A review of biopolymer-based wood consolidants for archaeological wood preservation

MRes Biomolecular Technology

Industrial Project and Dissertation BIOS4152- Literature Review

Jack Ayrton Crosby- Jack.crosby@nottingham.ac.uk

University of Nottingham, University of Nottingham, School of Biosciences, Sutton Bonington LE12 5RD

Supervisors:

Professor Stephen Harding - University of Nottingham

Contents

Abstract

Biopolymers have been considered as a source of potential consolidating or film-forming agents for many years, with the Oseberg artefacts attracting the interest approaches based upon cellulose, chitosan and other biopolymers. With a strong a strong platform now established in using our understanding of these polymers there is now interest in seeing what modifications can be made to the polymer chain, whether this be the methylation or the addition of hydroxypropyl groups, and what changes these may have on the consolidation of severely degraded and waterlogged archaeological wood samples. This review focuses on four separate polymers of interest, namely hydroxymethylcellulose, hydroxypropylcellulose, hydroxypropylchitosan and sporopollenin. Cellulose and Chitosan based consolidants have a strong basis as consolidants and have been applied in the past to the Oseberg and Mary Rose restoration projects. Sporopollenin, with its reputation as possibly the toughest natural polymer, is a dream consolidant that could permanently solve the Oseberg Problem but issues surrounding solvents and ligin/cellulose interactions remain a big issue.

1. Introduction

Biopolymers are long chains of repeating units called 'monomers'. Biopolymers themselves are defined as being polymers solely composed of one type of biomacromolecule, whether this be DNA, RNA, polysaccharides or proteins and can have a variety of properties suitable for use across a wide array of industries and applications. The use of biopolymers in a wide array of circumstances offers a few key benefits, namely when compared to their manufactured counterparts they are more defined and more complex, with the added bonus of biodegradability (Gowthaman et al., 2021 & Patachia & Croitoru., 2016). However, novel and existing biopolymers must be firmly characterised in terms of their biophysical properties and any relevant interactions they may have with relevant structures if they are to be employed successfully and reproducibly within bioarchaeology. More specifically, this involves the characterisation of the molecular weight or sedimentation coefficient distribution, partial specific volume, potential interactions with other target proteins/molecules and other hydrodynamic properties such as hydrodynamic radius and viscosity.

1.1. Experimental background

There are a wide variety of chemicals and processes that have been utilised in order to consolidate archaeological wood samples. In the past this has ranged from alum salts, petrochemical derived treatments such as polyethyleneglycol, copper ion complexes and naturally occurring compounds primarily in the form of biopolymers (Christensen et al., 2012). Each type of treatment and methodology employed has proven its benefits and drawbacks with some of the most important properties of a successful consolidant being its ability to penetrate a given organic material, whether

this be wood or textile-based, its environmentally friendliness, how safe + easy it is to work with for the conservator, how well it interacts with the sample and whether it is subject to seepage or evaporation back out of the sample.

To assess the suitability of potential bioconsolidants for use in preserving archaeological samples a primary mechanism of first-line assessment is the use of Analytical Ultracentrifugation (See papers by Lu et al., 2021; Wakefield et al., 2018; Wakefield et al., 2020a; Wakefield et al., 2020b; Wakefield., 2021). Analytical Ultracentrifugation involves the centrifugation of a sample at a much higher speed than is typically observed in a standard table-top centrifuge, up to speeds of 50,000 rpm and above (Harding., 2005 & Harding et al., 2010). The samples are observed via two optical methods, interference and absorption, and involve measurements in the change in light transmission through the cells in the centrifuge centerpiece over a number of hours to days (Harding., 2005 & Harding et al., 2010). What this allows is the separation of the physical properties of the analyte to be separated from other confounding factors that may significantly influence the results and is achieved using one of two main methodologies with an array of supporting instrumentation (Harding et al., 1992 & Harding et al., 2010).

The first is termed ‘Sedimentation Velocity’ whereby the sample is subject to a high speed over a relatively short time, typically less than 24 hours (Cole et al., 2008). This gives us a measure of how homogeneous the sample is, useful for characterising the reliability of a given samples results, as well as the confirmation and flexibility of the sample. Sedimentation equilibrium is run at a much slower speed and for a longer period of time and gives a highly precise and accurate measure of the samples molecular weight and the distributions within this parameter via the distribution within the sedimentation coefficient s (Cole et al., 2008 & Harding et al., 1992). This technique is widely used and posseses and important advantage over the complementary technique known as SEC/MALS (Size exclusion chromatography/Multiple angle laser light scattering), namely that no separation medium is required with potential problems of non-inertness (Harding et al., 1992 & Harding et al., 2010).

In terms of the optical detection using AUC or SEC-MALS, as most polysaccharides do not possess the aromatic rings that would allow for ultraviolet absorption, the ultra-violet detectors used to assess proteins and nucleic acids would not work (Harding et al., 1992 & Harding., 2005). Instead, modern analytical ultracentrifuges are equipped with interference optical systems which can register the change in equilibrium concentration distribution with distance (sedimentation equilibrium) or the change in concentration as a function of radial displacement and time (sedimentation velocity). Similarly SEC-MALS use refractometric optical detection systems, linked on-line downstream from the SEC column and light scattering cell. For both pieces of instrumentation an external refractometer can also be used to record the precise sample concentrations and is often initially employed due to assumed sample hydration (Harding et al., 1992 & Harding et al., 2010). In combination with AUC, the use of a Refractometer is used to take the concentrations of the samples, as polysaccharides do not possess the aromatic rings that would allow for IR absorption and thus necessitate a different piece of instrumentation (Harding et al., 2010).

More specifically, *sedimentation equilibrium* utilises the lower rotor speed in order to reach a point of equilibrium whereby there is no net force acting in either direction, with the rate of sedimentation is equilibrated with the force of back diffusion of the molecules being assessed. This has the effect of leaving the molecular weight of the samples as being the only property that can influence the migration of the sample throughout the cell. Once equilibrium has been reached there will be an exponential increase of sample concentration towards the cell base. This gives the molecular weight of the sample, either in terms of the *weight average* molecular weight by use of SEDFIT-MSTAR software (Schuck et al., 2014) and molecular weight distribution using Multisig software (Gillis et al., 2013). It can also provide the information about analyte non-ideality and association/dissociation of the analyte, see Creeth & Harding., 1982; Harding et al., 1992; Harding et al., 2010).

Sedimentation Velocity is based on similar principles. The higher speed at which SV is performed at, up to 50,000 rpm, does not generally allow for an equilibrium to be reached as is found within SE.

Instead, the high speed means the forces of back diffusion are much smaller in comparison and the solute is effectively concentrated at the cell base (Harding et al., 1992 & Harding et al., 2010). The concentration distribution will change with time and distance from the centre of rotation with the use of SEDFIT software (Schuck., 2000) to calculate the sedimentation coefficient s. What both methods can do is give an accurate measure of the molecular weight of an analyte and the distributions of this value or how polydisperse the analyte is, and the quantification of interactions of analyte to target protein/molecule, an example being the interactions between cellulose or chitosan and the desired consolidant (Harding et al., 1992 & Harding et al., 2010).

Another pair of techniques particularly useful for the hydrodynamic study of the consolidants is viscometry and "densimetry" i.e. use of a density meter. A viscometer allows for the measurement of the sample solution's viscosity and when combined with the concentration and density reading the intrinsic viscosity, a measure of conformation, swelling or "hydration" through the association with surrounding water or solvent, and, for non-spherical particles, a measure of molecular weight (Morris et al., 2014). The use of a density meter enables the characterisation of the density of the solvents/buffer and if measured as function of concentration, the partial specific volume (ml/g) with a variety of sample concentrations (Morris et al., 2014), in conjunction with the AUC and SEC-MALS this allows for a comprehensive hydrodynamic characterisation of the analyte (Gillis et al., 2014).

1.2. Oseberg artefacts

Recent research in the field has focused on the use of biopolymers, with recent efforts in the Oseberg project using these techniques and materials. Initially discovered in a burial mound in 1903, the Oseberg artefacts are a collection of Viking age primarily wooden artefacts and consist of a Viking ship, assorted wooden and metal artefacts and textiles as well as the remains of several sacrificial animals. These remain interned at the museum of cultural heritage in Oslo, Norway and prove to be

a valuable cultural asset to the country's cultural wealth (Wakefield., 2021). The conditions within this burial mound had meant that the artefacts were in remarkably good condition but remained waterlogged, a issue that presented itself when trying to preserve these artefacts, furthermore, some artefacts have already been lost, with most remaining artefacts that are still in good condition being made from oak, yew, pine and ash (Wakefield., 2021).

The initial conservation attempts concerned themselves with the use of Alum, initially developed as a consolidant method in Denmark in the 19th century, were deployed to conserve the Oseberg artefacts with good initial success (Cutajar et al., 2022 & McQueen et al., 2019). However, this effort was not as successful as was hoped in the long term, the limited penetration of the alum has left the artefacts with significantly weaker cores and with time has left all alum treated artefacts with a deteriorating condition to the production of acids as the alum breaks down (Cutajar et al., 2022 & Wakefield., 2021). The use of PEG, or Polyethylene Glycol has been documented since the 1960s, low molecular weight PEG samples are known to be soluble in both water and organic solvents, and with a proven ability to penetrate wood and prevent cracking it is a natural consideration for the application to the Oseberg artefacts. Its petrochemical-derived origins certainly act as a downside, with the tide turning against petrochemicals across most industries the appetite for cheap and easy solutions, often involving petrochemicals, is lower than it has been in the past (Christensen et al., 2012 & Wakefield., 2021).

Specifically to the applications to the Oseberg artefacts, it would seem that the Alum was not successful in penetrating past the first few millimetres, the limited penetration of the alum has left the artefacts with significantly weaker cores and with time has left all alum treated artefacts with a deteriorating condition to the production of acids as the alum breaks down (McQueen et al., 2019). The use of PEG, while suitable in some respects also poses its own issues. As such it is desired that there would be an alternative to these chemicals that are fully natural, environmentally friendly and safe to work with.

The Oseberg artefacts themselves are a collection of wooden, textile and iron-based artefacts found within the remains of a Viking age burial mount roughly 100 km southwest of Oslo, Norway. They remain to be some of the best-preserved Viking age artefacts and are an important part of Norway and England's shared cultural heritage, initially constructed in the early 9th century the ship was intended to be a burial chamber and provides an insightful glimpse into how the precursors to modern Norway and England treated their dead (Amberger & Braovac., 2015). The artefacts range from small wooden objects to the larger wooden structure of the ship, to textiles and tapestries and metal objects from jewellery and weapons (Amberger & Braovac., 2015). The importance of preserving these findings cannot be understated, as there is likely to never be such a well-preserved find in this manner regarding 9th century Norse burial mounds (Amberger & Braovac., 2015).

As such there have been a wide variety of methodologies employed when trying to preserve and enhance the conditions of these artefacts with one main consideration in mind, dealing with the waterlogged nature of the artefacts. The conditions of the Oseberg mount meant that the artefacts were waterlogged and kept in a low oxygen environment for over 1000 years. This had the bonus of being the main reason why the artefacts are relatively intact but also brings the main threat to their continued existence, as the removal of said artefacts into a more oxygen rich environment brings with it the serious threat of decomposition and structural damage with drying and resultant warping of the dimensions (Amberger & Braovac., 2015). One obvious way out of this issue is to dry out the samples and remove the driver of bacterial and fungal proliferation, this comes with the issue of shrinkage, cracking and embrittlement of the structures once the water is removed if the wood samples are not treated in some manner (Amberger & Braovac., 2015). The initial treatments in this regard were not as successful as was hoped, the limited penetration of the alum has left the artefacts with significantly weaker cores and with time has left all alum treated artefacts with a deteriorating condition to the production of acids as the alum breaks down (McQueen et al., 2019). This poses a few issues which can try to be addressed, as modern biotechnology progresses the ability to

manipulate naturally occurring polymers gets better and as such the ability to treat wooden artefacts follows.

2. Literature Review

In restoring and maintaining the tapestries and wooden artefacts like those found within the Oseberg findings there are several key factors that must be considered for them to be ideal for use and suitable for maintaining cultural artefacts that have a special significance tied to their existence and their continuation thereof. Firstly, the ideal consolidant would be one that possesses the necessary mechanical properties that would make it compatible with the artifacts that it is to be applied to. There are many different properties that fall under this umbrella with both viscosity and molecular weight being important considerations for how a consolidant may initially interact with the sample, with a must of any consolidant being the ability to penetrate and seep into the material whilst being able to adhere to the structure inside so that there is not seepage of the consolidant back out of the artefact, see Christensen et al., 2015. In this regard a higher molecular weight of > 5000 Da is typically desirable as biopolymers and other consolidants of this size seem to have a demonstrably improved ability to remain within the artefacts (see Christensen et al., 2015; Wakefield et al., 2018; Wakefield et al., 2020). By contrast the same paper by Christensen and coworkers also shows that a higher molecular weight of > 200 kDa has a decreased ability to penetrate the wood when compared to its lower molecular weight (< 20 kDa) counterparts, indicating a 'Goldilocks' zone of sorts, with full penetration of the samples being achieved with the larger Mw chitosan consolidants but lower Mw samples faring better or producing results in a much smaller timeframe.

This is further backed up by research involving by Harding & Wakefield et al at the National Centre for Macromolecular Hydrodynamics and Biophysics (NCMH) at Nottingham, working on the Oseberg

artefacts finding that lower molecular weights of sub 10 kDa typically function better than their larger counterparts (Lucejko et al., 2021; McHale et al., 2017; Wakefield et al., 2018; Wakefield et al., 2020a; Wakefield et al., 2020b; Wakefield., 2021). Another factor thought to aid in the uptake of the consolidant by the sample is the chemical similarity of the consolidant to the sample, while the previous use of petrochemical consolidants stands in stark contrast to this, the success in employing chitosan and other compounds chemically similar to cellulose and lignin, two major structural components of wood, has been suggested to be one of the main reasons behind their recent success (Christensen et al., 2015). In the past, petrochemical derived polymers had been utilised to preserve and restore waterlogged wood samples which while initially successful presented issues that many sought to solve by utilising greener consolidants that could avoid some of the issues.

The Mary Rose restoration project has encountered from the use of PEG based polymers with the polymers degrading into acidic products as well as the plasticising effects that the use of PEG can have on previously waterlogged wood. When a wood sample is already reaching an age and condition where its structure has been degraded to the point that there are significant concerns regarding its longevity the introduction of a chemical that could cause further damage later on, this is mirrored in the papers quoted for the issues with the warship Vasa and its conservation attempts within Sweden. These papers, namely Glastrup et al., 2006 and Mortensen et al., 2007 & 2009, show that the use of PEG in these wood samples has led to excessive degradation than what would have been expected. It was indicated that PEG polymers acted as solid-state ion transporters, further aiding in structural degradation. With sustainability issues and a perceived low eco-friendliness, the general appetite for the use of petrochemicals in conservation works has decreased significantly and has led to a further increase in the investigations of naturally sourced polymers.

A number of alternative substances, namely; Methylcellulose, hydroxypropylcellulose, hydroxpropylchitosan and sporopollenin show promise owing to their natural origins and similarities to the cellulose and lignin structures that make up the backbone of wood structure, with specific

interactions and properties detailed in *Table 1* below. Each have had their respective solution properties considered from what research is available and seem to provide a good avenue for further research. While sporopollenin has not been investigated regarding consolidation abilities, the reputation garnered as being a 'forever' polymer that has longevity far above and beyond most other natural polymers it makes an interesting point to see if this longevity can be brought to artefacts where this is a significant concern.

Table 1 *Comparative table of various previous and potential wood consolidants. C is cellulose, HC is hemicellulose, L is lignin, C/HC denotes interactions with both cellulose and hemicellulose. Viscosity and other hydrodynamic properties were excluded on the basis of lack of information or significant variance observed in literature values. (Brady et al., 2017; Durig & Karan., 2019; Humar et al., 2003a; McQueen et al., 2019; Peng et al., 2005) + Detailed in individual sections due to detail needed*

Properties of past and potential consolidants for archaeological wood

Treatment	Mw (g/mol)	Wood Interaction+	Organo-soluble	Water-soluble	Biodegradability
Alum Salt	474.39	C/HC	Limited	Soluble	Yes
Aminoethanol	61.08 – 61.10	HC & L	In ethanol	Soluble	Yes
Hydroxypropylcellulose	20,000 – 1,500,000	C	Insoluble	Soluble	Yes
Hydroxypropylchitosan	21,000 – 92,000	C/HC	Insoluble	Soluble	Yes
Methylcellulose	10,000 – 220,000	C	Soluble in methanol, ethanol & acetone	Soluble	Yes
Polyethylene glycol	200 – 10,000+	C/HC, L	Soluble	Soluble	No
Sporopollenin	Indeterminate	None known	In aminoethanol	Soluble	Yes*

*Certain moths can break down sporopollenin, see Luo et al., 2011.

2.1. Methylcellulose (Methocel 4AC)

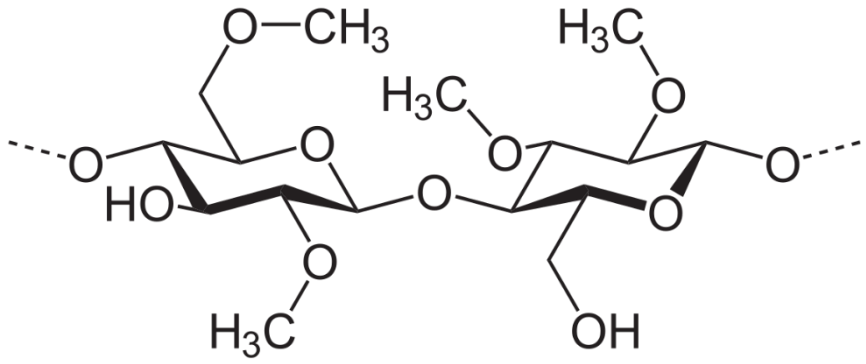


Figure 1. *Methylcellulose (Methocel 4AC) monomer unit. A synthetic polymer derived from cellulose by heating with a solution of sodium hydroxide and the addition of methylchloride. The addition of the methylgroups to the chain leads to an increased water solubility, with this being a consideration for ease of application to the Oseberg artefacts. Cellulose derivatives have been investigated in respect to archaeological wood consolidation (See Cipriani et al., 2010), with methylcellulose being largely ignored for consolidation purposes owing to its higher molecular weight.*

Methylcellulose, product name Methocel, shows promise for consolidation in waterlogged wood samples, see *Figure 1*. A derivative of cellulose, itself a polymer of glucose, and with other cellulose derivatives showing promise for the interaction with wood structures (See Wakefield et al., 2018 & Wakefield., 2021) there is also the hope that these successes can be translated across to this polymer despite a high molecular weight, shown in *Table 1*. Owing to the chemical similarity to cellulose and lignin of methylcellulose, it is generally regarded that interactions will be able to form between the degraded structures found in archaeological wood samples and strengthen the dimensional stability of the artefacts (See *Table* 1 & Miranda-Valdez et al., 2023), with the methylation of the cellulose polymers being noted as vastly increasing the solubility in both water and organic solvents (Shanks & Pardo., 2018). This could be indicative of ease of application and penetration of a methylcellulose consolidant. Interactions observed between cellulose products and lignin shows that methylcellulose

is able to form interactions with lignin and other cellulose polymers, giving a basis for interactions with archaeological wood to be present (Miranda-Valdez et al., 2023). Methocel 4AM is noted as being successfully used as a paint and wood consolidant, with 4AC grade being listed as being 'able to better penetrate fibres and structures', a property of use for wood consolidation (Preservation Equipment Ltd., nd). 4AC differs in its viscosity and its ability to form a paste to other grades of Methocel sold, which would indicate some possible difficulty in penetrating deeper into large samples but this is something that has not been investigated (Preservation Equipment Ltd., nd & Smith et al., 2022). This provides a basis for investigating Methocel 4AC for its suitability in archaeological wood preservation.

It does, however, possess a molecular weight above the upper limit values suggested by Wakefield and colleages in 2020 (Wakefield et al., 2020a & Wakefield et al., 2020b) to be nearly certain to penetrate deep into the wood samples, this raises issues with its potential use. However, successful utilisation in wood and paint consolidation indicates that it posseses the ability to penetrate deeper into wood samples than a Mw of > 100 kDa would otherwise indicate. In materials published by the Levantine foundation, it is shown that in cases of flaky paint remains an application of methylcellulose can save the structure of what is remaining with minimal changes to the colour. This source specifically relates to the topical applications, primarily by brushing, to painted surfaces and does not give any meaningful information as to how methylcellulose may interact with other samples. However, the use in this case illustrates that a 1 % Methylcellulose in a Water + 10 % ethanol solvent was appropriate for increased adhesion of paints to wood and other surfaces. Therefore, it follows that a similar concentration around the 1-5 % may also be investigated for consolidation for samples being submerged in the consolidant. This is a figure replicated in Wakefield and colleagues previous work, with sub 5-10% concentrations typically being considered successful for wood treatments in other cellulose/chitin based polymer solutions (Wakefield et al., 2018; Wakefield et al., 2020a; Wakefield et al., 2020b; Wakefield., 2021).

An issue that would seem to present itself within the literature is that the papers do not seem to have the same focus, namely to consolidate archaeological wood samples. This is not an issue in and of itself, however it does make translation of these properties across to this new application somewhat difficult, no estimations seem to have been carried out into the penetrative abilities of the Methocel solutions. As stated previously this could cause issues as Methocel typically possesses a mw far above a point at which uptake could be guaranteed but its previous use in surface level preservation (Cipriani et al., 2010) and proven interactions with lignin and other cellulose structures mean it could be a potential consolidant appropriate for the Oseberg artefacts, but only further research relating to this will give any substantial answers. Limited investigations into Methocel and other brands of Methycellulose have not detailed any hugely relevant findings, primarily down to differing aims of previous studies, however the previous use of other cellulose derivatives in a successful manner in water-logged wood samples, the successful deployment itself to past archaeological wood samples (See Feller et al., 1991 & Unger et al., 2001) and the properties explained previously make it a highly promising alternative of PEG.

2.2. Hydroxypropylcellulose

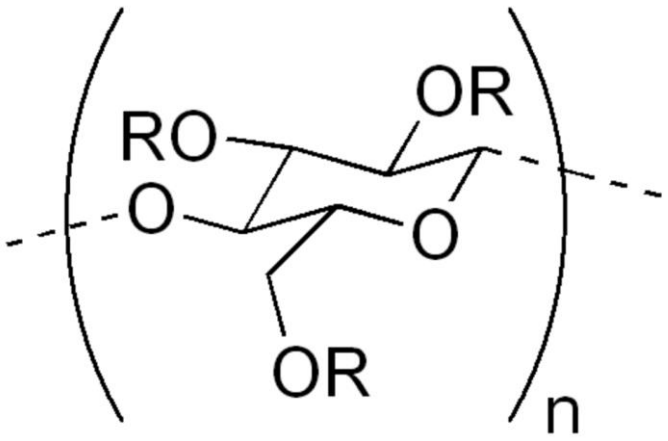


Figure 2 *Hydroxypropylcellulose monomer unit. Produced via the reaction of an alkali cellulose with propylene oxide at a high temperature and pressure, the addition of the Hydroxypropyl groups to the cellulose chain is also shown to increase water solubility, an important consideration for ease of application and penetration for consolidants.*

Another cellulose derived polymer, hydroxypropylcellulose results from the addition of a hydroxypropyl group into the cellulose polymers, seen *Figure 2*. Generally considered quite safe, hydroxypropylcellulose is utilised for synthetic tears for use in conditions where the tear ducts are damaged or are otherwise unable to produce tears (Luchs et al., 2010). This alone is an important consideration and a good sign that if this were to be utilised then the Conservatoires would be perfectly safe in doing so (Barty-King et al., 2021). The reported molecular weight range, see *Table 1*, is around 20,000 – 1,500,000 Da, with high levels of batch and manufacturer variation (Durig & Karan., 2019).

Klucel G, one of the product names for hydroxypropylcellulose (HPC), has a strong basis for use in consolidating deteriorating organic materials. As early as 1997, Gill and Boersma identified that in textile samples could be treated with HPC in an industrial methylated spirits solvent. Ever since there has been a consistent but low activity interest in the use of HPC in the conservation industry, some sources seem to indicate that it is more suitable for topical, film forming applications

Similar research conducted by the British Museum into the stability of Klucel G showed similar results, indicating a consensus on the suitability of HPC as a consolidant for archaeological organic (carbon based) artefacts. However, these papers illustrate a different issue related to the use of HPC, with it being noted that the application of HPC could lead to embrittlement of the sample and the treatment if such a sample was subject to accelerated aging. For Textile samples this could be an issue due to the need for flexibility within the fibres, however it is not stated what exactly is meant by accelerated aging, so other than pointing out this issue is present very little information is given into how it may be avoided. Other papers seem to indicate that proper uptake and 'curing' time

should be taken but this seems to vary wildly between samples, with some papers employing an uptake time of a matter of weeks with others employing upwards of several months (See Cutajar et al., 2023; Kucerova., 2012; Wakefield et al., 2018; Wakefield et al., 2020a; Wakefield et al., 2020b; Walsh-Korb & Avérous., 2019).

With regard its interactions with wood, an evaluation of Klucel G use on the physical properties of Sycamore wood samples led in 2022 by Hassan Sallam and colleagues showed that Klucel G treatment was able to elicit an improvement in the wood samples. This improvement was quantified on the basis that the wood samples would show a change in one of several mechanical and physical properties: Density, porosity, colour and water absorption. This paper lacks the methodology and the intent to have measured the change in strength in the wood (Against pressure/weight application) and focuses more on the change in colour and weight, however this paper is still useful as it illustrates the Klucel G samples interactions with Sycamore wood. For this particular wood sample, Klucel G was shown to have good penetration on samples with 5 x 5 x 5 cm dimensions. Sycamore is not a significant component of the Oseberg artefacts, however successful penetration of these samples and the similarity of Sycamore to wood types used in Oseberg indicate that a Klucel G consolidant could possibly be successful if applied to smaller wood fragments and artefacts thereof. It cannot be stated in this case how well these properties would translate over to archaeological samples, as the wood used in this case was only artificially aged in some cases, so whether the destruction in large part of the underlying cellulose and lignin structure would cause issues is unknown as of yet, but a promising avenue of further research.

Other sources published by the British museum would seem to indicate that Klucel G would be suitable for application to wood-based artefacts, however it is likely that the damage faced by the Oseberg artefacts is much worse than what was found in the samples tested (Shashoua et al., 1990). Therefore, it follows that the degree of interaction observed between the Klucel and the wood samples in this case can be assumed to be greater than what would be expected in the formerly

waterlogged and heavily degraded samples of the Oseberg findings (Gill & Boersma., 1997 & Shashoua et al., 1990). These findings remain unpublished by the British museum (Cited in Gill & Boersma., 1997. Cruickshank., 1995 would seem to cover a similar area but original papers seem unobtainable) and seem to refer to topical applications with painted wood objects, possibly indicating a better film forming property as opposed to consolidation (Gill & Boersma., 1997 & Shashoua et al., 1990). However, despite previous applications of Klucel G in a manner relevant to the artefacts found within the Oseberg collection it remains to be seen whether the increased viscosity of Klucel G compared to Klucel E or other similar products hampers its ability to penetrate the wood (Gill & Boersma., 1997). Typically, what is observed with consolidants (See Wakefield et al., 2018 & Wakefield., 2021) is that a high molecular weight and high viscosity leads to a significantly decreased ability of the consolidant to penetrate to the core of samples. Wakefield et al previously suggested that a molecular weight around the 5000 Da range or below is most suitable for certain penetration of wood samples but can suffer from seepage back out of the wood samples so the potential for a consolidant with a mw higher than this range that can penetrate samples effectively is of great interest (Wakefield et al., 2018 & Wakefield 2021). Walsh-Korb et al in 2022 show how hydroxypropylcellulose in the past has been used, but remains a molecular weight around 80000 Da. This points towards there being a basis for the successful use of polymers larger than a 5000 Da cutoff, often the polymers employed are hydrolysed to create a solution with a much lower mw than initially characterised, but this is not universal.

Overall, hydroxypropylcellulose is indicated to be a promising consolidant for archaeological wood samples. Evidence from Walsh-Korb and colleagues published in 2022 gives further validity to the exploration of polymers with what is typically considered to be a high molecular weight when regarding consolidants, with most of the surrounding literature indicating previous success with Klucel products at consolidating wooden and painted objects. This proves the suitability of Klucel or HPC in ideal conditions, providing a streamlined avenue of research into whether it will be suitable for archaeological consideration.

2.3. Hydroxypropylchitosan

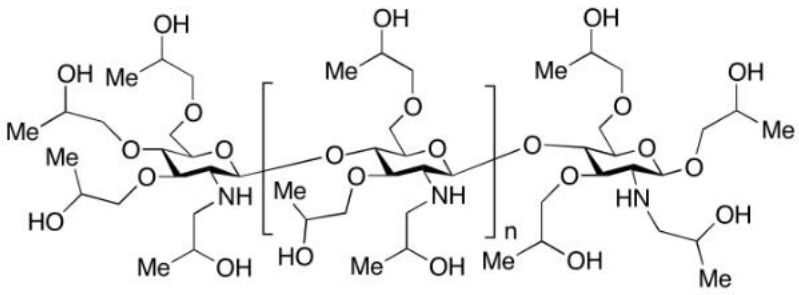


Figure 3 *Hydroxypropylchitosan structure, with square brackets denoting the monomer unit. Derived from chitosan, in turn the product of deacetylation of chitin, one of the most common biopolymers, the addition of the hydroxypropyl groups to the cellulose chain comes about by the application of propylene oxide to an alkali chitosan solution. Patachia & Croitoru outline previous uses of chitosan and derived polymers as consolidants, highlighting chemical similarity to cellulose and lignin as a key component of its success. This is also shown to increase water solubility, an important consideration for ease of application and penetration for consolidants. Image sourced from Toronto Research Chemicals H952740 entry.*

Our interest in hydroxypropylchitosan, seen *Figure 3,* following off the back of previous work that indicated chitosan and derived molecules could work as consolidants and film-formers for waterlogged wood samples (See Christensen et al., 2015 & Wakefield et al., 2020a). As such there has been an increased interest in investigating whether methylated or hydroxylated chitosan samples would exhibit an increased ability to penetrate wood or wool samples, whether they would lead to more or less observable colour change or whether there would be a restoration/increase in the dimensional stability and strength within treated samples (Wakefield., 2021). Based off Chitosan, in turn a natural polymer derived from chitin, a component of crustacean exoskeletons and second most common biopolymer after cellulose (Patachia & Croitoru., 2016). This makes it a sustainable source of consolidants as its source, crab shells, are often discarded as waste. In the past, interest in chitosan polymers was generated by the Oseberg and Mary Rose restoration projects, with chitosan

being looked towards due to its non-toxic, water solubility, metal ion chelation and antimicrobial properties (Christensen et al., 2013; Peng et al., 2005; Walsh et al., 2014; Walsh et al., 2017). Christensen showed in 2013 that chitosan would appear to be promising regarding the Oseberg artefacts and further work by Wakefield has further proven its potential in this regard.

Walsh-Korb et al in 2022 and papers published by the National Centre for Macromolecular Hydrodynamics at the University of Nottingham in conjunction with the National Museum of Cultural Heritage in Oslo (See Wakefield et al., 2018; Wakefield., 2020; Wakefield et al., 2020) have outlined the evidence behind the consideration of chitosan and derived polymers as consolidants. Walsh-Korb demonstrated that the use of chitosan as a consolidant could stabilise and strengthen the lignin structures within archaeological wood samples, which they define as being 'wood that has been excavated from waterlogged environments such as lacustrine pile dwellings, means of transportation, tools or war and trading ships'. It is likely that the samples used in this study mirror the quality and structure of those found within the Oseberg artefacts as well as those used in papers published by Wakefield at the NCMH, meaning that claims from this source are likely to be credible if substantiated elsewhere. Once issue with Walsh-Korbs paper is that no experiments were conducted to ascertain the nature/presence of interactions between the consolidants, with FTIR being typically used to assesses the interactions between a consolidant and the wood samples. Given the high level of similarity between chitosan and structural components like lignin and cellulose this is not a major concern, especially since other papers are able to fill in the gaps in this regard.

One place where there remains a clear gap in the literature is regarding the use of hydroxypropylchitosan. Initially disregarded due to its relatively high molecular weight the addition of a hydroxypropyl group to the polymer should lead to an increased water solubility, a property most useful for consolidants. In a similar manner Hamed & Hassan outlined the use of Hydroxypropylcellulose as a wood consolidant for scenarios remarkably similar to the Oseberg Project, the addition of the Hydroxypropyl groups to the cellulose polymers made the polymer more

plastic in nature. This is a bonus for consolidating as brittle wood samples could benefit very greatly from the addition of a consolidant that could add a level of flexibility and tensile strength that is sorely lacking from the Oseberg artefacts. Considerations for this are similar to the aforementioned Klucel G (hydroxypropylcellulose), there is a basis for using alike chemicals in a manner to what is intended for this project, with their similarity to lignin and cellulose being the crux of why they are ultimately deemed suitable to bring back the strength to the Oseberg artefacts. Other sources generally agree with previous literature in indicating that hydroxypropylchitosan would be a fairly safe avenue of further research for biopolymer consolidants (Hassan et al., 2021).

2.4. Sporopollenin and the use of Aminoethanol as a Solvent/treatment

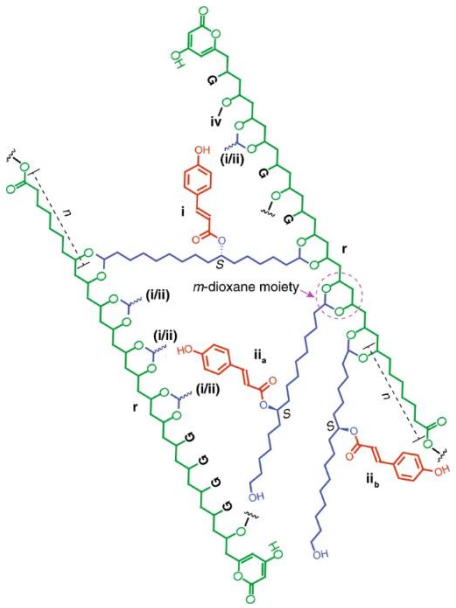


Figure 4. *Hypothesised structure of pine sporopollenin from Li et al., 2019. r units are polyketide-derived PVA-like units containing approx 38 carbons, at one end of the structure is an a-pyrone and on the other is a crosslink to an ester group. n represents the number of carbons given r is derived from a caprylic acid precursor of n = 8 , though this is noted to possibly be other even numbers*

depending on what fatty acids are utilised in synthesis of sporopollenin. G is glycerol-like moieties, i & ii are 7-O-p-coumaroylated C6 aliphatic units, iv is naringenin. Sourced from Li et al., 2019.

Sporopollenin is a biopolymer found in pollen exines and in the outer layers of plant spores (Mackenzie et al., 2015 & Southworth., 1974). Its structure has landed it the name the 'forever polymer' and makes it of interest to bioarchaeology as success in solubilising and applying a sporopollenin solution could effectively mean the end of the Oseberg problem, with the potential for this to spread to other works such as the Vasa or Mary Rose (Jardine et al., 2021 & Li et al., 2019). Primarily composed of an aliphatic-polyketide derived polyvinyl alcohol subunits and 7-O-p-coumaroylated C16 aliphatic units with high levels of crosslinking, visualised above in *Figure 4*. Being found widely throughout the plant world sporopollenin is very safe and requires very little in the way of environmental and safety considerations, with a notable drawback being the lack of evidence of any potential for interactions with cellulose or lignin structures. Given its durability alone it is worthy of consideration for wood consolidation as it could bring back the strength to the Oseberg artefacts on a permanent basis but with one big caveat, it is highly insoluble in most solvents (Lou et al., 20 & Southworth., 1974). A paper titled the solubility of pollen exines and some more recent literature show that aminoethanol is one of these solvents and is the most widely used for preparing sporopollenin solutions, but notes that other solvents are present but are not preferred (Li et al., 2019; Mackenzie et al., 2015; Southworth., 1974).

2.4.1 Aminoethanol

The most promising solvent is Aminoethanol, also known as 2-aminoethanol or ethanolamine, is an organic compound with the formula C_2H_7NO. It contains both an amine (NH_2) group and an alcohol (OH) group attached to the same carbon atom and has found widespread use in various industries and applications, including detergents, pharmaceuticals, cosmetics, and even in bioarchaeology as a wood consolidant and sporopollenin solvent. However, research on the use of aminoethanol in wood

consolidation, particularly by Humar et al at the University of Ljubljana, seems to have diminished since 2013 and its mechanism behind sporopollenin dissolution is largely unsolved.

Aminoethanol appears to interact with the wood structure, specifically the hemicellulose and lignin components, see *Table 1*. According to Humar et al in 2003 (Humar et al., 2003a), aminoethanol reacts with the C=O groups of hemicelluloses and the 1, 3, 4 benzene ring groups in lignin complexes, see *Table 1*. This interaction helps prevent the evaporation of aminoethanol from the treated wood and reduces the seepage of the solution back out. However, the complete mechanism of this reaction is not yet fully understood. It remains to be seen whether the Oseberg wood samples, known for their degradation, possess the necessary functional groups for the solvent to react. This area requires further research for the Oseberg artifacts.

One concern raised in the literature is the potential colour change caused by aminoethanol (Humar et al., 2003a & Humar et al., 2003b). While some sources suggest that this colour change could enhance the appearance of treated wood (Humar et al., 2003a & Humar et al., 2003b), it can pose problems when applied to painted or dyed artifacts like the Oseberg samples. The preservation goal for such artifacts is to maintain their original appearance, making any significant colour change undesirable. Previous studies have not focused strongly on this aspect, so exploring post-treatment colour modification becomes an important gap to address in the literature. Additionally, the compatibility of sporopollenins and aminoethanol with textiles remains unknown, as previous literature has not investigated changes in dimensional strength. Research in this area could shed light on the application of aminoethanol and sporopollenin in consolidating archaeological wood samples and potentially textiles.

The use of aminoethanol as a wood treatment raises some critical issues. While it has been successful in wood treatment (Humar et al., 2003a & Humar et al., 2003b), it has also been used for wood pulp dissolution. The concentration of aminoethanol required for wood pulp dissolution is not clearly specified, nor is the minimum concentration for treatment without causing dissolution. The

concentration of aminoethanol used must be tightly controlled, especially for unique artifacts that allow no margin for error. Studies on the structural integrity and lignin structure could be valuable in determining the exact effects of aminoethanol at safe concentrations suitable for conservation purposes. Literature, while vague, suggests that high concentrations of aminoethanol, potentially as high as 75%+ v/v, are used to break down lignin structures in wood pulp production with high temperatures of around 150 ^{O}C plus (Jimenez et al., 2008; Mikulski & Klosowski., 2021; Zhao et al., 2018). However, a concentration below 50% v/v might allow for the desired interaction demonstrated by Humar et al 2003a and 2003b without compromising the wood's structure, with Mikulski & Klosowski suggesting that any significant delignification is down to a co-treatment of choline chloride and heat treatment, further validating this potential approach.

This potentially indicates an avenue for wood preservation, with no such co-treatment or extreme temperatures being applied to the wood. This aspect requires further investigation, particularly considering the fragile nature of waterlogged wood and textile samples from the Oseberg site, with Humar's previous work using 20% concentrations (Humar et al., 2003a). Aminoethanol's mildly basic nature also makes it a potential buffer against future sulfuric acid production resulting from previous alum salt treatments of the Oseberg artifacts.

The treatment with aminoethanol introduces oxygen into the treated wood structures, but its potential impact on dimensional stability is not addressed in Humar et al., 2003. The paper does not mention any effects of aminoethanol on dimensional stability or increases in structural strength. Exploring the influence of aminoethanol on mechanical strength and dimensional stability in wood samples would be a fruitful avenue for future research. Additionally, the application of aminoethanol to previously waterlogged archaeological samples raises questions about the compromised wood structure's ability to uptake and retain the solvent., though this is something that has not been investigated.

Other research by Humar et al demonstrates the successful use of copper ethanolamine in preservative precipitation, where the conservative is precipitated into the wood structure, resulting in a highly leach-resistant preservation method (Humar et al., 2007a & Humar et al., 2007b). And although aminoethanol's fungicidal activity is noted to be relatively weak by Humar et al, it may still be a relevant property to consider. While this aspect is not a significant concern for the Oseberg project, it could be relevant in future related discoveries or for other artifacts. Aminoethanol serves to provide both great promise and great disappointment for hopeful application to the Oseberg artefacts, with a wide array of research indicating successful preservations in the past with equal research having been conducted in almost the opposite direction. It is likely due to the persistence of Humar and other researchers that there is a zone of interaction that does not illicit delignification, it can be assumed that the concentrations of aminoethanol in cases where wood pulping is intended are most likely above the minimum amount to break down the structure (Claus et al., 2005). Research by Claus et al also hints at aminoethanol being a somewhat selective delignification agent (Zhang et al., 2019), Wallis et al show that only a very small fraction of cellulose and hemicellulose structures are broken down by aminoethanol with papers by Wise et al and Wise & Harlow in the late 1930's further illustrating this. Other literature by Enkvist and Moilanen in 1949 comes to a similar conclusion with 'spruce wood could only be satisfactorily delignified by adding hydrogen sulphide at temperatures of around 100^{o}C', but still discovered that straw and hardwoods faced no issues for delignification.

Sporopollenin and aminoethanol hold promise as a wood consolidant, but several issues and unanswered questions surround its use with further research needed to fully understand its mechanisms, determine optimal concentrations, address dimensional stability concerns, and explore its compatibility with artifacts such as the Oseberg wood samples and textiles. Sporopollenin possesses great promise based off its extreme durability and longevity, but no substantial research has been carried out as to whether it would interact with lignin or cellulose, or indeed into determining its exact structure. Regarding the combination of sporopollenin and aminoethanol, the

challenges associated with using aminoethanol as a wood pulping agent need to be addressed when considering its application to artifacts like those from the Oseberg site. Sporopollenin holds promise as a long-lasting solution for the Oseberg artifacts, provided that potential interactions between sporopollenin and wood can be established through further research.

Almost paradoxically, the likely solvent for sporopollenin aminoethanol has a long track record of use with this being used as a wood preservative, but also has a basis in being used to break up the same structures it would be used to help conserve. A balanced evaluation of the benefits and drawbacks of aminoethanol is crucial for making informed decisions in conservation and preservation practices and whether a sporopollenin solution in aminoethanol would even be worth the potential risk of application to valuable artefacts.

3. Conclusion

A wide array of biopolymers have been used in recent years for preservation of waterlogged wood samples. These wood samples are characterised by their degradation in the ligin and cellulose structures, leading to a significant reduction in the dimensional stability of the wood structure.

Consolidants based on cellulose and chitin polymers already have an established record in use and have shown promise in regard to the Oseberg artefacts in the past, with great promise for modifications in these polymers to increase desirable properties such as solvent solubility. Proven interactions between similar polymers and lignin/cellulose structures gives a strong basis for assuming a similar interaction with Methocel and Klucel products.

Conversely, Sporopollenin provides an exciting avenue for further research with significant drawbacks. With no determination of any possible useful interactions and a lack of applications in a similar manner it remains to be seen whether this forever polysaccharide will be suitable for

consolidation or film forming. While the solvent, aminoethanol seems to have a basis in wood preservation its paradoxical use as a wood pulping agent raises the question of whether an aminoethanol solvent would salvage or slurry an archaeological wood sample, let alone whether it is suitable as a sporopollenin delivery system.

4. References

Amberger, M. and Braovac, S., 2015. Oseberg find. [online] Museum of Cultural History - UiO. Available at: https://www.khm.uio.no/english/research/previous-projects/saving-oseberg/oseberg-find/ [Accessed 1 May 2023].

Barty-King, C.H., Chan, C.L.C., Parker, R.M., Bay, M.M., Vadrucci, R., De Volder, M. and Vignolini, S., 2021. Mechanochromic, structurally colored, and edible hydrogels prepared from hydroxypropylcellulose and gelatin. *Advanced Materials*, *33*(37), p.2102112.

Brady, J., Dürig, T., Lee, P.I. and Li, J.X., 2017. Polymer properties and characterization. In *Developing solid oral dosage forms* (pp. 181-223). Academic Press.

Christensen, M., Kutzke, H. and Hansen, F.K., 2012. New materials used for the consolidation of archaeological wood–past attempts, present struggles, and future requirements. *Journal of Cultural Heritage*, *13*(3), pp.S183-S190.

Christensen, M., Larnøy, E., Kutzke, H. and Hansen, F.K., 2015. Treatment of waterlogged archaeological wood using chitosan-and modified chitosan solutions. Part 1: chemical compatibility and microstructure. *Journal of the American Institute for Conservation*, *54*(1), pp.3-13.

Cipriani, G., Salvini, A., Baglioni, P. and Bucciarelli, E., 2010. cellulose as a renewable resource for the synthesis of wood consolidants. Journal of Applied Polymer Science, 118(5), pp.2939-2950.

Claus, I., Kordsachia, O., Schröder, N. and Karstens, T., 2004. Monoethanolamine (MEA) pulping of beech and spruce wood for production of dissolving pulp.

Cole, J.L., Lary, J.W., Moody, T.P. and Laue, T.M., 2008. Analytical ultracentrifugation: sedimentation velocity and sedimentation equilibrium. *Methods in cell biology*, *84*, pp.143-179.

Creeth, J.M. and Harding, S.E., 1982. A simple test for macromolecular heterogeneity in the analytical ultracentrifuge. *Biochemical Journal*, *205*(3), p.639.

Cruickshank, P., 1995. *Starch and other carbohydrate adhesives for use in textile conservation: postprints*.

Cutajar, M., Braovac, S., Stockman, R.A., Howdle, S.M. and Harding, S.E., 2023. Evaluation of two terpene-derived polymers as consolidants for archaeological wood. *Scientific Reports*, *13*(1), p.3664.

Cutajar, M., Machado, F., Cuzzucoli Crucitti, V., Braovac, S., Stockman, R.A., Howdle, S.M. and Harding, S.E., 2022. Comparative hydrodynamic characterisation of two hydroxylated polymers based on α-pinene-or oleic acid-derived monomers for potential use as archaeological consolidants. *Scientific Reports*, *12*(1), p.18411.

Dürig, T. and Karan, K., 2019. Binders in wet granulation. In *Handbook of pharmaceutical wet granulation* (pp. 317-349). Academic Press.

Enkvist, T. and Moilanen, M., 1949. Ethanolamine sulfhydrate as a reagent for the release of lignin from spruce wood. *Svensk Papperstidning, 52(23),* pp.587-588.

Feller, R.L. and Wilt, M.H., 1991. *Evaluation of cellulose ethers for conservation*. Getty Publications.

Gill, K. and Boersma, F., 1997. Solvent reactivation of hydroxypropylcellulose (Klucel G®) in textile conservation: Recent developments. *The Conservator*, *21*(1), pp.12-20.

Gillis, R.B., Adams, G.G., Heinze, T., Nikolajski, M., Harding, S.E. and Rowe, A.J., 2013. MultiSig: a new high-precision approach to the analysis of complex biomolecular systems. *European Biophysics Journal*, *42*, pp.777-786.

Gillis, R.B., Rowe, A.J., Adams, G.G. and Harding, S.E., 2014. A review of modern approaches to the hydrodynamic characterisation of polydisperse macromolecular systems in biotechnology. *Biotechnology and Genetic Engineering Reviews*, *30*(2), pp.142-157.

Glastrup, J., Shashoua, Y., Egsgaard, H. and Mortensen, M.N., 2006, April. Degradation of PEG in the warship Vasa. In *Macromolecular symposia* (Vol. 238, No. 1, pp. 22-29). Weinheim: WILEY-VCH Verlag.

Gowthaman, N.S.K., Lim, H.N., Sreeraj, T.R., Amalraj, A. and Gopi, S., 2021. Advantages of biopolymers over synthetic polymers: social, economic, and environmental aspects. In *Biopolymers and Their Industrial Applications* (pp. 351-372). Elsevier.

Hamed, S.A.A.K.M. and Hassan, M.L., 2019. A new mixture of hydroxypropylcellulose and nanocellulose for wood consolidation. *Journal of Cultural Heritage*, *35*, pp.140-144.

Harding, S.E., 2005. Analysis of polysaccharides by ultracentrifugation. Size, conformation and interactions in solution. *Polysaccharides I: Structure, Characterization and Use*, pp.211-254.

Harding, S.E., Abdelhameed, A.S. and Morris, G.A., 2010. Molecular weight distribution evaluation of polysaccharides and glycoconjugates using analytical ultracentrifugation. *Macromolecular bioscience*, *10*(7), pp.714-720.

Harding, S.E., Rowe, A.J. and Horton, J.C., 1992. Sedimentation analysis of polysaccharides. *Analytical ultracentrifugation in biochemistry and polymer science*, pp.495-516.

Hassan Sallam, M., Marouf, M., El Hadidi, N. and Mohamed Ali, M., 2022. Evaluation of using Klucel G on the Physical properties of sycamore wood. International Journal of Multidisciplinary Studies in Architecture and Cultural Heritage, 5(1), pp.135-139.

Humar, M., Petrič, M., Pohleven, F. and Destpot, R., 2003a. Upgrading of spruce wood with ethanolamine treatment. European Journal of Wood and Wood Products, 61(1), pp.29-34.

Humar, M., Pohleven, F., Šentjurc, M., Veber, M., Razpotnik, P., Pogni, R. and Petrič, M., 2003b. Performance of waterborne Cu (II) octanoate/ethanolamine wood preservatives.

Humar, M., Žlindra, D. and Pohleven, F., 2007a. Fixation of Copper-Ethanolamine Wood Preservatives to Norway Spruce Sawdust. Acta Chimica Slovenica, 54(1).

Humar, M., Žlindra, D. and Pohleven, F., 2007b. Influence of wood species, treatment method and biocides concentration on leaching of copper–ethanolamine preservatives. Building and environment, 42(2), pp.578-583.

Jardine, P.E., Hoorn, C., Beer, M.A., Barbolini, N., Woutersen, A., Bogota-Angel, G., Gosling, W.D., Fraser, W.T., Lomax, B.H., Huang, H. and Sciumbata, M., 2021. Sporopollenin chemistry and its durability in the geological record: an integration of extant and fossil chemical data across the seed plants. Palaeontology, 64(2), pp.285-305.

Jiménez, L., Rodríguez, A., Serrano, L. and Moral, A., 2008. Organosolv ethanolamine pulping of olive wood: Influence of the process variables on the strength properties. *Biochemical Engineering Journal*, *39*(2), pp.230-235.

Kučerová, I., 2012. Methods to measure the penetration of consolidant solutions into 'dry'wood. *Journal of cultural heritage*, *13*(3), pp.S191-S195.

Li, F.S., Phyo, P., Jacobowitz, J., Hong, M. and Weng, J.K., 2019. The molecular structure of plant sporopollenin. Nature plants, 5(1), pp.41-46.

Lu, Y., Joosten, L., Donkers, J., Andriulo, F., Slaghek, T.M., Phillips-Jones, M.K., Gosselink, R.J. and Harding, S.E., 2021. Characterisation of mass distributions of solvent-fractionated lignins using analytical ultracentrifugation and size exclusion chromatography methods. Scientific Reports, 11(1), pp.1-12.

Łucejko, J.J., de Lamotte, A., Andriulo, F., Kutzke, H., Harding, S., Phillips-Jones, M., Modugno, F., Slaghek, T.M., Gosselink, R.J. and Braovac, S., 2021. Evaluation of soda lignin from wheat straw/sarkanda grass as a potential future consolidant for archaeological wood. Forests, 12(7), p.911.

Luchs, J.I., Nelinson, D.S., Macy, J.I. and LAC-07-01 Study Group, 2010. Efficacy of hydroxypropylcellulose ophthalmic inserts (LACRISERT) in subsets of patients with dry eye syndrome: findings from a patient registry. *Cornea*, *29*(12), pp.1417-1427.

Luo, S., Li, Y., Chen, S., Zhang, D. and Renner, S.S., 2011. Gelechiidae moths are capable of chemically dissolving the pollen of their host plants: first documented sporopollenin breakdown by an animal. PLoS One, 6(4), p.e19219.

Mackenzie, G., Boa, A.N., Diego-Taboada, A., Atkin, S.L. and Sathyapalan, T., 2015. Sporopollenin, the least known yet toughest natural biopolymer. Frontiers in Materials, 2, p.66.

McHale, E., Steindal, C.C., Kutzke, H., Benneche, T. and Harding, S.E., 2017. In situ polymerisation of isoeugenol as a green consolidation method for waterlogged archaeological wood. Scientific Reports, 7(1), p.46481.

McQueen, C., Łucejko, J.J., Flåte, I.M., Modugno, F. and Braovac, S., 2019. Ammonium alum in alum-treated wooden artefacts: discovery, origins and consequences. *Heritage Science*, *7*(1), pp.1-11.

Mikulski, D. and Kłosowski, G., 2022. Delignification efficiency of various types of biomass using microwave-assisted hydrotropic pretreatment. *Scientific Reports*, *12*(1), p.4561.

Miranda-Valdez, I.Y., Coffeng, S., Zhou, Y., Viitanen, L., Hu, X., Jannuzzi, L., Puisto, A., Kostiainen, M.A., Mäkinen, T., Koivisto, J. and Alava, M.J., 2023. Foam-formed biocomposites based on cellulose products and lignin. *cellulose*, pp.1-14.

Morris, G.A., Adams, G.G. and Harding, S.E., 2014. On hydrodynamic methods for the analysis of the sizes and shapes of polysaccharides in dilute solution: A short review. *Food Hydrocolloids*, *42*, pp.318-334.

Patachia, S. and Croitoru, C., 2016. Biopolymers for wood preservation. In Biopolymers and biotech admixtures for eco-efficient construction materials (pp. 305-332). Woodhead Publishing.

Peng, Y., Han, B., Liu, W. and Xu, X., 2005. Preparation and antimicrobial activity of hydroxypropylchitosan. *Carbohydrate Research*, *340*(11), pp.1846-1851.

Preservation Equipment Ltd. (n.d.). Methocel A4C Methylcellulose. *Preservation Equipment Ltd.* Available at: https://www.preservationequipment.com/Catalogue/Conservation-Materials/Adhesives/Methocel-A4C-Methylcellulose [Accessed 1st April 2023].

Schuck, P., 2000. Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. *Biophysical journal*, *78*(3), pp.1606-1619.

Shanks, R. and Pardo, I.M., 2018. Cellulose solubility, gelation, and absorbency compared with designed synthetic polymers. In *Cellulose-based superabsorbent hydrogels* (No. 2, pp. 1-26). Springer International Publishing.

Shashoua, Y, MacKay, C, and Zhang, J, Evaluation of Starch Pastes and Cellulose Ethers used in Textile Conservation, unpublished report, The British Museum, Department of Conservation (1990) pp1-13.

Smith, C.A., Lowe, B.J., Swart, J., Fuentes-Cross, P., Mistral, M. and Te Kanawa, R., 2022. Evaluating Five Consolidants for Black-Dyed Māori Textile Artefacts. *Studies in Conservation*, *67*(5), pp.271-288.

Southworth, D., 1974. Solubility of pollen exines. American Journal of Botany, 61(1), pp.36-44.

The Levantine Foundation., 2020. *Consolidation of flaking media - The Levantine Foundation*. Available at: http://www.thelevantinefoundation.co.uk/wp-content/uploads/2020/11/Consolidation-Handout.pdf (Accessed: 14 April 2023).

Unger, A., Schniewind, A.P., Unger, W., Unger, A., Schniewind, A.P. and Unger, W., 2001. History of wood conservation. *Conservation of Wood Artifacts: A Handbook*, pp.3-7.

Wakefield, J., 2021. *Natural polymers for consolidation of the Oseberg artefacts* (Doctoral dissertation, University of Nottingham).

Wakefield, J.M., Braovac, S., Kutzke, H., Stockman, R.A. and Harding, S.E., 2020a. Tert-butyldimethylsilyl chitosan synthesis and characterization by analytical ultracentrifugation, for archaeological wood conservation. European Biophysics Journal, 49, pp.781-789.

Wakefield, J.M., Gillis, R.B., Adams, G.G., McQueen, C.M. and Harding, S.E., 2018. Controlled depolymerisation assessed by analytical ultracentrifugation of low molecular weight chitosan for use in archaeological conservation. *European Biophysics Journal*, *47*, pp.769-775.

Wakefield, J.M., Hampe, R., Gillis, R.B., Sitterli, A., Adams, G.G., Kutzke, H., Heinze, T. and Harding, S.E., 2020b. Aminoethyl substitution enhances the self-assembly properties of an aminocellulose as a potential archaeological wood consolidant. European Biophysics Journal, 49, pp.791-798.

Walsh, Z., Janeček, E.R., Hodgkinson, J.T., Sedlmair, J., Koutsioubas, A., Spring, D.R., Welch, M., Hirschmugl, C.J., Toprakcioglu, C., Nitschke, J.R. and Jones, M., 2014. Multifunctional supramolecular polymer networks as next-generation consolidants for archaeological wood conservation. *Proceedings of the National Academy of Sciences*, *111*(50), pp.17743-17748.

Walsh, Z., Janeček, E.R., Jones, M. and Scherman, O.A., 2017. Natural polymers as alternative consolidants for the preservation of waterlogged archaeological wood. *Studies in Conservation*, *62*(3), pp.173-183.

Walsh-Korb, Z. and Avérous, L., 2019. Recent developments in the conservation of materials properties of historical wood. *Progress in Materials Science*, *102*, pp.167-221.

Walsh-Korb, Z., Stelzner, I., dos Santos Gabriel, J., Eggert, G. and Avérous, L., 2022. Morphological Study of Bio-Based Polymers in the Consolidation of Waterlogged Wooden Objects. Materials, 15(2), p.681.

Wise, L.E. and Harlow, W.M., 1938. Contributions to the chemistry of the plant cell wall. VII. The cellulose in the rhizomes of brake fern (Pteris Aquilina). American Journal of Botany, pp.217-219.

Wise, L.E., Peterson, F.C. and Harlow, W.M., 1939. The action ethanolamine on woody tissue. *Industrial & Engineering Chemistry Analytical Edition*, *11*(1), pp.18-19.

Zhang, L., Zhao, D., Feng, M., He, B., Chen, X., Wei, L., Zhai, S.R., An, Q.D. and Sun, J., 2019. Hydrogen bond promoted lignin solubilization and electrospinning in low cost protic ionic liquids. ACS Sustainable Chemistry & Engineering, 7(22), pp.18593-18602.

Zhao, Z., Chen, X., Ali, M.F., Abdeltawab, A.A., Yakout, S.M. and Yu, G., 2018. Pretreatment of wheat straw using basic ethanolamine-based deep eutectic solvents for improving enzymatic hydrolysis. *Bioresource technology*, *263*, pp.325-333.

5. Acknowledgments

I would like to Thank Professor Stephen E. Harding, Dr Jennifer Wakefield and Oliver Meeds for their assistance and guidance throughout the early stages of my research project and in particular for helping me construct this literature review.

New cellulose and chitosan-based polymer consolidants for archaeological wood consolidation

MRes Biomolecular Technology

Industrial Project and Dissertation BIOS4152- Project Report

Jack Ayrton Crosby- Jack.Crosby@nottingham.ac.uk

University of Nottingham, University of Nottingham, School of Biosciences, Sutton Bonington LE12 5RD

Supervisors:

Professor Stephen Harding - University of Nottingham

Contents

1. Abstract

Several biopolymers, namely hydroxypropylcellulose, hydroxypropylchitosan and methylcellulose have been assessed for their suitability as consolidants for archaeological wood samples coming from waterlogged low oxygen environments. In order to be deemed successful in this manner they should fit a number of parameters, the principle of which being the maintenance of the structural dimensions and original appearance of the artefact as well as the strengthening of the artefacts structure. Initial characterisation showed that all materials assessed had molecular weights above the established molecular weight to guarantee sample penetration. Testing with wood pieces of 2 x 2 x 1 cm and larger 20 x 5 x 4 cm pieces give a somewhat mixed indication for their efficacy. When cutting for sampling and microscopy treated samples faired significantly better than freeze dried and air-dried controls. When looking towards the FT-IR spectra and SEM results, very limited consolidation and penetration of the samples was observed, with only the PEG-2000 and Klucel G treatment showing a prevention of structural collapse. No real differences were observed in the FTIR spectras, with only the PEG-2000 spectra producing a peak ~ 2800 cm^{-1} that was not present in the control. Overall, these treatments do not seem to be very promising, with the control PEG treatment and Klucel G coming out as the best overall but with significant variance in the wood structure and strength both pre and post treatment it is difficult to say what affect the consolidants have had here. Further issues from freeze drying illustrate that none of the consolidant solutions stayed frozen with the freeze drier used, leaving the possibility of the water tension causing excess structural collapse. This could also have been followed by the removal of the consolidant by the vacuum, potentially explaining the results achieved.

2. Introduction

One of the critical challenges in bioarchaeological conservation is finding suitable consolidants for preserving archaeological samples. Historically various chemicals and processes including alum salts, petrochemical-derived treatments like polyethyleneglycol have been employed for consolidation. With issues with drying, colour change, stability and increasing demand for sustainability biopolymers have increasingly fit the criteria and have been the area of interest (Broda & Hill., 2021& Walsh-Korb et al., 2022).

The use of biological polymers in archaeology has a strong basis in recent years for the restoration of the strength and stability of wood-based artefacts. Previous attempts at the NCMH in Nottingham have used a variety of cellulose and chitosan-based polymers based off their chemical similarity to the cellulose and lignin structures. The primary concerns of any potential consolidant is twofold, with the molecular weight of a consolidant effects its ability to penetrate and interact with a wood sample. No concrete molecular weight limit exists as to what will prevent the penetration of the consolidant into the wood, so it is increasingly of interest to determine whether certain biopolymers will be suitable as consolidants and to what effect the molecular weight will have. Research has outlined that methylcellulose, hydroxypropylcellulose, hydroxypropylchitosan and sporopollenin should possess properties that would make them ideal considerations for consolidants., whether this be a previous use case in archaeology with surface consolidation, consolidating fibrous materials or the hope of harnessing the strength or similarity to cellulose and lignin. The waterlogged nature of many archaeological finds and specific to the Oseberg artefacts means that the underlying cellulose lignin structures are severely degraded, perhaps pointing towards the ease of penetration by larger polymer structures, thus bringing in polymers that had been discarded in the past due to uncertainty of sample penetration (Broda & Hill., 2021; Walsh-Korb et al., 2022; Wakefield., 2021).

To evaluate these potential consolidants for preserving archaeological samples, Analytical Ultracentrifugation (AUC) has emerged as a primary mechanism for first-line assessment. Involving centrifuging the sample at high speeds and utilizing interference and absorbance optic methods to observe the sample's behaviour over time. Two main methodologies within AUC, *Sedimentation Velocity* and *Equilibrium*, allow for precise measurements of molecular weight, distribution, and interactions with target proteins/molecules.

Methylcellulose, or brand name Methocel 4AC, has a basis in being used in surface consolidation of painted wooden objects. Materials from the Levantine Foundation establish the use of methycellulose as a surface consolidant, with the hope being that despite the higher molecular weight it should be able to penetrate the heavily degraded wood samples like those in the Oseberg artefacts. In a similar vein, the use of hydroxypropylcellulose or brand name Klucel G has a strong basis in consolidation with use by the British museum and previous research by Hassan Sallam and colleagues show that hydroxypropylcellulose has a good basis in being able to form interactions in carbon-based artefacts. Both cellulose based polymers carry the promise that due to their inherent similarity with the cellulose found in wood that they should be able to successfully form interactions with the degraded structure and consolidate the wood sample.

Hydroxyproplychitosan, a polymer derived from the crab shell polysaccharide chitin, shows great promise in being a wood consolidant. Previous research has shown that there are several different chitosan-based polymers that work well as film-formers and consolidants, with the hope that the addition of the hydroxypropyl group will make the polymer more soluble in water, an ideal property to have for a consolidant (Christensen et al., 2015). Molecular weights, again, remain above established boundaries that would guarantee penetration, but the degradation of the samples could make this a minor issue (Christensen et al., 2015).

Sporopollenin, a plant pollen exine, is renowned as being one of the toughest polymers found in the natural world has the potential to singlehandedly solve the Oseberg problem. With issues concerning

solubility and whether it would interact with cellulose or lignin structures it remains unclear as to whether sporopollenin is appropriate for use (Li et al., 2019).

A comprehensive characterisation of these polymers using AUC as well as a trial treatment will show whether the molecular weights are in the range to be concerned and whether this concern translates over to a lack of success in consolidating archaeological wood samples. The working hypothesis is that the wood degradation should allow for the discounting of the molecular weights in being such a crucial factor in sample penetration and make them at least somewhat suitable for use.

3. Methods

3.1. PBS Buffer preparation

Table 1. *PBS buffer solution properties for use in AUC runs (SV + SE) of all solutions analysed. Amounts refer to preparation of 1L PBS pH 7 0.1M preparation. *Reference pH solution measured at 6.69 on same pH meter.*

Theoretical pH	**Measured pH**	**disodium hydrogen orthophosphate dodecahydrate**	**potassium dihydrogen orthophosphate**	**sodium chloride**	**Density (g/cm^3)**	**Viscosity (P)**
7	6.66*	4.595 g	1.561 g	2.923 g	1.00334	0.01110

Standard pH 7.0 0.1M PBS buffer solutions were used throughout this investigation, being made up in quantities of 1 L and 2 L respectively.

3.2. Refractometer measurements and dn/dc measurements

With the samples being a polysaccharide, the only suitable method of concentration determination was via the use of a refractometer. For this, a ATAGO DD-7 Differential Refractometer was used. Initially, 5 mL aliquots of PBS buffer solution were injected into both the reference and sample ports, not pushing through the entirety of the solution. A simple mean average was taken of 3 readings to give an averaged Brix value for each sample concentration.

In some cases, it was necessary to determine the refractive index increment, dubbed dn/dc, as this was not known prior to investigation. In this case, undialysed samples of a known weight per unit solution followed the same procedure as above across a range of concentrations. Once these were completed a graph of Brix against estimated concentration was plotted with the gradient of the line of best fit being used to determine the refractive index increment using the following equation to get the dn/dc value of the sample.

$$Concentration = Brix \times \frac{\frac{dn}{dc} sample}{\frac{dn}{dc} sucrose} \times 10$$

The dn/dc value of sucrose is reported as being 0.15.

3.3. Density measurements and partial specific volume ($\bar{v}$) determination

For Density measurements an Anton Paar DMA 5000 Density meter was utilised for the measurements. Prior to measurements samples were left out on the lab side to wait for the sample temperature to reach equilibrium (approx. 20^{O}C), to reach consistency with results. Samples were pushed through the density meter in aliquots of 3 mL, taking care to avoid bubbles before taking a reading. Further aliquots of 3 mL were pushed through another two times from the same syringe to avoid bubbles and resultant artefacts. A simple mean average was taken of these 3 readings for each buffer solution used and each sample concentration ranging from 1 mg/mL to 5 mg/mL.

In cases where partial specific volume or $\bar{v}$ was not known, density readings were taken across a range of concentrations from 1 mg/ml to 5 mg/ml. These results were used to plot a graph of concentration against solution density, with the gradient and intercept being derived from this graph and used to calculate the partial specific volume using the equation below where ρ_0 is the intercept of the graph and dp/dc is the gradient of the line of best fit.

$$\bar{v} = \frac{(\frac{1}{\rho 0})}{1 - \frac{dp}{dc}}$$

3.4. Sedimentation Velocity

Sedimentation Velocity experiments were carried out using a Beckmann Coulter XLI analytical ultracentrifuge. 7 Cells of concentrations ranging from 0.3 – 1.0 mg/ml were used for each sample in PBS buffer 0.1M pH 7 (detailed *Table 1*) for a total of 7 cells per run. Reference and sample channels were filled to 400 µl into 12 mm pathlength double-sector epoxy cells with sapphire windows. Samples were run at 45,000 rpm at 20^{O}C overnight with scans being taken every 3 minutes for a total of 450 scans. SEDFIT software was used for analysis, giving a distribution of sedimentation coefficient (lgs(s) or g(s)). All sedimentation coefficients were normalised to standard conditions of the buffer density and viscosity at 20^{o}C. SEDFIT software (Schuck., 2000) was used for analysis with the c(s) distribution being used (Chaturvedi et al., 2018)

3.5. Sedimentation Equilibrium

As above, sedimentation equilibrium experiments were carried out using a Beckmann Coulter XLI analytical ultracentrifuge. 7 Cells of concentrations ranging from 0.4 – 1.1 mg/ml were used for each sample in PBS buffer 0.1 M pH7 (detailed *Table 1*) for a total of 7 cells per run. Reference and sample channels were filled to 100 µl into 12 mm pathlength double-sector epoxy cells with sapphire windows. Samples were run at variable speeds (typically 10,000 – 25,000 rpm) over a weekend with scans being taken every hour. SEDFIT MSTAR software was used for analysis giving the signal average + hinge point molecular weights and a less accurate molecular weight distribution (c(M)).

3.6. Wood preparation

4 large pieces of wood were supplied by Chas Jones for the purposes of this project. These pieces were initially cut down into more rectangular-esque shapes to ease excision of smaller, more workable pieces. During this process it was noted that the surface structures, what was presumably once the bark/outer rings of the wood, very closely resembled the peat that was initially surrounding the wood. This was in both in appearance and texture, with these areas closely resembling dirt/peat indicating very heavy levels of degradation and as such every effort was made to exclude these sections. The result was a total of 45 pieces of wood of dimensions ~ 2 x 2 x 1 cm with mostly uniform density.

3.7. Wood treatments

Out of the 45 pieces of wood obtained from the larger wood samples, only 37 were deemed to be acceptable with the remaining pieces having been identified as outliers by means of their dimensions, weight and density. Initially it was planned that there would be a total of 5 treatments with 7 pieces per treatment, however it was not considered when making the wood cubes that there would be a need for a air-drying and freeze drying comparison and as such previous pieces identified as undesirable were used for the PEG-2000 treatment.

Table 2. *Overview of selection for wood treatments.*

Treatment	Pieces
Hydroxypropylcellulose (80 kDa)	7
Hydroxypropylcellulose (110 kDa)	7
Hydroxypropylchitosan	7
PEG-2000	7*
Water (Freeze drying)	7
Water (Air drying)	7

**Due to limits of sample number wood pieces identified as anomalous were utilised for PEG-2000 treatments in order to reallocate to airdrying.*

3.8. Freeze drying

An Edwards Modulyo Freeze Dryer was used to dry out the wood samples. Samples were initially transferred to a -80^{O}C freezer overnight before moving to the freeze dryer. kept at a vacuum below 10 mBar and temperatures of -45^{O}C. Samples were freeze dried until a constant mass was obtained.

3.9. Moisture content determination

Wood pieces of measurements 2 x 1 x 1 cm were taken and placed in a 105^{O}C oven. The mass of each of the pieces was measured and put back into the oven until a constant mass was reached. The difference between the wet mass and the dry mass was taken and used to calculate the moisture content of the wood samples used in this investigation using the below equation.

$$Moisture\ content\ (\%) = 100\ \times \frac{Dry\ mass\ (g)}{Wet\ mass\ (g)}$$

3.10. Colourimetry

A Konica Minolta CM-700d spectrophotometer was used for the colour change determination using the CIELAB colour space. 5 readings were taken from the front and back sides of each sample piece of wood used throughout the consolidation testing. These were averaged on a per piece basis and used in the following formula to calculate the absolute colour change ΔE_H.

$$\Delta E_H = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

L represents lightness from black to white with a and b representing chromaticity.

3.11. Tape Test

For each piece in each treatment class, both control and consolidants, separate pieces 3M double sided Scotch tape were cut down into a 1 x 1 cm square, weighed and placed onto the front or back sides of each wood piece. These were gently pushed down 3 times on each side and then slowly removed before weighing again to get the weight difference.

3.12. Scanning electron microscopy & Energy-Dispersive spectroscopy

A Quanta 450 electron microscope was used for the environmental scanning electron microscopy images and EDS analysis. Samples were cut from each treatment type and secured into the microscope chamber, placing carbon tape connecting the samples to the platform to help dissipate any charging. A voltage of 11.5 KeV, spot size of 6.3 and magnifications of approx. 300, 500 & 1500x were used for the imaging. EDS was performed on samples anticipated to have a different elemental component.

3.13. Fourier-transformed Infrared specroscopy

A Nicolet IS50 FT-IR instrument was used for the FT-IR measurements. Wood pieces used in the control and treatments were cut/snapped in half, with 5 small fragments being taken from the middle of the sample and readings averaged on a per treatment basis. Pure consolidant samples in powder form were also measured 5 times and an average taken, with an approximate mass of 0.05 g per reading used. Readings were taken from one wood piece from each treatment, except for the control freeze dry sample where two pieces were used to show that readings were near identical within treatments.

4. Results

Some departures were made across the course of this project and for convenience the work carried out on each of the potential consolidants is outlined in *Table 3* below for convenience.

Table 3. *Materials included in the two main stages of the project. Characterisation includes materials that AUC SV/SE + SEC MALs was carried out on. Wood treatments refer to consolidants applied only to wood pieces as treatments*

	Inclusion in	
Wood treatment	**Characterisation**	**Wood Treatment**
Hydroxypropylcellulose*	✓	✓
Hydroxypropylchitosan	✓	✓
Methycellulose	✓	X
PEG-2000	X	✓
Sporopollenin	X	X
Deionised water**	N/A	✓

***Both 80 kDa ('Klucel E') and 110 kDa (Klucel G) were assessed for both characterisation and treatment.*

***** Inclusive of both freeze and airdrying*

4.1 Partial specific volume $\bar{v}$ determination

The Partial specific volumes, $\bar{v}$, was not known for the samples characterised as part of this study. Concentrations from 1 – 5 mg/ml had their respective densities measured and this was plotted and fitted with a linear line of best fit to determine the partial specific volume. Initially concentrations < 1 mg/ml had been used but these gave highly variable results which did not remain to be reliable upon later investigation. Similarly, a high level of variability was noted between density measurements on different days so effort was made to complete measurements of a given solution within one sitting.

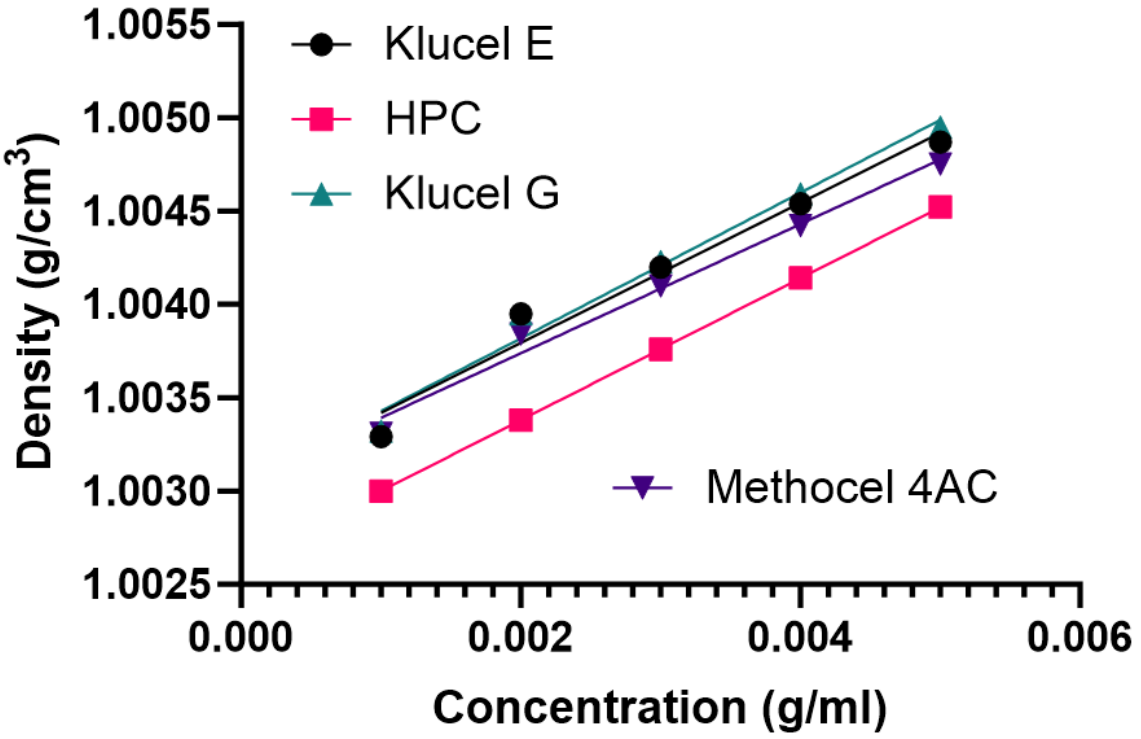


Figure 1. *Graph used to calculate partial specific volume from gradient and y intercept. HPC had a $\bar{v}$ = 0.619, Klucel E had $\bar{v}$ = 0.633, Klucel G had $\bar{v}$ = 0.600 and Methocel 4AC had $\bar{v}$ = 0.601. Vastly different results were obtained on different days across different concentrations, though 1 – 5 mg/ml yielded the most consistent results and as such these are what have been used.*

Partial specific volumes were determined to be around the 0.6 ml/g range, firmly within the range of what is expected of polysaccharides, derived from the graph shown *figure 1.*

4.2. Sedimentation velocity results

4.2.1. Hydroxypropylcellulose – 'Klucel E'

The results of the velocity analysis of hydroxypropylcellulose from Thermo Scientific (Dubbed Klucel E or HPCell for convenience) are consistent with a monodisperse system around 1.35 s, visualised in *figures 2 & 3*. Two sets of species are identified, with one of these being near zero and as such likely being an analysis artefact, shown *figure 3*. Small changes in the sedimentation coefficient are observed with increasing concentration as shown better in *Figure 3*, although it is not clear whether this is coming from a genuine increase in the observed sedimentation coefficient or whether it is coming from a more accurate analysis of the data, whether this be due to self-association or not. These results are consistent with a polydisperse polysaccharide with a significant sub 10 kDa component.

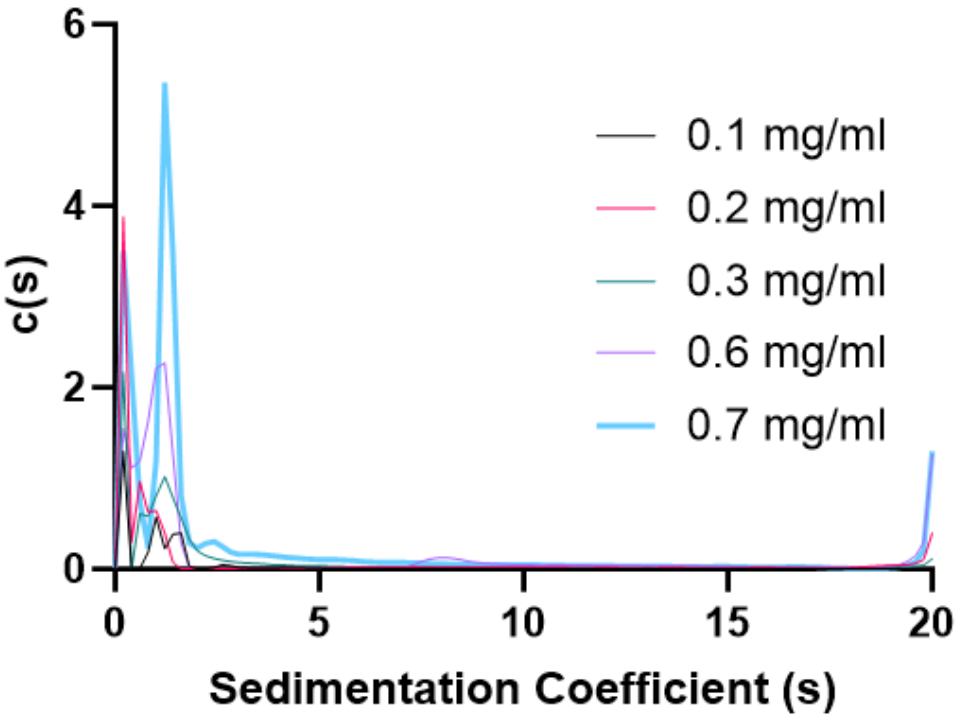


Figure 2. *Sedimentation coefficient results from sedimentation analysis of hydroxypropylcellulose (Klucel E) at 45,000 rpm, corrected to standard conditions. Upwards trend of data at 20 s is an artefact of the analysis and was not present if this axis was extended to 25,30 or 50 s.*

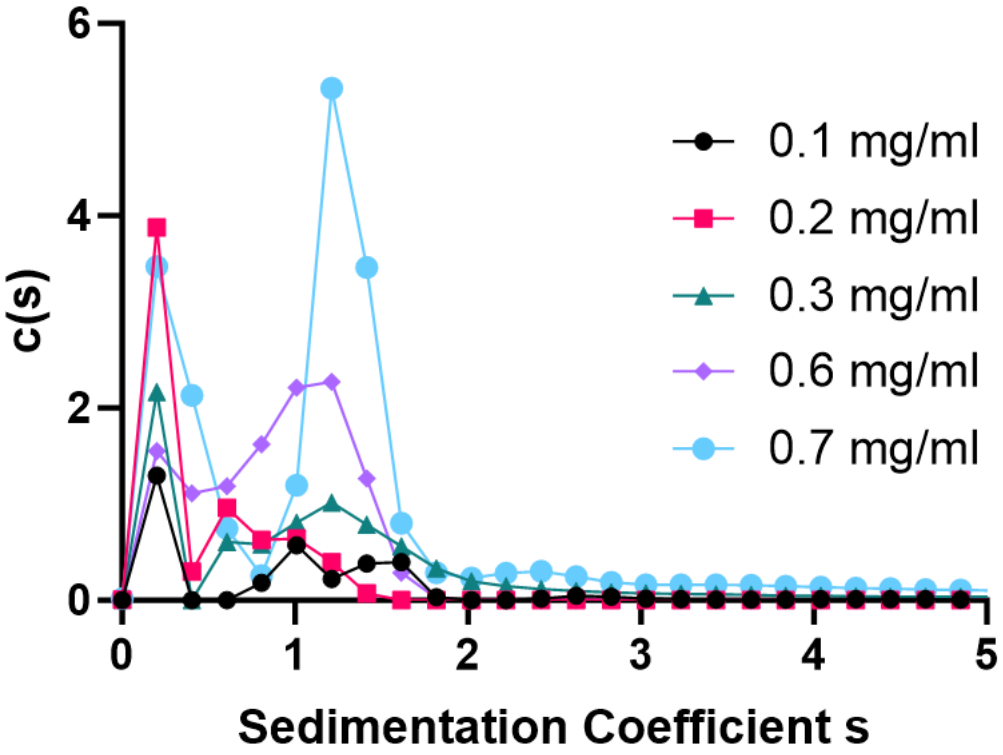


Figure 3. *Zoom in of sedimentation coefficient distributions corrected to standard conditions. Signals here accounted for > 90% of total signal in each concentration measured. Increasing peak amplitude around 1.35 s is noted with increasing concentration. It is likely near zero peaks are analysis artefacts and should be regarded as such.*

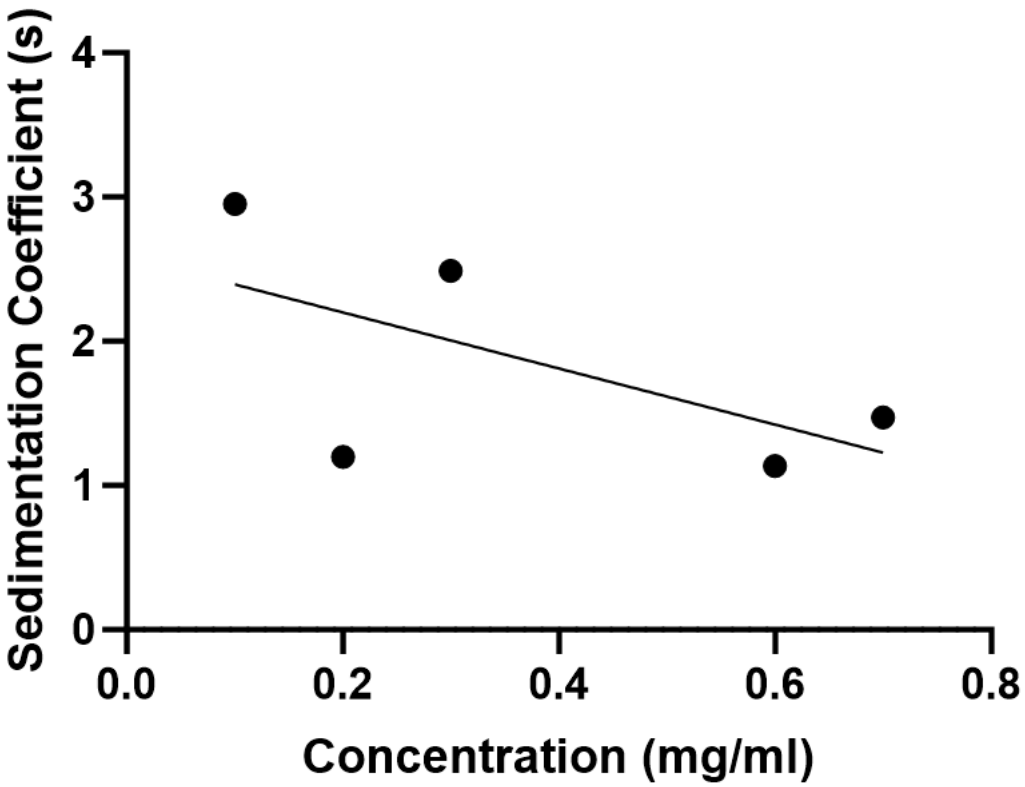


Figure 4. *Extrapolation of corrected sedimentation coefficients for each concentration of Klucel E. A very general trend of decline with increasing concentration indicates non-ideality. Values obtained from integration of main peaks from SEDFIT. Extrapolation back to zero concentration gives 2.64 s.*

4.2.2. Hydroxypropylcellulose – Klucel G

The results of the sedimentation velocity analysis of hydroxypropylcellulose Klucel G indicated that the system is polydisperse across a close range of 1 – 2.6 s, shown *figure 5 & 6,* with some near zero peaks that are most likely artefacts of the analysis, or possibly the result of the SEDFIT software being pushed to its limits by the presence of low molecular weight species. No real changes in sedimentation coefficient are observed with changing concentration, peaks fell within the same range with increasing amplitude found with increasing concentration as is expected. Extrapolating these results back to zero concentration to account for non-ideality in the system gets a result of 2.437 s, *figure 7*, a value not dissimilar to the results obtained owing to no real observable gradient in the fit to the data.

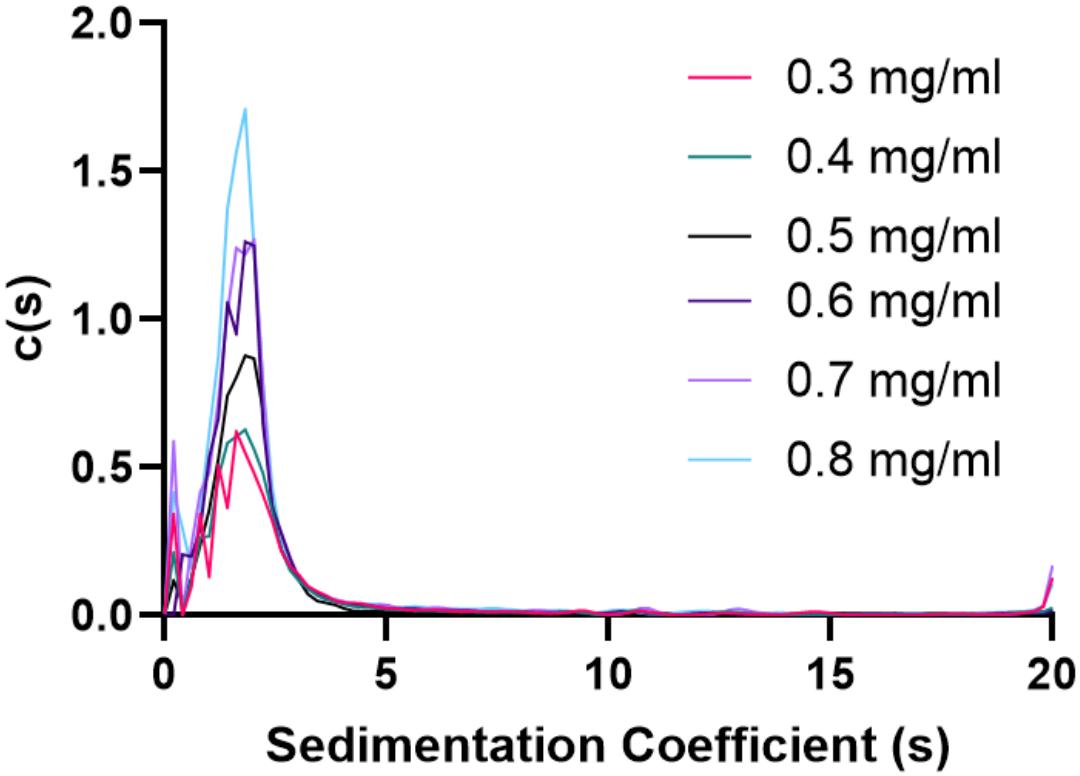


Figure 5. *Sedimentation coefficient results from sedimentation velocity analysis of hydroxypropylcelullose (Klucel G) at 45,000 rpm, corrected to standard conditions.*

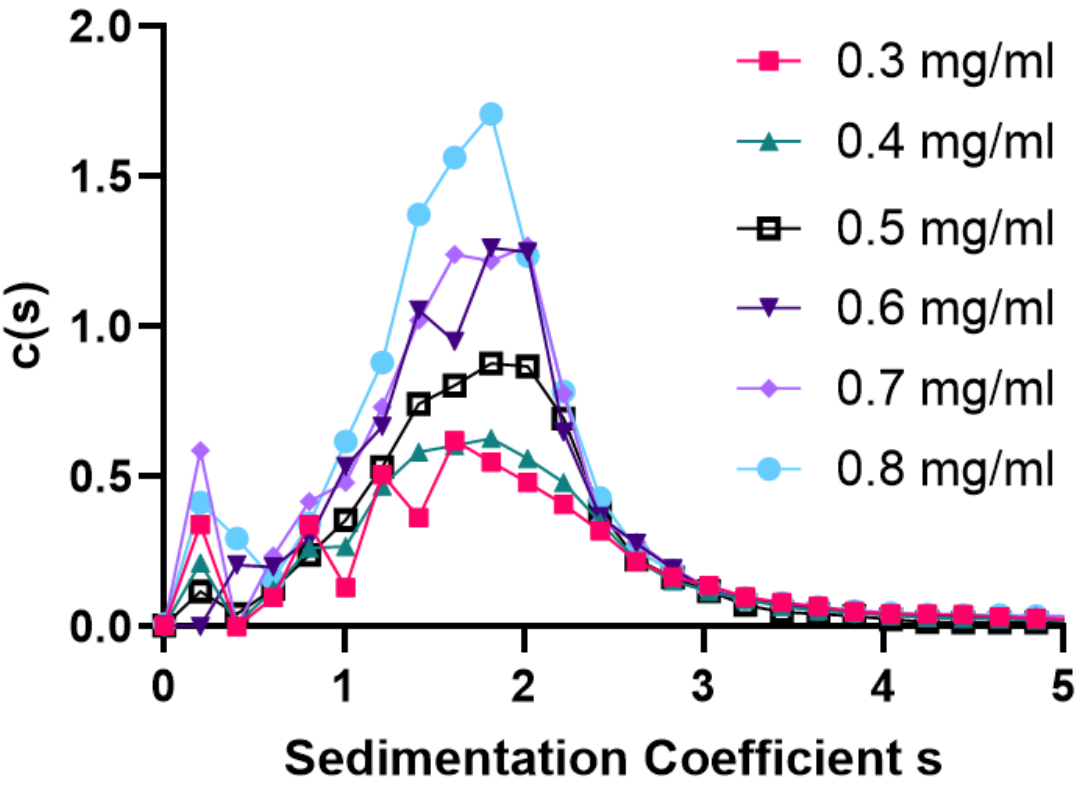


Figure 6. *Zoom in of sedimentation coefficient distribution corrected to standard conditions. Signals here accounted for upwards of 75% total signal in each concentration measured. No real change is noted with increasing concentration, indicating limited to no self-association with increasing concentration. An initial peaks around 0.25 – 1 s indicate the presence of sub 10 kDa species within the solution and potential artefacts of analysis.*

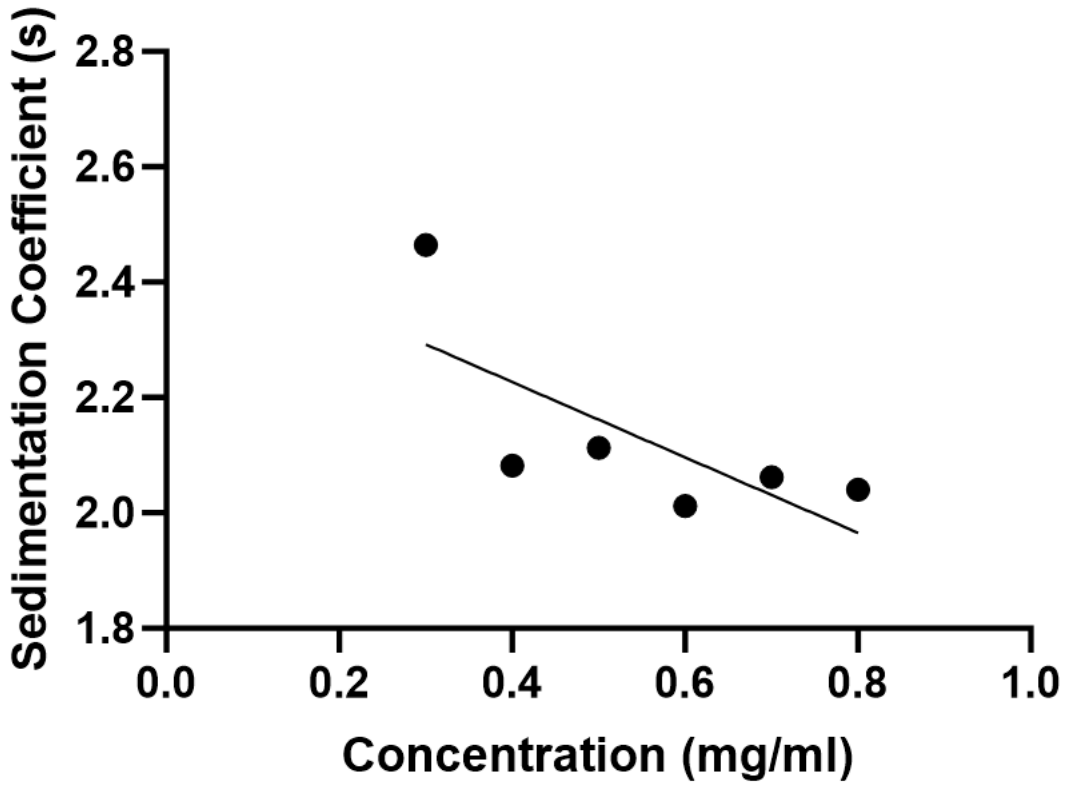


Figure 7. *Extrapolation of corrected sedimentation coefficients for each concentration of Klucel G. A very general trend of decline with increasing concentration indicates non-ideality. Values obtained from integration of main peaks from SEDFIT. Extrapolating back to zero concentration gives 2.488 S.*

4.2.3. Hydroxypropylchitosan

Sedfit analysis of hydroxypropylchitosan yielded consistent results across all concentrations measured, with a value around 2.8 s, visualised *figure 8 &9*. The polymer seems to be monodisperse with this being evidenced by the lack of multiple peaks. No self-association seems to be happening with increasing concentrations, visualised *figure 8*.

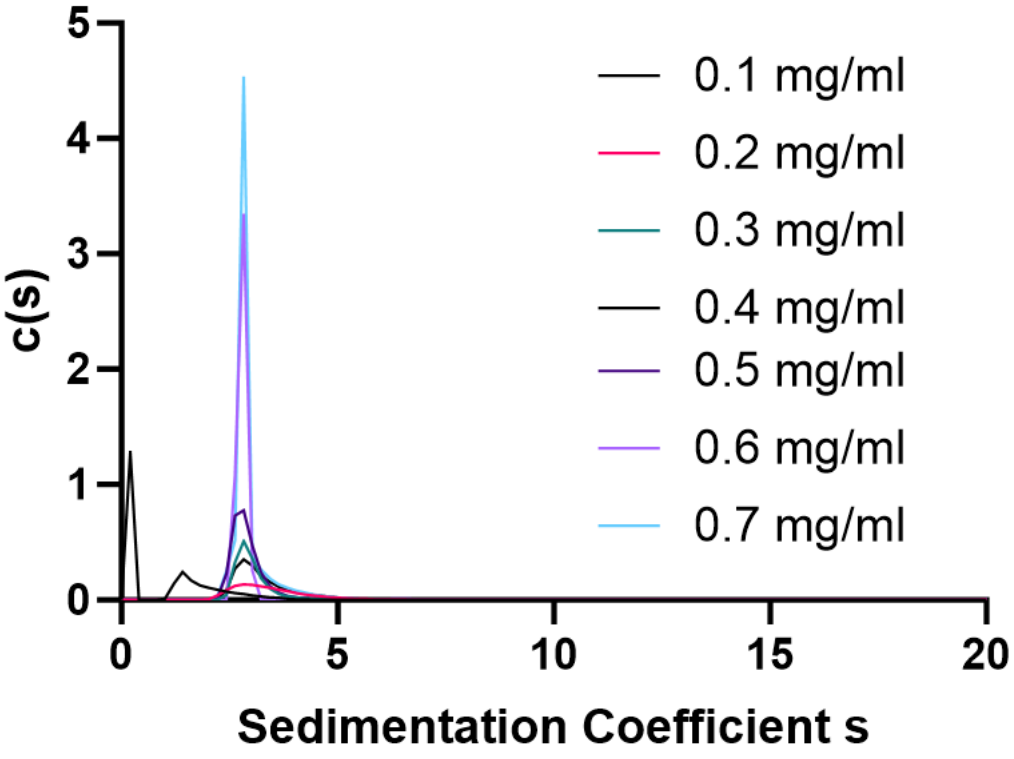


Figure 8. *Sedimentation coefficient distribution results corrected to standard conditions from sedimentation velocity analysis at 45,000 rpm.*

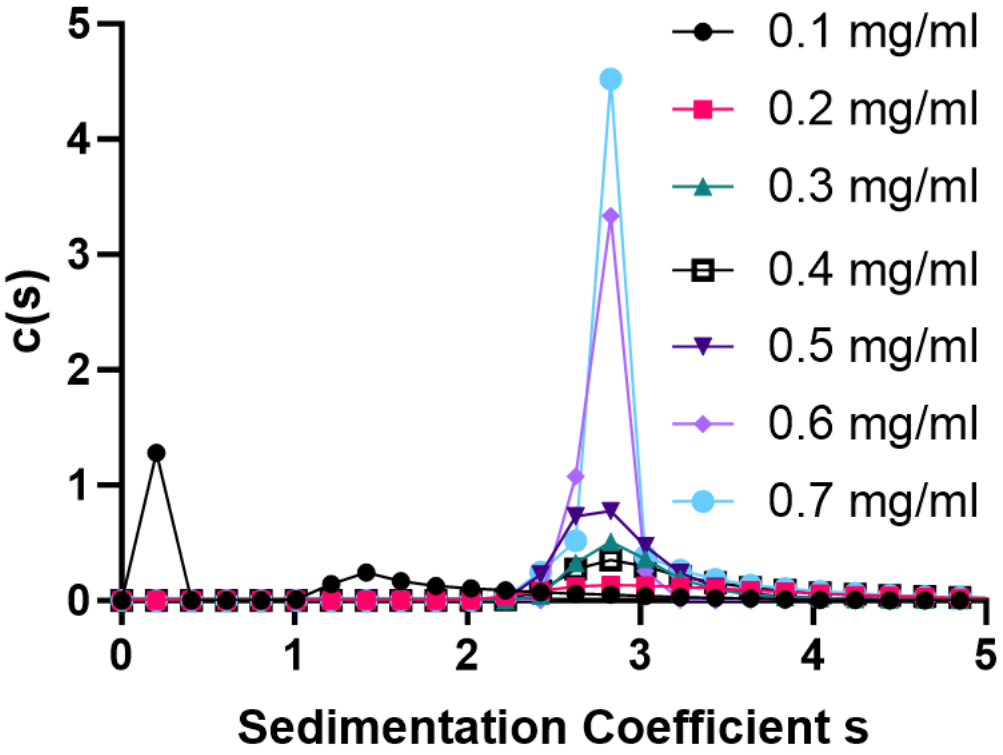


Figure 9. *Zoom in of sedimentation coefficient distribution corrected to standard conditions. Peaks are seen clustered around 2.8 s. A near zero peak is observed in 0.1 mg/ml, but otherwise no polydispersity is noted.*

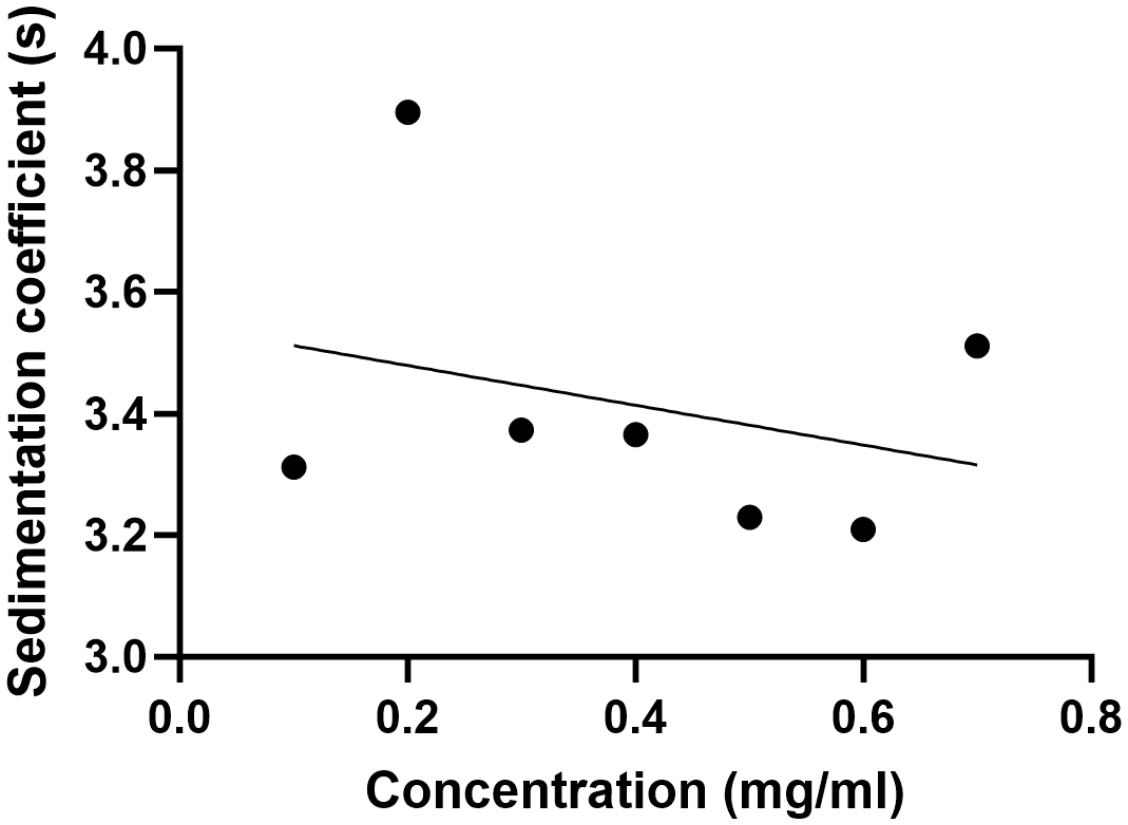


Figure 10. *Extrapolation of corrected sedimentation coefficients for each concentration of HPC. A general decline is noted with increasing non-ideality effects with increasing concentration. Extrapolation back to zero concentration gives 3.57 s.*

4.2.4. Methylcellulose – Methocel 4AC

SEDFIT analysis of the SV 45,000 rpm results initially indicate Methocel 4AC to be rather monodisperse. Peaks are clustered around ~ 2 – 2.4 s with some general broadening and shifting of the peaks up the x axis with increasing concentration. *Figure 11* shows some smaller peaks appearing above a value of 5 on the x axis, but this is likely an artefact of the analysis owing to these peaks possessing less than 5% of the total signal combined, *figure 12* shows that there is a slight shift of the peak centre up the scale, possibly being indicative of small levels of self-association. This is indicative of self-association of methylcellulose particles in this solution at these concentrations, forming larger complexes and thus giving a larger sedimentation coefficient readout. Seen in *Figure 13* below, extrapolating back to zero gives an s value of 1.994 s.

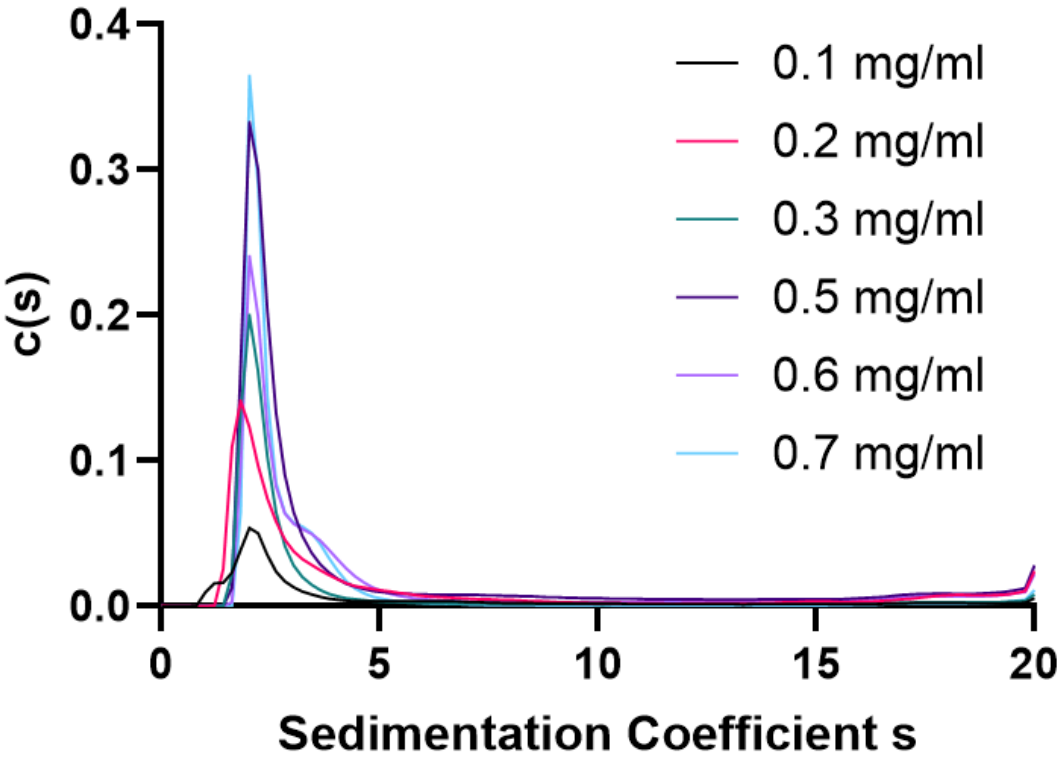


Figure 11. *Sedimentation coefficient results from sedimentation velocity analysis of methylcellulose (Methocel 4AC) at 45,000 rpm, corrected to standard conditions. Some slight upwards shifts are noted with increasing concentrations.*

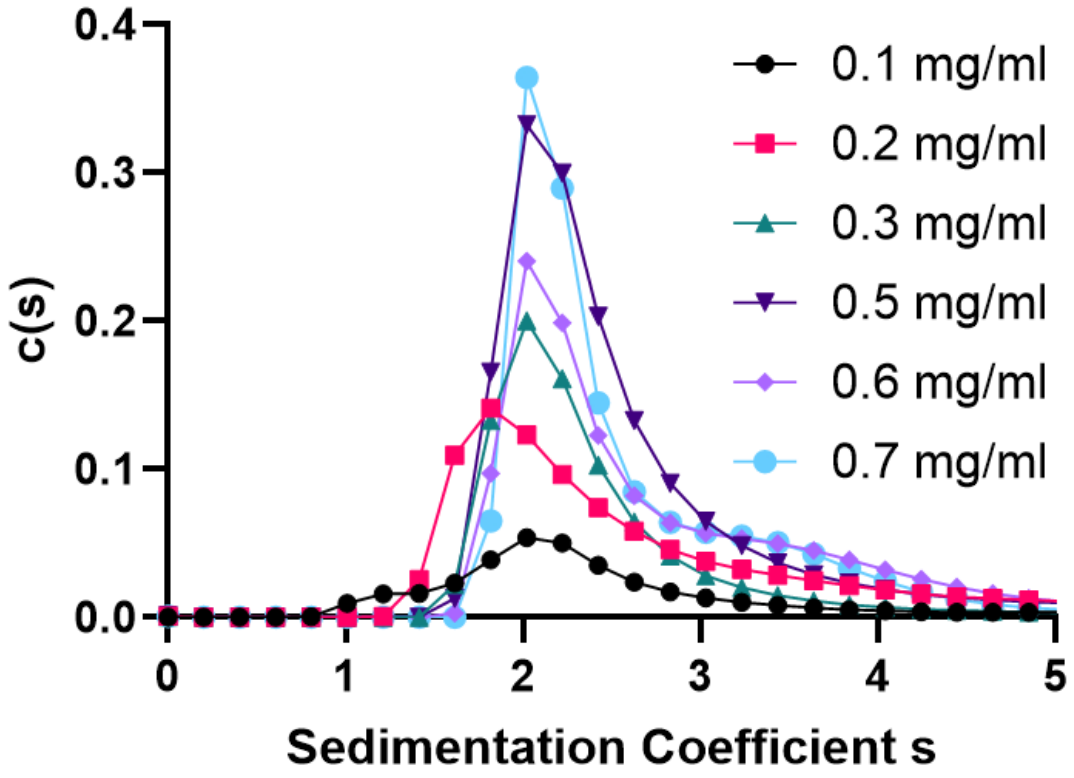


Figure 12. *Zoom in of sedimentation coefficient distribution corrected to standard conditions. Signals here accounted for upwards of 75% total signal in each concentration measured. A general shift of the peaks is observed with increasing concentration, both in slight broadening and shifting up the scale.*

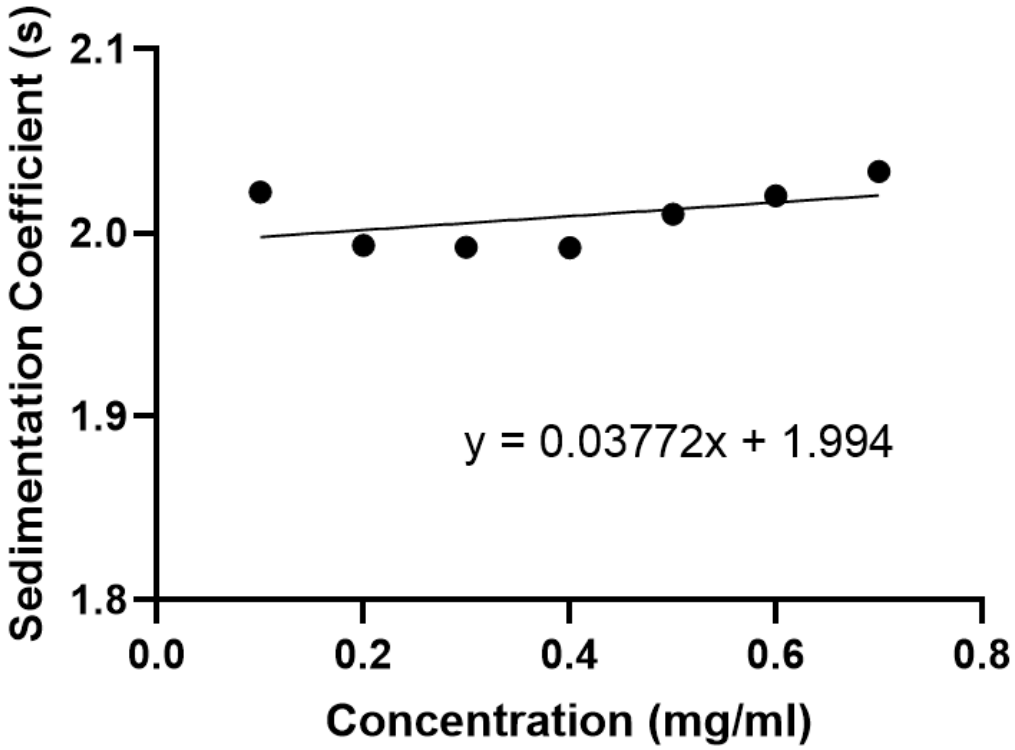


Figure 13. *Extrapolation of corrected sedimentation coefficients for each concentration of methylcellulose. A slight increase is observed with increasing concentration, confirming the previous figures of distributions showing a shift of the peak up the x axis. Values obtained from integration of main peaks from SEDFIT. Extrapolation back to zero concentration gives 1.99 s.*

4.3. Sedimentation equilibrium results

Table 4. *Summary of Sedimentation equilibrium results*

Sample	$M_{w,app}$ *(kDa)*	M_z **(kDa)**	**Polydispersity index** M_z/M_w	$M_{w,app}$ **Hinge (kDa)**
Hydroxypropylchitosan	92.80 ± 36.2	118.10	1.27	64.28
Klucel G	141.70 ± 63.3	175.30	1.23	106.20
'Klucel E'	52.16 ± 17.0	78.80	1.51	43.82
Methylcellulose	66.03 ± 18.2	120.02	1.82	55.83

4.3.1. Hydroxypropylcellulose – 'Klucel E'

The results show that hydroxypropylcellulose or 'Klucel E' is likely polydisperse, with non ideality being present within the samples when looking towards *figure 15*. The downwards slope shows the effects of non-ideality, which when compared to the signal baseline against r^2 graph shown in *figure 14* explains why this would be a linear line. Here, the effects of non-ideality are cancelling out polydispersity to give a linear graph. The apparent molecular weight at zero concentration, seen *table 4* is 52.16 ± 17.0 kDa.

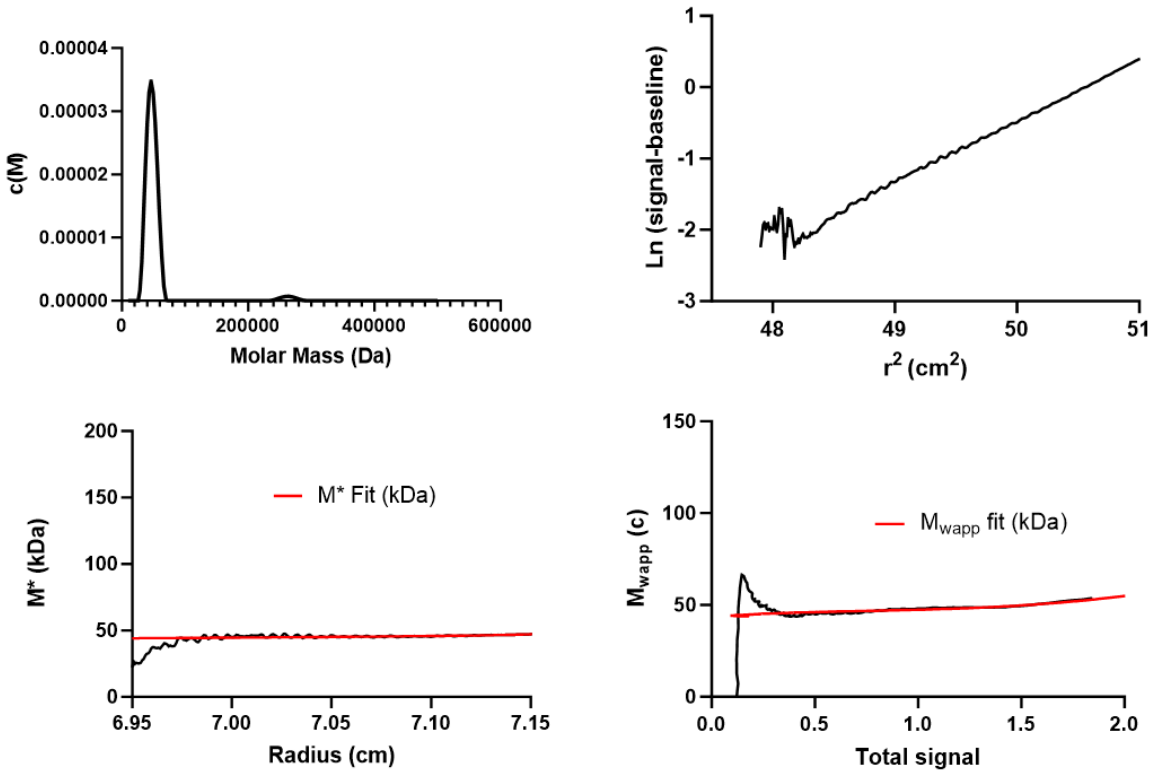


Figure 14. *MSTAR analysis results of 0.5 mg/ml hydroxypropylcellulose 'Klucel E' (SE was run at 17,000 rpm). From top left, the molecular weight distribution c(M) against Molar Mass (Da) shows two species at around 50 kDa & 260 kDa. Log concentration ln(c) vs. r^2 , where r is the radial distance from the centre rotation shows polydispersity and non-ideality with the curve mostly linear. M* plot shows the molecular weight tending towards ~ 50 kDa. Apparent molecular weight against total signal sees a general trend of increase to just above 50 kDa.*

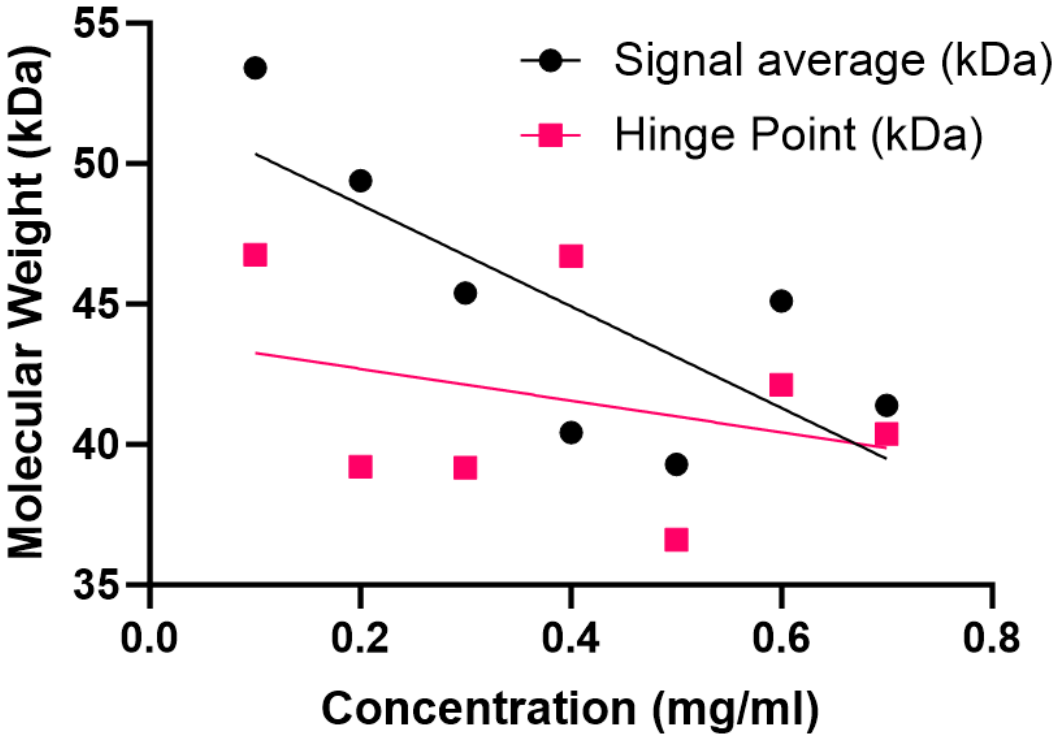


Figure 15. *Signal average and hinge point molecular weights against concentration, trends for both seem to indicate non-ideality as referenced by the decrease in molecular weight with increasing concentration. Molecular weights extrapolated back to zero concentration are summarised in table 4, being* 52.16 ± 17.0 kDa for signal average and 43.82 kDa for hinge point.

4.3.2. Hydroxypropylcellulose – Klucel G

The results show that Klucel G is polydisperse, with the signal baseline against r^2 graph sweeping upwards, shown *figure 16*. While not accurate the c(M) against molar mass shows two distinct peaks further confirming the polydispersity of the polysaccharide. The apparent molecular weight at zero concentration is 141.70 ± 63.3 kDa, see *figure 17* with the hinge point molecular weight being 106.20 kDa.

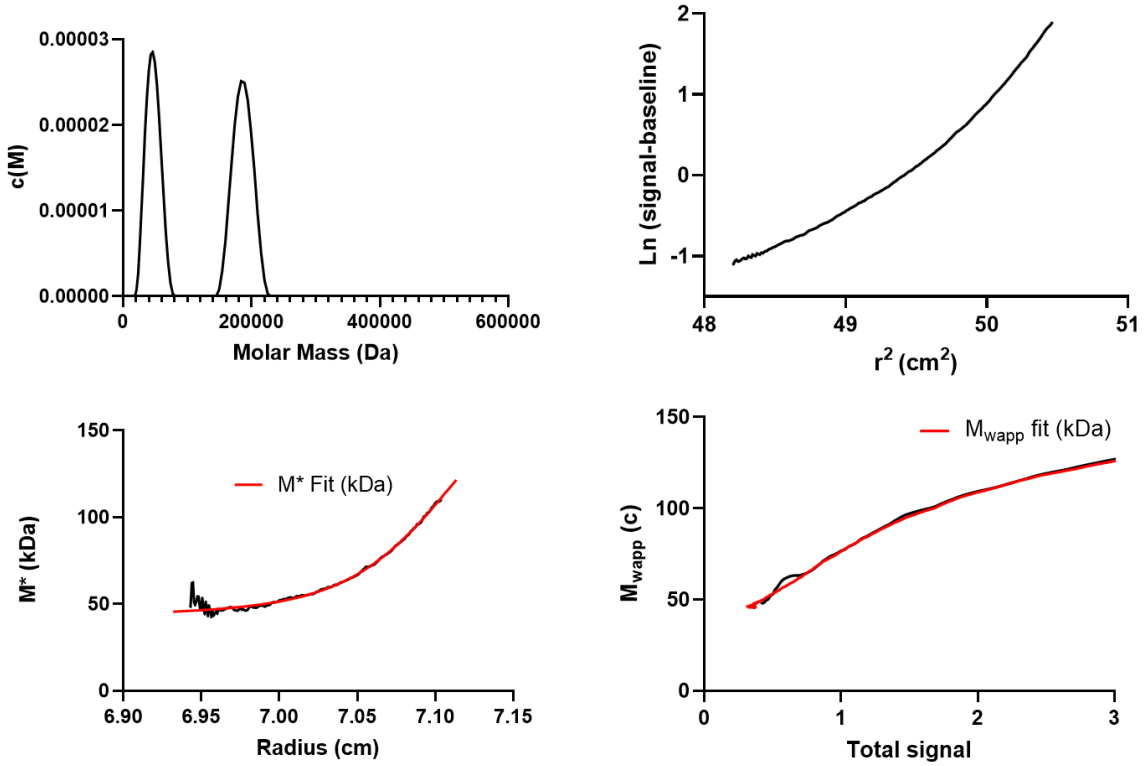


Figure 16. *MSTAR analysis results of 0.5 mg/ml hydroxypropylcellulose Klucel G (SE was run at 21,000 rpm). From top left, the molecular weight distribution c(M) against Molar Mass (Da) shows two species at around 50 kDa & 180 kDa. Log concentration ln(c) vs. r^2 , where r is the radial distance from the centre rotation shows polydispersity with the curve tending upwards. M* plot shows the molecular weight tending towards ~ 150 kDa but appears to level out earlier at around 50-60 kDa. Apparent molecular weight against total signal sees a general trend of increase to just below 150 kDa with a value of 120 kDa being reached.*

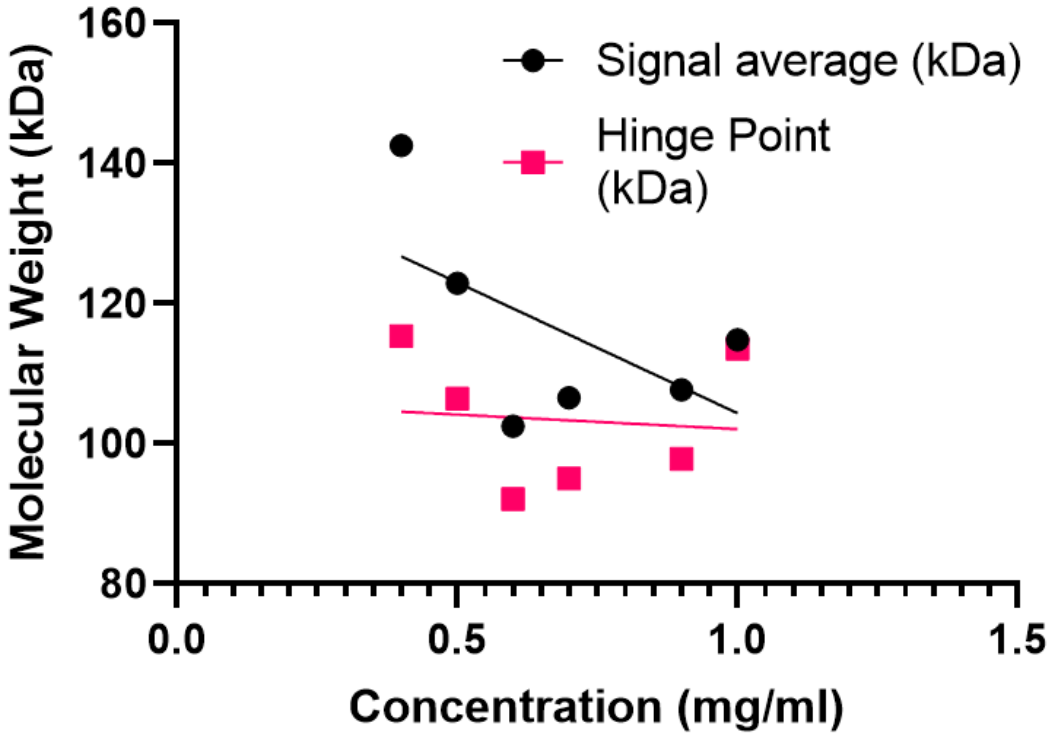


Figure 17. *Signal average and hinge point molecular weights against concentration. Trends for both seem to indicate non-ideality as referenced by the general decrease in molecular weight with increasing concentration. Results are skewed by several outliers but remain in the graph owing to the large standard deviation. Extrapolating back to zero concentration gives 141.70 ± 63.3 kDa signal average and 106.20 kDa hinge point.*

4.3.3. Hydroxypropylchitosan

The results show that hydroxypropylcellulose is polydisperse, with the signal-baseline graph sweeping upwards indicating polydispersity, see *figure 18*. The reported molecular weight obtained from extrapolating back to zero concentration is 92.80 ± 36.2 kDa for signal average and 64.28 kDa for the hinge point molecular weight, see *figure 19*.

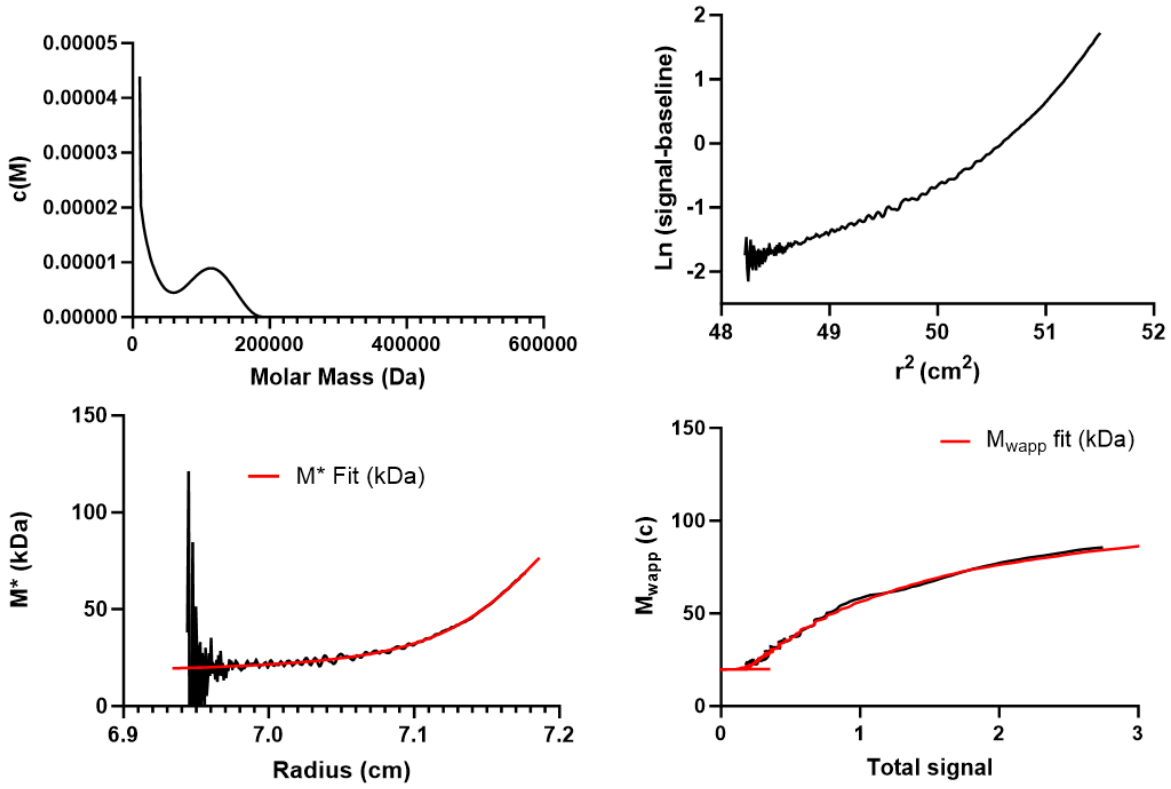


Figure 18. *MSTAR analysis results of 0.6 mg/ml Hydroxypropylchitosan (SE was run at 19,000 rpm). From top left, the molecular weight distribution c(M) against Molar Mass (Da) shows two species at around 20 kDa & 120 kDa. Log concentration ln(c) vs. r^2 , where r is the radial distance from the centre rotation shows polydispersity with the curve sweeping upwards. M* plot shows the molecular weight tending towards ~ 90 kDa. Apparent molecular weight against total signal sees a general trend of increase to just under 100 kDa.*

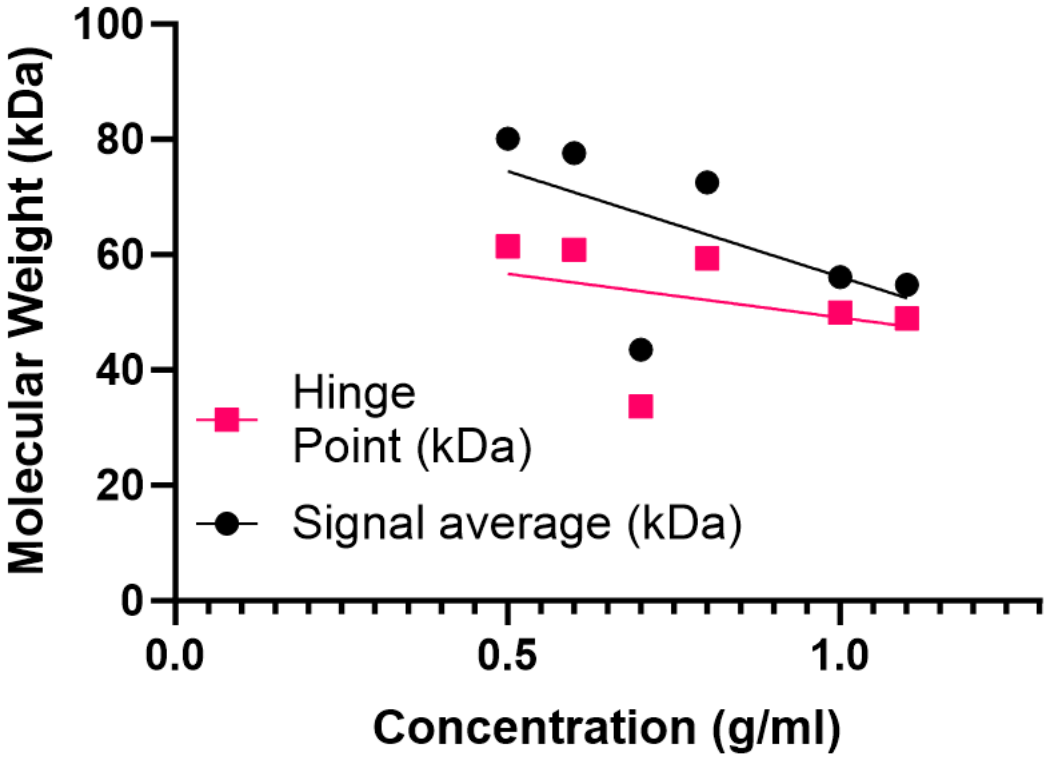


Figure 19. *Signal average and hinge point molecular weights against concentration, trends for both seem to indicate non-ideality as referenced by the decrease in molecular weight with increasing concentration. Extrapolating back to zero concentration gives 92.80 ± 36.2 kDa signal average and 64.28 hinge point molecular weights.*

4.3.4. Methylcellulose – Methocel 4AC

Methylcellulose Methocel 4AC was determined to be a polydisperse polysaccharide with a molecular weight ~ 66.03 ± 18 kDa as evidenced by the upwards sweeping Signal baseline graph, see *figure 20 & 21*. The MSTAR fit gives an upwards trend towards the end of the graph, potentially being indicative of sub 10 kDa species that stretch the limits of the analysis.

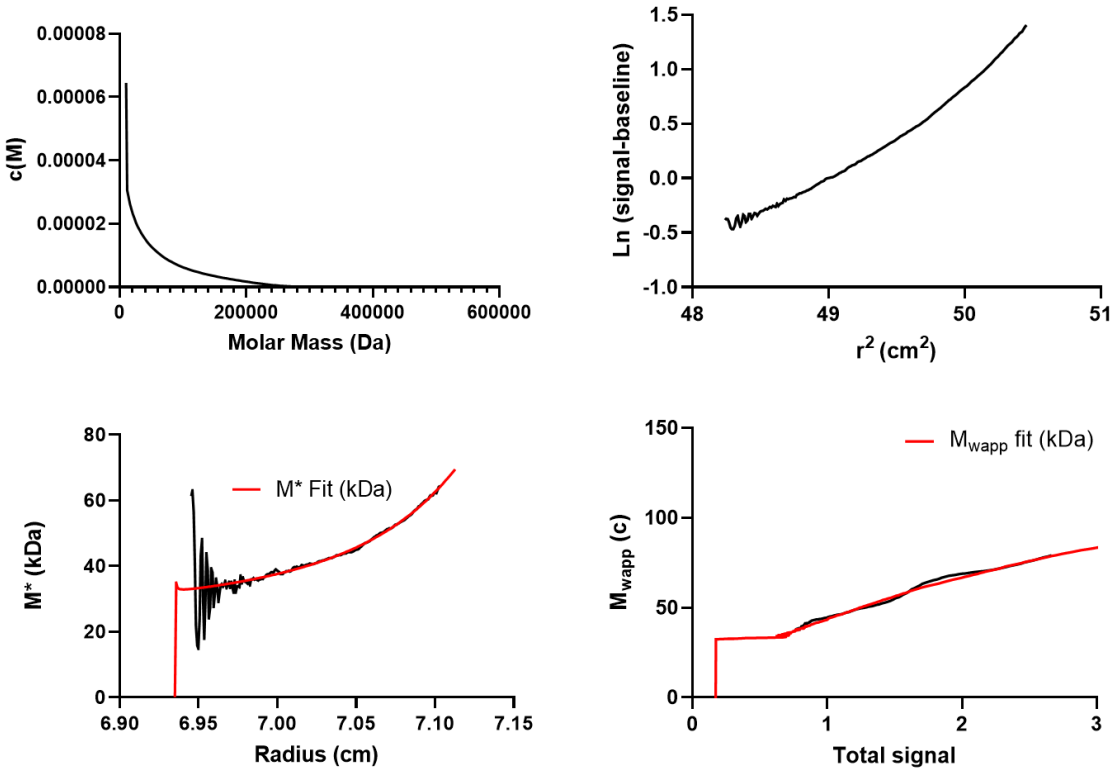


Figure 20. *MSTAR analysis results of 0.2 mg/ml methylcellulose (SE was run at 19,000 rpm). From the top left the molecular weight distribution c(M) vs. M showing two species peaking ~ 10 kDa & 110 kDa. The front peak is an artefact of analysis and/or possibly sub 10 kDa species. Log concentration ln(c) vs. r 2 , where r is the radial distance from the centre rotation which has an upward curve suggesting polydispersity. M* vs. r plot in black with the fit based on M* extrapolation giving Mw,app = 63.4 kD but trends upwards. local or point apparent molecular weight at radial position r plotted vs. local concentration c(r) for different radial positions; the red line is the fit and the black the raw data of Mw,app(c) vs. concentration (in total signal).*

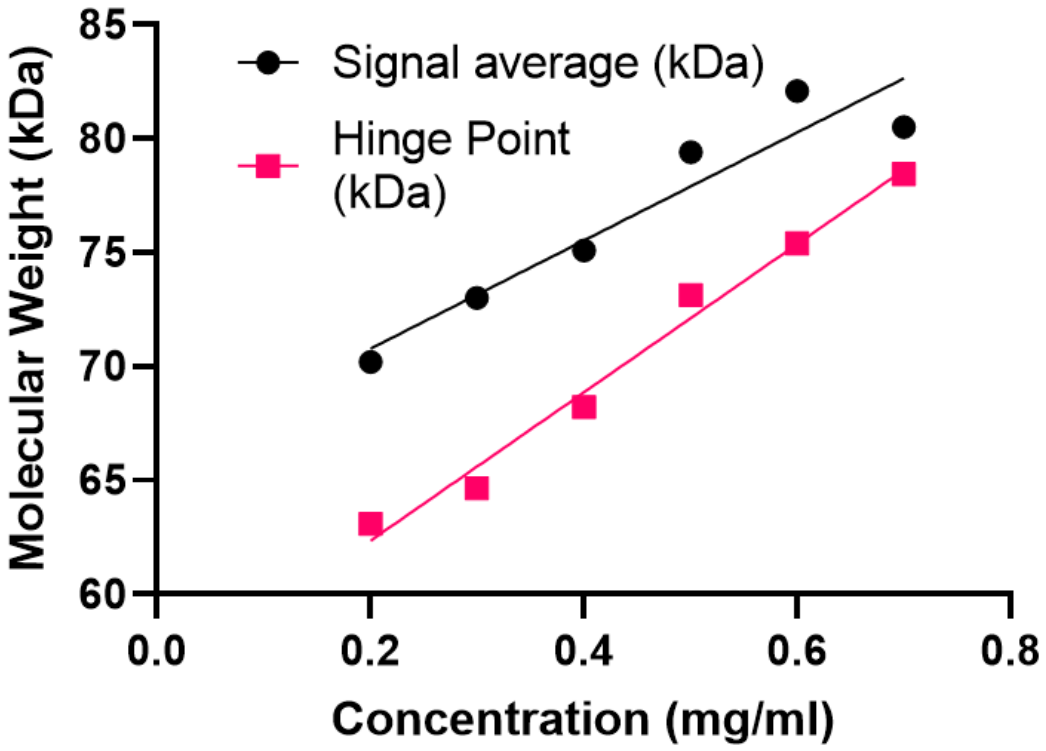


Figure 21. *Molecular weights of both Signal average and Hinge point results from AUC analysis. Results from Cell 1 (0.1 mg/ml) were excluded due to significant differences to other results. At zero concentration the Signal average molecular weight yielded 66.03 kDa and the Hinge point molecular weight yielded 55.83 kDa.*

4.4. Wood treatments and moisture content

The average wood moisture content was calculated to be 77.79% of the total mass of the waterlogged samples. There was a high degree of variability within these values, with some falling as low as 50% water content with others being as high as 91.22% water. Some of these differences can be explained through the lamination that plagued the larger segments of woods which ultimately had these resultant pieces cut from. There was a tendency for the higher water content samples to have split into several pieces upon being in the 105^{O}C for a number of days, potentially indicating that other pieces that remained intact could have held more water that was unable to boil off unless the overarching structure was compromised, however this is purely speculatory.

Table 5. *Changes in density and volume for each treatment group as well as the two control groups, denoted with an asterisk.*

Treatment	**% Change in Density**	**% Change in Volume**
Hydroxypropylchitosan	17.42	14.69
'HPCell' or 'Klucel E'	17.70	27.65
Klucel G	21.96	22.69
PEG-2000	23.89	38.95
Freeze dried*	-14.54	-31.88
Air dried*	41.21	-42.26

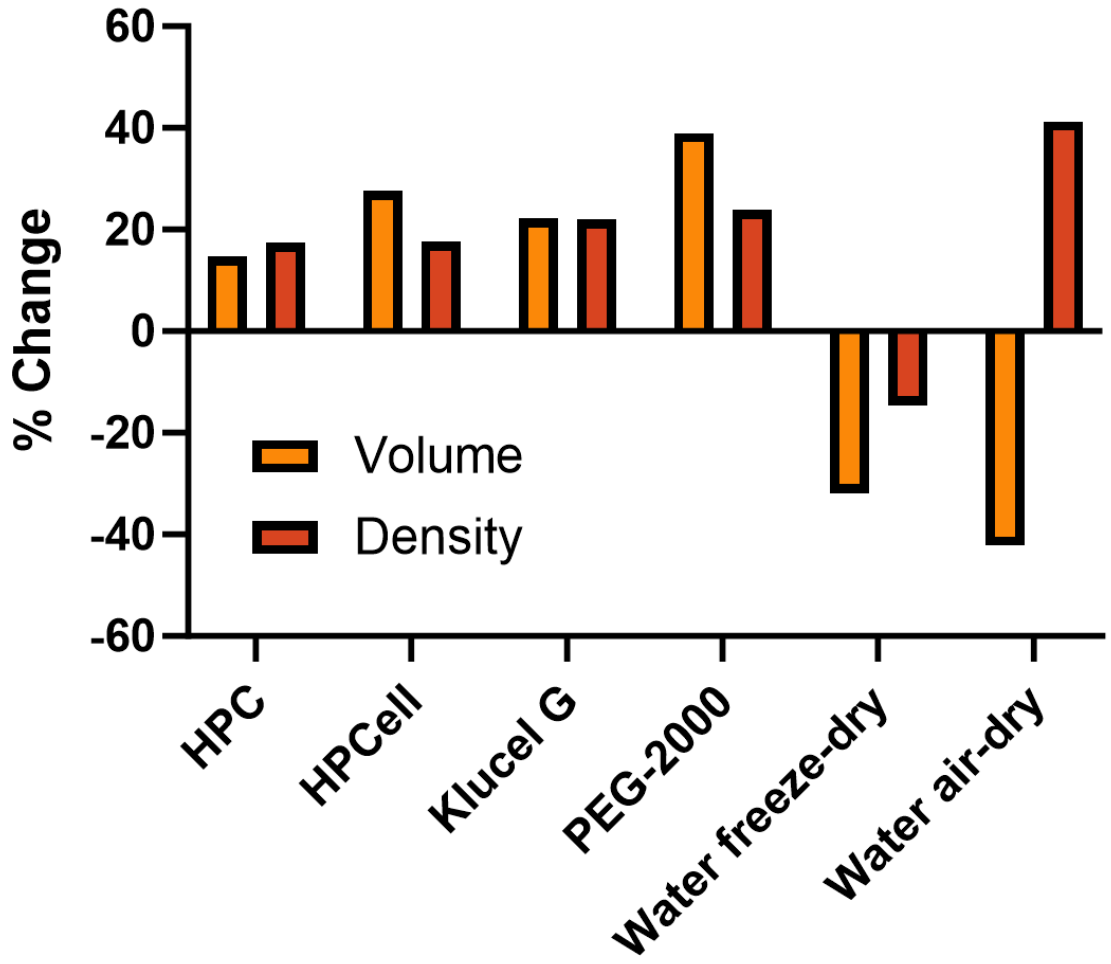


Figure 22. *Average percent change in density and volume of wood samples in each treatment group. All samples excluding control Air-dried and Freeze-dried underwent a large swelling of the wood samples, it is unclear as to why the freeze dried has swelled so much as it would be expected that it would shrink or stay equivalent to pre-treatment. All samples aside from the control freeze- and air-dried samples underwent a considerable density increase, air-dried increase was attributed to the volume reduction while freeze-dried is attributed to the greater loss of water in tandem with shrinking. Actual values are quoted in the table above.*

The densities and volumes observed pre and post treatments differed greatly. Control treatments of air- and freeze-drying exhibited volume loss in excess of 30 %, exacerbating the weakened structure and leading to an increase in the brittle nature of the samples, see *table 5 & figure 22*.

Utilising the tape test to see whether the consolidants could significantly alter the mass of wood that would be pulled off it was noted that the freeze dried performed the worst by a considerable margin.

Somewhat paradoxically the air dried samples performed the best, but this is down to the complete collapse of the wood structure (detailed in SEM section).

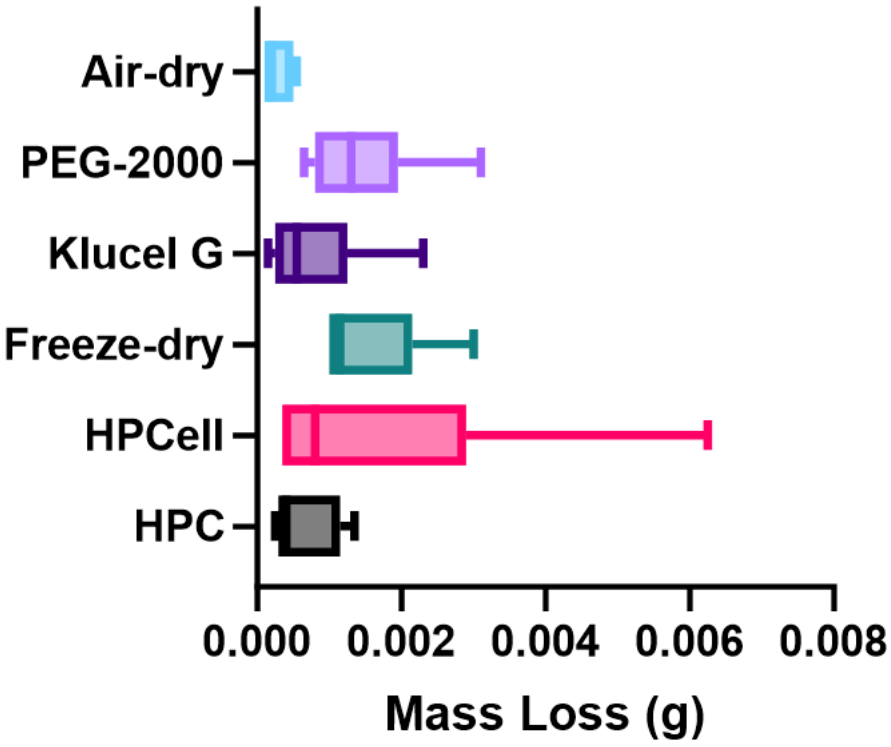


Figure 23. *Tape test results from wood treatments and controls. HPC and Klucel G gave significantly lower mass loss compared to the freeze dry control. High levels of variability are in part due to the tape breaking up surface structures by being too strong, as such poor performance of HPCell (Klucel E) is not necessarily reflective of poor consolidant performance. Similarly, Air dried samples fared better due to the much condensed and collapsed structure afforded to them.*

Tape test results seem inconclusive somewhat inconclusive given the real lack of difference in mass loss observed between treatments. All treatments aside from HPC and air-dried encompassed a large standard deviation relative to the mass of wood removed as shown in *figure 23* above. It was difficult to assess the effectiveness of the consolidants based off this test, leading to it not being very useful.

Analysis of the colour change in the SIE CIELABs colour space gave consistent results across all samples. All treatments including the air-dried control exhibited a ΔE value around 2-3, indicating that a consistent colour change was observed across all treatments and controls when comparing to the freeze dried. Comparisons were made to the freeze-dried samples on the basis of them being in good condition comparative to the air-dried, which exhibited a lot of collapse within the structure leading to a much darker colour than would have originally observed within the undamaged wood sample. Typically, a ΔE value > 3 is observable and recognisable by the naked eye with the range of values observed within the treatments typically falling below 3, seen below. This is typically identified as only being identifiable to the trained eye, and with some samples falling below this mark the treatments appear to have done a reasonably good job at preserving the original samples colour. (Faghihi et al., 2021).

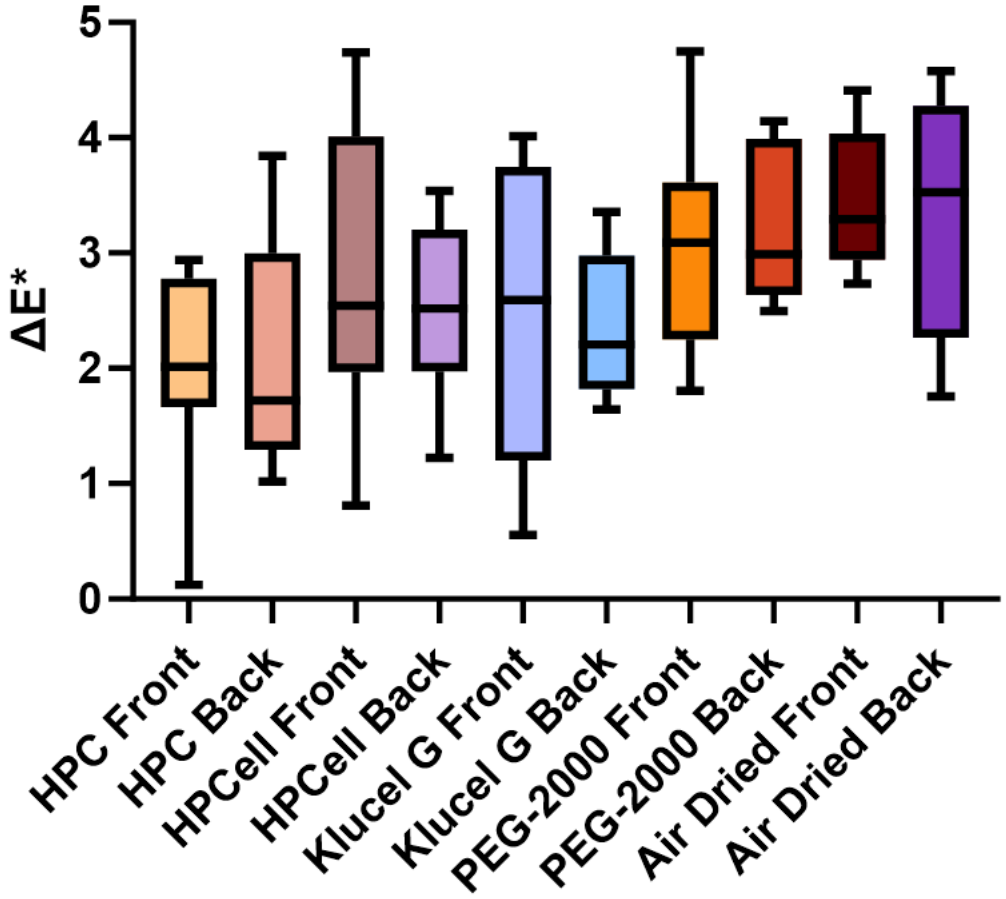


Figure 24. *Colour change of sample treatments standardised to the colour of freeze-dried wood samples. The colour change of both the front and back of pieces was averaged and compared to the average of the freeze-dried samples colour within the CIELABs colour space. Air dried was chosen not to be the standard of control for this as all other samples were freeze dried, making it a benign comparison, it is only included here for interest.*

4.5. SEM Imaging of Wood treatments

Overall, the SEM results show that the wood treatments assessed have not performed very well. A common feature to near all of the images obtained, is the collapse in part of whole of the wood structure. The air-dry control remains the worst example of this, as evidenced by the lack of any real observable structure within the wood towards the edge of the sample, visualised below in *Figure 25.*

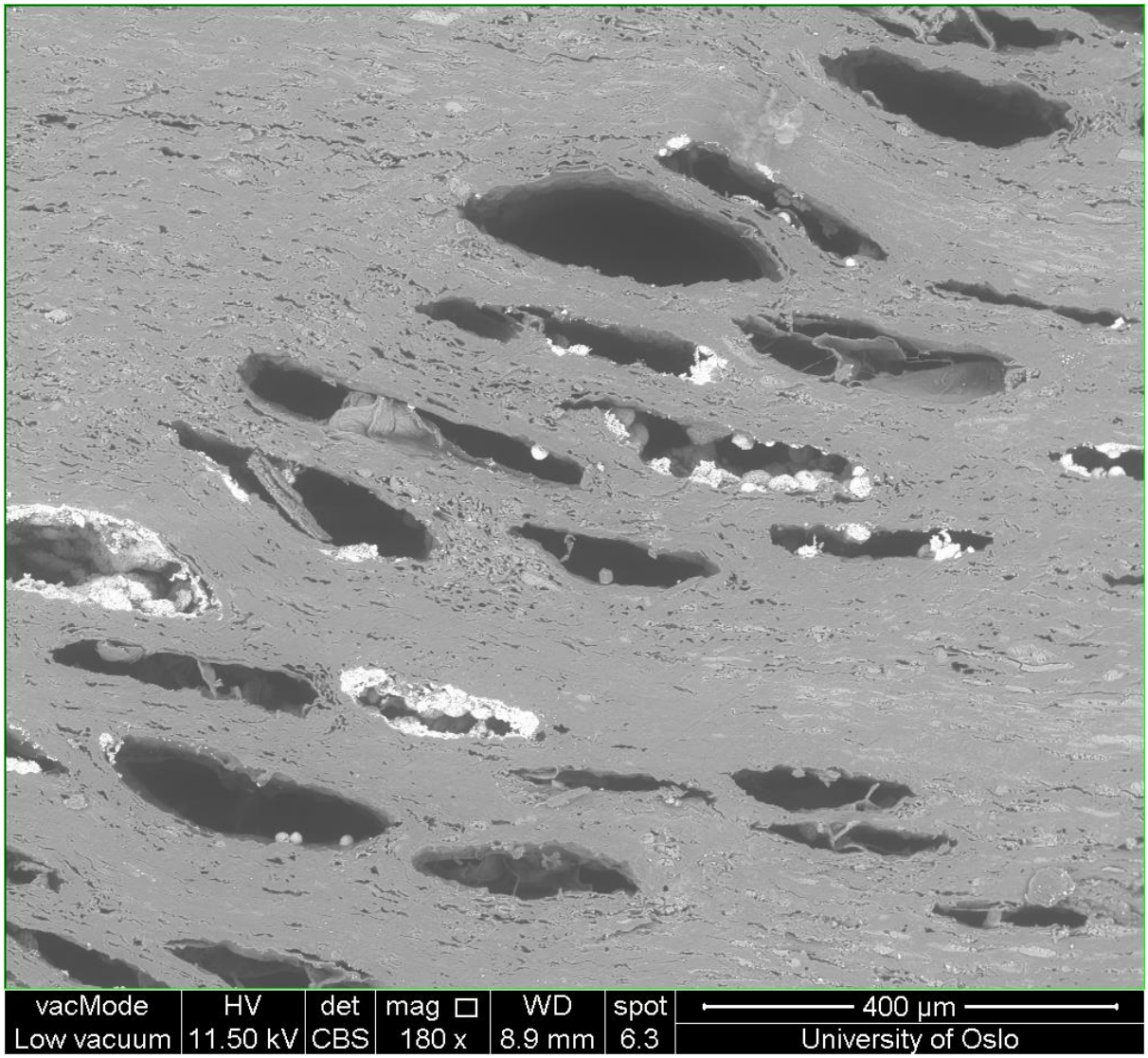


Figure 25. *Air dry wood SEM image. Taken from the edge of the sample, corresponding to the edge of the unbroken/unaltered sample of air dried wood. No real structure is observable here, it is possible this was affected by the cutting of the sample down to the original test size or even the further resizing to make it suitable for SEM. Species identification remains impossible given the degraded nature of the wood.*

The middle of the airdried sample shows a similar but different case, here the structure is better preserved. The structure is still heavily degraded and shows large areas of complete collapse surrounding some of the better-preserved structures shown in below in the middle of *figure 26*.

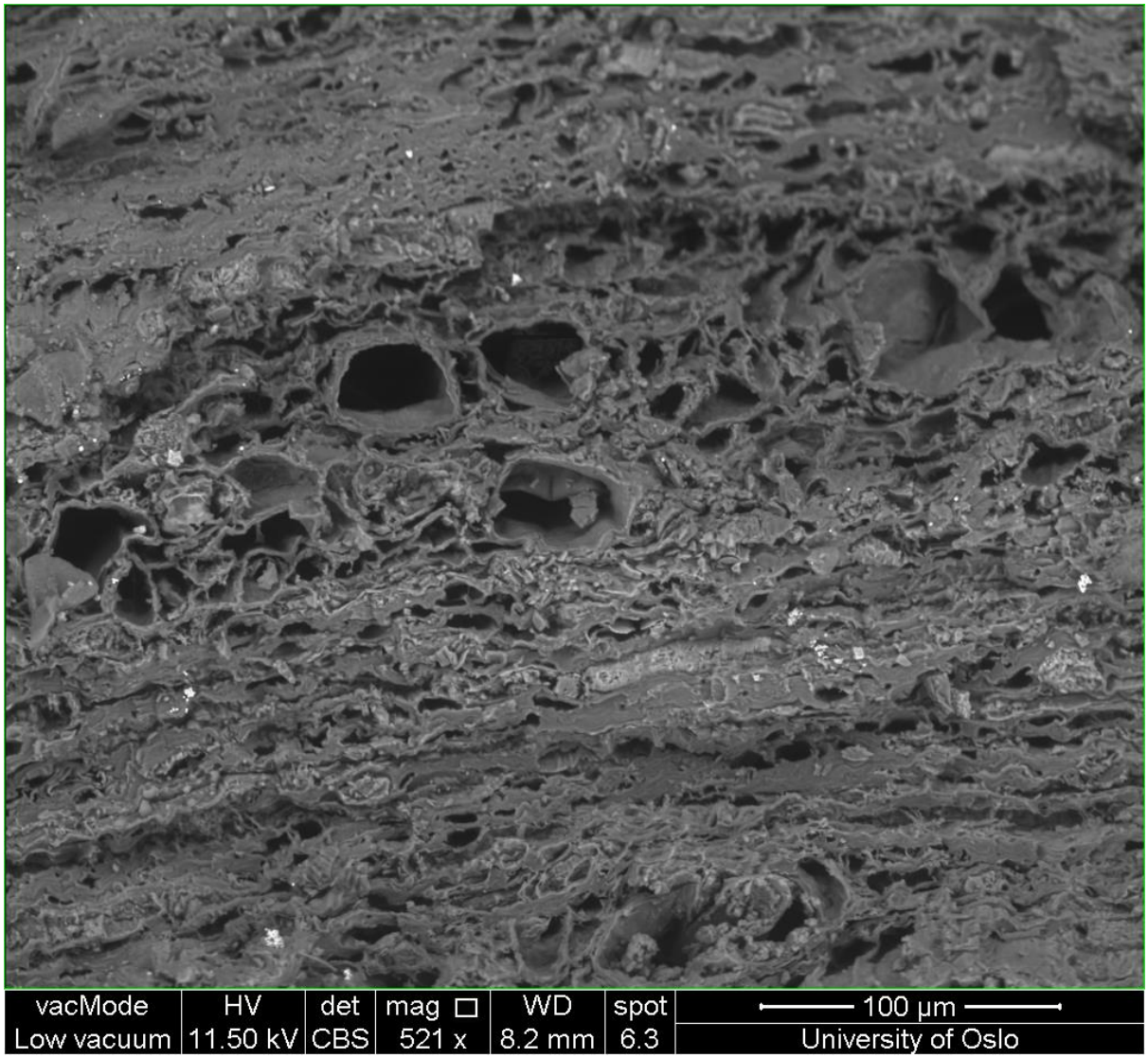


Figure 26. *Air dried wood SEM image. Image was taken from the centre of the wood, with the location being 'cherry picked' in order to give a better representation of the original wood structure. Otherwise the structure was completely collapsed into a homogeneous block of cellulose and its breakdown products.*

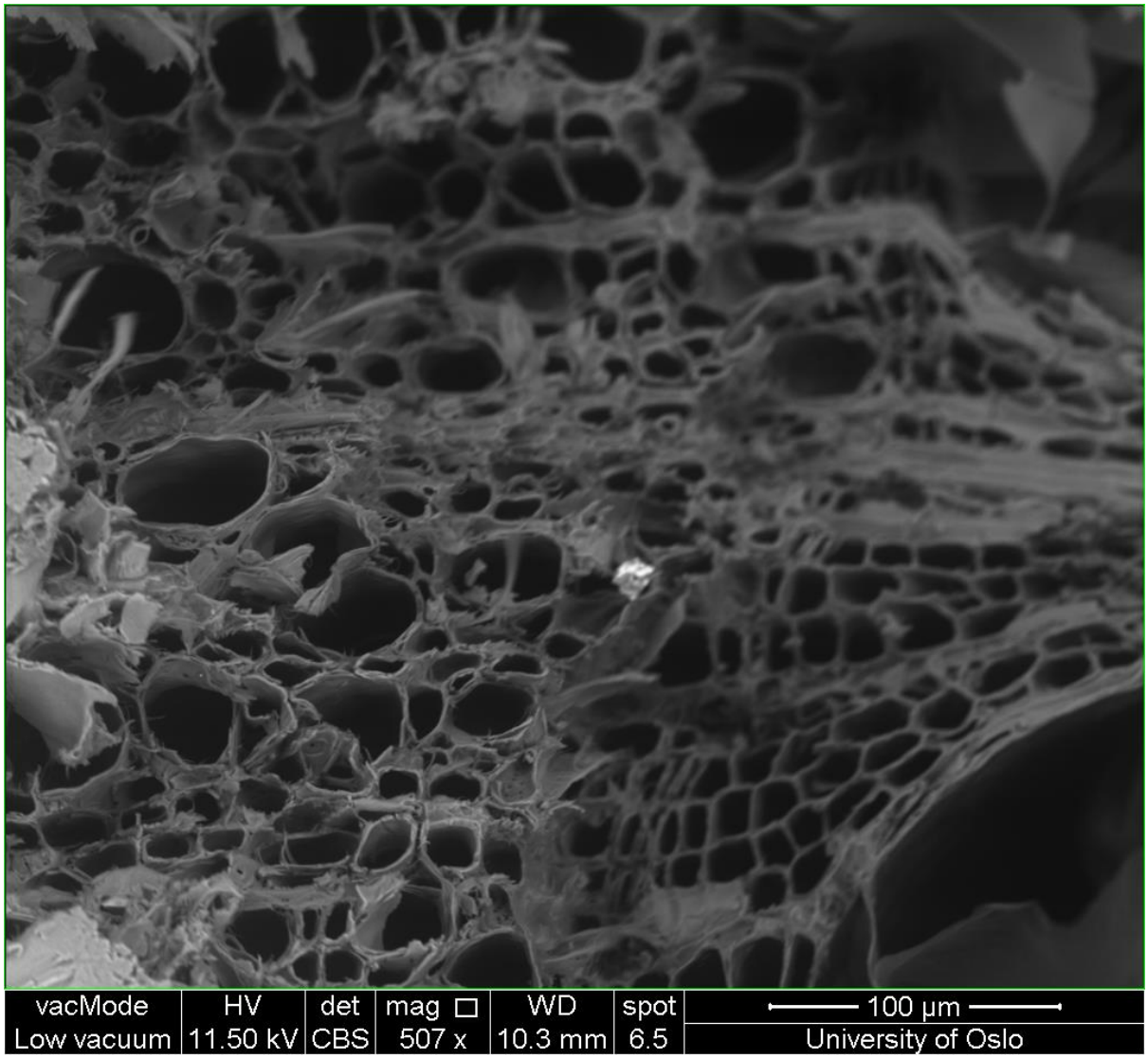


Figure 27. *Fresh oak sample SEM image. The oak supplied here was 'fresh' but appears to have suffered from some collapse, perhaps due to air drying of the sample, giving it some of the collapse as seen in the other treatments. Otherwise this is a good comparator for the treatments as it remains to be in much better condition and is likely to be the same species of wood found within the samples supplied for the purposes of this project.*

The fresh oak sample supplied by the University of Oslo shows a better representation of the intact wood structure that should be compared to the treatments performed during this project. As a baseline this gives a good comparator to the freeze dry control which faced much less collapse than the air dry and is most comparable with the fresh oak. As seen below in *Fig X* there is a great deal of similarity to both the freeze dried and the fresh oak samples, with some intact structures remaining visualised in the 2200x zoom image.

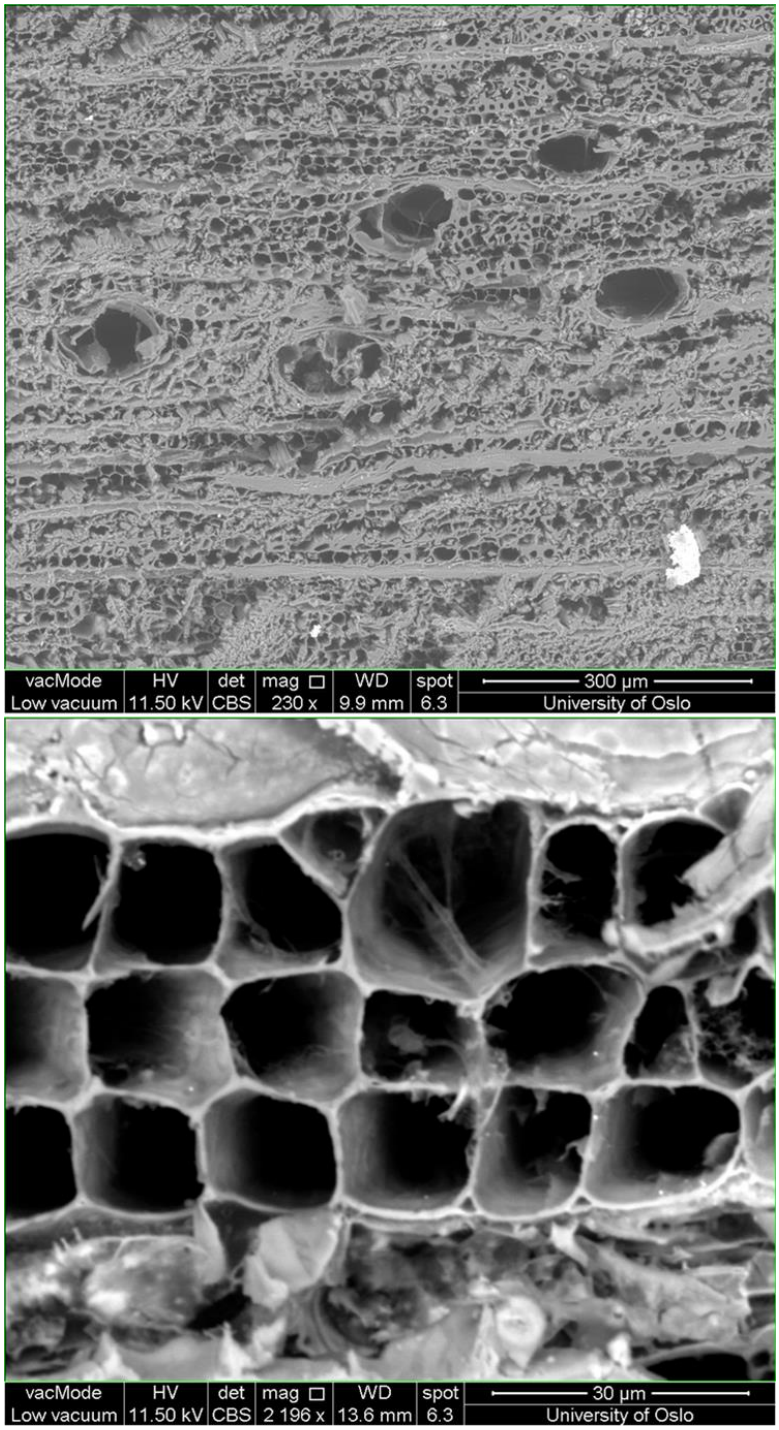


Figure 28. *SEM images of the freeze-dried wood samples. There was a high level of variability within this sample, with some structures, visualised at 230x, being very compact and showing signs of collapse. Others, shown in 2196x, show more intact structures of wood more indicative of the fresh wood sample show previously. It is noted that surface level degradation could also be attributed to the cutting and preparation of the sample for the SEM.*

The freeze-dried samples, *figure 28,* show that there are not as many well preserved structures as there is within the fresh oak structures, show below in *figure 27.* Structures seem both less and more organised in places, perhaps being indicative of damage during cutting.

Comparatively, the treatments are shown to be a mixture of successful structural preservation and absolute failure. HPCell or hydroxypropylcellulose seems to have failed to prevent the collapse of the wood structure, as is shown below in *figure 29* the remaining structure in the core of the wood sample is more condensed when compared to the freeze dried, or the fresh oak. It is difficult to determine the consolidation as it does not appear to be visible but remains in a better state than the air-dried samples.

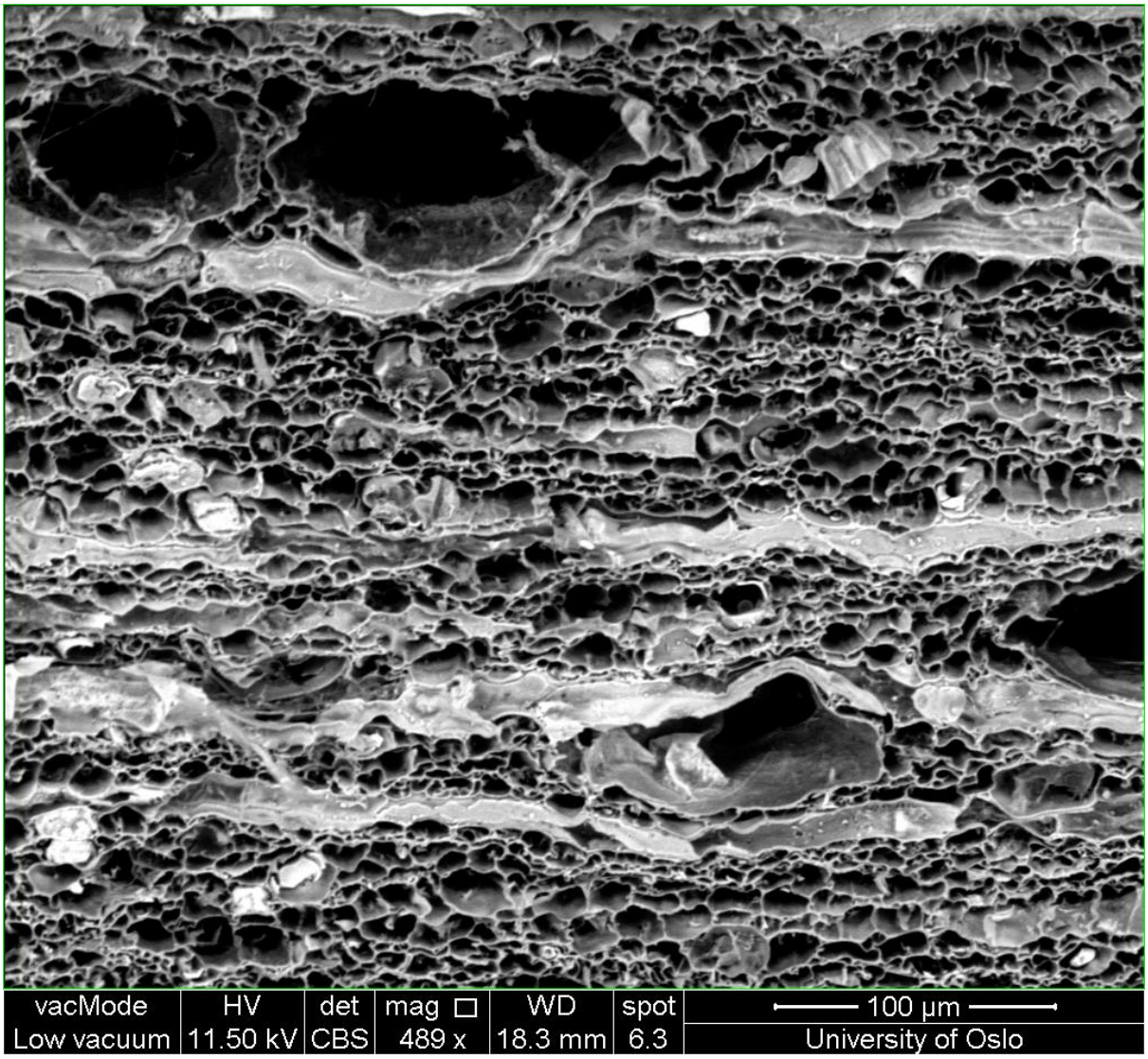


Figure 29. *SEM images of hydroxypropylcellulose treated wood, dubbed HPCell or 'Klucel E'. Significant collapse and shrinkage of microstructure is observed, in stark contrast with the swelling of the piece as a whole.*

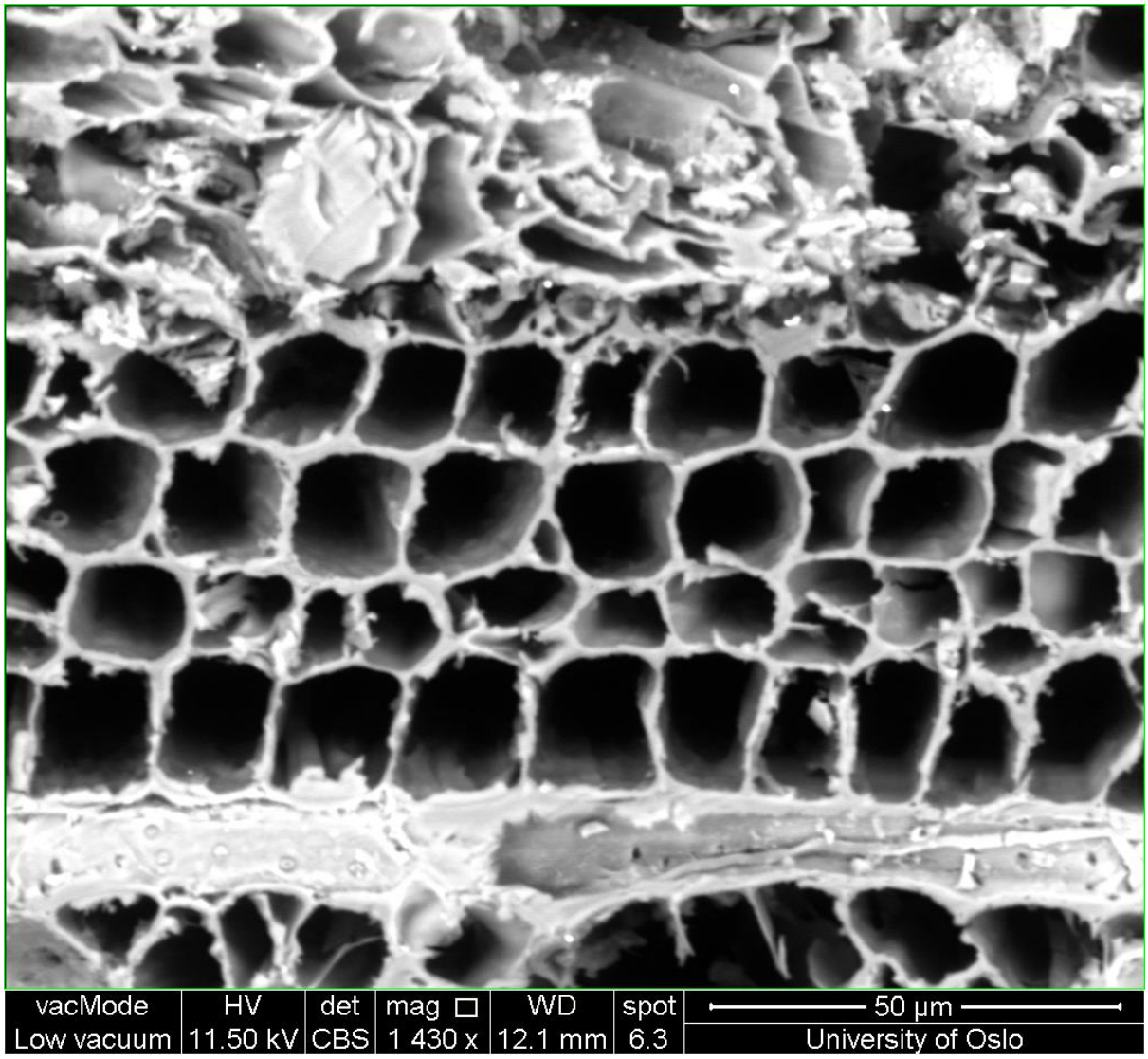


Figure 30. *SEM image of hydroxypropylcellulose Klucel G. The Klucel G treatment seems to have elicited by far the best-preserved wood structure out of all of the treatments and controls, structure remains even and ordered, with sections visualised with high damage being the result of sample preparation for the SEM. Even when compared to the fresh oak sample this remains in much better condition and could easily have been mistaken for the fresh sample were the images not named on creation.*

Klucel G seems to have faired remarkably well in the structural preservation, but no thickening of the structure was observed, see *figure 30*. The intact structures seem to still be symmetrical and ordered in nature, with large areas, visualised top of *figure 30*, believed to be the result of the cutting process. It was initially assumed that the quality of the structures observed here would be more

indicative of the fresh oak samples, as this appears to be the best-preserved sample by far.

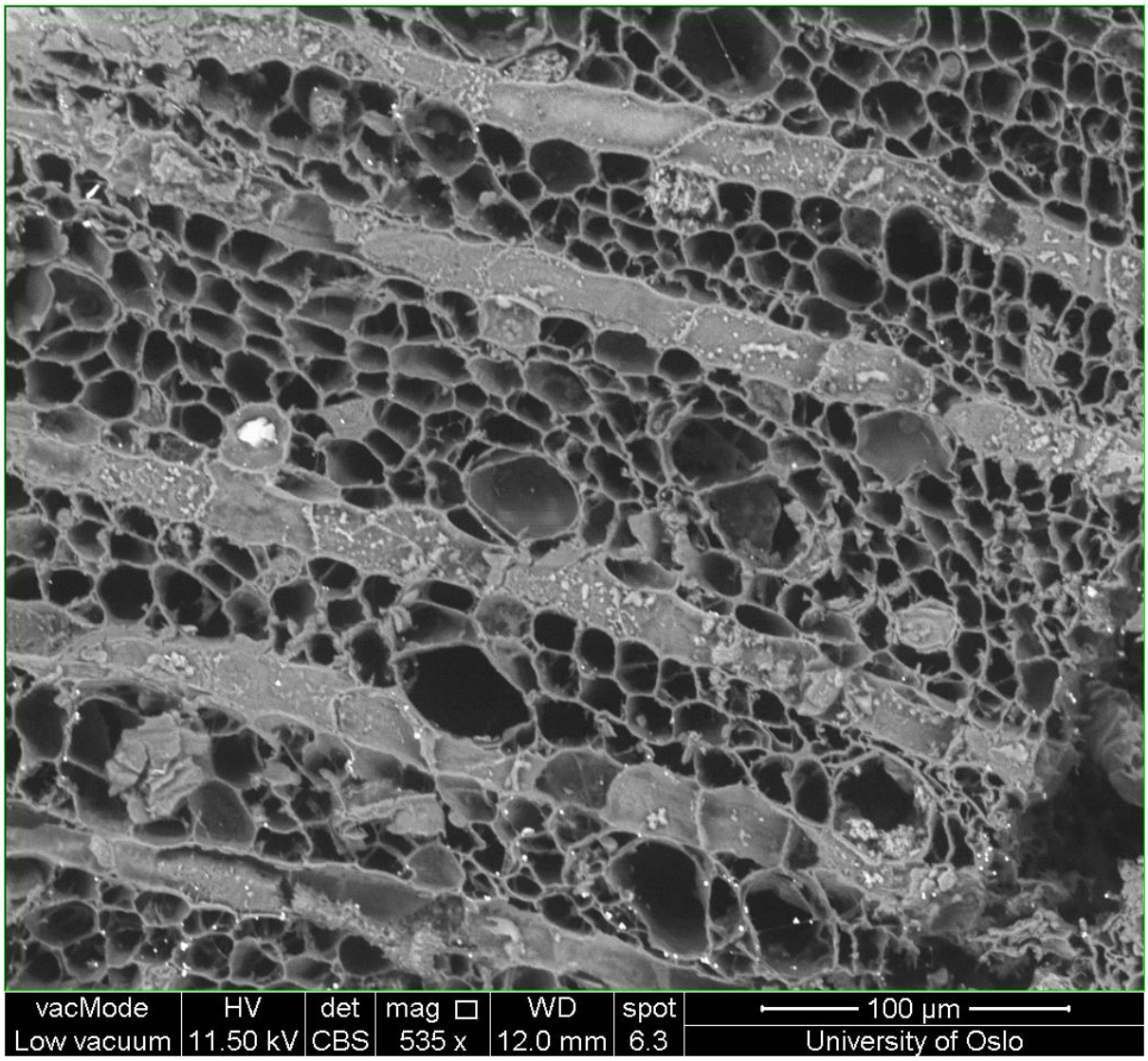


Figure 31. *SEM image of hydroxypropylchitosan treated wood centre. The structure seems to be more intact than the HPCell sample, but still suffers from uneven structures that have likely been partially collapsed due to the consistency of this appearance throughout the sample. A more irregular, inconsistent level of damage would typically be observed if this was the result of cutting damage.*

The wood structures in hydroxypropylchitosan treated pieces seem to be in better condition than the freeze-dried, with much less structural collapse but is in noticeably worse condition than the Klucel G treatment. EDS analysis was also conducted owning to the significantly higher nitrogen content, shown *figure 32 below.*

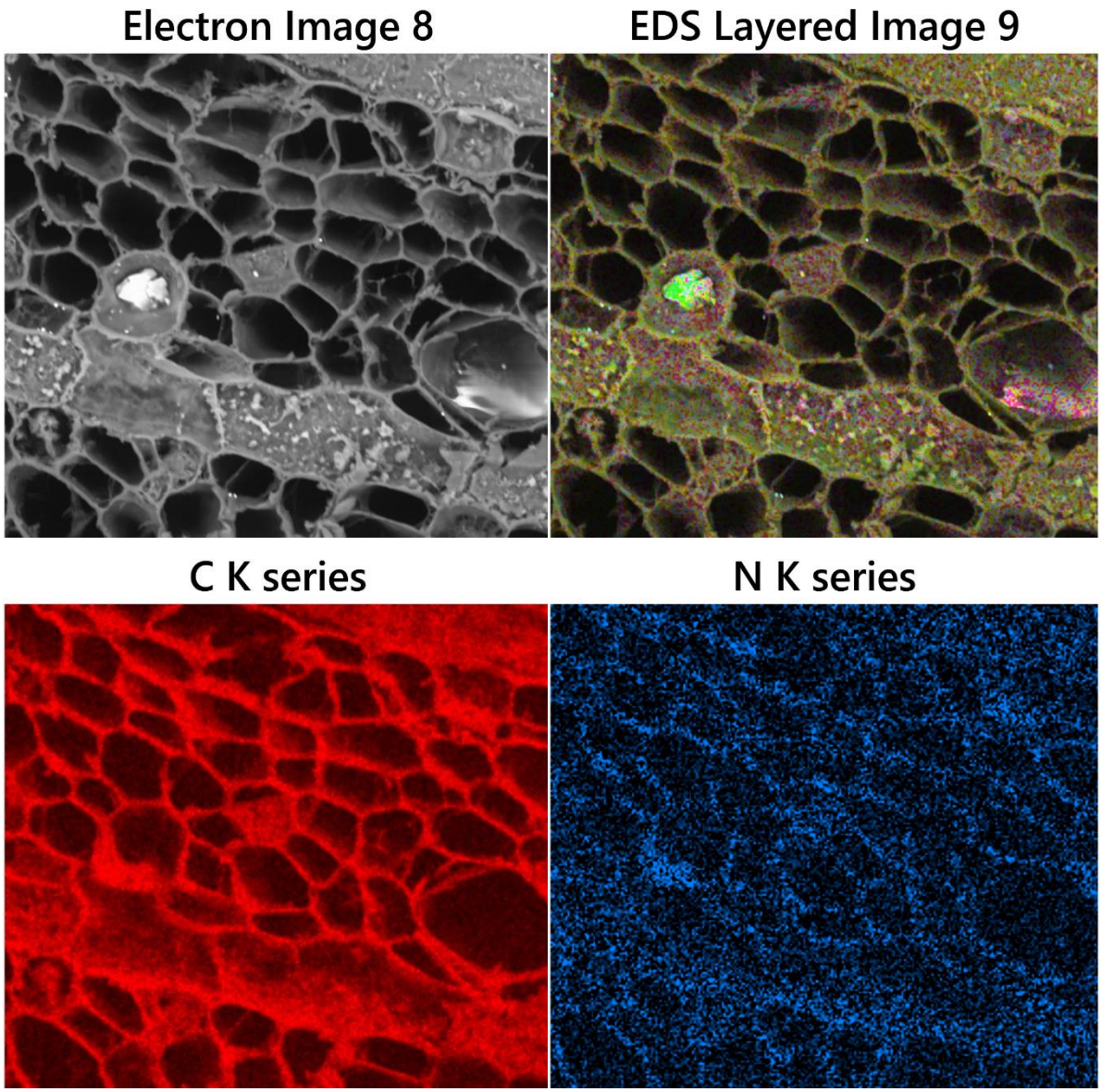


Figure 32. *Zoom in of SEM image of hydroxypropylchitosan treated wood piece. EDS (Electron dispersieve spectroscopy) was used for elemental analysis to determine nitrogen presence. Top left is the original SEM image, top right is EDS Layered image indicates a composite image of EDS results for all elements present within the sample, bottom left is C K series showing the presence of carbon atoms and bottom right is N K series showing the presence of nitrogen atoms. It is noted that no significant nitrogen component was found within the control or other treatments, indicating a successful penetration of HPC to the middle of the samples.*

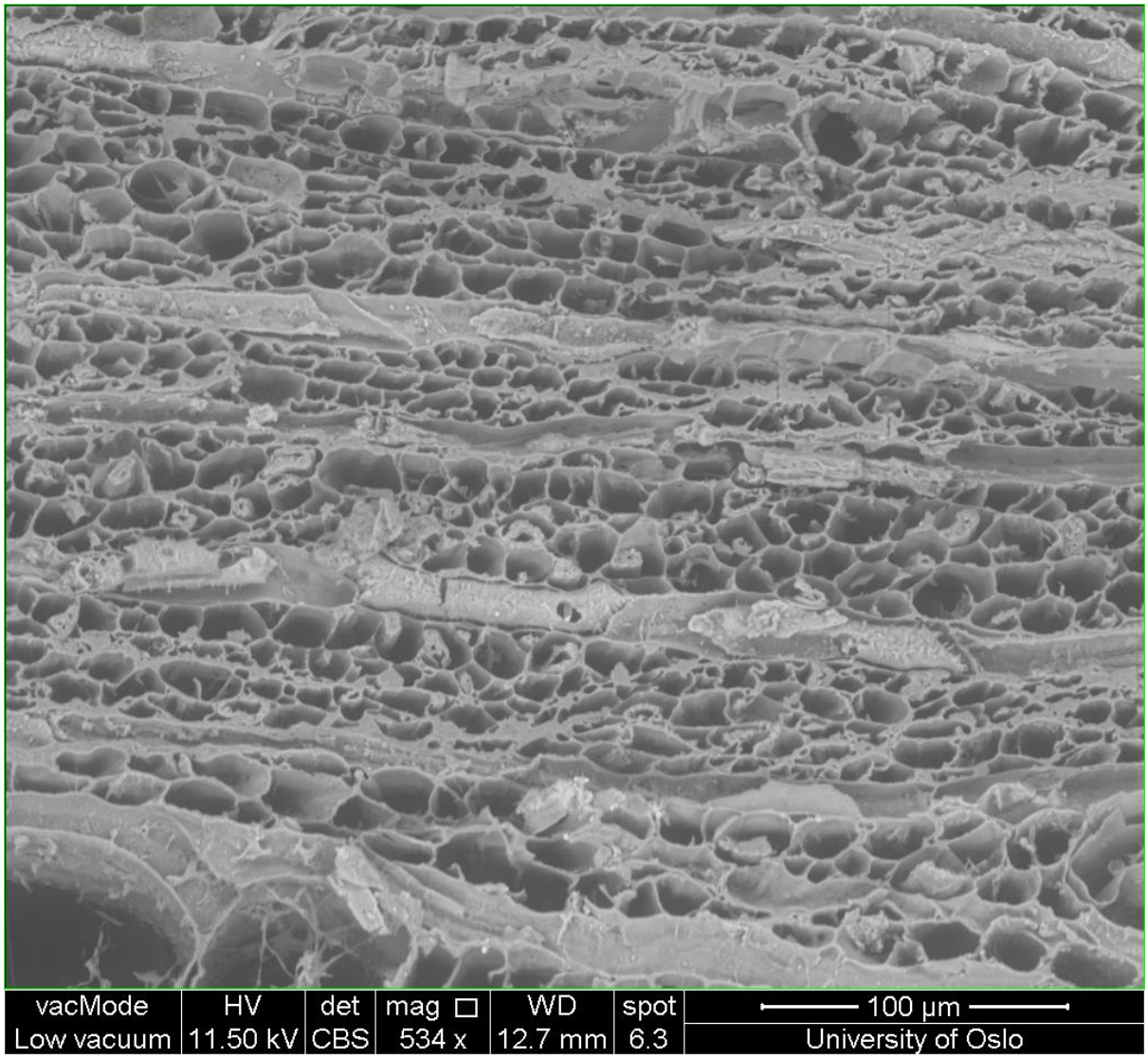


Figure 33. *SEM image of PEG-2000 treated wood centre. The treatment here seems to have given rise to at least some thickening of the structures, though some collapse is still noted in the middle of the image. Cell walls and wood structures seem thicker, with some features being filled in with the PEG treatment.*

The PEG-2000 treatment seems to have the most obvious consolidative abilities given the SEM images. Namely, the cell walls and wood structures seem to be visibly thicker meaning the PEG-2000 treatment has adhered and ideally interacted with the cellulose and lignin structures making up the wood.

4.6. FTIR spectra of wood treatments

Fourier transformed infrared spectroscopy

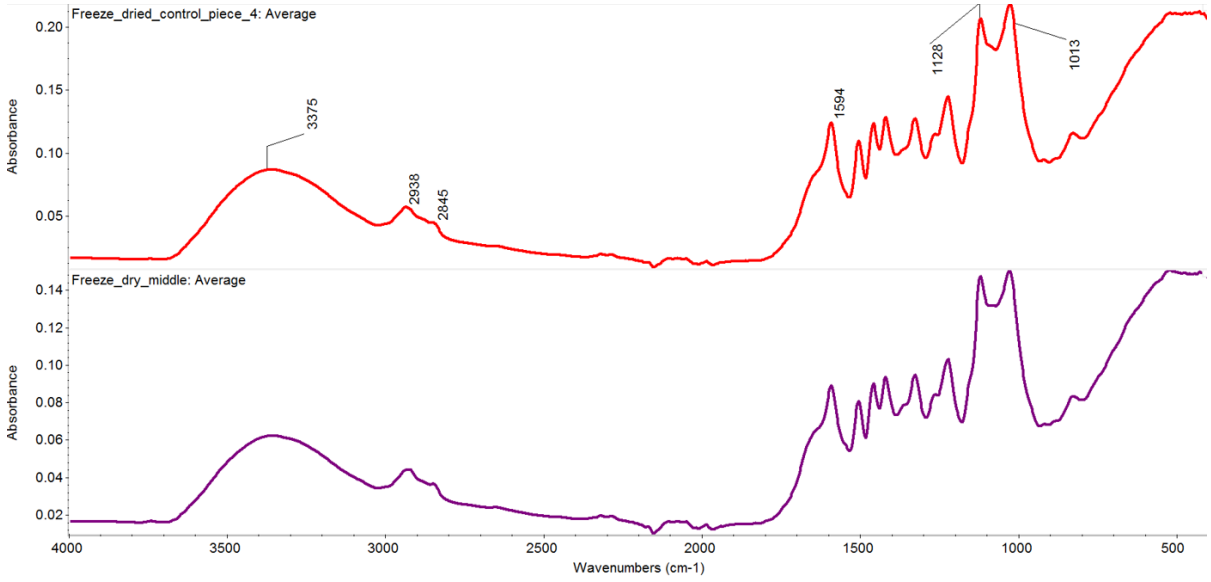


Figure 34. *IR spectroscopy results of two control, freeze dried pieces of sample wood. The spectra here is nearly identical, no major deviations in observed from piece to piece. Highlighted peaks represent key features of the spectra that represent the control wood for reference to other treatments.*

The FT-IR results are highly consistent across the treatments with the exception of the PEG-2000 treatments. There is a high level of similarity between treatments with no real significant deviations between the control samples and the treatments and even between the treatments themselves. The spectra for the freeze-dried wood is consistent with other archaeological samples that have been published, see Tamburini et al., 2017.

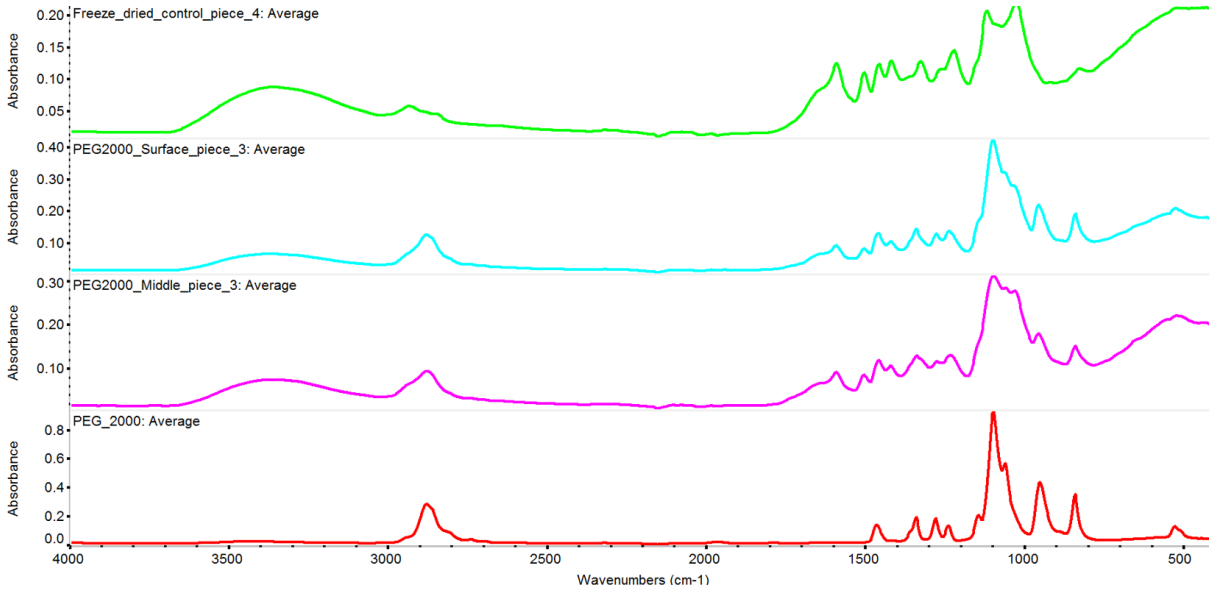
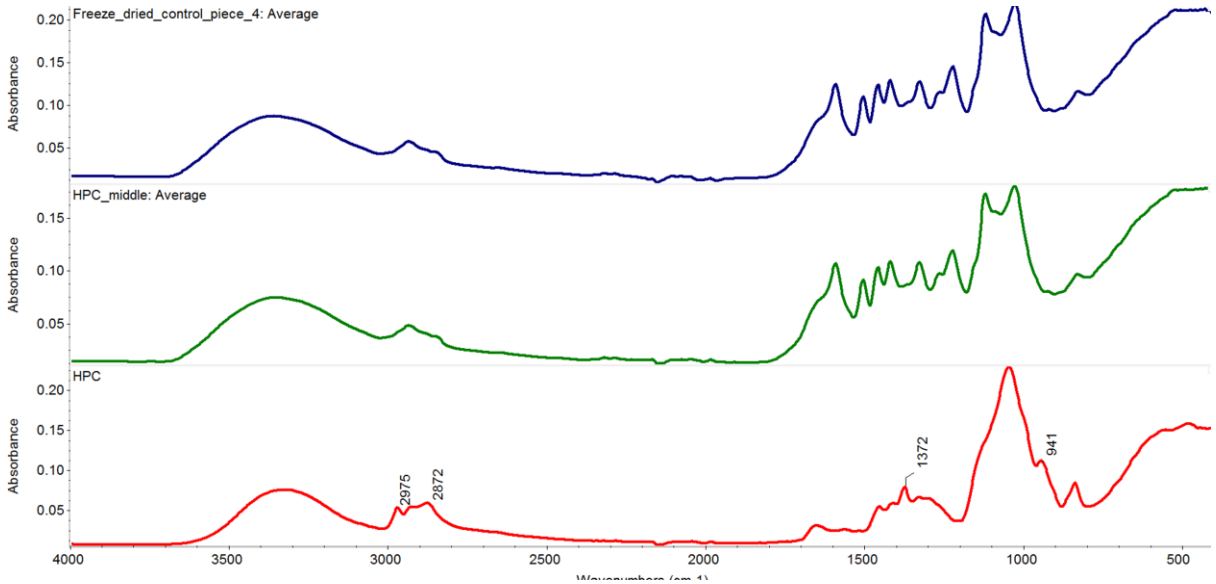
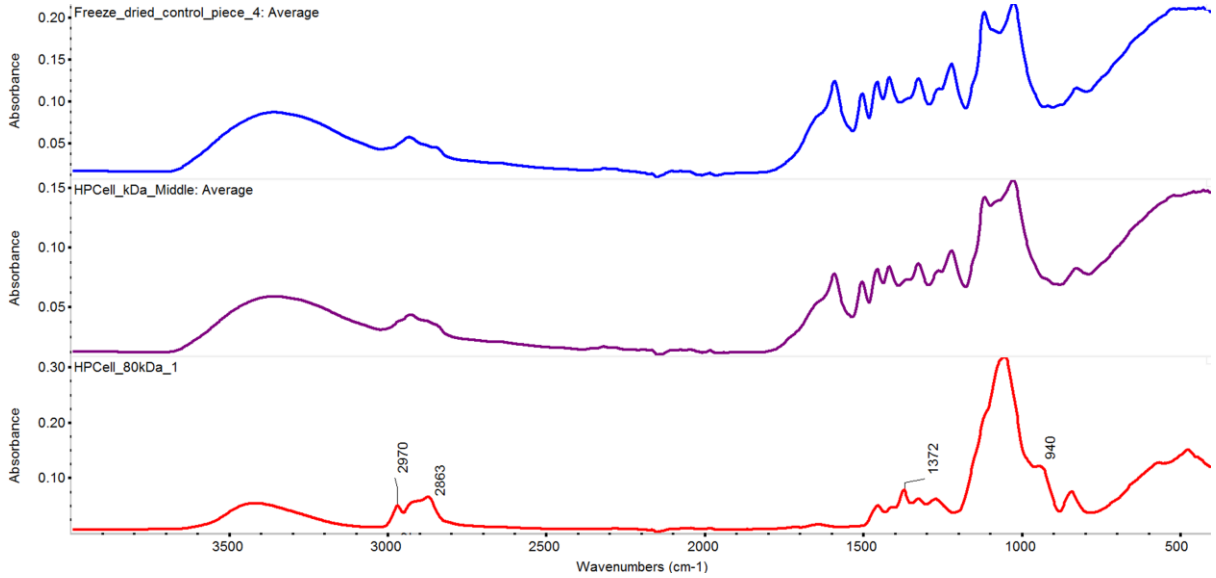


Figure 35. *FTIR spectra of Freeze dried control piece 4, surface + middle fragments of piece 3 of the PEG-2000 treatment and pure PEG-2000 sample for reference. A mild peak corresponding to PEG at around 2800 cm^{-1} us found within the surface and middle pieces of the wood treatment indicating successful penetration.*


Freeze_dried_control_piece_4: Average
HPC_middle: Average
HPC
Absorbance
2975
2872
1372
941
4000
3500
3000
2500
2000
1500
1000
500


Freeze_dried_control_piece_4: Average
HPCell_kDa_Middle: Average
HPCell_80kDa_1
Absorbance
2970
2863
1372
940
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

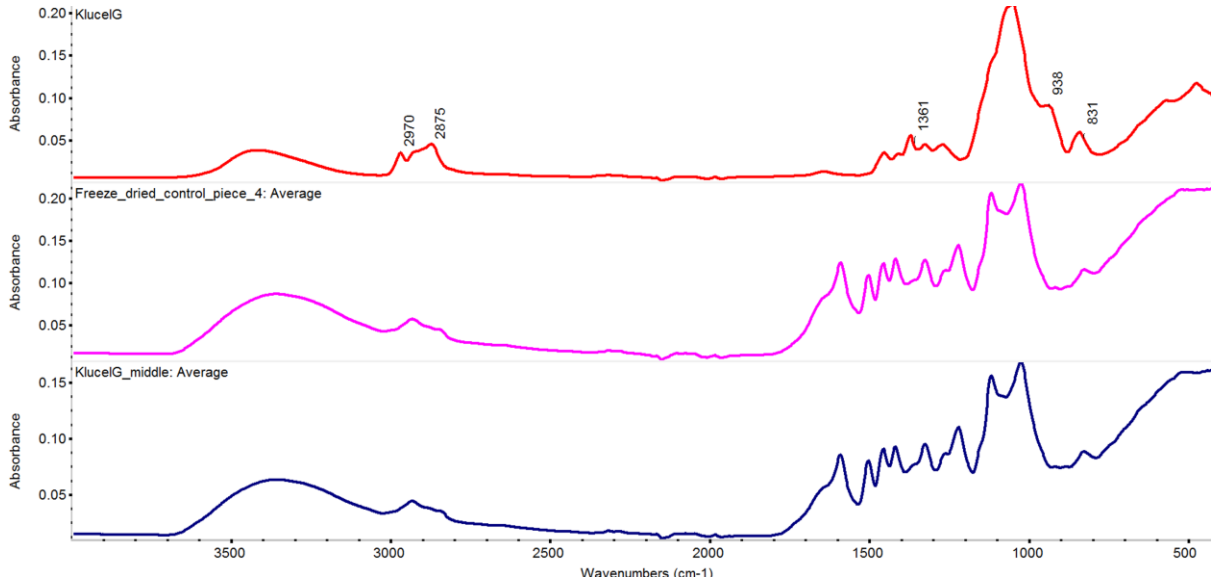

KluceIG
Freeze_dried_control_piece_4: Average
KluceIG_middle: Average
Absorbance
2970
2875
1361
938
831
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

Figure 36. *FTIR spectra's from top to bottom: Freeze dried control piece 4, HPC piece 2 middle, hydroxypropylchitosan pure, Freeze dried control piece 4, HPCellulose (Klucel E) piece 3 middle , Pure Klucel E, Klucel G pure sample, Freeze dried control piece 4 and Klucel G piece 3 middle. X axis correspond to wavenumbers in* cm^{-1}*. No real difference is observed between treatments despite differences between the pure consolidants and the freeze dried wood samples.*

There are several main features common across all of the samples, first is the OH stretching vibration around the 3330 cm^{-1} mark (Tamburini et al., 2017) that presents itself as the broak peak visualised in the left of the spectra's above. The regions with the most variability seem to be the 2800 – 3000 cm^{-1} regions, likely indicating a combination of C-H stretch in methyl and methylene groups (typically seen 2842 – 3000 cm^{-1}) and CH_2 or CH_2OH in cellulose from carbon 6 (typically seen 2835 – 2980 cm^{-1}) (See Schwanninger et al., 2004).

The region 500 – 2000 cm^{-1} shows the most differentiation compared to the pure consolidants. Hydroxypropylchitosan shows an absorbances at 941 & 1372 cm^{-1} that seem to differ from the treated wood and the controls, roughly corresponding to aliphatic CH stretch (1365-1370 cm^{-1}) with other peaks being difficult to distinguish and identify. Similar features are noted in the two hydroxypropylcelluloses (denoted HPCell and Klucel G), both having distinguishing absorbances at ~940 & 1360 cm^{-1} that aren't present within the respective wood treatments, see *figure 36*. It is unclear from these spectra whether the consolidants have penetrated to the centre of the wood, none of the characteristics specific to the hydroxypropylchitosan or either of the hydroxypropylcelluloses are present in any of the spectra's visualised above (Schwanninger et al., 2004).

PEG-2000 illicits a peak at 2800 cm^{-1} corresponding to the same peak observed in the NIST chemistry webbooks Polyethylene glycol entry. This shows up at a reduced amplitude in the corresponding wood treatment indicating PEG-2000 has penetrated to the centre of this samples, see *figure 35*.

5. Discussion

The initial characterisation of the polymers indicates that they are most likely inappropriate for the intended use, being the consolidation of archaeological wood samples. Sedimentation velocity analysis indicates that all assessed have a sedimentation coefficient distributed around 1.5-3 S, a value which is considered typical for polysaccharides. Work by G M Pavlov outlines the concentration dependence of sedimentation for polysaccharides, outlining a number of confounding factors that all play into the sedimentation coefficient observed. Given the variability observed in the molecular weights it would make sense to the layman that the sedimentation coefficients would differ in a similar manner, however this does not seem to be the case (Pavlov., 1997). Pavlov shows that differences in hydrodynamic diameter, contour length, persistence length and the molecular weight all play into the sedimentation coefficients observed via sedimentation velocity analysis (Pavlov., 1997). Owing to issues beyond the control of this investigation it was not possible to determine the hydrodynamic diameters or persistence/contour lengths of the polymers assessed, making it difficult to assess the effects of concentration dependence on the values obtained to an accurate degree, despite extrapolating back to zero concentration (Pavlov., 1997).

Sedimentation velocity analysis had also indicated that the polymers were monodisperse, with one main peak being present across all the polymers assessed. Some near-zero peaks were recorded across some concentrations, with this not being correlated to any particular polymer and more an artefact of the analysis presenting itself with a peak that is not representative of the polymer. It is possible that the high speed of the sedimentation velocity ~ 45,000 rpm did not lead to sufficient separation of components and was only possible at the lower speed the sedimentation velocity was run at.

The molecular weights obtained for the consolidants each fall above the values typically quoted to guarantee penetration and consolidation by a consolidant. Christensen et al in 2015 and other work by Wakefield et al in recent years (Wakefield et al., 2018 & 2020) shows that aqueous consolidants

typically need to have a very low molecular weight to guarantee penetration into wood samples, Christensen outlines that further research should be undertaken to determine exactly what this cutoff point is or whether it is purely a matter of a longer consolidation (Christensen et al., 2015), giving credence to the investigation of these consolidants even though they may have been disregarded as some by being too large.

In anticipation of this and using previous work (Christensen et al., 2015; Wakefield et al., 2018 & 2020) as a guideline a treatment time of two weeks was employed to see whether the potential consolidants assessed would penetrate and what the effects on the dimensional stability + strength would be. Considerable changes in density were observed in the consolidant treatments compared to the control samples, there are large increases in mean average density ranging from ~17% for hydroxypropylchitosan and hydroxypropylcellulose (denoted as HPCell or Klucel E) to as high as ~ 24% for the PEG-2000 treatments. Combined with large increases in volume the increase in density proves to be a promising sign that large amounts of the consolidants have penetrated right into the middle of the wood samples, as only surface consolidation would be limited to a minor density increase or even a decrease given the samples swelled by as much as 38% for the PEG-2000 treatment.

Swelling as much as is exhibited by these samples is not ideal under any scenario and would have the potential to destroy larger objects made up of larger wood structures, especially those made up of multiple interlocked/joined pieces of wood. While the ethos of preservation can differ on an individual and organisational level it is generally accepted that artefacts should be kept at their current conditions as best as possible. With such a large change in volume this alone can exclude these treatments from ever being viable for use by conservators, if this were to be used by conservators then it would need to be determined what exactly is causing the samples to swell which is difficult to discern.

Following on from the failure of dimensional maintenance and stability, the employment of the tape test hoped to give an idea of the success of consolidation and improvement of this brittle nature observed in the untreated samples. Indeed, it does appear that the treatments were a success. All

treatments when compared to the freeze-dried samples faired better in terms of mass loss and in terms of strength when handling and applying the tape to the samples. It was shown that the difference between the mean mass removed from each treatment was negligible, roughly equating to equal success in consolidating. Some issues were faced with the first batch of tape being too strong and tending to tear chunks or layers of wood off, showing the adhesive was far too strong for the samples to be differentiated from one another. There is no strong consensus for any physical or mechanical measurement/experiment to determine how well something has consolidated, with large numbers of conservators do not publish their work, so it is up to the individual experience of the investigator themselves which can vary wildly in experience and ideas (Drdácký & Slížková., 2013 & Wakefield., 2021).

The tape test has been successfully employed within the Saving Oseberg project before (see Wakefield., 2021) despite most research indicating its use in stone consolidation (Drdácký & Slížková., 2015). The operator, myself, used the same tape for each consolidant, and a consistent 1 cm^2 segment of tape was used and pressed against the wood three times with a consistent force though this was not measured. Previous attempts at using the tape test had also damaged areas of the samples, so effort was made to use different areas with a tape found to be more suitable across both the front and back of the wood pieces. As such it is likely that the results of this tape test have been reliable and accurate at measuring and determining the success of the consolidants in the wood samples used and shows that in some manner the treated wood samples are in better condition than the freeze-dried samples.

The tape test proves to be a somewhat subjective test that lacks the real scientific rigour of other experiments but still remains useful. Aside from initial issues faced with tape adhesive being too strong and thus completely tearing up samples once there was a more suitable tape in play it was able to give an idea of how well performing a consolidant was. The results of this showed that the best performing by far was the air dried, owing to the complete collapse of the structure leaving a stronger, denser

shell of what was initially present. The worst performing by far were the freeze-dried samples, with all treatments seeing an average mass loss significantly lower than what was observed in the freeze dried, giving a somewhat luke-warm indication that the consolidation has been successful.

The colourimetry conducted shows the consolidants have successfully managed to keep colour change incredibly minimal. With a ΔE of less than 3 across the board, the treatments applied would not have elicited a noticeable colour change for the untrained eye. Within this test the best performers were the hydroxypropylchitosan and the Klucel G treatments, although marginal, they managed to produce a mean change in colour less than other samples and less than the air-dried control, however this was deemed to be not statistically significant owing to the relatively large variability exhibited here. This was primarily put down to the colour gradient observed across a number of wood pieces that presented itself post freeze drying and has likely affected the results of the colourimetry for the worse. It is unclear what is responsible for this colour gradient, this was not noticeable pre-drying but it remains similar to some of the features observed within the larger wood chunk the piece was cut from. As preservation of an artefacts appearance is of high importance to conservators the relative success of the consolidants in only inducing a small colour change is a promising sign, although high variability in sample colour pre and post treatment hampers the significance of these results to a high degree.

Outside of the official testing, treated samples remained much stronger than the air-dried samples as was evidenced by frequent flaking and breakages observed within the freeze-dried samples that were not present within the treatments. Previous research outlined in the earlier stage of this investigation (Review Paper) showed that the consolidants assessed here had previous success in surface consolidation, (see Cipriani et al., 2010) the sample handling indicates that this has occurred across the treatments here with treatments showing considerably less flakiness when being handled when compared to the freeze-dried samples, although for the centre of the wood pieces it is less clear whether they have penetrated and consolidated.

When considering the penetration and consolidation of the wood samples centres, the FT-IR and SEM images give the best indication into what has happened. The FT-IR spectra's taken on their own would indicate that the consolidation was not successful. There was no real difference observed in the spectra's between treatments and the freeze dried control, with the exception of the PEG-2000 treatment. Pure consolidant samples indicate absorbances within the 2800 – 3000 cm^{-1} range that are not observed within the freeze-dried control samples nor do they seem to be present within the wood treatments with said consolidants. The petrochemical nature of the PEG-2000 treatment gives a distinct peak that is observable against the rest of the wood with ease, making identification of the PEG much easier than the other consolidants used, which elicited a clear peak at 2875 cm^{-1} that matches up to the pure PEG sample (NIST Chemistry WebBook., nd) . This does not mean that the consolidants did not penetrate the wood samples and this can be confirmed using a combination of experience handling and cutting the treated wood pieces and examining the SEM images. At the very least, the EDS analysis shows an increase in nitrogen presence within the centre of the sample that differed very greatly from the untreated freeze-dried samples, indicating a successful consolidation and penetration. Hydroxypropylchitosan has a significantly higher nitrogen content than cellulose and lignin structures found within wood, making this an easy confirmation of the sample penetration. The images also show the maintenance of the wood structure to a greater degree than what was observed in the freeze dried, though this was marred by damage from cutting the wood sample to fit it to the SEM platter.

The FTIR spectra of the other treatments, namely the two hydroxypropylcelluloses Klucel G & 'E' was equally as uninformative. Partially owing to the chemical similarity between the consolidants and the wood. Comparing these results to the SEM images, of Klucel Gas mentioned previously, has the best-preserved microstructure in the wood and stands in stark contrast to other consolidants within this investigation. The FTIR spectra does not show anything majorly different to the freeze-dried controlled spectra's which is to be expected to the high chemical similarity to cellulose and lignin structures.

Klucel G seems to have faired well when looking at the SEM images. Across all of the 6 treatments, 2 control and 4 consolidants, the Klucel G seems to have some of the best-preserved structures that even seemed to be in better condition than the structures observed in the supposedly 'Fresh' Oak sample supplied by the University of Oslo. This is not to say that the oak supplied was not fresh itself, but that the damage noted within the samples was similar to what was observed in some of the other samples visualised that had likely undergone drying with liquid water, leading to excess damage and shrinkage.

Another main issue that has plagued this investigation is the freezing point of the consolidants under a freeze dryer, specifically relating to the freeze drier used in this investigation. It was pointed out by conservators working at the University of Oslo that the PEG-2000 treatment was unlikely to have stayed frozen for the duration of the freeze drying despite being in a -80^{O}C freezer over a number of days prior. Further preliminary investigations into this showed that all consolidants investigated for wood treatments: two hydroxypropylcelluloses, hydroxypropylchitosan and PEG-2000 all did not remain fully frozen whilst within the freeze drier. This poses a very important issue that threatens the validity of the latter half of this investigation, namely if the consolidant solutions were not fully frozen then the lack of the consolidants observable in the FTIR spectra's and the damage observed in the SEM images could be put down to the surface tension of the solutions collapsing the wood structures leaving the wood in its present state. Unfortunately, this is not something that could be remedied at the University of Nottingham and as such any further investigations should make use of other freeze driers containing an insulated vacuum chamber that can hold a much lower temperature for the samples. With the freeze drier used having a non-insulated cooling/vacuum chamber and a condenser plate that appears to only keep the very bottom shelves somewhat cool it is unlikely this instrumentation has produced viable results for this investigation. Thus, any future attempts covering any of these materials would be better using a freeze drier with an insulated cooling/vacuum chamber.

The recency of this area of bioarchaeology makes it difficult to compare and contrast results with other alike polymers. Klucel E, a branded name product of hydroxypropylcellulose, has been trialled as a consolidant in the past in combination with varying levels of nanocellulose. Work by Hammed & Hassan in 2019 showed that Klucel E, or a hydroxypropylcellulose product around the 80 kDa range, could work as a consolidant in conjunction with the addition of nanocellulose polymers. Concentrations of around 1-2 % Klucel E seemed to have penetrated best, perhaps indicating that the concentrations used in this investigation of 4% w/v were too high for appropriate use. This research also talks of a significant accumulation of the consolidant on the vessel cell walls, even at the 1 % concentration (Hamed & Hassan., 2019). Not even in the outside and surface sections was this observed for either the Klucel G or the 'Klucel E' polymers. This could be related to the freeze-drying issue, whereby the surface tension of the water was enough to remove large portions of the consolidant from the wood samples, in combination with the higher treatment concentration used perhaps this gives the reason as to why both of the hydroxypropylcellulose polymers assessed have fallen shorter than hoped (Krorra et al., 2021; Mohamed & Ali., 2020; Mohamed., 2022; Pataki-Hundt et al., 2021). Other papers concerning the use of hydroxypropylcellulose, zinc oxide nanoparticles and nanocellulose seem to indicate successful consolidation when concerning paper, which is in turn primarily a cellulose-based product, giving at least some indication that interactions can form between Klucel G or E products and cellulose polymers (Krorra et al., 2021; Mohamed & Ali., 2020; Mohamed., 2022; Pataki-Hundt et al., 2021). This can only be left as speculation owing to the time constraints limiting the investigation no direct interaction studies could be conducted with the specific polymers used (Krorra et al., 2021; Mohamed & Ali., 2020; Mohamed., 2022; Pataki-Hundt et al., 2021).

For methylcellulose, owing to the previous literature outlining that its molecular weight is likely too high to be effective, but showing alike effectiveness to what is seen in the hydroxypropylcelluloses it is reasonable to assume that methylcellulose would work in some manner as a consolidant. Owning to time constraints and the lack of another methylated biopolymer for comparison and to assess what the addition of the methyl group does for conservation scenario's, it was simply not practical to carry

forward. As such we are left with the same conclusions that were reached in the review article produced as part A of this project, in that the Levantine foundation and other materials suggest that methylcellulose could be a potential consolidant (See Feller et al., 1991; Miranda-Valdez et al., 2013; Unger et al., 2001). It seems to have a successful basis in consolidating paints, flaky materials and other organic based objects but the molecular weight determined as part of this investigation makes it too high to guarantee success, and only further direct work on this can address its potential or whether it can interact with highly degraded wood samples.

For hydroxypropylchitosan, there is a strong background of research into the performance of various chitosan-derived polymers as consolidants for archaeological wood samples. The results here give an indication that, perhaps, if the freeze-drying had not been an issue then the hydroxypropylchitosan treatment could be more successful than it is. Given the properties demonstrated in this investigation, combined with its antimicrobial and metal ion chelating it would seem to be an appropriate consolidant for some artefacts (Christensen et al., 2015; Peng et al., 2005; Walsh et al., 2014; Walsh et al., 2017). Given the SEM images of the hydroxypropylchitosan treated pieces, as well as the FT-IR spectra's, it is unclear as to whether it has penetrated to the centre. No real thickening of the vessel cell walls or any other structural components is noted, nor indeed within any of the other treatments. However, a strong basis for the consideration of chitosan initially may be misguided as the substitution for the hydroxypropyl group comes on the nitrogen atom in the chitosan monomer, a location key for interactions with cellulose and lignin. It is certainly possible that the failures of hydroxypropylchitosan in this manner can be put down to this substitution leading to a loss or reduced rate of interactions with cellulose or lignin polymers. However, this is impossible to say given the apparent failure of the freeze drying and the lack of any interaction studies to prove this.

As was stated during the literature review section in part A, it was noted that there seemed to be a range of molecular weights that could be occupied by consolidants. This range, anywhere from 5 kDa to 20 kDa, with no clear limits or boundaries, plays a central role in interpreting the results of this

investigation (Christensen et al., 2015; Wakefield et al., 2018; Wakefield et al., 2020). Without the guarantee that the freeze-drying has not further damaged the wood and lacking in a true control to test for this, owing to the damaged nature of the fresh wood, the theoretical limit of what could work plays a significant role as to whether these consolidants do appear to have worked (Lucejko et al., 2021; McHale et al., 2017; Wakefield et al., 2018; Wakefield et al., 2020a; Wakefield et al., 2020b; Wakefield., 2021). Given the swelling of the samples, the density increases observed, the lack of a significant colour change, the molecular weights determined and the theoretical knowledge on whether these molecular weights would work it can be suggested that the consolidants have not been successful. Given how all the samples handled, while there was an improvement in the strength and structure compared to the air and freeze-dried this is all strictly relative and the lack of evidence in the SEM for vessel cell wall thickening in any of the treatments other than PEG-2000 which is already established as a treatment (Han et al., 2022 &Hunt et al., 2021).

6. Conclusion

Overall, the results of the investigation make it difficult to recommend any of the consolidants if nothing else is taken into consideration. With it now known that the consolidants would not stay frozen within the freeze drier used for this investigation the results of the consolidation testing remain unreliable as it is unclear. The role in which the consolidants played in failing to strengthen the wood samples is not possible to determine from these results and would require careful consideration of the testing process and closer cooperation with conservators to yield a higher quality of results than was achieved during this investigation. With the characterisation of the consolidants identifying them as likely being too high in molecular weight for an aqueous consolidant to successfully consolidate the results of this investigation confirm the initial characterisations assumptions that they would be mostly unsuitable for use in consolidating archaeological wood but must not be taken on face value due to the issues with freeze drying and ambiguity as to what role this has played in the failure to consolidate.

7. References

Abu Krorra, A., Noshy, W., Oun, A. and Abu Elleif, M., 2021. Evaluation of hydroxypropyl cellulose, zinc oxide nanoparticles and nanocellulose for tracing papers consolidation. *Advanced Research in Conservation Science*, *2*(1), pp.21-30.

Andrea Pataki-Hundt, A., Börngen, M. and Bretzendorfer, C. (2021) *ICOM-CC 19th triennial conference KLUCEL G*. Available at: https://www.researchgate.net/publication/354312207_ICOM-CC_19th_Triennial_Conference_Klucel_hydroxypropylcellulose_Its_impact_on_artificial_ageing_and_consequences_for_conservation_usage_-A_case_study (Accessed: 01 August 2023).

Broda, M. and Hill, C.A., 2021. Conservation of waterlogged wood—past, present and future perspectives. *Forests*, *12*(9), p.1193.

Chaturvedi, S.K., Ma, J., Brown, P.H., Zhao, H. and Schuck, P., 2018. Measuring macromolecular size distributions and interactions at high concentrations by sedimentation velocity. *Nature Communications*, *9*(1), p.4415.

Christensen, M., Kutzke, H. and Hansen, F.K., 2012. New materials used for the consolidation of archaeological wood–past attempts, present struggles, and future requirements. Journal of Cultural Heritage, 13(3), pp.S183-S190

Christensen, M., Larnøy, E., Kutzke, H. and Hansen, F.K., 2015. Treatment of waterlogged archaeological wood using chitosan-and modified chitosan solutions. Part 1: chemical compatibility and microstructure. *Journal of the American Institute for Conservation, 54(1),* pp.3-13.

Drdácký, M. and Slížková, Z., 2015. In situ peeling tests for assessing the cohesion and consolidation characteristics of historic plaster and render surfaces. *Studies in Conservation*, *60*(2), pp.121-130.

Faghihi, T., Heidarzadeh, Z., Jafari, K., Farhoudi, I. and Hekmatfar, S., 2021. An experimental study on the effect of four pediatric drug types on color stability in different tooth-colored restorative materials. *Dental research journal*, *18*.

Feller, R.L. and Wilt, M.H., 1991. Evaluation of cellulose ethers for conservation. *Getty Publications.*

Hamed, S.A.A.K.M. and Hassan, M.L., 2019. A new mixture of hydroxypropyl cellulose and nanocellulose for wood consolidation. *Journal of Cultural Heritage*, *35*, pp.140-144.

Han, L., Guo, J., Tian, X., Jiang, X. and Yin, Y., 2022. Evaluation of PEG and sugars consolidated fragile waterlogged archaeological wood using nanoindentation and ATR-FTIR imaging. *International Biodeterioration & Biodegradation*, *170*, p.105390.

Hunt, S., Grau-Bove, J., Schofield, E. and Gaisford, S., 2021. Effect of polyethylene glycol treatment on acetic acid emissions from wood. *Forests*, *12*(12), p.1629.

Li, F.S., Phyo, P., Jacobowitz, J., Hong, M. and Weng, J.K., 2019. The molecular structure of plant sporopollenin. *Nature plants*, *5*(1), pp.41-46.

Łucejko, J.J., de Lamotte, A., Andriulo, F., Kutzke, H., Harding, S., Phillips-Jones, M., Modugno, F., Slaghek, T.M., Gosselink, R.J. and Braovac, S., 2021. Evaluation of soda lignin from wheat straw/sarkanda grass as a potential future consolidant for archaeological wood. *Forests, 12(7),* p.911

McHale, E., Steindal, C.C., Kutzke, H., Benneche, T. and Harding, S.E., 2017. In situ polymerisation of isoeugenol as a green consolidation method for waterlogged archaeological wood. *Scientific Reports, 7(1)*, p.46481.

Miranda-Valdez, I.Y., Coffeng, S., Zhou, Y., Viitanen, L., Hu, X., Jannuzzi, L., Puisto, A., Kostiainen, M.A., Mäkinen, T., Koivisto, J. and Alava, M.J., 2023. Foam-formed biocomposites based on cellulose products and lignin. *cellulose, pp.1-14.*

Mohamed, W.S.A., 2022. Strengthening the Clumping Paper Properties using Hydroxypropyl Cellulose Applied on a Historical manuscript 13th Century AD. *Biointerface Research in Applied Chemistry, 13, pp. 375*

NIST Chemistry WebBook. *Polyethylene Glycol IR Spectrum* (nd) *Polyethylene glycol IR Spectrum*. Available at: https://webbook.nist.gov/cgi/formula?ID=B6009512&Mask=80#Top (Accessed: 14 August 2023).

Pavlov, G.M., 1997. The concentration dependence of sedimentation for polysaccharides. *European Biophysics Journal*, *25*(5-6), pp.385-397.

Peng, Y., Han, B., Liu, W. and Xu, X., 2005. Preparation and antimicrobial activity of hydroxypropylchitosan. *Carbohydrate Research, 340(11)*, pp.1846-1851.

Schuck, P., 2000. Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. *Biophysical journal*, *78*(3), pp.1606-1619.

Schwanninger, M.J.C.R., Rodrigues, J.C., Pereira, H. and Hinterstoisser, B., 2004. Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. *Vibrational spectroscopy*, *36*(1), pp.23-40.

Tamburini, D., Łucejko, J.J., Pizzo, B., Mohammed, M.Y., Sloggett, R. and Colombini, M.P., 2017. A critical evaluation of the degradation state of dry archaeological wood from Egypt by SEM, ATR-FTIR, wet chemical analysis and Py (HMDS)-GC-MS. *Polymer Degradation and Stability*, *146*, pp.140-154.

The Levantine Foundation., 2020. Consolidation of flaking media - The Levantine Foundation. Available at: http://www.thelevantinefoundation.co.uk/wp-content/uploads/2020/11/ConsolidationHandout.pdf (Accessed: 14 April 2023).

Unger, A., Schniewind, A.P., Unger, W., Unger, A., Schniewind, A.P. and Unger, W., 2001. *History of wood conservation. Conservation of Wood Artifacts: A Handbook*, pp.3-7.

Wakefield, J., 2021. Natural polymers for consolidation of the Oseberg artefacts (Doctoral dissertation, University of Nottingham).

Wakefield, J.M., Braovac, S., Kutzke, H., Stockman, R.A. and Harding, S.E., 2020a. Tertbutyldimethylsilyl chitosan synthesis and characterization by analytical ultracentrifugation, for archaeological wood conservation. *European Biophysics Journal, 49*, pp.781-789.

Wakefield, J.M., Gillis, R.B., Adams, G.G., McQueen, C.M. and Harding, S.E., 2018. Controlled depolymerisation assessed by analytical ultracentrifugation of low molecular weight chitosan for use in archaeological conservation. *European Biophysics Journal, 47, pp.769-775.*

Wakefield, J.M., Hampe, R., Gillis, R.B., Sitterli, A., Adams, G.G., Kutzke, H., Heinze, T. and Harding, S.E., 2020b. Aminoethyl substitution enhances the self-assembly properties of an aminocellulose as a potential archaeological wood consolidant. *European Biophysics Journal, 49, pp.791-798.*

Walsh, Z., Janeček, E.R., Hodgkinson, J.T., Sedlmair, J., Koutsioubas, A., Spring, D.R., Welch, M., Hirschmugl, C.J., Toprakcioglu, C., Nitschke, J.R. and Jones, M., 2014. Multifunctional supramolecular polymer networks as next-generation consolidants for archaeological wood conservation. *Proceedings of the National Academy of Sciences, 111(50),* pp.17743-17748.

Walsh, Z., Janeček, E.R., Jones, M. and Scherman, O.A., 2017. Natural polymers as alternative consolidants for the preservation of waterlogged archaeological wood. *Studies in Conservation, 62(3),* pp.173-183.

Walsh-Korb, Z., Stelzner, I., dos Santos Gabriel, J., Eggert, G. and Avérous, L., 2022. Morphological Study of Bio-Based Polymers in the Consolidation of Waterlogged Wooden Objects. *Materials*, *15*(2), p.681.

8. Acknowledgments

I would like to thank Professor Stephen Harding, Dr Jennifer Wakefield, Dr Susan Braovac, Dr Calin Steindal and Dr Hartmut Kutzke, for helping me with my work and supporting my trip to Oslo, without which this work would not be possible.