The Chemistry of Egg Binding Medium and Its Interactions with Organic Solvents and Water

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ABSTRACT. The aim of this study was to gain a deeper insight into the properties of egg, used as a binding medium, and its interactions with water and organic solvents. The research focused on egg tempera films prepared in July 2007 and on a tempera layer prepared by researchers of the Smithsonian's Museum Conservation Institute in 1995. It also included a sixteenth-century panel painting from the Pinacoteca di Siena. Two paint samples from this painting were analyzed by gas chromatography coupled with mass spectrometry and identified egg as the binding medium. Ethanol or acetone, isooctane, and water were used to test for leaching. In general, the main leaching occurs from the fresher samples: using solvents of different polarity (from isooctane to water), the lipid components are the most removed. It also was observed that pigmented layers are less affected by leaching phenomena than a layer without pigments, in particular for lipid components. Finally, it was noticed that unsaturated fatty acids were extracted mainly from fresh samples, with less from the 1995 tempera and nothing from the sixteenth-century painting. However, the study carried out on this painting showed that leaching is more pronounced for the lipid components, and amino acids were also detected. These results were unexpected because mild cleaning tests were believed not to affect a 500-year-old painting. These results might be regarded as guidelines to take into account for cleaning paintings.

INTRODUCTION

Egg tempera techniques employ the whole egg, or egg yolk and egg white separately, for binding media purposes, in which pigments are dispersed, sometimes combined with other materials, i.e., fig latex and cherry gum, depending on the painter's requirements. Egg tempera was traditionally used in the past, especially in the fourteenth and fifteenth centuries in Italian painting.

Because detailed studies are available on this topic (Thompson, 1936; Boon et al., 1997; Phenix, 1997), only a general description will be provided. Egg binding medium is made up of proteins, lipids, polysaccharides, and inorganic compounds. Egg white is a diluted water solution of proteins (mainly ovalbumin) and a small fraction of polysaccharides. Once applied, this material becomes insoluble.

Egg yolk contains mostly lipids (66% in terms of mass) and proteins, as well as small amounts of polysaccharides and inorganic compounds. Lipids are present in the yolk as triglycerides (neutral lipids), phospholipids, and cholesterol. Triglycerides, the main lipids, are the same type of compound as drying oils. However, in egg these lipids contain less unsaturated fatty acids compared to oils. The fatty acid distribution in yolk lipids is saturated 38%, monounsaturated 42%, and polyunsaturated 20%. Their drying properties are not as strong as oils, but they are subject to the same oxidative polymerization reactions that

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occur in linseed oil. One main difference is that egg yolk contains polymeric material (proteins). For this reason egg is considered a proteinaceous material, although this is not correct. In fact, this binding medium becomes extremely resistant when applied and aged because of the polymerization of its lipid component.

The lipids contained in egg yolk have the same characteristics as small molecules in a drying oil. A plasticizer action can then be hypothesized, and consequently, structural damage can result if the lipids are removed by using solvents during cleaning. The main preliminary work (Khandekar et al., 1994) seems to support this hypothesis. Complexity increases as the medium starts aging and undergoes chemical changes as a result of oxidation processes and interactions among the different components, with lipid oxidation and related radical reactions, cross-linking, and chain scission being the main reactions.

Shortly after the application of egg tempera, lipids can be extracted with several solvents: alcohols, ketones, and aromatic and chlorinated hydrocarbons. Consequently, the film undergoes contraction and shows superficial alterations. In artificial aging tests, some of these lipids have proved to be very mobile and tend to come to the surface spontaneously, and the most volatile ones tend to evaporate. As for the case of drying oils, the material may form a superficial patina at the interface with the eventual varnish, if present.

The lipid amount removable with solvents decreases considerably with aging. The mechanisms responsible for the film hardening during aging are fairly well known (Karpowicz, 1981; Boon et al., 1997; Phenix, 1997).

The resistance to different-polarity organic solvents and to water was checked on egg tempera samples made up of several pigments (white lead, vermilion, verdigris, azurite) and artificially aged (Khandekar et al., 1994). It was noted that hydrocarbons, such as dichloromethane, and acetone cause leaching of the lipid components on a recently formed film, whereas water causes film swelling. The extraction of these components causes film damage.

In past studies (White and Roy, 1998), the interaction of organic solvents with paint layers during cleaning operations (removal of a resinous varnish) of real paintings, including an egg tempera one, as well as that of unaged samples was explored. It was noted that the two solvents tested (2-propanone and 2-propanol [isopropyl alcohol]) did not appear to cause any leaching of the oil paints either from the unaged samples or the real paintings, not even in the case of the egg tempera painting.

Therefore, the aim of the present study was to gain a deeper insight into the properties of the egg binding medium, which was very important in Italian artistic tradition for several centuries, and its interactions with water and organic solvents. This research focused on studying (1) recent egg tempera films, prepared in our laboratory following the recipes in Cennino Cennini's treatise (Cennini, 1437) in July 2007 (Berzioli et al., 2009), (2) a tempera layer prepared by researchers at the Smithsonian's Museum Conservation Institute in 1995, and (3) a sixteenth-century painting from the Pinacoteca Nazionale in Siena. Two paint samples from this painting were analyzed by gas chromatography coupled with mass spectrometry (GC-MS), which identified egg as the binding medium.

The leaching of soluble components, caused by solvent application to the egg layers, was studied. Isooctane, acetone or ethanol, and water were applied by means of cotton swabs, lightly rolled over the egg tempera layers. The components extracted were identified by means of GC-MS. The color changes of the layers, before and after treatment with solvents, were studied by means of a multispectral scanner.

EXPERIMENTAL METHODS

Preparation of the Tempera Layers on Wood Panels

Following Cennino Cennini's description in his treatise (Cennini, 1437), a priming layer of four coats of animal glue and gypsum was applied onto several wooden panels. A binding medium made of egg yolk, cherry gum, and fig latex in a 10:10:1 ratio by volume (T) was then applied to rectangular sections of one panel. On another panel, layers of pigmented binding media of the same composition were prepared, one with vermilion (T-V) and one with minium (T-M). Two grams of each pigment were mixed with 2 mL of binding medium. The same layers (T, T-V, and T-M) were also applied onto glass slides for microscope observation. Once prepared, the panels were left to dry for about four months prior to the first solvent treatment.

Application of Organic Solvents and Distilled Water onto the Egg Tempera Layers

Ethanol or acetone, isooctane, and water were applied to the egg tempera layers by lightly rolling cotton swabs (previously rinsed in hexane, ethanol, and water and vacuum dried). Three drops of solvent were added to each swab and then rolled on the tempera for 15 s or 30 s on a 1 × 2 cm area. Afterward, the cotton swabs were transferred into vials and stored at 4°C.

Stereomicroscopic Investigation and Colorimetric analysis

The layers were observed under a stereomicroscope in order to monitor their morphology. The spectral reflectance factors were measured on fixed areas, and CIE L*a*b* values were calculated to obtain color specification of the surfaces. Lighting and observation conditions were 45/0° with a halogen lamp, and a D65 illuminating agent and CIE 1931 observer were considered. The spectrophotometric scanner (spectral region: 380–800 nm) used was developed by researchers of University of Parma (Antonioli et al., 2004).

GC-MS Analyses of Lipids, Proteins, and Cholesterol Fractions Extracted from Tempera Layers

For each tempera layer, cotton swabs (with solvents, i.e., ethanol, isooctane, and water, and without any solvent, i.e., dry swabbing, performed in triplicate tests) were analyzed for fatty acids, amino acids, and cholesterol. All procedures for analysis were repeated at least twice.

The chromatographic peak area of each analyte was integrated, corrected by a response factor, and expressed in relation to the internal standard (IS) in order to obtain quantitative information. Finally, the average analyte/IS ratio was calculated, and the average percentage of three runs was determined.

For cholesterol analysis, the cotton swabs used for cleaning were placed in a test tube for hexane extraction (1 mL of IS, 10 parts per million (ppm) stigmasterol in hexane, was added). Through a derivatization procedure the cholesterol was turned into trimethylsilylated cholesterol (Annaratone et al., 2009).

For analyses of lipids and proteins the cotton swabs were extracted for 30 minutes with 2 mL of the same solvent used for cleaning, under magnetic stirring in warm conditions (about 50°C). Then the cotton was removed and the solvent was evaporated under vacuum conditions. The following internal standards were added to the residue: 10 µL of a 1000 ppm heptadecanoic acid in hexane solution and 10 µL of a 1000 ppm norleucine in water solution. Thus, the samples were derivatized (Casoli et al., 2001). A gas chromatograph 6890N GC (Agilent Technologies) coupled to a Mass Selective detector (5973, Agilent Technologies) was used.

RESULTS AND DISCUSSION

Laboratory Tempera Samples

Ethanol, isooctane, and water were applied to the egg tempera layers by lightly rolling cotton swabs. Three drops of solvent were added to each swab and then applied to the tempera for 30 s on an area of 2 cm^2. Observation of the laboratory samples' surfaces (T, T-V, T-M) by means of stereomicroscope before and after the treatment with ethanol, isooctane, and water did not show significant differences. No mechanical abrasion of the surfaces of the egg tempera films was noticed.

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

All the cotton swabs, applied to the layers with each of the three solvents and without solvents, i.e., by dry swabbing, were analyzed by means of GC-MS. The chromatographic analyses showed the presence of fatty acids, amino acids, and cholesterol in all the cotton swabs analyzed. It was observed that the mechanical action was able to remove material. It was noticed that only isooctane applications caused a higher degree of extraction compared to that without solvents.

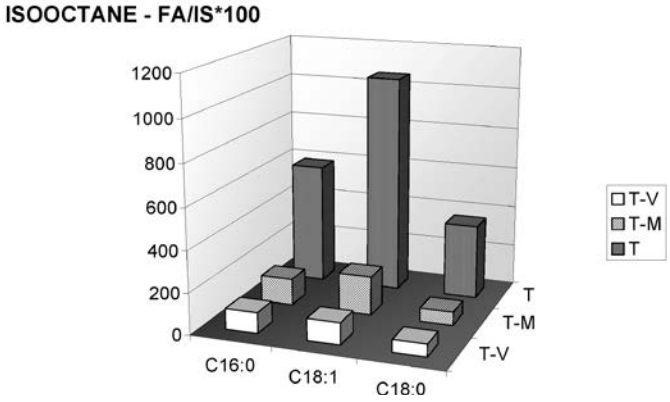

FIGURE 1. Histogram of fatty acids extracted by isooctane on the pigmented (T-V and T-M) and the unpigmented (T) tempera films. Each value represents the average percentage ratio of analyte to internal standard (IS). C16:0 is palmitic acid, C18:1 is oleic acid, and C18:0 is stearic acid.

Regarding fatty acid analysis, palmitic acid (C16:0), stearic acid (C18:0), and unsaturated oleic acid (C18:1) were identified. Figure 1 shows the trend of fatty acids extracted by isooctane applied on all the layers. The amount of oleic acid extracted from the T layer was about 100 µg, calculated by means of the internal standard.

The behavior of the three solvents related to cholesterol was then considered. The swabs without solvent extracted cholesterol at a trace level, in similar amounts to the application of water and ethanol. Isooctane was the solvent that removed the most cholesterol (Figure 2); some 50 µg of this compound were

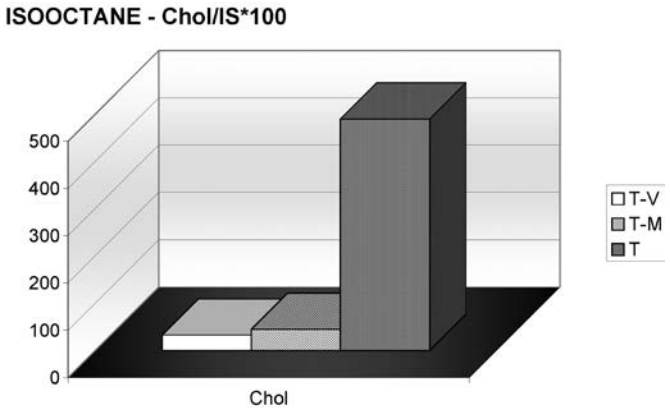

FIGURE 2. Histogram of cholesterol extracted by isooctane on the pigmented (T-V and T-M) and the unpigmented (T) tempera films. Each value represents the average percentage ratio of analyte to internal standard (IS).

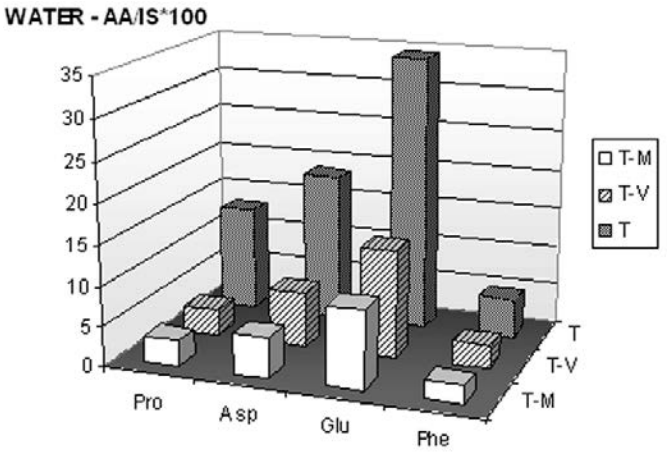

FIGURE 3. Histogram of some amino acids extracted by water from the tempera films. Each value represents the average percentage analyte to internal standard (IS) ratio. Pro = proline, Asp = aspartic acid, Glu = glutamic acid, and Phe = phenylalanine.

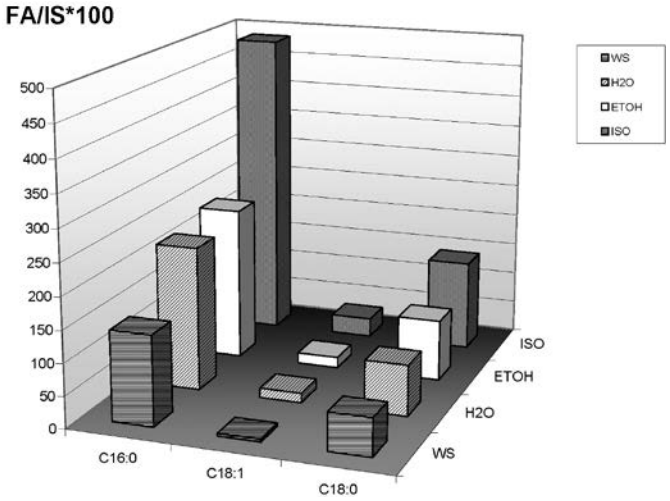

FIGURE 4. Histogram of fatty acids extracted by water (H_2O), ethanol (EtOH), and isooctane (ISO) and without solvents (WS) on the egg tempera layer from 1995. Each value represents the average percentage analyte to internal standard (IS) ratio.

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

The chromatographic results showed that the amino acids from proteinaceous material were only extracted by swabs with water. The histogram in Figure 3 shows the results of the proteinaceous fraction for the water application on layers, taking into account four amino acids: proline, aspartic acid, glutamic acid, and phenylalanine. The proteinaceous fraction extracted was very low in comparison to lipids (ratio of about 1:30).

The Smithsonian's Museum Conservation Institute Sample

A sample composed of egg tempera layered onto a Melinex polyester film, prepared in 1995, was also analyzed. The first GC-MS analyses carried out confirmed the presence of egg as a binding medium. Water, ethanol, and isooctane were applied by means of cotton swabs lightly rolled over the egg tempera layer for 15 s on an area of 1 cm^2.

Using the stereomicroscope, it was observed that the surface originally appeared greasy, but after solvent treatments, it lost this glossy appearance. The colorimetric analyses measured small variations due to solvent treatments, except for the case of ethanol applications where color changed in a visually perceptible way; the measured change was $\Delta E = 3.23$.

The GC-MS fatty acids analyses carried out on the cotton swabs (with and without solvents) showed palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0). The histogram in Figure 4 shows that isooctane is the best leaching agent, removing 25 ng of palmitic acid.

It is interesting to note that more saturated fatty acids were removed than oleic acid, in spite of the fact that oleic acid is more abundant than stearic acid in egg tempera. The saturated fatty acids were probably present on the surface because of a migration effect because the support of the film was not transpiring. This would also explain the original greasy surface.

A small amount of cholesterol was detected only in the cotton swabs with isooctane. From the proteinaceous fraction, the amino acids peaks are comparable to the analytical blank for all cotton swabs.

Case Study: A Painting from the Sixteenth Century

Cleaning tests were carried out on the painting *Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo* (Jesus at the Foot of the Cross, between Saint Peter, the Archangel Michael, and an Angel with the Small Tobia, and Saint Paul) by an unknown artist from around the middle of the sixteenth century (Figure 5). It is a small painting on wood (21.5 × 79.5 cm^2), probably part of an altar step, conserved in Pinacoteca Nazionale in Siena. The painted parts were all on gold: the board was gilded and then painted. This artwork appeared ideal for our purposes because it was done with egg tempera and it had not been varnished or restored.

The cleaning tests were carried out with three solvents with different polarities (water, acetone, and isooctane) in the same working conditions and on adjacent areas of the artwork in order to verify whether any leaching occurred because of the treatments. It was observed that isooctane was able to extract

FIGURE 5. Detail of the sixteenth-century painting *Caduta di Gesù sotto la croce, fra i Santi Pietro, Michele Arcangelo, Angelo col piccolo Tobia, e Paolo*, indicating one of the sampled areas.

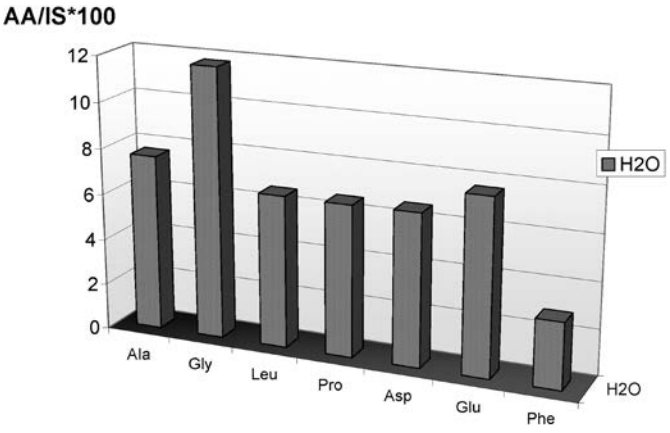

FIGURE 7. Histogram of amino acids extracted by water on the painting's surface. Each value represents the average percentage analyte to internal standard (IS) ratio.

both palmitic and stearic acids. With regard to cholesterol, in all the cotton swabs, its peak was comparable to an analytical blank. However, amino acids were detected in the water swabs. Figures 6 and 7 show the corresponding histograms. It can be seen that more fatty acids were extracted than amino acids.

From the results it can be inferred that isooctane, the most nonpolar solvent tested, removed the lipid fraction, whereas water partially removed the proteinaceous fraction. Acetone was observed to extract less fatty acid than the isooctane. As expected, unsaturated fatty acids were not detected; in fact, in this 500-year-old painting the polymerization process is complete.

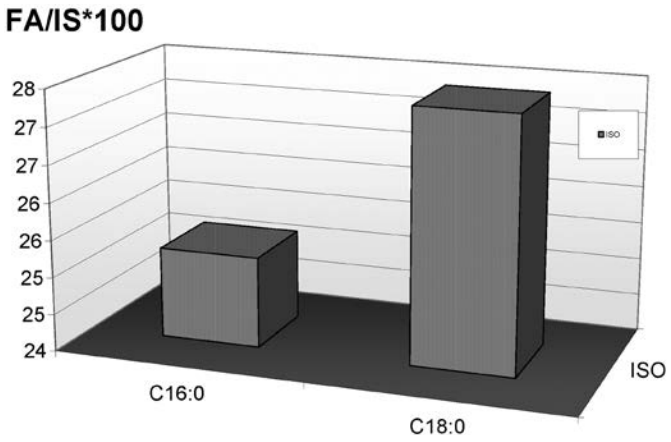

FIGURE 6. Histogram of fatty acids extracted by isooctane on the painting's surface. Each value represents the average percentage ratio of analyte to internal standard (IS).

However, the fact that both fatty acids and amino acids were extracted, even if the amounts extracted were about 50 times lower than those for the laboratory samples, was completely unexpected for a sixteenth-century painting.

CONCLUSIONS

Color changes, as a result of the interaction of organic solvents and water on egg binding medium carried out on laboratory samples, proved to be visually imperceptible, although changes were instrumentally measurable. Analyses of the cotton swabs applied without solvents showed the presence of fatty acids, amino acids, and cholesterol and confirmed that mechanical action alone was able to remove material. When solvents were used on the cotton swabs, the results depended on their polarity: isooctane removed mainly fatty acids and cholesterol, and water removed only amino acids. It was observed that the leaching ability of isooctane is 30 times greater than that of water.

It was confirmed that pigmented layers are less affected by leaching phenomena than pigment-free layers, particularly for lipid components. This finding may be linked to two factors: (1) The first is a physical-morphological factor; when the pigment is dispersed in it, the binding medium is obviously reduced. (2) The cations could bind to fatty acids, forming metal soaps and making the fatty acids less sensitive to leaching.

The analysis from all cotton swabs, with and without solvents, obtained from the egg tempera prepared at the Smithsonian's Museum Conservation Institute shows the presence of these fatty acids in decreasing order: palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). Cholesterol was removed in very small amounts relative to fatty acids. Apparently, no proteinaceous material was extracted. It was observed that the lipid

fraction is only present on the surface, rendering it hydrophobic; this was attributed to a migration effect because of the nonpermeable support. Interestingly, more saturated fatty acids were removed than oleic acid, in spite of the fact that oleic acid is more abundant than stearic acid in egg tempera. This is not surprising considering that unsaturated fatty acids become less mobile when they take part in the polymerization process.

The study on the sixteenth-century panel painting showed that the leaching phenomenon was greater for lipid components but that amino acids were also extracted. As expected, unsaturated fatty acids were not detected; in fact, in a 500-year-old painting the polymerization process is complete. The extraction content results were about 50 times lower compared to the laboratory samples. These results are unexpected because mild cleaning tests were believed to be safe and undamaging for a 500-year-old painting. These results might be regarded as guidelines for cleaning paintings.

Further research with other organic solvents and water solutions at different pHs is being carried out on laboratory panels and will also be tested on ancient tempera paintings.

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