# HISTORICAL TEXTILES AND THEIR CHARACTERIZATION

IVA REZIĆ

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Cambridge Scholars Publishing

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# PREFACE

This book focuses on gaining new insights into research on and the application of methodologies for the determination of the composition and degradation of historical textile materials. It presents a broad range of examples that can be found during the conservation and restoration of historical materials—from historical textiles on mummies to metal fibers and pearls.

Moreover, the book highlights the importance of a multidisciplinary approach as a part of complex tasks when only a relatively low amount of materials is available for analysis. In cases of the characterization of precious valuable and rare materials, only sensitive, selective, and reliable analytical procedures can be applied.

This book therefore presents sensitive and selective analytical procedures for the characterization of historical textiles, validated by certified reference materials. Current efforts to include optimized procedures and mathematical modeling in analytical chemistry, restoration and conservation, and materials characterization are also presented. These methodologies are applied to historical samples, where they have proved fundamental in unraveling the complex composition of samples with metalized yarns and pearls collected from historical samples.

As this book utilizes current scientific advances to better understand the principles of materials characterization, it is of broad general interest, but it will be of particular interest to the chemical, anthropological, and conservation-restoration communities. Moreover, it can offer particular support to interested audiences in historical materials preservation.

Historical textiles, as objects of particular value to cultural national heritage, belong to the group of the most sensitive materials that often need to be restored and preserved. The main reasons for damage to them include unsuitable climatic conditions, whether from light or from biological, physical, or chemical factors. The main goal of research into historical textile degradation and the need for restoration is to preserve the object and prevent its further destruction. The task with the greatest responsibility is to effectively clean objects of heterogeneous composition that contain fabrics combined with leather parts, bones, animal skin parchments, or wooden and metal parts. Before cleaning, proper material investigation needs to be performed in order to determine the morphology,

chemical and physical composition, and all other properties of the materials investigated. Without proper materials characterization, it is not possible to preserve valuable objects. Only after obtaining a full and deep insight into the composition of materials can historical items be cleaned, conserved, and restored appropriately. For example, after characterization and cleaning, damaged textiles often need to be attached to a material of similar structure and color. In addition, during the restoration of significant works of art, it is necessary to replace the missing parts and reconstruct the material in a completely identical way, that is, using the techniques originally applied and using similar embroideries and colors. All this is impossible without chemical analysis and characterization.

The characterization of historical materials that are highly valuable works of art with the goal to return them to their original condition is a very time consuming and arduous task, but it is an important step in preserving cultural and historical heritage. Methods chosen in the characterization depend upon material properties and analytical tasks. Therefore this book presents the most prominent methods of historical textiles characterization, as well as different real complex case studies which combine the investigation of textiles with metals, organic binding media (resins, waxes, and proteins), mummies, jewelry, and so on by using different methodologies for characterization.

# ACKNOWLEDGEMENTS

This book is dedicated to my precious teacher(s) Gyurme Chokyi Sengye Rabjam Rinpoche and Dilgo Khyentse Yangsi Rinpoche, who are an eternal inspiration in becoming a better person, and to my first teachers, mother Renata Penzar and father Josip Penzar, as well as to all mentors and professors I was privileged to work over many years until now: Prof. Ilse Steffan from the University of Vienna in Austria, Prof. Lidija Ćurković, Croatia, Prof. Ljerka Bokić and Marija Kaštelan-Macan from the University of Zagreb, and many others.

Many results could have not been discovered without the efforts of multidisciplinary teams and collaboration with dear coworkers from different institutions and universities, so my gratitude goes to all of them.

My deepest gratitude goes to the people who are the closest to my heart, to Jure Rezić and Ernest Meštrović, whose patience and support helped me during writing this book.

Last but not least, this book is for you, dear reader, I hope you will find it useful, providing advice, support, and inspiration that will help you in your everyday work.

# CHAPTER I

# HISTORICAL MATERIALS AND DEGRADATION

## 1.1. Historical textiles

Although the original term “textile” comes from the Latin word *textere*, which means *to weave*, today the term is used for fibers and for all products made of them by any processing technology, that is, spinning, weaving, knitting, lace, release, needlework, and so on. This means that the term textiles includes all linear and flat textile products and products made from them. Recently, the word *textiles* has been introduced as a synonym for textile [1]. The production of textiles in the history of humankind dates back to the beginning of civilization and is conditioned by the primary human need to find protection from the cold. Cotton, wool, linen, silk, and other textiles of natural origin were used in ancient Egypt, silk production was mentioned in China as early as 3,000 BC, and carpets over 2,000 years old have been found in Asian tombs [2].

Today, tapestries, embroideries, folk costumes, church items, toiletry bags, fans, hats, wallets, flags, and many other decorative and artistic items are often the subject of various restoration and conservation interventions. During restoration works on historical textiles, it is necessary to first determine the composition of the textile material, which may be of plant or animal origin (wool, hair, silk, cotton, linen, jute, hemp, grass, nettle), and then it is necessary to determine all the other components on the material (like metal components or other items, Figure 1). Animal fiber is mainly composed of protein and is more resistant to destruction than plant fiber, which is formed mainly of cellulose. Flax and cotton, for example, are very susceptible to the action of bacteria in humid conditions and rarely remain preserved in archaeological sites.

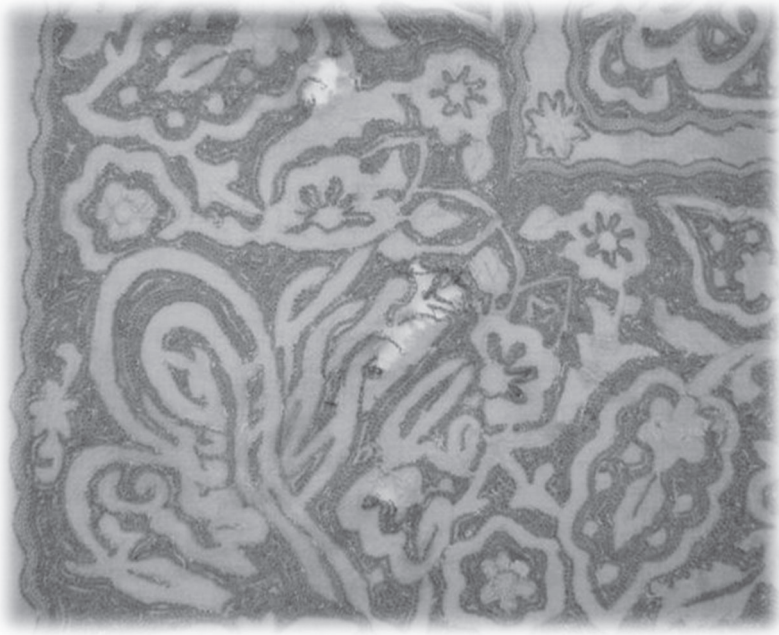


Figure 1.1. Historical textile before cleaning, analysis, conservation, and restoration.

All textile materials are subject to deterioration under the influence of various factors, such as light, insects, microorganisms, and dirty air, which individually or together cause significant losses in the strength of the material and its elasticity. Due to the loss of strength, textile materials in many cases are no longer able to function in their original purpose, for example, as a support for the painted layer of a work of art. “Outdated” textile backings are a big problem for restorers, who in the past often resorted to dubbing. Nowadays, however, this process is no longer considered satisfactory. Since the early 1970s, targeted research has attempted to examine the various causes of damage and to develop new procedures for the conservation and restoration of textile media [3].

Oxygen in the atmosphere affects all organic matter, including textiles. In contact with atmospheric oxygen, the organic matter in the canvas oxidizes. The process is unstoppable and at best can be slowed down. The oxidizing fabric loses its elasticity and becomes brittle, a process that is further accelerated by contact with drying oils (e.g., linseed or poppy oil) that absorb large amounts of oxygen during the “drying” process.

Prolonged exposure to normal atmospheric conditions will cause the decay of the textile material. The rate of decay varies depending on the environment and the nature of the material itself [2, 3, 4, 5].

The main factors that accelerate the decomposition of textile material can be classified into three groups:

**1. Organic substances:**

Since the textile is of organic origin, it is susceptible to attack by moths, bacteria, mold, and fungi that require a high degree of relative humidity or even condensation. In the case of decomposition of cellulose by the action of microorganisms, the fibers lose strength and elasticity, become brittle and disintegrate over time. Decomposition is greatest in situations that favor the growth of these organisms, such as moisture, heat, stagnant air, and contact of these materials with nutrients.

**2. Physical factors:**

Excessive heat causes drying and exposure to ultraviolet radiation causes weakening of the material, as well as photochemical decomposition of the underlying dyes. These factors are particularly harmful when high atmospheric humidity and pigments act as catalysts and accelerate the decomposition of textiles. The oxidation effect has UV and visible light, as well as electromagnetic radiation of the visible and ultraviolet part of the spectrum, which leads to photo oxidation, so UV filters are used for protection. In the case of physical factors, the influence of moisture is also very important. Like any organic matter, textiles rot and decompose under the influence of moisture. Of all the species, cotton is the most resistant material, but only if it is constantly in water, as otherwise it decays even faster than other types. Moisture resistance is increased by careful cleaning of the fibers and removal of natural adhesive materials. The greatest difficulty and consequence of exposure to moisture on textiles is created by shrinkage during wetting, and later (greater or lesser) stretching during drying. The problem is the harmonization of individual materials that often behave in opposite ways in the same time interval; as the textile material tightens, the preparation stretches, and vice versa. The preparation contains binders prone to swelling, such as glue, chalk, and bolus (inorganic binders), while the fibers of the fabric swell by receiving moisture. As a consequence, when water condenses with moisture on contact with a textile substrate, the swollen glutoline adhesive creates a gel-like mass that has only limited mechanical strength. Stresses occur that

can cause the painted layer to separate from the textile substrate. Wet cloth is also sensitive to greater cold—if wet fibers freeze, they become brittle. Ice, which has a larger surface area and volume than water, stretches the fibers from the weave.

**3. Chemical factors:**

Metals in direct contact with the canvas catalyze oxidation, which in old paintings is clearly visible at the edges over which they are stretched to the sub-frame (stretching edges), leading to a loss of color and corrosion [6]. Ocher preparations containing iron contribute to the accelerated deterioration of textile material. Exposure to harmful gases can also weaken the textile material. In some cases, these gases can interact with other substances in the atmosphere to turn into acids, which are the main cause of the decay of some textile materials. It should be noted that our civilization and industrialization contribute to the process of the accelerated decay of materials. Inorganic acids that enter the environment in exhaust gases (sulfuric and carboxylic acid) have a corrosive effect on cellulose. Depending on the location and equipment, the air in a museum or room where art objects are exhibited or stored also contains sulfur dioxide and dust particles. Sulfuric acid formed from sulfur dioxide destroys fibers. Dust particles that settle on the back of the material or in the gaps between the textile carrier and the sub-frame or coating accelerate the decomposition process. The microclimate of the space is very important in this process: because the thickness of the decorative frame in paintings or museum exhibits leaves a small air space between the wall and the museum object, there is a gradient in temperature and humidity. As a rule, the temperature behind the object is lower and the humidity higher. Climatic “inter-spaces” cause water to diffuse through the textile substrate and the image layer. This can cause cracks, separation of layers, and deformation. Patches on the canvas, inscriptions on the back, and similar materials increase the resistance to diffusion, which is one of the possible causes of their visibility on the face of the painting [3].

## 1.2. Textile fibres on historical materials

The fibers that make up the fabric should be identified before any processing of the material begins, especially if stain removal is required. Physical tests such as incineration (dry distillation) allow the rapid identification of the presence of animal fibers that do not burn but are extinguished with the rest of the clean charcoal, and during which a

pronounced smell of burnt hair is created. Fibers of plant origin burn easily, into the fine ash. Many fibers can be easily identified using a microscope. Animal hair, for example, is identified thanks to its cuticles and medullar cross-sections. Simple dyeing tests allow conservators to distinguish between different types of fibers.

According to their structure, all fibers are polymeric substances—chain macromolecules made of various polymers that can be formed either naturally (natural polymers such as cellulose, protein, or natural rubber) or synthetically in industrial plants. Textile materials most commonly found in archaeological sites are natural polymers of plant or animal origin: linen, cotton, hemp, wool, and silk.

The size of the cellulose macromolecule is expressed by the average relative molecular weight and the average degree of polymerization. During plant growth, cellulose macromolecules orient themselves in parallel along the fiber axis and come to a close position, whereby the hydroxyl groups of adjacent chains are cross-linked by hydrogen bonds and thus form crystal regions in the cellulose fibers. In addition to cellulose, plant fibers also contain lignin in woody fiber ingredients, wax, starch, sugar, and proteins. All impurities except wax, negatively affect the durability of the material. Processing, combing and rinsing remove woody ingredients as well as natural adhesives, which increases the quality of the fabric.

**Flax** is a natural cellulosic fiber obtained from the stem of a plant originating from the *Linaceae* family, and which blooms with small blue flowers (Figure 1.2).

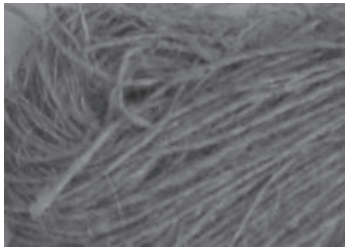


Figure 1.2. Flax natural cellulose fibers for historical textile restoration.

There are about 200 different species of this plant; the most important plant of the species for obtaining fiber is *Linum usitatissimum*. Flax fibers contain 65–90% cellulose and impurities: pectin, wax (which positively affects the fiber's resistance to moisture), starch, sugar, protein, lignin, and so on. This plant fiber is resistant to moderate alkaline conditions due to its

cellulosic composition, but is easily subject to damage in an acidic medium. Strong alkalis decompose internal pectin at higher temperatures, so the technical fiber decomposes into elemental properties [1]. Flax fiber is usually lighter in color, somewhat greenish gray. The finest types of linen are lighter, silvery yellow in color. Under standard conditions, it contains 12% moisture. By receiving moisture, the fibers become thicker, shorter, and firmer. Linen canvas is the highest quality type of painted surface carrier [3]. Moisture penetrates easily inside the linen material and causes changes in both the dimensions and weight of the material and the overall strength. Flax does not absorb dyes very well and is usually used in the bleached or unbleached state [8].

**Cotton** is a plant fiber obtained from the seeds of a plant of the genus *Gossypium*, of the family *Malvaceae* (Figure 1.3). According to its composition, cotton is almost pure cellulose (82–89%, Figure 1.3) with a lower percentage of wax (0.4 to 0.9%), pectin (0.6 to 1.1%), protein (1.0 to 1.8%), organic acids (0.5 to 0.9%), and minerals and ash (0.6 to 1.5%); 7–10% moisture is absorbed on the fiber. This fiber is the widest and most widely used textile fiber in the world. Historical sources state that cotton textiles date from around 5800 BC in the area of present-day Mexico. In Europe it has only been used since the Middle Ages, where it was introduced by the Arabs via North Africa and Sicily.

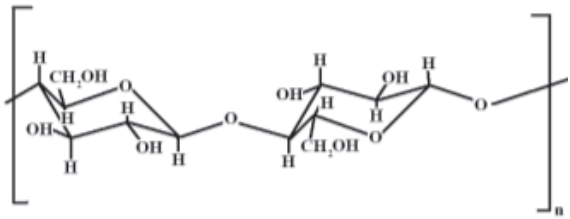


Figure 1.3. Chemical structure of cellulose polymers.

As the cotton does not have sufficient elasticity, the cotton cloth remains loose after stretching. An important property of cotton fabric is the fact that it tightens when wetting and applying the preparation, but stretches when it dries.

Figure 1.4. Cotton natural cellulose fibers for historical textile restoration.

Moreover, cotton fibers can also tolerate moderate alkaline conditions, but change greatly under the influence of acids. It does not transfer moisture like flax and has pronounced absorption properties, thanks to which it also has the property of good dye uptake [3].

Natural fibers of animal origin (wool, hair of various animals, and silk) are made of proteins whose macromolecules are composed of amino acids (Figure 1.5).

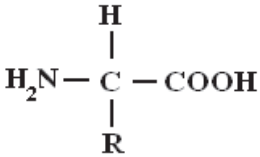



Figure 1.5. General formula of α-amino acids.

**Wool** is a natural protein fiber obtained from the fleeces of sheep. It is made up of the protein keratin (the most common amino acids are serine, glycine, leucine, arginine, glutamine, tyrosine, and cystine) [1]. Wool fibers better absorb moisture and receive dyes from plant fibers due to both their chemical and morphological structure. Wool fiber is not a solid fiber, and it is significant that in its wet state it loses even more strength (Figure 1.6).

Figure 1.5. Wool as natural protein fibers for historical textile restoration.

**Silk** is a thin and shiny natural fiber obtained by un-wrapping the cocoons of silkworms. We distinguish between two types of natural silk, cultivated and wild silk, which have almost identical chemical composition: they contain 70–82% fibroin, 18–30% sericine, 0.1–1% fat and waxes, and 1–1.4% pigment and ash [1]. Silk is as strong as steel wire of the same diameter [10] but is very sensitive to light and will be damaged if exposed to ultraviolet light.

The fabric that serves as a mechanical carrier of an image or as a base for another work of art (flags, banners, etc.) affects not only the durability but also the appearance of the work of art. The chemical and physical interactions of the components of the system used should be taken into account [11]. We can reliably come to a conclusion about the mutual chemical influences of different painting materials on the basis of knowledge about the components present on the textile material, so the analysis of these substances is very important.

In the case of conservation and restoration work, detailed documentation is first prepared on the material that needs to be processed. It is necessary to make an analysis of the material itself so that it is subjected to analytical procedures: after the physical-chemical analysis, the degree of damage to the external and internal structure of the object is determined. The structure of the tissue and the nature of the changes are then examined. Yarn analysis is performed and the condition of the object is determined with the help of micro photographs. Art and technical forms and elements in binder, decoration, and cut are examined. Proper preservation procedure includes careful documentation of the detected condition, cleaning and rinsing, replacement of necessary parts, sterilization, and proper storage and protection from undesirable atmospheric factors [13, 3].

Proper treatment of textiles usually requires the use of flat heated substrates and holders and other parts that can support sensitive textiles during rinsing, washing, and drying. Dust carries with it organic and mineral particles that settle on fabrics [14]. Alkaline reactions (from pH 7–9) caused by dust favor the development of fungi on the fabric. Most dirt can be removed from the textile material by rinsing in deionized water, with the addition of 0.4 to 1% ammonium hydroxide solution, and if necessary it is possible to add 1% neutral nonionic detergent (such as Lissapol N). During washing, some white materials can be bleached in 4% hydrogen peroxide solution. Sodium silicate and meta silicate can be used as stabilizers to be added to control peroxide decomposition. Stains caused by copper corrosion should be treated with 1–5% ammonium hydroxide solution. Stains caused by corrosion of silver should be treated with

potassium cyanide and then iodine, and washed with sodium thiosulfate. Textiles with iron stains should be washed with 5% hydrochloric acid, 5% oxalic acid, 10% hydrofluoric acid, 5% EDTA, 5% acetic acid, 5% lactic acid, and 2–10% ammonium citrate. In doing so, oxalic and hydrofluoric acids are most effective for removing iron stains from archaeological textiles. After any chemical treatment, intensive rinsing in deionized water removes all residual chemicals that can cause further destruction of the textile material over time. Textiles that cannot be cleaned with water (e.g., textiles containing water-soluble dyes) are recommended to be treated with organic solvents such as perchlorethylene or trichlorethylene and kerosene (white spirit). Although the disadvantages of cleaning with these solvents are pronounced (high treatment costs and the problem of the toxicity and flammability of these chemicals), there are many advantages to cleaning with organic solvents: they do not soften textile fibers as water does, which reduces the risk of material shrinkage and loss of shape; if they can only be treated this way, organic solvents are more efficient at removing grease, they are also mostly volatile so they dry quickly.

## 1.3 Consolidation of historical textiles

During conservation and restoration works, it is very often necessary to reinforce sensitive textile material by attaching it to a mesh base made of polyester, cotton material, glass fibers, or other material. Due to the lack of alternative solutions, until the early twentieth century weakened or destroyed textile supports were treated using fabric patches, adding edges or duplicating images. Duplication was a universal method for treating all types of damage, regardless of their size and extent. However, the discovery of modern synthetic adhesives for material restoration has led to a change in the way textile media is treated. Particularly sensitive textile materials are sometimes placed between plastic or glass surfaces. In most cases, thermo-stable adhesives (polyvinyl acetate, polyvinyl alcohol, acryloid B-72, or their emulsions) are used as a coating that is then thermally bonded to the textile. All damage and protrusions on the material must be additionally fixed with glue to prevent additional extraction of fibers. Various synthetic resins are also available to the preservative to consolidate the textile material, with emulsions and water-soluble resins being preferred because they do not dry as quickly so the preservatives have more time available to treat the material.

The resins most often recommended for the restoration and conservation of textile material are the following:

1. Polyvinyl alcohol (very soluble in water, dries with minimal shrinkage)
2. Polyvinyl acetate (V7, may shrink fibers due to resin shrinkage during drying)
3. Etulose (i.e., ethyl-hydroethyl cellulose, water soluble, very flexible)
4. Polymethacrylate
5. Acryloid B-72, 5% solution in toluene

### *1.3.1 Treatment of tears and holes*

In general, the space around a tear or hole will be stretched and deformed as a result of the relaxation of mechanical stress. Before this area is stabilized, it should be leveled and when this is done, depending on the extent to which the canvas is stretched, the edges of the crack when joining will overlap. The tear is then stabilized with Japan paper, smoothed and further treated. If the textile backing is stretched too much, the overlap is removed with a scalpel. There may be tears on the materials with which the edges can no longer be joined. After leveling between the two sides of the tear, a hole/gap remains. In extreme cases, the restorer will try to tighten the shrunken canvas along the tear with weights and wetting.

The process is not entirely simple as it can lead to the formation of fine cracks in the painted layer. When the edges of the tear are placed together again, they are fixed on the front with Japanese paper or sewn on the back. The tears are restored by sewing the torn threads, while the holes are filled with a piece of cloth. In case of large damages after the treatment of tears and holes, the textile material must be duplicated. Fabrics suitable for patches are those whose structure corresponds to the original wearer. Gauze and old canvas can also be used for patches. The patches are glued with glutoline glue, a mixture of flour and water, wax, lead white, synthetic resin or wax glue.

*Maruflage* is a term used today for a method of joining a textile material to a solid surface. *Maruflage* is used when there is great damage to textile carriers, unusual painting shapes, or protection from damage and deformation of the painted layer is required. Rigid supports more effectively stabilize a textile item made of duplicating material. Duplicate materials should be stable as they are exposed to light and air pollutants. They should be as strong as possible, possess isotropic characteristics (spread and contract equally in all directions), show minimal reactions to changes in humidity, and blend well with adhesives. Each duplicating cloth must be washed, leveled and free from irregularities. To flatten it, it

must be tensioned on a frame that is larger than the original image. These canvases usually weigh 300–450 g/m$^2$. Canvases were the only materials for duplication and patches until the 1970s [3].

### *1.3.2 Disinfection of historical textiles*

For successful treatment against moths and insects, infected textile materials should be placed in closed containers containing thymole crystals. The crystals should be evaporated so that the container is held above the bulb, and then a 0.5–1% solution of Lysol should be applied to the textile material by spraying. This treatment will remove most of the problems. Carbon disulfide can also be used instead of thymole crystals. The disinfectant solution can be prepared in the laboratory by mixing a 0.1% solution of Dowicide 1 (orthophenyl phenol), 68% ethanol, and 30% deionized water. This solution is effective for removing most bacteria, fungal spores, and surface molds.

Fungicides are used to control fungal tissue diseases. It is very harmful to store textile items in plastic bags and bags in damp rooms. The item can be exposed to protective chemicals in several ways: by immersion in a bath or, for example, by spraying objects. In addition, protection can be carried out by heating the object at a temperature of 60–70°C for about 30 minutes or by disinfecting with an IR lamp.

### *1.3.3 Preservation and protection of historical textiles*

Textile materials should be stored in conditions that limits exposure to atmospheric pollutants and ultraviolet rays. Relative humidity should be a maximum of 68% because an environment with relative humidity above 70% encourages the appearance of moths. Ideally, textiles should be stored in dark rooms with a low temperature of $10^0$C and low relative humidity (<50%). All rooms should be airy, well ventilated, and clean and well and properly lit. Dust should be removed from the room where the items are located. Moths and other insects should be repelled using para-dichloro-benzene inside the warehouse. This is very important for all woolen materials [15, 5].

## 1.4. Binding media on historical materials

The bonding material is the material that serves to strengthen the colored pigment and inorganic filler on the material, thus creating a solid and durable surface [2]. Each coating must meet many requirements, the

most important of which are the requirements for transparency of the coating, glossy or matte appearance, and appropriate protective properties [10]. Various natural materials meet the necessary conditions and have historically been used as a binder: proteins, carbohydrates, resins, and waxes and mixtures thereof. The true composition of a complex binder was often a closely guarded secret of the individual artist. Glues obtained from animal or fish organs, skin, or bones have been mostly used as binding materials on paper and as substrates in paintings [4]. Egg white was used to lighten the manuscript and as a binder for gold leaf [16], and egg yolk as a binder for tempera [2]. Casein can be found on coatings of colored furniture, textiles, and wall paintings [4].

**Carbohydrates** are organic molecules that are very widespread in nature, and are therefore used as binders, adhesives, and additives in all water-soluble art materials. The most commonly used are vegetable gums (gum Arabic, gum traganath, cherry gum), simple sugars and honey, or dextrin.

**Waxes** contain chain carbohydrates, acids, alcohols, and esters. They can come from a variety of sources: plants (carnauba wax, candelabra, or Japanese wax), animals or insects (beeswax, spermaceti, lanolin), and minerals and fossilized materials (ceresin, earth wax, or paraffin). They are used as waterproof materials, binders, and fillers in paintings and other works of art [4].

**Resins** are dried and hardened secretions from plants or trees that are made of isoprene parts and can be: monoterpenoids (turpentine oil, camphor, rose oil), sequiterpenes (shellac, elemi), diterpenes (canada balsam, rosin, sandarac, copal, kopaiba) or triterpene resins (dammar and mastic) [18]. Resins differ greatly in their properties such as appearance, odor, hardness, solubility, color, and color fastness [2]. They are mostly used as coatings on paintings and furniture, musical instruments, and gilding, or as components of complex bonding materials [4, 19, 20].

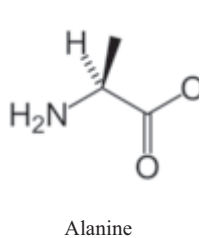
**Proteins** are organic molecules of high molecular weight, greater than 10,000 Daltons. They consist of sequences that form 22 amino acids interconnected by a peptide bond, which are characteristic of each protein. Proteins can be broken down into amino acids by hydrolysis [17].

**Protein binders** have been used since the time of ancient Egypt. Amino acids appear as monomeric units of protein in many natural materials found (eggs, glue, and casein) that have been used as a binding material. In order to be able to determine the amino acid composition of a protein, it is necessary to break the polypeptide chain by hydrolysis. Although this results in partial destruction of the indole ring in tryptophan and loss of ammonia from the side chain of amide groups in glutamine and

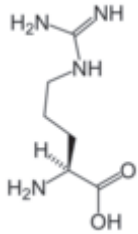
asparagine, and significant formation of glutamic and aspartic acid, it still allows the protein binder to be determined on the basis of the identification of other characteristic amino acids.

Amino acids are weak carboxylic acids that contain an amino group (-$NH_2$) [24]. Only those amino acids in which the amino and carboxyl groups (-COOH) are attached to the same carbon atom are biologically important [8]. Amino acids serve as starting materials for various other compounds needed by the body (enzymes, hormones, pigments) [25]. Essential are those that the body not only can synthesize but also must receive through diet [26–29].

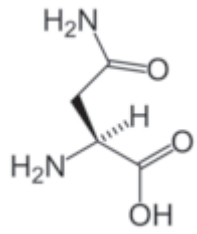
Amino acids are classified according to the position of the amino group on α, β, and γ amino acids. Amino acids that make up proteins can be divided into three different groups: amino acids that have polar but non-ionized side chains (glutamine, serine, threonine, tyrosine, and cysteine), amino acids that have acidic side chains (aspartic acid and glutamic acid), and amino acids that have basic side chains (lysine, arginine, and histidine) [30]. The chemical reactions of amino acids correspond to their functional groups: acylation (typical amino group reaction) and esterification (carboxyl groups). All amino acids (Figure 1.6) are amphoteric in nature, and their mutual differences in acid-base properties are very important for the functioning of proteins and peptides, as well as for the analysis of amino acid mixtures that we want to separate due to their proton-donor and acceptor properties [30].



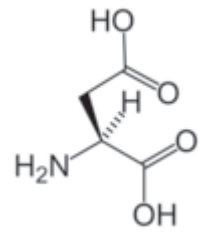
Alanine



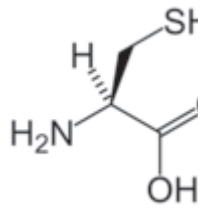
Arginine



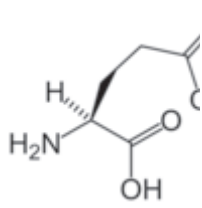
Asparagine



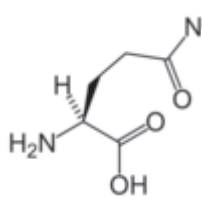
Aspartine acid



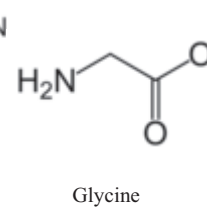
Cysteine



Glutamin acid



Glutamine



Glycine

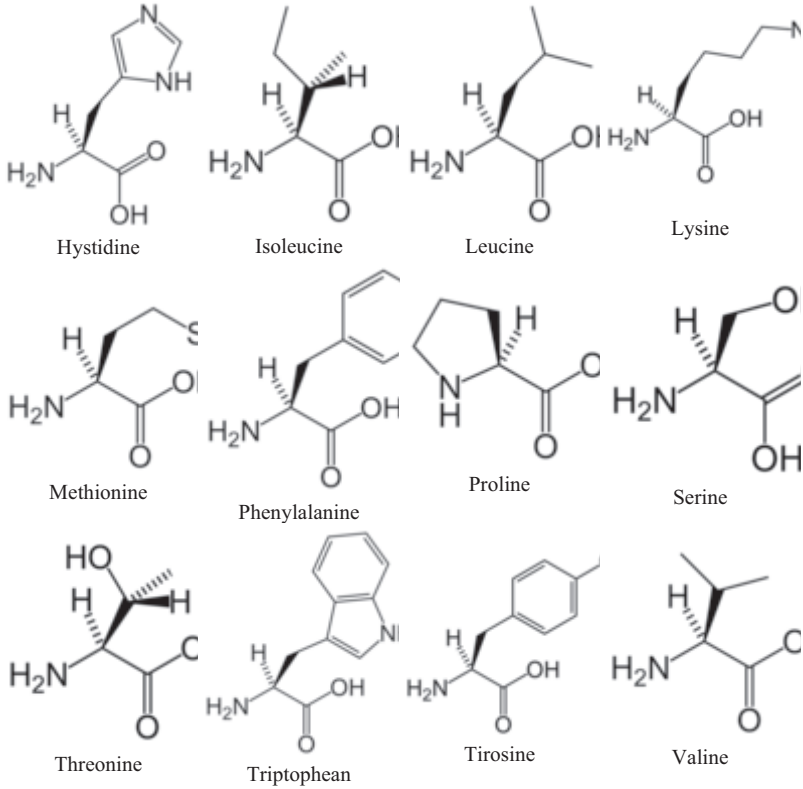
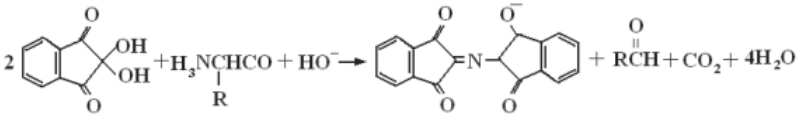



Figure 1.6. Aminoacids detected in historical materials from protein binders.

The presence of amino acids can be detected by the formation of a purple color with the reagent ninhydrine, which is often used to detect fingerprints [30]. All primary α-amino acids produce the same purple component as is presented in the following reaction:




Amino acid analysis is often performed today by ion exchange chromatography based on acid-catalyzed hydrolysis of peptide-linked amides [30–39]. The peptides were hydrolyzed by heating in 6 M

hydrochloric acid for 24 hours. The resulting mixture of amino acids is separated by ion exchange chromatography: at the outlet of the column they are mixed with ninhydrin and, depending on the intensity of the color or signal, are determined electronically. Unknown amino acids are identified by comparison with reference materials. This procedure can be performed automatically in an amino acid analysis instrument that has excellent sensitivity and can determine peptide samples in amounts of 10–5 to 10–10 g [30, 40, 41]. In addition to ion chromatography, paper chromatography [42, 43], inverted-phase thin-layer chromatography [44–48], high-performance liquid chromatography [49–53], and thin-phase thin-layer chromatography [44, 54–57] are of great importance in the analysis of amino acids and proteins and other analytical methods [58–61].

In addition to natural binders, which in history could only be used, today on historical textile materials can be found mixtures with new materials used in restoration and conservation work. From a conservation-restoration point of view, it is very important which bonding material is used on the textile material. Drying oxidizing oils act on textiles like water; there is shrinkage, but also darkening and partial transparency. When the oil dries, the canvas spreads again, but does not lighten—it becomes yellow-brown and loses transparency. As it ages, it darkens even more and transmits tanning to spreads. At the beginning of the drying process of fatty oxidizing oils, the oil absorbs oxygen and moisture from the air and increases the volume, which at this stage causes the fibers in the fabric to expand and shorten. In the next stage, it releases moisture and reduces the volume, leaving the fibers unevenly elongated. The drying process can take more than 10 years. Not all fatty oxidizing oils act equally on canvas; more harmful are those that are poorly cleaned, hot pressed, and slow drying. Linen is the most resistant to the influence of oil, while jute is not resistant due to the larger amount of woody materials. Turpentine oil evaporates quickly, so it does not affect the shrinkage and stretching of the fibers in the fabric. Alcoholic varnishes have the least harmful effect on canvas, which is why they are often mentioned for the protection of the reverse of a painting, but they have the disadvantage of yellowing over time [22]. Natural fabrics have excellent hygroscopic properties. The fiber in the fabric contains between 6 to 12% moisture. As moisture is lost, the fibers stretch and become loose. At a relative humidity of 20% to 95%, untreated canvas will shrink to 6.5%, while glue-impregnated canvas will spread between 1.5% and 5% [23, 3].

A list of binding media on historical materials is presented in Table 1 of the appendix at the end of this book.

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# CHAPTER II.1

# METHODS FOR THE CHARACTERIZATION OF HISTORICAL MATERIALS: SPECTROSCOPY AND DETERMINATION OF CHEMICAL COMPOSITION

## 2.1. The elemental composition of textile fibers

Historical textile materials are made of cotton, flax, or hemp and contain different elements. Their content varies significantly between many parameters, such as the time of the year of production, the soil, the amount of water used during producing, and, particularly, the chemical and physical parameters used [1–7]. The elemental composition of textile fibers is determined by using selected instrumental spectroscopic methods, such as flame absorption spectrometry (FAS or AAS), graphite furnace absorption spectrometry (GF-AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), or mass spectrometry (ICP-MS), to name only a few. Nevertheless, in order to obtain a full insight into the elemental composition of historical textile materials, the first step is to transform them from solid form into a liquid sample that can be introduced to the spectrometer. The digestion of materials can be performed by using wet digestion methodologies, the microwave digestion system, or dry mineralization procedures, as is shown in Figures 2.1, 2.2, and 2.3, respectively.

Figure 2.1 presents the protocol for the open system wet digestion of historical textile fibers. The sample is weighed (0.5 g) into the laboratory glass, heated on the hot plate with the addition of 10 mL of concentric nitric acid and 10 mL of hydrogen peroxide. The solutions were evaporated until dry and then diluted up to 10 mL using 1M of nitric acid. Therefore, the samples were prepared in 10 mL volumetric flasks before spectrometric investigation. Figure 2.2 presents the microwave digestion protocol with nitric acid; the sample is weighed into the microwave vessel, heated under pressure up to 250°C with the addition of 7 molar nitric acid and after 20 minutes the solid sample is digested, followed by preparation in 10 mL volumetric flasks before spectrometric investigation.

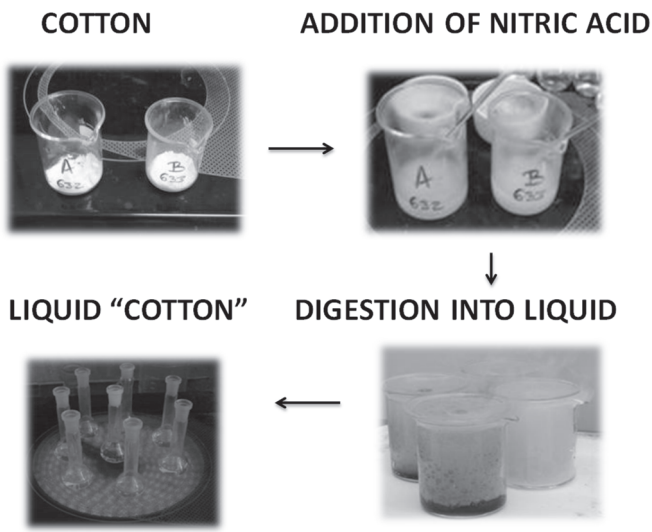



Figure 2.1. Open system wet digestion of historical samples by using 7 molar nitric acid and heating on a hot plate.

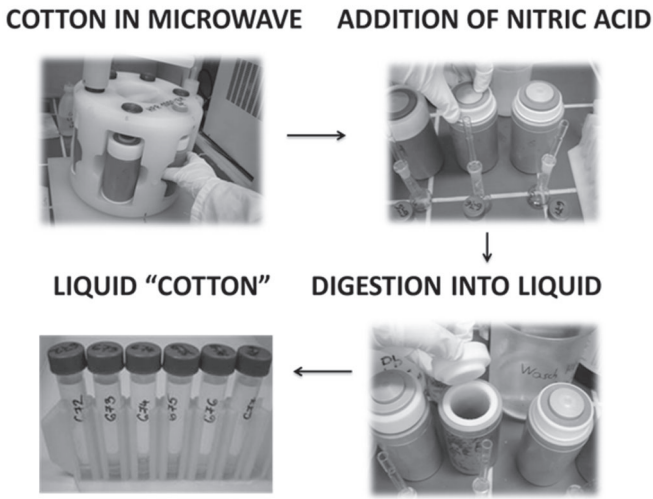



Figure 2.2. Microwave digestion of historical samples by using nitric acid and a closed microwave system.

After the digestion, the liquid analyte in the sample is introduced by a system of peristaltic pumps or an auto-sampler into the instrument, where the interaction between the source of energy (flame, plasma, or any other form) occurs.

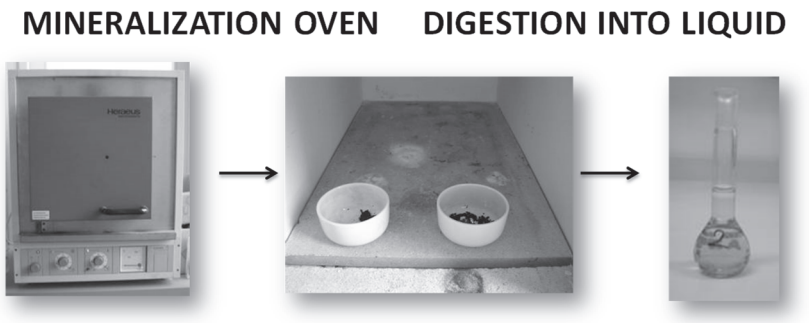



Figure 2.3. Dry mineralization of historical samples by using nitric acid and a closed mineralization oven: the sample is weighed (0.5 g) into the ceramic vessel, heated at 400°C with the addition of nitric acid and this is repeated several times until the solid sample is digested; digestion is followed by diluting the sample solution in 10 mL volumetric flasks before spectrometric investigation.

Pure textile materials, originating from cellulose polymers, are in their origin plant biological materials; thus, there are certain routine elements that are expected in samples. Table 1 lists the average elemental composition of such samples.

**Table 2.1. Macro, micro, and trace elements—the average composition of textile carriers [8].**

| *Element* | *% dry tissue* | *Element* | *% dry tissue* |
|---|---|---|---|
| H | ~ 50 | S | 0.02 |
| C | ~ 30 | Cl | 0.002 |
| O | ~ 25 | Fe | 0.0016 |
| N | 0.8 | B | 0.0016 |
| K | 0.2 | Mn | 0.0008 |
| Ca | 0.1 | Zn | 0.0002 |
| Mg | 0.06 | Cu | 0.00008 |
| P | 0.05 | Mo | 0.0000007 |

Several other elements (such as cobalt) are present in trace amounts or are important only for certain materials (like sodium, selenium, and silicone). Other elements that can occasionally be found in textile carriers (gold, aluminum, lead, mercury, strontium, cobalt, nickel, fluor, and uranium) are present from the painted cover [8, 9].

The mechanical properties of the textile carrier are influenced by the elemental composition of the yarns used for manufacturing. For example, potassium, calcium, and magnesium influence fiber processing friction: as the metal level increases, the friction properties decrease [10]. Problems are also reported during bleaching (and dyeing). Manganese and ferrous ions induce catalytic decomposition and degradation of cellulosic yarns [11].

Moreover, both manganese and ferrous ions are readily air-oxidized to compounds that cause yellowing. In addition, transition metals catalyze organic reactions and function as a mordant that strongly bonds many organic compounds to cotton [11]. For this reason, the textiles treated in those processes should be monitored for the presence of different metals (aluminum, calcium, copper, iron, magnesium, manganese, and titanium) and their presence has to be monitored by applying different methods [12].

Metals are most frequently found on textiles. The use of chromium based dyes is essential for fast black dyeing [13]. Cobalt, chromium, and occasionally copper and nickel form part of the most commonly used dyes. As a group, metal-complex dyes offer good overall fastness properties, but they are duller in shade than the strong acid and milling types [14]. Because of their wide application, metals are frequently present in waste that occurs after textile restoration and conservation together with other harmful organic solvents, which raises environmental concerns [15].

Cobalt and chromium dyes are applied particularly in dyes for wool: there is no wool mordant dye that does not use chromium compounds. It has been known for centuries that metallic compounds of aluminum, cobalt, copper, and iron are responsible for improvements in the properties of dyeing. These compounds became known as a mordant from the Latin word meaning *to bite*.

The mordant bites into the dye and the fiber and holds on. Around 1850, chromium salts were discovered, and as far as the mordant dyeing of wool was concerned, the other mordant metals fell rapidly behind. Today the chromium dye C. I. Mordant Black 11 is one of the most widely used dyes in the world [16]. Various salts of lead were used as pigments for millennia, since they give stable, brilliant colors [17].

### *2.1.1. Elemental composition of textile dyes*

In metal-dye complexes, metals have an important role: their metallic cations not only attract anions but also have electronic configurations that enable them to form complexes with a particular number of chemical groups—ligands. The maximum number of ligands is known as the coordination number (CN) and varies from metal to metal. Two metallic ions widely used in dye technology are the trivalent chromium and divalent copper ions ($Cr^{3+}$, $Cu^{2+}$) that have CN = 6 and CN = 4, respectively. Cobalt and nickel derivates are also used and have CN 6 and 4, respectively. Certain structures and configurations of individual dye molecules enable them to provide metal (mordant) ions with not only one but two, three, and even four ligands. Such dyes are known as chelating agents, from the Greek word for crab (claw) [15]. The most often applied—but unfortunately at the same time the most toxic among them—is chromium [18].

All the six common dye classes: acid, basic, direct, disperse, fiber reactive, and vat contain metals including chromium, arsenic, cadmium, mercury, copper, lead, and zinc. Usual concentrations of heavy metals in dyes are (in µg/g): 1.0 to 1.4 (As), up to 1.0 (Cd), 3.0–83.0 (Cr), 1.0–3.2 (Co), 33.0–110.0 (Cu), 6.0–52.0 (Pb), 0.5–1.0 (Hg), and 3.0–32.0 (Zn). Table 2 presents the average metal content of the selected dyes mentioned [19].

**Table 2.2. Average metal content of selected dyes in µg/g.**

| Metal | Acid dye | Basic dye | Direct | Disperse | Reactive | Vat dye |
|---|---|---|---|---|---|---|
| Arsenic | < 1 | < 1 | < 1 | < 1 | 1.4 | < 1 |
| Cadmium | < 1 | < 1 | < 1 | < 1 | < 1 | < 1 |
| Chromium | 9 | 2.5 | 3.0 | 3.0 | 24 | 83 |
| Cobalt | 3.2 | < 1 | < 1 | < 1 | < 1 | < 1 |
| Copper | 79 | 33 | 35 | 45 | 71 | 110 |
| Lead | 37 | 6 | 28 | 37 | 52 | 6 |
| Mercury | < 1 | 0.5 | 0.5 | < 1 | 0.5 | 0.1 |
| Zinc | < 13 | 32 | 8 | 3 | 4 | 4 |

Except for their important role in the formation of metal complex dyes, metals have many other functions: copper salts are used to fix direct dyes and to enhance the light fastness on nylon. Antimony improves the wash fastness properties of dyes on nylon fibers. Cationic dyes contain zinc as well as trace concentrations of mercury, cadmium, and arsenic as impurities from intermediates [20]. Zirconium, aluminum, and other salts are also used extensively in textile processing [21].

### *2.1.2. Toxicity of compounds on historical textiles*

Early in the sixteenth century, the alchemist-scientist Theophrastus von Hohenheim, known as Paracelsus, said: Only the dose makes a thing not a poison [22]. Although every substance must be considered toxic at some level (e.g., NaCl of 200 g), some substances are more toxic than others at a given dose. The determination of toxic elements of historical textile materials is very important not only for restorers and conservators, but also for the safety of the global population. Experimental studies on animals have revealed that cancers can be induced by several heavy metals including cadmium, chromium, nickel, lead, and zinc. In addition, epidemiological investigations have indicated that carcinogenesis in humans is associated with exposure to compounds of arsenic, cadmium, chromium, lead, and nickel as presented in Table 2.3 [1].

**Table 2.3: Effects induced by selected metals on organs and different systems in human body [23]**

| Element | Nervous | Cardio-vascular | Gastro-enteric | Endocrine | Immune |
|---|---|---|---|---|---|
| Al | + | | | | |
| As | + | | + | + | |
| Cd | + | + | + | | |
| Cr | | | + | | + |
| Co | + | + | + | + | + |
| Fe | + | | + | | |
| Mn | + | | | + | + |
| Hg | + | | + | | |
| Ni | | | | | + |
| Pb | + | + | + | + | + |
| Cu | + | | + | | |
| Tl | + | | + | + | |
| Zn | | | + | | |
| | Kidney | Liver | Lung | Blood | Skin |
| Al | | | + | | |
| As | | + | + | + | + |
| Cd | + | | + | | |
| Cr | + | | + | | + |
| Co | | | + | | + |
| Fe | | + | + | | |
| Mn | | | + | | |
| Hg | + | | + | | |
| Ni | | | + | | + |
| Pb | + | | | + | + |
| Cu | | | | + | |
| Tl | + | | + | | |
| Zn | | | | + | |

Today formal regulations, such as European Directive 67/548/EEC, classify the following substances as dangerous: any compound of antimony, arsenic, cadmium, chromium (VI), copper, lead, mercury, nickel, selenium, tellurium, thallium, and tin, including these metals in metallic form [23, 24]. The toxic effects of heavy metals on humans include damage to organs, disorders of the respiratory tract and lung diseases, dysfunction of the heart, blood, and blood-producing organs, disorders of the nervous system, skin diseases, and abnormalities in fertility and pregnancy. Accumulation of heavy metals in body tissues and binding to enzymes may disrupt the functioning of cells, which may also lead to the development of tumors or cancers [23].

**Arsenic** poisoning may cause disorders of the skin, respiratory tract, blood, blood-producing organs, and nervous system. It may also cause liver and kidney disorder but to a lesser extent. The compounds of arsenic may also induce cancer in the human body.

**Cadmium** is toxic even if present in trace amounts. Its chronic toxicity is manifested by *anosomia* as a result of nerve damage, kidney dysfunction, and emphysema and is a possible cause of lung cancer. Acute cadmium toxicity often leads to *pneumonitis* ranging from severe to fatal. Vomiting, diarrhea, and prostration are also symptoms of acute cadmium poisoning. It has been suggested that cadmium may play a role in the production of arteriosclerosis, hypertension, and cardiovascular disease. EU Directive 91/338EEC restricts the use of cadmium in pigments and paints.

Toxic **cobalt** over-consumption leads to symptoms such as depressed weight and appetite, anemia, polyethemia, hyperplasia of the bone marrow, reticelocytosis, and increased blood volume; several cases of heart failure have also been attributed to cobalt toxicity.

Excessive **copper** intake causes an accumulation of the substance in the liver and brain (Wilson's disease). Symptoms of this disease are nervous disorders, tremors, difficulty in swallowing, and stiff joints. It can be fatal within a few years of appearance if the symptoms are not treated. The other symptoms of copper toxicity include loss of weight, anemia, and jaundice associated with hemolysis. The hemolysis is related to a sudden release of accumulated copper in the liver. Hemoglobinemia and hemoglobinura may also occur.

**Chromium** toxicity results in poor growth and damage to the liver and kidneys. Chromate dust has been reported to be carcinogenic in humans. Chromium III can enter erythrocyte and bind the globins moiety of hemoglobin. Chromium VI penetrates biological membranes more easily than the trivalent and is therefore more toxic; it binds to the β globulins of

serum to sidrephilin. Cr VI is recognized as carcinogenic and genotoxic; it can also cause non-carcinogenic respiratory toxicity and sensitization.

**Lead** poisoning results in abdominal pain, anemia, and lesions of the central and peripheral nervous systems. The lesions of the central nervous system cause behavioral problems. The anemia is characterized by a larger than normal number of erythrocytes. Lead intoxication results in defective hemoglobin synthesis. Lead is classified as toxic to reproduction under European Council Directive 67/549/EEC.

**Mercury** is a cumulative poison because the body's ability to eliminate mercury is limited. Both elemental and organic mercury compounds can pass the blood-brain barrier and thus can induce central nervous system symptomatology. The symptoms of mercury intoxication vary from excessive salivation and diarrhea to tremors, ataxia, irritability, dizziness, moodiness, depression, and neurological disorders. *Minamata* disease in Japan is an example of an epidemic outbreak when local inhabitants consumed fish and shellfish from Minamata Bay into which a local factory had been dumping methyl mercury. Control of emissions of mercury to air, water, and soils is regulated under the EEC Directives 84/156/EEC. Directive 89/677/EEC bans mercury in paints, heavy-duty industrial textiles, and yarn.

**Nickel** is a highly toxic heavy metal that causes disorders of various bodily organs. They have been found to be carcinogenic in experimental animals and their compounds are a possible human carcinogen, as indicated by the epidemiological investigations [23]. Directive 94/27/EEC regulates the usage of Ni in objects that come in contact with the skin [24]. Nickel is the most important allergen today [25]. It affects approximately 15–20 % of woman and 2–5 % of men [26].

**Zinc** toxicity has been reported in humans after prolonged consumption, with symptoms of irritability, muscular pain, anorexia, and nausea. The symptoms of zinc toxicity in experimental animals are impaired growth, anorexia, anemia, internal hemorrhages, arthritis, and even death with a high enough intake [1]. A full risk assessment for zinc and its compounds is being conducted under EC 793/79.

To prevent those effects, different ecological textile standards are now in use: MST (Markenzeichen schadstoffgeprüfter Textilien), Öko Tex Standard (Internationale Gemeinschaft für Forschung und Prüfung auf dem Gebiet der Textilökologie), Clean fashion, Steilman, Commitextile, EC Approach, EPG (the European Product Guarantee), and the Ecomarc Scheme [27]. A number of other labels like MUT, GUT, GUW, TOX Proof, and Green Cotton are also in operation [1]. Suggested limits for toxic elements are listed in table 2.4.

**Table 2.4. Limits for heavy metals suggested by different ecological standards [28–31].**

| Heavy metals *μ/gmL* | Öko Tex | EPG | Eco-tex | TOX PROOF | M.U.T. |
|---|---|---|---|---|---|
| Antimony (Sb) | | - | - | 0.2 | - |
| Arsenic (As) | 0.2–1.0 | 0.01 | 0.01 | 0.2 | - |
| Cadmium (Cd) | 0.1 | 0.005 | 0.005 | 0.1 | - |
| Chromium III (Cr) | 1.0–2.0 | 0.1 | 0.1 | 1.0 | 0.5 |
| Chromium VI (Cr) | 0.0 | - | 0.0 | 0.0 | 0.1 |
| Cobalt (Co) | 1.0–4.0 | 0.2 | 0.2 | 1.0 | - |
| Copper (Cu) | 25–50 | 3.0 | 3.0 | 20.0 | 0.5 |
| Lead (Pb) | 0.2–1.0 | 0.04 | 0.04 | 0.8 | - |
| Mercury (Hg) | 0.02 | 0.001 | 0.001 | 0.02 | - |
| Nickel (Ni) | 1.0–4.0 | 0.2 | 0.2 | 1.0 | 0.5 |
| Zinc (Zn) | - | 5.0 | 3.0 | 20.0 | 2.0 |

According to the requirements of Öko Tex, goods are controlled for pH, fastness properties, formaldehyde, carcinogenic dyes, dyes that can break down into carcinogenic aryl amines or can cause allergic reactions to the skin, extractable harmful heavy metals, and halogenated carriers or contaminations with pentachlorophenol and pesticides [28]. The textile is categorized according to its utilization into products for babies, products in direct contact with skin, products without direct contact with skin, and decorative materials [29]. The limits for heavy metals vary depending upon the degree of the contact of a fabric to the consumer's skin as well as on the toxicity of the heavy metal [30, 1]. The limits do not involve the total amount of metals present in the fabric, but the part that can be extracted with artificial saliva or perspiration solutions used in the corresponding fastness tests [31]. The extractions are carried out in liquor to a goods ratio of 20:1 at $40^0$C for one hour, and the metal content is determined by means of spectroscopical methods such as atomic absorbance spectroscopy (AAS). It is important to say that according to current standards, the use of metals and metal complex dyes is not prohibited: their abandonment would result in a loss of some important shades like turquoise or brilliant green that use copper or nickel phtalocyanates, wet fastness in dyeing of wool or polyamide (no pre-metalized and after chroming dyes), and light fastness in case of violet, blue, or navy reactive and direct dyes [28]. However, in every case where a replacement is possible, the use of heavy-metal-based dyes and their compounds should be minimized or avoided by replacing them with safer alternatives [1].

### *2.1.3. Bleaching and metals on historical textiles*

Bleaching of textile surfaces with hydrogen peroxide, a reagent commonly used for this purpose, is the preliminary step to dyeing and other treatments. This process is well suited for the bleaching of natural fibers derived from cellulose, such as cotton or linen. It has an oxidizing effect based on the occurrence of radicals that dissolve collateral substances, but not the cellulose material. The textile fibers are bleached in widely varying processing stages as loose stock, yarns, or woven or knitted fabrics. Bleaching with hydrogen peroxide has many advantages: it provides a high bleaching effect, keeps fiber quality, is a single-stage performance, and has a cleaning as well as bleaching effect. Favorable pH for this process ranges from 10.5 to 12.0. Nevertheless, it has to be emphasized that at higher pH values, the decomposition of peroxide is too fast.

The disadvantage of this bleaching reagent is a possible material degradation that is catalytically caused by radicals induced with present heavy metals. Traces of heavy metals act as catalysts for $H_2O_2$ decomposition and $OH\cdot$ radicals can damage the material since reactions 1 and 2 occur:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- \quad (1)$$

$$H_2O_2 + OH\cdot \rightarrow HO_2\cdot + H_2O \quad (2)$$

The result of the presence of radicals is fiber damage that follows the decrease of polymerization. Very low levels of heavy metals can easily decompose hydrogen peroxide as is presented in table 2.5 and reported in the literature [32].

**Table 2.5. The decomposition of 10% solution of hydrogen peroxide in the presence of metal ions.**

| Metal ions on historical textiles | Percentage of Decomposed $H_2O_2$ | | |
|---|---|---|---|
| | 1 hour | 2 hours | 6 hours |
| Without any metal | 0.8 | 1.1 | 2.5 |
| With Cu–1 mg/kg | 10.8 | 35.2 | 80.8 |
| With Cu– 5 mg/kg | 38.7 | 93.8 | - |
| With Cu–10 mg/kg | 57.4 | 96.3 | - |
| With Fe–10 mg/kg | 2.6 | 4.6 | 13.9 |
| With Mn–10 mg/kg | 1.1 | 2.4 | 18.0 |
| With Ni–10 mg/kg | 0.8 | 1.8 | 3.8 |
| With Cr–10 mg/kg | 1.5 | 8.0 | 22.2 |

Because hydrogen peroxide is a highly reactive bleaching agent, it must be stabilized. Different reagents are used to remove copper, iron, manganese, cobalt, and other metal ions during textile processing. This results in better fabric whiteness, prevention of staining due to metal deposits, and prevention of fabric damage during bleaching caused by embedded metal fragments, mostly copper or iron. For that purpose sequestrates are usually used. Those are the chemicals that bind heavy metal ions in complexes and disable undesirable effects.

The problem is the decomposition of peroxide and other bleaching agents that occurs after bleaching. In some cases, that problem has been successfully solved with the application of enzymes decomposing bleaching agents; however, in the investigated treatment these were not applied because of their extremely high price. For this reason, it is extremely important to monitor the presence of heavy metals on textile materials during processing [33].

### *2.1.4. Spectroscopic Determination of Elemental Composition: AAS, GF-AAS, ICP-OES, and ICP-MS*

Elemental composition is determined by applying different methods [34–113]. The choice of particular spectroscopic method depends on many parameters [113–16]. Those are related to the amount of the sample available for analysis, the aggregate state of the sample, its volatility, and other chemical and physical parameters, but also to the analyte properties and its concentration ranges in the historical sample. Moreover, it depends on whether we are monitoring one element (in which case AAS and GF-AAS can be easily applied) [94–96] or many elements in the same sample (in that case, multi-elemental techniques such as ICP-OES or ICP-MS are needed) [108–11]. In addition, for complex analytical questions, it is useful to couple spectroscopic methods with a chromatographic approach such as chromatography [98–102]; TLC, HPLC, and GC (into HPLC-ICP-MS, or GC-MS, GC-ICP-MS) are a particularly useful combination [112–26].

Sample preparation steps depend upon the nature of the historical textile sample, especially on the analytical questions addressed [97, 101, 103, 106, 107, 109, 114–17]. For example, a very important question is whether we need the results of all macro, micro, and trace elements that are present in the sample, or whether we need to monitor special components due to their impact on the degradation of historical samples. Moreover, in cases where a historical textile consists of different fibers (for example metal threads that are small textile filaments wrapped around

a fragile textile core), the important analytical question concerns what is present in the sample and what is causing the degradation of the fragile historical materials (Figure 2.4).

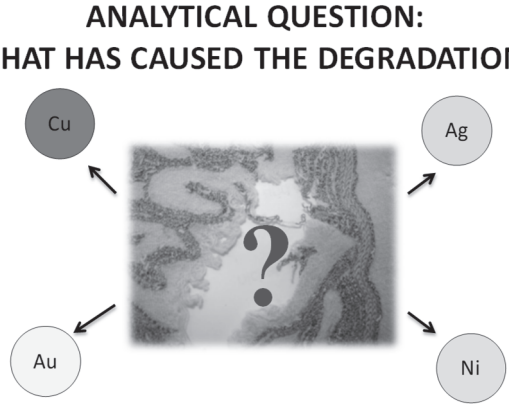



Figure 2.4. The relationship between the elemental composition of materials that are present on historical fibers and their degradation processes.

The historical development of spectroscopical methods starts in the late nineteenth century. Evidence has then began to accumulate that showed how classical Newtonian mechanics, which was completely successful on a macroscopic scale, was unsuccessful when applied to problems on an atomic scale.

In 1885 Balmer fit the discrete wavelengths of the hydrogen atom, which we now call "the Balmer series," to the empirical formula. There then followed the explanation of the hydrogen atom spectrum and the photoelectric effect from Planck, who described quantized energy in discrete packets [62]. This was a very important step forward since according to the classical physical model until then relied upon, the electrons could not circle around protons without their mutual final collision [63]. Another great step towards spectroscopic theory was introduced in 1906 when Albert Einstein applied this theory to the photoelectric effect. In 1924, he also introduced the concept of non-Euclidian space-time and explained the constant speed of light by publishing the *Special Theory of Relativity* [64].

The dilemma influenced Niels Bohr (1928), who offered his complementary principle and wrote that both particle theory and wave theory are equally valid. In addition, Planck assumed that electromagnetic energy is emitted or absorbed in discrete values, which means that energy

is discontinuous. In the case of the electrons in a free atom, four quantum numbers are used to identify the energy state of an electron: the principle quantum number $n$, the orbital angular momentum quantum number $l$, the orbital magnetic quantum number $m_l$, and the spin quantum number $m_s$.

The maximum number of electrons in the sub-shell is given by Pauli's exclusion principle, according to which no two electrons can have the same four quantum numbers. Heisenberg's uncertainty principle defines the property of nature on an atomic level. It states that the exact location and speed of a particle cannot be known at the same time, as well as that the particle's energy cannot be perfectly known. The motions of particles in a field are described by special functions called wave functions, Ψ. These wave functions are solutions of Schrödinger's equation:

$$\frac{-\hbar^2}{2\mu}\cdot\left(\nabla^2\Psi\right)+V(r)\cdot\Psi = E\cdot\Psi \tag{3}$$

where μ is the reduced mass of the particle, $\nabla^2$ a squared second derivative operator called the Laplace operator, *V(r)* the field, and *E* energy. The Laplace operator is defined as:

$$\nabla^2 \equiv \frac{\partial^2}{\partial x^2}+\frac{\partial^2}{\partial y^2}+\frac{\partial^2}{\partial z^2} \tag{4}$$

which means "the spatial rate of change in the spatial rate of change," and in this case is like acceleration. The two terms inside the brackets are "Hamiltonian": the first term of the Hamiltonian represents the kinetic energy and the second term the potential energy. The only possible wave functions Ψ that satisfy Schrödinger's equation represent stationary waves with definite energy. Because only certain solutions (states) are possible and each has a definite energy, Schrödinger's equation embodies quantization: only certain states are possible, only discrete (quantum) steps from one allowed state to another allowed state are possible and changes of state involve a definite change in energy.

Schrödinger's equation also embodies probability. The square of the electron wave function, that is, $\Psi^2$, at a particular point is the probability that the electron will be found near that point. The motion of each electron is now described by wave functions that satisfy Schrödinger's equation, plus a correction for relativity.

There are four key phenomena in which QED applies to spectroscopy: optical emission and absorption, reflection, refraction, and diffraction. Schrödinger's equation tells us that different electron structures and energies are possible for the same atom.

The atom may change to one of these other configurations by the absorption or emission of an amount of energy equal to the difference in the energies of the two configurations. A common mode for the change in atomic energy is the emission or absorption of a photon and this process is called an optical transition. A photon is a disturbance of the electromagnetic field; its energy is defined thus:

$$E = h \times v = hc/\chi \tag{5}$$

where $h$ is Planck's constant, ν is frequency, $c$ is the speed of light in a vacuum (299792458 m/s), and χ is wavelength.

Every element has its own characteristic set of energy levels and thus its own unique set of absorption and emission wavelengths [45].

It is this property that makes atomic spectrometry useful for element-specific analytical techniques.

Every element has its own fingerprint in emission energies and this makes it unique.

Some graphical presentations of different emission lines are shown in Figures 2.5 and 2.6.

Figure 2.5. Graphical presentation of the emission spectrum of hydrogen.

Figure 2.6. Graphical presentation of the emission spectrum of iron.

A spectral line is a dark or bright line in an otherwise uniform and continuous spectrum, resulting from an excess or deficiency of photons in a narrow frequency range. An emission spectrum consists of lines produced by irradiative de-excitation from excited levels; some elements generate more than 2000 lines, for example chromium generates 5000 lines and iron more than 6000 lines [65].

The intensity, I, of an emission line is proportional to the number of atoms, N*, populating the excited state. For a system in thermal

equilibrium, the population of the excited state is related to the total concentration of atoms, Na, by the Boltzmann distribution. For many elements at temperatures of less than 5000 K, the Boltzmann distribution for the ith excited state is approximated as [66]:

$$N^* = N\left(\frac{g_i}{g_0}\right)e^{-E_i/k_BT} \tag{6}$$

where gi and g0 are statistical factors accounting for the number of equivalent energy levels for the excited state and ground state, Ei is the energy of the excitated state relative to that of the ground state (E0=0), kB is Boltzmann's constant (1,3807×10-23 J/K), and T is the temperature in Kelvin.

When an atom absorbs a photon it is elevated to an excited state (Figure 2.7). This excited state will generally last for a limited time, typically $10^{-8}$ s. The atom can de-excite by emitting a photon.

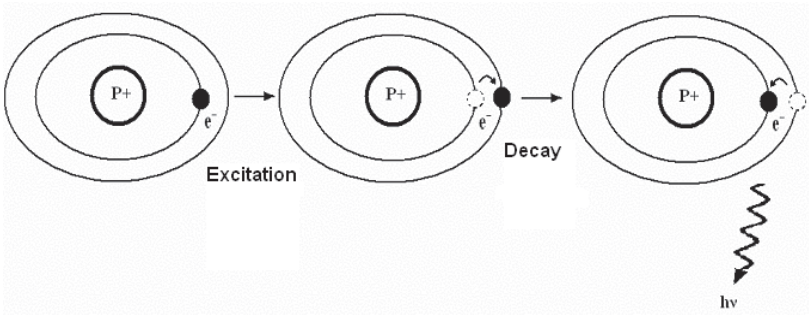



Figure 2.7. Bohr's model of the atom. As energy is adsorbed, an electron jumps to an orbital with a higher energy level. The atom may decay to a lower energy state by emitting a photon, *hv*.

Excitated states with lower energies have larger populations and, therefore, the most intense emission lines. Furthermore, emission intensity increases with temperature. The electromagnetic spectrum contains the range of all possible electromagnetic radiation, and it extends from frequencies used in the electric power grid (at the long-wavelength end) to gamma radiation (at the short-wavelength end).

### *2.1.4.1. Analytical techniques in atomic spectroscopy*

Spectrometric techniques are today the largest and the most important group of techniques in the instrumental chemical analysis of historical materials—including historical textiles. Their importance originates from their unique ability to obtain huge amounts of qualitative and quantitative information about compounds [67].

Spectroscopy can be defined as the study of the interaction between energy (originating from the specific source in the instrument), and the analyte in the sample. Spectroscopy is used for the identification of substances through the spectrum emitted from them or absorbed in them.

The type of spectroscopy depends on the physical quantity measured. For example, in atomic spectrometry, the sample is decomposed by intense heat into a cloud of hot gases containing free atoms and ions of interest. This technique is most commonly used for trace element analysis.

There are several different spectroscopic analytical methods applied in the analysis of historical samples:

In *atomic absorption spectrometry* (AAS), the light of a wavelength characteristic for the element of interest is shone through atomic vapor. Some of this light energy can then be absorbed by the atoms of that element. The amount of absorbed light energy is then measured and used to determine the concentration of the element in the sample.

In *optical emission spectrometry* (OES) the sample is subjected to extremely high temperature, which is high enough to cause not only dissociation into atoms but also significant amounts of collision excitation and ionization of the sample atoms. Once atoms or ions are in their excited states they can decay to lower states through thermal or irradiative (emission) energy transitions. The intensity of the light emitted at specific wavelengths is measured and used to determine the concentrations of the elements of interest.

One of the most important advantages of OES results from the excitation properties of the high temperature sources used in OES is that these thermal excitation sources can populate a large number of different energy levels for several different elements at the same time. The excited atoms and ions can emit their characteristic different emission wavelengths. The ability to measure emission from several different elements concurrently is advantageous. However, a disadvantage is that as the number of emission wavelengths increases, the probability for interferences that may arise from emission lines that are too close in wavelength to be measured separately also increases.

In *atomic fluorescence spectrometry* (AFS) a light source is used to excite atoms only for the element of interest through radiative absorption transitions. When these selectively excited atoms decay through radiative transitions to lower levels, their emission is measured to determine concentration, similar to in OES, and the selective excitation of the AFS technique can lead to less spectral interference than in OES. Nevertheless it is difficult to detect a large number of elements in a single run because the number of spectral excitation sources and detectors that can be used at one time is limited by the instrument performance.

Another technique related to three atomic spectroscopy techniques described above is *atomic mass spectrometry* (MS). Here, instead of measuring the absorption, the emission of the fluorescence of radiation from high-temperature-source mass spectrometry measures the number of singly charged ions from the elemental species within a sample.

This is similar to the function of a monochromator in emission and absorption spectrometry that separates the ions of various elements according to their mass-to-charge-ration in atomic mass spectrometry [68].

**Table 2.6. Classification of atomic spectral methods [68–93].**

| Irradiation source | Temperature, °C | Principle | Method title and abbreviation |
|---|---|---|---|
| **Flame** | 1,700–3,150 | Absorption<br>Emission<br>Fluorescence | Atom absorption spectrometry, AAS<br>Atom emission spectrometry, AES<br>Atom fluorescence spectrometry, AFS |
| **Electrothermal** | 1,200–3,000 | Absorption<br>Fluorescence<br>Emission | Electrothermal AAS<br>Electrothermal AFS<br>Electrothermal AES |
| **Inductively coupled plasma** | 6,000–8,000 | Emission<br>Fluorescence | Inductively coupled plasma spectrometry, ICP<br>Inductively coupled plasma fluorescence spectrometry |
| **Direct current plasma** | 6,000–10,000 | Emission | Direct current spectrometry, DCP |
| **Electrical ark** | 4,000–5,000 | Emission | Emission spectrometry with arc source |
| **Electrical spark** | 40,000 | Emission | Emission spectrometry with spark source |

### *2.1.4.2. Flame absorbance spectroscopy*

Absorbance is linear proportional to the concentration of the analyte, such is shown in the following equation:

$$A = a \times b \times c \quad (7)$$

where: *A* is the absorbance, *a* is the coefficient of absorption, *b* is the path length, and *c* is the concentration.

The sensibility of measurements is defined as the magnitude of the absorption signal that comes after the measurement of the specific sample concentration. In AAS flame absorption spectrometry (Figure 2.8) this parameter is expressed as the concentration of the element in m per liter that is needed to cause 1% of the absorption signal.

The detection limit is the lowest possible element concentration that can be determined by measuring the absorbance and observing the stability of the output signal. If the output signal is unstable, that is, there is a strong noise at the baseline, it is impossible to determine a low concentration sample. According to IUPAC, the detection limits are defined as the sample concentration that will have an absorption signal three times the noise magnitude, or three times the standard deviation of the blank. As noise makes up a significant part of the measured signal, it is not easy to pinpoint the detection limit. By definition, the accuracy obtained at the limits of detection is ± 33% of the relative standard deviation (RSD).

Atomic absorption instruments have an irradiation source that emits sharp atomic lines of the element we are determining. The source is a hollow cathode lamp that is designed to emit the atomic spectrum of the one particular element. The most common source of electromagnetic radiation is a hollow cathode lamp that is usually produced from a hollow cylinder made of a metal whose spectrum we want to produce.

For most elements, a hollow cathode lamp is a completely satisfactory source for the process of atomic absorption. In some special cases, such as testing volatile elements, the quality of the analysis is impaired. The absorption determination of such elements can be improved by using a more powerful and much more stable source such as an electrode-free discharge lamp.

Figure 2.8. Flame absorption spectrometry application for single element analysis of particular elements in the historical samples.

The optical parts of the instrument are a very important second part of the spectroscopic instrument. The aerosol sample contains particles of different sizes that come into the mixing chamber. Before entering the flame, the water evaporates, leaving solid particles that also need to be evaporated with the severance of chemical bonds between them.

Since the thermal energy of the flame causes the formation of certain chemical species, this parameter is important for process control. Some temperatures and flame properties are given in Table 2.7.

**Table 2.7. Flame properties: gas, oxidizing agent, temperature, and speed of combustion [93, 113].**

| Gas fuel | Oxidant | Temperature, °C | Maximum speed of combustion, cm s-1 |
|---|---|---|---|
| Natural gas | Air | 1,700–1,900 | 39–43 |
| Natural gas | Oxygen | 2,700–2,800 | 370–390 |
| Hydrogen | Air | 2,000–2,100 | 300–440 |
| Hydrogen | Oxygen | 2,550–2,700 | 900–1,400 |
| Acetylene | Oxygen | 3,050–3,150 | 1,100–2,480 |

### *2.1.4.3 Atomic emission spectrometry*

Atomic emission spectrometry (AES) is considered to be one of the oldest methods used for elemental analysis. The first observations of

emission were made using an alcohol flame at the beginning of the nineteenth century by Brewster, Herschel, Talbot, and Foucault. Their results were the basis for the work of Bunsen and Kirchhoff [69], which can be considered to be the real start of emission spectroscopy. In addition, to a Swedish agronomist named Lundergårdh is attributed the beginning of the modern era of flame photometry because his apparatus for elemental analysis of plants used pneumatic nebulization and a premixed air–acetylene flame [68].

AES is based on the production and detection of line spectra, which is emitted during the radiative de-excitation process of electrons (see Figure 2.7). Those go through a transition between upper excited levels and lower and ground levels, in which the electrons belonging to the outer shells of the atoms (called optical electrons) emit. A line spectrum is specific for every element and due to this the elements can be identified and quantified during the analysis: the selection of a line by a dispersive system allows the verification of the presence of this element and the determination of its concentration. An atomic emission spectrometer consists of a radiation source, a sample introduction system, an optical dispersive system, a detector, and electronics for data acquisition, processing, and editing.

The advantage of AES is in its universality and its multi-element capability: it is capable of both qualitative and quantitative analysis over a wide concentration area [34].

Radiation sources in AES methodology can be classified in two categories: sources for the analysis of solutions (flame or plasma) and sources for the direct analysis of solids (arc and spark). Commonly used flames include mixtures of propane-air, acetylene-air, and acetylene-oxygen flames, which exhibit temperatures of 2,200 °K, 2,500 °K, and 3,300 °K, respectively.

The AES process follows several steps: first, the sample in a liquid form is introduced into the flame, and after that it is desolvated, vaporized, dissociated, and atomized before being excited. Although flame emission spectrometry is today replaced by flame atomic absorption spectrometry, some low-cost systems are still commercially available for the determination of alkali and alkaline earth elements.

Plasma is a macroscopically neutral ionized gas. It has the same number of positive particles (ions) and negative particles (electrons). If a monatomic gas, $X$, is used, plasma is described by the following equilibrium:

$$X = \sum_{n=1}^{q} X^{n+1} + \sum_{n=1}^{q} ne \tag{8}$$

where $X^{n+1}$ is an ion with $n$ charges and $e$ represents an electron. Some properties of ideal gases such as pressure and volume still apply, in contrast to other properties such as viscosity and thermal conductivity, which significantly differ from those of ideal gases because of the presence of charged particles. Plasma is in a thermodynamic equilibrium if there are no temperature or density gradients for all particles [70].

In contrast to a flame, with this radiation source it is necessary to supply an external energy in the form of an electrical field in order to ionize the gas and to sustain the plasma. It will in turn transmit part of this energy to the sample for atomization and excitation.

Interestingly, the name "plasma" was given by the Nobel Prize winner I. Langmuir who produced the first plasma [71].

Different plasma systems are classified according to the kind of electrical field that is used to create and sustain the plasma:

- Direct current plasma (DCP) is obtained when a direct current field is established across electrodes.
- Inductively coupled plasma (ICP) is obtained when a high-frequency field is applied through a coil.
- Microwave-induced plasma (MIP) is obtained when a microwave field is applied to a cavity.

DCP was the first described and commercialized plasma. However, ICP is currently the most commonly used system because of its properties. In the case of ICP, a high-frequency generator (usually operating at 27 MHz or 40 MHz) is used to produce a high-frequency field through an induction coil. The power produced is of the order 700–1500 W. The power stability is a crucial parameter for avoiding any drifts in the properties of the plasma. The gas that is used to generate the plasma is usually argon, a mono-atomic element with a high ionization energy (15.76 eV) that is chemically inert.

Consequently, it emits a simple spectrum, has the capability to excite and ionize most of the elements of the periodic table, and does not form any stable compounds with the analytes. Argon is also the cheapest noble gas. It is purified from the air in which its concentration is approximately 1%. The limitation is the poor thermal conductivity of argon compared with molecular gases such as nitrogen and hydrogen. Normal argon consumption is in the range of 10–15 L/min. [34].

The plasma is created in a torch that provides electrical insulation between the plasma and the coil and confines and stabilizes the plasma for

the introduction of the sample. Because of the nature of the high-frequency field and the resultant skin effect, the energy from the generator is mainly deposited in the external part of the plasma. There is consequently a zone along the axis of the plasma where the viscosity is lower. This results in the creation of a central channel that facilitates the injection and confinement of the sample. Current torch designs make use of three concentric tubes: an outer one for confination and insulation of the plasma; an intermediate tube that serves to accelerate the plasma gas that is introduced between the outer and intermediate tubes; and the injector tube for the introduction of the sample (Figure 2.9).

As can be seen in Figure 2.9, the external tube is made of silica because of its refractivity and transparency to radiation. The observation of the plasma can be carried out perpendicularly to the axis of the plasma (radial viewing) or along the axis (axial viewing) [34]. The axial-viewed torch system has many advantages: for example, reducing interference, improving detection limits, and an achievable linear dynamic range [72].

Once the sample has reached the high temperature zone, the plasma has several functions: the first function is to remove the solvent from the aerosol (to desolvate), usually leaving the sample as microscopic salt particles. The next step involves the decomposition of the salt particles into a gas of individual molecules (vaporization) that are then dissociated into atoms (atomization).

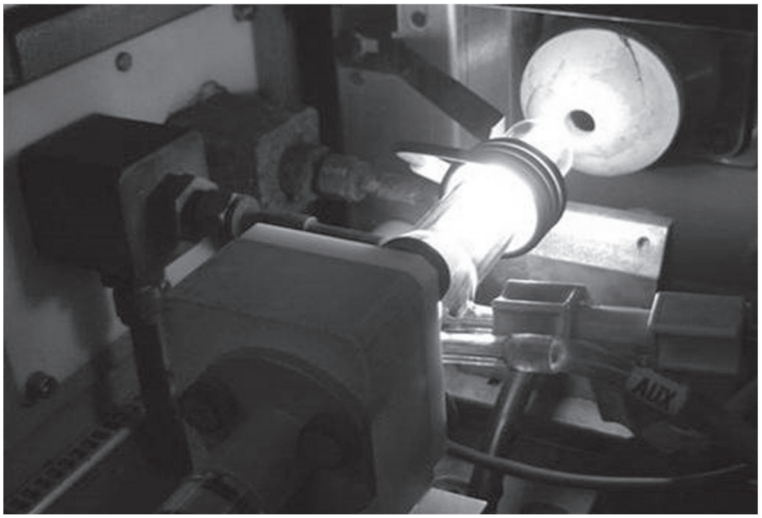



Figure 2.9. Inductively coupled plasma (ICP) torch with argon plasma, temperature regions 6,000–20,000°C.

The dissociation process in which molecular species (CaO, CaOH, KCl, LiOH) dissociate into atoms (MX ↔ M + X) can be described with:

$$K_d = \frac{n_m \times n_x}{n_{mx}} \tag{9}$$

where $n_m$ is the particle density. (Examples: BaOH, 2200 K, $E_d$= 4,7 eV, $K_d$= 2,5 × $10^{12}$ $cm^{-3}$;CaOH, 2200 K, $E_d$= 4,3 eV, $K_d$= 2,5 × $10^{13}$ $cm^{-3}$). In:

$$\log K_d = 20{,}274 + \frac{3}{2}\log\frac{M_m M_x}{M_{mx}} + \log\frac{Z_m Z_x}{Z_{mx}} + \frac{3}{2}\left(\log T\right) - 5400\frac{E_d}{T} \tag{10}$$

*M* represents molecular mass, *Z* partition function, and $E_d$ dissociation energy. It is obvious that the influence of temperature on the $K_d$ is significant:

$$Z(T) = \sum g_i e^{\frac{E_i}{kT}} \tag{11}$$

Once the sample aerosol has been desolvated, vaporized, and atomized, the plasma has two remaining functions: excitation and ionization. Ionization (M ↔ $M^+$ + $e^-$) of ionic species ($Na^+$, $CaOH^+$, $K^+$, $Ca^{++}$) is described with the Saha equation:

$$K_i = \frac{n_{m^+} n_{e^-}}{n_m} \tag{12}$$

in which $n_e$ is free electron density. (Example: at 2,500 K for K shows 50% ionization and $E_{ion}$= 4,34 eV, while for Na with 7% ionization, $E_{ion}$= 5,14 eV).

$$\log K_i = 15{,}684 + \log\frac{Z_{m_+}}{Z_m} + \frac{3}{2}\left(\log T\right) - 5040\frac{E_{ion}}{T} \tag{13}$$

All the above described processes are shown in Figure 2.10 [68].

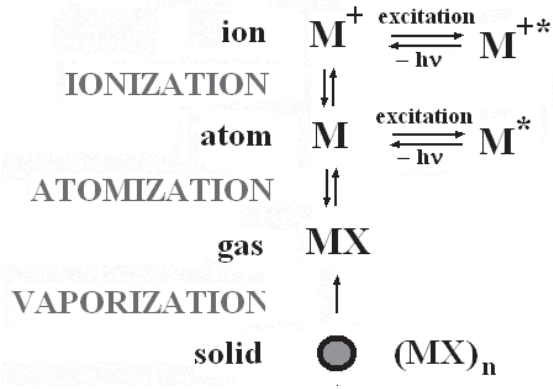



Figure 2.10. The process that takes place when a sample droplet is introduced into an ICP discharge.

While the exact mechanisms for excitation and ionization in the ICP are not yet fully understood, it is believed that most of the excitation and ionization processes in the ICP take place as a result of collisions of analyte atoms with energetic electrons.

The main advantage of the ICP over other emission sources is derived from its ability to vaporize, atomize, excite, and ionize efficiently and reproducibly a wide range of elements present in many different sample types. One of the important reasons for its superiority is in the high temperature within the plasma—the gas temperature in the center of the ICP is about 6,800 K [68].

Besides improving excitation and ionization efficiencies, the higher temperature of the ICP also reduces or eliminates many of the chemical interferences found in flames and furnaces.

According to the literature data [68], other electrical discharge emission sources (arcs, sparks, direct current plasmas, and microwave-induced plasmas) also have a high temperature and thus could be as efficient at excitation and ionization as the ICP. However, because of the ICP's combination of stability and freedom from sample matrix interferences, the ICP is a better source for atomic emission spectrometry than other electrical discharge sources. An arc is a stable electrical discharge of high current density (2A–30 A) and low burning voltage (up to 50 V) between two or more graphite electrodes. The shape of the plasma formed is related to the electrode gap and the shape, power, and composition of the sample. Sparks are intermittent, oscillating electrical discharges of high voltage and relatively low average current between electrodes. One electrode consists of the sample to be analyzed whereas

the other is usually made of tungsten. There is a wide variety of spark type that depends on the spark generator principle and characteristics.

An important feature of the ICP, unlike the majority of other emission sources, is that since the sample aerosol is introduced through the center of the ICP, it can be surrounded by the high temperature plasma for a comparatively long time (approximately two milliseconds). It is this long residence time of the analyte particles in the center of the plasma that is largely responsible for the lack of matrix interferences in the ICP. In addition, because the aerosol is in the center of the discharge and the energy-supplying load coil surrounds the outside of the plasma, the aerosol does not interfere with the transfer of the energy from the load coil to the discharge.

Although there are no interferences between the aerosol and the energy from the load coil, spectral interferences might occur. The most frequent interferences are presented in Figure 2.11, where horizontal lines present the wavelength in nanometers, and vertical lines the elements that emit the irradiation at particular wavelengths. Therefore, more emissions on particular wavelength and more interferences might be expected (if those elements are present in the same sample).

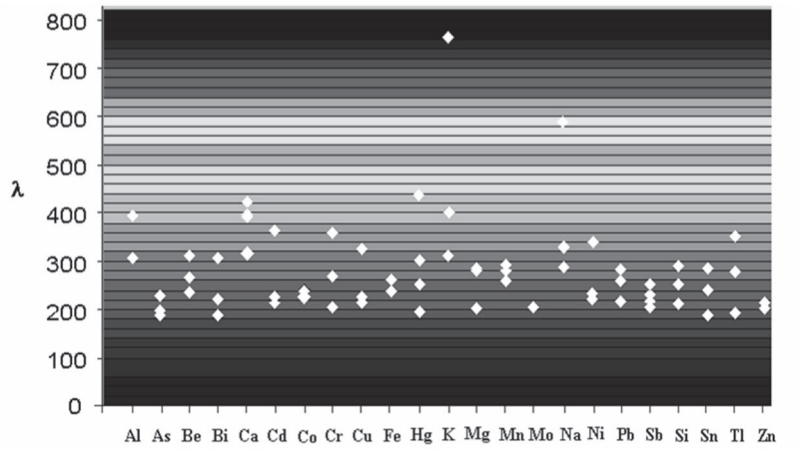



Figure 2.11. The overview of chosen spectral interferences on a combination of wavelengths in ICP.

In some other sources, as for example in direct current plasma, the sample travels around the outside of the discharge where it does not experience uniform high temperature for as long. In the arcs and sparks, the sample may commingle with the entire electrical discharge and interfere with the

production and sustaining of the discharge. These situations lead to the higher level of matrix effects and poorer stability that are often characteristic of non-ICP discharges [68].

The most important characteristics of emission spectrometry are its accuracy, precision, low limits of detection, large linear working range and absence of matrix effects. The precision (RSD) is as low as 1% or even less, and the limits of detection are in the 0.01–5 ng $mL^{-1}$ range for the best ICP OES systems (Figure 2.12). [68].

In specific cases, the interferences can be investigated by using special software programs that enable observing each emission line of each sample analyzed and comparing it with the reference materials (Figure 2.13)

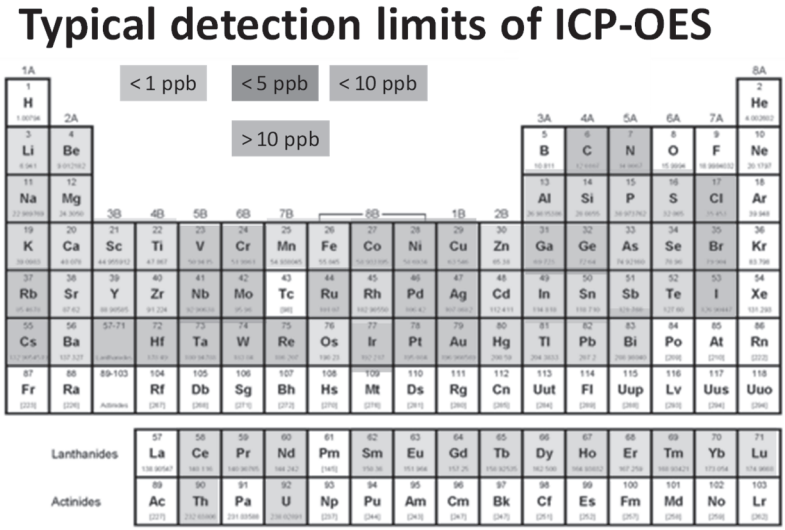



Figure 2.12. Typical detection limits in ICP-OES spectrometry.

As can be seen from Figure 2.12., the best limits of detection can be obtained for Ba, Be, Ca, Mn, Mg, and Ti. Alkali elements have relatively poor limits of detection because the temperature of the plasma is too high for them. Chemical interferences in plasmas are minimized because of the high temperature, although for elements like U, Fe, and Co it results in the emission of a line-rich spectrum that is a possible limitation of this method [68]. In other cases, in order to avoid spectral interferences, one needs to optimize the instrumental parameters, such as the nebulizer gas flow, as is shown in Figure 2.14.

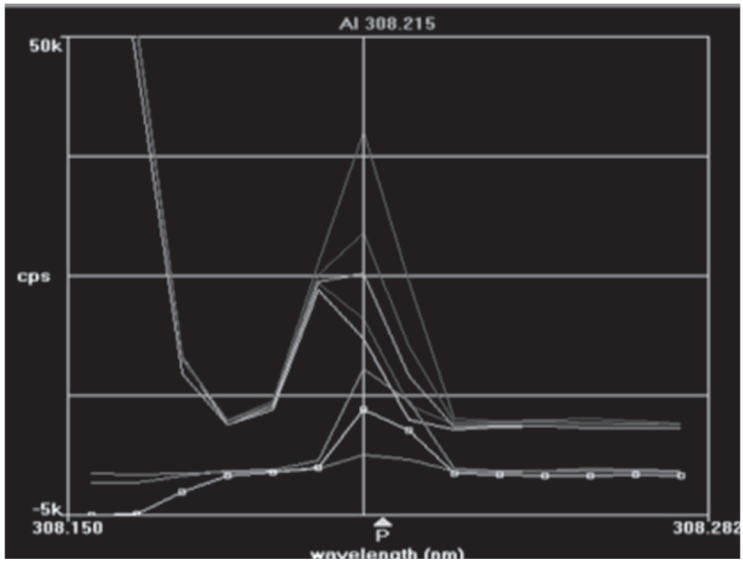



Figure 2.13. Monitoring the spectra of aluminum at the wavelength of 308.215 nm, comparison between reference materials (aluminum in standard solutions at 0.1, 0.5, 1.0, 1.45 ppm), blank solution (without Al), and sample solution of liquid cotton [94].

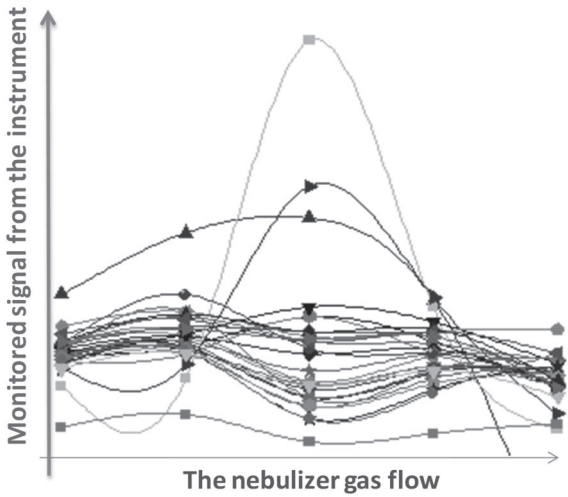



Figure 2.14. The optimization of nebulizer gas flow, monitoring the emission lines of many different elements in a sample [94].

Using a system with optimized parameters results in the best analytical performances. Among others, an important analytical parameter is the linearity range. When an analytical procedure offers a significantly larger linearity range, like ICP-OES, the sample preparation steps are easier than with other methodologies.

The linearity of ICP-OES is significantly greater than with other spectrometry methodologies. Moreover, the limits of detection are low enough to enable precise and accurate determination of micro and trace elements in historical textile samples.

The limits of detection of ICP-OES are presented in Table 2.8., and the limits of detection are presented in Table 2.9.

**Table 2.8. The limits of detection of ICP-OES with selected lines presented in bold [94].**

| Element | Recommended λ/nm | Limits of detection / working line (λ / nm) |
|---|---|---|
| Al | 396.152, 167.022 | 16.6 (308.215), 5.85 (237.312), 8.75 (396.152) |
| As | 197.197, 188.979 | 13.5 (188.979), 28.8 (193.696), 26.2 (197.197) |
| Ba | 455.403 | 1.0 (230.424), 0.5 (233.527), 4.9 (413.066) |
| Be | 313.107 | 0.2 (234.861), 4.3 (265.045) |
| Bi | - | 19.8 (190.178), 33.9 (222.825), 0.5 (206.170) |
| Ca | 317.933, 396.847 | 9.6 (315.887) |
| Cd | 228.802, 226.502 | 0.6 (226.502), 0.8 (228.802), 0.4 (214.438) |
| Co | 228.616, | 1.0 (228.616), 1.2 (230.786), 1.7 (238.892) |
| Cr | 267.716, 205.552 | 0.6 (205.560), 0.4 (267.716), 2.7 (357.896) |
| Cu | 327.396, 324.754 | 3.2 (224.700), 1.7 (324.754), 4.6 (327.396) |
| Fe | 259.940, 238.204 | 2.6 (238.204), 0.6 (239.562), 0.3 (259.940) |
| Hg | - | 4.0 (194.168), 25.8 (253.652), - (435.835) |
| K | 766.490 | - (404.721), - (310.205), 8.4 (766.491) |
| Mg | 285.213, 279.079 | 28.9 (202.582), 3.2 (279.079), - (279.553) |
| Mn | 257.610 | 13.3 (257.610), 35.9 (260.569), - (279.482) |
| Mo | 202.030 | 2.1 (202.030), 6.0 (203.844), 5.1 (204.598) |
| Na | 589.592, 330.237 | - (288.114), 105.1 (330.237), |
| Ni | 231.604, 221.648 | 2.2 (221.647), 2.6 (231.604), 6.3 (232.003), 31.31 (341.476) |
| V | - | 5.6 (270.412), 2.5 (292.402), 1.3 (309.311), - (310.230) |
| Pb | 220.353 | 30.3 (216.999), 6.9 (220.353), - (261.418) |
| Sc | - | 1.2 (337.215), 0.62 (357.253), 0.3 (361.384) |
| Se | 196.026 | 21.2 (196.026), - (203.985), - (235.435) |
| Si | 251.611 | 5.1 (212.412), 32.6 (221.667), 4.0 (251.611), 11.3 (288.158) |
| Sn | - | 6.0 (189.933), 40.5 (242.170), 10.6 (283.999) |
| Sr | 407.771, 421.552 | 11.9 (232.235), 0.1 (407.771), 0.1 (421.552) |
| Tl | 334.941 | 15.2 (190.800), 57.5 (276.787), 54.0 (351.924) |
| Zn | 206.200, 213.856 | 0.5 (202.548), 0.8 (206.191), 0.6 (213.856) |

**Table 2.9: Linearity of ICP-OES with limits of detection (LOD) [94].**

| Element | λ/nm | Linearity / µg / mL | LOD / µg/mL |
|---|---|---|---|
| Al | 308.215, (396.152) | 0.150 – 1000, (0.080 – 1000) | 0.01 |
| As | 188.979, (197.197) | 0.150 – 800, (0.300 – 1200) | 0.03 |
| Be | 313.107, (234.861) | 0.01 – 50, (0.01 – 50) | 0.0002 |
| Bi | 223.061, (306.772) | 0.200 – 500, (0.400 – 500) | 0.05 |
| Ca | 393.366, (422.673) | 0.0002 – 10, (0.150 – 2000) | 0.00005 |
| Cd | 226.502, (214.438) | 0.015 – 200, (0.010 – 100) | 0.003 |
| Co | 228.616, (237.860) | 0.040 – 300, (0.050 – 400) | 0.003 |
| Cr | 206.149, (205.560) | 0.020 – 300, (0.020 – 200) | 0.001 |
| Cu | 324.754, (224.700) | 0.020 – 200, (0.020 – 800) | 0.001 |
| Fe | 259.940, (240.49) | 0.020 – 100, (0.030 – 200) | 0.002 |
| Hg | 194.168, (187.060) | 0.100 – 500, (0.100 – 500) | 0.004 |
| K | 766.491, (404.721) | 0.300 – 800, (10 – 3000) | 0.08 |
| Mg | 279.550, (279.079) | 0.0002 – 10, (0.150 – 2000) | 0.00005 |
| Mn | 257.610, (294.920) | 0.005 – 300, (0.020 – 1200) | 0.0004 |
| Mo | 202.030, (281.620) | 0.020 – 300, (0.030 – 2000) | 0.004 |
| Na | 589.592, (330.237) | 0.100 – 500, (10 – 2000) | 0,02 |
| Ni | 231.604, (227.027) | 0.050 – 2000, (0.075 – 3000) | 0.001 |
| Pb | 220.353, (216.999) | 0.200 – 3000, (0.500 – 700) | 0.04 |
| Sb | 206.833, (187.120) | 0.150 – 500, (0.300 – 1000) | 0.03 |
| Si | 288.158, (251.611) | 0.100 – 2000, (0.050 – 800) | 0.008 |
| Sn | 189.933, (242.170) | 0.125 – 800, (0.400 – 100) | 0.01 |
| Tl | 190.800, (351.924) | 0.200 – 800, (1000 – 2000) | 0.04 |
| Zn | 213.856, (202.548) | 0.010 – 100, (0.150 – 100) | 0.003 |

Another excellent feature of ICP-OES methodology is the accuracy and precision of its measurements. In order to investigate instrumental performances, it is recommended to use certified reference materials (CRM). For example, one might use the certified reference material pure cotton and compare the results with the certified values. In this way, the accuracy of the methodology can easily be checked.

Tables 1.10 and 1.11 present such data. From those tables, it is obvious that not all elements have the same standard errors. The explanation is that all the elements that are present around the limits of detection and limits of quantification in a sample will have much worse results than micro and macro components in the sample.

The results of the certified reference materials can help the investigator during the method development. In this particular case, the results show which elements cannot be accurately and precisely determined, since they are present in very small amounts in the samples. Here, those elements are the following: cadmium, mercury, nickel, lead, and selenium.

**Table 2.10. Cotton certified reference material, analyzed by ICP-OES.** Accuracy data IAEA-V-9 mixed solution of 14 repeated microwave digestions [94].

| Element | Certified / μg/g | 95% Confidence Interval | Found / μg/g |
|---|---|---|---|
| Al | 44 | 13–53 | 43.49 |
| As | - | - | 3.84 |
| Ba* | 9 | 6–12 | 8.31 |
| Be | - | - | 0.08 |
| Bi | - | - | 0.23 |
| Ca* | 240 | 220–260 | 275 |
| Cd | 0.002 | 0.001–0.006 | 0.006 |
| Cr* | 0.11 | 0.08–0.14 | 0.12 |
| Cu* | 0.59 | 0.47–0.94 | 0.65 |
| Fe | 11 | 7–15 | 10.75 |
| K | - | - | 34.88 |
| Hg* | 0.06 | 0.04–0.08 | 0.09 |
| Mg* | 53 | 46–67 | 50.66 |
| Mn* | 0.15 | 0.12–0.21 | 0.15 |
| Mo* | 0.034 | 0.030–0.049 | 0.029 |
| Na* | 56 | 49–64 | 34.68 |
| Ni* | 0.09 | 0.07–0.18 | 0.06 |
| Pb* | 0.25 | 0.22–0.33 | 0.40 |
| V | 0.09 | 0.07–0.10 | 0.09 |
| Sc | 0.009 | 0.007–0.01 | 0.009 |
| Se | 0.015 | 0.008–0.018 | 0.022 |
| Si | - | - | 69.25 |
| Sm | 0.003 | 0.002–0.004 | 0.003 |
| Sr* | 0.65 | 0.54–0.96 | 0.65 |
| Zn | - | - | 1.60 |

* recommended elements by IAEA, others are reported for information purposes.

There are two approaches to how to improve the pure accuracy of these measurements:

- To apply another independent analytical procedure that offers significantly lower limits of detection, such as ICP-MS (presented in Figure 2.15).

- To add a standard element solution into the analyzed sample (to “spike” the sample) with the analyte of interest. In this way, the limits of detection and limits of quantification will stay significantly lower than the observed values.

Afterwards, it is important to ensure the interpretation of the obtained results is mathematically correct.

**Table 2.11. Certified reference materials: microwave digested RM#480 and LGC7162, n = 3.**

| CRM | RM#480 | | | LGC7162 | | |
|---|---|---|---|---|---|---|
| Element | Certified / µg/L | Found / µg/L | difference [%] | Certified / µg/L | Found / µg/L | difference [%] |
| **Al** | - | 4.10 | - | - | 477 | - |
| As | - | - | - | 0.28 | 0.0 | -100 |
| Ba | - | 16.26 | - | 107 | 0.96 | -9.1 |
| Be | - | 0.06 | - | - | - | - |
| Bi | - | 0.11 | - | - | 2.30 | - |
| Ca | 1700 | 1312 | -22.8 | 15300 | - | - |
| Cd | 68.6 | 0.17 | -99.8 | 0.17 | 0.19 | 11.8 |
| Co | - | - | - | 0.47 | 0.30 | -36.2 |
| Cr | - | 0.09 | - | 2.15 | 0.84 | -60.9 |
| Cu | - | 1.61 | - | - | 6.91 | - |
| Fe | 12.6 | 6.13 | -51.3 | 818 | 288 | -64.8 |
| Hg | - | - | - | 0.027 | 0.02 | -25.9 |
| K | 10.2 | - | - | 19600 | - | - |
| Mg | 350 | 176 | -49.7 | 3770 | 1906 | -49.4 |
| Mn | 4.90 | 3.86 | -21.2 | 171 | 131 | -23.4 |
| Mo | 49.5 | 0.70 | -98.6 | 0.32 | 0.60 | 87.5 |
| Na | 485 | 66.6 | -86.4 | - | 10.8 | - |
| Ni | - | - | - | 2.60 | 2.59 | -0.4 |
| Pb | - | - | - | 1.80 | 1.32 | -26.7 |
| V | - | 0.15 | - | - | 7.03 | - |
| Se | - | 0.61 | - | - | 0.54 | - |
| Sr | - | 4.29 | - | 64 | 46.98 | -31.9 |
| Sn | - | 3.59 | - | - | 0.04 | - |
| Zn | 6.90 | 6.46 | -6.4 | 24 | 21.13 | -11.3 |

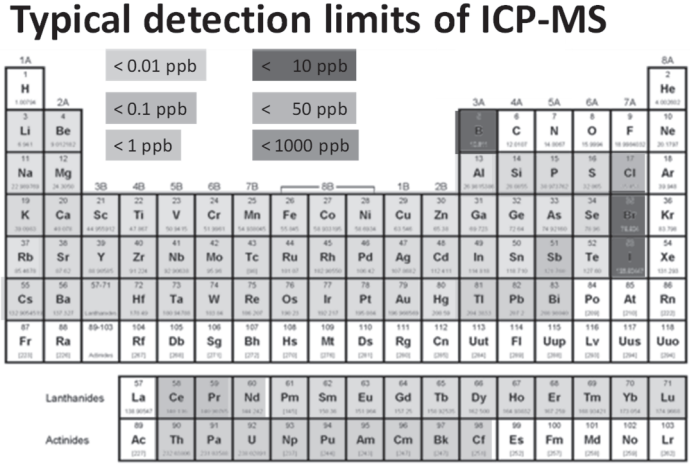



Figure 2.15. Typical detection limits in ICP-MS spectrometry.

Important criteria for selecting an analytical technique include sufficiently high limits of detection (limits of quantification), spatial-analytical working range, appropriate sample throughput, ease of interferences, user friendliness, and the available methodology.

It is necessary to know which elements are important, and at what levels they need to be determined, as well as how many samples and how much of a sample is available for the analysis.

Very often, historical materials are highly valuable and are available for analysis in extremely small amounts. Therefore only sensitive analytical techniques may be appropriate to trace the analyte of interest that is present in small amounts. Whereas macro-components can be easily determined, micro and trace amounts of a particular analyte of interest can present a challenge for restorers and conservators.

Several validation parameters are important.

First, the limits of detection and limits of quantification need to be low enough. Without adequate detection limit capabilities, lengthy analyte concentration procedures may be required prior to analysis. Typical limits of detection ranges for most usual spectroscopy techniques are shown in Figure 2.16. and in Table 2.12 [68].

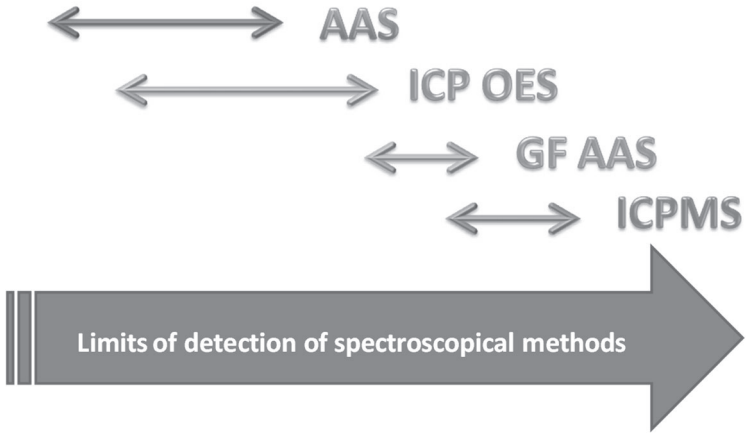



Figure 2.16. Typical detection limit ranges for the major atomic spectroscopy techniques [94].

It must be emphasized that some methodologies can be much improved by adjusting the instrumental parameters. For example, ICP-OES methodology offers two different ways of observing the emitting energy from the sample: axial and radial views and limits of detection are shown in Figure 2.16 and can be very different for particular elements.

Figure 2.16 presents a typical overview of selected spectroscopy techniques when compared according to limits of detection.

**Table 2.12. Detection limits of some elements reported with different spectroscopic methods, expressed in µg/L (ppb) [68, 94].**

| Element | **Flame AAS** | **GFAAS** | **ICP-OES** | **ICP-MS** |
|---|---|---|---|---|
| Ag | 1.5 | 0.005 | 0.6 | 0.002 |
| Al | 45 | 0.1 | 1 | 0.005 |
| As | 150 | 0.5 | 2 | 0.0006 |
| Au | 9 | 0.15 | 1 | 0.0009 |
| B | 1000 | 20 | 1 | 0.003 |
| Ba | 15 | 0.35 | 0.03 | 0.00002 |
| Be | 1.5 | 0.08 | 0.09 | 0.003 |
| Bi | 30 | 0.05 | 1 | 0.0006 |
| Ca | 1.5 | 0.01 | 0.05 | 0.0002 |
| Cd | 0.8 | 0.002 | 0.1 | 0.00009 |
| Co | 9 | 0.15 | 0.2 | 0.0009 |
| Cr | 3 | 0.004 | 0.2 | 0.0002 |
| Cu | 1.5 | 0.014 | 0.4 | 0.0002 |
| Fe | 5 | 0.06 | 0.1 | 0.0003 |
| Hg | 300 | 0.6 | 1 | 0.016[i] |
| Ir | 900 | 3.0 | 1 | 0.001 |
| K | 3 | 0.005 | 1 | 0.0002 |
| Li | 0.8 | 0.06 | 0.3 | 0.001 |
| Mg | 0.15 | 0.004 | 0.04 | 0.0003 |
| Mn | 1.5 | 0.005 | 0.1 | 0.00007 |
| Mo | 45 | 0.03 | 0.5 | 0.001 |
| Na | 0.3 | 0.005 | 0.5 | 0.0003 |
| Ni | 6 | 0.07 | 0.5 | 0.0004 |
| P | 75000 | 130 | 4 | 0.1 |
| Pb | 15 | 0.05 | 1 | 0.00004 |
| Pd | 30 | 0.09 | 2 | 0.0005 |
| Pt | 60 | 2.0 | 1 | 0.002 |
| Rb | 3 | 0.03 | 5 | 0.0004 |
| Sb | 45 | 0.05 | 2 | 0.0009 |
| Se | 100 | 0.05 | 4 | 0.0007 |
| Si | 90 | 1.0 | 10 | 0.03 |
| Sn | 150 | 0.1 | 2 | 0.0005 |
| Sr | 3 | 0.025 | 0.05 | 0.00002 |
| Te | 30 | 0.1 | 2 | 0.0008 |
| Ti | 75 | 0.35 | 0.4 | 0.003 |
| Tl | 15 | 0.1 | 2 | 0.0002 |
| V | 60 | 0.1 | 0.5 | 0.005 |
| Zn | 1.5 | 0.02 | 0.2 | 0.0003 |

Second, the linear working range is the concentration range that is important for the analysis. This is the range over which the quantitative

results are obtained. It is important to select a technique with an appropriate analytical working range since a wide working range reduces time and the steps needed for sample handling. This not only minimizes potential errors, but also enables the performance of a sensible analysis of precious historical materials [94].

Third, other parameters significantly influence the decision over which analytical methodology to use as a strategy for optimal analysis of historical materials. These are sample throughput, the cost of analysis, and the potential interferences that influence the expected result [95].

The highest number of samples that can be analyzed in one unit of time is called "Sample throughput." Regarding the number of samples, the methodologies are divided into two big groups: single element technologies (like AAS and GF-AAS), and multi-elemental techniques (such as ICP-OES and ICP-MS). ICP is a true multi-elemental technique with exceptional sample throughput that enables typical determination of more than 40 elements per minute in individual samples [96].

**Table 2.13. Comparison of the various spectroscopy techniques on parameter performances [68, 108–11].**

| Parameter | Flame AAS | GF–AAS | ICP–OES | ICP–MS |
|---|---|---|---|---|
| Detection limits | high ppb | Sub ppb | sub ppb–ppm | Sub ppt |
| Elements thoroughput | single element | Single element | Multi element | Multi element |
| Range | mid ppm | Low ppm | high ppm | Mid ppm |
| Precision | 0.1–1 % | 0.5–5 % | 0.1–2 % | 0.5–2 % |
| Elements applicable | to > 60 | to > 50 | to > 70 | to > 80 |
| Sample volume | 4–8 mL/min | 0.2–1 mL/min | 1–2 mL/min | 0.02–2 mL/min |
| Ease of use | very easy | more difficult | easy | more difficult |
| Operating costs | low | high | medium | High |

As can be seen from Table 2.13., there are many analytical parameters that influence the decision on the selection of a particular methodology. However, the most important are the speed of the analysis and the limits of detection. Therefore those two parameters are mutually compared as follows:
The Speed: ICP-OES & ICP-MS > AAS > GF-AAS
The limits of detection: ICP-MS > GF-AAS > ICP-OES > AAS
In cases where the selection is based on the analyte limits of detection, flame AAS and ICP-OES are favored for moderate to high levels, while GF-AAS and ICP-MS are favored for lower levels. ICP-OES and ICP-MS are multi-element techniques, favored in cases when large numbers of samples are to be analyzed [68].

### 2.1.4.4. FTIR, UV-VIS, and Raman spectroscopy

In analysis of functional groups, and not elements, other types of spectroscopic methods need to be involved in the analysis. Those are infrared spectroscopy, UV-VIS spectroscopy, and Raman spectroscopy. The advantage of those methodologies, unlike the before mentioned AAS, GF-AAS, ICP-OES, and ICP-MS, is that FTIR, UV-VIS, and Raman spectroscopy might be employed on solid samples that do not need to be transformed into liquid forms. Infrared spectroscopy (IR, FTIR) and Raman spectroscopy are methods that allow analysis of the fiber supra-molecule structure that included the crystalline and polymorphic phases of the cellulose. Therefore those can be applied in monitoring the degradation of historical textiles due to different parameters, and to obtain the data on supra-molecule structural changes in the degraded materials, for example on de-polymerization of the changes in the arrangement of macromolecules [107].

**Infrared spectroscopy** is a vibrational method, in which the analyte comes into interaction with infrared light. The obtained spectrum originates from molecules that absorb specific frequencies. Moreover, the FTIR ATR (attenuated total reflectance) method is a non-destructive process that enables the analysis of the surface of solid historical textile materials. The most important characteristic of the IR is the determination of functional groups. For example, the –OH group in cellulose polymers (such as historical cotton materials) are easily spotted around 3500 $cm^{-1}$ (Figures 2.17. and 2.18). Moreover, the changes that occur during degradation can be followed and the identification of the degradation procedure is possible.

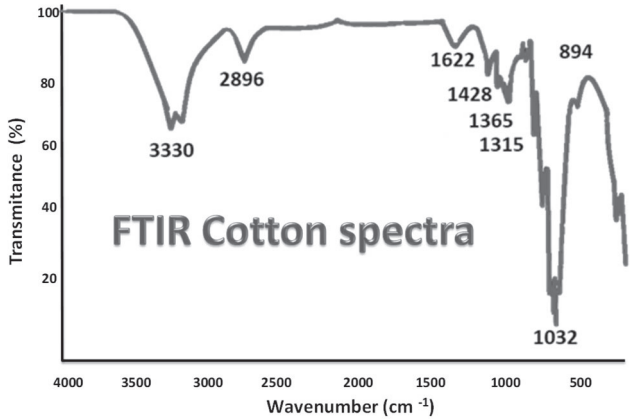



Figure 2.17. FTIR spectra of a cotton sample, with its functional groups.

The Fourier Transform Infrared spectroscopy technique applied to historical textiles shows resulting spectra with the specific absorption bands of organic compounds. For example, the resulting FTIR spectra show differences between samples that are mineralized in graves, and enable detection of the left organic matrix and the differences between samples. In addition, FTIR is useful in monitoring the degradation degree of aged textile fibers on a molecular level. By this, such methodology can be proposed as a method for dating cellulosic polymers.

There are several operating modes in FTIR spectroscopy. These are transmission, reflectance, or attenuated total reflectance (ATR) modes. First, the transmission mode has special requirements and restraints: it requires the removal of a sample and only very thin samples (as thin as 20 microns) are suitable for transmission. Therefore special sample preparation is needed that involves pressing and grinding procedures. It has to be emphasized that such sample preparation steps are destructive to the historical textile samples. Second, the attenuated total reflectance (ATR) mode needs to be pressed against a crystal window, which could be destructive to fragile historical textile samples. Third, the reflectance mode does not require sample removal or preparation; thus, this methodology is favorable because it is non-invasive and non-destructive. However, the drawback of the reflectance mode is that sometimes only surface analysis is possible. Figures 2.17–19 present the spectra of historical textile materials, with characteristic chemical groups.

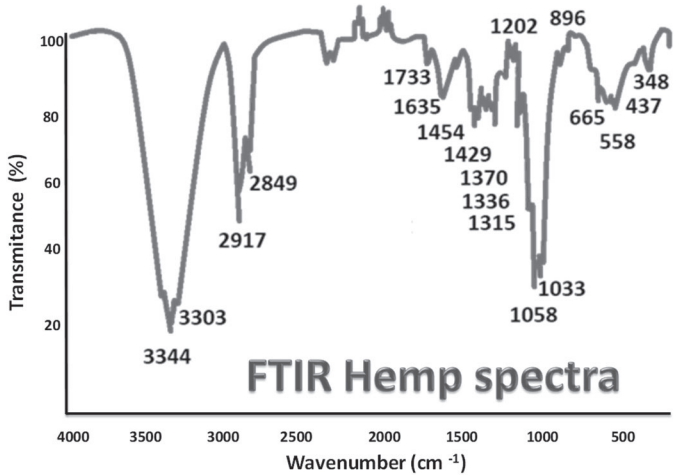



Figure 2.18. FTIR spectra of a hemp sample, presenting representative functional groups.

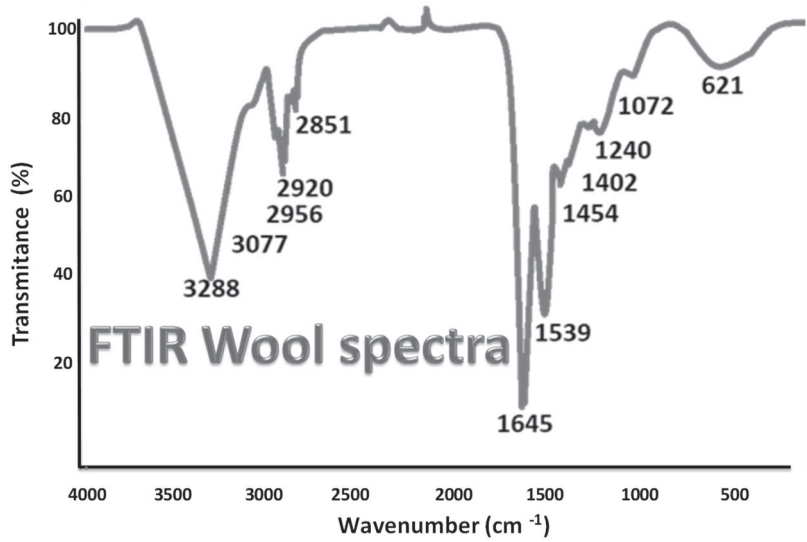



Figure 2.19. FTIR spectra of a wool sample, presenting representative functional groups.

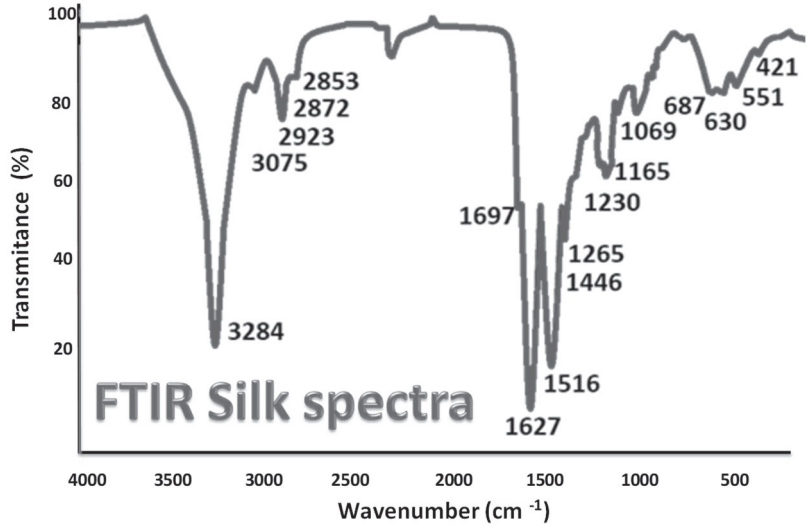



Figure 2.20. FTIR spectra of a silk sample, presenting representative functional groups.

The advantageous potential of the application of FTIR micro spectroscopy is that it can be a non-invasive and non-destructive characterization method that does not require permanent removal or destruction of historical textiles. Unfavorable environmental conditions such as temperature and moisture oscillations lead to the destruction of fragile textile fragments. These unfavorable environmental conditions induce physical, chemical, and microbiological degradation of materials. FTIR spectra such as those graphical presented in Figures 2.17–20 are recorded over a range of 4,000 to 500 $cm^{-1}$. The sample is placed on a holder in the central hole and FTIR spectra are obtained. The size of the sample can vary, but a relatively small amount of the sample is enough to perform the analysis (1 to 5 mm).

The resulting spectra have peaks that are the characteristics of the functional groups of polymers. For example, cellulosic polymers such as cotton, hemp, and flax have bands at 2,900 $cm^{-1}$ from stretching vibrations of C–H, while protein polymers such as wool and silk have distinguished bands at 3,275 $cm^{-1}$ assigned to the amide bonds N–H and 1,235 $cm^{-1}$ assigned to the peptide bonds. Table 2.14 presents functional groups in fibers of historical textiles and their obtained resulting peaks on FTIR spectra that are characterized by FTIR ATR mode spectroscopy.

**Table 2.14. Functional groups of historical textiles and their obtained resulting FTIR spectra peaks [107].**

| Wave number ($cm^{-1}$) | Functional groups in historical textiles |
|---|---|
| 3500 | -OH bond |
| 2900 | C–H bond |
| 1735 | C=O - hemp |
| 1595 | C=C lignin, hemp |
| 1365 | C–H bond, cellulose |
| 895 | C–O–C bond, cellulose |
| 3275 | N–H bond, proteinous textile |
| 1615 | C=O bond, proteinous textile |
| 1515 | N–H bond, proteinous textile |
| 1235 | C–N bond, proteinous textile |

**Raman spectroscopy** uses the monochromatic laser of different energy (thus different wavelengths from 229 to 1,064 nm), and the obtained spectra is the observation of obtained non-elastic scattering of the light that is reflected from the sample. In this manner, Raman spectra enable us to obtain information on the vibrations of molecules and about polymer vibrations. Therefore, the surface of historical textile materials can be easily analyzed.

**Raman spectroscopy** is used to identify the primary type (the chemical composition) of historical textile fibers. Therefore it can provide the blend percentages in the fabric. Raman spectrum is generated through collecting one hundred scans with a resolution of 4 $cm^{-1}$ to reduce the noise in the Raman spectra and then generating spectra for the samples and some of the standards. Afterwards a comparison between the sample and the standard spectra are performed to decide whether the two fabrics could have had the same origin.

Raman spectroscopy is based on the electromagnetic interactions of weak side-bands excited by the energy source of monochromatic light. The biggest advantage of this methodology is that Raman spectroscopy requires no sample preparation. Therefore it also has the potential of noninvasive analysis. The disadvantage to conservators, restorers, and chemists dealing with Raman spectroscopy of historical textile samples is that only a limited number of Raman spectra of natural dyes are available in the literature. Several improvements of routine Raman analysis have been offered to improve and enhance the analytical signals. These are nano-structured substrates like colloids, thin films, and small nano-composite materials that in combination with the solid phase micro-extraction enable conclusive identification of dyes from the surface of historical textiles. Figure 2.21 presents the graphical presentation of typical Raman spectra resulting from purple pigment after extraction from a cotton historical textile sample.

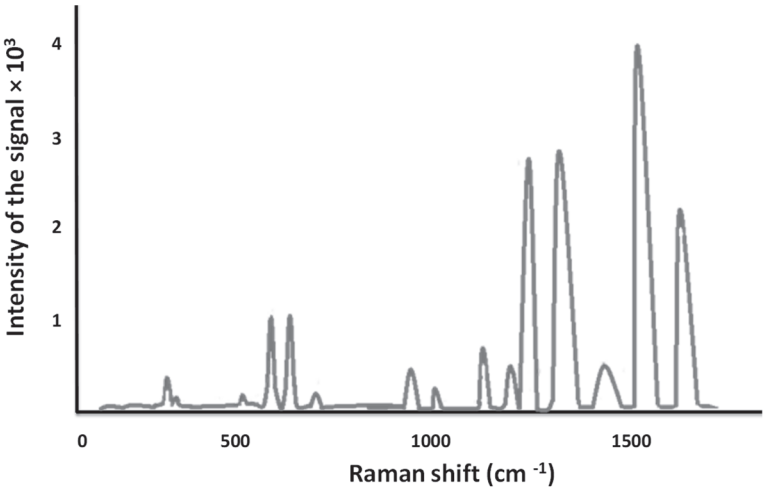



Figure 2.21. Raman spectra of a cotton historical textile sample, presenting peaks from purple pigment.

As can be seen from Figure 2.21, Raman spectra are useful in the detection of pigments on cellulose materials. Moreover, the differences between historical textile fibers of different origin can be observed. For example, historical fibers can be compared with modern cellulose. Such analysis will reveal the changes to historical samples caused by ageing and degradation, as well as surface impurities. For this reason, Raman spectroscopy is today widely applied in the analysis of historical textiles. Samples of interest cover a broad range of different materials, such as banners, religious textiles, military uniforms, theatre costumes, tapestries, and dresses, but also paintings of huge artistic value.

**UV-Vis** is principally used to study the color of the fibers; sometimes, it also provides information on the polymeric nature of the fibers. In UV-VIS, the reflectance accessory is used by the spectrophotometer in order to determine the exact color of a fabric sample. By following the recommended operating procedure, first the equipment is set to 100% reflectance background with a reference material disk covering the sample port of the integrating sphere. After the recording, in which the whole visible spectra is passed through (ranging from 350 nm to 800 nm), the position of the fabric sample is placed on the sample port and the final spectra is recorded. As was previously the case, by comparison between the wavelength characteristics of the referent materials and the unknown sample spectra, the chemist is able to decide the color and properties of the fibers and for example whether two of the historical textiles could have had the same origin. In this way, discrimination between cotton and woolen textile fibers is enabled.

In addition, the coupling of the **UV-visible-NIR micro-spectroscopy** for the analysis of historical textile fibers is one of the most common applications of the technique in the field of restoration and conservation analysis. Therefore, in the visible-NIR range, detailed spectral information is recovered from the sample, including information on the dyes and pigments used. Moreover, in the UV region, more information about dyes, resins, waxes, and other binding media is obtained. In particular cases when quantitative information is required, and not only a qualitative identification of the unknown analytes (dyes or pigments), the calibration curve is designed by using standard sample solutions of a known analyte concentration. Figure 2.22 presents the linear curve obtained for five standard samples of known concentration, called the “working standards” of purple iron complex dyes. The calibration curve is prepared in such a manner that samples with a known composition are prepared and measured, in order to obtain a calibration curve with the values of absorption for 1, 2, 3, 4 and 5 mg/L of iron complex. Afterwards the

obtained data are used to determine the composition of the unknown sample by interpolating its absorption data into the graphic presentation of the obtained data.

After creating a calibration curve, the UV-VIS spectra of the unknown sample is recorded. A typical UV-VIS spectrum of a purple textile dye is presented in Figure 2.23. As can be seen from this figure, different dye concentrations will result in different maximal absorbance value. This value is interpolated in the calibration curve, shown in Figure 2.22 and the quantitative data on the analyte is obtained.

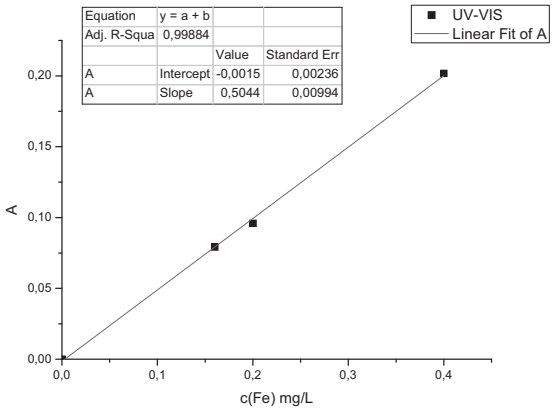



Figure 2.22. Calibration curve in UV-VIS spectrometry obtained at 452 nm with purple complex dye containing Fe ion; a relatively low linear analytical range is observed (between 0.0 and 0.5. mg/L), with relatively high limits of detection.

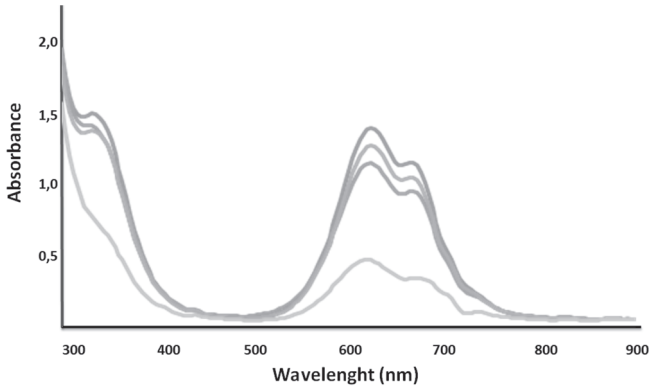



Figure 2.23. UV-VIS spectra of a purple textile dye of different concentration, recorded through the visible range.

UV-VIS, Raman, and FTIR spectroscopy are therefore complementary techniques and recommended procedures as their combination enables comprehensive analysis of historical textile fibers.

In addition, **fluorescence micro-spectroscopy** gives information on fiber dyes with the correct excitation. The most important advantage of this procedure is that it is a non-destructive methodology. Due to this property, and the fact that the sample preparation procedure requires minimal attention, UV-visible-NIR micro-spectroscopy has become an unavoidable approach in the analysis of historical textile materials.

The ability to perform accurate and precise analysis of historical natural fibers is of great importance to conservators and restorers of historical materials. Nevertheless, obstacles might be expected when analyzing samples of similar chemistry and similar microstructure. For example, historical degraded fibers such as flax and hemp can be challenging in this regard. When analyzing degraded materials, the most frequently used conventional microscopic techniques that enable measuring the morphology of fibers, including their width or the twist test, can result in unreliable results. In such cases, spectroscopic investigation might provide crucial data for distinguishing and differentiation between the samples. For example, FTIR–ATR methodology with polarized radiation will enable differentiation between flax and hemp through measurement of their characteristic sense and the angle of the wind of the fibers.

# CHAPTER II.2

# CHROMATOGRAPHY AND CHARACTERIZATION OF PAINTED LAYERS

## 2.1 Identification of layers

Identification of dyes and their binding media on historical textile materials is one of the most important analytical questions. Different groups of materials were applied on the surface of the textile carrier, and mixed with inorganic pigments in order to establish a long-lasting painted-over layer. Such materials include waxes, resins, sugars, and proteins that were mixed and used [97]. Their identification is essential for dating, restoring, and conserving artwork. Moreover, it is important not only for archeological and anthropological investigations but also for studying the history of the art globally. The importance of cultural artifacts, such as historical textiles, requires techniques that are reliable and precise. Moreover, it requires methods that are as indestructible as possible. The samples used are of microscopic origin, so the limits of detection and the quantification of chemical methods need to be appropriate [98].

The group of chromatographic procedures is a very important analytical group of instrumental methods that can provide accurate analysis of samples that are small and valuable [99]. Due to its excellent ability to separate different analytes in the sample, and to perform qualitative and quantitative determination of separated analytes, chromatography is an unavoidable step in the analysis of historical samples [100–116]. This chapter will present the procedures used in the analysis of waxes, resins, proteins, and other binding media from real case samples [103–7, 109, 114, 115].

Chromatography was invented by the Russian botanist Tswett in the early twentieth century. Tswett applied chromatography to separate the plant pigments chlorophyll and xanthophylls by passing the sample through a glass column filled with crushed Ca-carbonate. Chromatography applies different physical and chemical properties of materials in order to create a system with stationary and mobile phases. The sample is carried away by the mobile phases and the components in the sample are

separated on the basis of their unique properties. In order to occur, the separation of components in a sample requires an adequate difference in the strength of the physical interactions. Interactions that occur are the results of physical forces, or, for example, weak chemical bonds such as charge transfer, dipole–dipole interactions, and the formation of a hydrogen bond.

Consequently, by definition chromatography is a separation method based on the different distribution of sample components between two phases, one of which is stationary (stationary) and the other mobile (mobile).

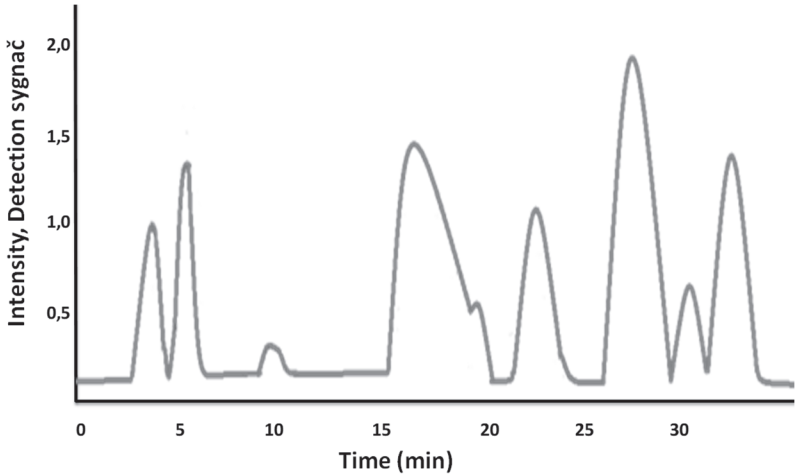
Therefore, chromatography is considered to be the most widely applied separation technique, used not only in the identification and analysis of unknown samples, but also in the isolation and purification of particular samples. Chromatographic methods are used to separate, identify, and quantify chemical constituents in complex mixtures.

The chromatographic process is optimized thorough the chromatographic system parameters in which, under the influence of the mobile phase, the components move through the stationary phase at different speeds and thus separate. The stationary phase is immobilized in a column or fixed on a solid surface so it can be solid or liquid. In addition, the mobile (moving) phase moves through the stationary phase and it can be liquid or gaseous. The criterion for selecting the mobile phase is the different solubility of the ingredients of the mixture, which ensures different movement of the ingredients and their efficient separation. By varying the mobile and/or stationary phases, the optimization of the chromatographic system is performed.

Chromatographic methods can be divided in several ways, according to the:

- stationary phase shape (plane or column chromatography)
- physical state of the mobile phase (gas and liquid chromatography)
- separation mechanism (ion exchange or size exclusion chromatography)

Chromatographic analysis enables separation, identification, and quantification of chemical compounds present in complex mixtures, and the result of such chromatographic analysis is a curve line with several peaks called the chromatogram (Figure 2.24). Each peak corresponds to a particular analyte of interest, impurity, or metabolic compound.




2.24. The chromatogram example of separated components in a historical sample.

As can be seen from Figure 2.24., the chromatogram has a series of symmetrical elution curves called "peaks." The ideal shape of the chromatogram peak would be a perfect Gauss curve, but in practice usually non-ideal peaks appear. This asymmetry is the result of the way in which a chromatogram peak is formed: the molecules of the sample move through the column or the thin plate in different non-ideal ways. Those processes are observed by many factors that influence the efficiency of the chromatographic process and the formation of peaks, such as distribution ratios, retention times $t_R$, the relationship between retention time and distribution ratio, and the analyte velocity and differential velocity.

The chromatographic process is therefore optimized by column resolution, which is actually a quantitative measure expressing the ability to separate two analytes in a column or on a thin plate. The complete separation of analytes A and B is possible only with a resolution $R_S$ higher than 1.5. It can be easily calculated that by lengthening the column length it is possible to achieve better resolution; however, this step has a disadvantage, as more time is required to achieve a better resolution. Optimization parameters reduce the height of the ceiling, increasing capacity factors and increasing the selectivity coefficient; the optimization techniques include reducing the grain size of the filler, the diameter of the column, and the thickness of the liquid layer; second, lowering the column temperature in GC; and, third, changing the composition of the mobile and stationary phases in all different types of chromatographic systems. In

conclusion, it is desirable to achieve the best possible resolution in the shortest possible time of analysis.

**Qualitative analysis** is performed on the basis of the read retention time, and in comparison with the standard. This enables the presence or absence of an ingredient to be determined in a mixture of known composition. Usually chromatography is combined with other techniques of greater identification ability in order to more accurately determine the composition of the mixture, including systems like GC-MS, HPLC-MS, GC-IR, and others, in which chromatography is used to separate the components, and spectroscopy is used to identify and quantify the components in the sample.

**Quantitative analysis** is based on a comparison of the height or area of the analyzed peak with the height or area of the peak of a standard sample solution. The analysis is based on the:

- peak height, the length of the vertical drawn from the maximum of the peak to the baseline of the peak, or the
- peak surface, in which the surface is measured by electronic integrators.

**Calibration** is, as in spectroscopy (explained in the chapter on UV-VIS spectroscopy), performed using standard samples in the following manner: a series of standard calibration solutions are prepared that are similar in composition to the test sample. Then the chromatogram is recorded for the standard solution, and the heights of the peaks or their surfaces are displayed depending on the sample concentration.

In **liquid chromatography**, the mobile phase is a liquid and the separation is carried out in a column or on a surface. The steps of the analysis include four steps:

1. Injection of the analytes.
2. Separation of the analyte in the column.
3. Elution (rinsing) of the components from the column when different ingredients come out of the column at different times.
4. Detection, which is performed by measuring different physical properties (refractive index, UV absorption, electrical conductivity) at the detector of the chromatographic system.

**High-performance liquid chromatography (HPLC)** has many advantages, because it is a sensitive, flexible method efficient in the

analysis of non-volatile and thermally sensitive compounds and is adoptable for a wide range of components in historical textile samples (amino acids, nucleic acids, sugars, organ metallic compounds, inorganic substances, etc.). HPLC is a useful method for identification of dyes used on historical textiles. The results obtained are helpful in dating the historical samples, which can be performed depending on the sort of dyes used, as well as their manufacturing date. Moreover, the qualitative analysis of the dyes used on samples of historical textiles enables placing their origin and time of production. In addition, precise and accurate analysis is necessary for proper conservation and restoration steps in order to preserve the precious and valuable historical textile samples. Although high-performance liquid chromatography is a highly specific and very sensitive separation technique, the sample preparation steps that include different extraction and separation steps could induce changes in the sample structure, and, more importantly, present a risk of sample loss. Moreover, the amount of the sample required for the analysis is a minimum of 5 mm of a historical textile thread, which makes this method not so ideal for the analysis of historical textiles. This is especially the case in samples of extremely high value that are so rare and precious that very little or no sampling at all is allowed.

Liquid chromatography is used not only to separate organic and inorganic substances, but also for the separation of ions, when it is called "**ion chromatography**." Ion chromatography allows the separation of ions or charged molecules, and as a method is based on the separation of the components of the mixture due to the action of Coulomb (ionic) forces. The stationary phase has ionic functional groups that react with ions from the analyzed mixture of the opposite charge. The ion chromatography method is divided into: CATION (where the stationary phase has negative-charged functional groups) and ANION (where the stationary phase has positively charged functional groups) ion chromatography.

In **gas chromatography (GC)**, the moving phase is a gas medium so it is always carried out in a column. Unlike liquid chromatography, in gas chromatography, the analyte does not react with the mobile phase and therefore its speed of movement through the column does not depend on the chemical structure of the mobile phase. Gas chromatography originated in developments in the 1950s when Archer J. P. Martin and Richard J. M. James developed a process by which samples were separated based on their solubility in two different solvents; in 1952, they received the Nobel Prize for their discovery. The analyte (such as natural indigo dye extracted from a historical textile) is in gas chromatography injected as a

liquid extract, which then due to the high temperature in the chromatograph turns into a gaseous state. The inlet temperature of the instrument is set 50°C higher than the boiling point of the weakest volatile component from the analyzed mixture. The mobile phase in gas chromatography is an inert gas that elutes the components of the mixture in a column filled with a stationary phase. Unlike liquid chromatography in gas chromatography, the analyte does not react with the mobile phase and therefore its speed of movement through the column does not depend on the chemical structure of the mobile phase. The stationary phase of the gas chromatography can be used for two different groups of components:

a) for separating components of low molecular weight or
b) for separating the high molecular weight components in which the liquid phase is applied to the surface of a solid support by adsorption or chemical bonding.

**Planar chromatography** has its first beginnings in the middle of the nineteenth century when in 1822 F. F. Runge was dripping different paint solutions onto filter paper. He noticed the separation of colors into concentric rings and called the phenomenon "capillary analysis." During the development of the chromatographic procedure, C. F. Schönbein immersed strips of filter paper in aqueous solutions of inorganic salts and found that in repeated experiments the same substance always stopped at the same place (1861). Partition chromatography occurred in 1944, when R. Consden, A. H. Gordon, and A. J. P. Martin tried to separate amino acids using organic solvents on water-impregnated filter paper. Planar chromatography can be performed on paper, or on some other thin layer.

**Paper chromatography** is applied for the identification of substances and for testing the purity of substances. It is a relatively fast method that requires a very small amount of material for the sampling.

**Thin layer chromatography** (TLC) uses different thin layers as a stationary phase (cellulose, silica gel, alumina) which are placed on a glass, plastic, or metal support. The mobile phase in both paper and thin layer chromatography is a solvent mixture containing organic and/or inorganic solvents. Thin layer chromatography originated in the 1950s with the work of Eegon Stahl.

The most important parameter in thin layer and paper chromatography is the $R_F$ value. It presents the ratio of the distance between the sample stain path and the solvent front. The spots can be identified by using different chemical reagents, or by sophisticated instruments such as a

video-densitometer. Thin layer chromatography is used in the routine analysis of binding media, which is present on historical painted textiles [101]. Although the detailed examples of the analysis of different samples on historical textiles will be presented in the second part of the book (through numerous real case examples), here an overview is presented of the most common chromatographic thin layer systems for the analysis of waxes, resins, and proteins.

## 2.2 Determination of waxes on historical materials

The stationary thin layer chromatography phase for the determination of waxes in the binding media on historical textiles is silica gel. The most useful method is to apply the stationary phase with the UV-indicator, so no additional visualization reagents will be necessary during the evaluation of the obtained chromatograms. The mobile phase contains the mixture of petroleum ether, diethyl ether, and acetic acid in volume rations 90:10:1. The visualization is performed under UV light of 254 nm, in a UV-cabinet, under a UV-lamp or by using a Videodensitometer's UV-lamp [103].

Table 2.15 presents the most common $R_F$ values of the components of different waxes. Values in this table can enable identification of unknown wax components extracted from historical textile materials. However, it has to be emphasized that thin layer chromatography is the only chromatographic procedure (together with paper chromatography) in which it is not possible to lead the process and to have an influence on pressure and temperature since the analysis is performed under laboratory conditions, usually outside the developing chamber. For this reason, the $R_F$ parameters might slightly change depending on room temperature and pressure.

Therefore, in every analysis, standard samples are individually analyzed and the spots and $R_F$ values are compared with the components that occur from the unknown sample on the same plate. For this reason, the values in Table 2.15 are useful for orientation purposes but should not be considered to be constant, unchangeable values [103].

**Table 2.15. $R_F$ parameters of reference wax samples under UV light.**

| Wax | Bees | Camellia | Carnauba | Japanese | Lanolin | Paraffin | Spermaceti |
|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 0.09 | 0.10 | 0.08 | 0.06 | 0.08 | *n.d.* | *n.d.* |
| 3 | *n.d.* | *n.d.* | 0.11 | 0.09 | *n.d.* | *n.d.* | *n.d.* |
| 4 | 0.14 | 0.16 | 0.15 | 0.14 | 0.13 | *n.d.* | *n.d.* |
| 5 | *n.d.* | *n.d.* | *n.d.* | *n.d.* | 0.19 | *n.d.* | *n.d.* |
| 6 | 0.30 | *n.d.* | *n.d.* | 0.33 | *n.d.* | *n.d.* | *n.d.* |
| 7 | 0.41 | 0.42 | *n.d.* | *n.d.* | *n.d.* | *n.d.* | *n.d.* |
| 8 | 0.50 | *n.d.* | *n.d.* | *n.d.* | 0.52 | *n.d.* | *n.d.* |
| 9 | *n.d.* | 0.69 | *n.d.* | *n.d.* | *n.d.* | *n.d.* | *n.d.* |
| 10 | *n.d.* | *n.d.* | *n.d.* | *n.d.* | 0.66 | *n.d.* | *n.d.* |
| 11 | 0.72 | *n.d.* | *n.d.* | *n.d.* | *n.d.* | *n.d.* | *n.d.* |
| 12 | 0.86 | *n.d.* | 0.85 | *n.d.* | *n.d.* | *n.d.* | 0.84 |
| 13 | *n.d.* | 0.90 | *n.d.* | *n.d.* | *n.d.* | *n.d.* | *n.d.* |
| 14 | 0.99 | 0.98 | *n.d.* | *n.d.* | 0.95 | n.d. | *n.d.* |
| 15 | *n.d.* | *n.d.* | *n.d.* | 1.00 | *n.d.* | 1.00 | *n.d.* |

**n.d. = Not detected, the component is below limits of detection.*

## 2.3 Determination of resins on historical materials

In thin layer chromatographic analysis of the resinous components of historical textiles, silica gel F256 is used as a stationary phase. Prior to the resin determination, the stationary phase needs to be washed in MeOH solvent and then activated by heating at 100°C for 60 minutes. Different mobile phases can be used for the separation and identification of resinous components, which are presented in Table 2.16 [101, 106].

**Table 2.16. Chromatographic conditions for the analysis of resinous binders on silica gel stationary phase, detection under UV light 254 nm, or visualization reagent SnCl2.**

| Components | Volumes | Developing/min | Detection |
|---|---|---|---|
| EtAc–n-Hexane | 30.0:20 | 25 | UV—254 nm, $SnCl_2$ |
| Petrol ether–EtAc-HCOOH | 93:7.0:1 | 50 | UV—254 nm, $SnCl_2$ |
| Petrol ether–Ether–HAc | 90:10:1 | 25 | UV—254 nm, $SnCl_2$ |
| Petrol ether–EtAc | 1.0:1.0 | 25 | UV—254 nm, $SnCl_2$ |

## 2.4 Proteins characterization and separation of amino acids from complex samples

Amino acid components from proteinous binders are the most complex analytes, due to their protein composition. Nevertheless, by careful choice of stationary and mobile phases, efficient separation can be achieved [97]. Table 2.17 presents the TLC systems for protein detection [117].

**Table 2.17. Chromatographic conditions for the analysis of resins binders, on silica gel stationary phase.**

| Components | Volumes | Developing time/min | Detection |
|---|---|---|---|
| $CHCl_3$–$CH_3OH$–17% $NH_3$ | 40:40:20 | 40 | Ninhydrine, isatine |
| n- BuOH–HAc–$H_2O$ | 80:20:20 | 80 | Ninhydrine, isatine |
| EtOH–$H_2O$–$NH_3$ | 90:8:2 | 60 | ninhydrine |
| $CHCl_3$–$CH_3OH$–HCOOH | 35:15:1 | 25 | ninhydrine |

# CHAPTER II.3

# MICROSCOPY

## 2.1 Preparation of micro cuts, determination of morphology, and physical-chemical properties

Historical textiles that are painted samples contain several layers of different materials that need to be carefully examined. For this reason, the cross cut of the sample needs to be carefully prepared and examined by microscopy methods (Figure 2.25), through the use of optical magnification or scanning electron microscopy (OM, SEM).

Microscopy is needed in order to perform the characterization of small fragments of different layers of historical materials [119–22].

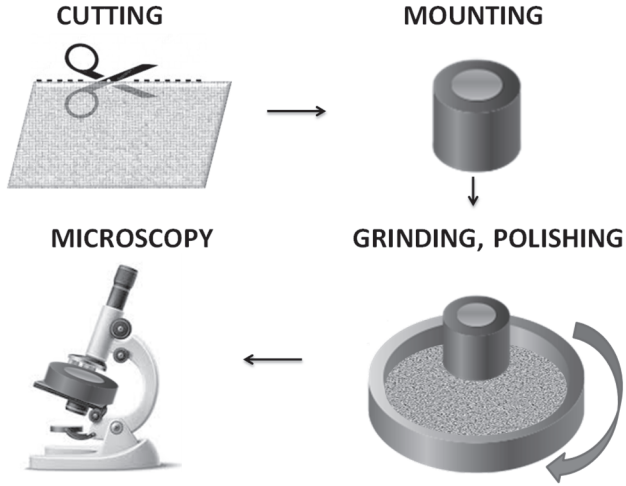



Figure 2.25. Preparation of sample for microscopic characterization including cutting, molding, grinding, and polishing.

All steps in the preparation of a sample cross cut for microscopy (Figure 2.25) need to be carefully performed; the basic routine process is the following:

1. The representative sample is chosen. The chosen specimen needs to have all its different layers present. Parts of the material that are heavily damaged cannot be used in this analysis, and the parts that are chosen need to be preserved both in horizontal and vertical cross cut directions.
2. The historical textile needs to be cut very carefully in order to avoid altering or destroying the sensitive structure of the material. In this regard one can apply different metal knifes and scissors, but the ceramic knife is necessary if the sample is to be analyzed not only by microscopy but also by spectroscopy techniques for trace elemental analysis of metals. Cutting with metal would result in contamination with trace metal amounts.
3. In cases where a historical textile sample contains metal fibers as wrapping around the fragile textile core, the process of cutting is delicate. This process can include some kind of abrasive saw and the application of coolant to cool the sample during cutting.
4. After the sample is cut to a desirable size, it is mounted in plastic or epoxy resin to facilitate handling during the grinding and polishing steps. The mounting media must be compatible with the sample and should not chemically alter the components present in the sample.
5. The grinding step with a series of progressively finer abrasive grits is applied to achieve a flat, smooth, and scratch-free surface.
6. Through polishing, the last thin layer is removed and a smooth reflective surface is left prepared for investigation. If the sample is metal fiber then etching in an appropriate acidic or basic solution may also be used for detecting the micro-structural details. Polishing involves the use of abrasives on an electrically powered wheel. Careful washing of the specimen is carried out prior to each stage of polishing. The procedure begins with the least fine grinding surface and ends with the final fine-grinding, with a rotation speed of 150 to 200 rpm. The specimen is initially held in one position on the wheel and can be rotated slowly.
7. Microscopical investigation of the sample cross cut is the last step of the procedure.

The initial microscopic investigation is performed under an optical or electron microscope. The first step is to use a stereomicroscope if possible, because it can reveal a three-dimensional scan of the specimen surface. The specimen is placed on the stage of the microscope where its surface needs to be perpendicular to the optical axis.

The choice of the microscopy technique depends on the characteristics of the sample. The following parameters are important: magnification, resolution, and the flatness of the field. The resultant magnification is the product of the magnifying power of the objective and that of the ocular. A Scanning Electron Microscope (SEM) has magnifications up to 20,000×, and a Transmission Electron Microscope (TEM) up to 100,000×. Therefore a TEM is applied in cases when a highly detailed micro-structural study is needed.

The preparation of the sample is followed by analysis of the microstructure under a microscope (light, polarization, or scanning electron microscope). The basic condition for a successful analysis of the structure of the material is the proper preparation of a representative sample, which is performed in two steps: selection of the sample, and preparation of the sample for analysis. As this analysis is a destructive method, due to the separation of the sample, in which the whole is disturbed both aesthetically and functionally, it is necessary that the separated sample completely (i.e., as much as possible) corresponds to the whole.

Procedures and methods that deform or destroy the microstructure with cutting blades (e.g., cutting with a flame, electrode, or cutting plates without intensive cooling) or that may contaminate the sample (e.g., cutting the sample with metal scissors before trace metal analysis) must not be used when sampling. It is therefore recommended to cut using a ceramic knife. If other cutting methods are used, the cutting should be done at a distance from that to be characterized, because the thickness of the deformed layer can range from 10 μm in ideal conditions to 900 μm in poor cutting parameters.

Furthermore, an important step in preparing the sample for microscopy is pouring the resin around the sample into a carrier, which is usually a flexible polymeric material. Due to the small dimensions of historical textile samples, their irregular shape and the need for insight into the microstructure of old and valuable materials at the edges of the samples, the samples must be fixed before analysis with special means that allow their acceptance and quality of preparation. Watering can be warm, pressing with granulate at elevated temperature, or cold with the help of two- or three-component means. Cold mixing of multi-component resins is most commonly used, so that the sample is not further damaged or altered. On the surface of the sample to be analyzed, in addition to the layer formed due to the cutting of the sample, there are also layers of oxides

(formed over many years on historical metal fibers), absorbed gases and various impurities, instead of pure oil or resin varnish (Figure 2.26).

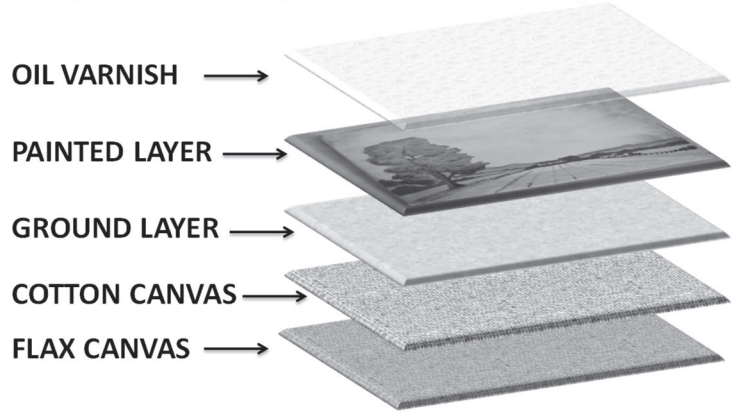



Figure 2.26. Presence of layers on painted historical textile.

As the contaminated zone mentioned prevents insight into the actual microstructure of the sample, it is necessary to remove it by grinding and polishing procedures. Grinding is the most aggressive procedure of material removal in the preparation of a sample for metallographic analysis. When sanding, the abrasive is attached to the substrate, usually paper. The sandpaper is graded according to the size of the abrasive grain, that is, according to the average number of abrasive grains per square inch. Polishing follows after sanding, in the final stage, to remove scratches and achieve a shiny and smooth surface. When polishing, the abrasive moves freely between the sample and the polishing cloth in the form of a paste or liquid that is applied to the substrate. Grinding and polishing can be performed both mechanically and manually.

Microstructure analysis is a surface analysis, which means that all measurements are performed on two-dimensional, flat samples or their projections (photographs). On the basis of this analysis, qualitative and quantitative data on the microstructure are provided. Qualitative analysis describes the pattern: what it looks like, which layers of the painted surface are present, which phases exist, potential damage. The task of quantitative analysis is to quantify the shape, size, quantity, and distribution of elements in the total volume of the sample, and to meaningfully connect the microstructure with the properties of the material.

## 2.2 Optical microscopy

When the cross-section of the sample is properly prepared, microscopical investigation can be performed. The first step is using optical microscopy to examine the sample and the sample of its fibers compared with the original historical sample, as well as the cross-section with different layers.

Figure 2.27 presents the cross cut of a banner from the nineteenth century with blue silk fibers and the painted and gilded layers that were taken of the sample under an optical microscope, 80 times enlarged. As can be clearly seen from this figure, the cross-section of the sample enables one to clearly distinguish between the historical textile carrier, inorganic and organic fillers that serve as the ground for the painted surface, the gilded overlay, and the finishing resin.

Such information is of great importance for conservators and restorers, who aim to conserve and restore the damaged historical material into its primary condition.

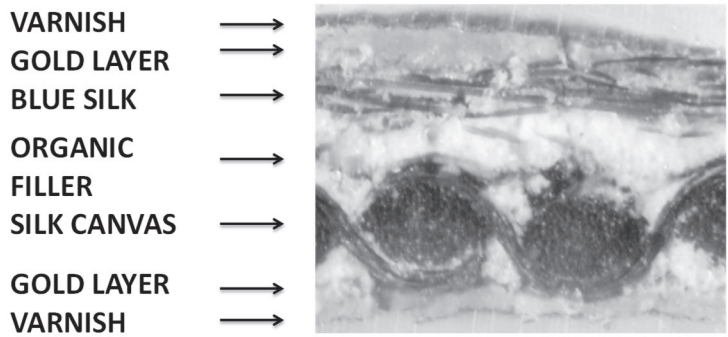



Figure 2.27. Microphotograph of the crossection of painted historical textile, magnification of 80×, revealing the presence of different layers such as varnish, gilded layer, blue silk canvas, organic filler, and vice versa [97].

Particularly interesting historical textiles can contain metal fibers or metallic parts on their surface. Metals can also be inspected by optical microscopy after performing a cross-section of samples of metal threads (metallic fibers, lamete). Such samples can have very different microstructures, which are revealed by measuring their hardness or etching the metal surface with 2% nitric acid in ethanol. The chemical resistance can be monitored by eluting the metal ions in special solutions, as well as by observing the changes in the masses of the samples investigated. For

example, metal ions such as $Fe^{3+}$, $Mn^{2+}$, $Ni^{2+}$, $Si^{4+}$, $Sn^{2+}$, and $Zn^{2+}$ are followed depending on the age of the different samples.

Since the results of chemical resistance of materials are most often expressed as the amounts of eluted ions per unit area, the areas of all metal fiber samples were calculated. The areas of the samples were calculated from their dimensions, and according to the expressions for the surface of the roller shell and the surface of the circle are:

$$P_L = 2r\pi\,(r+H) \tag{14}$$

$$P_K = r^2\pi \tag{15}$$

where $P_L$ is the surface of the roller sheath, $P_K$ is the area of the circle, $r$ is the radius, $H$ is the length, and $\pi$ is a constant number connecting the radius and the area of each circle (3,14…). The areas of the samples can be then calculated as the sum of the two areas of the circle and the area of one wrap ($Area = P_L+2P_K$), as shown in Table 2.18.

**Table 2.18. Calculation of metal fiber surfaces of five different metal fibers.**

| Sample | $r$, mm | $h$, cm | $P_L$, cm$^2$ | $P_K$,cm$^2$ ×10$^{-3}$ | *Area*, cm$^2$ |
|---|---|---|---|---|---|
| 1 | 1.07 | 100 | 33.6 | 9.0 | 33.6 |
| 2 | 0.81 | 100 | 25.4 | 5.1 | 25.5 |
| 3 | 0.61 | 100 | 19.2 | 2.9 | 19.2 |
| 4 | 0.41 | 100 | 12.9 | 1.3 | 12.9 |
| 5 | 0.28 | 100 | 8.8 | 0.6 | 8.8 |

Historical textiles with metal fibers usually have a copper core with a gold or silver outer layer. Such examples of the microphotographs of metallic samples recorded under the microscope are shown in Figure 2.28. Identification of surface gold nano-layers on historical alloys enable obtaining an insight into the valuable sample structure. Moreover, it enables detection of damaged parts of the sample in which the inner copper layer is exposed to environmental conditions causing further damage to the historical material.

Metallic samples, like pearls, buttons, and other small items that were used on historical decorative textile materials, dresses, church clothing, and military uniforms can be tested for their micro-hardness. This procedure is called the Vickers method, the principle of investigation for which is shown in Figure 2.29 [119].

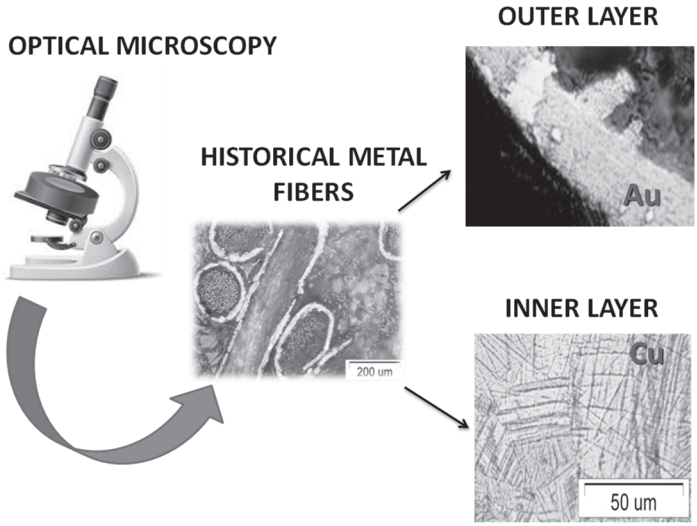



Figure 2.28. Microphotographs of metal fibers showing differences in the microstructure of their different layers: inner copper layer and outer gold surface.

Hardness is a mechanical property of a material that is defined as the resistance with which a body resists the penetration of another, harder body into its surface. Vickers hardness is defined as the ratio of the injection force F/N and the impression area S/mm$^2$ achieved by the injector on the surface of the test material.

A diamond embosser in the shape of a regular pyramid with a square base is usually used, as is shown in Figure 2.29.

After embossing the diamond pyramid, the dimensions of the embossed diagonals are determined, so the hardness is determined either from the tables or according to the given expression:

$$HV = 0{,}102\frac{2F\sin(136/2)}{d^2} = 0{,}1891\frac{F}{d^2} \tag{16}$$

where $F$ is the indentation force (N) and $d$ is the mean value of the imprint diagonal: $d = (d1+d2)/2$.

The Vickers test, developed in 1924 (Smith and Sandland), can be applied to all metal samples because it has one of the widest scales for expressing hardness. The unit used in this test is the HV number, that is, the Vickers pyramidal number defined by the load on the indenter. Before

determining the micro hardness, samples are prepared by a standard metallographic technique involving preparation of the cross-section of the sample.

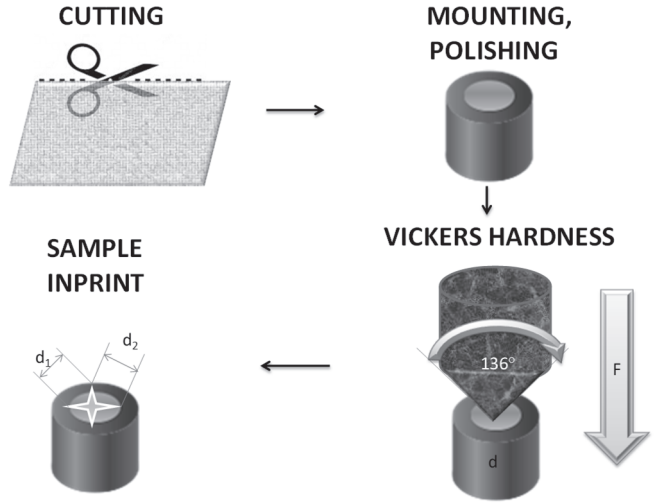



Figure 2.29. The principle of determination of micro hardness using the Vickers method [216].

All resistance tests of metal parts on historical textiles include two main parts: the test sample and the test environment. The aim of corrosion tests is to assess the chemical resistance of the material, and to monitor the amount of eluted ions (e.g., metal ions that cause further destruction and degradation of the material). The assessment of the chemical resistance of the material includes the following phases (Figure 2.30): material selection and sample preparation, exposure to aggressive media, characterization, and data selection and analysis [120].

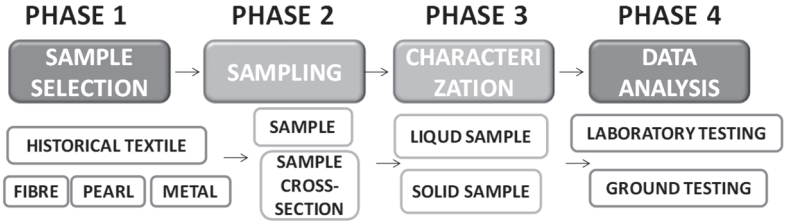



Figure 2.30. The assessment of the chemical resistance of the metallic parts on historical textile material.

The hardness of a metal fiber can confirm its presumed microstructure. Therefore, after the microscopic determination of the thickness of the layers and the cross-sectional shape of the samples of metal fibers in the polished state, the micro hardness of various layers—for example, various cores and casings—is determined. The hardness of the samples is determined at a load of 100 g (HV 0.1) on the cross sections of the samples. Micro hardness values are displayed as the mean of five consecutive measurements. In the case that all the samples of metal fibers have approximately the same chemical composition, differences in micro hardness values indicate that they do not have the same microstructure [121].

After determining the morphology, shape, and hardness of metal fibers, their chemical resistance can be monitored. The standard procedure according to standard procedure EN 1811:1998 is as follows: The durability test solution is prepared using 1.00 ± 0.01 g of urea, 5.00 ± 0.01 g of NaCl, and 940 ± 20 μL of lactic acid. After stirring the reagents, 900 mL of $H_2O$ is added to the mixture, and finally the pH is adjusted to 6.50 ± 0.10 with 25% NH4OH solution. The solution must be used within three hours of preparation. Samples should be degreased before testing so that each sample is gently immersed in an anionic surfactant solution (5 g of sodium dodecylbenzenesulfonate or sodium alkylarylsulfonate) for two minutes at room temperature. It is then washed with deionized water and dried. After degreasing, the samples must not be touched without protective gloves. This procedure is performed for all items used.

Degreased, dried, and weighed samples prepared in a series of three independent sampled parts of the original sample (n= 2 or 3) must be placed in a container with a solution of artificial sweat according to EN 1811:1998 E (approximately 1 mL of solution per $cm^2$ of sample), closed, and left without stirring in a thermostatic bath or oven at 37 ± 2 ºC for 168 hours (one week). The sample is then removed and washed in $H_2O$, after which the solution is quantitatively transferred to a volumetric flask previously washed with nitric acid. The eluted analyte is expressed in micrograms per square centimeter per week. For example, objects that come into direct and prolonged contact with human skin should not release more than 0.5 μg of nickel metal ions $cm^{-2}$ $week^{-1}$ according to the recommendations of many toxicologists. As an example of the results of persistence, graphical presentations are given in Figures 2.31 and 2.32. Figure 2.31 shows the amount of eluted zinc ions from metal fiber samples depending on the exposure time in the test solution and Figure 2.32 presents the comparison of the detected metal ions $Mn^{2+}$, $Ni^{2+}$, $Si^{4+}$, $Sn^{2+}$, and $Zn^{2+}$ that were eluted from the same metal fiber sample [122].

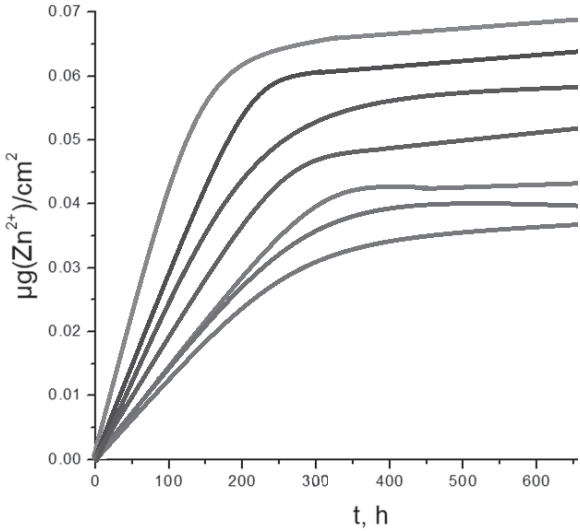



Figure 2.31. Graphical presentation of eluted metal ions $Zn^{2+}$ depending on time, presented in hours, on seven different metal samples.

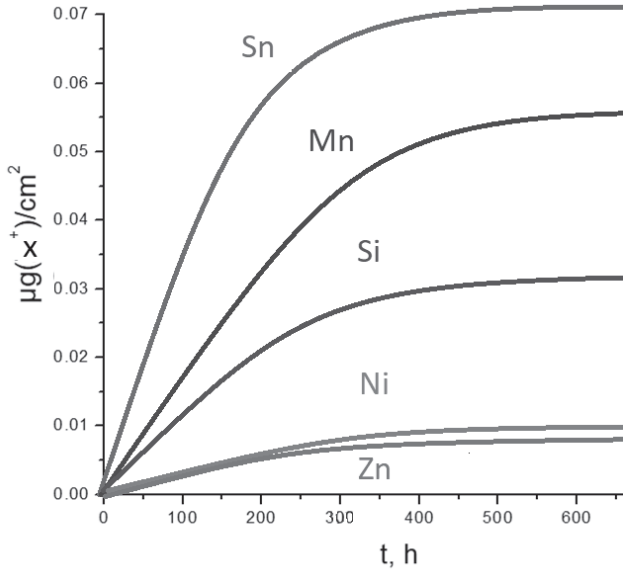



Figure 2.32. Detected metal ions $Mn^{2+}$, $Ni^{2+}$, $Si^{4+}$, $Sn^{2+}$ and $Zn^{2+}$ eluted from the same metal fiber sample.

As can be seen from Figure 2.32., the amount of eluted samples decreases in the following order: $Sn^{2+} > Mn^{2+} > Si^{4+} > Ni^{2+} > Zn^{2+}$. These results need to be confirmed from the data obtained after spectroscopic elemental analysis by using methods described in part 2.1 of this book [123–55].

The total amount of metal ions can be calculated as a sum of all eluted ions, and this parameter is important in order to determine the constant of the speed of corrosion of historical samples:

$$\left(\sum \mu g M^{n+} / cm^2\right)^2 = K_p \cdot t \tag{17}$$

where
$K_p$ ($\mu g^2 cm^{-4} s^{-1}$) presents the parabolic corrosion constant
$t$ (s) is the time of corrosion, and
$\sum \mu g M^{n+} / cm^2$ is the total amount of eluted metal ions per surface unit.

The values of the parabolic corrosion constant and the corresponding values of the correlation coefficients can be calculated from the slope of the linear curve of the graphical dependence of the square of the total amount of eluted ions on the exposure time for historical metals.

If the kinetic does not follow the parabolic dependence, it is defined as the linear relationship according to the

$$\sum \mu g M^{n+} / cm^2 = K_1 \cdot t \tag{18}$$

where
$K_p$ ($\mu g^2 cm^{-4} s^{-1}$) presents the linear corrosion constant
$t$ (s) is the time of corrosion, and
$\sum \mu g M^{n+} / cm^2$ is the total amount of eluted metal ions per surface unit.

In order to calculate the solubility of metal ions present in a metallic historical textile sample, the solubility is calculated according to:

$$\chi_i = \frac{A_i}{B_i} \tag{19}$$

where:
$A_i$ is the mass of eluted metal ion in artificial solution

$B_i$ is the total mass of metal ion in the sample
$i_x$ is the particular elements: metal ions of $Fe^{3+}$, $Mn^{2+}$, $Ni^{2+}$, $Si^{4+}$, $Sn^{2+}$, or $Zn^{2+}$

It can be noted that the metal ions' solubility is directly proportional to their standard electrode potentials ($E_0$). Some examples of standard metal potentials are given in Table 2.19.

**Table 2.19. Standard electrode potentials of metal ions eluted from fibers.**

| Metal | System | $E_0$, V |
|---|---|---|
| **Mn** | $Mn^{3+} + e^- \rightarrow Mn^{2+}$ | 1,5000 |
| **Fe** | $Fe^{3+} + e^- \rightarrow Fe^{2+}$ | 0,7710 |
| **Si** | $SiO_2 + 8H^+ + 8e^- \rightarrow SiH_4 + 2H_2O$ | 0,5160 |
| **Ni** | $Ni^{2+} + 2e^- \rightarrow Ni$ | -0,2570 |
| **Sn** | $Sn^{2+} + 2e^- \rightarrow Sn$ | -0,1375 |
| **Zn** | $Zn^{2+} + 2e^- \rightarrow Zn$ | -0,7626 |

In addition, the chemical resistance can be monitored through the mass loss of the samples, as well as through the decreasing of the thickness of the samples. A graphical presentation of the mass loss of different metal fibers is presented in Figures 2.33 and 2.34, and the changes in the thickness of the outer fiber diameter are shown in Table 2.20.

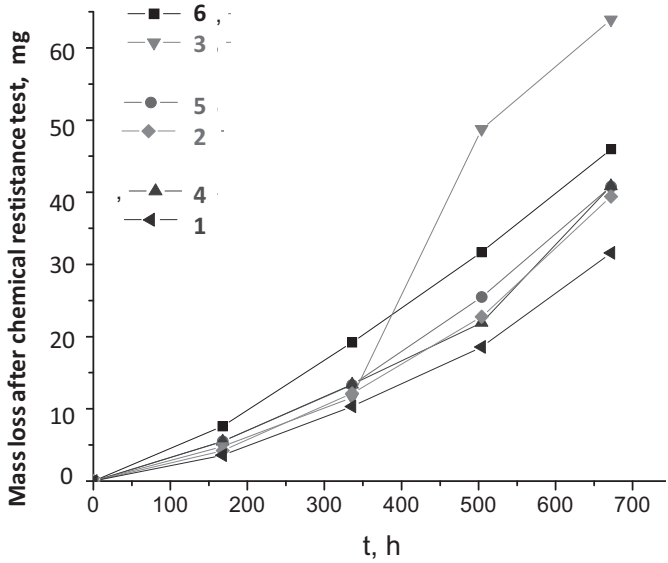



Figure 2.33. Mass loss of metal fibers during the investigation of chemical resistance.

Figure 2.33 shows significant differences between the mass loss of metal fibers with different morphologies. The metal fibers with the largest surface and the largest diameter have shown the biggest mass loss, and, in contrast, the smallest samples had the smallest mass loss.

Of particular note is sample 4, in which after 320 hours significant degradation occurred. This can be explained by the total decomposition of metallic fibers of small diameter, in which the corrosion process changed from linear to non-linear behavior.

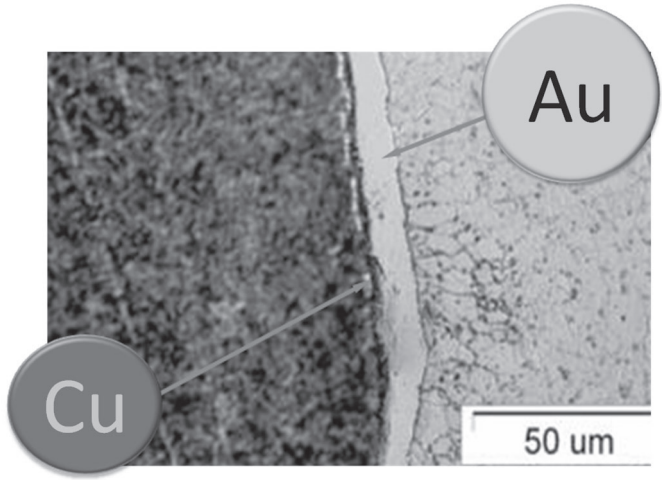



Figure 2.34. Typical difference between metal layers on historical metal fibers (metal threads: the copper inner layer is usually coated with an Au or Ag outer layer).

**Table 2.20. The changes in the thickness of the outer fiber diameter after corrosion and the investigation of chemical resistance.**

| Sample | Metal outer layer coating (d), μm | | d, μm |
|---|---|---|---|
| | before | after | |
| **1** | 7.65 ± 0.55 | 5.63 ± 0.67 | 2.02 |
| **2** | 5.00 ± 0.20 | 3.58 ± 0.42 | 1.42 |
| **3** | 5.16 ± 0.66 | 2.93 ± 0.34 | 2.23 |

As can be clearly seen in Figure 2.34, optical microscopy is useful in the identification of the morphology of historical textile fibers. In this part of the book, the morphology of metallic fibers was described by presenting the wonderful micro-world of metallic historical samples with golden or silver cover layers. Nevertheless, the most important usage of optical microscopy in the analysis of historical textiles is the identification of

textile fibers that are natural polymers of cellulose or protein structure. This analysis is presented in the following chapter.

## 2.3 TEM, SEM, and SEM-EDX investigations

Historical textile materials are beautiful and complex samples that need to be characterized and identified by different microscopic methods: light microscopy/optical microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

A full insight is obtained through the combination of different methods, and not by use of a single procedure, since every method has certain limitations. For example, optical microscopy has limited resolution and depth of field, while SEM enables only the investigation of the sample surface (and a thin layer underneath in which the electron beam can penetrate). Moreover, the degradation of historical textiles depends on many parameters that need to be characterized by microscopical methods. For example, dyeing materials and procedures, bleaching, wear, usage, and degradation influence the degradation of materials. Moreover, other materials in which historical textiles come into direct contact can be responsible for enhancing the degradation of textiles—from metals that enhance the corrosion processes to microbial presence, as well as the influence of the thick fillers of inorganic compounds that were used on paintings from medieval history.

Those questions are easily answered by applying OM, SEM, or TEM methodologies. High-resolution optical microscopy and TEM methodology enable the high-resolution study of textile fibers. These enable the observation of the cortex cells, the shape of pigment granules, and similar small items.

However, the SEM and TEM investigations require special sample preparation. The steps for preparing the historical textiles for SEM investigations are presented in Figure 2.35 (sample cross-section) and Figure 2.36 (sample fiber), while the procedure for TEM investigations is presented in Table 2.21 (Jørgensen and Frederiksen 2007).

From the problems related to TEM sample preparation, it can be concluded that high-resolution optical microscopy and SEM microscopy equipped with the EDX detector are very favorable tools for the determination of historical textiles, by enabling the detection of many different layers, coatings, and materials, as well as sample morphology and chemical characterization.

**Table 2.21. Sample preparation for TEM investigations**

| | |
|---|---|
| | SAMPLE REQUIREMENT |
| Sample yarns | 0.5 cm |
| | SAMPLE FIXATION |
| Solution | 0.5% glutaraldehyde + 2% paraformaldehyde |
| pH | 7.0 |
| Time | 15 hours |
| Temperature | 5°C |
| | SAMPLE RINSING |
| Solution | 0.1 M phosphate buffer |
| Time | 20 min |
| Repetition | 3 times |
| | SAMPLE 2nd FIXATION |
| Solution | 1% osmium tetroxide |
| pH | 7.0 |
| Time | 2 hours |
| Temperature | room temperature |
| | SAMPLE 2nd RINSING |
| Solution | 0.1 M phosphate buffer |
| pH | 7.0 |
| Time | 15 min |
| Repetition | 2 times |
| Water rinsing | 20 min |
| | SAMPLE DEHYDRATION |
| Solution | acetone |
| Concentration | 30 to 100% |
| Time | 20 min |
| | SAMPLE AND EMBEDDING |
| | SAMPLE EVALUATION |

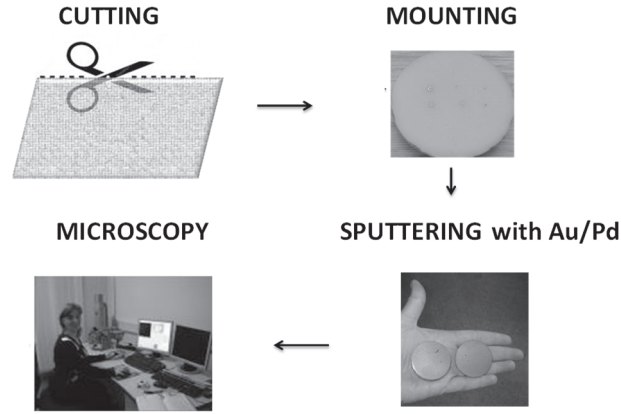



Figure 2.35. Sample crosscut preparation for SEM investigations.

As can be seen from Figure 2.35., in order to obtain the cross cut of the historical materials (no matter whether it is a historical textile fiber or a metallic part), it is necessary to mould it into the resin prior to the analysis. Nevertheless, when the surface of the horizontal sample needs to be investigated, this step is not necessary. In this case, only the sputtering with the Au or Pt thin layer is performed (Figure 2.36).

Sputtering enables the high-resolution SEM characterization of non-conductive samples. Moreover, the SEM enables observation of the cuticle cells on the fiber surface, even on historical and degraded items. If these results are compared with the TEM possibilities, it can be seen that cuticle cells present in historical mummies are lost during sample preparation for TEM analysis, by following many steps previously described in Table 2.21. Therefore SEM analysis is needed prior to the TEM. Nevertheless, the TEM enables one to monitor the granules of natural pigments that can be partially of completely dissolved, and the ultra structure of protein or the cortex of cellulose fibers.

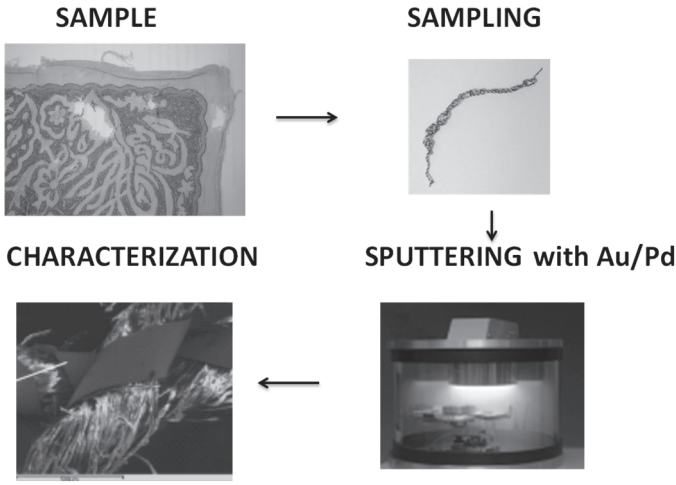



Figure 2.36. Historical textile fibre preparation for SEM investigations.

SEM analysis of historical textile fibers coated with metal stripes (called metal threads) for their morphology, chemical composition, and identification of core fibers is presented in Figures 2.37–2.39.

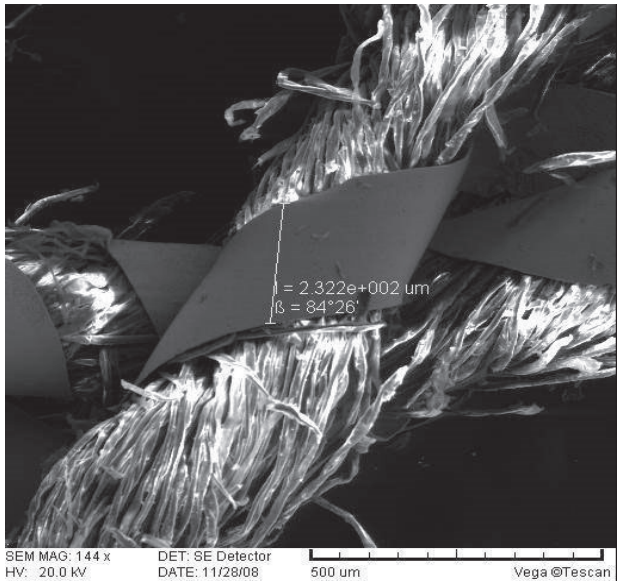



Figure 2.37. Historical textile metal fiber morphology detected on a cotton inner core characterized for its dimensions by SEM analysis.

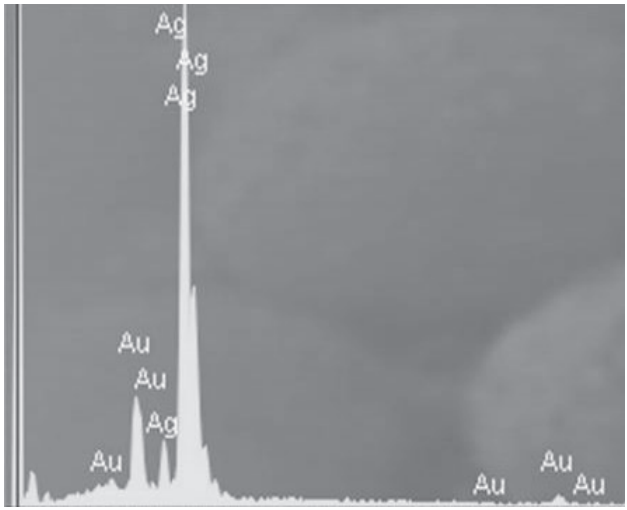



Figure 2.38. Historical textile metal fiber surface chemical composition obtained by EDX analysis after SEM characterization.

## 2.4 Identification of natural fibers on historical textiles

Figure 2.39 clearly shows the difference between the morphology of historical textile fibers and in this way enables their characterization.

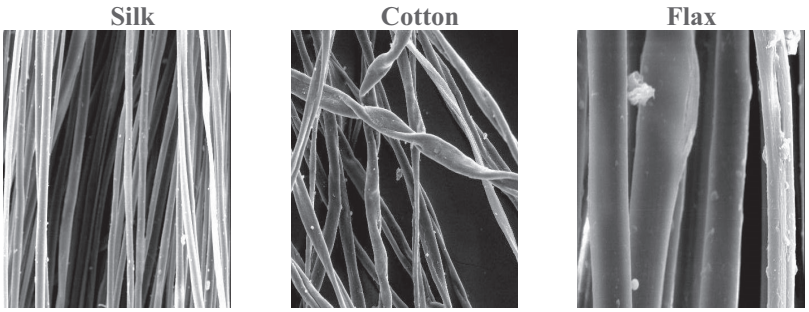



Figure 2.39. Identification of historical textile fibers using SEM microphotography, magnification 400×.

As can be seen from Figure 2.39, the silk fibers are plain, the cotton fibers have characteristic curves, and the flax fibers are larger and have characteristic morphological marks.

Through these morphological differences, historical textile fibers can be easily distinguished and identified. This can be performed by optical microscopy or SEM analysis (Figure 2.40). More detailed descriptions of historical fibers and figures for identification of unknown samples can be found in different Atlas books. In addition, specific chemical tests (like dry mineralization of dyeing) can be used.

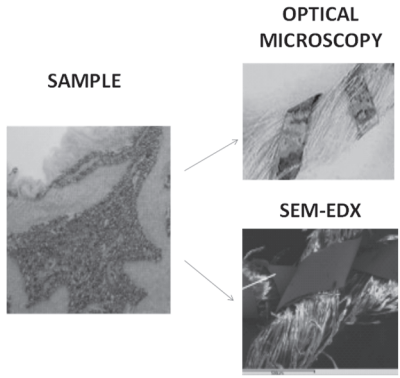



Figure 2.40. Identification of historical textile fibres using SEM microphotography.

## 2.5 Sample Preparation of Historical Textiles

### *2.5.1 Sampling, sample preparation and sample pretreatment*

The most important step of analysis, which is often emphasized in the chemical literature, is sampling and sample pretreatment. The reason for this is that errors performed in the first part of sampling cannot be removed in additional trials. Therefore, sample pretreatment is one of the key steps prior to the actual analytical determination and is also the most error-prone part of the analysis.

The goals of sampling and sample pretreatment are:

- to take up the representative sample without further destruction of valuable historical materials
- to isolate the analytes of interest from the matrix in the representative sample.

During this step the goal is to improve the:

- accuracy,
- detectability,
- reliability,
- reproducibility, and
- selectivity of the analysis as much as possible.

The sample preparation is dependent on the nature of the sample. There are many differences between the analysis of pure textile materials compared with the analysis of textile fibers wrapped with metal fibers (Figure 2.41), textiles from graves or mummies, or painted textile carriers of valuable artistic work.

Moreover, the analytical method to be used needs to be predefined in order to allow proper sampling and sample pretreatment. For spectroscopic investigation of macro, micro, and trace-element analysis, homogenization and drying are usually the first steps in the sample treatment. The second step could be followed by an extraction procedure.

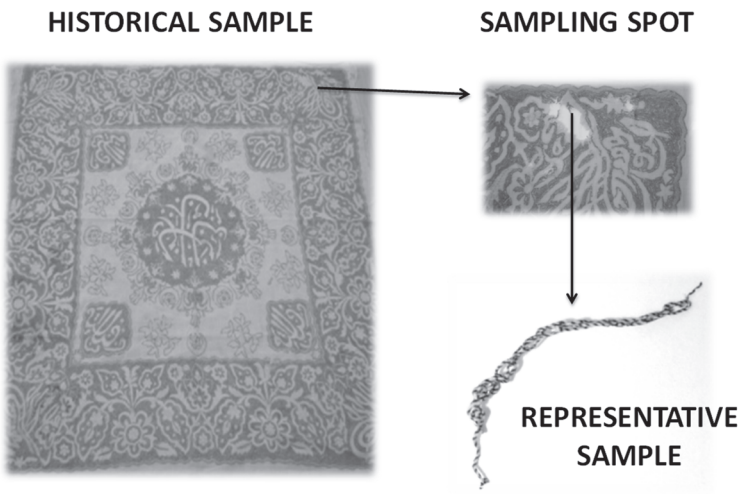



Figure 2.41. Sampling of historical textile material to obtain a representative sample that contains all the important information of the total sample.

Losses of analytes need to be prevented. Those can occur during the sampling, sample pretreatment, or sample processing, especially in cases where the sampling procedure is a complex task. In all cases, the goal of the sampling, the sample pretreatment, and the sample preparation is that the total amount of analyte in the original historical textile is transferred as efficiently as possible.

The general strategy for the sample pretreatment of historical textile materials prior to spectroscopic and chromatographic investigation includes:

1. Sampling and drying of the sample.
2. Digestion and dissolution and digestion of insoluble species.
3. Filtration, extraction, and hydrolysis.
4. Chromatographic separation and spectroscopic identification and quantification.

### *2.5.2 Digestion of historical textiles*

Digestion is a process in which a solid historical sample is decomposed into a form suitable for analysis (spectroscopic or chromatographic). [112]. Digestion is classified as either dry or wet ashing. In dry ashing, no liquid is involved, while in wet ashing, strong liquid acids are used—typically nitric acid, hydrochloric acid, hydrofluoric acid, or perchloric acid, or their

mixture [113]. For the elemental analysis of inorganic species, acid digestion and microwave-assisted digestion are recommended methodologies [34, 113].

Closed-vessel microwave heating techniques are the most often technique used for sample preparation. Microwaves, like all forms of electromagnetic radiation, have an electrical component as well as a magnetic component.

The microwave portion of the electromagnetic spectrum is characterized by wavelengths between 1 mm and 1 m, and correspond to frequencies between 100 and 5000 MHz, but the instruments applied in chemical laboratories use a fixed frequency of 2450 MHz (2.45 GHz) and are used for acid digestion (for AAS, ICP-OES, and/or ICP-MS determination), for hydrolysis of proteins (before the chromatographic determination of binding media on painted textile surfaces), for dry ashing or for solvent extractions (prior to the determination of dyes on historical samples). Organic matter is digested in an acidic reaction:

$$MeC + (CHN)_x + n\ HNO_3 \leftrightarrow MeNO_3 + CO_2 + H_2O + n\ NO_x \qquad (20)$$

Microwave energy has an energy of 0.0016 eV; it is strong enough only to rotate the molecules and does not cause breaking of the bonds between them. Two specific mechanisms of interaction between molecules of historical sample materials and microwaves occur:

1) dipole interactions and
2) ionic conduction.

Dipole interactions occur with polar molecules when the polar ends of a molecule tend to align themselves and oscillate in step with the oscillating electrical field of the microwaves. Therefore, the more polar samples, the more effectively it will be influenced by the microwave field.

Ionic conduction occurs since the ions can couple with the oscillating electrical field of the microwaves, so the effectiveness of microwave heating is a function of its concentration.

Conventional wet digestion has many drawbacks: it is carried out in vessels containing the sample as well as a large volume of decomposition reagents—from 15 to 100 mL. This mixture is heated for long periods using a hot plate, heating mantle, or oven. Moreover, there is a large potential for contamination of the sample, as well as the possible exposure of the analyst to corrosive fumes.

Closed-vessel microwave digestion offers many advantages: small amounts of reagents, typically 10 mL, and very fast results (10 to 15 minutes), high temperature, retention of volatile elements, better digestion,

low contamination possibilities, and low acid and solvent consumption, which is economically and ecologically favorable. The higher temperatures achieved in the closed system give microwave digestion a kinetic advantage over hot plate digestion, as described by the Arrhenius equation:

$$\frac{d\ln k}{dT} = \frac{E_a}{RT^2} \tag{21}$$

Integration of this equation gives:

$$\ln \frac{k_2}{k_1} = \frac{E_a}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{22}$$

In this expression, $k_1$ and $k_2$ are rate constants for the reaction of interest at $T_1$ and $T_2$, respectively, $E_a$ is the activation energy, and $R$ is the ideal gas constant. These equations show that the reaction rate increases exponentially with increasing temperature. This translates into approximately a 100-fold decrease in the time required to carry out a digestion at 175°C when compared to 95°C digestion.

Today standard microwave procedures have been developed and approved by numerous standard methods organizations. Twenty-one methods are currently approved, or in the process of being approved, by the Association of Official Analytical Chemists (AOAC), American Society for Testing and Materials (ASTM), the United States Environmental Protection Agency (US-EPA), Standard Method, and French and Chinese national methods for either microwave drying or microwave acid dissolution [35].

### *2.5.3 Extraction of historical samples*

Extraction is a process of moving one or more compounds from their matrix to another phase. Extraction techniques can be divided into:

1. Liquid extraction techniques:
Liquid / liquid and liquid / solid extraction
Soxhlet extraction
Pressurized liquid extraction
Microwave-assisted solvent extraction
Sonication-assisted solvent extraction
Supercritical fluid extraction

2. Trapping on solid (ad)sorbent:
- Solid-phase extraction
- Solid-phase micro-extraction
- Stir bar sorptive extraction
- Matrix solid-phase dispersion

For the extraction of inorganic species, solvent extraction, ion exchange, volatization, and precipitation are the most common techniques [34–37]. Mackenzie [37] has given a useful classification of the four basic classes of metal extractants and has compared these extractant classes on the basis of structure, extraction chemistry, and the metal species extracted. According to his work, which follows the paper by Sudderth and Kordosky [38], the four classes of metal extractants are: chelating extractants, ion pair extractants, neutral or solvating extractants, and organic acid extractants. For example, palladium and platinum can be extracted by alkyl amines and dialkyl sulfides [39], while nickel as well as neodymium can be extracted by di(2-ethyl-hexyl) phosphoric acid [40]. Cobalt can be extracted by alamine 336 in meta-xylene [41], copper by hydroxyoximes [42], and zinc and cadmium by an ion exchange process with the TPEN (N,N,N',N', tetrakis[2-pyridylmethyl] ethylenediamine) masking agent [43]. These metals are frequently used in dyes and pigments on historical textiles.

In liquid-liquid extraction, according to [34], the liquid sample is extracted with a solvent that is immiscible with the sample solvent, but in which the analytes of interest are soluble. The extraction can be performed manually in an extraction funnel, automatically in a sample vial, or in a continuous liquid–liquid extraction system. In liquid–solid extraction, analytes from a solid sample are extracted with the solvent. The extraction of an analyte from one phase into a second phase is dependent upon the partition coefficient of the analyte. The partition coefficient is dependent on the chemical nature of the analyte of the two solvents, as well as on the temperature. The increasing of the ionic strength or the addition of suitable additives can in many cases enhance the extraction.

In microwave-assisted solvent extraction, the term microwave refers to alternating current signals with frequencies between 300 MHz ($3\times10^8$ Hz) and 300 GHz ($3\times10^{11}$ Hz) [44]. In microwave-assisted solvent extraction (MAE), microwave radiation is utilized to heat the extraction solvent and the sample. The microwave energy affects molecules through ionic conduction and dipole radiation. For successful extraction, the solvent must absorb the radiation and pass it to the sample molecules in the form of the energy (heat). Suitable solvents are polar solvents like water,

methanol, or acetone that contain a high dipole molecule moment. Sometimes they are mixed with non-polar solvents like hexane or toluene. The MAE extraction is performed in a closed vessel system in which the pressure increases so the solvent is heated even above its atmospheric boiling point, which is the difference when compared with conventional heating. This type of microwave heating has a number of advantages over the conventional heating process: the heat is formed directly and rapidly in the sample so the energy is not wasted in heating the surrounding area (such as oil baths or furnaces). The entire volume of the vessel can be uniformly heated while selected volumes of the sample can be selectively heated. The extraction procedure is typically performed with 20 to 50 mL of solvents and the time of the extraction ranges from 10 to 40 minutes. MAE can be applied both for solid or liquid samples, although it is typically used for solids. It can be also applied for decomposing or oxidizing the organic compounds [34].

Basically, two types of MAE system can be used, an open-focused or a closed vessel system. In the open system, sample vessels are irradiated sequentially, while in the closed-vessel system the vessels are irradiated simultaneously. In a closed vessel, the temperature can be higher than the atmospheric boiling point of the solvent and the extraction, but this requires special apparatus. Some of these effects are derived from superheating or hot spots, well known effects for microwaves. Superheating is the phenomenon in which a liquid is heated to a temperature higher than its standard boiling point, without actually boiling. This can be caused by rapidly heating a homogeneous substance while leaving it undisturbed (so as to avoid the introduction of air bubbles at nucleation sites). Because a superheated fluid is the result of artificial circumstances, it is meta stable, and is disrupted as soon as the circumstances abate, leading to the liquid boiling very suddenly and violently [45]. The benefits of MAE are speed, economy, effectiveness, simplicity, and consistency due to precise software-based control of all reaction parameters, including time and extraction parameters. MAE is applicable to the extraction of different organic and inorganic compounds, and is adopted as a standard method in many cases [46, 47].

In ultrasound-assisted extraction (USE), also called sonication-assisted extraction (SAE), the acoustic vibrations are applied to the sample with frequencies of 20 kHz. When those vibrations are transmitted through the liquid, bubbles with a negative pressure are formed, and this phenomenon is known as cavitation [104–5].

Ultrasonic irradiation of aqueous solutions induces acoustic cavitation into liquid media: when an ultrasonic wave passes though a liquid, the

wave's oscillating pressure can cause a cavitation phenomenon that involves the generation, growth, oscillation, splitting, and implosion of numerous tiny gas bubbles called cavitation bubbles. As a result of cavitational bubble implosion, extreme temperatures and pressures are generated at the centre of the collapsed bubble, which results in solute thermolysis as well as the formation of hydroxyl radical and hydrogen peroxide. When a cavitating bubble collapses near the surface of a solid sample particle, microjets of solvent, propagated toward the surface at velocities greater than 100 $ms^{-1}$, cause pitting and mechanical erosion of the surface, which leads to particle rupture (i.e., disruption), and consequently, to smaller particle size [48]. Chemical compounds and particles are mechanically removed from the matrix surface and adsorption sites by the shock waves generated when the cavitation bubbles collapse. Furthermore, the implosion of the cavities creates microenvironments with high temperatures and pressures, which accelerate the extraction [34]. Ultrasound produces radicals in liquids due to the high temperatures and pressures experienced locally when a bubble collapses. Sonochemistry is performed by using a bath or with a high power probe. Cavitation enhances chemical reactivity in a number of systems. In addition, in reactions that use solids, ultrasound breaks up the solid pieces from the energy released from the bubbles created by cavitation collapsing through them. This gives the solid reactant a larger surface area for the reaction to proceed over, increasing the observed rate of reaction [49].

As in MAE, the SAE extraction system can be a static open-focused, closed, or dynamic system. The use of dynamic extraction can be advantageous, since the analytes are removed as soon as they are transferred from the solid matrix to the solvent. Moreover, in a dynamic system the sample is continuously exposed to the solvent [50]. The extraction typically requires 20–200 mL of solvent and the extraction time ranges from 2 to 20 minutes [51]. SAE can be used for both liquid and solid samples, and for the extraction of either inorganic or organic compounds [52]. Solid samples are deposited in a suitable vial and extracted for 15–30 minutes, so that the extract is separated from the solid residue. Potential problems are readsorption of the analyte onto the solid, heating of the sample and thus loss of the extraction solvent, and the decomposition of the analyte due to zones of high energy [34]. The following parameters that influence cavitation, and hence analyte extraction, are important: frequency and intensity of ultrasound, type of solvent, bubbled gas, and external temperature and pressure. One of the main drawbacks of an ultrasonic bath is that the ultrasound wave needs to cross the vessel sample container, which results in lower cavitation

efficiency and thus lower analyte extraction. Additionally, the ultrasonic electromagnetic transducer is often situated in the centre of the bath, as the cavitational efficiency is variable inside the bath [48].

One of the first applications of ultrasonic extraction to heavy metals dissolution for multi-element analysis was performed by Harper et al. [52]. In his investigation, a NIST SRM was subjected to ultrasonic irradiation in acid mixtures at different bath temperatures and durations, following a simplex optimization procedure. In a more recent study, Sanchez et al. [53] investigated the use of UE for the analysis of the heavy metals Cd, Cr, Cu, Mn, Ni, Pb, and Zn in several European reference materials. UE in aqua regia, followed by atomic spectroscopy, was used to determine the above heavy metals in BCR CRM 146: the values determined were all very close to the certified values.

A modified simplex optimization procedure was used to determine optimum bath temperatures and sonication periods according to [54]. UE in a mixture of aqua regia and hydrofluoric acid, followed by atomic spectrometry, was used to determine the same suite of metals in BCR CRMs 320 and 141. The values obtained from this investigation were comparable to the reference values, thereby demonstrating the utility of this extraction technique. Numerous studies have demonstrated the usefulness of ultrasound for effective extraction and the single or multi-element determination of heavy metals [55].

Ultrasonic extraction is today recognized as a useful method for analyte extraction from different biological and environmental samples, and more and more investigations are reported on UE heavy metals extraction. El Azouzi et al. [56] used the following liquid media in the USLE of several metals from mussel tissue using an ultrasonic bath: $H_2O$, HCl, $HNO_3$, $H_2O_2$, and their mixtures. The best reported recoveries were obtained for the combination of $HNO_3$, HCl, and $H_2O_2$ for Cd (95%), Cu (100%), and Cr (54%), the sonication time needed to be as long as 180 minutes. In contrast, Minami et al. [57] investigated the quantitative analyte extraction of Cd, Cu, Mn, and Pb from different biological materials in 1 M $HNO_3$ with only 5 minutes of sonication time.

Bermejo-Barrera et al. [58] reported a multivariate optimization of ultrasonic-bath-induced acid leaching for the determination of trace elements in seafood products by AAS. The variables studied by the authors were particle size, acid and/or oxidant reagent, acid leaching volume, exposure time to ultrasound, temperature of ultrasonic water bath, and frequency of the ultrasound energy. The elements for which optimal acid-leaching variables were researched were As, Cd, Co, Cr, Mn, Pb, Se,

Zn, Ca, Fe, Hg, and Mg. As a general rule the authors found that particle size could be considered not to be a significant parameter (sample size <30 and >300 μm). They also found the exposure time (range 10–120 minutes) to ultrasound not to be a significant parameter for all cases except for Se, so the sonication time of 10 minutes was chosen. Finally, they found that the temperature of the water-bath was not significant for most cases studied.

Other researchers have come to different conclusions: Santos et al. [59] found different arsenic extraction efficiencies from mussel samples when the sonication vessels were situated in different positions in the same ultrasonic bath. In the same way, Nascentes et al. studied the analyte extraction (Ca, Mg, Mn, and Zn) efficiency of vegetable samples in an ultrasonic bath as a function of horizontal and vertical positions, water volume, temperature, and detergent concentration (i.e., with detergent in the water the ultrasonic transmission performance is better performance) [60]. UE is a fast, inexpensive, and efficient alternative to conventional leaching processes [55]. It is expected that use of UE for sample preparation purposes in environmental analytical chemistry will become more widespread owing to its simplicity, ease of use, speed, and enhanced safety when compared with other, more traditional sample preparation procedures [61].

### *2.5.4 Electrochemical methods and determination of sample pH*

Electrochemical methods are, according to the definition, groups of analytical procedures in which data (e.g., concentration, activity) on a particular molecular species are obtained on the basis of mutually proportional electrical quantities, that is, with the help of electric voltage or electric charge. The difference in the flow of electric current, which is the charge flow, should be noted; however, whereas in metal conductors the charges are electrons, in electrolytes there is a flow of positive and negative ions in the solution moving in different directions towards the electrodes.

Electrolytes are substances that dissociate into ions in aqueous solutions and therefore conduct electricity, and the conductivity depends on all the electronic species present. For some examples of standard electrode potentials determined under standard conditions of 25°C, 1 atm pressure, and for 1 molar solutions presenting half reactions of reduction on cathode, see Table 2.22.

**Table 2.22. Standard electrode potentials determined under standard conditions.**

| Cathode (Reduction) | $E^{\circ}$/V | Cathode (Reduction) | $E^{\circ}$/V |
|---|---|---|---|
| $Li^{+}(aq) + e^{-} \leftrightarrow Li(s)$ | −3.04 | $Ni^{2+}(aq) + 2e^{-} \leftrightarrow Ni(s)$ | −0.23 |
| $K^{+}(aq) + e^{-} \leftrightarrow K(s)$ | −2.92 | $Sn^{2+}(aq) + 2e^{-} \leftrightarrow Sn(s)$ | −0.14 |
| $Ca^{2+}(aq) + 2e^{-} \leftrightarrow Ca(s)$ | −2.76 | $Pb^{2+}(aq) + 2e^{-} \leftrightarrow Pb(s)$ | −0.13 |
| $Na^{+}(aq) + e^{-} \leftrightarrow Na(s)$ | −2.71 | $Fe^{3+}(aq) + 3e^{-} \leftrightarrow Fe(s)$ | −0.04 |
| $Mg^{2+}(aq) + 2e^{-} \leftrightarrow Mg(s)$ | −2.38 | $2H^{+}(aq) + 2e^{-} \leftrightarrow H_2(g)$ | 0.00 |
| $Al^{3+}(aq) + 3e^{-} \leftrightarrow Al(s)$ | −1.66 | $Sn^{4+}(aq) + 2e^{-} \leftrightarrow Sn^{2+}(aq)$ | 0.15 |
| $Zn^{2+}(aq) + 2e^{-} \leftrightarrow Zn(s)$ | −0.76 | $Cu^{2+}(aq) + e^{-} \leftrightarrow Cu^{+}(aq)$ | 0.16 |
| $Cr^{3+}(aq) + 3e^{-} \leftrightarrow Cr(s)$ | −0.74 | $Cu^{2+}(aq) + 2e^{-} \leftrightarrow Cu(s)$ | 0.34 |
| $Fe^{2+}(aq) + 2e^{-} \leftrightarrow Fe(s)$ | −0.41 | $Fe^{3+}(aq) + e^{-} \leftrightarrow Fe^{2+}(aq)$ | 0.77 |
| $Cd^{2+}(aq) + 2e^{-} \leftrightarrow Cd(s)$ | −0.40 | $Ag^{+}(aq) + e^{-} \leftrightarrow Ag(s)$ | 0.80 |

The referent electrode is an electrode the potential of which is known and completely independent of the analyte concentration. The standard potential of an electrode is defined as the standard electromotive force of a cell in which one of the electrodes is a standard hydrogen electrode.

The ideal reference electrode should have the following properties: it has a known and constant potential, independent of the composition of the solution, its design is simple, and its potential does not change when small currents pass through it.

The standard hydrogen electrode is a universal reference electrode according to which the potentials of all other electrodes are expressed. It consists of platinum immersed in a solution of hydrogen ions of activity 1 through which hydrogen gas is passed under a pressure of 101 325 Pa. The redox potential of the standard hydrogen electrode is taken as 0. Due to the complexity of maintenance, the standard hydrogen electrode is often replaced with secondary reference electrodes whose potentials are determined in relation to the hydrogen electrode. Therefore, the calomel (*mercury(I)chloride*) and *silver/silver chloride* electrodes are the most commonly used reference electrodes.

The use of reference electrodes is extremely important in testing the physicochemical properties of historical textile materials; they are most often used for pH measurement and the measurement of individual ionic species with ion-selective electrodes. Ion-selective electrodes are electrochemical sensors whose potential (in conjunction with the corresponding reference electrode) depends on the logarithm of the activity of the test ion in solution. The glass pH electrode is the first and most commonly used ion selective electrode.

A pH value is expressed as the negative logarithm of the concentration, (i.e., the activity) of hydrogen ions in solution under standard conditions; the following equation is relevant to standard electrode potential:

$$E = E_0 - 0.059 \cdot pH \tag{23}$$

The contact pH of a historical textile sample is often measured at the location of the sampling and before sample preparation, prior to any other treatment procedure.

## 2.6 Validation of analytical procedure

Chemical analysis can be a qualitative analysis (determining which ingredients/materials are present in the sample) or a quantitative analysis (determining the amount of individual ingredients present in the sample). Instrumental methods of analysis are based on the interaction of molecules with different forms of energy and are divided into spectroscopic methods, chromatographic methods, electrochemical methods, thermal methods, and other methods. The most important methods that have application to historical textile materials are, as previously described, spectroscopic, chromatographic, electrochemical, and microscopic methods.

The choice of method is an extremely important step of the analysis. A large quantity of data on the material of the sample (origin, condition, type of material, etc.) are required to select the method of work and conduct the analytical process. The choice of method is conditioned by the nature of the sample material, the physical and chemical characteristics of the material, e.g., its corrosivity or the presence of microorganisms. It is extremely important to know the quantity of the sample available for analysis, and the place and time of the sampling. Therefore, the choice of analytical method depends on many different factors: the nature of the sample being tested, the nature of the analyte monitored in the sample, the analyte concentration (with 5–100% defining macro ingredients, 0.01–5% micro ingredients, and less than 0.01 % trace elements).

After selecting the analytical methods available, the analytical procedure consists of the following stages: defining the problem, obtaining a representative sample, preparing the sample for analysis, conducting the necessary chemical separations, measuring, calculating results, and presenting and interpreting data.

The analysis itself also has several important steps:

1) Setting the analytical task by studying the origin of the sample, the analysis plan, and the choice of the method of work.
2) Selection of a suitable validated method in accordance with the available sample quantity, the required duration, and the cost of the analysis.

3) Taking a representative sample, taking care not to contaminate the samples. In the case of taking samples of historical textile materials from graves, care should also be taken to protect the health of the persons conducting the sampling. Very often, such samples are subjected to chemical or physical processes prior to sampling that remove the microbiological hazard (such as radiation or bromination of the samples).
4) Sample preparation, moisture content control, dissolution, dissolution, extraction, analyte separation, and enrichment in biological materials.
5) Final measurement, interpretation of analytical information, and evaluation of measurement results.

The validation of the analytical procedure is the process of proving that an analytical method is acceptable for its intended purpose. In general, it includes specificity, accuracy, precision (repeatability, reproducibility), limit of detection, limit of quantitation, linearity, analytical working range, and robustness [73].

The **specificity** is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically these might include impurities, degradants, matrixes, and so on.

The **accuracy** of an analytical procedure expresses the closeness of agreement between the values that are accepted either as conventional true values or accepted reference values and the value found. It can be expressed in terms of absolute of relative systematic errors:

$$error_{xi} = \pm \left| x_i - \hat{X}' \right|$$
$$relative\ error_{xi} = \frac{\pm (error_{xi})}{\hat{X}'} \times (100) \tag{24}$$

where $x_i$ is the individual measured value and $\hat{X}'$ is the value held as true.

The **precision** of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision is usually expressed as the variance, standard deviation, or coefficient of variation of a series of measurements [74]. It is a measure of the spread in the observed analytical results due to random errors and is calculated from:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \overline{X})^2}{N-1}} \tag{25}$$

where $x_i$ is the individual measured value, $\overline{X}$ the mean of the measured values, and $N$ the number of measurements. The variance σ is defined as the standard deviation squared ($\sigma=s^2$). Precision is commonly reported as a relative standard deviation (RSD), which is the standard deviation expressed as a percentage of the mean value of the analytical results:

$$RSD = \frac{s}{\overline{X}} \times 100 \tag{26}$$

The relative standard deviation increases as the analyte concentration decreases [75]:

$$\%RSD = \pm 2^{(1-0.5\log C)} \tag{27}$$

RSD depends on the type of analysis performed and on the sample matrix: in the most rigorous quality control it should be less than 1%, in biological samples 10–15%, and in the textile samples 2–20 %. It also depends on the analyte concentration as is shown in table 8.

**Table 2.23. RSD limits according to the AOAC recommendations and medium efficiency.**

| Analyte in % | Analyte ratio | RSD (%) | Medium efficiency (%) |
|---|---|---|---|
| 100 | 1 | 1.3 | 98–102 |
| 10 | $10^{-1}$ | 1.8 | 98–102 |
| 1 | $10^{-2}$ | 2.7 | 97–103 |
| 0.1 | $10^{-3}$ | 3.7 | 95–105 |
| 0.01 | $10^{-4}$ | 5.3 | 90–107 |
| 0.001 | $10^{-5}$ | 7.3 | 80–110 |
| 0.0001 | $10^{-6}$ | 11 | 80–110 |
| 0.00001 | $10^{-7}$ | 15 | 80–110 |
| 0.000001 | $10^{-8}$ | 21 | 60–115 |
| 0.0000001 | $10^{-9}$ | 30 | 40–120 |

**Repeatability** expresses precision under the same operating conditions over a short interval of time, while **reproducibility** expresses the precision between laboratories**. Intermediate precision** expresses variations within laboratories: different days, different analysts, and different equipment, and so on [74, 75].

The **limit of detection** (LOD) is defined as the lowest amount of an analyte that can be detected in a sample. According to the International Union of Pure and Applied Chemistry (IUPAC), LOD is defined as follows: The limit of detection, expressed as the concentration $c_L$ or the quantity $q_L$ is derived from the smallest measure, $x_L$, that can be detected with reasonable certainty for a given analytical procedure [76]. The value of $x_L$ is given by the equation:

$$x_L = x_{bi} + ks_{bi} \tag{28}$$

where $x_{bi}$ is the mean of the blank measures, $s_{bi}$ is the standard deviation of the blank measures, and $k$ is a numerical factor chosen according to the confidence level desired, usually (3σ). In the past, IUPAC has recommended that k=3, although many atomic absorption spectroscopy and inductively coupled plasma optical emission practitioners still sometimes use k=2. A common misconception is that LOD is the smallest concentration that can be measured. Instead, it is the concentration at which we can decide whether the element is present by distinguishing a signal from the background. Several approaches for determining the LOD are possible depending on whether the procedure is non-instrumental or instrumental:

*1. Visual evaluation*:
LOD is determined from the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

*2. Signal-to-noise ratio*:
This determination is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected.

*3. Standard deviation of the response and the slope*:
The limit of detection may be expressed as:

$$LOD = \frac{3.3\sigma}{S} \tag{29}$$

where $\sigma$ is the standard deviation of the response and $S$ is the slope of the calibration curve.

The slope $S$ may be estimated in a variety of ways:

a) based on the standard deviation of the blank (measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses);
b) based on the calibration curve (a specific calibration curve should be studied using samples containing an analyte in the range of LOD). The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation. An alternative method of computing the LOD using the background equivalent concentration (BEC) and the RSD of the blank gives:

$$c_L = 3\ RSD_{blank} \times BEC/100 \tag{30}$$

The **limit of quantitation** of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. Quantitation is generally agreed to begin at a concentration equal to 10 standard deviations of the blank, and therefore LOQ = 3.3 LOD. Several approaches for LOQ determination are also possible, depending on whether the procedure is non-instrumental or instrumental. Visual evaluation, signal-to-noise ratio, or standard deviation of the response and the slope, as well as other approaches, may be acceptable.

The **linearity** of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

The **range** of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

The **robustness** is a measure of the method's capacity to remain unaffected by small but deliberate variations in method parameters. The ability to provide timely, accurate, and reliable data is central to the role of

analytical chemists.

The best way to minimize method problems is to perform adequate validation experiments during development [77].

Several protocols and guidelines for analytical method validation have been published, for example:

- the standard is that of the ISO (International Organization for Standardization, 1994),
- the IUPAC protocol (1993)
- the guide *The Fitness for Purpose of Analytical Methods* (Eurachem, 1998), or the
- ICH guidelines (International Conference on Harmonization, 1994). The core standard is ISO/IEC Guide 17025, *General Requirements for the Competence of Testing and Calibration Laboratories*.

The last emphasizes which methods should be validated: not validated methods, methods developed in the same laboratory, methods applied outside their intended usage, and methods the usage of which has been broadened [78].

This standard is linked to many other ISO standards, which are summarized in table 9, that give more detail in specific areas, for example, calibration, proficiency testing, statistics, and so on [34].

**Table 2.24: ISO standards related to analytical quality management.**

| ISO standard | Specific area |
|---|---|
| ISO 17025: 1999 | General requirements for the competence of testing and calibration laboratories |
| ISO 32: 1997 | Calibration in analytical chemistry and the use of chemical reference materials |
| ISO 33: 2000 | Use of certified reference materials |
| ISO 34: 2000 | General requirements for reference material procedures |
| ISO 5725: 1996/2001 | Accuracy (trueness and precision) of measurement methods and results |
| ISO 10011: 1990/1 | Guidelines for auditing quality systems |
| ISO 11095: 1996 | Linear calibration using reference materials |
| ISO 9000, 9001: 2000 | Quality management systems, fundamentals and requirements |

The ISO gives a definition of **reference material** (RM): an RM is a material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process. Its use may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

**A certified reference material (CRM)** is an RM characterized by a metrological valid procedure for one or more specified properties,

accompanied by a certificate that states the value of the specified property, its associated uncertainty, and a statement of metrological traceability [79]. The combined standard uncertainty for a CRM can be expressed by the equation [80]:

$$U_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2} \tag{31}$$

where uncertainties are given for characterization ($u_{char}$ ), homogenity testing ($u_{bb}$), short-term stability testing ($u_{sts}$, which describes stability during shipment) and long-term stability testing ($u_{lts}$, which describes stability during storage) [81].

Validation involves establishing documentary evidence that provides a high degree of assurance that a specific analytical process will consistently produce results meeting pre-determined requirements [82].

The main source of **uncertainties in analytical measurements** are random and systematic deviations. Systematic deviations (bias) represent a constant or multiplicative part of experimental deviation. Random deviations result from measurements of limited precision and can be reduced by replicate measurements. To characterize random deviations, probability approaches are used, where the measurements are considered to be random, independent events. The distribution of random data can be determined from their frequency in a predefined interval, also called "class." If the number of replicate measurements is increased to infinity and the class width is simultaneously reduced, a Gaussian (normal) distribution is obtained:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu_x}{\sigma}\right)^2} \tag{32}$$

where $f(x)$ is the probability function, $\sigma$ the standard deviation, $\mu_x$ the mean, and $x$ the measurement. Comparison of a sample mean with the true mean is based on Student's t-distribution:

$$t = \frac{\left|\bar{x} - \mu\right|}{s}\sqrt{n} \tag{33}$$

The confidence interval characterizes the range of the mean of a random variable in which an observation can be expected with a given probability P or risk α = 1 - P. As a statistical factor, the t-value from Student's distribution is used. The confidence interval for the mean is calculated for *f* degrees of freedom from:

$$\Delta x = \frac{t(1-\alpha/2; f)\cdot s}{\sqrt{n}} \tag{26}$$

To compare the variances of two random samples or their standard deviations, Fisher's F-test is applied ($F=s_1^2/s_2^2$), and to determine whether the population is normally distributed, the $\chi^2$ test is performed ($\chi^2=\sum(h_i-f_i)^2/f_i$) where $h_i$ is frequency and $f_i$ the expected frequency [34].

Chemometrics is a relatively new approach, which combines mathematical modeling and chemical data, but it has already had a huge impact on the spectroscopic field to such an extent that chemometrics software is nowadays integrated with spectroscopic laboratory and process instrumentation as a standard [83, 118]. Principal component analysis, partial least squares regression, spectroscopic imaging, and multivariate image analysis are just some of the most common examples from process analytical chemistry [84, 123–26]. The aims of applying optimization and design methods in analytical chemistry can be numerous. Typical factors in analytical chemistry comprise the pH value, reagent concentration, temperature, flow rate, solvent, elution strength, composition, irradiation, atomization time, or sputtering rate. The methods are developed to serve particular problematic historical samples, or to be more suitable for new laboratories, or new methodologies.

Typical responses include analytical figure-of-merits as well as objective functions that consist of combinations of different quality criteria. Systematic optimization procedures are carried out in the following sequence:

1.) **Choice of an objective function** that is composed of several criteria, such as selectivity, sensitivity, and precision. Objective function $Z_i$ is obtained by aggregation of quality criteria $z_i$ by a weighed sum where $w_i$ is weight of criterion *i*:

$$Z_i = \sum_{i=1}^{n} w_i z_i \tag{34}$$

2.) **Selection of the most important factors** that affect a given objective function on the basis of a simple experimental design, a screening design, by means of statistical tests.
3.) **Optimization.** To find the most suitable factor combination we can distinguish between simultaneous and sequential optimization approaches.

With simultaneous strategies the relationship between responses and factors is studied by running an experimental design, constructing a mathematical model, and investigating the relationship using so-called **response surface methods** (RSM).

Very often RSMs aim to judge this relationship graphically and the consequences are drawn from these plots. If the optimal point is desired it can be found by calculating the partial derivative with respect to the individual factors or by applying a grid search over the whole response surface. Sequential strategies of optimization are based on an initial design of experiments followed by a sequence of further measurements in the direction of the steepest ascent or descent. That is, no quantitative relationship between factors and responses is evaluated but the response surface is searched along an optimal (invisible) path [34].

The two strategies are exemplified in Figure 2.42. In the case of response surface methods, the response is described by a mathematical model (dotted contour lines of the response surface). By using search methods the response is measured along a search path, here along a Simplex path.

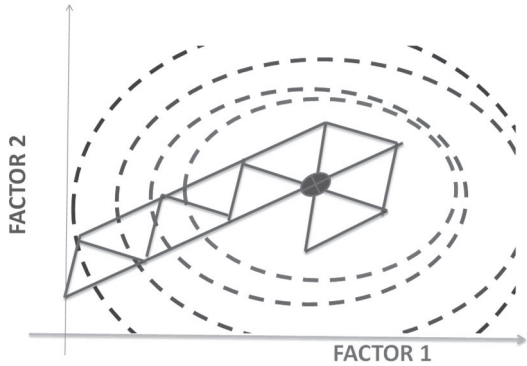



Figure 2.42. Comparison of two optimization procedures—response surface methodology and factor plot analysis.

## 2.7 Experimental design

The term "experimental design" is usually used to describe the stages of identifying the factors that may affect the result of an experiment, designing the experiment so that the effects of uncontrolled factors are minimized, and using statistical analysis to separate and evaluate the effects of the various factors involved [85, 124, 125]. Optimization of an analytical problem or of an analytical device has to be carried out by studying a limited number of factors. Very often it is easy to select the most important factors, but sometimes the effects of a particular one can only be presumed. Its effect has to be assured by a suitable screening experiment. Because of the complexity of most analytical problems, there can be additional factors that are either unknown or that cannot be controlled. Uncontrolled factors might be the impurity of reagents, the intoxication of an electrode surface, the instability of a plasma source, or the changing quality of a laboratory assistant's work. Since the study of all potential factors is usually prohibitive, the effect of selected factors will be investigated and the remaining factors should be kept as constant as possible.

During the optimization procedure, several other principles like the *replication* of measurements or their *randomization* (running the experiments in a random order) are important.

*Factorial experiments* are based on varying all factors simultaneously at a limited number of factor levels: this kind of experimentation is especially important at the beginning of an experimental study, where the most influential factors, their ranges of influence, and factor interactions are not yet known. Factorial experiments allow experiments to take place in the whole range of the factor's space. They reveal high precision for a minimum experimental effort, and they enable factor interactions to be detected.

*Confounding* of parameter estimation for different factors occurs if the factor combinations are highly correlated and, therefore, no difference between the factor effects can be detected. Confounding depends greatly on concrete experimental design. If, for example, the levels of two factors were changed in a constant ratio, it would not be possible to distinguish between the effects of these two factors.

*Symmetry*: factorial experiments should be partitioned in the whole factor space in a balanced manner. The same is true for replications in the experimental space. One reason for performing symmetrical experiments is the avoidance of confounded factor effects. In addition, symmetrical experiments may simplify data evaluation [34].

Designs on the basis of two levels for each factor are called screening designs. The most general design is a full factorial design at two levels. These designs are described as $2^k$ designs where the base 2 stands for the number of factor levels and *k e*xpresses the number of factors.

At a high factor number, the number of experiments increases dramatically and then a fractional factorial design is applied. For this experimental design, the number of experiments is reduced by a number *p* according to a $2^{k-p}$ design. If the effect of many factors is to be studied, numerous fractional factorial designs can be used. Concrete designs can be taken from tables and can be generated with most statistical software packages. Special designs for estimating only the main effects have been tabulated by Plackett and Burman.

In contrast to the simultaneous factorial design study, experimentation by varying one variable at a time is limited to the estimation of effects, and no interactions, that are common in analytical chemistry. What cannot be evaluated with screening designs are curved dependences, that is, more complicated relationships between responses and factors designs at 3 or more factor levels are needed.

In order to describe the relationship between responses and factors quantitatively, mechanistic or empirical models are used: these should be able to describe linear and curved response surfaces. (Three-level factorial designs ($3^k$) are called response surface designs.)

**Full factorial three-level designs** can be used for investigating a small number of factors (two or three), although their statistical properties with respect to symmetry or confounding of parameter estimates are less favorable than those known for the two-level designs. In the case of many factors the same problem as with two-level designs arises, that is, the number of experiments gets very high. These disadvantages led to the development of so-called optimal designs of which the central composite design and the Box-Benhken design are the most important [34].

**Central composite design** consists of a combination of a full or fractional factorial design and an additional design, often a star design. If the centers of both designs coincide they are called central composite designs. For the number of runs *r* we obtain:

$$r = 2^{k-p} + 2k + n_0 \tag{35}$$

where *k* is the number of factors, *p* is the number for reduction of the full design, and $n_0$ is the number of experiments in the center of the design. For example, for a design with three factors, the needed number of experiments is 15.

To estimate the experimental error, replications of factor combinations are necessary. For this reason the center point is usually run several times. A complete three-factor central composite design is depicted in Figure 23 (using Design Expert 6 software).

Except for the good statistical properties in the central composite design, one experimental disadvantage occurs: because of the star points outside the hypercube, the number of levels that have to be adjusted for every factor is actually five instead of three (like for instance in a conventional three-level design). In cases where the adjustment of levels is too difficult to be achieved, an alternative response surface design that can be chosen is the Box and Behnken design. In a Box–Behnken design, the experimental points lie on a hyper sphere equidistant from the center point [34].

In contrast to the central composite design, the factor levels have to be adjusted only at three levels. In addition, if two replicates are performed in the center of the three-factor design, the total number of experiments with the central composite design is 15 to 17. The disadvantage of Box–Bekhen designs is the representation of responses that depend on a single factor. This is because the corner points of the cube have not been measured but have to be computed by an appropriate response surface model [34].

Mixture designs are useful if mixtures such as eluents in liquid chromatography, or formulations in the pharmaceutical and textile industry, are under investigation. A special problem arises because additional relationships are held between the factors in an analytical investigation. The constituents of a mixture given in portions of weight, volume, or moles are confined to the assumption that the amount of the $N$ constituents sum up to 100% or (normalized) to 1. The most important mixture design for an analyst is based on a so-called $(k,d)$-lattice. For the $k$ factors, the lattice describes all experimental points having the factor levels 0, $1/d$, $2/d$ . . . $(d\text{-}1)/d$ or 1 [34]. Figure 25 shows a ternary mixture of water, acetonitrile, and hydrochloric acid used in the thin-layer chromatography experiment in which the lattice design was applied [86].

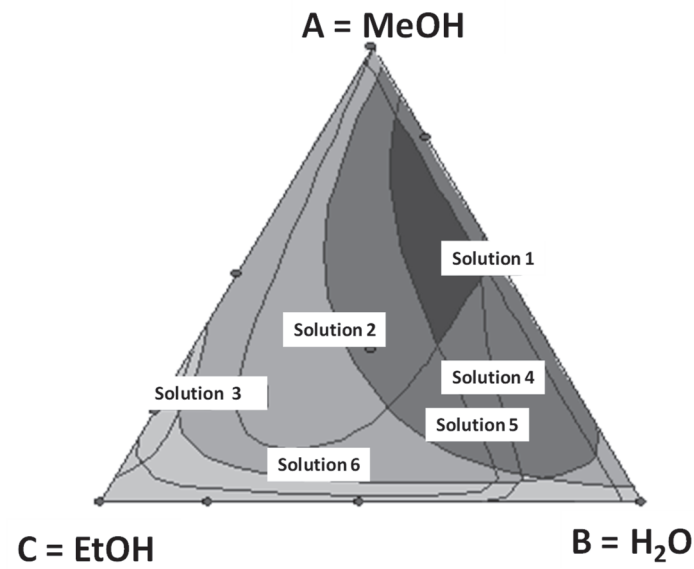



Figure 2.43. Lattice design for a ternary solvent mixture.

**Response surface methods** are useful for quantification and interpretation of the relationships between responses and factor effects. Those relationships in analytical chemistry can be based on physical or chemical models, generalized by statisticians as *mechanistic* models. Another approach would be empirical modeling, in which the parameters have no mechanistic meaning. General empirical models are second-order polynomials, where the response $y$ is related to the variables (factors $x$) as follows:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{l \le i \le j}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^{\,2} \tag{36}$$

with $k$ the number of factors (variables), $b_0$ the intercept parameter, and $b_i$, $b_{ij}$, $b_{ii}$ the regression parameters for linear, interaction, and quadratic factor effects. To estimate the parameters in equation 26, the experiments have to be carried out at three factor levels; this estimation is a general problem of least-squares estimation by linear models. The response surfaces based on the mathematical model can be explored graphically, as shown in Figure 2.44 [86].

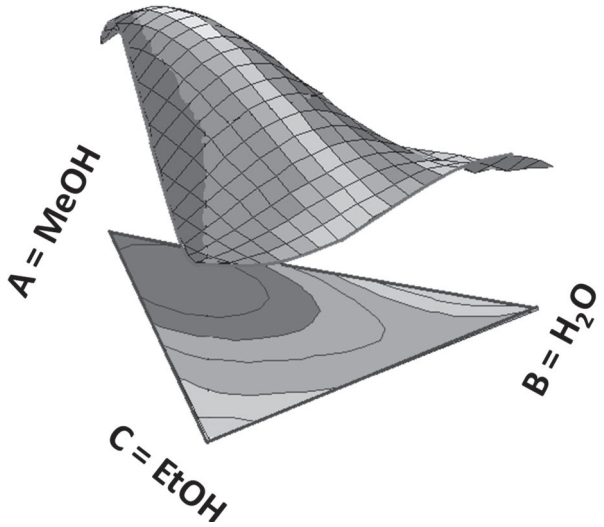



Figure 2.44. Surface and contour plots—example of response surfaces.

In the case of a large number of experiments that need to be carried out, it could be difficult to run the experiments under identical conditions, because strong systematic changes increase the overall experimental error. Their elimination can be accounted for if the changes are taken as a discrete event and the estimation of the time-dependent effects are confounded with the estimation of unimportant interactions such as a three-factor interaction [34].

Nedler and Mead developed the most common sequential optimization method based on the **simplex method**: a simplex is a geometric figure that has a number of vertices equal to one more than the number of factors. A simplex in one dimension is a line, in two dimensions, a triangle; in three dimensions, a tetrahedron; and in multiple dimensions, a hyper-tetrahedron. To find the steepest path along a response surface, an algorithm has to follow the a pattern that consists of designing an initial simplex, running the experiments at the initial vertices, and calculating the new vertex point by reflection of the vertex with the worst response. The simplex method has been the most used experimental optimization algorithm until now because of its main advantages: simplicity, speed, and good convergence properties. Problems with the simplex method arise if multimodal response surfaces are investigated (if several local optima exist). In such cases, the simplex will climb the nearest local maximum or minimum and the global optimum might be missed. Mathematical theory

provides more efficient optimization methods, such as the conjugate gradient method or Powell's method. These methods are used mainly in locating optima of mathematical functions and less often in experimental optimization [34].

In recent decades, several expert systems have been developed for practical applications in analytical chemistry and applied statistical methodologies. Existing expert systems have been explored for different purposes: the determination of appropriate statistical tests, regression analysis, and determination of the "best" experimental design. The Design Expert computer program is intended for scientific investigators and statisticians who must design and analyze complex experiments (multi-level experiments with nested factors, repeated measures, and both fixed and random effects). This system is "expert" in the sense that it is able to recognize specific types of complex experimental designs based on the application of inference rules to non-technical information supplied by the user. Secondly, it encodes the obtained and inferred information in a flexible general-purpose internal representation, for use by other program modules. This program also generates analysis of variance tables for the recognized designs and an appropriate computer programs runs files for data analysis, using the encoded information [87].

The Design Expert is a prototype expert system for the design of complex statistical experiments that can recognize randomized block designs, including lattice designs within embedded Latin squares, crossover designs, split plots, nesting, repeated measures, and covariates. It is written in an experimental programming language developed specifically for research in artificial intelligence [88].

Design Expert software version 6.0 (DX6) offers a wide variety of designs, possibilities to modify designs, evaluation capabilities, tools for response modeling, graphics for interpretation of results, and multiple response optimization. Among other designs there are two-level fractional and full designs, multilevel factorial designs, Taguchi orthogonal arrays, irregular fractions, Placket–Burman designs, response surface methods, mixture designs (such as simplex-lattice, simplex-centroid screening, and D-optimal), and combined mixture and process designs [89]. According to Alben [90], the optimization section in the DX6 program allows responses to be calculated for any combination of allowed experimental conditions. Because a large quantity of information on optimization can be produced in a very short time, the ability to visualize results is very important. In this program, results for each solution can be examined one at a time in a graph. Response surfaces and mixture designs in DX6 often use five or six levels per factor. Assigned values are not just whole integers, but also

include one-third and two-third fractions, in or beyond a specified range, to maintain geometric symmetry. Depending on the design, suggested values in a range from 2 to 4 may be 2, 2.33, 3, 3.33, 3.67, 4 or 1.66, 2, 3, 4, 4.34. These conditions are a clear departure from common experimental sequences, which vary logarithmically or by order of magnitude. The most basic decisions concern which design to use and how many levels to set for the known factors.

When performing an optimization procedure with the DX6 program, it is first necessary to specify the order of polynomials: first order polynomials only model linear behavior, while second order polynomials (called a *quadratic*) reveal two-component interactions. More complex interactions can be modeled by third-order polynomials. After this step, it is important to generate a "candidate set" with sufficient points to fit the specified model. For the mixture side of the problem, the formulators expect complex chemical problems, so they usually choose a third-order mixture model called a "special cubic." From the candidate set, the selection of the minimum number of points is performed to fit the model. The actual selection procedure involves computer-intensive matrix calculations designed to produce model coefficients of maximum possible precision [91].

A procedure for finding the optimal formulation for mixtures within DX6 is nicely described by M. Anderson and P. Whitcomb. The authors illustrate a relatively simple study on finding the optimal formulation of three surfactants as an example to show how to apply mixture design. They emphasize that with large numbers of factors only a small number of the runs need to be completed to produce estimates of main effects and simple interactions [92].

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# CHAPTER III.1

# CHALLENGES IN THE CHARACTERIZATION OF COMPLEX SAMPLES

## 3.1 Case Study 1: Investigation of Ag/Au nanolayers on metal historical fibers

Very often historical textiles contain metal fibers wrapped around the textile core. Such samples, called "metal threads," were very popular in the Middle Ages. They were used in decorative purposes on costumes, shoes, church clothing, decorative materials on dresses and jewelry, and military uniforms. [1] Due to the corrosive environment, metal fibers have a tendency to corrode. Therefore it is vital to characterize them prior to conservation to prevent the further deterioration of the textile material. During the characterization, different physical and chemical parameters of importance include the pH value of the surface, the presence of oxygen or corrosive reagents, and the environmental temperature and humidity [2].

Historical textiles with metallic wrapping around the core are very interesting archeological samples and can be found throughout history. One of the first historical alloys was samples from 4500 BC, found in a Vinča culture site in Serbia [3]. Vinča culture is considered to be responsible for the largest Neolithic settlement in Europe and one of the most advanced prehistoric cultures, dating back to more than 7000 BC.

The technology to produce the metal threads originated from China: the inside core made of textile fiber yarns (such as cotton, flax, hemp, wool, or silk) had an outside wrapping of metal strips with a dimension between 0.006 and 0.030 mm, and a thickness of 0.20–0.30 mm [4, 5].

This technology started spreading in the Mediterranean in the seventh and tenth centuries. Afterwards it conquered Europe (around the year 1000), and continued spreading around the world. The literature records that the oldest preserved example comes from the late antique era in Egypt [5]. Such very old and precious samples have had their surfaces exposed to many different chemical processes, for example corrosion and oxidation. Sometimes also adsorption of impurities, passivation, and diffusion

processes may have occured. The characterization of the surface layer is therefore important [6]. As was mentioned in the previous chapter, scanning electron microscopy equipped with an EDS detector (SEM-EDS) is a very convenient tool for the investigation of the sample surface. This methodology offers a fast and non-destructive procedure that can be applied to different surfaces, together with metal fibers: glass [7], dust [8], or ceramic parts [9, 10]. All these samples might remain almost completely preserved during the SEM investigation, which is one of the biggest advantages of this methodology [11–13]. Nevertheless, the fact that only the surface of the sample can be investigated presents a disadvantage and a significant drawback. Since the depth of the penetration of the electronic beam depends on the material that is under investigation, it can penetrate only the surface layers of the sample. For example, for soft materials such as aluminum samples, the penetration depth is around 1.25 μm, while for samples made from iron or gold, a much lesser depth than 1 μm is achieved [1]. For this reason, if information on the total chemical composition is needed, the combination of a SEM procedure with spectroscopic investigation is proposed.

With the spectroscopic method for the determination of elements present in the whole sample, and not only on the sample surface, the following methods are available:

The first choice would be to apply multi-elemental techniques: inductively coupled plasma-optical emission spectroscopy (ICP-OES) or inductively coupled plasma-mass spectroscopy (ICP-MS), which are faster due to their ability to analyze up to 70 elements in the same sample that have low limits of detection and a large linear working range.

The second choice is single element techniques: atomic absorption spectroscopy (AAS) or graphite furnace atomic absorption spectroscopy (GF-AAS), which are slower, but offer proper insight into particular elements of interest [1, 6, 14, 15].

These are used for quality control measurements, or for the determination of single elements, because the costs of the analysis are significantly lower [16, 17].

It must be emphasized that for the best results, a combination of special metallographic sample preparation with chromatographic separation or microscope techniques (OM, TEM or SEM) with spectroscopy AAS and ICP-OES is proposed [1, 18].

Chromatographic investigation provides the simultaneous separation of components in the sample, and the identification and quantification of different elements. Although the classical approach is to use chromatographic methods (such as liquid chromatography, gas

chromatography, or thin-layer chromatography) in the analysis of organic compounds, this example is one of the rare cases when thin-layer chromatography was applied in the analysis of metal samples. This methodology is very popular in conservation and restoration laboratories due to its many advantages [19–23]. With its fast investigation, low consumption of chemical and reagents, and wide variety of different stationary and mobile phases, this methodology allows one to obtain information on metals present in historical textile samples with metal threads. Moreover, the corrosion processes that occurred on the surface of such complex samples can only be determined by the combination of different analytical techniques, including chromatography, spectroscopy, and microscopic investigation [1, 24].

Case study 1 presents an investigation of Ag and Au nano-layers on Cu alloys used as metal threads originating from the nineteenth century (Croatia, Europe) [1]. Figure 3.1 presents the strategy that was used in planning and in the investigation.

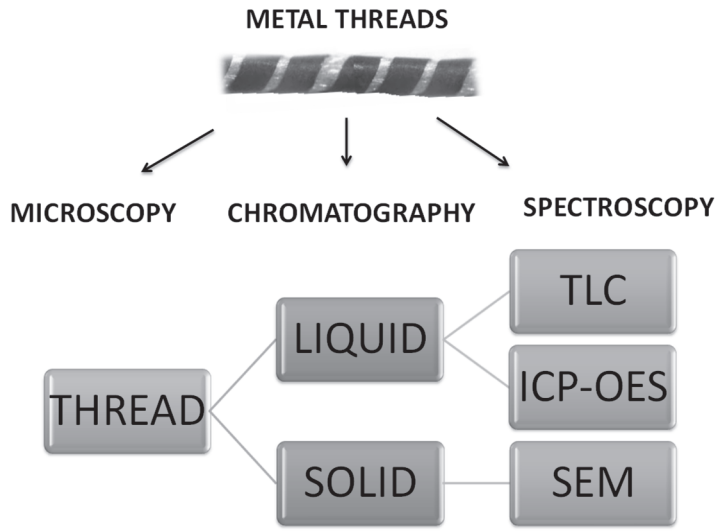


Figure 3.1. Methodology for investigation of Ag and Au nanolayers on Cu metal fiber alloys by combination of chromatographic, spectroscopic, and microscopic techniques.

As can be seen in Figure 3.1., complex historical textile samples require that the analytical procedure that will be used for their characterization is careful planned.

In this case study, the proposed strategy enables three different steps for obtaining information on:

- the simple separation and fast identification of metal ions by using thin layer chromatography,
- the quantitative analysis of total element analysis enabled by spectroscopy,
- the characterization of the sample surface by SEM and SEM EDX methods,

In the analysis of historical textiles with metal fibers, as well as in other different anthropological or archeo-metallurgical analysis, many different methods that are not destructive of the precious historical samples can be used. Such methods include:

- X-ray photoelectron spectroscopy [25],
- proton-induced X-ray emission spectrometry (PIXE) [26],
- fluorescence spectrometry [1, 25],
- a differential scanning calorimeter [27],
- scanning electron microscopy with energy dispersive X-ray spectroscopy [28, 29].

The threads of metal stripes in the form of thin sheets of metal alloys are found in archeological sites [29]. Due to their complex morphological composition, as well as their fragile textile core, their analysis can present a large analytical challenge.

The most important problem is usually related to the fact that only very small amounts of samples are available for analysis. Moreover, it has to be emphasized that such samples are precious and rare, and thus should be preserved as a valuable national heritage. Therefore only methods that are completely non-destructive, or, if that is impossible, methods that require very small sample amounts, should be applied for the analysis of such items. This chapter thus proposes the combination of chromatographic, spectroscopic, and microscopic analysis. The chosen protocol starts with preliminary investigation by means of thin layer chromatography (qualitative and quantitative TLC), continues with inductively coupled plasma-optical emission spectroscopy or mass spectroscopy (ICP-OES or ICP-MS), and finishes with scanning electron microscope with EDX detector (SEM-EDX) analysis. All these procedures have the advantage that they can be performed in a short time on small sample amounts, but with very good precision and repeatability.

The results of chemical characterization and historical materials investigation are very important since knowledge regarding long-term corrosion of historic metal objects can assist the work of conservators by providing essential answers on many important questions. Moreover, the

methodology described in this chapter may be used by anyone who needs information about the morphology and chemical composition of silver-plated and gilded-copper alloys, examples of which are not only found in historical sites.

### *3.1.1 Chromatographic investigation of historical threads*

Studies of historical textiles and other archeological artifacts improve our knowledge in the field of national heritage preservation, helping scientists prevent the deterioration of metals in museums [2]. The methods used in this work were thin-layer chromatography (TLC), inductively coupled plasma-optical emission spectrometry (ICP-OES), and scanning electron microscopy with EDS-ray diffraction (SEM-EDS).

This combination of instrumental methods should provide fast preliminary information on major and minor metal components that is useful in conservation and restoration [19]. The TLC enables sample preparation prior to ICP-OES and SEM-EDS analysis, which is beneficial before sample preparation, and before the preparation of standard solutions or before choosing the efficient instrumental parameters for particular samples. In addition, thin-layer chromatography can be used to determine the organic material that is present on historical samples in a form of natural binding media [20–22], as well as to analyze organic compounds such as amino acids resulting from proteinous binders [23]. This work therefore combines thin-layer chromatography with SEM-EDX and ICP-OES analysis of metals present in historical metal materials in order to perform qualitative identification and quantitative analysis of the metal analytes.

**Sample preparation:** samples of two metal lament alloys were taken from a historical textile (Figure 3.2). This historical material sample is a part of collection of historical materials preserved at the Faculty of Textile Technology, University of Zagreb. Both samples were prepared for analysis by dissolving 0.0500 g of the sample in 5 mL of concentric nitric acid.

Thin-layer chromatography (TLC) was applied as a simple, fast, and effective method for the determination of metals on historical samples. For visualization, several different reagents were applied: dimethylglioxime, quercetine, $NH_3$ vapors, and Alizarin red S.

The standard metal solutions needed in the development of the chromatographic system were spotted on 20 × 20 cm TLC cellulose pre-coated plates produced by Merck, Darmstadt, Germany. Samples were spotted by glass capillary; alternatively, Hamilton volumetric injections or

an automatic machine such as a Linomat can be applied. Development was, after the saturation of the chromatographic chamber with the vapors of the solvents, carried out in a Camag chromatographic chamber by the ascending technique to 8 cm distance. The mobile system acetonitrile–hydrochloric acid–water was chosen from the literature data and had the following volume ratios: 7.2 mL: 2.5 mL: 2.3 mL [19]. After the development, the plates were dried, sprayed with the detection reagents (dimethylglioxime, quercetine, alizarin red S), and exposed to ammonia vapors. The chromatograms with colored spots occurred after 37 minutes of development; the results are presented in Table 3.1.

Dimethylglioxime and quercetine were appropriate for detecting the majority of metals (dimethylglioxime for Ag, Al, Au, Co, Cr, Cu, Fe, Ni, and quercetine for Ag, Al, Au, Bi, Co, Cr, Cu, Fe, Ni), while $NH_3$ vapors revealed spots of Ag, Al, Au, Co, Cr, Cu, Fe, Ni, Mn, and Pb. Gold was visible only after exposing the plate to $NH_3$ vapors, while Alizarin red S revealed Zn, Pb, and Al. The proposed TLC method was applied for the identification of metals collected from two historical metal samples. Results showed that Au, Ag, Cu, and Ni are major and minor components in both samples, as was confirmed by SEM-EDX and ICP-OES technologies.

**Table 3.1. Detection of metal ions that were present on historical textiles by thin layer chromatography using different visualization reagents; after development by ACN: HCl: $H_2O$ (v/v = 72:25:23), on cellulosic pre-coated plates 20 × 20 cm.**

| Historical textile metal sample | Detected metals and their characteristic colors of spots | | | | |
|---|---|---|---|---|---|
| | Without indicator | Dimethyl-glioxime | Quercetine | $NH_3$ vapors | Alizarin red S |
| A | Ag, Cu | Ag, Au, Cu | Ag, Au, Cu | Ag, Au, Cu | Ag, Au, Cu |
| B | Ag, Cu | Ag, Cu | Ag, Cu | Ag, Cu | Ag, Cu |

Figure 3.2. Historical sample before cleaning, conservation, and restoration, embodied with metal threads used in chromatographic investigation.

Table 3.1 presents the obtained results of the investigation of metal threads that were composed of a textile core but that contained metal fiber wrapping around the core. After dissolution in nitric acid, the metals were analyzed by chromatographic separation and identification. The obtained TLC results of the investigated historical materials are compared in Table 3.1.

Therefore it can be concluded that the samples contained outer layers of silver and gold that were coated on the inside Cu core. Thus, chromatography enabled the efficient identification of metals in the samples. However, the full characterization of the historical samples can be performed only in combination with other spectroscopic and microscopic procedures. Therefore, the second step included testing of solid samples by ICP-OES, and the last step was the application of the SEM-EDS methodology.

### *3.1.2 Spectrometric investigation of historical metal threads*

The ICP-OES analytical procedure has many advantages, among the most significant of which are the following: low detection limits and the possibility of analyzing 70 elements in the same sample [6, 14–17].

Therefore it can be concluded that the combination of microscopy SEM-EDX and spectroscopy (such is ICP-OES or ICP-MS) is a very good analytical tool for monitoring metals present in historical textiles and the metal threads on them [18]. Nevertheless, their combination with thin-layer chromatography is, as far as the author knows, rarely used, but can be efficient in fast routine analysis.

**Table 3.2. Elemental composition in (w/w) of the outer layers of samples determined by EDS analysis.**

| Sample | Ag | Au | Cu |
|---|---|---|---|
| **A** | 83 | 17 | <LOD |
| **B** | 5 | <LOD | 95 |

**Table 3.3. Elemental composition in (w/w) of the outer layers of investigated samples determined by ICP-OES.**

| | w,% | | | | | |
|---|---|---|---|---|---|---|
| Sample | Ag | Au | Cu | Si | Sn | Zn |
| **A** | 0.1 | 0.02 | Rest | <LOD | <LOD | <LOD |
| **B** | 0.2 | <LOD | Rest | 1.4 | 0.02 | 0.02 |

** Other investigated metals were present under the limits of detection (< LOD).*

### *3.1.3 Microscopic investigation of historical threads*

Scanning electron microscopy with an EDS detector is a fast and non-destructive method. Therefore this methodology, as well as other high-resolution procedures (AFM, TEM, OM) are often applied in the analysis of historical textiles.

The most significant advantage of SEM-EDX is that it is a non-destructive procedure. For this reason, valuable historical textile samples can be returned to their original sites in museums and be preserved after the investigation.

Therefore it is no surprise that SEM-EDS was already reported to be an efficient tool for the analysis of alloys and glass [7], powder samples [8], and ceramics [9]. Such investigations are important since alloy composition strongly influences the material properties [10–13]. Nevertheless, it has to be emphasized that in addition to SEM-EDS, many other methodologies can be efficiently applied in the analysis of metals from historical materials.

The results of SEM-EDS are presented in Figure 3.3. These results were obtained from solid samples and present the elemental composition but *only* of the sample surface. It is crucial to understand that the electron beam does not penetrate deeply under the sample surface, which depends

on the material investigated. Soft metals like aluminum can be more deeply penetrated than others, but in all cases the results will be the average composition of the samples' surfaces. Therefore it *cannot* be expected that these results will be in full agreement with the preliminary results obtained by TLC, or the results obtained by ICP-OES methodology.

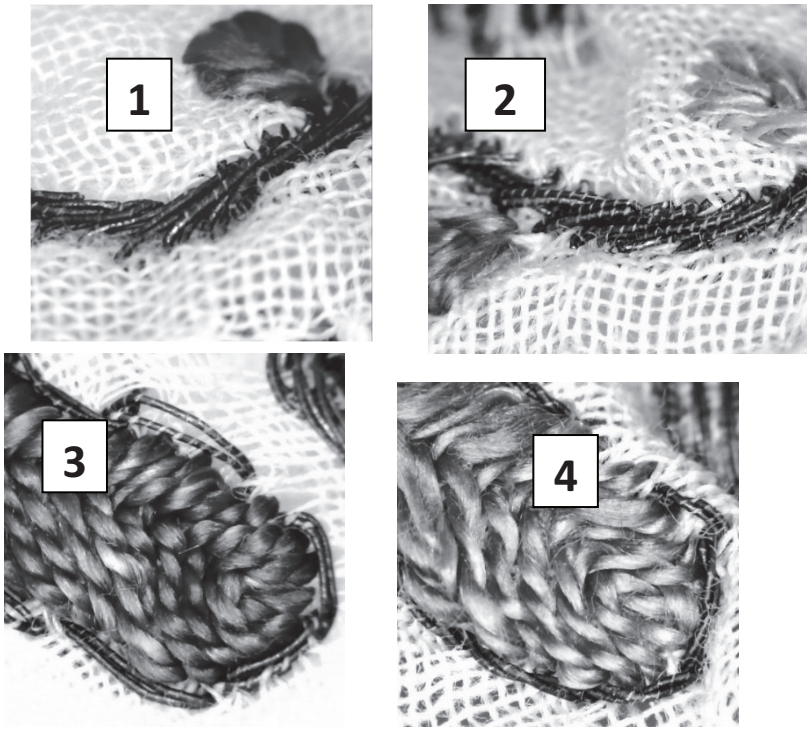


Figure 3.3. Historical samples with metal threads/fibers (containing Au and Ag nano-layers on Cu alloys) that was investigated by optical microscopy and SEM-EDS methodology, recorded under 100× magnification.

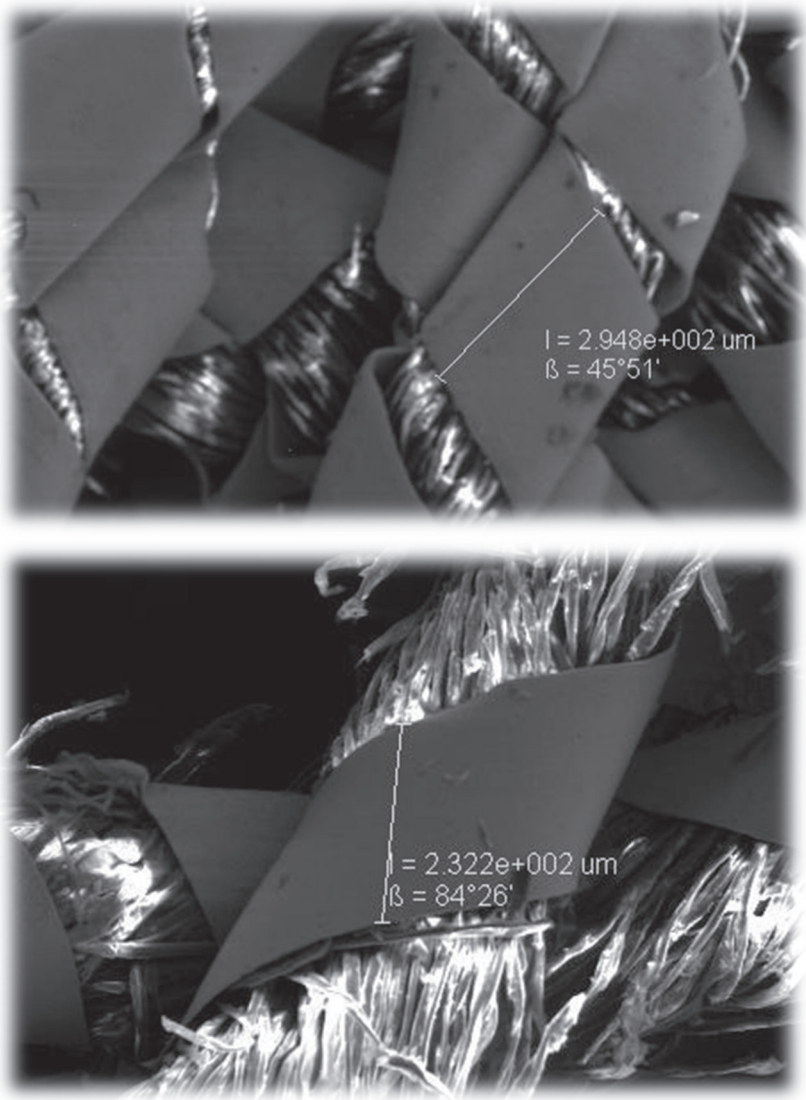


Figure 3.4. Determination of sample size by microphotography. Recorded under 1000× magnification.

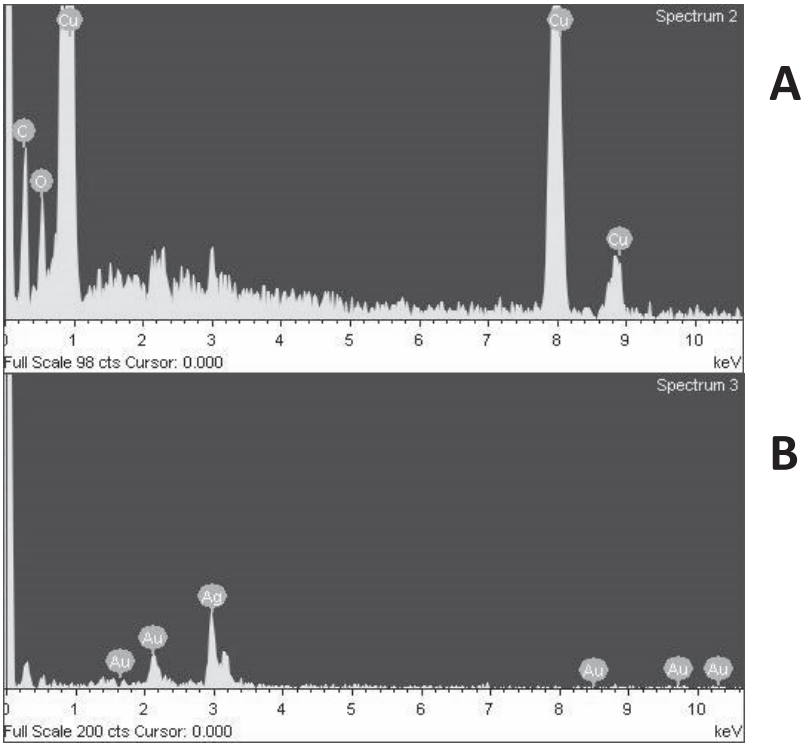


Figure 3.5. EDS investigation of layers on sample 1: A) Cu was detected as the inner part of the historical textile sample. As can be seen, it was detected without traces of protective Au and Ag outer layers, due to extensive corrosion that occurred on the surface of the samples. B) The other sample had preserved protective Au and Ag layers, so those could be easily detected.

The advantage of the SEM-EDS investigation was that it reveals the sample morphology (Figure 3.4). Moreover, as can be seen from Figure 3.5, it enables the fast and simple determination of the chemical composition of the sample surface. Last but not least, it can reveal the corrosion process if it has occurred somewhere on the sample surface.

The investigation of textile fibers in the inside core of historical textile materials wrapped with metal fibers can also be performed during an SEM-EDS study.

Figures 3.6 and 3.7 present microphotographs of the textile core fibers recorded under SEM microscopy during investigation of historical textiles.

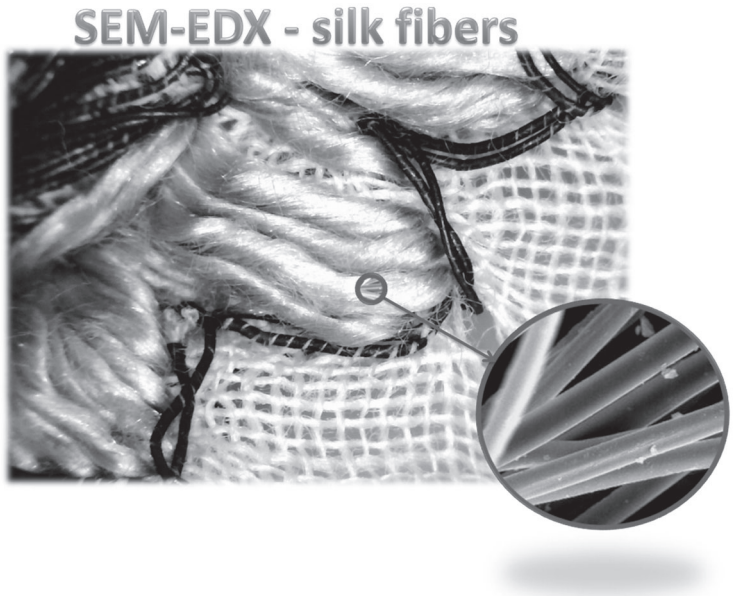


Figure 3.6. Silk fibers detected by SEM microphotography.

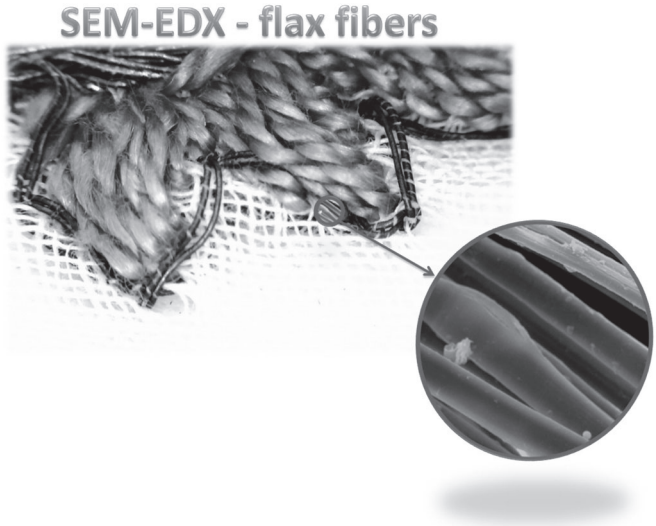


Figure 3.7. Flax fibers detected in the second sample by SEM microphotography.

As can be seen from Figures 3.6. and 3.7., during the microscopic investigation of the inner core, the results revealed that the inner textile fibers were made of silk and flax. In addition, SEM-EDS could be applied in the investigation of corrosion processes that occurred on historical metal materials, as will be explained in the second subchapter.

When comparing the results of the SEM-EDS investigations presented in Figure 3.5, it can be observed that they are completely in agreement with the preliminary results obtained by TLC that are listed in Table 3.1. The differences in methodologies are because it is possible to detect the presence of minor components in the surface of the samples by microscopic investigation.

For example, by using SEM-EDS methodology, the presence of Au in sample 1 was easily detected, which was not the case using TLC due to the corrosion of the samples. Therefore it can be concluded that SEM-EDS is a useful methodology for obtaining the relevant information required to understand corrosion processes and products formed on the surface of metals, as well as for the proper planning of the most adequate cleaning, conservation, and restoration steps [32].

The proposed methodology can be foreseen in developing new testing methods or in combination with automated instrumentation equipped with sophisticated mathematical models and chemometrical tools. Only through method development can we reach more precise results and faster investigations [23, 33].

The biggest drawback of TLC procedure is its high limit of detection of particular metal ions; thus, this methodology was unable to detect the micro-components in historical samples. On the contrary, ICP-OES and SEM-EDS provided information on the presence of trace elements, but SEM-EDS revealed only the presence of elements under the sample surface. Elements that were present under the upper layer of historical materials that could not be detected by SEM-EDS were metals like lead, nickel, manganese, silica, tin and zinc.

During the quantitative comparison of the detected elements a big difference occurred. For example, the differences in the results detected for gold (Au) obtained by SEM-EDS and ICP-OES were significant; for example, the first sample, A, which contained 0.12 wt% of silver, obtained SEM-EDS results much higher than the normal value (it was found to be around 83 wt %). It must be emphasized that this is because the Ag was present only in the outer layer sample of the historical textile. Furthermore, in the case of the same sample A, the gold was detected by microscopy and spectroscopy, but with strong disagreement between the obtained results: while SEM-EDS detected 17 wt % of gold, its real mass ratio was

only 0.02 wt %, as was proved by the spectroscopic investigation.

Nevertheless, SEM-EDS analysis is a very suitable methodology for the investigation of historical samples. First of all, it is a nondestructive method; second, it does not require tedious sample preparation and is therefore very convenient for the preliminary analysis of valuable historical samples [30, 31]. Moreover, it enables the determination of sample morphology, the characterization of the inner core of textile fibers, as well as the detection of the corrosion processes that occurred on historical metal threads. In addition, the results have shown that the proposed methodology is sufficient and efficient for providing the data necessary for the complete characterization of historical metal alloys.

The identification of nano-layers and the alloys used in different materials, as well as the characterization of inner materials and the investigation of corrosion process that have occurred on some historical samples can be used as model examples in the analysis of modern fibers and many different modern materials [34, 35]. After the characterization of historical samples, the documentation is performed, and information on the progress of corrosion processes is obtained in order to make decisions on the proper cleaning and preservation of valuable and precious samples [36, 37].

To conclude, this chapter presented the methodology for revealing the chemical composition of small metal samples collected from historical textile materials using TLC, ICP-OES, and SEM-EDS techniques. This combination was proved to be an efficient approach, while TLC revealed major compounds, ICP-OES provided a full insight into minor and trace elemental composition, and SEM-EDS detected sample morphology and surface structure. It also proved to be a non-destructive method that detected the spots of the corrosion processes that started on historical materials. The samples of interest were copper alloys containing thin layers of Ag and Au on their surfaces. The combination of all these chromatographic, spectrometric, and microscopic methods enabled fast, easy, and user-friendly investigation, and therefore can be recommended for laboratories and institutes that deal with the characterization of metals present in metal alloys in historical textile samples.

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# CHAPTER III.2

# CASE STUDY 2: CORROSION OF HISTORICAL METAL FIBERS

Historical textiles decorated with metal stripes, have been used for the decoration of historical textile materials. Therefore such materials were found in the production of clothing and as decorations on tapestry, embroidery, and lace [1]. The special combination of a metal strip that is carefully wound around a textile core fiber (made of natural fibers like cotton, flax, silk, and wool) is called a *thread*. Metal threads were made from metals (Ag, Au, and Cu) in combination with vegetable or animal fibers. Although there are many different methods of production, the most common was a technique in which the threads were drawn from a metal wire to the required diameter and length. These dimensions were as follows: the width was around 0.25 mm and the thickness was around 0.02 mm [2, 3].

Metal threads of this design can be found on many different decorative textiles, woven into the textile material. They rarely have a protective function, and much more frequently were signs of authority, wealth, and social and cultural importance. As we can detect, the metals most frequently used were gold, silver, and copper alloys; revealing the material's elemental composition can reveal its origin. For example, an alloy metal composition, as well as gold versus silver ratios, can be used as possible identifiers of origin and provenance.

Instrumental analysis of historical textiles that contain woven metal threads is very important for many different conservation and restoration steps of historical materials, including sampling, sample preparation, cleaning, and sample preservation [4, 5]. Accurate analytical methods provide full insight information on deterioration and corrosion processes. Such unwanted processes occur due to prolonged contact with different physical, chemical, and biological agents [6–9]. For example, a multi-elemental technique that is recommended for the analysis of metal threads is inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry [10–13]. Unfortunately, these methods

have one large drawback, and this is that they are destructive and demand the dissolution of the solid sample before the analysis can take place.

In contrast, microscopic investigation such is AFM, SEM, or TEM can be applied as a non-destructive methodology allowing the sample to be returned to the original material after the analysis [14–20]. The drawback of this approach is that the analysis is performed only on a specific and isolated part of the sample surface. Nevertheless, this information can be very useful in monitoring corrosion, providing an insight into the layer's composition and the corrosion processes occurring.

Therefore a highly sensitive analytical method is needed to properly characterize the chemical composition of ancient metal threads. The above mentioned complementary techniques provide a quantitative map of the elemental composition, which enables valuable information on the morphology of samples, such as the thickness of the fibers and metal threads, the composition of the metal coating, and the occurrence of heterogenic areas in samples, as well as the identification of surface coatings and corrosion products.

Most threads contain multiple layers: silver and gold over the copper or zinc bulk layers. This unique application of instrumental analysis is indispensable and the most efficient approach in the investigation of valuable historical materials. The characterization of materials provides data on the origin and fabrication of historical samples of metal threads, providing full insight into the cultural and historical significance of such materials. Moreover, it can be beneficial in the development of new techniques or the investigation of ancient metallurgy and gilding technology.

It was observed that due to corrosion processes, historical textile materials containing metals threads are usually in a worse condition than those that did not contain metals [5, 21–26]. This is the result of the corrosion products that cover the surface of historical materials leaving stains on and damage to the textile material. Moreover, because the metal threads are produced from copper covered with silver or gold, a galvanic corrosion occurs (Figure 3.8).

### *3.2.1. Corrosion of historical metal threads*

*Corrosion* is a general term used to describe a wide variety of interactions between a historical textile material and its surrounding environmental conditions that lead to a deterioration and lastly to full degradation. The most important interactions are those with the ambient oxygen, moisture, or water that cause the formation of oxide layers. Those

corrosive products may have negative effects on the fragile historical materials; thus, they should be carefully observed and removed from the materials.

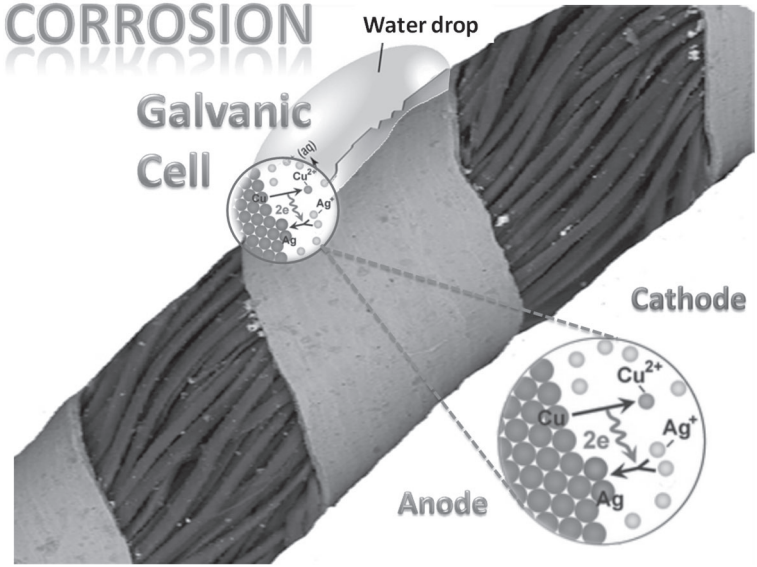


Figure 3.8. Corrosion and galvanic reaction on historical metal thread samples [28, 29, 30].

### *3.2.2. Galvanic reaction in historical metal fibers*

A galvanic reaction causes the corrosion of two metals in contact, whose driving force is a difference in their potentials. This is an electrochemical process in which one metal, in the presence of an electrolyte and another metal, corrodes due to the electrical contact. For the process of galvanic reaction to occur, one of the metals becomes the anode and corrodes faster than it would all by itself, while the other becomes the cathode. This process was discovered in the late part of the eighteenth century by Luigi Galvani and its principle was later put into a practical application by Alessandro Volta, Sir Humphrey Davy, and Michael Faraday.

In a metal pair, the less noble material is the anode where oxidation occurs. The more noble material is the cathode where reduction processes occur. Galvanic corrosion can be one of the most destructive forces to occur on historical metal fibers. A rapid reaction influences the thickness

loss of the dissolving anode. On the micro-structural level, different phases and micro-structural features can be subject to galvanic currents.

The corrosion of particular metals influences the state of historical objects. For example, silver corrodes in the presence of airborne sulfur molecules, and the copper corrodes under a variety of conditions. Therefore, one of the most critical parts of thread conservation work is a procedure of metal characterization that can prevent further damage due to pitting corrosion.

The corrosion depends on the chemical composition, morphology, and nature of the metal threads as well as on the environment to which it was exposed [27]. Cleaning methods need to be carefully chosen: for example, the methods that are suitable for metal filaments may be harmful to core yarns or to other ground materials. Since this is such a difficult task, some authors propose a laser cleaning process [21, 22]. Before classical or sophisticated laser cleaning, previous chemical characterization and chemical analysis of the metal composition is needed.

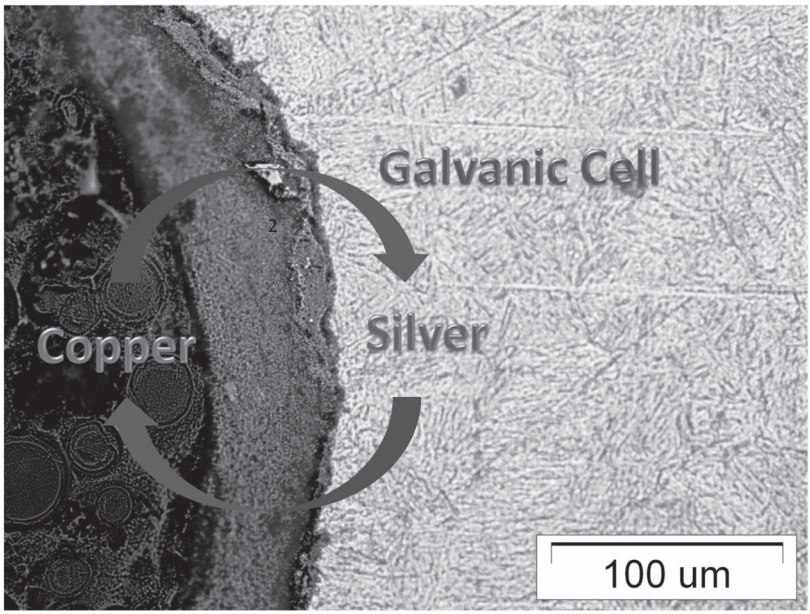


Figure 3.9. Cross cut of the copper/silver metal thread, recorded by optical microscopy [30] showing many potential spots for galvanic reaction.

Corrosion products leave stains and can damage precious historical materials. The most sensitive fibers are fragile silk and cellulose fibers. In ancient samples, silk and cellulose fibers may have decomposed through centuries of contact with corroded surfaces. Therefore the monitoring of galvanic corrosion processes needs to be performed with the goal of preserving cultural and national heritage. Chemists use sensitive and nondestructive techniques like SEM-EDS due to their sensitivity, determination of morphology of samples, and ability to perform qualitative and quantitative chemical analysis of samples without damaging historical items. The determination of alloying compounds presents a crucial step in the conservation and preservation of valuable historical materials. The goal of this chapter is to investigate the corrosion processes on metal threads collected from historical textile materials. Figures 3.9 and 3.10 show samples of metal threads taken from damaged areas of historical textiles. The required dimensions consist of threads five to seven mm in length weighing a few micrograms per sample (0.00100 to 0.30000 g of thin metal thread filaments per sample).

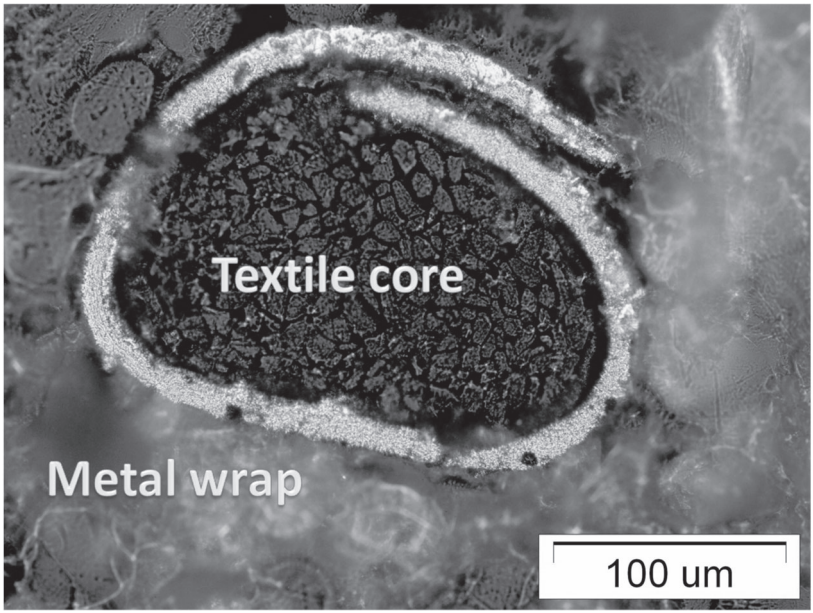


Figure 3.10. Cross cut of copper/gold metal thread, recorded by optical microscopy [30].

### *3.2.3. Pitting corrosion on historical samples*

The corrosion of metal threads is very problematic because its products may leave stains or ruin the textile carrier. A typical microphotograph of a corroded metal thread from a historical textile sample is shown in Figure 3.11. As can be clearly seen, the corroded surface has pits and holes, and is in direct contact with the core consisting of fragile textile fibers.

This is an example of pitting corrosion that occurred due to favorable conditions: the presence of the oxide layer on the surface made the material thermodynamically passive. Moreover, the environmental conditions provide aggressive ions such as sulfate and chloride due to humidity and air pollution.

Therefore it can be concluded that the corrosion of metal threads can be prevented if the valuable and precious materials are kept in special environmental conditions.

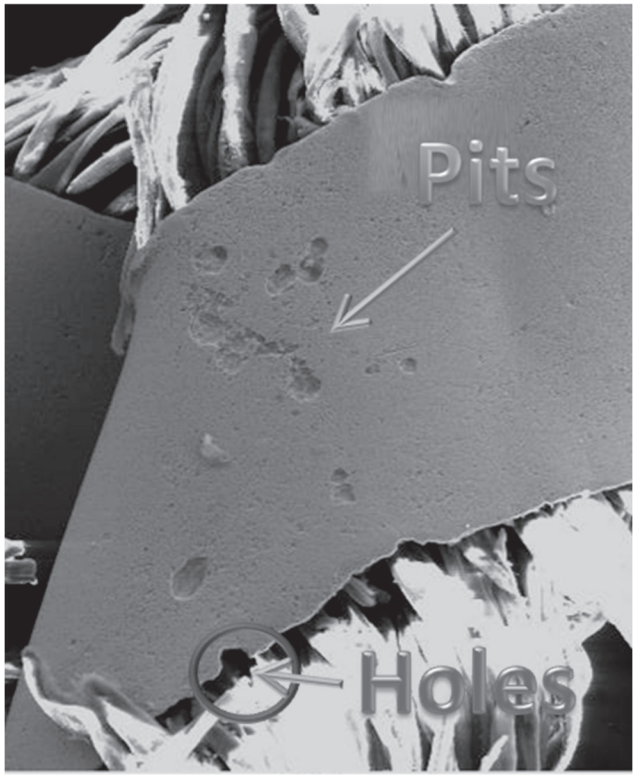


Figure 3.11. Pitting corrosion on historical metal thread.

The determination of the chemical composition of the corroded surface can reveal different layers, for example, copper in the ground layer and silver as the outer layer (Figure 3.8) or copper in the ground layer and gold in the outer layer (Figure 3.12). The most important information about the sample is obtained only after proper sample preparation, sample cleaning, and sampling procedures; these steps are the most important part of SEM-EDX. It is known that reproducible data with good statistics may be obtained only after proper sample preparation.

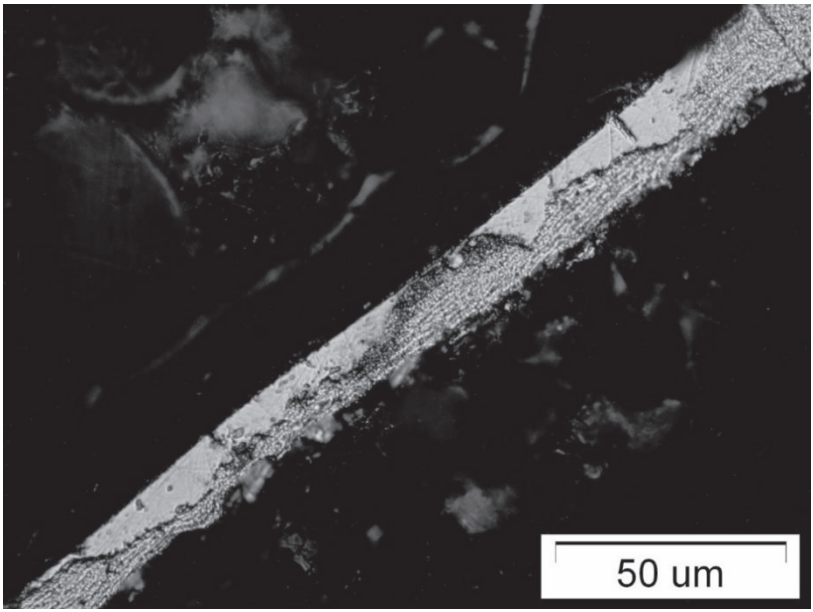


Figure 3.12. Cross cut of the copper/gold metal thread, recorded by optical microscopy [30] with a clear overview on the destroyed and corroded metal after corrosion processes.

In this chapter the problems with corrosion have been discussed, but it has to be emphasized that in some rare cases the products of corrosion may act as a protective layer covering the metallic parts on historical items. Nevertheless, in the majority of cases, the corrosion products present the biggest concern for textile conservators and restorers.

In addition, the results presented in this chapter show that AFM, TEM, or SEM-scanning electron microscopy equipped with the EDX detector (SEM-EDX) are suitable techniques for the analysis of metal threads. Such methods enable the determination of the morphology and chemical

composition of the samples. Moreover, the advantage of SEM-EDX is that it is a non-destructive method; thus, after performing the full analysis, the samples can be returned to the original historical textile sample.

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# CHAPTER III.3

# CASE STUDY 3: HOLY MUMMIES

The investigation of the holy bodies of two saints from the thirteenth and fifteenth centuries was probably one of the most intriguing tasks I have ever faced. Scientists are rarely in a position to search for answers beyond usual material paradigms. The most intriguing questions about life and death meet when investigating holy bodies. In many different parts of the world, as well as in different historical times, miracles were described and related to the mummified bodies of saints. Such an occurrence is called "incorruptibility." The question regarding performing research on such bodies is, Is it possible to detect parts of organic or inorganic substances that is preventing the corruptibility of the material body, or is there something else that cannot be described by current scientific paradigms?

The term "incorruptibility" describes the situation in which a human body does not go through the normal process of decomposition after death. This is most often explained as a sign that the individual is a saint (in **Roman Catholicism**). Parts of such saintly bodies, as well as whole mummified objects, go through detailed inspections in which the relics are sealed with wax and sent to Rome. Moreover, Catholic law allows the holy bodies of saints to be preserved under the altar, so that praying and Mass can be celebrated above the corpse.

The **Eastern Orthodox Church** makes a distinction between natural mummification and other cases that can be related to supernatural incorruptibility. Many Eastern Orthodox saints are incorrupt and are venerated: Saint Alexander of Svir, Saints Anthony, John, and Eustathios, Saint Dionysios of Zakynthos, Saint Elizabeth, Saint Gerasimus of Kefalonia, Saint Ioasaph of Belgorod, Saint Job of Pochayiv, Saint John Maximovitch of Shanghai and San Francisco, Saint John the Russian, Saint Nectarios of Aegina, Saint Parascheva of the Balkans, Saint Spyridon, and Saint Zosima. In the Catholic Church, such cases include those of Nicoloza Burza, Ivan Olinski, and Bernadette Soubirous.

It has to be emphasized, however, that embalmed bodies are not recognized as being incorruptible because the incorruptibility is seen as being distinct from good preservation of a body or from mummification. Incorruptible bodies have a sweet or floral, pleasant odor of sanctity.

Beyond Christianity, **Buddhism** speaks about **"tukdam,"** a term that is related to miracles, dying, and mummified corpses. For example, in Russia the exhumed body of Lama Dashi-Dorzho Itigilov has been miraculously resistant to decay for over 90 years, and science has reached no conclusions for the reasons behind the preservation. During a scientific investigation, it was found that the body is still very well preserved. The organs, such as the muscles, joints, and skin, are intact.

Similar miracles have been recorded in India, where, over the last 50 years 40 such cases of meditating Tibetan monks have been found mummified.

Croatia, a small and beautiful country in the heart of Europe, has a collection of 800 relics and one of the biggest collection of incorruptible mummified saints. In this work, two different mummified bodies of saints originating from 1200 AD and 1500 AD were investigated for the presence of any kind of binding media (proteins, sugars, waxes, resins, and oils). Methods used were a combination of chromatography, microscopy, spectrometry, and spectroscopy.

Mummies are human or animal remains that have been preserved in different cultures throughout history [1]. There are two varieties of mummification processes, natural and artificial [2]. Natural mummification is achieved by environmental factors that includes climate. In contrast, artificial mummification is an intentional procedure. The literature data shows that the first mummified bodies were produced by the Chincohoro ethnic group in northern Chile some 4000 years ago [3]. Mummies are found all over the world: in Egypt and Korea [4], Columbia [5], Peru [6], and other countries [7].

A distinct category of mummies are the bodies of saints that in the Roman Catholic tradition are called the Holy Incorruptible (Corpi Santi) and are venerated as relics. The majority of these mummies are located in Italy, but there are also many preserved in other countries (France, Poland, Croatia, etc.) [8, 41].

The Holy Incorruptible (Corpi Santi) relics that were a part of this investigation [41] were the bodies of St. Giovanni Olini and St. Nicolosa Bursa. These relics are just a part of a rich collection that is preserved in the church of St. Blaž in Vodnjan, Croatia (Figure 3.13). It is believed that the collection of *Corpi Santi* was brought to Croatia from Italy by Gaetano Grezler who donated his collection to the town in 1818. [9]. The MSCT

showed that these two relics are in exceptionally good state with preserved skeletons, inner organs, muscles, and tendons [10].

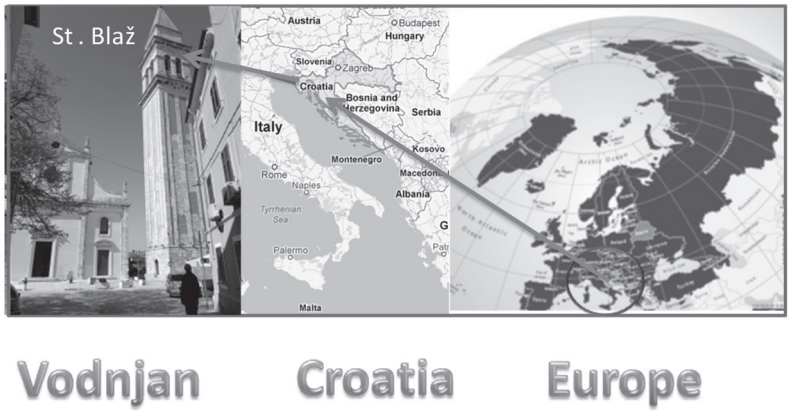


Figure 3.13. Vodnjan, the location of the collection of Holly Incorruptible relics, Croatia, Europe.

For thousands of years, the mummified remains of bodies kept their secrets. Today, although we have developed many sophisticated state-of-the-art chemical methodologies, we still have little knowledge of the true interpretation of the various ritual and hidden mysteries behind such relics. Many different teams of archeologists, chemists, anthropologists, priests, and other scientists have committed their time and effort to find answers to these really complex questions.

The mummification processes used since ancient Egypt involve removing the internal organs. However, some mummified corpses of saints have their internal organs intact. Moreover, there are no signs of desiccation: of wrapping the bodies in materials soaked in any kind of balm of plant extracts (such are oils, waxes or resins). It is known that some mummies could have been preserved naturally by being buried in dry desert sand. Such processes do not involve the help of chemical embalming. Nevertheless, this was not the case with the saints investigated here.

Therefore, our knowledge of mummification treatments needs to be widened; such goals can only be achieved by proper analyses. From revealing the materials used we can gain insight and information on the processes used and detect their origins, whether natural or not [9, 11, 39, 41]. In such treatments, non-destructive methodologies are a significant help.

In our published work [39], we have identified which analytical procedures are useful in the **identification of organic compounds**. These include UV-VIS, FTIR, and Raman spectroscopy, or mass spectroscopy coupled with chromatographic techniques [12, 13, 14]. It is not only information on the chemicals present that is important; the **processes of degradation** and their possible causes can also be identified through chemical investigation. The methods used are GC-MS [15–18] and pyrolises-GC [19, 20].

**Textile fiber identification**, which reveals the fiber's microstructure, is performed by FTIR [21] and optical microscopy. In cases of very valuable historical materials, this can be done on very small sample amounts by using SEM, TEM, and AFM microscopic techniques [22]. Combining this with the chromatographic procedure results in **lipids identification** from the mummified skin parts and the identification of a range of embalming reagents, polysaccharide sugars, diterpenoids from resins, or volatile compounds from aromatic plant extracts identified by GC and GC-MS [23–28]. Among these, the analysis of **antibacterial compounds** that help the mummification processes, such are diterpenoid molecules, is particularly interesting. However, it has to be emphasized that such procedures might be rather expensive. Only in rare cases can faster and less expensive methods be used [29–33].

A combination of inexpensive methodologies was described by I. Rezić and coauthors [39]. These researchers have shown that chemical investigation can be performed on very small fragments taken during the cleaning of holy bodies. The fragments belonged to two bodies, the first from St. Giovanni Olini and the second from St. Nikolosa Bursa (Figure 3. 14).

In Vodnjan, Croatia, in the church of St. Blaž, there is a unique collection of miraculous relics of saints and uncorrupted bodies of saints, kept together with relics from the Roman Empire more than 2300 years old. In addition, one can find items related to Jesus Christ, Saint Mary, and the 12 apostles. Nevertheless, the major attraction for science is the unexplained phenomena of the untreated incorrupt bodies of the following saints wholly preserved: Greek Archbishop St. Leon Bembo (died 1188), Venetian priest Giovanni Olini (1300), Benedictine nun Saint Niclosa Bursa (1512), and St. Paul Archbishop of Constantinople (250). Also preserved are incorrupt parts of Saint Sebastian (282), Constantinople martyr St. Barara (288), hermit Saint Mary of Egypt (522), and Constantinople patriarch St Eutih (583), a finger of St Antony Abbot (356), and a finger of a bishop from Egypt, St Basilius (379).

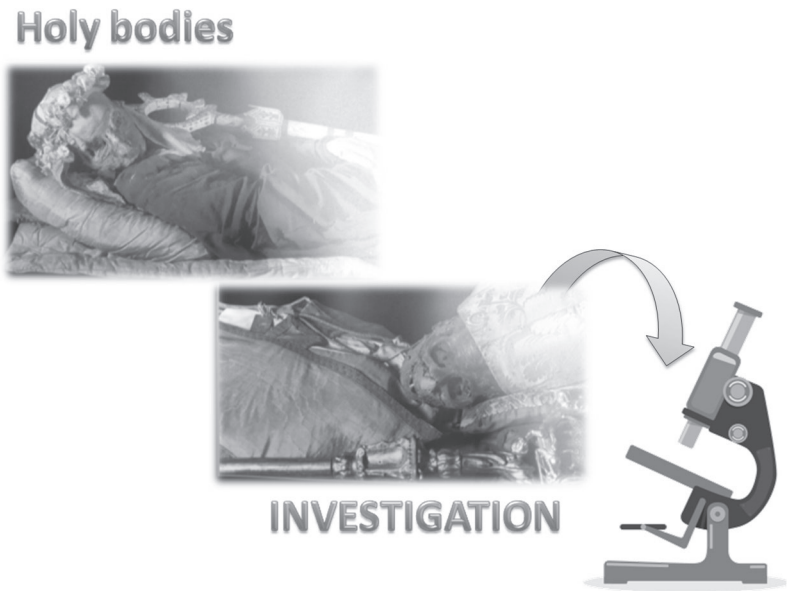


Figure 3.14. Holy bodies of St. Giovanni Olini (A) and St. Nikoloza Bursa (B), dated from 1300 and 1500, respectively, preserved in Vodnjan, Istria, Croatia, and originating from Venice, Italy [39]. St. Giovanni Olini was a priest who helped sick people during the plague without being ill. St. Nikolosa Bursa was a Benedictine. According to the literature data, she was able to predict the day of her death as Friday 23 April 1510.

An important part of **sampling the mummies** concerns **sterilization**. This is performed by using methyl bromide as sterilization reagents, after which the samples need to be cleaned and placed in boxes with an adequate climate. Samples that are well preserved are then divided for different investigative steps. During the sampling, special caution is taken over the methodologies that will be used for the identification of unknown materials. First, the solid samples are analyzed by means of an X-ray fluorescence (XRF) spectrometer and Fourier transformed infrared spectroscopy (FTIR). The results are presented in Figures 3.15. and 3.16.

Chromatographic investigation is performed on the chloroform extracts from sample fragments and on hydrolyzed proteinous samples. To define the unknown sample, reference materials of waxes, resins, oils, proteins, and sugars are used during the analysis. The plates after development are evaluated by comparing the colors of spots and their $R_F$ values to the known standard reference materials.

Table 3.4 presents various usual chromatographic systems that are used for the analysis and Tables 3.5–3.8 show the characteristic $R_F$ values of particular components.

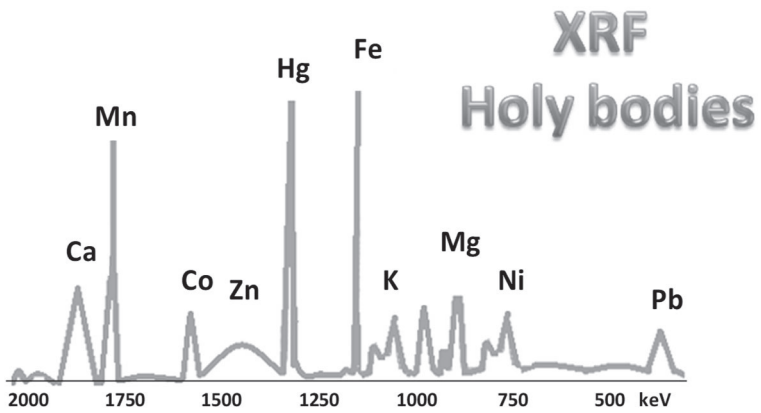


Figure 3.15. A) Schematic overview of the XRF Spectra that could be obtained from the mummies [for the real spectra please refer to 39]. As can be seen from the figure, it is expected that elements present in human skin will appear in the spectra of the sample.

**Table 3.4. Solid and mobile phases used in TLC investigation of the historical samples**

| | |
|---|---|
| Solid phase | Pre-coated cellulose plates or silica gel plates |
| Mobile phase | n-buthanol—acetic acid—water, acetonitrile—water, petroleum ether—diethyl ether—acetic acid |
| Visualization | Ninhydrine, iodine, UV-light |

**Table 3.5. Average $R_F$ values of proteinous components from historical textile materials [36, 38, 39].**

| Standard reference materials | $R_F$ value | Standard reference materials | $R_F$ value |
|---|---|---|---|
| Alanine | 0.3 | Leucine | 0.8 |
| Arginine | 0.2 | Lysine | 0.2 |
| Asparagines | 0.1 | Phenylalanine | 0.6 |
| Asparagine Acid | 0.2 | Praline | 0.3 |
| Cysteine | 0.1 | Serine | 0.2 |
| Glycine | 0.2 | Threonine | 0.3 |
| Glutamic Acid | 0.3 | Tyrosine | 0.5 |
| Hydroxyproline | 0.2 | Isoleucine | 0.7 |

**Table 3.6. Average $R_F$ values of sugar components from historical textile materials [36, 38, 39].**

| Standard reference materials | $R_F$ value | Standard reference materials | $R_F$ value |
|---|---|---|---|
| Arabinose | 0.4 | Ribose | 0.53 |
| Fucose | 0.5 | Xylose | 0.5 |
| Glucose | 0.3 | Cherry Gum | 0.0, 0.1, 0.3, 0.4, 0.5 |
| Galactose | 0.2 | Dextrin | 0.0, 0.1, 0.2, 0.3 |
| Galacturonic Acid | 0.1 | Gum Arabic | 0.0, 0.1, 0.3, 0.4, 0.6 |
| Mannose | 0.3 | Gum Tragacanth | 0.0, 0.3, 0.4, 0.5 |
| Rhamnose | 0.6 | Starch | 0,0, 0.2, 0.3 |

**Table 3.7. Average $R_F$ values of waxes components from historical textile materials [35, 36, 38, 39].**

| Wax standard | $R_F$ value(s) | Number of spots |
|---|---|---|
| Candelilla | 0.0, 0.1, 0.2, 0.4, 0.7, 0.9, 1.0 | 7 |
| Carnauba | 0.0, 0.1, 0.10, 0.2, 0.8 | 5 |
| Ceresine | 0.9, 1.0 | 2 |
| Earth wax | 0.0, 0.1 | 2 |
| Spermaceti wax | 0.8 | 1 |
| Beeswax | 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.9, 1.0 | 9 |
| Paraffin | 1.0 | 1 |

**Table 3.8. Average $R_F$ values of wax components from historical textile materials [35, 36, 38, 39].**

| Resin standard | $R_F$ value(s) | Number of spots |
|---|---|---|
| Amber | 0.0, 0.1, 0.2 | 3 |
| Colophony | 0.0, 0.1, 0.1, 0.20 | 4 |
| Congo copal | 0.0, 0.0, 0.1, 0.2 0.3 | 4 |
| Dammar | 0.0, 0.1, 0.1, 0.1, 0.1, 0.2, 0.2, 0.3, 0.4, 0.5, 0.8 | 11 |
| Dragons blood | 0.0, 0.1, 0.1, 0.1, 0.2, 0.2, 0.3, 0.4, 0.5, 0.8 | 10 |
| Elemi | 0.0, 0.1, 0.1, 0.1, 0.1, 0.2, 0.2, 0.3, 0.3, 0.4, 0.7, 0.7 | 12 |
| Mastic | 0.0, 0.1, 0.1, 0.1, 0.2, 0.2, 0.2, 0.3, 0.3, 0.4 | 10 |
| Myrrh | 0.0 | 1 |
| Shellac | 0.0, 0.1, 0.1, 0.2, 0.3 | 5 |
| Sandarac | 0.0 | 1 |

Chromatography performed on a thin layer is suitable for the simultaneous identification of many different compounds in historical textiles [30–33]. Although it is not a favorable technique in some laboratories, its advantages over column chromatography have to be emphasized since it does not demand purification prior to the chromatographic analysis [39]. As can be seen in Tables 3.5–3.8., many

different materials can be found on historical textiles. For example, trace amounts of caseine, animal glue, egg, and other substances will result in amino-acid detection. Such components are identified by comparison of the results of standard materials and the samples. Such analysis can show whether the samples of the mummies were treated with resins, waxes, or oils (TLC investigation). In addition, the chemical analysis will show the textile origin, and whether it was made of cotton, flax, or silk. In this work it was determined that the mummy of St. Ivan Ollini had a textile wrapping made of flax, while silk was present on the mummy of St. Nikoloza Bursa.

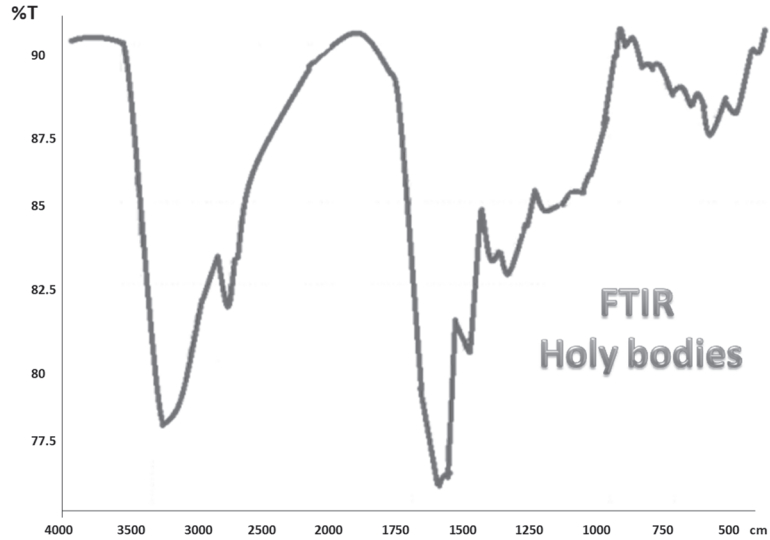


Figure 3.16. Schematic overview of the FTIR spectra that may be obtained from the mummies [for the real spectra please refer to 39].

It is sometimes hard to conclude whether the resulting components and balsams were applied to the holy bodies immediately after their death or many decades or hundreds of years afterwards. Nevertheless, the presence of chemical reagents will prove whether the mummies were treated with organic and inorganic materials during their conservation treatments many years after their death. The application of non-destructive methods (portable PIXE-alpha and portable XRD techniques [37]) allow a systematic investigation to be performed in situ, while their combination with different spectroscopic and chromatographic techniques enables us to

understand the technological conditions implemented for the mummification of the *Corpi Santi* [40, 41].

As can be seen from Figure 3.16, the most prominent lines were around 1250, 1400, 1550/1650, 2800, and 3450 cm. These parameters enable detection of the functional groups present, such as C-O-O, OH-, and others, and indicate the presence of resins, sugars, waxes, and proteins in the sample. For example, copal and shellac resins can be detected on the samples. The combination of microscopic, chromatographic, and spectrometric methods are the perfect tool for investigating the holy bodies of the mummies and other valuable historical textiles. Such protocols can therefore be recommended to archeologists, anthropologists, restorers, and conservators dealing with historical textile samples.

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# CHAPTER III.4

# CASE STUDY 4: IDENTIFICATION OF BINDING MEDIA ON HISTORICAL PAINTINGS, KNIGHTS, ARMOR, WEAPONS, JEWELRY

Some of the most valuable national historical textile materials are not only dresses and decorative materials, but also the painted layers beneath famous and precious paintings. Such materials are precious and available for analysis in very small fragments presenting a big chemical challenge. This challenge is even larger because there can be more than ten different layers on a small textile fragment. The chemical composition of such layers originates from a wide variety of different natural materials, including resins, waxes, sugars, proteins, and inorganic fillers or pigments.

Painters have developed many different formulas for efficient binding media to glue pigments to a textile carrier, and today sophisticated and state-of-the-art methodologies are applied in the analysis of such items.

There can be many different painted layers on historical textiles. For example, historical textiles produced of dyed fibers may have only one protective coating, made of natural materials (resins, oils, or waxes). Usually such materials are fixed on a wooden frame or background (Figure 3.17).


Figure 3.17. Historical textile made of dyed fibers, with only one protective coating on the surface.

The investigation of painting techniques and historical painted textile surfaces is multidisciplinary research. The expertise of conservators, restorers, artists, chemists, archeologists, and anthropologists meet on the thin border of expertise in the study and preservation of historical textile samples. And each of these perspectives brings new insights into the materials and methods used. Broad knowledge of the natural pigments and binding media that was used throughout history is enriched with new findings of recent state-of-the-art investigations.

It has to be emphasized, however, that the field of investigation of historical painted textiles is a rapidly growing field, due to the recent developments and progress of instrumental analytical methods, especially those that are non-destructive.

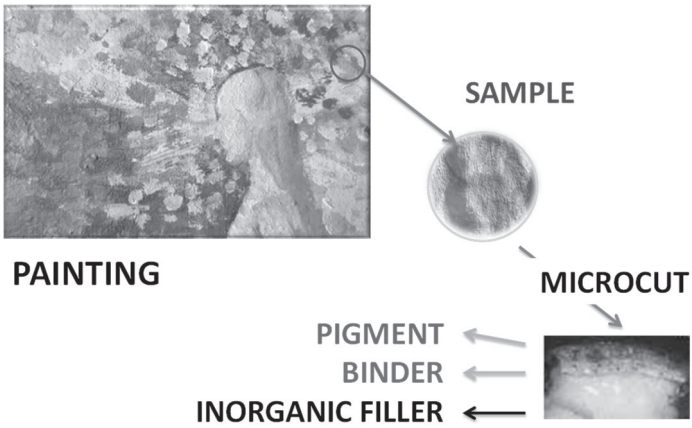


Figure 3.18. Historical textile with painted surface, containing multiple layers on its surface.

Certain basic steps in the process of studying historical painted textile samples are followed. These steps are as follows: identification of the materials used, authentication, and detailed chemical study of the sample. Many samples of painted historical textiles, wherever preserved (in museums or in private collections), have already been cleaned and restored, and sometimes even altered in comparison to their original state. Therefore, in certain cases, it will not only be the natural binding media that will be present on the sample.

In some samples of painted textiles, only a part of the supporting fabric is painted. Moreover, in other cases the textile surface is not stretched on a frame, remaining flexible, which makes the material even more fragile; as a result, severe damage can occur. The example of such complex historical textile banners will be described in this chapter.

In cases when valuable and precious historical textile samples need to be analyzed, only a small fragment of the original material can be sampled for the analysis. Therefore, powerful new extraction methodologies, such as ultrasound or microwave-assisted extractions, are applied in order to separate and pre-concentrate the analyte of interest. For the purpose of the identification of the natural binder composed of natural resins, ultrasound-assisted extraction can be applied.

A sample of a fragile silk banner from the nineteenth century will be used as a case study for analysis in this chapter; the target analysis will be testing for the presence of different resins. Briefly, extraction using an organic solvent (ethyl acetate) and sonication is used to extract the resinous materials from the historical textile banner. Ultrasound-assisted

extraction is a very efficient extraction procedure [1] that can be used for liquid or solid samples [2, 3]. The analysis takes place after the extraction using sonication performed by thin-layer chromatography where the mobile phase is a mixture of organic solvents, and the silica gel layer is a stationary phase.

Investigation of the painted layers and its binding media provides information about the methodology and technology by which it was produced. Moreover, it provides useful information on which kind of treatment and cleaning has been provided [4].

The analysis of natural resins can be problematic if a sample is available for analysis only in a very small amount. The next problem is that natural resins that are collected in nature as hardened plant extrusions can vary greatly in their physical properties. Those properties include differentiation in odor, shape, hardness, solubility, color, and color stability [5, 25]. Natural resins can often be found on the surface of historical textile samples because they were used as varnishes that increase the gloss of the treated textile surfaces [6, 7]. In the example considered here, the sample preparation involved careful sampling of unknown resin samples from the gilded textile banner (Figure 3.19).

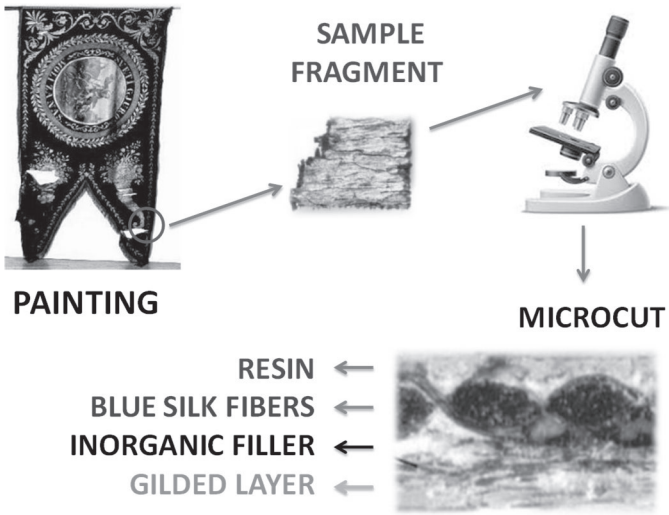


Figure 3.19. Sampling procedure, the spots determined for the sampling of the small textile fragment from the banner to analyze the resin [25].

As can be seen from Figure 3.19, the crosscut picture of the analyzed sample was taken under the microscope, with a magnification of 80 times,

in order to determine the layers applied. Moreover, the sampling procedure of the historical textile sample that contained the resinous layer was also performed under the microscope. The sample fragment marked with red letters was a size of 0.5–1.0 mm.

The historical textile sample was compared to different reference resin samples from the collection of natural resins. This collection contained dammar, colophony, shellac, dragon's blood, marasca tree resin, gummy mastic, copal, plum tree resin, cherry tree resin [25]. All samples were immerged in a sonication bath of 30 Hz for twenty minutes and extracted by five milliliters of organic solvent (ethyl acetate) under room temperature and a power of 400 W [10]. The advantage of the sonication process is its high efficiency and fastness. After the thin-layer chromatographic development, different chromatograms were obtained that belonged to different resinous samples. The results of chromatographic separation recorded under the Video Denzometer are shown in Figure 3.20 [25]. As can be seen from this figure, the analysis of the historical sample is performed by comparing the results obtained from the sample with the reference results of different resins. In this way, one can easily conclude which resin is on the sample, as was the case here.

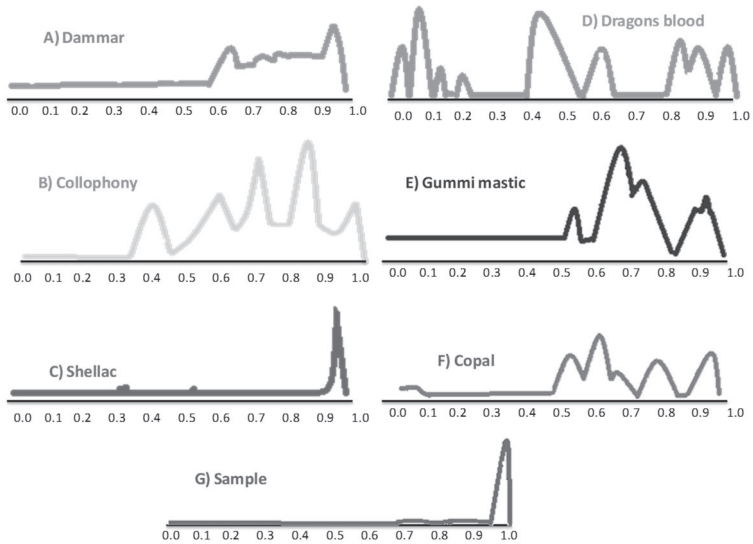


Figure 3.20. Determination of resins on a historical textile sample by thin-layer chromatography. The resin's chromatograms were captured under UV light on 254 nm with the green filter applied: a) dammar, b) colophony, c) shellac, d) dragon's blood, e) gummy mastic, f) copal, g) unknown sample.

The binder resin is identified by comparing the chromatograms of the reference materials and of the sample. As can be seen from Figure 3.20, the majority of standard resin materials can easily be distinguished from one another. The differences are obvious in the number and size of the chromatographic peaks, from which different resolution parameters can be calculated. Therefore, if we compare the unknown sample (g) with the standard shellac resin (c), the sample can be identified as shellac. Their chromatograms match perfectly.

The chemical resistance of a natural resin composition on a historical textile can be affected by other reagents used on the sample surface (inorganic fillers or pigments) [8]. Therefore the analysis of such materials is very important [9]. When performing chemical analysis, one has to have in mind that resins as samples are the most difficult to identify. Usually historical samples involve more components so therefore there are problems in obtaining complete separation. Moreover, some other components present such as furnishes, oils, or waxes can have acids or esters similar to those of resins.

**Table 3.9. The sophisticated methods useful in the analysis of the painted layers on historical textiles [26].**

| Methodology | Usage | Drawbacks of the methodology |
|---|---|---|
| Infrared reflectography | Identification of underdrawing | Thick over layer presents difficulties in identification |
| X-ray radiography | Study of underpainting, brushwork, composition and support materials | Difficult interpretation of organic materials and pigments with a low atomic number |
| X-ray tomography | Study of painted layers | Problematic interpretation of uneven layers; new methodology with high cost of analysis |
| IR, FTIR | Study of binder composition through functional groups, study of aging materials | Problematic analysis of very small and fragile heterogeneous samples |
| Microscopy | Study of cross-cut layers | Problems with the identification of corroded parts of the samples |
| IR Microscopy | Identification of materials used | Cross cut is necessary, which increases the cost of the analysis. Moreover, for positive identification, other procedures need to be available |
| Chromatography, TLC, GC, HPLC, IC and others | Binding media analysis | GC-MS and HPLC-MS are expensive methodologies. |
| SEM, SEM-EDX | Identification of sample surface, morphology, and composition | Only information on the particular depth is possible, problems arise with non-homogenous samples. |

In this case study, the investigation shows the examination of a very deteriorated model sample of a fragile silk banner that was painted and gilded on both sides. Due to thick inorganic filling, the silk rapidly deteriorated and decayed. The purpose of this chemical analysis was therefore to identify the unknown resinous binding materials, and to detect the further steps needed to be taken in the conservation and restoration of this complex material.

The characterization of the binding media of the 200-year-old silk banner was performed by thin-layer chromatography [11]. The method included analysis of amino acid standards, vapor-phase hydrolysis [12, 13, 14], and the separation and determination of components. The optimal ternary mobile phase used for the stationary cellulose phase for the characterization of proteinous binders was a mixture of butanol, acetic acid, and water. The volume rations were 60:19:21. Using this methodology, the binder was identified as an animal glue [25].

Glues made from the skin and bones of animals have been applied as binders since ancient Egypt. Egg white was used in old manuscripts, while egg yolk was a part of tempera paint. Casein was used in the painting of textiles [15] and complex binders contained a mixture of resins, waxes, and proteins [16]. For all these components, thin-layer chromatography is used [17–20]. The procedure is as follows: sample solutions are spotted on the plate using a Pt syringe or Linomat instrument. The development is carried out in the chromatographic chamber using the ascending technique and after the development, the plates are dried and sprayed with visualization reagents (such as, for example, nynhidrine, quercetine, and isoleucine). The chromatograms can be recorded on a sensitive CCD color video camera.

The parameter used for the identification is the $R_F$ value determined by the following equation:

$$Rs = \frac{lo \times (Rf2 - Rf1)}{0.5 \times (w1 + w2)}$$

where the $R_{Fi}$ presents the distance of the substance $i$ from the start and $w_i$ is the width of the spot of the substance $i$.

When sampling the binding layer from the surface of the historical textile, the procedure was carefully performed under the microscope. The focus was on ensuring that no silk parts from the ground layer were sampled. The reference materials used for the comparison were as follows: animal skin glue, fish glue, casein, whole egg, and a part of the egg (white

and yolk). For the hydrolysis, 0.35 mg of samples were mixed with the 6 N of hydrochloric acid at $110^0$C for 24 hours, and afterwards the samples were dissolved in 0.1 N HCl. [25] and analyzed by thin-layer chromatography. Table 3.9 presents the overview of the most frequently used methodologies in the identification of painted layers on historical textiles [26].

Several amino acids from protein binders have been detected and due to them the proteinous binders were identified. The aging influences the occurrence of some amino-acids—methionine and tyrosine decrease with aging, and the cystic acid increases. This information is crucially important for the preservation of national and cultural heritage, and modern science is able to contribute by means of highly sophisticated methods and instruments [25].

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# CHAPTER III.5

# CASE STUDY 5: ANALYSIS OF WAXES ON HISTORICAL MATERIALS

Analyzing the waxes in the binder composition applied to historical textiles makes for an intriguing analytical task. Moreover, the samples that are investigated are extremely precious pieces of art world heritage, such as for example Monet's *Water Lilies* painting. The results of such investigations are important since they will define future cleaning, conservation, and restoration work. The desire is that all procedures may be applied without damaging the artwork.

As was previously mentioned, real historical textiles often contain binders that are mixtures of waxes, resins, proteins, and sugars. For the purpose of wax characterization, thin-layer chromatography can be applied. The stationary phase is silica gel, and the mobile phase is a mixture of petroleum ether, diethyl ether, and acetic acid. Their ratios are 90, 10, and 1, respectively. The visualization of samples is done by using a UV lamp with a light of 254 nm. The waxes identified were carnauba, candelilla, and lanolin.

Waxes are waterproof, natural adhesive binders. Types of wax include beeswax, spermaceti wax, lanolin, carnauba wax, candelilla, and Japan wax. Ceresine, earth, and paraffin are associated with fossilized materials [1]. Historical textiles were often treated with waxes to achieve a soft touch [2]. Waxes are harder than other fats [3], and the sample of the carnauba palm is an especially hard wax [4].

Waxes are composed of long-chain hydrocarbons, esters, aldehydes, alcohols, diols, ketones, and triacylglycerols. For the separation and isolation of individual classes of waxes, thin-layer chromatography [6 –11] and high-performance liquid chromatography [12] are frequently applied.

For some purposes, the stationary phases of thin-layer chromatography are modified [13, 14], while in some other cases the mobile phases are changed [15, 21]. The advantage of thin-layer chromatography is that it is

a simple procedure for the analysis of waxes [16–19]. This case study presents the analysis of wax samples using thin-layer chromatography [20–22].

When sampling wax layers from historical textiles, one needs to be careful and patient. The most favorable manner of sampling is performed under optical microscopy. Different historical samples contain waxes, namely:

- paintings
- uniforms
- decorative textiles
- church clothing
- leather materials such as wallpaper, and
- precious samples without textile backgrounds such as metal armor.

The fragments of historical textile samples investigated were as follows: painting (painted surface depicting Saint Thomas originating in the seventeenth century); second, a fragment originating from leather wallpaper (early eighteenth century); and, last, a sample from metal surface armor (originating in the nineteenth century). For all the samples investigated, small fragments were taken for analysis and dissolved in chloroform, which is a non-polar organic solvent. During the sample preparation, the rule that non-polar materials are easily dissolved in a non-polar solvent needs to be taken into consideration.

The size of the sample should be at least 0.3 μg of wax binder per one mL of chloroform (or any other organic solvent), which entails a samples sized approximately between 0.5 and 1.0 mm.

Sample preparation includes preparing not only the historical materials but also the referent wax samples. For the purpose of a thorough investigation, one should test all referent wax materials that are available in the collection, such as candelilla wax, carnauba wax, lanolin, Japanese wax, spermaceti wax, beeswax, paraffin, and others.

The preparation of the sample has several stages and phases: 0.3 μg of the sample is mixed with 1 mL of chloroform. All wax samples need to be heated in sealed containers for at least 30 minutes at 35°C. Dissolved samples are applied to the thin-layer chromatography plate.

### *3.5.1 Thin-layer chromatography: application of the sample*

The stationary phase is a pre-coated plate, with dimensions of 20 × 20 cm, using Silica Gel 60 $F_{254}$ plates (Merck, Darmstadt, Germany). Aliquots of dissolved wax materials are applied by Pt syringe (Hamilton, Bonaduz, Switzerland) in the following order:

A) All referent materials such as candelilla, carnauba, lanolin, Japanese, spermaceti, bees, and paraffin.
B) Extracts from the historical samples (the painting, the wallpaper, and the armor) and of a blank extract were applied.

The position of the sample application should be 15 mm from the lower edge of the plate and in between the samples.

### *3.5.2 Thin-layer chromatography: development phase*

The development is performed in a chromatographic chamber (Camag, Merck, Germany). The length of time for the development depends on the chromatographic system: some are faster and others slower. In this case the development took 20 minutes. The solvent should pass up to a distance of 10 cm distance, in which time the capillary forces cause movements of the mobile phase that consist of different mixtures of solvents, the so-called ascending technique.

The chamber is saturated before the development so that the pure mobile phase is placed inside for at least 20 minutes. In this way, the vapors and the liquids are in thermodynamical balance. For the purposes of determination, the mobile phase is a mixture of petroleum ether, diethyl ether, and acetic acid, and their volume ratios are 90, 10, and 1 respectively.

### *3.5.3 Thin layer chromatography: identification and quantification of the samples*

Before the visualization, the developed plates are allowed to air dry. Chromatograms can be detected by some visualization reagents, or by using UV light. In this case, a UV light with $\lambda$ of 254 nm was used, by applying the green filter. This visualization of the spots on the chromatographic plate was chosen after several preliminary experiments based on recommendations from the literature [6, 9–11].

The identification of the unknown wax samples is done by comparing the $R_F$ parameters of the known reference materials and the unknown historical samples, as well as from the colors and positions of the spots on the chromatographic plate. The results of this investigation are presented in Table 3.10 [20] and in Figure 3.21.

**Table 3.10. The results of the thin layer investigation of the reference wax samples with their $R_F$ values [20].**

| Component | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Bees wax | 0.0 | 0.1 | 0.1 | 0.3 | 0.4 | 0.5 | 0.7 | 0.9 | 1.0 |
| Candelilla | 0.0 | 0.1 | 0.2 | 0.4 | 0.7 | 0.9 | 1.0 | | |
| Carnauba | 0.0 | 0.1 | 0.1 | 0.1 | 0.9 | - | | | |
| Japanese | 0.0 | 0.1 | 0.1 | 0.1 | 0.3 | 1.0 | | | |
| Lanolin | 0.0 | 0.1 | 0.2 | 0.2 | 0.5 | 0.7 | 1.0 | | |
| Paraffin | 0.0 | 1.0 | | | | | | | |
| Spermaceti | 0.0 | 0.8 | | | | | | | |

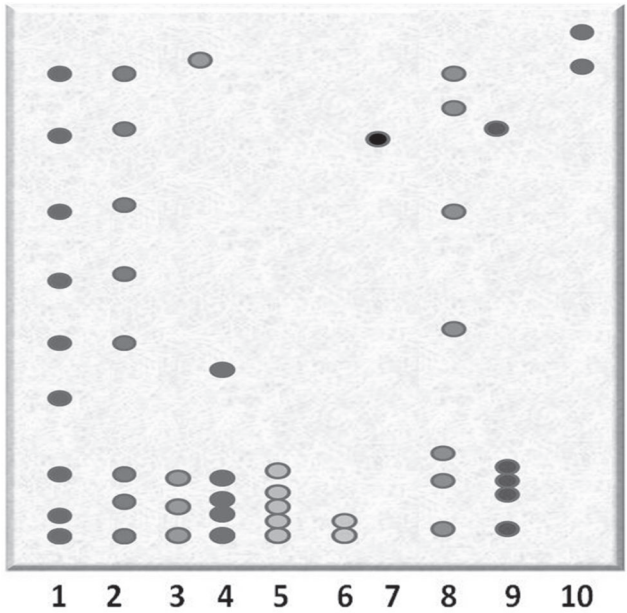


Figure 3.21. The graphical presentation of thin layer chromatographic detection of the wax samples present on historical textiles (1. beeswax, 2. bleached beeswax, 3. Earth wax, 4. Japan wax, 5. Montana wax, 6. rice wax, 7. spermaceti wax, 8. Candelilla wax, 9. carnauba wax, 10. ceresine wax) [1, 20].

The data presented in Figure 3.21 and in Table 3.10 are necessary for the determination of the unknown waxes present in samples of historical textiles. The historical materials that are analyzed together with the known wax sample materials are analyzed by thin-layer chromatography and the spots from different samples are identified on the basis of their number, location, and color. Moreover, as was previously emphasized, the differentiation can be calculated by $R_F$ parameters (see Table 3.10).

To conclude, the chromatographic system applied in the analysis of wax materials on historical textiles contains stationary phase silica gel 60 $F_{254}$ layers, while the mobile phase used contained a mixture of organic solvents (petroleum ether, diethyl ether, and acetic acid). The visualization of the spots on the chromatographic plates is performed by UV light on 254 nm (in a UV cabinet or using instrumentation such as a Videodenzitometer).

As can be seen from Table 3.10, for the purpose of the investigation of the three historical unknown wax materials, seven waxes were analyzed on the same chromatographic. The majority of the wax samples were clearly distinguished. This investigation therefore concludes that the wax materials in the unknown samples (from the seventeenth-century painting, the eighteenth-century wallpaper, and the 19$^{th}$ nineteenth-century armor) were candelilla, carnauba, and lanolin waxes.

The above-mentioned procedure was easily applied, fast, and efficient; thus, such a methodology is recommended to all restoration and conservation laboratories. Moreover, the methodology can be further optimized by changing the composition of the mobile and stationary phase. The thin-layer chromatographic procedure is therefore proposed as a useful tool for a prescreening methodology that is necessary for the identification of the wax composition in historical materials.

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# CHAPTER III.6

# CASE STUDY 6: SPOT TEST IDENTIFICATION OF UNKNOWN ELEMENTS IN PIGMENTS OF HISTORICAL SAMPLES

Metals applied on historical textile materials have to be determined in order to make a proper decision about the steps needed for cleaning, conservation, and restoration treatment of the investigated historical object. Spot test reactions are the quickest and the least expensive method available for this purpose. Spot test reactions are based on colored spots formed between the metal ions and the chemical reagents after dissolving the metal part in the appropriate solvents. Several chemical spot reactions for the determination of some of the most commonly present metals (copper, silver, iron, aluminum, and zinc) on metal threads applied on historical textile materials are presented in this work.

To conserve or restore an historical artwork, it is necessary to appreciate the nature of the basic materials used in its creation, to be able to identify the materials originally applied, as well as to understand the nature of gradual chemical and physical degradation that occurs within the various components of the system [1]. Metal threads were manufactured by drawing a metal wire to the required diameter, or by beating a thin sheet and then cutting to the required thickness. The metal was then wound around the core fiber yarn. [2] The metal threads applied on different valuable historical materials, like embroidery and woven textiles, were usually strips made from the noble metals in combination with vegetable or animal fibers or other organic materials, such as gut and paper (Figure 3.11).

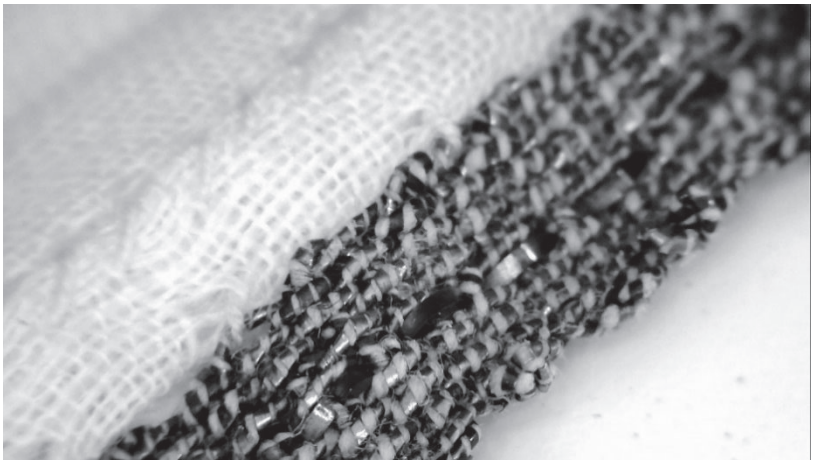

Figure 3.11. A variety of metallic strips used in combination with natural fibers in a type of metallic thread.

The metals used for this purpose historically were gold, silver, zinc, and copper, while today the main metal used for making metal threads is aluminum. The technology of solid metal strips includes manufacturing by cutting them from a foil or by flattening a wire. Short strips are joined by overlapping them around a fibrous core. The average width of the metal strips used is between 0.20 and 0.30 mm, and the average thickness varies between 0.006 and 0.030 mm. The morphology of metal threads has not varied appreciably over the centuries, but the materials and the technology have changed a lot. The fiber inside the thread could be made of cellulose or protein polymer, while today synthetic materials are mainly used for this purpose. The metals most commonly used are those that are chemically inert, reacting with other elements to form compounds only with difficulty. They are easy to draw, hammer, and form [3].

Gold, silver, and copper do not dissolve in dilute acids. Concentrated nitric acid dissolves copper and silver, but not gold. Gold can dissolve only under aggressive conditions such as a mixture of hydrochloric acid and nitric acid in a 3:1 volume ratio. Most metals are unstable and will change under different physical circumstances: dust, soil particles, fats, oils, calcareous deposits, and corrosion products of alloying elements (silver or copper) can cover the surface of gold threads, but will not constitute corrosion of gold [3].

The metals present in metal threads on valuable historical textile material may cause stains on sensitive materials like cotton or silk, or even damage to and the total degradation of the material. Materials that are

extremely sensitive to silver and silver gilt threads, which may tarnish, are wool and silk fibers. For this reason, determining the metal composition of metal threads applied on historical textiles is extremely important. The method chosen for the analysis are spot test reactions, which are fast and the most inexpensive. Also, such tests have excellent selectivity due to applied reagents and the fast and reliable information obtained about the analytes. The most important advantage is that spot test reactions require very small sample amounts, of only a few μg-mg, as only extremely small samples can be collected from valuable historical materials. For this reason, this research work was performed on the fragments of samples shown in Figure 3.12.

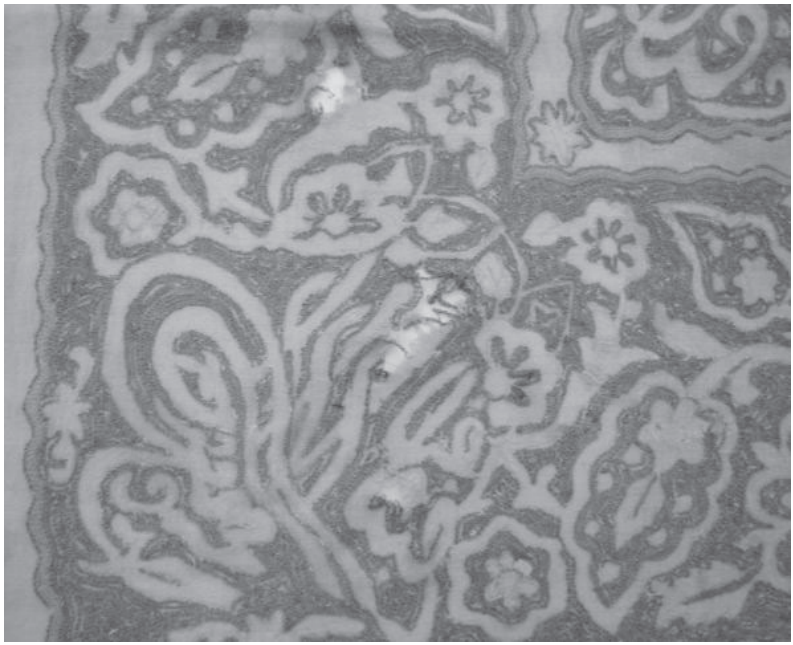

Fig 3.12. Metal threads on a historical textile.

For the sample preparation, the metals were carefully sampled and dissolved in appropriate diluted acids and afterwards analyzed by different chemical reagents. The solutions were used for color spot reactions (after the chemical reactions, different color complexes were formed), which allowed the qualitative determination (identification) of the metals.

Many modern analytical procedures may be applied while deeply investigating the nature of historical fibers and metal threads: time of flight

secondary ion mass spectrometry (ToF-SIMS), inductively coupled plasma-optical emission spectrometry (ICP-OES), or the scanning electronic microscope (SEM). Although those methods provide important information about the materials investigated [2, 4, 5] they are unfortunately extremely expensive and sometimes time-consuming procedures. This paper suggests spot test reactions—a method that can be applied in any restoration or conservation laboratory, on a small expanse of sample and in a short time, but with very good precision and repeatability. Spot test reactions are getting more attention today because, thanks to the development of new materials, they can be carried out on normal spot plates, reagents impregnated on filter papers, resins, ring ovens, gels treated with specific reagents, and other matrices [6].

Those colors are specific for each metal tested, and can be used for their determination. A diagram for the dilution and determination of all the metals investigated is presented in Figure 3.13. Figure 3.14 presents the different colors of metal complex solutions.

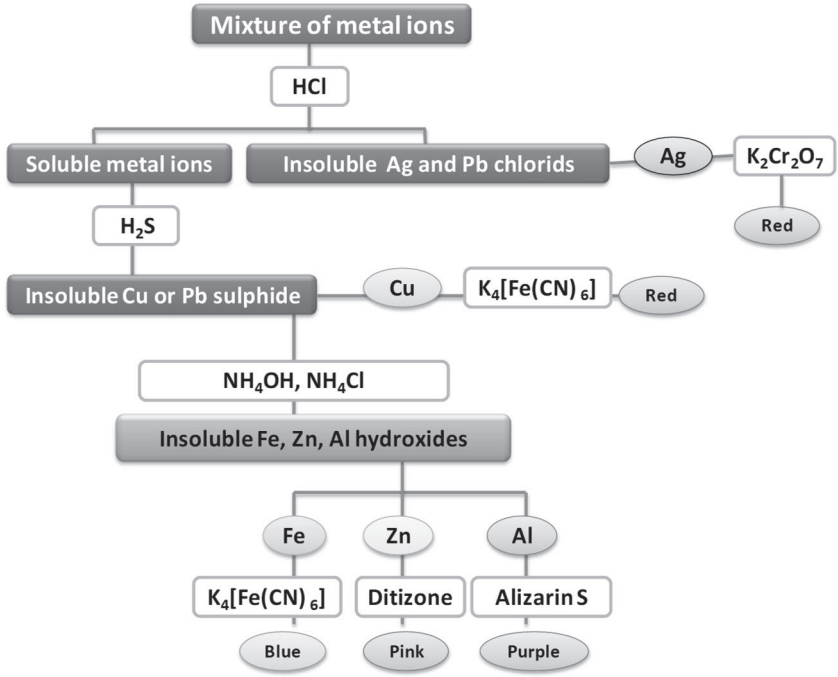


Figure 3.13. A diagram for the dilution and determination of all the metals investigated.

The results of the experiments are listed in tables 1 and 2. Table 1 presents the metals tested, their solvents, and the limits of detection, while Table 2 lists the reagents applied and the colors formed.

**Table 3.11. The limits of detection for the qualitative determination of metals in solutions from historical samples.**

| Element | Metal ion | Solvents | Limits of detection* |
|---|---|---|---|
| Ag | Silver | conc. nitric acid or hot conc. sulfuric acid | 0.15 μg / mL |
| Al | Aluminum | hydrochloride acid or hot alkali solutions | 0.02 μg / mL |
| Cu | Copper | conc. nitric acid or conc. sulfuric acid | 0.17 μg / mL |
| Fe | Iron | hydrochloride acid | 0.01 μg / mL |
| Zn | Zinc | solutions of mineral acids or alkali solutions | 0.08 μg / mL |

**Table 3.12. Spot test reaction colors of metals in solutions from metal threads on historical textiles.**

| Metal ion | Reagent | Color | Metal | Reagent | Color |
|---|---|---|---|---|---|
| Aluminum | NaOH, | White | Iron | $K_4[Fe(CN)_6]$ | Blue |
| Aluminum | $NH_4OH$+Alizarin S | Purple | Iron | $NH_4CNS$ | Red |
| Aluminum | Alizarin S | Violet | Silver | $Cl^-$ | White |
| Copper | $NH_4OH$ | Blue | Silver | $Br^-$ | Light yellow |
| Copper | $K_4[Fe(CN)_6]$ | Red | Silver | $I^-$ | Yellow |
| Zinc | Ditizone | Purple | Silver | $K_2CrO_4$ | Red |

In order to make future decisions about the cleaning, conservation, and restoration treatment steps of the historical object, it was extremely important to determine the metals present in the metal threads applied on the textile materials. Spot test reactions based on colored spots formed after dissolving the metal part in the appropriate solvent mixtures, for the determination of copper, silver, gold, iron, aluminum, and zinc on metal threads applied on historical textile materials, were presented in this work.

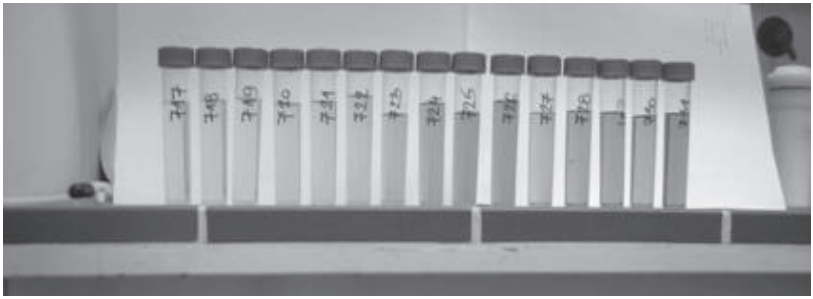

Figure 3.14. The variety of different colors of metal complex solutions.

The possible disadvantage of these tests is the problem of the partial destruction of the metal threads investigated, but since one textile fiber with a small part of the metal thread is sufficient for the analysis, this disadvantage is reduced. The advantages of spot test reactions are numerous: this method is fast, easily operated, and inexpensive, and therefore can be recommended as an optimal pre-screening method for metal determination to all textile restorers and conservators.

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# CHAPTER III.7

# CASE STUDY 7: IDENTIFICATION OF METAL THREADS BY SURFACE MAPPING

Metals are often part of valuable historical items. During aging, corrosion frequently occurs with highly negative consequences for valuable works of historical and cultural heritage. Therefore the monitoring of corrosion on such items is very important. Use of a scanning electron microscope equipped with an EDX detector (SEM-EDX) is a costly but unavoidable method for monitoring corrosion on valuable objects, since the analysis can be performed without the dissolution and destruction of the sample analyzed. Its usefulness is documented in this work, which presents examples of analyzing several different metal items collected from historical materials.

The corrosion process occurs on many historical metal items. Therefore the most important step during the conservation is monitoring the corrosion process to identify the metals present in the corroded parts, in order to understand the nature of the gradual chemical and physical degradation that occurs within the various components of the system [1]. The metal items are often present as metal threads applied onto different historical valuable materials. These threads were made from metals in combination with fibers. The average width of the metal strips used was between 0.20 and 0.30 mm, and the average thickness varied between 0.006 and 0.030 mm [2]. The metals most commonly used were those that are chemically inert, reacting with other elements to form compounds only with difficulty. For this purpose, gold, silver, zinc, copper, and their combinations or alloys that are easy to draw, hammer, and form were applied [3]. Most of these metals are unstable and will change under the different physical circumstances that occur on historical materials, such as dust, soil particles, fats, oils, calcareous deposits, and corrosion products of other alloying elements [3, 4].

The corrosion of metals present on valuable historical textile materials causes stains on sensitive materials like cotton or silk, or even damage to or the total degradation of old sensitive materials. The materials that are

most sensitive to silver and silver gilt threads are wool and silk fibers [3]. For this reason, determining the metal composition of metal threads applied onto historical textiles is extremely important.

The method presented in this work that was chosen for the analysis is a scanning electron microscope equipped with an EDX detector. It has excellent selectivity and sensitivity and can be applied as a non-destructible method of analysis, after which the historical metal part can be returned to the original material without changes in its physical or chemical state. The most important advantage is that the SEM-EDX requires only very small sample amounts. When working with the most valuable historical samples, only extremely small sample amounts need to be collected and provided for the analysis.

Many modern analytical procedures may be applied while deeply investigating the nature of historical fibers and metal threads. The most appreciated are time of flight secondary ion mass spectrometry (ToF-SIMS) and inductively coupled plasma-optical emission spectrometry (ICP-OES). Although those methods provide important information about the materials investigated, they are expensive and sometimes time-consuming procedures [2, 4–6].

The objective of this study was to evaluate the corrosion process and elements on the surface of the corroded parts of historical materials using SEM-EDX. The application of this method is recommended for fast pre-screening analysis before modern instrumental analysis methods are applied that are more expensive, sophisticated, and time consuming [7–16]. More importantly, the majority of analytical instrumental methods usually require sample dissolution and destruction prior the analysis, while SEM-EDX is non-destructible way of monitoring of corrosion. Therefore it was our intention to prove that SEM-EDX is a helpful, fast, and reliable method for monitoring corrosion on historical items.

The samples investigated were metal threads collected from three different historical textile materials from the collection of the Faculty of Textile Technology, University of Zagreb. The samples were collected from different historical materials: decorative material, uniform, and national costume. The samples were collected in such a way that the representative sample obtained contained all the properties of the whole sample. The samples analyzed are presented in Figure 3.15.

The monitoring of corrosion was performed using a scanning electronic microscope equipped with an EDX detector. The working instrumental parameters were: 25 mm working distance, 20 eV energy, and 1000–10000× magnification, and the detectors used were a back scatter detector and a detector of secondary electrons, with a collection time of 30 s.

Figure 3.15. Metal threads as a sample for monitoring corrosion processes.

0.05 g of metal threads was carefully sampled from historical materials under the microscope so that the textile carrier material would not be damaged even more [17–20]. Only a few metal stripes were taken for the analysis from places in which damage had already occurred, as shown in Figure 3.16. Figures 3.17–3.19. show different metal pearls and stripes found as decorative materials on historical textiles.

The SEM-EDX analysis was performed in two steps: first, the metal samples were analyzed using SEM in order to detect corrosion and degradation processes on the metal surfaces. Second, the EDX detector was used to obtain information on the chemical composition of the particular part of the corroded sample surface [21–25].

Figure 3.16. Fragment of metal thread as a sample point for monitoring corrosion processes.

Each analysis was repeated ten times in order to obtain reliable analytical data. The average chemical composition of the historical metal items investigated is presented in Table 3.13.

**Table 3.13. The average chemical composition of the historical metal items investigated.**

| Element | Weight % |
|---|---|
| C | 39.38 |
| O | 9.81 |
| Cu | 53.12 |
| Ag | 1.45 |
| Au | 0.86 |
| Mn | 0.57 |
| Si | 0.43 |
| Zn | 0.24 |
| Al | 0.19 |
| Ni | 0.17 |

As can be seen from the results obtained, samples were taken of copper as the major constituent. Much smaller amounts of Mn, Ni, Si, and Zn were detected inside the alloy, while Ag and Au were present as the outer layer on samples. It is important to emphasize that only SEM-EDX analysis could detect the elemental composition of the outer layer, since other instrumental methods analyze the sample after dissolution, and the results obtained present the average percentage of particular metals in the alloy. From the results obtained, decisions about the solvents for cleaning and conserving the historical items will be made.

For samples containing higher quantities of gold, cleaning with low acidic media is allowed. Figures 3.17–3.19 show different metal pearls and stripes found as decorative materials on historical textiles.

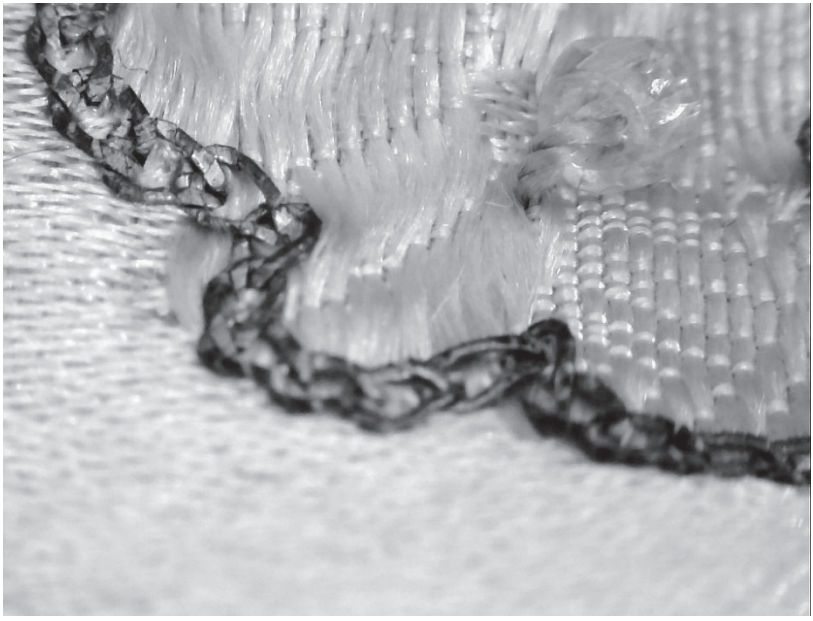

Figure 3.17. Metal threads used as decorative flower metal stripes.

SEM-EDS can be applied as a non-destructive method, which allows the sample to be returned to the original material after the analysis [14–17, 26, 28]. The drawback of SEM-EDS is that the results show the average chemical composition of only a specific analyzed part of the sample surface [18–19, 27–30]. Nevertheless, this information is useful in the complete characterization of the materials, providing an insight into the layer composition, morphology, and topology of samples and into the

corrosion processes.

In contrast to the previously mentioned analytical methods (AAS, GF-AAS, ICP-OES), which enable a full chemical analysis to be performed, a scanning electron microscope equipped with an EDS detector (SEM-EDS) is able to perform an analysis of a sample surface [44].

It is important to emphasize that the electron cloud of SEM-EDS penetrates only a few micrometers into the investigated sample surface. Therefore the results of qualitative and quantitative SEM-EDS analysis cannot be compared to the results of ICP-OES or AAS. But when all these results are combined, they provide a full insight into the sample's chemical composition, morphology, and structure. Such information may be very valuable in the analysis of historical samples [31–40].

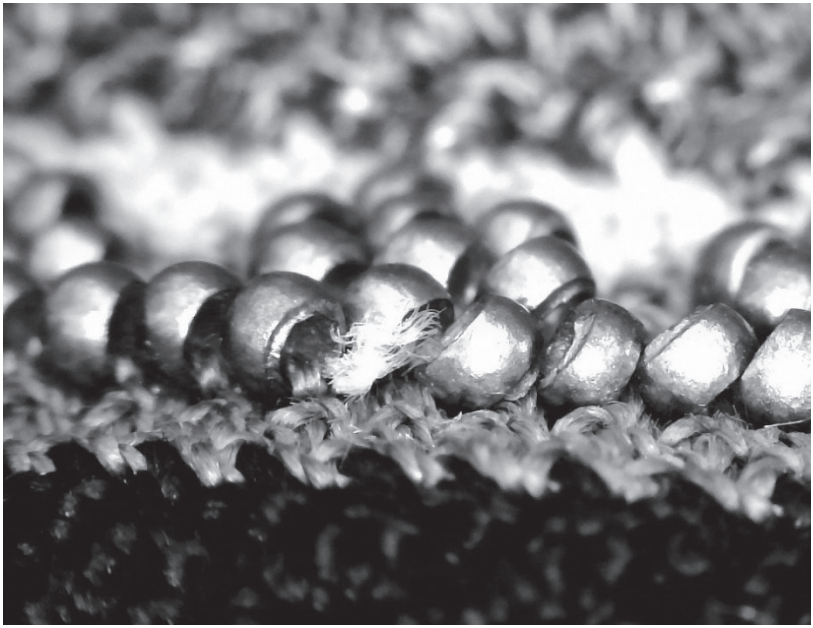

Figure 3.18. Metal pearls used as decorative materials on a historical textile sample.

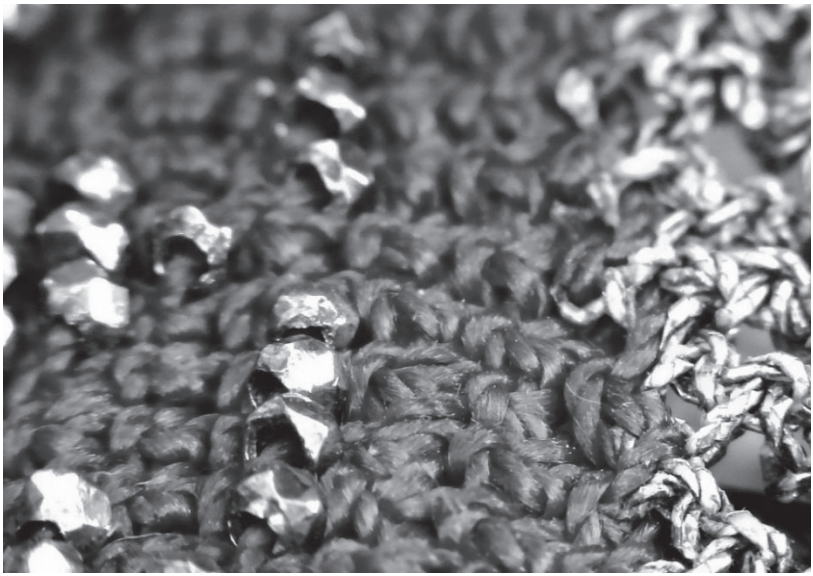

Figure 3.19. Metal pearls used as decorative materials on a historical textile sample.

This approach is a novelty in this field, since until now only SEM-EDS analysis was recommended as a non-destructive approach for the analysis of historical threads. According to our findings, it is better to use a few micrograms of samples and destroy them in order to have a full insight into the real chemical composition of samples than to have only SEM-EDS semi-quantitative data (quantitative data about the surface of the sample), and then to ruin the sample afterwards during the conservation procedure.

As was previously mentioned, SEM-EDS analysis is the chemical analysis of a sample surface [41–44]. Therefore, the quantitative SEM-EDS results of samples that have silver or gold layers on the surface of threads will show much higher quantities of those metals than are present in the average chemical composition obtained after ICP-OES and AAS analysis. Only with samples that have a unified chemical composition through their cross section, will SEM-EDS and ICP-OES (AAS) results be perfectly comparable.

In other cases, remarkable differences will occur. In order to make proper decisions about the cleaning, conservation, and restoration treatment steps of historical objects, it is extremely important to determine the metals present in the metal threads applied onto textile materials. Spot test reactions based on colored spots formed after dissolving the metal part

in the appropriate solvent mixtures, to determine copper, silver, gold, iron, aluminum, and zinc on metal threads applied onto historical textile materials, were presented in this work.

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# CHAPTER III.8

# CASE STUDY 8: ANALYSIS OF MUSICAL STRINGS

Playing musical instruments has been popular since ancient times. Today, it has been proved that music has therapeutic effects and that children who play musical instruments develop different abilities. According to the literature, it can be concluded that the guitar is today the most popular instrument in the world. Apart from players, the guitar has long been a subject of interest for scientists: physicists, chemists, and even scientists engaged in research on new materials. In the guitar and other stringed instruments, sound is created by the transverse vibration of a string, and is transmitted by the longitudinal vibration of air particles. The resulting sound (tone quality, warmth, roundness) depends on the material from which the wire is made, and the resonant body of the instrument.

The first and oldest instrument with strings was the harp, the ancestor of today's lute. The harp appears to us in the form of a lyre in a tomb in Ur approximately 4500 BC. Homer often mentions lyres, while Babylonian clay reliefs from 1900 BC show string instruments with two or more strings that are played with the right hand. A musical instrument preserved from 1500 BC, with a rounded body and a completely flat elongated neck, was found in an Egyptian tomb.

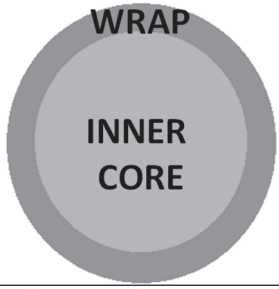
The first description of a guitar as we know it today comes from the New Testament Book of Revelation, which is known to have originated in the convent of San Miguel de Escalada near Leon in 926. Some sources state that the guitar was brought to Europe by the Arabs during their conquests between the eighth and thirteenth centuries. Guitars with six pairs of strings have been known since the fifteenth century, and lutes with four and five pairs of strings appeared in the sixteenth century. By the mid-seventeenth century, the five-string guitar was used in Italy, England, France, and Spain. The transformation of the guitar into the musical instrument we know today took place in France, where a musical notation was introduced, which replaced the old way of marking tones with tablatures. Then the guitar also lost its character as a soprano instrument because the sixth pair of strings (a quarter below the lowest previous pair)

was introduced. It was not until 1854 that Antonio Torres began making the guitars we use today. Over time, the principle of pairs of strings was abandoned, and the instrument was reduced to six individual strings, which we still use today: E, A, D, g, h, e. Great interest in the guitar was born in the late nineteenth century. Innovations since 1945 include wire made of artificial material (polyamide) and a growing interest in electronic sound amplification and the development of the electric guitar. Today, the most popular guitars are the electric guitar, acoustic guitar, and classical guitar. Of these, only the classical guitar uses metal fiber strings in combination with polymeric materials (carbon fibers, polyamides) while other instruments use exclusively metal strings in combination with metal fibers (steel, nickel, phosphor bronze).

Strings are the vibrating elements used in various string instruments, from the piano, guitar, and harp to the violin, viola, cello, and double bass. The strings can be of various lengths, and to get the tone it is very important that they are tense under a certain load, but that at the same time they can vibrate freely. They can be made of one material (steel, polyamide, natural fibers) or they can have a core made of one material wrapped in another fiber, in order to increase the mass of the wire, while maintaining a thin flexible structure. The strings of some violins, violas, and cellos are wrapped at the end with silk fibers to prevent damage. The color and pattern of the silk enable the wire to be identified because it determines the pitch and its manufacturer.

Wires having an inner core and an outer sheath can be made in several different geometries, which are shown in Table 3.14. The simplest embodiment has a sheath with a circular cross-section that is wrapped around a core with a circular cross-section. Such wires are simple to be produce so they are usually inexpensive. Unfortunately, they have many disadvantages: playing on such materials is difficult because the strings are often damaged, and the uneven surface leads to the unwanted appearance of a squeaky sound. The next shape used is a flat shell that has a much shallower profile, which allows for more comfortable playing, less damage to the material, and a better tone. The downside is the much less bright tone of the instrument. The semicircular profile of the casing is created by a compromise between the two previously described shapes: it is obtained by the subsequent alignment of the outer casing. The hexagonal shape has many advantages: due to the more compact structure, such wires are harder to destroy and wear and have a better tone. The only drawback is that they are less comfortable for the player’s fingers. They are often used for bass guitar.

**Table 3.14. Different possible geometric crosscuts of inner and outer musical strings.**

| Crosscut | Schematic overview |
|---|---|
| Circle |   |
| Hexagonal |   |
| Combination |   |

String instruments such as the violin, viola, and cello have four metal strings wrapped around a core; in the past, these strings were made of sheep intestines (some artists still use such strings today, which are extremely expensive). Today, the core is made of polymeric materials (polyamide, Kevlar, or carbon fiber) or pure metal. Around the core is wound one layer (sometimes several layers) of metal fibers of chromed stainless steel, aluminum, bronze (phosphor bronze), copper, nickel, gold, silver, tungsten, or other metals. Violin strings can sometimes be gold-plated and guitar strings silver-plated. Although these designs are much

more expensive, they are often used due to corrosion resistance and reduction of hypo-allergenicity.

Metal wires are significantly susceptible to corrosion and oxidation due to salt and moisture that comes into contact with them during use. Detailed scanning electron microscopy (SEM) tests can find links between metal wire damage and poorer instrument tone.

To reduce and prevent the corrosion of metal wires, many manufacturers apply a protective coating to the surface of the wire, which can be metal or polymer. Depending on the materials used, and the composition of the wire established, the tone and the color of the sound changes. Wires made from natural and artificial hoses have a warm sound similar to wires made of polymeric materials, which are specially designed to give a shiny and penetrating tone. Metal wires have the most penetrating tone. The choice of string depends on the type of music that is going to be performed on the instrument.

Guitar strings were also made from sheep intestines in the past. Such strings were found in ancient Egyptian sites on string instruments from the third dynasty. In the Middle Ages, such strings began to be wrapped in metal fiber. The oldest rules for their production that we have preserved today date from 1659 (E. Hartlib's poem *Ephemerida*), and in France they began to be mass-produced around 1675.

The method of production was as follows: the intestine of the lamb was well cleaned by rinsing with water (for several days). It was then left to stand in alkali to remove fats and waxes and all membranes other than muscle fibers. An alkaline solution was prepared by dissolving ash in water. Several cleaned fibers from the hose were then connected together (determining the final diameter of the future wire) and twisted on a drying frame, where it was fastened.

After the whole frame was filled, it was placed in an airy chamber where the wires were bleached with sulfur dioxide (which was prepared by burning sulfur in a bowl). At the end of this process, the wires were twisted again, air dried, and finally scratched with abrasives. The strings obtained by this classical procedure are still the most sought after and most expensive because they give the best tone to musical instruments. In some cases throughout history, silk has also been used as a fiber for the core of guitar strings.

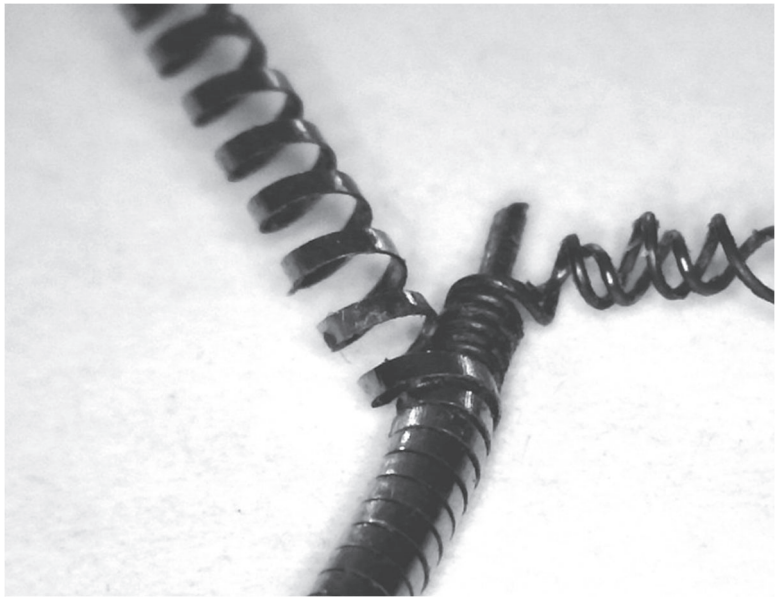


Figure 3.20. Microscopic picture of the several layers of a musical metal string.

Depending on the fiber in the core, guitar strings can be divided into two basic groups: either a combination of metal/metal fibers (for acoustic and electric guitars) or a combination of natural or artificial polymer/metal fibers (for classical guitars). Very often, after the 1970s, artificial polymers—polyamides—are used in classical guitar strings, which owe their strength to the chemical structure and additionally established hydrogen bonds between polymer chains.

The polyamide developed by Dupont had until then been used to make women's socks, parachutes, and fishing tackle, and after Albert Augustine tested its application for guitar strings, a number of advantages over natural materials were shown: while it is expensive, its surface is completely smooth and has significant wear resistance. The sound of a wire made of polyamide is not as brilliant as one made of natural materials. The sound of polyvinyldylene fluoride wire is most similar to naturally occurring polymers.

Recently, Kevlar (aramide fibers) and carbon fibers of extraordinary strength have also started to be used, while some artists are still happy to return to classic wires made of natural materials for special effects.

As mentioned earlier, the sound generated by the vibration of a wire is highly dependent on the material from which it is made.

In order to get a better insight into the distribution of chemical elements within the tested samples of metal fibers on electric guitar strings, it is necessary to perform a test of the samples using a scanning electron microscope with an EDS detector. For this purpose, cross-sections of the tested wires were made using common metallographic techniques (watering, grinding, polishing, etching), which are shown in Figures 3.21 and 3.22.

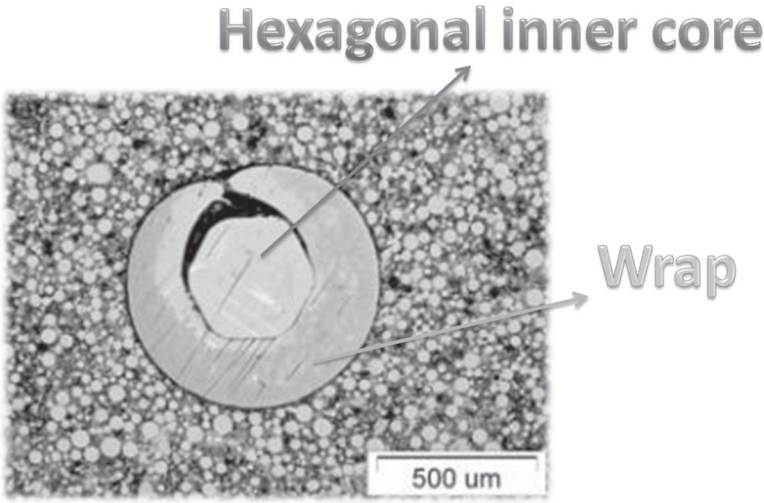



Figure 3.21. The microscopic figure of the sample cross cut with a hexagonal inner core.

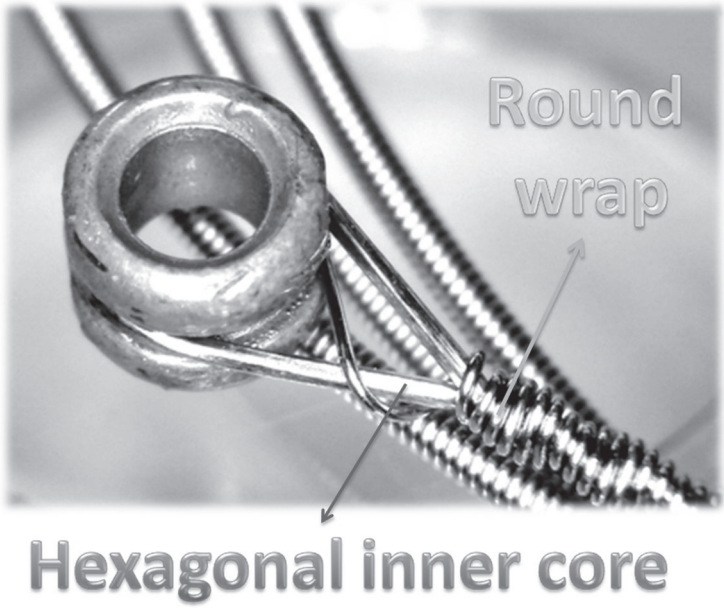



Figure 3.22. The microscopic figure of the sample with a hexagonal inner core, 100× magnification.

To determine the exact shape and cross-sectional dimensions of the wire samples, cross-sectional images of the samples were taken under a scanning electron microscope. SEM-EDS analysis of wire samples for three different electric guitar strings (namely E6, A5, and D4) showed that these samples consisted of two different parts: first, the inner core with a hexagonal cross-section was made of steel but contained a very thin layer of tin (the thickness of which was around 1 μm); second, steel wrapping materials containing a nickel layer of 5 μm. Moreover, the morphology of the samples was easily identified on the previously prepared cross sections of strings in resin holders. The cross-sections of the electric guitar strings differed in diameter: strings E6, A5, and D4 were 1.07 mm, 0.81 mm, and 0.61 mm in size, respectively.

The strings for a violoncello are even more complex. The microphotographs in Figures 3.23 and 3.24 show the complex morphology of the samples, the different wrapping materials, and the different morphology of each wrapping (using lamete, round cross cuts, and hexagonal cores). All these tests were performed with the obective of obtaining the most beautiful, clear, and strong sound from a string

instrument; thus, this case study is a beautiful example of the multidisciplinary nature of the research needed in order to obtain the most useful results.

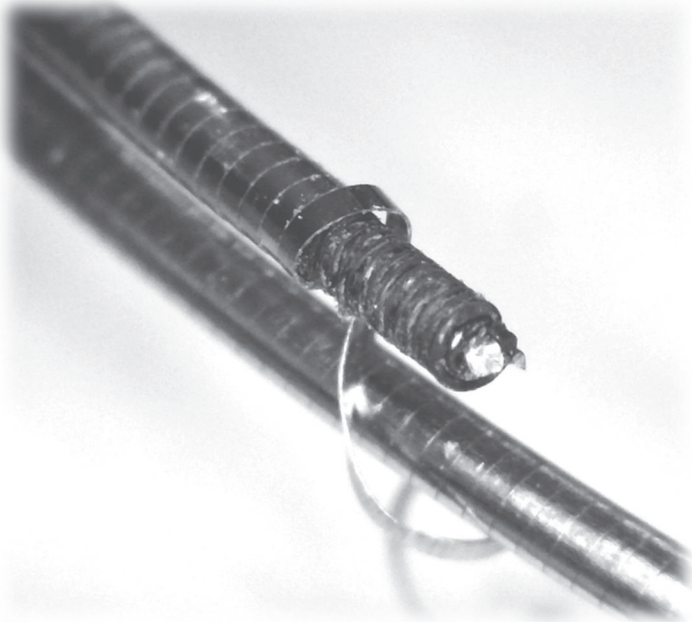


Figure 3.23. Microscopic view of the sample with three parts: hexagonal inner core and two different wrappings, the firs of the round cross section and the second flatted, 100× magnification.

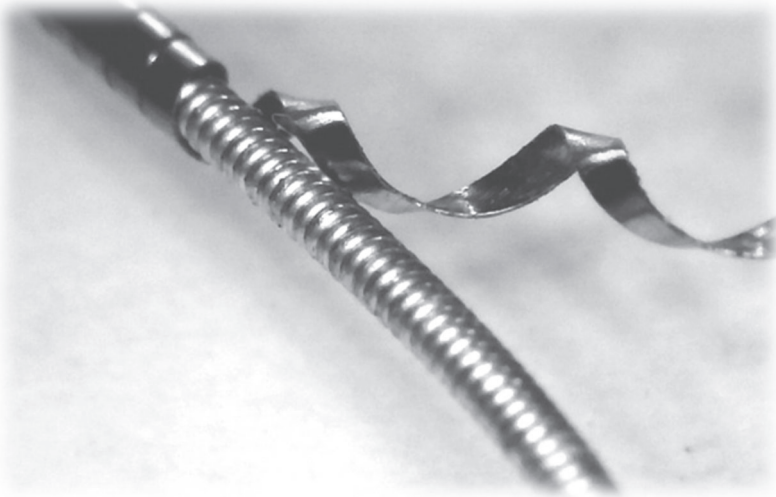


Figure 3.24. Microscopic view of the sample with two parts, inner and outer wrapping, 100× magnification.

Figure 3.25. Microscopic view of the sample with one material wrapped around its core, 100× magnification.

# Concluding Remarks

Historical samples are very precious materials. Their characterization is particularly complex since only relatively small amounts of materials are available for analysis. Therefore, only sensitive, selective, and reliable analytical procedures can be applied in the characterization of such materials. This book presents the current methodologies that have proved fundamental in unraveling the complex composition of metalized yarns and pearls collected from historical samples.

This book is therefore of a kind of interest to scientists dealing with the variety of interesting historical materials that it describes—from ancient mummies to knights and historical dresses and decorative textiles, jewels and pearls to musical instruments. The scientific findings that describe the characterization of materials detail complex procedures for gaining new insights into the application of methodologies for the determination of the composition and degradation of valuable hictorical materials.

Moreover, the book highlights the importance of multidisciplinary procedures involved in sampling, preserving, and conserving historical materials; in addition it presents modern sophisticated analytical procedures employed in materials characterization. Such methods cover microscopy, spectroscopy, and chromatography.

As this book utilizes current scientific advances to better understand the principles of materials characterization, it is of broad interest a wider public beyond the chemical, anthropological, and conservation-restorer communities. Moreover, it can offer particular support to a global audience interested in historical materials preservation.

# APPENDIX

## Table A1: List of binding media on historical materials

| Binder | Class | Chemical composition | Application |
|---|---|---|---|
| Aloe Resin | resin | vegetable resin | Used for gilding |
| Balms | essential oils + acids + resin | the acid may be, for example, benzoic acid | benzoin resin, peru balsam, canada balsam, tolu balsam |
| Benzoin | resin | benzoic acid, esters and terpenes | Soluble in alcohol. Used as part of varnishes. |
| Bitumen softer | bitumen | Hydrocarbon mixture | Dark, tough mineral sediment. Soluble in organic solvents |
| Egg white | protein | amino acids | Manuscript illumination, as an adhesive for gold leaves, and as a varnish for paint. |
| Cement | Mineral binder | 3 parts limestone (CaO) and 1 part clay ($SiO_2$, $Al_2O_3$, $Fe_2O_3$) | It is not a direct binder but serves to insulate wall surfaces before painting. |
| Ceresin | Mineral wax | with paraffin and rosin the appearance of beeswax | Use for impregnation, lubrication, and canning. |
| Damar | natural, | It is very compatible with other resins, waxes, and cellulose derivatives as a varnish. It is used for oil paints and enamels. It is cheaper than mastic, so mastic was often replaced with dammar. | |
| Dextrin | $(C_6H_{10}O_5)_n$ | Starch degradation product. Unlike starch, it is also soluble in cold water and precipitates with the addition of alcohol. It is very hygroscopic. The aqueous dextrin solution is very sticky. | |
| Elemi | Oil resin | essential oils somewhat sequiterpene and monoterpene | Compatible with vegetable, fish, and animal oils. Used in varnishes and water-colors. |
| Fragant | rubber resin | from the teagant plant | rubber resin that serves as an adhesive |

| | | | |
|---|---|---|---|
| Varnishes | oils + driers (meth oxides) | oils processed by cooking under the influence of air or with the addition of metal oxides | The most famous is the linseed oil varnish used for coating wood. Frequently used varnishes are obtained by adding resins and turpentine oil. They accelerate the drying of the surface. |
| Slaked lime | $Ca(OH)_2$ | Lime plaster. In the air, the plaster loses water, and with $CO_2$ from the air, the hydroxide turns into carbonate. | Living lime (CaO) is obtained by frying limestone CaCO3, and by adding water slaked lime is formed. |
| Gypsum | inorganic | $CaSO_4 \times 2H_2O$ | Mixed with water it gives a plastic mass. |
| Glycerine | alcohol | $C_3H_5(OH)_3$ | glue binder |
| Glutelin | cellulose glue, vegetable protein | insoluble in neutral solutions and water, highly soluble in dissolved acids and alkalis | The most important is gluten from wheat and contains the following proteins: albumin, globulin, glutei and gliding; with water it swells and turns into a sticky mass. It is not as perishable as glue or starch. |
| Tire | carbohydrate | dried juice of the plant | With cold water they give thick mucous liquids. |
| Gumi Arabica | resin | water-soluble resin, composed of Ca, Mg, and K salt of Arabic acid | As an adhesive and as an addition to inks and watercolors, and for the preparation of the most prized things. |
| Gumi gut | tire | Resin | poisonous resin, yellow watercolor paint |
| Gumi mastic | rubber resin | | Dissolves in alcohols and aryl CH, does not dissolve in aliphatic CH. |
| Egg | rubber resin | amino acids glutamine and aspergine, does not contain hydroxyproline | Whole egg, only egg white or only yolk can be used to prepare the binder. It is most often found in tempera (egg tempera). |
| Amber | protein | succinct acid, essential oils, etc. | Dissolves in hot linseed oil. It does not dissolve in any solvent. It served as a binder for the colors of Leonardo da Vinci and Rembrandt. |
| Japan wax | fossil resin from coniferous wood | | It is secreted by trees grown in Japan and China. It is very hard and brittle. It is used as a substitute for beeswax. |

| | | | |
|---|---|---|---|
| Potassium water glass | vegetable ointment with wax properties | $K_2O:SiO_2$=1:1.4-1.6 | It is used as a means of impregnating wood and fabrics and as an adhesive, and as a wetting agent and a means of fixing paint. |
| Camphor | potassium silicate | dicyclic ketone monoterpenes and diterpenes | It is obtained from camphor wood that grows in China, and serves mainly as a softener in the production of celluloid. |
| Canadian balm | resin | It contains a mixture of resin acids and volatile monoterpenes | Used as a solvent for oil paints and polishes, as putty for lenses, for microscopic preparations, and in the production of fine varnishes. |
| Kandelila | herbal | The main ingredient is hentriacontane, esters, resins, and myricyl alcohol | It is obtained from reeds that grow in Mexico and Texas. Yellow-brown, hard and stiff, it serves to harden other waxes, and as a substitute for carnauba and beeswax. |
| | wax | Most esters of ceratinic acid and myricyl alcohol and unsaturated OH acid at 12°C | It is shiny, hard, and is added to beeswax and other softer waxes to raise their melting point, hardness, shine, and resistance, to reduce the stickiness to dust, and the tendency of frost to crystallize it. |
| Carnauba | vegetable wax | amino acids, full of glutamic, proline, and hydroxyproline, does not contain glycine | It is obtained from milk, serves as a binder for wall and panel paintings, and for paints with which furniture and textiles are dyed. |
| Casein | protein | secretion of the insect Coccus Cerifus | It is whitish, hard, shiny and crystalline, odorless and tasteless, melting point 65–80°C. |
| Chinese wax | animal wax | esin acids, the most abundant abietic $C_{20}H_{30}O_2$ diterpenes | Soluble in turpentine oil, ether, acetone, miscible with fats, oils, insoluble in water. Serves as an additive to bath resins. |
| Rosin | resin | contains a mixture of resin acids, non-resinous substances, and diterpenes | It dissolves in alcohols and ketones. It is used for the preparation of oil varnishes. |
| | resin | | Looks like amber. Dammar and rosin are used instead. |

| | | | |
|---|---|---|---|
| He dug | of wood | contains esters of long-chain fatty acids and alcohols | It is extracted by washing the wool in detergent. It is yellowish in color, resistant to acids and alkalis, soluble in chloroform and ether, and insoluble in water |
| Lanolin | sheep's fleece wax | MgCl, MgO | Today it is used in the textile industry. It has the ability to resist moisture and has high strength. |
| Magnesium chloride and oxide | Inorganic binder | contains the most mastic acid $C_{23}H_{36}O_4$, little triterpene | Strongly intensifies colors. Compatible with vegetable oil and cellulose derivatives. Used for making fine varnishes. It is much more expensive than dammar. |
| Mastic | Natural resin of the aliphatic ester type | sucrose, glucose, and fructose | Rubber fragrant resin from wood, the smell is balsamic. |
| Honey | sugar | contains carbohydrates and terpenes | Used with lime and glue paints as an additional means of binding paint. |
| Mirtha | resin | Proteins, fats | very weak binder for azure paints |
| Milk | casein | | It is used for smoking and as a binder for paints. |
| Vinegar | | a mixture of resin acids and alcohols, esters, and essential oils. | Very similar to carnauba wax, it is obtained from palm trees in Brazil. |
| Olibanum (Aden) | soft resin | melting point 84°C | It is obtained by extraction with hot water from bituminous sediments. |
| Ouricury wax | herbal | saturated and unsaturated CH high molecular weight, liquid CH | It has a melting point of 500–800°C. It is resistant, stable and inert. It is crystalline. |
| Beeswax | wax | | It is currently the only material used in painting varnishes that is known to remain soluble in the mildest solvents. Adding beeswax to the varnish improves the solubility of the varnish. |
| Peru balm | wax | contains esters of palmitic acid and myrrh alcohol | soft, dark brown resin, soluble in alcohol, ether, chloroform, benzene |
| Beer | mineral wax | | poor binder for azure paints |
| Saccharose | animal wax | carbohydrates, alcohol | anti-drying binder with the addition of gelatin and egg white. |

| | | | |
|---|---|---|---|
| Sandarak | benzoic acid + oils + resin | glucose and fructose | It is used as a varnish for metals. In combination with mastic, it is used to obtain fine colors. Dissolved in linseed oil it serves as a coating for armor and bows. |
| Storax | beer | It contains the most sandaracolic acid 85%, a little diterpene, etc. | brown-green color, pleasant smell |
| Shellac wax | sugar | ester of cinnamon acid and styrene | Shellac contains only 3–8% shellac wax before purification. Shellac resin is soluble in alcohol, for putties, insulating material and sealing wax. |
| Shellac (resin) | soft resin | Mixture of lactides, lactones, esters, and ethers of alkyl hydroaromatic polyhydroxy acids | Natural resin produced by the insect *Tachardia lacca*. It darkens a lot and relatively quickly, so it is used for varnishing paintings. It is used to protect furniture, and it was once used as a thermoplastic adhesive. |
| Starch | ester | Soapy shellac is added to binders that are diluted with water to make it more water resistant after drying. | Insoluble in cold water, it dissolves colloidal in hot water and a starch glue is formed. Under the influence of higher temperatures, acids, or enzymes, diastase is broken down into simpler carbohydrates: dextrin, maltose and glucose. |
| Spermaceti wax | animal wax | glucose | It is found in the skull and sperm of a whale and is white transparent in color. Melting point 41–49°C. |
| Stearin | natural resin | contains mainly cetyl palmitate ester | It is similar to vegetable and animal waxes in appearance, but not in composition and properties. It is mainly used in the production of candles and cosmetics, but can be added to another wax to reduce stickiness. |
| Incense | polysaccharide | Products are from the 19$^{th}$ century. Produced by saponification of mainly animal fats and various oils. | Dried juice of the plant, yellow to reddish in color. |

| | | | |
|---|---|---|---|
| Turpentine | animal wax | contains terpene | Distillation separates the volatile essential part, oil, leaving a non-volatile resin-rosin, thinner for oil paints, and solvent for natural soft resins. |
| Tolu balm | animal synthetic wax | mixture of resin acids and essential oils (terpenes) | Semi-solid resin with a pleasant smell. |
| Glue | resin | contains both benzoic and cinnamic acid | addition to pigment paints, earth paints, for primers and glue for wood, cardboard and paper |
| Oils | coniferous wood balm | amino acids | cedar, poppy, olive, flax, walnut, castor, rosemary, rapeseed, fish, rose soybean, sunflower, teung oil |
| Venetian turpentine | oil, resin, acid | based on terpenes | as a solvent, it is obtained by distillation from turpentine |
| Sugarcane wax | proteins | monoterpenes | Similar to other vegetable waxes, it is produced by extraction from the scraps of sugar juice that remain in the production of sugar. |
| White spirit | oil | melting point 72–76°C | In artistic painting it must be colorless and completely volatile. It is a substitute for more poisonous turpentine. |
| Dragon's blood | resin | a mixture of aliphatic and aromatic hydrocarbons | Dark red amorphous resin, it is a pigment for varnishes and polishes. |
| Gelatin | vegetable wax | natural resin | Gelatin is purified glue. In painting, it is used for shiny gilding and forging paints, and for azure paints. |
| Egg yolk | amino acids | proteins, proline, hydrosiproline, and glycine | As a binder for tempera and for binding metallic paints; it preserves permanent shine and strength. |

**Table A.2. Limits of detection for ICP-OES, 20 elements in 1% $HNO_3$**

| Element | λ, nm | Standard deviation | Limit of detection, $\mu gcm^{-3}$ |
|---|---|---|---|
| Al | 237.312 | 0.0032 | 0.0097 |
| Al | 396.152 | 0.0034 | 0.0101 |
| As | 193.759 | 0.0015 | 0.0046 |
| Au | 191.964 | 0.0336 | 0.1007 |
| Au | 191.964 | 0.0075 | 0.0224 |
| Bi | 223.061 | 0.0021 | 0.0062 |
| Cd | 226.502 | 0.0001 | 0.0002 |
| Co | 228.616 | 0.0001 | 0.0003 |
| Cr | 205.552 | 0.0001 | 0.0003 |
| Cu | 324.754 | 0.0018 | 0.0054 |
| Fe | 259.940 | 0.0011 | 0.0032 |
| Mn | 257.610 | 0.0002 | 0.0006 |
| Mo | 202.030 | 0.0001 | 0.0003 |
| Ni | 231.604 | 0.0001 | 0.0004 |
| Pb | 220.353 | 0.0012 | 0.0036 |
| Sb | 206.833 | 0.0010 | 0.0031 |
| Si | 212.412 | 0.0008 | 0.0023 |
| Sn | 189.989 | 0.0005 | 0.0015 |
| V | 311.071 | 0.0011 | 0.0033 |
| W | 207.911 | 0.0018 | 0.0053 |
| W | 224.875 | 0.0055 | 0.0165 |
| Zn | 206.200 | 0.0003 | 0.0008 |

**Table A.3. Limits of detection for 20 elements in artificial sweat solution.**

| Element | λ, nm | Standard deviation | Limit of detection, $\mu gcm^{-3}$ |
|---|---|---|---|
| Al | 237.312 | 0.0088 | 0.0264 |
| Al | 396.152 | 0.0071 | 0.0213 |
| As | 193.759 | 0.0017 | 0.0051 |
| Au | 191.964 | 0.6683 | 2.0049* |
| Au | 191.964 | 3.3812 | 10.144* |
| Bi | 223.061 | 0.0043 | 0.0129 |
| Cd | 226.502 | 0.0001 | 0.0003 |
| Co | 228.616 | 0.0002 | 0.0006 |
| Cr | 205.552 | 0.0001 | 0.0003 |
| Cu | 324.754 | 0.0017 | 0.0051 |
| Fe | 259.940 | 0.0022 | 0.0066 |
| Mn | 257.610 | 0.0003 | 0.0009 |
| Mo | 202.030 | 0.0003 | 0.0009 |
| Ni | 231.604 | 0.0006 | 0.0018 |
| Pb | 220.353 | 0.0016 | 0.0048 |
| Sb | 206.833 | 0.0025 | 0.0075 |
| Si | 212.412 | 0.0195 | 0.0585 |
| Sn | 189.989 | 0.0015 | 0.0045 |
| V | 311.071 | 0.0016 | 0.0048 |
| W | 207.911 | 0.0058 | 0.0174 |
| W | 224.875 | 0.0266 | 0.0798 |
| Zn | 206.200 | 0.0011 | 0.0033 |

**Table A.4. Parameters of calibration curves for determination of 20 elements in 1% $HNO_3$.**

| Element | λ, nm | y = ax + b | | $R^2$ |
|---|---|---|---|---|
| | | a | b | |
| Al | 237.302 | 49.195 | -6.058 | 1.0000 |
| Al | 396.152 | 21.637 | 4.3927 | 0.9998 |
| As | 193.759 | 0.5802 | -0.669 | 0.9782 |
| Au | 191.964 | 7.9847 | 0.0228 | 1.0000 |
| Bi | 223.061 | 417.01 | 132.41 | 0.9994 |
| Cd | 226.502 | 298.82 | 50.401 | 0.9997 |
| Co | 228.616 | 203.67 | 20.429 | 0.9999 |
| Cr | 205.552 | 136.68 | 18.709 | 0.9991 |
| Cu | 324.754 | 59.909 | 5.2435 | 1.0000 |
| Fe | 259.940 | 347.31 | 88.169 | 0.9995 |
| Mn | 257.610 | 114.78 | 12.454 | 0.9999 |
| Mo | 202.030 | 135.67 | 29.459 | 0.9997 |
| Ni | 231.604 | 28.851 | 2.3494 | 1.0000 |
| Pb | 220.353 | 20.354 | -0.297 | 0.9997 |
| Sb | 206.833 | 49.033 | 7.1228 | 0.9999 |
| Si | 212.412 | 22.176 | 4.5632 | 0.9995 |
| Sn | 189.989 | 128.18 | 18.296 | 0.9999 |
| V | 311.071 | 3.3907 | 0.2640 | 0.9992 |
| W | 207.943 | 44.729 | 14.137 | 0.9994 |
| Zn | 224.826 | 7.5802 | -0.669 | 0.9782 |

**Table A.5. Parameters of calibration curves for determination of 20 elements in artificial sweat solution**

| Element | λ, nm | y = ax + b | | $R^2$ |
|---|---|---|---|---|
| | | a | b | |
| Al | 237.302 | 7.1116 | - 0.0934 | 1.0000 |
| Al | 396.152 | 52.069 | - 1.305 | 0.9995 |
| As | 193.759 | 25.556 | - 0.135 | 1.0000 |
| Au | 191.964 | 0.2878 | 0.9313 | 0.7866 |
| Bi | 223.061 | 10.057 | 0.6062 | 1.0000 |
| Cd | 226.502 | 427.48 | - 22.126 | 0.9999 |
| Co | 228.616 | 327.41 | - 15.242 | 0.9999 |
| Cr | 205.552 | 231.34 | - 9.1583 | 0.9999 |
| Cu | 324.754 | 175.18 | 8.0683 | 1.0000 |
| Fe | 259.940 | 62.1 | - 1.38 | 1.0000 |
| Mn | 257.610 | 417.44 | - 8.7167 | 1.0000 |
| Mo | 202.030 | 146.84 | - 4.7583 | 0.9999 |
| Ni | 231.604 | 142.81 | - 3.85 | 1.0000 |
| Pb | 220.353 | 30.774 | - 1.629 | 1.0000 |
| Sb | 206.833 | 21.253 | 0.6092 | 0.9998 |
| Si | 212.412 | 51.4 | 23.513 | 0.998 |
| Sn | 189.989 | 23.672 | - 0.2592 | 0.9999 |
| V | 311.071 | 138.31 | - 5.95 | 0.9999 |
| W | 207.943 | 5.2428 | - 2.2848 | 0.9596 |
| Zn | 206.200 | 49.6 | - 3.0133 | 0.9998 |

# About the Author

Assoc. Prof. Iva Rezić PhD PhD was until October 2020 a vice-dean for scientific research at the University of Zagreb, Department of Applied Chemistry, Faculty of Textile Technology, where she led a group of scientists in the field of chemical analysis and material sciences, and taught the courses “Analytical Chemistry,” “Instrumental Methods of Analysis,” “Physical Chemistry,” “Textile Chemistry,” and “Computer Method Design of Experiment.” She is a double doctor of science laureate: in the field of natural sciences (analytical chemistry) and technical sciences (materials engineering). She applies her professional competences in the management of projects and teams, with a current focus on the development of new materials and the analysis of old precious samples. She has published 56 scientific papers, 3 university textbooks, and 5 chapters in scientific books. For her work, she has been awarded with many prizes.