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An analytical and applicative approach to the *cleaning* of artworks

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1. Introduction

Cleaning is a critical operation aimed to recovering the legibility of the artwork (1963, C. Brandi, *Theory of restoration*), and it may involve, depending on the instances, removing soiling materials from surfaces (*surface cleaning)* or thinning/removing altered or unwanted layers (such as discoloured varnishes and coatings, retouches, overpaints, *patinas*, residual adhesives).

By its own nature, cleaning is thus one of the few inherently irreversible conservation processes, furthermore it is a recurring procedure, and it is strongly influenced by a variety of factors (i.e., cultural, historical, personal/subjective). It should be performed in the most selectively possible way, minimizing the risk of altering the chemical-physical and morphological properties of the surfaces to be preserved.

Therefore, it should only be performed when a sufficient degree of selectivity and control can be reasonably anticipated. Selecting appropriate and effective cleaning systems is only possible on the basis of the knowledge of the artwork itself, of the nature and the properties of its constituent materials. Before any treatment, an appropriate analytical campaign should provide the broadest possible information on the chemical components, their relative amount and location, the nature of soils retained on the surfaces, and their ageing characteristics. This body of information is crucial to defining the proper parameters the conservation materials should have, in terms of pH range, ionic concentration, requirement for gelling agents, surfactants or chelators, and preventing the uncritical use of standardized traditional “recipes”.

Lastly, the study of the interactions of all conservation materials with the artwork, i.e. permanence of residues, swelling and *leaching* phenomena of paint and ground layers, allows to ascertain the specificity of the treatment and to evaluate the risk factor involved in it.

As the first aim, this thesis addressed two kind of very fragile artworks, difficult to treat: plaster and wax sculptures. In general, they tend to be regarded as a sort of "lesser" form of art, as compared to paintings and there is an evident lack of scientific information on the treatments of these artworks. The purpose of the study was to search for selective methodologies for the *surface cleaning,* as a valid alternative to traditional methods which have severe limitations with regard to the potential interaction with their surfaces.

A further goal lied within the most known and studied artefacts, namely paintings. However, the paint binding medium selected, egg, has so far received much less attention than the oil-based media. This is in striking contrast with the importance that historically this binding medium had in the Italian painting tradition. More precisely, the egg binding medium has been often characterized with respect to its chemistry and film-forming characteristics; much less attention has been devoted to investigating the interactions of the cleaning materials with the egg medium, and understanding how they can affect its properties and its appearance. The susceptibility of this medium to *leaching* phenomena induced by water and organic solvents was thus evaluated.

In collaboration with Cesmar7 (*Centre for the Study of Materials for Restoration*), two important museums, the Galleria d'Arte Moderna in Milan and the Pinacoteca Nazionale in Siena, and The Gipsoteca Toschi in Parma, relying on the indispensable

collaboration of various conservators/restorers, an analytical and applicative research was conducted on both mocks up and actual artworks or their fragments.

With respect to these three different goals, this research allowed

- to develop an effective and innovative method for cleaning plaster by using Agarose-based gelling materials, respectful of the porous nature of the substrates;
- to prove the suitability of specifically designed aqueous solutions for cleaning wax artworks on laboratory-cast wax layers and on aged actual sculptures, characterized by altered surfaces;
- to evaluate the behaviour and the effects of water and organic solvents on unaged egg tempera films and on a 16^{th} century actual panel painting, yielding information on physical phenomena such as visual changes and swelling/leaching processes at various solvent polarities.

Various instruments were used for analyses, ranging from multispectral scanner (MSS) for colorimetric analysis to gas chromatography-mass spectrometer (GC-MS) for analyses of residues and components extracted during cleaning testing, Fourier transform-infrared spectroscopy (FT-IR) and scanning electron microscopy and X Ray microanalysis (SEM-EDX).

1.1 Cleaning of the artworks

Cleaning is perhaps the most important, delicate at the same time controversial process belonging to the conservation treatment of artworks.

Although a strict definition of cleaning would be the removal of dirt, grime, or other accretions (*surface cleaning*), in the conservation field, cleaning is used in the broader meaning to include thinning/removing altered or unwanted layers of materials without damaging or altering the chemical-physical properties of the surfaces to be preserved.
For instance, the materials to remove can be deposits (dirt and grime), additions (overpaints and retouches), altered patinas, discoloured or damaged varnish and coatings, gilding from polychrome artworks and stains, salts, and other accretions from surfaces or substrates of objects.

Furthermore, cleaning is one of the few conservation processes inherently irreversible that, if improperly performed, can be invasive and potentially lead to undesired effects.

During conservation plan, cleaning task is almost always required, it should be periodically performed and after that it should follow the other treatments; it is different than consolidation or protective treatments which are only necessary when the artworks have serious phenomena of deterioration and alteration.

Obviously, there is no single definitive cleaning methodology that can be applied to all objects and the conservator can choose between a lot of different methodologies. Indeed, an ideal cleaning methodology should be specifically designed for each

particular object giving to the conservator/restorer the greatest possible control over the cleaning procedure.

It would also not pose any potential health risk to the operator or potential damage to the environment.

Cleaning, first of all regarding paintings, has got serious implication in terms of both aesthetic and physical changes to the object being cleaned, so it has generated controversies and different schools of thinking. In addition, it has an important component due to the sensitivity and competence of conservator/restorer.

Summarizing, cleaning is a very complicated issue that presents theoretical (regarding the image), scientific (regarding the materials), and practical implications (regarding the operator).

In Italy, the theorization of restoration including cleaning dates back to 1960s and it remains the main reference. In 1963, the historian of art Cesare Brandi in the *Theory of Restoration* [1] defined the cleaning as a critical operation aimed to recovering the legibility of the artwork. For him, every conservation plan is set by esthetic instance and historical instance and he describes the material in a subordinate way than the image: "...which deals with the image epiphany". In 2002, Marco Ciatti [2] underlined the need to plan also the cleaning operation during the conservation campaign; in the same occasion, Giorgio Bonsanti [3] argued that cleaning operation can provide the access to the artworks surface, that is not only important for the study of the artwork, but it is also useful for the complete conservation project.

Although plays an important role in the cleaning operation, nobody defines a material instance: indeed, the aim of cleaning is the removal of altered materials that if are left on the artwork they can compromise its structural integrity.

In 2004 [4], it was affirmed that cleaning operation should be set inside the conservation project, that follows minimum intervention approach.

Since the beginning (1974) of the famous "cleaning controversy" [5], focused on the problem about the removal or not of paintings patina, two school of thinking have been defined: the "scientific cleaning" of English tradition, more objective and stronger, and on the other side the "differentiate cleaning" of Italian tradition, more subjective and less strong.

For the English school, as James Beck [6] recently has said, cleaning is performed to simplify the legibility of the artworks, for common people. This point of view doesn't consider neither of a material instance nor the historical instance of Brandi.

Considered that, the ideal cleaning approach has to combine the double nature of artwork: as an image and as a material (or better a complicated mixture of different materials); these parts are inevitably correlated. For instance, three factors due to the materials alter the image of an art object: the constituent materials, altered by degradation phenomena such as hydrolytic or oxidative processes; the materials intentionally added over the centuries to change the image of the art object for conservation purpose; the materials accidentally deposited on the surface over the time (dust, atmospheric pollutants), biological patina.

From a chemical point of view, ideal cleaning should act on one material – or treat several as one – (i.e., by dissolving or swelling) that is to be removed, leaving unchanged others that are to be preserved (the constituent materials). As a selective operation, cleaning procedure starts with the chemical analysis of the artwork materials and the knowledge of their distribution. Cleaning is a safe operation when the materials to remove have enough different properties (chemical composition, polarity, pH) to the

materials to be conserved. Otherwise, cleaning isn't inherently selective and it can be only attainable by using particular methods. Finally, in some cases, cleaning should not be carried out.

The work of art, especially the painting, is a complicated mixture of materials (pigments, binding media, coatings, fillers, etc..) that interact each other; furthermore, the materials are subject to aging and they are also modified for addition of conservation materials; like a solid/gas interfaces, surfaces are the most prone to aging and decay. It is difficult, or even impossible, to distinguish between original materials and conservation materials added after hand. So, the materials present into the artwork would be considered all together constituent materials.

The constituent materials are different but at the same time undistinguishable because they belong to the same layer, most of all the upper one. At the present, the materials, that were in origin different, are quite similar because of some properties such as polarity, pH value, solubility.

For example, it is known that deterioration of varnishes and binding media results in chemical changes such as cross-linking between chains of polymers, chain scissioning, oxidation of the main chains or side groups and the breakdown of molecules, often accompanied by the formation of highly oxidized products. These structural changes lead to an increase in the insolubility and polarity of the material, a reduction of the strength, and a change in color, among others [7].

The new compounds formation usually occurs by the chemical interaction of different materials.

For instance, the interaction of pigments and fatty-based binding media (such as oil and egg yolk tempera) may result in the production of metal soaps on the surface of

paintings which modifies their visible appearance and state of conservation. In fact, as the saponification progresses paintings appear darker due to the gradual loss of the pigment hiding power [8].

In practice, ideal cleaning is a very complex and delicate effort and it isn't always attainable.

In turn, it modifies the constituent materials.

Who has to perform the cleaning is the conservator/restorer. He can choose between a broad range of cleaning agents developed for particular tasks and he has to apply the best available procedure, some of which might compromise of the ideal design parameters, or the worst of the case the surface cleaning does not possible to performed. Based on their knowledge and experience, conservators/restorers develop a cleaning strategy to achieve the desired effect and that, it is hoped, will also fulfil broader parameters of conservation treatment (such as time schedules, resource limitations, etc..) and, further, meet established conservation standards.

In spite of a lot of progress done in the conservation field, nowadays a gap between conservator/restorer and conservation scientists persists.

Traditional cleaning methods available to the conservator/restorer include mechanical, noncontact (i.e., laser) and chemical removal (aqueous and nonaqueous formulations) [9].

The main mechanical means of removing is to use tools such as scalpel, with the aid of a magnifying loupe or a microscope. Abrasive methods that employed brick dust, ash and powdered resins and in same cases air have also been used. For mechanical removal of dirt mainly from organic objects, textiles and paper dye "eraser" are widely used to transfer soiling onto finely divided solids.

Over the past decade, lasers have been investigated and used as alternative to the more abrasive technique of sandblasting both for small-size cleaning of objects and for large-scale cleaning of architectural surfaces. Infrared visible lasers (Nd:YAG) are usually employed to remove surface dirt deposits, while ultraviolet excimer lasers are used for the removal of organic coatings. Lasers have been tested for cleaning paintings of dirt and varnish; however, they must be used with caution on painted surfaces until research provides a better understanding of their effects on pigments and substrates [10].

Since antiquity, aqueous cleaning methods have been usually applied to remove soil from surfaces (*surface cleaning*) [11] and also to swell/solve hydrophilic film-formers. These methods make use of water alone or water-based preparations of acids and alkali, soluble ions, soaps, detergents/surfactants and chelating agents.

Water itself, as a very polar solvent, is effective at dissolving hydrophilic materials and, because of its high dielectric constant, also ionic/ionisable materials. In the first case, water simply acts as a physical solvent by breaking intermolecular bonds and forming of hydrogen bonding and dipolar interaction; in the second one, water chemically reacts (with ionization/dissociation of acid/basic substances or even hydrolysis reaction) through the breaking down intramolecular (ionic) bonds and the formation of new bonds with water.

When simply water is not enough specific for cleaning purpose, its solvent power can be enhanced by adding some “active” components such as buffers, chelating agents and surfactants.

Acids/bases buffered solutions can ionize, respectively, basic/acid substances, especially when they are aged, oxidized and/or crosslinked; another ionic effect with specific ions (Na^+, K^+, etc..) can be useful and is required on these kind of films [11].

Tab. 1 Common buffering compounds [11].

Buffer	**pK_A**	**pH range**
Glycine/glycinate	2.35	1.0-3.7
Citric acid/citrate	3.12, 4.76	1.3-4.7
p toluenesulfonate/*p* toluenesulfunic acid	1.7	1.1-3.3
Formic acid/formate	3.75	2.8-4.6
Succinic acid/borate	4.2	3.0-5.8
Phenyl acetic acid/phenyl acetate	4.3	3.5-5.0
Acetic acid/acetate	4.77	3.7-5.6
Succinic acid/succinate	5.63	4.8-6.3
2-N-morpholinoethanesulfonic acid (MES)/ 2-N-morpholinoethanesulfonate	6.15	5.2-7.1
KH_2PO_4/borate	7.5	5.8-9.2
KH_2PO_4/ Na_2HPO_4	7.2	6.1-7.5
Triethanolamine/triethanolaminate	7.8	6.9-8.3
Diethylbarbiturate/diethylbarbituric acid	8.3	7.5-8.5
Tris(hydroxymethyl)aminomethane	8.08	7.2-9.0
N-tris(hydroxymethyl)methylglycine	8.13	7.2-9.0
N,N,bis(2-hydroxyethyl)glycine	8.35	7.4-9.2
Boric acid/borate	8.25	7.6-8.9
Glycine/glycinate	9.78	8.2-10.1
Ethanolamine/ethanolaminate	9.5	8.6-10.4
Carbonate/hydrogen carbonate	10.1	9.2-11.0
Na_2HPO_4/PO_4^{-3}	11.5	11.0-12.0

Aqueous solutions of chelating agents (such as citrates or ethylenediaminetetraacetic salts) are able to dissolve insoluble salts; finally, emulsioning/detergency of hydrophobic materials can take place in water by using surface-active agents (surfactants).

Among the most common group of aqueous cleaning solutions, simple ammoniated solutions and saliva, that provides ionic, surfactant, buffering, chelating and enzymatic effects, are still employed. Other primarily aqueous materials, and now totally outdated, have included various soap preparations, urine, wine, blood, milk, beer, solutions of potash and lye and sliced food such as potatoes and garlic.

Furthermore, to control the action of traditional aqueous cleaning agents, thickened or paste materials like soap mixture and, in the 1990s, poultices based on cellulosic materials have long been used.

The advantages of aqueous environment are obviously the absence of toxicity problem and the possibility to ideally design an cleaning system for any given conservation case. So, the selectivity of aqueous systems are generally grater than nonaqueous ones.

Since the mid-19th century, organic solvents have been the widely used technique especially for cleaning painted surfaces with the aim to dissolve layers (i.e., varnish, retouching, patinas) to be removed.

Alkaline (or acid) organic solvents chemically react by breaking down intramolecular chemical bonds, while neutral organic solvents have a physical action on intermolecular bonds; dipolar aprotic organic solvents exhibit both chemical and physical action.

The more common solvents used to clean paintings are ethanol, methanol, acetone, benzyl alcohol, xilene, toluene, mineral spirits, turpentine, and mixture of one or more of these.

Conservators involved in the pioneering design of cleaning methodologies developed empirical but effective solvent mixture at various points. The mixture usually varied in polarity to approximate the solubility parameters needed to solvate a particular material and became codified [12].

A similar approach is the Teas triangle method [13]. If materials to be removed are identified, the solvent blend that maximizes selectivity of the cleaning action can be chosen from Teas plots. These diagrams plot the bulk properties of the solvents as a function of the intensity of their intermolecular, noncovalent interactions (hydrogen bonding, dispersion forces, and dipolar interactions). Using this (or related tools), the

conservator can select what should be an appropriate solvent system to solvate the substance to be removed, starting by solvent with the minimum of polarity [14].

Solvents, usually applied with cotton-wool swabs or small brushes, do not provide control over capillary flow over the surface and through the structure of the object. Methods of controlling and manipulating solvent action have included "solvent-restrainers" and "dilutions" and wax-solvent pastes. These cleaning processes have limitations such as the problem of paste residue and the need to clear the residue with organic solvents.

In addition to lack of control, limitations of solvent cleaning are often the toxicity, the problem of removing hard insoluble layers of overpaint and the long term effect of leaching and swelling of the paint layers to preserve (mainly varnishes and binding media) [15]. One of the main negative side effects of swelling and leaching is the drastic decrease of both the mechanical strength and overall stability of the painting. Some traditional solvents are rarely used because newer cleaning methodologies provide less toxic and more effective alternatives.

The interaction of solvents and reactants and their effects on different artworks have lead to the search for selectivity and specificity of treatment materials with regards to the characteristics of the materials to which they are applied.

Today, a number of additional cleaning methodologies are available, based on (aqueous and nonaqueous) solvent gels, enzymes, rigid gels, resin soaps, among others. Each of these methods was developed following the principle of selectivity and they are fine-tuned to a specific cleaning task. However, these new methodologies have to be considered as an addition, rather than an replacement, to organic solvents and other traditional methods.

Gel-based aqueous cleaning systems, including organic solvent gels, were introduced in the 1980s by Richard Wolbers in response to the need for increased control, as an alternative to cleaning with free solvents [16].

Appling a liquid (water or solvents) to surface (especially when it is porous) is a dangerous action because of its high surface tension, scarce wetting power and high diffusion and capillarity. This can lead undesirable effects on water/solvent-sensitive materials within the internal layers of an object at the same time exhibiting limited surface action. On the contrary, entrapping the liquid cleaning agents in gel matrices consists of reduce surface tension, contact angle and increase the retention power and the viscosity of the system. According to the Washburn equation, the diffusion rate of the liquid immobilized within the polymeric gel network is significantly reduced, allowing its action to be better controlled. Another advantages of solvents gels are the control of the (organic) solvent evaporation rate and components originally immiscible (water, organic solvents, polymer, and surfactant) become miscible in gelled form and the gelled systems minimize human exposure to toxic organic solvents [17].

The high-viscosity Polyacrylic Acid derivatives are the gelled polymers usually employed for this purpose. Solvent gels are directly applied to a surface sometimes followed by mechanical action with a brush to increase gel-surface contact.

These gels developed by Wolbers have been mainly used to remove overpaints and varnishes from paintings and obviously for *surface cleaning* and in general they have solved many previously unsolvable cleanings problems. However, a disadvantage of the Wolbers system is the need of a clearing rinse after the gel application usually carries out by means of dry cotton swabs and swabs wetted with free solvent. By using free solvents and through making mechanical action, the cleaning can lead effect of leaching

on paint film. In addition, the possibility of the presence of any gel residue (or its non-volatile components) on the surface of an object remains, in particularly with the use of organic solvents. So to that follows the question of whether residue might have a detrimental effect on the state of preservation of the cleaned object.

Since 1990 [18], to address these problems, in particular the so-called "residue question", several studies have been undertaken and they have shown that gel residue is left onto a cleaned surface. For instance, Burnstock and Kieslich [19] demonstrated that even if solvent gel formulations containing Ethomeen is removed from a painted surface by solvents, residues of poly(acrylic acid)-based gel remain and the solvents used for clearance influenced the surface appearance of the painting. In a review, Lang [20] also dealt with this aspect of gel cleaning and underlined the lack of information about this issue. More recently, the in-depth Surface Cleaning-Gels research Project (1997-2004) found important answers regarding the residue question [9]. By making quantitative analysis of gel residue and by studying the chemical changes in surfactants during aging and the appearance of surfaces after cleaning, this broad research provided information on the gel retention rate of different gels and supports (ranging from oil painting to a very porous materials such as plaster) [21].

Resin soaps are another component of the aqueous cleaning system introduced by Richard Wolbers [11]. They consist of detergents based on triethanolamine salts of abietc and deoxycholic acids for removing oxidised resin varnish from oil paintings; they have structural similarity to triterpenoid resin components, which help to maximise non-covalent intermolecular interactions between soap and resin and provide a selectivity removing tools.

Other gels commonly used are the medium-viscosity Cellulose Ethers and the pseudo-plastic Xanthan Gum.

In 2003, Wolbers introduced the use of aqueous “rigid gels”[22] and a further study [23] has established that Agarose-based polymers, able to form high viscosity gels, are very effective and safe tools for cleaning porous supports. This “rigid gel” can deliver water (or aqueous solutions) in a very limited and controlled way, they require no post-treatment rinsing (because of their physical form and their limited adhesive strength) and they are able to draw into their molecular mesh particles that are dissolved once gels are applied on the surface of the artefact and water starts diffusing into it.

Because of their specificity, aqueous gel methods have been used for treating both painted and unpainted artworks allow conservators to solve not only the removal of soiling but also more problematic materials (coating, painting, adhesive, etc..) [24].

The gel-based cleaning technique allow to accommodate also highly selective cleaning agents such as enzymes [25], chelating agents [11], buffers, detergents and surfactants.

Among enzymes, the hydrolytic ones (Class III) are the most widely used in the conservation field. They catalyze selective cleavage of specific bonds within the proper substrates. So Proteases, Amylases, and Esterases hydrolyze, respectively, peptide bonds in proteins (i.e., animal glue and gelatines, Albumen, Casein), α-1,4-glucosidic bonds in Starch (i.e., flours, “vegetable glues”), and ester bonds in simple and complex esters (i.e., drying oils, fats, some waxes, some ester-containing synthetic resins).

With other approach, new studies have been focused on the making and using of high viscosity aqueous polymeric dispersions based on poly(vinyl alcohol)-borax matrices as cleaning agents for removal of oxidized coatings from paintings. Such

systems could be easily peeled from the surface onto which they are applied without leading to measurable amounts of surface residues [26]. The same researcher have also developed a new class of organogels ($PEICO_2$-based organogel) isothermally rheoreversible as cleaning tools directly applied onto the painted surface with effectiveness in removing different film-forming materials (i.e., dammar and acrylic polymeric resin). The properties of the original solution (exploited during the gel removal) are re-established after addition of a small amount of a weak acid [27]. Other researches have been based on the incorporation of magnetic, coated-ferrite nanoparticles into polyacrylamide gels adding functionality to a versatile system comprising oil-in-water microemulsions, aqueous micellar solutions, or xerogels that act as sponges. The ferrite particles allow the use of magnets both to place the gels precisely on a surface and to lift them from it after cleaning [28].

1.2 The artworks

1.2.1 Gypsum plaster

1.2.1.1 Historical uses of gypsum plaster

It is very common found plaster objects in museums (and their deposits) like original sculptures, fine copies or simple casts. The museum collections are composed of a lot of cases raging from variations, previously unknown pieces, curiosities, analogies and they may be models containing points of measurement, or primary works which still conserves marks due to sculptor tools. As “sacrifical” materials, plasters usually are phases of work production or its rendering in a different material, such as bronze. For these aspects, plasters should be considered more than a minor art how actually it is still thought.

Since antiquity, gypsum has been also used for the rendering of walls and as a mortar to join and consolidate bricks and stonework, especially in region characterized by a dry climate. It acts as a simple mortar without the necessity of adding any other inert components [29, 30]. In humid region, the use of gypsum, that is slightly water soluble, tends to be confined to indoor work and the ground layer of panel and canvas supports.

1.2.1.2 Physico-chemical properties of gypsum plaster

Gypsum plasters (or mortars) are prepared by calcining gypsum minerals or Selenite rocks ($CaSO_4.2H_2O$) at low temperature (128°C) [31]. Selenite crystallized in the monoclinic system and it is found in nature in the form of tabular glassy crystals of perfect cleavage; the crystals geminated in arrow-head form and in associations of

lenticular crystals, known as desert rose, are characteristic. Gypsum mineral is in the form of compact microcrystalline aggregates.

The hemi-hydrate obtained (pseudo-hexagonal crystals) rapidly sets through mixing with water and the speed of setting depends on the conditions under which the previous heating took place.

Actually, two kind allotropic of hemihydrate, having different rates of reaction with water, are known:

- α-hemihydrate is called the crystalline hemihydrate. It is formed by heating (97-106°C) at high pressure in the presence of water vapor, in autoclave. It is well crystallized (either needle-like or prismatic crystal) and it is not very porous. It reacts more rapidly with water.
- β-hemihydrate is called the microporous hemihydrate. It is formed in a dry atmosphere, in kilns; it has a soft and voluminous cryptocrystalline structure and it contains pores of relatively large dimensions. It reacts more slowly with water.

During setting reaction, the materials warm up and lose a little water by evaporation; this volume change is, however, offset by the expansion of the crystals caused by hydration, therefore the setting of gypsum takes place with a slight expansion (useful in making moulds).

The hemi-hydrate used to make moulds is known as *Scagliola* and it has a very fine granulometry and it comes from pure mineral of gypsum. Its time setting is 45 minutes.

The setting process occurs through several quick steps: firstly the dissolution of hemihydrate sulfate then, step by step, the re-hydratation and crystallization. In the end, a so weave network of acicular crystals to produce a kind felt results.

The amount of water necessary for setting process is 25% w/w, but to obtain a more fluid material the water amount practically used ranges from 65-75% to 85% w/w. The difference between water used and water necessary causes a higher water evaporation rate and then a widespread porosity (fig. 1). As a result, the mechanical strength of gypsum plaster can be sharply reduced.

Fig. 1 Detail of a plaster surface characterized by a widespread porosity.

In addition, no fillers are required to avoid contraction and cracking; setting is accelerated by adding to the gypsum dust or salts (i.e., sodium bisulfate, potassium sulfate) and is retarded when, for example, organic materials such as glue, milk or starch are present.

Finally, if the hydrate (or the hemi-hydrate) is heated above 163°C the remaining water is also eliminated and anhydrous calcium sulfate (α-soluble Anhydrite) is formed. By heating the gypsum beyond 250°C, up to 500°C and 600°C, insoluble anhydrite β is formed which is analogous to natural one. Anhydrite crystallized in the rhombic system and it is characterized by compact structure which is sometimes crystalline and saccharoidal. At around 1000°C hydraulic gypsum is obtained ($CaSO_4.nCaO$) and it is able to set after several weeks and in the end, at 1360°C gypsum melts and at 1375°C there is a complete dissociation in Calcium oxide, sulfur dioxide and Oxygen. Anhydrite may re-hydrate, especially the α-soluble Anhydrite, but rather slowly, giving a

contribute to the setting process. Transformation of hydrated gypsum into anhydrite can take place spontaneously in dry and hot climates (e.g in the Egyptian desert).

Tab.2 Forms of calcium sulfates and their properties [31].

Formula	Minerals	Form	Density (g/cm^3)	Water accounts	Water solubility (g/l) at 21°C	Hardness (Mohs scale)
$CaSO_4.2H_2O$	Selenite Gypsum	-	2.314-2.328	20.29%	2.4	1.5-2
$CaSO_4.0,5H_2O$	Bassanite	α, β	2.757 (α); 2639 (β)	6.21%	10	
$CaSO_4$	Anhydrite III α-soluble Anhydrite	α, β	2.44-2.45 (α)	0.02-0.05%	2.5	3-3.5
$CaSO_4$	Anhydrite II β-insoluble Anhydrite	B	2.899-2.985	0.00%	-	
$CaSO_4$	Anhydrite I	-	2.8-2.9	0.00%	-	

1.2.1.3 The conservation state of gypsum plaster

Because of its physico-mineralogical characteristics, plaster materials are able to absorb environmental humidity with dust, dirt deposit[1] and grease in a very quick way. The porosity, the quality and type of gypsum, production procedures such as water quantity, temperature, inner structures, are the main features. However, this natural degradation is less important than the consequences deriving from improper storage conditions.

The darkening of the surfaces can be also due to altered detaching materials[2] and oils applied when making replicas.

The artworks may be divided into those whose decay problems mainly involve the surface, affected by dust and dirt deposits more or less adhered or penetrated into the

[1] Generic particulate deposit is composed of inorganic elements (salts and metallic oxides from disaggregated minerals, cementing dusts, carbon compounds…), and organic compounds (hydrocarbons, pollutants, pollen…) which can function as cementing agents and maintain the particles cohesive.

[2] Detaching materials usually employed are lipids (linseed oil, olive oil, animal grease, oleine, tallow, vaseline, tall oil, coconut oil, and soaps, paraffin and stearin.

plaster, and those which are also structurally precarious, either fragmentary or chipped, with lacunae or loss of important elements.

Fig. 2 Deposit of insoluble dust on the surface of Incitamento alle barricate by Giuseppe Grandi from the Galleria d'Arte Moderna in Milan.

The present study mainly concerns with surface deterioration, usually in the form of a darkened appearance, with whiter marks due to abrasions or recent fractures, or noticeable brown spotting, caused by the internal presence of iron pieces which often provoke cracking and disconnections by increasing in volume in the presence of humidity. Any slight, more or less homogeneous coloration due to substances residual from casting are been taken into account.

Numerous sculptures have undergone operations of restoration in the course of the years, which have modified the layers of dirt, rendering them more tenacious and insoluble. Episodes of repainting, gessoing, filling in of strongly darkened areas are frequently encountered, as well as the presence of dark colorations, sometimes extended to adjacent areas to minimize intervention.

1.2.1.4 Traditional cleaning methods

Traditional methods employed to clean plasters may be divided into the following main categories: wet methods, peeling methods and dry methods [32].

The first one is based on the solvent action of water. It consists in applying water, alone or mixed with other solvents, soaps or detergents, with the aid of support materials (poultices, facing, or organic materials) followed by mechanical removal of dissolved dirt.[3] The results, even when cleaning is optimal, tend to leave the surface look like "pasty" and slightly smoothing.

The peeling method is based on delicate detachment of a thermoplastic coating[4] applied before to the surface by brush. The coating is usually made of a water-dispersed vinyl resin such as *Vinavil*®[5], whose viscosity does not permit penetration into the material while allowing capture of the dirt. The result can be very uniform but any previous interventions remain evident (like inappropriate cleaning which has left marks on the surface, differently colored fills and integration of lacunae, various types of spots penetrated into the plaster, darkened areas of porosity). However, this treatment is not suitable for plaster with cohesion problems or when plaster presents very elaborate surface with details and minute reliefs. Further negative factors are the acid pH of the dispersion, the possible presence of unknown substances in the formula (plasticizers, anti-fermentation, surfactant and dispersion additives, fillers) and the impossibility to perform any chemical action.

Dry methods include rubbing out and laser cleaning.

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[3] Some formulations are the so called "3A" (Ethyl Alcohol, Acetone, deionised water), "DA" (Dimethylformamide and Amyl Acetate), "DIDAX" (Dimethylformamide 35 ml, White Spirit 25 ml, Acetone 20 ml, Xylene 10 ml), "ABD" (water, Butylamine, Dimethylformamide), water and Ammonia in various percentages, water 70%, Peroxide 130 vol. 20%, Ammonia 10%, chelating substances such as Citric Acid and Trisodium Citrate with controlled pH, Marseilles soap in water solution, *Primal AC 33* applied with gauze, wood paste humidified with deionised water, mixed with Formamide and Carboxymethylcellulose. It may be noted that this list unfortunately contains numerous solvents problematic both for the health of the user and safety in the work environment because of their high level of toxicity, and for the structural integrity of the artefact to treat, because of their low degree of volatility, strong penetrating capacity into porous materials, and sometimes their acid or alkaline nature.

[4] Some formulations are *Vinavil NPC* and rubber latex.

[5] VINAVIL S.p.A., Via Valtellina, 63 - 20159 Milano, Tel. +39 02 695541, www.vinavil.it

Among the first class, sponges and erasers are used; their application has the disadvantage to leave grease residues to the surfaces.

The use of lasers (Q-switched Nd:YAG) to remove dust is widely employed on stone artwork conservation [33] but its application on plasters has just preliminarily been considered. For instance, a study [34], focusing on laboratory plaster samples constrained in temperature and testing different types of lasers irradiation, has shown that plasters cleaned by UV-laser (third harmonic of the Nd:YAG) underwent neither yellowing nor morphological or crystallographic changes, in contrast of an infrared wavelength (first harmonic of the Nd:YAG). Nevertheless, laser cleaning is not largely employed on plaster because generally it cause yellowish chromatic alteration and the laser ablation induces a fast rise in temperature in the medium (carrying out a possible modification of plaster phase between 100°C to 1200°C); furthermore its cost is still prohibitive and it is a time-consuming technique.

Among these methods, peeling methods and rubbing-out are the most widely used for cleaning of plaster.

As above described, traditional methods do not sufficiently guarantee the inalterability of the surfaces, or have proved ineffective in the presence of water drips or stains, or for removal of dirt solidified by patinas or residues of the manufacturing process.

1.2.1.5 Cleaning approach to the gypsum plaster

Among lithoid objects, plaster artifacts are certainly the most difficult to clean.

More than the nature of the dirt to remove (that requires aqueous methods), the critical parameters for evaluating any surface cleaner agents are the morphology, the color, the consistency, the porosity and the hygroscopicity of the gypsum.

The morphology of plaster objects is often irregular, even in terms of a small size (traces of workmanship) even for a tree-dimensional term, with jutting elements and negative areas, difficult to access during intervention. Plaster objects often are very large. These structural features induce to consider big areas to treat with important differences between flat planes.

The basic white color of constituent material can be altered by even the slightest dust deposit. It is necessary to discriminate among intentionally white surfaces and those which present various colorations (i.e., painting layers, patinas, or substances applied for detaching purposes) to be preserve as far as possible. The cleaning method must act gradually and in a selectivity way, layer-by-layer, in order to allow to choose the point of arrival, how far one should "insist" on going in the attempt to regain a surface free of spots, stains, darkened areas.

The surface layer is more compact than the internal ones, also because of a slight increase in volume and pressure exerted within the mould during casting: it is sensitive to all external influences, from humidity to any touching, and it is fragile in response to pressure, rubbing, abrasions.

The high degree of intrinsic porosity is a further critical point. This structure is able to absorb humidity, together with dust and dirt, and gypsum has got poor resistance also when in direct contact with water. The great water permeability of gypsum produces a loss of up to 1/3 of its mechanical properties in the case of saturation and the presence of even 1% of water in the pores of the material reduces the strength in compression to

some 40% of that of the dry material, because it acts as a lubricant for the movement of crystals [29]. Furthermore, the internal presence of cloth reinforcements, pins, or wooden and iron support elements, whose decay in the presence of humidity, may cause staining on the surface. Therefore, any wet treatment using free solvents constitutes a risk. It is impossible to be sure how much dissolved dirt will remain on the surface and how much will be dispersed instead into the porosity of the material, migrating towards the innermost layers and fixing there irreversibly, forming dark stains which cause the plaster to take on a certain "coloration".

Gypsum is not stable in atmosphere below the 50% of relative humidity at temperatures between 40-50°C or at rather lower temperatures if the surroundings remain dry over time.

Polar liquids act in the same way (but with a lesser degree) as water and non-polar liquids have no effect. Ammonia, that is commonly used in traditional cleaning, forms ammonium sulfate which is very soluble. Gypsum is also soluble in acids, in sodium thiosulfate, ammonium salts and glycerin and it is insoluble in ethanol.

Taking into account the critical parameters of the support than has to be preserved and recognizing the limits of traditional methods for *surface cleaning* of plaster, to identify an innovative method to remove superficial soiling or dirt penetrated inside the material was necessary.

The cleaning method should combine an aqueous methodology that release controlled amount of water, without the need to intervene in any way (i.e., mechanical action) on the surface to remove either the dirt or the substances used for cleaning (no rinse).

A theoretical answer was found in the aqueous rigid gels designed for application on three-dimensional plaster objects.

A recent study [23] have shown that rigid gels based on Agarose, such as Agar, is appropriate for cleaning polychrome artworks.

Agar is a polysaccharide able to form highly viscous gels and it is extracted from marine seaweed of the *Gelidiales* e *Gracilariales* orders. It accumulates in the cell walls of the *Agarophytes*, imbedded in the fibers of crystallized Cellulose, thus forming an important reserve for the plant. Agar is composed of two types of polysaccharides, Agarose and Agaropectin and only the first fraction is responsible for gelation, because of its high molecular weight (100.000-150.000 Daltons) and low percentage of sulphate groups (0.15%). Agarose is composed of disaccharide Agarobiose (fig. 3).

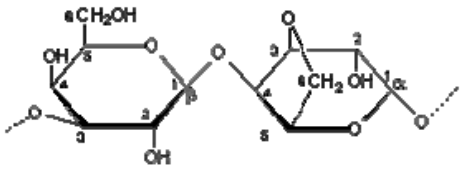


Fig. 3 Agarobiose is disaccharide of 1,3-β-D galactopyranose and 3,6-anhydro-α-L-galactopyranose through 1→4 glycosidic bonds.

By means of hydrogen bonding Agarose forms *physical gel:* a macroreticulate structure (fig. 4) occurs which make agar able to retain water and slowly released into the support, which is ideal for application in the field of surface cleaning.

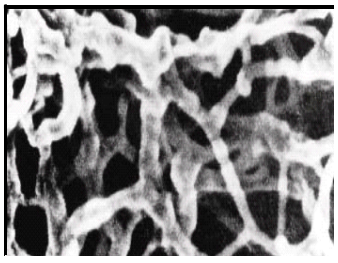


Fig. 4 Electron scansion microphotograph of 2% Agarose gel [35].

The gelation process of Agar and agarose is thermo-reversible: they in fact dissolve just by heating at 85°C and gel again when cooled, under 38°C; This process may be

repeated indefinitely. So, in solution they are in the form of random coil and in gelled form the chains of Agarobiose link each other taking on the form of double left helix. The two chains entwine so tightly that all spaces between them are closed, trapping water inside in a very effective way. The 4 terminations of 2 chains do not take part in the formation of helices, therefore remain in the coil form. This permits them to unite with other terminations, producing an extended 3-dimensional reticulum of helices containing water.The strength of the gel is related to the degree of reticulation (fig. 5). The gelling hysteresis (defined as the difference between the gelling temperature and the melting temperature) may reach 45°C.

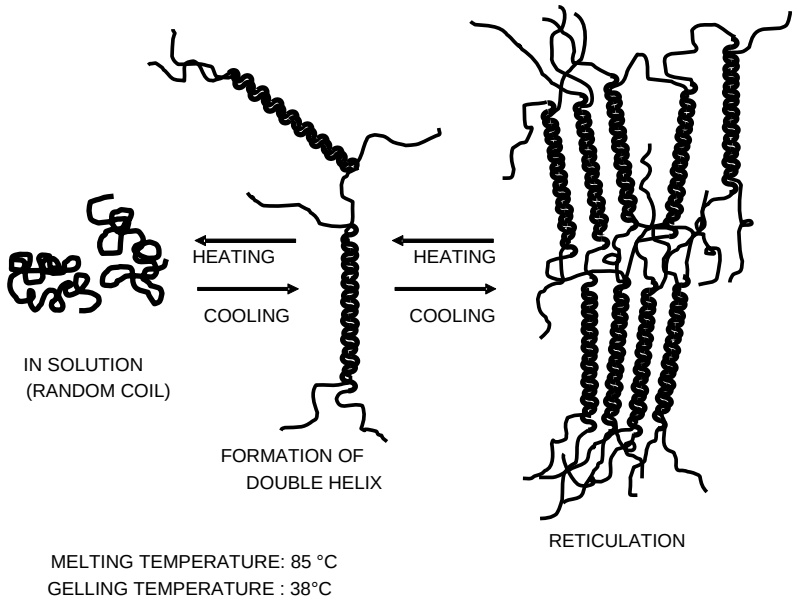


Fig. 5 Gelation mechanism of Agar [36].

Agarose gels are also highly porous. The *exclusion limit* (the greatest globular protein size that can traverse the gel in an aqueous solution) is 30,000,000 Daltons; the Agaropectin fraction instead will narrow the gel's reticulum pores and consequently also reduce the exclusion limit. Other properties of Agar gel are its capacity for syneresis, connected to the possibility for it to eliminate water contained in the reticulum mesh. Water release is accelerated by pressure which may be opportunely

applied to the surface of the gel: at 1% Agar or Agarose gel under such conditions is able to expel a considerable amount of the water accumulated in the cavities, meaning the elimination of up to 95% of the water in which Agarose or Agar has been dissolved. If the residual gel is re-emerged in water, it will return exactly to its initial state, which means that the structure of the gel has been maintained during the period of water loss (*gelling memory*).

Nevertheless Agar produces cloudier gels compared to clearness Agarose's, it is still to be preferred in the cleaning of artwork. Indeed, it is characterized by slower rate of discharge of water, as compared to Agarose. In additions, Agar is 10-20 times less expensive than Agarose.

The present research was focussed on the experimentations of these materials in order to develop an innovative methods for the *surface cleaning* of plaster. With analytical and applicative approach, a multidisciplinary study was carry out [32]; in particular, the need to the research initially had arised from a conservation require of the Galleria d'Arte Moderna in Milan that was undertaking a large campaign of recuperating, among that two hundred plaster pieces abandoned in storage far too many years ago were present. The state of conservation of several plasters, such as the famous plaster casts of the *Monumento alle Cinque Giornate* by Giuseppe Grandi, were mainly problematic: various past interventions had rendered the darkened appearance of the surfaces practically irreversible.

1.2.2 Waxes

1.2.2.1 Historical uses of waxes

Since antiquity, wax has found many uses and when mixed with other materials it was used for making sculptures. As a sculptor's material, wax-based formulations for finished artworks only occurred between the nineteenth and twentieth centuries.

It is known that wax played an important role during antiquity in technology, in the symbolic and artistic fields and in the preparation of cosmetics or medicinal commodities. For instance, the wax can be found in both humans and animals embalming formulations [37], in the materials for zoological findings conservation [38], in aqueous emulsions of encaustic painting [39] or in the wax-solvent pastes for cleaning of paintings, in adhesives, illuminant, sealing agents and waterproof coating to avoid, for example, the metal corrosion. In addition, it was also employed as writing ground and for lost wax casting, in the goldsmith's art, numismatics, and for the preparation of models to be transferred in stone, marble or metal.

Therefore the wax has been largely used by artists but nowadays only few artworks are preserved; they could be destroyed by the artist himself, recast, or rather be lost.

1.2.2.2 Physico-chemical properties of waxes

To know chemical composition of waxworks of art, that can provide insight into the natural or synthetic organic substances involved in sculpture making and the origin of the alterations observed, is of considerable importance for determining suitable methods for their restoration and preservation.

The wax-based sculptures are made by melting of waxes at low temperatures (below 100°C) in molds or by cold-molding (in pellets). The first one is more suited for the finished works of art and the second for the temporary [40] artworks, but both

techniques can be found in the same manufacture. The solidification occurs without withdrawal and, if wax is applied with organic solvent, it also takes place during solvent evaporation. The shape can always be changed by adding, by removing or by remodeling materials when wax is kept at a suitable temperature.

Although several materials (aliphatic compounds with different functional groups and with enough chain length to determine solid state) have a “waxy” character, in strictly chemical meaning the wax belongs to a specific family of natural compounds containing mixtures of esters (saturated fatty acids and long chain aliphatic alcohol), long chain hydrocarbons, alcohols and free fatty acids, sterols, terpenoids and their esters, in various amounts for each type of wax.

Natural waxes are amorphous substances and for this reason they are characterized by melting point and softening point. For instance, beeswax softens at 40-45°C and melts at 60-70°C. During manufacturing process, artificial waxes can also change their structure and assume a crystalline character.

Waxes have low cohesive and adhesive properties and, being non-polymer substances with low molecular weight, they are able to penetrate easily into the porosity of the material. In addition, waxes also have a high water repellency.

Although beeswax was the earliest waxy material exploited by men, many other natural substances have been used thereafter. Chinese insect wax, shellac wax, spermaceti and woolwax, from animal origin; carnauba, candelilla or Japan waxes, secreted by various plants, and fossil materials such as ozokerite or paraffin have also been employed in sculpture manufacture.

Following is the composition of the more important waxes found in the literature [41,42,43].

Animal waxes. Beeswax is synthesized by the bees (European bees: *Apis mellifera L.*, Asiatic bees: *Apis dorsata, Apis florea, Apis indica* and African bees: *Apis mellifera adansonii*) rather than collected from plants. Beeswax is golden yellow or light yellow and it presents a characteristic odour of honey. The composition of beeswax depends to some extent on the subspecies of the bees, the age of the wax, and the climatic circumstances of its production. However, this variation in composition mainly occurs in the relative amounts of the different components present, rather than in their chemical identity. It consists primarily of a mixture of esters of fatty acids and fatty alcohols, paraffinic hydrocarbons, and free fatty acids; minor amounts of free fatty alcohols are also present. On average, it consists of [43]:

- Free fatty acids (12-14%), most of which are saturated (ca. 85%) and have a chain length of C24-C34 (C24 as major compound).
- Free primary fatty alcohols (ca. 1%) with a chain length of C28-C36.
- Linear wax monoesters and hydroxymonoesters (35-45%) with chain lengths generally of C40-C48. The esters are derived almost exclusively from palmitic acid, 15-hydroxypalmitic acid, and oleic acid. The variation in total chain length of the ester is mainly the result of the different chain lengths of the alcohol moiety (C24-C34) (C24 as major compound).
- Complex wax esters (15-27%) containing 15-hydroxypalmitic acid or diols, which, through their hydroxyl group, are linked to another fatty-acid molecule. In addition to such diesters, tri- and higher esters are also found.
- Odd-numbered, straight chain hydrocarbons (12-16%) with a predominant chain length of C27-C33 (C27 as major compound). With increasing chain length, the

proportion of unsaturated species increases (above C33 only unsaturated species are present).

These are the main components, in fact, the beeswax is a mixture even more complex that also includes alkadienes, –trienes, diols, flavonoids, terpenes, hydroxy polyesters and other substances that have not yet been identified.

Bleaching the yellow beeswax with, for example, hydrogen peroxide, sulfuric acid, or sunlight, yields white beeswax (the so-called bleached beeswax). In case of bleaching with hydrogen peroxide, the melted wax is also treated with a bleaching earth or activated carbon to avoid the presence of peroxo compounds in the finished material.

Chinese insect wax is the product of secretion of *Coccus ceriferus* Farb. cultivated in China. It consists mainly of esters (83%) C48-C60, maximizing at C52. It is harder and clearer than beeswax.

Shellac wax or lac wax constitutes a by-product (3-4%) of crude shellac that is the resin obtained from the female of a species of insects (coccidi) that infest the trees. It is a hard and brittle substance. It contains only a small amounts of hydrocarbons, free alcohols (C28, C30, C32 and C34 in diminishing quantity), esters (C42-C68) with two broad bands peaking at C44 and C64.

Spermaceti wax is obtained from the head of the sperm whale *Physeter macrocephalus L.* and other cetacean species in the Pacific Ocean; today it is no longer produced and replaced by synthetic cetyl palmitate. It consists largely (65-95%) of cetyl myristate and palmitate (C32 and C30, respectively) and triglycerides (5-30%), alcohols (1-5%) and free fatty acids (0-3%).

Woolwax or lanolin is a greasy matter secreted by the sheep’s skin with protective and emollient function. It consists of hydrocarbons, esters of fatty acid (14-24%) (C14-C45),

aliphatic and free acids and alcohols, sterols (lanosterol and cholesterol) (45-65%) and terpenes (4-5%). After refining it is yellowish-brown, soft and oily.

Plant waxes. Carnauba wax is obtained by exudation of a Brazilian palm leaves (*Copernicia Cerifera*); it is fragile and usually colored (greenish, gray, yellow). This type of wax is mainly characterized by esters (85%) but its chemical composition includes fatty acid (3%), hydrocarbons and fatty alcohol and resinous compounds (5%) too, that give the wax hardness. It may often, in commerce, be adulterated with less expensive waxes such as paraffin. It is widely used as an additive to other waxes to give a harder and higher melting mixture.

Candelilla wax coats plants of *Euphorbia* spp. growing in Mexico and South America. It contains hydrocarbons (49%, C29-C33, particularly C31), followed by esters (28-29%), alcohols (main C31), free fatty acids (7-9%) and resins (12-14% triterpenoid esters). It is yellow or yellowish brown, hard and brittle. It is used with other waxes to harden them without raising the melting point [44].

Japan wax is obtained from the fruits of *Rhus* species growing in Japan and China. It is really a fat being composed of palmitic acid triglycerides and fatty acids (C4, C16). It is usually colored, is fragile and easily turn yellow for oxidation in air. It is used as a binding medium in crayons, such as softener for leather, as an additive to other waxes to increase the adhesive power.

Fossil and earth waxes. Ozokerite is wax deposit found in lignite beds in various parts of the world dating to Miocene. It contains alkanes (C18-60) with two broad bands peaking at C27 and C42 and the distribution of alkanes may depend on the natural processes of formation [45].

Paraffin is incorrectly defined a wax because it only contains high molecular weight hydrocarbons. It is white translucent material with laminar structure-crystalline and it is extracted from shale, lignite and petroluem. It consist of (40-90%) both even and odd-numbered *n*-alkanes (C20-36, C27 as major compound), iso-alkanes and cyclo-alkanes (C18-38) and the distribution of these components may slightly change depending on the raw matter. It was introduced for the first time in the second half of the nineteenth century in the manufacture of candles and wax modeling.

Other less used materials among plant waxes are Ouricuri wax, Esparto wax, Jojoba oil and peat waxes such as Montan wax, Ceresine, and Microcrystalline waxes are other fossil and earth waxes. There are also other types of synthetic waxes such as: low molecular weight polymers of ethylene, waxes obtained by hydrogenation of vegetable and mineral oils, silicone-based wax and ketone-based wax; in addition, Stearin wax represents the most important synthetic one. The commercial name Stearin identifies a mixture of palmitic and stearic acids (in various proportion depending on the industrial process of stearin making) synthetised by alkaline hydrolysis of animal fats in 1831 by the French chemist Chevreul [40]. It is more substantial than paraffin and, moreover, it is characterized by an high adhesive power. Stearin wax may be therefore be encountered as an ingredient of wax sculptures and models, either added deliberately especially to beeswax and paraffin, modifying the malleability of the wax mixture and decreasing its the cost.

In the table 3, other important characteristics of some of the above mentioned waxes are shown.

Tab. 3 Physico-chemical properties of several waxes. Saponification number is the amount of potassium hydroxide (KOH), in mg, necessary to neutralize fatty acids per g of fatty material; it is related to the amount of free fatty acids. Iodine number is a measure of the amount of iodine, in mg, that chemically react with 1 g of fatty material; it is an index of the total amount of unsaturation [41].

Type of wax	Saponification number (mg KOH)	Iodine number	Melting-point range (°C)
Beeswax	17-21	8-11	66-71
Chinese insect wax	11-15	1-2	80-83
Lanolin or woolwax			35-42
Spermaceti wax	1-3	3-4	42-50
Carnauba wax	4-8	12-15	82-86
Candelilla	16	14-37	67-79
Montan wax	23-27	10-16	76-92
Paraffina			46-68 (two peaks)
Ceresine		7-9	54-77
Microcrystalline waxes			70-90

For making sculptures, the most used wax is beeswax but it is often employed mixed with other waxes or additives in order to improve its property and color.

For instance, sculptors could add to beeswax the carnauba wax in small amounts to increase the hardness and melting point. In the nineteenth century synthetic materials, such as paraffin or ozokerite, were used to minimise the amount beeswax, an expensive resource; also Stearin, was also largely employed to change the malleability of beeswax as well as being cheaper.

In addition, beeswax frequently sold is adulterated by fatty materials such as lard and tallow (characterized by a small amount of C17 acid), vegetable fats (i.e., olive oil, linseed, walnut and poppy seed oils) with the aim to increase malleability and softness of wax. These fats are composed of saturated and unsaturated triglycerides and their aging results in partial hydrolysis of esters with the formation of palmitic and stearic acids, diglycerides and monoglycerides. This degradation is visible on the surface with a

whitish crystallization due to fatty acids and it also appears when there is stearin in the wax-based mixture.

Pine resin derivatives, especially colophony and turpentine, are the common organic additives in wax sculptures to harden and colour the material. Both resins are exuded naturally from the trunks of many species of *Pinus* [41]: turpentine corresponds to a fresh exsudate from pine trees whereas colophony is a solid residue obtained after distillation of fresh pine resin. They are composed of diterpenoid principally of pimarane and abietane compounds, that are almost exclusively acids.

The pimaradiene acids are pimaric, sandaracopimaric, isopimaric acids; as regards the abietadiene acids, abietic, neoabietic, laevopimaric and palustric acids are the more abundant. For their oxidation, degradation products as dehydroabietic acid and 7-oxodehydroabietic acid are formed.

The biomarkers of turpentine are pimaric, isopimaric, palustric and abietic acids and as degradation product the dehydroabietic acid. Colophony mainly contains dehydroabietic and dehydro-7-dehydroabietic acids, followed by pimaric, isopimaric, abietic and 7-oxodehydroabietic acids.

Pigments and dyes colour the material and they could be incorporated into the melted wax, dispersed in cool-diluents (i.e., turpentine) or wax can be layered by painting film as well as gilding and silvering. Table 4 lists the main pigments and dyes.

Tab. 4 The main pigments and dyes reported in the literature [40, 46, 47].

Color	Pigment and dye
Red	Ochre, madder, *Alcanna spuria,* dragon's blood, Vermilion, Red lead
Blue	Prussian blue, Azurite, Lazurite, Indigo
Green	Malachite, Verdigris.
Yellow	Ochre, orpiment, realgar, chrome yellow, saffron, fustic, turmeric
Black	Iron oxides, black carbon, Lamp black, pitch, aniline
White	Lead white, zinc white, gypsum, chalk, talc, kaolin

Finally, starch was used as an extender to minimise the amount of beeswax. It consists of polysaccharide of amylose (20-25%) and amylopectin (75-80%) and it is a reserve material of the vegetable kingdom occurring in roots, bulbs, tubers and seeds. Since the nineteenth century, its use has been attested. When adding in large amount, it can cause fragility to artwork.

Works of art may thus contain overlapped layers of different (in color and composition) waxes or heterogeneous mass of wax covered by a thin layer of homogeneous wax. The wax is easily combined with substrates such as wood, plaster, glass and slate. For example, since the mid-‘800 the wax has become component of formulations like the stearin-plaster. As support or as filling material were also used paper, cardboard, cloth and metal elements.

1.2.2.3 Ageing and deterioration of waxes

Most wax components are fully saturated materials and this results in considerable chemical stability.

Various alteration processes, including hydrolysis of esters and triacylglycerols, migration and crystallisation of *n*-alkanes and fatty acids, sublimation of the smallest *n*-alkanes but also oxidation and polymerisation (with decrease of unsaturated hydrocarbons) may occur. Degradation experiments of beeswax accelerated by temperature have also revealed chemical transformation of flavonoids (initially present in beeswax) into smallest phenolic compounds and the sublimation of palmitic acid formed by hydrolysis of wax esters [48]. It also known an increase in hydroxyacids (C16-C24, 15-hydroxydecanoic acid as major compound) and diols (C24-C32) (5:1) compared to the percentage of esters (mono, di- and tri-) [41]. Visually, the migration of

compounds towards the wax-based surface causes white crystallizations [45], the starch can cause mold growing and tallow and lard can lead to rancidity.

In general, these processes lead to an increase in acidity, an increase of polarity and rigidity of the wax.

These processes mostly depend on the environment of preservation or on the wax treatments.

First of all, it is known that heating (before use, either to mix it with additives or to increase its plasticity) induces the volatilization of the low molecular compounds, especially *n*-alkanes, and severe and irreversible modifications of the wax.

The light induces not significant modification to the wax, unless there are light-sensitive organic pigments in the wax pasty.

The humidity is not a critical parameter, because of wax hydrophobicity and no-hygroscopicy, although it can promote the hydrolysis of esters, and when alkaline, also the saponification. More the waxes are aged the stronger is the effect of alkaline pH, at the same time some waxes are more water-sensitive than others, such as lanolin which is able to absorb water at least two times its weight. The humidity also affects other materials associated with wax in the artwork, like wood or metals.

Nevertheless, having an high soiling retention [11] at room temperature, wax surfaces tend to become very dirty; this represents the major cause of degradation. The dust is incorporated into the wax because of its thermoplastic nature, forming blackish deposits. On the other hand, wax does not discolor much, although, of course, pigments or additives mixed with wax may fade.

From the conservator’s point of view, wax is easily broken and is subject to shrinkage, which often results in cracks and become rather more brittle with age.

1.2.2.4 Traditional cleaning methods

Cleaning is always necessary before carrying out repair work because of the undesirability of dirt particles becoming trapped in the repaired area, especially when a heated repair or filling is being undertaken. Traditional cleaning methods are based on the use of often toxics organic solvent (i.e., chloroform, toluene, xylene, naphtha, alcohol: acetone, ethanol, isopropyl alcohol, essence of turpentine) and other organic compounds (i.e., benzalkonium chloride) depending on the characteristics of wax [49].

It is the conservator's experience that waxes tend to become less quickly soluble with age and that waxes with a high pigment content are less quickly soluble than those without.

This cleaning methodology consists of lightly brush the solvent over an area of about one square centimeter and immediately drawn across the same area to take up the loosened dirt by using dry brush. Then, cleaned surface should not be touched and application should not take more than few seconds. If the operation must be repeated on the same area, it has to wait until the completely evaporation of the solvent and the hardness of the surface.

As can be seen in the Fig.6, beeswax is clearly an hydrophobic material, so apolar/low polar organic solvents (i.e., chlorinated hydrocarbons) dissolve the material while more polar solvents are not certainly able to solve it [50]; however the last ones are not considered out of the risk for cleaning purpose.

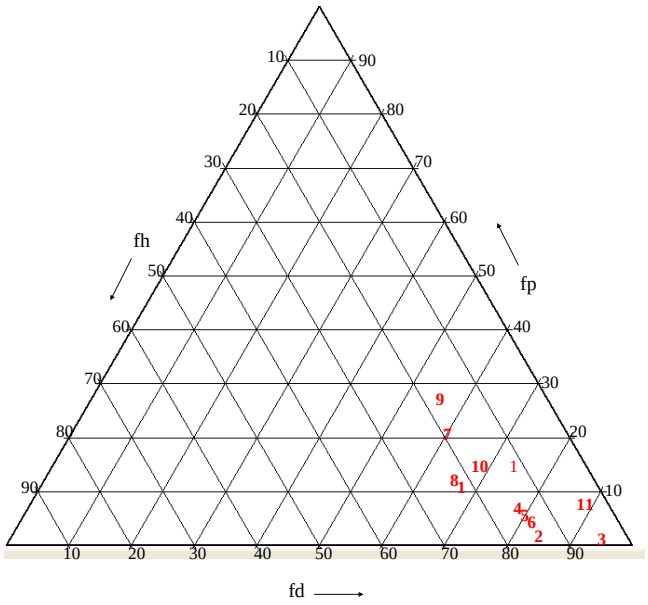


Fig. 6 solubility of beeswax [50]
[where fd=dispersion force contribution, fp=dipolar contribution, fh=hydrogen bonding contribution;
1=trichloromethane,
2=tetrachloromethane,
3=cyclohexane,
4=benzene,
5=toluene,
6=xylene,
7=dichloromethane,
8=trichlorethylene,
9=cyclohexanone,
10= 2,6-dimethyl 4-heptanone,
11=carbon disulphide].

In fact, solvents with medium-polarity (such as esters) may have a minimal interaction (i.e., *leaching*) with the more polar compounds of the wax such as fatty acids, fatty alcohols, terpenoid acids. Rapid volatilization is another propriety which is desirable in a cleaning agent, so that the surface quickly re-hardens and the penetration is not too deep.

Solvent cleaning of wax is obviously a very critical operation, and, in many cases, aqueous washing is quite sufficient. In any cases solvent cleaning should be preceded by aqueous cleaning to remove as much dirt as possible and thus reduce the amount of solvent cleaning necessary. In an applicative study, the mildest washing method used is distilled water with 2% Lissapol (non-ionic detergent) brushed over the surface with a soft sable. The wax is subsequently rinsed with distilled water, again using a sable brush for the purpose. Howards B30 spirit soap (Potassium oliate soap, containing Sextol) can be used in the same way as a 3-10% solution in water. Dilute ammonia is also very

effective, but strong solutions of ammonia should be avoided, as surface blanching, due to the saponification of the wax, can occur [51].

One traditional method of wax cleaning consists of massaging the surface of the wax with ordinary butter that removes the grime without softening the wax. The only problem is that the butter leaves a greasy film on the wax which is not only unsightly but would very quickly attract more dirt. This film, however, can easily be removed without harm to the wax by washing the surface with methanol. Many waxes have been disfigured by the another old cleaning method with turpentine, which has left a brown deposit on the surface of the wax because its high retention [52].

1.2.2.5 Cleaning approach to the wax

As seen above, it is preferable completely avoid the use of organic solvents for surface cleaning the wax artworks and choose an aqueous cleaning. Since water is the solvent with farthest polarity from wax, it can be considered, at least first, risk-free solvent for cleaning wax materials.

However, it should take in account that in a thermoplastic material such as wax, the separation between dusts and the surface to be preserved is not so clear, so it has to accept the idea of a possible heterogeneity in the level of cleaning.

In particular, the paraffins are considered inherently suited for an aqueous *surface cleaning,* while real wax containing free fatty acids (i.e., in the beeswax: 12-14%) are alkaline water-sensitive (it tends to ionize) although it has a very strong hydrophobic character (C22-34). In this form (anionic or salified) the fatty acid increases its hydrophilic character, being able to get a degree of water solubility. It is known that beeswax became disperdible at alkaline pH environment.

Finding in the literature different pK_A values about fatty acids present in the wax, it can be assumed an average value like $pK_A = 8$.[1] According to the Henderson-Hasselbalch equation for weak acids in solution, the safety pH range of aqueous solutions to be used for cleaning should be about 6-7; for pH=7 the risk of ionization reaches a maximum of 9% while for pH =6 of 1%.

Buffered solutions, that are able to maintain constant pH value during application, ensure an uniform action on the surface and a repeatability of the operations. Minimize the buffer concentration is necessary in order to reduce the problem of solid residues on the surface.

Another important parameter of aqueous solutions is the total ion concentration. Monovalent positive ions (i.e., Na^+, K^+, NH_4^+) are useful in aqueous solutions, because they may deliver ions bound to the acid groups of the surface and dust particles (also of acid nature) through ion exchange mechanisms. Too high ion concentration may represent a risk to fragile surfaces because osmotic phenomenon may occur for wetted surface; in the case of wax this problem is minimized. A conductivity close to 5 mS/cm can be chosen: this is the value proposed by Wolbers for surface cleaning of oil painting [11].

Taking into account the low wetting behavior of wax to aqueous solutions, the effectiveness of solutions in gelled form can also be considered.

Aqueous solutions containing chelator can be tested in order act on metal ions of soling materials (in form of oxides and salts from rock disintegration). The risk of interaction with pigments and metal soaps can be overcame without raising too much the pH of solutions and limiting the use chelator.

[1] Recently [53], the pK_A values of stearic acid (C18saturated), elaidinic acid (C18unsaturated), oleic acid (C18unsaturated), have been determined respectively in 10.15, 9.95 and 9.85, at least 1 unit higher than pH = 8 assumed. The range of safety may rise to 7-8.

Finally, the use of surfactants can also be explored (keeping low concentration, and choosing surfactants with high hydrophilic-lipophilic balance – HLB-) to remove the lipophilic dust such as hydrocarbons contained in air pollution. Attention has be paid because also waxes are lipophilic materials.

This study aims to first verify the applicability of aqueous solutions as *surface cleaning* agent by analyzing whether a chemical interaction with the wax compounds occurs; considering the parameters above mentioned, several aqueous solutions specifically designed for wax objects were tested, firstly on laboratory-cast reference wax layers then on wax-made sculptures.

Following (Tab.5) are reported the solutions tested both in free form and in gelled form.

Tab. 5 Five aqueous solution used for cleaning testing on wax objects

	pH Buffered solution	Conductivity (mS/cm)	Surfactant	Chelator
1	6	4,5	-	-
2	7	4,5	-	-
3	7	6,4	-	-
4	7	4,5	*Tween 20*	-
5	7	12,4	-	Citrate

The buffer was used at pH 7 compared to one application at pH 6. It was used a phosphate buffer at very low concentration at the same time effective. The conductivity has set to 4.5 cm/mS. For applications in gelled form, the xanthan gum, a neutral gelling agent with medium gelling strain (about 2000 mPas at 1.5% w/v) and pseudo-plastic character, has been chosen. The application of gelled solutions was followed by gel removal with dry swab then surface rinsing by wetted swab. The chelator citric acid salified with sodium hydroxide at low concentrations and with pH 6-7 was chosen.

*Tween 20 (*polyethoxylate) is the non-ionic surface-active with high HLB value employed at low concentrations.

1.2.3 Egg tempera

1.2.3.1 Historical uses of egg tempera

Egg tempera was traditionally used in past centuries, especially in the fourteenth and fifteenth centuries in Italian painting. It can be considered the main technique for painting on wooden panel.

Until the late of fifteenth century, egg tempera technique had widely employed when it was established oil paint technique, already known by the Flemish. A lot of information about egg temperas are accurately described in the "*Il Libro dell'Arte*"[54] by Cennino Cennini dates back the end of fourteenth century.

Although the egg tempera binding medium was so central to painting methods, it has received little attention from either the scientific (generally focused on the identification of egg in artworks) or conservation point of view, at least in comparison to drying oils and resins. The lack of knowledge concerning the ageing, the deterioration processes and the effect of conservation treatments (especially cleaning) on egg tempera paint films, limits the ability of the conservator to make informed judgments.

1.2.3.2 The chemistry of egg tempera

Egg tempera techniques employ the whole egg, or egg yolk and egg white separately, for binding media purposes, in which pigments are dispersed, sometimes combined with other materials (i.e., fig latex, cherry gum, resins, oil, etc..) depending on the painter's requirements [55]. It is recalled that egg tempera paint film belongs to a system even more complex that is the painting. Indeed, each painting is composed of a series of layers which, in the most elaborate form and moving from back to front,

usually consist of the support, the ground, the priming, the paint layer and a protective coating.

Egg binding medium is made up by proteins, lipids, polysaccharides and inorganic compounds and considering egg tempera in the manner of Cennino Cennini, it is mainly based on hen's egg yolk.

Egg yolk is a highly complex and heterogeneous substance.

At the microscopic level, the yolk contains particles (yolk granules, 0.3-1.6 μm, and yolk globules, 0.5-5 μm) suspended in a liquid plasma phase, where hydrophobic (lipid) and hydrophilic (protein) components cannot be simply considered separated as in an emulsion [39,56] as it has erroneously been regarded for many years in the conservation field [57].

Egg yolk mostly contains lipids (approx. 66% of dry weight) and proteins (approx. 35%), as well as small amounts of free carbohydrate (approx. 0,4%) and inorganic compounds (approx. 2,2%) [58]. Lipids in egg yolk can be divided into triglycerides (neutral lipids) (approx. 65–73%), phospholipids (approx. 23–32%) and cholesterol and its derivatives (approx. 4–6%)[58,59], present as associated with proteins or as particle aggregates or soluble complexes. Triglycerides, the main lipids, are the same type of compounds as drying oils being composed by fatty acids as Palmitic Acid (27%), Stearic Acid (9%), Oleic Acid (44%), Linoleic Acid (approx. 14%), and Linolenic Acid (0.5%) [43]; following in abundance, phospholipids mainly comprise lecithins such as Phosphatidylcholine (PC) and unsaturated fatty acids also occur in phospholipids fraction in which longer chain are more prevalent. However, in egg these lipids are low in unsaturation when compared to the composition of drying oils commonly used as binding media. Their drying properties are not as strong as oils, but they are subject to

the same oxidative polymerization reactions that occur in linseed oil. One main difference is that egg yolk contains polymeric material (proteins) and this fraction is largely responsible of the egg film-forming properties. The most important proteins among the 14 identified are: α-, β- and γ Livetins, Phosvitin (in which serine is the main abundant) α- and β Vitellin and Vitellenin. Among these, only Phosvitin is not linked to lipids. The overall amino acid weight composition of egg yolk is the following: Gly (3.5%), Ala (5.6%), Val (6.4%), Leu (9.2%), Iso(ile) (5.1%), Pro (4.5%), Phe (3.9%), Tyr (2.8%), Ser (9.1%), Thr (5.6%), Cys (1.9%), Met (2.3%), Arg (5.5%), Hist (2.4%), Lys (5.7%), Asp (11.5%), Glu (15.0%), Hpr (0.0%) [42]. For this reason, egg is grouped among proteinaceous material, although this is not correct.

Finally, the colour of egg yolk depends on the presence of fat-soluble pigments belonging to the families of carotenoids and xanthophylls; it is influenced by the hen's diet as Cennini empirically affirmed regarding the difference between town hen's eggs and farm ones [54].

Summarizing, in egg tempera paint the pigments are kept together by proteins as the stationary phase (the covalently polymer network molecules usually insoluble in solvents) and by lipids which have emulgating properties and represents the mobile phase (substances chemically bound and can be lost by evaporation or mobilised by aqueous or non-aqueous fluids) [60].

Freshly dried tempera films are reasonable flexible because of the mobile lipids that have plasticiser action (as small molecules in a drying oil); they are also readily swollen by water because of proteins and phospholipids. Lipids can easily be extracted from the combination with protein by several solvents: alcohols, ketones, aromatic and chlorinated hydrocarbons. Consequently, the film undergoes contraction and shows

superficial alterations like surface roughness. The lipid amount removable with solvents decreases considerably with ageing. In artificial ageing tests, some of these lipids have proved to be very mobile and tend to come to the surface spontaneously, and the most volatile ones tend to evaporate. As for the case of drying oils, the material may form a superficial patina at the interface with the eventual varnish, if present.

In particular, a model (Table 6) composed of three main stages of development of an egg tempera paint has been proposed by experimental findings: the initial and curing stage, the mature stage and the degradation stage [60].

Tab. 6 various stages of development of egg tempera paint by Boon, 1996 [60].

Initial and curing stage
Changes in the liquid state:
Reorganisation of the lipid and proteins due to emulsification with pigments.
Partial unfolding of proteins which stabilises the emulsion.
Hydration of the mineral phase and partial dissolution of metals
Physical drying:
Loss of water by evaporation.
Fusion of emulgated droplets into larger vesicles.
Aggregation of unfolded peptide chains into new 3D-networks (spaghetti effect).
Absorption of unfolded protein chains on mineral surfaces.
Chemical drying:
Autooxidation of polyunsaturated triglycerides and phospolipids.
Production of reactive aldehydes and dialdehydes from lipids.
Autooxidation of cholesterol.
Chemical modification of amino acids in the proteins: Deamidation, oxidation, beta-elimination, alkiylation.
Chain breaking reactions decrease the size of the proteins.
Mature stage
Formation of large network polyamides cross-linked by reactive lipid delivered compounds and radical condensation reactions. The original amino acid side chains have been modified in the chemical drying stage leading to loss of side chain speciation. The resulting polymer has lost most of its cationic sites due to deamidation, oxidation and alkylation processes. The acid polymer network is stabilised by metal ions from the mineral pigment phase.
Degradation stage
Loss of binding power by light induced and metal catalysed oxidation of the polymer system.

During the initial and hardening stage, physical and chemical drying take place at the same time; because lipoproteins are inherently unstable in the absence of water, the

proteins start to re-organisation (denaturation due to loss of water) possibly involving aggregation into 3D-networks stabilised by complex inter- and intra-molecular interactions. Due to the presence of sulphur-containing amino acids in egg yolk (i.e., cystine), new disulphide cross-links occur between proteins by means of sulfhydryl/disulphide interchange reactions. Other processes include complexing of metal ions from pigments by proteins, especially phosphate in phosvitin acting as ligands. This 3D-network is destabilized by oxidation and elimination reactions which create reactive sites for alkylation and cross-linking with reactive aldehydes from the autoxidation of lipids. For example, Cholesterol rapidly oxidise giving products such as 7-hydroxycholesterol and 7-ketocholesterol and it has been reported that the almost complete loss of cholesterol less than 20 years [61]. As regarding to triglycerides and phospholipids, Linoleate and Linolenate are oxides 10 and 20-30 times faster than Oleate, respectively, and the oxidation is catalysed by metal ions such as Fe^{3+}, resulting in their polymerisation. Lipid oxidation also results in immobilisation of unsaturated fatty acids, formation of low molecular weight products, volatiles, carbonyls, unsaturated compounds. The interaction of hydroperoxide and peroxide from lipids with proteins includes formation of protein radicals, cross-linking of protein radicals with lipids, polymerisation of protein-lipid products with further protein molecules. Lipids covalently bound to protein are not extractable by neutral organic solvents and complexes of protein and oxides proteins produced are insoluble.

Many of the lipids remain part of the mobile phase, because they can not form the reactive precursor structures required for incorporation in the main network structure.

The mature network becomes negatively charged as a result of the chemical changes in the side chains of the polypeptide chain which primarily affect the positively charged

amino acids. After aging, amino acids content decrease up to 25% for the high proportion of unstable amino acids in egg yolk proteins (histidine, tyrosine, methionine, cysteine) [62]. This is due to amino acid oxidation.

Studies by Khandekar on unpigmented and pigmented artificially aged egg yolk samples have shown that the retention of fatty acids in paint films has promoted by light ageing. Azurite and Vermilion have revealed to accelerate the cross-linking of egg yolk proteins compared to Lead White and Verdigris; in particular, the latter has promoted a severe breakdown of protein with hydrolysis. These data have been obtained from analysis (by GC/MS and/or electrophoresis) of organic solvent extracts after immersion. Oleic acid has been mainly extracted by chloroform from unaged samples and after light ageing its content has decreased, with more effect on azurite films. In addition, lipid extraction was, in many cases, accompanied by physical disruption of the paint films [63].

In other past study [64], the interaction of organic solvents with paint layers during cleaning operations (removal of a resinous varnish) of real paintings, as well as that of unaged samples, was explored. It was noted that the two solvents tested (2-propanone and 2-propanol isopropyl alcohol) do not appear to cause any leaching of the oil paints, neither on the most recent painting nor on the only egg tempera painting.

In conclusion, egg tempera dries and hardens to a tough and durable paint film whose features are influenced by lipids and proteins. Both components are involved in cross-linking processes leading to the formation of polymeric network (immobile phase) and that metal ions from pigments influence the curing process and deterioration one.
As above reported, there is little known of the proprieties of egg tempera paint film with regard to the effect of common cleaning agents and to their ageing.

This study has investigated the interaction of water and organic solvents with yolk egg binding medium, both freshly dried tempera films and real panting.

It is well known to contribute to risk in cleaning with solvents are both the physical swelling of the organic binder phase caused by sorption of solvent and leaching, i.e., the extraction of soluble, low molecular weight components of the organic binder phase.[15] To design a model system, as like oil paint one, to assess the effects of solvents on egg tempera film can be useful to conservator by increasing control during cleaning operations.

In particular, this research was focused on the study of the initial stage of supported freshly dried tempera films, unpigmented and pigmented; paint film of vermilion and minium and film of egg yolk medium alone, prepared following the recipes of Cennino Cennini, were treated by swab-rolling with distilled water, Ethanol and Isooctane. Stressing the chosen approach, the reference samples and the treatment methodology have been selected in order to simulate as close as possible the cleaning operation at the same time to perform it with standard procedures. Other similar studies have used to employ unsupported reference paint films (previously brushed onto a stiff and non-breathable polyester film) and to treat samples by immersion from few minutes to one hour [63].

An unsupported tempera layer prepared by researchers of the Smithsonian Museum Conservation Institute was investigated for comparison.

A cleaning case study on an ancient egg tempera painting, dating the sixteenth century, from the Pinacoteca Nazionale in Siena was then conducted.

2. Aim of the work

Aims of the present research were to develop and to evaluate specific materials and selective methodologies for the *surface cleaning* of plaster and wax artworks. Another purpose was to investigate the risk associated with the exposure of egg yolk-based tempera films to water and organic solvents during cleaning.

The research focussed on the experimentations of aqueous, Agarose-base rigid gels for *surface cleaning* plaster. Several tests were performed on generic samples of plaster, different mocks up and also on actual plasters in order to verify the effectiveness of the cleaning system, to identify the proper modes of preparation, application and removal from the plaster surfaces and to check the safety of the treatment. The latter testing aimed to measuring the diffusion of water released by gels, to check the cleaning efficiency by analyzing gel after treatment, to observe the treated surfaces in order to determine any chromatic or morphological changes and to detect potential residues from the treatment on and within the surfaces.

Actual plasters used in this study belong both to the Galleria d'Arte Moderna in Milan (such as the plaster casts of the *Monumento alle Cinque Giornate* by Giuseppe Grandi), San Colombano depository of Castello Sforzesco in Milan, and the Gipsoteca Toschi in Parma (such as *La Strega* by Cristoforo Marzaroli).

To verify whether specifically designed aqueous solutions could be suited for the *surface cleaning* of wax artworks, several solutions were firstly tested on laboratory-cast reference wax layers then on four fragile wax-made sculptures (dating back to the

period 1873-1947), from the Galleria d'Arte Moderna in Milan. GC-MS analyses of the cleaning cotton swabs, to detect possible wax components removed during the cleaning, were selected as a means of ascertaining the risk associated to these treatments.

This study has also investigated the interaction of distilled water, and organic solvents of different polarities, namely ethanol, acetone and isooctane, with egg yolk binding media, in the actual form of fresh, unaged tempera films and of an ancient panting. Tempera films used ranged from supported, unpigmented and pigmented samples to an unsupported tempera layer prepared by researchers of the Smithsonian Museum Conservation Institute. A cleaning case study on an ancient egg tempera painting, dating the sixteenth century, from the Pinacoteca Nazionale in Siena was conducted. *Leaching* phenomena induced by solvents were investigated by means of GC-MS. Changes in the surface and physical characteristics of reference tempera films, before and after treatment with solvents, were studied by means of stereomicroscope observation and multispectral scanner (MSS).

3. Gypsum plaster

3.1 Experimental

3.1.1 Materials

All the solvents were HPLC grade and used without any further purification and other chemicals used were commercial products from Sigma-Aldrich and Fluka. The following commercial standards were used: fatty acids (containing CC9, C14, C16:1, C16:0, C17, C18:2, C18:1, C18:0) from Merck, amino acids (containing Ala, Gly, Treo, Ser, Val, Nval, Leu, Isoleu, Nleu, Pro, Hpro, Asp, Glu, Phe) and monosaccharides (containing Sor, Xyl, Ara, Rha, Fuc, Glc, Man, Gal) from Sigma-Aldrich. For cleaning testing, Agar-Agar Powder (Food additive grade, Tang Freres), Multipurpose Agarose (Tebu-Bio), Agar (Fluka, 05040), Vinavil NPC Stella Bianca (VINAVIL S.p.A.), Rhodamin B (Kremer Pigmente), test strip *Merckoquant* ® (Merck KGaA) for Calcium *Test* (110083) and nitrate and nitrite reaction zone (110092), were used.

3.1.2 Preparation of plaster mocks up

Two sets of laboratory plaster were used. One set (plaster wafers) was prepared by pouring *scagliola* gypsum into teflon-coated metal moulds. Each sample weighed about 80 g, and had a truncated cone shape. It dates to 2007 and it was used 6 months later their setting (at RH 50-60% and temperature at 18-25° C). The lower base, that was in contact with the mould during drying, was the surface on which testing was performed. The other set came from a very porous tile dates to 2000. It was also made of plaster typically used for moulding without any additive and the upper surface, that was in contact with the air during drying, was considered for testing in order to maximize eventually damage due to the treatments.

In comparison with artworks, it is to note that these models lack the surface layer of "dirt", which acts as an interface between gel and gypsum.

3.1.3 Museum artworks

Several actual plasters were used such as models, plaster casts and plaster fragments. Fragments of plaster bas-reliefs, conserved for several decades in San Colombano depository (the location of the school housing the storage site, external to Milanese Museums) coming from the School of Applied Arts in the Castello Sforzesco in Milan, likely date from the early 20th century. Despite the fact that they are school exercise pieces, their technical characteristics and conservation history make them comparable to the sculptures conserved in the same site. They were characterized by superficial dust. Plaster casts *Monumento alle Cinque Giornate* by Giuseppe Grandi (Ganna, 1843 – Ganna,1894), coming from the Galleria d'Arte Moderna in Milan. For which, the sculptor worked for thirteen years, until 1894, the year of his death. These plaster casts appeared darkened, somewhat grey and yellowish. This appeared to be the result of various episodes: dust deposits, materials used to mask reconstruction operations, remains of decayed organic substances used as detaching agents or for re-painting, not according to the artist's desires but rather to mask restoration. From the same museum, several plaster casts of anatomical elements were considered. Two artworks, *La Strega* (about 1866) by Cristoforo Marzaroli (Salsomaggiore, 1836 – Parma, 1871) and a high-relief (school exercise piece) preserved at the Gipsoteca Toschi in Parma were also considered. *La Strega* (fig. 7) presents yellowish patina due to detaching materials applied when making it into bronze. The high-relief appears degraded by yellowish stains also rust-made crusts (fig. 8).

Fig. 7 *La Strega* (1866) by Cristoforo Marzaroli **Fig. 8 high-relief from the Gipsoteca Toschi**

3.1.4 Characterization of Agarose-based powders and plasters

Pure powders of Agar-Agar, Agarose and Agar were analyzed by means of FTIR-HATR and GC-MS. For GC-MS analysis in order to confirm their polysaccharide nature. Each sample was treated as follow. 1 mg of sample was transferred to a schlenk tube with 50 µl of a 0.01M Sorbitol solution as the internal standard. The sample was treated with 2M Trifluoroacetic acid (3ml) for six hours at 100°C. Afterwards, the sample is dried under vacuum on a heating plate equipped with magnetic stirrer at 40°C. After evaporation to dryness, to the hydrolysed residue are added 60 µl of a solution 2:1 in volume of Ethanethiol and Trifluoroacetic acid and the mixture is kept at room temperature for 40 minutes. After cooling, the solvent is evaporated under vacuum, and the residue is dissolved in 50 µl of Pyridine, 30 µl of Trifluoroacetic acid and 100µl of HMDS and kept at room temperature for one hour. The solvent is then evaporated under vacuum, and the residue is dissolved in 0.2 ml of Hexane. 1µl of the resulting solution is used for gas chromatographic analysis.

Plasters were analyzed by means of FTIR-HATR with the aim to confirm their gypsum nature. Complicated degradation (tab.7) materials were collected from Gipsoteca Toschi artworks and analyzed by FTIR and GC-MS.

Tab. 7 Description and location of samples collected from Gipsoteca Toschi artworks.

Artworks	General aspect	Lab reference	Location on the sculpture	Analysis
high-relief	yellowish stains	#1	the left shoulder of the figure	FT-IR GC-MS
La Strega	yellowish patina	#2	Back of the base	FT-IR
La Strega	Stucco residue	#3	Detached fragments near a negative area	FT-IR GC-MS

Samples #1 and #3 were analyzed for lipids and proteins. Each the samples were placed in schlenk tubes and internal standards were added: 100 µl of a 50 ppm heptadecanoic acid in hexane solution and 100 µl of a 5 ppm norvaline and of a 50 ppm norleucine in water solution. Thus, 1 ml of 4N hydrochloric methanol and 1 ml of hexane were added and the mixture was kept at 50°C for 2h under magnetic stirring. The hexane phase containing methyl esters of fatty acids was separated and transferred into vials. It was concentrated to dryness and afterwards dissolved in 1 ml of hexane. 1 µl of the extract

solution was injected in the gas chromatograph for fatty acids analysis. The residue of the methanol phase was dissolved in 2 ml of 6N hydrochloric acid and heated up to 100°C, this temperature was held for 5h with a magnetic stirrer. After drying 3 ml 2N hydrochloric isopropyl alcohol were added, this solution was warmed to 90°C for 1h. After evaporation of the solvent the residue was dissolved in 2 ml dichloromethane and 0,2 ml trifluoroacetic anhydride and it was left to react for 1h at 60°C (magnetically stirred). After cooling, evaporation of the solvent took place once more. Then the residue was rinsed with dichloromethane, concentrated and then re-dissolved in 1 ml of dichloromethane. 1 µl of the obtained solution was injected in the gaschromatograph. This derivation procedure had transformed the amino acids into their N-trifluoroacetyl-O-2-propyl esters [65]. Three runs were performed for each sample and the average of chromatographic peak areas were calculated and then corrected by a response factor. Fatty acids and amino acids detected were expressed in relation to the total amount of lipid and proteins, respectively, in order to obtain semi-quantitative information. To identify lipids and proteins qualitative analysis and relative amount of fatty acids and amino acids were considered.

3.1.5. Preparation, application and removal of Agarose-based gels

Agar (and Agarose) gels (2.5%-5% (w/v)) were prepared by dissolving and stirring Agar powder in distilled water above 80-85 °C for 15 minutes in a beaker; alternatively, the cold solution was poured into a lidded plastic container and brought to boiling in a microwave oven, mixing occasionally. The solution was left to cool to room temperature or, once a rigid material was obtained, the gel was re-dissolved in a microwave oven, so re-left to cool to room temperature. In both cases, once a semi-solid state material was obtained (at 40-45°C), the solution was uniformly brushed onto the surface to be cleaned; the gel have to be conserved in a thermos to slow down the cooling speed for working as long as the substance remains fluid and it is advisable to use Agar gels immediately after preparation.

According to the requirements of the specific treatment, the gel may be left on for a limited time (3 minutes) or until drying out of the gel and the operation can be locally repeated. In any case, the Agar film formed is then easily removed by peeling it from

the surface by means of wood stick or tweezers, especially when the gel remains is a solid crust not adhered to the surface, without rinsing the treated surface.

Fig. 9 Mode of preparation, application and removal of Agarose-based gels onto plaster surface.

3.1.6 Optical observations

The treated surfaces (with gels at 2.5% for 3, 20 and until dry out of the gel) of San Colombano plaster were observed by means of a 3D scanner and stereomicroscope, in reflected and raking light. The treated fragments were also observed Ultraviolet light (254 and 365 nm) to detect the eventual presence of surface residue.

Superficial chips and cross-sections obtained from the very porous tile treated with Agar gel 3% (for 3, 20 and until dry out of the gel), were also observed in scanning electron microscopy and X Ray Microanalysis (SEM-EDS).[1] For obtain cross-sections, plaster sample were kept in oven at 40°C for a night, then were impregnated with a bicomponent epoxy resin (100:28) and then cut with diamond blade. All the observations were compared with not treated surfaces and surfaces cleaned by using cotton swabs soaked in water.

3.1.7 Diffusion measurements

Diffusion measurements were performed on plaster mocks up. *Vinavil®,* Agar gels and water were stained with Rhodamin B. *Vinavil®* was applied to the surface of samples by brush and removed at the moment of greatest elasticity. Agar gels (2%, 3% and 4% at 40-45°C) were brushed onto the surface and removed after 3 minutes, 20 minutes and after dry out of the gel. Diffusion tests were also carried out on models held immersed

[1] Normal 8/81 esame delle caratteristiche morfologiche al microscopio elettronico a scansione (SEM)

in water for 72 hours and a water soaked cotton compress was kept on the surface for 3 minutes. After drying, treated samples cross-sections were observed under Ultraviolet light (254 nm).

3.1.8 Soluble salts extraction measurements

Preliminary soluble salts extraction testing was performed on wafers in which Sodium Nitrate and Sodium Nitrite (1% (w:w) to the gypsum) had been dissolved in water before adding powdered gypsum and then prepared as above mentioned. Agar gels were applied and removed after 3, 20 minutes and until dry out of the gels. The still damp gels were placed in contact with strips reactive for Nitrates and Nitrites detection.

3.1.9 Analysis of residues

Residue analysis were performed on the S. Colombano fragments. Two type of Agar and Agarose at 2.5% (w:v) gels were applied by brush and removed after 3, 20 minutes and until drying out of the gel. A sample was taken from each treated fragment; each sample had a surface area of 1 cm square and weighed about half a gram. Extraction from the powdered material was performed with distilled water at 100 °C for 30 minutes. The solutions obtained were filtered through paper filters and gradually dried out in a double boiler at 90° C. Each residue was homogenized in an agate pestle mortar and then divided into four parts equal in weight. One part was analyzed with FTIR-HATR, while the other three fractions were analyzed by GC-MS as above described. The chromatographic peak area of Galactose was integrated and expressed in relation to the internal standard (Sorbitol), in order to obtain quantitative information. Finally, the average analyte/IS ratio was calculated and the average percentage of three runs was made.

One untreated S. Colombano fragment was control tested and a hot extractions were carried out, first with Hexane and then with water; the solutions obtained, once concentrated, were analyzed with FTIR spectroscopy and GC-MS.

3.1.10 Analysis of gels after treatment

After peeling from tile surfaces, gels (3%) applied for 3, 20 min and until dry out of the gels on 4x2 areas were analyzed in order to discover if Ca^{++} ions due to the support

were removed during cleaning. A cleaning cotton swabs soaked in distilled water was rolled for 30 seconds on another 4x2 areas of the tile and it was also analyzed. For comparison a non treated gels was also considered. Calcium *Test* was performed with test strip suitable for rapid determination of calcium in aqueous media. Then, the gel were placed in a tube with 5 ml of distilled water and stirred for 30 min. The supernatant solutions were analyzed. Gels coming from Toschi plasters treatment were also analyzed with the aim to identify removed materials due to gel action.

3.1.11 Instrumentations

Optical observations were performed with a stereomicroscope (Optika) and 3D scanner (Alicona *InfiniteFocus*).[2] Morphology and composition data were obtained using a Jeol 6400 SEM (Scanning Electron Microscope) equipped with Oxford (Link) EDS (Energy Dispersive System) microanalysis (15 kV, 0.28 nA, electron beam about 1 mm in diameter, 60 s counting time, tungsten filament).

FTIR spectra (4000-400 cm^{-1}) were recorded on a Thermo-Nicolet Nexus spectrophotometer equipped with a Thermo-Smart Orbit HATR accessory (diamond crystal); resolution of 4 cm^{-1} and 120 scans. A gaschromatograph 6890N GC (Agilent Technologies), coupled to a MSD (Mass Selective Detector) detector 5973 (Agilent Technologies) with single quadrupole and split-splitless injector, was used for analysis. The mass spectrometer was operated in the EI positive mode (70 eV). The transport gas was Helium (flow 0.60 ml/min). Separation of components was done by means of a fused-silica DB-5 capillary column (Agilent J&W – USA) with a 0.25µm (30 m x 0.25 mm i.d.) methyl-silicone (5% phenyl) film and the injector was used in splitless mode. The splitless injector was set to 280°C with a 30 seconds purge off time. The MS transfer line was set to 280°C. MS spectra were recorded in TIC (Total Ion Current, mass range 45-450).

For the analysis of the monosaccharide derivates, the GC oven temperature program was: 165°C for 0 minutes, 2°C/minute to 190°C, 1°C/min. to 210°C, 20°C/min to 235°C. The complete run takes 34.75 minutes and the injector was used in split and in splitless mode 0.2.

[2] from the Department of Earth Sciences of the University of Pavia

For analyzing alteration materials, a Focus GC (Thermo Scientific) coupled to DSQ II (Thermo Scientific) with single quadrupole and split-splitless injector was used. The mass spectrometer was operated in the EI positive mode (70 eV). The transport gas was Helium. Separation of components was done by means of a fused-silica capillary column (RXI-5, Restek) with a 0.25µm (30 m x 0.25 mm x 0.25 µm) methyl-silicone (5% phenyl) film and the injector was used in splitless mode. This GC-MS was used for painting and leaching study of supported layers analyses.

Separation of the methyl ester of fatty acids was achieved following this temperature program: isothermal conditions at 80°C for 2 min, with 20°C/min heating up to 270°C and isothermal conditions at 270°C for 6 min (total run time 17.50 min). The mass spectra were collected in Total Ion Current (TIC; 40-500 m/z fragmentation rate). Separation of N-trifluoroacetyl-O-2-propyl esters amino acid derivatives was achieved following this temperature program: isothermal conditions at 60°C for 3 min, with 25°C/min heating up to 260°C and isothermal conditions at 260°C for 6 min (total run time 17.00 min). The mass spectra were recorded in Selected Ion Monitoring (SIM; 140, 126, 154, 153, 139, 168, 182, 166, 164, 184, 180, 198, 91, 190 m/z fragments).

3.2 Results and Discussion

3.2.1 Characterization of the materials

The characterization firstly carried on gelling materials based on Agarose, ranging from laboratory to food additive grades, confirmed their polysaccharide composition with the principle monosaccharide Galactose. FTIR spectra of the three materials taken into consideration are characteristic of polysaccharide materials (OH stretching at 3300 cm^{-1}, C-H stretching at 2800 cm^{-1}, intramolecularly bound water and C=O group at 1630 cm^{-1}, C-O stretching and C-OH and C-H bending at 1370 cm^{-1}, C-O-H bending at 1150 and 1040 cm^{-1}) (Fig.10). Gaschromatograms obtained showed the peak of the main monosaccharide constituting the gels: Galactose (Fig.11).

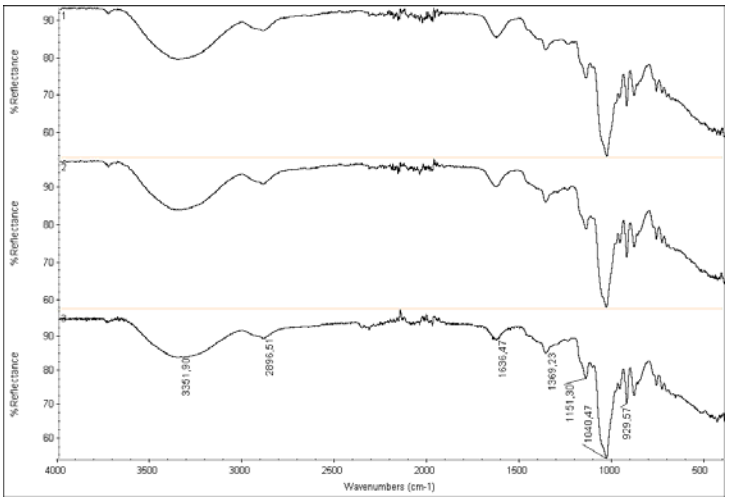


Fig. 10 FTIR spectra of pure powdered Agar (*Fluka*) (1), Agar (*Tang Freres*) (2) and Agarose (*Tebu-bio*) (3) used to clean plasters .

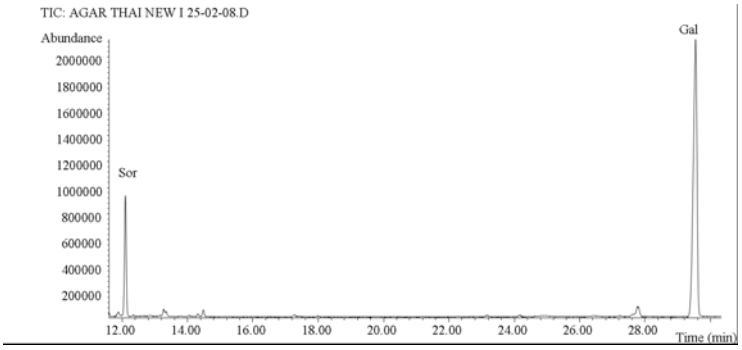


Fig. 11 Chromatogram of food additive grade Agar (*Tang Freres*) [Sor=Sorbitol (internal standar), Gal=Galactose].

FTIR spectra of the supports considered indicated the presence of bi-hydrated Calcium Sulphate (gypsum) (S-O stretching and bending at 1100 cm^{-1} and 1000 cm^{-1}, OH stretching at 3500, 3400, 1680 and 1620 cm^{-1}, fingerprint at 670 and 600 cm^{-1}).

FTIR spectra showed that the yellowish patina (#1) from the high-relief preserved at Gipsoteca Toschi is made of gypsum (3519, 3396, shoulder at 1618, 666, 595 cm^{-1}), calcium carbonate (Calcite) (CO_3^{2-} stretching at 1410, O-C-O bending at 873, fingerprint at 712 cm^{-1}) and calcium oxalate (C=O and C-O stretching at 1618 and 1321cm^{-1}). Hexane extracts spectra showed other organic substances such as proteins (N-H stretching at 3285 cm^{-1}, C-H stretching at 2917, 2849 cm^{-1}, Amide I (C-O) stretching 1644, Amide II (N-H) bending 1547 cm^{-1}), also confirmed by GC-MS data, and long chain hydrocarbons (C-H stretching at 3000-2800 cm^{-1}).

FTIR measurements of samples from *La Strega* by Marzaroli, showed that yellowish patina (#2) is composed of gypsum (3520, 3398, 1685, 1618, 1108, 668, 599 cm^{-1}), due to the support, calcium carbonate (Calcite) (1791, 1411, 871, 711 cm^{-1}) and lipids (CH_2 stretching at 2924 cm^{-1}, CH_3 stretching at 2953 cm^{-1}, C=O (ester) stretching at 1735 cm^{-1}) due to stucco employed as detaching material. Indeed, the latter (#3) spectra showed the presence of Calcite (1794, 1391, 869, 711 cm^{-1}), silicates (1008 cm^{-1}) and lipids (2922, 2852, 1735 cm^{-1}). Hexane extract spectra (2954, 2923, 2852, 1739, 1709, 1461, 1377, 1260, 799 cm^{-1}) showed a lipid like drying oil/ waxy material. This composition was confirmed by GC-MS (Fig.12). Laurate (3.6%), myristate (1.9%), palmitate (20.0%), linoleate (tr%) oleate (57.3%), stearate (17.2%) detected have indicated a semi-drying oil.

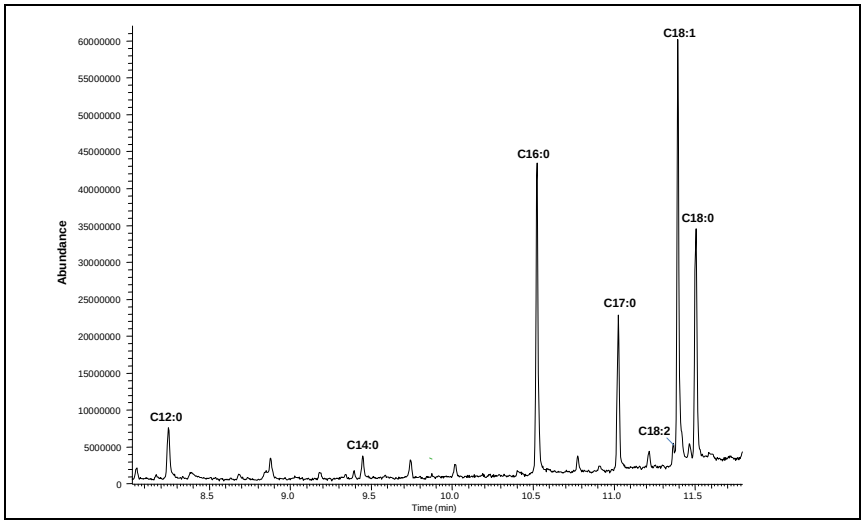


Fig. 12 Chromatogram of the lipid fraction of yellowish patina from *La Strega* by Marzaroli [C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, C17:0=Heptadecanoic Acid (internal standard), C18:2=Linoleic Acid, C18:1=Oleic Acid, C18:0=Stearic Acid].

3.2.2 Preparation, application, removal and efficacy of Agarose-based gels

Gels from 2.5% to 5% (w/v) were used depending on the type of application and the water-sensitivity of the object to be cleaned. Lower percentages form overly fluid gel which releases a greater amount of water, while very high concentrations gels appear too stiff and more difficult to apply. All kinds of Agarose-based materials tested performed in a very similar way. Having the same composition, the less expensive type, the food additive's, especially for cleaning large plasters with noticeable concentrations of dirt, was preferred.

The principle innovation in comparison to previous experiments with rigid gels [23] consisted in the modes of application, which took place in a fluid phase so that the gel may adhere to the sculptural volumes. Attention must be turned to the difference between the gelation temperature (38°) and the working-temperature (about 45°) in order to use a substance which has not yet solidified. The application is quite similar to that adopted for vinyl resins, but the density of the gel makes it even simpler and faster.

The relatively moderate gelation speed of the layer allows it to be brushed on the sculpture which can be turned around during application. This helped to achieve a single uniform layer free of joint lines or superimposed levels. Re-dissolving the gel after the first solidification, more homogeneous gels were obtained; this partially modified it, since the second heating, more rapid than the first needed to transform the powder into gel, made it more homogeneous and with higher water retention. Melting the gel again more than once produced no further variations. Heating time did not influence the results, although it was necessary to replace the quantity of water evaporated from the mixture.

All in all, preparation times were brief. For instance, about 10 min needed to produce 1 kg of gel, although this may vary depending on the quantity of material and temperature.

Regarding Agar gels conservation, it is advisable to use them immediately after preparation: Agarose and Agar, while stable in the dry powdered form, bio-deteriorate when gelled, because of their high water content and because it is practically impossible to maintain sterile conditions when working on art objects. This aspect necessitates a minimum of care during preparation (clean glassware), and in particular when handling, to avoid contamination of the fresh gel (use of gloves, clean implements, etc.). The gel blocks may be conserved in the refrigerator, better after sealing the surface with a plastic membrane as further protection.

In most applications to plaster sculptures, after only three minutes the plaster appeared clean with only a minimum release of water into the material. Practically, completing its own physical process of transformation from a fluid to a solid, Agar gel extracted dirt from a highly hygroscopic and permeable surface. In some cases, in order

to eliminate stains (such as caused by oxidation of iron pieces) penetrated into the plaster, or salts, it was left until it dries totally, so the water was totally "discharged".

Fig. 13 Giuseppe Grandi, *Incitamento alle barricate.* Elimination of stains caused by the oxidation of iron piece inside the arms, using Agar left until dry.

Beyond the kind of dirt, the time necessary for the cleaning was due to gelation time, related to the thickness of the layer brushed on and the dimensions of the area of intervention.

The operation was repeated locally on non-homogeneous surfaces with areas of particularly consistent deposits. This allowed to perform a layer-by-layer cleaning, starting from the outermost superficial one and it permitted to choose the cleaning level. By using even small brushes, precise application has performed. Observation of the gradual coloring of the gel, as it absorbs the dissolved materials, was often used to verify its action.

The Agar film formed on the surface was easily removable because its characteristic transparency and softness, associated with a high degree of elasticity. Eventual residues, which may remain near the edges of the areas treated, was detached spontaneously after drying. After evaporation, in fact, the film tended to separate naturally from the support. The surface freed from the gel did not suffer any manipulation, and totally conserved any minimum traces of workmanship and blade marks left by the scraper.

The cleaning by Agar resulted very effective, by attract both surface dirt and internal soiling that was partially absorbed and incorporated into the gel and in part forms a thin film of incohesive deposit on the inner face of the gel in contact with the surface.

By peeling of a vinyl-based coatings, successful in eliminating surface deposits, even those well adhered to the surface, was achieved but unable to remove the more resistant portion penetrated into the material structure of the plaster. At the same time, the treated surface appeared perfectly intact, whose most minute details and patinas were all preserved, and without color variations or saturation.

In particular, two cleaning testing showed interesting findings. They were performed on stains made of proteins (probably due to detaching materials), calcium oxalate and hydrocarbons (due to organic matter degradation and superficial dust, respectively) from an high-relief and oil-based yellowish patina of the sculpture *La Strega*.

Agar gels (3%, Fluka) applied for 5 min removed the yellowish stains of high-relief in a very effective way. After drying, the surface appeared “less clean” because the yellowish material resurfaced. In fact, the artifact was full of this substance. Subsequent applications allowed to achieve in-depth cleaning or, in this case, longer application time was required. Tests with cotton swabs soaked in deionized water evidenced that the material to be removed was completely water-soluble. After 2 min of swab-rolling, it was obtained similar effect to that of agar applied for 5 min. After drying, the area treated appeared a bit yellow and in order to obtain the effect comparable to treatment with agar for 20 min and 30 min, an other cotton-swab was applied. Finally, the surface treated with water, despite the same level of cleaning of Agar gels, resulted pasty and scraped.

Fig. 14 cleaning testing performed with cotton-swab (1), and Agar gel for 5 min (2), 20 min (3) and 30 min (4)

After 5, 10 and 30 min of gel application to yellowish patina of *La Strega*, the plaster did not appeared clean, as expected, but only lightened (Fig 15).

Fig. 15 Gel removing after 30 min of application.

Rolling-swabs test evidenced that the material to be removed was not water-soluble. 5 swabs soaked in deionized water obtained similar effect to Agar application after 30 min. Although lipophilic material was found in the gel after treatment (Fig.16), agar gel acted on hydrosuble compounds such as gypsum (and Calcite) which are strongly bound to this substance. In fact, as can be seen in the figure (Fig. 17) the material was also quite degreased and this may have facilitated the removal.

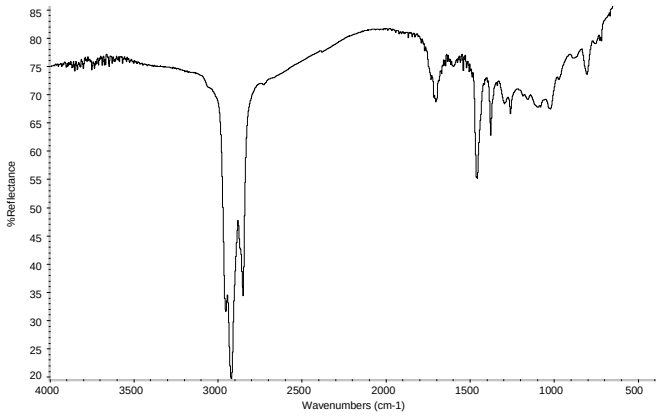


Fig. 16 FTIR spectra of hexane extract of the gel removed after 10 min from yellowish patina of *La Strega* by Marzaroli

Fig. 17 detail of the surface of the sculpture *La Strega*

3.2.3 Optical observations

From a visual point of view, no morphologic modifications and any evidence of damage resulted from the action of Agar gels. The marks and irregularities on the surfaces of the plasters were all conserved, especially in comparison of the application of water in free form that left the surface look like "pasty" and slightly smoothing (fig.18).

Fig. 18 cleaning testing with Agar (on the left) and rolling cotton swabs soaked in deionized water swab (on the right).

Visual control of Giuseppe Grandi's large models for the "*Monumento alle Cinque Giornate*", after more than a year from their restoration, have confirmed that the cleaning produce no visible transformations, nor yellow staining as frequently appear after various other types of intervention.

Observing the cleaned surfaces of San Colombano plaster by means of a 3D scanner (Fig.19) and stereomicroscope (Fig.20), streaks and porosity belonging to the natural surface conformation were preserved.

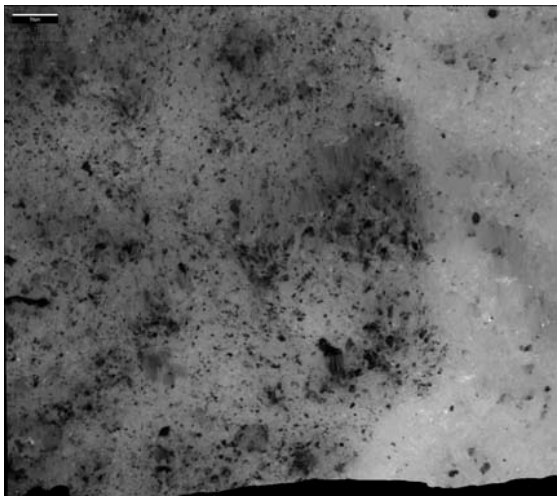


Fig. 19 cleaning testing on the surface of San Colombano fragments with 3D Scanner

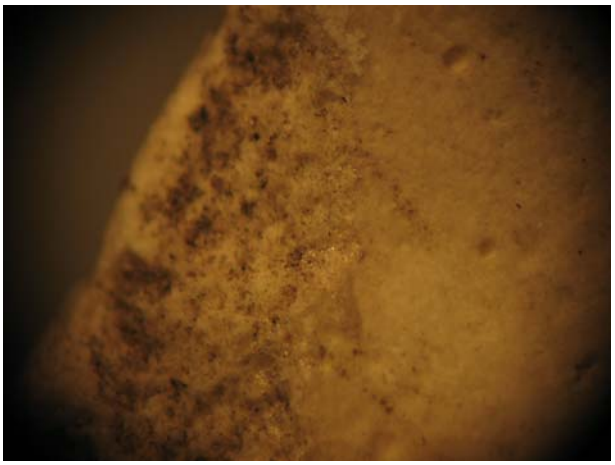


Fig. 20 detail under stereomicrocope (15x) of the edge of a San Colombano fragments treated with Agarose (Teu-bio) left until dry out

Along the edges of the fragments, thin dried films of gelling material, adhered or slightly penetrated, were sometimes observed. These films were easily lifted and taken off with a scalpel under the microscope. This was not surprising, given the size and shape of the samples which made the forming of little bits of film at the edges inevitable. It is likely the edges of the fragments were more subject to retaining gelling material and in those areas the greatest release of water during gel application occured; the central areas, in fact, appeared free of residue and unaltered morphologically. This aspect was minimized during the applications on the plaster sculptures, considering the operations were carried out on small adjacent areas, superimposed one on the other one. Eventual residual films were easily removed, simply by slightly humidifying them or with a light rubbing by finger.

Observation with UV light did not evidence fluorescent zones, except weak areas at the edges of the treated parts of the fragments. This slight fluorescence may be due

either to a dry, superficial residue of gel (fluorescent by nature) or to action of the water. To observe the effect of water alone, on a non-treated S. Colombano fragment two tests were carried out, letting 2 drops of distilled water drip on the surface, and gently rotating a cotton swab soaked in distilled water for 30 seconds. Once dry, the fragment was observed with the UV lamp. In both areas treated with water, a slight fluorescent halo was observed.

Observation of treated surfaces of a very porous tile showed similar results: all the porosity were preserved with a minimum change in the surface roughness (Fig.21).

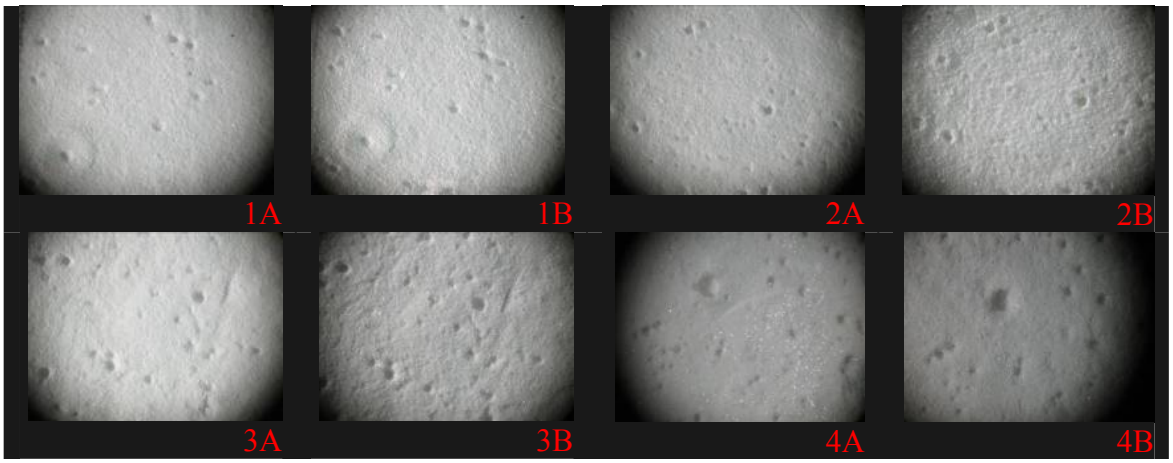


fig. 21 stereomicroscopical images of the surfaces of a very porous tile (70x) untreated (A) and treated (B) with agar 3% for 3 min (1), 20 min (2), until dry out of the gel (3) and by swab-rolling water for 30 sec (4).

Observation of cross-sections obtained from the same samples clearly showed the action of water released from the gel just below the surface level. More increase the gel application time more the effect of water was observed. Inside the porosity dry residues of gelling material were sometimes noticed. In contrast of this, water application in free form left more altered the surfaces.

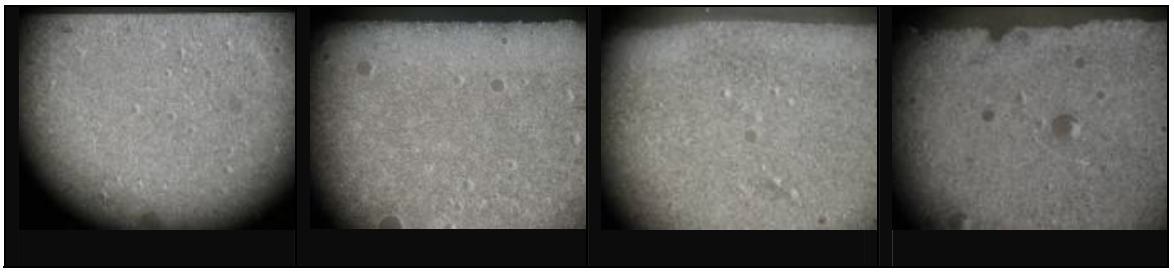


fig. 22 stereomicroscopal image of cross-sections (15x) not treated and treated with agar gel 3% for 3 min, 20 min, until dry out of the gel (from the left to right).

SEM-SEI images showed that the less organized surface of tile treated by Agar gels did not undergo any morphological important changes (Fig.23). Modifications of phase of gypsum occur for temperatures ranging between 100°C and 1200°C. The solubility of gypsum in water varies from 0.241 % at 0 °C, with a tendency to increase up to about 36-38 °C when it reaches a maximum of 0.25 %, which subsequently diminishes as temperature increases, becoming 0.222 % at 100 °C.[31] So the Agar treatment at 40-45°C can be firstly considered safe. Any re-crystallization of small crystals of gypsum can be occurred but it is difficult to distinguish crystals of new-formation than original crystals.

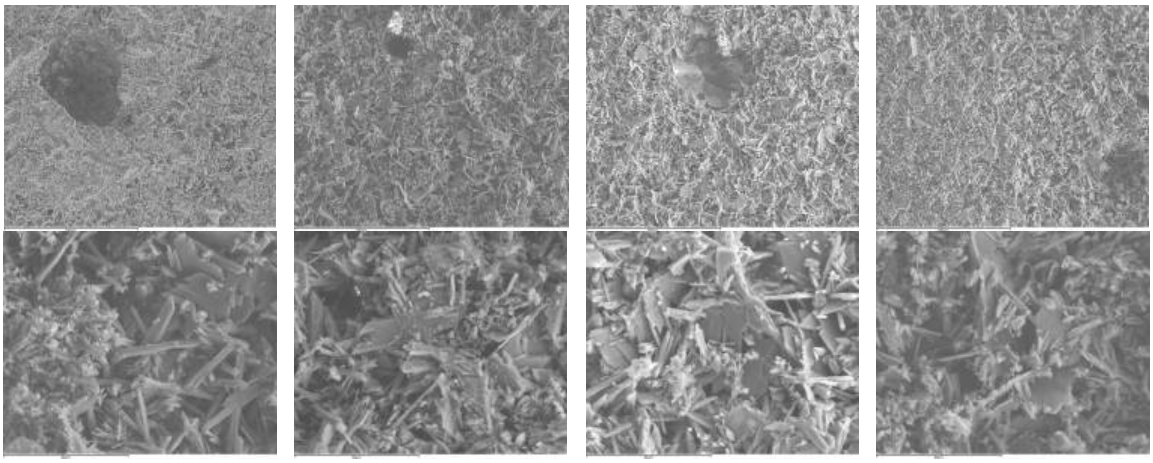


Fig. 23 SEM-SEI image of cross-sections (120x and 700x) not treated and treated with agar gel 3% for 3 min, 20 min, until dry out of the gel (from left to right).

3.2.4 Diffusion measurements

Observation of the cross-sections showed that *Vinavil*® demonstrates very low water diffusion. This film-producing product has a water content equal to 50% of its weight. Furthermore, among the various additives present in it, there are certainly surfactants added to stabilize the dispersion. These, thanks to their action on the surface tension of the water vehicle, contribute to limiting internal diffusion.

Agar gel at 3% in distilled water showed a diffusion of about 1.5 mm after a 3 min application, up to about 3 mm after 20 min and up to less than 10 mm after until dry out of the gels (Fig.24).

Fig. 24 cross-sections of plaster treated with agar 3% for 3 min, 20 min, until dry out of the gel (from the left to right) under Uv light. Water was tinted with rhodamin

More stiff agar gel (at 4% in deionized water) showed diffusion marking of about 1 mm after a 3 min application, which increased slightly up to about 2 mm after 20 minutes, while a 2% Agar gel in water after 20 min reached a depth of 4 mm. The same diffusion was obtained on the wafer which remained immersed in water for 72 hs, using the same 2% Agar gel again for 20 min. This test was carried out to control the behaviour of already impregnated gypsum, a situation which may sometimes be encountered when treating actual works. The 4% Agar gels, left on the surface until dry, marked the gypsum up to a depth of 7-8 mm. For the purpose of comparison, a thin layer of cotton soaked in water was left in contact with the surface for only 3 minutes, resulting in coloration of a 5-6 mm layer of gypsum.

As above described, the water diffuses in a controlled way just below the surface level. Varying density and times of application it is possible to design a cleaning according to the characteristics of the support.

3.2.5 Soluble salts extraction measurements

All the gels analysed revealed a presence of Nitrates and Nitrites. This preliminary test showed that Agar is able to absorb water-soluble substances contained in the substrate to which it was applied, assuming the potential use of Agar gel not only for surface cleaning, but also to extract soluble salts. The greater diffusion provided by longer application times allows acting more in depth.

3.2.6 Analysis of residues

FTIR spectra of the all the extracts only indicated the presence of bi-hydrated Calcium Sulphate (gypsum), coming from the support. GC-MS data of the extracts showed a very weak signals for Galactose, to the point where they may be considered traces. The average Galactose/Sorbitol (IS) ratio both for the pure materials and the extracts (for the latter, the relationships deriving from the tests performed were calculated averaging three repeated tests for each sample) is reported in the following table.

Tab. 8 Ratio between the areas of the chromatographic peaks for Galactose (Gal) and Sorbitol (SI), of the three pure materials and the extracts relative to the applications carried out.

	Agar (*Fluka*)	Agarose (*Tebu-Bio*)	Agar (*Tang Freres*)
		Gal/SI	
Pure powder (ca. 1 mg)	7.685	5.72	8.29
Gel, 3 minutes	0.008	0.01	0.11
Gel, 20 minutes	0.008	0.02	0.01
Gel, until dry	0.039	0.21	0.01

The values for the extracts from the fragments treated with Agar (*Fluka*) with 3 and 20 min applications are similar, being about 1000 times less than the same value for the

pure material; this ratio increases about 5 times for the dried out application. The ratios related to the applications with Agarose (*Tebu-bio*) increase together with the time of application, and for application until drying are about one time more than the applications of 3 and 20 min. For the applications of Agar of the food use variety (*Tang Freres*), the 3 min application is the one which releases the most in comparison with the other application times, although we are still speaking of infinitely small quantities. This value may be attributed to a particularly heterogeneous nature of the sample, while the behaviour of this material results totally comparable to the other two materials for micro-biological use. The results obtained have permitted to affirm that in all cases taken into consideration, the gel permeated into the supports is present in infinitely minute quantities. Relating to internal standard, the amount of Agar per cm square ranges from about 1 to 50 µg Agar and from about 2 to 40 µg for Agarose application.

3.2.7 Analysis of gel after treatment

Agar treatments performed with Agar gels (3%) applied for 3, 20 min and until dry out of the gels for 4x2 areas resulted to dissolve a small amount of Calcium from gypsum. Different times of application, as it can be seen in fig. 25, gave, more or less, comparable results. The amount of Ca^{++} ions removed resulted about 125-250 µg. Even a brief application of cotton swabs soaked in distilled water led to a small dissolution of gypsum.

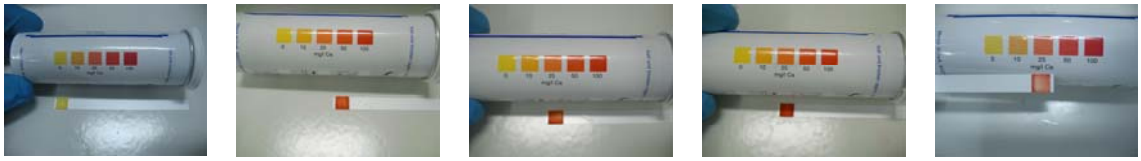


Fig. 25 calcium test of gel (from left to right) before treatment and after 3 min, 20 min, until dry out and application of cotton swab

3.3 Conclusions

A new procedure has been developed for surface-cleaning plaster, based on aqueous Agarose-based gels. The main innovation lies in the mode of application: brushed onto the surface during the cooling phase, at a temperature of about 45-50 °C, so as to adhere to the sculptural volumes. Depending on the type of application, Agar can be used in concentrations from 2.5 to 5 g in 100 ml deionized water and better results have been obtained re-dissolving the gel after the initial solidification. Cleaning tests have shown that Agar gels are effective on both soiling materials and stains penetrated into the plaster, generally with, respectively, only three minutes of application and until complete dryness of the gel, whereas are not effective for removal of lipid such as oils penetrated into the plaster. Preliminary testing also revealed that Agar gels are able to absorb water-soluble salts, such as Nitrates and Nitrites. The procedure may be repeated locally yielding a layer-by-layer cleaning and precise applications.

The Agar film formed on the artefact's surface is then easily lifted off.

Observation of treated surfaces has shown no morphological changes, and no evidence of damage, such as yellowing, was detected as a result from the action of Agar gels. Only a minimum change in the roughness was detected on a very porous tile surfaces.

Observation of the S. Colombano fragments sometimes has evidenced the presence of superficial residues of gel along the margins of the treated areas, where likely the greatest release of water occurred during gel application. This is not surprising, given the size and shape of the samples which made inevitable the forming of little bits of film at the edges. This problem was minimized during applications onto whole plaster sculptures: the "border effect" becomes irrelevant by slightly overlapping the different

applications on small adjacent areas. Furthermore, any residual film can be easily removed.

Diffusion measurements and observation of cross-sections from treated samples have shown that water diffuses from the gels in a controlled way just below the surface level. By varying gel density and application times it is possible to tailor the cleaning to the characteristics of the specific support

As far as the other, far more critical aspect is concerned, that of residues penetrated into the plaster, GC-MS analysis of the treated fragments, monitoring the Galactose peak, have shown that the gelling material is present only in trace amounts.

Concentration gradients, once water from the gel starts diffusing into the surface materials, can be hypothesized as the main factor ruling this process.

It appears that the exchange process between the two materials, plaster and gel, practically starts from the moment the gel is applied, with an initial strong attraction of the dirt towards the thickener, followed, once gelling has occurred, by the slow release of water into the plaster. For this reason, if cleaning is aimed only to removing the most superficial deposit layer, it will be sufficient to remove the film immediately after cooling. A further evidence for this is the finding that, when the gel was applied onto a very porous surfaces, free from soiling material, a small amount of dissolved gypsum migrated from the support into the gel.

4. Waxes

4.1 Experimental

4.1.1 Materials

All solvents were of HPLC grade and all other chemicals were of analytical grade. For cleaning testing, *Tween 20* (Polyoxyethylene (20) sorbitan monolaurate) CMC=0,06mM and HLB=16.7 from Sigma-Aldrich and *Vanzan* NF-C R.T. (Xanthan Gum) from Vanderbilt Company were used. Paraffin, unpurified beeswax and bleached beeswax were purchased from local market.

4.1.2 Laboratory wax layers preparation

Layers of an half cm of thickness made of paraffin, unpurified beeswax and bleached beeswax were prepared in Petri capsules by melting (fig.26). Once solid, the layers were aged under UV lamp for 24 h. Before and after layers aging, spectral reflectance factors were measured on fixed areas and CIE L*a*b* values were calculated for obtain color specification of the surfaces.

Fig. 26 Layers made of paraffin, unpurified beeswax and bleached beeswax, from left to right.

4.1.3 Galleria d'Arte Moderna sculptures

Four fragile sculptures (fig. 27-30) dating back to the late nineteenth and early twentieth century were considered. They were conserved at the Galleria d'Arte Moderna in Milan and stored out of the museum tour (Tab.9).

Tab. 9 Wax-made sculptures from the Galleria d'Arte Moderna in Milan

Sculpture title	Artist	Date	Dimension	Museum reference
Il ritratto del pittore Fortuny	Vincenzo Gemito	about 1873	(68 cm x 50 cm x 46 cm)	6258
Il vasaio	Vincenzo Gemito	1915	(76 cm x 26 cm x 42 cm)	6256
La madre dormiente	Raffaele Scorzelli	1947	(31 cm x 50 cm x 40 cm)	7379
Il velo	Pietro Cendali	1890-1910	(43 cm x 53 cm x 22 cm)	7343

Fig. 27 *Il ritratto del Pittore Fortuny* (about 1873) by Vincenzo Gemito

Fig. 28 *Il vasaio* (1915) by Vincenzo Gemito

Fig. 29 *Il velo* (1890-1910) by Pietro Cendali

Fig. 30 *La madre dormiente* (1947) by Raffaelle Scorzelli

Almost all artworks had been shaped by the artist and modelled in plaster and then covered with waxy coatings. In particular, Gemito in the *Il ritratto del pittore Fortuny* had shaped the sculpture using different in color and thicknesses waxes. A clear one is over the support and the upper one is brownish. *Il vasaio* represents a standing boy that holds a vase in his hands. Here the wax used seems to be yellowish and the vase, completely realized in waxy material, is slightly clearer. It was restored in 1975. *La madre dormiente*, that represents a female figure lying on her side, is a replica from a mold making in pieces. *Il velo,* that is signed by the artist, represents a woman face covered with a veil. It was donated to the Museum in the 1947. A very thin waxy coating with dark color covers the support.

Being covered by layered, incorporated dust and whitish patinas, they present a similar conservative state (fig. 31-34). Several deep cracks may be found in the waxy coatings to make them detached from the support and some deformation occurs.

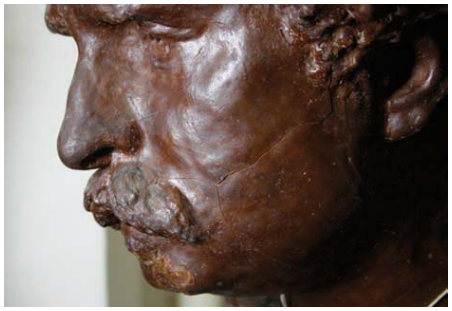


Fig. 31 detail of cracking down the cheek of *Il ritratto del Pittore Fortuny* (about 1873) by Vincenzo Gemito

Fig. 32 detail of the surface of *Il vasaio* (1915) by Vincenzo Gemito

Fig. 33 macro photo of the surface of *La madre dormiente* (1947) sculpture by Raffaelle Scorzelli

Fig. 34 detail of the surface soiling of *Il velo* (1890-1910) by Pietro Cendali

4.1.4 Characterization of wax

Micro-chips of waxy were collected from laboratory wax layers and from sculptures, they were observed under stereomicroscope and then analyzed by using GC-MS. From each sculpture were taken two samples: from the upper layer and from the inner one (tab. 10).

Tab. 10 list of the samples collected from the upper layer (1) and the inner layer (2) of sculptures

Sculpture title	Location of samples	Lab reference
Il ritratto del pittore Fortuny	On the back of the sculpture, near to a lacuna	CP1
		CP2
Il vasaio	On the back of the stand, in two areas different in colors	CV1
		CV2
La madre dormiente	On the back, near the head of the figure	CM1
		CM2
Il velo	On detached fragments	CVE1
		CVE2

1 mg of sample was transferred to a screw cap tube. The sample was treated with methanolic potash (5% w:v) (1 ml) for 1 hours at 80°C. After cooling to room temperature, to the sample was added Hexane (4 ml) and 50 ppm of Eicosane solution in Hexane (1 ml) as internal standard. Afterwards, the sample was shake and left to stand. Hexane solution wax separated by using Pasteur Pipette. To the methanol phase 6N hydrochloric acid solution (1 ml) was added. An extraction with diethilic ether (2ml) was finally performed. The Hexane and diethilic ether solutions were mixed. The solvents were then evaporated under nitrogen flux. The residue was dissolved in 100 µl of BSTFA and keep at 60°C for 30 minutes. 1µl of the resulting solution is used for gas chromatographic analysis. µFTIR-ATR (Attenuated Total Reflection) measurements were also performed on sculpture samples.

4.1.5 Preparation, application and removal of aqueous solutions

Five aqueous solutions specifically designed for wax objects were prepared and then firstly tested on laboratory reference wax then on wax sculptures.

1. Buffer solution at pH 6, conductivity 4,5 mS/cm

2. Buffer solution at pH 7, conductivity 6,4 mS/cm
3. Buffer solution at pH 7, conductivity 4,5 mS/cm
4. Buffer solution at pH 7, conductivity 4,5 mS/cm, surfactant *Tween 20*
5. Buffer solution at pH 7, conductivity 12,4 mS/cm, chelator Citrate

The first solution was prepared adding 1M NaOH to an aqueous solution of phosphoric acid 0.5% (w:v) up to pH 6. The conductivity was brought to a 4.5 mS/cm by dilution with water. The second solution was obtained from the first one by adding 1M NaOH up to pH 7 and conductivity 6,4 mS/cm. By adding distilled water to the second solutions it was obtained the third one with conductivity 4.5 mS/cm. The fourth solution was obtained adding five drops of surfactant to 25 ml of the latter solution. Adding 1M NaOH to an aqueous solution of phosphoric acid 0.25% (w: v) and citric acid 0.1% (w:v) up to pH 7 the fifth solution was prepared (conductivity at 12,4 mS/cm).

The solutions thus prepared were also thickened with *Vanzan* NF-C at 1.5% (w:v) at room temperature. The solutions one and four both in free and gelled form were tested on laboratory wax layers, while all solutions were applied on the sculptures. Cotton swabs (previously rinsed in hexane and water and vacuum dried) were soaked in solutions and then lightly rolled (10 rolls per treatment) on 1 cm square areas. Gel tests was consisted of application for a period of five minutes and the surface rinsing was performed rolling cotton swabs soaked in the respective solution. Afterwards, the cleaning cotton swabs were transferred into eppendorf and stored at 4°C. Following the procedure above described, each solution both in free and gelled form was analyzed by GC-MS in order to obtain the blank samples.

Tab. 11 cleaning testing by means of aqueous solution (1-5) in free from (S) and gelled from (G) on wax sculptures

Sculpture title	Location of cleaning testing	Lab reference
Il ritratto del pittore Fortuny	Base of the sculpture	1S-5S
		1G-5G
Il vasaio	Back of the base	1S-5S
La madre dormiente	left arm of the figure	1S-5S
		1G-5G
Il velo	Detached fragments	1S-5S
		1G-5G

4.1.6 GC-MS analyses of wax components extracted

Cleaning cotton swabs thus obtained were analyzed by GC-MS following the procedure above described. In order to verify eventually wax components removed during cleaning chromatograms of cleaning swabs were compared with wax samples ones (and the blank samples).

4.1.7 Instrumentations

A UV lamp (Hg lamp, 0.15 W/mq -measured at 10 cm height-, 254 nm, maximum temperature about at 25°C) and a stereomicroscope (Optika) were used. The spectrophotometric scanner (spectral region: 380–800 nm) used was developed by researchers of University of Parma [66]. Lighting and observation conditions were 45/0º with a halogen lamp and D65 illuminating agent and CIE 1931 observer were considered. FTIR microspectroscopic measurements were performed using a Nicolet Continuμm Microscope connected to the Nexus spectrometer, equipped with a crystal of silicon and a Mercury Cadmium Telluride (MCT) detector cooled by liquid nitrogen. Infrared spectra were acquired from 4000 to 650cm^{-1}, resolution of 4 cm^{-1} and 120 scans. A gaschromatograph 6890N GC (Agilent Technologies), coupled to a MSD (Mass Selective Detector) detector 5973 (Agilent Technologies) with single quadrupole and split-splitless injector, was used for analysis. The mass spectrometer was operated in the EI positive mode (70 eV). The transport gas was Helium. Separation of components was done by means of a fused-silica DB-5 capillary column (Agilent J&W – USA) with a 0.25μm (30 m x 0.25 mm i.d.) methyl-silicone (5% phenyl) film and the injector was used in split mode. For fatty acids, terpenoid acids and neutral compounds, alcohols, and hydrocarbons analysis the chromatographic oven was programmed as follows: 80°C, isothermal for 2 min, 10°C/min up to 200°C, 200°C, isothermal for 5 min, 20 °C/min up to 280°C, 280°C, isothermal for 20 min (total run time 43.00 min). The mass spectra were recorded in Total Ion Current (TIC; 40-500 m/z fragmentation rate).

4.2 Results and Discussion

4.2.1 Analyses of wax

Laboratory and museum samples were firstly analysed with the aim to identify the nature and the conservation state of waxy surface of the layers. The cleaning tests were performed on these surfaces.

4.2.1.1 Laboratory waxes

After solidification, the surfaces of wax-based laboratory layers (paraffin, unpurified beeswax and bleached beeswax) resulted compact and very homogenous. Multispectral scanner data (Tab. 12) measured only a small variation of colour due to UV ageing ($2<\Delta E<3$).

Tab. 12 CIELAB color differences measured on *L, *a**, and *b** coordinates. ΔE is the geometric distance between two points in the CIELAB space and it is calculated as follow: $\Delta E = \sqrt{(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2}$. In general, perceptibility differences occur for ΔE>3.**

wax lab layers	ΔE
Paraffin	2,51
Unpurified beeswax	2,29
Bleached beeswax	2,25

It is well known that UV radiation does not adequately represent the natural aging, but this method was used to induce photo-oxidation in a very short time.

Aged laboratory samples were analysed by GC–MS after extraction and trimethylsilylation in order to identify, in a single run, most of the solvent soluble molecular components present in the samples. GC-MS data of paraffin showed that it is characterised by a series of homologous linear alkanes, containing both even and odd carbon number from C22 to C29 (C23 as a major compound) and palmitic acid. Despite this composition is not perfectly in agreement with literature data about paraffin, its presence can be hypothesized with the addiction of a fatty material. In fact, the

distribution of *n*-alkanes in paraffin may slightly change depending on the raw petroleum matter distilled.

The chromatograms of unpurified beeswax from commercial source showed that it mainly consists of long-chain saturated (both even- and odd-numbered compounds C23-C33, except for C30 andC32) and unsaturated hydrocarbons (such as C26:1, C28:1, C30:1, C32:1, C33:1 and C34:1). Only palmitic acid was detected. The data revealed that it is made of a waxy material adulterated with synthetic wax likely paraffin and the chromatographic profile, without fatty alcohols and low amount of fatty acids, is not in agreement with literature data.

The chromatographic profile of bleached beeswax obtained (fig.35) showed that it is composed of a mixture of long chain hydrocarbons (ranging from C21 to C31 except C30 for saturated ones and monounsaturated ones are C30 and C33), alcohols (even-numbered compounds from C24 to C30, C24 as main component) and fatty acids (palmitic and stearic acids).

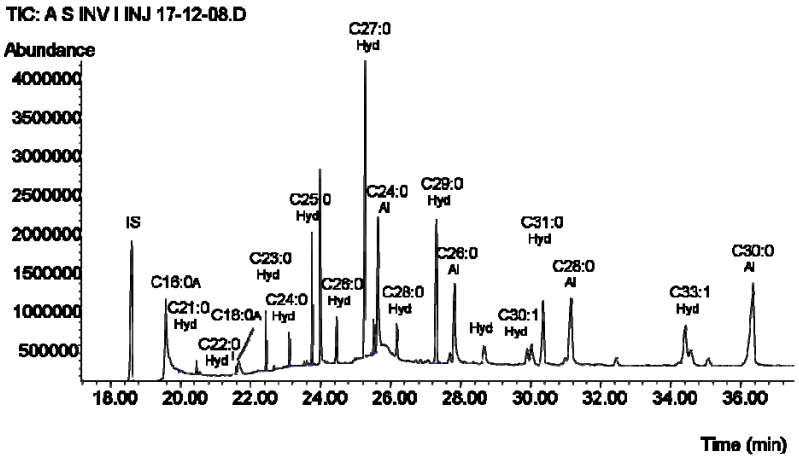


Fig. 35 Chromatogram of the bleached beeswax employed for a layer. [IS=internal standard (eicosane); A=fatty acid; Al=alcohol; Hyd=hydrocarbon]

4.2.1.2 Museum samples

Museum samples were observed under stereomicroscope, then analysed by μFT-IR (ATR) and GC–MS in order to detect wax components. By comparing the results of the

inner and upper layer samples, it was verified if the exposure to environment agents has led changes in the wax or if the artist had used different waxy preparations.

Under stereomicroscope, the surface of the samples (CP1, CP2) collected from *Il ritratto del pittore Fortuny* sculpture appeared covered by whitish crystallization (fig.36). FT-IR spectra (fig.37) did not show significant differences between CP1 and CP2 samples: they are characterized by absorption bands of long-chain hydrocarbons (CH_2 (a) stretching at 2916 cm^{-1}, (s) 2848 cm^{-1}, CH_3 (a) stretching at 2955 cm^{-1}, (s) 2870 cm^{-1}, CH3 (a) bending at 1472 cm^{-1}, (s) 1374 cm^{-1}, CH2 scissoring at 1463 cm^{-1}, CH2 rocking at 719, 729 cm^{-1}), acid and ester groups (C=O (acid) stretching at 1709 cm^{-1}, C=O (ester) stretching at 1735 cm^{-1}, and C-O stretching (ester) at 1170 cm^{-1}). Additional spectral features are associated with the CH bending vibrations (1350-980 cm^{-1}). GC-MS mainly indicated the presence of long-chain hydrocarbons and low amount of fatty acids, with differences between the two samples, suggesting a mainly paraffin composition (fig.38). In the upper sample (CP1), both even- and odd-numbered saturated hydrocarbons ranging from C21 to C33 and even-numbered unsaturated hydrocarbons (C26-C34), C27 as the major found components were found. In the CP2 sample, saturated hydrocarbons (C21-C30), C26 as the major component were found. Both samples contain palmitic and stearic acids and also in CP1 sample two hydroxyacids (C16) were detected.

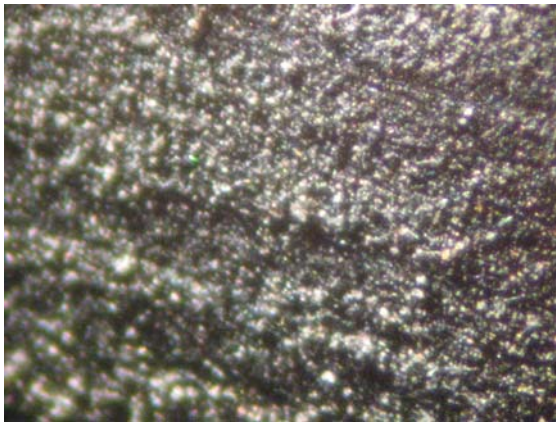


Fig. 36 Stereomicroscopical image (450x) of the surface of *Il ritratto del pittore Fortuny*. A white patina likely due to migration of saturated hydrocarbons is visible.

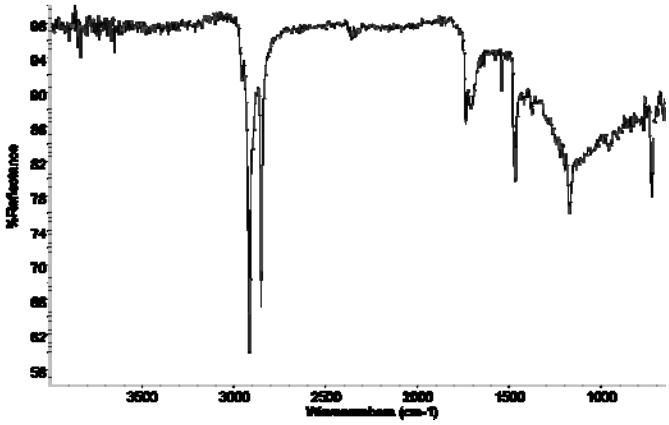


Fig. 37 Infrared spectra of CP1 sample from *Il Vasaio* sculpture. The absorption bands are typical of beeswax.

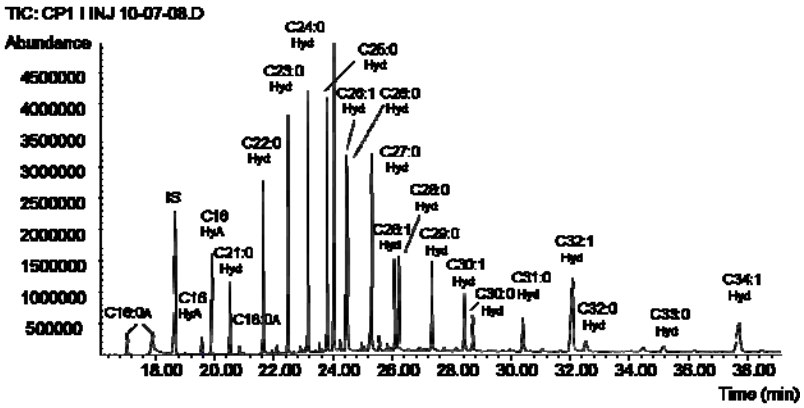


Fig. 38 Chromatogram of the sample CP1 taken off *Il ritratto del pittore Fortuny*. This sculpture is made of paraffin and lipidic material. [IS=internal standard (eicosane); A=fatty acid; Hya=hydroxyacid; Hyd=hydrocarbon; Cx:y=compound with x carbon atoms and y number of saturation]

Stereomicroscope investigation of the samples taken off *Il Vasaio* sculpture showed a very altered surface (fig.39). FT-IR spectra and GC-MS data obtained from two samples (CV1, CV2) did not show significant differences allowing to affirm that the wax consists of beeswax. FTIR spectra (fig. 40) are characterized by absorption bands of long-chain aliphatic hydrocarbons (CH_2 (a) stretching at 2916 cm^{-1}, (s) 2848 cm^{-1}, CH_3 (a) stretching at 2953 cm^{-1}, (s) 2865 cm^{-1}, CH_3 (a) bending at 1473 cm^{-1}, (s) 1376 cm^{-1}, CH_2 scissoring at 1462 cm^{-1}, CH_2 rocking at 719, 729 cm^{-1}), acid and ester groups (C=O (acid) stretching at 1710 cm^{-1}, C=O (ester) stretching at 1736 cm^{-1}, and C-O stretching (ester) at 1172 cm^{-1}). The chromatograms (fig. 41) revealed that it is a mixture of hydrocarbons (saturated and odd-numbered from C21 to C31, except C26 and C28, C27 as the main component), fatty acids (even-numbered compounds C18-C28, C24 as a

major one, and large amount of palmitic acid) and fatty alcohols (even-numbered compounds C24–C30, C24 as main compound). Hydrocarbons, probably with more than one unsaturation, oleic acid (C18:1) and compounds resulting from oxidation of fatty acids are also present.The predominance of palmitic acid and alcohols confirm this structure of esters from beeswax.

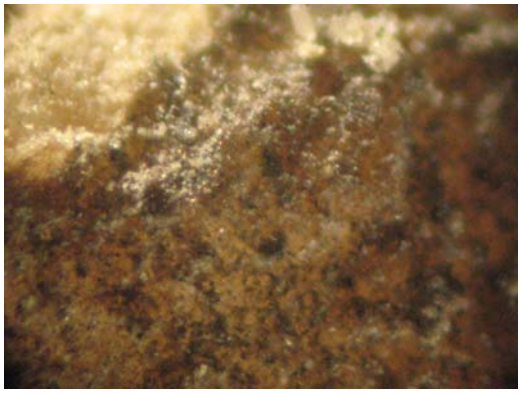


Fig. 39 Stereomicroscopical image (450x) of the surface of CV1 sample from *Il Vasaio*. The surface appears very altered.

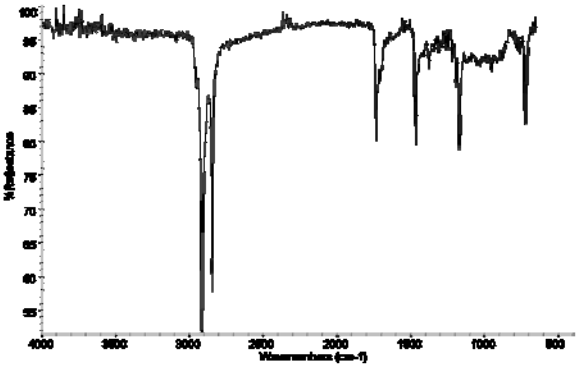


Fig. 40 Infrared spectra of CV2 sample from *Il Vasaio* sculpture. The absorption bands are typical of beeswax.

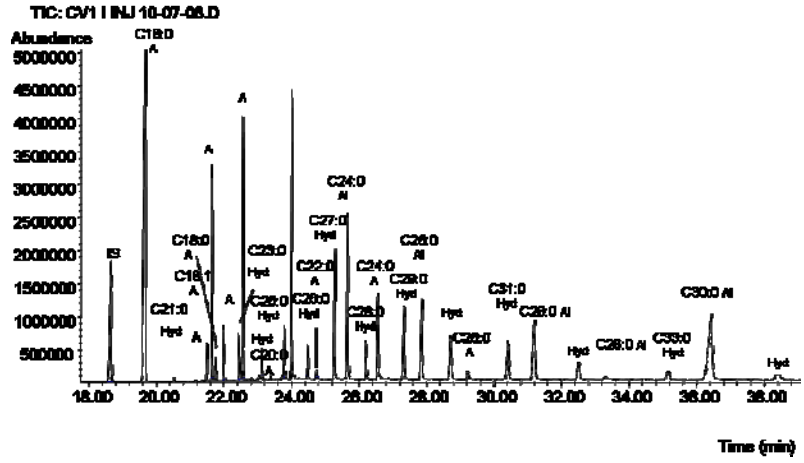


Fig. 41 Chromatogram of the sample CV1 taken off *Il vasaio*. This sculpture is made of beeswax. [IS=internal standard (eicosane); A=fatty acid;Al= fatty alcohol; Hyd=hydrocarbon; Cx:y=compound with x carbon atoms and y number of saturation]

Stereomicroscope observation of samples from *La madre dormiente* showed that the wax is clear and it seems to be composed of two overlapped layers. The surfaces appeared covered by dust material different in color and granulometry (fig.42). FT-IR spectra and GC-MS data obtained from CM1 and CM2 samples did not detected significant differences allowing to affirm that this waxartworks is made of paraffin. FTIR spectra (fig.43) present absorption bands of long-chain aliphatic hydrocarbons

with a solid and semi-crystalline structure (CH_2 (a) stretching at 2916 cm^{-1}, (s) 2848 cm^{-1}, CH_3 (a) stretching at 2955 cm^{-1}, (s) 2871 cm^{-1}, CH_3 (a) bending at 1472 cm^{-1}, (s) 1377 cm^{-1}, CH_2 scissoring at 1462 cm^{-1}, CH_2 rocking at 719, 729 cm^{-1}). The chromatograms have revealed that it is made of a typical mixture of hydrocarbons of paraffin (both even and odd-numbered *n*-alkanes from C21 to C34, C27 as the main component) (fig.44).

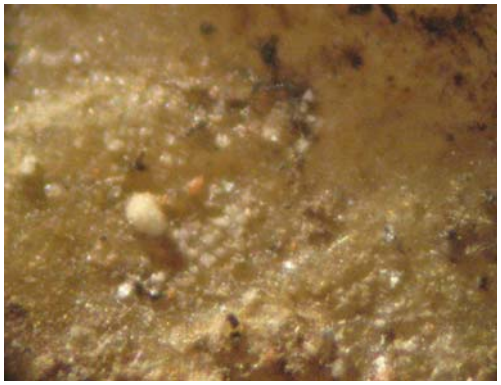


Fig. 42 Stereomicroscopical image (450x) of the surface CM1 sample from *La madre dormiente*. The surface appears very dirty.

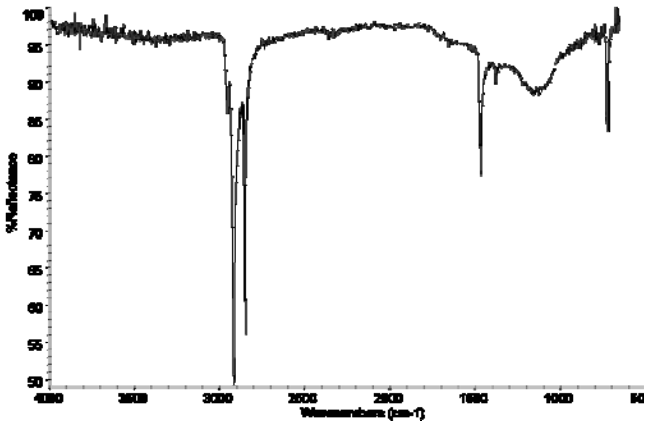
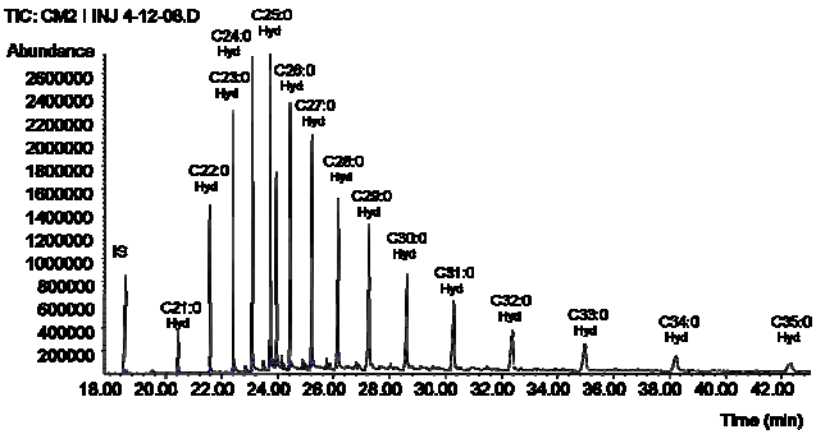


Fig. 43 Infrared spectra of CM1 sample from *La madre dormiente* sculpture. The absorption bands are typical of paraffin.

Fig. 44 Chromatogram of the sample CM2 taken off *La madre dormiente*. This sculpture is made of paraffin. [IS=internal standard (eicosane); Hyd=hydrocarbon; Cx:y=saturated compound with x carbon atoms and y number of saturation]



By observing *Il Velo* artwork, two different waxy layers did not identified rather than a very thin superficial and dark film. Stereomicroscope investigation on CVE1 and CVE2 samples showed that the surfaces are characterized by dark stains and heterogeneous areas (fig.45). FTIR spectra (fig.46) of both samples registered absorption bands of long-chain aliphatic hydrocarbons (CH_2 (a) stretching at 2916 cm^{-1}, (s) 2848 cm^{-1}, CH_3

(a) stretching at 2955 cm^{-1}, (s) 2871 cm^{-1}, CH_3 (a) bending at 1473 cm^{-1}, (s) 1378 cm^{-1}, CH_2 scissoring at 1463 cm^{-1}, CH_2 rocking at 718, 730 cm^{-1}, CH bending 1300-980 cm^{-1}) and FTIR of CVE1 sample also presents broad bands at 1750-1550 cm^{-1} and 1300-1000 cm^{-1} due to acid and ester groups .The chromatograms of both samples revealed hydrocarbon as major components (fig. 47). They are characterized by chain length of C21-C34, C26 as the main compound in the CVE1 and ranging from C21 to C33, C25 as principal component in the CVE2 sample. As it was preliminarily found by means of FTIR analyses, acid compounds were identified such as palmitic, stearic, oleic acids (C16:0, C18:0 e C18:1) and dehydroabietic acid. The latter is the main degradation products of conifer derivatives such as colophony and it is not unusual found resins in waxy mixture. Despite the slight differences in the hydrocarbons profile, it can be argued that the wax used for *Il velo* is a paraffin into which were added fats and a terpenic resin.

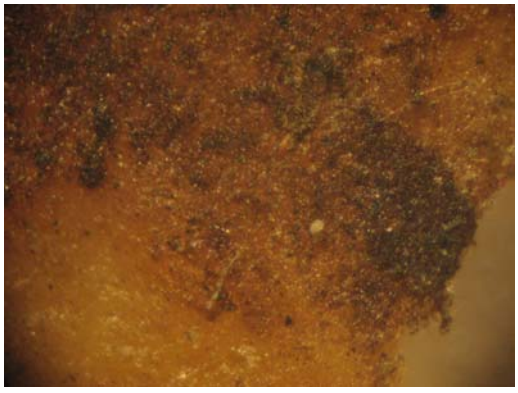


Fig. 45 Stereomicroscopical image (70x) of the surface CVE1 sample from *Il Velo*. The surface seems very heterogeneous.

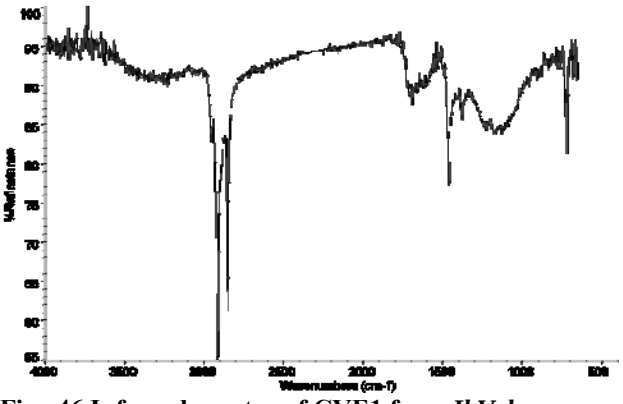


Fig. 46 Infrared spectra of CVE1 from *Il Velo* sculpture.

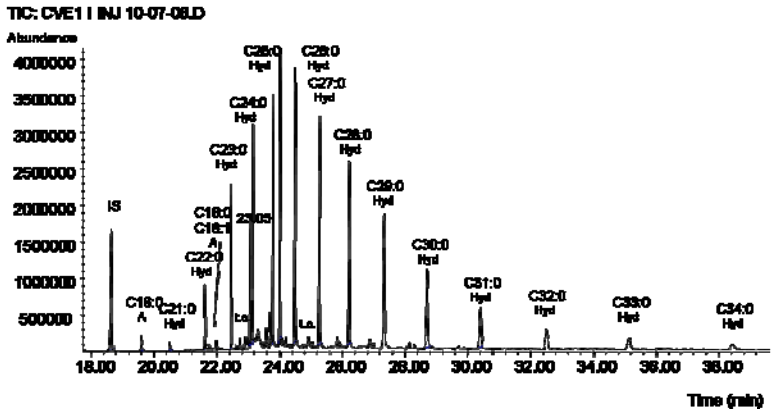


Fig. 47 Chromatogram of the sample CM2 taken off *La madre dormiente*. This sculpture is made of paraffin. [IS=internal standard (eicosane); Hyd=hydrocarbon; Cx:y=saturated compound with x carbon atoms and y number of saturation]

Tab. 13 reports the results of the GC-MS characterization of waxy-material above mentioned.

Tab. 13 Composition of the wax from reference layers and wax sculpture

	Type of wax	**Components of the upper layer**
Paraffin lab layer	Paraffin and fatty material	fatty acids, hydrocarbons
Unpurified beeswax lab layer	Paraffin and fatty material	fatty acids, hydrocarbons
Bleached beeswax lab layer	Like beeswax composition	fatty acids, hydrocarbons, alcohols
Il ritratto del pittore Fortuny Sculpture	Paraffin and fatty material	fatty acids, hydrocarbons
Il Vasaio sculpture	Beeswax	fatty acids, hydrocarbons, alcohols
La madre dormiente sculpture	Paraffin	Hydrocarbons
Il Velo sculpture	Paraffin, fatty material, terpenic resin	fatty acids, hydrocarbons, terpenic resin

4.2.2 Application, removal and efficacy of aqueous solutions

The cleaning tests were carried out on laboratory wax layers then on the surfaces of sculptures preserved at the Galleria d'Arte Moderna in Milan.

Two aqueous solutions, ideally considered the mildest and the most aggressive ones for cleaning wax, were applied, both in free and gelled form, on the homogenous and varying in composition lab layers. The first solution is the Buffer #1 (pH 6, conductivity 4,5 mS/cm) and the second one is the Buffer #4 (pH 7, conductivity 4,5 mS/cm, surfactant *Tween 20).* Cleaning tests did not show changes on the treated surfaces. All solutions proposed in this study (in addition to buffer #1 and #4, buffer#2 - pH 7, conductivity 6,4 mS/cm - , buffer #3 - pH 7, conductivity 4,5 mS/cm - , buffer #5 - pH

7, conductivity 12,4 mS/cm, chelator Citrate-) were used for cleaning test on small areas of sculpture surfaces (fig. 48-51).

Fig. 48 cleaning tests on *Il Ritratto del pittore Fortuny*

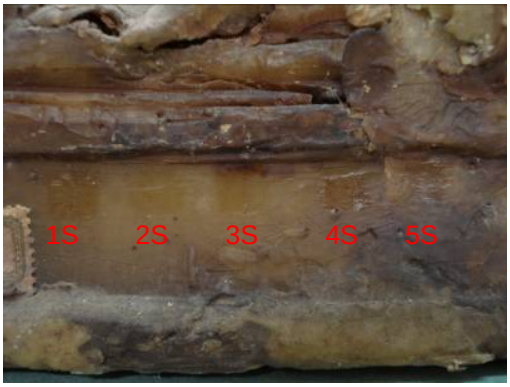


Fig. 49 cleaning tests on *Il vasaio*

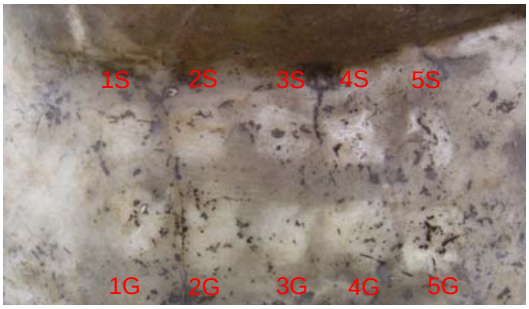


Fig. 50 cleaning tests on la Madre dormiente

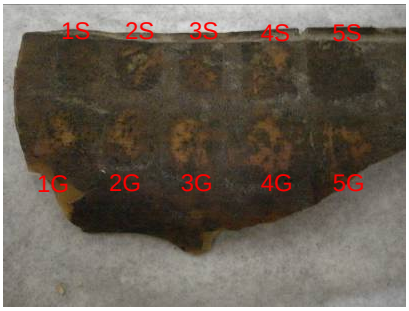


Fig. 51 cleaning tests on *Il velo*

The observation of the treated areas allowed to preliminarily consider the efficacy of the cleaning. The comparison of treated areas on Il *ritratto del Pittore Fortuny* was very difficult due to the dark colour of the wax. The cleaning performed on *Il Vasaio* seemed to be in order of efficacy from 1S to 4S solution, while 5S resulted less evident. Gelled solutions showed a sort of bleaching effect, especially solutions with surfactant and chelator, than solutions in free form on *La Madre Dormiente* sculpture. Although it is difficult to compare various tests, 4G treatment on *Il Velo* surface showed major efficacy.

4.2.3 GC-MS analyses of wax components removed during cleaning

Cleaning cotton swabs were analyzed by means of GC-MS in order to verify eventually wax components extracted and/or removed during treatment.

GC-MS data of lab layer showed that no wax components were removed during treatment suggesting that no chemical interaction occurred.

As above reported, the wax of *Il ritratto del pittore Fortuny* is composed of saturated and unsaturated hydrocarbons with a small amount of fatty materials such as palmitic, stearic and hydroxyl acids. GC-MS data showed that only fatty acids from wax were removed during cleaning with gelled solutions (Fig. 52) with less amount for the gelled solution #3 (Buffer solution at pH 7, conductivity 4,5 mS/cm, *Vanzan*) and #5 (Buffer solution at pH 7, conductivity 12,4 mS/cm, chelator Citrate, *Vanzan*). Cleaning removed only acids components from a hydrocarbon-based wax probably due to chemical interaction with a fatty material used as a protective layer.

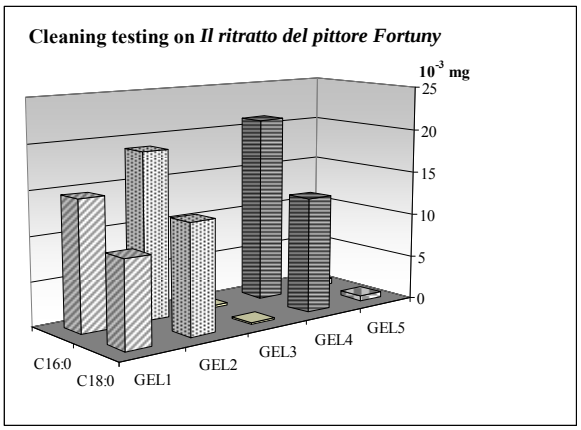


Fig. 52. Histogram of fatty acids removed from *Il ritratto del Pittore Fortuny* sculpture during cleaning testing with gelled aqueous solutions. Each value represents the amount (in mg) of analyte removed calculated by using internal standard at known concentration. [C16:0=palmitic acid; C18:0=stearic acid; GEL1= Buffer at pH 6, conductivity 4,5 mS/cm, *Vanzan*; GEL2= Buffer at pH 7, conductivity 6,4 mS/cm, *Vanzan*; GEL3= Buffer at pH 7, conductivity 4,5 mS/cm, *Vanzan*; GEL4= Buffer at pH 7, conductivity 4,5 mS/cm, surfactant *Tween 20, Vanzan*; GEL5=Buffer at pH 7, conductivity 12,4 mS/cm, chelator Citrate, *Vanzan*].

Il Vasaio sculpture is made of beeswax, a mixture of hydrocarbons, fatty acids, fatty alcohols and esters. The chromatograms showed that wax hydrocarbons such as C21, C23, C25, C26 were (fig.53) removed during cleaning probably due to a mechanical action during swabs-rolling. Among the different solutions tested, the solution with

surfactant seemed to be the more aggressive, followed by the solution with the chelating agent.

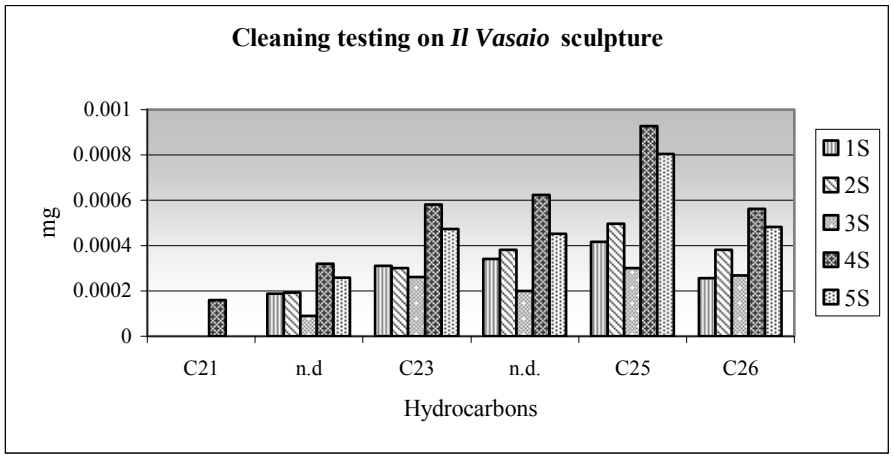


Fig. 53 Histogram of components removed by *Il Vasaio* sculpture during cleaning testing with aqueous solutions. Each value represents the amount (in mg) of analyte removed calculated by using internal standard at known concentration. [Cx=*n*-alkenes with x carbon atoms; n.d=hydrocarbons not identified; 1S= Buffer at pH 6, conductivity 4,5 mS/cm; 2S= Buffer at pH 7, conductivity 6,4 mS/cm; 3S= Buffer at pH 7, conductivity 4,5 mS/cm; 4S= Buffer at pH 7, conductivity 4,5 mS/cm, surfactant *Tween 20*; 5S=Buffer at pH 7, conductivity 12,4 mS/cm, chelator Citrate].

La madre dormiente wax is exclusively made of hydrocarbons and the surface appeared very deteriorated. The more aggressive solution seemed to be the 5S with chelator, while 1S the mildest (fig.54). It is not clearly the behavior of the gels, but in general they showed greater interaction with the wax than solutions in free from. The presence of only hydrocarbons on the cleaning swabs may simply be due to the mechanical action of rolling. Unlike solutions, gels remained in contact with the surface for several minutes, and more action was needed to remove them. This may explain the greater amount of hydrocarbons found in gel cleaning swabs.

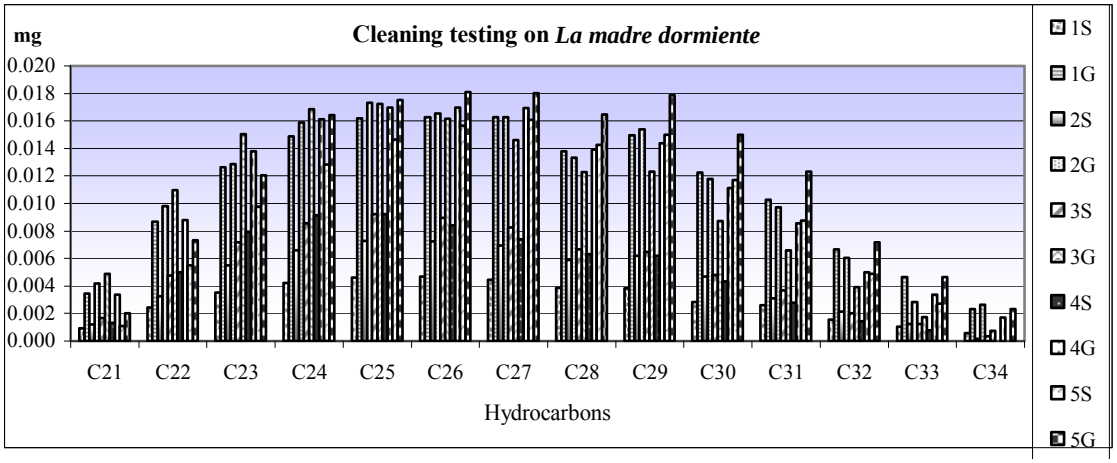


Fig. 54 Histogram of components removed by *La madre dormiente* sculpture during cleaning testing with aqueous solutions. Each value represents the amount (in mg) of analyte removed calculated by using internal standard at known concentration. [Cx=*n*-alkenes with x carbon atoms; 1S= Buffer at pH 6, conductivity 4,5 mS/cm; 2S= Buffer at pH 7, conductivity 6,4 mS/cm; 3S= Buffer at pH 7, conductivity 4,5 mS/cm; 4S= Buffer at pH 7, conductivity 4,5 mS/cm, surfactant *Tween 20*; 5S=Buffer at pH 7, conductivity 12,4 mS/cm, chelator Citrate; 1G-5G=the same solutions above mentioned in gelled form].

The surface layer of the sculpture *Il velo* is made of a mixture hydrocarbons, fatty material and resin terpenic. These compounds were found in all cleaning swabs (Fig.55). With regard to fatty acids, the more aggressive solution was 4S with the surfactant, followed by relative gel (4G). Hydrocarbons were more removed from the gel applications especially the solution 4G with surfactant and 5G with chelator. The removal of hydrocarbons was due to a mechanical action, while the removal of acids may be due to a chemical interaction. Instead, the compounds related to terpenic resin were only found in the solution at pH7 and conductivity 6.4 mS / cm in gelled form (3G).

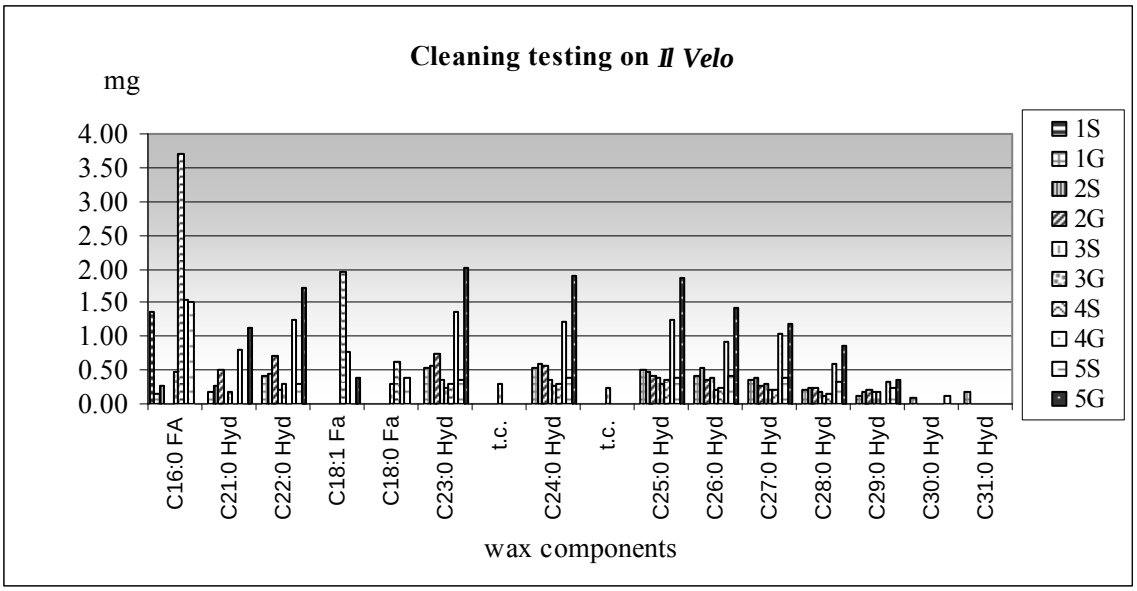


Fig. 55 Histogram of components removed by *Il Velo* sculpture during cleaning testing with aqueous solutions. Each value represents the amount (in mg) of analyte removed calculated by using internal standard at known concentration. [Cx:y=compound with x carbon atoms and y number of saturation; Fa=fatty acid; Hyd=hydrocarbon; t.c.=terpenic compounds; 1S= Buffer at pH 6, conductivity 4,5 mS/cm; 2S= Buffer at pH 7, conductivity 6,4 mS/cm; 3S= Buffer at pH 7, conductivity 4,5 mS/cm; 4S= Buffer at pH 7, conductivity 4,5 mS/cm, surfactant *Tween 20*; 5S=Buffer at pH 7, conductivity 12,4 mS/cm, chelator Citrate; 1G-5G=the same solutions above mentioned in gelled form].

Tab. 14 reports the results of cleaning swabs analyses obtained.

Tab. 14 List of components present in the upper layer and removed during cleaning

	Components of the upper layer	**Components removed**	**Observations**
Paraffin lab layer	Hyd, FA	-	
Unpurified beeswax lab layer	Hyd FA	-	
Bleached beeswax lab layer	Hyd, FAs,Al	-	
Il ritratto del pittore fortuny sculpture	FAs, Hyd	FAs	gel 4G is the most aggressive while the activities of gels 3G and 5G are very low
Il vasaio sculpture	FAs, Hyd, Al	Hyd	solution 4s is the most aggressive followed by 5s
La madre dormiente sculpture	Hyd	Hyd	1s is mildest aqueous solution, and 5s the most aggressive; the gels show greater interaction in comparison with the free-solutions
Il velo sculpture	FAs, Hyd, terpenic components	FAs, Hyd, terpenic resin	solution 4s is the most aggressive toward fatty acids, while the hydrocarbons are sensitive to gels, in particular 4g and 5g

4.3 Conclusions

The GC-MS data obtained from the analysis of the cleaning cotton swabs on the laboratory-cast wax layers have shown that no wax components were removed during treatment, thus proving that this type of cleaning is chemically safe.

This finding confirms that using aqueous solutions at pH lower than the pK_A value of the fatty acids present in the wax (or about pH=7), ionization of the fatty acids is minimized, and by retaining mainly hydrophobic character they are practically insoluble in water. In addiction, the aqueous solution that was potentially more aggressive (#4, pH 7, conductivity 4.5 mS/cm, with the surfactant) was found to free from chemical risk.

The GC-MS data obtained from the cleaning cotton swabs used on the actual wax sculptures, have shown that aqueous solutions interacted with wax surfaces removing both hydrocarbons and fatty acids, and to a higher extent so for gel applications.

Under a stereomicroscope, the artworks surfaces before treatment appeared altered, desegregated and covered by crystallized whitish material, due to the well known alteration process of migration of alkanes and fatty acids towards the wax-based surface.

This finding has suggested that, in our tests, wax components were removed by mechanical rather than chemical (ionization/solubilization) action, and this is also confirmed observing the perfectly compact surface of the laboratory layers.

The relatively more aggressive behavior of gels, as compared to free solutions, can be explained as a result of greater mechanical interaction due to longer time of application

and of the presence of the thickener Xanthan gum, which may have some emulsifying action on wax components.

The chemical properties of the constituent acidic materials do not represent the only variable that rules the selectivity of the cleaning for this kind of artworks: the morphology of the surface plays also an important role, and it can represent an intrinsic risk.

In this context, it is difficult to foresee a different mode for applying aqueous solutions, that could provide no mechanical action, yielding a completely “safe” cleaning.

Observing (under adequate magnification) the wax surfaces prior of any intervention, appears to be a necessary step, in order to making any decision about the feasibility of the cleaning treatment.

On an aged wax surface, some degree of interaction inevitably has to be expected, once the decision to clean has been taken.

5. Egg tempera

5.1 Experimental

5.1.1 Materials

All solvents were of HPLC grade and all other chemicals were of analytical grade. The following commercial standards were used: fatty acids (containing CC9, C14, C16:1, C16:0, C17, C18:2, C18:1, C18:0) from Merck, amino acids (containing Ala, Gly, Treo, Ser, Val, Nval, Leu, Isoleu, Nleu, Pro, Hpro, Asp, Glu, Phe) from Sigma-Aldrich. Animal glue, gypsum, wooden panels, egg, cherry gum, fig latex, vermilion, minium were purchased from local market.

5.1.2 Tempera layers preparation

Following Cennino Cennini's description in his treatise [54] a priming layer of four coats of animal glue and gypsum was applied onto several wooden panels. Binding medium made of egg yolk-cherry gum-fig latex in a 10:10:1 ratio by volume (T) was then applied to rectangular sections of one panel. On another panel, layers of pigmented binding media of the same composition were prepared, one with vermilion (T-V) and one with minium (T-M). Two grams of each pigment were mixed with 2 ml of binding medium. The same layers (T, T-V, T-M) were also applied onto glass slides for microscope observation. Prepared in July 2007, the panels were left to dry for about four months, prior to the first solvent treatment. An unpigmented egg yolk layer (fig. 56) was prepared by researchers of the Smithsonian Museum Conservation Institute in August 1995. It is made up of thick film with high proportion of egg yolk brushed onto a stiff polyester film (Melinex/Mylar).

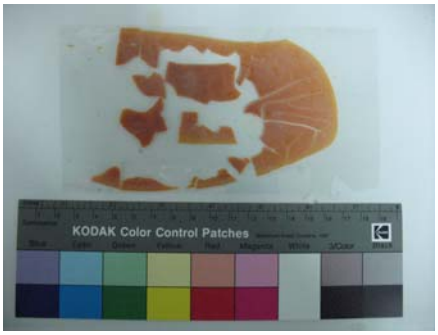


Fig. 56 egg yolk layer prepared by researchers of the Smithsonian Museum Conservation Institute in August 1995. It is made up of thick film with high proportion of egg yolk brushed onto a stiff polyester film (Melinex/Mylar).

5.1.3 Easel painting on wood

The painting "Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo" [Jesus at the foot of the cross, between Saint Peter, the Archangel Michael, and an angel with the small Tobia, and Saint Paul] (fig. 57) is attributed to an unknown artist, a follower of the painter Riccio, sometimes defined as "Monache di Santa Marta" [Sisters of Saint Martha] because who had painted was perhaps not a single artist but a workshop, maybe the same sisters of the Saint Martha convent. It dates to around the middle of the 16th century. It is a small painting on wood (21.5 x 79.5 cm), probably part of an altar step, conserved at Pinacoteca Nazionale in Siena. The painted parts were all on gold: the board was gilded and then painted. This artwork is presumably made of egg tempera and it had not been varnished or restored.

Fig. 57 The 16th century painting "Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo" preserved at the Pinacoteca Nazionale in Siena

5.1.4 Binding medium identification

Before solvents treatment, samples from painting and from Smithsonian layer were characterized with the aim to confirm their yolk egg nature.

Two paint micro-samples (Siena1, Siena2) (fig. 58) were collected from the painting and then analyzed by μFTIR-ATR (Attenuated Total Reflection), to obtain information on the class of organic and inorganic substances, and by GC-MS, in order to identified the lipid and protein fraction of the organic binder.

Fig. 58 points of Siena1 and Siena2 sampling from "Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo" painting

For analyses of lipids and proteins, the samples were placed in schlenk tubes and internal standards were added: 10 μl of a 1000 ppm heptadecanoic acid in hexane solution and 10 μl of a 100 ppm norvaline and of a 1000 ppm norleucine in water solution. Thus, 1 ml of 4N hydrochloric methanol and 1 ml of hexane were added and the mixture was kept at 50°C for 2h under magnetic stirring. The hexane phase containing methyl esters of fatty acids was separated and transferred into vials. It was concentrated to dryness and afterwards dissolved in 150 μl of hexane. 1 μl of the extract solution was injected in the gas chromatograph for fatty acids analysis.

The residue of the methanol phase was dissolved in 2 ml of 6N hydrochloric acid and heated up to 100°C, this temperature was held for 5h with a magnetic stirrer. After drying 3 ml 2N hydrochloric isopropyl alcohol were added, this solution was warmed to 90°C for 1h. After evaporation of the solvent the residue was dissolved in 2 ml dichloromethane and 0,2 ml trifluoroacetic anhydride and it was left to react for 1h at 60°C (magnetically stirred). After cooling, evaporation of the solvent took place once more. Then the residue was rinsed with dichloromethane, concentrated and then re-dissolved in 150 μl of dichloromethane. 1 μl of the obtained solution was injected in the

gaschromatograph. This derivation procedure had transformed the amino acids into their N-trifluoroacetyl-O-2-propyl esters [65].

Three runs were performed for each sample and the average of chromatographic peak areas were calculated and then corrected by a response factor. Fatty acids and amino acids detected were expressed in relation to the total amount of lipid and proteins, respectively, in order to obtain semi-quantitative information. To identify the lipids qualitative analysis and relative amount of fatty acids were considered. To identify the proteins, the relative amino acid contents of the samples, together with a set of reference samples (the other proteinaceous material that may be present in a ancient painting)[67] were subjected to multivariate data analysis according to the PCA method. Multivariate statistical analysis PCA, was performed using SPSS 16.0 and the variables taken into consideration were the relative percentage contents of the 8 amino acids (Ala, Gly, Leu, Pro, Hpro, Asp, Glu, Phe) [68].

Two micro-samples were collected from Smithsonian layer and then analyzed by GC-MS, in order to confirm its egg composition for fatty acids, amino acids (sample 1= 0.76 mg) and cholesterol (sample 2= 0.45 mg) fractions. For analyses of lipids and proteins the procedure are the same above mentioned. For cholesterol analysis, sample 2 were placed in a test tube and dissolved in 4 ml of hexane, adding 50 µl IS (100 ppm stigmasterol in hexane) and 2 ml of methanolic potash (5% w:v). After mixing, the hexane phase was drained into a silica column and then discarded. Ethyl acetate (4 ml) was poured over the column thus containing the sterol fraction. After evaporation to dryness 0,6 ml of Hexamethyldisilazane and 0,3 ml of Trimethylchlorosilane were added to the residue. The sample thus obtained was injected (1 µl) for chromatographic analysis. This derivation procedure transforms cholesterol into trimethylsilylated cholesterol [69].

5.1.5 Solvents treatment

Ethanol, isooctane and distilled water were applied to the egg tempera layers by lightly rolling cotton swabs (previously rinsed in hexane, ethanol and water and vacuum dried). Three drops of solvent were added to each swab and than rolled on the tempera for 30 seconds on a defined area (1.5 cm x 2 cm) on supported layers and for 15 seconds on unsupported layer on 1 cm square areas (fig. 59). Applications by using dry cotton-

swabs were also performed. All the applications were performed in triplicate tests. Afterwards, the cotton swabs were transferred into vials and stored at 4°C. Cleaning testing on the painting was carried out by swab-rolling acetone, isooctane and distilled water on the same conditions and in close areas.

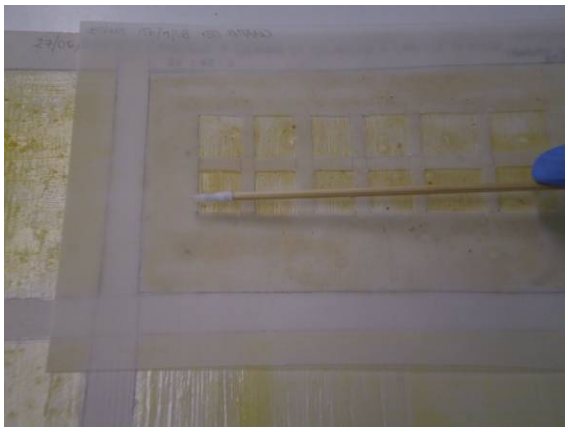


Fig. 59 cleaning testing on unpigmented egg tempera layer (T)

5.1.6 Stereomicroscopic investigation

Before and after solvent application, the layers were observed under stereomicroscope in order to monitor their morphology.

5.1.7 Colorimetric analysis by MSS

Before and after layers treatment, spectral reflectance factors were measured on fixed areas and CIE L*a*b* values were calculated for obtain color specification of the surfaces.

5.1.8 GC-MS analyses of lipids, proteins and cholesterol fractions extracted

Each cleaning cotton swab (with and without solvents, performed in triplicate tests) was analysed for fatty acids, amino acids and cholesterol. All procedures for analysis were repeated at least twice. The chromatographic peak area of each analyte was integrated, corrected by a response factor and expressed in relation to the internal standard (IS), in order to obtain quantitative information. Finally, the average analyte/IS ratio was calculated and the average percentage of three runs was made. For cholesterol analysis, the cotton swabs used for cleaning were placed in a test tube to be extracted by hexane, and 1 ml of IS (10 ppm stigmasterol in hexane) was added. As above reported, through

a derivatization procedure the cholesterol was turned into trimethylsilylated cholesterol. For analyses of lipids and proteins the cotton swabs were extracted for 30 minutes with 2 ml of the same solvent used for cleaning, under magnetic stirring in warm condition (about 50 ºC). Then the cotton was removed and the solvent evaporated under vacuum conditions. The following internal standards were added to the residue: 10 µl of a 1000 ppm heptadecanoic acid in hexane solution and 10 µl of a 1000 ppm norleucine in water solution. Thus, the samples were derivatized with the same procedure above mentioned.

5.1.9 Instrumentations

Observation were performed with stereomicroscope from Optika. The spectrophotometric scanner (spectral region: 380–800 nm) used was developed by researchers of University of Parma [66]. Lighting and observation conditions were 45/0º with a halogen lamp and D65 illuminating agent and CIE 1931 observer were considered. FTIR microspectroscopic measurements were performed using a Nicolet Continuµm Microscope connected to the Nexus spectrometer, equipped with a Mercury Cadmium Telluride (MCT) detector cooled by liquid nitrogen and a crystal of silicon. Infrared spectra were acquired from 4000 to 650cm^{-1}, resolution of 4 cm^{-1} and 120 scans. A gaschromatograph 6890N GC (Agilent Technologies), coupled to a MSD (Mass Selective Detector) detector 5973 (Agilent Technologies) with single quadrupole and split-splitless injector, was used for analysis. The mass spectrometer was operated in the EI positive mode (70 eV). The transport gas was Helium. Separation of components was done by means of a fused-silica DB-5 capillary column (Agilent J&W – USA) with a 0.25µm (30 m x 0.25 mm i.d.) methyl-silicone (5% phenyl) film and the injector was used in splitless mode. This GC-MS was used for painting and leaching study of supported layers analyses. For analysing Smithsonian layer, a Focus GC (Thermo Scientific) coupled to DSQ II (Thermo Scientific) with single quadrupole and split-splitless injector was used. The mass spectrometer was operated in the EI positive mode (70 eV). The transport gas was Helium. Separation of components was done by means of a fused-silica capillary column (RXI-5, Restek) with a 0.25µm (30 m x 0.25 mm x 0.25 µm) methyl-silicone (5% phenyl) film and the injector was used in splitless mode. This GC-MS was used for painting and leaching study of supported layers analyses. Separation of trimethylsilylated cholesterol was achieved following this

temperature program: isothermal conditions at 240°C for 3 min, with 20°C/min heating up to 280°C and isothermal conditions at 280°C for 15 min (total run time 20.00 min). The mass spectra were recorded in Selected Ion Monitoring (SIM; 458, 368, 484, 394 m/z fragments). Separation of the methyl ester of fatty acids was achieved following this temperature program: isothermal conditions at 80°C for 2 min, with 20°C/min heating up to 270°C and isothermal conditions at 270°C for 6 min (total run time 17.50 min). The mass spectra were collected in Total Ion Current (TIC; 40-500 m/z fragmentation rate). Separation of N-trifluoroacetyl-O-2-propyl esters amino acid derivatives was achieved following this temperature program: isothermal conditions at 60°C for 3 min, with 25°C/min heating up to 260°C and isothermal conditions at 260°C for 6 min (total run time 17.00 min). The mass spectra were recorded in Selected Ion Monitoring (SIM; 140, 126, 154, 153, 139, 168, 182, 166, 164, 184, 180, 198, 91, 190 m/z fragments).

5.2 Results and Discussion

5.2.1 Binding medium identification

Before solvents treatment, samples from painting "Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo" and from Smithsonian Museum Conservation Institute were characterized with the aim to confirm their yolk egg nature.

FTIR spectra collected from Siena1 (fig. 60) showed that gypsum (S-O stretching 1108 cm^{-1}, OH stretching at 3524, 3339, 1683 and 1614 cm^{-1}, fingerprint at 667 cm^{-1}) belongs to inorganic component of the ground layer and calcium oxalate (C=O and C-O stretching at 1645 and 1323 cm^{-1}), Calcite (CO_3^{2-} stretching at 1413, O-C-O bending at 874 cm^{-1}), silicate (Si-O-Si stretching at 1031 cm^{-1}) and organic compounds (C-H stretching at 2928 cm^{-1}) are present in the painting layer. In addiction, FTIR spectra recorded on Siena2 sample (fig.61) showed absorption bands of proteins (Amide I (C-O) stretching 1637, Amide II (N-H) bending 1541 cm^{-1}) and Kaolin (O-H stretching at 3688, 3619 cm^{-1}, Si-O-Si stretching at 1001 cm^{-1}, Si-O stretching at 912 cm^{-1}).

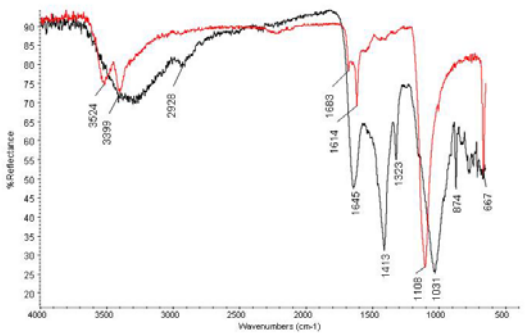


Fig. 60 ATR spectra of red surface (black line) and white ground (red line) of sample Siena1.

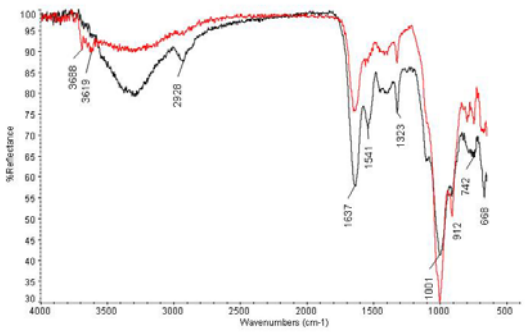


Fig. 61 ATR spectra of red sample named Siena2.

GC-MS analyses of the fatty materials showed palmitic acid and stearic acid in both samples. For example, the chromatogram of the sample Siena1 is reported in figure 62. The absence of azelaic acid may exclude the use of drying oil as a binding medium. The analysis of protein fraction showed amino acids in both samples (fig.63).

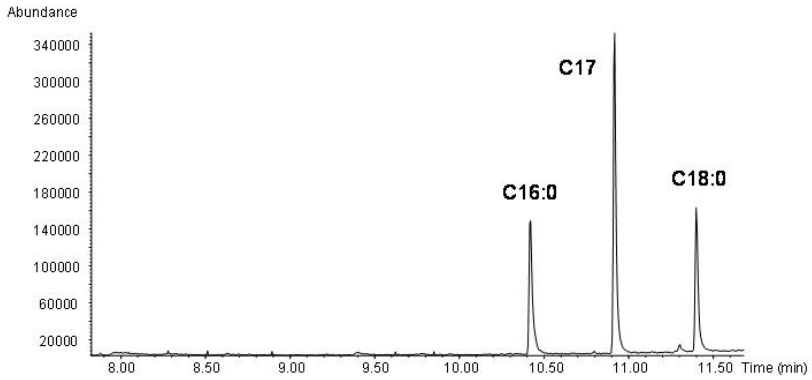


Fig. 62 Chromatogram of fatty fraction of Siena1 sample. [C16:0=palmitic acid, C17=heptadecanoic acid (internal standard), C18:0=stearic acid].

To identify the proteinaceous binding medium, the amino acid percentage content of the samples was subjected to multivariate data analysis according to the PCA method. The resulting score plot (fig.64) highlighted that Siena samples probably consist of a mixture of egg and animal glue proteins. This finding is confirmed with the results from fatty fraction, characteristic of an aged egg yolk and with the presence of the specific marker hydroxyproline for animal glues.

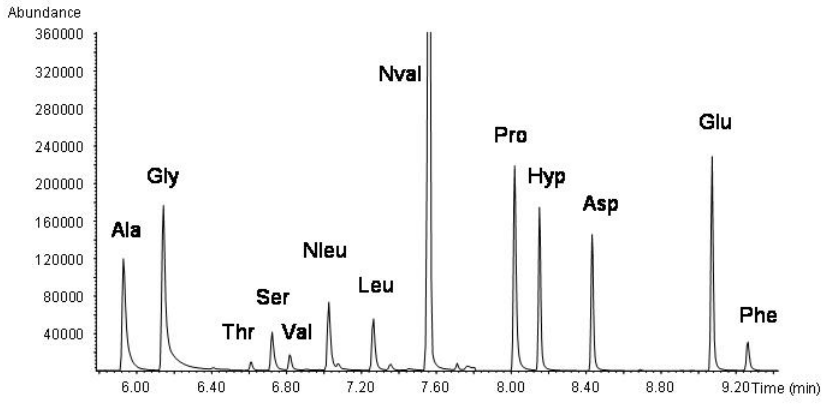


Fig. 63 GC-MS profile of the Siena2 sample. [Ala=alanine, Gly=glycine, Thr=threonine, Ser= serine, Val=valine, Nleu=Norleucine (internal standard), Leu=leucine, NVal= norvaline (internal standard), Pro=proline, Hyp=hydroxyproline , Asp= aspartic acid, Glu=glutamic acid, Phe=phenylalanine].

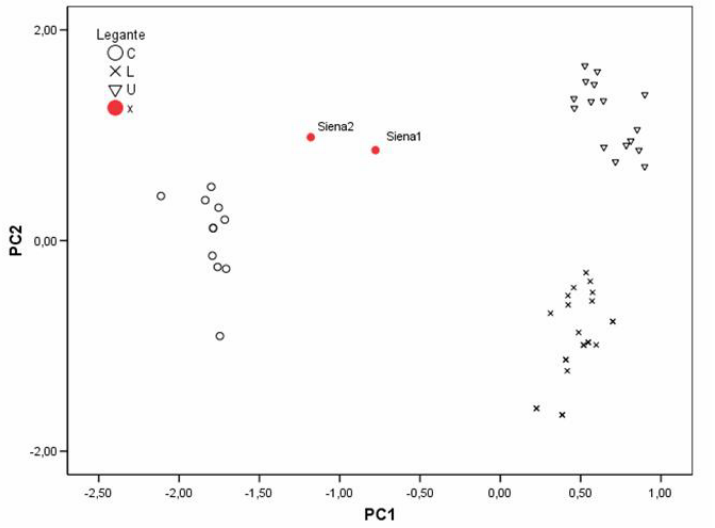
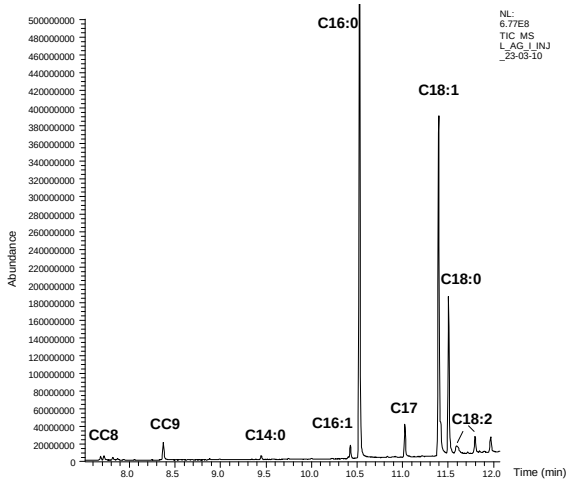


Fig. 64 PCA Score plot of the reference samples previously analysed in this laboratory and belonging to the paint reference collection of the Opificio delle Pietre Dure, Florence and the samples Siena1 and Siena2.

The binding medium identified allowed to indicate that the "Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo" is made of egg yolk and the priming layer presumably contains a mixture of gypsum and animal glue, suggesting the use of tempera painting. This painting showed a late use of egg painting technique, because in the second half of the sixteenth century oil painting had already established.

The GC-MS data from Smithsonian Museum Conservation Institute sample confirmed its egg yolk composition. In particular, the chromatograms of the fatty fraction (Fig. 65) showed palmitic acid, oleic acid, stearic acid, azelaic acid, myristic acid, suberic acid, palmitoleic acid and two isomers of linoleic acid in quite agreement with typical proportion found in a fresh yolk. Compared to literature data the composition differs only by a larger amount of palmitic acid than oleic acid, but the decrease of oleic acid may be due to ageing processes (confirmed by the presence of dicarboxylic acids) in a layer of 15 years old. Fatty fraction showed also (fig. 66) cholesterol compound. The analysis of protein content showed the following amino acids: alanine, glycine, threonine, serine, valine, leucine, proline, aspartic acid, glutamic acid, phenylalanine (fig. 67). To identify the proteinaceous binding medium, the relative

contents of 8 amino acids [alanine (7,98), glycine (6,67), leucine (9,56), proline (8,07), hydroxyproline (0,00), aspartic acid (21,86), glutamic acid (39,13), phenylalanine (6,73)], together with a set of reference samples were subjected to multivariate data analysis according to the PCA method resulting that sample1 is made of egg proteins (fig. 68).



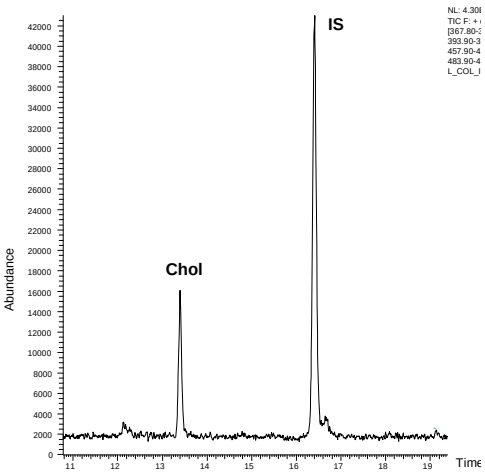


Fig. 65 and 66 Chromatograms fatty fraction of samples from Smithsonian layer [CC8=suberic acid; CC9=azelaic acid;C14:0=myristic acid; C16:1= palmitoleic acid; C16:0= palmitic acid; C17=heptadecanoic acid (internal standard); C18:1= oleic acid; C18:0= stearic acid;C18:2=isomers of linoleic acid; Chol=cholesterol; IS=stigmasterol, (internal standard)]Considering the main fatty acids the follow is their relative percentage C14:0 (0.4%) C16:1 (1.2%), C16:0 (47.8%), C18:1 (32.8%), C18:0 (14.3%), C18:2 (1.6%), C18:2 (1.9%)

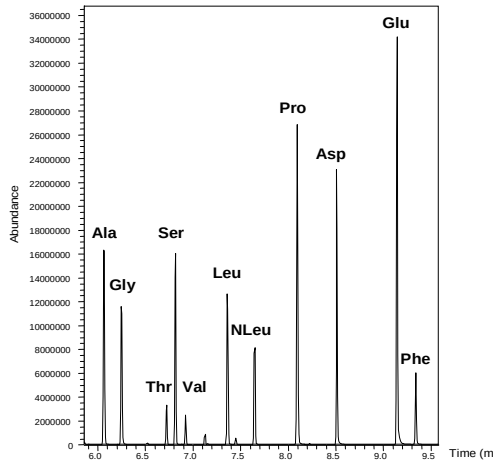


Fig. 67 chromatogram of proteinaceous fraction of sample1. [Ala=alanine, Gly=glycine, Thr=threonine, Ser= serine, Val=valine, Leu=leucine, Nleu=Norleucine (internal standard), Pro=proline, Asp= aspartic acid, Glu=glutamic acid, Phe=phenylalanine].

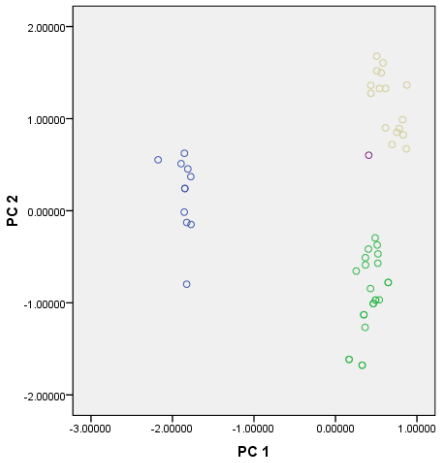


Fig. 68 PCA Score plot of the reference samples belonging to the paint reference collection of the Opificio delle Pietre Dure, Florence and the samples1 from Smithsonian layer. The blue, yellow, green clusters represent samples made of animal glue, egg and milk proteins, respectively.

5.2.2 Stereomicroscopic investigation

Observation of the laboratory samples layers surfaces (T, T-V, T-M) by means of stereomicroscope before and after the treatment with ethanol, isooctane, and water did not show significant differences. No mechanical abrasion of the surfaces of the egg tempera films was noticed (Fig.69, 70).

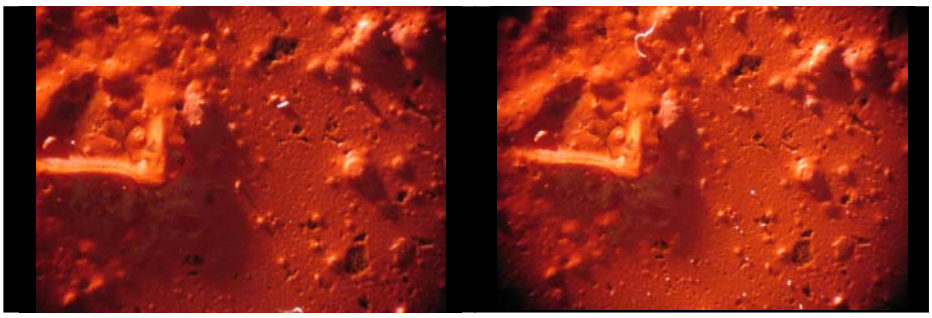


Fig. 69 Stereomicrosopical images obtained in raking light of the cast layers: binding medium-Vermilion before (left) and after (right) ethanol treatment

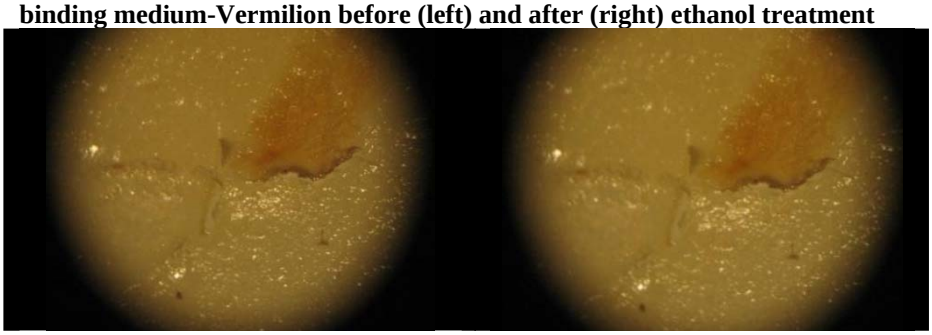


Fig. 70 Stereomicrosopical images obtained in direct light of the cast layers: binding medium before (left) and after (right) water treatment

The observation of Smithsonian Museum Conservation Institute sample surfaces showed that the surface originally appeared greasy, but after solvent treatments, it lost this glossy appearance (fig. 71), especially after application of isooctane. The dry treatment (by swab-rolling without solvent) isooctane also induced a slight decrease in gloss.

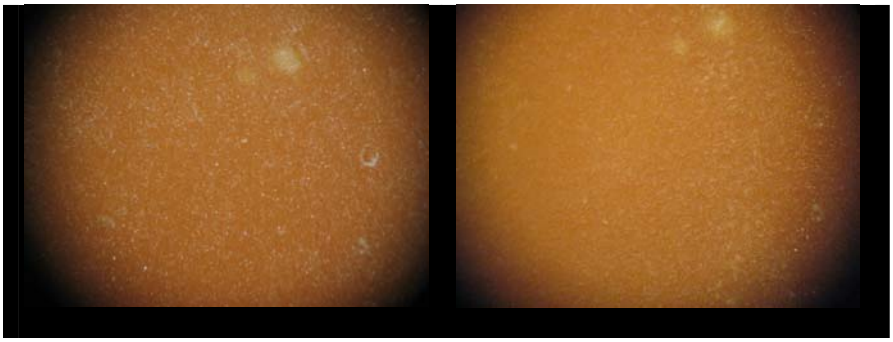


Fig. 71 Stereomicrosopical details of the Smithsonian Museum Conservation Institute sample before (left) and after (right) isooctane treatment

5.2.3 Colorimetric analysis by MSS

The spectral reflectance factor and the coordinate values (CIE L*a*b*) were measured before and after the treatments. Data collected (fig. 72, 73, 74) from laboratory samples layers surfaces (T, T-V, T-M) showed that for the binding medium layer (T), organic solvent treatments caused a very slight increase of the spectral reflectance factor, probably due to an insignificant thinning of the layer or a slight brightening Instead, the application of water to the layer T showed a very slight decrease of the spectral reflectance factor. One explanation could be that the surface may have darkened because of the dust collecting in time. It is interesting to notice that the two pigmented layers showed the same behaviour after ethanol, isooctane and water treatments. It was noted that in the 600-700 nm interval the spectral reflectance factor decreased after treatment: this might be due to slight darkening of the layer caused by solvent action. Nevertheless all these measured effects were not visually perceptible.

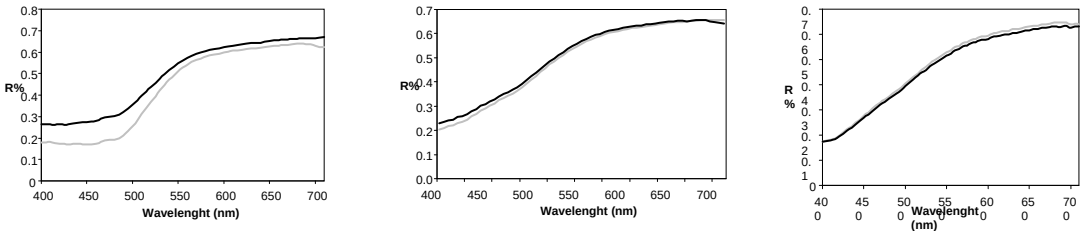


Fig. 72 Spectral reflectance factor of T layer before (grey line) and, starting from the left to right, after ethanol, isooctane and water treatment (black line)

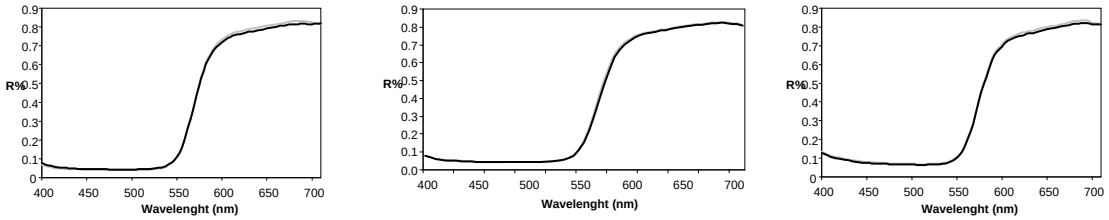


Fig. 73 Spectral reflectance factor of T-M layer before (grey line) and, starting from the left to right, after ethanol, isooctane and water treatment (black line)

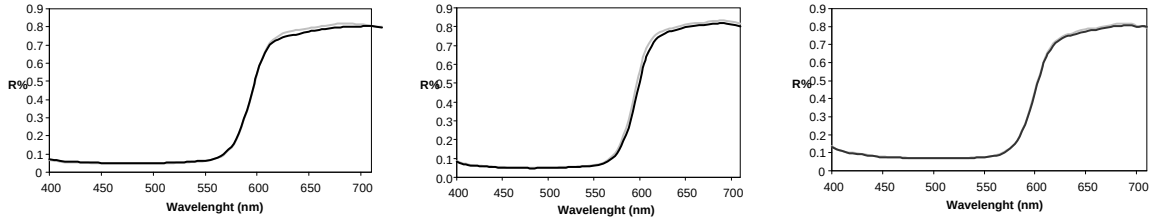


Fig. 74 Spectral reflectance factor of T-V layer before (grey line) and, starting from the left to right, after ethanol, isooctane and water treatment (black line)

The colorimetric analyses on Smithsonian Museum Conservation Institute sample did not measure significant changes due to solvents treatments (fig. 75) (for water application ΔE =1.61, isooctane ΔE=2.70) except a small variation for the case of ethanol applications where colour changed in a visually perceptible way (ΔE= 3.23).

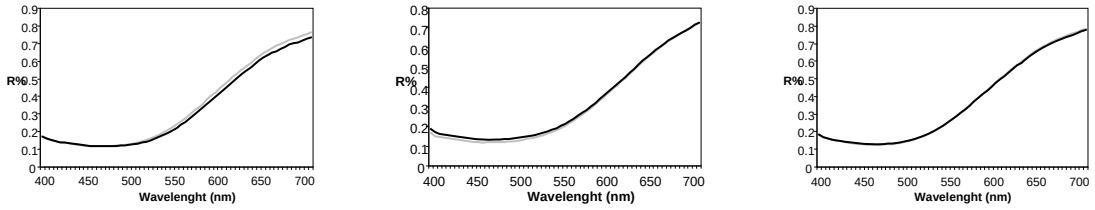


Fig. 75 Spectral reflectance factor of Smithsonian layer before (grey line) and after (black line) (starting from the right) ethanol, isooctane and water treatment

5.2.3 GC-MS analyses of lipids, proteins and cholesterol fractions extracted

All the cotton swabs, applied to the layers with and without solvents, were analysed by means of GC-MS.

The chromatographic data of laboratory samples layers surfaces (T, T-V, T-M) showed that fatty acids, amino acids and cholesterol are present in all the cotton swabs analysed. It was observed that the mechanical action was able to remove material. It was noticed that only isooctane applications caused a higher degree of extraction compared to that without solvents. Regarding fatty acids analysis, palmitic acid, stearic acid and unsaturated oleic acid (C18:1) was identified. Fig. 76 shows the trend of fatty acids extracted by isooctane applied on all the layers. The amount of oleic acid extracted from the T layer was about 100 micrograms, calculated by means of the internal standard.

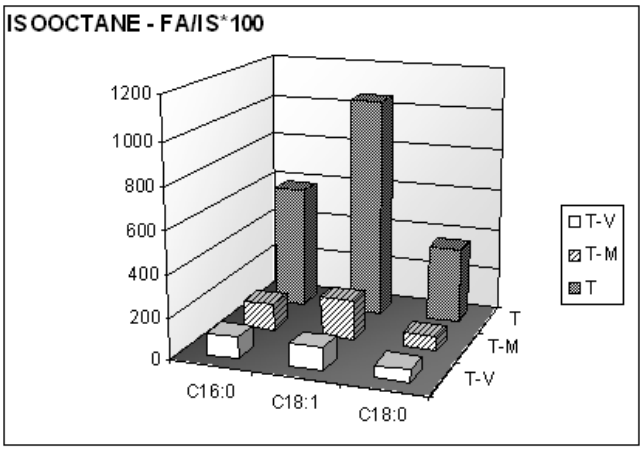


Fig. 76 Histogram of fatty acids extracted by isooctane on the pigmented (T-V and T-M) and the unpigmented tempera films (T). Each value represents the average percentage ratio analyte/internal standard (IS). Where: C16:0 is palmitic acid; C18:1 oleic acid, and C18:0 stearic acid.

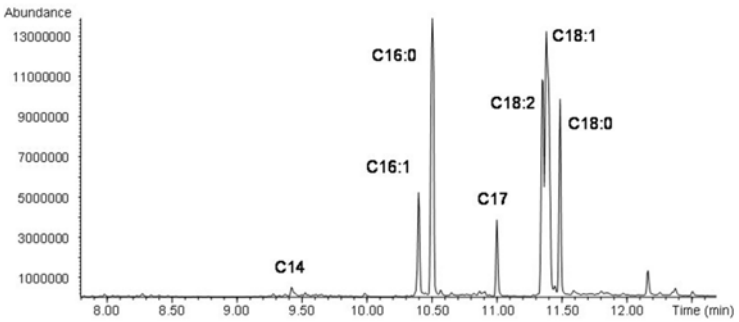


Fig. 77 Chromatogram of the fytty acids extracted by isooctane from the binding medium layer [myristic acid (C14), palmitoleic acid (C16:1), palmitic acid (C16:0), eptadecanoic acid (C17), linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0)].

The behaviour of the three solvents related to cholesterol was then considered. The swabs without solvent extracted cholesterol at trace level, in similar amounts to the application of water and ethanol. Isooctane was the solvent that most removed cholesterol (Fig. 78), some 50 micrograms of this compound were detected in the swabs

from the binding medium alone, while a small amount was observed in the pigmented layers (ratio about 10:1).

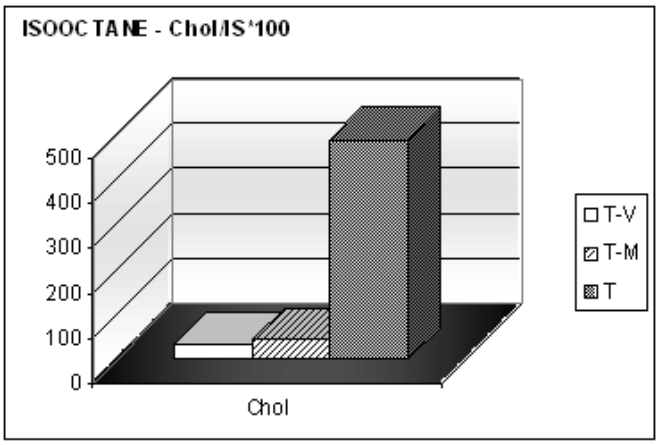


Fig. 78 Histogram of cholesterol extracted by isooctane on the pigmented (T-V and T-M) and the unpigmented tempera films (T). Each value represents the average percentage ratio analyte/internal standard (IS).

The chromatographic data showed that the amino acids from proteinaceous material were only extracted by swabs with water. The histogram of Fig. 79 reports the results of the proteinaceous fraction for the water application on layers taking into account four amino acids: proline, aspartic acid, glutamic acid and phenylalanine. It was noticed that the proteinaceous fraction extracted was very low in comparison to lipidis (ratio about 1:30).

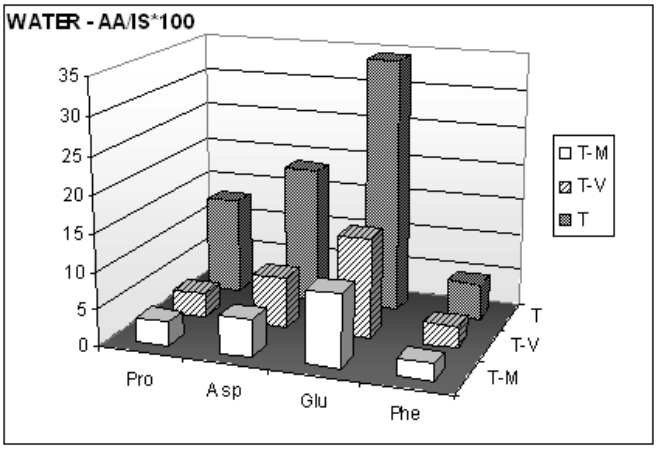


Fig. 79 Histogram of some amino acids, where: Pro= proline; Asp= aspartic acid; Glu= glutamic acid; Phe= phenylalanine, extracted by water from the tempera films. Each value represents the average percentage analyte/internal standard (IS) ratio.

The GC-MS fatty acids analyses carried out on the cotton swabs (with and without solvents) about Smithsonian Museum Conservation Institute sample showed palmitic

acid, oleic acid and stearic acid. The histogram of Fig. 80 shows that isooctane is the best leaching agent, removing 25 nanograms of palmitic acid.

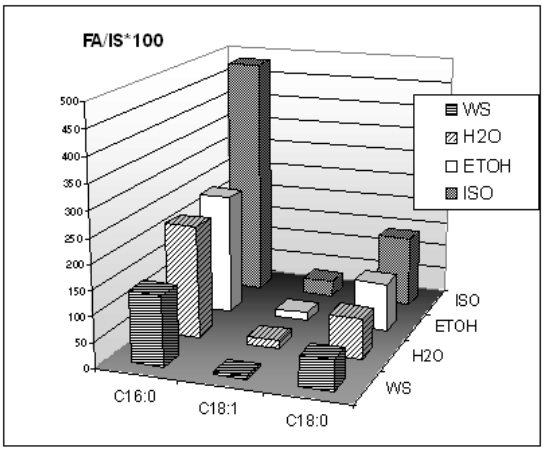


Fig. 80 Histogram of fatty acids extracted by water (H_2O), ethanol (EtOH), isooctane (W8) on the egg tempera layer dated 1995. Each value represents the average percentage analyte/internal standard (IS) ratio.

It is interesting to note that more saturated fatty acids were removed than oleic acid, in spite of the fact that oleic acid is more abundant than stearic acid in egg tempera. Probably the saturated fatty acids were present on the surface due to a migration effect, because the support of the film was not transpiring. And this would also explain the original greasy surface. Only in the cotton swabs with isooctane employed was a small amount of cholesterol detected. From the proteinaceous fraction, the amino acids peaks are comparable to the analitical blank, for all cotton swabs.

Data from cleaning tests carried out on the painting "Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo" with the three solvents having different polarity (water, acetone, isooctane) showed that isooctane was able to extract both palmitic and stearic acid. With regards to cholesterol, it was observed that, in all the cotton swabs, its peak was comparable to an analitical blank. However, amino acids were detected in the water swabs. Histograms (fig. 81, 82) show the corresponding data. It can be seen that more fatty acids were extracted than amino acids.

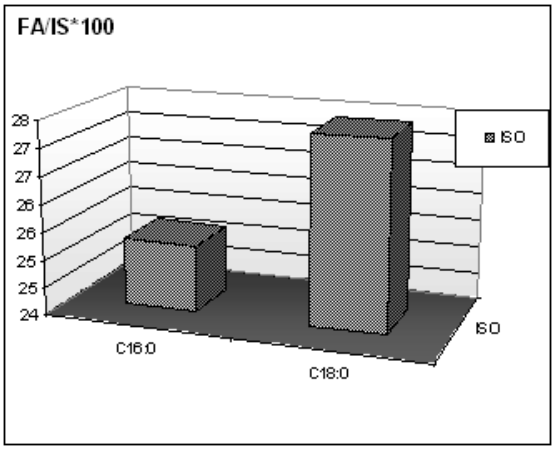


Fig. 81 Histogram of fatty acids extracted by isooctane on the painting surface. Each value represents the average percentage ratio analyte/internal standard (IS).

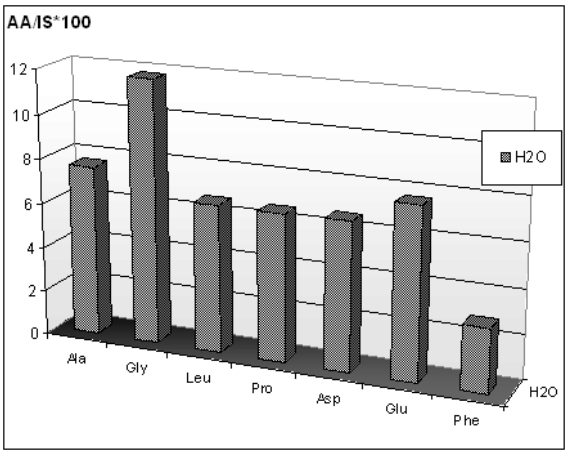


Fig. 82 Histogram of amino acids extracted by water on the painting surface. Each value represents the average percentage analyte/internal standard (IS) ratio.

From the results it can be inferred that isooctane, the most polar solvent of the ones tested, removed the lipid fraction, while water partly removed the proteinaceous fraction. It was observed that acetone could extract less fatty acids than the isooctane. As expected, unsaturated fatty acids were not detected: in fact in the five hundred year old painting the polymerisation process is completed.

However, the fact that both fatty acids and aminoacids were extracted, even if the amounts extracted were about fifty times lower to that for the laboratory samples was totally unexpected for 16th century painting.

This painting needed a surface cleaning. The analysis showed that the painting contains water-sensitive materials (such as proteins of the ground and painting layers and kaolin from gilding layer) and also apolar solvents-sensitive compounds (such as the fatty fraction of the binding medium). Therefore, aqueous surface cleaning rather a cleaning with water in the form of emulsion in an apolar solvent could not be performed. A dry *surface cleaning* by using a not grease eraser had been carried out representing the safest at the same time efficient cleaning method.

Fig. 83 detail of the “Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo”during surface cleaning with a not grease eraser.

5.3 Conclusions

Morphological and color changes, as a result of the interaction of organic solvents and water on egg binding medium carried out on laboratory samples, proved to be visually unperceptible (although changes were instrumentally measurable by MSS). Analyses of the cotton swabs applied without solvents to these samples, showed the presence of fatty acids, amino acids and cholesterol and confirmed that mechanical action alone was able to remove material. When solvents where used on the cotton swabs, the results depended on their polarity: isooctane extracted mainly fatty acids and cholesterol, water extracted only amino acids. It was observed that the leaching ability of isooctane is far greater, some thirty times, than that of water.

It was confirmed that pigmented layers are less affected by leaching phenomena than unpigmented layers, in particular for lipidic components. This finding may be linked to two factors:

1. A physical-morphological factor: due to the presence of the pigment, the total amount of binding medium is obviously lower;

2. The metal cations from the pigments could react with fatty acids forming metal soaps making them less sensitive to leaching.

The analysis from all cotton swabs, with and without solvents obtained from the egg tempera prepared at the Smithsonian Museum Conservation Institute, show the presence of fatty acids in decreasing order: palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1). Cholesterol was removed in very small amount with respect to fatty acids. Apparently no proteinaceous material was extracted. It was observed that the lipidic fraction is the only present on the surface, rendering it hydrophobic and this

was attributed to a migration effect, because of the nature of the samples, that had been originally cast onto a non-permeable , plastic support. Interestingly, saturated fatty acids were removed more than oleic acid, in spite of the fact that oleic acid is more abundant than stearic acid in egg tempera. This is not surprising considering that unsaturated acids become less mobile when they take part in polymerization process.

The study on the 16th century panel painting showed that the leaching phenomenon was greater for lipid components, and that amino acids were also extracted. As expected, unsaturated fatty acids were not detected: it is reasonable to hypothesize that in a five hundred year old painting the polymerization process is completed. The amount extracted was about fifty times lower in comparison to the laboratory samples. These results are unexpected, because mild cleaning tests were believed to be safe and undamaging for a five-hundred-year-old painting. This finding was crucial for the decision that the conservator had to make about surface-cleaning the painting: no aqueous solution would be use, but rather the mild mechanical action of a powdered, lean eraser, gently brushed onto the surface.

6. Conclusions

Firstly, this research allowed to develop an effective and innovative method for the *surface cleaning* of plaster by means of aqueous Agarose-based rigid gels, respectful of the porous nature of the substrates, which are potentially quite sensitive to treatment with water. The performance of the method was evaluated by monitoring various parameters: the rate of release and diffusion of water, the effectiveness of cleaning, the structural integrity and the appearance of the treated surfaces, and the presence of residual material; taking also into account the time required for application and the limited costs for materials, the system can eventually be defined suitable for the cleaning treatment of plaster artworks [32].

The second line of this research, addressing the aqueous cleaning of wax artworks, has demonstrated a limited risk of interaction, from a chemical point of view. When working on compact surfaces, like those of laboratory-cast wax layers, neither appreciable modification of the surface, nor chemical interaction with the wax components could be detected. However, on aged, desegregated surfaces of actual wax sculptures, covered by whitish exudate material, small amounts of wax components were detected in the swabs used for the application of free solutions, as well as in the gels; simple mechanical, rather than chemical action, can be held responsible for this interaction. On an aged, weathered wax surface, some degree of interaction inevitably has to be expected, once the decision to clean has been taken [70].

Lastly, the study on the effects of water and organic solvents on fresh egg tempera films than demonstrated that color changes were visually rather imperceptible. As for the leaching effect, it depended on the polarity of the solvents, in a different way and to a different extent on pigmented and unpigmented films. A study on an actual 16th century panel painting, showed that the paint layer was still susceptible – unexpectedly – to leaching; this finding has provided useful guidelines for the conservation treatment [71].

7. References

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