Rigid Gels and Enzyme Cleaning

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ABSTRACT. Hydrolytic enzymes and polysaccharide-based rigid gels represent two very helpful cleaning tools for a variety of polychrome artworks: moveable paintings, wooden polychrome sculptures, paper and library materials, and in some instances mineral or inorganic supports. In addition, they can be used for other tasks that are not, strictly speaking, the cleaning of a painted surface, but rather more structural interventions, such as the removal of aged film formers, such as adhesives and consolidants. Rigid gels have several advantages over conventional fluid gels, such as delivering water or aqueous solutions in a controlled way, being able to draw into their mesh any particles dissolved upon their application, and generally not requiring posttreatment rinsing. Hydrolytic enzymes, by acting selectively on specific substrates under mild conditions, can often represent a valid alternative to conventional acids and alkalis that is safer to the artwork and less hazardous to the conservator's health. Many successful applications have been conducted; however, a systematic study still remains to be done, with the aim to better understand the influence of various parameters that affect the enzyme activity and how they can be tailored to the specific needs of the artwork. The information we currently rely on is too often related to the biochemical and biological fields, rather than to the materials of the artworks.

INTRODUCTION

Water can be, depending on the situation, either a desirable or a strictly necessary medium for the cleaning of objects. The aqueous approach, originally developed by Wolbers (2000) over the past 30 years, can often be a successful alternative to the use of more toxic and less selective organic solvents for removing varnishes and overpaints, and it is strictly necessary for surface cleaning polychrome artifacts.

Water, by itself, can be a physical solvent for hydrophilic film-forming materials, but it also possess the unique feature of being a good medium for chemical reactions to occur, namely, ionization and/or dissociation of materials, i.e., acidic or alkaline film formers, or even hydrolysis. Furthermore, water is the right environment for two processes: chelation and emulsification or detergency. However, water has a potentially dangerous action on a painted surface: although exhibiting a limited action on the actual surface, it has high diffusion and capillarity, which can exert undesirable effects on materials within the internal layers of a painting.

Increasing the viscosity of water by means of hydrophilic polymers, the so-called gelling agents, is the strategy most commonly used to limit the amount of water released, particularly when dealing with water-sensitive materials and layers. Many different polymers have been used for this purpose: the well-known cellulose ethers like Klucel, the

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pseudoplastic xanthan gum (i.e., Vanzan NF-C), and the high-viscosity polyacrylic acid derivatives (Carbopols and Pemulens).

A common feature is the need of a clearing rinse after the gel has been applied and removed with a dry cotton swab. The clearing rinse uses water to ensure proper removal of the hydrophilic gelling agents' residues. On particularly fragile surfaces, this final rinse could create a problem, considering that the gel had originally been selected just to avoid the use of free water. To avoid the drawback of these conventional fluid gels, the so-called rigid gels have been used, with the great advantage that, in general, they do not require an after treatment because of their physical form and their limited adhesive strength.

RIGID GELS

In 2003, the use of rigid agar gels was introduced by Richard Wolbers during one of his cleaning courses organized in Italy. Preliminary applications onto painted surfaces seemed very promising, and it was decided to further study their use on paintings, from both an analytical and a practical point of view (Campani et al., 2007).

At a later time, the field of study was broadened to other artifacts, such as wooden objects, plaster sculptures, and mural paintings (Anzani et al., 2008). Parallel to this study, the Istituto Centrale per il Restauro e la Conservazione del Patrimonio Archivistico e Librario (ICPAL) in Rome developed applications of similar gelling materials more suited to paper and library materials (Iannuccelli et al., 2004; Iannuccelli and Sotgiu, 2009). The latter area has grown in importance, and our separate experiences have merged into a common effort: in 2009 Cesmar7 started a joint research program with colleagues from ICPAL in Rome, the Università degli Studi in Parma, and the Opificio delle Pietre Dure in Florence that has already produced some interesting results, such as the "Study Day on Aqueous Rigid Gels" (Gel rigidi acquosi nel restauro) held in Rome on 16 June 2010.

The structure and chemistry of agarose and agar have been described elsewhere (Armisén and Galatas, 2000; Campani et al., 2007); here it is simply recalled that the polysaccharide complex agar, composed of galactose, dissolves in hot water above 85°C; once the solution is cooled below 37°C–39 °C, the polymer molecules tightly assemble into a regular mesh, capable of retaining a large amount of water, forming a so-called rigid gel. When applied to a porous surface, the gel can gradually release this water, a physical process termed syneresis.

Generally, concentrations of 2–5 g of agar in 100 mL of water are used, depending upon the type of application and the sensitivity to water by the treated object. Different varieties of agar are commercially available, ranging from laboratory to food additive grades, and in our experience all types tested performed in a very similar way.

Agarose is the purest galactose-derived gelling material; it produces clear, more transparent gels than the clouded agar gels, a particularly important feature when treating delicate supports, such as paper. However, because of its high price (10–20 times more expensive than agar) it is unlikely to be used in conservation. Furthermore, for the specific structural characteristics of some artworks, the "poorer" agar is still to be preferred because of its slower rate of syneresis compared to agarose. Agaropectin, the other polysaccharide component of agar, is a sulfated polymer, and the bulky sulfate groups decrease the pore sizes within the polymer network, thus slowing down the discharge rate of water. Besides applications for the cleaning of surfaces, rigid agar gels in many instances can perform as true humidification systems, capable of controlled-release of water for whatever conservation purpose.

Extensive analytical testing was done on very porous model supports using gas chromatography–mass spectrometry (GC-MS). The chromatographs always showed a minute peak for galactose, indicating that only trace amounts of agar deriving from the gel had permeated into the porous supports (Campani et al., 2007).

As an example, Figure 1 shows the application of a rigid agar gel onto an eighteenth-century polychrome wooden cross, painted in egg-tempera medium, from the Balkan area. The whole surface had been coated with a pigmented layer of animal glue, now strongly altered and discolored. Water was necessary for its removal, and the safest way of using it could only be in gel. However, even with thick Carbopol gels, the rinsing phase affected the paint layer because of the presence of some very hydrophilic components within the paint layer, likely a polysaccharide gum additive. A rigid agar gel, applied for just 1–1.5 minutes, delivered the right amount of water to swell the proteinaceous material, which was then completely removed by the simple action of a dry cotton swab.

FIGURE 1. Application of a rigid agar gel to a tempera-painted wooden cross to remove a discolored proteinaceous coating.

According to the requirements of the specific treatment, the gel may be left on for a limited time, until the amount of water necessary to obtain the desired results has been released or until the water has been totally "discharged." In the latter situation, what remains is a solid crust, which is easy to remove because it does not adhere to the surface. Observation of the gradual coloring of the gel as it absorbs the dissolved materials is often used to verify its action. Concentration gradients, once water from the gel starts diffusing into the surface materials, can be hypothesized as the main factor controlling this process.

In other cases, the surface material will not completely dissolve, such as when intrinsically hydrophilic substances gradually lose this characteristic upon aging and oxidation or when they cross-link with other materials. Then the usefulness of the gel may be identified by the swelling of the material, enough to soften it and aid its removal with a bland mechanical action. This type of action does entail treatment subsequent to gel application, but certainly to a far lesser degree than the washing procedure required when using traditional gels made from cellulose ethers or polyacrylic acid, which tend to adhere to the surface.

If, instead of simply gelling water, an aqueous solution containing acids or bases, chelating agents, or surfactants is used, a more specific action on certain materials can be obtained, such as (1) ionizing acid or basic substances (animal glues; certain polysaccharides, especially when aged; and lipophilic materials, such as natural resins, oils, waxes, especially when aged and/or oxidized), by using bases or acids, (2) dissolving insoluble salts (such as surface patinas composed of salts of fatty acids on oil paintings or caseinates) by using chelating agents such as citrates or EDTA (ethylenediaminetetraacetic acid) salts, and (3) emulsifying or dispersing hydrophobic materials, both oily and fatty (oils and natural resins), by using surfactants. Regarding their conservation, it must be remembered that these gels are aqueous-based materials and therefore have a tendency to develop mold. This requires a minimum of care during preparation (clean glassware), particularly when handling, to avoid contamination of the fresh gel (use of gloves, clean implements, etc.). The gel blocks may be stored in a refrigerator after sealing their surface with a plastic membrane for further protection.

The nature of these rigid gels also represents the main limit to their use: since they are rigid, it may be difficult to obtain good contact on surfaces that are not perfectly flat, even if the gel thickness is decreased. This problem stimulated our curiosity: might a different application method for the agar be developed that would allow it to be distributed over the irregular surfaces of three-dimensional objects, terra-cottas, plaster objects, and marble sculptures? On the basis of preliminary applications on plaster sculptures at the Galleria d'Arte Moderna in Milan, a new procedure was developed: once agar has dissolved in water at the proper melting temperature, the solution is cooled down to only 40°C–45°C, so that a semisolid state is achieved, allowing it to be brushed onto the surface to be cleaned.

In most applications to plaster sculptures, after only three minutes the plaster appeared to be clean with only a minimum release of water into the material. In some instances, to eliminate stains penetrated into the plaster or salts, it may be useful to leave the gel on until it dries entirely. In any case, the agar film formed on the surface can then be easily removed. Depending on the specific treatments, the appropriate agar concentration range is 2.5–5 g in 100 mL of deionized water. The best results were obtained by redissolving the gel after the initial solidification, as this partially modifies it since the second heating, which is faster than the first one needed to transform the powder into gel, renders the gel more homogeneous, with better water retention properties. These applications were also monitored by Fourier transform infrared spectroscopy and GC-MS analysis; the results again indicate that only trace amounts of agar deriving from the gel permeated into the plaster (Anzani et al., 2008). The new methodology permits cleaning plaster objects in an efficient and innovative way while remaining respectful of the porous nature of the substrates, which are potentially quite sensitive to treatment with water.

A further development that led to a broader area of application consisted of using a different kind of agar with a lower gelling temperature, i.e., 28°C–30°C. The semisolid state, required for the brush-on application, is then reached at 35°C. This lower temperature is safer for application to organic supports such as wood and canvas. For example, polychrome wood ceiling elements have been treated according to this procedure. The agar-substitute Phytagel, with a 27°C–31°C gelling temperature, is also perfectly appropriate for this type of application; furthermore, its higher flexibility and transparency in some instances represent a great improvement over agar.

HYDROLYTIC ENZYMES

Class III enzymes, according to the 1960s Enzyme Committee classification, namely, hydrolytic enzymes, or simply hydrolases, catalyze selective cleavage of specific bonds within the proper substrates. Among the hydrolases, proteases, amylases, and esterases hydrolyze peptide bonds (-CO-N-) in proteins, α-1,4-glucosidic bonds (-O-C-O-) in starch, and ester bonds (-CO-O-) in simple and complex esters, respectively (Nelson and Cox, 2009). The most typical feature of enzymes is the molecular recognition of the proper kind of substrates, i.e., the one most similar to the structure of the active site of the enzyme, and selectivity results from this feature that is unmatched by more conventional reagents, such as acids and alkalis, or even by organic solvents.

Since the 1970s, these enzymes have found application in conservation treatments (Bellucci and Cremonesi, 1994; Buttazzoni et al., 2000; Wolbers, 2000; Cremonesi, 2002, and references therein) for the removal under controlled conditions of such film-forming materials as animal and fish glues and gelatins, albumin, and casein; starch and starch-containing materials, e.g., flours; vegetable glues; drying oils; fats; some waxes; and some ester-containing synthetic resins (Bellucci et al., 1999).

When these film formers become largely insoluble in water (for hydrophilic materials) or in organic solvents (for lipids),

upon aging, a chemical action is required for their removal. If ionization or dissociation carried out by acids and alkalis under moderate-pH conditions (about 5–9 for polychrome surfaces) is not sufficient, the same acids or alkalis must be used under hydrolytic conditions, i.e., higher concentration, higher pH, and longer application times, with a greater risk to the artwork's integrity. In these cases, the hydrolytic action of enzymes can be a more effective and safer alternative.

Figure 2 illustrates a representative enzyme application. A small polychrome stone sculpture showed a strongly discolored appearance, caused by the alteration of materials that had been repeatedly applied over it for consolidation and outdoor protective purposes as well as for "brightening up" the colors. With aging, these mixed materials had become intractable: among traditional methods, only concentrated ammonia solutions could remove them, yielding a very blanched and abraded surface.

FIGURE 2. Application of an enzyme solution, absorbed onto a cellulose tissue, on a polychrome stone sculpture to remove a discolored oily and proteinaceous coating.

After characterizing the materials by GC-MS as linseed oil and animal glue, a bacterial lipase, effective at a pH of 7–7.5, and a bacterial protease, with an optimal pH of 7.5, were chosen. Free-enzyme solutions, containing 0.5–1 g enzyme in 100 mL of 50 mM phosphate buffer, were applied in sequence onto the surface, supported on pure cellulose tissues, kept in contact with the surface, and warmed to about 35°C for 15–20 minutes. After this, the tissues were removed, and the surface was cleared with artificial saliva (freshly prepared by dissolving at 37°C 0.1 g mucin in 100 mL of distilled water). It is important to stress that for an enzymatic treatment cleaning is carried out in an aqueous medium within a "safe" pH range and in a basically nontoxic environment (if precautions are taken to avoid skin contact and inhalation of the enzymes in the powder form and skin contact once the solutions are made.)

Enzymes have been used, depending on the circumstances, as free solutions, absorbed on tissues, or in a gelled form. Solutions may also be gelled with agarose and agar since the dimensions of the pores in the gel network structure are large enough to even allow movement of the enzyme protein macromolecules. To use them this way, however, it is necessary to slightly vary the preparation procedure because enzymes are thermolabile: the rigid gel is prepared as already described, but using only two-thirds the prescribed amount of water; the remaining one-third, buffered to the correct pH, will be used to prepare the enzyme solution. The latter is then quickly mixed into the cooling agar solution once its temperature is down to 45°C–50°C.

Many enzymatic applications in cleaning treatments have been carried out throughout the years on different kinds of objects (paintings on canvas and panel, mural paintings, paper artifacts, stone sculptures), yielding in general good results. In all instances, the enzymatic treatment was chosen precisely because more conventional materials were either too slow or produced unsatisfactory results from the point of view of the visual appearance (incomplete removal, not homogeneous, blanched, etc.) or required conditions that were deemed risky to the structural integrity of the artwork (excessive polarity or pH, too long an application time, limited selectivity) and/or were hazardous to the conservator's health.

However, our understanding of how hydrolases work on artifacts is still largely incomplete, and there is need for a systematic study. Far too much of the information available is taken from the biochemical and biological fields and is simply considered valid and applicable to the conservation field as well. However, in many cases, this is only a rough approximation.

In a biological system the catalytic process is very efficient. When enzymes are taken out of their biological "protective" environment and used as mere chemical reagents, e.g., when applied to the surface of artworks for cleaning purposes, a large part of this efficiency is lost. Optimal reaction conditions, such as pH and temperature, may not be used for cleaning conditions, and furthermore, inhibitors may be present on the artwork, such as salts, metal ions, or other molecules that, by binding to the proteinaceous structure of the enzyme, induce changes in its

conformation. These changes may bring about a decrease in or even the total loss of the enzymatic activity.

For instance, heavy-metal ions, such as lead, mercury, antimony, and cadmium, are known to be powerful enzyme inhibitors under homogeneous phase catalysis, i.e., when they are in the form of salts, dissolved in the same aqueous medium as the enzyme. These same metals are found within many ancient pigments, for example, lead white, minium, orpiment, and cinnabar, just to mention a few. However, when part of a paint layer, particularly an oil-bound layer, these metal ions may be not bioavailable to the enzyme if the pigment particles are fully coated by a binder film. Therefore, under these circumstances, they may not exert enzyme inhibition.

CONCLUSIONS

Rigid agar gels have proven suitable conservation tools for treating various kinds of objects: for cleaning soiled surfaces and, when applied still in a semisolid state, even for soiled objects. Acting as "molecular sponges," these gels can draw into their tight polymer mesh the particles dislodged from the surface and generally require no posttreatment rinsing.

When gels are used over the hydrophilic film-forming materials to be removed, simply varying the application time allows the transfer of the proper amount of water and swelling of the material to the point that removal can then occur by gentle mechanical action. In this fashion, rigid gels are most useful on the verso of paintings, when residual lining adhesive has to be removed with a limited amount of water, and on paper.

These treatments are safe for the artwork. Analytical studies have revealed that only trace amounts of polysaccharides are transferred into the porous objects treated with agar gels.

Hydrolytic enzymes represent powerful means for tackling difficult tasks in cleaning, minimizing the risk for the artwork: in an aqueous medium under mild-pH conditions, aged, insoluble protein-based film-forming materials, starch-based glues, and oil-bound overpaints can often be effectively removed with proteases, amylases, and esterases, respectively. Many successful treatments have been conducted on various supports, and the enzyme's catalytic action has been assisted and monitored by analytical techniques. However, a systematic study of how hydrolytic enzymes work on artworks remains to be carried out, with a focus on optimizing the parameters that are crucial to catalytic activity in relation to the materials that are present on the artwork.

REFERENCES

Anzani, M., M. Berzioli, M. Cagna, E. Campani, A. Casoli, P. Cremonesi, M. Fratelli, A. Rabbolini, and D. Riggiardi. 2008. *Gel rigidi di agar per il trattamento di pulitura di manufatti in gesso—Use of Rigid Agar Gels for Cleaning Plaster Objects*. Translation by D. Kunzelman. Quaderni Cesmar7, No. 6. Padua: Il prato.

Armisén, R., and F. Galatas. 2000. "Agar." In *Handbook of Hydrocolloids*, ed. G. O. Phillips and P. A. Williams, pp. 22–40. Boca Raton, Fla.: CRC Press.

Bellucci, R., and P. Cremonesi. 1994. L'uso degli enzimi nella conservazione e nel restauro dei dipinti. [The Use of Enzymes in the Conservation and Restoration of Paintings.] *Kermes, arte e tecnica del restauro*, 21:45–64.

Bellucci, R., P. Cremonesi, and G. Pignagnoli. 1999. A Preliminary Note on the Use of Enzymes in Conservation: The Removal of Aged Acrylic Resin Coatings with Lipase. *Studies in Conservation*, 44(4):278–281. http://dx.doi.org/10.2307/1506657.

Buttazzoni, N., A. Casoli, P. Cremonesi, and P. Rossi. 2000. Preparazione e utilizzo di gel enzimatici, reagenti per la pulitura di opere policrome. [Preparation and Use of Enzymatic Gels as Reagents for the Cleaning of Polychrome Objects.] *Progetto Restauro*, 16:11–19.

Campani, E., A. Casoli, P. Cremonesi, I. Saccani, and E. Signorini. 2007. *L'uso di agarosio e agar per la Preparazione di "gel rigidi"—Use of Agarose and Agar for Preparing "Rigid Gels"*. Translation by D. Kunzelman. Quaderni Cesmar7, No. 4. Padua: Il prato.

Cremonesi, P. 2002. *L'uso degli enzimi nella pulitura di opere policrome*. [The Use of Enzymes in Cleaning of Polychrome Works.] 2nd ed. I Talenti 4. Padua: Il prato.

Iannuccelli, S., and S. Sotgiu. 2009. "L'impiego di gel rigidi polisaccaridici per il trattamento di pulitura di opere grafiche a stampa." [The Use of Polysaccharide Rigid Gels for the Cleaning Treatment of Prints.] In *Lo stato dell'arte: Proceedings of the 7th National Congress Italian Group of the International Institute for Conservation*, pp. 1–8. Turin, Italy: IGIIC Gruppo Italiano.

Iannuccelli, S., S. Sotgiu, and M. Missori. 2004. "La chinea di Papa Clemente VIII del 1598: Pulitura del supporto cartaceo, tecniche di lavaggio e diagnosi ottica dei risultati." [The White Horse of Pope Clement VIII from 1598: Cleaning of the Cardboard Support, Washing Technique and Visual Diagnosis of the Results.] In *Lo stato dell'arte: Conservazione e Restauro, Confronto di Esperienze; Proceedings of the 2nd National Congress Italian Group of the International Institute for Conservation*, pp. 124–135. Turin, Italy: IGIIC Gruppo Italiano.

Nelson, D. L., and M. Cox. 2009. *Lehninger Principles of Biochemistry*. 5th ed. New York: W. H. Freeman.

Wolbers, R. C. 2000. *Cleaning Painted Surfaces: Aqueous Methods*. London: Archetype Publications.