PRETREATMENT, PROCESSING, AND PRODUCTION OF HYDROGELS USING NANO-SIZED BIOMASS

by

LAUREN MCCOY

(Under the Direction of Suraj Sharma)

ABSTRACT

Society as a whole produces an excessive amount of waste and thinks it's ok to do so. Government regulations encourage this due to high standards and lots of regulation, so there is a lot of biomass that is unused wasting away. This is especially true for the paper and cotton industries. This research aims to take the biomass and create hydrogels by producing nano-sized particles created from the biomass. These hydrogels are versatile and do not create excessive amounts of additional waste. They are pretreated using sustainable materials, carboxymethylcellulose (CMC), and use homogenization to produce nanofibrillated hydrogels. The biomasses used in this research were from kraft pulp, cotton noil and silk sources. After being produced into hydrogels, their rheological, morphological, and thermal properties were analyzed. This research also suggests several potential applications such as biomedical sutures, textile dyeing, and composites that these hydrogels can be applied to.

INDEX WORDS: Hydrogel, Sustainability, Nanomaterials, Homogenization, Pretreatment, Biomass

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DEDICATION

I dedicate this thesis to my husband, Matthew, my parents, Russell and Susan, and my sister, Alison. Thank you for your support, encouragement, and love throughout the years. You all have truly been an inspiration to me and continued to push me to become the greatest version of myself. I would have not been able to accomplish so much without you all. Thank you from the bottom of my heart. I love you guys!

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“Being strengthened with all power according to his glorious might so that you may have great endurance and patience, and giving joyful thanks to the Father, who has qualified you to share in the inheritance of his holy people in the kingdom of light.” Colossians 1:11-12

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CHAPTER 1

INTRODUCTION

With the world's population continuously growing, space is becoming ever more limited, factory production and output is increasing due to higher demands, thus, increasing the amount of human and factory waste on the environment. This is a problem everyone should be concerned with because the more waste and less space there is, the more health and environmental problems we will see arise. Because of quality standards for products, production processes, factory operation needs, and the wastefulness of mankind, there is a substantial amount of waste material in the current manufacturing processes in most industries. The refining and processing steps are so selective that the amount of material that is deemed non-usable in regards to treating raw materials or that is generated during production is sizable. The amount of usable material is substantially less than un-usable material, therefore, creating a surplus of unused material that is thrown away, causing greater landfill issues, burned, which introduces carcinogens into the air, or that is stored in a facility, wasting space. With an abundance of waste material, comes a need to find uses of and applications for those materials that do not create additional waste and that are environmentally friendly. The majority of the materials are just biomass with no discovered use yet.

From the Egyptian papyri to the development of paper by the Chinese, cellulose based biomass has been present in history, but not to the full capacity of its abilities[7].

Scientists have been studying cellulose for over 150 years, but in recent times due to technology advances, have really begun to see the potential that cellulose biomass has to offer. Biomass and waste have recently been a priority for researchers because of the environmental problems manufacturing is causing and because of the excess of waste and limited amount of physical space. There are two cellulosic waste materials that have sparked a significant amount of interest due to their availability: kraft pulp from woody materials used in paper production and cotton noil from the cotton plant. Specifically in the process of paper milling, there is a excess of waste pulp produced during the course of making paper products that is deemed un-fit for paper processing and paper products. Another example is that of cotton noil. Cotton noil is a byproduct from the cotton yarn manufacturing process. The noil is generated during the combing process when the noil is combed out because it is too short to use for cotton yarn. The noil can be expended in other useful ways. This research seeks to create hydrogels from such waste material that can then be used for a variety of applications ranging from textile dyes to biomedical sutures. The majority of this research focuses on the pretreatment methods of the waste materials, cellulose and cotton noil. Pretreating the lignocellulosic byproducts can modify the hydrogel characteristics, and therefore, enhance the natural properties of the base materials. **Figure 1** demonstrates the structure of the natural fiber cell that cellulose based materials are found in.

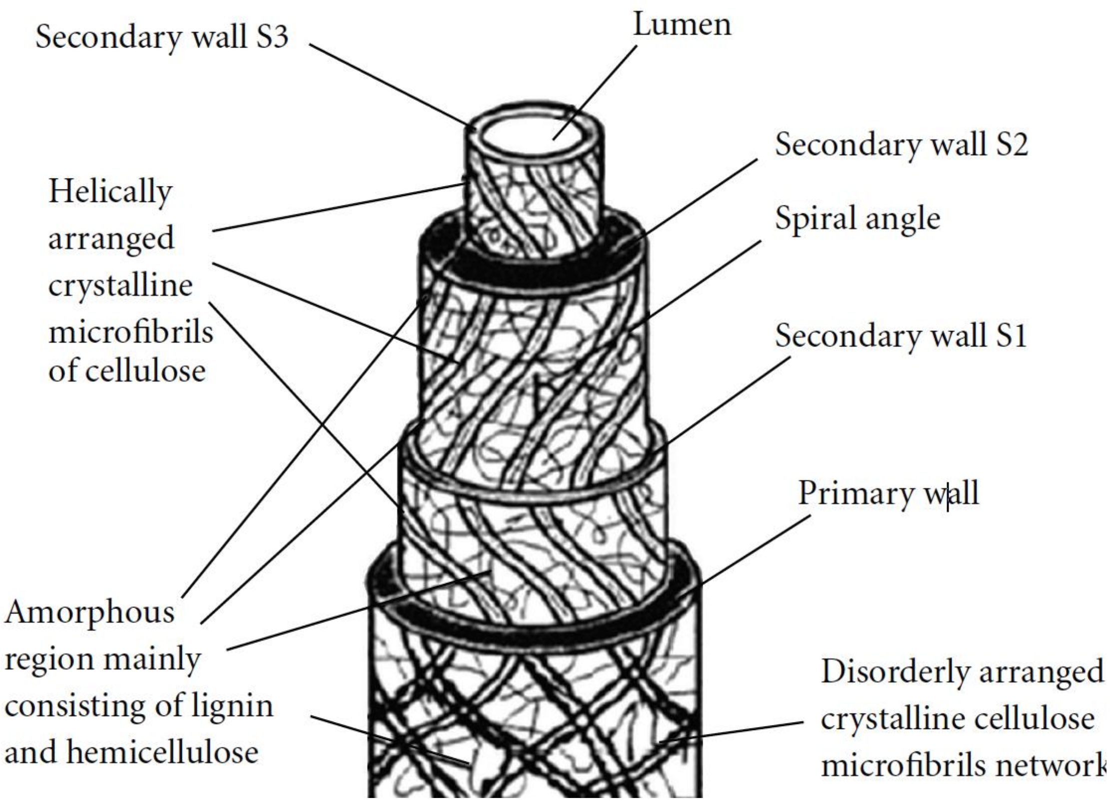


Fig. 1. Structure of natural fiber cell. Reprinted with permission from[4]

Cellulose is located within the cell walls of the plant cells and is found along with many other amorphous and crystalline regions like as seen above. Because there are so many additional parts of a plant, the cellulose has to be extracted. This is called pretreatment and it's why there are so many pretreatment methods developed and being developed.

In research, there are several pretreatment methods that are commonly used: TEMPO oxidation, caustic soda treatment, and carboxymethylation. The research presented in this manuscript used pretreatment methods developed and optimized in the research lab. Once the additional amorphous and crystalline regions of the plant are removed, the cellulose is then ready to be processed and produced into a hydrogel. There are various mechanical methods that include mills, microfulidizers, and homogenizers for defibrillation. This research used the method of homogenization. Homogenization was used over microfluidization because homogenizers use multiple chambers for splitting, or defibrillating, materials, whereas microfluidizers only use one direct chamber. Homogenizers work using a process called cavitation. The mixture of cellulose and liquid is poured into the feed hopper and pushed through a roughly 1 mm hole at a high pressure, high shear force, high temperature where it is sheared down to nano-sized cellulose fibrils. The material passes through two chambers where a small steel ball shears the material due to the pressure the cellulose mixture is being pushed through, producing a homogeneous hydrogel upon finishing the homogenization process. Microfluidization, however, works by pushing the material through one long zig-zagged chamber where the material bounces off the harsh angles and is sheared down to nano-sized material, also producing a homogeneous hydrogel as well. Both the hydrogels produced from homogenizers and microfluidizers are comparable in characteristics, but for this research, a homogenizer was used (**Figure 2**).

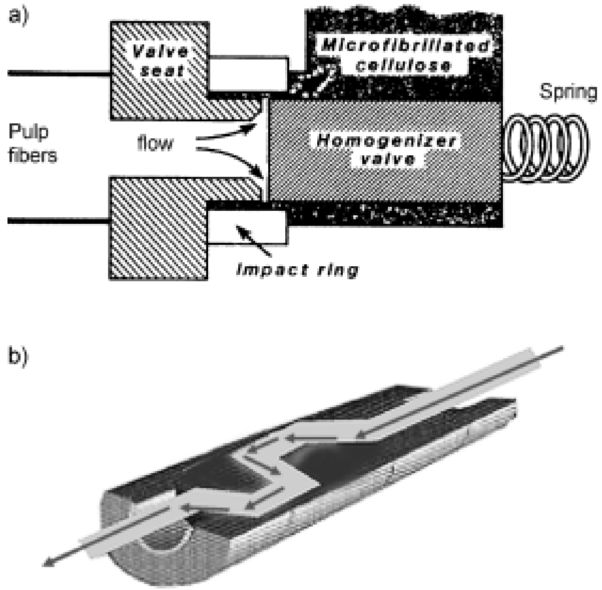


Fig. 2. Homogenizer schematic (top) vs microfluidizer schematic (bottom)
Reprinted with permission from[5]

Another material that has potential to become a useful hydrogel, silk, is also briefly studied in order to characterize its gelling capabilities as a nanostructured material. The research aims to find out if the developed pretreatment methods are comparable to the commonly used methods such as TEMPO oxidation, caustic soda, or carboxymethylation, in order to make sure the fabricated hydrogel is performing up to standards and current products. Pressure plays a large part in the gelling process, and the effect of pressure is also to be studied. After using lignocellulosic materials, the research aims to be able to apply the processing to protein-based materials and any other biomass. Though there are plenty of useful ways to pretreat lignocellulosic byproducts currently, the majority of them use harsh chemicals. This research uses natural materials, and materials that are substantially less harmful to the user, scientist, or environment.

HYPOTHESES

- The hydrogel pretreatment and production method developed and optimized in this research can be applied to any cellulose based lignocellulosic byproduct as well as produces a hydrogel that is characteristically comparable to current pretreatment and production methods of cellulosed based materials such as TEMPO oxidation, caustic soda treatment, and carboxymethylation.

- Hemicellulose content affects gelling time and temperature.

OBJECTIVES

- To determine if the pretreatment method developed and optimized in this research to make hydrogels from cellulosic byproducts is comparable to current, popular pretreatment methods
- To determine if pressure influences gelling capabilities of cellulosic hydrogels
- To determine if the pretreatment and processing methods can be applied to other cellulosic based materials, not just kraft pulp
- To determine if the pretreatment and processing methods can be applied to protein based materials such as silk or wool

LIMITATIONS

- Only one concentration of material to gel ratio, 2%, will be studied. Results could vary depending on the concentration of the gel studied.
- The pretreatment methods used for comparison are only some of the common ones used in current research. This research does not cover every single method that is currently published.

CHAPTER 2

REVIEW OF LITERATURE

HYDROGELS

Hydrogels are hydrophilic biomaterials[8]. They can be natural or synthetic and have been historically used in tissue regeneration because their physical properties are similar to the extracellular matrices in human tissue. They are a series of 3D hydrophilic polymer networks and can absorb up to hundreds of times their dry weight due to the crosslinking between polymer chains[9]. They are extremely permeable, retain large amounts of water, have a low coefficient of friction, and have been used not only in the medical field, but in agricultural, food, and pharmaceutical industries as well[10]. There are two classes of hydrogels, physical and chemical[11]. Physical hydrogels have weak connections and are reversible when chains break, but chemical polymers are permanently bonded using covalent bonds and are much stronger than the physical class. Hydrogels also get their strength and class from their production method. Various types of copolymerization starting from different starting polymers can change not only the characteristics of the hydrogel, but what end uses it can be used for. As mentioned before, hydrogels can be made from natural materials like polysaccharides or from synthetic materials like vinyl acetate, ethylene glycol, or lactic acids. Their structures can be amorphous, semicrystalline or hydrogen-bonded in physical structure. Recently, hydrogels have been produced to be stimulus responsive, where the hydrogels react to the environment around them[12]. Hydrogels have many uses and many capabilities. They can be produced through different ways from a variety of materials. This makes them a viable option to use when using a versatile polymer such as cellulose.

CELLULOSE

Cellulose, the one of the most abundant natural polymers found on Earth, is an "inexhaustible raw material that has been a key source of sustainable materials" for an ever growing range of applications [5]. Since the Chinese discovered the papermaking process in 150 B.C., cellulose has been of interest to scientists despite the lack of general knowledge about its characteristics and potential. It has been extensively researched and developed for over 150 years and has been previously used as an energy source, for building materials, for clothing, and for paper like materials. However, it has currently shown characteristics to be used in biomedicine and other novel applications, especially since scientists have demonstrated the ability to produce nanocellulose[7]. Cellulose, a chain polymer, is comprised of the glucose units, β-D-glucose, and are found in 6-membered rings, pyranoses, that are joined by single oxygen atoms between the C-1 and C-4 of adjoining rings (**Figure 3**).

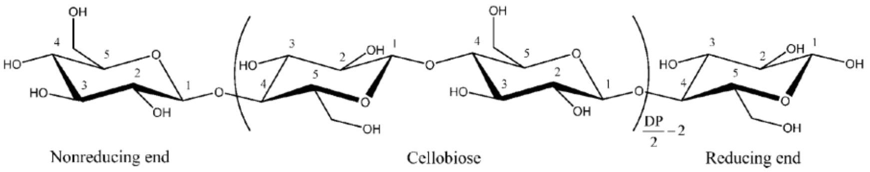


Fig. 3. Chemical structure of cellulose Reprinted with permission from[1]. Copyright (2009).American Chemical Society.

Cellulose has presented itself as a desirable sustainable biomass, not only because it is naturally abundant, can be extracted from many natural sources, and has historically demonstrated its versatility, but it has also many scientifically desirable qualities. The advantageous qualities of cellulose include: abundance in nature, biodegradable, nonabrasive, cost effective to produce and obtain, low weight, nontoxic, and low density[4]. The toxicity studies of cellulose have been conducted comparing it to table salt, but when it produced to be nanocellulose, the toxicity of it is not known, but thought to still be relatively low. Cellulose should not be inhaled in large quantities, especially cellulose nanocrystals (CNC) because they can cause some self-aggregation in the body of animals. In regards to cellulose nanofibrils (CNF), there have been no reports of cytotoxicity and inflammatory effects, as well as chromosome or DNA damage have not been detected in mouse and human macrophages. However, there have been some reports of low acute environmental toxicity, which was performed by kinetic luminescent bacteria testing. Bacterial cellulose (BC) also displays no cytotoxicity due to observations of osteoblast cells and endothelial cells in mice, but the study noted that not enough testing and current research is available to definitively say that there is no cytotoxicity in BC. It also provides many favorable biological properties such as its biocompatibility with the human body, only entreating minimal, if any, adverse foreign body responses *in vivo* because it is not readily digestible by the human body due to its lack of cellulolytic enzymes [13]. It should be noted, though, that different pretreating and production processes of refining cellulose or cellulose derivatives can change the biocompatibility of cellulose. This is due to the nature of the chemicals involved in these processes. The mechanical properties of cellulose as well as cellulose nanoparticles have to offer are influenced by several factors including the chemical composition and location in the plant, the matureity of the plant, how it is extracted, any defects in its source, and soil and weather conditions of the source [2]. Cellulose microfibrils demonstrate a longitudinal modulus of 100 GPa, while CNC exhibits one of 130 GPa. CNCs also exhibit a Young's modulus of 70 GPa with a density of 1.5-1.6 g cm^{-3} which is higher than glass fibers and very similar to Kevlar, but could potentially be stronger than steel. Some extraction methods and pretreatment methods can use harsh chemicals that should not be digested by the human body. Cellulose can be extracted from a variety of sources ranging from bacteria to wood pulp to fibrous plants. Once extracted from its natural sources, studies have shown that producing nanocellulose from cellulose sources is more beneficial to the user and produces a stronger, better material to work with. Nanocellulose is typically found in three different forms: CNC, CNF, and BC. Each exhibits different qualities and has a specific set of characteristics.

CELLULOSE NANOCRYSTALS (CNC)

CNC has several names including whiskers and nanocrystalline cellulose [5]. They are rodlike and rigid in structure, having few amorphous regions, low flexibility, and dimensions averaging 5-70 nm in width and 100 nm to several microns in length, which is typically shorter and thinner than MFC **(Figure 4)**. A defining characteristic of CNC is the crystallinity, which is higher in CNCs than other types of cellulose. Its crystallinity is approximately 70% with 30% amorphous structures[14]. CNC is

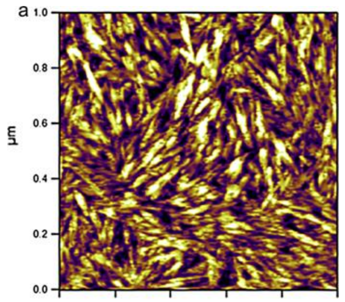


Fig. 4. AFM image of CNC rods. Reprinted with permission from[6]

generated from removing amorphous sections of purified cellulosic sources, such as wood, plants, animals, bacteria, and algae, by acid hydrolysis using mineral acid. The mineral acid used in CNC production plays a major role in the characteristics the CNC will have. When using HCl, the crystals that are produced demonstrate poor colloidal stability. If stability from strong electrostatic repulsions between sulfate ester groups on the surface are desired, sulfuric acid is a better choice. Another advantage using sulfuric acid is that it contributes to the hydrophilic nature of CNC because it enhances the hydroxyl groups in native cellulose with the sulfate ester that is introduced during hydrolysis. CNC produced using sulfuric acid tend to be more stable over a larger range of pH values, whereas CNC produced using HCl produce neutral CNC that has poor dispersion in water. In general, CNC have some dispersion properties in aqueous based mixtures and organic solvents with dielectric constants (DMSO and ethylene glycol, for example). However, it should be noted that when introduced into highly hydrophobic solutions, there is substantial aggregation of CNCs. Despite the many beneficial and great natural characteristics of cellulose nanocrystals, the need to implement surface modification to enhance and add desirable qualities was identified. The biggest challenge scientists faced with surface modification regarding CNC was that the modification needed to be performed without dissolving the CNC. Chemical surface modification methods that have proved successful include but are not limited to, silylation, acylation, carboxylation, esterification, fluorescein-5’-isothiocyanate labeling, polymer grafting using PCL, polyethyleneglycol, or polystyrene, and cationic surface functionalization. Colloid surface surfactant adsorption proved to be successful in improving stability of the nanoparticles in organic solvents. This is an alternative to chemical surface modification.

Once the appropriate surface modifications have been performed, the CNC can then be made into its desired end use. CNCs have been mostly used as fillers for composite films. They are useful for such because of their high aspect ratio and their crystallinity[1]. CNC has also successfully been produced into hydrogels, flexible packaging for food products, and a unit for drug delivery.

Though there are many advantages to using CNCs, there are several disadvantages that should be reviewed. CNCs are costly to harvest due to the chemical processing that must occur for extraction of the cellulose from the raw material. Because of their processing methods, they have a limited availability because of time needed to produce and materials necessary for production. They also have a low degradation temperature of 230 °C, so any processing for products it is to be used in must be performed under 200 °C. Lastly, though there are a variety of methods of extraction, some being sustainable, and others not so much, there is a significant amount of money needed to product crystalline cellulose.

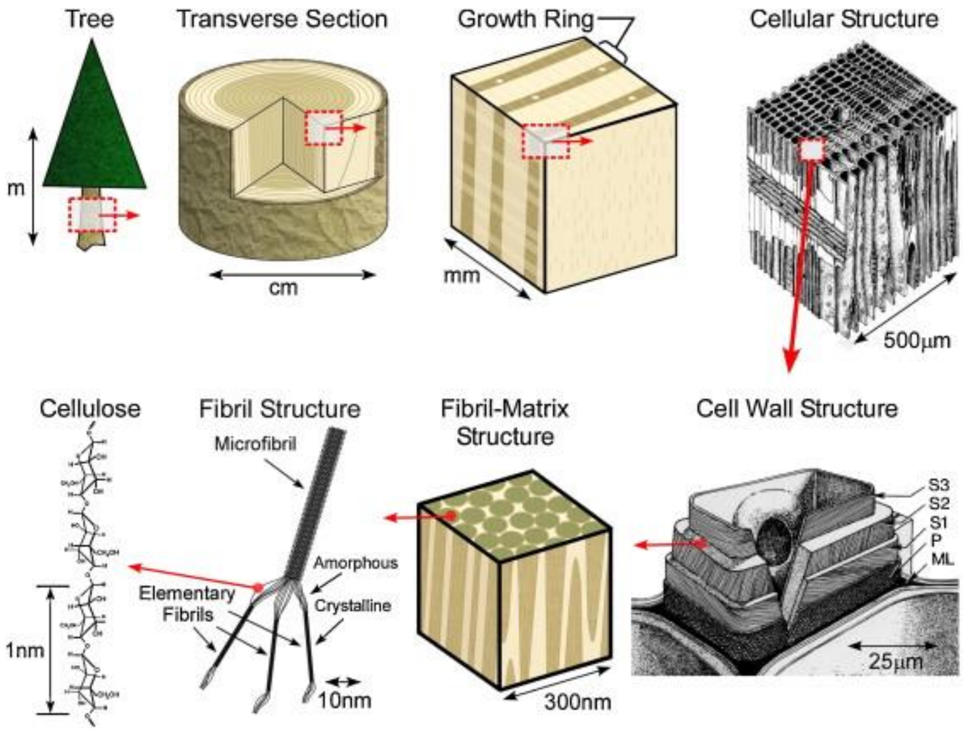


Fig. 5. Hierarchical structure of wood spanning from tree to cellulose. Reprinted with permission from[2].

CELLULOSE NANOFIBRILS (CNF)

Cellulose nanofibrils are the second type of nanocellulose (**Figure 5**). **Figure 5** is a good depiction of the process from initial source to cellulose polymer. Cellulose has to be extracted from the cell wall, which includes primary and secondary walls, hemicellulose, lignin, pectin, and other amorphous regions[4]. CNF are typically sourced from wood, sugar beet, potato tuber, hemp, flax, and cotton [5]. CNFs are 5-60 nm in diameter and can span up to several microns in length. Extracted mainly from trees, cellulose extracted from softwoods tends to be between three and four times as long as cellulose extracted from hardwoods[15]. The particle size is important because the size directly influences the properties of the material and the ease and ability of production[16]. Because CNF are typically found within a woody source, they have to be delaminated in order to be removed from any noncellulosic materials, like hemicellulose and lignin, and treated with high-pressure homogenization in order to achieve nano sizes, which is indicated by diameters less than 100 nm, in gel form[17]. The woody fibers' walls are between 1 and 5 microns thick and have distinct, defined layers: the primary wall and the secondary wall. From the fiber wall, the microfibers or "elementary fibrils" can be extracted as long as the source is one of natural cellulose. Hemicellulose, though its role in cellulose is not entirely known and understood in current research, is a polysaccharide that comes in several forms: xylan, glucronoxylan, xyloglucan, and several others [18]. Hemicellulose from softwood sources tend to be composed of galactoglucomannan and glucuronoxylan from hardwood sources. It is amorphous in structure and differs in structure from cellulose simply by number of glucose monomers found (500-3,000 instead of 7,000+). Hemicelluloses have demonstrated a good use in scaffolding, cell adhesion, cell proliferation, and a reinforcing agent in tissue engineering [19]. Hemicelluloses have biological activities such as antioxidative properties, antitumor properties, and immunomodulating properties that were developed for biomedical applications. Composites with substantial concentrations of hemicellulose have been known to have an enhanced amount of mechanical properties but have seen a reduction in thermal expansion [20]. Hemicellulose in CNFs can be up to 30% depending on the origin of the material and the processing method used [18]. Being negatively charged in nature, hemicellulose causes repulsion between cellulose fibrils but provides adhesion between nanofibers or microbirbils which are the combination of cellulose and hemicellulose[18, 20]. Found both on the surface of fibrils and within the internal structures of fibrils, hemicellulose can be easily hydrolyzed by a diluted acid or base. Fibrillation is negatively affected by the drying of pulp because drying the pulp strengthens the internal bonds of the cellulose polymer chains. However, the more hemicellulose present, the less effect drying has on fibrillation and the cellulose polymer because hemicellulose helps retain water within the structure. Pulps that have a higher concentration of hemicellulose typically have higher yields of fibrillation [21]. Also, the degree of hornification is directly correlated with the hemicellulose content in the pulp [20]. Hornification is the process of strengthening the cellulose polymers through a series of wetting and drying cycles. Lastly, because hydrogels are the most common way hemicelluloses are utilized in the biomedical field and other areas, the properties of the hydrogel are also directly affected by the mechanism of how hemicellulose is included in the gel [19]. This is crucial to understand because the different types of hemicellulose from different sources can make a difference on the integrity of both the individual fibrils and the hydrogel in production. Understanding which type of cellulose being used and the potential properties it can affect positively or negatively should be considered before producing a hydrogel for specific purposes. CNFs do not require a substantial amount of water to form gels, which is why they are commonly used to make hydrogels. CNFs have shown no evidence of cytotoxicity, DNA damage, or chromosome damage in its purest form, but have shown little to some cytotoxicity when modified with PEI or CTAB [13]. In industry, the primary raw material to extract CNFs from are woody materials[22-23]. These materials yield the best results and seem to be the easiest raw material to work with. This is because the geometric properties of the CNF's are dependent on the extraction method and the raw material[24]. CNFs have high mechanical strength, high elastic modulus, low thermal expanding, have self-bonding and self-assembling characteristics, and can be produced by homogenization, microfluidization, or sonication. They are also strong, tough, and have a large surface area with good surface functionality [22]. The hydroxyl surface functionality is easily modified, which allows ionic groups to be easily introduced. This is good for surface modification which is beneficial in Chemical-physical reactions[22-23]. Any additional chemical treatments changes and determines the crystal structure, degree of crystallinity, surface chemistry, morphology, and aspect ratio[23]. This is important when determining the end use of the material and what functions it should serve. CNFs react strongly with water, which is why they are used in gelling [24]. They bind well with water and have a large potential for swelling, which is a key part of the gelling process. Even though CNFs offer many positive characteristics, it should be noted that there are several characteristics that could potentially hinder research. CNFs from woody sources are susceptible to bacteria and fungi in gel form because they are suspended in a majority of water and the wood sources themselves commonly have impurities[22-23]. The impurities that are introduced lower thermal stability. Despite these cons, CNFs have proven to be a very useful material that has lots of potential to expand into all areas of research. They have been used in photonic crystals, barrier films, shape-memory polymers, and drug delivery systems, just to name a few [22].

BACTERIAL CELLULOSE

Bacterial cellulose is also found in cell walls of plants, but can also be secreted extracellularly in various bacterial species such as *Acetobacter G. xylinus*[25]. They have the ability to form acids in high yield[5]. They are randomly assembled and ribbon shaped. They are typically less than 100 nm wide, and tend to bundle in straight, uniform dimensions (**Figure 6**).

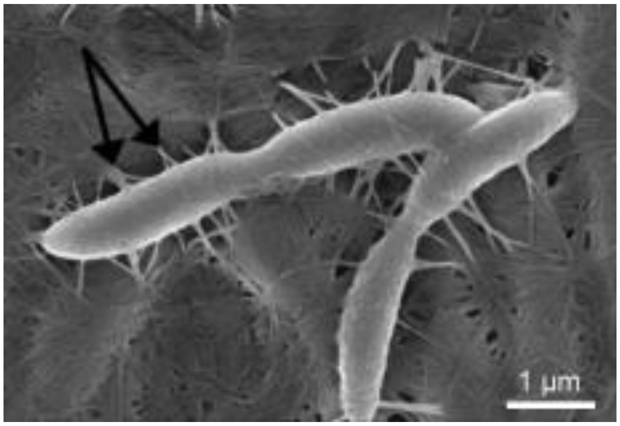


Fig. 6. Bacterial cellulose forming from *Gluconacetobacter* bacteria. Reprinted with permission from[5].

They are grown in cultures and extracted from the bacteria in which they are produced. The largest difference between bacterial cellulose and other cellulose sources is their nanofiber architectures due to differing extraction methods. Simple purification leaves the bacterial cellulose with no impurities. They are typically used in biomedicine due to their low toxicity and biocompatibility, but there are drawbacks to this type of cellulose. It is expensive to produce and requires a substantial amount of resources in order to keep it alive and producing in large quantities.

HORNIFICATION

Another concern, though a mild one, when using CNF is the effect of hornification on the initial starting material, once it has been extracted from its natural source. Hornification is a technical term coined by the wood pulp and paper industry that suggests stiffening of the cellulose polymer occurring when the lignocellulose materials are dried or when water is removed[26]. Research shows that when the fibers are repeatedly dried and dried again, a significant loss of large pores occurs, which also reduces the surface area. Hornification changes the properties of the pulp by not only reducing the pores and pore sizes, but with added aggregation of fibrils higher crystallization, and some natural crosslinking due to the lack of water present[26-27].

Hornification is performed using series of wetting cycles and drying cycles and can be used as a pretreatment method in CNF production[28].

Understanding the process of hornification is essential to this research. The hornification pretreatment method that some researchers use to produce CNF can be used in addition to other mechanical or chemical pretreatments. The advantage the hornification pretreatment method has over the other pretreatments discussed is that it helps naturally strengthen the cellulose to become a natural performance polymer. With wetting and drying cycles implemented, hornification can help make CNF have even more applications within the industry.

PRETREATMENT METHODS

Pretreatment is a crucial step in the production of nanocellulosic hydrogels. The raw materials used, though a variety is used, frequently are in a form with unwanted or non-useful constituents. That is where pretreatments come in. The purpose of pretreatment methods is to eliminate unwanted structures to get the purest form of the raw material possible. Also, certain pretreatment methods introduce chemical additives that make processing easier, change structural properties, or allow for modifications to occur. Though there are countless different pretreatments, several continuously occur in literature.

The first of these methods is carboxymethylation. Carboxymethylation is the addition of negatively charged carboxymethyl groups introduced into cellulose in its pulp form [29]. It uses surface modification chemistry to achieve its tailored nanofibrils [30]. For the cellulose to be able to separate into nanofibrils, an electrostatic repulsion is created in the reaction that encourages the separation. Monochloroacetic acid provides the carboxymethyl groups in the reaction while ethanol, and methanol are used as solvents [29]. Once the reaction is completed, sodium hydroxide is used to neutralize any hydrochloric acid formed in the reaction. The treated pulp is then washed with acetic acid and deionized water. Carboxymethylation pretreatments are high in energy and leave the treated cellulose with carboxymethyl groups attached to the cellulose [29-30]. Structural modifications and additions such as carboxymethyl groups should be considered when choosing a pretreatment because carboxymethyl groups are not always necessary or wanted.

Another commonly used pretreatment, caustic soda, is an alkali solution with nonmercerizing conditions that is used to bust up any aggregations of fibrils within the raw materials [31]. An added benefit to choosing caustic soda for pretreatment is that it acts as a surface modifier that is low in cost [32]. Though some research uses caustic soda concentrations of NaOH at high concentrations such as 5% [31] most research finds that using a NaOH solution of only 1% is sufficient in yielding results [32-34].

Enzymatic or acid hydrolysis have also proven their worth in current research. It is commonly seen as the current most environmentally friendly pretreatment method [35-36], [37]. However, both enzymatic hydrolysis and acid hydrolysis both use a sulfuric acid treatment during processing. Sulfuric acid helps the reaction with the ester occur within the mechanism. Hydrolysis is typically used to extract ethanol for fuel from cellulose sources and is extracted in crystalline form. Hydrolysis does very well at breaking down hemicellulose and other amorphous regions so that the crystalline structures may be exposed yet undamaged [36, 38-39]. Enzymatic hydrolysis is deemed as a cheaper hydrolysis and pretreatment alternative to chemical pretreatments [37]. Using enzymatic hydrolysis could also provide benefits of enhancing fiber strength, decreasing the amount of beating time in the refining process, and strengthening interfiber bonds during fibrillation [37].

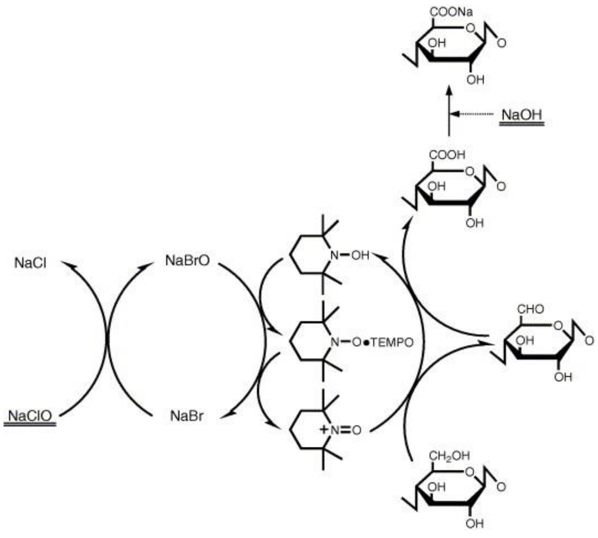


Fig. 7. TEMPO-oxidation of cellulose. Reprinted with permission from[3]

The last and most commonly used pretreatment to be considered, though there are many more being studied, is TEMPO oxidation (**Figure 7**). This is a simple and "green" form of pretreatment that uses TEMPO, a stable nitroxyl radical [25]. TEMPO, which stands for 2,2,6,6-tetramethylpyperidine-1-oxy, is the conversion of polysaccharides to their corresponding polyuronic acids using catalytic oxidation [3]. Carboxylate and aldehyde functional groups are introduced into solid cellulose where the reactions occur at C6 during TEMPO oxidation [3, 30]. This cost effective pretreatment method converts hydroxymethyl groups to their carboxylic forms [25]. Only the hydroxyl groups of the polysaccharide are oxidized during the reaction, which will only be successful if NaBr and NaOCl are present. TEMPO treated cellulose can easily be converted into composites or films, but the different variations in cellulose raw materials can cause different properties and products to be obtained from TEMPO oxidation [3, 25]. In order to produce cellulose nanofibrils with a width of 3-20 nm, the optimal conditions of a pH of 10 and a temperature between 0 and 10°C should be maintained during and after the reaction [25]. In conclusion, there are many different types of pretreatment methods for cellulose before mechanical processing. It is important to tailor the pretreatment method to the desired properties and abilities of the end goal of the cellulose and its derivative.

COTTON BYPRODUCTS

Cotton is an extremely important crop for people all over the world. It is an annual shrub that is grown north and south of the equator in tropical and subtropical regions [40]. In 2003, there was an annual production of more than 23 million tons per year of cotton [41]. In 2010, 42 million metric tons of cotton lint were produced [42]. Cotton production, use, and waste seems to be growing at a fast pace year by year. Natural fibers like cotton create such a draw because they are cheap, abundant, renewable, and biodegradable along with their ability to be grown in such an abundance of places [42]. Cotton fibers have been a great source of cellulose nanostructures because they are 88-96% cellulose [41-42]. There are many different varieties of cotton, which produce many different waste products(like linters and combers aka noil) due to strict processing and ginning standards [40]. Linters are another byproduct of cotton yarn production. They are also short fibers that are 80% cellulose when dried. In 2010, around 2.5 million metric tons were produced [40, 42]. The main differences between linters and staple cotton, lint, are that linters are shorter (2-6 mm), thicker (6-12 μm), and are round in lumen shape. Combers or noil are a waste product of mills that are 100% staple fibers and noted as good for paper manufacturing [40]. Though there is an abundance of cotton waste and byproducts, there is not a substantial amount of research on these materials. They have the potential to be a useful and successful material due to their high cellulose content and natural properties.

SILK

Silk has historically been used in the biomedical field for centuries. This natural material can be extracted from both worms and spiders. Silk is a protein that is composed of many different combinations of amino acids and monomers [43]. Spiders typically produce one of four common amino acid sequences in their silk: poly-Ala, poly-Ala-Gly, GPGXX, and GGX [44]. There can be eight different types of spider silk, but only one type of silkworm silk [45]. Spider silk is extracted from webs, while silkworms spin their silk into cocoons. Both have little length change in water and are hydrophobic in solid states. The both are thermally stable at both very low and very high temperatures. Silk has exhibited natural properties such as high tensile strength, extensibility, and biological compatibility [45]. Silk from spider sources and silk from a worm source tend to differ in composition, structure, and properties. This is also directly affected by the diet and conditions the insects or spiders are in. Silk, in general, can be modified to have specific biodegradation times dependent on its use [46-47]. An important aspect to remember when using silk is that the majority of it, from any source, has to be degummed in order to remove sericin [46]. The degumming decreases the fiber strength of silk, but provides a more pure form because sericin is a glue-like protein.

CHAPTER 3

WOOD BASED CELLULOSIC BIOMASS

INTRODUCTION

I would first like to dedicate this chapter to the late Dr. Turbak. This research conducted in this chapter was inspired from his work with nanocellulose[48]. Cellulose, the most abundant, naturally occurring polysaccharide, is the major component of the cell wall in its most natural form[49]. Its long, linear shape comes from unbranched glucose polymers bonded by β-glycosidic linkages[49-51]. The linked β-glycosidic bonds are called anhydroglucose units, and when two of those are linked together, they become an anhydrocellobiose[15, 52]. That is the repeating unit of the cellulose polymer, a polymer which typically has 500+ glucose units and can be specifically identified by the forward and backward alternating positions of the ring oxygens[15, 50]. Because of the β- linkages, cellulose has the unique ability to fold and form sheet-like structures unlike other forms of glucose or other cellulose derivatives[49]. Cellulose bonds break most commonly at the β-glycosidic linkages[51]. The folding creates intermolecular hydrogen bonds between the cellulose polymer chains, giving cellulose the ability to be insoluble in water even though the polymer is strongly hydrophilic (**Figure 8**)[49].

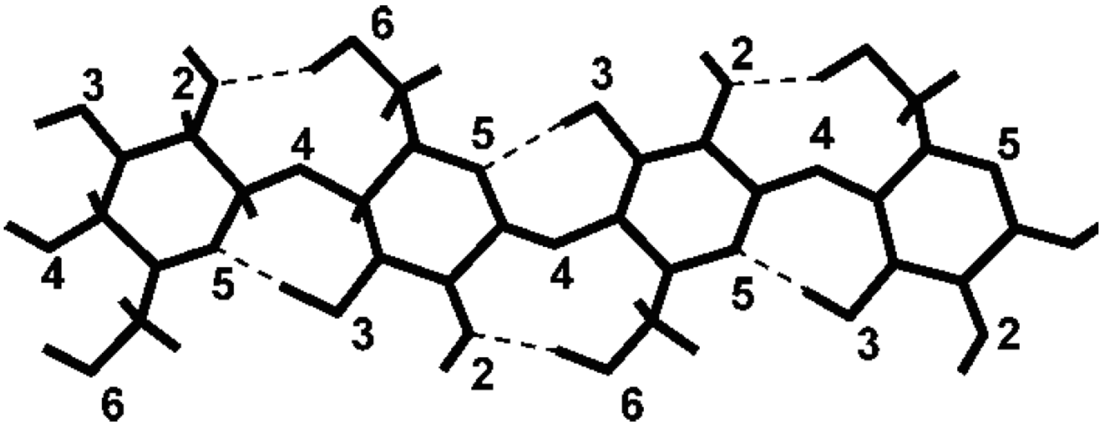


Fig. 8. Intramolecular hydrogen-bonding network in a representative cellulose structure. Reprinted with permission from[1]. Copyright (2009).American Chemical Society

Cellulose, especially from woody sources, is semicrystalline, with the degree of crystallinity ranging from 40-70% in its natural form[15, 52]. Literature also confirms that cellulose has regular, repeating structures that are microcrystalline[49]. The crystallinity of cellulose is dependent on the type of raw material, the age and season of harvest, and the nutrients it received while growing. One of the downfalls to the structure of cellulose is that it is not easily dissolved, especially in water[53]. Recently, however, it was proved that ionic liquids possess the abilities to physically dissolve cellulose. This is important because historically researchers have had trouble extracting and pretreating fibrillated cellulose in an environmentally manner. The following methods discussed in this chapter, varying molecular weight CMCs, thermal treatments, and glycerol, provide more chemically safe and environmentally friendly pretreatment methods for cellulose.

PROCESSING

The first step in the production of wood based nanocellulosic hydrogels was the processing of the raw material. This step is vital because initial particle size is important for not only flow properties, but also has a direct effect on ease of production[16]. The raw material was received in large, long pulp sheets that were waste kraft pulp from paper plants. The cellulose pulp sheets (pre-bleached, from pine trees) were provided by Weyerhaeuser (Dacula, GA) (**Figure 9**).

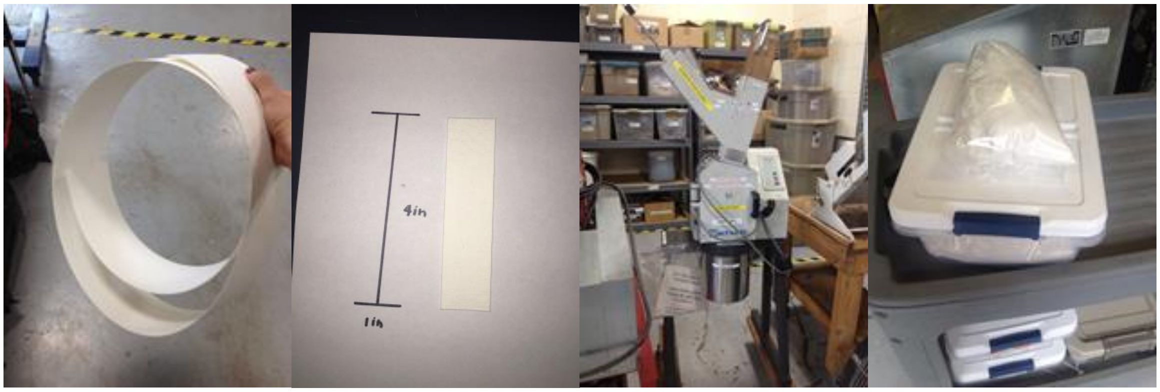
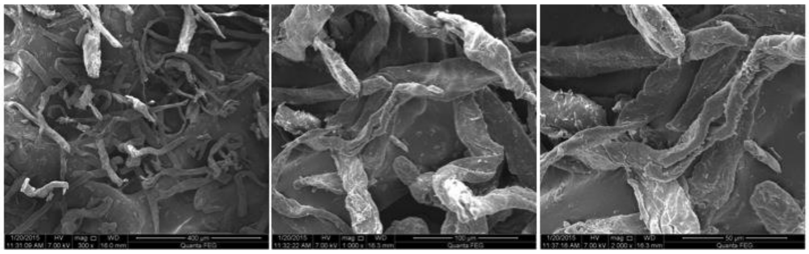


Fig. 9. From left to right: Cellulose pulp sheets, cellulose pulp sheets cut into 1inx4in pieces, knife mill, three-time milled cellulose powder ground

The sheets were first cut into 1 inch by 4 inch strips to fit into the hopper of the knife mill. Those strips were then run through a Restech SM2000 (Haan, Germany) knife mill in order to be ground into a fine powder using a 250 μm mesh sieve. The grinding process was repeated three times in order to remove any aggregations of pulp sheet left and to ensure that all of the material was a uniform, fine powder. When the research first started, the original powder production process was to use a hammer mill to reduce initial particle size then run the powder through a knife milling process, but the hammer mill used significantly more power and energy than the knife mill did. Also, the amount of time the hammer mill required for operation was significant, and combined with operation of the knife mill, a large amount of both time and energy had been consumed. Therefore, the hammer milling process was removed because the powder produced from just using the knife mill was comparable in both size and performance as the powder that had been through both the hammer milling and knife milling processes.

Once ground, the powder was stored in a desiccator in order to keep the powder dry. The powder was placed in an oven at 60°C at least 24 hours before use. That step is not critical to the production of the hydrogels, but the material that had been in the oven did not absorb as much water from the air and was blended into the other components of the gels easier. This is due to the hydrophilic nature of cellulose and the parallel intermolecular hydrogen bonds between the chains that allow for the excessive intake of water and swelling in size[49]. It made production of the hydrogel much smoother because the oven dried powder did not clog the machine. Cellulose can swell to at least double its original size, so it was important to keep the starting particles dry, moisture free, and as small as possible (**Figure 10**). The smaller the starting particle, the smoother and better it mixed in the slurry and went through the homogenizer for the gelling process. It also kept the size more uniform and homogeneous in both the powder and the final gel product. The processing of the powder did not seem like a crucial step, but it ended up being very important to the hydrogel pretreatment and production process. After the kraft pulp was cut, ground, and dried, it was ready to be pretreated before being produced into a hydrogel.

IDEAL



MOISTURIZED (or swollen)

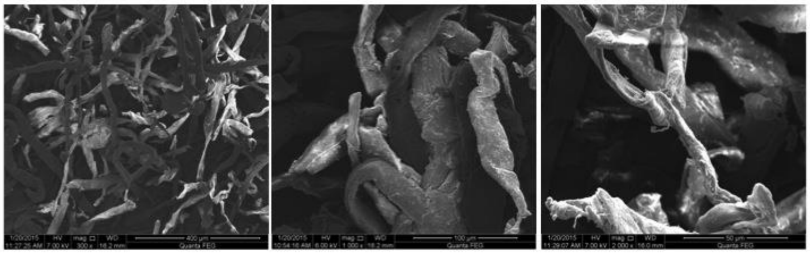


Fig.10. (Top) SEM images of what the cellulose pulp powder should ideally look like versus (bottom) images of moisturize or swollen cellulose pulp powder due to exposure to humidity

PRETREATMENT METHODS

Pretreatment of the ground powder was the second and arguably, the most important step in the gelling process. The pretreatment adds or removes active groups for chemical modification, affects the amount of aggregates present, and directly affects the properties and potential end uses of the hydrogel[54]. Pretreatments can be chemical or physical and historically have been very detrimental to the environment and hazardous to the researcher[5, 30-31, 35, 38, 54]. The pretreatments this research used were natural, safe, and cost effective. Carboxymethylcellulose (CMC) was the pretreatment additive of choice because CMC is a salt derivative of cellulose, allows for mechanical shearing to occur easier, and has negative charging, which repels with the negative charging of the hydroxyl groups on the cellulose chains. The natural repelling between the two allowed for the bonds to be broken easier through mechanical shearing from homogenization. The gels produced from the pretreatments discussed in the following paragraphs are safe, sustainable, and provide the end user a variety of potential end uses. The pretreatments on the wood based cellulose pulp were as follows: 90,000 molecular weight CMC, 250,000 molecular weight CMC, and 700,000 molecular weight CMC. Three control hydrogels were also produced: a completely cold run, a treatment with no thermal treatment and no CMC, a treatment with a thermal treatment and 90,000 molecular weight CMC. Lastly, glycerin was added to the NFC to see if the NFC was capable of being plasticized. The goal of these pretreatments was to see if the molecular weight of the CMC caused a change in characteristics of the hydrogels, to see if a thermal treatment developed in the lab influenced the integrity of the hydrogels produced, and to see if the NFC had plasticizing abilities. **Table 1** provides an overview of all of the pretreatments used in the research.

Table 1. Overview of Pretreatment methods and materials tested

Method	Purpose	Materials Tested
Addition of varying molecular weight of Carboxymethylcellulose (CMC)	Enhances mechanical shearing, repelling of hydroxyl groups in cellulose because of negative charge	Kraft pulp Cotton noil
Thermal Treatment	Thermal treatments encourage delamination	Kraft pulp Cotton noil
Addition of Glycerol	Glycerol gives NFC plasticizing effects	Kraft pulp

Carboxymethylcellulose

Carboxymethylcellulose (CMC) was the first choice of pretreatment methods for study. Because it is a derivative of cellulose, it is also not harmful and has a low toxicity. Commonly used in food packaging, CMC does not cause harm to the human if consumed. However, CMC and cellulose are not digested, absorbed, or degraded in the human body[55]. CMC was purchased from Sigma Aldrich (MO, USA) and was used as received, with no additional modifications to the powder. The average molecular weights of the CMCs used to produce variations of the pretreatments of the nanofibrillated cellulose gel (NFC) were 90,000, 250,000, and 700,000 respectively. A mechanical blender (Waring Commercial, CT) was used to blend cellulose powder and CMCs with water for better dispersion of the materials and mixing of the slurry. The slurry refers to the mixture of powder and CMC. The blender ran at a speed of 22,000 RPM. The ratio of CMC to water was fixed to 0.0025% and the ratio of cellulose powder to water was varied to various weight percent from 0.5 to 5%. The ratio to determine the amounts for each batch is X% NFC = X g of cellulose pulp powder: 0.25% CMC: 100 ml DI water. For example, if you want to produce 500 mL of 2% NFC, you will use 1.25g of CMC, 10g of cellulose pulp powder, and 500 ml of DI water. The CMC was first added to water at 65°C in temperature and blended at 22,000 RPM for 2-5 minutes. The cellulose pulp powder was then added to that mixture and blended for an additional 2-5 minutes **(Figure 11).**

Fig. 11. From left to right: Carboxymethylcellulose (CMC), cellulose pulp powder, blender with the slurry mixture

The mixing of the two can be done simultaneously without disturbing the integrity of the hydrogel production. The slurry was then moved to a beaker, where it continued to be mixed at a constant agitation of 600-700 RPM on a hot plate. The slurry was brought up to a temperature of 100°C then cooled to 80°C. This allowed for the delamination process to begin, or the separation of cellulose fibrils from hemicellulose, to occur due to the loosening and breaking of bonds between cellulose polymer chains and the adhesion between cellulose and hemicellulose[56-57]. The slurry was then blended once again for 2-5 minutes before being poured into the hopper of the homogenizer. The blending of the slurry was used to ensure that there was no settling of the cellulose in the slurry and that it was a homogenous mixture before being poured into the hopper of the homogenizer. Failure to have a homogenous mixture resulted in clogging the homogenizer or busting parts inside of the machine because the increased pressure from the homogenizer could not push the aggregations, big clumps, through the small orifices. If the slurry was left without agitation during the delamination phase, the cellulose would separate from the water and settle. For that reason, the blender was added to the slurry production process, a modification of the original process. CMC acted as a lubricant because it is believed to reduce friction between cellulose fibrils in the mixture as well as help break the bonds between the cellulose and hemicellulose. Though CMC is a salt that is derived from cellulose, constant agitation was required to mix it thoroughly with cellulose and DI water because CMC dissolves readily in cold water, not hot water.

Thermal Treatments

Two treatments were compared to the standard treatment of 2% thermally treated 90,000 NFC, in order to determine any differences on the effect of heat. The first treatment followed the standard procedure up until the delamination process. The slurry was blended then poured immediately into the hopper of the homogenizer. No heating and cooling steps were performed, but CMC was present in the slurry. The second treatment followed the standard process, but no thermal treatment was performed and no CMC was added to the slurry. The difference between the two treatments was the addition of CMC to one of the treatments. Neither of the two treatments was subjected to any thermal treatment, except the initial heating of the machine, which will be described in the "Production of Hydrogels" section. The purpose of having two non thermally treated slurries, one with CMC and one without, is to determine if the delamination process, the thermal treatment, has an effect on the characteristics of the gel. By having one with CMC and one without CMC it can be determined if the thermal treatment had the effects or the CMC did.

Control

The control used in this research was a hydrogel that was not subjected to any thermal treatment or any warming of the machine prior to homogenization and using room temperature DI water for slurry production. This is also referred to as the cold study because all materials and equipment were at room temperature. The molecular weight of CMC used was 90,000. The same ratios of cellulose powder, DI water, and CMC were used in this method as described in the "Carboxymethylcellulose" section. The blending process was also kept the same.

PRODUCTION OF HYDROGELS

Producing the Hydrogel

An APV 1000 homogenizer (SPX, Poland) was used for homogenization of the hydrogels. The slurry containing cellulose pulp powder and CMC was blended for 2-5 minutes before being poured into the hopper of the homogenizer, which had an internal temperature of at least 65°C. This was performed by either circulating hot water through the machine until the slurry was ready to be poured in or turning up the pressure in the machine to heat the water while the slurry was being produced. Both methods worked the same and did not differ in end results of the hydrogels. The homogenization process delaminated cellulose fibers into nanofibrils *via* mechanical shear force under high pressure. Delamination is the process of separating layers, so in this case it meant the peeling of the layers of cellulose aggregates into individual fibrils. That also reduced the amount of hemicellulose attached to the cellulose fibrils in the final NFC material because of the shearing and the high temperature and pressure applied, which broke some of the bonds between the cellulose and amorphous hemicellulose. There was still hemicellulose present within the hydrogels, but not as much directly connected with the cellulose.

The number of cycles that were necessary to produce NFC depended on the percentage of gel that was being produced. For example, 0.5% NFC took around 30 minutes to reach the gelling point of at least 65°C. However, 2% NFC took roughly 10 minutes. The higher the pressure applied, the shorter the gelling time. This was due to the fact that more material in the slurry caused more friction and shearing between the fibrils, which increased the temperature quicker and also caused the physical phase change quicker. Since 2% was the standard percentage used for the rest of the research, the average time of homogenization was 10 minutes that occurred at an average of 740 bar of pressure applied (**Table 2**).

Table 2. Gelling process for 2% NFC with 90k CMC and thermal treatment

Time (minutes)	Temperature (°C)	Pressure (bar)
0	53.1	10
5	65.5	730
10	77	730

This was equivalent to 2 cycles of approximately 5 minutes for the material to pass completely through the machine.   However, the higher the concentration, the less time it took for the gelling to occur due to the larger presence of materials in the slurry (**Figure 12**).

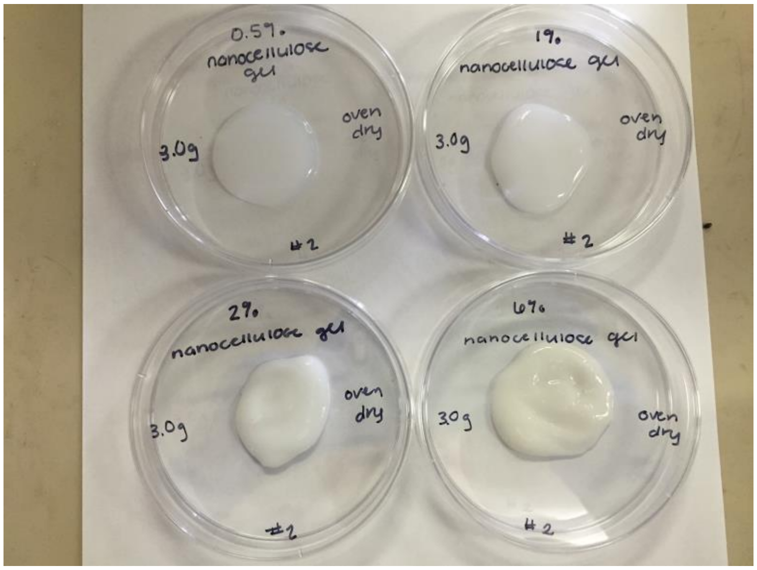


Fig. 12. Visuals of varying concentrations of NFC: 0.5% (top left), 1% (top right), 2% (bottom left), and 6% (bottom right).

The more material present in the slurry, the more shearing and defibrillation that occurred. This lead to the hydrogels with a higher concentration to gel quicker. The gelling time difference due to the concentration of powder in the slurry was being sheared down and delaminated during the homogenization process. The slurry was considered to be a homogenized, finished gel once the temperature of it reached between 65°C and 80°C. Letting the homogenization process continue to process until 80°C did not damage the material or change the abilities of the hydrogel. There were no visible differences in the material at 65°C or 80°C+. The completed gel was placed into a glass jar to cool and stored in a refrigerator. It was important to keep the hydrogel in a sealed jar in a cool environment because any exposure to ambient conditions speeds up the ability of the gel to mold. The shelf life of the material was 6 months to 1 year. The final product was white in color and thick in consistency.

Choosing a Standard

Because there were so many gels being produced during this research, it was imperative to pick a standard for comparison. Taking into account ease of production, what was being commonly used by others, and literature, it was decided that the hydrogels would be produced at 2% concentration of cellulose pulp. The standard 2% NFC gel also involved using 90,000 molecular weight CMC. This was identified as the standard because of the ease of processing. The identification of a standard allowed for comparison of results among all studies and all materials involved in the research. Multiple concentrations of NFC were produced, but because 2% was desired by other researchers, specifically for textile dyeing purposes, the NFC, 2% became the most produced, and therefore, the standard (**Figure 13**). The graph indicated that as the concentration of cellulose powder in the slurry was increased, so was the viscosity. This was due to the increase in shearing capabilities that the hydrogels had due to the increase in initial powder. These gels were produced without blending. The 0.5% NFC (aka NCG) took the longest time to produce, while 5% NFC took a much shorter time. All of the NFCs at different concentrations still underwent a physical change, but the time at which those happened occurred earlier in the process as the initial powder concentration increased.

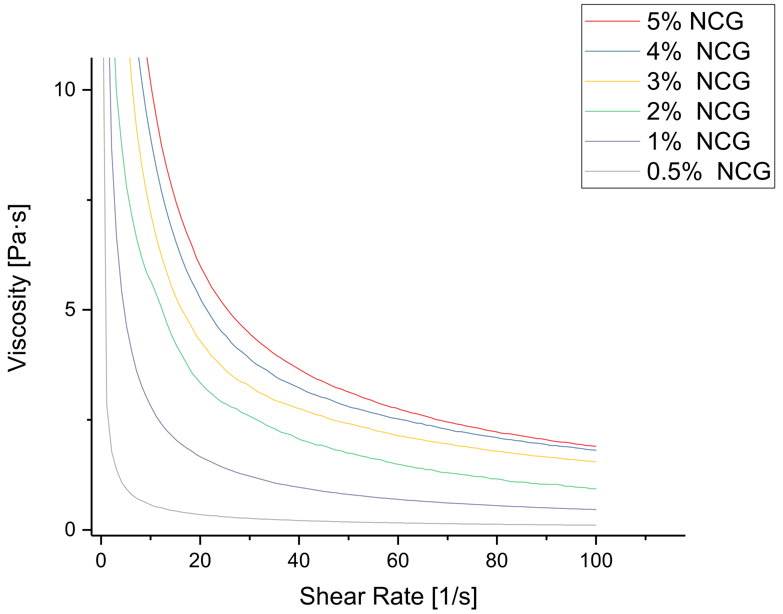


Fig. 13. Graphical representation of the viscosity changes from the changes of the concentration of cellulose pulp powder in the NFC.

STUDIES

Carboxymethylcellulose Study

This main purpose of this study was to identify characteristic differences between 2% thermally treated NFC with different molecular weight CMCs. CMC not only acted as a lubricant for the cellulose fibrils during mechanical shearing, but because of its anionic nature, acted as a natural repellent between itself and the cellulose fibrils, also making mechanical shearing easier. The molecular weights that were used were 90,000, 250,000, and 700,000 respectively. Each hydrogel was produced using the methods described in sections “Carboxymethylcellulose” and “Producing the Hydrogel”. These three NFCs were compared to each other using AFM, SEM, viscosity, and XRD characterization methods (see “Characterization” section).

The first aspect analyzed was ease of production and visual inspection. The gels looked the same, but there were some differences in production. The 90,000 mw, blended smoothly, and was easy for production. 250,000 mw CMC was the most difficult to incorporate in production. It blended smoothly and the homogenization process was simple and quick. 700,000 mw was the easiest CMC to incorporate within the slurry mixture. The CMC did not want to blend smoothly with the cellulose and formed large aggregations, even when the blender was used. **Tables 3-5** provided the processing times and pressures for the varying molecular weight CMCs.

Table 3. Processing of 90,000 mw CMC for 2% thermally treated NFC

Time (min)	Temperature (°C)	Pressure (Bar)
0	49.8	10
5	62.0	690
10	73.6	730
15	81.8	740

Table 4. Processing of 250,000 mw CMC for 2% thermally treated NFC

Time (min)	Temperature (°C)	Pressure (Bar)
0	49.7	250
5	71.4	720
10	81.3	720

Table 5. Processing of 700,000 mw CMC for 2% thermally treated NFC

Time (min)	Temperature (°C)	Pressure (Bar)
0	41.6	10
5	52.7	750
10	67.6	740

By the processing methods defined for this research, AFM imaging did not show a significant difference of the fibrils after homogenization with each of the hydrogels

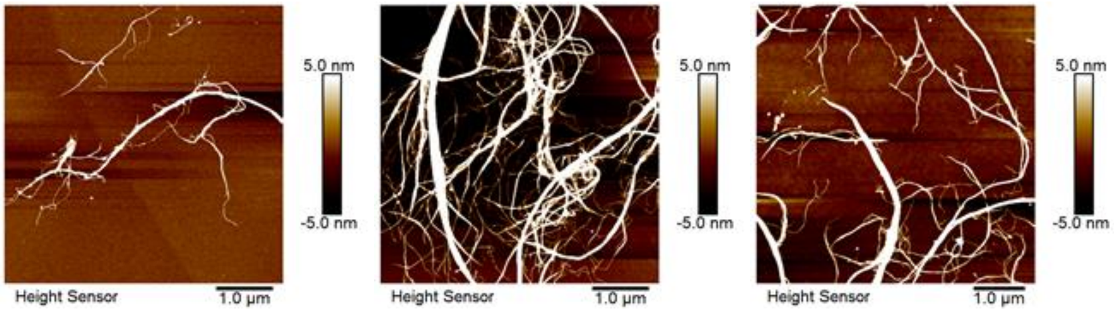


Fig. 14. (from left to right) AFM images of individual NC fibrils homogenized without CMC, CMC with 90k average molecular weight (Mw), and CMC with 250k Mw

(**Figure 14**). There images with CMC treatments show thicker bundles of fibrils, this is due to the fact that CMC is used as a thickener, has a high affinity for the intake of water, and acts as glue between single fibrils within the hydrogels. It also acts to mechanically loosen up the bonds between the amorphous parts of the cell wall and because it is negatively charged, act as a repellent to the negatively charged hydroxyl groups, also creating space between the amorphous structures and the cellulose fibrils. CMCs have been used as gelling agents in materials. This was an important factor when determining which anionic salt to use for production. SEM imaging of films made from the NFCs with different CMC molecular weights also demonstrates the visual differences on a larger basis of the effect of CMC (**Figure 15**).

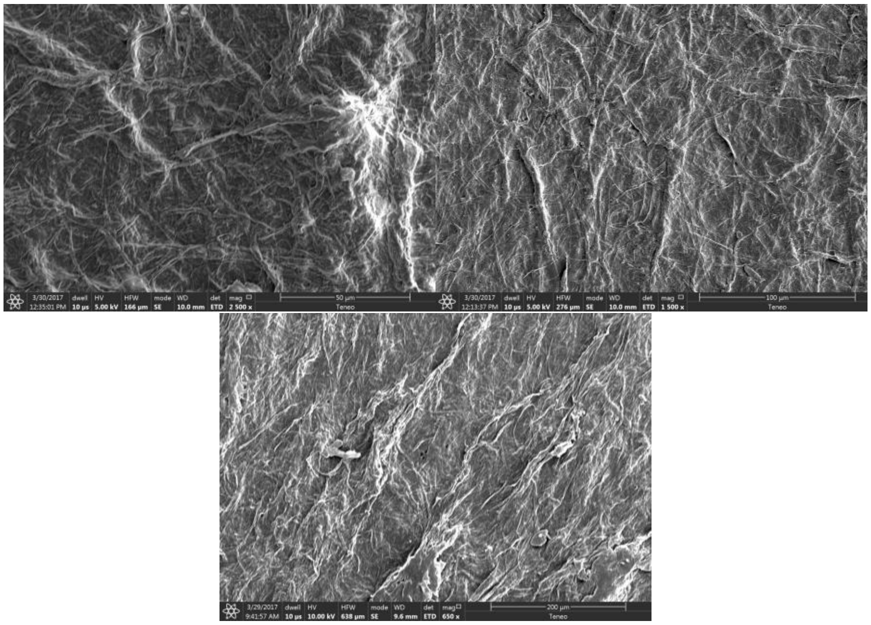


Fig. 15. SEM imaging of 2% NFC with 90,000 mw CMC (top left), 250,000 mw CMC (top right), and 700,000 mw CMC (bottom)

The films are uniform with some thicker aggregations of fibrils weaved together. Each of the three SEM images visually represents a thin film that has distinct nanofibrils present. While the NFC with 90,000 CMC is at the highest magnification (2,500x), it also has the least amount of aggregates. The NFC with 250,000 CMC, at a magnification of 1,500x, has a smooth matrix of fibrils with a few more aggregate ridges. The NFC with the 700,000 CMC has the largest amount of aggregate ridges. It was also the most difficult to produce. Because the molecular weight was higher, the CMC particles were larger and made processing with the cellulose pulp powder difficult. Even though the look and feel of the hydrogels may appear to be similar, the viscosity told a different story (**Figure 16**).

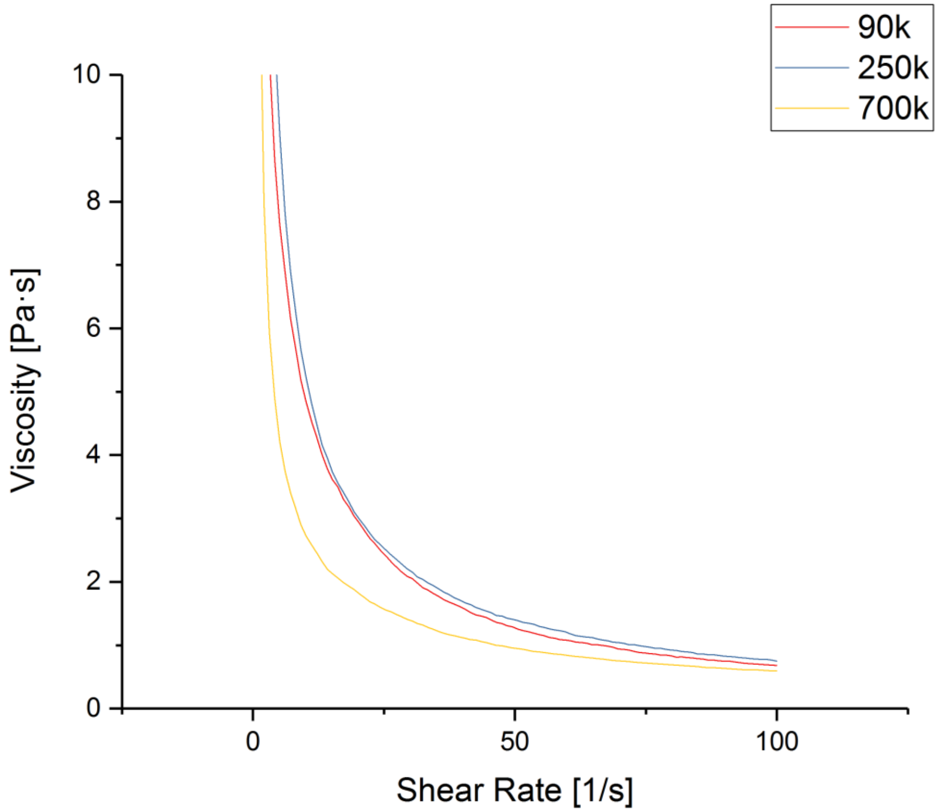


Fig. 16. Viscosity of 2% nanocellulose gel made with different molecular weight CMC's as a function of shear rate: 90k, 250k, and 700k.

The graph indicated that all of the hydrogels produced are shear thinning and thixotropic which is confirmed with literature[15]. This is due to the fact that the viscosity is reduced as time and constant pressure were applied and that the gel continued to thin as the pressure and shearing was applied. The NFC with no CMC demonstrated the highest viscosity overall, with the NFC with 700,000 CMC having the lowest viscosity, significantly less than the others. The NFC with 250,000 CMC was slightly more viscous than the standard NFC with 90,000 CMC. The zeta potential of the NFCs made from 90,000, 250,000, and 700,000 molecular weight CMCs are -46.2 mV, -56.2 mV, and -60.7 mV respectively. They confirm that the NFCs are stable, are anionic (due to the negative charges of the reactive hydroxyl groups), and differ slightly from each other due to the molecular weights of the CMCs. Yet, it is obvious that they are still related because of the closeness in values. XRD provided relevant information regarding the crystallinity changes of the NFCs during delamination and homogenization (**Figure 17**). The peaks are similar because they are comprised of the same materials, only the molecular weight of the CMC is different.

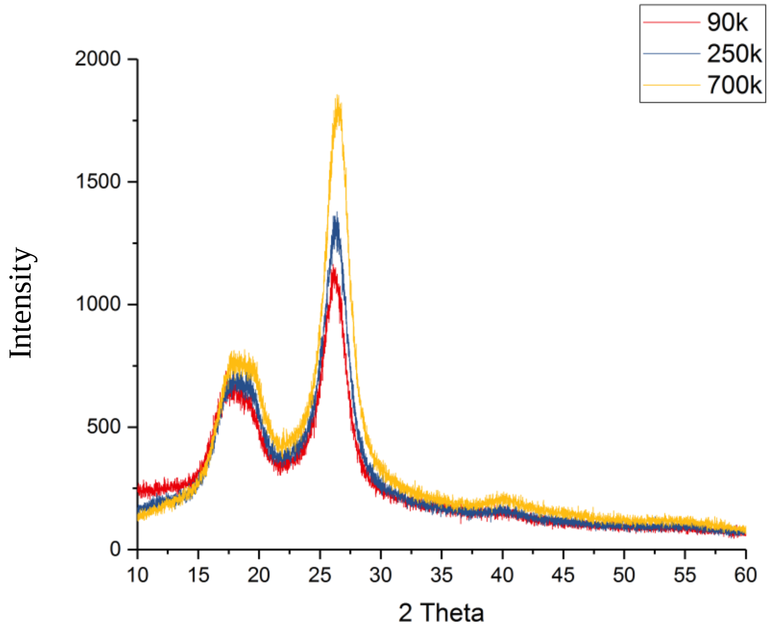


Fig. 17. XRD Spectra of NFC with varying mw CMCs. They have the characteristic peaks of cellulose with slightly varying intensities.

The crystallinity index (CI) and % crystallinity could be determined from the XRD spectra using the equation found under XRD in the characterization section (**Table 6**).

Table 6. % Crystallinity and CI of NFC with varying CMC pretreatments

	NFC w/ 90,000 CMC	NFC w/ 250,000 CMC	NFC w/ 700,000 CMC
% Crystallinity	60.71	63.12	69.19
CI	0.353	0.416	0.555

Because the spectra are almost identical, the values should not be substantially different. The % crystallinity should vary a little due to the difference in molecular weight from the CMCs, but not substantially. The above table confirms. The percent crystallinity of the three hydrogels with varying molecular weights increased as the molecular weight of the CMC increased. The CMC helped preserve the microcrystalline structures within the cellulose fibrils. This is important to understand when varying the molecular weight of the CMC. It should be noted that the second peak on the the 2Θ plane was shifted approximately 4° because the second peak on cellulose typically falls around or below 22 and this was at 26. The machine was calibrated before use, but the 4° difference could be due to mechanical error or sample preparation error.

The purpose of this study was to figure out if there were significant differences in the NFCs produced from varying molecular weight CMCs. While there are some differences between the gels in terms of processability, the differences were so insignificant that ease of production is not a significant driving factor. The viscosities were slightly different, not enough to truly affect the flow, though. Also, the amount of crystalline microstructures was not substantially high or different. This also helps confirm that the molecular weight of the CMC is not an important factor within the NFC production. Lastly, the amount of aggregations in the films produced was also varying, but not significantly, as seen from SEM and AFM imaging. Overall, it can be concluded that varying the molecular weight does not significantly affect the integrity of the NFC.

Thermal Treatment Study

The thermal treatment study, or lack of thermal treatments, with the addition of CMC served to determine if the delamination process, the heating of the slurry to 100°C then cooling to 80°C had an effect on the integrity and basic characterizations of the gel. The study was conducted by comparing the 2% NFC hydrogels with no thermal treatment and no CMC and no thermal treatment with 90,000 molecular weight CMC against the standard, which is thermally treated with 90,000 molecular weight CMC.

It took the production of the NFC without both CMC and thermal treatment about 5 minutes longer to reach the appropriate gelling temperature than it took the standard 2% NFC. The NFC that was produced without the thermal treatment but with CMC took the same amount of time, 10 minutes, as the standard production method. They were easier to produce because of the time factor. There was the initial blending of the materials to make the slurry, but then the slurry went right into the homogenizer without any of the delamination, or thermal treatment, process. This reduced the total time of production by about 45 minutes since the slurry did not have to heat to 100°C then cool to 80°C. Once produced, the NFCs without thermal treatment, including the cold run NFC, all had the same end color as the standard, but appeared to be less viscous (**Figure 18**). The graph indicated that the non-thermally treated NFCs were less viscous than the standard 2% NFC that was thermally treated and had 90,000 molecular weight CMC. The NFCs produced for this study are also consistent with the standard,

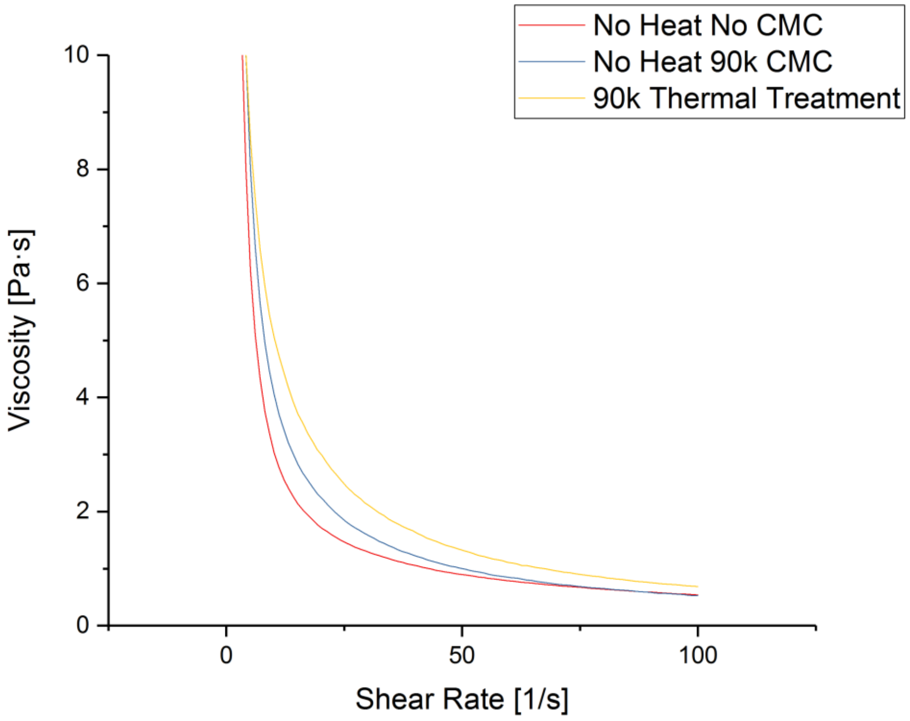


Fig. 18. Viscosity graph of the non-thermally treated NFCs compared to the standard 2% thermally treated 90,000 mw CMC NFC

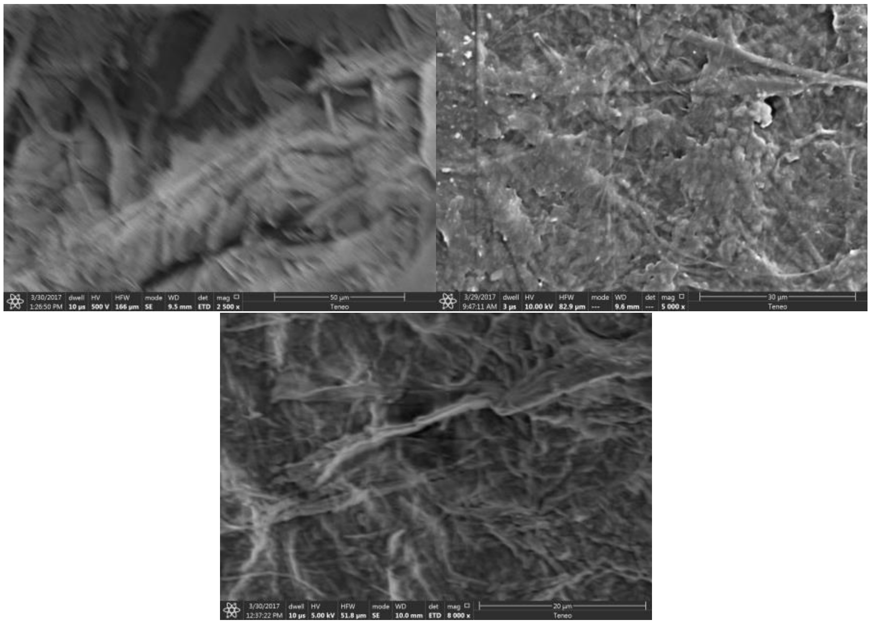


Fig. 19. SEM imaging of 2% NFC produced by no thermal treatment and no CMC (top left), no thermal treatment and 90,000 CMC (top right) and the standard (bottom)

which is shear-thinning. Visually, the NFCs all appeared to look very similar, but once viewed by SEM, it was evident that there were significant differences, like there were with viscosity (**Figure 19**). The cold run NFC had significantly more aggregation and a very uneven surface area. This was due to the lack of heat present from not only the slurry, but the machine itself. This could also be said for the NFC produced using no thermal treatment and no CMC. The fibrils in that image, however were much longer and thicker than those of the cold run. The NFC produced using no thermal treatment and 90,000 CMC had the thinnest fibrils, but was very uneven on the surface and appeared to have large aggregates. Confirming that these NFCs were consistent with cellulose-based materials, the zeta potentials were all negative due to the surface charge and reactivity of cellulose, which is negative because the more mechanical processing that occurs, the stronger the negative charge[58]. The no thermal treatment no CMC NFC had a charge of -41 mV; the no thermal treatment 90,000 mw CMC NFC had a charge of -64 mV. These were once again compared to the charge of the standard NFC, -46 mV. The spike in the NFC produced with no thermal treatment and 90,000 CMC can be attributed to the introduction of CMC adding more reactive sites to the cellulose that would normally be diminished when heat is introduced. The other two have much lower charges because of that.

Cellulose is predominately amorphous with some crystalline structures within. XRD and FTIR spectra shed more light as to what happened to the crystallinity with the different pretreatments (**Figures 20 and 21**)[59].

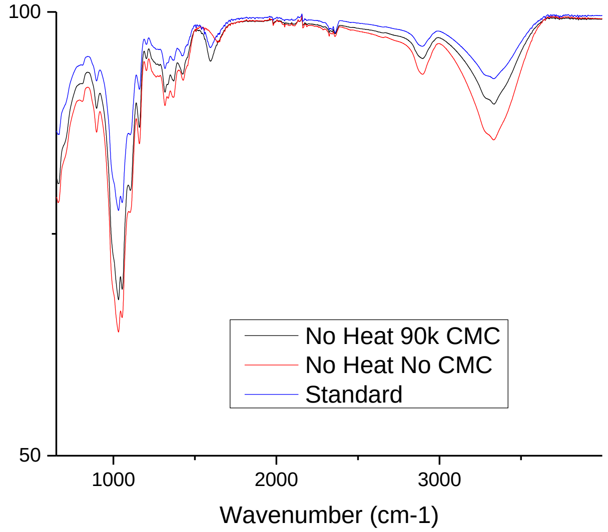


Fig. 20. FTIR Spectra of varying NFCs without thermal treatment compared to the standard NFC

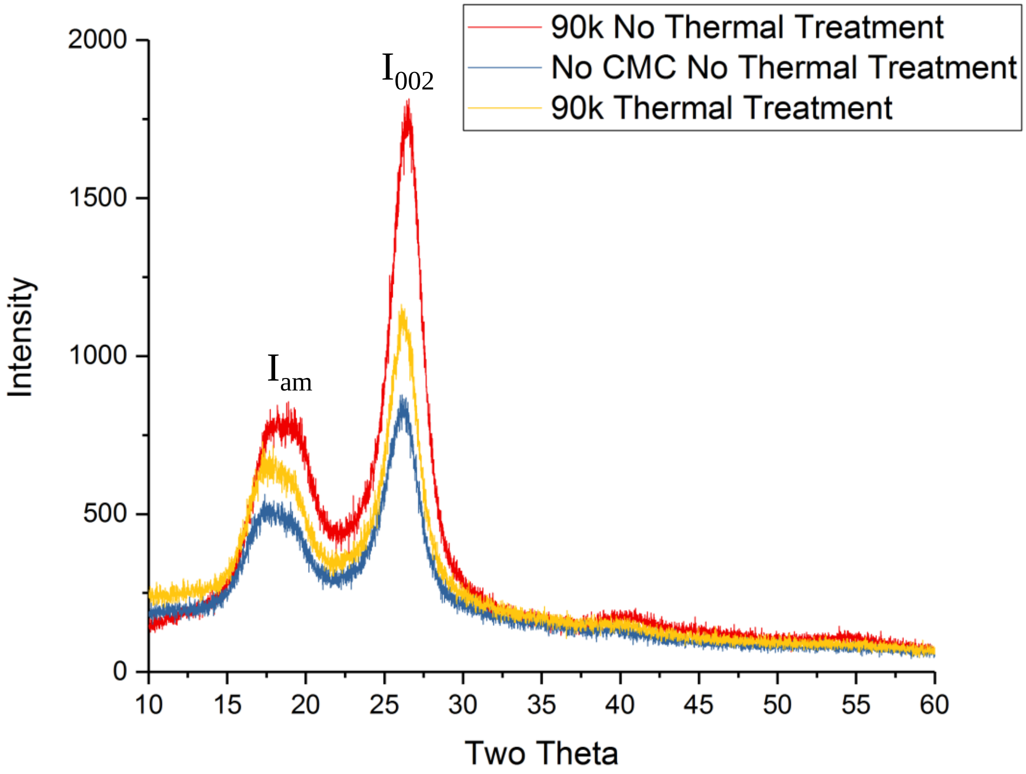


Fig. 21. XRD spectra of NFCs without thermal treatments compared to the standard NFC and the cold run

The FTIR (**Figure 20**) is typical of a cellulose based structure[37]. The crystalline index of each of the NFCs produced are found from the peaks above; the method used was described in the characterization section (**Table 7**).

Table 7. Crystalline Index and % Crystallinity for NFCs without thermal treatment, cold run and standard NFC

Treatment	Crystalline Index	% Crystallinity
No Thermal Treatment No CMC	0.386	61.97
No Thermal Treatment 90k CMC	0.630	73.00
Thermal Treatment 90k CMC	0.353	60.71

The NFC produced with no thermal treatment and 90,000 CMC had the highest % crystallinity. When comparing the two gels without thermal treatment, the addition of CMC impacted the crystallinity. The presence of CMC without thermal treatment helped preserve the crystalline structures within the gel. However, when the non thermally treated NFC with 90,000 CMC was compared to the standard NFC with thermal treatment and 90,000 CMC, the crystallinity decreased. This could be due to the thermal treatment removing some of the crystalline structures that were preserved with just CMC and no thermal treatment.

The purpose of this study was to discover if thermally treating the NFC and warming the machine and initial water made a difference on the processing and integrity of the NFC produced. Processing and the actual homogenization did not seem to make a big enough difference in the structures of the gels, but once imaging and crystallinity were considered, there was a big difference. There was some reduction in crystallinity that could have been because of CMC and thermal treatments. Their addition or removal from the process did not make the cellulose more or less crystalline, but broke down some of the crystalline sections within the slurry and displaced them. Thermal treatments should continue to be a part of the NFC production, even if it does use a significant amount of energy.

Fig. 22. Intermediary samples taken in 5 minute intervals from time 0 minutes to time 25 minutes.

Intermediary Study

Intermediary samples were taken from the production of 2% gel every 5 minutes during the homogenization process (**Figure 22**). This was conducted to determine the physical and chemical changes throughout the entire homogenization process. The 5-minute intervals were due to the cycle times, which were approximately 5 minutes each. Roughly 15 ml of sample was taken every 5 minutes. Visually, it can be seen that there were significant changes in the slurry from start to finish. In the first vial, the cellulose and water separated because the cellulose is much heavier and denser than the water. In the last vial, there was no separation and the slurry was a finished hydrogel. The center vials pictured showed less and less separation of the slurry, a thickening of the slurry, and the physical change of the slurry into a hydrogel. The viscosity of each of the intermediary samples was taken in order to determine the rheological differences within the changes of the standard NFC (**Figure 23**).

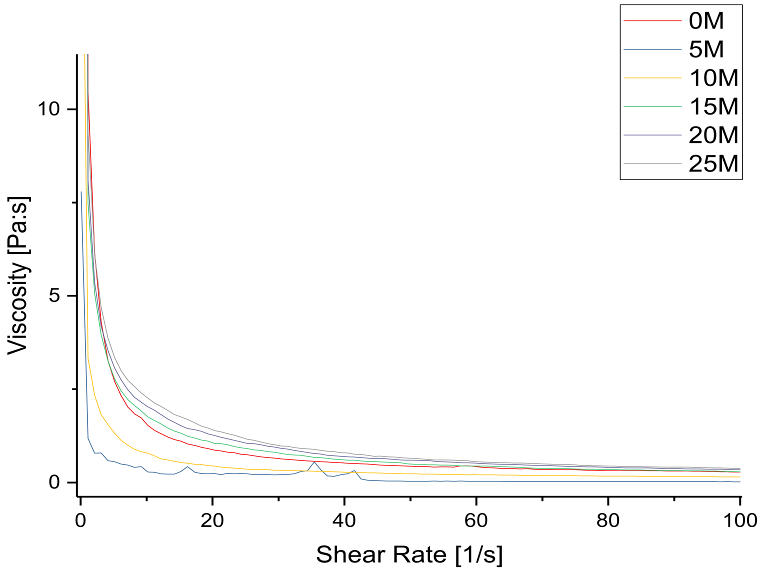


Fig. 23. Graphical representation of the viscosity changes from the start of the homogenization process until the final product is made

The viscosity graph showed that the more time the slurry had in the homogenizer, the more viscous the NFC became. This can also be attributed to the shearing of the fibrils into nano-sized materials. The first 5 minutes of the process are where the slurry was most unstable. This is because the slurry had aggregations and the orifice size in the homogenizer was small and larger material took a longer time to pass through. This study provided information regarding the physical changes that happen to the standard NFC during homogenization. The slurry started off as an unstable shear thinning liquid that became more viscous and stable as more pressure and cycles of homogenization occurred. At around 10 minutes is when the standard NFC was considered complete. The graph showed that there was not a significant enough difference in viscosity from 10 minutes to 25 minutes. This correlates with the notes that were made during production that there are no significant changes to the integrity and characterization of the NFC after it reaches 65°C.

Hemicellulose Study

Hemicellulose has been a debated aspect of cellulose research for many years. Its role within the natural source and its usefulness are still debated and not understood[20, 60]. Hemicellulose has been said to make isolating cellulose fibrils difficult[61]. There are many types of hemicellulose that are hard to extract making up a large percentage of the dry mass within the natural cellulose source[19, 60, 62]. This study was conducted to see if there was a loss of hemicellulose after the powder was treated and produced into a hydrogel. The hornification process, a procedure that dealt with the drying of powder, also affected the hemicellulose content. This study served to test an indirect method of determining hemicellulose content in the initial powder as well as after it had been through the homogenization process. This method also provided an insight into the hornification process because the powder was dried before and after. Since the process of hornification strengthens and solidifies bonding within the powder and NFCs, it is important to know its role in the NFC production process. A secondary way of determining the hemicellulose content in a pretreated NFC is to take the water retention value (WRV)[20]. This experiment was conducted on the cellulose pulp powder before and after it was produced into NFC with three varying pretreatments: no thermal treatment and no CMC; thermal treatment and no CMC; and thermal treatment and 90,000 CMC (**Tables 8-10**).

Table 8. WRV of 2% NFC with no thermal treatment and no CMC

Measurement	Value
W_0	0.9447 g
W_1	0.2702 g
WRV	249.63%

Table 9. WRV of 2% NFC with thermal treatment and no CMC

Measurement	Value
W_0	0.6004 g
W_1	0.2655 g
WRV	126.14%

Table 10. WRV of 2% NFC with thermal treatment and 90k CMC

Measurement	Value
W_0	0.6239 g
W_1	0.2538 g
WRV	145.82%

It was discovered that thermally treating the slurry does significantly reduce the WRV, meaning that there is less hemicellulose present or bonded to the cellulose. This is because hemicellulose is amorphous and helps retain water[20]. It should be noted, though, that when CMC was introduced into the thermally treated NFC, the WRV actually increased instead of decreased. This is because CMC is a gelling agent and acts as glue, which could have rebound some of the hemicellulose and cellulose within the slurry before homogenization. The introduction of thermal treatments reduced the WRV because there was more swelling within the fibrils. When there was CMC, there was a slightly less reduction of water. This was because CMC is a gelling agent and helps retain water. Therefore, there would be more water retained than without CMC.

Overall, this study provided an insight, though was not conclusive about the role of hemicellulose in NFC. It is important to understand that hemicellulose has the tendency to help cellulose retain water and pretreating or removing the hemicellulose from the NFC will reduce the amount of water that can be retained. By deductive reasoning, one can say that if the WRV value decreases, so does the hemicellulose content.

Effects of a Plasticizer Study

The ability of NFC to be modified is not short of possibilities. Because of the hydroxyl groups, it was easy to add a modification to the NFC. The purpose of the plasticizer study was to see if the NFC had the ability to demonstrate plasticizing effects when a plasticizer, glycerin, was added in various percentages to the NFC slurry. The NFC with glycerin was produced as the standard was, except glycerin was added in the slurry phase when the cellulose pulp powder and CMC were added. They were produced with 90,000 molecular weight CMC and were subjected to the thermal treatment before homogenization. The only differing variable was the addition of the glycerin in the slurries. The NFCs that had the addition of glycerin were produced with 10% (wt: cellulose powder wt), 20% and 30% glycerin added. They were compared with the standard 2% NFC in order to have a baseline to determine the effects of adding glycerin. They were tougher to produce than the standard NFC because of the thickness of the glycerin. The more glycerin added, the harder and more viscous the initial slurry was. The glycerin added to the slurry caused the homogenizer to clog more easily than in production of the 2% NFC with no glycerin in it. Even though they were more difficult to produce, the end viscosity of the NFCs with glycerin was less than the standard NFC (**Figure 24**).

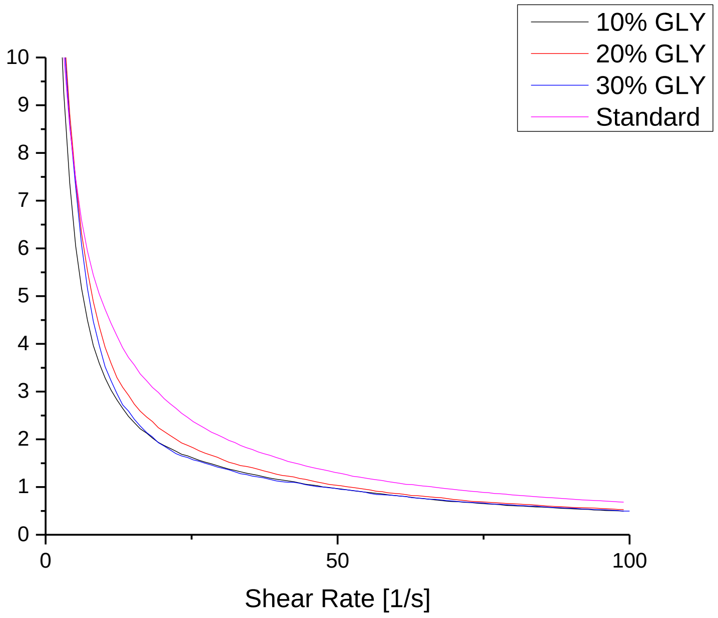


Fig. 24. Viscosity graph of Standard NFC compared to NFC with glycerin addition

The viscosity did not change much with the different percentages of glycerin added, though. This is important to know for future use. If a NFC plastic was to be created, the viscosity would not be an issue dictated by the amount of glycerin added. XRD was also taken on the three NFCs with glycerin added (**Figure 25**). This helped with phase identification of crystalline materials within the NFCs and provided the crystalline index as described in the characterization section (**Table 11**).

Table 11. Crystalline Index of NFCs with glycerin

	Crystalline Index	% Crystallinity
NFC with 10% Glycerin	0.531	68.10
NFC with 20% Glycerin	0.521	67.60
NFC with 30% Glycerin	0.607	71.80

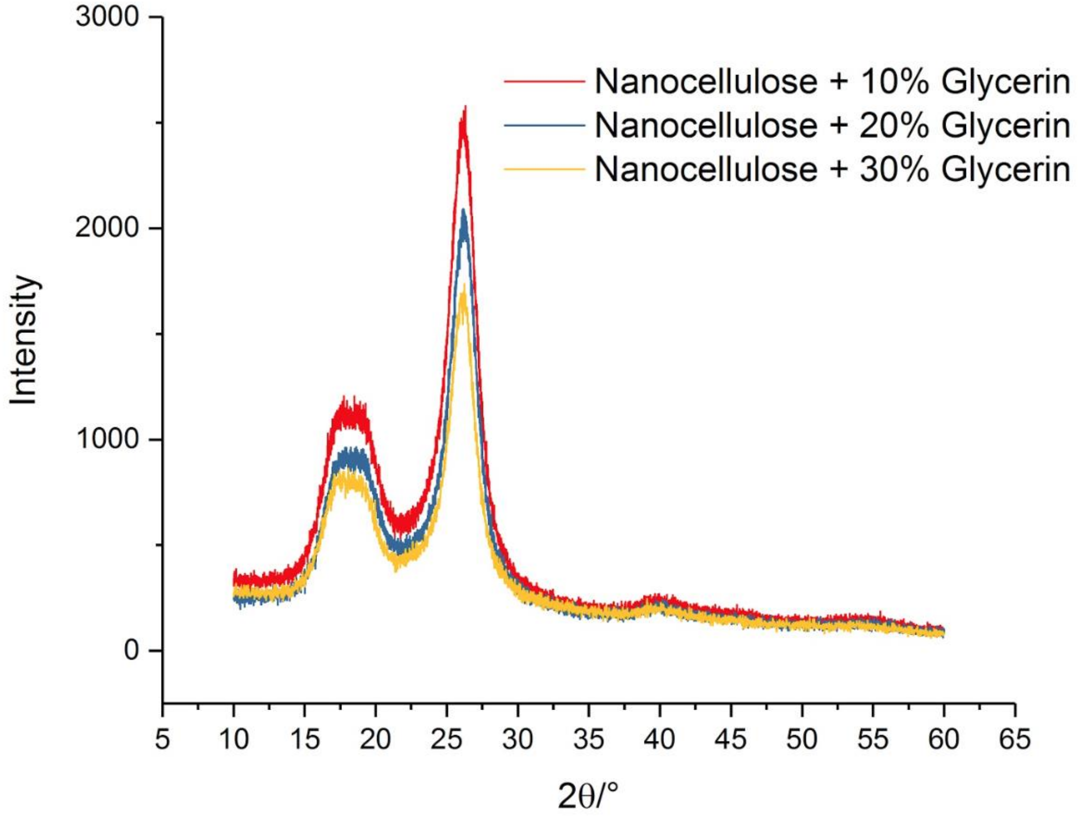


Fig. 25. XRD Spectra of NFC with added glycerin

The addition of 30% glycerin shifted the spectrum to the left. This could indicate that the amount of crystalline structures in the NFC increases at 30% glycerin added into the slurry. This could have also been due to a machine error since the samples were prepared the same way. This was the most difficult of the NFCs to produce, so the crystallization process could have begun with the initial agitation of the slurry in the blender.

Once the NFCs were made, it was important to get a visual of the structures of the fibrils so that the morphology could be understood (**Figure 26**). All three of the NFCs with glycerin formed very smooth films. All had long fibrils visible, but they were all homogenous and smooth. This would be very useful in plastics and producing molds in

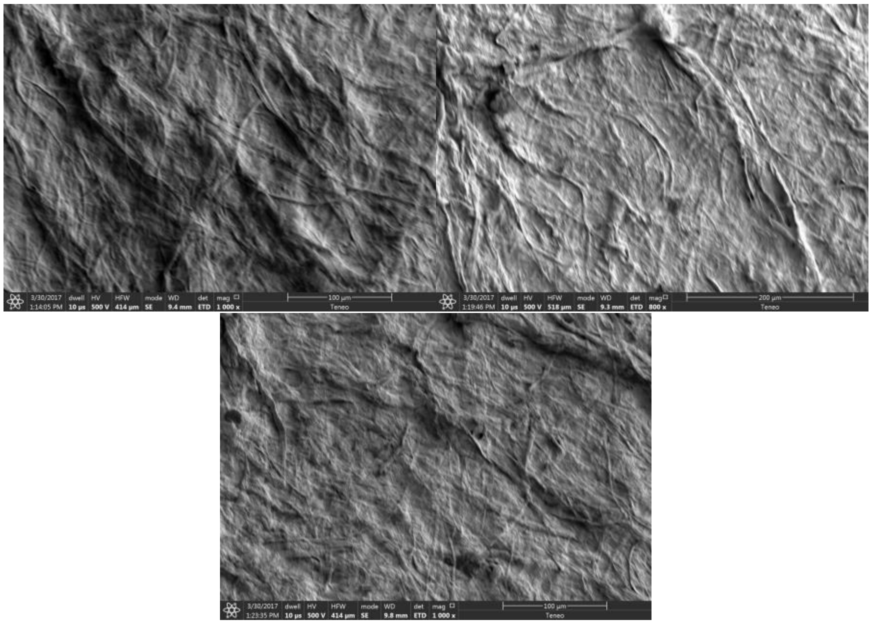


Fig. 26. SEM imaging of 2% NFC produced with 10% added glycerin (top left), 20% added glycerin (top right), and 30% glycerin (bottom)

the future because the mixtures were smooth and would be better for molding. Water contact angle also confirms this (**Figure 27**).

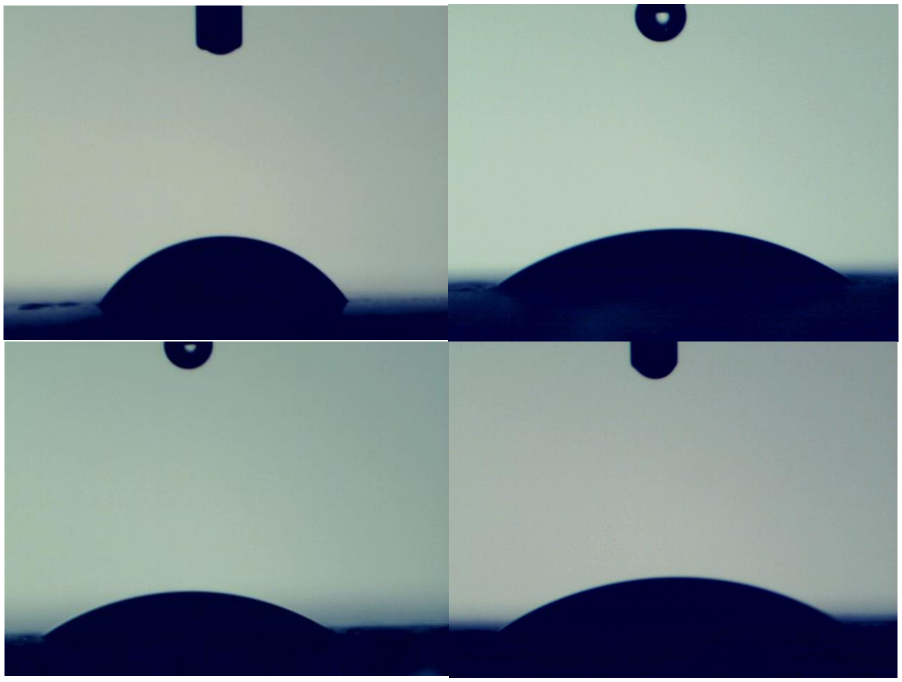


Fig. 27. Water contact angle of standard NFC (top left), NFC with 10% glycerin (top right), NFC with 20% glycerin (bottom left), and NFC with 30% glycerin (bottom right)

Table 12 provided the actual angles and the standard deviation from the measurements. This indicated that though still all hydrophilic in nature, the NFC with no glycerin added was the least hydrophilic, with the droplet forming a semicircle instead of almost flattening like on the other films treated with glycerin. This could be due to the mixture of glycerin and CMC because both are highly absorbent of water. Though, there was not a significant difference in results with the addition of the various concentrations of glycerin.

The addition of glycerin offered promising results in the application of using NFCs as coating or films, especially in food production. A significant amount of glycerin was not needed in order to get significant results. This is important because this was not a wasteful amount of materials. Further research should be conducted with the use of glycerin in NFCs. An emphasis should be put on strength and durability because of the natural strength of cellulose fibrils and the added strength that is induced due to the addition of a plasticizer.

Table 12. Water contact angle results from NFCs in glycerin study and accompanying standard deviation

Water Contact Angle Results				
	Standard NFC	NFC w/ 10% GLY	NFC w/ 20% GLY	NFC w/ 30% GLY
WCA	56.18	33.72	36.22	34.56
SD	2.39	4.62	3.99	2.99

CHARACTERIZATION

X-Ray Diffraction (XRD) XRD measurements were performed on a Bruker D8 Advantage. The diffracted intensity of Cu Kα radiation was measured in a 2θ range between 10° and 60°. The scan speed was .1second at .01 increments and the machine operated at 35 kV and 40 mA. The crystallinity index was also calculated using the results of XRD. The following equation was used:

$$CI = (I_{002} - I_{am}) / I_{002}$$

where, I_{002} is the intensity for the peak at the 2θ value of 22° and I_{am} is the intensity at the 2θ value of 18°. The % crystallinity was calculated using the following equation:

$$\%\, Crystallinity = \frac{I_{002}}{I_{002} + I_{am}} * 100$$

The samples were prepared by making thick films on glass slides. It should be noted that the value had to be taken at a 2θ value of 26° instead of 22° because there was a shift in the machine used.

Scanning Electron Microscopy (SEM) A field emission SEM was used. Samples were coated with gold using a vacuum sputter-coater to reduce charging. The samples were prepared by using freeze-dried NFC. The process required 20 ml of 2% NFC to be placed in a test tube and stored in a freezer overnight. It was then placed into a vacuum-sealed freeze dryer (Labconco Freezone 4.5) for approximately 48 hours at -50°C below 0.05 mbar.

Thermogravametric Analysis (TGA) TGA measurements were performed on a Mettler Toledo TGA. All materials were studied using nitrogen gas and a temperature range from 25°C to 800°C at 10°C/minute. The samples were oven dried at 60°C and ground back into a powder for TGA.

Viscosity The rheological properties of the gel were determined using an Anton-Parr MCR 302 Rheometer (VA, USA). Approximately 1 ml of NC gel was placed on the plate with a 1 mm gap between the rotating and base plates. The shear rate ranged from 0.1 to 100 s^{-1} with the plate set at 25°C. For the thicker hydrogels, the flat plat was used.

The less viscous samples required the conical base. Each sample was replicated three times.

Atomic Force Microscopy (AFM) AFM measurements were performed using a Bruker Dimension Icon. Samples were prepared using one drop of 100x diluted NC gel and was spin coated onto a silicon wafer that had been pretreated with PEI and piranha solutions and baked at 120°C for 20 minutes.

Fourier Transform Infrared Spectroscopy (FTIR) FTIR was measured using a Nicolet iS10 FTIR (Thermo Scientific). The samples were prepared by using freeze-dried NFC. The process required 20 ml of 2% NFC to be placed in a test tube and stored in a freezer overnight. It was then placed into a vacuum-sealed freeze dryer (Labconco Freezone 4.5) for approximately 48 hours at -50°C below 0.05 mbar.

Zeta Potential Measurements were conducted using a Malvern Zetasizer Nano Zs. Samples were prepared by diluting one drop of NC gel 100x with DI water and filling a cuvette with the diluted gel.

Water Retention Value (WRV) WRV served as an indirect method of identifying hemicellulose content. Pulp was dispersed into water at 0.5 wt % and boiled for 5 minutes. After boiling, 50g of the suspension was centrifuged for 15 minutes and filtered using a Buchner funnel until the water was removed. The wet powders were weighed (W_0). Then, the powders were dried at 110°C for 5 hours and weighed again (W_1). The values were then plugged into the following equation to get the water retention value[20]:

$$WRV\% = \frac{W_0 - W_1}{W_1} * 100$$

This test was performed on 2% NFC with no thermal treatment and 90,000 mw CMC, 2% NFC with no thermal treatment and no CMC, and the standard 2% NFC.

CHAPTER 4

COTTON BASED BIOMASS

INTRODUCTION

Cotton has been a staple plant of the United States for hundreds of years. From its cultivation to its ginning, cotton has been and still is a part of Americans' lives[41]. One of the major downfalls of the process of producing cotton fibers is that there is a lot of byproducts and material that are leftover due to the high standards the industry puts on cotton production[41]. Some of the leftover materials are cotton linters and the trash that is found with it. There is substantial research done on cotton linters, but there is nothing on cotton noil[36, 40-42, 63]. Cotton noil are the untreated, unbleached, surplus of short, staple fibers removed after the cotton is removed from the stalk. There is more of this waste than other cotton byproducts. Though it is not heavy in weight, it is large in volume and takes up a substantial amount of space. There is a need to figure out a use for this byproduct because there is plenty of potential for it to become something useful. Since cotton is 90+% cellulosic, cotton noil became a viable material to apply the homogenization and hydrogel production processes to[42].

PROCESSING

The cotton noil (**Figure 28**) was provided by Buhler Yarns (Jefferson, GA) was ground on a Wiley Mill using 20, 40, and 60 mesh sieves. The material went through one pass for each sieve and was stored The material was not pre-bleached like the cellulose

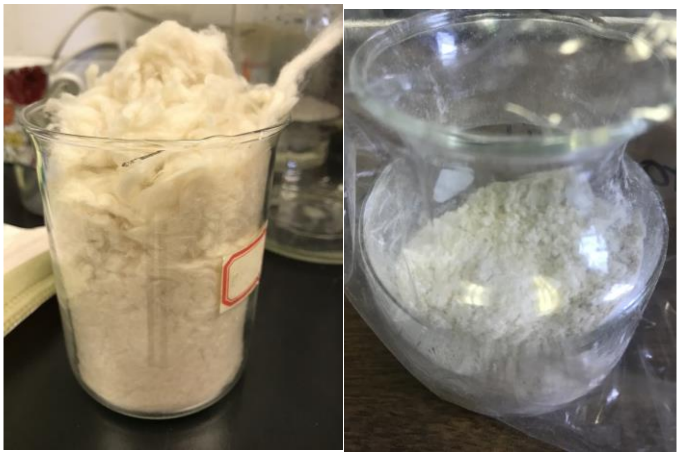


Fig. 28. Cotton noil as received and fully ground cotton noil powder

kraft plup sheets, and this material arrived in a fibrous form instead of in sheets. Once ground, the powder was stored in an oven at 60°C until use. The materials were easy to mill and formed a very fine powder. **Figure 29** provided the thermal degradation of the cotton noil powder as it was subjected to substantial amounts of heat. The majority of the degradation occurred around 350°C and completely degraded the entire sample.

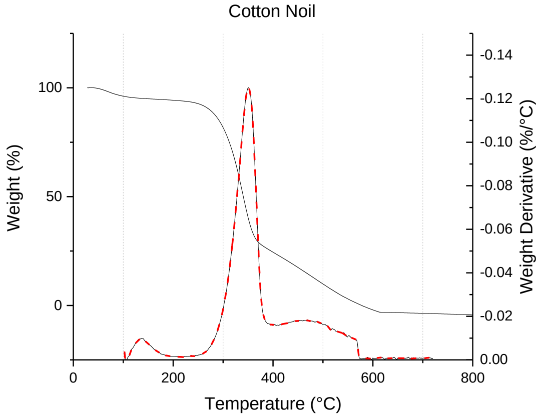


Fig. 29. TGA analysis and weight derivative of cotton noil raw material

The sieves used were standard sized mesh sieve which was approximately 250 µm. There was a significant loss of material from start to finish due to the fineness of the powder. It slipped through the small cracks in the mill and into the air. A mask and glasses were worn when milling the cotton noil because of the sensitivity to the senses the powder caused. No health risks are known, but there are not any toxicity studies done on cotton noil.

PRETREATMENT METHODS

Carboxymethylcellulose

CMC was purchased from Sigma Aldrich (MO, USA) and was used as received, with no additional modifications to the powder. The average molecular weight of the CMC used to produce the pretreatment of the cotton noil gel (CNG) was 90,000. A mechanical blender (Waring Commercial, CT) was used to blend the ground cotton noil and CMC with water for better dispersion of the materials and mixing of the slurry. The slurry refers to the mixture of powder and CMC. The blender ran at a speed of 22,000 RPM. The ratio of CMC to water was fixed to 0.0025%. The ratio to determine the amounts for each batch is X% CNG = X g of cotton noil pulp powder: 0.0025% CMC: 100 ml DI water. For example, if you wanted to produce 500 mL of 2% CNG, you would use 1.25g of CMC, 10g of cotton noil powder, and 500 ml of DI water. The CMC and cotton noil powder were blended with 65°C DI water at 22,000 RPM for 2-5 minutes. The slurry was then moved to a beaker, where it continued to be mixed at a constant agitation of 600-700 RPM on a hot plate. The slurry was brought up to a temperature of 100°C then cooled to 80°C. This allowed for the delamination to occur, just as in the processing of NFC. It was still necessary to have the delamination process for cotton noil because though this material is not 100% cellulosic, there is a majority of cellulose in it. The slurry was then blended once again for 2-5 minutes before being poured into the hopper of the homogenizer.

Thermal Treatments

Two treatments were compared to the standard treatment of 2% 90,000 mw CMC, in order to determine any differences on the effect of heat. The first treatment followed the standard procedure that was described in the previous section up until the delamination process. The slurry was blended then poured immediately into the hopper of the homogenizer. No heating and cooling steps were performed, but CMC was present in the slurry. The second treatment followed the same process, but no thermal treatment was performed and no CMC was added to the slurry. The difference between the two treatments was the addition of CMC to one of the treatments. Neither of the two treatments was subjected to any thermal treatment.

PRODUCTION OF HYDROGELS

Producing the Hydrogels

An APV 1000 homogenizer (SPX, Poland) was used for homogenization. The slurry containing the cotton noil powder and CMC was blended for 2-5 minutes before being poured into the hopper of the homogenizer, which had an internal temperature of at least 65°C. The homogenization process delaminated cellulose fibers into nanofibrils *via* mechanical shear force under high pressure. This was performed just as it was in the NFC process. The same theory of delamination can be applied to CNG because cotton noil is made predominately of cellulose. The entire gelling process took approximately 10 minutes (**Table 13**).

The slurry was considered to be a homogenized, finished gel once the temperature of it reached of at least 65°C and 80°C, just as in NFC production. Letting the homogenization process continue to process after 65°C did not damage the material or

Table 13. Demonstrates a typical homogenization process for CNG

Time (minutes)	Temperature (°C)	Pressure (Bar)
0	51.9	20
5	69.2	730
10	59.5	710

change the integrity of the hydrogel. There were no visible differences in the material at 65°C or 65°C+. The completed gel was placed into a glass jar to cool and stored in a refrigerator until it was used for characterization purposes. It was important to keep the hydrogel in a sealed jar in a cool environment because any exposure to ambient conditions sped up the ability of the gel to mold. The final product was tan in color and thick in consistency. The thicker the consistency, the less homogenous the gel looked. At higher concentrations of noil powder it looked grainy. CNG was produced at 2% (wt:vol) and 8% (wt:vol) using 90,000 mw CMC as well as with and without thermal treatment. This provided a range of gels for comparison with each other and with CNF produced in the previous chapter.

Production of the CNGs was much easier than the NFCs. The powder was not quite so hydrophilic because of the increase of other constituents in the cotton noil powder and the lack of pre-bleaching on it. The structure is more amorphous than it is crystalline. This allowed for the powder to not swell significantly, like the cellulose pulp powder, and blend easier with the CMC and the DI water. This was true for all four of the gels made with cotton noil powder as their base. The slurries produced reached 100°C much faster than the slurries of NFC and the same was true for cooling to 80°C . The overall time it took from the slurry to finished gel was much less than the NFC. Higher concentrations could be produced of CNG over NFC. Production of NFC had to be capped at around 5% while CNG was produced at 8% with ease. This is due to the shortness of these staple fibers where as NFC materials are long fibrils.

STUDIES

Rheological Study

This study focused on the rheological properties of the CNG. Four different CNGs were produced in order to see if changing thermal treatments or concentrations of raw material would make a difference on the integrity of the gel. This would be beneficial for characterization and for developing future uses of the material once its characteristics are fully understood. The viscosity gave the first insight as to what could be determined in the rest of the characterization (**Figure 30**). The 8% CNG had the highest viscosity, which is to be expected, because it had the highest concentration of raw material within its slurry. The least viscous was the 2% CNG. Unlike NFCs, the two hydrogels that were not thermally treated had higher viscosities than the standard CNG. All of the CNGs produced were shear thinning and thixotropic. In terms of just

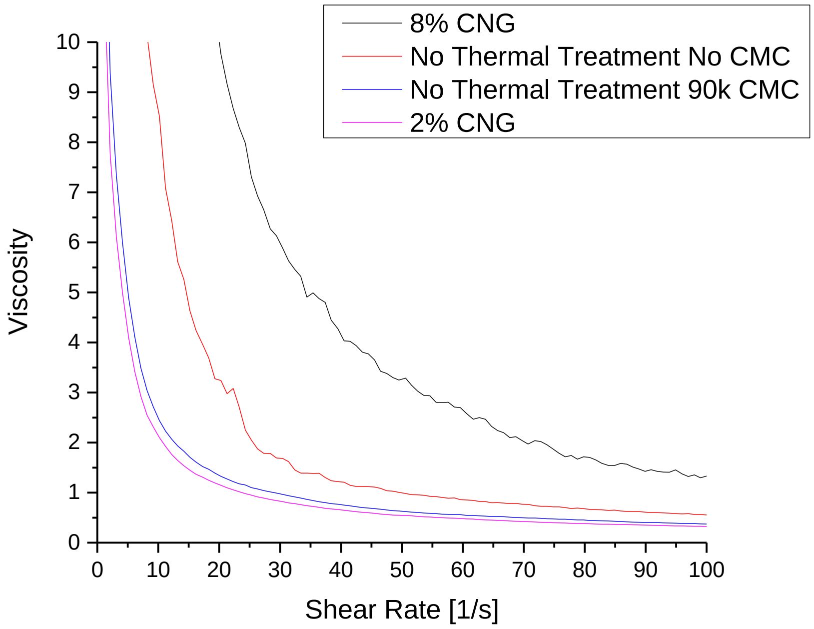


Fig. 30. Viscosities of the treated CNGs

rheological characterization, the most viscous non-thermally treated CNG was the CNG without the CMC.

By just comparing rheological results, cotton noil at 8% is the best choice to produce a viscous hydrogel without much energy or additives because of the short production time, and high viscosity. However, if using a lesser concentration is necessary, no pre-thermal treatment is necessary to delaminate the cellulose in the cotton noil to still produce a very viscous stable, shear thinning gel that does not require significant production time or energy use from the machinery. The CNGs at the lower concentrations (2%) would still be a viable option for composites. So, it can be concluded that with 8% cotton noil gel being the most viscous and 2% cotton noil gel being the least viscous, the viscosity increased as the concentration increased. However, the 2% cotton noil gels without thermal treatments had higher viscosities than the 2% cotton noil gel with thermal treatment. The 2% cotton noil gel without thermal treatment and 90k CMC had a lower viscosity than the 2% cotton noil gel without both thermal treatment and CMC. This lead to the conclusion that CMC not thermal treatment influenced viscosity in cotton noil hydrogels.

Morphological Study

To understand on a smaller scale what the CNGs produced looked like, SEM imaging was taken to get structural views and an idea of what a film or coating would like when produced using cotton noil as a base for a hydrogel (**Figure 31**). The baseline for the films was the 2% CNG with 90,000 CMC, top left of Figure 14. It, like 2% NFC demonstrated a smooth and flat surface with visual fibrils. The 8% CNG, however, had a significant amount of aggregates and thicker bundles of fibrils. This confirms what was visually seen during and after production of the 8% CNG. The two hydrogels produced without any thermal treatment favored each other, both having significantly more aggregations than the 2% thermally treated 90,000 CMC, but much less than the 8% CNG. However, the CNG produced with 90,000 CMC and no thermal treatment did appear to be more homogenous than the CNG without CMC. So, it was concluded that as

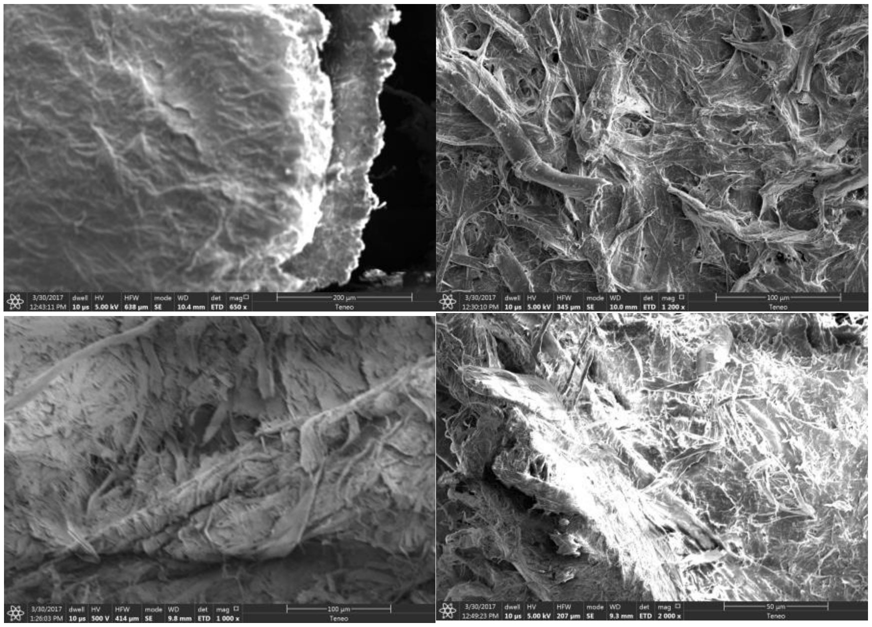


Fig. 31. SEM imaging of 2% CNC produced by 90,000 CMC (top left), 8% CNC produced by 90,000 CMC (top right), 2% no thermal treatment and no CMC (bottom left) and 2% no thermal treatment and 90,000 CMC (bottom right)

concentration increased, the films became less smooth and contained more aggregations within the films. The addition of the thermal treatments also reduced aggregations within the films.

The zeta potential of the CNGs produced are as follows: -57 mV for 2% CNG, -57.8 mV for 8% noil, -48.1 mV for CNG with no thermal treatment and no CMC, and -56.5 for CNG with no thermal treatment and 90,000 CMC. These should all be negatively charged, as they are, because they are anionic and cellulose based materials are negatively charged[58]. These results suggested that thermal treatments and concentration do not impact the charge because the zeta potentials of the 2% cotton noil gel with thermal treatment and 90,000 CMC was almost identical to that of the 8% cotton noil gel with thermal treatment and 90,000 CMC. Their charges were similar to the charge of the 2% cotton noil gel with no thermal treatment but with 90,000 CMC. The only major difference in charges was when the CMC was introduced into the hydrogel. The lack of CMC in the hydrogel increased the charge y almost 10 mV. So, it can be concluded that the addition of CMC showed a difference in charge.

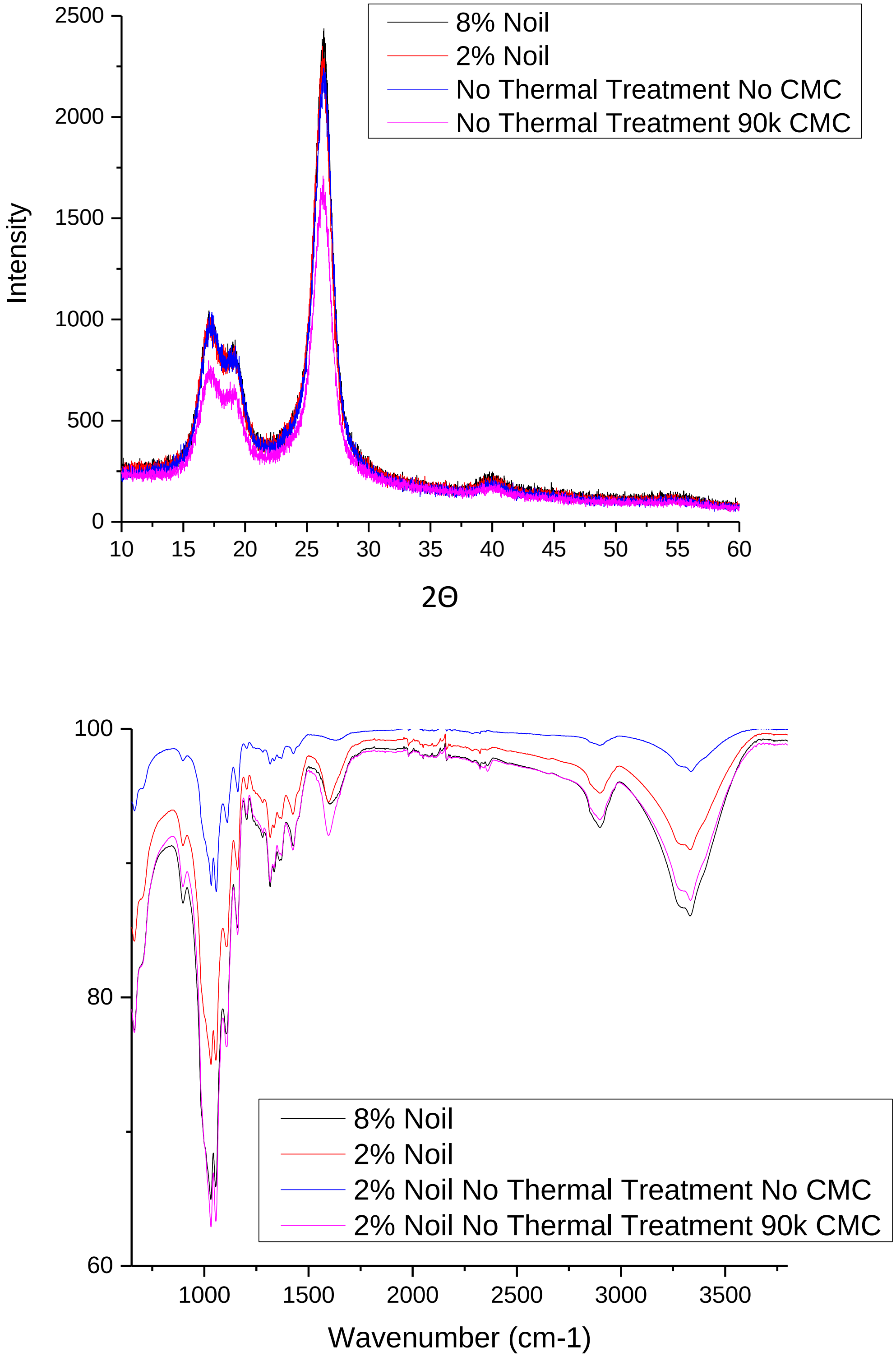


Fig. 32. XRD Spectra (top) and FTIR Spectra (bottom) of CNGs

The XRD results of CNGs favor the spectra of the NFCs (**Figure 32, Table 14**). This confirmed by the results of other cellulose based materials[64]. However, because there are impurities and trash in the cotton noil raw material, there are two peaks in the first curve on the CNG peaks where as the CNF only has one smooth curve. The results from FTIR also resemble that of the NFCs, but there are also some added dips where the impurities can be identified. Also, it was concluded that neither thermal treatment, CMC, or concentration significantly effected crystallinity within cotton noil hydrogels.

Table 14. % Crystallinity and CI of CNFs

	8%	2%	CNF w/ No Thermal Treatment No CMC	CNF w/ No Thermal Treatment 90k CMC
% Crystallinity	70.05	70.73	71.01	69.86
CI	0.572	0.586	0.592	0.568

Thermal Properties Study

So, in conclusion, thermal treatments should be a staple part of the CNG production process. The melting and degradation temperatures confirmed that the heating and cooling process as well as the end time for the gelling should be as they are[65]. Any higher temperatures would not change the integrity of the gel and would be a waste of resources.

Hemicellulose Study

Water retention value was conducted as an indirect method for determining the amount of hemicellulose present in the CNG (see characterization section). The WRV describes the amount of water intake before and after a boiling process. The WRV was taken of both the powder and the thermally treated powder from the hydrogel production

(**Tables 15 and 16**). Those values produced the difference in the initial and final ability of the powder to retain water. These values indirectly describe how much hemicellulose is removed within the processing because hemicellulose helps retain water. The powder has a low water retention value because there was only a small amount of reduction in hemicellulose content from the wet to dry powders.

Table 15. WRV of cotton noil powder

Measurement	Value
W_0	0.3847
W_1	0.2152
WRV	67.41%

Table 16. WRV of thermally treated cotton noil gel with 90,000 CMC

Measurement	Value
W_0	0.5967
W_1	0.2354
WRV	153.48%

CONCLUSION

Overall, cotton noil proved to be a good cellulose based material to use. This cotton byproduct, though does not have a current use, has the characteristics to be produced into a sustainable and durable hydrogel that is comparable to other hydrogels from cellulose based materials. SEM analysis showed smooth films. The gels were easier to produce than the NFC hydrogels produced in the previous chapter and could be produced at higher temperatures and concentrations than the NFCs. The cotton noil was not pretreated in its raw form, unlike the cellulose kraft pulp, so that allowed for the ability to do more pretreatments in the future. Thermal degradation indicated that the materials were stable at high temperatures. WRV confirmed that pretreatment and mechanical processing through homogenization changed the ability of the material to retain water, also indicating that there was a reduction of hemicellulose and other trash being released from the cellulose within the noil.

CHARACTERIZATION

X-Ray Diffraction (XRD) XRD measurements were performed on a Bruker D8 Advantage. The diffracted intensity of Cu Kα radiation was measured in a 2θ range between 10° and 60° for cellulose materials and a 2θ range between 5° and 40° for silk materials. The scan speed was .1second at .01 increments and the machine operated at 35 kV and 40 mA. The crystallinity index was also calculated using the results of XRD. The following equation was used[33, 35, 37, 57, 66]:

$$CI = (I_{002} - I_{am}) / I_{002}$$

where, I_{002} is the intensity for the peak at the 2θ value of 22° and I_{am} is the intensity at the 2θ value of 18°. The % crystallinity was calculated using the following equation:

$$\%\ Crystallinity = \frac{I_{002}}{I_{002} + I_{am}} * 100$$

The samples were prepared by making thick films on glass slides. It should be noted that the 2θ value of 26° was used instead of 22° because there was a shift in the machine used.

Scanning Electron Microscopy (SEM) A field emission SEM was used. Samples were coated with gold using a vacuum sputter-coater to reduce charging. The samples were prepared by using freeze-dried NFC. The process required 20 ml of 2% NFC to be placed in a test tube and stored in a freezer overnight. It was then placed into a vacuum-sealed freeze dryer (Labconco Freezone 4.5) for approximately 48 hours at -50°C below 0.05 mbar.

Thermogravametric Analysis (TGA) TGA measurements were performed on a Mettler Toledo TGA. All materials were studied using nitrogen gas and a temperature range from 25°C to 800°C at 10°C/minute. The samples were oven dried at 60°C and ground back into a powder for TGA.

Differential Scanning Calorimetry (DSC) DSC measurements were performed on a Mettler Toledo DSC using nitrogen gas and a temperature range from 40°C to 180°C at a rate of 10°C /minute. The samples were oven dried at 60°C and ground back into a powder for DSC.

Viscosity The rheological properties of the gel were determined using an Anton-Parr MCR 302 Rheometer (VA, USA). Approximately 1 ml of NC gel was placed on the plate with a 1 mm gap between the rotating and base plates. The shear rate ranged from 0.1 to 100 s^{-1} with the plate set at 25°C. For the thicker hydrogels, the flat plat was used. The less viscous samples required the conical base. Each sample was replicated three times.

Fourier Transform Infrared Spectroscopy (FTIR) FTIR was measured using a Nicolet iS10 FTIR (Thermo Scientific). The samples were prepared by using freeze-dried NFC. The process required 20 ml of 2% NFC to be placed in a test tube and stored in a freezer overnight. It was then placed into a vacuum-sealed freeze dryer (Labconco Freezone 4.5) for approximately 48 hours at -50°C below 0.05 mbar.

Zeta Potential Measurements were conducted using a Malvern Zetasizer Nano Zs. Samples were prepared by diluting one drop of gel 100x with DI water and filling the cuvette.

Water Retention Value (WRV) WRV served as an indirect method of identifying hemicellulose content. Pulp was dispersed into water at 0.5 wt % and boiled in water for 5 minutes. After boiling, 50g of the suspension was centrifuged for 15 minutes and filtered using a Buchner funnel until the majority of the water was removed. The wet powders were weighed (W_0). Then, the powders were dried at 110°C for 5 hours and weighed again (W_1). The values were then plugged into the following equation to get the water retention value[20]:

$$WRV\% = \frac{W_0 - W_1}{W_1} * 100$$

CHAPTER 5

APPLICATIONS AND FUTURE WORK

NFC

The NFC produced in this research was a very versatile product. It can be used for a variety of applications in a variety of field. It can also be modified to make variations of one application. NFC is currently being used in textile dyeing[67]. It is also in the developmental stages of a biomedical suture and 3D scaffolding using a melt extrusion method into an ethanol bath (**Figure 33**).

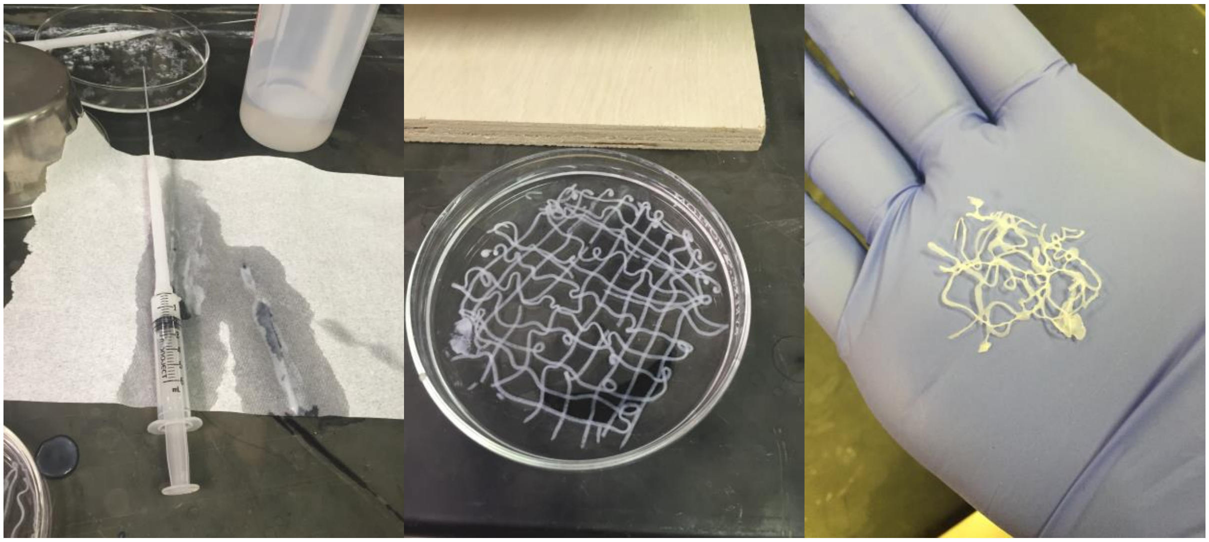


Fig. 33. From left to right: Syringe and glass pipet for self-assembling NFC fibrils, self-assembling fibers in an ethanol bath, and a precursor to a 3D scaffold

Our method for this was inspired by a paper exploring the potential biomedical applications of nanocellulose[68]. A glass pipet was melted and cut to size in order to produce a smaller orifice size that was roughly less than 1 mm. It was left to cool before being used. This took precision and needed to be repeated until the thin, uniform orifice size was formed. The NFC was gathered in a syringe and pushed through the glass pipet. It was heated for a few seconds, just to before the boiling point, at the base of the thin part of the syringe and the expanded part then pushed through the thin tube directly into an ethanol bath in a glass dish (**Figure 33**). The NFC was allowed a solvent exchange once it was in the ethanol for approximately 15 minutes then dried for 2-3 hours in ambient conditions. This solvent exchange allowed for the water in the NFC to be exchanged with ethanol so that increased strength and can be achieved. The solvent exchange and drying process was repeated 3 times. The more times the solvent exchange occurs, the stronger the fibers appeared to be. There was also a certain degree of self-assembly in the fibers. The solvent exchange and drying process appeared to promote the fibers to reassemble into straight lines with thin fibrils and nodes where the attachment occured**.** It was then shaped into a crude 3D-scaffold. Once automation could be achieved, the fibers will be more uniform and tighter in position together. Formal characterization testing has not been done, but it is planned for in future studies.

Another application that is in the preliminary production stage is that NFC could be used as a filler for cement. A mixture of 4 g of NFC and 12g of cement powder were mixed and let to set. Both samples appeared to be fairly similar in physical and visual looks, but there is no data to compare the two yet. These are also future works and applications of NFC. There are commercial standards for cement and roadways that would be used and that are available for testing the standard cement and well as the NFC cement for comparison.

A future application that is still in the planning stage is the ability of NFC at a low concentration be used to develop a bioink with a mixture of pigments and NFC for digital printing and textile digital printing. Food applications, pharmaceutical applications, and nutraceutical applications are being developed using NFC as a coating for extended release properties.

Lastly, these processing, pretreatment, and production methods considered in this research have the potential to be used for more than just lignocellulosic or natural materials. Time permitting, the processing, pretreatment, and production methods described above will be used on synthetic materials. Future studies should include producing plastics and molds from the NFC with the addition of glycerin. There could be a big potential market for this type of material. NFC has already been used in some food packaging[4-5, 69]. However, the method described in this research offers a better alternative. Also, tensile testing should be performed on the gels once they are formed into a mold or shape. This would help determine more and better uses for the gel. Scaling up is also in the direct future. This process should not be complicated because the ratios can be scaled easily and all of the materials are easy to gather in large quantities. Increasing the production capacity will allow more application based research to occur and will be one of the first large scale NFCs to be produced other companies such as Exilva, Blue Goose Biorefineries, or Bioplus produce and sell their nanocellulosic hydrogels or nanocrystalline hydrogels, but they are not produced on a large scale. This is very important to the future of nanocellulose hydrogels because large scale production would allow for many people to use the gel and for many more uses of it to be discovered.

COTTON NOIL

Cotton noil has the potential to be a filler or a composite. Because it is tan in nature, any addition of pigments may be distorted because the raw material was tinted to begin with. However, a bleaching agent or a synthetic pigment may be the best options for dyeing purposes. There are no toxicity studies for cotton noil and very little data about the properties of cotton noil. These should be pursued before any applications are developed using cotton noil because of the lack of current information about the material. There is little known about cotton noil. Extensive research has been performed on cotton linters and other byproducts of cotton[40, 42]. Even though these fibrils are not bleached, they could be easily bleached and used for a dyeing technique as well. Additionally, this gel has shown that it can be produced at higher concentrations than traditional nanocellulose hydrogels, therefore, it should continue to be produced at high concentrations to understand the full potential abilities and end uses of cotton noil.

CHAPTER 6

OTHER POTENTIAL MATERIALS

SILK

The silk was purchased from TKB Trading Company and was used as received. It was stored in a sealed bag away from sunlight to prevent any deterioration or degradation. The material was used in the same condition it arrived in and was not treated before the pretreatment processes it underwent. It was cosmetic grade silk and was commonly used in facial moisturizers, makeup powders, and other lotions that have luster and soothing skin properties. Silk has also been used historically in biomedical applications for sutures and wound healing purposes[44-47].

PRETREATMENT METHODS

Carboxymethylcellulose

CMC was purchased from Sigma Aldrich (MO, USA) and was used as received, with no additional modifications to the powder. The average molecular weight of the CMCs used to produce the pretreatment of the cotton noil gel (CNG) was 90,000. A mechanical blender (Waring Commercial, CT) was used to blend the ground cotton noil and CMC with water for better dispersion of the materials and mixing of the slurry. The slurry refers to the mixture of powder and CMC. The blender ran at a speed of 22,000 RPM. The ratio of CMC to water was fixed to 0.0025%. The ratio to determine the amounts for each batch is X% CNG = X g of cotton noil pulp powder: 0.0025% CMC:

100 ml DI water. For example, if you want to produce 500 mL of 2% CNG, you will use 1.25g of CMC, 10g of cotton noil powder, and 500 ml of DI water. The CMC and cotton noil powder were blended with 65°C DI water at 22,000 RPM for 2-5 minutes. The slurry was then moved to a beaker, where it continued to be mixed at a constant agitation of 600-700 RPM on a hot plate. The slurry was brought up to a temperature of 100°C then cooled to 80°C. This allowed for the delamination to occur, just as in the processing of NFC. The slurry was then blended once again for 2-5 minutes before being poured into the hopper of the homogenizer.

Thermal Treatments

One additional treatment was prepared to compare to the standard treatment of 2% 90,000 mw CMC, in order to determine any differences on the effect of heat. The silk gel was produced with no thermal treatment and no CMC in order to see if any changes occurred. The only heating occurred was the warming of the machine which was standard of the hydrogel production.

PRODUCTION OF HYDROGELS

Producing the Hydrogel

An APV 1000 homogenizer (SPX, Poland) was used for homogenization. The slurry containing the silk powder and CMC was blended for 2-5 minutes before being poured into the hopper of the homogenizer, which had an internal temperature of at least 65°C. The homogenization process delaminated cellulose fibers into nanofibrils *via* mechanical shear force under high pressure. This was performed just as it was in the NFC and CNG processes. The entire gelling process took approximately 35 minutes (**Table 17**).

Time (minutes)	Temperature (°C)	Pressure (Bar)
0	47.8	20
5	52.4	650
10	63.3	660
15	71.2	660
20	77.0	670
25	81.3	690
30	85.6	690
35	88.3	700

Table 17. Demonstrates a typical homogenization process for silk gel

The slurry was considered to be a homogenized, finished gel once the temperature of it reached between 65°C and 80°C. Letting the homogenization process continue to process after 65°C did not damage the material or change the integrity of the hydrogel. There were no visible differences in the material at 65°C or 65°C+. The silk did not form a gel like the other two materials studied in this research. Visually, the consistency changed, and there was some physical changes, but the slurry never truly thickened up. It was still more of a liquid than it was a gel. The completed gel was placed into a glass jar to cool and stored in a refrigerator. It was important to keep the hydrogel in a sealed jar in a cool environment because any exposure to ambient conditions sped up the ability of the gel to mold, especially being a protein based material. The final product was tan/purple in color and thin in consistency. Silk gel was produced at 2% (wt:vol) and 5% (wt:vol) using 90,000 mw CMC as well as without thermal treatment. There was not a significant difference between the two. This provided a range of gels for comparison with each other and with CNF and CNG produced in the previous chapter. The following studies looked at the 2% silk with 90,000 CMC and thermal treatment and 2% silk gel without CMC and thermal treatment in order to determine if pretreating and thermally treating made any difference at all on the silk mixture.

STUDIES

Morphological Study

The silk gel performed differently than the other gels produced in this research, see rheological study and refer to previous chapters. The initial particle size was much smaller to begin with, averaging 3 microns and was milled using a 1250 mesh sieve[70]. Visually, the silk gels are much smoother and have smaller particles in length but not width than cellulose based materials (**Figure 34**).

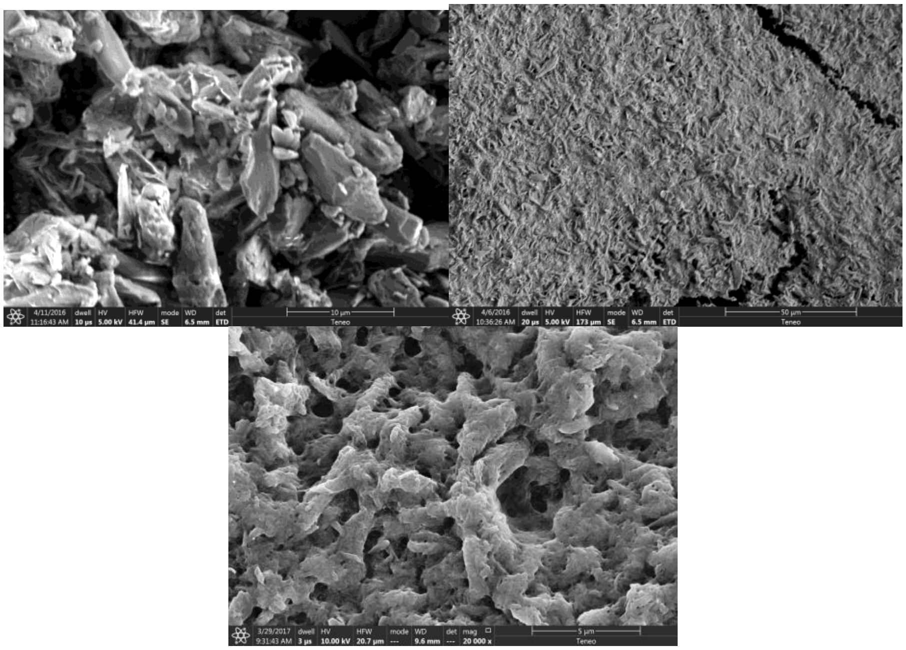


Fig. 34. Silk gel production from initial powder (top left), 2% silk gel after freeze drying (top right), and 2% silk gel with no thermal treatment and no CMC (bottom)

The zeta potential of the two silk gels were -50.4 mV for 2% silk gel and -45.7 for 2% silk gel with no thermal treatment and no CMC. These negatively charged gels have lots of active hydrogen bonds that encourage the negative charge and the ease of bonding with this protein.

CHARACTERIZATION

Scanning Electron Microscopy (SEM) A field emission SEM was used. Samples were coated with gold using a vacuum sputter-coater to reduce charging. The samples were prepared by using freeze-dried silk gel. The process required 20 ml of 2% NFC to be placed in a test tube and stored in a freezer overnight. It was then placed into a vacuum-sealed freeze dryer (Labconco Freezone 4.5) for approximately 48 hours at -50°C below 0.05 mbar.

Zeta Potential Measurements were conducted using a Malvern Zetasizer Nano Zs. Samples were prepared by diluting one drop of gel 100x with DI water and filling the cuvete.

LYOCELL

Lyocell was ground using the Wiley Mill with the 60 mesh sieve. It was then stored in an oven at 60°C. It is a potential material to be produced into a hydrogel because it is a synthetic biomass left over from the production of Rayon. It is a waste product/by-product of the rayon processing methods, so it fits the criteria of using a waste material to create a sustainable hydrogel. The processing discussed in this research has proved successful for natural polymers and proteins, so the next step is to try it on a synthetic material. Because this is regenerated cellulose, lyocell seemed like the next logical step to ease into synthetic materials[71].

REFERENCES

1. Habibi, Y. L., Lucian A.; Rojas, Orlando J., Cellulose Nanocrystals: Chemistry, Self-Assembly, and Applications. *Chem. Rev.* **2009**, 22.

2. Dufresne, A., Nanocellulose: a new ageless bionanomaterial. *Materials Today* **2013,** *16* (6), 220-227.

3. Saito, T.; Okita, Y.; Nge, T. T.; Sugiyama, J.; Isogai, A., TEMPO-mediated oxidation of native cellulose: Microscopic analysis of fibrous fractions in the oxidized products. *Carbohydrate Polymers* **2006,** *65* (4), 435-440.

4. Kalia, S.; Dufresne, A.; Cherian, B. M.; Kaith, B. S.; Avérous, L.; Njuguna, J.; Nassiopoulos, E., Cellulose-Based Bio- and Nanocomposites: A Review. *International Journal of Polymer Science* **2011,** *2011*, 1-35.

5. Klemm, D.; Kramer, F.; Moritz, S.; Lindstrom, T.; Ankerfors, M.; Gray, D.; Dorris, A., Nanocelluloses: a new family of nature-based materials. *Angew Chem Int Ed Engl* **2011,** *50* (24), 5438-66.

6. Xu, Y.; Atrens, A. D.; Stokes, J. R., Rheology and microstructure of aqueous suspensions of nanocrystalline cellulose rods. *J Colloid Interface Sci* **2017,** *496*, 130-140.

7. Turbak, A. F. Birth of Nanocellulose *Over the Wire* [Online], 2011. http://www.naylornetwork.com/ppi-otw/newsletter.asp?issueID=22333.

8. Park, H.; Kim, M. H.; Yoon, Y. I.; Park, W. H., One-pot synthesis of injectable methylcellulose hydrogel containing calcium phosphate nanoparticles. *Carbohydr Polym* **2017,** *157*, 775-783.

9. Portnov, T. S., Tiberiu R.; Zilberman, Meital, Injectable hydrogel-based scaffolds for tissue engineering applications. *Reviews in Chemical Engineering* **2016,** *33* (1), 16.

10. Oliveira, J. P.; Bruni, G. P.; Lima, K. O.; Halal, S. L.; Rosa, G. S.; Dias, A. R.; Zavareze, E. D., Cellulose fibers extracted from rice and oat husks and their application in hydrogel. *Food Chem* **2017,** *221*, 153-160.

11. Paleos, G. A. What are Hydrogels? 2012.

12. Mehrali, M.; Thakur, A.; Pennisi, C. P.; Talebian, S.; Arpanaei, A.; Nikkhah, M.; Dolatshahi-Pirouz, A., Nanoreinforced Hydrogels for Tissue Engineering: Biomaterials that are Compatible with Load-Bearing and Electroactive Tissues. *Adv Mater* **2017,** *29* (8).

13. Lin, N.; Dufresne, A., Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal* **2014,** *59*, 302-325.

14. Picker, K. M. H., Stephen W., Characterization of the Thermal Properties of Microcrystalline Cellulose by Modulated Temperature Differential Scanning Calorimetry. *Journal of Pharmaceutical Sciences* **2002,** *91* (2), 8.

15. Nechyporchuk, O.; Belgacem, M. N.; Bras, J., Production of cellulose nanofibrils: A review of recent advances. *Industrial Crops and Products* **2016,** *93*, 2-25.

16. Horiba Instruments, I., Why is Particle Size Important? In http://www.horiba.com/us/particle, Horiba Instruments, I., Ed. HORIBA Instruments, Inc.: 2012.

17. Chinga-Carrasco, G., Cellulose fibers, nanofibrils, and microfibrils: The morphological sequence of MFC components from a plant physiology and fibre technology point of view. *Nanoscale Research Letters* **2013**.

18. Arola, S.; Malho, J. M.; Laaksonen, P.; Lille, M.; Linder, M. B., The role of hemicellulose in nanofibrillated cellulose networks. *Soft Matter* **2013,** *9* (4), 1319-1326.

19. Liu, J.; Chinga-Carrasco, G.; Cheng, F.; Xu, W.; Willför, S.; Syverud, K.; Xu, C., Hemicellulose-reinforced nanocellulose hydrogels for wound healing application. *Cellulose* **2016,** *23* (5), 3129-3143.

20. Iwamoto, S. A., Kentaro; Yano, Hiroyuki, The Effect of Hemicelluloses on Wood Pulp Nanofibrillation and Nanofiber Network Characteristics. *Biomacromolecules* **2007,** *9* (3), 1022-1026.

21. Vallejos, M. E.; Felissia, F. E.; Area, M. C.; Ehman, N. V.; Tarres, Q.; Mutje, P., Nanofibrillated cellulose (CNF) from eucalyptus sawdust as a dry strength agent of unrefined eucalyptus handsheets. *Carbohydrate Polymers* **2016,** *139*, 99-105.

22. Sacui, I. A.; Nieuwendaal, R. C.; Burnett, D. J.; Stranick, S. J.; Jorfi, M.; Weder, C.; Foster, E. J.; Olsson, R. T.; Gilman, J. W., Comparison of the properties of cellulose nanocrystals and cellulose nanofibrils isolated from bacteria, tunicate, and wood processed using acid, enzymatic, mechanical, and oxidative methods. *ACS Appl Mater Interfaces* **2014,** *6* (9), 6127-38.

23. EMPA, New process for manufcaturing nanocellulose: Using nanocellulose to create novel composite materials. *Science Daily* **2011**.

24. Dimic-Misic, K.; Ridgway, C.; Maloney, T.; Paltakari, J.; Gane, P., Influence on Pore Structure of Micro/Nanofibrillar Cellulose in Pigmented Coating Formulations. *Transport in Porous Media* **2014,** *103* (2), 155-179.

25. Habibi, Y., Key advances in the chemical modification of nanocelluloses. *Chem Soc Rev* **2014,** *43* (5), 1519-42.

26. Fernandes Diniz, J. M. B.; Gil, M. H.; Castro, J. A. A. M., Hornification?its origin and interpretation in wood pulps. *Wood Science and Technology* **2004,** *37* (6), 489-494.

27. Giacomozzi, D. E. J., Olli, Drying Temperature and Hornification of Industrial Never-Dried Pinus radiata Pulps. 1. Strength, Optical, and Water Holding Properties . *Biosources* **2015,** *10* (3), 17.

28. Ballesteros, J. E. M.; Santos, S. F.; Mármol, G.; Savastano, H.; Fiorelli, J., Evaluation of cellulosic pulps treated by hornification as reinforcement of cementitious composites. *Construction and Building Materials* **2015,** *100,* 83-90.

29. Arvidsson, R.; Nguyen, D.; Syanstrom, M., Life Cycle Assessment of Cellulose Nanofibrils Production by Mechanical Treatment and Two Different Pretreatment Processes. *Environmental Science & Technology* **2015,** *49* (11), 6881-6890.

30. Chinga-Carrasco, G.; Syverud, K., Pretreatment-dependent surface chemistry of wood nanocellulose for pH-sensitive hydrogels. *J Biomater Appl* **2014,** *29* (3), 423-32.

31. Larsson, P. T.; Salmén, L., Influence of cellulose supramolecular structure on strength properties of chemical pulp. *Holzforschung* **2014,** *68* (8).

32. Abraham, E.; Deepa, B.; Pothan, L. A.; Jacob, M.; Thomas, S.; Cvelbar, U.; Anandjiwala, R., Extraction of nanocellulose fibrils from lignocellulosic fibres: A novel approach. *Carbohydrate Polymers* **2011,** *86* (4), 1468-1475.

33. V.S. Karande, A. K. B., G.B. Hadge, S.T. Mhaske, and N. Vigneshwaran, Nanofibrillation of Cotton Fibers by Disc Refiner and Its Characterization. *Fibers and Polymers* **2011,** *12* (3), 399-404.

34. Savadekar, N. R.; Karande, V. S.; Vigneshwaran, N.; Bharimalla, A. K.; Mhaske, S. T., Preparation of nano cellulose fibers and its application in kappa-carrageenan based film. *Int J Biol Macromol* **2012,** *51* (5), 1008-13.

35. Jain, P.; Vigneshwaran, N., Effect of Fenton's pretreatment on cotton cellulosic substrates to enhance its enzymatic hydrolysis response. *Bioresour Technol* **2012,** *103* (1), 219-26.

36. Silverstein, R. A.; Chen, Y.; Sharma-Shivappa, R. R.; Boyette, M. D.; Osborne, J., A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresour Technol* **2007,** *98* (16), 3000-11.

37. Hassan, M. M., A; Hassan E; Oksman, K, Effect of pretreatment on properties of nanopaper sheets. *Wood and Fiber Science* **2010,** *42* (3), 362-376.

38. Morán, J. I.; Alvarez, V. A.; Cyras, V. P.; Vázquez, A., Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose* **2007,** *15* (1), 149-159.

39. Zhao, H.; Kwak, J.; Conradzhang, Z.; Brown, H.; Arey, B.; Holladay, J., Studying cellulose fiber structure by SEM, XRD, NMR and acid hydrolysis. *Carbohydrate Polymers* **2007,** *68* (2), 235-241.

40. Sczostak, A., Cotton Linters: An Alternative Cellulosic Raw Material. *Macromolecular Symposia* **2009,** *280* (1), 45-53.

41. Jeihanipour, A.; Taherzadeh, M. J., Ethanol production from cotton-based waste textiles. *Bioresour Technol* **2009,** *100* (2), 1007-10.

42. Morais, J. P.; Rosa Mde, F.; de Souza Filho Mde, S.; Nascimento, L. D.; do Nascimento, D. M.; Cassales, A. R., Extraction and characterization of nanocellulose structures from raw cotton linter. *Carbohydrate Polymers* **2013,** *91* (1), 229-35.

43. Hardy, J. G.; Römer, L. M.; Scheibel, T. R., Polymeric materials based on silk proteins. *Polymer* **2008,** *49* (20), 4309-4327.

44. Kluge, J. A.; Rabotyagova, O.; Leisk, G. G.; Kaplan, D. L., Spider silks and their applications. *Trends Biotechnol* **2008,** *26* (5), 244-51.

45. Hakimi, O.; Knight, D. P.; Vollrath, F.; Vadgama, P., Spider and mulberry silkworm silks as compatible biomaterials. *Composites Part B: Engineering* **2007,** *38* (3), 324-337.

46. Allardyce, B. J.; Rajkhowa, R.; Dilley, R. J.; Atlas, M. D.; Kaur, J.; Wang, X., The impact of degumming conditions on the properties of silk films for biomedical applications. *Textile Research Journal* **2015,** *86* (3), 275-287.

47. Vepari, C.; Kaplan, D. L., Silk as a Biomaterial. *Prog Polym Sci* **2007,** *32* (8-9), 991-1007.

48. Turbak, A. F. S., Fred W.; Sanberg, Karen R. Microfibrillated Cellulose. 1983.

49. Lindhorst, T. K., *Essentials of Carbohydrate Chemistry and Biochemistry*. Wiley-VCH: Federal Republic of Germany, 2000; p 217.

50. Hart, H. C., Leslie E.; Hart, David J.; Hadad, Christopher M., *Organic Chemistry: A Short Course*. 12 ed.; Houghton Mifflin Company: Boston, MA, 2007; p 577.

51. Flory, P. J., *Principles of Polymer Chemistry*. Cornell University Press: Ithaca, New York, 1953; p 672.

52. Coseri, S., Cellulose: To depolymerize... or not to? *Biotechnol Adv* **2017,** *35* (2), 251-266.

53. Wang, S.; Lu, A.; Zhang, L., Recent advances in regenerated cellulose materials. *Progress in Polymer Science* **2016,** *53*, 169-206.

54. Paakko, M.; Ankerfors, M.; Kosonen, H.; Nykanen, A.; Ahola, S.; Osterberg, M.; Ruokolainen, J.; Laine, J.; Larsson, P. T.; Ikkala, O.; Lindstrom, T., Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels. *Biomacromolecules* **2007,** *8* (6), 1934-41.

55. *Carboxymethyl cellulose (packaging) and Sodium carboxymethyl cellulose*; U.S. Food and Drug Administration: https://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm261244.htm, 1973.

56. Bharimalla, A. K.; Deshmukh, S. P.; Patil, P. G.; Vigneshwaran, N., Energy Efficient Manufacturing of Nanocellulose by Chemo- and Bio-Mechanical Processes: A Review. *World Journal of Nano Science and Engineering* **2015,** *05* (04), 204-212.

57. Boufi, S.; Chaker, A., Easy production of cellulose nanofibrils from corn stalk by a conventional high speed blender. *Industrial Crops and Products* **2016,** *93*, 39-47.

58. Dimic-Misic, K. R., Juuso; Maloney, Thad C.; Gane, Patrick A.C., Gel structure phase behavior in micro nanofibrillated cellulose containing in situ precipitated calcium carbonate. *Journal of Applied Polymer Science* **2016**.

59. French, A. D. C., M.S., Cellulose polymorphy, crystallite size, and the Segal Crystallinity Index. *Cellulose* **2012,** *20*, 5.

60. Yang, H.; Yan, R.; Chen, H.; Lee, D. H.; Zheng, C., Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel* **2007,** *86* (12-13), 1781-1788.

61. Yue, Y.; Han, J.; Han, G.; Zhang, Q.; French, A. D.; Wu, Q., Characterization of cellulose I/II hybrid fibers isolated from energycane bagasse during the delignification

process: Morphology, crystallinity and percentage estimation. *Carbohydr Polym* **2015,** *133*, 438-47.

62. Li, X.; Sun, C.; Zhou, B.; He, Y., Determination of Hemicellulose, Cellulose and Lignin in Moso Bamboo by Near Infrared Spectroscopy. *Sci Rep* **2015,** *5*, 17210.

63. Li, F. B., Paolo; Bollani, Monica; Maccagnan, Andrea; Piergiovanni, Luciano, Multi-functional coating of cellulose nanocrystals for flexible packaging applications. *Cellulose* **2013,** *20*, 13.

64. Mori, R. H., Toshio; Takagishi, Toru, Changes in Dyeability and Morphology of Cotton Fiber Subjected to Cellulase Treatment. **1996**.

65. Hatakeyama, H. H., Tatsuko, Interaction between water and hydrophilic polymers. *Thermochimica Acta* **1997,** *308*, 19.

66. Chaker, A.; Boufi, S., Cationic nanofibrillar cellulose with high antibacterial properties. *Carbohydr Polym* **2015,** *131*, 224-32.

67. Minko, S. S., Suraj; Hardin, Ian; Luzinov, Igor; Daubenmire, Sandy Wu; Zakharchenko, Andrey; Saremi, Raha; Kim, Yun Sang Textile dyeing using nanocellulosic fibers. 2016.

68. Rees, A.; Powell, L. C.; Chinga-Carrasco, G.; Gethin, D. T.; Syverud, K.; Hill, K. E.; Thomas, D. W., 3D Bioprinting of Carboxymethylated-Periodate Oxidized Nanocellulose Constructs for Wound Dressing Applications. *Biomed Res Int* **2015,** *2015*, 925757.

69. Korhonen, J. T.; Kettunen, M.; Ras, R. H.; Ikkala, O., Hydrophobic nanocellulose aerogels as floating, sustainable, reusable, and recyclable oil absorbents. *ACS Appl Mater Interfaces* **2011,** *3* (6), 1813-6.

70. TKB Trading, L. *Micro Fine Silk Powder*.

71. Adak, B.; Mukhopadhyay, S., A comparative study on lyocell-fabric based all-cellulose composite laminates produced by different processes. *Cellulose* **2016,** *24* (2), 835-849.