Microscopy for Paper Conservation: Comparing Various Adhesives and Examining Wheat Starch Paste Preparation Methods

INTRODUCTION

Paper conservators are very particular about their paste-making recipes, each arguing that their method of cooking paste is the most effective. There are a myriad of variables in preparation. Some choose to pre-soak their starch, others debate the methods of sieving, working and kneading the paste after it is cooked. Perhaps the most controversial question is about the merits of cooking paste with a cook and stir, a double boiler, or using a microwave oven. Storage methods for paste, whether in the refrigerator, in a syringe, or under water, are equally contested. This project is made up of a number of small experimental vignettes, each exploring a facet of the use and preparation of wheat starch paste in book and paper conservation.

The Andrew W. Mellon Heritage Science for Conservation (HSC) research project at The Sheridan Libraries of Johns Hopkins University[1] has not only enhanced dialogue between conservators and conservation scientists, but enabled conservators to explore various conservation questions using analytical and diagnostic equipment. A number of secondary research projects were developed alongside the primary research of the HSC post-doctoral fellows. This particular research project began by exploring the Zeiss Axio Imager M1m compound microscope, employing reflected light (bright field and dark field) and fluorescence microscopy, in order to further elucidate some of the methods and materials used by conservators in the preparation of wheat starch paste.

Wheat Starch Granular Structure

The wheat endosperm has a cellular structure. Each cell is filled with starch granules, surrounded by proteins, minerals, coloring matter and enzymes, contained in thin cellulose walls (Olkku and Rha 1978). On a molecular level, starch consists of amylose (linear chains of α-D-glycopyranose units joined by 1–4 linkages), and

amylopectin (a non-random 1–6 branching of the 1–4 linked α-D-glycopyranose units, having one of the highest molecular weights of naturally occurring polymers) (Singh *et al.* 2003). Purified wheat starch used for preparing conservation adhesives has approximately a 25%(w/w) amylose content (Van Steene and Masschelein-Kleiner 1980). This equates to several million amylopectin molecules per granule, and a much larger number of the smaller amylose molecules (Chaplin 2010). Amylopectin is oriented radially within the starch granules, with concentric amorphous and crystalline regions, in such a way that as the granule radius increases, so does the number of polymer branches to fill the radius (Chaplin 2010). The crystalline regions are clusters of double helices formed by hydrogen bonding between outer strands of the amylopectin; these crystalline regions cause starch granules, when viewed under crossed polarization filters, to exhibit their characteristic anisotropic cross pattern. Amylose strands in the granules also form hydrogen-bonded double helices, but these do not result in high enough order to exhibit birefringence (Tester and Morrison 1990). The average diameter of wheat starch granules ranges from 2–40μm; it is the larger granules, from 15–40μm (Belard *et al.* 2009), some say 30–40μm (Van Steene and Masschelein-Kleiner 1980), that are used in the preparation of starch paste for conservation activities.

Theory of Paste Cooking

The theoretical background for this research was found largely in the arena of food science, where there has been, and continues to be, a great deal of investigation into the properties and cooking of various starches. In granule form, starch is insoluble in water, briefly forming a suspension before settling out completely (Van Steene and Masschelein-Kleiner 1980). When the suspension is heated, however, the granules will slowly and reversibly take up water, until they reach the temperature of the onset of gelatinization. Gelatinization is, by the narrowest definition, the thermal disruption of crystalline structure within the starch granules marked by the loss of birefringence (Tester and Morrison 1990). More broadly,

the term gelatinization is used to include the swelling of the granules and the leaching of soluble amylose components out of the intact granules. Alternately, the term pasting is used to refer to the change in viscosity just before, during and after strict gelatinization (Zeng *et al.* 1997).

Gelatinization can be broken down into numerous small steps. First, wheat starch granules swell tangentially. This swelling, under the right temperatures and concentrations, can expand the starch granules up to 30 times their original size without disintegration (Singh *et al.* 2003). Initially a reversible hydration in the weakly bound and amorphous regions of amylopectin, the swelling continues irreversibly in the crystalline amylopectin regions, as well as in the strands of amylose. In these crystalline regions, the hydrogen bonds between molecular components of starch are replaced by hydrogen bonds with water and the hydroxyl groups along the starch polymer chains (Singh *et al.* 2003). In the case of amylose, hydration takes the polymer chains from a state of suspension into solution; this transition results in the increased translucency observed during paste cooking. This solubilized amylose is found both within the still intact starch granules, and also exuded outside the starch grains.

The viscosity of paste has been shown experimentally to reach a maximum after granule swelling ceases. This indicates that it is the release of exudates from granules, rather than simply the swelling of the granules, that is responsible for viscosity. In SEM images of flash frozen cooking starch, the exudates can be seen entangling with themselves and with the intact granules (Miller 1973). Above the point of maximum viscosity, as cooking proceeds, all cohesive forces within the granules are lost, the granules fragment, and the viscosity of the paste decreases (Christianson *et al.* 1981).

Swelling of the starch granules begins at 45–55°C, with a loss of birefringence in the range of 50–55°C. By 65–67°C, the paste is almost completely gelatinized (Belard *et al.* 2009). Increases in viscosity and tack continue linearly up to 85°C, above which the granules begin to disintegrate completely, with a corresponding drop in tack above 92°C (Tester and Morrison 1990). Stirring is necessary during paste preparation to evenly distribute heat and ensure complete and even gelatinization. Hot starch paste is a mixture of granules, granule fragments, and colloidially- and molecularly-dispersed starch molecules (Olkku and Rha 1978).

Upon cooling, the properties of starch are governed by a process called retrogradation, which is the reassociation of starch molecules into an ordered structure. Hydrogen bonding between the molecular chains is restored. Amylose molecules align to form aggregates, with the association of 40–70 glucose units in each (Singh *et al.* 2003). The recrystallization of amylopectin also occurs, but to a much lesser degree than in the original native starch grains. This reordering is associated with an increase in viscosity. The resulting gel has a three-dimensional structure held together by the now aggregated amylose chains that were leached out of the starch grains during gelatinization. In gels with highly swollen, intact granules, there is a high proportion of water still held in the granules. This system requires only a small fraction of leached amylose to form a continuous network. If the granules were not sufficiently swollen, or were swollen to the point of granule fragmentation, a much higher proportion of amylose would be required to hold the gel network together (Olkku and Rha 1978). Hence it is possible to both under or overcook starch paste: insufficient or incomplete gelatinization leads to insufficient exudate to fully gel and tack, whereas rupturing all of the granules will result in a paste of low cohesiveness.

According to one study, day-old starch is already about 50% retrograded, while three-day-old paste is about 90% retrograded (Miller and Root 1991). The effects of retrogradation on adhesion are not well understood, but while studying furunori (aged, highly retrograded starch, known to be less tacky than fresh starch; employed in the mounting of scrolls), researchers have found no significant decrease in adhesiveness of highly retrograded paste (Daniels 1988).

Aside from the clear changes made by sieving and kneading on a macroscopic level, on a microscopic level, it is possible that these actions are rupturing additional starch granules. Swollen starch granules are susceptible to shear disintegration, as well as mechanical and thermal breakdown (Olkku and Rha 1978). Some level of fragmentation may result in the release of additional exudate.

EXPERIMENTAL

The project consists of a number of small experiments, each examining a facet of how adhesives, primarily wheat starch paste, are prepared, used and stored in the context of book and paper conservation. Each small experiment is couched in terms of a question, followed by a short account of the theoretical background behind the question, the experimental design, the photomicrograph image results, and finally, any conclusions or follow-up research resulting from the experimentation.

Experiment #1: Adhesive Films Coating Paper Fibers

Question—How do different adhesive films dry on a "real life" surface, such as paper?

Background—Book and paper conservators use a number of different adhesives, consolidants and sizing agents, each with a different set of working properties. As an introduction to what is visible under the microscope, a number of these adhesives were coated onto paper supports to observe their film-forming characteristics, while examining the interaction of adhesive and substrate.

Experimental—Twelve adhesives, consolidants and sizing agents (1: Control (no coating), 2: Wheat starch paste (double boiler), 3: Wheat starch paste (microwave), 4: 2% Methyl cellulose A4M, 5: 2% Methyl cellulose A15C, 6: Jade 403 PVA, 7: Lascaux 360HV acrylic, 8: Lascaux 498HV acrylic, 9: 2% Klucel G in ethanol, 10: Cellugel (hydroxypropylcellulose in isopropanol), 11: 2% Gelatin USP, and 12: Gum Arabic (Winsor & Newton)) were brushed onto the surface of two papers: one Western paper (Rives Lightweight Buff, 115gsm, 100% cotton rag) and one Japanese paper (Ikeda #29, 22gsm, 100% Kozo). The appearance of the control was compared to the various adhesives, consolidants and sizing agents with reflected light brightfield illumination under 200X magnification.

Conclusions and Future Experimentation—The image results can be seen in figures 1a and 1b. The thermoplastic adhesives (PVA and the Lascaux acrylics) form a film coating the entire surface of the paper fibers, unlike the other coatings, which settle into the interstices. The sizing agents and consolidants (the cellulose ethers, gelatin and gum Arabic) seem to bridge between adjacent paper fibers. Wheat starch paste falls somewhere between these two characteristics, forming more of a film over the paper fibers than the cellulose ethers, but also seeming to penetrate between the individual fibers more than the thermoplastic adhesives.

Experiment #2: Mixed Adhesive Films

Question—Do wheat starch paste, methyl cellulose and polyvinyl acetate (PVA) form compatible films when mixed?

Background—Paper conservators often mix various adhesives together to get a blend that tweaks desired working properties. For instance, methyl cellulose and wheat starch paste are mixed for an adhesive with "more slip." Particularly in book conservation, wheat starch paste or methyl cellulose adhesives are mixed alone, or in combination, with Jade 403 PVA to acquire longer working times, while maintaining the flexible character of the PVA film. By examining these mixed adhesives microscopically, it is possible to see if these various components will form homogeneous films, providing insight into whether these adhesives are truly miscible.

Experimental—Pure films of fresh double boiler wheat starch paste (WSP), 2% A4M methyl cellulose (MC) and full strength Jade 403 (PVA) were cast on microscope slides, by laying two "guide tracks" of double sided tape down onto microscope slides and using a razor blade resting on the tape to cast films with a smooth surface and even thickness. This film casting technique was also employed for the films cast in the experiments #3–6. Mixed films

of 1:1 WSP:MC, 1:1 MC:PVA, 1:1 PVA:WSP, and 1:1:1 WSP:MC:PVA were also cast on microscope slides. Photomicrographs were recorded under bright field illumination at 200X magnification.

Conclusions and Future Experimentation—The image results can be seen in figure 2. Wheat starch paste forms a significantly more dimensional film than either of the smooth PVA and very smooth MC films. 1:1 mixtures of wheat starch with either of the other two adhesives, or a 1:1:1 mixture of all three adhesives, result in uniform films, with character similar to that of a wheat starch paste film. The 1:1 mixture of PVA and MC form a film with a strange reticulation pattern, perhaps indicating that the two adhesives are not fully miscible. It would be very interesting to follow this observational research with research testing the flexibility, durability, reversibility and adhesive strength of these pure and mixed films.

Experiment #3: Paste Swell

Question—Does soaking dried starch prior to cooking visibly swell (hydrate) the granules?

Background—The food science literature and even some conservation articles seem to indicate once starch granules are dried, they do not rehydrate until heated. Most authors state that uncooked starch in water will always simply settle out (Van Steene and Masschelein-Kleiner 1980; Light 1980). Several studies indicate that starch swelling really only occurs at the onset of gelatinization T=45-55°C (Tester and Morrison 1990). This may be due to the method of dry starch manufacture. One method of industrially drying starch in involves taking raw starch (after extraction from wheat flour) from a moisture content of 42.3% down to 6.4% by exposure to hot air at a temperature of 60–70°C. Under such conditions, the surfaces of the starch grains are altered, meaning that they do not absorb water as easily as starch that remains in the wet state (Belard *et al.* 2009). Nonetheless, the additional step of soaking prior to cooking is frequently referenced in paste preparation methods as a means to swell the starch granules. It is possible that the practice of soaking starch prior to cooking paste was adopted from Japanese paste preparation methods where the intent not to swell the starch grains, but rather to decant the soaking water to remove unnecessary impurities (Belard *et al.* 2009).

Experimental—The pre-soak time recommended in paste preparation recipes ranges from not at all, to 20 minutes, to several days. This experiment compromised by soaking precipitated wheat starch granules (Zin Shofu) in deionized water (1:4 or 20% v/v) for 1 hour at room temperature. The excess water was then decanted, and the soaked
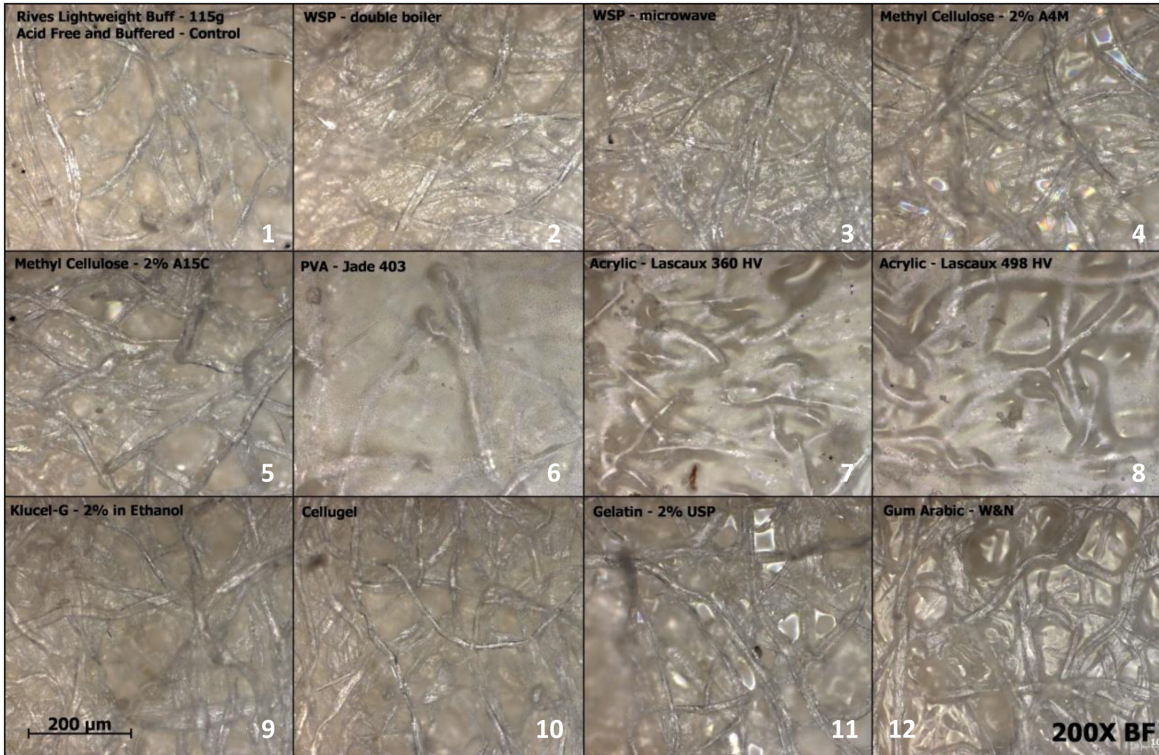
Fig. 1a. Coatings on Rives Lightweight Buff, 115gsm, 100% cotton rag fiber paper, viewed with 200X magnification under reflected light bright field illumination
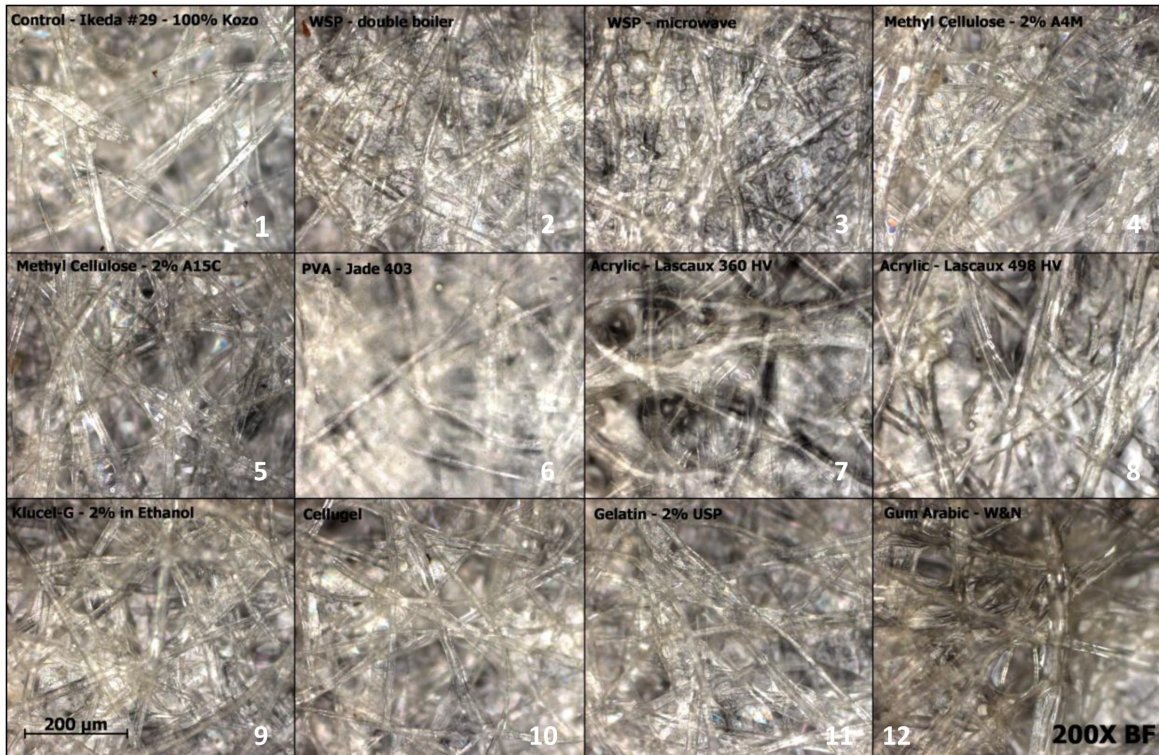

Fig. 1b. Coatings on Ikeda #29, 22gsm, 100% kozo fiber paper, viewed with 200X magnification under reflected light bright field illumination
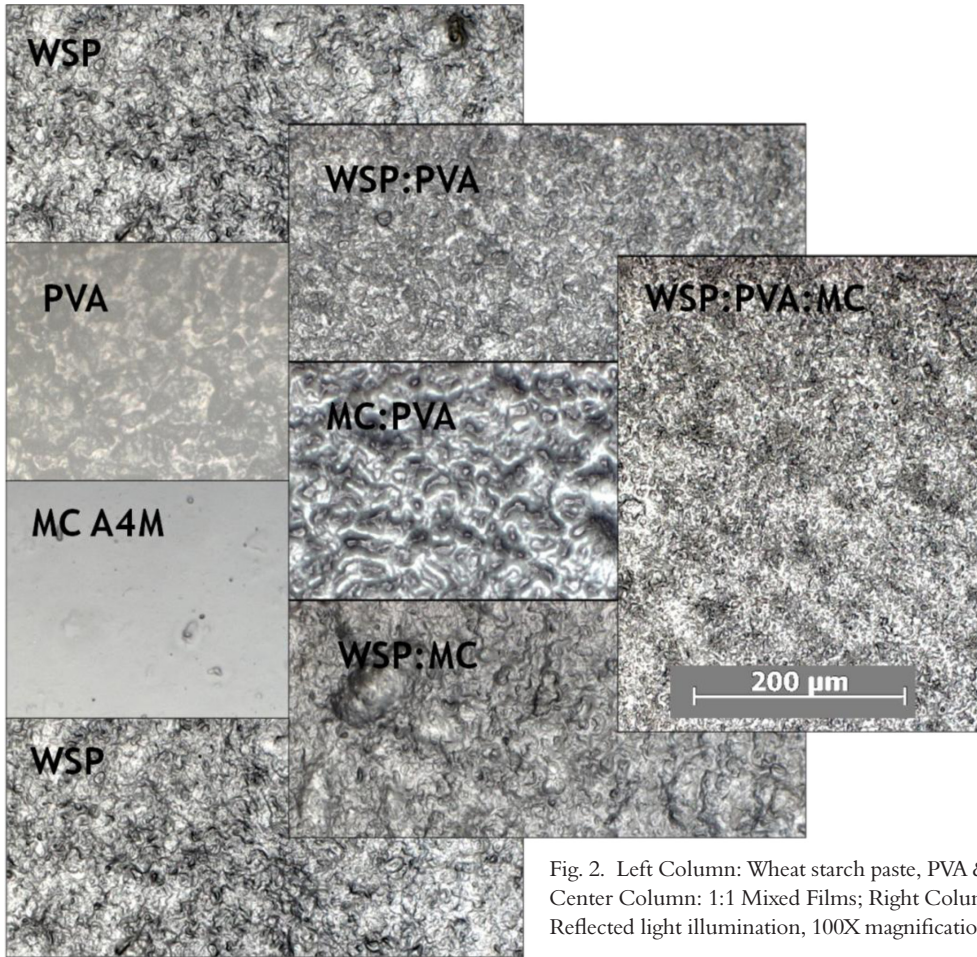
Fig. 2. Left Column: Wheat starch paste, PVA & Methyl Cellulose pure films; Center Column: 1:1 Mixed Films; Right Column: 1:1:1 Mixed Films; Bright field, Reflected light illumination, 100X magnification
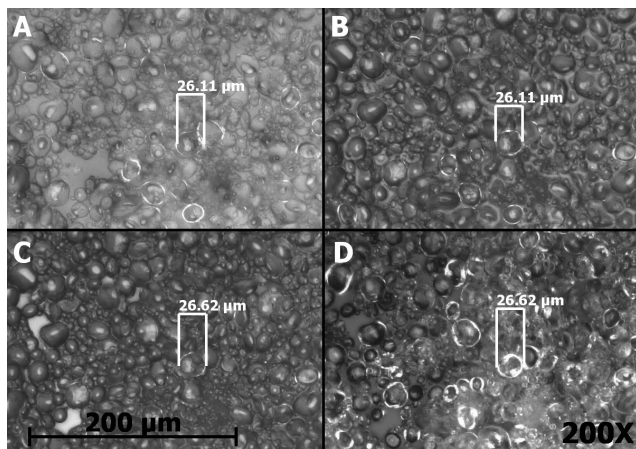

Fig. 3. A: starch granules after 1 hour soaking; B-C: starch granules drying; D: dry starch granules. Bright Field; Reflected Light; 200X magnification

granules were mounted on a microscope slide. The granules were observed under bright field reflected light illumination at 200X magnification. Photomicrographs were taken while they dried, and the images of the soaked starch compared to dry starch to assess visible change.

Conclusions and Future Experimentation—The image results can be seen in figure 3. The diameter of the paste granules does not appear to change during soaking at room temperature. While this does not rule out the possibility of some hydration at a molecular level, it does imply that significant pre-swelling of starch granules in excess water at room temperature does not occur.

Experiment #4: Microwave vs. Double Boiler

Question—Is there a significant difference in the granularity and film-forming characteristics of paste prepared by microwave and double boiler cooking methods?

Background—This is a question of expediency versus more traditional preparation methods. On a macroscopic

level, the consistency of paste prepared in a microwave is observably different than that prepared by the slower cook-and-stir or double boiler cooking methods. Looking to the food science literature, an explanation can be found in the fact that starch gelatinization occurs in a relatively narrow range of temperatures. In slower, stirred cooking methods, the granules are likely to swell uniformly. In a microwave there is more likely to be a broad range of temperatures in different locations ("hot spots") and therefore a varying degree of granular swelling (Light 1990). Paste, in what is considered its most adhesive format (i.e. at the point just after peak viscosity is reached), consists of a mixture of swollen granules, granule fragments, exudate from swollen granules, and uncooked granules (Miller *et al.* 1973; Singh *et al.* 2003). Undercooked paste will exhibit slightly swollen granules that are still highly intact. In overcooked paste, the majority of granules will have ruptured, with a high degree of fragmentation. Cooking time, temperature and mechanical shear all affect the degree of paste swelling. If the temperature is too low throughout the entire cooking period, starch will not swell sufficiently, even over an extended cooking period. If the paste is cooked for too long or at too high a temperature, the starch will break down completely.

In a previous study of paste, Miller and Root (1991) note that microbial growth is greater on microwave paste, attributing this to the fact that the water that was used in paste preparation was not disinfected by microwave cooking. They also examined microwave paste under crossed polarization, and found incomplete gelatinization, as exhibited by remaining birefringence (Miller and Root 1991).

Experimental—
- Microwave Paste—15mL of Zin Shofu wheat starch in 60mL deionized water was combined in a 150mL Pyrex beaker, which was first sterilized by immersion in boiling water. The starch suspension was placed in the microwave (Magic Chef MCD 766W, 2450MHz) at full power for 30 seconds before stirring with a glass stir rod. Cooking continued first for 15 seconds, followed by 10 second increments, where the paste was alternately microwaved and stirred. At each stirring point the temperature was recorded in degrees Celsius using a mercury thermometer and a small sample cast onto a microscope slide in the method described in Experiment #2. The total cooking time was 75 seconds, with a peak temperature of 82°C. Cooking was judged complete when the paste became translucent, with a smooth texture.
- Double Boiler Paste—15mL of Zin Shofu wheat starch in 60mL deionized water was combined in a

sterilized 150mL Pyrex beaker and set into a sauce pan of hot water on a hotplate element. The water in the saucepan was brought to a simmering boil around the continuously stirred beaker. A mercury thermometer monitored the temperature of the paste mixture. Samples were cast at regular intervals on microscope slides at temperatures similar to those recorded in the microwave paste, until the paste took on a creamy, translucent texture, just after peak viscosity was reached. Cooking proceeded for a total of 14 minutes, with a peak temperature of 85°C.
- Working the Paste—Both pastes were cooled to room temperature before an additional film of each was cast (direct cast) followed by films which had been sieved through plastic mesh three times. The final films were of sieved paste which had been kneaded with the addition of a small amount of deionized water to a very smooth, thick, creamy texture using a synthetic bristle paint brush against a glass surface. Photomicrographs of each of the cast films were recorded at 100X magnification, under bright field reflected light.

Conclusions and Future Experimentation—The image results can be seen in figures 4a and 4b. Similar temperatures are reached by both cooking methods. Greater granularity does remain in microwave paste, but both films, when sieved and kneaded, are quite uniform. Future experimentation should involve testing the adhesive tack of the different preparation methods. A Fourier transform infrared spectroscopy (FTIR) study of starch paste gels shows that changes in the spectra are visible throughout the gelatinization and retrogradation processes (Wilson *et al.* 1988). FTIR could also be used in a future study to monitor various paste preparation methods throughout cooking.

Experiment #5: Paste Storage
Question—How does the storage method affect the structure of cast films of paste over time?

Background—There is great diversity represented in the conservation literature regarding the storage recommendations for paste. The question of storage is a balance of maintaining working properties of paste, while also trying to keep microbial growth at bay. Older recipes often recommend the addition of fungicides (Mucci 1975; Baker 1984), but these cause concern both for the health of the conservator and for the potential long-term effects on artifacts. Other recipes focus on means of sterilizing storage containers by boiling or UV irradiation. Some seek to exclude air, by storing under water or in a syringe, in order to limit the introduction of mold spores. Many recipes recommend refrigeration as the only way to keep
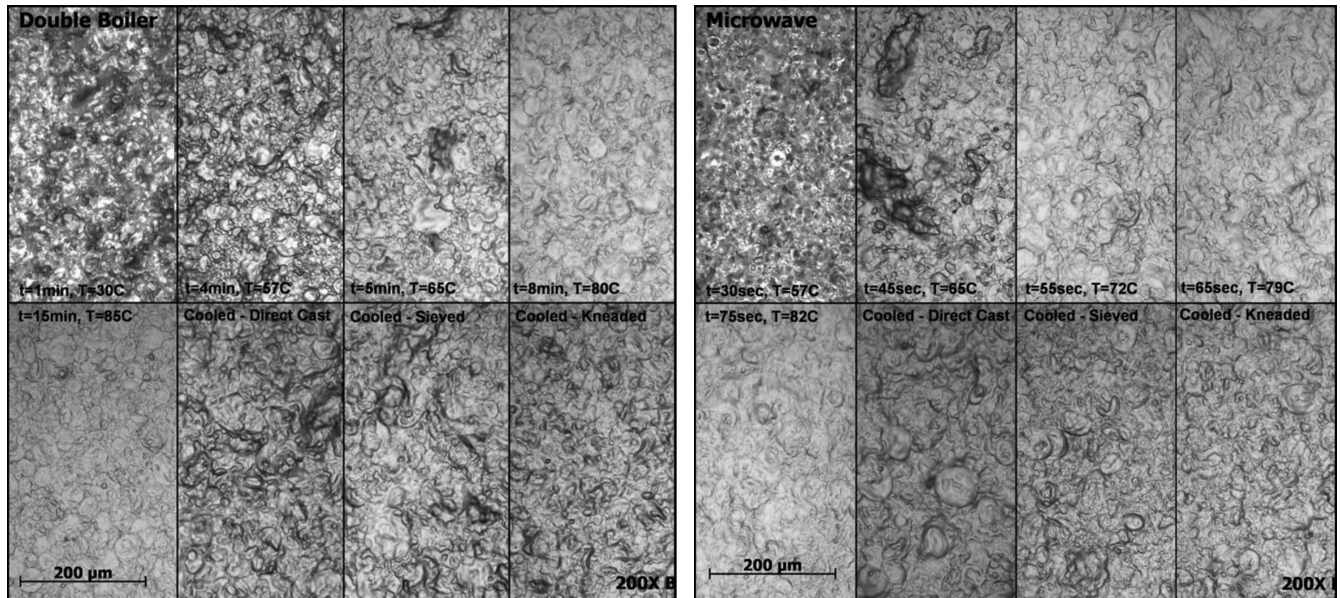
Fig. 4a. Double boiler prepared paste films cast during cooking while temperature was monitored. The final three films of cooled paste are direct cast, sieved through a plastic mesh and kneaded with a brush, respectively. Bright field, reflected light illumination, 200X magnification

Fig. 4b. Microwave prepared paste films cast during cooking while temperature was monitored. The final three films of cooled paste are direct cast, sieved through a plastic mesh and kneaded with a brush, respectively. Bright field, reflected light illumination, 200X magnification

paste for several days, while others state that refrigeration, while still being ineffective at preventing mold growth, speeds the retrogradation of paste, and therefore should be avoided at all costs (Miller and Root 1991). While this statement regarding retrogradation may be true, there is no indication that increased retrogradation affects the adhesive nature of paste. Indeed, furunori, a highly retrograded paste valued for its lower viscosity and flexibility and used primarily by Japanese scroll mounters, has been found to retain a high level of adhesiveness (Daniels 1988).

Experimental—A batch of double boiler paste was prepared according to the method described in Experiment #4. The paste was cooled to room temperature, and then the batch was divided into the following five storage arrangements:

1. Refrigerator, in a Pyrex beaker sealed with Parafilm.
2. Refrigerator, covered in deionized water in a Pyrex beaker sealed with Parafilm.
3. Dark location at room temp. (inside an opaque container), in a Parafilm-sealed Pyrex beaker.
4. Covered in deionized water, dark location at room temp. in a Parafilm-sealed Pyrex beaker.
5. Luer-lok syringe, loaded from the back end of the syringe, and excluding all air through the syringe tip.

All of the storage containers were sterilized in boiling water prior to use. Paste samples were cast onto microscope slides in three ways: direct cast (straight out of the storage container), sieved (worked three times through a fine plastic mesh strainer), and kneaded (after sieving, a few drops of deionized water were worked into the paste by kneading with a brush to a smooth, creamy consistency). The samples were cast on the first day (from fresh room temperature paste), as well as after the 4th, 7th, 12th, 15th and 21st day of storage. The resulting films were observed under bright field reflected light illumination at 200X magnification.

Conclusions and Future Experimentation—After seven days of storage (see figure 5a) each of the five pastes could be worked (sieved, kneaded) to the point where they exhibited virtually identical film-forming properties. After kneading, they also all resembled the initial freshly prepared paste. Observations of the pastes after this point are summarized in table 1.

From day 1 to day 12, it seemed that kneading and sieving each served to equalize the properties of the pastes, regardless of differences brought out by storage method. In general, the pastes stored without water over top were a little stiffer, although they were similar in texture to the syringe-stored paste. The pastes stored under water became less and less cohesive as time passed. No mold
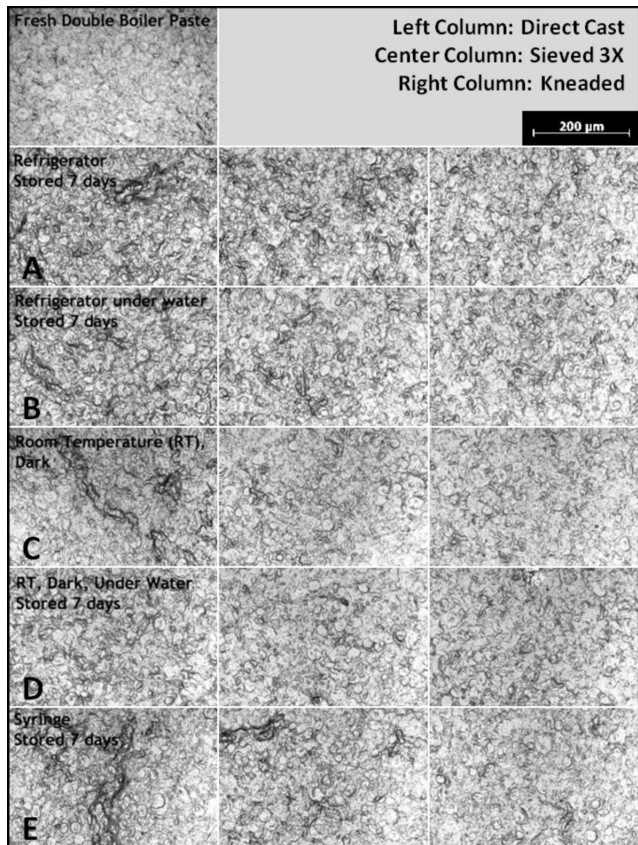
Fig. 5a. Fresh paste compared to the same batch of paste after seven days of storage using five storage methods. Stored paste was cast directly onto microscope slides (top row), sieved through plastic mesh (middle row) & kneaded with distilled water and a paint brush (bottom row). Bright field, reflected light illumination with 200X magnification
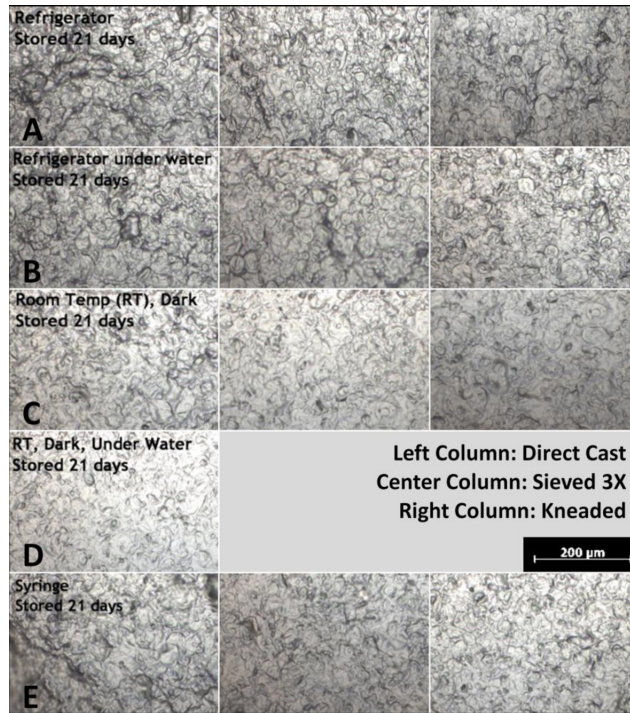

Fig. 5b. The same batch of paste as seen in figure 5a, after 21 days of storage. Bright field, reflected light illumination with 200X magnification. Paste D was no longer capable of being sieved or kneaded

	Day 12	Day 15	Day 21 (see figure 5b)
Paste A (refrigerator)	Good tack, gelled, casts a nice film.	Good tack, casts a good film when worked, gelatinous in direct cast.	Good tack, casts a good film when worked, gelatinous in direct cast.
Paste B (refrigerator, under water)	Slightly watery, mealy, doesn't cast a cohesive film until it is worked.	A little granular/watery, good tack, works (sieves/kneads) well.	A little granular/watery, good tack, works (sieves/kneads) well.
Paste C (room temperature, dark)	Smooth, good tack.	Visible mold (yellow), with mycelia throughout. Still kneads and sieves well, good remaining tack.	More visible mold, granules less defined than previous.
Paste D (room temperature, dark, under water)	Very watery, slight odor, weak tack.	Water and paste phases indistinct, no sign of mold (visible or microscopic). Sour odor.	Paste mixed completely with water. Very low cohesion. Smells very strongly. Cannot effectively sieve or knead. No sign of mold (visible or microscopic). Smell bacterial in origin?
Paste E (syringe)	Thick, but sieves and kneads well, good tack.	Thick, but sieves and kneads well, good tack.	Thick, still works well, but with slight odor; mold was evident under magnification.

Table 1. Observed properties of aged paste, as stored by five separate methods

mycelia were observed without the simultaneous presence of visible mold, with the exception of the syringe paste on day 21. The smell of aged paste cannot necessarily be attributed to mold, as odorous paste did not necessarily contain mycelia; bacterial growth is another likely cause. The presence of mold did seem to disrupt the structural integrity of intact granules in the paste films (see figure 5b, column C, which exhibits less granularity than the other pastes).

In future experimentation, it would be useful to explore how the adhesive tack of the paste films correlates to these visual observations.

Experiment #6: Observation of Mold

Question—After removing visible mold growth from a batch of paste, is the remaining paste still viable?

Background—When there is only one repair to make at the end of the week, and your batch of paste is just the smallest bit moldy; it may be tempting to reach around the visibly moldy paste for the "fresh" paste underneath. However, based on previous studies (Miller and Root 1991), it is likely that mold has contaminated the entire batch of paste.

Experimental—Naturally aged paste was monitored daily until the first observable signs of mold. A sample was taken from a portion of this paste that was exhibiting no visible mold, and mounted on a microscope slide. This paste was observed at 200X magnification under bright field and dark field illumination, as well as by fluorescence microscopy (emission filter: LP 470nm, excitation filter: BP 395–440nm, beam splitter: FT 460nm).

Image Results—See figure 6

Conclusions and Future Experimentation—Microscopic examination of a batch of visibly moldy paste clearly demonstrates that even portions of this paste without mold visible to the naked eye have mycelia distributed throughout. Daily examination of the five different storage methods (see experiment #5), however, revealed no such mycelia until they were also accompanied by visible mold somewhere on the batch. Even a batch of paste that began to smell bad didn't necessarily show any microscopic mycelia, indicating that bacterial growth, rather than mold, may be responsible for the odor. Paste with mold mycelia also seemed to exhibit fewer intact granules than the same paste earlier in its aging properties, supporting the theory that mold may affect the adhesive nature of paste.
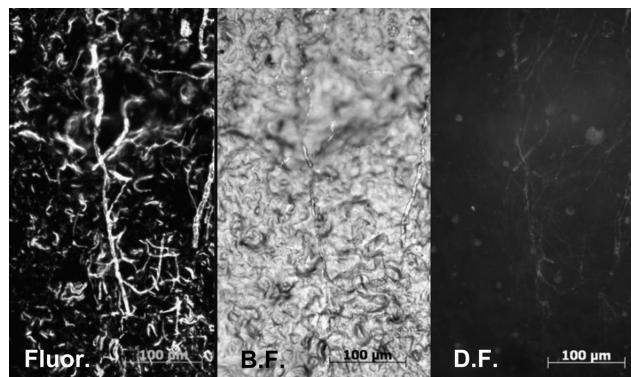

Fig. 6. Paste examined at first sign of visible mold, sampled from a non-visibly moldy portion of the paste batch. Mold mycelia observed with reflected light fluorescence, bright field & dark field illumination, 100X magnification

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NOTE

1. For full color images see http://www.library.jhu.edu/departments/preservation/hcs/. The research presented in this paper was originally exhibited as a poster at AIC 2010 in Milwaukee.

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