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**GREEN STRATEGIES FOR THE CLEANING OF WORKS OF ART  
SETTING UP OF AN ANALYTICAL PROTOCOL FOR THE  
EVALUATION OF CLEANING**

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## Abstract

In last decades, the safety of both artworks and restorers during cleaning operations, has been the subject of many research works, aimed at the development of gel-systems that confine the solvent action to the very surface thus preventing its absorption into the paint layers and minimizing operators' exposure.

In this research work, a totally sustainable approach has been presented for the cleaning of artworks. In particular new organogels made of bio-compatible components, were evaluated as cleaning tool for the removal of paint varnishes. The gels consist of a polymer derived from renewable bio-materials, poly(-3hydroxybutyrate), and different green solvents ( $\gamma$ -valerolactone GVL, ethyl lactate EL, dimethyl carbonate DMC). These gels, previously characterized in order to investigate thermal and mechanical properties, were tested for the removal of natural and acrylic varnishes over different binding medium (linseed oil, egg and glue). The gels were first tested on painting mock-ups, not aged and aged, then validated on real paintings. Their cleaning capabilities were compared to methods traditionally employed in restoration (e.g. dimethyl sulfoxide, benzyl alcohol acetone, carbopol-gel). A specific protocol of cleaning evaluation was designed for assessing varnish removal capability, residues releasing and solvent retention by means of Optical Microscope observations of paint cross sections in visible and ultraviolet light,  $\mu$ FTIR-Attenuated Total Reflection surface analyses and Head Space-Solid Phase Micro Extraction coupled with GC-MS analyses. These new totally safe and biodegradable organogels demonstrated good varnish cleaning capabilities that, in conjunction with their easy preparation, use and waste, represent a "green" and safe approach for both paintings and painting restorers.

## Introduction

Recently, the lack of standardized procedures in restoration practices, together with the tacit use of harmful substances, has enhanced the demand to build up regulations and recommendations in matter of safeness at international level. The need of “sustainability” in restoration practices became the actual proposal.

During the last decades, a great attention was paid for the development of cleaning systems less invasive for the artwork matter. Numerous studies reported in scientific literature, revealed the implicit difficulty to predict the long-time effect of cleaning due either to the use of aggressive methods, and the complexity of the substrate, such as paintings. Many critical issues are related to cleaning practices, for instance the alternative physical and chemical interactions between the solvent and the organic binder of the paint layer.

Traditional cleaning treatments, aimed at the removal of aged protective coatings from the surface of paintings, are carried out by wet chemical cleaning. A cotton swab soaked with organic solvents are rolled-on the surface. The solvency power of solvent, assisted by the mechanical action of the swabbing, ensured a good removal ability. Concerning this method, several drawbacks were successively highlighted, first of all the lack of selectivity of the cleaning system and the diffusion of the solvent among the surface and substrate. Unfortunately, despite the demonstrations of the risk for the artwork and also for the restorer, due to the exposure to volatile harmful substances, this method is still widely employed.

Recently, researches have been focalized in the development of gelled systems capable to enhance the selectivity of their action by localizing the solvent in the very superficial layer, decreasing its diffusion.

Is evident how the most scientific production was aimed at the improvement of cleaning methods taking into account mainly the risk for the artwork. Aspects such as safeness of the operator potentially exposed to harmful substances and also environmental issue has not been extensively addressed.

The aim of this thesis was the development and evaluation of new green gels for the cleaning of painted surfaces. These gels are based on poly-(3hydroxybutyrate) bio-polymer, produced from biomass through bacterial processes, and different green solvents.

The topic of the research, arose from the potentiality to obtained gel-systems fully bio-compatible, non-toxic and biodegradable starting from renewable bio-sources. Indeed the novelty of the work is the total sustainability of the entire cleaning process, from the sources of gel components to totally safe application and waste of the product.

The project involved other two research groups of the University of Bologna. The research group held by Prof. E. Tagliavini (Centro Interdipartimentale di Ricerca Industriale Energia Ambiente, Ravenna) and the group held by Prof. L. Giorgini (Centro Interdipartimentale di Ricerca Industriale per la Meccanica

Avanzata e i Materiali, Bologna). The promising results obtained from the formulated gels, found the enthusiastic interest of restorers, personally careful about safeness issue.

During this thesis, each gel will be presented separately. Preparation procedure, analytical characterization of mechanical and thermal properties and cleaning performance evaluation will be defined for each formulation. Advantages of the proposed approach will be discussed in comparison with traditional cleaning systems. Moreover varnish removal capability, residues releasing and solvent retention were selected as performance indicators and evaluated by means of a protocol specifically defined using non-invasive and microdestructive techniques, as required in Cultural Heritage field.

Furthermore, this research would highlight the relevance of complementary information obtained by a multi-analytical approach in order to exhaustively evaluate a cleaning treatment.

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## Chapter 1. THE CLEANING OF WORKS OF ART

In the “Theory of restoration” of the art historian Cesare Brandi (Brandi, 1977), who pointed out the basis of modern restoration, the cleaning is defined as a critical operation aimed at the recovery of the legibility of the artwork. Based on Brandi’s definition, in Italy the term “cleaning” means a traditional intervention aimed at the partial or total removal of extraneous material deposited over the surface during time or added as protective coating or successive restorations. Thus the kind of materials that is possible to find over a surface can have several origin. It could be a material more or less coherent such as grime and dust, deposited to the surface as a consequence of exposure or conservation conditions or an intentionally added such as overpainting or protective varnish layer. In any case everything that with ageing can disfigure the aesthetic appearance of the original surface have to be periodically removed and substituted.

According to the modern conception of conservation and restoration of works of art (P. Baglioni, Berti, et al., 2013), that tends to the “minimum intervention principle”, all the interventions are regulated by few basic principles:

1. Chemical compatibility between restoration material and the surface
2. Inertness of new material toward the original one
3. Chemical and physical stability of the restoration material in order to avoid further unknown reactions and interactions during time.

As a restoration intervention, the cleaning have to follow these principles and thus the methods employed have to avoid interferences with the original surface minimizing the action to the material that have to be removed. The situation becomes more critical when the layer to be removed is similar, physically and chemically, to the underlying paint layer.

Cleaning methods are generally choose on the basis of material to be removed. Superficial deposits of dirt and grime are removed by mechanical methods, using brush or rubber, or physical methods such as laser cleaning. A more complicated intervention is required in the cleaning of multilayered surface, for instance a painting. In this case many factors have to be consider before restoration. Thickness and chemical composition of the layer to be removed, together with the composition and porosity of the layer underneath that have to remain untouched, are all factors that influence the choice of the most appropriate cleaning method. Commonly the removal or thinning of superficial layers, like varnishes or overpainting, are carried out by chemical methods.

The cleaning can dramatically alter the appearance of the surface and its mechanical properties. Indeed this issue has been for long time debated as demonstrated by the copious scientific production from the 1950s until nowadays. The main aspect that interested scientists community over the world concerns the risk to damage the original material during cleaning as effect of solvents. Systematic studies carried during 1950s and 1960s gained a better knowledge of mechanisms and effect of cleaning.

In the course of this thesis, the attention will be focused on the removal of varnish from painting surfaces carried out by chemical methods. Criticisms and problems concerning this cleaning approach will be extensively show below.

## **1.1 THE MATERIALS OF PAINTING ARTWORKS**

Before starting the dissertation about cleaning is important to figure out what a painting is made of, from a material point of view, and which are the most critic challenges for a cleaning intervention.

A painting could be seen as an overlapping of several layers composed by organic and inorganic materials mixed together to create the artistic image. Ancient recipes for artistic technique and materials preparation, were meticulously reported by Cennino Cennini in “The book of the art” (Cennini, 1982) dated back the end of fourteenth century, used still nowadays as a reference. The support, the main structural layer of a painting, was principally canvas, wood or wall. Over the support a ground layer was applied, generally composed by gypsum and animal glue, in order to receive the drawing of the painting and also act as a barrier between the paint and the support. The painted surface was applied on the top of the ground by applying the mixture of pigments and binding medium. A range of materials were used as paint binders over the years: egg white, egg yolk, casein, animal glue, arabic gum and oils. Finally the varnish layer was put externally either for the protective and the aesthetical function for enhance the saturation and brilliance of colors (de la Rie, 1987).

Principal binders medium, linseed oil and egg-tempera, together with tepernic and acrylic varnishes will be described below in terms of chemical composition and ageing phenomena.

### **1.1.1 LINSEED OIL BINDER, AGEING AND DEGRADATION**

The oil technique, based on the use of drying oils as binding medium in painting, was largely employed in north Europe since 13<sup>th</sup> century. The major exponents of this technique were Flemish, while in Italy the period of diffusion of oil paint was 15<sup>th</sup> century.

Oils are complex mixtures of organic and inorganic materials, in which triglycerides are the main components, sterols and vitamins are contained in lower amount. Triglyceride is a glycerol molecule bound to three fatty acids chains by esters bonds. The principal fatty acids present in triglycerides are: palmitic (C16), stearic (C18), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3). Despite the relative proportion of these acids varies according to oil sources, is possible to figure out typical values, as reported in the **table 1** (Colombini & Modugno, 2009).

The dryness property of oil lies in the high amount of unsaturated fatty acids, in particular the polyunsaturated linoleic and linolenic. The process consists in polymerization reactions that start from free radical mechanism and involves the oxidation and subsequent cross-linking of fatty acid chains, producing a complex three-dimensional network (Bonaduce et al., 2012; Wexler, 1964). During drying and ageing,

alternative reactions of chains scission occurred, leading to the production of smaller molecules. These molecules are low molecular weight and have a very little tendency to polymerize and crosslink, but can further oxidize producing several species such as acids, aldehydes, ketones and alcohols. Among these compounds, the most volatile evaporate from the film during drying while the others, higher and less volatile, remain covalently bonded to the glyceride network. Another reaction that can occur during ageing is the hydrolysis of ester linkages which can produce fatty acids and diglyceride and monoglycerides and free glycerol, with further hydrolysis reactions (Erhardt, Tumosa, & Mecklenburg, 2005).

**Table 1.3** Fatty acid percentage composition of some fresh vegetable oils and of animal lipids

Oil	Palmitic acid (esadecanoic acid)	Stearic acid (octadecanoic acid)	Oleic acid (9-octadec- enoic acid)	Linoleic acid (9,12-octa- decadienoic acid)	Linolenic acid (9,12,15- octadecatri- enoic acid)	Elaeostearic acid (9,11,13- octadecatri- enoic acid)	Ricinoleic acid (12- hydroxy-(Z)- 9-octadec- enoic acid)	Gondoic acid (11- eicosenoic acid)	Erucic acid (13-docose- noic acid)
Linseed	6-8	3-6	14-24	14-19	48-60	—	—	—	—
Walnut	3-7	0.5-3	9-30	57-76	2-16	—	—	—	—
Poppyseed	8-12	2-3	12-17	55-65	3-8	—	—	—	—
Olive	8-18	2-5	56-82	4-19	0.5-1	—	—	—	—
Sunflower	5-6	4-6	17-51	38-74	—	—	—	—	—
Castor	1-2	1-2	3-6	4-7	—	—	83-89	—	—
Tung	3-5	2-4	8-11	12-15	0-3	75-85	—	—	—
Palm	43-46	4-10	35-40	7-10	—	—	—	—	—
Rapeseed	2-6	1-3	20-30	17-22	6-10	—	—	13-16	20-40
Hen's egg	25-27	9-12	38-44	13-15	0-1	—	—	—	—
Lard	20-27	13-19	37-45	7-10	0-1	—	—	—	—

**Table 1.1** Fatty acids percentage contained in some vegetal and animal lipids (Colombini & Modugno, 2009).

Mechanisms of oil ageing are complex and several aspects are still under examination. Several factors influence the types of reaction and the rate at which they take place. One of the most important is the interaction of the binder with pigments. Even if pigment effects are complex and poorly understood, for certain pigments the catalytic or inhibiting effects were pointed out (Rasti & Scott, 1980; Wexler, 1964). Pigments contained into the paint layer show a great influence on polymerization extent. Metals that show a catalytic effect are Cu, Co, Pb, Mn, Fe, on the contrary pigments that retard the polymerization are vermilion (HgS) and black carbon (Cremonesi, 2000). Cations of certain pigments (e.g. zinc, copper and lead) react with hydrolyzed fatty acids forming metal carboxylates, which have been extensively investigated (Mazzeo et al., 2008; Plater et al., 2003). The natural degradation processes of lipids can be accelerated or modified if the material is exposed to oxidizing conditions or to high temperatures, for instance drying oil were often pre-polymerized by heating before use as paint binders. Thus, the nature of degradation products depends on the composition of the original material, on the treatment of the material before or during its use and the presence of interacting factors (Colombini & Modugno, 2009).

The chemical changes in the drying paint film are accompanied by physical changes, including an increased stiffness and brittleness resulting from the loss of volatile component from the film, thought to have plasticizing role in the film, in combination with an increased cross-linking and the formation of ionic

interactions. In the degradation stage, the paint film is expected to become more brittle and therefore increasingly sensitive to mechanical stress (van der Berg, van der Berg, & Boon, 1999).

Old paint films are consistently different from young films. Oil paint becomes more polar on ageing and consequently more sensitive to polar solvents, including water. Oxidation is not the only process that contributes to the increased polarity of old oil film. Hydrolysis of glyceride ester polymer network is another significant factor in the chemical alteration of oil binder due to ageing. De-esterification/hydrolysis of the cross linked polyesters network of a dried oil film, leads to the formation of a residual cross-linked polymeric fraction that contains functional groups in long chains fatty acids, increasing significantly their solubility in polar solvents and hydrogen-bonding solvents (Raymond White & Works, 1998). The increased ionic character of the residual polymer phase may leads to coordination with multivalent metal ions, either at pigment surfaces or dissolved in the organic phase. This is suggested as a significant contribution to cohesion of the paint film, increasing compactness and stiffness (Phenix, 2002b).

### **1.1.2 EGG BINDER, AGEING AND DEGRADATION**

Protein substances such as egg yolk, glair, casein, animal glue and their mixtures with oils, resins, gums, etc., have been used as binding media since very early times. In some cases they showed very high stability in various atmospheric conditions, compared with the yellowing and brittleness of aged oils and resins (Karpowicz, 1981).

Egg-tempera medium is the central painting technique employed in Italy during 14<sup>th</sup> and 15<sup>th</sup> century, followed by the assessment of oily technique. It can be considered the main technique for wooden panel paintings, but despite its artistic importance, it received less attention than oil.

According to ancient recipes egg-tempera medium may employ the whole egg or egg yolk and egg white separately. Sometimes others compounds such as vegetable gum, oils etc. were added (Thompson 1936). First systematic studies on egg were carried out by Karpowicz (Karpowicz, 1981), regarding the ageing of proteinaceous materials. In a protein medium, the amino acids are joined together by peptide bonds to form a long chain with three-dimensional structures. These structures, together with the sequence of amino acids in the chain are responsible of differences in chemical and physical properties of proteins and are unique for different proteins. The three-dimensional conformation is stabilized by bonds between side-chain groups of amino acids: hydrogen bondings, disulphide links, hydrophilic and electrostatic interactions (Karpowicz, 1981).

Hens egg contains both lipidic and proteinaceous fractions. The egg white, albumen, is prevalently formed by water (88-89%), then protein (10.5%), carbohydrate (0.5%) and inorganic ions (0.5%). Proteins of albumen have been extensively studied and the ovalbumin it was point put as the prevalent amino acid (54%) (Phenix, 2010). Egg yolk is a highly complex and heterogeneous matrix containing oily substances, water and compounds with emulsifying properties. The majority of yolk is composed of lipids (66%), present as

triglycerides, phospholipids and cholesterol compounds. Like as oil, the dominant fatty acids in egg yolk are palmitic, stearic, oleic and linoleic acids (Phenix, 2010). The lipids contained in egg 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.

In a freshly dried tempera film, the mobile lipid fraction acts as a plasticizers conferring reasonably flexibility to the film. The other main characteristic of a fresh egg films is the hydrophilic character due to the presence of proteins and phospholipids. Thus a young egg-tempera paint may be easily damage both by water and solvents. In fact in one hand water can readily swell the layer and in the other the majority of lipid can be easily extracted by suitable organic solvents such as ketones, chlorinated solvents, alcohols, hydrocarbons (Phenix, 2010).

Cleaning tests performed on artificially aged tempera films, proved the mobility of some lipids, as previously seen for oil binder, to migrate to the surface spontaneously and the most volatile tend to evaporate. Thus the risk of lipids extraction by some organic solvents became very probable leading to compaction of the paint film and surface roughness (Phenix, 2010). Kandekar (Khandekar, Phenix, & Sharp, 1994) tested different-polarity organic solvents and water in order to evaluate the egg-tempera resistance in artificially aged samples made up of several pigments. It was noted that hydrocarbons such as dichloromethane, and acetone cause leaching of the lipidic components on a freshly dried film, whereas water causes film swelling. Moreover a recent investigation on the effect of aqueous and organic cleaning on fresh egg tempera tester, revealed the extraction of egg amino acids by water treatment. Clearly the amount of the extracts is small compared with the extraction of lipids by organic solvents, but is not negligible. In the same investigation amino acids resulted extracted by water also in a XVI century tempera painting (A Casoli, Berzioli, & Cremonesi, 2010).

Protein drying properties are not as strong as oils, due to the less content of polyunsaturated fatty acids. Despite the presence of hydrophilic proteins, egg binder becomes extremely resistant once aged because of the polymerization of lipid component and possibly the formation of mixed aggregates between proteins and lipids.

Young egg paint contains large amounts of water that once applied as a paint medium, evaporates quickly. During drying the protein continuously loses water to reach an equilibrium with the surrounding atmosphere. The loss of water leads to denaturation process in which consistent alteration of at least tertiary and quaternary protein structures occurred as a consequence of the disruption of the hydrogen bondings of the globular proteins (Khandekar et al., 1994). In fact because the content of water decreases, empty spaces between molecules arise, forcing them to fill the spaces and simultaneously to rearrange the structure possibly involving lipid-protein aggregations (Phenix, 2010) (Karpowicz, 1981). Therefore the drying and the

initial curing of egg involves the disruption of the native conformation of proteins and decrease the sensitivity to water.

Several processes may contribute to the initial hardening and immobilization of protein components include complexing of metal ions and aggregation with lipids. According to Karpowicz (Karpowicz 1981) several possible reactions may play an important role, such as:

1) lipids oxidation, by direct autoxidation or photoinduced, catalized by metal ions (e.g.  $\text{Fe}^{3+}$  from pigment). Lipids oxidation results in polymerization of triglycerides and phospholipids, formation of cross linked lipid matrix, immobilization of unsaturated fatty acids, formation of low molecular weight compounds, volatiles, carbonyls, unsaturated compounds, oxidation of cholesterol; 2) formation of insoluble complexes of protein and oxidised lipids covalently bonded, resistant to extraction by neutral organic solvents; 3) decomposition of amino acids, amino acids oxidation, cross linking; 4) reactions catalized by metal ions such as copper, cobalt, iron, manganese; 5) hydrolysis of phospholipids, condensation of proteins with carbohydrates, formation of metal ions protein complexes.

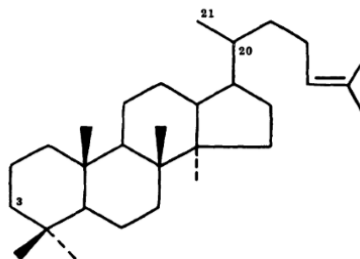
Once the paint layer is applied, the chemical reactions described start and continue through the centuries. Different reaction pathways could predominate depending on the amino acids content, initial structural variations and accessibility of chemically active side-chain groups. Some of the ageing effects are evidently deteriorative, but some of them increase the durability of paint during in time. Sulphur-containing proteins such as egg and casein, are the most labile and their crosslinking and oxidation is very probable. Animal glue (which has no sulphur amino acids) may be expected to be relatively stable, which is not surprising, considering the well preserved reversibility to water treatments of aged gesso grounds. (Karpowicz 1981).

### **1.1.3 TERPENIC AND ACRYLIC VARNISHES**

Varnish is generally a thin protective film applied over the painted surface, thus have to appear transparent in order to allow the visualization of the underneath paint layer. During time varnishes become progressively yellowish or brownish because of oxidative phenomena that leads to the formation of yellow chromophores (J. S. Mills & White, 1994). The chemical oxidation of molecules together with the mechanical disruption of protective film are spontaneous ageing phenomena, due to the interaction with atmosphere, which made necessary the periodical removal and substitution of varnish. Brittleness, change in solubility, blooming, cross-linking and permeability to moisture are only some of the properties of varnishes that have been extensively studied (de la Rie, 1987).

Natural resins such as mastic and dammar were used since ancient time as a component for varnishes on paintings (Gettens, 1966). They are exudates from certain Pinaceae trees and are mainly composed by terpenoids, which include the class of triterpenoids composed by 30 atoms of carbons per molecule (J. S. Mills & White, 1994; J. Mills & White, 1977). Terpenoids oxidize rapidly when applied as thin films causing hazing,

cracking and yellowing (de la Rie, 1987). Natural Mastic and dammar belong to this class and their were employed as picture varnishes because showed less yellowing and more durability than many conifer resins. Moreover they are readily solvent-soluble than the leguminous copals (J. Mills & White, 1977) and show a good solubility in solvents, such as oil of turpentine.



**Figure 1.1** Dammarane skeleton (J. Mills & White, 1977)

As a natural exuded product, resins have a complex and varied composition that often changes considerably with time due to oxidation or polymerization. In fact once exuded as a viscous liquid substance, subsequently it hardens by evaporation of volatiles or by the partial polymerization of some components (J. Mills & White, 1977). Mastic resin is a complex mixture of triterpens with a portion of polymeric hydrocarbon. Dammar resin, introduced in the nineteenth century, is generally considered to be more stable than other natural resin (de la Rie, 1987).

During centuries both materials and procedures of varnish production were evolved. Before the discover of distillation techniques, the resins were solubilized by boiling in drying oils (commonly linseed oil) making the so called “oil resinous varnish” were commonly used in the Middle Ages. In the 16<sup>th</sup> century, resins started to be solubilized in volatile solvents, called “spirit varnishes”. At present spirit varnishes consisting of dammar or mastic dissolved in oil of turpentine are probably the most popular natural resin varnishes (de la Rie, 1987).

The ageing process of oil-resinous varnishes were strongly problematic from a conservative point of view. They rapidly developed a yellow or even brown color and became insoluble by cross-linking. Moreover the high thickness of the layer and its contraction during drying were further problems. Because the hard polymerization of oil cross-linked network, oil-resinous varnish became insoluble and its dissolution required the use of strong reactive solvents, enhancing the risk to damage the paint layer underneath (Campanella, 2007; J. S. Mills & White, 1994).

The oil-resinous varnish were successively substituted by solvent-varnishes, more reversible during time. In fact the resin is applied as a colloidal solution in an organic or mixture of solvents and dry by evaporation of the solvent, forming a film on the surface. With ageing the small molecules of resin are not further cross-linked and is therefore possible to resolubilize the varnish film by applying solvents, as in cleaning. They were first mentioned in sixteenth century sources in Italy and widely used in both southern and northern Europe in the seventeenth century (de la Rie, 1987).

From the second half of the 20<sup>th</sup> century, synthetic polymers were introduced as replacements for the unstable, natural resin varnishes. At that time the concept of reversibility was extremely diffused and new products were commercialized as more resistant to ageing and easily removable. In fact due to dangers of frequent cleaning procedures, an increasing interest on more stable coating materials, that keep their transparency and remain soluble in solvents of low polarity were assessed (de la Rie, 1987). Thanks to their wide variety of formulations, synthetic polymers were employed as varnishes, media, consolidating agents and adhesives for paintings or as fillers in stone materials and archaeological objects (Osete-Cortina & Doménech-Carbó, 2006).

Unfortunately, also synthetic polymers undergo degradation similarly to natural resins, resulting in the decrease of their solubility and in the significant alteration of their visual aspect, mainly yellowing (P. Baglioni, Chelazzi, Giorgi, & Poggi, 2013).

Several acrylic polymers, in particular Acryloid B72 (commercialized as Paraloid B72<sup>®</sup> by Rohm & Haas, ethylmethacrylate/methacrylate copolymer) started to be employed as paint medium after the second world war as an alternative to alkyd resins and drying oils. Acrylic polymers were used and are still extensively used mainly either as consolidant and coating for paintings, perhaps to a larger extent in the United States than in Europe. Some of the acrylics cause problems because they become insoluble due to cross-linking (de la Rie, 1987).

## **1.2 THE CRITICAL ISSUES ABOUT CLEANING**

The main critics concerning the cleaning methods in restoration of paintworks, regard principally three risk-factors: the risk of damage irreversibly the paint layer during cleaning procedure, the risk linked to the employment of harmful or toxic substances for the human health, the potential risk for the environment.

The potential harmful action of a cleaning solvents (organic solvent and water-system) towards the paint layer were extensively debated. A number of studies reported in literature on the removal of varnishes from paintings demonstrated the risk for the original paint to be altered by the interaction with solvent. What arose from investigations was mainly the possibility for paint surface to be swollen and softened by the cleaning solvent and leached of some plasticizer components, affecting the optical and mechanical properties of the paint layer such as the loss of pigment and the brittleness of the surface.

In the 1950s and 1960s a number of scientific studies lied with the effects of organic solvents on cleaning, gaining a more precise understanding on their mechanisms and effects. Of particular relevance were the phenomena of swelling and leaching of oil binder described in detail by Stolow (Stolow, 1963) and further by Phenix (Phenix, 2002a, 2002b; Phenix & Sutherland, 2001).



During decades, systematical studies were carried out on “cleaning methods” in order to develop systems capable to reduce the risk of solvent-paint layer interaction or propose softer cleaning agents such as aqueous solutions.

### 1.2.1 THE SWELLING PHENOMENON

Swelling is a physical phenomenon of softening of the organic binder due to the sorption of solvent during cleaning. It is an immediate and tangible effect and if extends to a significant degree, the binding power of the paint medium is reduced and the pigment is vulnerable to removal, for example, by mechanical action of the cleaning swab during varnish removal (Phenix, 2002a; Phenix & Sutherland, 2001).

Over the years many approaches were adopted to measure the swelling of paint film. Generally they can be grouped into two categories: determination of dimension change either in the plane of the film or in the perpendicular direction, and measurement of weight due to solvent sorption.

In his studies, Stolow carried out precise measurements of swelling, making a foundation for model descriptions of the risk in cleaning processes for more than 40 years (Phenix & Sutherland, 2001; Stolow, 1963). In fact, the degree of swelling was used as indicator of the magnitude of interaction between the solvent and the organic phase of the paint (Phenix & Sutherland, 2001). Solvents exhibited a trend according to their molecular volume and viscosity: those of low viscosity such as acetone and benzene, produced much more rapid swelling than more viscous solvents such as isobutyl alcohol. Solvents with low molecular volume, such as methanol, showed rapid diffusion and swelling (Phenix & Sutherland, 2001).

Ageing of the paint film demonstrated to have a significant influence on swelling extent. In a severely aged paint films, the mass of organic phase diminished as a consequence of loss of volatile products in combination with the increased cohesiveness conferred by internal electrostatic and H-bonding forces. Thus aged films show only low magnitudes of swelling in solvents compared to their young counterparts. Swelling may be a phenomenon of risk mainly in young or medium/rich oil paints. (Phenix, 2002b). Concerning swelling power, Phenix pointed out that solvents vary significantly both in rate at which they cause swelling and in their ultimate swelling power (**Table 1.2**) (Phenix, 2002a).

As shown in the **Table 1.2** reported, aliphatic ester solvents fall generally into the low-moderate swellers. The cyclic  $\gamma$ -butyrolactone is on the border with group of low-swelling solvents. Simple aliphatic alcohols seem to increase the capacity to swell with increasing polarity and decreasing molecular size. Among these, benzyl alcohol, widely used in aqueous formulations to enhance the cleaning activity, is in the class of high-swelling solvent. Strong dipolar solvents, such as dimethyl sulfoxide, it is in border between high and moderate sellers (Phenix, 2002b).

Table 3. Classification of Solvents in Terms of Ultimate Swelling Power and Rate of Swelling Action

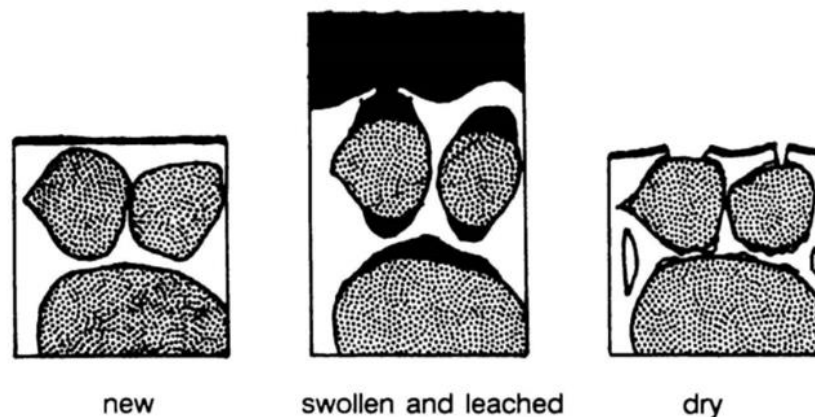
Category: Magnitude of Maximal Swelling	Solvent	Category: Rate of Swelling	Solvent
<b>Low-swelling solvents</b> 16, < 6% swelling 17, < 4% swelling	perfluorodecalin <i>iso</i> -octane 1,8-cineole white spirit  <i>γ</i> -butyrolactone (borderline +)	<b>Very slow</b> 16, $t_{1/2} \geq 50$ mins. 17, $t_{1/2} \geq 100$ mins.	cyclohexanol tributylphosphate perfluorodecalin spirit of turpentine <i>γ</i> -butyrolactone  1,8-cineole diacetone alcohol <i>iso</i> -octane <i>iso</i> -butanol
<b>Low-moderate-swelling solvents</b> 16, ca. 6–12% swelling 17, ca. 4–9% swelling	<i>iso</i> -butanol (borderline -) di- <i>n</i> -butyl ether (borderline -)  methoxypropyl acetate ethoxypropyl acetate methyl <i>iso</i> -butyl ketone ethylbenzoate <i>n</i> -butyl acetate propylpropanoate ethylacetoacetate diethylcarbonate acetone butanone (MEK) pentan-2-one  ethylpropanoate (borderline +) methyl <i>iso</i> -propyl ketone (borderline +) <i>tert</i> -butylpropanoate (borderline +) spirit of turpentine (borderline +)	<b>Slow</b> 16, $t_{1/2} = 20$ –50 mins. 17, $t_{1/2} = 40$ –100 mins.	butan-1-ol butan-2-ol propan-2-ol benzyl alcohol hexafluorobutanol ethylacetoacetate white spirit  methylcyclohexyl acetate (borderline +)
<b>High-moderate-swelling solvents</b> 16, ca. 12–18% swelling 17, ca. 9–15% swelling	tetrachloromethane (borderline -) tributylphosphate (borderline -)  1,2-dichloroethane 1,1,1-trichloroethane methylcyclohexyl acetate diacetone alcohol methoxypropanol  dimethylsulphoxide (borderline +)	<b>Intermediate</b> 16, $t_{1/2} = 8$ –20 mins. 17, $t_{1/2} = 20$ –50 mins.	diethylcarbonate (borderline -)  2-ethoxyethanol propan-1-ol methoxypropanol methoxypropyl acetate ethoxypropyl acetate <i>tert</i> -butylpropanoate ethylbenzoate methyl <i>iso</i> -butyl ketone dimethylsulphoxide  di- <i>n</i> -butyl ether (borderline +) propylpropanoate (borderline +)
<b>High-swelling solvents</b> 16, ca. 18–22% swelling 17, ca. 15–21% swelling	anisole (borderline -)  dichloromethane 2-ethoxyethanol 2-methoxyethanol cyclopentanone <i>N</i> -methylpiperidine	<b>Fast</b> 16, $t_{1/2} = 3$ –8 mins. 17, $t_{1/2} = 6$ –20 mins.	1,1,1-trichloroethane (borderline -)  tetrachloromethane ethylpropanoate <i>n</i> -butyl acetate ethanol 2-methoxyethanol <i>N</i> -methylpiperidine <i>N</i> -methylformamide methyl <i>iso</i> -propyl ketone  anisole 1,4-dioxane butanone (MEK) pentan-2-one pentan-3-one cyclohexanone cyclopentanone toluene
<b>Very high swelling solvents</b> 16, > ca. 22% swelling 17, > ca. 21% swelling	acetylacetone (borderline -) <i>N</i> -methylformamide <i>N</i> , <i>N</i> -dimethylformamide <i>N</i> -methyl-2-pyrrolidone benzyl alcohol hexafluorobutanol 2,2,2-trifluoroethanol trichloromethane (chloroform) cyclohexanone	<b>Very fast</b> 16, $t_{1/2} \leq 3$ mins. 17, $t_{1/2} \leq 6$ mins.	2,2,2-trifluoroethanol (borderline -) xylene (borderline -)  <i>N</i> -methyl-2-pyrrolidone <i>N</i> , <i>N</i> -dimethylformamide methanol dichloromethane trichloromethane (chloroform) 1,2-dichloroethane acetone acetylacetone

Table 1.2 Classification of solvents in terms of swelling power (Phenix, 2002a).

### 1.2.2 THE LEACHING OF SOLUBLE COMPONENTS

Leaching describes the extraction of soluble components of the organic binder and in contrast to swelling, is less immediately tangible, it has a potential risk in long-term. As consequence of the extraction of low-molecular weight components, which show plasticizing properties in the film, the primary risks of leaching are the embrittlement of the film and the alterations of the optical properties of the paint surface increasing mattness (**Figure 1.2**).

The most detailed analyses of solvent extracts (unpolymerized triglycerides, those containing palmitic and stearic acid, monounsaturated oleic acid, in combination with lower molecular weight oxidation products such as dicarboxylic acids, aldehydes and hydroperoxides) from pigmented and unpigmented oil paint, were carried out by Stolow. Quantitative measurements of the extracts were determined either from weight changes in extracted paint samples or using chromatographic analysis of extracted fatty acids by (Antonella Casoli, Di Diego, & Isca, 2014; Khandekar et al., 1994; Phenix & Sutherland, 2001; Raymond White & Works, 1998). A magnitude of extractable components was determined and referred as a general indicator of risk in cleaning treatments.



**Figure 1.2** Leaching and swelling phenomena on paint layer (Michalski, 1990).

Important parameters linked to the risk of swell and leach the paint layer are related to the retention-diffusion of solvent applied in cleaning.

### 1.2.3 SOLVENT DIFFUSION

Diffusion of solvent was reviewed by Michalski. He makes important distinction between two forms of solvent penetration: capillary penetration and diffusion. The first one is primarily influenced by porosity of the paint structure and by the viscosity and surface tension of the solvent. Its action is fast and does not have much impact on a paint film but provides greater surface access for diffusion to occur. Film porosity increases on ageing and as a consequence of leaching. Solvent penetration occurs by simple diffusion of the solvent through the binder medium following Fick's law, thus diffusion is only controlled by solvent concentration (Michalski, 1990; Phenix & Sutherland, 2001).

Is principally a physical phenomenon, the rate is mainly driven by superficial tension and viscosity of the solvent. A liquid with high superficial tension and low viscosity will show high retention. Indeed among the most retentive solvents are water, amines, amides. On the contrary, a low superficial tension will show an high wettability and a scarce penetration (Cremonesi, 2000).

#### 1.2.4 SOLVENT RETENTION

Retention is the concentration of solvent in paint film and is governed by the speed of diffusion of the solvent outwards, as in drying or de-swelling process. As a basic principle, a rapid diffusion was often exploited by restorers to prevent harmful levels of solvent in the paint layer. De-swelling processes are slower than swelling because of the affinity of the paint film binder for solvent. The initial step involve the evaporation of liquid solvent at the surface (controlled by solvent evaporation) and a later step of loss of absorbed solvent from the paint (Phenix & Sutherland, 2001).

In scientific literature, just few studies were published concerning the solvent retention in cleaning practice. The lack of studies confirms the difficulties in measure this phenomenon and the complexity of existing variables either of the substrate and the solvent. Reported investigations measured the retention of solvents labeled with C14 isotope and applied by syringe drop and swab to the surface. The study was conducted by Dauchot-Dehon in 1973 and results revealed that the loss of solvent approximately follows an exponential pattern and traces of solvent were found to be left after many days. The most famous study on retention is ascribable to Masschelein-Kleiner in 1981 (Masschelein Kleiner, 1994; Masschelein Kleiner & Deneyer, 1981) where gravimetric measurements were carried out and curves of solvents evaporation-retention were drawn. This scheme is widely followed by conservators around the world. Is a sperimental method by which solvents were classified according to the time taken to evaporate from the sample and also to their rate of penetration, based theoretically on measurement of viscosity and surface tension (Phenix & Sutherland, 2001). Solvent sclassified as: a) high retentive (glycoles and tueprntine essence), high (alochol superior, ammide, ammine), medium (alcholo, ketons, ester, some aromatic hydrocarbons), weak (saturated hydrocarbons and chlorinated, esters, acetone) (Cremonesi, 2000).

As a consequence of leaching and swelling phenomena by solvent absorption, also physical and mechanical effect may arise from cleaning procedure. Changes in physical properties of paint film due to solvent leaching were studied by McGlinchey in 1995 using Differential scanning calorimetry analyses (Phenix & Sutherland, 2001). Hedley investigated the effects of solvents on mechanical properties of paint film using thermal mechanical and dielectric analysis (Hedley, Odlyha, Burnstock, Tillinghast, & Husband, 1990) and observed an increasing stiffness as a results of leaching and a change in the surface morphology (erosion) as a consequence of swabbing and immersion in solvents, observed by SEM. Changes in thickness, color and gloss of a variety of oil paint samples due to solvents exposure were measured by Erhardt and Tsang n 1990 (Erhardt et al., 2005; Tsang & Erhardt, 1992). A more recent study reported the long-term damage of cleaning

solvents on oil paint by plotting the influence of the solvents on the elasticity modulus of the oil paint (Fuesers & Zumbühl, 2008).

#### 1.2.5 TOXICITY AND ENVIRONMENTAL ISSUE

Still in the second half of 20<sup>th</sup> century, solvents with a high or medium level of toxicity were commonly used, e.g. butyl amine, pyridine, carbon tetrachloride and formic acid (Signorini, 2010). Thus the research of less toxic materials and a more reasonable and cautious use of traditional ones, such as diethanolamine and hydrocarbons, were undertaken and are currently ongoing.

In Italy, toxicity became a great concern in the 1990s and it was the main reason for initiating the information campaign on the new methods and techniques both in conservation schools and among restorers and conservators. When the campaign started, the high toxicity level of nitro diluents (a mixture of organic solvents, mainly toluene, acetone, dichloropropane, isobutyl acetate, cellosolve and isopropyl or isobutyl alcohol, in varying proportions) and of butyl amine was clearly recognized. Dimethyl sulfoxide and dimethylformamide and n-methylpyrrolidone were suggested instead of more toxic solvents, because they are polar aprotic solvents with similar characteristics and solubility ability (Cremonesi, 2000). DMSO was soon adopted as a “universal” solvent because its capability to quickly dissolve old varnishes and oil retouches as well as its lower toxicity (Signorini, 2010). But because the long retention time inside the paint layer and its very poor selectivity, it was suggested to use only if necessary and in mixtures with more volatile solvents and/or in a gel.

Toxicity and environmental issues are related not only on the use of certain chemicals or substances, but also on the way they are employed. Volatile solvents, for instance, induce a potential risk for the healthiness of restores because the majority of private studios are not equipped with adequate air-aspiration systems. Indeed frequently artworks must be restored in situ and in indoor environments where ventilation and other protection devices are not always available.

Moreover the correct disposal of chemical waste requires the specific treatment for special waste, more expensive and not always properly followed by small restoration studios (Cremonesi, 2000).

### **1.3 CLEANING METHODS: STATE OF THE ART**

Traditional cleaning method for the removal of coatings and overpainting employed wet chemicals applied with cotton swab rolled on the surface (Raymond White & Works, 1998). Thanks to the believed easy and predictable action due to the efficacy of the solvent assisted by the mechanical action of the swab, this method was for long time the only protagonist for the cleaning of paintings.

As a consequence of the extensive research works, mainly carried out from the second half of 20<sup>th</sup> century, the potential risk of using organic solvents arose. The serious hazards principally linked to the

phenomena of swelling and leaching of the binder medium were extensively investigated and still nowadays the absence of these phenomena are considered parameters for a “safe” cleaning method. These studies contributed to the further ongoing development of new cleaning methods aimed to enhance the selectivity of the cleaning agent to the very superficial coating by reducing its diffusion to the underneath paint layer and thus the interaction with it. Many research works focused on viscous solutions and gelled-systems for obtain a confinement of cleaning action reducing mechanical stress and excessive solvent release.

At the end of 20<sup>th</sup> century, thanks to the increasing sensibility to healthiness issue, alternative approaches to organic solvents were presented. The most toxic solvents employed in the cleaning were substituted with less harmful solvents and aqueous-methods were proposed as not toxic and eco-compatible alternatives.

Despite many efforts and proposals of cleaning methods, more respectful of human health and artwork matter as well, nowadays the search for sustainable practices and methods in restoration of Cultural Heritage become prominent. Thanks to the great diffusion of the green chemistry principles in most of the sector of chemistry and chemistry-applied, the development of a sustainable approach is primarily based on circular economy. Indeed the use of renewable sources, such as biomass, and the reducing of waste, either in the production and application steps, become the actual challenge for conservation.

### **1.3.1 TRADITIONAL WET CLEANING METHOD**

As previously mentioned, traditional method adopted for the cleaning of paintings, is the use of cotton swab soaked in pure or mixture organic solvents. The cleaning action of this method results from the dissolution of the film by solvent jointed to the mechanical action of the swabbing.

Concerning the peculiar application in artworks conservation field, solvents have to be selected on the basis of some requirements: a) good selectivity in cleaning, b) reasonable volatility, c) scarce flammability, d) low toxicity, e) chemical inertness (Rosati, 1991).

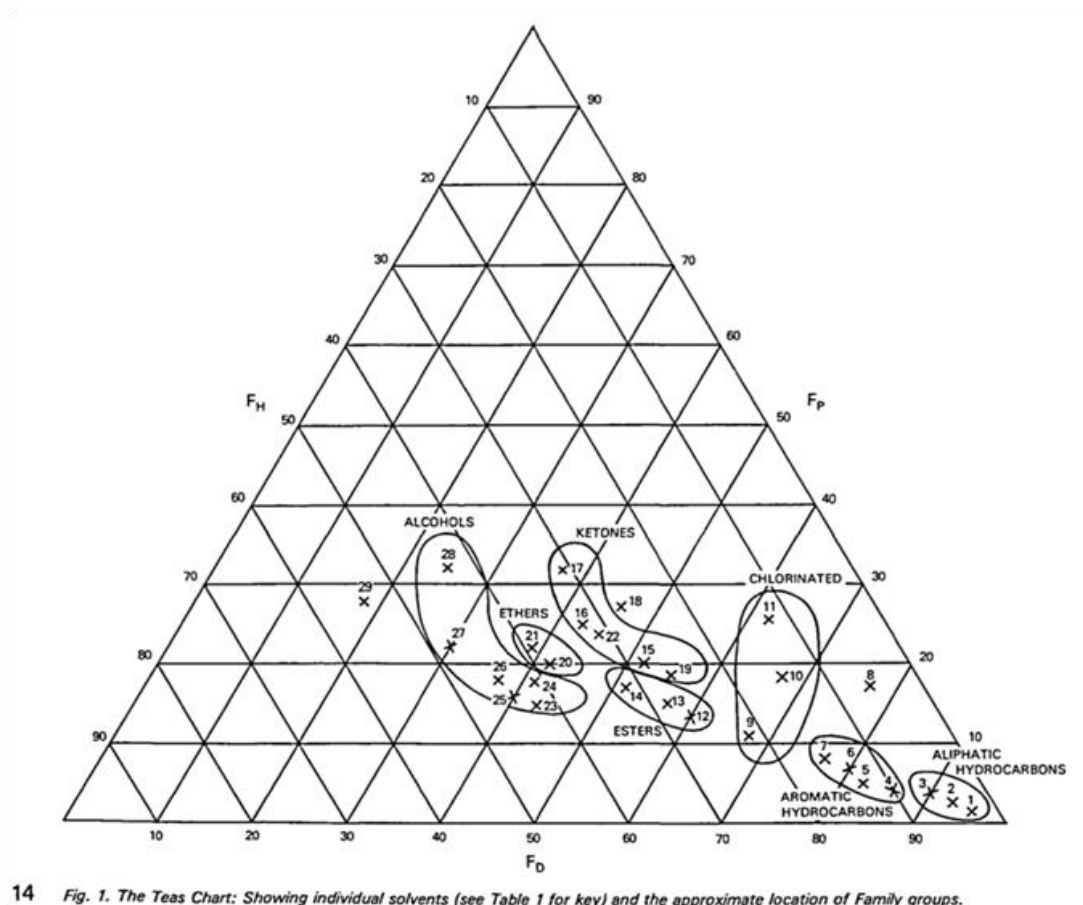
The principles of solvents selection were extensively described by Hedley (Hedley, 1980) starting from the concept of “like dissolves like”. A solution is basically defined as a homogeneous mixture of the molecules of solvent with those of solute. This means that for dispersion to occur the solute-solute and solvent-solvent intermolecular attractive forces must be broken down and solvent have to be able to disperse solute molecules. One of the best conditions for solubility occurs when the forces in the solvent are very similar to the forces in the solute.

The three categories of intermolecular forces involving electrostatic attraction between dipoles are: 1) dispersion forces ( $F_D$ )— present in all molecules, they result from the formation of temporary dipoles due to the motion of electrons. They are weak forces and predominate in hydrocarbons; 2) polar forces ( $F_P$ ) — these forces exist between molecules with permanent dipoles or in which a permanent dipole in one molecule induces a dipole in a neighbor; 3) Hydrogen bonding forces ( $F_H$ ) — these arise when a hydrogen

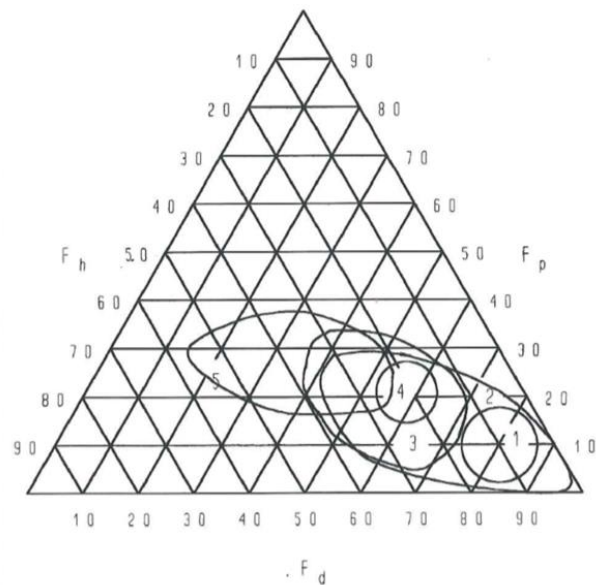
atom is bonded to a strongly electronegative atom like oxygen. A very strong dipole is created which can form a movable bond with a neighboring electronegative atom. All the three internal forces are involved in the cohesive energy density, the energy required to separate the attractive forces which hold the molecules together in the liquid state. These forces are measured as the energy required to vaporized a certain volume of solvent in liquid state (Hedley, 1980).

In the Teas Chart (1960s), solvents are located on the basis of  $F_D$   $F_P$   $F_H$  values, thus different solvent families show different location. This chart is commonly employed by restorers as a reference concerning solvent power and mixture power prediction. In fact solvents close to each other, will have similar power solvency. On the basis of “like dissolves like”, also coating materials such as varnishes, oil, wax etc., were plotted defining the region of solubility of certain natural materials reported in **Figure 1.4** (Cremonesi, 1997).

Because the susceptibility of painting materials to acidic or alkaline environment (the ground layer composed by gypsum and glue is susceptible in acidic environment as well as for cellulosic material, oily binder is susceptible to organic bases such as ammine), neutral organic solvents were the most employed in cleaning of polychrome substrate. Among these saturated and unsaturated hydrocarbons, aromatic and aliphatic, terpenic, chlorinated, esters, ketones, eters and alcohols were plotted in the chart.



**Figure 1.3** Teas Chart showing the approximate location of solvents families on the basis of polar forces ( $F_p$ ), dispersion forces ( $F_d$ ) and H-bonding forces ( $F_h$ ), relative values (Hedley, 1980).

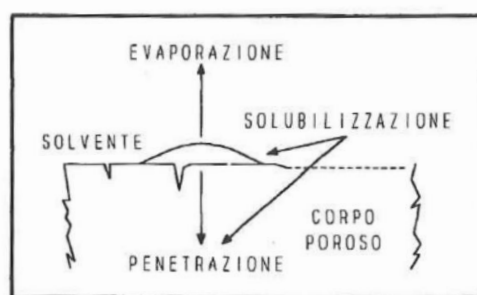


**Figure 1.4** Solubility areas of wax (1), oils (2), natural resins (3), aged oils (4), polysaccharides and proteins (5), plotted in Teas chart (Cremonesi, 1997).

Generally low  $F_D$  value corresponds to apolar materials, such as wax or synthetic varnish not oxidized. On the contrary extremely aged natural varnish has high  $F_D$  value.

In addition to the power solvency of a solvent, other factors have to be taken into account. Indeed when we consider a wet solvent applied on the surface of a polychrome artifact, such as painting, several factors play important roles at the same time, for instance the porosity of the surface and the diffusion rate of the solvent. Thus a portion of the solvent will tend to evaporate according to its volatility, and a portion will penetrate and spread across the surface toward internal layers (Cremonesi, 2000). Evaporation and diffusion are influenced each other thus it is not easy to predict the volatility of the solvent once applied over a porous surface and thus in which grade it will interact with the paint layer (**Figure 1.5**).

So it is important to underline that solvent power is not the only factor to be considered in cleaning. Rate of action, evaporation and diffusion need to be taken into account. More specifically: the evaporation is a physical phenomenon and is related to boiling point and vapor tension; diffusion/penetration is a physical phenomenon and is related to superficial tension and viscosity (Cremonesi, 2004).

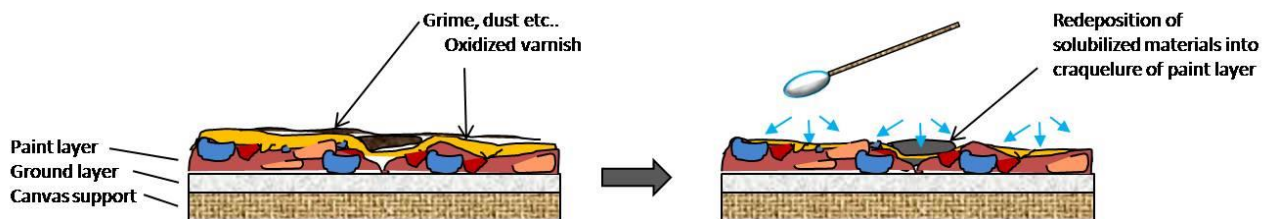


**Figure 1.5** Application of a solvent over a porous substrate (Cremonesi, 1997)



For the aforementioned difficulty in the prediction of liquid solvent action, once applied in cleaning practice, the principal drawback of the wet cleaning is the poor “selectivity” of the method. The solvent diffuses towards the substrate with unpredictable penetration depth reaching easily the paint layer. The interaction with the binder medium during time, may lead to visible alteration of the surface (as explained in the previous sections). Moreover the diffusion and re-distribution of the solubilized material across the porosity (**Figure 1.6**) is an important risk to be considered in case of particularly porous or damaged surface (P. Baglioni, Berti, et al., 2013; Antonella Casoli et al., 2014; J. Domingues, Bonelli, Giorgi, & Baglioni, 2014).

The low control of cleaning performance of a liquid solvent, together with the scarce environmental and healthiness security, lead to the search of new approaches.



**Figure 1.6** On the left, a schematic representation of uncleaned painting stratigraphy is reported; on the right the stratigraphy after swab cleaning, diffusion of solvent through cracks of the paint layer, redeposition of solubilized material on the surface and into craquelure happened.

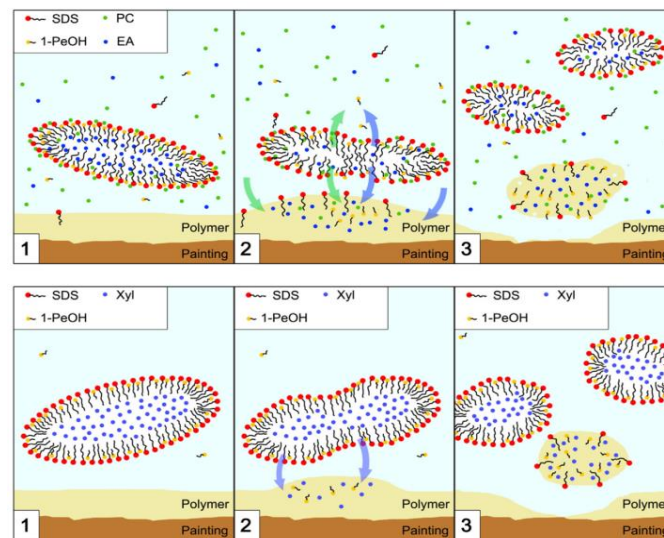
### 1.3.2 THE AQUEOUS APPROACH AND BIOCLEANING

With the aim to find alternatives to the use of commonly accepted toxic solvents, in 2000s water-based methods were proposed by Wolbers and later by Cremonesi in Italy.

Aqueous cleaning systems consists in the use of detergents and surfactants as a components able to lower the surface tension of the liquid enhancing the wettability and cleaning efficacy of water. The co-presence of these components demonstrated a satisfactory cleaning action (Cremonesi, 2004; Khandekar, 2000; Wolbers, 2000). Surfactants can be selected on the basis of two important parameters, the critical micellar concentration (CMC) and the hydrophilic-lipophilic balance (HLB). The latter is selected according to the hydro/lipo-philic character of the material to being removed and the extent of its polymerization. Several classes of surfactants are available for restoration practice but the most recommended are non ionic, such as polyethoxylates (e.g. Brij 35, TritonX100). Respect to ionic products, these class shows a low critical micellar concentration, thus a small amount of surfactants is required to forming the micelle, minimizing the risk to leave residues after cleaning. In fact, the major drawback of these cleaning method is that further clearances are required in order to eliminate surfactant residues (Cremonesi, 2004).

Recently nano-structured fluids such as micellar solutions and microemulsion were proposed as versatile cleaning systems with low environmental impact and toxicity. Nanostructured fluid act as a “container” for the solubilized material, limiting its diffusion into the paint layer (**Figure 1.7**). In particular, microemulsions, compared to micellar solutions, show a more controlled cleaning action due to the presence

of the solvent phase immiscible in the continuous phase (generally oil in water or water in oil). Thus the continuous phase can be hydrophilic (o/w) or hydrophobic (w/o) allowing a control in the spreading of the continuous phase into the artefacts to be treated. Moreover the spreading of the solubilised material into the porous matrixes may be limited, because solubilisation or swelling occurs into the core of nanodroplets and/or at the droplet interface (M. Baglioni et al., 2014, 2015; M. Baglioni, Berti, Teixeira, Giorgi, & Baglioni, 2012; P. Baglioni, Berti, et al., 2013; P. Baglioni & Giorgi, 2006; Emiliano Carretti, Fratini, Berti, Dei, & Baglioni, 2009; Emiliano Carretti, Giorgi, Berti, & Baglioni, 2007; Giorgi, Baglioni, Baglioni, & Berti, 2010; Grassi et al., 2007).



**Figure 1.7** Schematic representation of mechanism of interaction between the nanostructured system and the polymer coating (P. Baglioni, Berti, et al., 2013).

In biocleaning, microorganisms such as certain bacterial cells and enzymes are employed as “green” and safe method for the removal of inorganic patina and organic coatings (Lustrato, Alfano, Andreotti, Colombini, & Ranalli, 2012; Pereira et al., 2013; Ranalli et al., 2005). In particular hydrolases enzymes are able to catalyze hydrolytic processes of certain macromolecules, such as proteins and polysaccharides and lipids. The enzymes most used in cleaning belong to the class of lipase, capable to hydrolyze the ester-bond in triglycerides. The break of polymerized network leads to the formation of small molecules such as mono/di-glyceride or glycerol and respectively 1,2 or 3 molecules of fatty acids, extractable by aqueous medium. Generally lipase enzyme are employed for the removal of oily (aged and not aged) layer, for instance overpainting, but also application for the removal of acrylic varnish is reported in literature (Bellucci, Cremonesi, & Pignagnoli, 1999; Cremonesi, 1999). Enzymatic cleaning is considered a very specific cleaning method due to the extremely selectivity of the enzyme in the recognition of the specific substrate. Unfortunately, they required specific application condition such as a buffered solution and a range of temperature. Moreover enzymatic activity can be inhibited by certain heavy metals contained in pigments.

In conclusion, water-based solutions were enthusiastically adopted by restorers mainly because the total safety for the health of conservators. Moreover the possibility to hold the solution into a gelled network, allowed the reduction of the cleaning agent penetration and in this way a better control of the application.

Soon the limits of aqueous systems began to arise. As reported above, the application of water-based solutions requires the clearance of residues after treatment, moreover certain substrates may show a critical sensibility to water. In fact, even if water is a totally safe solvent for human health, it is a high polar solvent for materials. Water plays a double role: as a medium of reactions and as a solvent with high polarity and high capability of penetration into the microporosities of the substrate. Water proved to be particularly harmful for not aged protein-based materials like animal glue (used in ground preparation or as a binder) and egg-tempera medium (Karpowicz, 1981) and polysaccharides. The main effect caused by water is the swelling of some hydrophilic binders with the consequent contraction and wrinkle of the layer. In some cases may leach out small amount of amino acids from not aged film (A Casoli et al., 2010; Khandekar et al., 1994) or even inorganic pigments soluble in water, leading to the dissolution and migration of metallic ions (P. Baglioni, Bonelli, et al., 2015; Michalski, 1990). Investigations on modern art, reported the particular water-sensibility character of modern oil paintings date from the 1950s and 1960s (Burnstock, van der Berg, de Groot, & Wijnberg, 2006; Tempest, Burnstock, Saltmarsh, & van den Berg, 2013; Tsang & Erhardt, 1992).

For the aforementioned risks, water-based solutions cannot be used indiscriminately over all kind of surfaces and this is the major limit for this totally safe and eco-sustainable cleaning system.

### **1.3.3 THE GELLED SYSTEMS**

The use of gelling agents overcame some drawbacks related to the use of common wet cleaning, by enhancing the control of solvent diffusion and consequently its selectivity. From liquid state to viscous gel, the diffusion of the solvent is more focused on the very surface, thus its cleaning action is localized and more selective. Moreover they become a very useful tool when a prolonged contact with the surface is necessary and/or the mechanical action should be avoided (Stavroudis, Doherty, & Wolbers, 2005).

According to the definition, a gel is a material composed of subunits that are able to bond with each other obtaining a network of macroscopic dimensions. A gel has the mechanical properties characteristic of a solid, even though it is structurally disordered and indeed may contain a high volume fraction of liquid solvent (Jones, 2002). If the gel consists of linear segments joined together at cross link points, and the linear sections are flexible and long enough to be considered as random walks, the mechanical properties may be described by the theory of rubber elasticity. Gels may also go through a glass transition: gels with a high density of short rigid segments are likely to be glassy.

Gels are divided into two classes: 1) chemical gels - the bonds linking the subunits are covalent chemical bonds, 2) physical gels – the subunits are linked by physical interactions.

The most common gelled-systems employed in restoration belong to the class of physical gels, sometimes known as thermoreversible gels, since usually the physical interactions are disrupted by increasing the temperature. While chemical gels are recently proposed by researchers as higher performer container for cleaning agent.

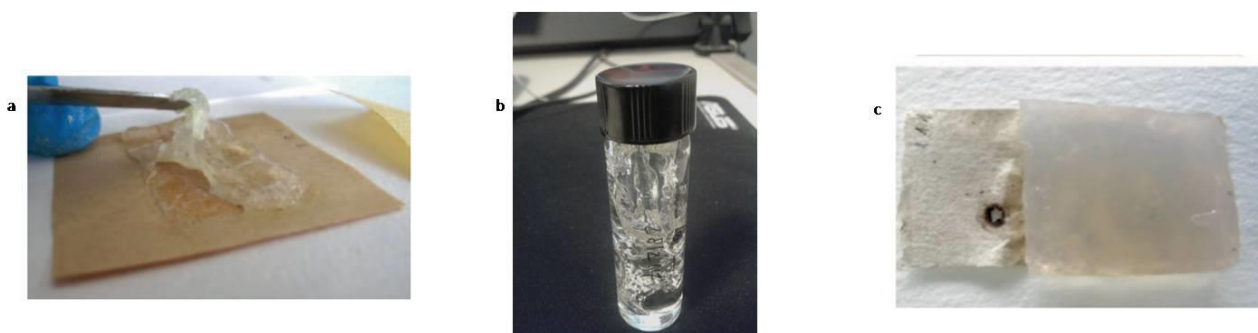
The first gel-systems employed in the cleaning of paintings were “thickeners”, polymers able to swell in the solvent increasing the viscosity of the solution. By thickening the solution, a reduction of solvent diffusion is achieved. The most employed materials in restoration are polysaccharide-based and synthetic polymers, described below.

Cellulose derivatives such as eters (methyl-, idroxypropyl-, methylhydroxypropyl-cellulose), were among the most employed in restoration. They are able to enhance the viscosity of the solution by forming a poultice. They are only soluble in water or polar organic solvents. Among the advantages in using cellulose ethers, is the low price of materials and the easy preparation. In the other hand they show a scarce power to retain the solvent and a loose consistence that tends to release residues on the surface (**Figure 1.8-a**). For these reasons the application of polysaccharide gels required an accurate removal followed by several clearance washings for the complete removal of residues. Moreover the residues can be attacked by microorganisms and are unstable to UV radiation (Cremonesi, 1997).

Among synthetic gelling agents, polyacrylic-based formulations were successfully introduced by Richard Wolbers in 1990s (Wolbers, 2000). The gelator is the polyacrylic acid which, at low pH, exists in a macromolecule folded conformation due to the H-bondings among protonated acidic groups (**Figure 1.8-b**). The addition of a strong basic component (e.g. polyethoxylate amine or inorganic base) made the acidic polymer partially salified, allowing the conversion to carboxylate anions. Then, the repulsive inter- and intra-molecular interactions between the negatively charged groups force the unfolding of the chains and a randomcoil conformation. In this way the solubilisation is maximised and the formation of hydrogen bonding between different chains favoured, forming the 3D physical network that constitutes the skeleton of the gel (P. Baglioni, Berti, et al., 2013).

Wolbers’s gel, commercialized as Carbopol® (polyacrylic acid) and Ethomeen® (poly-ethoxylated cocoalkylamine), has been widely used from restored around the world and still finds effective application (Emiliano Carretti, Grassi, et al., 2009). The main advantages of Carbopol gel, compared to polysaccharide-base gel, lies in the higher thickener properties and in the detergent/surfactant properties of Ethomeen component. This latter in fact is an alkaline surfactant that can be choose on the basis of the polarity of the solvent that has to load. Thus Carbopol-system can form a highly viscous solution either with polar and less polar solvent. Unfortunately it was demonstrated that residues of the surfactant remained in the substrate after cleaning, entailing a dramatic risk due to its alkaline nature and high penetrative capability (Burnstock & White, 2000; Antonella Casoli et al., 2014; Khandekar, 2000; Stulik et al., 2000, 2009).

Other polysaccharide compounds are agar agar and gellan gum, the latter mainly reported for the cleaning of paper and manuscripts (Mazzuca, Micheli, Carbone, et al., 2014; Mazzuca, Micheli, Cervelli, et al., 2014). Agar is a complex polysaccharide composed of two different polymers: agarose, a linear-chain, neutral, and high-molecular weight polymer responsible for the gelation; and agaropectin, the same basic structure containing methyl, sulphate, and pyruvate substituent groups. At high temperature agar can form, with water or water-based detergents, a “rigid” gel capable to hold a great amount of water within its macro-reticulate structure (**Figure 1.8-c**). The adhesiveness to the surface is low, thus it generally require no aqueous clearance procedures (Cremonesi, 2015). Gellan gum is a linear, anionic heteropolysaccharide produced by microorganisms. As well as agar is capable to gelifying water-solutions, in the presence of calcium salts, forming an hard and rigid hydrogel. Moreover it is homogenous, transparent, and stable to pH variations (Iannuccelli & Sotgiu, 2010; Mazzuca, Micheli, Carbone, et al., 2014). Because of the rigidity of the “rigid” network, these gels are less prone to stick to the surface and thus to leave gel residues after cleaning. However, the water retention capability is not enough to treat water sensitive paintings and the excessive wetting surface can result in an undesired colors leaching (P. Baglioni, Berti, et al., 2013; J. A. L. Domingues et al., 2013).



**Figure 1.8** Images of physical gels: **a)** hydroxypropylcellulose Klucel® gel, **b)** Carbopol-Ethomeen® gel, **c)** Agar rigid gel. Images a) and b) come from (P. Baglioni, Chelazzi, & Giorgi, 2015).

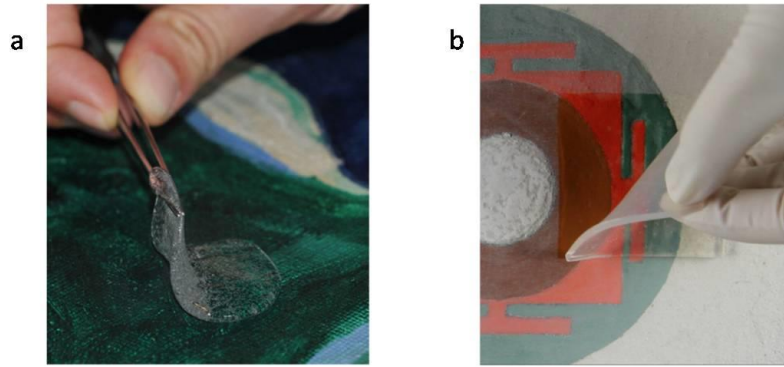
Summarizing the main drawbacks of these existing physical gels, lie in the scarce power to retain the solvent and the tendency to leave gel residues on the surface after application. The gelation process for these thickeners is a physical phenomenon, the polymer chains swell and weak interactions stabilized the highly viscous dispersion. From a rheological point of view they cannot nether be considered properly gels but “gel-like” systems . Because of the weak interactions among polymer chains, the network is not strong enough to be removed without leave residues. Several washings with water or solvent, even if less polar compared to the cleaning solvent, are required. The clearance procedure exposes the already treated surfaces to further risks linked to the application of solvent in a free-state (Cremonesi, 2004).

In last decades, a great contribution to the development of confining systems for the cleaning of paintings was carried out. Of particular interest are the polyvinylalcohol-based gels developed by Carretti (Angelova et al., 2011; E Carretti, Matarrese, Fratini, Baglioni, & Dei, 2014; Emiliano Carretti et al., 2010;

Emiliano Carretti, Dei, Weiss, & Baglioni, 2008; Emiliano Carretti, Grassi, et al., 2009; Grassi et al., 2007; Natali et al., 2011). Polyvinyl alcohol (PVA) is a biodegradable and biocompatible polymer soluble in water, by the addition of different cross-linkers is able to form chemical gel (for instance by adding glutaraldehyde) or physical gel through H-bonding with hydroxyl groups. Aqueous solutions of PVA in presence of boron ions form highly viscous systems thermoreversible due to cross-linking intra and intermolecular between PVA chain and ions (Angela, Berrie, Getaldi, Kerr, & Weiss, 2013; E Carretti et al., 2014; Emiliano Carretti et al., 2010; Natali et al., 2011). PVA-borax formulation does not have the rheological characteristic to being defined properly a gel, but a “high viscosity polymer dispersion” (P. Baglioni, Berti, et al., 2013; Emiliano Carretti et al., 2010). The advantages of PVA-borax gel are the good elasticity and viscosity that confer a good contact with the surface and “peelable” properties that do not required further clearance after application (**Figure 1.9-a**). Moreover the visco-elastic properties are tunable and it can be loaded with aqueous detergent solutions or organic solvent as co-solvents. It found application, for instance, in the removal of varnish from tempera paint layer (E Carretti et al., 2014; Emiliano Carretti et al., 2010).

Further improvements in the retentive capability and cohesive forces of the gel were obtained with the development of “chemical gels”. In this class, the bonds that link the subunits are covalent chemical bonds. This property leads to higher network cohesion and less probability to leave gel residues over the surface after cleaning (Pizzorusso et al., 2012). An example of chemical gel applied for the cleaning of paintings is the acrylamide/bis-acrylamide hydrogels (P. Baglioni, Chelazzi, et al., 2013; Pizzorusso et al., 2012). Another hydrogel system is based on semi-interpenetrating poly-hydroxyethyl methacrylate linked with polyvinyl pyrrolidone (pHEMA/PVP)(J. A. L. Domingues et al., 2013) (**Figure 1.9-b**). The HEMA polymer confers mechanical strength to the network while PVP the hydrophilicity (J. Domingues et al., 2014). Semi-interpenetrated polymer network, compared to acrylamide-hydrogel, are more hydrophilic and more retentive, indeed suitable for the cleaning of water-sensitive surfaces such as hydrophilic soiling over acrylic tempera and adhesives from canvas paintings (J. Domingues, Bonelli, Giorgi, Fratini, & Baglioni, 2013). Moreover they can load either with aqueous formulation and polar solvents.

A completely water-free system was recently developed by Baglioni. It consists of a chemical organogel based on the polymerization of methyl methacrylate (MMA) and a cross-linker (usually a divinyl-group molecule) in a solvent solution. The gel showed the capability to load organic solvents of different polarities and to be efficaciously applied on the surface of canvas painting samples for the removal of varnish layer (P. Baglioni, Bonelli, et al., 2015).



**Figure 1.9** Chemical gels **a)** polyvinylalcohol-borax, **b)** semi-interpenetrating poly-hydroxyethyl methacrylate linked with polyvinyl pyrrolidone (Giorgi, Dei, Ceccato, Schettino, & Baglioni, 2002).

As conclusion for this brief review on materials and methods used for the cleaning of artworks, is important to highlight the evolution, during time, towards a more respectful solutions either for artwork-material, human healthiness and environment.

Scientific research gave and gives a great contribution to the understanding of phenomena and mechanisms of alteration and interaction with the aim of being a “objective” support in the development of new methods. Because of the enormous parameters which affected a cleaning procedure, such as factors coming from materials and interactions with the environment, is still difficult to certainly predict the performance of cleaning on a extremely complex substrate like as multilayered painting. Thus the direction is to develop a method capable to maximize the solvent action to the specific layer to be removed and minimize the interaction with others. Moreover the increasing demand of totally safe solutions arose also in conservation field, where restorers are directly expose to harmful vapors and substances, often without the adequate systems of protection. Finally according to the principle of circular economy, from renewable to biodegradable is surely the direction of the future!

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## Chapter 2. EXPERIMENTAL

In the following section the analytical techniques employed both for the evaluation of the cleaning performances and for the gels characterization will be presented. Moreover the preparation of paint laboratory reconstructions, employed as testers, will be reported.

### 2.1 THE ANALYTICAL TECHNIQUES

Concerning the evaluation of cleaning performances, an analytical protocol was specifically tailored according to the methodological approach required in Cultural Heritage field. Thus the employment of either non-invasive and micro-disruptive techniques allowed the exhaustive investigation of treatment efficacy and effects on the painted surface. The role of scientific investigations is to inform, guide and improve the art and craft of cleaning practice to help minimize the risk to objects and maximize the benefits of picture being displayed to their best advantage (Phenix & Sutherland, 2001).

Gel characterization, investigation of gelation process and mechanical properties were carried out with calorimetric analysis, rheological measurements, diffractometric data.

#### 2.1.1 OPTICAL MICROSCOPY (OM)

Samples observation under Optical Microscope allows the magnification of particulars otherwise not visible. In particular employing a microscope equipped with both visible and ultraviolet light (UV), enable the distinction of certain materials. The examination of paintings by UV light started since the 1920s. The range closest to visible light (UV 320-400nm) is the most useful for the examination of easel paintings. This range has the ability to excite visible fluorescence in many organic (and a few inorganic) materials, including varnishes, paint media and some pigments. With this ability to render some materials layer more visible, UV examination may clarify the distribution of the varnishes and binders in a multilayered stratigraphy. Moreover can provide valuable information about the materials, pigments and media and their ageing. Indeed fluorescence of resin varnishes intensifies as the film ages and yellows because the oxidation processes results in increased absorption of the longer wavelengths of UV energy and of the shorter wavelengths of visible light. (AA.VV., 2012; Bitossi, Giorgi, Salvadori, & Dei, 2005; de la Rie, 1982; Pereira et al., 2013; Ploem & Tanke, 1987; Sandu, Schäfer, Magrini, Bracci, & Roque, 2012).

In this study, OM was employed for the observation of cross sections, conveniently prepared, in order to evaluate the thickness of the layers (especially varnish), efficacy and homogeneity of the varnish removal after cleaning, effect of cleaning and interaction with the paint layer underneath, stratigraphy of unknown samples and distribution of components.

Paint cross-sections were prepared by using double embedding system with KBr salt and synthetic polyester resin (Prati, Sciutto, Catelli, Ashashina, & Mazzeo, 2013). This preparation modality allowed the

visualization and the analysis on the external varnish layer without any interference with the synthetic resin for the embedding.

Photomicrographs of standard and historical samples were obtained by using an Olympus (Olympus Optical, Tokyo, Japan) BX51 microscope equipped with an Olympus DP70 digital scanner camera. A 100-W halogen projection lamp and a Ushio Electric (USHIO Inc, Tokyo, Japan) USH102D ultraviolet (UV) lamp were employed for the acquisition of visible and fluorescent images, respectively.

### **2.1.2 $\mu$ FTIR SPECTROSCOPY IN TOTAL ATTENUATED REFLECTION (ATR)**

Fourier-Transform Infrared (FTIR) spectroscopy is an absorption technique useful for the easy and fast identification of chemical compounds providing information about the chemical bonding or molecular structure of material, whether inorganic or organic. It finds application in the identification of a huge variety of materials in Cultural Heritage field analyzing binding media, varnishes, grounds and pigments (Bitossi et al., 2005; Derrick, Stulik, & Landry, 1999; J. S. Mills & White, 1994; Stuart, 2007) and their possible degradation products (Erhardt, Tumosa, & Mecklenburg, 2005; Lazzari & Chiantore, 1999; Manzano, Navas, Checa-Moreno, L.Rodríguez-Simón, & Capitán-Vallvey, 2009; R. Mazzeo et al., 2008; Plater et al., 2003; Ren, Atlasevich, Baade, Loike, & Arslanoglu, 2016). Analyses carried out in total attenuated Reflection (ATR) allow to obtain information about the chemical composition of the matter without any preparation of the sample. ATR is satisfied by three conditions: 1- the IR radiation passes from an higher refractive index medium (the crystal) to lower index medium (the sample), 2- the ATR crystal is in contact with the sample surface, 3- the radiation incidence angle is higher than the critical angle. Once satisfied these conditions, the incident IR radiation enters into a superficial area of the less dense medium (the sample) as an evanescent wave. The penetration depth of this wave is a function of wavelength, refractive index of materials and incidence angle of the radiation. Because the sample (less dense medium) absorbs the evanescent wave in correspondence of the wavelength of absorption bands, the technique is named Total Attenuated Reflection (Skoog, Holler, & Crouch, 2009).

In this research  $\mu$ FTIR in total attenuated reflectance (ATR) mode was performed (1) directly over the surface after treatment, without any sample preparation, in order to assess non-invasively the removal of the varnish and the releasing of cleaning residues and (2) in cross section for the identification and localization of organic and inorganic components into a multilayered sample, by mapping a small area (Joseph et al., 2010; R. Mazzeo et al., 2008; Rocco Mazzeo, Joseph, Prati, & Millemaggi, 2007; Prati, Sciutto, Bonacini, & Mazzeo, 2016; Reffner & Martoglio, 1995). Identification of components was assigned by comparison with reference spectra reported in literature (Derrick et al., 1999; Doménech-Carbó, 2008; Doménech-Carbó, Comenech-Carbó, Gimeno-Adelantado, & Bosch-Reig, 2001; Meilunas, Bentsen, & Steinberg, 1990; J. S. Mills & White, 1994; J. Mills & White, 1977; Osete-Cortina & Doménech-Carbó, 2006; Tomasini, Siracusano, & Maier, 2012).

A Thermo Nicolet (Thermo Fisher Scientific, Waltham, MA, USA), iN™10MX imaging microscope, fitted with a mercury-cadmium-telluride (MCT) detector cooled by liquid nitrogen, was used for spectroscopic analyses. Measurements were performed using a slide-on ATR objective, equipped with a conical germanium crystal, in the range 4,000–675 cm<sup>-1</sup>, at a spectral resolution of 4 cm<sup>-1</sup>. (1)  $\mu$ ATR spectra were acquired on the surface of the mockup before and after the treatment with an optical aperture of 200x200  $\mu$ m<sup>2</sup> relative to an investigation area of about 50  $\mu$ m<sup>2</sup> for each point of analysis. (2) Mapping analyses were performed on the historical sample to characterize the stratigraphic composition of the painting layer and varnish by adjusting the numerical aperture according to the thickness of the layers under investigation.

Moreover FTIR analyses in reflection-absorption spectroscopy (RAS) on gold plate, were performed for the analysis of neat liquid solvents  $\gamma$ -Valerolactone, ethyl lactate, dimethyl carbonate, acetone, dimethyl sulfoxide, benzyl alcohol and the plasticizer triethyl citrate in order to assess the residues of solvent. Peak assignment was carried out by comparison with literature references (Aparicio & Alcalde, 2009; Silverstein & Webster, 2001).

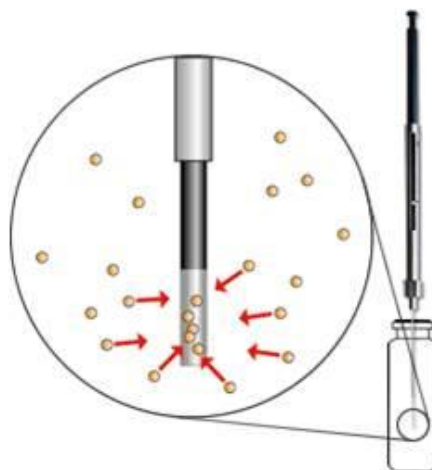
### 2.1.3 HEAD SPACE-SOLID PHASE MICREXTRACTION -GC/MS (HS-spme)

Gas chromatography mass spectrometry is a very useful molecular technique employed for the analysis of organic components in mixture. Indeed separation of components into the chromatographic column is coupled with mass spectrometer analyzer allowing the identification of low amount of analyte. In this study Headspace Solid Phase Microextraction (HS-SPME) sampling method was used for the extraction of low volatile components from solid matrix (a fragment of paint layer). It consists in an equilibrium sampling method among the concentration of the analyte in the sample, in the headspace above the sample and in the polymer coating on the fiber. According to the partitioning equation of analytes between the sample matrix and the extraction medium (eq.1), the amount of analyte absorbed by the coating at equilibrium is linearly related to its concentrations in the sample

$$n = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + V_s} \quad (\text{eq.1})$$

where  $n$  is the mass of an analyte absorbed by the coating;  $V_f$  and  $V_s$  are the volumes of the stationary phase (coating) and the sample, respectively;  $K_{fs}$  is the partition coefficient of the analyte between the coating and the sample matrix; and  $C_0$  is the initial concentration of the analyte in the sample.

Sensitivity of SPME method can be improved by increasing the fiber coating thickness ( $V_f$ ), the equilibrium time or increasing the  $K_{fs}$  (by changing the fiber coating or optimizing the temperature of extraction)(Wercinski & Pawliszyn, 1997).



The method is considered simple, rapid, solvent-free (thus sustainable) and very sensitive. Can be used for the extraction of a wide range of analytes (volatile and semivolatile) from a different range of matrices. Moreover the selectivity can be varying by changing the coating or the thickness of the fibre (Colombini & Modugno, 2009; Lai, Corbin, & Almirall, 2008; Louch, Motlagh, & Pawliszyn, 1992; Turner, 2013; Wercinski & Pawliszyn, 1997; Zhang & Pawliszyn, 1993; Zhang, Yang, & Pawliszyn, 1994).

In this research the sampling method HS-SPME was employed for the evaluation of solvent retention into the substrate after cleaning treatments. Analysis were performed by directly exposing a Carboxen-Polydimethylsiloxane (CAR/PDMS) fibre into the headspace of sealed vial containing the sample. The sample consisted in a fragment (about 1 mg) collected from the surface of the painting mockup after fixed time from cleaning treatments (1, 24 hours and 3 days in some tests). Then the sample was placed into 20ml HS vial, spiked with 1 µg of internal standard ( $\gamma$ -butyrolactone) and sealed with a silicone/PTFE septa and aluminium cap (Thermo Fisher Scientific, Waltham, MA, U.S.A.). The SPME fiber was inserted into the headspace vial and the sample was thermally heated to 150°C for 15, 30, 45 and 60 min. This preliminary kinetic experiment allowed to determine the optimal time of exposure (30 min) at which all further experiments were performed (Ghidotti, Fabbri, & Hornung, 2016; Rombolà et al., 2015). After reaching the extraction time, the fibre was inserted into the injector of a 5977 Agilent gas chromatograph connected to a 7820A Agilent quadrupole mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.). Analytes were thermally desorbed at 250°C for 10 min and separated with a DB-FFAP polar column (30 m length, 0.25 mm i.d, 0.25 µm film thickness), using helium as carrier gas. The thermal program was: 100°C to 250°C at 10°C min<sup>-1</sup>. The abundances of the individual compounds were quantified from the m/z 86 for  $\gamma$ -butyrolactone, 100 for  $\gamma$ -valerolactone, 75 for ethyl lactate, 45 for dimethyl carbonate, 79 for benzyl alcohol, 63 for dimethyl sulfoxide mass chromatograms. Calibration curves were performed for each solvent in the concentration range 500–10000 ppm and were drawn by regression method. The areas corresponding to this range of concentration of standard solutions are in accordance with those obtained by sample measurements. The curves, obtained by applying the same procedure described above to standard solutions, showed a good linear response ( $R^2 > 0.9$ ) in the considered concentration range.



### 2.1.4 LASER 3D MICROPROFILOMETRY

Laser scanning microprofilometry was applied after cleaning treatment for monitoring the morphology of the surface (Fontana et al., 2015). An area of 3x4 cm, step 50  $\mu\text{m}$  was analyzed in Cimabue's painting. Depth resolution and trasversal resolution of the technique employed was 1 and 20  $\mu\text{m}$ , respectively.

### 2.1.5 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Aimed at the characterization of thermal behaviour of gels, Differential Scanning Calorimetry (DSC) were performed by two successive heating cycles in order to check the thermoreversibility of the systems. The TA Instrument DSC Q2000 Modulated apparatus equipped with a RCS90 cooling system was used. Measurements were carried out on about 5 mg samples adopting the temperature programs: I scan heating from 0°C to 100°C for gels (in order to being below the temperature of gelation 130°C) and from 40°C to 180°C for pristine P(3)HB; II scan controlled cooling; III scan heating as previously. Heating/cooling rate at 10°C/min under nitrogen atmosphere. Melting temperature ( $T_m$ ) and melting enthalpy ( $\Delta_m$ ) were obtained from thermograms and cristallinity values ( $X_c$ ) were calculated by the following equation  $X_c = \Delta H_{\text{PHB}} / \Delta H_{0\text{PHB}} * 100$ , where  $\Delta H_{\text{PHB}} = \Delta H / \% \text{wtPHB} * 100$ , and  $\Delta H_{0\text{PHB}}$  is 146 J/g the melting enthalpy for a theoretical 100% crystalline PHB (Barham, Keller, Otun, & Holmes, 1984).

### 2.1.6 WIDE-ANGLE X-RAY SCATTERING (WAXS)

Wide-angle X-ray diffraction contributes to the elucidation of the molecular organization especially when microcrystals are formed in agel-system . The long distance spacing ( $d$ ) obtained from XRD represents the longest repeat distance in the ordered structures by molecular self-assembly, which may provide insight into the packing of small molecules in either an extended or a bent conformation (Du, Zhou, Shi, & Xu, 2015).

In this study WAXS investigation was performed in order to attribute the endothermic transitions revealed by DSC analyses. Measurements were carried out at room temperature with a PANalytical X'Pert PRO diffractometer equipped with an X'Celerator detector. A Cu anode was used as X-ray source (K radiation:  $\lambda = 0.15418 \text{ nm}$ , 40 kV, 40 mA), and  $\frac{1}{4}^\circ$  divergence slit was used to collect the data in  $2\theta$  range from  $2^\circ$  to  $60^\circ$ .

### 2.1.7 RHEOLOGY

The basic principle of oscillatory rheometry is to measure the response of the material to an applied oscillatory stress, which is quantified by the elastic properties, such as  $\eta^*$  (complex viscosity),  $G'$  (storage or elastic modulus), and  $G''$  (loss modulus or viscosity) (Du et al., 2015; Jones, 2002; Stokes & Frith, 2008). Oscillatory shear measurements were carried out on a Paar Physica UDS200 rheometer working at 25°C ( $\pm 0.1^\circ\text{C}$  Peltier temperature control system) using plate-plate geometry (25mm diameter). Frequency sweep

measurements were carried out at 5% strain. The storage and loss moduli and complex viscosity were measured over the frequency range 0.1 to 100 Hz.

### **2.1.8 GEL PERMEATION CHROMATOGRAPHY (GPC)**

In order to evaluate the stability of the gel during time, measurements on the average molecular weight of the polymer were determined by size exclusion chromatography (SEC) in a time frame of 11 weeks. Samples were dissolved in chloroform and a HPLC Lab Flow 2000 apparatus was used, working with a 1 mL/min flow, equipped with an injector Rheodyne 7725i, a Phenomenex Phenogel 5u 10E6A column and a Refractive Index detector Knauer RI K-2301. Each sample was previously filtered with a 0.45  $\mu\text{m}$  porosity Teflon filter and sample injection volume was set to 20  $\mu\text{L}$ . Calibration curves were obtained using several monodisperse polystyrene standards in the range between 0.2 and 3 MDa.

### **2.1.9 THERMOGRAVIMETRIC ANALYSIS (TGA)**

The capacity of the gel network to retain the solvent and thus reduce its evaporation rate, was evaluated by thermogravimetric analysis. TA Instruments model STD-600 was used. Analyses on gels (about 25 mg) and neat solvents (sample weight about 25 mg) were performed under nitrogen flow, an isothermal run at 40°C for 90 min was selected as the most similar to the exposition condition of the restorer during cleaning practice.

### **2.1.10 SCANNING ELECTRON MICROSCOPY (SEM)**

Morphology of the dry gels were taken with a Scanning Electron Microscope (SEM) ZEISS EVO 50 EP in Environmental mode with  $\approx 100$  Pa pressure in the chamber.

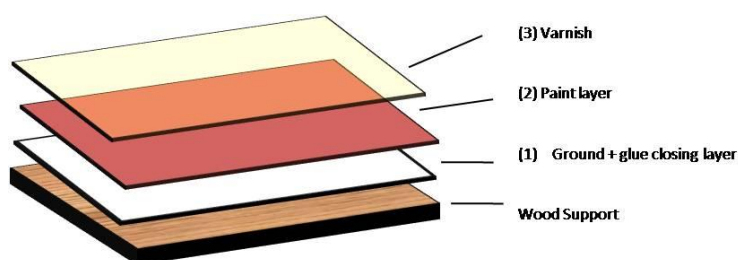
### **2.1.11 BIODEGRADABILITY ESSAY**

The biodegradation of the gel 1-9 (PHB-GVL-TEC) was determined by a ready biodegradability test in an aerobic aqueous medium according to the OECD guideline 301F, "Manometric respirometry" (Samorì et al., 2016). The test medium was prepared by adding specific concentrations of mineral components from stock solutions (potassium and sodium phosphates plus ammonium chloride, calcium chloride, magnesium sulfate and iron (III) chloride) to distilled water. The bacterial inoculum, derived from an activated sludge taken from a treatment plant receiving domestic sewage located in Ravenna, Italy, was aerated in mineral medium for 5 days at the test temperature. The biodegradability tests were carried out in bottles for 28 days at  $20 \pm 2^\circ\text{C}$ . Each compound and glucose (reference compound) were tested in duplicate, run in parallel with a blank (containing only inoculum). The test concentration and the corresponding Theoretical Oxygen Demands (ThOD) of each compound have been reported in Table 5s. The ThOD values for PHB and gel 1-9 were calculated on the basis of the carbon content determined by using an elemental analyzer (Thermo

Scientific, Flash2000, Organic Elemental Analyzer) by means of the flash combustion technique, whereas for GVL, TEC and glucose according to the OECD protocol. The consumption of oxygen was determined by measuring the change in pressure in the apparatus. Evolved CO<sub>2</sub> was absorbed in a solution of KOH. The amount of oxygen taken up by the microbial population during biodegradation of the test substance (corrected for uptake by blank inoculum) was expressed as a percentage of ThOD.

## 2.2 SAMPLES PREPARATION

Paint laboratory reconstruction were prepared with a complete stratigraphy as ancient reported.



The ground layer consists on a mixture of gypsum and rabbit glue. First the glue (2g) is melted in hot water (18 ml), then when the solution is homogeneous 32g of gypsum are added and homegenized. Over the ground layer a glue closing layer is applied, melting glue in water with a ratio 1:2.

Three different kind of paint layer were prepared by using different medium: egg, linseed oil and glue. The egg tempera binding medium is obtained by mixing egg white (previously foaming and deposited again), egg yolk and water in the ratios 1:1:0.5. Then about 1.5 ml of binder is mixing with 2 g of pigment. Oil painted surface was prepared just adding 1g of pigment to about 0.5 ml of linseed oil. Glue binder was obtained by melting rabbit glue (6%wt) in water; then about 0.6 ml of binder were mixed with 1g pigment.

Two kinds of varnishes were applied over different binding media: dammar and Paraloid B72<sup>®</sup> varnishes. Dammar resin was dissolved in turpentine (1g of resin in 2.5 ml of solvent), while Paraloid was dissolved in acetone (solution at 15%).

The mock-ups were used for the cleaning tests here reported 1 year after their preparation (not-aged mock-ups).

Aged standard samples were prepared in 1970s applying several layers composed by silicate-based pigments mixed with an oily binder and an acryli medium. The exact composition of each layer was not documented because of out the purpose of this research. In January 2016, a dammar varnish was applied on the surface of the mockup and then artificially aged using the following protocol: 336 h in a dark chamber at 50°C and 30- 80 RH, followed by 336 h in a light chamber with a fluorescent daylight tube (10,000 lux) at 23°C.

### **2.3 MATERIALS**

All solvents and chemicals used in this study were obtained from Sigma-Aldrich (purities  $\geq 98\%$ ) and used without purification. Standard P(3)HB was purchased from Biomer (DE). Carbopol® UTREZ 21 and Ethomeen® C25, Klucel G, Japanese paper were purchased from CTS. Paraloid®B72, dammar resin, malachite and red burnt ochre pigment were purchased from Kremer. Silica abrasive cards used in the preparation of paint cross- section were purchased from Micro-Surface Finishing Products Inc., Wilton, IA.

Painting mockups was kindly provided by the Cultural Heritage Agency, Ministry of Education, Culture and Science of Amsterdam (The Netherland) in the frame of the European project “Iperion”.

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## Chapter 3. GREEN PHB-BASED ORGANOGELS FOR THE CLEANING OF PAINTINGS

During time, the increasing attention towards healthiness and environmental issues (Sheldon, 2014) has gone ahead in all sectors of chemistry, until reach the field of chemistry applied to Cultural Heritage. Indeed the use of not standardized restoration procedures, together with the employment of hazardous or toxic substances, have been the basis for further research of more “green” and safe solutions. The particular interest, in conservation field, for this issue is demonstrated by the growing number of international conferences and meetings proposed in the last few years (Balliana, Ricci, Pesce, & Zendri, 2016). They prove the demand to strengthen regulations and recommendations in matter of safeness and security for both the human healthiness and the environment in restoration practices at international level, aimed at the “sustainability in restoration”.

Concerning the specific issue of painting cleaning, the protagonists of sustainability become methods which employ non toxic substances and with low environmental impact. Despite the great development of “safe” cleaning methods based on aqueous approach or bio-approach (e.g. enzymatic cleaning), drawbacks related to their application limits are still present. Recent scientific researches proved that gel-system is the promising direction for cleaning, thanks to the enhanced selective and controlled cleaning action. Moreover they reduce the evaporation of the solvent and thus the risk of inhalation is limited.

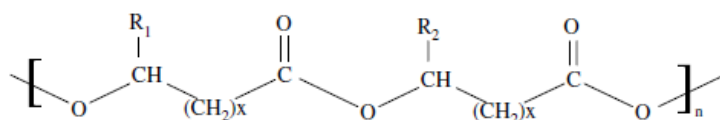
In the following sections, totally sustainable approaches will be presented for the cleaning of paintings. Several gelled-systems based on poly-3hydroxybutyrate (PHB) bio- polymer and different green solvents ( $\gamma$ -valerolactone, ethyl lactate and dimethyl carbonate) will be discussed as a cleaning tool for the removal of coatings from painted surfaces. Cleaning tests for the removal of natural varnish laboratory paint reconstructions and real canvas paintings will be extensively reported. The cleaning performances were evaluated by the analytical protocol described in Chapter 2, employing both microdestructive and non-invasive techniques as required in Conservation field. The cleaning capability of the PHB-gels were compared to traditional solvents applied by swab and in a gelled form in order to evaluate advantages and disadvantages of the new systems respect to the consolidate and widely used cleaning methods. Varnish removal efficacy, morphological alteration of the paint layer and retention of the solvent were the parameters adopted for the overall evaluation of the cleaning performances.

### 3.1 PHB bio-polymer

In recent years, materials from renewable and sustainable resources have been developed for in several sectors thanks to their environment friendly aspect. Biodegradable polymers such as poly-hydroxyalkanoates (PHAs) received large attention thanks to their biocompatibility and biodegradability properties (Scalioni, Gutiérrez, & Felisberti, 2017). They have attracted commercial interest as plastic materials because of their similarities in physical properties with synthetic polymers such as polypropylene,



but they presents useful properties such as biodegradability and biocompatibility (Akaraonye, Keshavarz, & Roy, 2010; Somleva, Peoples, & Snell, 2013). PHAs are linear polyesters that are synthesized and intracellularly accumulated as inclusions in a wide variety of bacteria, through the aerobic conversion of various carbon sources (Bengtsson, Pisco, Johansson, Lemos, & Reis, 2010; Samorì et al., 2015). Their composition is governed by two main factors: the bacteria strain and the carbon source used to grow the bacteria (Cervantes-Uc et al., 2014).



**Figure 1.** General structure of polyhydroxyalkanoates<sup>6</sup> ( $R_1/R_2 =$  alkyl groups  $C_1-C_{13}$ ,  $X = 1-4$ ,  $n = 100-30000$ ).

**Figure 3.1** General formula of Poly-hydroxyalkanoates (Akaraonye et al., 2010)

Poly(3-hydroxybutyrate), (PHB) is a homopolymer belongs to PHA family and in particular to the class of short chain length (scl) polymers, consisting in 3-5 carbon atoms containing monomers. A sustainable scale production of PHB from renewable feedstocks such as oil palm, potato, tobacco, flax, rice, sugarcane, switch grass, and other plants is reported in literature (Scalioni et al., 2017). These biopolymers are produced intracellularly by bacteria through aerobic fermentation of various carbon sources and are completely biodegradable under aerobic and anaerobic conditions. Their elastomeric/ thermoplastic properties are tunable according to actual co-monomer composition. The most efficient method for the extraction of the polymer from the bacterial cell, is gained by a variety of organic solvents such as chlorinated solvents (chloroform and dichlorometane are the best performers). But in order to limit the use of hazardous solvents, in the past few years, some green alternatives were found such as ketones, cyclic or alkyl carbonate (Fiorese et al., 2009; Samorì et al., 2015). Recently has been found that lactones, such as  $\gamma$ -valerolactone and  $\gamma$ -butyrolactone, are able as well to dissolve PHB forming a gel-like material (Samorì et al., 2016).

PHB polymer is crystalline, quite brittle and stiff, due to the high degree of crystalline phase arising from the high stereoregularity of biosynthesized molecules. Is a thermoplastic material with a melting point of 180°C and on cooling slowly from the melt, crystallizes to form large spherulites. Alternatively it may be quenched to a glassy state with  $T_g$  5°C or precipitated from dilute solution to produce thin lamellar crystals (Barham, Keller, Otun, & Holmes, 1984). Its physical and mechanical properties are strongly affected by the aging history, since its crystallization is slow and characterized by a lower nucleation rate compared with the growth rate, which results in mechanical fragility (Scalioni et al., 2017).

### 3.2 PHB-GVL-TEC gel

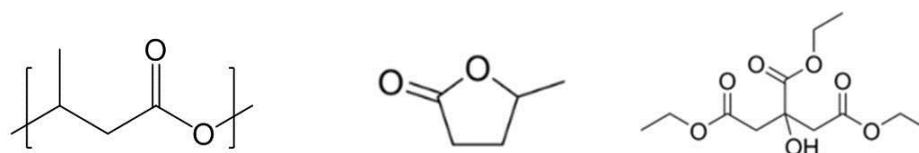
A totally safe and bio-based gel will be presented and discussed as a cleaning material for painting. The gel-like material (PHB gel) was made using three component derive from bio-renewable sources: PHB

polymer as gelling agent (described in the previous section 3.1),  $\gamma$ -valerolactone as a green solvent and triethyl citrate as a plasticizer. Several formulations were investigated in terms of mechanical properties in order to find the most suitable for the application in cleaning. Then the most promising was tested on laboratory paint reconstructions for the removal of terpenic varnish applied as a protective coating on a oil painted surface. The evaluation of the cleaning performances was carried out by the protocol defined in Chapter 2. Finally the “validation” of the cleaning efficacy was performed on real painting analyzed before and after cleaning.

Characterization and application results of this formulation were in part reported in the article “The Green Attitude in Art Conservation: Polyhydroxybutyrate-based Gels for the Cleaning of Oil Paintings”, *Chemistry Select*, Samorì et al. (2016), 1 (15).

### 3.2.1 THE BIO-BASED COMPONENTS OF THE GEL

$\gamma$ -valerolactone (GVL) is a 5 carbon cyclic ester (4 carbons and 1 oxygen) in the ring (**Figure 3.2**). It can be produced from lignocellulosic biomass, is non-toxic and biodegradable (Alonso, Wettstein, & Dumesic, 2013; Horváth, 2008). It is presented as colorless liquid and has a high boiling point, is stable in water and in the presence of air at normal conditions and does not decompose or degrade with time (Alonso et al., 2013; Fegyverneki, Orha, Láng, & Horváth, 2010). Its toxicity is low and the main risk is flammability; however, the low volatility of GVL makes the flammability risk at normal conditions low. The main properties of GVL are reported in the **Table 3.1**.



**Figure 3.2** Chemical formula of PHb (left), GVL (centre), TEC (right).

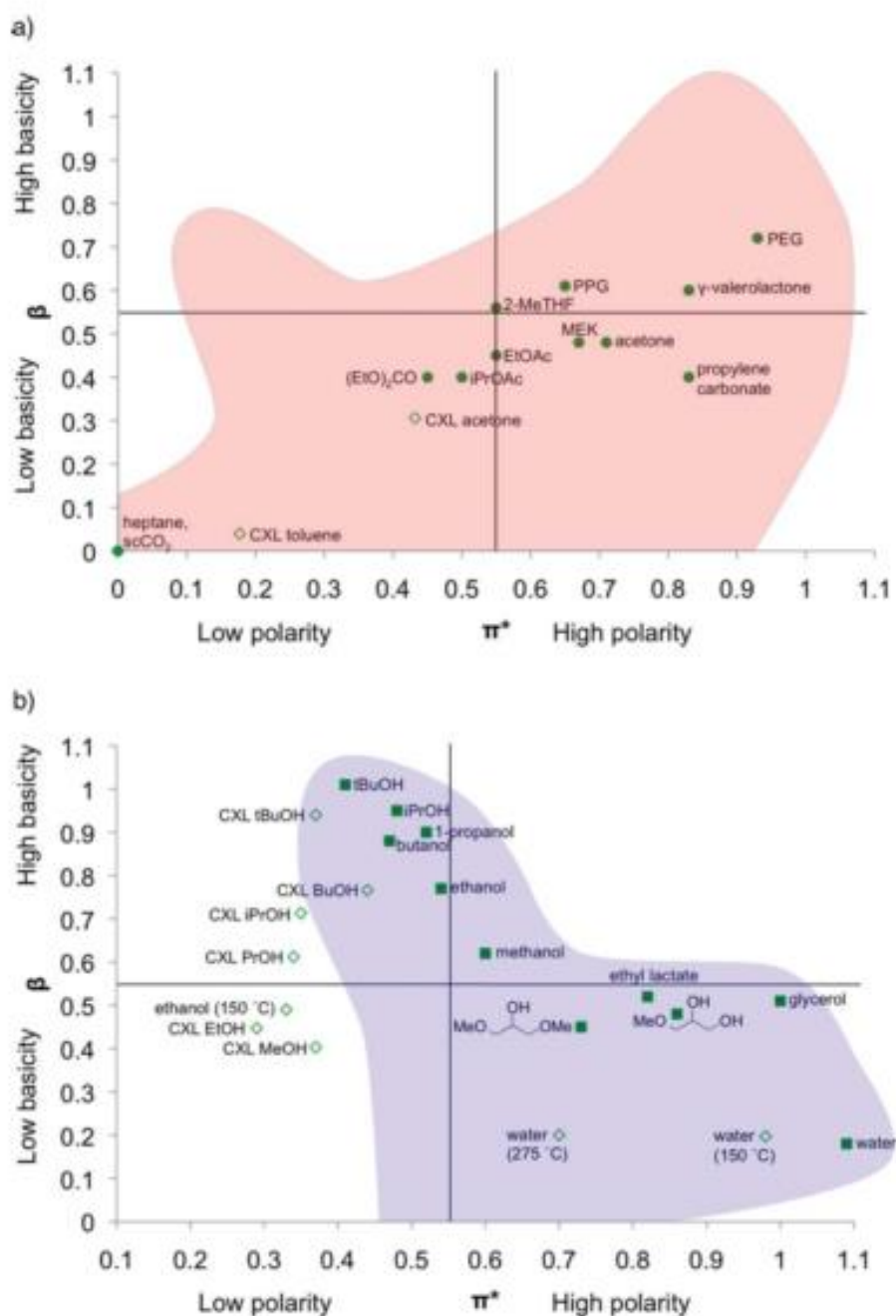
**Table 3.1** Main properties of GVL (Alonso et al., 2013).

Property	Value
CAS-No	108-29-2
Formula	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>
MW (g mol <sup>-1</sup> )	100.112
Refractive index (n <sub>20</sub> /D)	1.432
Density (g mL <sup>-1</sup> )	1.05
Flash point (°C)	96
Melting point (°C)	-31
Boiling point (°C)	207–208
Solubility in water (%)	100
$\Delta H_{\text{vap}}$ (kJ mol <sup>-1</sup> )	54.8
$\Delta_c H^\circ_{\text{liquid}}$ (kJ mol <sup>-1</sup> )	-2649.6

Due to its functionality/reactivity is an excellent candidate as a solvent (Alonso et al., 2013; Fegyverneki et al., 2010) and according to Jessop (Jessop, 2011), it is considered a green aprotic solvent

showing an high polarity and high polarizability properties and a medium H-bond accepting ability (**Figure 3.3**).

Concerning the use of GVL as a solvent in cleaning practices, it shows several advantages either for the restorer, for the environment and for the artwork as well. The low toxicity, low vapor pressure and chemical stability of GVL, lead this solvent to be “safe” for the operator also in in-door environment, limiting the risk of inhalation and volatile compounds emission. Moreover, as a green solvent, it presents two great advantages for the environmental issue: it is obtained from biomass and it is biodegradable.



**Figure 3.3** The graph shows (a) green aprotic solvents, (b) green protic solvents plotted according to their  $\pi^*$  (polarity and polarizability) and  $\beta$  (basicity or hydrogen-bond accepting ability) values. GVL states in the area of aprotic solvents with high  $\pi^*$  and  $\beta$  values (Jessop, 2011).

For the specific application in the cleaning of artworks, the use of GVL was never been reported in literature until now. Thus the investigation of the performance of this solvent become extremely challenging thanks to its non-toxicity and the capability to be gelled into the network of PHB polymer.

$\Gamma$ -valerolactone is a cyclic ester, therefore a cleaning efficacy of the same family is expected. Esters are aprotic polar solvent considered penetrative but low-retentive. They are commonly used in the removal of not-aged natural varnishes and synthetic varnishes (Cremonesi, 2004). Moreover, according to Jessop plot, GVL may show good removal capability of both aged and not aged coating thanks to its high polarity. Its dissociation ability, comparable to non-green aprotic solvent dimethyl sulfoxide and dimethylformamide is expected thanks to its high dielectric constant of 36,47 at 25°C (DMSO and DMF dielectric constants are respectively 48.9 and 36.7) as reported in literature (Aparicio & Alcalde, 2009; Ismalaj et al., 2014).

Even if the prediction of solvent diffusion and retention into the paint layer is more complex as expected (as previously underlined in Chapter 1), the high boiling point and enthalpy of vaporization together with a very low vapor pressure of 0.6 KPa at 25°C (Horváth, Mehdi, Fábos, Boda, & Mika, 2008) predict a scarce volatility. However the risk of swell the paint layer is expected to be similar to  $\gamma$ -butyrolactone that was inserted by Phenix in the category of low swellers solvents (a more detailed explanation is reported in Chapter 1).

The capability of a solvent to being gelled leads to better control in the solvent releasing and slow down its evaporation, reducing the risk of inhalation for the operator. The penetration and retention of the solvent into the paint layer is reduced, as well as the mechanical stress applied to the surface. Indeed the solvent is left in contact with the surface without rubbing or rolling any cotton swabs.

Triethyl citrate (TEC) is known as an environmentally friendly biodegradable plasticizer, non-toxic and bio-based (Scalioni et al., 2017). This plasticizer was added in the gel formulation to enhance the handling properties (Choi & Park, 2004; Samorì et al., 2016; Scalioni et al., 2017) (**Figure 3.2**).

### 3.2.2 GEL PREPARATION AND CHARACTERIZATION

PHB gel is easily obtained by thermal process of solubilization of PHB powder in GVL solvent at 130°C for few minutes. The formation of gel occurred on cooling the solution, the same process is reported in literature using dimethylformamide as solvent (Turchetto & Cesko, 1995). Experimental evidences on time of heating (1, 15 and 30 minutes) proved that this parameter slightly influence the texture of the gel obtained, decreasing the polymer molecular weight as time of heating increased (Samorì et al., 2016). Since 1 minute heating-time demonstrated to be enough for the formation of gel-like material, without remarkably affecting the polymer molecular weight, this condition was selected as a synthesis time.

Several gels were prepared at different PHB/GVL ratio, with/without the addition of TEC and characterized by calorimetric, diffractometric and rheological analysis in order to investigate the physical-mechanical properties and find the most suitable formulation for the cleaning requirements: an easy

handling and a good contact with the surface without stick. All the formulations tested are reported in the table below (**Table 3.2**).

Sample name	PHB (mg)	GVL (ml)	TEC (ml)	Heating time (min)
1-6	70	1	0.02	1
1-9	100	1	0.02	1
1-12	140	1	0.02	1
1-9noTEC	100	1	-	1

**Table 3.2** PHB gels formulations investigated.

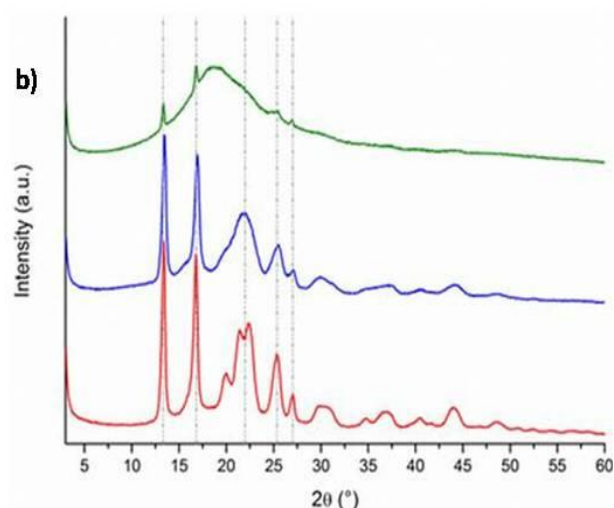
To get an insight into gelation behavior, Differential Scanning Calorimetry was performed on PHB gels formulations reported in **Table 3.2** and pristine PHB powder (**Figure 3.4-a**). All recorded thermograms showed the presence of a single endothermic transition both in the first and second heating scan, either in the gels and in the pristine polymer. This phenomenon means a reversible process. The degree of crystallinity ( $\chi_c$ ) was evaluated as  $\chi_c = \Delta H_{PHB} / \Delta H_0 * 100$ , where  $\Delta H_0$  is the enthalpy of fusion of reference PHB (146 J/g).

Wide-angle X-ray scattering (WAXS) measurements allowed the attribution of these thermal transitions on PHB crystalline phase. WAXS analysis were recorded on dry and wet gels and on pristine PHB. As reported in **Figure 3.4-b** for 1–9 formulation, all samples displayed reflections typical of a crystalline phase besides the amorphous broad signal. By comparing such reflections with those of pristine PHB powder, is possible to assign the peaks to crystal phase of the polymer. In dried gel, the broadening peak is possibly due to crystallites size decreasing or lattice distortion.

**a)**

Table 1. Thermal properties of wet gels and pristine PHB.							
Sample	PHBfraction (%wt)	1 <sup>st</sup> scan			2 <sup>nd</sup> scan		
		T <sub>m</sub> (°C)	$\Delta H_m$ (J/g)	$\chi_c$ (%)	T <sub>m</sub> (°C)	$\Delta H_m$ (J/g)	$\chi_c$ (%)
PHB	100	173	99.6	70	172	91.1	64
1-6	6.1	78	4.8	53	75	4.8	54
1-9	8.5	81	8.8	71	76	7.4	59
1-12	11.5	81	11.5	68	78	9.3	55
1-9noTEC	8.7	81	7.1	56	75	5.4	42

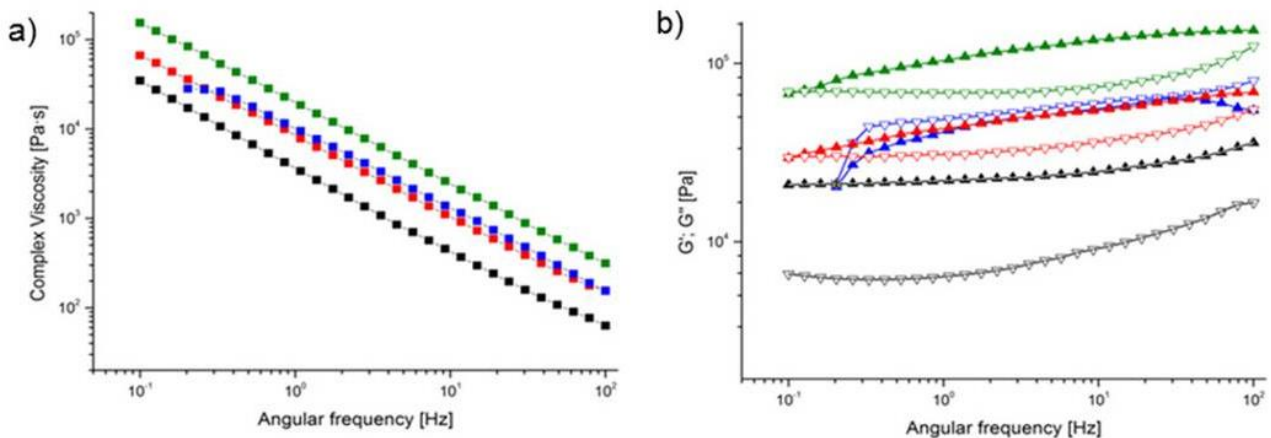
**b)**



**Figure 3.4 (a)** Thermal properties of PHB-gels formulations and pristine PHB, melting temperature (T<sub>m</sub>), melting enthalpy ( $\Delta H_m$ ) and crystallinity ( $\chi_c$ ) are reported; **(b)** WAXS diffractograms of pristine PHB (red), 1-9 dried gel (blue) and 1-9 wet gel (green) (Samori et al., 2016).

From this information is possible to describe the gels as a thermoreversible physical gel stable up to 70°C. The network is based on crystallization of PHB upon cooling of a homogeneous GVL solution.

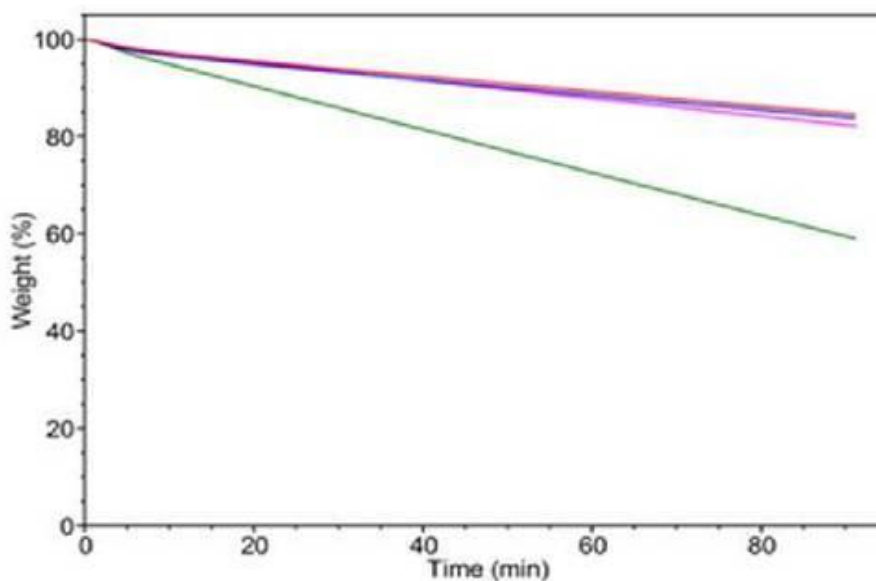
The mechanical properties of gels were assessed with low amplitude oscillatory shear measurements. As shown in **Figure 3.5-a** the complex viscosity ( $\eta^*$ ) of all analyzed materials steadily decreased with the frequency, the lower value is reached for the formulation with lower polymer content (1-6 in black color). The storage and loss moduli ( $G'$  and  $G''$ ) do not show a similar strong frequency dependence (**Figure 3.5-b**). All samples, except for 1–12, showed a behaviour like gels, thus  $G' > G''$  over the range of applied frequencies suggesting that gel strength is significantly influenced by polymer concentration. Indeed increasing the polymer content, the gel resulted strengthened to a certain extent, above which relaxation phenomena predominate ( $G'' > G'$  for 1–12) at this extent the behavior of the material is more plastic and not gel-like. The plasticizing effect of TEC was clearly observed comparing 1–9 and 1–9noTEC results, in absence of plasticizer the moduli  $G'$  and  $G''$  are slightly higher. Thus the formulation without TEC resulted more rigid.



**Figure 3.5** Complex viscosity **(a)** and storage and loss moduli **(b)** respectively  $G'$  ( $\blacktriangle$ ) and  $G''$  ( $\blacktriangledown$ ), carried out with rheometric oscillatory shear measurements. Samples 1-6 (black), 1-9 (red), 1-12 (blue) and 1-9noTEC (green) are reported (Samori et al., 2016).

The capacity of gel to slow down the solvent evaporation was evaluated by thermogravimetry Analysis (TGA), comparing the weight loss of neat GVL to the gels during an isothermal run at 50°C (**Figure 3.6**). Results demonstrated that the evaporation rate of solvent is consistently decreased when embedded into the gel network. Moreover the actual polymer content appeared to have a negligible influence on the phenomenon, at least in the applied conditions.

Among the analyzed gel formulations, the content of polymer did not show a dramatic influence in the solvent evaporation (and possibly its retention capability), but strongly affected the mechanical properties of the gel. Thus the formulation 1-9 was selected as the best candidate for cleaning gel and further analyzed.



**Figure 3.6** TGA isothermal run at 50°C. Weight loss percentage of neat GVL (green), 1-6 (purple), 1-9 (blue) and 1-12 (red) are reported in comparison. Evaporation rates of gels are evidently slower than neat solvent (Samori et al., 2016).

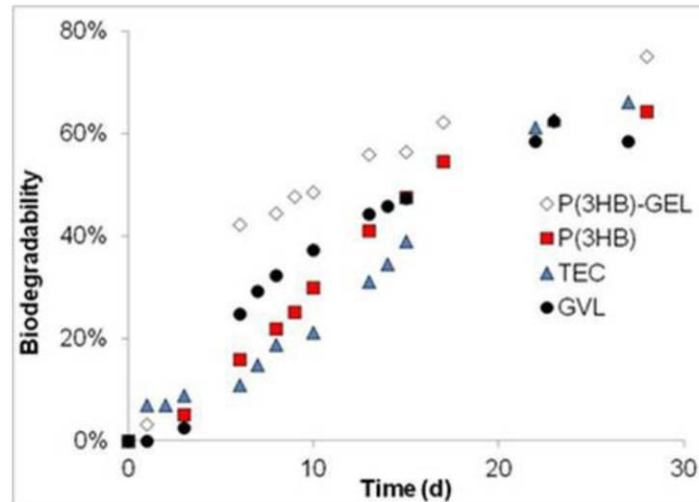
Gel permeation chromatography (GPC) was performed in order to assess the shelf life of the gel over a period of time of 11 weeks (**Table 3.4**). Results showed that the average molecular weight of polymer ( $M_w$ ) is not significantly affected over the aging time at room temperature. This evidence suggests that the gel can be stored and used in an acceptable time-frame.

**Table 3.4** Shelf life of 1-9 gel in time. The average molecular weight of polymer ( $M_w$ ) is measured at different ageing time with GPC (Samori et al., 2016).

Sample	Ageing (days)	$\overline{M}_w$ (MDa)
PHB	0	0.85
1-9/0	0	0.84
1-9/7	7	0.84
1-9/14	14	0.86
1-9/21	21	0.84
1-9/28	28	0.88
1-9/77	77	0.89

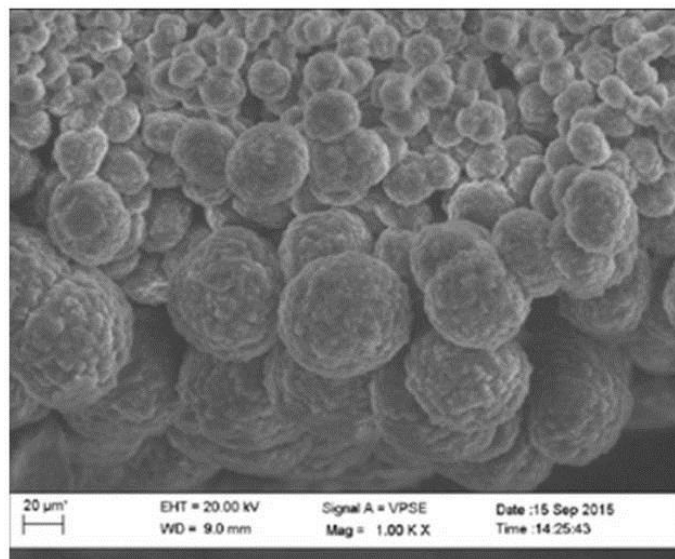
Biodegradation of gel, pristine P3HB, neat GVL and TEC were determined by a ready biodegradability test in an aerobic aqueous medium according to the OECD guideline 301F, “Manometric respirometry” (the protocol is specified in section 2.1.11). From the test, the gel resulted to be readily biodegradable in water according to the OECD guidelines, reaching a final biodegradation value of 75% after 28 days (**Figure 3.7**), higher than the single components (64% for PHB, 58% for GVL and 66% for TEC).





**Figure 3.7** Biodegradability test of 1-9gel (P3HB-GEL) and its single components over 28 days (Samorì et al., 2016).

Morphology of the dried gel was investigated by electronic microscopy (SEM). As shown in **Figure 3.8** is consistent with the spherulitic arrangement of polymer chains in pristine PHB, such as reported in literature (Barham et al., 1984).

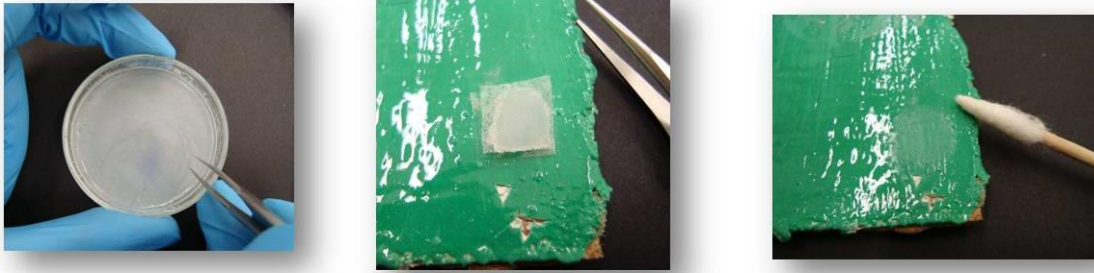


**Figure 3.8** Micrograph of dried 1-9 gel acquired with SEM at 1000x. the morphology of the gel is spherulitic such as pristine PHB (Samorì et al., 2016).

### 3.2.3 APPLICATION AND GEL-RESIDUES EVALUATION

A piece of gel, cut in the desired size and embedded between two sheets of rice paper (Japanese paper), was applied over the varnish and left 5 minute acting without applying any kind of mechanical stress. After application a dry cotton swab was gently rolled on the treated surface for the complete removal of varnish (**Figure 3.9**).

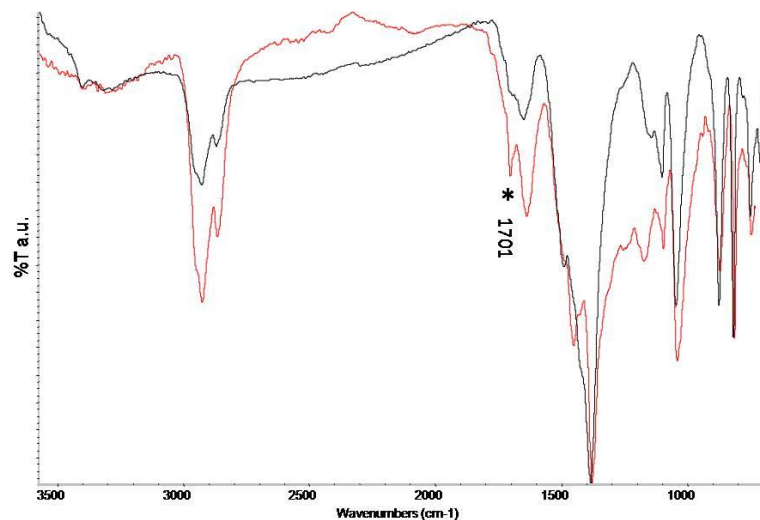




**Figure 3.9** PHB gel applied over the surface of a mockup for cleaning test.

The time of application was selected as reasonably long enough to allow the easy swelling and removal of the varnish without interacting with the paint layer. FTIR-ATR spectra, acquired on the surface treated at different times 2.5 and 5 minutes (**Figure 3.10**) suggested a possible tunable cleaning efficacy of the system related to the time of application. Indeed after 5min application the residue of varnish (marked with asterisk at  $1701\text{ cm}^{-1}$ ) is lower compared to 2.5min. This evidence suggests a controllable cleaning action of the gel.

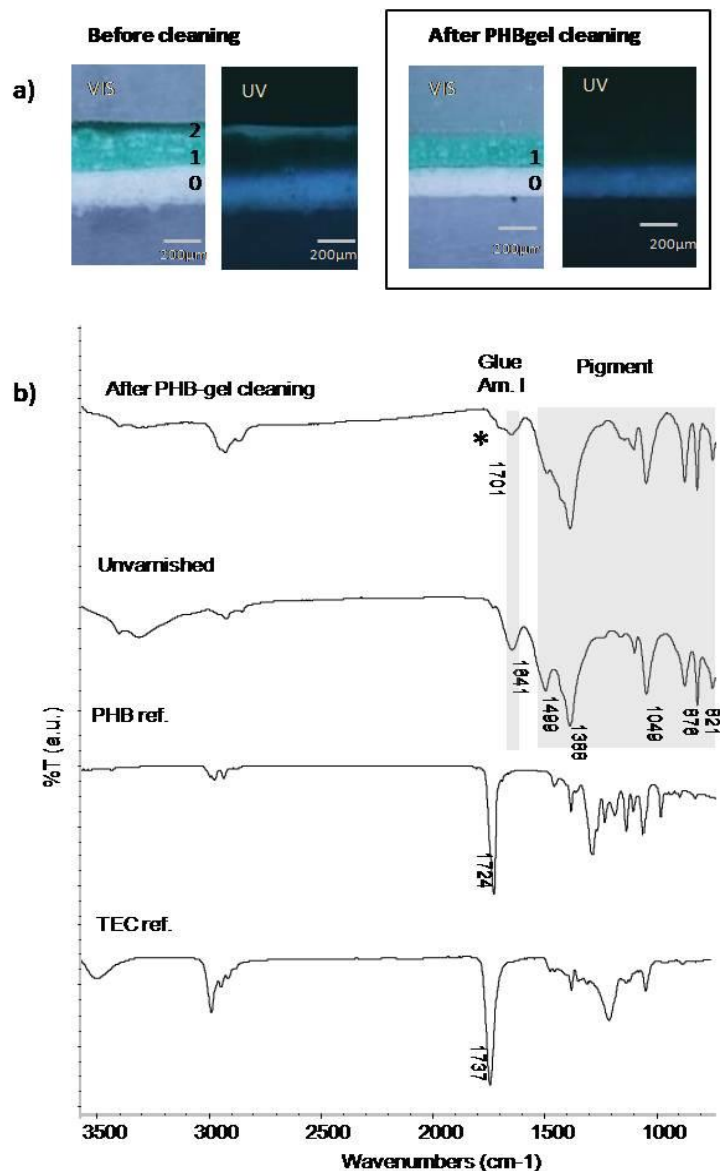
First cleaning tests of PHB-GVL-Tec gel (PHB gel) were performed on dammar varnish applied on a proteic binder layer (rabbit glue mixed with inorganic pigment) in order to assess eventually the presence of gel residues after application. Indeed animal glue binder does not contain infrared absorption bands in the region of ester bonds  $1725\text{--}1750\text{ cm}^{-1}$  and thus the overlapping with the main characteristic peaks of gel components ( $1724$  and  $1737\text{ cm}^{-1}$  for PHB and TEC respectively) was avoided.



**Figure 3.10** ATR spectra after PHB gel treatment applied for 2.5 min (red) and 5 min (black). Peak at  $1701\text{ cm}^{-1}$  corresponds to the diagnostic peak of dammar varnish. Therefore a reduction of varnish residue is observed for longer time application.

The mock-up was prepared, according to ancient recipes, with the following stratigraphy: (0) ground layer, (1) paint layer composed by malachite pigment  $\text{Cu}_2(\text{CO}_3)(\text{OH})_2$  and rabbit glue binder, (2) dammar varnish (thickness  $35\text{ }\mu\text{m}$ ). In **Figure 3.11-a** cross sections of microsamples collected before and after PHB

gel cleaning, are reported. After gel application for 5 minutes the varnish appeared removed, indeed any fluorescent layer is visible over the paint layer. The spectrum collected after gel cleaning, shows the characteristic absorption bands of glue binder, mainly at  $1641\text{ cm}^{-1}$  (C=O stretching, amide I), and malachite pigment at  $1499, 1388, 1049, 876, 821\text{ cm}^{-1}$  (**Figure 3.11-b**). A small band at  $1701\text{ cm}^{-1}$  is associated to the characteristic vibration of carbonyl bond in Dammar resin, revealing the presence of a negligible residue of varnish. No residues of PHB and TEC are clearly visible in the spectrum (respectively at  $1724$  and  $1737\text{ cm}^{-1}$ , due to carbonyl stretching of ester bonds) suggesting that the application of the gel was effective in the varnish removal without release detectable residues.



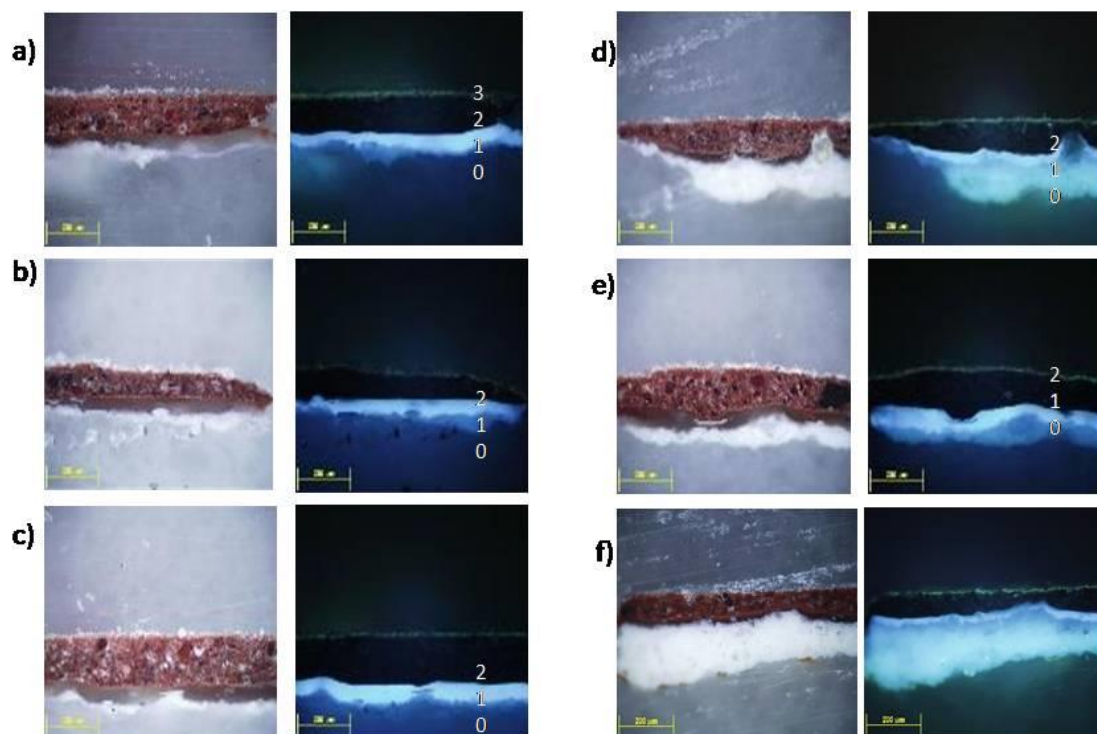
**Figure 3.11 (a)** Optical microscopy cross sections before and after cleaning in Visible and UV light. Before cleaning the varnish layer (2) is visible, after cleaning it is removed. **(b)** FTIR-ATR spectrum of the areas cleaned and unvarnished compared to reference spectrum of PHB and TEC (respectively from the top to the bottom); no detectable residue of gel components remained after cleaning.

### 3.2.4 CLEANING PERFORMANCE EVALUATION ON DAMMAR VARNISH

Aimed at the evaluation of the cleaning performance of PHB gel, tests on paint laboratory reconstructions were performed. Cleaning tests on not-aged and aged dammar varnish applied on a painted oil layer were carried out in comparison with neat GVL and other solvents commonly used in cleaning practices (acetone and dimethyl sulfoxide). The action of PHB gel was evaluated following the analytical protocol described in Chapter 2, by the employing optical microscopy and spectroscopic techniques for the varnish removal evaluation and HS-spme method for the retention of the solvent.

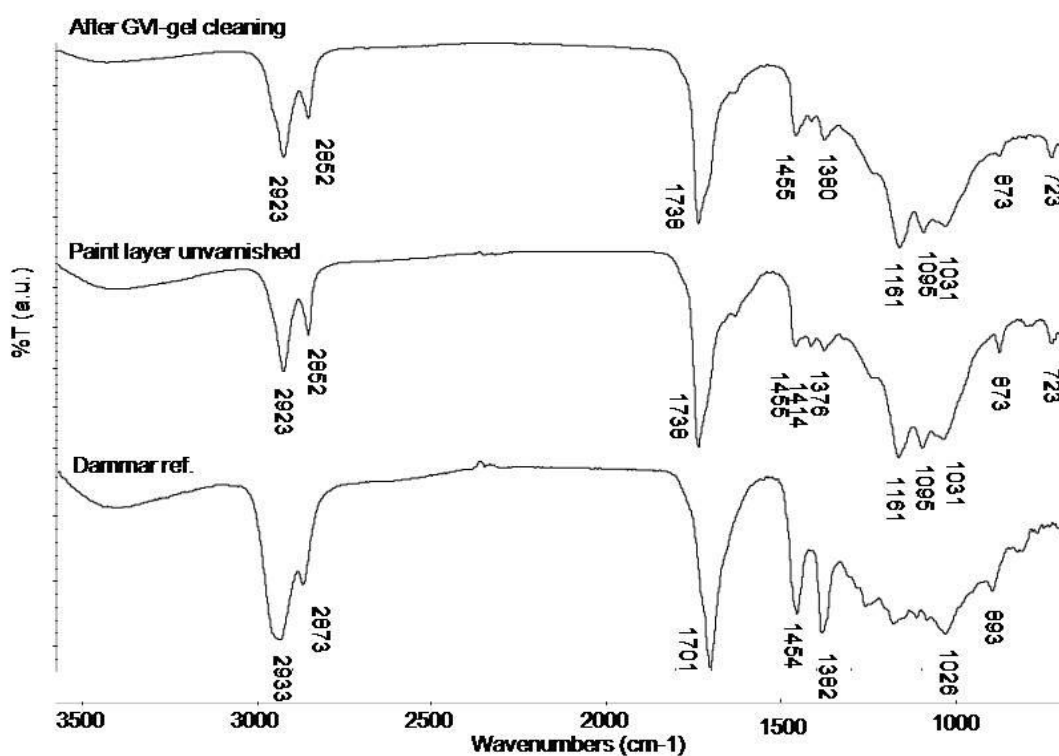
#### 3.2.4.1 NOT AGED OIL PAINT LAYER

Cleaning tests of PHB gel compared to GVL, acetone and DMSO applied by swabs were performed on dammar varnish not-aged sample. As showed in micro-cross sections in **Figure 3.12-a**, the mock up is composed by several layers. The upper varnish layer of 10-15  $\mu\text{m}$  (3), the paint layer of burnt red ochre and linseed oil medium (2), a glue closing layer (1) and the ground layer (0). After PHB gel cleaning (**Figure 3.12-b**) the varnish layer, observable in UV light, appears removed. A slight fluorescent thin layer of about 2  $\mu\text{m}$  is present, but it is due to the oil binder as observable in the cross section of unvarnished sample in **Figure 3.12-f**.



**Figure 3.12** Microphotographs of cross sections in visible and UV light obtained with optical microscopy (obj 20X); **(a)** before cleaning, numbers correspond to dammar varnish, linseed oil and ochre pigment paint layer, glue closing layer and ground layer (respectively from 3 to 0); **(b)** after PHB gel, **(c)** GVL swab, **(d)** acetone swab, **(e)** DMSO swab cleaning. After all treatments a consistently reduction of varnish is achieved; **(f)** cross section on unvarnished area, the fluorescent layer is due to oil binder.

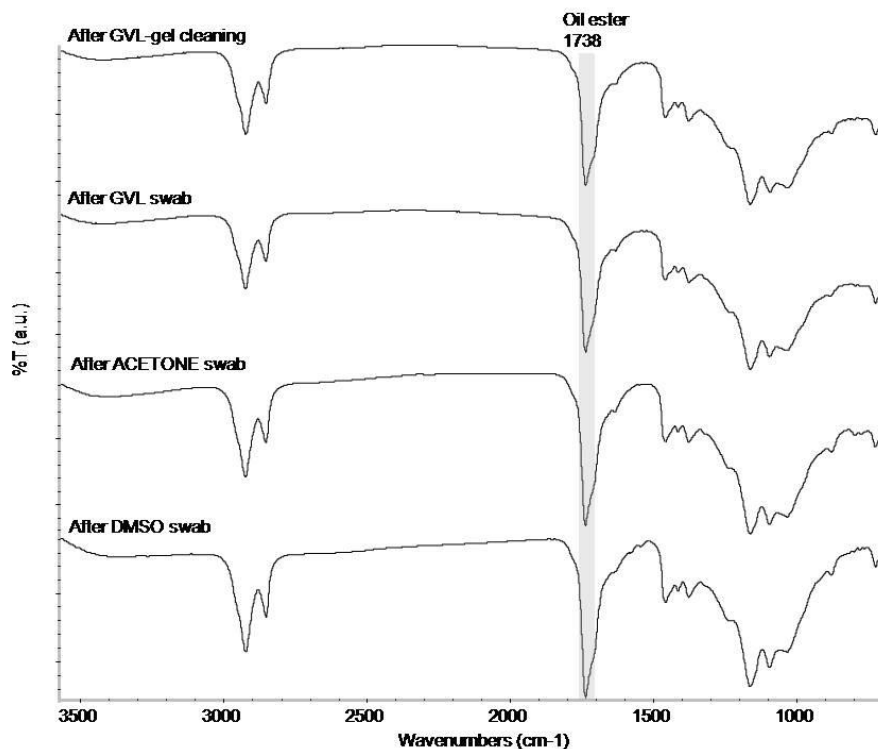
ATR analysis performed after treatment showed the prevalent signals of the paint layer, while the most intense peak associated to dammar resin, at  $1701\text{ cm}^{-1}$ , is not clearly distinguishable (**Figure 3.13**). Because of the possible overlapping of C=O absorption at  $1701\text{ cm}^{-1}$ , and C-H aliphatic chains vibration at  $1455$  and  $1382\text{ cm}^{-1}$  of the resin, with those of the paint layer (respectively at  $1738$  and  $1455\text{ cm}^{-1}$  due to oil binder and  $1376\text{ cm}^{-1}$  due to pigment), the presence of negligible residues of varnish after cleaning cannot be ruled out.



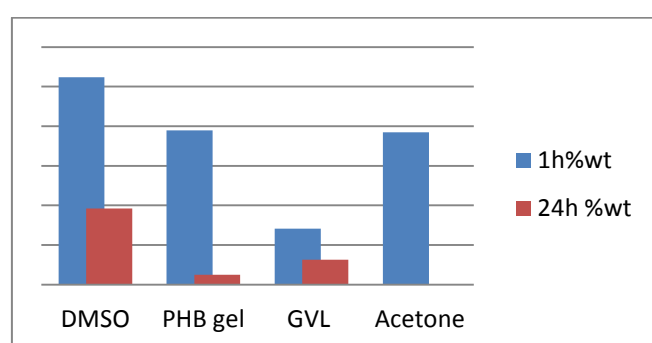
**Figure 3.13** FTIR-ATR spectra of area cleaned with PHB gel (on the top), unvarnished area (in the middle) and dammar varnish (on the bottom). Spectrum after cleaning is comparable to unvarnished area.

By comparing the spectra, reported in **Figure 3.14**, acquired directly over the areas after different treatments, some considerations can be done. The removal capability of PHB gel can be considered comparable to GVL applied by swab (**Figure 3.14-a,b**) thus even if the solvent is confine into the gel network, its cleaning capability is not reduced. A comparable removal capability is demonstrated also for acetone and DMSO, therefore GVL can be considered an effective solvent as well as “the universal” DMSO. Despite comparable results were obtained by all the systems, in terms of varnish removal capability, very different data arose regarding the retention of solvents into the paint layer. HS-spm data after 1 and 24 hours from the treatment are reported in the table below (**Table 3.5**). Despite data after 1h, in which the amount of solvent in the area treated with PHB gel is higher compared to GVL swab, after 24 hours this amount decreases highly until reaches a value lower than neat solvent. This behavior may suggest that the gel is capable to retain the solvent in the very surface therefore it can evaporate easier (so after 1h we

observe a high value that tends to decrease quickly after 24h). Moreover the values of both PHB gel and GVL are between acetone and DMSO, suggesting that GVL tends to be a little retained by the paint medium (as expected due to its low volatility) but is far from the high-retentive value of DMSO.



**Figure 3.14** ATR spectra after PHB gel, GVL, acetone and DMSO applied by swab (respectively from the top to the bottom). The peak related to ester bond of the oil medium at 1378 cm<sup>-1</sup> is highlighted. A comparable cleaning efficacy is demonstrated in all the systems tested.



Cleaning system	1h %wt	24h %wt
PHB gel	0.779 ± 0.241	0.050 ± 0.007
GVL swab	0.282 ± 0.082	0.152 ± 0.087
Acetone swab	0.769 ± 0.243	n.d.
DMSO swab	1.048 ± 0.195	0.384 ± 0.169

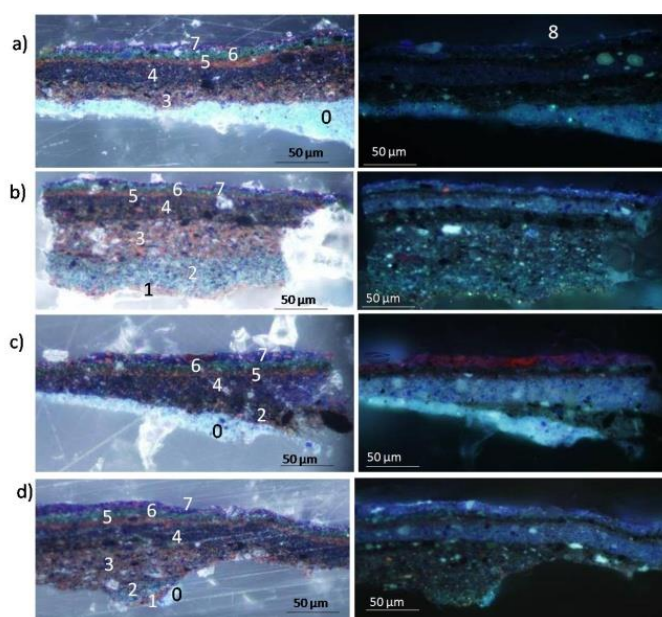
**Table 3.5** Amount of solvent retained by the substrate after 1 and 24 hours from the cleaning treatments. Data gained from HS-spmc-GC/MS analyses. (n.d.= not detected)

### 3.2.4.2 AGED OIL PAINT LAYER

Because the aging of materials, due to time and environment-interactions, may dramatically change the physical and mechanical properties of a painting as well as the chemical composition (as previously explained in Chapter 1), the performance of a new cleaning method could be unpredictable. Thus, further cleaning tests were performed on aged mock-up in order to assess the performance of PHB gel over a more oxidized, and thus more polar, substrate. A comparison with neat GVL applied by cotton swab and DMSO gelled with a traditional thickener product (Klucel®, hydroxypropyl cellulose) was considered. Both the gels (PHB-GVL and Klucel-DMSO) were applied for 5 minute embedded in Japanese paper. Klucel gel was prepared by mixing 1ml of solvent in 80mg of Klucel powder at room temperature.

The standard sample was prepared in 1970 applying several layers of silicate-based pigments mixed with an oily binder. The layer of dammar varnish was applied successively and artificially aged (the protocol of ageing is reported in section 2.2).

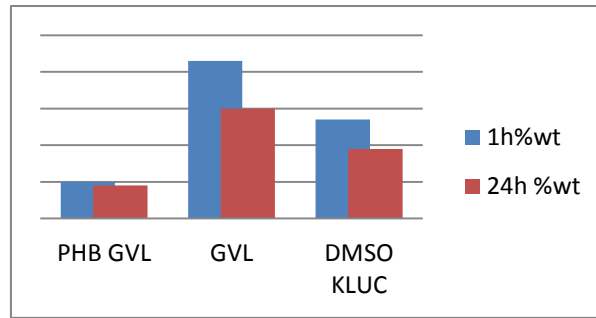
In **Figure 3.15** cross sections of the sample before and after cleaning are reported. Either after PHB gel and GVL swab the varnish coating of about 5  $\mu\text{m}$  was removed (respectively **Figure 3.15-b,d**). As well varnish removal occurred with DMSO-Klucel treatment (**Figure 3.15-c**) but in this latter case an interaction with the paint layers probably happened as demonstrated by the different fluorescence visible in UV light.



**Figure 3.15** Cross sections of dammar aged mockup in visible (on the left) and UV light (on the right). Before cleaning **(a)** the thin varnish layer is labeled as '8'; after PHB gel **(b)**, DMSO-Klucel gel **(c)**, GVL swab **(d)**.

In **table 3.6** HS-spmc method results are reported for PHB gel, GVL neat applied by swab and DMSO gelled with Klucel. Comparing values after 1 and 24 hours of PHB gel with GVL neat, the advantages in using the gel-system are evident. Moreover high retentive values for DMSO are observable, as expected, even if applied in a gelled form.



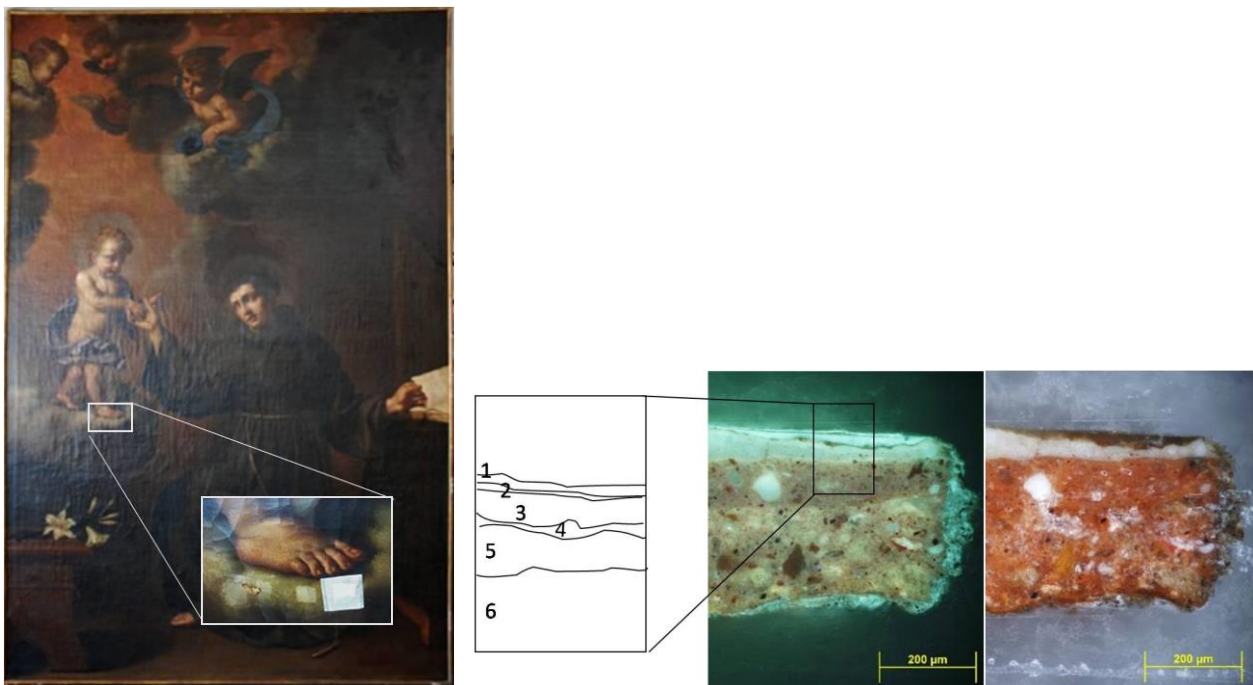


Cleaning systems	1h %wt	24%wt
PHB-gel	0.102 ± 0.040	0.089 ± 0.011
GVL swab	0.427 ± 0.029	0.284 ± 0.094
DMSO- Klucel	0.274 ± 0.028	0.188 ± 0.087

**Table 3.6** Amount of solvent retained by the sample after 1 and 24 hours from the cleaning, evaluated by HS-spm-GC/MS analyses. PHB gel results are lower than both GVL and DMSO-Klucel.

### 3.2.5 CASE STUDY: OIL PAINTING “SAN ANTONIO”

PHB gel was finally “validated” on a real oil painting represented San Antonio and attributed to Benedetto Gennari (XVII century, Ravenna, Italy), exhibited in the San Francesco Church in Ravenna, Italy. Gel efficacy was evaluated in comparison with traditional acetone and DMSO applied by swab.

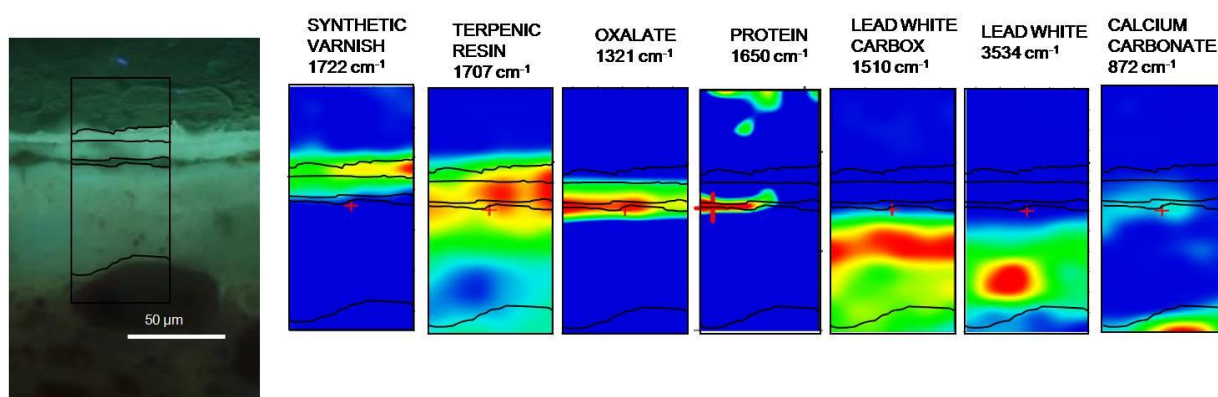


**Figure 3.16** Oil painting “San Antonio” attributed to Benedetto Gennari (XVII century, Ravenna, Italy). Into the square a magnification of the area where PHB was applied is shown. **On the right** cross section of the sample before cleaning in UV and visible light (optical microscopy obj. 20X). Two varnish layers (1 and 3) can be distinguished in UV microphotograph.

Micro samples of real painting were collected before and after cleaning and the gel performance was evaluated by observation of cross section and  $\mu$ FTIR-ATR analyses. A full characterization of the paint stratigraphy was achieved by mapping an area of the cross section before cleaning.

In **Figure 3.16** the complex stratigraphy of the painting is reported: (1) external varnish of 3-5  $\mu$ m; (2) grime layer 3  $\mu$ m; (3) varnish layer of 10  $\mu$ m; (4) not fluorescent deposit layer 3-5  $\mu$ m; (5) paint layer 20-30  $\mu$ m; (6) ground layer >20  $\mu$ m.

The overlapping of several thin layers, close to the spatial resolution limit, together with the presence of degradation products made the spectroscopic analyses hard to perform. Anyway  $\mu$ FTIR-ATR mapping was performed in cross section using an aperture of 60x60  $\mu$ m and a step size 10x10  $\mu$ m in x and y direction. Map profiles allowed the chemical characterization of the layers reported in **Figure 3.17**. The more external coating layer (1) results a synthetic varnish associated to the band at 1722 $\text{cm}^{-1}$ ; due to the very thin layer and maybe the lack of well distinguishable marker it was not been possible to define the composition of layer (2). Layer (3) corresponds to dammar recognized by map profile at 1707 $\text{cm}^{-1}$ ; layer (4) showed the presence of proteinaceous material (profile at 1650  $\text{cm}^{-1}$ ) and oxalates (1321  $\text{cm}^{-1}$ ) suggesting that maybe it was the original varnish layer degraded and replaced by dammar first and synthetic varnish later. Finally the paint layer (5) is constituted by lead white and a degraded lipidic binder, as the presence of lead carboxylates suggested (peak profile 1510 $\text{cm}^{-1}$ ).

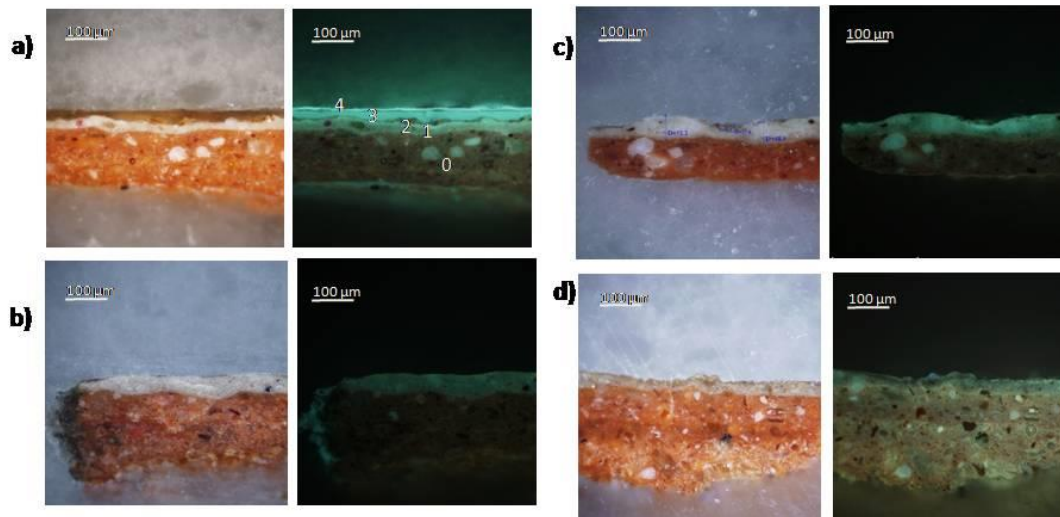


**Figure 3.17**  $\mu$ FTIR-ATR map profiles of the sample before cleaning. A compositional characterization of layers is achieved.

Cleaning tests with PHB gel applied for 5 minutes was performed and compared to traditional solvents acetone and DMSO applied by swab. **Figure 3.18** reported the cross sections before and after treatments. Cross section microphotographs after PHB gel cleaning (**Figure 3.18-b**), allowed to reveal the satisfactory removal of both the varnishes, the synthetic and the natural one, without any interference with the paint layer. Similar efficacy were obtained with acetone and DMSO applied by swab (**Figure 3.18-c,d**), even if in this latter case an interaction with paint layer cannot be excluded. Indeed, after DMSO the paint



layer appeared darkened (in visible light image) maybe due to dissolution and penetration of dissolved components into the paint layer. Indeed this phenomenon is one of the drawbacks assigned to traditional wet cleaning method by swab (as reported in Chapter 1).



**Figure 3.18** Cross section in visible and UV light of area (a) before cleaning, (b) after PHB gel, (c) acetone swab and (d) DMSO swab.

### 3.2.6 DISCUSSION

With the aim of develop a sustainable approach for the cleaning of paintings, all the three main aspects concerning restoration practices have been considered: the healthiness of the operator, the low environment impact of the product and the low risk of damage the artwork.

A fully non-toxic, bio-based and biodegradable organogel was developed. The gel is composed by three components: the polymer poly-(3hydroxybutyrate) (PHB) derive from renewable sources, the solvent  $\gamma$ -valerolactone (GVL) derived from lignocellulosic biomass and the plasticizer triethyl citrate (TEC).

Characterization analyses revealed that the gel is thermoreversible and the network is due to the crystallization of PHB. Among several formulations, the best performer in terms of stability during time and mechanical properties, has been selected and tested in the removal of varnish layer from painted surfaces.

From an applicative point of view it shows several advantages: the gel slow down the evaporation of the solvent thus a more controlled cleaning action is achieved and the risk of inhalation is reduced. Moreover the network retains the solvent, reducing the excessive spread and diffusion through the paint layer. Its preparation is fast and easy, its application and waste as well.

Cleaning tests carried out on aged and not-aged natural varnish (dammar resin) applied on oil binder paint layer, demonstrated an effective action of varnish removal without affecting the paint layer underneath and without leaving residues of gel-components. The cleaning performance has been evaluated in comparison with solvents and methods commonly used in restoration practices. The application on real

painting “San Antonio” (XVII) confirmed the capability to remove both natural and acrylic varnish with any drawbacks.

### **3.3 GVL-PHB gel**

#### **3.3.1 GEL PREPARATION**

PHB-GVL gel is prepared following the same process described in section 3.2.2. A fixed amount of PHB (9%wt) is added to  $\gamma$ -valerolactone at 130°C and stirred for 1 minute in a petri dish. As the transparent solution cooled down to room temperature, the gelation occurred homogeneously, without form any liquid or solid separation phase. In this formulation, the use of TEC plasticizer is excluded. Analyses results previously reported, showed that the elimination of plasticizer does not affect neither the thermoreversibility of the gel, nor its biodegradability. The only influence is, as expected, on the mechanical properties.

This formulation was tested on both terpenic (dammar resin) and acrylic (Paraloid B72®) varnish applied over three different paint binders: egg, linseed oil and rabbit glue in order to investigate the capability of remove natural and synthetic varnishes and to establish if any dangerous interaction with the organic binder happened. Moreover testing the removal capability of either dammar and paraloid is extremely useful since they are among the most used as restoration varnishes.

In the following sections only tests on egg-mock up will be presented as comparison for the real case, the tempera painting of Cimabue.

#### **3.3.2 CLEANING EVALUATION ON DAMMAR VARNISH**

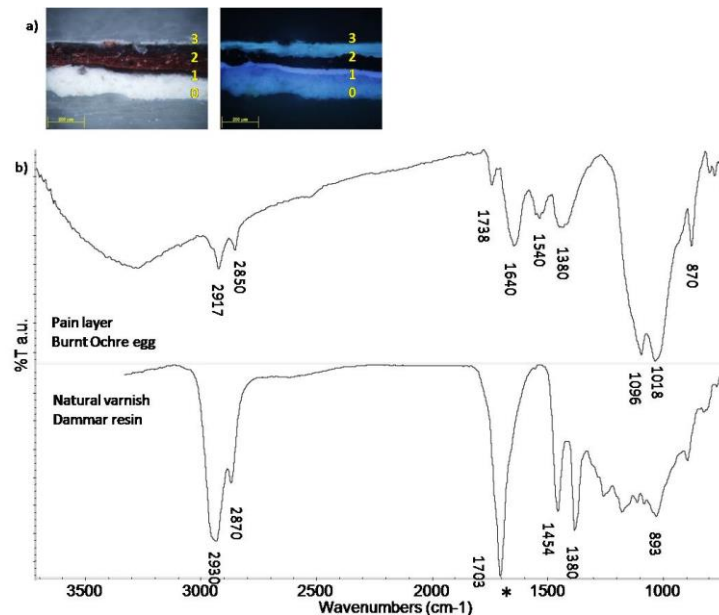
In order to overview the performance of PHB-GVL gel, it was compared to GVL neat and Benzyl alcohol (BAL). The latter is used in this case as a reference solvent commonly used in restoration, effective in varnish removal but dangerous because highly retentive. Both the solvents were applied by swab and in Carbopol-Ethomeen gel as a comparative gel-system generally used by restorers (a more detailed explanation about solvents and methods commonly employed in restoration practices is reported in section 1.3). Carbopol-Ethomeen gel was prepared by mixing 1 ml of Ethomeen C25, 100 mg of Carbopol UTREZ 21, 5 ml of solvent and 0.5 ml of deionized water.

According to the analytical protocol of cleaning evaluation, the varnish removal capability was assessed by micro-cross sections observation in visible and ultraviolet light under the optical microscopy and FTIR-ATR analyses collected directly over the surface after treatment. CG/MS analyses with HS-spm sampling method were carried out either for obtained data on the retention of the solvent by the substrate, and for the capability of PHB to hold and retain the solvent into its network during cleaning. In particular, this latter investigation was performed comparing PHb-GVL gel to GVL-Carbopol gel.

Tests on mockups covered by dammar varnish applied over an ochre pigment mixed with egg binder is discussed below. The importance of testing the new cleaning method on egg-tempera painting is extremely

important because is considered a delicate surface with high porosity and high risk of leaching both lipidic and proteic components (a more detailed explanation of leaching phenomenon is reported in section 1.2.4)

As shown in **Figure 3.19-a**, the mock up is composed by the overlapping of several layers: (3) terpenic varnish thickness 50-70  $\mu\text{m}$ , (2) egg binder mixed with burnt red ochre, (1) glue closing layer, (0) ground layer composed by gypsum and glue. FTIR-ATR spectra of paint layer is characterized by typical vibrational bands of egg at 1738  $\text{cm}^{-1}$  (C=O stretching of ester bonds belong to the lipidic component of egg), 1640 and 1540  $\text{cm}^{-1}$  (respectively C=O stretching of amide I and N-H bending of amide II, belonging to the proteic component). Peaks at 1380 and 870  $\text{cm}^{-1}$  due to the presence of calcite, are assigned respectively to  $\text{CO}_3^{2-}$  stretching and C-O-C bending vibrations, 1096-1018  $\text{cm}^{-1}$  to silicates (Si-O and Si-O-Si stretching). Dammar varnish is characterized by the most intensive peak of carbonyl at 1703  $\text{cm}^{-1}$  (C=O stretching). Other characteristic peaks, mainly for their shape, are at 1454 and 1380  $\text{cm}^{-1}$  due to vibrations of aliphatic chains in the molecules (C-H bending).



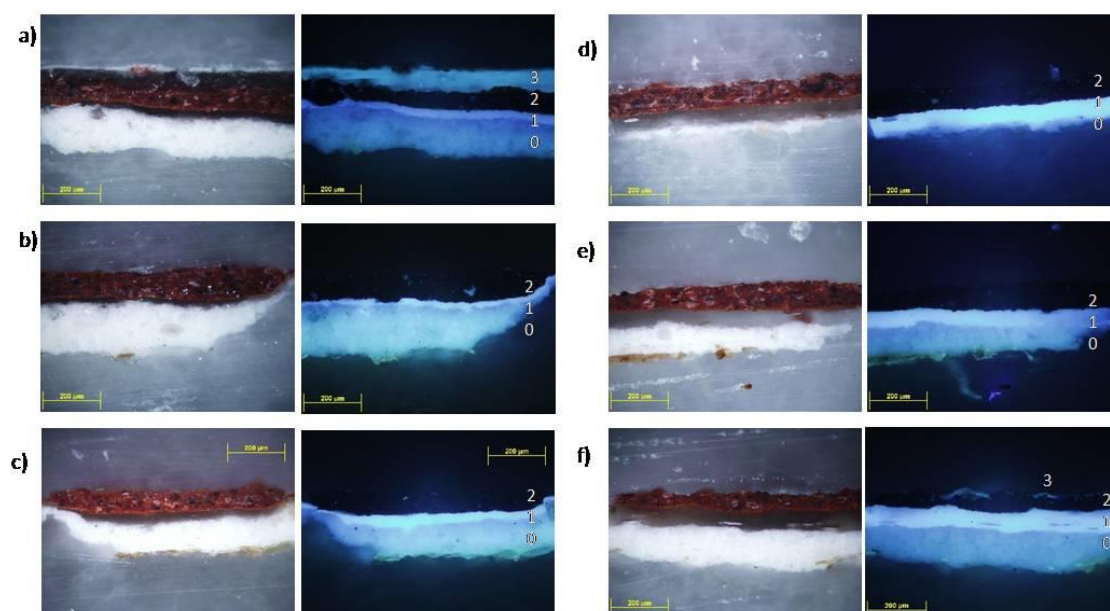
**Figure 3.19 (a)** cross section before cleaning, numbers correspond to varnish layer (3), paint layer (2), closing layer (1) and ground layer (0); **(b)** FTIR-ATR spectra of paint layer (on the top) and varnish layer (on the bottom), the main characteristic peak of dammar used as reference is labeled with asterisk at 1703  $\text{cm}^{-1}$ .

PHB-GVL was applied for 5 min over the surface of dammar layer and its action was compared to GVL and BAL applied with swab, and to GVL and BAL gelled with Carbopol. Several considerations can be reported regarding the cleaning ability of the PHB-GVL gel and the capability of PHB polymer to retained the solvent.

**Figure 3.20** shows the cross section before cleaning and before treatments. Both in PHB-GVL and GVL swab cleaning (respectively **Figure 3.20-b,c**), the varnish appears successfully removed without any damaging for the underneath paint layer. As well for the other treatments, the varnish resulted removed

more or less homogeneously. Only after BAL-Carbopol cleaning some varnish residues are still present (**Figure 3.20-f**).

ATR spectra acquired over the surface after PHB-GVL cleaning, confirmed the good removal ability of the gel (**Figure 3.21**). Indeed the signals can be assigned to the egg medium and ochre pigment of the paint layer as shown in **Figure 3.21-a,b**. The diagnostic peaks of dammar resin and the PHB polymer labeled in **Figure 3.21-c,d** are not clearly detected, but cannot be completely excluded because the possible overlapping of several functional groups belonging both to binder (egg ester bond at  $1742\text{ cm}^{-1}$ ), varnish (carbonyl group at  $1703\text{ cm}^{-1}$ ) and polymer (PHB ester bond at  $1724\text{ cm}^{-1}$ ). Thus possible residues of varnish can be considered negligible, while residues of the solid component of the gel (PHB) can be ruled out taking into account investigation reported in chapter 3.2.3.

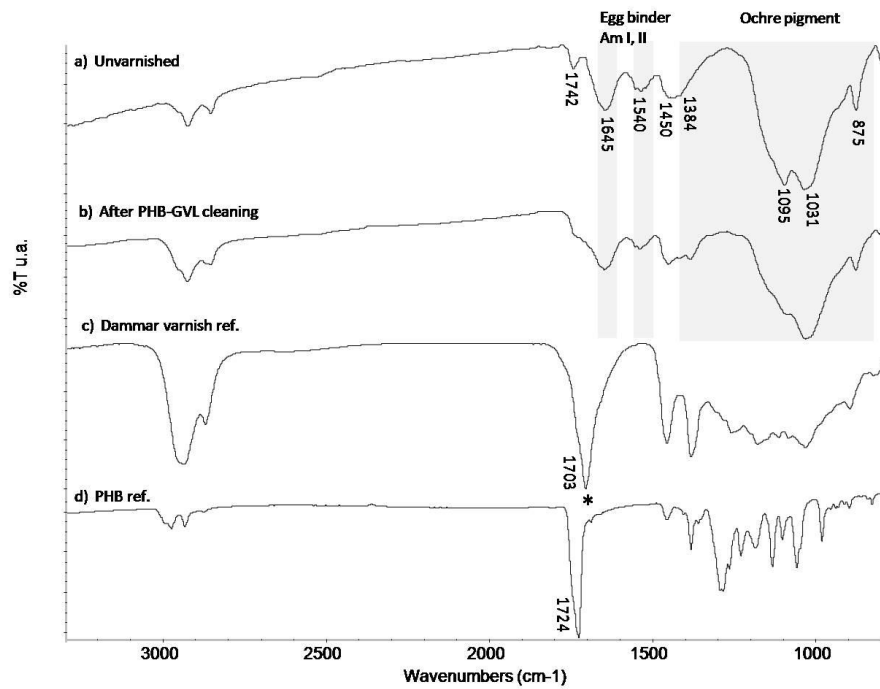


**Figure 3.20** Microphotographs of cross sections in visible and UV light (objective magnification 20X) of mockups composed by dammar varnish applied on egg medium paint layer. **(a)** Before cleaning a thick layer of varnish is present, after **(b)** PHB-GVL gel, **(c)** GVL swab, **(d)** BAL swab, **(e)** GVL-Carbopol gel, **(f)** BAL-Carbopol gel. In all the cases the varnish is removed or reduced consistently.

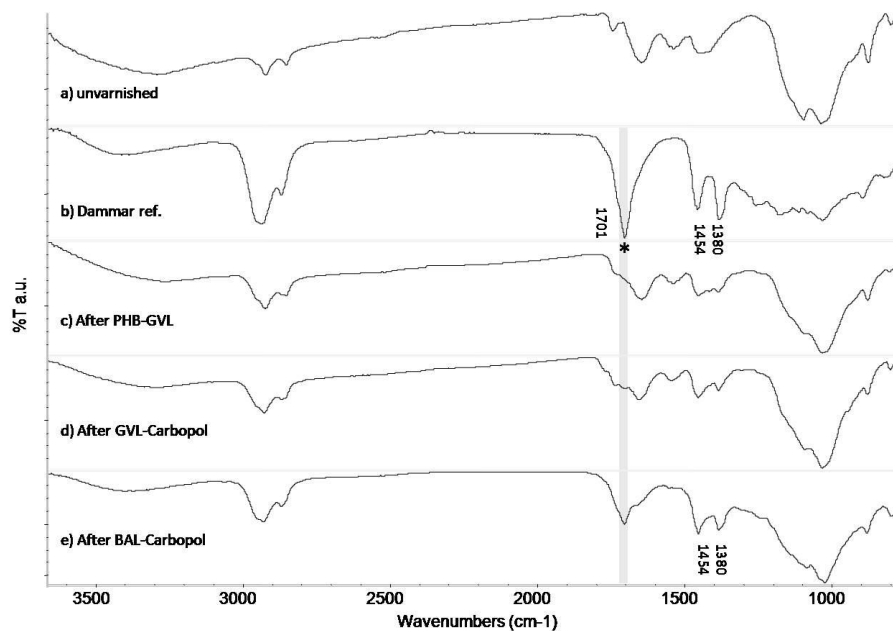
ATR spectra collected after GVL-Carbopol revealed the peak at  $1703\text{ cm}^{-1}$  ascribable to varnish residues (**Fig 3.22-d**), even if any fluorescent layer is visible under microscope (**Fig 3.20-e**). ATR spectra on area treated with BAL-Carbopol revealed as well the present of major varnish residues (**Fig 3.22-e**), as demonstrated mainly by the presence of evident carbonyl peak at  $1703\text{ cm}^{-1}$  and the absence of amide II of egg binder at  $1540\text{ cm}^{-1}$ . Indeed fluorescent residues, over the surface after treatment, were clearly visible under UV light in cross section (**Fig 3.20-f**).

Spectroscopic and microscopy results allow to confirm the very good cleaning capability of PHB-GVL gel, comparable to the action of neat solvent GVL and BAL, but with the advantage to do not apply any

mechanical stress to the surface during cleaning. The application of this gel can be considered also for the most delicate and sensitive surfaces. On the contrary Carbopol-Ethomenn gel do not show the same removal efficacy, indeed residues of varnish are detectable. Moreover do not ensure a homogenous cleaning action over the treated surface probably due to the lack of good contact with the surface.



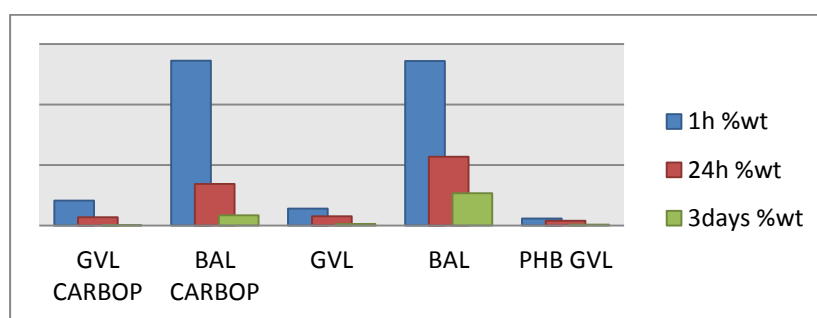
**Figure 3.21** ATR spectra of unvarnished area **(a)**, area cleaned by PHB-GVL gel **(b)**, dammar varnish **(c)** and pristine PHB polymer **(d)** are reported. No evident residues of varnish and polymer are detected after cleaning; the characteristic peak of dammar resin is labeled with asterisk at  $1703\text{ cm}^{-1}$ .



**Figure 3.22** ATR spectra representative of **(a)** unvarnished area, **(b)** dammar reference, **(c)** PHB-GVL cleaning, **(d)** after GVL-Carbopol gel, **(e)** BAL-Carbopol gel. The main peak of varnish is labeled with asterisk at  $1701\text{ cm}^{-1}$ . Residues of varnish remained in after GVL-Carbopol and BAL-Carbopol.

A more exhaustive evaluation of the cleaning systems tested was achieved by spme-GC/MS analyses, performed after 1h, 24 and 3 days from cleaning. Data reported in Table 3.7 proved that using PHB-GVL gel the amount of solvent retained by the substrate after cleaning is very low, thus the risk to damage the paint layer is reduced. As expected the highest values are obtained by BAL-systems (both swab and gel), and these can be considered the threshold limits of a dangerous retentive solvent. All the other systems involving GVL are very far from these limit ensuring a “safe” treatment.

Comparison among PHB-GVL and GVL-Carbopol was performed in order to evaluate the strength of the gels to retain the same solvent. Data after 1h showed that the amount of solvent after PHB-GVL cleaning, is less than are treated with GVL-Carbopol gel. This difference is maintained also after 24 h, until reach a comparable value after 3 days. Results suggested that PHB may retain more efficaciously the solvent GVL compared to Carbopol system.



Cleaning systems	1h %wt	24h %wt	3 days %wt
GVL-Carbopol	0.411 ± 0.025	0.138 ± 0.020	0.063 ± 0.003
BAL-Carbopol	2.725 ± 1.299	0.686 ± 0.475	0.471 ± 0.171
GVL swab	0.282 ± 0.040	0.155 ± 0.043	0.121 ± 0.026
BAL swab	2.721 ± 0.478	1.137 ± 0.383	0.615 ± 0.534
PHB-GVL gel	0.117 ± 0.031	0.077 ± 0.020	0.078 ± 0.014

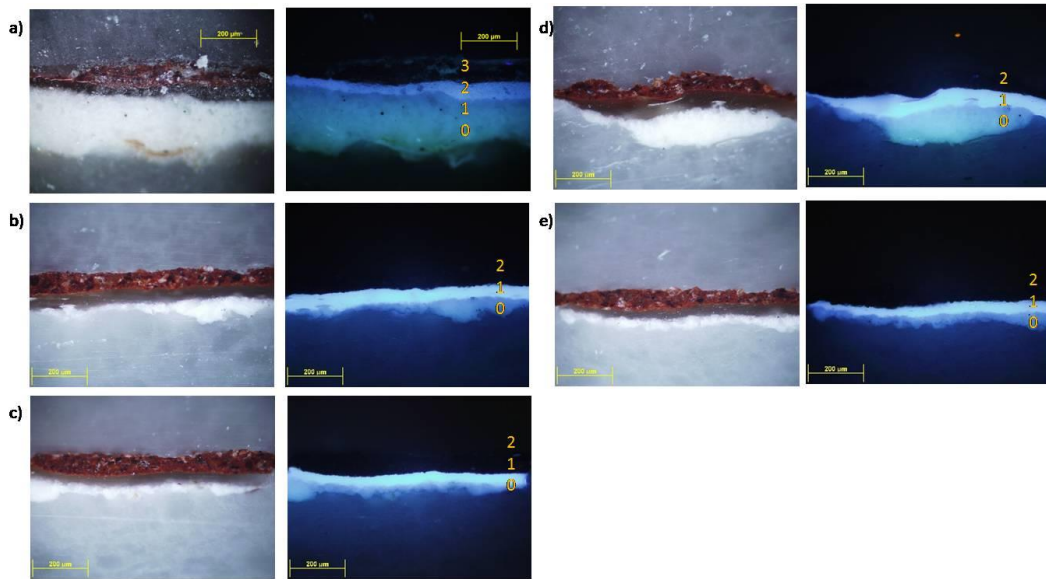
**Table 3.7** Amount of solvent retained by the substrate after 1h, 24h and 3 days from the cleaning. The data carried out with Hs-spme-GC/MS analyses.

### 3.3.3 CLEANING EVALUATION ON ACRYLIC VARNISH

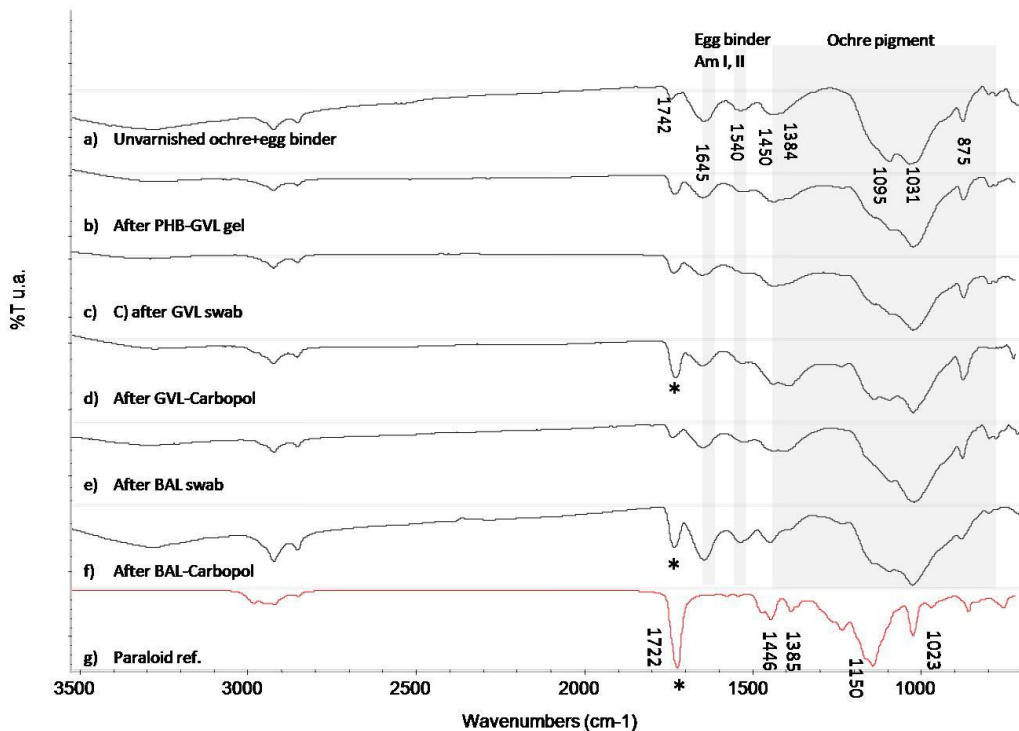
As for the previous testes carried out on dammar varnish, here results of removal capability of acrylic varnish (Paraloid B72) are reported. The efficacy of PHB-GVL gel was evaluated on a mockup, in comparison among neat solvents GVL and BAL and Carbopol gels (GVL-Carbopo and BAL-Carbopol). For all the gelled systems the time of application is 5 minutes.

The mockup stratigraphy consists in varnish layer (Paraloid) 15-20 µm thick (3) applied over a paint layer (2) made of burnt ochre pigment mixed with egg binding medium, a glue closing layer (1) and the ground layer of gypsum and glue (0). In Figure 3.23 cross sections, in visible and UV light, before cleaning (Figure 3.23-a) and after treatments are reported. Is possible to observed that a removal of the coating layer is achieved by

all the systems tested. A more detailed investigation of removal efficacy is performed by FTIR-ATR on the treated surfaces (**Figure 3.24**).



**Figure 3.23** Cross sections of paraloid-egg binder mock up, before cleaning (a) the varnish layer is about 15-20 µm, after PHb-GVL gel (b), GVI swab (c), GVL-Carbopl gel (d) and BAL-Carbopol gel (e) the varnish layer is removed.



**Figure 3.24** ATR spectra acquired on unvarnished area, after every cleaning treatment and on varnish are reported. The main peak of paraloid at  $1722\text{ cm}^{-1}$  is labeled with asterisk. All the spectra collected after treatments reported the characteristic bands ascribable to the paint layer; after GVL-Carbopol gel (d) and BAL-Carbopol (f) an evident residue of varnish remained.

FTIR-ATR spectra reveal that after each treatment a consistent reduction of the varnish layer is achieved. In fact, every spectrum reported the characteristic absorption bands of egg binder and ochre



pigment belonging to the paint layer, mainly amide I and II of binder (respectively at  $1645$  and  $1540\text{ cm}^{-1}$ ) and silicates in the range  $1100\text{-}1030\text{ cm}^{-1}$ . Possible very thin layer of varnish, not visible in optical microscopy, cannot be excluded by spectra because the presence of ester band of egg at  $1742\text{ cm}^{-1}$  that may overlapped with those of varnish at  $1722\text{ cm}^{-1}$ . Moreover Paraloid is a common synthetic product used as a protective coating and as a consolidating agent, thus its possible penetration into the paint layer cannot be excluded. Clearly detectable residues of varnish are present after GVL-Carbopol and BAL-Carbopol gel (**Figure 3.24-d,f**) suggesting a less effective cleaning efficacy compared to the other systems.

### 3.3.4 CASE STUDY: TEMPERA PAINTING OF CIMABUE

From tests carried on mock-ups, the use of gel PHb-GVL resulted more advantageous than traditional methods. In fact despite the cleaning efficacy is good and comparable to the other systems, the gel is able to retain the solvent avoiding excessive diffusion, moreover any mechanical stress is applied over the surface during cleaning.

Once pointed out the advantages in using PHB-GVL, the gel was tested as a cleaning agent on a real tempera painting, “The Majesty” attributed to Cimabue (XIII), currently exposed in the Church Santa Maria dei Servi in Bologna (**Figure 3.25**).



**Figure 3.25** Cimabue’s painting “The Majesty” (XIII). The two images on the right show respectively the application of the gel.

The cleaning efficacy was evaluated by comparison before/after cross sections and by the non-invasive technique Laser scanning 3D microprofilometry, performed directly in-situ, allowing the inspection

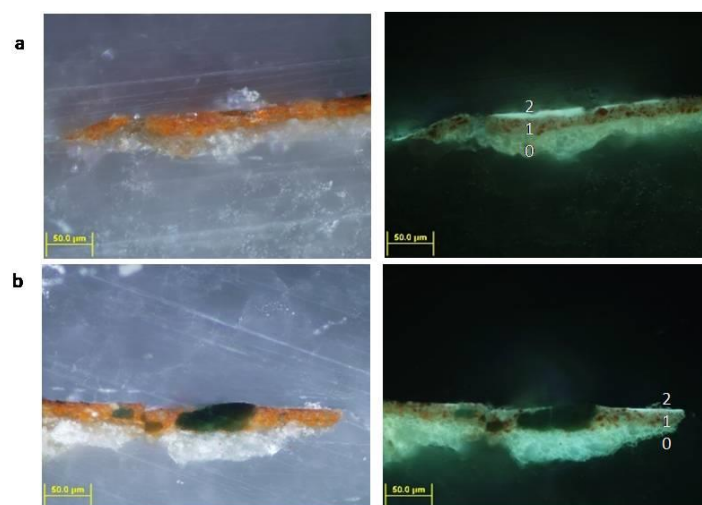


of the superficial morphology (Fontana et al., 2015). The gel was applied and left in contact with the surface for 5 minute. After removal microprofilometry technique was performed between treated and untreated area in order to verify if any morphological alteration happened due to cleaning. Image reported in **Figure 3.26** shows the topographic map in grazing light (on the right) with in evidence the area cleaned with PHB-GVL gel (into the square). Any morphological difference with the untreated area are visible, suggesting that the gel action was extremely superficial and soft without any affection of the original roughness of the painting layer.



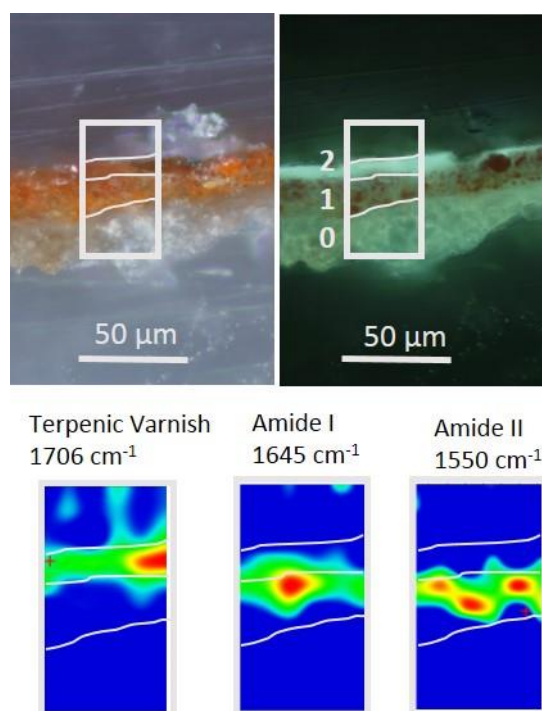
**Figure 3.26** Laser scan microprofilometry on Cimabue. The grey scale image represents the topographic image in simulated grazing light between uncleaned and cleaned area (inside the yellow square) No morphological modification is observed after gel cleaning.

Observation of cross sections of microsamples collected in the treated area before and after cleaning, allowed to assess a consistent varnish layer reduction. As showed in **Figure 3.26** the initial layer of 5-8  $\mu\text{m}$  was reduce to 0-2  $\mu\text{m}$  after gel application (**Figure 3.26-b**).



**Figure 3.26** Micro cross section in visible and UV light before (a) and after gel cleaning (b). A consistent reduction of varnish layer is achieved after cleaning.

Complete information on chemical composition of the coating layer was gained with  $\mu$ FTIR-ATR mapping on cross section. ATR map profiles (**Figure 3.27**) reveal that the varnish is composed by a natural terpenic resin, as defined by the carbonyl diagnostic peak at  $1706\text{ cm}^{-1}$  for dammar resin. The distribution of the amide I and II bands at  $1645$  and  $1550\text{ cm}^{-1}$  in the painting layer, are characteristic for proteins and they are consistent with the use of egg, the typical painting binder used at Cimabue times.



**Figure 3.27** ATR-FTIR map profiles of varnish layer (2) characterized by carbonyl peak at  $1706\text{ cm}^{-1}$  and paint binder (1) by proteic peaks of amide I and II (respectively at  $1645$  and  $1550\text{ cm}^{-1}$ )

### 3.3.5 DISCUSSION

The formulation without plasticizer has been tested for the cleaning of both terpenic and acrylic varnishes applied on egg-tempera painting. The possible sensitivity of egg binder, either to water and organic solvents and the fragility of this paint layer, allowed to investigate the performance of PHB-GVL gel on critic surfaces. Thus cleaning tests on mockups were performed in comparison with neat solvent applied by swab and Carbopol-gel, referred as a traditional gelling-agent employed in restoration. Finally a cleaning test on Cimabue's tempera painting (XIII) was performed and evaluated in terms of removal capability and morphological alteration of the surface.

Gel performance was demonstrated effective in the removal of both natural and acrylic coatings, any alteration of the egg paint layer was observed, despite the critical young binder, indeed topographic analysis carried out on Cimabue's painting confirmed the superficial and gentle action of the method proposed.

Experimental data of solvent retention by the paint layer suggested that GVL tends to be retained, also because its low volatility, anyway it cannot be considered a high retentive solvent as comparison with

“dangerous” benzyl alcohol evidenced. Using PHB-GVL the release of solvent during cleaning is reduced, and thus the risk to interact with the surface is reduced as well. Moreover retention data suggested that PHB polymer is more effective in retain GVL than Carbopol system.

### **3.4 PHB-EL and PHB-DMC GELS**

Other green solvents, capable to form a gel-like material with PHB polymer were considered. The possibility to investigate several cleaning gels increases the range of choice in terms of efficacy an application for cleaning purposes. Moreover the low volatility of  $\gamma$ -valerolactone drove the research to find new alternatives employing solvents more volatile as to decrease further the retention in the paint layer. The green solvents used in the new gels were ethyl lactate and dimethyl carbonate. PHB-based gels were tested for the removal of both terpenic and acrylic coatings applied over mockup painted layers. In order to investigate the effects of cleaning on painting binder, several mockups were prepared using three different binding medium: egg, linseed oil and rabbit glue, mixed with burnt ochre pigment.

In the following section a description of the solvents will be provide and the evaluation of their cleaning performances for the removal of not-aged and aged terpenic varnish applied on oil-binder mockup will be discussed.

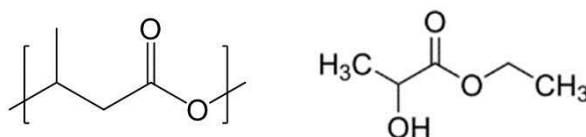
#### **3.4.1 PHB-EL GEL PREPARATION AND CHARACTERIZATION**

The gel is made of two components, the bio-polymer poly(3-hydroxybutyrate) (PHB) largely discussed in section 3.1, and the solvent ethyl lactate (EL).

Ethyl lactate is an important member of lactate esters family thanks to its readily biodegradability and solvent properties (Aparicio, Halajian, Alcalde, García, & Leal, 2008; Pereira, Silva, & Rodrigues, 2011; Salerno, Clerici, & Domingo, 2014). It is found naturally in small quantities in a wide variety of foods, including wine, chicken, and some fruits and can be produced from carbohydrate feedstock since it is produced from ethanol and lactic acid obtained by fermentation of biomass. Is 100% biodegradable, easy to recycle, non-corrosive and non-carcinogenic (Clary, Feron, & van Velthuisen, 1998) . It finds application as a green solvent in several sectors, such as organic synthesis, pharmaceutical preparations, as flavour chemical and in fragrance. It appears particularly attractive as solvent for the industry, thanks to its high capability to dissolve nitro and ethyl cellulose, gums, oils, dyes and many resins (Pereira et al., 2011). Moreover, ethyl lactate has replaced solvents such as toluene, acetone and xylene, in several chemical processes resulting in a much safer workplace (Lomba, Giner, Zuriaga, Gascón, & Lafuente, 2014).

Ethyl lactate is a polar protic solvent (Jessop 2011) characterized by high solvency power, high boiling point (155°C), low vapor pressure and low surface tension. EL molecule is characterized by the vicinity of

hydroxyl and carbonyl groups that give rise to remarkable intra-molecular hydrogen bonding (Aparicio et al., 2008).



**Figure 3.28** chemical formula of PHB polymer and ethyl lactate, respectively on the left and right.

PHB-EL gels were prepared by adding the polymer to the solvent at 110°C and stirring for 2 minutes. Initially the polymer is dispersed in the solvent, after heating become a viscous solution, homogeneous and transparent and as is cooled at room temperature a gel-like material is formed. A similar gelation process as PHB-GVL gel was supposed.

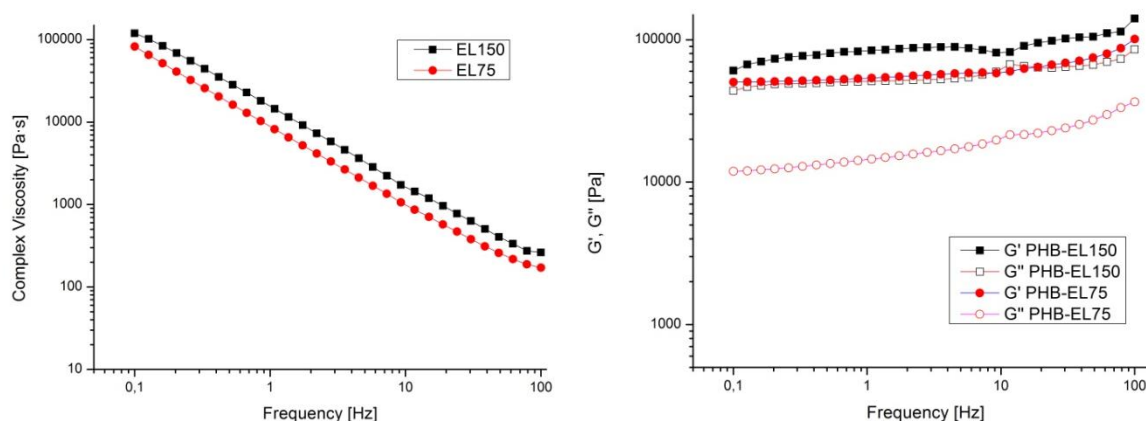
Two formulations were prepared and analyzed: PHB 7%wt (PHB-EL75) and PHB 13%wt (PHB-EL150).

Results of DSC analyses, performed on both gels formulations, are reported in the table below. In all the analyzed samples, only one endothermic transition was observed during first and second scans (**Table 3.8**). As for GVL-gels, discussed in the previous section 3.2.2, the endothermic peak was attributed to the melting of crystalline phase of PHB in the gel, even if it occurred at lower temperature. Also in the second scan of heating an endothermic transition occurred, related to the melting of crystals formed during cooling. This evidence clearly suggest the thermoreversibility of PHB-gels. The melting temperature ( $T_m$ ) is slightly dependent on the amount of polymer present in the formulation.

**Table 3.8** Thermal properties of PHB-EL75 and PHB-EL150 gels acquired by DSC analyses.

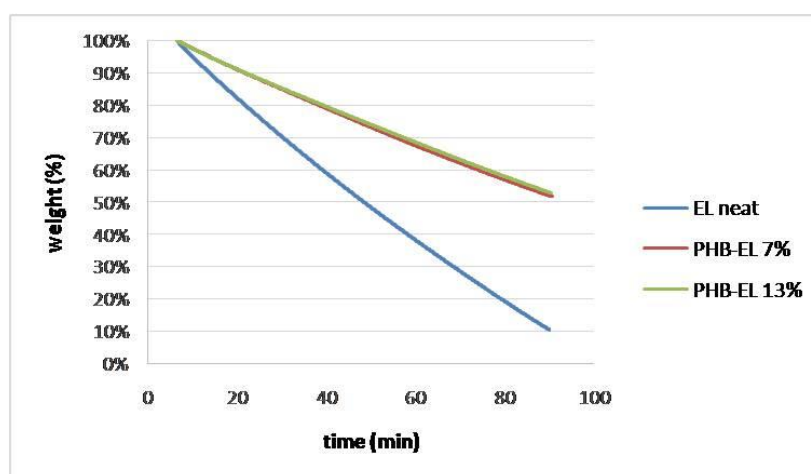
Sample	PHB (%wt)	I scan			II scan		
		$T_m$ (°C)	$\Delta m$ (J/g)	$X_c$ (%)	$T_m$ (°C)	$\Delta m$ (J/g)	$X_c$ (%)
PHB	100	173	99.6	70	172	91.1	64
PHB-EL75	7	89	7.8	78.7	88	6.3	63.6
PHB-EL150	13	91	7.6	40.9	90	7.9	42.6

Rheological data acquired in oscillatory range, reveal that the complex viscosity of both EL gels decreases as frequency increases (**Figure 3.29**) and the contribution of conservative modulus (storage modulus  $G'$ ) is higher than dissipative modulus ( $G''$ ). This behavior in accordance with gel material.



**Figure 3.29** Rheological data of EL gels. On the left the complex viscosity is reported; on the right storage modulus ( $G'$ ) and loss modulus ( $G''$ ) are plotted as frequency function.

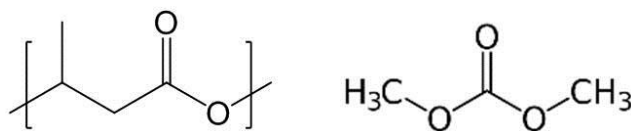
In **Figure 3.30** the evaporation curves of neat solvent ethyl lactate and PHB-EL gels are reported. The curves were obtained by Thermogravimetric Analysis (TGA) using an isothermal run at 40°C for 90 minutes. As expected, the curves show a higher evaporation rate for EL neat solvent compared to gels. Thus also in this case the gel network is capable to retain the solvent slowing its evaporation rate.



**Figure 3.30** Evaporation curves of ethyl lactate neat solvent (blue), PHB-EL75 (red) and PHB-EL150 (green) obtained by TGA isothermal run at 40°C for 90 min.

### 3.4.2 PHB-DMC GEL PREPARATION AND CHARACTERIZATION

PHB-DMC gel is prepared with two components: PHB polymer and dimethyl carbonate solvent (DMC). Dimethyl carbonate is classified both as a non-toxic and environmentally friendly chemical which does not cause emissions of volatile organic compounds in the atmosphere (Rivetti, 2000; Tundo & Selva, 2002). DMC is an alkyl carbonate, classified as a flammable liquid, does not have irritating or mutagenic effects either by contact or inhalation. It exhibits a versatile and tunable chemical reactivity depending on the experimental conditions. The main physical properties of DMC are reported in **table 3.9**.



**Figure 3.31** chemical formula of PHB polymer and dimethyl carbonate, respectively on the left and on the right.

**Table 3.9** Main physical and thermodynamic properties of dimethyl carbonate (Tundo & Selva, 2002)

**Table 2. Some Physical and Thermodynamic Properties of DMC**

mp (°C)	4.6
bp (°C)	90.3
density ( $D^{20}_4$ )	1.07
viscosity ( $\mu^{20}$ , cps)	0.625
flashing point (°C, O. C.)	21.7
dielectric constant ( $\epsilon^{25}$ )	3.087
dipole moment ( $\mu$ , D)	0.91
$\Delta H$ vap (kcal/kg)	88.2
solubility H <sub>2</sub> O (g/100 g)	13.9
azeotropic mixtures	with water, alcohols, hydrocarbons

The gel PHB-DMC was prepared dissolving the polymer in DMC at 100°C for 60 minutes under stirring, inside a closed tube in order to increase the temperature over the boiling point of the solvent. Gelation process happened as the gel cooled to room temperature.

Data from differential scanning calorimetry, reported in **Table 3.10**, evidenced that the melting temperature ( $T_m$ ) of the gel is lower than pristine polymer, but also lower than GVL/EL gels. This may suggest a stronger interaction among DMC and PHB polymer, compared to other solvents.

**Table 3.10** Thermal properties of PHB-DMC gel compared to pristine PHB. Data acquired by DSC analyses

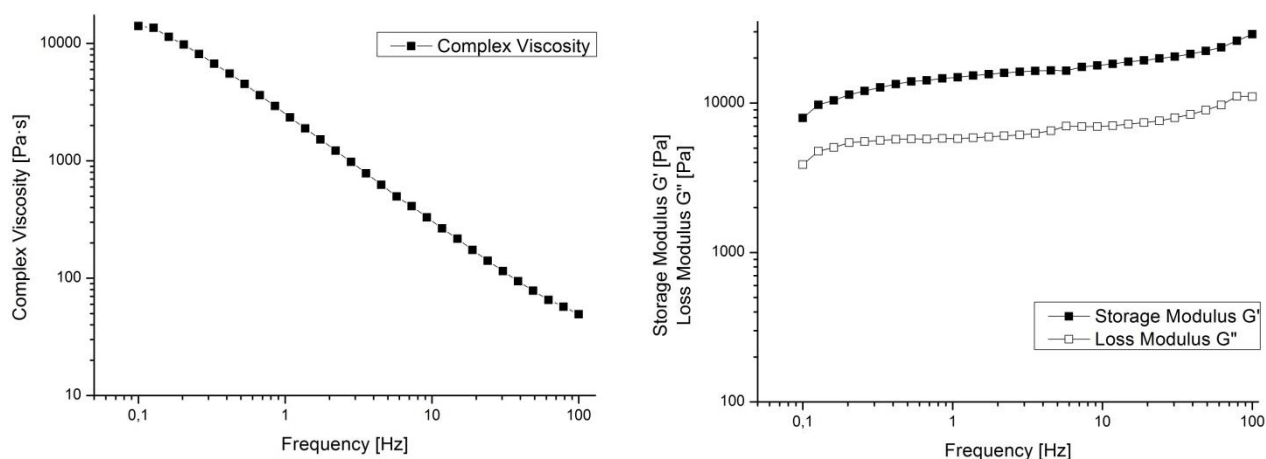
Sample	PHB (%wt)	I scan			II scan		
		$T_m$ (°C)	$\Delta m$ (J/g)	Xc (%)	$T_m$ (°C)	$\Delta m$ (J/g)	Xc (%)
PHB	100	173	99.6	70	172	91.1	64
PHB-DMC	9	76	10.2	81.7	64	7.5	60.1

Rheological data reveal a reduction of complex viscosity by the increasing the angular frequency of the applied oscillatory stress (**Figure 3.32** graph on the left). The elastic component of the system (storage modulus  $G'$ ) and the viscous component (loss modulus  $G''$ ) satisfy the typical condition for gel-material:  $G' > G''$  (**Figure 3.32** right). Indeed this behavior indicates good mechanical properties to the gel and good handling.

Despite the supposed strong interaction between the solvent and the polymer, DMC does not seem to degrade the polymer in a consistent way. In fact the evaluation of molecular weight during a range of time of 10 weeks (ageing time), performed by GPC analyses on PHB-DMC gel, reveals that it remains constant (**Table**

**3.11).** The same result was obtained for PHB-GVL (1-9) formulation, suggesting again the possibility to store and use the product in a reasonable time frame once prepared.

The extremely fast evaporation of dimethyl carbonate, did not allow, to performed thermogravimetric analyses on liquid solvent in order to compare the evaporation rate of gel to neat solvent.



**Figure 3.32** Rheology data on oscillator range. Left: complex viscosity of PHB-DMC gel related to frequency; right: storage modulus ( $G'$ ) and loss modulus ( $G''$ ).

**Table 3.11** Measurements on molecular weight pf PHB-DMC gel aged up to 11 weeks.

Sample Name	Ageing (days)	Mw (Da)	PDI
PHB Pristine	-	850000	3.6
PHB-DMC	7	840000	4.6
PHB-DMC	14	880000	4.3
PHB-DMC	21	860000	3.8
PHB-DMC	28	900000	3.5
PHB-DMC	77	830000	4.7

### 3.4.3 CLEANING EVALUATION ON DAMMAR VARNISH

The cleaning efficacy of the new gels PHB-EL75, PHB-EL150 and PHB-DMC was evaluated on oil mockup for the removal of not-aged and aged dammar varnish.

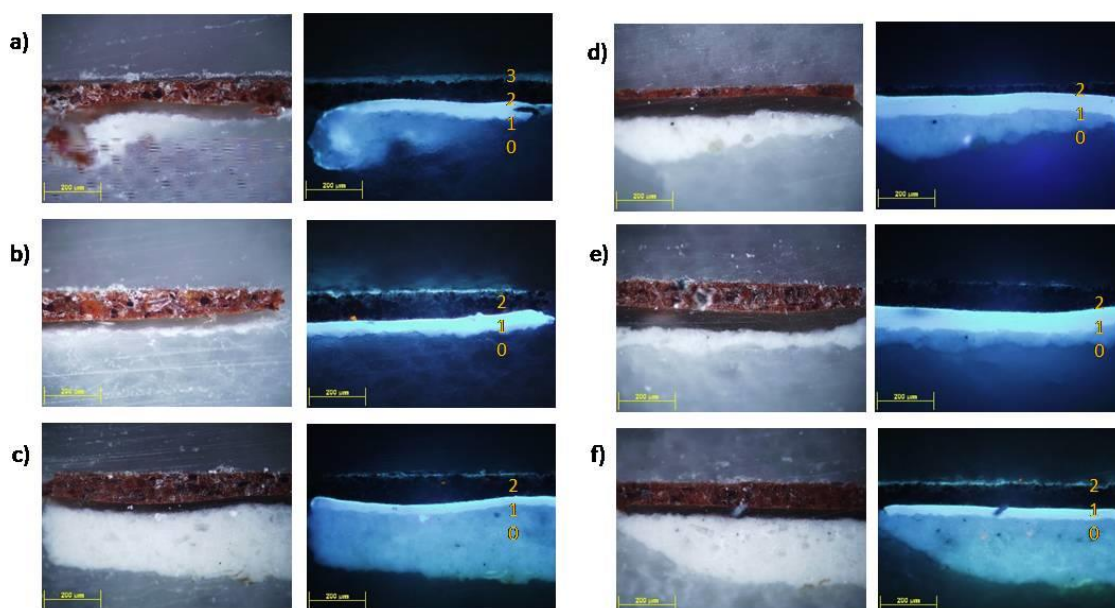
The gels were applied following the same procedure described for the other gels. They were embedded between 2 sheets of rice paper and left on the surface for a contact time of 5 minutes. The cleaning capabilities were compared to neat solvents, ethyl lactate and dimethyl carbonate, applied by cotton swab and benzyl alcohol. Regarding the varnish removal capability, only gels results are reported, while for the evaluation of the retention risk, a comparison with the high retentive solvent benzyl alcohol both in wet form (BAL) and gelled with Carbopol (BAL-Carbopol) was considered.



### 3.4.3.1 NOT AGED OIL PAINT LAYER

The paint reconstruction is composed by an external layer of dammar varnish (about 10-15  $\mu\text{m}$  thick), applied over a paint layer prepared with red burnt ochre and linseed oil medium (**Figure 3.33-a**). Microphotographs of cross sections in visible and UV light after cleaning, revealed that in all cases the fluorescent layer (visible in UV light) on the upper part of the stratigraphy is reduced and in some cases not present, such as after EL and DMC applied with swab (**Figure 3.33-d,e**). As previously seen in **Figure 3.11-f**, the oil binder tends to form a thin layer on the very surface, maybe pushed by the weight of the pigment grains. Thus the thin fluorescent layer of about 2  $\mu\text{m}$ , observable mainly after gels application (**Figure 3.33-b,c,f**) could be due to the preservation of this oil layer, on the contrary neat solvent applied by swab tends to remove it. ATR spectra led to a better consideration regarding the varnish removal of the systems.

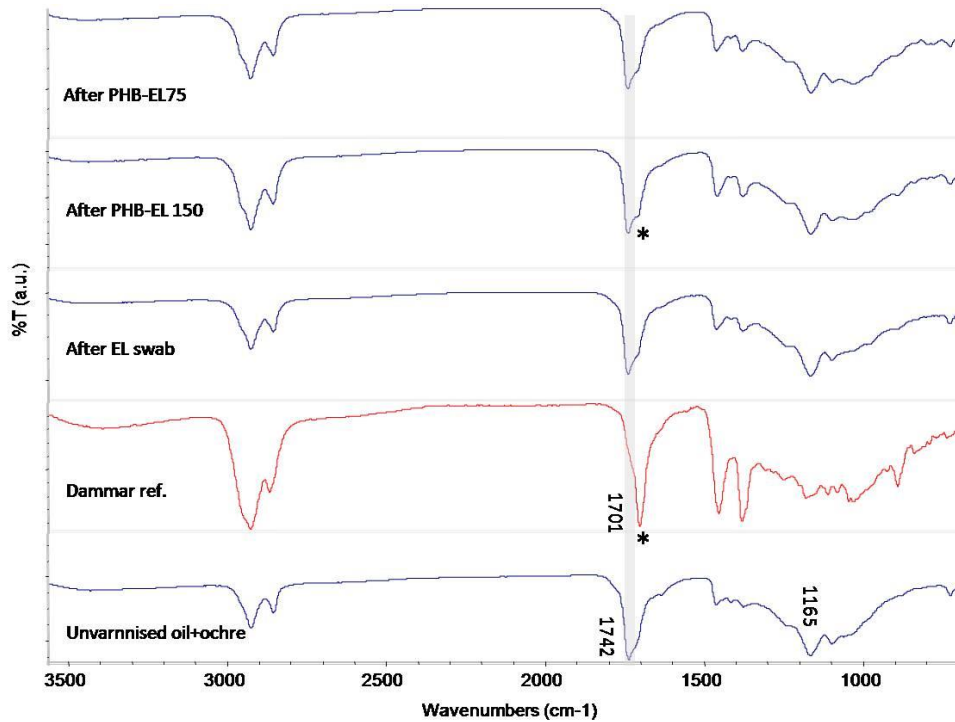
Spectra reported in **Figure 3.34** confirmed that the removal of the varnish was achieved with all the systems. Spectra after cleaning are totally comparable with the spectrum of the unvarnished area, confirmed by the presence of the main oil peak at  $1742\text{ cm}^{-1}$  (stretching C=O ester) and peak at  $1165\text{ cm}^{-1}$  (stretching of C-O-C bond of linseed oil ester). Probably residues of varnish are still present after PHB-EL150 gel as reported in the spectrum.



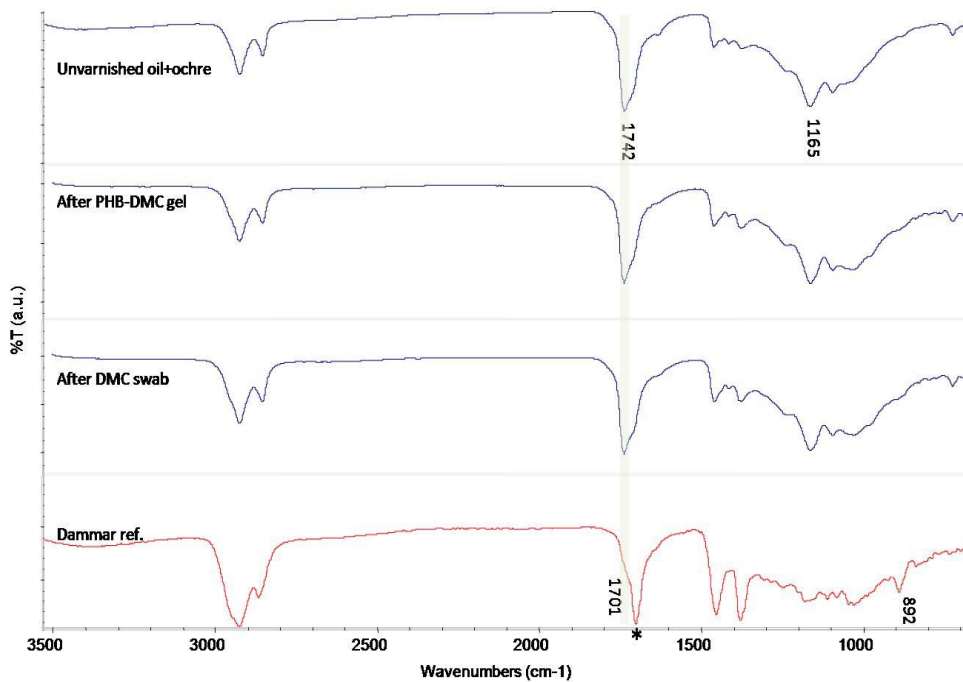
**Figure 3.33** Cross sections in visible and UV light (OM objective 20X) of mock up composed by: varnish dammar of 10-15  $\mu\text{m}$  (3), paint layer of oil and ochre (2), glue closing layer (1) and ground layer (0). Before cleaning (**a**), after PHB-EL150 (**b**), after PHB-EL75 (**c**), after EL swab (**d**), after DMC swab (**e**), after PHB-DMC (**f**).

Cleaning tests performed by PHB-DMC gel and DMC swab, did not show any clearly detectable residue of varnish, as demonstrated by ATR spectra in **Figure 3.35**. In this case as well the spectra after treatment are consistent with the spectrum of unvarnished layer.





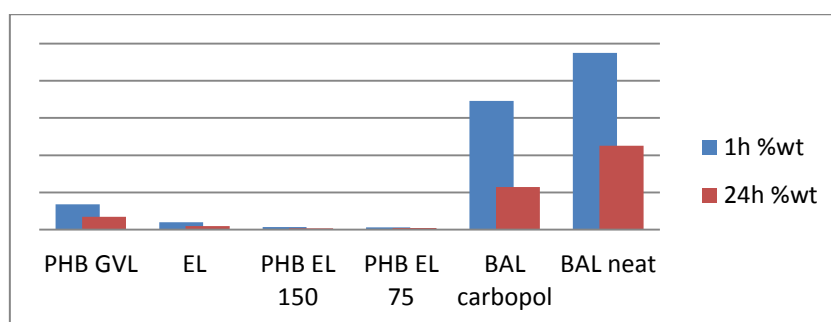
**Figure 3.34** ATR spectra reported in Transmittance % (a.u.) collected on the treated, untreated and unvarnished areas. Varnish peak used as reference is labeled with asterisk at  $1701\text{ cm}^{-1}$ , while reference peak for oil binder is highlighted at  $1742\text{ cm}^{-1}$ . All the spectra acquired after cleaning are consistent with the unvarnished spectrum; possible residues of varnish remained after PHB-EL150.



**Figure 3.35** ATR spectra expressed in Transmittance % (a.u.) referred to unvarnished area, after PHB-DMC cleaning, after DMC swab and varnish, respectively from the top to the bottom. The main peak of varnish is labeled with asterisk at  $1701\text{ cm}^{-1}$ , while the main peak of oil binder is highlighted at  $1742\text{ cm}^{-1}$ .

The cleaning efficacies of EL-gels and DMC-gel respect to EL and DMC solvents, respectively, can be considered comparable in terms of varnish removal. Observing the microscopy cross sections after cleaning, possibly a leaching effect of the oil binder by neat solvents may be considered.

The amount of solvent retained by the substrate after cleaning, was evaluated in comparison with data collected after PHB-GVL, BAL swab and BAL-Carbopol cleaning in order to evaluate the solvent less retained and the “range of safety” of the new methods. Data reported in **Table 3.12** show that cleaning treatments performed with gel systems release less solvent on the surface compared to swab method. Indeed the amount of solvent detected after 1 and 24h after EL-gels is less than EL swab. This evidence suggests a confining action of the solvent due to the polymer network, minimizing the diffusion and retention by the substrate. After DMC, both gel and swab, no residues of solvent was detected, possibly due to the fast evaporation of this solvent. By comparing EL results with PHB-GVL this latter is more persistent and less volatile. Finally comparing with benzyl alcohol treatments, extremely lower values were obtained, classifying also EL- and DMC-systems as “safe” from the risk of high retention in the paint layer.



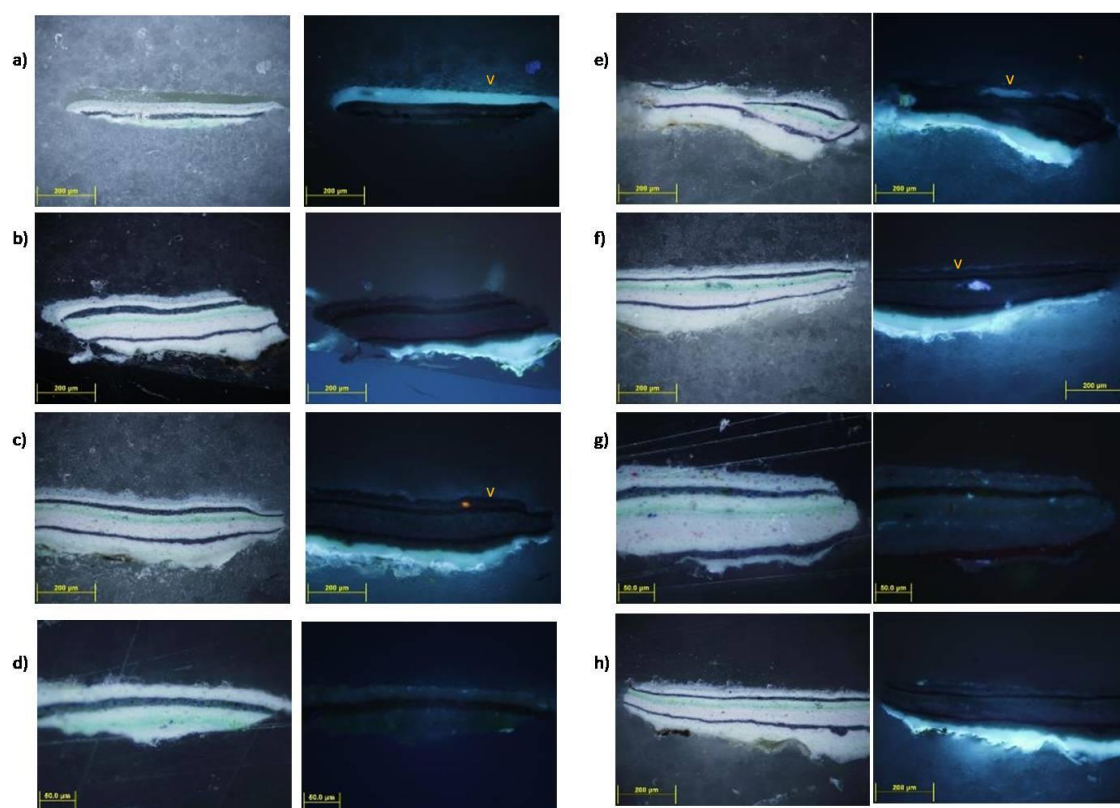
Cleaning systems	1h %wt	24h %wt
PHB-GVL	1.356 ± 0.781	0.685 ± 0.255
EL swab	0.399 ± 0.043	0.194 ± 0.001
PHB-EL150	0.135 ± 0.003	0.067 ± 0.022
PHb-EL75	0.130 ± 0.028	0.088 ± 0.021
BAL-Carbopol	6.926 ± 1.453	2.285 ± 0.160
BAL swab	9.498 ± 3.605	4.505 ± 1.159
PHB-DMC	n.d.	n.d.
DMC swab	n.d.	n.d.

**Table 3.12** Solvent fraction retained by the oil paint layer (not aged sample) after 1 and 24 hours from the cleaning treatments. Data acquired by Hs-spme-GC/MS method, [n.d. = not detected].

### 3.4.3.2 AGED ACRYLIC PAINT LAYER

The artificially aged mockup was used as a tester for the cleaning evaluation of the new PHB-based gels. Ageing process is the same described in section 3.2.4.2. The varnish layer (dammar varnish) is about 50  $\mu\text{m}$  thick and the paint layer is composed by carbonates and silicates pigments mixed with an acrylic binder.

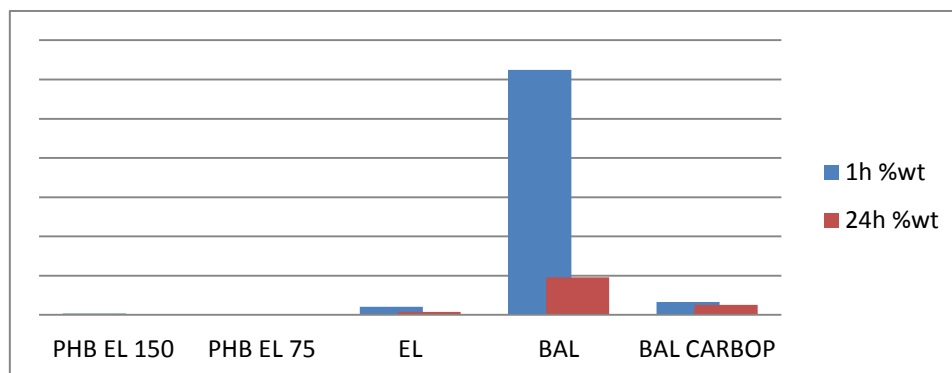
In **Figure 3.36**, microstratigraphy before and after cleaning are reported. The varnish layer applied is very thick (50 $\mu\text{m}$ ). How is possible to observed in UV light images, after EL and DMC neat solvents applied by swab the varnish can be totally removed (**Figure 3.36-b,d**), while after PHB gel-systems some residue remained (**Figure 3.36-c, e, f**) ranging from 5 to 20  $\mu\text{m}$ . This suggest that both the green solvents are effective in the removal of aged dammar varnish but in swab-cleaning method a mechanical action is applied on the surface favoring the removal. Benzyl alcohol, as expected, showed a good removal both in wet and gelled cleaning (**Figure 3.36-g,h**).



**Figure 3.36** Micro cross sections photographs acquired with optical microscopy (obj 20X) under visible and UV light of the aged mockup. (a) before cleaning, (b) after DMC swab, (c) PHb-DMC gel, (d) EL swab, (e) PHB-EL75, (f) PHB-EL150, (g) BAL swab, (h) BAL-Carbopol. The presence of varnish is labeled with 'V' in UV light images.

Retention investigation, after 1 and 24 hours from treatments, was performed and results are reported in **Table 3.13**. As seen in previous case of not-aged sample, the values obtained after gelled-systems are minor compared to swab cleaning method. Therefore PHB-EL gels are capable to retain the solvent limiting its diffusion into the substrate. Difference between the two formulation (PHB-EL75 and PHB-EL150)

are negligible in terms of retention, while after DMC-systems no solvent was detected suggesting a complete evaporation. The values reach after benzyl alcohol treatments are extremely high compared to ethyl lactate and dimethyl carbonate, as expected.



Cleaning systems	1h %wt	24h %wt
PHB-EL150	0.170 ± 0.126	0.039 ± 0.017
PHB-EL75	0.108 ± 0.043	0.045 ± 0.005
EL swab	1.045 ± 0.571	0.373 ± 0.228
BAL swab	31.214 ± 9.662	4.806 ± 1.277
Bal-Carbopol	1.638 ± 1.171	1.296 ± 0.225

**Table 3.13** Solvent fraction detected by Hs-spme-GC/MS method in the substrate (acrylic layer aged) after 1 and 24 hours from the cleaning.

#### 3.4.4 CASE STUDY: OIL PAINTING “MALE PORTRAIT”

The three new gels formulations (PHB-EL75, PHB-EL150 and PHB-DMC) were tested in a oil painting “Male portrait” (XIX century, unknown artist) coming from Holland (**Figure 3.37**). The PHB-based green gels were applied for the fixed time of 5 minutes and their efficacy was compared to the same solvent applied by swab (ethyl lactate and dimethyl carbonate). As a comparison among new and traditional methods, also a tests with benzyl alcohol applied by swab and gelled with Carbopol-Ethomeen were performed.

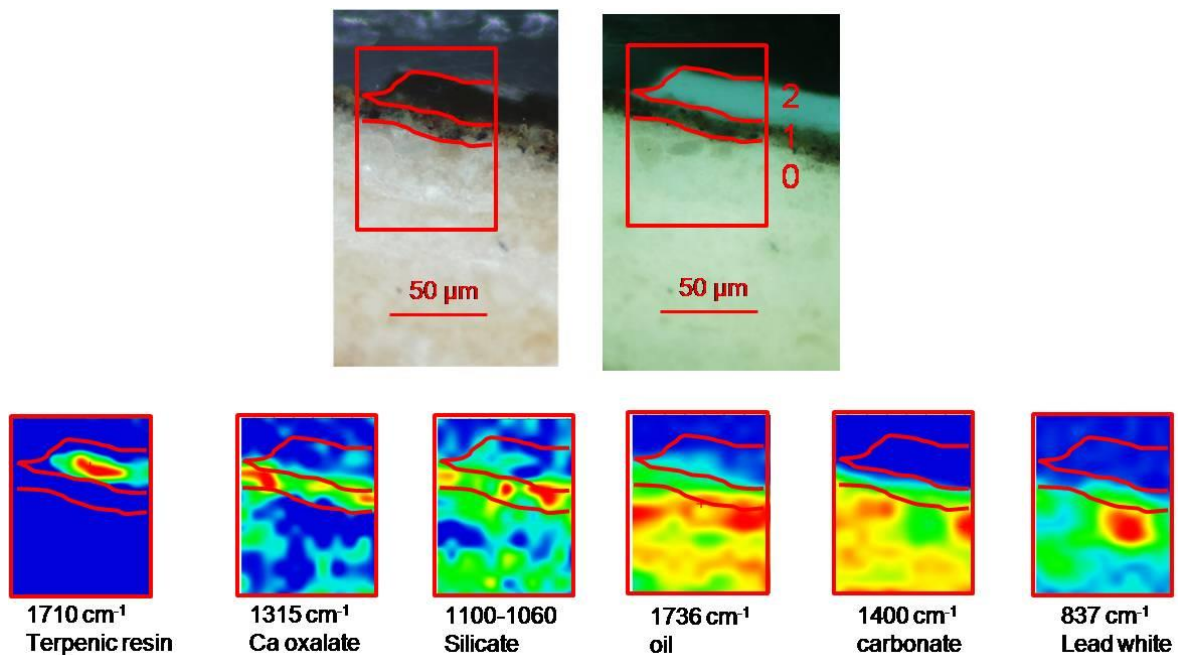
Particularly interesting for this case study is the evaluation among the new methods and the traditional one both in terms of efficacy and retention of the solvent. In fact this is the first real painting where spme-GC/MS was applied.

Cross section of the painting sample collected from the surface before cleaning is reported in **Figure 3.38**. The stratigraphy is composed by an external varnish layer of 15 µm thick (reported as layer number 2), then the paint layer of about 10 µm (layer number 1) and finally a ground layer (number 0). µATR mapping performed in cross section (aperture 40x40 µm, step 7 µm) allowed the attribution of the chemical composition of the layers. Profiles of the map are reported in **Figure 3.38** and showed that the coating layer is the terpenic varnish (map profile at 1710 cm<sup>-1</sup>, associated to C=O stretching vibration in terpenic resin).

The paint layer is constituted by calcium oxalate (map profile at  $1315\text{ cm}^{-1}$  due to the symmetrical stretching of C-O), silicates (profile at  $1100\text{-}1060\text{ cm}^{-1}$  assigned to Si-O-Si stretching vibration) and oil medium (profile peak at  $1736\text{ cm}^{-1}$ , stretching of C=O in ester). Thus probably the paint layer is constituted of an earth pigment and oil medium, consistent with the oil technique most employed in Holland, and some degradation products since the presence of oxalate. Finally the ground layer is composed by calcium carbonate and lead white (respectively peaks profile at  $1400\text{ cm}^{-1}$  and  $837\text{ cm}^{-1}$ ) and oil medium.



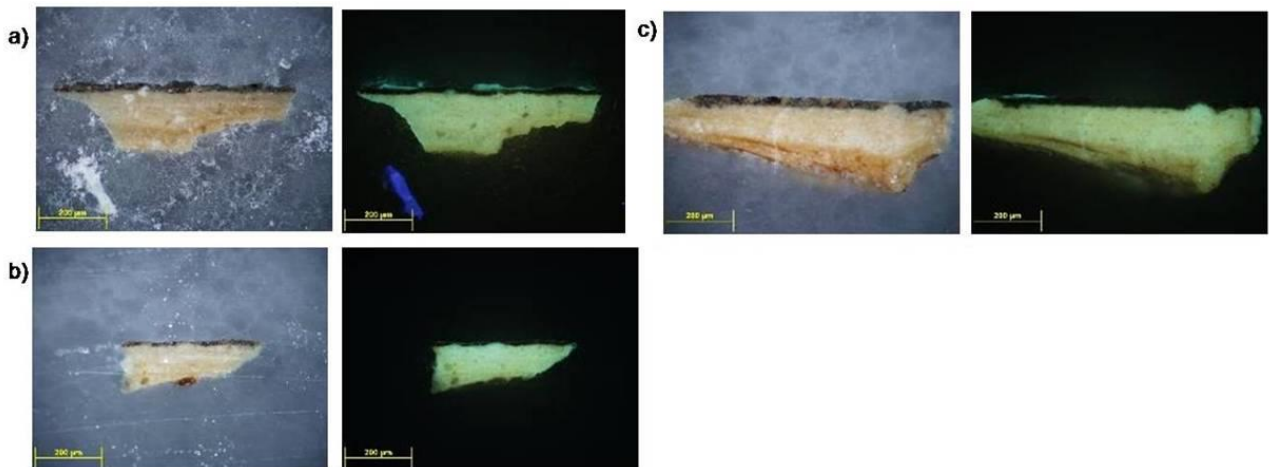
**Figure 3.37** On the left the painting “Male portrait” (XIX, Holland) during restoration practices; on the right the application of the gels.



**Figure 3.38** On the top, cross section in visible and UV light (Optical Microscopy, objective 50X) before cleaning, the stratigraphy is composed by varnish layer (2), paint layer (1) and ground (0). On the bottom, chemical peak profiles of the layers obtained by micro-ATR mapping in cross section.

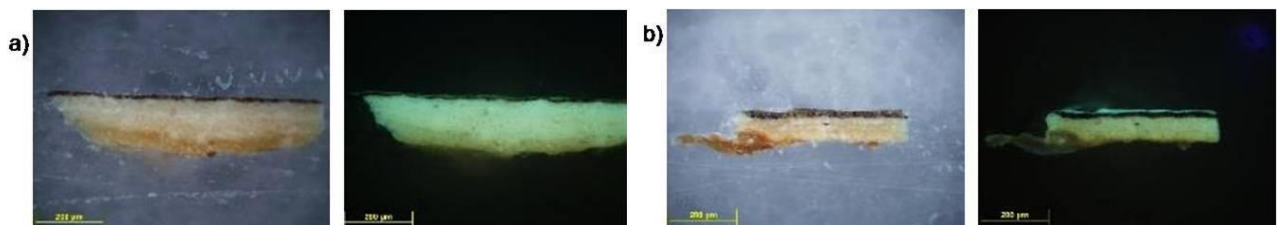


Micro samples were collected from areas after each cleaning and investigated in cross section under Optical Microscopy in visible and UV light in order to evaluate the efficacy of the treatments and eventually the thickness of the varnish residues. Microphotographs of the cross sections are reported below. Considering the ethyl lactate-systems, the most effective removal was achieved by PHB-EL75 gel and EL applied with swab (**Figure 3.39-b,c**). After gel a varnish residue of about 2  $\mu\text{m}$  thick is observed, while the swab cleaning leads to a spot residue. Different is the case after PHB-EL150 gel, in fact a quite non effective removal is observed (**Figure 3.39-a**).



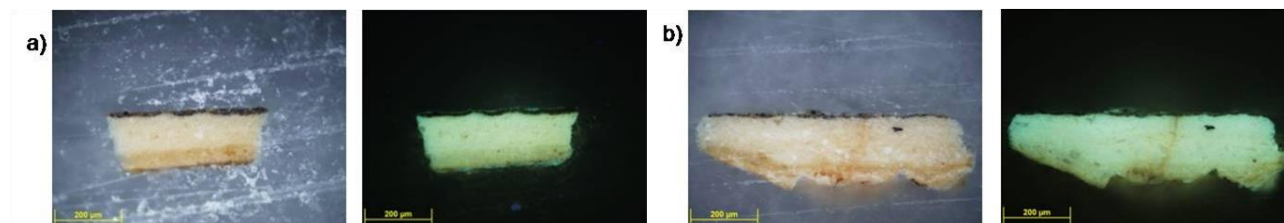
**Figure 3.39** Microphotographs of real oil painting after cleaning. Cross section in visible and UV light after (a) PHB-EL150 gel, (b) PHB-EL75 gel, (c) EL swab. Evident residues of varnish remained after PHB-EL150 gel cleaning.

Interesting is the cleaning efficacy of PHB-DMC gel compared to dimethyl carbonate neat solvent applied by swab. In fact in the latter the varnish was very little thinned (**Figure 3.40-b**) while after the application of DMC gel for 5 minutes, the reduction of the varnish is consistent (3-4  $\mu\text{m}$ ) (**Figure 3.40-a**). This evidence may be explained by the long contact time between the solvent in a gelled form and the varnish. Indeed DMC is a very volatile solvent and sometimes it required more time or more applications for a good removal, but using the solvent as a gel allowed a longer contact time action.



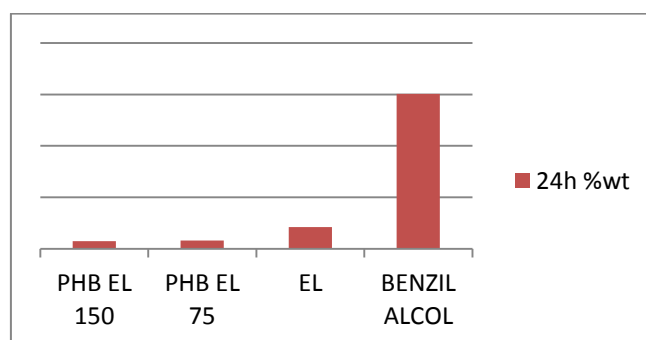
**Figure 3.40** Microphotographs of cross sections in visible and UV light after (a) PHB-DMC gel, (b) DMC swab. Evident residues of varnish remained after cleaning with swab.

Cleaning performances of benzyl alcohol performed in a gelled form (BAL-Carbopol) and by swab (BAL) are reported in **Figure 3.41**. After swab cleaning the varnish is removed, after BAL-Carbopol gel a consistent reduction is obtained (about 4  $\mu\text{m}$ ).



**Figure 3.41** Microphotographs of cross sections in visible and UV light after (a) BAL-Carbopol gel, (b) BAL swab. In both cases a satisfactory cleaning action was achieved.

Retention studies carried out after 24 hours from the cleaning (**Table 3.14**), confirmed the results obtained until now. Indeed using PHB-gels instead of neat solvent applied by swab allows a more controlled release of solvent and thus reduce the retention risk. Moreover the comparison with benzyl alcohol, here reported as a high retentive solvent, confers to ethyl lactate and dimethyl carbonate the “totally safeness” of the cleaning also for the artwork.



Cleaning systems	24h %wt
PHB-EL150	0.076 ± 0.033
PHB-EL75	0.081 ± 0.049
EL swab	0.212 ± 0.169
PHB-DMC	n.d.
DMC swab	n.d.
BAL swab	1.506 ± 0.917

**Table 3.14** Amount of solvent detected by HS-spme-GC/MS method in the paint layer (oil painting “Male Portrait”, XIX) after 24h from the cleaning.

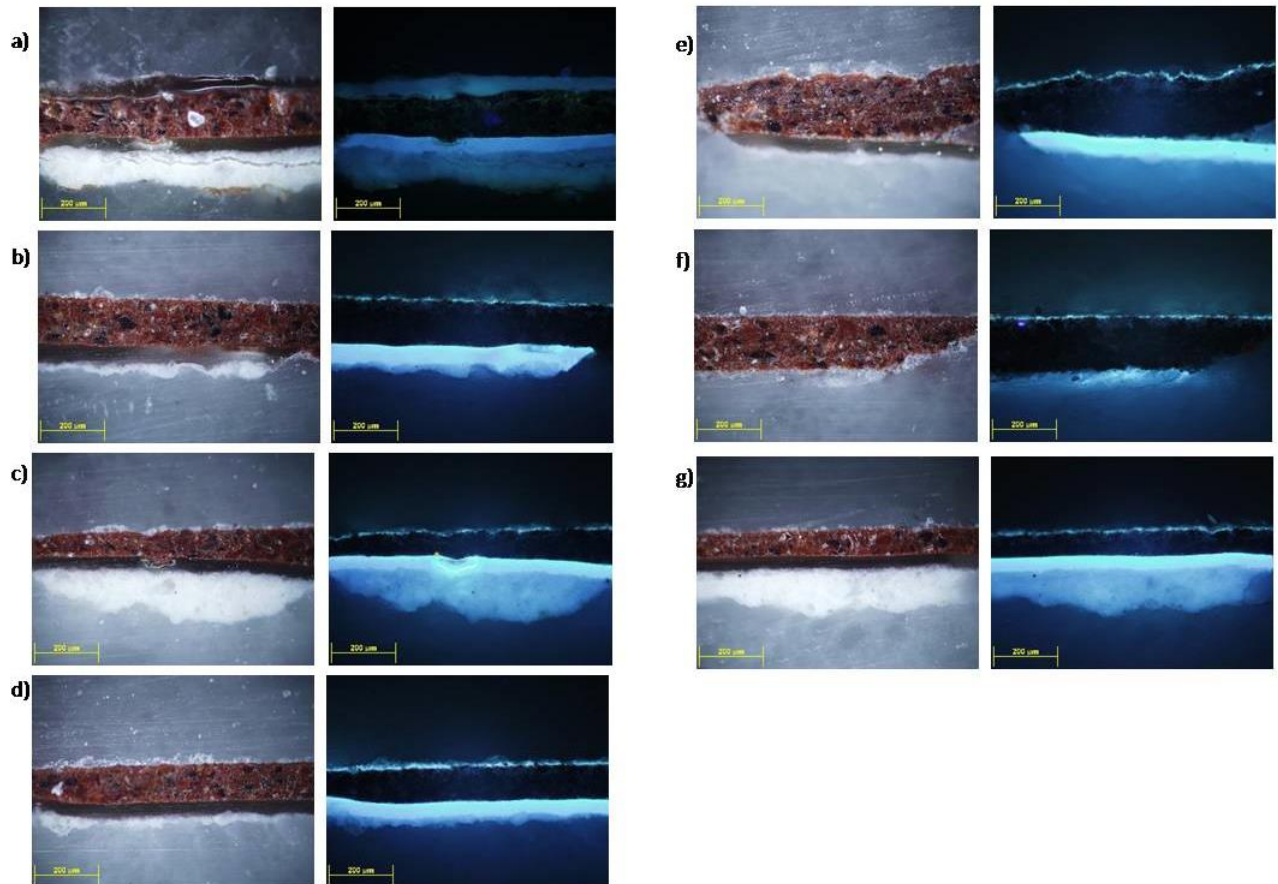
### 3.4.5 CLEANING EVALUATION ON ACRYLIC VARNISH

Ethyl lactate and dimethyl carbonate gels and neat solvent applied by swab, were evaluated for the removal of synthetic coating from an oily mockup. In fact because the wide use of synthetic polymers, such as acrylic resin, for protective and consolidating purposes, is important to test the capabilities of the new systems in the removal of Paraloid B72®.

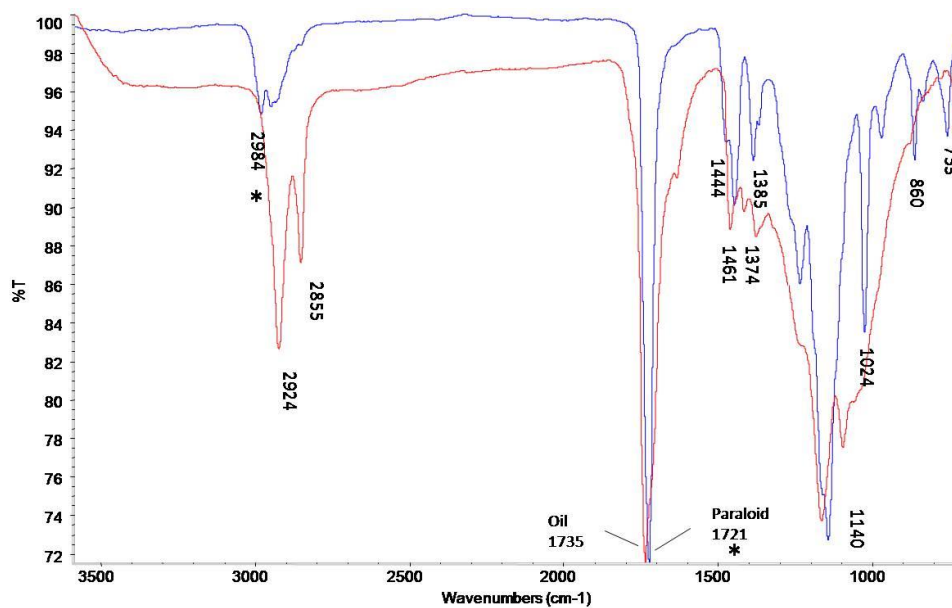
Cleaning tests were performed on mockups composed by three different binder, linseed oil, animal glue and egg, mixed with the same burnt red ochre. Here only results on paraloid applied over oily-binder will be show. Paraloid varnish tends to penetrate into porous layer, indeed ATR analyses in cross section of glue and egg binders, revealed the presence of the varnish through the paint layer also in a not-cleaned sample (spectra not reported). Using oil binding media the porosity of the layer is reduced and no penetration occurred.

As shown in **Figure 3.42** the mockup is composed by the acrylic varnish layer Paraloid of about 40  $\mu\text{m}$  thick applied over a linseed oil binder mixed with red ochre pigment. The cleaning systems tested were PHB-EL150 gel, PHB-EL75 and PHB-DMC gels applied for 5 minutes, compared to EL and DMC neat solvents and acetone applied by swab as cleaning performance reference (in fact acetone is the solvent employed for the varnish preparation). Observing the optical microscopy cross sections in UV light, is possible to notice that after all cleaning treatments, a thin fluorescent layer ( $< 3\mu\text{m}$ ) remained on the surface. But by optical observation in is not possible to establish if the fluorescence is due to a residue of varnish or to the binder. In fact as previously seen in oily-medium sample not varnished, a superficial fluorescence is present as well. ATR spectra acquired on the treated surfaces, became a very useful tool to establish if any varnish residue remained. Because the partial overlapping of some vibration frequencies present on both varnish and paint layer, as evidenced in **Figure 3.43**, residues of paraloid will be identified mainly by most prominent characteristic peak at  $1721\text{cm}^{-1}$  due to the carbonyl stretching vibration of ester group and  $1240 - 1024\text{ cm}^{-1}$  respectively of asymmetric and symmetric C-O stretching of ester group. Further diagnostic peaks are in the range of aliphatic chain stretching vibration (  $-\text{CH}_2-$  stretching at  $2984\text{ cm}^{-1}$ ). ATR spectra reported in **Figure 3.44** reveal that only in one case the cleaning allowed to the total removal of the varnish layer. Indeed spectrum after PHB-DMC gel (**Figure 3.44-e** ) present the typical absorption bands of oil binding medium, mainly recognizable at  $2924$ ,  $2855$  and  $1735\text{ cm}^{-1}$ , while acrylic bands of the varnish at  $2984$ ,  $1024$ ,  $860\text{ cm}^{-1}$  are not detectable. This evidence suggest that DMC is a good solvent for this kind of synthetic polymer, but because its high volatility is not efficient enough applied by swab. On the contrary, applied in a gelled-form allowed a better and longer contact with the substrate. This different observed for DMC systems, in not clearly evidenced among EL-gels and EL neat solvent. In this case the difference in cleaning performance is not so remarkable. Anyway, PHB-EL150 and PHB-EL75, showed a comparable cleaning capability to acetone applied by swab and here considered as a solvent reference.

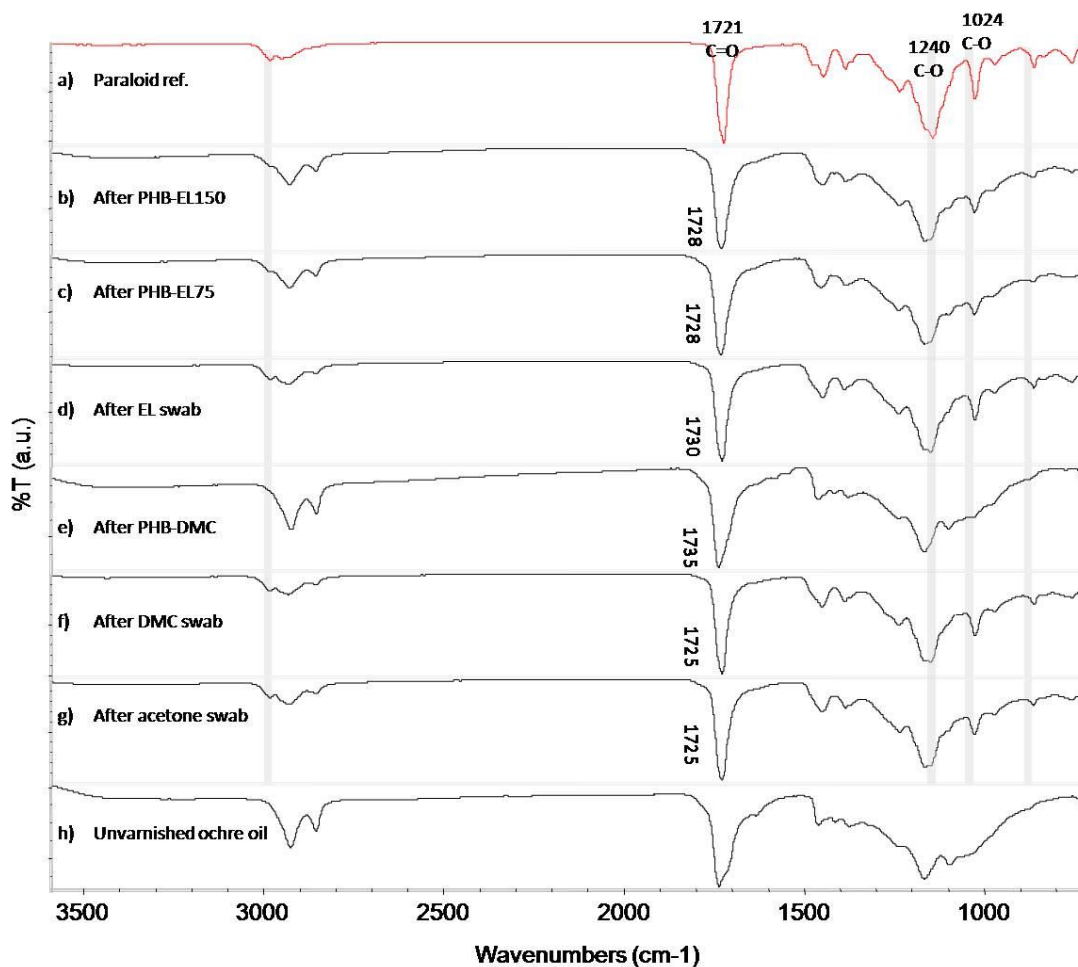




**Figure 3.42** Microphotographs in cross section (Optical microscopy obj. 20X) of paranoid oil-binder mockup; (a) before cleaning the varnish layer is about 40-50 µm, after (b) PHb-EL150 gel, (c) PHb-EL75 gel, (d) EL swab, (e) PHB-DMC gel, (f) DMC swab, (g) acetone swab.



**Figure 3.43** ATR spectra on paraloid B72 varnish layer (blu) and oil-ochre paint layer (red). Diagnostic peaks of varnish are marked with asterisk at 2984, 1721, 1024  $\text{cm}^{-1}$ .



**Figure 3.44** ATR spectra collected over the surface before cleaning and after cleaning on paraloid varnish an oil-ochre paint layer mockup. (a) Paraloid varnish reference spectrum, (b) after PHB-EL150 gel 5min, (c) after PHB-EL75 gel 5min, (d) EL swab, (e) PHB-DMC gel 5min, (f) DMC swab, (g) acetone swab, (h) unvarnished area. Only after PHB-DMC gel the varnish is clearly removed. After all the other treatments a comparable cleaning efficacy is showed.

### 3.4.6 DISCUSSION

New PHB-based gels were prepared and tested as cleaning tool for the removal of varnish coating from painting in restoration practices. Gels are composed by the bio-polymer poly-(3-hydroxybutyrate) and two different green solvents: ethyl lactate and dimethyl carbonate. With ethyl lactate, two formulations were prepared containing different amounts of polymer (PHB-EL75 and PHB-EL150), while for dimethyl carbonate only one formulation was considered (PHB-DMC). Analytical characterization of the gels carried out with calorimetric and rheological measurements evidenced the thermoreversible behavior of these materials. As previously observed for GVL gels, the gelation process can be associated to the recrystallization of the PHB crystalline phase, possible in smaller aggregates.

Cleaning tests performed on not aged and aged natural terpenic varnish demonstrated a satisfactory removal capability of EL/DMC-gels. In some cases, the action of gel compared to its respective green solvent,

is less pronounced but this lies in a more gentle action without apply any mechanical stress to the surface. The evaluation of the amount of solvent retained by the substrate after cleaning, allowed to consider PHB-based gels totally safer for the artwork as well. Indeed the solvent is retained by the polymer structure reducing its diffusion into the paint layer. Moreover, due to its high evaporation rate, dimethyl carbonate gel did not show any solvent residue after treatment. Comparable results were obtained on real oil painting "Male portrait", both in terms of cleaning ability and in terms of solvent retention. Moreover the possibility to work with a completely non toxic and not harmful system, either for human health and environment, are further advantageous aspects to be considered in restoration practices.

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## CHAPTER 4. Conclusion

The cleaning of artworks is one of the most critical intervention aimed at the removal/reduction of superficial degraded layers such as protective coatings, not-original overpainting, incoherent materials (e.g. dust and grime). The physical and chemical alteration of materials is a natural phenomenon due to the interaction with the surrounding environment. Once alteration causes aesthetical modification of the artistic information or mechanical damage, compromising the stability of the layers underneath, the removal and substitution of such degraded materials is required.

The cleaning can dramatically alter the appearance of the surface and its mechanical properties. Several studies mainly during 1950s highlighted the complexity of this procedure, not only because the chemical composition and the state of conservation of the substrate, but mainly because of the traditional methods employed. Indeed regarding the cleaning of paintings, several risks must be taken into account: the risk to alter the original surface by using chemicals too aggressive, the risks for the healthiness of the restorer/conservation by using toxic or harmful substances, the risk for the environment related to the not correct waste disposal of such products.

During decades cleaning methods evolved in the direction to reduce the interaction with the underneath layers as much as possible. Indeed the traditional method of wet cleaning, which employed a cotton swab soaked with organic solvents, has been (quite) substituted by gelled-systems. Gels allow a better control of the cleaning action by reducing the lateral and vertical diffusion of the solvent and by increasing the contact time with the surface. In this way the material to be removed is slowly swelled and no mechanical stress is required for its removal.

During 1990s also toxicity became an important issue in restoration practices, inducing the introduction of "safer" approaches such as water-based solutions and enzymatic methods. Despite many efforts were paid for the definition of alternative strategies to the wide use of harmful organic solvent, several applicative drawbacks still remained (e.g. water-sensitive substrate, scarce control of the cleaning action, controversial efficacy).

Nowadays the increasing attention towards both healthiness and environmental issues has gone ahead also in the field of chemistry applied to Cultural Heritage. The use of not standardized restoration procedures has demanded to strengthen regulations and recommendations in matter of safety and security for both the human healthiness and the environment in restoration practices at international level, aimed at the "sustainability in restoration".

This research work was aimed at the development of sustainable strategies for the cleaning of paintings. Totally safe organogels, based on poly-(3-hydroxybutyrate) biopolymer (PHB) as gelling agent and several green solvents ( $\gamma$ -valerolactone, ethyl lactate, dimethyl carbonate), were produced and evaluated as cleaning tools for the removal of varnishes from painted surfaces. Each gel component, derived from

renewable sources, are non toxic and biodegradable. According to green chemistry principles, sustainability starts from the source of the materials employed.

The analytical characterization of PHB-gels, carried out with differential scanning calorimetry and wide-angle X-ray diffraction, allowed to investigate the process and factors responsible of gelatin process. Indeed all the systems are thermoreversible, physical interaction between polymer chains and solvents link the 3D network in a gel-like structure. The solubilization of PHB in such solvents (GVL, EL and DMC) occurred only at high temperature (130-110°C depends on the solvent considered) and required a very short time, 1-2 minutes. Only DMC required 60 min to dissolve the polymer. Gelation process occurred as the solution is cooled down to room temperature by the recrystallization of polymer aggregates, as confirmed by diffractometric analyses on wet and dry gels. For most of the formulations analyzed, rheological measurements showed the mechanical behavior typical of gels: the conservative modulus (storage modulus  $G'$ ) is higher than dissipative forces (loss modulus  $G''$ ). Therefore such materials show viscoelastic properties which confer a good handling and mechanical properties suitable for the application on painting surfaces. In correspondence of high polymer content in the formulation, an inverse situation is observed, gels show more plastic behavior. These gels appear more rigid, but this characteristic allow to be applied without stick to the surface.

The cleaning efficacy of the proposed methods was tested for the removal of natural (terpenic) and acrylic varnishes, applied over different binding media (linseed oil, egg, animal glue) in order to evaluate interactions with the binder, if any, during cleaning. Moreover the tests were carried out on not aged and aged mock-ups purposely prepared and finally validated on real paintings.

Studies reported in literature on the long-time effects of cleaning, demonstrated how is difficult to predict and evaluate exhaustively a cleaning action because different phenomena occurs at the same time, at the interface between the substrate and the solvent. Therefore dissolution, evaporation and penetration become factors not easily predictable in a porous matrix such as painting. The irreversible long-time damage of the painted surface becomes a real risk that science in conservation have to evaluate. For this reason an analytical protocol for the overall evaluation of the new PHB-gels was specifically tailored, in compliance with conservation requirements. Thus the use of non-invasive or micro-disruptive techniques with the maximum information with minimum sampling, was satisfied.

The protocol consists of optical microscopy equipped with visible and ultraviolet light,  $\mu$ -FTIR spectroscopy in total attenuated reflection mode (ATR) and headspace-spme coupled with GC-MS technique (HS-spme). In particular observation of micro-cross sections under microscopy in vis/UV light allowed the definition of the layers thickness and the evaluation of varnish removal efficacy. Moreover damaging interactions with the paint layer, such as chromatic alteration and mixing of layers, can be revealed by this technique.  $\mu$ -ATR technique allowed the collection of an high number of spectra over the treated surface, in a non-invasive way. Thus the efficacy of the cleaning and the residues of varnish or the not volatile gel

components were evaluated by this spectroscopic technique. In order to improve the sensitivity of the analytical protocol, achieving information about the retention of the solvent into the paint layer, the HS-spmc methods was successfully introduced. Up to now, retention studies mainly referred to a gravimetric method introduced in 1980s (curves of retention-evaporation), obtained experimentally and based on solvents volatility. In this research, the introduction of HS-spmc method allowed the quantification of the amount of solvent retained by the paint layer after cleaning, and thus indirect information about retention can be obtained. Moreover indirect information about the retentive power of the gel-system can be achieved as well, allowing the construction of a relative “safer” scale for cleaning treatments.

The overall cleaning performance evaluation of the three gel-systems considered (GVL-/EL-/DMC-PHB) gels has been satisfactory. The green solvents employed belong to ester family and thus a good removal capability for both natural and synthetic varnish was predictable. Moreover esters are considered low retentive solvent and low swellers. Being neutral solvents a physical mechanism of solubilization was expected. Experimental data on cleaning efficacy confirmed the theoretical suggestions.

GVL-PHB gels showed a cleaning efficacy comparable to GVL neat solvent and reference solvents (acetone and dimethyl sulfoxide used as traditional solvents in cleaning) for the removal of natural and acrylic varnishes. Moreover the confinement of the solvent into the PHB network allowed the excessive diffusion into the paint layer reducing the retention risk. PHB demonstrated to be more effective than traditional gel-system (Carbopol-ethomeen gel) to retain GVL solvent and control its release. Cleaning tests carried on real paintings with oily and tempera binders, confirmed the gentle and controlled cleaning action of GVL-PHB gels, avoiding mechanical stress on delicate surface and allowing an effective reduction/removal of the varnish layer.

EL-PHB gels, both the formulation containing different amount of polymer, were effective in the removal of aged and not aged natural and acrylic varnishes without any damage of the paint layer. According to the varnish thickness, in some cases the application of neat EL solvent seemed to remove more efficaciously than gel, but retention investigations confirms the capability of PHB gel to retain and confine the solvent. Thus, from an applicative point of view, more than one application of the PHB-gel is preferred to the mechanical and less controlled application of wet swab.

DMC-PHB gel, due to the extremely high volatility of the solvent, is the only systems (among the here considered) with less retention risk. The ability of the gel to remove the varnish layer is enhanced compared to DMC neat solvent. Indeed the PHB network has the ability to entrap the solvent slowing down its evaporation and thus allows a longer contact time with the layer to be removed.

From cleaning evaluation data, GVL gels seem to be the most effective in the removal of both terpenic and synthetic coatings, at least the most reproducible system. GVL is an aprotic polar solvent with high dielectric constant, thus a strong solubilization of material was expected. But because its low volatility, it remains into the paint layer still after few days. Retention values associated to GVL are very far from the



values of an higher retentive solvent such as dimethyl sulfoxide or benzyl alcohol, but are not null. For these reason systems that employ more volatile solvents, such as ethyl lactate and dimethyl carbonate, may be considered “safer” for the artwork.

The confinement of these solvents into the polymer matrix of PHB polymer, allows the reduction of the evaporation rate of the solvent, as demonstrated by thermogravimetric analyses. This effect is advantageous both for the operator because the risk of solvent inhalation is reduced and for the artwork because by enhancing the contact time with the surface a more gentle removal is performed. Moreover the use of these gel systems allow a more localized and controlled solvency action avoiding the spread and redistribution of solubilized material which will tend to migrate into the gel.

In conclusion the development of new PHB-based gels, allowed the definition of a totally sustainable cleaning approach. All the gel components come from renewable sources, are non toxic and biodegradable. Gels preparation is easy and fast, their application as cleaning agent demonstrated a satisfactory action, effective, localized and controlled. The employment of several green solvents allowed the possibility to choose and select the most promising on the basis of the substrate to be treated. Finally the gels are biodegradable, thus completely in accordance with circular economy principles.

About the evaluation of cleaning performances, a tailored protocol has been developed in order to consider all the aspects concerning the cleaning risks for the artwork. This protocol was applied also to a real painting, showing good accordance with tester data.

Further improvements of the proposed systems are currently in progress by exploring solvent mixtures and new formulation for the removal of apolar coatings such as wax-based varnish applied on metals and stones artifacts.