ABSTRACT

ZHU, YIDAN. The Development of Membranes Made with Blends of Soy Protein and Other Natural Biopolymers using a Novel Solvent System and Stabilized with Glutaraldehyde. (Under the direction of committee chair Richard Kotek, PhD.)

Using the novel ED/KSCN solvent system developed in previous studies, a simpler, environmentally friendlier method was developed to produce membranes using cellulose, proteins, and other polymers. In contrast to current industrial methods that use process that are relatively expensive with toxic or dangerous solvents, the new system eliminated majority of those concerns. Previously it was discovered that a blend of cellulose and soy protein membranes can be produced using this system, but due to its poor water resistance the membranes required chemical stabilization via crosslinking.

Soy protein concentrate was used to develop nonporous membranes with other natural biomaterial to make a composite membrane with good physical properties. Glutaraldehyde was used as the crosslinking agent to stabilize the molecular structure of the blended membranes. Results showed that nonporous membranes were produced that are strong, flexible, and the exposure to the crosslinking agent shown structural and thermal improvement of the membranes. This resulting blend of biopolymer membranes with improved physical abilities can be useful for food packaging, filtration systems, or even medical applications.

The Development of Membranes Made with Blends of Soy Protein and Other Natural Biopolymers using a Novel Solvent System and Stabilized with Glutaraldehyde

by
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APPROVED BY:

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Samuel Hudson, PhD	Martin King, PhD

DEDICATION

This work is dedicated to:

First and foremost, my mother Chunmei Li, who gave me life, hope, compassion, understanding, support and all the love in the world to help me go through life, who had taught me countless lessons in life and will continue to do so.

My grandparents, to my two grandmothers and one grandfather who had all passed away in the recent days, thank you for raising me up, thank you for making me who I am, thank for your constant belief in my abilities and your never ending love. And to my last living grandfather, thank you for being so strict and pushed me to my limit, for teaching me so much in academia and in life, I will do all I can to make my family proud.

My family and friends, for without your constant support I would not be who I am today, I would not be as driven and humble, and grateful to all that life has given me. Thank all of you for being there when I am down and needed help, and for being there to share all the joy in life. You all made my life colorful and I have learnt so much beyond words could describe.

Finally, to my advisor Dr. Kotek and fellow group members, thank you for your constant support and knowledge and guidance. You all had made my academic experience one that changed who I am for the better, I am truly humbled to be in the presence of so many brilliant minds, and forever grateful for all the support and understanding that I received during difficult times.

BIOGRAPHY

Yidan Zhu was born in Jilin City, in the Jilin providence in China. She grew up with her grandparents, who were aerospace engineers, and great teachers, and have taught her much as a child. She attended two different elementary schools, and learnt Chinese calligraphy, traditional Chinese painting, poetry, and Olympic math.

The life-changing event came in the summer of 1999, where she arrived in Charleston, SC and begun her life in America with her mother. Although unable to speak English, she was enrolled in middle school after being in the country for two weeks, and became an honor-roll student after just 2 years.

After graduating from high school ranking #13 among over 660 students, she attended Clemson University and earned her BS in Polymer Fiber Chemistry. During her undergraduate days she picked up Japanese and did study abroad in Kyoto, Japan for a semester, and gained much knowledge and appreciation for different language and culture. She also participated in many organizations and assumed leadership positions. After induction into the national honorary textile fraternity Phi Psi she was able to interact with students, professors, and professionals from many institutions and industries.

Post graduation from Clemson University, she was accepted into North Carolina State University Graduate School in 2010 and begun her pursue for a Master's in Textile Chemistry, under the direction of Professor Richard Kotek.

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CHAPTER 1. Introduction

In the world of natural polymers, cellulose (**Figure 1.1**) is considered to be the most abundant. Cellulose have been used throughout history, and in the current market, it is also widely used as a biodegradable and renewable polymer [1]. Cellulose can be found in wood, flax, and most famously in cotton. The recognition of cellulose as a chemical compound traced back to the 19th century, with the name officially given by a French agriculturist Anselme Payon in the 1830s. Scientists did not recognize the molecular structure of this linear 1-4-β glucose polymer until almost a century later (1930s). [1]

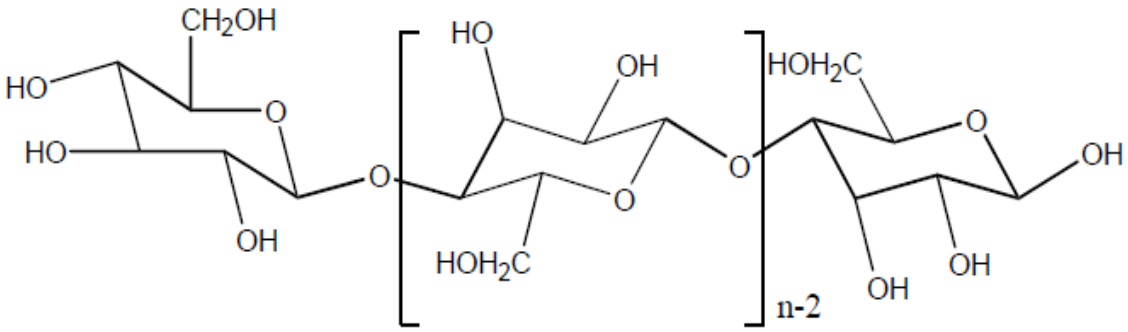


Figure 1.1 Chemical Structure of Cellulose [2]

Due to its large abundance and attractive properties**,** like its renewability, biodegradability, biocompatibility in conjunction with low density, high strength and high stiffness, cellulose have been a very popular natural polymer in the market of today. [3] Various types of polymer materials can be derived from cellulose, such as cellulose acetate, cellulose nitrate and more. [4]

Cellulose processing is time-consuming and typically requires a single solvent system. One of the most well-known methods is called the “Lyocell Process” by directly dissolving the cellulose using N-methylmorpholine N-oxide. Even though this process has proven to be a commercial success, it still cannot replace all applications the rayon process can accommodate, and the NMMO solvent require extra attention since NMMO is a strong oxidizing agent. [5] To overcome this challenge, Professors Cuculo and Kotek at North Carolina State University were able to develop a new solvent system composed of ethylene diamine and potassium thiocyanate (ED/KSCN). This new solvent system is very simple and complete dissolution of cellulose can be achieved in a short period of time under low processing temperature. [6] This method of dissolution will be discussed in greater detail in following chapters.

The other material of interest is soy protein, which is extracted from soybeans. These soybean products can be categorized by the concentration of protein: soy flour (SF), soy protein concentrate (SPC), and soy protein isolate (SPI). Chemically, the soy flour contains ~55% protein and 32% carbohydrate and has the lowest purification requirement of the three. Soy protein concentrate (SPC) contains ~70% protein and 18% carbohydrates, and finally the soy protein isolate (SPI) contains ~90% protein and 4% carbohydrates. [7]

It is difficult to present a structure of the soy protein because its sequence of amino acids is very lengthy. Majority of the soy protein are used in food, although they have been used more in industrial applications such as composites, adhesives, plastics and others in recent years. For example, protein-based adhesives have become more popular because they

serve as a good replacement for petroleum-based adhesives, but their resistance to water is very poor. [8]

Although soy protein seems to be a promising replacement for some of the petroleum-based materials in the market, their applications are limited due to their low structural integrity in water. Soy protein is made up by a collection of isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptohan, valine, etc. [8] Soy proteins are soluble in water, so to overcome this problem, the chemical structure of soy proteins must be stabilized.

The objective of this study is to produce strong cellulose/soy protein nonporous films using the new ED/KSCN solvent. To stabilize the structural integrity of the films in the hopes of future fiber production, glutaraldehyde was chosen as the crosslinking agent in hopes of producing strong, uniform, nonporous membranes that can be adapted into fiber production for mass production. The resulting physical and chemical properties of the films were analyzed using various analytical instruments and procedures. This study was supported by the NC Soybean Producers Association and the NC farmers to see if strong membranes can still be obtained using lower grade soy protein, to explore and expand the applications for soy proteins.

CHAPTER 2. A Review of Cellulose and Soy Dissolution Technology

2.1.1 Introduction

Cellulose (as seen in **Figure 1.1**) is a linear glucose polymer where the 1,4-β-linked glycosidic bonds that form cellobiose residues make up the repeating units of the cellulose chain. Because of this, their polymer structure can stack neatly forming crystalline order held together by hydrogen bonds. [9] The high density of hydroxyl groups in cellulose will form hydrogen bonds due to the existence of intra- and intermolecular forces, which make cellulose insoluble in water. [10] To make cellulose into membranes, films, or a single-filament fiber, it is necessary to dissolve the cellulose. Due to insolubility in water, alternative methods to dissolve cellulose are needed.

There are many methods one can use to achieve cellulose dissolution, although most systems require multiple solvents and baths which increase the cost and the time for production. It is important to note that cellulose has multiple polymorphic forms, cellulose I, II, III, and IV. Cellulose I is native cellulose, which can be found in wood and cotton. Cellulose II is made by soaking cellulose in strong alkali solutions or by dissolving it in the viscose process. These regenerated cellulose products are often referred to as rayon in fiber form and cellophane in film form. Cellulose III is obtained by treating cellulose with ethylamine, and finally cellulose IV is made by glycerol ($CH_2(OH)CH(OH)CH_2(OH)$) treatment or alkali treatment at high temperature. It is difficult for the polymorphic forms of cellulose (II, III, and IV) to revert back to cellulose I, but it can be accomplished by partial hydrolysis. **(Figure 2.1.1)** [11]

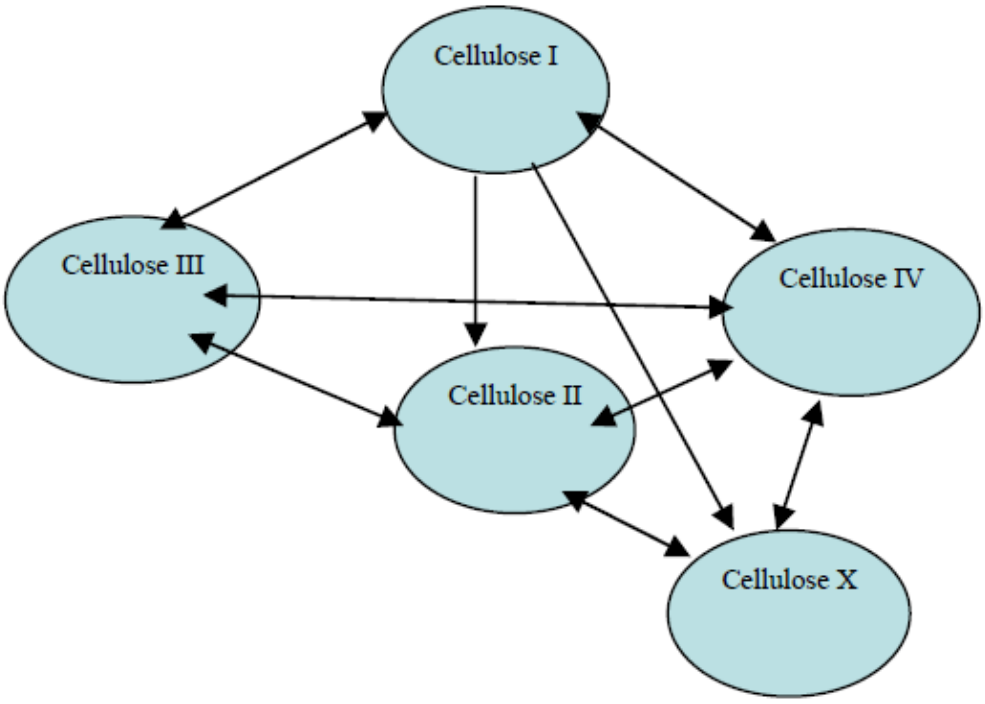


Figure 2.1.1 Possible Cellulose Crystalline Transitions. [12]

In recent years worldwide, there has been much research and development devoted to a new cellulose solvent system, and there have been many publications proposing novel systems. This literature review will present some of the most practiced solvent dissolution systems; explain how the chemistry works, and how they are used in commercial processing systems for cellulose or cellulose derivatives.

2.1.2 Traditional Rayon Viscose Process – Caustic Soda Solution

The first U.S. commercial rayon fiber produced can be traced back to 1910, and was produced by Avtex Fibers Inc., formally known as the FMC Corporation and American Viscose. According to the Federal Trade Commission, rayon fibers are classified as "A manufactured fiber composed of regenerated cellulose, in which substituents have replaced not more than 15% of the hydrogen of the hydroxyl groups." [13]

In the viscose process, natural cellulose will be dissolved to create this regenerated cellulose through several steps of chemical processing. The processes includes: 1. Steeping – under right condition, can also be used for purifying and mercerizing the cellulose; cellulose is treated in caustic soda [$(C_6H_{10}O_5)_n + nNaOH \rightarrow (C_6H_9O_4ONa)_n + nH_2O$] prior to aging. 2. Aging – effect the desired degree of polymerization, aged through exposed to oxygen. 3. Xanthation – converts cellulose to soluble form for spinning: aged cellulose mixed with carbon disulfide to form cellulose xanthate ($(C_6H_9O_4ONa)_n + nCS_2 \rightarrow (C_6H_9O_4O\text{-}SC\text{-}SNa)_n$). **(See Figure 2.1.2)** 4. "Ripening" - where the viscose is allow to ripen by resting the viscose for a period of time prior to spinning ($(C_6H_9O_4O\text{-}SC\text{-}SNa)_n + nH_2O \rightarrow (C_6H_{10}O_5)_n + nCS_2 + nNaOH$). Finally 5. Spinning – shaping the dissolved cellulose xanthate into desired forms. [13,14] Post extrusion, the viscose will be exposed to a sulfuric acid bath that results in the formation of rayon filaments.

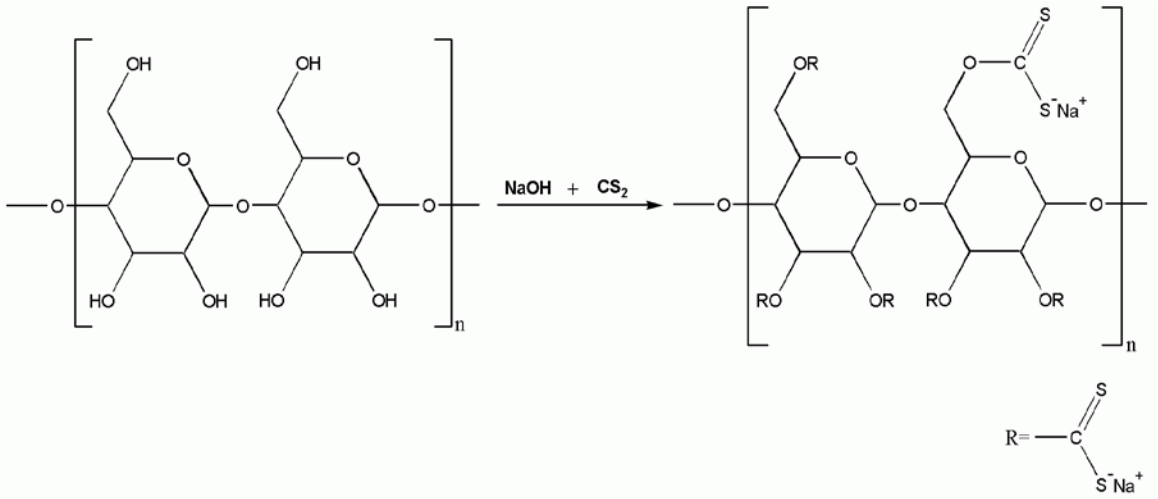


Figure 2.1.2 Cellulose (left) treated with alkali and carbon disulfide to yield viscose. [15]

Typical wood pulp cellulose may have an initial degree of polymerization of 1100, but the rayon resulting from it may only have a degree of polymerization of 350. [14] Properties may be further altered during the lay down and spinning processes. If exposed in water, the swelled cellulose fibers can have lower tenacity than cotton fibers. This traditional rayon process is known to be very damaging to the environment, caused by the uses of many harmful chemicals. In the recent years different solvent systems have been investigated and studied to overcome the environmental challenges.

2.1.3 The Lyocell Production Process

Lyocell is another type of regenerated cellulose fiber that resulted from dissolving pulps. The technology for Lyocell staple fiber was developed by a rayon supplier Courtaulds Fibers (now Acorddis Cellulosic Fibers). According to the Federal Trade Commission, Lyocell fiber is defined as "A cellulose fiber obtained by an organic solvent spinning process where 1) 'organic solvent' means a mixture of organic chemicals and water, and 2) 'solvent spinning' means dissolving and spinning without the formation of a derivative."[16] Unlike the traditional rayon process, the Lyocell process is much simpler and more environmentally friendly. [16,17]

The raw materials for Lyocell fibers also come from wood pulp harvested from trees. This raw cellulose is soaked in an amine oxide solvent **(N-methylmorpholine-N-oxide)** and is dissolved in NMMO hydrate. Following the dissolution, the solution is filtered and spun to produce filaments into a water bath where the fibers are obtained via coagulation while the amine oxide solvent is recovered and reused. Following extrusion, the coagulated fibers underwent scouring and lubrication, and it is here where the solvent and water are recovered for reuse. **(See Figure 2.1.3)** [17,18]

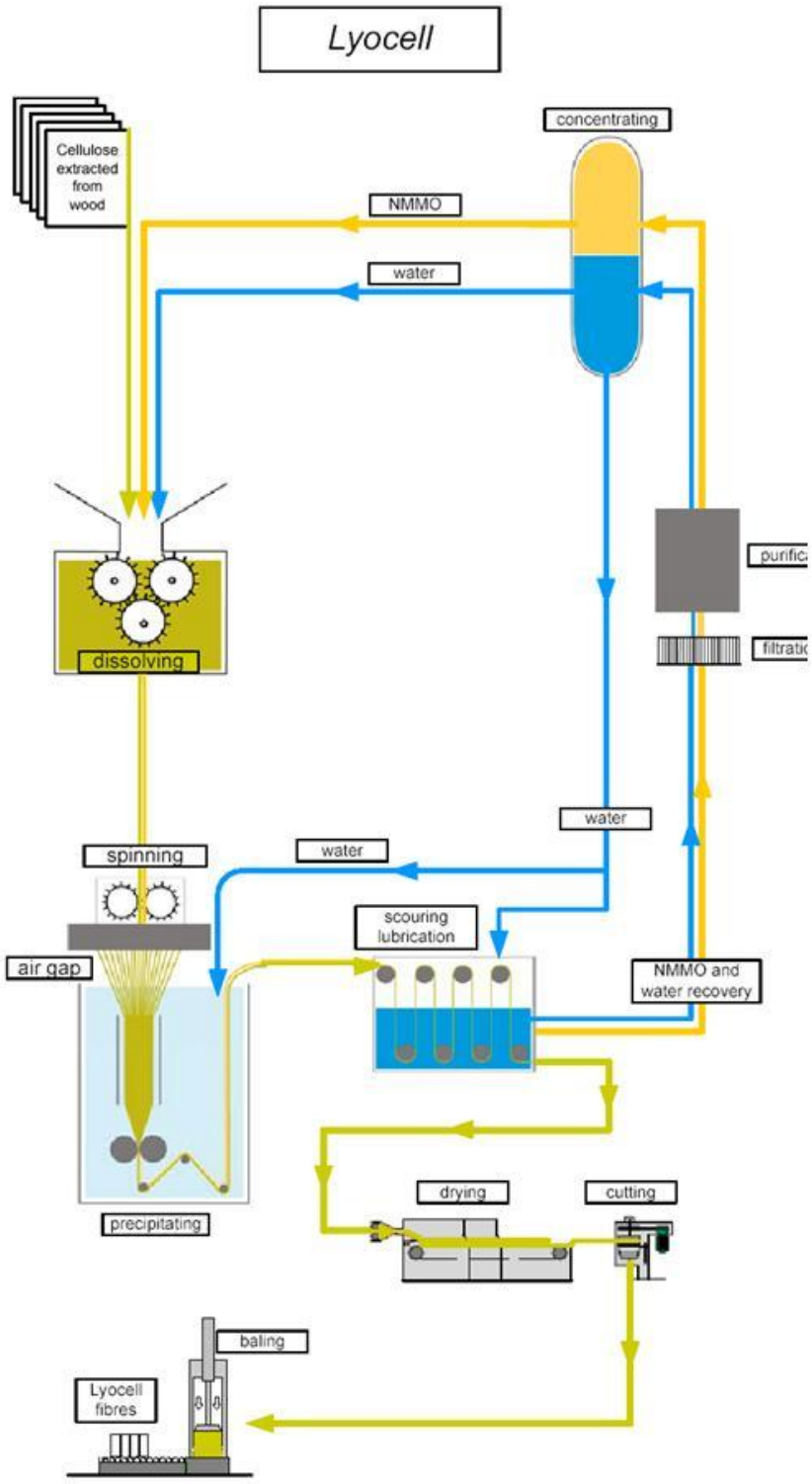


Figure 2.1.3 Graphic Representation of the Lyocell Process. [19]

N-Methylmorpholine-*N*-oxide, or simply NMMO **(See Figure 2.1.4)** can be directly applied to cellulose without any derivatization, and the solvent is 99.5% recycled even on an industrial scale. Although this makes the Lyocell process sounds ideal and simple, since NMMO is also a strong oxidant, a labile compound, and a solid at room temperature, the NMMO solvent system currently practiced in the industry actually requires numerous chemical processes and energy to keep NMMO in the liquid state.

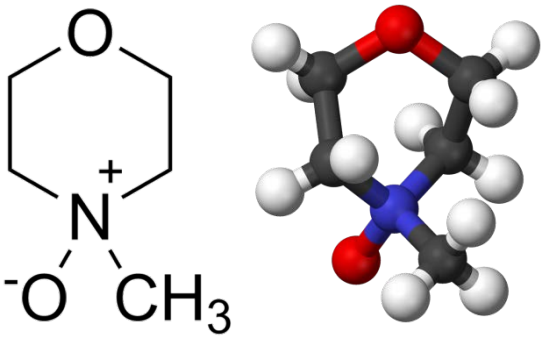


Figure 2.1.4 Chemical Structure of *N*-Methylmorpholine-*N*-oxide (NMMO). [20]

To prepare the NMMO solvent system, additional chemicals are required. The most widely used chemical is *n*-propyl gallate (PG), where it is used as an NMMO stabilizer to control the degree of polymerization of the cellulose in the mixture. This mixture is placed in a closed-container and is heated at 130º C under constant stirring. Complete dissolution of cellulose can be achieved in less than 30 minutes, and said time can be reduced further under optimum conditions. [17,18]

Aside from the necessity of using additional chemical and processing steps, there are still many hazards associated with the Lyocell process. Due to the nature of NMMO, the

increasing consumption of NMMO can lead to degradation of cellulose polymer chains, which can have negative effects on strength, thermo-degradation, hydrophilicity, etc. The degradation of cellulose and overuse of NMMO can also increase chromophore formation (discoloration). Most importantly NMMO's chemical instability can result, in rare cases, explosions. However, even though there are all these negative effects associated with the Lyocell process, the NMMO solvent system is still being used because of its capability of dissolving high concentration (up to 50%) of cellulose and the process is fast, which is very appealing for industrial production. [20]

2.2.1 Introduction of Novel Solvent System

In the recent years, scientists have been investigating new methods to dissolve cellulose, in particular, for a safer, more efficient method. Among many different types of solvent systems that were created, the dissolution of cellulose in ethylenediamine (ED)/salt solvent is of particular interest for this research.

In the past, John A. Cuculo et al. have been studying solvent systems involving ammonia/ammonium thiocyanate and analogous solvent system using hydrazine/salt and ethylenediamine/salt. These different solvents were all capable of cellulose dissolution. Of the three types of solvent systems, the hydrazine/salt can be toxic and carcinogenic, and the ammonia/ammonium thiocyanate, similar to the NMMO solvent system, can cause some chemical degradation to cellulose, and can also be relatively volatile. The ethylenediamine/salt system on the other hand, does not alter the chemical structure of cellulose and is much less dangerous, giving this solvent system the potential to be developed for commercial use. [21]

In the following sections of this chapter, a review of ethylenediamine/salt solvent system will be presented, particularly the ethylenediamine/potassium thiocyanate (KSCN) solvent system. Previous North Carolina State University graduate students have performed in-depth research on this solvent system to create the optimal conditions for cellulose, starches, and protein dissolution. The mechanism of said solvent system and the role of the salt on dissolution will be described, as well as the analytical methods to provide the evidence of how the ED/KSCN solvent system can effectively dissolve cellulose and proteins.

2.2.2 Dissolution of Cellulose/Soy Protein in ED/KSCN Solvent System

The behavior of cellulose varies with different ethylenediamine (ED)/salt solvent systems. In a paper published by Min Xiao and Margaret W. Frey in 2007, they exposed cellulose to four different ED/salt solvent systems: ED/potassium thiocyanate (KSCN), ED/potassium iodide (KI), ED/ sodium thiocyanate (NaSCN), and ED/sodium iodide (NaI). The solution of ED/salt was prepared and chilled in a freezer at -20ºC in various concentrations, and a polarized light microscope was used to analyze the result and help determine the degree of dissolution. Other tests, like FTIR spectroscopy and wide angle X-ray diffraction, were also conducted, and from their experiment, they were able to conclude that ED/KSCN possessed the best dissolving ability to a wide range of celluloses (low molecular weight to high molecular weight) **(See Figure 2.2.1)**. [22]

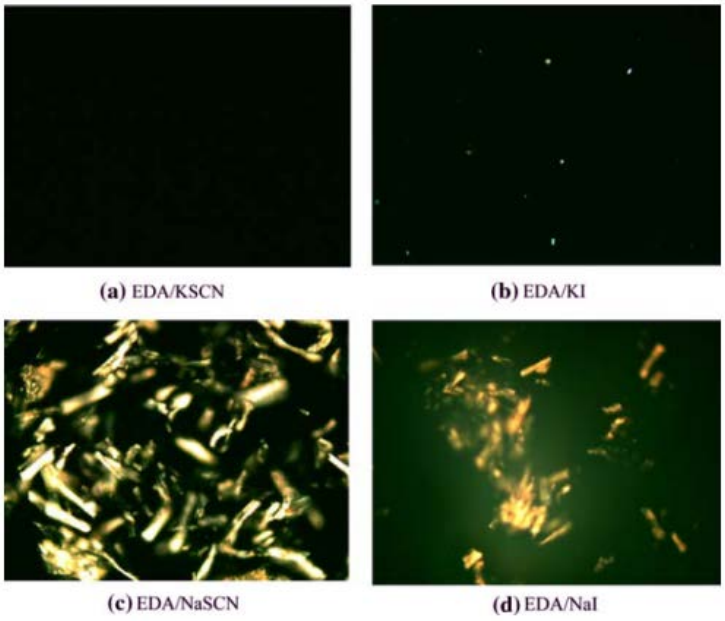


Figure 2.2.1 Polarized light microscopy images of 3 wt% of cellulose dissolution in various ED/salt solvent systems.[22]

In the book by Kenji Kamide “Cellulose and Cellulose Derivatives – Molecular Characterization and its Applications,” the author described that amines can effectively swell cellulose without dissolving it. Ethylenediamine and other amines can cause the cellulose structure to undergo rearrangement, and different metal and counter ions create different degrees of swelling:

$$Zn^{+2} > Li^{+} > Ca^{+2} > Mg^{+2} > Ba^{+2} > Na^{+} > NH_4^{+} >$$
$$K^{+}SCN^{-} > I^{-} > PO_4^{-3} > Br^{-} > Cl^{-} > NO_3^{-} > SO_4^{-2} > ClO_3^{-}$$

Figure 2.2.2 Metal and counter ion in order of decreasing ability to swell cellulose. [10]

A separate study of the ED/KSCN solvent system was conducted by Hudson and Cuculo, where they started with ammonia and ammonium thiocyanate for fiber production, and their student Hattori continued the study of cellulose dissolution in hydrazine and thiocyanate salts, and compared the results with the data from the ammonia/ammonium thiocyanate solvent system. [23] A follow up study was continued by Hattori et al. where they investigated cellulose dissolution with ethylene diamine/thiocyanate systems. Here they used four types of thiocyanate salts: calcium (Ca^{+2}), sodium (Na^{+}), lithium (Li^{+}), and potassium (K^{+}). From their studies it was discovered that the calcium and lithium thiocyanate salt solvents did not dissolve the cellulose at al. The sodium and the potassium thiocyanate salts were able to dissolve the cellulose in liquid crystal forms of the solution. Hattori et al. also determined to use water as the coagulant for fibers and film production, although they were not attempted. However, they did expose a small amount of dissolved cellulose to different coagulants, such as water, methanol, 2-propanol, etc. and analyzed their crystalline

structures using x-ray. Cellulose II was the result from the water coagulant, while methanol, 2-propanol, and acetone resulted in the production of cellulose that was primarily amorphous. [21]

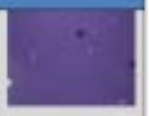
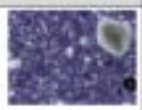
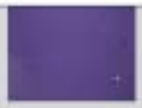
Using these findings as a reference, Hyun Jik Lee studied the dissolution of cellulose in the ED/KSCN solvent system while trying to find the optimized ratio of ED vs. KSCN that can make useable fiber/films with good physical properties. Lee dissolved sample cellulose fibers with a gradual increase in weight percent of KSCN in the ED solvent until maximum solubility was achieved. Viscosity of the dissolutions were studied, and the maximum solubility was achieved using 44 wt% KSCN in ED at room temperature, but it was discovered that cellulose dissolved most efficiently at temperature between 80-100ºC with the salt concentration ranges from 30-40 wt%. Further experiments allowed Lee to conclude that ideal cellulose dissolution using ED/KSCN was achieved at 90-100 ºC and the ratio of ED to KSCN was 65:35 wt% under constant agitation. The cellulose fibers produced had excellent mechanical properties as compared to commercialized cellulose fibers, such as rayon and Lyocell. [6]

The ED/KSCN solvent system was further studied by Dr. Eugene Douglass, where materials other than cellulose, such as starches and proteins were exposed to the solvent system. Dr. Douglass created porous membranes and nonporous films using a combination of cellulose and starch/protein while dissolving them in the ED/KSCN 65:35wt% solvent system and various coagulants (water, methanol, propanol, and acetone) and studied the physical and mechanical properties of the products. [24]

He observed that cellulose began to swell between 15 to 30 minutes of the dissolution process, and complete dissolution can be achieved somewhere between 2-4 hours depending on the concentration of cellulose. When waxy maize starch is added to the mix, the swelling occurred almost immediately, possibly caused by the decrease in cellulose concentration. After 30 minutes it appears that majority of the cellulose was dissolved, leaving starch particles behind. After 120 minutes, Douglass observed under the microscope that most of the crystallites are gone. The membranes produced from cellulose and starch also had good physical properties, although not as high as the pure cellulose membranes. [24]

Finally Dr. Douglass performed a series of dissolutions of cellulose and proteins. By combining cellulose with soy protein isolate (~90% protein content); complete dissolution was achieved within 120 minutes. Unlike the cellulose/waxy maize starch combination, where after 120 minutes starch particles can still be observed, majority of the cellulose/soy protein were dissolved after only 45 minutes, and after 120 minutes the solution became essentially artifact free (**See Table 2.2.1**). The solution was studied using thermogravimetric analysis (TGA) to determine if it was a mixture or a compatible blend between the cellulose and the protein. The data from the TGA indicated a shift to a lower temperature range, indicated an ease of decomposition that arise due to the solvation of the soy protein in the cellulose as they dissolve, then coagulation of the cellulose blend converting the structure back to a solid **(See Figure 2.2.3)**. The decrease in char level showed the presence of more pure carbon material that is a different structure from the final material of the pure cellulose membrane, proving that the cellulose and the protein had truly blended together as a new mixture. [24]

Table 2.2.1 Visual time elapse dissolution study via cross polarization microscopy of different cellulose blends in solvent. [24]

Time (minutes) \ Material	15	30	45	60	90	120
Cellulose						
Cellulose / Waxy maize Starch						
Cellulose / Soy protein						

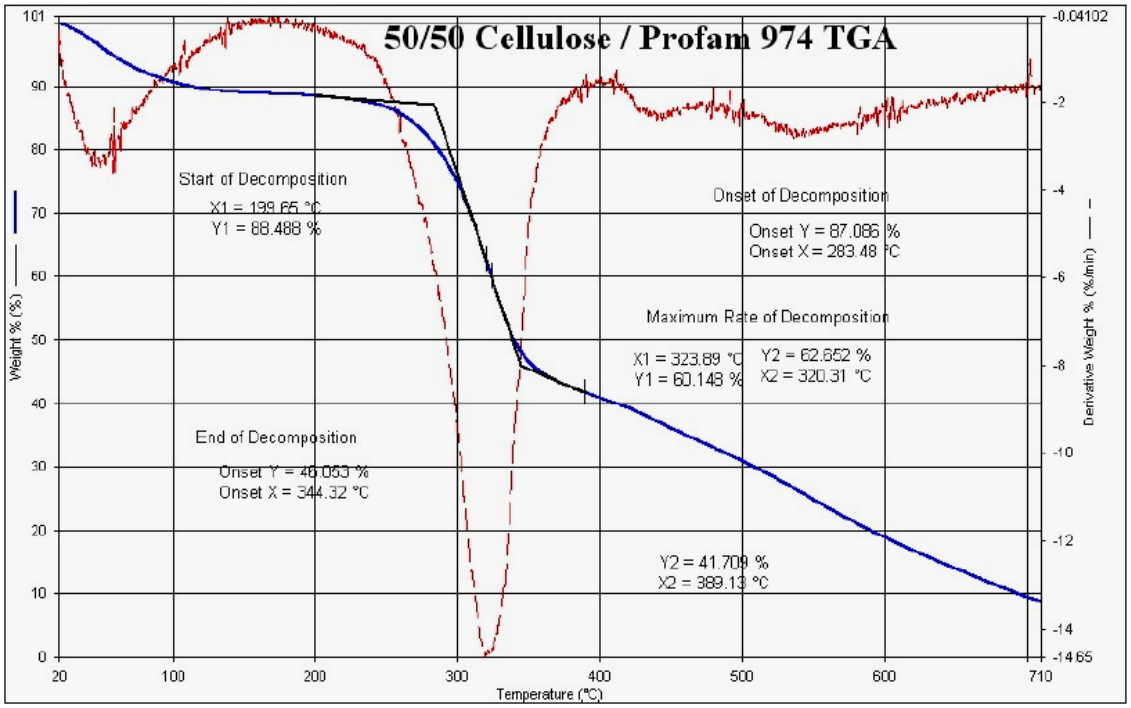


Figure 2.2.3 TGA curve for Douglass cellulose/Profam 974 (SPI) membrane. [24]

Post dissolution, Douglass created nonporous films using the cellulose/soy protein mixture with the methanol coagulation bath. He did use other coagulation baths as well, such as water, acetone, and propanol. However, the films with the best properties and least wrinkles were those that used methanol as the coagulant. By comparing mechanical testing to observe the tensile moduli of the cellulose/soy protein films to the pure cellulose films, Douglass observed that by incorporating soy protein into the membrane, the tensile modulus had improved by more than 200%.

Table 2.2.2 Comparison of tensile properties between cellulose and cellulose/soy protein blend membranes. [24]

Samples	Tensile modulus (kgf/mm^2)	Failure stress (kgf/mm^2)	Failure strain (%)	Thickness (mm)
Cellulose membrane	75 ± 12	2.5 ± 1.2	36 ± 12	0.047 ± 0.015
Cellulose / brim membrane	157 ± 52	3.2 ± 1.6	27 ± 12	0.029 ± 0.003
Cellulose / Profam 974 membrane	200 ± 75	4.7 ± 1.2	16 ± 8	0.026 ± 0.001
Cell / PF 40%	220 ± 53	5 ± 2	29 ± 12	0.026 ± 0.001
Cell / PF 30%	204 ± 74	4.3 ± 2.3	27 ± 12	0.031 ± 0.005
Cell / PF 20%	195 ± 69	2.4 ± 1.8	20 ± 12	0.034 ± 0.003

Based on the results obtained from Douglass, producing films using cellulose and soy protein was made very appealing. By using less cellulose and incorporating it with very low-cost soy proteins, the physical properties of the membranes can improve drastically. There is a main challenge associated with these blended films, and that is their poor resistance to water. Soy protein is water soluble, and after exposure to water, the structural integrity of the films rapidly decreases, which affects the strength and the potential application of said films. If this were to be used in fiber production, it would make it very difficult for commercial applications, and thus further studies to learn how to stabilize the structural integrity are needed.

CHAPTER 3. A Review of Cellulose and Protein Membrane Production Technology

3.1 Introduction

A membrane often describes a layer of material that serves as a barrier between two phases. This selective barrier can remain impermeable to specific particles, molecules, or substances when exposed to a driving force. [25] In certain situations, a membrane can be a film or a coating that can protect one material from another. Often in industry, nonporous membranes are referred to as films. Membranes can be made from many different types of materials depending on the desired application.

For many years, cellulose has been used as a raw material for the manufacturing of membranes and fibers. Due to the fact that cellulose is the most abundant polymer among the raw materials found in nature, the usage for cellulose are high with cellophane as the most commonly known cellulose-based membrane and is used from food packaging, gift wraps, protection sleeve/scovers, etc.

Various types of cellulose have been used in different applications. Majority of the membranes made from cellulose are those of some soluble derivatives of cellulose, such as nitrocellulose or cellulose acetate dissolved in common solvents and then molded/cast into the desired material. Materials like rayon and Lyocell that were introduced in previous chapters are all cellulose derivatives (cellulose xanthate and cellulose acetate). [10, 26]

In recent years, protein-incorporated membranes have become a popular field of research for many, because of their potential in the medical field for promoting the would-healing process. In addition, due to the high biocompatibility properties of proteins, industry

has also became interested in producing biodegradable films for packaging and other applications.[27] The following sections of this chapter will provide some examples of the methods that are being used/researched for membrane production using cellulose and proteins.

3.2 Cellulose Membrane Preparation Methods

Because cellulose is the most abundant and renewable biopolymer on Earth, it has become a primary chemical resource to produce renewable, biodegradable, and biocompatible materials. Although they are biodegradable, due to the high density of hydroxyl groups in the cellulose structure that forms intramolecular and intermolecular hydrogen bonds in solid-phase cellulose, they are insoluble in water or in aqueous alkaline solutions. [28]

One of the most popular examples of cellulose-based membranes is cellophane. Cellophane is a thin, transparent sheet made from regenerated cellulose. Using cellulose from wood pulp or cotton linters, the English chemist Charles F. Cross and Edward J. Bevan patented viscose. A decade later, a Swiss chemist designed a machine to allow mass production of these cellulose viscose films. To make these membranes, the carefully ripened viscose is piped to the casting machine and extruded through a slit into an acid coagulation bath, allowing the film to reconvert to cellulose. Before the films can be made, they will go through several series of baths where the film is washed and bleached and then treated with softening materials such as glycerol and then finally coated with moisture-proofing material. [29]

Traditional cellophane manufacturing process is very similar to the viscose rayon and the Lyocell process. Purified cellulose is dissolved in NMMO under elevated temperature and constant agitation. But instead of extruding the dissolved cellulose through small holes to produce rayon fibers, they are extruded through a thin slit, producing a thin membrane and is precipitated (or “solidified”) in the coagulation bath of diluted sulfuric acid and sodium

sulfate. Post coagulation, the membranes will go through a few more processes to remove the solvent, and the excess water and solvent can be recycled and reused. [30]

Obtaining cellophane using the Lyocell process is popular because the solvents can be recycled and is considered a “green” process. NMMO has a strong electronegative oxygen atom that can break the hydrogen bonds between cellulose chains and form new hydrogen bonds to produce viscose cellulose solutions. [31] It was noted earlier that although the NMMO process is “green,” the process of removing solvents still leads to lots of unwanted chemical wastes, and thus initiated a new wave of research to develop a truly environmentally friendly solvent system.

To modify the Lyocell process, Khare et.al explored alternatives for membrane coagulation. Using NMMO and dimethylacetamide (DMAc)/lithium chloride (LiCl), such solvents offer certain advantages such as the absence of chemical byproducts and their reusability. Cellulose produced *via* the Lyocell process results in fibers of higher tenacity, higher modulus, lower shrinkage and lower tenacity & modulus reduction in wet state. [32]

A clear homogeneous cellulose solution was obtained by adding 45wt% NMMO, various amount of DMSO, 5 wt% deionized (DI) water, and 4-12 wt% of cellulose together and heated to 135ºC in a flask in a silicone oil bath under constant stirring for 20 minutes. They then coagulated the material in acetone (~5 minutes), followed by fresh acetone for 20 minutes, isopropanol for another 20 minutes, and then heptane for 24 hours. Porous films were obtained from this method, although morphologies and other details were not provided in the publication. [32]

Many different approaches have also been studied. One such is to partially dissolve/degrade cellulose with NaOH aqueous solutions developed by Yu Cao and Huimin Tan. They prepared the dissolved cellulose at –5º C for 8 hours and then centrifuged at 9000G for 30 minutes at 4º C for 30 minutes. For cellulose membrane production, a 4.8 wt% cellulose solution was used and the casted solution coagulated in aqueous H_2SO_4 solutions at 10ºC. Microporous cellulose membranes were obtained that contained cellulose II crystalline. [30] Some membrane properties are presented in **Figure 3.2.1**.

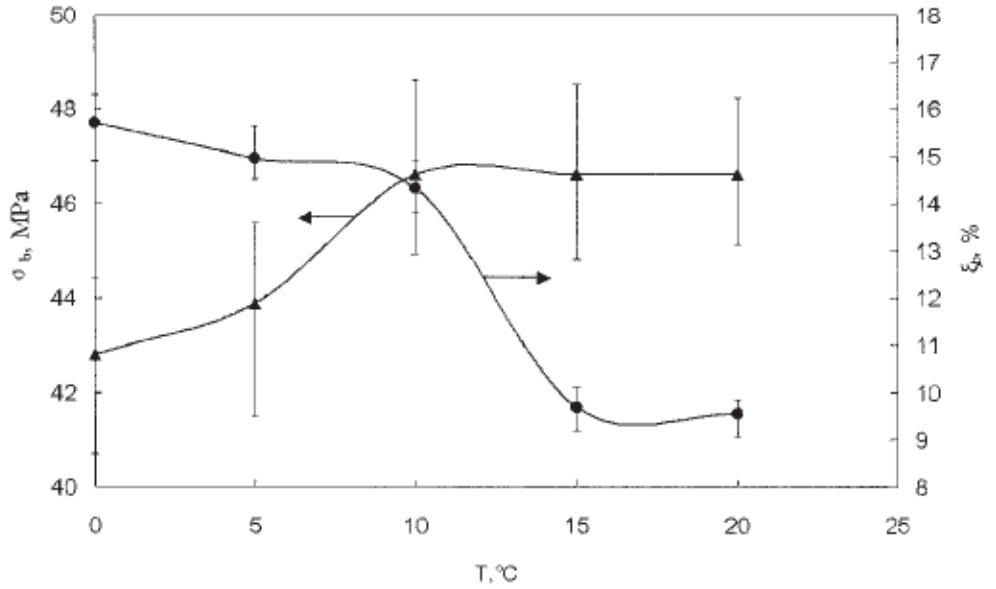


Figure 3.2.1 Tensile strength (▲) and breaking elongations (●) of membranes at various coagulation temperatures in 10 wt% H_2SO_4 aqueous solution for 10 min. [30]

Although microporous membranes were produced, this process does not solve the poor environmental properties that the NMMO solvent system faces. The use of sulfuric acid is damaging to the environment, and the low preparation temperature (below freezing point) made this process impractical for large scale production.

In another study Xiaopeng Xiong, Wei Zheng et al. reported a new method of preparing cellulose membranes by impregnation of chitosan into a regenerated cellulose membrane using a NaOH/thiourea aqueous solution for dissolving the cellulose. This group hoped that by combining chitosan with the cellulose membrane, the functional amino groups on chitosan can improve the affinity or absorptive performances of the membrane **(See Figure 3.2.2)**. [33, 34]

The cellulose solution was prepared by immersing the cellulose acetate in a 6/5 wt% NaOH/thiourea aqueous solvent, followed by storing the mixture at -12ºC for 8+ hours. After this the mixture was vigorously stirred for an hour and centrifuged at 4000 rpm for 25 minutes. Separately the chitosan powders were dissolved in 2 wt% acetic acid aqueous solution and served as a coagulation bath for the casted cellulose solution. [33]

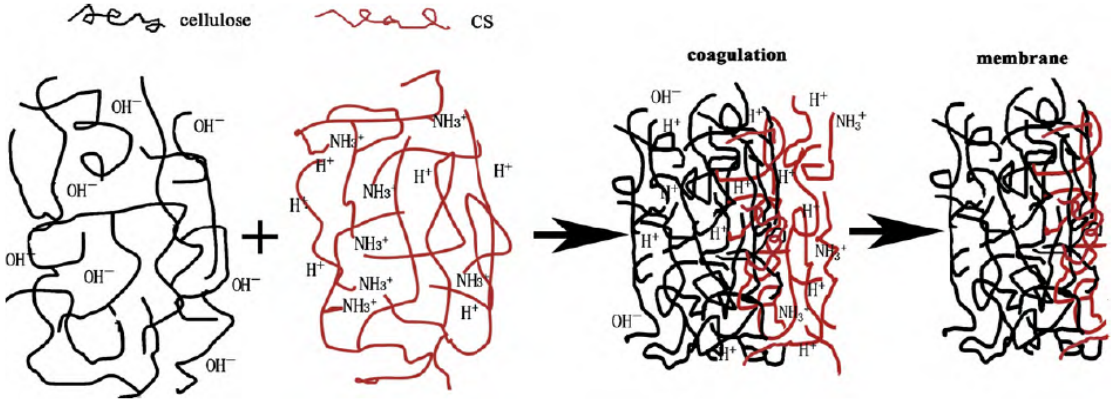


Figure 3.2.2 Cellulose + Chitosan (CS) membrane preparation schematic. [33]

Using this method, Xiong et. Al successfully prepared cellulose/chitosan blend membranes, and the chitosan molecules were able to penetrate into the membrane, and the entanglement between the cellulose and chitosan polymer chains resulted in a denser

homogeneous porous structure with improved strength. Differential scanning calorimetry (DSC) was conducted to compare pure cellulose and the cellulose/chitosan membranes. The blended membrane showed **(Figure 3.2.3)** the glass transition of incompletely mixed chitosan at 205ºC, but also improved the thermal decomposition temperature of the membrane. [33]

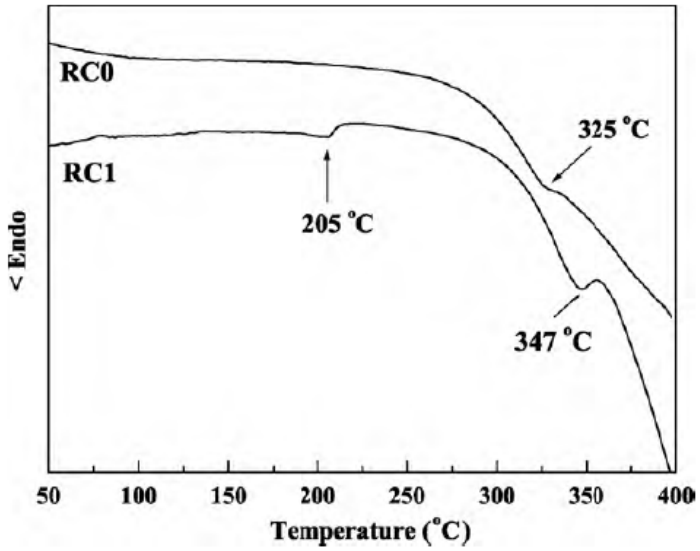


Figure 3.2.3 DSC thermograms of RC0 (pure cellulose membrane) and RC1 (cellulose membrane containing chitosan).

The dissolution process used by Xiong et. al showed that cellulose can be dissolved in alkali solutions other than NMMO and membranes with good strength were achievable. However, similar to another process mentioned above, the solution is still not ideal environmentally, not to mention the process is quite time-consuming. The dissolution is prepared below the freezing point and separation by centrifuge is also impractical for industrial production Even though this study showed promising results of an alternative

solvent system, a more time/energy efficient, industrial production-friendly process still needs to be explored.

3.3 Protein and Cellulose/Protein Blend Membrane Preparation Methods

Protein membranes are being explored for their biocompatibility and biodegradability that make them very promising for wound-healing processes. Soy proteins have been investigated for the biomedical field in forms of hydrogels, membranes, or incorporate into another substance, such as membranes or fibers. [35]

Beyond the vast potential soy protein held in biomedical research, there is also a great deal of interest for soy protein in food packaging and textiles. Since the 1930s, soy proteins have been considered to be a natural material capable of producing textile fibers and films, and in 1986 Guilbert et. al were able to produce flexible, smooth, and clear films out of soy proteins. Since then, soy protein-based edible films have received considerable attention for their excellent film-forming abilities and barrier properties against oxygen. [35-37]

Soy protein-based films have been commercially produced using soy protein concentrate (SPC) and soy protein isolates (SPIs). Chemically, SPI contains 90% protein and 4% carbohydrates, and SPC contains 70% protein and 18% carbohydrates. Other soybean products are soy flour (SF) and rough soy hulls: SF contains about 55% protein and 32% carbohydrates and soy hulls typically consist of >30% protein, and is the least expensive soy product. [38] Commercial SPCs are prepared by removing alcohol-soluble nonprotein compounds from defatted meal with 60-80% alcohol solution and SPIs are obtained from defatted soy meal by alkali extraction followed by acid precipitation (pH 4.5).[39]

To produce soy protein films, the denaturation of protein is needed followed by surface dehydration. Denatured proteins can form films through disulfide crosslinking and hydrophobic bonds. Most soy protein-based films are prepared from SPI, but during

denaturation, acid precipitation decreases the nitrogen solubility of soy proteins, the solubility of SPI is limited. To increase the solubility alkaline condition is needed by adding ammonia water or NaOH, but insoluble particles can still be detected in final product. [40]

S.Y. Cho et. al in 2007 published their method of producing edible soy protein films, by dissolving glycerol and SPI samples in DI water and adjusted the pH of the solution from 7.0 up till 10.0 with 1.0 mol/L of acetic acid solution and ammonia water. The mixture was heated at 90ºC for 10 minutes and then poured onto Teflon™ film coated glass plate. Soy protein films were obtained after the mixture was left overnight for drying/evaporation. [41]

Table 3.3.1 Mechanical properties of membrane processed soy concentrate (MSC) and soy protein isolate (SPI) films at various film-forming solution pHs. [41]

Sample	pH	Thickness (μm)	Tensile strength (MPa)[1]	Elastic modulus (MPa)[1]	Elongation (%)[1]
MSC	7.0	76.2 ± 7.0^{cd}	7.6 ± 1.4^{ab}	214.7 ± 49.2^{a}	73.7 ± 24.4^{a}
	8.0	72.3 ± 4.3^{de}	7.2 ± 1.0^{ab}	210.1 ± 29.9^{a}	54.4 ± 12.7^{bc}
	9.0	78.9 ± 4.0^{bc}	7.3 ± 0.7^{ab}	208.4 ± 34.7^{a}	66.2 ± 27.5^{ab}
	10.0	68.1 ± 3.6^{e}	7.7 ± 1.0^{ab}	216.7 ± 32.0^{a}	42.0 ± 6.3^{c}
SPI	7.0	79.8 ± 10.4^{bc}	6.9 ± 0.9^{b}	178.3 ± 29.0^{b}	39.9 ± 10.0^{c}
	8.0	77.1 ± 3.7^{bcd}	8.3 ± 1.1^{a}	234.4 ± 24.2^{a}	51.2 ± 18.6^{bc}
	9.0	82.7 ± 6.6^{ab}	8.2 ± 0.6^{a}	231.5 ± 16.8^{a}	43.7 ± 11.6^{c}
	10.0	85.9 ± 4.6^{a}	8.4 ± 0.9^{a}	237.3 ± 30.1^{a}	50.8 ± 16.9^{bc}

[1]Means in the same column with the same letter are not significantly different ($P<0.05$) by Duncan's multiple range test.

The data presented above indicate that with increasing film-forming solution pH, stronger films can be obtained. It is also observed that the elongation % of SPI is much lower than SPC until the film-forming solution reached pH value of 10.0. Post tensile testing, the

group also performed water vapor permeability (WVP) tests for the MSC and SPI films produced. The WVP values of MSC and SPI films ranged from 2.13-2.88 ngm/m^2s Pa and 3.20-3.67 ngm/m^2s Pa respectively. There were no significant differences detected among the films obtained with different film solution pHs. [41]

The method presented by Cho et. al is relatively simple, and the dissolution process is rather fast (30 minutes). However, to obtain the film the casting mixture needed to be evaporated for several hours, and there is the risk that rapid evaporation will decrease the uniformity of the films produced. The pH adjustment can also be inconvenient for film making, although it is not a factor when producing SPC films. [41]

Typically, pure soy protein films have poor strengths. To counteract this, they are often mixed with another polymer. One of these combinations is cellulose/soy protein membranes. Both are natural materials, are affordable, abundant and possess excellent biocompatibility that has made them very appealing for biomaterials as well as food packaging. [30, 35-36]

Interested in using cellulose to perform ultrafiltration and other biomedical functions, Chen et. al developed a method to prepare microporous membranes from cellulose/soy protein. The group wanted to incorporate soy protein isolate (SPI) into cellulose in the hopes that the addition of protein on the surface may increase cell growth there. [42]

Using the solvent system developed by Xiong et. al that was mentioned earlier, [33] the group used NaOH, thiourea, H_2SO_4 and other chemicals for their membrane preparation. Cotton was immersed in 6 wt% NaOH, 5 wt% thiourea solution, kept at -8ºC for 12 hours, then thawed and stirred below 20ºC for an hour to obtain the cellulose solution. For the soy

protein isolate (SPI), the same solvent was used at room temperature to obtain a slurry with ~10 wt% SPI content, then the SPI slurry was mixed with cellulose solution at different ratios: 90:10, 80:20, 70:30, 60:40, 50:50 (cellulose: SPI by weight). The mixture was put through a centrifuge at 10 ºC and was then cast. The coagulation bath used is a diluted H_2SO_4 solution (5 wt%) and transparent membranes were generated after 5 minutes and named CS1-n. Prior to drying, some of the membranes were hydrolyzed with 5 wt% NaOH solution at room temperature for 12 hours, washed, then dried in air and was labeled CS2-n for comparison purposes. [42]

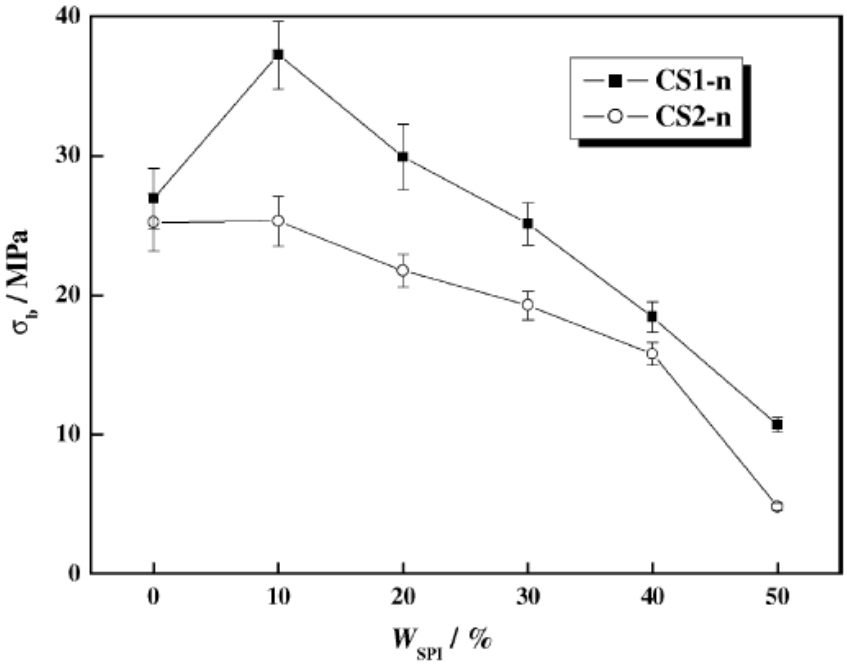


Figure 3.3.1 Tensile strength (y-axis) of the two series of cellulose/soy protein membranes. [42]

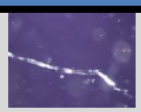
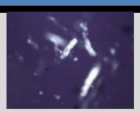
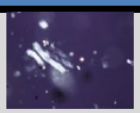
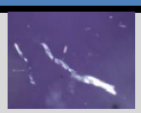
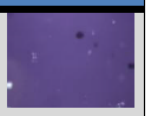
Chen et. al. were able to successfully produce microporous cellulose/soy protein membranes, and by treating some with NaOH-hydrolysis, the resulted membrane had higher

tensile strength as indicated in **Figure 3.3.2.** The group reported that as the concentration of SPI increases, the pore size also increased. The ultrafiltration rate of the hydrolyzed membranes (CS2-n) also showed improvement over pure cellulose. Although the NaOH/thiourea solvent system can produce promising results for dissolving cellulose, the soy protein was prepared separately and then mixed in later. Such a process is doable in industry, yet the conditions for cellulose dissolution are still costly. [41]

Dr. Douglass, a recent North Carolina State University graduate student, conducted a series of research on the dissolution and membrane preparation of cellulose and cellulose/starch, cellulose/protein blends. Using the novel solvent system developed by another NC State graduate Mr. Hyun Jik Lee and Dr. Douglass were able to successfully dissolve cellulose within a few hours.

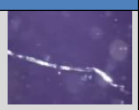
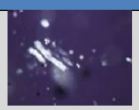
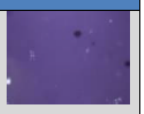
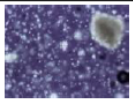
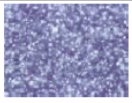
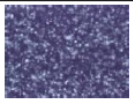
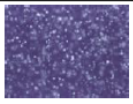
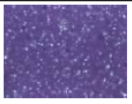
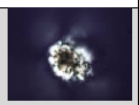
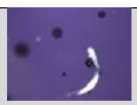
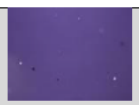
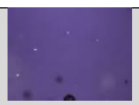
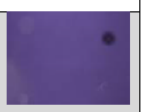
The solvent was prepared using ethylenediamine (ED) and potassium thiocyanate salt (KSCN) at a 65:35 weight ratio. Prior to dissolution, cellulose and soy protein samples were vacuum dried at elevated temperature (60-80ºC) for several hours. Dr. Douglass prepared 7 wt% dry cellulose into the solvent, under constant agitation and nitrogen atmosphere at 90-100ºC. After 4 hours a complete dissolution was achieved. Visual study of the dissolution process was conducted using cross polarization microscopy to monitor the dissolution process, and it was noted that after 120 minutes the cellulose had almost completely dissolved. **(See Table 3.3.2)** [24]

Table 3.3.2 Time elapse visual study of cellulose dissolution in ED/KSCN solvent. [24]

Time (minutes) \ Material	15	30	45	60	90	120
Cellulose						

Using the same solvent, Douglass was able to successfully dissolve waxy maize starch, chitosan, and soy proteins. Instead of dissolving the soy protein separately as proposed in other literature, this solvent system was able to dissolve the dried cellulose and soy protein powder together at the same time. Since the concentration of cellulose is reduced, the dissolution was achieved in a shorter time, as seen in **Table 3.3.3,** and under lower temperature (70-80ºC). [24]

Table 3.3.3 Time elapse visual study of the dissolution of cellulose and other cellulose blends in ED/KSCN solvent. [24]

Time (minutes) \ Material	15	30	45	60	90	120
Cellulose						
Cellulose / Waxy maize Starch						
Cellulose / Soy protein						

To make the membranes, Dr. Douglass explored a variety of coagulants such as water, acetone, and methanol, as inspired by the studies conducted by Khare et. al: 5-10 minutes initial coagulation in acetone, then 20 minutes in a fresh acetone bath, followed by 20 minutes soaking in isopropanol and finally heptane for 24 hours. The results gave membranes with nano pores. For bigger pore sizes, Douglass modified the soaking process and replaced the initial coagulant with water, which was able to produce microporous cellulose films, though the physical strength had decreased some. [34]

Of the various membrane preparation methods reviewed, the method employed by Dr. Douglass is the simplest and overall most energy efficient**.** The solvent system developed by Mr. Lee is mostly a clean solvent consisting of amine and salt. Dissolution can be achieved in a short period of time in a single step instead of multiple steps suggested by Xiong, Chen, and others.

CHAPTER 4. A Review of Glutaraldehyde Crosslinking to Stabilize the Structure of Cellulose/Soy Protein Membranes

4.1 Introduction

Cellulose is the most abundant natural polymer and can be used to make various products from membranes, filters, packaging materials, fibers, textiles, and many more. Cellulose can exist in several phases (cellulose I, II, III, IV) and each type of cellulose can be obtained *via* different treatments. Regenerated cellulose (cellulose II) can form fibers such as rayon and Lyocell, or films. Cellulose films have been used for food packaging (cellophane) and in the medical field as ultra-filtration membranes or wound-dressing materials. [1-7, 10]

As mentioned in previous chapters, because of the molecular structure of cellulose, which is a linear syndiotatic homopolymer composed of D-anhydroglucopyranose units (AGU) linked together by β-(1,4)-glycosidic bonds. The large presence of hydrogen bonds allow very strong, water-resistant films to be produced **(See Figure 4.1.1)**.

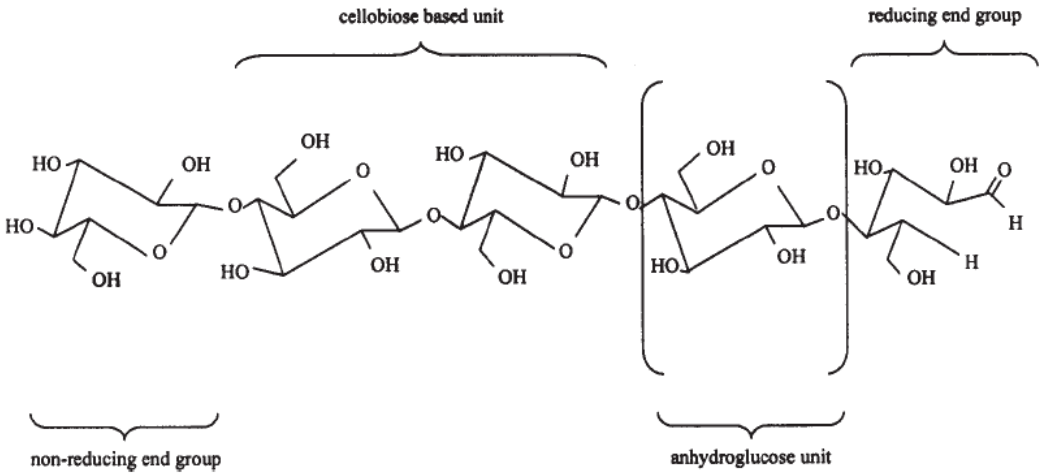


Figure 4.1.1 Molecular structure of cellulose. [32]

Another naturally abundant polymer that is widely used is soy protein. Most of the soy protein is used in food and feed applications since the 1930s, [35-37] but in the recent decades they are also being used for industrial applications such as composites, adhesives, plastics, wound-dressing, and other applications. Since petroleum based polymers have issues such as limited resources, environmental and health concerns, natural polymers are being researched more to replace some of the petroleum polymers. [8]

There are still many challenges protein-based materials need to overcome: one of the major drawbacks being their poor structural resistance to water, which can lead to loss of strength or adhesive abilities. Proteins are made from a combinations of 20 different amino acids, which have many functional groups like –OH, and -NH, -SH, that are susceptible to chemical interactions. Although these functional groups contribute to their solubility in water, they may also leads to loss of functional and/or structural integrity if exposed to water. If a crosslinking agent is introduced to the protein, it might be possible to overcome their weak structural resistance to water. [8, 43]

One of the most commonly used crosslinking agent is a dialdehyde known as glutaraldehyde. This chapter will review the effects of using glutaraldehyde as a crosslinking agent by providing methods of crosslinking, the chemical mechanisms of the crosslinking process, analytical results to prove the occurrence of crosslinking such as mechanical test, thermoanalysis, etc. and why glutaraldehyde is favored over other crosslinkers.

4.2 Behaviors of Glutaraldehyde in Aqueous Solutions

In order to know how to achieve successful crosslinks, knowing the chemical mechanism of the process is very important. But to fully understand or predict the crosslinking mechanism, it is crucial to understand how the crosslinking agent behaves. The structures of glutaraldehyde (GA) in aqueous solution have been studied by many and it was observed that GA is not limited to the monomeric form. [44]

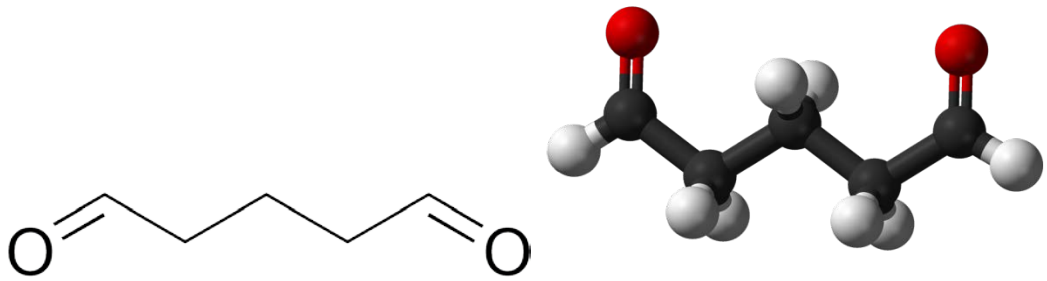


Figure 4.2.1 Basic chemical structure of glutaraldehyde (GA). [45]

The basic structure of glutaraldehyde is provided in the figure above. According to a chronic toxicity summary released by the state of California, glutaraldehyde is frequently used as a disinfectant and sterilizing agent (2% solution), component in leather tanning solutions, and as an intermediate for sealants, resins, dyes, and electrical products. [46] Glutaraldehyde is soluble in water, and often used in solutions of various concentrations.

Based on studies conducted in the past 50 years, Migneault et. al have combined the majority of its possible structures into a single figure **(Figure 4.2.2)**. [44] In 1962, Aso and Aito discovered that glutaraldehyde polymerizes spontaneously in aqueous solutions at room temperature without the need of a catalyst during their study of the polymerization of glutaraldehyde using cationic catalysts. [47, 48]

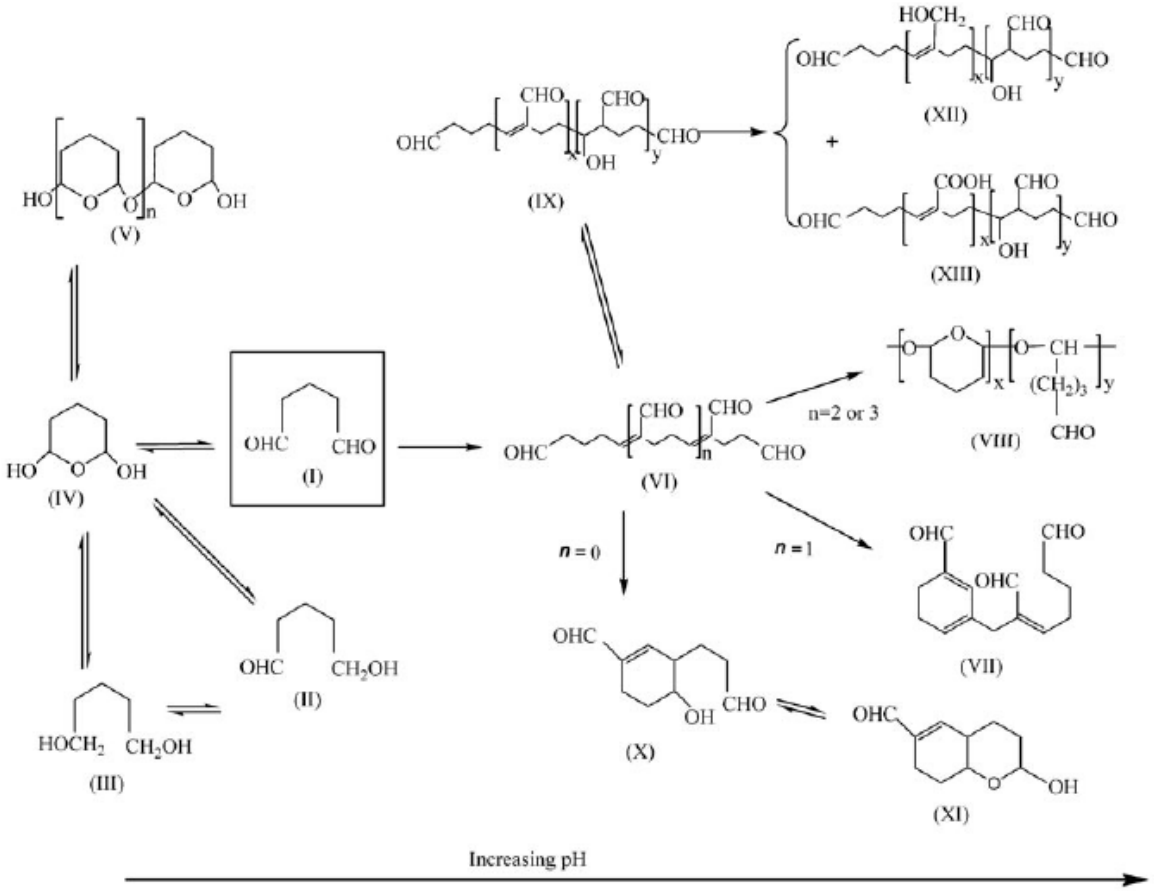


Figure 4.2.2 Summary of possible glutaraldehyde structures in aqueous solution. [44]

Later in 1968, Richards and Knowles studied glutaraldehyde behaviors using proton nuclear magnetic resonance (^{1}H-NMR) and found that the solution did not consist mostly dimer or trimer, but rather a mixture of its polymeric forms (structure VI) and oligomers. There were still small amounts of monomers present, and the authors concluded these commercial solutions were largely "polymeric and contained significant amounts of α,β-unsaturated aldehydes" (structure VI) [49].

In the years after this finding, many studies resulted in different structures of glutaraldehyde in solution depending on the pH of the solution. In 1992, Kawahara et. al

pointed out that the studies conducted by Richards and Knowles were done in deuterated water, which differs from protonated water used in commercial solvents. They investigated the molecular structure of commercial glutaraldehyde solvents by using UV absorption and light scattering. In their study they found that the solvent actually contained a large quantity of the cyclic hemiacetal polymeric structures (structure V) and with further dilution the polymerized glutaraldehyde will slowly convert to monomers, and using ^{1}H-NMR they discovered that, regardless of concentration of glutaraldehyde, the α,β-unsaturated structures (structure VI) was negligible. [50]

4.3 Crosslinking Mechanism between Glutaraldehyde and Protein

Proteins are made from a combination of 20 possible amino acids, and majority of the amino acids that makes up proteins contains functional groups like –OH, and –NH. Soy protein contain reactive amino acids such as cysteine, arginine, lysine, hystidine, etc. that makes it susceptible to crosslinking with other molecules. As mentioned earlier, one of the biggest challenges faced with producing cellulose/soy protein membranes is that the proteins have poor water resistance, which could compromise the functional and/or structural integrity. [8, 38, 43]

There are many ways to crosslink proteins, with the most used agents being aldehydes, especially formaldehyde and glutaraldehyde being the most popular choices. [51] In a study conducted by Migneault et. al, they stated that the linear, 5-carbon dialdehyde compound (glutaraldehyde) has had many success because of its commercial availability and low cost, but most importantly its high reactivity. They reported that a series of aldehydes were used to crosslink collagen, from formaldehyde to dialdehydes having chain length of two to six carbons (glyoxal, malonaldehyde, succinaldehyde, glutaraldehyde, and adipaldehyde). They concluded that the reactivity in this series was maximized at five carbons, indicating that glutaraldehyde is the most effective crosslinking agent in the series. [44, 52] Many researches of glutaraldehyde (GA) as a crosslinking agent for stabilizing proteins have been conducted in the past, and researchers found that GA reacts with the amine groups in proteins to form intermolecular crosslinks, especially in alkaline condition. [49, 53]

Crosslinking is a specific chemical modification where two components can be joined together by a covalent bond, and GA polymer can couple two amino groups to form amino residues through a Schiff's base reaction. [8] Even though the overall crosslinking mechanism is known, there has been no consensus on the reaction of the proteins with GA. This can be caused by the diversity of the protein structure, and also the various structures that naturally occurs in GA solutions (**shown in Figure 4.2.2**).

There have been suggestions where the GA reacts with the α-amide group of glycine and the α- and ε-amide groups of lysine, although lysine will be the only one to have a free amino group to react with GA. Another suggestion is that the GA reacts with the protein through an aldol condensation followed by a crosslinking reaction. However, some have noticed that after exposing GA to protein might cause a change in color, indicating the occurrence of this reaction. [49, 54] In the studies conducted by Chabba et. al, the crosslinked soy protein concentrate (SPC) resin resulted in increasing strength, reduced moisture absorption and improved thermal stability. It was noted by the researchers that it is difficult to assess the average degree of crosslinking because of the complexity of the chemistry. But the evidence from examining the SPC resin, *ie.,* improved tensile strength, decreased moisture absorption, the immediate viscosity increase for the SPC resin mixture and finally thermogravimetric analysis (TGA) (**See Figure 4.3.1**) showed improved thermal degradation, was deemed enough proof that the crosslinking had occurred **(See Figures 4.3.2 and 4.3.3)**. [7, 38]

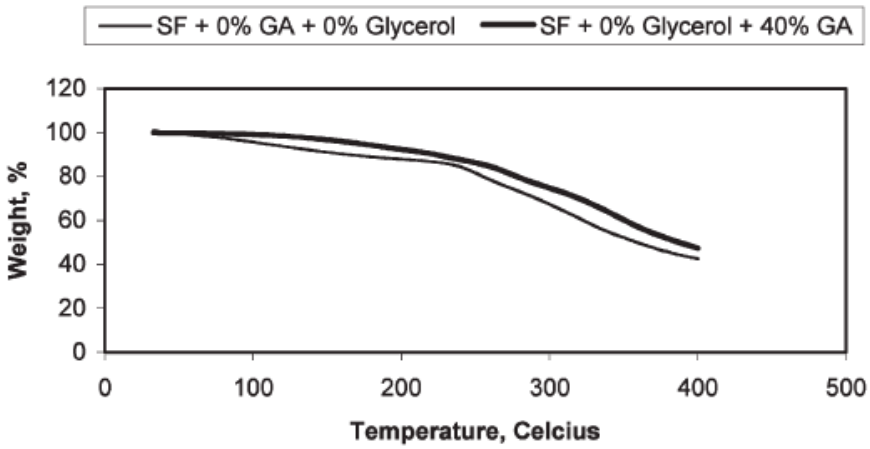


Figure 4.3.1 TGA thermograms of soy flour (SF) and soy flour with GA (CSF) resins. [7]

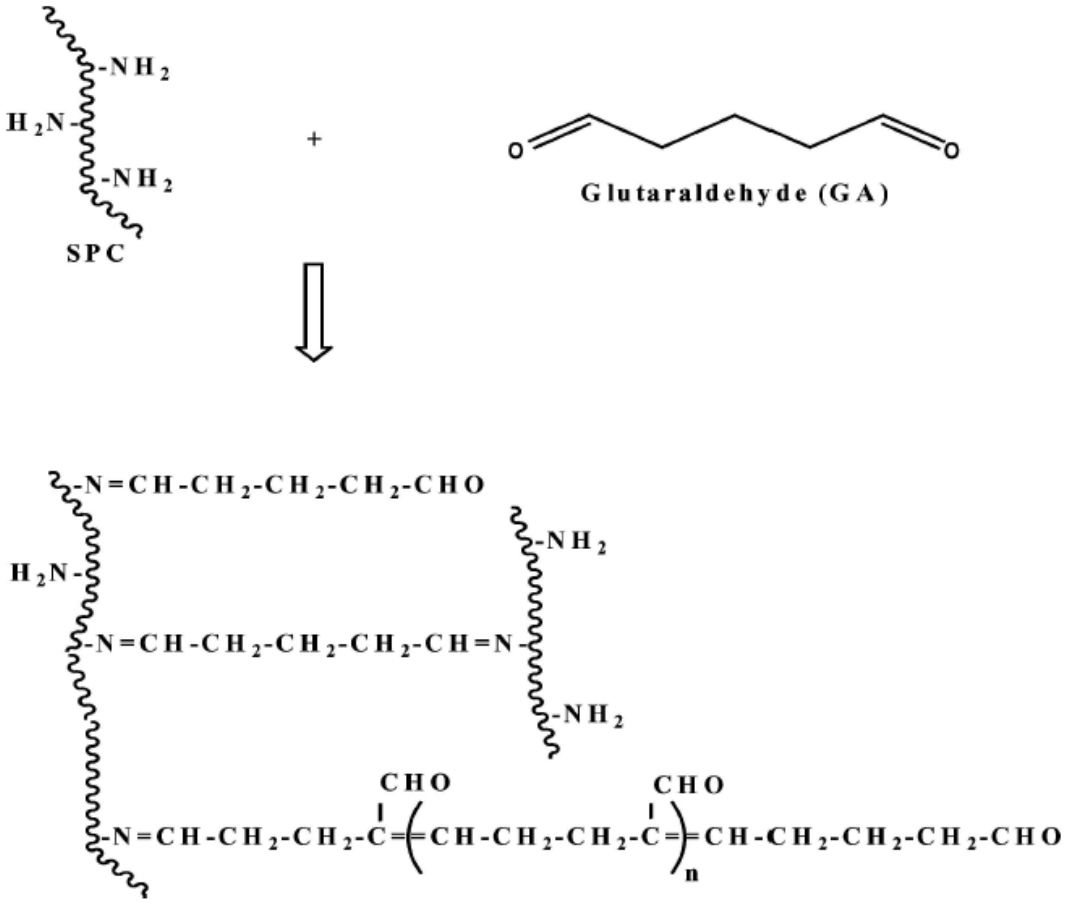


Figure 4.3.2 Proposed crosslinking mechanism between soy proteins and GA. [38]

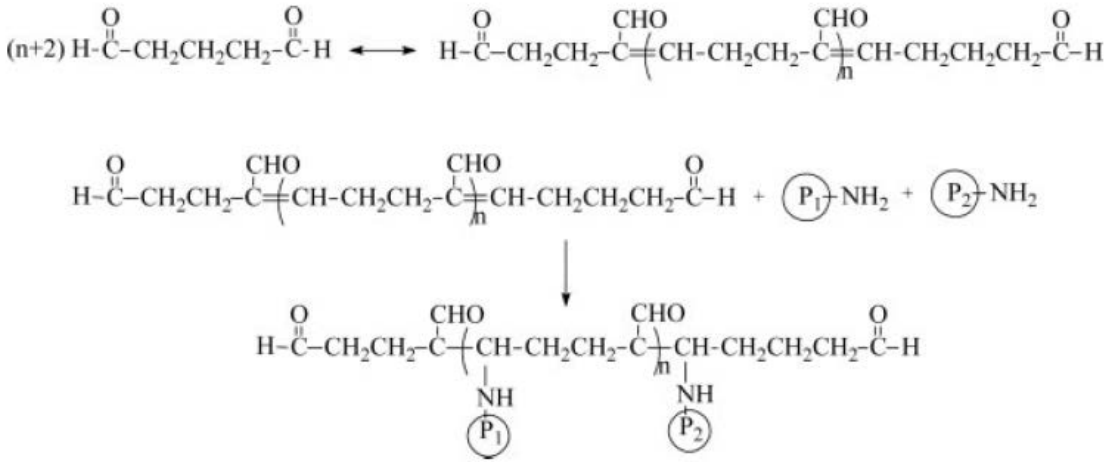


Figure 4.3.3 Another proposed crosslinking mechanism between SP and GA. [8]

Typical methods of producing cellulose membranes involve casting the cellulose solution, followed by coagulation. To crosslinking the mixture of soy protein and/or cellulose, different techniques were used by various researches to expose the material to glutaraldehyde (GA).

S.K. Park et. al used soy protein isolate (SPI, ~90% protein content) by alkali extraction (pH 9.0), followed by acid precipitation (pH 4.5). The SPI polymers were washed, spray dried, and then 3.4 mL of 2.5% GA was added into 150mL of 10% SPI solution (wt/vol, in 0.1M phosphate buffer, pH 7.5) and stirred for 30 minutes. Final mixture was kept at room temperature for 12 hours, and then freeze dried. [55]

Another crosslinking method was deployed by Chabba et. al using soy protein concentrate (SPC), to produce the GA modified SPC membranes, SPC was mixed with distilled & deionized water in 1:15 weight ratio with predetermined amount of GA to the SPC/glycerin mixture (glycerin serves as a plasticizer) while in a water bath. Solution was pre-cured at 70º C for 27 minutes under constant stirring, then resin was dried at room

temperature after casting on Teflon® coated glass plate and dried at 35ºC for 20 hours. The dried SPC resin sheets were then cured by hot pressing in Carver hydraulic hot press, at 120ºC for 25 minutes under 7MPa of pressure. [7]

Finally, Luo et. al produced cellulose/soy protein isolate membranes coagulated from a modified method that was mentioned back to the production of cellulose membranes. [30] The group prepared an aqueous solution as a solvent for cellulose using sodium hydroxide (7 wt% and 12 wt%), urea, and distilled water. The solvent was pre-cooled to -13ºC prior to the addition of cellulose (3.5 wt%) and the mixture was stirred for 5 minutes until a transparent solution was obtained. The SPI (10 wt%) used was also dispersed in the sodium hydroxide/urea solvent at room temperature and then added to the cellulose mixture. This mixture was stirred at room temperature for 30 minutes, degassed at 10º C by centrifugation for 10 minutes at 7500 × *g*, and finally cast on a glass plate to produce a gel sheet with a 200 μm thickness. The membrane was coagulated in 5 wt% acetic acid solution for 5 minutes, and the membranes were then washed with distilled water for 12 hours. The group used the membranes for a biocompatibility study. After a long term *in vivo* implantation study, the films were taken out and fixed in 2.5 wt% glutaraldehyde in phosphate buffer (pH 7.4) and then post-fixed in 1wt% of OsO_4. This is not a direct crosslinking method, but the method of producing the membrane is worth mentioning. [56]

In all of the mentioned methods, the cellulose and soy protein solutions were prepared separately, and the glutaraldehyde applied directly to the dissolved soy proteins. In the studies conducted by Douglass, using the ED/KSCN solvent system, he was able to dissolve the soy protein and the cellulose simultaneously in the same flask. Dissolution was achieved

within 2-3 hours and the membranes were cast on a glass plate, followed by coagulation using methanol. Even though Douglass did not expose the film to glutaraldehyde, strong, uniform films were obtained using this very simple method. Just by blending the two biopolymers to produce the films had already shown an improvement in strength compared the pure cellulose membranes, as seen in **Table 4.3.1.**

[24]

Table 4.3.1 Tensile test comparison between pure cellulose and cellulose/SPI (Profam 974) membranes. [24]

Samples	Tensile modulus (kgf/mm^2)	Failure stress (kgf/mm^2)	Failure strain (%)	Thickness (mm)
Cellulose membrane	75 ± 12	2.5 ± 1.2	36 ± 12	0.047 ± 0.015
Cellulose / brim membrane	157 ± 52	3.2 ± 1.6	27 ± 12	0.029 ± 0.003
Cellulose / Profam 974 membrane	200 ± 75	4.7 ± 1.2	16 ± 8	0.026 ± 0.001

Of all the methods described above, the membrane preparation method used by Douglass is the simplest and most efficient: No cooling below room temperature and no excessive centrifugation needed. However, even though others exposed the soy protein to glutaraldehyde during dissolution, the same cannot be applied to this method. The ED/KSCN solvent system used contains amine groups that can also react with glutaraldehyde, so a

different crosslinking method is needed. The new method will be investigated during experimental procedures, which the following chapters will describe.

CHAPTER 5. Development and Characterization Methods of Membranes

5.1 Materials

The solvent used for dissolution was made using 35 wt% reagent grade potassium thiocyanate (KSCN) and 65 wt% reagent grade 98%+ ethylene diamine (ED), both provided by Sigma-Aldrich. Prior to use, the KSCN was placed in vacuum oven at 50ºC for 24 hours to remove excess moisture. The weighed samples were combined in a flask and a magnetic stir bar was used to stir the mixture under low heating until the KSCN is completely dissolved into the ED and the solvent was allowed to equilibrate.

The cellulose used for all experiments was Buckeye VFC SR-2711 acetate grade pressed, refined and bleached wood pulp with ~400 degree of polymerization. The cellulose board was cut and then ground into fine pieces for optimal dissolution. All cellulose samples were dried in vacuum oven overnight at elevated temperature (50-60ºC) prior to dissolution to remove excess moisture.

Another “cellulose” sample was used to observe its film-making properties. The sample was labeled “GP 50” that was provided by the North Carolina Soy Council. Even though this is labeled as a type of “cellulose”, this fine white powder obtained during the soy bean purification process does not actually contain any cellulose, as discovered by the analysis performed by the soy council.

Various soy samples were used in the project. All soy samples were provided by the North Carolina Soy Council. The soy samples were: Profam 974 (soy protein isolate), Arcon F (soy protein isolate), and rough soy hull samples A and C.

The coagulation bath used ACS reagent grade methanol (≥99.8%) provided by Sigma-Aldrich. The crosslinking agent glutaraldehyde (GA) reagent grade, 25 wt% in H_2O was also obtained from Sigma-Aldrich.

Membrane-casting tools included polyester plastic films, glass surface casting table, and the metal casting bar (5 mil – 50 mil) were provided by Byk-Gardner. A three-neck round bottom flask provided by Pyrex® to perform the dissolution of soy protein and other polymers.

5.2 Experimental Procedures of Membrane Formation

Using the novel solvent system ED/KSCN, Dr. Douglass was able to demonstrate that successful dissolution can be obtained when combining soy protein and cellulose. To verify the method of membrane production and for comparative purposes, pure cellulose membranes were made first. The following membranes: cellulose/soy protein, cellulose/GP 50, cellulose/soy hull pulps A, and cellulose/soy hull pulps C, were made for the purpose of the actual research. The method of dissolution is almost identical, so the production procedures are combined for this section.

5.2.1 Dissolution of Cellulose

To dissolve the cellulose, 5g, 6g, and 7g of dried, ground cellulose pulps were used in 3 different dissolution processes. Following the addition of the material, 95g, 94g, and 93g of the solvent ED/KSCN was added into the three-neck round bottom flask respectively. A Teflon© blade attached to a long ground glass stirring rod attached to an elector motor was inserted into the center neck, a water-cooled condenser was placed on one of the side necks for the purpose of condensing the ED back into the flask, and the last opening was plugged with a rubber (or a glass) stopper.

The stirring rod was fitted into the flask at an adequate length to allow the Teflon© blade to be turned for stirring. The flask was then immersed in a glycol oil bath at 90ºC, where the material was stirred for 3-4 hours until complete dissolution. The dissolved cellulose was then let to cool down slowly, and then reheated to 90ºC to pour the dissolved cellulose into a glass container for membrane casting use. The residual material was cleaned

up using water, which coagulated the dissolved cellulose and was easily disposed of in the trash.

5.2.2 Dissolution of Cellulose/Soy Protein Concentrate

Methods to dissolve the blend of cellulose and soy protein were very similar to the cellulose dissolution process. The ratio between the cellulose and the soy protein concentrate (SPC) was 50 wt%: 50 wt%; 3g of cellulose was used for every 3g of SPC, following experiments of Dr. Douglass, for data comparison and analysis.

The material set up is identical to cellulose dissolution; the duration of stirring was reduced to between 2-3 hours of stirring in the heated oil bath at 80-90ºC. After initial dissolution, solution was again cooled down gradually followed by another reheating procedure before transferring the solution into a glass container for membrane casting. Residual solution disposal method was the exact same as for the pure cellulose solution.

5.2.3 Dissolution of Cellulose/Soy Hulls and GP50

As Mentioned above, the methods of dissolution were very similar to the cellulose dissolution process. Here, due to concerns for the low quality of soy hulls (> 15% soy protein) and GP50, the ratio was adjusted to 75 wt%: 25 wt% so that adequate-strength films may be produced. For all dissolutions, 4.5g of cellulose was used with 1.5g of soy hull pulp samples or GP50, and 94g of ED/KSCN was poured into the three-neck round bottom flask.

The set up of the stir-bar and condenser are identical to the previous dissolution procedures above. Oil bath temperature was kept at 80-90ºC and the stirring process took

between 2 to 5 hours, due to the presence of some large chunks of soy hull pulps that were unable to break apart/ground prior to the dissolution. The majority of dissolution was achieved after 2 hours of stirring at elevated temperature. Once again the solution was cooled down gradually prior to reheating and placed in a glass flask for membrane casting use. The residual solution in the flask and on the stir bar was coagulated using water and disposed of in the trash.

5.3 Experimental Procedures of Membrane Formation

Membranes were obtained using a casting bar followed by solution coagulation. The membrane formation procedures for all different solution blends were identical, so this section will not be broken down into separate sections for each blend.

Using a Byk-Gardner Casting Table, a single sheet of polyester film was placed on top of the glass surface for the substrate. The casting bar had a casting thickness range of 5 – 50mil, but through experimentation it was determined that 20 – 25 mil was the best casting thickness. Methanol was used as the coagulant, as well as the solvent for the following soaking baths.

It was noted by Dr. Douglass in previous studies that any thickness above 30mil would be too large for coagulation, because it traps the ED/KSCN solvent blend in the system, prevents extraction due to the tight morphology and porous membranes are produced. This research focused on non-porous films, which can be produced using thickness of 20-25 mil (1mil = 0.0254 mm).

Glass containers with the solutions were heated to allow a steady flow, and a small amount was placed on the PET film. Using the casting bar, the solution was dragged from top to bottom, forming a thin layer atop the PET films. The PET film with the solution was removed from the glass casting table and placed into the methanol coagulation bath. Coagulation of the solution can be observed within the first 10 seconds, but to ensure the film is thoroughly coagulated, they were left in the bath for 5-10 minutes.

Followed the initial coagulation, the cast membranes automatically separate from the PET film, thus the PET films were removed from the bath and disposed of. Post initial

coagulation time, the films were then placed in a new batch of methanol solution and were soaked for 20 minutes. This step was repeated 3 times total to allow for the removal of the majority of the ED/KSCN solvent. Finally, the films were placed in a fresh batch of methanol solution and left to soak overnight.

Individual films were removed from the final methanol bath and placed between two sheets of glass. Separating each film is a thin sheet of Teflon film (for cellulose, PET films were used for best results), and finally a brick was placed on top of the upper layer glass, to ensure an even pressure is applied throughout. The stack of films with the brick on top was then placed in a vacuum oven, where it was dried at ambient temperature in the vacuum oven for 24+ hours, then dried for an additional 24+ hours at 40-50ºC. Depending on the thickness of the films, films were kept in vacuum oven for longer periods of time, until they were even, uniform, and dry.

5.4 Methods of Crosslinking using Glutaraldehyde

Several attempts of crosslinking were made. A known amount of glutaraldehyde (GA) was added during coagulation, during final soaking, and finally a series of dried films were exposed to different amount of GA for various lengths of times.

5.4.1 Glutaraldehyde Exposure during Coagulation

Five weight percent of GA with respect to soy protein was used. Approximately 3g of soy proteins were used for an entire batch of the films, 0.6 mL of GA solution (25 wt% in water) was applied using a micropipette into the methanol coagulation bath. After the cellulose/SPC solution was casted into a film, the films were placed in the GA-containing coagulation bath.

5.4.2 Glutaraldehyde Exposure during Final Soaking

Using the same amount of GA as above, 5 wt% of GA was measured with respect to amount of soy protein used, and the crosslinking agent was added to the final methanol soaking bath to a new batch of cellulose/SPC films. Using a micropipette, 0.6 mL of 25 wt% in water GA solution was added into the bath. After the third 20 minutes soaking, the films were placed into and left overnight in the crosslinker-containing bath.

5.4.3 Glutaraldehyde Reaction with Soy Proteins

A systematic method was devised to observe the reaction between GA and the soy protein. Instead of soy protein concentrate (~ 70% protein); soy protein isolate (~90%

protein) was used for its higher purity. The soy protein isolate (SPI) powders were dried at 60ºC in a vacuum oven overnight prior to use.

Two sets of 6 test tubes were prepared: first set consisted of test tubes each containing 10mL of deionized distilled water, and the second set contained 10 mL of 10 wt% soy protein isolate solution in each tube **(See Figure %.4.1)**. Each tube was labeled: **C** – control, **1%** - 1 wt% GA to protein, **3%** - 3 wt% GA to protein, **5%** - 5 wt% GA to protein, **7%** - 7 wt% to protein, and **10% -** 10 wt% to protein. The protein here referred to the amount of SPI used, and the tubes that only contained water had the exact same amount of GA added as those with proteins. Each test tube in set 2 contained 1g of SPI, and the amount of GA needed was calculated and is listed below:

1% = 40 µL of GA in 25 wt% H_2O solution

3% = 120 µL of GA in 25 wt% H_2O solution

5% = 200 µL of GA in 25 wt% H_2O solution

7% = 280 µL of GA in 25 wt% H_2O solution

10% = 400 µL of GA in 25 wt% H_2O solution

Using micropipettes, the measured amount of GA was added into each labeled test tube in both sets. Images were taken of the test tubes at: 0 minute, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 5 hours, 24 hours, and 5 days. Any observed changes in behaviors were documented.

Figure 5.4.1 Image of the test tube experiment set-up. (L) Test tubes filled with 10 mL of water. ® Test tube filled with 10 wt% soy protein isolate in 10 mL water.

5.4.4 Glutaraldehyde Exposure to Dry Films

Another series of systematic studies of crosslinking the cellulose/SPC films were conducted. This time the GA is exposed to previously dried films to observe its effects. The dried films were exposed to different amount of GA solution for different times. After exposing the films to the crosslinking agent, the films were dried and re-examined.

The films were divided into 4 groups, each group contained 3 samples. Four shallow plates were prepared, each containing a small amount of water and a calculated amount of GA. The small amount of water was to prevent too much dilution of the GA, and the volume of GA used was calculated based on the weight of the films.

For each plate, 3 films were weighed together, and the amount of the 25 wt% GA solution used was calculated based on the total weight of the membranes. Dried films were

exposed to a solution of 1 wt%, 3 wt%, 5 wt%, and 10 wt% of GA for 10 minutes, 20 minutes, and 30 minutes. Listed below were the initial conditions of the experimental set up.

Table 5.4 Experiment Set-up Condition and Calculation for GA needed.

Plate #	Combined weight of membranes (g)	Calculated amount of GA needed (µL)	Actual Amount of GA used (µL)
1 – 1 wt% GA	0.7437	29.748	29.8
2 – 3 wt% GA	0.7138	85.656	85.7
3 – 5 wt% GA	0.6856	137.12	137.1
4 – 10 wt% GA	0.7361	294.44	294.4

Once the calculation was completed and the correct amount of GA was added into each plate, 3 dried cellulose/SPC films were placed into each solution. After every 10 minutes, a film from each plate was extracted and labeled 30 minutes **(See Figure 5.4.2).** After 30 minutes all the films were removed, the films were sandwiched between sheets of Teflon, and then vacuum dried for further analysis.

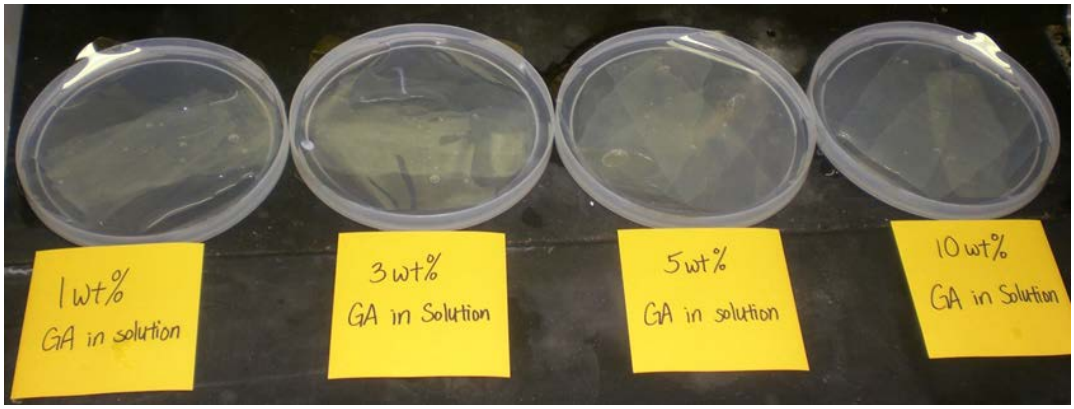


Figure 5.4.2 – Image of the set-up of the Experiment. Each plate contained a known amount of GA with 3 cellulose/SPC films.

5.5 Analytical Instruments used for Membrane Characterization

For the analysis of these membranes, several analytical instruments were used. This section will list the analytical instruments used and the functions they served. The results of the tests performed can be found in the following chapter.

5.5.1 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was employed to examine the surface and the cross-sections of the membranes. Using SEM it is possible to examine the porosity of the membranes, the thickness, as well as any surface characteristics. The SEM used for this research was the Hitachi S-3200 Scanning Electron Microscope, under standard vacuum conditions with a 5 kV potential difference used for measurement. Images from 100x up to 10,000x were captured and used for this study. The SEM Revolutions software was used to analyze the resulting micrographs.

5.5.2 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used to obtain infrared spectra of the membranes. The instrument used for this experiment was the Thermo Fisher Nexus 470 FTIR with Continuum Microscope and ORBIT/OMNI ATR. The tip of the sensor was made of diamond, and to analyze the obtained IR spectra, its OMNI software was used to measure peak intensity, compare spectra, as well as examination of the chemical components of the membranes.

5.5.3 Tensile Tests

Tensile tests were performed in the physical testing laboratory, where the mechanical properties of the produced membranes were measured and analyzed. The actual test was performed using a MTS Q-Test/5 Universal Testing Machine with a 5 lb. load cell (on one occasion a 250 lb. load cell was used), set at 50 mm gauge length, a speed of 10 mm/min, following an adapted method for the appropriate ASTM test method for polymer films (ASTM D882 with a 5 lb. load cell). Membranes were conditioned in the testing lab for 24 hours prior to testing, and individual membrane/sample thickness were measured using a Thwing-Albert Thickness tester following ASTM D1777 test method to obtain the proper thickness for the Q-test software. Membrane samples were prepared with a width of ½ inch using sharp razor blades to ensure a clean cut guided by a metal ruler. The lengths of the samples were between 3-6 inches long, and some of the shorter samples had some excess at the tip to extend the length to ensure a steady grip and prevent slippage during testing.

5.5.4 Thermogravimetric Analysis

The Thermogravimetric Analyzer (TGA) was used for comparative purposes, as well as the determination of structural differences between raw materials and any produced membranes. TGA was performed using a Perkin-Elmer TGA device, under Nitrogen atmosphere, at a heating rate of 20ºC/minutes going from room temperature (25ºC) up to 800ºC. For each analysis, 5-8 mg of samples of the material were prepared, and post analysis, the resulting curve obtained was examined and studied using the Pyris software package that accompanied the Perkin-Elmer TGA device.

5.5.5 Water Absorption Test

Water absorption tests were performed to observe the water pick-up weight by the dried membranes upon soaking for 24 hours. The initial dry weight was compared to the wet weight of various batches of membranes to calculate the percent of water pick up. Different types of films were then compared with each other to observe any trend or pattern that matches previous research.

CHAPTER 6. Results and Discussion

6.1 Characterization of Pure Cellulose Membranes

6.1.1 Membrane Formation

The dissolution process had been carefully studied by Dr. Douglass previously, using an optical microscope to observe the rate of dissolution of cellulose, starch, and proteins in the novel ED/KSCN solvent. In the thesis written by Dr. Douglass, he stated that to achieve complete dissolution of 7 wt% pure cellulose in solution, it would take somewhere between 2 to 4 hours. [24] It was also described in the same dissertation that the entire dissolution process were carried out under nitrogen atmosphere to prevent unwanted oxidation.

During the initial coagulation attempt, the parameters stated by Dr. Douglass were followed. Pure cellulose dissolution was attempted under nitrogen atmosphere, and the glycol oil bath was elevated to 90ºC using a thermometer to help maintain the bath at that temperature. However, it was not discovered until after 90 minutes after the start of the dissolution process that the nitrogen tank supply was low and that the system was no longer purged in nitrogen atmosphere. Despite this the experiment was carried out until full dissolution was achieved.

While Dr. Douglass stated that dissolution can be carried out between 2 to 4 hours, for pure cellulose, a full dissolution should be carried out close to 4 hours or even longer. After stirring in heated oil bath for 3 hours there were still some small traces of cellulose observed in the flask. Only until almost 4 hours did the dissolution seemed complete, and a clear, light yellow colored viscose solution was obtained.

It was uncertain at that time whether the absence of the nitrogen gas purge would have caused any undesired results that would alter the physical or chemical behaviors of the membranes. The solution was cooled gradually over night to a gel-form. The following day the gelled solution was heated gradually at lower temperature to remove any trapped air bubbles. It was heated for almost 2 hours until a good flow was achieved, allowing the solution to be cast into membranes.

Two different types of coagulation bath were used to determine which would result in better membranes: water or methanol. Using a 10”x10” glass dish, the coagulant was filled to about 1” – 2” deep. Membranes were casted on the Byk-Gardner Casting Table with a smooth glass surface. Membranes were produced using the 25 mil casting bar provided by Byk-Gardner. It was discovered by Hyun Lee that with very thin fibers a methanol coagulation bath was sufficient to remove the majority of the solvent blend without hurting the physical properties of the fibers. For the sake of comparison, the first batch of cellulose solution was coagulated in water as well. [6]

The amount of cellulose dissolved was also investigated. The 7 wt% concentration originally used by Dr. Douglass was very viscous and made it difficult to cast the membranes. To find a better condition, batches of 3 wt% and 5 wt% cellulose membranes were made. The lower concentration of cellulose present reduced the viscosity of the solution, and it was at a level of viscosity where the solution poured onto the casting table would not begin drying rapidly to form bumps after casting. The dissolution time was also reduced, while the 7 wt% solution required 4+ hours to complete, the 5 wt% took roughly 3 hours and the 3 wt% only took a little over 2 hours.

Once the cast membranes were placed into the coagulation bath, solid films formed within very short periods of time. During this coagulation process, the membranes began to curl up from the edges until gradually the membrane itself begin to show wrinkles. This observation was not described by Dr. Douglass, thus additional action was taken. Using a clean, flat piece of glass to press on top of the films with a sheet of PET film between the glass and the actual membrane, flatter, more uniform membranes were obtained.

After the membranes had been coagulated for 5 minutes, as proposed by Dr. Douglass, it was noted that the membranes coagulated in methanol were transparent, while those coagulated in water appeared opaque. The films were then removed from the coagulation bath and placed in the first of the 3 soaking baths. During the membrane transfer, the water-coagulated membrane was very fragile, and if too much force was used the membrane would tear and eventually fall apart. The methanol-coagulated films however, had better physical and structural integrity, with less wrinkling of the edges observed and their handling was much easier.

Even during the soaking (washing away more ED/KSCN solvent), films not under pressure had a tendency to curl up, so the flat glass was used throughout the entire process: three 20-minutes soaking sessions and soaking in the final bath overnight. Following that process the membranes were removed from the bath, and sandwiched between thin PET films atop a glass plate. To further ensure the production of uniform membranes, another glass was placed on top, finally topped with a brick to constantly apply pressure to the films while they were dried in the vacuum oven for 24 hours in ambient temperature, and a further 24 hours at 40ºC.

After the 48 hours of drying, membranes were removed from the oven. However, upon the removal of the glass and the PET films, it was discovered that the membranes did not dry up completely, and the membrane exposed to air begun to shrink rapidly. The remaining membranes were then put back into the oven for additional drying. From multiple attempts, it was concluded that cellulose dissolution using this solvent and condition is best achieved after 4 hours, methanol as the coagulant resulted in better membranes, and the drying time is best carried out 24 hours in ambient temperature, 24 hours at 40ºC, and an additional 24 hours drying in ambient temperature **(See Figure 6.1.1)**. (More drying at elevated temperature can result in smaller films with very hard edges)

Figure 6.1.1 Actual image of pure cellulose membrane.

6.1.2 Membrane Characterization

After perfecting the process to allow production of more uniform membranes, they were characterized using various instruments and techniques. It was noticed that the initial appearance of these membranes are very transparent with no noticeable difference in color than those produced previously by Dr. Douglass. This indicated that our initial assumption that the absence of nitrogen purge in this case did not make a difference was probably a good one. The risk for undesired oxidization was also suppressed thanks to the relatively low heating temperature (~90ºC). However, to be completely certain, such conclusion was not drawn until the membranes were carefully characterized.

6.1.2.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was performed to characterize the morphology of the membranes, and to confirm that nonporous membranes were produced using this solvent system. Images were taken from the cross-sectional view and the surface at various magnifications **(See Figures 6.1.2.1a,b)** .

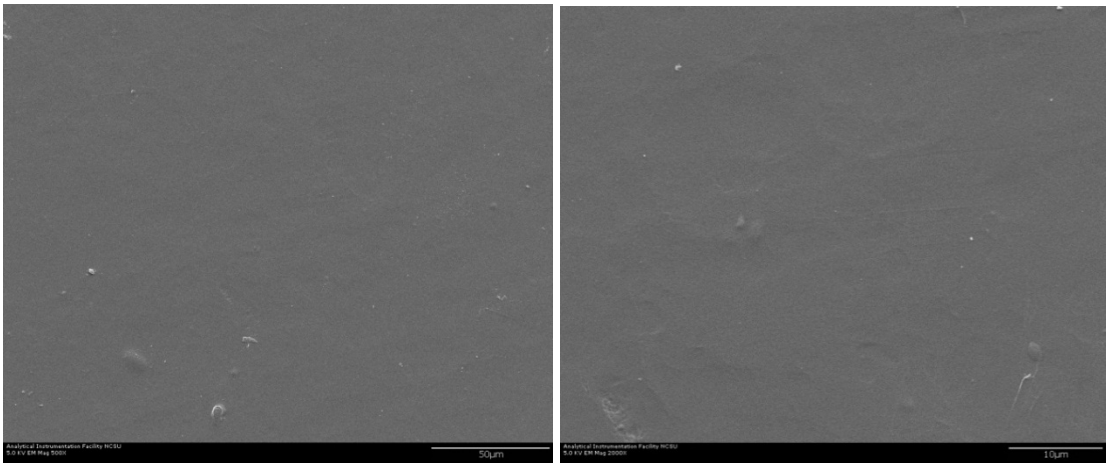


Figure 6.1.2.1a SEM imaging of the cellulose membrane surface. (L) 500x magnification; (R) 2000x magnification.

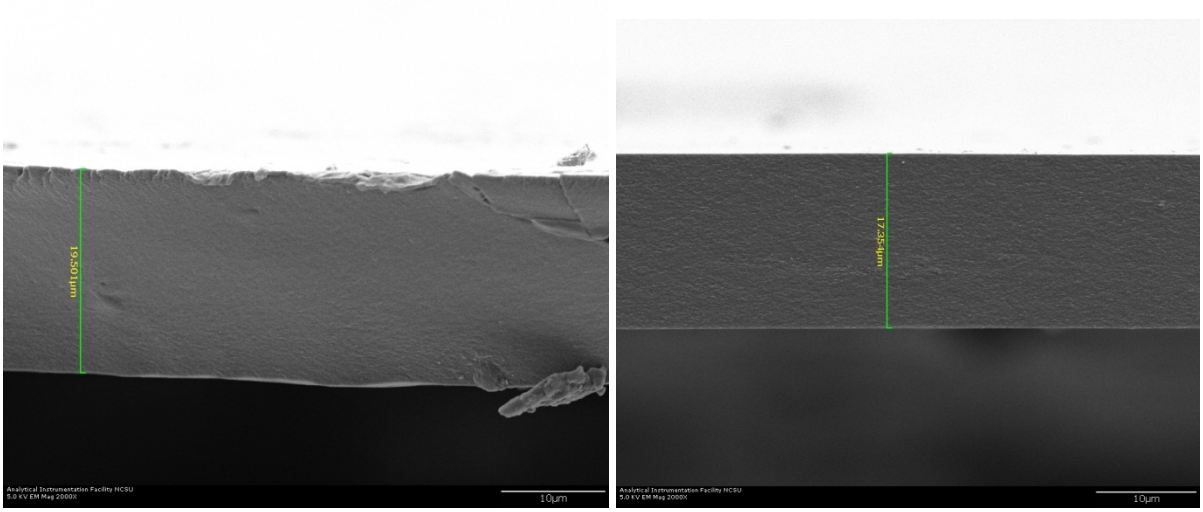


Figure 6.1.2.1b Cross-sectional SEM image of two cellulose membranes. (L) 2000x magnification, 19.501µm; (R) 2000x magnification, 17.354µm.

From the images obtained, SEM helped to verify that the cellulose membranes produced were indeed nonporous. Although there are still some small bumps or scratches observed on the surface, the nonporous membranes (or films) were relatively uniform.

6.1.2.2 Fourier Transform Infrared Spectroscopy (FTIR Spectroscopy)

To verify that the dissolution process do not need to be carried out under nitrogen atmosphere, a set of dissolutions were made under constant nitrogen exposure and cast into membranes so they could be compared with the ones made without nitrogen. From their physical appearances there were no any noticeable differences, thus FTIR was used to analyze the membranes and to see if any unwanted reactions had occurred that could result in new IR absorption peaks.

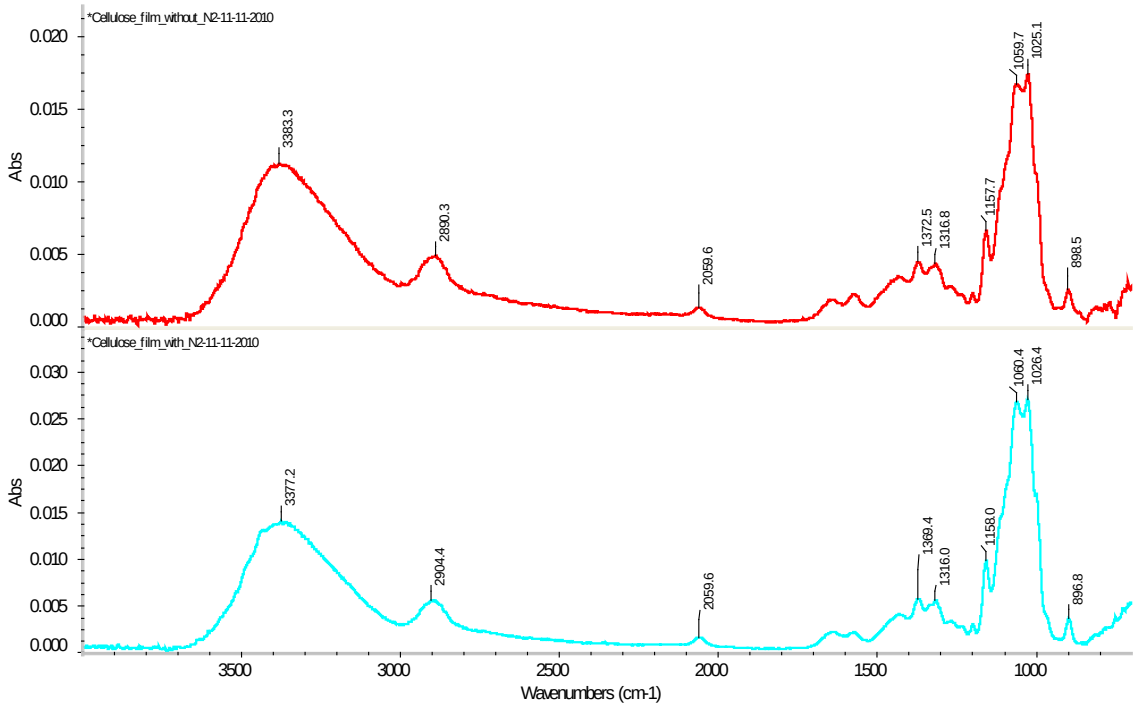


Figure 6.1.2.2 FTIR spectrums of cellulose membranes made without N_2 (top) and with N_2 (bottom).

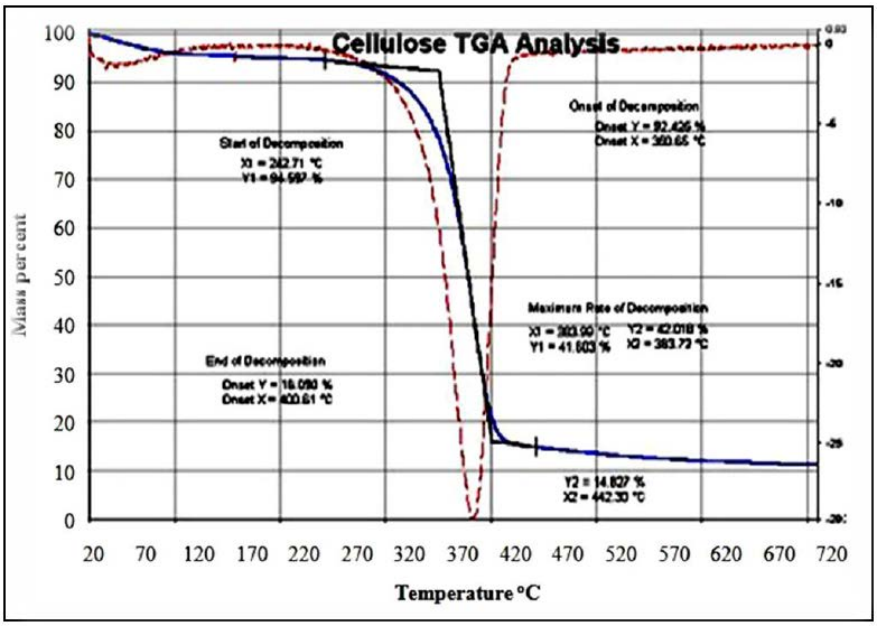
As seen above, the FTIR spectra of the two are nearly identical. The major functional groups like –OH, -CH sp^3, -C-C-, -C-O-, and –C-OH are all present, with nearly identical band widths and intensities. From this it was concluded that a nitrogen atmosphere was not necessary during the dissolution process.

6.1.2.3 Thermogravimetric Analysis (TGA)

TGA was performed to determine the morphology and the thermal properties of the membranes. In the studies conducted by Dr. Douglass, he noted that there is a significant difference in the TGA curve between the cellulose pulp and the regenerated membrane. From two TGA studies he was able to identify the curve shifted towards a lower overall

decomposition that indicated an ease of decomposition from dissolving the cellulose and then regenerating it into a different cellulose structure via coagulation. [24]

a)



b)

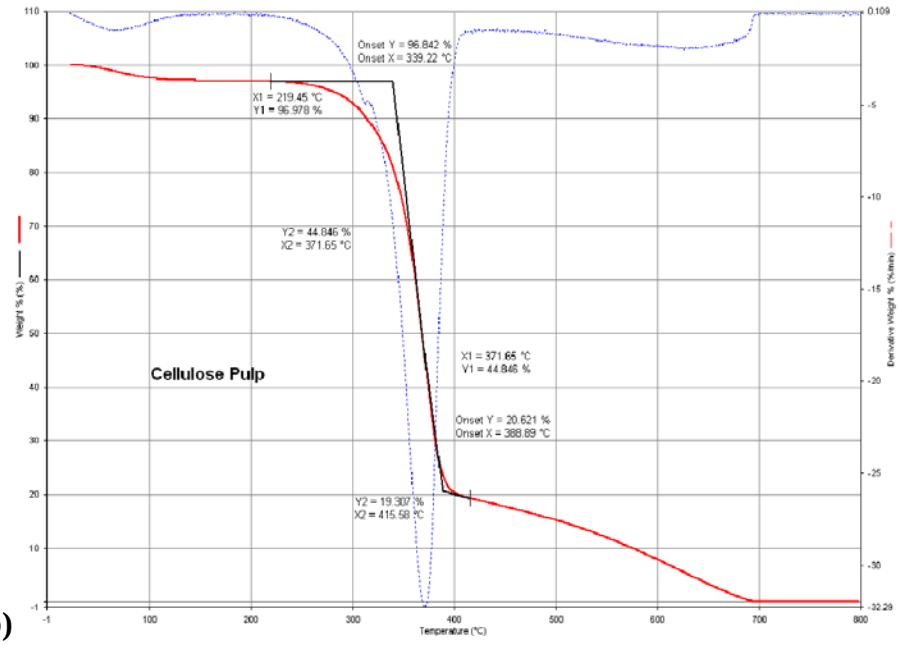
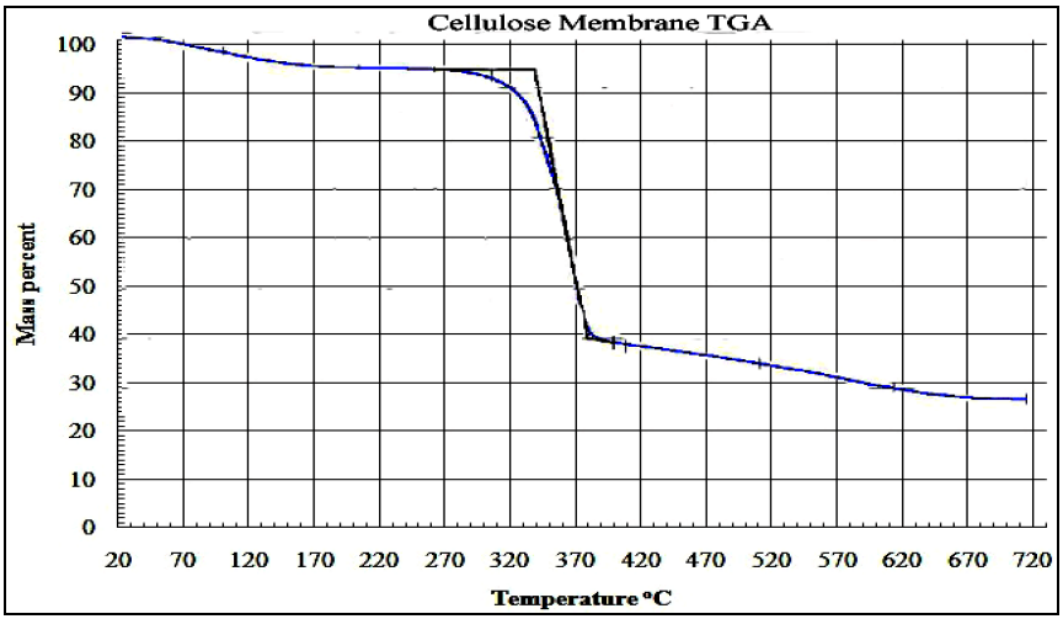


Figure 6.1.2.3a TGA analysis curve of raw cellulose: (a) Data from Dr. Douglass [24], (b) data obtained from raw cellulose samples for current membranes.

a)



b)

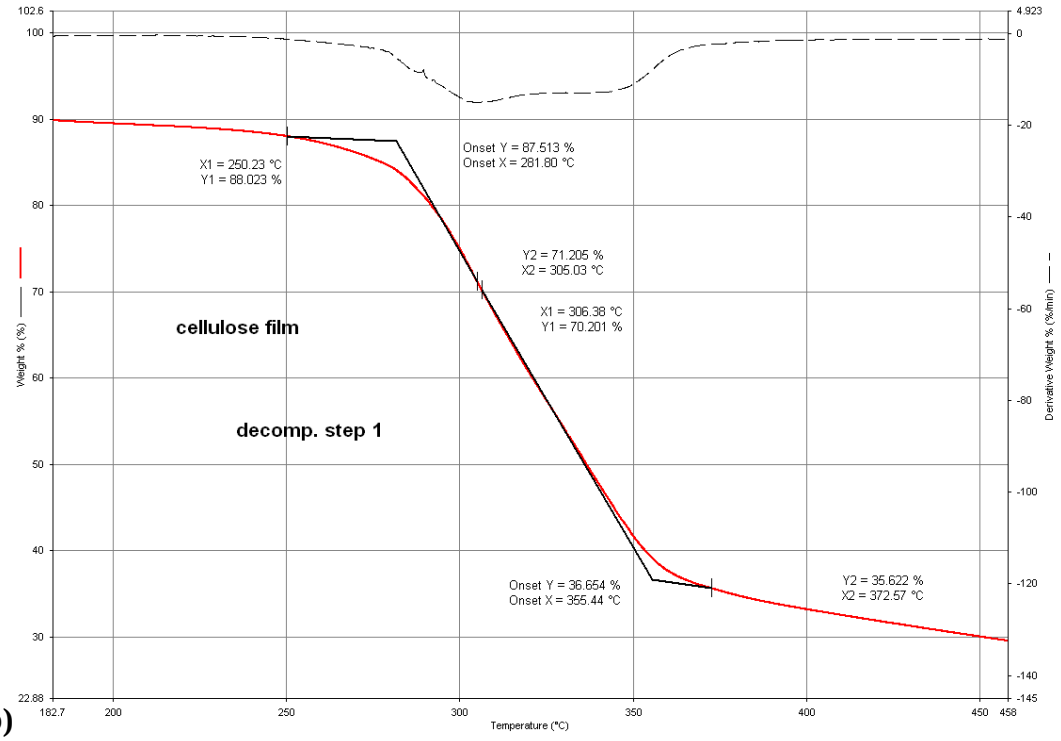


Figure 6.1.2.3b – TGA analysis curve of pure cellulose membrane: (a) produced by Dr. Douglass [24], (b) newly attempted membranes.

By comparing above the TGA graphs that were conducted by Dr. Douglass and the current samples, the new membranes exhibit a similar trend in comparison to the pure cellulose pulps. The beginning of decomposition and the end of decomposition shifted about 20ºC to lower temperatures. Membranes made with the modified procedures in comparison to those made by Dr. Douglass also have a slightly lower end of decomposition temperature.

Another interesting observation was made between the cellulose pulp TGA graph and the cellulose membrane TGA graph: after the rapid decomposition, little to none of the cellulose pulp remains, while the membranes are left with 30%-20% ash remaining. This char level suggests that a different crystal structure resulted from regenerating cellulose.

6.1.2.4 Water Absorption Test

Water absorption tests were conducted to see the amount of water the membrane can pick up. The study was performed by weighing the dried membrane, then after swelling in deionized, distilled water for 24 hours; finally measure its wet mass to determine the percent of wet mass increase. Such a test was also performed by Dr. Douglass to characterize the cellulose membranes he produced, and all results are presented below.

Table 6.1.2.4 A comparison of water absorption results

Material	Dry mass (g)	Wet mass (g)	Wet mass increase (%)
Wet cellulose membrane (coagulated & kept wet) [24]	0.28 (after)	4.70 (before)	94 decrease, 1580 increase
Cellulose membrane a [24]	0.75	1.34	79
Cellulose membrane b [24]	0.49	1.03	110
Cotton control membrane [24]	0.21	0.42	100
6 wt% Cellulose membrane (no N_2)	0.3074	0.3516	14.38

It was noted the membranes produced using the new method resulted in a much lower water pick-up percentage than those created by Dr. Douglass. The main reason for this difference is that the membranes that were tested by Dr. Douglass are porous membranes, and the presence of the pores improved the wet pick-up by trapping more water inside the pores. Even though Dr. Douglass created nonporous cellulose membranes, he did not conduct any water absorption tests. But it does appear that the nonporous cellulose membrane do not pick up nearly as much water. Even though cellulose is hydrophilic, the regenerated cellulose membranes do not pick up as much water. The purpose for this test for the new experiment is to use it for comparison to the cellulose/soy protein blend membranes, and those that were exposed to the crosslinking agent glutaraldehyde (GA) to observe the changes.

6.1.2.5 Tensile Tests

Tensile tests were conducted to characterize the mechanical properties of the membranes, particularly their modulus, failure stress and failure strain. For each test 20-30

samples were prepared (sample preparation procedure was described in chapter 5), all tests were conducted using a 5 lb. load cells. Khare *et. al* produced cellulose membranes that had a tensile modulus of 1.6 ± 0.6 GPa (163.1 kgf/mm^2 ± 61); with failure stress of 64.9 ± 18.3 MPa and failure strain of 6.5 ± 1.5%. Dr. Douglass used the new solvent system and was able to produce non-porous membranes with similar tensile properties. The table below shows the tensile properties of cellulose membranes newly produced with the small modifications from those of Douglass in comparison to those produced by Khare *et al.* and by Douglass.

Table 6.1.2.5 A comparison of tensile tests to works of Khare *et al.* and Douglass

Sample	Tensile Modulus (kgf/mm^2)	Failure Stress (kgf/mm^2)	Failure Strain (%)	Thickness (mm)
Khare [32]	163.1 ± 61	6.62 ± 1.9	6.5 ± 1.5	Unknown
Douglass nonporous [24]	165.5 ± 16	5.36 ± 0.7	26.2 ± 10.1	0.047 ± 0.015
Douglass Porous [24]	33.0 ± 9.3	0.59 ± 0.17	3.9 ±1.4	Unknown
Zhu 3 wt% nonporous	191.50 ± 44.72	5.83 ± 2.34	13.3 ± 13.4	0.0173 ± 0.0021
Zhu 5 wt% nonporous	260.89 ± 27.54	4.65 ± 0.63	15.1 ± 4.5	0.034 ± 0.004

Comparing the above data, the membranes made without the presence of nitrogen, and with extra pressure applied to improve their uniformity resulted in increased modulus in comparison to those produced by Khare *et al.* and Douglass. The failure strain decreased compared to the nonporous membranes produced by Douglass, which is inversely related to the increase in modulus. The higher modulus indicated that the regenerated cellulose

molecules are more uniform and closely packed, and when the polymer chains are more closely packed, the freedom for the movement between polymer chains are reduced, which results in a decrease in stress and strain.

The standard deviation for the 3 wt% nonporous is rather high; this was due to some samples that had snapped at or near the grip. This breaking at the grip caused some of the data to have either very low values or the program could not detect a value, and resulted in high deviations. Khare *et al.* produced their porous regenerated cellulose with NMMO and water, while the new films and those prepared by Douglass were using the ED/KSCN solution produced by Hyun Jik Lee. This solution does not result in very strong porous membranes, but it is a much cleaner method and the nonporous membranes have exhibited relatively good strength, especially when constant pressure was applied throughout the membrane formation process.

6.1.3 Conclusions

Cellulose membranes were produced for future comparative purposes with the cellulose/soy protein blend membranes, as well as for getting familiar with the membrane producing procedure. Using the novel ED/KSCN solvent system developed recently, this new process by comparison has a lower toxicity, potentially decreases the cost and improves overall efficiency.

Cellulose dissolution is achieved by disintegration of the crystalline regions followed by the swelling of the amorphous regions. It was observed that the ED causes the cellulose to swell while the KSCN provided the charge balance that allowed the material to flow at

elevated temperature. Although accidental, it was discovered that the dissolution process can be carried out without purging the entire system with nitrogen gas and can still produce membranes with the same chemical make-up. By applying even pressure throughout the membrane forming process, the overall uniformity of the membranes improved, giving smooth, transparent membranes were produced with improved mechanical properties.

During dissolution, the 7 wt% cellulose solution had a somewhat too high viscosity and made it more difficult to form uniform membranes. To overcome this, 3 wt% and 5 wt% cellulose solutions were used. The reduced amount of cellulose required less time to achieve complete dissolution, and the solution gave enough flow so that they will not create as much bumps during membrane casting. After conducting tensile tests with 20-30 samples each, it was observed that 5 wt% cellulose created very uniform cellulose membranes and had higher tensile strength in comparison to the 3 wt% cellulose membranes and the 7 wt% cellulose membranes produced previously by Dr. Douglass.

6.2 Characterization of Cellulose/Soy Protein Concentrate Membranes

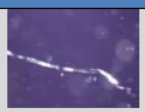
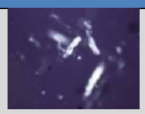
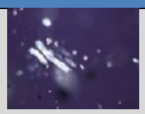
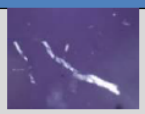
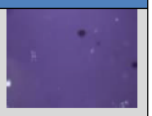
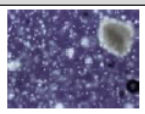
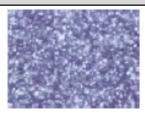
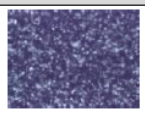
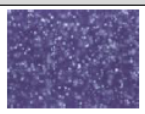
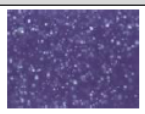
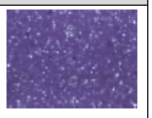
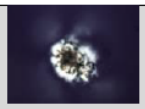
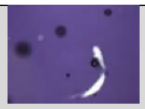
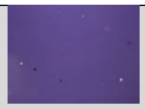
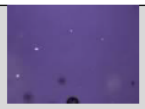
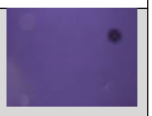
6.2.1 Membrane Formation

Studies were conducted by attempting to produce films with cellulose and soy protein. Soy protein isolate (~90% protein) was used previously by Dr. Douglass, but the objective here was to see if good strength membranes can be made using less purified proteins, since SPI is more costly. Because of this, soy protein concentrate (SPC) (~70% protein) was used in place of SPI and the membranes made were carefully characterized.

The membrane forming process for cellulose/SPC membranes are nearly identical to those previously described for cellulose. During the dissolution process, 3g of cellulose and 3g of SPC were used. Due to the presence of the SPC, the time required to achieve complete dissolution was reduced to 2-2.5 hours. The temperature required to carry out the dissolution was also lowered to between 75-80º C to prevent the soy protein from charring if exposed to high temperature for long period of time or both.

Dr. Douglass had conducted visual dissolution studies of cellulose and cellulose/protein blends **(See Figure 6.2.1),** and he observed that after 120 minutes, soy protein had completely blended into the solution. With the study he conducted as reference, and by visually observing that all cellulose and SPC were no longer visible in the beaker after an hour, that the dissolution time of 2-2.5 hours was more than sufficient.

Table 6.2.1 Time elapse visual study of dissolution with cellulose and a variety of blends in ED/KSCN solvent using cross polarization microscopy. [24]

Time (minutes) \ Material	15	30	45	60	90	120
Cellulose						
Cellulose / Waxy maize Starch						
Cellulose / Soy protein						

When left to cool gradually the cellulose/SPC solutions turned into gels like all of the cellulose solutions. To liquefy, low heat was applied again prior to membrane casting. Using the same glass casting table provided by Byk-Gardner, and using a 20 mil casting bar, a wet, uniform film was formed on the surface. This film was removed from the casting table and placed in a methanol coagulation bath followed by several more baths of methanol for the removal of any solvent still present. Similar to the pure cellulose films, the cellulose/SPC membranes coagulated to a decent strength within several seconds. Similar curling at the edges was also observed, thus the same tactic was applied as before, where a piece of uniform glass was pressed on top of the membranes with a layer of PET film in between to apply a uniform pressure, reducing the amount of curling.

Instead of the clear, transparent membranes that were produced from cellulose, the cellulose/SPC blended membranes appeared opaque with an off-white color. The drying

process was carried out at ambient temperature and low heating temperatures similar to before, and a brick was also used this time to ensure an evenly distributed pressure is applied to the films during the drying process, in order to reduce the amount of shrinking and wrinkling to a minimum. The pure cellulose films was kept dried by placing films between a single sheet of PET, however, the cellulose/SPC films had a tendency to stick to the PET and so sheets of Teflon were used in its place.

For comparison purpose, a batch of 6 wt% pure soy protein solution was made in the hopes of forming some membranes and to compare their properties to the cellulose/SPC blends. The dissolution was complete within 2 hours at 75º C and had the consistency that is just slightly thicker than water. Even after cooling down overnight, the solution did not turn into a gel at room temperature. After heating it back up at a low temperature for membrane formation, membranes were casted using the 20mil casting bar.

The cast membrane was placed into the coagulation bath, and within seconds the membrane coagulated and separated itself from the PET film. After coagulating the membranes for 5-10 minutes in methanol, they were transferred into the first of the three 20 minute methanol soaking baths. It was observed during this transfer that the films, although coagulated, were extremely fragile. Any slight movement caused the film to fall apart into small pieces. Being very careful, little over half of all the membranes cast were placed into the final soaking bath to soak overnight (the rest were lost or destroyed during transferring between soaking baths).

Even after soaking in methanol overnight, the membranes were still extremely fragile. During the transfer of the films between sheets of Teflon for the drying process, more films

fell apart. Afterwards only a handful of membranes remained and were placed in the dryer, because how thin the films were, the drying process took much more time because they soaked up more moisture and the Teflon sheets were more closely together, preventing the moisture from leaving the membranes. The dried films obtained afterwards were too thin for successful removal from the Teflon sheets, and could not be characterized. However, it was discovered that pure protein films can be made, but unfortunately using the current membrane forming technique cannot result in films that can actually be used for any purpose.

6.2.2 Membrane Characterization

Once the cellulose/SPC membranes were produced, flat, opaque, off-white nonporous films were produced. These films then went through a series of characterizations using various analytical instruments. The image below is of the dried membranes. It can be observed that around the edges there are still some wrinkles present, but overall membrane thickness was relatively uniform with very low deviation.

Figure 6.2.2 Actual images of dried cellulose/SPC blend membranes.

6.2.2.1 Fourier Transform Infrared Spectroscopy (FTIR Spectroscopy)

FTIR spectra were obtained and used to compare with the FTIR spectra of the pure cellulose membranes. The functional groups of the soy protein are predominately –OH and –NH groups, and only small differences between the pure cellulose membranes and the cellulose/protein blend membranes were observed from the FTIR.

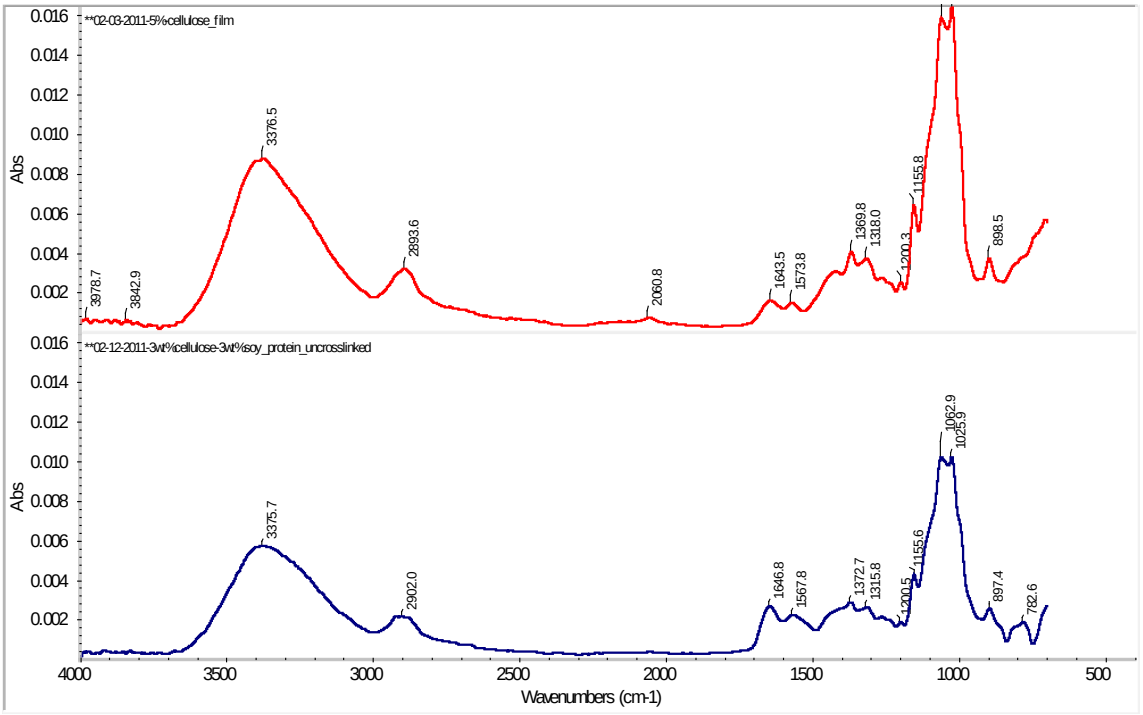


Figure 6.2.2.1 FTIR spectrum of (top) 5w t% cellulose membrane and (bottom) 3wt %cellulose, 3 wt% SPC membrane

Majority of the spectra looks very similar, although the peak intensities of the cellulose/SPC membranes are lower than those of the pure cellulose membranes. It is also observed that there is a new peak showing at 782.8 cm^{-1} in the fingerprinting region that was not observed in the pure cellulose membranes. R – NH_2 primary amines can have a band

between 860-760 cm^{-1}, so this newly observed peak at 782.8 cm^{-1} could be an indication of the presence of primary amine groups.

6.2.2.2 Water Absorption Test

Water absorption test was conducted to see how much water the dried membranes can pick up. Soy proteins have been reported to be water-soluble [35-37, 39] and have relatively high water-pick up properties. Again membranes were weighed prior and after soaking in water for 24 hours and the % weight gain were calculated.

Table 6.2.2.2 Comparison of water absorption results

Material	Dry mass (g)	Wet mass (g)	Wet mass increase (%)
Douglass Wet Cellulose membrane (coagulated & kept wet) [24]	0.28 (after)	4.70 (before)	94 decrease, 1580 increase
Douglass cellulose membrane a [24]	0.75	1.34	79
Douglass cellulose membrane b [24]	0.49	1.03	110
Douglass cotton control membrane [24]	0.21	0.42	100
Zhu 6 wt% cellulose membrane (No N_2)	0.3074	0.3516	14.38
Zhu cellulose/SPC membrane	0.2213	0.4903	121.55

From the water absorption test it was noted above that the cellulose/SPC membrane was able to pick up much more water, with a wet mass increase of 121.55%. It was also observed that after the dried membrane had been soaking in water for 24 hours, the film still remained intact, and even after removal from water that some strength still remained. The film did appear more transparent however, indicated that some protein had escaped into the

water. To solve or reduce this from occurring, it is necessary to crosslink the film to stabilize the protein so that it would remain in the film. It is noted here that a more systematic method was devised in another section, and by weighing the dry membranes before and after water exposure did resulted in overall weight loss which can support this hypothesis.

6.2.2.3 Oxygen and Water Barrier Tests

Some of the soy protein membranes were sent to the US Army Research Laboratory at Natick, MA to have water and oxygen barrier testing conducted thanks to their interest in the possibilities of using the cellulose/soy membranes in military food packaging. The conditions and results of the tests are provided below.

The oxygen barrier test was carried out at 0% relative humidity at 23ºC at 1 atmosphere of pressure. The oxygen transmission rate (OTR) of cellulose/SPC film was measured at 4.53 cc/m^2 per day. It was provided by the Army research laboratory that typical high density polyethylene (HDPE) has an OTR value of $100cc/m^2$ per day while nylon 6 has an OTR value between 1-4 cc/m^2 per day under dry condition. From these values it was noted that the blended films have better barrier to oxygen than HDPE but not quite as low as nylon. However, due to the protein's affinity for water, the OTR value is likely to increase with the rise in relative humidity.

Water barrier test was also conducted by the Army researchers at Natick, MA, and the testing was conducted at 1 atmosphere of pressure with 90% relative humidity at 37ºC. The water vapor transmission rate (WVTR) of the cellulose/SPC films was measured to be 829.7 g/m^2 per day. This high value was expected due to the water sensitivity that cellulose and soy

proteins have, because they are both are hydrophilic. The laboratory also provided some data for comparison, and the WVTR value for HDPE is about 1-5 cc/m^2 per day. It was also stated by the laboratory that materials having similar WVTR values are mostly biodegradable materials, such as polylactic acid (PLA) and polyhydroxybutyrate (PHB).

6.2.2.4 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was performed with the same testing procedure used on the cellulose membranes, with the specific methods and parameters described back in Chapter 5. The TGA data obtained from the cellulose/SPC blended membranes were compared to the pure cellulose membranes. Dr. Douglass also did some study with cellulose/soy protein isolate (SPI), which were also used to compare to the blended membranes using a lower grade protein (lower protein content). If the crystalline structure is different, the thermo-degradation of the film will also be different; the amorphous region will degrade first, and the nature of the crystalline region will affect the decomposition curve as well. [24]

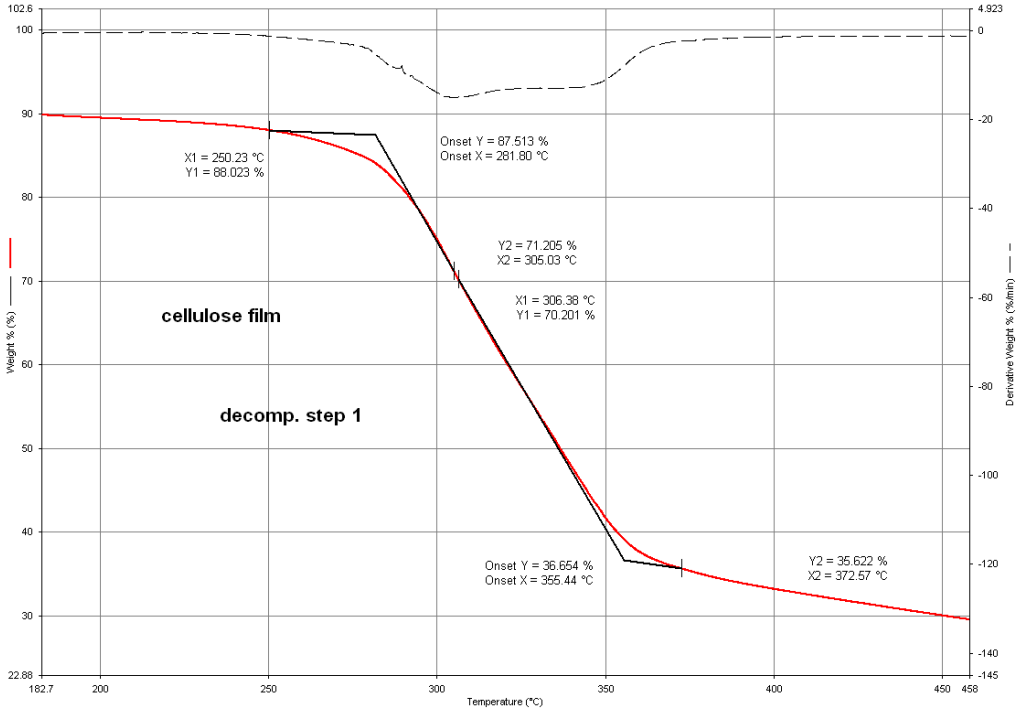


Figure 6.2.2.4a TGA curve of cellulose membrane

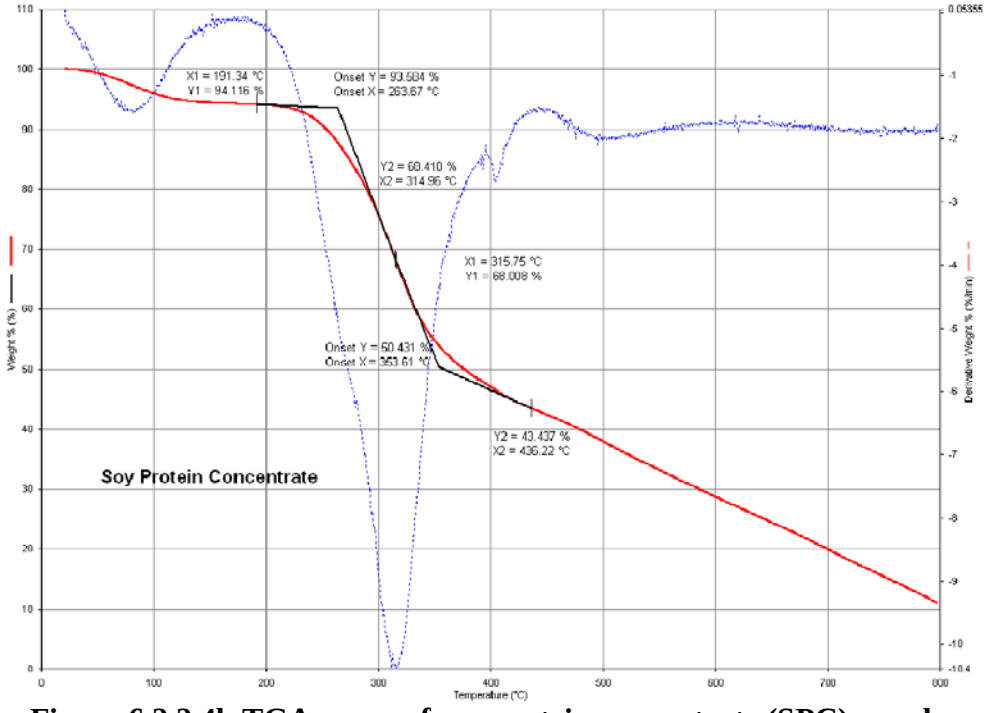


Figure 6.2.2.4b TGA curve of soy protein concentrate (SPC) powder

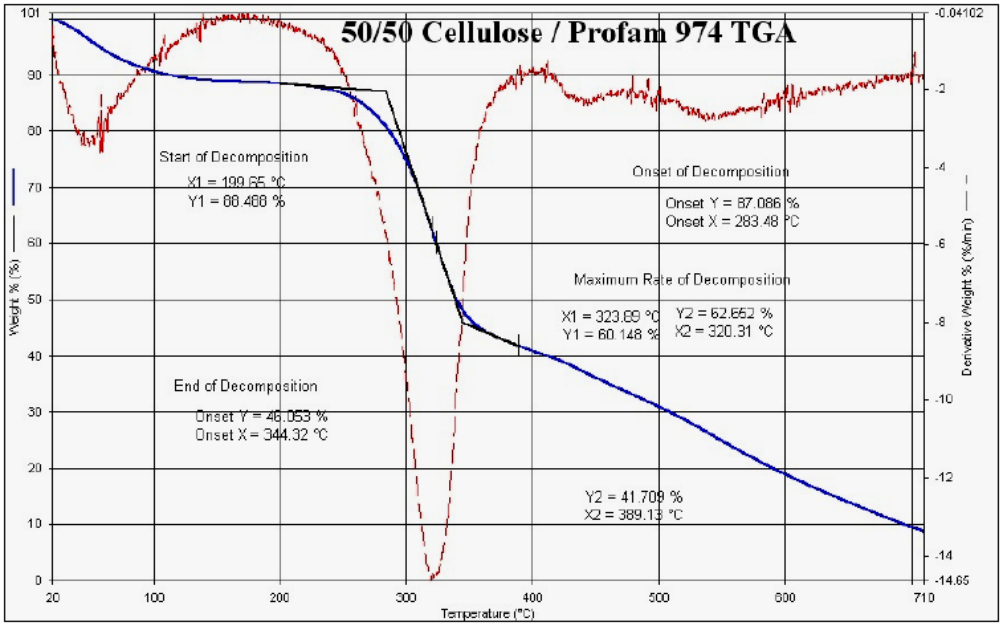


Figure 6.2.2.4c TGA curve of Douglass cellulose/soy protein isolate (SPI) membrane [24]

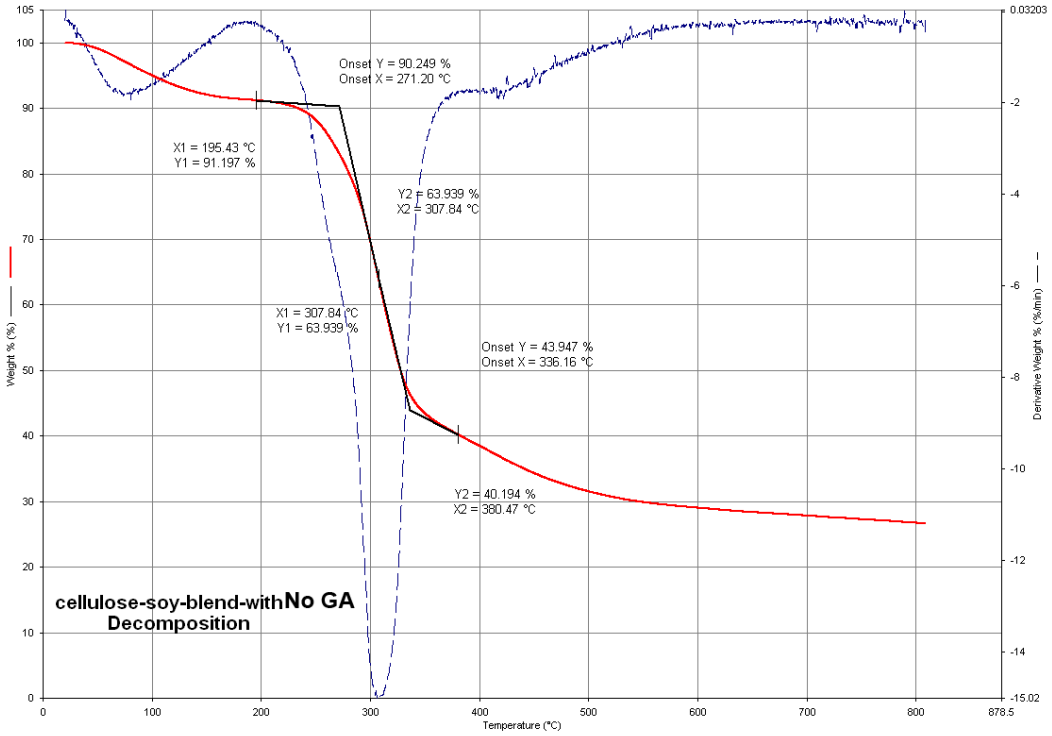


Figure 6.2.2.4 TGA curve of 50:50 cellulose/SPC membrane

Table 6.2.2.4 Beginning and end of rapid decomposition temperatures of the TGA curve

Sample	Onset temperature and wt% of material remain)	Offset temperature and wt% of material remain)
Zhu cellulose membrane	250.23ºC (88.023%)	372.57ºC (35.622%)
Zhu SPC powder	191.34ºC (94.116%)	436.22ºC (43.437%)
Douglass cellulose/SPI membrane	199.65ºC (88.488%)	344.32ºC (46.053%)
Zhu cellulose/SPC membrane	195.43ºC (91.197%)	380.47ºC (40.194%)

From the data seen above, it was observed that pure cellulose membrane rapidly degrades at a higher temperature than the cellulose/soy protein membranes by a little over 50ºC. However, the end temperature of this rapid decomposition for the regenerated cellulose membrane is lower than the pure SPC membrane. But once the soy protein and cellulose were dissolved and regenerated, the films produced had an initial decomposition temperature that is closer to the value of the protein, and the decomposition temperature is closer to the cellulose membrane degradation temperature. Even though this is observed, there were no significant differences in thermo-degradation between the cellulose/SPI membrane and the cellulose/SPC membrane.

Judging from the TGA curves, it can be assumed that as temperature is elevated, the protein component begins to decompose first, but the cellulose portion completely degrades prior to the complete degradation of soy protein is achieved. The weight percent of the remaining sample for all protein-contained TGA graphs show a steeper slope of degradation post the rapid decomposition, where the last 15-30% of the soy protein is slowly degraded until only the remaining charred pure carbon material remains. Although it is uncertain if this

is a true blend, but the data suggest that at least the cellulose and soy proteins had achieved certain degree of miscibility.

6.2.2.5 Tensile Tests

Two different sets of cellulose/SPC membranes were used to conduct the tensile tests with each set containing between 20-30 membranes. All samples were conditioned in the testing lab for 24 hours prior to testing, samples were cut to ½ inch wide and the length varied. The load cell used was 5 lb. and the results are compared below to those cellulose/SPI membranes produced by Dr. Douglass.

Table 6.2.2.5 List of tensile test data of cellulose and cellulose/protein blend membranes

Samples	Tensile modulus (kgf/mm^2) CV(%)	Failure stress (kgf/mm^2) CV(%)	Failure strain (%) CV(%)	Thickness (mm) CV%
Douglass 7 wt% cellulose membrane	165.5 ± 16 (9.7%)	5.36 ± 0.7 (13.1%)	26.2 ± 10.1 (38.5%)	0.047 ± 0.015 (31.9%)
Zhu 3 wt% cellulose membrane	191.50 ± 44.72 (23.4%)	5.83 ± 2.34 (40.1%)	13.3 ± 13.4 (100.7%)	0.0173 ± 0.0021 (12.1%)
Zhu 5 wt% cellulose membrane	260.89 ± 27.54 (10.6%)	4.65 ± 0.63 (13.5%)	15.1 ± 4.5 (29.8%)	0.034 ± 0.004 (11.7%)
Douglass 3 wt% cellulose/ 3 wt% SPI	157 ± 52 (33.1%)	3.2 ± 1.6 (50%)	27 ± 12 (44.4%)	0.029 ± 0.003 (10.3%)
Zhu 3 wt% cellulose/ 3 wt% SPC a	255.79 ± 19.21 (7.5%)	4.40 ± 0.27 (6.1%)	9.3 ± 2.8 (30.1%)	0.0347 ± 0.0023 (6.6%)
Zhu 3 wt% cellulose/ 3 wt% SPC b	256.78 ± 20.27 (7.9%)	3.72 ± 0.52 (14.0%)	13.3 ± 10.7 (80.4)	0.0329 ± 0.0030 (9.1)

Based on the data obtained, the newly produced cellulose/SPC membranes had much higher tensile modulus than those cellulose/SPI membranes produced by Dr. Douglass. This could have happened due to the overall uniformity of the membranes that improved with the modified membrane formation method. All the solutions made here (excluding the ones belonging to Dr. Douglass) did not require the usage of nitrogen. Although the modulus had increased by 35-45% (after values were normalized), the strain for one set of the cellulose/SPC membranes had a high coefficient of variance of 80.4%. Similar to what happened to the 3 wt% cellulose membranes, this large difference was most likely contributed to some of the samples breaking at the grip.

Here once again the failure strain had decreased in comparison to those samples produced by Dr. Douglass. This too is inversely proportional to the increase in tensile modulus; the higher modulus meant the polymer (or oligomer) chains had become more orientated, and the chain alignments are more orderly, which also meant the decrease in lower failure strain value.

6.2.3 Conclusions

Uniform membranes were produced by dissolving 3g of cellulose and 3g of Arcon F SPC, and complete dissolution can be achieved between 2-2.5 hours. Methanol also proved to be an effective coagulant for the dissolved blend. Resulting membrane from this blend appears opaque with an off-white color. Membranes made from pure protein solution were attempted, but the resulting membranes were extremely fragile and deemed not useful for applications, at least not using the current preparation method.

Since the blend membranes contain soy protein, which is hydrophilic, the results from water absorption and water vapor barrier results were expected and supported. The pure cellulose membranes only had a 14.38% wet mass increase after 24 hours of soaking, while the cellulose/SPC blend membranes had a 121.55% of wet mass increase. It was noticed that even after 24 hours of soaking the membrane did not fall apart, though we suspect some proteins were lost in water. The results from the water barrier test conducted by the Army researchers also show that the cellulose/SPC films have a high water vapor transmission rate value similar to many biodegradable materials. Under dry conditions the film also has a good oxygen barrier property, though we suspect that the oxygen transfer rate would likely increase as the relative humidity rises.

Thermogravimetric analysis shown that the cellulose/SPC membranes had an initial decomposition temperature closely resembling SPC, yet its end of rapid decomposition temperature is closer to that of pure cellulose membranes. The TGA curve is of a single curve with one rapid drop, and the fact that this blended membrane adapted thermo properties of both cellulose and SPC is a strong indication that some miscibility was achieved**,** suggesting that if the films can be successfully crosslinked, the resulting rapid decomposition curve will shift to the right.

Cellulose/SPC membranes produced using the current method resulted in membranes that were superior in uniformity and tensile modulus. The tensile moduli of the blended membranes were very similar to the 5 wt% cellulose membranes, and even though SPC was used and had lower protein content than SPI, it was still possible to obtain strong membranes. After normalizing the values, the tensile strength of the newly produced blended membranes

had a 35-45% increase in tensile modulus compared to the cellulose/SPI membranes produced by Dr. Douglass. However, the resulting increase in modulus also caused the lower value obtained in failure strain, most likely due to the improved molecular orientations of the polymer chains.

6.3 Characterization of Membranes Exposed to Glutaraldehyde

6.3.1 Glutaraldehyde Exposure Attempts

To stabilize the soy protein when exposed to water, the membrane needs to be crosslinked. By looking through literature, it was decided that glutaraldehyde would be the most suitable, for it is a popular crosslinking agent used for proteins and no catalysts are required for the reaction. [44, 47-52] Although the exact reaction mechanisms have not reached a common consensus, it was suspected that this 5-carbon dialdehyde can react to the several potential functional groups in protein such as –OH, –SH, and especially –NH. [8, 38]

Although no examples were found on crosslinking a membrane containing cellulose and soy protein, we made a few attempts to crosslink the cellulose/SPC membranes. Initially a small amount of GA measured at 5 wt% to the amount of proteins and cellulose used (6g) was calculated and added into the coagulation bath. After further study it was decided that exposing the membrane during coagulation did not cause effective crosslinking, because the wet membrane still contained a large amount of ED/KSCN solvent, and was GA had the opportunity to react with the ED on top of the cellulose and SPC, not to mention the GA was greatly diluted in the coagulation bath.

In order to let the GA react mostly to the protein and cellulose, it is necessary to remove most of the solvent from the membrane. The second attempt to expose the membranes to GA was during the final soaking bath, where the majority of the solvent had been removed. Same amount of GA was added into the soaking bath as previous, and the films were left in there overnight. Upon inspection the following day **(See below)**, the color of the methanol soaking bath, as well as the color of the membranes had changed.

Figure 6.3.1.1 – Image of the membranes in final soaking bath with GA, observed after leaving it overnight.

The change in color had been mentioned in the literature indicating the possibility of GA reacting with a polymer. [49, 54] The membranes were then dried in hope that successful crosslinking had occurred. It was noted that since the bath is made of methanol, there was also a big chance that the GA might have reacted with methanol as well, and this might have affected the actual crosslinking.

After more careful study of the literature, it was eventually decided that in order to ensure that the GA react only to the cellulose and the soy proteins that made up of the membrane, it is better to expose the membranes to GA in a small amount of DI water after

the membranes were dried. This removes the opportunity for the GA to interact with methanol, and the majority of the ED/KSCN solvent system would have been removed from the membranes by that point. To test this theory, 2 dried membranes was exposed to 5 wt% of membrane weight of GA and used for initial examination. A systematic study to expose the membranes to GA was conducted separately and more information will be provided in detail in a separate section.

6.3.2 Membrane Characterization

The two dried membranes that were exposed to the GA in a small bath of DI water were dried after 2 hours of exposure to see if this procedure could result in successful crosslinking prior to the systematic study that followed.

6.3.2.1 Fourier Transform Infrared Spectroscopy (FTIR Spectroscopy)

FTIR spectra were once again taken using the same machine and software. The obtained spectrum is compared in **Figure 6.2.3.1** with those of the cellulose and the cellulose/SPC membranes that were not exposed to GA.

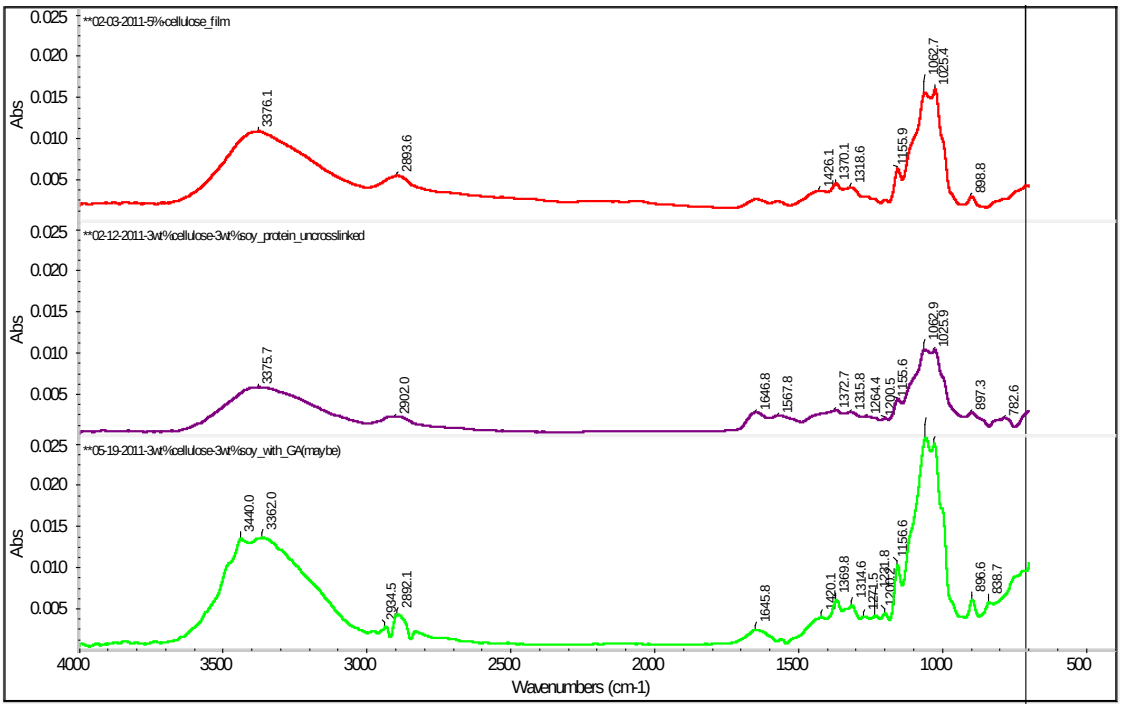


Figure 6.3.2.1a FTIR spectrum of (top) cellulose membrane, (middle) cellulose/SPC membrans (no GA), and (bottom) cellulose/SPC membrane with GA

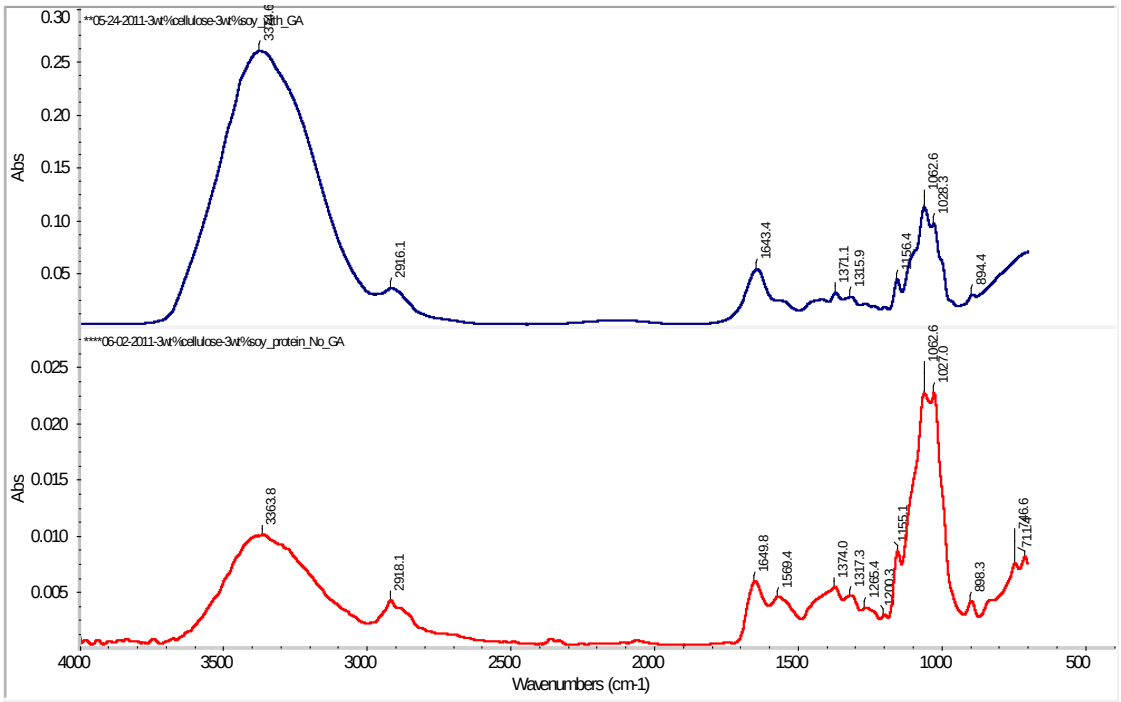


Figure 6.3.2.1b FTIR spectrum comparison of (top) blend membrane with GA and (bottom) blend membrane without GA

Here it is observed that the membranes exposed to GA did not result in an IR spectrum that is too different from the cellulose membrane or the blend membrane with no exposure to GA. The primary –OH group for the membranes exposed to GA has a much higher intensity than the blended membranes without GA, and is closer in intensity to that observed in pure cellulose membranes. The orientation of the functional groups caused the difference in intensities since light will be absorbed/reflected differently as a result. Another interesting thing is that the peak for the cellulose/SPC no GA membrane located at 1569.4cm^{-1} is gone from the membrane that was exposed to GA. Both the cellulose membrane and the blended membrane have two small peaks there as seen in the spectrum, but after coming in contact with GA that area of the graph only has a single peak. This is a good indication that some of the $–NH_2$ primary alkyl amide groups had been reacted with the aldehyde groups.

6.3.2.2 Tensile Tests

The two dried cellulose/SPC membranes that were exposed to GA were prepared for tensile testing. Since there were only two membranes, the sample size was reduced to less than 10. This test was conducted just to see if there have been any changes in the tensile strength of the membranes after exposure to GA.

Table 6.3.2.2 List of tensile test data of various membranes

Samples	Tensile modulus (kgf/mm^2) CV(%)	Failure stress (kgf/mm^2) CV(%)	Failure strain (%) CV(%)	Thickness (mm) CV%
Douglass 7 wt% cellulose membrane	165.5 ± 16 (9.7%)	5.36 ± 0.7 (13.1%)	26.2 ± 10.1 (38.5%)	0.047 ± 0.015 (31.9%)
Zhu 3 wt% cellulose membrane	191.50 ± 44.72 (23.4%)	5.83 ± 2.34 (40.1%)	13.3 ± 13.4 (100.7%)	0.0173 ± 0.0021 (12.1%)
Zhu 5 wt% cellulose membrane	260.89 ± 27.54 (10.6%)	4.65 ± 0.63 (13.5%)	15.1 ± 4.5 (29.8%)	0.034 ± 0.004 (11.7%)
Douglass 3 wt% cellulose/ 3 wt% SPI	157 ± 52 (33.1%)	3.2 ± 1.6 (50%)	27 ± 12 (44.4%)	0.029 ± 0.003 (10.3%)
Zhu 3 wt% cellulose/ 3 wt% SPC a	255.79 ± 19.21 (7.5%)	4.40 ± 0.27 (6.1%)	9.3 ± 2.8 (30.1%)	0.0347 ± 0.0023 (6.6%)
Zhu 3 wt% cellulose/ 3 wt% SPC b	256.78 ± 20.27 (7.9%)	3.72 ± 0.52 (14.0%)	13.3 ± 10.7 (80.4)	0.0329 ± 0.0030 (9.1)
Zhu 3 wt% cellulose/ 3 wt% SPC with GA	292.55 ± 15.43 (5.3%)	4.24 ± 0.40 (9.4%)	9.8 ± 6.6 (68.3%)	0.0289 ± 0.0014 (4.8%)

By comparing the above data, the membranes that were exposed to GA did show an improvement in overall tensile modulus, with similar thickness as the ones produced by Dr. Douglass, the modulus increased almost 200%. As a result the failure strain is reduced; which was explained previously because of the increase in polymer chain orientation. There were two samples that broke at the grip and caused the large variation in the failure strain value. Although no definite conclusion was drawn at this point, this improvement in tensile strength is a good indication that potential crosslinking had occurred.

6.3.2.3 Water Absorption Test

After FTIR spectra were obtained, the remains of the two membranes were placed in DI water and soaked overnight. The dry weight was measured prior to the test and 24 hours after the membranes were removed, and the wet weight was recorded. Below is a table that has all the information recorded. Note that the data of the cellulose membranes obtained from Dr. Douglass were of porous membranes, thus the wet mass increase is very high. It was unfortunate that he did not provide any data for the non-porous membranes.

Table 6.3.2.3 Comparison of water absorption results of various membranes

Material	Dry mass (g)	Wet mass (g)	Wet mass increase (%)
Douglass wet cellulose membrane (coagulated & kept wet) [24]	0.28 (after)	4.70 (before)	94 increase, 1580 increase
Douglass porous cellulose membrane a [24]	0.75	1.34	79
Douglass porous cellulose membrane b [24]	0.49	1.03	110
Douglass cotton control membrane [24]	0.21	0.42	100
Zhu 6wt% cellulose membrane	0.3074	0.3516	14.38
Zhu cellulose/SPC membrane	0.2213	0.4903	121.55
Zhu cellulose/SPC membrane with GA a	0.1045	0.1963	87.85
Zhu cellulose/SPC membrane with GA b	0.3251	0.5785	77.94

By comparing the data listed above, it appears that the two blend membranes that were exposed to GA resulted in a lower wet pick-up value. In the study conducted by Chabba

et. al, they also observed that the moisture absorption of the crosslinked material decreased, which corresponds to what is observed here. [7, 38] Even though the amount of wet mass increase is still relatively high in comparison to the pure nonporous cellulose membrane, the wet pick-up did decrease when compared to the cellulose/SPC membrane, from 121.5% to between 87-77%. This is another good indication that a certain degree of crosslinking did occur.

6.3.2.4 Glutaraldehyde Exposure Study

After initially exposing two dried cellulose/SPC membranes to GA in DI water for a period of time, promising results were obtained. To further this study, a more systematic method was developed. First a set of test tubes were used to observe the reaction of GA in a solution of soy protein isolate, and then another set exposed a series of dried cellulose/SPC membranes to various concentration of GA for different times.

6.3.2.4a Test Tube Study – Glutaraldehyde with SPI

The test parameter for this test tube study was described in detail in Chapter 5, section 5.4.3. A series of test tubes were prepared, and with the 10 wt% SPI solution in DI water, different concentrations of GA were added and images taken after a certain amount of time had passed.

Table 6.3.2.4a Series of images of the test tube study at different GA exposure time

Image 1: No GA

Image 2: Initial contact with GA

Image 3: 15 minutes after contact with GA

Image 4: 30 minutes after contact with GA

Table 6.3.2.4a Continued

Image 5: 1 hour after contact with GA

Image 6: 2 hours after contact with GA

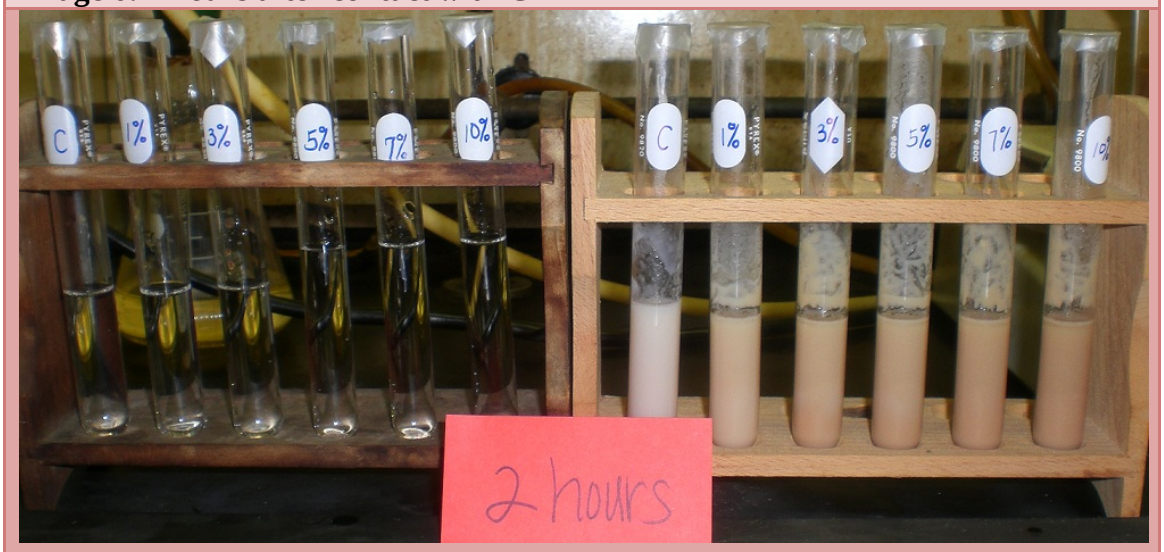


Table 6.3.2.4a Continued

Image 7: 3 hours after contact with GA

Image 8: 5 hours after contact with GA

Table 6.3.2.4a Continued

Image 9: 24 hours after contact with GA

Image 10: 5 days after contact with GA

By examining the above images, it was observed that the moment GA was injected into the test tubes that contained SPI the color of the SPI solution had already changed. A small trace of yellow can be observed in the tubes, especially obvious in the 10 wt% GA tube. After 15 minutes of exposure, the discoloration can clearly be observed. It appears that

the higher the concentration of GA present, the more severe the discoloration became. If let the material settle, the tubes with more GA present also seemed to be more densely packed, as it can be observed in several of the images. After letting the tubes set for periods of times, the change in color became more severe, while the control remained off white.

The tubes on the left containing only DI water did not have any color changes, because there is nothing for the GA to crosslink with. It was pointed out by studies conducted by Aito, Migneault, Knowles and several others that in solution, GA could potentially form up to 13 different structures, and one of the most common is that the GA would form a polymer. [44, 47-49]

Based on this study, it was concluded that GA will react rapidly with the soy protein, and the higher the concentration, the more discoloration will result. Higher concentration of GA also led the proteins to pack more densely together. This gives an idea for the crosslinking of cellulose/SPC films with GA, as it appears that the needs to expose the membranes to the crosslinker do not require long exposure time.

6.3.2.4b Membranes Study – Glutaraldehyde with Cellulose/SPC Films

Based on the observations from the test tube analyses, a set of crosslinking methods was developed. Several dried cellulose/SPC membranes were prepared for exposure to certain amounts of GA for periods of 10, 20, and 30 minutes. Amounts of GA used were calculated based on the weight of the dried membrane, and all percentages were calculated in relation to weight. The detailed experimental set up can be found in Chapter 5, section 5.4.4.

A set of films were exposed to 1 wt%, 3 wt%, 5 wt%, and 10 wt% concentration of GA for 10, 20, and 30 minutes. Weights of the membranes were measured and after the exposure time the wet membranes were removed, placed between Teflon sheets, and vacuum-dried for further testing.

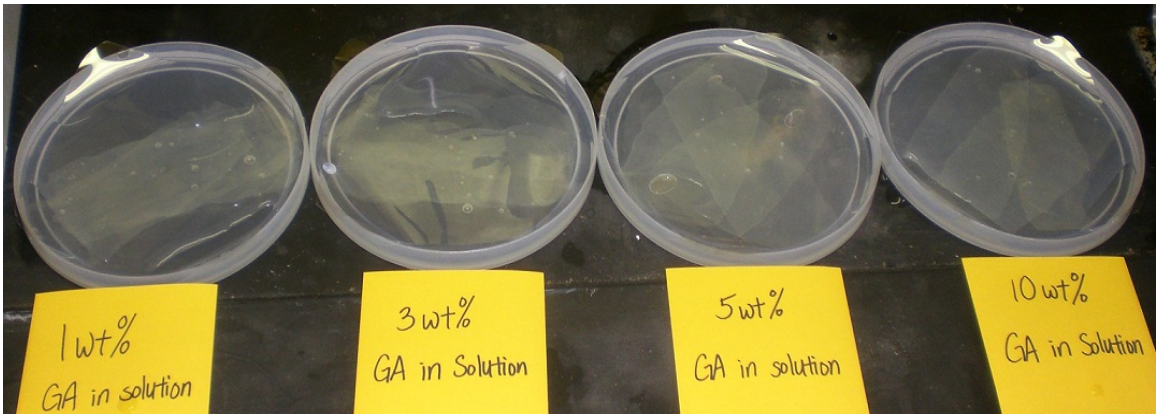


Figure 6.3.2.4b-1 Initial Exposure of films in small plates containing GA

Table 6.3.2.4b-1 Images of Membranes after exposure to GA

Image 1: Membranes exposed to GA for 10 minutes

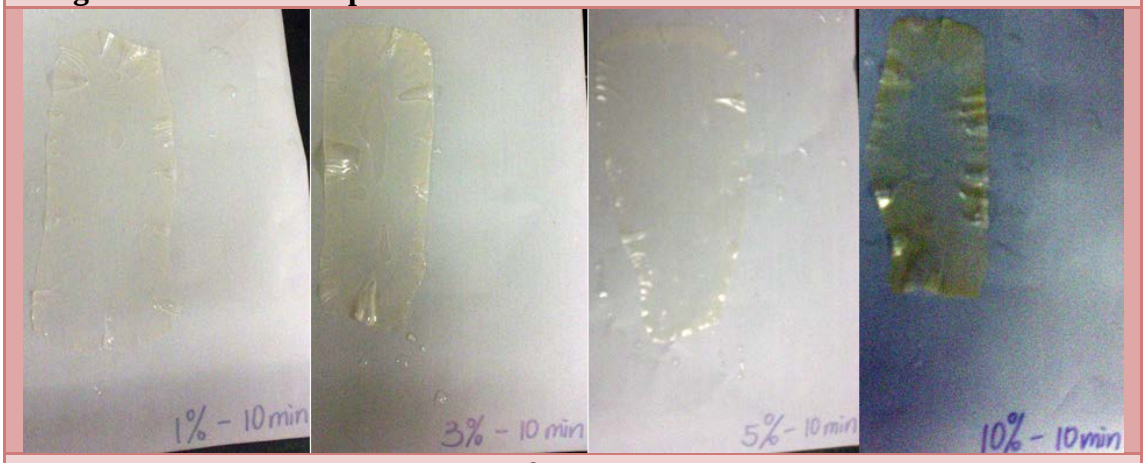
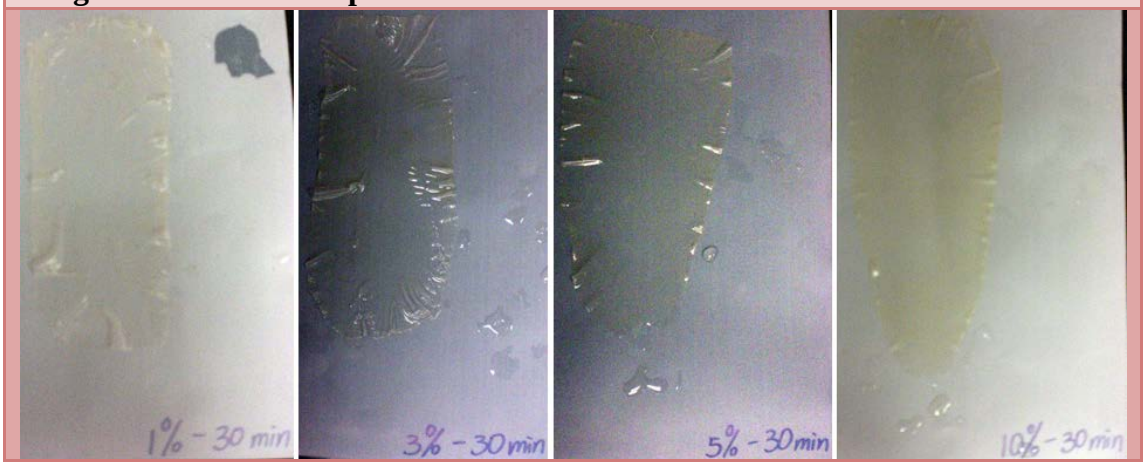


Image 2: Membranes exposed to GA for 20 minutes

Image 3: Membranes exposed to GA for 30 minutes



Although the quality of the above images are not very high, it can be seen that the membranes that were exposed to 10 wt% GA have more discoloration when compare to the membranes that were only exposed to 1 wt% GA, which corresponds to the observations made with the test tube experiment. This was more easily observed once the membranes were dried.

Table 6.3.2.4b-2 Images of dried membranes after GA exposure

Image 1: Membranes exposed to GA for 10 minutes

Image 2: Membranes exposed to GA for 20 minutes

Image 3: Membranes exposed to GA for 30 minutes


It was also observed that the membranes became more transparent in comparison to before soaking in GA solutions. After the removal of the films, the GA containing bath in the plates had some discoloration. It was not very noticeable in the 1 wt% and the 3 wt% plate, but in the 5 wt% and especially the 10 wt%, the solution is now a light shade of yellow.

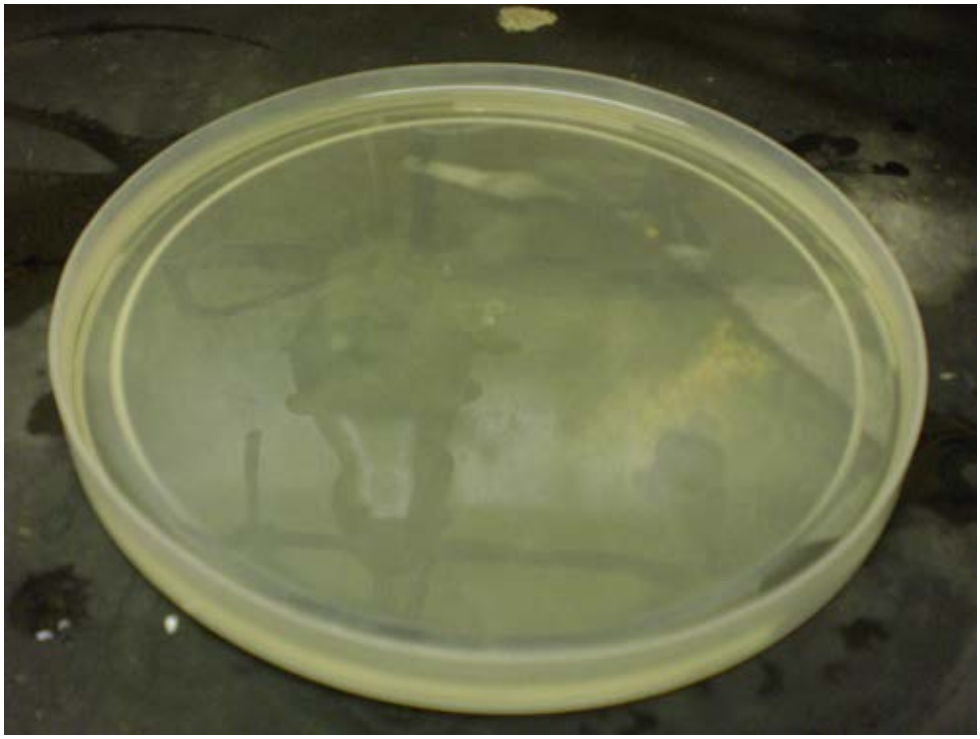


Figure 6.3.2.4b-2 Image of the 10 wt% GA containing bath turned yellow after experiment

This change in color could be related to the films appearing more transparent, suggesting that some protein escaped from the film and into the bath, and once they reacted with the GA, yellowing became the result. To verify this, after the membranes were dried, the weight of the membranes was taken again and the results are given below.

Table 6.3.2.4b-3 List of weight loss of membranes after GA exposure

Membrane groups	Initial dry weight of combined membranes (g)	Dry weight after exposure of combined membranes (g)	Weight loss (%)
1 – 1 wt% GA	0.7437	0.6265	15.76
2 – 3 wt% GA	0.7138	0.6042	15.35
3 – 5 wt% GA	0.6856	0.5785	15.62
4 – 10 wt% GA	0.7361	0.6364	13.54

By measuring the sets of membranes after drying again, it is certain that weight loss did occur, and the suspicion of protein escaping into the GA soaking bath is now verified. The percent of weight loss was very consistent, although the films soaked in 10wt% GA bath was able to reduce the amount of protein from escaping, but this reduction is not significant. Unfortunately, the films lost their uniformity during the soaking and the drying process, and because the films were too wrinkled, accurate tensile tests could not be conducted. Instead, TGA was conducted to analyze their thermal decompositions.

6.3.2.5 Thermogravimetric Analysis (TGA)

Since the films are not very uniform due to the large amount of wrinkles, it is difficult to obtain accurate tensile data from them. Thermogravimetric analysis was conducted on these films to see if the exposure to GA will alter the thermal behavior of these membranes. If crosslinking had indeed occurred, then the TGA curve should shift towards the right to higher temperatures assuming more energy is required to decompose the cross-linked membranes.

Table 6.3.2.5a Images of TGA graphs of membranes in crosslinking study

Image 1: Cellulose/SPC membrane exposed to 1 wt% GA for 10 minutes

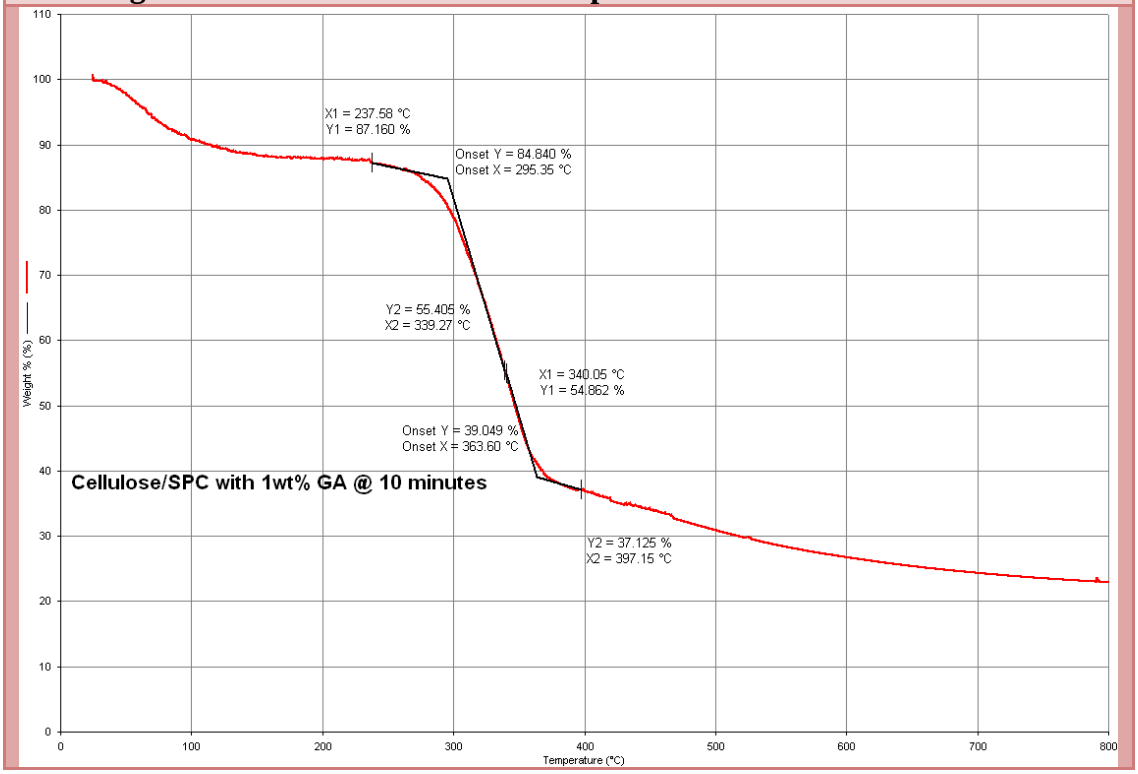


Table 6.3.2.5a Continued

Image 2: Cellulose/SPC membrane exposed to 1 wt% GA for 20 minutes

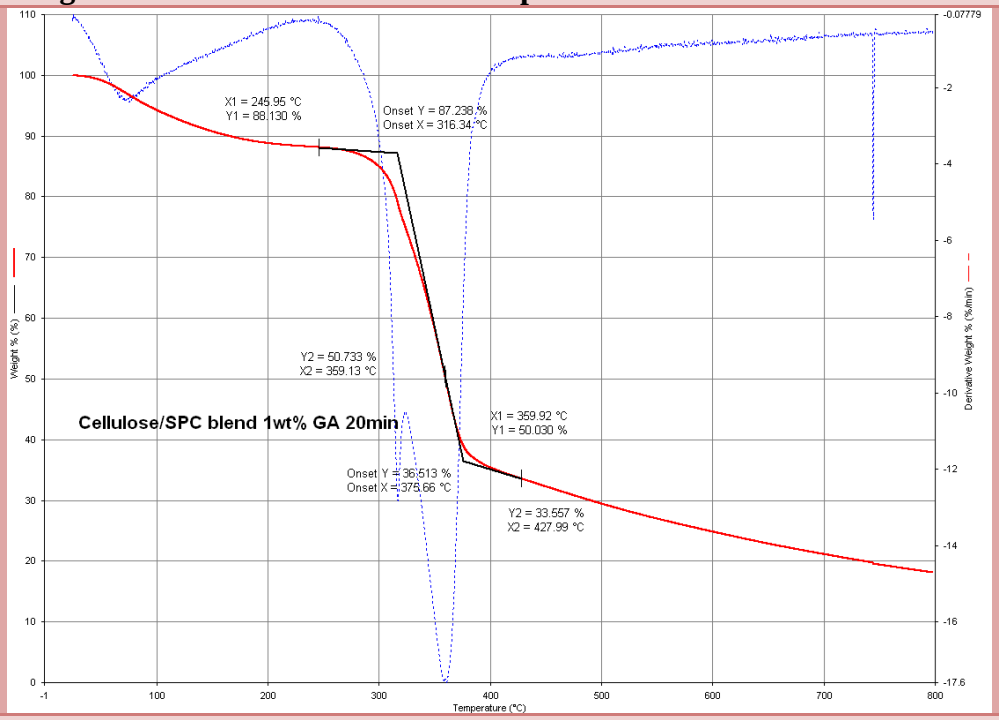


Image 3: Cellulose/SPC membrane exposed to 1 wt% GA for 30 minutes

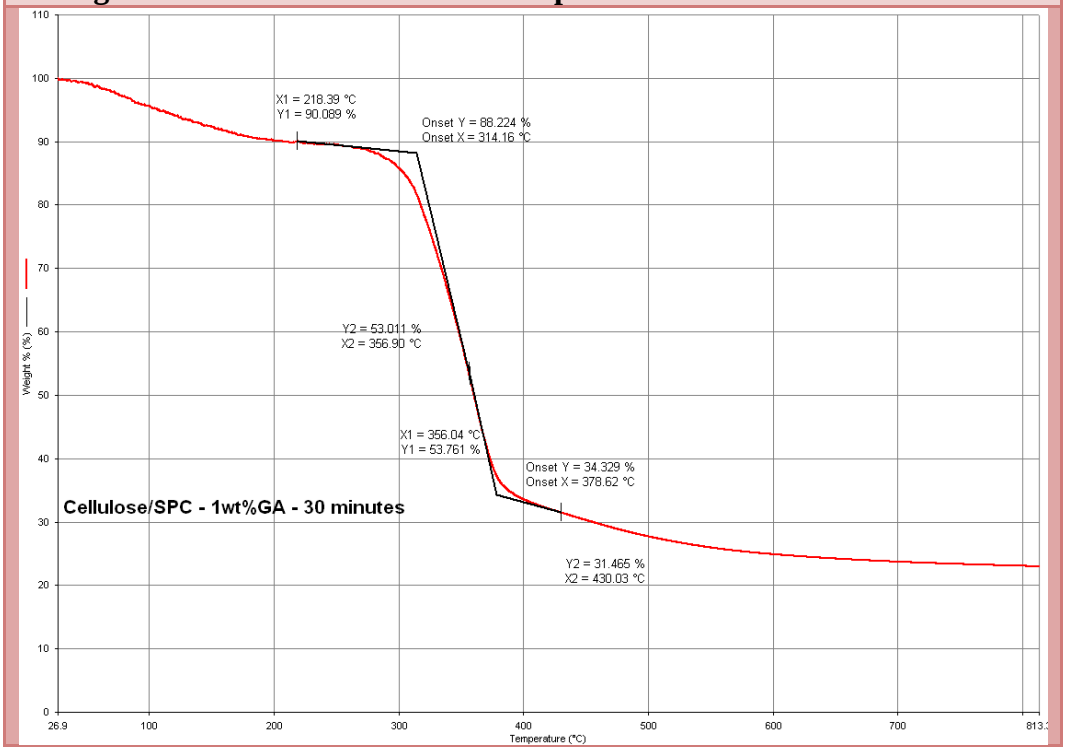


Table 6.3.2.5a Continued

Image 4: Cellulose/SPC membrane exposed to 3 wt% GA for 10 minutes

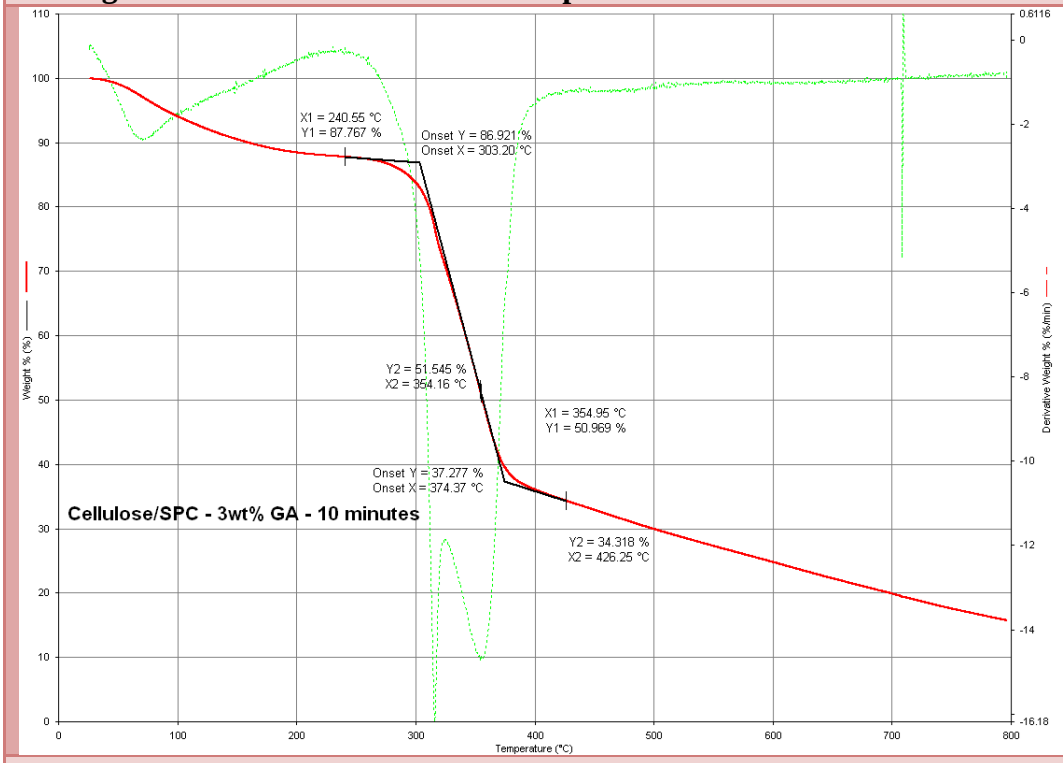


Image 5: Cellulose/SPC membrane exposed to 3 wt% GA for 20 minutes

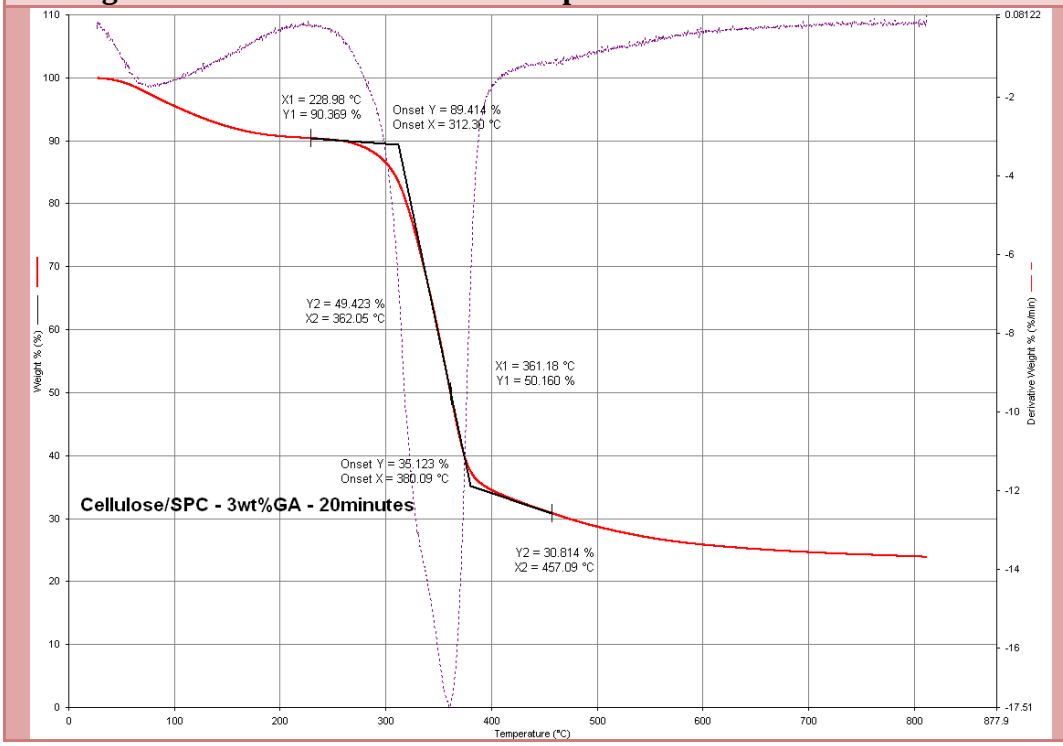


Table 6.3.2.5a Continued

Image 6: Cellulose/SPC membrane exposed to 3 wt% GA for 30 minutes

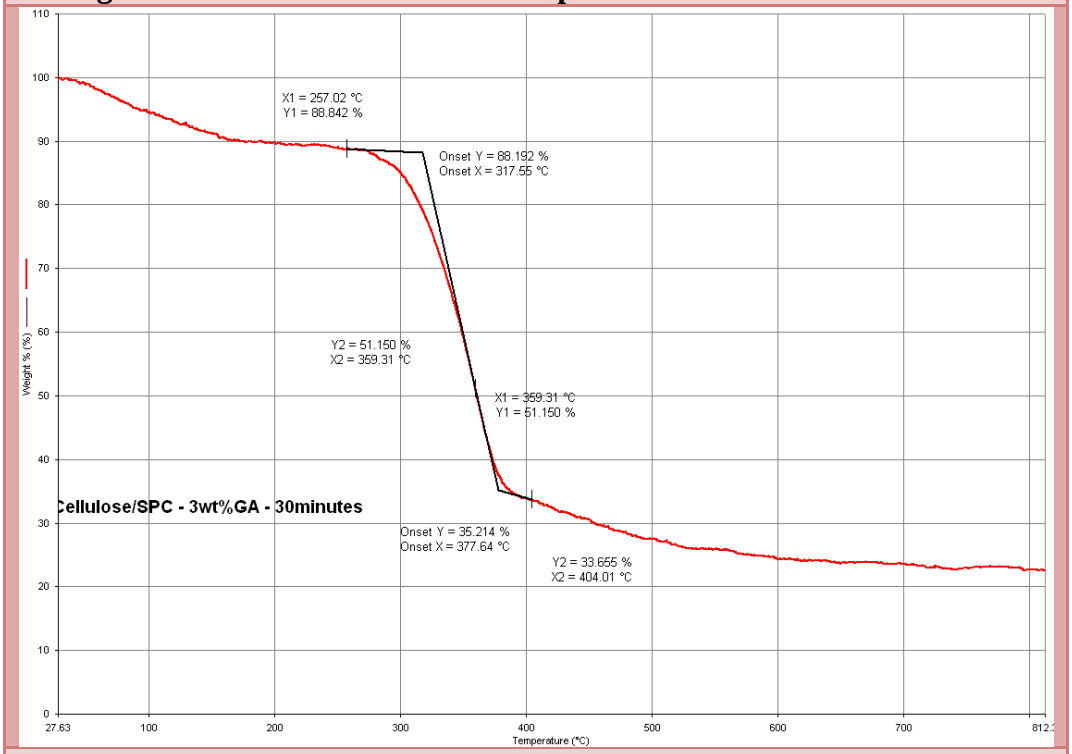


Image 7: Cellulose/SPC membrane exposed to 5 wt% GA for 10 minutes

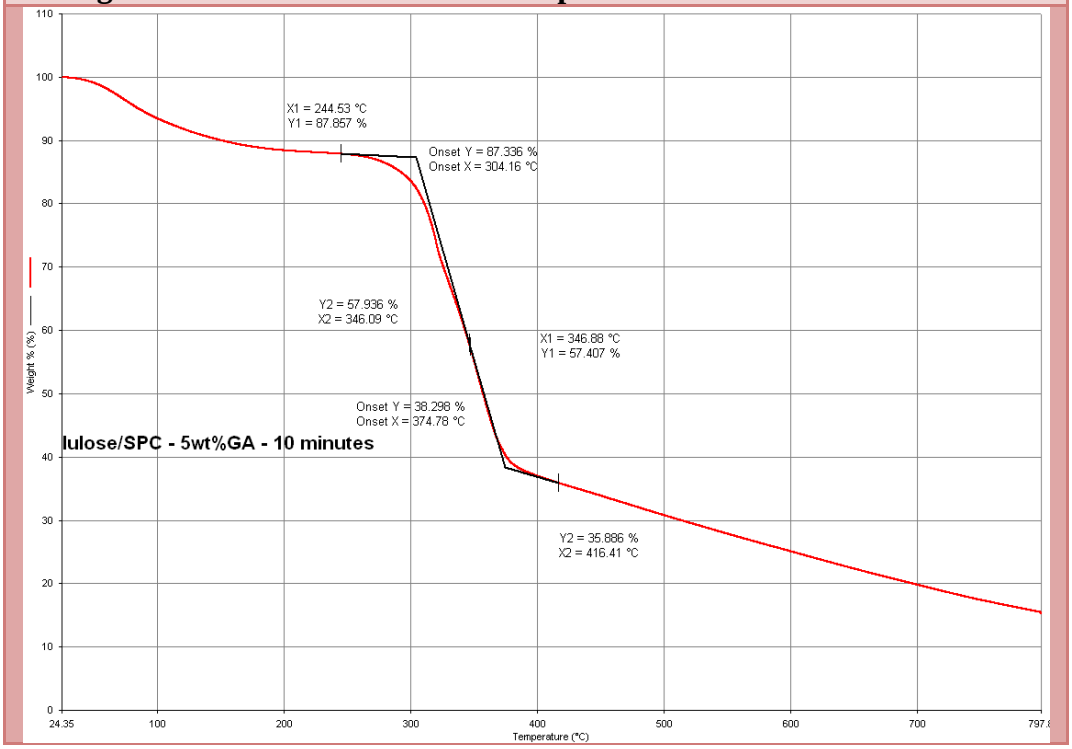


Table 6.3.2.5a Continued

Image 8: Cellulose/SPC membrane exposed to 5 wt% GA for 20 minutes

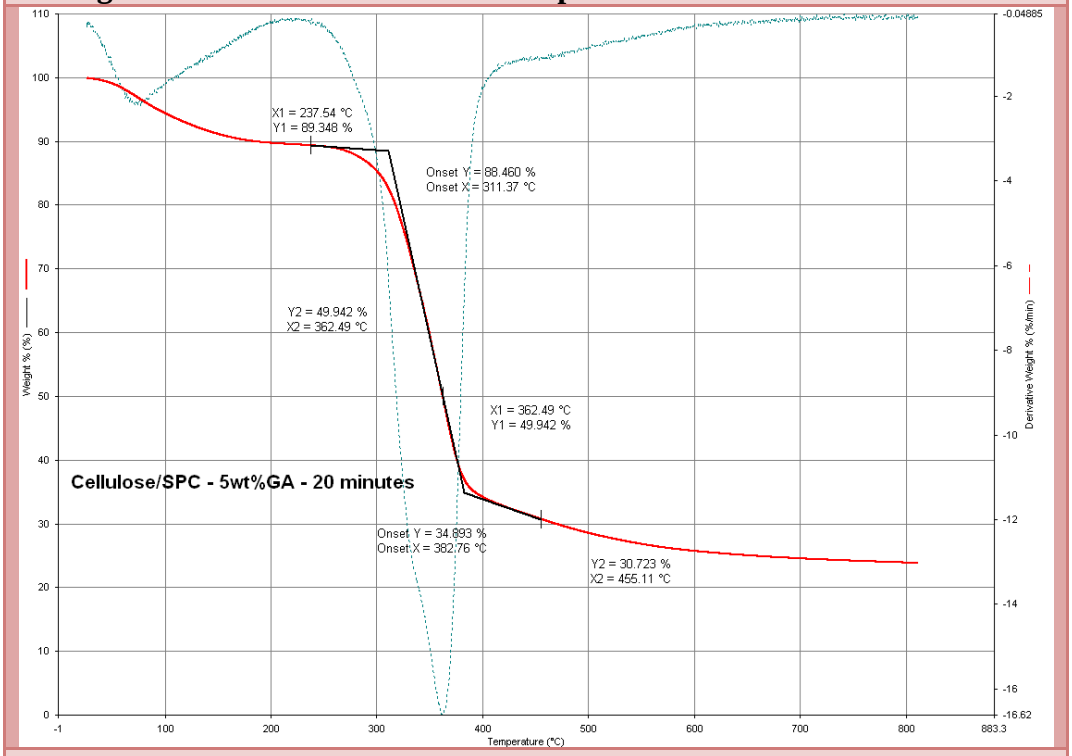


Image 9: Cellulose/SPC membrane exposed to 5 wt% GA for 30 minutes

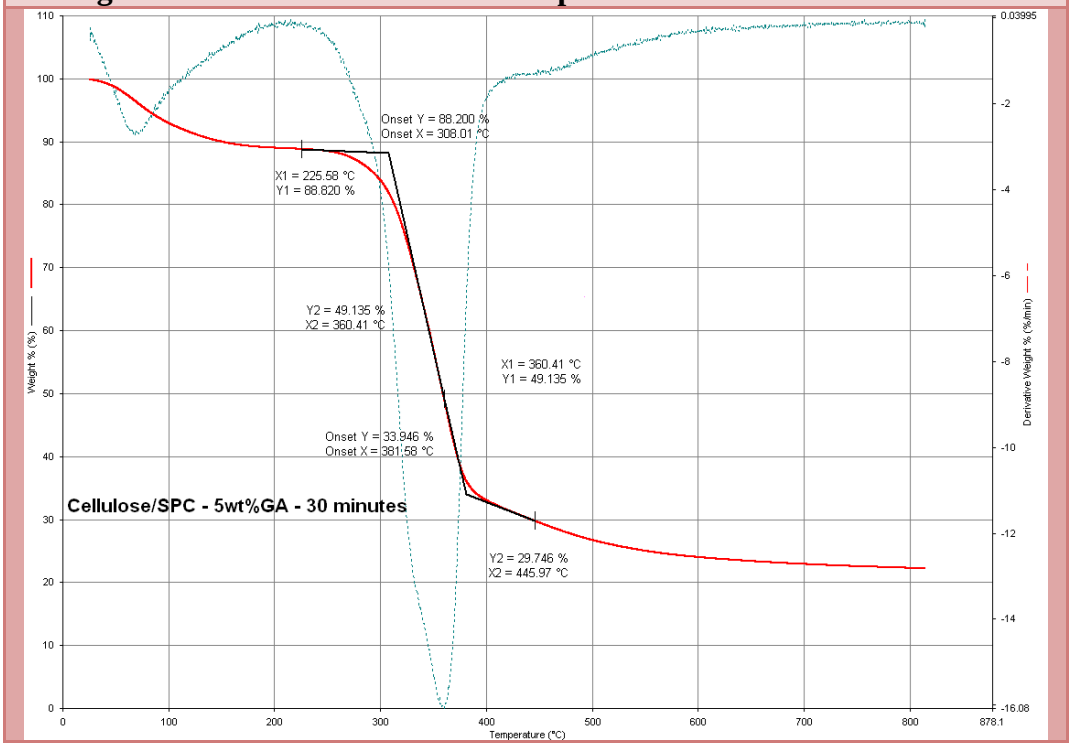


Table 6.3.2.5a Continued

Image 10: Cellulose/SPC membrane exposed to 10 wt% GA for 10 minutes

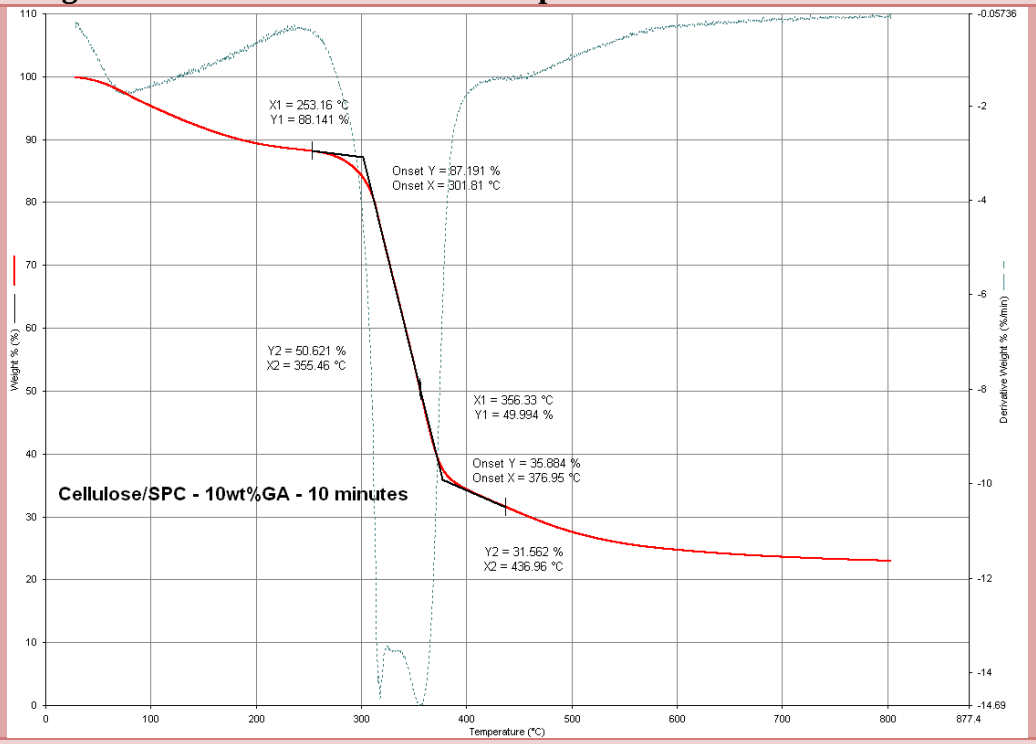


Image 11: Cellulose/SPC membrane exposed to 10 wt% GA for 20 minutes

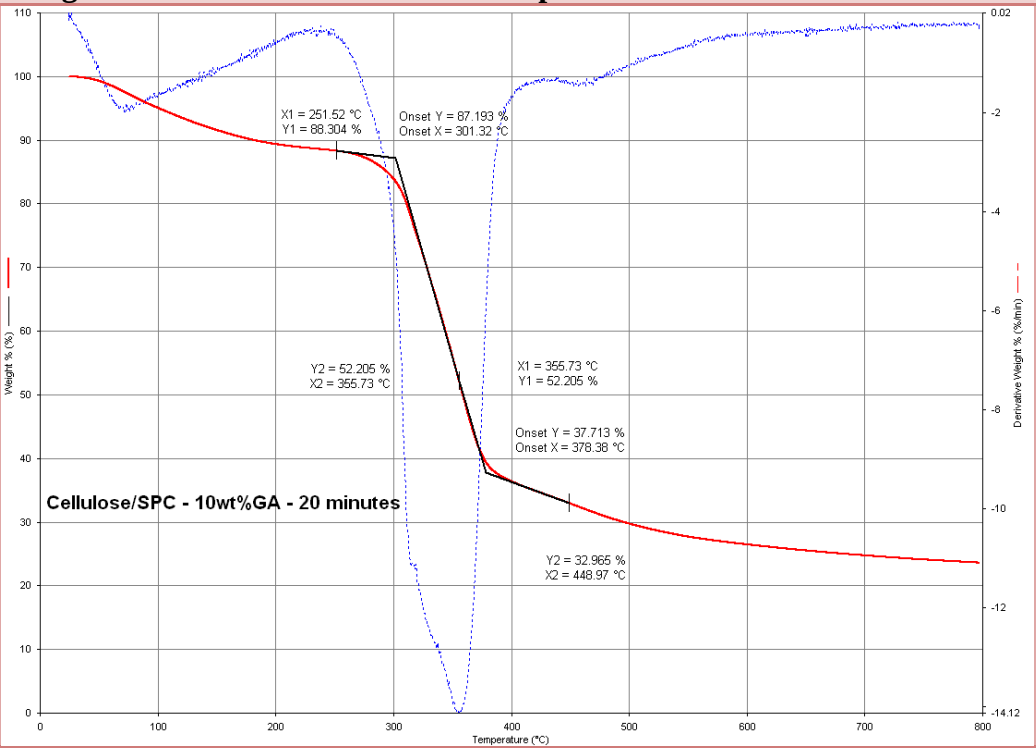


Table 6.3.2.5a Continued

Image 12: Cellulose/SPC membrane exposed to 10 wt% GA for 30 minutes

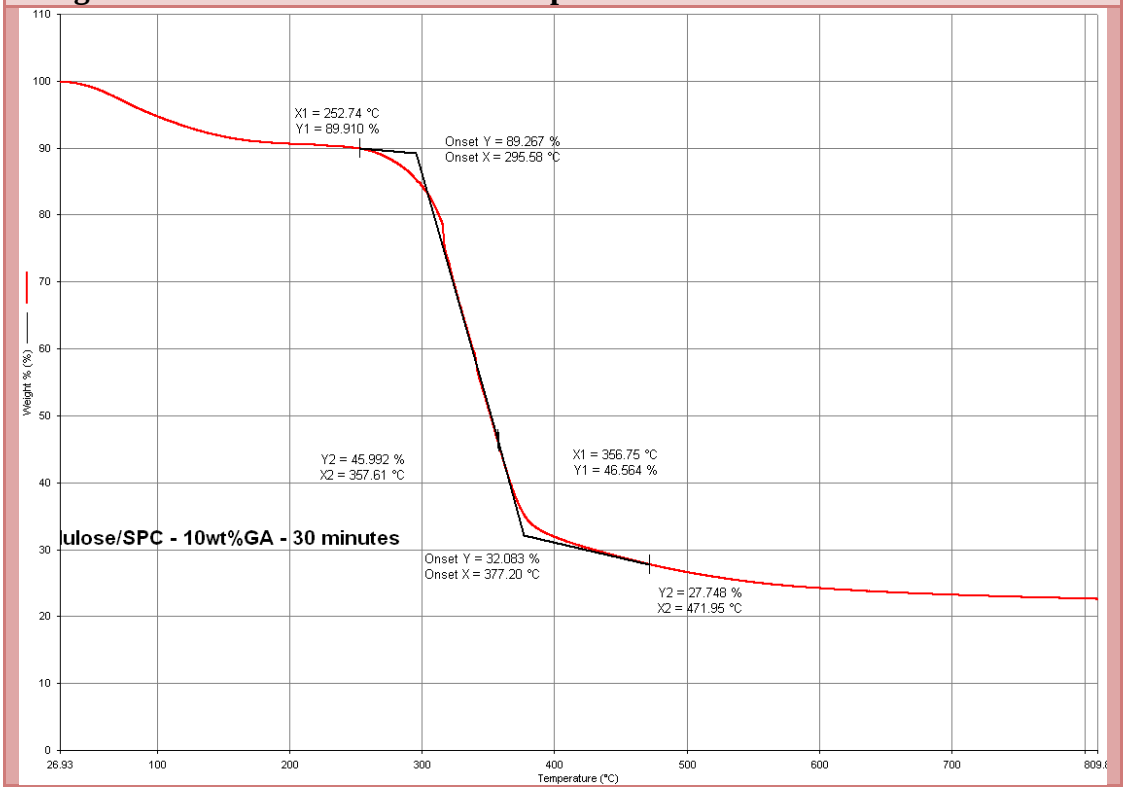


Table 6.3.2.5b List of Initial Decomposition and end decomposition temperatures

Sample	Onset temperature and remaining wt% of material)	Offset temperature and remaining wt% of material)
Zhu cellulose membrane	250.23ºC (88.023%)	372.57ºC (35.622%)
Zhu SPC powder	191.34ºC (94.116%)	436.22ºC (43.437%)
Douglass cellulose/SPI membrane	199.65ºC (88.488%)	344.32ºC (46.053%)
Zhu cellulose/SPC membrane	195.43ºC (91.197%)	380.47ºC (40.194%)
Zhu cellulose/SPC 1 wt% GA @ 10 minutes	237.58ºC (87.160%)	397.15ºC (37.125%)
Zhu cellulose/SPC 1 wt% GA @ 20 minutes	245.95ºC (88.130%)	427.99ºC (33.557%)
Zhu cellulose/SPC 1 wt% GA @ 30 minutes	218.39ºC (90.089%)	430.03ºC (31/465%)
Zhu cellulose/SPC 3 wt% GA @ 10 minutes	240.55ºC (87.767%)	426.25ºC (34.318%)
Zhu cellulose/SPC 3 wt% GA @ 20 minutes	228.98ºC (90.369%)	457.09ºC (30.814%)
Zhu cellulose/SPC 3 wt% GA @ 30 minutes	257.02ºC (88.842%)	404.01ºC (33.656%)
Zhu cellulose/SPC 5 wt% GA @ 10 minutes	244.53ºC (87.857%)	416.41ºC (35.886%)
Zhu cellulose/SPC 5 wt% GA @ 20 minutes	237.54ºC (89.348%)	455.11ºC (30.723%)
Zhu cellulose/SPC 5 wt% GA @ 30 minutes	225.58ºC (88.820%)	445.97ºC (29.746%)
Zhu cellulose/SPC 10 wt% GA @ 10 minutes	253.16ºC (88.141%)	436.96ºC (31.562%)
Zhu cellulose/SPC 10 wt% GA @ 20 minutes	251.52ºC (88.304%)	448.97ºC (32.965%)
Zhu cellulose/SPC 10 wt% GA @ 30 minutes	252.74ºC (89.910%)	471.95ºC (27.748%)

By looking at the TGA curves and the decomposition data listed in the table above, the rapid decomposition temperatures have indeed increased from the cellulose/SPC that was not exposed to GA. The overall TGA curves have shifted towards the right compared to the

non-crosslinked blended membranes, indicating that the energy required to degrade the material to pure carbon has increased, which was contributed by the polymer network that was formed after exposing the membrane to the crosslinking agent.

By first comparing the membranes to the concentration of GA exposure, the membranes that were exposed to 10 wt% GA have higher beginning and end of rapid decomposition temperatures, indicating that more energy is required to break down the polymer matrix. The membranes were then compared by their exposure time to GA, with one or two exceptions, the membranes that were exposed to the GA for 30 minutes had higher decomposition temperatures. These results combined with previous studies, seem to make it is safe to say that the membranes had indeed been crosslinked, and that the membranes exposed to 10 wt% GA required more energy to degrade. Particularly the membranes that were exposed to 10 wt% GA for 30 minutes, indicating that membrane had a higher degree of crosslinking than the rest.

6.3.3 Conclusions

Previous sections had proven that it was possible to produce nonporous membranes from cellulose and soy protein concentrate with good strength. Although Dr. Douglass claimed that the cellulose/soy protein blend membranes are stronger than pure cellulose membranes, the current production show that the strength of the two types of membranes are very similar. However, lower quality soy protein can still produce strong membranes and can reduce the cost of production further, as well as allow this biocompatible membrane to find use in where an affordable biodegradable material with a cellulose matrix is required.

One of the biggest challenges faced by these membranes is that the proteins tend to dissolve in water and that the membrane can lose a lot of its structural integrity after exposure to water. To overcome this problem, the polymer matrix must be stabilized using a crosslinking agent. After exposing the crosslinking agent GA at various stages of the membrane production, it was deemed best to expose the dried membrane to GA, removing other factors that could potentially react with GA, such as the ED in the solvent and the methanol in the coagulation bath.

Membranes that were exposed to GA post drying were characterized and it was discovered that the overall tensile modulus had improved and the wet pick-up percent had been reduced, corresponding to the literature describing the results from successful crosslinking.

A series of crosslinking studies were conducted, and it was discovered that GA causes color change when in contact with SPC, and the reaction occurs very rapidly. Another series of dried membranes were exposed to different baths containing different concentration of GA for various period of time, and during the process some protein had escaped from the membrane and then reacted with GA in the bath, causing the bath color to turn yellow. Various tests show that crosslinking had indeed occurred, and those membranes exposed to higher concentration of GA had a higher degree of yellowing. After conducting Thermogravimetric analysis on these membranes, it was observed that membranes exposed to 10 wt% GA had higher degree of crosslinking, with the highest being the one soaked in 10 wt% GA bath for 30 minutes.

CHAPTER 7. Conclusions

Cellulose is the most abundant polymer found in nature. In the past cellulose has been used in paper, packaging, food, biomedical applications and more. Soy proteins are another cheap and abundant natural biopolymer extracted from soy beans, and can be used in food, packaging, and also in medical applications because of its biocompatibility.

Many scholars found that cellulose can form useful porous and nonporous membranes, one of the most common examples being cellophane. Traditional regenerated cellulose is made from the viscose rayon or NMMO process, but these processes generate wastes and can be quite harmful to the environment. Several years ago, a new solvent system was developed by Cuculo and Kotek using a blend of ED and KSCN.

This new solvent system is a much simpler, and cellulose dissolution is carried out in one step and membranes could be obtained by simple coagulation. It was investigated by Dr. Douglass that methanol was a suitable coagulant, and strong, uniform cellulose membranes were developed. Douglass also investigated and discovered that the solvent can dissolve a variety of polysaccharides and proteins. By combining cellulose and soy protein, it was discovered that it could produce membranes with improved strength.

Under the support of the NC Soybean Producer Association, more research was conducted to observe their membrane forming abilities and to see if this could create more applications for soy proteins, especially if the possibility of using lower quality soy protein (lower protein purity) to create good quality membranes.

Dissolutions method were replicated, but it was discovered accidentally that the dissolution process is not required to be conducted under nitrogen atmosphere, which could make this process easier for industry adaptation and reduce the cost. Using a 20 or a 25 mil casting bar, uniform, nonporous membranes were produced. During membrane coagulation, the membranes tend to curl up around the edges, and the same behavior was observed during the following washing and soaking process, so a weight was used to apply constant pressure throughout the membrane-forming process.

Once the membranes were removed from soaking bath, they were best kept between sheets of PET films, and cellulose/soy protein membranes were best kept between Teflon sheets because they stick to PET films. During the drying process, a sheet of glass and a brick was placed on top to further apply even pressure to ensure the production of uniform membranes. The drying process was modified from the original method, which did not always result in dry membranes. The membranes are now kept in a vacuum oven to dry for 24 hours at room temperature, 24 hours at 40ºC, and an additional 24 to 48 hours drying at room temperature.

By adapting this modified method, the membranes produced showed improvements in uniformity and in mechanical properties. SEM was used to verify that the membranes were indeed nonporous. The nonporous cellulose membrane had the best mechanical properties when it was made with 5 wt% cellulose, and the overall tensile modulus improved by over 50%. The cellulose/SPC blend membranes produced also showed improvement from the cellulose/SPI membranes made by Douglass, showing 35-35% tensile modulus improvement. However, instead of the previous finding that cellulose/soy protein can result in membranes

with better physical properties was proven false, since the newly produced membranes show physical properties that were relatively the same. It is worth noting however, that the soy protein used here was SPC instead of SPI, which have a lower concentration of protein, and this could be the cause for no significant improvement over cellulose membranes. Even though there was no significant improvement, it did show that using a lower quality soy protein can still produce strong membranes.

Using the water absorption test, it was observed that pure cellulose membranes did not pick up as much water as the cellulose/soy protein blend membranes. The percent of wet pick up of the blend membrane was over 8 times the amount the cellulose membrane picked up, however the soy protein is not stable in water, and to solve/reduce this problem, glutaraldehyde was picked as the crosslinking agent to stabilize the membrane.

A series of studies were conducted to observe the reaction between glutaraldehyde and soy protein. It was noted that the glutaraldehyde reacts almost instantaneous with the soy protein, and the result from the reaction causes the protein to change color from off-white to a shade of yellow. Due to the presence of other molecules that could potentially react with glutaraldehyde, the best time to expose the glutaraldehyde to the membranes was determined to be post vacuum drying of the membranes.

Different concentrations of glutaraldehyde were exposed to the cellulose/SPC membranes for different lengths of time. By analyzing the samples using TGA, it was shown that the membranes exposed to 10 wt% cellulose had the biggest increase in degradation temperature, with the ones exposed for 30 minutes having the highest numbers (highest degree of crosslinking). Two test samples were used prior to the systematic study; the

samples showed a slight improvement in physical properties and the amount of wet absorption was also reduced from the membranes that were not exposed to GA. Even though the crosslinking agent had reacted with the membrane and proof of crosslinking was observed, some protein still left the membrane and escaped into the diluted GA solution. This was proved from observations made by the color change in the GA solution, as well as the measured weight loss after the films were dried once again.

In the end, the membrane production methods were improved, resulting in cellulose/soy protein films that have better uniformity and good physical properties. Strong, uniform nonporous membranes were obtained even when using lower grade soy protein. Glutaraldehyde was used to stabilize the structural integrity of the blended membranes, and as a result the membranes observed improvement in thermal degradation, strength, reduced water weight pick-up, and chromophore formation from the reaction of aldehyde and amines.

These membranes can be used for wound-dressing, filtration, or food-packaging applications. Further characterization and crosslinking studies are still needed to improve the stability of these materials and their reproducibility. This simple and novel system could also be modified to make it appropriate for mass production and even fiber production. There are lots of potential for these biomaterials in the future, thus more research and studies will improve the versatility and applicability of the cellulose/soy protein membranes.

References

1. Bauer-Wu, Susan, and Georgia M Decker. “Introduction.” *Seminars in oncology nursing* 28.1 (2012): 1.

2. T. P. Nevell, S. H. Zeronian, Cellulose Chemistry and Its Applications, 1985.

3. Klemm, Dieter et al. “Cellulose: fascinating biopolymer and sustainable raw material.” *Angewandte Chemie (International ed. in English)* 44.22 (2005): 3358-93.

4. Zugenmaier, Peter. “Cellulose Derivatives 6.1.” *Cellulose* (2008): 175-206. Print.

5. Manian, Avinash P., Hartmut Ruef, and Thomas Bechtold. “Spun-dyed Lyocell.” *Dyes and Pigments* 74.3 (2007): 519-524. Web. 29 Jan. 2012.

6. Lee, Hyun Jik. *Novel Cellulose Solvent System and Dry Jet Wet Spinning of Cellulose/ED/KSCN Solutions*. Master of Science Dissertation. North Carolina State University. 2007.

7. Chabba, Shitij, and Anil N. Netravali. “‘Green’ composites Part 1: Characterization of flax fabric and glutaraldehyde modified soy protein concentrate composites.” *Journal of Materials Science* 40.23 (2005): 6263-6273. Web. 3 July 2011.

8. Wang, Ying et al. “Soy Protein Adhesion Enhanced by Glutaraldehyde Crosslink.” *Polymer* (2006): 130-136.

9. Davis, Mark et al. "Changes in Cellulose Morphology of Pretreated Yellow Poplar During Enzymatic Hydrolysis." The 24th Symposium on Biotechnology for Fuels and Chemicals in Gatlinburg, TN. National Bioenergy Center, National Renewable Energy Laboratory.(2002) Retrieved from: http://www1.eere.energy.gov/biomass/pdfs/po32125.pdf

10. Kamide, Kenji. “Solubilization and Structural Factors Governing Solubility and the Dissolved State Solubilization of Cellulose by the Steam."*Cellulose and Cellulose Derivatives Molecular Characterization and its Applications.* Elsevier (2005). 549-616.

11. Sperling, L.H. "Chapter 6.3.4. Polymeric Forms of Cellulose." *Introduction to Physical Polymer Science, 4th Edition.*Wiley Publication (2006). 253.

12. Sangwatanaroj, Usa. *The Mechanism of Dissolution of Cellulose in the Ammonia/Ammonium Thiocyanate Solvent.* Master of Science Dissertation. North

Carolina State University. 1995.

13. "Rayon Fiber (Viscose)." American Fiber Manufacturers Association/Fiber Economics Bureau. (2012) Retrieved from: http://www.afma.org/f-tutor/rayon.htm

14. Mitchell, R. L. “Viscose Processing of Cellulose.” *Industrial and Engineering Chemistry* 41.10 (1949): 2197-2201. Print.

15. Rayon_synth.png. Image obtained from: http://upload.wikimedia.org/wikipedia/commons/7/78/Rayon_synth.png (2012).

16. "Lyocell Fiber." American Fiber Manufacturers Association/Fiber Economics Bureau. (2012). Retrieved from: http://www.fibersource.com/f-tutor/Lyocell.htm

17. Smith, Joyce Ann. "Lyocell - One Fiber, Many Faces." *Ohio State University Extension Fact Sheet, Consumer and Textile Science.* (2012) Retrieved from: http://ohioline.osu.edu/hyg-fact/5000/5572.html

18. Rosenau, Thomas et al. "The Lyocell Process: Cellulose Solution in N-Methylmorpholine-N-oxide (NMMO) Degradation Processes and Stabilizers." 12th International Symposium on Wood and Pulping Chemistry. (2003). June 9-12, Madison, Wisconsin, p. 305-308.

19. "Lyocell." *CIRFS - European Man-made Fibres Association.* Brussels, Belgium.(2010). Retrieved from: http://www.cirfs.org/Portals/0/2009%20Lyocell%20pag55.JPG

20. " *N*-Methylmorpholine *N*-oxide." *Wikipedia - The Free encyclopedia.* (2012). Image retrieved from: http://en.wikipedia.org/wiki/N-Methylmorpholine_N-oxide

21. Hattori, Kazuyuki et al. "New Solvents for Cellulose. II. Ethylenediamine/Thiocyanate Salt System." *Polymer Journal*. 36.2. (2004): 123-130.

22. Xiao, M., & Frey, M. W. The role of salt on cellulose dissolution in ethylene diamine/salt solvent systems. *Cellulose*, *14*.3. (2007): 225-234.

23. Hattori, Kazuyuki et al. “New Solvents for Cellulose. Hydrazine/Thiocyanate Salt System.” *Journal of Polymer Science.* Part A, Polymer Chemistry. 40.4. (2002): 601-611.

24. Douglass, Eugene Farley. *The Development of Cellulose Blend Membranes using Cellulose and other Natural Biopolymers using a Novel Solvent System.* Doctor of Philosophy Dissertation. North Carolina State University. 2010.

25. Zydney Al, Zeman LJ. *Microfiltration and Ultrafiltration: Principles and Applications.* New York: CRC; 1996.

26. Woodings, C. *Regenerated Cellulose Fibres.* Cambridge, UK: Woodhead Publishing; 2001.

27. Peles, Zachi, and Meital Zilberman. “Novel soy protein wound dressings with controlled antibiotic release: mechanical and physical properties.” *Acta biomaterialia* 8.1 (2012): 209-17.

28. Jarvis, Mike. "Chemistry: Cellulose Stacks Up." *Nature*. (2003). 426. 611-612.

29. "Cellophane." *Encyclopædia Britannica.* (2012). Retrieved from: http://www.britannica.com/EBchecked/topic/101586/cellophane

30. Cao, Yu, and Huimin Tan. “Preparation and properties of microporous cellulose membranes from novel cellulose/aqueous sodium hydroxide solutions.” *Journal of Applied Polymer Science* 102.1 (2006): 920-926.

31. Jie, Xingming et al. “Influence of drying method on morphology and properties of asymmetric cellulose hollow fiber membrane.” *Journal of Membrane Science* 246.2 (2005): 157-165.

32. Khare, Vivek P et al. “Synthesis and Characterization of Dense and Porous Cellulose Films.” *Polymer* 105.3. (2007) 1228-1236.

33. Xiong, Xiaopeng et al. “A pH-sensitive regenerated cellulose membrane.” *Journal of Membrane Science* 363.1-2 (2010): 96-102.

34. C.X. Liu, R.B. Bai, "Preparation of Chitosan/Cellulose Acetate Blend Hollow Fibers for Adsorptive Performances." *Journal of Membrane Science* 267 (2005): 68-77.

35. Guilbert, S. "Technology and Application of Edible Protective Films In M. Mathlouthi (Ed.)" *Food Packaging and Preservation.* New York: Elsevier Applied Science Publishers Ltd. (1986). 371-394.

36. Miller, K. S., J. M. Korchta. "Oxygen and Aroma Barrier Properties of Edible Films." *Trends in Food Science & Technology.* 8 (1997): 228-237.

37. Brandenburg, A. H. et. al. "Edible Films and Coatings for Soy Protein." *Journal of Food Science.* 58. (1993): 1086-1089.

38. Chabba, S., G. F. Matthews, and a. N. Netravali. "'Green' composites using cross-linked soy flour and flax yarns." *Green Chemistry* 7.8 (2005): 576.

39. Mounts, T.L., W.J. Wolf, and W.H. Martinez. "Processing and Utilization. In J.R. Wilcox (Ed.)" *Soy beans: Improvement, Production, and Uses.* Madison, WI: American Society of Agronomy. (1987). 819-860.

40. Gennadios, A. et. al. "Effect of pH on Properties of Wheat Gluten and Soy Protein Isolate Films." *Journal of Agricultural and Food Chemistry.* 41. (1993): 1835-1839.

41. Bartolo, L. De. et. al. "Evaluation of Cell Behavior Related to Phyco-chemical Properties of Polymeric Membranes to be Used in Bioartificial Organs." *Biomaterials.* 23. (2002): 2485

42. Chen, Y. "Physical properties of microporous membranes prepared by hydrolyzing cellulose/soy protein blends." *Journal of Membrane Science* 241.2 (2004): 393-402.

43. Reddy, Narendra et al. "Effect of Glutaraldehyde Crosslinking Conditions on the Strength and Water Stability of Wheat Gluten Fibers." *Macromolecular Materials and Engineering* 293.7 (2008): 614-620.

44. Migneault, Isabelle et al. "Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking." *BioTechniques* 37.5 (2004): 790-6, 798-802.

45. Glutaraldehyde_structue.png, Glutaraldehyde-3D-balls.png. Images obtained from: http://en.wikipedia.org/wiki/Glutaraldehyde (2012).

46. "Chronic toxicity summary." Determination of Noncancer Chronic Reference Exposure Levels Batch 2A. *Pathology.* December (2000): 131-136.

47. Aso, C. and Y. Aito. "Intramolecular-intermolecular polymerization of glutaraldehyde." *Bulletin of Chemical Society of Japan.* 35. (1962): 1426.

48. Aso, C. and Y. Aito. "Polymerization of bifunctional monomers. II. Polymerization of glutaraldehyde." *Macromolecule. Chemistry.* 58. (1962): 195-203.

49. Richards, F.M. and J.R. Knowles. "Glutaraldehyde as a protein cross-linkage reagent." *Journal of Molecular Biology.* 37. (1968). 231-233.

50. Kawahara, J. et. al. "The structure of glutaraldehyde in aqueous solution determined by ultraviolet absorption and light scattering." *Analytical Biochemistry.* 201. (1992): 94-98.

51. Wong, S.S. *Chemistry of Protein Conjugation and Cross-Linking.* CRC Press: Boca Raton, FL, 1991.

52. Bowes, J. H. and C.W. Cater. "The Interactions of aldehydes with collagen." *Biochimica et Biophysica Acta.* 168. (1968): 341-352.

53. Blass, J et. al. "Monomeric glutaraldehyde as an effective crosslinking reagent for proteins." *Journal of American Leather Chemistry Association.* 71.3. (1976): 121-132.

54. Hiramoto, R. and A.F.S.A. Habeeb. "Reaction of proteins with glutaraldehyde." *Archives of Biochemistry and Biophysics.* 126. (1968): 16-26.

55. Park, S K, D H Bae, and K C Rhee. "Soy Protein Biopolymers Cross-Linked with Glutaraldehyde." *Journal of the American Oil Chemists' Society.* 77.8. (2000): 879-884.

56. Luo, Li-Hua et al. "Physical properties and biocompatibility of cellulose/soy protein isolate membranes coagulated from acetic aqueous solution." *Journal of biomaterials science. Polymer edition* 19.4 (2008): 479-496.

57. Mansfield, Richard G. "Polypropylene in the Textile Industry", *Plastics Engineering*, June 1999, 30.

APPENDIX

Side Studies with Soy Proteins

To study some other potential for soy protein and the lower quality soy hull pulps, some side studies were conducted for comparison and observational purposes. Below are two of the side studies conducted that involved soy-related products.

Soy protein isolate-containing polypropylene fibers

With the world becoming more and more environmentally conscious, more and more biopolymers or blends of polymer with biopolymers are becoming popular. Having one of the biggest market shares of all polymers, polypropylene (PP) has been used in many applications from the woven to the nonwoven, and many other industries.

The question of whether soy protein can be blended in with polypropylene during extrusion was brought up, because if successful, the polypropylene fibers containing soy protein can be a big selling point to promote polypropylene as a "greener" fiber, and also boost the demand for soy proteins. However, like cellulose, soy proteins are prone to char at elevated temperature. Typically the industry processing temperature for polypropylene is around 250-270ºC, well above the melting temperature of PP (between 130-170ºC) [57]. This high processing temperature can be problematic for soy protein, since charring can occur at a much lower temperature in comparison.

Due to industry interest, a very small study was conducted just to see whether it is possible to incorporate soy protein into PP fibers without too much discoloration, and at what processing temperature could this be achieved.

High viscosity PP chips were provided by Exxon Mobil, and the Arcon F SPC was used for mixing. The target was to mix 15 wt% of protein into the PP chips during extruding, so a small amount of soy protein and PP chips were measured and mixed together. The instrument used was the ATLAS Laboratory Mixing Extruder, which is a miniature extruder with a minimum of operating parameters (rotor temperature and heater chamber temperature and the out-put rate of the extruder). Next to the machine is a little cylinder that is used to wind the extruded fiber, and the speed can be adjusted accordingly.

The system was purged with some PP chips and once the system was cleared, the measured amount of soy protein and PP chips were placed into the metallic funnel. Initially the extruding temperatures were set to 250ºC, and the resulting fiber turned from clear and transparent to dark brown, the dark protein particles can be observed visually below on the clear fiber, which is a good indicating that the proteins had charred at that temperature.

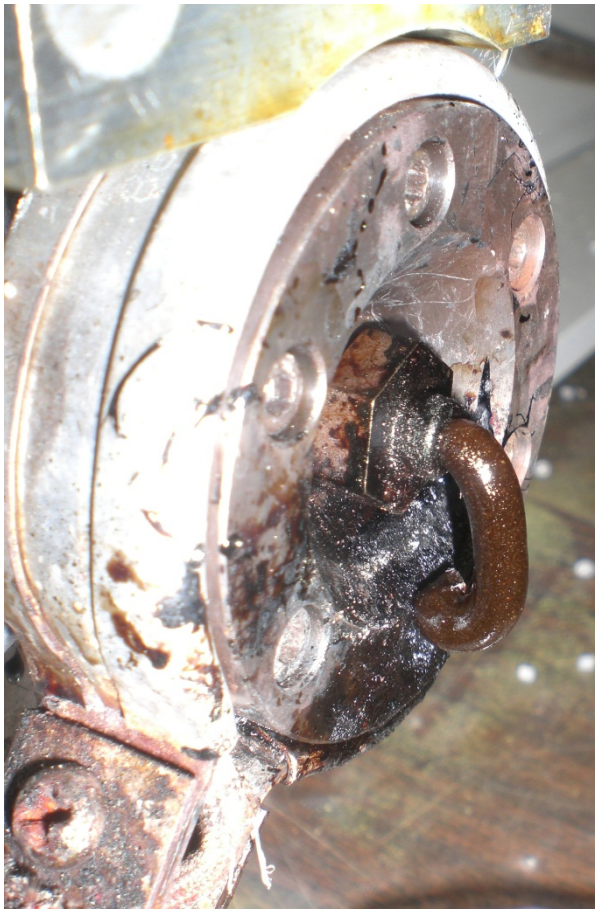
a)  b)

Figure Appendix-1. Images of the a) PP+SPC melt mixture and b) the dark, grainy fiber obtained after drawing

To reduce the chance for the protein to char, the rotor and the heater temperature was reduced. As the temperature was reduced, the molten PP chips became less dark, but they were all still different shades of brown even when the temperature was reduced to ~200ºC. Extruder and heater temperatures were further reduced until 180ºC and as seen below the color of the melted PP and SPC turned yellow. The rotor speed had to be turned up

drastically because there was no longer sufficient amount of flow for the fiber drawing process to happen. Pressure had to be applied directly into the chip funnel to induce more flow, and a small amount of fibers were obtained.

Figure Appendix-2. The color of the melted PP chips and SPC powder at 180ºC

Further reduction of the extruding temperature was attempted, but anything below 180ºC only resulted in clumps of melted polymer and there was not enough flow in the melted polymer chips for any fibers to be drawn.

After the fibers were drawn, a small sample was placed under SEM for observation, since the protein powder was just mixed with the PP chips and not melted and truly blended

into the PP chips, as well as the observation that burnt soy proteins were seen scattered unevenly throughout the PP fibers, it was suspected that individual grains of soy protein should be able to be observed from the SEM, as seen below.

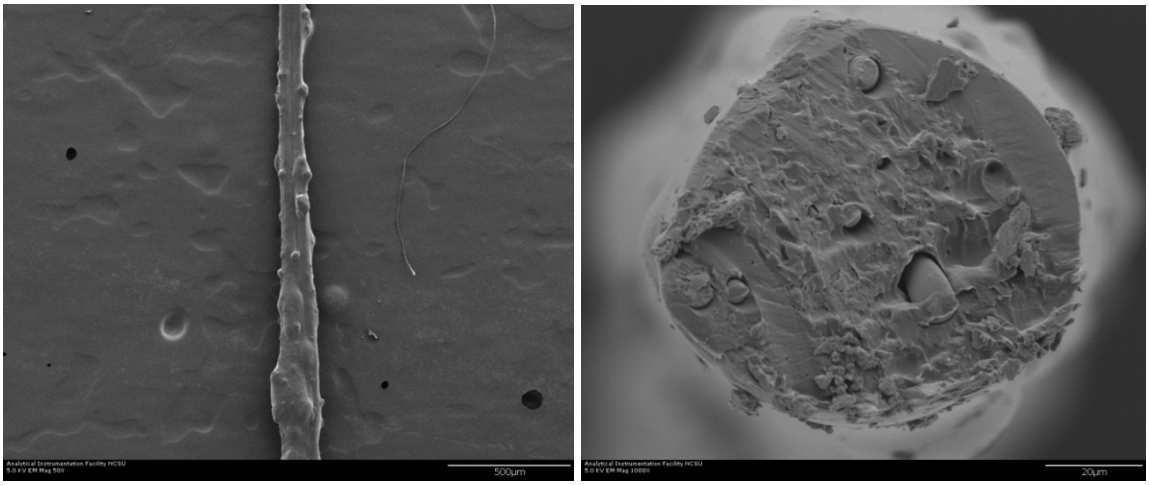


Figure Appendix-3. (left) Longitudinal view of the SPC containing PP at 50x magnification, and (right) cross-sectional view of the fiber at 1000x magnification

The images obtained from SEM show that the SPC grains were distributed unevenly throughout the fiber, also observed from the cross-sectional view, and there seems to be some microfibers forming within the fiber. The bumps created by the soy protein can be seen in the background. This brief study was conducted to observe if soy protein could be mixed with PP to produce a fiber that is within acceptable color range, and this experiment proved that such is not possible, because the temperature required to produce said fibers is not applicable to industry.

Membrane Formation Study of Cellulose & GP50, Soy Hull Pulps

Earlier experiments explored the potential of producing cellulose/soy protein membranes using lower quality soy protein (cheaper cost), and it was found that cellulose/SPC membranes can be produced with very good strength. Some lower quality soy protein samples as well as a sample of "cellulose" that is removed during the extraction/purification of the soy proteins were provided, and their membrane forming abilities were investigated.

Courtesy of the NC Soybean Producers Association, two samples of soy hull pulps (>15% protein) and a sample called "GP50" were provided. The sample GP50 is the "cellulose" that is extracted during the soy protein refinement process. Courtesy of Solae LLC, they analyzed the sample using Englyst's NSP method, and were able to identify the components of the material **seen in Appendix-1.**

Table Appendix-1 Components of the GP50 "cellulose"

Dietary Fiber and sugar components of soluble and Insoluble Non-Starch Polysaccharide (NSP)

Dietary Fiber Component	Non-Starch Polysaccharide, % dB		
	Soluble	Insoluble	Total
Cellulose		11.6	11.6
Non-cellulosic Polysaccharide:			
Rhamose	0.7	1.1	1.8
Fucose	0.5	1.3	1.8
Arabinose	3.7	9.2	12.9
Xylose	1.2	3.5	4.7
Mannose	0.3	0.9	1.2
Galactose	7.5	19.5	27
Glucose	0.4	0.4	0.8
Uronic Acid	6.8	9	15.8
Total	21.1	56.5	77.6

As seen here, the glucose content only took up 0.8% of the total non-cellulosic polysaccharide. The rest of the GP50 is made of 9.1% protein and the rest moisture. Even though this sample claims to be “cellulose”, it does not contain much cellulose at all.

All the samples were vacuum dried at 60ºC for at least 24 hours. The two soy hull samples closely resembled wet mud, so they were dried for three more days until majority of the moisture was removed from them. Since the quality of these samples are low and are mostly consisted of oligomers instead of polymers, they amount of cellulose used was increased and all the samples were prepared as: 4.5g cellulose with 1.5g of sample. One of the challenges was that the dried soy hull pulps were in large chips **(See below)**, and even using a pestle and mortar, it was still difficult to break the samples apart.

Figure Appendix-4. Images of actual samples

The solvent used for dissolutions were the same (ED/KSCN), and the cellulose/GP50 mixture was completely blended after 3 hours. The soy hull pulp A and C took little over 4

hours to dissolve, though some large chunks of samples were still present. After dissolution, the solution was left to cool overnight, reheated, and then stored in glass jars **(See below).**

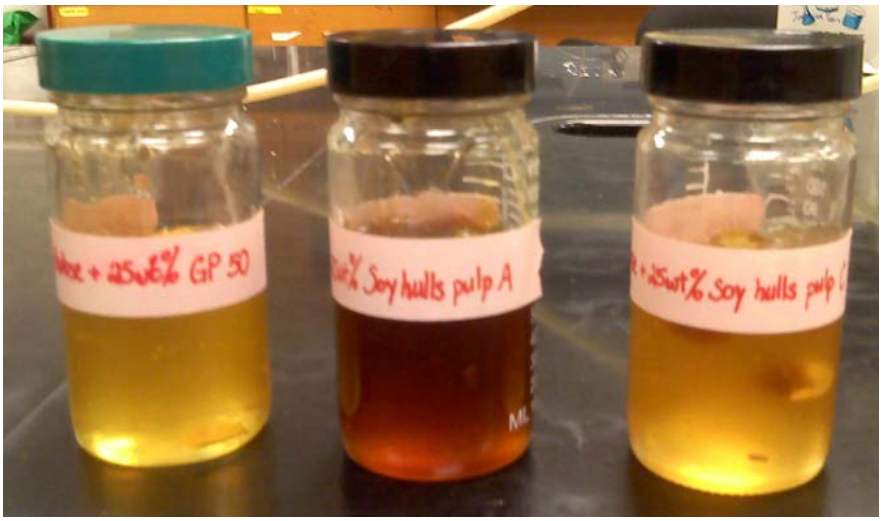


Figure Appendix-5. Images of dissolved samples, large chunks of soy hull pulps

Nonporous membranes from these solutions were obtained by using a 25 mil casting bar on the flat glass surface followed by coagulation, methanol soaking baths, followed by drying in vacuum ovens for 24 hours at ambient temperature, and 24 hours at 40ºC, and another 24 hours of drying at ambient temperature.

After membranes were obtained, they were taken to the physical testing lab for conditioning prior to conducting the tensile tests. Membranes were cut into ½ inch wide, 3-5" long strips, with 3-4 thickness readings per individual strip. Initially a 5 lb. load cell was used, but the limit was detected within seconds of running the machine. After trying 1 sample per membrane, it was determined that the weight of the load cell needed to be increased. The next available weight was a 250 lb. load cell, following the ASTM standard for film testing

using a 250 lb. load cell, tensile test data was obtained and the units were converted so they can be accurately compared with previous experiments.

Table Appendix-2 Tensile Test Data of various soy protein blend membranes

Sample	Tensile modulus (kgf/mm^2) CV(%)	Failure stress (kgf/mm^2) CV(%)	Failure strain (%) CV(%)	Thickness (mm) CV(%)
Douglass 7 wt% nonporous cellulose	165.5 ± 16 (9.7%)	5.36 ± 0.7 (13.1%)	26.2 ± 10.1 (38.5%)	0.047 ± 0.015 (31.9%)
Zhu 5 wt% cellulose membrane	260.89 ± 27.54 (10.6%)	4.65 ± 0.63 (13.5%)	15.1 ± 4.5 (29.8%)	0.034 ± 0.004 (11.7%)
Zhu cellulose + GP50	205.95 ± 21.73 (10.5%)	3.86 ± 0.74 (19.1%)	34.9 ± 6.5 (18.6%)	0.0567 ± 0.011 (19.4%)
Douglass 3 wt% cellulose / 3 wt% soy blend membrane	157 ± 52 (33.1%)	3.2 ± 1.6 (50%)	27 ± 12 (44.4%)	0.029 ± 0.003 (10.3%)
Zhu 3 wt% cellulose / 3 wt% soy blend membrane	255.79 ± 19.21 (7.5%)	4.40 ± 0.27 (6.1%)	9.3 ± 2.8 (30.1%)	0.0347 ± 0.0023 (6.6%)
Zhu cellulose + soy hull A	155.59 ± 37.34 (23.9%)	2.82 ± 0.56 (19.8%)	32.7 ± 9.2 (28.1%)	0.1198 ± 0.0312 (26%)
Zhu cellulose + soy hull C	167.78 ± 42.9 (25.5%)	3.35 ± 0.68 (20.3%)	38.8 ± 9.1 (23.4%)	0.0920 ± 0.0075 (8.1%)

By comparing the above data, it seems that good-strength membranes can be produced with these these crude soy protein materials. However, 75% of the solution was

made of cellulose, and that could be the major contributor to the resulting high strength. It is also worth noting that the thicknesses had a very large standard deviation, not to mention the thicknesses of the membranes are either doubled or tripled those of films made from pure cellulose or cellulose/SPC blend.

In conclusion, the films resulting from using these lower grade samples could result in decent strength membranes, but mainly due to the presence of a majority of cellulose. Dried membranes were much less uniform and much thicker than films made from cellulose and cellulose/SPC blended membranes. We anticipate that if the soy hull samples were carefully ground prior to use, the uniformity of the membranes would have been improved.