

**CELLULOSE FIBER DISSOLUTION IN SODIUM  
HYDROXIDE SOLUTION AT LOW TEMPERATURE:  
DISSOLUTION KINETICS AND SOLUBILITY  
IMPROVEMENT**

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# CELLULOSE FIBER DISSOLUTION IN SODIUM HYDROXIDE SOLUTION AT LOW TEMPERATURE: DISSOLUTION KINETICS AND SOLUBILITY IMPROVEMENT

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS . . . . .	iii
LIST OF TABLES . . . . .	vii
LIST OF FIGURES . . . . .	viii
LIST OF SYMBOLS OR ABBREVIATIONS . . . . .	xi
SUMMARY . . . . .	xiii
I INTRODUCTION . . . . .	1
1.1 Objectives . . . . .	1
1.1.1 Objective I: Mechanism Study . . . . .	2
1.1.2 Objective II: Solubility improvement . . . . .	3
1.1.3 Objective III: Hemicellulose and Lignin effect . . . . .	3
1.2 Lignocellulosic Materials . . . . .	4
1.2.1 Lignocellulose Components . . . . .	4
1.2.2 Molecular Structure of Cellulose . . . . .	5
1.2.3 Supramolecular Structure of Cellulose . . . . .	7
1.3 Review of Cellulose Dissolution . . . . .	16
1.3.1 Cellulose Solvents . . . . .	16
1.3.2 Cellulose Dissolution in Alkali Solution . . . . .	20
1.3.3 Solution Structure of Cellulose . . . . .	31
1.4 Enzymatic Hydrolysis of Cellulose . . . . .	33
1.5 Novelty and Significance . . . . .	35
1.6 Main Foci . . . . .	36
II DISSOLUTION KINETICS . . . . .	38
2.1 Introductions . . . . .	38
2.1.1 Review of Chemical Reaction Kinetics . . . . .	39
2.1.2 Thermal Analysis of Cellulose-NaOH/urea-water System in Literature . . . . .	41

2.2	Experimental . . . . .	43
2.2.1	Materials and Chemicals . . . . .	43
2.2.2	Low Temperature Reactor Design . . . . .	44
2.2.3	Sample Preparation . . . . .	45
2.3	Characterization Methods . . . . .	49
2.4	Results and Discussions . . . . .	50
2.4.1	Solubility . . . . .	50
2.4.2	Decrystallization Kinetics . . . . .	53
2.4.3	Correlation of Overall Solubility with Decrystallization Process	66
2.4.4	The Limit of Cellulose Solubility . . . . .	66
2.4.5	The Effect of Phase Change During Dissolution . . . . .	67
2.4.6	The Effect of NaOH Concentration . . . . .	68
2.4.7	NaOH Adsorption on Cellulose . . . . .	74
2.4.8	The Effect of Urea Addition . . . . .	75
2.4.9	Other Effects . . . . .	77
2.4.10	Possible Dissolution Mechanism . . . . .	78
2.5	Conclusions . . . . .	80
III	ENZYMATIC HYDROLYSIS AIDED COTTON DISSOLUTION . . . .	81
3.1	Introduction . . . . .	81
3.2	Experimental . . . . .	84
3.2.1	Materials and Chemicals . . . . .	84
3.2.2	Sample Treatments . . . . .	84
3.2.3	Characterization . . . . .	85
3.3	Characterizations and Discussions . . . . .	86
3.3.1	Solubility . . . . .	86
3.3.2	Morphology Change . . . . .	90
3.3.3	Crystallinity and Crystal Size . . . . .	91
3.3.4	Degree of Polymerization . . . . .	96

3.4	Conclusions . . . . .	98
IV	HEMICELLULOSE AND LIGNIN EFFECTS . . . . .	100
4.1	Introduction . . . . .	100
4.1.1	Hemicellulose . . . . .	100
4.1.2	Lignin . . . . .	102
4.1.3	The Basics of HPLC . . . . .	103
4.2	Experimental . . . . .	103
4.2.1	Materials and Chemicals . . . . .	103
4.2.2	Sample preparation . . . . .	103
4.2.3	Characterization . . . . .	104
4.3	Results and Discussions . . . . .	105
4.3.1	Observations . . . . .	105
4.3.2	Solubility Change . . . . .	105
4.3.3	Sugar Component Change . . . . .	106
4.3.4	Water Retention Change . . . . .	108
4.3.5	Degree of Polymerization Change . . . . .	108
4.4	Conclusions . . . . .	109
V	CONCLUSIONS . . . . .	114
VI	FUTURE WORK . . . . .	118
	REFERENCES . . . . .	121

## LIST OF TABLES

1	Degree of Polymerization . . . . .	6
2	Unit Cell Parameters of Cellulose Polymorph . . . . .	12
3	Conventional and New Cellulose Solvents . . . . .	17
4	DP <sub>v</sub> and possible cellulose I dissolution threshold with various solutions	27
5	Example of Spread Sheet of Kinetics Calculation . . . . .	64
6	Activation Energy for Decrystallization . . . . .	64
7	Typical Crystal Change During Dissolution . . . . .	66
8	Temperature Peak . . . . .	70
9	Crystallinity & solubility in NaOH/urea . . . . .	95
10	Crystallinity and crystal size . . . . .	95
11	Sugar Components of Sample . . . . .	107

## LIST OF FIGURES

1	Model of Wood Fiber Cell Wall . . . . .	5
2	Molecular Structure of Cellulose . . . . .	6
3	Scale of Cellulose . . . . .	7
4	Bragg Diffraction Theory . . . . .	10
5	XRD of Cellulose During Alkalization and Regeneration . . . . .	11
6	Crystal Structures of Cellulose I . . . . .	13
7	Crystal Structure of Cellulose II . . . . .	14
8	Fringed Fibril Model . . . . .	15
9	Phase Diagram of NaOH in Water . . . . .	21
10	Phase diagram of cellulose-NaOH-water system . . . . .	23
11	Unit Cell Model for Na-Cellulose I . . . . .	25
12	Sliding Chain Model . . . . .	30
13	Schematic Dissolution Process . . . . .	31
14	Schematic Formation of Carbamate . . . . .	31
15	Solution Structure of Cellulose . . . . .	32
16	Project Plan . . . . .	37
17	DSC Spectra of Cellulose-NaOH-water Solution . . . . .	42
18	Experimental Setup for Dissolution Kinetics Study . . . . .	47
19	Phase Diagram of Ethylene Glycol in Water . . . . .	48
20	Picture of Samples After NaOH/urea Treatment . . . . .	49
21	Solubility of Cotton Linter in 6%NaOH/4%urea Solution . . . . .	51
22	Solubility of Cotton Linter in 6%NaOH Solution Without Urea . . . . .	51
23	Sampling Time and Temperature . . . . .	52
24	Urea Contribution to Solubility . . . . .	52
25	Crystal Type Change at $T_c = -11^\circ\text{C}$ . . . . .	54
26	Crystal Type Change at $T_c = -15^\circ\text{C}$ . . . . .	55
27	Crystal Type Change at $T_c = -20^\circ\text{C}$ . . . . .	56

28	Plot of Crystal Type Change During 4% NaOH Solution Dissolution Without Urea, $T_c = -15^\circ\text{C}$ . . . . .	57
29	Crystallinity of Cotton Residues Treated in 6%NaOH solution . . . . .	58
30	Crystallinity of Cotton Residues Treated in 6%NaOH/4%urea Solution . . . . .	58
31	The Reaction Order of Cellulose Decrystallization . . . . .	60
32	Plot of Eyring Equation . . . . .	62
33	Plot of Gibbs Free Energy . . . . .	63
34	Plot of Instantaneous Reaction Rate . . . . .	63
35	Crystallite Size Change during Dissolution . . . . .	65
36	Correlation of Overall Solubility with Decrystallization Process . . . . .	67
37	Temperature Profiles at $T_c = -8^\circ\text{C}$ , $-11^\circ\text{C}$ (a) and $T_c = -15^\circ\text{C}$ and $-20^\circ\text{C}$ (b) . . . . .	69
38	NaOH Concentration Effects on Dissolution Kinetics . . . . .	70
39	Lever Rule on NaOH-water Phase Diagram . . . . .	72
40	Hydrodynamic Diameter of Hydrates . . . . .	73
41	Plot of NaOH Concentration Change During Dissolution . . . . .	76
42	The Effect of Urea Addition . . . . .	77
43	The Effect of Different Diameters of Sample Container . . . . .	78
44	Solubility of cotton in NaOH/urea solution . . . . .	87
45	Yield of Enzyme Hydrolysis . . . . .	88
46	Solubility of Enzyme Pretreated Cotton Linter . . . . .	89
47	Morphology Change of Cotton Fibers . . . . .	92
48	XRD of Samples . . . . .	93
49	Crystal Structure Change of Cotton Linter . . . . .	97
50	DP changes . . . . .	99
51	Picture of Various Lignin Content Samples . . . . .	105
52	Overall Weight Loss of Lignin Content Samples After NaOH/urea Treatment . . . . .	106
53	Sugar Components . . . . .	108
54	Glucan Components Change during Dissolution Process . . . . .	109

55	Sugar Component Change during Dissolution Process . . . . .	110
56	Sugar Component Change during Dissolution Process, cont. . . . .	111
57	Canadian Standard Freeness Test . . . . .	112
58	DP Change with Bleaching Treatment . . . . .	113

## LIST OF SYMBOLS OR ABBREVIATIONS

$^{13}\text{C}$ CP – MAS NMR	$^{13}\text{C}$ cross-polarization magic angle spinning nuclear magnetic resonance.
$2\theta$	Bragg scattering angles.
<b>AGU</b>	Anhydroglucose unit.
<b>CBH</b>	Cbellobiohydrolase.
$d$	The interplanar distance.
$\Delta G^\ddagger$	Change of Gibbs free energy of activation of decrystallization.
$\Delta H^\ddagger$	Change of enthalpy free energy of activation of decrystallization.
$\Delta H_{interaction}$	The enthalpy change during the solvation of the polymer macromolecules.
$\Delta H_{mixing}$	The enthalpy change during the mixing of the solvated polymer molecules with solvent to give an infinitely diluted solution.
$\Delta H_{transition}$	The enthalpy change during the transition of the amorphous regions from a vitreous to a highly elastic state.
$\Delta S^\ddagger$	Change of entropy free energy of activation of decrystallization.
<b>DI water</b>	Deionized water.
$DP$	Degree of polymerization.
<b>DSC</b>	Differential scanning calorimetry.
<b>EG</b>	Endoglucanase.
<b>FTIR</b>	Fourier transform infrared spectroscopy.
$h$	Planck's constant.
<b>HEC</b>	O-(2-hydroxyethyl)cellulose.
$k$	The Arrhenius reaction rate.
$k_B$	Boltzmann's constant.

$\Delta H_{fusion}$	The enthalpy change during the transition of the solid polymer to a hypothetical, highly elastic liquid state which corresponds to disintegration of the crystalline regions.
$T_m$	Melting temperature.
<b>ML</b>	Middle lamella.
<b>NMMO</b>	N-methylmorpholine-N-oxide.
$R$	The gas constant.
$S$	The solubility degree.
$T$	The system absolute temperature.
$W_o$	The original weight of cellulose.
$W_r$	The weight of undissolved cellulose residue.
<b>XRD</b>	X-ray diffraction.

## SUMMARY

Cellulose is a material that does not melt at the temperature lower than its degradation temperature. Strong intra- and inter-molecular hydrogen bonds in cellulose prevent its molecules from dissolution in most common solvents. Although there are a number of approaches to produce regenerated cellulose, such as viscose rayon, cuprammonium cellulose, Lyocell fibers, the market is shrinking due to the environmental and economic feasibility concerns of these methods. Some other processes, such as using organosolvents and ionic liquid can also dissolve cellulose, but the high cost and organic solvent recovery problems hinder their further applications in a large scale. For these reasons, cellulosic materials are regarded as un-moldable materials. Because of the un-moldable identity, wood and cotton fibers are difficult to be refabricated as other thermosetting and thermoplastic polymers. Therefore, fundamental understanding of the cellulose dissolution chemistry in aqueous solution is particularly interested by cellulose chemists. If an effective, economic and environmentally friendly cellulose dissolution method can be developed, a new platform for producing moldable cellulosic intermediate materials will be created providing new opportunities for using cellulosic materials as a renewable and sustainable engineering polymers.

Effective cellulose solvents should be able to break down these forces and for this purpose, sodium hydroxide is the most popular solvent. NaOH can cause cellulose to swell and can even dissolve cellulose in a narrow range of the phase diagram. It was found that for cellulose with low to moderate degree of polymerization, the maximal solubility occurs with 8 ~ 10% soda solution. In recent years, researchers found that sodium hydroxide with urea at cold temperature can dissolve cellulose better than

sodium hydroxide alone. However, the lack of sufficient understanding of the NaOH and NaOH/urea dissolution process significantly constrains its applications. In order to fully understand the cellulose dissolution in alkali system, there are several aspects of problems that need to be addressed. Our focus in this study is in the interaction of cellulose with alkali solution at low temperatures, the improvement of its solubility, and the effect of hemicellulose and lignin.

Our results from kinetic analysis showed that the dissolution of cellulose in NaOH/urea solution has strong dependence on the temperature of reaction. There is a critical temperature for reaction to take place in the studied solution system. The optimum NaOH concentration in this study is at 6% for high molecular weight cotton linter. The Gibbs free energy of cellulose decrystallization in 6% NaOH with and without urea had a turn point at temperature  $-15^{\circ}\text{C}$ . Above and below this temperature, the contribution of adding urea to enhance the solubility of cellulose is opposite.

For solubility improvement, we used enzymatic hydrolysis as a pretreatment to alkali dissolution. The results showed that the enzyme pretreatment greatly enhanced the dissolution degree of cellulose in NaOH/urea solution with much shorter time. Enzyme treatments did not change crystal type and crystal size, slightly increased crystallinity of cellulose and the molecular weight reduced rapidly. High crystallinity did not necessarily result in low solubility, or at least crystallinity alone could not explain the difficulty of cellulose dissolution.

In the study of the effect of hemicellulose and lignin on cellulose dissolution, we found that due to the low lignin content of our raw material, its influence on cellulose dissolution is not significant; the responses of different sugar components in hemicellulose to the dissolution solvents were quite different, in that xylan was quickly removed but mannan were sustained. The bleaching chemicals of sodium chlorite can not only remove lignin, but also degrade cellulose. The solubility increase observed was due to the reduction of cellulose molecular weight rather than the decrease of

lignin amount.

In a summary, we have gained new insight into mechanism of the cellulose dissolution in alkali solution with or without urea, especially its dependency on temperature. We also explored a new way to enhance the cellulose solubility and found that a certain component of hemicellulose may have special connection to cellulose that cannot be easily broken by chemical treatments, possibly leading lead to the difficulty of wood fiber dissolution.

# CHAPTER I

## INTRODUCTION

### *1.1 Objectives*

Cellulose has a long history in the pulp and paper industry, and nowadays, it draws more and more research interest in textile, material science and biomedical engineering. Not only is it an abundant resource and easily renewable, but it also has special characteristics such as hydrophilicity, chirality, biodegradability, broad chemical modifying capacity and its capability of forming versatile semicrystalline fiber morphologies. However, handling and molding of cellulose is difficult. Because of the rigidity of the cellulose chain and the large amounts of network connecting hydrogen bonds, cellulose belongs to neither thermoplastic nor thermosetting polymers, and is hardly dissolvable in general inorganic or organic solvents. Therefore, the difficulty of cellulose dissolution becomes one of the major limitations of its application. Development of better cellulose dissolving systems is of special importance in cellulose industry and other fields.

In recent years, the NaOH and NaOH/urea solution system has been found as good direct solvents for fully dissolving low molecular weight but partially dissolving high molecular weight cellulose. Comparing with traditional cellulose dissolution systems, it was reported that the NaOH/urea dissolving method is rapid, environmentally friendly and cost effective. However, some conflict results and mechanisms were reported in literature, the lack of sufficient understanding of the NaOH and NaOH/urea dissolution process significantly constrains its applications.

In order to fully understand the cellulose dissolution in alkali system, there are several aspects of problems need to be addressed:

1) The understanding of cellulose from molecular level and supermolecular level, including the hydrogen bonding between molecules, the amorphous cellulose and the crystalline structure, and the pore structure.

2) The behavior of alkali solution with or without urea at low temperature and the interaction between cellulose and aqueous sodium hydroxide and urea and the dissolution mechanism.

3) To extend the application to the general plant lignocellulosic materials, we have to understand the major components of them, that is, hemicellulose and lignin, their chemical structure, their connection with cellulose and their influence in cellulose dissolution.

This problem cannot be fully unveiled unless all above issues are addressed. Our focus in this study is in the interaction of cellulose with alkali solution at low temperatures, the improvement of its solubility, and the effect of hemicellulose and lignin. Specifically, we have three objectives:

#### **1.1.1 Objective I: Mechanism Study of Cellulose Interaction in NaOH and NaOH/Urea Solution Systems**

The cellulose-NaOH/urea-water dissolution mechanism study is the main focus of this project. A thorough kinetics study in terms of cellulose decrystallization during dissolution was performed, through which approximate activation energy was calculated. The effects of low temperature, NaOH and urea concentration were studied. Based on observations from experiments, a possible cellulose dissolution mechanism was proposed. New insight was gained in the cellulose-NaOH-water system. The questions being answered include: the reason that the low temperature works better for cellulose dissolution in alkali system; optimum dissolution temperature, and optimum NaOH and urea concentration.

### **1.1.2 Objective II: Solubility Improvement via Enzymatic Hydrolysis Pretreatment**

The solubility of high molecular weight cellulose is too low according literature to have practical application. Therefore, enzymatic hydrolysis pretreatment was introduced to increase this solubility.

The difficulty of cellulose dissolution is due to its long range ordered crystalline regions. To dissolve cellulose, the solvent molecules (here are NaOH and Urea molecules) have to be able to get access into the core of the cellulose crystal. Therefore, the size of the crystal, in both the lateral and the longitudinal direction and the cellulose chain packing pattern are the determining factors in cellulose dissolution.

Cellulases were utilized to partially degrade cellulose molecular weight. The cellulase treated cellulose was then interacted with NaOH and urea in aqueous solvent to obtain various cellulose dissolution degrees. This is based on a known fact that cellulases, especially endoglucanases, have the preference to attack cellulose from the cross section direction, leading to a reduction of the degree of polymerization. It was expected that, with the synergistic effect of cellulases and alkali, the solubility of cellulose could be greatly increased.

### **1.1.3 Objective III: Fundamental Understanding of the Effects of Hemicellulose and Lignin on the Cellulose Dissolution in NaOH/urea Solution**

Hemicellulose and lignin are two major components in wood fibers. In native structure, hemicelluloses are embedded in plant cell walls, binding to cellulose to form a network of cross-linked fibers, while lignin fills the spaces in the cell wall between cellulose and hemicellulose. The structural relationship of these three components dictates the influence of hemicellulose and lignin on cellulose dissolution. This effect was also investigated in this study.

## ***1.2 Lignocellulosic Materials***

### **1.2.1 Lignocellulose Components**

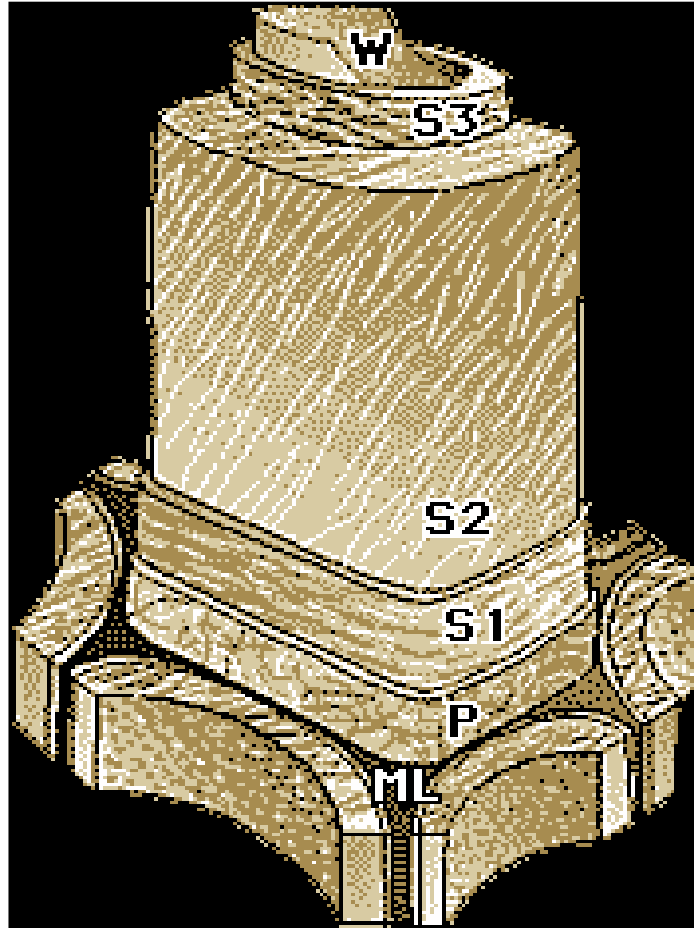
Lignocellulosic materials refers to plant material that is composed of cellulose, hemicellulose, and lignin. In nature, cellulose is the structural material for plant cell wall and generally exists in the form of lignocellulose. Nowadays, lignocellulosic material is of highly interest to researchers in the field of biofuel and bioenergy. Cellulose is an environmentally friendly and renewable biomaterial. Two statistical data will give us the idea that how important this biomaterial is to our daily life: it constitutes around  $1.5 \times 10^{12}$  tons of the total annual biomass production and approximately 2% (3.2 million tons in 2003) were used for the production of cellulose regenerate fibers and films, as well as for the synthesis of a large number of cellulose esters and ethers[53]. Wood pulp is the most important raw material source for the processing of cellulose, which is mostly used in paper industry.

Cellulose is the main structural material in plant cell wall[36], which contributes 45% of wood mass. The less abundant components are hemicellulose and lignin; the former takes about 35% in hardwoods and 25% in softwoods, while the latter takes up 21% in hardwoods and 25% in softwoods respectively[118].

A typical wood fiber cell wall model is shown in Figure 1. It is constituted by middle lamella(ML), the primary cell wall(P), and the secondary cell wall 1, 2 and 3(S1, S2 and S3). The middle lamella is a layer rich in pectins. This outermost layer forms the interface between adjacent plant cells and glues them together. The primary cell wall is generally a thin, flexible and extensible layer formed while the cell is growing. The secondary cell wall is a thick layer formed inside the primary cell wall after the cell is fully grown. It is not found in all cell types. In some cells, such as found xylem, the secondary wall contains lignin, which strengthens and waterproofs the wall. The structural cellulose exists mostly in the S2 layer. Researchers determined the mechanical interaction between cellulose microfibril and

matrix substance in wood cell wall X-ray diffraction(XRD)[1].

Cellulose structures, at both molecular level and supermolecular lever will be reviewed in the following sections. Hemicellulose and lignin will reviewed in Chapter 4.



**Figure 1:** Model of Wood Fiber Cell Wall

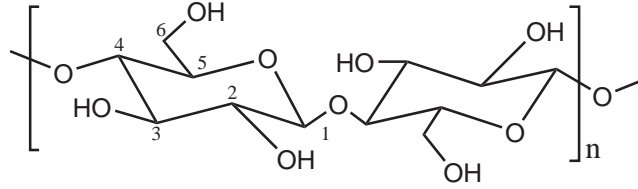
### 1.2.2 Molecular Structure of Cellulose

Cellulose has the basic molecular format of  $C_6H_{10}O_5$ , which is also called Anhydroglucose unit (AGU). The cellulose molecule is linked in the form of  $\beta$ -1, 4-glucan. Figure 2 is the schematic molecular structure of cellulose.

**Table 1:** Degree of Polymerization[53, 118]

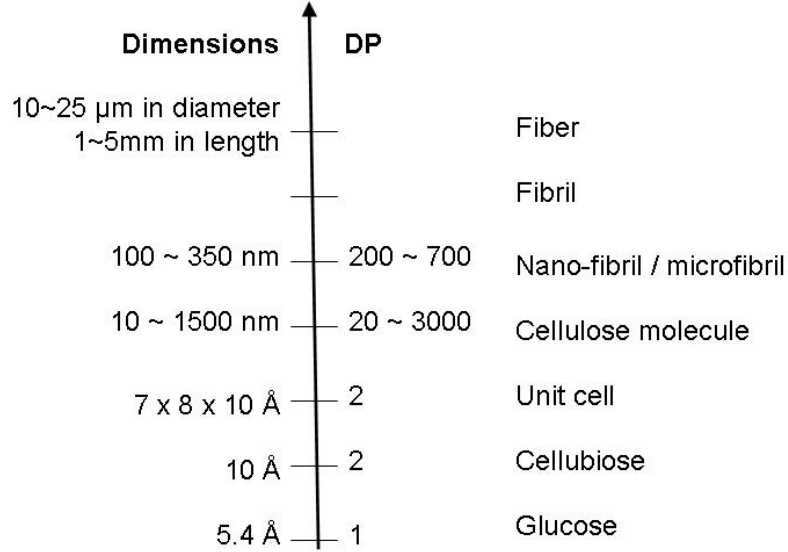
Degree of Polymerization Values (Weight Average)	
Purified cotton linters	1000 ~ 3000
Commercial wood pulps	600 ~ 1500
Regenerated cellulose (e.g. Rayon)	200 ~ 600
Microcrystalline cellulose (e.g. Avicel)	150 ~ 300
Crystallites	50 ~ 100
Microfibril	250 ~ 1000

The polymer chain length is expressed as the number of AGUs or degree of polymerization,  $DP$ . This  $DP$  value varies with the cellulose origins and treatment history (see Table 1).

**Figure 2:** Molecular Structure of Cellulose

Generally, 20 ~ 30 repeating units give all cellulose properties. Figure 3 is a rough scale of cellulose from the AGU to fiber.

Each cellulose chain has two ends, one with an original C4-OH group is called the nonreducing end and the other with an original C1-OH is called the reducing end. Additional carbonyl and carboxy groups can be introduced onto cellulose by chemical treatments, such as by bleaching chemicals[106]. Many characteristic properties are determined by the molecular structure, including hydrophilicity and degradability. The multiple OH groups on cellulose molecule and its linear structure enable the formation of crystalline fiber bonded by extensive hydrogen bonds.



**Figure 3:** Scale of Cellulose

### 1.2.3 Supramolecular Structure of Cellulose

Besides cellulose molecular level, the understanding of cellulose at the supramolecular level is also important to the dissolution mechanism study[46, 52, 51]. This includes crystalline types[86, 90], crystallinity, crystallite size[85, 89, 88], and pore sizes and structures, which are reviewed in the following paragraphs.

#### 1.2.3.1 Hydrogen Bonds in Cellulose Crystal

Cellulose itself is a hydrophilic material[10]. The reason of its difficulty of dissolution in aqueous solution is due to the existing of large quantities of hydrogen bonds which group cellulose chains together to form a network. There are two kinds of hydrogen bondings: intermolecular, and intramolecular hydrogen bondings. Two major intramolecular hydrogen bonds in native cellulose were proposed[36]. One is between the C3 hydroxyl groups and the pyranose ring oxygen of an adjacent glucose residue ( $03 - \text{H} \cdots 05'$ ) with bond length of 2.707  $\text{\AA}$ [140]. The other one is between the C2 OH and the C6 oxygen of a neighboring glucose residue ( $02 - \text{H} \cdots 06'$ ) with bond

length of 2.802 Å. The major intermolecular hydrogen bond is between the C6 – OH and C3 oxygen (O6 – H ··· O3') along the b axis with bond length of 2.874 Å. Besides hydrogen bonding, van der Waals force is also important for cellulose network forming, especially between the non-polar sheets. Computer modeling of cellulose crystallite shows that van der Waals force contributes to the major part of lattice energy between the non-polar sheets [122].

The hydroxyl groups in cellulose are arranged in radial orientation while the aliphatic hydrogen atoms in axial positions. Therefore, strong interchain hydrogen bonding between neighboring chains are easily formed and between cellulose sheets, some weaker hydrophobic interaction exist[42]. Researchers proposed that the cellulose resistance to acid hydrolysis is due to the hydrophobic face of cellulose sheets where a dense layer of water formed near the hydrated cellulose surface[77] and the resistance to enzyme hydrolysis is due to the inter-chain hydrogen bonding[92].

To fully dissolve cellulose, the inter-cellulose and inter-sheet dispersive force have to be broken at least. Many cellulose solvents are designed for this purpose, for an example, sodium hydroxide is the most popular one.

#### *1.2.3.2 XRD and Cellulose Crystal Structure*

Cellulose crystalline structure has been extensively studied by researchers[4, 3, 125]. The major characteristic techniques used to study the crystal structure of cellulose include  $^{13}\text{C}$  cross-polarization magic angle spinning nuclear magnetic resonance  $^{13}\text{C}$  CP – MAS NMR[5, 3, 4, 50, 49, 56, 57], FTIR[75, 81, 94], and XRD[15, 31, 41, 63, 65, 73, 80, 92, 93, 95, 114]. The comparison between Wide angle X-ray and solid state  $^{13}\text{C}$ -NMR studies of cellulose alkalization was discussed by Fink[32]. The results obtained from these different techniques are not the same in that NMR and FTIR are sensitive to the chemical environment of polymer chain, while XRD is sensitive to the crystal lattice structure. Because of this, XRD can be viewed as a direct tool

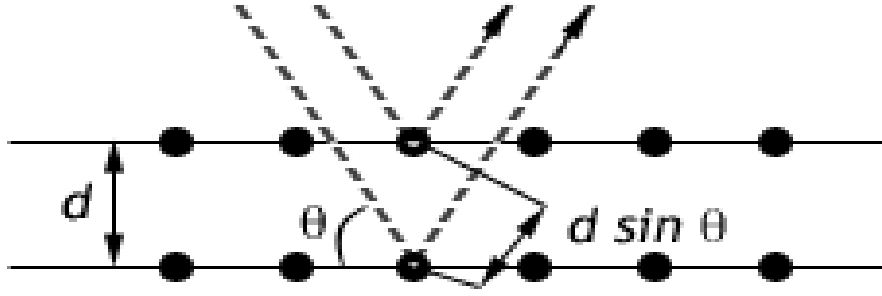
to investigate crystalline structure while NMR and FTIR are indirect. In this study, XRD was used as the main characteristic technique, therefore, a brief review of this technique and the characteristic spectra of cellulose is necessary.

X-ray scattering techniques are a family of non-destructive analytical techniques which reveal information about the crystallographic structure, chemical composition, and physical properties of materials and thin films. These techniques are based on observing the scattered intensity of an x-ray beam hitting a sample as a function of incident and scattered angle, polarization, and wavelength or energy.

X-ray diffraction finds the geometry or shape of a molecule using x-rays. X-ray diffraction techniques are based on the elastic scattering of x-rays from structures that have long range order. The most comprehensive description of scattering from crystals is given by the dynamical theory of diffraction. Powder diffraction (XRD) is a technique used to characterize the crystallographic structure, crystallite size (grain size), and preferred orientation in polycrystalline or powdered solid samples. Wide angle X-ray scattering (WAXS) is a technique concentrating on scattering angles ( $2\theta$ ) larger than  $5^\circ$ .

The Bragg formulation of X-ray diffraction (also referred to as Bragg diffraction) was first proposed by William Lawrence Bragg and William Henry Bragg in 1913 in response to their discovery that crystalline solids produced surprising patterns of reflected X-rays (in contrast to that of, say, a liquid). They found that in these crystals, for certain specific wavelengths and incident angles, intense peaks of reflected radiation (known as Bragg peaks) were produced. The concept of Bragg diffraction applies equally to neutron diffraction and electron diffraction processes.

W. L. Bragg explained this result by modeling the crystal as a set of discrete parallel planes separated by a constant parameter known as the interplanar distance ( $d$ ). It was proposed that the incident X-ray radiation would produce a Bragg peak if their reflections off the various planes interfered constructively.



**Figure 4:** Bragg Diffraction Theory

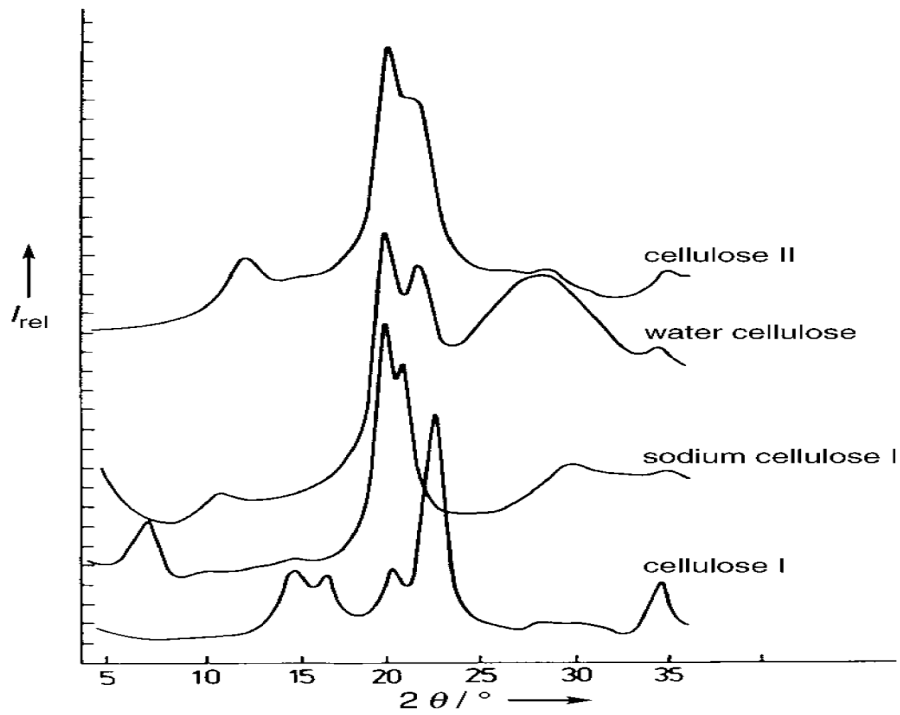
The mechanics is shown in Figure 4. As the wave enters the crystal, some portion of it will be reflected by the first layer, while the rest will continue through to the second layer, where the process continues. By the definition of constructive interference, the separately reflected waves will remain in phase if the difference in the path length of each wave is equal to an integer multiple of the wavelength. the path difference is given by  $2d \sin \theta$ , where  $d$  denotes the interplanar distance. This gives the formula for what is known as the Bragg condition or Bragg's law:

$$2d \sin \theta = n\lambda \quad (1)$$

Waves that satisfy this condition interfere constructively and result in a reflected wave of significant intensity.

The typical patterns of cellulose treated with alkali is shown in Figure 5[31], where native cellulose has the pattern of cellulose I, during alkali treatment, an intermediate product of Na-cellulose I is formed, after the removal of alkali by washing with water, water-cellulose pattern is obtained which turns to cellulose II pattern after drying. It is remarkable that Na-cellulose I has the similar crystal pattern as cellulose II.

Native cellulose form crystalline microfibrils or bundles of cellulose chains of  $2 \sim 5$  nm in diameter for higher plant celluloses and  $15 \sim 30$  nm for algal celluloses[38]. The inside of crystalline domains cannot be accessed by water at room temperature.



**Figure 5:** XRD of Cellulose During Alkalization and Regeneration[31]

Atalla and VanderHard proposed in 1984 that the native cellulose is a composite of two distinct crystalline allomorph,  $I_\alpha$  and  $I_\beta$  [6]. The composition of  $I_\alpha$  and  $I_\beta$  varies with cellulose source. It is well known now that the  $I_\alpha$  is dominant in bacterial and algal celluloses, while  $I_\beta$  is the main crystalline allomorph in higher plants. Figure 6 shows the crystal structure of cellulose  $I_\alpha$  and cellulose  $I_\beta$ .  $I_\alpha$  has a triclinic cell with three dimensions of  $a$ ,  $b$ , and  $c$  of 6.717 Å, 5.962 Å, and 10.400 Å respectively and the  $\alpha$ ,  $\beta$ , and  $\gamma$  angles of 118.08°, 114.80° and 80.37° respectively.  $I_\beta$  has the  $a$ ,  $b$ ,  $c$  dimensions of 7.784 Å, 8.201 Å, and 10.380 Å respectively. Its  $\gamma$  angle is 96.5° [92, 93].  $I_\beta$  is more stable than  $I_\alpha$ .  $I_\alpha$  can be turned into  $I_\beta$  through annealing.

Besides native cellulose I, there are other cellulose polymorph: cellulose II, III and IV. Among these celluloses, cellulose II has the lowest lattice energy and is the most stable form. Cellulose I, III and IV all can be transformed to cellulose II under certain conditions. Cellulose II has a space group of  $P2_1$  and its crystal structure(see Figure 7)

is based on a two-chain monoclinic unit cell with antiparallel packing[63, 64, 86]. The unit cell dimension of cellulose II is  $a = 8.10(3) \text{ \AA}$ ,  $b = 9.03(3) \text{ \AA}$ ,  $c = 10.31(5) \text{ \AA}$ ,  $\gamma = 117.10(5)^\circ$ . Researchers proposed an additional  $O2 - H \dots O2'$  intermolecular bond along the ab diagonal and suggested it contributing to the high stability of the cellulose II structure[61].

Among all the cellulose polymorph, the cellulose I and cellulose II are most important types and are relevant in the alkali treatment. Cellulose III is produced with liquid ammonia treatment, and the cellulose IV is obtained through annealing cellulose III. Their unit cell parameters are listed in Table 2.

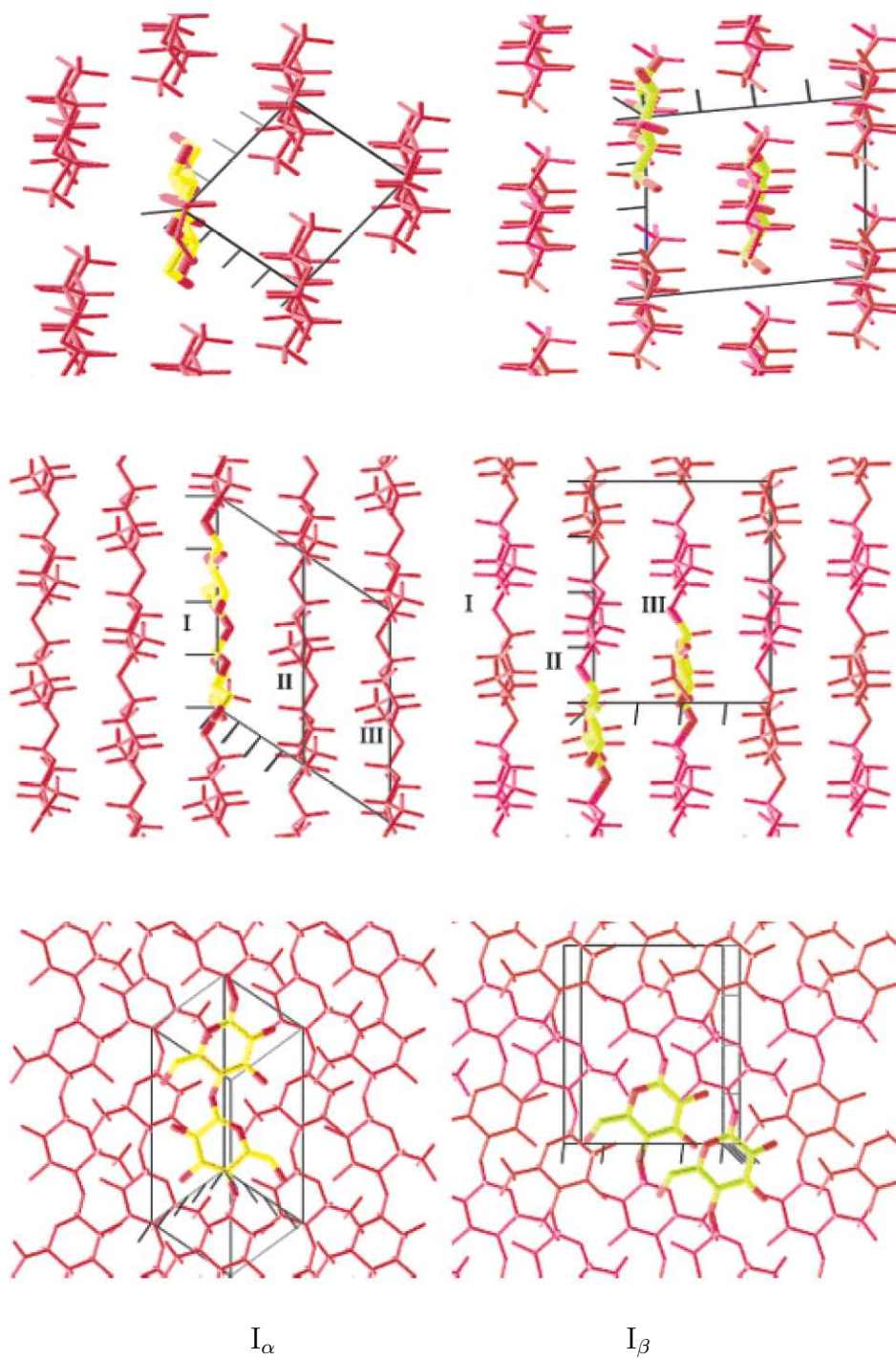
**Table 2:** Unit Cell Parameters of Cellulose Polymorph[26, 140]

Polymorph type	Number of chains	Axes( $\text{\AA}$ ) and angles( $^\circ$ )					
		a	b	c	$\alpha$	$\beta$	$\gamma$
$I_\alpha$	1	6.74	5.93	10.36	117	113	81
$I_\beta$	2	7.85	8.27	10.38	90	90	96.3
II	2	8.10	9.05	10.31	90	90	117.1
$III_1$	2	10.25	7.78	10.34	90	90	122.4
$IV_1$	2	8.03	8.13	10.34	90	90	90
$IV_2$	2	7.99	8.10	10.34	90	90	90

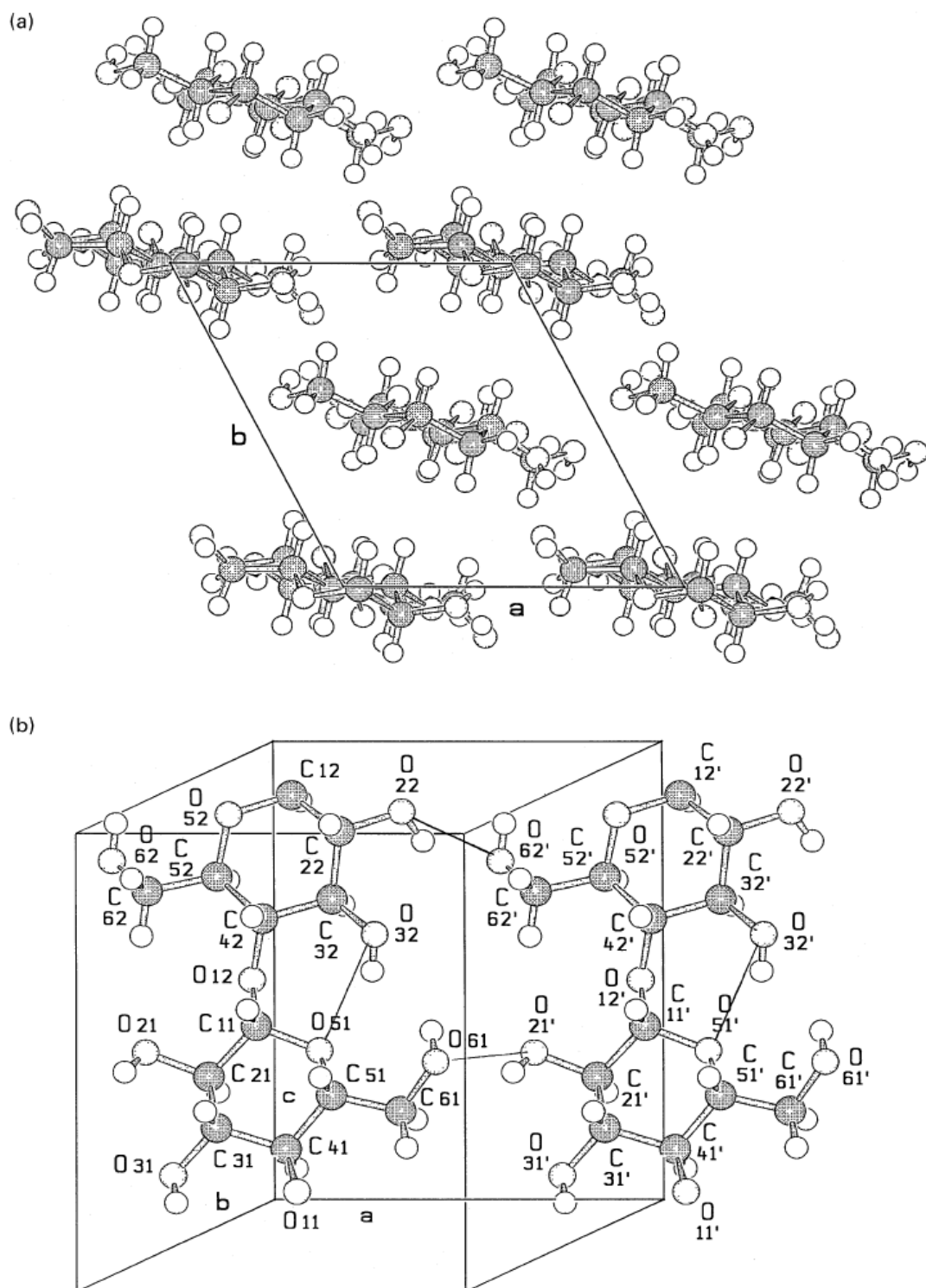
a, b and c correspond to the axes of unit cell;  $\alpha, \beta$  and  $\gamma$  are the angles between b and c, a and c, and a and b, respectively

Based on the lateral dimensions of cellulose fiber, its morphology can be defined by elementary fibrils(1.5~3.5 nm), microfibrils(10 ~ 30 nm), and microfibrillar bands(the order of 100 nm)[53]. The length of microfibrils is several hundreds of nanometers.

A well accepted cellulose supermolecular structure is that the crystalline parts and the amorphous parts are alternating in the longitudinal direction of fiber. The ratio of crystalline part to amorphous part is 300 AGUs to 4 ~ 5 AGUs[91], which is 150 nm to 2 nm by length if considering that each AGU is about the size of 5  $\text{\AA}$  and the alignment of all the AGUs is at the longitudinal direction. As a comparison, the crystalline nano-whiskers obtained by acid hydrolysis method, has a dimension of



**Figure 6:** Crystal structures of cellulose  $I_{\alpha}$  and  $I_{\beta}$ [92, 93].

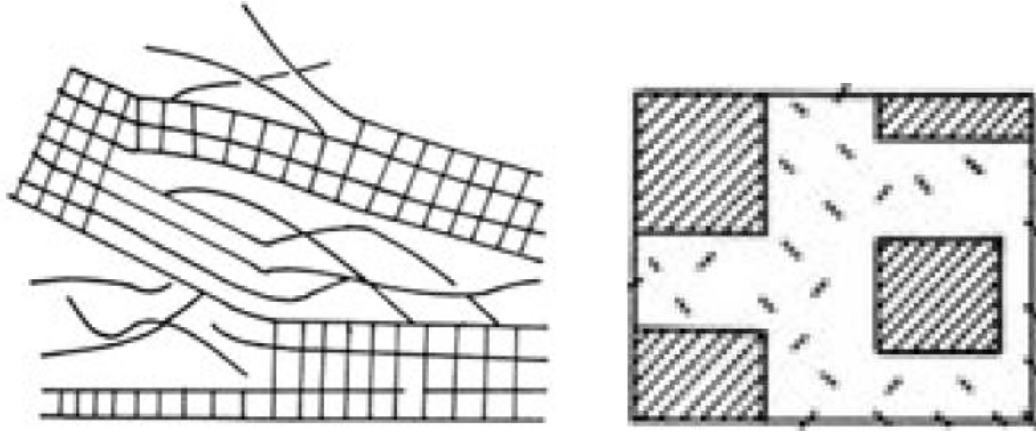


**Figure 7:** Crystal Structure of Cellulose II[86]

100 ~ 300nm length and 10 ~ 20nm in diameter[23, 24, 29].

#### 1.2.3.3 Cellulose Microfibrillar Model

Many models have been proposed to represent the supramolecular structure of cellulose microfibrils. Among them, the fringed fibrillar model(Figure 8) is well accepted to describe the structure of microfibrils and the partial crystalline structure of cellulose[31].



**Figure 8:** Fringed Fibril Model[31]

#### 1.2.3.4 Pore Structure and Pore Sizes

Native cellulose fiber possesses pores or void spaces of different ranges of dimensions, for examples, intra-fibrillar pores of range from 2 ~ 7 nm cellulose induced by microfibril inefficient packing, and large pores of up to 150 nm that may present between hydrogen bonding fibrillar network[102]. The pore sizes and void ratios vary from different cellulose origin and as well as from different treat history. These pores are important to dissolution because it provides spaces for the diffusion of solvent chemicals into fibers. Approximately 3 ~ 4% of void volume were estimated in native cellulose based on density measurements and 0.7 ~ 3.4% were reported for cotton through small angle XRD[41]. Both alkali mercerization and amonia treatments reduced the void fraction. Drying process reduces the internal active surface areas and

probably lead to the formation of new hydrogen bonds on the amorphous and highly swollen surface area of the fibrils[62, 58]. Generally, native cellulose has more of the larger pores while regenerated cellulose has most of colloidal size pores[58].

### ***1.3 Review of Cellulose Dissolution***

Now we have the basic knowledge about cellulose structure, let's take a brief review of cellulose dissolution. This part includes the introduction of some conventional cellulose solvents, a comprehensive review of the alkali solution with or without urea, and the solution structure of cellulose.

#### **1.3.1 Cellulose Solvents**

Table 3 lists some conventional and new cellulose solvents, including organic, and inorganic ones. Sulfuric acid hydrolysis is now widely used to yield cellulose whisker microcrystals. It is believed that sulfuric acid destroys the amorphous regions of the cellulose fibers and allows the grafting of sulfate groups on the surface that stabilize the aqueous whisker suspensions by electrostatic repulsion[21]. Alkali, including LiOH and NaOH, can be used to swell and activate cellulose. Alkaline metal complex are mostly used for  $DP_v$  analysis. Alkaline Xanthogenation is also well known as traditional viscose rayon process, but because the high toxicity, it is gradually replaced by other process, for an example, the lyocell production, which uses organic solvent N-methylmorpholine-N-oxide(NMMO), is a partially industrialized process. Because Rayon viscose method and NMMO lyocell method are two mostly applied dissolution method for cellulose dissolution, more reviews will be given in the following paragraphs.

##### *1.3.1.1 Viscose Method*

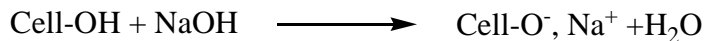
In 1894, Charles Frederick Cross, Edward John Bevan, and Clayton Beadle patented their artificial silk, which they named *viscose*, because the reaction product of carbon

**Table 3:** Conventional and New Cellulose Solvents[5]

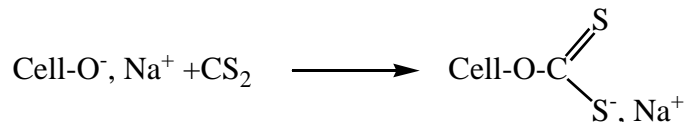
Category	Solvent	Remarks
Acid	$> 52\% \text{H}_2\text{SO}_4$ $> 85\% \text{H}_2\text{SO}_4$	Partial hydrolysis Partial hydrolysis
Alkali	6%LiOH 6% – 9%NaOH	Needs pretreatment of cellulose Needs pretreatment of cellulose
Alkaline metal system complex	$\text{Cu}(\text{NH}_3)_4(\text{OH})_2$ [cuoxam] $\text{Cu}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_2(\text{OH})_2$ [cuen] $\text{CO}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_2(\text{OH})_2$ $\text{Ni}(\text{NH}_3)_6(\text{OH})_2$ $\text{Cd}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_3(\text{OH})_2$ [cadoxen] $\text{Zn}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_3(\text{OH})_2$ Fe/3(tartaric acid)/3NaOH [EWNN]	Cuprammonium rayon production Standard solvent for $DP_v$ measurement  Transparent solution  Relatively stable
Alkaline Xanthogenation	$\text{CS}_2/\text{NaOH}$	Dissolves cellulose, forming Viscose rayon production system
Inorganic salt	$> 64\% \text{ZnCl}_2$ $> 50\% \text{Ca}(\text{SCN})_2$	Dissolves cellulose by heating at $100^\circ\text{C}$ Dissolves cellulose by heating at $100^\circ\text{C}$
Organic solvents	$\text{Cl}_3\text{CHO}/\text{DMF}$ $(\text{CH}_2\text{O})_x/\text{DMSO}$ $\text{N}_2\text{O}_4/\text{DMF}$ , $\text{N}_2\text{O}_4/\text{DMSO}$ LiCl/DMAc, LiCl/DMI $\text{SO}_2/\text{amine}/\text{DMSO}$ $\text{CH}_3\text{NH}_2/\text{DMSO}$ $\text{CF}_3\text{COOH}$ (trifluoroacetic acid: TFA) $(\text{Bu})_4\text{N}^+ \text{F}^- 3\text{H}_2\text{O}/\text{DMSO}$ ca. 80% N-methylmorpholine-N-oxide/ $\text{H}_2\text{O}$	Dissolves cellulose, forming chloral hemiacetals at all cellulose-OH Dissolves cellulose, forming (poly)-methylol hemiacetals at cellulose-OH Dissolves cellulose, forming nitrite ester work on all hydroxyl groups Stable; needs pretreatments of cellulose Unstable; gives stable amorphous regenerated cellulose Dissolves cellulose, forming complex Dissolves cellulose, forming TFA ester work on C6-OH group: volatile solvent Dissolves cellulose with $DP < 650$ Uses for lyocell production
Others	$\text{NH}_4\text{SCN}/\text{NH}_3/\text{water}$ $\text{N}_2\text{H}_4$	Forms mesophase states Explosive

DMF: N,N -dimethylformamide, DMAc: N,N -dimethylacetamide, DMI: N,N -dimethylimidazolidinone, DMSO: dimethylsulfoxide, Bu: butyl-.

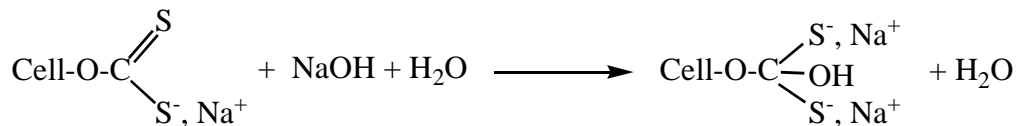
disulfide and cellulose in basic conditions gave a highly viscous solution of xanthate. This method was adopted to produce Rayon fiber, a regenerated fiber used in textile industry. The method is able to use wood (cellulose and lignin) as a source of cellulose while the other methods need lignin-free cellulose as starting material. This makes it cheaper and therefore it was used on a larger scale than the other methods. This process can be divided into three major steps: alkali activation, xanthation and dissolution. First, cellulose fibers from spruce, pine, or other cellulosic materials need to be soaked in 17 ~ 20% NaOH solution at room temperature for a few hours to convert native cellulose into alkali-cellulose.



Cellulose fibers are swollen in this process and activated for other chemical treatment. After removing extra NaOH solvents by pressing, the alkali cellulose further need to be shredded into small pieces and aged under controlled temperature for almost two days. During aging, cellulose will be degraded into desired degree of polymerization which determines the viscosity of viscose solution. Then the alkali cellulose will be mixed with carbon disulfide ( $\text{CS}_2$ ) for xanthation for hours.



The resulting cellulose derivative is cellulose xanthate, which is dissolvable in dilute NaOH solution (under 6%).



The solution formed is very viscous. The dissolution is due to the large xanthate substitution on cellulose which can break the hydrogen bonds between cellulose molecules. The viscose can be used to form fibers, thin films, or sponges, etc., depending on the formation process. After formation, the regeneration process will be applied by precipitating cellulose xanthate into coagulation bath. In this process, cellulose will be regenerated into cellulose II, and carbon disulfide and  $\text{H}_2\text{S}$  will be released.

Although this viscose process has been industrialized for a long time, the hazardous chemicals (mainly  $\text{CS}_2$  and  $\text{H}_2\text{S}$ ) released from this process is a huge environmental problem that cannot be solved simply by optimization and improvement of the viscose process. Alternative method is needed to replace this process.

#### *1.3.1.2 Lyocell Process*

Beginning in 1980s, a new solvent system based on NMMO monohydrate was industrialized which is known as Lyocell process. NMMO is believed to be a 'direct' solvent of cellulose which means it can dissolve cellulose without prior activation or derivatization. NMMO is well known as an oxidizing agent in organic chemistry, it can dissolve cellulose due to its strong N-O dipole. The dissolution procedure is much simpler comparing with viscose process. First, about 8~23% of conventional cellulose fibers are dispersed into 50% of NMMO in water to make a slurry, then this suspension is concentrated at higher temperature (60°C) at reduced air pressure until NMMO monohydrate is made (with water content of 13.3% and melting point of 74°C). This monohydrate is proved to be a better solvent for cellulose than pure NMMO. After dissolution, the cellulose/NMMO/water solution can be used for fibre and film formation through different processes. The structure formation of regenerated cellulose materials from NMMO-solutions was studied by Fink[33].

Lyocell fibers have some improvements over the conventional viscose fibers in the

properties such as wet and dry strength, modulus of elasticity, wearing properties, gloss and touch, but inferior in wet fibrillation.

The chemistry of the NMMO/cellulose system is complex, there are multiple reaction pathways in the system, thus the process is hard to control. The good knowledge of the potential reactions in the cellulose/NMMO/water system is important for its safe and effective application.

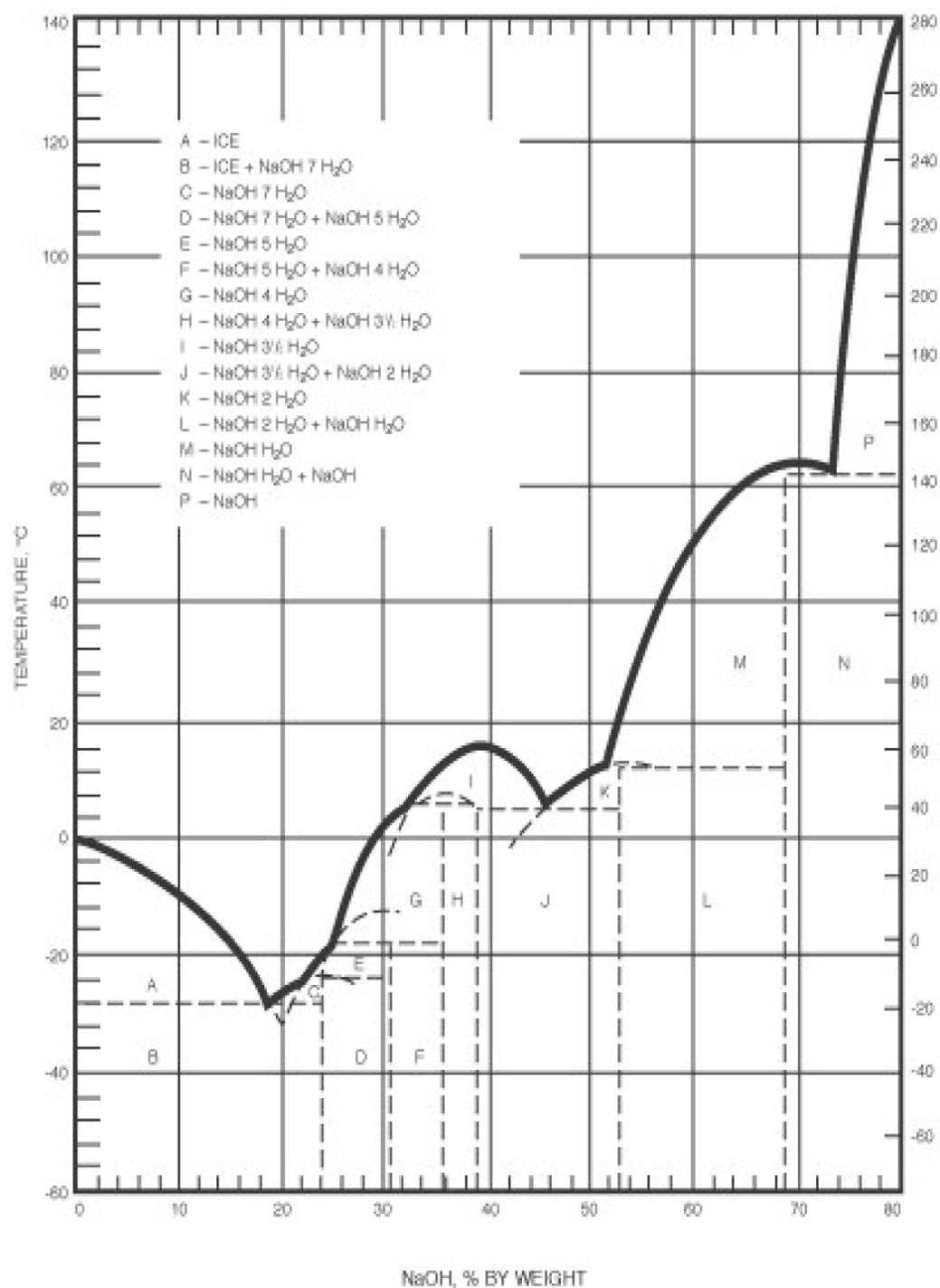
Due to the inherent problems of the above viscose process and lyocell process, researchers are still working to find and optimize environmentally friendly and low cost cellulose solvents. In recent years, alkali solution becomes a good option.

### 1.3.2 Cellulose Dissolution in Alkali Solution

To understand cellulose dissolution in alkali solution, we need to know, firstly the alkali solution itself without cellulose, especially at low temperature; secondly, NaOH interaction with cellulose; thirdly, the effect of additive, such as urea. In the following paragraphs, relevant literatures are reviewed.

#### 1.3.2.1 *NaOH-Water Solution*

Sodium hydroxide, or caustic soda, is a common strong base. It is largely used in the pulp and paper industry. The characteristics of NaOH aqueous solution has been widely studied. NaOH-water can form various hydrates (Figure 9) depending on the NaOH concentration and temperature. The range of NaOH weight percentage in water is from 0 to 80% in this diagram, while the weight concentration used most in the alkali treatment of cellulose is from 0 to 30%. The concentration we interested in this study is in the range of 0 ~ 20%. According to the diagram, in this range, the temperature of phase change will decrease with the increase of NaOH concentration. In a slow cooling process, ice will occur at phase change without any sodium hydroxide hydrate formed. If the temperature is dropped rapidly enough to under  $-34^{\circ}\text{C}$ , then an eutectic mixture of ice and  $\text{NaOH}\cdot 7\text{H}_2\text{O}$  will be formed.



**Figure 9:** Phase diagram of NaOH in water[44]

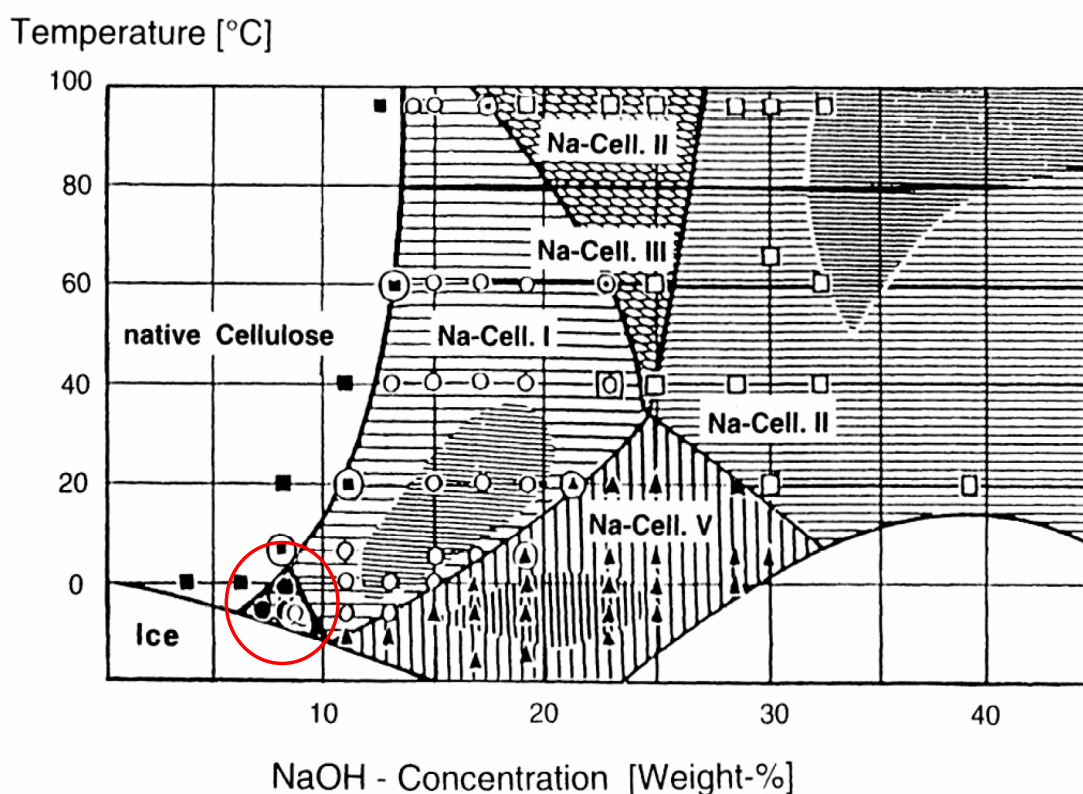
### 1.3.2.2 Mercerization and Na-Cellulose

In 1850, Mercer filed a patent to treat cotton fabric with concentrated sodium hydroxide and obtain luster surface[79]. Decades later, Lowe found that cotton fabric can be more lustrous if applying tension while treating with alkali[71]. This method is called tension mercerization while the Mercer method is called slack mercerization. Most changes in the fine structure caused by mercerization decrease with the application of increasing tension, and decreased swelling of fibers under tension hinders the change to cellulose II[35].

Sarko's group did a series of investigations on the mercerization of cellulose[88, 43, 87, 90, 95, 96]. They monitored the ramie cellulose mercerization process under X-ray diffraction and presented the evidence of intermediate structures: Na-cellulose I, IIA, IIB, III, and IV. They showed that for the crystal transformation to occur, there has to have some amorphous region in the starting cellulose, and the transformation is divided into several steps: in the first step, the alkali solution readily enters the amorphous regions that exist as the interface regions between the crystallites. They defined a vector from cellulose reducing end to the non-reducing end to express the direction of cellulose chain. If one direction is called 'up', the opposite direction is called 'down', as shown in Figure 12. With equal probability, the crystallite chains are arranged in 'up' and 'down' direction, with amorphous regions containing chains with both directions. Due to the interaction with NaOH solution, the cellulose chains in amorphous region are rearranged into antiparallel Na-cellulose I while crystalline regions are hardly affected at this time. When the swelling of cellulose continues, cellulose chain mobility is enhanced. In cellulose I, as the formation of Na-cellulose I proceeds, the crystalline cellulose gradually diminish in size. Aravindanath published the evidence of the presence of cellulose I and cellulose II in the same cross section of partially mercerized cotton fibers[2].

Figure 10 is the earliest cellulose-NaOH-water phase diagram plotted by Sobue[119].

In different NaOH concentration and temperature combination, cellulose would interact with NaOH in different way to form different complex which are marked as Na-cell I, Na-cell II and Na-cell III. Noticeably, there is a small triangle region marked as cellulose Q. This is for NaOH concentration between 6% to 10% and temperature from  $-10^{\circ}\text{C}$  to  $4^{\circ}\text{C}$ . Cellulose are highly swollen in this region. Later, researchers found that in this region, NaOH can be a direct solvent of cellulose. There are many on-going researches to investigate the dissolution behavior in this region.



**Figure 10:** Phase diagram of cellulose-NaOH-water system  
[119]

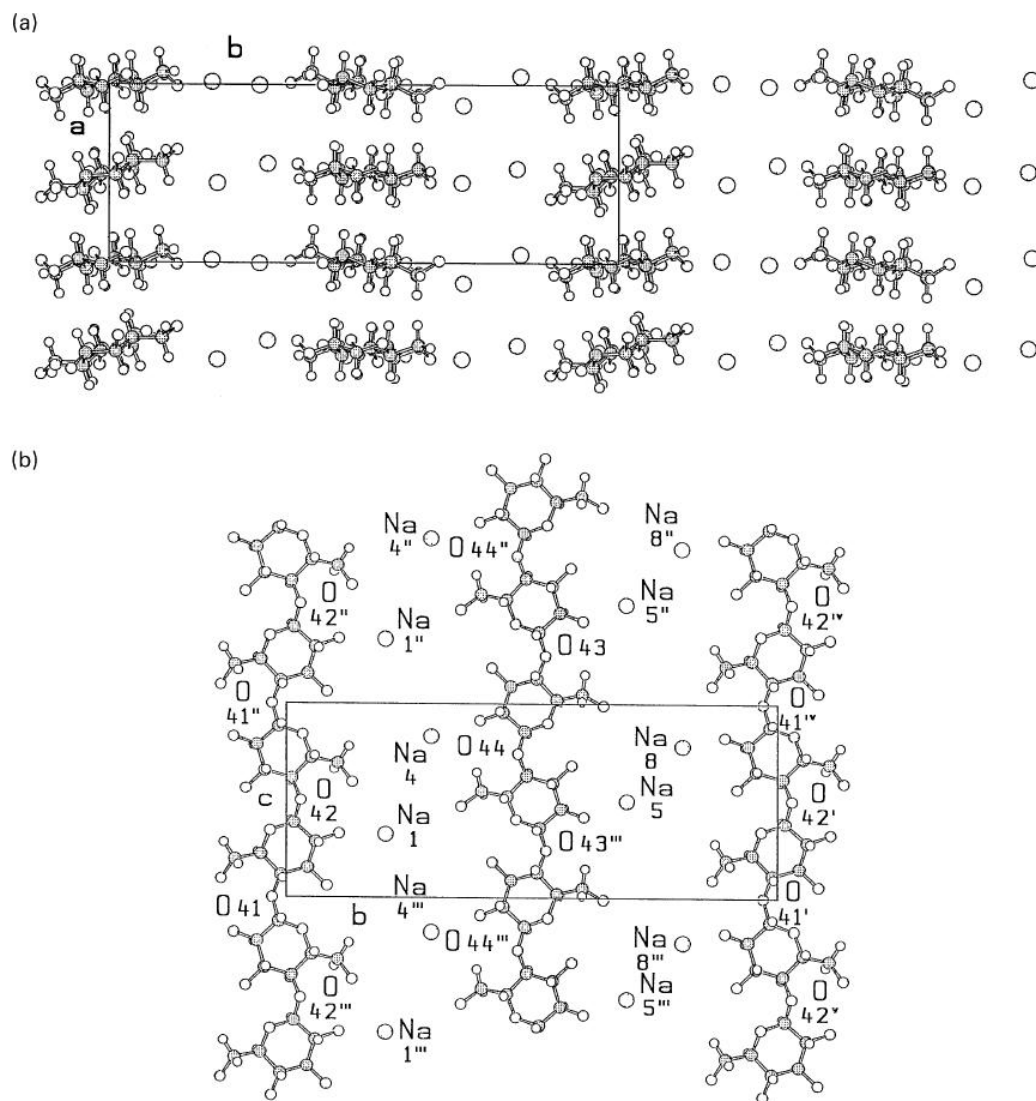
NaOH can cause cellulose to swell and in a narrow range of the phase diagram, even can dissolve cellulose. It was found that for low to moderate DP cellulose, the maximal solubility occurs with 8 ~ 10% soda solution. Soda hydrates can penetrate

the amorphous area of cellulose, then solvate to cellulose and destruct the neighboring crystalline regions. The alkali solubility of cotton cellulose cannot be correlated entirely with its apparent amorphous content[50]. A regenerated sample prepared from a cuprammonium solution having a 94% amorphous content was totally soluble in 10% NaOH while a powdered cellulose obtained by ball milling to a similar amorphous content of 92% had an alkali solubility of only 58%. The alkali solubility of cellulose was shown to have a higher correlation with the reduction in intramolecular hydrogen bonding than with the apparent amorphous content[50, 49, 51].

Literatures suggest that the formation of Na-cellulose complex is an interim reaction step from crystalline cellulose I to cellulose II. Fink treated cotton linters at room temperature and found out that NaOH concentration had to be higher than 10% for the start of lattice transformation from cellulose I to Na-cellulose I. Cellulose I and Na-cellulose I coexisted between NaOH concentration of 10 ~ 14%[30]. Fink proposed a new concept assuming that crystalline part and amorphous part of cellulose had different adsorption capacity for NaOH, with the maximum of 0.5 mol of NaOH adsorption per AGU for crystalline part and a variable adsorption on amorphous part depending on concentration of NaOH, which maximized at 1.9 mol per AGU.

Zugenmaier treated ramie fibers with aqueous NaOH, and obtained crystalline fibers of Na-cellulose I with unit cell dimension of  $a = 8.83 \text{ \AA}$ ,  $b = 25.28 \text{ \AA}$ ,  $c = 10.29 \text{ \AA}$ , all angles  $\alpha = \beta = \gamma = 90^\circ$ [140] (See Figure 11). According to Zugenmaier, Na ions only have strong polar interactions with O2 and O6. There are no intrasheet hydrogen bonds because the large Na ions and probably some water separate the cellulose chains.

Roy[108] proposed a model of the structure of cellulose-soda solution at low temperature. Soda hydrates are composed of a “core” bounded with  $9\text{H}_2\text{O}$  and a “shell” of amorphous water which is depending on the soda concentration. Other water in the solution is free water. Cellulose only exists at the soda hydrate area, not in the



**Figure 11:** Unit Cell Model for Na-Cellulose I[140]

free water part. The preferential interaction sites of NaOH with cellulose are C2 and C3 hydroxyl groups. The morphology of Mercerized cotton cellulose was studied by Kolpak [55].

Isogai investigated the cellulose dissolution in aqueous NaOH with particular attention on the effects of crystalline form and molecular weight [48]. Their procedure requires to freeze cellulose-NaOH mixture at  $-20^{\circ}\text{C}$  to form a tight solid mass. They found: firstly, cellulose with  $\text{DP}_v \leq 200$  could easily be dissolved in the NaOH solution; secondly, most regenerated cellulose can be dissolved in NaOH solution; thirdly, cellulose I with a higher than leveling-off DP can only be partially dissolved; fourthly, the existence of lignin will reduce the solubility, whereas hemicellulose does not affect much. They suggested that the long range order in solid cellulose, that is, a dimension over 100 nm, is the determining factor preventing cellulose dissolution in caustic soda solution.

#### *1.3.2.3 Sodium Hydroxide and Urea solution*

In recent years, Zhang's research group did many investigations on cellulose dissolution in NaOH/urea solution and the potential applications. They found that certain compositions of NaOH/urea and NaOH/thiourea are good solvents for cellulose[12, 131, 134, 137, 136, 139]. These recommended compositions are 6%NaOH/4%Urea, 7%NaOH/12%urea and 6% NaOH/5%thiourea. They also explored novel fibers[13, 111, 132, 130] and new composites from this solution[45, 109, 110, 127, 135]. It was shown that LiOH/urea, NaOH/urea and NaOH/thiourea sorts of aqueous solutions are non-derivatizing solvents for cellulose to form true solution. The mechanism they proposed is that NaOH hydrates - urea hydrates - free water - cellulose form a special complex in the solution. NaOH or LiOH destroys the inter- and intra- hydrogen bonds between cellulose molecules. Urea hydrates function as hydrogen bonds donor and receptor between solvent molecules and prevents the reassociation of cellulose

**Table 4:**  $DP_v$  and possible cellulose I dissolution threshold with various solutions

$DP_v$	$M_w, 10^{-4}$	Remarks
100	1.58	
200	3.16	9%NaOH dissolution
300	4.74	
425	6.7	6%NaOH/4%urea
500	7.9	6%NaOH/5%thiourea
600	9.48	
700	11.06	7%NaOH/12%urea
800	12.64	
900	14.22	
2000	31.6	
2500	39.5	4.2%LiOH/12%urea

molecules, thus leading to molecular dissolution of cellulose. Zhou[139] prepared O-(2-hydroxyethyl)cellulose(HEC) in 6%NaOH/4%urea aqueous solution and examined the characteristic of this solvent system as a new homogeneous reaction medium for the esterification of cellulose. Structural changes and activation of cellulose by caustic soda solution with urea was studied by Kunze [59].

Both of Zhou’s and Isogai’s work suggest that there exists an upper DP limit of cellulose for each solution system, those cellulose with a higher DP value that is beyond the corresponding upper limit can not be dissolved in the solution. Table 4 lists the upper  $DP_v$  threshold of cellulose to be dissolved in selected solvents according to literatures. We can see that the higher the  $DP_v$  of cellulose, the stronger the solvents required to dissolve cellulose. According to their definition, the dissolution state is that a clear solution of cellulose being attained. Most commercial wood pulps and original cotton linters have  $DP_v$  values of about 800, this may partially explain why these cellulose cannot be easily dissolved in 6%NaOH/4%urea or 7%NaOH/12%urea solutions.

#### 1.3.2.4 Dissolution Mechanisms

##### 1. Cellulose Dissolution *vs.* Cellulose Hydrolysis

Cellulose hydrolysis, also called cellulose degradation[99], is to break down molecular weight by cutting in the middle of cellulose molecule or from the two ends of cellulose to obtain low molecular weight cellulose or even cellulobiose or glucose. Many chemical and biochemical methods are used for this purpose, for examples, enzyme hydrolysis and acid hydrolysis[60, 78]. Cellulose dissolution is different from cellulose hydrolysis because dissolution is to break the inter- and intra- molecular hydrogen bonds, destroy the long range order of cellulose crystal to form cellulose molecular solution. Cellulose dissolution is more similar to solid melting. Cellulose dissolution may need certain degree of hydrolysis as pretreatment for high molecular weight cellulose, however this may not be necessary for small to moderate molecular weight cellulose. Over hydrolysis to too small cellulose molecules will induce big mass loss and is not desired for the pretreatment in the case of cellulose dissolution. The ultimate goal of dissolution study would be to dissolve cellulose without using hydrolysis pretreatment.

The second difference between cellulose dissolution and hydrolysis is that the dissolved cellulose, which is in the form of macromolecular solution, can be regenerated and reformed into fiber or other shapes, while complete hydrolyzed cellulose, or more exactly, small sugar molecules, can not be regenerated back into macromolecules of cellulose.

The third difference is that fiber dissolution does not chemically consume solvent, but hydrolysis does. Most of the chemicals added to dissolve cellulose can be recycled.

##### 2. Thermodynamics

Cellulose dissolution in alkali solution, in a sense, is to change cellulose crystal structure from cellulose I to cellulose II. Because cellulose II has lower lattice energy, in another word, it is more stable than cellulose I, which is metastable, the dissolution process is thermodynamically favorable.

The fact that the cellulose dissolution process favors lower temperature states that the overall dissolution process is exothermic. Cellulose dissolution can be considered as a multi-stage process, including, firstly, the melting of cellulose crystalline region and transition of amorphous regions to elastic state; secondly, solvation of cellulose macromolecules; and the last stage is the mixing of the solvated cellulose with solvents. The decrystallization process is entropy-driven[102].

### 3. Folding Model *vs.* Chain Sliding Model

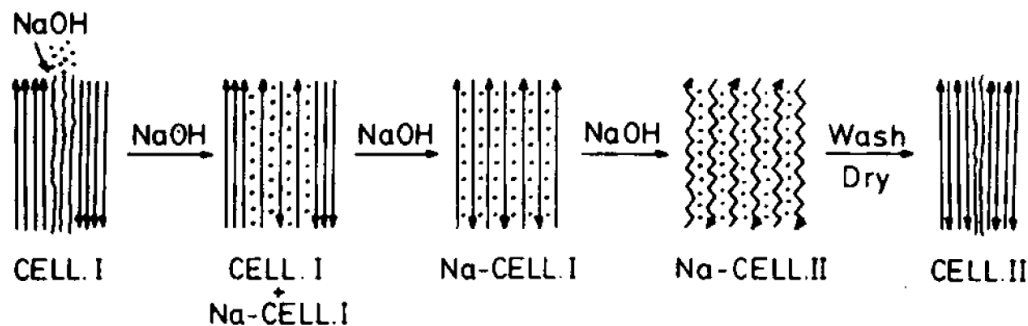
The structural change during cellulose dissolution in alkali solution is of high interest to researchers[19]. The transformation mechanism from parallel cellulose I to antiparallel cellulose II during mercerization is still not well understood. Some researchers proposed one possible explanation that during alkalisation, sodium hydroxide disrupts the hydrogen bonds in cellulose I and swells the fibers, so the cellulose molecules can be mobile enough to fold chains forming the stable antiparallel conformation[117]. Another possible mechanism is due to the sliding of hydrogen-bonded sheets of chains[55, 96].

### 4. Derivative *vs.* Non-derivative

There are two mechanisms to explain the cellulose-NaOH/urea dissolution system, one believes it's non-derivatizing, the other holds that derivative reaction occurred in the system.

#### **Non-cellulose derivative mechanism.**

Zhang's group did investigation on the mentioned dissolution system[12]. Their

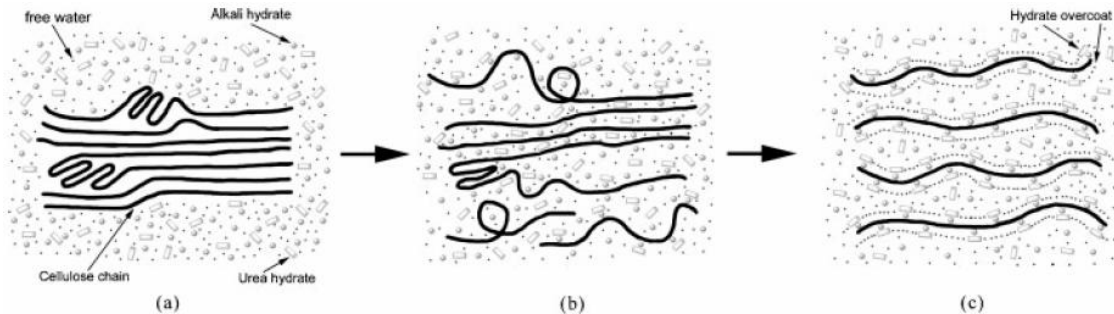


**Figure 12:** Sliding Chain Model of Cellulose Mercerization  
[96]

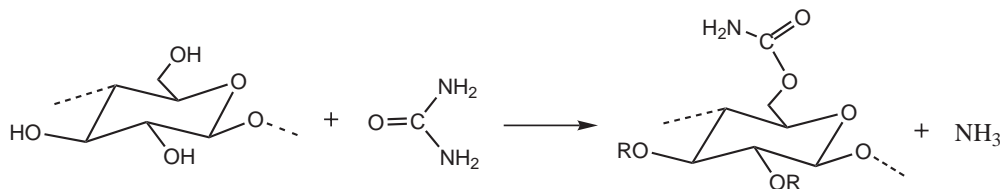
results from DSC suggested that certain amount of NaOH hydrates were bound to cellulose, while the urea hydrates and free water were not.  $^{13}\text{C}$  NMR spectra of the cellulose solutions indicated that an interaction of urea with NaOH existed in the solution. Also, they compared the chemical shifts in NaOH/urea-cellulose system with cellulose in cadoxen, DMAc/LiCl, NMMO/DMSO, and NaSCN/D<sub>2</sub>O, which are known as non-derivatizing solvents and no new peak was found, indicating an absence of derivatization. Figure 13 is a schematic dissolution mechanism they proposed. The mechanism is that NaOH hydrates - urea hydrates - free water - cellulose form a special complex in the solution. NaOH or LiOH destroys inter- and intra- hydrogen bonds between cellulose molecules and urea hydrates function as hydrogen bonding donor and receptor between solvent molecules and prevent the reassociation of cellulose molecules, thus leading to molecular dissolution of cellulose.

**Cellulose derivative mechanism**(See Figure 14).

A patented Carbacell technology used alkalined and pre-ripened cellulose, similar pretreatment as for viscose rayon technology, followed by derivation by concentrated urea and dissolution with sodium hydroxide solution.  $^{13}\text{C}$ -CP/MAS solid state NMR was used to characterize the structural change of cellulose in the CarbaCell process.



**Figure 13:** Schematic Dissolution Process[12]



**Figure 14:** Schematic formation of carbamate[53]

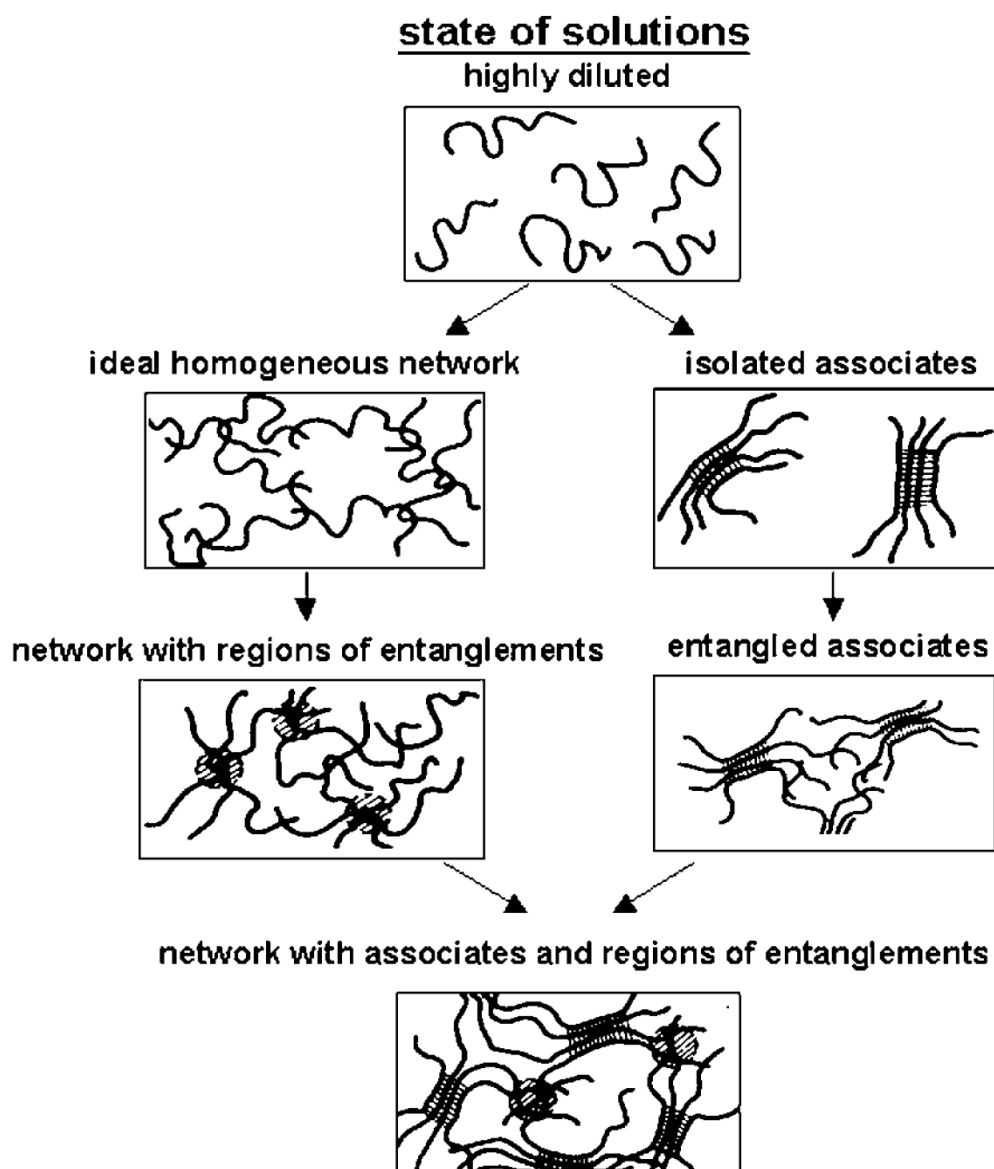
Kunze[59] used caustic soda with urea to activate cellulose. His research showed that single NaOH or urea solution has zero or only minor effects on cellulose structural change. He pointed out that there was a unique urea-NaOH-cellulose complex which was distinct from sodium cellulose I or II. Cellulose II can be partially reconverted to cellulose I by concentrated urea after pretreatment of cellulose with NaOH. Cellulose I and II coexisted in the bi-solvent system of NaOH/urea. At low temperature ( $-25^{\circ}\text{C}$ ), the activation effect is better than that under room temperature.

The difference in the non-derivative and the derivative system is that the urea concentration for non-derivative one is low (lower than 10%) while the derivative system use concentrated urea that can be up to 40%.

### 1.3.3 Solution Structure of Cellulose

To understand the cellulose structure in solution is important to investigate the cellulose dissolution mechanism. It is also related to practical application, such as the shaping of cellulose from spinning solutions. Over the past decades, intense researches

have been done to study the structure of cellulose in solution. Figure 15 is potential solution structure of cellulose[53], which may depend on the type of solvent, polymer concentration, chain length distribution, and the type, pattern, and degree of cellulose substitution.



**Figure 15:** Solution Structure of Cellulose and Cellulose Derivatives[53]

As a rule, cellulose derivatives with free OH groups are not molecularly dispersible[53].

Highly diluted solutions are usually used for fundamental study of solution structure and gel solution is used on the application side. The solution state of cellulose in NMMO was studied, the results substantiated the presence of bimodal molecule aggregations with up to 1000 chains, which can be attributed to incomplete dissolution of crystal structures of the starting cellulose material[105]. The smaller aggregates have the average of 50~100 molecules and the larger ones have 250~1000, the former can be correlated to crystallite while the latter can be correlated to microfibril[33].

### ***1.4 Enzymatic Hydrolysis of Cellulose***

Enzymatic hydrolysis was utilized in this study as a pretreatment to improve the cellulose solubility in alkali solution. Here a brief review was given on the basics of cellulases(the enzymes that can degrade cellulose), and the practices of combining enzymatic hydrolysis with alkali treatments.

- **Cellulase and Enzymatic Hydrolysis of Cellulose**

The enzyme we used in the study is Celluclast. Celluclast is a commercial cellulase complex. It is obtained from *Trichoderma reesei* strains. Celluclast contains endoglucanases (EG) I and II), and cellobiohydrolases (CBH) I and II. This Celluclast is used in paper industry mainly for the improvement of drainage. Bhat did a comprehensive review on cellulose degrading enzymes and their potential industrial application in 1997 [9]. Ramos [103, 104] did research on the effect of *Trichoderma* cellulases on the fine structure of a bleached softwood kraft pulp. All the enzymes he used, including Celluclast, have high hemicellulose activity and can remove most of the residual hemicellulose from softwood kraft pulp. In the process of enzyme hydrolysis, the reduction of DP can only be observed at the early stages induced by a random attack of endoglucanases at amorphous regions. CBHs can remove cellubiose residues from the end of cellulose chains, therefore, can gradually, but only slightly, decrease

the degree of polymerization. The enzyme prefers to attack the crystalline at (002) diffraction plane [15], so it can be applied to cut the length of crystallite. Ramos and his coworkers didn't find much effect of enzyme treatment on cellulose crystallinity, but they suggest that the crystallinity change can be observed after longer incubation time.

The effect of enzyme concentration on the rate of the cellulose hydrolysis was studied by Sattler[115]. An enzyme loading of 10 FPU/g substrate is the most commonly used dosage for saccharification process.

Hayashi[40] produced nano-ordered short elements of cellulose  $I_\beta$  after enzymatic hydrolysis of *Cladophora* microcrystalline cellulose. Cellulose  $I_\alpha$  was easily removed by enzyme hydrolysis, leave  $I_\beta$  crystalline elements of 350 nm long. The enzymatically produced short elements have a narrower DP distribution of around 690. Their research indicated that the supermolecular structure of cellulose microcrystalline fiber, such as the distribution of  $I_\alpha/I_\beta$ , may have influence on the production of  $I_\beta$  short elements during the enzyme hydrolysis.

- Synergistic Effect of Cellulase and alkali solution.

Eremeeva etc.[27] combined enzyme treatment with a two-stage sodium hydroxide treatments to fractionate cellulose as well as investigating the molecular characteristics of cellulose in enzyme hydrolysis. They reported that the DP reduction of the alkali-insoluble fraction was small, whereas the changes for the soluble fraction were large. They also found that the alkali soluble part and insoluble part kept the same ratio during the enzyme hydrolysis. Rahkamo[101] tried the similar method but to improve the solubility of sodium hydroxide.

### ***1.5 Novelty and Significance***

The alkali solution with urea is a promising new solvent for direct cellulose dissolution. The understanding of this system is of on-going interest to researchers. This project mainly focus on the fundamental research on the cellulose dissolution kinetics. The results provided new insight into the dissolution mechanism. Also, for practical use, enzymatic hydrolysis was introduced in as a pretreatment to alkali dissolution. Through this method, the cellulose solubility in sodium hydroxide/urea solution was greatly increased.

This project provided not only a low cost and environmental benign means to overcome the recalcitrance of cellulose, but also presented a scenario for new materials engineering, i.e., solution spinning and molding technology to using cellulose based nanocomposite, a new approach never reported to date. Currently, the cellulose based composites were basically from cellulose derivatives such methyl, hydropropyl, or hydropropyl methyl cellulose, or cellulose acetate, but not original cellulosic materials. Although methyl, hydropropyl, or hydropropyl methyl cellulose and cellulose acetate are water- or solvent-soluble, these derivatives have low mechanical strength with low crystallinity. They do not form strong hydrogen bonding as unmodified cellulose. Furthermore, cellulose modification will undoubtedly increase the cost and technique difficulties. The cellulose dispersed at molecular level will exhibit intrinsically different characteristics in comparison with those in bulk or associates form. For example, the cellulose dissolved in molecular state will more easily enter the intergallery space of the platelet nanoclay, allowing cellulose/nanoclay nanocomposite construction. The new material may possess unexpected properties by introducing nanoclay in cellulose matrix. Thus, the project possesses the following unique characters:

1. Novel dissolving technique and medium for cellulose dissolution with cost effectiveness and environmentally friendliness;

2. Wide availability for a range of cellulose sources;
3. Dispersion at molecular level;
4. Possibility to regenerate into novel cellulose based engineering material with facile technique approaches.

## **1.6 Main Foci**

As shown in the project plan(Figure 16), the whole research work was divided into three parts:

- Cellulose dissolution kinetics

Cellulose dissolution in NaOH and NaOH/urea was compared; dissolution kinetics in dependence of temperature was discussed in thorough in Chapter II.

- Enzyme pretreatment aided cellulose dissolution

From literature and our preliminary work, only low molecular weight cellulose, that is, with DP under 200, can be fully dissolved in NaOH solution. While for regular wood fiber, the cellulose molecular weight is usually higher than 800. The solubility of this wood fiber in NaOH solution is around 20%, which is not acceptable for application. So one focus for this study is to use enzyme pretreatment to enhance the cellulose dissolution. The detailed description was in Chapter III.

- Hemicellulose and lignin effect on dissolution degree

Hemicellulose and lignin, other than cellulose are two major components of wood fibers. Their influence on cellulose dissolution in NaOH/urea solution was discussed in Chapter IV.

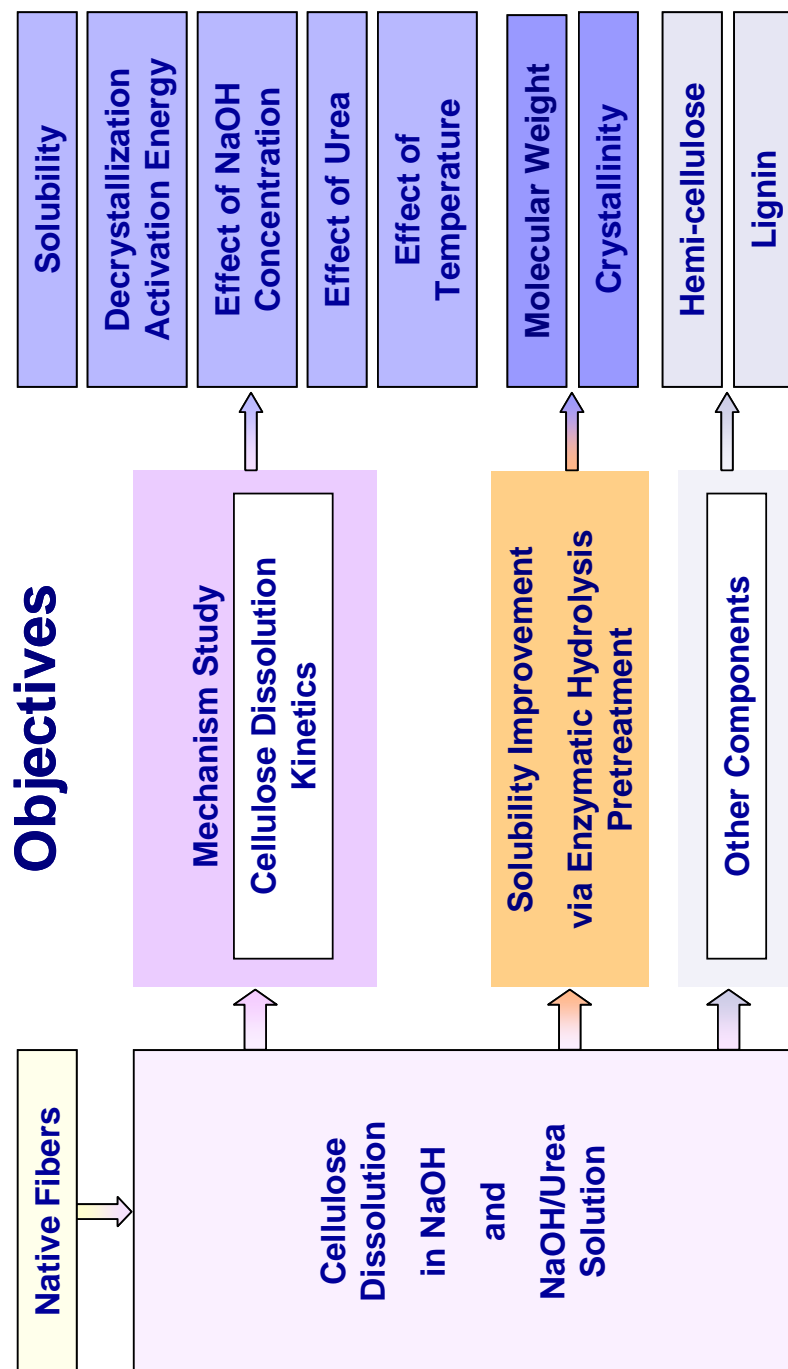


Figure 16: Project Plan

## CHAPTER II

### DISSOLUTION KINETICS

The study of dissolution kinetics is important in order to understand cellulose dissolution mechanism in NaOH with or without urea at low temperature. In this part, a customized low temperature reactor was designed to perform reaction with high efficiency and repeatability. High molecular weight cotton linter was used and dissolution residue was characterized by Powder X-Ray Diffraction to study the crystal change during dissolution. The observed reaction rate of decrystallization of cellulose was obtained and the activation energy for cellulose decrystallization in NaOH solution was derived using Eyring equation. The effect of urea additive was discussed. New insight of the dissolution process in low temperature was proposed.

#### ***2.1 Introductions***

The key to cellulose dissolution is to break the hydrogen bonds between cellulose molecules. This can be done with chemical methods and mechanical methods or both. The chemicals used for cellulose dissolution include CS<sub>2</sub>, NMMO, LiCl, sodium hydroxide and so on. Sodium hydroxide is the most commonly used chemical for this purpose. The use of high concentration of sodium hydroxide to treat cotton fabric has a long history since its inventor Mercer filed the patent in 1850. And the mechanism of NaOH interact with cellulose has been of interest to researchers since then. In 1939, Sobue published cellulose-NaOH-water phase diagram based on ramie cellulose and found that only at a narrow phase window, i.e., NaOH concentration of 6% to 10%, at temperature of  $-5$  to  $-10^{\circ}\text{C}$ , could NaOH dissolve cellulose. Ever since then, researchers have performed extensive study on the dissolution mechanism. However, partly due to the limitation of characterization techniques and analysis method, more

importantly due to the lack of complete knowledge of the supermolecular structure of cellulose and the alkali behavior at low temperature, there is no widely accepted explanation yet. [53], [131]

Phase diagram for sodium hydroxide solution was published in 1942[44], which is important to understand the phase behavior of NaOH at low temperature. In recent years, researchers did some fundamental study on the structure and rheological properties of cellulose(MCC)-sodium hydroxide solution at low temperatures[25, 107, 108].

In the study of cellulose dissolution mechanism, Ramos proposed that, since cellulose dissolution in alkali solution is a heterogeneous reaction involving the disintegration of the crystalline part of cellulose, its decrystallization kinetics can be investigated through monitoring crystallinity change of the undissolved residue[102]. It was shown that the activation energy can be analyzed through Eyring equation[102].

### 2.1.1 Review of Chemical Reaction Kinetics

The reaction of dissolution of crystalline cellulose into molecular dispersion can be divided into several stages: firstly, the transition of the solid polymer to a hypothetical, highly elastic liquid state which corresponds to disintegration of the crystalline regions ( $\Delta H_{fusion}$ ) and transition of the amorphous regions from a vitreous to a highly elastic state ( $\Delta H_{transition}$ ); secondly, solvation of the polymer macromolecules ( $\Delta H_{interaction}$ ) and thirdly, mixing of the solvated polymer molecules with solvent to give an infinitely diluted solution ( $\Delta H_{mixing}$ )[76, 83]. The total enthalpy of cellulose dissolution is given by

$$\Delta H_{Dissolution} = \Delta H_{fusion} + \Delta H_{transition} + \Delta H_{interaction} + \Delta H_{mixing} \quad (2)$$

The only endothermic term of Equation 2 is  $\Delta H_{fusion}$ , which is associated with breaking the hydrogen bonds in crystalline regions. The other terms are exothermic

and related to interactions between cellulose hydroxyl groups and the solvent system. The overall process of cellulose dissolution is exothermic and is favored by lower temperature[76, 83].

To investigate the mechanism of the cellulose dissolution in alkali solution, we try to understand how its elementary step-decyrstallization proceed at whatever rates are observed, and how to control the rate and/or direction of the reaction. Let’s take a brief review of reaction kinetics.

We are interested in kinetics study because there is relationship between kinetics and thermodynamics. Since the earliest rates studies, analogies have been drawn between rates and equilibria. In fact, kinetics and stoichiometry are directly related for elementary steps. This suggests that there is a sort of analogy between the amount of product formed at equilibrium and the rate of formation of products. Two most applied equation for kinetics study are the Arrhenius Equation and the Eyring equation. In this study, the dissolution kinetics is investigated through Eyring equation.

The Eyring equation also known as Eyring-Polanyi equation in chemical kinetics relates the reaction rate to temperature. It was developed almost simultaneously in 1935 by Henry Eyring, M.G. Evans and Michael Polanyi. This equation follows from the transition state theory (aka, activated-complex theory) and contrary to the empirical Arrhenius equation, this model is theoretical and based on statistical thermodynamics[100].

The general form of the Eyring-Polanyi equation somewhat resembles the Arrhenius equation:

$$k = \frac{k_B T}{h} e^{-\frac{\Delta G^\ddagger}{RT}} \quad (3)$$

where  $\Delta G^\ddagger$  is the Gibbs free energy of activation,  $k_B$  is Boltzmann’s constant, and  $h$  is Planck’s constant.

It can be rewritten as:

$$k = \left( \frac{k_B T}{h} \right) \cdot \exp \left( \frac{\Delta S^\ddagger}{R} \right) \cdot \exp \left( -\frac{\Delta H^\ddagger}{RT} \right) \quad (4)$$

To find the linear form of the Eyring-Polanyi equation:

$$\ln \frac{k}{T} = \frac{-\Delta H^\ddagger}{R} \cdot \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R} \quad (5)$$

where

$k$  = reaction rate constant

$T$  = absolute temperature

$\Delta H^\ddagger$  = enthalpy of activation

$R$  = gas constant

$k_B$  = Boltzmann constant

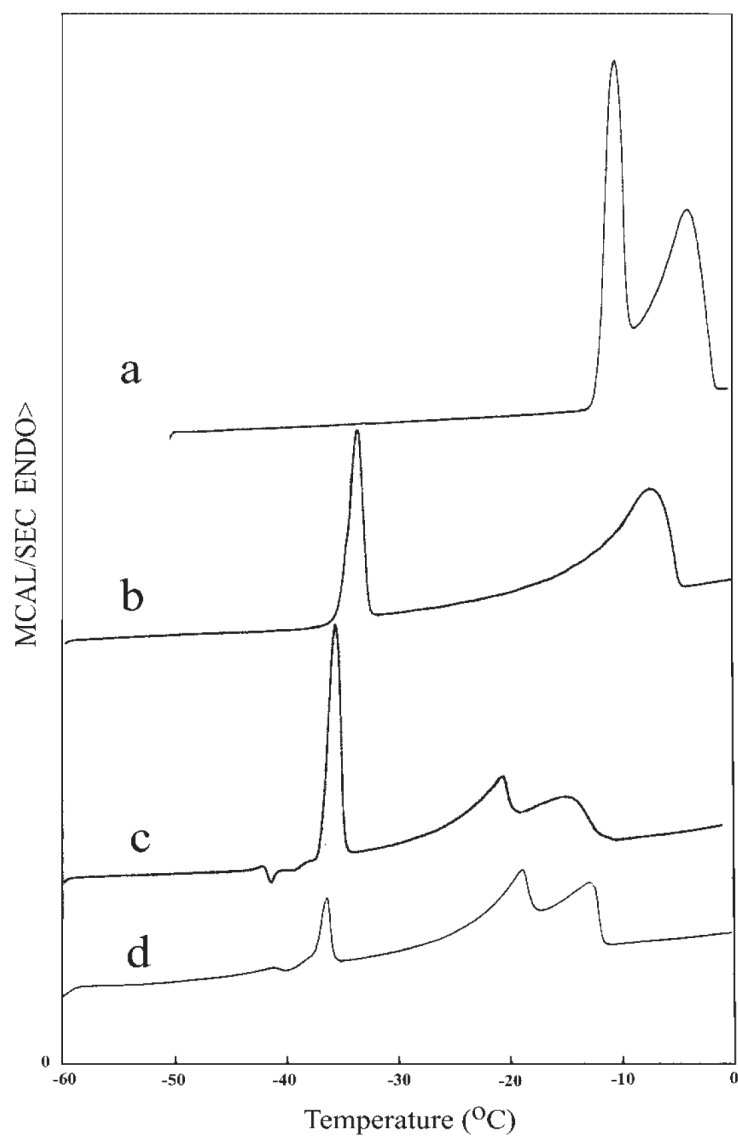
$h$  = Planck's constant

$\Delta S^\ddagger$  = entropy of activation

Reaction rates can be obtained after performing experiments of cellulose dissolution in NaOH at different temperatures. The plot of  $\ln(k/T)$  versus  $1/T$  gives a straight line with slope  $-\Delta H^\ddagger/R$  from which the enthalpy of activation can be derived and with intercept  $\ln(k_B/h) + \Delta S^\ddagger/R$  from which the entropy of activation is derived.

### 2.1.2 Thermal Analysis of Cellulose-NaOH/urea-water System in Literature

Cellulose-NaOH/urea-water system was studied by other researchers using differential scanning calorimetry (DSC)[12]. Figure 17 shows their results. The tests were conducted firstly decreasing the sample temperatures to  $-60^\circ\text{C}$ , then increasing temperature by providing heat flow at a certain rate. The peak locations are the corresponding melting temperatures for specific phases. For urea-water solution(a), the two peaks



**Figure 17:** DSC Spectra of Cellulose in NaOH and NaOH/urea Solution[12] a) urea-water, b) NaOH-water, c) NaOH/urea-water, d) cellulose-NaOH/urea-water

were assigned for free water and urea hydrate at melting temperature of  $-4^{\circ}\text{C}$  and  $-10.5^{\circ}\text{C}$  respectively. The two peaks appeared while melting the 7%NaOH/12%urea solution(b) were of free water( $T_m = -7.5^{\circ}\text{C}$ ) and sodium hydroxide hydrate ( $T_m = -33.8^{\circ}\text{C}$ ) respectively. Curves (c) and (d) are for 7%NaOH/12%urea and 4% cellulose in 7%NaOH/12%urea respectively. Comparing these two thermograms, there are only slight difference for the melting points of free water, urea hydrate and sodium hydroxide hydrate, suggesting that the existing of cellulose did not change much of the melting points of the solution components. There is a large decrease for the peak of sodium hydroxide hydrate after adding cellulose, suggesting bonding between cellulose and sodium hydroxide hydrate. The melting temperature of free water for curve (d) is  $-15.3^{\circ}\text{C}$ .

It is remarkable that these data are obtained through melting method, which is a different process from frozen process (the method used in this study). As will be shown in this study in XRD spectra of cellulose dissolution during cooling process, cellulose structure changes from type I to type II. This may affect the melting points of varies species in the solution mixture. This method, however, still can give important thermal information on the cellulose-NaOH/urea system.

## **2.2 *Experimental***

### **2.2.1 Materials and Chemicals**

The cellulose material used in this study was cotton fibers(Procter & Gamble Company) with measured  $\text{DP}_v$  value of 800. Urea, in crystal form, is from Aldrich and NaOH in 50% solution is from VWR. The chemicals were used without further treatments.

Despite that many researchers have stated that high DP cellulose cannot be dissolved in NaOH solution, the selection of high DP cotton fibers versus low DP cellulose for kinetic study has the following advantages:

1. due to the existence of large percentage of undissolved residue, the degree of dissolution can be easily monitored by weighting method.
2. native cellulose in high plants all have high DP, their dissolution behavior would be quite different from low DP cellulose. Investigating high DP cellulose is more meaningful for dissolution of wood fibers.
3. The undissolved residue can be used to monitor crystallinity decrease during dissolution process.

### **2.2.2 Low Temperature Reactor Design**

The cellulose dissolution kinetics was studied under a series of low temperatures from 0 to  $-20^{\circ}\text{C}$ . In our preliminary work, cellulose samples were put into a freezer directly for cooling. Although this method is very simple, it has many problems, for example, samples were cooled by forced air which has low thermal conductivity comparing with liquidus cooling bath (water has 23 times the thermal conductivity that air has), as a result it usually takes from several hours to days to freeze a sample. Considering the large sample sizes required for kinetic study, cooling in the freezer is very inefficient. Furthermore, the temperature in the freezer cannot be easily adjusted and controlled, and the temperature profile in a freezer is hard to be monitored. Therefore, we designed a customized low temperature reactor for this study.

The design of the low temperature reactor requires low cost, high efficiency and good repeatability. The design included a commercial plastic cooler as a cooling bath container as well as insulator, a customized sample container holder that can place fifteen 15ml plastic centrifuge tubes at a time, a mechanical stirrer set at constant stirring speed to ensure uniform heat convection in the cooling bath, two thermocouples, one of which is to monitor the temperature in the cooling bath( $T_c$ ), the other one is to record the temperature in the sample of cellulose solution( $T_s$ ), and a computer to display and the same temperature recordings. The uniqueness of this reactor

is the real-time temperature measurement and recording in samples throughout the whole cooling process. This is realized by a thermal couple with temperature resolution of  $0.1^{\circ}\text{C}$  and a recording frequency of 3 readings per second. This method has the advantages over the traditional cooling method for cellulose treatment in NaOH at low temperature. In traditional cooling method, cellulose sample was put into a cooling equipment for some time, and then taken out, what happens in between is a black box. This new reactor recorded what a cellulose sample experienced in terms of temperature changes so that its solubility and crystallinity can be directly correlated to its real temperature experience.

### 2.2.3 Sample Preparation

#### 2.2.3.1 NaOH dosage on cellulose

According to Egal[25], the limit of cellulose that can be dissolved in NaOH solution is as the same weight percentage of NaOH solution, that is, if we have 6% NaOH solution in water, the maximum cellulose concentration that can be dissolved solution would be 6%. They stated that since cellulose can only be dissolved in a narrow window of cellulose concentration from 6% to 10%, the maximum cellulose percentage that can be dissolved in various concentration of NaOH would be 10%. Based on this one-to-one NaOH to cellulose weight ratio, since NaOH has the  $M_w$  of 40 g/mol and cellulose AGU has  $M_w$  of 162 g/mol, the NaOH to cellulose molar ratio would be 4 to 1. In another word, the NaOH dosage has to be greater than 4 mol per mol of AGU for cellulose to be dissolved.

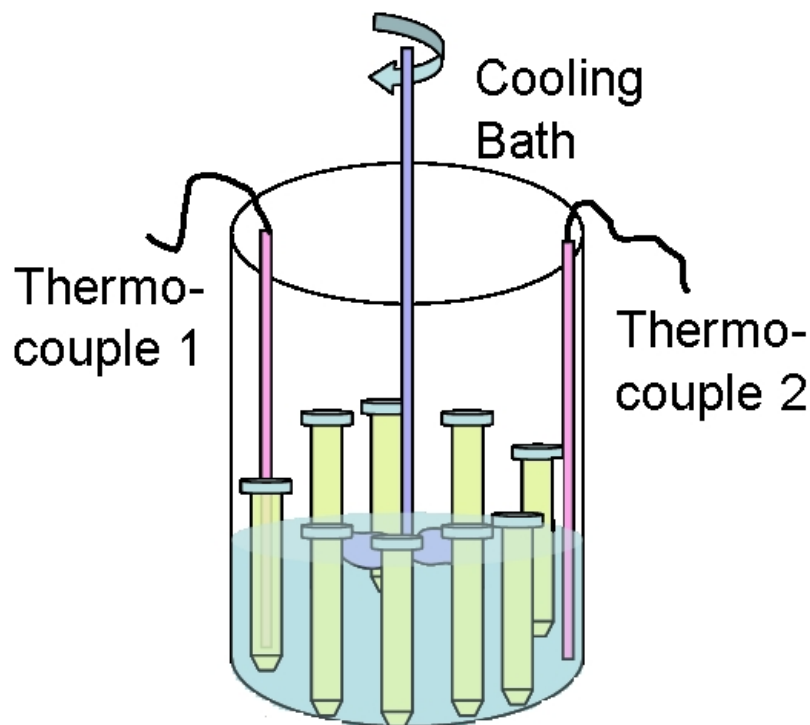
In all of our kinetics experiments, without specification, 0.250 g (0.00158 mol AGU) cotton linter was weighted and put into 10 g of 6wt% NaOH (0.015 mol). So the NaOH dosage per cellulose AGU is 9.49 mol, which is much greater than the dosage minimum of 4 mol [25]. The saturated adsorption of NaOH on cellulose molecule is  $2 \sim 3$  mol according to literature.

### 2.2.3.2 Experimental setup

The cellulose dissolution in NaOH solution is a heterogeneous reaction, including both solid phase and liquid phase. The cellulose solid content in NaOH solution in this study is 2.5%, which were suspended as a slurry. At this concentration, the kinetic study can not be done in a single batch reactor where the total amount of reactants was put in and one small aliquot was sampled out at each time, because uniform sampling cannot be ensured. Therefore, multiple independent sample tubes filled with the same amount of chemical reactants were used for the kinetics study. In this way, when sampling, taking out one sample tube will have negligible influence on the rest of the samples. Also noticing that during cooling, no stirring was provided inside of the sample tubes, so the heat transfer in the samples is dominated by heat diffusion. The independence of each sample was ensured by using same type of sample tubes, same amount of reactants, and uniform stirring in the cooling bath outside of the sample tubes.

The design of the cooling bath is indispensable in order to obtain consistent data. The cooling bath was set up as shown in Figure 18. Thermocouple 1 records the temperature within the sample tube and thermocouple 2 reads the temperature in the cooling bath. These temperature can be shown and recorded simultaneously through a computer connected to the thermocouple data analyzer. The cooling liquid was prepared according to the phase diagram of ethylene glycol water solution [20](see Figure 19).

We prepared 23%, 25%, 30%, and 38% of ethylene glycol in water to obtain the corresponding target bath temperature( $T_c$ ) of  $-8^{\circ}\text{C}$ ,  $-11^{\circ}\text{C}$ ,  $-15^{\circ}\text{C}$ , and  $-20^{\circ}\text{C}$ . The  $0^{\circ}\text{C}$  experiment was performed in ice-water bath. Dry ice was used to cool the ethylene glycol-water solution to the phase equilibrium temperature. For each sample, 0.25g of cotton linter was weighted, put into a 15ml plastic centrifuge tube, then, 10g of 6% NaOH or 6% NaOH/4%urea solutions was added, mixed well, and stayed in room

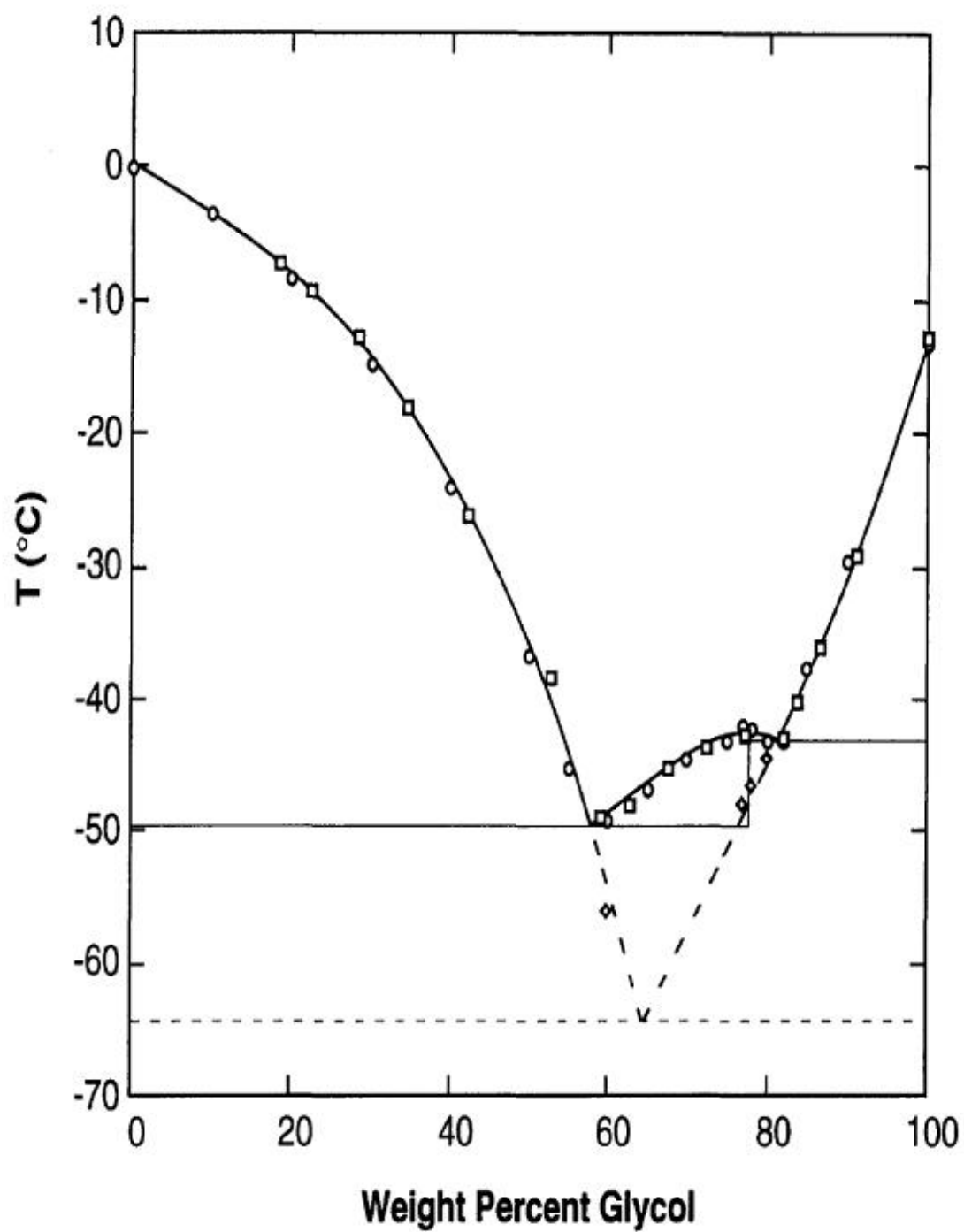


**Figure 18:** Experimental Setup for Dissolution Kinetics Study

temperature for one hour before submerging into cooling bath. Then all the samples were quickly moved into cooling bath. The samples were taken out of cooling bath sequentially and submersed water bath at room temperature immediately, until fully thawed. The dissolved cellulose and undissolved part were separated using centrifuge at 2500rpm. The undissolved residue was washed with deionized water(DI water) extensively till the system had been neutralized to  $pH = 7$ . Then the samples were pre-freezed in freezer followed by freeze drying. The dried samples were stored at room temperature for further characterizations.

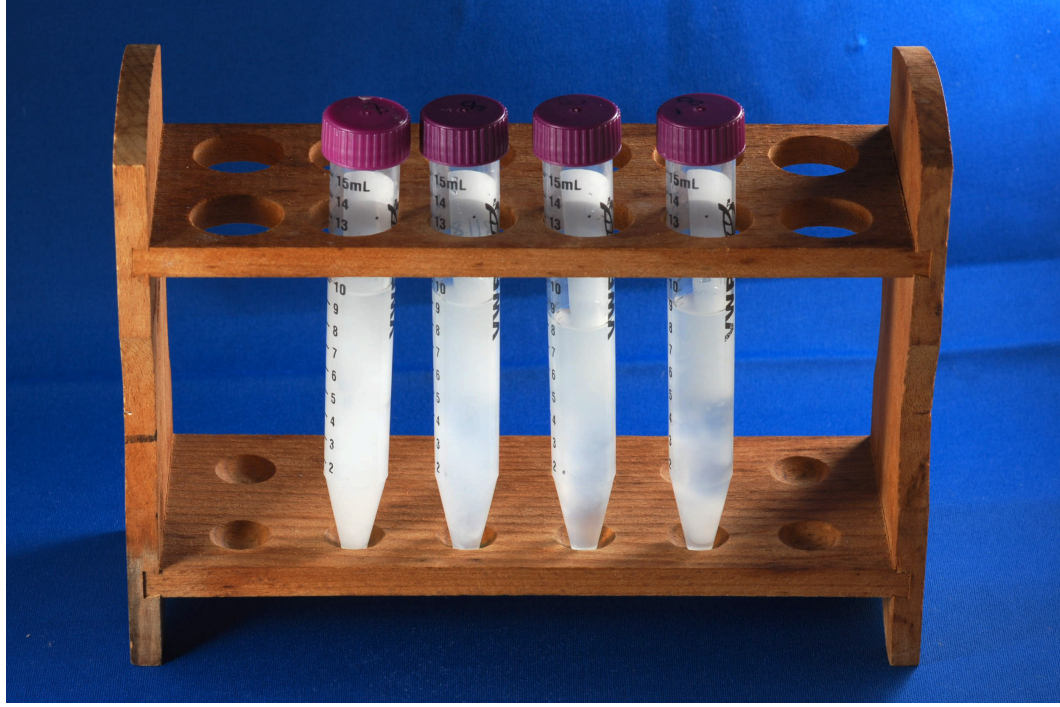
#### 2.2.3.3 Observations

The appearance of the samples after alkali treatment under various temperatures were shown in Figure 20. From the left to the right, the samples are from NaOH/urea treatments under cooling bath temperatures of  $-8$ ,  $-11$ ,  $-15$  and  $-20^{\circ}\text{C}$  respectively. We can see that the transparency change from opaque pulp slurry to transparent gel,



**Figure 19:** Phase Diagram of Ethylene Glycol in Water[20]

indicating the increase of dissolution degree.



**Figure 20:** Picture of Samples After NaOH/urea Treatment Under Different Temperatures: from Left to Right,  $-8$ ,  $-11$ ,  $-15$  and  $-20^{\circ}\text{C}$  respectively.

### 2.3 Characterization Methods

- Solubility

The dissolution degree of cellulose was defined as the weight of dissolved cellulose (the difference between the original sample weight and the undissolved residue) divided by original oven dried weight of cellulose

$$S = \frac{W_o - W_r}{W_o} \times 100\% \quad (6)$$

where  $S$  is the solubility degree,  $W_r$  is the weight of undissolved cellulose residue, and  $W_o$  is the original weight of cellulose.

- XRD

The crystallinity and crystallite size were characterized using Powder X-Ray

Diffraction (XRD). The Bragg angle was scanned from 5 to 40°. The X-Ray source is CuK $\alpha$  with wave length of 0.154nm.

- Titration

NaOH adsorption on cellulose was measured by titration with dilute hydrochloride acid.

## ***2.4 Results and Discussions***

In this study, the overall dissolution was characterized by solubility. The decrystallization, as part of the dissolution process, was monitored by the crystallinity of the undissolved residues. The reaction rate and activation energy of decrystallization were analyzed and discussed. The effect of temperature, urea additive, concentration of NaOH and other effects were also discussed.

### **2.4.1 Solubility**

As stated by literature[102], cellulose dissolution in solvents is a complex process, including several steps. In this study, the overall dissolution kinetics was followed by solubility which is defined in Equation 6, or in another word, the weight loss of solid cellulose. The results were presented in Figure 21 and Figure 22. Figure 21 is for cellulose treated in 6%NaOH/4%urea solution and Figure 22 is in 6%NaOH solution. The cooling bath temperature set ranged from 0°C to −25°C. In this range, the solubility data can be divided into three types: at 0°C, the weight loss is at about 3%. Considering reasonable loss during washing, there is almost no cellulose dissolution at 0°C. At −8°C and −11°C, the solubility slightly increased to about 7% during the alkali treatment. For temperature −15°C and −20°C, the weight loss greatly and rapidly increased and plateaued at the range of 25% to 32%. These huge change in solubility is very interesting. It suggests that between temperature −11°C and −15°C, different reaction mechanism is involved in.

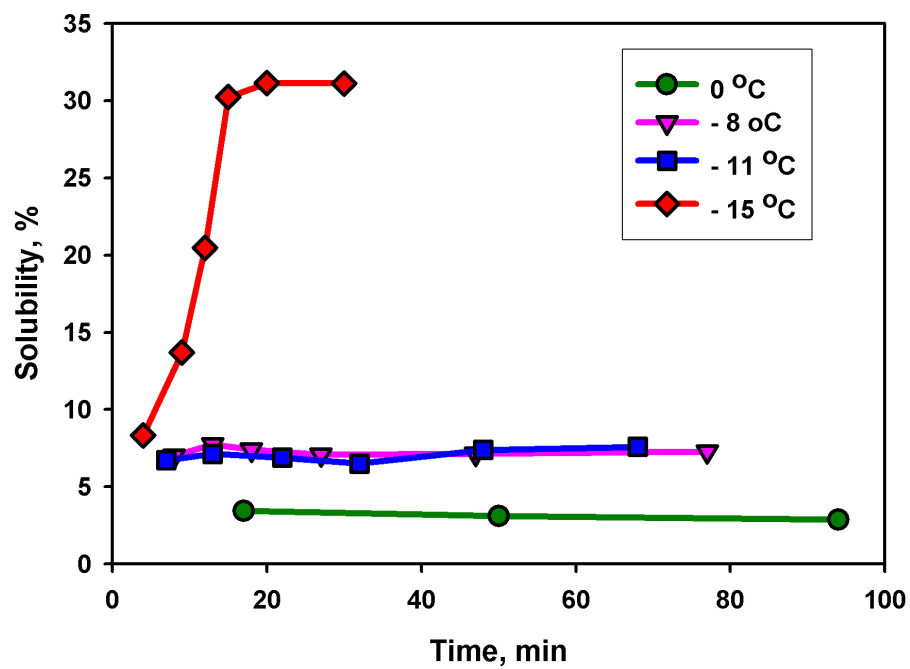


Figure 21: Solubility of Cotton Linter in 6%NaOH/4%urea Solution

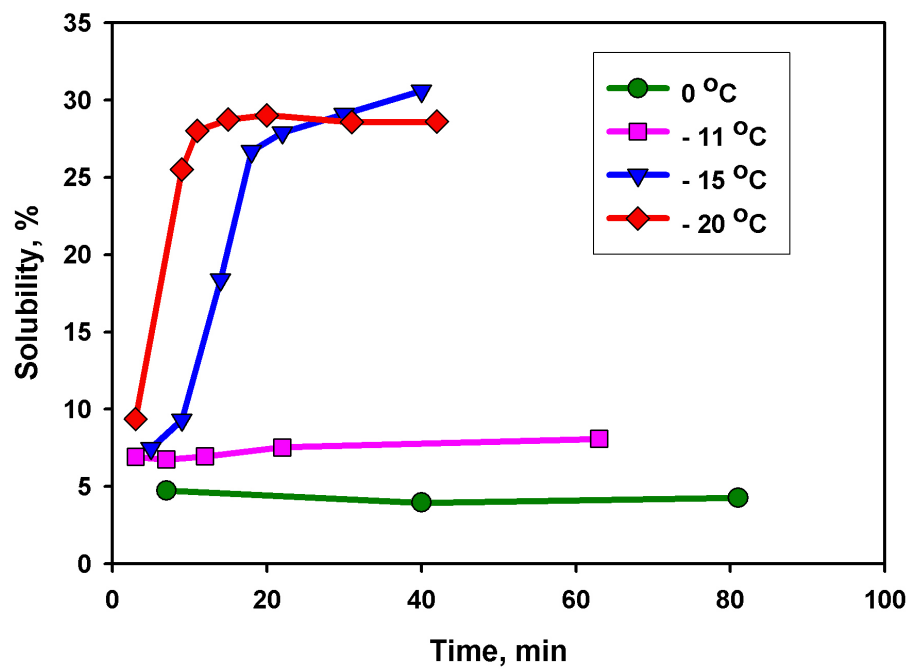
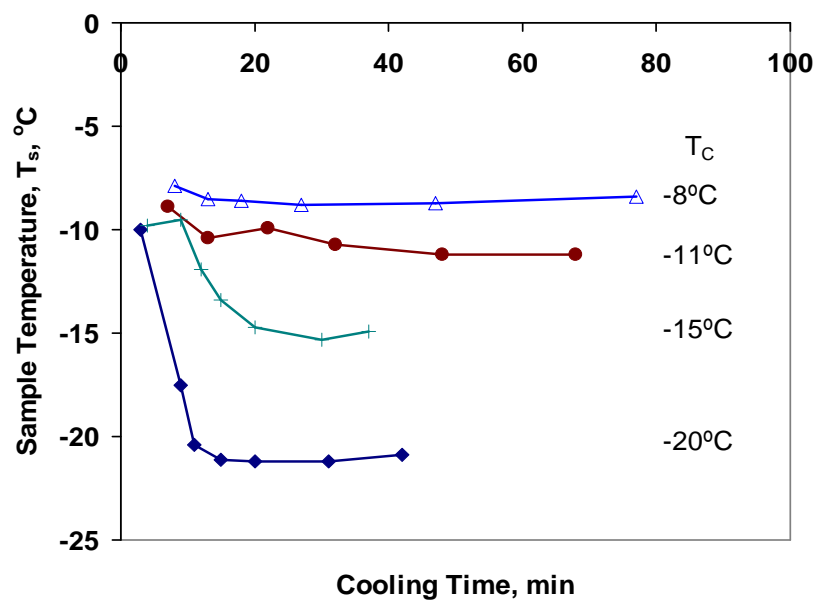
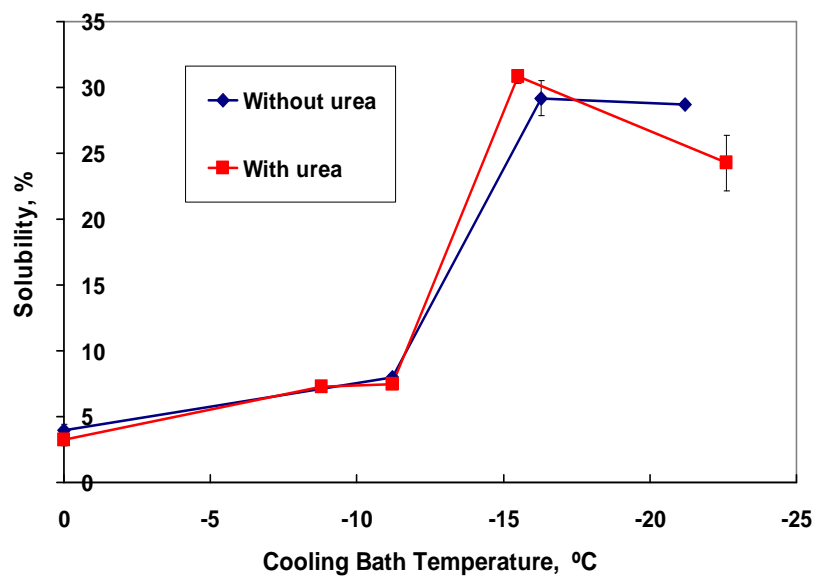


Figure 22: Solubility of Cotton Linter in 6%NaOH Solution Without Urea



**Figure 23:** Sampling Time and Temperature, each line represents an experimental run of cellulose dissolution and each data point records the sampling temperature and time.



**Figure 24:** Urea Contribution to Solubility

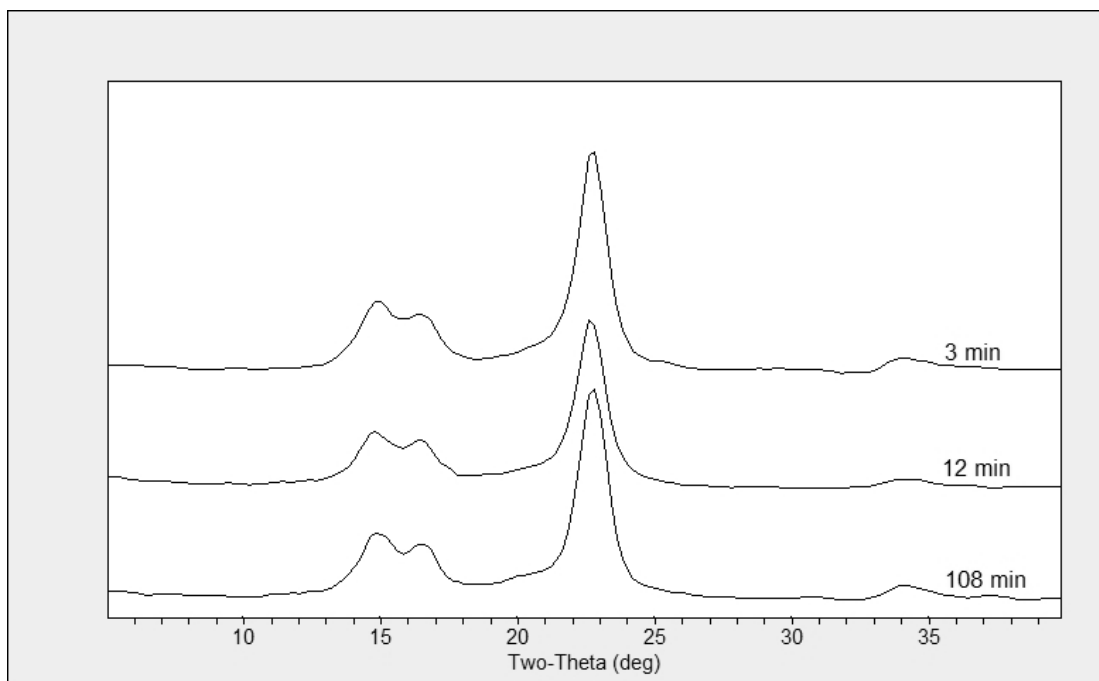
Figure 23 showed the sampling time and corresponding sample temperatures. After samples were placed into the cooling bath, the sample temperature quickly dropped to cooling bath temperature within 10 to 20 minutes. Since at different cooling bath temperatures, the maximum cellulose solubility plateaued at different values. These maximum solubility values with regard to cooling bath temperature were plotted in Figure 24. As we can see that from cooling temperature 0 to  $-11^{\circ}\text{C}$ , cellulose solubility are the same for both alkali solution with and without urea. While at  $-15^{\circ}\text{C}$ , slightly higher solubility was obtained in alkali solution with urea, however, at  $-20^{\circ}\text{C}$ , solubility in alkali solution without urea was higher.

#### **2.4.2 Decrystallization Kinetics**

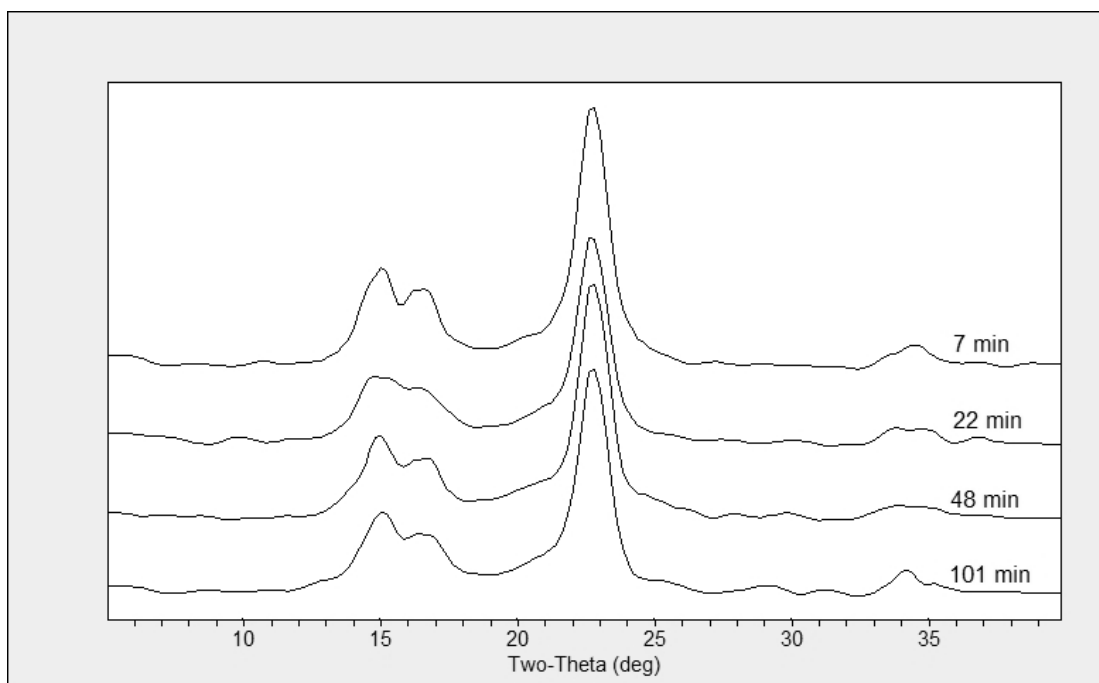
It was shown in other's work that cellulose dissolution can be investigated through monitoring the crystallinity change of undissolved cellulose due to treatment with solvent[76, 102]. It is believed to be a reliable indicator of the transformation of crystalline cellulose into a highly elastic state. In this study, the crystallinity change of undissolved residue was measured by Powder X-Ray Diffraction. The procedures for data analysis were shown and results were presented and discussed.

##### *2.4.2.1 Crystal Type Change*

The changes of cellulose crystal type due to alkali treatment in various temperatures were shown in Figure 25 to Figure 28. Each plot corresponds to a cellulose dissolution experimental run in a specific cooling bath temperature  $T_c$ . For cooling bath temperature higher than  $-11^{\circ}\text{C}$ , no crystal type change was observed (plots, which are not shown here, for  $0^{\circ}\text{C}$ ) and  $-8^{\circ}\text{C}$  are similar to that of  $-11^{\circ}\text{C}$ ), while for temperature  $-15^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$ , gradual change of cellulose crystal structure from cellulose I to a mixture of cellulose I and cellulose II, at last to cellulose II were identified.

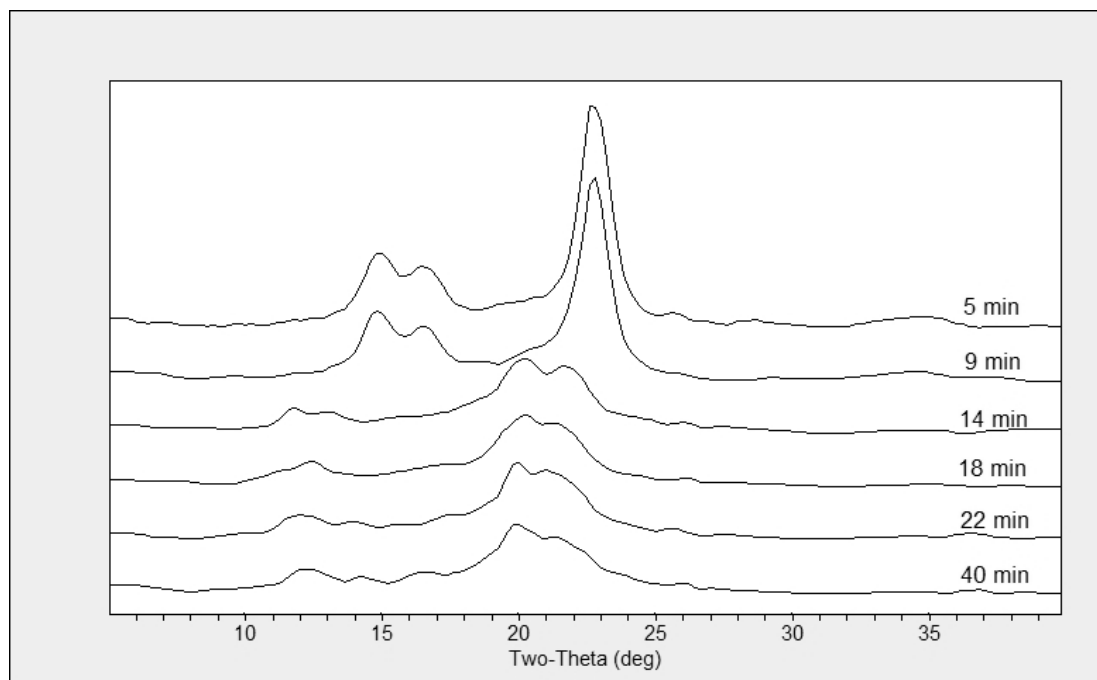


a) Without urea

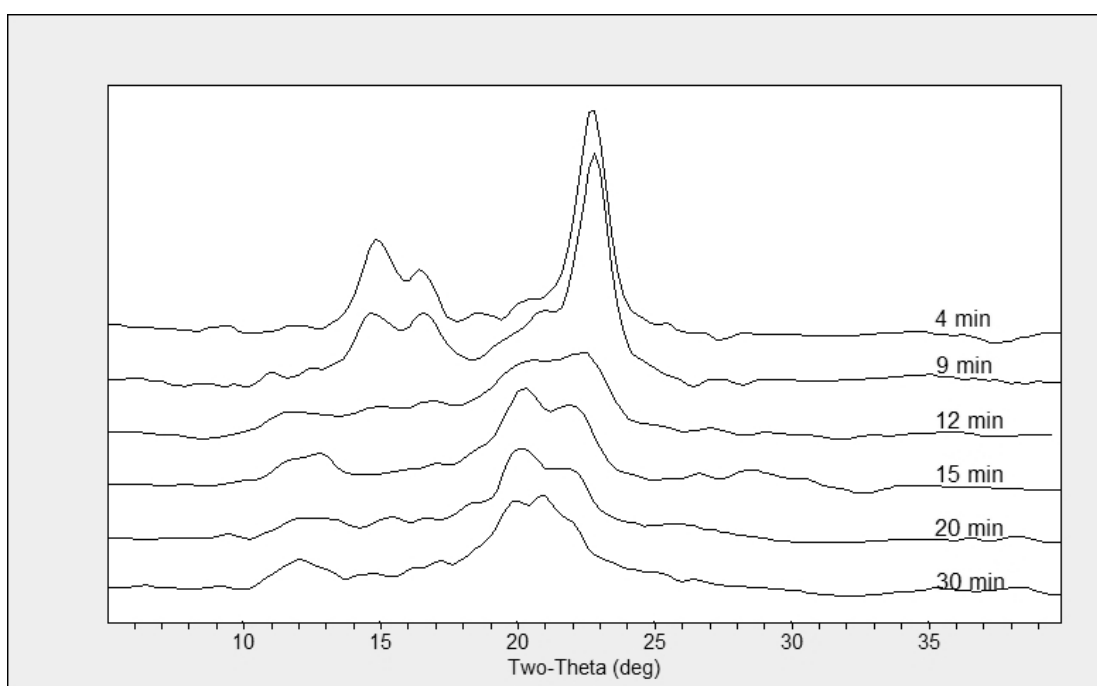


b) With urea

**Figure 25:** Crystal Type Change at  $T_c = -11^\circ\text{C}$

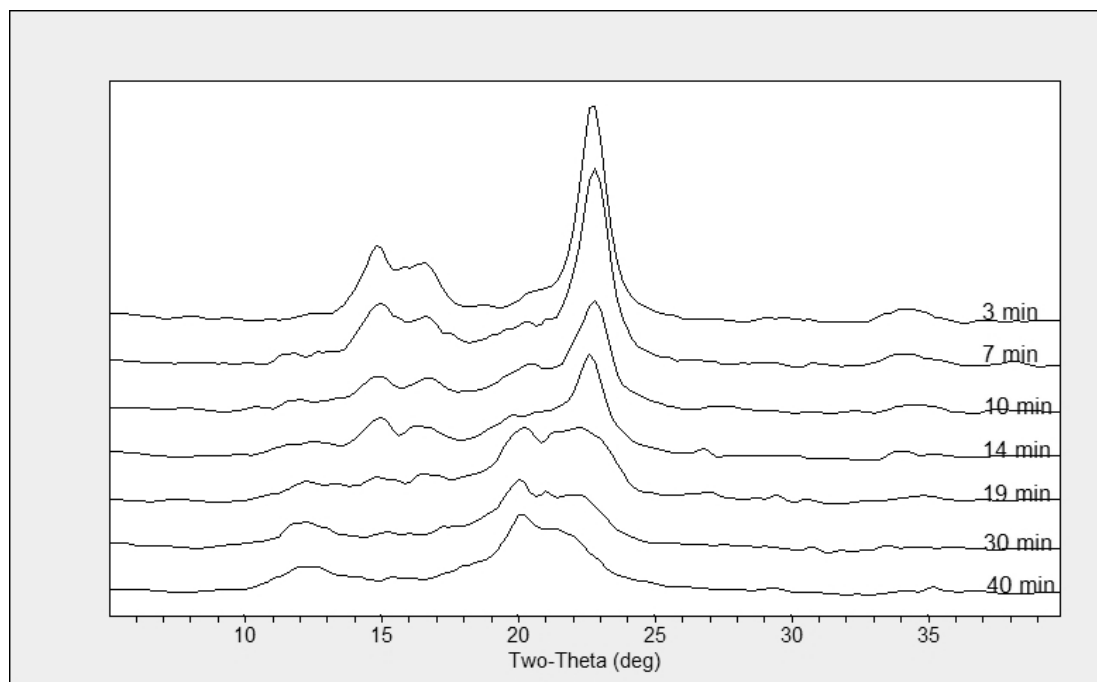


a) Without urea

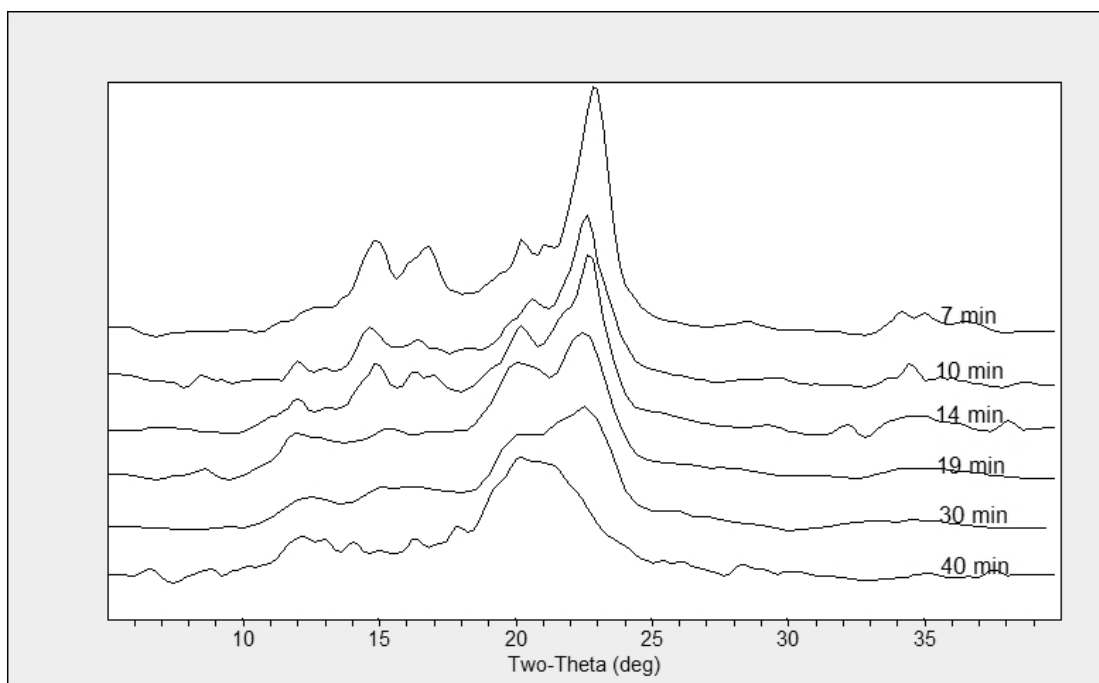


b) With urea

**Figure 26:** Crystal Type Change at  $T_c = -15^\circ\text{C}$

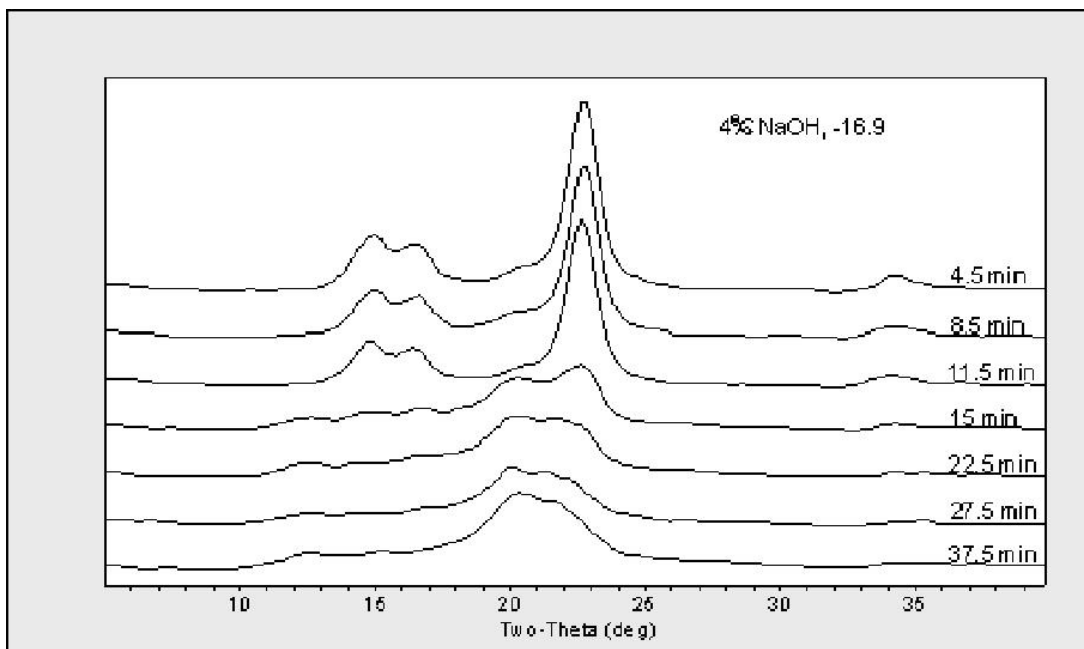


a) Without urea



b) With urea

**Figure 27:** Crystal Type Change, With Urea,  $T_c = -20^\circ\text{C}$



**Figure 28:** Plot of Crystal Type Change During 4% NaOH Solution Dissolution Without Urea,  $T_c = -15^\circ\text{C}$

#### 2.4.2.2 Crystallinity Change

The XRD spectra were further analyzed using Jade 8 software, through which crystallinity and crystal size data were obtained. The crystallinity change due to alkali treatment with and without urea were plotted in Figure 29 and Figure 30, respectively. We can see that the trends in crystallinity change agree well with the change in solubility.

#### 2.4.2.3 Reaction Rate and Activation Energy

Because the dissolution process in our designed experiments was a non-isothermal process, the crystallinity decrease occurred while the sample temperature  $T_s$  was in continuing drop (data not shown). The crystallinity at a specific point was not only a function of time, but also a function of temperature. The observed reaction rate and the activation energy were all calculated based on the non-isothermal system. The procedure was explained in the following paragraphs.

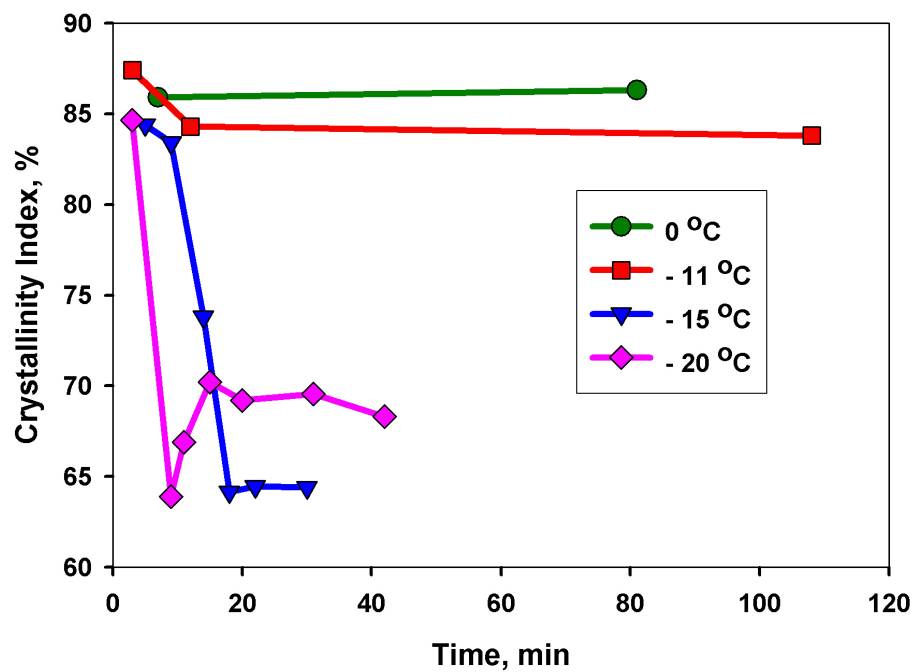


Figure 29: Crystallinity of Cotton Residues Treated in 6%NaOH solution

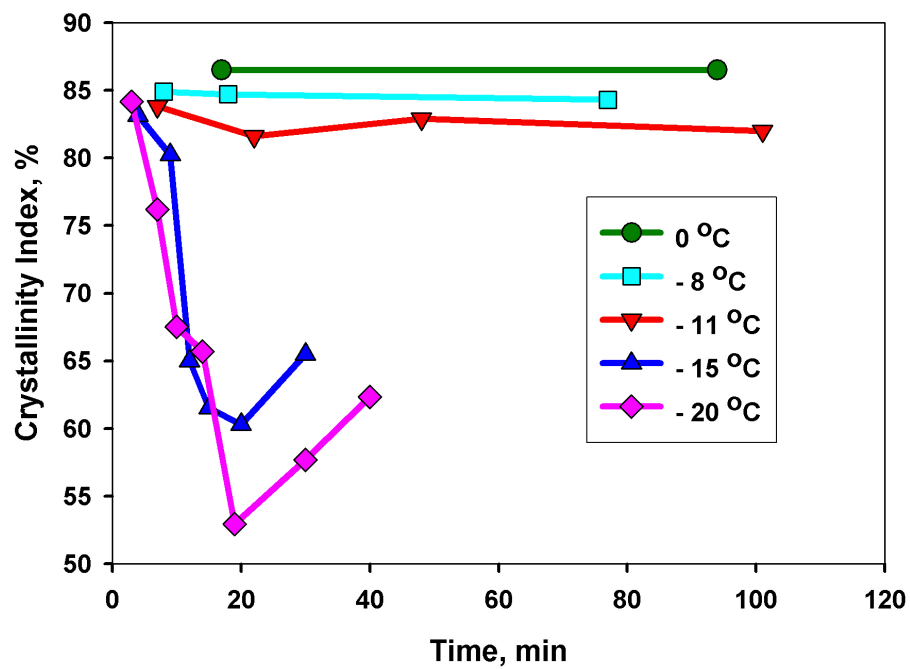


Figure 30: Crystallinity of Cotton Residues Treated in 6%NaOH/4%urea Solution

Since we have already obtained the XRD spectra, after basic data analysis, for example, smoothen the spectra, baseline correction, and peak fitting etc., using a commercial peak analysis software(Jade<sup>TM</sup> 8), we achieved the statistical data for each characterized crystalline cellulose peak. Then, the degree of crystallinity was calculated through Equation 7, where  $I_{cr}$  was the crystallinity of undissolved residue,  $I_{min}$  was the minimum intensity at bragg angle  $18^\circ$  and the  $I_{max}$  was the maximum intensity found at  $22.7^\circ$ . This is a simple and fast method to get crystallinity from XRD spectra. Recall that the crystallinity  $I_{cr}$  derived here is for the undissolved residue, the crystallinity for the initial sample  $I_{cw}$  can be obtained by multiply it with instantaneous weight factor of  $W_t/W_o$  assuming all the dissolved cellulose are amorphous.

$$I_{cw} = \frac{W_t}{W_o} \times I_{cr} \quad (7)$$

where

$$I_{cr} = 1 - \frac{I_{min}}{I_{max}}$$

is the crystallinity for undissolved cellulose

$I_{min}$  is the minimum intensity between bragg angle  $17 \sim 18^\circ$  for cellulose I and  $14 \sim 15^\circ$  for cellulose II,

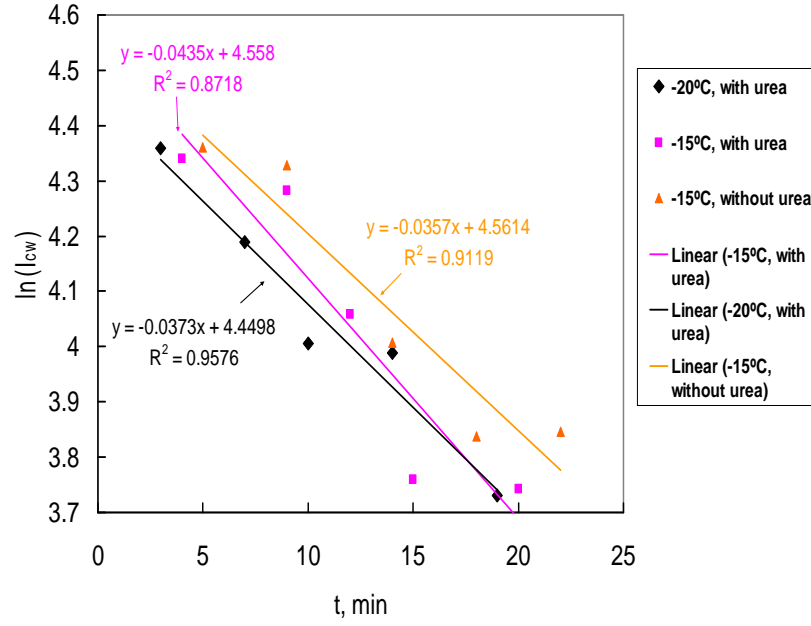
$I_{max}$  is the maximum intensity between bragg angle  $20 \sim 22^\circ$ .

$I_{cw}$  is the crystallinity for the whole cellulose sample,

$W_t$  is the weight of cellulose at treatment time  $t$ ,

and  $W_o$  is the initial weight of each sample.

The cellulose decrystallization can be treated as a pseudo-first-order reaction according to literature[76, 102]. To check this assumption, we plotted  $\ln I_{cw}$  vs. time  $t$  to see if we could get a linear relation. Figure 31 showed that this assumption still holds in our solution system. get the reaction rate constant from Equation 8. Note



**Figure 31:** The Reaction Order of Cellulose Decrystallization

that since we are dealing with a non-isothermal cooling process, both the crystallinity  $I_{cw}$  and reaction rate  $k_{obs}$  are time dependent.

$$-\frac{dI_{cw}}{dt} = k_{obs} \times I_{cw} \quad (8)$$

After we got the rate constant, activation enthalpy and entropy can be derived by Eyring equation (Equation 9) for non-isothermal reaction

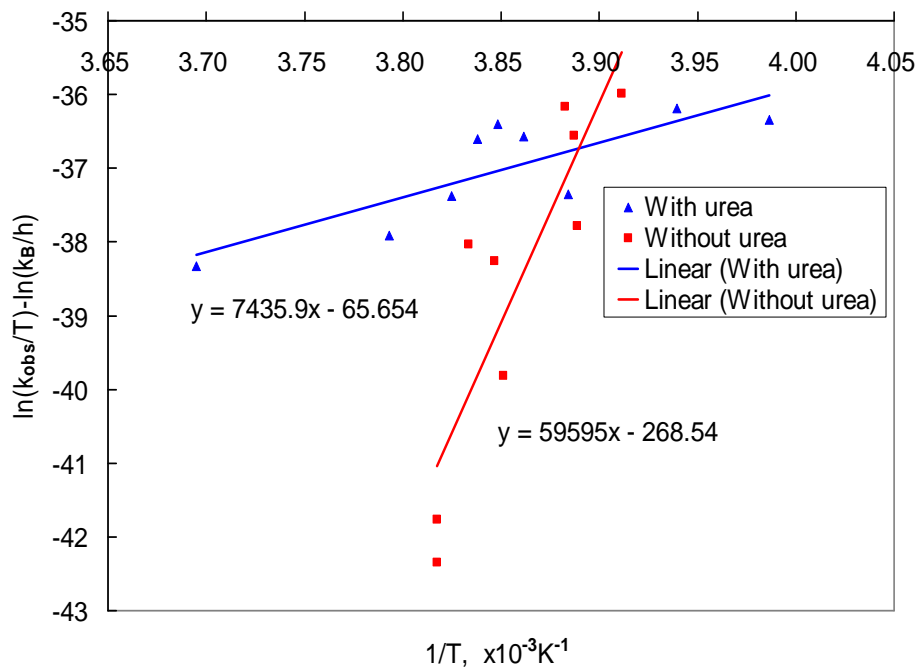
$$\ln \frac{k_{obs}}{T} = \frac{-\Delta H^\ddagger}{R} \cdot \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R} \quad (9)$$

An example of the spread sheet of calculation was listed in Table 5. Plot  $\ln \frac{k_{obs}}{T} - \ln \frac{k_B}{h}$  versus  $\frac{1}{T}$  (Figure 32) and perform Linear regression. Straight lines can be identified with slope  $\frac{-\Delta H^\ddagger}{R}$  from which the enthalpy of activation was derived and with intercept  $\frac{\Delta S^\ddagger}{R}$  from which the entropy of activation is derived. The Gibbs free energy can be calculated (Equation 10).

$$\Delta G = \Delta H - T \cdot \Delta S \quad (10)$$

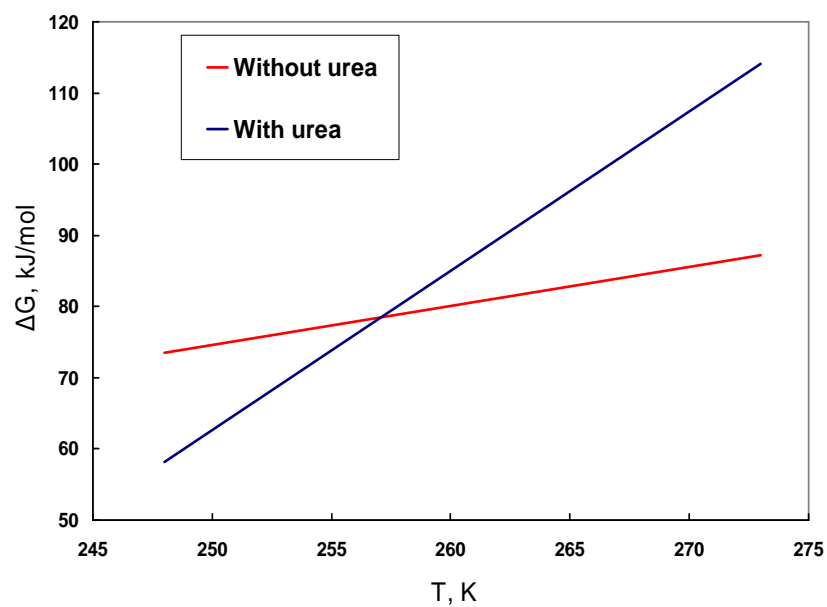
The free energy data were listed in Table 6. The calculated enthalpy and entropy for cellulose to break crystal are  $-61.82$  kJ/mol and  $-0.5458$  kJ/K mol for alkali treatment with urea and  $-495.47$  kJ/mol and  $-2.2326$  kJ/K mol for treatment without urea respectively. At  $-15^\circ\text{C}$ , the Gibbs free energy was  $79.01$  kJ/mol for dissolution with urea and  $80.55$  kJ/mol for that without urea. The negative enthalpy indicated that the decrystallization reaction is exothermic, favoring lower temperature. However, the negative entropy determined the positive Gibbs free energy in this reaction. In Ramos' work, they dissolved cellulose in a lithium Chloride/N,N-Dimethylacetamide solvent system and obtained a cellulose(avicel) decrystallization enthalpy of  $-1.82$  kcal/mol ( $-7.62$  kJ/mol) and entropy of  $-83.05$  cal/Kmol ( $-0.347$  kJ/K mol). Lithium Chloride/N,N-Dimethylacetamide solvent system is an ionic direct solvent of cellulose. NaOH solution with or without urea at temperature range from  $0 \sim -10^\circ\text{C}$  is also believed to be a direct solvent of cellulose. Comparing with these solvent systems, their enthalpy and entropy (absolute values) have the order of  $\text{LiCl} \leq \text{NaOH/urea} \leq \text{NaOH}$ . However, since entropies of decrystallization for all these solvent systems are negative, their contribution to Gibbs free energy are also negative. From the point of view of Gibbs free energy, LiCl is a better direct solvent than sodium hydroxide. To show the contribution of enthalpy and entropy to Gibbs free energy, a diagram (Figure 33) of Gibbs free energy verses temperatures was drawn for sodium hydroxide solution with and without urea. Interestingly, two straight lines of Gibbs free energy crossed at the temperature of  $257.1\text{K}$  ( $-15^\circ\text{C}$ ). When temperature is higher than  $-15^\circ\text{C}$ , NaOH/urea has lower free energy than NaOH solution without urea, which is better for the decrystallization reaction. When the temperature is lower than  $-15^\circ\text{C}$ , NaOH solution might be better in terms of the free energy of decrystallization. However, the lowest cooling bath temperature we investigated

was at  $-20^{\circ}\text{C}$ . There are not enough data below  $-15^{\circ}\text{C}$  region to support a conclusion. The cooling bath temperature need to be extended to a lower one in order to make the trend clear. Because both enthalpy and entropy are negative in the alkali solution, when temperature increases, the power of entropy in Gibbs free energy increases and the enthalpy decreases relatively.

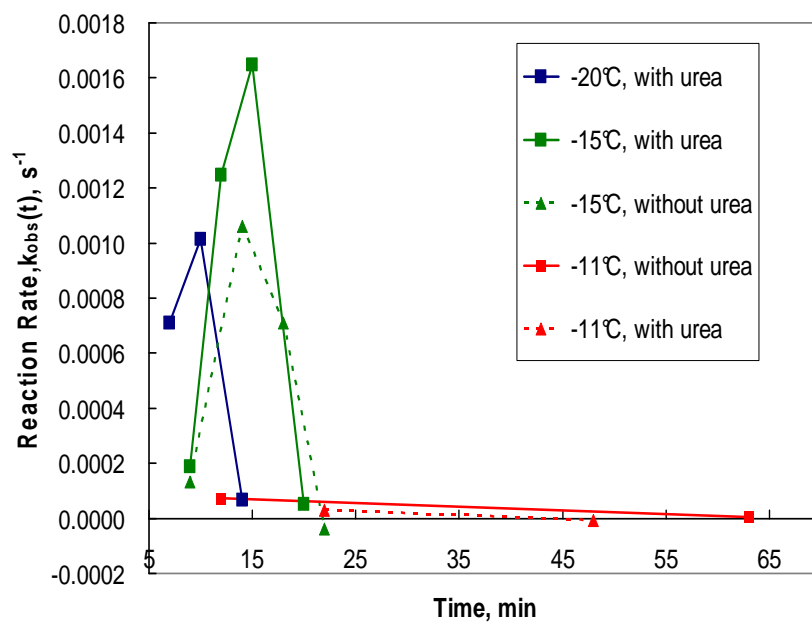


**Figure 32:** Plot of Eyring Equation

Because the dissolution process is non-isothermal, the reaction rate is not a constant, but depends on the instantaneous time and temperature (Figure 34). The highest reaction rate occurred in the NaOH/urea treatment at  $-15^{\circ}\text{C}$ .



**Figure 33:** Plot of Gibbs Free Energy for Cellulose Decrystallization in Alkali Solution



**Figure 34:** Plot of Instantaneous Reaction Rate

**Table 5:** Example of Spread Sheet of Kinetics Calculation

Time,s	Temp,k	Ic(t)	dIc/dt	k(t)	1/T	lnk(t)h/kBT(t)
180	265.15	78.177	0.00377	/	/	/
420	258.95	65.907	-0.05112	0.000637	0.00386	-36.674
600	253.85	54.892	-0.06119	0.000852	0.00394	-36.365
840	251.55	53.997	-0.00373	5.60296E-05	0.00397	-39.077
1140	250.85	41.674	-0.04107	0.000692	0.00398	-36.5602

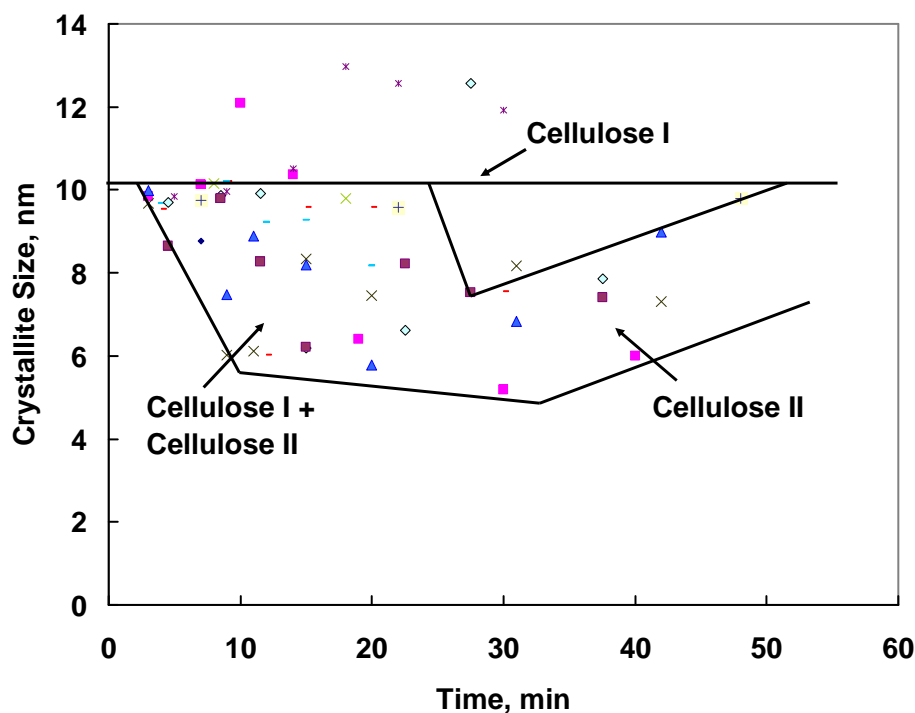
**Table 6:** Activation Energy for Decrystallization

Treatment	Slope	Intersect	$\Delta H, kJmol^{-1}$	$\Delta S, kJK^{-1}mol^{-1}$	$\Delta G$ at $-15^{\circ}C, kJmol^{-1}$
With urea	7435.9	-65.654	-61.82	-0.5458	79.01
Without urea	59595	-268.54	-495.47	-2.2326	80.55

#### 2.4.2.4 Crystallite Size Change

Table 7 listed the data for a typical cellulose crystal change during dissolution in NaOH solution with or without urea. Two crystal size data were listed, one is obtained from peak analysis software, the other is calculated using Scherrer equation[85]. The results were comparable. The overall crystal size data were plotted in Figure 35. Except for one run, all other data fell into the following regions:

1. For samples run at temperature 0,  $-8$  and  $-11^{\circ}\text{C}$ , crystallite sizes were about 10nm, and cellulose type is cellulose I.
2. For samples under  $-15$  and  $-20^{\circ}\text{C}$  NaOH or NaOH/urea treatment, the crystal sizes drooped from 10nm to 6nm at the first 20 minutes, cellulose types in this region were a mixture of cellulose I and cellulose II. After the crystal sizes reduced to the minimum, it gradually increased as the cellulose type changed to cellulose II completely.



**Figure 35:** Crystallite Size Change during Dissolution

**Table 7:** Typical Crystal Change During Dissolution

Time, min	$2\theta$	d, Å	XS, nm software	XS, nm calc.	CrI	Type
3	22.806	3.8961	10.4	9.843	84.15	I
7	22.839	3.8905	10.7	10.127	76.2	I+II
10	22.59	3.9329	12.9	12.086	67.5	I+II
14	22.673	3.9187	11.0	10.371	65.7	I+II
19	22.62	3.9278	6.6	6.412	52.95	I+II
30	22.674	3.9185	5.3	5.179	57.7	II
40	20.231	4.3858	6.2	6.002	62.35	II

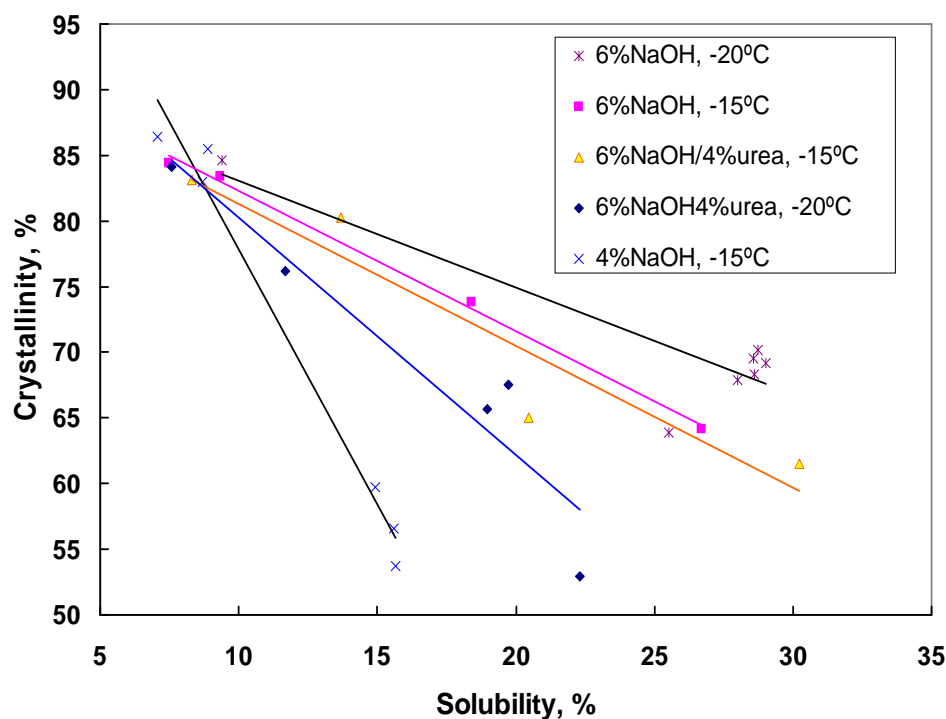
### 2.4.3 Correlation of Overall Solubility with Decrystallization Process

The reaction of dissolution of crystalline cellulose into molecular dispersion can be divided into several stages: firstly, the transition of the solid polymer to a hypothetical, highly elastic liquid state which corresponds to disintegration of the crystalline regions ( $\Delta H_{\text{fusion}}$ ) and transition of the amorphous regions from a vitreous to a highly elastic state ( $\Delta H_{\text{transition}}$ ); secondly, solvation of the polymer macromolecules ( $\Delta H_{\text{interaction}}$ ) and thirdly, mixing of the solvated polymer molecules with solvent to give an infinitely diluted solution ( $\Delta H_{\text{mixing}}$ ) [76, 83]. It is widely accepted that the recalcitrance of cellulose is due to its highly ordered crystalline region which was bonded by hydrogen bonding and van der Waal forces. Therefore, we would expect that the decrystallization process will be directly correlated to the dissolution degree, which was shown in Figure 36. We can see that the decrease of cellulose crystallinity is linearly correlated to the increase of solubility in various cooling temperatures.

### 2.4.4 The Limit of Cellulose Solubility

In this study, the highest solubility of cotton linter in NaOH/urea solution is 31%, while in NaOH solution is 25% to 30%. The solubility is very low for any applications. The reason of this low solubility include:

1. The molecular weight of cellulose is high, at very beginning of dissolution, a



**Figure 36:** Correlation of Overall Solubility with Decrystallization Process

slight amount of cellulose dissolved in solution would induce huge viscosity increase, lead to gelation which sterically hindered further dissolution.

2. the higher the molecular weight, the higher the interaction between polymer-polymer chains.
3. the crystalline part is hard to be accessed by chemicals.

#### 2.4.5 The Effect of Phase Change During Dissolution

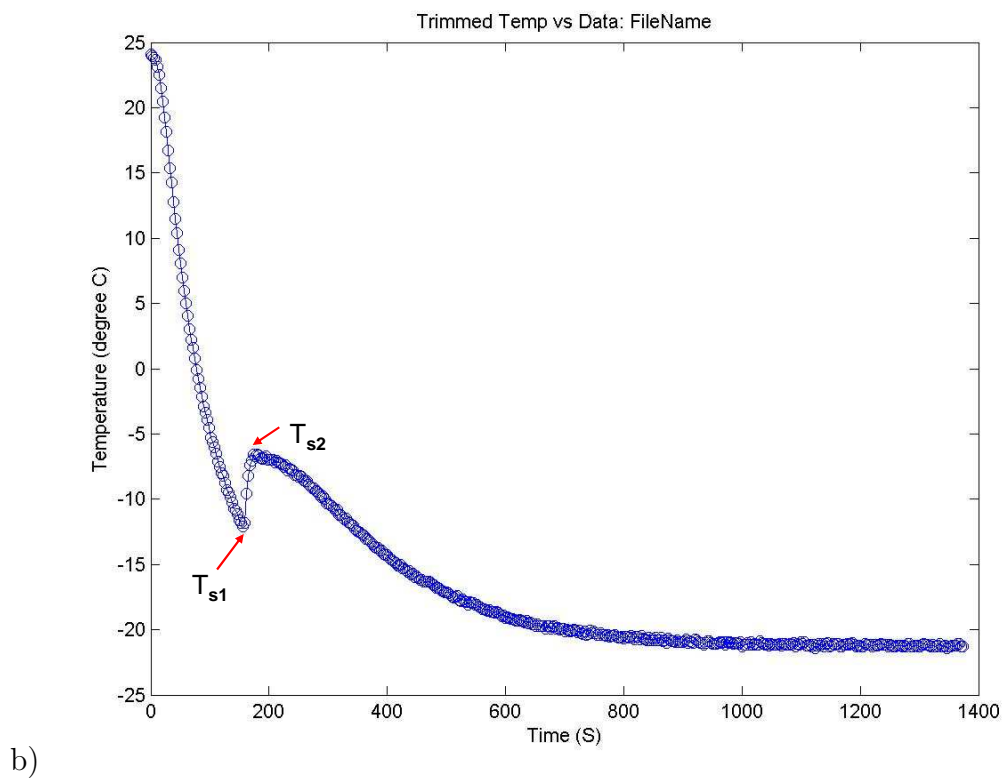
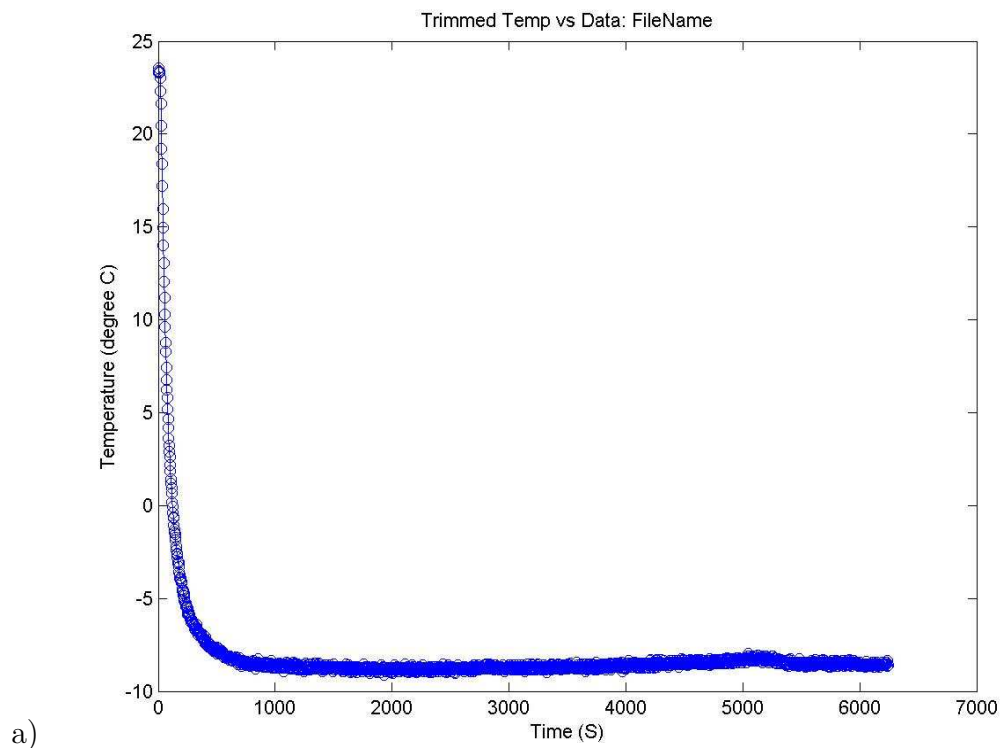
From the Gibbs free energy, the cellulose dissolution in NaOH solution favors lower temperature (Figure 33). However, the solubility increase with respect to temperature was not a gradual change (Figure 21 and Figure 22). The solubility result suggests that some other effects may have important impact. After analyzing the recorded temperature profiles of sample ( $T_s$ ) (Figure 37), we found a phenomena that was related to the abrupt change of solubility.

Figure 37 shows temperature profiles in the sample ( $T_s$ ) at certain cooling temperatures ( $T_c$ ). These temperature profiles were clearly divided into two types: 1. smooth  $T_s$  temperature dropping profile(Figure 37a) for  $T_c -8^\circ\text{C}$  and  $-11^\circ\text{C}$ , 2. sharp peak in  $T_s$  (Figure 37b) at cooling temperature  $T_c -15^\circ\text{C}$  and  $-20^\circ\text{C}$ .

After examining the temperature peaks(Figure 37 b), it was found that although temperature peaks started at various temperature  $T_{s1}$ , they ended at similar temperature  $T_{s2}$ . The data was read and listed in Table 8. For cellulose-NaOH-water mixture, the average end point was  $-6.51^\circ\text{C} \pm 0.125^\circ\text{C}$  and for cellulose-NaOH-urea-water mixture, this value was  $-7.85^\circ\text{C} \pm 0.495^\circ\text{C}$ . These peaks are typical crystallization peaks where the minimum temperature on the peak is the supercooling temperature and the end temperature is the real freezing (or melting) temperature. To assign the phase change peak, we compared our data with DSC data from literature (Figure 17) which showed melting temperature of  $-13^\circ\text{C}$  for free water in cellulose (in the -7%NaOH/12%urea system), and phase diagram for NaOH-water system (Figure 9), which showed around  $-6^\circ\text{C}$  of icing temperature for 6%NaOH in water. We conclude that the freezing temperature belongs to the free water in the cellulose (in the -6%NaOH/4%urea-water system). We also noticed that the phase change temperature of the mixture with urea is lower than the mixtures without urea which can be explained by melting point depression when additional chemical is added. How could this free water phase change affect the solubility? This is discussed in the following sections of *The effect of NaOH concentration* and *NaOH adsorption on Cellulose*.

#### 2.4.6 The Effect of NaOH Concentration

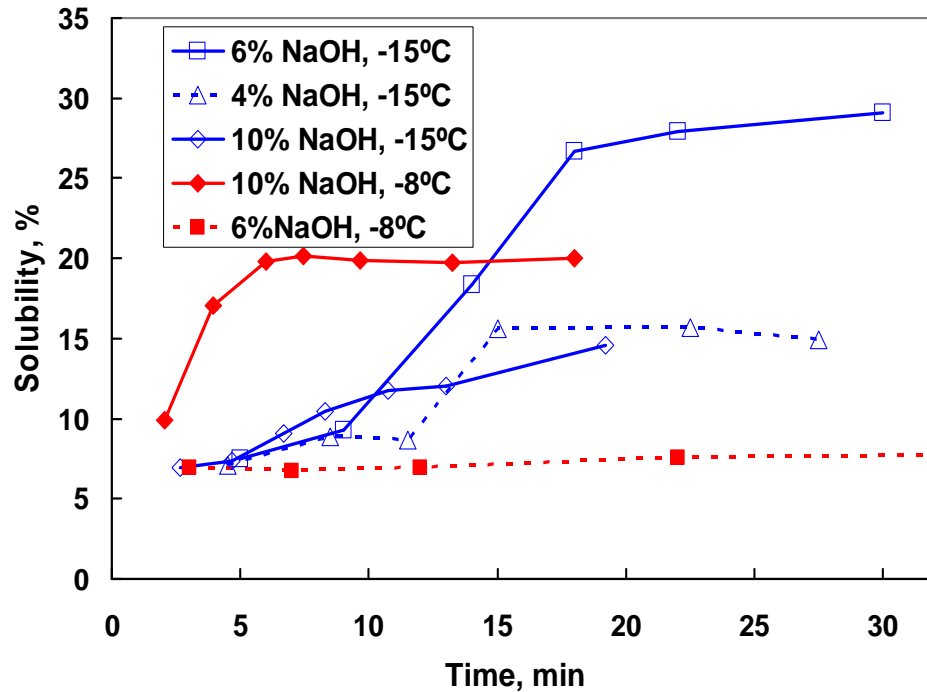
The effect of different concentration of NaOH solution on cellulose dissolution kinetics was evaluated by comparing solubility of cellulose in 4%, 6% and 10% NaOH solution(Figure 38). Comparing blue lines for 4%, 6% and 10%NaOH at cooling bath temperature of  $-15^\circ\text{C}$ , 6% NaOH concentration gave the highest solubility of 30%.



**Figure 37:** Temperature Profiles at  $T_c = -8^\circ\text{C}$ ,  $-11^\circ\text{C}$ (a) and  $T_c = -15^\circ\text{C}$  and  $-20^\circ\text{C}$  (b)

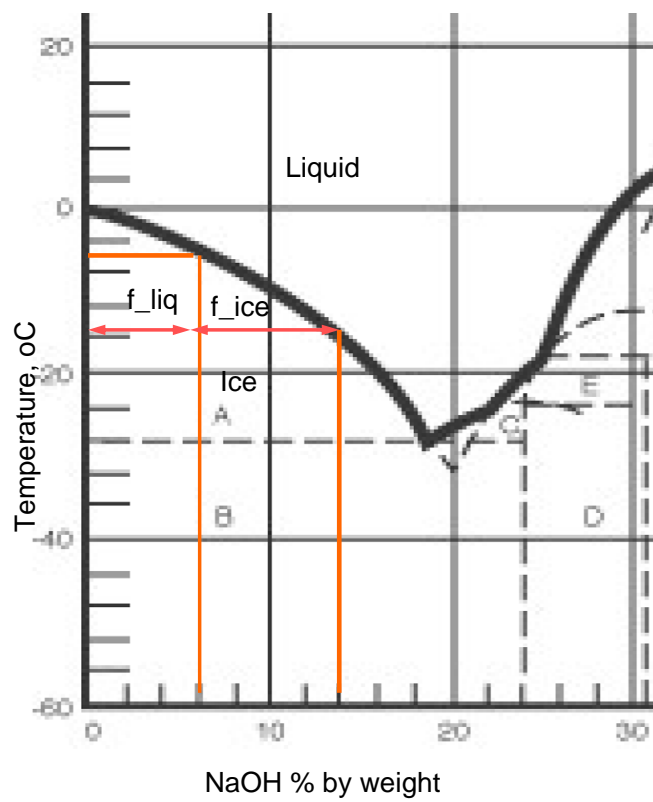
**Table 8:** Temperature Peak

Urea	Cooling Bath $T_c$ , °C	Supercooling $T_{s1}$ , °C	Phase Change $T_{s2}$ , °C
0%	-16.3	-9.98	-6.64
	-22.17	-15.78	-6.38
	-21.9	-15.28	-6.44
	-21.14	-11.8	-6.6
	-11.45	/	/
4%	-22.28	-8.15	-7.24
	-22.03	-16.49	-7.7
	-21.15	-18.16	-8.61
	-15.21	-12.59	-7.78
	-11.03	/	/
	-8.5	/	/

**Figure 38:** NaOH Concentration Effects on Dissolution Kinetics

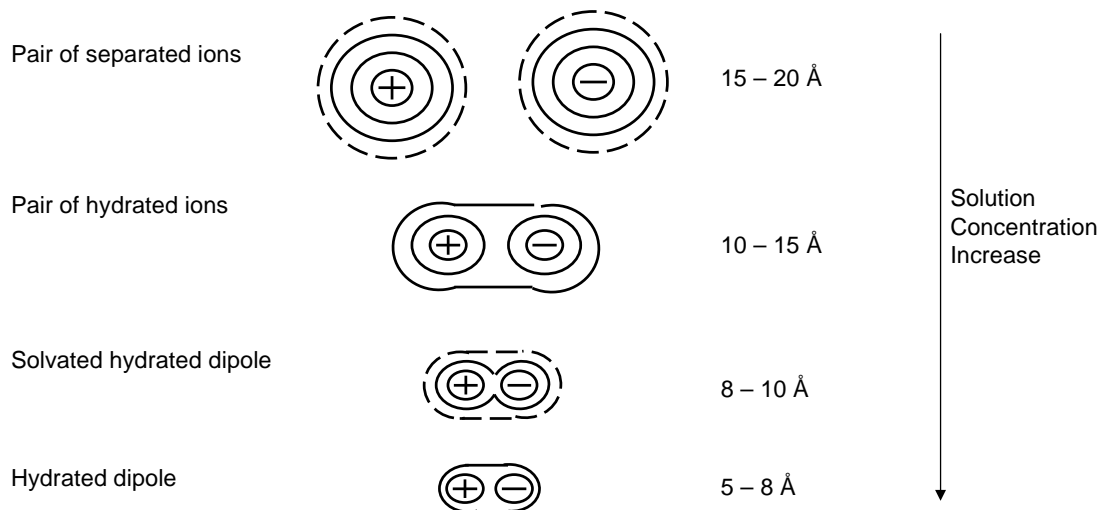
At the same temperature, 4% NaOH only dissolve 15% cellulose. Comparing another pair, 10% NaOH and 6% NaOH at  $-8^{\circ}\text{C}$ , 10% NaOH got 20% solubility while only 7 to 8% for 6% NaOH at the same temperature, and the shapes of curves are apparently different, which suggested decrystallization took place in 10% NaOH but not in 6%NaOH at this temperature. Also, it is remarkable that the solubility in 10% NaOH at  $-8^{\circ}\text{C}$  is much lower than that of 6%NaOH at  $-15^{\circ}\text{C}$ . In a summary of the comparison between all different concentrations, there exists an optimum condition of alkali concentration and cooling temperature, the best one found in this study is 6% NaOH at  $-15^{\circ}\text{C}$ . Kunze stated that in the activation of cellulose using caustic soda solution, the decrease of temperature acts similar to the increase of NaOH concentration at room temperature[59]. Our experiment results are in partly agreement with their results. Their experiments investigated two temperatures: 0 and  $-25^{\circ}\text{C}$ , which was not adequate to show the effect of temperatures.

To explain the effect of NaOH concentration on cellulose dissolution from a different angle, let us revisit the NaOH-water phase diagram (Figure 39) and apply lever rule to analyze NaOH-water behavior at low temperature. Starting with 6%NaOH in water solution, when the solution temperature drops to  $-6^{\circ}\text{C}$ , ice will appear. As the temperature continuously dropping, more ice produced, resulting a change of the NaOH concentration in liquid phase. When temperature falls to  $-15^{\circ}\text{C}$ , the weight fraction of ice (from Figure 39) to liquid is  $f_{ice} = 4/7$ , and  $f_{liq} = 3/7$ . Because part of the water freezed, the NaOH concentration in liquid phase increase, the concentration of NaOH in liquidus water is 14% at this point. Thus we can see NaOH concentration greatly increased from 6% to 14% by cooling and phase change. In a cellulose-NaOH-water system, during phase change, the concentration of NaOH in the immediate environment of solid cellulose will increase. This concentration change may contribute to cellulose dissolution. In next section, experiments were designed to prove that the adsorption of NaOH on cellulose was actually increased during phase



**Figure 39:** Lever Rule on NaOH-water Phase Diagram

transition.



**Figure 40:** Hydrodynamic Diameter of Hydrates with Respect to Solution Concentration.

To understand why the increase of NaOH concentration would increase the cellulose dissolution, let us imagine a pair of ions in solution (Figure 40). Researchers found that hydrodynamic diameter of ion hydrates were associated with the concentration of ion solution. As in Figure 40. Generally, when solution concentration is low, pairs of separated ions exist and are surrounded by large amount of water molecules. When the concentration becomes higher, hydrated ions will be formed with lower hydrodynamic diameter. For NaOH solution, the turn point is at concentration of 9% [126]. Recall that cellulose crystal is very densely packed, with a crystallite diameter of only 10 nm and intersheet distance of about 10 Å. When the alkali concentration is too low, the hydrodynamic diameter of NaOH ions will be too large to penetrate the crystalline region of cellulose. However, if the alkali concentration is too high, the hydration of alkali ions is also insufficient to break hydrogen bonding. In that case, cellulose will not be dissolved but mercerized. In this study, the concentration of NaOH solution changed from 6% to 14% during cooling, passed the changing

point concentration of 9%. This may explain why cellulose solubility was suddenly increased during cooling process. According to the NaOH-water phase diagram, the temperature to phase change temperature to get 9% NaOH in liquid is  $-9^{\circ}\text{C}$ . This means the cooling temperature of 6% NaOH solution has to be lower than  $-9^{\circ}\text{C}$ . The great solubility change observed in this study occurred in the temperature range of  $-11^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$ , which is in good agreement with literature, considering our system included cellulose.

#### **2.4.7 NaOH Adsorption on Cellulose**

Several literatures stated that cellulose can be dissolved in a narrow range of NaOH concentration, from 6% to 10% at low temperatures about  $-5^{\circ}\text{C}$ . Therefore NaOH concentration is very important for cellulose dissolution. As discussed in sections *The Effect of Phase Change During Dissolution* and *The Effect of NaOH Concentration*, for 6% NaOH solution, the increase of NaOH concentration in the liquidus phase was induced by the free water phase change. This observation might be an explanation of sudden increase of solubility. Because NaOH has interaction with cellulose, when cellulose fiber was put into NaOH solution, it was expected that the concentration of NaOH in liquid phase and the concentration at cellulose solid surface would be different from the initial NaOH concentration. Will this concentration on cellulose surface keep constant during the whole dissolution process or will it change continuously? What is the real concentration of NaOH on cellulose surface? An experiment was designed to check the NaOH adsorption on cellulose.

To enlarge the amount of NaOH adsorption on cellulose, we doubled the cellulose solid content in alkali solution in this experiment. Samples and tests were done as following:

Put 0.5 g cotton linters into 10 g of 6% NaOH solution, mixed well and submerged into cooling bath at  $-15^{\circ}\text{C}$  for a series of time. (In this experiment, the cooling

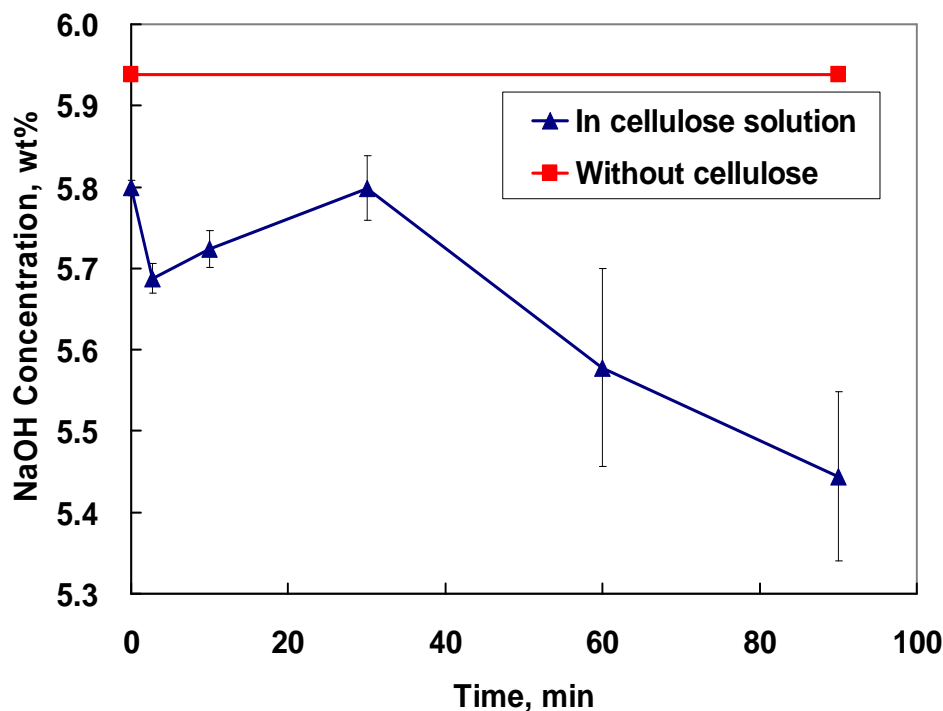
bath was placed in a freezer without mechanical stirring, therefore, the temperature dropping rate in the sample was expected to be slower comparing with those in previous experiments. Samples were taken out and the dissolved part and undissolved residue were separated by centrifuge at 2500rpm. The solution were collected and its NaOH concentration were tested through titration with hydrochloride acid. The results were in Figure 41.

Overall, the concentration of NaOH in the titrated samples were lower than that in the initial NaOH solution. The reduction in the NaOH concentration in the titrated sample means there is adsorption of NaOH on cellulose, and the larger reduction, the higher the adsorption amount. It can be seen that after mixing of cellulose with NaOH water solution at room temperature, the concentration of NaOH in solution was immediately reduced from 5.94% to 5.8%, indicating a small amount of NaOH was adsorbed onto cellulose fiber. After a period time of fluctuation, the concentration rapidly dropped to 5.45% at 90min, and the appearance of the sample turned to transparent gel, which indicated good dissolution.

The data in Figure 41 provided evidence that during the cooling process, free water phase change induced higher liquid NaOH concentration in the immediate environment of solid cellulose, resulting in higher adsorption of NaOH on cellulose, which leading to the abrupt solubility change.

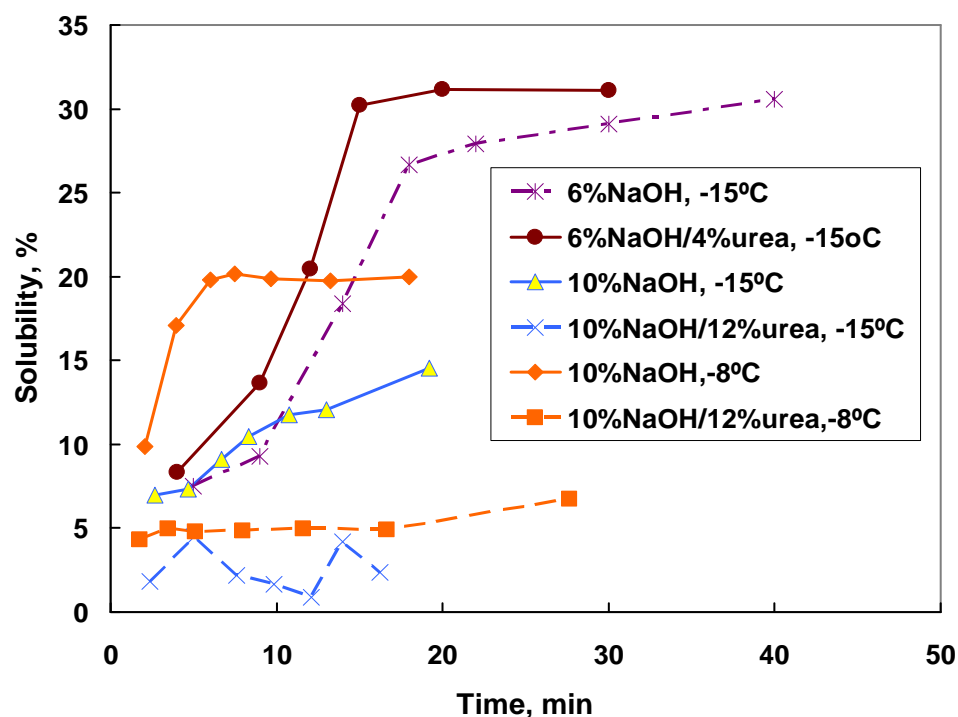
#### **2.4.8 The Effect of Urea Addition**

Many researchers reported that the addition of urea in NaOH solution can increase the cellulose solubility or induce the transform of crystalline polymorph. Some even reported that no crystalline change can be observed in their designed experiments without the addition of urea. Although the role of urea in cellulose dissolution in alkali solution is not well understood, many have agreed that there is no interaction between urea and cellulose molecules when using NaOH/urea as a direct solvent of



**Figure 41:** Plot of NaOH Concentration Change During Dissolution, at  $-15^{\circ}\text{C}$  cellulose.

In our study, we found that the effect of urea to cellulose dissolution was complicated. Firstly, at low addition amount (6% NaOH and 4% urea), there was no significant effect from our solubility data (see Figure 24), actually no effect when the cooling temperature is higher than  $-11^{\circ}\text{C}$ , and with positive effect at temperature  $-15^{\circ}\text{C}$  and negative effect at temperature  $-20^{\circ}\text{C}$ . Secondly, according to our Gibbs free energy analysis (Figure 33), comparing with 6% NaOH solution, the addition of 4% urea had two fold function: when temperature was higher than  $-15^{\circ}\text{C}$ , the addition of urea reduced Gibbs energy; while when temperature was lower than  $-15^{\circ}\text{C}$ , the introduction of urea increased Gibbs energy. Thirdly, as shown in Figure 42, the addition of high concentration of urea (12%) into 10% NaOH solution greatly suppressed the cellulose solubility at both cooling temperatures of  $-8^{\circ}\text{C}$  and  $-15^{\circ}\text{C}$ .



**Figure 42:** The Effect of Urea Addition

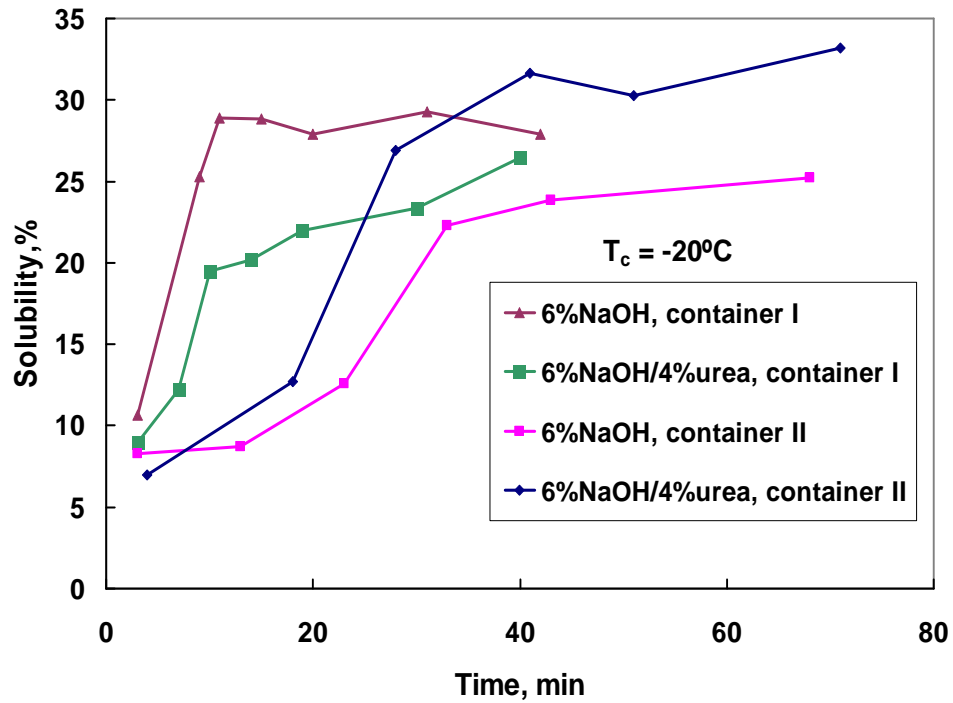
#### 2.4.9 Other Effects

Other than the above discussed effects, many other effects are also important but not included in this study due to the time limit, for example, the origin of the cellulose material, the initial cellulose crystallinity, crystal sizes, pore structures, treatment histories, etc.. These can be investigated in future work.

For the current kinetics study, two more things still need to be mentioned. As noticed in the experimental design, no shear force was provided inside of sample holder, which means the heat transfer in sample was pure conduction, therefore the diameters of sample holders were expected to affect the kinetics. Two different sizes of sample containers were used for study. All the previously presented data were from same kind of sample container (container I) of 15ml centrifuge tube with diameter of 1.2 cm, while container II was 50ml centrifuge tube with diameter of 2.5 cm. With

the same amount of total reactants, it took longer to get the same level of solubility in the sample tubes with larger diameter, which was shown in Figure 43.

As shown in our experiments, cellulose dissolution happened without providing shear force in the sample container during the whole freezing and thawing process. We don't know that if applying shear force can further increase the solubility. This can be included in the future work.



**Figure 43:** The Effect of Different Diameters of Sample Container

#### 2.4.10 Possible Dissolution Mechanism

Based on XRD spectra(Figure 25 to Figure 28) and the NaOH adsorption behavior(Figure 41), the cellulose decrystallization process can be divided into three stages:

1. cellulose I crystallinity slightly decreased without change of crystal type.
2. crystallinity further decreased with partial crystal type changes, the crystal type was a mixture of cellulose I and cellulose II.

3. crystallinity went to a minimum when all the cellulose I changed to cellulose II, after that cellulose II crystallinity slightly increased.

The crystallinity change conforms with NaOH adsorption data, where NaOH had an immediate adsorption on cellulose when mixed. As temperature drops, the adsorption fluctuated for a short time, then followed by a rapid adsorption. These three stages can be explained by the NaOH interaction with cellulose.

At stage 1, NaOH was mostly adsorbed on loosely ordered region. Although at this stage, the crystalline structure was almost intact, the NaOH and water molecules that made the less ordered region swollen will also affect the highly ordered crystalline region. We can imagine that a lot of small wedges are inserted into the less ordered region and the power and the quantity of these small wedges is related to NaOH and water amount in this region. Therefore, the initial NaOH concentration in solution is important in that it determines the initial alkali adsorption amount. Urea almost has no effect in this stage.

At stage 2, as temperature drooped below the freezing point of free water in the solution system, ice started to form, resulting in the increase of NaOH concentration in the liquid phase, and causing the increase of the adsorption of NaOH on cellulose fiber. Besides, NaOH hydrate becomes more stiffer at lower temperature, thus more easily penetrate crystalline cellulose. Moreover, the crystal growth in free water induced a rapid volume expansion that can break hydrogen bonds and separate cellulose molecules in the highly ordered region. The addition of urea can reduce the Gibbs free energy for cellulose decrystallization. As NaOH gradually entered the crystalline part, cellulose crystallinity quickly decreased. An intermediate structure of Na-cellulose I with larger unit cell is formed and this structure will change to cellulose II with smaller unit cell after washing and drying.

At stage 3, all the cellulose I has been changed to Na-cellulose I although still a major amount of cellulose are not dissolved. The solubility of cellulose does not

further increase at this stage. One possible explanation of the ceiling solubility is the gelation of cellulose solution at thawing process. This also suggests that the cellulose solution in sodium hydroxide in the studied condition is not a true molecular dispersion. Association of crystalline structure of cellulose still exist. The gelation might be formed by entangling the associates, and preventing more cellulose associates being dispersed into the solvent. The higher the cellulose molecular weight, the larger possibility of the existence of this crystalline associates and the larger possibility of gelation. At this stage, urea probably could delay the gelation.

## ***2.5 Conclusions***

To achieve the best dissolution degree, multiple parameters, including cooling bath temperature, initial NaOH concentration, urea addition and concentration, need to be considered simultaneously and optimized for each cellulose origin. The dissolution of cellulose in NaOH/urea solution has strong dependence on the temperature of reaction. There is a critical temperature for reaction to take place in the studied solution system. The addition of urea in our system is not very significant comparing with sodium hydroxide alone. The optimum NaOH concentration in this study is at 6% for high molecular weight cotton linter. The Gibbs free energy of cellulose decrystallization in 6% NaOH with and without urea had a turn point at temperature  $-15^{\circ}\text{C}$ . Above and below this temperature, the contribution of adding urea to enhance the solubility of cellulose is opposite.

## CHAPTER III

# ENZYMATIC HYDROLYSIS AIDED COTTON DISSOLUTION

As found in Chapter of *Dissolution Kinetics*, the cellulose solubility in NaOH solution with or without urea is only 20 ~ 30%. The solubility is hard to increase without an appropriate pretreatment. In this chapter, enzymatic hydrolysis was used to pretreat cotton linters. Its effect on cellulose dissolution was discussed.

### 3.1 *Introduction*

Cellulose has basic molecular unit of  $C_6H_{10}O_5$  and is linked in the form of  $\beta - 1, 4 -$  glucan. Each chain unit of cellulose contains three hydroxyl groups which make cellulose a hydrophilic material. However, cellulose is hard to be dissolved in aqueous solutions due to the existing of large quantities of inter- and intra-molecular hydrogen bonds and considerable van der Waals forces between the non-polar groups.

Cellulose is a material that does not melt at the temperature lower than its degradation temperature. Strong intra- and inter-molecular hydrogen bonds in cellulose prevent its molecules from dissolution in most common solvents. Although there are a number of approaches to produce regenerated cellulose, such as viscose rayon, cuprammonium cellulose, Lyocell fibers, the market is shrinking due to the environmental and economic feasibility concerns of these methods. Although other processes, such as using organosolvents and ionic liquid can dissolve cellulose, the high cost and organic solvent recovery problems hinder their further applications in large scale. For these reasons, cellulosic materials are regarded as un-moldable materials. Because of the un-moldable identity, wood and cotton fibers are difficult to be refabricated as

other thermosetting and thermoplastic polymers. If an effective, economic and environmentally friendly cellulose dissolution method can be developed, a new platform for producing moldable cellulosic intermediate materials will be created, which provides new opportunities for using cellulosic materials as a renewable and sustainable engineering polymers.

Sodium hydroxide is a simple chemical that can swell cellulose in a certain concentration, and even can dissolve cellulose at high NaOH concentration. The dissolution mechanism is that soda hydrates can penetrate the amorphous area of cellulose, and can also destruct the neighboring crystalline regions. It was found that for low to moderate degree of polymerization (DP) of cellulose, the maximal solubility occurs with 8 ~ 10% soda solution. However, NaOH alone has never been used as a solvent for dissolving cellulose in industrial applications because of the chemical degradation of cellulose, high soda concentration and chemical recovery problems. Cellulose dissolution in aqueous NaOH was studied with particular attention on the roles of crystalline form and molecular weight in other's works[48].

In recent years, researchers found that sodium hydroxide with urea at cold temperature can dissolve cellulose better than sodium hydroxide alone. Zhang's research group investigated the cellulose dissolution chemistry in NaOH/urea solution, and found that certain compositions of NaOH/urea and NaOH/thiourea are good solvents for cellulose [12, 131, 137, 136, 138, 139, 134]. According to their research, the sodium hydroxide and urea aqueous solutions are non-derivatizing solvents for cellulose to form true solution. The proposed mechanism is that NaOH hydrates - urea hydrates - free water - cellulose form a special complex in the solution. NaOH destroys inter- and intra- hydrogen bonds between cellulose molecules and urea hydrates function as hydrogen bonds donor and receptor between solvent molecules and prevent the reassociation of cellulose molecules, thus leading to molecular dissolution of cellulose. Laszkiewicz's [66], Isogai and Atalla's[48] and Zhou et al.'s works

[134, 136] suggested that there exists an upper DP limit of cellulose for each solution system, and those cellulose with a higher DP value beyond the corresponding upper limit can not be dissolved in the solution.

To make cellulose fiber more accessible for NaOH/urea dissolution, a pretreatment is necessary. There are a variety of methods of pretreatment: physically, such as ball-milling[1, 72, 112], high pressure steaming[98], extrusion, expansion, and high energy radiation[54]; chemically, for example, alkali[101], acids, gases, oxidizing agents, cellulose solvents; biologically, enzymes or fungi[120].

Enzymatic hydrolysis on cellulosic materials is widely studied[9, 17, 14, 15, 16, 18, 129, 123, 133, 7, 8, 28, 37]. The effects of all kinds of pretreatments on enzymatic hydrolysis were investigated[69, 113, 67]. Physicochemical properties of chemically and enzymatically modified cellulosic surfaces was studied by Buschle[11].

The enzyme that can degrade cellulose is called cellulase. Cellulase, an enzyme, can be used to cut high molecular weight cellulose. Bhat [9] did a comprehensive review on cellulose degrading enzymes and their potential industrial application. The effect of *Trichoderma* cellulases on the fine structure of a bleached softwood kraft pulp was studied by Ramos et al. [104, 103]. All the enzymes used in their study, including Celluclast, have high hemicellulose activity and can remove most of the residual hemicellulose from softwood kraft pulp. Celluclast works on crystalline cellulose by the synergistic action of endoglucanases and exoglucanases. The endoglucanases randomly locate surface reaction sites on cellulose, create a new reducing and non-reducing chain end by inserting a water molecule in the  $\beta - (1, 4)$  bond. Meanwhile exoglucannases reacts on cellobiose to reduce the end product inhibition[42]. In the process of enzyme hydrolysis, the reduction of cellulose DP can only be observed at the early stages induced by a random attack of endoglucanases at amorphous regions. Cellobiohydrolases (CBHs) can remove cellobiose residues from the end of cellulose

chains, therefore, can gradually, but only slightly, decrease the degree of polymerization. Ramos and his coworkers didn't find much effect of enzyme treatment on cellulose crystallinity, but they suggested that the crystallinity change can be observed after longer incubation time. The enzyme prefers to attack the crystalline at (002) diffraction plane [15], so it can be applied to cut the length of crystal.

It is well known that molecular weight, crystal size and crystal packing pattern will affect the dissolution of polymer in a solution. However, it is not clear if the molecular weight and crystal properties have the same effect on the dissolution using NaOH alone and NaOH/urea solution. It is also unknown how high the dissolution degree can be achieved with NaOH/urea solution after enzymatic pretreatment. In this study, enzyme was utilized as a tool to tailor cellulose molecules to the desired molecular weight to facilitate their dissolution in NaOH/urea solution. We will also show the advantages of the two-stage cotton fibers dissolution using enzyme and NaOH/urea solution. The comparisons with NaOH/urea treatment and NaOH treatment alone will be discussed.

## **3.2 *Experimental***

### **3.2.1 Materials and Chemicals**

The cellulose material used in this study were cotton fibers(Procter & Gamble Company). Celluclast 1.5 L enzyme is a gift from Novozymes North America, Inc. It has 45 filter paper units (FPU) and 6.5 units of  $\beta$ -glucosidase per gram of liquid and a density of 1.1989 g/ml. Urea, in crystal form, is from Aldrich and NaOH in 50% solution is from VWR. The chemicals were used without further treatments.

### **3.2.2 Sample Treatments**

#### **1. NaOH treatment**

For NaOH treatment, the cotton linters were mixed with various concentrations of NaOH aqueous solutions to make a 2% slurry at room temperature, and then

put into freezer at  $-15^{\circ}\text{C}$  for 72 hours. After the samples were solid frozen, they were taken out from the freezer and stirred at room temperature for 10 minutes after the thaw of the solid. The dissolved part and undissolved part were separated by centrifuge at 7000 rpm. The residue part was washed with deionized (DI) water adequately and then oven dried and stored in a desiccator. The dissolved part was regenerated by neutralizing alkaline with hydrochloric acid, then oven dried and weighted.

## 2. NaOH/Urea treatment

For NaOH/urea treatment, the cotton linters were mixed with 6%NaOH and 4% urea at room temperature to make a 2% cellulose slurry, then put into freezer at  $-15^{\circ}\text{C}$  for various time. The frozen samples were taken out and subjected to the same treatment as in NaOH treatment.

## 3. Celluclast treatment followed by NaOH/Urea

Cotton linters were used in enzymatic pretreatment and dissolution experiments. The linters were first mixed with pH 4.5 acetate buffer to make a 5% slurry, then pre-heated to  $50^{\circ}\text{C}$  through water bath, after that, celluclast was added at the dosage of 0.4 ml/g substrate. The reaction was carried out with mixing under the speed of 200rpm. The treating time was varied from 0.5 hr to 4 hours. After the treatment, the reaction was immediately stopped by submerging into boiling water and followed by filtration and extensive washing with DI water. The washed samples were vacuum oven dried for 48 hours at  $60^{\circ}\text{C}$ . Then the samples were stored in a desiccator and were subjected to NaOH/urea treatment later.

### 3.2.3 Characterization

- Dissolution Degree of Cellulose

The dissolution degree of cellulose was defined as the weight of dissolved cellulose (the difference between the original sample weight and the undissolved residue) divided by original oven dried weight of cellulose(Equation 6).

- Degree of polymerization of Cellulose Residue

The molecular weight of cellulose residue was calculated through the correlation between DP and viscosity using the equation[47]

$$DP^{0.905} = 0.75[\eta] \quad (11)$$

and the viscosity was measured using Tappi Test Method T230 om-94[97].

- Morphology Change of Cellulose Residue

Morphology change was observed by polarized microscope.

- Crystallinity and crystal size of Cellulose Residue

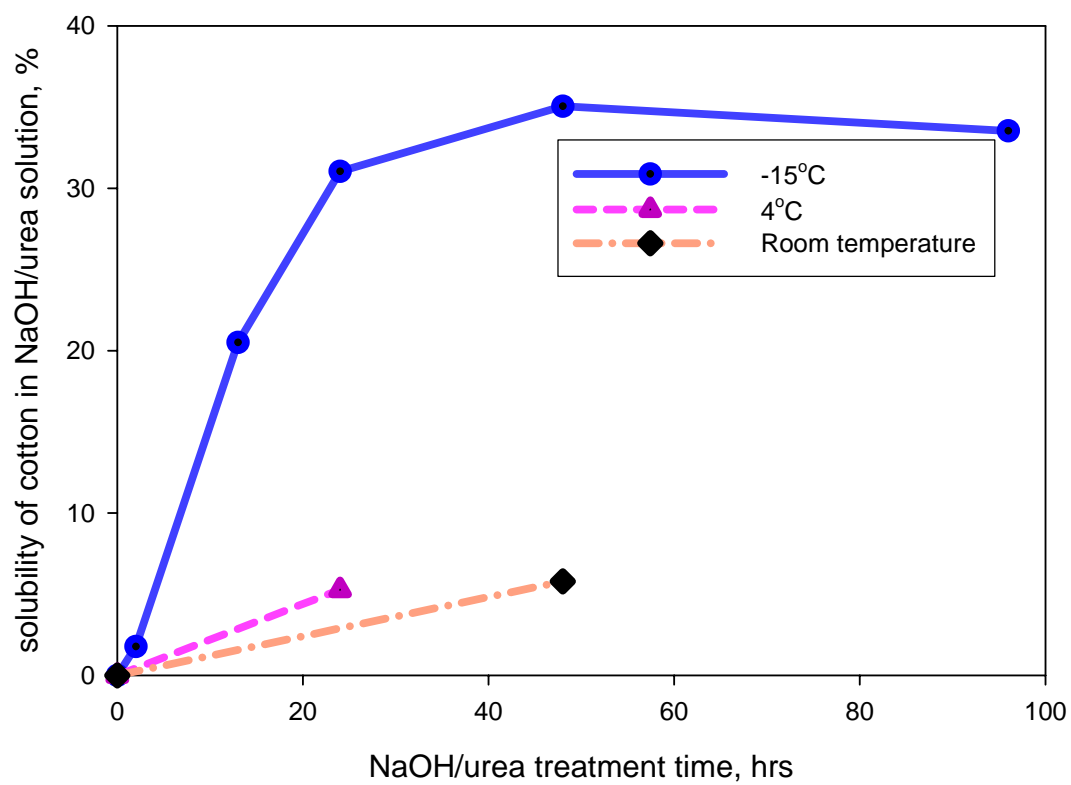
The crystallinity and crystal size were characterized using Powder X Ray Diffraction. The bragg angle was scanned from 5 to 40°. The X-Ray source is  $CuK\alpha$  with wave length of 0.154nm.

### ***3.3 Characterizations and Discussions***

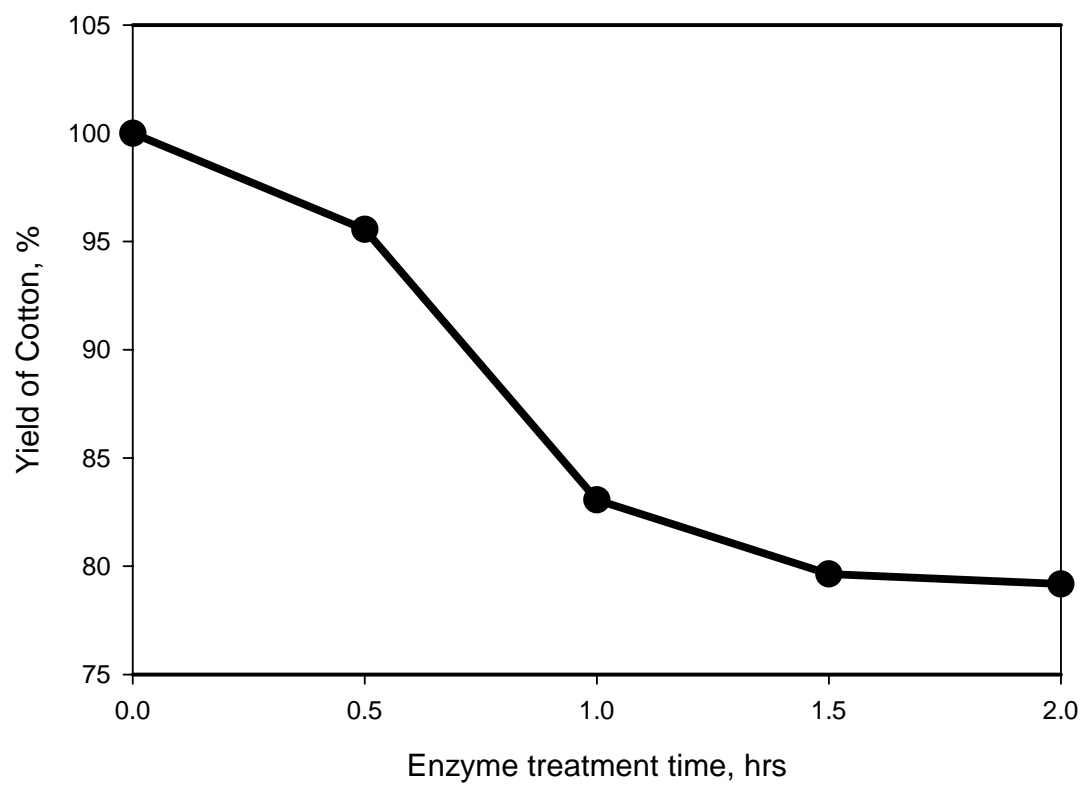
#### **3.3.1 Solubility**

Isogai and Atalla [48] reported that 9% NaOH solution could dissolve cellulose with DP lower than 200. Zhang's group claimed that cotton linters with DP lower than 400 can be rapidly dissolved in 6%NaOH/4%urea within 5 minutes after freezing for 12 hours [12]. However, for the common wood fibers and cotton linters which have much higher DP, i.e. above 800, considerable amount of cotton residues were observed by naked eyes after NaOH/urea treatment. The solubility of the cotton linter (DP 850) was shown in Figure 44.

Figure 44 is the effects of treating time and temperature on the solubility of cotton linter in NaOH/urea solution. After 24 hours treatment by soaking the cotton liner

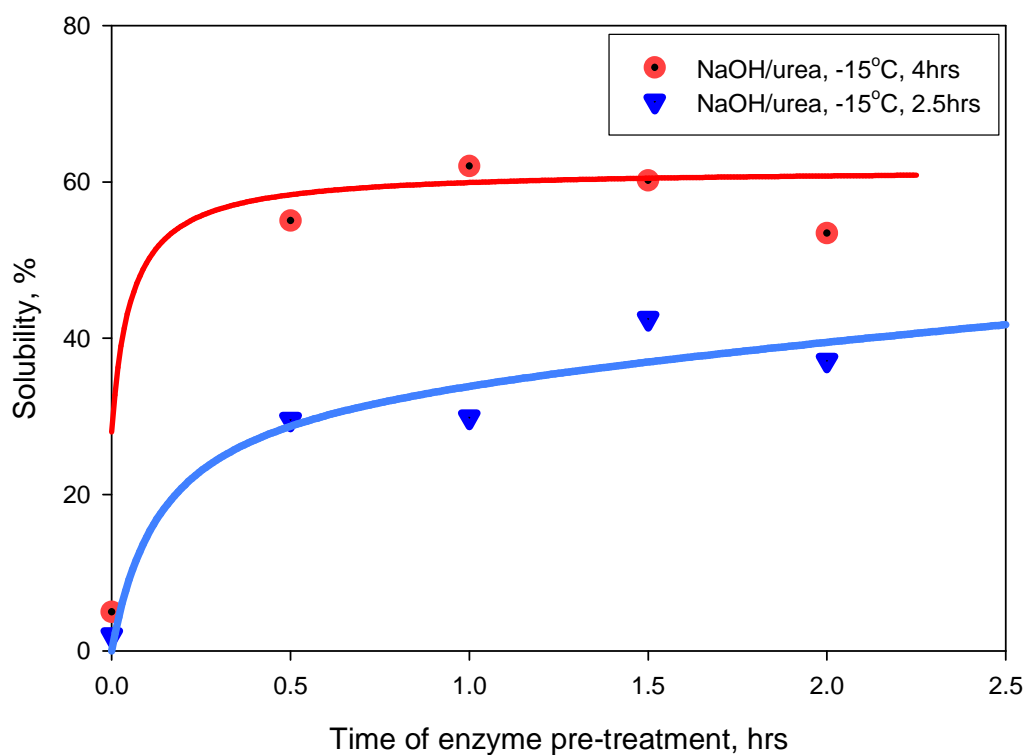


**Figure 44:** Solubility of cotton in NaOH/urea solution



**Figure 45:** Yield of Enzyme Hydrolysis

(DP of 790) samples in the solutions at different temperatures, the solubility is only about 3 percent for room temperature treatment and 5 percent for 4°C temperature treatment, while at  $-15^{\circ}\text{C}$ , the solubility was dramatically enhanced to 31 percent. A significant effect of soaking time on the dissolution degree was also observed for NaOH/urea up to 48 hours treatment at  $-15^{\circ}\text{C}$ . However, the longer treatment time beyond 48 hours could not further improve the solubility, which suggests the up limitation of the dissolution under this condition. The highest solubility obtained in 6%NaOH/4%urea solution is 35%, which means 65% of cotton linters were undissolved. The limit of the dissolution degree may be due to crystallinity, crystal size, and DP of cellulose. In this study, various DPs of cellulose were obtained by enzyme pretreatment of cotton linter.



**Figure 46:** Solubility of Enzyme Pretreated Cotton Linter in NaOH/urea Solution as a Function of Enzymatic Hydrolysis Time.

The yield of cellulose after enzyme hydrolysis was measured by the weight loss of cotton linter. The data were shown in Figure 45. The yield dropped quickly to 82 percent during the first hour of treatment, then went to a much slower rate, suggesting that there are two different underlying mechanisms. The yield was about 95 percent after half an hour treatment.

The effects of enzyme pre-treatment on the solubility of cellulose in NaOH/urea solution were studied and shown in Figure 46. In 2.5 hours and 4 hours of NaOH/urea treatments, the solubility increased from around an estimated 5 percent to 30 percent and 60 percent respectively. The estimated 5 percent, which was read from Figure 44, was based on the same NaOH/urea treatment time both for enzyme pretreated and un-pretreated samples. The maximal solubility was increased from 30% to 65%. The experiment results showed that, the solubility dramatically enhanced with enzyme pretreatment and the dissolution time largely shortened comparing with the data without enzyme pretreatment. It was also shown that it was also shown that cellulose solubility was increased rapidly during the first hour of enzymatic pretreatment, and then changed slowly up to 2 hrs of treatment time. The reason for reaching the maximum solubility in a very short pretreatment time is not clear.

### **3.3.2 Morphology Change**

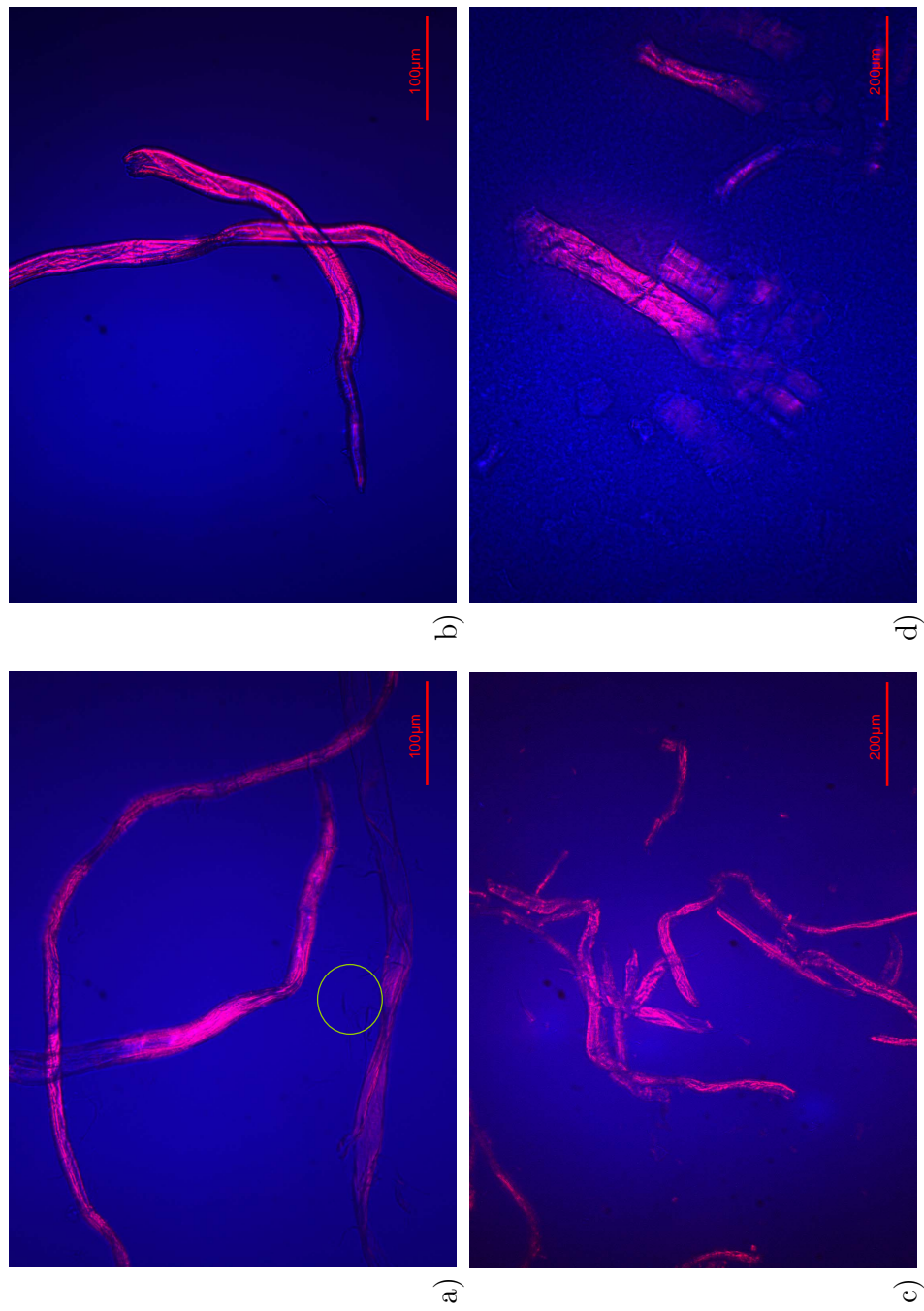
Figure 47 shows the morphology change of cotton linter due to enzyme and NaOH/urea treatment observed by polarized optical microscope. Enzyme treatment did not change the shape of fiber except smoothening the fiber surface by removing fibrillars (shown in Figure 47a and b) and shortening the fiber length. However, the treatment of the mixture of cotton linter and NaOH/urea solution at frozen temperature remarkable changed the fiber shape and size. Clearly, the freezing treatment of cotton linter and NaOH/urea solution still plays a dominating role in the fiber dissolution even after enzyme pretreatment, as compared in Figure 47c and d. The fibers were

highly swollen and most of the cellulose crystals have diminished (Figure 47d).

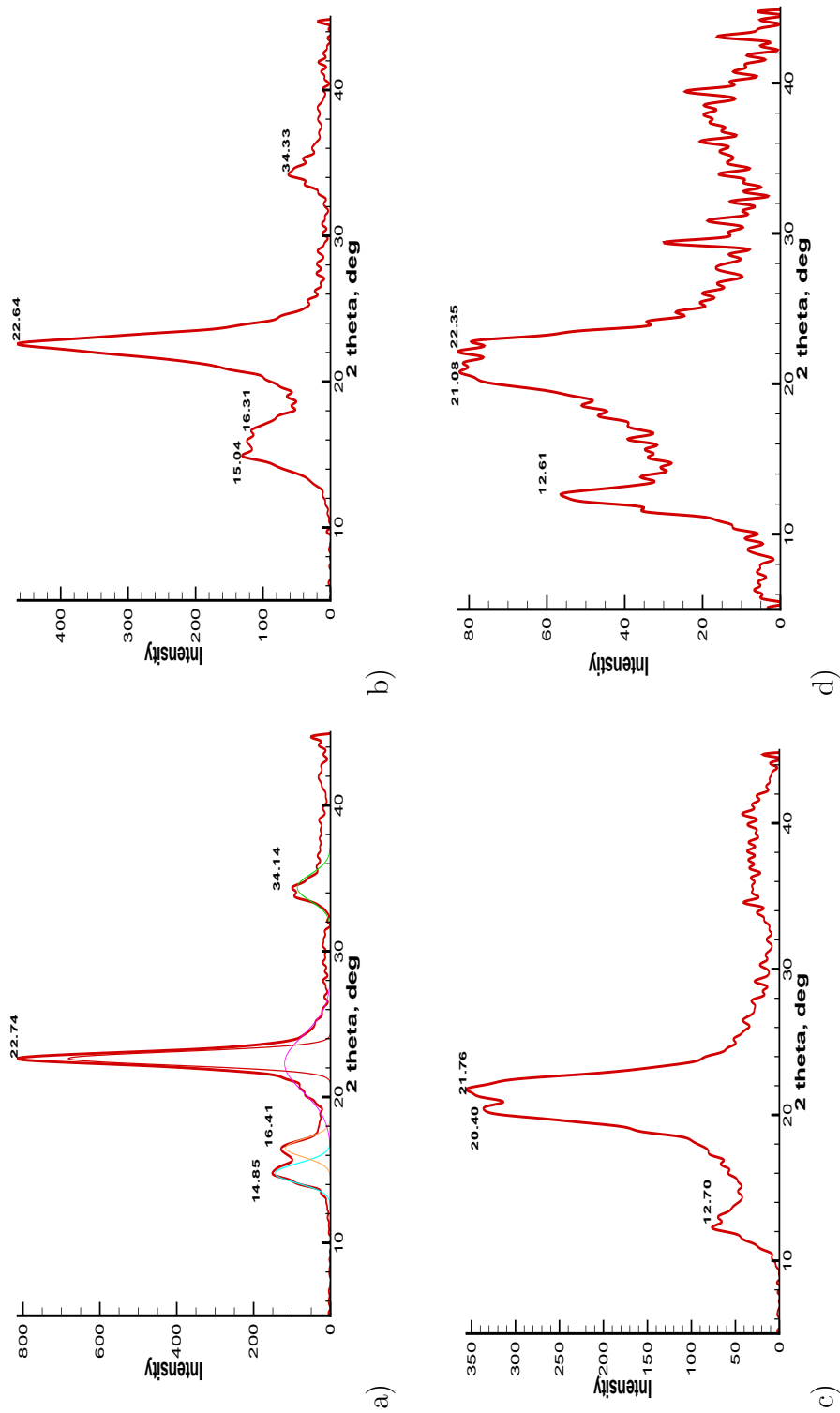
### **3.3.3 Crystallinity and Crystal Size**

Crystallinity obtained from Powder X Ray Diffraction data is calculated according to Martin and Segal method[116]. This method is fast and easy. It uses the height of (200) peak and the minimum between (200) and (110) peaks, assuming that Intensity of (200) represents both crystalline and amorphous part while the minimum intensity at the mentioned location is for amorphous part only.

Commercial cellulose fibrous from Sigma was used as standards to test the repeatability and accuracy of crystallinity analysis by XRD. Good repeatability was obtained with crystallinity of  $93.3\% \pm 0.2\%$ .



**Figure 47:** Morphology change of cotton fibers. a) cotton linter b) enzyme treatment c) enzyme pretreatment followed by NaOH/urea treatment at room temperature and d) enzyme pretreatment followed by NaOH/urea treatment at cold temperature



**Figure 48:** XRD of Samples. (a) is standard cellulose from Sigma; (b) is enzyme treated sample; (c) and (d) are undissolved and regenerated cellulose from NaOH/urea solution, respectively.

Figure 48 was XRD spectra of pure Sigma cellulose (a), enzyme treated bleached kraft softwood fiber (b), undissolved (c) and regenerated bleached kraft softwood fiber (d) from direct treatment of NaOH/urea solution. Pure Sigma cellulose is in cellulose I crystal type, same as native cellulose, and enzyme treatment did not change the crystal type, however after NaOH/urea treatment, the crystal type changed from cellulose I to cellulose II for both the undissolved residue and the regenerated cellulose. The crystal size was calculated using Scherrer equation (Equation 12) [85].

The crystal size was calculated using Scherrer equation[85]

$$L(hkl) = \frac{k\lambda}{B\cos\theta} \quad (12)$$

where  $L$  is the crystal dimension at the  $(hkl)$  lattice plane of diffraction,  $\lambda$  is the wavelength of X ray,  $k$  is Scherrer's constant, and  $B$  is the half width of peak.

The DP and crystallinity index of cellulose from different sources and their solubility in NaOH/urea solution were summarized in Table 9. It can be seen from Table 9 that pure Sigma cellulose, which has the lowest DP and the highest crystallinity index, gave the highest solubility among all the samples. Enzyme treatment did not obviously decrease the crystallinity of cotton linter, but did improve the solubility significantly. Ramos [103] also found that for high molecular weight wood fiber, enzyme treatment did not obviously affect crystallinity. Based on the results of our experiments, it can be concluded that DP of cellulose plays a more important role in cellulose dissolution in NaOH/urea solution than cellulose crystallinity does, and cellulose with high crystallinity does not necessarily lead to low solubility.

The XRD spectra of assorted treated cotton linters were shown in Figure 49, where we can see that for enzyme treated sample, the basic shape of spectra in a) did not change except a slight increase of the intensity of the (200) peak, corresponding to a slight increase of crystallinity. This can be explained by that the endoglucanase component in Celluclast attacks the amorphous area of cellulose, leaving this area

**Table 9:** Crystallinity & solubility in NaOH/urea  
Crystallinity of different fibers and their solubility in the NaOH/urea solution

Fiber	CrI	Solubility
Cotton linter	95.4	20% – 30%
Bleached SW fiber	88.8	20% – 30%
Sigma cellulose	97.3	> 90%
Enzyme treated cellulose	95.8-96.2	> 70%
Undissolved cellulose from NaOH/urea solution	94.3	
Regenerated cellulose from NaOH/urea solution	74.7	

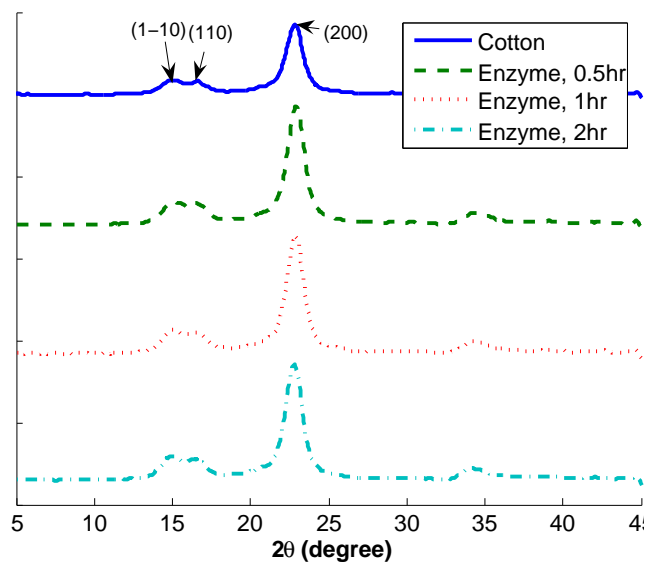
**Table 10:** Crystallinity and crystal size  
Crystallinity and crystal size change of cotton with enzyme treatment

Samples	d-spacing (nm)			Crystallite dimensions (nm)			CrI
	(1 $\bar{1}$ 0)	(110)	(200)	(1 $\bar{1}$ 0)	(110)	(200)	
Cotton	0.592	0.532	0.389	5.44	5.45	6.97	95.4
Enz 0.5hr	0.586	0.529	0.387	5.22	5.76	6.54	96.2
Enz 1hr	0.593	0.534	0.389	5.32	5.33	7.47	95.9
Enz 1.5hr	0.595	0.535	0.391	5.45	5.46	6.82	96.1
Enz 2.0hr	0.595	0.535	0.390	5.28	5.29	6.82	95.8
6% NaOH –15°C, 72hr	0.722	0.436	0.402	3.11	4.61	4.96	
14% NaOH –15°C, 72hr	0.722	0.442	0.406	4.62	5.76	5.34	
NaOH/urea, –15°C, 5.5hr	0.589	0.531	0.388	4.90	6.22	6.28	94.3
NaOH/urea, –15°C, 2hr	0.594	0.537	0.389	4.60	3.50	6.80	

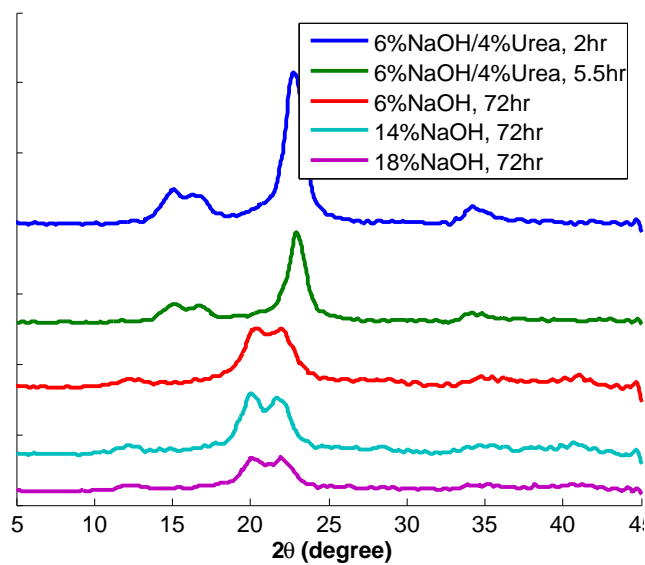
exposed for CBHs to react, but this process is relatively slow so that we can only observe small increase of the crystallinity after two hours of enzymatic treatment (See Table 10). The effect of enzyme treatment on NaOH and NaOH/urea treated cotton residues was investigated and no crystal type change was found as shown in Figure 49a, comparing with NaOH and NaOH/urea treated cotton residues. For short time NaOH/urea treatments, crystal type change was not observed. However, for the samples under 72 hours of treatments by different concentrations of NaOH, the crystal type change from cellulose I to cellulose II was observed and the peaks shifted to lower Bragg angles, suggesting that the crystallite sizes decreased with the NaOH treatments. The calculated data were summarized in Table 10, showing the d-spacing of crystal lattices, crystal dimensions perpendicular to (10), (110) and (200) lattice planes and the crystallinity index for cotton linters. The d-spacing and crystal dimension of these diffraction planes did not change for enzyme treated cotton linter and also for NaOH/urea treatment of up to 5.5 hours. As a comparison, for 6% and 14% NaOH freezing treatment after 72 hours, d-spacing changed from 0.592, 0.532 and 0.389 to 0.722, 0.436 and 0.402 for cotton treated by 6% NaOH, suggesting that crystal type has changed from cellulose I to cellulose II. Meanwhile, crystal size apparently decreased.

### 3.3.4 Degree of Polymerization

Comparing with enzyme treatment, the effects of NaOH and NaOH/urea treatment on DP of cellulose were also investigated (Figure 50b and c). The time required to reduce cellulose DP to 650 by the enzyme treatment was 2 hrs. However, it took 24 hours for the NaOH/urea approach to achieve the same deduction reduction of cellulose DP. This showed that cellulose DP reduction by enzyme treatment was much faster than that by NaOH/urea treatment. The effect of NaOH concentration on cellulose DP was shown in Figure 50c. With higher NaOH concentration, greater



a) Enzyme Treatment



b) NaOH, NaOH/urea, NaOH-enzyme and NaOH/urea-enzyme treatment

**Figure 49:** Crystal structure change of cotton linter prepared by a) enzyme treatment b) NaOH, NaOH/urea, NaOH-enzyme and NaOH/urea-enzyme treatment

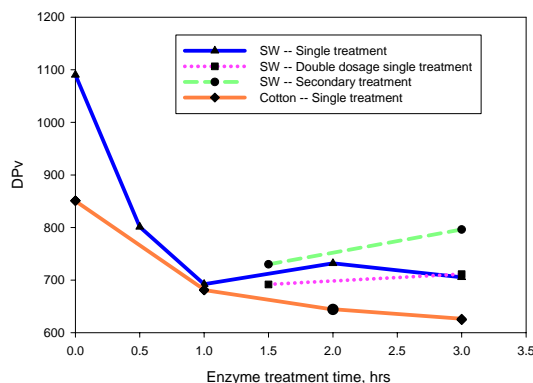
cellulose degradation was observed.

The effect of molecular weight on the solubility of is shown in Figure 50d. It is shown that the solubility decreased as the degree of the polymerization increased for the cellulose treated by NaOH/urea solution for 2.5 and 4 hours.

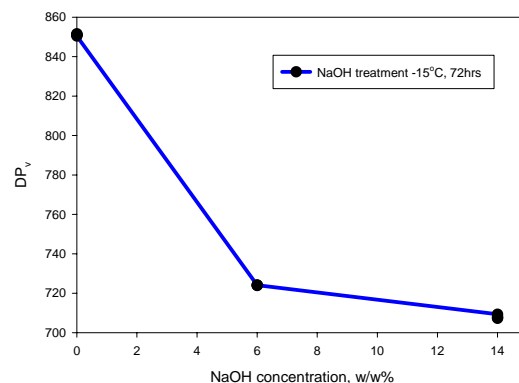
Although both enzyme and NaOH/urea treatments can degrade cellulose, their roles in the two step treatment are different. Cellulase attacks the cellulose crystal from (002) lattice plane, cutting cellulose molecules. This effect is dominant especially at the initial stage of enzyme treatment, which is shown in Figure 50a. NaOH/urea solution interacts with cellulose crystal to break the inter- and intra- cellulose hydrogen bonds to obtain individual cellulose molecules. The enzyme pretreatment reduces the length of cellulose crystal, thus let it be easily penetrated by NaOH and urea solution.

### ***3.4 Conclusions***

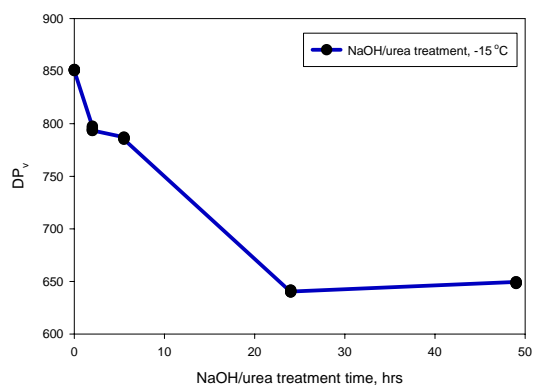
Enzyme pretreatment greatly enhanced the dissolution degree of cellulose in NaOH/urea solution with much shorter time. Enzyme treatments did not change crystal type and crystal size, slightly increased crystallinity of cellulose, but reduced the molecular weight rapidly. High crystallinity did not necessarily result in low solubility, or at least crystallinity alone could not explain the difficulty of cellulose dissolution.



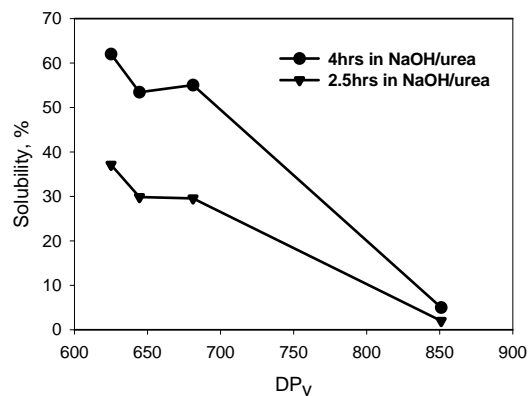
a)



b)



c)



d)

**Figure 50:** DP changes with (a) various time of enzyme treatment at the dosage of 0.4 ml enzyme per gram of oven dried cotton fiber (b) various time of 6%NaOH/4%urea treatment at 2% solid contents of cotton fiber in solution (c) various concentrations of NaOH treatment at 2% solid contents of cotton fiber in solution for 72 hours (d) the solubility changes in 6% NaOH/4%urea solution as a function of degree of polymerization tailored by enzyme pretreatment as in (a).

## CHAPTER IV

### HEMICELLULOSE AND LIGNIN EFFECTS

It has been proved that wood fibers are very difficult to be dissolved in NaOH solution with or without urea. The main reason is in the cellulose structure itself, including the tight packing of crystalline cellulose. Other than that, the influence of two other major wood fiber components, i.e., hemicellulose and lignin, can not be neglected. In this part, unbleached southern pine was treated to obtain different lignin and hemicellulose contents in order to investigate their effects in cellulose dissolution.

#### **4.1 *Introduction***

The majority source of cellulose is from wood, thus it is indispensable to study the dissolution of wood as whole instead of separated cellulose. The main components in both hardwoods and softwoods are cellulose (45%), hemicellulose (35% in hardwoods and 25% in softwoods) and lignin (21% in hardwoods and 25% in softwoods) [118]. Cellulose has been reviewed thoroughly in the first chapter. A brief review of hemicellulose and lignin will be given in the following section.

##### **4.1.1 Hemicellulose**

The predominant hardwood hemicelluloses are partially acetylated acidic xylans, a small percentage of hardwood is also composed of mannan. The predominant softwood hemicelluloses are galactoglucomannans.

Hemicelluloses are polymers with much shorter chains compared to cellulose, with a degree of polymerization of 80~200 comparing with 600~3500 for cellulose. Hemicellulose interacts with cellulose and through hydrogen bonds and covalent bonds

respectively. Their general formulae are  $(C_5H_8O_4)_n$  and  $(C_6H_{10}O_5)_n$  and their chemical elements, besides glucose, include two other hexoses – mannose and galactose, and two pentoses – xylose and arabinose. The structures of these hemicelluloses vary a lot. These nonglucose units often display distinctly different reactivities from the glucose residue because of their different ring structures or hydroxyl configurations. They are generally more reactive than cellulose and can be selectively removed from cellulosic substrates. Most studies on the reaction of wood hemicelluloses, except for structural analysis, were largely associated with the delignification or biomass component separation process.

Although hemicelluloses occurring in plant tissues are likely to be amorphous[121], they, like cellulose, are capable of forming strong hydrogen bonds and have a tendency to crystallize after certain degradation resulting in removal of side chain units. The presence of strong hydrogen bonding between acetyl and the neighboring hydroxyl groups were thought to be a significant factor in reducing the accessibility of xylan[124].

Xylan in solution has a strong tendency to deposit on the surface of cellulose fibers as a result of strong hydrogen bonding. In alkaline pulping, a significant portion of the residual xylan was very resistant to alkaline extraction or heterogeneous acid hydrolysis. It was suspected that the adsorbed xylan, being low in uronic acid content, might have cocrystallized on the cellulose surface[128]. It was suggested that the O-3H...O5' intramolecular hydrogen bond occurring in cellulose was probably also present in the xylan[74]. The presence of galactose side chains in galactoglucomannans certainly would contribute to their water solubility and probably prevent them from aligning together to form strong hydrogen bonds. Partially degraded glucomannans containing few side chains are capable of crystallizing. The true mannan is known to coexist of two crystalline forms, I and II[34, 84]. Both are stabilized by intra- and inter-molecular hydrogen bonds. A similar O-3H...O5' intramolecular hydrogen bond

was observed in both mannan and cellulose.

A considerable amount of hemicelluloses are lost during chemical pulping process because of its easy degradability. Hemicellulose can be isolated from lignocellulosic matrix by alkaline hydrolysis of ester linkages.

#### **4.1.2 Lignin**

Lignin exist mainly in the middle lamella of wood fibers, functioning as glue to hold fibers together. Some lignin also exist in the cross-section of fiber. Lignin has a three dimensional network structure.

Lignin usually plays a negative role in the chemical utilization of lignoclelulosic materials and must be modified, partially degraded, or completely removed depending on the end uses of the final products. Lignin appears to be amorphous, occurring in plant tissues or in isolation forms, and like cellulose and hemicelluloses has a high tendency to form hydrogen bonds. Although the approximate contents of major lignin linkages are now well understood, the chemical structure of lignin still cannot be precisely defined.

After chemical pulping process, the obtained pulp still have roughly hemicellulose (20%) and lignin (less than 5%) without bleaching. The effects of this chemical components on the overall solubility of cellulose fiber are not well studied.

In this chapter, this chemical effects of solubility in NaOH/urea solution are studied by varying the lignin content of softwood pulp. Solubility of cellulose are calculated after accounting in the weight losses of hemicellulose and lignin during the sample preparation processes.

The characterization and the utilization of hemicellulose and lignin are of high interest to many researchers[22, 68, 70, 38]. The influence of hemicellulose and lignin on cellulose dissolution or degradation were studied by many researchers[39, 22, 82].

### 4.1.3 The Basics of HPLC

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, molecules being analyzed, and the solvent(s) used. With a ultraviolet(UV) detector and a standard sugar monomer curve, the sugar components of an Polysaccharide sample can be determined.

## 4.2 *Experimental*

### 4.2.1 Materials and Chemicals

Raw materials (Sample I) was unbleached pulp from southern pine with lignin content of 3.88%. Sodium chlorite and sodium acetate were used as bleaching chemicals.

### 4.2.2 Sample preparation

Two 20 g oven dried pulp were weighted, put into 500 ml flasks, then added 250 ml preprepared sodium chlorite solution (60 g/l sodium chlorite and 60 g/l sodium acetate), soaked for three days (Sample II) and six days (Sample III) respectively. Then the samples were washed with DI water for several times, followed by extraction with 0.15 N NaOH twice, and again washed with DI water thoroughly. After that, the samples were freeze-dried and stored for further experiments.

The dissolution experiments were done in 6%NaOH/4%urea at cooling bath temperature of  $-15^{\circ}\text{C}$  with same procedure as in kinetics study.

### 4.2.3 Characterization

#### 4.2.3.1 *Water Retention*

Canadian freeness tests were carried out to check the water retention capability change induced by bleaching process.

#### 4.2.3.2 *Lignin and Sugar Contents*

High-performance liquid chromatography (HPLC) with Ultraviolet spectroscopy detector was used to quantify the carbohydrate components of the samples.

Chemical components of pulp were characterized by HPLC. Weight  $0.175g \pm 0.001g$  of sample I, II and III pulp into 50 ml digestion tubes. Add 1.5 ml 72% sulfuric acid, occasionally stirring for 1 hour at 30°C, then diluted to 3% sulfuric acid by with addition of 42 ml of DI water. The sample was then autoclaved for one hour at 121 setting. Then filter through a 934-AH Whatman filter(24mm circle) with gooch crucible. The hydrolyzed solid residue were dried and weighted to calculate the lignin content. The filtrate was made up to 50 ml. The filter with lignin residue was dried and weighted. Pipette 1ml of aliquots and 1ml of 1mg/ml fucose into 25ml volumetric flasks, then make up with water. Standards of various concentrations are also prepared from dilution of stock standard mix.

#### 4.2.3.3 *Solubility*

The dissolution degree of cellulose was defined as the weight of dissolved cellulose (the difference between the original sample weight and the undissolved residue) divided by original oven dried weight of cellulose(Equation 6).

#### 4.2.3.4 *Molecular Weight*

The molecular weight of cellulose residue was calculated through the correlation between DP and viscosity using the Equation 11[47] and the viscosity was measured using Tappi Test Method T230 om-94[97].

### 4.3 Results and Discussions

#### 4.3.1 Observations

Figure 51 showed the appearance of samples I, II and III (left to right) after NaOH/urea treatment. There was clearly a huge transparency change, indicating the increase of dissolution degree. The color of samples changed from brown to white, indicating the removal of lignin due to the bleaching process.

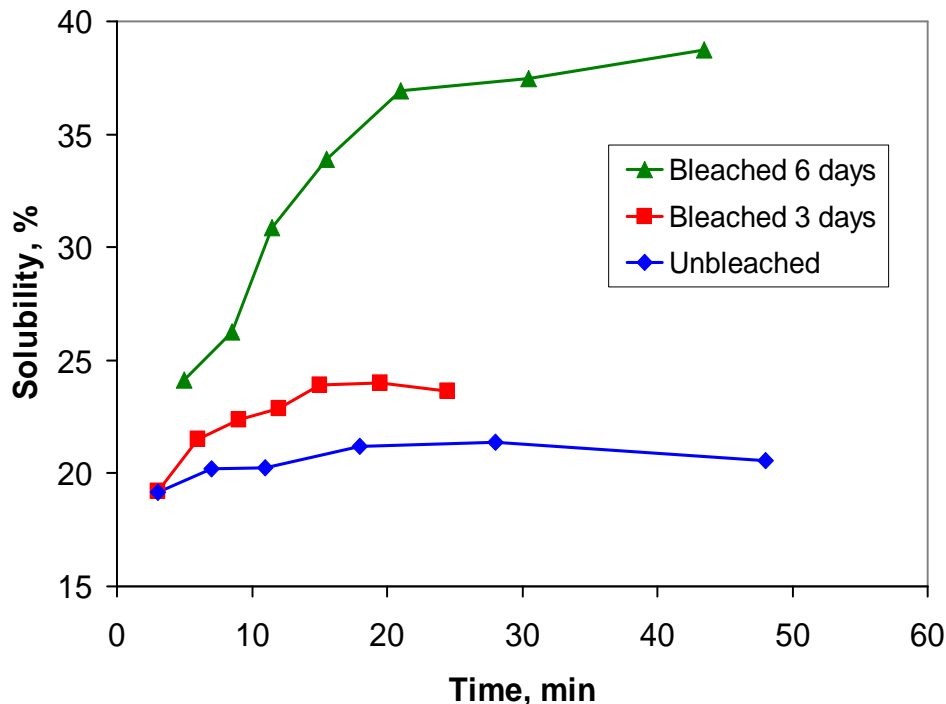


**Figure 51:** Picture of Various Lignin Content Samples Treated by NaOH/urea Solution, from left to right, sample without bleaching, bleached for 3 days and bleached for 6 days, respectively.

#### 4.3.2 Solubility Change

The overall solubility data was shown in Figure 52. For unbleached sample, the solubility was kept at around 20%, with negligible change. From sample I to sample II, solubility was increased from 20% to 23%; while from sample II to sample III, the solubility was significantly increased from 23% to about 40%. Different from the

solubility of cotton linter in alkali solution, the solubility of softwood sample started at a higher value of 20%. Sugar content tests were done to explain the reason.



**Figure 52:** Overall Weight Loss of Lignin Content Samples After NaOH/urea Treatment

#### 4.3.3 Sugar Component Change

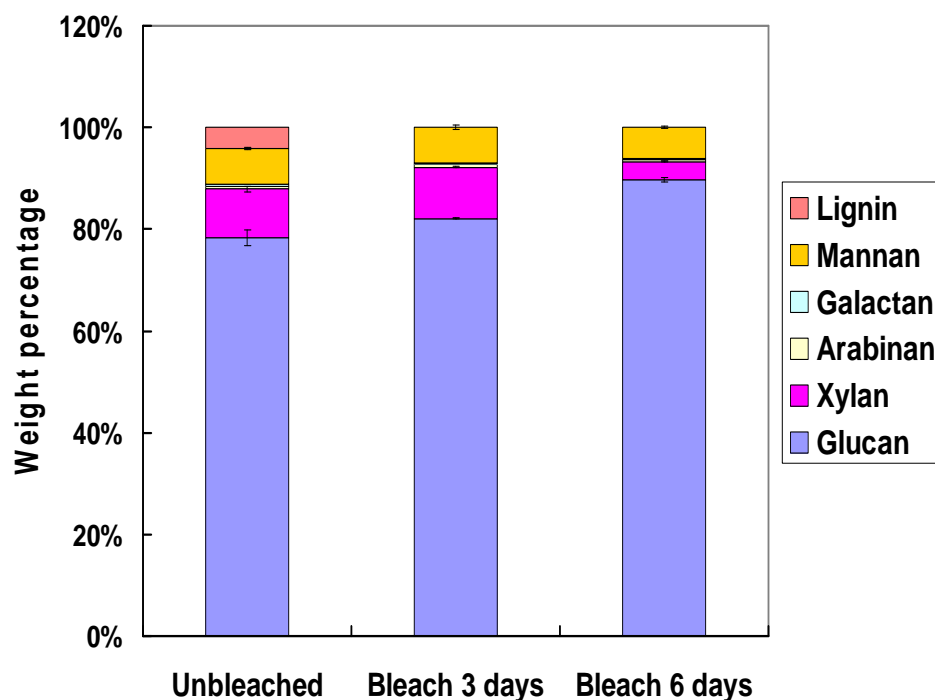
Table 11 listed sugar components of samples before and after bleaching. We can see that actually, all the lignin was removed from sample after bleaching treatments. So the huge increase of solubility from sample II to sample III was not due to lignin reduction, and the small change from sample I to sample II, indicating that the lignin influence, if any, is very small in this study. In order to show more clearly the contribution of each sugar content to the wood fiber, the relative contents were plotted in Figure 53. The sugar contents were all normalized to 100%, so that we can compare. For unbleached and 3-days bleached samples, the sum of the sugar contents excluding glucan (which is a basic unit for cellulose) is about 20%. This is about the

same as the solubility for sample I and initial solubility of sample II in Figure 52, indicating that the high initial solubility of softwood fiber is due to the hemicellulose which has major sugar contents of xylan and mannan. Also noticed from Figure 53 for sample III is that a considerable amount of xylan has been removed by bleaching process, which may contribute to the solubility increase by exposing cellulose for chemical access[1]. Mannan was almost intact in the bleaching process.

To understand the effect of the alkali solution on individual wood fiber components, the undissolved residue were brought to sugar component test. The results were graphed in Figure 54 for Glucan, and Figure 55 and Figure 56 for other components. Glucan has opposite change from xylan, mannan, Arabinan and Galactan in that its proportion increased while other four components decreased. This can be easily explained by the removal of the those minor components. Interestingly, the percentage decrease rate of xylan and mannan, which are two major components for hemicellulose, were very different. Xylan percentage in wood fiber quickly decrease during alkali dissolution, while the percentage of mannan sustained. Mannan survived in both the bleaching treatment and the alkali treatment. We don't know whether this is partly the reason for the low solubility of softwood in alkali solution. Future work need to be done.

**Table 11:** Sugar Components of Sample

Samples	Percentage of Sugar Components					
	Glucan	Xylan	Arabinan	Galactan	Mannan	Lignin
I	69.56	8.70	0.58	0.39	5.99	3.88
II	73.60	9.03	0.56	0.26	6.69	0
III	74.58	9.17	0.51	0.21	6.34	0



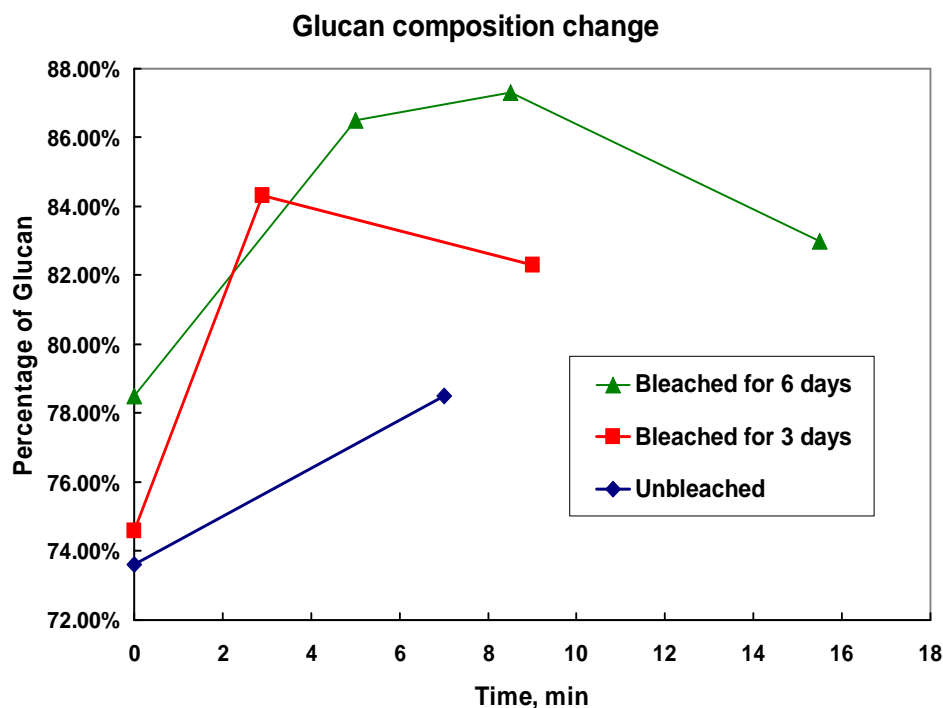
**Figure 53:** Sugar Components

#### 4.3.4 Water Retention Change

The bleaching treatment may affect the porosity of samples, and this may contribute to the solubility increase. This effect was tested by Canadian Standard Freeness. The result was shown in Figure 57. Surprisingly, there is almost no change between sample I, II and III, thus eliminating the porosity change as one reason for the solubility increase.

#### 4.3.5 Degree of Polymerization Change

Since we have shown that the DP value of fibers is a major factor for cellulose dissolution, the DP value of sample I, II and III were also checked using viscosity method. The result in Figure 58 showed great decrease of DP from sample I (1594) to sample III (642). And the DP value of sample III fell into the range of dissolution capability of 6%NaOH/4%urea. This DP drop may be the major reason of the huge increase

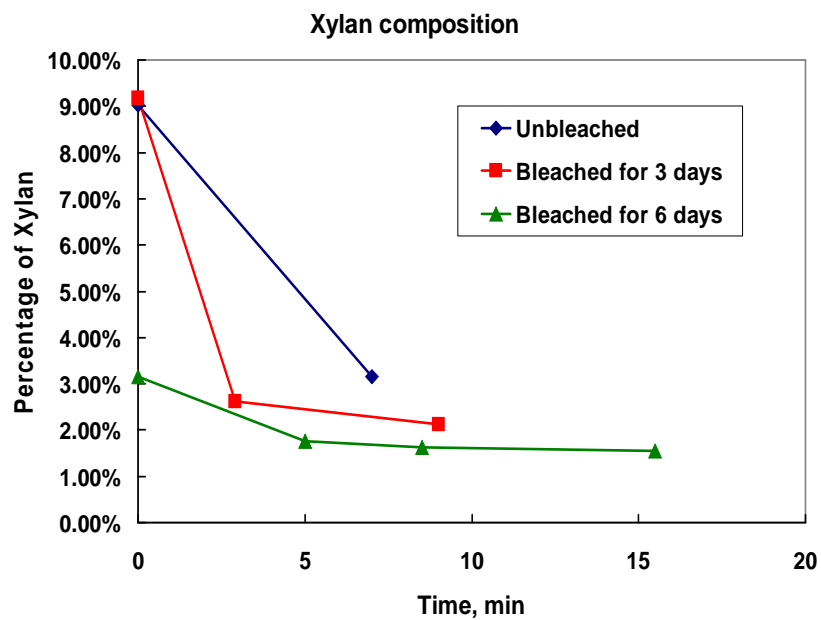


**Figure 54:** Glucan Components Change during Dissolution Process

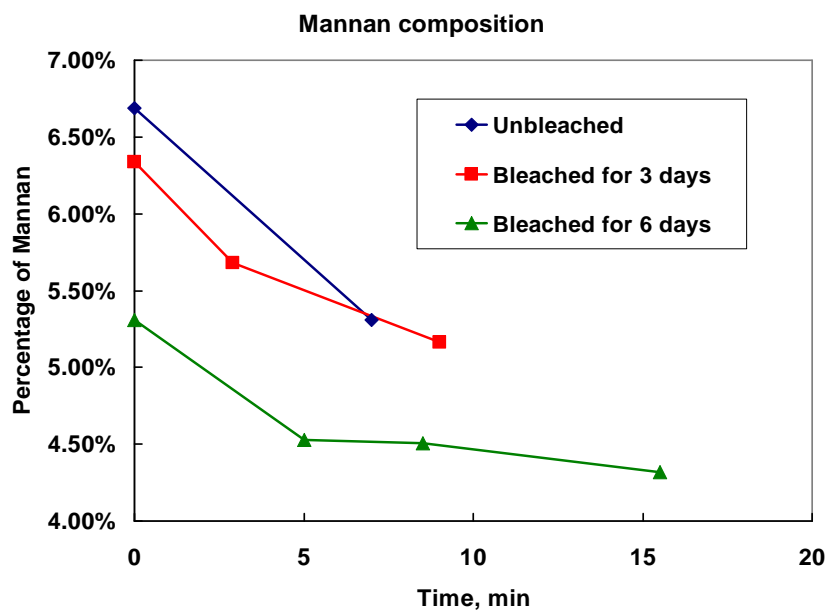
the solubility in this case.

#### 4.4 Conclusions

The bleaching chemicals of sodium chlorite can not only remove lignin, but also degrade cellulose. Due to the low initial lignin content in the original sample, the effect of lignin was not significant in this study. The solubility increase observed was due to the the reduction of cellulose molecular weight rather than the decrease of lignin amount. Hemicellulose can be easily dissolved at the very initial stage of mixing with alkali, thus does not affect the solubility much during the dissolution. Mannan was resistant to bleaching chemicals and alkali solution. the Future work need to be done to investigate the effect of Mannan on wood fiber dissolution in alkali solution.

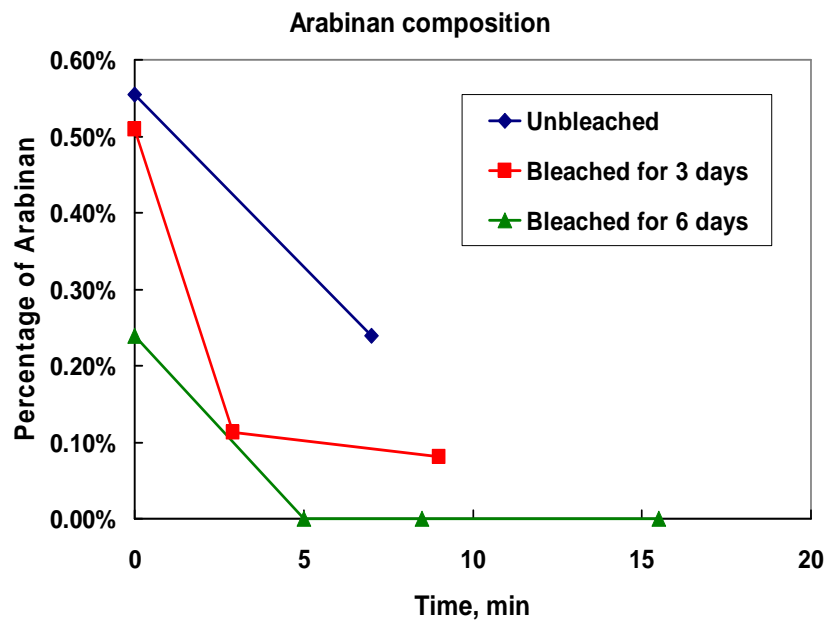


a)

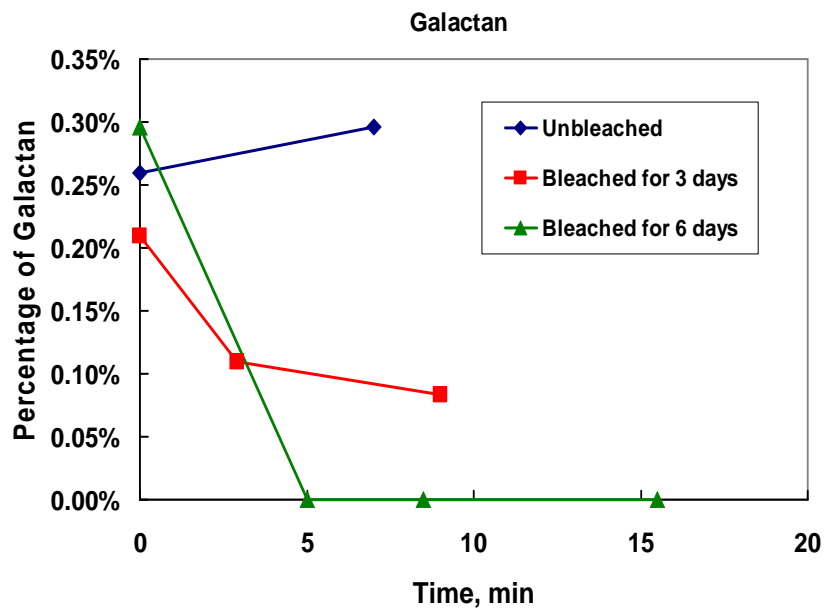


b)

**Figure 55:** Sugar Component Change during Dissolution Process

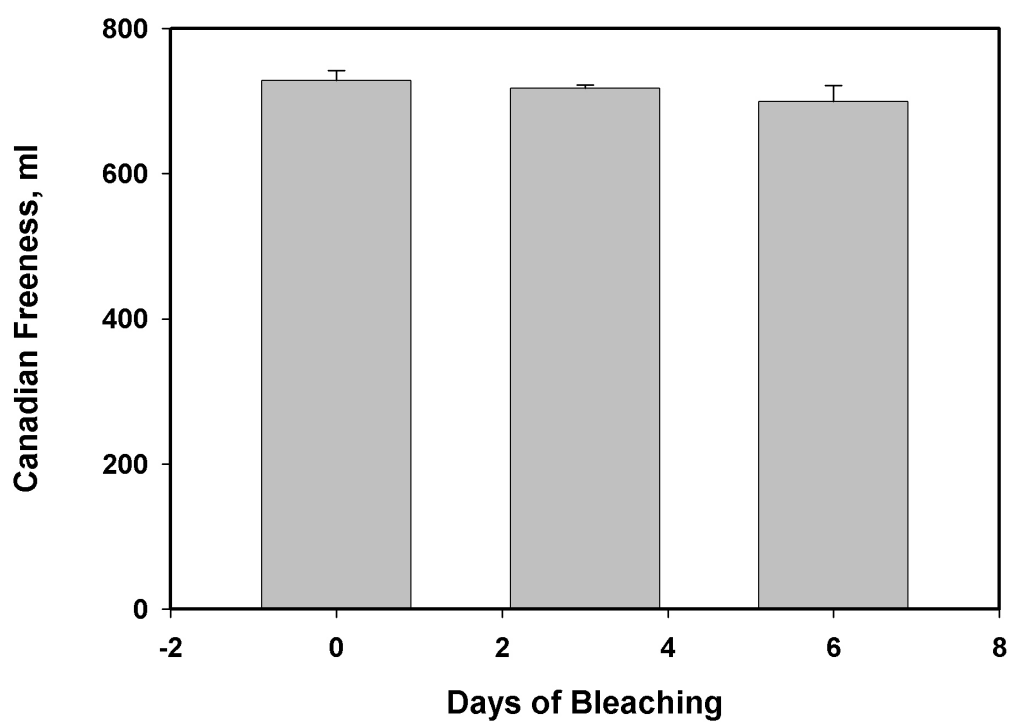


c)

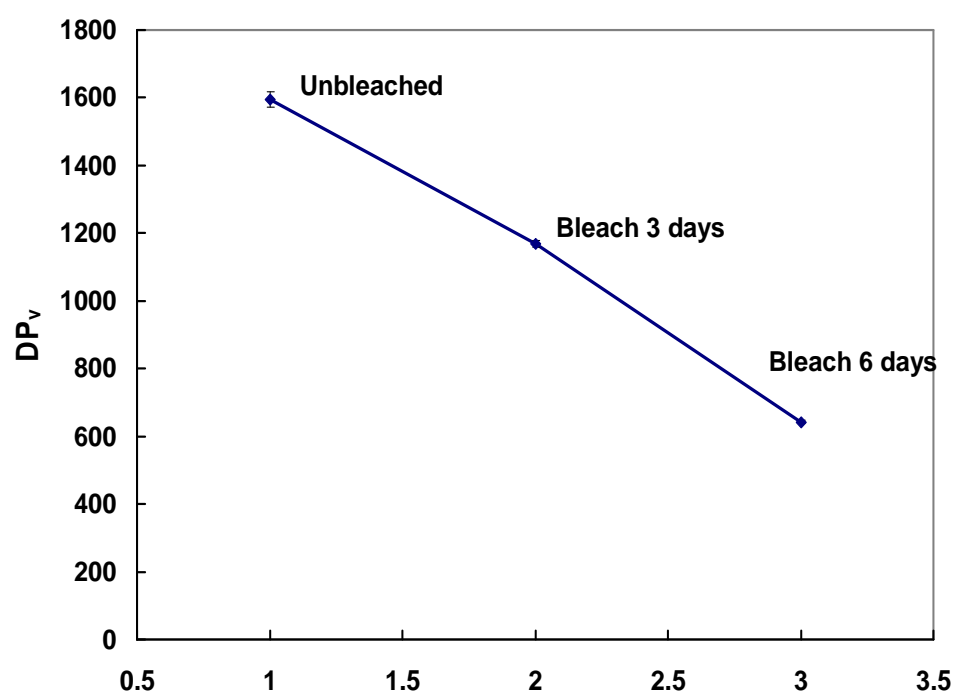


d)

**Figure 56:** Sugar Component Change during Dissolution Process, cont.



**Figure 57:** Canadian Standard Freeness Test



**Figure 58:** DP Change with Bleaching Treatment

## CHAPTER V

### CONCLUSIONS

The work performed in the present thesis tackles both fundamental scientific questions and practical problems. The following major tasks were conducted:

- The kinetics and the activation energy for decrystallization of cotton cellulose dissolution in 6% NaOH and 6%NaOH/4%urea solution were evaluated.
- The influences of dissolution chemistry and conditions, including NaOH concentration, urea addition, and temperature were studied.
- A combination of enzymatic hydrolysis pretreatment of cellulose following by NaOH dissolution was proposed and investigated.
- The effect of wood fiber components, hemicellulose and lignin was also studied.

Based on the experimental design and results from above tasks, the following conclusions were achieved.

#### 1. Low temperature reactor design

A customized low temperature reactor was designed for dissolution kinetics study. Cooling bath temperature can be adjusted by varying the weight ratio of ethylene glycol and water based on the phase diagram. The temperature change within sample was also monitored and recorded by an accurate thermocouple. The designed system can be easily used in other heterogeneous reaction in which a liquid bath is needed for heating or cooling, and in which uniform sampling cannot be ensured in a traditional batch reactor. The designed reactor can provide repeatable data with high efficiency and low cost.

## 2. Dissolution kinetics

The dissolution of high molecular weight and high crystallinity cellulose in 6% NaOH and 6%NaOH/4%urea solution was studied. The cellulose dissolution degree with respect to temperature change was thoroughly studied. The crystal type, crystallinity, and crystal size change during the dissolution process were also investigated. Through applying Eyring equation in transition state theory, the activation energy for the decrystallization was obtained for cellulose dissolution in 6% NaOH and 6%NaOH/4%urea solution.

Solubility data and the decrystallization data can be linearly correlated well. Both have shown the strong dependence of the cellulose dissolution at low temperature. An optimum temperature of around  $-15^{\circ}\text{C}$  was found. For temperature above this, solubility was only 7 to 8 percent; while at the optimum temperature, it increased sharply to 30 percent. Below this temperature, the solubility slightly decreased from 30 to 25 percent. It was shown that the phase change of the free water in the solution at low temperature played an important role on the dissolution degree of cellulose. Due to the freezing of free water, the concentration of NaOH in the rest of liquid phase was increased. Also the adsorption of NaOH on cellulose was increased, which leads to larger swollen of cellulose and break of the crystalline hydrogen bonds, hence to a higher solubility.

The effect of urea additive on the dissolution system was not remarkable in the studied conditions. Different activation energy for 6% NaOH and 6%NaOH/4%urea solution was found. At temperature of  $-15^{\circ}\text{C}$ , the two solution system had the same activation energy. Above that temperature, the addition of urea can decrease the activation energy while below that temperature, resulting in an increase in the cellulose dissolution. However, the addition of urea would increase the activation energy at the temperature lower than  $-15^{\circ}\text{C}$ , which is

not favorable for cellulose dissolution. At high alkali concentration (10%), the addition of urea (12%) greatly suppressed the cellulose solubility.

The effect of NaOH concentration was studied for 4% NaOH, 6% NaOH and 10% NaOH. Cellulose can be dissolved in 4% NaOH, but with much lower solubility than that of 6% NaOH. Although cellulose dissolution is faster in 10% NaOH than in 6% NaOH and 4% NaOH, the maximum solubility it reached is lower than that in 6% NaOH solution.

### **3. Solubility improvement with the aid of enzymatic hydrolysis pretreatment**

It was found that due to the high molecular weight, the cellulose could not be fully dissolved in alkali solution. In this study, enzymatic hydrolysis with commercial cellulase was used to break down cellulose molecular weight while maintain its crystal structure. The dissolution behavior of enzyme pretreated cotton fibers in NaOH/urea solution was investigated. The experimental results indicated that although the crystallinity of cotton linter almost did not change during the enzymatic pretreatment, the solubility of cellulose in cold NaOH/urea solution increased from 30 percent for original cotton fibers to 65 percent for enzymatic treated fibers, which was mainly attributed to the reduction of cellulose's molecular weight by the enzymatic treatment. Moreover, the dissolution time was also greatly shortened by the enzymatic pretreatment. The results suggest that the effect of crystallinity of the cellulose on the cellulose dissolution in NaOH/urea solution is much less than that of molecular weight.

### **4. Hemicellulose and lignin effect**

In the study of the effect of hemicellulose and lignin on cellulose dissolution, no significant influence of lignin on the cellulose was found. However, we would like to be careful on this conclusion because the difference in the lignin contents

for the fibers we used in this study is insignificant. Therefore, the results may not be applied to other cellulose fibers with high lignin contents. The responses of different sugar components in hemicellulose to the dissolution solvents were quite different, in that xylan was quickly removed but mannan was sustained. The bleaching chemical of sodium chlorite can not only remove lignin, but also degrade cellulose. The solubility increase observed may be due to the reduction of cellulose molecular weight rather than the decrease of lignin amount.

In summary, we have successfully fulfilled our three objectives in dissolution mechanism study, solubility improvement and the fundamental study of the effect of hemicellulose and lignin. This is not a completion of the whole research on cellulose dissolution in alkali system. Many factors have not been included in current study and a few of them are listed in the future work chapter.

## CHAPTER VI

### FUTURE WORK

Just as that have been mentioned in the very beginning of the project introduction, in order to fully understand the cellulose dissolution in alkali system, there are several aspects of problems need to be addressed:

1) The understanding of cellulose from molecular level and supermolecular level, including the hydrogen bonding between molecules, the amorphous cellulose and the crystalline structure, and the pore structure.

2) The behavior of alkali solution with and/or without urea at low temperature and the interaction between cellulose and aqueous sodium hydroxide and urea and the dissolution mechanism.

3) The understanding of the major components in general plant lignocellulosic materials, for example, hemicellulose and lignin, their chemical structure, their connection with cellulose and their influence in cellulose dissolution.

This problem cannot be fully unveiled unless all the above are addressed. In our study, we have gained new insight into mechanism of the cellulose dissolution in alkali solution with or without urea, especially on its dependence on temperature. We also explored a new way to enhance the cellulose solubility. We found that a certain component of hemicellulose may have special connection to cellulose that cannot be easily broken by chemical treatments and this may partly lead to the difficulty of wood fiber dissolution.

As to the whole dissolution system of cellulose in alkali solution, there are still many unknown aspects that we are interested in, but can not carry out in the present study due to the time limitation. We would like to name a few of them, and put into

the future work.

- Improvement on the current study

The dissolution kinetics can be further refined in the following area:

- 1) Improve the low temperature reactor to make it more reliable, adjust cooling bath to obtain either fast kinetics or slow kinetics.
- 2) The current activation energy obtained is just an estimation, and should not be taken as quantitatively meaningful. More better controlled experiments need to be designed to minimize both the systematic error and randomized error.
- 3) The effect of temperature, NaOH concentration, and urea concentration can be investigated in a wider range in order to find the real optimum combination.
- 4) The mechanism of dissolution in the molecular and supermolecular level should be further investigated.
- 5) The effect of high content Lignin as in wood chips need to be investigated. The effect of individual component of hemicellulose, such as xylose and mannose, need to be distinguished and studied separately.

- Investigation in other effects

Other than the above discussed effects, many other effects are also important but not included in this study for the time limit, for example, the origin of cellulose material, initial crystallinity, crystal size, pore structure, treatment history, etc.

- Application of the Cellulose Solution

The characteristics of the obtained cellulose solution need to be studied. The possible applications need to be explored, such as making film, sponge, nanocomposite, etc.

The knowledge we have gained in this study on the activation energy of cellulose decrystallization in dilute NaOH solution with or without urea and the temperature dependence behavior will guide us to the deeper understanding of cellulose dissolution mechanism. The low temperature reactor designed will expedite future study of the dissolution system. The method of pretreatment with enzymatic hydrolysis to increase cellulose solubility opened a new way to the potential application of cellulose solution.

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