakes from a mock-up panel were investigated (Doherty *et al.* 2011).

In addition, Londero *et al.* coupled laser ablation to the microscope of the Raman spectrometer and they ablate few picograms of the analytes from solid samples and deposited them directly on a SERS active substrate. This procedure allowed also to perform reflectance and fluorescence measurements (Londero, Lombardi, and Leona 2013a) and it permitted to record SER spectra from samples ablated from a dyed Egyptian leather (Londero, Lombardi, and Leona 2013b) and from an alizarin lake detached from a mock-up painting layer and mounted in cross section (Londero, Lombardi, and Leona 2013c).

4.4.4 INVESTIGATED DYES AND DYESTUFFS

Alizarin shows a very high SER efficiency, therefore alizarin and alizarin-containing (natural or synthetic) dyestuffs and lake pigments were widely exploited to compare the efficiency of different substrates and conditions of analysis, as well as to set up new analytical procedures (Baran *et al.* 2009; Cañamares *et al.* 2004; Chen, Vo-Dinh, *et al.* 2006; Chen, Vo-Dinh, *et al.* 2006; Doherty *et al.* 2011; Fazio *et al.* 2013; Lofrumento *et al.* 2013; Londero, Lombardi, and Leona 2013c; Shadi *et al.* 2004; Whitney, Van Duyne, and Casadio 2006; Whitney, Casadio, and Van

Duyne 2007). Also carmine lake was employed to test new procedures (Doherty *et al.* 2011; Fazio *et al.* 2013; Whitney, Casadio, and Van Duyne 2007). Moreover, the spectral features of alizarin, the variability of its SER spectra with different conditions and the possibility of distinguishing alizarin from purpurin were explored in several works (Baran *et al.* 2009; Brosseau, Gambardella, *et al.* 2009; Cañamares *et al.* 2004; Jurasekova *et al.* 2010; Shadi *et al.* 2004; Van Elslande, Lecomte, and Le Ho 2008).

In-depth investigation were also carried out on other pure dyeing molecules such as: apigenin (Jurasekova *et al.* 2006; Jurasekova *et al.* 2008; Corredor *et al.* 2009), berberine (Leona and Lombardi 2007), carminic acid (Brosseau, Gambardella, *et al.* 2009; Brosseau, Casadio, and Van Duyne 2011; Cañamares *et al.* 2006; Lofrumento *et al.* 2013; Whitney, Casadio, and Van Duyne 2007), crysin (Corredor *et al.* 2009), curcumin (Brosseau, Rayner, *et al.* 2009; Cañamares, Garcia-Ramos, and Sanchez-Cortes 2006), ellagic acid (Bruni *et al.* 2011), flavone and its hydroxy derivatives (Teslova *et al.* 2007), 3-hydroxyflavone (Wang *et al.* 2007), kaempferol (Jurasekova *et al.* 2006), laccaic acid (Cañamares and Leona 2007), lawsone (Heo *et al.* 2011), luteolin (Jurasekova *et al.* 2006; Corredor *et al.* 2009), proto-berberine alkaloids (Cañamares, Lombardi, and Leona 2008), quercetin (Jurasekova *et al.* 2006). Moreover, Leona *et al.* (2006) published a database of SER spectra of seventeen pure dyeing molecules obtained with the Lee and Meisel colloid aggregated with potassium nitrate.

In addition, SER spectra were obtained also on several natural dyestuffs: *Allium cepa* (Jurasekova *et al.* 2010), *Baccharis* specie (Jurasekova *et al.* 2010), *Bixa orellana* (Casanova-González *et al.* 2012), brazilwood (Casanova-González *et al.* 2012; Fazio *et al.* 2013; Whitney, Van Duyne, and Casadio 2006), buckthorn berries (Mayhew *et al.* 2013), Cape jasmine (Brosseau, Gambardella, *et al.* 2009; Cañamares *et al.* 2010), cochineal (Casanova-González *et al.* 2012; Jurasekova *et al.* 2010; Whitney, Van Duyne, and Casadio 2006), dragon's blood (Fazio, Trusso, and Ponterio 2013), dyers' broom (Bruni *et al.* 2011; Jurasekova *et al.* 2010), kermes (Leona 2009; Whitney, Van Duyne, and Casadio 2006) lac dye (Chen, Vo-Dinh, *et al.* 2006; Leona 2009; Whitney, Van Duyne, and Casadio 2006; Whitney, Casadio, and Van Duyne 2007), logwood (Casanova-González *et al.* 2012), madder (Chen, Leona, *et al.* 2006; Brosseau, Gambardella, *et al.* 2009; Bruni, Guglielmi, and Pozzi 2010; Doherty *et al.* 2011; Leona 2009; Whitney, Van Duyne, and Casadio 2006), old fustic (Jurasekova *et al.* 2010), pomegranate (Bruni *et al.* 2011), saffron (Anantha, Stokes, and Vo-Dinh 1996), sepia melanin (Centeno and Shamir 2008), stil de grain (Mayhew *et al.* 2013), sumac (Bruni *et al.* 2011), Syrian rue (Pozzi, Shibayama, *et al.* 2013), turmeric (Mayhew *et al.* 2013),Tyrian purple (Bruni, Guglielmi, and Pozzi 2010; Van Elslande, Lecomte, and Le Ho 2008), weld (Bruni *et al.* 2011; Jurasekova *et al.* 2008; Mayhew *et al.* 2013). Furthermore, a database of the SER spectra of twenty-five dyestuffs and nine pure molecules was reported by Bruni *et al.* (Bruni, Guglielmi, and Pozzi 2011) employing the Lee and Meisel colloid aggregated with sodium perchlorate.

As previously underlined, in some cases mock-ups of dyed textiles, lake pigments and varnishes containing the above-listed dyes were prepared and then analyzed through SERS. It should also be noted that, besides the above mentioned papers about the identification of purpurin and alizarin, very few works were carried out on the SERS analysis of mixtures. In particular, Jurasekova *et al.* (2010) investigated a linen fiber dyed with cochineal and indigo employing laser photoreduction to coat the sample with silver nanoparticles; as a result of this SERS analysis of a textile mock-up, only signals attributable to carminic acid were recorded, while no peaks belonging to indigotin were

found. Moreover, Whitney *et al.* (2007) employed AgFONs in order to determine whether mixtures of red dyes in solution (e.g. lac dye and alizarin, purpurin and alizarin, purpurin and lac dye) were identifiable with this SERS substrate, obtaining conclusive results.

4.5 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS OF NATURAL DYES

High Performance Liquid Chromatography is still the reference technique for the analysis of natural dyes in the field of cultural heritage, as it allows a characterization of most, if not even all, of the molecules that contribute to the final color of the sample. As a consequence, it allows to discriminate the specific species that have been employed to color the artwork. The main issue of the technique stands principally on the amount of sample required to perform the analysis, i.e. few milligrams. The minute mass of sample required allows to consider HPLC investigation as micro-invasive. Nevertheless, even few milligrams of thread may be a large sample when artworks such as very minute needle works are considered. In addition, the coloring matters have to be extracted from the sample, whether a textile or a lake, and dissolved in an appropriate solvent to be injected in the instrument. Hence, the extraction step is critical to get good HPLC results and it is strongly dependent on the chemical class of the compound of interest (Degano *et al.* 2009). In 2008, Rosenberg reviewed the state-of-the-art of liquid chromatography applied to the analysis of natural dyestuffs, with particular concern to the coupling of this separative technique with mass spectrometry. In addition, Degano *et al.* (2009) examined more in general the analytical methods employed at the time for the characterization of organic dyes in artworks. Since a detailed review of the literature about HPLC analyses of natural dyes in art and archaeology is far from the aim of this dissertation, the following paragraphs are intended to give only a general overview of the principal features of the technique, referring to the cited works for a further deepening. The application of HPLC to specific classes of molecules or historic materials is therefore not discussed here and it will be dealt with whenever it will be useful for the subjects of the following chapters of the thesis.

4.5.1 HPLC: EXTRACTION PROCEDURES

In the last thirty years, several extraction procedures have been proposed and developed to treat historical and archaeological samples in order to extract dyes to be analyzed by the detectors coupled to an HPLC system. In general, the extraction step was set up with different solvents heated at variable temperatures for a certain time. This step was followed by the evaporation of the solvent and the reconstitution of the extract with a solvent that must be compatible with stationary and mobile phases of the separation system, and suitable to dissolve the dyes (Rosenberg 2008).

As for the extracting solutions, Wouters (1985) employed HCl 3N for the extraction of anthraquinones from textiles or a solution of HCl:MeOH:H_2O 2:1:1 (v/v/v) (Wouters and Verhecken 1989). Orska-Gawryś *et al.* (2003) replaced methanol with ethanol, which has a higher boiling point and thus allows a better extraction of the dyes. These methods are known as "harsh methods" as they hydrolize the glycosydes from the dye molecules and may also degrade several among the most labile chromophores.

In order to characterize also the glycosylated species, which often allow the identification of the dyestuffs, several mild extraction procedures were proposed. Specifically, the use of HF for the extraction of anthraquinone mordant dyes was suggested (Sanyova and Reisse 2006). Moreover, Zhang and Laursen (2005) tested the use of formic acid/methanol 5:95 (v/v) and of 0.001 M H_2EDTA:acetonitrile:methanol 2:10:88 (v/v/v) and they concluded that the first procedure was preferable for wool, while the latter gave better results for silk samples (Rosenberg 2008). Marques *et al.* (2009) employed 0.2 M oxalic acid:methanol:acetone:water (0.1:3:3:4, v/v/v/v). In addition Vanden Berghe *et al.* (2009) proposed a second extraction after the procedure by Wouters employing ethyl acetate.

Hydrophobic indigoids were extracted with DMF or DMSO (Hofenk de Graaff 2004), while brominated indigoids of Tyrian purple were effectively treated with pyridine (Wouters 1991). Differently, Puchalska *et al.* (2004) and Pawlak *et al.* (2006) combined DMSO with HCl. The partial dissolution of red dyewoods was proposed by Surowiec *et al.* (2004) using ethyl acetate, chloroform or carbon tethrachloride.

Comparative works of different extraction procedures were carried out by Valianou *et al.* (2009), Manhita et al. (2011) and Wouters *et al.* (2011).

4.5.2 HPLC: *INSTRUMENTATION AND CONDITIONS OF ANALYSIS*

Reverse-phase high performance liquid chromatography (RP-HPLC) is the most used technique for the identification of natural dyes (Hofenk de Graaff 2004; Wouters and Verhecken 1989). Generally, a C_{18} or a C_8 column is used, even though some authors preferred other types of columns. The most common solvents employed in the mobile phase are water, methanol, acetonitrile (Rosenberg 2008). Gradient elutions with two or more solvents were proposed by Valianou *et al.* (2009), Surowiec *et al.* (2006) and by Peggie *et al.* (2008). Solvents programs are usually employed with the addition of acids to suppress the ionization of the analytes; the most used acids are TFA and formic acid (Halpine 1996). HPLC is usually coupled to a UV-Vis detector (Wouters 1985), working in the 200-600 nm range, while recently also diode array (DAD) and mass spectrometric (MS) detectors have been introduced (Degano *et al.* 2009). In addition, fluorimetric detectors were employed (van Bommel *et al.* 2007). As regards the coupling of HPLC with MS, soft ionization methods are chosen, such as atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) (Rosenberg 2008; Degano *et al.* 2009). These two ionization methods are complementary, as APCI works better for species having medium molecular weights and moderate to medium polarities, while ESI is specific for medium to high polarity molecules up to ionic species. As most natural dyestuffs are polar, ESI is usually the favored method (Rosenberg 2008), while for less polar indigoid compounds APCI is preferred (Degano *et al.* 2009). In addition, the use of multidimensional mass spectrometers allows to isolate one particular ion from the first MS dimension and to induce its fragmentation to record the fragment ion spectrum. Another approach to increase information content is the use of high resolution MS, which permit to reconstruct the molecular formula of the investigated compounds with a higher degree of accuracy (Rosenberg 2008).

4.6 OTHER MICRO-INVASIVE TECHNIQUES FOR THE ANALYSIS OF NATURAL DYES

Other micro-invasive techniques were employed to characterize natural dyestuffs, both on reference materials and on artworks, even though their application in the field of dye analyses in objects from the cultural heritage environment is not as diffused as HPLC and, more recently, SERS. Some of these technique are intrinsically invasive techniques (e.g. chromatographic techniques), which cannot be employed without the detachment of a sample and often are also destructive techniques (e.g. the sample is destructed during the analysis. In addition, potentially non-invasive techniques, as Raman spectroscopy, were employed to investigate small samples with a micro-invasive approach.

The use of traditional micro-Raman spectroscopy in art and archaeology was reviewed by Vandenabeele *et al.* (2007), who highlighted the sporadic use of this technique for the identification of dyestuffs, especially with visible excitation wavelengths, mainly due to the fluorescence issues that SERS overcome. Besides this, the only dye that does not exhibit fluorescence, indigo, was investigated by means of Raman spectroscopy to characterize its main constituents (Karapanayiotis *et al.* 2004). In addition, Bell *et al.* (1997) and Burgio and Clark (2001) in their libraries reported the Raman spectra obtained with visible excitation wavelengths of powders of some dyes: purpurin, berberine, gamboge, Indian yellow, saffron, carminic acid and indigo. On the contrary, Near-Infrared Raman spectroscopy, is more effective in reducing the fluorescence of natural dyes and obtaining an analytical response. In particular, two libraries of Raman spectra of natural dyes with 1064 nm excitation wavelength have been published, one employing a Fourier-Transformed equipment (Burgio and Clark 2001) and the other with a dispersive instrument (Schmidt and Trentelman 2009). In addition, FT-Raman was employed to characterize brazilin and brazilein (de Oliveira *et al.* 2002) in the different tree species used to obtain the dyestuff generally called brazilwood (Edwards, de Oliveira, and Nesbitt 2003). Finally, FT-Raman was exploited as a supplementary technique in some SERS investigations (Baran *et al.* 2009; Bruni, Guglielmi, and Pozzi 2011; Cañamares *et al.* 2006; Cañamares, Lombardi, and Leona 2008; Leona and Lombardi 2007). Another promising technique that allows to eliminate the fluorescence is subtracted-shifted Raman spectroscopy (SSRS), which was firstly proposed by Bell *et al.* (2000) for the identification of the dyeing molecules of *Phellodendron amurense* in the first ever printed document and it was later used for the investigation of Tyrian purple (Osticioli, Zoppi, and Castellucci) and red natural dyes and lakes (Rosi *et al.* 2010), both on reference samples and historical materials. The purple dye orchil was analyzed too with SSRS and it was successfully identified on a 9th century manuscript and a 16th century map (Rosi *et al.* 2013). Moreover, also shifted excitation Raman difference spectroscopy (SERDS) proved to be effective for the analysis of dyes (Osticioli, Zoppi, and Castellucci).

In some cases, Fourier-Transformed Infrared Spectroscopy (FTIR) was employed as a complement of other techniques for the characterization of dyestuffs. The technique is considered as micro-invasive as samples prepared in KBr pellets or diamond cells were investigated. In particular, it supported the above-cited FT-Raman characterization of brazilwood and its principal dye molecules (de Oliveira *et al.* 2002), as well as several investigations carried out by means of SERS (Anantha, Stokes, and Vo-Dinh 1996; Bruni, Guglielmi, and Pozzi 2011; Centeno and Shamir 2008;

Leona and Lombardi 2007). In fewer cases, it was employed for the identification of dyes working directly on the fiber (Gillard *et al.* 1994; Martoglio *et al.* 1990). In particular, Martoglio *et al.* identified the dyestuffs employed for six Peruvian textiles of the Paracas culture, while Gillard *et al.* built a library of FTIR spectra analyzing different natural fibers dyed with twelve dyes and five mordants and they applied various algorithms for the automatic identification of unknown samples, succeeding in the identification of indigo in an historical sample. In addition, FTIR was used to identify the use of saffron in Persian manuscripts (Barkeshli 1999; Barkeshli and Ataie 2002).

Dye molecules and natural dyestuffs were also investigated by means of voltammetry by Doménech-Carbó *et al.* (2010). Moreover, nuclear magnetic resonance and mass spectrometry were employed to characterize the compounds found in *Nucella lapillus* (Clark and Cooksey 1997), a Tyrian purple-containing mollusk, and the Al(III)/brazilwood complex (Wongsooksin *et al.* 2008).

As for intrinsically invasive techniques, gas chromatography coupled to mass spectrometry (GC-MS) was employed to study the ageing of dyes and textiles (Ahn and Obendorf 2004; Ahn and Obendorf 2007; Colombini *et al.* 2007). Also thin layer chromatography was employed in some works (Wallert and Boytner 1996; Wongsooksin *et al.* 2008).

Additional invasive analyses on dyed fibers were carried out in order to identify the mordants. In particular, induced coupled plasma with optical emission spectrometry (ICP-OES), flame atomic absorption spectrometry (FAAS) and scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) proved to be the most useful techniques for the characterization of mordants (Manhita, Ferreira, Vargas, *et al.* 2011).

CHAPTER 5
SURFACE-ENHANCED RAMAN SCATTERING (SERS)
FOR THE ANALYSIS OF NATURAL DYES

The information that can be gained by means of FORS for the identification of natural dyes (chapter 3) may need to be complemented by more selective micro-invasive approach, as specific molecular information cannot be achieved by non-invasive techniques. The choice of the micro-invasive technique on which this research is focused was indicated by the need of satisfying some requirements peculiar of the conservative mission of Laboratorio Analisi Scientifiche (LAS), which hosted the present research work. Firstly, the selected technique should enable the analysis on micro-samples, as sampling is always a destructive operation and thus ought to be reduced at the least. Secondly, the instrumentation employed for the research should be available among those of the laboratory, as this would enable the identification of natural dyes as part of the routine work of LAS and, in addition, cut down the costs of the analysis. Among the analytical techniques that can be considered for dyes analysis, ,SERS answered all these conditions, as a very limited amount of sample is needed, as described in chapter 4, and a Raman spectrometer has been recently purchased by the laboratory. In addition, this technique is presently among the most innovative approaches in the field of analytical chemistry for cultural heritage and thus still needs to be developed and optimized for a number of different applications.

As thoroughly discussed in paragraph 4.4, SERS has been applied to the investigation of natural dyes according to various approaches. As far as the present research work is concerned, in a preliminary stage some among the proposed syntheses of silver substrates and sample pre-treatments were tested on reference dyes and reference samples of dyed fibers in order to seize the preparation of the substrate and to verify the applicability of the different approaches. Afterwards, the research focused on the application of on-the-fiber extractionless SERS, which involved also the Art Institute of Chicago (Illinois, US) and the group of Prof. Richard P. Van Duyne at Northwestern University (Evanston, Illinois, US). This work is of particular interest for LAS and also for the Art Institute of Chicago as it would allow to strongly limit the amount of sample required for the analysis with respect to SERS on extracts. Three different substrates, characterized by means of UV-Vis spectroscopy and scanning electron microscopy, were concentrated to obtain the so-called silver colloidal pastes and tested on reference samples of textiles mordanted with different mordant and dyed with natural dyestuffs.

The use of silver colloidal pastes was also tested for the analysis of purple parchments, already discussed in paragraph 3.2. The application of SERS for the analysis of colored parchments has never been tackled before. In this case, SERS analyses of reference samples were performed employing different approaches, using both extraction and extractionless procedures, with the aim of achieving an accurate identification of the dyestuff by treating the least possible amount of sample.

Furthermore, the application of silver colloidal pastes for the identification of painting lakes was tested directly on stratigraphic cross sections obtained by mounting in epoxy resin fragments detached from historical artworks (painted wooden statues and mural paintings).

This chapter describes and discusses the whole experimental work about SERS. In particular, the first section deals with the spectroscopic and morphological characterization of the SERS substrates selected for on-the-fiber SERS and with the analytical work on textile reference samples. An in-depth presentation encompasses the systematic work for on-the-fiber extractionless SERS. The second section reports the various tests for SERS analysis of purple dyestuffs on parchment. On the other hand, the experimental work for the identification of lakes in cross sections is reported in paragraph 6.9.

5.1 EVALUATION AND OPTIMIZATION OF SERS PROCEDURES FOR ON-THE-FIBER EXTRACTIONLESS ANALYSIS OF TEXTILE DYES

The substrates selected for on-the-fiber extractionless SERS were the two most popular chemically reduced silver colloids employed for SERS analysis in the field of cultural heritage, that are synthesized following the procedures reported by Lee and Meisel (1982) and by Leopold and Lendl (2003). Both the colloids were concentrated through centrifugation in order to obtain silver colloidal pastes, as proposed by Brosseau *et al.* (Brosseau, Gambardella *et al.* 2009). The addition of sodium perchlorate as aggregating agent to the silver colloidal paste obtained from the Lee and Meisel colloid was also tested, modifying the procedure suggested by Bruni *et al.* (Bruni, Guglielmi, and Pozzi 2011). The merit of the pastes for on-the-fiber SERS was tested by coating and analyzing reference samples of dyed textiles. In order to characterize the substrates, the localized surface plasmon resonance of the colloids and the pastes was measured with different instrumentation in transmittance and reflectance mode. In addition, a morphological characterization of the colloids, of the pastes and of the coating on the investigated fibers was undertaken by means of high-resolution scanning electron microscopy at Northwestern University. The reference samples selected to test the efficacy of the different pastes for on-the-fiber SERS were four natural fibers (e.g. wool, silk, cotton and flax) mordanted with different mordants and dyed with cochineal or brazilwood. The systematic SERS investigation of these samples allowed also to ascertain the variability of the SERS signals for these two dyestuffs. Part of the results discussed in this chapter has been reported in a recent paper, published in Analyst (Idone *et al.* 2013).

This section illustrates the procedures employed to synthesize and concentrate the colloids, their characterization and the results obtained for on-the-fiber analysis of the reference samples.

5.1.1 MATERIALS AND METHODS

Hydrochloric acid, nitric acid, methanol, silver nitrate, sodium perchlorate, hydroxylamine hydrochloride, sodium citrate and sodium hydroxide were purchased from Carlo Erba reagents (Arese, Italy). Analytical grade carminic acid was obtained from Sigma Aldrich (St. Louis, USA). Brazilwood powder extract and chopped brazilwood (from *Caesalpinia echinata* Lamark) were provided by Kremer Pigments Inc. (New, York, USA). Ultra High Quality (UHQ) water was obtained by a Millipore (Darmstadt, Germany) Direct-q 3 system.

5.1.1.1 SUBSTRATES

All the glassware employed for the synthesis of the colloids was washed with *aqua regia* (HCl:HNO_3, 3:1, v/v) and then rinsed tenfold with ultrapure water (Chen *et al.* 2006). The synthesis procedures should be performed in the dark and the colloids should be stored in shielded bottles at room temperature. Also silver colloidal pastes were conserved in the dark at room temperature.

The colloid of Lee and Meisel was synthesized following the standard procedure of the Van Duyne's group, which encompasses some modifications to the original procedure proposed by the authors (Lee and Meisel 1982). In particular, 0.09 mg of silver nitrate were added under magnetic stirring (600 rpm) to 500 mL of heated UHQ water. When the solution was vigorously boiling, 10 mL of a 1 % solution of sodium citrate (0.1 g in 10 mL of UHQ water) were added and the solution was allowed to boil for exactly thirty minutes. The solution was then let to cool at room temperature. The beaker should be kept covered during the whole procedure in order to avoid evaporation. The degassing phase with nitrogen while heating, originally proposed in Van Duyne's group, was eliminated as it proved not to influence the colloid characteristics (Christa Brosseau, personal communication).

The Leopold and Lendl colloid was prepared following the original procedure (Leopold and Lendl 2003), selecting the reagents ratio in order to get the most monodispersed distribution, as investigated by the authors. In particular, 10 mL of a 10^{-2} M solution of silver nitrate (0.0422 g in 25 mL of UHQ water) were added while magnetically stirring (900 rpm) to a $1.67 * 10^{-3}$ M and a $3.33 * 10^{-3}$ M solution of hydroxylamine hydrochloride and sodium hydroxide (0.0121 g of $NH_2OH*HCl$ and 0.01412 g of NaOH in 100 mL of UHQ water, respectively). The stirring was maintained for some seconds.

Both the colloids were concentrated by centrifuging 1 mL of the colloid for fifteen minutes at 3600 rcf. After each centrifugation cycle, the surnatant was discarded and additional 1 mL of colloid were added to the tube. This procedure was performed ten times and about 10 mg of a dense silver colloidal paste were obtained in each tube.

In order to further aggregate the silver colloidal paste obtained from the Lee and Meisel colloid, a 1.8 M solution of sodium perchlorate was prepared (2.204 g of $NaClO_4$ in 10 mL of UHQ water). The addition of the aggregating agent was tested both before and after centrifugation. In the first case, the solution of sodium perchlorate was added to the colloid while stirring with a volume ratio of 1:24. This addition resulted in a very scarce amount of silver colloidal paste, which was mainly deposited onto the tube walls and it was very difficult to be recovered with a micropipette. In the

latter case, 2 µL of solution were added to the silver colloidal paste obtained after the centrifuging cycles.

Silver colloidal pastes obtained by centrifugation of the Lee and Meisel and the Leopold and Lendl colloids will be henceforth referred to as LM paste and LL paste, respectively, while the LM paste with addition of sodium perchlorate will be called Na-LM paste. Colloids were synthesized and centrifuged the day before analysis in order to check the effectiveness of freshly prepared silver colloidal pastes.

5.1.1.2 REFERENCE SAMPLES

Reference samples were obtained from the reference sets employed for FORS analysis. In particular, samples of wool and silk mordanted with alum, alum and cream of tartar or alum, cream of tartar and iron(II) sulfate dyed with cochineal or brazilwood were considered. Samples dyed with different concentrations of the dyestuff were also analyzed. In addition, samples of cotton and flax fibers mordanted with alum and gambier (*Uncaria gambir* Roxb.), alum and sodium carbonate or alum, sodium carbonate and acetic acid and dyed with brazilwood were investigated. No reference samples of cochineal dyed on plant fibers were considered as this dyestuff poorly works on such fibers. A list of the considered samples with details about their dyeing procedure is reported in Table A1.1 of Appendix 1. In addition, a more restricted number of samples was analyzed systematically with LM and Na-LM pastes on different days thus employing silver colloidal pastes obtained from different batches of colloid preparation to check reproducibility of results. Table A1.1 in Appendix 1 accounts also for these samples.

Stock solutions of 10^{-3} M carminic acid in methanol were prepared in order to verify the effectiveness of the pastes in enhancing the Raman signals from less complex matrices. SERS analysis of carminic acid was also employed to obtain an indication of the expected signals on cochineal dyed fibers, while for brazilwood 0.1 g of brazilwood powder extract were dissolved in 25 mL of bidistilled water. A second extract was obtained by soaking overnight 0.5 g of chopped brazilwood in 50 mL of bidistilled water, then boiling for one hour and filtering.

5.1.1.3 INSTRUMENTATION

Localized Surface Plasmon Resonance (LSPR) was investigated by recording absorbance spectra on silver colloids properly diluted with UHQ water with a Jasco (Easton, US) V-550 UV-Vis spectrophotometer in the range 300-600 nm.
LSPR of LM paste was instead investigated in the diffuse reflection mode employing a Perkin Elmer Lambda 900 UV-Vis-NIR (Waltham, US) equipped with an integration sphere, with 1 nm spectral resolution. The colloid was deposited onto a microscope slide and reflectance spectra were recorded in the 350-700 nm range. Reflectance spectra of the LM paste were also collected by means of an Ocean Optics (Dunedin, FL) SD2000 fiber optically-coupled spectrometer, with a probe diameter of about 2 mm. In addition, reflectance spectra of Na-LM paste were recorded with a Corona45Vis spectrophotometer by Zeiss (Oberkochen, Germany) employing the 0°/0° geometry. No reflectance spectrum of the LL paste could be recorded, as the paste was transparent.

SEM imaging of the silver colloids and the silver colloidal pastes was performed on an Hitachi (Tokyo, Japan) S-4800-II c-FEG SEM. Additional SEM imaging was performed on a LEO Gemini 1525 microscope operating at 3 kV for the fiber samples and 7 kV for both the silver colloidal paste and the silver colloids samples. Environmental SEM (FEI Quanta 600F sFEG ESEM) imaging was performed on the flax fibers in order to circumvent the low conductivity of the samples without applying extra coating material. The microscope was operated at 12 kV in low vacuum mode, with a pressure of 1.20 Torr (water vapor).

SERS measurements were performed at the Art Institute of Chicago (Illinois) with a Jobin Yvon Horiba Labram 300 confocal Raman microscope and at Laboratorio Analisi Scientifiche (Aosta, Italy) with a Renishaw (Stonehouse, Great Britain) inVia micro-Raman spectrometer.

The Jobin Yvon Horiba Labram 300 confocal Raman microscope operated with the excitation line of a He-Ne laser (λ_{ex}=632.8 nm), focused through a 100x Olympus objective onto the samples. Spectra were recorded in the 100-1700 cm^{-1} range.

SERS measurements with the Renishaw inVia micro-Raman spectrometer were performed using the excitation wavelength of a 632.8 nm laser and a 100X Leica (Wetzlar, Germany) microscope objective to focus the laser beam onto the sample. Spectra were recorded in the 100-1800 cm^{-1} range.

Power at the samples was kept very low (never exceeding 300 µW) in both instruments by a series of neutral density filters in order to avoid any thermal damage.

The SER spectra obtained with the Renishaw in-Via instrument were normalized in order to exclude the influence of integration time (10 s) and effective laser power on signal intensities. Laser power was measured using a ThorLabs (Dachau, Germany) 110D power meter provided with a S121C Si detector.

Further details about the instrumentations are given in Appendix 2.

5.1.1.4 SAMPLE PREPARATION

For SEM investigation, silver colloids and silver colloidal pastes were dropped on a silicon wafer fixed by means of a carbon tape to a stub specimen mount. As for natural fiber, 1-2 mm long wool, cotton and silk fibers were affixed on carbon tape which was placed on an SEM stub specimen mount, while the flax fibers were placed on a silicon wafer mounted onto an SEM stub specimen mount. The fibers were then drop coated with silver colloidal paste or silver colloids. The substrate was spread evenly with a gold wire and allowed to dry before obtaining SEM images.

Blank samples for SERS measurements were prepared by dropping 5 µL of silver colloidal paste on a pre-cleaned microscope slide and allowing them to dry. Moreover, 5 µL drops of the considered reference solutions (e.g. carminic acid and brazilwood extracts) were put on a microscope slide rapidly followed by addition of 5 µL of the selected silver colloidal paste. Reference dyed fibers were preliminarily rinsed with methanol in order to remove any unmordanted dyed that possibly remained after the dyeing process and dried under the laboratory hood. Fibers were cut into small fragments (< 1 mm in length), deposited on a pre-cleaned microscope slide and drop-coated with 0.5 µL of the selected silver colloidal paste. The paste was gently brushed on the fiber with a golden wire.

Raman investigation was performed the same day as sample slide preparation. A minimum of five measurements were performed on each sample, in order to check the reproducibility of the obtained spectra and account for possible inhomogeneity of the dyeing. Different portions of the same sample were analyzed on different days, thus employing silver colloidal pastes obtained from different batches of colloid preparation to check reproducibility of results. In some cases, the prepared samples were re-analyzed after some days to verify the efficacy of the enhancement on aged samples. In addition, some samples dyed with cochineal were employed for further tests with LM paste. In particular, two samples of wool and one of silk were immersed in water for some hours before being coated in order to swell the fibers and, hence, to favor the penetration of the paste into the fiber. Then, some fibers of a wool sample were rinsed with bidistilled water after being coated with LM paste to try to dissolve citrate. Finally, the effectiveness of silver colloidal pastes for on-the-fiber extractionless analysis was tested versus on-the-fiber SERS with hydrolysis and SERS on extracts. For this purpose, two samples of wool dyed with cochineal were expressly pre-treated before being coated with the three considered silver colloidal pastes. In particular, for on-the-fiber hydrolysis, the fibers were exposed to HCl vapors for fifteen minutes, while extracts were prepared following the procedure by Wouters and Verhecken (1989). In particular, the samples were kept in a solution of HCl:MeOH:H$_2$O (3:1:1, v/v/v) at 90°C for ten minutes, then dried under a nitrogen flux and recovered in methanol.

5.1.2 RESULTS AND DISCUSSION

5.1.2.1 SPECTROSCOPIC CHARACTERIZATION OF THE SUBSTRATES

Absorbance and reflectance spectra of the considered colloid and colloidal pastes are reported in Figures 5.1-5.6. LSPR of LM colloid was investigated by recording absorbance spectra of different batches, which were measured after 1, 2, 4, 9, and 18 days from the synthesis (Figure 5.1).The minimum dilution for colloids was of 1:10 v/v. The same batch was measured after 1 and 18 days in order to determine the spectral changes due to possible degradation of the colloid. The spectra of batches B-D showed a maximum at 424-429 nm (Figure 5.1, green, blue and cyan spectra), while in the case of batch A the maximum was positioned at 446 nm (Figure 5.1, magenta spectrum) and did not present a shift when measured after 18 days (Figure 5.1, red spectrum). A shoulder at 355-360 nm was found in all the spectra of LM colloid. The absorbance spectrum of the supernatant (Figure 5.1, black spectrum) was recorded without dilution and showed a less intense maximum at about 405 nm, while the position of the shoulder was comparable with that registered in colloid samples. The absorbance spectrum of the Na-LM colloid (Figure 5.2, red spectrum), obtained from batch A, presented a shoulder at about 355 nm too, while in this case the position of the absorption maximum was not identifiable as the spectrum presented a broad absorption band in the visible region. The spectrum of the supernatant (Figure 5.2, black spectrum) showed similar but less intense features. Finally, the maximum of absorbance of LL colloid was positioned at 413 nm (Figure 5.3, red spectrum), while the supernatant (Figure 5.3, black spectrum) presented a maximum at 406 nm.

Diffuse reflectance spectra of LM paste (Figure 5.4) presented a structured absorption band with minima at about 450 and 540 nm, while the minimum of the reflectance spectrum was found at

about 480 nm (Figure 5.5). As for Na-LM paste, an absorption band was found at about 530 nm (Figure 5.6).

The spectral features of the considered colloids are consistent with previously published data. In particular, the main peak of LM colloid, observed also in the aggregated colloid (e.g. Na-LM colloid), is due to the dipolar localized plasmon resonances of the colloid, while the shoulder at lower wavelength is a contribution of the quadrupolar resonance (Le Ru and Etchegoin 2009). As expected, the position of the maximum absorption, which mainly depends on the synthesis details, is found between 400 and 450 nm (Cañamares *et al.* 2005; Cañamares *et al.* 2008; Dement'eva *et al.* 2008; Larmour, Faulds, and Graham 2011; Le Ru and Etchegoin 2009; Munro *et al.* 1995; Pillai and Kamat 2004). In addition, a variability among the position of the absorption maxima over different batches was previously reported by Cañamares *et al.* (2005). The asymmetric aspect of the band and its broadening are influenced by inhomogeneity in size and shape of the nanoparticles (Le Ru and Etchegoin 2009). On the contrary, the very broad band observed in Na-LM colloid is mainly due to aggregation, which also causes a red-shift of the maximum (Le Ru and Etchegoin 2009), barely observable in Figure 5.2. Also the data obtained for LL colloid are consistent with the literature, as the absorption maximum was usually found at 400-430 nm (Cañamares *et al.* 2005; Cañamares *et al.* 2008; Larmour, Faulds, and Graham 2011; Leopold and Lendl 2003). The presence of a less intense maximum in the spectra obtained from the supernatant indicates that a smaller quantity of nanoparticles was still suspended and did not deposit at the bottom of the tube. Moreover, the position of the absorption band in the reflectance spectra of LM and Na-LM pastes indicates that a bathochromic shift occurred when concentrating the colloid, similarly to what is observed for the addition of an aggregating agent. The presence of two absorption bands in the spectra of the LM paste collected in diffuse reflectance might suggest a dichroic behavior of silver nanoparticles, which dimensions are slightly lower to the wavelengths of the radiation employed for the analysis. It should also be added that the position of the maximum of absorbance indicates the resonance wavelength, that is the wavelength where the largest SERS enhancement is expected (Le Ru and Etchegoin 2009). Thus, silver colloidal pastes generally present a resonance wavelength closer to the excitation wavelength of the most common lasers employed in the field of cultural heritage analysis (532 and 633 nm). However, in the case of aggregated colloidal solutions, where a small number of high-enhancement regions are found, the resonance wavelength can be a bad indicator of the wavelength where the maximum enhancement occurs (Le Ru and Etchegoin 2009).
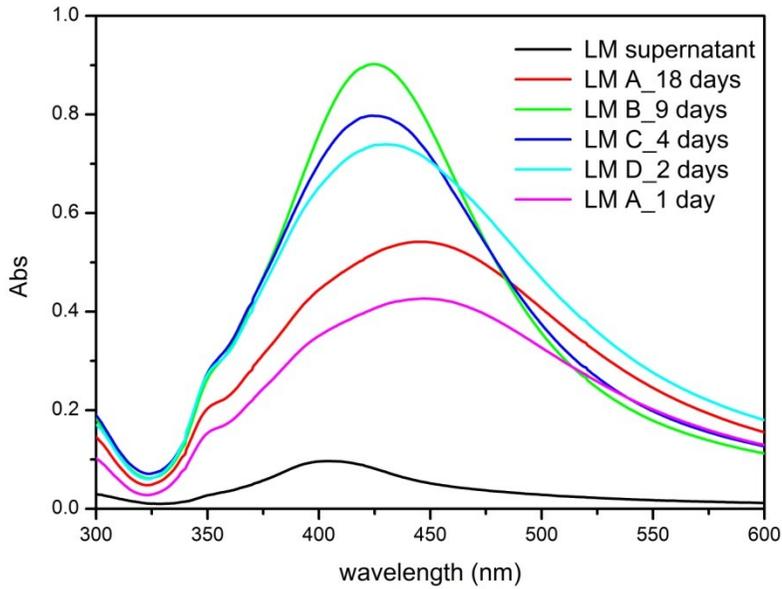
Figure 5.1: Absorbance spectra of Lee and Meisel colloid batches (A-D) recorded after 1-18 days from the preparation and of the supernatant solution removed after the centrifugation of the colloid (black spectrum).
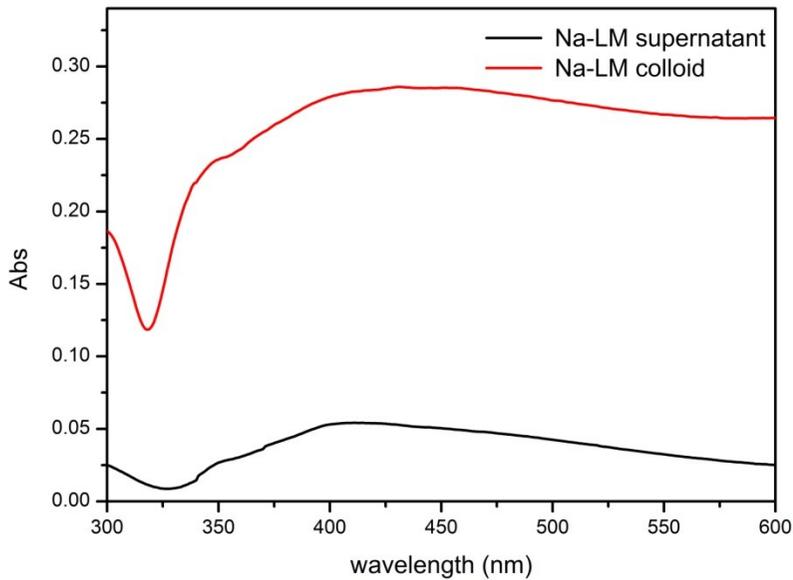

Figure 5.2: Absorbance spectra of Lee and Meisel colloid added with sodium perchlorate (red spectrum) and of the supernatant solution removed after the centrifugation of the colloid (black spectrum).
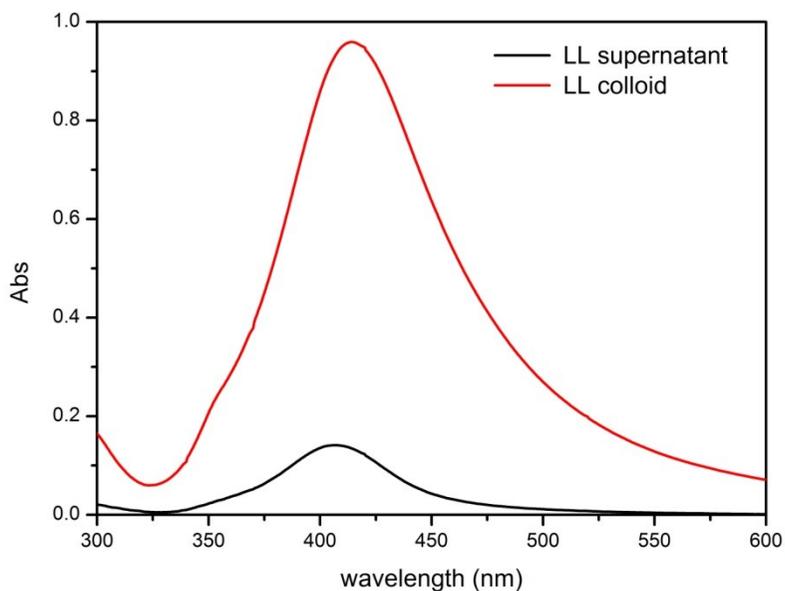
Figure 5.3: Absorbance spectra of Leopold and Lendl colloid (red spectrum) and of the supernatant solution removed after the centrifugation of the colloid (black spectrum).
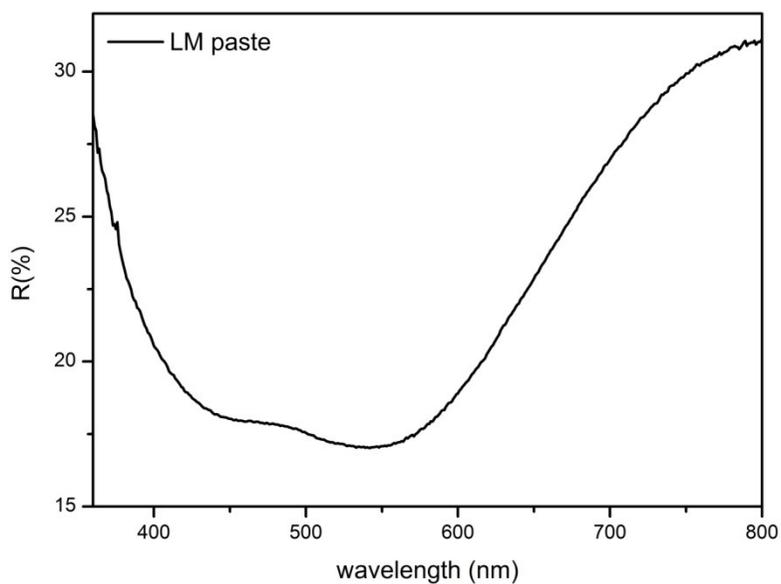
Figure 5.4: Diffuse reflectance spectrum of the paste obtained from the Lee and Meisel colloid.
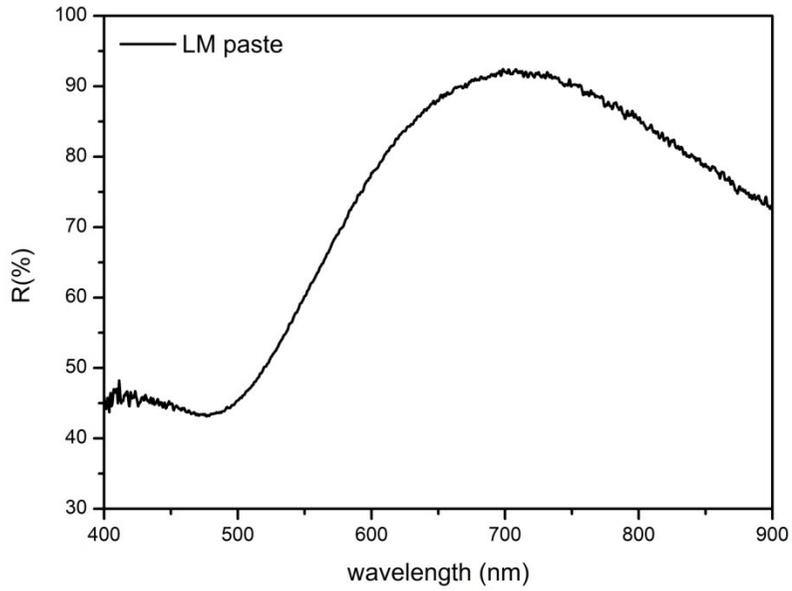
Figure 5.5: Reflectance spectrum of the paste obtained from the Lee and Meisel colloid.
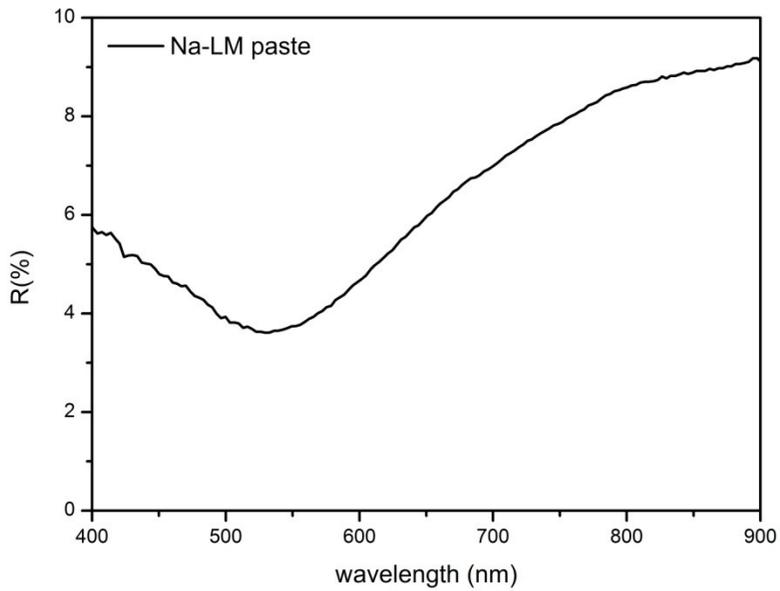
Figure 5.6: Reflectance spectrum of the paste obtained from the Lee and Meisel colloid added with sodium perchlorate.

5.1.2.2 MORPHOLOGICAL CHARACTERIZATION OF THE SUBSTRATES

Secondary electron images of LM paste (Figure 5.7) showed that it consists mostly of spherical nanoparticles, along with rods and even wires (aspect ratio comprised between 3 and 16). Spheres diameter ranged from 17 to 100 nm with an average diameter of 39±17 nm (based on measuring the diameter of 40 particles). The length of the low axis of the rods was measured to range between 18 and 58 nm, while the long axis was measured to range between 143 and 457 nm. As expected, the composition in size and shape of the LM colloid (Figure 5.8) did not differ from the LM paste. Only the nanoparticle concentration differed drastically. Figures 5.7 and 5.8, recorded on LM colloid and LM paste, clearly show that the paste is much more concentrated than the initial colloidal solution and that randomly oriented nanoparticle superlattices can be observed. The presence of small particles in the LM colloid can be correlated with the broadening of the absorbance band in the left part of the spectrum (Figure 5.1), while at longer wavelengths the absorption is due to the presence of rods and aggregates (Cañamares *et al.* 2005). The LM nanoparticles in the silver colloidal paste created mostly planar layers (Figure 5.7) where individual nanoparticles are distinct and clearly visible. In fact, citrate ions adsorbed on the LM nanoparticles imparts high superficial negative electric charge (Munro *et al.* 1995), which is responsible for the formation of rod-shaped structures in the colloidal solution and prevents particles fusing together in the centrifuged paste. It is also worth mentioning the presence of organic matter (the darker 100 nm spots) in the LM colloid that was not observed in the paste. This is consistent with the fact that remaining surfactant in the colloidal solution was removed by collecting the supernatants in the several centrifugations during the preparation of the paste.

Silver nanoparticles in Na-LM paste (Figure 5.9) maintained shape and dimensions of those of the LM paste, but the tridimensional morphology of the paste appeared more complex, with fairly disordered clusters of particles and inhomogeneous holes. In addition, Na-LM colloid presented the same morphology observed in the Na-LM paste, but in this case the dimensions of the clusters was smaller. The morphology of the aggregated colloid is consistent with micrographs reported by Cañamares *et al.* (2005) for LM colloid aggregated with KNO_3.

Silver particles of the LL paste (Figure 5.10) looked quite different in shape when compared with LM ones; in fact, centrifugation led to fusion of the silver nanoparticles in a honeycombed fashion. Moreover, these particles were slightly larger than the LM paste ones, with a mean diameter of sixty-eighty nanometers. The LL colloid presented only spherical particles, which sometimes aggregate into dimers, trimers, some multimers and higher order aggregates of the order of half a micron, as previously demonstrated by Cañamares *et al.* (2005). These results showed that centrifugation led to coalescence of the silver particles of LL colloid. The fusion of the LL silver nanoparticles eliminates hot spots between two particles and decreases SERS activity. In addition, Cañamares *et al.* (2005) observed that the lower potential existing on the LL nanoparticles with respect to LM explains why they are more easily aggregated.

The SEM observation of the coating obtained with different silver colloidal pastes on different natural fibers was intended to investigate the interaction between the fiber and the substrate. For this reason, the coating of the fibers investigated through SEM might not be appropriate to obtain SER signals from the dyes. It should also be added that the aspect of the coated fibers observed by

means of optical or high-resolution electron microscopy can be rather different. The higher magnification reached by electron microscopy and the conductivity of silver nanoparticles indeed allow to better visualize them when employing a SEM.

When observing the relative ability of the pastes to coat the fibers of animal or plant origin, flax consistently appeared to have the most uncoated areas. In general, the most uniform coating for all types of fibers was provided by Na-LM paste. At high magnification the different modalities of coating of the fibers of the different pastes appeared evident: LM paste gave a smooth, homogeneous and relatively continuous coating of the fiber composed of spheres and rods, while Na-LM paste showed limited areas that are partly un-coated and presented a more textured, three-dimensional geometry of the silver spheres (Figure 5.11). LL paste both on cotton and wool fibers gave the worst overall coating of the fibers, with many uncoated areas and showed mostly fused spheres giving a highly textured surface (Figure 5.12). These specific morphological features were consistently observed over multiple trials with different fibers and colloidal pastes over several days.

The micromorphology of the silver nanoparticles coating of LM paste should be discussed in greater detail. In particular, the LM paste formed a relatively dense nanoparticle coating on the wool fibers (Figure 5.13A). High magnification observation of the samples (Figure 5.14) allowed us to confirm that the structural integrity of the nanoparticles forming the paste is maintained. In particular, what may appear as a smooth film at low magnification actually consisted of thick multilayers of colloids whose shape and size were the same as the particles from the silver colloidal paste. Along this smooth film though, sparse islands were observed. Thin covered areas on the wool sample consisted of aggregated nanoparticles islands. The observation of islands and not film suggest some dewetting on the wool fiber surface. The coating of the silk fibers (Figure 5.13B) with the LM paste was observed to be excellent, both at low and high magnification (Figure 5.14B). Only very few bare areas were observed by SEM. The cotton samples (Figure 5.13C) have excellent coverage both at low and high magnification (Figure 5.14C). Flax fibers (Figure 5.13D) coated with the LM paste showed charging in the SEM image due to lower nanoparticle coverage. Due to charging effects, the flax fiber sample was re-imaged with an environmental SEM (eSEM, Figure 5.14A). The coverage of flax fibers differed drastically from the silk and cotton samples. Similar to the wool sample, at low and high magnification (Figure 5.14D) it can be seen that the LM paste forms islands on the flax fiber whose size varies with thickness and may form film-like structure in areas with thickest coverage. While the majority of wool, cotton, and silk fibers were largely covered with silver colloidal paste, nanoparticles coverage is low on the flax fiber with respect to the other types of fibers studied here.

Furthermore, in order to compare the relative coating of fibers when using LM colloid versus LM paste, flax fibers were imaged after having been treated with these two substrates (Figure 5.15). Due to relatively low coverage of nanoparticles, there was poor conductivity of the fibers resulting in images with high charging effects in the non-environmental SEM. Therefore, the eSEM was used to image these samples. Differences in the nanoparticle coverage were clearly observed between the LM paste and the LM colloid. While islands of aggregated nanoparticles were observed on the

LM paste coated sample, none was observed on the LM colloid coated sample. Comparison of the two samples illustrates therefore the superiority of the Ag colloidal paste at coating the fibers.

15.0kV 8.2mm x60.0k SE(U) 12/5/2011   500nm
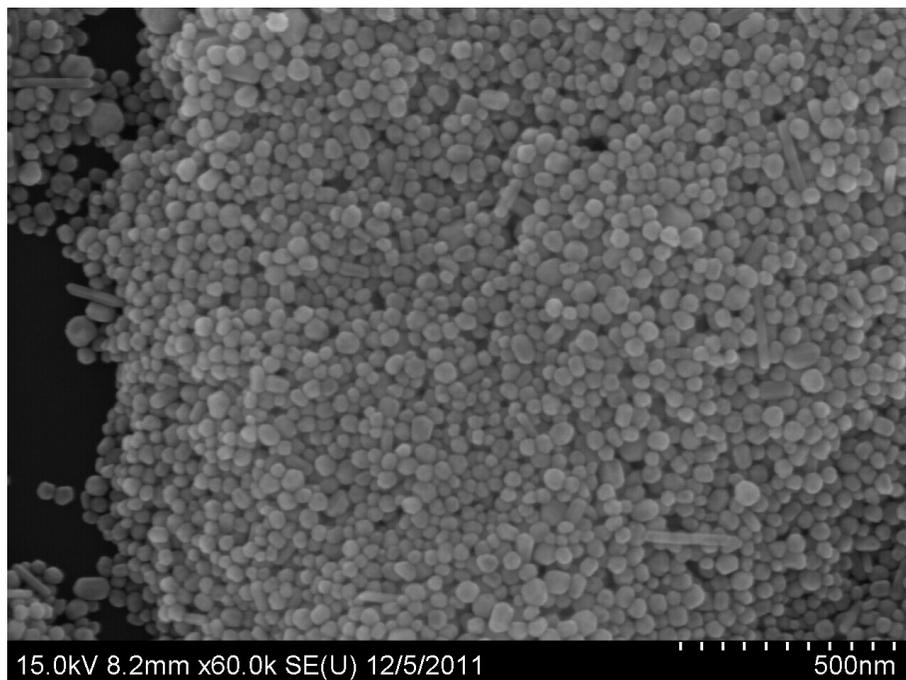
Figure 5.7: SEM micrograph of the silver colloidal paste obtained from the Lee and Meisel colloid, 60.000X.

Mag = 80.54 K X   300nm*   EHT = 7.00 kV   Signal A = InLens   Date :9 Apr 2013
                                     WD =   4 mm   Photo No. = 6927   Time :11:46
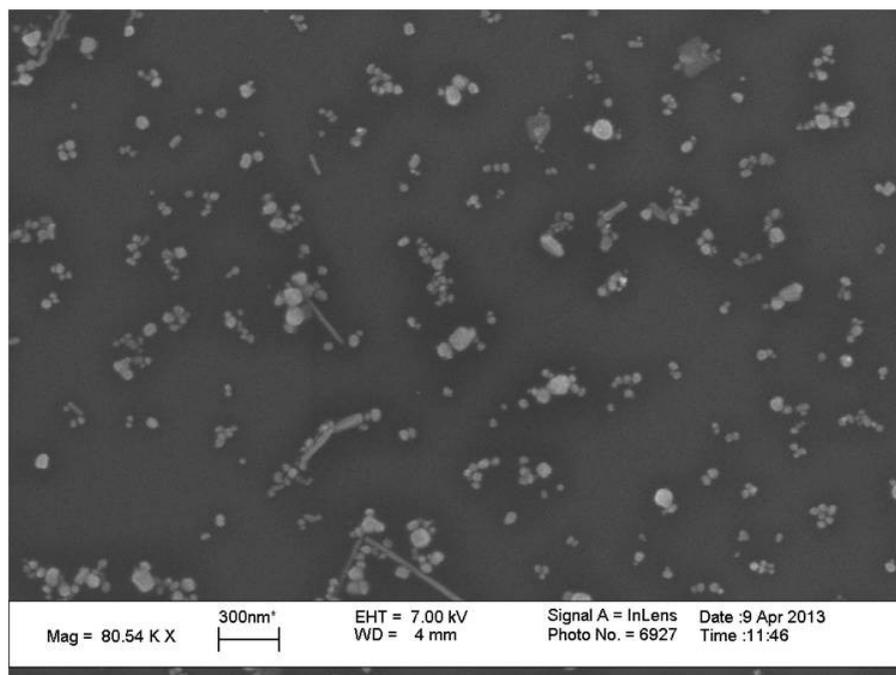
Figure 5.8: SEM micrograph of the Lee and Meisel colloid, 80.000X (courtesy of Anne-Isabelle Henry).
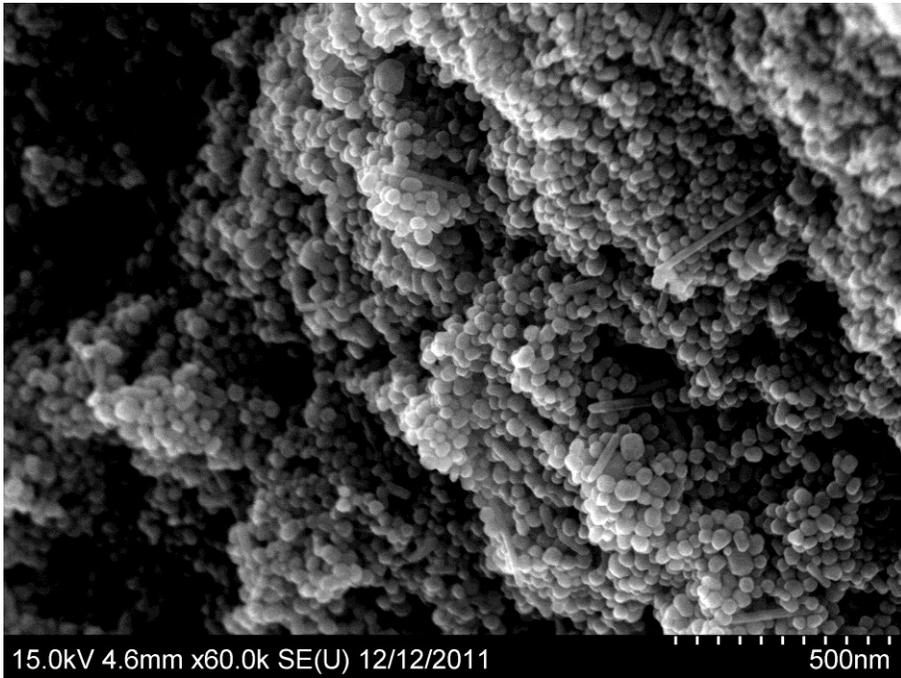
Figure 5.9: SEM micrograph of the silver colloidal paste obtained from the Lee and Meisel colloid aggregated with sodium perchlorate, 60.000X.
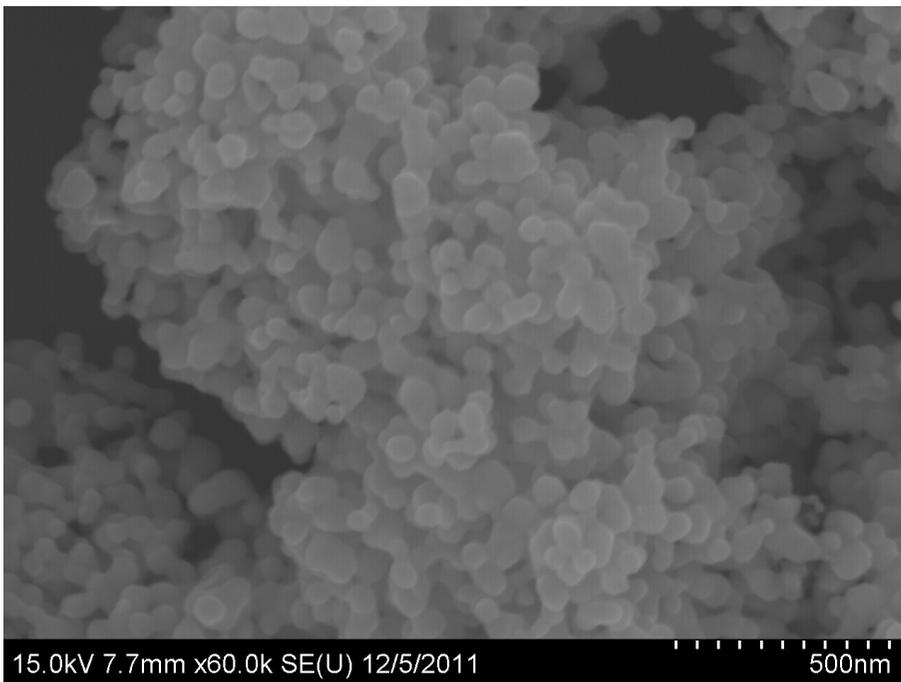

Figure 5.10: SEM micrograph of the silver colloidal paste obtained from the Leopold and Lendl colloid, 60.000X.
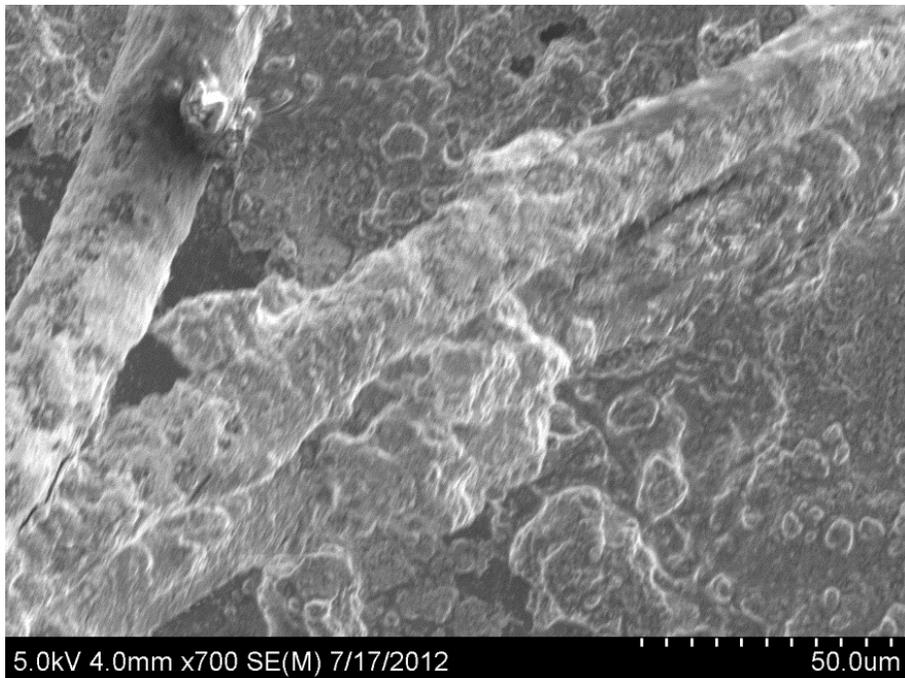
Figure 5.11: SEM micrograph of wool fibers coated with the silver colloidal paste obtained from the Lee and Meisel colloid aggregated with sodium perchlorate, 700X (courtesy of Lisa Backus).
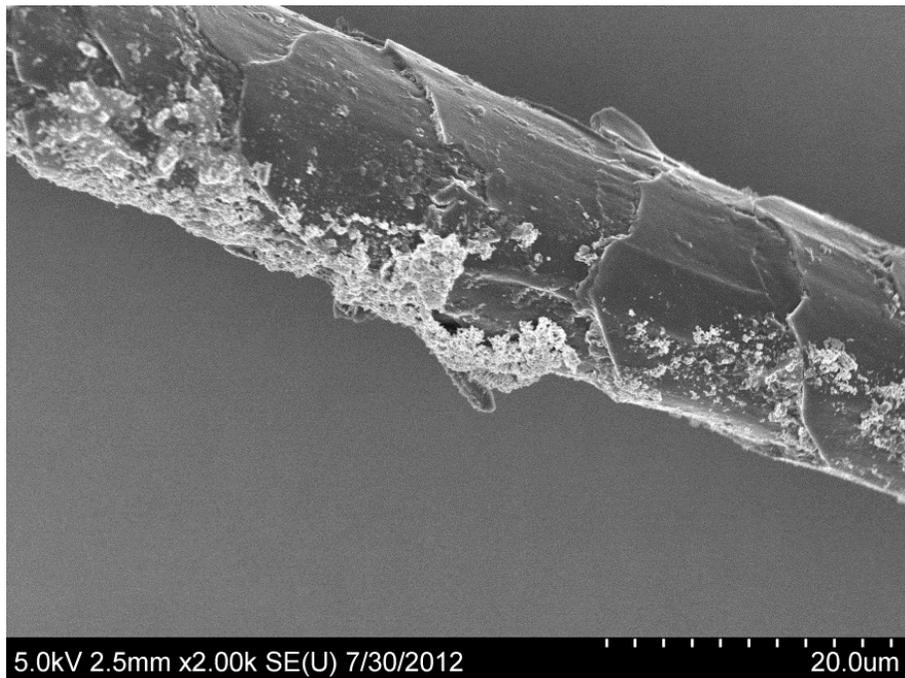
Figure 5.12: SEM micrograph of wool fibers coated with the silver colloidal paste obtained from the Leopold and Lendl colloid, 2.000X (courtesy of Lisa Backus).
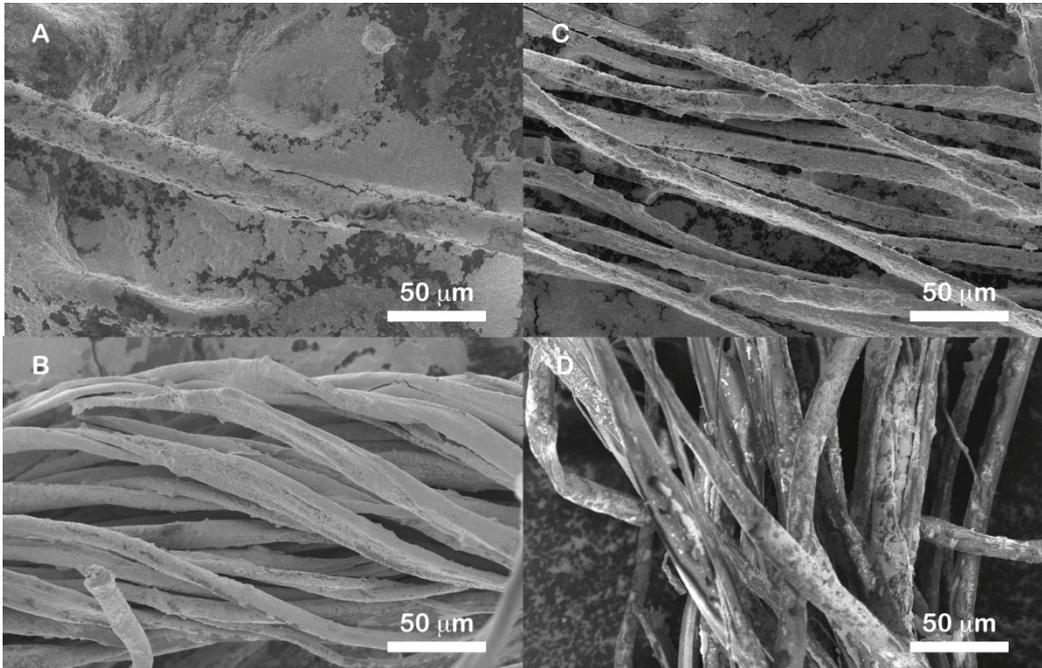
Figure 5.13: SEM micrographs of natural fibers coated with the silver colloidal paste obtained from the Lee and Meisel colloid: (A) wool, (B) silk, (C) cotton and (D) flax (Idone *et al.* 2013).
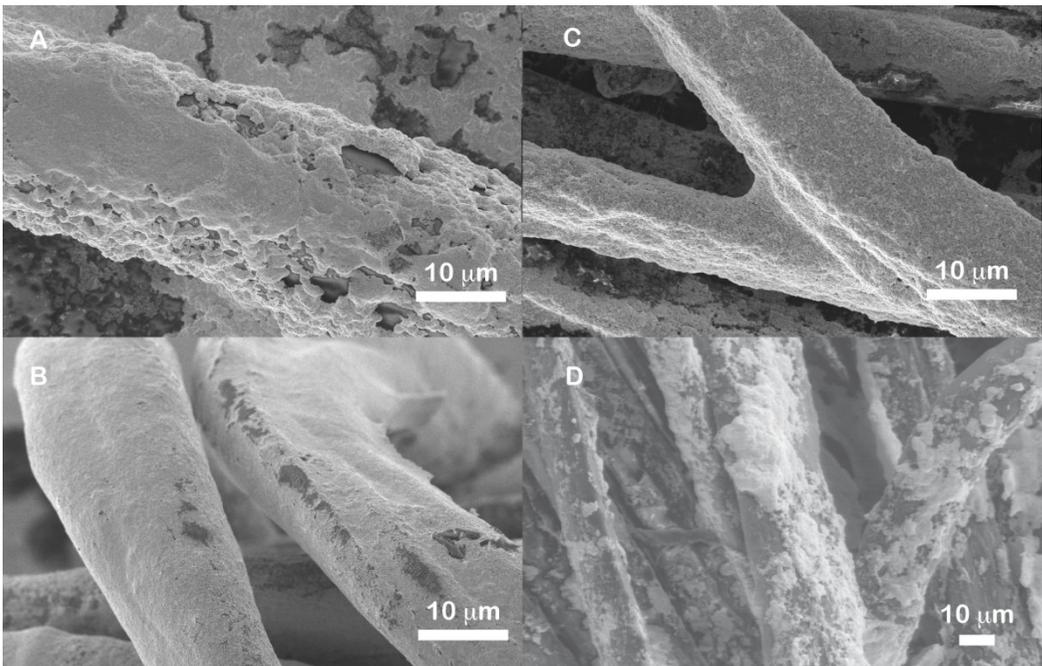
Figure 5.14: SEM micrographs at high magnification of natural fibers coated with the silver colloidal paste obtained from the Lee and Meisel colloid: (A) wool, (B) silk, (C) cotton and (D) flax (Idone *et al.* 2013).
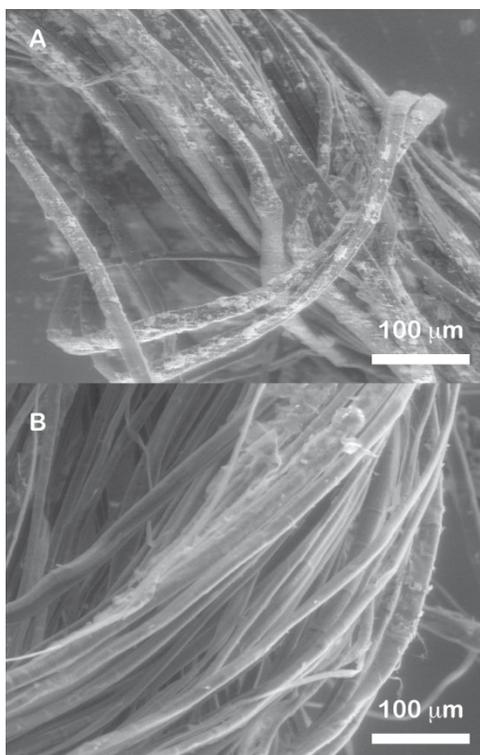
Figure 5.15: ESEM micrographs of flax fibers coated with (A) silver colloidal paste and (B) silver colloids (Idone *et al.* 2013).

### 5.1.2.3 ON-THE-FIBER EXTRACTIONLESS SERS

SER spectra recorded from LM and Na-LM pastes (Figure 5.16, red and green spectra) showed the presence of peaks at about 230, 690, 730, 810, 930, 1045, 1300, 1350, 1400 and 1470 cm^{-1}, while LL paste (Figure 5.16, black spectrum) presented only a peak at about 230 cm^{-1}. Among these signals, the very intense peak at about 230 cm^{-1} was attributed to the ionic species adsorbed onto the metal surface (Cañamares *et al.* 2005), while other signals represent vibrational modes of the citrate or its degradation products. Spectra of Na-LM paste did not display additional peaks, although these were mentioned in the literature for a number of other aggregating agents (Yaffe and Blanch 2008). Intensity and shape variations of the colloid's peaks for different measurements and different batches of silver paste are coherent with the results given by Larmour *et al.* (2011).

As expected, when the analysis was performed without silver colloidal paste, intense fluorescence dominated the spectrum. Moreover, as discussed in section 5.1.2.2, silver colloids were not effective in coating the textile fibers and thus no enhancement of the Raman signals of the dyes was observed when dropping them onto the fibers. The absorption spectra of the main dyeing molecules of cochineal and brazilwood (Favaro *et al.* 2002; Wongsooksin *et al.* 2008) and reflectance spectra of the dyed reference fiber samples (Figure 3.5 and Figure 3.7) display main peaks in the green region (500–550 nm). Since a 633 nm laser was employed in the present study,

the enhanced signal is not attributed to resonance effects. In addition, LL paste proved to be effective only when analyzing the reference dye solutions, while SER analysis of reference dyed textile fiber samples did not show any signals that could be attributed to the dye components, also when a coating of the fiber was achieved. On the contrary, both LM paste and Na-LM paste were effective in enhancing the signals of the dye molecules of cochineal and brazilwood that impregnate the reference fibers. Besides some specific cases discussed in the following paragraphs, the intensity of the above listed spurious signals deriving from the pastes was generally much lower than those of Raman signals arising from the dyes.

In general, the achievement of an enhancement of the Raman signal from the dye analytes, both in solution and in solid form, is dependent on many factors, among which the colloid synthesis plays a major role. It was indeed observed that silver nitrate suffers degradation after some months and thus care must be taken in substituting the reagent whenever the enhancing capability of the synthesized nanoparticles is diminished.

SER spectra of carminic acid in methanol (Figure 5.17) presented a very intense peak at about 1300 cm^{-1}, while an intense peak and a medium peak were observed at 1200 cm^{-1} and 460 cm^{-1}, respectively. Some measurements presented also peaks at about 1075(w), 1420 (w) cm^{-1}. These features are comparable with data for carminic acid reported in literature (Brosseau, Gambardella, *et al.* 2009; Brosseau, Casadio, and Van Duyne 2011; Cañamares *et al.* 2006; Leona, Stenger, and Ferloni 2006; Lofrumento *et al.* 2013; Whitney, Casadio, and Van Duyne 2007). The intense peak at 1300 cm^{-1} is assigned to C–OH bending modes (Bruni, Guglielmi, and Pozzi 2011) and it includes to a certain extent the contribution of the citrate used to prepare the nanoplasmonic substrate. Spectra obtained with LM and Na-LM pastes were comparable (Figure 5.17, red and green spectra), while spectra of carminic acid solutions treated with LL paste were more noisy and showed a shift of the characteristic signals of the dye towards higher Raman shifts (Figure 5.17, black spectrum).

SERS measurements of the solution obtained from the brazilwood powder extract presented fairly reproducible spectra, where only a peak at 460 cm^{-1} appeared clearly identifiable. In this case, the enhancement of the signals from the dye might be prevented by some species employed to prepare the powder, possibly an inorganic material. On the contrary, SER spectra of the extract prepared from chopped brazilwood mixed with LM or Na-LM pastes (Figure 5.18, red and green spectra) were more reproducible and allowed the identification of a very intense peak at about 465 cm^{-1} and of two intense peaks at 1350 and 1540 cm^{-1}. The position of these latter peaks could not be precisely identified as they were often shifted, possibly because they are due to the convolution of many peaks. Other peaks were found at 1160 (w) and 1200 (w) cm^{-1}. As remarked by Bruni *et al.* (Bruni, Guglielmi, and Pozzi 2011), the first intense peak at about 460 cm^{-1} is due to ring deformation. The peak centered at 1350 cm^{-1} superimposes to the signal from the citrate. Also in this case, SER spectra obtained with the LL paste presented a shift in some peaks towards higher wavenumbers (Figure 5.18, black spectrum). Data were consistent with published SER spectra of brazilwood (Casanova-González *et al.* 2012; Fazio, Trusso, and Ponterio 2013; Whitney, Van Duyne, and Casadio 2006) and with FT-Raman spectra of brazilwood (Edwards, de Oliveira, and Nesbitt 2003) and its main dyeing molecules, brazilin and brazilein (de Oliveira *et al.* 2002).

As for the SERS analysis of dyed fibers, the centrifugation procedure and the sample preparation proved to influence the possibility of obtaining signals from the dyes. In fact, controlling the manual removal of the supernatant was practically impossible and thus, at the end of ten centrifuging cycles, more or less concentrated tubes of paste were obtained. This resulted in some variability of the morphology of the coated samples when observed under the optical microscope coupled to the Raman spectrometer, which sometimes led to the impossibility of obtaining SER signals pertinent to the analyte. In addition, variations in the sample preparation, like the quantity of sample or the interval between the deposition of the drop of paste and the brushing with the golden wire, affected the morphology of the coating too. As a rule of thumb, the most intense signals were obtained from areas that appeared coated by a thin film of silver nanoparticles under the optical microscope, whereas poor signals were obtained from islands showing a thicker coating. In the case of LM paste, a number of areas where intense SER signals were recorded were generally found, while for Na-LM paste these areas were scarcer. Moreover, obtaining a proper coating with Na-LM paste was harder than with LM paste.

Fibers that were soaked in water before being coated proved in some cases to be over-coated, while in other cases SER spectra presented lower quality as opposed to spectra recorded on non-soaked samples (Figure 5.19). Similarly, the rinsing of the sample after coating was not effective, as often a poor coating of the fibers was observed and also when a SER spectrum could be recorded to weaker signals of the citrate corresponded a weakening of the signals from the dyestuff.

The hydrolysis treatment of wool fibers dyed with cochineal proved to be more effective in the case of a less dyed sample, whose signal intensity increased after the treatment (Figure 5.20, black and red spectra), while in the case of a sample containing a higher quantity of dyestuff, SER signals after hydrolysis were weaker (Figure 5.20, green and blue spectra). Moreover, no SER signals could be recorded from the extracts obtained following the Wouters procedure from the fragments of the same samples employed for the hydrolysis. Since the intent of this work was not to optimize the use of silver colloidal pastes with hydrolysis or extracting methods, no further tests were performed.

Figures 5.21 to 5.23 show SER spectra obtained from the same reference sample coated with LM paste or Na-LM paste that are representative of the trend shown by the spectra obtained from the whole set of reference samples. SER spectra obtained from samples dyed with cochineal (Figure 5.21) showed a very strong peak at 1300-1305 cm^{-1} and a less intense one, which sometimes becomes a shoulder, variable between 1200 and 1225 cm^{-1}. Other signals can be found at 460 (m), 1083 (w), 1425 (m), 1515 (w), 1630 (w) cm^{-1}. These signals can be attributed to cochineal and to its major dyeing molecule, carminic acid, even though a shift of the peak at 1200 cm^{-1} was observed. Also in this case, a contribution of the citrate could be included in the intense peak at 1300 cm^{-1}.

The main peaks recorded for samples dyed with brazilwood (Figure 5.22-5.23) were at about 465, 1350 and 1560 cm^{-1}, consistently with what observed for the analyzed brazilwood extract; weaker signals were detected at about 1020, 1090, 1160, 1190, 1310, 1495 cm^{-1}, together with a fingerprint region between 500 and 900 cm^{-1}. These peaks can be attributed to the main dye components of brazilwood. The peak at about 1350 cm^{-1} superimposed to the signal from the citrate.

In general, SER spectra collected on the same sample were reproducible in terms of position of the peaks, while the relative intensity of the signals varied. This intensity variation is possibly due to the proximity of the dye near the SERS substrate or the varying concentration of the dye and the silver colloidal paste at different locations on the fiber. Overall, the SERS enhancement is highly inhomogeneous in aggregated dried surfaces and the weaker peaks can remain undetected in some of the repeated measurements performed on a same sample. Besides the presence of spurious signals from the silver pastes, the spectra obtained from the silver-coated fibers (Figures 5.21-5.23) did not show additional peaks that can be attributed to the proteinaceous or glycosidic fiber substrate. Moreover, the comparison between SER spectra recorded on a same reference sample coated with LM paste or Na-LM paste indicated that signals intensities for both cochineal and brazilwood are comparable in the case of animal fibers (Figures 5.21 and 5.22). On the other hand, SER spectra of brazilwood on plant fibers (Figure 5.23) showed an increase of the intensity of the signals when the fiber is coated with Na-LM paste, suggesting that the glycosidic nature of the fiber favors the enhancement of the Raman signals when silver nanoparticles are aggregated by sodium perchlorate.

The spectra recorded on samples mordanted with alum and cream of tartar did not present additional features with respect to samples mordanted with alum only (Figure 5.24, black spectrum). The same considerations can be done for the sample of wool mordanted with alum, cream of tartar and iron(II) sulfate dyed with cochineal, even though in this case the intensity of the peaks is lower (Figure 5.24, red spectrum) despite this sample presents color coordinates similar to those of the most intense samples not mordanted with iron(II) sulfate (Table A1.1, Appendix 1). In the case of brazilwood, no SER spectra with characteristic peaks were recorded on the sample mordanted with iron(II) sulfate. On the other hand, intense peaks pertinent to the dyeing molecules of brazilwood were obtained from a sample of flax and cotton mordanted with alum and gambier (Figure 5.25). Although gambier is a tannic dyestuff which can be also employed as a mordant, no additional peaks attributable to gambier were found, possibly because brazilwood molecules at the surface prevent the interaction between the silver nanoparticles and the tannins of gambier.

As for the sensitivity of the method, the enhancement due to the coating with LM paste or Na-LM paste allowed to detect the Raman signals of the dye even in weakly dyed fibers: in fact, good SER spectra of light red samples dyed with cochineal in a bath previously used to dye other samples were successfully collected (Figure 5.26), although the signals of the dye were obviously less intense than those recorded for reference samples with higher color saturation. In the case of brazilwood, the identification of the signals in samples dyed with a second bath was more difficult.

Finally, some samples dyed with brazilwood prepared with fresh pastes were analyzed five or seven days after their preparation. The recorded spectra still showed the diagnostic peaks of brazilwood, in spite of a diminished intensity and a worse reproducibility if compared with spectra recorded the day after the preparation (Figure 5.27).
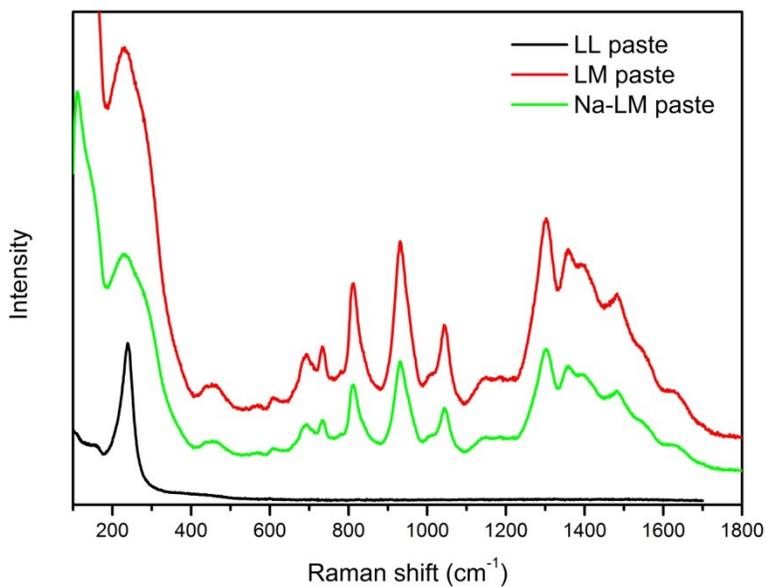
Figure 5.16: SER spectra of LL paste (black spectrum), LM paste (red spectrum) and Na-LM paste (green spectrum).
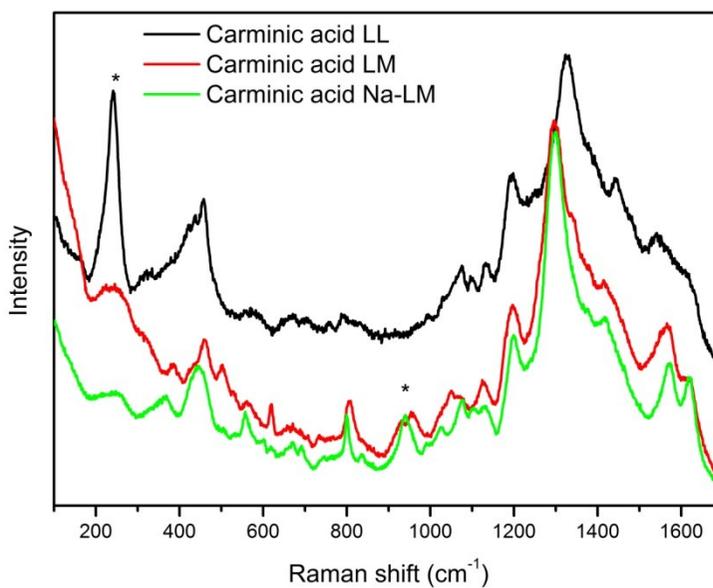
Figure 5.17: SER spectra of carminic acid in methanol with LL paste (black spectrum), LM paste (red spectrum) and Na-LM paste (green spectrum); asterisks indicate peaks belonging to the pastes; spectra were stacked for ease of comparison.
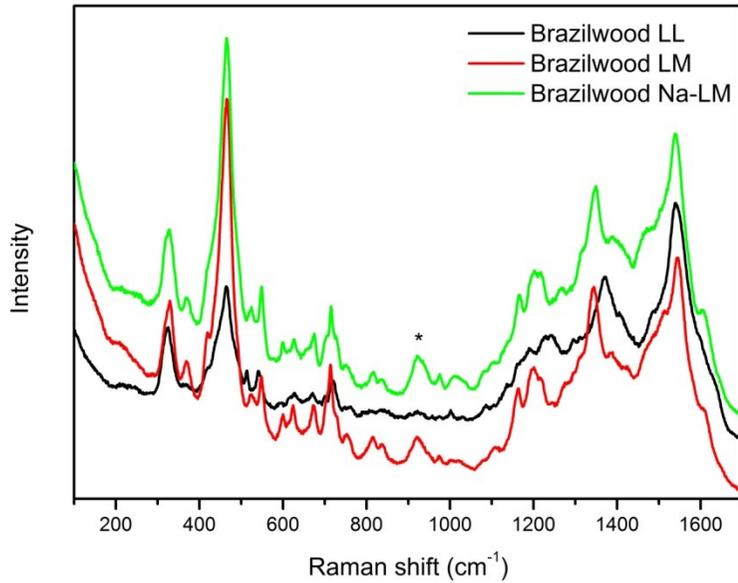
Figure 5.18: SER spectra of a water extract from chopped brazilwood with LL paste (black spectrum), LM paste (red spectrum) and Na-LM paste (green spectrum); asterisks indicate peaks belonging to the pastes; spectra were stacked for ease of comparison.
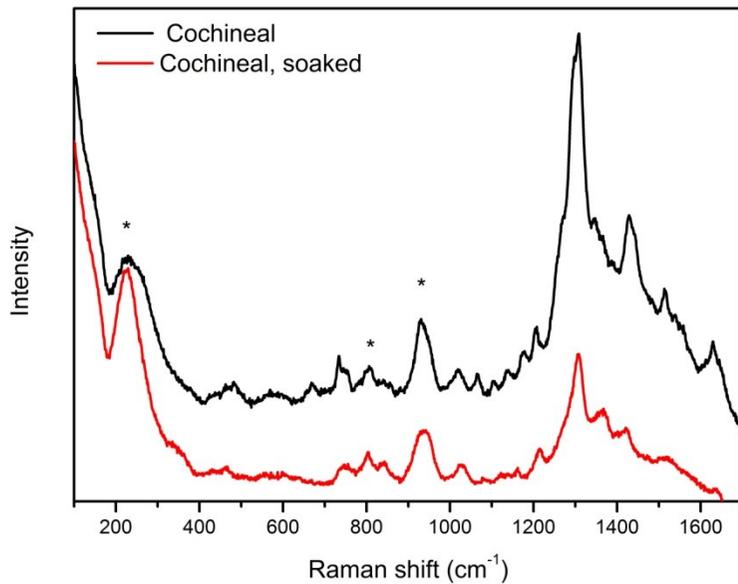
Figure 5.19: SER spectra of silk fibers mordanted with alum and cream of tartar and dyed with cochineal coated with LM paste: fiber not soaked in water before coating (black spectrum); fiber soaked in water before coating (red spectrum); asterisks indicate peaks belonging to the pastes.
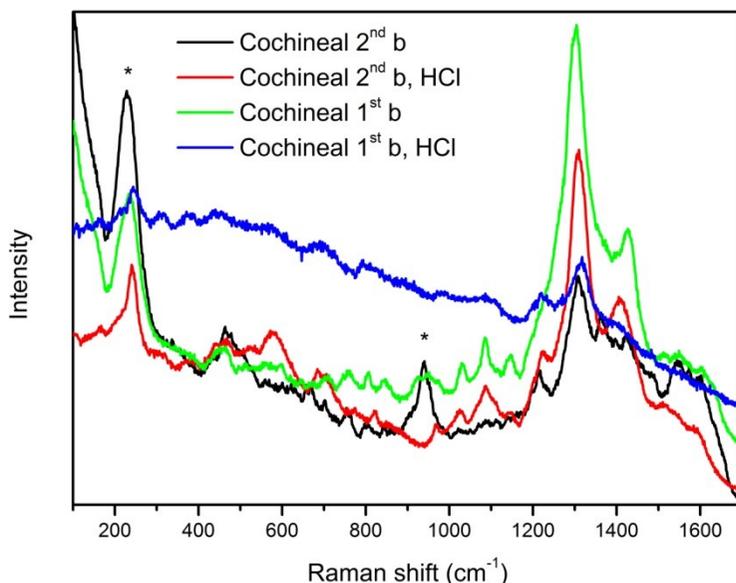
Figure 5.20: SER spectra of wool fibers mordanted with alum and cream of tartar and dyed with cochineal:); 2nd bath, Na-LM paste (black spectrum); 2nd bath, previously treated with HCl, LM paste (red spectrum);); 1st bath, LM paste (green spectrum); 1st bath, previously treated with HCl, LM paste (blue spectrum); asterisks indicate peaks belonging to the pastes; spectra stacked for ease of comparison.
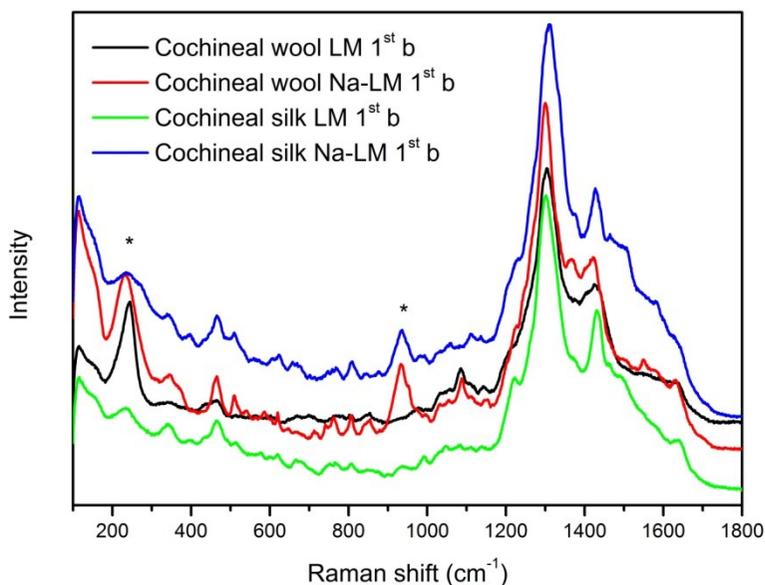
Figure 5.21: SER spectra of animal fibers mordanted with alum and cream of tartar and dyed with cochineal in 1st bath: wool, LM paste (black spectrum); wool, Na-LM paste (red spectrum); silk, LM paste (green spectrum); silk, Na-LM paste (blue spectrum); asterisks indicate peaks belonging to the pastes; spectra were normalized according to the integration time and the effective laser power and stacked for ease of comparison.
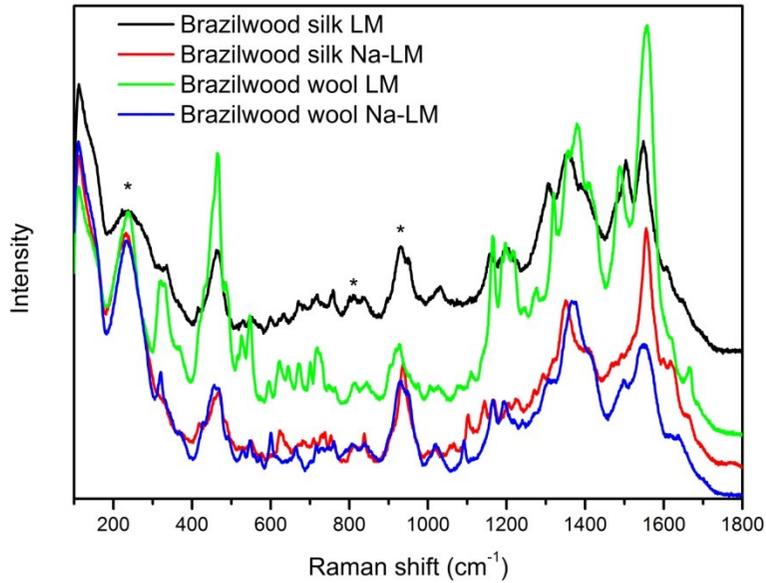
Figure 5.22: SER spectra of animal fibers mordanted with alum and cream of tartar and dyed with brazilwood in 1st bath: silk, LM paste (black spectrum); silk, Na-LM paste (red spectrum); wool, LM paste (green spectrum); wool, Na-LM paste (blue spectrum); asterisks indicate peaks belonging to the pastes; spectra were normalized according to the integration time and the effective laser power.
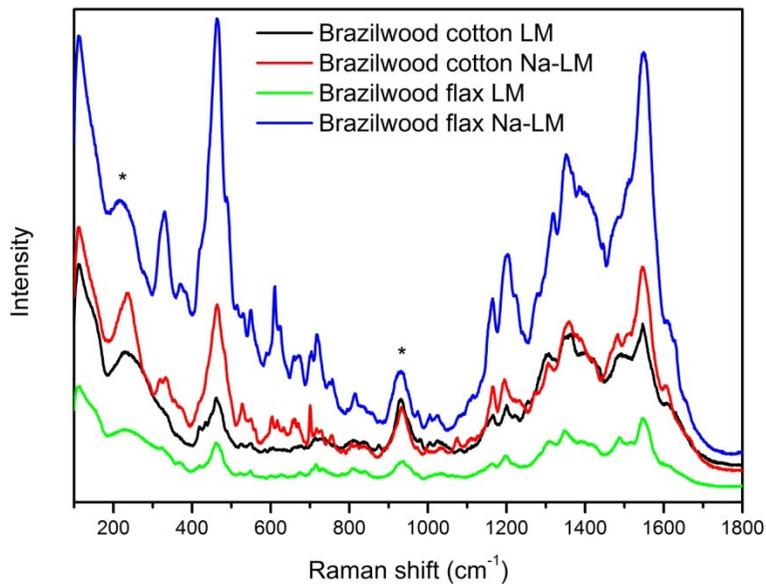
Figure 5.23: SER spectra of plant fibers mordanted with alum and cream of tartar and dyed with brazilwood in 1st bath: cotton, LM paste (black spectrum); cotton, Na-LM paste (red spectrum); flax, LM paste (green spectrum); flax, Na-LM paste (blue spectrum); asterisks indicate peaks belonging to the pastes; spectra were normalized according to the integration time and the effective laser power.
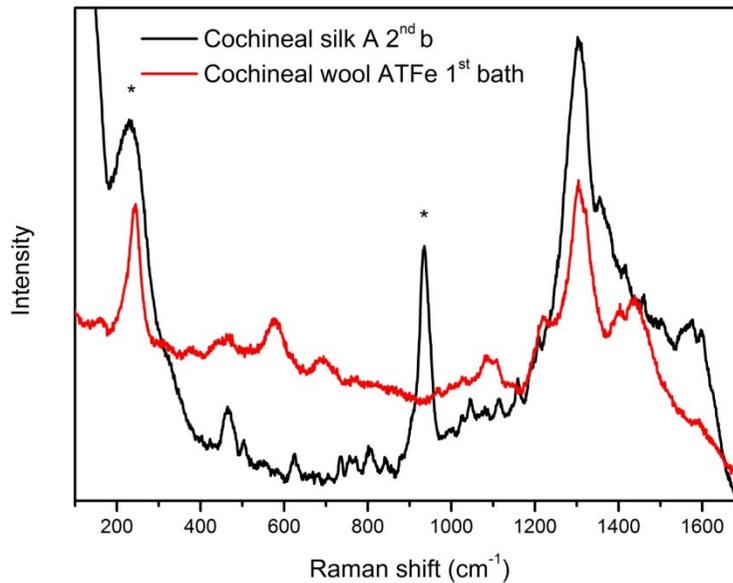
Figure 5.24: SER spectra of animal fibers dyed with cochineal: silk mordanted with alum, 2nd bath, Na-LM paste (black spectrum); wool mordanted with alum, cream of tartar and iron(II) sulfate, 1st bath, LM paste (red spectrum); asterisks indicate peaks belonging to the pastes.
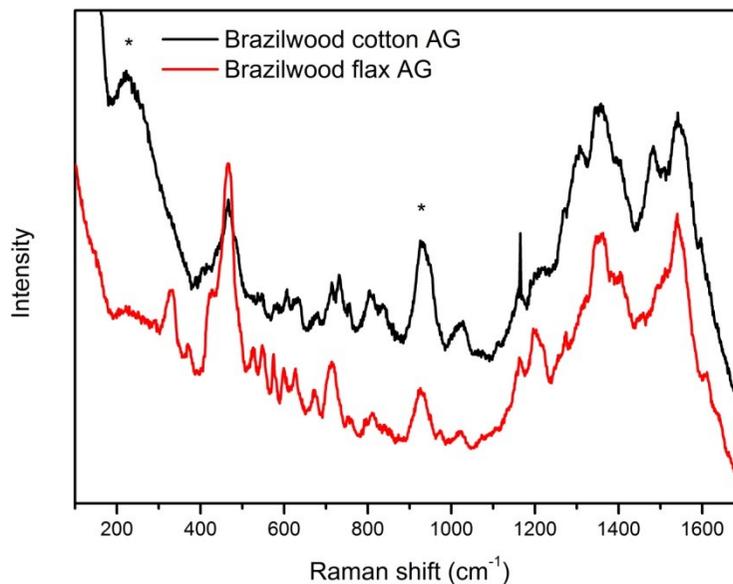
Figure 5.25: SER spectra of plant fibers mordanted with alum and gambier and dyed with brazilwood in 1st bath: cotton, LM paste (black spectrum); flax, Na-LM paste (red spectrum); asterisks indicate peaks belonging to the pastes.
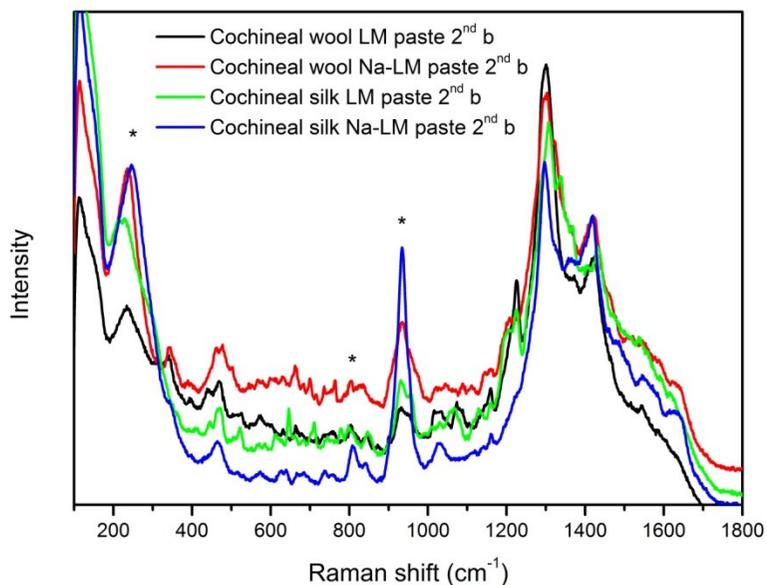
Figure 5.26: SER spectra of animal fibers mordanted with alum and cream of tartar and dyed with cochineal in 2nd bath: wool, LM paste (black spectrum); wool, Na-LM paste (red spectrum); silk, LM paste (green spectrum); silk, Na-LM paste (blue spectrum); asterisks indicate peaks belonging to the pastes; spectra were normalized according to the integration time and the effective laser power.
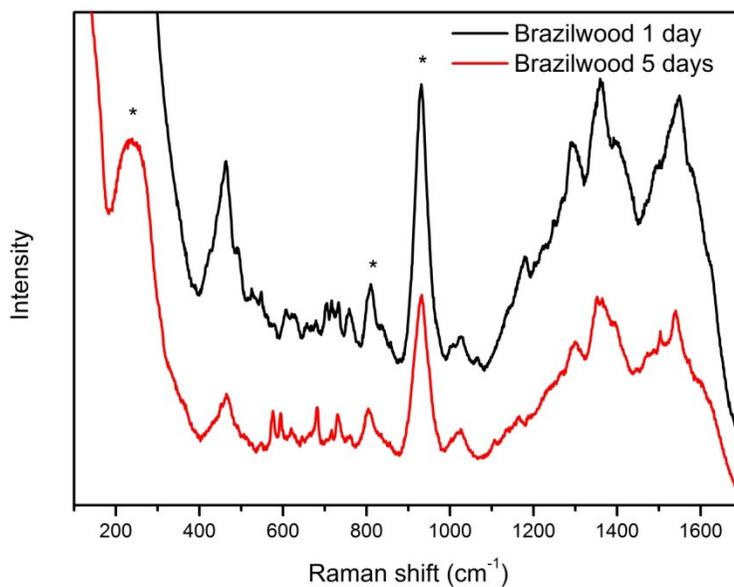
Figure 5.27: SER spectra of cotton fibers mordanted with alum, cream of tartar and acetic acid and dyed with brazilwood in 1st bath: spectrum recorded 1 day after the preparation of the sample, LM paste (black spectrum); spectrum recorded 5 days after the preparation of the sample, Na-LM paste (red spectrum); asterisks indicate peaks belonging to the pastes.

5.1.3 CONCLUSIONS

This work investigated the suitability of different chemically reduced silver colloids for the direct extractionless analysis of dyed fibers. A paste was obtained by centrifugation of the colloids and the SERS substrate was applied directly on the fiber sample. This method previously proved to be able to detect the dyes in fibers, pastels and watercolors (Brosseau, Gambardella, *et al.* 2009; Brosseau, Rayner, *et al.* 2009; Brosseau, Casadio, and Van Duyne 2011; Wustholz *et al.* 2009). The two most popular silver colloids, prepared following the Lee and Meisel (1982) and the Leopold and Lendl (2003) procedure, respectively, were considered. Furthermore, the addition of sodium perchlorate as an aggregating agent was tested for the Lee and Meisel colloid, modifying a procedure reported by Bruni *et al.* (2010).

Silver colloidal pastes were employed to analyze reference fibers dyed with cochineal and brazilwood following different dyeing procedures. In addition, the spectroscopic and morphological characterization of the colloidal pastes supported the results obtained from SERS analyses. The interaction between the silver colloidal pastes and the fibers was also investigated by examining through a high-resolution scanning electron microscope the different natural fibers coated with the considered pastes.

Results showed that colloidal pastes derived from the Leopold and Lendl synthesis are unsuitable for the direct, extractionless analysis of dyed fibers. In fact, due to the lower surface potential of LL colloids, the process of centrifugation leads to fusing of the nanoparticles to obtain a honeycombed structure where most if not all hot-spots are lost, with dramatic decrease of SERS activity. These features resulted in a very inhomogeneous coating, as fibers coated with LL paste showed most uncoated areas. On the other hand, pastes obtained after the Lee and Meisel synthesis gave intense and reproducible SERS spectra of dyes used to color reference textiles. LM pastes are recommended for on-the-fiber SERS, as they provide a suitable coating that allows detection of signals of cochineal and brazilwood of both plant and animal origin. The addition of sodium perchlorate proved to enhance the dye signals with respect to LM paste when analyzing dyed plant fibers. Despite this, coating the fibers with Na-LM pastes was generally more difficult and fibers coated with these pastes displayed often lesser areas where good SER signals could be registered as respect to LM pastes. These issues can derive from the different aggregation of the silver nanoparticles, documented with high-resolution SEM images.

Attempts for obtaining a better coating by soaking the fibers in water were not successful, as a over-coating or less intense SER signals were observed in soaked samples. Trials to remove the citrate by rinsing the fibers after being coated did not give good results too, since together with the citrate peaks also the SER signals belonging to the dyes diminished. Besides, the hydrolysis of the samples before coating with silver colloidal pastes presented ambiguous results, which are confirmed by the work by Pozzi *et al.* (2012), who reported that carminic acid generally shows better SER spectra without pre-treatment. Finally, silver colloidal pastes proved to be not suitable for the analysis of extracts obtained from the Wouters procedure, as no SER signals were recorded.

SERS measurements of reference dyed fibers confirmed that the type of fiber does not affect the position of the diagnostic peaks for the considered dyes, as previously highlighted by Jurasekova *et al.* (2010) for wool and flax dyed with madder or cochineal investigated through SERS employing silver nanoparticles obtained by laser photoreduction. Also the use of different mordants does not influence the recorded spectra, even though in the case of a wool sample mordanted with alum, cream of tartar and iron(II) sulfate and dyed with cochineal the intensity of the signals was lower than that of a sample with similar color coordinates not mordanted with iron. Furthermore, no additional peaks were found also in cotton and flax fibers mordanted with gambier, a tannic dye also employed as a mordant, and dyed with brazilwood, possibly because brazilwood developed better interactions with silver nanoparticles and was located at the surface. In addition, silver colloidal pastes enabled the detection of carminic acid even in weakly colored fibers, while in the case of brazilwood this identification was more arduous. The possibility of registering SER spectra after several days from the coating of the fiber was also ascertained, although measurements undertaken on aged slides resulted in weaker SER signals of the dyes.

In conclusion, this work provided an in-depth characterization of the morphology of different silver colloidal pastes obtained through centrifugation of chemically reduced silver colloids, as well as of their coating on natural fibers. SER measurements highlighted the suitability of both LM and Na-LM pastes for on-the-fiber extractionless non-hydrolysis SERS, while LL pastes proved to be ineffective for this application. Despite the higher signal-to-noise ratio obtained on plant fibers coated with Na-LM pastes, the use of LM pastes should be preferred as they usually provide a better coating and a higher number of hot-spots. These considerations were further confirmed by some tests on historical samples, where the worse SER spectra were recorded when the coating was obtained with Na-LM pastes. As a matter of fact, SER signals were obtained on several samples taken from historical textiles by employing LM pastes, as reported in paragraphs 6.5, 6.6 and 6.8.

5.2 EVALUATION OF SERS PROCEDURES FOR THE ANALYSIS OF PURPLE PARCHMENTS

The analytical work for the non-invasive identification of colorant in purple codices described in paragraph 3.2 required to be integrated by a more selective micro-invasive study which would allow to better differentiate the different dyestuffs. Surface-enhanced Raman scattering is a suitable technique to set up a micro-invasive procedure for colored parchments as the minimum amount of sample necessary for this technique is in accordance with the conservative issues of these precious artworks. In fact, sampling is seldom allowed for manuscripts, and when this is the case the fragment detached should be as smallest as possible. The dyestuffs selected as possible candidates on the basis of information obtained from ancient treatises, already discussed in paragraph 3.2, were investigated through SERS after being coated with silver colloidal pastes obtained upon centrifugation of the Lee and Meisel colloid. In addition, the reference samples of dyed parchment were employed in order to test the best procedure for SERS analysis of minute fragments of parchment. Fragments of the parchments were drop-coated and analyzed with an *in situ* extractionless non-hydrolysis approach (e.g. without any pre-treatment) and after hydrolysis with hydrochloric acid. In addition, small portions of the reference dyed parchment were treated with drops of different extracting solutions at room temperature; both the parchment and the dried drop of solution were coated with silver colloidal pastes and analyzed. Some of the extracting solutions were also employed to extract the dyes from minute fragments of parchment following procedures proposed in the literature for HPLC or SERS analyses.

5.2.1 MATERIALS AND METHODS

Hydrochloric acid, nitric acid, methanol, N,N-dimethylformamide, formic acid, ethylenediaminetetraacetic acid, silver nitrate, sodium citrate were purchased from Carlo Erba reagents (Arese, Italy). Analytical grade orcein was obtained from Sigma Aldrich (St. Louis, USA). Ultra High Quality (UHQ) water was obtained by a Millipore (Darmstadt, Germany) Direct-q 3 system. The provenance of the dyestuffs, their extraction and the preparation of the dyed parchment have been described in paragraph 3.2.1. In addition, a cotton swatch dyed with folium provided by Kremer Pigmente GmbH & Co. (Aichstetten, Germany) was also considered.

5.2.1.1 SILVER COLLOIDAL PASTES

Silver colloidal pastes were prepared following the procedure described in paragraph 5.1.1.1. In this case, the volume of synthesized colloid was diminished in order to dispose of a smaller amount of colloid not employed for the centrifuging cycles. In particular, 0.036 g of silver nitrate were dissolved in 200 mL of heated UHQ water and 4 mL of a 0.1% solution of sodium citrate were added when the solution was vigorously boiling. The pastes were prepared fresh every week and employed up to two-four days after their preparation. The enhancement ability of the pastes was tested on stock solutions of water extracts of common dyestuffs (e.g. madder, logwood) and on a reference sample of wool mordanted with alum and cream of tartar and dyed with cochineal. In some cases, silver colloidal pastes were diluted with 20 or 50 μL of UHQ water in order to obtain a concentration of silver nanoparticles that enabled the development of a suitable coating of the sample for SERS analysis.

5.2.1.2 INSTRUMENTATION

SERS measurements were performed with a Renishaw (Stonehouse, Great Britain) inVia micro-Raman using 532 and 632.8 nm excitation lasers, 1800 lines per mm grating and a 100X Leica (Wetzlar, Germany) microscope objective to focus the laser beam onto the sample. Power at the samples never exceeded 300 µW. Spectra were recorded in the 100-1800 cm^{-1} range; in some cases the range was extended to 100-3500 cm^{-1}. Further details about this instrumentation are given in Appendix 2.

5.2.1.3 SAMPLE PREPARATION

All the considered dyestuffs and analytical grade orcein were analyzed after drop-coating 0.5 µL of silver colloidal paste on a small quantity of powder and mixing with a golden wire. The water extract from madder roots was instead analyzed directly by mixing 2 µL of the extract with the same volume of silver colloidal paste. All the samples were also investigated through traditional Raman spectroscopy, without the addition of silver colloidal pastes. The parchments were coated with 0.5 µL of silver colloidal paste, as well as the fragments of fibers from the cotton swatch. As for the fragments of parchments subjected to hydrolysis, they were exposed to hydrochloric acid vapors for ten minutes in a micro-chamber before being coated. In addition, small portions (less than 1x0.5 mm) of parchment were cut from the reference samples, deposited onto a microscope slide and treated with 0.5 µL of extracting solution at room temperature. Pure N,N-dimethylformamide, pure formic acid and a solution of hydrochloric acid:methanol:water (2:1:1) were considered for this purpose. After some minutes, the parchment was removed from the drop and coated with the silver colloidal paste, while the drop of solution was allowed to evaporate and then mixed with 0.5 µL of silver colloidal paste. Extracts from the parchment dyed with folium or orchil were obtained following different procedures reported in the literature (Hofenk de Graaff 2004, manh; Manhita *et al.* 2011; Rosi *et al.* 2013; Wouters and Verhecken 1989; Zhang and Laursen 2009). Small fragments of parchment (about 2x0.5 mm) were deposited in a micro-vial and 50 µL of the selected solution were added. Table 5.1 reports the experimental conditions for each procedure. The liquid was then evaporated to dryness and 50 µL of methanol were added to the residues. 2 µL of the extract were dropped onto a microscope slide and mixed with the same volume of silver colloidal paste. The extraction with HCl:MeOH:H$_2$O (2:1:1, v/v/v) was also employed on the cotton swatch dyed with folium.

Table 5.1: Experimental conditions for the extraction of dyes from colored parchments.

Extracting solution	Temperature (°C)	Time (min)	Orchil parchment	Folium parchment
HCl:MeOH:H$_2$O (2:1:1, v/v/v)	90	10	X	X
FA	40	30	X	X
FA:MeOH (1:1, v/v)	40	30	X	X
FA:MeOH (5:95 v/v)	40	30	X	X
FA	T$_{room}$	24h	X	X
EDTA 0.1% in H$_2$O:DMF (1:1, v/v)	100	30	X	X
DMF	60	30	X	X

HCl=hydrochloric acid, MeOH=methanol, H$_2$O=water, FA=formic acid, EDTA= ethylenediaminetetraacetic acid, DMF= N,N-dimethylformamide.

5.2.2 RESULTS AND DISCUSSION

5.2.2.1 SERS OF PURPLE DYESTUFFS

SER spectra of **madder** were recorded both with the 532 and the 633 nm excitation wavelengths (Figure 5.28, black and red spectra). Intense peaks were found at 342, 1186, 1288, 1329, 1448 and 1461 cm^{-1}, while weaker peaks were positioned at 309, 391, 418, 450, 478, 582, 632, 660, 682, 763, 830, 898, 942, 1013, 1047, 1161, 1548 and 1625 cm^{-1}. These peaks correspond to tabulated data for alizarin and purpurin (Leona, Stenger, and Ferloni 2006; Lofrumento *et al.* 2013; Whitney, Van Duyne, and Casadio 2006), the two main dyeing molecules of madder and are reproducible over different trials. The shift between data recorded with the 532 and the 633 nm laser was always below 8 cm^{-1}.

The SER spectra of **kermes** lake recorded with 633 nm laser (Figure 5.28, blue spectrum) showed peaks at 1135 (sh), 1203, 1273, 1295 (sh), 1310 (s), 1361 (s), 1425 (s), 1466, 1485, 1507 and 1559 cm^{-1}, which presented a good reproducibility even though some intensity variations were observed in the main peaks. The position of the peaks did not vary according to the excitation wavelength, apart from a slight shift of the peak at 1425 cm^{-1} towards higher wavenumbers when the sample was excited with the 532 nm laser (Figure 5.28, green spectrum). The results are consistent with previously reported data (Leona, Stenger, and Ferloni 2006; Whitney, Van Duyne, and Casadio 2006).

The spectra recorded on a lake obtained from **Armenian cochineal** did not present peaks that could be attributed to carminic acid, the main dyeing molecule of this dyestuff, or to other coloring matters.

When excited with the 532 nm wavelength, a powder extract of **Tyrian purple** (Figure 5.29, red spectrum) revealed the presence of strong peaks at 306, 629, 1253, 1580 and 1625 cm^{-1}, while medium peaks were found at 385, 693, 937, 1302, 1316, 1363, 1446 and 1699 cm^{-1} and weak peaks were positioned at 123, 186, 190, 575, 757, 1050, 1105, 1192 and 1212 cm^{-1}. The Raman spectrum recorded with the 633 excitation wavelength (Figure 5.29, green spectrum) presented fewer and less intense signals, recorded at 307, 386, 629, 691, 760, 1252, 1304 and 1581 and 1624 cm^{-1}, in good agreement with the signals obtained with the 532 nm laser and with the literature (Bruni, Guglielmi, and Pozzi 2010; Van Elslande, Lecomte, and Le Ho 2008). No signals belonging to indigoid dyes were instead registered after coating the powder of Tyrian purple with silver colloidal pastes. This might be due to the steric hindrance of the bromine atoms which prevent the interaction between the -NH groups of 6,6'-dibromoindigotin and the silver nanoparticles, as reported by Bruni *et al.* (2010).

Analytical grade **orcein** presented identifiable signals also when analyzed through traditional Raman spectroscopy. In particular, peaks at 201, 432, 475, 524, 586, 625, 821, 1073, 1184, 1276, 1408, 1520 and 1643 cm^{-1} were identified (Figure 5.30, black spectrum). Slight shifts were found in the SER spectra of this compound (Figure 5.30, red spectrum) as respect to the Raman spectrum, namely for peaks at 478, 597, 631, 1078, 1193, 1523, 1647 cm^{-1}, while additional peaks were found at 891, 1234, 1317, 1549 cm^{-1}. SER spectra of **orchil** were recorded both with 532 and 633 nm

excitation wavelength. Spectra recorded with 633 nm wavelength (Figure 30, green spectrum) presented strong peaks at 520, 625, 1408 and 1643 cm^{-1}, while other peaks were found at 425 (w), 474 (m), 718 (w), 825 (w), 1188 (m), 1311 (m), 1517 (w), 1592 (w), 2923 (m) and 2969 (sh). It should be observed that the peaks in the 2900-3000 cm^{-1} region were not recorded in any of the spectra of orcein. Moreover, spectra recorded with 532 nm excitation wavelength (Figure 5.30, blue spectrum) displayed a residual fluorescence in the 1100-1650 cm^{-1} region, nevertheless peaks were identifiable at 475, 520, 619, 726, 756, 827, 989, 1074, 1185, 1274, 1322, 1347, 1373, 1420, 1526, 1552, 1579 and 1643 cm^{-1}. The spectra recorded on orchil are in good accordance with spectra of orcein, even though some additional peaks were observed, mainly in the spectra recorded with 532 nm excitation wavelength (e.g. 726, 756, 989, 1347, 1373, 1579 cm^{-1}). In addition, the SER spectra recorded on orcein were comparable with the only published SER data obtained with the 785 nm excitation wavelength (Leona, Stenger, and Ferloni 2006), while no SER spectra were reported before for orchil. Finally, SER spectra of orchil were compared with subtracted shifted Raman spectra, both obtained with the 533 nm excitation wavelength (Rosi *et al.* 2013). Even though the relative intensities of the peaks of SRR and SER spectra were often different, all the major peaks observed in the SRR spectrum were found in the SER spectra with slight shifts, except for a peak at 1497 cm^{-1}, which was not present in the SER spectra, and for a wider shift of the 652 cm^{-1} SRR peak, found at 619-629 cm^{-1} for all the spectra of orcein and orchil investigated in this work.

SER spectra obtained on **folium** powder with the 633 nm excitation wavelength (Figure 5.31, red spectrum) presented strong peaks at 637, 1466 and 1641 cm^{-1} while less intense peaks were found at 433 (w), 504 (sh), 517 (m), 551 (w), 578 (m), 595 (sh), 658 (sh), 690 (w), 749 (w), 1032 (w), 1135 (w), 1478 (sh) and 1575 (w). Weaker signals were observed when hitting the sample with the 532 nm laser (Figure 5.31, black spectrum), anyway the main peaks were observed with shifts inferior than 8 cm^{-1}. SER spectra of cotton fibers dyed with folium (Figure 5.31, green spectrum) presented less numerous peaks if compared with SER spectra obtained on folium powder. Despite this, the main signals found in folium powder were in any case present at 579, 638, 1465, 1486 and 1641 cm^{-1} with weaker signals at 373, 429, 474, 503, 701, 1318, 1557 cm^{-1}. Also the extract of cotton presented the main SER peaks registered for folium powder (Figure 5.31, blue spectrum), at 578, 643, 1470 and 1645 cm^{-1}, while weaker signals were at 505, 1030, 1295, 1591 cm^{-1}. To the best of the author's knowledge, no previous SER spectra of folium are reported in literature, thus the spectra recorded during this experimental work can be considered the first SER spectra obtained on this dyestuff. As no molecular information is available about *Chrozophora tinctoria* (L.) A. Juss., no spectroscopic considerations about the vibrational modes that determine the peaks can be made.
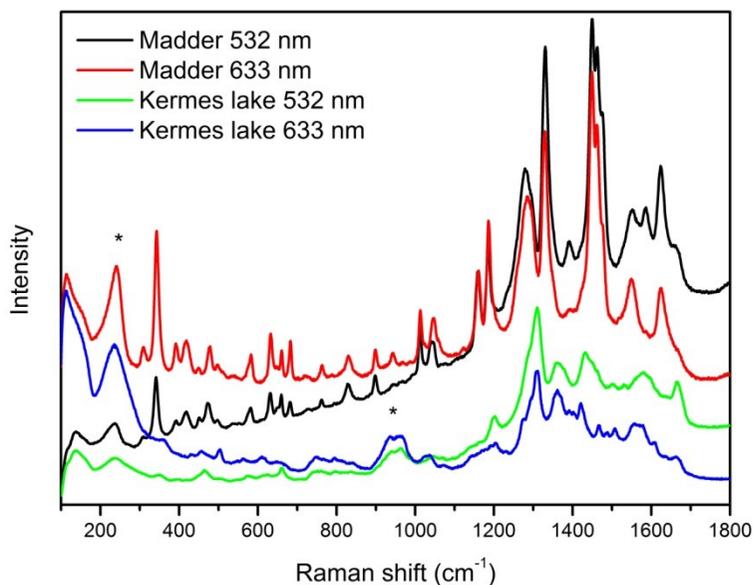
Figure 5.28: SER spectra of: madder, λ$_{ex}$= 532 nm (black spectrum); madder, λ$_{ex}$= 633 nm (red spectrum); kermes lake, λ$_{ex}$= 532 nm (green spectrum); kermes lake, λ$_{ex}$= 633 nm (blue spectrum);); asterisks indicate peaks belonging to the paste.
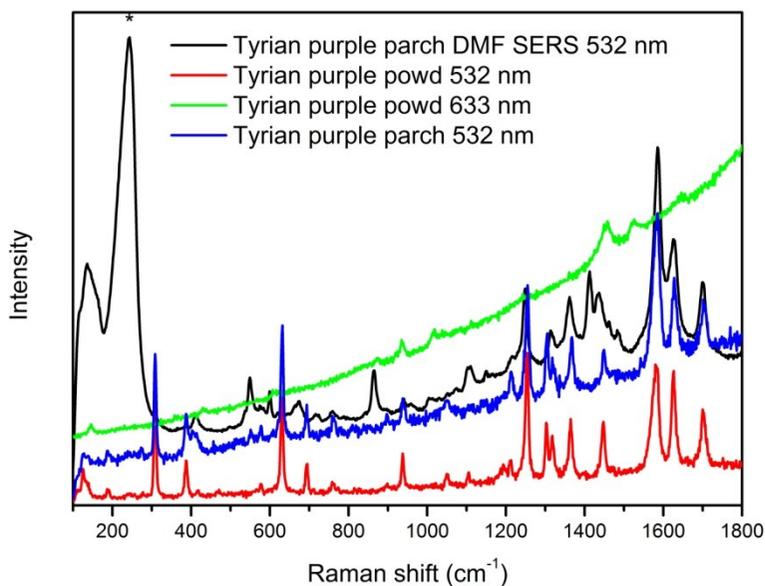
Figure 5.29: Raman and SER spectra of Tyrian purple: DMF drop on dyed parchment coated with LM paste, λ$_{ex}$= 532 nm (black spectrum); powder, λ$_{ex}$= 532 nm (red spectrum); powder, λ$_{ex}$= 633 nm (green spectrum); parchment, λ$_{ex}$= 532 nm (blue spectrum); asterisks indicate peaks belonging to the paste; blue and red spectra were multiplied by 20, green spectrum was multiplied by 2, spectra were stacked for ease of comparison.
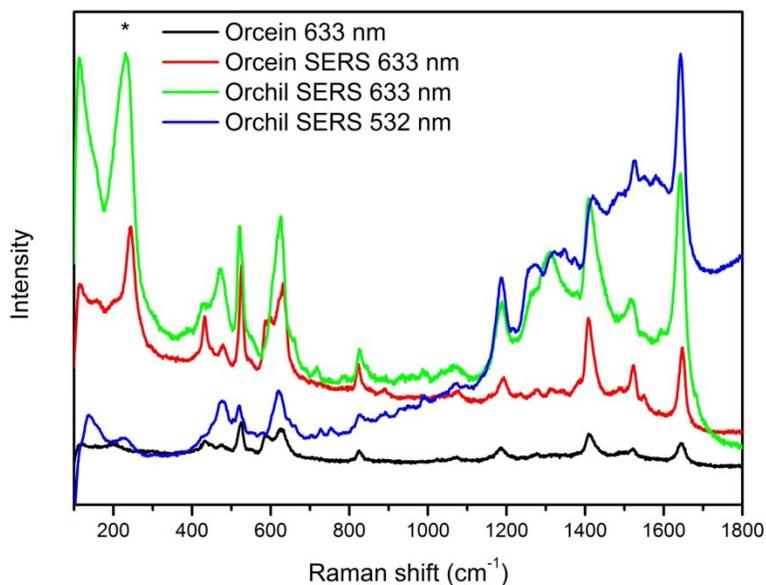
Figure 5.30: Raman and SER spectra of orcein and orchil: orcein, Raman λ_{ex}= 633 nm (black spectrum); orcein, SERS, λ_{ex}= 633 nm (red spectrum); orchil, SERS, λ_{ex}= 633 nm (green spectrum); orchil, SERS, λ_{ex}= 532 nm (blue spectrum); asterisks indicate peaks belonging to the paste; spectra were stacked for ease of comparison.
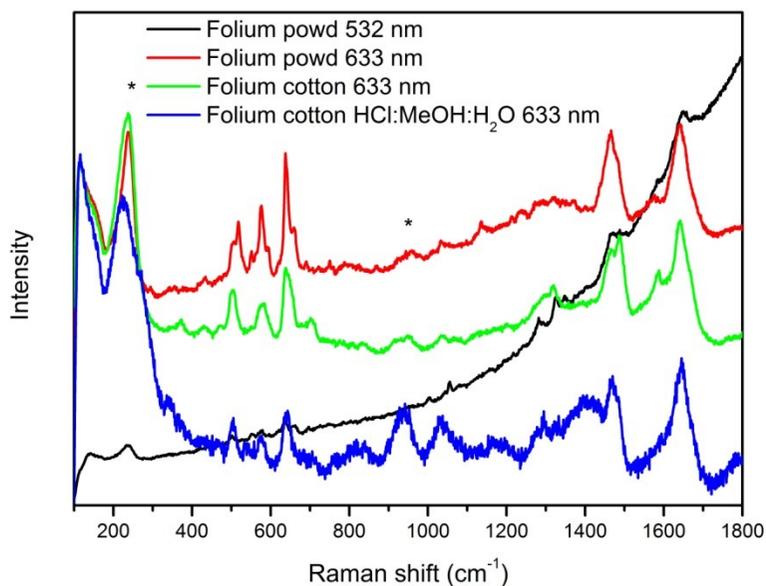
Figure 5.31: SER spectra of folium: powder, λ_{ex}= 532 nm (black spectrum); powder, λ_{ex}= 633 nm (red spectrum); cotton, λ_{ex}= 633 nm (green spectrum); HCl:MeOH:H$_2$O extract from cotton, λ_{ex}= 633 nm (blue spectrum); asterisks indicate peaks belonging to the paste; black and green spectra were multiplied by 2, blue spectrum was multiplied by 5; spectra were stacked for ease of comparison.

5.2.2.2 PROCEDURES FOR THE ANALYSIS OF PURPLE PARCHMENTS

Traditional Raman measurements of parchment dyed with **Tyrian purple** presented a high fluorescence background in the case of parchments provided by Dr. Rolf Haubrichs, while the Raman spectra of the parchment dyed in our laboratory presented identifiable peaks (Figure 5.29, blue spectrum). Those peaks were well related with previously discussed peaks observed on the powder extract of Tyrian purple (Figure 5.29, red and green spectra). On the other hand, parchments directly coated with silver colloidal pastes did not show SER signals attributable to the coloring matters of Tyrian purple. The pre-treatment with a drop of DMF was instead effective in enhancing some signals from a goat parchment dyed with fresh glands of *Hexaplex trunculus* (Linnaeus, 1758) (Figure 5.29, black spectrum). In this case, only some SER signals were attributed to Tyrian purple, namely the peaks at 1104, 1248, 1313, 1361, 1585, 1625 and 1699 cm^{-1}, while other peaks at 413, 549, 599, 674, 789, 864, 1031, 1053, 1071, 1110, 1413 and 1436 cm^{-1} were not assigned. The presence of a number of spurious peaks confirms the above mentioned poor interaction of 6,6'-dibromoindigotin with silver nanoparticles and thus Raman signals of other competing species could be enhanced on complex matrices such as parchments. The results obtained reveal that the accurate identification of Tyrian purple is strongly dependent on the features of the parchment, as in some cases traditional Raman spectroscopy could be employed, while in other cases none of the proposed approaches were effective in obtaining signals from the dye. It should also be noted that, despite the impossibility of registering good SER signals with *in situ* extractionless non-hydrolysis SERS, the coating with silver nanoparticles allowed to verify under the optical microscope the presence of very dissimilar morphologies of the coated and uncoated areas, which seemed mainly due to the production technique and the finishing of the parchment, apart from the small differences in the coating previously discussed for textile fibers (5.1.2.3).

Silver colloidal pastes directly spread onto the parchment dyed with **orchil** were effective in enhancing the signals of the dye (Figure 5.32), even though their intensity was lower than what observed for the powder sample. In particular, the peaks below 800 cm^{-1} were more intense, while weaker signals were found in the 1000-1700 cm^{-1} region. The position of the peaks was in quite good accordance with that of powder orchil: 419 (w), 461 (w), 476 (w), 522 (s), 602 (sh), 619 (s), 630 (sh), 658 (w), 818 (m), 1186 (w), 1410 (m), 1526 (w) and 1645 (m). Furthermore, no results were obtained after pre-treating fragments of the parchment with drops of extracting solutions at room temperature, even though in the case of pure formic acid the drop turned rapidly to a reddish color. In reverse, some signals could be identified in the SER spectra obtained from the extract of the parchment kept in formic acid at room temperature for twenty-four hours. Despite the low signal-to-noise ratio of these spectra, peaks at 515, 617, 1412 and 1643 cm^{-1} could be identified, which correspond to the main peaks of orchil. Very weak signals were instead recorded on the other extracts and on the parchment subjected to hydrolysis with HCl, therefore in these cases the dye could not be correctly identified. Also in the case of orchil the coating of the parchment proved to be a major issue, as several trials should be made after obtaining a correct coverage of the sample.

Folium parchment coated with silver nanoparticles displayed diagnostic signals both when excited with the 532 and the 633 nm laser. SER spectra recorded employing the 633 nm excitation

wavelength (Figure 5.33, red spectrum) presented the stronger signals at 1467, 1483 (sh) and 1641 cm^{-1}, with medium peaks at 503, 572 and 640 cm^{-1} and weak peaks at 370, 583 (sh), 595 (sh), 684, 1000, 1033, 1068, 1117, 1289, 1319, 1555 cm^{-1}, most of which corresponding to the SER peaks observed for powder folium. The spectra recorded with the 532 nm excitation wavelength (Figure 5.33, black spectrum) presented higher signals if compared with the SER spectra of the powder excited with the same laser. In particular, peaks were recorded at 472 (w), 494 (w), 638 (m), 728 (w), 1072 (w), 1127 (w), 1240 (w), 1322 (w), 1465 (s), 1487 (s), 1577 (w) and 1652 (s). Also in this case, folium could be correctly identified. Spectra obtained from a drop of DMF incubated on the parchment for some minutes and then coated with silver colloidal pastes (Figure 5.33, green spectrum) presented the stronger signals at 578, 1476 and 1645 cm^{-1}, while weaker signals were located at 506, 844, 1043 and 1284 cm^{-1}. All but the peak at 844 cm^{-1} could be attributed to folium after comparison with the SER spectra recorded on powders and cotton fibers. No SER spectra could be recorded with all the other tested procedures.
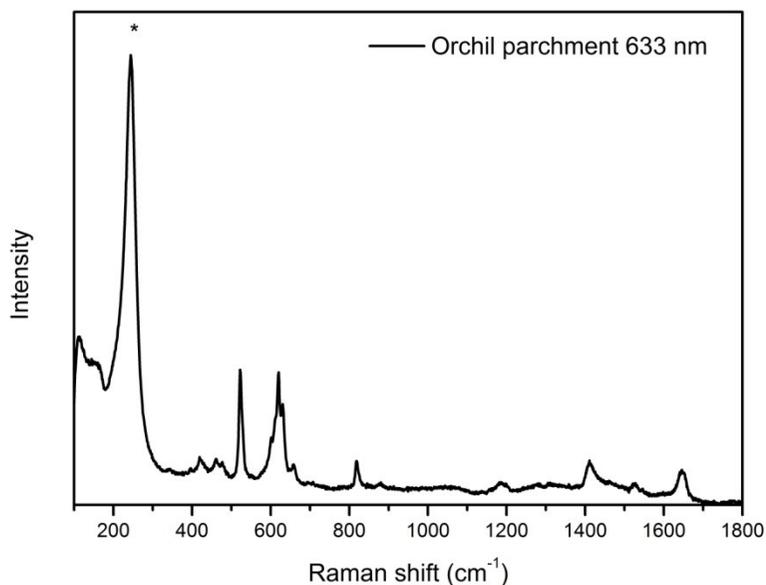
**Figure 5.32: SER spectrum of orchil parchment, λ$_{ex}$= 633 nm; asterisks indicate peaks belonging to the paste.**
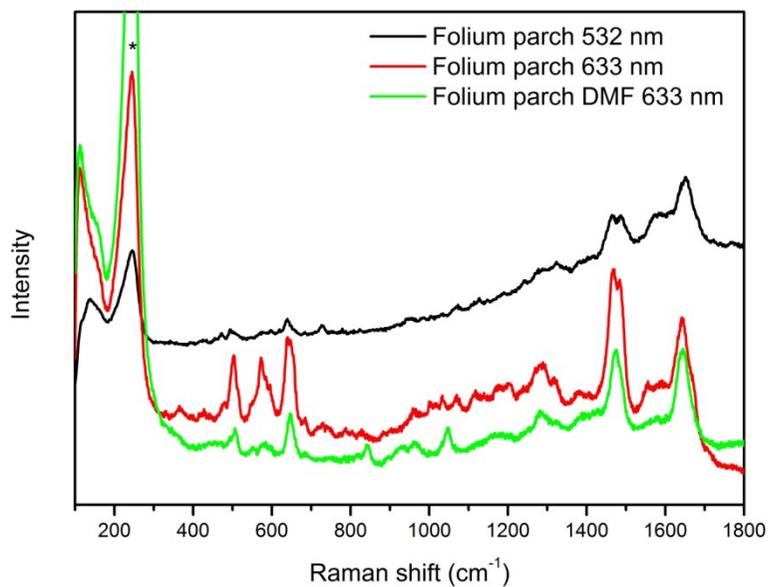
Figure 5.33: SER spectra of folium parchment: λex= 532 nm (black spectrum); λex= 633 nm (red spectrum); DMF drop on dyed parchment, λex= 633 nm (green spectrum); asterisks indicate peaks belonging to the paste; spectra were stacked for ease of comparison.

5.2.3 CONCLUSIONS

In this work, a number of dyestuffs employed, alone or with indigo, to color purple parchments of the so-called purple codices, were investigated through SERS. SER signals were obtained from madder, kermes, Tyrian purple, orchil and folium while no peaks attributable to its coloring matters were recorded for Armenian cochineal.

In the case of orchil, no previous spectra were recorded on this extract from various lichens, even though Leona *et al.* (2006) reported the SER spectrum of synthetic orcein, the main dyeing molecule of orchil, obtained with the 785 nm excitation wavelength. The comparison between the recorded data and the tabulated peaks reported in that work allows to confirm that orchil is mainly composed by orcein, or orcein has the best SERS cross-section and interaction with silver nanoparticles among its main molecules, even though slight differences were observed.

Furthermore, no records were found in the literature of previous SER analyses on folium, the extract from *Chrozophora tinctoria* (L.) A. Juss, and thus the above reported data can be considered the first obtained from this dyestuff through SERS. The position of the peaks was confirmed over multiple trials performed with two different excitation wavelengths (e.g. 532 and 633 nm) and on different substrates (e.g. extract in powder, dyed textile, dyed parchment, extract from the dyed parchment). Unfortunately, the molecular structure of the coloring matters contained in folium is still unknown and thus the data could not be supported by an assignment of the various SER signals to specific vibrational modes of the molecule(s).

The measurements also evidenced the complexity of obtaining SER spectra from Tyrian purple, as previously reported by Bruni *et al.* (2010). In fact, good Raman spectra could be obtained on the powder extract without coating the sample with silver nanoparticles, while no signals were recorded after the coating. The same observations were valid when analyzing dyed parchment, as for one reference sample no coating was necessary to obtain a signal, while traditional Raman measurements on the other samples showed only a high fluorescence background. In these cases, some results were obtained after treating the parchment with a drop of DMF and then coating the dried drop with silver nanoparticles, while no spectra were obtained with the extractionless non-hydrolysis approach. Nevertheless, also in the case of DMF treatment a number of spurious signals not attributable to the dye were observed.

SER signals were successfully recorded with the extractionless non-hydrolysis approach both on parchments dyed with orchil and folium. In the first case, worse quality spectra were obtained from the parchment as respect to the SER spectra recorded on the powder extract. In reverse, SER spectra of the parchment dyed with folium presented signals with high signal-to-noise ratio, even better than those recorded on the powder extract as regards the 532 nm excitation wavelength. No results were obtained on fragments of parchment hydrolyzed with hydrochloric acid, while weak signals were recorded on a formic acid extract from the parchment dyed with orchil and medium signals were obtained from a drop of DMF incubated in a fragment of parchment dyed with folium.

In general, obtaining a proper coating on parchment was arduous, undoubtedly more than for dyed textiles, and the process was strongly influenced by the morphology of the parchment surface. As for Tyrian purple, the type of parchment influenced the correct identification of the dye. In addition, the pre-treatments with drops of extracting solutions and the extraction procedures showed ambiguous results. In the first case (e.g. extraction at room temperature directly on the microscope slide with drops of solution) the failed extraction might be due to a low affinity of the molecules with the extracting solution. This means that only dyes with high affinity with the solution can be extracted with this method and thus it is unlikely to be transferred to historical samples, where the dye is not known. In the second case (extraction in a vial at a controlled temperature for a determined amount of time) the lack of SER signals might be due to the very restricted amount of sample selected for these tests, that often did not even color the solution. In addition, some extracting solutions might have a scarce affinity with the silver nanoparticles, as previously observed in the case of the here employed procedure by Wouters and Verhecken (1989) with a solution of $HCl:MeOH:H_2O$ (2:1:1, v/v/v) (5.1.2.3).

In conclusion, the most likely dyestuffs employed to color the parchment of purple codices were investigated through SERS and their spectral features successfully ascertained or confirmed. Several procedures to obtain SER spectra from dyed parchments were also tested on mock-ups dyed with some of the considered dyestuffs. The results indicated that the morphology and the complexity of the matrix under analysis both play a major role in allowing the identification of the dyestuff. Unfortunately, a standard procedure cannot be proposed, as different approaches proved to be effective depending on the dyestuff considered and on the type of parchment. The availability of a fragment of unusually large dimension taken from a precious 6th century purple codex (the so-called *Codex Brixianus*) allowed to re-perform some of the discussed tests directly on a historical sample. Those results confirmed the issues encountered for SER analysis of the reference samples, even though the identification of the dye was achieved after several trials thanks to the comparison with the SER spectra discussed in this chapter. The results of the non-invasive investigation of *Codex Brixianus* as well as the analytical procedure undertaken for SERS analysis will be discussed in paragraph 6.7.

6.1 SPECTROMETRIC AND CHROMATOGRAPHIC SURVEY OF AN EMBROIDERED PANEL FROM BOSCO MARENGO

Chromatographic techniques, in particular high performance liquid chromatography coupled with spectrophotometric and/or mass detectors, are well established in the field of dyes identification, since they provide reliable and in-depth information about the dyeing molecules that can be extracted from different historical samples (Rosenberg 2008). A number of papers has been already published on this topic and previously discussed in this work (4.5). Moreover, the comparison of the results obtained by means of chromatography with those obtained with UV-Vis spectroscopy proved to be a very valuable approach for the understanding of degradation paths of natural dyestuffs such as madder, saffron and flavonoid-based dyestuffs (Ferreira *et al.* 2002; Vickackaite *et al.* 2004; Clementi *et al.* 2007).

In the present case, the use of liquid chromatography was aimed at assessing the applicability of the non-invasive procedure for the identification of textile natural dyestuffs developed in this work (3.1) on real historical samples. In particular, an embroidered textile panel from the parish of Bosco Marengo (Italy), was considered. The accurate identification of the dyestuffs by means of fiber optics reflectance spectrometry in the visible range (Vis-FORS) was tested by high performance liquid chromatography coupled with a diode array detector and mass spectrometry (HPLC-DAD-MS) performed on samples taken from the same areas investigated through the non-invasive technique. The results of this work have been reported together with part of the results of the experimental work for the identification of natural dyes in textiles, described in paragraph 3.1, in a recent paper, published in Dyes and Pigments (Gulmini *et al.* 2013).

6.1.1 MATERIALS AND METHODS

THE EMBROIDERED TEXTILE PANEL

The embroidered panel under investigation was hung in the church of Santa Croce in Bosco Marengo (Alessandria, Italy). It is part of a series of six textile panels and it was used to cover one of the pilaster strip of the church's central nave. The scientific investigation was carried out during its restoration and, after the conservation treatments, the panel will be moved to a museum in order to prevent further damages. The embroidery is made in half cross stitch with colored silk yarns on a flax cloth. The details are obtained with a smaller stitch on a black silk canvas sewn to the flax cloth. A thick glue layer was spread in the rear of the cloth, probably at the time of its production, in order to give stiffness to the panel.

The dimensions and the quality of the embroidery are peculiar. Because of the lack of documentary evidences no information on provenance, on the commissioner or on the date of

execution of this artwork are available. According to the restorer's experience, its production can be possibly dated to the end of the 16th or the beginning of the 17th century AD, while the provenance was not definable with the information at her disposal.

The textile panel represents the Deposition of Christ and it is organized in three scenes enclosed in medallions; the entire depiction is surrounded by a colorful floral decoration with fruits (Figure 6.1.1). The upper part of the textile shows an angel with a scroll, while in the central one the Deposition of Christ is illustrated. In the lower part of the panel, a pelican with his chest ripped open feeds his chicks with his own blood; the pelican represents Christ himself and his charity and two angels are at his sides.

The overall conservation state was good, except for a deformed area, some bare areas that required integration and a general extended fading of colors, due to the extended exposition to light which caused photo-degradation.

Vis-FORS measurements were carried out on seventeen colored areas of the cloth, both from the *recto* and the *verso*. Figure 6.1.1 reports the positions of the investigated areas. Samples for HPLC-DAD-MS analyses were taken from the back of the cloth by cutting small portions of the selected colored threads (Figure 6.1.2). A total of fourteen samples were analyzed.

INSTRUMENTATION

Vis-FORS spectra were recorded using a Corona45Vis spectrophotometer by Zeiss. The geometry of the fiber optics was set to 0°/45°x2 or 0°/0° depending on the dimensions of the area under analysis. Further details about this instrument are reported in Appendix 2.

HPLC-DAD-MS analysis were performed *on* 1-2 mg of the yarns sampled from the historical cloth. The samples were treated in centrifuge tubes in order to extract the colorants from the substrate fibers. An orange-pink sample was subdivided into two portions which were extracted with different solvents. The extraction procedures here employed follow, with slight modifications, those indicated in previous works (Manhita *et al.*; Karapanagiotis and Chryssoulakis 2005; Zarkogianni, Papliaka, and Tsatsaroni 2009; Valianou, Karapanagiotis, and Chryssoulakis 2009) and are reported in Gulmini *et. al.* (2013). For sake of completeness, the procedure is also reported below.

Red and a portion of the orange-pink samples were extracted with 0.5 mL of concentrated hydrochloric acid/water/methanol (2:1:1 v/v/v) by immersing the tubes into a boiling water bath for 10 minutes.

Yellows and a portion of the orange pink sample were extracted with 0.5 mL of 5 mol·L^{-1} formic acid/water/methanol (2:1:1 v/v/v) by immersing the tubes into a boiling water bath for 10 minutes. Then 0.25 mL of a solution of EDTA 0.5·10^{-3} mol·L^{-1} were added and the heating was further prosecuted for 5 minutes. This procedure (henceforth named FA-EDTA) was also employed as extraction step for blue and green samples (see below).

Blue and green samples were subjected to subsequent extractions with different solvents. The extracts obtained from each solvent were analyzed separately.

Blue samples were treated with 0.5 mL of dimethylformamide (DMF) by immersing the tubes into a water bath at 60°C for 30 minutes. The fibers were then recovered from the organic solvent, transferred into a clean centrifuge tube and extracted as the yellow samples with the FA-EDTA procedure indicated above.

Green samples were subjected to the FA-EDTA extraction, then the fibers were recovered and transferred into a clean tube, where they were treated with DMF as the blue ones.

After having picked out the fibers from the tubes with tweezers, the extracts were evaporated to dryness under a gentle stream of nitrogen at 65°C; then the residues were dissolved with 0.5 mL $H_2O:CH_3OH$ (1:1 v/v) and centrifuged at 4000 rpm for 15 min. Finally, the upper clear solutions were transferred into glass vials to be injected into the HPLC system. HPLC conditions are reported in Appendix 2.

Figure 6.1.1: Embroidered panel from Bosco Marengo. Position of the FORS measurements; asterisks indicate areas analyzed on the reverse side of the textile.

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Figure 6.1.2: Reverse side of a light green area sampled for HPLC-DAD-MS analyses.

6.1.2 RESULTS AND DISCUSSION

Table 6.1.1 summarizes the dyes extracted from the panel and identified by HPLC-DAD-MS analyses. Moreover, key information for their identification is also reported. Results of HPLC-DAD-MS are considered here in view of their relevance for the discussion of results obtained by FORS.

Reflectance spectra of blues were recorded on two areas of the front side and from one of the reverse side of the cloth. All the spectra showed the features attributed in the methodological work (3.1.2.1) to a blue indigoid vat dye (Figure 6.1.3). Besides this, some slight differences could be observed by comparing the spectra obtained in the *recto* with those recorded on the *verso*. In particular, the minimum at about 660 nm of indigoid dyes was slightly blue-shifted (5-10 nm) in spectra from the front side (AEM02, AEM03), while the position of the maximum in the blue-violet region varied of more than 50 nm (the *verso* spectrum, AEM16, is shifted towards the violet region). In addition, two relative minima at about 540 and 820 nm were observed in the less faded area (e.g. *verso*), whereas these signals were no longer detectable in the faded portions. The identification of the dyestuff performed through reflectance spectroscopy was confirmed by HPLC-DAD-MS analyses, which indicated the presence of indigotin in the three investigated blue samples (Table 6.1.1).

Red and red-pink areas AEM06 and AEM05 (Figure 6.1.4) investigated in the *recto* showed the same spectral features (Figure 6.1.5, black and red spectra). In particular, both the spectra presented an absorption band structured in two sub-bands at about 525 and 565 nm, features that were attributed to scale insects dyes. Also a relative maximum in the blue region (460-465 nm) and an inflection point at 600-605 nm were observed. Differently, the reflectance spectrum recorded on the *verso* of an orange area (Figure 6.1.5, green spectrum, AEM13) showed a single absorption band at 520-525 nm, a maximum at 470-475 nm and an inflection point at 575-580 nm. The position of the absorption band suggested here the possible use of brazilwood (mordanted with

alum), although a yellow dyestuff should have been added to the red brazilwood in order to achieve the orange hue.

HPLC-DAD-MS analyses identified carminic acid as the main coloring matter of red and red-pink areas, thus confirming and further specifying the attribution given by reflectance spectroscopy. As far as the orange sample was concerned, the chromatographic analyses revealed the presence of genistein and luteolin and of some of their glycosylated forms and derivatives (Table 6.1.1) along with brazilein. Genistein and luteolin, together with their related compounds, are yellow dyes that can be found in dyer's broom (*Genista tinctoria* L.), while brazilein is one of the main red colorants contained in brazilwood (1.3.2).

The reflectance spectra recorded on the front side of the textile on yellow areas (AEM01, AEM04) did not reveal any characteristic spectral features (Figure 6.1.6, black and red spectra) that may suggest the use of saffron and other yellow carotenoids. Therefore the yellow color of the yarns shall be attributed to flavonoids or curcuminoids (3.1.2.3). On the contrary, the yellow area investigated in the *verso* (AEM17) showed different spectral features (Figure 6.1.6, green spectrum), with an absorption band at about 650 nm. This feature is not characteristic of yellow dyes but it suggests the presence of a small amount of an indigoid blue dyestuff in the area mixed with an unknown flavonoid or curcuminoid yellow dyestuff. HPLC-DAD-MS measurements undertaken on some yellow samples showed the presence of the same compounds revealed in the orange sample (Table 6.1.1), thus pointing to the use of dyer's broom.

Spectra recorded on deep green (AEM08, AEM14), light green (AEM09, AEM15) and green-blue (AEM07) areas of the front (AEM07, AEM08, AEM09) and the reverse side (AEM14, AEM15) of the panel (Figure 6.1.7) showed similar spectral features in the red region, that are an absorption band at about 650-660 nm and an inflection point at 705-720 nm. On the contrary, the relative maximum in the blue-green region varied from about 500 to 565 nm, and this accounts for their different color. In particular, light green areas showed bathocromic shifts of the reflectance maxima (Figure 6.1.7, green and cyan spectra). Moreover, an additional feature could be identified in the spectra recorded on the *verso* (Figure 6.1.7, blue and cyan spectra), that is the presence of a second inflection point at about 480-490 nm. The absorption band and the inflection points can be related to the use of an indigoid blue dyestuff, while the relative maxima in the blue-green region depend on the presence of a yellow dyestuff. Since no absorption bands were evidenced in the 420-450 nm region, it is possible to confine the employed yellow dyestuff to flavonoids or curcuminoids. The data obtained through HPLC-DAD-MS ascertained the presence of indigotin, luteolin and genistein (with their related compounds), thus confirming the use of double dyeing (3.1.2.6), where a blue indigoid dyestuff was superimposed on yarns dyed yellow by dyer's broom.

Deep brown (AEM12), light brown (AEM11) and green-brown (AEM10) areas showed reflectance spectra with no identifiable spectral features (Figure 6.1.8), except for the green-brown area, in which an absorption band at about 655 nm was observed. This feature indicates the presence of an indigoid blue dyestuff to obtain the greenish hue, but no attribution can be made for the brown dyestuff, as in the other two brown areas. In this case, no samples were taken in order to perform HPLC-DAD-MS measurements.

Table 6.1.1: Dyes revealed via HPLC-DAD-MS on extracts obtained from threads sampled from the decorative historical panel. Absorption maxima, molecular ions and fragment ions obtained by collision induced dissociation (CID) are reported. (Gulmini *et al.* 2013)

Compound	Ionization mode	$[M + H]^+$ or $[M-H]^-$ *(m/z)*	CID fragment ions *(m/z)*	λ_{max} *(nm)*
Genistein methyl ether glucoside	ESI+	447	285	255, 323
Luteolin glucoside	ESI+	449	287	228, 276, 347
Genistein glucoside	ESI+	433	271	260, 325
Genistein methyl ether	ESI+	285	270, 242, 229	256, 326
Luteolin	ESI+	287	241, 153	253, 346
Genistein	ESI+	271	253, 243, 215, 153	260, 325
Dc II	ESI+	667	649, 635, 607, 564, 536, 465, 463	238, 285, 434
Carminic Acid	ESI+	493	355, 379, 373	276, 495
Brazilein	ESI-	283	265, 268	244, 280, 330, 445
Indigotin	ESI+	263	235, 219, 207, 132	244, 285, 610
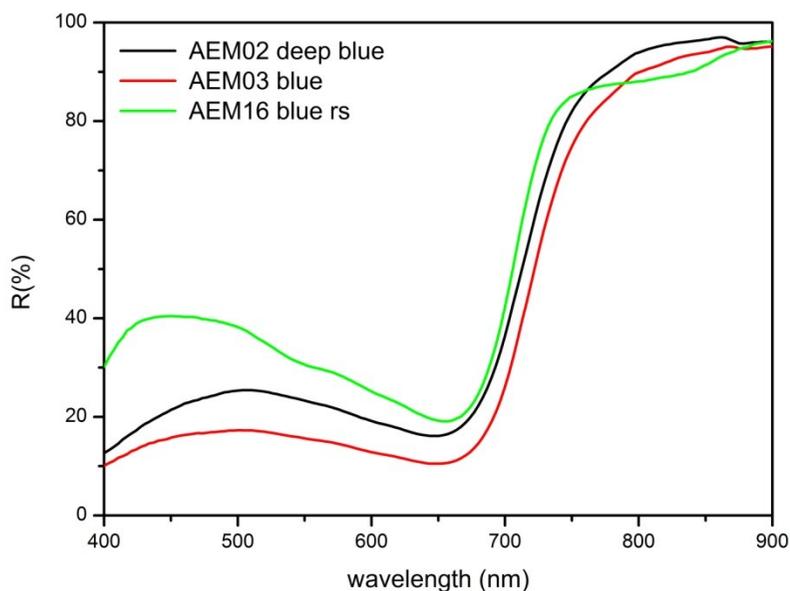
Figure 6.1.3: Reflectance spectra of blue areas investigated in the embroidered panel Black and red spectra were recorded on the front side, green spectrum was recorded on the reverse side.

Figure 6.1.4: Detail of the embroidered panel. The red pink area of the flower was analyzed by means of reflectance spectroscopy.
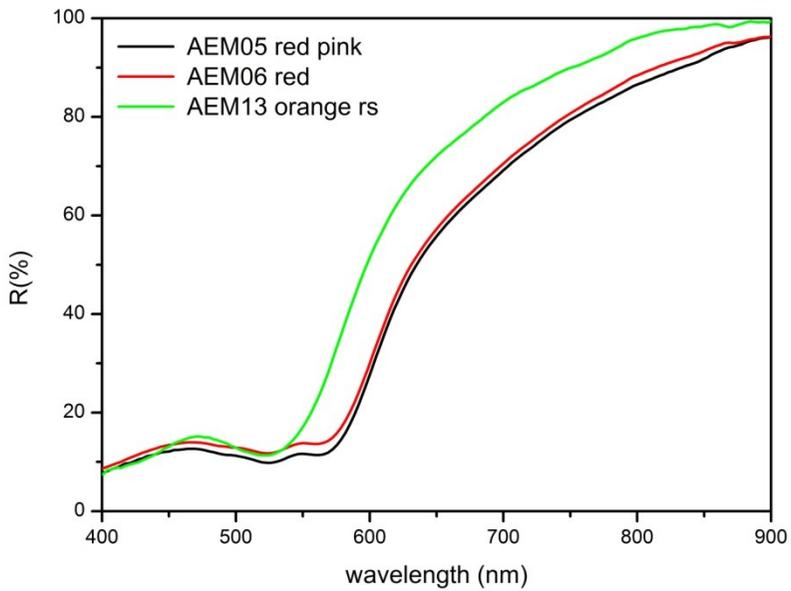
Figure 6.1.5: Reflectance spectra of red, red pink and orange areas investigated in the embroidered panel. Black and red spectra were recorded on the front side, green spectrum was recorded on the reverse side.
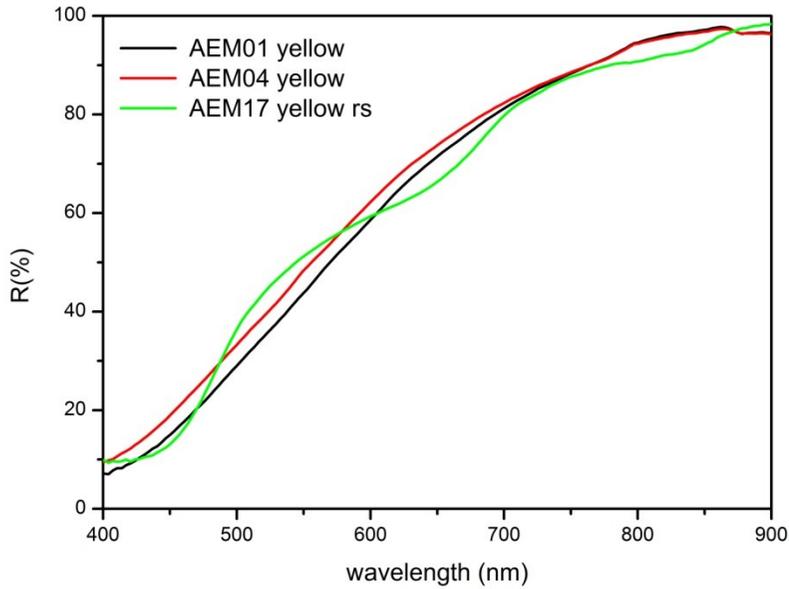
Figure 6.1.6: Reflectance spectra of yellow areas investigated in the embroidered panel. Black and red spectra were recorded on the front side, green spectrum was recorded on the reverse side.
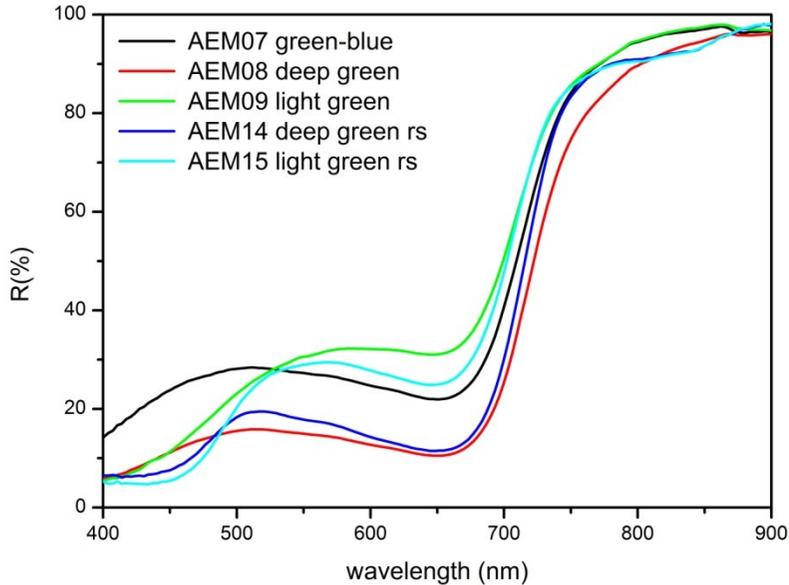
Figure 6.1.7: Reflectance spectra of green and green-blue areas investigated in the embroidered panel. Black, red and green spectra were recorded on the front side, blue and cyan spectra were recorded on the reverse side.
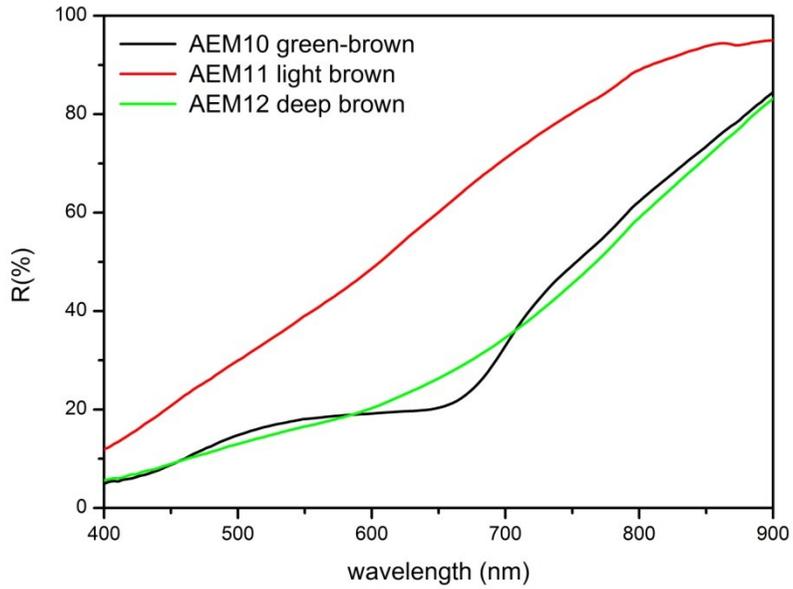
Figure 6.1.8: Reflectance spectra of brown and green-brown areas investigated in the embroidered panel. All the spectra were recorded on the front side.

6.1.3 CONCLUSIONS

In this work the identification of most of the dyestuffs used to obtain the various colors of the textile panel from Bosco Marengo was achieved. Moreover, the results obtained with the non-invasive technique (fiber optics reflectance spectroscopy) were compared with those found with a micro-invasive approach (HPLC-DAD-MS). In particular, reflectance spectroscopy proved to be able to recognize red and blue species also on historical samples, thus validating the procedures employed and the considerations drawn for mock-ups of dyed textiles (3.1). The results obtained by the non-invasive approach on the historical textile were confirmed thanks to HPLC-DAD-MS analyses, which identified cochineals (e.g. carminic acid-rich scale insects) in the red portions of the panel, in accordance with the indication obtained by means of reflectance spectroscopy. Indigoid dyestuffs were properly identified through FORS in blue, green, green-blue, green-yellow and yellow areas. FORS investigation was able to highlight the presence of an additional yellow dye in green areas, and its identification was performed by HPLC-DAD-MS measurements (with the exception of green-brown areas where no samples were taken). The red dye used to obtain an orange color was identified as brazilwood by both the techniques.

As for yellow dyestuffs, the possibility of verifying the presence of carotenoid dyes established in mock-ups was confirmed for historical objects. In fact, the absence of the characteristic absorption band of carotenoids in the reflectance spectra of yellow, orange and green areas was confirmed by HPLC-DAD-MS results that revealed the presence of flavonoids (genistein and luteolin together with their related compounds) and identified dyer's broom as the yellow dyestuff employed for all the investigated areas. The recorded reflectance spectra on brown areas did not give raise to any characteristic features and, since no samples were taken from brown areas, the employed dyestuffs remained unidentified. In addition, the comparison between spectra recorded in the front and in the reverse side of the textile evidenced the loss of spectral features due to fading on the side exposed to light; anyway, in this case the dyestuffs could be correctly identified also using spectra recorded in the front side. These considerations stressed the need of performing multiple measurements on historical textiles, in order to enlarge the set of spectra that may show useful features for the identification of the dyestuff. In conclusion, the non-invasive approach through FORS succeeded in recognizing blue and red dyestuffs employed in ancient textiles, whereas for yellow ones, the lack of spectral features limits the diagnostic power of the technique.

6.2 NON-INVASIVE TECHNIQUES FOR THE INVESTIGATION OF THE PALETTE OF PERSIAN MANUSCRIPTS

The expression "Persian manuscripts" indicates a wide group of handwritten paper books of diverse provenances, dates and subjects that are written in Persian language. In fact, the influence of Persian Empire led to the production of manuscripts with common linguistic-textual features also over the borders of political influence (Piemontese 1989). The taste for the chancellery, courtier and academic Persian models was acquired by the rulers of the Empire of Turkish-Mongol origins and widespread along the Iranian plateau as far as ottoman Turkey and the Indian Mughal Empire (Figure 6.2.1). Since the Persian Era, writing had an enormous importance, because it represented the state and the religion; this role is further stressed with the advent of Muslim religion, as the Koran is the holy book from which writing is born. As a consequence, calligraphers were highly considered in the Persian society and were asked to move across and over the Empire together with painters, paper-makers and other artisans connected to the production of manuscripts, thus creating a common taste for books in a large geographical area. The art of Persian manuscripts has been surviving for over a millennium and came to its end only at the beginning of 20th century, but the golden era of these artworks lasts from the 14th to the 17th century AD.

The access to ancient treatises about illumination, calligraphy and paper decoration is limited due to the lack of translations in European languages of most of the known literature. Only two books are available in an English translation: *Qanun us-Suvar*, written by Sadiqi Bek at the end of 16th century and *Gulistan-i Hunan*, by Quadi Ahmad, dated at the 17th century (Purinton and Watters 1991). The first deals with theoretical and practical aspects of painting and describes some recipes and techniques for illuminations. The latter provides a detailed description of the atelier (*ketab-khaneh*) of prince Ibrahim Mirza. Two interesting works by Mandana Barkeshli and co-workers allow to the European scholars the access to a review of non-translated treatises about Persian painting and decoration of manuscripts (Barkeshli 2007; Barkeshli, Ataie, and Alimohammadi 2008). In particular, the authors cite and describe, among others, the content of the three most interesting works about paper dyeing, all dated to the 16th century AD: *Golzari Safa*, by Ali Seyrafi, *Adab al-Mashgh*, by Baba Shah Isfahani and *Savad al-Khat*, by Ahmad Majnoon Rafigh Heravi (Barkeshli, Ataie, and Alimohammadi 2008). Of main interest is also a book by Porter in which he fully discusses the Indo-Persian technical literature about the subject from the 12th to the 19th century (Porter 1994).

Books were made of paper, as the technique of paper making was imported from China centuries earlier and papers were already used for documents in the 7th century AD, while it is known that a paper factory was settled in Samarqand in the 8th century (Loveday 2001). Paper was made of flax with the eventual addition of hemp in the region of actual Iran, while in India it was produced with cotton and hemp. Paper was then sized in order to be able to receive the colors; common sizing products were starch (rice or wheat), plant mucilage (rice, cucumber seeds, fleawort…), animal or vegetal glues (fish, Arabic gum, serish), sugar or fruit syrups, such as melon or grapes (Barkeshli 2003).

Books were often finely decorated, with the use of colored and also precious metal inks and stunning illuminations (Figure 6.2.2). Compared to contemporaneous western manuscripts, the use of paper decoration was widely diffused. Paper decoration included paper dyeing, marbling of paper (Figure 6.2.3), called *abri* in Persian, the use of stencils and silhouetted papers and *zarafshani*, that is gold-flecking (Porter 1994; Blair 2000). In particular, paper dyeing (Figure 6.2.4) was undertaken for aesthetic and functional purposes, as reported in *Golzar-e Safa*, by Seyrafi (AD 1545): "Paper; once dyed is better, for white surely harms the eyesight" (Barkeshli, Ataie, and Alimohammadi 2008). The practice of dyeing paper was already used in Maghreb in the 13th century AD, while it has been known in Iran from the beginning of the 14th century (Blair 2000). A whole chapter of Porter's book (1994) reports the recipes for dyeing paper, but also other authors indicate the dyestuffs used in Persian manuscripts (Purinton and Watters 1991; Barkeshli, Ataie, and Alimohammadi 2008). It is possible from the above mentioned sources to have an outlook of the colorants, inorganic and organic, that are said to be employed for the production of Persian manuscripts. Table 6.2.1 reports a list of the employed dyestuffs according to literature.

Scientific works undertaken before 1991 are reported by Purinton and Watters (1991). The analytical techniques used in those works were microscopy, energy dispersive X-ray analysis, laser analysis and X-ray diffraction and identified only inorganic pigments. More recent investigations were carried out by means of optical microscopy and microchemical analysis (Barkeshli 2007), multispectral analysis (Isacco and Darrah 1993), Fourier transformed – infrared spectroscopy (Barkeshli 1999; Barkeshli 2003) and Raman spectroscopy to identify fibers, sizing materials and colorants. In particular, Raman spectroscopy has been widely used in recent years, sometimes in combination with other techniques, and allowed the assessment of the inorganic palette for many manuscripts (Hayez *et al.* 2004). By means of this latter technique indigo, the only non-fluorescent dyestuff, has been identified in several works, employed alone or mixed with inorganic pigments (Clark and Mirabaud 2006; Slogget 2007; Burgio *et al.* 2008; Muralha, Burgio, and Clark 2012). Moreover, Indian yellow was found on Indian illuminations of the 17th century and on a 19th century copy of a older "Anatomy of the body" (Ravindran *et al.* 2011; Ciomartan and Clark 1996). The only successful acquisition of a Raman spectrum of a carmine lake on a 17th *Shah Namah* (Book of Kings) is reported in a recent work by Muralha *et al.* (2012) while in other studies the presence of several organic colorants to obtain violet, purple, yellow and green hues is only supposed due to high fluorescence of the recorded Raman spectra (Clark and Mirabaud 2006; Bruni *et al.* 2001; Ciomartan and Clark 1996). In addition, Fourier transformed – infrared spectroscopy was used to identify the use of saffron in green areas obtained with verdigris to avoid paper corrosion caused by this green pigment (Barkeshli 1999; Barkeshli and Ataie 2002).

Persian manuscripts are conserved in many western countries libraries; in particular, Angelo Piemontese presented an inventory of all the Persian manuscripts stored in Italian libraries (1989), even though he stated that only 10% of the books were classified at that time and, as a consequence, known.

In this work, nine Persian manuscripts stored in different libraries of Northern Italy, most of them described by Piemontese, have been analyzed by means of non-invasive techniques, such as portable microscopy, fiber optics reflectance spectroscopy (FORS) and portable X-ray fluorescence

(XRF). The aim of the analytical work was to identify the whole colorants palette, but the presence of organic colorants allowed also the verification of the possibility of transferring the methodology used for identification of textile dyestuffs by means of FORS (3.1) to a different substrate (e.g. paper).

The results obtained during the investigation of Persian manuscripts were included in a recently published paper about the characterization of miniature paintings by means of FORS (Aceto, Agostino, *et al.* 2014).

Table 6.2.1: Dyestuffs employed on Persian manuscripts according to literature. (Purinton and Watters 1991; Porter 1994; Barkeshli 2007; Barkeshli, Ataie, and Alimohammadi 2008)

COLOR	DYESTUFFS
RED	Kermes
	Cochineal
	Madder
	Lac
	Sappanwood
	Safflower
YELLOW	Saffron
	Gamboge
	Indian yellow
	Safflower
	Turmeric
	Persian berries
ORANGE	Henna
BROWN	Pomegranate
BLUE	Indigo
	Sunflower
GREEN	Indigo + yellow (dye or pigment)
	Verdigris + saffron

Figure 6.2.1: Geographical distribution of Persian manuscripts.
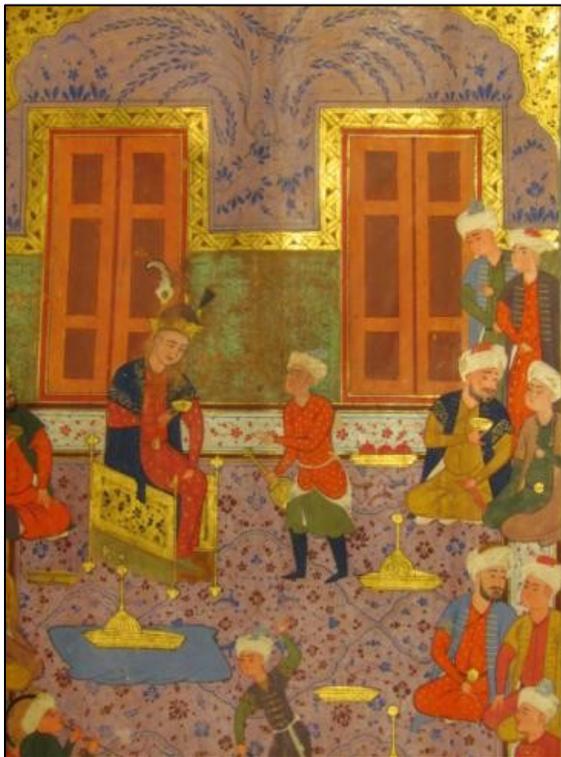
Figure 6.2.2: Illumination on page 118r of Ms. Cast22, conserved at Biblioteca Nazionale Braidense in Milano.

Figure 6.2.3: Marbled paper, p. 6v of Ms. Or101, conserved at Biblioteca Reale in Torino.

Figure 6.2.4: dyed paper, p. 5 of Ms. 3574PP, conserved at Biblioteca Nazionale Universitaria of Bologna.

6.2.1 MATERIALS AND METHODS

INVESTIGATED MANUSCRIPTS

The nine investigated manuscripts date from 15th to the 18th century and their provenances cover a wide geographical area. In particular, five of them were surely produced in the Persian Empire, while two manuscripts come from Turkey and one from the Mughal India. The provenance of a book is doubtful, as it could be produced in the Persian Empire or in Mughal India.

The manuscripts are presently stored in four different libraries in Northern Italy: Biblioteca Nazionale Braidense in Milano (2 manuscripts), Biblioteca Nazionale Universitaria in Torino (1 manuscript), Biblioteca Nazionale Universitaria in Bologna (1 manuscript) and Biblioteca Reale in Torino (5 manuscripts). The subjects, dimensions and quality of the artworks are diverse; in particular, some of them show a broad decorative apparatus, with illuminations, decorated titles, dyed papers and colored inks, while others are less refined. Table 6.2.2 summarizes the principal features of each of the analyzed manuscripts.

Table 6.2.2: Principal features of the analyzed manuscripts.

CODE	TITLE/SUBJECT	PROVENANCE	DATE	PAGES	PAPER	ILLUMINATIONS	INK(S)	LIBRARY
Cast22	*Xamsa* (poetical pentalogy)	Siraz (Iran)	1540-1580	380	white	24	black	BB
Cast23	*'Iyār i dāniš* (Widsom's measure)	Mughal India	1st half 17th	195	white	16	black, red	BB
a.I.60	Koran	Iran	15th		white		black, red	BNU
3574PP	*Gulšan i rāz* (Mistery's florilegium)	Herat (Afghanistan)	Beginning of 16th	15	colored	-	black	BIBO
Or37	*Sabri* (Collection ofTurkish poems)	Turkey	?	55	colored	-	black	BRT
Or40	*Mantik-ut-tair* (Birds' language)	Bagdad / Tabriz (Iran)	Half of 15th	207	white	6	black	BRT
Or81	*Dīvān i Şābir* (Collection of Şābir's poems)	Istanbul (Turkey)	1476-1477	158	white	-	black, blue, gold	BRT
Or101	*Safīna* (The ark)	Iran?	1503	222	colored	2	black, gold	BRT
Or113	Calligraphy and peinture album	Mughal/Persian	1760-1761	50	white	24	Black	BRT

Mock-ups were obtained by dyeing pieces of paper with some of the dyestuffs employed in Persian manuscripts, in order to compare them with the recorded spectra, in addition to the comparison with FORS spectra of dyed textile fibers (3.1). In particular, paper dyed with safflower red was obtained with the following procedure. Safflower florets were washed in a cloth with bi-distilled water until all the yellow soluble dyestuffs were removed. Then, Na_2CO_3 was mixed to the washed florets with a proportion of 10% in weight compared to the initial weight of the dry florets. Water was added while stirring and the the solution was filtered. Then, drops of lemon juice were added until the solution turned to red and the paper was put into the bath at room temperature. In some cases, the paper was put in the solution before adding the lemon juice. In addition, also saffron and henna were dyed directly on paper.

INSTRUMENTATION

The transportation of the valued manuscripts was forbidden by the curators of the libraries, as was the sampling from the books. Due to these limitations, the techniques to be employed on the manuscripts were selected on the base of their portability and non-invasiveness. Another issue that was taken into account was the limited access in terms of time, that required to favor the less time-consuming techniques, thus avoiding the use of, for example, portable Raman spectroscopy. When possible, the measurements with the different techniques were performed on the same areas.

In order to highlight the morphology of the areas under investigation, a visual analysis was performed with the help of a portable microscope. The observation permitted the evaluation of the presence of mixtures of pigments, whose identification is fundamental for the interpretation of spectral data, and of altered areas, which can respond with anomalous signals. The microscope used is a Dino-Lite.

Fiber Optics Reflectance Spectrometry analysis were performed in the range 360-1100 nm with an AvaLight-HAL source and a AvaSpec-USL2048XL spectrometer. The integration time was set at 4.5 ms with accumulation of 200 spectra. The system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 7.

Portable X-Ray Fluorescence measurements were carried out with an EDXRF Thermo NITON spectrometer XL3T-900 GOLDD model. Total time of analysis was set to 120s. The instrument was held in position with a moving stage allowing micrometric shifts, in order to reach the desired probe-to-sample distance; the stage was laid on a sturdy tripod. In order to avoid interference from the underlying pages, several sheets of cellulose acetate were put below the analyzed page. Cellulose acetate is totally harmless for the conservation of paper and colorants and does not contain elements that can be revealed by XRF measurements. The obtained spectra were processed with the commercial software WinAxil, derived by the academic software QXAS from IAEA.

Further technical details about the employed instrumentations are reported in Appendix 2.

6.2.2 RESULTS AND DISCUSSION

A total of 400 reflectance spectra and 100 XRF spectra were recorded on the nine analyzed manuscripts. Those data allowed the identification of both the inorganic and organic pigments, but only the results about colorants deriving from natural dyestuffs are extensively discussed.

ILLUMINATIONS

Although most of the analyzed blue areas proved to be painted with lapis lazuli, a characteristic spectrum with a minimum of absorption centred at 655-658 nm was observed in several blue areas (Figure 6.2.5, black spectrum). The XRF spectra of those areas did not reveal any key-elements, thus confirming the attribution to the organic pigment indigo. This dye was found alone in manuscript Or113, while the increase of lead counts in XRF spectra acquired in light blue areas of manuscript Cast23 suggests the mixture of indigo with lead white to lighten the color. The presence of a second minimum at 535 nm in two pages of manuscript 3574PP indicated the use of a mixture of indigo and safflower red to obtain a particular hue (Figure 6.2.5, blue spectrum); microscope images confirmed the presence of small red areas (Figure 6.2.6).

Red and pink areas were mainly obtained with vermillion, minium or red ochre. Some reflectance spectra of red or pink areas of Cast23, Or40 and Or113 could be attributed to minium but the slight increase of reflectance in the red region was not coherent with the single presence of this pigment, which might be mixed with a dye to obtain lighter hues. Unfortunately, the presence of minium did not allow a thorough investigation of such spectra in order to identify the bands related to the dye.

On the contrary, three dark red areas on manuscript Or113 exhibited reflectance spectra with a band structured in two sub-bands at 520-530 and 560-570 nm (Figure 6.2.7, red spectrum); XRF spectra were not collected in these areas. The position of the bands allowed the identification of scale insects dyes as the coloring matter. As previously discussed (3.1.3), it is not possible to distinguish between the different scale insects dyes using reflectance spectroscopy; from the historical point of view, the most likely species to be found in the analyzed manuscripts are kermes (*Kermes vermilio* Planchon) Armenian cochineal (*Porphyrophora hamelii* Brandt) and Indian lac (*Kerria lacca* Kerr).

The increase of signals attributable to lead lines in XRF spectra of lighter (red and pink) areas permitted to hypothesize the use of lead white mixed with scale insect dyes for two areas of manuscripts Cast23 and A.I.60.

In addition, certain pink illuminated areas of 3574PP exhibited a reflectance spectrum with a band at about 530 nm and an inflection point at about 580 nm (Figure 6.2.7, green spectrum), thus identifying the coloring matter as safflower red.

On the contrary, some reflectance spectra of illuminated areas of Cast22 and Or113 did not show spectral features attributable to any of the considered red pigments or dyes, probably because of the poor saturation of the colors considered.

Orpiment was found to be the main yellow colorant in the analyzed manuscripts. Instead, some yellow areas of Or113 and 3574PP showed reflectance spectra different from the paper (Figure 6.2.8, red spectrum), but XRF measurements present no signals different from the paper background so it can be hypothesized that the unknown colorant is a dye. Since reflectance spectroscopy does not allow to discriminate yellow dyes (3.1.2.3), only the use of carotenoids (e.g. saffron) can be excluded as they show recognizable spectral features.

The use of portable microscopy has been essential to investigate green hues, as some of them proved to be composed by a mixture of blue and yellow grains. For instance, many areas in five of the investigated manuscripts (Cast22, Cast23, Or40, Or101, Or113) had a minimum of reflectance at 650-658 nm (Figure 6.2.9, red and green spectra), assigned to indigo, while XRF spectra displayed As and S lines, ascribable to yellow pigment orpiment, and did not exhibit elements attributable to blue pigments. Therefore, the mixture of these green areas is composed by indigo and orpiment. Other areas showed the presence of inorganic green pigments: atacamite, malachite, verdigris. Only a light green area of 3574PP presented a reflectance spectrum with no diagnostic spectral features, possibly due to the poor state of conservation of the color.

Most of the brown areas were obtained with ochre or minium mixed with another colorant, for which the results did not allow to make an attribution.

Reflectance spectra in purple areas of the different manuscripts (found in all but Or37 and 3574PP) showed a similar trend, with a characteristic absorption band structured in two sub-bands at 530-550 nm and 560-590 nm (Figure 6.2.10, black spectrum). The absence of signals attributable to mineral pigments in the corresponding XRF spectra allowed to suppose the presence of a dye. As already underlined (3.1.2.2), these spectral features are distinctive of scale insect dyes; as to the attribution to a particular specie or genus, the same considerations aforementioned for reds are valid.

The reflectance spectrum of a purple area of manuscript Or113 presented a different trend: two bands at about 525 and 650 nm and an inflection point at 705 nm (Figure 6.2.10, green spectrum). The latter two features are distinctive of indigo, while the first band can be attributed to a red dye, that is in some cases well defined into sub-bands, thus confirming the use of scale insects dyes, while in other cases the lack of definition makes a more in-depth attribution arduous.

The use of a carbon based black pigment is supposed for the analyzed black areas. A black corroded area of manuscript 3574PP is possibly made with a ferrogallic pigment, although there are no historical proofs of the use of this pigment in the Persian area.

All the white areas are composed by a lead white pigment.

Silver, lapis lazuli or a mixture of lead white and a carbon based black pigment were found in some grey areas. On the contrary, the reflectance spectra of some other grey areas on manuscripts Cast23 and Or113 exhibited a minimum at 650-656 nm (Figure 6.2.5, red spectrum), which suggest the use of indigo in mixture with other dyes or pigments to obtain a grey hue.

All manuscripts showed golden areas that proved to be composed of true gold. XRF analysis suggested that organic glues were used below gold leaves, differently from the use on Western manuscripts in which gypsum, Armenian bole or cinnabar were employed.

DYED PAPERS

Blue papers of Or101 were characterized by a minimum of reflectance at 656 nm (Figure 6.2.5, green spectrum), thus allowing to identify indigo as the employed dye.

Pink papers of Or37, Or40, Or101 and 3574PP showed a reflectance minimum at 527-532 nm and an inflection point at 572-580 nm (Figure 6.2.7, black spectrum), while no detectable signals different from the uncolored paper were found in the XRF spectra. The above mentioned features can be attributed to the dye safflower red.

Manuscript Or101 has some yellow colored papers, for which XRF measurements deny the presence of inorganic pigments, while reflectance spectra do not display spectral features different from the undyed paper (Figure 6.2.8, black spectrum). Also XRF spectra of yellow papers of manuscript 3574PP do not show specific signals, but in that case reflectance spectra are different from the uncolored paper (Figure 6.2.8, green spectrum). The coloring matter can be a yellow dye, but for its attribution to a specific dye one must take into account the considerations mentioned in paragraph 3.1.2.3.

Reflectance spectra of green papers of Or37 and 3574PP showed the presence of a maximum of reflectance at about 550 nm and an absorption band at about 652-660 nm (Figure 6.2.9, black spectrum). The lack of signals different from the uncolored paper in the XRF spectra permitted to suppose the use of indigo with an unidentified yellow dye, which, as previously underlined, can be hardly identified through reflectance spectroscopy.

A purple dyed flower decoration has been found in manuscript Or101. The reflectance spectra presented a band structured in two sub-bands at about 545 and 585 nm (Figure 6.2.10, red spectrum), attributable to scale insect dyes.

Black papers of Or101 were obtained with a carbon based black pigment, whereas for those of 3574PP a ferrogallic pigment has been employed.

INKS

Black inks are generally composed of a carbon based pigment, while reds are made of vermillion, blue of azurite and golden writings of true gold.
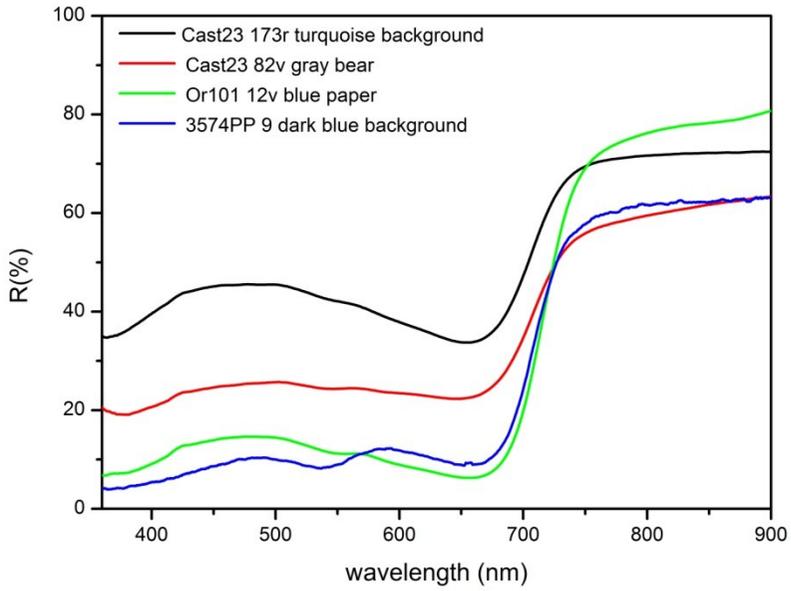
Figure 6.2.5: reflectance spectra of blue and gray areas recorded on different manuscripts. Painted areas: black, red and blue spectra. Dyed areas: green spectrum.

Figure 6.2.6: Micrograph of a blue area of Ms. 3574PP obtained with a mixture of a blue and a red colorant.
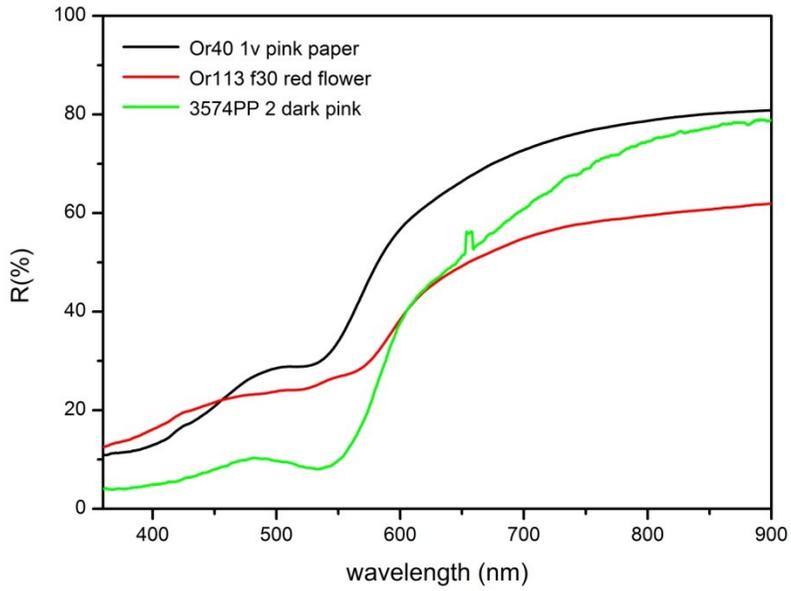
Figure 6.2.7: reflectance spectra of red and pink areas recorded on different manuscripts. Painted areas: red and green spectra. Dyed areas: black spectrum.
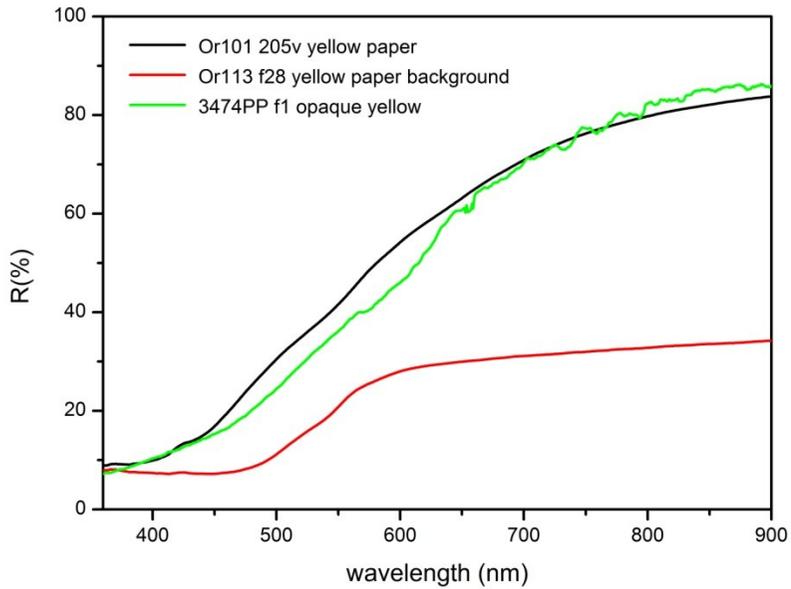
Figure 6.2.8: reflectance spectra of yellow areas recorded on different manuscripts. Painted areas: red spectrum. Dyed areas: green spectrum. Paper background: red spectrum.
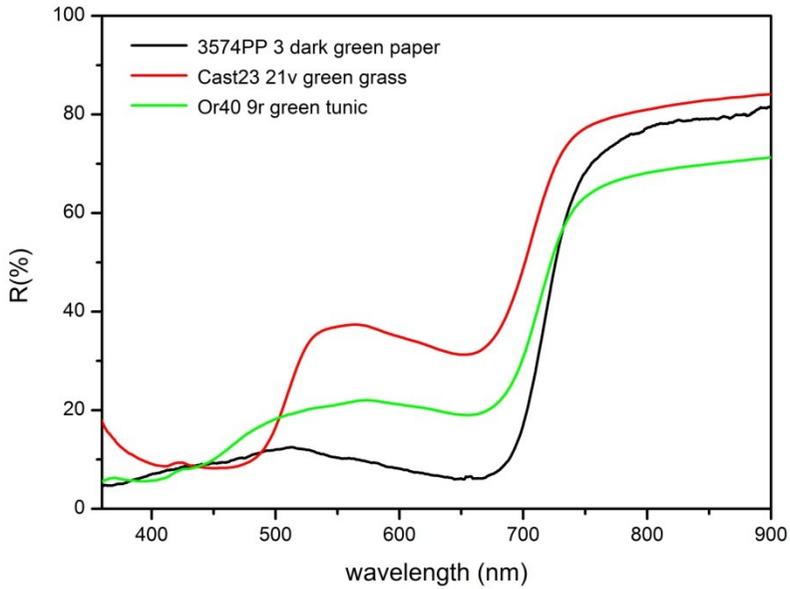
Figure 6.2.9: reflectance spectra of green areas recorded on different manuscripts. Painted areas: red and green spectra. Dyed areas: black spectrum.
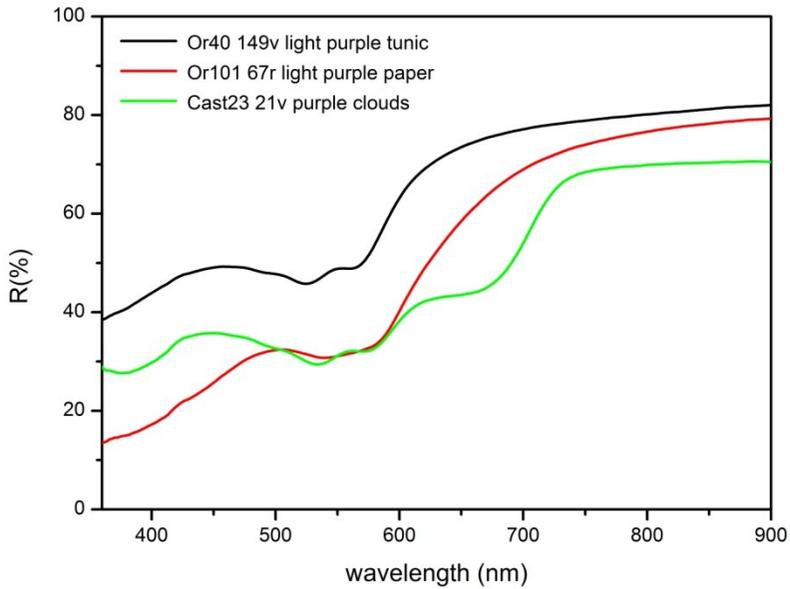
**Figure 6.2.10: reflectance spectra of purple areas recorded on different manuscripts. Painted areas: black and green spectra. Dyed areas: red spectrum.**

6.2.3 CONCLUSIONS

A list of the identified pigments and dyes in the illuminated scenes is shown in Table 6.2.3. The palette appears limited and homogeneous throughout the three centuries that cover the analyzed manuscripts (15th-18th). In fact, while in Western countries the number of employed colorants increased during these centuries, the same trend could not be observed in the Persian area.

Persian painters mostly used high-priced pigments, such as lapis lazuli, cinnabar (or vermillion), orpiment, silver and gold. In particular, lapis lazuli is the most employed blue pigment, also in mixture with less precious pigments to obtain light blue and grey hues. There is no evidence of a hierarchy between this pigment and the other blue such as indigo, traditionally less valued in contemporary European art and for this reason used for less important parts of the paintings. The reasons for these different tendencies in Persian and European miniature art could be found in the major importance given to this art in Persian countries but also to the diverse pigment value among the two cultures. In effect, miniature art benefited of a high esteem in Persian countries during the considered centuries, as it represented the power of the Royal family, in spite of the subject of the book. Moreover, the proximity with Sar-e-sang ores in Badakhshan and the abundance of sulphur-containing rocks in Iran could contribute to a different consideration of lapis lazuli and orpiment respectively. In fewer cases, a deep blue was obtained blending two dyes: safflower and indigo.

As Barkeshli *et al.* (2008) previously indicated, the use of a mixture of blue (indigo) and yellow (orpiment or a yellow dye) to obtain green colors was preferred. The results about the use of indigo with orpiment to achieve green hues is in accordance with previous results obtained by means of Raman spectroscopy (Clark and Mirabaud 2006; Muralha, Burgio, and Clark 2012). The identification of atacamite is in agreement with previous analytical works (Purinton and Watters 1991; Burgio *et al.* 2008; Muralha, Burgio, and Clark 2012) but historical sources did not mention this copper chloride, that might be a degradation product of a different copper based pigment.

Browns were mainly created with ochre, while for pinks and grays there is a certain variability of recipes, with the use of precious materials too (e.g. silver, lapis lazuli). The unique white pigment employed is lead white, also employed to lighten the colors. Golden areas were all of true gold, which was applied in powder.

The pre-eminence of inorganic pigments is possibly due to their better stability, even though for purple hues there is an exclusive employ of dyestuffs, mostly scale insects. The lack of identification of madder in all the manuscripts is in disagreement with Wulff, who affirmed that red vegetal dyes were used as lakes in Persian manuscripts (Purinton and Watters 1991).

Most of the analyzed inks were black, but in some manuscripts there were also blue, red and golden inks, while in none of them were found silver inks (Table 6.2.4). Following the description of the preparation of black inks given in Qadi Ahmad's book *Gulistan-i Hunan* it seems likely that black inks used in the manuscripts were lamp black or starch black. Red and blue inks were composed respectively by vermillion (or cinnabar) and azurite, while all the golden writings proved to be made of true gold. The exclusive individuation of blue pigment azurite in the ink of

manuscript Or81 (15th century) confirms the hypothesis by Laurie about the ending of the use of this pigment in the 15th century in the Persian area, in spite of the presence of rich copper carbonate mines in the North of Iran (Purinton and Watters 1991).

Papers were exclusively dyed with organic dyes (Table 6.2.5), with the exception of black papers that were dyed with a carbon based pigment and, in one case, with a ferrogallic pigment. In particular, blue papers were obtained with indigo, red papers with scale insect dyes and yellow papers with unidentified yellow dyes. Greens were created by a mixture of indigo and an unknown yellow dye, with the possible use of the complex technique of double dyeing. Finally, pink papers were found to be dyed with safflower red, which was, according to Porter (1994), one of the most used dyes for paper dyeing. To the best of the author's knowledge, this is the first identification with scientific techniques of safflower red on Persian manuscripts.

In conclusion, the application of different complementary non-invasive techniques allowed the identification of most of the pigments and dyes used for illuminations, inks and dyed papers in the considered manuscripts. The work partially confirms previous analytical efforts on Persian manuscripts, extending the number of analyzed artworks and permitting a comparison with historical sources. As for natural dyes, they proved to be more widely used as previously reported, not only for paper dyeing but also for illuminations, and it was possible to identify the use of indigo, scale insect dyes and safflower red, while yellow dyes were only hypothesized. Moreover, this work underlined the extensive use of indigo in mixture with other dyes (e.g. scale insects dyes, safflower red, yellow dyes) or orpiment and allowed the first identification of safflower red on Persian manuscripts. Finally, the possibility of using the results from the database of reflectance spectra recorded on dyed textiles mock-ups for the identification of dyestuffs on paper is established.

Table 6.2.3: Identified pigments and dyes in the illuminated scenes.

COLOR	COLORANT(S)	Cast22	Cast23	Or40	Or81	Or101	Or113	Or37	Al60	3574PP
blue	Lapis lazuli	X	X	X	X	X	X		X	X
	Indigo						X			
	Indigo + safflower									X
light blue	Lapis lazuli + lead white	X					X		X	
	Indigo + lead white		X							
	Indigo + ?						X			
red	Vermillion	X	X	X	X	X				X
	Minium	X	X							X
	Red ochre	X	X				X			
	Minium + ?		X							
	Scale insects						X			
pink	Vermillion + lead white					X	X			
	Red ochre + lead white		X				X			
	Scale insects + lead white		X						X	
	Vermillion + ?			X						
	Minium + ?	X		X			X			
	Scale insects + ?						X			
	Safflower			X				X		X
	Red dye			X						
orange	Vermillion + ?			X		X	X			
	Minium + ?			X		X	X		X	
	Red ochre + ?						X			
yellow	Orpiment	X	X	X		X	X			
	Yellow dye						X			X
green	Indigo + orpiment	X	X	X		X	X			
	Atacamite		X				X			
	Verdigris			X	X	X				
	Malachite			X			X			
	Copper green						X			
	Indigo + yellow dye									X
	Unidentified green									X

COLOR	COLORANT (S)	Cast22	Cast23	Or40	Or81	Or101	Or113	Or37	AI60	3574PP
brown	Ochre + ?	X	X	X		X				
	Minium + ?	X					X ?			
purple	Scale insects	X	X	X	X	X	X		X	
	Indigo + red dye						X			
black	Carbon pigment	X	X			X				
	Ferrogallic pigment									X
white	Lead white	X	X				X		X	
grey	Carbon pigment + lead white		X	X						
	Lapis lazuli + ?	X								
	Indigo + ?		X				X			
	silver	X		X				X		
	Pigment					X				
	Carbon pigment + ?						X			
metals	gold	X	X	X	X	X	X	X	X	X

Table 6.2.4: Identified pigments on the analyzed inks.

COLOR	COLORANT (S)	Cast22	Cast23	Or40	Or81	Or101	Or113	Or37	AI60	3574PP
blue	Azurite				X					
red	Vermillion		X						X	
black	Carbon pigment		X					X	X	
gold	Gold				X	X				

Table 6.2.5: Identified pigments and dyes for analyzed colored papers.

COLOR	COLORANT (S)	Cast22	Cast23	Or40	Or81	Or101	Or113	Or37	AI60	3574PP
blue	Indigo					X				
pink	Safflower			X		X		X		X
yellow	Yellow dye					X				X
green	Indigo + yellow dye							X		X
purple	Scale insects					X				
black	Carbon pigment					X				
	Ferrogallic pigment									X

6.3 NON-INVASIVE SURVEY ON DYEING MATERIALS OF COPTIC TEXTILES FROM THE COLLECTION OF MUSEO EGIZIO OF TORINO

The term "Coptic textiles" indicates a series of woven objects produced in Egypt from 30 to 1300 AD with common stylistic features and culture but coming from different historical periods (e.g. Roman, Byzantine and Islamic). They were obtained exploiting the skilled weaving techniques of the era of the Pharaohs but introducing the use of wool, a fiber prohibited in the previous historical period since it was considered impure by Egyptian priests. Wool was preferred for decorations as it is easier to be dyed as opposed to flax and fills up the textile, while flax was employed for the structure given that it is a strong, long, glossy and smooth fiber and creates fine textiles.

Coptic textiles have been investigated mainly by liquid chromatography even though the first work on this subject dates to 1935, when René Pfister employed chemical reactions in order to identify dyes on Coptic textiles (Pfister 1935). In particular, high performance liquid chromatography was employed by Wouters in several analytical investigations on Coptic textiles (Wouters 1985; Wouters 1993; Wouters 1994), in which he identified the use of a number of dyes, namely weld, madder, wild, madder, indigotin, cochineals, kermes, indigotin, lac, cutch and Tyrian purple. Moreover, a comparison between different detection methods for HPLC (UV-Vis , fluorescence and mass spectroscopy) was made by a group from the University of Warsaw (Orska-Gawrys *et al.* 2003; Surowiec *et al.* 2003; Szostek *et al.* 2003; Trojanowicz *et al.* 2004). These works identified the presence of madder, armenian cochineal, lac dye, indigotin, tannins, weld and flavonoid dyes in the analyzed Coptic samples. The paper by Trojanowicz and co-workers (2004) also reports all the previously identified dyes on Coptic textiles where a thorough record of the blend of dyes employed to obtain the different colors is indicated. In addition, another work reports the use of HPLC-PDA for the identification of madder, indigo and a mixture of the two dyestuffs to obtain a purple color in Coptic textiles (Abdel-Kareem, Alawi and Mubarak 2010). Moreover, extractionless SERS was employed on an archaeological Coptic textile (6th-8th century AD) with laser photoreduced silver nanoparticles (Jurasekova *et al.* 2010); the authors identified the presence of alizarin in the sample.

The Coptic collection of the Museo Egizio of Torino includes about 250 textiles. Many of them are decorative parts that have been cut out from tunics or fragments deriving from larger textiles, although the collection also encompasses whole tunics and headdresses. The conditions of the textiles are largely inhomogeneous: some of them are excellently preserved whereas others are in bad conditions possibly due to restorations that were undertaken at the beginning of the past century.

In order to shed light on provenance, date of production, conservation and materials, and to highlight possible similarities or differences among the fragments, a selected set of Coptic textiles presently stored for studying and cataloguing is the object a multidisciplinary project that will consider the textiles from an iconographic and technological point of view. In particular, the project is focused on the study of textiles fibers and weaving technologies under the optical microscope, as well as on the application of non-invasive and invasive analytical techniques in order to investigate the dyeing materials.

A first limited set of materials was investigated at Fondazione Centro di Conservazione e Restauro "La Venaria Reale" by means of fiber optics reflectance spectrophotometry as the textiles were the object of a restoration thesis. Hereafter, a non-invasive screening was performed *in situ* with UV-Vis-NIR reflectance spectrophotometry and portable molecular fluorescence equipped with fiber optics on a larger number of textiles from Museo Egizio in order to identify common spectral features that would point to common dyeing materials.

6.3.1 MATERIALS AND METHODS

COPTIC TEXTILES

The collection of Coptic textiles from Museo Egizio of Torino contains objects from the Roman, the Byzantine and the Islamic period, dating from the 3rd to the 11th century AD (Donadoni Roveri 1987). Fifty textiles have been already conferred to the new-established Fondazione Museo delle Antichità Egizie di Torino, while the other two hundred are actually under study for the inventory before passing to the foundation. Even though, if compared with other remarkable collections like that of Museo Egizio of Firenze, the collection has a limited number of objects, it is exceptional for the quality and the richness of the textiles. Part of the objects comes from the Drovetti's collection while others are indicated as "Gift from Cairo's Museum" and possibly derive from Antinoe's archaeological site. Other objects were bought by the archaeologist Ernesto Schiapparelli in 1898 from the Franciscan fathers of Akhim, an Egyptian center renowned for the production of textiles, and in 1901 in Cairo, possibly from the monastery of Deir Mairi Girgis, next to Thebes. More, a restricted number of objects come from Ashmunein's excavations of 1903-1904 by Schiapparelli and his co-workers Breccia and Biondi. Finally, some textiles derive from an exchange with Museo Civico of Torino, to which they were donated by Guimet.

Textiles are woven with flax and wool, the structure being realized in plain weave fabric. They are clothes (tunics and shawls, Figure 6.3.1a), accessories (purses and hats, Figure 6.3.1b) and interior textiles (blankets and carpets). In some cases, the fragmentary appearance of the objects makes the attribution difficult. Decorations are realized employing colored monochrome (mainly red-brown) or polychrome wool threads, whether woven, attached or, rarely, embroidered. The subjects of the decoration come from the Hellenistic and Christian traditions, with geometric (Figure 6.3.2) and plant themes on medallions and stripes with interlaced plants, series of animal and human figures (Figure 6.3.3). The nature of the employed dyestuffs is known only from historical sources (Hofenk de Graaff 2004; Cardon 2007). Analyses were performed on 140 objects.

INSTRUMENTATION

A Multichannel spectrometer system ZEISS MCS 600 equipped with halogen lamp CLH 600 and two detectors, MCS 601 UV-NIR and MCS 611 NIR 2.2, was employed to perform FORS measurements of the Coptic textiles at Centro Conservazione e Restauro "La Venaria Reale". The range of analysis varied according to the geometry of the probes: 350-2200 nm with of 0°/45°x2 geometry and 350-1100 nm for 0°/0° geometry.

The second set of textiles was investigated through a AvaLight-HAL Tungsten Halogen light source and a AvaSpec-USL2048XL-USB2 spectrometer, with a 45°/45° geometry. In all measurements the distance between probe and sample was kept constant to 1 mm. To visualize the investigated area

on the sample, the probe contained a Framos (Agrate Brianza, Italy) WEB1315SI model digital micro camera, connected to PC via USB. Fiber Optics Reflectance Spectrometry analysis were performed in the range 360-1100 nm, the integration time was set at 30 ms with an integration of 50 spectra for a total acquisition time of 1.5s for each spectrum. The system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 7.

Spectra were all reported in the range 400-900 nm for ease of comparison. About 450 reflectance spectra were recorded on the analyzed objects.

Portable Molecular fluorescence was performed on samples from Museo Egizio with an Ocean Optics Jaz model spectrophotometer using the 365 nm Jaz-LED internal light source. The spectrophotometer is working in the range 191–886 nm and the investigated area on the sample is 1 mm diameter. In all measurements the sample-to-probe distance was kept constant to 1 mm (corresponding to focal length) with aid of a small black cylinder inserted on top of the probe, in order also to exclude contributions from external light. Instrumental parameters were as follows: 2 s integration time, 3 scans for a total acquisition time of 6 s for every spectrum. The system is managed with SpectraSuite software under Windows 7. About 350 fluorescence spectra were recorded on the analyzed objects.

Further technical details about the employed instruments can be found in Appendix 2.

Figure 6.3.1: tunic (a, inventory code suppl. 1702) and woollen hat (b, inventory code suppl. 1709).

Figure 6.3.2: orbiculus decorated with geometric themes (inventory code suppl. 17343).

Figure 6.3.3: fragment of fabric decorated with animals and human figures (inventory code suppl. 17441).

6.3.2 RESULTS AND DISCUSSION

Most of the reflectance spectra from **red** and **pink** areas showed a structured absorption band with two sub-bands showing maxima in the range 500-550 nm, whose position permitted to distinguish between madder (Figure 6.3.4, red spectrum) and scale insects dyes (Figure 6.3.4, black spectrum). When the reflectance minima of the band cannot be identified, molecular fluorescence spectra allowed the identification of the dye through the position of the maximum of intensity taking 600 nm as a reference, as maxima lower than 600 nm are attributed to madder (Figure 6.3.5, red spectrum) while scale insects dyes show maxima higher than this wavelength (Figure 6.3.5, purple spectrum). Features below 500 nm in the fluorescence spectra are associated with the textile (Figure 6.3.5, green spectrum). In particular most red and pink areas showed the presence of madder (Figure 6.3.5, black spectrum), while some red were obtained with scale insects dyes (Figure 6.3.5, blue spectrum). The presence of madder was identified also in all the analyzed **orange** areas, while the use of a yellow dyestuff was hypothesized in order to obtain the color but the employed techniques were not able to give further information about its nature.

Every **blue** area showed the same spectral features when analyzed through reflectance spectroscopy (Figure 6.3.6, blue spectrum). In particular, a minimum at about 660 nm was clearly identifiable together with an inflection point at 700-730 nm. These features can be unequivocally attributed to indigotin, thus pointing to the use of an indigoid vat dyestuff. Since the reflectance spectrum of this coloring matter is so peculiar and constant, fluorescence spectra are redundant for its identification.

The spectra of **purple** areas showed the presence of the above-mentioned absorption band related to red dyes, that was attributed to madder in most cases (Figure 6.3.7, red spectrum), whereas fewer spectra were consistent with the use of scale insects dyes (Figure 6.3.7, blue and black spectra). Most of the spectra presented also a marked minimum at about 660 nm (Figure 6.3.7, red and blue spectra), attributed to the blue dye indigo, while a few areas were only dyed with scale insects dyes (Figure 6.3.7, black spectrum). In the case of double dyeing with red and indigo, the fluorescence maxima of red dyes are possibly suppressed by the presence of indigo, thus preventing the correct identification of the red dye by means of portable molecular fluorescence.

Reflectance spectra from **yellow** areas showed the presence of inflection points between about 475 and 570 nm, while the correspondent fluorescence spectra presented a maximum at about 515-535 nm. Despite the presence of such spectral features, yellow dyestuffs still remained unidentified.

A minimum at about 660 nm and an inflection point at 700-730 nm were observed in every reflectance spectrum recorded on a **green** area (Figure 6.3.6, black and red spectra), whereas variable features were identified in the blue-green region (e.g. below 600 nm). In particular, relative maxima and minima in this latter region varied of more than 100 nm. These features are consistent with the use of a double dyeing of a yellow and a blue dye but as it is possible to ascertain the presence of a blue indigoid vat dyestuff, the yellow dye cannot be identified, although the employ of a carotenoid dye (e.g. saffron) can be excluded.

Brown areas showed in some cases the presence of the absorption band of madder and indigotin (Fig. 8, red spectrum), while in other spectra madder was possibly employed with another dye to obtain a brown color (Figure 6.3.8, blue spectrum). In addition, some spectra presented a different trend, most likely attributable to a tannic dye (Fig. 8, black spectrum). Fluorescence spectra were not useful to identify this latter dye, as only features attributable to the textile were recorded (Figure 6.3.9).

Most of the reflectance spectra of **black** areas presented a characteristic minimum of reflectance at about 660 nm together with a reflectance around 0% in the visible range (Figure 6.3.10, red spectrum), thus indicating the use of a very deep indigoid dyestuff for most of the black areas. Only in one case, the black hue was obtained with another unknown dye (Figure 6.3.10, black spectrum), which remained unidentified. Also in this case, no characteristic features in the fluorescence spectra were found in order to identify the dye.
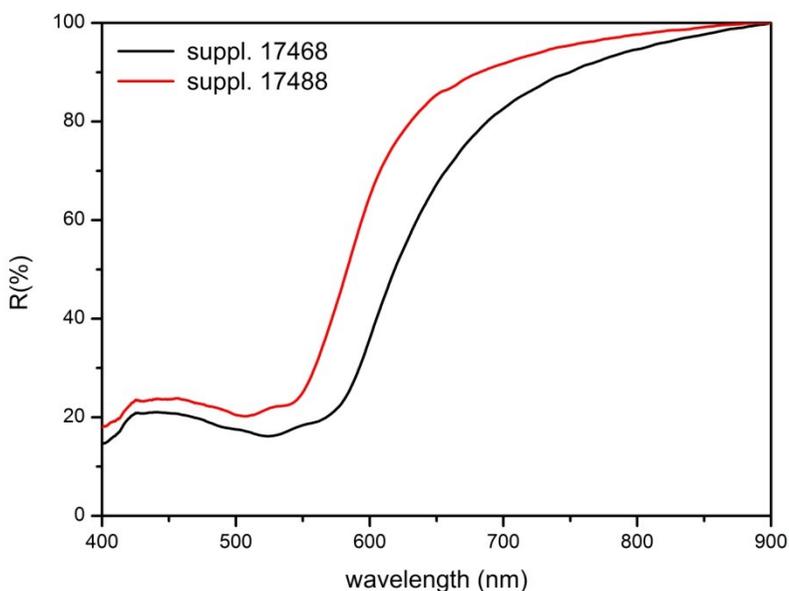
Figure 6.3.4: reflectance spectra of red areas from Coptic textiles; spectra were normalized to 100% reflectance for ease of comparison.
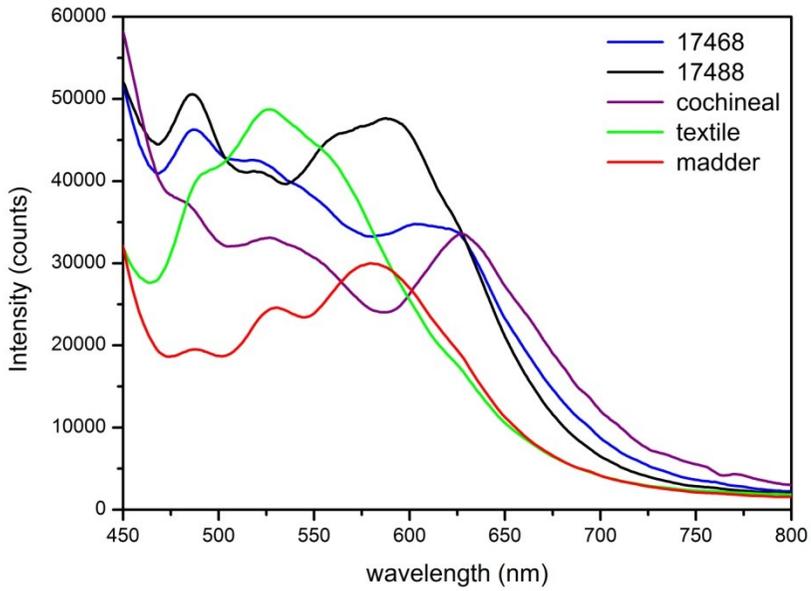
Figure 6.3.5: molecular fluorescence spectra of red areas from Coptic textiles (blue and black spectra) compared with reference spectra of cochineal (purple spectrum), madder (red spectrum) and with a spectrum recorded on an undyed textile (green spectrum).
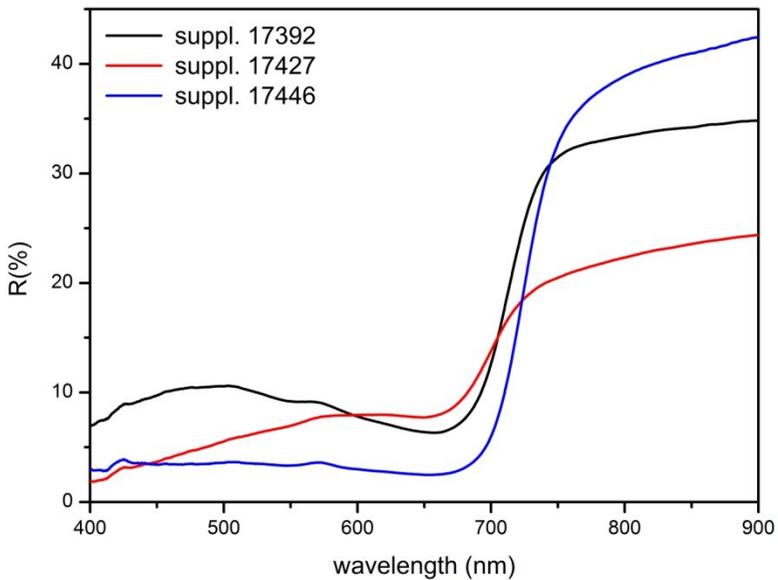
Figure 6.3.6: reflectance spectra of blue (blue spectrum) and green (black and red spectra) areas from Coptic textiles.
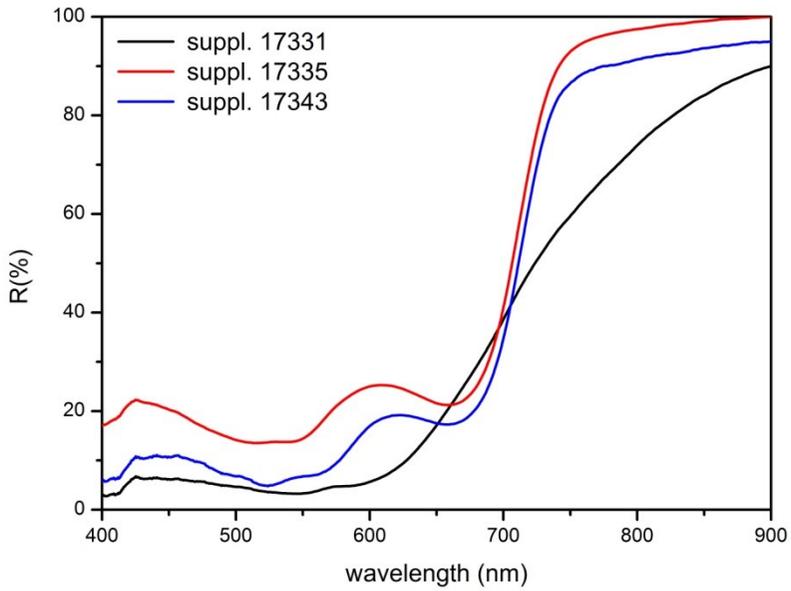
Figure 6.3.7: reflectance spectra of purple areas from Coptic textiles; spectra were normalized to 100% reflectance and stacked for ease of comparison.
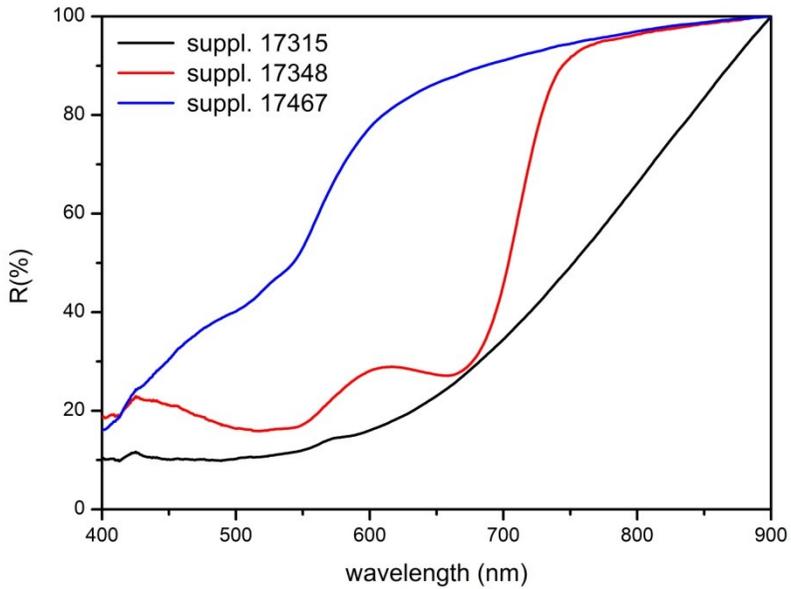

Figure 6.3.8: reflectance spectra of brown areas from Coptic textiles; spectra were normalized to 100% reflectance for ease of comparison.
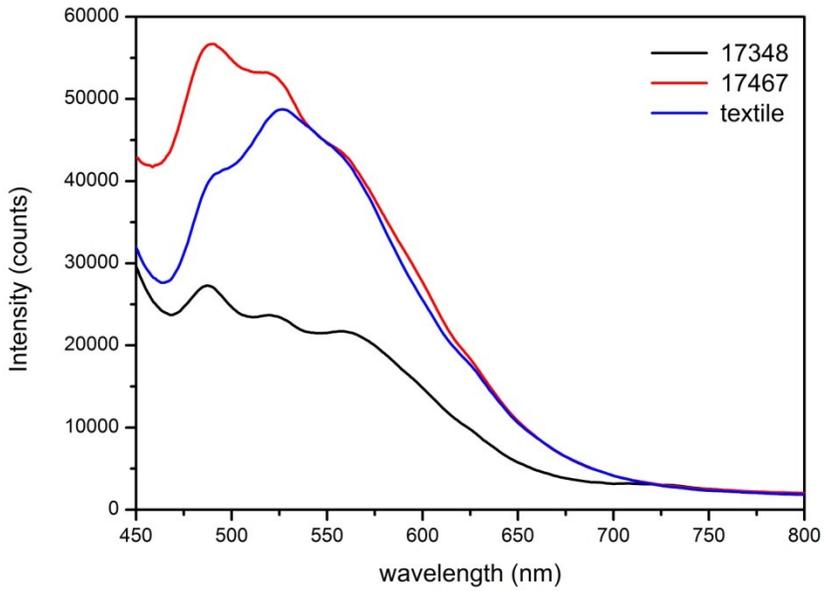
Figure 6.3.9: molecular fluorescence spectra of brown areas from Coptic textiles (black and red spectra) compared with a spectrum recorded on an undyed textile (blue spectrum).
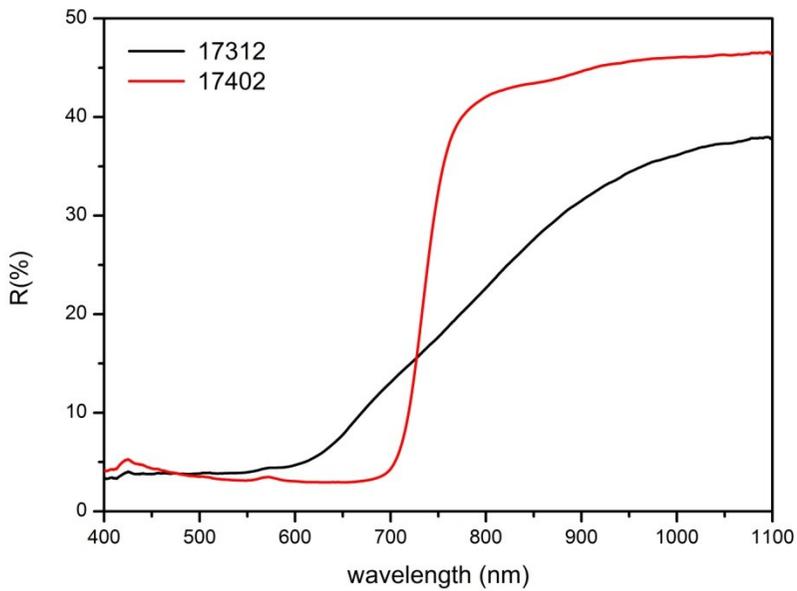

Figure 6.3.10: reflectance spectra of black areas from Coptic textiles.

6.3.3 CONCLUSIONS

Non-invasive investigations on Coptic textiles from Museo Egizio of Torino allowed to highlight the different combinations of dyes exploited to obtain the various colors. In particular, most of the investigated red and pink areas showed the presence of madder, while few reds were obtained with scale insects. Also orange colors were obtained employing madder, with the addition of an unidentified yellow dyestuff. Moreover, all the blues were obtained by using vat dyes from indigoid dyestuffs. Purple colors were mainly obtained using madder and a blue indigoid dyestuff, but in few cases dyes from scale insects, by themselves or combined with an indigoid dyestuff, were preferred. As previously highlighted, non-invasive measurements were not effective in identifying yellow dyestuffs, while for all the green areas it was possible to ascertain the presence of an indigoid blue vat dyestuff that was superimposed to a yellow (unidentified) dyestuff. In addition, brown colors were created with several dyestuffs: a blend of madder and an indigoid dyestuff or an unknown dye (a tannic dye?) were used for most of the areas, while in some cases madder, possibly mixed with another dye, was employed. Finally, black areas were, in general, obtained employing an indigoid dyestuff even though one textile was dyed with other coloring materials (tannins?).

Reflectance spectroscopy was effective in exhibiting the spectral features of blue and red dyestuffs, but in some cases the accessory use of molecular fluorescence allowed to discriminate madder and scale insects dyes in ambiguous spectra. Unfortunately, the discerning ability of this latter technique between anthraquinone reds from plants or animals failed in presence of indigoid molecules, thus preventing the use of this technique in most of the purple and brown areas.

The results obtained are consistent with previous analytical evidences about the coloring matters of Coptic textiles. In particular, the comparison with the table reported by Trojanowicz *et al.* (2004) confirms that madder is the mostly employed red dyestuff while the use of scale insects dyes is less widespread. The exclusive use of indigoid species for blue colors is also verified. In addition, most of the attribution to double dyeing in this work were already identified on other Coptic objects. On the contrary, the identification of scale insects, employed by themselves or with indigoid dyestuffs, to obtain purple colors was never reported before. Furthermore, the analyzed black areas did not show the presence of madder, as reported by Wouters (1993).

In conclusion, this work underlined that robust information can be obtained by performing a large number of analysis on the same object and by considering together the information obtained from two analytical techniques. The results obtained with the non-invasive survey will permit to set up the sampling plan in order to deepen the investigation on the various dyeing materials by means of micro-invasive techniques.

6.4 NON-INVASIVE SURVEY ON OTTOMAN TEXTILES FROM MUSEO DI ARTE ORIENTALE OF TORINO

The Ottoman Empire lasted for more than six hundred years (1299-1922 AD) and reached its apex in 16th century under the reign of the Emperor Suleiman the Magnificent. At this time, the Ottoman Empire covered an area of more than five million square kilometers, including Eastern European countries, Ukraine, Greece, modern Turkey, Near Eastern countries, part of North Africa and Saudi Arabia, Persia and other Central Asian countries. Textile arts were advanced there and woven carpets from Turkey and Persia were particularly appreciated also outside the Empire. Fabrics were often embellished with the use of metallic threads to give the objects the shining aspect of gold and silver.

Few scientific works have been published about analyses on Ottoman textiles. In particular, no records were found of the employ of non-invasive techniques on these objects or of a comprehensive study of a whole collection. Only punctual works on selected samples performed with micro-invasive techniques were reported. HPLC measurements were performed on wool and silk textiles of Benaki Museum (Athens, Greece) from the Mamluk period (1250-1517), in which the identified molecules allowed to hypothesize the wide use of an indigoid dyestuff (indigo or woad), while in other cases madder, cochineal, lac dye and weld were supposed to be used (Valianou, Karapanagiotis, and Chryssoulakis 2009). Another work (Petroviciu *et al.* 2012) reported the identification by means of high performance liquid chromatography (HPLC) of madder in fifteen Oriental knotted carpets from 15th-17th century. Moreover, *in situ* extractionless SERS employing silver colloidal pastes was tested for the first time with concern to an historical textile on a carpet from Turkey (Istanbul or Brussa), dating to the late 16th - early 17th century, revealing the use of lac dye (Brosseau, Gambardella *et al.* 2009). In addition, thirty Kaitag textiles, from the region of Daghestan (Northern Caucasus), dating from the 16th to the 19th century, were analyzed with a multitechnique approach employing also visible reflectance spectroscopy, surface-enhanced Raman scattering and high performance liquid chromatography and revealed the use of madder, indigo, weld, tannins and *Rhamnus* species dyestuffs (Pozzi *et al.* 2012).

In this work, the Ottoman textiles from the collection of Museo d'Arte Orientale of Torino were investigated through non-invasive techniques in order to characterize the colorants employed. In particular, fiber optics reflectance spectrometry was used to identify the dyestuffs employed on fiber threads of the textiles, while the complementary use of portable X-ray fluorescence spectrometry allowed to ascertain the nature of metallic threads, black and white areas. The results allowed to set up a sampling plan in order to get more information about the dyestuffs by means of micro-invasive measurements.

6.4.1 MATERIALS AND METHODS

OTTOMAN TEXTILES

The Museo d'Arte Orientale of Torino stores a collection of twenty-six Ottoman textiles, dating from the 16th to the 19th century AD. They are mainly woven fragments from interior textiles made of silk and metallic threads, decorated with colored geometric and plant themes (Figure 6.4.1 a-b). Only in two fabrics animals (ISt/1, Figure 6.4.2) and human figures (ISt/11), respectively, are portrayed. In general, the provenance is known (e.g. Persia or Turkey), but in some cases also the precise place of production could be determined, as for five textiles from Brussa, in Turkey. Table 6.4.1 reports a summary of the main features of each of the analyzed textiles. According to the date, the place of production and the decorative themes, the textiles can be divided into two main groups: textiles of the 16th-17th century from Turkey (group A, ISt/7, ISt/8, ISt/12- ISt/24) and textiles of the 18th century from Persia (group B, ISt/2- ISt/5, ISt/9). Six of the investigated textiles cannot be classified into these two groups and will be treated singularly in the discussion of the results: ISt/1, ISt/6, ISt/10, ISt/11, ISt/25 and ISt/26.

INSTRUMENTATION

Portable X-Ray Fluorescence measurements were carried out with an EDXRF Thermo NITON spectrometer XL3T-900 GOLDD model, equipped with a silver tube. Total time of analysis was set to 120s. The obtained spectra were processed with the commercial software WinAxil, derived by the academic software QXAS from IAEA.

Fiber Optics Reflectance Spectrometry analysis were performed in the range 360-1100 nm with an AvaLight-HAL source and an AvaSpec-USL2048XL spectrometer. The integration time was set at 4.5 ms with accumulation of 200 spectra. The system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 7. A total of 230 spectra were recorded in order to characterize all the colors found in the 26 textiles.

Further technical details about the employed instruments are reported in Appendix 2.

Table 6.4.1: Summary of the main features of the Ottoman textiles from the collection of Museo d'Arte Orientale of Torino.

CODE	DESCRIPTION	PROVENANCE	DATE	MATERIALS
ISt/1	Carpet fragment	Persia	Half of 16th century	Wool, silk
ISt/2	Fabric	Persia	18th century	Silk, golden threads
ISt/3	Placemat	Persia	18th century	Silk, golden threads
ISt/4	Placemat	Persia	18th century	Silk
ISt/5	Fabric	Persia	17th-18th century	Silk, silver and golden threads
ISt/6	Fabric	Persia	16th-17th century	Silk, silver threads
ISt/7	Pillow cover	Turkey	16th-17th century	Silk
ISt/8	Fabric	Persia	17th century	Silk, silver threads
ISt/9	Fabric	Turkey	End of 18th century	Silk, silver threads
ISt/10	Fabric	Persia	18th-19th century	Silk, cotton
ISt/11	Fabric	Persia	16th century	Silk
ISt/12	Pillow cover	Turkey	18th century	Silk, silver and golden threads
ISt/13	Fabric	Turkey, Brussa	17th century	Silk, silver and golden threads
ISt/14	Pillow cover	Turkey	17th century	Silk, silver and golden threads
ISt/15	Pillow cover	Turkey	17th century	Silk, silver threads
ISt/16	Pillow cover	Turkey	17th century	Silk, silver threads
ISt/17	Pillow cover	Turkey	16th-17th century	Silk, golden threads
ISt/18	Fabric	Turkey, Brussa	16th-17th century	Silk, silver threads
ISt/19	Fabric	Turkey, Brussa	17th century	Silk, silver and golden threads
ISt/20	Fabric	Turkey, Brussa	16th century	Silk
ISt/21	Fabric	Turkey	16th century	Silk, silver and golden threads
ISt/22	Pillow cover	Turkey	16th century	Silk, silver threads
ISt/23	Pillow cover	Turkey	16th century	Silk, silver threads
ISt/24	Pillow cover	Turkey, Brussa	17th century	Silk, silver and golden threads
ISt/25	Ottoman embroidery	Turkey	17th-18th century	Chenille embroidery
ISt/26	Khema	Turkey	16th century	Brocade

Figure 6.4.1: textile ISt/24, Brussa (Tukey), 17th century (a); textile ISt/18, Brussa (Turkey), 16th-17th century (b).

Figure 6.4.2: textile ISt/1, Persia, half of 16th century.

6.4.2 RESULTS AND DISCUSSION

Table 6.4.2 and 6.4.3 report a summary of the results obtained on the analyzed textiles. In particular, spectra from **red** areas presented in general two absorption bands at about 520-525 and 560 nm (Figure 6.4.3, red spectrum), attributable to scale insects dyes. These features were found in textile ISt/5 from group A, in all the objects from group B containing reds, except ISt/7 (Figure 6.4.3, blue spectrum) and in textile ISt/26. On the contrary, red areas from the above cited ISt/7 could be possibly attributed to madder, while areas from singular textiles ISt/10 and ISt/25 were not attributed. Also **pink** areas from textiles ISt/9 (group A), ISt/1 and ISt/25 showed bands at 520-525 and 560 nm, thus confirming the use of scale insects dyes for these hues. In addition, pink areas of ISt/10 and ISt/11 presented different spectral features, in particular spectra of ISt/11 (Figure 6.4.3, black spectrum) showed an absorption band at about 570-575 nm, not attributable to any known pink or red dyestuff.

The features of spectra from **yellow** areas were not sufficient to identify the employed dyestuffs, even though the presence of a carotenoid dyestuff could be excluded. In one case, textile ISt/10, the presence of a band at about 660 nm allowed to ascertain the presence of an indigoid blue vat dyestuff, even though this dye might not be added by superimposition to the yellow dyestuff in order to modify the color, but possibly was registered as the yellow spot was too small to cover the whole probe area. Moreover, XRF measurements on two objects from group B (ISt/21 and ISt/23) highlighted the presence of silver and gold plus iron, copper and lead for ISt/23, thus pointing to the employ of a silver alloy to obtain the yellow colors.

Orange areas were found on ISt/3 (group A), ISt/1 and ISt/11. In the latter two cases, two weak absorption bands at 515 and 540 nm, were observed (Figure 6.4.4), thus allowing to hypothesize the presence of madder, while for ISt/3 no characteristic spectral features were recorded.

All the analyzed **blue** areas from textiles of group A, group B and from textiles ISt/1, ISt/10, ISt/25 and ISt/26 showed the presence of an absorption band at about 645-660 nm (Figure 6.4.5, blue spectrum), typical of indigotin. As a consequence, the areas were all obtained with the use of a blue indigoid vat dyestuff.

Green areas from textiles of group A, group B and textiles ISt/1, ISt/10, ISt/25 and some areas of ISt/11 presented reflectance spectra with an absorption band at about 650-660 nm and a relative maximum at about 500-550 nm (Figure 6.4.5, red spectrum). The first spectral feature indicates that indigotin is present in the textile but the position of the relative maximum suggests the use of a second dyestuff to obtain a green color. These areas were therefore obtained employing an unidentified yellow dyestuff above which an indigoid blue vat dyestuff was superimposed. Different spectra were obtained from green areas of ISt/26 and from some areas of ISt/11 (Figure 6.4.5, black spectrum). In particular, the absorption band was found at about 610-620 nm, thus pointing to the use of the so-called indigo carmine, obtained by mixing indigo or woad paste with sulphuric acid. It should also be pointed out that green areas analyzed on ISt/26 are from the frames of the textile and so they might be added later to the artwork.

Spectra from **purple** areas of ISt/1 and ISt/26 showed absorption bands at 525, 560 (Figure 6.4.6, blue spectrum) and in some cases also at 660 nm (Figure 6.4.6, black spectrum), that allowed to assume the use of scale insects dyes sometimes with a blue indigoid vat dyestuff superimposed. In reverse, spectra from ISt/3 (group A) showed only a band at about 560-570 nm (Figure 6.4.6, red spectrum), that was not attributed.

Brown areas were analyzed on two textiles from group B and from ISt/11. Spectra of ISt/7 showed the presence of a band at about 600 nm, while in the other cases the spectra presented a slope towards the infrared region. Spectra were not assigned, but in the case of ISt/11, XRF measurements revealed the presence of silver, gold and copper, thus allowing to hypothesize the use of metallic threads realized with an alloy of these three precious metals that partially degraded over time.

Gray areas presented reflectance spectra with no characteristic spectral features but in some textiles from groups A and B XRF measurements revealed the presence of precious metals, mainly silver with copper or gold or both. These gray areas were therefore obtained by employing silver alloys threads.

Also reflectance spectra from **black** areas showed the characteristic trend of iron-gall pigments, confirmed by XRF measurements which presented high counts of iron.

In addition, XRF measurements from **white** areas revealed always the presence of silver, mainly with copper and gold; in some cases, also iron and lead were found. Areas of textile ISt/15 contained also mercury.

Finally, reflectance spectra from golden areas showed an inflection point at about 450-500 nm and a rapid increase of reflectance towards the red region. Moreover, XRF measurements identified silver, gold and copper in all the analyzed areas, whereas iron and lead were found less frequently. Textile ISt/11 revealed also the presence of nickel.

Table 6.4.2: Identified dyestuffs on the investigated Ottoman textiles (part I). Empty boxes indicate colors not found in the textiles.

CODE	RED	PINK	YELLOW	ORANGE	BLUE
ISt/1		Scale insects dyes	?	?	Indigotin
ISt/2					Indigotin
ISt/3			?	?	Indigotin
ISt/4			?		Indigotin
ISt/5	Scale insects dyes		?		Indigotin
ISt/6	Scale insects dyes		?		
ISt/7	?				Indigotin
ISt/8	Scale insects dyes				Indigotin
ISt/9		Scale insects dyes			
ISt/10	madder	Madder	Indigotin + ?		Indigotin
ISt/11		?	?	Madder + yellow	
ISt/12	Scale insects dyes				
ISt/13	Scale insects dyes				
ISt/14	Scale insects dyes				
ISt/15	Scale insects dyes				
ISt/16					
ISt/17	Scale insects dyes				
ISt/18			?		Indigotin
ISt/19	Scale insects dyes				Indigotin
ISt/20					
ISt/21	Scale insects dyes		Ag, Au		
ISt/22					
ISt/23	Scale insects dyes		Ag, Fe, Au, Cu, Pb		Indigotin
ISt/24	Scale insects dyes				
ISt/25	Madder	Scale insects dyes	?		Indigotin
ISt/26					Indigotin

Table 6.4.3: Identified dyestuffs on the investigated Ottoman textiles (part II). Empty boxes indicate colors not found in the textiles.

| CODE	GREEEN	PURPLE	BROWN	GRAY	BLACK	WHITE	GOLD
ISt/1	Indigotin + yellow	Scale insects dyes (+ indigotin)		?		?	
ISt/2	Indigotin + yellow						Ag, Fe, Au, Cu, Pb
ISt/3	Indigotin + yellow	? 565				Ag, Cu, Au	Ag, Cu, Au
ISt/4							Ag, Cu, Pb
ISt/5					Fe	Ag, Cu, Au	
ISt/6						Ag, Fe, Cu, Pb	
ISt/7			?	?			
ISt/8						Ag?	?
ISt/9				Ag, Au, Cu	Fe		
ISt/10	Indigotin + yellow				Fe		
ISt/11	Indigotin/indigo carmine+ yellow		Ag, Au, Cu		Fe		Ag, Au, Cu, Ni
ISt/12	Indigotin + yellow					Ag, Fe, Au, Cu, Pb	Ag, Fe, Au, Cu, Pb
ISt/13	Indigotin + yellow						?
ISt/14	Indigotin + yellow				?	Ag, Fe, Au, Cu, Pb	
ISt/15						Ag, Cu, Au, Hg	?
ISt/16							
ISt/17	Indigotin + yellow					Ag, Fe, Au, Cu, Pb	
ISt/18	Indigotin + yellow			Ag, Au			
ISt/19	Indigotin + yellow					Ag, Cu, Au	
ISt/20							
ISt/21				Ag, Cu			
ISt/22							
ISt/23	Indigotin + yellow						
ISt/24			?			Ag, Fe, Au, Cu, Pb	
ISt/25	Indigotin + yellow				Fe	?	
ISt/26	Indigo carmine+ yellow	Scale insects dyes (+ indigotin)				?	Ag, Au, Cu, Pb
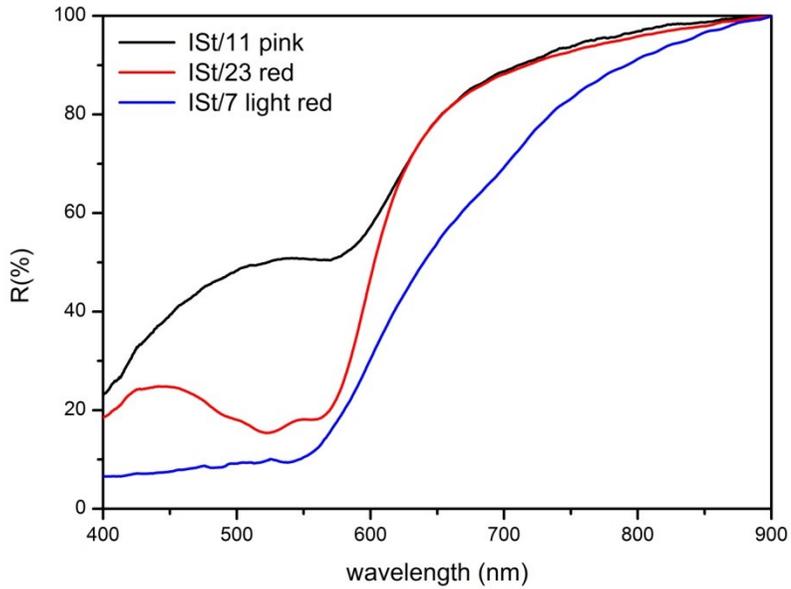
Figure 6.4.3: reflectance spectra of red and pink areas from Ottoman textiles; spectra were normalized to 100% reflectance for ease of comparison.
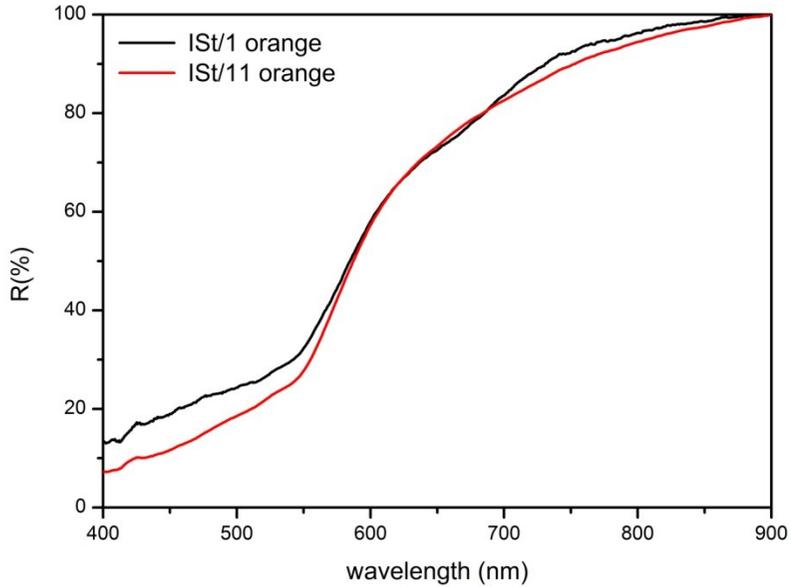
Figure 6.4.4: reflectance spectra of orange areas from Ottoman textiles; spectra were normalized to 100% reflectance for ease of comparison.
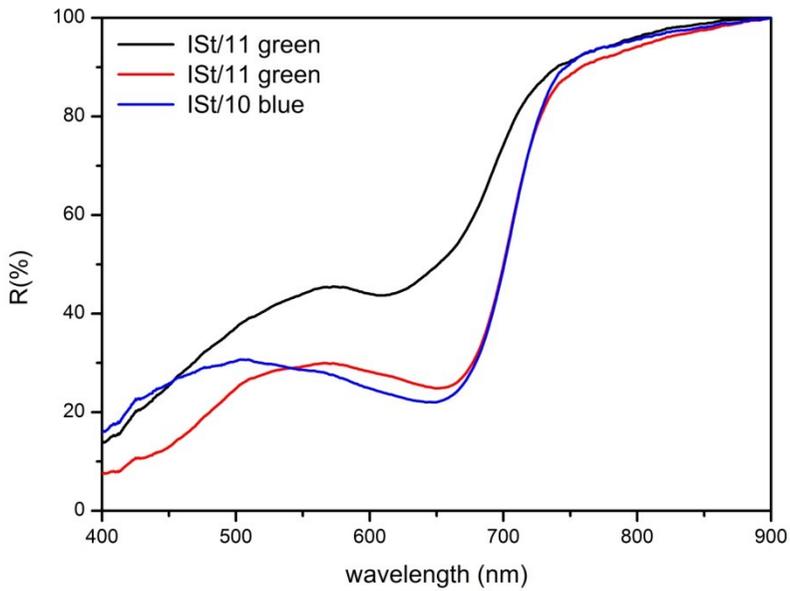
Figure 6.4.5: reflectance spectra of green and blue areas from Ottoman textiles; spectra were normalized to 100% reflectance for ease of comparison.
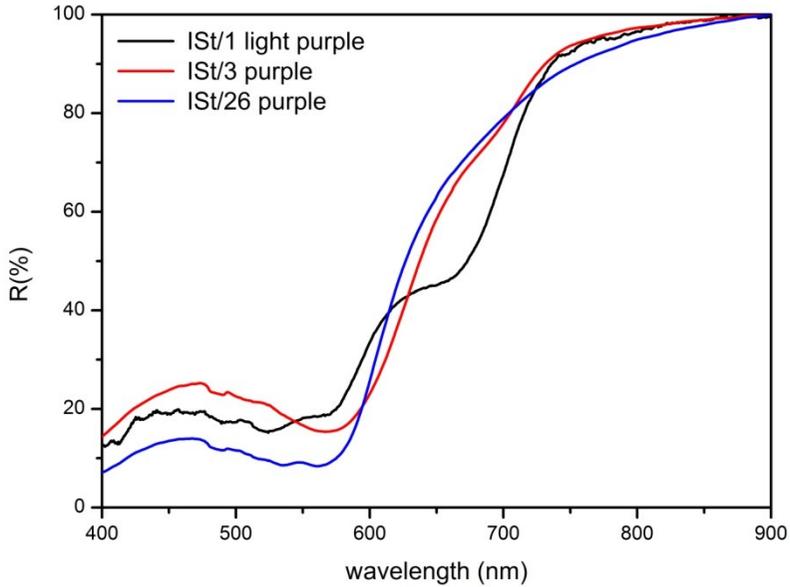

Figure 6.4.6: reflectance spectra of purple areas from Ottoman textiles; spectra were normalized to 100% reflectance for ease of comparison.

6.4.3 CONCLUSIONS

This work allowed to characterize the materials employed to color the Ottoman textiles from the collection of Museo d'Arte Orientale of Torino. In general, the textiles proved to be colored by employing dyes or metallic threads.

Most of the colored areas obtained with the use of a red dye (red, pink, purple), by itself or mixed, proved to be composed of scale insects dyes. Unfortunately, a further deepening of their nature could not be performed with reflectance spectroscopy. In addition, some red and orange areas were possibly attributed to madder, while other red, pink, purple and orange areas were not attributed. On the contrary, no evidences of the use of red dyestuffs to obtain brown colors were found.

The employ of indigotin (probably from indigo) was widespread, as this dye was used to obtain blue, purple (with a red dyestuff) and green (with a yellow dyestuff) areas. No indigotin bands were highlighted in black areas. Moreover, the green areas of two textiles (ISt/11 and ISt/26) presented spectral features attributable to indigo carmine that was invented only in 1740. As both textiles are dated to 16th century, the use of this dye should be confirmed by micro-invasive measurements while advices from conservators might be taken about the possibility that these green parts are not original.

Also in this case, yellow dyestuffs were not identified even though in some areas XRF measurements revealed that they were obtained with metallic threads. XRF measurements were useful also to ascertain the wide use of precious metals to obtain gray, white and golden areas, while the presence of iron in XRF spectra from black areas would point to the use of iron-gall pigments to obtain the color. It should be mentioned that the color of the areas obtained with metallic threads could have been modified over time, thus not allowing to ascertain the original color of the investigated area.

In conclusion, this preliminary survey allowed to discriminate between different materials and to set up an effective sampling plan that is detailed in Table 6.4.4, that will be carried out in the next months. As for textile ISt/11, two samples from pink and green areas are indicated as the different columns of human figures (Figure 6.4.7) showed different spectral features. Samples will be investigated mainly through SERS and, when possible, a comparison with HPLC-MS measurements will be carried out.

Table 6.4.4: sampling plan for the Ottoman textiles from the collection of Museo d'Arte Orientale of Torino. Only textiles selected for sampling are reported in the table.

CODE	RED	PINK	YELLOW	ORANGE	BLUE-PURPLE	GREEN	GRAY/SILVER	BLACK	GOLD
ISt/1	X		X	X	X	X	X		
ISt/2						X			X
ISt/5	X							X	
ISt/6	X						X		
ISt/7	X								
ISt/9							X	X	
ISt/10	X	X				X		X	
ISt/11		XX	X	X		XX		X	X
ISt/13	X					X	X		X
ISt/17							X		
ISt/21	X						X		X
ISt/25	X	X				X			
ISt/26	X					X			X

Figure 6.4.7: textile ISt/11, Persia, 16th century.

6.5 Spectroscopic investigation of the tapestry depicting the Deposition from the Cross from Museo del Duomo of Milano

Tapestries are textiles composed by warps and wefts decorated with scenes; the support is given by the warps, while the illustration is created by the wefts. Tapestries were usually woven in series with uniform decoration to be employed on the walls of entire rooms of rich residences. These artworks were also used to adorn external settings and they were often paraded due to their portability (Viale Ferrero 1963). Tapestries were produced in different steps: initially a small sketch was prepared by a painter, then a board of the dimensions of the tapestry was designed, finally the tapestry-weavers transposed the board to the textile. In order to create a tapestry, many tapestry-weavers were involved, often learned in the realization of different details, such as human figures, animals, grass (Nicastro 2012).

Previous analytical works on historical tapestries aimed at the investigation of the ageing of textile fibers by means of time-of-flight secondary ion mass spectrometry (Batcheller *et al.* 2006) and of both fibers and dyes by high performance liquid chromatography (Degano *et al.* 2011). As for the identification of dyes, orcein and indigo were identified in a Renaissance tapestry by means of *in situ* fluorimetry (Clementi *et al.* 2006; Clementi *et al.* 2009). Other works reported the use of high performance liquid chromatography coupled with different detectors to identify a mixture of indigo and weld on a supposed Italian tapestry (Pawlak *et al.* 2006), safflower on a 16th century Italian tapestry (Degano, Łucejko, and Colombini 2011) and a synthetic blue dye on a 19th century Japanese tapestry (Puchalska *et al.* 2004). Moreover, a red thread from a Renaissance Netherlandish tapestry was investigated with surface-enhanced Raman scattering using extractionless hydrolysis with hydrofluoric acid and allowed the identification of alizarin (Leona, Stenger, and Ferloni 2006). An area of the same tapestry was also subjected to extraction of a red dye with a polymeric hydrogel and subsequently the gel was coated with the Lee and Meisel silver colloid (1982); the dye was identified as alizarin in this case also (Leona *et al.* 2011).

In this work, a unique and very complex small scale devotional tapestry depicting the Deposition from the Cross is investigated. The tapestry is presently owned by the Museo del Duomo di Milano and possibly produced in Bruxelles at the very beginning of the 16th century and has been recently undergone restoration. Non-invasive and micro-invasive techniques were employed to detect the materials that were used originally to produce this very important tapestry and to investigate those that were employed in subsequent restorations, in order to confirm the production date, investigate the conservation history of the textile and gain information that can guide the restorers. In particular, this work discusses the role of spectroscopic techniques (FORS and SERS) in the identification of the colorants, while the identification of the fibers and the restoration materials different from colorants is reported elsewhere (Nicastro 2012). Molecular analyses were in some cases supported by elemental analysis performed through portable X-ray fluorescence spectrometry.

6.5.1 MATERIALS AND METHODS

THE TAPESTRY DEPICTING THE DEPOSITION FROM THE CROSS

The tapestry depicting the deposition from the cross (Figure 6.5.1) owned by the "Museo del Duomo" of Milano was probably woven in Bruxelles between 1500 and 1510 AD. The date of production was attributed according to stylistic considerations, as the first written document that mentions the tapestry is an inventory note that testifies that it was owned by the Milano cathedral and dates to 1653 (Nicastro 2012). The art of tapestry-weaving was as its apex in the city of Bruxelles in the 14th century, but already at the beginning of the 13th century Bruxelles' tapestries were requested from all over Europe. These richly decorated panels appeared in Italy after the second half of the 15th century. Unfortunately, only few of these masterpieces are still present within the Italian artistic heritage since most of them were sold or disappeared during lootings and wars (Forti Grazzini 1990).

The here considered small scale tapestry was produced for devotional use according to the typical Flemish iconography that influenced the tapestry production in the area until about 1520 AD. In particular, it was woven on a cartoon inspired by the paintings of Rogier van der Weyden by largely employing very precious materials such as gold and silver and richly colored silk or wool wefts. Dense warps (12 threads per centimeter) and wefts (up to 25 threads per centimeter) yield an extreme sharpness of the scene while silver- and gold- wrapped weft treads, along with copious silk fillings, enhance lighting. All these technological characteristics are peculiar of pre-Renaissance tapestries. The high-quality of the tapestry is also testified by the use of dyes that have maintained intact hues over centuries; finishing touches with painting enhance the final effect, as found in tapestries that were produced before 1525 (Schneebalg Perelman 1961).

The masterpiece was probably woven for Cardinal Georges I d'Amboise (Forti Grazzini 1988), the primate of Rouen and Prime Minister of King Louis XII of France. He ordered the tapestry for the Milano cathedral at the time of the French domination, when he was frequently in the town (Cattaneo, Brivio, and Cinotti 1976). The tapestry was hanged for centuries in the chapter house during the Good Friday. From 1953 it is instead exposed in the museum of the Milano cathedral.

At the beginning of the restoration, the tapestry appeared blurred by a diffuse deposition of dust; in addition, weathering products fully obscured the peculiar brightness of the metallic threads. Holes and missing weft particularly affected the portions obtained with silk, while open slits were present in the upper part of the tapestry, where tensions due to hanging were more intense. Past restorations heavily modified some of the original parts; in particular, all the edges were removed and substituted, as far as the upper part of the tapestry is concerned, by a gallon. Moreover, possibly as a consequence of an accidental heavy damage on the central part of the scene, embroidered or/and painted portions were added. These were set onto the tapestry by means of seams, embroidery or employing an adhesive. The tapestry is presently mounted on a plain weave fabric anchored to the tapestry itself through sewing and patching (Nicastro 2012). Unfortunately, documents describing past restorations, if ever existed, are missing and the materials employed, as well as the original ones, are unknown. As a result of the various interventions, the textile is now a very complex mix of fibers, dyes, pigments and supports, and represents an intriguing task for art historians and restorers.

SAMPLE PREPARATION

Sampling was not allowed on the tapestry, nevertheless a tiny red silk thread from an original area of the tapestry detached due to soft brushing during the cleaning treatment and was consequently considered in order to obtain more detailed molecular information about the dyes employed on red silks. The detached sample was 2 mm long and less than 0.1 mg in mass. The sample was therefore too small to allow the application of analytical protocols involving high performance liquid chromatography coupled with mass spectrometry. The application of surface-enhanced Raman analysis was therefore considered.

The sample was then gently washed with some drops of methanol on a microscope slide, rinsed with double distilled water and allowed to dry. This mild pre-treatment of the fiber was selected following the suggestion of a textile conservator (namely Cinzia Oliva) as it would remove part of the contaminants and leave the dyed fiber unaltered. Afterwards, single fibers were divided and cut with a scalpel from the thread. Few of them were coated with 0.5 µL of silver colloidal paste (5.1.1.1). In this way, three different sub-samples for SER measurements were obtained even from such a small sample.

INSTRUMENTATION

X-ray fluorescence spectra were acquired with a Tracer III-SD (Bruker), equipped with a rhodium anode and a palladium split, employing an integration time of 60s at 15kV. The spectra were recorded in order to determine the composition of some metallic threads and painted areas, as well as to verify the presence of overlying pigment on the dyed textile. A total of eighteen XRF spectra were collected.

FORS measurements were performed using a Multichannel spectrometer system ZEISS MCS 600 equipped with halogen lamp CLH 600 and a MCS 601 UV-NIR detector with 0°/0° geometry. The front side of the artwork was investigated in forty-five areas, thirty-two original and thirteen from previous restorations (Figure 6.5.1). Also thirteen colored threads used to fix the painted portions on the tapestry, visible in the back side of the panel, were investigated.

SERS measurements were carried out with a Renishaw InVia spectrometer coupled with a Leica DM2500 M optical microscope, using an excitation wavelength of 633 nm with a magnification objective of 100X. The acquisition time was set to 10s while the laser power onto the sample never exceeded 400 µW.

Further technical details about the employed instrumentations are given in Appendix 2.

Figure 6.5.1: front side of the Deposition from the Cross tapestry from Museo del Duomo of Milano; investigated areas by means of fiber optics reflectance spectroscopy: white dots and numbers identify original areas, red dots and numbers identify restored areas.

6.5.2 RESULTS AND DISCUSSION

RED, PINK, PURPLE

Reflectance spectra obtained from red and pink silks found on original and restored areas (both on the front and on threads of the back side of the tapestry) showed the presence of an absorption band structured into two sub bands at about 525-540 and 550-590 nm (Figure 6.5.2, red spectrum), which are distinctive of scale insects dyes, as previously discussed (3.1.2.2). Further information about the species employed cannot be obtained with this non-invasive technique (3.1.3). In two cases (AFX14 and AFX15), the dyed silk was overpainted with a blue pigment, that showed high counts of iron in the XRF spectrum. FTIR analyses on a micro-sample allowed to identify the colorant as Prussian blue (Nicastro 2012). On the contrary, original red areas made of wool showed reflectance spectra with an absorption band blue-shifted compared with those registered on red silk (Figure 6.5.2, black spectrum); the sub-band positions allowed the identification of madder as the employed dyestuff. The employ of twists of wool and silk dyed with the above cited red dyestuffs produced subtle shades which gave a three-dimensional aspect to the figures, as superbly demonstrated in the dress of Mary of Cleofa (Figure 6.5.3) (Nicastro 2012). A red restoration plain wave fabric made of cotton was dyed with a scale insect dye, onto which an unidentified pigment was spread. Pink cotton fabrics were painted (and not dyed) with vermillion that was identified by XRF. Finally, some purple threads of silk from the back side of the tapestry showed spectral features that are attributed to anthraquinones, although the presence of synthetic dyes cannot be excluded in this case.

Silver coating obtained on the red silk sample showed the presence of numerous nanoislands (Figure 6.5.4). Some SER spectra presented peaks that could not be attributed to a known dye, possibly due to the presence of some residual contaminants after the pre-treatment, but in others the presence of an intense peak at about 1300 cm^{-1} with a medium peak at about 1220 cm^{-1} (Figure 6.5.5) allowed the identification of carminic acid. Since this coloring matter is not the main constituent of scale insect dyes kermes and lac, the attribution given by FORS can be further narrowed only to the carminic acid-rich scale insects dyes, that are Mexican, Armenian or Polish cochineal (1.3.2, Wouters and Verhecken 1989).

YELLOW

Only yellow areas made of original silk were investigated through reflectance spectroscopy. Since the identification of yellow dyestuffs is critical with this technique (3.1.2.3), the only consideration that can be made is the exclusion of carotenoid yellow dyestuffs for the production of yellow colors.

ORANGE

Original orange silks and wool showed faint bands in the 500-550 nm region that would possibly point to the presence of madder (Figure 6.5.2, green spectrum); the employ of a yellow dyestuff to obtain the color was also suggested. The possible use of madder instead of a scale insect dye on silk in those cases might be due to lower consideration given to double dyeing, as yellow dyestuffs were often less expensive than scale insect dyes (except for saffron that was excluded for the above given reasons) thus "blemishing" the precious red dye. On the contrary, the choice of

madder could have had only dyeing reasons, as this dyestuff has a natural red-orange hue, while scale insects dyes are more purplish-red.

BLUE

Reflectance spectra recorded on original areas made of blue silk and wool showed the presence of an absorption band at about 660 nm (Figure 6.5.6, black spectrum), which allowed the attribution to an indigoid blue vat dyestuff. The analysis of painted areas on wool and cotton proved that the indigoid dyes was partially covered with Prussian blue. Moreover, restoration areas made of silk on the front and on the back side showed in some cases an absorption band at about 600 nm (Figure 6.5.6, red spectrum), which is consistent with the use of an indigoid dyestuff treated with sulphuric acid, called Saxon blue or indigo carmine (1.3.1, 3.1.2.1); in other cases the dyes used for the restoration areas remained unidentified.

GREEN

Original green silk presented the absorption band of indigoid dyestuffs (Figure 6.5.7, black spectrum) with a bathochromic shift in the maximum of reflectance due to the presence of an unidentified yellow dyestuff (not a carotenoid one). Differently, the spectra from silk restoration areas on the *recto* and the *verso* of the artwork showed the absorption band of Saxon blue or indigo carmine (Figure 6.5.7, red spectrum), always mixed with a yellow dyestuff. In addition, blue and blue-green inserts of silk were dyed with Saxon blue and a yellow dyestuff and painted with Prussian blue, while for cotton the dyeing was obtained with an indigoid blue vat dyestuff.

GRAY AND BROWN

The reflectance spectrum of a gray wool area showed the spectral features of indigotin (Figure 6.5.7, blue spectrum). The use of woad is mentioned in ancient treatises that described dyeing techniques employed in Bruxelles at the beginning of 16th century in combination with an iron-gall brown pigment. Spectra of brown areas did not show characteristic spectral features, but the compromised conservative state of the brown threads permitted to hypothesize the use of iron and tannins, as those materials made the fibers fragile.

METALLIC THREADS

Golden metallic threads investigated through portable XRF proved to be composed mainly of silver with lower counts of copper and, in some cases, iron, nickel and calcium thus allowing to suggest the use of a silver-copper alloy; the golden aspect of the threads could be achieved with a very thin gold foil, as confirmed by some of the spectra recorded, in which gold is found (Figure 6.5.8). In reverse, silver threads were mainly constituted by silver. XRF measurements on the metallic gallon on the upper part of the tapestry showed the presence of copper, thus proving the use of less precious materials for restoration works compared to the original parts of the artwork.
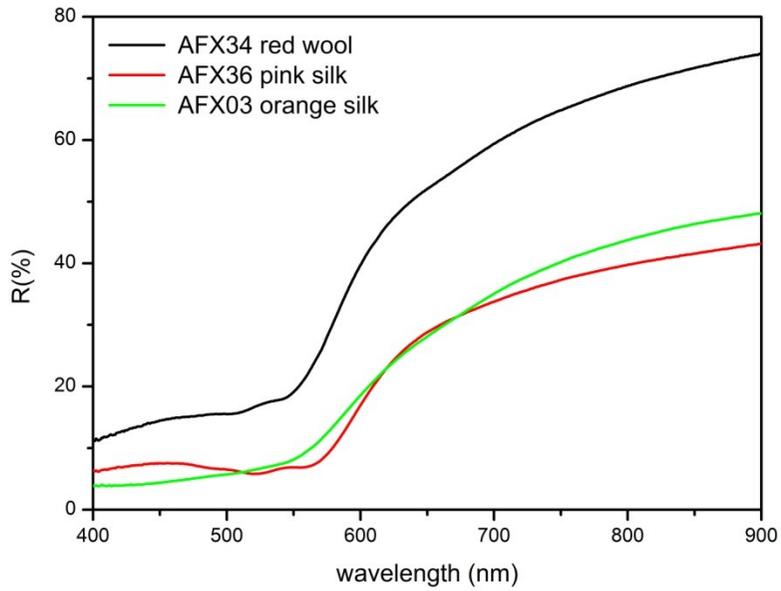
Figure 6.5.2: reflectance spectra of red, pink and orange areas on wool or silk from the front side of the tapestry; the black and the red spectra were recorded on original areas; the green spectrum was recorded on a restored area.

Figure 6.5.3: detail of the tapestry in which Mary of Cleofa is represented.
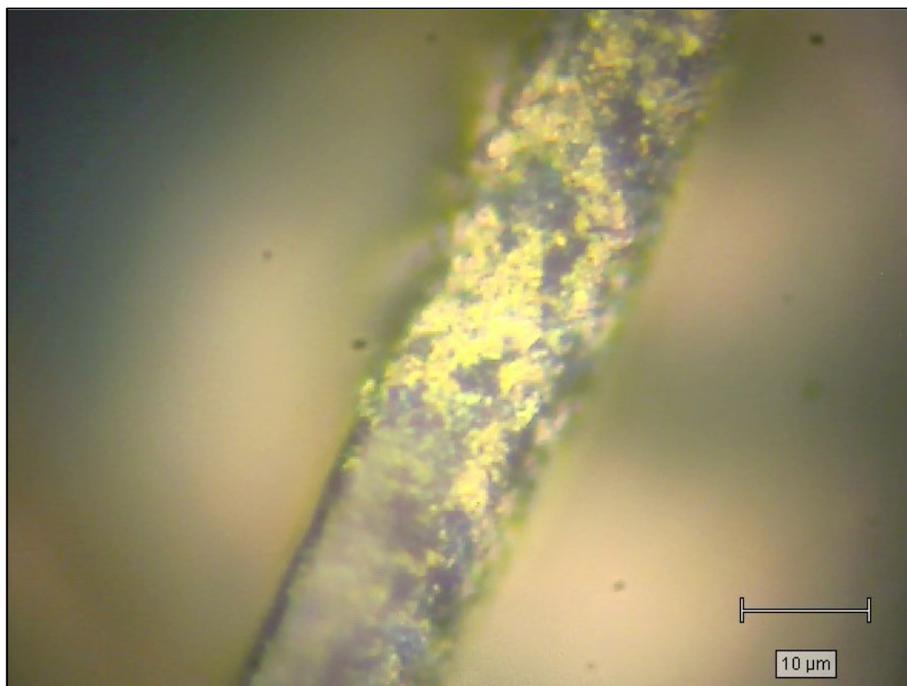
Figure 6.5.4: micrograph (100X) of the silver coating of a red silk fiber from the tapestry.
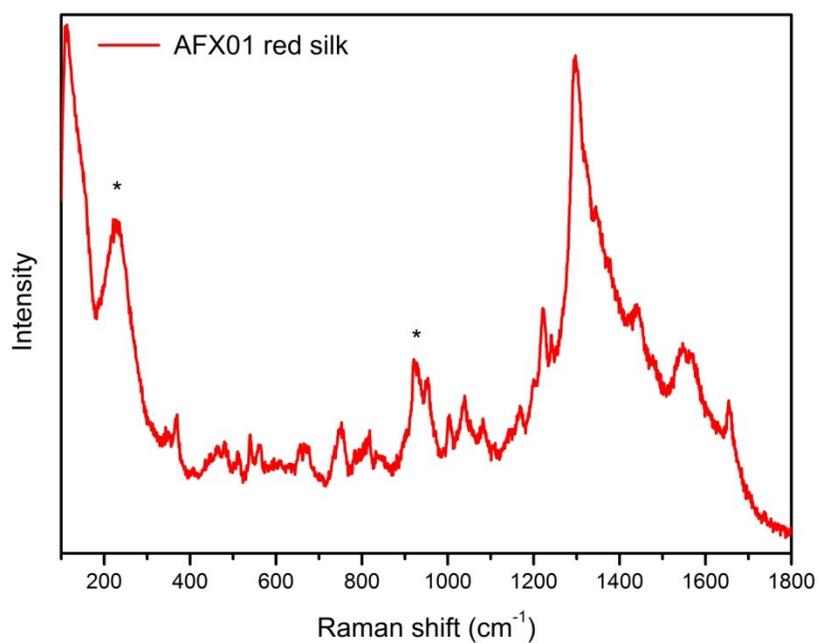

Figure 6.5.5: SER spectrum of a red silk fragment from the tapestry; the spectrum was recorded on the nanoisland in the center of Figure 4; asterisks indicate peaks arising from silver colloidal paste.
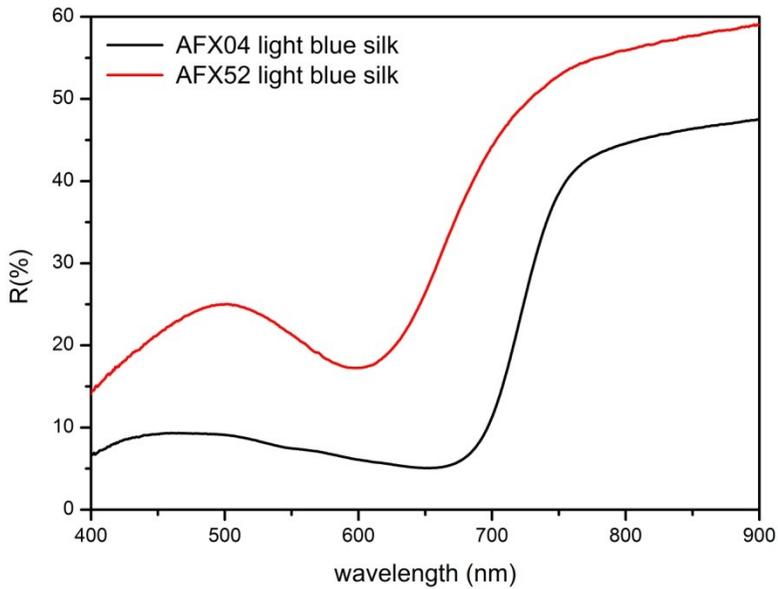
Figure 6.5.6: reflectance spectra of blue areas on silk; the black spectrum was recorded on an original area from the front side of the tapestry; the red spectrum was recorded on a restored area in the reverse side of the artwork.
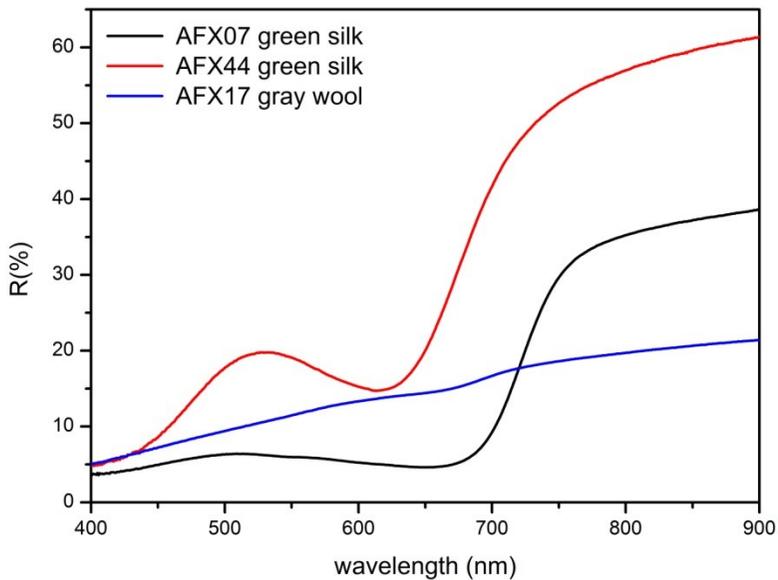

Figure 6.5.7: reflectance spectra of green and areas on silk or wool; the black and the blue spectra were recorded on original areas from the front side of the tapestry; the red spectrum was recorded on a restored area in the reverse side of the artwork.
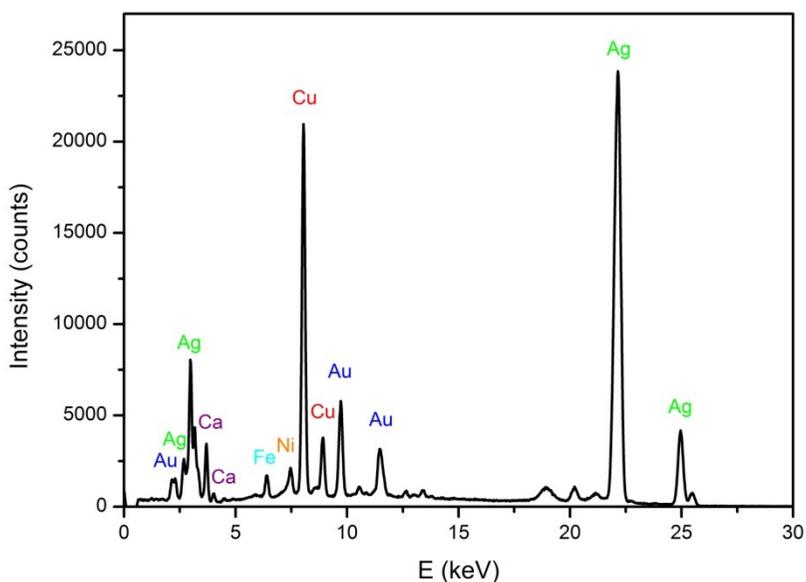
Figure 6.5.8: X-ray spectrum from a golden metallic thread of an original part of the artwork.

6.5.3 CONCLUSIONS

This work allowed the identification through spectroscopic techniques of most of the dyestuffs employed on original and restoration parts of the Deposition from the Cross tapestry. Table 6.5.1 summarizes the identified dyestuffs according to the type of fiber (silk, wool, cotton). In particular, reflectance spectroscopy proved that red silks and wools were dyed with different dyestuffs: scale insects dyes for silk and madder for wool. In the first case, a vivid effect is obtained by combining the natural shining of silk with the intense color of coccid dyes.

SER measurements from a very small thread of red silk confirmed the attribution and better defined the possible species employed, as the presence of the characteristic peaks of carminic acid excluded the use of kermes or lac dye (e.g. species that are not rich in carminic acid), thus pointing to the use of cochineals. The determination of the cochineal species would be of main interest since the supposed date of production of the tapestry is only some years before the first importation of Mexican cochineal into Europe (1512) (Bellucci *et al.* 1991). Unfortunately, a further deepening in the nature of dyestuff is not possible by means of SERS, and it is very critical for ancient samples even through the identification of minor components by means of high performance liquid chromatography (Wouters and Verhecken 1989; Karapanagiotis *et al.* 2005), since the composition of different cochineals is very similar and because photodegradation of the different chemical species would have changed the quantitative pattern of the anthraquinones originally present in the dyestuff.

Orange colors were possibly obtained by mixing madder with an unidentified yellow dyestuff, both on silk and on wool. The dyestuff employed for blue was mainly composed of indigotin, though it was used as vat dye in original threads while it was treated with sulphuric acid to obtain Saxon blue in restoration parts. This latter blue dye was produced only from 1740 (Hofenk de Graaff 2004), thus placing the restoration work after this date. The same differences between original and restoration parts could be found in green areas obtained from a blue indigoid dyestuff and an unknown yellow dyestuff. In addition, indigotin was found also on a gray area.

Portable X-ray fluorescence favored the identification of some pigments spread on some areas of the tapestry and on inserts. In particular, a diffuse exploitation of Prussian blue was found, while some pink areas were obtained with vermillion. It is worth observing that cotton was exclusively used for inserts painted with pigments. In addition, XRF measurements allowed the identification of the main elements for the production of metallic threads: golden threads were obtained with a silver-copper alloy covered with a golden foil, while silver threads were mainly made of silver and the subsequently added gallon of the upper part of the tapestry was made by copper threads.

In general, original materials were highly-priced, while restoration parts were obtained from cheaper dyestuffs and metals, with the addition of sewed parts painted with inorganic pigments instead of being dyed. The determination of the main coloring matter by means of SERS in such a small and ancient sample confirmed the applicability of the proposed procedure with silver colloidal pastes (Idone *et al.* 2013), even though the presence of other species in the matrix made the registration of a spectrum of the dyeing molecules hard. The information yielded by the instrumental investigation was of particular interest for museum's curators due to the beauty and great art-historical relevance of the tapestry. In fact, the overall mapping of the original and restoration materials substantially deepened the knowledge of this very complex textile. Moreover, analytical data were exploited by the restorers to plan their restoration preserving the original materials and some of the subsequent additions that are now fully part of the tapestry itself.

Table 6.5.1: summary of the colorants identified through reflectance spectroscopy and portable XRF; empty boxes correspond to situations not found on the textile.

HUE	SILK				WOOL		COTTON
	Original	*Not original*	*Reverse side*	*+ pigment*	*original*	*+ pigment*	*Not original + pigment*
RED	Scale insects dyes Not identified	Scale insects dyes	Scale insects dyes	Scale insects dyes + Prussian blue	Madder Not identified		Scale insects dyes + ?
PINK	Scale insects dyes		Scale insects dyes Not identified		Not identified		vermillion
PURPLE			Antraquinonic dye				
YELLOW	Not carotenoids						
ORANGE	Madder + yellow dye Not identified				Madder + yellow dye		
BLUE	Indigoid dye	Not identified	Indigo carmine Not identified	Indigoid dye (original) + indigo carmine + Prussian blue	Indigoid dye	Indigoid dye + Prussian blue	Indigoid dye + Prussian blue
BLUE-GREEN							Indigoid dye + yellow dye + Prussian blue
GREEN	Indigoid dye + yellow dye		Indigo carmine + yellow dye +	Indigo carmine + yellow dye + Prussian blue			
GRAY					Indigoid dye + tannins?		
BROWN	Not identified						

6.6 Non-invasive and micro-invasive analyses on two copes from the Cathedral of Aosta

The *Museo del Tesoro* of the Cathedral of Aosta occupies the Gothic deambulatory behind the major altar and the adjacent chapel of the relics. It illustrates through the conserved objects the evolution of art, history and faith in Valle d'Aosta from the 1st to the 19th century AD. Among the conserved artworks, of particular value are a roman cameo, several Gothic sculptures from the 13th-14th centuries and the big case-reliquary of San Grato. Moreover, Romanesque and Gothic stained glass windows, precious goldsmith's art objects, funerary monuments, paintings, vestments and wooden sculptures are exposed in the museum.

Among the conserved objects, two magnificent liturgical vestments are displayed in the cabinets of *Museo del Tesoro*. These long mantles open in front and fastened at the breast with a band or clasp are known in Latin as "pluviali" and properly named "copes".

The first cope, together with a chasuble and chalice veil made with the same fabric, is among the most remarkable ones of the diocese of Aosta. The cope was assembled most likely after the 17th century with parts and swatches of different provenance, date and quality. In particular, the date of production and the provenance of the crimson velvet mantle still remain uncertain. The cope was investigated through non-invasive and micro-invasive techniques as a sampling from the red velvet brocade mantle was authorized. A second 17th century polychrome velvet cope was investigated by means of non-invasive analyses.

6.6.1 Materials and methods

15TH CENTURY COPE

The first analyzed cope, as mentioned above, is composed of several parts (Figure 6.6.1) with different provenances and dates of production (Zidda 2010; Appolonia *et al.* 2012). In particular, the mantle (153x305 cm) is a combination of six portions of a crimson silk velvet brocade with golden metallic threads. The place of production of the mantle is still controversial, since different scholars suggested comparisons with 18th century Morelli's artworks or Rogier Van Der Weiden's designs. Moreover, no records of the cope were found in the archives before 1837 inventory. In addition, a recent work (Oliva and Zidda 2009) demonstrated that it could be produced both in Europe or in Near as well as Far East. On the straight part of the mantle a stole (17x300 cm) is sewn. The stole is composed by ten embroidered tiles on an unbleached linen cloth. The tiles portray Saints or Apostles on aediculae and are possibly collocated in the 15th century French-Piemontese or Flemish schools. In the middle of the stole a collar (16x38 cm) from a fragment of a cope's cowl is attached. The center of the collar is embroidered with the scene of the Enthronement of the Virgin, of a German production from the beginning of the 16th century; two inserts of a yellow decorated Florentine border with the Ascension of Christ on a red background (16th century) are stuck on the sides of the scene. Finally, a shield-shaped cowl (42x48 cm) representing an Annunciation of Tuscany style and German or French technique, dated to the end of the 15th or the beginning of the 16th century, is fixed to the collar, embroidered on a linen cloth with polychrome silk and metallic threads. Most of the embroidered parts of the cope were re-

integrated and modified with headlong and inaccurate interventions (Oliva and Zidda 2009), while the mantle presents three different linings whose originality and dating are doubtful. The identification of the red dyestuffs of the velvet would possibly help in the individuation of the date and place of production of the mantle. In addition, an indication of the possible dating could be supported also by the determination of the technique of production of the metallic threads, as it is known that in Italy membranous gold ceased to be used in 15th century and was replaced by the use of metal foils wrapped on a silk thread core.

Since the cope could not be removed from its horizontal case, which can be opened only from one side, reflectance spectroscopy was performed only in areas that could be reached by the operator with the help of the fiber optics probe. For the same reasons, only the metallic threads of the mobile cowl could be analyzed by means of portable X-ray fluorescence. The presence of a lining strongly limited the access to the cope for sampling, then it was possible to take only a sample under the hem at the right border of the mantle. The sample is constituted by textile and metallic threads (Figure 6.6.2 a). In particular, the textile presents red and pink threads when observed under the stereo-microscope: the hair of the velvet is red-colored while the warps that support the fabric are pink. Moreover, the metallic threads are composed by a metallic platen wrapped on a silk thread. The platen appears golden in the outer side while it is silvery in the inner side (Figure 6.6.2 b-c).

HORNS OF PLENTY COPE

The second investigated artwork is a cope (148x290) realized in a so called "garden" polychrome velvet with horns of plenty (Figure 6.6.3, Oliva and Zidda). Different finishing were employed to give a tri-dimensional effect to the decoration; in particular, the velvet was left uncut in some areas, while in others the threads were as usual cut to display the hair. It is part of a series of four copes with similar design recorded in an 19th century inventory of the Cathedral (after 1860) and clearly found an homologous in another cope from the Cathedral of Aosta, studied in greater detail on the occasion of the exhibition "Textilia Sacra" (Cataldi Gallo 2000). Though, the presence of horns of plenty is of particular concern, since this kind of velvets was produced until the third quarter of 19th century in France. Nevertheless, stylistical and technical considerations allow to locate the production of the two copes in Torino or in Genova, which is the place where "garden" velvets were created at first (Oliva and Zidda),while the date of production is situated between 1660 and 1680. The cope is also represented in a 18th century painting conserved in the Sacristy of the Cathedral depicting San Grato (Zidda 2010).

The presence of a horizontal case prevented most of the non-invasive measurements, thus only some colored areas of the upper part of the cope were analyzed through fiber optics reflectance spectrophotometry. The golden galloon which fringes the cope was investigated through portable X-ray fluorescence. No sampling was allowed on the artwork.

SAMPLE PREPARATION

The threads from the sample taken from the 15th century cope were divided according to the color and analyzed separately. The samples were gently washed with some drops of methanol on a microscope slide, rinsed with bidistilled water and let to dry (6.5.2). Afterwards, single fibers were

divided and cut with a scalpel from the thread. Few of them were coated with 0.5 μL of silver colloidal paste (5.1.1.1). In this way, several sub-samples for SER measurements were prepared.

INSTRUMENTATION

X-ray fluorescence spectra were acquired with a Tracer III-SD (Bruker) with an integration time of 60s at 15kV. The spectra were recorded in order to determine the composition of some metallic threads on the copes.

A Multichannel spectrometer system ZEISS MCS 600 equipped with halogen lamp CLH 600 and two detectors, MCS 601 UV-NIR and MCS 611 NIR 2.2, were employed to perform FORS measurements (appendix II); a geometry of 0°/45°x2 or 0°/0° was used depending on the dimensions of the area under analysis. The range of analysis varied according to the geometry of the probes: 250-2200 nm with of 0°/45°x2 geometry and 350-1100 nm for 0°/0° geometry. A total of forty-nine spectra were recorded on the 15th century cope, among which ten from undoubtedly restored area. In some cases, measurements were repeated on the same area with both the geometries. On the contrary, FORS measurements on the horns of plenty cope were performed only on original areas, since no restored parts were apparent. Thirteen spectra were collected from colored curly and plain velvets. Spectra are all reported in the range 400-900 nm for ease of comparison.

SERS measurements were carried out with a Renishaw InVia spectrometer coupled with a Leica DM2500 M optical microscope, using an excitation wavelength of 633 nm with a magnification objective of 100X. The acquisition time was set to 10s while the laser power onto the sample never exceeded 200 μW.

HPLC-DAD-MS analyses were performed on portions of the samples investigated through SERS. Dyes were extracted from the textile following the traditional procedure reported by several authors (Surowiec, Quye, and Trojanowicz 2006; Karapanagiotis *et al.* 2005; Zarkogianni, Papliaka, and Tsatsaroni 2009; Valianou, Karapanagiotis, and Chryssoulakis 2009), that is extraction with hydrochloric acid (37%, w/w):methanol:water 2:1:1 (v/v/v). Briefly, 1 mL of extracting solution was put on a test tube with about 2 mg of the sample and kept at 90°C for 10 minutes; then, the solvent was evaporated under nitrogen stream at 60°C and 0.5 mL of methanol:water (1:1, v/v) were subsequently added. The solution is left to cool at room temperature, the tube is centrifuged at 4000 rpm and 200 μL were employed for HPLC-DAD-MS measurements. HPLC conditions are reported in Appendix 2.

Scanning Electron Miscroscopy coupled to Energy Dispersive Spectrometry was performed at Centro Conservazione e Restauro "La Venaria Reale" using a Zeiss EVO60 coupled to EDX Oxford PentaFET microprobe in order to investigate the composition of the metallic threads. The threads were analyzed directly as well as mounted in cross sections, polished up to 1 μm and coated with a graphite layer.

Micro- X-ray fluorescence measurements were performed with a Roentgenanalytik Systeme μXRF EAGLE III XPL in order to ascertain the composition of the metallic threads of the 15th century cope. A cross section and an unwrapped platen from the cope were analyzed; the platen was investigated on both sides.

Further technical details about the employed instrumentations are given in Appendix 2.

Figure 6.6.1: the vestment composed as a cope, assembling 15th and 16th century elements.
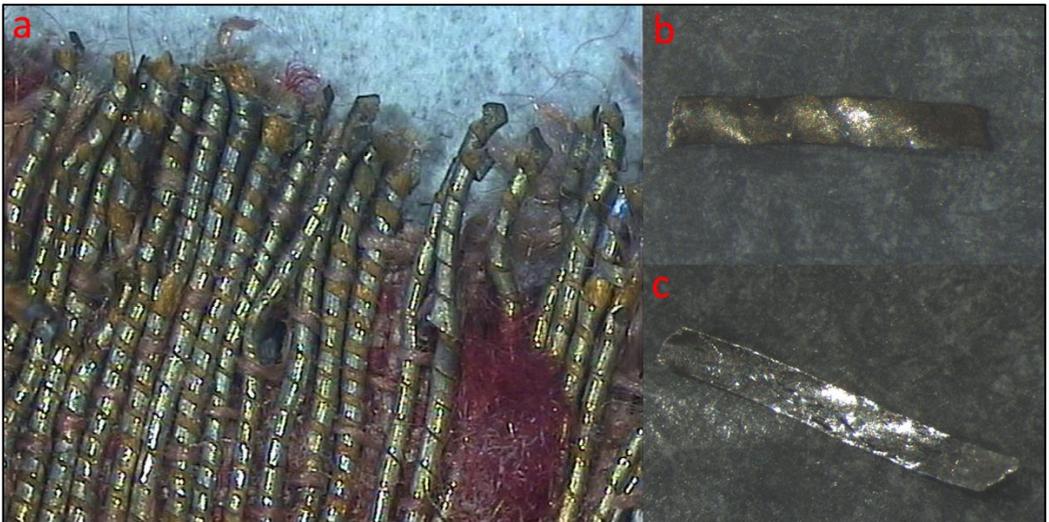

Figure 6.6.2 stereomicrograph of: a detail of the sample from the 15th century cope, 6X (a); the external side of the metallic platen of the brocade, 18X (b); the internal side of the metallic platen of the brocade, 18X (b).

Figure 6.6.3: the 17th century polychrome velvet cope.

6.6.2 RESULTS AND DISCUSSION

15TH CENTURY COPE

METALLIC THREADS.

Portable XRF measurements on the fringes of the cowl revealed the presence of copper, thus allowing to identify this metal as the main component of the employed metallic threads. Moreover, the metallic threads employed to embroider the cowl proved to be obtained with an alloy of copper, silver and gold.

Back-scattered images obtained on the cross sections of the metallic threads identified a brighter area (e.g. composed by heavier elements) of about 1-2 µm in the outer side of the platen, whose total thickness is about 20 µm (Appolonia *et al.* 2012). Concentration profiles of the cross sections evidenced the presence of higher counts of gold in that area, while the darker part of the platen are mainly made of a silver-copper alloy. Moreover, the investigation of the whole thread (e.g. not mounted in cross section) highlighted the presence of parallel stripes on the external surface of the platen (Figure 6.6.4), while the cutting edge is heterogeneous. In particular, an external thinner layer is partially superimposed to a thicker internal layer, at the interface between the two layers, a darker area, in which lighter elements are revealed (Si, Ca, Cl, Al, K, Fe, Mg), is observed (Figure 6.6.5). EDS measurements together with morphological observations allowed to hypothesize that the platen was obtained putting a golden foil (or gold-silver-copper foil) on a thicker platen made of a silver-copper alloy (Nicodemi and Mapelli 2009). The platen was then cut into ribbons and wrapped onto the silk thread; the superimposion of the gold foil to the silver-copper platen is possibly due to cutting, while the presence of lighter elements could be due to the deposition of atmospheric particulate. Also the parallel stripes could indicate a processing technique, namely the polishing of the platen (Nicodemi and Mapelli 2009).

Micro-XRF measurements confirmed the presence of the elements revealed by EDS and allowed a quantification of the major elements with an error lower than 2% in weight of the element. In particular, the measurement of an area of about 10 μm^2 revealed 88.1% of silver, 6.6% of copper and 3.7% of calcium, with minor quantities of sulphur, iron and lead. The analysis of the platen identified high quantities of calcium on both sides, possibly due to the presence of dirt. The golden side is composed of 78.5% of silver, 15.6 of calcium, 3.0% of copper and 0.7% of gold. On the contrary, in the silver side the quantities of calcium are higher (24.9%), while silver content is 70.8%, copper is 3.1% and gold is found in minor amounts with an high error. Sulphur, iron and lead were also revealed on both sides of the platen.

DYES.

Red dyes were analyzed through FORS in areas from the mantle (Figure 6.6.6, black and red spectra), the stole (Figure 6.6.6, blue spectrum) and the decorated border (Figure 6.6.6, green spectrum). All the spectra presented an absorption band structured in two sub-bands at 525-530 and 565-570 nm, thus pointing to the use of scale insects dyes. Spectra recorded on areas of the mantle covered by the cowl (Figure 6.6.6, red spectrum) showed darker hues making the interpretation of the spectral features more arduous than in areas exposed to light-fading (Figure 6.6.6, black spectrum).

The coating obtained on red fibers (Figure 6.6.7) allowed to record several SERS spectra attributable to dyes (Figure 6.6.8). In particular, a shoulder or a peak at 1200-1220 cm^{-1} and of an intense peak at about 1300 cm^{-1} were attributed to carminic acid; in some spectra, also weaker peaks of carminic acid at 460 and 1425 cm^{-1} are observed. It should be also noted that the shifts in the major peak are consistent with data reported in previous works (Cañamares *et al.* 2006; Leona, Stenger, and Ferloni 2006; Whitney, Casadio, and Van Duyne 2007; Lofrumento *et al.* 2013; Pozzi *et al.* 2012). SERS measurements thus confirmed the attribution to scale insects dyes obtained by FORS and allowed to exclude the use of kermes and lac dye (1.3.2): a cochineal dye was employed for the mantle of the cope.

HPLC measurements showed the presence of a major compound with absorption maxima at 276 and 495 and molecular ion of 493 m/z (cf. Table 6.1.1), corresponding to carminic acid. These data confirmed the results obtained by SERS. The presence of dcII compound, with absorption maxima at 238, 285 and 434 nm and molecular ion of 667 m/z (cf. Table 6.1.1) was instead not found in the analyzed sample. The absence of dcII permit to exclude the use of Mexican cochineal, and to point to the possible use of Polish cochineal, which does not contain this compound (Wouters and Verhecken 1989).

FORS measurements on **pink** original (Figure 6.6.9, red and green spectra) and restored areas (Figure 6.6.9, black and blue spectra) of the stole revealed two different trends in the recorded spectra. In fact, some original and restored areas showed a clear absorption band at about 520 nm (Figure 6.6.9, black and green spectra), while others did not evidence diagnostic spectral features (Figure 6.6.9, red and blue spectra). In the first case, the spectra correspond to those recorded on brazilwood dyed mock-ups.

SERS measurements performed on the pink threads of the mantle (Figure 6.6.10, red, purple and blue spectra) revealed the presence of very intense peaks at about 460, 1350 and 1545 cm^{-1}, while less intense peaks were found at about 1160, 1200, 1310 and 1495 cm^{-1}. The spectra are quite

reproducible in terms of position and intensity of the peaks. The correspondence with major and medium peaks of the SER spectra of mock-ups dyed with brazilwood (Figure 6.6.10, black spectrum) is good, as well with some of their minor features (for example shoulders at 410, 1605 and 1390 cm^{-1}). The correspondence of the peaks registered in mock-ups with published data has been already discussed (5.1.2.3). HPLC measurements revealed a compound with absorption maxima at 242 and 449 nm and molecular ion of 283 nm (cf. Table 6.1.1), corresponding to brazilein, and of a second compound with absorption maxima at 256, 306 and 331 nm and molecular ion of 243 m/z. This second compound was previously identified by several authors (Karapanagiotis and Chryssoulakis 2005; Karapanagiotis *et al.* 2005; Manhita *et al.* 2011; Manhita *et al.* 2013) as the type C compound, a degradation product of brazilwood. Also in this case, HPLC measurements confirmed the results obtained by means of SERS.

Blue areas were investigated on the stole (Figure 6.6.11, green spectrum) and on the collar (Figure 6.6.11, black spectrum) and were all characterized by reflectance spectra with an absorption band at 640-660 nm, assigned to indigotin. Most of the investigated areas presented one or more bands in the red region from 550 to 590 nm, thus allowing to hypothesize the use of a blue indigoid vat dyestuff with a red unidentified dyestuff, as the bands were so weak that a more precise attribution was prevented. In some blue and **blue-violet** areas where the bands were more intense (Figure 6.6.11, blue spectrum), the presence of a scale insect dye was suggested. No differences were found between the original and the restored areas of the stole. The absorption band of indigotin was found also on **green** and **yellow-green** areas (Figure 6.6.11, red spectrum), while no information could be obtained for **yellow** dyes, except that they are not carotenoids. It should also be noted that all the reflectance spectra from original yellow areas of the stole presented the absorption band of indigotin, while this band was not observed on restored areas.
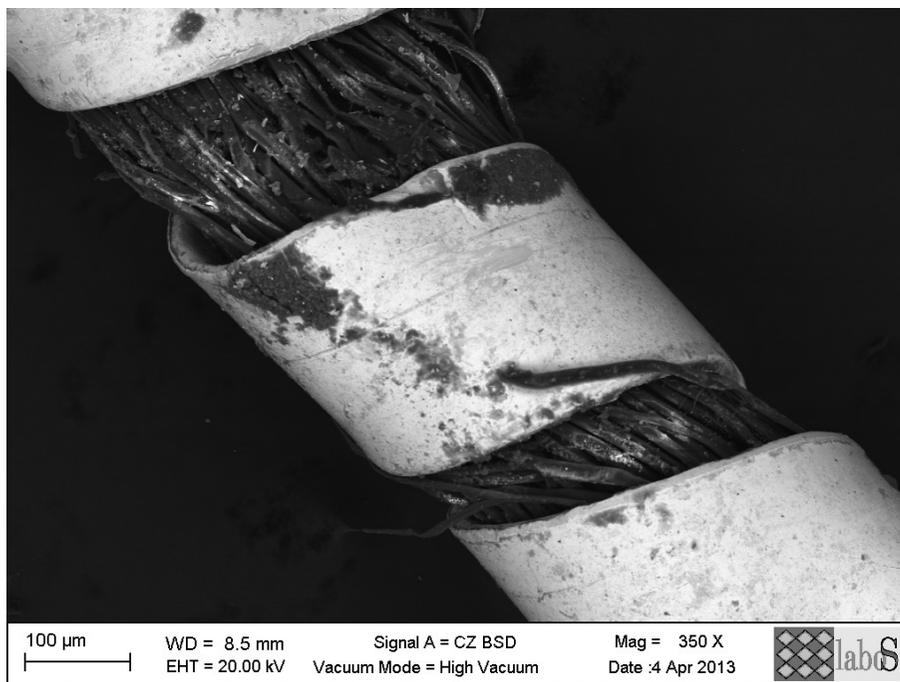

Figure 6.6.4: micrograph obtained with backscattered electrons of a metallic thread from the 15th century cope, 350X.

202
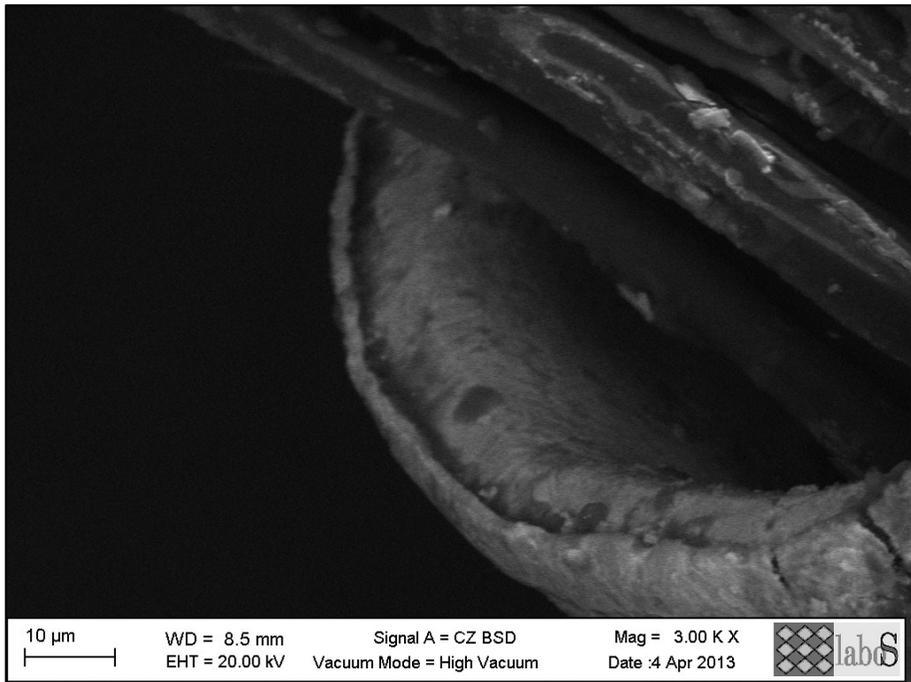
Figure 6.6.5: micrograph obtained with backscattered electrons of the cutting edge of a metallic thread from the 15th century cope, 3000X.
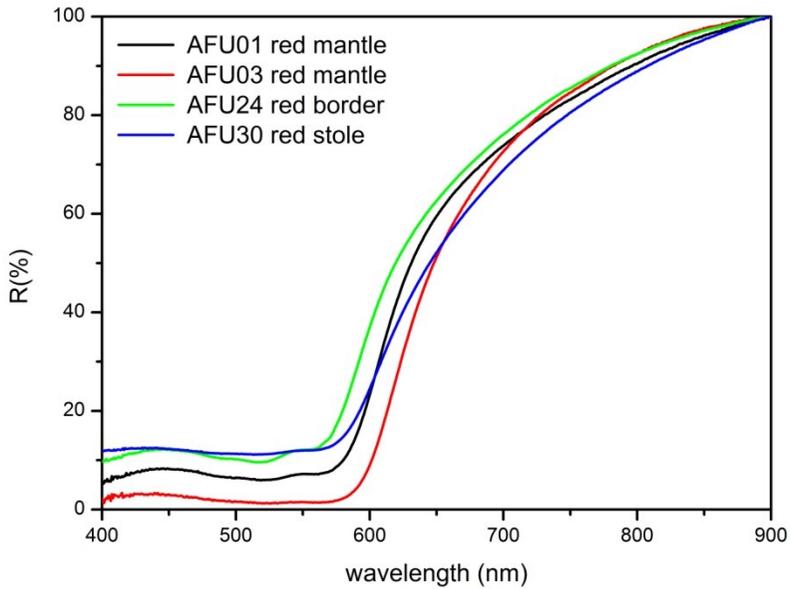

Figure 6.6.6: reflectance spectra from original red areas of the 15th century cope. Black and red spectra were recorded on the velvet mantle, green spectrum was recorded on the decorated border, blue spectrum was recorded on the stole.

203
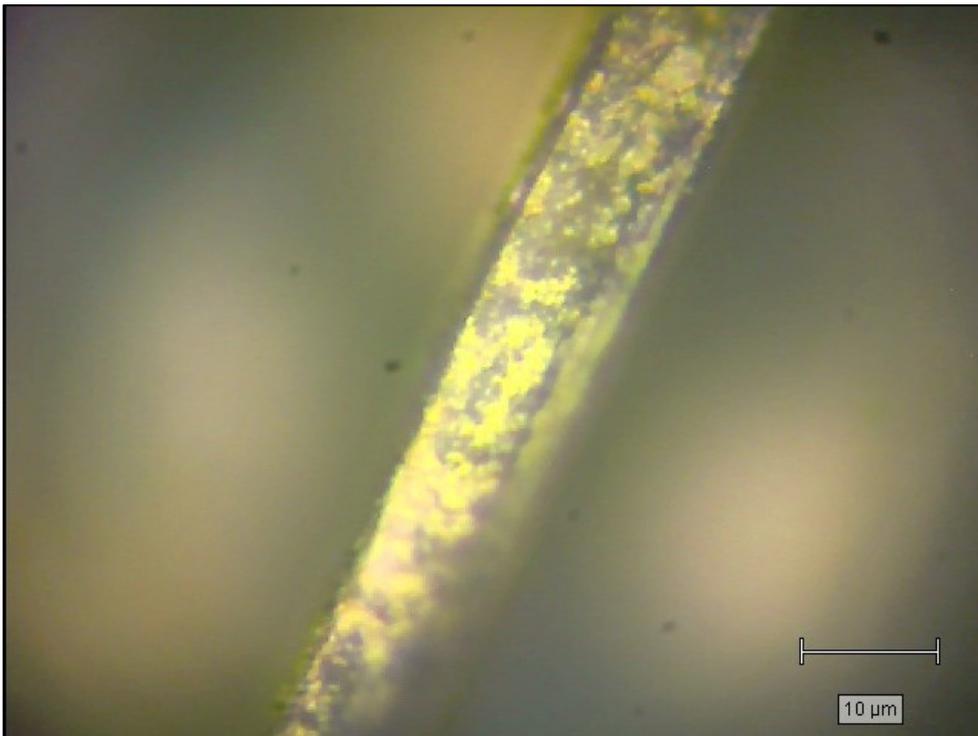
Figure 6.6.7: micrograph (100X) of the silver coating of a red silk fiber from the mantle the 15th century cope.
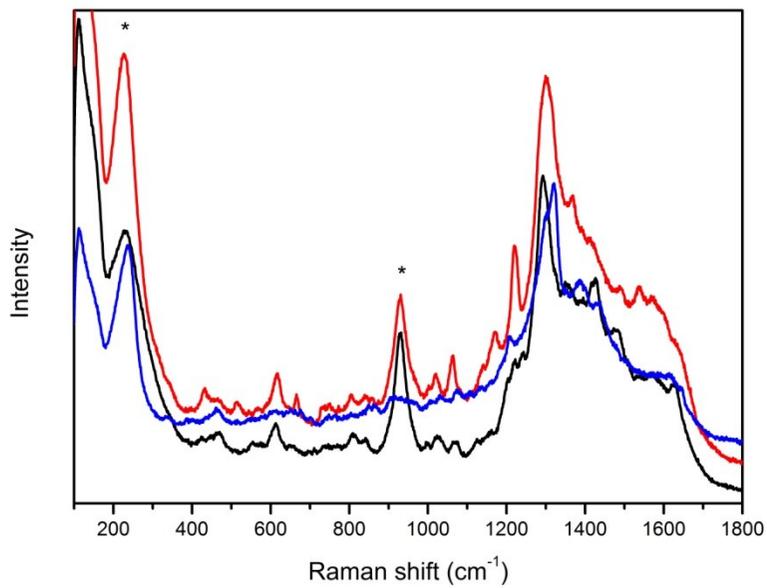

Figure 6.6.8: SER spectra of the red silk threads from the mantle of the 15th century cope; asterisks indicate peaks arising from silver colloidal paste.
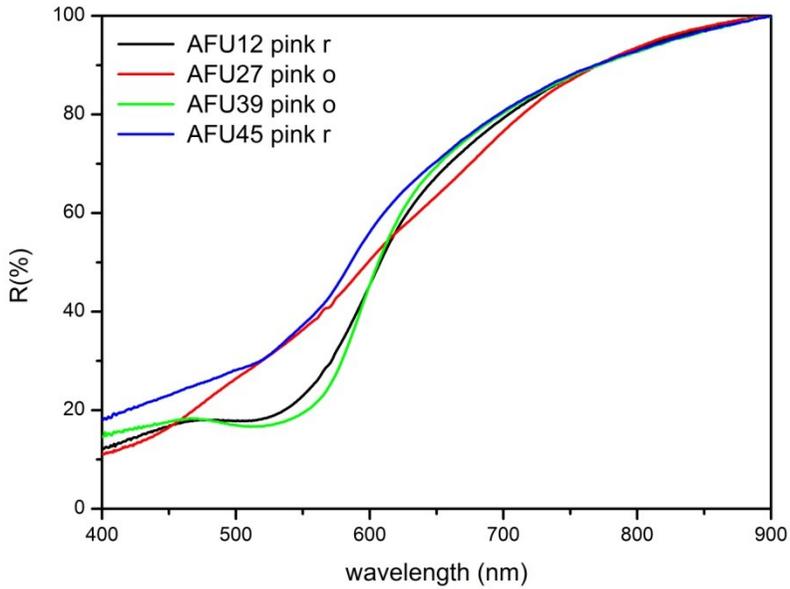
Figure 6.6.9: reflectance spectra from pink areas of the stole of the 15th century cope. Red and green spectra were recorded on original areas, black and blue spectra were recorded on restored areas.
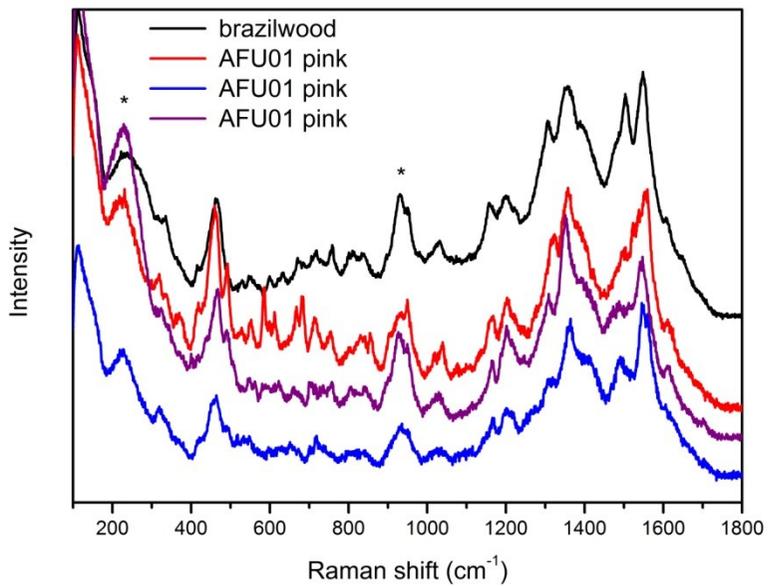
Figure 6.6.10: SER spectra of the pink silk threads from the mantle of the 15th century cope (red, blue and purple spectra) compared with a SER spectrum from a silk mock-up dyed with brazilwood (black spectrum); asterisks indicate peaks arising from silver colloidal paste.
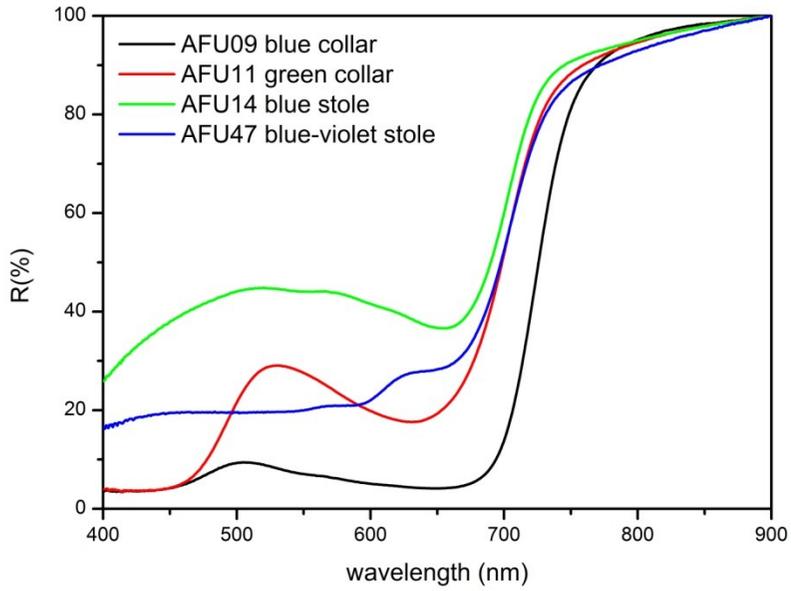
Figure 6.6.11: reflectance spectra from blue, blue-violet and green areas of the 15ᵗʰ century cope. Black and red spectra were recorded on the collar, green and blue spectra were recorded on the stolon. Green spectrum was recorded on a restored area.

XRF measurements performed on the galloon of the "garden" cope with horns of plenty revealed that it is mainly composed by silver, while minor quantities of gold, copper, calcium and iron were also revealed.

Red areas investigated by FORS (Figure 6.6.12, red spectrum) revealed the presence of a structured absorption band at about 525 and 570 nm, thus allowing to identify the presence of scale insects dyes. On the contrary, spectra from **pink** areas (Figure 6.6.12, blue spectrum) showed an absorption band at 515-520 nm and an additional band at 655-660 nm. The first band was attributed to brazilwood while the second one indicates that this red dyestuff was possibly mixed with an indigoid vat dyestuff. **Green** areas presented reflectance spectra with an absorption band at 640-650 nm (Figure 6.6.12, black spectrum), attributed to indigotin. Also in this case, no information could be obtained for **yellow** dyestuffs. Moreover, no spectral differences were found among the two finishings of the velvet.
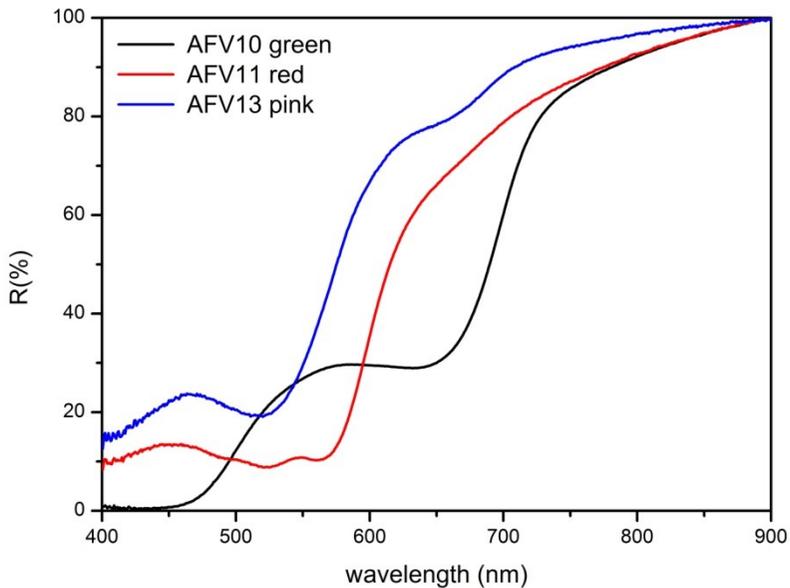

Figure 6.6.12: reflectance spectra from original areas of the 17th century cope. Black spectrum was recorded on a green area, red spectrum was recorded on a red area, blue spectrum was recorded on a pink area.

6.6.3 CONCLUSIONS

This comprehensive study allowed to ascertain the attribution of dyes and the composition of some metallic threads in two historical copes. In particular, a cope from *Museo del Tesoro* of the Cathedral of Aosta possibly dated to the 15th century obtained assembling several fragments of different provenances and dates was investigated by non-invasive techniques (portable XRF and FORS). In addition, a sample was taken from the crimson silk velvet mantle brocade with golden metallic threads and fully investigated in order to deepen the identification of dyestuffs and metals. Sampling from this part of the cope was of particular interest as the mantle had an unknown conservative history and no information was found as regards its place and date of production. In particular, technological aspects of the production of the metal threads as well as their composition and the identification of the red dyestuffs would allow to compare the data with a previous work on ottoman textiles (Sardjono 2007) and to possibly confirm the recent attribution of this textile to an Islamic production (Oliva and Zidda 2009). In addition, non-invasive measurements were performed on a 17th century cope conserved in *Museo del Tesoro*.

The results obtained on the first cope clarified the production technique of the golden metallic threads of the brocade. In particular, they were obtained wrapping a metallic platen on a silk thread; the platen was made by superimposing a thin gold (or gold-silver-copper) foil on a thinner silver-copper platen and then polished. The comparison with previous data on ottoman textiles (Sardjono 2007) underlined some differences, as ottoman metallic threads proved to be gilded on both sides. Moreover, ottoman metallic threads were composed of nearly pure gilded silver (Cu<1%), while in this case silver forms an alloy with copper.

As for the identification of the dyestuffs employed on the first cope, reflectance spectra indicated the use of scale insects dyes for reds and brazilwood for pinks. In addition, the practice of double dyeing was widespread in the different parts of the cope as most of blue, blue-violet, green, green-yellow and yellow colors were obtained with this technique. In particular, the addition of red (identified with scale insects dyes in some cases) to a blue indigoid vat dyestuff for blue colors and of a blue indigoid vat dyestuff to a yellow unidentified dye for yellow colors was quite unusual. On the contrary, the superimposion of a blue indigoid vat dyestuffs to red dyestuffs (scale insects dyes) or yellow dyestuffs (unidentified) to obtain purple and green shades was a common practice. It is interesting to observe that original yellow dyestuffs presented the addition of the indigoid dye while restored part did not show features attributable to indigotin.

SERS and HPLC-DAD-MS measurements performed on red and pink threads of the cope allowed the identification of carminic acid for the red sample and of brazilwood for the pink sample. The identification of carminic acid was of particular interest as it confirmed and deepened the results obtained by FORS. In particular, SERS measurements identified a carminic acid-rich scale insects and HPLC-DAD-MS measurements confirmed the presence of high quantities of carminic acid. Furthermore, the absence of dcII compound was ascertained by this technique, thus allowing to possibly identify the employed dyestuff as Polish cochineal. Moreover, the use of threads with different functions dyed with different dyestuffs is reported for ottoman velvets (Sardjono 2007); scientific investigations revealed the employ of various red dyestuffs but no record of previous identification of brazilwood were found. In reverse, Mexican cochineal and brazilwood were

identified on several 17th and 18th century Romanian religious velvet brocade by means of HPLC-DAD and HPLC-MS (Petroviciu *et al.* 2012).

Non-invasive measurements on the second cope allowed to identify the composition of the galloon (e.g. silver-gold-copper alloy) and the dyestuffs employed to obtain red, pink and green colors that decorate the cope. In particular, reds were all obtained with scale insects dyes and pinks with brazilwood and an indigoid blue vat dyestuff; this latter was also employed on a yellow dyestuff to obtain green colors.

In conclusion, this work demonstrated that a number of information about the materials and the techniques employed on complex historical objects can be obtained with non-invasive measurements supported by the sampling of only one fragment from a selected and representative area. Additional information was given to art historians in order to achieve a more detailed contextualization of the investigated copes. Moreover, the possibility of performing HPLC-DAD-MS and SERS on the same historical sample allowed to cross-link the data and to verify the effectiveness of the procedure for *in situ* extractionless SERS.

6.7 Non-invasive and micro-invasive investigation on a 6TH century purple codex

Purple codices are among the most relevant and prestigious book productions of Late Antique and Medieval age. They usually contained texts from Holy Writings written with golden or silver inks on parchment colored in a purple hue.

In this work, the dyestuffs that were possibly employed to color purple codices have been discussed in paragraph 3.2. In addition, a systematic investigation of the spectroscopic features of these dyestuffs was carried out by means of fiber optics reflectance spectrometry and surface-enhanced Raman scattering and is reported in paragraphs 3.2 and 5.2, respectively. As for SERS, different approaches for the analysis of dyed parchments were also compared.

Previous analytical works mainly employed high performance liquid chromatography to identify Tyrian purple (Karapanagiotis *et al.* 2006; Koren 2006; Koren 2012) but in some cases also SERS was used on archaeological objects (Bruni, Guglielmi, and Pozzi 2010; Van Elslande, Lecomte, and Le Ho 2008). Moreover, orchil was analyzed by means of fluorimetry (Clementi *et al.* 2006; Clementi *et al.* 2009) and subtracted shifted Raman spectroscopy (Rosi *et al.* 2013). The first molecular information on folium was obtained by means of SERS in this work and are reported in the above mentioned paragraph 5.2.

As for manuscripts, bromine would have been identified in a miniature painting of the Barberini Gospels codex (Vatican City, Biblioteca Apostolica Vaticana, ms. Barb. lat. 570) (Porter, Chiari, and Cavallo 2002; Porter 2008); in that case, a single occurrence of bromine was found overall the codex and the presence of Tyrian purple was never confirmed with other direct techniques such as Raman spectroscopy or FT-IR. It must be noted that in other early Medieval codices analysed by Porter, bromine was not found at all. Aceto *et al.* identified bromine in the 6th century AD purple codex known as Vienna Genesis (Vienna, Österreichische Nationalbibliothek, Cod. Theol. Gr 31) but analysis with UV–visible diffuse reflectance spectrophotometry did not confirmed the presence of Tyrian purple in the same context (Aceto *et al.* 2012). Apart from these studies, very few other analytical works were performed on purple codices. In the pioneering work by Thomas and Flieder on the Sinope Gospels purple codex (Paris, Bibliothèque Nationale de France, ms. Suppl. gr. 1286), GC–MS analysis carried out on a parchment fragment did not evidence the presence of Tyrian purple (Thomas and Flieder 1980); authors indicated instead turnsole as the dye used. Similar results were obtained by Roger in her non-invasive investigation on the Godescalc Evangelistary (Paris, Bibliothèque Nationale de France, ms. Nouv. Acq. lat. 1203) in which the author used XRF and spectrofluorimetry analysis on the purple parchment (Roger 2007); results showed that Tyrian purple was absent and turnsole was again suggested as the most probable candidate for the purple color. Finally, Rosi *et al.* and Eveno *et al.* both identified orchil on a fragment of purple parchment taken from 9th century codex known as Bible de Théodulphe, conserved in the Cathedral in Le Puy-en-Velay (France) (Rosi *et al.* 2013; Eveno, Délit, and Nowik 2003).

The information gained within the methodological work of this dissertation, together with some additional measurements carried out on mock-ups (3.2.1) by means of portable techniques (portable optical microscopy, X-ray fluorescence spectrometry and portable molecular spectrofluorimetry), was exploited to shed light on the materials and techniques employed on a 6th

century purple codex, the so-called *Codex Brixianus* from Biblioteca Civica Queriniana (Brescia, Italy). Analyses on the codex were firstly performed *in situ* in totally non-invasive way with X-ray Fluorescence Spectrometry (XRF), UV–visible diffuse reflectance spectrophotometry with optical fibers (FORS), spectrofluorimetry and optical microscopy. Results were compared with those obtained from parchment painted or dyed with various purple dyes. In a second time, sampling from the manuscript was authorized by the curators of the library. The sample was then investigated by means of SERS with several different approaches.

The results obtained with micro-invasive techniques on this artwork have been published, together with considerations on the results obtained on mock-ups, in a recently published work in Spectrochimica Acta Part A (Aceto, Idone *et al.* 2014).

6.7.1 MATERIALS AND METHODS

CODEX BRIXIANUS

The manuscript under analysis is held in the Biblioteca Civica Queriniana at Brescia and is known as *Codex Brixianus*. The *Codex Brixianus* was produced in Northern Italy in Gothic realm, possibly in Ravenna, at the beginning of 6th century AD; it was acquired by Biblioteca Civica Queriniana in 1797. It contains 418 folios, all of them of purple color (Figure 6.7.1). It was written in uncial script with silver ink and golden titles; according to stylistic investigation, two different scribes were at work on it. The codex contains the four Gospels written in the antejeronimian version, that is St. Matthew, St. John, St. Luke and St. Mark (Ferraglio 2010; Lowe 1938; D'Agostino 1994); similarities with the Gothic version have been observed in some points of the text. In the lower margin of each folio, concordance tables of the Gospel text are present.

SAMPLE PREPARATION

The sample from *Codex Brixianus* (Figure 6.7.2) was divided into several sub-samples in order to test different approaches for the identification of the coloring matter through SERS. In particular, one minute fragment (less than 1 mm^2) was directly coated with 0.1 µL of silver colloidal paste. In addition, a 2 µL drop of different solutions was deposited on three more fragments in order to favor the partial extraction of the dyes; the parchment was removed after about one minute and 0.5 µL of silver colloidal paste were added to both the parchment and the liquid. One fragment was treated with dimethylformamide, one with a solution of hydrochloric acid:methanol:water (2:1:1 v/v) and the latter with formic acid. Also, two bigger fragments of about 1x1 mm were subjected to extraction procedures with formic acid or formic acid:methanol 15:85, slightly modifying the indications reported by Manhita *et al.* (2011) and by Brosseau *et al.* (Brosseau, Gambardella *et al.* 2009), respectively. In particular, 200 µL of formic acid were dropped in a micro-vial containing one fragment and heated at 40°C for one hour. In reverse, 200 µL of a solution of formic acid:methanol (15:85 v/v) were added to the second fragment and heated at 50°C for one hour. Then, the parchment was removed and the solutions allowed to cool. From each extract, a 2 µL aliquot was mixed to the same volume of silver colloidal paste and let to dry. 5 µL of each extract were deposited on two different clean microscope slides and allowed to dry on the same area until a total amount of 25 µL of extract with formic acid and 50 µL of extract with

formic acid:methanol 15:85 were dried onto the slide surfaces; 2 μL of silver colloidal paste were then added to the dried extract. Finally, blank samples were prepared by mixing 2 μL of the methanol, formic acid or dimethylformamide with 2 μL of silver colloidal paste.

INSTRUMENTATION

A Dino-Lite (Naarden, The Netherlands) AM413TL-FVW model optical microscope was employed to take digital images from the parchment. The microscope allows magnification in the range 20x–90x.

XRF measurements were performed with an EDXRF Thermo (Waltham, USA) NITON spectrometer XL3T-900 GOLDD model, equipped with Ag tube. Working distance was set to 2 mm, total time of analysis was 240 s. The obtained spectra have been processed with the commercial software Win-Axil, derived by the academic software QXAS from IAEA.

FORS analysis was performed with an Avantes AvaSpec-ULS2048XL-USB2 model spectrophotometer and an AvaLight-DH-S-BAL balanced deuterium–halogen light source. The investigated area on the sample had a 3 mm diameter. In all measurements the distance between probe and sample was kept constant to 1 mm. To visualize the investigated area on the sample, the probe contained a Framos (Agrate Brianza, Italy) WEB1315SI model digital micro camera, connected to PC via USB. The instrumental parameters were as follows: 30 ms integration time, 50 scans for a total acquisition time of 1.5 s for each spectrum. The whole system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 7. Spectra were all reported in the range 250-900 nm in Log (1/R) coordinates (apparent absorbance) in order to better the diagnostic spectral features.

An Ocean Optics Jaz model spectrophotometer equipped with a 365 nm Jaz-LED internal light source was employed to measure molecular fluorescence spectra. The investigated area on the sample is 1 mm diameter. In all measurements the sample-to-probe distance was kept constant to 1 mm (corresponding to focal length) with aid of a small black cylinder inserted on top of the probe, in order also to exclude contributions from external light. Instrumental parameters were as follows: 2 s integration time, 3 scans for a total acquisition time of 6 s for every spectrum. The system is managed with SpectraSuite software under Windows 7.

SERS measurements were carried out in the range 100-1800 or 100-3800 cm^{-1} with a Renishaw InVia spectrometer coupled with a Leica DM2500 M optical microscope, using an excitation wavelength of 633 nm with a magnification objective of 100X. The acquisition time was set to 10-20 s with 1-10 accumulation. Laser power onto the sample exceeded 300 μW.

Further technical details about the employed instrumentations are given in Appendix 2.

Figure 6.7.1: two folios of Codex Brixianus, Biblioteca Civica Queriniana, Brescia.

Figure 6.7.2: stereomicroscope image of the darker side of the sample detached from Codex Brixianus, 6X.

6.7.2 RESULTS AND DISCUSSION

NON-INVASIVE MEASUREMENTS

The purple color of the parchment of *Codex Brixianus* is highly variable in terms of saturation and brightness among the different sheets. Portable microscopy revealed that the parchment did not show the presence of pigment grains (Figure 6.7.3a), contrary to what observed in a mock-up painted with Tyrian purple (Figure 6.7.3b). This observation allowed to exclude the hypothesis that parchment was dyed with a pigment or a lake, which would leave colored particles on the parchment. The hypothesis that an organic dye dispersed in water was spread on the parchment is supported by the different color of the sides of each page, which would appear more homogeneous if immersed in a dye-bath to be colored. It is also true that the sides of the parchments always present some differences as they derive from an internal and an external part of the animal. Since it has not been possible at present to verify whether these inner differences could lead to such color variations when parchment is dyed, the hypothesis of the application of a dye with a procedure different than traditional dyeing cannot be confirmed but should be considered as the most likely.

XRF measurements on different pages of the manuscript revealed the presence of the signals of bromine. This element was considered in some works as an indirect evidence of the presence of Tyrian purple, as it is present in 6,6′-dibromoindigotin, the main coloring matter of this precious dye. Besides this, recent data obtained on various orcinol-containing lichens and from *Chrozophora tinctoria* Juss, still unpublished, revealed that bromine is found also in these species, thus preventing to point to a single purple dyestuff.

Despite the different aspect of the pages in the manuscript, the results of FORS analysis were relatively homogeneous within the codex. Two similar, though not identical, spectral behaviors could be singled out on the basis of FORS spectra (Figure 6.7.4, black and red spectra). In both cases two absorption maxima were present, occurring at 555 and 579 nm; in some spectra (Figure 6.7.4, black spectrum) a marked shoulder arose in the 650–700 nm range as well as two inflection points. The FORS spectra obtained from *Codex Brixianus* were compared with those measured from the standard preparations of the colorants considered and already discussed in paragraph 3.2.2. From the comparison of the spectra, it is apparent that Tyrian purple, if present, is not the main colorant on parchment, as the apparent absorbance maximum of Tyrian purple at 530 nm (Figure 6.7.4, purple spectrum) does not match with the two absorption bands from the parchment of *Codex Brixianus* (Figure 6.7.4, black and red spectra). At the same time, the colorant/colorants responsible for purple color are not to be sought for among anthraquinonic dyes, characterized by a double absorption band among 510 and 565 nm. There is, on the contrary, a good matching against the spectrum of folium, the extract from *Chrozophora tinctoria*, according to the absorbance maxima at 545 and 580 nm and to the general shape of the spectrum (Figure 6.7.4, green spectrum). The use of folium in miniature painting is cited on several treatises (Baroni 2012; Brunello 1971; Dodwell 1961) and it is therefore a reliable candidate. The presence of orchil can be suggested as well, however, as its absorption maxima occur at wavelengths close to those of *Codex Brixianus* (Figure 6.7.4, blue spectrum). Orchil too is cited in treatises (Travaglio 2012a; Travaglio 2012b) and moreover it has been recently identified in the miniatures of the Book

of Kells (Dublin, Trinity College, ms. 58)(Romani *et al.* 2008). Finally, it must be discussed the shoulder occurring between 650 and 700 nm in the spectra from some folios (Figure 6.7.4, black spectrum). This shoulder, resembling the typical absorption band of indigoids colorants, could be a clue of the presence of Tyrian purple and could suggest the use of a double dyeing procedure. It could be hypothesized that this shoulder occurred in folios where the main dyestuff (folium or orchil) had been partially faded, making the layer of Tyrian purple dye more apparent.

To support the identification of folium or orchil as the main colorant on the parchment of Codex Brixianus, spectrofluorimetric analysis was performed. The spot diameter of spectrofluorimetry probe is 1 mm and it is therefore narrower than that of FORS and XRF, nevertheless results were fairly constant among areas with different saturation. Differently from what reported in 3.2.2 for the analysis of mock-ups with FORS, in the case of spectrofluorimetry some differences were found in the spectral responses obtained from painted and dyed parchment reference samples. In analyzing paints, the contribution from parchment is less apparent (Figure 6.7.5, green and purple spectra); this can be easily explained since spectrofluorimetry is a superficial technique and the light source used (a LED source centered at 365 nm) is strongly absorbed by the superficial layer, in this case the paint, and can hardly reach the support. In dyed samples, instead, parchment and dye molecules lay on the same layer and both contribute to the spectral response (Figure 6.7.5, red and blue spectra). Apart from this, though, the spectral features are similar in terms of fluorescence maxima. Fluorescence spectra from the parchment of Codex Brixianus (f. 32r) and from the standard preparations of the colorants considered and analyzed with FORS are reported in Figure 6.7.6, while Table 6.7.1 reports a summary of the fluorescence maxima identified in the analyzed areas, together with maxima from standard colorants. In particular, the maximum at 487 nm, present in all spectra, should be ascribed to the parchment (Figure 6.7.6, black spectrum). In agreement with the literature (Romani *et al.* 2008), no significant spectral features were found for Tyrian purple (Figure 6.7.6, purple spectrum). Results from fluorescence analysis substantially confirmed the fact that dyes other than Tyrian purple are present on parchment, according to the apparent presence of fluorescence-active compounds. In this case, the fluorescence maxima and the general shape of the spectra suggest that the best matching with the Codex Brixianus is found from orchil (Figure 6.7.6, blue spectrum) more than from folium (Figure 6.7.6, green spectrum).

Table 6.7.1: spectral features of fluorescence spectra on standard paints, Codex Brixianus and parchment.

| sample	λ_{max1}	λ_{max2}	λ_{max3}
Cochineal std.	487 nm	529 nm	639 nm
Kermes std.	487 nm	584 nm	618 nm
Madder std.			603 nm
Tyrian purple std.	487 nm		
Folium std.	488 nm	518 nm	595 nm
Orchil std.	487 nm	522 nm	624 nm
Codex Brixianus (f.32r)	487 nm	522 nm	623 nm
Parchment	487 nm		
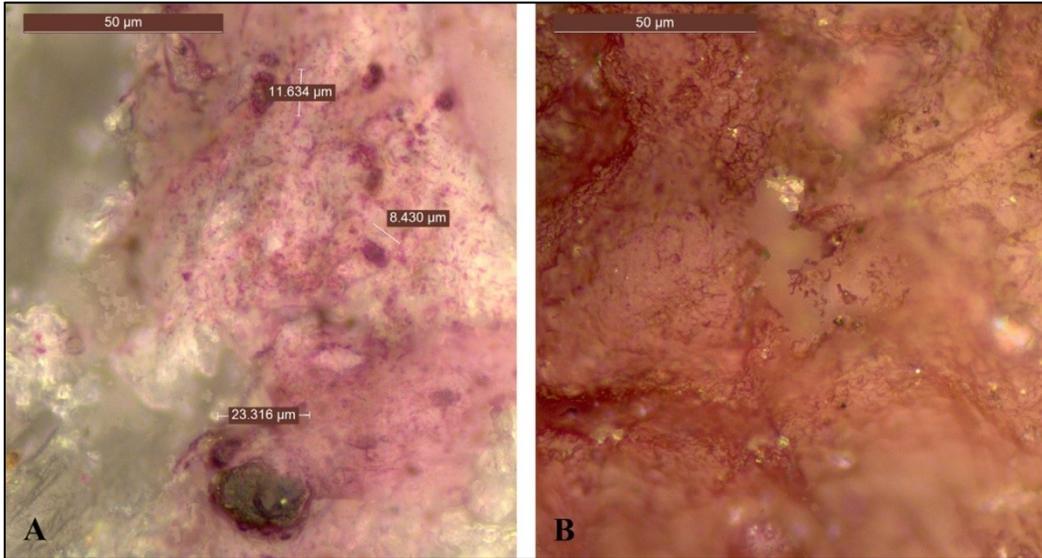
Figure 6.7.3: portable microscope image from a mock-up of parchment painted with Tyrian purple in Gum Arabic (A) and the parchment of *Codex Brixianus* (B), 50X (Aceto, Idone, *et al.* 2014).
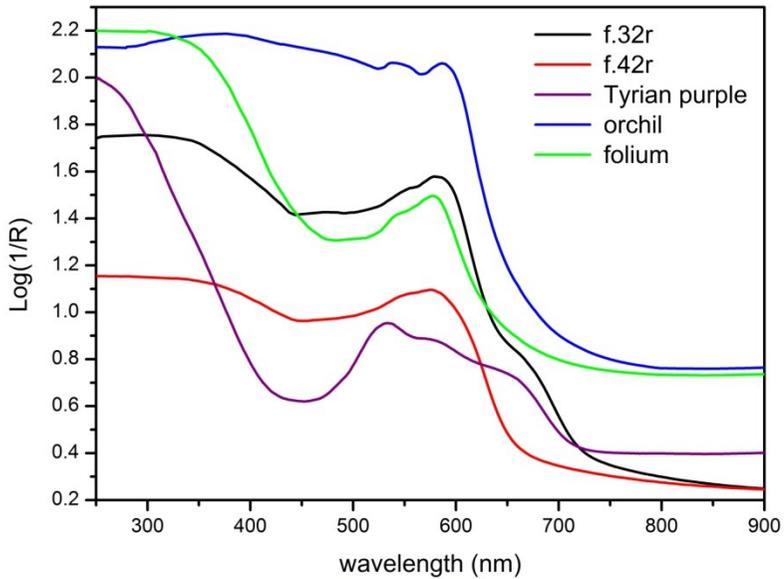

Figure 6.7.4: reflectance spectra reported in Log(1/R) coordinates of: two folios of the parchment of Codex Brixianus (f.32r and f.42r, black and red spectrum, respectively), standard paints on parchment of Tyrian purple (purple spectrum), orchil (blue spectrum) and folium (green spectrum).
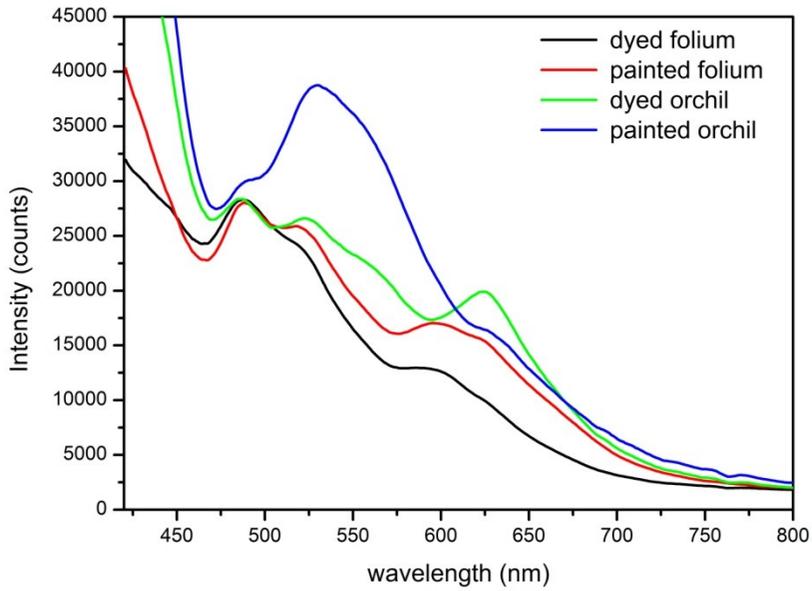
Figure 6.7.5: fluorescence spectra of: dyed folium (black spectrum), painted folium (red spectrum), dyed orchil (green spectrum), painted orchil (blue spectrum).
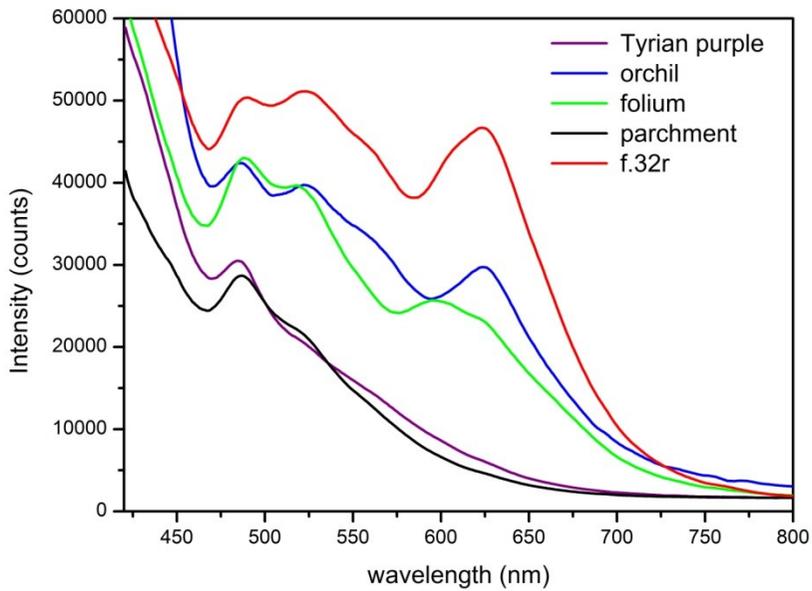

Figure 6.7.6: fluorescence spectra of: Tyrian purple (black spectrum), orchil (blue spectrum), folium (green spectrum), parchment (black spectrum), folio 32r from Codex Brixianus (red spectrum).

SER MEASUREMENTS

The coating of the fragments of parchment with silver colloidal paste was affected by the morphology of the parchment itself as the surface was rugged and nanoislands developed hardly. Despite this, some SER measurements were tried in some coated areas of treated and untreated parchments but they generally led to very poor spectra, with a very strong peak of silver at about 230 cm^{-1} and no identifiable peaks of other molecules. The features of the recorded spectra might be possibly due to the fact that the nanoparticles fixed to the parchment in the depressions, thus generating a thick layer of silver which prevented the enhancement of the signals of the underlying molecules.

SER spectra of the prepared blanks (Figure 6.7.7) did not show any additional signals when compared to the unmixed silver colloidal paste (black spectrum) in the case of methanol and dimethylformamide (green and red spectra), while in the case of formic acid (blue spectrum) the peaks attributable to citrate and nitrate in the silver colloidal paste were attenuated but other peaks were recorded at about 765, 1340 (sh), 1530 and 2840 cm^{-1}. The spectra recorded on drops of dimethylformamide in which a minute fragment of *Codex Brixianus* was soaked revealed variable intense peaks, none attributable to any known dyestuff. In this case, dimethylformamide possibly extracted other unknown species in the matrix of parchment. On the contrary, SER spectra obtained on the drop of hydrochloric acid:methanol:water (Figure 6.7.8, black spectrum) showed a low signal-to-noise ratio but some peaks were anyway identified at 528, 616, 657, 1004, 1031, 1414 and 1646 cm^{-1}. Also in spectra recorded on the drop of formic acid (Figure 6.7.8, red spectrum), despite their poor quality, peaks at about 620, 1415 and 1640 cm^{-1} were identified; in some of the repeated measurements, additional peaks at about 520, 1005 and 1520 cm^{-1} were found.

SER measurements carried out on the 2 μL drop and the 50 μL dried drop of extract with formic acid:methanol 15:85 (v/v) did not evidence any signals different from those of the silver colloidal paste. It should be noted that the extract was completely uncolored under visual examination, thus it is possible that the extracting solution was ineffective for the considered system parchment/dyestuff. On the contrary, high signal-to-noise ratio SER spectra were collected on the 2 μL pale pink extract obtained upon extraction with formic acid (in which case the extract appeared reddish), while in the case of the 25 μL of the same extract dried poor quality spectra were recorded. In the first case, very reproducible peaks at 368vw, 443w, 479m, 519m, 558vw, 621s, 761w, 825m, 1182s, 1278vw, 1336vw, 1414s, 1488m, 1640s cm^{-1} were observed in several SER spectra (Figure 6.7.9) collected on the silver coated area. In the second case, the peak of silver was very strong if compared with the other peaks; however, more variable signals were found (Figure 6.7.8, blue spectrum), with peaks at about 620, 732, 957, 1005, 1325, 1400 being the most intense ones. The lower intensity and quality of the signals on a larger amount of extract might be attributed to the lack of interaction between the dried extract and the silver colloidal paste deposited on the surface of the microscope slide.

The peaks obtained on the extract of formic acid were compared with the results obtained on the reference spectra obtained from the possible dyestuffs and discussed in greater detail in paragraph 5.2.2. In particular, the SER spectra of the extract from Codex Brixianus were in more than good comparison with natural orchil and orcein. Slight shifts were observed in the peaks at

1414 and 1488 cm^{-1}, possibly due to a partial degradation of the coloring matter in the parchment. In addition, some peaks obtained with the other above mentioned analytical approaches could be as well attributed to orchil, i.e. the peaks at about 528, 620, 1414, 1646 cm^{-1}. SER spectra from Codex Brixianus were also compared with a published SER spectrum of orcein obtained with an excitation wavelength of 785 nm (Leona, Stenger, and Ferloni 2006); in this case, a good agreement was found with the tabulated peaks at 472, 522, 550, 615, 825, 1182, 1407 and 1642 cm^{-1}. In addition, subtracted-shifted Raman spectra of standard orcein and of two historical purple parchments were compared with the recorded spectra (Rosi *et al.* 2013). In this case, the different intensities of the peaks might be due to the preferential enhancement of some vibrational modes given by silver nanoparticles, as previously discussed for the results obtained on reference samples (5.2.2). Despite this, the position of the peaks, especially between the historical samples, is in good agreement.
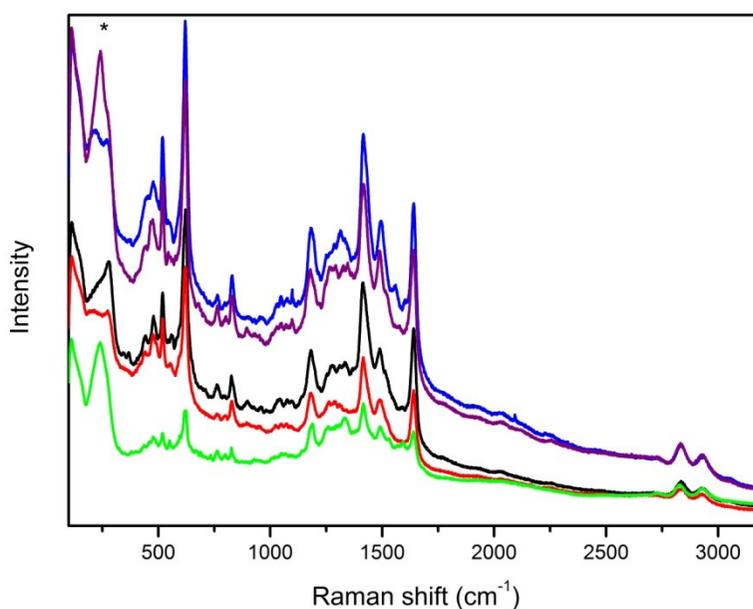
Figure 6.7.7: SER spectra of: dimethylformamide (green spectrum), methanol (red spectrum), formic acid (blue spectrum), silver colloidal paste without any addition (black spectrum); asterisks indicate peaks arising from silver colloidal paste. Spectra were stacked for ease of comparison.
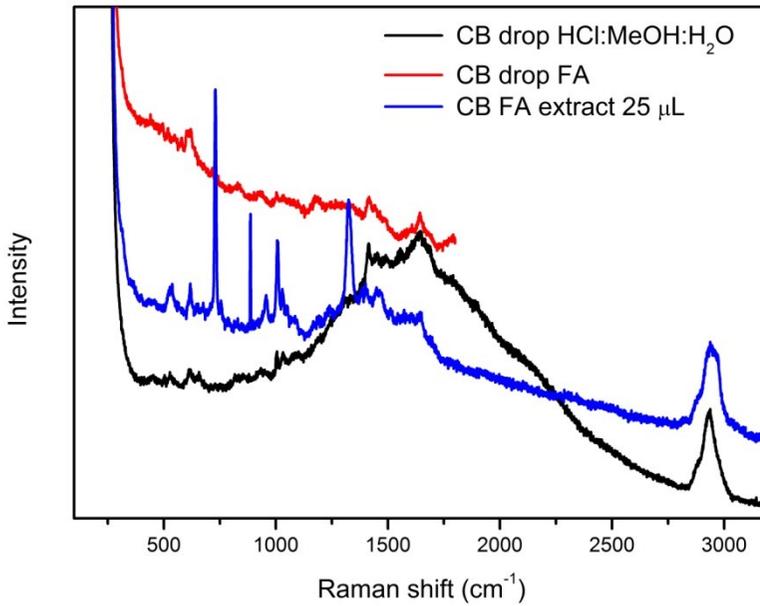
Figure 6.7.8: SER spectra from the parchment of Codex Brixianus: extracted with a drop of hydrochloric acid:methanol:water 2:1:1 (black spectrum), extracted with a drop of formic acid (red spectrum), 25 μL of extract obtained with formic acid for one hour at 40°C and then dried (blue spectrum). Spectra were stacked for ease of comparison.
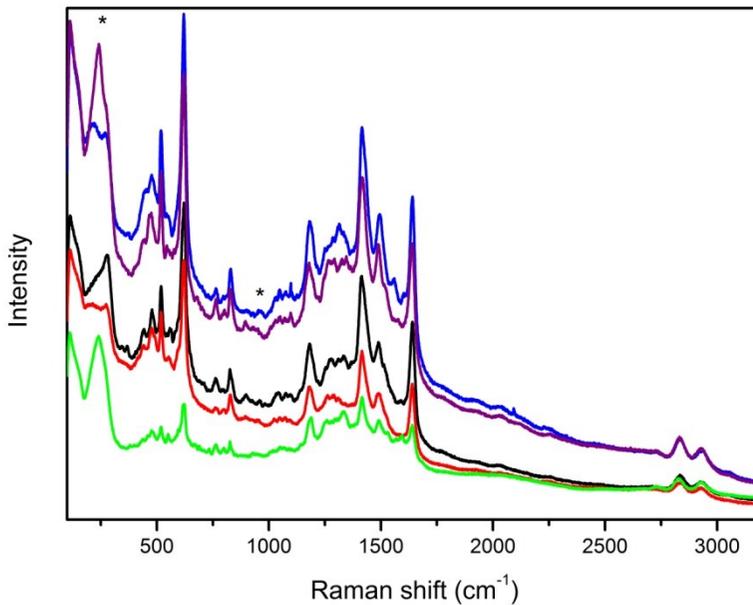
Figure 6.7.9: SER spectra of 2 μL of an extract from the parchment of Codex Brixianus obtained with formic acid for one hour at 40°C; asterisks indicate peaks arising from silver colloidal paste.

6.7.3 CONCLUSIONS

In this work, a thorough investigation of the purple dyestuffs employed to color the parchment of a 6th century codex was undertaken. In particular, non-invasive investigations were carried out *in situ* employing portable technique, while SER measurements using different approaches were performed on minute fragments detached from a unique sample.

Portable microscopy revealed that the surface of the parchment did not show the presence of grains, thus excluding the possibility of the use of Tyrian purple employed as a pigment as well as of lakes obtained from soluble dyestuffs and pointing to the use of a dye dispersed in water. Despite this, it was not possible to determine if the parchment was properly dyed or only spread with the solution. Moreover, X-ray fluorescence measurements showed the presence of bromine that is indeed part of the molecular structure of Tyrian purple but is also found in orchil and folium as an impurity. The comparison of reflectance spectra of *Codex Brixianus* with those obtained on mock-ups of painted and dyed parchments allowed to exclude the use of Tyrian purple and antraquinonic dyes as the main dyestuffs and pointed to a major use of folium or orchil. In addition, the presence in some spectra of features usually attributed to indigoid dyestuffs allowed to hypothesize that Tyrian purple might be used in double dyeing with the other dye. A further support to the results obtained by FORS was given by spectrofluorimetry, since also the fluorescence spectra identified orchil as the most reliable candidate.

Several approaches were tested in order to obtain good SER spectra from the unique sample detached from the precious manuscript. Minute fragments were cut from the sample and coated with silver colloidal paste as they were or after treatments with different extractive solutions. The best results were obtained from only 2 μL of a formic acid extract of the parchment. In this case, intense and reproducible signals were recorded and allowed the identification of the main dyestuff as orchil. Also other approaches allowed to record spectra in which some peaks could be attributed to orchil, but in these cases the signal-to-noise ratio was low and additional peaks were found. The results obtained thus confirmed the suggestions given by non-invasive techniques and allowed to make the first molecular identification through SERS of a dyestuff employed on purple codices.

6.8 IDENTIFICATION OF DYES FROM FORTUNY TEXTILES USING DIRECT EXTRACTIONLESS, NON-HYDROLYSIS SURFACE-ENHANCED RAMAN SCATTERING

Mariano Fortuny y de Madrazo (Granada, Spain, 1871 - Venice, Italy 1949) was the son of a painter and was trained himself as a painter too. He worked in the Palazzo Pesara-Orfei in Venice, which is now home of the Fortuny musem, for most of his life with his companion Henriette Negrin and they created together astonishing textiles and dresses, appreciated by the European elite of the time. In 1919 Fortuny formed an additional company, called *Società Anonima Fortuny*, which produced interior textiles, mostly made of cotton, with some mechanical printing. Mariano Fortuny was a veritable "Renaissance man" in early 20th Century Europe and despite numerous patents, Fortuny's specific recipes and processes remain a mystery. In fact, from the literature, images of the studio, and extant textiles, equipment and pattern books, it is clear that Mariano and Henriette mixed their own dyes and dyed their own textiles, often in multiple and successive baths. Fortuny is also thought to have employed a wide variety of surface decoration techniques including, color removal, direct printing, photo-lithography, stenciling, and a mixed method of painting and hand and screen printing. Blending of historical patterns and Fortuny's technical innovations result in textiles both reminiscent of the past and the contemporary. For fifty years Mariano Fortuny and Henriette Negrin continued to develop new and innovative techniques and after they passed away, Fortuny's company along with his "secret" technology passed on to Countess Elsie Lee Gozzi, the American distributor of Fortuny's creations. Today their production is still a guarded secret within the company. As a result, this artist, who has been called "the magician of Venice" (Kunst 1985), continues to interest and mesmerize textile historians, conservators, and enthusiasts through the beauty and complexity of his creations.

The Art Institute of Chicago houses an important collection of over 58 costumes and textiles produced by the various incarnations of Mariano Fortuny's creative enterprises. The collection of the Art Institute includes Delphos gowns (a hallmark production and symbol of refined extravagance, whose innovative mechanically produced pleated silk was patented in 1909), silk velvet coat, printed silk velvet panels, and several printed cotton lengths. The technical process of creating the intricate, yet visually stunning decoration of these textiles is not well documented and has been little studied so far.

In addition, uncertainty still exists on whether the claim that Fortuny did not use synthetic dyes is true, at a time when the chemical industries were flooding the market with bright and attractive new industrial products. Only one previous example of analysis of Fortuny dyes is known (Pritchard 2001). This study used absorption spectrophotometry and thin layer chromatography and identified indigo, brazilwood, cochineal, and a blend of yellow dyes, most likely old fustic with quercitron bark, on a limited number of samples. However, the techniques used do not provide unambiguous molecular information for identification of the colorants.

As previously underlined (4.4.2) there are only a few published works dedicated to direct on-the-fiber analysis without the need of extraction or hydrolysis pre-treatments. In particular, a method for on-the-fiber analysis using silver nanoparticles fabricated by laser photoreduction was proposed (Jurasekova *et al.* 2008; Jurasekova *et al.* 2010). This method was used to detect

flavonoids in weld-dyed wool, silk textiles, and linen fibers. Recently, Brosseau *et al*. developed a method to perform on the fiber detection of dyes using silver nanoparticle colloidal pastes (Ag colloidal pastes). This method has proven to be very versatile and enabled the detection of highly fluorescing colorants from historical samples such as fibers, pastels, and watercolors (Brosseau, Casadio, and Van Duyne 2011; Brosseau, Gambardella *et al.* 2009).

In the present work, extractionless non-hydrolysis SERS is used for the direct molecular identification of colorants in selected examples from the Art Institute collection in order to shed further light on Fortuny's textiles.

The results of this work, together with a selection of the methodological work, were reported in a recently published paper in Analyst (Idone *et al.* 2013).

6.8.1 MATERIALS AND METHODS

FORTUNY TEXTILES

Six samples were taken from the two productions of Fortuny's lifetime. 1-2 mm long threads were cut from pink (AIC accession number 1978.173f) and purplish-red (AIC accession number 1978.173e) pleated silk swatches of fabric for Delphos gowns (Figure 6.8.1). Another sample was taken from the ground fabric of a coral printed silk velvet swatch (AIC accession number 1978.174). These three swatches were given to the museum by Countess Elsie Lee Gozzi, who also confirmed that they were produced by Fortuny at the Palazzo Pesara-Orfei (Countess Lee Gozzi 1978). A fourth sample was taken from the silk pile of a red velvet panel based on a 15th century Venetian design (AIC accession number 1986.47, Figure 6.8.2). This sample bears the *Mariano Fortuny Deposé* stamp of textiles produced at the Palazzo Pesara-Orfei in Venice. Two samples were also taken from cotton panels from the *Società Anonima Fortuny* production. The weft thread from a red panel almost entirely covered with printed metallic flakes was analyzed (AIC accession number 1987.375.2). The second sample was from the weft of another red panel printed with a 15th century Italian silk design (AIC accession number 1985.681). Both of these latter textiles have the *Società Anonima Fortuny* stamp in the selvage (Figure 6.8.3).

In addition, three cotton printed panels produced by *Società Anonima Fortuny* in the 1970s, after Mariano Fortuny's death, were sampled. Samples were taken from the purple weft of panel "Glicine" (AIC accession number 1978.170), from red-brown threads of panel "Carnavalet" (AIC accession number 1978.171) and from the red-pink weft of the panel "Corone" (AIC accession number 1978.184).

SAMPLE PREPARATION

Small portions (about 0.5 mm in length) of a single fiber were cut with a scalpel and deposited on a pre-cleaned microscope slide. A 0.5 μL aliquot of the silver colloidal paste (5.1.1.1) was pipetted onto the fiber and gently brushed on the fiber with a golden wire. In some cases, samples were gently washed with some drops of methanol on a microscope slide, rinsed with bidistilled water and let to dry in order to remove some of the contaminants on the fibers.

INSTRUMENTATION

SERS measurements were carried out with a Renishaw InVia spectrometer coupled with a Leica DM2500 M optical microscope, using an excitation wavelength of 633 nm with a magnification objective of 100X. The acquisition time was set to 10s while the laser power onto the sample never exceeded 300 µW. The SER spectra were normalized in order to exclude the influence of integration time and effective laser power on signal intensities. A minimum of five measurements were performed on each sample, in order to check the reproducibility of the obtained spectra and account for possible inhomogeneity of the dyeing. Further technical details about this instrumentation is given in Appendix 2.

Figure 6.8.1: pleated silk swatches for Delphos gowns (Art Institute of Chicago, AIC accession number 1978.173 a-h). Samples were taken from swatch e and f.

Figure 6.8.2: red velvet panel based on 15th century Venetian design (Art Institute of Chicago, AIC accession number 1986.47).
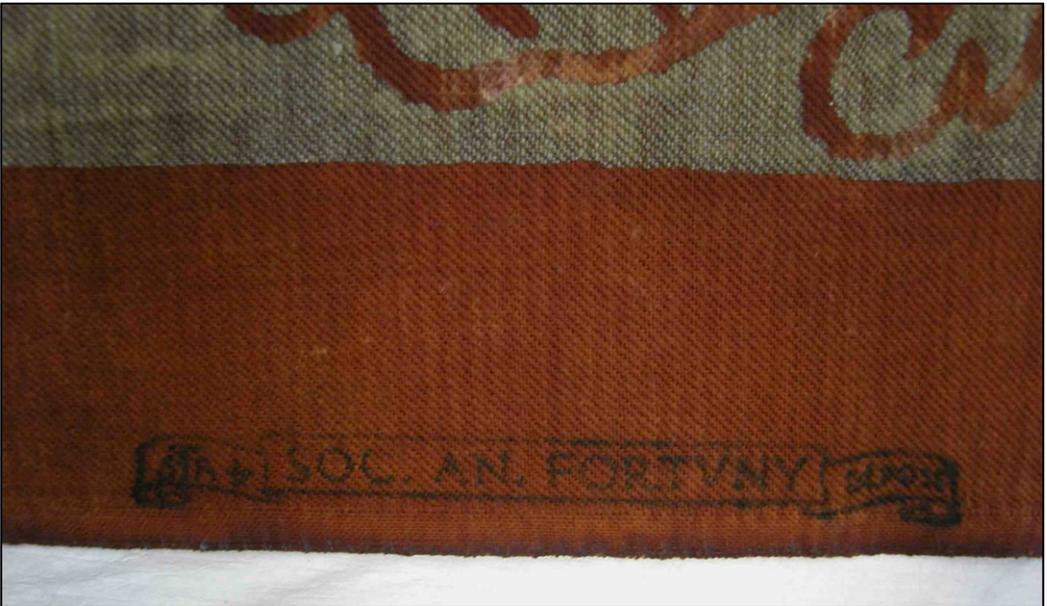

Figure 6.8.3: detail of Società Anonima Fortuny stamp in the selvage of a red cotton panel printed with a 15th century Italian silk design (Art Institute of Chicago, AIC accession number 1985.681).

6.8.2 RESULTS AND DISCUSSION

SER spectra of the sample of purplish-red silk (1978.173e, Figure 6.8.4) generally had an intense peak at about 1300 cm^{-1} and a shoulder, appearing as a distinctive peak in some of the repeated measurements, at about 1225 cm^{-1}. The spectra also showed the presence of medium intensity peaks at about 460 and 1425 cm^{-1}. These peaks were attributed to carminic acid (5.1.2.3) therefore the dyestuff employed to color this purplish-red sample can be identified as cochineal. The spectra obtained from this sample are generally reproducible, while the peak at 460 cm^{-1} remains sometimes undetected.

SER signals of the dye molecules were not detected from a very light pink pleated silk sample 1978.173f. Here, the lack of signal could possibly be a consequence of a very low concentration of dye. In this case, extraction of the coloring matter and analysis in solution (Bruni, Guglielmi, and Pozzi 2011) might be beneficial. However, the limited amount of sample prevented the possibility of performing further analytical attempts. On the other hand, pre-treatment with HF to release dye molecules from the complex used for mordanting would not be effective as it has been demonstrated to hydrolyze silk fibers (Pozzi *et al.* 2012).

The spectra obtained from the sample of coral printed silk velvet (1978.174, Figure 6.8.5) displayed bands that can be attributed to the presence of brazilwood (5.1.2.3). While variable features were observed in the region around the peak at 1350 cm^{-1}, the relative intensity and shape of the major peaks at 460 and 1560 cm^{-1} were generally rather reproducible.

SER spectra recorded from the sample of a weft thread of the red cotton panel (1987.375.2, Figure 6.8.6) and the sample of silk pile of the red velvet panel (1986.47, Figure 6.8.7) were very similar although the intensity varied among the different measurements performed on the fibers belonging to the same sample. In general, the spectra showed intense peaks at 460, 1300 or 1560 cm^{-1}, while less intense signals could be detected at about 1020, 1080, 1090, 1160, 1190, 1205, 1350 and 1425 cm^{-1}. Some of these peaks could be attributed to cochineal whose main component is carminic acid (e.g. 1080, 1205, 1300, 1425 cm^{-1}) while the others arised from brazilwood (e.g. 1020, 1090, 1160, 1190, 1350, 1560 cm^{-1}). The peak at about 460 cm^{-1} could be interpreted as a sum of contributions to the spectrum arising from ring deformations of the dye molecules deriving from both dyestuffs. To the best of the author's knowledge, this is the first time that dyeing with multiple dyes were detected on historical dyed fibers using on-the-fiber, extractionless SERS. The variability of the spectral response for double-dyed samples is evident in some representative spectra from these two samples reported in Figure 6.8.6 and Figure 6.8.7.

SER spectra of the sample of cotton weft thread from a red panel (1985.681, Figure 6.8.8) showed a very intense peak at about 1300 cm^{-1}, medium intensity peaks at 460, 1205 and 1560 cm^{-1} and a weak one at 1080 cm^{-1}. Repeated SER measurements showed in this case fairly reproducible spectra. Most of the signals could be attributed to cochineal. However, the presence of a peak at 1560 cm^{-1} and the intensity of the peak at 460 cm^{-1} suggested that brazilwood also contributes to the final hue of the sample.

SER spectra from *Società Anonima Fortuny*'s production of the 1970s (1978.170, 1978.171 and 1978.184) did not show signals different from those recorded on the blank of silver colloidal paste (5.1.2.3). As the presence of a protein-based layer was determined by previous Fourier-Transformed Infrared Spectroscopy measurements (Francesca Casadio, personal communication), a mild pre-treatment with methanol and water was attempted in order to remove it. Unfortunately, no results were obtained also on the treated fibers.
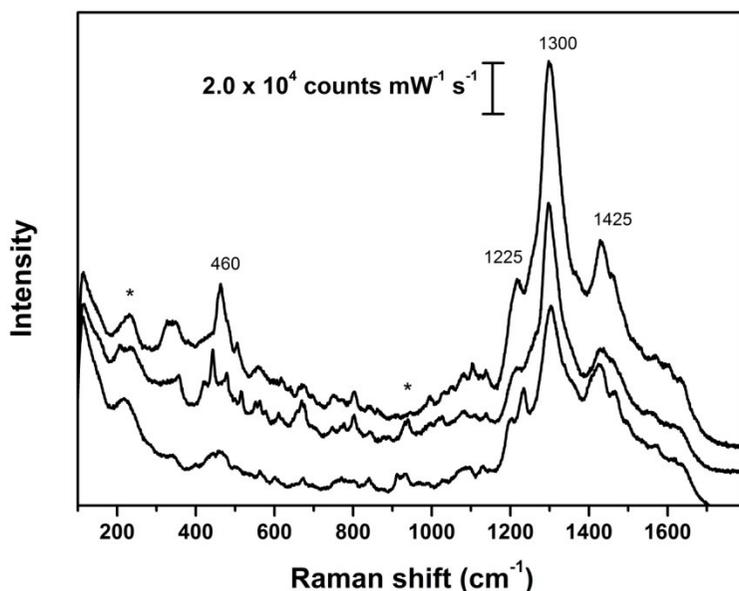
Figure 6.8.4: SER spectra obtained on a sample from a purplish-red pleated silk swatch from Delphos gowns (AIC accession number 1978.173e); asterisks indicate peaks arising from silver colloidal paste. SER spectra obtained using λ_{ex} = 633 nm (Idone *et al.* 2013).
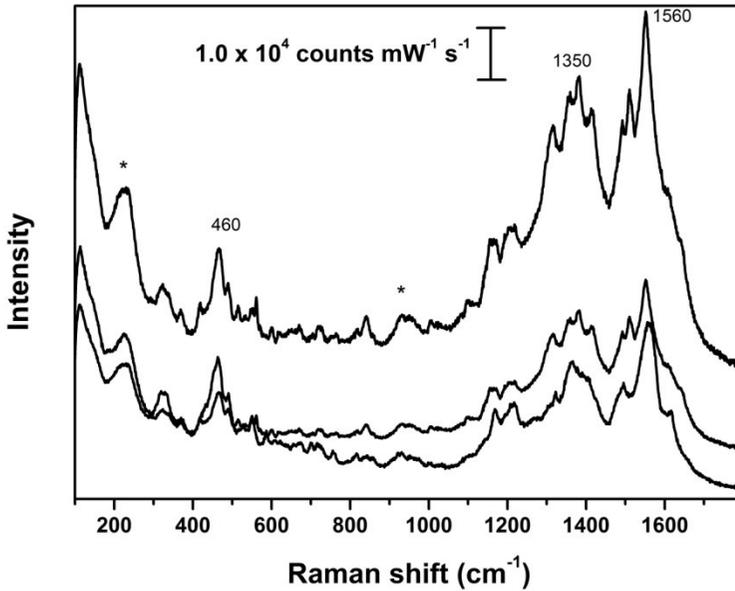
Figure 6.8.5: SER spectra obtained on a sample from the gound fabric of a coral printed silk velvet swatch (AIC accession number 1978.174); asterisks indicate peaks arising from silver colloidal paste. SER spectra obtained using λex = 633 nm (Idone *et al.* 2013).
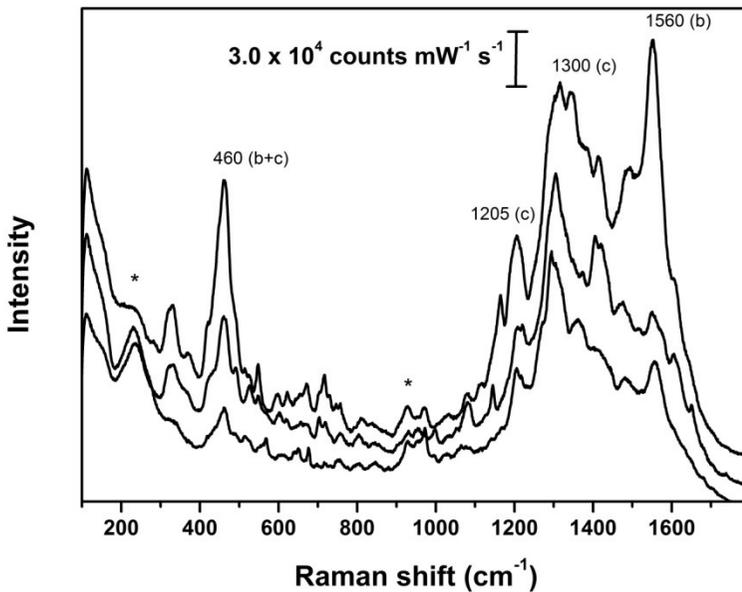
Figure 6.8.6: SER spectra obtained on a sample from the weft thread of a red panel (AIC accession number 1987.375.2); asterisks indicate peaks arising from silver colloidal paste; (b) and (c) indicate the most diagnostic peaks for brazilwood and cochineal, respectively. SER spectra obtained using λex = 633 nm (Idone *et al.* 2013).
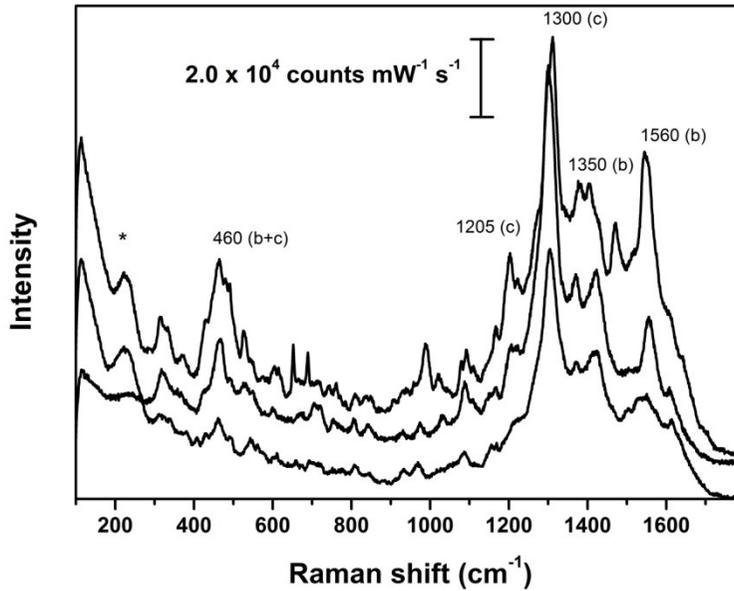
Figure 6.8.7: SER spectra obtained on a sample from the silk pile of a red velvet panel (AIC accession number 1986.47); asterisks indicate peaks arising from silver colloidal paste; (b) and (c) indicate the most diagnostic peaks for brazilwood and cochineal, respectively. SER spectra obtained using λ_{ex} = 633 nm (Idone *et al.* 2013).
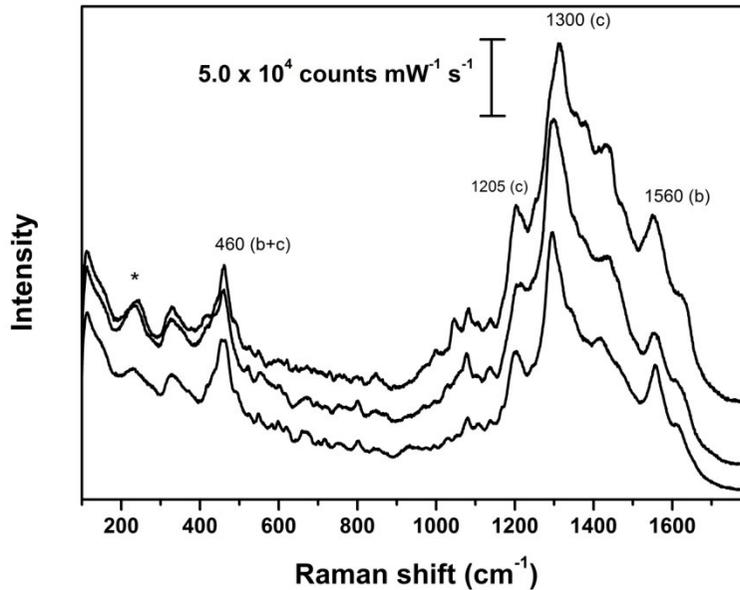
**Figure 6.8.8: SER spectra obtained on a sample from the weft thread of a red panel (AIC accession number 1985.681); asterisks indicate peaks arising from silver colloidal paste; (b) and (c) indicate the most diagnostic peaks for brazilwood and cochineal, respectively. SER spectra obtained using λ_{ex} = 633 nm (Idone *et al.* 2013).**

6.8.3 CONCLUSIONS

In this work, the proposed procedure for extractionless non-hydrolysis surface-enhanced Raman scattering with silver colloidal pastes (5.1) was tested on an important corpus of historical Fortuny textiles. The SERS analysis provided precise molecular identification of the red dyes used by Mariano Fortuny's different enterprises. In fact, the exclusive use of natural dyestuffs was confirmed for the examined samples belonging to the textiles created during his lifetime. On the contrary, the dyes used to produce a very pale pink pleated silk swatch from Delphos gowns and three cotton printed panels, produced several years after his death, were not identified.

Cochineal and brazilwood were consistently found in five of the silk velvets and cotton fibers that were analyzed. In particular, a red-purplish thread from a pleated silk swatch of Delphos gowns was dyed only with cochineal, while a coral printed silk velvet swatch was dyed with brazilwood by itself; both the textiles were produced at Palazzo Pesara-Orfei in Venice. In addition, a skilful use of combinations of such dyes (possibly obtained through multiple baths) was put in place to obtain a variety of different hues on three panels made of silk or cotton from both Palazzo Pesara-Orfei and *Società Anonima Fortuny*.

The lack of identification of the dyes in the second swatch from Delphos gowns was possibly due to the very low concentration of dye that might be used to obtain such a pale pink. Differently, a previous identification, by means of FTIR, of a proteinaceous coating of the sampled cotton panels dating to the Seventies, would possibly point to a change of materials or technologies of production after Mariano Fortuny's death. Unfortunately, the residual amount of these samples was too low to allow to pursue different approaches for the identification of the dyes.

In conclusion, this work allowed to verify the effectiveness of the use of silver colloidal pastes for direct extractionless non-hydrolysis SERS on historical textiles. Red dyestuffs employed by Mariano Fortuny during his lifetime were identified. Furthermore, this was the first time that the direct, extractionless, non-hydrolysis SERS method was used to identify two dyes simultaneously on a historical sample.

6.9 SURFACE-ENHANCED RAMAN SCATTERING FOR THE IDENTIFICATION OF RED LAKES ON POLYCHROMIES

Surface-enhanced Raman scattering can bring an important contribution to the identification of the materials found in polychromies (e.g. statues, mural paintings, easel paintings), as it makes possible the investigation of lakes, which are not detectable with other micro-invasive techniques commonly employed in this field (optical microscopy, microchemical tests, conventional Raman spectroscopy). Moreover, as previously underlined (chapter 4), the technique is not destructive and requires a smaller sample in comparison with HPLC analyses. Paragraph 4.4.2 discusses the different approaches for the preparation of samples for SERS analyses. As respects lakes, it is worth recalling that *in situ* approaches were proposed by Leona (2009) and Pozzi *et al.* (2012) with extractionless hydrolysis, while Brosseau *et al.* (Brosseau, Rayner *et al.* 2009; Brosseau, Casadio, and Van Duyne 2011) employed silver colloidal pastes directly on very small samples of pastels and watercolors. In addition, a pink lake from the Greco-Roman period was investigated by Van Elslande *et al.* (2008) using a silver colloid obtained with the synthesis procedure by Creighton *et al.* (1979). Moreover, the use of active hydrogels for the analysis of red lakes from a mock-up panel was proposed by Doherty *et al.* (2011). Besides the possibility of analyzing grains expressly sampled from the painting, the opportunity of gaining molecular signals from the lakes embedded in cross sections is particularly interesting. Sections of painting layers mounted in epoxy resin are in fact very useful during the diagnostic phases preliminary to restorations and their preparation is routinary in laboratories devoted to scientific investigations on paintings. It is therefore not surprising that the analytical task of analyzing dyes directly on cross sections has started to be considered by the most advanced laboratories dealing with samples from the cultural heritage, as testified by a paper by Oakley *et al.* (2011) about the investigation of cross sections from two 18th century oil paintings with partially concentrated Lee and Meisel colloid and by some works presented at the latest international congress on the application of Raman in Art and Archaeology (held in Ljubljana, September 2013). In particular, three different approaches were reported there: the Metropolitan Museum (New York, United States) suggested the separation of dyes from lake grains by means of laser ablation and their deposition on a SERS active substrate for their identification (Londero, Lombardi, and Leona 2013b; Londero, Lombardi, and Leona 2013c), while the Institute for the protection of Cultural Heritage of Slovenia (Ljubljana, Slovenia) presented the use of a new substrate stabilized with hydroxypropyl cellulose for the investigation of a mock-up sample of madder lake (Retko, Ropret, and Cerc Korosec 2013). A third approach, also presented during the congress, is described in this case study.

The Laboratorio Analisi Scientifiche (LAS, Aosta) has been working on polychromies for thirty years and it has stored up to now thousands of cross sections, mainly from artworks conserved in Valle d'Aosta. Cross sections are in fact employed within a diagnostic procedure that follows different steps and allows the characterization of the materials used in the different the layers. A sampling plan is elaborated after a careful examination of the results of the preliminary non-invasive survey, which may include portable X-ray fluorescence measurements, fiber optics reflectance spectrometry analyses and portable microscopy observations. Samples mounted in cross sections are subjected to observation under an optical microscope followed by micro-chemical tests, which allows the identification of some of the colorants and the determination of the chemical class of

binders. When the attribution is doubtful, the cross sections are further analyzed through infrared spectroscopy in the attenuated total reflectance mode (ATR) or by means of micro-Raman spectroscopy. This latter technique has been acquired only very recently by LAS (2011) allowing the systematic and unambiguous identification of inorganic pigments in cross sections (Odisio 2012), while previously Raman measurements were performed only on selected samples at Università degli Studi del Piemonte Orientale thanks to the collaboration with Dr. Maurizio Aceto. As a consequence, due to the lack of suitable analytical techniques in the LAS, the identification of lakes in the polichromies considered there has not been possible so far. Therefore, the presence of lakes in some of the cross sections was only hypothesized according to indirect evidences such as the lack of XRF signals attributable to key-elements in inorganic pigments, or high fluorescence in the Raman spectra. The use of reflectance spectroscopy for the identification of dyes, as proposed for other materials (chapter 3) is not of general application for paintings, due to the recurring habit of mixing lakes with pigments in polychrome works and to the presence of multiple layers.

The possibility of performing SERS on cross sections is therefore crucial for the diagnostic work of LAS, since it would allow to fully cover all the tasks related to the identification of painting materials. In particular, it would allow the identification of painting lakes by verifying the hypothesis based on indirect evidences, and thus it will open the way to new historical and artistic considerations on the investigated works of art. Moreover, the employ of preexisting samples mounted in cross sections will not damage further the object and it will give access to samples from artworks that cannot be further sampled due to conservation issues as well as to restoration processes undergone in the mean time.

This work reports and discusses the results obtained by analyzing cross sections from different polychromies (wooden statues and mural paintings) that were investigated in LAS with the previously mentioned techniques in the last five years. Cross sections were selected on the basis of the possible attribution of one of their layers to a red lake and analyzed through micro-Raman spectroscopy in order to exclude the presence of an inorganic pigment. Cross sections showing high fluorescence when exposed to the different laser beams of the micro-Raman equipment were treated with silver colloidal pastes and investigated by means of surface-enhanced Raman scattering.

The results obtained by means of SERS analysis have been reported in a paper, recently submitted to Journal of Raman Spectroscopy (Idone *et al.* submitted).

6.9.1 MATERIALS AND METHODS

CROSS SECTIONS

Cross sections were selected from five different wooden statues and a mural painting. A brief description of the artworks and of the chosen cross sections is reported below.

VIRGIN WITH CHILD WOODEN STATUE

The painted wooden statue of the Virgin with child (Figure 6.9.1 a) is conserved in the parish museum of La Salle (Aosta, Italy); the statue previously stood in the Chapel of Natività della Vergine in La Salle. The statue is part of a homogeneous group of 13th century statues of Virgins in throne. The recent restoration (2012) of the art object revealed the presence of a re-painting layer on the red mantle. One sample from this red mantle (AGH16), already mounted in cross section was selected for Raman investigations.

CHRIST ON THE CROSS WOODEN STATUE

The wooden statue of Christ on the cross (Figure 6.9.2 a) is dated to the 15th century and is of unknown provenance. It is conserved in the cathedral of Santa Maria Assunta in Aosta (Italy). The presence of a red lake was suggested in previous investigations (Cheney 2008) on some purple-red remnants in one of the many (eight) layers that were observed in a cross section obtained from a sample taken on the cheek of Christ (AAH02).

SAINT BISHOP WOODEN STATUE

The Saint Bishop wooden statue (Figure 6.9.1 b) is presently kept in the cathedral of Santa Maria Assunta in Aosta (Italy) but it was previously stored in the Chapel of Madonna delle Nevi in Ayas (Aosta, Italy). The statue is made of chiseled walnut wood and dates to the 16th century. Previous investigation on cross sections of painting layers indicated the possible presence of a lake in the left red glove of the Saint (AAC06) and in a thick red layer (50 μm) on its left red shoe (Cheney 2008).

SANT'ORSO WOODEN STATUE

The wooden statue representing Sant'Orso (Figure 6.9.2 b) was conserved in the chapel of San Rocco (Aosta) before its restoration and diagnostic investigation (Agostoni 2009). Archive and historical researches ascertained that this statue was part of the major altar of Collegiata dei Santi Pietro e Orso in Aosta, the masterpiece of local Late Gothic production, possibly designed by Antoine de Lonhy. The artworks that composed the altar are now conserved in several museums, castles and chapels in Valle d'Aosta and Piemonte. The statue underwent several reshuffles: the head is at present detached from the body of the Saint while the hand and the bird on Sant'Orso's shoulder are not original. The use of lakes was hypothesized in the bottom pale pink layer (30-100 μm) of a sample from the right hand of Sant'Orso (cross section ACF07), which also presented an external pink layer, not attributed. Also for a pink homogeneous layer (25 μm) on cross section ACF13, prepared from a sample of the skin of the face of the Saint, the presence of a lake was supposed.

SAINT PETER WOODEN STATUE

Saint Peter wooden statue (Figure 6.9.3 a) was found in chapel of San Rocco, together with the statue of Sant'Orso (Agostoni 2009). Similar stylistic and historical considerations led to its attribution to the liturgical décor of the altar of Collegiata dei Santi Pietro e Orso in Aosta. The pastoral staff of the statue is possibly more recent as usually it is not attributed to Saint Peter's iconography; on the contrary, some golden remnants suggested the presence of handcuffs, typical features of this Saint. The pink older layers of cross sections ACG01 and ACG03, sampled respectively from the forehead and the neck of Saint Peter, were supposed to be obtained with a lake. Other two pink layers of section ACG03 were investigated.

SAINT ANNE MURAL PAINTING

The altar of the left nave of the *Santi Pietro e Orso* church in Aosta frames a mural painting Saint Anne next to the Virgin with the Child (an iconographic arrangement named "Sant'Anna Metterza") re-painted by Carlo Morgari in XXth century. The dismantling of the baroque wooden altar for conservation purposes in 2007 evidenced the presence of an underlying larger mural painting dated to the 16th century (before 1576, Figure 6.9.3 b). A previous scientific work (Da Pra 2009) underlined the presence of several layers on cross sections obtained from the artwork in which the use of lakes was suggested. Raman measurements were undertaken on three cross sections from a fragment sampled on the face of a woman at the right of Saint Anne (ABI36), from the dark background above Saint Anne (ABI40) and from the green dress of Saint Anne (ABI61). In particular, cross section ABI36 was composed of five different layers among which the second from the bottom (25 µm thick) presented purple-red particles (with white particles) attributed to a lake. In section ABI40, the layer underneath the dirt (40 µm) showed the presence of red, white and orange inhomogeneous grains; red grains were supposed to be composed by a lake. A thick white layer (50-60 µm) with orange, purple and black particles was observed also on section ABI61 under the green layer Sainte Anne's mantle.

SAMPLE PREPARATION

All the cross sections, mounted in epoxy resin during previous investigation campaigns, were polished with diamond pastes down to 3 µm from Struers (Ballerup, Denmark) in order to remove any residues of the previous microchemical tests. The cross sections possibly containing lakes were drop coated with silver colloidal pastes (5.1.1.1). In the present work, the paste was tested as is, or after dilution with UHQ water. Several trials were performed in order to select the suitable dilution of the paste, as discussed in the next section. The cross sections were re-polished after each trial, until signals from the dyes emerged from the background. In particular, SERS signals from the dyes were obtained after having diluted 10 mg of silver colloidal paste (e.g. the content of a tube after 10 centrifugation cycles) with 2 ml of UHQ water and drop-coated the cross section with 0.5 µL of the substrate. The laser beam was then focused on the selected pigment grains when a dry coating of silver nanoparticles was obtained.

INSTRUMENTATION

Micrographs of the cross sections were obtained with a Polyvar Pol petrographical microscope coupled with a Leica DC3000 camera.

Raman measurements were carried out with a Renishaw inVia instrument, observing the cross sections both in the bright field and in the dark field modes. Traditional micro-Raman and SER measurements were all performed at 100X magnification with the 633 nm excitation wavelength. Micrographs of the silver coating on some cross sections were collected using the camera coupled to the Raman instrument.

Further technical details about the employed instrumentations are given in Appendix 2.

Figure 6.9.1: wooden statues of the Virgin with child (a) and of a Saint Bishop (b).

Figure 6.9.2: wooden statues of Christ on the cross (a) and Sant'Orso (b).

Figure 6.9.3: wooden statue of the Saint Peter (a) and mural painting of Saint Anne (b).

6.9.2 RESULTS AND DISCUSSION

SILVER COATING OF CROSS SECTIONS

The main issue of this approach consisted in achieving a suitable silver coating on the complex surfaces under investigation, since the coating plays a major role in promoting signal enhancement (4.3, 5.1.2.3). It was indeed observed that an excessive coating prevented the possibility of observing the different layers on the cross section, thus making arduous to correlate the signals to a specific pigment grain. Besides, SER signals of the dyes were obtained from pigment grains that showed deposited clusters of aggregated silver nanoparticles, similarly to what observed for textiles (5.1.2.3). This topic was addressed also in the previously cited work by Oakley *et al.* (2001), who observed that different areas of a same sample may exhibit excellent enhancement or fluorescence only owing to the heterogeneous coverage of the sample. For the above mentioned reasons, the coating should be a compromise between the possibility of producing an adequate number of silver nano-islands and the need of permitting the observation of the stratigraphy of the sample. After several trials, the procedure that produced the higher number of clusters without obscuring the sample involved the use of a diluted silver colloidal paste as reported in the paragraph 6.9.1. Figure 6.9.4 a-d shows the morphology of the coating that allowed us to detect SERS signals from the lake pigment grains by keeping the sample visible through the microscope coupled with the spectrometer. In addition, the dilution of the paste partially removes the citrate ions adsorbed on the silver nanoparticles and significantly reduces the intensity of the spurious SERS signals from the substrate (Leona 2009).

MICRO-RAMAN AND SER MEASUREMENTS

VIRGIN WITH CHILD WOODEN STATUE
Micro-Raman measurements of cross section AGH16 (Figure 6.9.4 a) revealed that the red-orange remnants of the re-painting layer were constituted of vermillion, while the high fluorescence of the underlying purple-red layer prevented the recognition of the pigment. SER measurements undertaken on this cross section proved to be affected by numerous spurious signals produced by the matrix. For this reason, various measurements were needed so as to acquire spectra distinctive of the lake. In particular, the cross section was re-polished and coated five times and a total of fourteen spectra with peaks not belonging to the silver colloidal paste were collected. It should be noted that the quality of the recorded spectra is lower than the good signal-to-noise ratio observed in spectra recorded from historical textiles (cf. Figure 6.5.5, 6.6.8, 6.6.10) and only few of the spectra showed signals that can be attributed to natural dyes (Figure 6.9.5). The spectra of cross section AGH16 showed the presence of a number of peaks, among which the most intense ones were found at 1288, 1325, 1438 and 1472 cm^{-1}. The attribution was performed by comparing the spectra with SER spectra collected on reference materials and with published spectra of red dyestuffs or dyeing molecules. In particular, recorded spectra were assigned to a madder-type dyestuff rich in alizarin and/or purpurin. By comparing the most intense signals from the cross section with those obtained on commercial purpurin (Figure 6.9.5, red spectrum) and alizarin (Figure 6.9.5, green spectrum) it appeared that the peaks at 1288, 1325 and 1472 cm^{-1} could be possibly attributed to both the chemical species. On the other hand, the peak at 1438 cm^{-1} was only observed in spectra recorded on purpurin. More in detail, as far as the peak at 1288 cm^{-1} is

concerned, it should be noted that alizarin and purpurin showed the signal at 1295 cm^{-1}, possibly because of an effect due to the complex matrix under investigation.

Other weaker SERS features that were observed in the spectra recorded on the cross section (1212 and 1584 cm^{-1}) could be attributed to alizarin or purpurin on the base of the data obtained from pure compounds (purpurin 1206 and 1583 cm^{-1}; alizarin 1208 and 1583 cm^{-1}), while the peak at 1232 cm^{-1} arose most probably from purpurin (1227 cm^{-1} on the pure compound). Peaks centered at 1423 (strong) and 1457 cm^{-1} (medium) that are present in the SER spectrum of commercial alizarin, were not detected in SER spectra recorded on the cross section under investigation.

SER spectra reported in the literature for alizarin and purpurin (Leona, Stenger, and Ferloni 2006; Whitney, Van Duyne, and Casadio 2006; Lofrumento *et al.* 2013) confirmed overall the above-discussed attribution of signals to alizarin, purpurin, or both of them, and further stressed the fact that the lack of some of the specific signals of alizarin, as well as the presence of the peak at about 1230 cm^{-1} that was indicated as a marker for purpurin, would exclude the presence of alizarin at detectable levels in the pigment. Alizarin is expected to show lower detection limit compared to purpurin when SER analysis is employed (Jurasekova *et al.* 2010; Van Elslande, Lecomte, and Le Ho 2008), therefore the results point to the use of an alizarin-poor plant dyestuff, thus ruling out the use of common madder (*Rubia tinctorum* L.). By focusing on plants that grow in Mediterranean/European area, the most probable candidates are wild madder (*Rubia peregrina* L.), Lady's bedstraw (*Galium verum* L.) and dyer's woodruff (*Asperula tinctoria* L.), in which alizarin is found at trace levels while purpurin is among the main coloring matters (Hofenk de Graaff 2004; Cardon 2007).

CHRIST ON THE CROSS WOODEN STATUE

Raman spectra of cross section AAH02 (Figure 6.9.6 a) showed a high fluorescence. Even though a number of nanoislands with morphology consistent with that observed in cross section AGH16 (Figure 6.9.4 d) was observed after coating the cross section with diluted silver colloidal paste (Figure 6.9.6 b), and despite many attempts, SER spectra did not reveal peaks that could safely identify one or more dyeing matters.

SAINT BISHOP WOODEN STATUE

The high fluorescence of Raman spectra previously obtained suggested the presence of a purple-red lake in painting layers of the sections AAC06 and AAC09. As in the case discussed above, different silver coatings followed by SER measurements did not lead to the possibility of identifying the coloring matters here. Many Raman SER signals were obtained, but the vibrational patterns of the scatters stimulated by the laser could not be attributed to red natural dyes.

SANT'ORSO WOODEN STATUE

Raman spectra recorded from the bottom layer of cross section ACF07 showed very strong peaks at 122, 152 and 549 cm^{-1} (Figure 6.9.7, black spectrum), that could be attributed to the red oxide of lead (II,IV), called minium or red lead (Bell, Clark, and Gibbs 1997), while the red grains of the second layer showed the presence of peaks at 145, 324, 343, 357, 380, 825, 839, 848 cm^{-1} (Figure 6.9.7, red spectrum), which characterize the presence of phoenicochroite (Frost 2004), a red lead chromate (Pb_2CrO_4). Also other cross section from this statue (ACF13) gave spectra that could be

attributed to inorganic pigments without the need of employing silver nanoparticles. In particular the presence of vermilion (HgS) was highlighted through its characteristics signals at 254, 285 and 343 cm^{-1} (Bell, Clark, and Gibbs 1997).

SAINT PETER WOODEN STATUE

Raman measurements on the orange layer of section ACG01 generated spectra that indicate the presence of vermillion (Figure 6.9.7, blue spectrum). Similarly, the three flesh-colored layers of section ACG03 were also mainly colored by vermillion.

SAINT ANNE MURAL PAINTING

Spectra recorded with traditional Raman measurements on cross sections ABI36, ABI40 and ABI61 only showed a high fluorescent background. Only for section ABI36 (Figure 6.9.8) the silver coating allowed the enhancement of signals that could be attributed to the presence of a natural dyestuff, even though the signal-to-noise ratio was poor. The most intense peaks in the spectra recorded on the cross sections were found at 1208, 1298, 1310, 1362 and 1464 cm^{-1}, while weaker signals were positioned at 384, 907, 1136, 1263, 1495, 1506, 1515, 1568 and 1600 cm^{-1} (Figure 6.9.9, black spectrum). The SER spectrum of a kermes lake (Figure 6.9.9, red spectrum), already discussed in paragraph 5.2.2.1, showed peaks consistent with those obtained by analyzing cross section ABI36 (e.g. 1135 (sh), 1203, 1273, 1295 (sh), 1310, 1361, 1466, 1485, 1507 and 1559 cm^{-1}), even though in some cases a slight shift is observed. In addition, also a reference spectrum obtained from a water extract of lac dye[4] (*Kerria lacca* Kerr, 1782) was considered (Figure 6.9.9, blue spectrum). The comparison between this latter spectrum and the one obtained on the cross section highlighted a poor match, since one single peak at 1462 cm^{-1} fits the signals obtained from the cross section, while other peaks showed shifts that excluded the presence of lac dye.

Further indications derive from an overview of published SERS data for kermesic acid (Leona, Stenger, and Ferloni 2006) and aqueous extracts of *Kermes vermilio* insects (Whitney, Van Duyne, and Casadio 2006) that confirmed the attribution of the above cited peaks to kermes (*Kermes vermilio* Planchon, 1864, 1.3.2) and underlined some variability within the intensities of the peaks at about 1420 and 1460 cm^{-1}. Moreover, literature data also indicate that the weak peak at 384 cm^{-1} could be attributed to kermesic acid, thus definitely supporting the characterization of the red grains in the section as a kermes lake pigment.

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[4] Purchased from Kremer Pigmente.
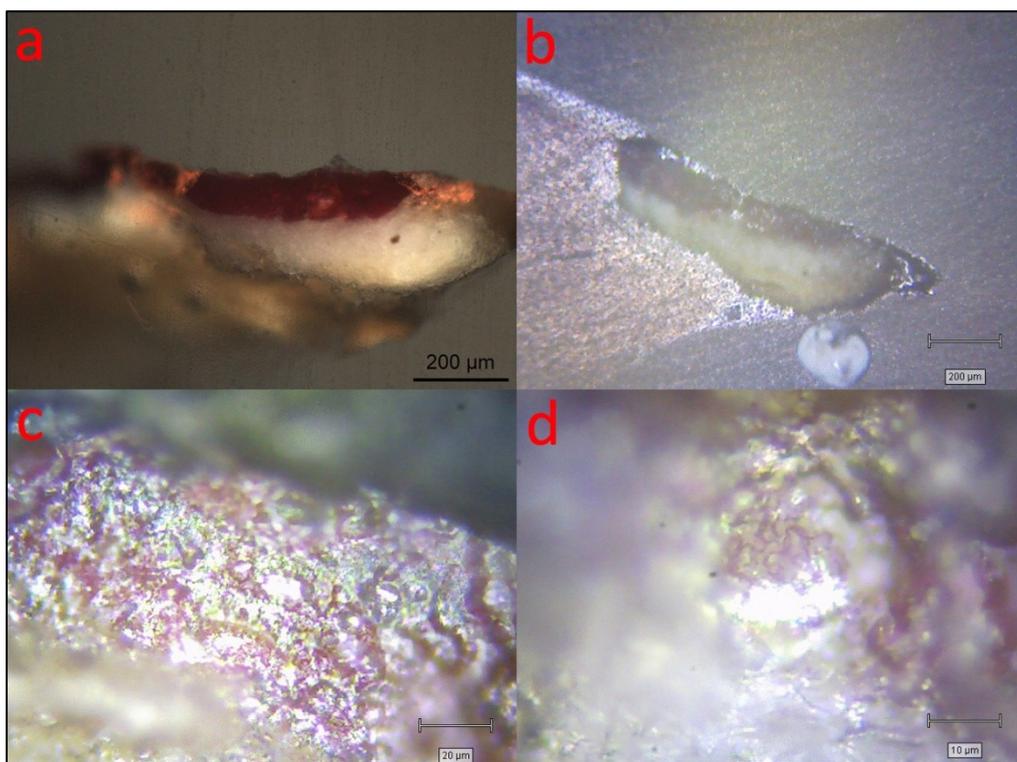
Figure 6.9.4: micrographs of cross section AGH16 from the wooden statue of the Virgin with child: uncoated, 20X (a); coated with silver colloidal paste, 5X (b); coated with silver colloidal paste, 50X (c); coated with silver colloidal paste, 100X (d).
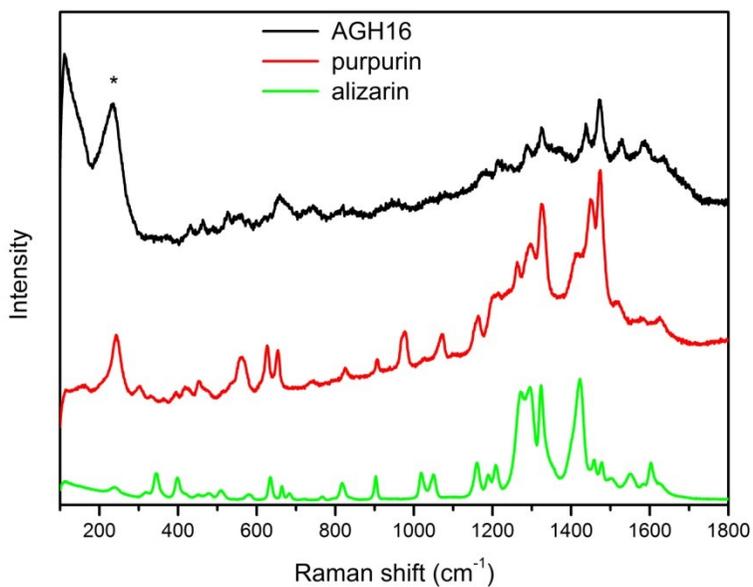
Figure 6.9.5: SER spectra of: cross section AGH16 from the wooden statue of the Virgin with child (black spectrum); a standard reference of purpurin (red spectrum); a standard reference of alizarin (green spectrum); asterisks indicate peaks arising from silver colloidal paste; spectra of purpurin and alizarin were divided by two for ease of comparison.
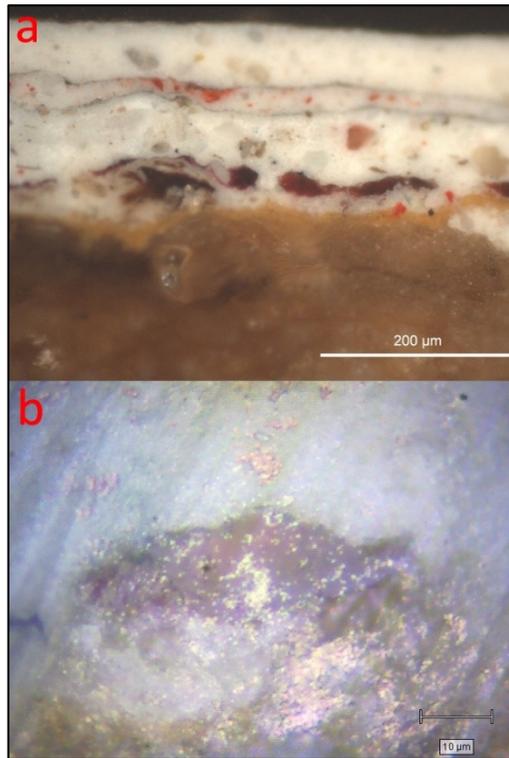
Figure 6.9.6: Micrographs of cross section AAH02: uncoated, 20X (a); coated with silver colloidal paste, 100X (b).
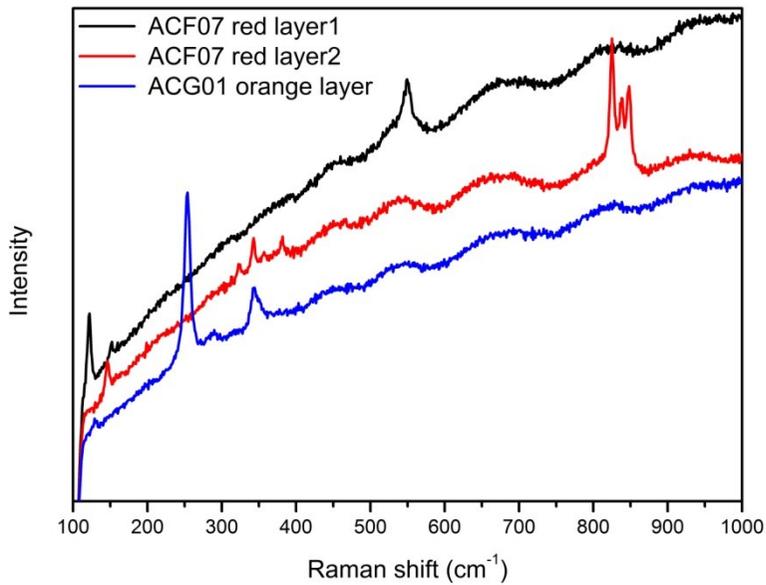
Figure 6.9.7: Raman spectra of: cross section ACF07 from Sant'Orso wooden statue, first red layer (black spectrum) and second red layer (red spectrum); cross section ACG01 from Saint Peter wooden statue, orange layer (blue spectrum); spectra were stacked for ease of comparison.
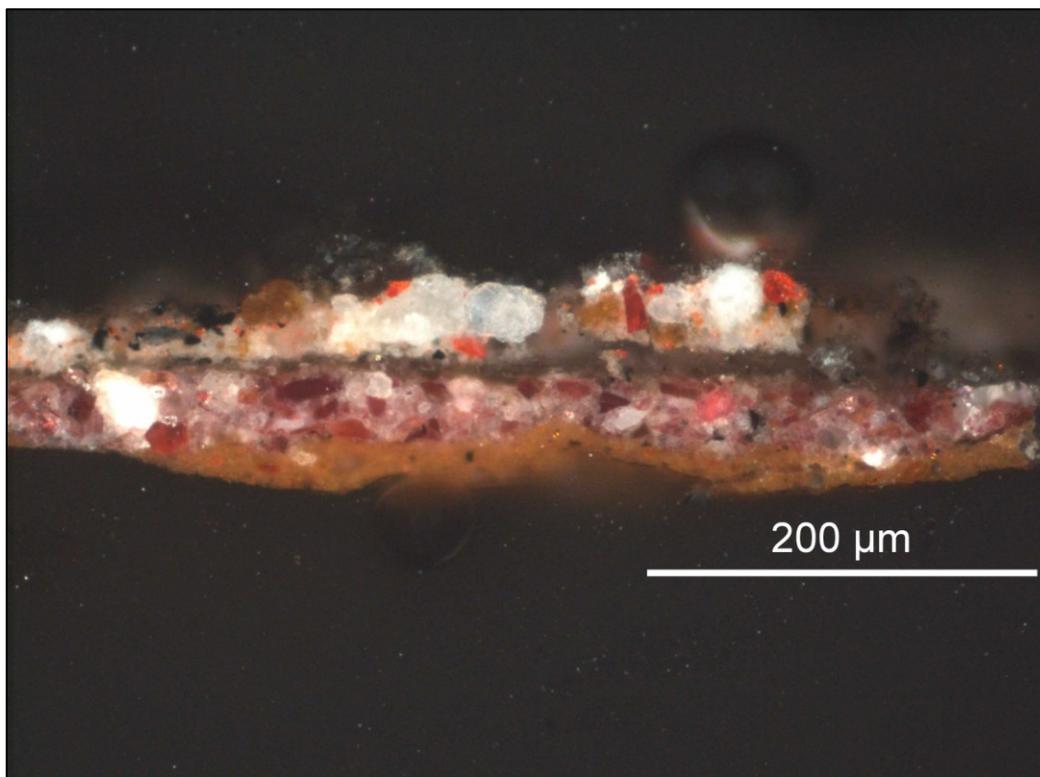
Figure 6.9.8: Micrograph of cross section ABI36 from Saint Anne mural painting, 20X.
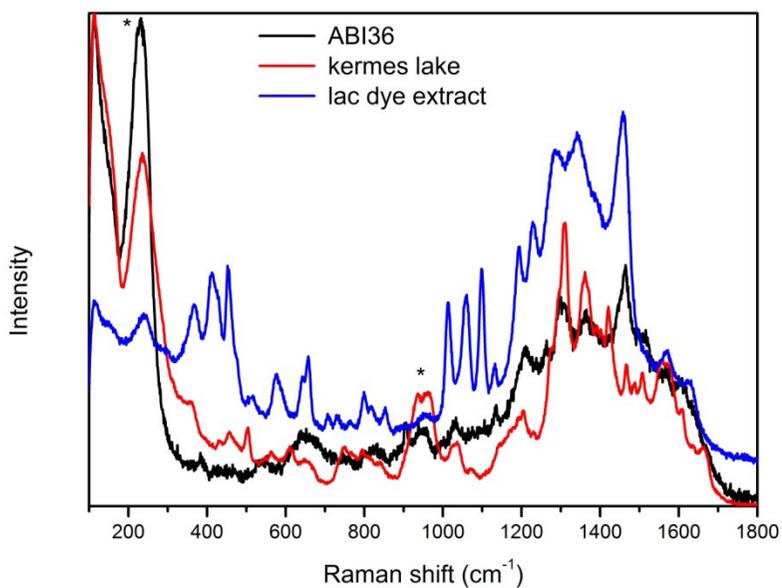
Figure 6.9.9: SER spectra of: cross section ABI36 from the mural painting of Saint Anne (black spectrum); a reference of kermes lake (red spectrum); a reference of lac dye extract (blue spectrum); asterisks indicate peaks arising from silver colloidal paste; spectra were normalized and stacked for ease of comparison.

6.9.3 Conclusions

This work proposed a new approach for the identification of lakes on cross sections from polychromies. A first nucleus of cross sections from previous analytical works of Laboratorio Analisi Scientifiche was investigated through traditional Raman spectroscopy in order to highlight signals from red pigments. In the cross sections obtained from samples of Sant'Orso and Saint Peter statues, the presence of vermillion, red lead or phoenicochroite were identified. On the contrary, red pigments in the other analyzed cross sections did not present signals attributable to inorganic pigments and showed a high fluorescence background when analyzed through traditional Raman spectroscopy, suggesting the possible presence of red lakes. These cross sections were coated with properly diluted silver colloidal pastes to perform SER measurements. Silver coating appeared critical and several trials should be made to achieve both the formation of an adequate number of nanoislands and a correct visualization of the layers of the cross section. Still, the recording of SER signals from the layers under investigation was demanding. Difficulties may arise from both the presence in the sample of several compounds that can compete for the adsorption sites on the silver nanoparticles and from a scarce interaction between silver nanoparticles and the lake grains. Despite these limitations, in two samples, one from a wooden statue and one from a mural painting, it was possible to record SER spectra with signals attributable to dyeing molecules. In particular, cross section AGH16 from the Virgin with child wooden statue presented SER spectra attributable to purpurin, thus hypothesizing the use a lake from a madder-type dyestuff rich in purpurin, while cross section ABI36 from the mural painting of Saint Anne showed peaks comparable to those observed for kermes scale insects. Generally speaking, it was noticed that the signal-to-noise ratio of SER spectra recorded on the samples from the wooden statue is lower than that of SER spectra of historical textile samples and it was even lower in the case of the cross section from the mural painting. As a consequence, the registration of spectra from these samples was a time-consuming work. Since the identification of lakes in cross sections is of main interest for LAS and for all the analytical laboratories working in the cultural heritage field, it would be worth trying to overcome the above indicate issues by exploring other approaches, in order to optimize the procedure. In particular, various silver colloids that will better interact with the samples (4.3) should be tested. Moreover, the possibility of microsampling through laser ablation (Londero, Lombardi, and Leona 2013a), also employing laser beams designed for other application such as LIBS, would represent a further step of the research.

The PhD work discussed in this dissertation aimed to give a general contribution to the advance of the knowledge in the field of analytical chemistry for the cultural heritage, namely for the analysis of natural dyes in historical and archaeological artworks. In particular, among the major objectives one was to fill some gaps found while consulting the literature and another was to set up highly sensitive and selective procedures for the identification of the dyes through a non-invasive or a micro-invasive approach. To this aims, both mock-ups and historical artefacts or archaeological finds were considered. In these cases, the collaboration with museums and institutions devoted to the preservation of cultural heritage was of paramount relevance.

Fiber optics reflectance spectroscopy (FORS) in the visible range is indeed a rather employed technique for the preliminary non-invasive investigation of natural dyes on textiles or paintings. Nevertheless no systematic work dealing with the possible variability of the signals of dyestuffs employed on different textile fibers or according to different dyeing procedures (mordants and concentration of the dye) has been published yet. Also in the case of surface-enhanced Raman scattering (SERS), whose application is more and more increasing in the field of dyes identification, no methodological works have been carried out in order to verify whether different fibers, mordants or concentration of the dye may affect the SER signals of the investigated dyes.

Among the SERS substrates, silver colloids are the most popular in the field of dyes analysis in samples from the cultural heritage. In particular, silver colloidal pastes, proposed by Brosseau *et al.* (Brosseau. Gambardella *et al.* 2009), proved to effectively enhance the signals of the dyeing molecules embedded in a number of matrices. Also here, a characterizaion of the interaction between the paste and the coated fibers was not available.

These gaps were filled by the methdological work carried out during the PhD research, whose main results were published in two papers: Gulmini *et al.* 2013 and Idone *et al.* 2013. Besides the methodological work, many case studies were considered in order to verify the validity of the developed analytical procedures also on historical and archaeological objects.

A part of the research work was devoted to elucidate the dyes that could have been employed for the production of the so-called purple codices. These were characterized by means of FORS and SERS and the data obtained on mock ups were employed to investigated a precious 6th century codex, as discussed in (Aceto, Idone, *et al.* 2014) as respects the non-invasive investigations. Finally, SERS was also employed to identify the red painting lakes in samples mounted in cross sections obtained from polychromies (Idone *et al.*, submitted).

The investigation of a number of mock-ups of dyed wool and silk by means of FORS allowed to confirm that only red and blue dyestuffs can be identified with this technique, while the spectral features of yellow dyes are not diagnostic for their identification. Moreover, the identification of the dyestuff proved not to be influenced by the fiber and by the mordant, even though lighter and darker colors often determine the loss of the diagnostic features for the considered dyes. Among the red and blue dyestuffs, it is not possible to discriminate within the indigoid blue dyestuffs

indigo and woad and within the red dyestuffs obtained from the *Coccoidea* family (e.g. cochineals, kermes and lac dye).

On the other hand, FORS measurements carried out on dyed and painted parchments allowed to verify that the signals of the considered purple and red dyes used in the past to produce purple parchments were not influenced by the technique of production (e.g. painted *versus* dyed). FORS allowed to distinguish among Tyrian purple, madder, kermes/Armenian cochineal and folium/orchil, while the discrimination between these two couples of dyes was somewhat difficult, as they presented very similar spectral features.

FORS investigation of the 6th century purple codex allowed to indicate that orchil and folium are the most likely candidates to color the parchment. The investigation of this codex was supported by other non-invasive analyses (e.g. portable microscopy, portable micro-fluorimetry and X-ray fluorescence spectrometry) carried out on the same mock-ups investigated by FORS, which confirmed the results obtained by FORS. The same techniques were also employed to complement the FORS analyses on the other investigated historical and archaeological objects.

In some cases it was possible to compare the results obtained by FORS measurements carried out on historical an archaeological textiles with data obtained through more selective micro-invasive techniques (HPLC-MS or SERS). These allowed to validate FORS as a useful technique for the preliminary analysis of dyed textiles. In particular, FORS proved to be a valuable preliminary analytical tool in the case of the analysis of vast collections, as in the case of Coptic textiles from Museo di Antichità in Torino and of Ottoman textiles from Museo Civico d'Arte Orientale - MAO in Torino and Persian manuscripts from several libraries in the North of Italy. It indeed allows to perform a high number of analysis in a limited amount of time and thus to plan the sampling for further micro-invasive analyses by avoiding redundant sampling. The results obtained during the investigation of Persian manuscripts were included in a recently published paper about the characterization of miniature paintings by means of FORS (Aceto, Agostino, *et al.* 2014).

The experimental work about SERS was mainly intended to the optimization of a procedure for the analysis of very small samples of textile fibers through on-the-fiber extractionless non-hydrolysis SERS with silver colloidal pastes. Three different colloidal pastes, obtained by centrifugation of chemically reduced silver colloids, were tested on mock-ups of natural fibers (from animals and plants) mordanted with different mordants and dyed with cochineal or brazilwood. Several tests were performed in order to obtain a coating that could favor the enhancement of the dye signals. In addition, the pastes and the colloids were characterized by means of reflectance or absorption spectroscopy in the UV-Vis range and high-resolution scanning electron microscopy. SERS measurements highlighted the poor performances of the paste obtained from the colloid of Leopold and Lendl (2003) for on-the-fiber analytical approach, while both the pastes obtained from Lee and Meisel (1982) colloid, with or without addition of sodium perchlorate as an aggregating agent, determined the enhancement of scattering signals for most of the investigated reference samples. Due to a greater ease in obtaining SER signals with silver colloidal pastes obtained from the colloid of Lee and Meisel without the addition of the aggregating agent, these were selected to be employed on historical samples.

The SERS analysis of fragments detached from an important collection of Mariano Fortuny textiles at the Art Institute of Chicago, dating to the first decades of the 20th century, allowed to verify the suitability of the proposed procedure for the analysis of historical samples (Idone *et al.* 2013). In addition, the investigation shed light on the dyes employed by that artist and allowed the first simultaneous identification of two dyes by means of SERS in a historical textile. SERS was also employed to investigate a red fragment from a 16th century precious tapestry from Museo del Duomo in Milano and red and pink threads from the mantle of a cope of controversial dating (15th-17th century) owned by Museo del Tesoro of the cathedral of Aosta, allowing to identify the dyes. In the case of the fragments from the cope, the identification was supported by the comparison with HPLC-DAD-MS results. Several SER spectra obtained from these approximately 500-year-old samples presented signals not attributable to any dyes, possibly deriving from contaminants.

Furthermore, the investigation of the dyes employed to color purple parchments allowed to record SER signals from all the considered dyes, with the only exception of Armenian cochineal, which was only available as a painting lake of unknown bulk composition. To the best of the author's knowledge, SER spectra of natural orchil and of folium have not been reported before. In reverse, the tested procedures, with or without extraction of the dye, for the SERS analysis of mock-ups of purple parchments presented ambiguous results and hence a standard procedure for the analysis of these materials could not be proposed. In some cases, SER spectra of the considered dyes from the dyed parchments were however successfully collected.

Moreover, the availability of a quite large sample detached from the so-called *Codex Brixianus* permitted to perform further tests directly on minute fragments of the historical sample. Identifiable peaks were obtained with various approaches, among which the SERS analysis of a drop of formic acid extract coated with silver colloidal pastes gave the best results. The comparison with the SER spectra recorded on the considered dyes allowed to identify orchil as the main colorant. This was the first time that SERS was employed to identify a dye in a historical parchment.

Finally, the application of silver colloidal pastes for the analysis of red painting lakes in samples mounted in cross sections allowed to identify two lakes used in a 13th century painted wooden statue and in a 16th century mural painting. This work allowed to underline that obtaining an interaction between the substrate and the analyte (e.g. a suitable coating) is a critical step towards the recording of signals of the dyes in cross sections. In addition, some contaminants in the sample matrix competed with the dyes for the absorption onto the substrate and thus most of the recorded SER spectra presented spurious signals attributable to these contaminants.

The research work undertaken during my PhD represents a further step towards the general applicability of SERS to the analysis of dyes in complex matrices like textiles, manuscripts and polychromies, although it is far to be concluded. Several problems were faced, despite many arose in the course of the experimental work and some of them still remain unsolved. Several issues indeed concerned the general application of SERS to various matrixes of interest in the cultural heritage field. In particular, the contamination of historical and archaeological samples is major issue when dealing with ancient objects and is at present the greater obstacle to the application of extractionless SERS, which, as emerged in the course of the experimental work, strongly diminishes the amount of sample required for the analysis compared to SERS on dye extracts.

For this reason, the set-up of micro-invasive pre-treatments that allow to physically separate the dye from the other compounds contained in the sample is highly recommended, as recently demonstrated by Londero *et al.* (2013b), who achieved it by integrating laser ablation with SERS. Also the optimization of substrates devoted to absorb and enhance the signal of selected species would allow a substantial advance of the field and also possibly push towards the identification of different dyes in the same sample. The possibility of discriminating through SERS the various dyes deriving from the natural source would permit to distinguish among different raw materials, and also to identify mixtures of dyestuffs.

This is presently possible only with HPLC separations. Nevertheless, this goal will be possibly achieved in the future through SERS after more in-depth studies about the interaction between the probes and the substrate.

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APPENDIX 1: MOCK-UP SAMPLES

The mock-up samples considered for the optimization of the FORS and SERS procedures for the analysis of textile dyes were obtained following the traditional dyeing techniques employed in the past. This appendix reports in Table A1.1 a list of the codes of each investigated sample, the indication of the set of provenance, of the concentration employed and the measured color coordinates, when available. In addition, the column "notes" indicates whether the samples were investigated through FORS, SERS or by means of both techniques. The code of the first column of Table A1.1 contains all the available information about the dyeing procedure employed for the preparation of the sample. Color coordinates were collected on the reference samples by means of a Minolta (Tokyo, Japan) CM-700d Spectrophotometer employing the D65 standard illuminant, the CIE 1964 10° standard observer and setting the measuring aperture to 3 mm. Color coordinates in the CIE L*a*b* color space were calculated as the mean of five measurements obtained on different portions of the same sample.

The code of the samples reported in the first column of Table A1.1 can be read as follows:
- the first three letters indicate the dyestuff (Alk=alkanet; Ann=annatto; Blo=bloodred webcap; Bra=brazilwood; Bro=dyer's broom; Buc=common buckthorn; Coc=cochineal; Fus=old fustic; Gal=galls; Hen=henna; Ind=indigo; Log=Logwood; Mad=madder; Que=quebracho; SaB=Saxon blue; San=sandalwood; Saf=saffron; Saw=safflower; Shi=shikon; Tur=turmeric; Wal=walnut husks; Wel=weld; Woa=woad); in the case of double dyed samples, the second dyestuff is indicated after an underscore;
- the letters placed after the first underscore indicate the mordant/additive employed (Aa=acetic acid; Al=alum; C=cream of tartar; Cr=potassium dichromate; Cu=copper(II) sulfate; Fe=iron(II) sulfate; G=gambier; Na=sodium carbonate; Sn=tin(II) chloride) or, in the case of vat dyes, the number of subsequent baths performed (1=1 bath; 2=2 baths; 3=3 baths); the number zero (0) indicates that the dyestuffs was employed directly, without the addition of mordants;
- the first letter placed after the second underscore indicates the process to extract the dye (s=soaked; n=not soaked), while the first number indicates the number of the exhaustion bath employed (1=1st bath, i.e. not employed to dye other fibers before; 2=2nd bath, i.e. bath employed one time before; 3=3rd bath, i.e. bath employed two times before); then, the capital letter indicates the fiber (S=silk; W=wool; C=cotton; F=flax) and the following lower case letter indicates the manufacturing (f=flock; t=thread; c=cloth); the final number is a progressive number for the samples dyed with a same procedure;
- the letter x placed at whatever position indicates that the information is not known.

In addition, the set of provenance is indicated in the second column of Table A1.1 with the code shown below:
- the letter P indicates the set of samples coming from the group of Prof. Maria Perla Colombini of Università degli Studi di Pisa;

- the letter M indicates the set of samples dyed by myself and the other participants to two courses of natural dyeing at "Associazione Colore e Tintura Naturale Maria Elda Salice" in Milano;
- the letter T indicates the set of samples dyed at Università degli Studi di Torino by several bachelor's and master's students of the former Corso di Studi in Scienza e Tecnologia per i Beni Culturali during their experimental thesis;
- the letter "L" indicates the sets of samples dyed at Laboratorio Analisi Scientifiche, L1 samples were dyed about twenty years ago, while L2 samples were dyed by myself.

Samples from sets T and L2 were obtained following the indications reported by Maria Elda Salice in her book "La tintura naturale", 1979, Milano: Sonzogno.

In Table A1.1, empty boxes indicate unavailable information.

Table A1.1: Main features of the mock-up samples of dyed textiles investigated through FORS and/or SERS in the experimental work of the PhD research (Chapter 3, Chapter 5)

Code	Set	Dyestuff with respect to the textile fiber (wt%)	Color coordinates			Notes
			L*	a*	b*	
Alk_0_xxSc1	P					FORS
Alk_0_xxSc2	P					FORS
Alk_0_xxWt3	P					FORS
Ann_AlC_s1Wf1	L2	50%				FORS
Ann_0_s1Wf2	L2	50%				FORS
Ann_AlC_s1Wf3	L2	50%, Na_2CO_3 120%				FORS
Ann_AlC_s1Wf4	M	40%				FORS
Ann_AlC_s1Wt5	M	40%				FORS
Ann_AlC_s1Sc6	M	40%				FORS
Ann_AlC_s2Wf7	M	40%				FORS
Ann_AlC_s2Wt8	M	40%				FORS
Ann_AlC_s2Sc9	M	40%				FORS
Bra_Al_xxWt1	P		32.2	38.0	25.6	FORS
Blo_AlC_s1Wf1	M	10%				FORS
Blo_AlC_s1Wt2	M	10%				FORS
Blo_AlC_s1Sc3	M	10%				FORS
Blo_AlC_s2Wf4	M	10%				FORS
Blo_AlC_s2Wt5	M	10%				FORS
Blo_AlC_s2Sc6	M	10%				FORS
Bra_AlC_s1Wf2	T	20%	49.8	40.3	31.8	FORS; SERS
Bra_AlC_s1Wf3	T	20%	54.0	41.8	32.2	FORS; SERS
Bra_AlC-n1Wf4	T	10%	50.8	38.2	29.0	FORS; SERS
Bra_AlC_n1Wf5	M	30%	47.5	39.9	16.3	FORS; SERS
Bra_AlC_n1Wt6	M	30%	48.0	40.2	16.1	FORS; SERS
Bra_AlC_n1Sc7	M	30%	52.4	36.0	5.6	FORS; SERS
Bra_AlC_n2Wf8	M	30%	58.1	39.0	15.1	FORS; SERS
Bra_AlC_n2Wt9	M	30%	63.5	35.3	14.3	FORS; SERS

Code	Set	Dyestuff with respect to the textile fiber (wt%)	Color coordinates			Notes
			L*	a*	b*	
Bra_AlC_n2Sc10	M	30%	68.0	29.4	2.8	FORS; SERS
Bra_AlCFe_s1Wf11	T	20%	59.2	14.5	19.6	FORS; SERS
Bra_AlCFe_s1Wf12	T	20%	59.2	23.3	21.8	FORS
Bra_AlCFe_n1Wf13	T	10%	54.8	15.4	18.4	FORS
Bra_AlNa_n1Cc14	M	extract 4%				SERS
Bra _AlNa_n1Fc15	M	extract 4%				SERS
Bra _AlG_n1Cc18	M	extract 4%				SERS
Bra _AlG_n1Fc19	M	extract 4%				SERS
Bra _AlNaAa_n1Cc22	M	extract 4%				SERS
Bra _AlNaAa_n1Fc23	M	extract 4%				SERS
Bro_Al_xxWt1	L1	400%				FORS
Buc_Al_xxWt1	P					FORS
Coc_AlC_s1Wt1	M	15%	33.2	44.6	6.8	FORS; SERS
Coc_AlC_s1Sc2	M	15%	31.6	42.2	1.2	FORS; SERS
Coc_AlC_s2Wf3	M	15%	45.7	40.9	1.2	FORS; SERS
Coc_AlC_s2Wt4	M	15%	50.2	37.5	0.7	FORS; SERS
Coc_AlC_s2Sc5	M	15%	51.8	37.5	-5.3	FORS; SERS
Coc_AlC_s3Wf6	M	15%	69.4	20.8	10.4	FORS
Coc_AlC_s3Wt7	M	15%	74.0	19.2	9.5	FORS
Coc_AlC_s3Sc8	M	15%	79.9	11.9	6.1	FORS
Coc_AlC_s1Wf9	T	10%	26.6	28.8	-5.6	FORS; SERS
Coc_AlC_n1Wf10	T	10%	29.1	31.1	-5.5	FORS; SERS
Coc_AlC_xxWt11	L1	10%	28.6	36.7	3.7	FORS; SERS
Coc_AlC_xxWt12	L1	5%	49.9	34.8	2.3	FORS; SERS
Coc_Al_s1Wf13	M	15%	32.9	37.9	-1.0	FORS
Coc_Al_s1Wt14	M	15%	35.8	35.6	-2.6	FORS
Coc_Al_s1Sc15	M	15%	30.9	36.3	-1.4	FORS
Coc_Al_s2Wf16	M	15%	55.9	28.6	-1.7	FORS; SERS
Coc_Al_s2Wt17	M	15%	58.2	28.3	-2.0	FORS; SERS
Coc_Al_s2Sc18	M	15%	59.5	27.0	-7.6	FORS; SERS
Coc_AlCFe_s1Wf19	T	10%	28.0	20.2	-3.6	FORS; SERS
Coc_AlCFe_n1Wf20	T	10%	31.4	19.5	-4.3	FORS
Fus_AlC_s1Wf1	T	25%	73.3	4.2	42.4	FORS
Fus_AlC_n1Wf2	M	extract 3%	57.6	10.3	52.4	FORS
Fus_AlC_n1Wt3	M	extract 3%	60.3	7.4	51.2	FORS
Fus_AlC_n1Sc4	M	extract 3%	53.5	14.7	43.9	FORS
Fus_AlC_n2Wf5	M	extract 3%	71.8	5.6	39.6	FORS
Fus_AlC_n2Wt6	M	extract 3%	69.8	6.6	36.9	FORS
Fus_AlC_n2Sc7	M	extract 3%	69.1	10.2	34.1	FORS
Fus_AlC_xxWt8	L1	200%	45.0	17.1	44.6	FORS

Code	Set	Dyestuff with respect to the textile fiber (wt%)	Color coordinates			Notes
			L*	a*	b*	
Fus_Al_xxWt9	P		45.8	16.4	46.1	FORS
Gal_AlC_s1Wf1	L2	250%				FORS
Gal_AlC_xxWt2	L1					FORS
Gal_FeC_s1Wf3	L2	300%				FORS
Hen_AlC_n1Wf1	L2	50%				FORS
Hen_0_n1Wf2	L2	50%				FORS
Ind_1_n1Wf1	T	15%	35.8	-5.4	-15.2	FORS
Ind_2_n1Wf2	T	15%	25.2	-1.4	-15.1	FORS
Ind_2_n1Wt3	L1		37.0	-6.2	-17.3	FORS
Ind_3_n1Wf4	T	15%	18.6	1.8	-12.1	FORS
Ind_x_xxWt5	P		28.0	-2.9	-17.3	FORS
Log_AlC_s1Wf1	M	30%	17.2	3.0	-9.4	FORS
Log_AlC_s1Wt2	M	30%	17.5	4.6	-12.1	FORS
Log_AlC_s1Sc3	M	30%	19.8	2.5	-2.9	FORS
Log_AlC_s2Wf4	M	30%	28.0	6.2	-13.3	FORS
Log_AlC_s2Wt5	M	30%	32.8	6.0	-13.3	FORS
Log_AlC_s2Sc6	M	30%	34.9	5.6	-4.6	FORS
Log_AlC_s3Wf7	M	30%	46.4	6.4	-9.7	FORS
Log_AlC_s3Wt8	M	30%	50.7	5.3	-8.2	FORS
Log_AlC_s3Sc9	M	30%	64.9	3.8	-0.9	FORS
Log_AlC_n1Wf10	T	100%	20.7	8.4	-4.4	FORS
Log_AlC_xxWt11	L1	50%	21.6	3.5	-3.1	FORS
Log_Al_s1Wf12	M	30%	16.3	5.0	-10.8	FORS
Log_Al_s1Wt13	M	30%	16.6	6.5	-13.2	FORS
Log_Al_s1Sc14	M	30%	20.0	5.4	-6.6	FORS
Log_Al_s2Wf15	M	30%	33.7	8.9	-14.6	FORS
Log_Al_s2Wt16	M	30%	35.8	8.6	-14.3	FORS
Log_Al_s2Sc17	M	30%	36.1	9.2	-9.7	FORS
Log_Al_s3Wf18	M	30%	53.1	7.1	-9.6	FORS
Log_Al_s3Wt19	M	30%	54.2	6.6	-8.9	FORS
Log_Al_s3Sc20	M	30%	63.1	5.9	-4.3	FORS
Log_Al_xxWt21	P		21.9	3.6	-2.3	FORS
Log_AlCFe_s1Wf22	T	100%	18.7	2.8	-4.8	FORS
Log_Cr_s1Wf23	T	100%	15.8	-0.4	-2.2	FORS
Mad_AlC_s1Wf1	T	100%	55.9	32.5	26.2	FORS
Mad_AlC_s1Wf2	T	100%	55.1	34.6	31.8	FORS
Mad_AlC_s1Wf3	T	100%	32.5	38.3	29.1	FORS
Mad_AlC_n1Wf4	M	80%	29.1	34.8	20.6	FORS
Mad_AlC_n1Wt5	M	80%	30.1	36.3	22.7	FORS
Mad_AlC_n1Sc6	M	80%	40.5	37.2	19.5	FORS

Code	Set	Dyestuff with respect to the textile fiber (wt%)	Color coordinates			Notes
			L*	a*	b*	
Mad_AlC_n2Wf7	M	80%	63.0	25.2	24.6	FORS
Mad_AlC_n2Wt8	M	80%	53.7	29.3	24.5	FORS
Mad_AlC_n2Sc9	M	80%	69.1	11.3	27.0	FORS
Mad_AlC_xxWt10	L1	100%	37.0	44.7	29.1	FORS
Mad_AlC_xxWt11	L1	50%	36.6	41.2	26.7	FORS
Mad_Al_n1Wf12	M	80%	28.3	39.4	22.2	FORS
Mad_Al_n1Wt13	M	80%	29.7	40.1	23.0	FORS
Mad_Al_n1Sc14	M	80%	39.3	41.1	24.6	FORS
Mad_Al_n2Wf15	M	80%	52.6	32.3	22.4	FORS
Mad_Al_n2Wt16	M	80%	55.4	32.1	23.3	FORS
Mad_Al_n2Sc17	M	80%	69.3	13.0	22.4	FORS
Mad_Al_xxWt18	P		34.2	43.9	29.5	FORS
Mad_AlCFe_s1Wf19	T	100%	52.0	21.8	21.2	FORS
Mad_AlCFe_s1Wf20	T	100%	44.1	17.3	13.1	FORS
Mad_AlCFe_s1Wf21	T	100%	24.6	21.1	14.4	FORS
Que_AlC_n1Wf1	M	extract 3%				FORS
Que_AlC_n1Wt2	M	extract 3%				FORS
Que_AlC_n1Sc3	M	extract 3%				FORS
Que_AlC_n2Wf4	M	extract 3%				FORS
Que_AlC_n2Wt5	M	extract 3%				FORS
Que_AlC_n2Sc6	M	extract 3%				FORS
SaB_Al_n1Wf1	M	with H_2SO_4, 20 drops	59.4	-16.2	-11.0	FORS
SaB_Al_n1Wt2	M	with H_2SO_4, 20 drops	59.3	-16.3	-10.8	FORS
SaB_Al_n1Sc3	M	with H_2SO_4, 20 drops	75.7	-6.4	0.1	FORS
SaB_Al_n1Wf4	M	with H_2SO_4, 40 drops	43.8	-16.6	-17.6	FORS
SaB_Al_n1Wt5	M	with H_2SO_4, 40 drops	44.7	-16.8	-17.2	FORS
SaB_Al_n1Sc6	M	with H_2SO_4, 40 drops	63.5	-10.9	-4.8	FORS
SaB_Al_n1Wf7	M	with H_2SO_4, 60 drops	38.6	-15.5	-19.3	FORS
SaB_Al_n1Wt8	M	with H_2SO_4, 60 drops	40.2	-16.0	-19.0	FORS
SaB_Al_n1Sc9	M	with H_2SO_4, 60 drops	58.2	-11.5	-3.9	FORS
Saf_AlC_s1Wf1	T	25%	69.7	21.6	85.5	FORS
Saf_AlC_n1Wf2	T	25%	68.4	18.6	82.4	FORS
Saf_Al_xxWt3	L1	3.5%	75.8	7.8	51.4	FORS
Saf_Cu_s1Wf4	T	10%	63.0	-4.1	51.9	FORS
Saf_0_s1Wf5	T	25%	70.2	11.9	75.4	FORS
Saf_0_n1Wf6	T	25%	71.0	12.1	74.3	FORS
Saf_0_xxWt7	P		80.2	3.2	42.9	FORS
San_AlC_s1Wf1	L2	100%				FORS
San_AlC_s1Wf2	L2	10%				FORS
Saw_AlC_s1Wf1	L2	50%				FORS

Code	Set	Dyestuff with respect to the textile fiber (wt%)	Color coordinates			Notes
			L*	a*	b*	
Saw_AlC_s1Wf2	T	100%				FORS
Saw_AlC_n1Wf3	T	100%				FORS
Saw_Al_xxWt4	L1	100%				FORS
Shi_0_xxWc1	P					FORS
Shi_0_xxSc2	P					FORS
Tur_AlC_s1Wf1	T	50%	65.9	20.9	85.2	FORS
Tur_AlC_n1Wf2	T	50%	70.4	10.9	82.2	FORS
Tur_AlC_n1Wf3	T	50%	68.5	19.0	88.7	FORS
Tur_Cu_n1Wf4	T	50%	53.7	7.9	55.3	FORS
Tur_Cu_n1Wf5	T	40%				FORS
Tur_0_s1Wf6	T	50%	65.7	14.5	75.9	FORS
Tur_0_n1Wf7	T	50%	64.7	16.6	78.4	FORS
Tur_x_xxWt8	L1	50%	71.2	6.8	52.3	FORS
Wal_AlC_n1Wf1	M	100%				FORS
Wal_AlC_n1Wt2	M	100%				FORS
Wal_AlC_n1Sc3	M	100%				FORS
Wal_AlC_n2Wf4	M	100%				FORS
Wal_AlC_n2Wt5	M	100%				FORS
Wal_AlC_n2Sc6	M	100%				FORS
Wal_0_xxWt7	L1	400%				FORS
Wel_AlC_s1Wf1	T	400%	75.1	3.5	36.3	FORS
Wel_AlC_s1Wf2	T	400%	79.3	-0.1	47.8	FORS
Wel_AlC_n1Wf3	T	400%	74.5	0.6	40.7	FORS
Wel_AlC_n1Wf4	T	400%	70.2	2.6	46.6	FORS
Wel_Al_s1Wf5	M	100%	80.9	-3.2	45.6	FORS
Wel_Al_s1Wt6	M	100%	79.9	-1.5	47.7	FORS
Wel_Al_s1Sc7	M	100%	85.9	-0.8	21.0	FORS
Wel_Al_s2Wf8	M	100%	85.1	-3.6	30.2	FORS
Wel_Al_s2Wt9	M	100%	88.0	-3.6	29.9	FORS
Wel_Al_s2Sc10	M	100%	89.4	-2.0	20.6	FORS
Wel_Al_xxWt11	P		69.4	3.2	58.0	FORS
Wel_SnC_s1Wf12	T	100%	77.4	-0.2	52.7	FORS
Wel_SnC_n1Wf13	T	100%	65.4	2.0	45.6	FORS
Woa_1_n1Wf1	T	15%	65.7	-7.5	5.9	FORS
Woa_2_n1Wf2	T	15%	55.3	-9.6	-6.9	FORS
Woa_2_n1Wf3	T	33%	47.8	-7.7	-10.8	FORS
Woa_3_n1Wf4	T	15%	58.4	-10.3	-5.2	FORS
Woa_3_n1Wf5	T	33%	41.2	-7.2	-15.0	FORS
DOUBLE DYEING						
Coc_ind_x1_Wt1	L1	5%, ?				FORS
Coc_ind_x1_Wt2	L1	10%, ?				FORS

Code	Set	Dyestuff with respect to the textile fiber (wt%)	Color coordinates			Notes
			L*	a*	b*	
Fus_SaB_AlC_1Wf1	M	Extract 3%, with H_2SO_4 15 drops				FORS
Fus_SaB_AlC_1Wt2	M	Extract 3%, with H_2SO_4 15 drops				FORS
Fus_SaB_AlC_1Sc3	M	Extract 3%, with H_2SO_4 15 drops				FORS
Fus_SaB_AlC_2Wf4	M	Extract 3%, with H_2SO_4 15 drops				FORS
Fus_SaB_AlC_2Wt5	M	Extract 3%, with H_2SO_4 15 drops				FORS
Fus_SaB_AlC_2Sc6	M	Extract 3%, with H_2SO_4 15 drops				FORS
Fus_ind_AlC_1Wf1	T	330%, 15%				FORS
Fus_ind_xx_Wt8	L1					FORS
Ind_wel_AlC_1Wf1	T	13%, 330%				FORS
Log_fus_Cr_1Wf1	T	30%, 40%				FORS
Log_res_Cr_1Wf1	T	30%, 40%				FORS
Mad_ind_x1_Wt1	L1	100%, ?				FORS
Saf_ind_Al_Wt1	L1					FORS
Saw_ind_Al_Wt1	L1					FORS
Tur_ind_0_Wt1	L1					FORS
Wel_SaB_Al_1Wf1	M	100%, with H_2SO_4 15 drops				FORS
Wel _SaB_Al_1Wt2	M	100%, with H_2SO_4 15 drops				FORS
Wel _SaB_Al_1Sc3	M	100%, with H_2SO_4 15 drops				FORS
Wel _SaB_Al_1Wf4	M	100%, with H_2SO_4 25 drops				FORS
Wel _SaB_Al_1Wt5	M	100%, with H_2SO_4 25 drops				FORS
Wel _SaB_Al_1Sc6	M	100%, with H_2SO_4 25 drops				FORS
Wel _SaB_Al_2Wf7	M	100%, with H_2SO_4 25 drops				FORS
Wel _SaB_Al_2Wt8	M	100%, with H_2SO_4 25 drops				FORS
Wel _SaB_Al_2Sc9	M	100%, with H_2SO_4 25 drops				FORS
Wel _ind_AlC_1Wf1	T	80%, 15%				FORS

APPENDIX 2: INSTRUMENTATION

This appendix details the features of the instrumentation employed for the experimental work of the PhD research.

FIBER OPTICS REFLECTANCE SPECTROSCOPY (FORS)

AVANTES INSTRUMENTATION

The AvaLight-HAL (Avantes, Apeldoorn, The Netherlands) is a tungsten/alogen lamp with emission from 360 to 2500 nm. The AvaLight-DH-S-BAL (Avantes, Apeldoorn, The Netherlands) is a balanced deuterium–halogen light source with a wavelength range of 190–2500 nm. The AvaSpec-USL2048XL-USB2 is a 2048 pixel CCD detector ranging from 200 to 1160 nm with a spectral resolution of 0.09-20 nm. The integration time can be selected by the operator in the range between 2 µs and 20 s. Depending on the features of the monochromator (slit width 50 µm, grating of UA type with 300 lines/mm) and of the detector, the best spectra resolution was 2,4 nm calculated as FWHM. Either the light sources can be coupled to the detector. The selected source and the detector are both connected with fiber optic cables to an Ocean Optics (Dunedin, Florida) R-400 model reflection probe or to an Avantes FCR-7UV200-2-1,5x100 model reflection probe (Apeldoorn, The Netherlands). In this configuration, light is sent and retrieved with a unique fiber bundle positioned at 45° from the surface normal, in order not to include specular reflectance. The spot area is 3 mm with the first probe and 1 mm with the second probe. The white calibration is made with the 98% reflective reference title WS-2 (Avantes, Apeldoorn, The Netherlands). In some cases, the distance between probe and sample was kept constant to 1 mm. To visualize the investigated area on the sample, the probe contained a Framos (Agrate Brianza, Italy) WEB1315SI model digital micro camera, connected to PC via USB. The whole system is managed by means of AvaSoft v. 8 dedicated software, running under Windows 7. These instrumentations are owned by Dipartimento di Scienze e Innovazione Tecnologica, Università degli Studi del Piemonte Orientale "Amedeo Avogadro".

CORONA 45VIS SPECTROPHOTOMETER

Corona45Vis spectrophotometer (Zeiss, Oberkochen, Germany) is equipped with a stabilized halogen source A10. The lamp has a voltage of 10 V and a power 20 W; its average lifetime is of about 3000 hours. The spectrometer is a single beam diode array with a MMS 1 polychromator and 256 diodes and a spectral resolution of 3.3 nm, operating in the 380-1000 nm range. The wavelength accuracy is less than 0.5 nm, while the reflection accuracy is less than 0.2%. Hellma fiber optics are employed in order to obtain two different measurement geometries. In particular, the 2X45°/0° geometry can be achieved by employed the expressly designed probe shown in Figure A2.1. In this case, a Y-shaped fiber optic cable is employed to carry the incident light from the source to the sample with a 45° angle, while a linear cable is used to collect the reflected light at 0°. The 0°/0° geometry is obtained by employing a unique Y- shaped cable connected both to the source and the spectrometer. A SphereOptics (Uhldingen, Germany) diffuse reflectance standard made of highly reflecting PTFE is used as calibration target. The instrument is managed with AspectPlus dedicated software, running under Windows. This instrumentation is owned by Laboratorio Analisi Scientifiche, Direzione Ricerca e Progetti Cofinanziati, Regione Autonoma Valle d'Aosta.
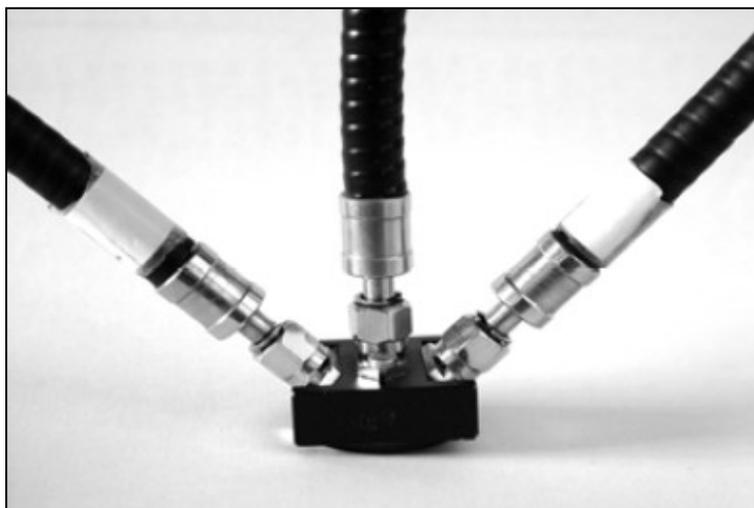

Figure A2.1: Particular of the probe employed for some of the FORS measurements.

MULTICHANNEL SPECTROMETER SYSTEM MCS 600

The Multichannel spectrometer system MCS 600 (Zeiss, Oberkochen, Germany) is equipped with the halogen lamp CLH 600 and two detectors, the MCS 601 UV-NIR spectrometer and the MCS 611 NIR 2.2 spectrometer. The CLH 600 is a halogen source operating in the range 360-2400 nm, has a power of 10 W, a color temperature of approximately 2900 K and a mean lifetime of 5000 hours. The MCS 601 UV-NIR spectrometer is equipped with a MCS polychromator and 1024 diodes. It operates in the 190-1015 nm range with a spectral resolution (calculated as half width 1/10 max) of 3 nm, a wavelength accuracy of less than 0.5 nm at wavelengths inferior than 500 nm and of 1.0 nm at wavelengths higher than 500 nm. The minimum integration time is of 12 ms. Coupled with the CLH 600 lamp it has a measurement range of 380-1000 nm. The MCS 611 NIR 2.2 spectrometer is equipped with a PGS polychromator and 256 diodes. It is cooled through a 2 stage Peltier cooling. It operates in the 910-2200 nm range with a spectral resolution (calculated as half width 1/10 max) of 18 nm and a wavelength accuracy of less than 1.0 nm. The minimum integration time is of 0.1 ms. Coupled with the CLH 600 lamp it has a measurement range of 910-2150 nm. The spectrometers were employed separately or coupled. In particular, Hellma fiber optics were employed in order to obtain a 0°/2x45° geometry with the probe shown in Figure A2.1. In this case, a fiber optics cable brings the incident light from the source at 0° to the sample, while each of the cables at 45° is connected to one spectrometer. The 0°/0° geometry was instead employed by operating with only the MCS 601 UV-NIR spectrometer in the 380-1000 nm range employing a Y-shaped fiber optics cable connected both to this spectrometer and the light source. A SphereOptics (Uhldingen, Germany) diffuse reflectance standard made of highly reflecting PTFE is used as calibration target. The instrument is managed with AspectPlus dedicated software, running under Windows. This instrumentation is owned by Laboratorio Analisi Scientifiche, Direzione Ricerca e Progetti Cofinanziati, Regione Autonoma Valle d'Aosta.

UV-VIS SPECTROSCOPY

Absorbance spectra of silver colloids were recorded with a Jasco (Easton, US) V-550 UV-Vis spectrophotometer in the range 300-600 nm. This instrumentation is owned by Dipartimento di Chimica, Università degli Studi di Torino.

Diffuse reflectance spectra of the silver colloidal pastes were recorded with a Perkin Elmer Lambda 900 UV-Vis-NIR (Waltham, US) equipped with an integration sphere, with 1 nm spectral resolution in the 350-700 nm range. This instrumentation is owned by Dipartimento di Chimica, Università degli Studi di Torino.

PORTABLE MOLECULAR SPECTROFLUORIMETRY

The Ocean Optics (Dunedin, Florida, USA) Jaz model spectrophotometer was employed to measure molecular fluorescence spectra. The instrument is equipped with a 365 nm Jaz-LED internal light source; a QF600-8-VIS/NIR fiber fluorescence probe is used to drive excitation light on the sample and to recover emitted light. The spectrophotometer is working in the range 191–886 nm; according to the features of monochromator (200 lm slit width) and detector (2048 elements), the spectral resolution available is 7.6 nm calculated as FWHM. The investigated area on the sample is 1 mm diameter. In all measurements the sample-to-probe distance was kept constant to 1 mm (corresponding to focal length) with aid of a small black cylinder inserted on top of the probe, in order also to exclude contributions from external light. The system is managed with SpectraSuite software under Windows 7. This instrument is owned by Dipartimento di Scienze e Innovazione Tecnologica, Università degli Studi del Piemonte Orientale "Amedeo Avogadro".

POTABLE X-RAY FLUORESCENCE

EDXRF THERMO NITON SPECTROMETER

The EDXRF Thermo (Waltham, USA) NITON spectrometer XL3T-900 GOLDD model is equipped with Ag tube (max. 50 kV, 100 µA, 2 W), large area SDD detector, with an energy resolution of about 136 eV at 5.9 keV. The analyzed spot has an average diameter of 3 mm or 8 mm and is focused by a CCD camera, with a working distance of 2 mm. The instrument can be held in position with a moving stage allowing micrometric shifts, in order to reach the desired probe-to-sample distance; the stage is laid on a sturdy tripod. The obtained spectra were processed with the commercial software WinAxil, derived by the academic software QXAS from IAEA. This instrumentation is owned by MAIN group, Dipartimento di Chimica, Università degli Studi di Torino.

EDXRF BRUKER TRACER III-SD

The tracer III-SD produced by the Bruker Corptoration (Billerica, Massachussets, US) is equipped with a Rh tube and a palladium split, X-flash SDD detector (10 mm^2), with an energy resolution of 145eV at 100 000 counts. The accelerating voltage can be set between 10 and 40 kV, while the anode current can be varied from 0.05 to 60 µA. This instrumentation is owned by Laboratorio Analisi Scientifiche, Direzione Ricerca e Progetti Cofinanziati, Regione Autonoma Valle d'Aosta.

MICROSCOPY

PORTABLE MICROSCOPE

The potable microscope used is a Dino-Lite (Naarden, The Netherlands) AM413T-FVW PRO. The microscope allows a magnification in the range 20X-90X and can also register images under the UV light. This instrumentation is owned by Dipartimento di Scienze e Innovazione Tecnologica of Università degli Studi del Piemonte Orientale "Amedeo Avogadro".

OPTICAL MICROSCOPE

The optical microscope employed to observe some of the samples before the analysis is a Polyvar Pol (Reichert-Jung, Reichert, Austria) equipped with 2X, 5X, 10X, 20X, 40X and 80X magnification objective. It allows to observed the sample both in the dark field and bright field mode and to collect images by means of a Leica DC3000 camera. The image acquisition is managed by ImageProPlus, running under Windows. This instrumentation is owned by Laboratorio Analisi Scientifiche, Direzione Ricerca e Progetti Cofinanziati, Regione Autonoma Valle d'Aosta.

SCANNING ELECTRON MICROSCOPES

To record the images of colloids and silver colloidal pastes three different instrumentation were employed. In particular, a S-4800-II c-FEG SEM (Hitachi, Tokyo, Japan) equipped with a Super ExB filter and a resolution of 1.0 nm at 15 kV was employed; the accelerating voltage was set to 5.0-15.0 kV, the emission current was 10 µA. Also a LEO Gemini 1525 microscope operating at 3 kV or 7 kV was used. Environmental SEM (FEI Quanta 600F sFEG ESEM) imaging was performed in some cases in order to circumvent the low conductivity of the samples. The microscope was operated at 12 kV in low vacuum mode, with a pressure of 1.20 Torr (water vapor). These instrumentations are owned by the EPIC facility, Northwestern University (Evanston, Illinois, US).

In addition, the metallic threads of the cope described in paragraph 6.6 two instrumentations were employed: a Zeiss (Oberkochen, Germany) EVO60 and a Stereoscan S-360 by Cambridge Instruments were both equipped with an EDX Oxford PentaFET microprobe. The first instrument can operate at variable pressure (20-200 Pa), while the second operates in vacuum (10^{-4}-10^{-5} Torr). The maximum accelerating voltage is of 50 kV and 40 kV, respectively. The Zeiss EVO60 is owned by Centro di Conservazione e Restauro "La Venaria Reale", while the Stereoscan S-360 is owned by Dipartimento di Scienze della Terra, Università degli Studi di Torino.

RAMAN SPECTROSCOPY

LABRAM 300 HORIBA JOBIN YVON

The Horiba Jobin Yvon (Longjumeau, France) Labram 300 confocal Raman microscope is equipped with Andor multichannel Peltier cooled open electrode charge-coupled device (CCD) detector (Andor DV420-OE322; 1024x256), BXFM open microscope frame (Olympus), edge filters, and a 1800 lines/mm dispersive grating. The excitation line of a He-Ne laser (λ_{ex}=632.8 nm) is employed, focused through a 100x Olympus objective. Power at the samples could be controlled by a series of neutral density filters. This instrument is owned by the Art Institute of Chicago (Illinois, US).

RENISHAW INVIA

The Renishaw InVia spectrometer is coupled with a Leica DM2500 M optical microscope. Three excitation wavelengths are available: 532 nm, 632.8 nm and 785 nm. The instrument is equipped with edge filters, which cut the signals before about 100 cm^{-1}. Two dispersive gratings of 1800 lines/mm and 1200 lines/mm are employed to send the signal to the air-cooled CCD detector (400 pixels). The first grating is employed for measurements with the visible excitation wavelengths (e.g. 532 and 632.8 nm), while the second is employed for the NIR excitation wavelength (e.g. 785 nm). The Leica DM2500 M is a confocal optical microscope equipped with 5X, 10X, 50X and 100X magnification objective. A 50X objective with long working distance is also mounted on the microscope. It is possible to observe the sample under reflected or transmitted light, in dark field and in bright field mode. The instrument is also equipped with an automatic calibration system which allow to perform measurements on the same point with different excitation wavelengths. The maximum power at the sample is of about 77 mW for the 533 nm laser, 7.7 mW for the 632.8 nm laser and 230 mW for the 785 nm laser. The power of each lasers can be diminished by employing a series of filters. Spectra can be collected in the 100-5000 cm^{-1} range approximately. The instrument is optimized for acquisition with the 100X objective, which was employed for most of the measurements. The spot diameter with this objective is of about 2 μm. The whole system is managed with the dedicated software Wire 3.4, running under Windows. This instrumentation is owned by Laboratorio Analisi Scientifiche, Direzione Ricerca e Progetti Cofinanziati, Regione Autonoma Valle d'Aosta.

MICRO-X-RAY FLUORESCENCE

The µXRF EAGLE III XPL from Roentgenanalytik Systeme is equipped with a tungsten cathode and a rhodium anode. The voltage can be set from 10 to 50 kV, while the intensity of current can vary from 20 to 1000 µA. The EDS Si(Li) detector has an area of 30 mm^2. The analysis can be performed with a 10X or a 100X magnification. The minimum lateral resolution is of 30 µm. The system is managed by means of the VISION32 software, running under Windows. This instrument is owned by Centro Interdipartimentale per lo studio degli amianti e di altri particolati nocivi "Giovanni Scansetti", Università degli Studi di Torino.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Separation was performed with a Phenomenex (Torrance, USA) Synergi C18 column, 150 mm × 2.1 mm with 3 µm particle size injecting a volume of 10 µL. Gradient elution with acetonitrile/water (5/95 to 100/0 in 30 min) was employed at a 200 µL/min flow rate; the mobile phase also contained formic acid (0.15% v/v) in order to keep the acidic chromophores in their neutral form. The column was mounted on a modular system consisting of a Dionex Ultimate 3000 HPLC (Dionex, Milan, Italy) coupled with a Surveyor PDA UV detector and a LTQ Orbitrap mass spectrometer (Thermo Scientific, Rodano, Italy) equipped with an atmospheric pressure interface and an ESI ion source; nitrogen was used as sheath and auxiliary gas. The tuning parameters adopted for the ESI source were: source voltage 4.5 kV, capillary voltage 21.00 V, tube lens voltage 80 V for positive ion mode acquisition and source voltage 3.5 kV, capillary voltage -18.00 V, tube lens voltage -69.14 V for negative ions mode. The heated capillary temperature was maintained at 300°C. The MS/MS CID experiments were performed in the data dependent mode using an isolation width of 1.0 and normalized collision energy of 35%. The system was managed by means of the Excalibur software, running under Windows. This instrumentation is owned by Dipartimento di Chimica, Università degli Studi di Torino.