Investigation of Foxing Reduction in Works on Paper with Enzymes and Chelators in Agarose Gels

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Introduction

Foxing is a prevalent problem for paper-based materials in museums, libraries, and private collections. These stains usually appear as a cluster of circular or "snowflake"-shaped brown spots. While some attribute foxing to pigmented compounds associated with fungi and their metabolic processes, others cite the oxidation of metal particles. Still others believe foxing is a combination of the two. Both fungal spores and metal inclusions may be inherent to a sheet of paper from its manufacture or introduced at a later date from its surrounding environment. Under the premise that foxing is composed of both metal and fungal components, this experiment examined the combined and independent effects of the chelator N,N′-bis (2-hydroxybenzyl) ethlyenediamine-N,N′-diacetic acid (HBED) and the enzyme chitinase delivered in a 3% w/v agarose gel. Naturally foxed samples were attained from a mid-19th-century book donated to the Winterthur/University of Delaware Program in Art Conservation (WUDPAC) for student experiments. The pages are comprised of primarily cotton and bast fibers, treated with a gelatin size. Chosen for similar foxing damage and for as consistent manufacture as possible, eight adjacent pages were removed from the book for the experiment.

Characterization

Using an additional sample taken from the same suite of book pages, a histological staining procedure was performed using a wheat germ agglutinin (WGA)-Texas Red® dye conjugate to probe for fungal material in the foxed samples. WGA has a strong affinity for chitin, the primary component of fungal cell walls. As this attachment alone does not produce a visible indicator, WGA may be coupled with any number of histological stains that fluoresce when exposed to specific ranges of electromagnetic radiation. In this experiment, WGA was coupled with the dye Texas Red® and applied to the sample in a 1:8 dilution with phosphate buffered saline (PBS), allowed to react, and then rinsed with additional PBS to clear any unreacted material. Texas Red® is excited at ~595nm and emits at 615nm [1]. Fluorescence resonance energy transfer (FRET) was used to excite the dye when exposed to near-ultraviolet radiation (400-440nm) and highlight areas where chitin components are found. *Fig. 1.*

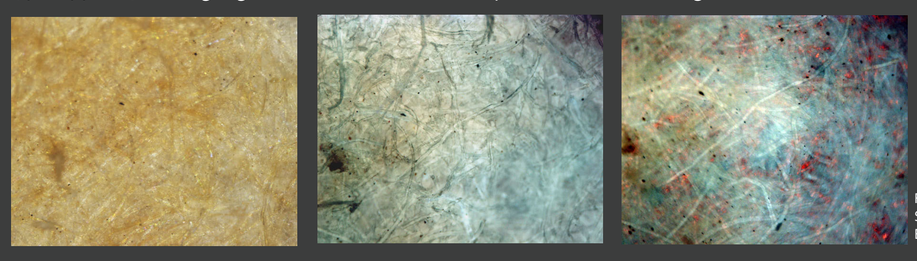

Fig. 1. Wheat Germagglutinin-Texas Red Stain (1:8, Phosphate Buffer Solution), From Left to RIght: Visible Light, UV Light (before staining), UV Light Exposed (after staining with Red positive); Image and stain Courtesy R. Wolbers

To determine the contribution of metal oxidation to foxing present in the paper samples, semi-quantitative x-ray fluorescence (XRF) spectroscopy was performed using a handheld Bruker Tracer III-SD XRF spectrometer with a rhodium tube; an oblong spot size measuring 0.5cm x 1cm; and fitted with a 25µm Ti/305µm Al filter. The spectrometer was operated at 40 kV high voltage; 9.6 µA anode current; and 60 seconds live time irradiation. These measurements determined the relative metal ion concentrations between the foxed versus non-foxed areas. The iron and calcium peaks are of particular interest in this study given that the foxed areas consistently exhibit higher concentrations of these metals. *Fig* 2.

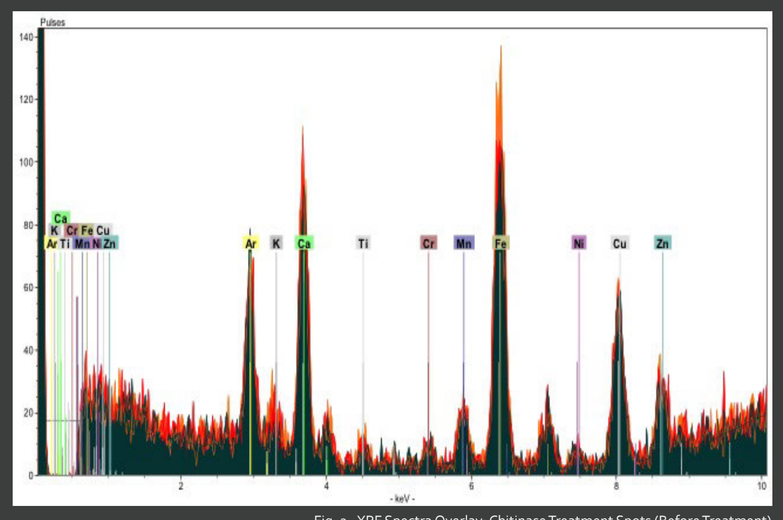

Fig. 2. XRF Spectra Overlay: Chitinase Treatment Spots (Before Treatment) Red/Orange Spectra are of three foxed areas; Dark Teal Spectra is an unfoxed area

Treatment Procedures

With the presence of fungal material and higher iron concentration confirmed, a targeted treatment was developed to address both contributing factors to the foxing observed in the samples selected for this experiment. The chelator N,N′-bis (2-hydroxybenzyl) ethlyenediamine-N,N′-diacetic acid (HBED) was selected as it is a potent iron chelator, forming highly stable, water-soluble complexes over a broad pH range of 2.5 to 11, functions as an indicator by turning pink when it has complexed with iron [2], and has a high stability constant of 39.01 with Fe (III) [3]. To address the fungal component, the enzyme chitinase was selected as it catalyzes hydrolysis of chitin in fungal cell walls without disturbing cellulose. This study examined the results of 16 samples: the following five treatments, each of which were tested for 60-, 90-, and 120-minute intervals, and a control. All but the control and blank agarose gel were buffered to pH 6.5 with sodium citrate/citric acid. The gels were cast to a depth of 2mm and punched into 3-mm diameter discs.

Fig.3. Installing gel disc for treatment using a template to register the treatment spot

Treatment Variations

- Control (bathing protocol only)
- Blank gel
- Chelator-only gel
- Enzyme-only gel
- Chelator-enzyme gel ("hybrid gel")
- Chelator-enzyme solution (direct application by micropipette)

All samples were humidified prior to treatment and blotter washed for three hours, with one blotter change after the two hours, using Winterthur Filtered Tap Water (pH~7, 30-50 ppm calcium ion concentration). Refreshed on a new damp blotter, the gel discs were applied using templates to map placement. Following treatment, the samples were cleared in a 20-minute immersion bath and dried

Data

The samples were documented before and after treatment using visible and UV (320-400nm) photography. The visible light photographs were taken to document the aesthetic changes of the treatment variations and the ultraviolet photographs to record changes in relative autofluorescence and/or absorption. To assess the efficacy of the treatments, color shift and metal concentration were measured before and after treatment using a Minolta CR-221 colorimeter and the Bruker handheld XRF spectrometer. Three foxed spots and one control spot were selected per sheet, taking care to select sites that were even in overall tone (i.e. non-nucleated spots) and exclude printing ink. Color measurements were taken from all samples before and after treatment. The ΔE values for each treatment reflect an average of the three spots treated on each sample. Due to time limitations, before and after treatment XRF data was collected for the control and the 120-minute samples only. Templates were created for each of the 16 samples to facilitate gel application as well as registration of the colorimeter and handheld XRF unit.

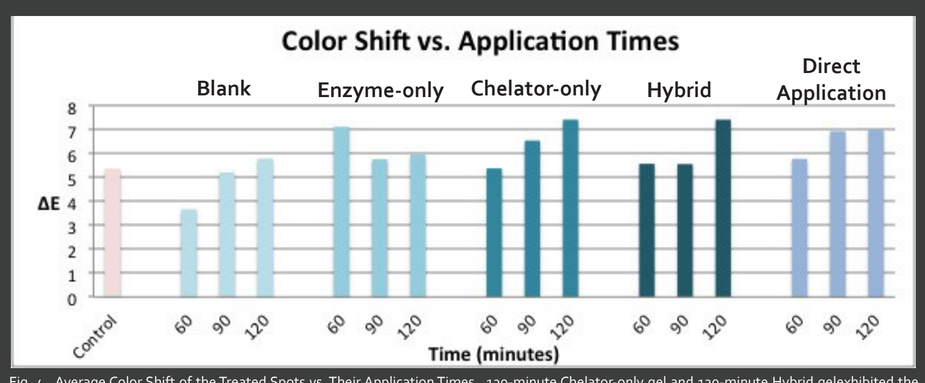

Fig. 4. Average Color Shift of the Treated Spots vs. Their Application Times. 120-minute Chelator-only gel and 120-minute Hybrid gelexhibited the greatest olorr Shift with a ΔE of 7.42. All treatments tended towards lighter, greener, and bluer shifts.

Overall, an increase in sheet brightness was observed under visible light and an overall decrease in autofluorescence, due to the extraction of oxidized degradation products, under ultraviolet radiation. While the majority of treatments did not result in an increase in autofluorescence, the 120-minute applications of the enzyme-only gel and the hybrid gel result in areas of increased autofluorescence corresponding to gel disc application. A review of the colorimetry data collected reveals that all of the treatments variations result in significant color shifts (ΔE > 3) with increased L* values, decreased a* values, and decreased b* values corresponding to lighter, greener, and bluer color shifts respectively. The majority of the variations yielded greater shifts in color change with increased treatment time [Fig.4]. The greatest shifts were produced by the 120-minute treatments with the chelator-only and hybrid gels, each producing a ΔE of 7.42. All 120-minute treatments reduced the metal concentration in foxed areas [Fig. 5]. Of the gels, the enzyme-only gel removed the greatest quantity of iron, and the chelator-only gel removed the least. The hybrid gel also removed less iron than the enzyme-only alone gel. In all samples, a decrease in both calcium and iron was observed after treatment.

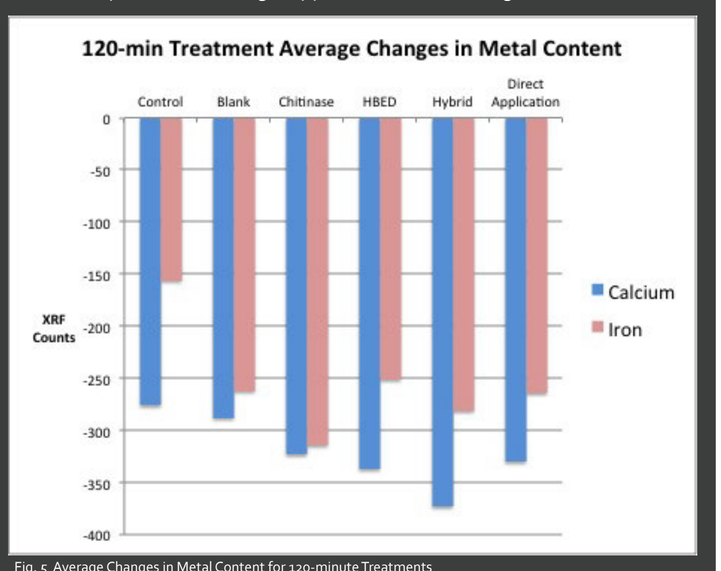

Fig. 5 Average Changes in Metal Content for 120-minute Treatments

Discussion

While efforts were taken to select consistent samples that were naturally aged, the treated spots still have variation in metal content, staining materials, and levels of oxidation. Undoubtedly, all of these variations in the paper and foxing impact the performance of each treatment tested. With this consideration, trends rather than conclusions are drawn from the data collected. These trends are viewed as points of departure for future experiments and continued research.

In all samples, a decrease in both calcium and iron was observed after treatment. It is believed that the decrease in calcium is a result of the blotter wash and/or immersion bath clearance protocols. This conclusion is founded on an observed decrease in calcium concentration in all samples, including the control and those treated with the blank, deionized water gel. Further, HBED has a low stability constant (9.29) with calcium relative to iron [2]. The significant iron loss in the enzyme-only treatment may indicate the release of iron-containing materials that are trapped in the cell. If the iron is contributing to fungal attachment to the paper, the HBED may not be able to fully access the iron due to the larger molecules blocking the metal ion, thus impeding its chelating performance. The hybrid gel is performing slightly better than the chelator-only gel, but not as well as the enzyme-only gel, perhaps indicating an interaction between the HBED and chitinase that was not anticipated.

Conclusion

This experiment was designed to test treatment of naturally foxed spots that contain both iron and fungal components using an HBED-chitinase solution delivered in a 3%-agarose gel. In this study, it appears that both HBED and chitanase contribute to reduction in iron content in the paper as well as the desired color shift. Longer treatment intervals result in greater reduction of foxing, however clearance methods need to be refined to ensure complete removal of chitinase after treatment. Further, the rigid-gel delivery system facilitates local treatment by restricting lateral flow observed in direct application and leads to more efficient use of materials.

Case Study: Lithograph Treated with the Hybrid Chelator-Enzyme Gel

Fig. 6 Case Study, Before Treatment

Fig.. 7. Case Study, After Treatment

In light of the positive results of the experimental treatment, a lithographic print was selected from the program's study collection for full treatment using the hybrid enzyme-chelator gel. The print was first dry surface cleaned and attachments and adhesive residues reduced on the verso. Following 30 minutes of humidification, the print was blotter washed for a total duration of three hours. After one hour, hybrid gels were applied locally to foxed areas of the print.

References: [1] Life Technologies. 2014. Wheat germagglutinin, Texas Red®-x. http://www.lifetechnologies.com/order/catalog/product/W21405 (accessed 01/15/14). [2] L'Eplattenier, F., I. Murase, and A. E. Martell. 1967. New multidentate ligands. vi. chelating tendencies of n,n′-di(2-hydroxybenzyl) ethylenediamine-n,n′-diacetic acid. In Journal of the American Chemical Society 89: 837-43. [3] Ma, R., R.J. Motekaitis, and A.E. Martell. 1994. Stability of metal ion complexes of n,n′-bis(2-hydroxybenzyl)ethylenediamine-n,n′-diacetic acid. In Inorganica Chimica Acta 224: 151-55.

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