A Preliminary Investigation into the Application of Agarose-Enzyme Gels in Textile Conservation

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Abstract

Introduction of the gelling agent agarose into the textile discipline has provided another vehicle for the application of localised cleaning. Application both within and outwith the textile discipline has included various solvents, chelating agents and enzymes. Various aspects of an agarose application can be adapted such as concentration, gel height and treatment duration. However, presently there are limited publications specific to application of agarose on a textile substrate. Available literature has indicated that application of agarose on textiles is heavily impacted by the fibre type and requires different parameters from cross-disciplinary use. The aim of this study is to determine the optimal application parameters of an agarose-α amylase gel for the removal of heavily accreted wheat starch from a cotton substrate. Investigation of three concentrations of agarose and three treatment durations on both thermally aged and unaged samples indicated that localised application of agarose-α amylase was effective. Evaluation of the efficacy of the treatment indicates that the optimum application parameter is the application of 3% agarose gel. The findings did not suggest a difference in efficacy between different treatment durations or sample age. Further research could be undertaken to investigate barriers to control lateral water movement and application parameters for other fibre substrates.

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No acknowledgement would be complete without mentioning my family and partner. Thank you for your unwavering support and encouragement. This would not have been possible without you.

Part 1: Introduction and Background

Chapter 1: Introduction

1.1 Introduction

The application of poultices for the controlled introduction of cleaning solutions, such as solvents, chelating agents or enzymes, to a substrate can be seen throughout the field of conservation. Introduced to the textile discipline in the early 90s, it has predominately been utilised in localised cleaning treatments to remove soiling, impurities and adhesive residues. The application of cleaning agents in this manner allows soiling and staining found on textile objects with sensitive components, such as dyes, painted surfaces and water soluble decorative elements, to be addressed with lower risks.

The efficacy of poultice treatments relies on understanding and controlling several physical principles relating to capillarity and water movement.[1] The main principles include: diffusion, evaporation, capillary action and equilibria[2]; all of which affect the poultice system, the substrate, the solvent and the surrounding environment. Briefly explained, when a wet poultice is applied to a textile substrate, the solvent held in the poultice diffuses into the textile until equilibrium of the solvent held in the two materials is reached. The diffusion of solvent into the substrate acts on solubilise soiling within the textile. As the solvent is lost from the top of the poultice through evaporation, the capillary action of the poultice draws the solvent, along with any solubilised materials, out of the textile substrate.[3] The upward movement of the solvent will continue until a new equilibrium has been reached between the poultice, substrate and atmosphere.[4] The key to the success of a poultice treatment is that the capillary forces must be stronger in the poultice than the substrate to ensure that the solvent is effectively drawn upwards and does not spread into the fabric beyond the area of poultice application.[5]

The materials used to form poultices for conservation have included paper pulp, fabric, clays[6] and in recent decades, viscosity modifiers and gels.[7] The adoption of viscosity modifiers and gels into the conservation field has provided a plethora of options for tailoring cleaning

[1] Shawna Lemiski, "An Investigation of Poultice Materials for Textile Conservation," *Textile Conservation Newsletter Supplement*, 1998, 5.
[2] Karen Thompson, "Sepiolite Poulticing-An Alternative for the Cleaning of Textiles," *Conservation News*, no. 53 (1994): 49.
[3] Lemiski, "An Investigation of Poultice Materials for Textile Conservation," 5.
[4] Ibid.
[5] Ibid.
[6] Ibid., 4.
[7] Emma Schmitt and Sarah Foskett, "Gelling in Theory and Practice : An Examination of Agarose Gels in Textile Conservation," *Textile Specialty Group Postprints* 26 (2016): 157.

solutions and localised cleaning applications. The rapid integration of these new materials into conservation practice across a range of disciplines has outraced research and publications on refining their application to different substrates.

1.2 Aims

This present study intends to determine if the use of an agarose-alpha amylase gel is an effective method of removing accreted wheat starch residue from cotton fabrics in the practise of textile conservation. It aims to:

1. Evaluate the current literature and methodologies on agarose and enzyme use for conservation of textiles and other materials
2. Determine if an agarose-alpha amylase gel is effective and practical for removing accreted wheat starch residues
3. Establish which concentrations and methodologies enhance the ability of this gel cleaning system

This paper will begin with a literature review and discussion of agarose and enzyme use in conservation, followed by an experimental exploration of agarose-alpha amylase gels on both aged and unaged cotton substrates. The experiment is designed to determine what concentration of agarose is most effective in removing accreted wheat starch adhesive with limited and controllable wetting of the substrate. Additionally, factors such as duration and weighting of gels that may enhance the treatment will be evaluated. The enzyme treatments will be evaluated through weight change, movement of water and visual and tactile evaluations of the adhesive removal.

Chapter 2 Background

2.1 Wheat Starch

2.1.1 The Conservation Context

The removal of aged wheat starch is an issue commonly seen in both paper and textile conservation. Traditional applications can be seen on the reverse of embroideries, banners and flags[8] as large clumps of adhesive or as a layer that has permeated into the interstices of the weave. The nature and historical application of wheat starch results in areas of heavy creasing, distortion, discolouration and embrittlement. Removal of wheat starch is sometimes necessary to increase the stability of the objects.

2.1.2 Composition and Properties

Natural starches consist of two types of molecules: amylose and amylopectin (Fig 1).[9] Both of these molecules are constructed of glucose monomers; however, these units are linked differently resulting in distinct molecular structures and physical properties. Amylose is a highly linear polymer, with monomers linked through α-1,4 glycosidic bonds.[10] Amylose forms strong water-resistant films and contributes to the gelling of solutions upon cooling.[11] Amylopectin is a larger, branched molecule. The monomers are joined in the same manner as amylose, but only short chains are formed which join together to form the branched shape.[12] This polymer forms weak water-sensitive films and can be dissolved in cold water to form a viscous solution.[13] The

[8] Gillian Bott, "Amylase for Starch Removal from a Set of 17th Century Embroidered Panels," *The Conservator* 14, no. October (1990): 23–29, doi:10.1080/01410096.1990.9995053; Nobuko Shibayama and Dinah Eastop, "Removal of Flour Paste Residues from a Painted Banner with Alpha-Amylase," *The Conservator* 20, no. December (1996): 53–64, doi:10.1080/01410096.1996.9995103; Vivien Chapman, "Amylase in a Viscous Medium: Textile Applications," *The Conservator* 10, no. October (1986): 7–11, doi:10.1080/01410096.1986.9995011; Shirley Ellis, "A Passage in the Life of a Palampore: Conservation," *Journal of the Canadian Association for Conservation = Journal de l'Association Canadienne Pour La Conservation et La Restauration* 34 (2009): 21–28.

[9] Velson Horie, *Materials for Conservation: Organic Consolidants, Adhesives and Coating*, 2nd Edition (Oxon: Routledge, 2011), 221.

[10] David Grattan et al., "The Characterization of Enzymes for Use in Paper Conservation," in *Conservation of Library and Archive Materials and the Graphic Arts*, ed. Guy Petherbridge (The Institute of Paper Conservation and the Society of Archivists, 1987), 16.

[11] Horie, *Materials for Conservation: Organic Consolidants, Adhesives and Coating*, 223.

[12] Grattan et al., "The Characterization of Enzymes for Use in Paper Conservation," 16.

[13] Horie, *Materials for Conservation: Organic Consolidants, Adhesives and Coating*, 223.

ratio of these components varies depending on the source of the starch and determines the properties of the paste and resulting film.[14]

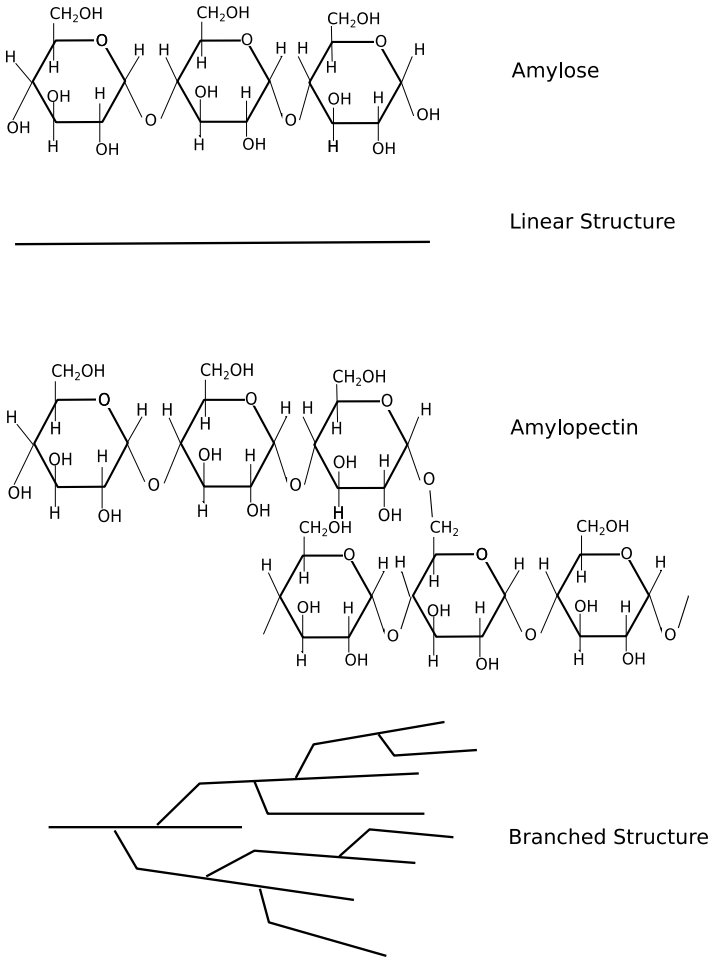

Figure 1 Diagram of amylose and amylopectin polymers, showing both the molecular composition and simplified polymer structure (Adapted from Horie 2011 and Down 2015. All diagrams drawn using Inkscape (open source vector graphics software) unless otherwise captioned.

2.1.3 Preparation of Starch Paste

Starch paste is made through heating starch granules in water to its gelatinization temperature (55-80°C), at which the granules swell and burst.[15] The amylose component which is located on the periphery of the granule dissolves first and the amylopectin swells and forms a network. This increases the viscosity of the solution. Continued stirring and heating at

[14] Jane Down, *Adhesive Compendium for Conservation* (Ontario: Canadian Conservation Institute, 2015), 45.
[15] Horie, *Materials for Conservation: Organic Consolidants, Adhesives and Coating*, 222.

gelatinization temperature distributes the starch components, eventually forming a smooth paste. On cooling, the amylose molecules cross link through hydrogen bonding and the mixture forms a gel.[16] On standing these molecules can become stabilised, forming stronger hydrogen bonds, a process known as retrogradation.[17] Retrograded starch is suggested to be resistant to "enzyme attacks".[18]

2.1.4 Removability

As starch films are not water soluble, the application of water in the form of ultrasonic mist, steam or a hot water bath will only reswell the starch. This allows for mechanical removal of the resulting gel or failure to adhered layers; however, complete removal of the adhesive cannot be achieved using only water.[19] Solvents such as N-methyl-2-pyrrolidone and dimethyl sulphoxide have been used to dissolve starch but these have been identified as harmful to health and likely to disrupt and weaken the substrate.[20] The most effective method of removing wheat starch residues is through the use of enzymes.

2.2 Enzymes

2.2.1 Enzymes in Conservation

Enzymes have been applied in conservation since the early 70s. They have been charmingly documented as an "enzyme scalpel",[21] no doubt in reference to the specific nature of the catalytic molecule. The specificity of these molecules comes from their complex polypeptide structure. Each enzyme consists of complex chains of amino acids that are configured and arranged to form a substrate-specific active site. This site engages with the substrate catalysing a reaction and then releasing the enzyme-converted molecules, the products of the reaction. Enzymes can be reaction-specific, so that only a particular type of reaction, such as the breaking of a specific bond, is catalysed.[22] This is a particular advantage as its specificity and speed allows for short contact times during conservation treatment.

[16] Down, *Adhesive Compendium for Conservation*, 44.
[17] Horie, *Materials for Conservation: Organic Consolidants, Adhesives and Coating*, 223.
[18] Ibid.
[19] Ágnes Tímár-Balázsy and Dinah Eastop, *Chemical Principles of Textile Conservation* (Oxford: Butterworth-Heinemann, 1998), 317.
[20] Down, *Adhesive Compendium for Conservation*, 50.
[21] Ø. Wendelbo and B Fosse, "Protein 'Surgery' A Restoring Procedure Applied on Paper," *Restaurator* 1, no. 4 (1970): 245–48.
[22] Grattan et al., "The Characterization of Enzymes for Use in Paper Conservation," 15.

The most useful group of enzymes to conservation are the hydrolases, which form two products from a substrate by hydrolysis, the cleaving of molecules through the addition of water.[23] These enzymes are capable of breaking down natural biopolymers such as proteins, starch.[24]

2.2.2 Alpha Amylase

The enzyme most commonly used to remove wheat starch is the hydrolase, α-amylase, also known as 1,4-α-D-glucan-glucanohydrolases. The identification number assigned to this enzyme by the Enzyme Commission, according to the reaction that it catalyses, is E.C 3.2.1.1.[25]

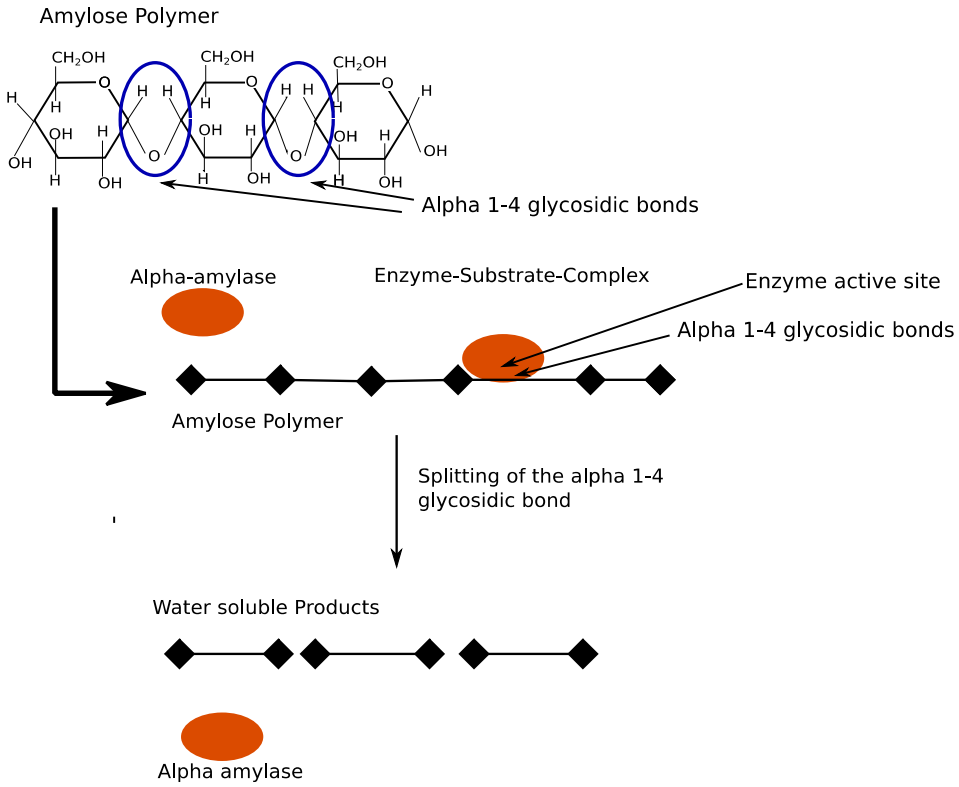

Figure 2 Simplified diagram of the effect of α-amylase on the amylose polymer.

This enzyme specifically breaks down starch molecules by hydrolysing the 1,4 glycosidic linkages at random points over the linear sections of the starch polymer (Fig 2). As the monomers

[23] Richard Wolbers, *Cleaning Painted Surfaces: Aqueous Method* (Archetype Publications Ltd, 2000), 128.
[24] Ibid., 128–29.
[25] "Enzyme Entry: EC. 3.2.1.1: Alpha-Amylase," *ExPASY: Bioinformatics Resource Portal*, accessed July 7, 2018, https://enzyme.expasy.org/cgi-bin/enzyme/enzyme-search-ec.

of both amylose and amylopectin are joined by these linkages, α-amylase is able to break down these polymers. The resulting sections of the starch polymer become readily soluble in water and are too short to retain adhesive capabilities.[26]

Applications of α-amylase within paper and textile disciplines have included immersion, spot cleaning and poulticing. The performance of the enzymes depends on several factors: the activity of the enzyme, the purity of the enzyme, the precise composition of the substrate, pH and temperature.[27] Experimental investigations have been undertaken to evaluate these parameters and overall, enzyme treatments have been found to be a powerful method of addressing starch residues that is safer for both the conservator and object. However, one of the main limitations to the usefulness of enzymes is the necessity for application to occur in aqueous environments. Modifications to localised application methods have been introduced to address this issue. A more recent method of enzyme application is through the incorporation of the enzyme into a gel.

2.3 Agarose

2.3.1 Introduction

A gel that has risen to prominence within paintings and paper conservation is the gelling agent, agarose. 'Gel' is a generic term for a high viscosity dispersion of finely divided solid particles, known as colloids.[28] Within a liquid medium, the colloidal particles interact to form a rigid matrix, which results in little flow or movement when no force is being applied.[29] Agarose is the main gelling component derived from agar, a hydrocolloid polysaccharide extracted from red algae of the class Rhodophyceae.[30] Agarose has been widely used in biomedical science and microbiology. Within conservation applications, it has been used as a rigid gel poultice that enables greater control over the introduction of moisture and leaves minimal residue.[31] Its transparent rigid gel formation allows the conservator to observe the progress of the treatment and to tailor the gel to a specific shape. Its theoretically neutral charge means that it is less likely

[26] Harold M. Erickson, "Usage Recommendations Fro Alpha-Amylases: Maximizing Enzyme Activity While Minimizing Enzyme-Artifact Binding Residues," *The Book and Paper Group Annual* 11 (1992): 25.
[27] Grattan et al., "The Characterization of Enzymes for Use in Paper Conservation," 15.
[28] Richard Wolbers, "Terminology and Properties of Selected Gels," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (London: Archetype Publications Ltd, 2017), 386, 388.
[29] Ibid., 386.
[30] R. Armisen, F. Galatas, and S. A. Hispanagar, "Agar," in *Handbook of Hydrocolloids*, ed. G. O. Phillips and P. A. Williams, 2nd Editio (Cambridge: Woodhead Publishing, 2009), 83.
[31] Jeffrey Warda et al., "Analysis of Agarose , Carbopol , and Laponite Gel Poultices in Paper Conservation Author (s): Jeffrey Warda , Irene Brückle , Anikó Bezúr and Dan Kushel Source : Journal of the American Institute for Conservation , Vol . 46 , No . 3 (Fall - Winter , Pu," *Journal of the American Institute for Conservation* 46, no. 3 (2007): 272.

to interact with other molecules, enabling a broad range of additives including water-miscible solvents, chelators, surfactants and enzymes.

2.3.2 Structure and Properties

Agarose is a linear polysaccharide composed of agarobiose monomers. Agarobiose is a monomer made of α-(1,3)-D-galactose and β-(1,4) -(3,6)-anhydro-L-galactose rings (Fig 3).

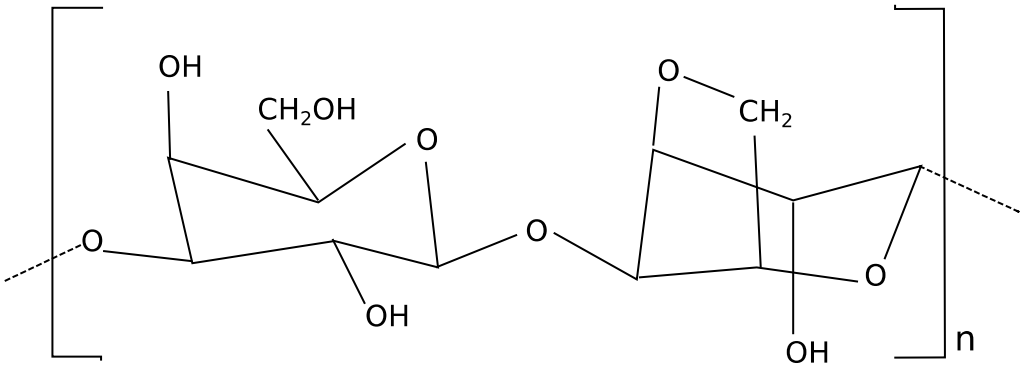

Figure 3 The agarobiose monomer, α-(1,3)-D-galactose and β-(1,4) -(3,6)-anhydro-L-galactose (Adapted from Armisen 1997).

Insoluble in cold water, agarose can be prepared by adding the powdered form to water and gradually bringing the mixture to 80-100°C.[32] At this temperature, the polymer chains become solvated. As it cools, the randomly orientated chains shift to double helix conformations, through the formation of hydrogen bonds, and interlink to form a three-dimensional matrix, creating a rigid gel (Fig 4).[33] The rigid gel structure enables the formation of the pores and gel matrix which aids capillary action and diffusion. This structure is crucial for its use in conservation as it allows agarose gel to function as a poultice.[34]

[32] Yana Van Dyke, "Enzymes in Paper Conservation," in *Art, Biology, and Conservation: Biodeterioration of Works of Art*, ed. Robert J. Koestler et al. (New York: The Metropolitan Museum of Art, 2003), 168.
[33] Struther Arnott, A. Fulmer, and W. E. Scott, "The Agarose Double Helix and Its Function in Agarose Gel Structure," *Journal of Molecular Biology* 90 (1974): 270.
[34] Yana Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (London: Archetype Publications Ltd, 2017), 101.

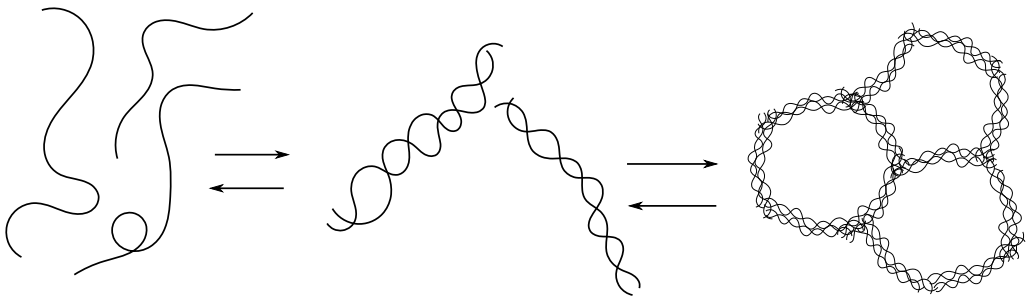

Figure 4 The gelling phases of agarose from left to right: agarose polymer solvated in liquid phase, the double helix forming and crosslinking as the gel cools, forming a rigid gel matrix (Adapted from Arnott et al. 1974).

The nature of the resulting gel is adjusted through the concentration of agarose, described by dry weight to volume of water. At higher concentrations, there are more polymer chains and in turn more interlinking regions available, creating an extensive and dense matrix.[35] A lower concentration results in an open matrix formed by fewer polymer chains. The extent of the matrix affects the amount of water in the gel and also the speed of diffusion of water through the gel.[36] Gels with a higher concentration contain less water and have a slower diffusion rate; conversely, gels with a lower concentration contain more water and have a faster diffusion rate. This has been supported by analytical investigations that connected higher concentration gels with lower amounts of free water, resulting in less water removed through capillary action into a substrate.[37]

2.3.3 Conservation Application

A significant number of publications can be found within paper and paintings conservation on the application methodologies of agarose as a gel poultice. Publications on agarose present a range of applications with an agarose concentration range of 0.5-5%, however the most commonly applied concentrations are 1-2%.[38] The application of lower concentrations suggests that gels with higher water content are preferred and successful in these applications.

[35] Ibid.
[36] Ibid.
[37] Moira Bertasa et al., "A Study of Commercial Agar Gels as Cleaning Materials," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (London: Archetype Publications Ltd, 2017), 11–18.
[38] Warda et al., "Analysis of Agarose , Carbopol , and Laponite Gel Poultices in Paper Conservation Author (s): Jeffrey Warda , Irene Brückle , Anikó Bezúr and Dan Kushel Source : Journal of the American Institute

Though textile-specific publications and cases studies are still limited, interest in the agarose medium within the textile discipline is growing. A recent significant body of work undertaken by Schmitt has contributed towards establishing parameters for the application of agarose onto textile substrates.[39]

These publications highlighted the lack of discipline-specific published resources as a major barrier to use, despite the interest generated through the success in other disciplines. Her study shows that there is great potential for the use of agarose as a poultice in textile conservation but stresses the need for more research, evaluation of risks and the importance of object-based testing.[40] This present study aims to add to this body of work by providing a starting point for the usage of agarose-enzyme gels in textile conservation.

for Conservation , Vol . 46 , No . 3 (Fall - Winter , Pu"; Yana Van Dyke, "Practical Applications of Protease Enzymes in Paper Conservation," *Book and Paper Annual* 23 (2004): 93–107; Amy Hughes and Michelle Sullivan, "Targeted Cleaning of Works on Paper: Rigid Polysaccharide Gels and Conductivity in Aqueous Solutions," *The Book and Paper Group Annual* 35 (2016): 30–41.

[39] Schmitt and Foskett, "Gelling in Theory and Practice : An Examination of Agarose Gels in Textile Conservation"; Emma Schmitt, "An Examination of the Working Properties of Agarose Gels for Textile Conservation" (University of Glasgow, 2014); Emma Schmitt, "Gelling Predictions: The Challenges of Taking Research into Practice," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (Archetype Publications Ltd, 2017), 92–95.

[40] Schmitt, "Gelling Predictions: The Challenges of Taking Research into Practice," 95.

Chapter 3: Conservation Literature Review

3.1 Introduction

This literature review provides an outline of the use of agarose gels and enzymes in conservation, focusing primarily on publications produced from paper and textile disciplines. The scope of the literature is limited to texts written in English, from European and North American authors. Publications produced from conservation symposia, peer-reviewed journals and unpublished dissertations are included. The aim of this review is to provide background for the present research specifically regarding the considerations for agarose concentration, water movement and trends and concerns in enzyme treatment.

3.2 Agarose in Conservation

Agarose, like many of the other gel cleaning systems, was widely introduced into the painting discipline by Richard Wolbers in the late 2000s. To date there are still limited published works by Wolbers. Early publications[41] were intended to be core texts for guiding conservators in formulating aqueous cleaning solutions for varnished surfaces but do not include discussions of agarose. The case studies provided are specific to the paintings discipline but the theory regarding consideration of the immediate and long-term effects of cleaning is relevant to all conservation practice.

Over the last decade, Wolbers has disseminated information via workshops which have taken place over a range of locations in North America, Europe and Australia. Informal publications do arise from these workshops in the form of reviews[42] and YouTube videos.[43] Whilst these are accessible, the workshops tend to cover a whole range of topics pertaining to aqueous cleaning and, as a result, specific methodology and techniques are rarely given in detail. With such limited access to information and published sources regarding the application of materials such as

[41] Wolbers, *Cleaning Painted Surfaces: Aqueous Method*.
[42] Yadin Larochette, "Wolber's World: A Review of a Textile Wet-Cleaning Workshop Held in Oaxaca, Mexico," *WAAC Newsletter* 34, no. 1 (2012): 24–26; Alan Derbyshire, "Wolbers' Course- A Review," *V&A Conservation Journal*, no. 35 (2000); Rebecca Pavitt, "Cleaning of Painted Surfaces- Wolbers Strikes Again!- A Workshop Review," 2012, https://www.iiconservation.org/node/3216; Sarah Foskett, "A Review of 'New Methods of Bathing and Stain Removal for Textiles' with Richard Wolbers," 2017, http://textileconservation.academicblogs.co.uk/a-review-of-new-methods-of-bathing-and-stain-removal-for-textiles-with-richard-wolbers/.
[43] "'The Use of Gels in Aqueous Conservation of Paper' by Richard Wolbers- 5 Part Series," *Icon Book & Paper Group*, 2013, https://www.youtube.com/watch?v=mu7_nS-zF1c&t=621s.

agarose, it is not surprising that there are limited case studies on their applications in both paper and textile disciplines.

Paper conservators have undertaken investigations to further understand how agarose interacts with and affects a paper substrate. Unlike painted surfaces, both paper and textile substrates are porous. Similarities in their interaction with water and the resulting concerns of distortion, tidelines and the risk to water-sensitive components allow textile conservators to draw heavily from paper conservation publications to inform the use agarose.

3.2.1 Conservation and Water Movement of Agarose

Within the paper and textile disciplines, publications specifically discussing agarose began to appear in the late 2000s. A key source that discussed the technical aspects of the material was published by paper conservators, Warda et al.[44] The authors discussed agarose ageing and residues compared to other new poulticing materials. The authors were critical of the agarose medium, voicing concern for rapid water movement and the inability of agarose to suspend removed soiling. However, the experimental phase indicated that agarose residue did not contribute to discolouration of the paper substrate. The authors concluded that flexibility of the agarose medium shown in paintings conservation and the lack of harmful residue was promising and encouraged continued investigation of this medium.

Conservators Shaeffer and Gardiner presented a similar survey of agarose and six other materials used for localised cleaning of textiles.[45] The authors highlighted the strength of agarose, which reduced the total solvent volume needed for treatment and reduced mechanical action. Shaeffer and Gardiner also emphasised the importance of controlling the delivery of the solution to reduce water movement into the substrate through adjustment of the agarose concentration. Warda et al. had also observed this but had not discussed the manipulation of concentration. Additionally, Shaeffer and Gardiner offered the disclaimer that properties of textile substrates such as weave, fibre density, sizing and fibre type will affect the action of the poultice.

A recent significant body of work specifically addresses this last consideration; unlike paper, where the substrate is predominantly cellulosic, textiles include a range of fibre types, each of which have different and complex interactions with water. Schmitt's investigation which resulted

[44] Warda et al., "Analysis of Agarose , Carbopol , and Laponite Gel Poultices in Paper Conservation Author (s): Jeffrey Warda , Irene Brückle , Anikó Bezúr and Dan Kushel Source : Journal of the American Institute for Conservation , Vol . 46 , No . 3 (Fall - Winter , Pu."

[45] Elizabeth Shaeffer and Joy Gardiner, "New and Current Materials and Approaches for Localised Cleaning in Textile Conservation," *Textile Specialty Group Postprints* 23 (2013): 109–23.

from her dissertation research, investigates the application of agarose gels on different textile substrates[46]. She brought together cross disciplinary information, treatment trends and product choice, and highlighted the limited available research on agarose both within the textile discipline and the general conservation field. Schmitt suggested that the limiting factors in agarose application were lack of information on preparation techniques, expense of the materials, testing time required, and, finally, the lack of clear information on the cleaning action of agarose and how to control it.[47] Her research concluded that a different range of concentrations is needed for textile substrates than for applications in paper and painting, as the capillary action of textile fibres is comparatively stronger. Additionally, she noted that different fibre types had different optimal gel application ranges, which minimised the extent of water movement whilst still allowing the liquid carried in the gel to interact with the substrate. Schmitt's papers contextualised agarose application for the textile discipline while clearly underlining that continued research is required.

3.2.2 Case Studies

Few case studies are available on textile applications of agarose.[48] The lack of introductory publications is seen in these case studies, as the bulk of these publications focus on establishing application parameters through preliminary testing of gel concentration, gel preparation methods and formulation of cleaning solutions. The application concentrations are cited from paper conservation and through interdisciplinary discussion. Rapid water movement was noted in by both Duffus, Younger and Benson and Sahmel et al. This was addressed through pre-emptive application of a cyclododecane (CDD) barrier[49] and remedial applications of agarose gels to tidelines.[50]

The publications that were produced from the *Gels in the Conservation of Art* conference[51] included a textile conservation case study that is particularly relevant to this present research; Schmitt builds on her existing body of work by applying the agarose treatment parameters that

[46] Schmitt, "An Examination of the Working Properties of Agarose Gels for Textile Conservation"; Schmitt and Foskett, "Gelling in Theory and Practice : An Examination of Agarose Gels in Textile Conservation"; Schmitt, "Gelling Predictions: The Challenges of Taking Research into Practice."
[47] Schmitt, "An Examination of the Working Properties of Agarose Gels for Textile Conservation," 6–17.
[48] Philippa Duffus, Sophie Younger, and Sarah Benson, "Agar Paper: Trails and Tribulations with Agarose Gel," in *Joined Up Thinking: Textiles and the Historic Interior-Forum of the ICON Textile Group*, ed. Alison Fairhurst (Birbeck College, London, 2014), 36–44; Katherine Sahmel et al., "Removing Dye Bleed from a Sampler: New Methods for an Old Problem," *Textile Specialty Group Postprints* 22 (2012): 76–90.
[49] Sahmel et al., "Removing Dye Bleed from a Sampler: New Methods for an Old Problem," 76.
[50] Duffus, Younger, and Benson, "Agar Paper: Trails and Tribulations with Agarose Gel," 40.
[51] Appendix 1

she had previously determined for the treatment of wool sampler.[52] The author provided a detailed discussion of the formulation of the agarose treatment and critically evaluated the process. The treatment included the consecutive application of two agarose gels at 2.5%. The second gel resulted in alarmingly rapid water movement, necessitating remedial intervention. Schmitt's paper highlights the difficulties of transferring theory into practice. The concentration that was applied had been identified as suitable to the substrate, however, it was difficult to predict the effect of additional variables which a real object brings to the treatment, along with the application of multiple gels which had not been explored in her previous publications.

A review of key technical publications and object-based applications suggests a need for more up to date technical publications on the properties of agarose. The case studies show the engagement of conservators with different levels of agarose application. A parameter that was continually tested across all case studies was the concentration of the gels, through which water movement can be adjusted. This serves to emphasise it as a key parameter and one that needs to be tailored to each treatment. This present paper intends to continue investigating this variable for agarose-enzyme application with the additional parameter of gel duration which has not been evaluated.

3.3 Enzymes in Paper and Textile Conservation

This section will review conservation literature specifically regarding the application of α-amylase to paper and textiles, with the aim of highlighting the application trends and concerns of enzyme use. Identification of these patterns will aid the evaluation of the agarose-enzyme treatment investigated in this present study.

3.3.1 Enzymes and Paper Conservation

Enzyme usage first appeared in the conservation literature in the 1970s. In the conclusion of the earliest publication on α-amylase,[53] the authors noted that the application of enzymes in non-aqueous solutions was being investigated so that their application could be extended to objects with water-sensitive components. Four decades later, this is still a major issue in enzyme treatments and key to this present study. The publication by Segal and Cooper in 1977 is still heavily cited. The article provides a valuable record of the early concerns of immersion enzyme application. These concerns included the denaturation and rinsing of enzymes, the effect of

[52] Schmitt, "Gelling Predictions: The Challenges of Taking Research into Practice."
[53] Judith Segal and David Cooper, "The Use of Enzyme to Release Adhesives," *The Paper Conservator* 2, no. 1 (1977): 47–50.

enzymes on the physical properties of the paper substrate, and the risk to water-sensitive components. The article emphasised the field's enthusiasm towards enzyme applications but also recognised the need for further investigation for this method that is "quick, safe, clean and cheap."[54]

The 1980s saw several publications from the paper conservation discipline that focused specifically on refining knowledge of the properties of different enzymes used in practice.

Publications focused on quantifying the effect of temperature, pH, concentration, purity, shelf life and the permeability of enzymes through different kinds of paper.[55] A seminal publication by DeSantis emphasised the need for conservators to engage with enzymological literature as a method of clarifying conservation applications; this was of particular importance for stating optimum pH and temperature ranges in immersion treatments.[56] Investigation into adapting immersion treatments for objects with water-sensitive components, with the addition of buffers and solvents were also investigated.[57] This increased the stability of the water-sensitive components but required adjustment of other application parameters such as pH, temperature and immersion duration, which negatively impacted the substrate.[58]

3.3.2 Enzymes and Textile Conservation

Similarities in the target soiling and concerns for water-sensitive elements have allowed textile conservators to directly apply enzyme treatment processes and parameters from paper conservation.[59] Publications from the textile discipline have included both immersion and localised enzyme treatments. These articles also emphasised the value of enzyme specificity within an immersion treatment that had been highlighted in paper conservation literature.

[54] Ibid., 48.
[55] Grattan et al., "The Characterization of Enzymes for Use in Paper Conservation."
[56] Pia C. DeSantis, "Some Observations on the Use of Enzymes in Paper Conservation," *Journal of the American Institute for Conservation* 23, no. 1 (1983): 7–27.
[57] David Cooper, Carolyn King, and Judith Segal, "The Use of Enzymes in Partially Non-Aqueous Media," in *Conservation of Library and Archive Materials and the Graphic Arts*, ed. Guy Pethebridge (The Institute of Paper Conservation and the Society of Archivists, 1987), 25–30.
[58] Ibid., 28.
[59] Chapman, "Amylase in a Viscous Medium: Textile Applications"; Bott, "Amylase for Starch Removal from a Set of 17th Century Embroidered Panels"; Shibayama and Eastop, "Removal of Flour Paste Residues from a Painted Banner with Alpha-Amylase."

Several authors corroborated DeSantis' note on the flexible range of enzymes[60] and that application conditions could be adapted so that the treatment is safer for textile substrates.[61]

Investigations concerning immersion treatments with textiles specifically underlined the importance of considering duration. Shibayama and Eastop noted that, similar to a wet cleaning, enzyme treatments should not exceed two hours.[62] This parameter had not been defined in any paper conservation literature which is perhaps reflective of the nature of wet cleaning process in these two disciplines: a common procedure in paper conservation and rarer in the textile discipline.

3.3.3 Localised Applications

Preliminary investigations into localised enzyme treatments can be seen throughout the 70s and 80s in both disciplines. Application methods included blotting paper infused with enzymes and a range of viscous, thixotropic media such as methyl cellulose, a cellulose ether, and laponite, a synthetic silicate.[63] These case studies were all presented as successful enzyme treatments, however, evaluation of these methods was brief, and it is not clear how successful each of these media were as a localised treatment. Conversely, it was observed that application using these media resulted in slower enzyme penetration,[64] and for some media adjustment of pH was necessary for the enzyme to be effective.[65]

Recent publications indicate the continued interest in both disciplines in localised enzyme applications. These papers include comparative studies and also the invention and evaluation of a prefabricated enzyme-infused poultice, the Albertina Compress.[66] These publications indicate that whilst localised enzyme treatments are effective they are not as successful as immersion treatments; their value as a treatment option lies with water-sensitive objects. Additionally, limitation of water movement was highlighted as a continuing concern in textile applications. The

[60] DeSantis, "Some Observations on the Use of Enzymes in Paper Conservation," 20.
[61] Bott, "Amylase for Starch Removal from a Set of 17th Century Embroidered Panels," 27; Shibayama and Eastop, "Removal of Flour Paste Residues from a Painted Banner with Alpha-Amylase," 55.
[62] Shibayama and Eastop, "Removal of Flour Paste Residues from a Painted Banner with Alpha-Amylase," 55.
[63] Matthew Hatton, "Enzymes in a Viscous Medium," *The Paper Conservator* 2, no. 1 (1977): 9; Shelley Fletcher and Walsh Judith, "The Treatment of Three Prints by Whistler on Fine Japanese Tissue," *Journal of the American Institute for Conservation* 18, no. 2 (1979): 118–26.
[64] Hatton, "Enzymes in a Viscous Medium," 9.
[65] Chapman, "Amylase in a Viscous Medium: Textile Applications," 9.
[66] Ingrid Schwarz et al., "The Development of a Ready-For-Use Poultice for Local Removal of Starch Paste by Enzymatic Action," *Restaurator* 20, no. 3–4 (1999): 225–44, doi:10.1515/rest.1999.20.3-4.225.

application of interleaving barriers and CDD to limit lateral moisture spread was suggested for further testing.[67]

The review of literature on the application of enzymes in conservation highlights the strength in the specificity of enzymes which allows conservators to apply targeted treatments. However, application of enzyme treatments to both paper and textile substrates with water-sensitive components needs further refining to control the movement of moisture.

3.4 Agarose-Enzyme Applications

The core body of research on agarose-enzyme gels in paper conservation has been published by Yana van Dyke.[68] Her publications provide a comprehensive discussion on enzyme selection, treatment temperatures, enzyme effects on paper substrate, enzyme residue and post-treatment rinsing. These key points are reflective of the main concerns in both paper and textile disciplines. The same research is available in three different publications, spanning 2003-2017, making it widely accessible and commonly cited. Two of van Dyke's publications mainly focus on the selection and efficacy of different proteinases, which were evaluated through both quantitative and qualitative methods.[69] The application of agarose-enzyme treatments is discussed through the treatment of an Indian miniature painting.

The author's most recent publication was presented at the *Gels in Conservation of Art* conference. This publication focuses specifically on the preparation of agarose gel. A thorough summary of the preparation, optimal agarose concentration and strengths of agarose was given. The author suggested the application of 400 units/mL of enzyme in an agarose gel with a concentration range of 1-1.2%, however, no treatment duration is given. The author also noted that agarose gels at 2% were comparable to control gels with no enzymes.[70] Successful application on other paper substrates following the same method were cited. Similar to other paper conservation publications on agarose applications, risk of substrate swelling, and planar distortion was noted. Little was suggested to remedy this aside from planning remedial intervention such as terminating the treatment and the application of weights when drying the substrate.

[67] Rachael Pixie-Ann Collinge, "The Use of Enzymes in Textile Conservation: A Preliminary Investigation into Localised Application Techniques" (Universtiy of Southampton, 2004), 54.
[68] Van Dyke, "Enzymes in Paper Conservation"; Van Dyke, "Practical Applications of Protease Enzymes in Paper Conservation"; Van Dyke, "Agarose-Enzyme Gels in Paper Conservation."
[69] Van Dyke, "Enzymes in Paper Conservation," 160–66.
[70] Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," 103.

Within the textile discipline, there has only been one publication that mentions the application of agarose-enzyme gels on a textile substrate. Ellis discussed it as a treatment option that she did not pursue in an object-based publication. [71] Agarose-alpha-amylase gel was tested at 1% concentration for the removal of wheat starch residues on the reverse of a cotton palampore with gilding and water-sensitive components. The author did not provide a citation for the parameters of agarose gel, however, personal communication with paper conservators was referenced. Evaluation of the gel was brief and noted that whilst the treatment was effective, the wetting out caused by the 1% concentration gels was considered unacceptable. The conservator felt that 1.5% gels might be more successful, but due to time and cost constraints the agarose-enzyme gel was not tested further.

The available literature suggests that there is some potential in the application of agarose-enzyme gels. However, due to the lack of published case studies, it is difficult to determine application parameters and to predict the treatment outcomes. Highlighted in the literature is the potential of water damage; both authors discussed above noted this as a risk to the success of the treatment. Additional considerations mentioned in previous agarose and enzyme publications, such as enzyme and gel residues, enzyme transport rate, enzyme denaturation and adjustment of treatment temperature, have not been thoroughly considered for this combined application method.

3.5 Conclusion

The conservation literature shows that both paper and textile disciplines have engaged with the application of agarose and enzymes in the continued quest to perfect localised cleaning. For the textile discipline, publications on agarose applications are limited, preliminary in nature and rely heavily on cross disciplinary publications. This is also seen in publications on enzymes, but to a lesser extent, largely due to its incorporation into the textile discipline in the 80s. However, it is important to note that, less investigation into enzyme application has taken place within the textile discipline than paper.

Research and case studies evaluating the localised treatments of both agarose and enzymes for textile substrates continue to report on concerns about the excess movement of water. This reflects the direct application of parameters from the paper discipline and emphasises the need for the identification of application parameters specific to textile substrates. The investigations undertaken by Shaeffer and Gardiner and Schmitt stress the differences in interaction with

[71] Ellis, "A Passage in the Life of a Palampore: Conservation."

agarose gels both between textile and paper substrates and across different fibre types. Future experiments providing further details of the interaction between agarose and different textile substrates will add significantly to the literature, as will the continued publication of object specific case studies. Research on the physical and chemical effects of agarose residue on textiles is also needed. This present study intends to add to this body of knowledge by identifying the optimal parameters such as concentration and treatment duration for the application of an agarose-alpha amylase gel on a cotton substrate.

Part 2: Experimental Phase and Results

This section provides an overview of the aims of the experimental phase of this research and describes the chosen variables, justifying why each was selected. It draws on the parameters set by other agarose applications within paper and textile disciplines.

This experiment intends to investigate the efficacy of agarose-alpha amylase gels on cotton samples with areas of wheat starch adhesive. The following questions are addressed:

- Do agarose-α amylase gels effectively remove thick accreted wheat starch from cotton substrates?
- What concentration allows for optimal starch removal and least movement of water into the substrate?
- What duration of gel application is optimal?
- Is there a difference in efficacy when the gels are applied on thermally aged samples versus unaged samples?

Chapter 4: Materials

4.1 Cotton

4.1.1 Fabric Choice

Previous investigations into the application of agarose gels on textile substrates showed that the effect varied greatly depending on fibre type.[72][73] In order to thoroughly evaluate the efficacy of different applications of the agarose-enzyme gel, only a single fibre substrate was selected. Water movement of agarose gels has been investigated on linen, cotton, wool and silk. Compared with other fibres, cotton substrates showed a moderate range of tidelines and wicking out. The suggested optimal concentration of agarose applications on cotton was 2.5%.[74] As this concentration was the closest to recommended concentrations of agarose-enzyme gel applications found in paper conservation (1-1.2%),[75] cotton was selected as the substrate.

4.1.2 Structure and Properties

The absorbent nature of cotton fibre is due to both the microstructure of cellulose fibres and the molecular structure. The cotton polymer consists of the natural polymer cellulose which is built up of many anhydro-β-D-glucose units.[76] The chemical properties of cellulose depend on the functional groups present on the anhydro-β-D-glucose molecule. The most influential component to the nature of the cellulose polymer is the hydroxyl or -OH group. Due to the high number of small side groups, cellulose fibres have many intra and intermolecular hydrogen bonds that form between the hydroxyl groups in the closely packed polymer chains.[77] This results in a highly polar and crystalline fibre.[78] The cellulose polymers are packed together forming bundles, which unite to form microfibrils which are packed further together into macrofibre. The spaces between these different levels of cotton polymers form a network of fine and coarse capillaries which allows the movement of liquids through cotton fibre, contributing to cotton's hydroscopic nature.

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[72] Schmitt, "An Examination of the Working Properties of Agarose Gels for Textile Conservation," 67–69.
[73] Schmitt and Foskett, "Gelling in Theory and Practice : An Examination of Agarose Gels in Textile Conservation," 164.
[74] Ibid.
[75] Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," 104.
[76] Ágnes Tímár-Balázsy and Dinah Eastop, *Chemical Principles of Textile Conservation* (Oxford: Butterworth-Heinemann, 1998), 20.
[77] Ibid., 20.
[78] E.P.G. Gohl and L.D. Vilensky, "The Cellulosic Fibres," in *Textile Science*, Second Edi (Melbourne: Longman Cheshire, 1983), 45.

Consideration of cotton's hydroscopic nature is important as application of localised poultices needs to balance out the strong capillary action of this substrate.

4.2 Agarose

The agarose used for this experiment was B160-100 from Fisher Scientific, a molecular biology grade low EEO gel with a gelation temperature range of 34.5°C-37.5°C.[79] It was selected for this present study as it has low gelation temperature suitable for enzyme addition[80] and has been widely used in other conservation publications, allowing for comparison of the results. This present dissertation will test three concentrations of agarose, 1.2%, 2% and 3%. The selection of these concentrations was informed by paper conservation case studies and the preliminary testing phase.[81] A control gel with the lowest agarose concentration but without any enzymes was also tested.[82]

4.3 Alpha Amylase

The enzyme used for this study was A6814 from Sigma Aldrich (E.C. Number: 3.2.1.1, Lot# SLBN2925V): α-Amylase derived from *Bacillus* sp. in powder form with $\leq$400mg units/mg protein (Lowry). The unit definition provided by Sigma Aldrich states that one unit will liberate 1.0mg of maltose from starch in 3 mins at pH 6.9 at 20°C.[83] PH ranges for this enzyme are 5.0-10.0, with the optimum range being pH 6.0-7.0. The optimum temperature range is 65-75°C.[84] The activity level of the chosen α-amylase is comparable to other α-amylase applications in textile conservation.[85]

[79] "Agarose (Low-EEO/Multi-Purpose/Molecular Biology Grade), Fisher BioReagents," *Fisher Scientific*, accessed June 14, 2018, https://www.fishersci.co.uk/shop/products/agarose-low-eeo-multi-purpose-molecular-biology-grade-fisher-bioreagents-3/p-24089#?keyword=agarose.
[80] Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," 101.
[81] Appendix 2
[82] The lowest concentration was selected as the control gel, as would be able to hold the highest amount of moisture. This was likely to cause the most amount of swelling in the wheat starch and possible softening in the adhesive layer.
[83] "α-Amylase from Bacillus Sp.," *Sigma-Aldrich*, accessed June 15, 2018, https://www.sigmaaldrich.com/catalog/product/sigma/a6814?lang=en&region=GB.
[84] Ibid.
[85] Bott, "Amylase for Starch Removal from a Set of 17th Century Embroidered Panels"; Ellis, "A Passage in the Life of a Palampore: Conservation."

Chapter 5: Preparation

5.1 Introduction

This chapter describes the preparation of the materials for the experimental phase, which took place prior to and throughout this phase. A preliminary testing phase also took place before the experimental phase which informed the testing parameters. Preliminary testing took approximately two weeks, preparation approximately a week and the experimental phase four weeks.

5.2 Sample Preparation

5.2.1 Cotton Lawn

Samples were prepared from white plain weave cotton lawn from Whaley's Bradford (CD18, Batch:034300/117, approximately 86g/m^2). The fabric was scoured twice in a domestic washing machine at 90°C for 150 mins with no detergent. It was then ironed to prevent wrinkles and to maintain a uniform flat surface. To ensure uniform tension in the samples, the selvedge was removed. Two different sample sizes were cut from the fabric: 9 x 8 cm and 8 x 8 cm. The larger sample size was to allow the samples to be stitched to the oven racks to facilitate thermal ageing.

5.2.2 Wheat Starch Application

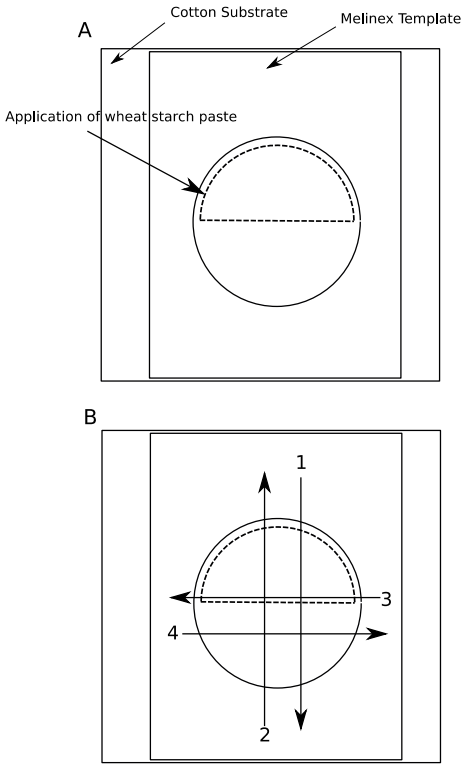
The application method was determined during the preliminary testing stage[86] with these aims:

- To apply a heavy accreted wheat starch layer of a consistent weight across all samples, to recreate conditions reported in textile conservation case studies.[87]
- To maintain a flat sample surface.

Prepared wheat starch[88] was applied to the center of each cotton sample using a Melinex®[89] template with a centred circular hole, approximately 36mm in diameter. This was designed to be ~4mm smaller than the diameter of the gels, in order to account for the wicking tendency of the

[86] Appendix 2
[87] Bott, "Amylase for Starch Removal from a Set of 17th Century Embroidered Panels," 23.
[88] Appendix 3
[89] DuPont brand of polyester films

substrate. This would then create an adhesive area close in dimension to that of the moulds used for making the agarose gels. The application method is illustrated below (Fig 5 and 6)



1) Premade wheat starch was diluted 2:1 with deionised water, to achieve a consistency that could be measured out with a syringe

2) 1ml of wheat starch was measured out with a syringe

3) The wheat starch was applied to the top half of the Melinex template into the outline of a semi-circle, to evenly distribute the paste (A)

4) The paste was then spread out using a damp brush in four motions, two in each direction of the weave to create an even layer of adhesive (B)

5) The template was then removed from the fabric substrate and the edges of the cotton samples were pinned down to reduce distortion and wrinkling of the fabric

Figure 5 Diagram of the wheat starch application process

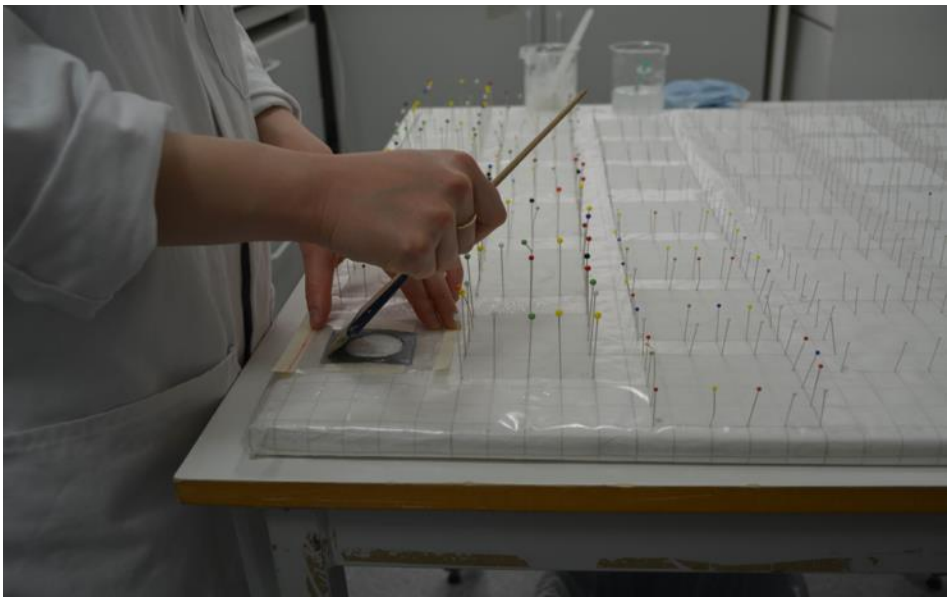


Figure 6 Brushing of the wheat starch using a *Hake* brush

The template and brush were cleaned after each application using deionised water. The template was dried completely, and excess water was removed from the brush through blotting on paper towels, to prevent excess water diluting the wheat starch. Once dry the samples were weighed and photographed. The samples that were not aged were stored in airtight bags at room temperature until the experimental phase (approximately 10 days).

5.2.3 Thermal Ageing

To evaluate the efficacy of wheat starch removal and inform applications on object treatments, it was necessary to test both fresh and aged starch. Due to the time constraints on the current investigation, it was not possible to naturally age wheat starch; instead, degradation was simulated through the application of the Arrhenius equation through thermal dark ageing, accelerating oxidation and promoting crosslinking of the adhesive.[90]

Prepared 9 x 8 cm samples were artificially aged using a Kendo Heraeus UT 6 P oven at 80°C for 7 days. These conditions were selected as they are similar to other studies investigating enzyme efficacy and the aged adhesive and consolidant properties.[91]The relative humidity could not be controlled. Two lengths of polyester thread were stitched into the top edge of each sample, to tie the samples to the metal racks in the ageing oven. The samples were arranged staggered, in order to encourage even air flow around the samples and spread across the two layers of racks. The samples were placed in the oven at ambient temperature, before the oven was turned on, to allow the samples to come up to temperature with the oven. The temperature display was checked periodically, and it appeared to be stable. Once the ageing period was completed, the oven was turned off and the samples were left in the oven with the door ajar overnight, allowing them to slowly come to room temperature (Fig 7). The aged samples were weighed again after removal from the oven.

[90] Robert L. Feller, *Accelerated Aging: Photochemical and Thermal Aspects* (Los Angeles: The Getty Conservation Institute, 1994), 143–66.

[91] Warda et al., "Analysis of Agarose , Carbopol , and Laponite Gel Poultices in Paper Conservation Author (s): Jeffrey Warda , Irene Brückle , Anikó Bezúr and Dan Kushel Source : Journal of the American Institute for Conservation , Vol . 46 , No . 3 (Fall - Winter , Pu"; Schwarz et al., "The Development of a Ready-For-Use Poultice for Local Removal of Starch Paste by Enzymatic Action"; Lisa Cumming and Jane Colbourne, "The Conservation of Mrs Marton, an Eighteenth-Century Pastel and Gouache Portrait by Daniel Gardner," *The Paper Conservator* 22 (1998): 38–47, doi:10.1080/03094227.1998.9638607.

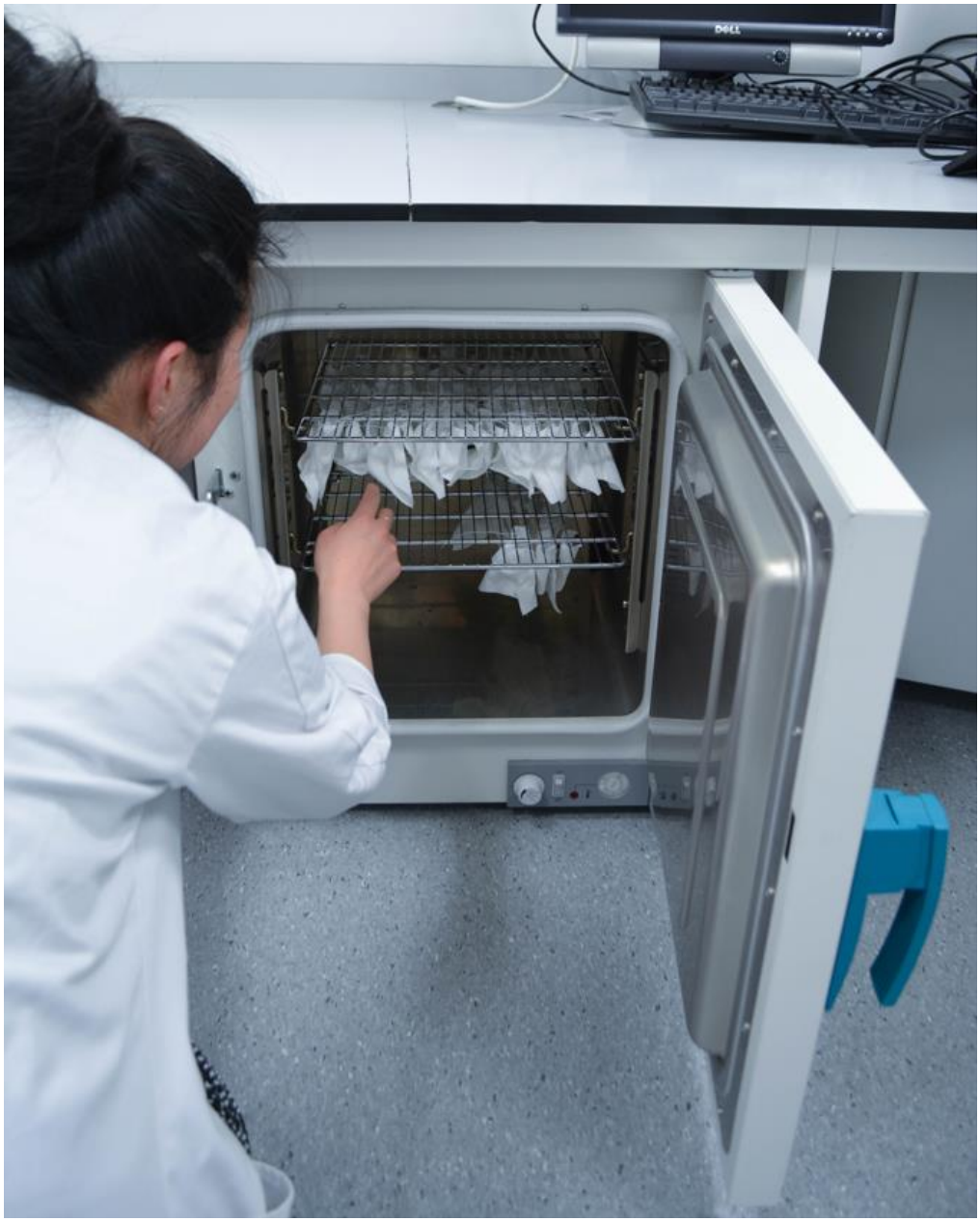


Figure 7 Removing the aged samples from the ageing oven

5.2.4 Sample Randomisation

To minimise any effect of variation in weave, uneven starching and uneven temperature in the ageing oven, all samples were randomised using Microsoft excel and split into 24 groups.

5.2.5 Appearance of Wheat Starch

The dried starch exhibited two distinct characteristics: deep weft direction cracks and smooth bubbled areas (Fig 8).

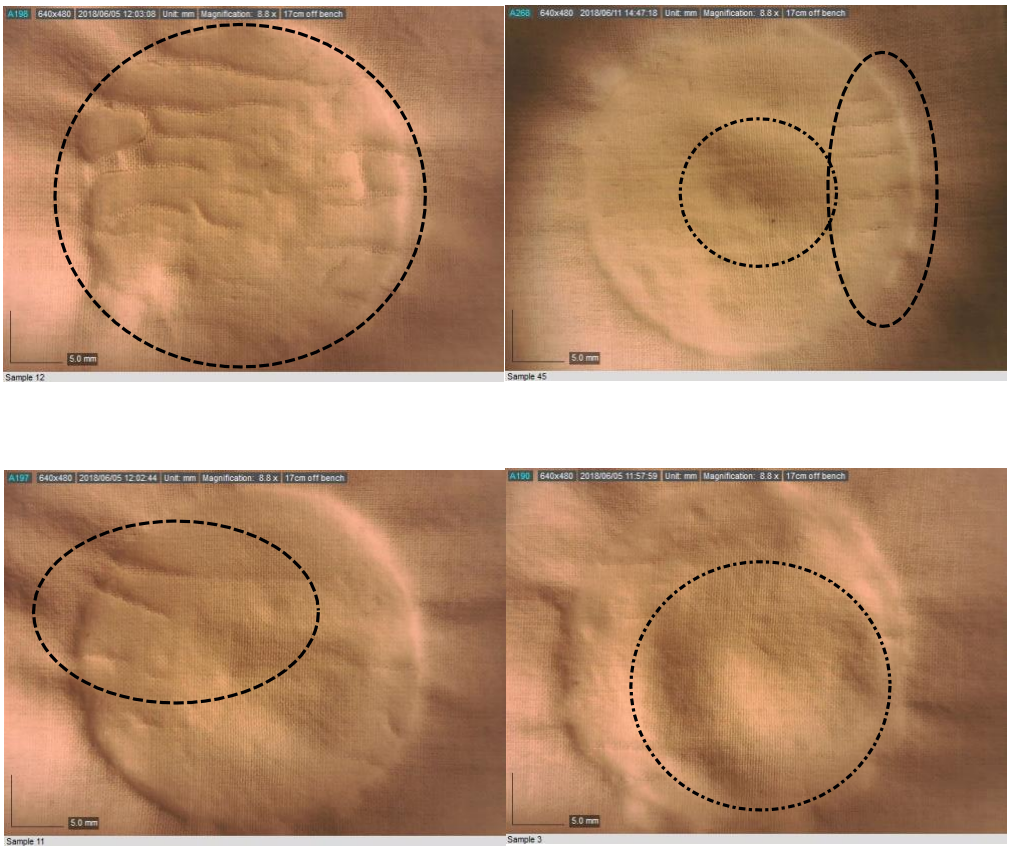


Figure 8 Different applications of wheat starch, highlighting characteristic areas

These characteristics were seen across all samples without any distinct patterns or trends and regardless of the drying location of the samples or time and date of the application (Refer to Table 1).

Table 1: Summary of the number of samples exhibiting different wheat starch characteristics

Wheat Starch Appearance	**Number of Samples**
Weft direction cracks throughout adhesive area	25

Weft direction cracks around the edges of the adhesive area, rest of adhesive smooth but not bubbled	37
Smooth and bubbled	35
Combination of weft cracks and bubbled areas	23
Total	**120**

The most common characteristic was weft direction cracking. The extent of cracking varied from small cracks located at the edge of the adhesive to cracks that extended the entire diameter and throughout the adhesive area. As all samples were starched with the same batch of wheat starch, these characteristics are suspected to be a result of a combination of human error and uneven tension in the weave structure. The presence of cracks only in the weft direction might be due to uneven tension in the weave, with more tension in the warp of the cotton fabric resulting in cracks in the perpendicular direction. This variation in wheat starch added an unexpected variable to the experiment. The appearance of the wheat starch was recorded before application of the gel.

5.3 Preparation of Gels

The agarose gels were made using a silicon macaron baking mould. To maintain a gel height of 5mm, Melinex® strips were cut, marked at a height of 5mm and shaped into rings to sit inside the indents of the mould. The strips were used to ensure consistent heights of the gels.

The gels were prepared by weighing out and dissolving agarose powder in 90% of the total volume of deionised water. The suspension was heated in a beaker on a hot plate with occasional stirring until it became a clear homogeneous solution. This was around 86°C for lower concentrations (1.2%) and around 95°C for higher concentrations (2-3%). A glass slide was placed on top of the beaker during the heating of the higher concentrations to reduce water loss.

Once the agarose had dissolved it was left to cool to 55°C with occasional stirring. The agarose was left to this temperature, as suggested by van Dyke, to allow for the addition of alpha amylase enzymes without risk of denaturing.[92] The remaining 10% of the total volume of water

[92] Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," 102.

was mixed with the α-amylase at 400 units/ml, the ratio recommended for agarose application in paper conservation.[93] The mass of enzyme was calculated for total volume of 50mL of deionised water as follows:

$$Enzyme = \frac{Concentration(volume) \times Total\ Volume}{Activity\ Solid}$$

$$= \frac{400\ units \frac{activity}{ml} \times 50\ ml}{400\ units/mg}$$

= 50mg

= 0.05g of alpha amylase

To limit the variables within this present study, the same units/ml of α-amylase was used across all gel concentrations. The enzyme mixture was then poured into the beaker containing agarose, then swirled to combine. It was noted that pouring the enzyme mixture along the side of the beaker instead of directly into the center aided the mixing process. Once combined, the mixture was poured into the silicon tray and left to set. This took approximately 30 minutes. A total volume of 50mls made approximately 10 gels.

5.4 Preparation of Iodine/Potassium Iodide Indicator Solution

Iodine/potassium iodide solution is a commonly used indicator of starch. Interaction between starch and iodine results in the formation of colour. It is sensitive to small amounts of starch, as little as 1μg, and is also able to indicate changes in degree of polymerization.[94] When starch is present, the indicator solution stains blue, indicating the presence of amylose. The other component of starch, amylopectin, can also be identified, however, it is much less intense in colour. When starch polymers are hydrolysed by α-amylase, both polysaccharides gradually lose the capacity to stain with iodine.[95] The blue amylose colour becomes purple, red, light brown and then finally disappears. The use of this indicator solution in this present experiment was intended

[93] Ibid., 103.
[94] J. M. Bailey and W. J. Whelan, "Physical Properties of Starch: I: Relationship between Iodine Stain and Chain Length," *The Journal of Biological Chemistry* 236, no. 4 (1961): 969.
[95] Ibid.

to track the changes in residual wheat starch and starch lifted by the agarose-enzyme gel. The indicator solution was made following the instructions provided in *Material Characterization Tests for Objects of Art and Archaeology.*[96] Two lots of indicator solution were made over the duration of the experimental period following the same recipe. Indicator solution was tested on starched cotton samples before it was applied to test samples.

[96] Nancy Odegaard, Scott Carroll, and Werner S. Zimmt, *Material Characterization Tests for Objects of Art and Archaeology*, 2nd ed. (London: Archetype Publications, 2000), 128–29. Appendix 4

Chapter 5: Methodology

6.1 Testing Process

Three concentrations of agarose (1.2%, 2% and 3%) and three treatment durations (60, 80 and 100 minutes) were tested during the experimental phase. The sample groups were broken into twenty four groups (Table 2) with five replicates for each sample group to increase the sample size and statistical viability.

Table 2: Summary of the sample groups

	Duration					
Concentration	**A: 60 Minutes**		**B: 80 Minutes**		**C: 100 Minutes**	
1: 1.2%	1A- Aged	1A- Unaged	1B- Aged	1B- Unaged	1C-Aged	1C- Unaged
2: 2%	2A- Aged	2A- Unaged	2B- Aged	2B- Unaged	2C- Aged	2C- Unaged
3: 3%	3A- Aged	3A- Unaged	3B- Aged	3B- Unaged	3C- Aged	3C- Unaged
4: 1.2% with no enzymes	4A- Aged	4A- Unaged	4B- Aged	4B- Unaged	4C- Aged	4C- Unaged

A single concentration and duration were tested per day on both aged and not aged samples. A fresh set of gels was made each day. The aged samples were tested in the morning and the not aged in the afternoon. The gels were kept at room temperature for the entire day.[97]

The fabric samples were placed on a Melinex® sheet and the widest points of the applied wheat starch were measured in the warp and weft direction. The nature of the dried adhesive was also described and recorded.

A single glass slide weighing 73.50g was used to weight all gels for the first 30 seconds of their application. As the applications for each sample group were staggered, the same weight could be applied to all of the gels. Schmitt had noted that good contact was achieved with low

[97] During the testing for concentrations 2 and 3 the agarose gels for the not aged samples were kept in a sealed plastic bag at room temperature. This was to prevent loss of water through evaporation, which was noticed during the testing of concentrations 1 and 4. This will be discussed further in the following chapters.

concentrations of gel, as they exhibited more flexibility and conformed to the substrate, and that weighting for the entire duration of treatment was unnecessary.[98] However, preliminary testing showed that, due to the three-dimensional nature of the wheat starch, initial weighting was required for all concentrations to encourage the gel to conform to the surface of the wheat starch.[99]

The gels were selected at random and lifted out the of the silicon tray with a flat metal spatula; this was the easiest way to move the gels without marking them. They were placed centred on the wheat starch and weighted with the glass slide. Once the slide was removed, the widest point of the water movement was measured in both warp and weft directions at 30 seconds, 1, 3, 5, 10, 15, 20 minutes and then in ten-minute intervals until the end of the treatment.[100] This measurement was taken to track the lateral wicking of the water into the substrate. The gels were then lifted off the substrate by first running the spatula around the edge of the gel, then lifting and slowly peeling the gel back.

Both the gel and the substrate were photographed and then the indicator solution was applied. The gel and substrate were photographed immediately after the application of the indicator and approximately 10 minutes after application to record any colour changes of the indicator solution. The gel and substrate were examined, and the following information recorded:

- Percentage of wheat starch removal
- The nature of any remaining wheat starch
- The colour of the indicator solution on both substrate and gel and any changes that occurred

6.2 Measurements

The degree of efficacy of the agarose-enzyme gel was evaluated by measuring the movement of water and the change in weight of starched cotton samples. This section will describe how data was collected and processed for each of these variables.

[98] Schmitt, "An Examination of the Working Properties of Agarose Gels for Textile Conservation," 94.
[99] Appendix: gel testing
[100] The frequency of these time measurements was informed by the preliminary testing phase which showed that water movement occurred rapidly in the first 10-15 minutes after gel application. Appendix

6.2.1 Water Movement

The total water movement was measured in both warp and weft directions for each sample at the same time intervals. This data was evaluated as the mean total water movement, which was calculated by:

$$Total\ Water\ Movement_{Warp} = B_{Warp} - A_{WS\ Warp}$$

$$Total\ Water\ Movement_{Weft} = B_{Weft} - A_{WS\ Weft}$$

where A_{WS} was the measurement of the widest warp and weft dimensions of the wheat starch and B was the widest warp and weft dimensions of the water movement at the end of the treatment (Fig 9).

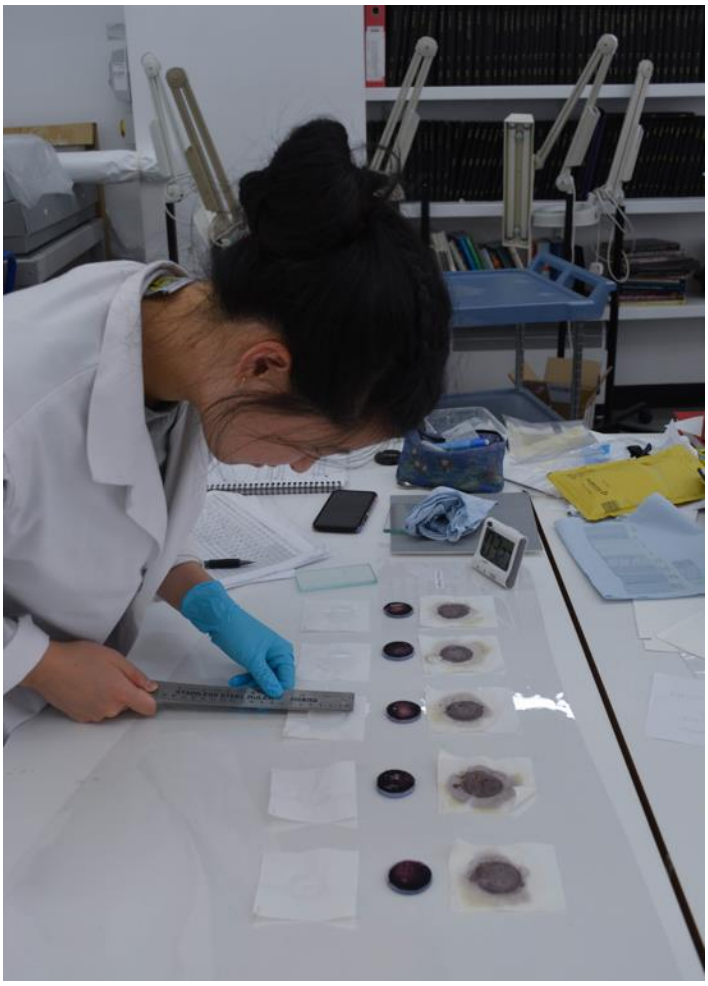


Figure 9 Measuring the water movement in the cotton samples

Water movement was also evaluated through the rate of water movement. The rate, in mm per minute, was calculated by dividing the average water movement measurement for each sample group, taken 20 minutes after application by 20.

6.2.2 Weight

The weight of each sample was measured throughout the sample preparation and testing process on the same balance and recorded to three decimal points. Due to cotton's high affinity for water sorption, the weight of the samples was likely to vary depending on the RH.[101] To reduce this effect, all samples were weighed when they were completely dry, and the before-treatment weighing was completed in two groups: aged and not aged.

Weight change of the samples was measured by mean absolute weight change; The mean was used to account for variations in sample weight, wheat starch application and weave of the substrate. The use of absolute values, which represent the magnitude of the number without regard for the sign, aided the presentation of the control groups' weight change.

This was calculated by:

$$|\Delta\, Weight\ (A - B)|$$

Where A is the weight of the sample before treatment and B is the weight after treatment.

6.2.3 Statistical Analysis

The total water movement and weight change were compared through analysis of variance (ANOVA) using the statistical software R, version 3.1.2. The total water movement data was analysed with Multivariate Analysis of Variance (MANOVA) and the weight change data with Three Factor ANOVA. These tests were selected as they best suited the nature of the variables. Both of these methods test whether the independent variables, in this study the concentration of agarose gel, the duration of the treatment and the age of sample, can explain the variance in the dependent variables, which was the total water movement and weight change of samples.

[101] Callum A. S. Hill, Andrew Norton, and Gary Newman, "The Water Vapor Sorption Behaviour of Natural Fibres," *Journal of Applied Polymer Science* 112 (2009): 1524–37.

The ANOVAs generate P-values, which indicate the probability of obtaining these results by chance; the smaller the P-value, the less likely it is that the variation seen in the data is just random error, and the more likely it is that the independent variables have contributed significantly to that variation. P-values <0.05, 0.01 and 0.001 are considered significant, very significant and highly significant respectively.[102]

[102] For another explanation on P-values see: Margaret J. Smith, Thomas Hugh Flowers, and Frances J. Lennard, "Mechanical Properties of Wool and Cotton Yarns Used in Twenty-First Century Tapestry: Preparing for the Future by Understanding the Present," *Studies in Conservation* 60, no. 6 (2015): 378, doi:10.1179/2047058414Y.0000000144.

Chapter 7: Results and Discussion

7.1 Introduction

This chapter summarises and discusses the results of the experimental phase. These experiments evaluated the efficacy of the agarose-enzyme gels at removing accreted wheat starch paste. Three concentrations of agarose gels with enzymes and one control gel were tested on aged and unaged cotton substrates for three application durations (Table 3).[103]

Table 3: Summary of the gel application parameters. The shaded groups are the control agarose gels (#4), which did not contain any enzymes.

Sample Group Name		Agarose Concentration (%)	Application Duration (mins)
Aged (A)	Unaged (N)		
1A-A	1A-N	1.2	60
1B-A	1B-N	1.2	80
1C-A	1C-N	1.2	100
2A-A	2A-N	2	60
2B-A	2B-N	2	80
2C-A	2C-N	2	100
3A-A	3A-N	3	60
3B-A	3B-N	3	80
3C-A	3C-N	3	100
4A-A	4A-N	1.2	60
4B-A	4B-N	1.2	80
4C-A	4C-N	1.2	100

The results from the experimental phase are presented in text, tables, and graphs. Firstly, the general trends seen in the two outcome variables, water movement and weight change, will be summarised. These outcomes will then be further discussed in three sections divided by the application parameters: the effect of concentration, of duration and of ageing. Qualitative

[103] Representative images and samples can be found in Appendices 7 and 11 respectively

evaluations of the treatment will also be discussed, including gel clearance, gel properties, residual adhesive and tactile evaluation. Additional exploratory analysis such as starch indicator solution and UV photography will be evaluated in the final section.

7.2 General Trends in Total Water Movement

Water movement was evaluated to determine how effectively agarose gels of different concentrations act as a localised treatment on a cotton substrate. The water movement of each sample group was evaluated in two directions, warp and weft (Section 6.2.1).

The majority of sample groups, 22 out of 24 groups, showed more total water movement in the warp direction than the weft. This pattern was not seen in cotton samples from Schmitt's investigation.[104] The difference between the two directions of the weave observed here is suspected to be related to the weft direction cracks seen in the dried applied wheat starch (Section 5.2.5). It is suspected that differential tension in the weave created stronger capillary action in the warp direction, contributing to greater water movement in this direction.

Though this trend was seen in the average total water movement, there was variation across the replicates. Additionally, the movement of the water was irregular in both the warp and weft directions; as the averaged water movement was measured from the widest points, the variation in each direction is not reflected. The variation seen may be due to the varied nature of the applied wheat starch, as discussed in the section 5.2.5. Areas of thicker and raised starch exhibited less water movement and areas of thinner starch exhibited more.

7.3 General Trends in the Rate of Water Movement

Similar to the total water movement values, the warp and weft measurements that were taken throughout the duration of the treatment were averaged across all replicates (n=5). All samples showed similar general patterns of water movement, regardless of concentration, duration or age. Accelerated water movement was observed in the first 20 minutes of the gel application, followed by deceleration and stabilisation. The initial acceleration of water movement is characteristic of water absorption by a dry textile, the rate of water movement slows down as moisture in the textile reaches equilibrium.[105]

[104] Schmitt, "An Examination of the Working Properties of Agarose Gels for Textile Conservation," 41–52.
[105] Tímár-Balázsy and Eastop, *Chemical Principles of Textile Conservation*, 15.

Some samples showed a decrease of the water movement measurement, as areas that had been wetted out started drying before the end of the application (Sample group 4C in Fig 10). This will be discussed in the application duration section.

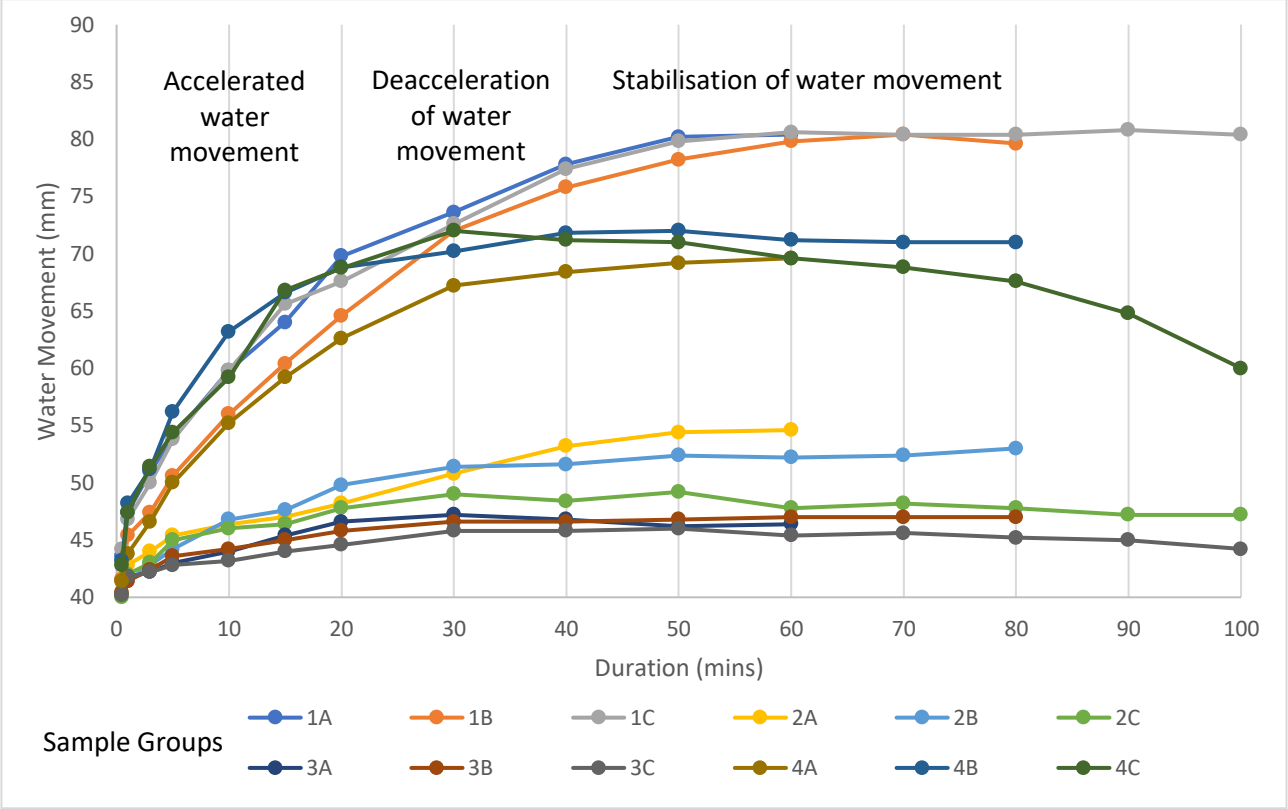


Figure 10 Average water movement in the warp direction of aged sample groups, showing the overall pattern of water movement

7.4 General Trends in Weight Change

The weight change of samples was used to measure the loss of wheat starch to quantify the efficacy of the enzyme action. All samples groups 1-3, regardless of duration and age, exhibited weight loss after the application of the agarose-enzyme gels. The samples in group 4 showed weight gain after the application of control gels likely due to the absorption of water from the gel by the starch polymers. The weight loss seen in samples from group 1-3 and the weight gain in the control group indicated that agarose-enzyme gels have an effect on reducing accreted wheat starch on a cotton substrate.

7.5 Effect of Concentration

Three concentrations of agarose-enzyme gels were evaluated to determine the most effective localised enzyme treatment for the removal of accreted wheat starch.

7.5.1 Total Water Movement

The degree and rate of water movement were clearly associated with the concentration of the agarose gels. The greatest total water movement was seen in sample group 1, treated with 1.2% agarose-α amylase gels, and the least movement was seen in group 3, treated with 3% gels (Table 4).

Table 4: Summary of the total water movement seen across all the samples

Water Movement	Sample Groups
Most water movement	1 (1.2%)
More water movement	4 (1.2% Control)
Less water movement	2 (2%)
Least water movement	3 (3%)

The effect of concentration is further substantiated by statistical analysis. MANOVA analysis indicates a strongly significant difference in total water movement between the different concentrations (P-value 2.2e-16). This strongly suggests that total water movement is affected by concentration.

7.5.2 Rate of Water Movement

The concentration of the agarose-alpha amylase gels also affected the rate of water movement. Samples from groups 1 and 4 (control group) showed the most rapid rate of water movement (Fig 11 and 12).[106]

[106] Due to their similarities, this section only contains two graphs of the water movement for 100 minute applications.

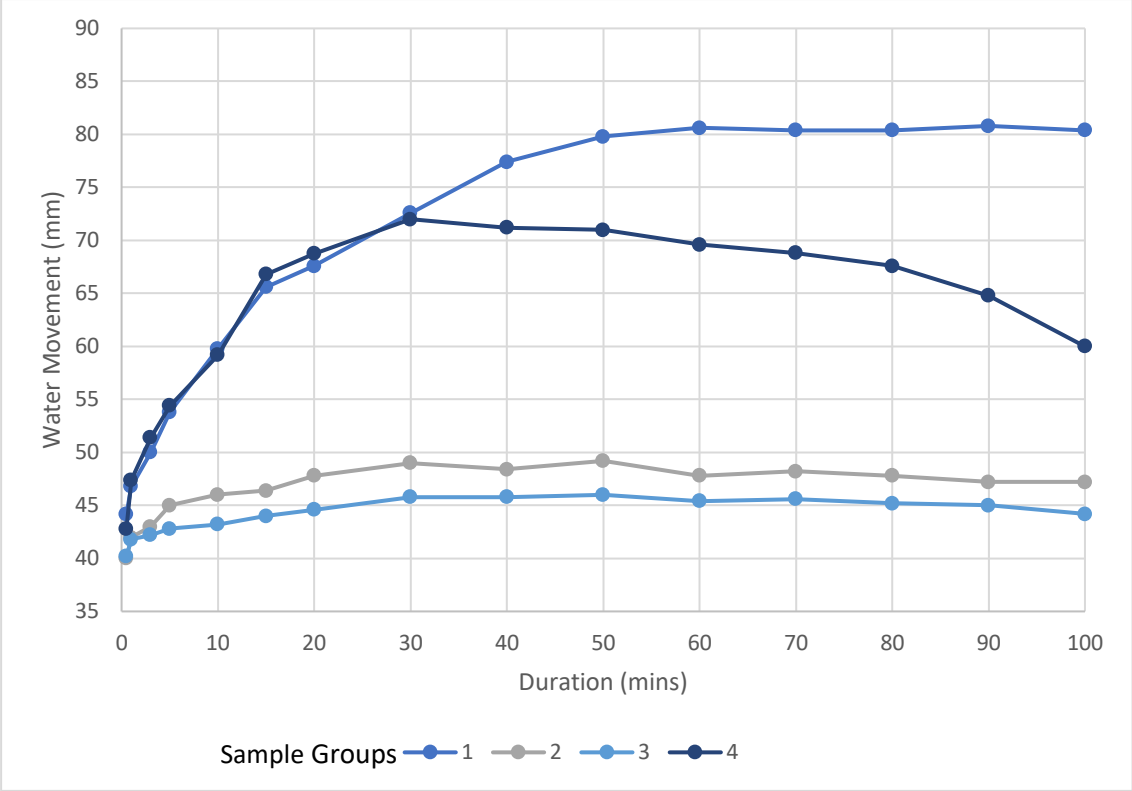


Figure 11 Average water movement in the warp direction of aged samples over 100 minutes (Duration C)

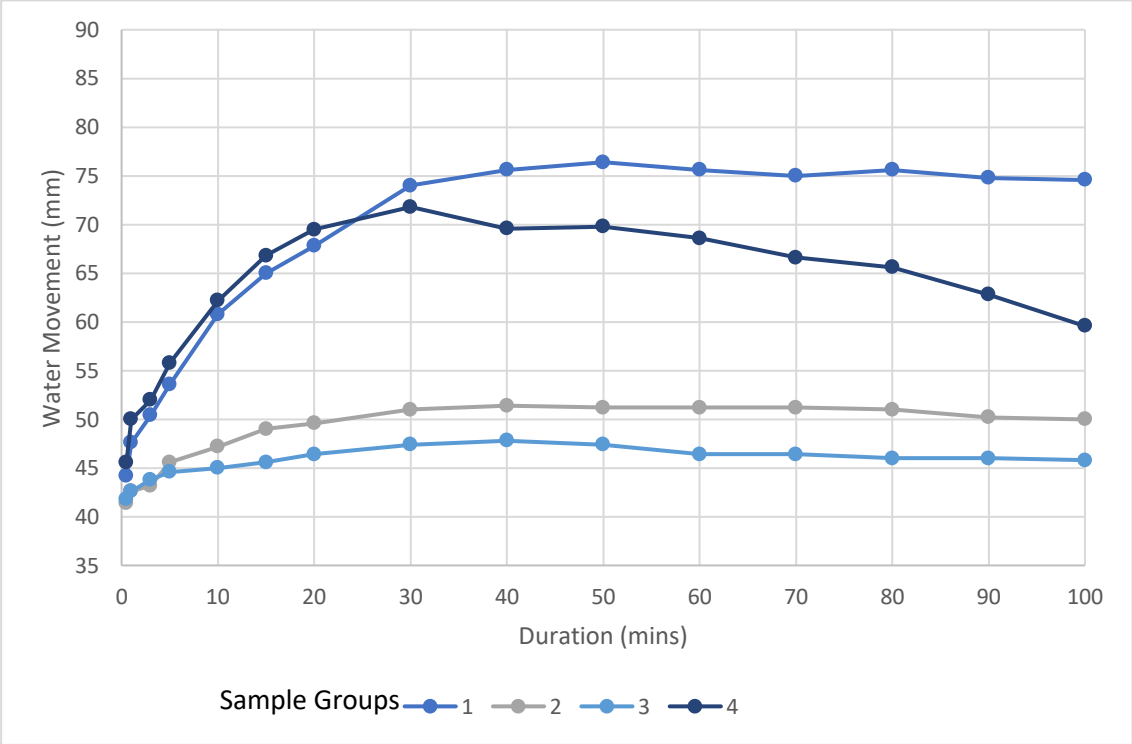


Figure 12 Average water movement in the weft direction of aged samples over 100 minutes (Duration C)

For most samples, the rate of water movement is similar for groups 1 and 4 in the first 20 minutes. After this period, water movement continued to increase in group 1, but appeared to slow and plateau in group 4 (Fig 11 and 12).

Table 5: Summary of the average rate of water movement (mm/min) seen in the first 20 minutes of gel application

		Rate of water movement (mm/min)			
Sample Groups		**1**	**2**	**3**	**4**
Aged Samples	**Warp direction**	3.37	2.43	2.28	3.34
	Weft direction	3.47	2.45	2.36	3.31
Unaged Samples	**Warp direction**	3.21	2.48	2.34	3.13
	Weft direction	3.21	2.51	2.38	3.08

.

Samples from groups 2 and 3 wetted out more slowly but in a similar pattern (Table 5). Both concentrations showed a faster rate of water movement in the first 20 minutes, with group 2 showing greater water movement in all samples than group 3 (Fig 11 and 12). Both groups showed very little change in water movement for the rest of the treatment.

The difference seen in the total and rate of water movement across the four groups directly corresponded to the concentration of the agarose gels. For gels of lower concentration, groups 1 and 4, a more open gel matrix is formed with larger pores, resulting in greater total water movement along with rapid initial water movement. The open matrix allows for water to diffuse easily from gel to substrate, where capillary action of the cotton fibres is stronger than that of the gel matrix.

For gels of high concentrations, groups 2 and 3, a denser matrix with smaller pores is formed, which allows water to diffuse more slowly from the gel. The capillary action of the cotton fibres is still stronger than that of the gel but at these concentrations, the difference between these surfaces is smaller and the movement of the water slower.

7.5.3 Weight Change

The concentration of agarose-alpha amylase gels also had a clear effect on the weight change of the samples. As discussed in section 7.4, samples in groups 1-3 showed weight loss, indicating the loss of wheat starch, after the application of the gel. Samples in group 4 showed weight gain, indicating the increase of weight in the wheat starch. The data were converted to absolute value (Fig 13) to aid interpretation.

Within groups 1-3, group 1 showed the least wheat starch lost after the treatment (Fig 13). Groups 2 and 3 showed a wide range of weight change, possibly due to the variation in the wheat starch application. The greatest weight change was seen in sample groups 2B-N and 3A-A (Fig 13). However, on average, samples in group 3 showed the greatest weight change. Samples in group 4 showed a wide range of values, again likely due to the variation in starch application.

Group 1 was the only group that showed a consistent pattern across aged and unaged samples, where increases in duration decreased the change in weight (Fig 13). This suggests that longer applications of gels at 1.2% are less effective as an enzyme treatment as they removed less wheat starch. This observation will be discussed further in the following section on the effect of duration.

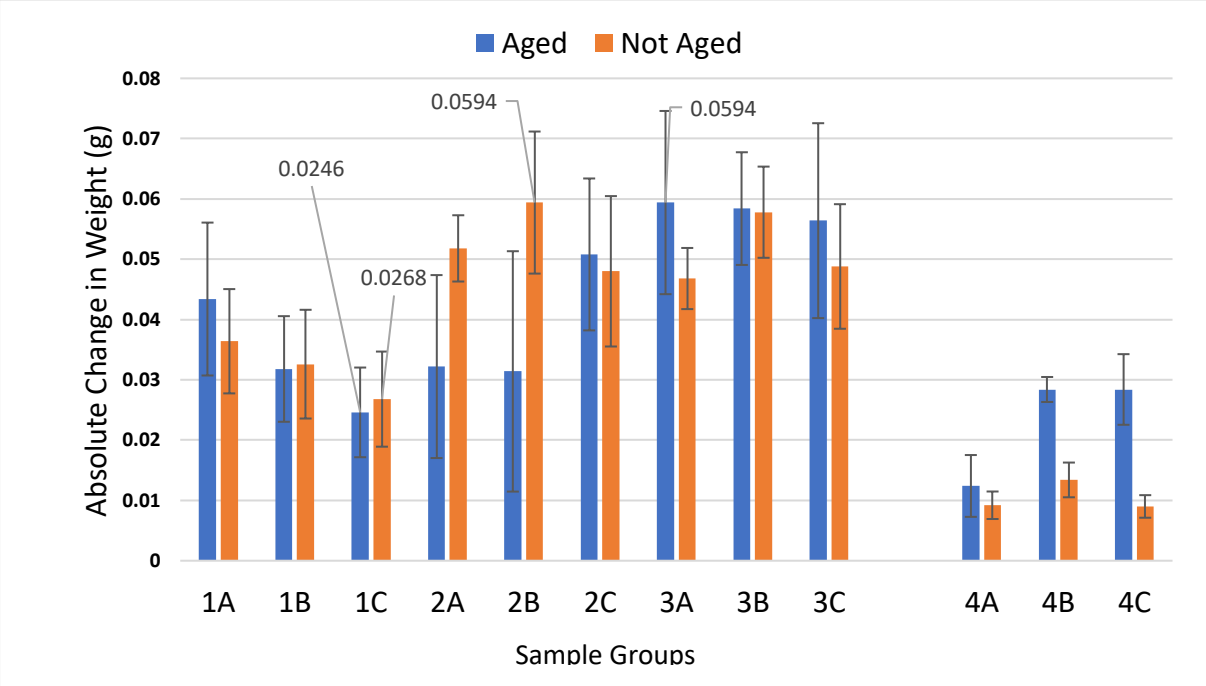


Figure 13 Absolute weight change of all sample groups, the least and the greatest weight change in groups 1-3 are highlighted

Statistical analysis indicated that different concentrations of agarose-alpha amylase gel affected weight change of the samples. Three Factor ANOVA showed that there was a strongly significant difference in weight change between the different concentrations (P-value <2e-16).[107]

The weight change data showed that the agarose-alpha amylase gel reduced wheat starch in all of the applied concentrations, 1.2%, 2% and 3%. Applications of gel at 2% and 3% were more successful at removing starch than at 1.2%, with 3% being the most successful.

7.5.4 Conclusion

The concentration of agarose is a significant parameter for the application of agarose-alpha amylase gels on a cotton substrate. Statistically significant differences can be seen between the different concentrations for both outcome variables. Application of agarose-alpha amylase gels at 3% showed the least and slowest water movement and the most effective enzyme treatment.

[107] Appendix 5

7.6 Effect of Duration

The agarose-alpha amylase gels were applied at three different durations: 60 minutes (duration A), 80 minutes (B) and 100 minutes (C).

7.6.1 Total Water Movement

The application duration had some effect on the total water movement. For the aged sample groups, the water movement was similar between duration A and B (Fig 14 and 15) For the unaged samples of group 1 and 4, duration B showed the most total water movement (Fig 15). For the unaged samples of groups 2 and 3, little difference in total water movement was observed across all durations (Fig 15). Statistical analysis of the data indicates that there is a significant relationship between water movement and duration (P-value 5.7e-05).

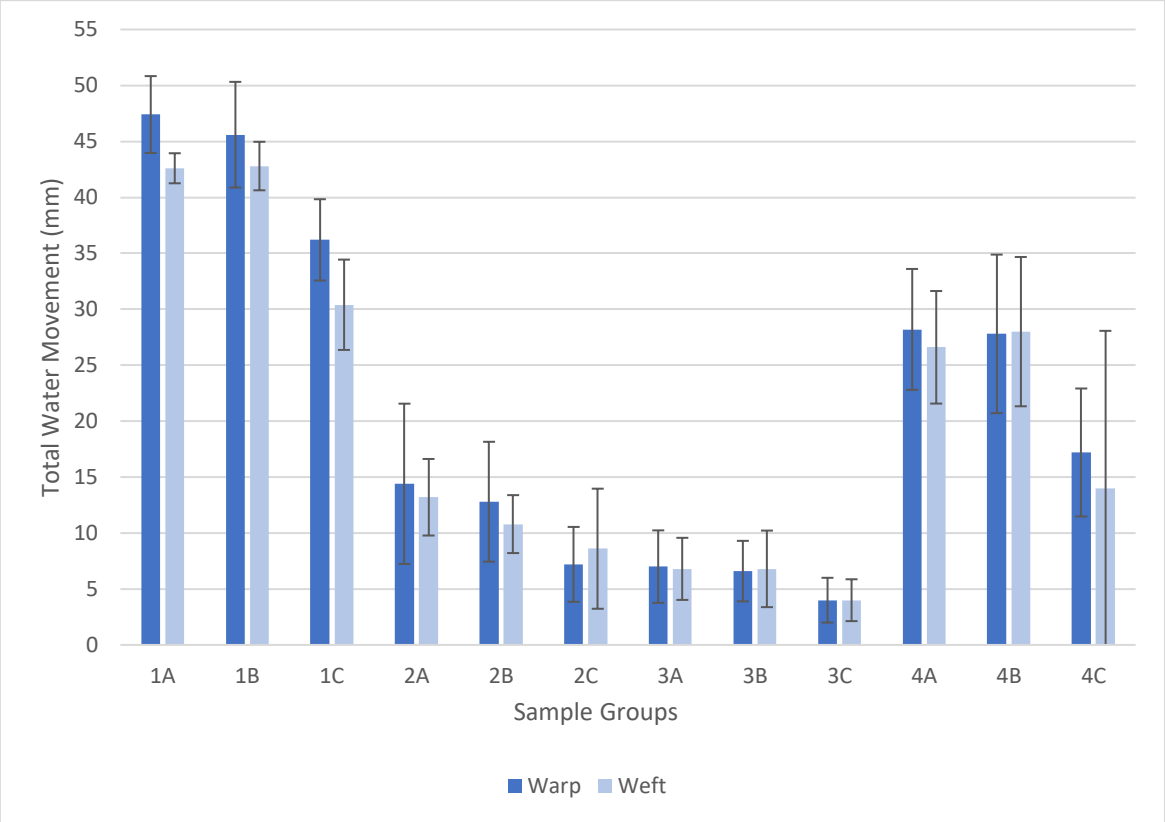


Figure 14 Average total water movement of aged sample. Durations: A= 60 minutes, B= 80 minutes and C= 100 minutes

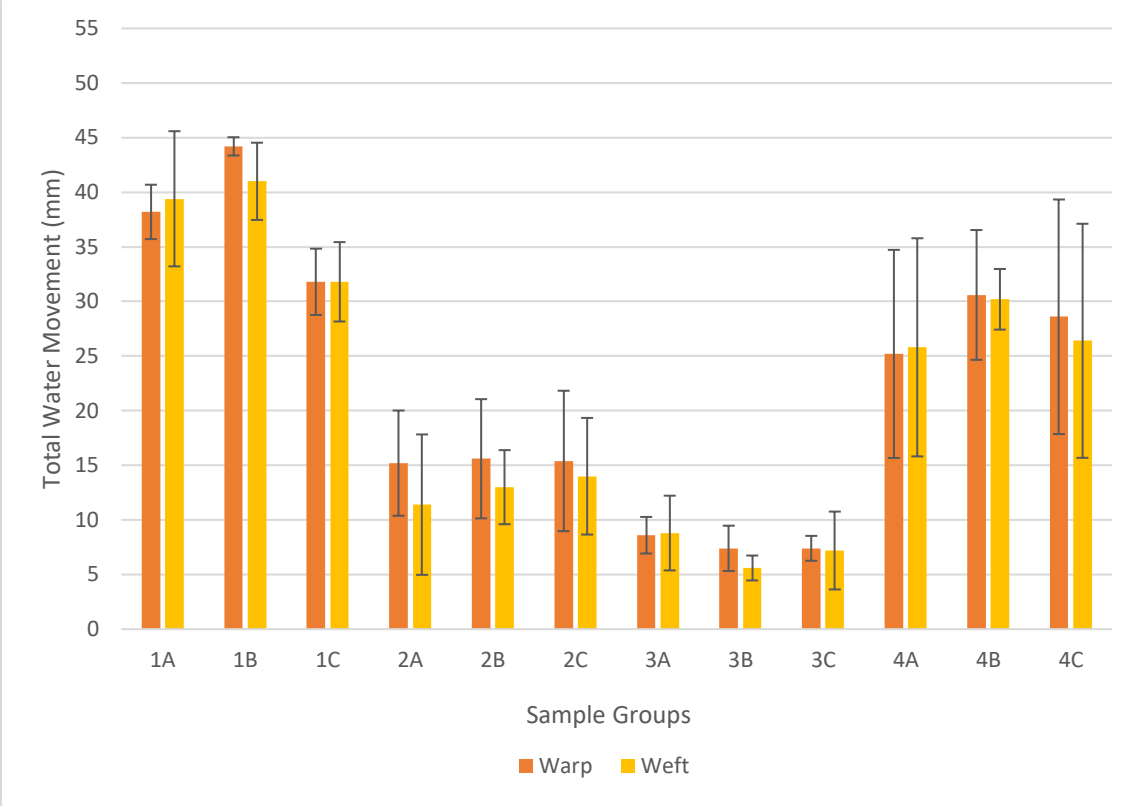


Figure 15 Average total water movement of unaged samples. Durations: A= 60 minutes, B= 80 minutes and C= 100 minutes

As discussed in a section 7.2, in many of the sample groups the total water movement for samples treated for duration C is much lower than the same sample group treated for durations A and B. Importantly, this does not reflect the maximum range of water movement of these sample groups, but that an application duration of 100 mins in the environmental conditions[108] in this experimental phase resulted in the edges of the cotton samples drying before the termination of the treatment.

7.6.2 Rate of Water Movement

In general, different application durations had minimal effect on the rate or pattern of water movement (Figs 16-19)[109].

[108] Appendix 6
[109] As mentioned above, due to the similarities across the sample, only one set of graphs have been shown.

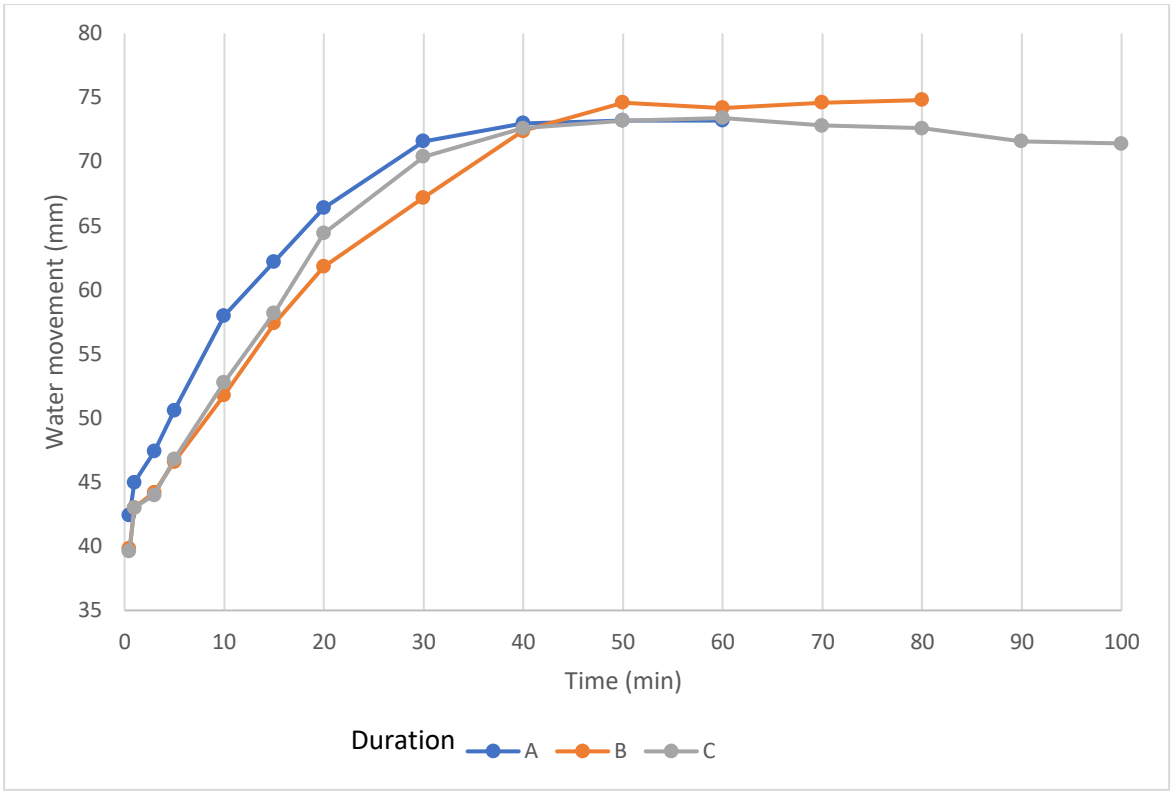


Figure 16 Average water movement in the weft direction of unaged samples in group 1, concentration 1.2%

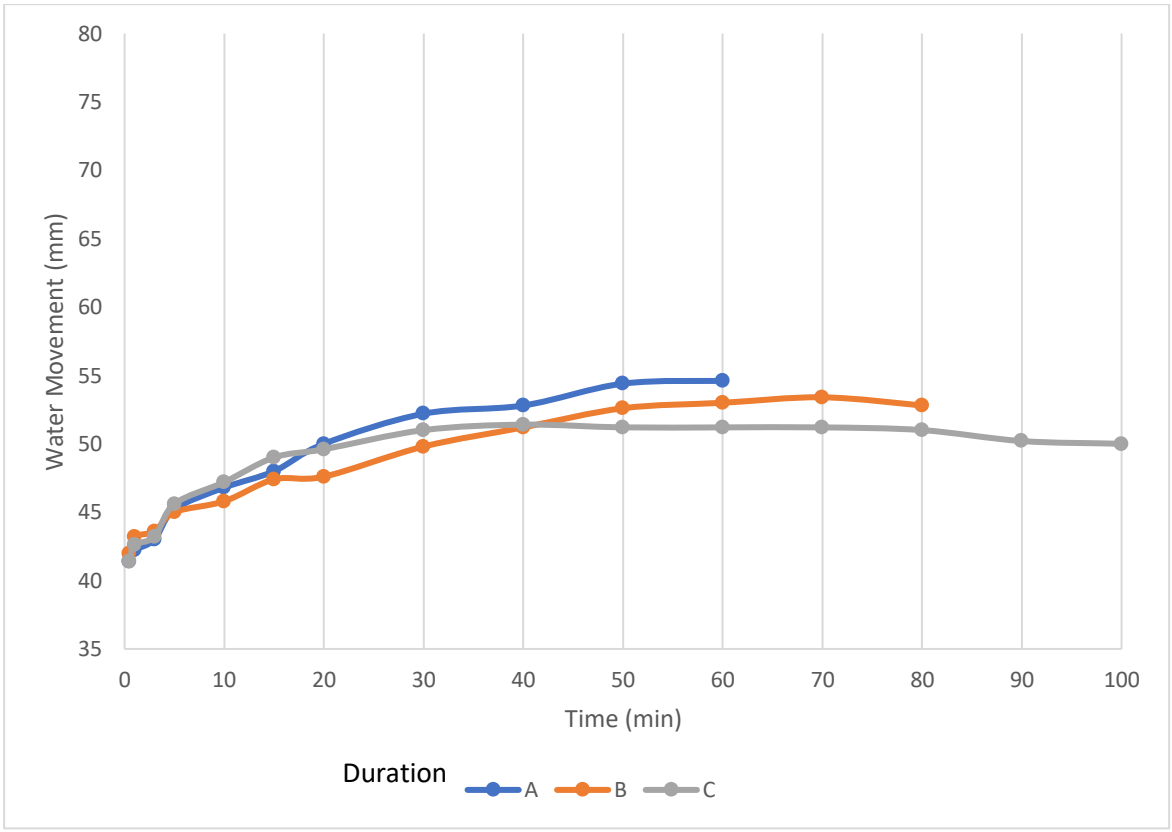


Figure 17 Average water movement in the weft direction of unaged samples in group 2, concentration 2%

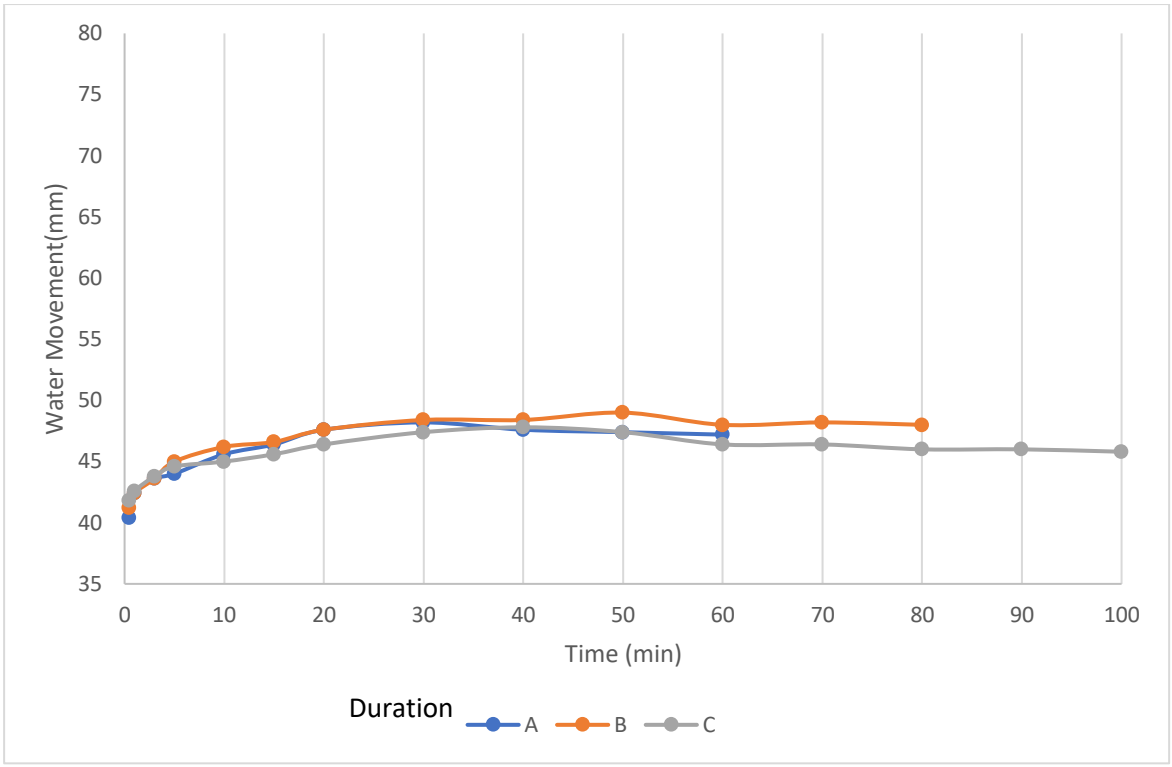


Figure 18 Average water movement in the weft direction of unaged samples in group 3, concentration 3%

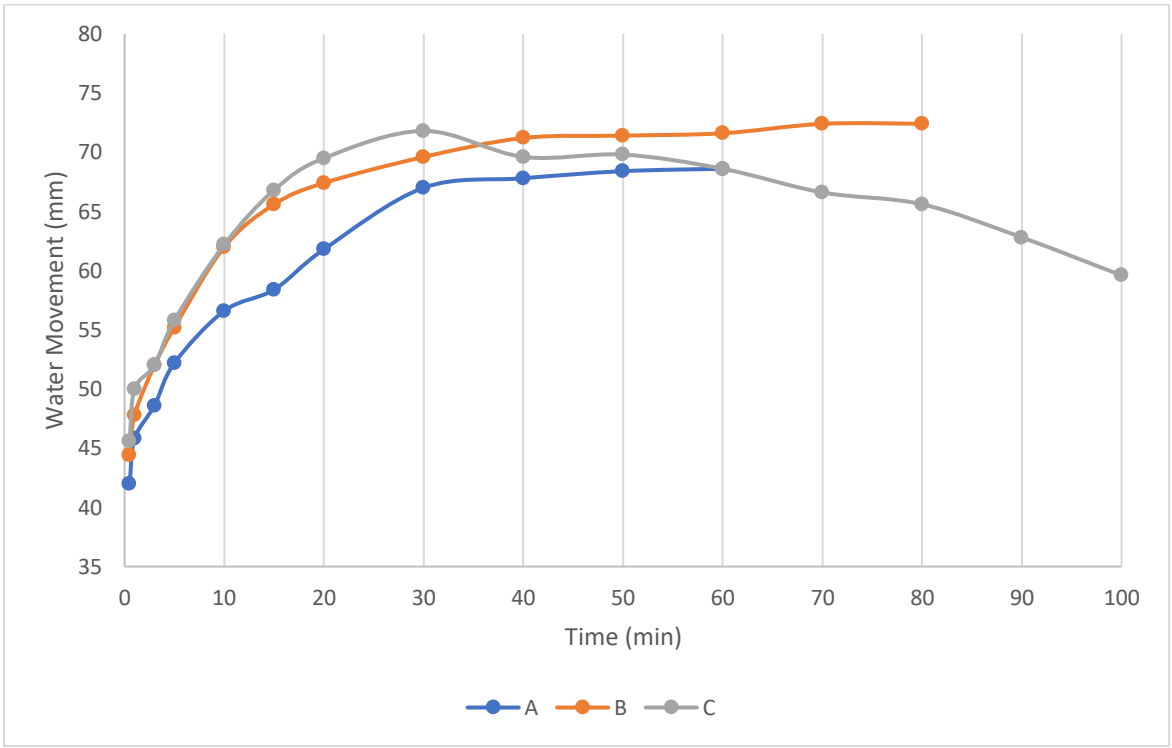


Figure 19 Average water movement in the weft direction of unaged samples in group 4, control gels, concentration 1.2%

This dataset highlights the difference between the maximum water movement of the samples and the total water movement. As discussed previously, some samples treated for 100 minutes showed a decrease of water movement towards the end of the treatment period. This was seen most clearly in groups 1 and 4 (Fig 16 and 19). This may be due to the faster rate of water movement seen in these two sample groups, resulting from the open gel matrix which allowed the water to move into the substrate to a greater extent in a shorter amount of time. This rapid water movement is suspected to cause the gel matrix to collapse, which reduces its function as a poultice. The gel would not be able to exert any upward pull to keep the water in the substrate.

7.6.3 Weight Change

Duration had varied effects on the weight change of the samples. As discussed in 7.5, only samples from group 1 showed a distinct pattern (Fig 20). The weight change of the samples decreased with the increased duration of gel application. This trend is seen consistently across all samples in group 1 regardless of age.

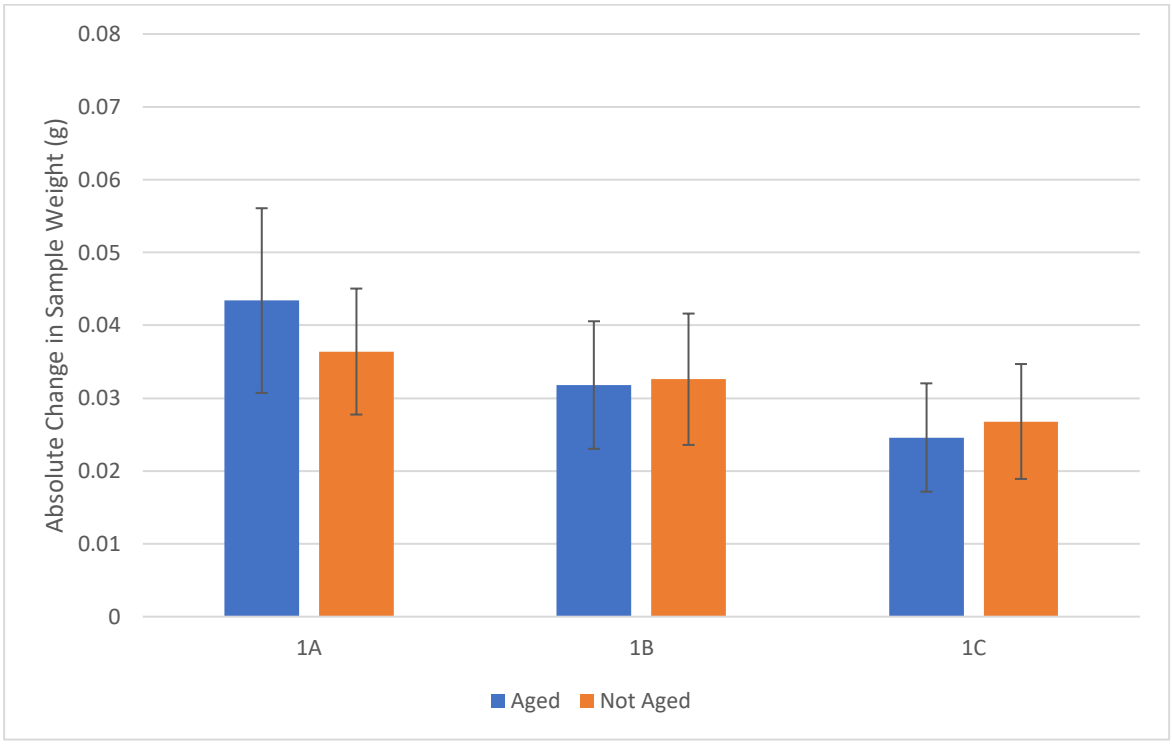


Figure 20 Absolute weight change of samples from group 1

The occurrence of weight loss shows that the agarose-alpha amylase gel is effective when applied at 1.2%, the suggested concentration from paper conservation sources.[110] The decrease of efficacy with increased duration could be due to the low concentration of the gel. As discussed above, the capillary action of the cotton substrate results in rapid loss of the water from the gel. This causes the matrix to collapse, resulting in the loss of the poultice function, which reduces the amount of wheat starch that can be removed.

Application duration did not have a clear impact on weight change across all the samples from group 2, 3 and 4. Statistical analysis of the data did not show a significant difference between the different weight change samples treated for different durations (P-value 0.245).

7.6.4 Conclusion

The duration of gel application did not appear to be a significant parameter. A statistically significant outcome was only seen for the total water movement, which appeared lower in the longest application duration. Duration did not appear to have a clear effect on the efficacy of the gel, which was consistent across groups 1-3.

[110] Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," 106.

7.7 Effect of Ageing

The purpose of ageing half of the samples was to investigate the difference in efficacy of applying agarose-alpha amylase gels to aged cotton and wheat starch. However, the significance of this parameter was affected by error in the methodology, namely the inappropriate storage of the gels prior to application, see section 5.3. As such only the effect on samples in group 2 and 3 are reliable. This section will summarise the effect of ageing seen in these two groups.

7.7.1 Total Water Movement

The total water movement of samples was not significantly different across samples of different age (P-value 0.254). However, for samples in group 2 and 3, there does appear to be a consistent pattern between aged samples and unaged samples. In the aged samples in group 2 and 3, the total water movement is similar between duration A and B and smallest for samples treated for duration C. For the unaged samples in these groups, the total movement appeared to be similar across all durations.

Additionally, for all durations in both sample groups, the total water movement was greater in unaged samples than aged samples (Fig 21). However, it is important to note the wide range of results, as indicated by the standard error bars.

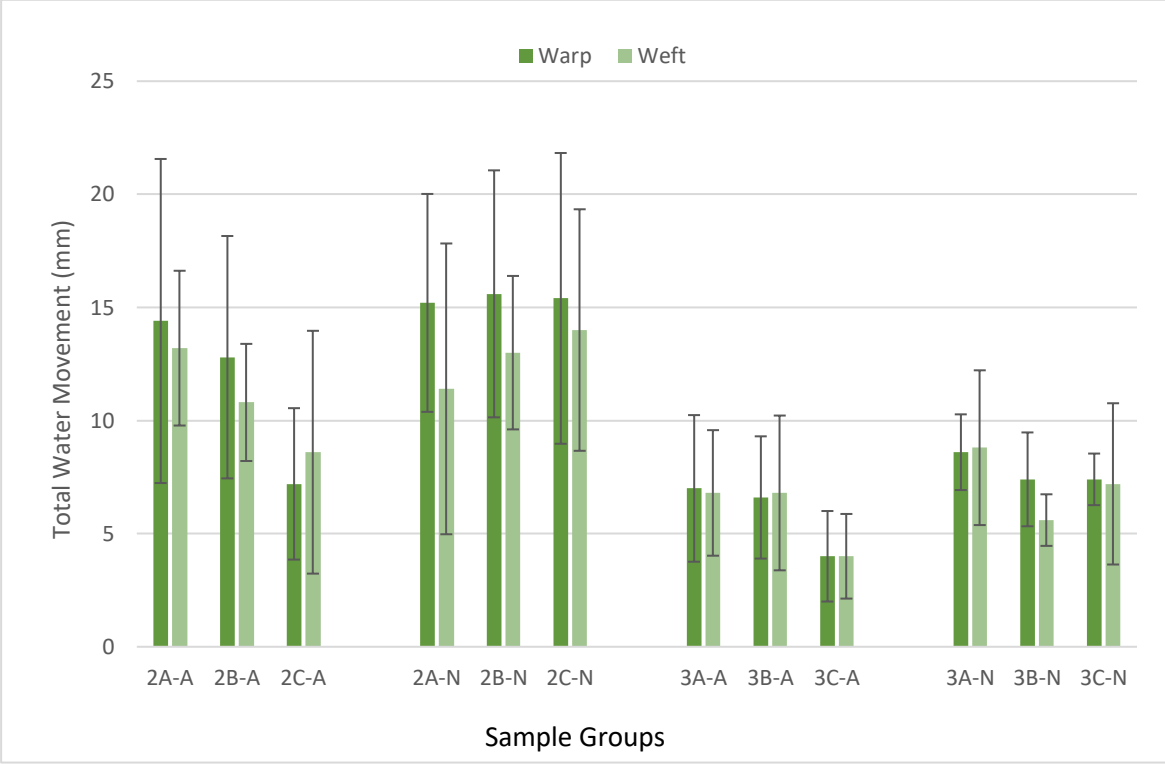


Figure 21 Average total water movement for all samples in group 2 and 3 (A: Aged samples, N: Unaged samples)

7.7.2 Rate of Water Movement

Overall the samples did not show a difference in the rate and pattern of wetting out between groups of different age.

7.7.3 Weight Change

The ageing of the samples appeared to have some effect on the weight change of samples in group 2 and 3. For unaged samples, a pattern of weight change can be seen in both groups in that more weight change is seen in duration B and less in duration A and C (Fig 22 and 23). However, the aged samples do not show any consistent pattern across the two groups. Statistical analysis of the weight change data indicates that there was only a weakly significant difference between the aged and unaged samples (P-value 0.011).

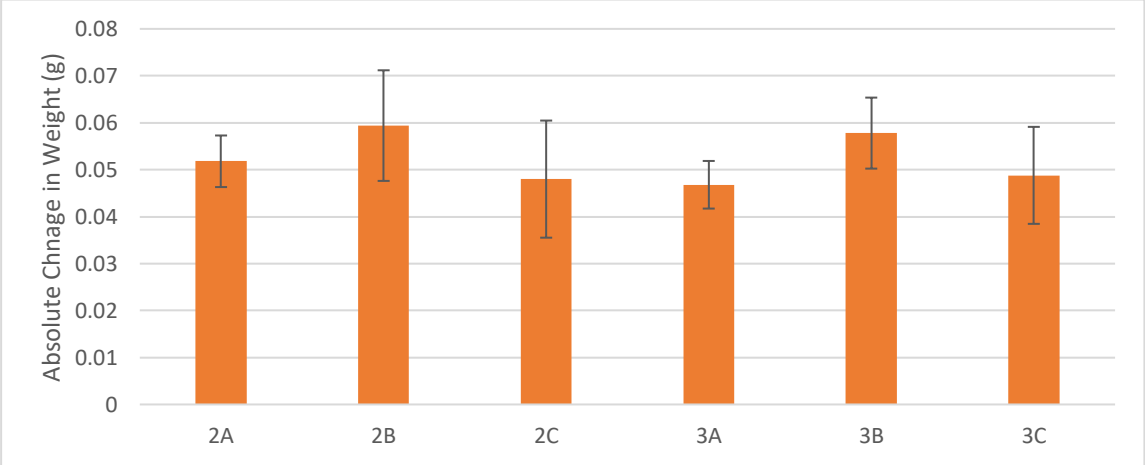


Figure 22 Absolute weight change of not aged samples treated in groups 2 and 3

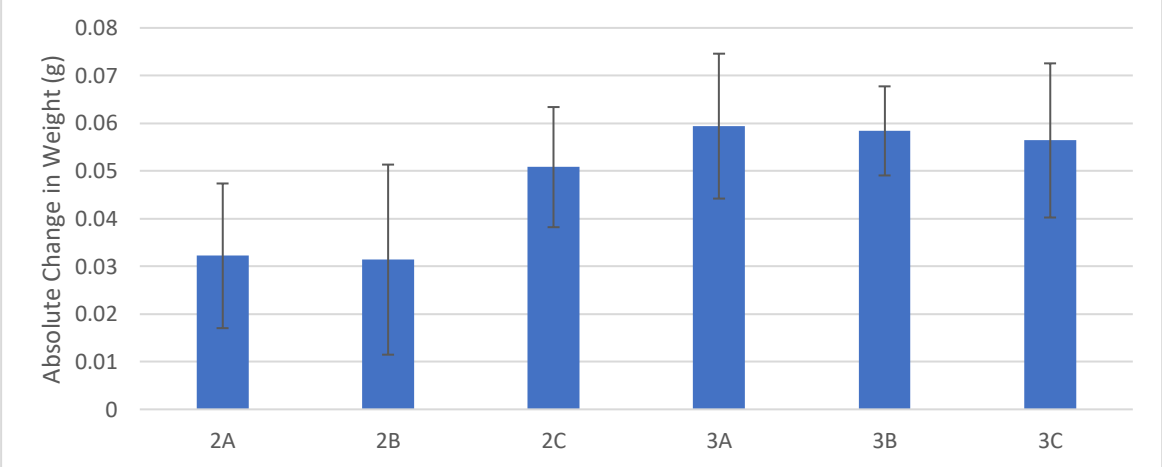


Figure 23 Absolute weight change of aged samples treated in groups 2 and 3

7.7.4 Conclusion

Within the current experiment, it is unclear what effect ageing had on the efficacy of the removal of the wheat starch from the cotton substrate. Patterns of water movement and weight change are present between some of the aged and unaged samples in groups 2 and 3, but there are no consistent trends and there are no strong statistically significant relationships.

7.8 Qualitative Analysis

The following observations discuss the practical aspects of the agarose-alpha amylase application.

7.8.1 Visual Analysis of the Removal of Wheat Starch form the Cotton Substrate

The residual wheat starch adhesive remaining on the substrate after the removal of the gel was visually analysed for each sample. This was recorded to evaluate if the observed success of the agarose-alpha amylase treatment corroborates quantitative measurement. The amount of residual wheat starch on each sample was ranked by the percentage remaining on the substrate (Table 6).

Table 6: Ranking of Gel Efficacy

Gel Efficacy	Percentage of Adhesive Remaining on Cotton Substrate
Great	<5%
Good	5-10%
Fair	10-25%
Poor	25-50%

Overall, the visual analysis of wheat starch removal corresponded with the results of change in weight of the samples. Samples in group 1 showed a range of adhesive removal from great to poor. As discussed in the previous section on duration, the samples showing poor clearance were treated at the longest duration, 100 minutes. Samples in group 2 and 3 also showed a range of great to fair removal, reflecting the variation in weight change seen in these groups.

The samples in group 4, treated with the control gel, showed no wheat starch clearance. The adhesive appeared swollen after the removal of the gel. This was observed across all samples in the group, regardless of duration or age; visual analysis alone could not detect the variation shown in the weight change data.

7.8.2 Gel Properties

During the preparation of the agarose gels, the rate of gelatinization and viscous properties of the solvated solutions were distinct between higher concentrations and lower concentrations. Gels made at higher concentrations, group 2 and 3, needed to be taken to slightly higher temperatures than gels made at lower concentrations, group 1 and 4, for the agarose powder to completely solvate. Due to the addition of enzymes, all concentrations were cooled to the same temperature. The cooled solutions for groups 2 and 3 were distinctly thicker than for groups 1 and 4. This increased the difficulty of pouring the solution into the moulds for the gels at a higher concentration. In order to make the same amount of gels required for each experimental session, the overall volume of mixture for groups 2 and 3 had to be increased.

The different concentrations also influenced the flexibility of the resulting gels. Group 1 and 4 gels were very flexible and soft. After application on the cotton substrate, these gels retained impressions of the weave and accreted wheat starch. Group 2 and 3 gels were less flexible but still able to maintain strong contact with the substrate during treatment due to the initial weighting and regular finger tacking.

7.8.3 Gel Removal

The ease of gel removal from the substrate was also impacted by the concentration of the gels. Although all gels were made to be a height of 3mm, the height of the gel changed throughout the treatment duration. Similar to previous studies[111], a rapid depression of the center of the gel was seen in all applications within the first few minutes. As discussed previously, the concentration affects the gel matrix which impacts the movement of water into the substrate. Gels with lower concentrations lost more water and showed a significant reduction in height. These gels were difficult to lift off the substrate, whilst maintaining the contact between the gel and the wheat starch adhered to it (Fig 24).

[111] Schmitt, “An Examination of the Working Properties of Agarose Gels for Textile Conservation,” 53–55.

Figure 24 Removal the agarose gel at the end of the treatment

7.8.4 Nature of Residual Wheat Starch

The wheat starch remaining on the substrate exhibited different properties across the different groups. Ingroup 1, the remaining wheat starch had a wet, soft, paste-like nature. This made the adhesive difficult to remove from the substrate cleanly. For samples from groups 2 and 3, the wheat starch was film-like, very easy to separate from the substrate and lifted off cleanly. For group 4, the adhesive had softened, and it was possible to insert tools, such as metal spatulas, into the adhesive layer. However, it was difficult to lift off any section of the adhesive and mechanical action was needed to lift any section of the starch where it was adhered to the substrate fabric. Minimal difference was seen between aged and not aged samples.

7.8.5 Tactile Comparison

Tactile comparison between the sample groups after treatment corresponded to the weight change data, group 3 feeling the most flexible with the largest weight loss and group 4 the least flexible with the least weight loss (Table 7).

Table 7: Ranking of the Sample Flexibility

Flexibility of Sample	Sample Groups
Most Flexible	3
More Flexible	2
Less Flexible	1
Least Flexible	4

Additionally, the not aged samples for all concentrations were less flexible than the aged samples, both before and after treatment.

7.9 Exploratory Analysis

The following results are considered exploratory analysis as the methods utilised have not been used to analysis agarose or amylase application in this manner. Additionally, these analyses could not feasibly be applied to object treatments, however the observations highlight interesting aspects of this treatment.

7.9.1 Iodine/Potassium Iodide Indicator

As discussed previously, the iodine/potassium iodide indicator was used to highlight undigested starch. The indicator served to enhance the contrast between the substrate and the starch, emphasising the location and amount of residual adhesive. This was to aid characterisation of the breakdown of the accreted adhesive.

The iodine/potassium iodide indicator provided two interesting observations on the substrate. Firstly, it provided a link between areas of applied adhesive of different thicknesses and areas of increased residual adhesive. In group 1 samples, the residual adhesive directly corresponded to thin areas of applied wheat starch that had appeared bubbled (Fig 25). Conversely, in samples in group 2 the residual adhesive corresponded to severely cracked areas of the applied adhesive (Fig 26).

Secondly, colour change of the indicator was observed 5-10 minutes after application. Initial application of indicator on residual adhesive on the samples after treatment was purple in colour, shifting to pink after a few minutes (Fig 27). This indicates the hydrolysation of the polysaccharides, from either amylose or amylopectin to smaller components.[112] This colour change was not seen on samples treated with the control gels. This is suggestive of the presence and continued action of enzymes on the substrate even after the removal of the gel. As the indicator solution was only applied to areas of adhesive residue, it is not known if enzymes were present on other areas of the substrate; it is possible that enzymes were present on all areas wetted out by the gel.

[112] Bailey and Whelan, "Physical Properties of Starch: I: Relationship between Iodine Stain and Chain Length," 969.

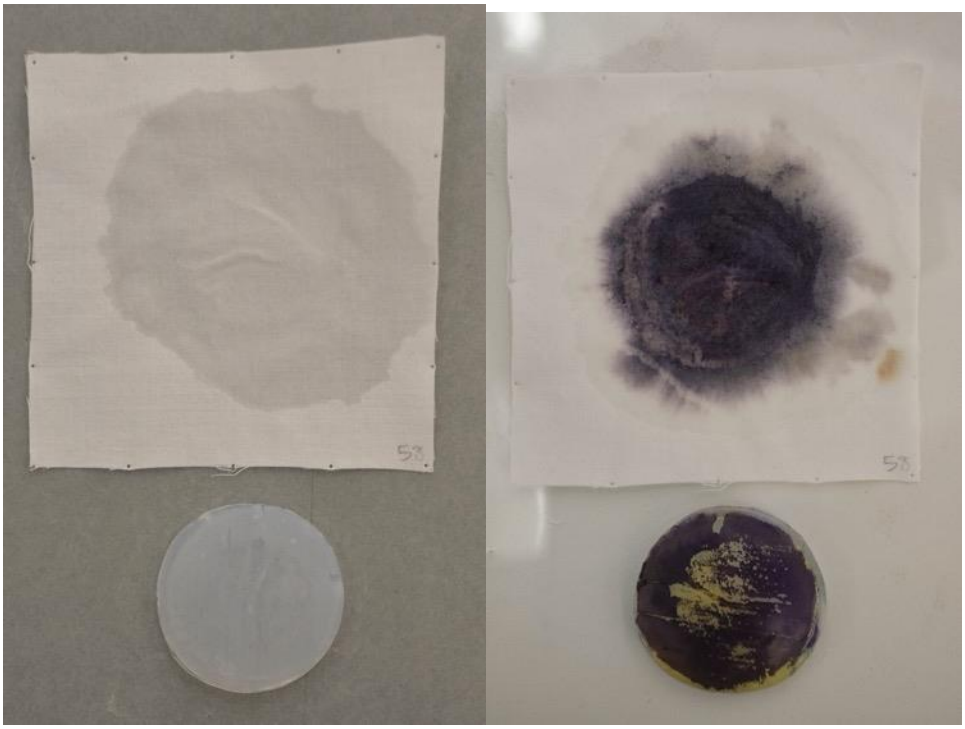


Figure 25 Example of samples treated in group 1 showing bubbled application of starch, immediately after the removal of the gel (left) and the same sample after the application of indicator solution

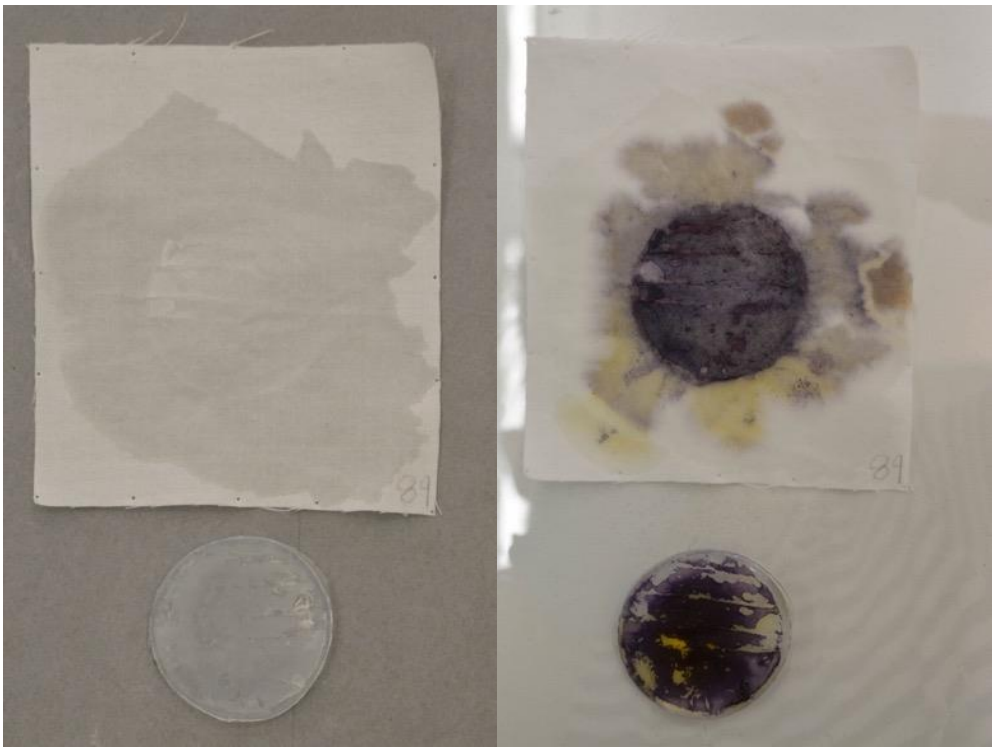


Figure 26 Example of sample treated in group 2 showing a severely cracked application of starch, immediately after the removal of the gel (left) and the same sample after the application of indicator solution

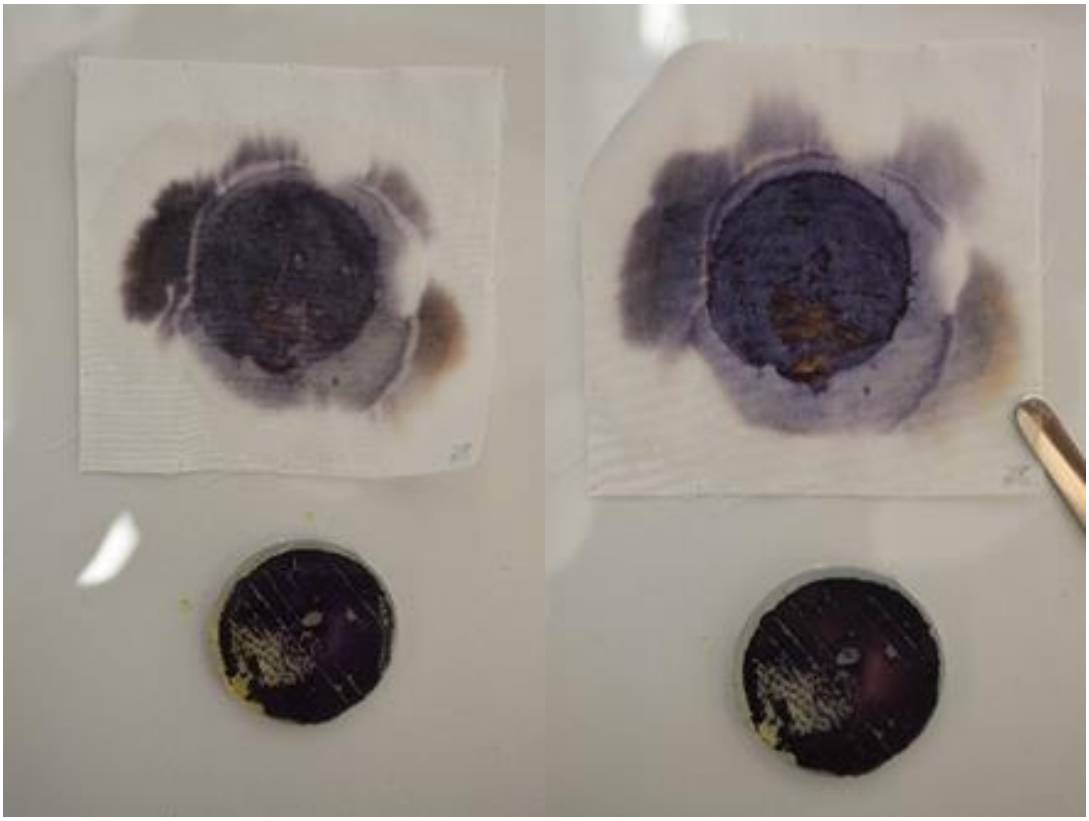


Figure 27 Example of the colour change seen in the wheat starch on the gel and cotton substrate. The darker purple colours seen directly after the application of indicator (left) and 10 minutes after application with areas of wheat starch becoming lighter purple and pink.

7.9.2 UV Photography

All samples were photographed under UV fluorescence with the intention of identifying areas of undigested starch. Organic materials, such as starch, can have a bright white fluorescence that would show on a non-fluorescing cotton fabric substrate.[113] Wheat starch that has been broken down into smaller molecules should not fluoresce. Under UV illumination, 20 out of 24 sample groups showed a white fluorescent tideline. The most distinct tidelines were seen in group 1B-Aged (Figure 5). These bright white tidelines corresponded with the tidelines that were visible under ambient lighting (Fig 28 and 29)

[113] Nick Umney and Shayne Rivers, *Conservation of Furniture* (Oxford: Butterworth-Heinemann, 2003), 610–11.

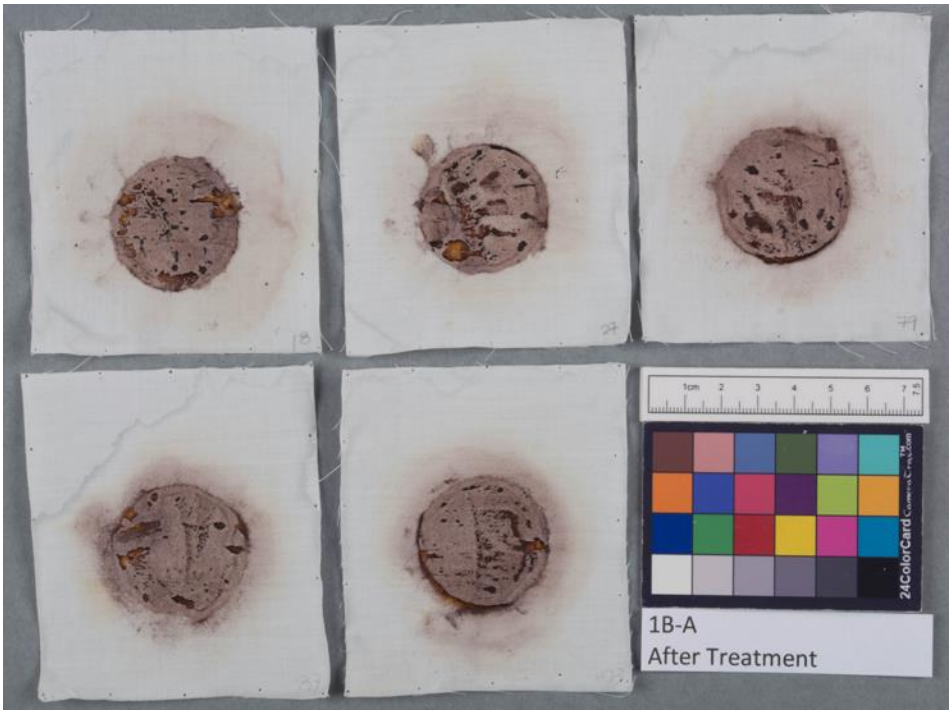


Figure 28 After treatment of aged samples treated with concentration 1 and 80 minutes and stained with indicator solution, under photography studio lights

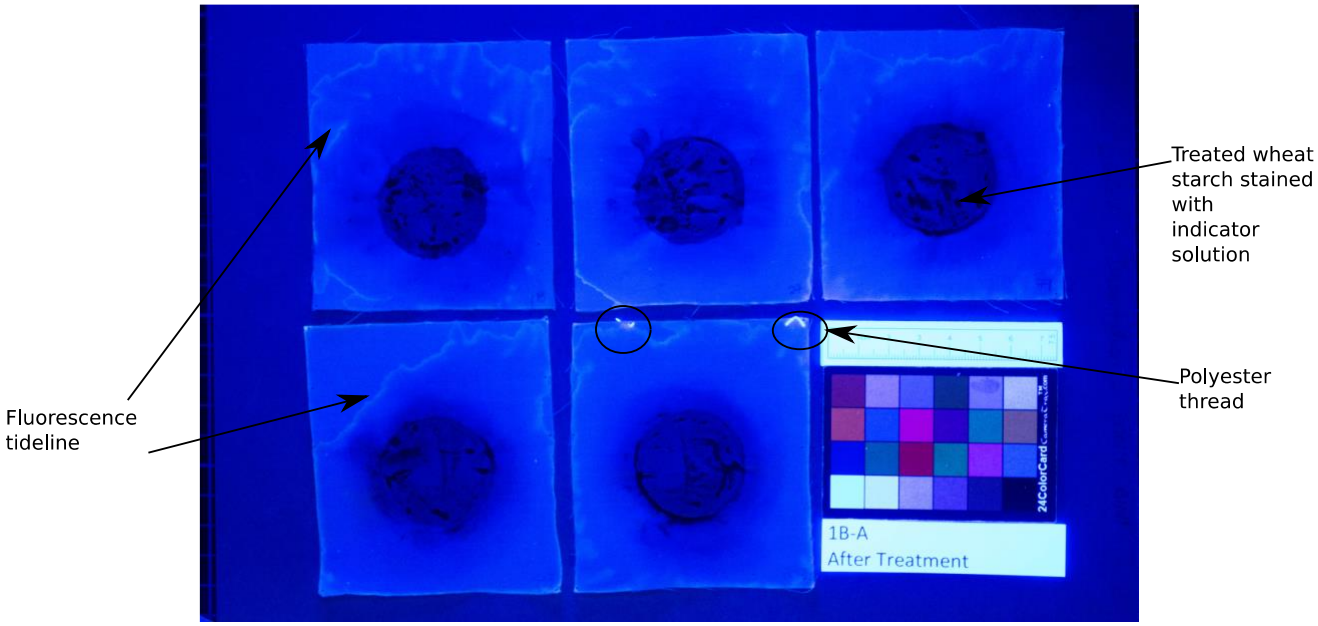


Figure 29 After treatment of aged samples treated with concentration 1 and 80 minutes and stained with indicator solution, under UV lights, the two bright lines on the top of the central lower sample are remnants of the polyester thread used during the thermal ageing process

The areas that were highlighted under UV were stiff to the touch, which was suggestive of areas of starch moved by water from the gels and deposited at the boundaries of water movement.

The fluorescence was strongly apparent on all samples. Overall, it was more distinct on samples treated with concentration 1, followed by concentration 2. It was fainter on samples treated with concentration 3 and for one group, none was seen (Figure 30 and 31). No auto-fluorescence was seen on samples treated with the control gel (Figure 32).

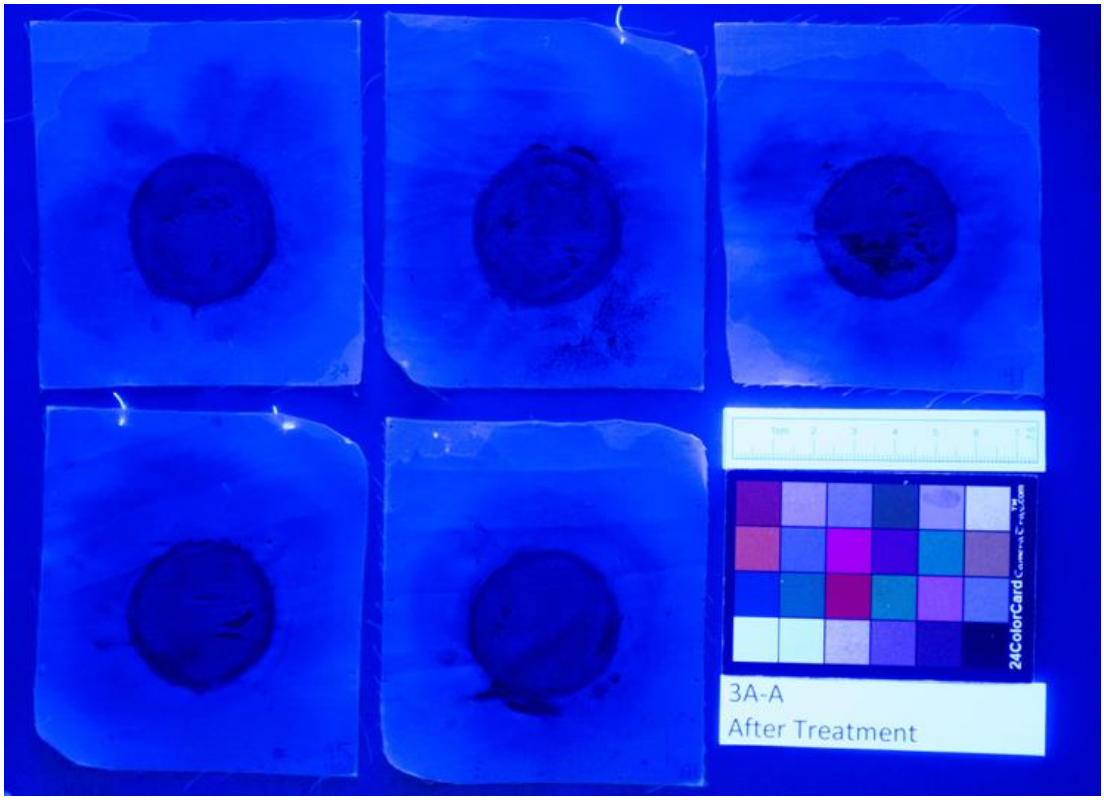


Figure 30 After treatment of aged samples treated with concentration 3 and 60 minutes and stained with indicator solution, under UV lights, the two bright lines on the top of the lower left sample are remnants of the polyester thread used during the thermal ageing process

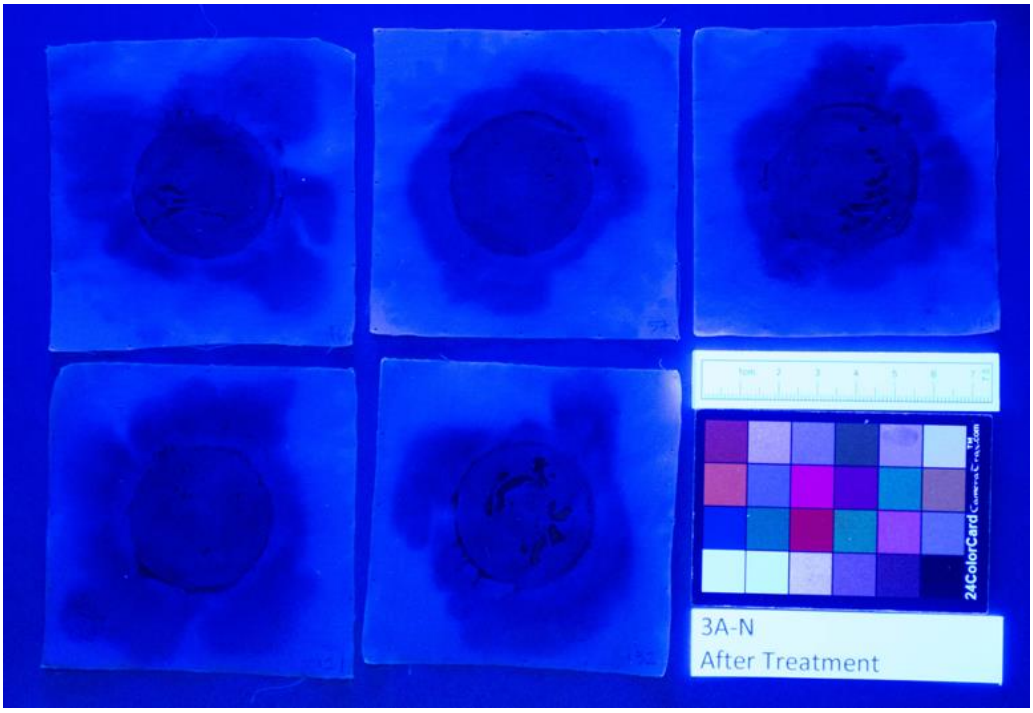


Figure 31 After treatment of aged samples treated with concentration 3 and 60 minutes, and stained with indicator solution, under UV lights, showing minimal fluorescence

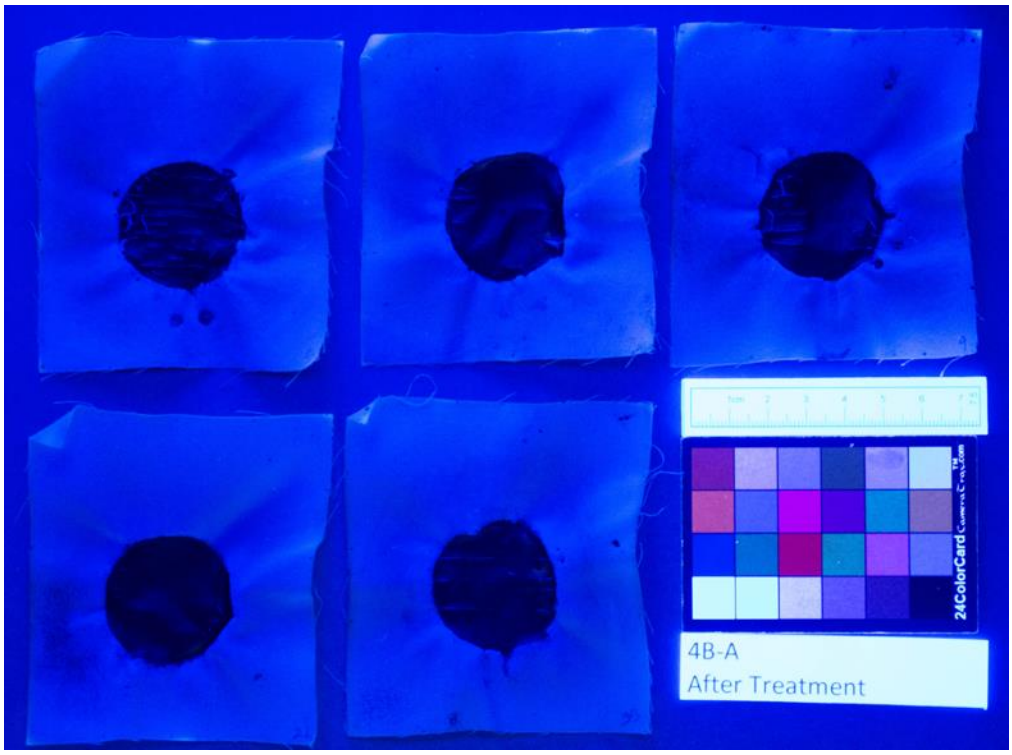


Figure 32 After treatment of aged samples treated with concentration 4, control gel and 80 minutes and stained with indicator solution, under UV lights, showing no fluorescence

It was felt that fluorescence from some samples might be obscured by the iodine/potassium iodide indicator. In order to specify the cause of the auto-fluorescence, untreated samples and samples treated with control gels that were not stained with indicator solution were also photographed under UV (Figure 10). However, the untreated samples did not show any fluorescence and control treated gels showed a faint line around the edge of the tideline (Figure 10). This result suggested that water movement alone caused some auto-fluorescence.

Although the auto-fluorescence seen on many of the samples corresponded with the tidelines resulting from water movement and also retained a starched feel, it was not possible to determine its cause. It is suspected to be related to the starch, enzymes, deionised water or a finishing product on the cotton or a combination. Further testing and analysis are required.

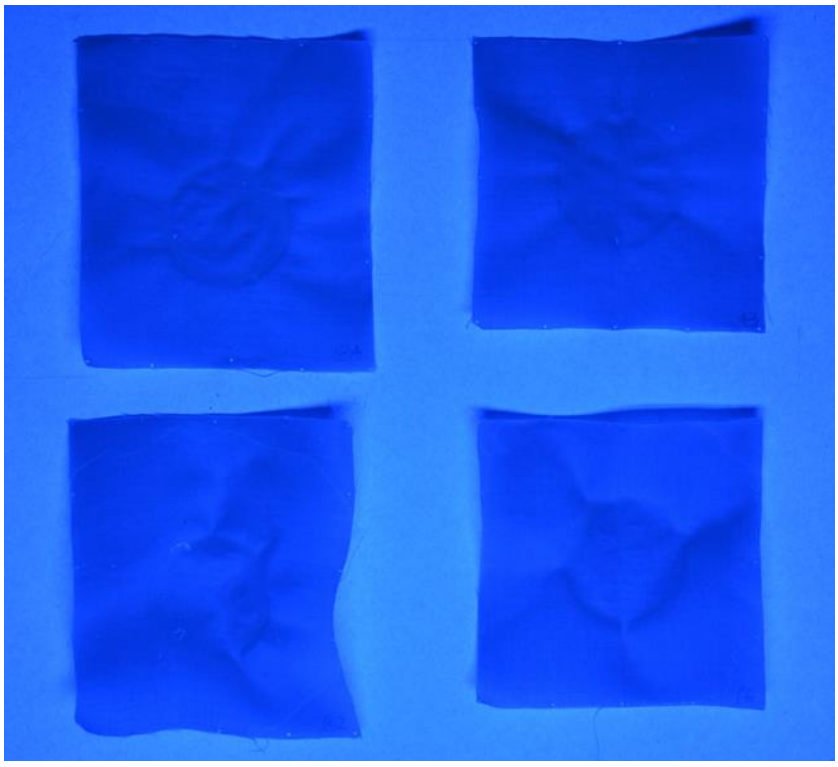


Figure 33 Unstained samples under UV lights: untreated samples, aged (top left), not aged (top right), control treated samples, aged (bottom left) and not aged (bottom right), showing faint fluorescence

Chapter 8: Conclusion

8.1 Introduction

The primary aim of this dissertation was to evaluate the efficacy of agarose-alpha amylase gels for the removal of accreted wheat starch from a cotton substrate. To this end, a review of conservation literature on agarose and enzyme application, of publications from both textile and other conservation disciplines, was conducted. Evaluation of these sources highlighted the lack of literature specific to the application of agarose gels to a textile substrate, along with the trends and concerns for the application of alpha amylase. The experimental phase of this dissertation investigated three application parameters of agarose-alpha amylase gels and how variations in these parameters impacted the success of the treatment. The main conclusions from quantitative and qualitative evaluations and exploratory analysis will be summarised in this chapter. The limitations of this present study and suggestions for future research will also be discussed.

8.2 Experimental Findings

8.2.1 Application Parameters

The application parameters concentration, duration and age of the samples were shown to have varying effects on the efficacy of the agarose-alpha amylase treatment. However, the findings showed that agarose-alpha amylase gels can effectively remove heavily accreted wheat starch adhesives. Recommended application concentrations drawn from paper conservation literature suggested an optimum range of 1-1.5% agarose concentration for effective enzyme action. However, in this present study, successful adhesive removal was seen across all concentrations (1.2-3%). Evaluation of water movement and weight change showed that the most effective concentration for a cotton substrate was 3%. The efficacy of this concentration was not affected by different durations or sample age.

8.2.2 Qualitative Evaluations

The qualitative evaluations of the treatment included visual analysis of the wheat starch removal, gel properties, ease of gel removal, the nature of residual wheat starch and tactile comparison of the cotton samples after treatment. These evaluations encompass practical aspects of the application of agarose gel on a textile substrate. These application considerations have not been discussed in detail in conservation literature.

The significant influence of agarose concentration on the ease of application was emphasised through these findings. As discussed previously, gels made with a lower concentration of agarose are comparatively more flexible and conform well to substrate surfaces. However, they are more difficult to remove from the substrate and thus are less effective at clearing wheat starch from the substrate. Gels with higher concentrations are slightly less flexible but are easier to remove, allowing for a more effective removal of the adhesive from the substrate. These observations corroborate the use of higher concentration agarose gels, such as 2-3%, for applications on a cotton substrate.

8.2.3 Exploratory Analysis

Further analysis of the samples included the application of iodine/potassium iodide indicator solution and UV photography. These were considered exploratory analysis as these methods had not previously been used in this way within textile applications. Whilst these analyses could not feasibly be applied to object treatments, they highlight a key issue that would be beneficial to investigate further: the question of residue, both of agarose and enzyme. Various publications are available in conservation literature that investigate these residues, however, presently there are no investigations specific to textile substrates. As residues of both agarose and enzymes have been noted in paper conservation, it is pertinent to investigate the amount of residues left on textile substrates, given their hydroscopic nature.

8.3 Limitations to Present Study

Aspects of the methodology of this present study have introduced two limitations to the interpretation of the results from this present study. Firstly, the distinctly different characteristics of the dried wheat starch introduced an unintended variable to the study. Although it reduced the consistency of the test substrate, significant findings were still identified. Additionally, it showed how accreted soiling with different thickness affected the movement of the water. This is an aspect of agarose treatment that has not been addressed in conservation literature.

Secondly, the gels applied to the unaged samples in groups 1 and 4 were incorrectly stored prior to application. As such, comparison of the results from the aged groups with those from the unaged groups is not entirely reliable. Whilst this does affect the application parameters recommended in this dissertation, it would be useful to continue to investigate the impact of textile age on the action of agarose gels.

8.4 Further Research

8.4.1 Barriers to Water Movement

Water movement was seen to some extent across all concentrations of agarose within this present study and has also been reported in textile conservation literature regarding agarose applications. Experimental investigation into addition of barriers to lateral water movement, such as cyclododecane, should be continued to improve the control of agarose applications on textile substrates.

8.4.2 Different Textile Substrates

As highlighted in Schmitt's body of work, different fibre types greatly impacted the effect of agarose gel. The results presented in this dissertation are specific to the removal of accreted wheat starch from a cotton lawn substrate. Investigation into application parameters of agarose-alpha amylase gels onto other textile substrates, such as silk, would allow this method to be applied more widely across textile collections. Additionally, as emphasised in Schmitt's most recent publication[114], the transition of theory to object treatments is complex. With the addition of dyes, finishes, weave, age and assorted soiling, it is difficult to predict how applications of agarose-enzyme gels will react. The continued publication of object-based investigations from the textile discipline will enable conservators to gain an understanding of the nature of agarose gels and guide them in formulating future treatments.

[114] Schmitt, "Gelling Predictions: The Challenges of Taking Research into Practice."

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Appendix 1 Review of Gels in Conservation of Art

Most publications regarding gel applications stem from the conference, *Gels in the Conservation of Art* held in London in October 2017. This was the first interdisciplinary conference focused on the current theory, research and practice on the use of gels#. The diversity of contributions spanned several countries, covering a range of disciplines. The presentations were broken into five sections, each focusing on different gel materials and methods. Papers pertaining to agar and agarose from the first section titled: *Polysaccharides: Agar, Gellan, Xanthan and Methyl cellulose* are discussed below.

Of the 31 papers presented in this section, 18 discussed technical properties or applications of agar and agarose. The geographical range of authors included Europe, North America and Japan. The disciplines from which they were written from included: stone, paper, paintings, textiles and heritage science (Table 1). Many of the authors had previously published papers on gels, however, there were also pieces published by emerging professionals in the field. Even with the large scope and diversity, only one paper covered agarose application to textiles.

Author	**Country**	**Discipline**	**Article Type**	**Material**
Arrighi, Quarato and Rossi	Italy	Organics Conservation	Case Study: Treatment of a Fijian Barkcloth to locally reduce soiling	Agar
Barbisan and Dupont	France	Paper Conservation	Case Study: Treatment of a charcoal drawing with extensive tidelines, included investigation into adjusting pH and conductivity of water used to make the gels	Agar, Agarose and Gellan Gum
Barkovic, Diamond and Cross	UK	Paintings Conservation	Case Study: Treatment of water stained paintings in the presence of sensitive size layer p(EA/MMA)	Agar
Bazemore	UK	Paper Conservation	Investigation of calcium phytate gel using agar gel as a vehicle	Agar
Bertasa et al.	Italy	Stone Conservation	Comparative study of four different raw agar products	Agar

Cremonesi and Casoli	Italy	Heritage Science	Investigation of agar properties and gel cleaning action	Agar Hydrogels
Delattre, Bouvet and Le Bourg	France	Paper Conservation	Case Study: Treatment of albums of drawings with dots of animal glue, included comparative testing of different washing methods	Gellan Gum and Agar
Hughes	USA	Paper Conservation	Comparative study of different methods of measuring surface pH on a paper substrate (cold extraction pH, surface pH and agarose plugs)	Agarose
López, Herrera and Martínez	Mexico	Paper Conservation	Case Study: Treatment of a 20th Century poster for the removal of adhesive residue	Agar
Markevičius et al.	Norway, USA, Denmark and Lithuania	Paintings and Paper Conservation	Investigative application of IMAT heater to improve the efficiency of enzyme treatments	Agarose and Gellan Hydrogels
Miller, Whitby and Garside	UK	Paper Conservation	Investigation of phytate gels as vehicles for calcium phytate treatment	Agarose and Gellan Hydrogels
Pasnak	Norway	Paper Conservation	Investigation into the application gellan gum and agar with different custom chelating agents	Deacylated Gellan Gum and Agarose/Agar
Sánchez and Martínez	Mexico	Paper Conservation	Case Study: Treatment of a 19th Century document with a combination of rigid gels and soft gels	Agarose and Carbopol
Sawicka, O'Toole and Ara	UK	Paintings Conservation	Case Study: Treatment of a water sensitive oil paint surface	Agar
Schmitt	USA	Textile Conservation	Case Study: Application of agarose gel for localised cleaning of a sampler	Agarose
Sullivan et al.	USA	Paper Conservation	Comparative residue study and evaluation of treatment variables for minimising deposits	Gellan Gum, Methyl Cellulose and Agarose

Tamura and Takagi	UK/Japan	General Conservation	Discussion of sustainable use of agar/agarose in conservation	Agar and Agarose
van Dyke	USA	Paper Conservation	Introduction of application of agarose-enzyme gels on works on paper	Agarose (Food Grade)

Table 1: Brief Summary of the publications reviewed from Gels in the Conservation of Art

The experimental papers can be broken up into two main groups. The first consists of investigations that build on previous research on agar and agarose gels. One provides a more in-depth understanding of the properties and cleaning action of the different gels,[115] while another is a comparison and evaluation of the gels against commonly used materials for pH measuring and the effect of the addition of different cleaning agents.[116] A particular topic that was mentioned across several papers and was the main focus of one paper was the investigation of residues of agarose gels on different paper substrates.[117] The conclusion contradicts the early findings of Warda et al. showing that none of the gels tested (gellan, agarose and methyl cellulose) are residue less. The level of residue is also dependent on the type of substrate. These investigations on the cores properties of agar and agarose reflects the gaps present in the previous literature. The continued investigation into residues emphasis this as a key concern.

The second group of investigative articles explore and evaluate new applications of agar and agarose. These can be further broken down into applications of two key additives: enzymes and calcium phytate.[118] These articles emphasised the flexibility of agar and agarose gels as a

[115] Paolo Cremonesi and Antonella Casoli, "Thermo-Reversible Rigid Agar Hydrogels: Their Properties and Action in Cleaning," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (London: Archetype Publications, 2017), 19–28; Moira Bertasa et al., "A Study of Commercial Agar Gels as Cleaning Materials," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (London: Archetype Publications Ltd, 2017), 11–18.

[116] Amy Hughes, "Measurements of Surface PH of Paper Using Agarose Gel Plugs: A Feasibility Study," in *Gels in the Conservation of Art*, ed. Lora V. Anglova et al. (London: Archetype Publications, 2017), 62–66; Ekaterina Pasnak, "Washing Works of Art on Paper Using Rigid Hydrogels Containing Chelating Agents," in *Gels in the Conservation of Art*, ed. Lora V. Anglova et al. (London: Archetype Publications, 2017), 135–37.

[117] Michelle Sullivan et al., "Rigid Polysaccharide Gels for Paper Conservation: A Residue Study," in *Gels in the Conservation of Art*, ed. Lora V. Anglova et al. (London: Archetype Publications, 2017), 42–50.

[118] Yana Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," in *Gels in the Conservation of Art*, ed. Lora V. Angelova et al. (London: Archetype Publications Ltd, 2017), 101–6; Tomas Markevičius et al., "Cold, Warm, Warmer: Use of Precision Heat Transfer in the Optimization of Hydrolytic Enzyme and Hydrogel Cleaning Systems," in *Gels in the Conservation of Art*, ed. Lora V. Anglova et al. (London: Archetype Publications, 2017), 67–72; Zoë Miller, Gayle Whitby, and Paul Garside, "Investigating the Ability of Phytate Gel Systems to Treat Iron Gall Ink at the British Library," in *Gels in the Conservation of Art*, ed. Lora V. Anglova et al. (London: Archetype Publications, 2017), 77–81; Avery Bazemore, "Chelating Soluble Iron (II)

medium. Additionally, they provided detailed methodology of these application whilst addressing issues such as maximising the efficiency of the treatments, reducing the risks of the treatment and the volume of reagents needed.

Two of these publications specifically discussed the importance of controlling the wetness of the agarose through the adjustment of concentration. Miller et al. tested both 2 % and 4% agarose gels on different paper substrates and reported that 2% was too wet and # 4% gels left significant tidelines.[119] However, van Dyke's article suggests a concentration range of 1-1.2% and simply advises the need for preparation and intervention should there be alterations to the surface texture, planar distortions, disruption to sensitive media or tideline formation.[120] Water movement is emphasised as a concern by both of these articles however, neither publication present any further discussion on any other factors of the substrate or application parameters that may address water movement.

Aside from experimental publications, several case studies were also presented in this section. Practical methodologies and application parameters across a range of treatment types and substrates were presented. The majority of articles discussed the testing conducted for their particular object type, their reason for proposing gel treatments, and critical evaluations of the results. Overall these case studies introduce a significant range of knowledge to literature discussing gel applications. The case studies present a range of gel concentrations and durations of treatment using a variety of solutions followed by results that are evaluated and presented in an accessible manner.

The majority of case studies discussed treatments where agar was applied and not agarose. For many of the case studies it is not clear why agar was selected. Some articles cited previous publications from paper conservation where several agar applications are available. For some articles the properties cited for material selection are shared by both agarose and agar, or the terms agar and agarose were used interchangeably. This suggests that further clarification is required to distinguish the intrinsically different properties of these materials to inform treatments. It is not clear what drives material selection between these gels, it is suspected to be influenced by accessible literature and the availability and cost of the materials.

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from Iron Gall Ink Using Calcium Phytate in Agar Gel," in *Gels in the Conservation of Art*, ed. Lora V. Anglova et al. (London: Archetype Publications, 2017), 116–18.

[119] Miller, Whitby, and Garside, "Investigating the Ability of Phytate Gel Systems to Treat Iron Gall Ink at the British Library," 80.

[120] Van Dyke, "Agarose-Enzyme Gels in Paper Conservation," 104–5.

The most relevant case study to this present study is the only publication written from the textile conservation discipline. Schmitt builds on her existing body of work by applying the agarose treatment parameters that she had previously determined, for the treatment of sampler. Detailed preparation and methodology was given along with critical evaluation of the treatment. The treatment was not successful as the object required remedial intervention due to water movement. Schmitt's paper highlights the difficulties of transferring theory into practice, as each object or even area of an object presents a series of variables that are difficult to determine, control and to predict their effect on the success of the treatment.

Appendix 2- Preliminary Testing

This appendix will briefly summarise the preliminary testing that guided the determination of the testing parameters of the experimental phase.

Part 1: Wheat Starch Application

The aim of this section of preliminary testing was to determine a method of accurately measuring wheat starch and applying a thick even layer whilst reducing keeping the fabric samples flat. Initially different methods were tested for measuring out set amounts of wheat starch and maintaining the set amount left on the substrate. However, as sample weight was key to evaluating the efficacy of the treatment, the weight of each sample was tracked throughout the experimental phase. The focus of wheat starch application was then focused on ease of replication.

Application methods tested included:

- Measuring of wheat starch with measuring spoon
- Measuring of wheat starch with different sized pipettes
- Smoothing out of wheat starch using a piece of Melinex, Teflon folder, bone folder and *Hake* brush
-

Additional actions considered were:

- Wetting of the fabric samples to reduce distortion due to the rapid uptake of water from the applied wheat starch
- Pinning the samples out to maintain tension when drying. Other methods were tested which included weighting with and without Reemay.

Samples of different tests were weighed after they were dried and the method that was felt that the most replicable was measuring of wheat starch using a 1mL syringe, applying the adhesive onto wet fabric that was wetted out evenly using a dahlia spray. The adhesive was then brushed to form an even layer.

Part 2: Application Parameters

Determination of the testing parameters for the experimental phrase was influenced by available literature and preliminary tests.

Specific concentrations of agarose were drawn specifically from publications by van Dyke and Schmitt. The application range suggested by van Dyke was very narrow 1-1.2% and did the include Schmitt's suggested concentration range for textiles 2.5-4%. Testing of 1, 2, 2.5 and 4% were conducted to establish how application of agarose in this manner functioned. Application of 1% wetted out the entire sample within 20 minutes of application which was felt to be

unacceptable, instead the highest concentration suggested by van Dyke was to be tested. Application of 4% gels showed minimal wetting however, after 60 minutes of weighted application the gel removed minimal wheat starch <10% and the remaining wheat starch was still very difficult to mechanically remove. This was considered an unsuccessful application. The concentrations to be tested were decided to be 1.2, 2, 3% with a control gel of at 1.2%.

Different durations of the 1% gels were also tested from 20 minutes to 60 mins. The gel appeared to be most effect when applied for longer than 60 minutes, as this was seen in the gel with the lowest concentration the test durations was decided to be 60, 80 and 100 minutes.

Reports of application of agarose vary in the application of weights to gels. Wolbers maintain that it is not necessary and capillary action of the gel alone is enough. However, Schmitt found that, though weighting was not necessary for all concentration, the application of weights and finger tacking improved the contact of the gel with the textile substrate and reduced water movement. During these initial tests some samples were weighted, and some were not. It was found that weighting for the entire duration did not appear necessary, but an initial weighting helped establish the contact between the gel and the cotton.

Appendix 3- Wheat Starch Preparation

The wheat starch recipe for the experimental phase of this dissertation was adapted from an AIC recipe.[121] This recipe has been used previously by the author and was selected on the basis of producing an even smooth paste.

The recipe is as follows

1) Wheat starch powder (from Fisher Scientific) and deionised water was measured out to a 1:4 v:v ratio using a 250ml glass beaker. For measuring both wheat starch and water the beaker was filled to the top. The components were measured into a non-stick saucepan.
2) The mixture was heated on a hot plate set to medium for 70 minutes, stirring regularly with a silicon spoon. The mixture begins to become thicker and translucent after 30 minutes. At this point the mixture was stirred vigorously and continuously making sure to scrape the bottom and sides of the pot regularly.
3) Once the paste had been cooked for the set time and was shiny, translucent and dried from the top edges of the pan and spoon in sheets it was taken off the heat.

Making the wheat starch on the hotplate (left), wheat starch that is shiny and translucent, nearing the end of the cooking period, note the dried layers of adhesive pulling away from the top edges of the pot (right)

4) Once cool the starch paste was sieved once through a fine horsehair sieve and stored in a lidded glass jar that had been wiped down with ethanol, to reduce fungal growth.
5) The volume of wheat starch used yielded approx. 200g of wheat starch paste.

[121] Linda Barone et al., "BPG Adhesives Recipes and Tips," *AIC Book and Paper Group*, accessed June 3, 2018, http://www.conservation-wiki.com/wiki/BPG_Adhesive_Recipes_and_Tips#Funori_.28Japanese_Seaweed_Adhesive.29.

Appendix 4 Iodine/Potassium Iodide Indicator

The iodine/potassium iodide (KI_3) indicator solution used in the experimental phase was made following the recipe from *Material Characterization Tests for Objects of Art and Archaeology*[122] and is follows:

1) Add 0.9 g of potassium iodide to 5mL of deionised water and then add 0.04g of iodine to the KI solution.
2) When the iodine is fully dissolved dilute the mixture to 35mL with deionised water.

Two batches of indicator solution was made during the experimental phase.

[122] Nancy Odegaard, Scott Carroll, and Werner S. Zimmt, *Material Characterization Tests for Objects of Art and Archaeology*, 2nd ed. (London: Archetype Publications, 2000).

Appendix 5 Statistical Analysis

This appendix includes the raw data used for ANOVA testing in the statistical software R along with the results generated

Total Water Movement:
Raw Data

Sample	Ageing	Concentration	Duration	WM Warp	WM Weft
1	Aged	1	60	44	42
2	Aged	1	60	49	44
3	Aged	1	60	44	44
4	Aged	1	60	52	41
5	Aged	1	60	48	42
6	Aged	2	60	6	8
7	Aged	2	60	15	14
8	Aged	2	60	18	12
9	Aged	2	60	24	17
10	Aged	2	60	9	15
11	Aged	3	60	3	4
12	Aged	3	60	9	10
13	Aged	3	60	4	4
14	Aged	3	60	9	7
15	Aged	3	60	10	9
16	Aged	4	60	28	21
17	Aged	4	60	32	28
18	Aged	4	60	20	23
19	Aged	4	60	34	34
20	Aged	4	60	27	27
21	Aged	1	80	45	41
22	Aged	1	80	38	45
23	Aged	1	80	46	44
24	Aged	1	80	49	44
25	Aged	1	80	50	40
26	Aged	2	80	10	7
27	Aged	2	80	18	14
28	Aged	2	80	5	11
29	Aged	2	80	17	12
30	Aged	2	80	14	10
31	Aged	3	80	11	6
32	Aged	3	80	7	5
33	Aged	3	80	5	3
34	Aged	3	80	4	12

35	Aged	3	80	6	8
36	Aged	4	80	24	32
37	Aged	4	80	19	18
38	Aged	4	80	28	30
39	Aged	4	80	38	35
40	Aged	4	80	30	25
41	Aged	1	100	39	29
42	Aged	1	100	41	24
43	Aged	1	100	33	33
44	Aged	1	100	35	34
45	Aged	1	100	33	32
46	Aged	2	100	5	2
47	Aged	2	100	7	5
48	Aged	2	100	6	8
49	Aged	2	100	13	14
50	Aged	2	100	5	14
51	Aged	3	100	5	6
52	Aged	3	100	2	1
53	Aged	3	100	3	4
54	Aged	3	100	7	5
55	Aged	3	100	3	4
56	Aged	4	100	16	15
57	Aged	4	100	19	20
58	Aged	4	100	8	-9
59	Aged	4	100	23	29
60	Aged	4	100	20	15
61	Unaged	1	60	35	42
62	Unaged	1	60	38	42
63	Unaged	1	60	42	39
64	Unaged	1	60	38	29
65	Unaged	1	60	38	45
66	Unaged	2	60	13	12
67	Unaged	2	60	17	3
68	Unaged	2	60	9	17
69	Unaged	2	60	15	7
70	Unaged	2	60	22	18
71	Unaged	3	60	11	9
72	Unaged	3	60	7	10
73	Unaged	3	60	9	12
74	Unaged	3	60	9	10
75	Unaged	3	60	7	3
76	Unaged	4	60	12	13

77	Unaged	4	60	28	32
78	Unaged	4	60	35	38
79	Unaged	4	60	32	27
80	Unaged	4	60	19	19
81	Unaged	1	80	43	41
82	Unaged	1	80	45	36
83	Unaged	1	80	44	46
84	Unaged	1	80	45	41
85	Unaged	1	80	44	41
86	Unaged	2	80	7	9
87	Unaged	2	80	17	16
88	Unaged	2	80	21	12
89	Unaged	2	80	19	17
90	Unaged	2	80	14	11
91	Unaged	3	80	5	6
92	Unaged	3	80	9	7
93	Unaged	3	80	6	6
94	Unaged	3	80	10	4
95	Unaged	3	80	7	5
96	Unaged	4	80	30	35
97	Unaged	4	80	23	28
98	Unaged	4	80	33	29
99	Unaged	4	80	28	30
100	Unaged	4	80	39	29
101	Unaged	1	100	29	33
102	Unaged	1	100	34	29
103	Unaged	1	100	34	27
104	Unaged	1	100	28	35
105	Unaged	1	100	34	35
106	Unaged	2	100	8	6
107	Unaged	2	100	18	17
108	Unaged	2	100	13	12
109	Unaged	2	100	25	20
110	Unaged	2	100	13	15
111	Unaged	3	100	7	5
112	Unaged	3	100	8	5
113	Unaged	3	100	7	10
114	Unaged	3	100	6	12
115	Unaged	3	100	9	4
116	Unaged	4	100	33	36
117	Unaged	4	100	11	12
118	Unaged	4	100	26	18

119	Unaged	4	100	36	32
120	Unaged	4	100	37	34

MANOVA

```
Error: sample
                                Df  Pillai approx F num Df den Df    Pr(>F)
Aging                            1 0.02847   1.3920      2     95   0.25359
Concentration                    3 0.91067  26.7517      6    192 < 2.2e-16
 ***
Duration                         2 0.24051   6.5613      4    192 5.711e-05
 ***
Aging:Concentration              3 0.15295   2.6498      6    192   0.01715
 *
Aging:Duration                   2 0.11404   2.9026      4    192   0.02308
 *
Concentration:Duration           6 0.17450   1.5294     12    192   0.11635
Aging:Concentration:Duration     6 0.08241   0.6876     12    192   0.76231
Residuals                       96
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Weight Change Data
Raw Data

	Weight Change	Aged	Concentration	Duration
1	0.044	Aged	1	60
2	0.052	Aged	1	60
3	0.059	Aged	1	60
4	0.028	Aged	1	60
5	0.034	Aged	1	60
6	0.044	Aged	1	80
7	0.035	Aged	1	80
8	0.031	Aged	1	80
9	0.029	Aged	1	80
10	0.02	Aged	1	80
11	0.023	Aged	1	100
12	0.028	Aged	1	100
13	0.015	Aged	1	100
14	0.035	Aged	1	100
15	0.022	Aged	1	100
16	0.024	Aged	2	60
17	0.02	Aged	2	60

18	0.052	Aged	2	60
19	0.045	Aged	2	60
20	0.02	Aged	2	60
21	0.011	Aged	2	80
22	0.042	Aged	2	80
23	0.009	Aged	2	80
24	0.052	Aged	2	80
25	0.043	Aged	2	80
26	0.06	Aged	2	100
27	0.063	Aged	2	100
28	0.048	Aged	2	100
29	0.031	Aged	2	100
30	0.052	Aged	2	100
31	0.067	Aged	3	60
32	0.042	Aged	3	60
33	0.074	Aged	3	60
34	0.07	Aged	3	60
35	0.044	Aged	3	60
36	0.053	Aged	3	80
37	0.048	Aged	3	80
38	0.072	Aged	3	80
39	0.063	Aged	3	80
40	0.056	Aged	3	80
41	0.056	Aged	3	100
42	0.066	Aged	3	100
43	0.077	Aged	3	100
44	0.035	Aged	3	100
45	0.048	Aged	3	100
46	-0.015	Aged	4	60
47	-0.006	Aged	4	60
48	-0.008	Aged	4	60
49	-0.015	Aged	4	60
50	-0.018	Aged	4	60
51	-0.028	Aged	4	80
52	-0.026	Aged	4	80
53	-0.027	Aged	4	80
54	-0.031	Aged	4	80
55	-0.03	Aged	4	80
56	-0.033	Aged	4	100
57	-0.036	Aged	4	100
58	-0.026	Aged	4	100
59	-0.022	Aged	4	100

60	-0.025	Aged	4	100
61	0.035	Unaged	1	60
62	0.028	Unaged	1	60
63	0.033	Unaged	1	60
64	0.051	Unaged	1	60
65	0.035	Unaged	1	60
66	0.033	Unaged	1	80
67	0.048	Unaged	1	80
68	0.027	Unaged	1	80
69	0.026	Unaged	1	80
70	0.029	Unaged	1	80
71	0.03	Unaged	1	100
72	0.023	Unaged	1	100
73	0.039	Unaged	1	100
74	0.023	Unaged	1	100
75	0.019	Unaged	1	100
76	0.046	Unaged	2	60
77	0.054	Unaged	2	60
78	0.048	Unaged	2	60
79	0.06	Unaged	2	60
80	0.051	Unaged	2	60
81	0.075	Unaged	2	80
82	0.049	Unaged	2	80
83	0.053	Unaged	2	80
84	0.051	Unaged	2	80
85	0.069	Unaged	2	80
86	0.062	Unaged	2	100
87	0.041	Unaged	2	100
88	0.04	Unaged	2	100
89	0.036	Unaged	2	100
90	0.061	Unaged	2	100
91	0.051	Unaged	3	60
92	0.038	Unaged	3	60
93	0.049	Unaged	3	60
94	0.048	Unaged	3	60
95	0.048	Unaged	3	60
96	0.066	Unaged	3	80
97	0.047	Unaged	3	80
98	0.056	Unaged	3	80
99	0.064	Unaged	3	80
100	0.056	Unaged	3	80
101	0.059	Unaged	3	100

102	0.035	Unaged	3	100
103	0.058	Unaged	3	100
104	0.042	Unaged	3	100
105	0.05	Unaged	3	100
106	-0.009	Unaged	4	60
107	-0.009	Unaged	4	60
108	-0.013	Unaged	4	60
109	-0.007	Unaged	4	60
110	-0.008	Unaged	4	60
111	-0.011	Unaged	4	80
112	-0.017	Unaged	4	80
113	-0.012	Unaged	4	80
114	-0.016	Unaged	4	80
115	-0.011	Unaged	4	80
116	-0.011	Unaged	4	100
117	-0.006	Unaged	4	100
118	-0.01	Unaged	4	100
119	-0.009	Unaged	4	100
120	-0.009	Unaged	4	100

Three Factor ANOVA

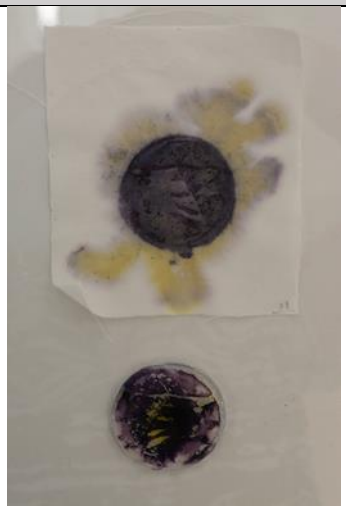
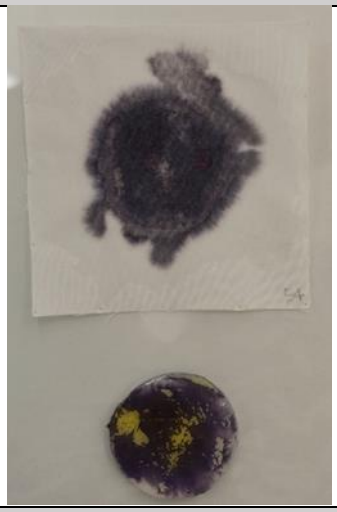
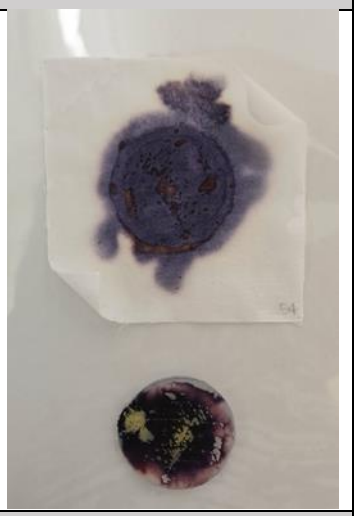
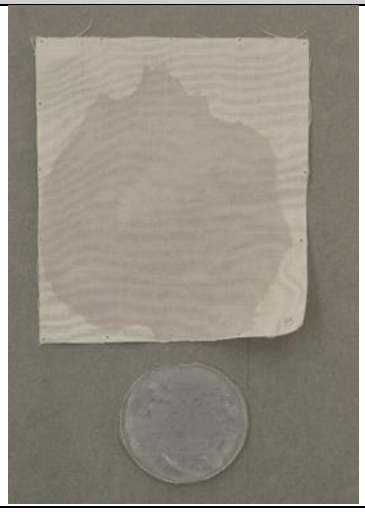
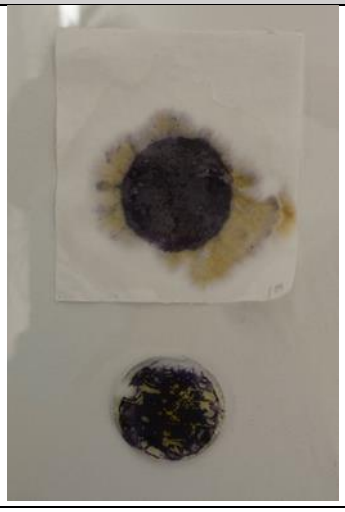
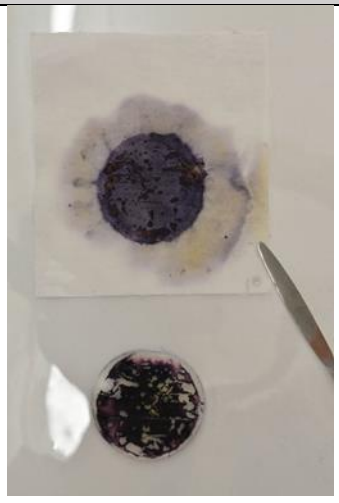
```
> summary(weights.aov)

Error: sample
                            Df  Sum Sq  Mean Sq F value   Pr(>F)
Aging                        1 0.00069 0.000691   6.709   0.0111 *
Concentration                3 0.09125 0.030415 295.210  < 2e-16 ***
Duration                     2 0.00029 0.000147   1.428   0.2449
Aging:Concentration          3 0.00253 0.000844   8.196 6.51e-05 ***
Aging:Duration               2 0.00056 0.000280   2.718   0.0711 .
Concentration:Duration       6 0.00174 0.000290   2.815   0.0144 *
Aging:Concentration:Duration 6 0.00136 0.000227   2.205   0.0490 *
Residuals                   96 0.00989 0.000103
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

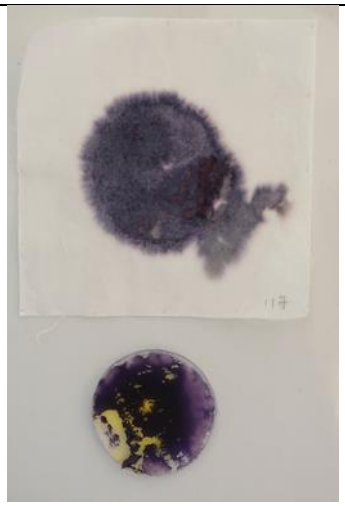
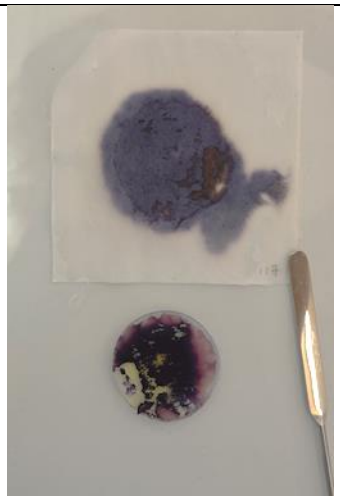
Appendix 6 Environmental Conditions

This appendix presents the environmental conditions of the laboratory in which the experimental took place. These measurements were taken using a Hanwell Humbug Temperature and Humidity Data Logger. These conditions is suspected to have some effect on the results obtained in the experimental phase however it was not analysed within this current study.

Date	Morning		Afternoon		Sample Group Tested
	Temperature °C	RH %	Temperature °C	RH %	
19 June	24	45	24	49	4A
20 June	24	49	24	49	4B
21 June	24	39	24	39	4C
27 June	23	70	23	70	1B
28 June	27.6	42.5	30	29	1A
29 June	25.8	38.1	27.2	35	1C
2 July	25.8	38.1	27.2	35	2A
3 July	25.5	42	28.5	33.7	2B
4 July	26	39.9	28.4	38.8	2C
5 July	26.5	39.3	27.4	31.2	3B
6 July	25.6	30.9	26.7	32.1	3C
9 July	25.8	39.4	27.2	36	3A

Appendix 7 After Treatment Photos of Representative Samples

1A-A			
			
1A-N			
54			
1B-A			
			
1B-N			

117			
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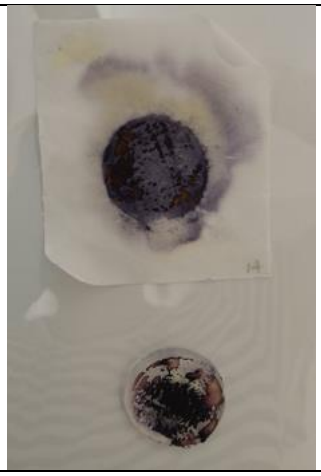
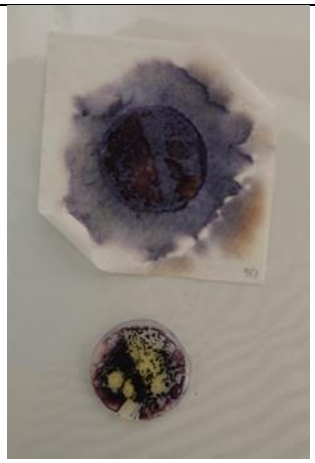
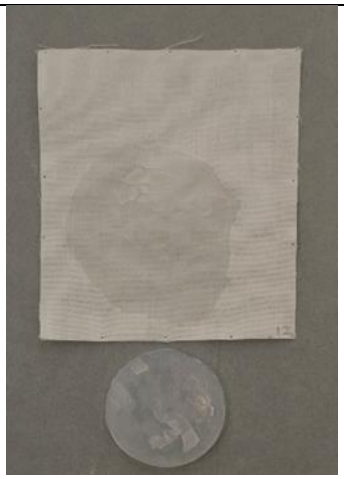
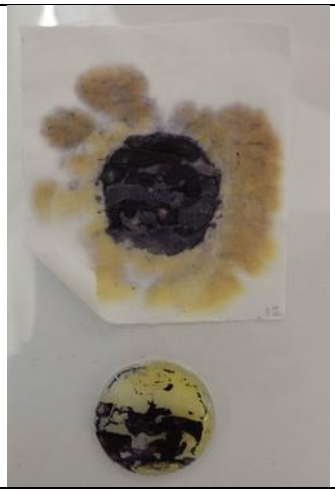
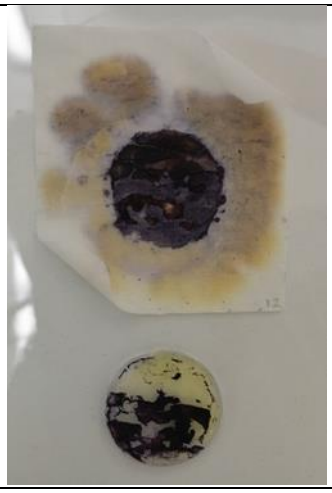
14			
1C-N			
40			
2A-A			

12			
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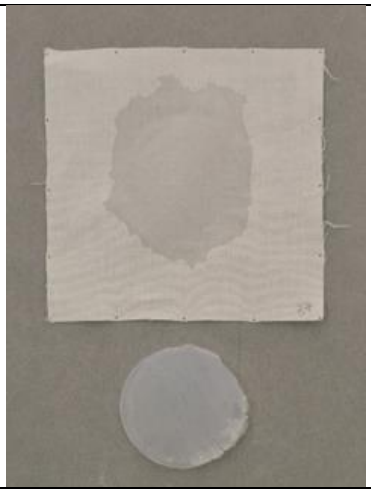
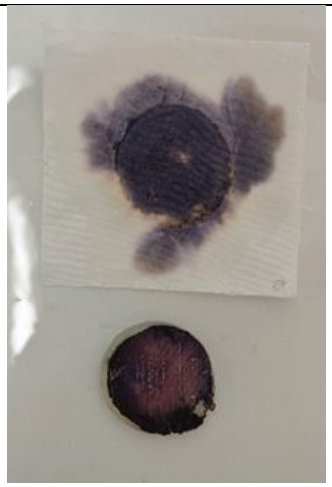
37			
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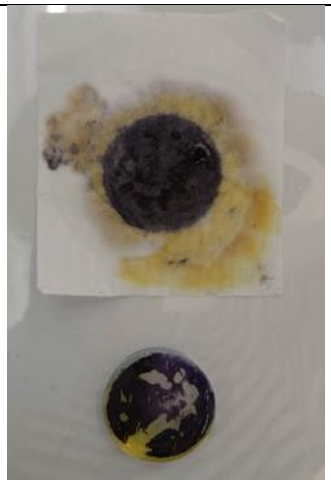
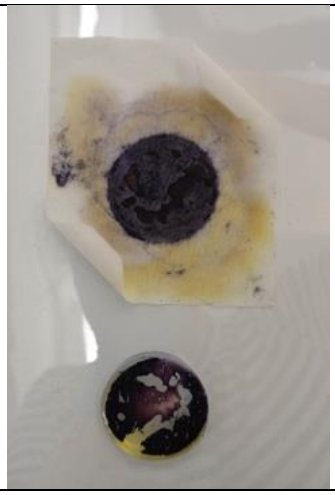
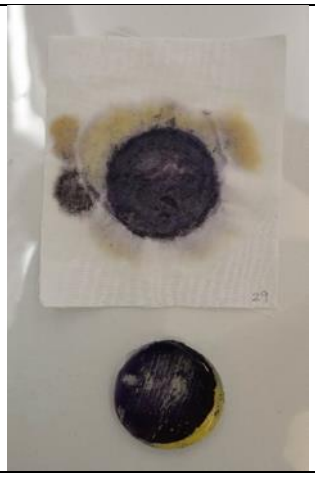
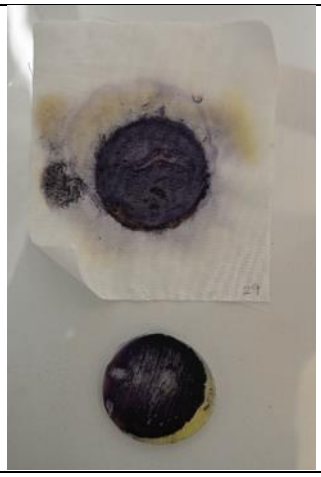
15			
2B-N			

50			
2C-A			
29			
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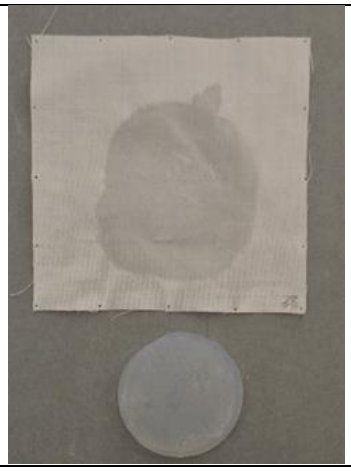
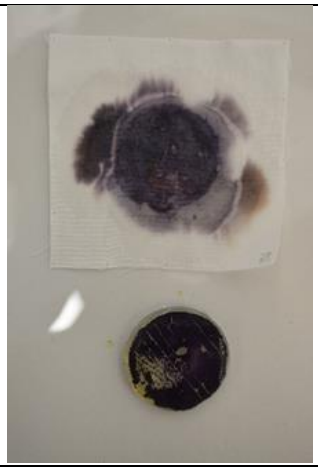
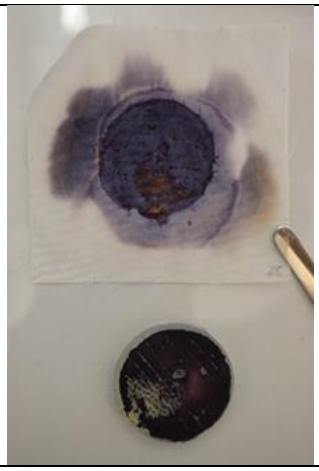
38			
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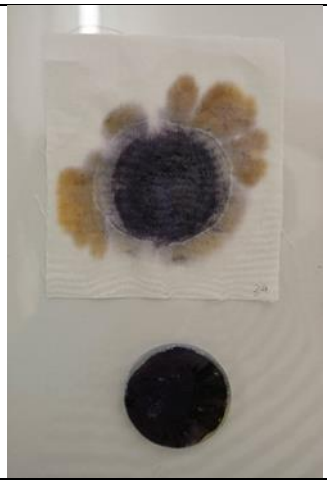
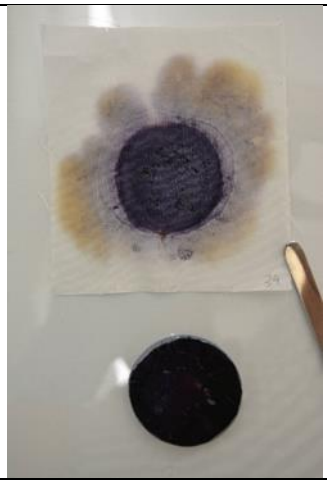
34			
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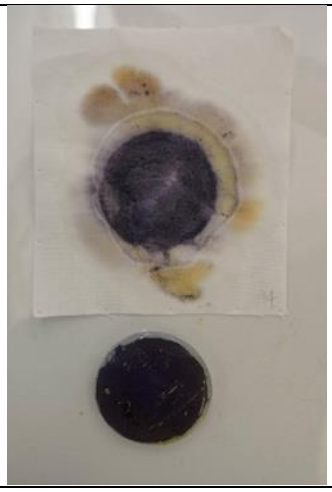
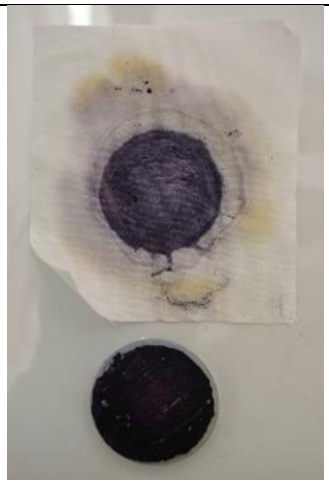
46			
3B-A			
94			
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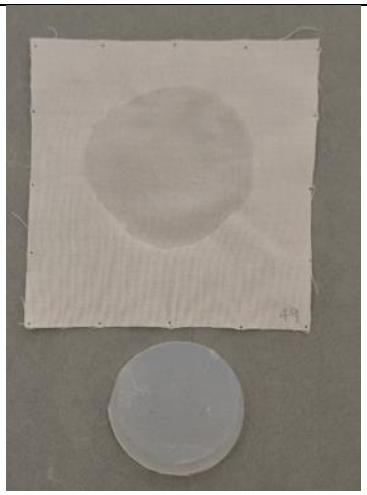
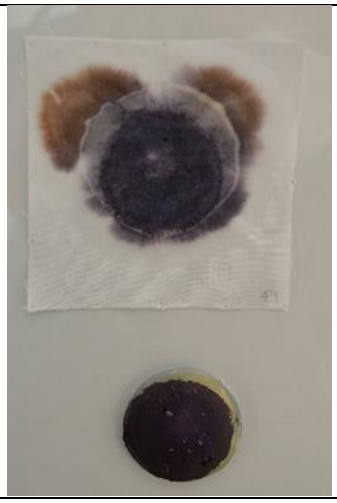
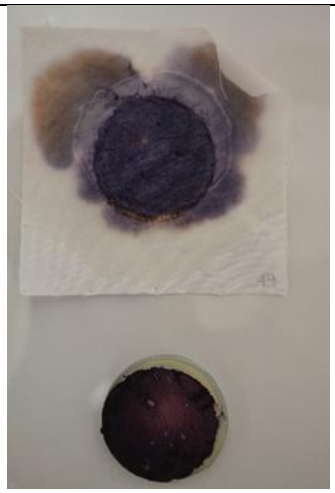
49			
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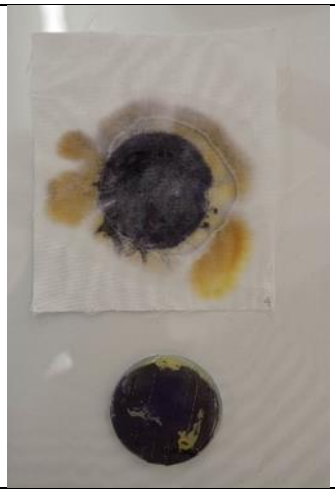
4			
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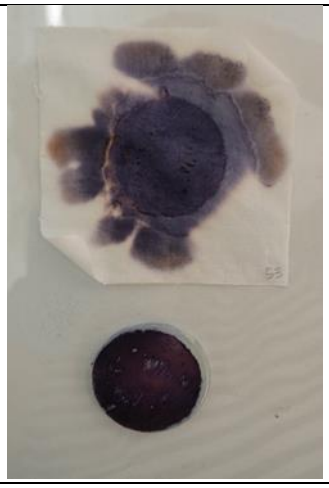
53			
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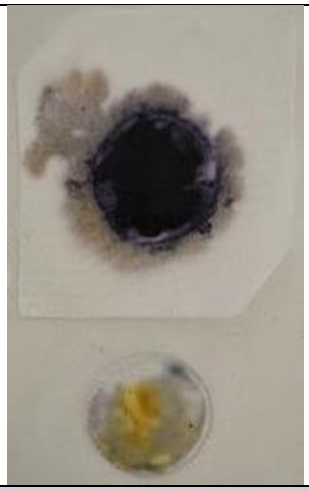
31			
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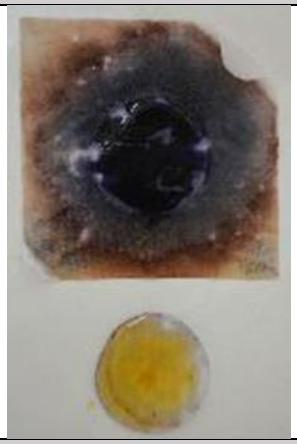
112			
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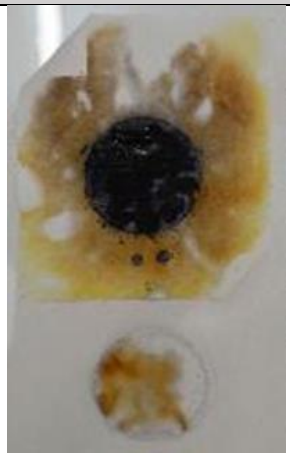
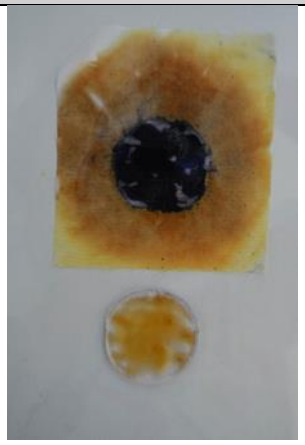
5			
4B-N			

42			
4C-A			
10			
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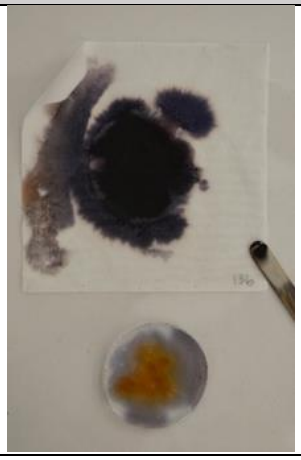
136			

Appendix 8 Materials Sources

Agarose
BP160-100
Molecular Biology Grade
Low EEO/Multipurpose
Lot: 166539
Fisher Scientific UK,
Bishop Meadow Road,
Loughborough, Leicester, LE11 5RG
UK
Tel: 44 1509231166
www.fisher.co.uk

Wheat Starch
Laboratory Regent Grade
Lot: 1666369
Fisher Scientific UK,
Bishop Meadow Road,
Loughborough, Leicester, LE11 5RG
UK
Tel: 44 1509231166
www.fisher.co.uk

α-Amylase
A6814-1MU
≥400 units/mg protein (Lowry)
Lot: SLBN2925V
Molecular Biology Grade A9539
Sigma-Aldrich Company Ltd.
The Old Brickyard
New Road Gillingham Dorset, SP8 4XT
UK
Tel: 44 1747 833000
www.sigmaaldrich.com/united-kingdom.html

Macaroon mould
Lakeland
18A Buchanan Galleries Buchanan St.
Glasgow G1 2FF
UK
Tel: 44 0141 331 1112 www.lakeland.co.uk

Cotton lawn

Whaleys Bradford Ltd.
Harris Court
Great Horton, Bradford
West Yorkshire
BD7 4EQ
UK
Tel: 44 (0) 1274 576718 www.whaleys-bradford.ltd.uk

Iodine
Anhydrous beads
Sigma Aldrich Company Ltd.
The Old Brickyard
New Road Gillingham, Dorset, SP8 4XT
UK
Tel: 44 1747833000
www.sigmaaldrich.com/united-kingdom.html

Potassium Iodide
Anhydrous beads
Sigma Aldrich Company Ltd.
The Old Brickyard
New Road Gillingham, Dorset, SP8 4XT
UK
Tel: 44 1747833000
www.sigmaaldrich.com/united-kingdom.html

Appendix 9 Representative Samples

Age	Unaged
1A	
1B	
1C	

Aged **Unaged**

2A

2B

2C

Aged	Unaged
3A	
3B	
3C	

Aged **Unaged**

4A

4B

4C

Aged	**Unaged**
Control Gel- 60 minutes	Control Gel-60 minutes
Untreated	Untreated