Chemical Modification of Poly-α-1,3-Glucan

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

Sustainability is becoming a primary consideration in the development of new materials. Specifically, renewability and biodegradability are desirable properties for materials in a variety of applications, including packaging and personal care products. α-1,3-glucan is a biodegradable polysaccharide found naturally in fungi and bacteria. It can also be synthesized enzymatically from sucrose to have a controlled structure and molecular weight. Similar to cellulose, α-1,3-glucan has a high degree of crystallinity which hinders its interaction with other materials. The objective of this research is to investigate the chemical modification of α-1,3-glucan with the goal of changing its properties so it would become suitable to be manufactured into a valuable material.

α-1,3-glucan, dissolved in N,N-dimethyl acetamide/lithium chloride, is functionalized with octenylsuccinic anhydride. Reaction conditions are varied and their effects on the structural and thermal properties of the esterified α-1,3-glucan are explored. Modified α-1,3-glucan samples are also qualitatively analyzed for melt flow behaviour and interaction with oil and water mixtures.

The optimal reaction conditions for maximizing the degree of substitution and minimizing chain degradation consist of a temperature of 50 °C and a molar ratio of octenylsuccinic anhydride to anhydroglucose units of 3:1. Increasing the reaction temperature was found to cause degradation of the polysaccharide backbone. The addition of pyridine is found to increase the degree of substitution and alter the extent of chain degradation depending on the reaction conditions. A decrease in lithium chloride concentration leads to an increase in degree of substitution and increased molecular weight loss. Esterified α-1,3-glucan samples do not show thermal transitions in calorimetry measurements but samples with sufficient extent of modification flow under the simultaneous application of heat and force. Due to the amphiphilic nature of octenylsuccinic anhydride, α-1,3-glucan modified at elevated temperature shows potential as an emulsifying agent.

α-1,3-glucan continues to show promise as a renewable and biodegradable material. Chemical modification via functionalization of the hydroxyl groups has been shown to successfully alter the material properties. Additional experimentation is recommended to further explore the effects of catalyst and lithium chloride concentrations on the reaction efficiency and material properties. Continued investigation of the emulsifying capacity should be performed to verify the conclusions drawn from preliminary testing.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Full Name
AGU	Anhydroglucose unit
DMAc	N,N-Dimethyl acetamide
DMSO	Dimethyl sulfoxide
DP_n	Number average degree of polymerization
DP_w	Weight average degree of polymerization
DS	Degree of Substitution
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared (spectroscopy)
KBr	Potassium bromide
LiCl	Lithium chloride
M_n	Number average molecular weight
M_w	Weight average molecular weight
M_z	Third moment molecular Weight
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance (spectroscopy)
OSA	Octenylsuccinic anhydride
PDI	Polydispersity index
SEC	Size exclusion chromatography
TGA	Thermogravimetric analysis
XRD	X-ray diffraction
% w/v	Percent by weight per volume in grams per millilitre

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND & MOTIVATION

Natural polymers are integral to our lives in applications that we may not think twice about, such as wool and cotton for clothing, cellulose in paper, and latex for rubber applications. It was not until 1920 when Hermann Staudinger theorized the true nature of polymers as molecules composed of repeating units – or monomers – connected via covalent bonds into long chains [1]. However, 55 years previously, in 1865, cellulose was first acetylated by Paul Schutzenberger, to produce what would later become the cellulose acetate used in camera film, aircraft dope, and fabric fibers [2]. Despite cellulose acetate, one of first human-made thermoplastics, being a renewable and biodegradable material, it was quickly replaced by petroleum-based polymers throughout the 20th century [3]. Bakelite, a phenol-formaldehyde resin, was the first synthetic polymer used as a thermoset for a variety of applications [3, 4]. In the decades following World War I, the synthesis and production of polymers increased dramatically, including the creation of new materials like polystyrene, polyvinyl chloride, and polyethylene – all of which are non-renewable and non-degradable [3]. Polymers were and are still favoured for their durability, tunability, low cost, and light weight making it easy to use in a wide variety of applications. They have thus replaced alternative materials like metals, glass, and ceramics.

However, during the onset of wide polymer usage in 1900s, the idea of plastic waste was not at the forefront. Decades later we are now dealing with the consequences of plastic pollution globally. In 2010, 275 million tonnes of plastic waste were produced in 192 coastal countries, with 4.8 to 12.7 million tonnes estimated to have entered the world's oceans [5]. As conventional plastics are non-degradable, the pervasive plastic waste is persistent and can remain in land and marine environments for thousands of years [3]. Since recycling infrastructure is still limited in Canada, it is desirable to create biodegradable plastics that can replace conventional plastics in vital applications that can then be composted in households or in industrial composting facilities.

In addition to the plastic pollution crisis, climate change is having drastic negative effects around the world, from worsening air quality to increasing frequency and strength of natural disasters [6]. Conventional plastics are produced from fossil fuel by-products and therefore provide financial incentive for fossil fuel corporations to continue extracting and burning fossil fuels. This establishes a need for biodegradable plastics to be made from renewable resources. Hence, key attributes like renewability and biodegradation found in natural polymers are becoming increasingly relevant as our society begins to prioritize sustainability.

Current available biodegradable plastics include poly (butylene adipate-*co*-terephthalate) (PBAT), polybutylene succinylate (PBS), polylactic acid (PLA), polyhydroxy alkanoates (PHAs), cellulose acetate, and thermoplastic starch. PLA, acetate, and starch are all sourced from renewable sources but have limitations on their properties. For example, PLA has a low glass transition temperature, making it susceptible to deformation at low to moderate temperatures and the properties of starch are highly dependent on its structure which can vary significantly between starch sources [7, 8, 9, 10].

Alternatively, α-1,3-glucan is a polysaccharide produced via enzymatic synthesis where sucrose is fed to *E.coli* containing the gene for a glucosyltransferase enzyme in an alkaline aqueous medium [11, 12]. The product of this enzymatic polymerization is unbranched and has a controlled chain length, allowing for its thermal, structural, and mechanical properties to be consistent between batches. Like starch and cellulose, α-1,3-glucan has a very high melting point above its thermal degradation temperature. So, strategies for their chemical modification or plasticization are required in order to be thermoprocessable.

1.2 PROJECT OBJECTIVES

The objective of this research project is to investigate the chemical modification of α-1,3-glucan with the goal of changing its properties so it would become suitable to be manufactured into a valuable material. There are multiple potential applications of polysaccharides like α-1,3-glucan, including as biodegradable plastics or as emulsion stabilizers in food and cosmetic industries ([9, 13, 14, 15].

At the onset of this project, the objective was:

- To alter the properties of α-1,3-glucan by chemical modification to produce a thermoprocessable material which could be used as an alternative to conventional plastics.

Meeting this objective involves increasing the hydrophobicity, disrupting the crystallinity, and depressing the melting point of α-1,3-glucan without causing extensive degradation of the polysaccharide backbone.

As the project progressed, the objective was modified to include:

- To alter the properties of α-1,3-glucan by chemical modification to produce an amphiphilic molecule to be used as an emulsion stabilizer in personal hygiene applications.

1.3 SCOPE

The research project involved exploration of the modification of α-1,3-glucan with a variety of reagents using a variety of methods. A concept map summarizing these explorations is provided in Figure 1.

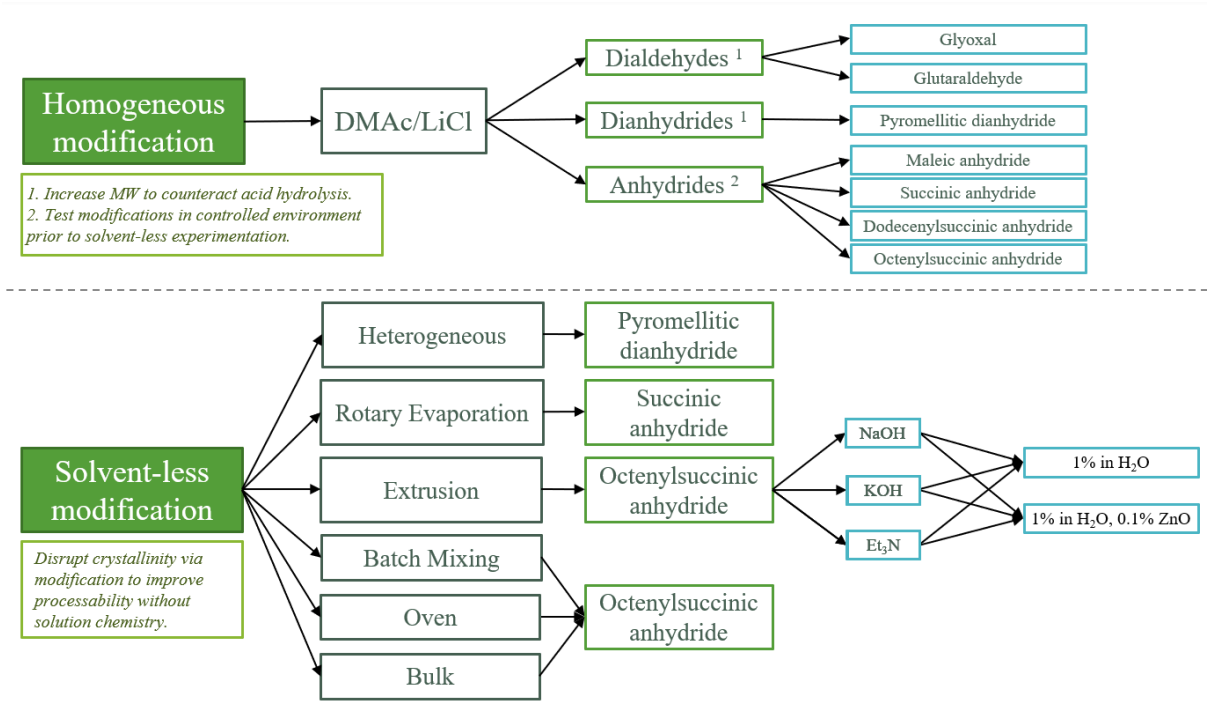


Figure 1. Concept map of the chemical modification of α-1,3-glucan.

The scope of this thesis involves the chemical modification of α-1,3-glucan with octenylsuccinic anhydride and subsequent chemical and thermal analysis. Although some modifications were performed in solid-state, they did not perform as expected. The primary focus of this research was to explore the reaction in solution. Experiments were performed to evaluate the effect of time, temperature, and concentration of octenylsuccinic anhydride on the degree of substitution and molecular weight of α-1,3-glucan.

The chemical modification of α-1,3-glucan is a fairly new research topic. Several challenges were encountered throughout experimentation; therefore, this thesis also contains a description of these obstacles and how they could impact the validity of the results presented herein.

1.4 THESIS OUTLINE

This thesis contains six chapters covering the background, results, and conclusions of this project. Chapter 1 covers the motivating factors and scope of the project. Chapter 2 contains a thorough literature review of information and research relevant to the project. Chapter 3 describes the materials and experimental methods of the project. Chapter 4 presents the analysis of the structure and properties of the polysaccharide α-1,3-glucan. Chapter 5 covers the results of homogeneous modification of α-1,3-glucan and presents a discussion of the validity of the results. Chapter 6 summarizes the conclusions drawn from the results, and recommendations for future work on the project. Appendices are included for additional information. Preliminary results of solid-state esterification of α-1,3-glucan are presented in Appendix I. Appendix II includes the derivation of equations used for analysis of the experimental results, and Appendix III contains additional figures and data not included in the main body of Chapter 5.

CHAPTER 2
LITERATURE REVIEW

2.1 SUSTAINABILITY AND THE PRINCIPLES OF GREEN CHEMISTRY

Environmental protection and sustainability are becoming primary factors in the development of new materials. Sustainability in plastics is typically concentrated on the consideration of the feedstock and degradability of the material. There are four categories of plastics based on these considerations. They include petroleum-based non-degradable plastics, petroleum-based biodegradable plastics, renewable non-degradable plastics, and renewable biodegradable plastics. With the environmental impacts of oil extraction and plastic pollution becoming ever more evident, there is incentive for renewable biodegradable plastics to enter the market. Life cycle assessments are becoming more commonplace and show the need to consider the holistic impact of a material from cradle to grave or cradle to cradle. It is therefore vital to consider the substances and energy used during material development to better understand the safety and sustainability of a material.

Green chemistry is the design of chemicals and syntheses that reduce or eliminate negative impacts on humans and the environment. Paul Anastas and John Warner created the 12 principles of green chemistry in 1998 to define green chemistry and to provide guidelines on the areas of chemical processes that can be re-engineered to be safe and green from synthesis to disposal [16]. The 12 principles are summarized in Table 1.

Table 1. Principles of Green Chemistry as defined by Anastas and Warner [17, 16].

Principle	Description
Waste Prevention	Reduce the ratio of waste to functional material produced; reduce solvent usage, side reactions. $E-factor = \frac{kg_{waste}}{kg_{product}}$
Atom Economy	Maximize the number of atoms used during transformation of the reagents to the product $Atom\ Econ. = \frac{MW_{atoms\ used}}{MW_{reactants}} \times 100\%$
Less Hazardous Chemical Syntheses	Eliminate the use and production of substances that are toxic to humans and the environment
Designing Safer Chemicals	Design safer chemicals to maintain function and efficacy of the product while minimizing their toxicity; requires understanding of chemistry and toxicology
Safer Solvents and Auxiliaries	Eliminate auxiliary substances where possible or replace with benign materials where required
Design for Energy Efficient	Reduce energy usage by performing reactions at ambient temperature and pressure, eliminating separation steps where possible, and reducing steps to minimize pumping energy usage
Use of Renewable Feedstocks	Use renewable feedstocks where technically and economically practical
Reduce Derivatives	Minimize derivatization steps such as blocking, protection and deprotection steps; enzymes can be useful for improving selectivity and catalyzing reactions
Catalysis	Select recyclable catalytic reagents instead of stoichiometric reagents to reduce the E-factor of the process
Design for Degradation	Design materials which will degrade into non-toxic, non-persistent products after their functional lifespan
Real-Time Analysis for Pollution Prevention	Use real-time feedback to prevent hazards, waste, and pollution in the case of process issues; use analytical methods for in-line process monitoring and control
Inherently Safer Chemistry for Accident Prevention	Use benign chemical methods and syntheses to prevent accidents and to create safer working environments; choose substances to minimize the potential for accidental release, explosions, and fires

The 12 principles of green chemistry aim to reduce the negative environmental and health impacts of chemical synthesis, processes, and disposal. They allow for the design of lab-scale and industrial processes which ensure safe working conditions, a healthy environment, and sustainable development. It is therefore crucial to consider these principles when performing new chemical modifications and considering their use beyond the lab environment.

2.2 POLYSACCHARIDES

Polysaccharides are natural biopolymers composed of repeating units of monosaccharides. These monosaccharides are ring structures composed of carbon, hydrogen, oxygen, and sometimes nitrogen. Typical monosaccharides include glucose, as found in starch and cellulose, and glucosamine, as found in chitin. Common between these monosaccharides is the presence of hydroxyl groups as the primary functional groups. These allow for the creation of glycosidic linkages between monomers as well as for hydrogen bonding between the resulting polysaccharide chains. When two monosaccharides react via a condensation reaction between the hydroxyl groups, a disaccharide is formed; for example, glucose combines with fructose to form sucrose. When 3 – 10 monosaccharides are combined by the same reaction, an oligosaccharide is formed. As more monosaccharides are added to the oligosaccharide chain, the molecule becomes a polysaccharide.

There is a large variety of natural sources for polysaccharides, including plants and trees, fungi, algae, and bacteria. Plants provide starch and cellulose which are the two most common polysaccharides in use today. However, alginates from algae and other α- and β-glucans from fungi are of increasing interest for their unique material properties [1]. Polysaccharides can also be produced synthetically; for example, α-1,3-glucan can be synthesized enzymatically from sucrose in industrial bioreactors [13].

Table 2 lists some examples of the historical and current uses of polysaccharides.

Table 2. Chemical structures and uses of some common polysaccharides.

Polysaccharide	Structure	Use
Starch [18]	α-1,4-glucan α-1,6-glucan	Food Paper coatings Adhesives Biodegradable packaging • Loose-fill protection (foamed starch) • Flexible films (polyolefin blends)
Cellulose [19]	β-1,4-glucan	Textiles • Cotton • Rayon (regenerated cellulose) Packaging • Thermoplastic (acetylated cellulose) Other • Wood • Paper, cardboard
Fungal Polysaccharides [20]	α- and β-glucans	Food Cosmetics Medicinal applications • Anti-tumour • Anti-inflammatories • Immunomodulators
Bacterial Polysaccharides [21]	α- and β-glucans Complex heteropolysaccharides	Food additives Pharmaceutical carriers

2.2.1 STRUCTURE

The properties of polysaccharides are defined by their structural features. The structural features of note are the monosaccharide composition, the location and configuration of the glycosidic linkage, and the presence of branching. Nomenclature of glycosidic linkages is defined by their configuration and by the carbons which they bond together. The configuration of the bond is named for the placement of the linkage relative to the plane of the ring which is in turn determined by the placement of the hydroxyl group on the anomeric carbon (C_1) on the original monosaccharide. When the hydroxyl group at the anomeric carbon is in the axial position, the hydroxyl group is in the α-position and the glycosidic linkage formed with it is an α-bond. Conversely, when the

hydroxyl group is in the equatorial position, it is in the β-position and forms a β-bond. Polysaccharides, when found in nature, are often not homogeneous in their composition and may contain branches caused by a mixture of glycosidic linkages. For example, α-1,3-glucans in nature may contain α-1,4 and α-1,6 bonds [22].

2.2.2 SOLUBILITY

Small molecules like monosaccharides can be solubilized as individual molecules as they are released from the crystal structure into the solvent. With time, the molecules become evenly dispersed throughout the solvent, creating a homogeneous solution. To solubilize a polymer, the extensive polymer-polymer interactions must be replaced by polymer-solvent interactions [23]. As polymer-polymer interactions are replaced by the polymer-solvent interactions, the polymer coil expands and allows more solvent to interact with it, continuing until the coil has expanded fully [23].

Some polysaccharides are soluble in water at moderate or elevated temperatures while others, like cellulose, are insoluble in water and most organic solvents. A suitable solvent for a polysaccharide will be capable of forming polymer-solvent bonds which are stronger than the intermolecular hydrogen bonding present in the polysaccharide. A good solvent will replace these intermolecular interactions, in both the amorphous and crystalline regions of the polysaccharide, causing the polymer to expand throughout the solution [23]. This causes increased viscosity of the solution.

2.3.2.1 THETA SOLVENT

In a good solvent, the polymer-solvent interactions are energetically favourable, so the coil expands. In a poor solvent, the polymer-polymer interactions are favourable so the coil collapses. In a theta solvent, neither the polymer-solvent nor polymer-polymer interacts are favoured, so there is no excess chemical potential of mixing between the polymer and solvent [24]. Therefore, the polymer chain acts in an ideal manner. For a polymer-solvent combination, the temperature can be adjusted until this condition is satisfied. This temperature is called the theta temperature for that specific polymer-solvent pair [25].

Using a theta solvent allows for measurements of polymer properties to be independent of the solvent [24]. For example, when the identification of relationships between the solution behaviour and polymer characteristics – such as Mark-Houwink parameters – is performed under theta conditions, the measurements reflect the polymer behaviour as modeled by random or ideal chain models [26].

2.3.2.2 SOLVENTS OF INTEREST

There are two primary categories of solvents: derivatizing and non-derivatizing. Derivatizing solvents form covalent bonds with the polymer to dissolve it while non-derivatizing solvents solubilize the polymer without affecting its chemical structure [27].

One of the most common solvents for polysaccharides is N,N-dimethylacetamide (DMAc) with lithium chloride (LiCl). DMAc is a polar aprotic solvent and despite its ability to hydrogen bond with the polysaccharide via the carbonyl oxygen and amide nitrogen atoms, this hydrogen bonding is insufficient to cause dissolution of the polysaccharide [27]. It is instead proposed that the chloride ion of LiCl interacts with the polysaccharide hydroxyl protons and thus disrupts the intermolecular hydrogen bonding of the polymer system [28, 29]. The chloride ion is associated with a Li^+(DMAc) cationic complex. The bulking effect caused by charge repulsions and the size of the complex improves the ability of the solvent to penetrate the polymer system to dissolve the polysaccharide (Figure 2) [27, 28]. DMAc/LiCl is capable of dissolving cellulose, which is difficult to dissolve given its high degree of crystallinity, so it is likely to be good solvent for many other polysaccharides [26].

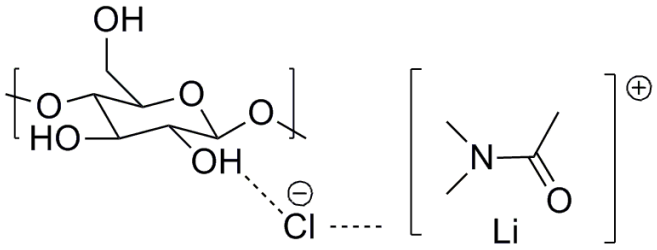


Figure 2. Interaction between cellulose and DMAc/LiCl causing dissolution, reprinted with permission under the Creative Commons Attribution 4.0 International License [27].

More recently, ionic liquids have been of interest as non-volatile, green solvents for polysaccharides. Ionic liquids are large salts with low melting points comprised of an organic cation and an organic or inorganic ion. The most common ionic liquid used to dissolve polysaccharides is 1-butyl-3-methyl imidazolium chloride (BMIMCl), but others include pyridinium, ammonium, and phosphonium based salts [27]. The primary drawbacks to the implementation of ionic liquids at industrial scale are the high cost and difficult recovery process due to their low volatility.

2.3.2.3 STRUCTURE & SOLUBILITY

Polysaccharides vary in their molecular weight, monosaccharide composition and glycosidic linkage configuration, degree of branching, and surface charge. These structural differences cause differences in functional properties including solubility in water and organic solvents, thermal properties, and mechanical properties.

For example, pullulan is soluble in cold water, some starches are soluble in hot water, while cellulose is insoluble in water except at high temperature and pressures. The hydroxyl groups on polysaccharides give the polymers a strong affinity for water but also for each other [23]. Depending on the strength of polymer-polymer interactions, water as a solvent may replace the hydrogen bonds throughout the material, replace the bonds only in the amorphous regions, or not replace any of the bonds in the material. In the latter case, the intramolecular interactions dominate the system, and the material does not dissolve [23]. Instead, the polymer chains aggregate until they precipitate, or undergo gelation.

Therefore, improved solubility of the polysaccharide in water is dependent on the polysaccharide having structural features which inhibit intermolecular association and/or reduce the strength of the intermolecular hydrogen bonds [23]. These structural features include branching, charged groups, molecular weight, and stereoregularity. Branching prevents the polysaccharide chains from packing closely together, thus reducing their ability to form strong, regular intermolecular bonds. Charged groups on the polysaccharide backbone, like carboxylate or sulfate groups, cause the chains to repel each other due to electrostatic effects and to interact more strongly with the

water instead. However, changes in pH can shield the electrostatic effects and cause the polysaccharide to precipitate out of solution [23].

In contrast, polysaccharides with poor solubility have strong intermolecular association caused by high molecular weight and linear conformations [23]. Chains with large molecular weights have a larger excluded volume causing strong intermolecular association and reduced solubility relative to polysaccharides with low degrees of polymerization (< 20) which are typically water-soluble [23]. High molecular weight polymers also have a longer dissolution time due to the slow rate of disentanglement of long chains from the aggregate. As well, materials with high polydispersity have a higher rate of dissolution due to the presence of chains of varying lengths, where smaller chains dissolve faster, reducing the average time required to dissolve a sample of set mass [23]. In contrast to polysaccharides with branching, linear polysaccharide chains, especially those which assume a ribbon conformation instead of a random coil or helix, have a dense packing structure resulting in strong intermolecular association and low solubility [23].

2.2.3 CELLULOSE

Cellulose is a polysaccharide formed via β-linkages between carbons 1 and 4 on glucose monosaccharides (Figure 3).

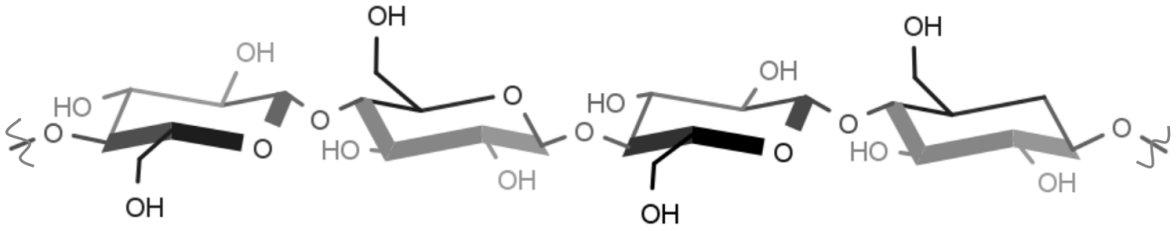


Figure 3. Structure of cellulose, β-1,4-glucan.

Like other polysaccharides, crystallinity in cellulose is caused by hydrogen bonding between the hydroxyl and acetal groups of the glucose-based backbone. However, cellulose has a higher degree of crystallinity than many polysaccharides due to its chain conformation. The β-linkage in cellulose occurs between two equatorial hydroxyl groups, which allows the backbone to remain in a linear conformation without applying strain to the glycosidic bonds [30]. This bond energy is further reduced as every other glucose unit is flipped, creating a repeating structure of two glucan

units called cellobiose. This means that cellulose is a stereoregular, linear polymer with a ribbon-like conformation capable of hydrogen bonding at regular intervals along its backbone. This results in a strong crystal structure with tight chain packing.

Cellulose is a polymorphic material, meaning that it can be converted between several allomorphs, or crystal structures. These crystal structures are defined by their unit cell dimensions and chain configuration which are affected by differences in the hydrogen bonding patterns between allomorphs.

Natural cellulose is found as the allomorph Cellulose I but there are two subtypes, α and β. Natural cellulose always contains both Cellulose Iα and Iβ which can coexist in the same microfibril [30]. The relative quantities of each subtype vary between the organism with bacteria and algae containing cellulose rich in Iβ and higher plants rich in Iα [30]. Cellulose Iα can be converted to Iβ via high temperature alkaline treatment which can then be converted to Cellulose II, III, and IV [30]. As shown in Figure 4, Cellulose Iβ can be reversibly converted to Cellulose II, III, or IV by regeneration, treatment with ammonia or ethylenediamine, or high temperature treatment with glycerol, respectively. High temperature treatment of Cellulose III with glycerol irreversibly produces Cellulose IV. And Cellulose IV can be converted back to Cellulose Iβ or II.

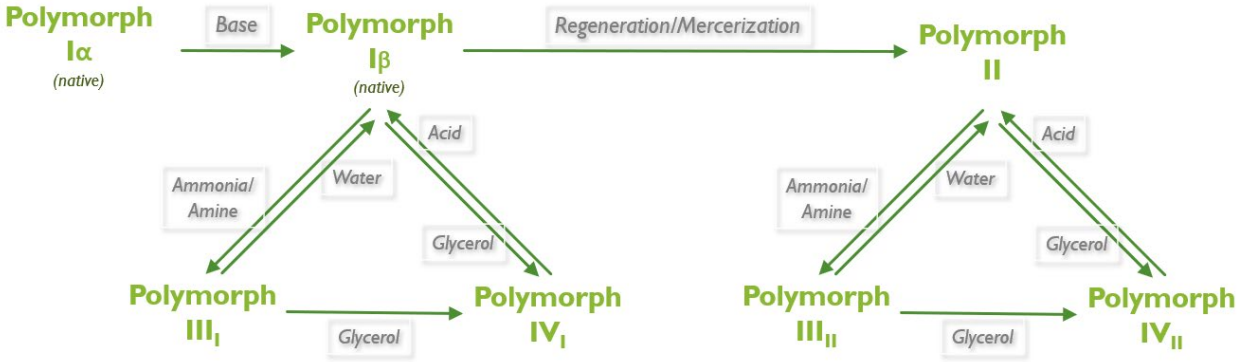


Figure 4. Conversion between cellulose allomorphs, adapted from Ciolacu et al [30].

The conformation of cellulose causes the hydroxyl groups to extend from the glucose rings at equatorial positions, allowing them to hydrogen bond with adjacent chains [30]. The hydrogen bonding pattern varies between cellulose allomorphs as summarized in Table 3. Hydrogen bonding

is described by the oxygen atoms involved where the oxygen atoms are labelled by the adjacent carbon.

Table 3. Unit cell structure and hydrogen bonding patterns for cellulose allomorphs [30, 31].

Allomorph	Morphology	Intrasheet Bonding	Intersheet Bonding
Iα	1-chain triclinic, parallel chains	$O_3 – O_5$ $O_3 – O_6$ $O_2 – O_6$	van der Waals
Iβ	2-chain monoclinic, parallel chains		
II	2-chain monoclinic, antiparallel chains	$O_3 – O_5$ $O_2 – O_6$ $O_2 – O_2$	$O_3 – O_6$
III_I	2-chain monoclinic, parallel chains	$O_3 – O_5$ $O_3 – O_6$ $O_2 – O_6$	$O_2 – O_6$
III_{II}	2-chain monoclinic, antiparallel chains		
IV_I	2-chain orthorhombic, parallel chains	*— Limited information —*	
IV_{II}	2-chain orthorhombic, antiparallel chains		

The crystal morphology affects the cellulose properties, including the rigidity of the fibrils and accessibility of its hydroxyl groups for chemical modification. For example, the vulnerability of the allomorphs to enzymatic depolymerization is dependent on the hydrogen bonding patterns and strength, where the rate of degradation from highest to lowest is as follows: amorphous cellulose > III_I > IV > III_{II} > I > II [32].

Cellulose I is favoured for its low cost, high rigidity and toughness and is used predominantly as a fiber or filler for composite materials [30]. Cellulose II has less crystallinity than cellulose I and so has increased water absorption capacity; it is used as film, fibers, and as additives for cosmetics [30]. Cellulose III has reduced crystallinity due to the swelling caused by ammonia or amine treatment and thus is favoured for derivatization of cellulose for various applications [30].

The hydrogen bonding patterns affect which hydroxyl groups are available for modification and their reactivity. Due to the flexibility of C_6, O_6 is often most accessible for modification and reacts

first, followed by O_2. O_3 is generally less reactive because it is already interacting with multiple groups via inter- and intra-molecular hydrogen bonds [30].

Despite the information known regarding the structure and dimensions of the unit cells for different cellulose allomorphs, the polysaccharide crystals are not perfectly regular. Instead, there exist defects in size and orientation of the molecules [30]. This hinders determination of exact morphologies and chain alignment via analytical methods. There is also disagreement concerning the existence of a distinct Cellulose IV allomorph with some research suggesting that Cellulose IV_I is Cellulose Iβ with lateral disorder [33, 34].

2.2.4 STARCH

Starch is a polysaccharide composed of glucose connected via α-1,4 and α-1,6 glycosidic linkages as seen in Figure 5. The different linkages give starch a more complex structure composed of two distinct fractions: amylose and amylopectin. Amylose is linear α-1,4-glucan while amylopectin is a branched polymer containing α-1,4 and α-1,6 linkages.

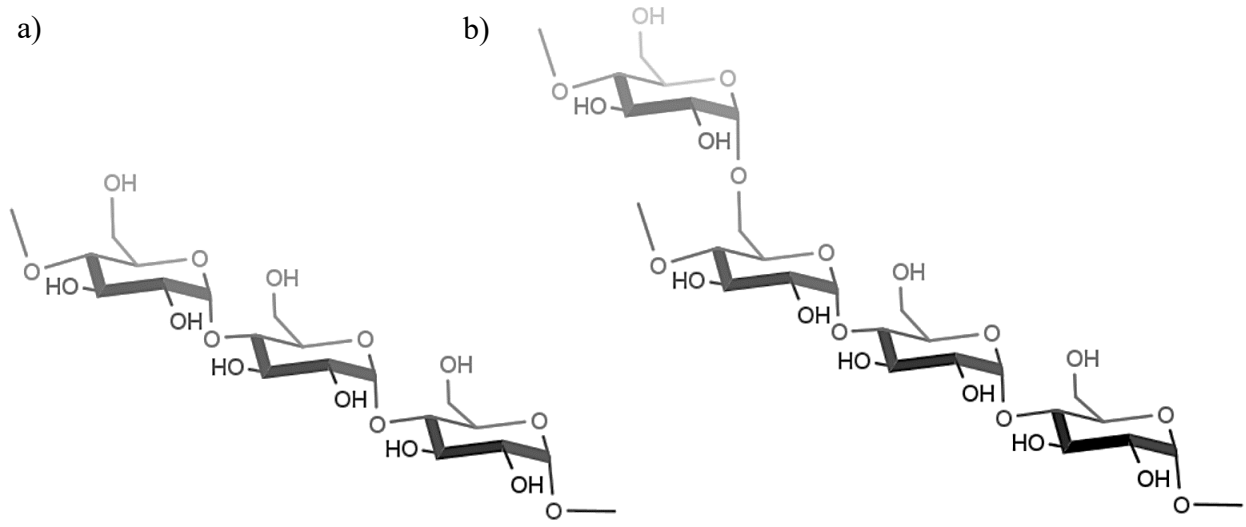


Figure 5. Structure of starch components **a)** amylose and **b)** amylopectin.

Amylopectin forms double helices with hydrogen bonding between chains and thus produces crystalline regions in the polysaccharide. The amorphous region contains branches from amylopectin as well as amylose [7]. Starch granules are arranged in concentric layers of semi-crystalline and amorphous regions [35]. The morphology, size, and degree of crystallinity depend

on the starch source, composition of amylose and amylopectin, and the molecular weight of the components [7, 8, 9]. Native starch typically has a degree of crystallinity between 14% and 45% [36].

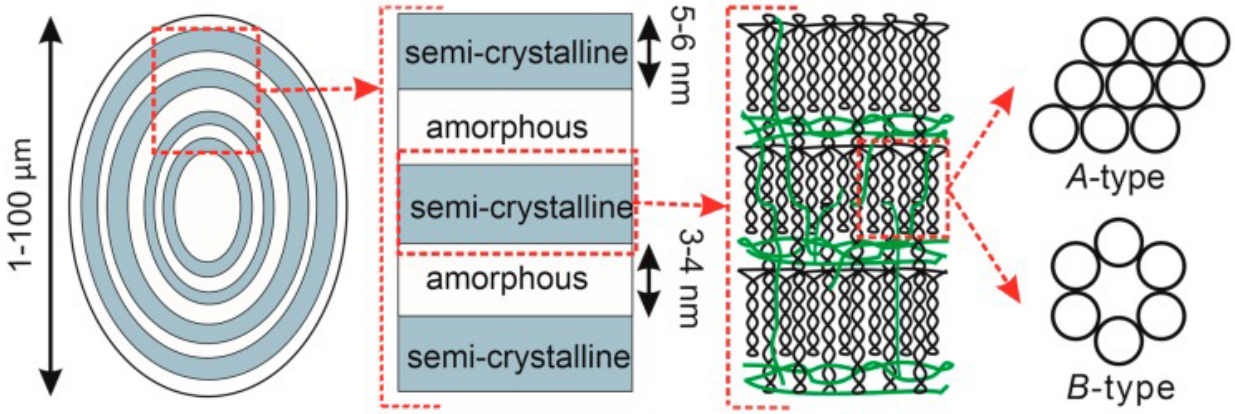


Figure 6. Crystalline structure of starch showing amylopectin double helices (black lines) and amylose single helices (green lines), reprinted under the terms of the Creative Commons Attribution 4.0 International License [7].

Starch crystalline regions present in three polymorphs, labelled as A-type, B-type, and C-type starches [37]. Descriptions of each type and their unit cell parameters are summarized in Table 4.

Table 4. Unit cell structure for starch polymorphs [38].

Polymorph	Morphology	Unit Cell Dimensions
A	Monoclinic unit cell, left-handed parallel-stranded double helices	a = 11.90 Å b = 17.70 Å c = 10.52 Å α = β = γ = 90°
B	Hexagonal unit cell, 6 left-handed parallel-stranded double helices	a = b = 18.50 Å c = 10.40 Å α = β = 90° γ = 120°
C	Coexisting B-type core with A-type surrounding crystallites	a = b = 18.50 Å c = 10.47 Å α = β = 90° γ = 120°

A-type starch is generally found in cereal starches like corn and rice [39]. Due to the dense packing of its crystal structure, the hydroxyl units are protected, giving it low reactivity. B-type starch usually contains a higher amylose content such as the starch found in fruits and root vegetables [39]. C-type starch can be found in legumes and plant roots [39].

The starch structure affects the properties of plasticized starch. If granules remain intact after processing, the mechanical properties of stiffness, strength, and elongation are negatively impacted [40]. High-amylose starches lead to materials with high strength and stiffness due to chain entanglement of the long linear chains [41]. High-amylopectin starch materials have higher flexibility and elongation due to the high molecular weight and presence of branching [42, 40]. Plasticized starch materials can undergo ageing after processing brought about by changes in the crystal structure, called retrogradation. The degree of crystallinity of starch increases with time after processing, particularly in humid environments. The increase in crystallinity can negatively impact properties like elongation but can initiate physical crosslinks in the material, leading to higher strength and stiffness [43].

2.2.5 LIMITATIONS

The desirability of polysaccharides to be used as alternative materials to conventional, petroleum-derived materials is hindered by the processes required to transform them into substances with the properties required for the selected application. Plant material is complex and contains a variety of polysaccharides, carbohydrates, and other molecules. Retrieval of starch and cellulose from plant material therefore requires extraction, purification, and treatment steps. Additionally, the structure of these polysaccharides is dependent on the source from they are extracted. There is a strong relationship between structural properties and material properties. Therefore, variations in molecular weight and branching, for example, can have significant impacts on the strength and thermal stability of the final treated product.

2.3 α-1,3-GLUCAN

α-1,3-glucan is the polysaccharide used in this research project and has been receiving increased interest in recent years due to its potential as an immunological agent, as a hydrogel, and as a filler in composite materials.

2.3.1 STRUCTURE

α-1,3-glucan is an isomer of starch and cellulose. It is a glucose-based polysaccharide composed of repeating monomers called anhydroglucose units (AGU) connected via glycosidic linkages between carbons 1 and 3 in axial conformations. To reduce strain on the glycosidic bond, every other monomer flips orientation as seen in Figure 7. This allows the polysaccharide backbone to remain in a linear conformation, contributing to tight chain packing and increased crystallinity.

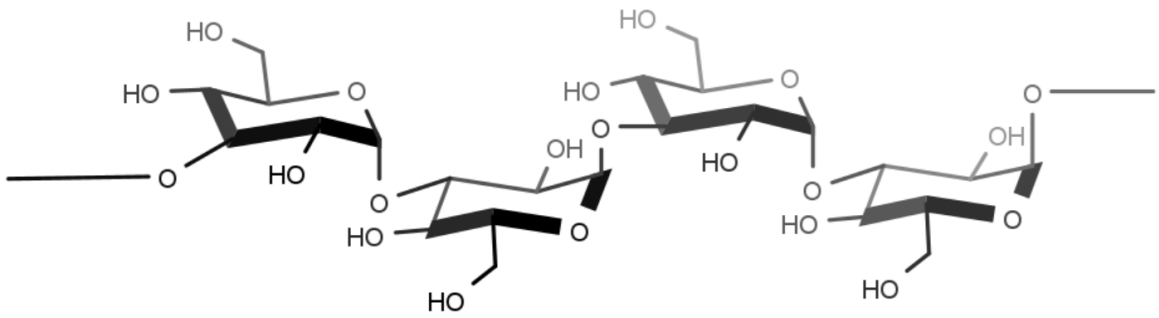


Figure 7. Structure and conformation of α-1,3-glucan.

Like other polysaccharides, crystallinity in α-1,3-glucan is strengthened by hydrogen bonding. Due to the linear conformation of the glucan chain and its tendency to assume a ribbon-like conformation, hydrogen bonding can occur at regular intervals along its backbone. This contributes to an increased degree of crystallinity. Hydrogen bonds can be formed between hydroxyl and acetal groups of the same polymer backbone or between distinct chains.

The chemical structure and chain conformation has a significant effect on the solubility, thermal properties, and mechanical properties of α-1,3-glucan.

When extracted from natural sources, the chemical structure of α-1,3-glucan can vary significantly between sources. This variation occurs in chain length and in the presence and frequency of branching via 1,6 glycosidic linkages [13, 44]. When synthesized enzymatically, the degree of polymerization can be controlled from 400 to 2000 monomer units based on the desired application [45].

2.3.2 SOURCES

α-1,3-glucan can be found in a variety of organisms including fungi and bacteria, with fungi being the most common historical source. More recently, bacteria have been used to produce α-1,3-glucan in a controlled environment.

Fungi have been used in eastern parts of the world for centuries due to their proven medicinal properties [46]. These properties, including anti-tumour and anti-inflammatory effects, are provided by a variety of α- and β-glucans found throughout the mycelia and fruiting bodies of fungi [46]. α-1,3-glucan is one of these components but has not been investigated as thoroughly due to its poor water solubility. With poor water solubility it is both difficult to extract the polysaccharide from the plant material and difficult for the polysaccharide to reach a target area for medical treatment as it cannot dissolve in the blood stream.

The location and quantity of α-1,3-glucan within the layers a fungal cell wall is dependent on the location of the cell wall within the fungi and on the species of fungi. The role of α-1,3-glucan in fungi is disputed; initially, it was hypothesized that α-1,3-glucan may contribute to mycelial

growth or structural reinforcement, but in cases where it was removed from the fungus, there was insignificant impact on these properties [22, 47].

α-1,3-glucan can also be found in yeasts and bacteria [11]. These bacteria can be found in human saliva as *Streptococcus mutans* and Streptococcus *salivarius* where it contributes to dental plaque build-up and subsequent tooth decay [44]. *S. mutans* used a glucansucrase enzyme to convert sucrose into a biofilm of α-1,3-glucan while *S. salivarius* uses sucrose to produce an α-1,3-glucan capsule around itself [44]. The gene that *S. mutans* uses to produce the glucansucrase enzyme can be isolated and copied into *Escherichia coli* to produce α-1,3-glucan in a controlled environment [11].

2.3.3 EXTRACTION & PURIFICATION FROM FUNGI

Polysaccharides are not found as pure entities in nature. Rather, they are found in combination with other polysaccharides, carbohydrates, and proteins. Therefore, α-1,3-glucan must be extracted from the plant material and purified from the other organic compounds that have been extracted simultaneously. The primary methods of extraction are conventional solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, and enzyme-assisted extraction.

Conventional solvent extraction is a favourable process for the removal of polysaccharides and other carbohydrates from organic material due to the low energy requirements, simple equipment requirements, and low toxicity of the common solvents used. These common solvents are water, acetone, and ethanol or methanol, wherein methanol is avoided due to its harmful health effects [20]. However, due to the poor solubility of α-1,3-glucan in common organic solvents, it must be extracted using an aqueous alkali system. Alternatively, α-1,3-glucan can be isolated as the insoluble fraction of a mixture and subsequently purified.

Microwave-assisted extraction (MAE) follows the same procedure as conventional solvent extraction with microwave radiation to improve the process efficiency. MAE can be used for bioactive compounds and thermally unstable compounds as it increases the rate of heating of the solvent to reduce the overall time that these compounds are exposed to high temperatures. It also reduces the solvent requirements for the process. It can be used for 2-phase extraction of compounds and can increase the yield of the desired polysaccharides in each phase [48]. MAE

efficiency depends on the dielectric constants of the desired materials, the polarity of the solvents, and the solvent-to-solids ratio [49].

Ultrasound-assisted extraction (UAE) produces cycles of high and low pressure in the medium via sound waves [20]. The pressure cycling causes disruption of intermolecular interactions and can disintegrate cells in the plant material. This disruption accelerates the rate at which the desired organic materials are released into the solvent [50]. UAE is a rapid, inexpensive, and efficient improvement to conventional solvent extraction [20]. The efficiency of the extraction process is dependent on the ultrasonic power and the solvent-to-material ratio.

Enzyme-assisted extraction (EAE) of polysaccharides is gaining favour due to its reduced need for time, solvent volume, and energy while increasing yield for a variety of substances, including polysaccharides, oils, pigments, and other medicinal compounds [20]. EAE works by hydrolyzing the cell wall to release the desired compounds from within the cell and the cell wall. Common enzymes include cellulase, hemicellulase, and pectinase derived from bacteria and fungi, and they can be used in combination to disrupt the cell wall based on the cell wall composition [51]. Given that enzymes are selective, this extraction method can increase purity of the desired polysaccharide, thus reducing the energy and time requirements for further isolation. Enzyme-assisted extraction is particularly beneficial for compounds with poor solubility as it can reduce the chain length to improve solubility in the extraction solvent; however, this results in shorter polysaccharides which may be undesirable, depending on the application.

In a typical extraction of α-1,3-glucan from fungi, the mushroom fruiting bodies are pulverized to prepare the initial sample for extraction. Extraction is performed using one or a combination of multiple methods described above. The resulting solids contain a mixture of various polysaccharides, proteins, phenolic compounds, monosaccharides, and amino acids [20].

Since the sample after extraction contains multiple compounds, several purification steps may be required to obtain the final polysaccharide. Purification can be performed using chemical methods, chromatographic methods, or membrane filtration.

First, the sample can be extracted in methanol to remove small molecules like monosaccharides. Alternatively, small molecules can be removed with dialysis [52].

Then, proteins can be removed via one or a series of several methods. These methods include the salting out method, the Sevag method, the enzymatic method, the distribution precipitation method, and quaternary salt precipitation method [20]. The salting out method uses ammonium sulfate to increase intermolecular interactions between proteins, causing them to aggregate and precipitate out of solution. The Sevag uses chloroform, *n*-butanol, and trifluoracetic acid to denature the protein then uses ethanol to precipitate it from the sample solution, whereas the enzymatic method uses a protease to the same end. The enzymatic method may be preferred due to its low toxicity. The distribution precipitation method involves the addition of an alcohol to the sample solution to precipitate out remaining proteins or other polysaccharides. The quaternary ammonium salt method is similar to the salting out method except it uses a larger salt, like hexadecyltrimethylammonium bromide, to precipitate the protein impurities [20].

Column chromatography is used in many areas of chemistry to separate compounds with similar properties based on their interaction with the column. Minute differences in properties of the substances, including polarity and size, affect the strength of their interaction with the column, causing a difference in elution times. For example, if the desired compound is least polar out of the mixture of compounds, it will interact weakly with a polar column, allowing it to pass through the column quickly, thus decreasing its elution time. Therefore, the first compound removed from the column would be the target substance. Chromatography is a time- and cost-intensive process relative to the chemical methods and so is not favoured unless necessary to separate the compounds.

Membrane filtration operates similarly to the chromatographic methods where smaller molecules will pass through the membrane while larger molecules will remain in the original solution. Several membranes can be used in series to allow for multiple separations based on molecular weight. Additionally, the membrane pore size can range from the micro-range to nano-range, allowing for highly selective differentiation of compounds. However, membrane filtration is not useful on its own if multiple polysaccharides have similar molecular sizes. In this case, membrane filtration can be used in combination with chromatographic methods to purify the target material.

The purified polysaccharide is then analyzed to determine the monosaccharide composition, the glycosidic linkage configuration, the molecular weight, and the presence of structural features

including branching and substituted groups [20]. This analysis aids in defining the expected properties of the α-1,3-glucan.

2.3.4 ENZYMATIC SYNTHESIS

Recently, α-1,3-glucan has been synthesized in a controlled environment using a glucansucrase enzyme from the GH70 class of the enzyme family Glucosyltransferase J [11, 12]. The DNA which expresses the gene responsible for this enzyme is cloned from *S. salivarius* into *E. coli* [53]. The E. coli bacteria is cultured in an aqueous sucrose medium at pH 5.5 and 30 °C where sucrose is consumed by the bacteria to produce α-1,3-glucan and fructose [53]. The fructose is washed away while the α-1,3-glucan precipitates out of solution. The resulting polysaccharide is unbranched and has a controlled chain length [53].

2.3.5 CRYSTALLINITY

Crystallinity is defined as the degree of structural order of a material. In polymers, crystallinity is caused by alignment of the polymer chains. Alignment of the chains occurs readily in a polymer that demonstrates regularity in its structure and conformation.

Due to the linearity, high persistence length, and extensive hydrogen bonding network of its polymer chains, α-1,3-glucan has a strong crystal structure [11]. This crystal structure defines the properties of the polysaccharide and can be described by its morphology.

The morphology of a polymer describes the size, shape, and interactions of the polymer chains. For crystalline regions, this can include characterization of the single polymer crystal in terms of its method of growth, presence of chain folding into lamellae or bundles and the thickness of these structures. Or, in terms of the aggregation of crystals into a larger crystalline structure involving the configuration of the crystals, and the dimensions of the larger structure. The morphology is described by the type and dimensions of the crystal unit cell and by the three-dimensional shape of the polymer aggregates.

2.3.5.1 GLUCAN POLYMORPHS

Like cellulose, α-1,3-glucan is a polymorphic material. α-1,3-glucan has 4 polymorphs dependent on the environment and treatment history of the polysaccharide. Polymorph I is found in native α-1,3-glucan [22, 54, 55]. During the extraction process, α-1,3-glucan is dissolved and precipitated, causing a change in the crystalline structure. There is therefore limited information regarding the structure of Polymorph I as it is difficult to analyze its structure without extracting it. The precipitated α-1,3-glucan is in the hydrated form, denoted by Polymorph II [22, 54]. Polymorphs I and II can be dried to form Polymorph III, which is the dehydrated form [22, 54]. The dehydration of Polymorph II is reversible but the drying of Polymorph I is not [54]. Acetylation and subsequent deacetylation of Polymorph II forms Polymorph IV, called the regenerated form [56]. Solubilization in DMAc/LiCl and precipitation into ethanol via a wet-spinning process produces a fiber with crystalline conformation and dimensions similar to those of Polymorph IV and is therefore likely to have the same crystal structure [57]. A schematic of these transformations can be found in Figure 8.

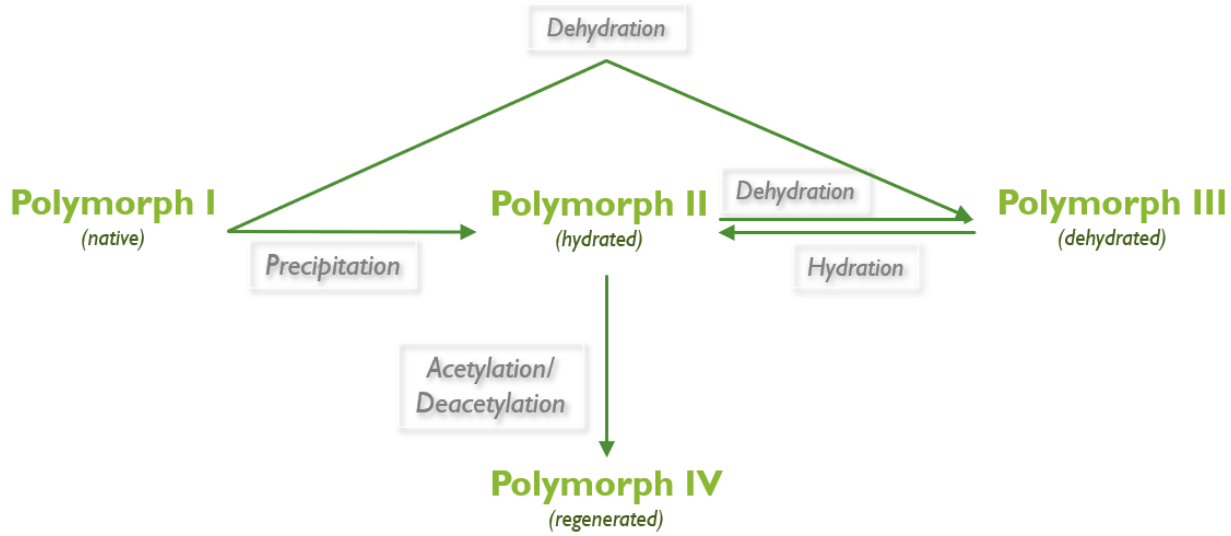


Figure 8. Schematic of the transformations between α-1,3-glucan polymorphs.

The unit cell of α-1,3-glucan contains extended chains in a 2_1 helical conformation in an orthorhombic unit cell [56]. The number of chains and the dimensions of the unit cell depend on the crystalline polymorph. This information is summarized in Table 5. For α-1,3-glucan, *c* is the axis that lies along the fiber axis. When α-1,3-glucan was produced via enzymatic synthesis, the *b*

axes of the hydrated and dehydrated forms were doubled, resulting in a unit cell with 4 chains instead of 2 chains like the hydrated and dehydrated forms extracted from fungi and bacterial sources [58].

Table 5. Conformation and unit cell dimensions of the α-1,3-glucan polymorph crystal structures.

Polymorph	Conformation	Unit Cell Dimensions	Water Molecules
I – Native	*Unknown*		
II – Hydrated [54]	2 chains, 2_1 helical	a = 10.04 Å b = 9.63 Å c = 8.35 Å	4
III – Dehydrated [54]	2 chains 2_1 helical	a = 9.14 Å b = 8.65 Å c = 8.35 Å	0
IV – Regenerated [59]	4 chains 2_1 helical	a = 16.46 Å b = 9.55 Å c = 8.44 Å	–
II – Hydrated (synthetic) [58]	4 chains 2_1 helical	a = 9.98 Å b = 19.02 Å c = 8.43 Å	8
III – Anhydrous (synthetic) [58]	4 chains 2_1 helical	a = 9.21 Å b = 17.15 Å c = 8.41 Å	0

Polymorph I is the most common polymorph found in nature despite it being less energetically stable than Polymorph II. Polymorph IV is suspected to be the most stable as its crystal structure is not affected by the relative humidity of its environment, unlike Polymorphs II and III [56]. Polymorph IV contains an extensive hydrogen bonding network between α-1,3-glucan chains to form sheets and between sheets as well. There is significantly less hydrogen bonding in Polymorphs II and III, but there is limited information regarding its detailed structure [54]. The dehydrated polymorph is less ordered than the hydrated polymorph [58].

2.3.5.2 GLUCAN AGGREGATE MORPHOLOGY

The polymer chains of α-1,3-glucan synthesized *in vitro* present in two primary morphologies shown in Figure 9. The dominant morphological form is composed of wavy aggregated fibrils which contain α-1,3-glucan chains folded over each other causing tight packing and a strong crystalline region [58]. The secondary form is composed of lamellae arrangements at the edge of the α-1,3-glucan crystal structure. There are very few lamellae and the molecular weight of the chains which form lamellae is significantly lower than the molecular weight of the fibrillar chains so there is limited information regarding the lamellar dimensions and quantity [58].

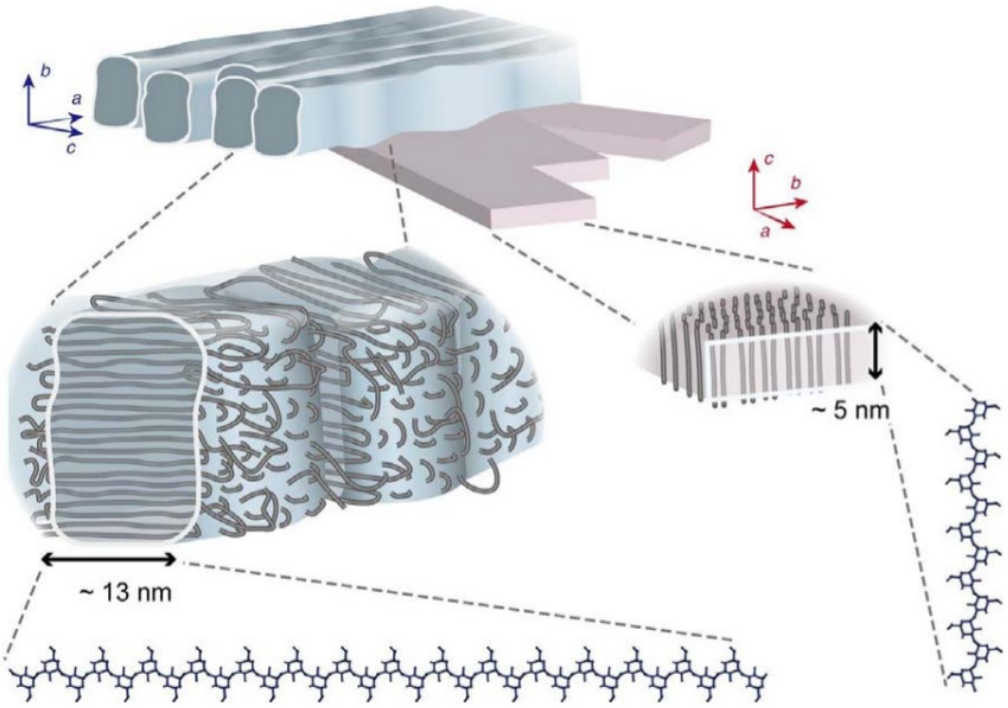


Figure 9. Morphology of α-1,3-glucan showing aggregated fibrils (blue, left) and short lamellae (pink, right), reprinted with permission from Elsevier [58].

The presence and conformation of the fibrils and lamellae were found via Transmission Electron Microscopy (TEM) where the width and thickness of the fibrils could be measured from the imaging as approximately 13 nm and 20 nm, respectively [58]. The length of the fibrils was unmeasurable due to difficulties in finding the edges along the aggregate but were estimated to be between 0.2-1.0 μm [58]. The dimensions of the lamellae were measured to be 100-500 nm in length, 30-40 nm in width, and 5 nm in thickness.

2.3.6 PROPERTIES

α-1,3-glucan obtains poor solubility in water as the degree of polymerization increases [53]. It is insoluble in most common organic solvents and has limited solubility in sodium hydroxide [11]. Solubilization of α-1,3-glucan in aqueous alkali systems causes eventual degradation of the polymer backbone. The polysaccharide is soluble in solvents composed of lithium chloride or lithium bromide dissolved in dimethyl sulfoxide (DMSO) or N,N-dimethylacetamide (DMAc) [11].

Many glucan polysaccharides found in fungi are medicinally active ingredients with anti-inflammatory, anti-tumour, and/or immunomodulatory properties [22]. Due to its poor water solubility, α-1,3-glucan has reduced biological activity compared to other fungal glucans as it cannot dissolve into the blood stream [22]. It has therefore not been studied as extensively as other glucans until recently where chemically modified α-1,3-glucan has been of interest due to its increased solubility [60].

In nature, α-1,3-glucan aids in metal absorption into fungi by biosorption due to its porosity, low crystallinity, and large number of hydroxyl functional groups on its surface [61]. This allows fungi to be used as bioremediation tools for wastewater and soil treatment. Natural α-1,3-glucan has lower crystallinity than synthetic α-1,3-glucan due to the presence of branching caused by α-(1,4) and α-(1,6) bonds distributed throughout the molecule [22].

Like many long-chain polysaccharides, α-1,3-glucan has a melting temperature above its thermal degradation temperature of over 300 °C [11]. For this reason, it cannot be melted without burning the material and therefore cannot be processed as a thermoplastic without modification or plasticization.

Since α-1,3-glucan cannot be melted in its native state, many mechanical properties like tensile strength and impact resistance are not measurable. However, enzymatically synthesized α-1,3-glucan has low backbone density, high reinforcing surface area, and has more rigid polymer chains compared to cellulose [11]. Research is ongoing to explore the rheological properties of α-1,3-glucan when dispersed in water [62].

Exploration of the behaviour of enzymatically synthesized α-1,3-glucan in dodecane-in-water emulsions found that the particle morphology of the polysaccharide affects the microstructure, stabilization mechanism, and strength of the resulting emulsion [63]. Microcrystalline glucan, which has a platelet morphology, reduces the interfacial tension in an emulsion and produces emulsions with smaller droplet size compared to α-1,3-glucan wetcake and fibrids [63].

α-1,3-glucan can be regenerated by wet spinning from dimethyl acetamide/lithium chloride, or it can be chemically modified to tune its properties [11, 57]. For these reasons and due to its biobased and biodegradable nature, α-1,3-glucan is a significant polysaccharide of interest.

2.4 CHEMICAL MODIFICATION OF α-1,3-GLUCAN

In its native form, α-1,3-glucan is not processable due to its strong crystal structure resulting in a melting temperature above its thermal degradation temperature. However, due to presence of hydroxyl groups along its backbone, α-1,3-glucan can be chemically modified to produce a material with enhanced properties, including melt processability, water solubility, water sorption as a hydrogel, and improved medicinal properties.

α-1,3-glucan has three hydroxyl groups available for substitution at carbons 2, 4, and 6 as shown in Figure 10. For many modifications, the hydroxyl group at carbon 6 is expected to be most reactive due to the greater flexibility of the carbon 5-6 bond and the reduced steric hindrance relative to the hydroxyl groups located at carbons 2 and 4 within the glucose ring.

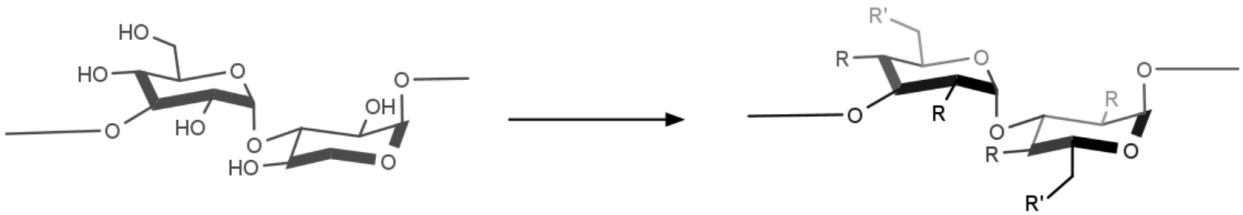


Figure 10. Simplified schematic for the chemical modification of α-1,3-glucan.

2.4.1 AMINATION & AZIDATION

Amination of α-1,3-glucan has been explored recently to determine its applicability for medical applications as a hydrogel. In 2020, He et al. aminated α-1,3-glucan to 6-amino-6-deoxy-α-1,3-glucan, also known as aminoglucan, via bromination at carbon 6 with triphenylphosphine-*N*-bromosuccinimide (NBS-Ph_3P), azidation of bromoglucan with sodium azide, and reduction of azidoglucan with sodium borohydride [64]. The degree of substitution of the aminoglucan was 0.97 with 67% loss in molecular weight [64]. The amination decreased the thermal stability of α-1,3-glucan by 100 °C [64]. Aminoglucan was soluble in aqueous solutions at pH less than 11 but insoluble in common organic solvents. Due to the good solubility of aminoglucan in alkaline conditions, it can be crosslinked with ethylene glycol diglycidyl ether (EGDE) to form a hydrogel with high compressive moduli and no syneresis upon breaking [64].

A similar process has been performed by He et al. where α-1,3-glucan is brominated, azidated and crosslinked, then reduced [65]. This process used NBS-Ph_3P for bromination, sodium azide for azidation, 1,8-nondyine and a copper catalyst for crosslinking, and sodium borohydride for reduction [65].

2.4.2 SULFATION

Sulfation of α-1,3-glucan has been performed to improve its water solubility and to increase its biological activity. The bioactivity of a polysaccharide increases both due to its ability to dissolve in bodily fluids and due to the presence of sulfate groups which are known to enhance anti-inflammatory, antiviral, antitumor, and immunomodulatory activity [60]. In 2001, Bao et al. produced sulfated α-1,3-glucan by adding the sulfating agent, pre-prepared from pyridine and chlorosulfonic acid, to α-1,3-glucan suspended in DMF at ambient temperature [66]. The reaction mixture was stirred for 30 minutes before neutralization and purification [66]. The degree of substitution was varied by adjusting the reaction time and temperature, as well as the ratio of the sulfating agent to anhydroglucose units. The highest degree of substitution obtained was 2.3 from the reaction of 8 sulfating agents to 1 anhydroglucose unit at 95 °C for 2 h [66]. All sulfated samples with a degree of substitution of 1.1 and higher were soluble in cold water, but the apparent molecular weight decreased with increasing degree of substitution [66].

In 2005, Zhang et al. sulfated α-1,3-glucan by first dissolving it in DMSO/LiCl at ambient temperature and adding pyridine then chlorosulfonic acid dropwise [67]. Reactions were performed at 60 °C for 2 h [67]. The maximum degree of substitution obtained was 0.81 but with significant molecular weight loss [67]. Sulfation was found to improve water solubility and to change the conformation of α-1,3-glucan in solution. Neat α-1,3-glucan is water-insoluble and takes the form of a slightly extended random coil chain in DMSO/LiCl while sulfated α-1,3-glucan is water soluble and forms a semi-stiff chain in water [67].

2.4.3 CROSSLINKING

E. I du Pont de Nemours and Company published a patent in 2016 on films formed from crosslinked α-1,3-glucan [68]. The patented process covers the solubilization of α-1,3-glucan in a caustic solution, addition of boric acid or sodium borate to the solution, and the subsequent preparation of films from the solution [68]. The objective of the research was to develop a α-1,3-glucan film similar to that of cellophane (cellulose xanthate) with a reduced number of steps and with safer chemicals [68]. Crosslinking could improve the processability of α-1,3-glucan solutions via extrusion as crosslinked materials can undergo greater extensional forces without breakup.

Several methods were followed for the crosslinking of α-1,3-glucan with variations in the concentrations of α-1,3-glucan and borate ions, in the source of borate ions, and in the order of material additions to the caustic solvent [68]. The caustic solvent used was potassium hydroxide in water, but the researchers expect similar results from the same process in sodium hydroxide or tetraethyl ammonium hydroxide in water [68].

The concentration of α-1,3-glucan in the solution was varied between 6.6 and 10% and the molar ratio of borate ions to anhydroglucose units (α-1,3-glucan monomers) was varied between 0.001 to 0.3 [68]. These concentrations aimed to achieve light crosslinking defined as 1 to 6 borate ions per α-1,3-glucan chain [68]. The source of borate ions was boric acid or sodium borate [68]. The alkalinity of the solution was maintained above a pH of 11 [68].

The fundamental method utilized involved preparing a slurry α-1,3-glucan in water, adding a concentrated base solution to the slurry, and mixing the mixture until the α-1,3-glucan dissolved [68]. Borate ions were either dissolved in the water used for the slurry preparation, dissolved in

the concentrated base solution, or were added to the solution containing dissolved α-1,3-glucan as a powder [68]. Films were formed by casting the solution onto a substrate using a rod coater or a draw down coater [68]. Although not attempted by the researchers, it is expected that films could be similarly prepared by extrusion through a slot die [68].

During film formation, the steps may include preliminary drying, coagulation with water, alcohols, or acids, washing, air-drying, and peeling [68]. The coagulation step removes the solvent but may also remove some unreacted borate ions. The presence of borate ions during film formation may reduce crystallization and lower mobility of the α-1,3-glucan chains in the presence of water, thus producing films with improved clarity and flexibility [68].

Characterization of the solutions and films involved viscosity measurements, molecular weight distribution measurements, film thickness, and mechanical testing to determine tensile strength, toughness, and maximum strain [68]. The reported mechanical testing results presented a sample with a maximum tensile stress of 50 MPa, a maximum strain of 24%, and a toughness of 10 MPa [68]. These values were determined for a crosslinked sample formed via a method where α-1,3-glucan was dissolved in 7.5% aqueous potassium hydroxide solution and boric acid was added after dissolution at a molar ratio of borate ions to anhydroglucose units of 0.006:1 [68].

2.4.4 ETHERIFICATION

As described in a patent submitted by E. I du Pont de Nemours and Company in 2015, etherification of α-1,3-glucan can be performed with organic compounds, including alkyl, hydroxyl alkyl, and carboxy alkyl groups [69]. While the patent predicts extension of the methods used for numerous other substituents, the methods have been tested for the following substituents: methyl, ethyl, hydroxyethyl, hydroxypropyl, carboxymethyl, and dihydroxypropyl [69]. The substituent chemical formulae and associated etherification reagents are summarized in Table 6. Allowable reaction conditions for etherification include heating between 25-200 °C, pressure at ambient or elevated conditions, alkaline pH between 11 and 13, and reaction time between 2 and 20 h [69]. The patent predicts a degree of substitution between 0.05 and 3 is possible for etherification of α-1,3-glucan with a variety of etherification agents [69]. No thermal or mechanical properties were described in the patent. Etherification can be used to tune the

properties of α-1,3-glucan for a variety of applications including as fibers for textiles, thermoplastics, or for medicinal uses.

Table 6. Etherification agents and substituents for α-1,3-glucan [69].

Group	Substituent	Chemical Formula	Etherification Agent
Alkyl	Methyl	—CH_3	Dialkyl sulfates, dialkyl carbonates, alkyl halides, iodoalkanes, alkyltriflates
	Ethyl	—CH_2CH_3	
Hydroxy Alkyl	Hydroxyethyl	—CH_2CH_2OH, —$CH(OH)CH_3$	Alkylene oxides, hydroxyalkyl halides
	Hydroxypropyl	—$CH_2CH_2CH_2OH$, —$CH_2CH(OH)CH_3$, —$CH(OH)CH_2CH_2CH_3$	
	Dihydroxypropyl	—$CH_2CH(OH)CH_2OH$, —$CH(OH)\ CH_2CH_2OH$, —$CH_2CH_2CH(OH)OH$	Dihydroxyalkyl halides
Carboxy Alkyl	Carboxymethyl	—CH_2COOH	Haloalkylates

2.4.5 OXIDATION

As described in US Patent 9,695,253 submitted by E. I du Pont de Nemours and Company in 2017, the hydroxyl groups on α-1,3-glucan can be oxidized to aldehydes or carboxylic acids in the case of the hydroxyl group on carbon 6, or to a ketone in the case of the groups on carbons 2 and 4 [70]. Increasing oxidation time or the amount of oxidizing agent causes increased extent of oxidation, potentially causing the hydroxyl group at carbon 6 to transform to a carboxylic acid and the hydroxyl groups at carbons 2 and 4 to transform into aldehydes or carboxylic acids, causing opening of the glucose ring. The oxidizing agent is typically a N-oxoammonium salt, including TEMPO (2,2,6,6-tetramethylpiperidine 1-oxyl) oxoammonium salts (Figure 11) [70].

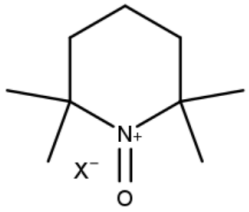


Figure 11. Unsubstituted 2,2,6,6-tetramethylpiperidine 1-oxyl oxoammonium salt.

Oxidation of α-1,3-glucan occurs heterogeneously in aqueous medium at neutral or acidic pH [70]. The patent describes methods of oxidation occurring between 18 °C – 40 °C from 1 to 48 h [70]. Oxidized α-1,3-glucan can be used as an additive to oral care compositions, detergents, and other personal care substances [70].

2.4.6 ESTERIFICATION

The most common method of modification of α-1,3-glucan to date is the esterification of the hydroxyl groups and it has been performed with reagents of varying alkyl chain lengths.

Published in 2016 and 2017, Puanglek et al. esterified α-1,3-glucan via two methods with reagents containing alkyl chain lengths from 1 carbon to 8 carbons: acetic, propionic, butyric, valeric, hexanoic, octanoic (Figure 12) [13, 53]. In Method A, dried α-1,3-glucan was dissolved in a solution of lithium chloride (LiCl) in dimethyl acetamide (DMAc), then pyridine and an acid anhydride with the desired alkyl chain length were added and reacted at 60 °C for 96 h. In Method B, trifluoroacetic anhydride was mixed with the desired carboxylic acid, then the dried α-1,3-glucan was added at 50 °C and stirred for 3 h. Using both methods A and B, Puanglek produced α-1,3-glucan esters with a degree of substitution of 3 for all reactions. All samples had improved thermal stability with a degradation temperature above 420 °C and melting temperatures above 200 °C [13]. The modification changed the material solubility profile, allowing films to be produced via solvent casting in chloroform. However, the use of an acid in Method B caused significant chain degradation resulting in low molecular weight materials with unmeasurable tensile properties as the films were too brittle or too soft. The tensile properties of the films produced via Method A showed decreasing tensile strength, increasing elongation at break, and decreasing Young's Modulus with increasing alkyl chain length [13]. Based on these results,

researchers suspect that with a higher starting molecular weight of α-1,3-glucan, thermoplastics with improved mechanical properties could be obtained using Method A [13].

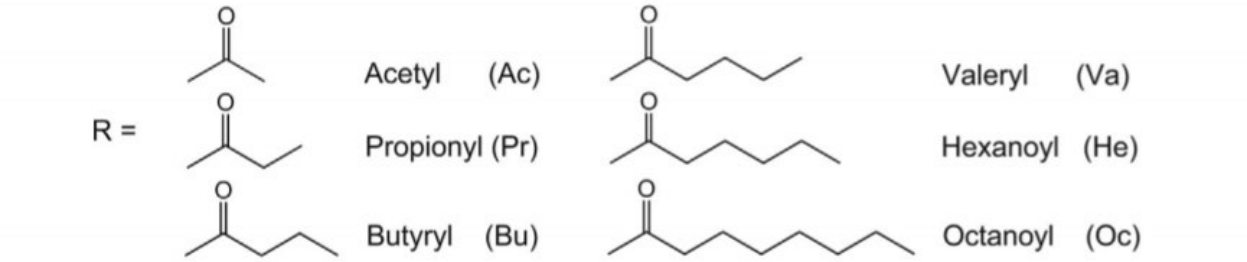


Figure 12. Alkyl chain substituents used for the esterification of α-1,3-glucan by Puanglek et al, reprinted with permission from Elsevier [13].

In 2016, E. I du Pont de Nemours and Company was granted a patent (US 9278988) for the esterification of α-1,3-glucan and the preparation of films from α-1,3-glucan esters [71]. The patent covers α-1,3-glucan modified with individual acid anhydrides or a mixture of anhydrides, including acetic, propionic, butyric, pentanoic, hexanoic, heptanoic, octanoic, and phthalic anhydrides [71]. The esterification proceeds in the presence of an acid anhydride, an organic acid, and an acid catalyst, with and without the presence of a solvent like dichloromethane. The temperature and time of each step in the procedure can vary between 15 – 80 °C and 30 minutes – 3 days, respectively [71]. Esterification with a variety of anhydrides produces modified α-1,3-glucan with a degree of substitution between 0.05 to 3 [71]. Films of the esterified α-1,3-glucan samples were formed by solvent casting from acetone, but can also be performed from other solvents, including ethanol, tetrahydrofuran, and dichloromethane [71].

Following this, E. I du Pont de Nemours and Company published a patent in 2019 for the functionalization of α-1,3-glucan and α-1,3-1,6-glucan with acyl halides, acid anhydrides, and vinyl esters [72]. The modifications target degrees of substitution between 0.001 and 3. The esterifying agent and polysaccharide were contacted in solution, then heated at a temperature between 30 °C and 175 °C [72]. Solvents used for the modifications included dimethyl acetamide, acetone, methyl ethyl ketone, and tetrahydrofuran [72]. The modified glucan was not isolated from the reaction solution but could instead be blended with other polymers for subsequent fiber spinning or solvent casting [72].

In 2020, E. I du Pont de Nemours and Company published a patent for the esterification of α-1,3-glucan with cyclic organic anhydrides, including maleic anhydride, itaconic anhydride, and 2-octenylsuccinic anhydride [73]. The esterification can be performed above the melting temperature of the anhydride wherein the mixture is stirred at the desired temperature for more than 1 hour. Alternatively, the anhydride and polysaccharide can be dispersed in an aqueous alkaline solution with a pH in the range of 7.5 – 10.0 [73]. The patent also covers esterification performed in organic solvents, including alcohols, dichloromethane, and tetrahydrofuran [73]. While the patent covers modifications which produce esterified α-1,3-glucan with a degree of substitution between 0.001 and 3, the reported values were limited to maleation with a maximum degree of substitution of 0.14 [73].

In 2020, Gericke et al. esterified α-1,3-glucan with a benzoate functionality with the goal of producing a biodegradable stationary phase for chromatographic separation of enantiomers [74]. In Method A, dried α-1,3-glucan was dissolved in DMAc/LiCl, benzoyl chloride was added with or without pyridine and the reaction occurred over 20 h at 70 °C. In Method B, a solution of benzoic acid and carbonyldiimidazole in DMAc was added to α-1,3-glucan dissolved in DMAc/LiCl, and the reaction occurred over 20 h at 70 °C. The addition of pyridine in Method A caused a significant reduction in the extent of chain degradation from the hydrogen chloride produced as a by-product. The maximum degrees of substitution were 1.96 for Method A without pyridine, 1.72 for Method A with pyridine, and 1.5 for Method B [74]. The reduction in degree of substitution relative to esterification with alkyl substituents is likely due to the bulky nature of the benzoate moiety.

Geitel et al. described 3 methods of esterification of α-1,3-glucan with long-chain fatty acids: lauric (C_{11}), palmitic (C_{15}), and stearic (C_{17}) acid [14]. The goal of the modification was to produce an α-1,3-glucan ester with thermal stability but reduced melting temperature to improve its applicability for hot-melt adhesives. In Method A, α-1,3-glucan was dissolved in DMAc/LiCl with *p*-toluenesulfonic acid chloride (TsCl) and the desired acid and stirred at 100 °C for 4 h. In Method B, a solution of carbonyldiimidazole (CDI) and the acid in DMAc was added to α-1,3-glucan dissolved in DMAc/LiCl. The resulting solution was stirred for 4 h at 100 °C. In Method C, oxalyl chloride was added to pre-cooled dimethyl formamide (DMF) at -20 °C and stirred until gas evolution ceased, at which point the desired acid was added and stirred at -20 °C for 15 minutes.

The DMF solution was added to α-1,3-glucan dissolved in DMAc/LiCl and stirred at 100 °C for 4 h.

The long chains of the fatty acid substituents reduced the hydrophilicity of the α-1,3-glucan chain, resulting in improved solubility in non-polar solvents and reduced solubility in polar solvents, including DMAc, DMF, and DMSO [14]. The α-1,3-glucan esters produced with TsCl or oxalyl chloride/DMF melted with heating between 40 and 200 °C with the melting range reducing with increased degree of substitution, longer substituent chain, and lower molar mass [14]. α-1,3-glucan esters produced with CDI activation decomposed without melting. Pressure/shear-tests were performed with wood to test the applicability of the esters as adhesives. It was found that a longer chain substituent produces higher strength adhesive and that esters produced with TsCl were stronger than those produced with oxalyl chloride/DMF [14]. However, the use of TsCl increases the number of side reactions which occur during α-1,3-glucan ester synthesis. The bonding strength of all samples was lower than the required strength for thin or thick wood joints. The maximum degree of substitution was 2.2 for the reaction with lauric acid, using TsCl or ImCl as the activating agent, and reacting for 4 h at 100 °C with a molar ratio of anhydroglucose unit to acid to activating agent of 1:5:5 [14].

Fukata et al. produced ester derivatives of α-1,3-glucan using the Puanglek method with trifluoroacetic anhydride and linear, iso-branched, and tert-branched carboxylic acids (Figure 13) [45]. The goal of this modification was to form a processable material with a reduced melting temperature relative to α-1,3-glucan esters with shorter alkyl chain substituents. Dried α-1,3-glucan was added to pre-mixed trifluoroacetic anhydride and the desired carboxylic acid. The reaction was stirred at 50 °C for 1 h, at which was the α-1,3-glucan ester was completely dissolved in the reaction medium.

The maximum degree of substitution found for all esterification reactions was 3. Clear, colourless films of all samples were prepared via solvent casting in chloroform. Films were tested for tensile strength, elongation at break, and Young's modulus. Both linear and branched ester derivatives showed that increasing substituent chain length results in lower tensile strength and higher elongation at break [45]. Young's modulus was higher for branched derivatives with the same number of carbons in the substituent as the linear derivative. Esterification increased the thermal

degradation temperature; however, esterification with branched reagents showed increasing melting temperature with increasing side chain length, opposite to the trend with linear reagents [45].

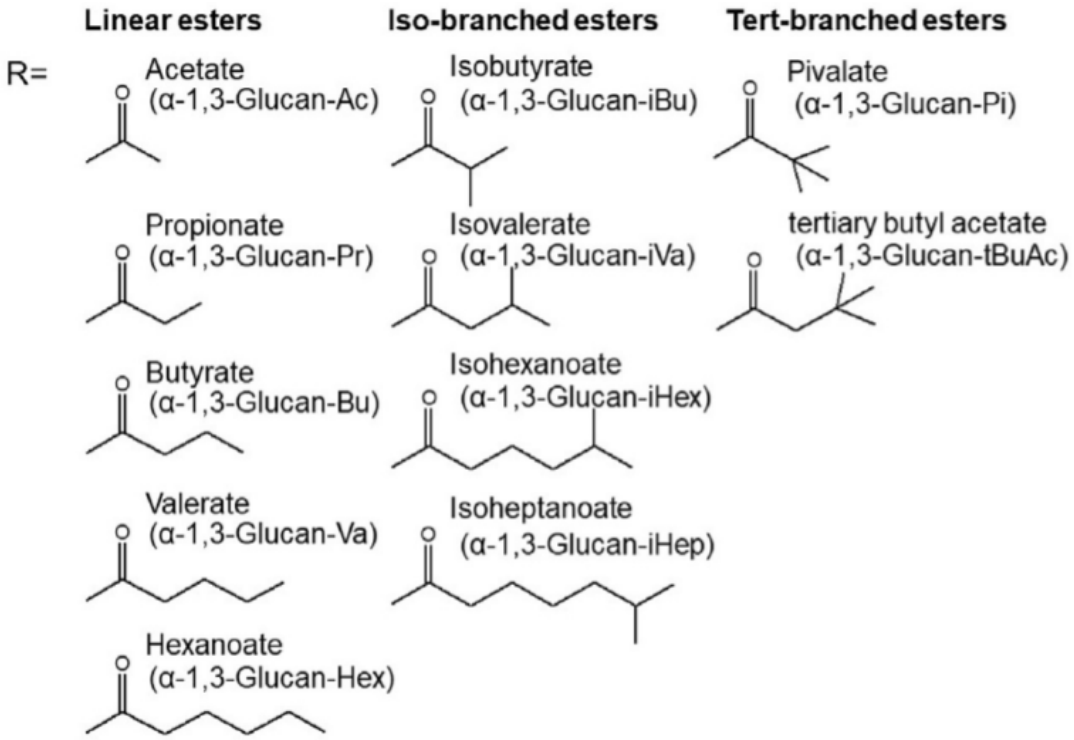


Figure 13. Alkyl chain substituents used for the esterification of α-1,3-glucan by Fukata et al, reprinted with permission from Elsevier [45].

Esterification of α-1,3-glucan with succinic anhydride has recently been performed in the solid-state with a target degree of substitution of 1 [75]. Exploration of the reaction was first performed at the microscale in a thermogravimetric analysis (TGA) crucible, then transferred to the laboratory scale in a rotary evaporator. Reaction of α-1,3-glucan with succinic anhydride for 14 h at 130 °C with and without $NaPO_2H_2 \cdot H_2O$ catalyst produced a material with a melting temperature around 340 °C [75]. It is theorized that the catalyst protects the succinic anhydride from sublimation and thus causes an increase in the degree of substitution from 0.37 to 0.70 [75].

2.4.7 COMPARISON OF MODIFICATIONS

A summary of the methodologies of the chemical modifications on α-1,3-glucan and their associated degrees of substitution is provided in Table 7.

Esterification is the primary method used for the synthesis of a polysaccharide-based thermoplastics. One of the primary challenges associated with chemical modification of α-1,3-glucan for thermoplastic applications is degradation of the polysaccharide backbone caused by hydrolysis. This has been shown to be significant for esterification, as well as for sulfation, and amination reactions [45, 53, 60, 74, 14, 66, 64]. Pyridine has been shown to reduce the extent of hydrolysis by neutralizing the acidic functionality produced during esterification [53, 74]. The researchers expect that increasing the molecular weight of the enzymatically synthesized α-1,3-glucan will improve the resulting mechanical properties, including tensile strength and Young's Modulus [13]. This is under investigation by Puanglek et al [13].

The secondary challenge is performing chemical modifications without extensive use of solvents, time, and material. Currently, all chemical modifications that have been outlined in this section require dissolution or dispersion of α-1,3-glucan, modification, followed by precipitation and washing. Each of these steps are solvent- and time-intensive and therefore cannot be economically scaled up to an industrial scale. This severely limits the applicability of α-1,3-glucan as a replacement for conventional, petroleum-based thermoplastics.

Table 7. Summary of the chemical modifications of α-1,3-glucan.

Modification	Temperature	Time	Reagents	DS [a]	Source
Amination	70 °C/ 70 °C/ 60 °C	2 h/ 48 h/ 48 h	Triphenylphosphine-*N*-bromosuccinimide/ sodium azide/sodium borohydride	0.97	[64]
Sulfation	25 – 95 °C	2 – 8 h	Chlorosulfonic acid + pyridine	2.3	[66]
Crosslinking	25 °C	3 h	Borate ions	–	[68]
Etherification	25 – 200 °C	2 – 20 h	*See Table 6*	2	[69]
Oxidation	18 – 40 °C	1 – 48 h	TEMPO oxoammonium salt	–	[70]
Esterification	60 °C	96 h	Acid anhydride + pyridine	3	[13]
	50 °C	3 h	Carboxylic acid + trifluoroacetic anhydride	3	[13]
	15 – 80 °C	1 – 72 h	Organic acid + acid anhydride + acid catalyst	3	[71]
	30 – 175 °C	–	acyl halide/acid anhydride/vinyl ester	2.5	[72]
	Not specified	> 1 h	Cyclic organic anhydride	0.14	[73]
	70 °C	20 h	Benzoyl chloride + pyridine	1.96	[74]
	70 °C	20 h	Benzoic acid + carbonyldiimidazole	1.5	[74]
	100 °C	4 h	Acid + *p*-toluenesulfonic acid chloride	2.2	[14]
	100 °C	4 h	Acid + carbonyldiimidazole	1.4	[14]
	100 °C	4 h	Acid + oxalyl chloride + dimethylformamide	2.2	[14]
	50 °C	1 h	Carboxylic acid + trifluoroacetic anhydride	3	[45]
	130 °C	14 h	Succinic anhydride + $NaPO_2H_2 \cdot H_2O$	0.7	[75]

[a] *Maximum Degree of Substitution achieved*

CHAPTER 3
MATERIALS AND METHODS

3.1 MATERIALS

α-1,3-glucan (Nuvolve^{TM}) was provided by International Flavours & Fragrances. It was produced via enzymatic synthesis with glucosyltransferase. α-1,3-glucan used for homogeneous modifications contained 90% solids by weight, an average particle size of 15 μm, and a degree of polymerization of 741. Dried α-1,3-glucan was stored in a vacuum oven at 22 °C ± 2 °C and 10^{-2} bar. α-1,3-glucan used for solid-state modifications contained 43% solids by weight in water and an expected average degree of polymerization of 800. This sample is also referred to as wetcake. Wetcake was stored in the refrigerator at 4 °C. N,N-dimethylacetamide (DMAc, 99%), anhydrous lithium chloride (LiCl, 98%), dimethyl sulfoxide (DMSO, 99.7%), 2-octenylsuccinic anhydride (OSA, cis- and trans-mixture, 95%), and potassium bromide (KBr, FTIR grade) were purchased from Thermo Fisher Scientific. Deuterated dimethyl sulfoxide (DMSO-d6, 99.8% with 0.03% TMS), deuterated trifluoracetic acid (TFAA-d, 99.5%), and acetone (99%) were purchased from Sigma-Aldrich. Anhydrous ethyl alcohol (EtOH, 99%) was purchased from Greenfield Global.

3.2 METHODS

Homogeneous esterification. Dried α-1,3-glucan powder (1 g, 6 mmol anhydroglucose unit (AGU)) was dispersed in DMAc (10 mL) at 120 °C for 1 h under nitrogen in an oil bath. The oil bath temperature was reduced to 80 °C, the cooling water in the condenser was turned on, and LiCl (1.25 g) was added with additional DMAc (15 mL); the mixture was stirred until both the α-1,3-glucan and LiCl dissolved (approximately 30-60 minutes). The oil bath temperature was adjusted to the desired reaction temperature and once the temperature stabilized, OSA was added at the desired molar ratio to the AGU. The reaction solution was stirred at the reaction temperature for 24 h. For analysis of the reaction kinetics, reaction solutions were stirred for 72 h and 1 mL samples were taken after 1 h, 2 h, 3 h, 4 h, 5 h, 24 h, 48 h, and 72 h. The solution was cooled to room temperature, and the modified polysaccharide was precipitated. For samples with low degree of modification, the solution was precipitated into ethanol, dried, and purified by Soxhlet extraction

with acetone. For samples with high degree of modification, the solution was precipitated into water, dried, dissolved in acetone, and re-precipitated in water; this process was repeated until the sample was insoluble in acetone and could then be purified by Soxhlet extraction with acetone. Purified samples were stored in an auto-desiccator at 13% ± 7% R.H. and 22 °C ± 2 °C.

Solid-state modifications were performed on α-1,3-glucan via three primary methods. Due to the limited conversion achieved via these experiments, the methods and results are provided in Appendix I.

Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker Avance 300 MHz spectrometer. Samples were prepared for analysis by dissolving 10 – 20 mg of sample in 1 mL of a solution of 3% LiCl in DMSO-d_6. 6-8 drops of deuterated trifluoroacetic acid (TFAA-d) were added to suppress the signals from unmodified hydroxyl group protons. ^{1}H NMR spectra (32 scans) were recorded at room temperature (22 °C). TMS was used to calibrate the spectra at a chemical shift (δ) of 0 ppm. Assignment of the peaks for neat and modified polysaccharide is discussed below.

Fourier-transform infrared (FTIR) Spectroscopy was performed in a Bruker Tensor 27 spectrometer. KBr pellets were prepared by grinding the sample to a fine powder, mixing with KBr, and pressing with 8 tons of pressure for 2 minutes. Spectra were recorded at room temperature with a resolution of 4 cm^{-1} using 64 scans.

X-ray diffraction (XRD) measurements were performed on a Bruker D8 diffractometer using steel sample holders. Measurements were taken with the following parameters: 2 mm divergence slit, 2 mm antiscatter slit, 0.2 mm detector slit, 0.03° step size, 2 seconds per step, and a 2θ range of 5 – 50°.

Size exclusion chromatography (SEC) was performed to determine the molecular weights and molecular weight distributions of the polysaccharide samples. The equipment included a Waters Alliance™ 2695 separation module coupled with a Waters 2414 differential refractometer, a Wyatt Heleos II multiangle light scattering photometers, and a Wyatt ViscoStar differential capillary viscometer. Measurements were performed through styrene-divinyl benzene columns with a 0.11% LiCl/DMAc mobile phase, a flow rate of 0.5 mL/min, an injection volume of 100 μL, and

a temperature of 50 °C. Samples were dissolved at 0.5 mg/mL in 2% LiCl/DMSO, shaken overnight at room temperature, filtered through a 5 μm filter, then run through the triple-detector chromatograph. Modified samples were assumed to have the same refractive increment (dn/dc) as unmodified α-1,3-glucan. The measurements were performed by the industrial sponsor, IFF [76].

Dynamic viscosity measurements were obtained in a Brookfield viscometer with spindle 00 in a cylindrical chamber. Samples were prepared by dissolving 0.16 g of polysaccharide in 16 mL of 5% LiCl/DMSO at 70 °C overnight. Measurements were performed at 22 °C ± 2 °C at shear rates between 1 and 123 s^{-1}.

Thermogravimetric analyses (TGA) were performed to examine thermal degradation of the neat and modified polysaccharide. A TA Instruments TGA Q500 was used to heat clean, tared platinum pans containing a precise amount in the range 10-20 mg of polysaccharide from 25 °C to 600 °C under nitrogen at a rate of 20 °C/minute.

Differential scanning calorimetry (DSC) was used to check for the thermal transitions of glass transition temperature and melting temperature using a TA Instruments DSC Q2000. DSC analyses were performed on 5-10 mg of polysaccharide in closed aluminum pans with a ramping rate of 10 °C/minute for a heating cycle from 30 °C to 160 °C to -50 °C to 160 °C to 30 °C. The temperature limit was defined as 20°C – 30 °C less than the onset of degradation observed in TGA.

Hot pressing of samples was performed to observe for flow and/or melting of samples. Ground samples were placed on Teflon sheets between steel plates, placed in the hydraulic press at 100 °C for 2 minutes without applying pressure, then for 2 minutes with 2 tons of pressure applied. Samples were removed and checked for signs of flow or degradation. The press temperature was increased by 10 °C and the process was repeated until melting or thermal degradation occurred.

Wetting tests were performed on samples which flowed under hot pressing. Using a Pasteur pipette, a droplet of water was gently released onto the sample film, a photograph was taken, and the wetting behaviour was qualitatively observed (contact angle was observed visually, not measured).

Preliminary emulsion testing was performed on all modified samples to explore their interaction in water and oil phases. Dried sample (0.09 g) was added to water (3 g) in a 20 mL vial. The vial was capped and agitated manually. Mineral oil (1 g) was added to the mixture, the vial was capped again and agitated manually. After agitation was ceased, visual observations were recorded at 0 seconds, 30 seconds, 1 minute, 5 minutes, and 30 minutes. Additional oil (1 g at a time) was added, and the agitation and observation processes were repeated.

CHAPTER 4
RESULTS AND DISCUSSION – PROPERTIES OF α-1,3-GLUCAN

The goal of this chapter is to improve the understanding about the nature of α-1,3-glucan to support the research in subsequent chapters. As discussed in the literature review, the solubility of α-1,3-glucan is a topic still under investigation. This is a relevant aspect for the chemical modification due to the crystalline regions of the polysaccharide. The structure and properties of α-1,3-glucan were examined and measured to understand the chemical composition, molecular weight distribution, crystallinity, thermal properties, and solubility of the material as well as to create a baseline onto which the modified samples could be compared. The degree of substitution was determined by proton nuclear magnetic resonance spectroscopy, the crystallinity was analyzed by X-ray diffraction, the molecular weight distribution was found by size exclusion chromatography, transition temperatures were observed by differential scanning calorimetry, and the thermal stability was measured by thermogravimetric analysis. The polysaccharide solubility was tested in N,N-dimethyl acetamide and lithium chloride.

4.1 CHARACTERIZATION

The chemical composition of polysaccharides and their degree of substitution can be measured with NMR. Often, deuterated water (D_2O) is used to suppress hydroxyl proton signals for neat and modified polysaccharides [77, 78, 79]; however, the addition of D_2O to esterified α-1,3-glucan samples in DMSO-d_6/LiCl caused the samples to irreversibly precipitate out of the deuterated solvent. Here, TFAA-d did not have the same effect and so was added to the solutions of α-1,3-glucan in DMSO-d_6/LiCl prior to analysis by ^{1}H-NMR. As seen in the spectra of α-1,3-glucan in Figure 14, TFAA-d effectively suppressed the strong signal of residual water at a shift of 3.7 ppm.

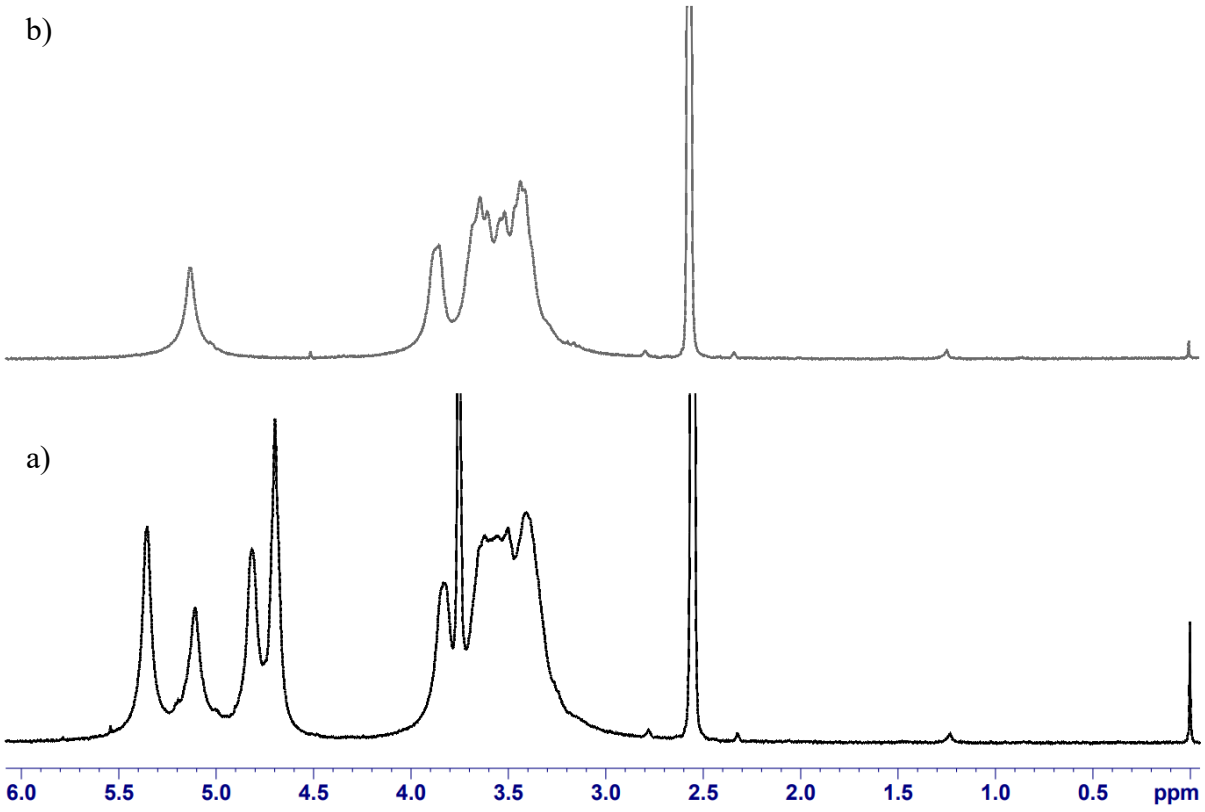


Figure 14. [1]H-NMR spectra of α-1,3-glucan **a)** without TFAA-d and **b)** with TFAA-d.

Peaks from [1]H-NMR spectra were assigned to α-1,3-glucan based on the literature where α-1,3-glucan was analyzed via 1D and 2D NMR [80, 81]. Peak assignments can be found in Table 8 where protons are numbered based on the carbon to which they are bonded, as labelled in Figure 15.

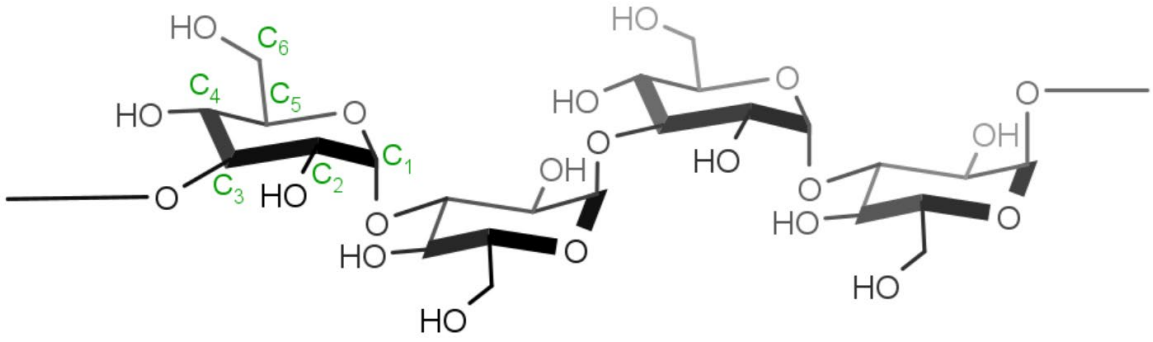


Figure 15. Structure of α-1,3-glucan with carbon numbers assigned for NMR analysis.

Table 8. Assignment of peaks from NMR spectra for α-1,3-glucan.

Chemical Shift (ppm)	Assignment
3.40	H2, H4
3.58	H3, H6a, H6b
3.84	H5
4.62	OH-C6
4.77	OH-C2
5.11	H1
5.27	OH-C4

Solvent: Deuterated dimethyl-d_6 sulfoxide (DMSO-d_6)

Fourier transform infrared (FTIR) spectra are shown in Figure 16 and the peaks are assigned to the functional groups of α-1,3-glucan in Table 9. FTIR spectra were obtained for powder samples of α-1,3-glucan as received and after dissolution and precipitation from a solution of 7.3 wt. % LiCl in DMAc. Based on the similarity of the two spectra, dissolution in DMAc/LiCl and subsequent precipitation does not affect the key functional groups of α-1,3-glucan.

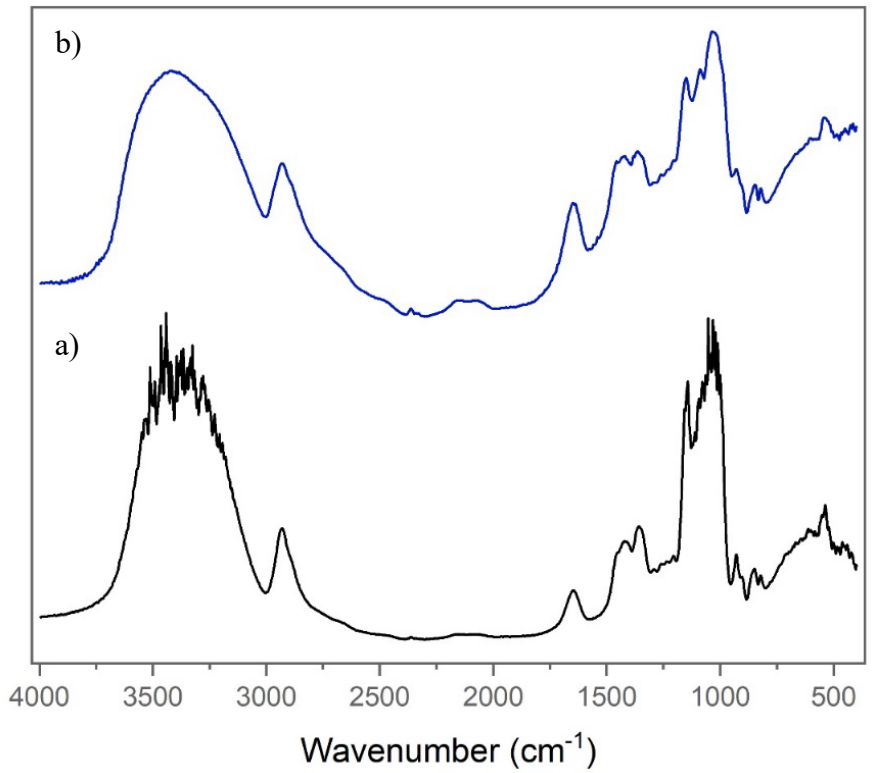


Figure 16. FTIR spectra of α-1,3-glucan **a)** before dissolution, **b)** after dissolution and precipitation from DMAc/LiCl.

Table 9. Assignment of peaks from FTIR spectra for α-1,3-glucan.

Wavenumber	Strength	Functional Group	Assignment
3000 – 3700 cm^{-1}	Strong, broad	O-H stretching, inter- and intra-molecular bonded	O-H at C2, C4, C6
2935 cm^{-1}	Medium, narrow	Alkane C-H stretching	C-H at C1, C2, C3, C4, C5, C6
1650 cm^{-1}	Weak, narrow	H-O-H bending	Residual bound water
1300 – 1500 cm^{-1}	Medium	Alkane C-H bending	C-H at C1, C2, C3, C4, C5, C6
		Alcohol O-H bending	O-H at C2, C4, C6
950 – 1190 cm^{-1}	Strong, broad	C-O stretching	-O- in ring (C3-C5) and in glycosidic linkage (C1-C3)

The crystallinity was investigated with X-ray diffraction. Powder samples of α-1,3-glucan were analyzed by XRD three times. The first sample was analyzed as received, the second sample had been dissolved and precipitated from DMAc/LiCl, and the third sample had been dispersed and

recovered from DMAc. The diffractograms are provided in Figure 17, where the shift and reduction in intensity of the peaks after treatment can be observed. The diffractogram of α-1,3-glucan contains three distinct crystalline peaks at 2θ values of 9.5°, 17.95°, and 21.35°.

After dissolution in 7.3% LiCl/DMAc and precipitation, the intensity of the crystalline peaks was significantly reduced. Additionally, there is a downward shift of the first crystalline peak and an upward shift of the second crystalline peak. After dispersion in DMAc and subsequent filtration, the first crystalline peak is significantly reduced, the second peak is again shifted upward and there is an additional small peak found at a 2θ value of 14.24°. This is expected as it has been shown that the treatment method of α-1,3-glucan affects its crystalline structure [22, 54]. An amorphous sample of α-1,3-glucan was not available for analysis in order to determine the degree of crystallinity of the α-1,3-glucan samples; however, the polysaccharide supplier provided an estimate of the degree of crystallinity of dried α-1,3-glucan powder at 55%.

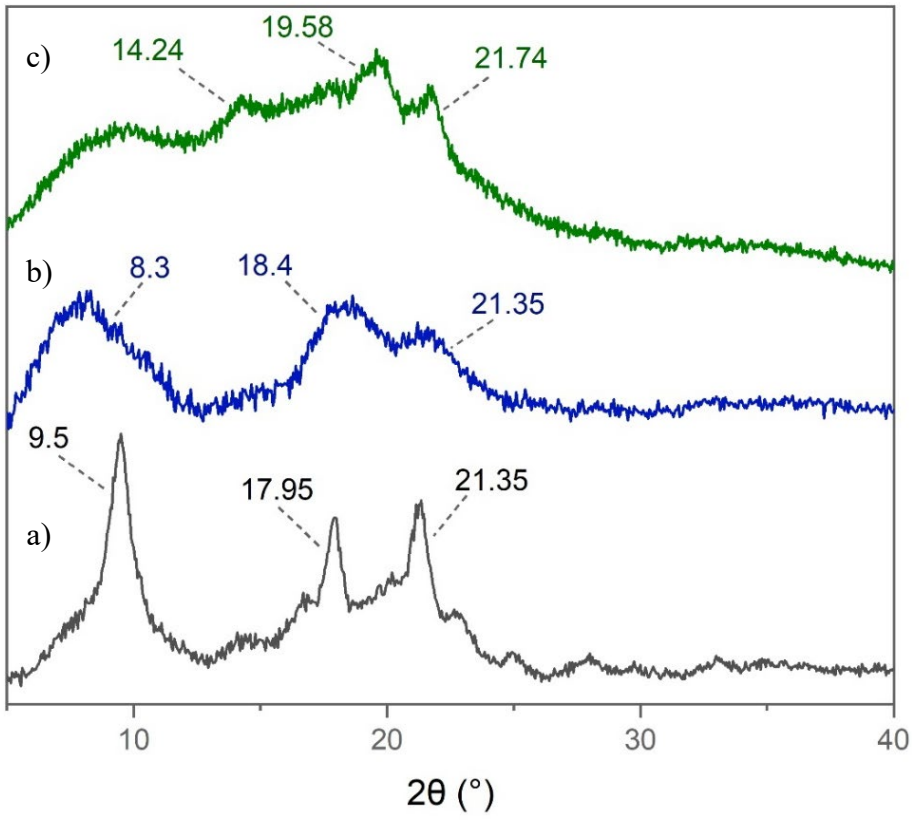


Figure 17. XRD spectrum of **a)** α-1,3-glucan as received, **b)** α-1,3-glucan dissolved and precipitated from DMAc/LiCl, and **c)** α-1,3-glucan dispersed and recovered from DMAc.

The molecular weight distribution of unmodified α-1,3-glucan was investigated with size exclusion chromatography (SEC). The molecular weight distribution trace is provided in Figure 18. α-1,3-glucan produced by the enzymatic process described in Chapter 2.3 has a bimodal distribution due to the presence of insoluble oligomers in the product mixture that are not washed away. These oligomers can be seen in the molecular weight distribution curve at a molar mass around 1500 g/mol. Key molecular weights of α-1,3-glucan are provided in Table 10 alongside the polydispersity index, the intrinsic viscosity, and the weight-average degree of polymerization.

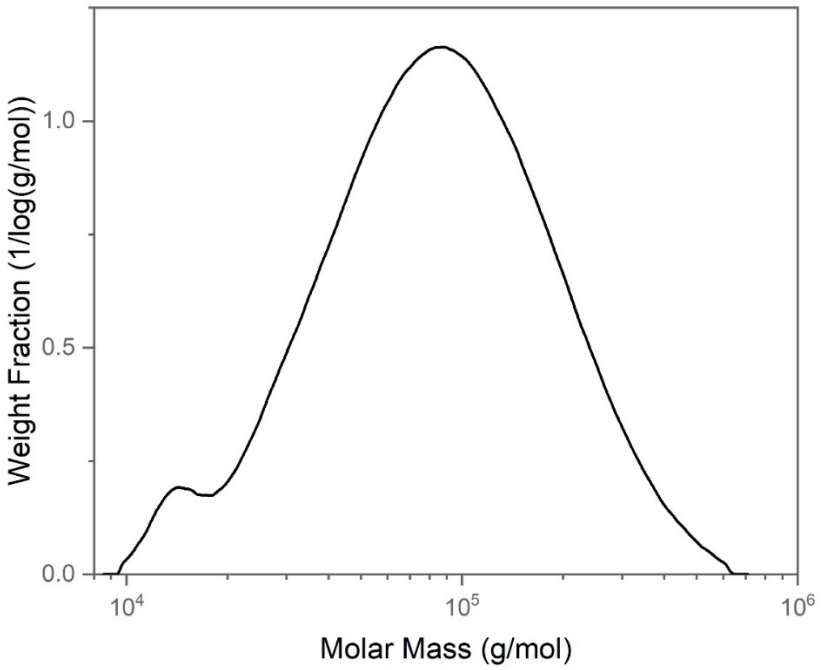


Figure 18. Molecular Weight Distribution of α-1,3-glucan.

Table 10. Molecular weights, polydispersity index, intrinsic viscosity, and degree of polymerization of α-1,3-glucan.

M_n [a] (kDa)	M_w [b] (kDa)	M_z [d] (kDa)	PDI [c]	[η] [e] (dL/g)	DP_n [f]	DP_w [g]
64.2	120.1	208.5	1.871	1.905	396	741

[a] *Number-Average Molecular Weight (from SEC)*
[b] *Weight-Average Molecular Weight (from SEC)*
[c] *Polydispersity Index (M_w/M_n)*
[d] *Third Moment Molecular Weight (from SEC)*
[e] *Intrinsic Viscosity (from SEC)*
[f] *Number-Average Degree of Polymerization*
[g] *Weight-Average Degree of Polymerization*

Dynamic viscosity was used throughout the project with the intent to estimate the effect of solubilization and modification on the molecular weight of α-1,3-glucan. The reliability of this is discussed in Chapter 5. It is important to note that a reduction in dynamic viscosity may also be caused by reduced solubility in the measurement solvent (DMSO/LiCl). α-1,3-glucan was analyzed in solution three times. The first measurement was performed on α-1,3-glucan as received, the second measurement was performed on α-1,3-glucan that had been dissolved in a solution of 7.3% LiCl in DMAc, stirred at 80 °C for 24 h, and subsequently precipitated into ethanol, and the third measurement was performed on α-1,3-glucan that had been dissolved in a solution of 7.3% LiCl in DMSO, stirred at 80 °C for 24 h, and subsequently precipitated into ethanol.

As can be seen in Figure 19, the dynamic viscosity is reduced significantly by the solubilization process in both DMAc/LiCl and DMSO/LiCl. Since this effect is less pronounced in DMAc/LiCl, reactions in Chapter 4 were performed in this solvent system instead of in DMSO/LiCl. Since viscosity measurements were performed in DMSO/LiCl for all samples throughout the project, it is possible that the process of solubilizing samples in DMSO/LiCl for analysis causes some degradation of the sample as well.

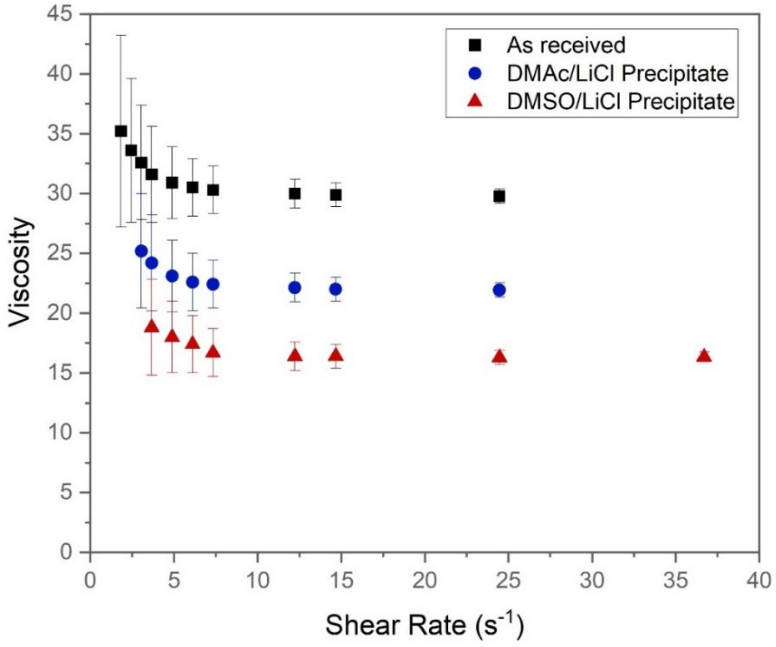


Figure 19. Dynamic viscosity measurements of α-1,3-glucan before and after dissolution and precipitation from DMAc/LiCl or DMSO/LiCl.

Differential scanning calorimetry (DSC) was used to look for the key transition temperatures of α-1,3-glucan. As shown in in Figure 20, there were no clear melting or crystallization temperatures found in the range analyzed. There is a step change at -19 °C which could indicate a glass transition temperature. Increasing the ramp rate could improve the resolution and thus confirm this value as the T_g for α-1,3-glucan.

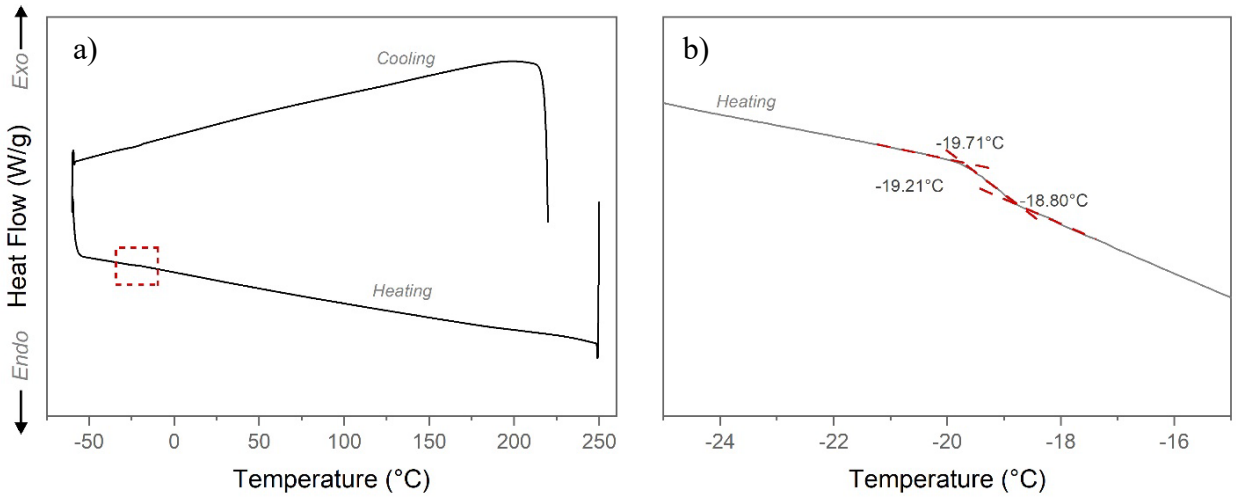


Figure 20. a) DSC curve for α-1,3-glucan as received (dried) **b)** region marked with dashed box (a) with determination of T_g.

Thermogravimetric analysis (TGA) was used to determine the degradation temperature of α-1,3-glucan. α-1,3-glucan has a degradation onset temperature of 308 °C (Figure 21, solid black line) and contains approximately 7% moisture when stored in a vacuum oven at 10^{-2} bar and 22 °C ± 2 °C. The degradation temperature is unaffected by dissolving α-1,3-glucan in 7.3% LiCl/DMAc and precipitating into ethanol (Figure 21, dashed blue line). The residual sample mass at 600 °C for the sample which was dissolved and precipitated may be due to incomplete washing of LiCl from the precipitate [82].

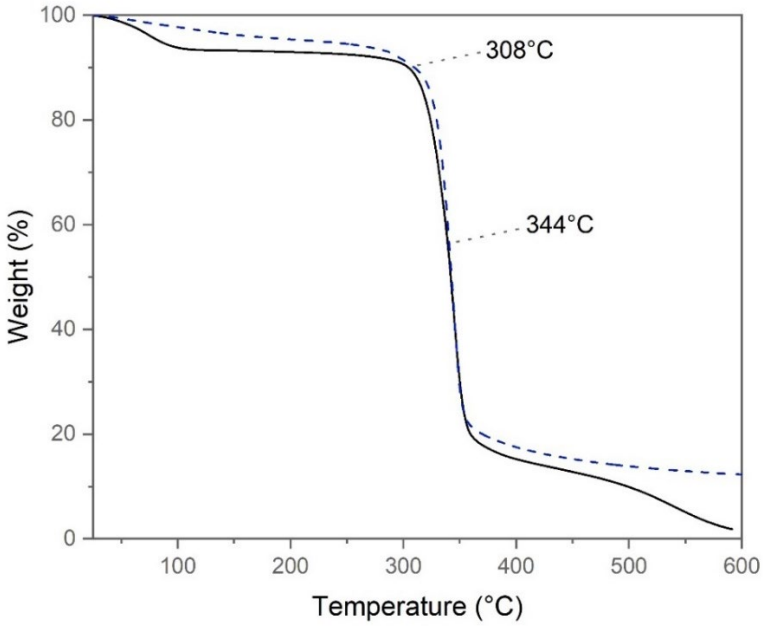


Figure 21. TGA curves of α-1,3-glucan as received (solid black line) and after dissolution and precipitation from DMAc/LiCl (dashed blue line).

4.2 SOLUBILITY

Solubility tests were motivated by a change in solubility of the polysaccharide over the time of usage. As will be discussed in Chapter 5, initial modifications of α-1,3-glucan were performed at 4% by weight in DMAc with 7.3% LiCl as found in literature [14]. However, after successful, reproducible modification of α-1,3-glucan with octenylsuccinic anhydride in this solvent system, the unmodified α-1,3-glucan no longer dissolved completely in 7.3% LiCl/DMAc. Experiments were then repeated in 5% LiCl/. Later, the solubility of α-1,3-glucan was again tested in 7.3% LiCl/DMAc and was found to be soluble. This inconsistency was concerning so further solubility testing was performed.

Primary solubility testing was performed in this laboratory where it was concluded that a solvent system consisting of lithium chloride (LiCl) in N,N-dimethyl acetamide (DMAc) was optimal for dissolving α-1,3-glucan [83]. To further explore the solubility of α-1,3-glucan in this solvent system, a set of tests were performed as shown in Table 11. Note that samples 1.2, 2.1, and 3.1 are identical and most closely resemble the solvent system and method followed for the solution-state modifications described in Chapter 4.

Table 11. Experimental design to explore effects of concentrations and method on solubility of α-1,3-glucan in DMAc/LiCl.

Sample	Order	Temperature (°C)	Concentration (% w/v)	Method
1. Effect of LiCl Concentration on Solubility				
1.1	Glucan + DMAc	120	4	α-1,3-glucan and DMAc stirred at 120 °C without cooling water for 30 minutes to remove water, LiCl added at 80 °C and mixture observed until transparent solution was obtained.
	LiCl	80	2.5	
1.2	Glucan + DMAc	120	4	
	LiCl	80	5	
1.3	Glucan + DMAc	120	4	
	LiCl	80	7.5	
2. Effect of α-1,3-glucan concentration on solubility				
2.1	Glucan + DMAc	120	4	α-1,3-glucan and DMAc stirred at 120 °C without cooling water for 30 minutes to remove water, LiCl added at 80 °C and mixture observed until transparent solution was obtained.
	LiCl	80	5	
2.2	Glucan + DMAc	120	7	
	LiCl	80	5	
2.3	Glucan + DMAc	120	10	
	LiCl	80	5	
3. Effect of solubilization method on solubility				
3.1	Glucan + DMAc	120	4	See samples 1.2 and 2.1
	LiCl	80	5	
3.2	LiCl + DMAc	80	4	LiCl and DMAc stirred at 80 °C until all LiCl dissolved, then α-1,3-glucan added and mixture observed until transparent solution was obtained.
	Glucan		5	
3.3	Glucan + LiCl + DMAc	80	4 (glucan) 5 (LiCl)	α-1,3-glucan, LiCl, and DMAc stirred at 80 °C and observed until transparent solution was obtained.

The dissolution observations for the primary tests are summarized in Table 12. From experiments 1.1 to 1.4, it can be concluded that increasing the concentration of lithium chloride increases the time required to solubilize α-1,3-glucan completely. Additionally, the maximum LiCl concentration to dissolve 4% α-1,3-glucan rests between 7.5% and 10%. The midpoint of tested LiCl concentrations that solubilized α-1,3-glucan was 5%, so this concentration was used to test the concentrations of α-1,3-glucan which could be dissolved. The maximum concentration of α-1,3-glucan which can be solubilized by 5% LiCl/DMAc is close to 7%. The experiment with 7% α-1,3-glucan in 5% LiCl/DMAc almost achieved complete dissolution except for a few small particles which could only be seen upon close observation. The experiment was repeated to confirm this, and the behaviour of α-1,3-glucan in 5% LiCl/DMAc was found to be consistent.

The solubilization process of α-1,3-glucan varies in literature [13, 74, 14], so experiments 3.1 to 3.3 were performed to determine if the order of mixing was important for the solubility. At constant LiCl and α-1,3-glucan concentrations of 5% and 4%, respectively, it was found that the order did not affect the solubility, but it did affect the time required to achieve a solution. Adding α-1,3-glucan first requires 30 minutes to disperse the polysaccharide, then 30 minutes to dissolve after LiCl is added; adding LiCl first requires 15 minutes to dissolve the LiCl, then 75 minutes to dissolve α-1,3-glucan once it has been added; and adding LiCl and α-1,3-glucan at the same time requires 90 minutes to dissolve both the salt and the polysaccharide. The first method was selected for the chemical modification of α-1,3-glucan in this project because it allows water to be removed during the first heating stage, improving the interaction of α-1,3-glucan with lithium chloride, and potentially reducing hydrolysis of the polysaccharide during the modification.

Table 12. Qualitative results of the primary experiments on the solubility of α-1,3-glucan in DMAc/LiCl.

Sample	Concentrations (% w/v)	Dissolved (Yes/No)	Observations
1.1	4% α-1,3-glucan 2.5% LiCl	Yes	Complete dissolution within 15 minutes
1.2/2.1/3.1	4% α-1,3-glucan 5% LiCl	Yes	Complete dissolution within 30 minutes
1.3	4% α-1,3-glucan 7.5% LiCl	Yes	Complete dissolution within 75 minutes
1.4	4% α-1,3-glucan 10% LiCl	No	Small solids still present at 120 minutes
2.2	7% α-1,3-glucan 5% LiCl	No	Small solids still present at 120 minutes
2.3	10% α-1,3-glucan 5% LiCl	-	Not tested since 7% did not dissolve
3.2	4% α-1,3-glucan 5% LiCl	Yes	Complete dissolution within 75 minutes
3.3	4% α-1,3-glucan 5% LiCl	Yes	Complete dissolution within 90 minutes

Based on the positive results observed for experiment 1.1, a secondary set of experiments was performed to determine the minimum amount of lithium chloride required to dissolve 4% α-1,3-glucan by weight, and subsequently to determine the maximum amount of α-1,3-glucan that could be dissolved in this weight percentage of lithium chloride. These experiments are described in Table 13 and the qualitative observations are summarized in Table 14.

Table 13. Determination of the solubility limits of α-1,3-glucan in DMAc/LiCl.

Sample	Order	Temperature (°C)	Concentration (% w/v)	Method
4.1	Glucan + DMAc	120	4	Glucan and DMAc stirred at 120 °C without cooling water for 30 minutes to remove water, LiCl added at 80 °C and mixture observed until transparent solution was obtained.
	LiCl	80	0.5	
4.2	Glucan + DMAc	120	4	
	LiCl	80	1	
4.3	Glucan + DMAc	120	7	
	LiCl	80	1	
4.4	Glucan + DMAc	120	7	
	LiCl	80	2.5	
4.5	Glucan + DMAc	120	10	
	LiCl	80	2.5	

From experiments 4.1 to 4.5, it was determined that the minimum concentration of LiCl needed to solubilize 4% α-1,3-glucan is 1% in DMAc. The maximum concentration of α-1,3-glucan solubilized by 1% LiCl/DMAc is between 4% and 7% with the mixture with 7% showing worse solubility in 1% LiCl/DMAc (Table 14, Experiment 4.3) compared to in 5% LiCl/DMAc (Table 12, Experiment 2.2). However, 7% α-1,3-glucan is soluble in 2.5% LiCl/DMAc (Experiment 4.4).

Due to this variation of α-1,3-glucan solubility with LiCl concentration, it is likely that at 1% LiCl/DMAc, there is an insufficient quantity of LiCl ions to disrupt the hydrogen bonding network of all of the α-1,3-glucan chains, and at 5% LiCl/DMAc, the increased concentration of LiCl causes an increase in the mixture viscosity, worsens the quality of the mixing, and reduces the ability of the polysaccharide chains to be broken apart and solubilized. 10% α-1,3-glucan is not soluble in 2.5% LiCl/DMAc (Experiment 4.5). Based on the consistency of the precipitates in Experiment 4.5, it appears that the solvent has dissolved the majority of the α-1,3-glucan and has swollen the remaining, undissolved solids.

Table 14. Qualitative results of the secondary experiments on the solubility of α-1,3-glucan in DMAc/LiCl.

Sample	Concentrations (% w/v)	Dissolved (Yes/No)	Observations
4.1	4% α-1,3-glucan 0.5% LiCl	No	Opaque mixture at 120 minutes
4.2	4% α-1,3-glucan 1% LiCl	Yes	Complete dissolution within 15 minutes
4.3	7% α-1,3-glucan 1% LiCl	No	Opaque mixture with no signs of improvement at 45 minutes
4.4	7% α-1,3-glucan 2.5% LiCl	Yes	Complete dissolution within 60 minutes
4.5	10% α-1,3-glucan 2.5% LiCl	No	Transparent with colourless, swollen precipitates at 120 minutes

As described previously, the solubility of α-1,3-glucan in 7.3% LiCl/DMAc was found to be inconsistent without any contamination or significant structural changes found by NMR and SEC. The positive solubility result of α-1,3-glucan in 7.5% LiCl/DMAc (Table 12, Experiment 1.3) was unexpected. So, the solubility test in 7.3% LiCl/DMAc was repeated using the set up for a chemical modification where α-1,3-glucan is stirred in DMAc at 120 °C for 1 h, then LiCl is added at 80 °C and stirred until dissolution with nitrogen gas flowing through the system during the entire process. After 1 h, the mixture was colourless and showed only one phase, but the clarity was reduced compared to its behaviour in the past and compared to Experiment 1.3. After stirring for 48 h at room temperature, the clarity was further reduced. Experiment 1.3 was repeated using this method and again produced a positive solubility result.

The solubility of α-1,3-glucan in 7.3% LiCl/DMAc behaved consistently for the first 16 months of use, after which it became inconsistent. α-1,3-glucan behaved consistently in 5% LiCl/DMAc during the time of use which was a span of approximately 3 months. It is therefore recommended that the solubility be periodically tested to ensure its consistency. The validity of the results of the solubility tests is limited by the use of qualitative observations over quantitative and by the number of repetitions performed for each test. The difference between defining a mixture as a solution versus a dispersion was based on visual observation only, and differences observed by the human

eye were not detected by the camera. In future testing, the use of a turbidimeter for preliminary measurements and of dynamic light scattering for fine determinations is recommended.

CHAPTER 5
RESULTS AND DISCUSSION – MODIFICATION OF α-1,3-GLUCAN WITH OCTENYLSUCCINIC ANHYDRIDE

The goal of this chapter is to investigate the esterification of α-1,3-glucan with octenylsuccinic anhydride in a homogeneous system (Figure 22). The effects of reaction time and temperature, the concentration of OSA, the presence of pyridine, and the concentration of LiCl are explored by measurement and analysis of the structure and properties of the modified α-1,3-glucan samples. It is expected that the degree of substitution and molecular weight can be controlled by the reaction conditions. The degree of substitution was determined by proton nuclear magnetic resonance spectroscopy, the crystallinity was analyzed by X-ray diffraction, the molecular weight distribution was found by size exclusion chromatography, transition temperatures were observed by differential scanning calorimetry, and the thermal stability was measured by thermogravimetric analysis. Qualitative observations were made for the wetting behaviour of pressed films and the behaviour of the samples in water and oil mixtures as described in Chapter 3.

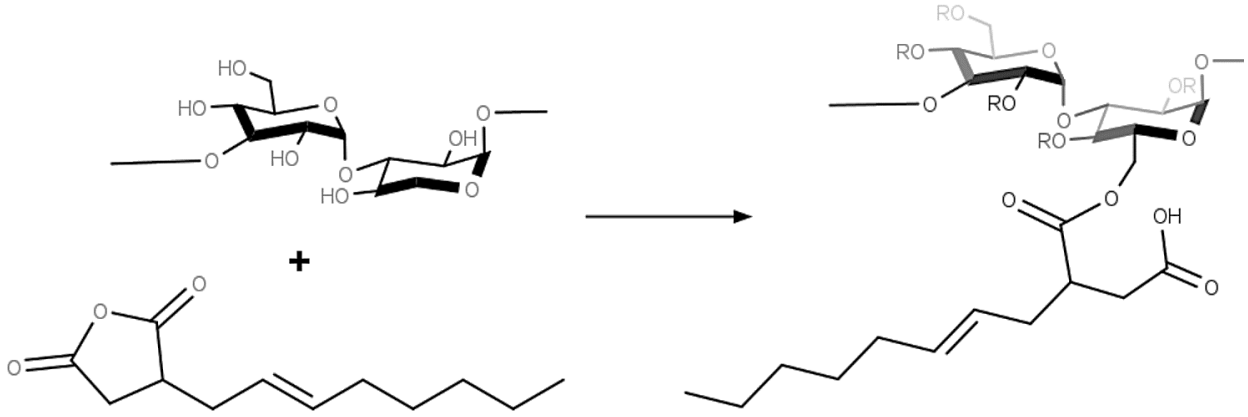


Figure 22. Esterification of -1,3-glucan with octenylsuccinic anhydride.

5.1 EXPERIMENTAL DESIGN

α-1,3-glucan was modified with octenylsuccinic anhydride (OSA) in N,N-dimethyl acetamide (DMAc) and lithium chloride (LiCl) with varying reaction conditions. As outlined in Table 15, the reaction time was varied between 4 h, 24 h, and 72 h, the reaction temperature was adjusted as 30 °C, 50 °C, and 70 °C, and the molar ratio of octenyl succinic anhydride to anhydroglucose units (AGU) was varied from 1:1, 3:1, up to 6:1. Initially, the experimental design included a full factorial design with three levels selected for each variable (time, temperature, and molar ratio of OSA to AGU). However, due to time and resource constraints, several reactions were omitted as denoted by N.E. in Table 15. Instead, to explore other factors on the reaction of α-1,3-glucan, additional experiments described in Table 16 were performed. These experiments involved increasing the temperature to 110 °C, adding pyridine to the reaction solution, or changing the concentration of LiCl.

The modified α-1,3-glucan samples are named using the molar ratio of OSA:AGU, the reaction temperature, the reaction time, and any additional conditions used. For example, the sample "3·50-24H-PYR" was produced from a reaction solution with a molar ratio of OSA:AGU of 3:1, a temperature of 50 °C, a reaction time of 24 h, and the reaction system contained pyridine.

Table 15. Primary experiments to explore the effect of time, temperature, and concentration of OSA (molar ratio OSA:AGU) on the esterification of α-1,3-glucan.

Constant: Temperature = 70 °C		**Low OSA:AGU** ***1:1***	**Moderate OSA:AGU** ***3:1***	**High OSA:AGU** ***6:1***
Short time	***4 h***	1·70-4H	3·70-4H	NE
Intermediate time	***24 h***	1·70-24H	3·70-24H	NE
Long time	***72 h***	1·70-72H	3·70-72H	NE
Constant: OSA:AGU = 3:1		**Short Time** ***4 h***	**Intermediate Time** ***24 h***	**Long Time** ***72 h***
Low Temperature	***30 °C***	N.E.	3·30-24H	NE
Moderate Temperature	***50 °C***	3·50-4H	3·50-24H	3·50-72H
High Temperature	***70 °C***	3·70-4H	3·70-24H	3·70-72H
Constant: Time = 24 h		**Low Temperature** ***30 °C***	**Moderate Temperature** ***50 °C***	**High Temperature** ***70 °C***
Low OSA:AGU	***1:1***	1·30-24H	1·50-24H	1·70-24H
Moderate OSA:AGU	***3:1***	3·30-24H	3·50-24H	3·70-24H
High OSA:AGU	***6:1***	NE	6·50-24H	N.E.

NE = No experiment carried out
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

Table 16. Additional experiments to explore the effect of temperature, catalyst, and LiCl concentration on the esterification of α-1,3-glucan.

Sample	OSA:AGU	Temperature (°C)	Time (h)	Notes
1·110-24H	1:1	110	24	High temperature
1·70-24H-PYR	1:1	70	24	Pyridine
3·50-24H-PYR	3:1	50	24	Pyridine
3·70-24H-2.5% LiCl	3:1	70	24	2.5% LiCl
3·70-24H-7.3% LiCl	3:1	70	24	5% LiCl

Sample label: ratio·temperature·time; example: 3·50-24H-PYR has a molar ratio OSA:AGU 3:1, 50 °C, 24 h, and pyridine

5.2 CHEMICAL STRUCTURE

The peaks from the spectra obtained via FTIR in Figure 23 were assigned to distinct functionalities, including ester and hydroxyl groups in Table 17. After esterification, the spectra contained an additional peak at 1718 cm^{-1}. This peak has been assigned as the carbonyl peak involved in the ester linkage between α-1,3-glucan and the OSA substituent. The spectra in Figure 23 show that the peak height increases with degree of substitution, supporting the assignment as an ester carbonyl group. The FTIR spectrum for pure OSA shows a carbonyl group at approximately 1785 cm^{-1}. The lack of peaks at 1785 cm^{-1} and 1865 cm^{-1} in the spectra for modified α-1,3-glucan is evidence that the purification steps are successful in removing unreacted OSA from the system.

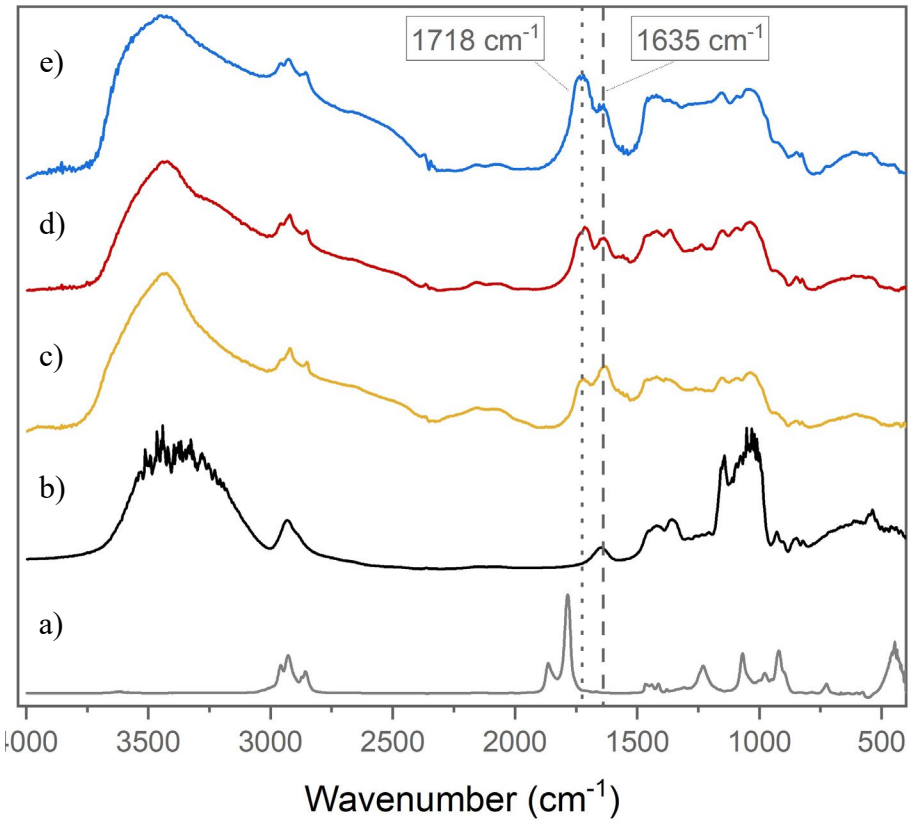


Figure 23. FTIR spectra for **a)** octenylsuccinic anhydride, **b)** neat α-1,3-glucan, and α-1,3-glucan modified to varying degrees: **c)** 1·70-24H (DS = 0.10), **d)** 3·50-24H (DS = 0.17), **e)** 3·70-24H-2.5% LiCl (DS = 0.84). DS calculated by NMR.

Table 17. Assignment of peaks from FTIR spectra for neat and modified α-1,3-glucan.

Neat α-1,3-glucan			
Wavenumber	**Strength**	**Functional Group**	**Assignment**
3000 – 3700 cm^{-1}	Strong, broad	O-H stretching, inter- and intra-molecular bonded	O-H at C2, C4, C6
2935 cm^{-1}	Medium, narrow	Alkane C-H stretching	C-H at C1, C2, C3, C4, C5, C6
1650 cm^{-1}	Weak, narrow	H-O-H bending	Residual bound water
1300 – 1500 cm^{-1}	Medium	Alkane C-H bending Alcohol O-H bending	C-H at C1, C2, C3, C4, C5, C6 O-H at C2, C4, C6
950 – 1190 cm^{-1}	Strong, broad	C-O stretching	-O- in ring (C3-C5) and in glycosidic linkage (C1-C3)
Modified α-1,3-glucan-OSA			
Wavenumber	**Strength**	**Functional Group**	**Assignment**
3000 – 3800 cm^{-1}	Strong, broad	O-H stretching, inter- and intra-molecular bonded	O-H at C2, C4, C6 O-H on OSA carboxylic acid end
2935 cm^{-1}	Medium, narrow	Alkane C-H stretching	C-H at C1, C2, C3, C4, C5, C6
2361 cm^{-1}	Weak, narrow	O=C=O stretching	Carbon dioxide
1720 cm^{-1}	Strong, narrow	Ester C=O stretching	Ester linkage between α-1,3-glucan and OSA
1635 cm^{-1}	Medium narrow	H-O-H bending	Residual bound water
1300 – 1500 cm^{-1}	Medium, broad	Alkane C-H bending Alcohol O-H bending	C-H at C1, C2, C3, C4, C5, C6 O-H at C2, C4, C6
950 – 1190 cm^{-1}	Medium, broad	C-O stretching	-O- in ring (C3-C5), in glycosidic linkage (C1-C3), and in ester linkage (α-1,3-glucan-OSA)

The reaction of OSA with α-1,3-glucan leads to the functionalization of the polysaccharide. The maximum theoretical degree of substitution would be 3 when all hydroxyl groups of the α-1,3-glucan have reacted with OSA. The degree of substitution is measured by NMR. Peaks from ^{1}H-NMR spectra were assigned to α-1,3-glucan in Table 18 based on literature where α-1,3-glucan was analyzed via 1D and 2D NMR [80, 81]. Here, peaks were assigned to OSA by comparison with the spectrum of pure OSA and with literature of starch modified with OSA [77]. Protons are numbered based on the carbons to which they are bonded as shown in Figure 15.

NMR spectra were collected in DMSO-d_6 with TFAA-d. TFAA-d was added to replace the exchangeable hydroxyl group protons, thus removing their signals from the spectra and simplifying the integration required to calculate the degree of substitution. Spectra are included in Figure 14 for neat α-1,3-glucan and for modified α-1,3-glucan with varying degrees of substitution. In these figures, it is possible to see the evolution of OSA peaks, particularly at shifts of 0.83 ppm, 1.22 ppm, and 5.1 – 5.4 ppm, with increasing degree of substitution.

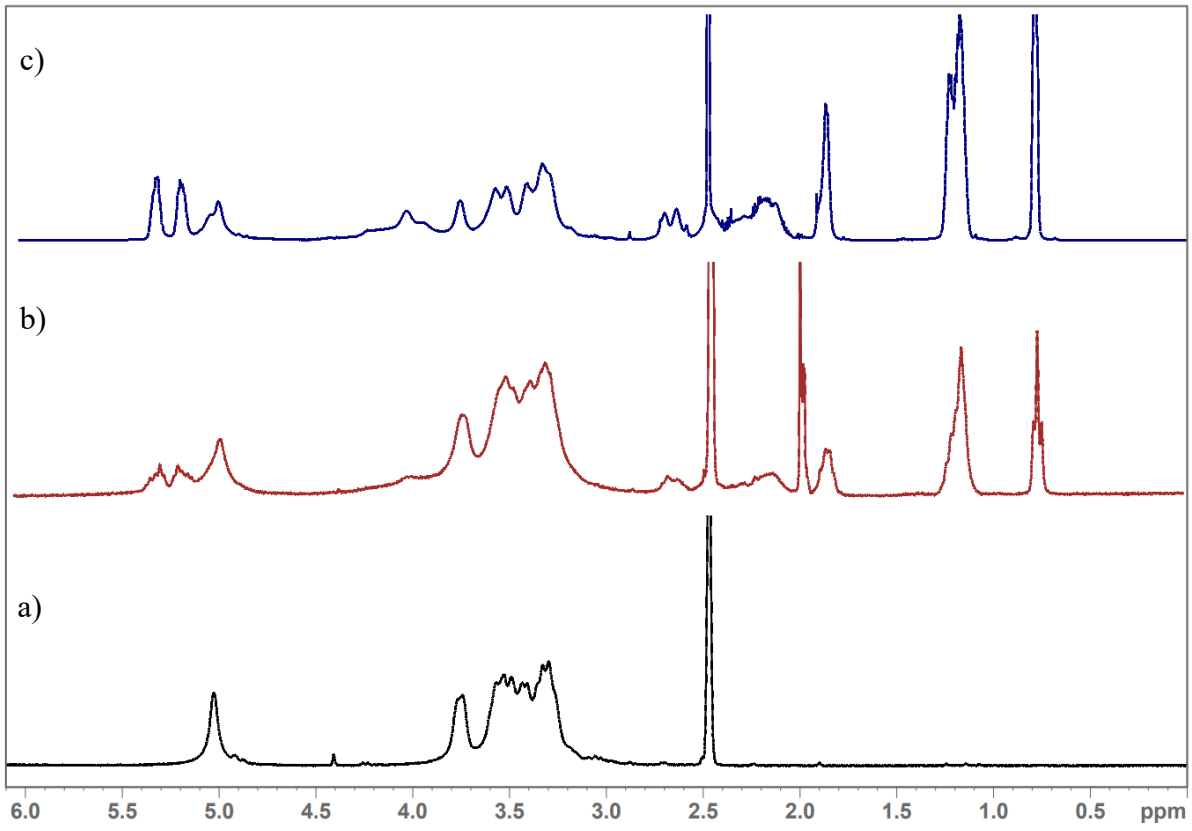


Figure 24. ^{1}H-NMR spectra of **a)** neat α-1,3-glucan, and α-1,3-glucan modified to varying degrees: **b)** 3·50-24H A (DS = 0.17), **c)** 3·70-24H B (DS = 0.59).

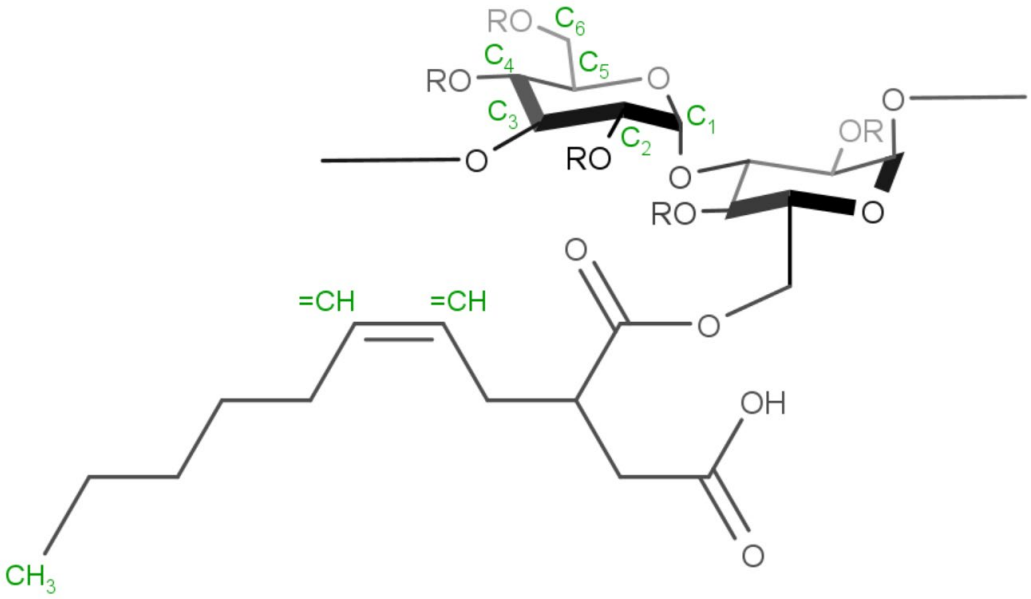


Figure 25. Structure of modified α-1,3-glucan with carbon numbers assigned for NMR analysis.

Table 18. Assignment of peaks from NMR spectra for neat and modified α-1,3-glucan.

α-1,3-glucan [a]		**Modified α-1,3-glucan-OSA [b]**	
Chemical Shift (ppm)	**Assignment**	**Chemical Shift (ppm)**	**Assignment**
3.40	H2, H4	0.83	CH_3 (OSA)
3.58	H3, H6a, H6b	1.22	$-CH_2$ (OSA)
3.84	H5	3.32	H2
4.62	OH-C6	3.36	H4
4.77	OH-C2	3.45	H6b
5.11	H1	3.54	H6a
5.27	OH-C4	3.60	H3
		3.81	H5
		5.07	H1
		5.30	=CH (OSA)
		5.39	=CH (OSA)

[a] *Solvent: Deuterated dimethyl-d_6 sulfoxide (DMSO-d_6)*
[b] *Solvent: Deuterated dimethyl-d_6 sulfoxide (DMSO-d_6) and deuterated trifluoroacetic-d acid (TFAA-d)*
Note: peak at chemical shift of 2.50 ppm is due to residual DMSO-d_5.

The degree of substitution for the polysaccharide can be found in two ways using integration of ^{1}H-NMR spectral peaks. In the first method (Equation 1), the terminal methyl peak of OSA (δ = 0.83 ppm) is compared against the anomeric proton (H1) of α-1,3-glucan (δ = 5.07 ppm). In the second method (Equation 2), the terminal methyl peak is compared against all ring protons of α-1,3-glucan (δ = 3.32, 3.36, 3.45, 3.54, 3.60, 3.81 ppm).

$$DS_1 = \frac{\frac{I_{CH_3}}{3}}{I_{H_1}} \qquad (Equation\ 1)$$

$$DS_2 = \frac{\frac{I_{CH_3}}{3}}{\frac{I_{RingH}}{7}} \qquad (Equation\ 2)$$

The second method is more robust as it is less affected by noise in the baseline. However, the two methods should produce similar DS values. Despite this, there were discrepancies in degrees of substitution using the first and second methods, with the first method giving DS values of at least 15% higher at low DS, and up to 160% higher at elevated DS. This may be caused by the presence of water or residual ethanol in the sample, improper baseline selection, or inadequate noise-to-signal ratio. Degrees of substitution for modified α-1,3-glucan using the second calculation method (Equation 2) can be found in Table 19. ^{13}C-NMR could be used in future experiments to confirm the degree of substitution with greater confidence.

The degree of substitution is significantly lower than the target DS for all samples. The target DS is defined by the molar ratio of OSA:AGU input to the system; for example, a ratio of 1:1 targets a DS of 1 and a ratio of 3:1 targets a DS of 3. Esterification of starch with OSA also often attains low DS values [15]. Starch-OSA is often used an additive in the food industry; the OSA concentration is limited by the U.S. Food & Drug Administration to be less than 3 wt. % and the target DS is therefore low (DS < 0.023) [84]. Starch esterification is commonly performed in aqueous alkaline conditions where starch grains are swollen to improve contact with OSA. However, other starch modifications have been performed with OSA for other applications with DS values from 0.3 up to 1.2 [85, 86, 87, 88]. The esterification of starch with OSA in DMAc/LiCl has not been reported.

In comparison, complete esterification (DS = 3) of α-1,3-glucan has successfully been performed in the literature with a variety of reagents [13, 45, 53]. These modifications were performed on α-1,3-glucan with a similar molecular weight (M_w of 120 – 200 kDa) to the polysaccharide used in this project (M_w of 120 kDa) with acid anhydrides [13, 45, 53].

Table 19. Summary of the degree of substitution (calculated with Equation 2) for homogeneously modified α-1,3-glucan samples.

Sample	Molar Ratio [a]	Temperature (°C)	Time (h)	DS [b]
α-1,3-glucan	-	-	-	*0*
1·30-24	1:1	30	24	0.03
1·50-24	1:1	50	24	0.05
1·70-4	1:1	70	4	0.09
1·70-24	1:1	70	24	0.10 [c]
1·70-72	1:1	70	72	0.29 [d]
1·110-24	1:1	110	24	0.34
3·30-24	3:1	30	24	0.06
3·50-4	3:1	50	4	0.07
3·50-24	3:1	50	24	0.19
3·50-72	3:1	50	72	0.33 [d]
3·70-4	3:1	70	4	0.20
3·70-24	3:1	70	24	0.68 [d]
3·70-72	3:1	70	72	1.24 [d]
6·50-24	6:1	50	24	0.36
1·70-24-PYR	1:1:1	70	24	0.39
3·50-24-PYR	3:3:1	50	24	1.5
3·70-24H-2.5% LiCl	3:1	70	24	0.84
3·70-24H-7.3% LiCl	3:1	70	24	0.35

[a] Octenyl succinic anhydride:Anhydroglucose unit or Octenyl succinic anhydride:Pyridine:Anhydroglucose unit
[b] Degree of Substitution (from NMR)
[c] Average value taken from multiple experiments with good agreement
[d] Average value taken from multiple experiments with poor reproducibility
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

Size exclusion chromatography experiments were performed on a subset of modified α-1,3-glucan samples to obtain molecular weights and molecular weight distributions. Samples were selected based on availability at the time of analysis and based on having a wide variety of reaction conditions and degrees of substitution in order to best understand their effects on the degradation of α-1,3-glucan. Where experiments were repeated, the sample closest to the midpoint of the degree of substitution values was chosen for SEC analysis. Therefore, in Table 20, the listed degrees of substitution are specific to the sample analyzed by SEC, instead of an average as listed in Table 19. Plots of the molecular weight distributions for each sample are provided in Appendix III.

Table 20. Summary of the molecular weights, polydispersity, and degree of polymerization for neat and modified α-1,3-glucan.

Sample	DS [a]	M_n [b] (kDa)	M_w [c] (kDa)	M_z [e] (kDa)	PDI [d]	[η] [f] (mL/g)	DP_n [g]	DP_w [h]
α-1,3-glucan	*0*	*64.2*	*120.1*	*208.5*	*1.87*	*1.905*	*396*	*741*
1·30-24	0.03	71.8	137.8	259.4	1.92	2.115	427	819
1·70-24	0.10	55.6	92.1	148.3	1.66	1.490	304	502
1·70-72	0.20	44.3	81.9	157.3	1.85	1.167	217	401
1·110-24	0.34	15.6	18.9	22.6	1.21	0.295	67	81
3·30-24	0.06	71.7	141.4	259.9	1.97	2.172	411	810
3·50-24	0.19	69.0	132.9	237.6	1.93	1.981	342	658
3·50-72	0.50	81.5	155.2	288.8	1.90	1.888	305	581
3·70-24	0.48	78.3	129.3	217.6	1.65	1.682	298	492
3·70-72	1.4	89.9	153.0	269.4	1.70	1.383	197	335
1·70-24-PYR	0.39	78.7	144.9	278.3	1.84	1.808	323	594
3·50-24-PYR	1.5	120.8	218.5	405.1	1.81	1.724	253	458
3·70-24H-2.5% LiCl	0.84	82.4	150.3	263.6	1.82	1.616	243	444
3·70-24H-7.3% LiCl	0.35	61.5	129.7	298.1	2.11	1.407	261	550

[a] *Degree of Substitution (from NMR)*
[b] *Number Average Molecular Weight (from SEC)*
[c] *Weight Average Molecular Weight (from SEC)*
[d] *Polydispersity Index (M_W/M_n)*
[e] *Third Moment Molecular Weight (from SEC)*
[f] *Intrinsic Viscosity (from SEC)*
[g] *Number-Average Degree of Polymerization*

[h] Weight-Average Degree of Polymerization
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

The degree of substitution found by NMR and the molecular weight found by SEC were compared directly to determine if any overarching correlations exist. In Figure 26 and Figure 27, the degree of substitution is compared to the number average molecular weight (M_n) and the weight average molecular weight (M_w), respectively.

As expected, there is minimal correlation between the degree of substitution and the molecular weight since the extent of degradation is expected to be strongly affected by the reaction temperature and time which are not distinguished in these figures.

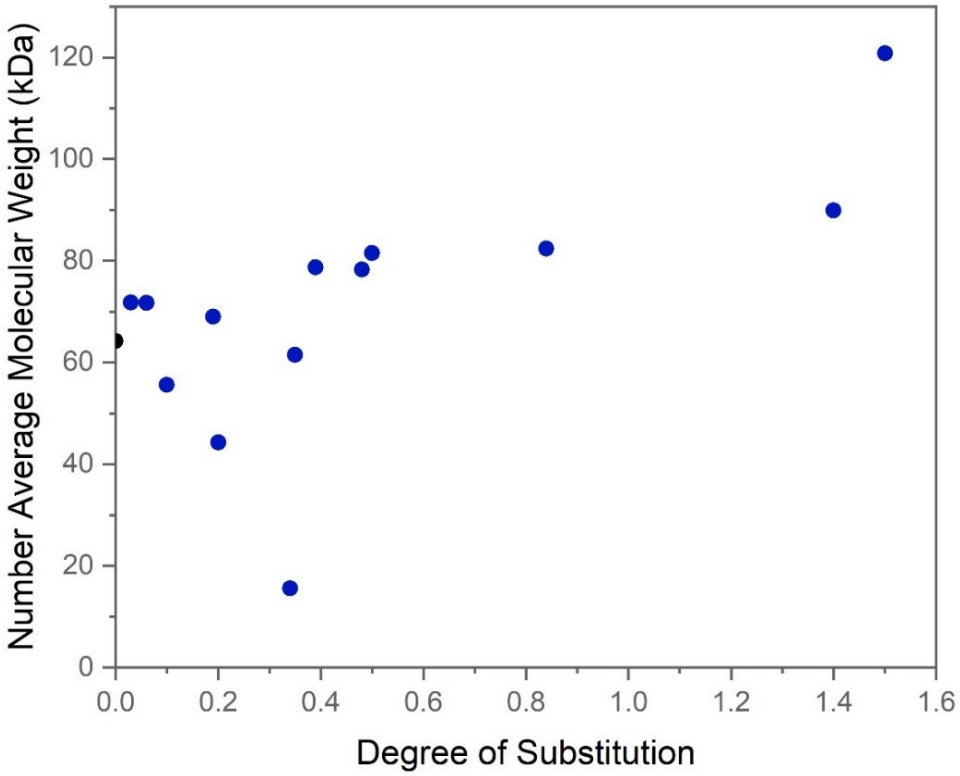


Figure 26. Number average molecular weight (M_n) compared against the degree of substitution for modified α-1,3-glucan.

Using the degree of substitution and Equation 3, the theoretical weight average molecular weight was calculated for each sample. The theoretical values are included in Figure 27 to show both degradation and discrepancies between the values. The weight average degree of polymerization

was calculated using Equation 4 and is also included in Figure 27 to show the decreasing trend of polysaccharide chain length with increasing degree of substitution. Since the theoretical molecular weight and degree of polymerization are calculated using the degree of substitution, the values are subject to the uncertainty associated with the determination of the degree of substitution by NMR.

$$M_{W\,Theoretical} = \frac{M_{W\,glucan}}{MW_{AGU}}(DS \times MW_{OSA} + MW_{AGU}) \qquad (Equation\ 3)$$

$$DP_w = \frac{M_w}{DS \times MW_{OSA} + MW_{AGU}} \qquad (Equation\ 4)$$

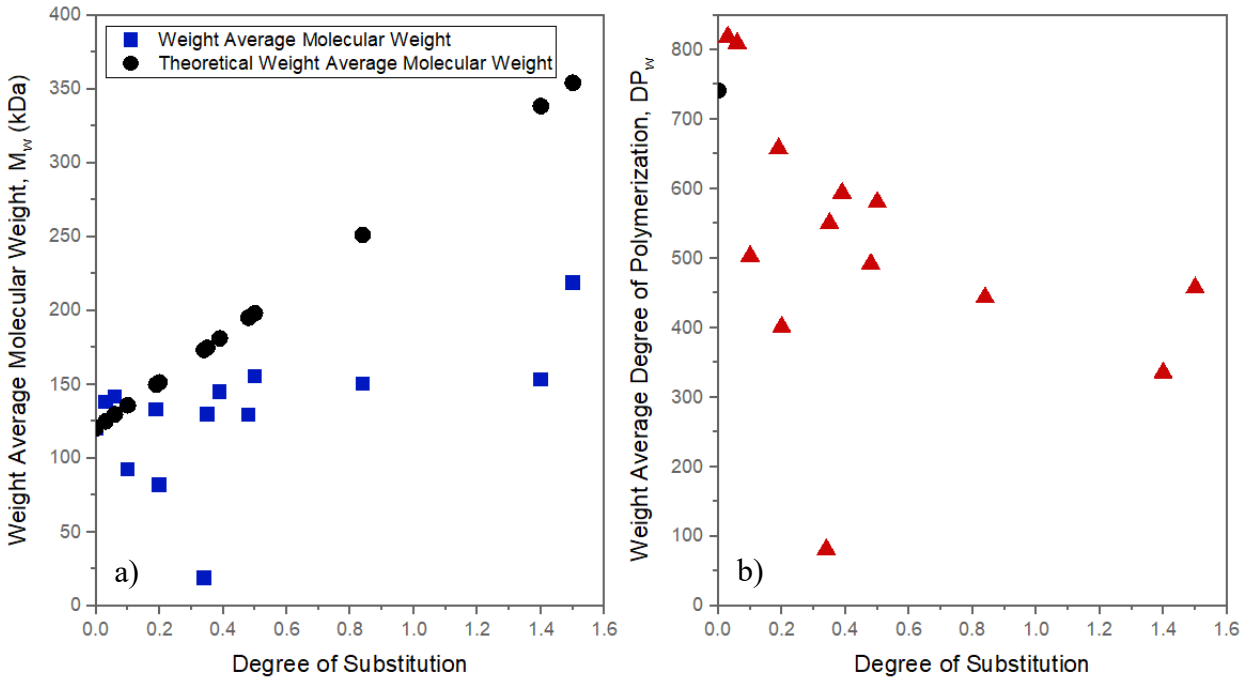


Figure 27. a) Weight average molecular weight (M_w) and **b)** Weight average degree of polymerization (DP_w) compared against degree of substitution for modified α-1,3-glucan.

Where the measured M_W is less than the theoretical value, it is assumed that this is primarily a result of degradation during the modification. A higher measured value may be a result of long chain branching or crosslinking initiated by the modification. In both cases where the measured value is lower or higher than the theoretical value, it may be that the degree of substitution could have been affected by experimental uncertainty.

Some discrepancies are expected due to the manual nature of selecting a baseline for both NMR and SEC analyses. Samples with higher molecular weights than expected did not exceed the theoretical molecular weight by more than 10%. In contrast, significant extents of degradation can be observed in samples 1·70-72, 1·110-24, and 3·70-72 with 46%, 89%, and 55% reductions in molecular weight relative to the theoretical value, respectively. The correlations of molecular weight loss and reaction conditions are discussed below.

Table 21. Theoretical weight average molecular weights and degrees of substitution for modified α-1,3-glucan.

Sample	DS [a]	M_w [b] (kDa)	Theoretical M_w [c] (kDa)
α-1,3-glucan	*0*	*120.1*	*120.1*
1·30-24	0.03	137.8	124.8
1·70-24	0.10	92.1 [d]	135.7
1·70-72	0.20	81.9 [d]	151.3
1·110-24	0.34	18.9 [d]	173.1
3·30-24	0.06	141.4	129.5
3·50-24	0.19	132.9 [d]	149.7
3·50-72	0.50	155.2 [d]	198.1
3·70-24	0.48	129.3 [d]	194.9
3·70-72	1.4	153.0 [d]	338.4
1·70-24-PYR	0.39	144.9 [d]	180.9
3·50-24-PYR	1.5	218.5 [d]	354.0
3·70-24H-2.5% LiCl	0.84	150.3 [d]	251.1
3·70-24H-7.3% LiCl	0.35	129.7 [d]	174.7

[a] *Degree of Substitution (from NMR)*
[b] *Weight Average Molecular Weight (from SEC)*
[c] *Weight Average Molecular Weight (estimated using Equation 3)*
[d] *Molecular weights less than theoretical values by more than 10%*
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

Dynamic viscosity measurements were performed on all samples to serve as an indicator of the molecular weight of the polysaccharide. It was assumed that a lower viscosity indicated a reduction in molecular weight; however, as mentioned in Chapter 4, a lower viscosity may be evidence of

reduced solubility caused by a significant change in the structure and properties of α-1,3-glucan. Viscosity measurements are provided in Appendix III and separated to explore the effect of the reaction conditions on the viscosity. All modifications caused a notable reduction in the dynamic viscosity but the trends in dynamic viscosity did not agree with the trends in molecular weight as measured by SEC.

Using the average of the viscosity measurements between 7 to 15 s^{-1} for each sample, the dynamic viscosity was compared to the weight average molecular weight in Figure 28 to determine whether it was an adequate measure of degradation. There is a poor correlation between viscosity and molecular weight which cannot reliably be used to monitor degradation using only viscosity measurements. Samples with similar molecular weights have significantly different viscosities and it appears that only significant degradation correlates well with reduced viscosity as seen by the data point on the lower left of Figure 28.

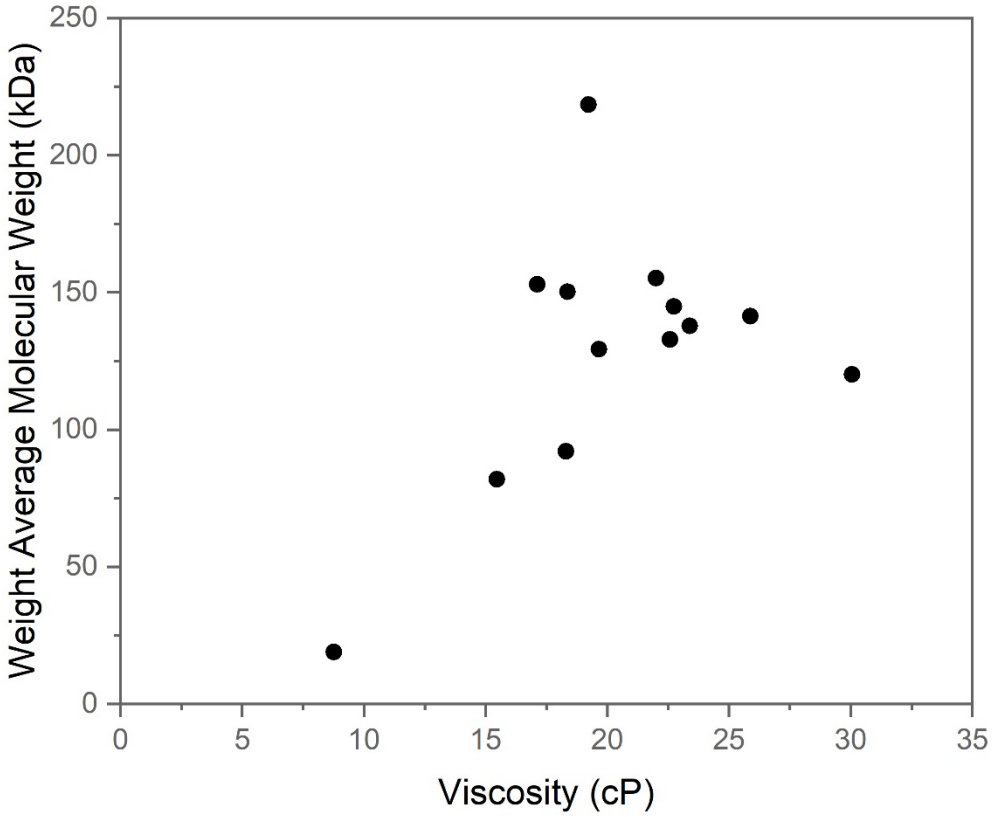


Figure 28. Comparison of weight average molecular weight (M_n) and dynamic viscosity of modified α-1,3-glucan.

5.3 THERMAL PROPERTIES

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to quantify the thermal properties of neat and modified α-1,3-glucan. As the temperature range for DSC measurements was selected based on the degradation temperature for each sample, TGA was performed first. Upon heating, all of the samples foamed as shown in Figure 29. This may be a result of DMAc or OSA trapped within the sample particles. Due to this property, it was crucial that the temperature range for DSC stayed well below the degradation temperature to avoid the sample lid opening and the sample pan falling out of the holder.

Figure 29. Example sample material after undergoing heating for thermogravimetric analysis.

TGA provides a curve of weight change versus the chamber temperature. In subsequent sections, these curves will be used to explore the relationship between the reaction conditions and the degradation temperature of the product. Commonly, the temperature at 5% weight loss is used as the degradation temperature but in some of the samples analyzed this temperature was low and the mass loss can likely be attributed to moisture in the samples. The degradation onset temperature can also be used as a measure of thermal stability and these values are provided in Table 22; however, the onset temperature is less easily identifiable than the inflection point. Figures

containing identification of the onset temperature are provided in Appendix III. Therefore, the temperature at which the rate of degradation was the fastest, denoted as the inflection point or T_{MAX}, was assumed to be more reliable and was thus the primary temperature used for comparison of the samples. For all modified samples analyzed, the inflection point was reduced by at least 77 °C compared to unmodified α-1,3-glucan. This reduction in thermal stability is noted visually in Figure 30. It has been found that the thermal stability of cellulose increases with increasing degree of crystallinity [89, 90, 91]. Similarly, decomposition of amorphous starch was found to occur more quickly at lower temperatures compared to semi-crystalline starch [92]. Therefore, the reduced thermal stability may be a result of a reduction in the crystallinity of the α-1,3-glucan caused by modification with OSA. From Figure 30, it can also be observed that there remains significant sample mass (> 20%) for most samples at 600 °C. This is an indication that the purification step may be ineffective in removing LiCl from the modified α-1,3-glucan samples since LiCl evaporates above 800 °C [82]. Increasing the temperature range for TGA measurements could confirm this. In future experiments, purification should include washing with water or methanol to improve the removal of LiCl from samples.

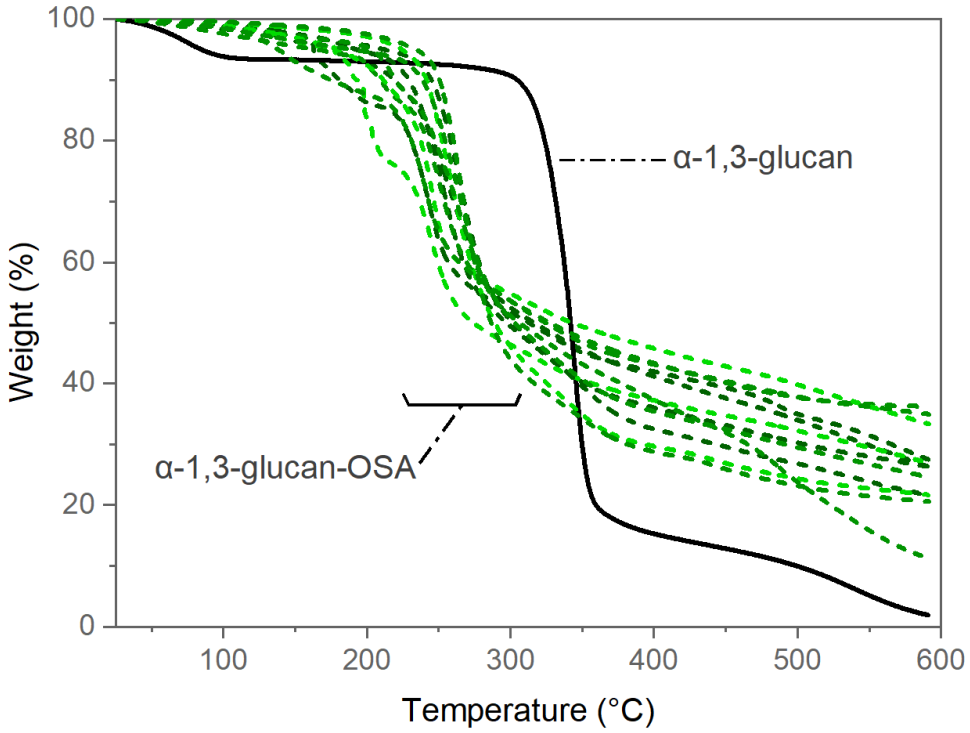


Figure 30. TGA curves for neat α-1,3-glucan (solid black line) and modified α-1,3-glucan (dashed green lines).

The assigned maximum degradation temperature, T_{MAX}, has been compared with the degree of substitution and the weight average molecular weight in Figure 31 and Figure 32, respectively. There is no clear correlation between T_{MAX} and the DS. T_{MAX} weakly correlates positively with the molecular weight of modified samples. Overall, the range of degradation temperatures is narrow and samples of similar molecular weight display different thermal stabilities within the narrow range.

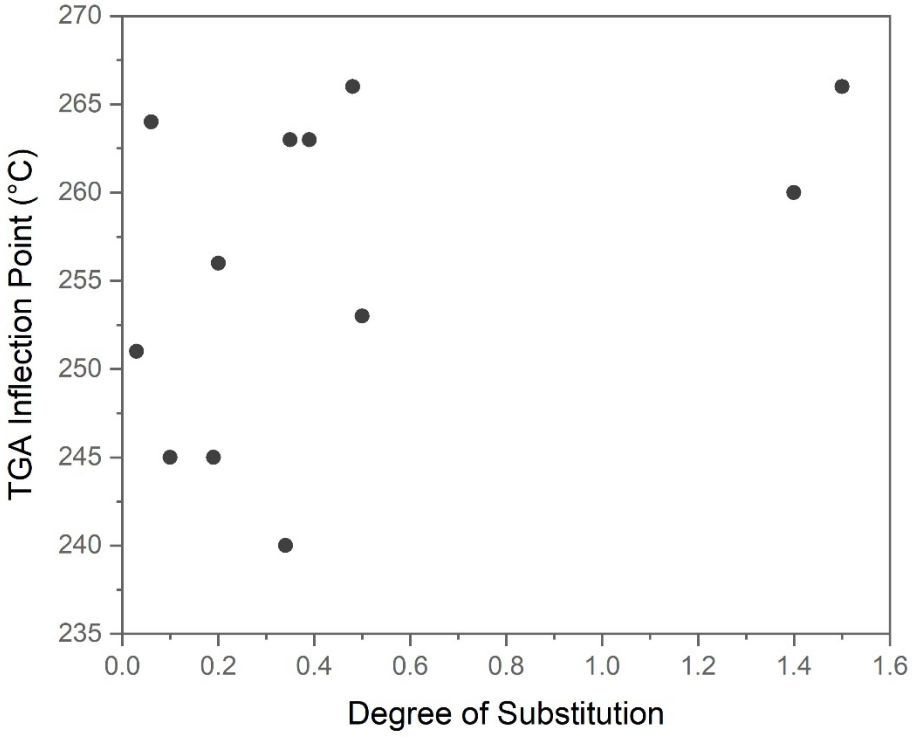


Figure 31. Comparison of the maximum degradation rate from TGA and the degree of substitution (DS) of modified α-1,3-glucan.

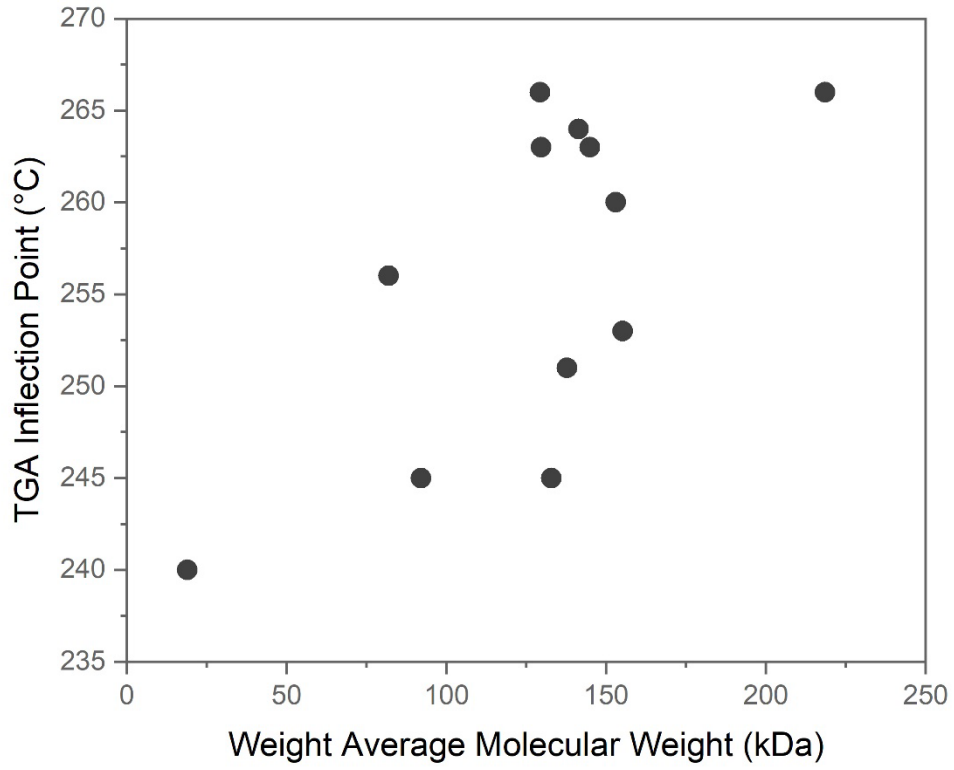


Figure 32. Comparison of the maximum degradation rate from TGA and the weight average molecular weight (M_W) of modified α-1,3-glucan.

Once thermal stability analyses were completed, calorimetry was performed between -50 °C and 160 °C. The maximum temperature was selected to avoid the foaming observed in Figure 29. DSC curves of heat flow versus temperature did not show any key transition temperatures (melting, crystallization, glass transition) but are provided in Appendix III for reference. Since no transition temperatures were found, the sample pans were pried open after analysis to observe any changes in the appearance of the samples. These observations are summarized alongside the TGA results in Table 22. Most samples were unchanged; however, samples 3·30-24, 3·50-24, 3·50-24-PYR, and 3·70-24H-2.5% LiCl underwent a colour change from a light colour to a darker colour. This could indicate some thermal degradation which is supported by the low 5% mass loss temperatures of 3·30-24, 3·50-24, and 3·70-24H-2.5% LiCl. Despite the lack of transition temperature shown in the DSC curves, samples 3·50-72, 3·70-72, and 3·50-24-PYR showed some indication of flow as multiple solid particles were input to each DSC pan but the final materials were single pieces of material.

Table 22. Summary of thermal analyses for modified α-1,3-glucan.

Identifier	DS [a]	$T_{5\%}$ (°C)	T_{onset} (°C)	T_{MAX} (°C)	DSC Observations
α-1,3-glucan	*0*	*84*	*308*	*344*	*Unchanged*
1·30-24	0.03	165	196	251	Unchanged
1·50-24	0.05	204	204	267	Unchanged
1·70-24	0.08	180	183	245	Unchanged
1·70-72	0.20	184	188	256	Unchanged
1·110-24	0.34	138	212	240	Unchanged
3·30-24	0.06	178	178	264	Colour change (white to amber)
3·50-24	0.17	175	220	245	Colour change (yellow to amber)
3·50-72	0.40	192	212	253	One piece
3·70-24	0.48	211	232	266	Unchanged
3·70-72	1.39	224	208	260	One piece
6·50-24	0.36	163	214	242	Unchanged
1·70-24-PYR	0.39	190	225	263	Unchanged
3·50-24-PYR	1.50	234	240	266	One piece, colour change (yellow to amber)
3·70-24H-2.5% LiCl	0.84	159	159	162	Colour change (yellow to amber)
3·70-24H-7.3% LiCl	0.39	217	232	263	Unchanged

[a] Degree of Substitution (from NMR), sample-specific
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

5.4 HOT PRESSING & WETTING TESTS

Hot pressing of samples was performed at between 100 °C and 200 °C; by 160 °C, all of the samples which showed flow properties were successfully pressed into films. Increasing the temperature caused sample discolouration, indicating the onset of thermal degradation. The exact temperature at which each sample flows with pressure is difficult to determine precisely due to the wide temperature fluctuations experienced in the hydraulic press. Table 23 provides descriptions of the samples pressed at 160 °C shown in Figure 33. Based on the appearance of the films, a

degree of substitution greater than 0.50 is needed to consistently obtain a sample which can be pressed into a smooth continuous film. The primary exception to this is sample 1·110-24H which forms a film with a DS as low as 0.34. For samples 3·50-24H A and B, it appears that the combination of heat and pressure is sufficient to cause the individual particles to combine to some extent but is insufficient to form one continuous film.

Table 23. Descriptions of hot-pressing behaviour of modified α-1,3-glucan samples shown in Figure 33.

Sample	DS [a]	Film
1·110-24H	0.34	*a)* Amber film with trapped air
3·50-24H A	0.17	*b)* Some flow behaviour, individual particles still visible
3·50-24H B	0.20	*c)* Some flow behaviour, individual particles still visible
3·50-72H	0.50	*d)* Smooth continuous film
3·50-24H-PYR	1.50	*e)* Continuous film with some imperfections
3·70-24H A	0.38	*f)* Some flow behaviour, individual particles visible at edge
3·70-24H B	0.59	*g)* Smooth continuous film
3·70-24H C	1.24	*h)* Smooth continuous film
3·70-24H D	0.48	*i)* No significant flow behaviour
3·70-24H E	0.74	*j)* Continuous film, texture caused by Teflon film
3·70-72H A	1.39	*k)* Continuous film with some imperfections
3·70-24H-2.5% LiCl	0.84	*l)* Smooth continuous film

[a] *Degree of substitution (from NMR)*
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

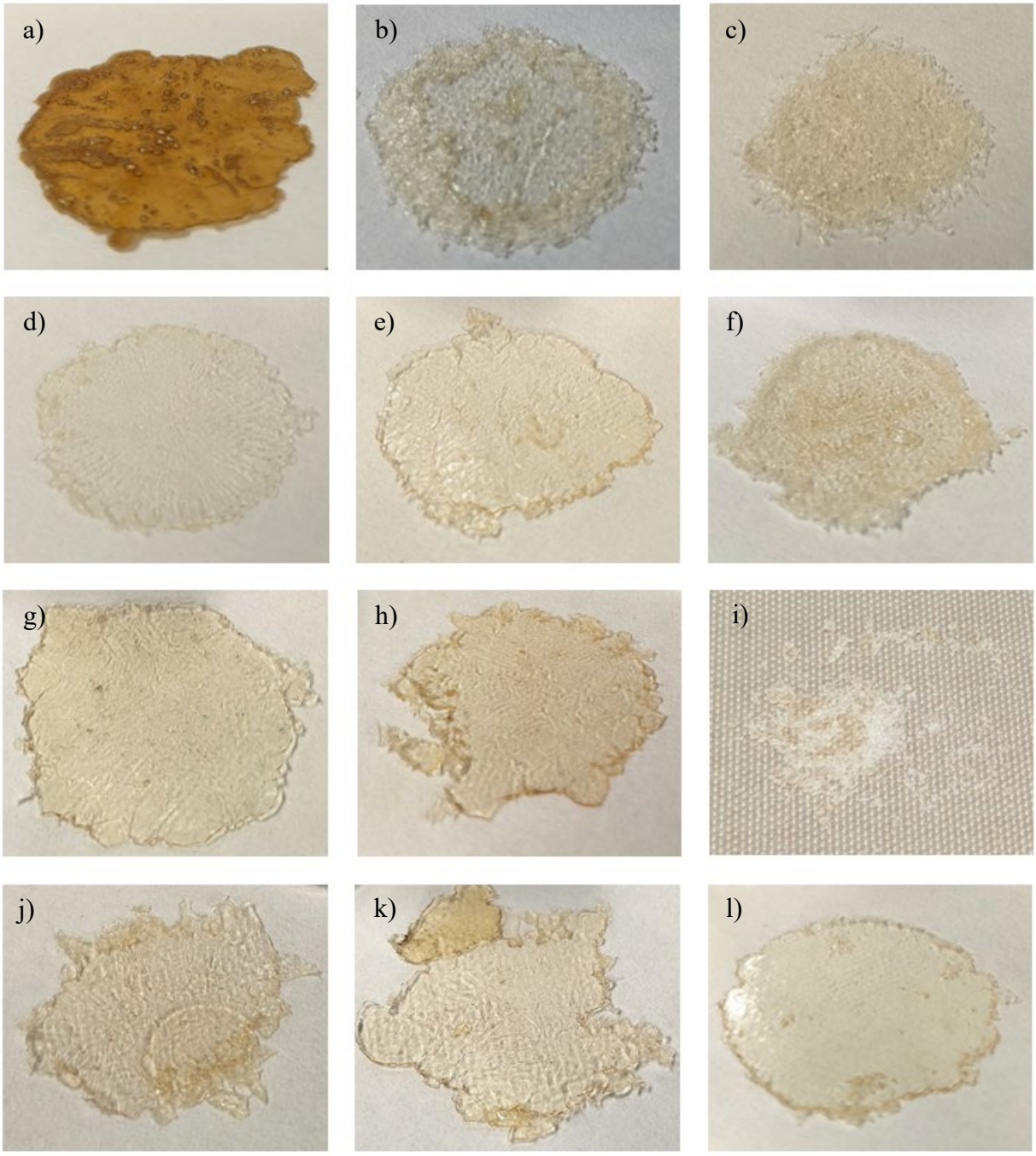


Figure 33. Films formed by hot pressing modified α-1,3-glucan samples at 160 °C: **a)** 1·110-24H, **b)** 3·50-24H A, **c)** 3·50-24H B, **d)** 3·50-72H, **e)** 3·50-24H-PYR, **f)** 3·70-24H A, **g)** 3·70-24H B, **h)** 3·70-24H C, **i)** 3·70-24H D, **j)** 3·70-24H E, **k)** 3·70-72H, **l)** 3·70-24H-2.5% LiCl

After pressing was found to be successful, the formation of tensile bars was attempted. However, the films were too brittle to be removed from the tensile bar mold without fracturing.

The pressed samples in Figure 33 were then wetted with water as a preliminary test of the hydrophobicity. The water droplet behaved similarly on all pressed samples and maintained a contact angle less than 90 °C for both lower (Figure 34) and higher (Figure 35) modified samples.

Figure 34. Water droplet on modified α-1,3-glucan sample 1·110-24H (DS = 0.34).

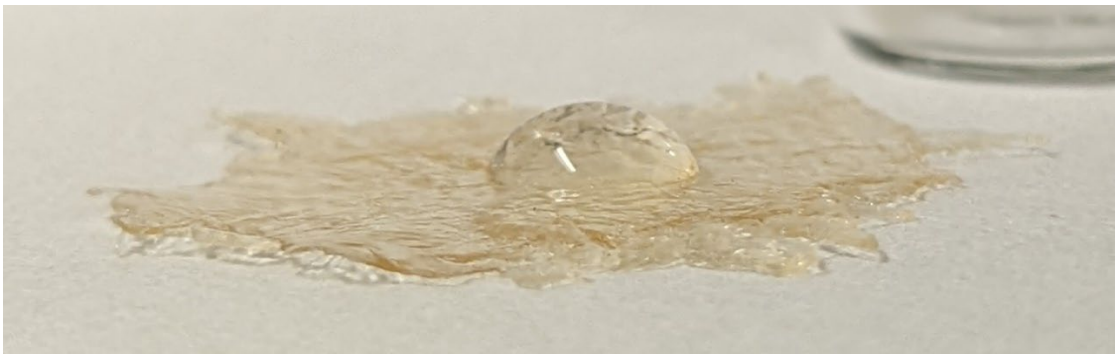


Figure 35. Water droplet on modified α-1,3-glucan sample 3·70-24H E (DS = 0.74).

The water droplets were stable on the films, but when the films were blotted dry, the appearance of the film where the droplet had been placed was significantly changed. As seen in Figure 36, the film became white and opaque. This is evidence that while the droplets appeared stable, there was some interaction occurring between the sample and water.

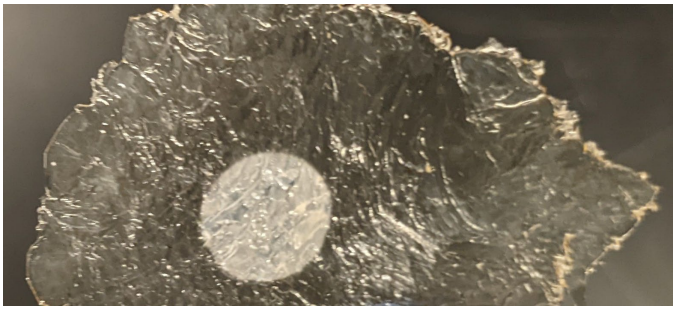


Figure 36. Example sample after wetting test with water.

Due to the brittleness of the pressed samples and their interaction with water, the current modified α-1,3-glucan is not yet a viable polymer for packaging applications.

5.5 PRELIMINARY EMULSION TESTING

Since the film behaviour is insufficient for packaging applications, the secondary objective of the esterification of α-1,3-glucan is to create an emulsifier for personal care applications.

First, the behaviour of each sample was explored in water and mineral oil separately as described in Table 24. None of the samples interacted with mineral oil, settling quickly after manual agitation of the vial. Sample 1·110-24H interacted well with water, changing colour upon addition of water and beginning to disperse, then creating a stable foam phase on the surface of the water (Figure 37). The mixture in Figure 37 has a lower sample concentration than those used for the emulsion tests and is included only for evidence of the foam layer. Samples 3·70-24H and 3·70-72H also showed positive interaction with water as the colour of the solids changed.

Table 24. Behaviour of modified α-1,3-glucan in water and mineral oil.

Sample	Behaviour in Water	Behaviour in Oil
1·30-24H	Particles settled	Particles settled
1·70-24H	Particles settled	Particles settled
1·70-72H	Particles settled	Particles settled
1·110-24H	Particles settled and slowly dissolving; colour of solids getting lightening, water turning opaque; significant foaming after agitation	Particles settled
3·30-24H	Particles settled	Particles settled
3·50-24H	Particles settled	Particles settled
3·50-72H	Particles mostly settling, some particles floating	Particles settled
3·70-24H	Particles mostly settling, some particles floating, lightened colour of solids	Particles settled
3·70-72H	Particles mostly settling, some particles floating, lightened colour of solids	Particles settled

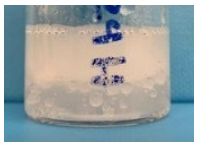
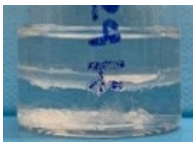
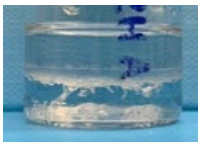
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

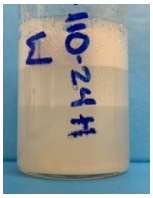
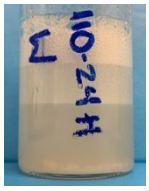
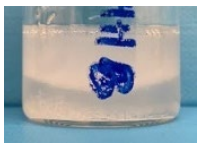
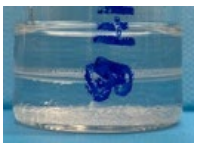
Figure 37. Modified α-1,3-glucan sample 1·110-24H in water.

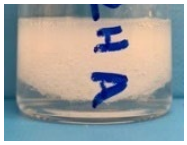
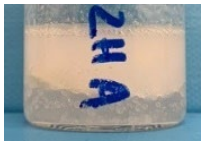
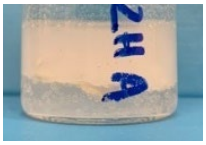
Mineral oil was then added to each sample in water, 1 g at a time, and agitated prior to observation. For simplicity, images and qualitative observations are provided in Table 25 for the mixtures with 3 g of oil added, making a 50/50 oil/water ratio. The remaining observations and images are provided in Appendix III.

There are promising interactions with the oil and water mixtures with all samples initially showing positive interactions with both phases. For many samples (1·30-24H, 3·30-24H, 3·50-24H, 3·50-72H, 3·70-24H, 3·70-72H), after a settling time of 30 minutes, solid particles remained at the interface. Additionally, droplets of oil in water were found for several samples (1·30-24H, 1·70-24H, 1·70-72H, 3·30-24H, 3·50-24H, 3·70-72H). However, the most promising sample for emulsifying ability was sample 1·110-24H as the addition of the sample to water created a stable foam on the liquid surface, and the addition of oil resulted in a phase of small droplets which remained beyond a settling time of 48 h. This can most easily be seen in Figure 38. After 48 h, the other samples all showed significant clarification of both phases, and while some solids remained suspended at the interface, the water and oil phases separated completely.

Table 25. Qualitative observations of modified α-1,3-glucan samples in 50/50 water/oil mixtures.

Sample	Observations			
	1.5 min	**5 min**	**30 min**	**48 h**
1·30-24H				
	Top: small bubbles *Middle:* opaque liquid with droplets *Bottom:* translucent liquid with large droplets and settled solids	*Top:* small bubbles *Middle:* opaque liquid *Interface:* droplets *Bottom:* on translucent liquid with large droplets and settled and suspended solids	*Top:* opaque liquid *Interface:* solids, droplets *Bottom:* translucent liquid with droplets and suspended solids	*Top:* transparent liquid *Interface:* solids *Bottom:* transparent liquid with settled solids
1·70-24H				
	Top: bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled solids		*Top:* opaque liquid *Bottom:* translucent liquid with settled solids	*Top:* transparent liquid *Bottom:* transparent liquid with settled solids
1·70-72H				
	Top: bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled and suspended solids and droplets		*Top:* opaque liquid *Interface:* solids, droplets *Bottom:* translucent liquid with suspended droplets and solids	*Top:* transparent liquid *Interface:* solids *Bottom:* transparent liquid with settled solids

Sample	Observations			
	1.5 min	**5 min**	**30 min**	**48 h**
1·110-24H				
	Top: foam *Middle:* opaque liquid composed of small droplets *Bottom:* opaque liquid with settled solids			*Top:* opaque liquid composed of small droplets *Interface:* some solids *Bottom:* translucent liquid with settled solids
3·30-24H				
	Top: bubbles *Middle:* opaque liquid with large droplets *Interface:* solids, droplets *Bottom:* translucent liquid with settled solids	*Top:* bubbles *Middle:* opaque liquid *Interface:* solids, droplets *Bottom:* translucent liquid with settled solids	*Top:* translucent liquid *Middle:* opaque liquid *Interface:* solids, droplets *Bottom:* transparent liquid with settled solids and droplets	*Top:* transparent liquid *Interface:* some solids *Bottom:* transparent liquid with settled solids
3·50-24H				
	Top: bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled solids and droplets	*Top:* bubbles *Middle:* opaque liquid *Interface:* a few solids, small droplets *Bottom:* translucent liquid with settled solids and droplets	*Top:* opaque liquid *Middle:* *Interface:* a few solids, small droplets *Bottom:* translucent liquid with settled solids and droplets	*Top:* transparent liquid *Interface:* some solids *Bottom:* transparent liquid with settled solids

Sample	Observations			
	1.5 min	**5 min**	**30 min**	**48 h**
3·50-72H				
	Top: bubbles *Middle:* opaque liquid *Interface:* solids, droplets *Bottom:* transparent liquid	*Top:* opaque liquid *Interface:* solids, droplets *Bottom:* transparent liquid		*Top:* transparent liquid *Interface:* solids *Bottom:* transparent liquid, some settled solids
3·70-24H				
	Top: opaque liquid with small droplets *Interface:* solids, small droplets *Bottom:* transparent liquid		*Top:* opaque liquid *Interface:* solids *Bottom:* transparent liquid	*Top:* transparent liquid *Interface:* solids *Bottom:* transparent liquid
3·70-72H				
	Top: bubbles *Middle:* opaque liquid *Interface:* solids *Bottom:* translucent liquid with suspended droplets	*Top:* opaque liquid *Interface:* solids *Bottom:* translucent liquid with suspended droplets		*Top:* transparent liquid, some small bubbles on vial walls *Bottom:* transparent liquid with settled solids and a few bubbles

Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

Figure 38. Modified α-1,3-glucan sample 1·110-24H in water and mineral oil.

The preliminary emulsion tests provided evidence that α-1,3-glucan modified with OSA may prove to be an effective emulsifier. However, to better determine the emulsifying abilities of the samples, it is recommended that future formal testing of water/oil emulsions: use samples which have been more finely ground to increase their available surface area to interact with the two phases, use a homogenizer to improve the dispersion of the solids in the first phase and the mixing of the two phases together, and use lower concentrations of solid samples. For example, the mixtures in Table 25 contain 0.09 g of sample in 6 g of liquid whereas the mixture in Figure 38 contains approximately 0.01 g of sample in 13 g of liquid. There remain a few settled solids at this concentration, but the clarity improves the observation process. The reduced concentration may also indicate a greater emulsifying capacity of modified α-1,3-glucan than expected.

5.6 EFFECT OF REACTION CONDITIONS

The degree of substitution was the primary quantitative value targeted from experimentation. Therefore, it was important to determine the relationship between the reaction conditions and the degree of substitution of modified α-1,3-glucan.

To determine the optimal reaction conditions for future experimentation, the weight-average molecular weight was compared against the reaction conditions of time, temperature, OSA:AGU ratio, LiCl concentration, and presence of pyridine. The theoretical molecular weight based on the degree of substitution is included with the measured values to monitor polymer degradation. The weight-average molecular weight is used for comparisons because its measurement by the light

scattering detector of the chromatograph is more accurate than the number-average molecular weight. Additionally, the weight-average molecular weight is more sensitive to changes in the molecular weight as a result of degradation.

5.6.1 EFFECT OF REACTION TIME

The degrees of substitution for α-1,3-glucan modifications performed at 50 °C and 70 °C at OSA:AGU ratios of 1:1 and 3:1 with varying reaction time are shown in Figure 39. For these reactions, one experimental setup was created for each temperature and concentration combination used (one setup for 1·70, one for 3·50, and one for 3·70), and 1 mL samples were drawn out of the reaction solution at 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 24 h, 48 h, and 72 h. The samples were precipitated into ethanol and purified with acetone before NMR analysis. Due to the small sampling size, no further analyses were performed. Additional reactions were repeated for 24 h and 72 h to check for reproducibility of the modifications. In Figure 39, different colours and shapes of the data points are used to differentiate between reaction setups. The blue squares indicate the first sampling experiment performed as described for 72 h (S1), the red circles indicate the second sampling experiment performed for 24 h (S2, only Figures 25a and 25b), and the green triangles indicate individual 24 h and 72 h reactions performed without sampling.

Considering first Figure 39a for the modification performed at 70 °C and with an OSA:AGU of 3:1, the degree of substitution overall increases with time, with the rate lowering after 48 h. However, there is an anomaly in the first sampling experiment (blue squares) where the DS drops significantly at the 5 h mark, then increases again for 24 h, 48 h, and 72 h samples. In the second sampling experiment (red circles), the DS starts higher and unexpectedly shows a slower rate of reaction in the first 4 h. Additionally, when modifications were repeated for 24 h and 72 h, the degree of substitution varied significantly between repetitions. This is discussed further in Chapter 5.8.

Similarly, in Figure 39b for the modification performed at 50 °C with an OSA:AGU of 3:1, the degree of substitution increases inconsistently with time. In the first sampling experiment (blue squares), the degree of substitution increased drastically at the 5 h mark, decreased at 24 h, increased again at 48 h, then decreased significantly at 72 h. In the second sampling experiment

(red circles), the DS increased suddenly at 2 h, decreased at 3 h, then continued increasing up to 24 h. When repeating the 24 h and 72 h experiments, the DS varied significantly at 72 h.

In Figure 39c for the modification performed at 70 °C with an OSA:AGU of 1:1, the degree of substitution increased with time, with a sudden increase between 48 h and 72 h. Due to time and resource constraints, the sampling experiment was not repeated. Individual repetitions of the 24 h and 72 h modifications showed inconsistencies between the DS of the sampling experiment and of the repeated experiments, with the individual reactions showing a lower degree of substitution.

Discrepancies were found for all of the reactions described above but the inconsistency does not appear to be a direct result of the act of sampling as the degrees of substitution from the sampling experiments are not consistently higher or lower than the individual experiments. For the samples with anomalies, the samples were re-purified and NMR analysis was performed again with no change in the calculated DS value. It may be possible that the reaction is reversible, and that the production of carboxylic acid functionalities causes cleavage of the OSA from the α-1,3-glucan, causing a significant reduction in DS for some samples. However, it is unlikely to have the drastic result observed in Figure 39. Although the sampling does not have a consistent effect on the degree of substitution, it is possible that it introduces water to the system and thus affects the reaction progression. This may account for discrepancies between the sampling and individual experiments at 24 h and 72 h but does not account for the variation in DS throughout one sampling experiment.

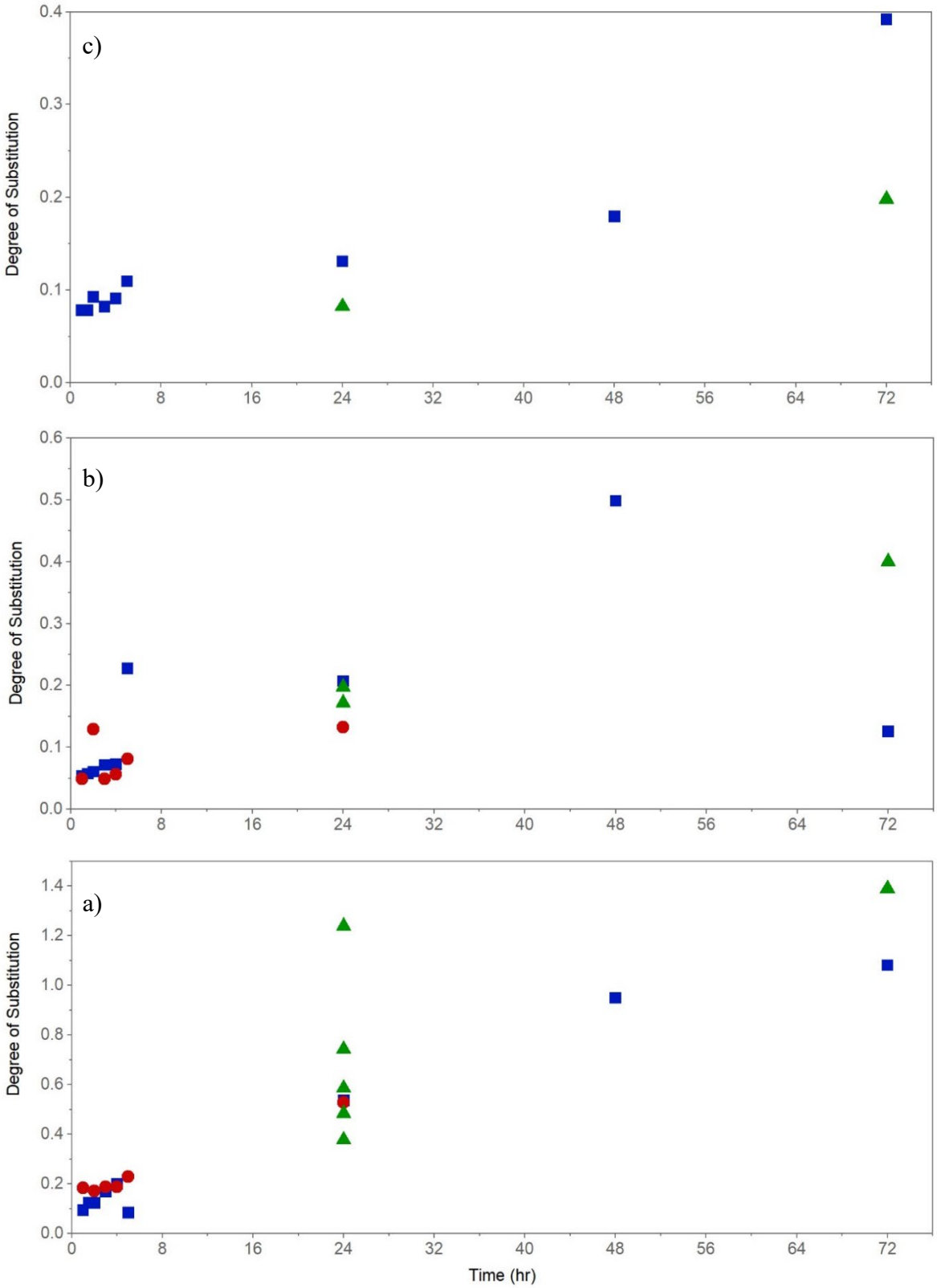


Figure 39. Degree of substitution as a function of time for modified α-1,3-glucan at **a)** OSA:AGU = 3:1, T = 70 °C, **b)** OSA:AGU = 3:1, T = 50 °C, and **c)** OSA:AGU = 1:1, T = 70 °C.

In Figure 40, modifications performed at 50 °C and 70 °C with OSA:AGU ratios of 1:1 or 3:1 for 24 h or 72 h show different correlations with the weight average molecular weight and degree of polymerization depending on the reaction conditions. For a ratio of 1:1 at a temperature of 70 °C, the molecular weight decreases with increasing reaction time. With a low ratio of OSA:AGU, the modification obtains a low DS and so has a lower expected molecular weight than more highly modified samples, but the elongated reaction time causes additional reduction of the molecular weight compared to the theoretical value. For a ratio of 3:1 at a temperature of 70 °C, the molecular weight increases with time due to the increase in degree of substitution but remains significantly below the theoretical value. Similarly, for a ratio of 3:1 at a temperature of 50 °C, the molecular weight increases with time; however, the molecular weight of these reactions at reduced temperature (50 °C) show a closer relationship with the theoretical molecular weight and a higher degree of polymerization. Therefore, increasing the reaction time increases the extent of degradation of the polysaccharide and the effect is more pronounced at elevated temperature (70 °C).

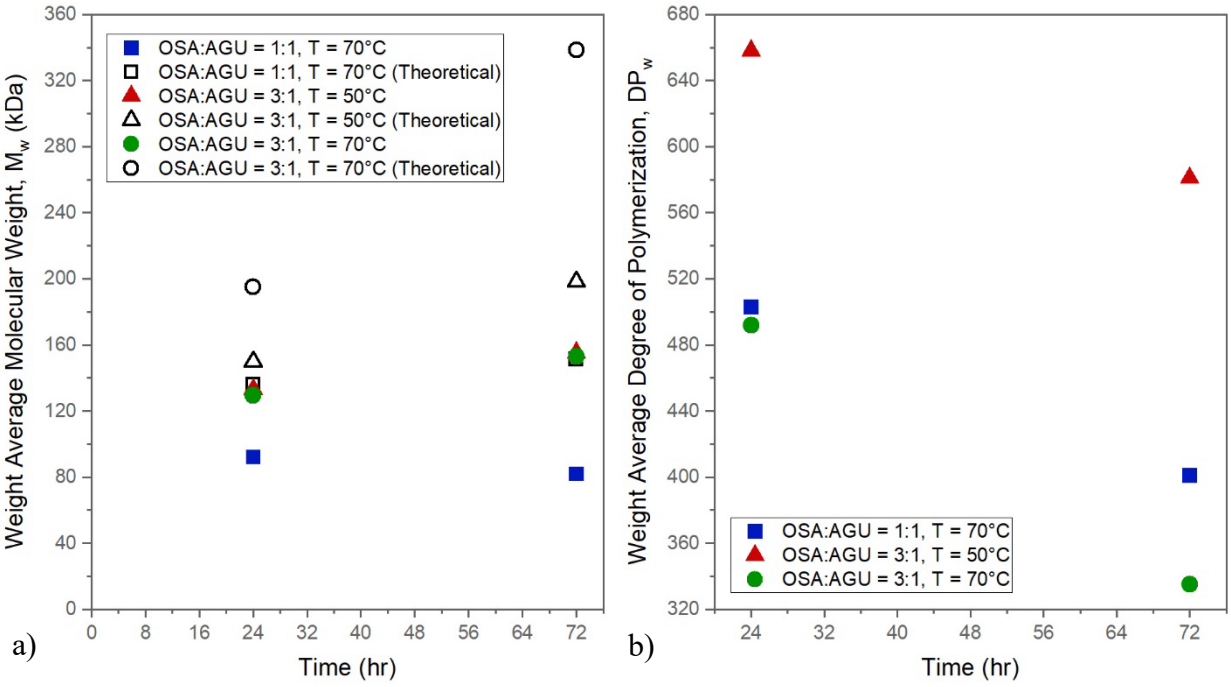


Figure 40. Correlation of **a)** weight average molecular weight (M_w) and **b)** weight average degree of polymerization (DP_w) with reaction time for the modification of α-1,3-glucan.

In Figure 41, the effect of reaction time on the sample degradation is explored. There is no clear correlation between reaction time and the inflection point: at a reaction temperature of 50 °C, the sample with a longer reaction time has a higher thermal stability (253 °C), whereas at a reaction temperature of 70 °C, the sample with a longer reaction time has a slightly lower thermal stability (260 °C) than that of the shorter time (266 °C). The TGA curve for sample 3·50-24 (OSA:AGU of 3:1, temperature of 70 °C, and time of 24 h) shows an early degradation onset at approximately 176 °C which may be a result of residual DMAc or OSA evaporating as they have boiling points of 165 °C and 168 °C, respectively.

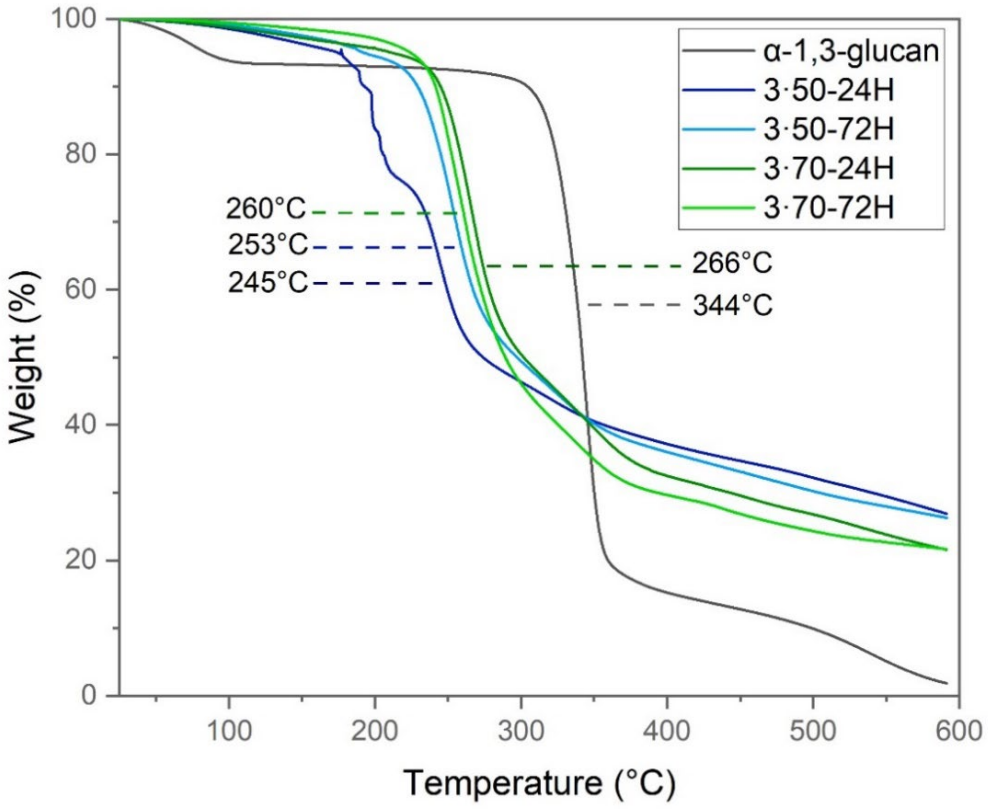


Figure 41. TGA curves of samples to show effect of reaction time on the thermal stability of α-1,3-glucan.

The effects of reaction time on the structural and thermal properties of α-1,3-glucan are summarized in Table 26.

Table 26. Effect of reaction time on degree of substitution, molecular weight distribution, and thermal stability of modified α-1,3-glucan.

Sample	DS [a]	M_n [b] (kDa)	M_w [c] (kDa)	PDI [d]	M_z [e] (kDa)	[η] [f] (mL/g)	DP_n [g]	DP_w [h]	T_{MAX} (°C)
α-1,3-glucan	*0*	*64*	*120.1*	*1.87*	*208.5*	*1.905*	*396*	*741*	*344*
1·70-24	0.10	55.6	92.1	1.66	148.3	1.490	304	503	245
1·70-72	0.20	44.3	81.9	1.85	157.3	1.167	217	401	256
3·50-24	0.19	69.0	132.9	1.93	237.6	1.981	342	658	245
3·50-72	0.50	81.5	155.2	1.90	288.8	1.888	305	581	253
3·70-24	0.48	78.3	129.3	1.65	217.6	1.682	298	492	266
3·70-72	1.4	94.9	153.0	1.70	269.4	1.383	197	335	260

[a] Degree of Substitution (from NMR)
[b] Number-Average Molecular Weight (from SEC)
[c] Weight-Average Molecular Weight (from SEC)
[d] Polydispersity Index (M_w/M_n)
[e] Third Moment Molecular Weight (from SEC)
[f] Intrinsic viscosity (from SEC)
[g] Number-Average Degree of Polymerization
[h] Weight-Average Degree of Polymerization
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

5.6.2 EFFECT OF REACTION TEMPERATURE

Modifications performed for 24 h at an OSA:AGU of 1:1 and 3:1 and varying temperature are compared in Figure 42. The degree of substitution has a positive correlation with reaction temperature up to a temperature of 110 °C.

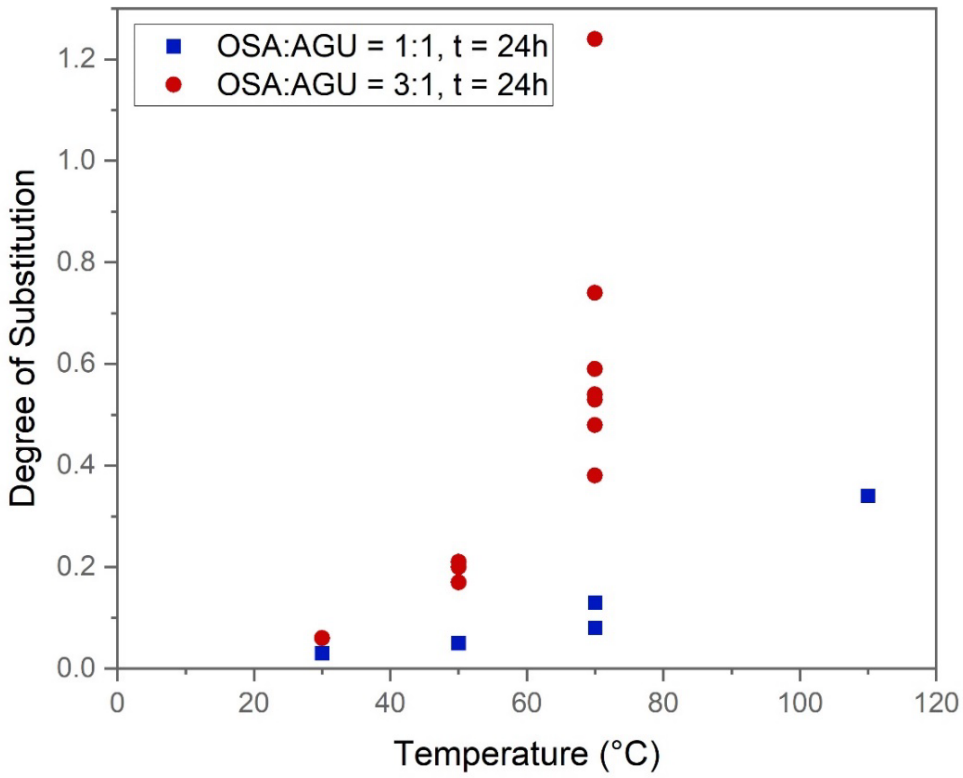


Figure 42. Degree of substitution as a function of temperature at varying ratios of octenylsuccinic anhydride to anhydroglucose units.

The relationship between molecular weight and reaction temperature is explored in Figure 43. The molecular weight and degree of polymerization decrease with increasing reaction temperature. The effect is similar for OSA:AGU ratios of 1:1 and 3:1. At a reaction temperature of 30 °C, the degree of polymerization is higher than the degree of polymerization of unmodified α-1,3-glucan. This may be due to experimental uncertainty in the degree of substitution and molecular weight distribution. Due to the lack of high molar mass tails on the molecular weight distributions of these samples in Figure 44, it is unlikely that long-chain branching or crosslinking has occurred at 30 °C.

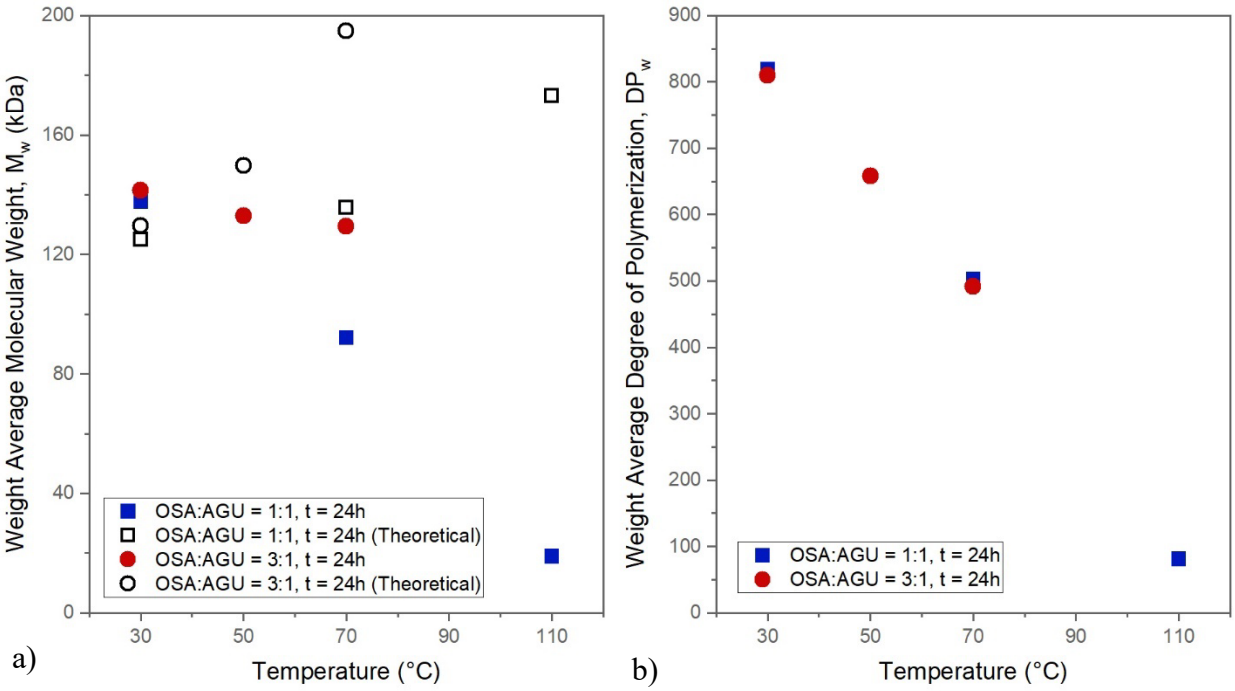


Figure 43. Correlation of **a)** weight average molecular weight (M_w) and **b)** weight average degree of polymerization (DP_w) with reaction temperature for the modification of α-1,3-glucan.

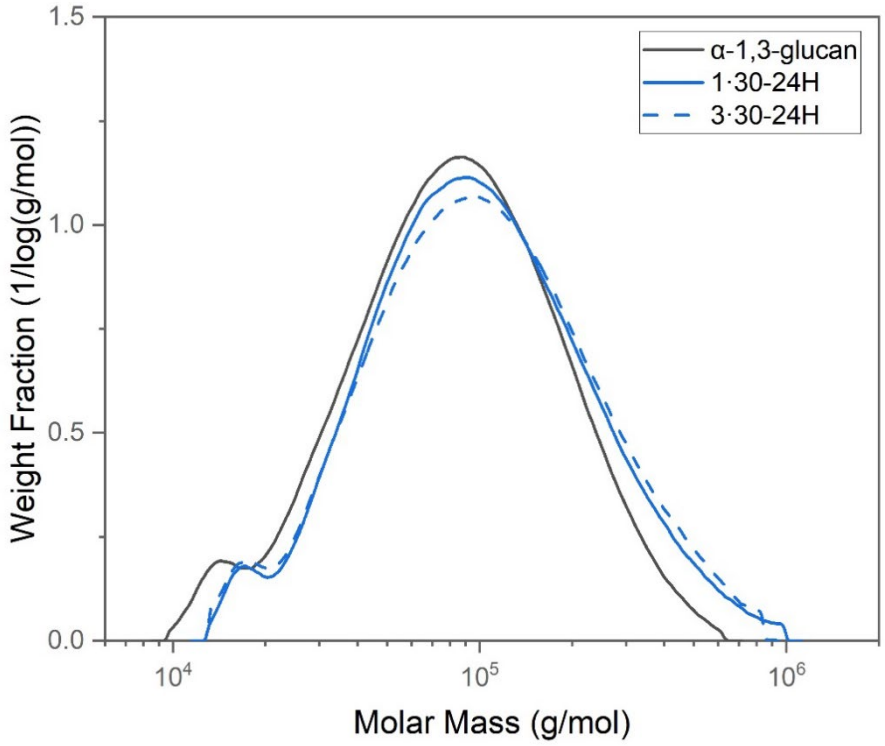


Figure 44. Molecular weight distribution traces for α-1,3-glucan modified at 30 °C.

The relationship between reaction temperature and thermal stability is shown in Figure 45 and Figure 46 for OSA:AGU ratios of 3:1 and 1:1, respectively. Decreasing the reaction temperature from 70 °C to 50 °C at an OSA:AGU ratio of 3:1 causes a reduction in the thermal stability, while further decreasing the temperature from 50 °C to 30 °C correlates with increased thermal stability. This may be due to reduced hydrolysis at lower reaction temperatures, thus creating a product that maintained a higher molecular weight which increases its degradation temperature. At a molar OSA:AGU ratio of 1:1, decreasing or increasing the reaction temperature from 50 °C reduces the thermal stability of the product. Of note, the modification at 110 °C shows a marked mass loss starting around 120 °C.

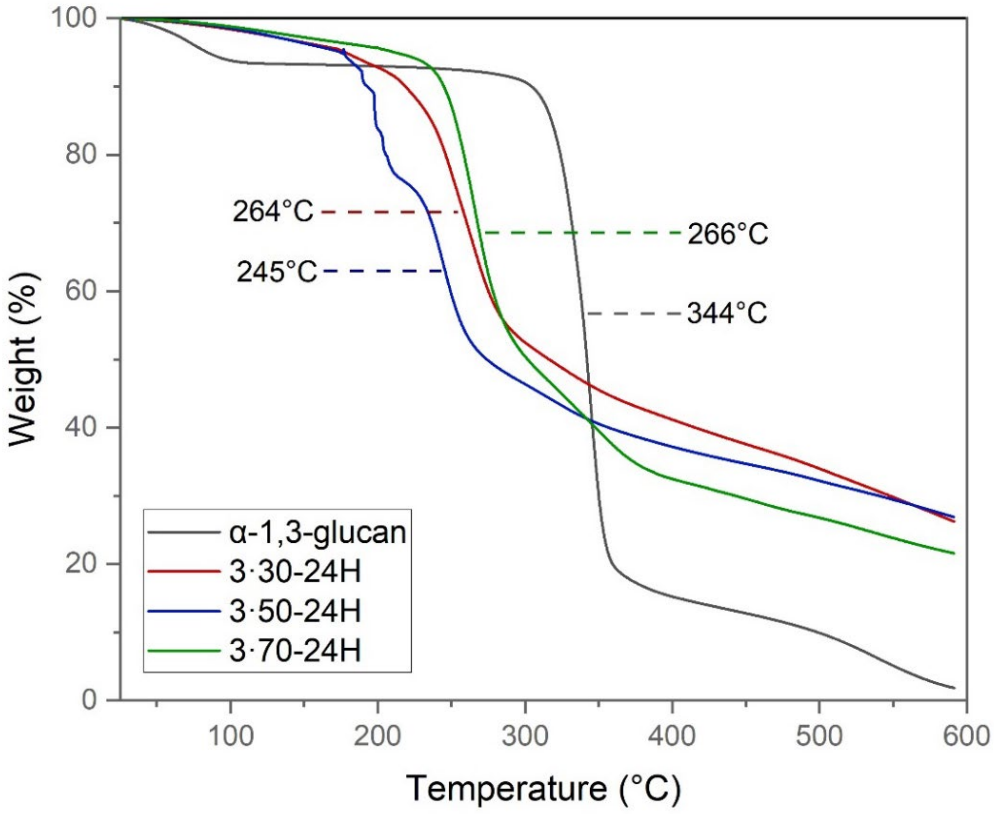


Figure 45. TGA curves of samples to show effect of reaction temperature on the thermal stability of α-1,3-glucan modified with an OSA:AGU of 3:1.

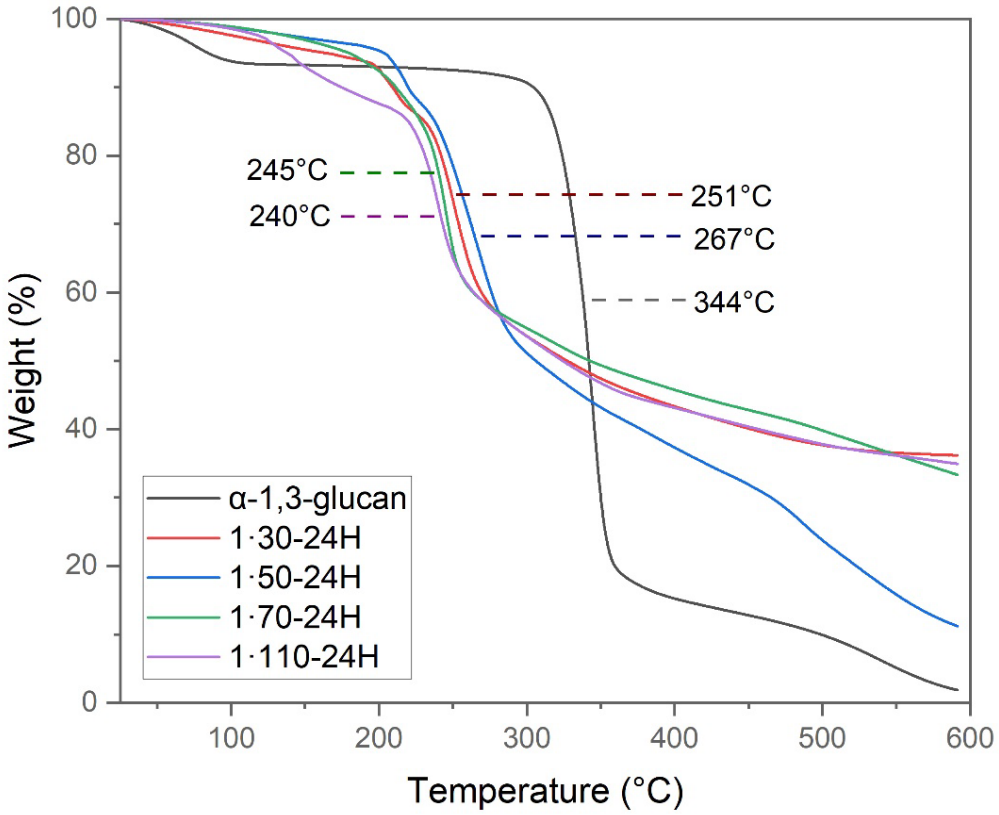


Figure 46. TGA curves of samples to show effect of reaction temperature on the thermal stability of α-1,3-glucan modified with an OSA:AGU of 1:1.

The effects of reaction temperature on the structural and thermal properties of α-1,3-glucan are summarized in Table 27. Of note, the significant reductions in weight-average molecular weight, third moment molecular weight, intrinsic viscosity, and polydispersity of the sample modified at 110 °C (1·110-24H) all indicate degradation of the polysaccharide backbone.

Table 27. Effect of reaction temperature on degree of substitution, molecular weight distribution, and thermal stability of modified α-1,3-glucan.

Sample	DS [a]	M_n [b] (kDa)	M_w [c] (kDa)	PDI [d]	M_z [e] (kDa)	[η] [f] (mL/g)	DP_n [g]	DP_w [h]	T_{MAX} (°C)
α-1,3-glucan	*0*	*64.2*	*120.1*	*1.87*	*208.5*	*1.905*	*396*	*741*	*344*
1·30-24	0.03	71.8	137.8	1.92	259.4	2.115	427	819	251
1·70-24	0.10	55.6	92.1	1.66	148.3	1.490	304	503	245
1·110-24	0.34	15.6	18.9	1.21	22.6	0.295	67	81	240
3·30-24	0.06	71.7	141.4	1.97	259.9	2.172	411	810	264
3·50-24	0.19	69.0	132.9	1.93	237.6	1.981	342	658	245
3·70-24	0.48	78.3	129.3	1.65	217.6	1.682	298	492	266

[a] *Degree of Substitution (from NMR)*
[b] *Number-Average Molecular Weight (from SEC)*
[c] *Weight-Average Molecular Weight (from SEC)*
[d] *Polydispersity Index (M_w/M_n)*
[e] *Third Moment Molecular Weight (from SEC)*
[f] *Intrinsic viscosity (from SEC)*
[g] *Number-Average Degree of Polymerization*
[h] *Weight-Average Degree of Polymerization*
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

5.6.3 EFFECT OF OSA:AGU

Modifications performed for 24 h at reaction temperatures of 30 °C, 50 °C, and 70 °C and various ratios of octenylsuccinic anhydride to anhydroglucose units are compared in Figure 47. The degree of substitution increases with increasing OSA:AGU ratio. The DS experiences a greater increase from increasing the temperature from 50 °C to 70 °C than from increasing the OSA:AGU from 3:1 to 6:1. However, it is important to note the physical characteristics of the reaction solution for sample 6·50-24: Due to the high concentration of OSA required to achieve this ratio, the OSA did not completely dissolve within the 24 h reaction and also appears to have caused the α-1,3-glucan to precipitate out of the solution. At the completion of the 24 h reaction, there was a transparent gel-like precipitate in the reaction mixture. This precipitate was isolated and purified separately. It was expected that due to the precipitation, it would have a significantly lower degree of substitution, but when analyzed by NMR showed a similar degree of substitution to the remaining solution (DS of 0.29 versus 0.36 for the solution). Despite having similar degrees of substitution, it is possible that the poor solubility of both the polysaccharide and the OSA contributed to poor

availability of their functional groups for a reaction to occur. Therefore, the effect of OSA concentration above an OSA:AGU ratio of 3:1 is difficult to ascertain from the available data.

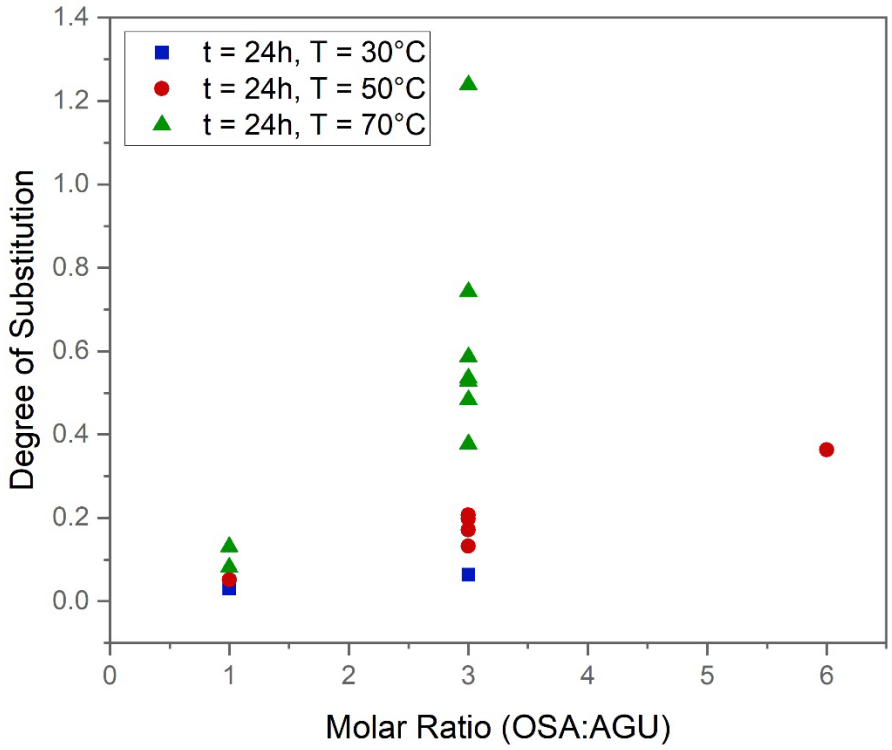


Figure 47. Degree of substitution as a function of the molar ratio of octenylsuccinic anhydride to anhydroglucose units at varying temperatures.

In Figure 48, the weight average molecular weight and weight average degree of polymerization are compared with the ratio of OSA:AGU for modifications performed at 30 °C and 70 °C. There is an increase in the molecular weight with increasing OSA concentration. This increase is caused by an increased extent of modification which increases the molecular weight of each repeat unit; however, increasing the OSA concentration correlates with a minor decrease in the degree of polymerization with less than 12 repeat units lost at each temperature. Therefore, at 30 °C and 70 °C, the ratio of OSA:AGU has an insignificant effect on the extent of polysaccharide degradation.

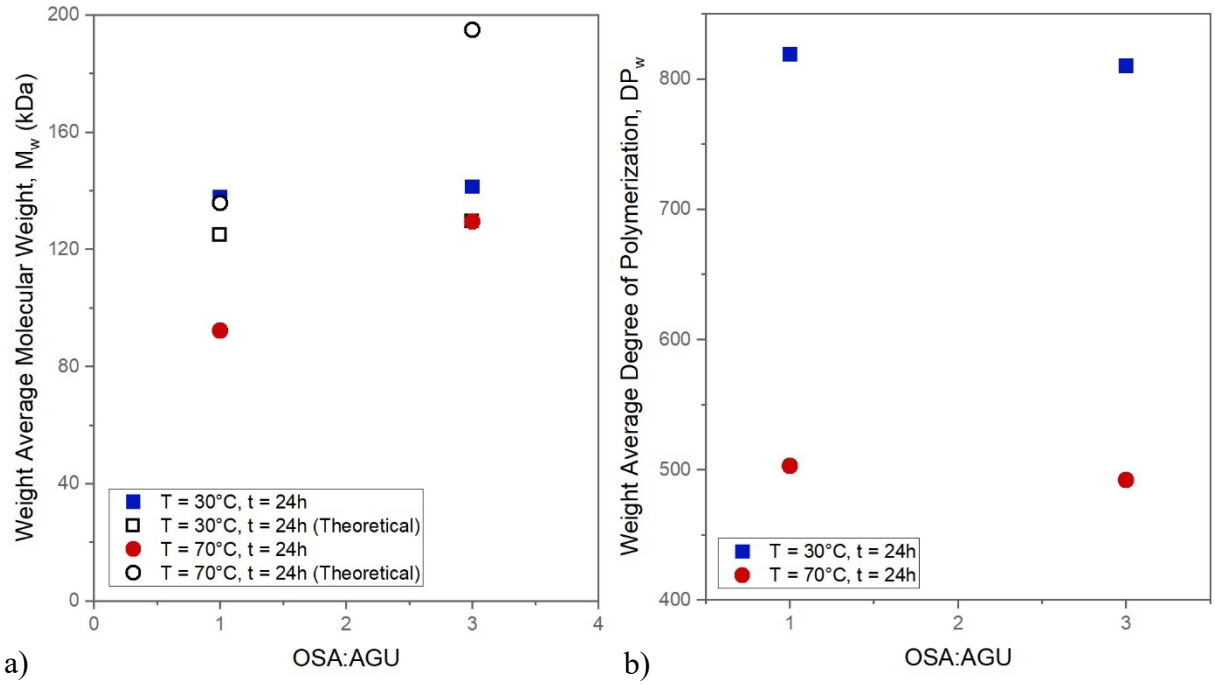


Figure 48. Comparison of **a)** weight average molecular weight (M_w) and **b)** weight average degree of polymerization (DP_w) with OSA concentration for the modification of α-1,3-glucan.

In Figure 49, the TGA curves for samples modified at 50 °C and 70 °C are compared to explore the effect of OSA:AGU ratio on the thermal stability of the final product. There is no clear relationship as the samples with a ratio of 1:1 at 50 °C and 3:1 at 70 °C have the highest degradation temperatures.

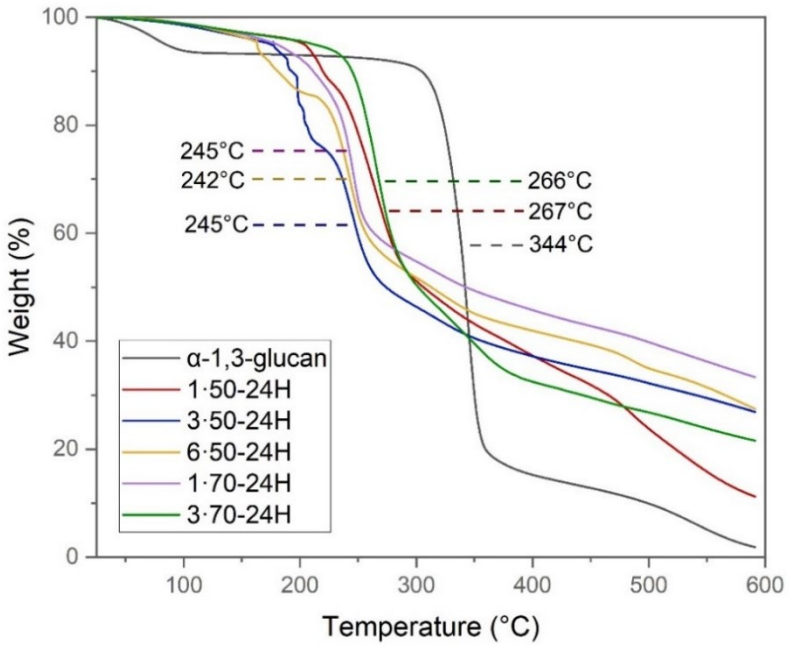


Figure 49. TGA curves of samples to show effect of OSA concentration on the thermal stability of α-1,3-glucan.

The effects of OSA:AGU molar ratio on the structural and thermal properties of α-1,3-glucan are summarized in Table 28.

Table 28. Effect of OSA:AGU molar ratio on degree of substitution, molecular weight distribution, and thermal stability of modified α-1,3-glucan.

Sample	DS [a]	M_n [b] (kDa)	M_w [c] (kDa)	PDI [d]	M_z [e] (kDa)	[η] [f] (mL/g)	DP_n [g]	DP_w [h]	T_{MAX} (°C)
α-1,3-glucan	*0*	*64.2*	*120.1*	*1.87*	*208.5*	*1.905*	*396*	*741*	*344*
1·30-24	0.03	71.8	137.8	1.92	259.4	2.115	427	819	251
3·30-24	0.06	71.7	141.4	1.97	259.9	2.172	411	810	264
1·70-24	0.10	55.6	92.1	1.66	148.3	1.490	304	503	245
3·70-24	0.48	78.3	129.3	1.65	217.6	1.682	298	492	266

[a] *Degree of Substitution (from NMR)*
[b] *Number-Average Molecular Weight (from SEC)*
[c] *Weight-Average Molecular Weight (from SEC)*
[d] *Polydispersity Index (M_w/M_n)*
[e] *Third Moment Molecular Weight (from SEC)*
[f] *Intrinsic viscosity (from SEC)*
[g] *Number-Average Degree of Polymerization*
[h] *Weight-Average Degree of Polymerization*
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

5.6.4 EFFECT OF PYRIDINE

Modifications of α-1,3-glucan were performed with pyridine to catalyze the reaction as well as to prevent degradation of the polysaccharide backbone. Pyridine was added to the reaction system for 24 h modifications performed with an OSA:AGU ratio of 1:1 and temperature of 70 °C or a ratio of 3:1 and a temperature of 50 °C. As can be observed in Figure 50, pyridine has a positive effect on the degree of substitution.

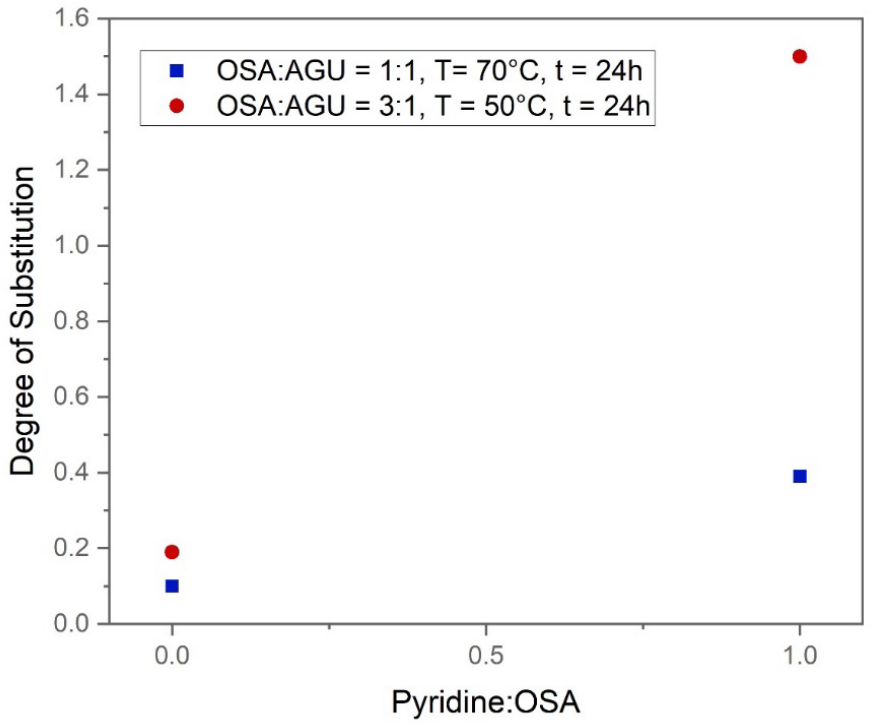


Figure 50. Degree of substitution correlated with the ratio of pyridine to octenylsuccinic anhydride.

As shown in Figure 51, the molecular weight is positively correlated with the addition of pyridine for both reactions. However, at an OSA:AGU ratio of 3:1 and a temperature of 50 °C the increase in molecular weight is primarily caused by the increased degree of substitution noted in Figure 50. The addition of pyridine in these conditions causes significant hydrolysis of the polysaccharide backbone evidenced by the reduction in calculated degree of polymerization in Figure 51. At a low OSA:AGU ratio of 1:1 and a temperature of 70 °C, pyridine has a positive effect on the degree of polymerization, providing evidence that the addition of pyridine reduced polysaccharide degradation in these reaction conditions. It is possible that at lower temperatures and higher

OSA:AGU ratios, pyridine catalyzes a competing reaction which produces carboxylic acid functionalities and results in the hydrolysis of the polysaccharide. The addition of pyridine should be explored at additional reaction conditions to separate the effects of reaction temperature and OSA:AGU ratio.

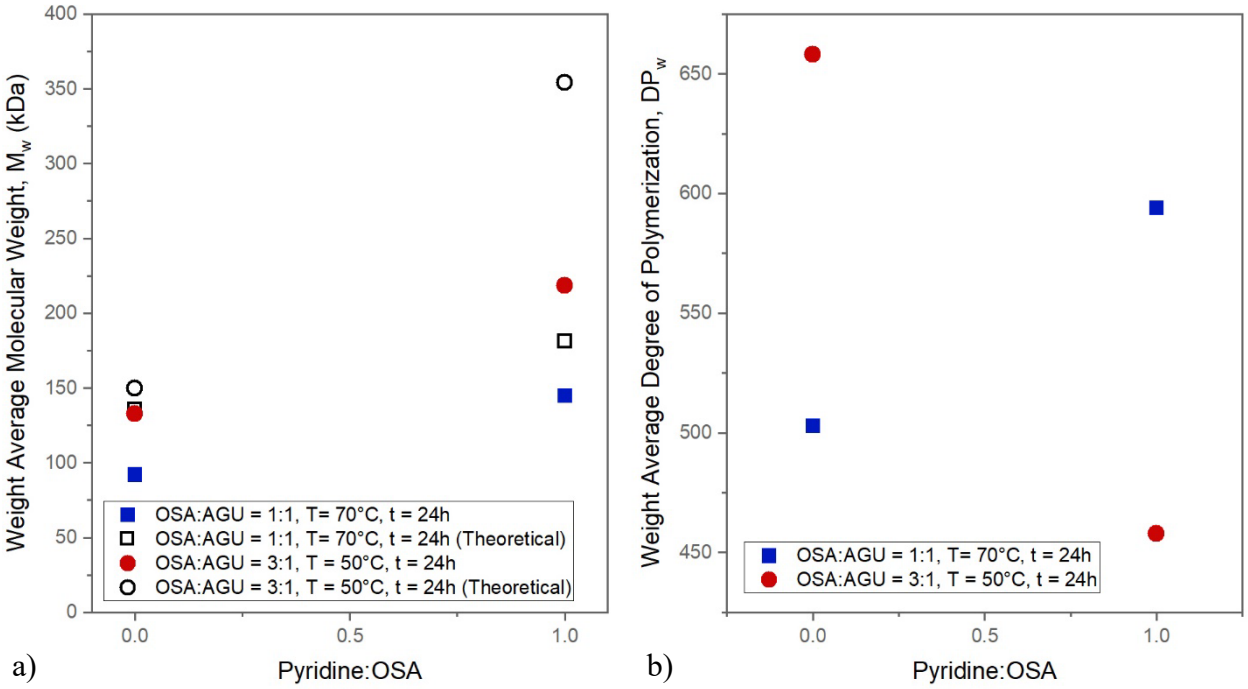


Figure 51. Comparison of **a)** weight average molecular weight (M_w) and **b)** weight average degree of polymerization (DP_w) for the modification of α-1,3-glucan with and without pyridine.

As shown in Figure 52, the addition of pyridine to the reaction system at the selected reaction conditions (OSA:AGU ratio of 1:1 and temperature of 70 °C; OSA:AGU ratio of 3:1 and temperature of 50 °C) improves the thermal stability of the modified product by approximately 20 °C. Therefore, pyridine has increased the degree of substitution, reduced the extent of degradation at an OSA:AGU ratio of 1:1 and a temperature of 70 °C, and improved the thermal stability of α-1,3-glucan modified with octenylsuccinic anhydride.

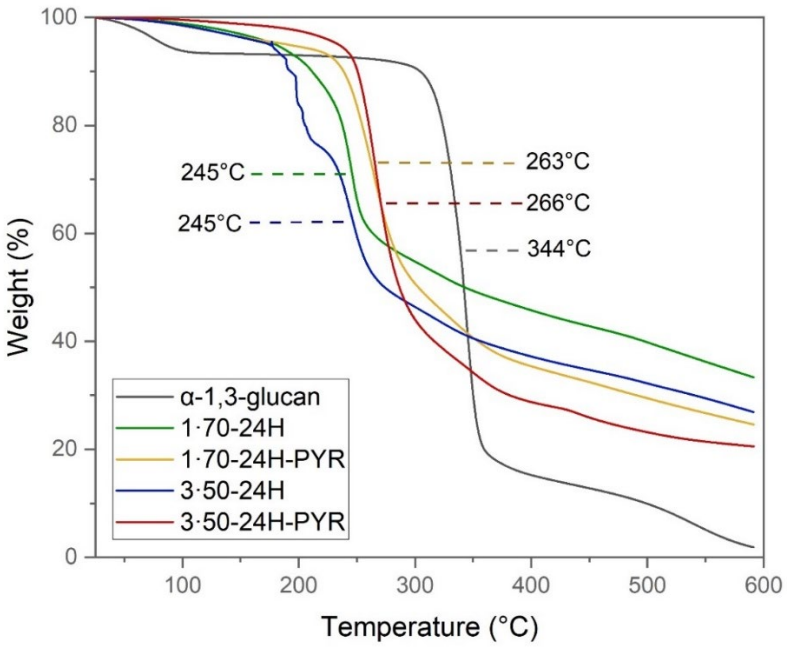


Figure 52. TGA curves of samples to show effect of pyridine on the thermal stability of α-1,3-glucan.

The effects of reaction time on the structural and thermal properties of α-1,3-glucan are summarized in Table 29.

Table 29. Effect of pyridine on degree of substitution, molecular weight distribution, and thermal stability of modified α-1,3-glucan.

Sample	DS [a]	M_n [b] (kDa)	M_w [c] (kDa)	PDI [d]	M_z [e] (kDa)	[η] [f] (mL/g)	DP_n [g]	DP_w [h]	T_{MAX} (°C)
α-1,3-glucan	*0*	*64.2*	*120.1*	*1.87*	*208.5*	*1.905*	*396*	*741*	*344*
1·70-24	0.10	55.6	92.1	1.66	148.3	1.490	304	503	245
1·70-24-PYR	0.39	78.7	144.9	1.84	278.3	1.808	323	594	263
3·50-24	0.19	69.0	132.9	1.93	237.6	1.981	342	658	245
3·50-24-PYR	1.5	120.8	218.5	1.81	405.1	1.724	253	458	266

[a] *Degree of Substitution (from NMR)*
[b] *Number-Average Molecular Weight (from SEC)*
[c] *Weight-Average Molecular Weight (from SEC)*
[d] *Polydispersity Index (M_w/M_n)*
[e] *Third Moment Molecular Weight (from SEC)*
[f] *Intrinsic viscosity (from SEC)*
[g] *Number-Average Degree of Polymerization*
[h] *Weight-Average Degree of Polymerization*
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

When α-1,3-glucan reacts with octenylsuccinic anhydride, the hydroxyl oxygen from the polysaccharide attacks the carbonyl carbon, creating an intermediate with a positively charged oxygen in the ester functionality shown in Figure 53. This is unstable and may push the esterification to reverse before the carboxylate group takes the hydrogen.

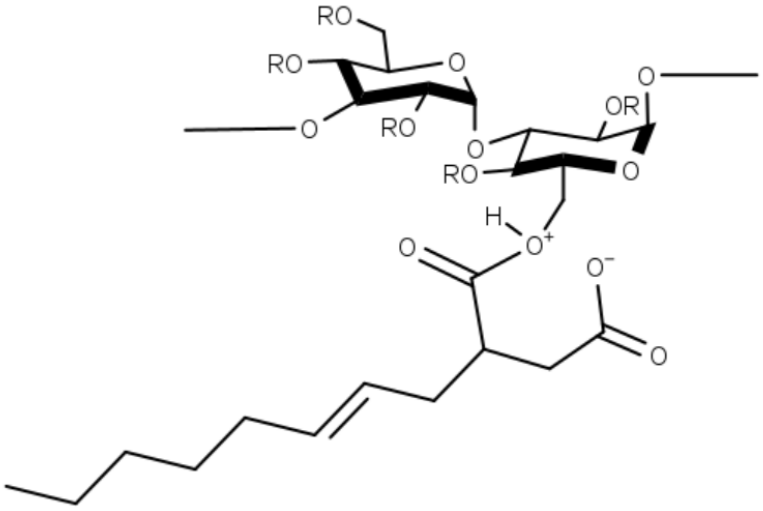


Figure 53. Reaction intermediate for the esterification of α-1,3-glucan.

Pyridine is added to quickly deprotonate the ester oxygen creating a positively charged species which ionically interacts with the carboxylate functionality as seen in Figure 54. This pushes the esterification forward while removing carboxylic acid functionalities in the solution which could lead to degradation of the polysaccharide backbone. This was found to be successful in this project with the addition of pyridine improving the degree of substitution and molecular weight for α-1,3-glucan samples modified at select reaction conditions (Figure 50, Figure 51). However, pyridine is flammable, toxic, and potentially carcinogenic and is therefore not a favourable chemical to use [93].

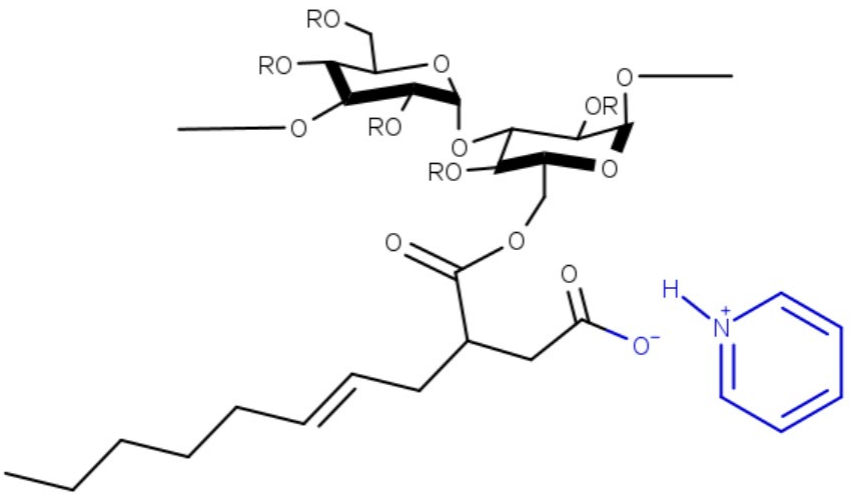


Figure 54. Modified α-1,3-glucan with pyridinium and carboxylate functionalities.

5.6.5 EFFECT OF LITHIUM CHLORIDE CONCENTRATION

Initially, all experiments were performed in a solvent system composed of 7.3% LiCl by weight in DMAc. When the behaviour of the neat polysaccharide in the solvent changed, the solvent system was changed to 5% LiCl in DMAc. This caused an increase in the degree of substitution, and so an additional data point at 2.5% LiCl in DMAc was obtained to explore the effect of LiCl on the modification of α-1,3-glucan with octenylsuccinic anhydride. As evident in Figure 55, increasing the concentration of LiCl from 2.5% to 7.3% causes a significant reduction in the degree of substitution from 0.84 to 0.35.

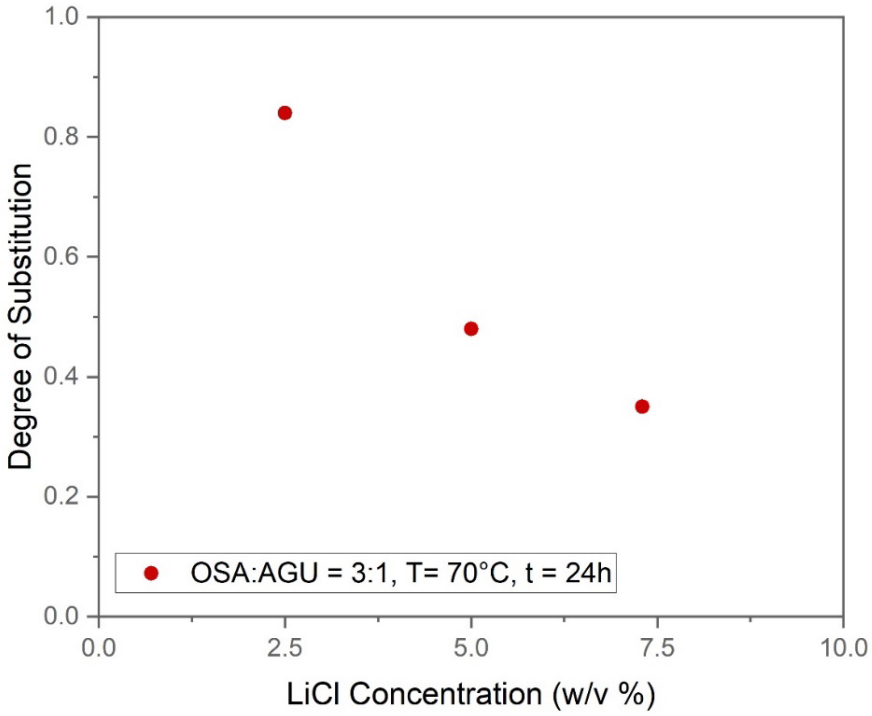


Figure 55. Degree of substitution correlated with the concentration of lithium chloride used in the solvent system.

The molecular weight is compared against LiCl concentration in the reaction solution in Figure 56 for the modification performed at 70 °C for 24 h with an OSA:AGU of 3:1 (3·70-24H). Increasing the amount of lithium chloride in the solvent system correlates with a higher degree of polymerization indicating a reduction in the extent of chain degradation. The molecular weight distribution for this set of reactions is provided in Figure 57. The distributions show a shift of the peak to lower molar mass with increasing LiCl concentration. For the sample prepared in 7.3% LiCl/DMAc, the peak molar mass is lower than for the other samples, but the distribution shows a high molar mass tail. This tail is reflected in the increased polydispersity of 2.11 in Table 30 and may indicate some crosslinking. Crosslinking is a possibility due to the bifunctional nature of octenylsuccinic anhydride.

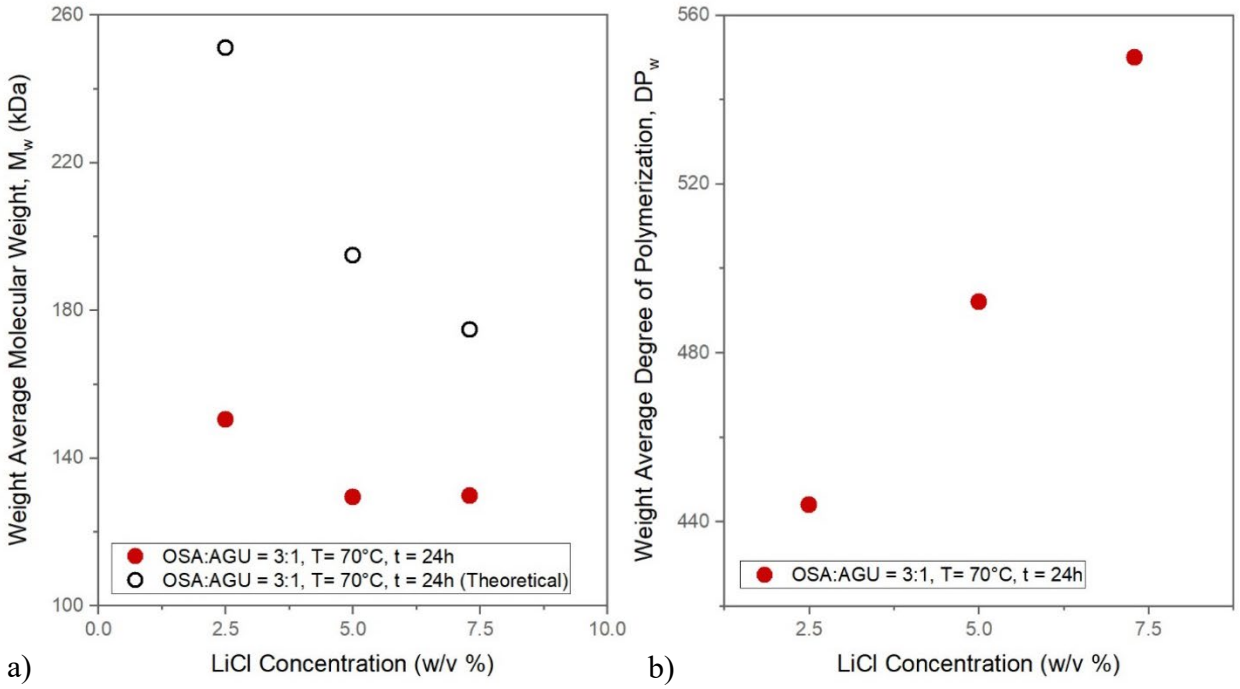


Figure 56. Correlation of **a)** weight average molecular weight (M_w) and **b)** weight average degree of polymerization (DP_w) with LiCl concentration for the modification of α-1,3-glucan.

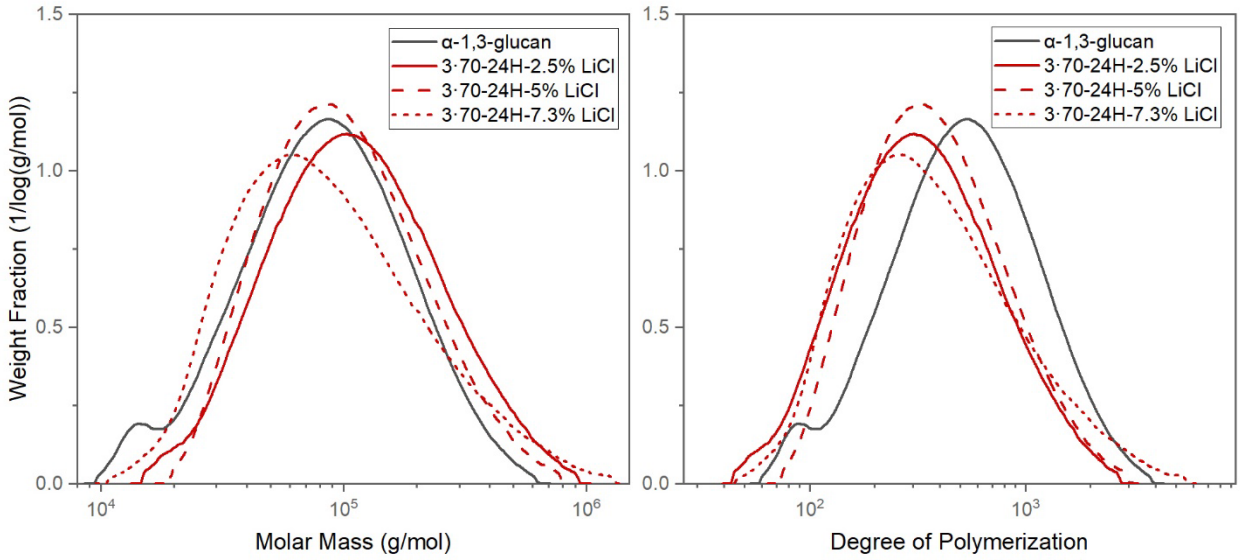


Figure 57. Molecular weight distribution traces for modified α-1,3-glucan to explore the effect of LiCl concentration.

The concentration of lithium chloride appears to have minimal effect on the thermal stability of modified α-1,3-glucan as observable in Figure 58. The sample modified in 2.5% LiCl/DMAc

undergoes a sudden weight loss at 162 °C. Due to the steep slope, it is suspected that the weight change may be a result of evaporation of residual DMAc or OSA or loss of the sample off of the pan during analysis. There is a second weight loss with a point of inflection at 244 °C which is expected to be closer to the true degradation temperature of the sample. The sample should be measured by TGA again to verify its true thermal stability.

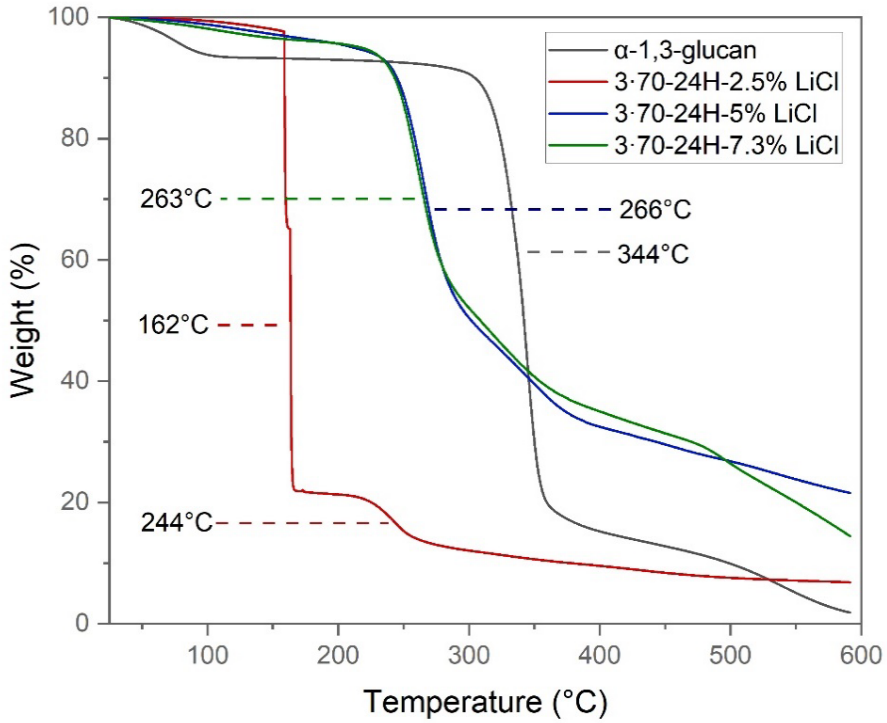


Figure 58. TGA curves of samples to show effect of LiCl concentration on the thermal stability of α-1,3-glucan.

The effects of reaction time on the structural and thermal properties of α-1,3-glucan are summarized in Table 30.

Table 30. Effect of lithium chloride concentration on degree of substitution, molecular weight distribution, and thermal stability of modified α-1,3-glucan.

Sample	DS [a]	M_n [b] (kDa)	M_w [c] (kDa)	PDI [d]	M_z [e] (kDa)	[η] [f] (mL/g)	DP_n [g]	DP_w [h]	T_{MAX} (°C)
α-1,3-glucan	*0*	*64.2*	*120.1*	*1.87*	*208.5*	*1.905*	*396*	*741*	*344*
3·70-24H-2.5% LiCl	0.84	82.4	150.3	1.82	263.6	1.616	243	444	162
3·70-24-5% LiCl	0.48	78.3	129.3	1.65	217.6	1.682	298	492	266
3·70-24H-7.3% LiCl	0.35	61.5	129.7	2.11	298.1	1.407	261	550	263

[a] *Degree of Substitution (from NMR)*
[b] *Number-Average Molecular Weight (from SEC)*
[c] *Weight-Average Molecular Weight (from SEC)*
[d] *Polydispersity Index (M_w/M_n)*
[e] *Third Moment Molecular Weight (from SEC)*
[f] *Intrinsic viscosity (from SEC)*
[g] *Number-Average Degree of Polymerization*
[h] *Weight-Average Degree of Polymerization*
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

5.6.6 OPTIMAL REACTION CONDITIONS

Based on NMR and SEC results, chemical modifications performed at 50 °C for 24 h with an OSA:AGU ratio of 3:1 showed the optimal combination of degree of substitution and molecular weight. Further adjustments to the reaction conditions including increasing the reaction time at low temperature and adding pyridine may cause further improvements in these values to increase the degree of substitution without increasing the extent of chain degradation. If 2.5% LiCl/DMAc is used as the reaction solvent, it may be possible to increase the concentrations of both α-1,3-glucan and octenylsuccinic anhydride. This could initiate an interesting investigation into the effect of absolute concentration on the degree of substitution, molecular weight, and thermal properties of esterified α-1,3-glucan.

Significant variation in degrees of substitution were obtained for repeated samples as observed in Figure 42 and Figure 47. This is discussed further in Chapter 5.8. Due to the uncertainty associated with the degrees of substitution, and therefore associated with the calculated degrees of polymerization, the trends observed between the reaction conditions and the structure of modified

α-1,3-glucan may be unreliable. Therefore, the reproducibility of the esterification must be improved prior to continuing the optimization of the reaction conditions to obtain the desired properties for the target application.

5.7 DETERMINATION OF ACTIVATION ENERGY

The sampling experiments described in Chapter 5.6.1 for experiments undertaken at a molar ratio of OSA:AGU of 3:1 and temperatures of 50 °C and 70 °C were used to explore the reaction kinetics of the esterification of α-1,3-glucan with octenylsuccinic anhydride.

The degrees of substitution and conversion of OSA for these experiments are summarized in Table 31. The conversion of OSA was calculated by Equation 5 using the initial concentration of OSA and the degree of substitution. The derivation of this equation is included in Appendix II.

$$X = \frac{DS \times [AGU]_0}{[OSA]_0} \times 100\% \qquad (Equation\ 5)$$

The conversion of OSA is low, leading to poor reaction efficiency. Furthermore, low degrees of substitution measured by NMR are more sensitive to experimental uncertainty as a result of the manual selection of boundaries for peak integration. Therefore, at short reaction times, small variations in degree of substitution are expected. However, there exist outlier data points as described in Chapter 5.6.1. These points are distinguished in Table 31 and were omitted from subsequent reaction rate analysis. The data for the duplicate sampling experiments are included to show the discrepancies in reaction progression between experiments. All sampling experiments were used for reaction kinetics analyses to establish a range of potential activation energies for the esterification.

Table 31. Degrees of substitution and OSA conversion for sampling experiments at 50 °C and 70 °C.

3·50 S1 [a]			3·70 S1 [a]		
Time (h)	**DS [b]**	**X [c] (%)**	**Time (h)**	**DS [b]**	**X [c] (%)**
1	0.054	1.80	1	0.095	3.18
1.5	0.058	1.92	1.5	0.124	4.14
2	0.061	2.03	2	0.124	4.13
3	0.072	2.39	3	0.169	5.64
4	0.073	2.43	4	0.200	6.66
5	0.227 [d]	7.58 [d]	5	0.085 [d]	2.82 [d]
24	0.207	6.91	24	0.536	17.9
48	0.498	16.61	48	0.950	31.7
72	0.126	4.19	72	1.081	36.1
3·50 S2 [a]			**3·70 S2 [a]**		
Time (h)	**DS [b]**	**X [c] (%)**	**Time (h)**	**DS [b]**	**X [c] (%)**
1	0.049	1.64	1	0.183	6.11
2	0.129 [d]	4.30 [d]	2	0.171	5.69
3	0.049	1.63	3	0.188	6.26
4	0.056	1.88	4	0.189	6.30
5	0.082	2.72	5	0.229	7.64
24	0.132	4.42	24	0.527	17.6

[a] *S denotes a sampling experiment*
[b] *Degree of Substitution (from NMR)*
[c] *Conversion of OSA*
[d] *Outliers which did not follow reaction trends (see Chapter 5.6.1)*

Using the degree of substitution, the concentration of OSA at each sampling time was determined via Equation 6. The calculation requires the assumption that OSA is only consumed by reaction with α-1,3-glucan. The validity of this assumption is discussed in Chapter 5.9.

$$[OSA] = [OSA]_0 - DS \times [AGU]_0 \qquad (Equation\ 6)$$

The order of a reaction can be determined by finding the best fit for the relationship between OSA concentration and time.

For a rate law of the form in Equation 7 where k is the rate constant, [] denotes concentrations of OSA and hydroxyl groups (−OH), and *m* and *n* indicate the reaction order of OSA and hydroxyl groups, respectively, the simplest relationship involves a reaction that is zeroth or first order in OSA and zeroth order in hydroxyl groups (*n = 0*).

$$rate = k[OSA]^m[-OH]^n \qquad (Equation\ 7)$$

The integrated form of the rate laws for the zeroth and first order reactions are provided in Equation 8 and 9, respectively, where t denotes the reaction time.

$$[OSA] = -k_0 t + [OSA]_0 \qquad (Equation\ 8)$$

$$\ln[OSA] = -k_1 t + \ln[OSA]_0 \qquad (Equation\ 9)$$

It is also possible that the rate law is first order in both OSA and the hydroxyl groups. Since the molar ratio of OSA:AGU input to the reaction system is 3:1, the molar ratio of OSA:−OH is 1:1. Therefore, the initial concentrations of OSA and hydroxyl groups are equivalent, they are consumed at the same rate, and the rate law thus reduces to the law for a reaction of second order in one reagent (*m = 2*). The integrated form of the second order rate law is provided in Equation 10.

$$\frac{1}{[OSA]} = k_2 t + \frac{1}{[OSA]_0} \qquad (Equation\ 10)$$

The integrated rate laws are plotted in Figure 59. If a rate law is appropriate for the reaction, the plot should show a linear relationship. The equations of linear fit and associated R^2 values are provided in Table 32. R^2 is not a sufficient indicator of fit for these plots. While the R^2 values are above 0.90, the plots in Figure 59 do not show linear relationships. Non-linear relationships are most obvious for the sample 3·70 S1. The non-linearity indicates that the exponents of the rate law equation may not be integers. This may be a result of side reactions occurring in the system. Further, the curvature of the non-linearity progresses in the opposite direction for the sample 3·50. Therefore, the selectivity of the competing reactions likely varies with temperature. Alternatively, due to the bulky nature of the OSA substituent and the difference in environments of the hydroxyl groups, it is likely that the esterification proceeds at different rates at each functional group of the

AGU. If the reaction occurs preferentially at one functional group, once the concentration of that functional group is reduced, the reaction rate will reflect the altered reactivity of the polysaccharide towards OSA. To further explore this possibility, detailed NMR analysis of the samples without TFAA-d is required.

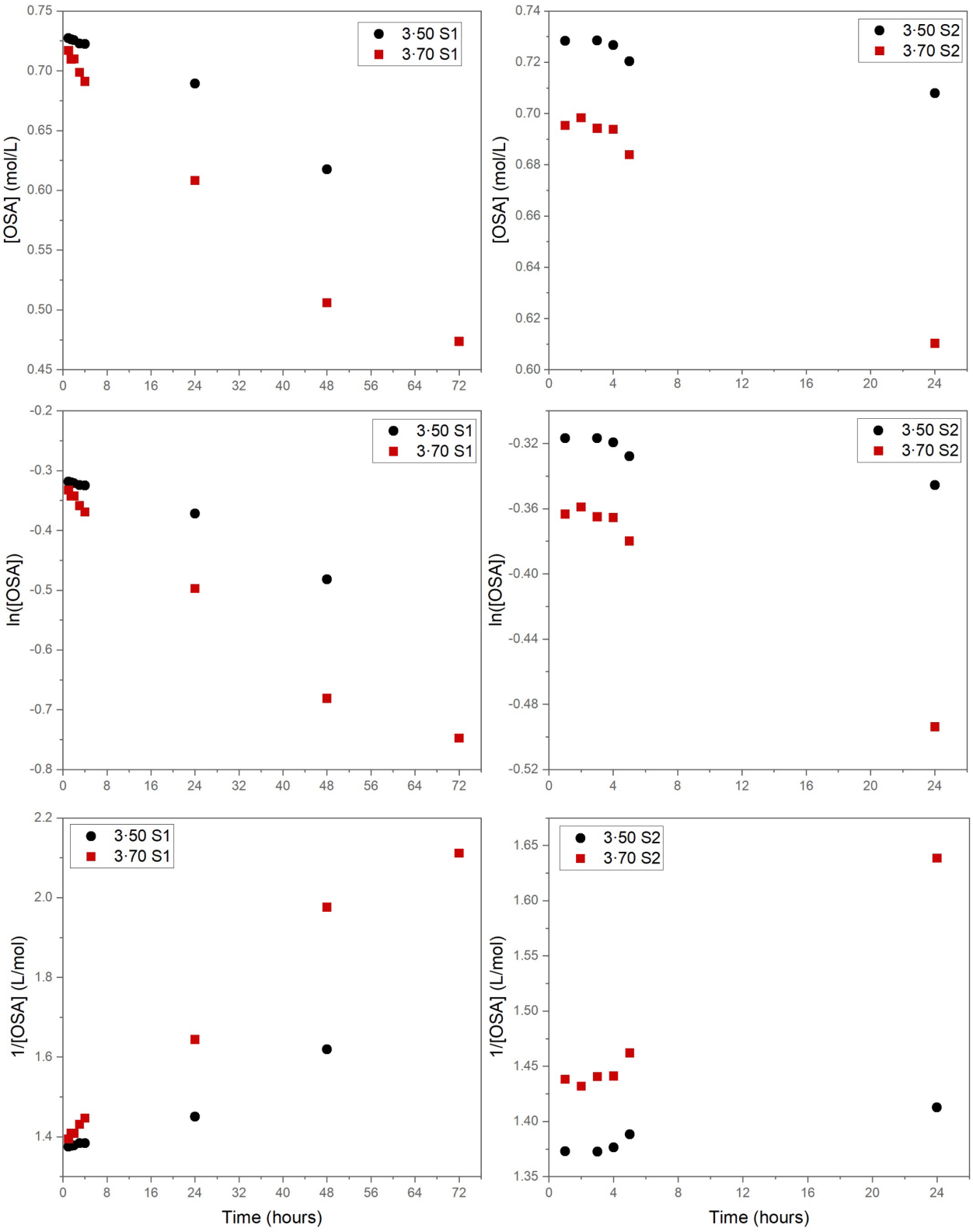


Figure 59. Integrated rate laws of zeroth, first, and second order reactions in OSA for the esterification of α-1,3-glucan.

Table 32. Fitted linear relationships for zeroth, first, and second order rate laws of α-1,3-glucan esterification with OSA.

	Zeroth Order	First Order	Second Order
3·50 S1	$[OSA] = -0.0022t + 0.7313$	$\ln[OSA] = -0.0033t - 0.3120$	$\frac{1}{[OSA]} = 0.0050t + 1.3647$
	$R^2 = 0.98$	$R^2 = 0.98$	$R^2 = 0.97$
3·50 S2	$[OSA] = -0.0009t + 0.7290$	$\ln[OSA] = -0.0012t - 0.3161$	$\frac{1}{[OSA]} = 0.0017t + 1.3717$
	$R^2 = 0.92$	$R^2 = 0.92$	$R^2 = 0.93$
3·70 S1	$[OSA] = -0.0036t + 0.7097$	$\ln[OSA] = -0.0061t - 0.3395$	$\frac{1}{[OSA]} = 0.0106t + 1.3976$
	$R^2 = 0.97$	$R^2 = 0.98$	$R^2 = 0.99$
3·70 S2	$[OSA] = -0.0039t + 0.7048$	$\ln[OSA] = -0.0060t - 0.3488$	$\frac{1}{[OSA]} = 0.0092t + 1.4155$
	$R^2 = 0.99$	$R^2 = 0.99$	$R^2 = 0.99$

If the relationships are assumed to be linear, the esterification rate constant can be determined from the equation of the line of best fit since it is equivalent to the slope. The Arrhenius equation in Equation 11 defines the relationship between the rate constant and the temperature where k is the rate constant, A is an empirical pre-exponential factor, E_a is the activation energy, R is the universal gas constant, and T is the temperature

$$k = Ae^{\frac{E_a}{RT}} \qquad (Equation\ 11)$$

Since the sampling experiments were performed with identical reaction conditions but at different temperatures, and assuming that the pre-exponential factor is independent of temperature, the rate constants can be compared to find the esterification activation energy using Equation 12.

$$E_a = \frac{R \ln\left(\frac{k_{T_2}}{k_{T_1}}\right)}{\frac{1}{T_1} - \frac{1}{T_2}} \qquad (Equation\ 12)$$

The uncatalyzed esterification of cellulose in DMAc/LiCl has been found to follow a second-order reaction mechanism, first order in both cellulose and the esterification reagent which included acid anhydrides of carbon chain length 1 to 6 [94, 95]. To simplify the analysis here, the esterification of α-1,3-glucan with octenylsuccinic anhydride is assumed to follow a similar second-order mechanism. The second-order rate constants for the sampling experiments and the associated activation energies are provided in Table 33. The rate constants and activation energies calculated for zeroth and first order rate laws for the four sampling experiments can be found in Table 41 in Appendix III.

Table 33. Rate constants and activation energy for the second-order esterification of α-1,3-glucan with OSA.

Rate Order	Sample	Rate Constant, k	Sample	Rate Constant, k	Activation Energy, E_a ($J \cdot mol^{-1} \cdot K^{-1}$)
Second (k in $L \cdot mol^{-1} \cdot s^{-1}$)	3·50 S1	0.0050	3·70 S1	0.0106	3.5×10^4
			3·70 S2	0. 0092	2.8×10^4
	3·50 S2	0. 0017	3·70 S1	0.0106	8.3×10^4
			3·70 S2	0. 0092	7.7×10^4

There are significant discrepancies in the rate constants determined for the sampling experiments at 50 °C which has a significant effect on the calculated activation energy. The experiment reproducibility is further explored in Chapter 5.8. For the sake of this discussion, the first sampling experiment at 50 °C (3·50 S1) is assumed to be more reliable due to the greater number of data points and to the improved agreement between DS of the 24 h sample (0.21) and the DS of other 24 h reactions performed with the same reaction conditions (0.17, 0.20). Therefore, by isolating the analysis to 3·50 S1, 3·70 S1, and 3·70 S2, the activation energy is estimated to be between 2.2 x 10^4 and 3.5 x 10^4 $J \cdot mol^{-1} \cdot K^{-1}$.

As discussed above, the relationships in Figure 59 are non-linear. This imparts significant uncertainty on the calculated rate constants and activation energy. Future reaction kinetics investigations could be adjusted to improve the reliability of the calculated values. First, the sampling experiments could be repeated under pseudo-first order conditions with a significant excess of one reagent. Second, under the assumption that OSA is being consumed in competing

reactions, the OSA concentration in solution is likely less than the value calculated from the degree of substitution. Therefore, the concentration of OSA could be measured directly. Potentiometric titration can be used to measure the number of carboxylic acid groups in solution. The reaction between OSA and α-1,3-glucan produces one carboxylic acid end while the reaction between OSA and water produces two carboxylic acid functionalities. The degree of substitution determined by NMR could be compared with the measured carboxylic acid content to determine the amount of free octenylsuccinic acid in solution. The progression of the degree of substitution and production of octenylsuccinic acid may then be analyzed to estimate the rate constants and activation energies for the two competing reactions. The recommendation ignores the existent of other competing reactions occurring in the system but would provide more reliable conclusions regarding the reaction kinetics.

5.8 REPRODUCIBILITY

The first modifications performed in solution with octenylsuccinic anhydride were performed without the use of nitrogen. After repeating select experiments, it was found that the experiments were not reproducible. The potential contributors to the inconsistency were theorized to be fluctuations in the oil bath temperature, improper measurement of reagents due to equipment error related to the analytical balance and Eppendorf pipette, impure reagents, contaminated glassware, and the varying humidity of the fume hood. Upon further experimentation and analysis of the reagents, it was determined that the primary contributing factor must be the humidity affecting the moisture content of the reaction system. Therefore, the experimental setup was redesigned to include a nitrogen bubbler. Nitrogen was used to purge the glassware before the addition of reagents and flowed through the setup throughout the modifications. The adjustment to the experimental setup proved successful in improving the reproducibility of the esterification in 7.3% LiCl/DMAc. However, when the solvent was changed to 5% LiCl/DMAc, inconsistencies in the degree of substitution arose.

The degrees of substitution for the sample 3·70-24H produced in 7.3% LiCl/DMAc and 5% LiCl/DMAc are provided in Table 34 alongside their associated measurements errors, averages, and standard deviations. The measurement error was propagated from an assumed 10% error on the integration of the peaks for H1 and H2 to H6 using Equation 13. The error for each degree of

substitution was propagated to the average using Equation 14, while the standard deviation was found using Equation 15 where x is the degree of substitution.

$$\delta_{DS} = DS \times \sqrt{\frac{\left(0.10 \times I_{H_1}\right)^2 + \left(0.10 \times I_{H_2-H_6}\right)^2}{I_{RingH}^2}} \qquad (Equation\ 13)$$

$$\delta_{DS\ average} = \sqrt{\sum \delta_{DS}^2} \qquad (Equation\ 14)$$

$$S_x = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \qquad (Equation\ 15)$$

For repeated modifications performed in 7.3% LiCl/DMAc, the error propagated from the assumed 10% integration error is larger than the standard deviation, indicating that the integration error is less than assumed. Both values are low, indicating a narrow spread of degree of substitution as desired. In contrast, the propagated error for the modifications performed in 5% LiCl/DMAc is smaller than the standard deviation, implying that the assumed error on the integration should be more conservative. Additionally, the standard deviation is large and since the degree of substitution has a significant effect on the material properties of the final product, the modifications performed in 5% LiCl are not sufficiently consistent.

Table 34. Degrees of substitution and propagated errors for α-1,3-glucan modifications performed in 7.3% and 5% LiCl/DMAc.

Sample	Degree of Substitution	Propagated Error	Average	Standard Deviation	Propagated Error
3·70-24H-7.3% LiCl A	0.36	0.03	0.42	± 0.06	± 0.08
3·70-24H-7.3% LiCl B	0.39	0.03			
3·70-24H-7.3% LiCl C	0.53	0.04			
3·70-24H-7.3% LiCl D	0.45	0.04			
3·70-24H-7.3% LiCl E	0.38	0.03			
3·70-24H-7.3% LiCl F	0.40	0.03			
3·70-24H-5% LiCl A	0.38	0.03	0.6	± 0.3	± 0.18
3·70-24H-5% LiCl B	0.59	0.05			
3·70-24H-5% LiCl C	1.2	0.1			
3·70-24H-5% LiCl D	0.48	0.04			
3·70-24H-5% LiCl E	0.74	0.07			
3·70-24H-5% LiCl S1	0.54	0.05			
3·70-24H-5% LiCl S2	0.53	0.05			

Note: S1 and S2 denote sampling experiments.

The degrees of substitution are provided visually in Figure 60 and Figure 61 where the spread of DS values has widened with the change in solvent.

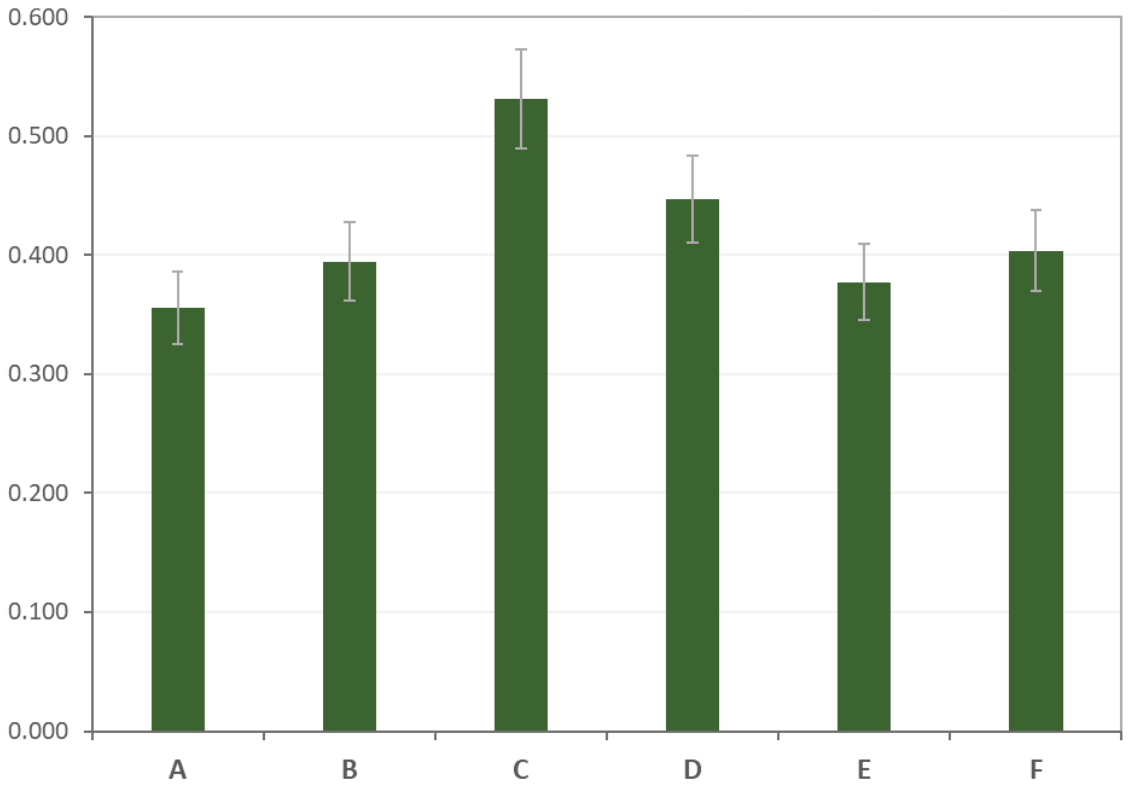


Figure 60. Degrees of substitution for repeated α-1,3-glucan modifications 3·70-24H-7.3% LiCl.

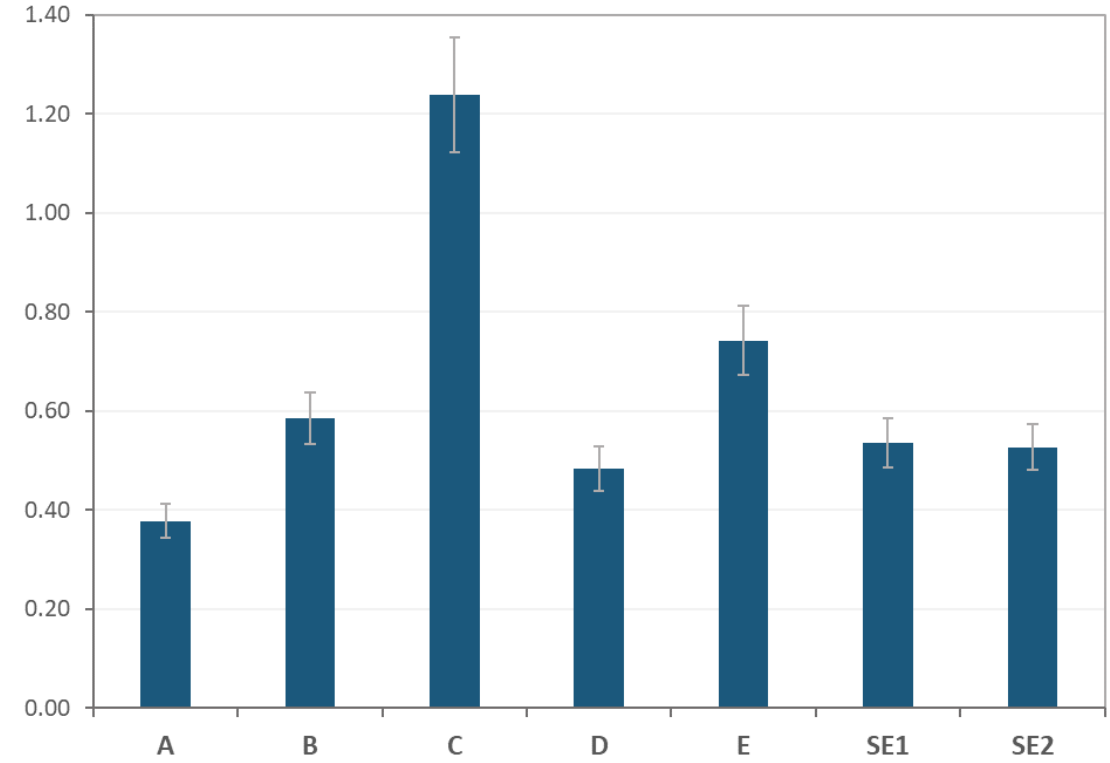


Figure 61. Degrees of substitution for repeated α-1,3-glucan modifications 3·70-24H-5% LiCl.

While the reproducibility of the modifications performed in 7.3% LiCl/DMAc is desirable, the inconsistency of the solubility of α-1,3-glucan in this solvent system prevents its use as the reaction solvent. It should also be considered that the change in reproducibility may not be a result of the solvent change but rather a change in the neat polysaccharide as the reduction in reproducibility of modifications coincided with a reduction in the reproducibility of the α-1,3-glucan solubility.

5.9 COMPETING REACTIONS

The molar ratios of OSA:AGU were selected based on the target degree of substitution. For example, a ratio of 1:1 targets a DS of 1 and a ratio of 3:1 targets a DS of 3, or complete substitution of the hydroxyl groups on α-1,3-glucan. However, with the reaction conditions used in this project, the targeted degree of substitution was never reached. Separate experiments, denoted as *mock reactions*, were performed to determine if there were competing reactions occurring in the reaction solution between OSA, water, LiCl, and DMAc. The experiments were performed following a similar methodology to the α-1,3-glucan modifications but with the polysaccharide omitted.

The experiments outlined in Table 35 were performed at 70 °C for 24 h. Water was added at a 1:1 molar ratio to OSA. An image of the final solutions is provided in Figure 62.

Table 35. Material concentrations for mock reactions to determine potential side reactions.

Experiment	Solvent	LiCl Concentration (% w/v)	OSA Concentration (% w/v)	Water Concentration (% w/v)	Nitrogen
1.1	DMAc	7.3	15.6	1.34	No
1.2	DMAc	7.3	15.6	0	Yes
1.3	DMSO	7.3	15.6	0	Yes
2.1	DMAc	0	15.6	0	Yes
2.2	DMAc	0	15.6	1.34	No

Figure 62. Mock reaction solutions for (left to right) experiments 1.1, 1.2, 1.3, 2.1, and 2.2.

Based on the appearance of the reaction solutions, removing water from the systems containing LiCl (experiments 1.1, 1.2, and 1.3) increases the extent of side reactions causing a colour change. The colour change was also associated with a reduction in the solution viscosity. In contrast, removing LiCl (experiments 2.1 and 2.2) from the system eliminates all colour change even in the presence of water. Colour change is not the only indicator of a reaction, so it is likely that OSA is still reacting with water to form octenylsuccinic acid (Figure 63).

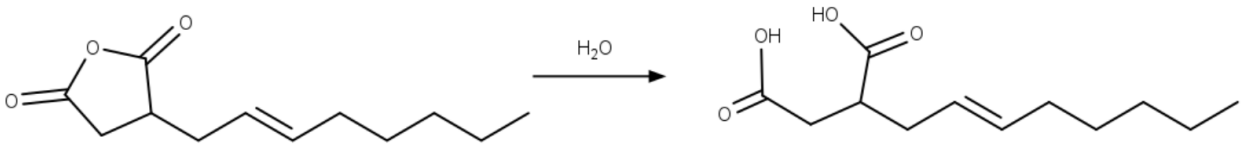


Figure 63. Transformation of octenylsuccinic anhydride to octenylsuccinic acid in the presence of water.

The solutions were analyzed by ^{1}H-NMR and FTIR to determine if any structural changes occurred. The ^{1}H-NMR spectra in Figure 64 are difficult to analyze due to the DMAc peaks which obstruct OSA peaks between 1.9 – 2.1 ppm and 2.75 – 3 ppm. Compared to the spectrum of pure OSA, the spectrum for experiment 1.1 is missing the peaks for OSA ring protons at between 3.05 ppm and 3.55 ppm but has additional peaks between 2.05 ppm and 2.31 ppm. The peaks for the

alkene protons have experienced an upfield shift. This confirms that OSA is undergoing a reaction despite the lack of colour change. The spectra for experiments 1.2, 2.1, and 2.2 show downfield shifts for the ring protons and the alkene protons at 5.4 ppm.

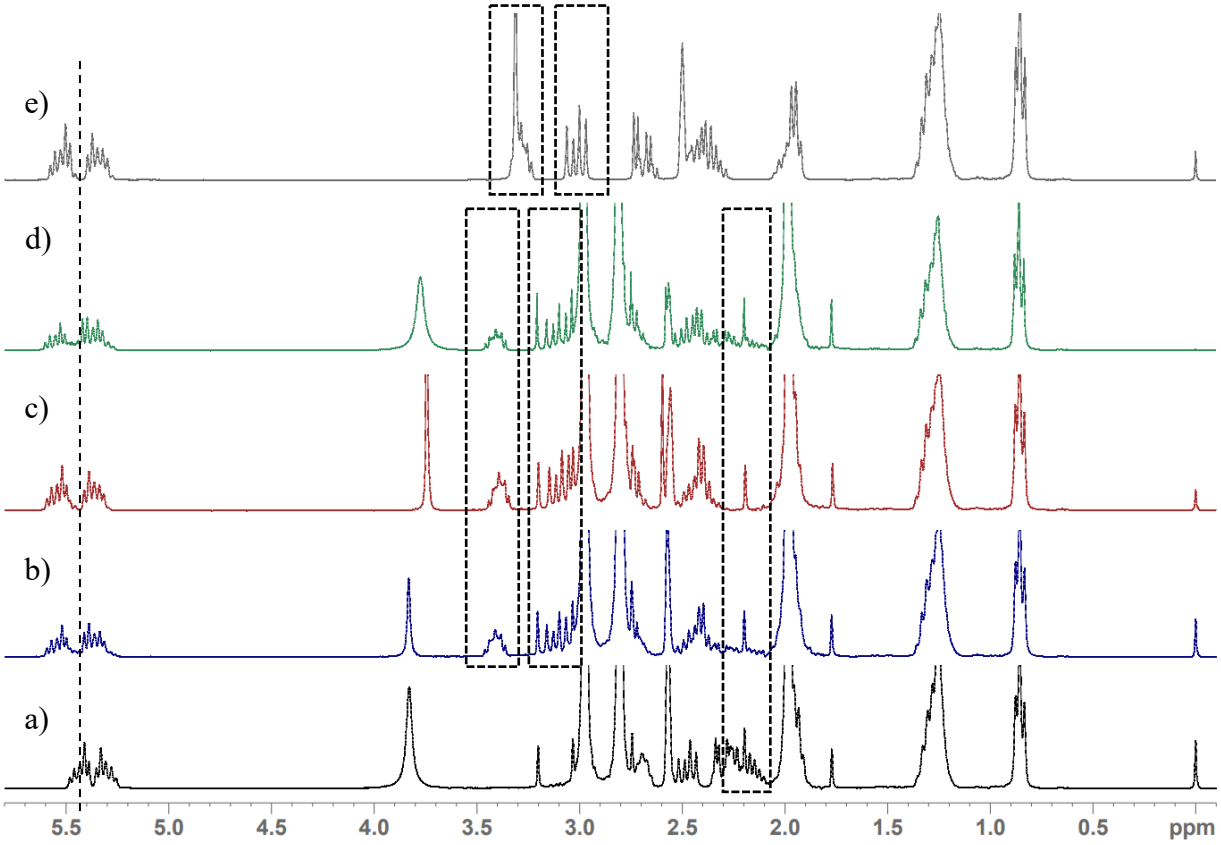


Figure 64. ^{1}H-NMR spectra of mock experiments **a)** 1.1, **b)** 1.2, **c)** 2.1, **d)** 2.2, and of **e)** pure OSA.

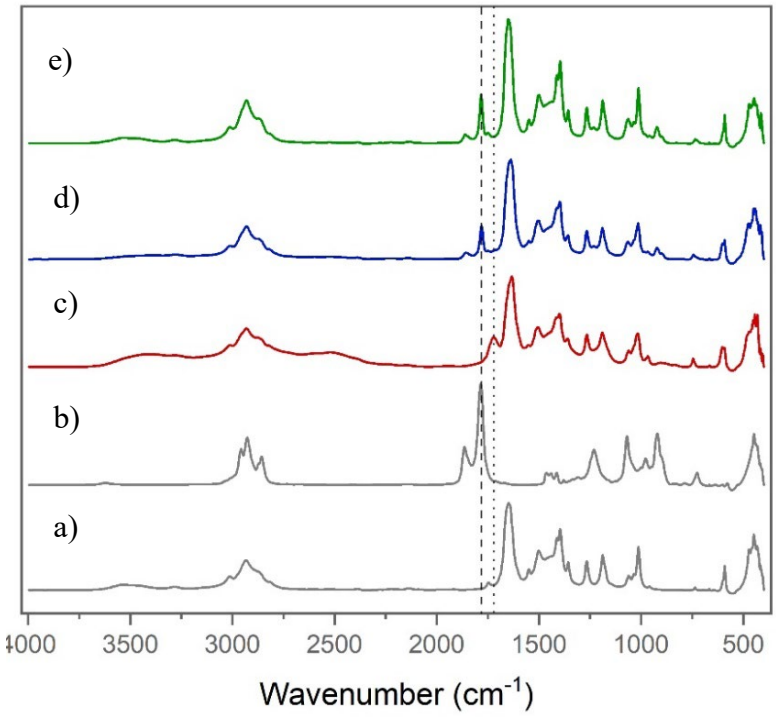


Figure 65. FTIR spectra of **a)** DMAc, **b)** OSA, and of mock experiments **c)** 1.1, **d)** 1.2, **e)** 2.1.

The FTIR spectra in Figure 65 show that for experiment 1.1, the OSA carbonyl peaks at 1786 cm^{-1} and 1860 cm^{-1} have either been eliminated or shifted to a lower wavenumber and thus obstructed by the DMAc peak. This may be caused by a ring-opening reaction between OSA and water. Experiments 1.2 and 2.1 do not contain water and have retained these carbonyl peaks, providing further evidence of the existence of a reaction with water in experiment 1.1.

Further analysis was not performed to determine other reaction products from the competing reactions in the system. The apparent degradation occurring in experiment 1.2 and 1.3 in the presence of LiCl is concerning and likely occurs during the modifications of α-1,3-glucan as well. Performing these mock reactions at 50 °C may find that a lower temperature reduces the extent of this reaction. LiCl has a significant impact on the behaviour of the materials in solution; this is likely related to the correlation between degree of substitution and LiCl concentration with a reduced LiCl concentration leading to a higher DS (Figure 55). The behaviour found in experiment 1.2 compared to experiment 2.1 indicates that performing esterification on α-1,3-glucan dispersed in DMAc without LiCl could prove effective for increasing the DS in amorphous regions of the polysaccharide.

5.10 EVALUATION OF α-1,3-GLUCAN ESTERIFICATION USING THE PRINCIPLES OF GREEN CHEMISTRY

The principles of green chemistry were outlined in Chapter 2. A preliminary evaluation of the homogeneous esterification of α-1,3-glucan with OSA using these principles is summarized in Table 36. Suggestions to reduce the environmental and health hazards of the modification are included in the right-most column. Transferring the modification to the solid-state environment would have a significant effect on the E-factor, environmental and health hazards, and energy efficiency of the process. Additionally, selecting an alternative catalyst to pyridine could make the modification safer by eliminating the use of flammable materials. To reduce the E-factor, recycling of the reaction, precipitation, and washing solvents should be explored. Recycling of DMAc will be difficult due to the similarity in boiling point with OSA, but ethanol and acetone may be recycled more easily due to their low vapour pressures.

Table 36. Evaluation of the homogeneous esterification of α-1,3-glucan using the 12 Principles of Green Chemistry.

Principle	Evaluation	Comments
Waste Prevention	$E-factor = \frac{kg_{waste}}{kg_{product}} = 664$	High E-factor due to solvent requirements of precipitation & purification processes and due to low conversion
Atom Economy	$A.E. = \frac{MW_{atoms\ used}}{MW_{reactants}} \times 100\% = 100\%$	All OSA and α-1,3-glucan atoms present in product
Less Hazardous Chemical Syntheses	α-1,3-glucan: no known hazards OSA: irritant LiCl: irritant DMAc: toxic, combustible Pyridine: toxic, flammable	Alternative solvents and catalysts should be explored to minimize hazards
Designing Safer Chemicals	α-1,3-glucan-OSA: unknown toxicity	Toxicity profile likely similar to starch-OSA (low toxicity at low DS) [96, 84]
Safer Solvents and Auxiliaries	DMAc: toxic, combustible, high vapour pressure, poor recyclability	Perform modification in heterogeneous or solid-state environment

Principle	Evaluation	Comments
Energy Efficiency	Ambient pressure, elevated temperature, multi-step procedure (solubilization, reaction, precipitation, purification)	Reduce reaction temperature, perform modification in heterogeneous or solid-state environment
Renewable Feedstocks	α-1,3-glucan: renewable OSA: non-renewable DMAc: non-renewable LiCl: non-renewable Pyridine: non-renewable	Use alternative solvents or perform modification in solvent-less environment Explore modification with biomass-derived anhydrides [97]
Reduce Derivatives	No derivatization steps	Not applicable
Catalysis	Pyridine: not consumed	Explore recycling of catalyst to reduce E-factor
Design for Degradation	α-1,3-glucan: biodegradable α-1,3-glucan-OSA: unknown degradability	Biodegradation tests should be performed on future materials produced via esterification of α-1,3-glucan with OSA
Real-Time Analysis	Not applicable	Not applicable
Safer Chemistry for Accident Prevention	Solvent: toxic Reagents: varied toxicity, pyridine is flammable Process: ambient pressure, elevated temperature, requires nitrogen gas (inert)	Explore alternative solvents, perform modification in solid-state environment, select safer catalyst

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

α-1,3-glucan was successfully esterified with octenylsuccinic anhydride in solution. Overall, the reaction efficiency was low with a maximum reaction efficiency of 50% for the sample formed in the presence of pyridine with an OSA:AGU molar ratio of 3:1, and a reaction temperature and time of 50 °C and 24 h, respectively. Due to the bulky nature of OSA, a high degree of substitution would be difficult to achieve.

As described in Chapter 5, the degree of substitution was increased by elongating the reaction time, increasing the reaction temperature, and increasing the concentration of OSA. Additionally, decreasing the concentration of LiCl and adding pyridine individually had a positive impact on the DS. However, despite increasing the degree of substitution, the relationship between these factors and chain degradation of α-1,3-glucan is more complicated.

Increasing the reaction time increases the extent of degradation of the polysaccharide backbone at all reaction conditions. Increasing the reaction temperature causes increased degradation. Increasing the OSA:AGU molar ratio from 1:1 to 3:1 has an insignificant effect on the extent of degradation. The addition of pyridine successfully reduced chain degradation at an OSA:AGU ratio of 1:1 and a reaction temperature of 70 °C but had a negative effect on the molecular weight at an OSA:AGU ratio of 3:1 and a temperature of 50 °C. Reducing the LiCl concentration to from 7.3% to 2.5% in DMAc increased the extent of degradation.

Based on NMR and SEC results, chemical modifications performed at 50 °C for 24 h with an OSA:AGU ratio of 3:1 showed the optimal combination of degree of substitution and molecular weight. However, increasing the reaction time at a lower reaction temperature may prove to produce superior results if a sufficiently high degree of substitution can be obtained. Adjustments in the concentrations of lithium chloride and pyridine may be performed to further explore their effect on the degree of substitution and molecular weight of modified α-1,3-glucan.

The dynamic viscosity measurements of samples in 5% LiCl/DMSO were found to not be a reliable predictor of chain degradation. There were limited correlations between the reaction conditions

and the thermal properties of the modified samples. All modified samples had reduced thermal stabilities relative to unmodified α-1,3-glucan. The films formed by hot pressing the samples of sufficient DS were too brittle to be formed into tensile bars for mechanical measurements. Instead, preliminary emulsion testing showed positive interactions between the selected samples and oil/water mixtures.

The solubility of α-1,3-glucan was found to be variable throughout this project. While the experiments presented in Chapter 4.2 provide some insight into the viable lithium chloride concentrations that can be used to solubilize α-1,3-glucan, there remains concern over the inconsistency of the solubility over the timeline of the project. It should be expected that future experiments may experience similar irregularities.

Despite the renewability and biodegradability of α-1,3-glucan as a starting material, the health and environmental impacts of the current modification procedure are significant. This is mostly caused by the large quantities of solvent required for the reaction, precipitation, and purification steps. Therefore, investigation of the solid-state modification of α-1,3-glucan with OSA should be a priority.

6.1 RECOMMENDATIONS

While α-1,3-glucan was successfully esterified with octenylsuccinic anhydride, this research project provides only a start to the potential experiments and analysis that can be performed going forward. Further improvements to the reaction conditions may be made by varying the lithium chloride concentration, adjusting the concentration of α-1,3-glucan, and exploring alternative catalysts and varying catalyst concentrations to reduce chain degradation. The reactions performed in this project were not found to be reproducible and as such, further analysis should be conducted to determine the cause. Examples of analysis and experiments could include measurement and control of the solution water content to quantify the effect of water on the reaction, drying of dimethyl acetamide prior to use, and measuring solvent and reagent amounts by mass instead of by volume. Other adjustments to the experimental setup could include using dimethyl sulfoxide instead of dimethyl acetamide or replacing lithium chloride with another salt such as lithium bromide or calcium chloride dihydrate. If the reaction continues to show poor reproducibility,

esterification of α-1,3-glucan with octenylsuccinic anhydride may not be a feasible modification for commercial manufacture of value-added α-1,3-glucan materials.

If experiments show improved reproducibility and materials are obtained with desirable properties for the target applications, the esterification of α-1,3-glucan with octenylsuccinic anhydride should be transferred to the solid-state to reduce solvent-usage and waste. In transferring the modification, the reaction conditions will require adjustments to produce materials at high yield, high conversion, and with the desired properties.

The current samples show promising behaviour in water and oil mixtures. To continue this analysis, emulsions should be formally prepared using a homogenizer with larger sample volumes. The interaction of the samples with water can also be explored using contact angle measurements.

Puanglek et al. homogeneously esterified α-1,3-glucan with complete substitution of the hydroxyl groups using acid anhydrides of varying chain length [13]. The resulting samples showed clear melting points in DSC thermograms, had improved thermal stability over unmodified α-1,3-glucan, and formed colourless, transparent films when solvent casted in chloroform. The films were sufficiently flexible to be formed into tensile bars for mechanical testing. It is recommended that the experiments performed by Puanglek et al. are repeated to reproduce their positive results. It is possible that in repeating their experiments, ideas may arise to improve the consistency and behaviour of the experiments performed in this research project.

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APPENDIX I
SOLID-STATE MODIFICATION OF α-1,3-GLUCAN WITH OCTENYLSUCCINIC ANHYDRIDE

Homogeneous chemical modifications require large quantities of solvent for the reaction, precipitation, and purification steps. This becomes cost- and material-intensive when scaled up for industrial production. Therefore, it is desirable to perform reactions in heterogeneous or solid-state conditions to reduce the amount of solvent and the number of steps involved in the modification. Heterogenous modification of α-1,3-glucan was performed to explore the reaction efficiency of the esterification in a solvent-less state. The goal of these exploratory heterogeneous modifications was to modify α-1,3-glucan close to a degree of substitution of 1. A degree of substitution around 1 should allow the material to melt and to be thermoprocessed. Extrusion and batch mixing were employed to apply shear to the material to provide access to the hydroxyl groups to be functionalized by OSA. Due to the strong crystalline structure and high degree of crystallinity of α-1,3-glucan, the esterification is expected to occur primarily in the amorphous regions of the polymer.

AI.I MATERIALS

α-1,3-glucan (Nuvolve^{TM}) was provided by International Flavours & Fragrances where it was produced via enzymatic synthesis with glucosyltransferase. α-1,3-glucan wetcake used for solid-state modifications contained 43% solids by weight, an average degree of polymerization of 800, and was stored in the refrigerator at 4 °C. 2-octenylsuccinic anhydride (OSA, cis- and trans-mixture, 95%) was purchased from Thermo Fisher Scientific. Deuterated dimethyl sulfoxide (DMSO-d6, 99.8% with 0.03% TMS), deuterated trifluoracetic acid (TFAA-d, 99.5%), and acetone (99%) were purchased from Sigma-Aldrich.

AI.II METHODS

Solid-state modifications were performed on α-1,3-glucan wetcake via three primary methods.

Extrusion. α-1,3-glucan wetcake (43% solids, 57% water) was manually mixed with water and octenylsuccinic anhydride, then fed to a Haake Minilab conical twin-screw extruder operating at 60 RPM and 25 °C or 85 °C. The extrudate was collected, ground using a mortar and pestle, then placed in an oven at the desired reaction temperature for 24 h. The product was then washed with acetone via Soxhlet extraction, dried, and analyzed by NMR to determine the degree of substitution.

Batch Mixing. α-1,3-glucan wetcake (43% solids, 57% water) was manually mixed with octenylsuccinic anhydride, then fed to a Haake Polylab QC Internal Mixer operating with roller rotors. The mixing was performed at 25 °C or 70 °C for 30 minutes and the torque was monitored throughout. The solid mixture was collected and placed in an oven at the desired reaction temperature for 24 h. The product was then washed with acetone via Soxhlet extraction, dried, and analyzed by NMR to determine the degree of substitution.

Manual mixing. α-1,3-glucan wetcake (43% solids, 57% water), or α-1,3-glucan powder, was manually mixed with octenylsuccinic anhydride, then placed in an oven at the desired reaction temperature for 24 h. Some samples were dried between mixing and heating in a vacuum oven to reduce the extent of reaction between OSA and water. The product was washed with acetone via Soxhlet extraction, dried, and analyzed by NMR to determine the degree of substitution.

AI.III RESULTS

Due to the limited success achieved in solid-state modifications using the above methods, characterization of the samples was constrained to NMR analysis only. The degree of substitution for each sample is provided in Table 37.

Table 37. Processing and reaction conditions, and degrees of substitution for α-1,3-glucan modified in solid-state.

Sample	OSA:AGU	$T_{Processing}$ (°C)	$T_{Reaction}$ (°C)	$t_{Reaction}$ (h)	Catalyst	DS
0.1EXT25-R70-24H	0.1:1	25	70	24	-	0.013
0.7EXT25-R70-24H	0.7:1	25	70	24	-	0.035
0.9EXT25-R70-24H	0.9:1	25	-	24	-	0.034
0.1EXT25-R22-24H-NaOH	0.1:1	25	22	24	1% NaOH	0.019
0.1EXT25-R70-24H-NaOH	0.1:1	25	70	24	1% NaOH	0.023
0.1EXT25-R22-24H-NaOH/ZnO	0.1:1	25	22	24	1% NaOH 0.1% ZnO	0.011
0.1EXT25-R70-24H-NaOH/ZnO	0.1:1	25	70	24	1% NaOH 0.1% ZnO	0.016
1.2EXT25-R70-24H	1.2:1	25	70	24	-	0.032
0.1EXT25-R70-24H-KOH	0.1:1	25	70	24	1% KOH	0.020
0.1EXT25-R22-24H-KOH	0.1:1	25	22	24	1% KOH	0.010
0.1EXT25-R70-24H-KOH/ZnO	0.1:1	25	70	24	1% KOH 0.1% ZnO	0.021
0.1EXT25-R22-24H-KOH/ZnO	0.1:1	25	22	24	1% KOH 0.1% ZnO	0.005
0.1EXT25-R70-24H-Et3N	0.1:1	25	70	24	1% Et3N	0.005
0.1EXT25-R22-24H-Et3N	0.1:1	25	22	24	1% Et3N	0.004
0.1EXT25-R70-24H-Et3N/ZnO	0.1:1	25	70	24	1% Et3N 0.1% ZnO	0.004
0.1EXT25-R22-24H-Et3N/ZnO	0.1:1	25	22	24	1% Et3N 0.1% ZnO	0.003
0.1EXT85-R70-24H	0.1:1	25	70	24	-	0.043
0.1EXT85-R22-24H	0.1:1	85	22	24	-	0.001
0.1EXT85-0H	0.1:1	85	-	0	-	0.005

Sample	OSA:AGU	$T_{Processing}$ (°C)	$T_{Reaction}$ (°C)	$t_{Reaction}$ (h)	Catalyst	DS
0.1BATCH25-R70-24H	0.1:1	25	70	24	-	0.004
0.1BATCH25-R22-24H	0.1:1	25	22	24	-	0.003
0.1BATCH70-R70-24H	0.1:1	70	70	24	-	0.004
3MAN22-R30-24H	3:1	-	30	24	-	0.005
1MAN22-R70-24H	1:1	-	70	24	-	0.18
3MAN22-R70-2H	3:1	-	70	2	-	0.005
3MAN22-R70-24H	3:1	-	70	24	-	0.015
3MAN22-R90-24H	3:1	-	90	24	-	0.037
3MAN22-R110-2H	3:1	-	110	2	-	0.008
3MAN22-R110-24H	3:1	-	110	24	-	0.012

Sample Names: (OSA:AGU molar ratio)(processing method)(processing temperature)-(reaction temperature)-(reaction time)

The maximum degree of substitution found by NMR was 0.18 for sample 1MAN22-R70-24H. However, the degree of substitution was variable throughout the mixture. Due to the high density of OSA, when mixing is ceased during the reaction, the liquid quickly settles to the bottom of the beaker. So α-1,3-glucan undergoes a greater extent of reaction at the base of the system than at the top surface.

The primary limitation to solid-state modification of α-1,3-glucan is the high degree of crystallinity and strength of its crystal structure. An additional limitation for the esterification reaction with OSA is the high molecular weight of OSA. When adding sufficient OSA to achieve a degree of substitution around 1, the reaction system has a low viscosity and so processing by extrusion and batch mixing does not impart enough shear to provide considerable access of OSA to the hydroxyl groups. Better success may be found with a smaller reagent molecule.

APPENDIX II
EQUATION DERIVATIONS

AII.I DEGREE OF SUBSTITUTION

Degree of Substitution:

$$DS = \frac{\#\ OSA}{AGU}$$

$$\#\ OSA = \frac{Terminal\ Methyl\ Protons\ from\ NMR}{3} = \frac{I_{CH_3}}{3}$$

$$\#\ AGU = \frac{All\ ring\ protons\ from\ NMR}{7} = \frac{I_{H_1} + I_{H_2-H_6}}{7} = \frac{I_{RingH}}{7}$$

$$DS = \frac{\frac{I_{CH_3}}{3}}{\frac{I_{RingH}}{7}}$$

An NMR spectrum is provided in Figure 66 with the integration of the AGU ring protons (2.9 ppm – 4.6 ppm, 4.8 – 5.2 ppm) and of the OSA terminal methyl protons (0.8 – 1.0 ppm) included. The degree of substitution can then be calculated from the integrated values:

$$DS_{sample} = \frac{\frac{1.000}{3}}{\frac{0.555 + 4.911}{7}} = 0.43$$

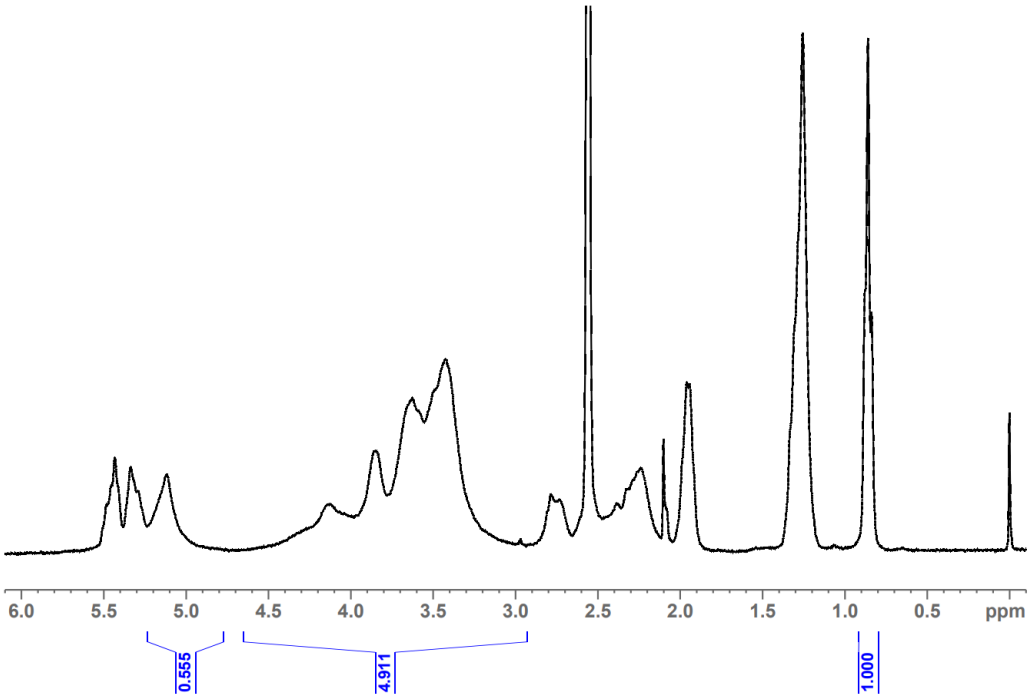


Figure 66. [1]H-NMR spectrum for sample calculation of degree of substitution for α-1,3-glucan-OSA.

AII.II CONVERSION

OSA Concentration:

$$[OSA] = [OSA]_0 - DS \times [AGU]_0$$

Where the initial concentrations of OSA and AGU are found by:

$$[OSA]_0 = \frac{\frac{V_{OSA,0}\rho_{OSA}}{MW_{OSA}}}{V_{solution}}$$

$$[AGU]_0 = \frac{\frac{m_{\alpha-1,3-glucan}}{MW_{AGU}}}{V_{solution}}$$

OSA Conversion:

$$X = \frac{[OSA]_0 - [OSA]}{[OSA]_0} \times 100\%$$

$$X = \frac{DS \times [AGU]_0}{[OSA]_0} \times 100\%$$

AII.III DEGREE OF POLYMERIZATION AND MOLECULAR WEIGHT

Degree of Polymerization:

For simplicity of calculation, assume modified samples have one fraction of completely modified anhydroglucose units (DS = 3) and one fraction of unmodified units (DS = 0)

$$DP_w = \frac{Weight\ Average\ Molecular\ Weight}{\left(\left(Fraction_{modified\ AGU}\right)\left(MW_{modified\ AGU}\right) + \left(Fraction_{unmodified\ AGU}\right)\left(MW_{unmodified\ AGU}\right)\right)}$$

$$Fraction_{modified\ AGU} = \frac{DS}{3}$$

$$Fraction_{unmodified\ AGU} = \frac{3 - DS}{3}$$

$$MW_{unmodified\ AGU} = MW_{AGU}$$

$$MW_{modified\ AGU} = MW_{AGU} + 3 \times MW_{OSA}$$

$$DP_w = \frac{M_w}{\frac{DS}{3}\left(MW_{AGU} + 3 \times MW_{OSA}\right) + \frac{3 - DS}{3}\left(MW_{AGU}\right)}$$

$$DP_w = \frac{M_w}{DS \times MW_{OSA} + MW_{AGU}}$$

Theoretical Molecular Weight:

For simplicity of calculation, assume modified samples have one fraction of completely modified anhydroglucose units (DS = 3) and one fraction of unmodified units (DS = 0)

$$M_{W\,Theoretical} = DP_{w\,glucan}\Big(\big(Fraction_{modified\ AGU}\big)\big(MW_{modified\ AGU}\big) + \big(Fraction_{unmodified\ AGU}\big)\big(MW_{unmodified\ AGU}\big)\Big)$$

$$DP_{w\,glucan} = \frac{M_{w\,glucan}}{MW_{AGU}}$$

$$M_{w_{Theoretical}} = \frac{M_{w\,glucan}}{MW_{AGU}}\left(\frac{DS}{3}(MW_{AGU} + 3 \times MW_{OSA}) + \frac{3 - DS}{3}(MW_{AGU})\right)$$

$$M_{w_{Theoretical}} = \frac{M_{w_{glucan}}}{MW_{AGU}}(DS \times MW_{OSA} + MW_{AGU})$$

AII.IV ACTIVATION ENERGY

OSA Concentration:

$$[OSA] = [OSA]_0 - DS \times [AGU]_0$$

−OH Concentration:

$$[OH] = [OH]_0 - \frac{DS}{3} \times [OH]_0$$

Where the initial concentration of −OH is found by:

$$[OH]_0 = 3 \times [AGU]_0$$

Zeroth-order rate law:

$$[OSA] = -k_0 t + [OSA]_0$$

First-order rate constant:

$$\ln[OSA] = -k_1 t + \ln[OSA]_0$$

Second-order rate constant:

For second order in OSA or first-order in OSA and first order in −OH groups with equivalent initial concentrations:

$$\frac{1}{[OSA]} = k_2 t + \frac{1}{[OSA]_0}$$

Activation Energy:

Using Arrhenius law where k is the rate constant, A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is the reaction temperatures in Kelvin:

$$k = Ae^{\frac{E_a}{RT}}$$

Rate constants for the same reaction at different temperatures can be compared and isolated to solve for activation energy:

$$\frac{k_{T_2}}{k_{T_1}} = \frac{A_{T_1}e^{\frac{E_a}{RT_1}}}{A_{T_2}e^{\frac{E_a}{RT_2}}}$$

The pre-exponential factor is assumed to be independent of temperature:

$$\frac{k_{T_2}}{k_{T_1}} = \frac{e^{\frac{E_a}{RT_1}}}{e^{\frac{E_a}{RT_2}}} = e^{\frac{E_a}{RT_1} - \frac{E_a}{RT_2}}$$

$$\frac{E_a}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) = \ln\left(\frac{k_{T_2}}{k_{T_1}}\right)$$

$$E_a = \frac{R\ln\left(\frac{k_{T_2}}{k_{T_1}}\right)}{\frac{1}{T_1} - \frac{1}{T_2}}$$

AII.V ERROR PROPAGATION

Error on Degree of Substitution:

Assume 10% error on integration of each NMR peak and propagate to DS value:

$$\frac{\delta_{H_1}}{I_{H_1}} = \frac{\delta_{H_2-H_6}}{I_{H_2-H_6}} = 0.10$$

$$\delta_{RingH} = \sqrt{\delta_{H_1}^2 + \delta_{H_2-H_6}^2}$$

$$\delta_{RingH} = \sqrt{\left(0.10 \times I_{H_1}\right)^2 + \left(0.10 \times I_{H_2-H_6}\right)^2}$$

$$\delta_{DS} = DS \times \sqrt{\left(\frac{\delta_{RingH}}{I_{RingH}}\right)^2}$$

$$\delta_{DS} = DS \times \sqrt{\left(\frac{\sqrt{\left(0.10 \times I_{H_1}\right)^2 + \left(0.10 \times I_{H_2-H_6}\right)^2}}{I_{RingH}}\right)^2}$$

$$\delta_{DS} = DS \times \sqrt{\frac{\left(0.10 \times I_{H_1}\right)^2 + \left(0.10 \times I_{H_2-H_6}\right)^2}{I_{RingH}^2}}$$

Propagated Error on Average Degree of Substitution:

$$\delta_{DS\ average} = \sqrt{\sum \delta_{DS}^2}$$

Standard Error on Average Degree of Substitution:

$$S_x = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

AII.VI E-FACTOR & ATOM ECONOMY

E-Factor:

$$E - Factor = \frac{mass_{waste}}{mass_{product}}$$

The E-factor was calculated for the four samples in Table 38 with an average yield of 80%. The waste produced during the process was defined as:

$$Waste = Unreacted\ Reagents + Reaction\ Solvent + Catalyst + Purification\ Solvent$$

Table 38. Material usage and waste production of select α-1,3-glucan modifications.

	1·70-24H	**3·70-24H**	**1·70-24H-PYR**	**3·50-24H-PYR**
Reaction Inputs				
α-1,3-glucan (g)	1	1	1	1
DMAc (g)	23.4	23.4	23.4	23.4
LiCl (g)	1.25	1.25	1.25	1.25
OSA (g)	1.30	3.89	1.30	3.89
Pyridine (g)	0	0	0.49	1.46
Precipitation & Purification				
Ethanol (g)	387	387	387	387
Acetone (g)	119	119	119	119
Product				
Sample (g)	0.80	0.80	0.80	0.80
OSA Conversion (%)	8	20	52	39
Process				
Waste	533	531	533	531
E-Factor	**666**	**664**	**667**	**664**

Atom Economy:

$$Atom\ Economy = \frac{MW_{atoms\ used}}{MW_{reactants}} \times 100\%$$

The reaction between α-1,3-glucan and OSA uses all atoms from both reagents to produce α-1,3-glucan-OSA. Therefore, the atom economy for the esterification is 100%.

APPENDIX III

ADDITIONAL DATA FOR CHAPTER 5

AIII.I NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

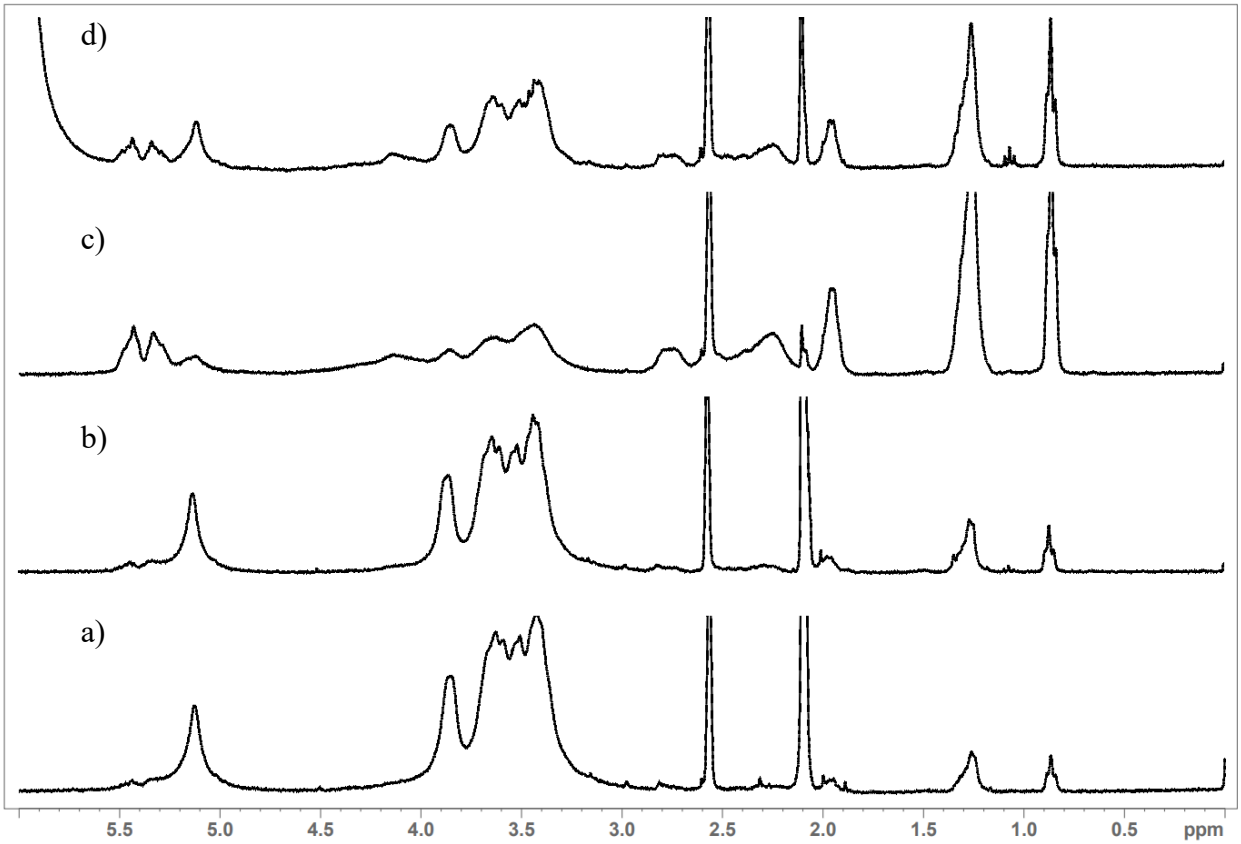


Figure 67. ^{1}H-NMR spectra for **a)** 1·30-24H, **b)** 1·50-24H, **c)** 1·70-24H, and **d)** 1·110-24H.

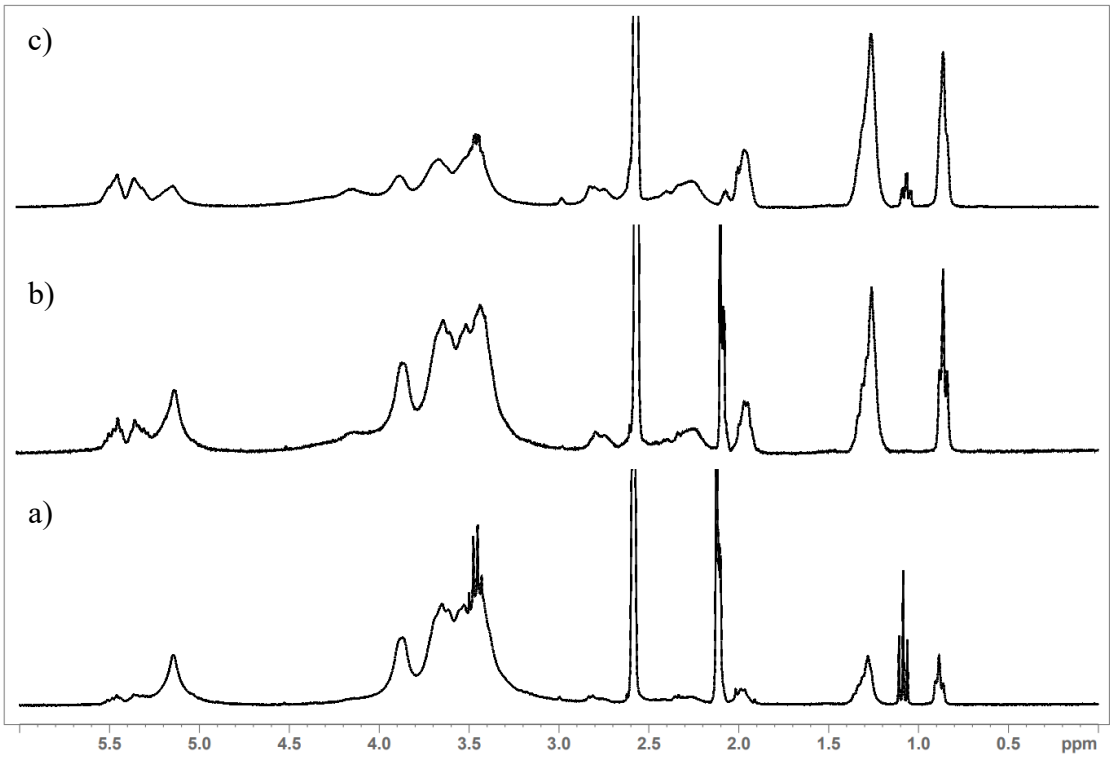


Figure 68. ^{1}H-NMR spectra for **a)** 3·30-24H, **b)** 3·50-24H, and **c)** 3·70-24H.

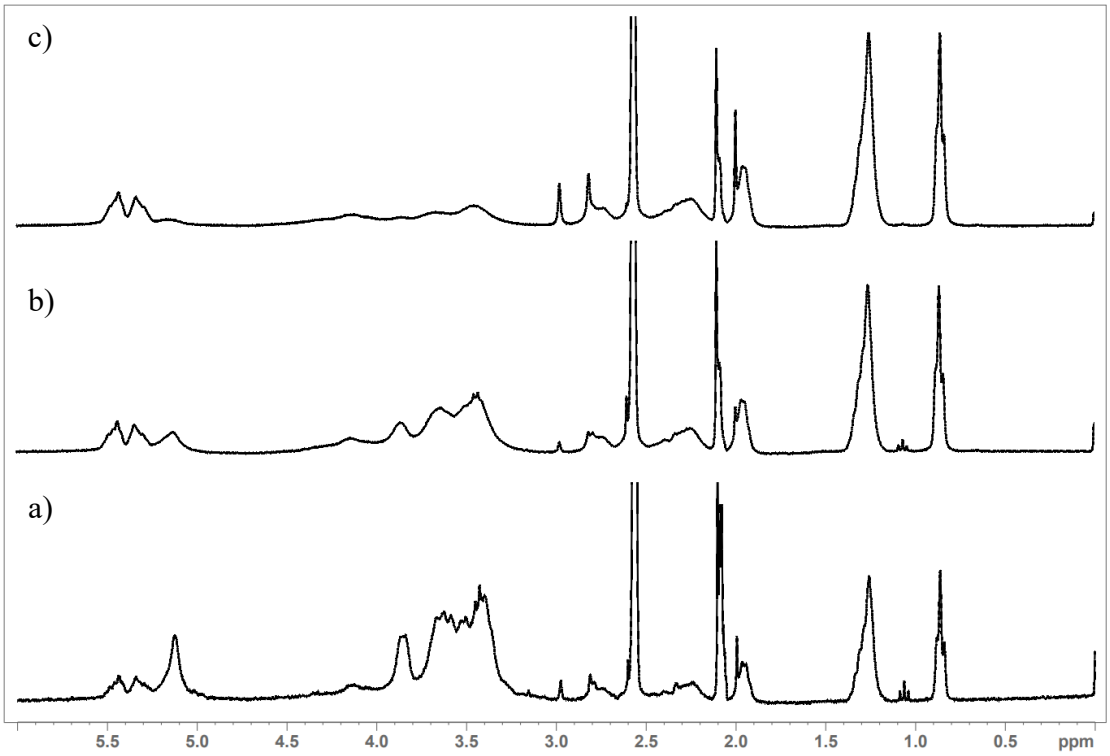


Figure 69. ^{1}H-NMR spectra for **a)** 1·70-72H, **b)** 3·50-72H, and **c)** 3·70-72H.

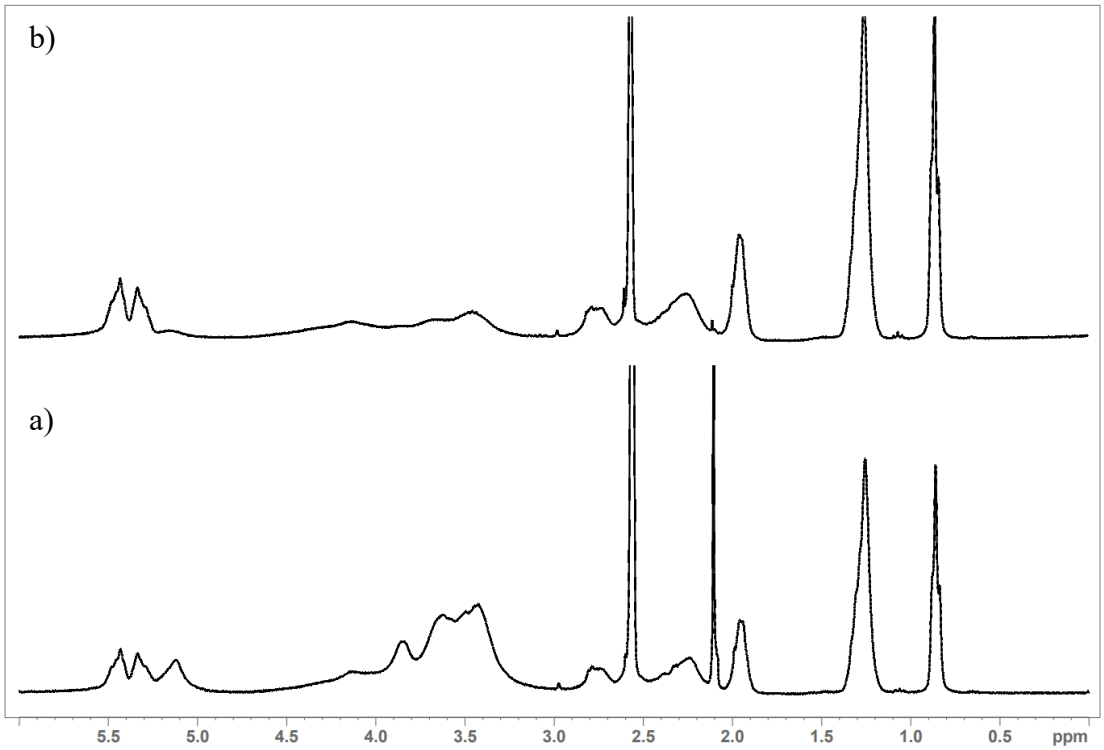


Figure 70. ^{1}H-NMR spectra for **a)** 1·70-24H-PYR, and **b)** 3·50-24H-PYR.

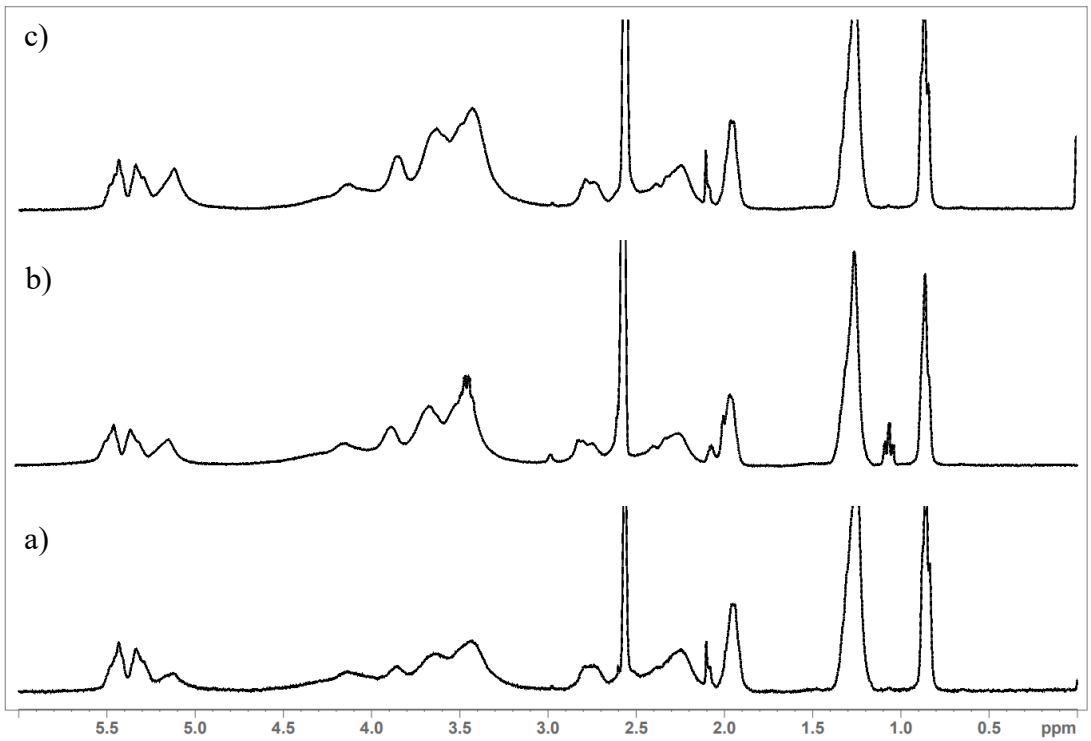


Figure 71. ^{1}H-NMR spectra for **a)** 3·70-24H-2.5% LiCl, **b)** 3·70-24H-5% LiCl, and **c)** 3·70-24H-7.3% LiCl.

AIII.II SIZE EXCLUSION CHROMATOGRAPHY

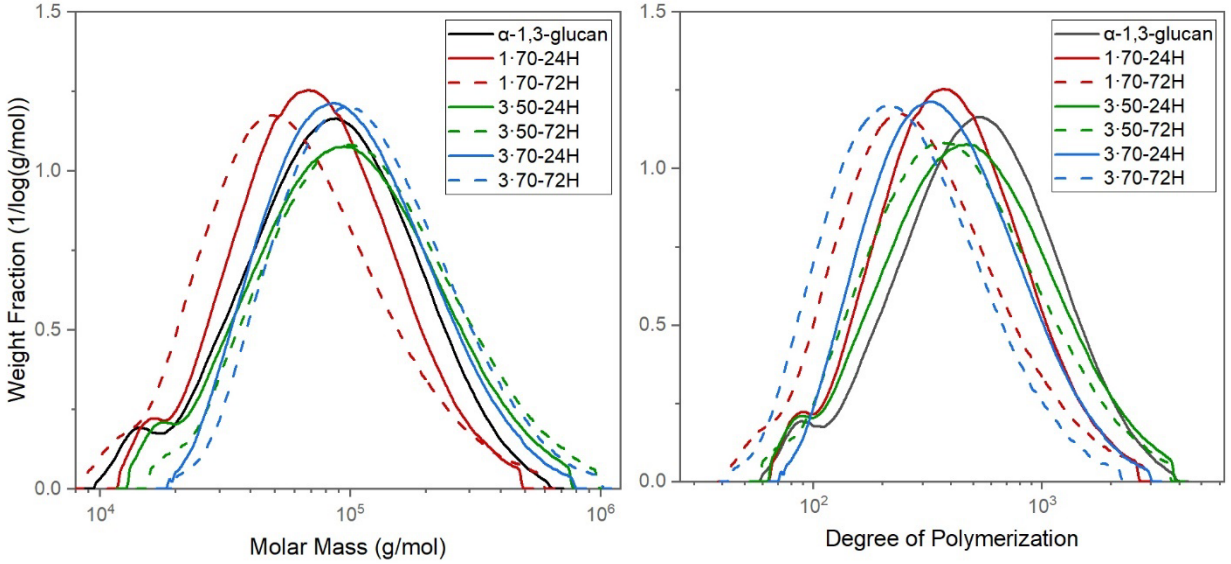


Figure 72. Molecular weight distribution traces for modified α-1,3-glucan to explore effect of reaction time.

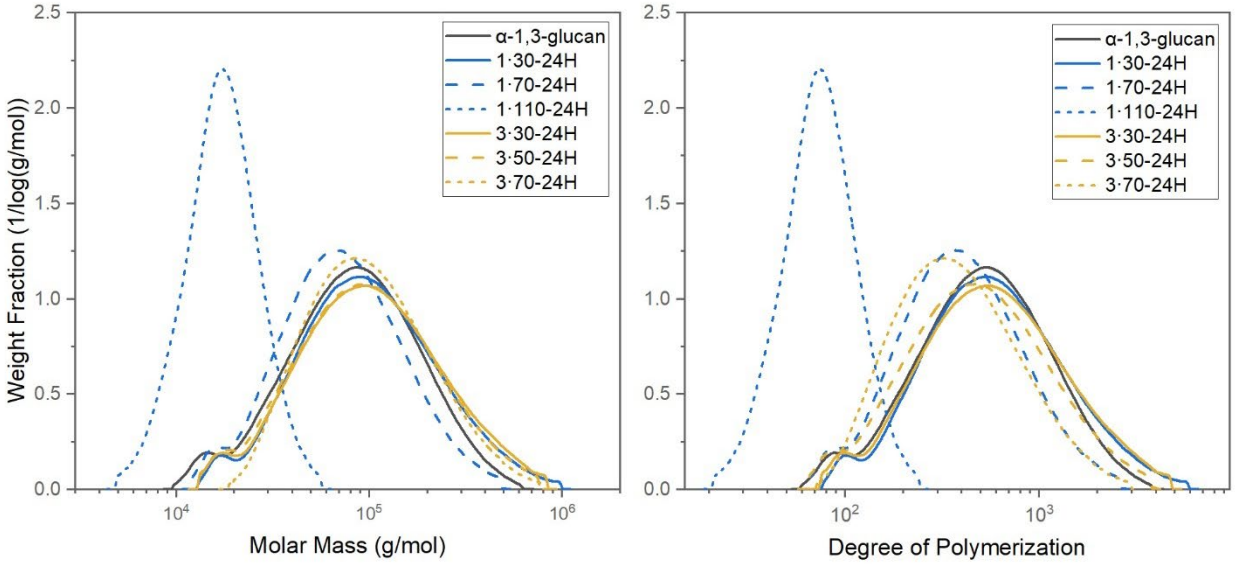


Figure 73. Molecular weight distribution traces for modified α-1,3-glucan to explore effect of temperature.

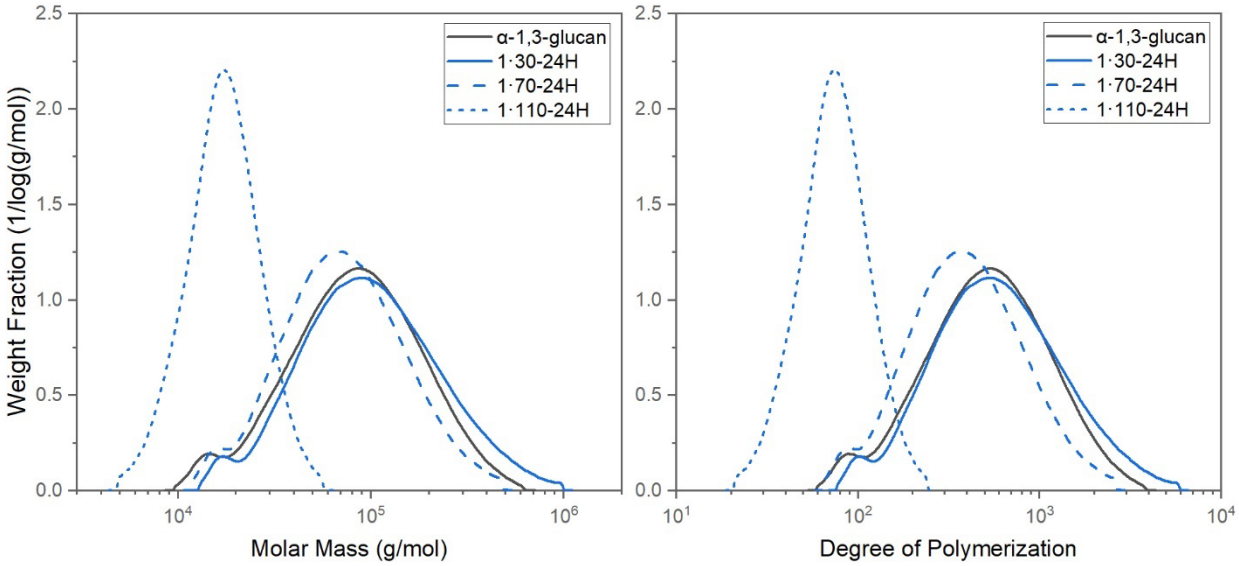


Figure 74. Molecular weight distribution traces for modified α-1,3-glucan to explore the effect of temperature at an OSA:AGU ratio of 1:1.

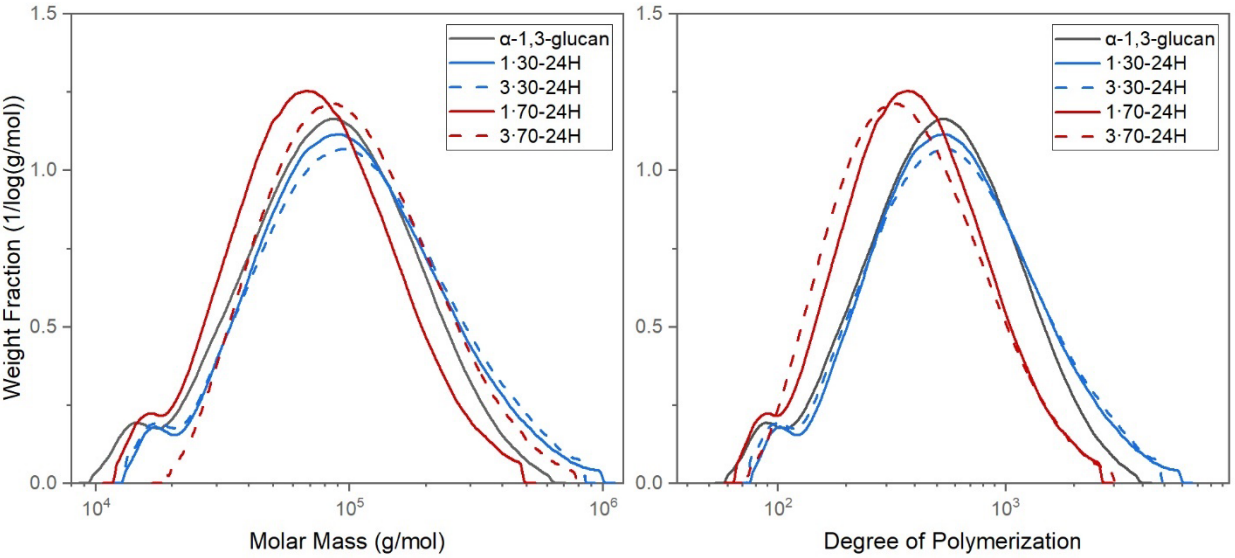


Figure 75. Molecular weight distribution traces for modified α-1,3-glucan to explore the effect of OSA:AGU ratio at a temperature of 30 °C and 70 °C.

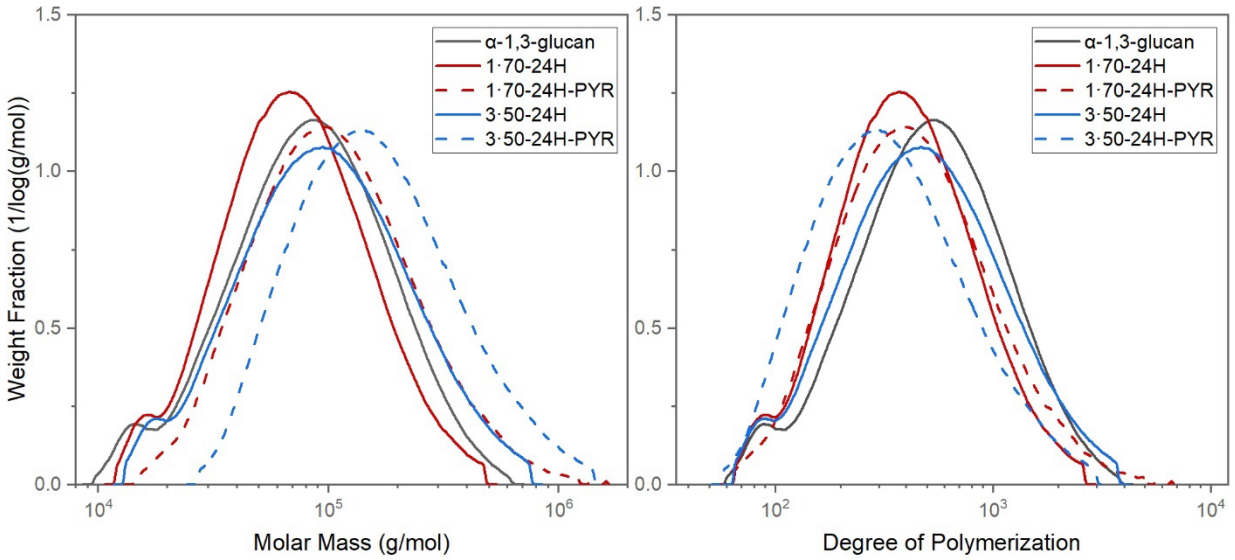


Figure 76. Molecular weight distribution traces for modified α-1,3-glucan to explore the effect of pyridine.

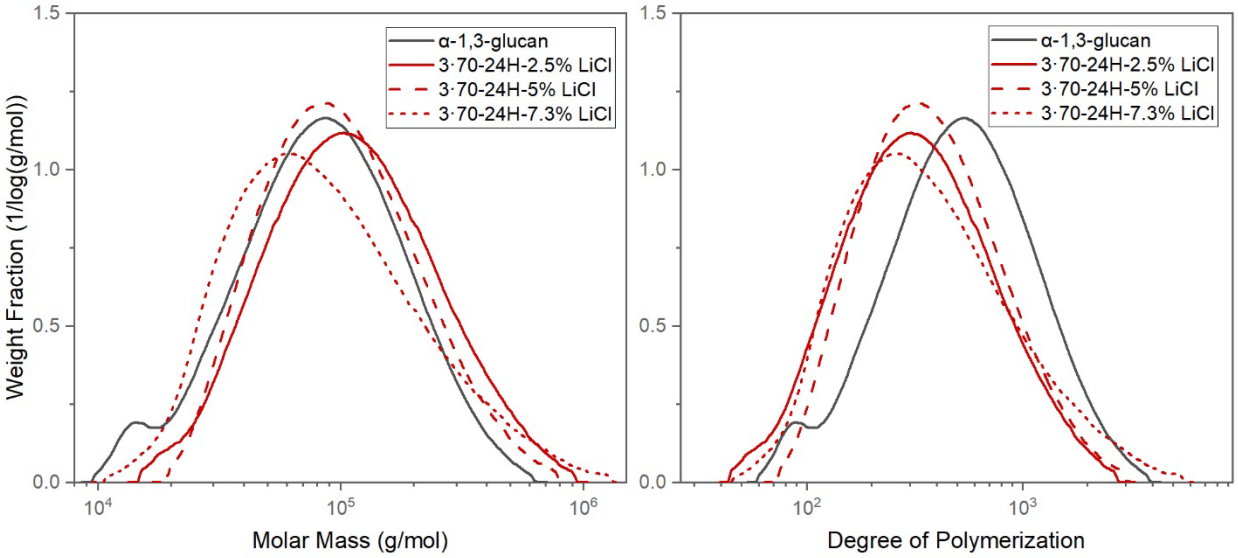


Figure 77. Molecular weight distribution traces for modified α-1,3-glucan to explore the effect of LiCl concentration.

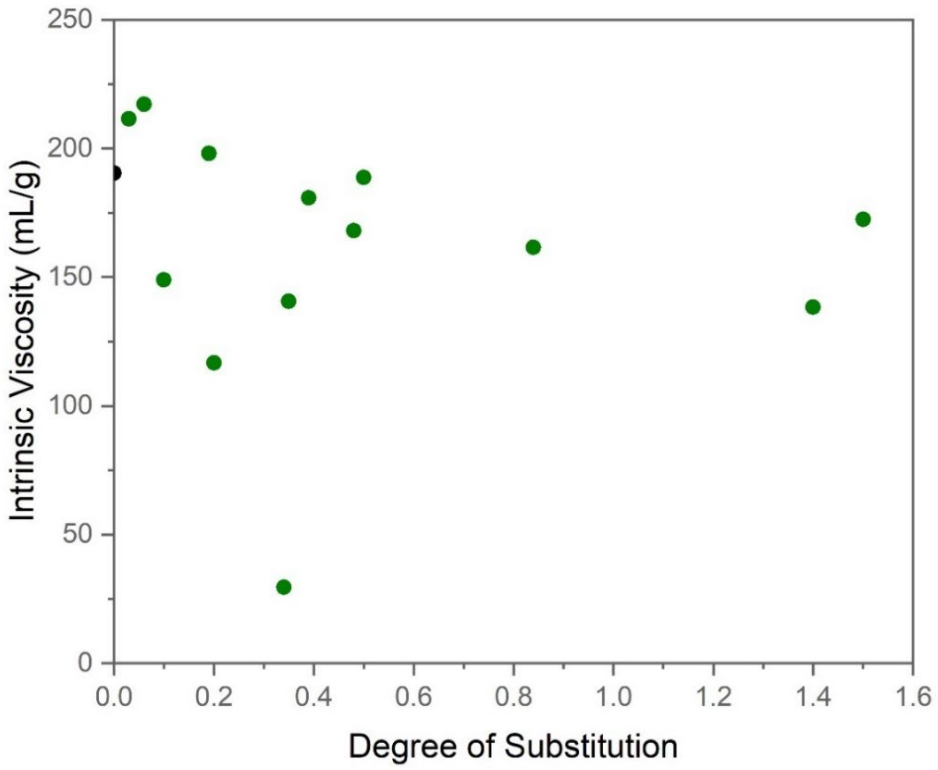


Figure 78. Intrinsic viscosity as a function of degree of substitution.

AIII.III DYNAMIC VISCOSITY

Figures 72 to 75 display the dynamic viscosity measurements of modified α-1,3-glucan samples separated to explore variations in viscosity compared with the reaction conditions.

Figure 79 shows the viscosity of modifications performed at varying temperatures for an OSA to AGU ratio of 1:1 and 3:1. Observing each molar ratio separately, the viscosity decreases with increasing reaction temperature for an OSA:AGU ratio of 1:1 while for an OSA:AGU of 3:1, the viscosity only clearly decreases upon increasing the temperature above 50 °C. From SEC measurements, increasing the temperature consistently increased the extent of degradation and the effect was more pronounced for an OSA:AGU ratio of 1:1.

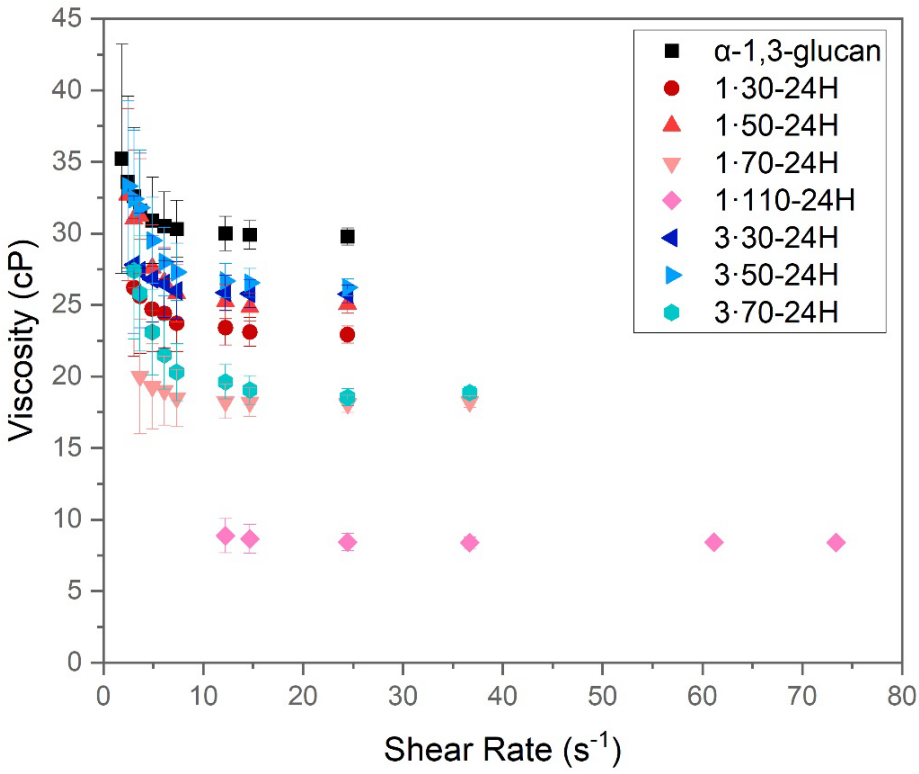


Figure 79. Dynamic viscosity measurements of samples to show effect of reaction temperature on the degradation of α-1,3-glucan.

The effect of reaction time on the sample viscosity is shown in Figure 80 where modifications were performed at 50 °C and 70 °C with OSA:AGU of 1:1 and 3:1. Observing each molar ratio separately, the viscosity decreases with increasing reaction temperature for an OSA:AGU ratio of 1:1 while for an OSA:AGU of 3:1, the viscosity only clearly decreases upon increasing the temperature above 50 °C. For all experiments, a longer reaction time caused a marked reduction in the dynamic viscosity.

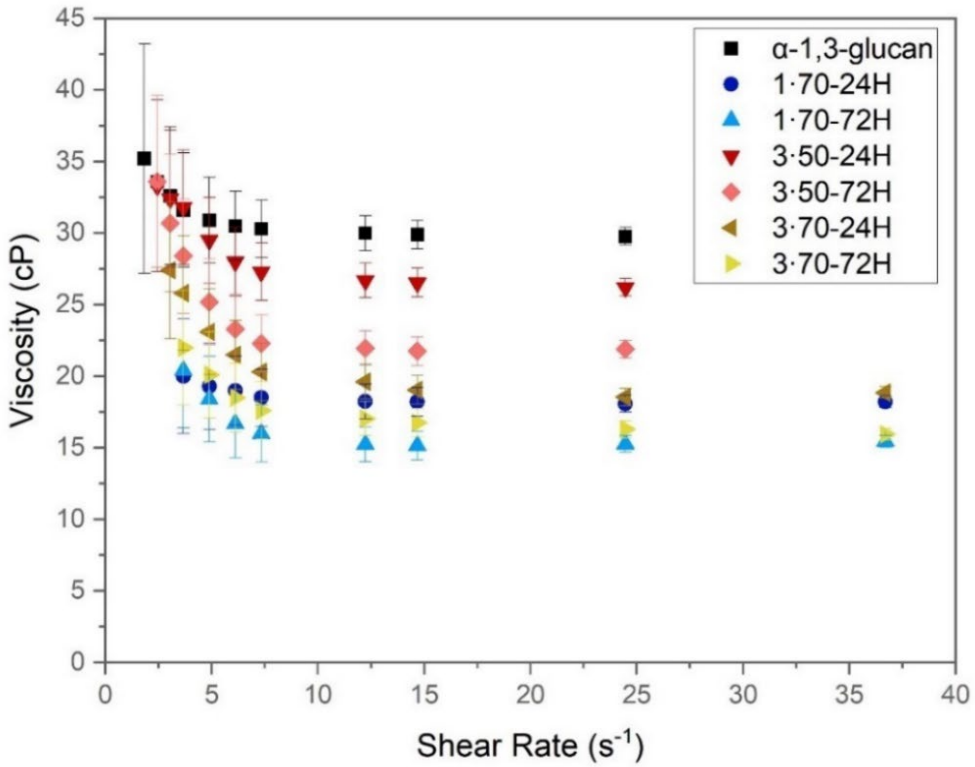


Figure 80. Dynamic viscosity measurements of samples to show effect of reaction time on the degradation of α-1,3-glucan.

In Figure 77, the effect of OSA concentration (or OSA:AGU) on the sample viscosity in solution is shown where adjusting the OSA:AGU from 1:1 to 3:1 causes a slight increase in the viscosity, but an increase from 3:1 to 6:1 causes a reduction in the dynamic viscosity.

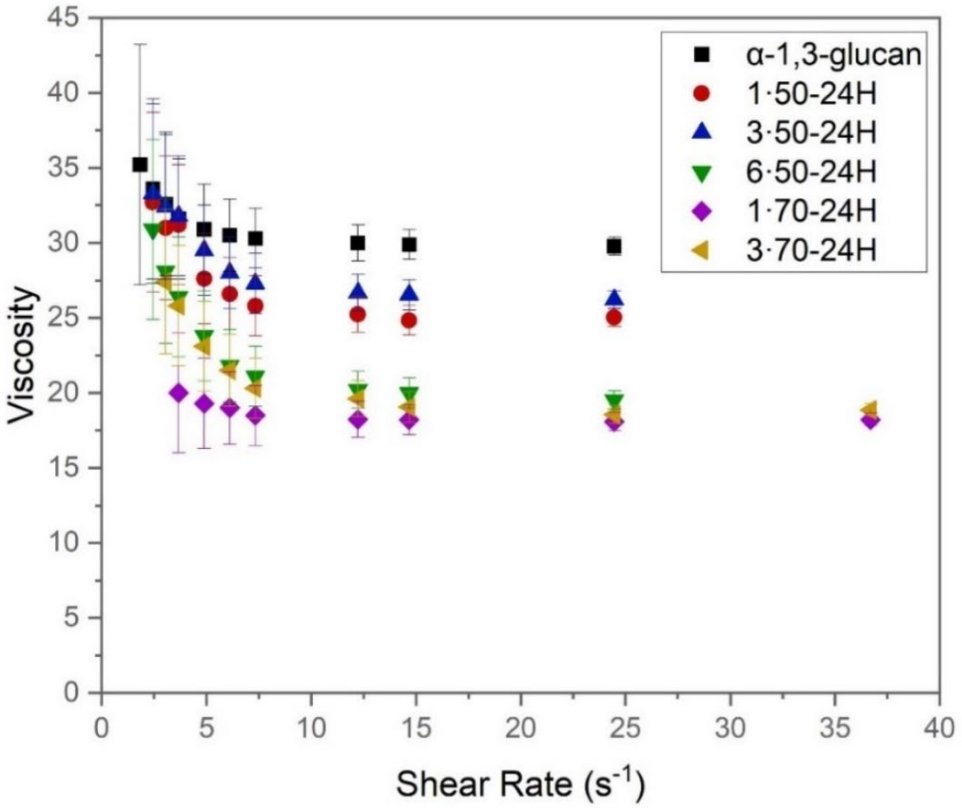


Figure 81. Dynamic viscosity measurements of samples to show effect of OSA concentration on the degradation of α-1,3-glucan.

Pyridine was added to the reaction solution in an attempt to prevent degradation of the polysaccharide backbone during modification. In Figure 82, the addition of pyridine correlates with a reduced decrease in viscosity relative to neat α-1,3-glucan at an OSA:AGU of 1:1; however, at an OSA:AGU of 3:1, the addition of pyridine caused a further reduction in viscosity. The viscosity measurements do not correlate well with the molecular weight measurements which showed that the addition of pyridine was effective in increasing the degree of substitution and reducing the extent of degradation.

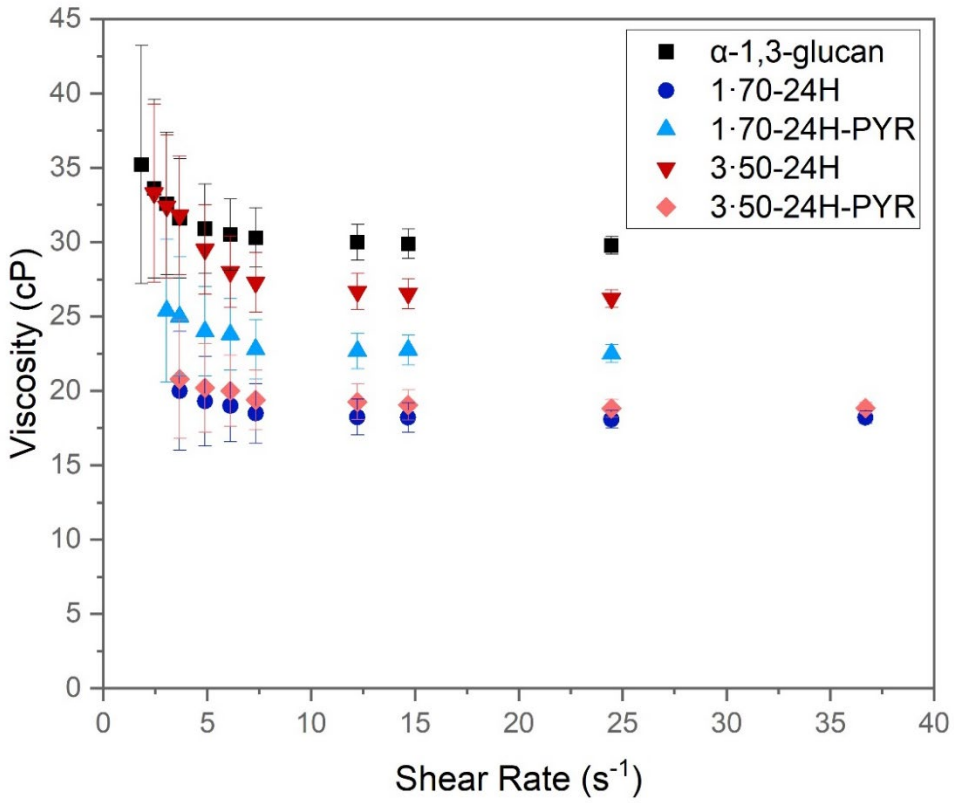


Figure 82. Dynamic viscosity measurements of samples to show effect of pyridine on the degradation of α-1,3-glucan.

AIII.IV THERMOGRAVIMETRIC ANALYSIS

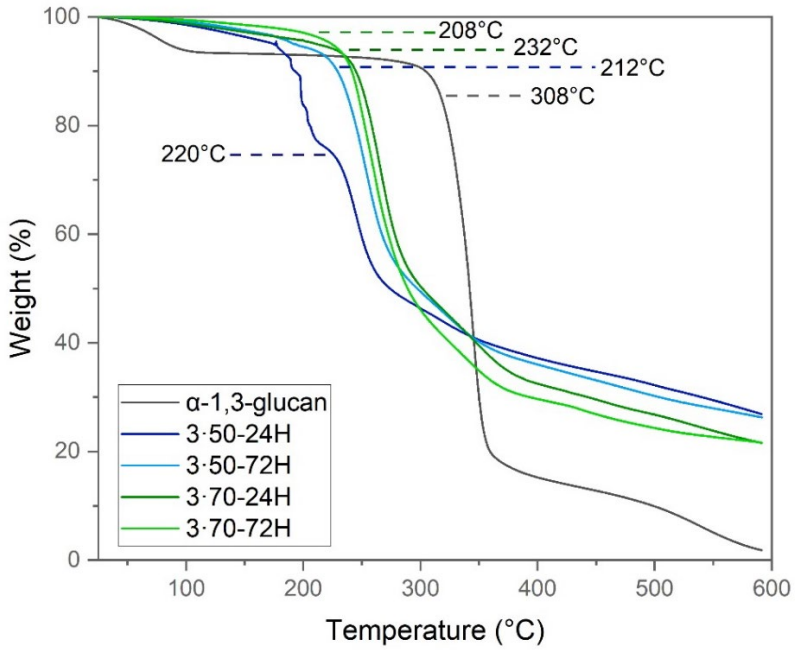


Figure 83. TGA curves of samples to show effect of reaction time on the thermal stability of α-1,3-glucan with degradation onset temperature identified.

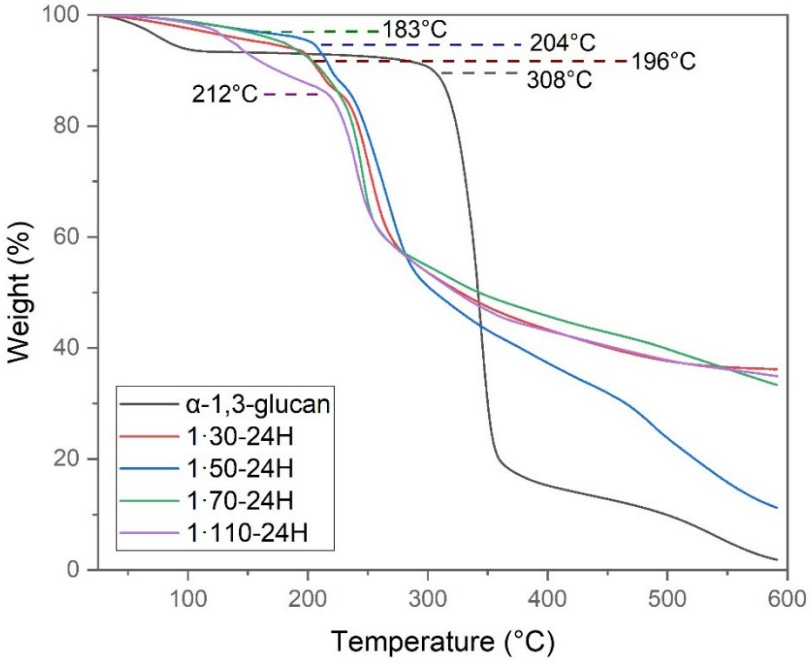


Figure 84. TGA curves of samples to show effect of reaction temperature on the thermal stability of α-1,3-glucan modified with an OSA:AGU of 1:1 with degradation onset temperature identified.

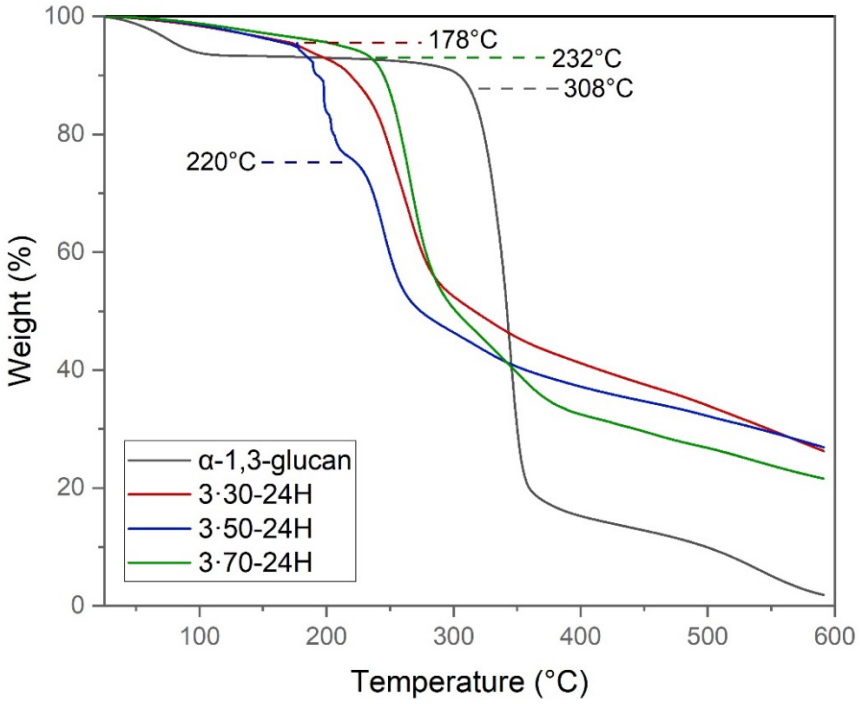


Figure 85. TGA curves of samples to show effect of reaction temperature on the thermal stability of α-1,3-glucan modified with an OSA:AGU of 3:1 with degradation onset temperature identified.

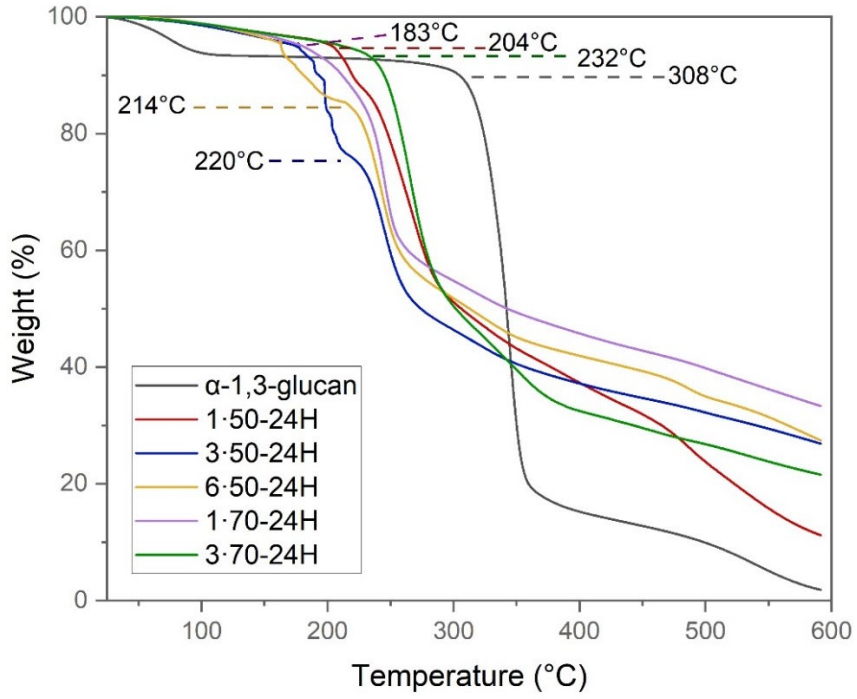


Figure 86. TGA curves of samples to show effect of OSA concentration on the thermal stability of α-1,3-glucan with degradation onset temperature identified.

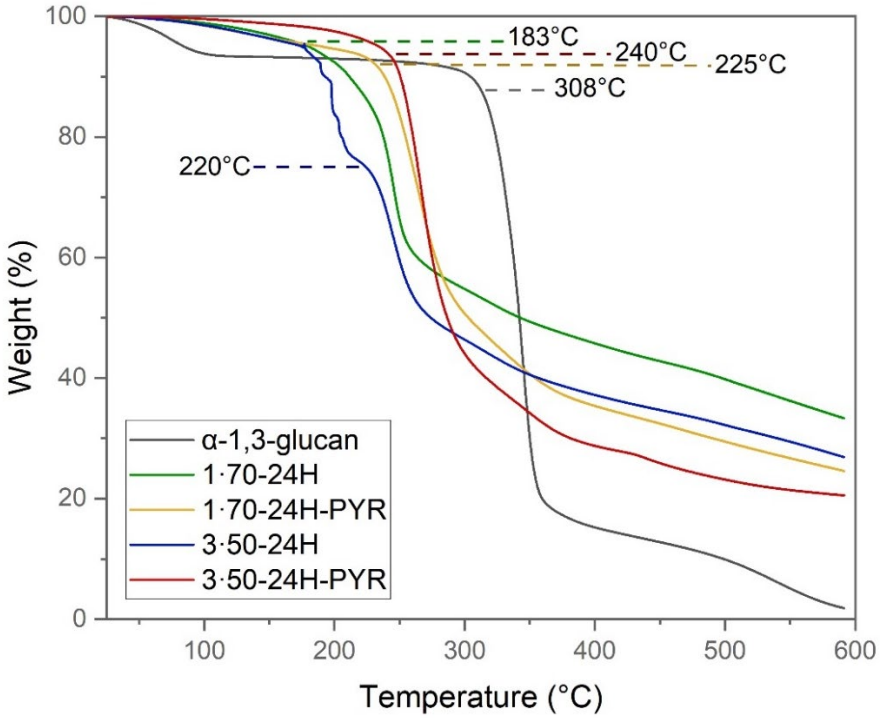


Figure 87. TGA curves of samples to show effect of pyridine on the thermal stability of α-1,3-glucan with degradation onset temperature identified.

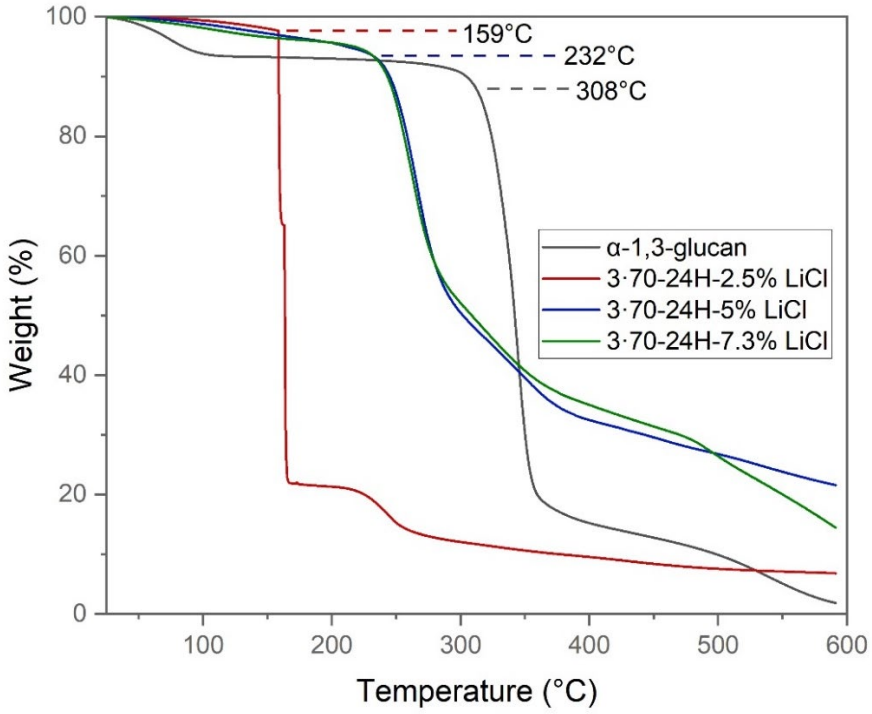


Figure 88. TGA curves of samples to show effect of LiCl concentration on the thermal stability of α-1,3-glucan with degradation onset temperature identified.

AIII.V DIFFERENTIAL SCANNING CALORIMETRY

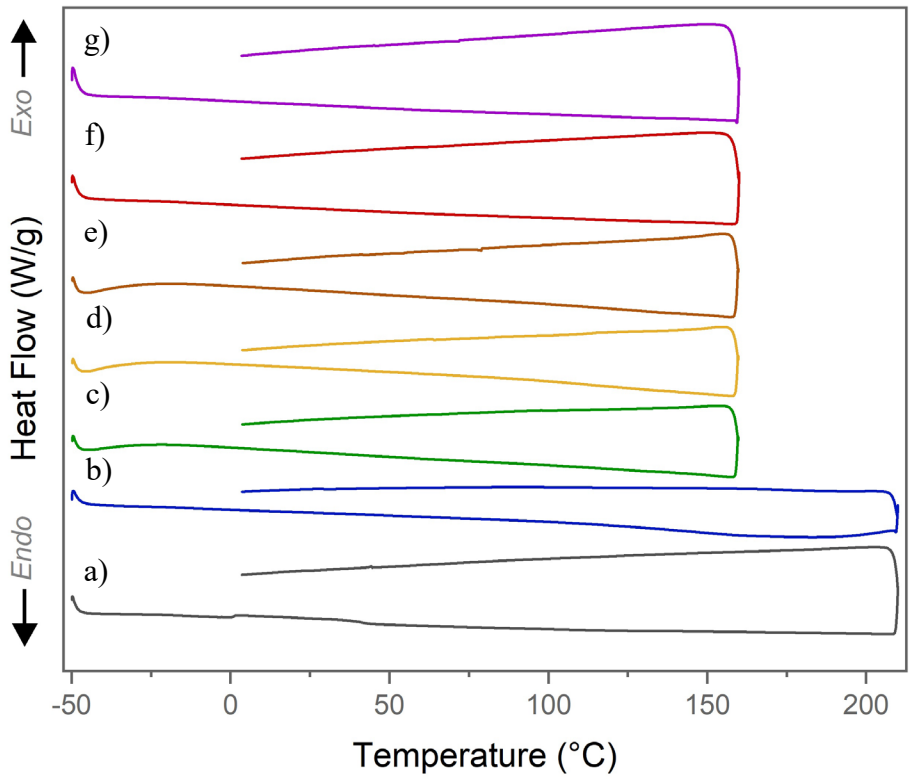


Figure 89. DSC curves of samples to show effect of reaction time on the thermal properties of α-1,3-glucan: a) neat α-1,3-glucan, b) 1·70-24H, c) 1·70-72H, d) 3·50-24H, e) 3·50-72H, f) 3·70-24H, g) 3·70-72H.

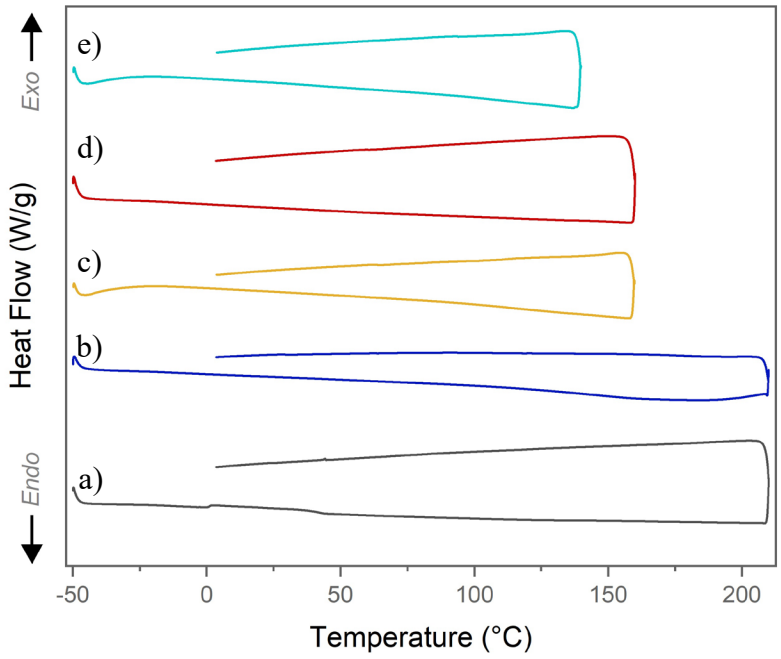


Figure 90. DSC curves of samples to show the effect of OSA concentration on the thermal properties of α-1,3-glucan: a) neat α-1,3-glucan, b) 1·70-24H, c) 3·70-24H, d) 3·50-24H, e) 6·50-24H.

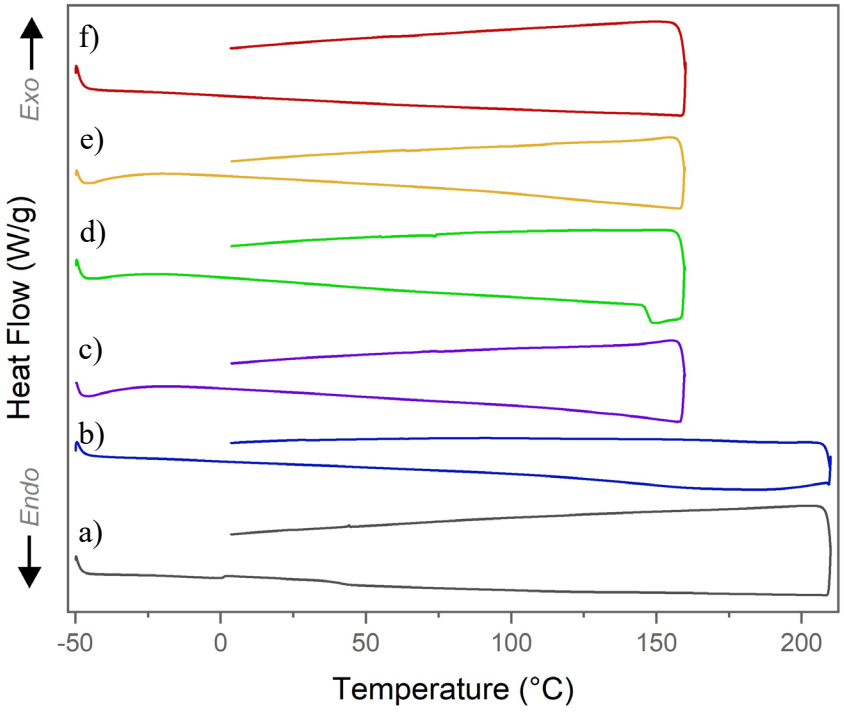


Figure 91. DSC curves of samples to show the effect of reaction temperature on the thermal properties of α-1,3-glucan: a) neat α-1,3-glucan, b) 1·70-24H, c) 1·110-24H, d) 3·30-24H, e) 3·50-24H, f) 3·70-24H.

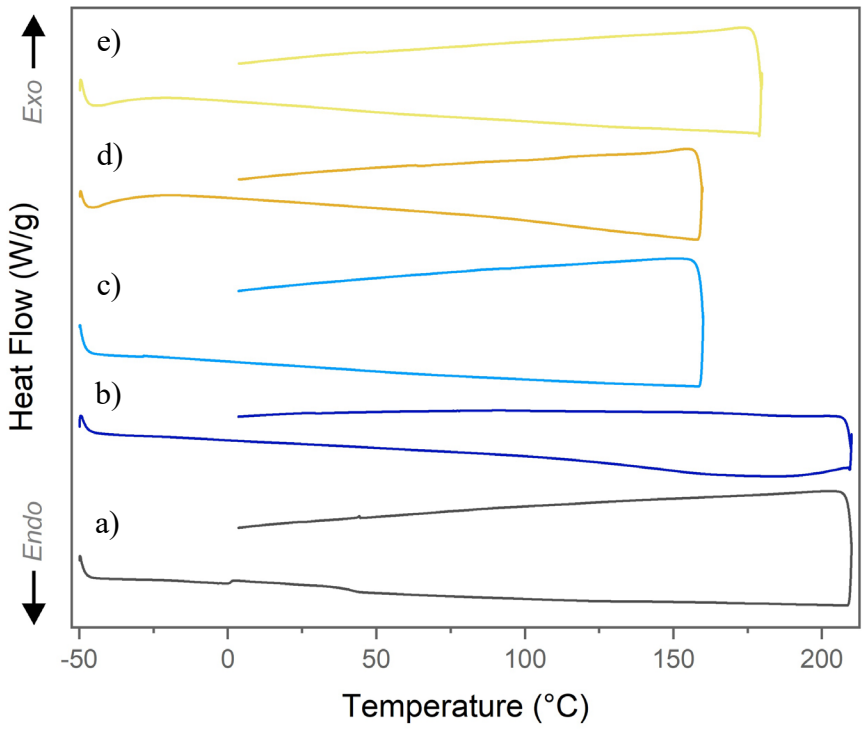


Figure 92. DSC curves of samples to show the effect of pyridine on the thermal properties of α-1,3-glucan: a) neat α-1,3-glucan, b) 1·70-24H, c) 1·70-24H-PYR, d) 3·50-24H, e) 3·50-24H-PYR.

AIII.VI PRELIMINARY EMULSION TESTING

Table 39. Qualitative observations of all oil/water mixtures with modified α-1,3-glucan samples for a settling time up to 30 minutes.

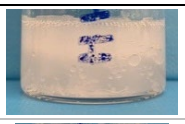
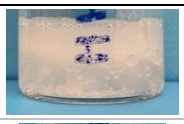
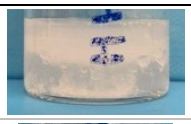
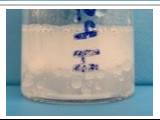
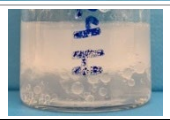
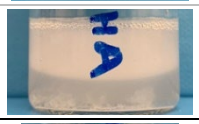
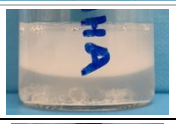
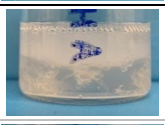
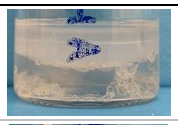
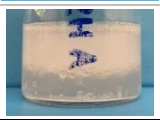
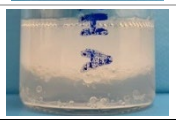
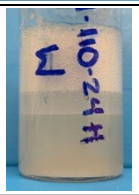
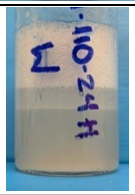
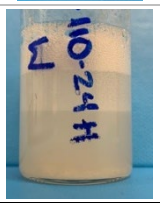
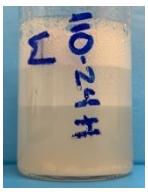
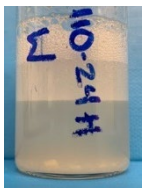
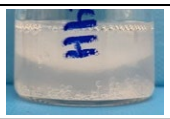
Sample	Oil Added	Observations		
		1.5 min	**5 min**	**30 min**
1·30-24H	2 g	*Top:* small bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with large droplets and settled solids		*Top:* opaque liquid with droplets *Bottom:* translucent liquid with large droplets and settled solids
	3 g	*Top:* small bubbles *Middle:* opaque liquid with droplets *Bottom:* translucent liquid with large droplets and settled solids	*Top:* small bubbles *Middle:* opaque liquid *Interface:* droplets *Bottom:* on translucent liquid with large droplets and settled and suspended solids	*Top:* opaque liquid *Interface:* solids, droplets *Bottom:* translucent liquid with droplets and suspended solids
1·70-24H	2 g	*Top:* bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled solids		
	3 g	*Top:* bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled solids		*Top:* opaque liquid *Bottom:* translucent liquid with settled solids
1·70-72H	2 g	*Top:* bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled and suspended solids and droplets		*Top:* opaque liquid *Bottom:* translucent liquid with suspended droplets and solids
	3 g	*Top:* bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled and suspended solids and droplets		*Top:* opaque liquid *Interface:* solids, droplets *Bottom:* translucent liquid with suspended droplets and solids

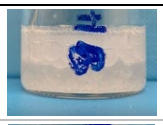
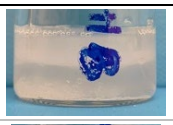
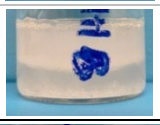
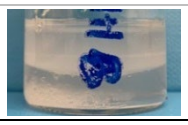
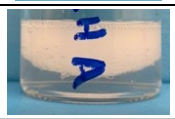
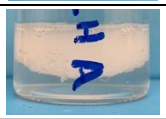
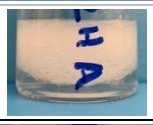
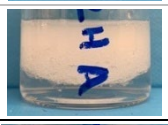
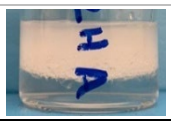
Sample	Oil Added	Observations		
		1.5 min	5 min	30 min
1·110-24H	2 g	*Top:* foam *Middle:* opaque liquid composed of small droplets *Bottom:* opaque liquid with settled solids		
	3 g	*Top:* foam *Middle:* opaque liquid composed of small droplets *Bottom:* opaque liquid with settled solids		
3·30-24H	2 g	*Top:* bubbles *Middle:* opaque liquid with large droplets *Bottom:* opaque liquid with settled solids	*Top:* bubbles *Middle:* opaque liquid with large droplets *Bottom:* opaque liquid with settled solids and suspended droplets	
	3 g	*Top:* bubbles *Middle:* opaque liquid with large droplets *Interface:* solids, droplets *Bottom:* translucent liquid with settled solids	*Top:* bubbles *Middle:* opaque liquid *Interface:* solids, droplets *Bottom:* translucent liquid with settled solids	*Top:* translucent liquid *Middle:* opaque liquid *Interface:* solids, droplets *Bottom:* transparent liquid with settled solids and droplets
3·50-24H	2 g	*Top:* bubbles *Middle:* opaque liquid with large droplets *Bottom:* translucent liquid with settled solids and droplets	*Top:* bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled solids and droplets	
	3 g	*Top:* bubbles *Middle:* opaque liquid *Bottom:* translucent liquid with settled solids and droplets	*Top:* bubbles *Middle:* opaque liquid *Interface:* a few solids, small droplets *Bottom:* translucent liquid with settled solids and droplets	*Top:* opaque liquid *Middle:* *Interface:* a few solids, small droplets *Bottom:* translucent liquid with settled solids and droplets

Sample	Oil Added	Observations		
		1.5 min	**5 min**	**30 min**
3·50-72H	2 g	*Top:* bubbles *Middle:* opaque liquid *Interface:* droplets, solids *Bottom:* transparent liquid		
	3 g	*Top:* bubbles *Middle:* opaque liquid *Interface:* solids, droplets *Bottom:* transparent liquid	*Top:* opaque liquid *Interface:* solids, droplets *Bottom:* transparent liquid	
3·70-24H	2 g	*Top:* bubbles *Middle:* opaque liquid *Interface:* small droplets, solids *Bottom:* transparent liquid,		
	3 g	*Top:* opaque liquid with small droplets *Interface:* solids, small droplets *Bottom:* transparent liquid		*Top:* opaque liquid *Interface:* solids *Bottom:* transparent liquid
3·70-72H	2 g	*Top:* bubbles *Middle:* opaque liquid with suspended solids, small droplets *Bottom:* translucent liquid with small, suspended droplets		
	3 g	*Top:* bubbles *Middle:* opaque liquid *Interface:* solids *Bottom:* translucent liquid with suspended droplets	*Top:* opaque liquid *Interface:* solids *Bottom:* translucent liquid with suspended droplets	

Note: due to technical difficulties images and observations after 1 g of oil were lost and are thus omitted.
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

Table 40. Photographs of all oil/water mixtures with modified α-1,3-glucan samples for a settling time between 1.5 and 30 minutes.

Sample	Oil Added	Photographs		
		1.5 min	5 min	30 min
1·30-24H	2 g			
	3 g			
1·70-24H	2 g			
	3 g			
1·70-72H	2 g			
	3 g			
1·110-24H	2 g			
	3 g			
3·30-24H	2 g			
	3 g			

Sample	Oil Added	Photographs		
		1.5 min	5 min	30 min
3·50-24H	2 g			
	3 g			
3·50-72H	2 g			
	3 g			
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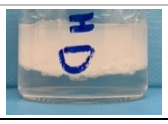
	3 g			
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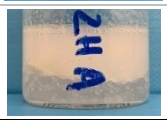
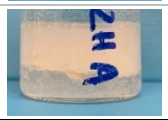
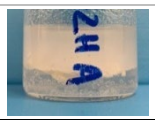
	3 g			

Note: due to technical difficulties images and observations after 1 g of oil were lost and are thus omitted.
Sample label: ratio·temperature·time; example: 1·70-4H has a molar ratio OSA:AGU 1:1, 70 °C, 4 h

AIII.VII DETERMINATION OF ACTIVATION ENERGY

Table 41. Rate constants and activation energies for the esterification of α-1,3-glucan with OSA.

Rate Order	Sample	Rate Constant, k	Sample	Rate Constant, k	Activation Energy, E_a ($J \cdot mol^{-1} \cdot K^{-1}$)
Zeroth (k in $mol \cdot L^{-1} \cdot s^{-1}$)	3·50 S1	0.0022	3·70 S1	0.0036	2.2×10^4
			3·70 S2	0.0039	2.5×10^4
	3·50 S2	0.0009	3·70 S1	0.0036	6.4×10^4
			3·70 S2	0.0039	6.8×10^4
First (k in s^{-1})	3·50 S1	0.0033	3·70 S1	0.0061	2.8×10^4
			3·70 S2	0.0060	2.7×10^4
	3·50 S2	0.0012	3·70 S1	0.0061	7.3×10^4
			3·70 S2	0.0060	7.2×10^4
Second (k in $L \cdot mol^{-1} \cdot s^{-1}$)	3·50 S1	0.0050	3·70 S1	0.0106	3.5×10^4
			3·70 S2	0. 0092	2.8×10^4
	3·50 S2	0. 0017	3·70 S1	0.0106	8.3×10^4
			3·70 S2	0. 0092	7.7×10^4