ABSTRACT

Yu, Yang. Novel Membranes Made from Blend of Cellulose/Gluten Using Ethylenediamine/Potassium Thiocyanate Solvent System. (Under the direction of committee chair Dr. Richard Kotek)

Current industrial methods for dissolution of cellulose in making regenerated cellulose products are relatively expensive, toxic and dangerous and have environmental problems come with the hazard chemical wastes. To solve these problems, a novel ED/KSCN solvent system was developed, that is cheaper, ecofriendly and highly efficient. These properties of the solvent system were verified in previous studies. The ED/KSCN solvent system was proven to be a suitable solvent for fabricating cellulose (blended with other polymers) membranes. Those membranes were uniform with good mechanical properties.

Gluten was used to develop nonporous membranes with cellulose. Composite membranes with good physical and mechanical properties are studied. The improved method of casting membranes provides better membranes than former researchers. Results showed membranes properties are influenced by the ratio of cellulose/gluten. The SEM images show all membranes are uniform and nonporous. The FTIR spectra prove cellulose and gluten are completely dissolved and miscible in the ED/KSCN solvent system. The ED/KSCN can be totally extracted from membranes by methanol. TGA and WAXS reveal the crystallinity of membranes change with different ratio of cellulose/gluten. Tensile properties show the resulting cellulose/gluten blend membranes have good and controllable mechanical properties. The water absorption test indicates that higher gluten concentration render membranes more hydrophobic. Overall, cellulose/gluten blend membranes made in this work are nonporous, uniform, strong, flexible, ecofriendly and renewable. All these properties demonstrate potentials of cellulose/gluten blend membranes for food packaging and medical applications.

The Novel Membranes Made from Blend of Cellulose/Gluten Using Ethylenediamine/Potassium Thiocyanate Solvent System

by
Yang Yu

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Textile Engineering

Raleigh, North Carolina
2016

APPROVED BY:

_____	_____
Dr. Richard Kotek Committee Chair	Dr. Samuel M Hudson
_____	_____
Dr. Ericka Ford	Dr. William Oxenham

DEDICATION

This work is dedicated to:

First and foremost, my parents, Pingyuan Yu and Ruijing Liu, who gave me life, love, knowledge, hope, understanding and support to help me face and solve problems in my life.

Second, my fiancee, Shenyu Zhang, who always considered me as her hero and gave me all her love, supporting me, encouraging me, staying by my side and cheering for me. She was the powder for me to go through all the troubles. I will never let her down and prove I am her Mr. Right.

My family and friends, without your companion, I would never be who I am today; without your help, I would not be successful; without your support, I would never be such stronger.

Finally, my advisor, dear Dr. Kotek and my group members, thanks for you constant support and patient guidance. Because of you, I became mature, stronger, a more professional scholar and a better man.

Because of all of you, my life became brighter and much more colorful. Thank you.

BIOGRAPHY

Yang Yu was born in Qianxi, a small but very beautiful town, in Hebei province in China. Yang spent his whole childhood in a beautiful village called Pan Jiakou, where is famous for its attractive views, reservoir and the Ming Great Wall. He had a carefree childhood and finished his primary school study in Pan Jiakou.

When finishing the primary school study, in 2005, Yang left his parents, who could not work because of their work, to Tianjin for junior and senior high school study, alone. He lived with his grandpa. A teenage boy, studying in a totally new and strange environment, first time felt lonely and helpless. He knew he had no choice but being independent and stronger. Yang studied in Tianjin for six years. In these six years, he faced countless problems, fell down and got up, again and again. He became mature and stronger than people at same age.

In 2011, passing the College Entrance Examination, Yang was enrolled in College of Textile of Jiangnan University. During the three years he stayed in Jiangnan University, Yang not only learnt lots of knowledge of textile, but also made lots of contributions to his college. Yang joined in the Student Union and performed outstandingly. After working in the Student Union for two years, finally he was elected to be the chairman. Under his leading, the Student Union became one of the most popular student organizations.

In 2014 summer, which was the special year for Yang, he joined in the 3 + X program held by the College of Textiles of both Jiangnan University and NC State University. That summer, Yang became a student of NCSU. He never imagined that he could have a chance to study aboard. This opportunity changed his life. He was so lucky that he could join in Dr.

Richard Kotek's group. Under the guidance of Dr. Kotek, Yang learnt lots of new knowledge and grew up, becoming a real and qualified master student.

ACKNOWLEDGMENTS

- My parents, Pingyuan Yu and Ruijing Liu
- My fiancee, Shenyu Zhang
- Dr. Richard Kotek
- Dr. Ericka Ford
- Dr. Samuel M Hudson
- Dr. William Oxenham
- Mr. Eugene Douglass
- Ms. Lilli Myers
- Ms. Birgit Andersen
- Ms. Judy Elson
- Mr. Charles Mooney
- Ms. Teresa White
- My research group: Ramiz Boy, Ashish Virmani, Yan Jiang, and Mesbah Najafi

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CHAPTER 1 INTRODUCTION

A membrane is a thin layer of material which acts as a selective barrier between different phases to allow the chosen materials to pass through but stops others. Such "chosen materials" can be specific particles, molecules, ions or other substances.[1] Membranes can also be called films or coating to protect one thing from another. Membranes have a very broad range of end uses in daily life and industry, such as packaging and protector for food, coating on furniture and books, dialysis, ultrafiltration and fractionation of mixtures and so on. Most membranes made from polymers such as PE, PP, PVC, PA and PU, which are all petroleum-based polymers. Those polymers consume great amount of petroleum resources and are nondegradable or taking long time to degrade in the land which causes sever environmental problems. Because of the limitation of petroleum resources and environment problems, biopolymers, like cellulose (derivatives), starch and proteins, have been considered as alternatives for nondegradable petroleum-based polymers because of their abundance, renewability, low cost and good biodegradability.[2] The strong need for development of new eco-friendly materials has led scientists to study the properties of membranes made of degradable biopolymers. In this work, cellulose and gluten protein were chosen as raw materials to produce membranes.

Cellulose is the most common polymer on the earth which exists in primary cell wall in plants serving as the structural component. Cellulose is considered as a promising and recommended material with good biodegradability, low cost, abundance and excellent properties. Cellulose is kind of glucose polymer with a molecular structure with linear 1-4-β

linkage, shown in Figure 1. However, the potential applications of cellulose are limited because it cannot be melted to fabricate into a desired form or to be dissolved in a common solvent, because of existence of strong intra- and intermolecular hydrogen bonding[3], cellulose usually degrades before melting. Therefore, scientists have paid more attention to finding new efficient solvents for cellulose. Among all new solvent systems, N-methylmorphorine-N-oxide (NMMO), LiCl/dimethylacetamide (LiCl/DMAC) and aqueous base solvent systems are very popular with high efficiency.[4-6] However these solvent systems may produce chemical waste which can cause environmental problems. Especially, the NMMO solvent system is a thermal instable and strong oxidizing agent which is very dangerous.[7] To solve these problems, Professors Cuculo and Kotek at North Carolina State University had developed a new solvent system composed of ethylenediamine and potassium thiocyanate (ED/KSCN).[8] This solvent system allows for dissolution of cellulose in a shorter period of time at lower temperature which has been verified by other researchers. This ED/KSCN solvent system was used in this work to dissolve raw materials.

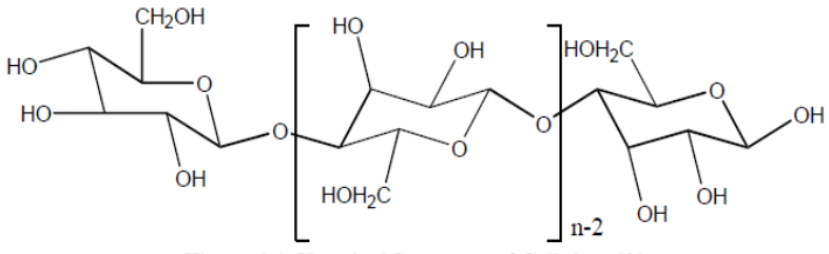


Figure 1. Chemical structure of cellulose[9]

Gluten is the name of a special kind of protein which can be found in grains, such as wheat. Gluten proteins play a vital role in determining the unique baking property and mechanical properties of wheat flour by controlling water absorption capacity, cohesivity, viscosity and elasticity on dough.[10] Because large amount of disulfide bonds exist between molecular chains gluten proteins are water-insoluble. There is no specific chemical structure of gluten proteins because gluten proteins are all mixtures have extremely long molecular chains. Gluten consists of two main components, gliadin and glutenin, with same amount in gluten. Both of them have unique properties.[11] Gliadins have little elasticity and are less cohesive than glutenins but they strongly contribute to the viscosity and extensibility of the dough system. Compared to gliadins, glutenins are cohesive and elastic and are responsible for strength and elasticity of the dough system.[10,11]

Blending is a useful and important method to develop new materials for polymers. When cellulose is combined with other polymers, hydroxyl groups in cellulose facilitate the formation of intermolecular hydrogen bonds between molecular chains of cellulose and other polymers leading to good miscibility and novel functions and properties.[12] Lilli Myers, and undergraduate student started this project first, just as a final project for a class, but the data was not reliable. I improved the whole procedures and designed my experiments, continuing this project. In this work, membranes were made from cellulose/gluten blend, which was dissolved in ED/KSCN solvent system. The objective of this work is to develop novel cellulose/gluten blend membranes and study how different cellulose/gluten concentration influence the properties of membranes and the potential applications of them. Transparent,

uniform and thin membranes were made. The physical and chemical properties of membranes were characterized by using different analytical instruments and procedures.

CHAPTER 2. A REVIEW OF CELLULOSE

2.1 Introduction

Cellulose is one of the most abundant natural organic polymer materials on the earth and has already been used for hundreds of applications for thousands of years. It is the main component of plants, serving to maintain their structure, and is also present in bacteria, fungi, algae and even in animals. [13]

"Cellulose is a polysaccharide composed of glucosidic rings linked through oxygen bridges with a repeat unit having three hydroxyl groups and an acetal linkage."[14] Because of strong hydrogen bonds existing between the hydroxyl groups of adjacent chains, cellulose polymer structure stacks neatly, forming a sheet shape. That is the reason why cellulose is a highly crystalline, insoluble polymer that degrades before melting. [13,15,16] The molecular structure of cellulose is shown in Figure 2 and 3.

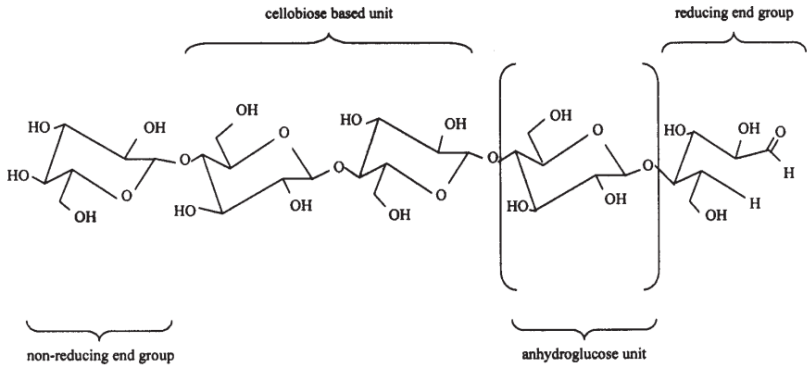


Figure 2. Molecular structure of cellulose. Cellulose is a linear syndiotactic homopolymer composed of D-anhydroglucopyranose units (AGU) linked together by β-(1-4) glycosidic bonds. If the dimer cellobiose is considered as the basic unit, then cellulose can be considered as an isotactic polymer of cellobiose[14]

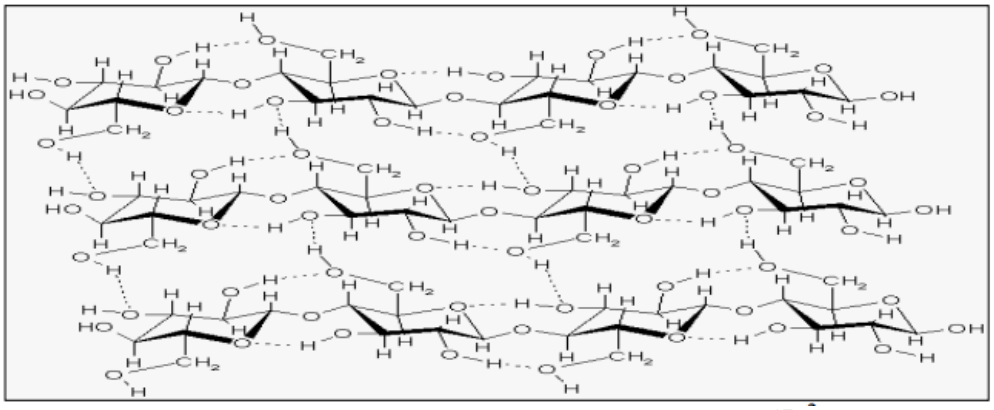


Figure 3. Molecular structure of cellulose[17]

It is important to mention that cellulose has six polymorphs: I, II, III_1, III_{11}, IV_1 and IV_{11}, as shown in Figure 4.[18-20] Under specific experimental conditions, these six polymorphs can convert to each other. Cellulose I, so called native cellulose, is the main form which is found in nature (plants). Cellulose II is the second most extensively studied form, can be obtained from cellulose I by regeneration and mercerization. Celluloses III_1 and III_{11} are formed from celluloses I and II, respectively, by treatment with liquid ammonia or some amines and the subsequent evaporation of excess ammonia. That is a reversible process. Polymorphs IV_1 and IV_{11} may be prepared by heating celluloses III_1 and III_{11} respectively, to 206℃, in glycerol.[13]

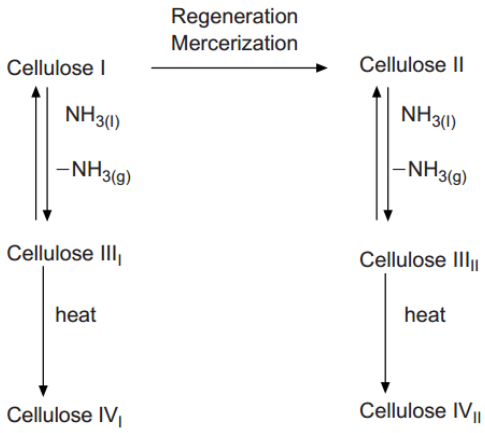


Figure 4. Interconversion of the polymorphs of cellulose[13]

Since cellulose is a high abundant, relatively low price, biodegradable, sustainable natural polymer, it has attracted scientists to study it for hundreds of years. There is a strong reason for using cellulose as the main raw material to make useful products. Cellulose has been used as a raw material for manufacturing membranes and fibers for many years. Because cellulose is not able to be dissolved in common solvents and casting by standard methods, and the relatively low degradation temperature (cellulose degrades before melting) the melt processing is not an option.[14] Nowadays, the most popular method to make cellulose products is dissolution processing. Therefore, to use cellulose to make membranes or fibers or other products, it is necessary to find an efficient way to dissolve it.

A number of studies has been conducted in recent years to improve the cellulose dissolution. Even though most solvent system can provide a good efficiency of cellulose dissolution, they still require multiple chemicals and reagents which can increase the cost and

time for production and some of them can cause contamination to the environment that is not desired. This review will describe: a) the most useful novel solvent systems, b) how they are used, b) dissolution mechanisms, and finally c) advantages and disadvantages of these systems.

2.2 Dissolution of Cellulose

2.2.1 The Lyocell Production Process

The Lyocell production process is currently the most popular and commercially used method to dissolve cellulose. N-Methylmorpholine-N-oxide monohydrate (NMMO with 13.3% water content) is the solvent for dissolution of cellulose. Technically, the Lyocell process is a method that uses NMMO monohydrate as a solvent for direct dissolution of cellulose in industrial cellulose fiber, film and related products-making.[21] In contrast to the viscose process, NMMO in aqueous solution is capable of physically dissolving cellulose without and derivatization, complexation or special activation.[22] Also, the Lyocell process is simple and ecofriendly.

NMMO is produced by oxidation of the ternary amine N-methylmorpholine with hydrogen peroxide, as shown in Figure 5.[23] The basic properties of NMMO have been mentioned in detail in K. E. Perepelkin's research.[24] The molecular weight of NMMO is 115.2; the melting point (anhydrous NMMO) is 170℃, the saturated vapor pressure is 0.35 mm Hg at 25℃, 6.5 mm Hg at 91℃; the initial decomposition temperature is ≥ 100-110℃, and the intensive decomposition temperature is ≥ 130-140℃. NMMO is hygroscopic and forms hydrates.

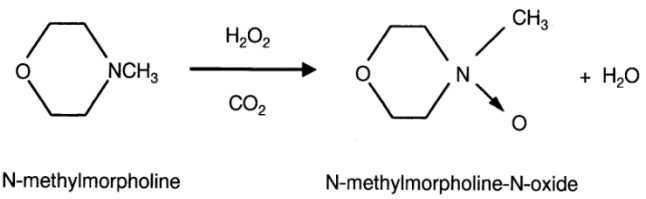


Figure 5. Formation of N-Methylmorpholine-N-oxide (NMMO).*[23]*

Fink et al., discussed specific steps of manufacture of regenerated cellulose materials (e.g. Fibers and films) by NMMO-route: [25]

- Preparation of a homogeneous concentrated solution (dope) of cellulose (dissolving pulp) in an NMMO-water mixture.
- Extrusion of the highly viscous spinning dope at elevated temperatures through an air gap into a precipitation bath (dry jet-wet spinning process).
- Coagulation of the cellulose fibers/films in the precipitation bath.
- Washing, drying and posting-treatment of the cellulose fiber/film.
- Recovery of NMMO from the precipitation and washing bath.

Figure 6 shows the basic stages of Lyocell process: dissolution, preparation of solutions, spinning, washing, finishing and drying the fibers. It clearly shows that after dissolution, the spinning dope must be filtered and degassed. Then fibers are spun into water bath, coagulated and subsequently washed. Following extrusion, finishing and drying steps are carried out. The advantage of this process is recycling of NMMO.[23]

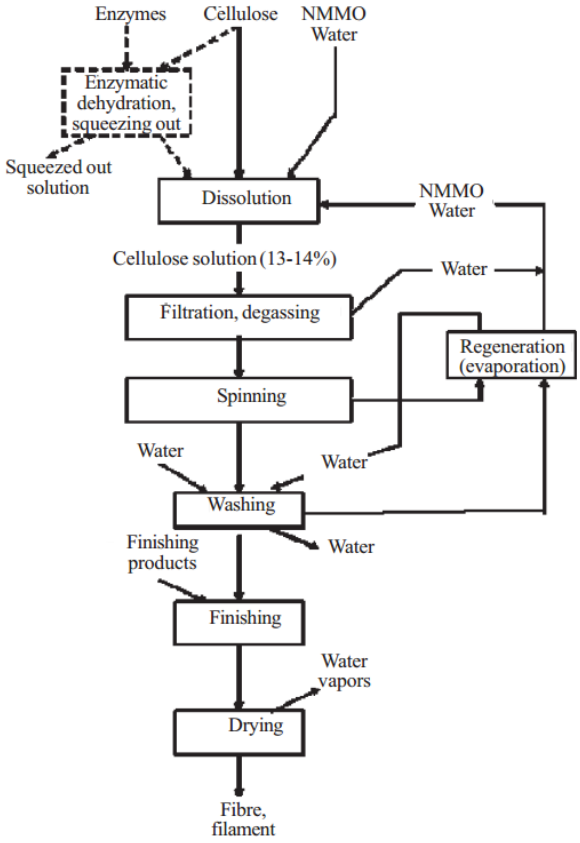


Figure 6. Diagram of manufacture of Lyocell fibers[24]

Similar to the stages shown in the Perepelkin's paper, Fink et al., designed a dry jet-wet fiber spinning process, as shown in Figure 7. In this progress, the process temperature is elevated from 90 to 120℃. The solution consists of 8-20% cellulose, 75-80% NMMO and 5-12% water. The exact composition of solution is influenced by cellulose concentration.[25] Both two methods have the same features namely, the distance between spinning nozzle is too short to adjust for fiber properties. The short distance can cause rapid coagulation of fiber in water bath altering mechanical properties.

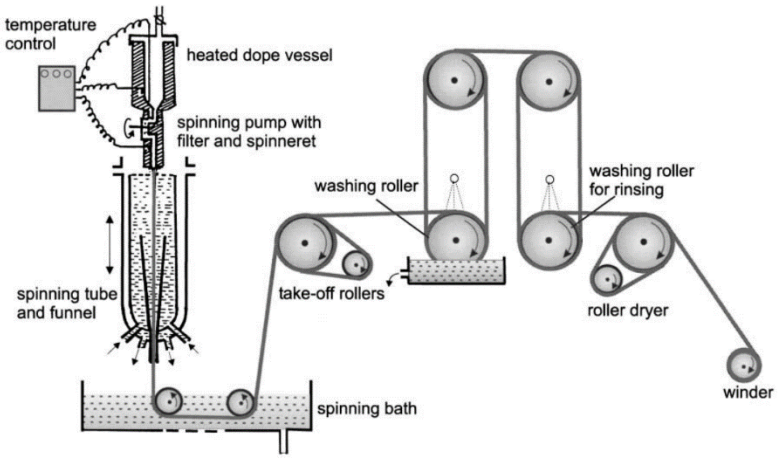


Figure 7. Scheme of the dry jet-wet fiber spinning process for cellulose-NMMO solution[25]

Fink et al., also proved that Lyocell fibers have more regular shape of cross-section (circular) than those made by viscose process (lobulated). Besides the cross-section shape, the Lyocell fibers also have better quality. The surface and cross-section of Lyocell fibers are more uniform with clearer fibrils in fiber structure. Both characteristics are shown in Figure 8.

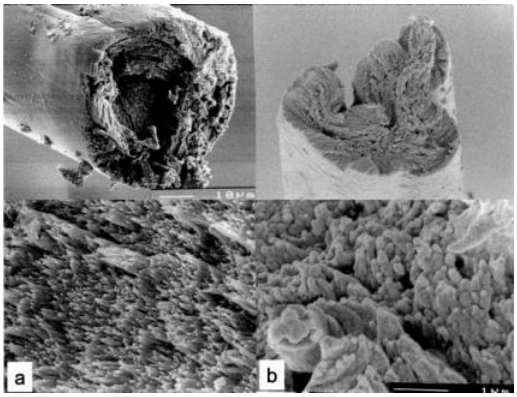


Figure 8. SEM micrographs of fracture surfaces: (a) NMMO, (b) Viscose[25]

Fink et al., reported that not only fibers but two-dimensional films can be made by using the blow extrusion process. As shown in Figure 9 cellulose solution is generally extruded out through the ring nozzle to form a round tube. Then the tube downwards to water bath at working temperature from 80 to100℃. The regenerated cellulose film is obtained by folding and drawing over a roller followed by washing, post-treatments and drying.

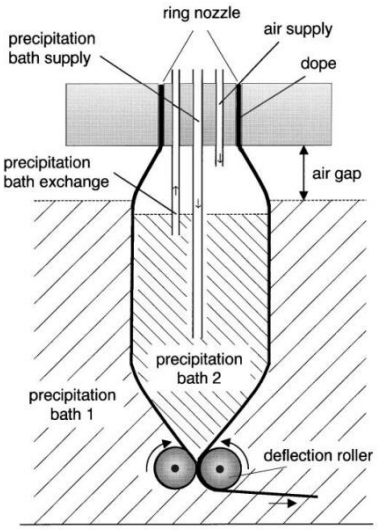
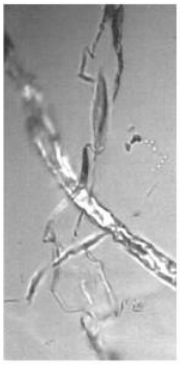
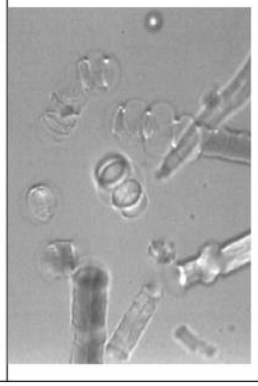
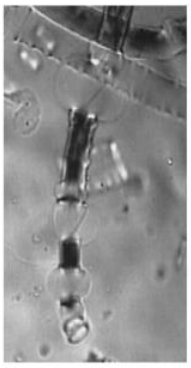


Figure 9. Scheme of the blow-extrusion process for cellulose film formation[25]

During the process, there are many parameters that can influence film properties, for instance, pulp type, draw-down and blow-up ratios, processing temperature, solution parameters, inner and outer air gap, nozzle width and precipitation media. Cellulose dissolution is a very important for the blow extrusion as it is significantly affected especially by the polymer concentration. A high water content can limit dissolution process (Table 1). The full cellulose dissolution occurs at the narrow range of water content (Figure 10).

Table 1. Different swelling and dissolution mechanisms for cotton and wood fibers in NMMO-water mixtures at various water content[21]

Content of water	< 17%	19 – 24%	25 – 35%	> 35%
Swelling and dissolution mechanism	Dissolution by disintegration in spindle (Mode 1)	Swelling by ballooning, dissolution (Mode 2)	Swelling by ballooning, no dissolution (Mode 3)	Homogeneous swelling, no dissolution (Mode 4)
10 µm	Wood fibre 	Wood fibre 	Wood fibre 	Cotton fibre 

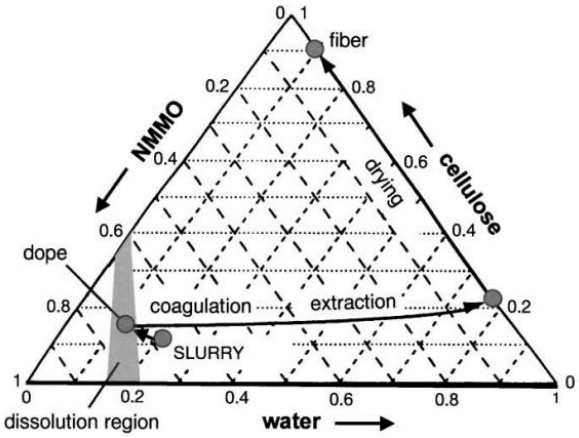


Figure 10. NMMO-water-cellulose phase diagram in preparation of spinning solution and fibers[23]

Table 2 clearly shows the Lyocell films have better mechanical properties than viscose Cellophane films.

Table 2. Mechanical properties of cellulose films[25]

Property	Extrusion-blown film	Cellophane for comparison
Strength (MPa)		
Longitudinal	100–300	125
Transversal	50–200	75
Elongation (GPa)		
Longitudinal	5–40	25
Transversal	2–30	75
Elastic modulus (GPa)		
Longitudinal	2–8	4.7–5.4
Transversal	2–8	3.7
Film thickness (μm)	5–100	20–40

Undeniably, the Lyocell process is not perfect. It has a number of negative side effects such as degradation of cellulose, temporary or permanent discoloration of the resulting fibers, decreased product performance, accelerated decomposition of NMMO, increased need of stabilizers (NMMO is labile), increased chromophore formation, and decreased chemical stability, even leading to explosion.[26] However, even though there are all these negative effects associated with the Lyocell process, the NMMO solvent system is still being popular because of its capability of dissolving cellulose up to 50% of and the process is fast, which is very appealing for industrial production.

2.2.2 Dissolution Mechanisms of Wood Cellulose Fibers in NaOH–Water Solvent System

In Moigne and Navard studied the dissolution mechanisms of wood fibers in NaOH-water solvent systems and identified various types of cellulose samples with different dissolution capacities.[27]

Five wood pulps with different degree of polymerization (DP) and pre-treatment were studied (Table 3). Cellulose samples were dissolved in 8% NaOH aqueous solution at the concentration of 1 wt% cellulose. The solution was mixed by a rotary overhead mixer for 2 hours at -6℃ and 1000 rpm. After pulps were dispersed thoroughly, the solutions were centrifuged without further treatments following the experimental protocol shown in Figure 11. The centrifugation process was separated into three steps to separate the large, medium and small insoluble parts and the clear solution fraction, I_1, I_2, I_3 and S, respectively.[27]

Table 3. Properties of the wood-based cellulose samples used for fractionation by centrifugation[27]

Samples	Origin	Pre-treatment	DP	Fibre/particle diameter (μm)
SE DP 403	Spruce (Borregaard)	Sulphite + steam explosion (SE) 15 bars, 100 s	403	15–30
SE DP 360	Spruce (Borregaard)	Sulphite + steam explosion (SE) 15 bars, 100 s	360	15–30
Avicel PH 101	Wood origin unknown	Hydrolysis (MCC)	170	5–100
Bleached sulphite pulp	Spruce (Borregaard)	Sulphite	400	15–30
PH Kraft pulp	Mixed hardwood	Pre-hydrolysed (PH) kraft	2,000	15–30

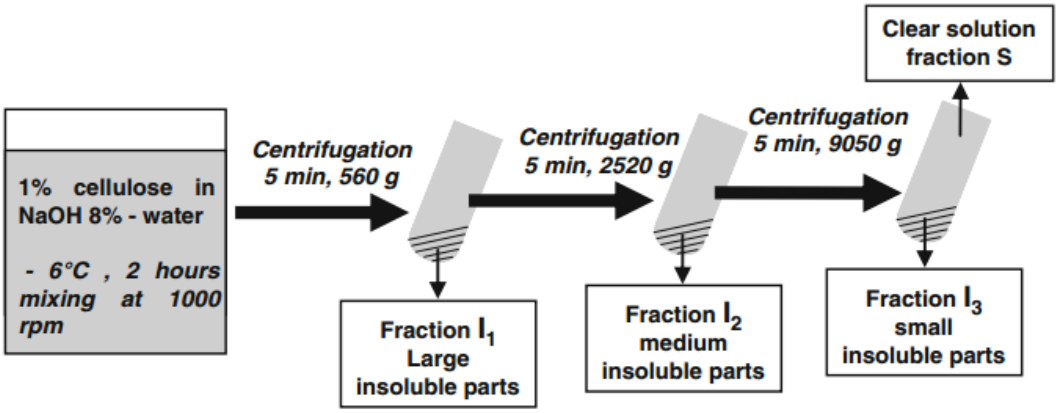


Figure 11. Centrifugation protocol in three steps to fractionate the insoluble fractions (I_1, I_2, I_3) and the clear solution fraction (S) in SE DP 360 and SE DP 403/8%NaOH aqueous solution[27]

Then the resulting fractions (I_1, I_2, I_3 and S) of each sample were observed by optical microscopy and transmission electron microscopy. Also, molecular weight distribution, carbohydrate composition and cellulose II content were measured.

Under the optical microscope ballooned fibers, highly swollen fibers, highly swollen sections and flat rings were observed in I_1. (Figure 12) With the increasing times of centrifugation, the size of cellulose in I_2, I_3 and S decreased. In I_3, only small fragments (10~50μm) were observed. In the clear solution, no insoluble part were observed.

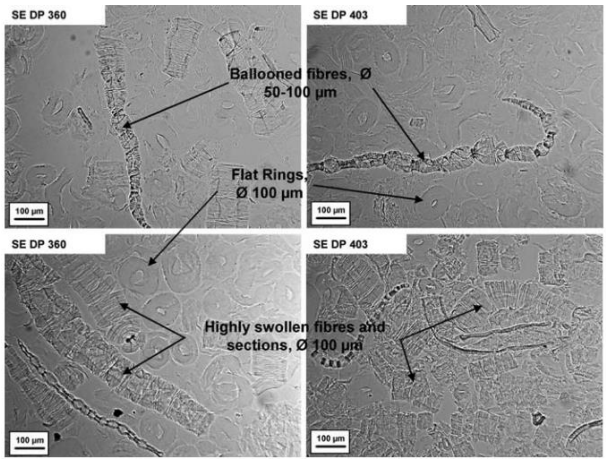


Figure 12. Optical microscopy images of the fraction I1 from the SE DP 360 and SE DP 403/8% NaOH aqueous solution. Ballooned fibres, highly swollen fibres, highly swollen sections and flat rings are observed[27]

In Figure 13, the PH Kraft pulp shows the worst solubility in 8% NaOH aqueous solutions. The insoluble parts account for more than 80%. It can be attributed to the highest degree of polymerization. The SE DP 403, non-agitated, shows three time higher insolubility than the agitated one. It proves agitation can improve the dissolution efficiency.

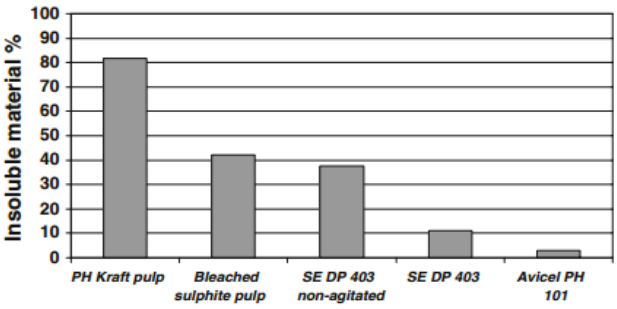


Figure 13. Amounts of insoluble material in 8% NaOH aqueous solution of PH Kraft pulp, bleached sulphite pulp, SE DP 403 (non-agitated) and Avicel PH 101[27]

By dissolving and coagulation of various cellulose samples, Moigne and Navard proved that cellulose I (in native cellulose/control sample) was converted to cellulose II. The evidence is shown in Table 4. There was no cellulose II in the control sample but the after dissolved, the composition of residues was almost 100% cellulose II which was converted from cellulose I. (Table 4)

Table 4. Cellulose II content of original samples, insoluble fractions and recovered fractions (S_r) for SE DP 360, SE DP 403 pulps and Avicel PH 101[27]

Samples	% Cellulose II
SE DP 360 wood pulp	0
SE DP 360 fraction I_1	92
SE DP 360 fraction I_2	88
SE DP 360 fraction I_3	92
SE DP 360 fraction S_r	94
SE DP 403 wood pulp	0
SE DP 403 fraction I_1	98
SE DP 403 fraction I_2	96
SE DP 403 fraction I_3	94
SE DP 403 fraction S_r	97
Avicel PH 101	0
Avicel PH 101—fraction I	100
Avicel PH101—fraction S_r	98

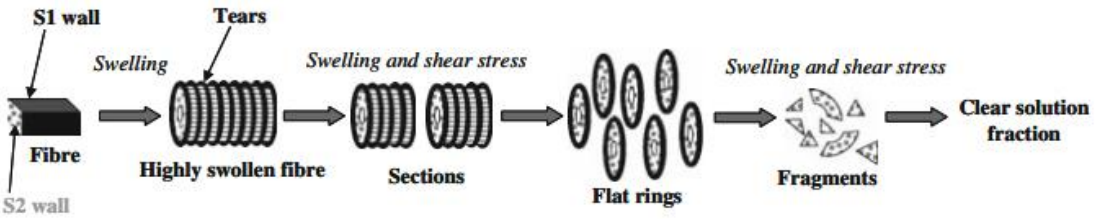


Figure 14. Schematic representation of the dissolution steps of wood pulp fibers in 8% NaOH aqueous solution[27]

Moigne and Navard summarized the dissolution steps of wood cellulose fibers in 8% NaOH aqueous solutions that is schematically represented in Figure 14. The multiple steps

involve fiber swelling and shearing that leads to the formation of small sections, flat rings, small fiber fragments and eventually the clear cellulose solution.

Even though the 8% NaOH aqueous solvent system showed the dissolution ability, this system cannot achieve the complete cellulose dissolution. It shows lower efficiency than the NMMO-water system. In recent year scientists have developed a new solvent system that consists of ethylenediamine (ED)/potassium thiocyanate (KSCN) that has the higher efficiency, lower cost and is eco-friendly.

2.2.3 The ED/KSCN Solvent System

Among all different types of novel solvent systems, the ethylenediamine (ED)/salt solvent system shows an outstanding dissolvability for cellulose and is used in this research.

ED proved that it can swell cellulose and facilitate the dissolution of cellulose. However, it is too difficult to dissolve cellulose completely until a salt is added. The ions generated from salts can interact with cellulose that cause swelling on cellulose fibers.[28] The ability of various cations and anions to cause swelling are shown in Figure 15 by a decreasing sequence.

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

Figure 15. Metal and counter ion order of decreasing ability to swell cellulose[29]

Because the type of salt can determine the efficiency of dissolution of cellulose, to find a salt with the outstanding swelling ability, Min Xiao and Margaret W. Frey set up a series of experiments. They tried to dissolve cellulose in four different ED/salt solvent systems: 1) ED/potassium thiocyanate (KSCN), 2) ED/ sodium thiocyanate (NaSCN), 3) ED/sodium iodide (NaI) and 4) ED/potassium iodide (KI). After making the ED/salt solution with different salt concentration, it was chilled in at -20℃ and then the cellulose with certain weight was mixed in. During the series of experiments, many tests were carried out, such as electrical conductivity test, NMR measurement of solution, FTIR spectroscopy and wide angle X-ray diffraction. Under the help of a polarized light microscope, comparing the results of all the

solutions, it was clear that the ED/KSCN solvent system showed the best efficiency of dissolution of cellulose. (Figure 16)[28]

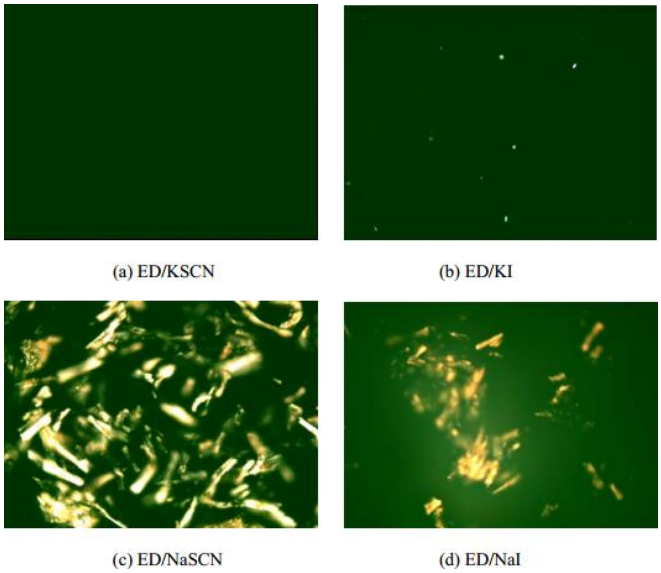


Figure 16. Polarized light microscopy images of 3 wt% of cellulose dissolution in various ED/salt solvent systems[28]

Based on the previous work by Dr. Kotek's group, Hyun Jik Lee designed an experiment to: a) study the dissolving ability of ED/KSCN solvent system for cellulose, b) compare it to ED/NaSCN solvent system and c) find the best ratio of ED to KSCN.[8] Lee prepared solutions with increasing weight percent of salts until the maximum solubility of KSCN and NaSCN in ED was observed. The maximum solubility of KSCN and NaSCN at ambient temperature is 44 and 46 wt%. Then the known weight of cellulose was mixed in at high temperature (60~70℃) and temperature cycling technique was also used. The solubility of cellulose in different solutions is shown in Table 5. It appears that ED/NaSCN solvent system could not

dissolve cellulose completely and some undissolved polymer particles remained. In contrast to the latter solvent, the ED/KSCN solvent system showed excellent dissolving ability to cellulose when salt concentration was between 30 to 40%. Later Lee increased the processing temperature and observed that the ideal dissolving ability of ED/KSCN at 90 to 100℃ and salt concentration of 35%. His studies proved that cellulose fiber and films produced under these conditions had improved mechanical properties.[8]

Table 5. Solubility of VFC Cellulose (DP 450) in Different Ratio of ED/salt Solvents: X (No Solution), O (Solution), Δ (Partially Dissolved Solution)[8]

Ratio of ED/Salt	ED/KSCN		ED/NaSCN	
	High Temp.	Temp. Cycling	High Temp.	Temp. Cycling
85/15	X	X	X	X
80/20	X	X	X	X
75/25	X	O	X	X
70/30	O	O	X	X
65/35	O	O	Δ	Δ
60/40	O	X	Δ	Δ
50/50	No Solvent	No Solvent	No Solvent	No Solvent

Douglass used the ED/KSCN solvent system and verified that it was an efficient system to dissolve cellulose.[17] In his research, not only cellulose, but also starches and proteins fully dissolved in the ED/KSCN solvent system. Douglass used this solvent system (ED/KSCN 65:35 wt%) to dissolve the mixtures of cellulose/starch and cellulose/protein, to produce porous and nonporous membranes. Those membranes were coagulated in different coagulants such as water, methanol, propanol and acetone. Then the physical and mechanical properties were studied.[17]

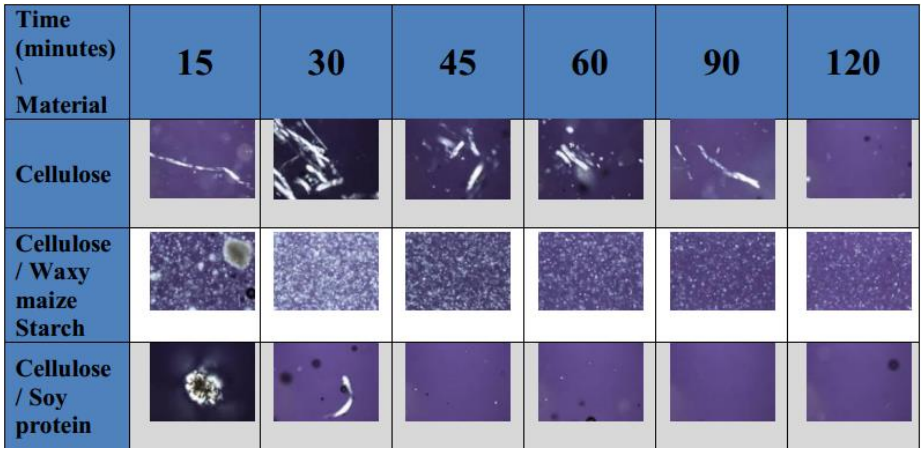


Figure 17. Time elapse visual study, using cross polarization microscopy, of dissolution of cellulose variety blends in solvent[17]

Figure 17 shows dissolution states of different raw materials in the ED/KSCN solvent system at different period of time. Three kinds of raw materials were used, cellulose, cellulose mixed with waxy maize starch and cellulose mixed with soy protein. The results showed that the soy protein had the best solubility with cellulose in the solvent system. In the dissolution study, Douglass observed that cellulose fibers started swelling between 15 and 30 minutes, most fibers disappeared after 90 minutes and finally, cellulose fibers were dissolved completely after around 4 hours. The time consumed during dissolution was dependent on the cellulose concentration. Interestingly, the dissolution of mixture of waxy maize starch and cellulose started immediately which could be attributed to decreasing content of cellulose. The cellulose fibers were swollen fast and disappeared after 30 minutes and some crystalline starch particles were still present. Two hours later, all crystallites were gone which was shown under the microscope. When cellulose was mixed with soy protein solution, swelling started after 15

minutes and the polymer solution became homogenous without any artifacts (such as swollen fibers or crystalline structures). The ED/KSCN solvent system showed efficient dissolution for blends of cellulose and other materials such as starch and proteins.[17]

Following the Douglass work, Zhu[30] studied mechanical properties of non-porous films made of cellulose/soy protein concentrate blend using ED/KSCN solvent system. The similar properties were observed. In her research, the crosslinking agent, glutaraldehyde, was also added to stabilize the structure molecular structure of the blended membranes. After comparing the mechanical properties of blended films with and without glutaraldehyde, the results showed that those film treated by glutaraldehyde had much better mechanical properties which should be attributed to the structural improvement. The studies not only showed the efficiency of dissolving ability of ED/KSCN solvent system to cellulose/proteins, but also proved that cellulose/protein blend can be used to produce films which can be useful for food packaging, filtration systems, or even medical applications.[30]

CHAPTER 3. REVIEW of GLUTEN PROTEINS

3.1 Introduction

Proteins are large biomolecules with a large number of functions in living organisms – animals and plants.[31] For example, collagen, which is the most abundant protein (accounts 25% or more total body protein) found in higher vertebrates, serves as the main structural protein of connective tissues; elastin is also an important component of tissues forming skin, blood vessels and lungs; enzymes are the necessary part in the body that can catalyze thousands of important chemical reactions essential to life.[31] Many kinds of proteins can be found in plants, for example, soy protein, which exists in soybeans used as source of edible oil; rapeseed protein, which is hiding in rape seeds which is also a source of edible oil and gluten, which is commonly found in wheat used to make bread and noodles. Gluten is one of the most common proteins with multiple useful properties in human life and it is discussed specifically in this review. Proteins have a complex 3D structure using 20 common amino acids (Table 6) as blocks. The protein formation can be separated into four main stages, primary, secondary, tertiary and quaternary structures (Figure 18). The primary structure is polypeptide chain formed by dehydration condensation of amino acids. Then the amino acid residues interact within localized domains so that folding, bending and coiling are occurred and the polypeptide chain forms a 3D structure (secondary structure). The secondary structure keeps folding and coiling back and forth with distance sections (side chains) of the polypeptide chain, forming a more complex 3D structure (tertiary structure). Finally, interactions between subunits, or individual polypeptide chain in multi-chain proteins occur and the quaternary structure is

formed. The amino acid sequence and structure of polypeptide chain serve as an ID number determining the properties of protein which are unique for each kind of protein.[31]

Amino Acid	Three Letter	One Letter
Alanine	Ala	A
Cysteine	Cys	C
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y

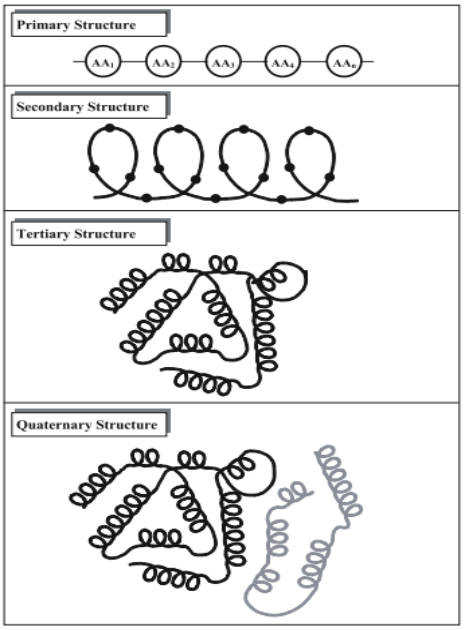


Table 6. **Figure 18.**

Table 6. One- and Three-Letter Abbreviations for the 20 Common Amino Acids.[31]
Figure 18. Schematic illustration of the four levels of protein structure.[31]

Gluten is the name of a kind of protein which can be found in wheat. The wheat gluten proteins are of immense importance in food industry because their properties support the processing of wheat flour to produce bread, cakes, pasta, noodles and a range of other foods that are the main part of our daily meals. So wheat gluten proteins are very common but very important in daily life.[11]

As a protein, gluten proteins consist of a couple of amino acids, cysteine, proline, glutamine, glycine, tyrosine and serine. Proline and glutamine acid account for more than 50%

in gluten. Proline is a kind of hydrophobic amino acid that has hydrophobic chains which make gluten more hydrophobic. Those amino acids can form polypeptide chains by dehydration condensation which is the primary structure of gluten proteins. Each kind of gluten protein has a unique amino acid sequence and amount of those amino acids. However, it is difficult to present a structure of the gluten protein because the amino acid chain is too long to be measured.[10]

Gluten proteins show many properties that can be taken advantages of. Gluten proteins play a vital role in determining the unique baking quality and mechanical properties of wheat flour like water absorption capacity, cohesivity, viscosity and elasticity on dough.[10] Simply, because of gluten proteins, flour can form doughs with water and keep its shape and influence chewy texture of the final food. Abundant resources, low cost, good biodegradability and suitable properties mentioned above have become the reason why scientists have spent hundreds of years on studying gluten proteins.

The first report about isolation of gluten was published by Dr. Jacobo Beccari in 1745.[32] Because of his contribution, the properties of gluten have attracted more and more scientists to be engaged in studying gluten proteins.

In Dr. Beccari's work, it showed that wheat flour consists of two fractions, one of which was water-soluble amylo (starch) which had characteristics similar to sugars, and the other one was glutinin (gluten) which was water-insoluble similar to substances of animals.[33] After gluten was been found, scientists focused on studying solubility of gluten. Gluten proteins were found not soluble in water but was largely dissolved in acetic acid and was partially soluble in

alcohol/water mixture, with roughly similar fractions being present in grains of barley and rye.[10,11,32]

Previous studies laid the foundation and provided the basis for the systematic analysis of T.B. Osborne in the research of wheat proteins. From the experiments, Osborne concluded that proteins in wheat gluten could be separated into four groups, defined by the extraction sequence in a series of solvents. The first two fractions were water soluble albumins and globulins dissolved in dilute salt solutions. The third fraction was called gliadin which was extracted with alcohol/water solution. The last fraction which was dissolved in dilute acid of alkali was called gluteni.[34] Both gliadin and glutenin are the main components of gluten proteins. These two components are being present in approximately equal amount in gluten.[11] From the results concluded by Osborne, it is clear that gluten proteins are far away from pure substance but a kind of mixture. That is one of the reason why there is no specific chemical structure of gluten. In this review, the gliadin and glutenin proteins are mentioned specifically.

3.2 Gliadins

Gliadin and glutenin are important contributors to the rheological properties of dough. Both of them have their own unique and divergent characteristics. Both fractions consist of numerous, partially closely related protein components which are high in glutamine and proline contents.[10]

Gliadins have worse elasticity and are less cohesive than glutenins but they strongly contribute to the viscosity and extensibility of the dough.[10,11] Gliadins are soluble in alcohol that was observed by Osborne. Early studies of gluten proteins were presented that the gliadins comprised mainly monomeric proteins and the molecular weight of gliadins ranging from 30,000 to 50,000.[35-37] Later, in the further studies, with the help of electrophoresis and peptide mapping, the similar structures of both kinds of proteins were revealed by scientists in characterizations gliadins and glutenin monomers (disulphide-reduced glutenin).[38-42] The standard separation and classification for wheat gliadin subunits is electrophoresis which is based on their electrophoretic mobility at low pH. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method is convenient and widely used to separate proteins nowadays.[43,44] By this method, four groups of bands can be resolved, as shown in Figure 19.

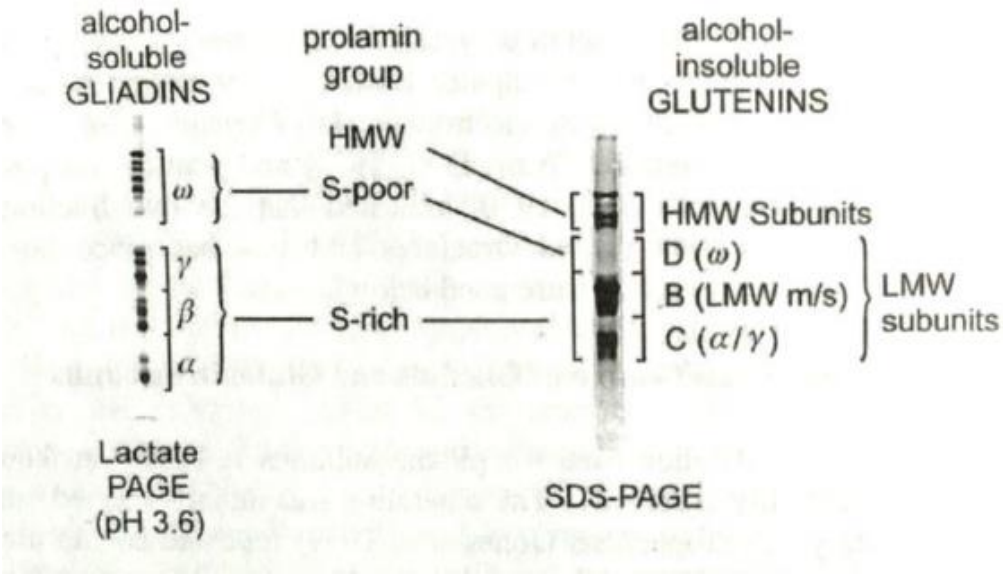


Figure 19. The classification and nomenclature of wheat gluten proteins separated by SDS-PAGE and electrophoresis at low pH. The D group of LMW subunits are only minor components and are not clearly resolved in the separation shown[11]

Table 7. Characterization of gluten protein types[46]

Characterisation of gluten protein types

Type	MW × 10^{-3}	Proportions[a] (%)	Partial amino acid composition (%)				
			Gln	Pro	Phe	Tyr	Gly
ω5-Gliadins	49–55	3–6	56	20	9	1	1
ω1,2-Gliadins	39–44	4–7	44	26	8	1	1
α/β-Gliadins	28–35	28–33	37	16	4	3	2
γ-Gliadins	31–35	23–31	35	17	5	1	3
x-HMW-GS	83–88	4–9	37	13	0	6	19
y-HMW-GS	67–74	3–4	36	11	0	5	18
LMW-GS	32–39	19–25	38	13	4	1	3

[a]According to total gluten proteins.

These four groups of gliadins are named: α-gliadins (fastest), β-gliadins, γ-gliadins and ω-gliadins (slowest), which are defined by resolved speed.[45] Although gliadins can be classified into these four kinds, the amount of ω-gliadins is much lower than the other three. So α-gliadins, β-gliadins and γ-gliadins are the major components. (Table 7)[46] Though gliadins are

considered to be monomeric proteins, there are still some polymeric gliadins which can be shown by SDS-PAGE at low pH with similar mobility of low molecular weight (LMW) units of glutenins.[47,48]

3.3 Glutenins

Compared to gliadins, glutenins are more cohesive and elastic and are responsible for strength and elasticity of the dough system.[10,11] Glutenins were proved that they were polymeric proteins with vary sizes and molecular weight ranging from about 500,000 to more than 10 million.[49] Disulphide bonds present in both gliadins and glutenins as interchain crosslinks. Glutenins have more disulphide bonds than gliadins providing stable structures.[50] The existence of large amount of disulphide bonds is the reason why the solubility of glutenins is limitted. So to dissolve glutenins, reduction of disulphide bonds to convert glutenin polymers to monomers is necessary. The reduced glutenins have similar solubility in alcohol to gliadins. By early analyses of SDS-PAGE, reduced glutenin units were separated into four groups of bands called A, B, C and D units (same way as naming gliadins), as shown in Figure 20. The A subunits have molecular weight over 100,000, however, the B and C subunits are similar to the α/β/γ-gliadins and the D subunits are similar to ω-gliadins.[51,52] Because of the different magnitudes of molecular weight, the A subunits is also called high molecular weight (HMW) subunits and the B, C and D subunits are called low molecular weight (LMW) subunits.[53,54] A model of disulphide interchain linked LMW glutenin subunits (LMW-GS) and HMW glutenin subunits (HMW-GS) is shown in Figure 21.[46] The variation in types and amount of HMW and LMW glutenin subunits affects the rheological properties of the dough system by affecting the MW distribution of gluten proteins.[55,56]

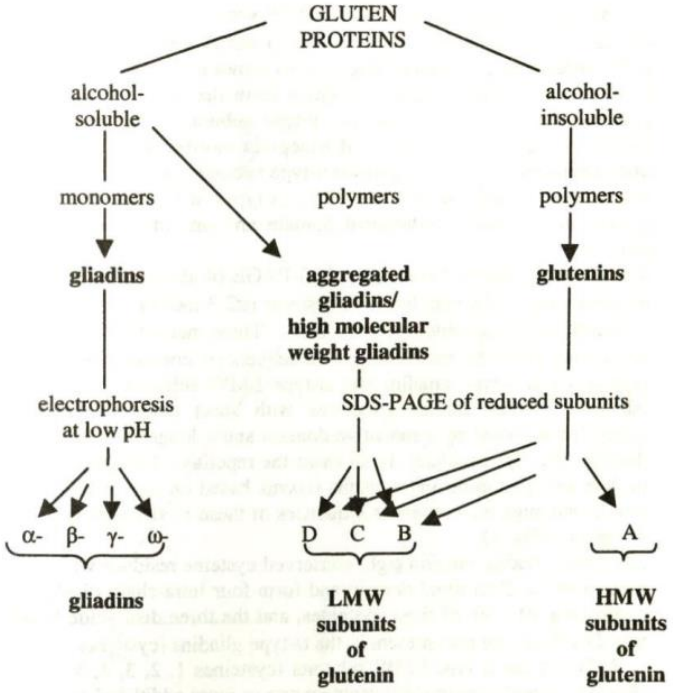


Figure 20. The "classical" nomenclature for wheat gluten proteins[11]

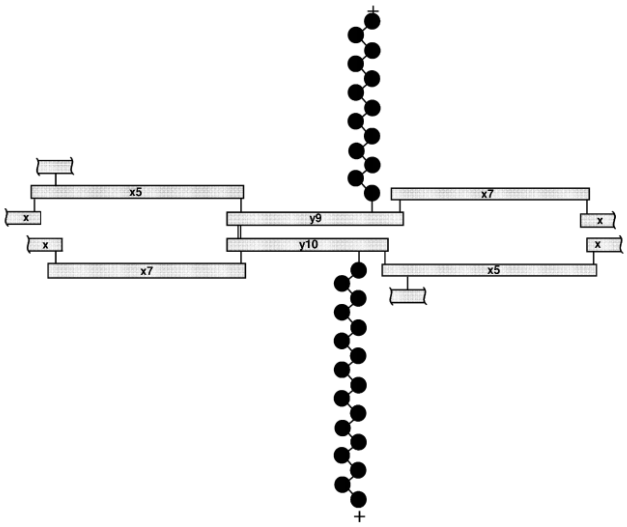


Figure 21. A model double unit for the interchain disulphide structures of LMW-GS (●) and HMW-GS (□)) of gluten polymers[46]

Previous studies have revealed that gluten is a mixture of hundreds of proteins which are monomers or oligomers and polymers linked by intermolecular disulphide bonds. Gluten proteins vary with proportions, properties, structures, amino acid sequences and genotypes of components. Gluten proteins have high level of polymorphism which means that characterizing wheat gluten proteins using classical biochemical approaches can be a challenging task. Because of the extremely long amino acid chains, and complex structures of the chains, the specific structure of gluten proteins can’t be drawn.

3.3 Extraction of Gluten Proteins

Giving the unique properties to doughs, it is not surprising that gluten has been gained attention by the food industry. That interest has extended to the commercial separation of gluten from the starch and soluble proteins of flour.

Gluten can also be defined by the rubbery mass that remains when dough is washed to remove starch granules and water-soluble constituents.[10] So gluten protein is normally obtained by thorough kneading of flour powder or dough under a stream of water. After thoroughly washing, the dried substances remains 75-80% protein, 5-10% lipids, 5-15% starch residues and very small amount of salts.[57]

Gluten can be extracted from flour by two ways: 1) kneading the flour, followed by agglomerating the gluten into an elastic network; 2) kneading a dough, and then washing out the starch. Starch granules disperse in cold water, sieved by a mesh with fine holes. After sieving, the gluten is left and then dried and ground to powder. If a saline solution is used instead of water, a purer protein could be obtained, with more impurities departing to the solution with the starch. If starch is the prime product in the flour, cold water is the favored solvent because the relatively lower cost.[56]

Under different conditions, different methods can be used to extract gluten from flour. In home or restaurant cooking, a wheat flour dough is made first. Then the dough is kneaded under water until the starch is washed out. However, in industrial production, making the dough first is not necessary, a slurry (salt solution) of wheat flour is kneaded vigorously by machine until the gluten is separated from other impurities and agglomerates into a mass (elastic network structure). This mass is collected by centrifugation, then transported through

several stages. About 65% of the water in the wet gluten is removed by means of a screw press. The remainder is sprayed through an atomizer nozzle into a drying chamber, where it remains at an elevated temperature a short time to evaporate the water without denaturing the gluten. After thoroughly drying, the dried substances contains 75-80% protein, 5-10% lipids, 5-15% starch residues and very small amount of salts. In the final step, the collected gluten is sifted and milled to produce a uniform product.[56,58]

3.4 Applications of Gluten and Factors Influencing Mechanical Properties of Membranes

3.4.1 Gluten Applications and Feasibility of Forming Films

Early studies have proved the advantages of gluten proteins for using as membranes and plastics because of their abundant resources, low cost, good biodegradability and suitable properties. Gluten membranes have been used in food and non-food applications (and potentials) protective coatings, films (e.g. edible), adhesives, surfactants, ingredients and thermoplastic material.[59-62] Most of these end uses relate to its cohesive, viscoelastic and solubility properties.

Most of the information of commercial gluten films has only been reported in patents which are trade secrets. Objective and quantitative data on film-forming procedures and properties are scarce. Only some researchers reported a few methods to make gluten dispersion and to cast films that are similar to each other:[63-65] A typical procedure and experimental steps are:

(1) Weigh a specific amount of gluten.

(2) Weigh a specific amount of solute (ethanol/water solution) and plasticizer (e.g. glycerol) and mix together. The pH is adjust to high (or low) value.

(3) Dissolve gluten in the mixture and stir it and heat for a period of time.

(4) The film-forming solution is spread onto a Plexiglass (coated) leveled surface.

(5) The cast films are then dried at room temperature or in oven.

Studies of various researchers showed that the factors affecting properties of gluten membranes can be: gluten concentration, ethanol concentration, pH of film-forming solution, drying time and temperature, type of reagent, treatment. Those factors can affect mechanical

properties, opacity, water solubility and water vapor/gas permeability of films.[47,66] The details will be given in the subsequent review.

3.4.2 Effects of Gluten Concentration, Ethanol Concentration and pH on Various Film Properties

Gontard et al, used response surface methodology (RSM) to determine the influence of some film-forming conditions on edible gluten film properties.[61] The effects of gluten concentration, ethanol concentration and pH on film-forming solution on various properties were studied. Obvious effects were observed in different conditions of pH and ethanol concentration, affecting film opacity, solubility and water vapor permeability.[62]

When preparing gluten films, the solution was made by dissolving gluten in absolute ethanol, acetic acid and water. The concentration of gluten and ethanol and the pH of the solution was adjusted based on needs. Glycerol was added as plasticizer to prevent the film from becoming brittle. The mixture was held under magnetic stirring at 40℃. Then solution was immediately used for casting forms by pouring and spreading it on to a plexiglass plate. The film was dried in a ventilated oven at 30℃ to constant weight. Before films used for experiments, they are equilibrated at 56% RH.

Opacity, solubility in water, water vapor permeability and mechanical properties were measured in different condition of pH and concentration.

Figure 22 (a) shows the relationship of opacity, solution pH and ethanol concentration. Film opacity increased with the increase of ethanol concentration especially at ethanol concentration above 50% and pH above 4.[62] Gontard et al, attributed this phenomenon to

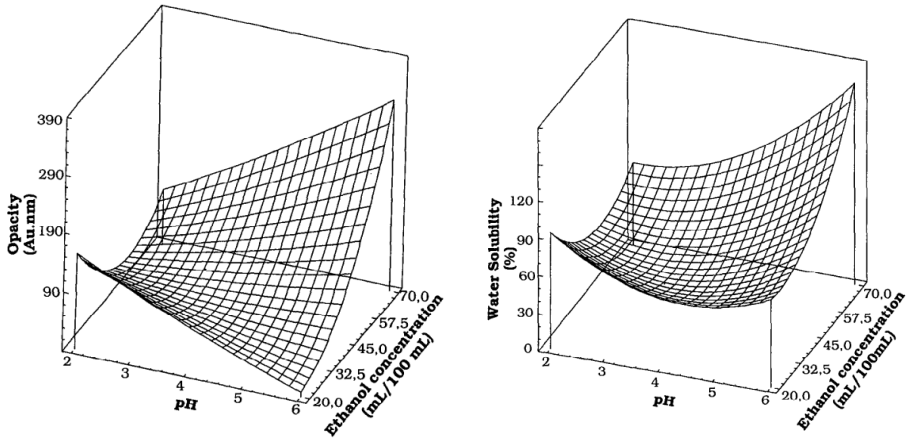
precipitation of glutenins because of insolubility in ethanol and pH 4 was not low or high enough to dissolve gluenins. High ethanol concentration caused gliadins to dissolve better and forming transparent films. The effects of pH on opacity was strongly dependent on ethanol concentration. At ethanol concentration over 35%, film opacity decreased with a decreasing pH. This was related to the improvement of dissolution of glutenins.

Water resistance is important to edible films used to protect foods where water is unavoidable. As shown in Figure 22 (b), solution pH and ethanol concentration were main factors affecting film water solubility.[62] The high ethanol concentration (above 50%) at pH 5-6 resulted in high film solubility because gliadins, which account for about 50% in gluten, were almost dissolved totally. Also, at low pH, film solubility was high, too, because most glutenins were dissolved. Gontard et al, concluded that the best range of conditions that could result in the lowest film solubility with a minimum value (about 40% after 24 hours) was varying between ET = 40%-pH= 2 and ET= 20%-pH = 5.

Water vapor permeability is also an important property for edible films. The shape of response surface (Figure 22 (c)) reflected that low pH with high ethanol concentration or high pH with low ethanol concentration could result in low water vapor permeability.[62] Gontard et al, pointed out that water vapor permeability of films was dependent on the number of polar groups the polymer contained. So at low pH, glutenins were unfolded and hydrophilic groups were exposed which could help water vapor to migrate through films.

The response surface for film puncture strength, deformation and relaxation coefficients are shown in Figure 22 (d-f).[62] Gluten concentration and solution pH are the most important factors determining the mechanical properties. Figure 22 (d) shows that film puncture strength

decreased with a decreasing pH when gluten concentration was high (above 7.5g/100mL). The weakest film was made at the lowest pH which was the result of broken of linkages between protein chains. If pH was fixed, puncture strength was improved with increasing gluten concentration from 7.5 to 12.5 g/mL. It could be explained by the increasing number of linkages between protein chains. Figure 22 (e) describes that puncture deformation of film with high gluten concentration decreased with decreasing pH. The deformation was sensitively affected by pH. The evidence is puncture deformation decreased dramatically when pH was below 4. Figure 22 (f) clearly shows that gluten film is a viscoelastic material with a relaxation coefficient value between 0.3 and 0.87. From the experiment, the highest relaxation coefficient films were made under condition of pH 4 and gluten concentration was 7.5%.



(a) (b)

Figure 22a. Response surface for the effect of pH and ethanol concentration of the film-forming solution on (a) film opacity; (b) water solubility at a constant gluten concentration of 7.5 g/700 mL[62]

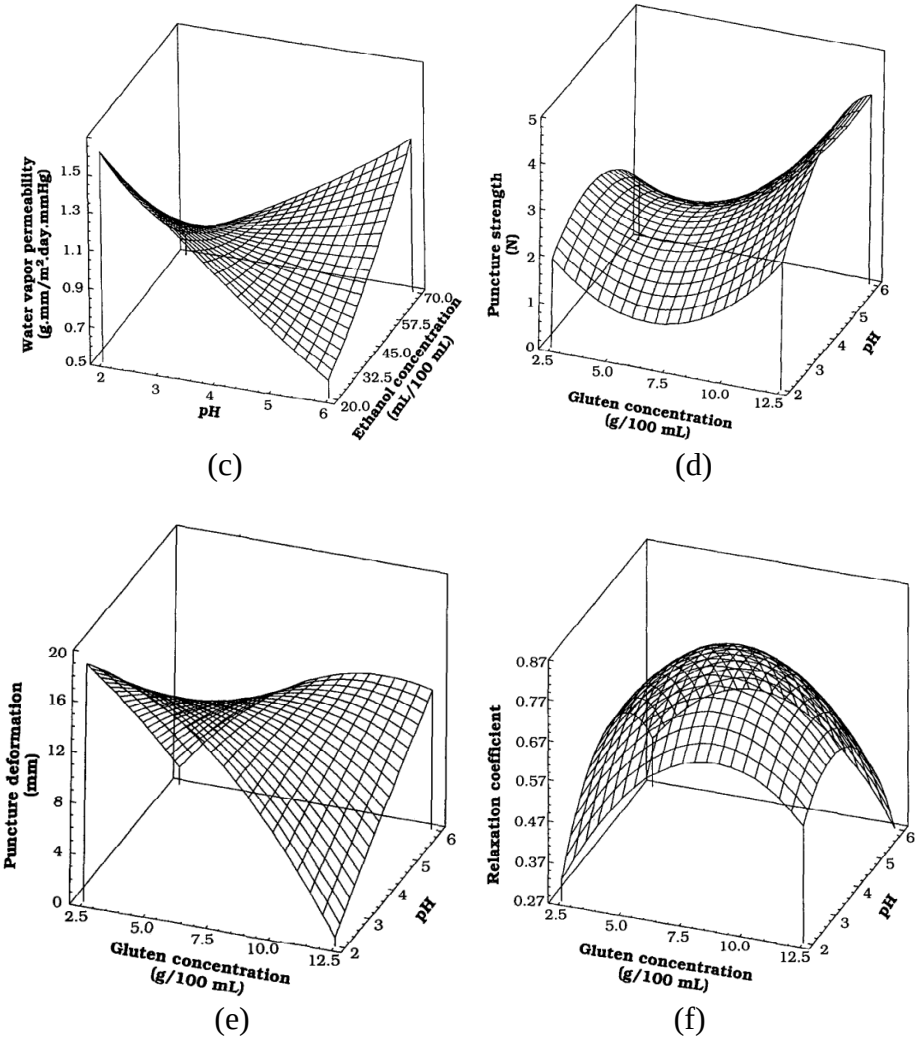


Figure 23b. Response surface for the effect of pH and ethanol concentration of the film-forming solution on (c) water vapor permeability at a constant gluten concentration of 7.5 g/700 mL; Response surface for the effect of pH and gluten concentration of the film-forming solution on film (d) puncture strength; (e) puncture deformation; (f) relaxation coefficient at a constant ethanol concentration of 45 mL/100 mL[62]

Like Gontard et al, Kayserilioglu et al, did a similar research but proposed new conclusions. In the research, film-forming solutions with pH of 4, 6 and 11 was made by using

HCl, NaOH and NH_3.[64] Same experiments were applied to the films and the data was collected in Table 8. From this Table, by comparing with the films prepared at pH 4 and 6, those prepared at pH 11 showed significantly higher TS (tensile stress) values, if the base was NaOH. NH_3 did little help to basic properties.

Table 8. Moisture content, thickness, tensile strength (TS), elongation at break (E) and water vapor transfer rate (WVTR) of gluten films under different process conditions[64]

Film treatment	Acid/base	pH	Moisture[1)] [%]	Thickness[2)] [mm]	*TS*[3)] [MPa]	*E*[4)] [%]	WVTR[5)] [g × 10^2/m^2 × 24 h]
Legend water	HCl	4	9.6 ± 0.1	0.340	2.1 ± 0.4	261.4 ± 25.0	7.1 ± 0.7
		6	9.2 ± 0.1	0.365	1.8 ± 0.7	324.2 ± 54.0	6.9 ± 1.0
	NaOH	11	10.3 ± 0.3	0.335	5.2 ± 0.7	170.1 ± 74.0	6.2 ± 0.1
Ethanol	HCl	4	8.9 ± 0.1	0.367	1.8 ± 0.0	250.5 ± 40.0	8.6 ± 1.9
		6	10.4 ± 0.1	0.408	1.7 ± 0.6	238.2 ± 66.0	6.0 ± 0.2
	NaOH	11	10.6 ± 0.0	0.270	7.4 ± 0.7	188.7 ± 50.0	6.4 ± 0.4
	NH_3	11	11.6 ± 0.2	0.316	2.2 ± 0.2	225 ± 27.0	–

[1, 5)] n = 2, mean ± SD. [2–4)] $n \geq 4$, mean ± SD.

3.4.3 Effects of Type Reagent and Different Treatments on Gluten Film Properties

Besides gluten concentration, ethanol concentration and pH on film-forming solution were important factors influencing gluten film properties, Gennadios et al, pointed out that type reagent and different treatments could affect gluten film properties, too.[61]

Gennadios et al, set up a rigorous experiment with 7 groups of gluten films, 1 control group and 6 experiment groups. The control group was prepared from the solution by dissolving gluten in water/ethanol mixture with glycerol as plasticizer under basic condition. The experiment groups were made from modified film-forming solution (Film 1-3) and by different treatments on control films (Film 4-6):[61]

Film 1. Heavy mineral oil (increase film water resistance) was added to the control solution before heating.

Film 2. Sodium sulfite (0.2 g) was added to the control solution before heating. Sodium sulfite is a reducing agent to break linkages between protein chains, increasing chain mobility and chances of bonding upon drying.

Film 3. Small amount (13.33%) of gluten was replaced by hydrolyzed keratin protein.

Film 4. Control films were soaked in 15% (w/w) lactic acid solutions for 20 sec to introduce a tanning effect on the films.

Film 5. Control films were soaked in 1 M aqueous calcium chloride ($CaC1_2$) solution for 20 sec and then immediately submerged in distilled water for 10 sec to remove excess solution. This bonded the divalent calcium cations with pairs of negatively charged sites on polypeptide chains, promoting crosslinking in the film structure.

Film 6. Control films were soaked for 20 sec in a buffer solution with a pH of 7.5, a value corresponding to the isoelectric point of wheat gluten. Insolubilization of the wheat gluten protein at its isoelectric point was the reason for applying this treatment.

Thickness and surface density, mechanical properties, water vapor permeability and oxygen permeability were tested. The data of physical properties were shown in Table 9. The data of barrier properties, such as water vapor permeability and oxygen permeability, were shown in Table 10.

Table 9. Mean and Standard Deviation Values for Thickness, Surface Density, Tensile Strength, and Elongation at Break of Various Wheat Gluten-Based Films[61]

Treatment Number	Film Treatment	Thickness (μm)	Surface Density (mg/cm^2)	Tensile Strength (MPa)	Elongation at Break (%)
	Wheat gluten (control)	127 ± 11 b	14.9 ± 1.2 b	2.6 ± 0.2 c	237.9 ± 21.9 d
1	Wheat gluten with mineral oil	125 ± 11 b	15.0 ± 1.0 b	2.2 ± 0.3 d	267.2 ± 40.1 c
2	Wheat gluten with sodium sulfite	128 ± 8 b	14.7 ± 1.0 b	2.9 ± 0.4 b	192.3 ± 24.9 f
3	Wheat gluten with hydrolyzed keratin	119 ± 9 c	15.2 ± 1.1 b	1.7 ± 0.2 e	313.5 ± 34.5 b
4	Control soaked in 15% (w/w) lactic acid	136 ± 10 a	17.0 ± 0.7 a	1.4 ± 0.3 f	417.0 ± 41.5 a
5	Control soaked in 1*M* calcium chloride	127 ± 10 b	15.2 ± 0.8 b	3.8 ± 0.9 a	162.2 ± 40.3 g
6	Control soaked in pH 7.5 buffer	127 ± 9 b	14.9 ± 1.1 b	2.8 ± 0.4 b	215.0 ± 30.3 e

[a] Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

Table 10. Mean and Standard Deviation Values for Water Vapor Permeability and Oxygen Gas Permeability of Various Wheat Gluten-Based Films[61]

Treatment Number	Film Treatment	WVP[b] ($\times 10^{-11}$ g/m·sec·Pa)	O_2GP[c] (amol/m·sec·Pa)
	Wheat gluten (control)	5.6 ± 0.3 c	2.0 ± 0.2 b–d
1	Wheat gluten with mineral oil	4.1 ± 0.1 e	1.7 ± 0.2 d
2	Wheat gluten with sodium sulfite	6.1 ± 0.4 b	2.1 ± 0.2 bc
3	Wheat gluten with hydrolyzed keratin	4.3 ± 0.3 e	0.3 ± 0.1 e
4	Control soaked in 15% (w/w) lactic acid	5.4 ± 0.3 c	2.1 ± 0.2 bc
5	Control soaked in 1*M* calcium chloride	4.8 ± 0.4 d	1.9 ± 0.1 cd
6	Control soaked in pH 7.5 buffer	4.8 ± 0.2 d	2.3 ± 0.3 b

[a] Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
[b] Evaluated at 23°C with 11.1% rh on one side of the films and 0% rh on the other.
[c] Evaluated at 23°C and 0% rh, where 1 amol = 10^{-18} mol.

From Table 9, films containing mineral oil (Film 1) showed a little decrease on tensile strength. Adding sodium sulfite (Film 2) or soaking in pH 7.5 solution (Film 6) only resulted in the films a little bit stronger than control film. Hydrolyzed keratin played a role as plasticizer to gluten (Film 3) that caused decreasing in tensile strength and increasing in elongation. Film

4, which was soaked in lactic acid solution, was the thickest. This could be attributed to deposition of lactic acid on film surface which could be proved by increased surface density. Also lactic acid worked like a plasticizer leading to the highest elongation but lowest tensile strength. By soaking in aqueous calcium chloride solution (Film 5), films obtained the highest tensile strength but the lowest elongation (an increase of almost 50%).

From Table 10, mineral oil (Film 1) and partial substitution of gluten with hydrolyzed keratin (Film 3) showed the best positive influence on water resistance and decreased water vapor permeability. Film 5 and 6 showed smaller reductions (about 15%) on water vapor permeability. In contrast, adding sodium sulfite (Film 2) resulted in films with increased water vapor permeability. Oxygen permeability of all films prepared in this study was low, especially Film 3, which contains keratin, was the only one with lower oxygen permeability (by about 80%) than that of the control film.

Each experiment group showed a conclusion:

Film 1. Mineral oil could increase film water resistance.

Film 2. Sodium sulfite can improve tensile strength of films. Sodium sulfite is a reducing agent to break linkages between protein chains. Chain mobility and chances of bonding upon drying was increased. Eventually, tensile strength increased.

Film 3. Addition of hydrolyzed keratin protein was good for water vapor and oxygen barrier properties. It indicated that linkages developed between molecular chains.

Film 4. Lactic acid showed its advantage as a plasticizer in gluten film forming process.

Film 5. $CaC1_2$ could promote crosslinking between protein structures so that the mechanical properties of gluten films could be improved.

Film 6. Adjusting pH of the bath could influence the mechanical properties and water vapor permeability.

CHAPTER 4. A REVIEW of BLENDS of CELLULOSE (DERIVATIVES) with WHEAT GLUTEN and OTHER BIOPOLYMERS

4.1 Composite Biofilms of Cellulose Acetate Phthalate and Wheat Gluten

It is well known that both cellulose (derivative) and gluten proteins are natural biopolymers which are abundant, cheap, biodegradable and renewable. Because of their good properties, both cellulose (derivative) and gluten proteins are good choices for making edible films to protect foods.

To develop a kind of edible biofilms, Fakhouri et al, did a research on composite films made of wheat gluten and cellulose acetate phthalate blend.[67] The films were prepared from different thickness and component concentrations. Water vapor and oxygen permeability, water and acid solubility and mechanical properties were tested. The results showed that the mixture improved film properties which were better than each of the individual component completely.

The gluten films ware produced from a solution of vital wheat gluten, absolute ethanol, distilled water and glycerol at pH 10 which was adjusted by ammonium hydroxide. After the solution was mixed completely by magnetic stirring at 70℃, it was casted evenly on a Teflon-coated glass surface and dried at room temperature for 24 hours.[67]

In this research, the cellulose acetate phthalate (CAP) films were prepared from a solution of CAP, sodium phosphate, glycerol. The way to produce film was same as that to made gluten films. Solution was spread evenly over Plexiglas plates in aliquots of 8mL, 12mL and 16mL, which could control the thickness of film.

The composite films were made from a solution mixed by the vital wheat gluten film-forming solution and CAP film-forming solution at ratios of 1:1, 1:4 and 4:1 under magnetic stirring. Then made films following the way of producing gluten films. Solution was spread evenly over Plexiglas plate.[67]

Table 11. Water vapor permeability and solubility in water and acid of the film[67]

Film	Water vapor permeability (gmm/m^2dkPa)*	Solubility in water (%)*	Solubility in acid (%)*
CAP:glu (1:4)/8ml	-	-	-
CAP:glu (1:4)/12ml	-	-	-
CAP:glu (1:4)/16ml	12.19 ± 0.25^{a}	100.00^{a}	50.69 ± 1.64^{b}
CAP:glu (1:1)/8ml	3.75 ± 0.17^{b}	100.00^{a}	100.00^{a}
CAP:glu (1:1)/12ml	3.83 ± 0.13^{b}	100.00^{a}	100.00^{a}
CAP:glu (1:1)/16ml	4.11 ± 0.20^{b}	100.00^{a}	100.00^{a}
CAP:glu (4:1)/8ml	3.81 ± 0.01^{b}	100.00^{a}	100.00^{a}
CAP:glu (4:1)/12ml	3.64 ± 0.0^{b}	100.00^{a}	100.00^{a}
CAP:glu (4:1)/16ml	3.76 ± 0.26^{b}	100.00^{a}	100.00^{a}
CAP/8ml	6.03 ± 0.35^{ab}	100.00^{a}	30.50 ± 1.56^{c}
CAP/16 ml	10.05 ± 0.47^{a}	100.00^{a}	33.14 ± 1.87^{c}
Gluten	8.61 ± 1.03^{ab}	22.70 ± 4.10^{b}	-

*Mean and standard deviation of replicates. $^{a-c}$ Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

The data of films with the highest concentration of gluten was not collected because of the brittleness. Table 11 shows that the water vapor permeability composite films increased with the increase of gluten concentration. The composite films (1:1, 4:1) with all thicknesses had much better water barrier properties than both cellulose and gluten only films. Both composite and cellulose films were totally soluble in water. The composite films were dissolved completely in acid except the one with the highest gluten concentration with 50% solubility in acid.[67]

Table 12. Mechanical properties and oxygen permeability of the films[67]

Film	Tensile strength (MPa)*	Elongation at break (%)*	Oxygen permeability ($cm^3\mu m/m^2dkPa$)*
CAP:glu (1:4)/8ml	-	-	-
CAP:glu (1:4)/12ml	-	-	-
CAP:glu (1:4)/16ml	1.32 ± 0.11^{e}	4.06 ± 0.78^{e}	-
CAP:glu (1:1)/8ml	7.57 ± 0.18^{de}	4.65 ± 1.63^{de}	-
CAP:glu (1:1)/12ml	10.89 ± 2.19^{cd}	5.27 ± 0.85^{cde}	-
CAP:glu (1:1)/16ml	16.18 ± 3.11^{bc}	4.91 ± 1.01^{de}	22.21 ± 1.23^{b}
CAP:glu (4:1)/8ml	12.87 ± 2.41^{cd}	4.79 ± 1.96^{de}	-
CAP:glu (4:1)/12ml	22.60 ± 1.63^{ab}	6.79 ± 0.98^{bd}	-
CAP:glu (4:1)/16ml	23.78 ± 5.12^{a}	8.82 ± 0.68^{b}	-
CAP/8ml	11.08 ± 3.29^{cd}	7.86 ± 1.23^{bc}	-
CAP/16 ml	11.76 ± 4.31^{cd}	8.60 ± 1.36^{b}	-
Gluten	5.25 ± 0.24^{de}	215.30 ± 12.20^{a}	41.02 ± 0.86^{a}

*Mean and standard deviation of replicates. [a-e] Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

Table 12 demonstrates that composite films, tensile strength decreases with increasing gluten concentration that were still higher than both cellulose and gluten films. Tensile strength increased with increasing thickness for both composite and cellulose films. With the increase of gluten concentration elongation at break decreased. The highest elongation of composite films were still smaller than those cellulose and gluten films. The thicker films showed higher tensile strength and elongation at break in both composite and cellulose films. Because of the brittleness, oxygen permeability in only one group of composite films (1:1, 16mL) and gluten films could be measured. It showed better oxygen barrier property of composite films than gluten only films.[67]

4.2 Biocomposites from wheat gluten and hydroxyethyl cellulose

Because synthetic polymers are generally not biodegradable, research efforts of natural polymers dramatically increased in recent years. These materials have a number of advantages but a high moisture content and low mechanical properties limit their usage in some applications. To solve these problems, scientists tried to use fibers as fillers into plant proteins matrixes to produce biocomposites with better mechanical properties and water resistance. Because of the abundant resources, low cost, good biodegradability and suitable properties, Song et al, used wheat gluten (WG) and hydroxyethyl cellulose (HEC) as raw materials blended with glycerol to make better ecofriendly biocomposites by thermo-molding at 120℃.[68] The effects of HEC concentration on moisture absorption, glass transition and mechanical properties were investigated respectively.

Wheat gluten (WG, protein content ≥75 wt%) and highly substituted hydroxyethyl cellulose (HEC, degree of substitution from 60 to 70 wt%) and glycerol were hand-mixed in a mortar and then mixed on a three-rolling mixer at room temperature. Then the mixed material was thermo-molded at 120℃ under 10 MPa for 5–30 min. The ratio of WG to glycerol was 7 to 3 in weight while the HEC content was controlled from 0 to 31.8wt% with respect to the total mass of the biocomposites.[68]

Figure 23 shows moisture absorption of WG/HEC/glycerol biocomposites at RH of 75%. As can be seen in this Figure, the HEC content increase from 0 to 23.1% caused a significant decrease of moisture value from 14.5 to 9.6%.[68]

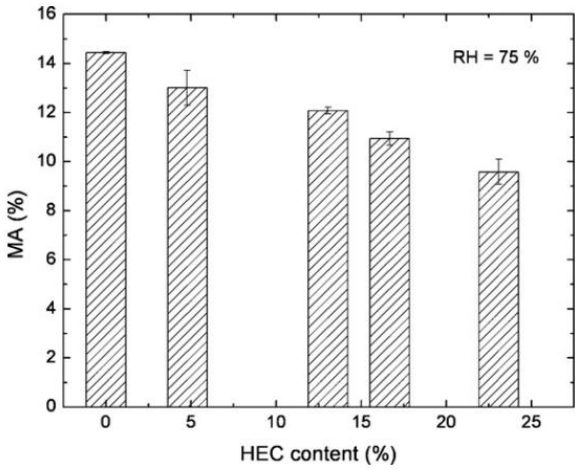


Figure 24. Influence of HEC content on moisture absorption (MA) of the biocomposites thermo-molded at 120℃ for 5 min[68]

As shown in SEM micrographs (Figure 24), gluten and cellulose fibers retained their original shapes in composite samples. SEM micrographs taken at the tensile break surface show the tough fracture with delamination and corrugation. Spherulite-like domains with different sizes are observed in both gluten only and the composite materials. The larger domains are HEC particles and the smaller domains are gluten particles. The formation of microfibrils are observed which are caused by uniaxial delamination. Those microfibrils can lead to cavitation in materials. The SEM micrographs reflects that the introduced HEC doesn't affect the morphology of the gluten matrix.[68]

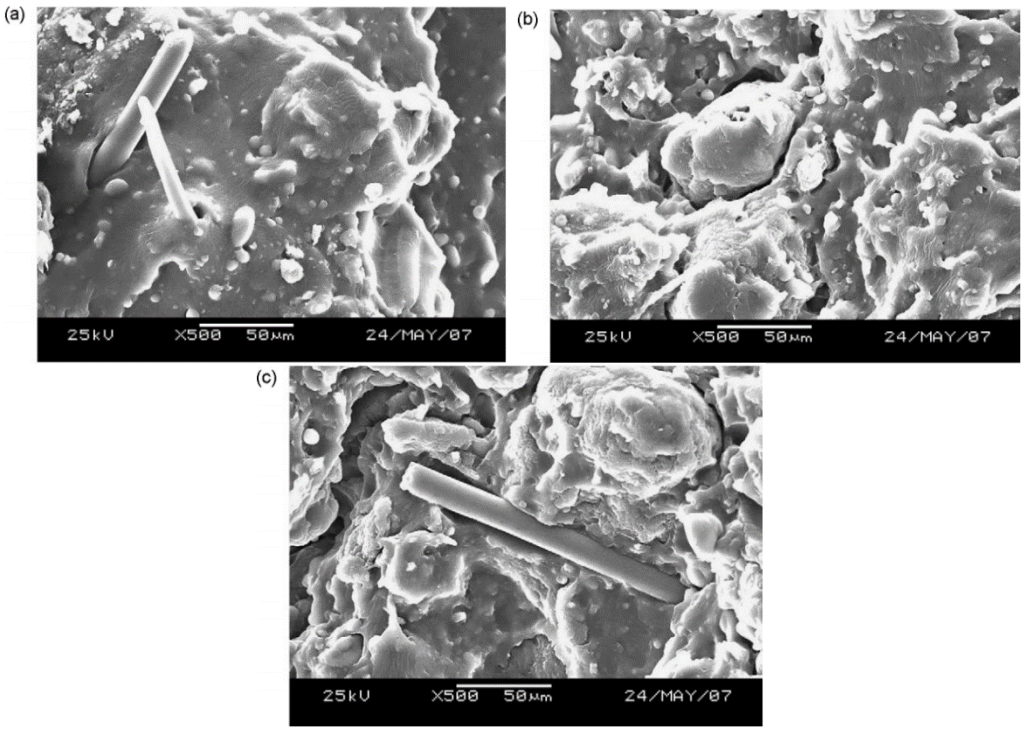


Figure 25. SEM micrographs taken at the tensile break surfaces of the WG/HEC/glycerol biocomposites with a composition of a) 7/0/3, b) 7/1/3 and c) 7/3/3[68]

The strain-stress relationship of samples thermo-molded at 120℃ for 5 min is shown in Figure 25. The gluten-only bioplastic exhibits the best extensibility but the lowest breaking stress. With the increase of HEC content, stress increases but strain decreases. In Figure 26, it shows that Young's modulus and tensile strength increase when HEC content increases. However, the strain at break shows a decrease trend when HEC content increases. The results suggest that HEC filler can positively enhance the biocomposite because HEC can form hydrogen bonding and entangle with gluten proteins.[68]

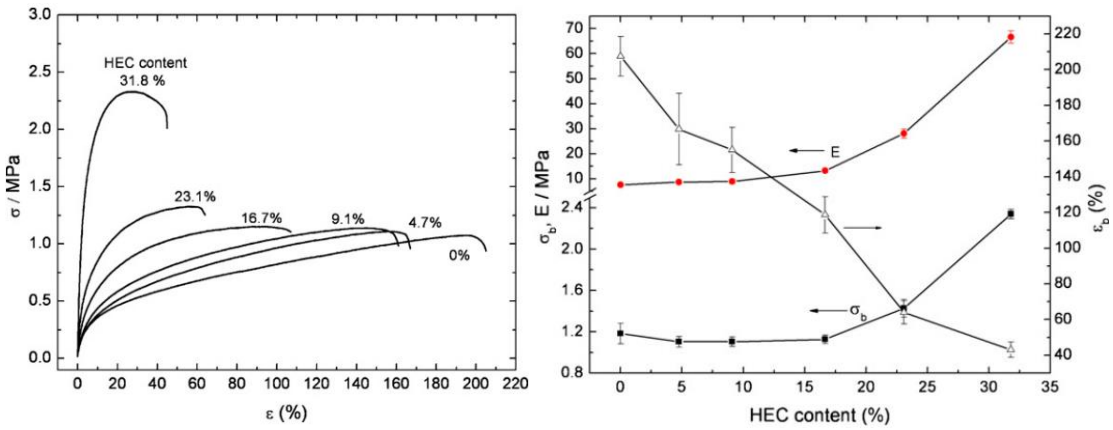


Figure 26 (left). Stress–strain (δ-ε) relationship of the biocomposites thermo-molded at 120℃ for 5 min[68]

Figure 27 (right). Influence of HEC content on Young's modulus (E), tensile strength (δ_b) and strain at break (ε_b) for the biocomposites thermo-molded at 120℃ for 5 min[68]

Figure 26 shows storage modulus E' and loss factor tanδ as a function of temperature T for the biocomposites thermo-molded at 120℃ for 5 min. In Figure 27, E' decreases dramatically and tanδ shows two peaks with an increasing temperature. The higher temperature peaks are related to T_g of gluten proteins and the lower temperature peaks are related to T_g of glycerol in the gluten matrix. The data is also collected in Table 13. The peak value of tanδ decreases gradually with increasing HEC content. The T_g of both gluten and glycerol vary a little bit with an increasing HEC content.[68]

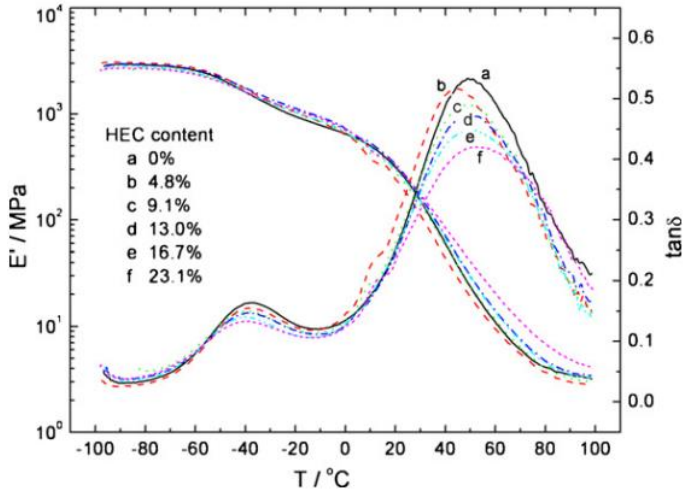


Figure 28. Storage modulus (E) and loss factor (tanδ) as a function of temperature T for the biocomposites thermo-molded at 120℃ for 5 min[68]

Table 13. Glass transition temperatures and corresponding tanδ maximum values for the gluten-rich and the glycerol-rich phases of the biocomposites thermo-molded at 120℃ for 5min[68]

HEC content (wt%)	T_{g1} (°C)	Tanδ at T_{g1}	T_{g2} (°C)	Tanδ at T_{g2}
0	49.2	0.533	−36.7	0.163
4.8	44.0	0.516	−38.2	0.155
9.1	50.0	0.489	−39.8	0.149
13.0	49.2	0.473	−38.3	0.147
16.7	50.4	0.448	−40.0	0.140
23.1	53.3	0.417	−39.0	0.133

The relationship between molding time and E, δ_b and ε_b is shown in Figures 28 and 29 shows the influence molding time on tanδ. The increasing molding time from 5 to 30 min causes increasing of tensile strength and Young's modulus but the strain remains almost unchanged. It is obvious that the tanδ decreases with increasing molding time from 5-30 min.

It can be ascribed to the increasing crosslinking density of wheat gluten network and explain the increase of E and δ_b for the composite.[68]

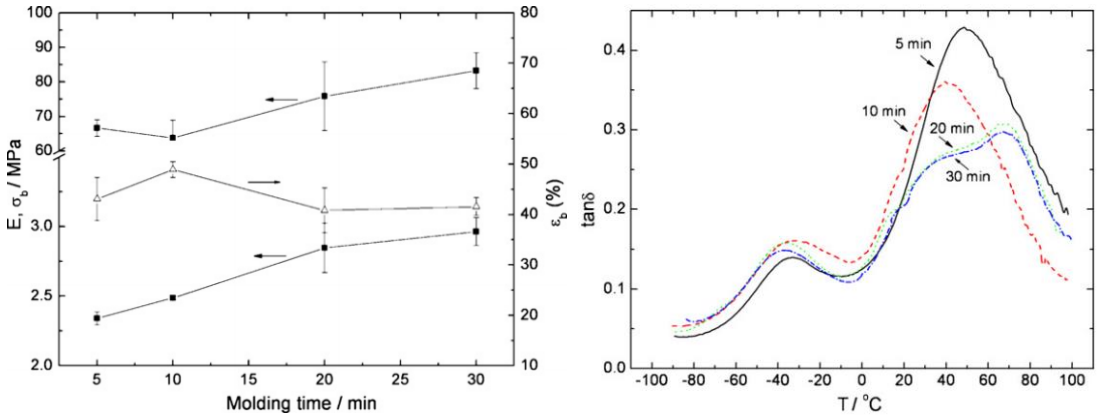


Figure 29 (left). Influence of molding time on Young's modulus (E), tensile strength (δ_b) and strain at break (ε_b) for the biocomposites containing 31.8 wt% HEC.[68]
Figure 30 (right). Influence of molding time on loss factor tanδ curve for the biocomposites containing 31.8 wt% HEC.[68]

4.3 Wheat Gluten/Methylcellulose Binary Blend Film

It has been proved that films made from a solution of wheat gluten and cellulose blend show better properties than those films made of one of both material individually. So the composition of the film-forming solution is an important factor. Besides that, the method used to produce films can also affect film properties. Zuo et al., set up experiments to compare the properties of wheat gluten and methylcellulose binary blend films made by casting and compression molding, respectively.[69] Morphology, tensile properties and water permeability were investigated. The results showed that the casting films had better mechanical properties, process ability and barrier properties than molded films.

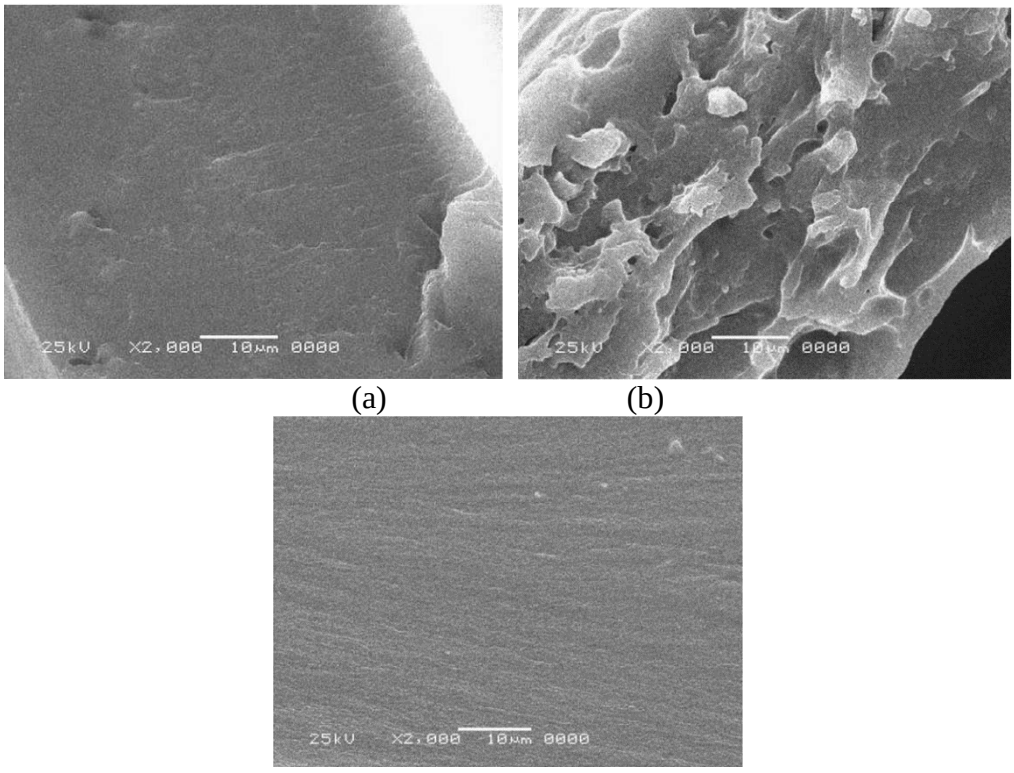
Two groups of films produced by casting and compression molding were made as follows:

Casted films: Wheat gluten (protein content ≥75%) and methylcellulose (MC) powders were dissolved in 13wt% aqueous ammonia under continuous magnetic stirring. Glycerol was added into the mixture as a plasticizer. The ratio of mass of methylcellulose to wheat gluten, x_{MC}, was controlled and varied from 0 to 1. Films were made of 40 g film-forming solution by poured onto plastic Petri dish with an inner diameter of 90 mm and dried at room temperature followed by detaching at 100℃ and 125℃, respectively, for 30 min to crosslink the protein phase.

Compression molded films: WG, MC and glycerol were mixed on a three-rolling mixer at room temperature. Then mixture was thermo-molded at 125℃ and 15 MPa for 10 min.

Because the plasticization effect of glycerol to MC without water was poor, the mixture at x_{MC} >0.5 could not form coherent mixture thus it could not be cast into films.

Figure 30 (a-c) show the SEM micrographs of casting film with different MC ratio. The WG- (x_{MC} = 0) and MC-only (x_{MC} = 1) films show a smooth fracture surface and homogeneous phase (Figure 8a and c). However the fractured surface of blend films show rough and phase-separated heterogeneous morphologies (Figure 9b) which reflects these two materials are not miscible or incompatible.[69]



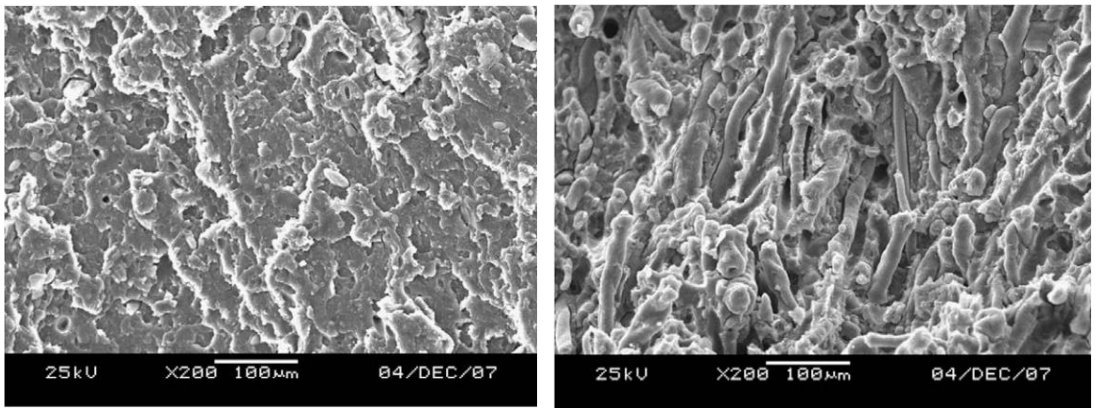
(a) (b)

(c)

Figure 31. SEM images of the casting films with (a) x_{MC} = 0, (b) x_{MC} = 0.2 and (c) x_{MC} = 1[69]

Figure 31 (a, b) show the SEM images of the compression molded films, WG-only (x_{MC} = 0) and WG/MC blend (x_{MC} = 0.3). Both images show rough fracture surface, displaying

tough fracture with delamination. In WG/MC blend films, the fiber-shaped domains, which are observed in the matrix, are MC particles.



(a) (b)

Figure 32. SEM images of the molded composites with (a) x_{MC} = 0 and (b) x_{MC} = 0.3[69]

Table 14 shows the relationship between H, E, δ_b and ε_b of the casting blend film and x_{MC}, treated at 100℃ and 125℃, respectively. Thickness of both films decreased with increasing x_{MC} and the change of film treated at 125℃ was greater than those treated at 100℃. The value of E, δ_b and ε_b of both two kinds of films increased when x_{MC} increased. It was observed that the E and δ_b value of films treated at 125℃ were higher than those treated at 100℃ which could be related to the increase in cross-linking density at higher temperature.

Table 14. Effects of mixing ratio x_{MC} on thickness H, Young's modulus E, tensile strength δ_b and strain at break ε_b for the casting films annealed at 100 C and 125 C, respectively[69]

x_{MC}	100 °C				125 °C			
	H (μm)	E (MPa)	σ_b (MPa)	ε_b (/%)	H (μm)	E (MPa)	σ_b (MPa)	ε_b (%)
0	98.8 ± 2.5	60.5 ± 9.3	1.7 ± 0.1	19.2 ± 4.8	106.0 ± 15.1	187.0 ± 39.5	5.2 ± 1.4	10.2 ± 3.8
0.20	91.5 ± 4.8	123. ± 7.1	4.7 ± 0.2	25.0 ± 2.0	117.0 ± 18.4	160.3 ± 28.8	6.8 ± 0.7	25.4 ± 2.5
0.30	85.0 ± 4.1	166.5 ± 22.4	9.0 ± 1.1	30.8 ± 1.5	86.5 ± 5.9	189.6 ± 12.3	9.1 ± 0.8	26.2 ± 3.9
0.40	95.0 ± 5.0	159.9	8.0 ± 0.3	28.9 ± 1.1	76.8 ± 14.2	326.8 ± 4.2	15.7 ± 0.3	30.6 ± 1.1
0.51	79.7 ± 4.0	261.0 ± 42.7	13.6 ± 1.4	28.6 ± 2.4	88.7 ± 9.9	284.4 ± 29.0	16.1 ± 1.5	33.3 ± 3.4
0.60	83.2 ± 6.4	403.9 ± 34.3	21.6 ± 1.2	37.1 ± 5.5	84.7 ± 9.2	484.5 ± 53.5	20.2 ± 2.2	40.0 ± 4.5
0.70	64.7 ± 6.4	415.8 ± 29.3	20.6 ± 1.4	34.2 ± 3.8	83.3 ± 11.5	578.7 ± 45.5	25.5 ± 1.6	27.3 ± 1.1
0.80	53.0 ± 2.6	443.9 ± 19.2	21.2 ± 1.8	28.2 ± 2.1	78.3 ± 15.3	642.6 ± 44.3	23.8 ± 0.6	17.8 ± 3.7
0.90	63.0 ± 5.3	508.8 ± 102.1	27.4 ± 5.0	36.6 ± 7.1	75.0 ± 13.2	710.3 ± 46.8	38.2 ± 3.4	42.3 ± 3.9
1.00	77.3 ± 4.6	609.9 ± 84.3	44.0 ± 0.2	41.0 ± 9.21	60.0 ± 14.1	680 ± 12.48	45.9 ± 1.8	39.3 ± 1.4

Table 15 shows how x_{MC} influenced the E, δ_b and ε_b of compression molded films. The E value of the sample showed a decreasing trend when addition of MC was lower than 15 wt%. It could be attributed to poor plasticization of MC with glycerol. Furthermore, because of the filler effect of MC fibers in WG matrix, the E value increased slightly. The value of δ_b and ε_b decreased with an increasing x_{MC} because of the poor interfacial interaction between MC fiber and WG matrix. It is common for composites to break at the weak interface which was proved in Figure 35b. By comparison between casting blend films and compression molded, the casting blend films had better mechanical properties than those compression molded films.

Table 15. Influences of mixing ratio x_{MC} on Young's modulus E, tensile strength δ_b and strain at break ε_b for the molded composites[69]

x_{MC}	E (MPa)	σ_b (MPa)	ε_b (%)
0	34.4 ± 3.8	4.6 ± 0.3	157.2 ± 22.0
0.08	19.4 ± 2.7	3.0 ± 0.5	130.9 ± 26.5
0.15	19.1 ± 1.1	2.5 ± 0.3	93.8 ± 12.4
0.30	25.6 ± 2.7	2.3 ± 0.2	33.8 ± 5.3
0.50	27.7 ± 5.2	1.4 ± 0.4	10.2 ± 2.6

The moisture absorption (MA) and water vapor permeability (WVP) of casting blend films at different conditions were shown in Table 16. At 29% RH, MA did not change obviously. But at 87% RH, MA increased rapidly from17% to 33% when x_{MC} increased from 0 to 1. MA increased with increasing RH.[69]

Because of the brittleness of WG film, the measurement of WVP failed. The WVP value of blend films and MC film were collected in Table 16. WVP at 87% changed a little while WVP increased at 29% and 55% RH with increasing x_{MC}. But it is clear that at same x_{MC}, WVP increased rapidly with increasing RH. So the addition of MC decreased the water barrier properties of casting films. Casting blend films with lower MC content had better water barrier properties.

Table 16. Influences of mixing ratio x_{MC} on MA and WVP of the casting films thermally treated at 125 C[69]

x_{MC}	MA (wt%)		WVP (10^{-11} g m^{-1} s^{-1} Pa^{-1})		
	29% RH	87% RH	29% RH	55% RH	87% RH
0	7.5 ± 0.8	17.3 ± 2.4	ND[a]	ND[a]	ND[a]
0.20	/	/	1.97 ± 0.42	7.58 ± 0.40	26.06 ± 4.75
0.30	5.0 ± 1.3	17.6 ± 3.2	3.08 ± 0.78	7.90 ± 1.14	22.55 ± 5.20
0.40	/	/	2.45 ± 0.48	7.90 ± 1.43	24.05 ± 5.19
0.51	/	/	4.46 ± 0.55	10.18 ± 1.65	24.73 ± 5.30
0.60	4.3 ± 0.9	22.8 ± 0.2	4.20 ± 0.78	10.04 ± 1.56	23.20 ± 4.29
0.70	5.5 ± 0.3	21.8 ± 1.4	4.02 ± 0.50	8.55 ± 1.24	17.86 ± 2.44
0.80	3.5 ± 0.9	25.0 ± 2.4	5.27 ± 0.53	14.88 ± 2.83	27.50 ± 3.10
0.90	/	/	5.97 ± 0.30	9.52 ± 1.49	19.27 ± 2.04
1.00	6.4 ± 0.8	33. 4 ± 2.5	9.07 ± 0.38	16.81 ± 2.60	30.38 ± 3.94

[a] WVP of the WG film is not detectable due to the film crack during measurement.

The casting WG/MC blend films show better mechanical properties than the compression molded WG/MC composite films. The mechanical properties were significantly improved with

an increasing x_{MC}. WVP also increased with an increasing x_{MC}. By controlling x_{MC}, the water barrier properties could be controlled. The blending method was proved to be the efficient way to make edible films with tailored mechanical and moisture barrier properties.[69]

4.4 Cellulose/Soy Proteins and Starch Blend Films from ED/KSCN Solvent System

As shown in previous chapter, the interface between gluten particles and a cellulose derivative serves as weak point lowering mechanical properties of composite products. To solve this problem, researchers have done a lot of work to study the properties of films produced by dissolving cellulose (derivatives).

The ED/KSCN solvent system proved to be a useful and efficient solvent for cellulose. Douglass and Zhu used this solvent system to dissolve cellulose, soy protein or starch to make films.[17,30] The results showed that the cellulose/soy protein blend could be dissolved completely in ED/KSCN solvent and the films had good mechanical properties that could be used as food package, filter and other uses.

7 wt% of cellulose or cellulose blend (cellulose/soy proteins or cellulose/starch) was completely dissolved in ED/KSCN (65/35) solvent by heating a flask in an oil bath at 90℃ for 2 to 4 hours. Then the polymer solution was poured on a polyester film on a glass casting board and casted by using a 20 or 30 mil casting bar. After casting, the film was moved into the methanol bath for coagulation followed by drying in the oven.[17,30]

During dissolution the solubility of each sample was studied. (Figure 32) Cellulose and cellulose/soy protein blend were completely dissolved in ED/KSCN solvent after 90 minutes while some insoluble particles of waxy maize starch were still in the solvent. The ED/KSCN solvent system showed the outstanding dissolving ability to cellulose and soy protein.[17]

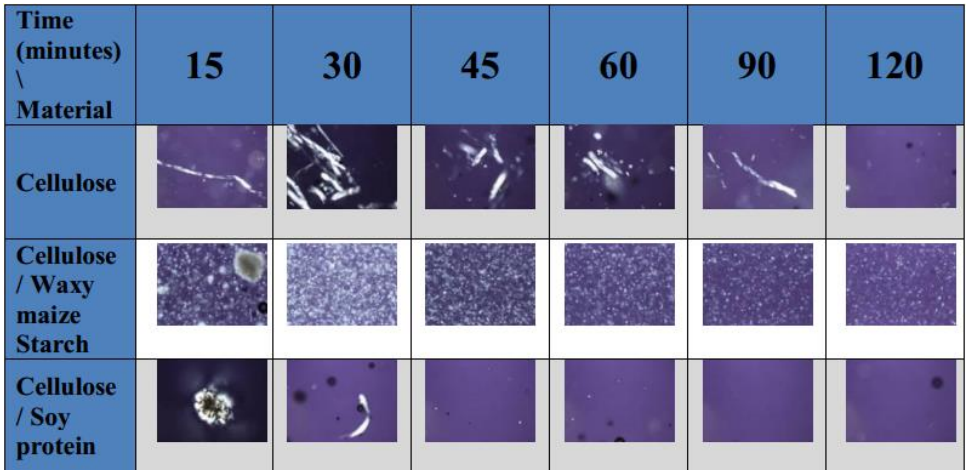
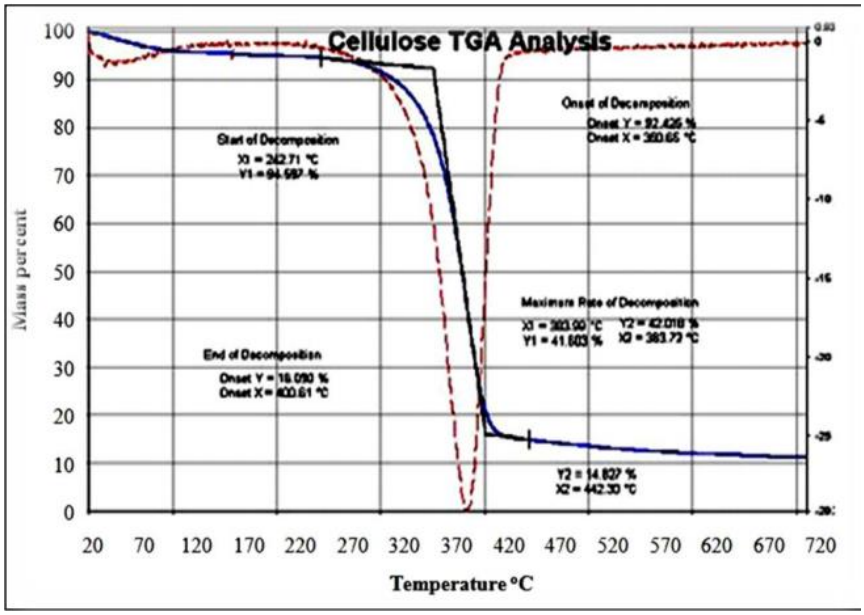


Figure 33. Time elapse visual study, using cross polarization microscopy, of dissolution of cellulose variety blends in solvent[17]

Thermogravimetric analysis (TGA) and wide angle X-ray scattering (WAXS) were performed to study and detect the changes of crystalline structure of raw materials and membranes. Figure 33 (a & b) shows the TGA curves for wood pulp and cellulose membrane made by Douglass. Comparing these two curves confirms that wood pulp shows a higher onset temperature (351℃) and offset temperature (400℃) as well as a lower char content than cellulose membranes. These differences may be caused by formation of a new cellulose structure during film coagulation in methanol. Indeed, this suggestion can be supported by WAXS images shown in Figure 34 (a & b). Figure 34 (a) for native cellulose sample represents cellulose I with peaks at 16, 17 and 23^{o} 2θ. Figure 34 (b) shows the curve of regenerated cellulose membrane with different peaks at around 13 and 20-22^{o} 2θ that can be assigned to cellulose II.[17]

(a)



(b)

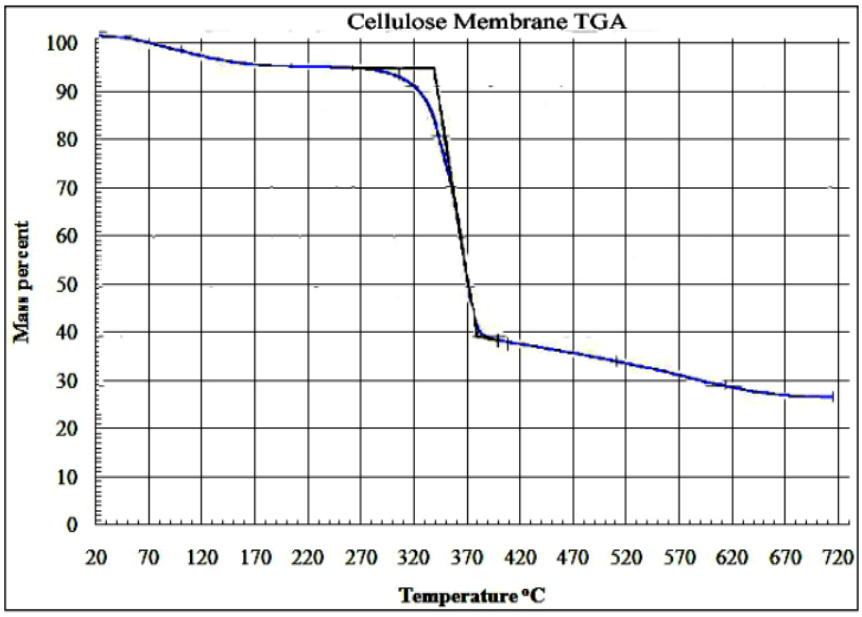
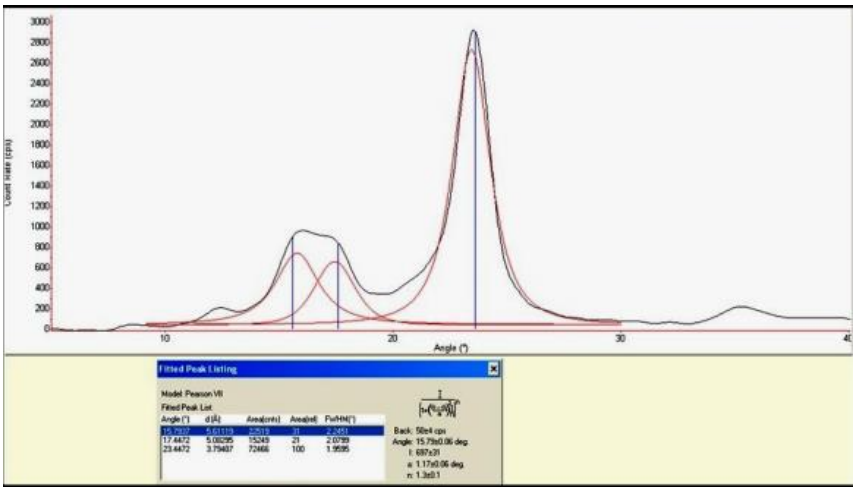


Figure 34. TGA analysis curve for (a) raw wood pulp; (b) TGA analysis curve for Douglass cellulose membrane[17]

(a)



(b)

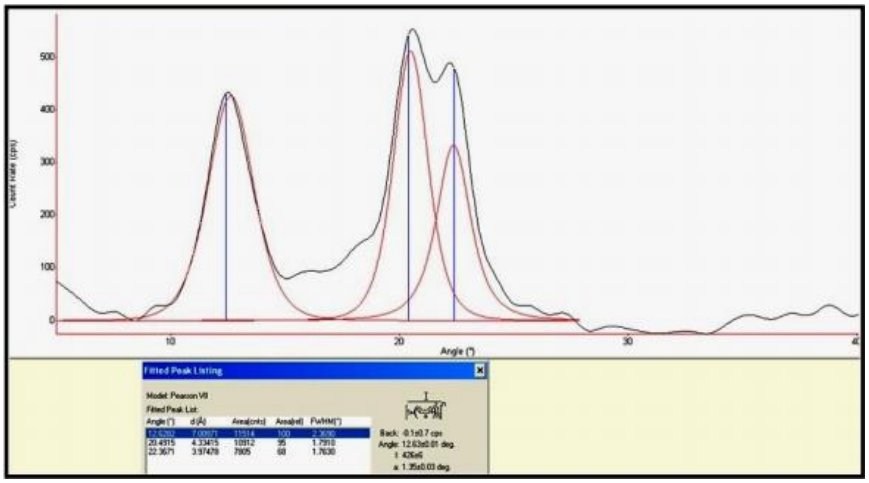


Figure 35. WAXS curve of raw pressed cellulose from (a) refined wood pulp; (b) WAXS curve of Douglass cellulose membrane[17]

The tensile test data of films made by Douglass and Zhu are shown in Table 17.[30] All the samples with different composition had good mechanical properties. Because Yidan provided extra pressure on films to improve the uniformity during coagulation, those films had a higher modulus and a lower strain at break than Eugene Douglass's films. In Yidan's experiment, the

cellulose blend films had a higher modulus but a lower failure strain than cellulose films. The soy protein concentrate (SPC) had a stronger effect on mechanical properties than soy protein isolate (SPI). The results showed that soy protein could influence the mechanical properties of cellulose films and cellulose/protein blend could be used to produce films with good mechanical properties. Yidan also used glutaraldehyde (GA) to crosslink blend films. The crosslinked films showed improved mechanical properties.[30]

Table 17. List of tensile test data of various membranes[30]

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%)

In Yidan's research, the water absorption and water/oxygen barrier properties were measured to determine the usage of cellulose/protein films in various applications. The results reflected that the SPC containing films could pick up a large amount of water and soy protein could be slightly extracted by water. The cellulose/SPC film showed very low oxygen transmission rate (OTR), 4.53 cc/m^2 per day and very high water vapor transmission rate

(WVTR), 829.7 g/m^2 per day. The WVTR value increased with an increasing amount of absorbed water.[30]

The studies of Douglass and Zhu provided good examples for producing films from cellulose/protein blend dissolved in ED/KSCN solvent system. The ED/KSCN solvent system was proved to be an efficient solvent to dissolve cellulose and soy protein. The films made in these studies had good mechanical properties. Their work promoted the interest in studying properties of cellulose/protein blend films.[17,30]

CHAPTER 5. DEVELOPMENT and CHARACTORIZATION METHODS of MEMBRANES

5.1 Materials

The Buckeye VFC wood pulp was used as the raw material with a degree of polymerization (DP) of around 600. The pulp sheet was cut into thin slices and then ground into fine powder followed by drying in an oven for 24 hours to remove the moisture prior to experiments.

The gluten protein was Arise® 8000, provided by MGP. It is a wheat protein isolate prepared by removing starch from wheat flour and carefully drying the remaining high protein fraction to retain the native viscoelastic properties. This gluten protein consists of protein (≥94%), moisture (≤8%) and ash (≤2%). The sample was dried before used.

The ED/KSCN (65/35) solvent system was chosen to dissolve cellulose and cellulose/gluten blend because this system has been proved as an outstanding system with high dissolution efficiency, low cost and ecofriendly properties by Douglass's and Zhu's study. This solvent consisted of 65 wt% reagent grade ≥99% ethylene diamine (ED) and 35 wt% reagent grade potassium thiocyanate (KSCN). Both chemicals were provided by Sigma-Aldrich. KSCN was dried in an oven at 60℃ overnight to remove moisture which is necessary. The correct amounts of ED and KSCN were mixed in a flask with magnetic bar at a low temperature (60℃) and heated in the oil bath until KSCN was completely dissolved in ED. The ED/KSCN solvent was allowed to equilibrate after dissolution.

Reagent grade methanol (≥99.8%) provided by Sigma-Aldrich was used for making coagulation bath.

Membrane-casting tools: a casting board with a glass plate, polyester films for holding substrate, and a casting bar with thickness ranging from 5-50 mil.

Solvent dissolution tools: a Pyrex® three-neck round bottom flask to perform the dissolution of gluten protein and other polymers, a water-cooled condenser for condensation of ED, a stirring system consisted of a Teflon© blade, a glass rod and an electric motor.

5.2 Experimental Procedures of Raw Materials Dissolution

The ED/KSCN solvent system was chosen to dissolve cellulose and gluten protein because it was proved as an efficient, low-cost and ecofriendly solvent system by Douglass's and Zhu's studies.[17,30] First of all, solubility of gluten in the ED/KSCN solvent system was verified to make sure it could be dissolved. Then cellulose-only membranes were cast to verify the method of making membranes and the dissolution efficiency of ED/KSCN solvent system. Because Douglass and Zhu only proved the solubility of soy protein in ED/KSCN, solubility and film-forming ability of gluten protein in this system was necessary. Then the cellulose/gluten protein blend membranes with a different ratio of raw materials, 90/10, 80/20, 70/30 and 60/40 were produced. The same procedures were used to dissolve cellulose and gluten protein and coagulate these polymers.

5.3 Dissolution of Cellulose

Before dissolving cellulose, 6 g of fine and dried cellulose powder and 94 g ED/KSCN solvent was weighted. Then the dried powder was added into a three-neck round bottom flask followed by the addition of ED/KSCN solvent. The left neck was connected to water-cooled condenser. A Teflon© blade attached to a long ground glass rod connected with an elector motor was inserted into the center neck for the purpose of stirring and mixing the solvent thoroughly. The right neck was plugged by a glass (or rubber) stopper. During stirring, the flask was immersed and heated in glycol oil bath at 90ºC, which was proved the best temperature for cellulose dissolution. Thermometer was used to monitor the temperature from time to time. The mixture was stirred for 3-4 hours until complete dissolution was achieved.

When cellulose was dissolved completely, the heater was turned off and the solution was poured it into a glass container for film-forming use. The flask was cleaned with cold water to coagulate and discard the remaining polymer residue.

5.4 Dissolution of Gluten Protein

To study the solubility and film-forming ability of gluten, the correct amount of gluten was dissolved in ED/KSCN solvent to make 6, 10, 20, 30, 40 and 50 wt% polymer solutions. Because the procedure to dissolve cellulose and gluten protein are exactly same, it will not be described again.

5.5 Dissolution of Cellulose/Gluten Protein Blend

The first step of dissolution of cellulose/gluten protein blend was preparation of the physical mixture of cellulose and gluten protein powder. Four 6 g samples with different ratio of cellulose to gluten protein, namely 90/10, 80/20, 70/30 and 60/40 were prepared. Dried cellulose and gluten protein powder were weighed out separately and mixed together before adding to the three-neck round bottom flask. It was important to avoid the aggregation of gluten protein that is not good for mixing, when the ED/KSCN solvent is added. The equipment and temperature set up were same as for cellulose dissolution. Dissolving time was a little bit shorter, around 3 hours. A clear polymer solution was poured into a glass container for storage. The procedure for cleaning the flask was the same as for cellulose.

5.6 Experimental Techniques of Membrane Formation

All membranes were always casted with a casting bar on the casting board followed by coagulation in methanol bath and drying in a vacuum oven.

The casting board was placed on the flat workbench. A polyester film was put on the surface of casting board for holding the substrate. Air bubbles between polyester film and casting board surface should be eliminated. The casting bar had a casting thickness range from 5 to 50 mil. The best casting thickness for casting cellulose/gluten protein blend films was 20 – 25 mil and 25 mil was used in the experiments. However, the best casting thickness for casting cellulose-only films is 30 mil that was used in the experiments. It was noted in Douglass's research that if the thickness of cellulose-only film was lower than 30 mil, the membrane was too brittle and broke when coagulated; if the thickness was higher than 30 mil, the film was too thick to coagulate completely and the ED/KSCN solvent was trapped in the film and it could not be extracted completely.

Prior to pouring on the polyester film, the glass container with film-forming solution was heated in the oil bath at 90℃ to allow it to flow smoothly. The solution was poured into the casting bar on the polyester film carefully from left to right with the constant speed. Then the bar was dragged instantly and slowly from top to bottom also with the constant speed. Then a thin and flat wet membrane was formed on the polyester film. The PET film with wet membrane was peeled off from casting board and immersed in the prepared methanol bath for coagulation. A polyester film was laid on the top of immersed films to provide uniform pressure on the coagulated films to prevent the curls on four sides and make membranes flat.

The cast membranes were packed together in the methanol bath. Coagulation of solution layers could be observed after 30 seconds. To coagulate thoroughly, films were immersed in methanol for about 20 minutes and the coagulated films were separated from PET films automatically. Then PET film were removed and disposed. To remove the trapped ED/KSCN solvent, after initial coagulation, those films were placed into a new methanol bath and soaked for another 20 minutes. After repeating this step 3 times and soaking membranes in a new methanol bath overnight any ED/KSCN residue could be completely eliminated. It is important to note that the glass container with cellulose solution was always put back to oil bath after to keep it warm and to ensure its low viscosity and fluidity.

After extracting any traces of ED/KSCN solvent, membranes were removed from methanol bath and packed between glass plates. Films were separated by thin sheets of Teflon film. Air bubbles were squeezed out carefully. Films were packed neatly to avoid wrinkles. A sandwich like structure were was formed from bottom to top: glass plate/Teflon film/samples film/ Teflon film/samples film/ Teflon film/samples film/……/glass plate. To provide a more uniform pressure, more glass plates could be used in this structure. Finally this sandwich structure was laid between two bricks with extra weight on the top.

The packed films were left and dried at ambient temperature for 24 hours and then moved to a vacuum oven and dried at 50℃ for 2 or 3 days. The time for drying was dependent on the film thickness. When drying was finished, the packed films were cooled to the ambient temperature for another 24 hours before separating sample films from the “sandwich”. If separating films were immediately moved out from the oven, those films shrank very fast and

a number of winkles were formed. Winkles must be avoided because they create weak and stress concentration points thus lowering mechanical properties.

Before casting membranes, viscosity of solutions was measured by a Brookfield viscometer to study the effect of an increasing gluten amount. These membranes were tested by following methods. Scanning Electron Microscopy (SEM) was used to detect fine non- or porous structure of cross sections and surface characteristics. Fourier Transform Infrared Spectroscopy (FTIR) was used to examine the chemical components. Thermogravimetric Analysis (TGA) was used for comparative purposes, and determination of structural differences between the raw materials and membranes with different composition. Wide angle X-ray scattering (WAXS) was used for determination of initial or residual crystalline structures and to support the results of TGA. Tensile and permeability tests were applied for end use practicality and viability of membranes.

5.7 Characterization methods

5.7.1 Viscosity measurement

The DV-E Brookfield viscometer by was used to determine viscosity of film-forming solutions with different compositions. Containers with solution were heated in an oil or water bath, at around 95℃, to maintain the liquid state. During measuring, containers were kept in the bath to maintain temperature constant. Because the solutions were very viscous, the number 7 spindle (for the highest viscosity range) was chosen and the speed was set up at 100 rpm. Each solution was measured 3 times. The test was used to study the variation of viscosity of film-forming solutions with an increasing amount of gluten protein mixed with cellulose.

5.7.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was utilized to characterize the surface and cross-sections (before and after break) of membranes. Porosity, thickness and any surface/cross-sections characteristics were studied. The SEM micrographs were obtained on a Hitachi S-3200 Scanning Electron Microscope under standard vacuum conditions of 5 kV potential difference. Representative micrographs are reported with 500x to 10,000x magnification are reported in this work. The Revolutions software used was used to analyze the resulting micrographs.

5.7.3 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was carried out to characterize the chemical components of the membranes. The test was performed on a FTIR Thermo Fisher iS50 machine with a diamond sensor. The ORBIT/OMNI ATR software was used to measure peak intensity and examine the chemical components of membranes.

5.7.4 Thermogravimetric Analysis (TGA)

The thermo-gravimetric analysis (TGA) was performed for characterizing the thermal behavior of raw materials and produced membranes. The TGA test was performed on a Perkin-Elmer TGA device, under a Nitrogen atmosphere, and a heating rate of 20ºC/min from 25ºC (initial ambient condition) to 700ºC. 5-8 mg samples of each material were prepared for testing. The final TGA curves were analyzed using the Pyris software package that came with the Perkin-Elmer device.

5.7.5 Wide Angle X-ray Scattering (WAXS)

Wide angle X-ray scattering (WAXS) was performed on Philips XLF ATPS XRD 1000 machine with OMNI Instruments from 5 to 40 2θ, to give a graphic representation of the results. It was used to characterize the crystalline structures of raw materials and membrane with different compositions.

5.7.6 Tensile Tests

Tensile tests were performed in the conditioned physical testing laboratory. All tests were completed on a MTS Q-Test/5 Universal Testing Machine with a 250 lb load cell, 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). Membrane samples were prepared into a 70 mm long and 1/2 inch wide strips. All samples were conditioned in the lad for 24 hours before testing. Prior to tensile test, thickness of each sample was measured by a Thwing-Albert Thickness tester following ASTM D1777 test method to obtain the proper thickness for the Q-test software. Samples were between rubber grips in the 250 lb probe to prevent slippage of samples during testing.

5.7.7 Water Absorption Test

Water absorption test was conducted to study the hydrophilicity of dried membranes and calculate the amount of absorbed water. Before testing, membranes should be dried and weighed. Then membranes were moved into deionized and distilled water, soaking for 24 hours. Following, the wet membrane was weighed. The extra water on the surface of membrane should be quickly and carefully removed with tissue. The increasing weight percent of water could be calculated with the help of wet mass. Membranes with different gluten concentration were tested. Each kind of sample was measured with at least three membranes. All kinds of membranes were compared to each other to observe trend or pattern.

CHAPTER 6. RESULTS and DISCUSSIONS

6.1 Characterization of Cellulose-only Membranes

6.1.1 Membrane Production

The choice of dissolution solvent system and dissolution process for cellulose had been systematically studied by Lee and Douglass.[8,17] Lee proved that the ED/KSCN solvent system is an effective solvent for cellulose and Douglass used an optical microscope to observe the rate of dissolution of cellulose, starch, and proteins in the ED/KSCN solvent system.[8,17] Douglass also pointed out that the dissolution of 6-7 wt% cellulose would take 2-4 hours around 90℃.[17] In the further study done by Yidan Zhu, theories of Lee and Douglass were used and verified.

The first two steps of making membranes, dissolution and casting membranes, were following Zhu's procedures stated in her paper. The nitrogen gas, preventing unwanted oxidation, was not applied because it was proved unnecessary by Zhu.[30] The membranes made from solutions which were made under or not under nitrogen atmosphere showed almost same properties.

It was mentioned by Douglass that the complete dissolution of pure cellulose could be achieved between 2 to 4 hours. However, in Zhu's work, the full dissolution of cellulose was achieved after 4 hours or even longer. In my experiments, the perfect dissolution was completed after at least 4.5 or even 5 hours. The solution was usually transparent light yellow. Then the solution was cooled down to room temperature. Later, cooled solution was reheated to around 120℃ and poured into a glass container to storage. The reason why the polymer

solution was reheated at 120℃ instead of 90℃ stated in Douglass's and Zhu's papers was because the viscosity of the solution at 90℃ was too high for the solution to flow out from the flask. In this case the large amount of solution was left on the wall of the three-neck flask. A higher temperature caused a viscosity decrease of the solution and improved the solution fluidity. When pouring solution into the container, air was always trapped in it. So before using the solution it was heated around 120℃ for 30 minutes to get rid of air bubbles that could cause pores formation in membranes. In Zhu's experiment, the solution was heated at a lower temperature for about 2 hours to remove trapped air. However, this was not suitable for this experiment because heating it too long caused the formation of a thin liquid ED layer on the top of the solution. As a result a good membrane could be not produced.

Membranes were casted on the Byk-Gardner Casting Table with a smooth glass surface by using a 25 mil casting bar also provided by Byk-Gardner. Douglass stated that 25 mil was too thin for cellulose to form membranes because of brittleness.[17] But in this work, membranes casted by 25 mil bar showed very good properties which were compatible to his 30 mil membranes.

After casting, membranes should be moved into methanol bath to coagulate membranes and extract ED/KSCN solvent from membranes as mentioned in Douglass's and Zhu's work. However, during this coagulation process, edges of membranes started curling up and winkles showed up. Zhu noticed that but Douglass did not. Zhu put glass plate on the top of membranes to provide even pressure to membranes to make them flat. The reason why membrane showed obvious curling and winkles was the temperature dropped dramatically. It is an easily understandable physical phenomenon. In this work, membrane were not moved into methanol

bath until it was cooled down to room temperature. The temperature of membranes was close to the methanol bath, and wrinkles were still existed but not obvious. A polyethylene film or glass plate could be used to reduce winkles.

After coagulating for 5 minutes, the appearance of membrane changed from transparent to opaque and methanol turned into light yellow that was the sign of extraction of ED/KSCN solvent. After 20 minutes, membranes were transferred to a new methanol bath soaking for 20 minutes. After repeating this process twice membrane was soaked for 24 hours. Eventually, ED/KSCN was perfectly removed and membranes were back to be transparent after membrane soaking in methanol for the additional 48 hours.

Membranes should be packed neatly without air between membranes and Teflon sheet (How to pack membranes is mentioned in the last experimental section). No more than 4 membranes should pack between two glass-plates because it was difficult to reduce winkling on edges. Packed membranes were dried in ambient temperature in the vacuum oven, and a further 3-4 days at 50℃.

Following drying, packed membranes were left in the hood at room temperature for 24 hours before pealing them from Teflon sheets. In order to avoid membrane shrinking they cannot be directly taken out from the oven at 50℃ and stored in the humid environment at a low temperature.

After many attempts, the new procedure for making films was adapted from the Douglass's and Zhu's methods. The new procedure can make decent and uniform films. Then cellulose-only membrane made in this work is shown in Figure 35.

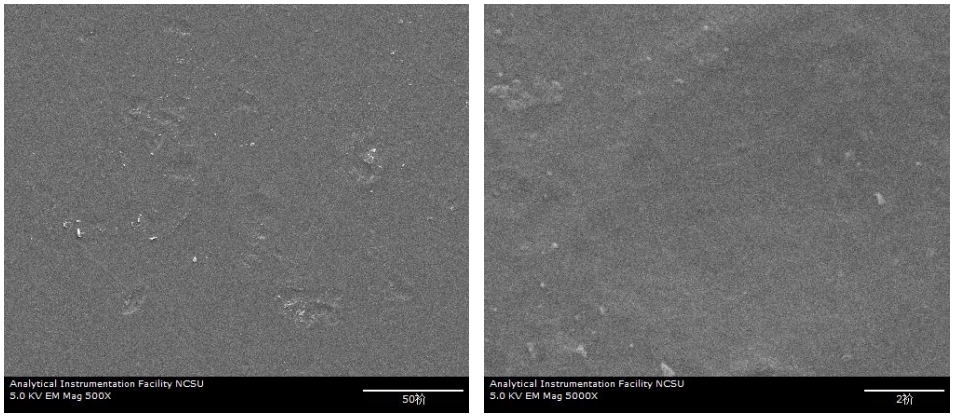
Figure 36. Cellulose-only membrane

6.1.2 Membrane Characterization

Many uniform membranes were made by using the improved procedure. Those membranes were tested to characterize their properties using various techniques and instruments. Viscosity measurement, scanning electron microscopy, Fourier transform infrared spectroscopy, thermogravimetric analysis, wide angle X-ray scattering, tensile tests and water absorption test were carried out in this study.

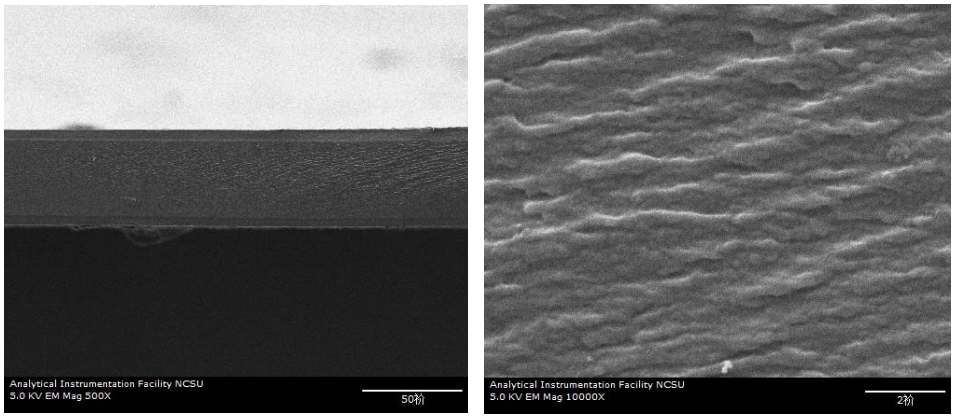
6.1.2.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was performed to characterize the morphology of the cellulose membranes ant to verify the membranes made in this work were uniform and nonporous. Both surface and cross-section were scanned at various magnifications and shown in Figure 36 (a-d).



(a) **(b)**

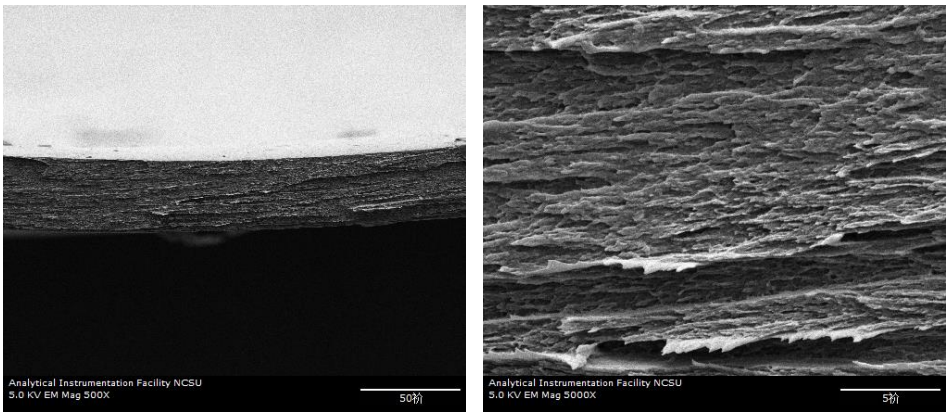
Figure 37a. SEM images of surface of cellulose membranes (unstretched) at (a) 500x and (b) 5000x magnifications



(c) **(d)**

Figure 38b. SEM images of cross-section of cellulose membranes at (c) 500x and (d) 10000x magnifications

As shown in Figure 36 (a-d), the surface of the cellulose membranes is flat and no pores are visible. The Figure 36 (c) and (d) show the morphology of the cross-section of unstretched cellulose membranes at 500x and 10000x magnifications. Those cross-sections are also very even and nonporous. So it is easy to draw a conclusion that cellulose membranes made in this work were uniform and nonporous, which verify the conclusion drawn by Douglass and Zhu.



(a) **(b)**

Figure 39. SEM images of cross-section of cellulose membranes (stretched to break) at (a) 500x and (b) 5000x magnification

Not only unstretched membranes but also stretched membranes were tested. Figure 37 (a) and (b) show the cross-sections of cellulose membranes stretched to break. Comparing with the cross-sections of unstretched membranes, the stretched membranes had a little bit rougher cross-section that was caused by stretching. The rougher cross-sections mean that the crystallinity of cellulose membranes was not high because high crystallinity could cause membranes broken abruptly and the broken surface should be smooth. The change of crystallinity will be proved by WAXS tests.

6.1.2.2 Fourier Transform Infrared Spectroscopy

Membranes were formed by dissolving solid polymer powder and coagulating back cast thin liquid films. Before dissolution, cellulose showed a fibrous structure (white powder) that was destroyed during dissolution process. After back to solid state, there were no trace of

fibrous structure (transparent membranes) existed in membranes which is shown in SEM images. Because the structure of cellulose changed, it was necessary to verify whether side reactions happened or not. Thus FTIR was used to analyze those cellulose-only membranes to see if any side reactions occurred and ED/KSCN left in the membranes which could result in new IR absorption peaks.

(a)

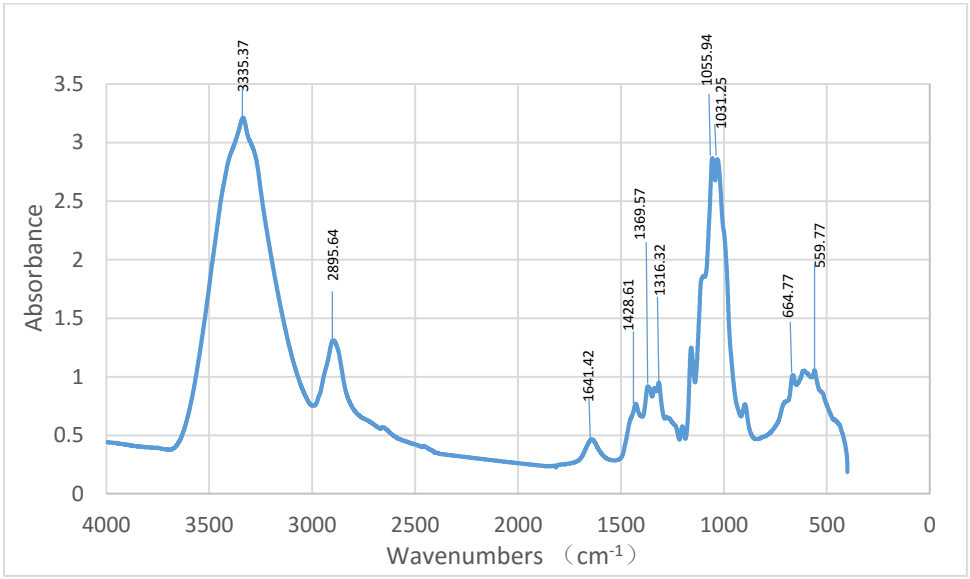
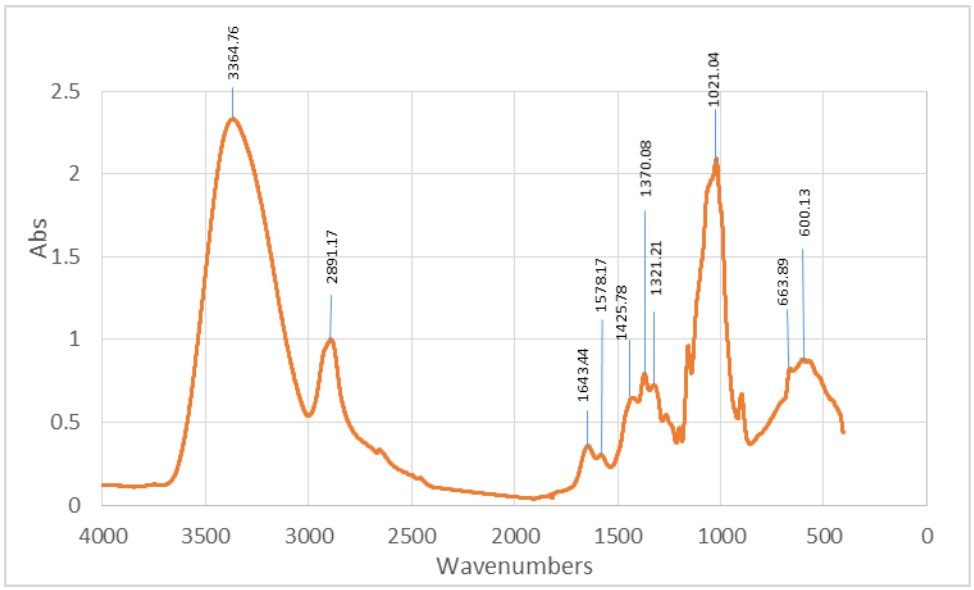


Figure 40a. FTIR spectra of (a) cellulose powder

(b)



(c)

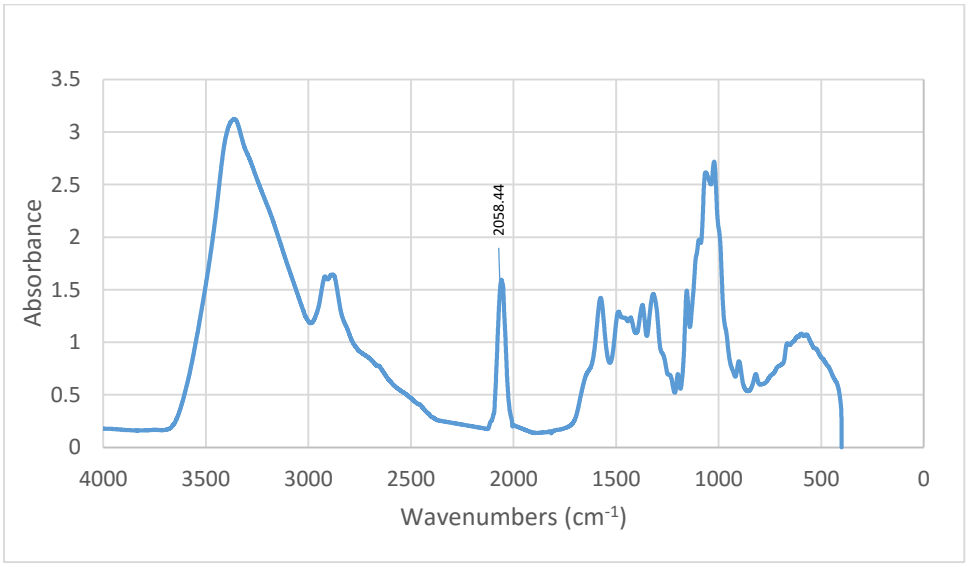


Figure 41b. FTIR spectra of (b) cellulose-only membranes with no ED/KSCN present; (c) cellulose-only membranes with much ED/KSCN present

(d)

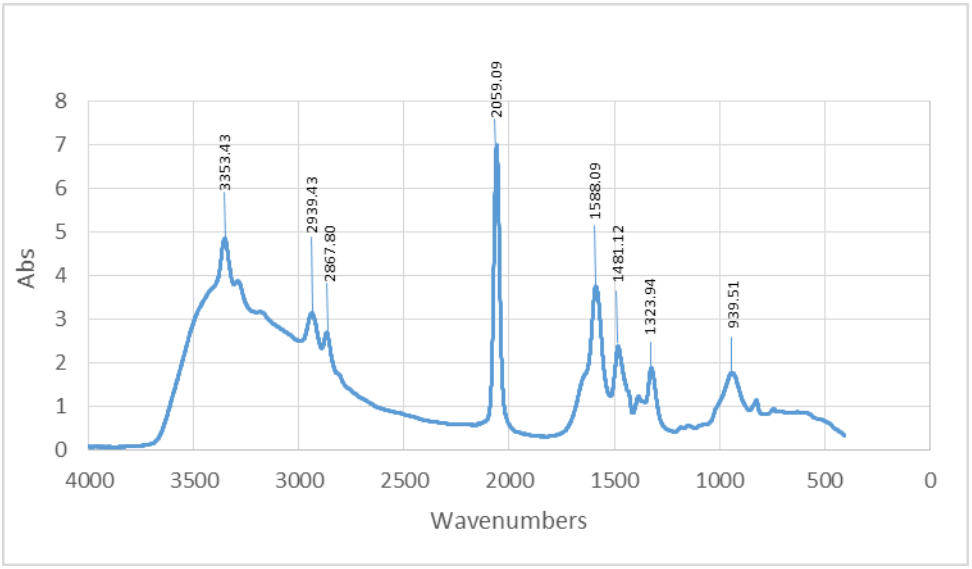


Figure 42c. FTIR spectra of (d) ED/KSCN solvent system

As seen above, Figure 38 (a) and (b) are correspondingly FTIR spectra of cellulose powder (raw material) and cellulose-only membranes. These two spectra are nearly identical. Both FTIR spectra show peaks at same wavenumbers with nearly identical band width and intensities which represent the major functional groups like -OH, -C-C-, -C-O- and -C-OH, which is similar to the results in Zhu's paper.[30] It is easy to draw a conclusion that there was no side reactions occurred during membranes forming. It was just physical change between raw material and final products. There is a new peak shown at 1578.17 cm^{-1} which is caused by ED residue. It can be attributed to that time of coagulation is not long enough. Cellulose is relatively thicker than the blend membranes (shown in Table 22) may need longer time to remove all solvent.

Figure 38 (d) is the FTIR spectra of ED/KSCN solvent which was used to dissolve cellulose and should be totally removed from membranes during coagulation. It is clear that there is an obvious peak with high intensity shown at 2059 cm^{-1} which represents -SCN (2140-1990 cm^{-1}) group which should not in the spectra of cellulose membranes. In the spectra of cellulose membranes (Figure 38 (b)), there is no peak exists between 2140-1990 cm^{-1} of wavenumbers. That means ED/KSCN solvent in membranes were perfectly washed out during coagulation process and methanol is an efficient agent for washing ED/KSCN solvent as stated in Douglass's work.

Figure 38 (c) shows a FTIR spectra of a cellulose-only membrane but with an obvious peak of -SCN group. That means ED/KSCN solvent was not clearly washed. The sample was light yellow which is also the sign of ED/KSCN solvent existing. So the sample was soaked in methanol for 2 days but the change was not obvious. It was assumed that maybe the ED and KSCN left in the lab were aged, causing they were difficult to be washed out of membranes. So new ED and KSCN were bought and used. This time, membranes were colorless and the peak of -SCN group disappeared from FTIR spectra. Clearly, there had to be something wrong with the old ED and KSCN. So when using ED/KSCN solvent, it is necessary to check the quality of raw materials.

6.1.2.3 Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) was performed to study the weight loss that can be linked the morphology changes of cellulose membranes. It was used to characterize the crystal structure of the membranes and raw materials, comparing and telling the differences between

them. In Douglass's work, it was stated that the crystalline structure of cellulose membranes and powder should be obviously different because the cellulose I, which is the natural form in cellulose, is converted to cellulose II by dissolution and coagulation.[17] Different crystal structure can cause different thermo-degradation behaviors because the amorphous regions will degrade first and the crystal regions will degrade later and differently.

Both raw material (cellulose powder) and final products (cellulose-only membranes) were tested. The TGA graphs are shown in Figure 39 and 40.

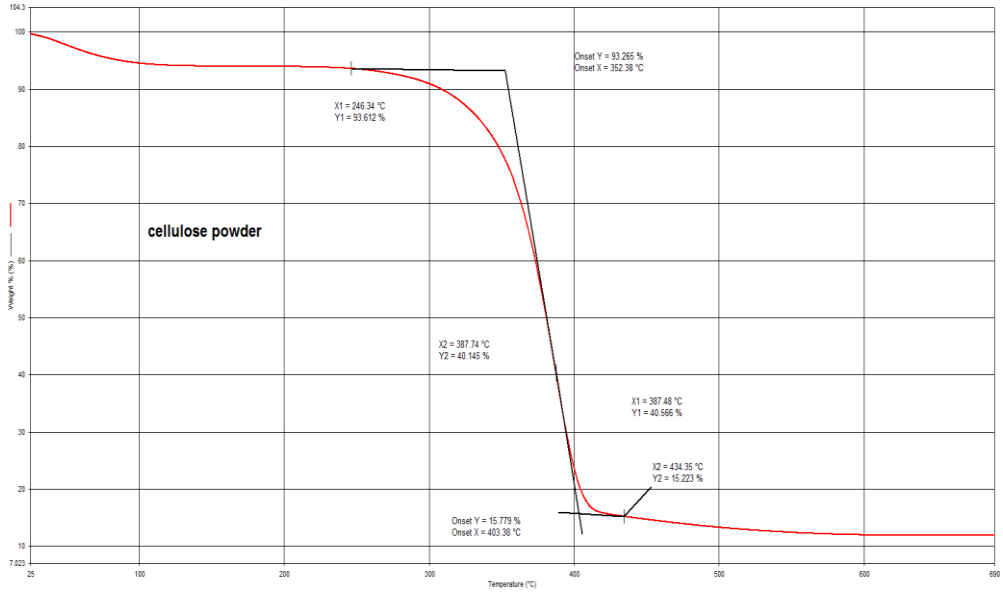


Figure 43. TGA analysis curve for raw cellulose fibers

Figure 39 clearly shows that the degradation starts at 352.38℃and ends at 403.38℃. Because the TGA test is performed under nitrogen, water is removed first and other

degradation byproducts followed. Eventually, the pure carbon is remained as ash which accounts for around 11 wt%. It shows a very low as level.

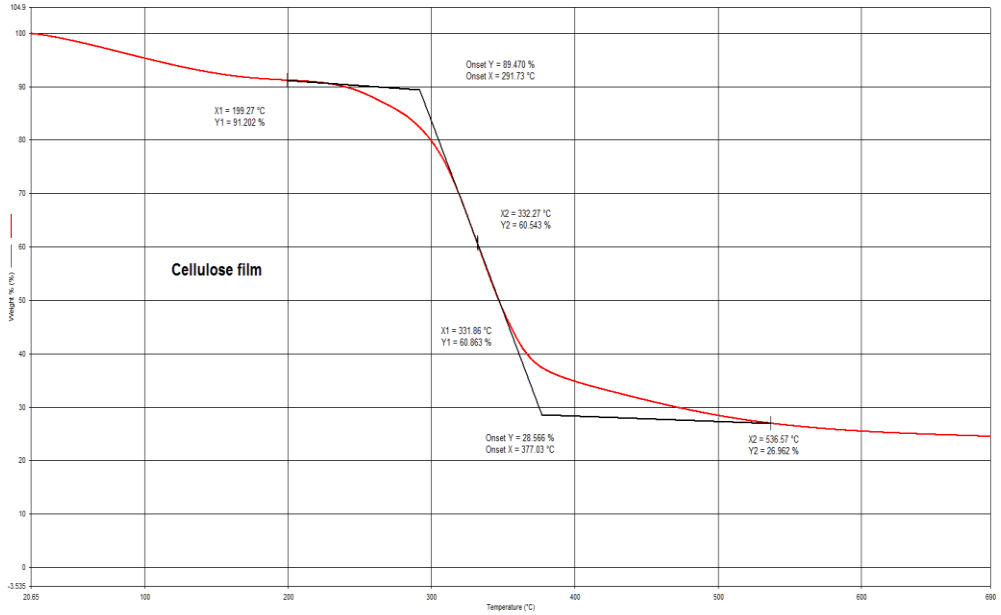


Figure 44. TGA analysis curve for cellulose membrane

From Figure 40, comparing with cellulose powder, cellulose-only membranes had a lower onset temperature of 291.79℃ and offset temperature of 377.03℃. It is obvious that the crystalline structure of cellulose-only membranes and cellulose powder are different. This difference can be attributed to the dissolution of cellulose, which changed the crystalline structure, and then the coagulation of regenerated cellulose converting its structure to a different solid structure. Also, cellulose membranes and cellulose powder left different wright percent of pure carbon which is an evidence for different crystalline structures. The following WAXS test will support the TGA results.

6.1.2.4 Wide-Angle X-ray Scattering (WAXS)

Wide-angle X-ray scattering was applied to characterize the crystallinity of cellulose before and after processing to confirm the structural differences, as shown in Figures 41 and 42.

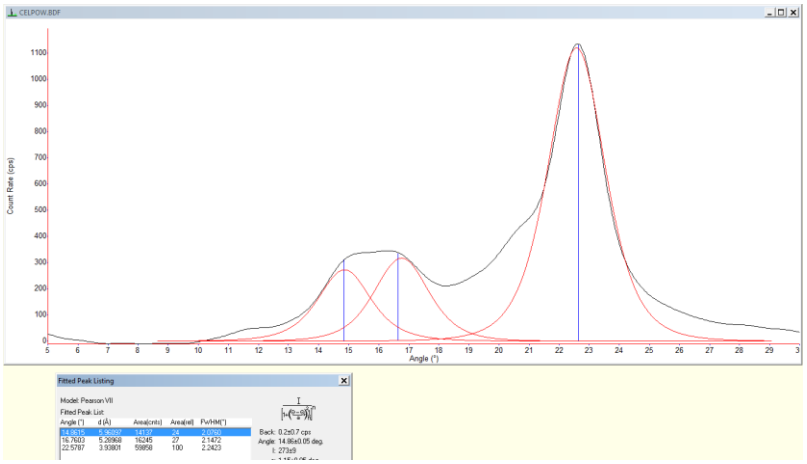


Figure 45. WAXS curve of raw pressed cellulose from refined wood pulp

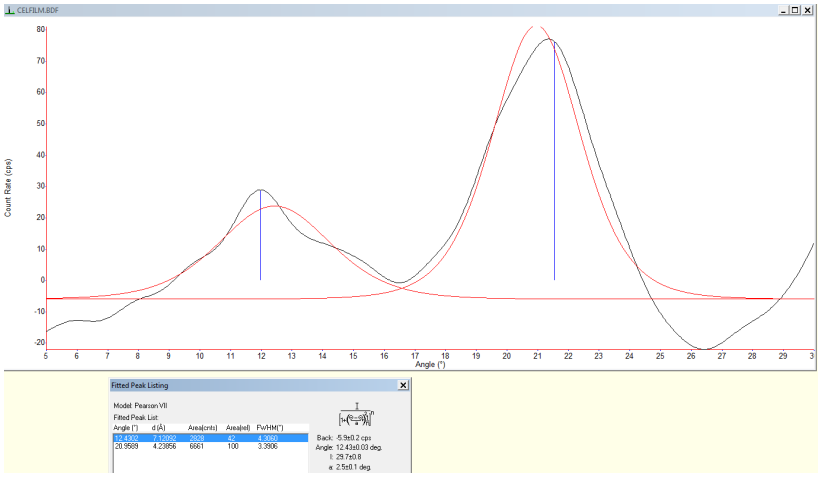


Figure 46. WAXS curve of Yang cellulose membrane

Figure 41 shows a typical cellulose I structure with peaks at 15, 17 and 23 2θ. The spectra of the membranes made in this work (Figure 42) shows an obviously different curve. The peaks of membranes have been shifted down to around 12, and 21-22 2θ. That represents a cellulose II structure similar to the results by Douglass and Cao[17,70] when they made a solution of cellulose, and then coagulated the solution back to solid with different processes. Those peaks have been spread out making a more amorphous appearance.

This verification of the results by earlier scientists proves dissolution of cellulose can convert cellulose I to cellulose II.[27] Also, the differences of TGA results for samples are explained.

6.1.2.5 Tensile Properties

Tensile properties are very important properties for studying membranes which are highly related to the potential usages for the membranes. During the tests, tensile modulus, break stress and elongation at break were paid more attention to. The data was collected and shown in Table 18.

Table 18. Comparison of tensile properties of cellulose membranes from works of Douglass[17] and Zhu[30]

Cellulose membranes from different works	Tensile Modulus (kgf/mm^2) & CV (%)	Break Stress (kgf/mm^2) & CV (%)	Elongation at Break (mm) & CV (%)	Thickness (mm) & CV (%)
Douglass 7 wt% cellulose nonporous membrane	165.5 ± 16 (9.7)	5.36 ± 0.7 (13.1)	26.2 ± 10.1 (38.5)	0.047 ± 0.015 (31.9)
Douglass cellulose 7 wt% porous membrane	33.0 ± 9.3	0.59 ± 0.17	3.9 ±1.4	Unknown
Zhu 3 wt% cellulose nonporous 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 nonporous membrane	260.89 ± 27.54 (16.6)	4.65 ± 0.63 (13.5)	15.1 ± 4.5 (29.8)	0.034 ± 0.004 (10.3)
Yu 6 wt% cellulose nonporous membrane	239.50 ± 15.49 (6.47)	3.58 ± 0.39 (10.98)	17.14 ± 4.52 (25.96)	0.076 ± 0.005 (6.35)

Porous membranes have relatively poor tensile properties than nonporous membranes. As shown in Table 18, the nonporous membranes had good tensile properties namely, tensile modulus of 165.5 ± 16 kgf/mm^2; with break stress of 5.36 ± 0.7 kgf/mm^2 and elongation at break of 26.2 ± 10.1 mm. In contrast, those porous membranes had poor tensile properties than the nonporous ones. It can be attributed to pores in those membranes which served as defects, causing stress concentration and making membranes break easily. Also because of those pores, the tensile properties of porous membranes are very difficult to control.

The tensile properties of nonporous cellulose membranes are influenced the concentration of cellulose. Comparing those membranes made by Zhu, 5 wt% membranes had higher tensile

modulus (239.5 ± 15.49 kgf/mm^2). It is difficult to compare break stress and elongation at break of these two kinds of membranes because the CV values of 3 wt% membrane are very high, 40.1% of break stress and 100.7% of elongation at break, which means the data is not reliable enough. By comparison between Zhu's 5 wt%, Yu's 6 wt% and Douglass's 7 wt% nonporous membranes, Zhu's and Yang's membranes showed very close values of all parameters and higher tensile modulus but a lower break stress than Douglass's membranes. It could be attributed to the increased modulus, which meant that the regenerated cellulose molecules were packed closer, orderly and uniformly. The neatly packed polymer chains had limited deformation and freedom for movement which could act as the reason for decrease of break stress (brittle) and elongation at break.

The data of Zhu's 3 wt% nonporous cellulose membranes had much higher coefficient of variation (CV) value than others indicated lower reliability of those samples. Because all membranes were made manually, it was extremely difficult to make membranes perfect. The elongation at break and thickness are the most difficult to control and replicate. Using thickness as an example, both concentration of cellulose and the way to pack membranes before drying can influence the thickness of membranes, especially the later one. Membranes will shrink during drying which can thickness change. If membranes are packed neatly, the shrinkage will be reduced and membranes will be relatively thinner. If not, membranes will shrink a lot and membranes will be relatively thicker. Another reason for a relatively lower properties is that the viscosity of solution with high cellulose concentration was too high to cast membranes uniformly.

There are other data with high standard variation (STDEV) and CV in Table 18. Those could be caused by many reasons, like breaking at the grip, causing very low values; winkles and trapped air bubbles, resulting in stress concentration and slip of samples during test.

6.1.2.6 Water Absorption Test

Water absorption is an important property for studying cellulose membranes which is related to the potential usage. Water absorption test was conducted to study the hydrophilicity of dried cellulose membranes calculation how much water was absorbed. Douglass did same experiment in his work to characterized cellulose membranes. All the data were collected and showed in Table 19.

Table 19. Comparison of water absorption of cellulose-only membranes made by Yu and Douglass[17]

Membranes	Dry mass (g)	Wet mass (g)	Wet mass increase (%)
Douglass wet cellulose membrane (coagulated & kept wet)	0.28 (after)	4.70 (before)	94 decrease, 1580 increase
Douglass cellulose membrane a	0.75	1.34	79
Douglass cellulose membrane b	0.49	1.03	110
Douglass cotton control membrane	0.21	0.42	100
Yu 6wt% cellulose membrane	0.3547	0.5147	45

It is obvious from Table 19 that those cellulose-only membranes produced by the improved method in this work showed a much lower water pick-up percentage than those made

by Douglass. Most of Douglass's membranes showed higher than 100 wt% water pick-up and there was one kind membrane that showed 1580% increase. This a very dramatic difference could be attributed to the porous structure of Douglass's membranes which could provide much more place to trap more water inside membranes. However, membranes made in this work were nonporous, which could be supported be the SEM images, with less space for water. Douglass had made nonporous membranes but no water absorption test was conducted. The regenerated cellulose membranes showed a low water absorption even though cellulose is a kind of hydrophilic material, which could be attributed to the structure change.

Soaking for 24 hours was enough for membranes to absorb water. After measuring wet mass, membranes were put back to the water, soaking for another 24 hours. The wet mass of 48-hour-soak membranes showed almost the same mass. The wet membranes were intact and some strength still remained. All membranes were dried after soaking. Those dried membranes were intact with good mechanical properties.

The purpose of this test was to serve as the control group for comparison to the cellulose/gluten blend membranes.

6.1.3 Conclusions

Cellulose membranes were effectively made by using the improved method. Those cellulose-only membranes were produced for comparative purposes and verification. The ED/KSCN solvent system, which is a novel solvent for cellulose, was used to dissolve cellulose because of relatively higher efficiency, lower cost and toxicity. By characterizing cellulose-

only membranes made in this work and comparing with the results of Douglass's and Zhu's the following conclusions can be drawn:

1) The ED/KSCN (65/35) solvent system is an effective solvent system for cellulose and can be perfectly removed from the membrane-forming solution by soaking in methanol bath.
2) The improved membrane production method, providing extra and even pressure, neatly packing membranes, can provide more uniform membranes.
3) Cellulose cast by 25mil bar can form decent membranes with good mechanical properties.
4) Scanning electron microscopy for characterization of membrane structure, surface and cross-sectional area (before and after stretched) was performed. All images showed that all membranes were nonporous.
5) Fourier transform infrared spectroscopy was performed to detect the chemical components of cellulose powder and regenerated membranes. Nearly the same spectra prove that no chemical side interactions occurred during producing membranes.
6) The results of thermogravimetric analysis showed a lower degradation temperature for regenerated membranes than for the raw cellulose powder. This happened because the cellulose I in cellulose powder was converted to cellulose II during dissolution and the methanol coagulation of thin films.
7) Wide-angle X-ray scattering showed the evidence for celullose structural change during making regenerated membranes from cellulose powder. Native cellulose I

was converted to cellulose II that supports the results of TGA.

8) Cellulose membranes studies by Douglass, Zhu and the data from this work prove that the cellulose concentration and membrane structure can obviously influence tensile properties. Since membranes are made manually it is difficult to make membranes perfectly. The membranes made in this work show good tensile properties and were more uniform as confirmed by a lower value of the coefficient of variation.
9) Comparing the water absorption of cellulose membranes made by Douglass and in this work, membranes made in this work showed a lower water absorption. Membranes after drying still maintained good properties.

6.2 Characterization of Cellulose/Gluten Membranes

6.2.1 Membrane Production

To study the properties of cellulose/gluten membranes, membranes with different composition were made. The objective here was to study the variation (if any) of properties of membranes with different composition.

Before making membrane-forming solutions, it was necessary to verify the solubility of gluten in the ED/KSCN solvent system. 10 g gluten was added to 90 g ED/KSCN solvent and stirred for 3 hours under 85℃. After 3 hours, a clear yellow liquid was obtained. No residue was observed even placed in ambient condition for 24 hours. So the solubility of gluten in the ED/KSCN solvent system was proved.

The membrane forming process for cellulose/gluten membranes were nearly the same as described for the cellulose-only membranes. Four groups of raw materials, cellulose/gluten powder mixture containing 6 g polymer, were prepared. The ratio of cellulose and gluten in mixtures were 90/10, 80/20, 70/30 and 60/40. Because of the presence of gluten, the dissolution time was shorter (around 3 hours) and the temperature was lower (around 85℃) to avoid damages of gluten. The membrane casting, coagulation and drying processes were the same as for making cellulose-only membranes. Eventually, thin, uniform and a little bit opaque membranes were obtained.

For comparison purpose, gluten-only membranes were trying to make. The 6 wt% gluten-only solution was made first. However, the solution viscosity was too low to form wet membranes. Following that, 6, 10, 20, 30, 40 and 50 wt% solutions were made to find qualified viscosity to form wet membranes. Only 40 and 50 wt% solutions were suitable. After casting,

wet membranes were moved in the methanol bath for coagulation. However, the wet films couldn't be coagulated to form a membrane. After extraction of ED/KSCN solvent, cracks appeared on all edges just like unconnected powder. So the gluten-only membranes could not be formed by the method in this work. Blend membranes with different gluten concentration made in this work are shown in Figure 43.

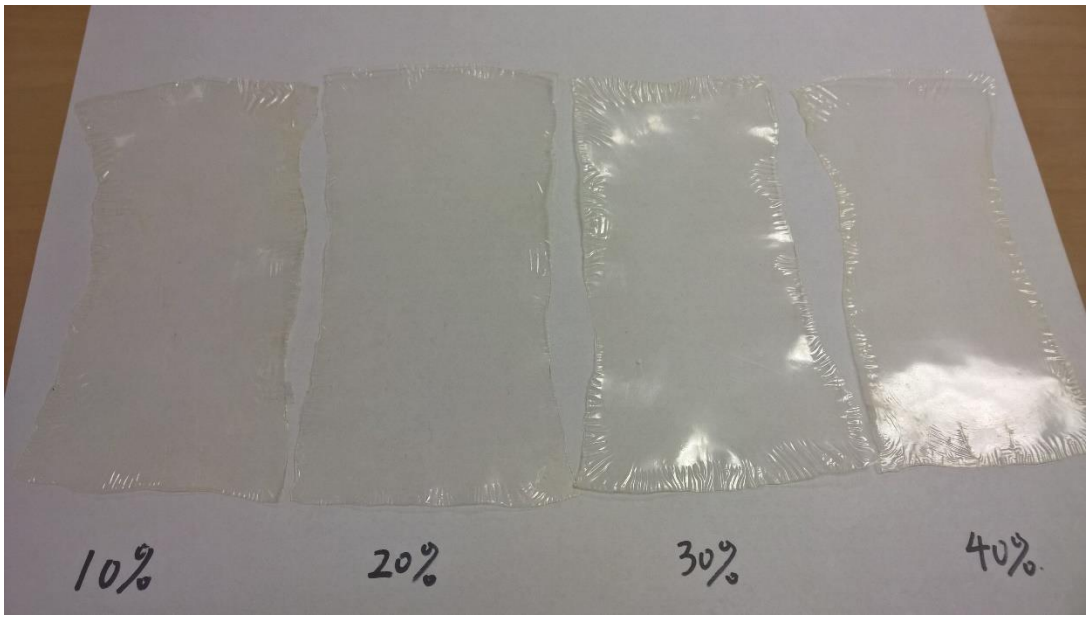


Figure 47. Cellulose/gluten blend membranes with different gluten concentration

6.2.2 Membrane Characterization

6.2.2.1 Viscosity Measurement

There are many parameters or factors that play important roles in dissolution of cellulose, for instance, salt type, salt concentration, cellulose molecular weight and cellulose concentration. The molecular weight is also related to a number of properties of materials. Generally, cellulose materials with a higher molecular weight have better mechanical properties like higher tensile properties and modulus.[8] If the cellulose material is in liquid state, higher molecular weight can cause higher viscosity because of the entanglement of polymer chains which limit chains' mobility.

In this work, the viscosity of solution of cellulose with different gluten concentration of 0, 10, 20, 30 and 40%, were measured. The data were collected and are shown in Table 20.

Table 20. Viscosity of cellulose solutions with different gluten concentration

Cellulose/gluten ratio	Torque (%)	Viscosity (cP)
100/0	65.30	26120
90/10	58.70	23480
80/20	47	18800
70/30	36.10	14440
60/40	28.80	11520

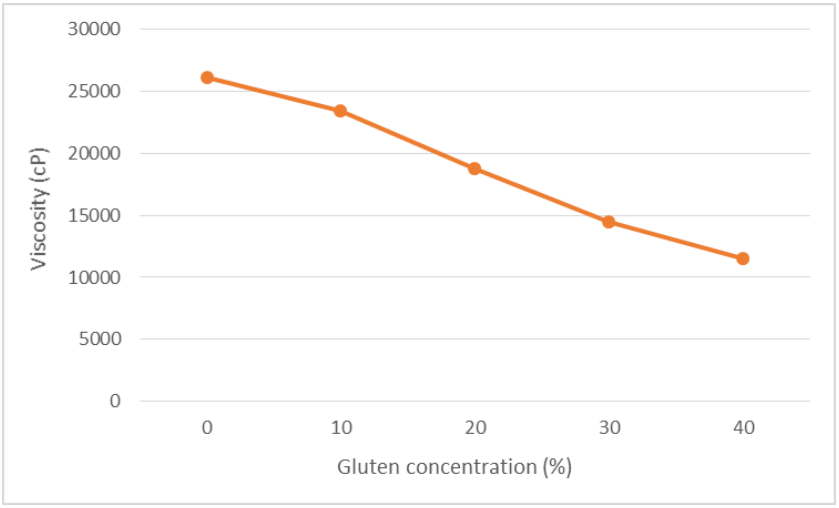


Figure 48. Viscosity of cellulose/gluten solutions with a different gluten concentration

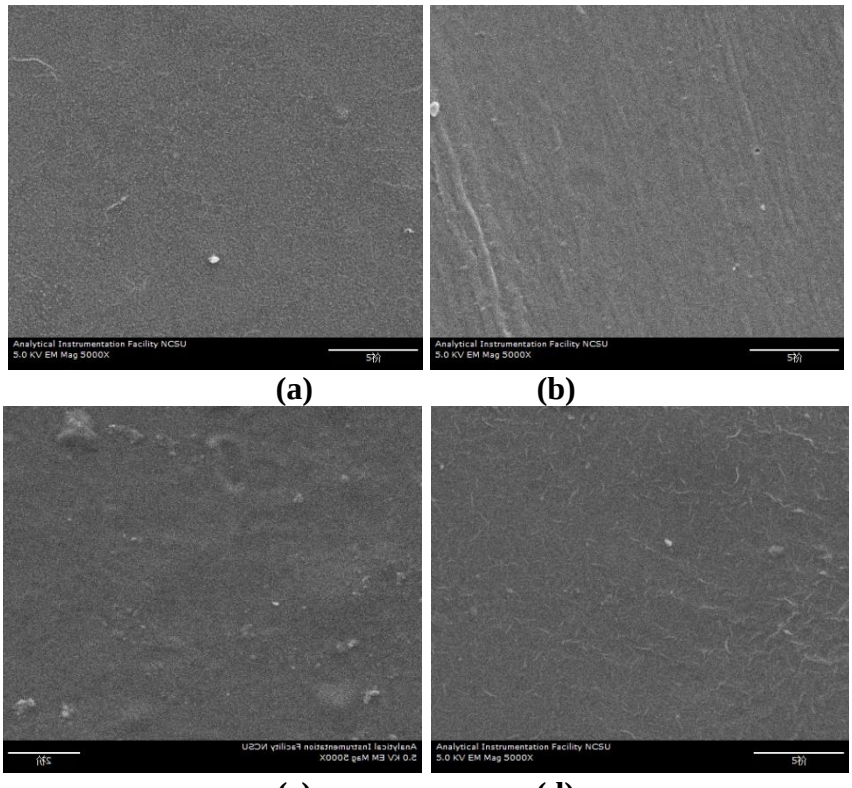
In our test, the spindle #7, the smallest one for the highest range of viscosity was chosen. The rotation speed was set at 100 rpm. In Table 20 "torque" represents the resistance of spindle while rotating in the polymer solution. Higher torque values mean a higher resistance in the solution. The cP is the unit for viscosity; 1 cP = 1 mPa*s. The value of torque and cP are proportional to each other.

As seen in Figure 44, the cellulose-only solution showed the highest torque of 65.30% and viscosity of 26120 cP. With the increase concentration of gluten, the torque and cP value dropped gradually. In the end the cellulose solution with 40% gluten showed the lowest torque of 28.80% and viscosity of 11520 cP. Both torque and viscosity decreased by 55.9%. It can be explained that as the gluten concentration increased the average molecular weight of the blend material or crytallinity decreased. As the result the polymer chains in the solution were less

entangled and the solution viscosity was lower. The decrease of viscosity can lead to a decrease in mechanical properties of membranes which will be discussed later.

6.2.2.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was performed to characterize the morphology of the cellulose membranes to verify that the membranes made in this work were uniform and nonporous. Both surface and cross-section (before or after stretched to break) were scanned at various magnifications and shown in Figures 45 (a-d).



(a) **(b)**

(c) **(d)**

Figure 49. SEM images of surface of cellulose/gluten membranes with (a) 10, (b) 20, (c) 30 and (d) 40% gluten concentration at 5000x magnification

It is very clear from Figure 45 (a-d), that the surface of blend membranes with all different gluten concentration were uniform and nonporous. Those white spots are dust or trash.

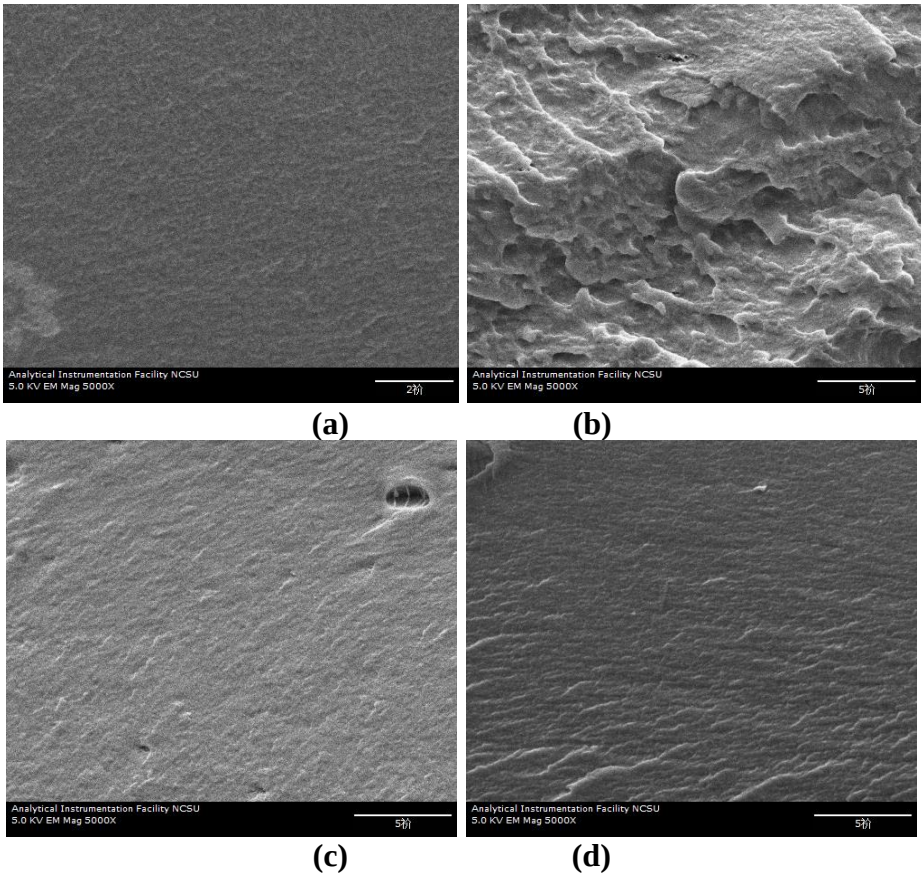


Figure 50. SEM images of cross-section of cellulose/gluten membranes (unstretched) with (a) 10, (b) 20, (c) 30 and (d) 40% gluten at 5000x magnification

Figures 46 (a-d) show very even cross-section areas which represent blend membranes with all different gluten concentration that were uniform and nonporous. The cross-section shown in Figure 46 (b) is not flat which was caused by fracture during cutting in liquid

nitrogen. Because membranes were made manually, it was difficult to make them perfect. The pore in Figure 46 (c) was caused by trapped air during casting.

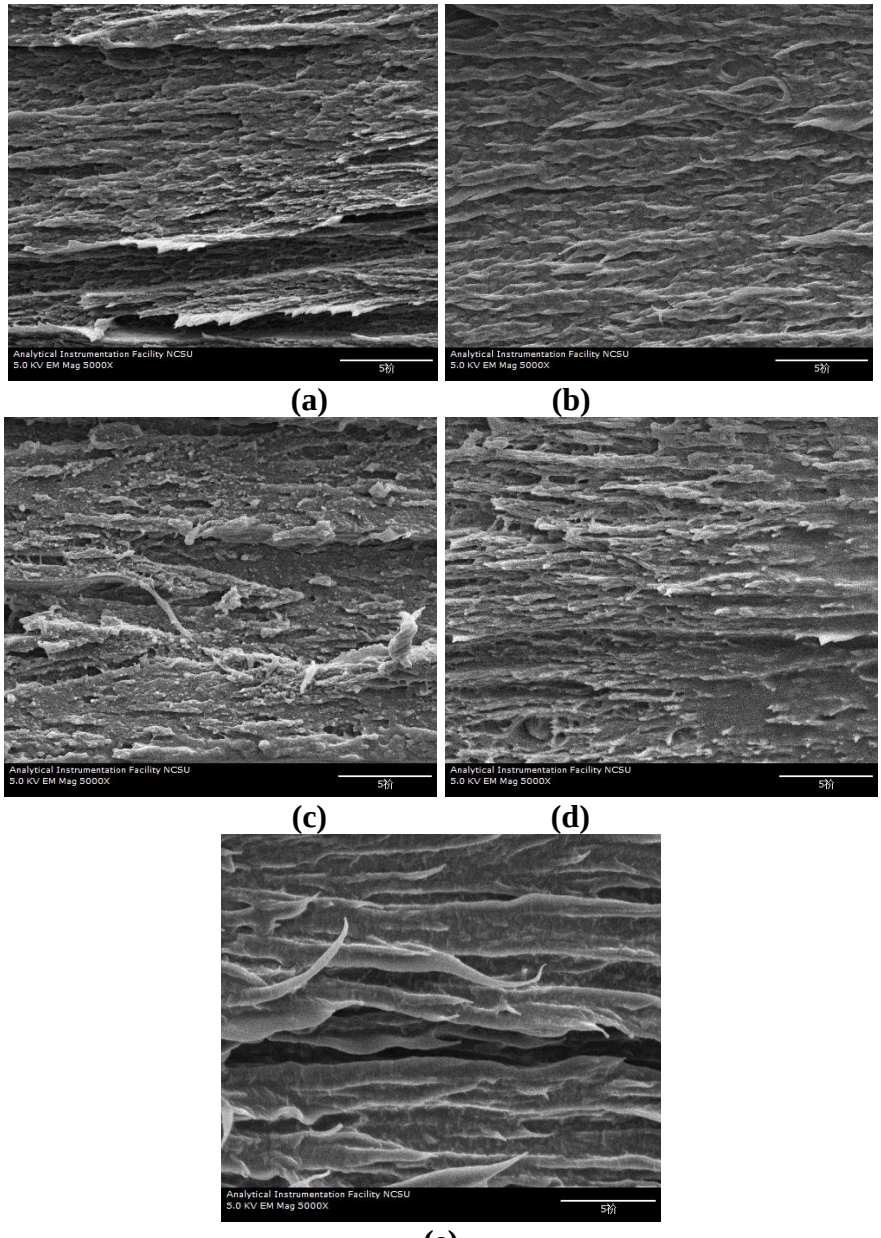


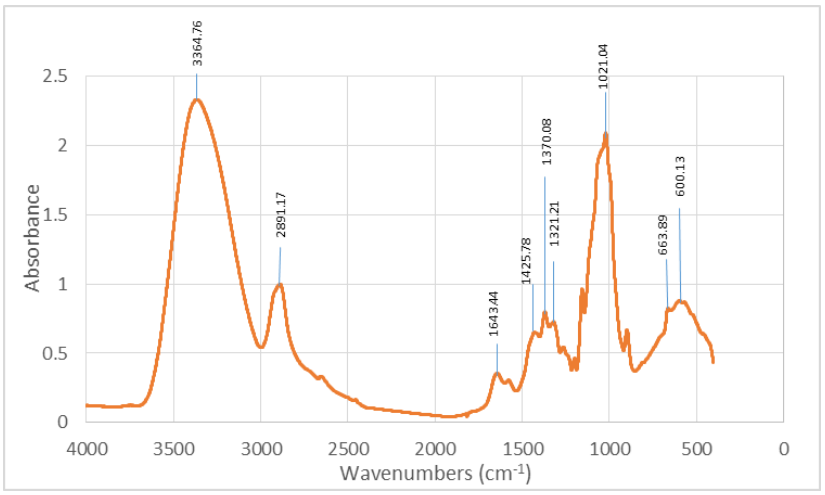
Figure 51. SEM images of cross-section of cellulose/gluten membranes (stretched to break) with (a) 0, (b) 10, (c) 20, (d) 30 and (e) 40% gluten at 5000x magnification

As documented in Figure 47, the stretched-to-break cross-section of membranes become rougher and the fibrous fractures formed on the cross-section area, especially for membranes with 40% gluten. This can be attributed to a decrease in membrane crystallinity which was caused by addition of gluten. This will be proved by WAXS tests. Furthermore, the decrease in crystallinity of membranes could influence the tensile properties of membranes which will be discussed in tensile properties section.

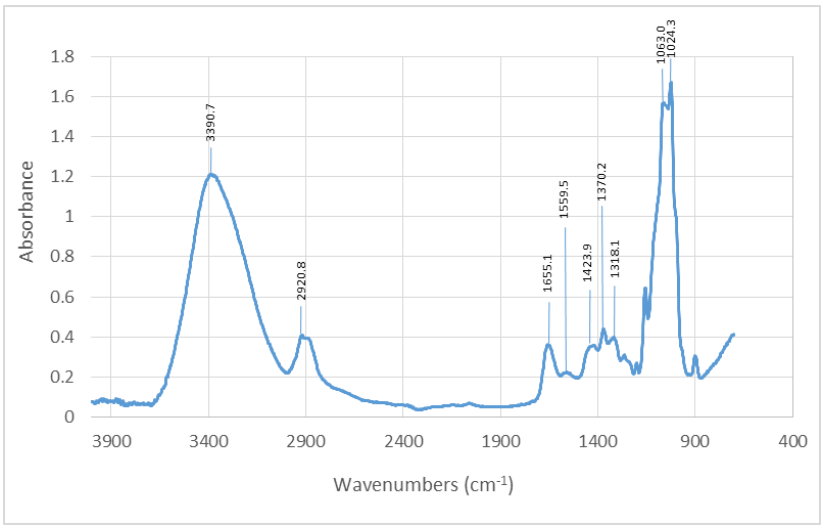
It is very important to point out that from the fracture surface of all membranes in those SEM images, and there is no fibrous cellulose present and no separation of cellulose and gluten can be visible at a high gluten content. As the evidence indicates for the complete dissolution of cellulose and gluten in ED/KSCN solvent system, SEM images prove that both polymers are compatible and perfectly blended together.

6.2.2.3 Fourier Transform Infrared Spectroscopy (FTIR Spectroscopy)

The predominately functional groups of gluten proteins are -NH, -OH, -CH, amide groups and C-C. The FTIR spectra of cellulose/gluten blend membranes with different composition were obtained and used to compare with that one of cellulose-only membrane. To get more obvious differences, the FTIR spectra of membranes with the highest amount of gluten were used to compare. Also it was necessary to verify whether any chemical reactions occurred in blend systems during membrane forming process. Because spectra of membranes with different gluten concentration are very close to each other, to show the obvious differences between pure cellulose and cellulose/gluten blend membranes, the FTIR spectra of cellulose membranes with 0, 20 and 40% gluten are shown in Figure 48 (a-d).



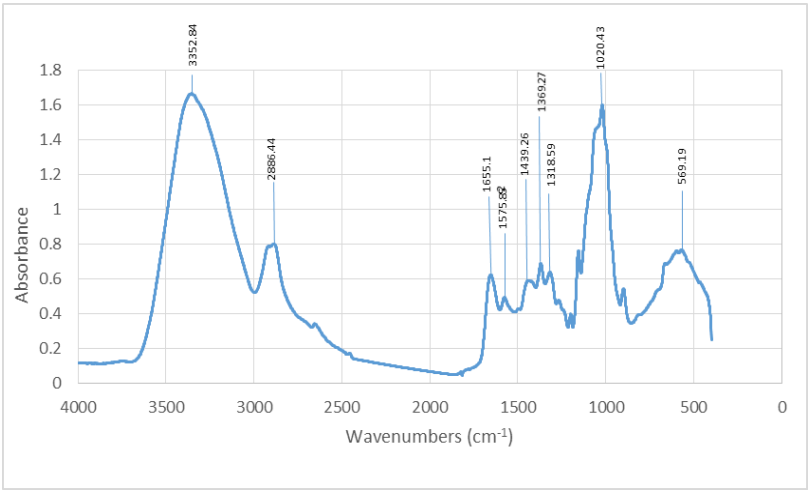
(a)



(b)

Figure 52a. FTIR spectra of cellulose membranes with (a) 0, (b) 20 % gluten

(c)



(d)

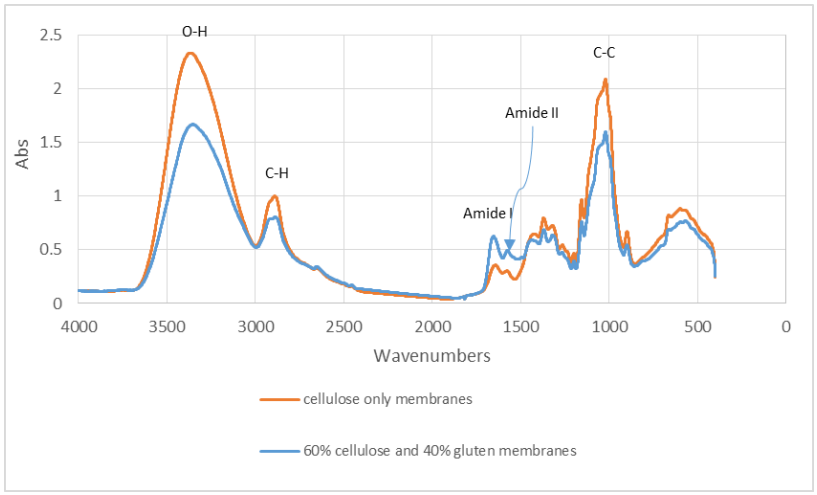


Figure 53b. FTIR spectra of cellulose membranes with (c) 40% gluten; (d) combination of (a) and (c)

Figure 48 (a-d) show the FTIR spectra of cellulose-only membranes and cellulose/gluten membranes. The same number of peaks with different intensities and the very similar shapes are present in these Figures. It is very clear from Figure 48 (d), that all peaks are almost at same wavenumbers and no new peak appears. With the increasing of gluten concentration, the

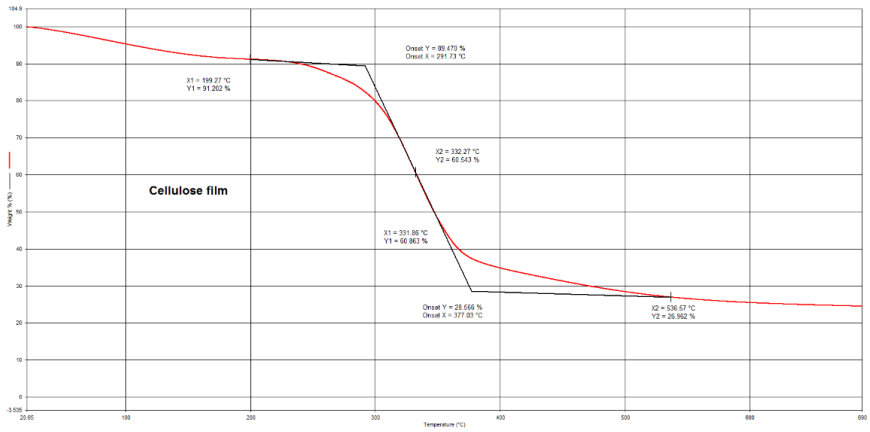
intensity of most peaks decreased without shifting but two obvious peaks present at 1651 and 1537cm^{-1}. Those two peaks represent amide I and amide II groups which belong to gluten, because gluten is a kind of protein which is made of amino acids. Hamee[71] and Guo[72] got the same peaks at close wavenumbers. Cellulose doesn't have amide group as gluten does. So the addition of gluten increased intensity of the peaks of amide I and amide II groups. There are no new peaks appearing which means no new bonds formed and no undesired side reactions occurred during dissolution and coagulation processes.

The decrease of peak intensity can be attributed to the decrease of cellulose concentration. The decrease even a little bit broadening of -OH peak can also be attributed to the hydrogen bonding between -OH and -NH groups.

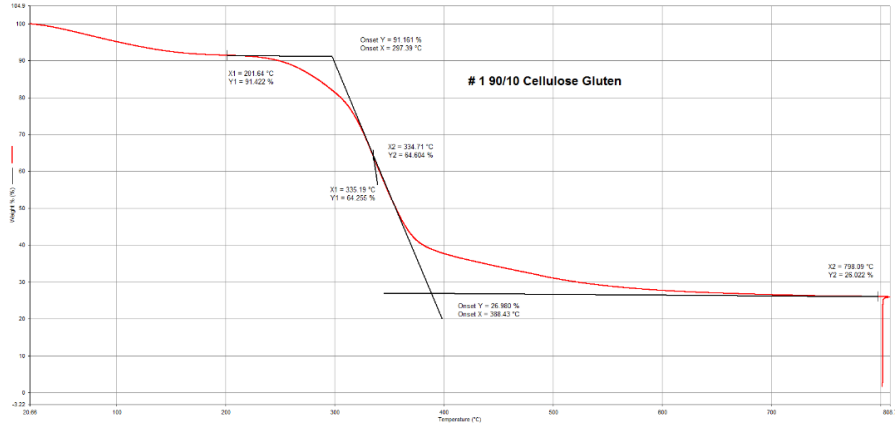
6.2.2.4 Thermogravimetric Analysis (TGA)

The Thermogravimetric Analysis (TGA) was performed to study the morphology of the membranes. It was used to characterize the crystal structure of the membranes with different gluten concentration, comparing and telling the differences between them. Different crystal structure can cause different thermo-degradation behaviors.

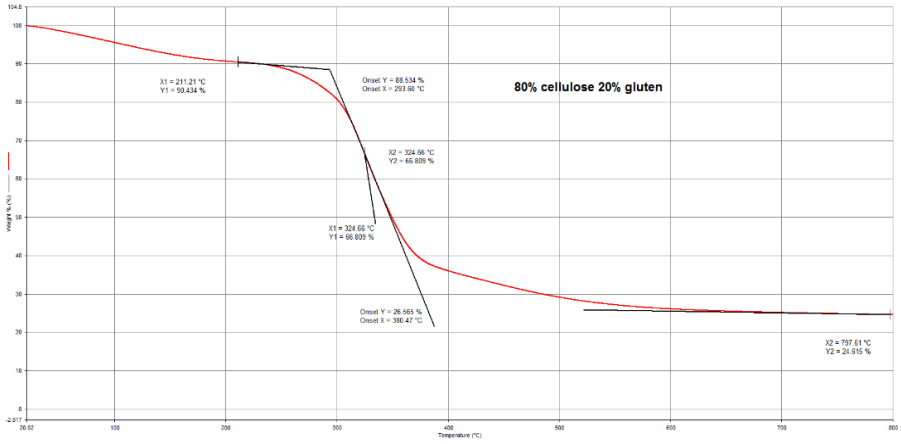
Membranes with 0, 10, 20, 30 and 40% gluten were tested. The TGA graphs are shown in Figure 49 (a-e).



(a)

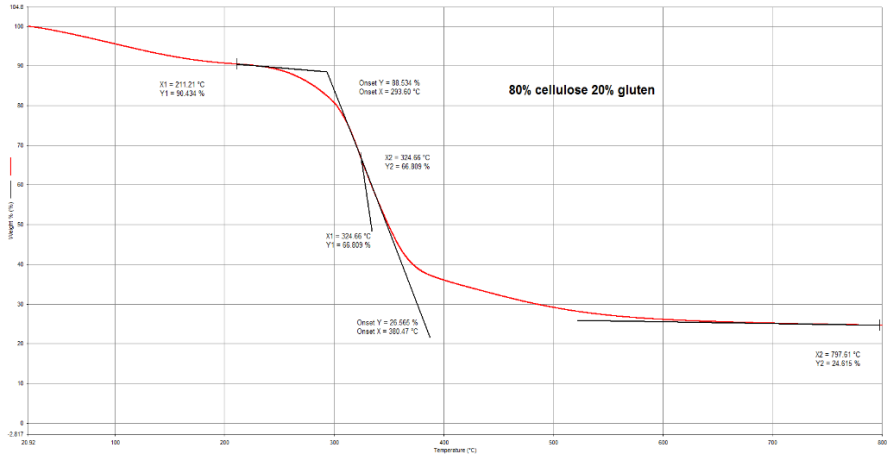


(b)

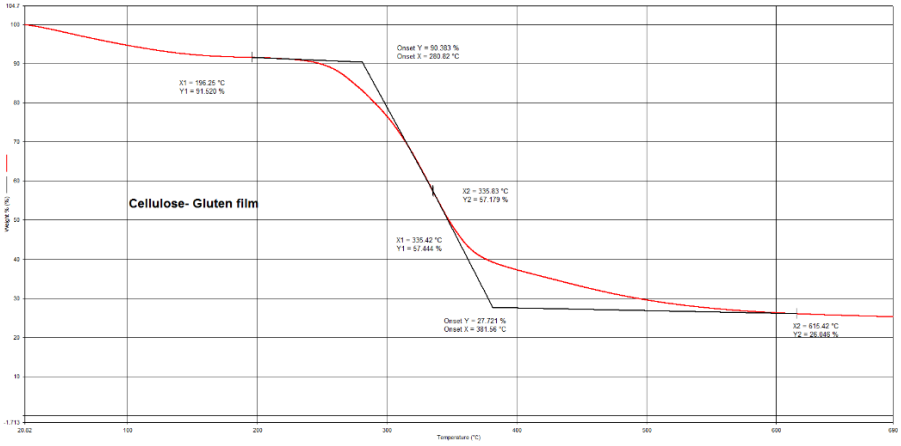


(c)

Figure 54a. TGA curves of cellulose membranes with (a) 0, (b) 10 and (c) 20% gluten



(d)



(e)

Figure 55b. TGA curves of cellulose membranes with (d) 30 and (e) 40% gluten

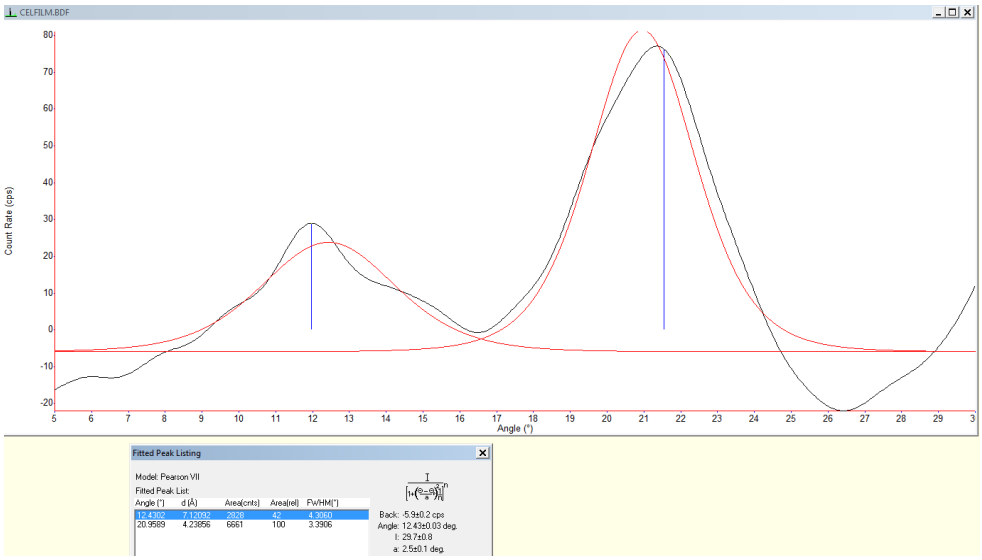
Table 21. Onset and offset decomposition temperatures and char levels of the TGA curve

Cellulose/gluten ratio	Onset temperature (℃)	Offset temperature (℃)	Char level (%)
100/0	291.73	377.03	25
90/10	297.39	388.43	26
80/20	293.6	380.47	24
70/30	297.09	393.42	23
60/40	280.82	381.56	26

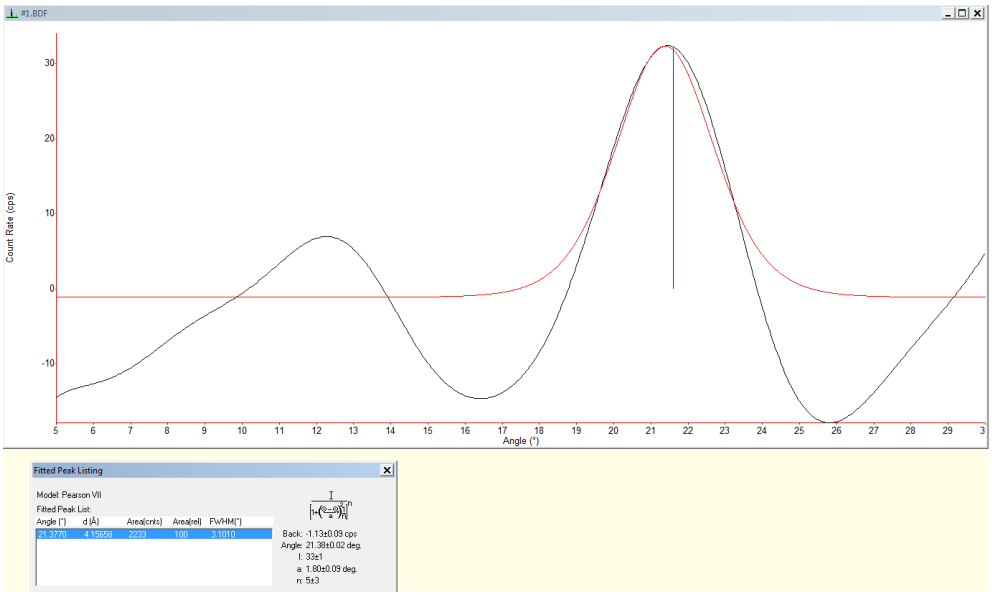
The onset and offset temperature of wood pulp (264.34℃ and 403.38℃) are higher than those of gluten (199.69℃ and 391.23℃). As shown in Figures 49 (a-e) and Table 21, with the increase of gluten concentration the onset and offset temperature increase but are still very close to each other. Comparing the char level, membranes with the different gluten concentration have very close char levels which means cellulose and gluten are miscible. By analyzing the decomposition rates, those curve only one curve which also means cellulose and gluten are miscible. This is similar to the result of the Douglass's work.

6.2.2.5 Wide-Angle X-ray Scattering

Wide-Angle X-ray Scattering was performed to detect morphological features of cellulose/gluten blend membranes with different gluten concentration and to show the structural differences. The degree crystallinity is an important parameter to influence physical and mechanical properties of materials. The WAXS spectra of membranes with 0, 10, 20, 30 and 40% gluten were shown in Figure 50 (a-e).



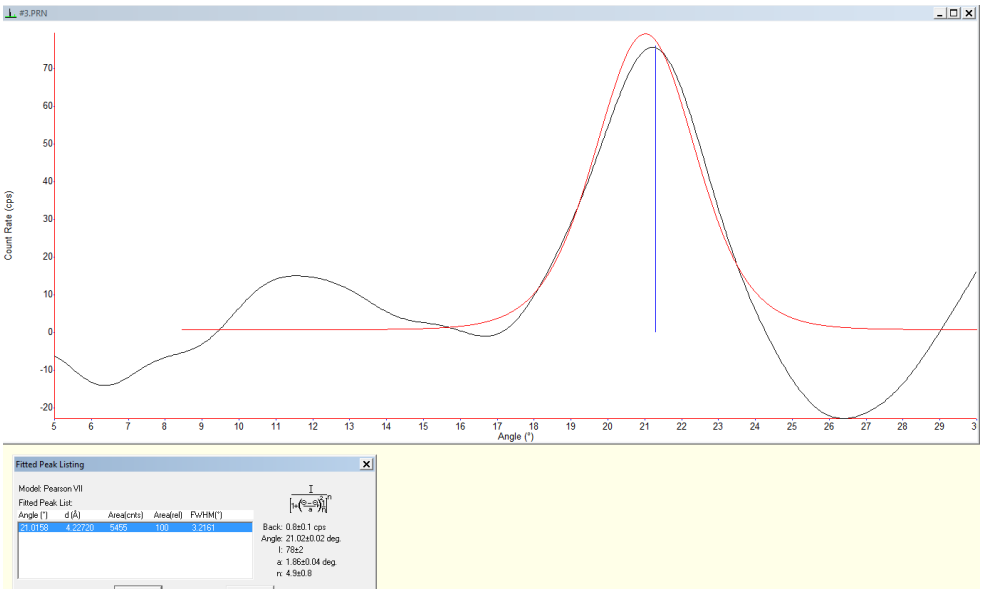
(a)



(b)

Figure 56a. WAXS curves of cellulose/gluten blend membranes with (a) 0, (b) 10% gluten

(c)



(d)

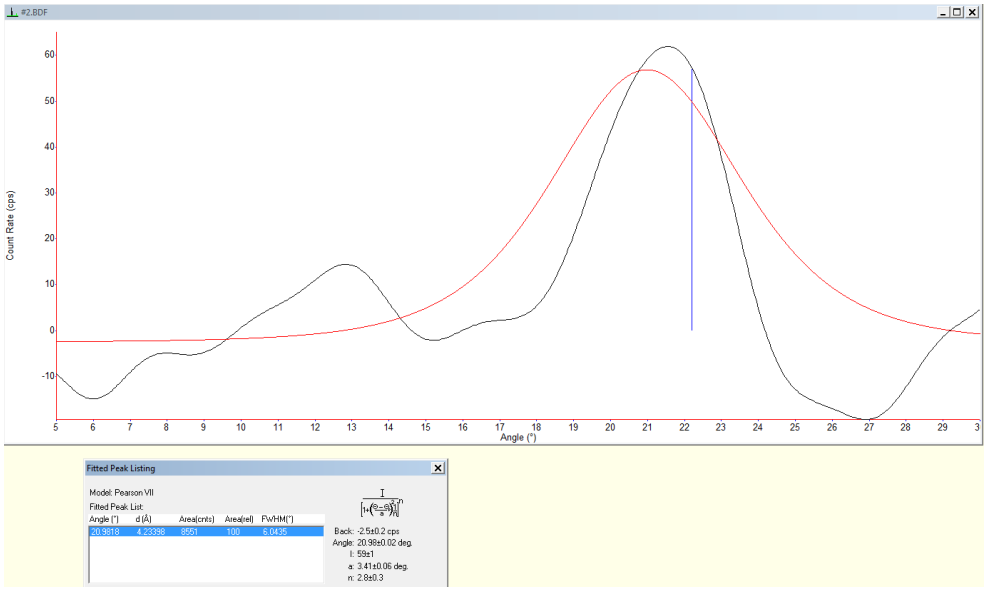


Figure 57b. WAXS curves of cellulose/gluten blend membranes with (c) 20, (d) 30% gluten

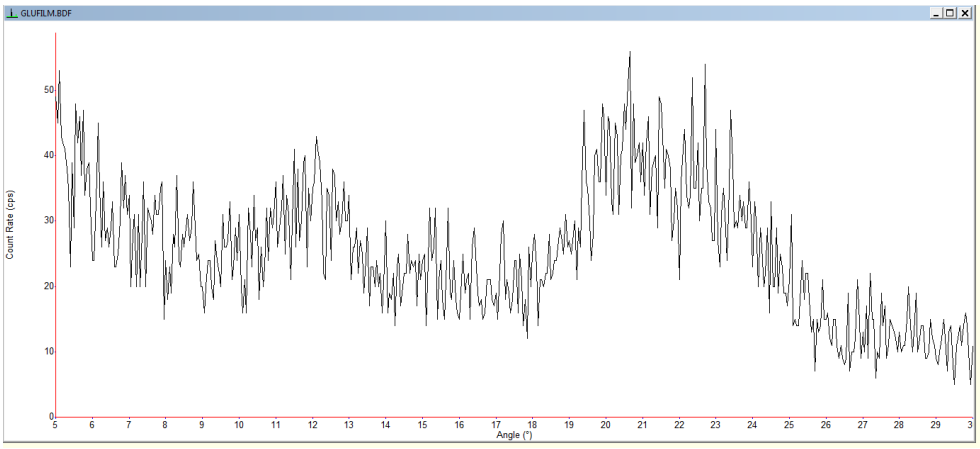
(e) 

Figure 58c. WAXS curves of cellulose/gluten blend membranes with (e) 40% gluten

Generally, with the increase of gluten concentration, the crystallinity of membranes decreased. Figure 50 (a-e) show the WAXS curves of cellulose/gluten blend membranes with different gluten concentration. Figure 50 (a) shows the curve of cellulose II which was converted from cellulose I by dissolution and then coagulation, which verified by Douglass and Cao.[17,70] WAXS curves of all the membranes with different gluten concentration have the same peaks at around 13 and 21-22º 2θ that mean cellulose II exists in all these membranes. However, the peaks are spread out with an increase of gluten concentration. We can probably assume that as the peak are sharper the higher crystallinity the membrane increase.[73] So with the increasing concentration of gluten, the crystallinity of membranes decreased, because of the rearrangement of macromolecules during dissolution and regeneration. The ED/KSCN solvent dissolved crystalline form of cellulose by destroying inter- and intramolecular hydrogen bonds between the cellulose molecules.[73] During the regeneration (coagulation),

(some of) hydrogen bonds reformed again. The addition of gluten prevented the formation of hydrogen bonds so the crystallinity decreased and the amorphous area increased. Figure 55 (e) shows the WAXS "curve" of cellulose/gluten blend membranes with 40% gluten. The intensity for 40% gluten membranes was too low to form a curve which means the crystallinity of the membrane was very low.

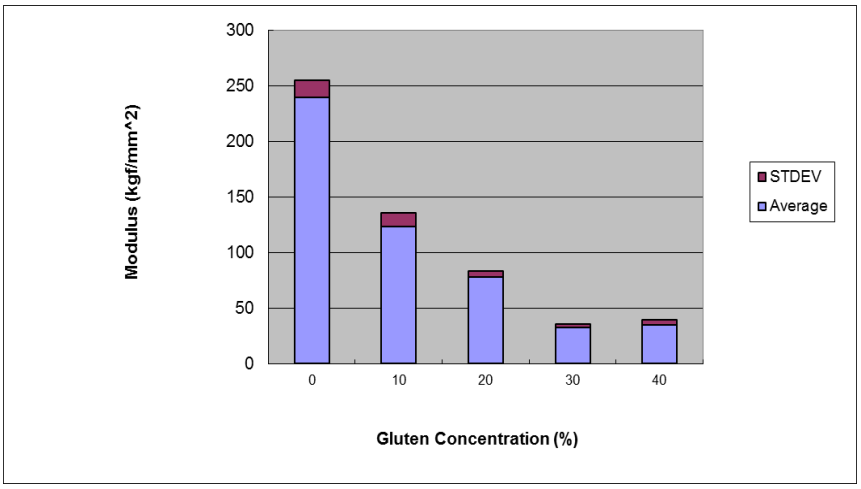
6.2.2.6 Tensile properties

Tensile properties are very important properties for studying cellulose/gluten blend membranes which are highly related to the potential usages for the membranes. Cellulose membranes with different amount of gluten of 10, 20, 30 and 40%, were tested. During the tests, tensile modulus, break stress and elongation at break were paid more attention to. The data was collected and shown in Table 22.

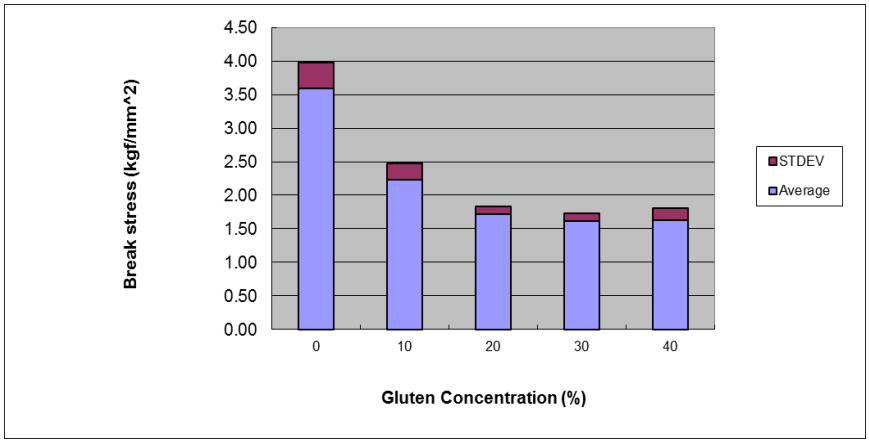
Table 22. Comparison of tensile properties of cellulose membranes with different gluten concentration

Cellulose/gluten ratio	Tensile Modulus (kgf/mm^2) & CV(%)	Break Stress (kgf/mm^2) & CV(%)	Elongation at Break (mm) & CV(%)	Thickness (mm) & CV (%)
100/0	239.50 ± 15.49 (6.47)	3.58 ± 0.39 (10.98)	17.14 ± 4.52 (25.96)	0.076 ± 0.005 (6.35)
90/10	123.44 ± 12.2 (9.88)	2.23 ± 0.24 (10.68)	19.32 ± 4.24 (21.94)	0.066 ± 0.002 (3.3)
80/20	77.42 ± 6.05 (7.81)	1.48 ± 0.1 (6.72)	19.58 ± 3.25 (16.6)	0.062 ± 0.001 (1.12)
70/30	32.75 ± 2.76 (8.42)	1.61 ± 0.12 (7.66)	22.30 ± 2.59 (11.59)	0.050 ± 0.001 (2.44)
60/40	34.53 ± 4.4 (12.73)	1.62 ± 0.18 (11.03)	28.51 ± 4.1 (14.38)	0.046 ± 0.003 (6.08)

(a)



(b)



(c)

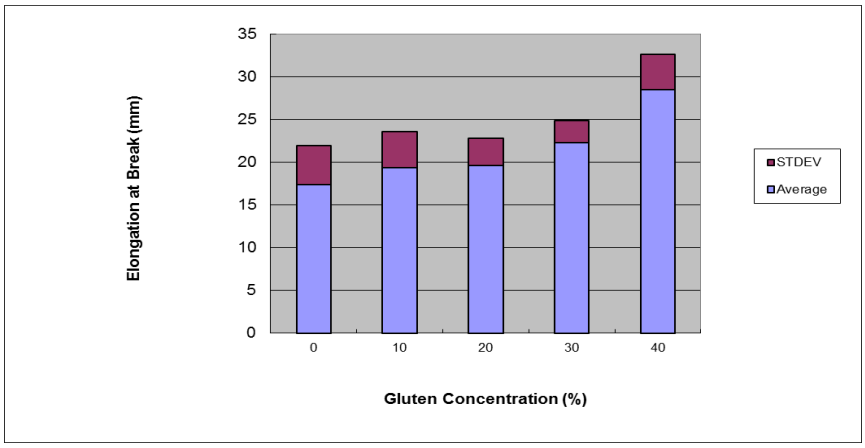


Figure 59a. (a) Modulus, (b) tensile stress and (c) elongation (mm) at break of cellulose membranes with different amount of gluten

(d)

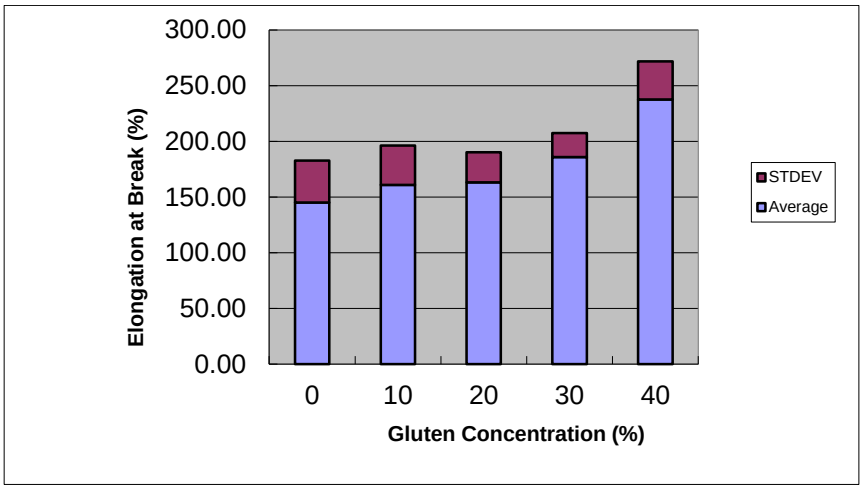


Figure 60b. (d) elongation (%) at break of cellulose membranes with different amount of gluten

Figure 51 (a-d) clearly present how gluten concentration influences tensile modulus, break stress and elongation at break respectively and trend is obviously observed.

The cellulose-only membranes had the highest tensile modulus of 239.5 ± 15.49 kgf/mm^2 and break stress of 5.36 ± 0.7 kgf/mm^2 but the lowest elongation at break of 26.2 ± 10.1 mm (145%). With the addition of gluten, at low gluten concentration, the modulus and stress dramatically decreased, because the addition of gluten increased the amorphous phase content and decreased the degree crystallinity. At last, the blend membranes with the highest gluten amount of 40%, showed the lowest tensile modulus (34.53 ± 4.4 kgf/mm^2). The membranes with 20 and 30% gluten showed mechanical properties between the cellulose-only and the 40% gluten membranes.

So it is plausible to draw a final conclusion that with the increase of gluten tensile modulus and break stress decrease and the elongation at break increases. This means that the addition of gluten can alter the mechanical properties and can increase the processability of cellulose membranes because of the increased elongation at break. In Table 22, the elongation at break of all groups of samples show very high STDEV and CV value. This effect was obviously influenced by the sample morphology. Wrinkling and air bubbles were sometimes responsible for these effects. Air bubbles in polymer solutions were difficult to eliminate during membranes casting.

6.2.2.7 Water Absorption

Water absorption is an important property for studying cellulose/gluten blend membrane which is related to the potential usages. Water absorption test was conducted to study the hydrophilicity of dried cellulose membranes and water sorption. Gluten proteins have been reported to be water-insoluble. Samples were prepared and tested in the same way as testing cellulose-only membranes. All data of different samples were collected and shown in Table 23.

Table 23. Comparison of water absorption results of cellulose-only and cellulose/ gluten blend membranes

Cellulose/gluten ratio	Dry mass (g)	Wet mass (g)	Wet mass increase (%)
100/0	0.3547	0.5147	45
90/10	0.3966	0.5380	36
80/20	0.2932	0.3965	35
70/30	0.2827	0.3715	31
60/40	0.2951	0.3588	22

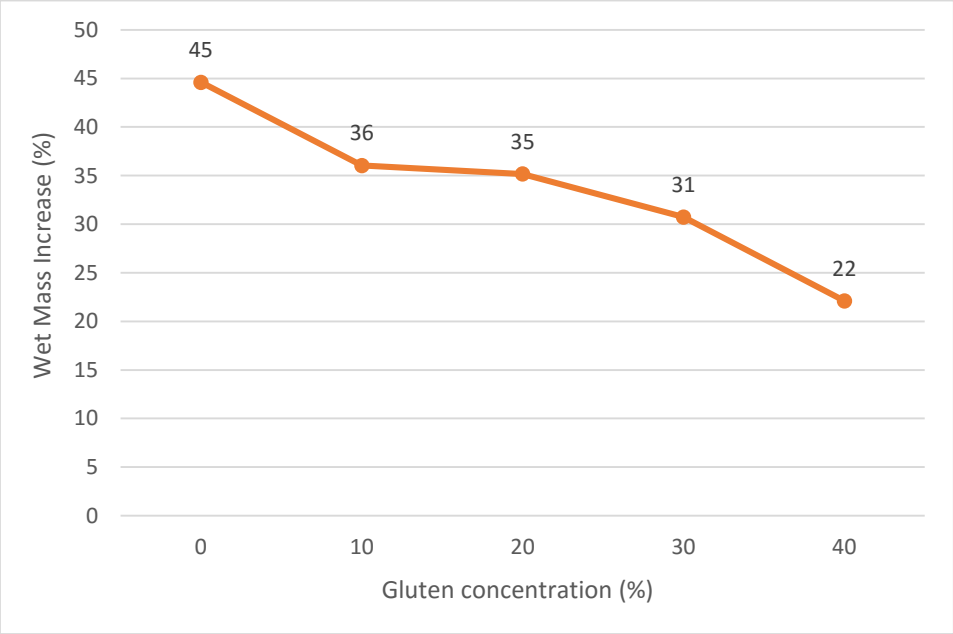


Figure 61. Wet mass increase of cellulose membranes with different gluten concentration

The data in both Table 23 and Figure 52 show a decreasing trend of water pick up by cellulose membranes with an increasing gluten amount. After soaking for 24 hours, the wet mass increase of cellulose-only membranes was 45%. At the gluten content of 40% water absorption decreased 51% to 22%. It means that gluten is not as hydrophilic as cellulose and a

higher percentage of gluten can cause a lower water absorption. It is because of the decrease of amount of –OH (cellulose concentration decreases) and increase of hydrophobic chains (increase of gluten.)

It was noticed that all membranes were intact after soaking in water for 24 hours or even 48 hours, and some strength was kept. Since gluten is water-insoluble, there was no traces of gluten in water. All wet membranes were dried and were intact with good physical and mechanical properties. The water absorption test showed the potential of cellulose/gluten membranes to serve as a food packaging films.

6.2.3 Conclusions

Uniform membranes made by solution of cellulose/gluten blend with the different ratio of 90/10, 80/20, 70/30 and 60/40, were tested by the same characterization methods as for used cellulose-only membranes. Viscosity of each blend solution and membrane-forming ability of gluten-only membrane were studied as well. The results shown in this chapter show the influence of different gluten concentration on the properties of blend membranes. Several conclusions can be summarized as follows:

1) The ED/KSCN (65/35) solvent system is a highly efficient solvent for gluten. Complete dissolution can be obtained after 3 hours.
2) To obtain a gluten solution with a film forming viscosity for casting, the gluten concentration must be increased to 40%. However gluten-only membrane can’t be made by the method in this work which is attributed to low polymer chain entanglements.

3) The results of viscosity measurement show that viscosity of cellulose/gluten blend solution decreases with an increasing gluten concentration. It can be attributed to decrease in the average molecular weight or crystallinity.
4) Methanol is proved to be an effective coagulant for the solution of cellulose/gluten blend.
5) SEM images show the blend membranes made in this work are uniform and nonporous. The SEM images of cross-sectional area of stretched-to-break membranes show perfect blend of cellulose, and gluten and increase in amorphous region with an increasing gluten concentration.
6) The results in FTIR tests prove that there is no chemical side interaction occurred between gluten, cellulose and ED/KSCN. The peak intensity of amide groups increased because of gluten addition. The peaks wavenumber did not shift. The results prove that cellulose and gluten are perfectly blended. There is no peak for -SCN group implying that ED/KSCN solvent residue was completely removed with methanol.
7) The TGA results show different thermo-degradation behavior for cellulose membranes with different gluten concentration that can be linked to the formation of cellulose II in the blend membranes.
8) The WAXS tests prove that addition of gluten can influence the crystal structure. The cellulose II peaks still remains. Furthermore, with the increase of gluten concentration, the amorphous region increases.
9) The cellulose/gluten blend ratio can obviously influence the tensile properties of membranes. As the gluten content increases the elongation at break of membranes

increases thus improving the processability of cellulose membranes. The increase of cellulose can improve mechanical properties of gluten. With the increase of cellulose, the modulus and stress of blend membranes increase. The results of tensile test shows that the tensile properties can be controlled by adjusting the ratio of cellulose/gluten blend.

10) Water absorption test shows the relationship between water absorption and gluten concentration. With the increase of gluten concentration to 40%, the amount of absorbed water decreases to 22% for the cellulose membranes. The membranes soaked in water for 24 even 48 hours, still have integrity and some mechanical properties. After drying membranes show mechanical properties.

The properties of regenerated cellulose/gluten blend membranes were systematically studied. The good properties show potential uses of the regenerated cellulose/gluten blend membranes in food packaging or medical end uses. Both cellulose and gluten are biodegradable and renewable material with low price and abundant resources, so the cellulose/gluten blend membranes are worthy for industrial use in the future.

CHAPTER 7. GRAND COCLUSIONS

Both cellulose and gluten are abundant, low cost, biodegradable, renewable polymers found in nature. The novel membranes made of regenerated cellulose/gluten blend are produced in this work by improved method that used in Douglass's and Zhu's work. The influence of the ratio of cellulose/gluten to the properties of membrane is systematically studied. The overall conclusions are drawn:

1) Cellulose and gluten can be efficiently dissolved by the ED/KSCN solvent system. With the increase of gluten concentration, the dissolution time decreases.
2) Methanol is an effective coagulant for cellulose/gluten blend membranes. Also methanol can remove ED/KSCN from membranes perfectly.
3) The improved membrane-producing method can make more uniform membrane.
4) No chemical side interactions occurred during membrane production.
5) Cellulose and gluten are compatible and perfectly blended together.
6) Membranes made in this work are uniform and nonporous.
7) The blend membranes have higher elongation at break and improved water barrier properties.
8) The properties of blend membranes are related to the ratio of cellulose/gluten blend which can influence the molecular weight, crystal structure and other factors. So the properties of blend membranes can be controlled by adjusting the ratio of cellulose/gluten concentration.
9) The cellulose/gluten blend membranes can have potential applications like food

packaging even medical applications because of their good properties, sustainability, low cost, biodegradability and they are ecofriendly.

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