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Ionic Liquids as Solvents for Catalytic Conversion of Lignocellulosic Feedstocks

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Ionic Liquids as Solvents for Catalytic Conversion of Lignocellulosic Feedstocks

By

Sean Joseph Dee

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Chemical Engineering in the Graduate Division of the University of California, Berkeley

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Professor John F. Hartwig

Fall 2012
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Abstract

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Doctorate of Philosophy in Chemical Engineering

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Professor Alexis T. Bell, Chair

The deconstruction and upgrading of lignocellulosic biomass dissolved in ionic liquids was studied as a potential alternative route to products traditionally synthesized from petroleum. While domestic biomass is a cheaper, lower carbon emission, alternative feedstock to petroleum, its utilization requires the selective deconstruction of the biopolymer to monomeric sugars and upgrading of the sugars to higher value products. Since biomass is soluble in ionic liquids, there is the opportunity to do both the deconstruction and secondary upgrading using “one-pot” homogeneous catalysis.

The primary focus of this work was to understand the kinetics of both biomass deconstruction and secondary sugar chemistry in ionic liquids. Biomass is a complex collection of molecule that consists of three primary components, cellulose, hemicellulose, and lignin. Since cellulose is the primary component, accounting for roughly 45 wt% of the raw biomass on a dry basis, initial studies aimed to understand the hydrolysis of dissolved cellulose to its sugar residue glucose. Using microcrystalline Avicel cellulose as a model, the rate laws and activation energies of cellulose hydrolysis and glucose dehydration were determined in the ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]). No evidence of oligosaccharides was observed, suggesting that hydrolysis occurs preferentially at chain ends and is irreversible. Gradually adding water to the reaction solution, so as not to precipitate cellulose but also limit the secondary dehydration of the resulting glucose to 5-hydroxymethyl furfural (5-HMF), significantly increased glucose yield and limited production of degradation products (humins). Several mechanisms were proposed to explain the effects of water, and possible routes to humin formation.

While understanding the reactivity of model compounds is important to the development of biomass conversion technologies, it is critical to understand how the components of biomass react in their native form. An investigation was carried out to compare the reactivity of cellulose and hemicellulose model compounds to both pretreated and miscanthus grass in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]). Activation energies of model compounds were compared with the native component in raw biomass. Significant rate decreases in hydrolysis of the cellulosic and hemicellulosic
components in raw biomass compared to Avicel and Xylan from Birchwood were attributed to the interaction of lignin with the biopolymers in raw biomass. However, reaction of two pretreated substrates with varying degrees of delignification showed that the presence of lignin did not have a detrimental effect on hydrolysis, but instead suggested that breaking the raw biomass macrostructures is the key to improving hydrolysis of the biopolymers. Gradual water addition strategies further improved saccharine yield, but left the cellulosic component incompletely hydrolyzed. This unhydrolyzed cellulosic component could be further converted by varying the temperature, acid concentration, or performing a second hydrolysis on the reactor residue.

After demonstrating the ability to generate sugars in high yield from miscanthus, we investigated the selective conversion of glucose to 5-HMF in ionic liquids using metal chlorides. Chromium chloride, CrCl₂, has been proposed to isomerize glucose to fructose, which is rapidly dehydrated to 5-HMF in imidazolium chloride ionic liquids without an added catalyst. We began by studying the kinetics of fructose dehydration in [Emim][Cl] and investigating the effects of CrCl₂ on the dehydration to 5-HMF. Then the kinetics of glucose isomerization to fructose using CrCl₂ were characterized and compared to the rate and activation energy for fructose dehydration. Using the data for fructose, a model for the kinetics of fructose dehydration and isomerization in the presence of CrCl₂ was developed. When the model was applied to glucose, it failed to describe the large conversion of glucose and small yields of 5-HMF in the initial reaction period. The accuracy of the model could be improved by including an intermediate in the glucose to fructose dehydration, which highlighted the need to characterize intermediates and products formed during dehydration using more detailed spectroscopic techniques than chromatography.

Finally, we conducted in-situ ¹³C NMR experiments to characterize the intermediates and products formed and to understand the effect of the ionic liquid solvent on glucose dehydration to 5-HMF catalyzed by metal chlorides in [Emim][Cl]. Glucose dissolved in [Emim][Cl] exhibited higher equilibrium concentrations of the furanose and acyclic isomers of glucose, compared to glucose dissolved in H₂O. These isomers were observed to undergo dehydration more rapidly than the pyranose isomers of glucose. The rate of anomerization was also found to be faster in [Emim][Cl], a process that may facilitate ring opening in several proposed mechanisms for glucose dehydration. In situ catalytic studies were conducted using WCl₆, which concluded that fructose is not formed as the reactive intermediate, but rather glucose first undergoes partial dehydration, before it is transformed from its unreactive aldose form to the more reactive ketose form. Using these observations, combined with studies of glucose dehydration catalyzed by H₂SO₄ several mechanisms were proposed to explain the progressive dehydration of glucose to 5-HMF using metal chlorides.
To my parents John and Mary Dee:
for everything they sacrificed to get me to Berkeley.

To my wife Maureen:
for everything she sacrificed to get me out.
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Abbreviations

Ar | Arabinose
[Bmim][Cl] | 1-butyl-3-methylimidazolium chloride
[Bmim][OAc] | 1-butyl-3-methylimidazolium acetate
[Emim][Cl] | 1-ethyl-3-methylimidazolium chloride
CB | Cellobiose
CMR | Symyx Core Module Robot system
C1, C2, Cn, position | Carbon at ‘n’ location in a molecule by IUPAC standards
C5, C6 Sugar | Carbohydrate containing 5 or 6 carbons
DFT | Density functional theory
DMSO | Dimethyl Sulfoxide
DP | Degree of Polymerization
E_A | Activation Energy in Arrhenius Rate law, \( k = Ae^{-E_A/RT} \)
EDA | Ethylene Diamine
[Emim][Tos] | 1-ethyl-3-methylimidazolium tosylate
FID | Flame Ionization Detector
GC | Gas Chromatography
HPAEC | High Performance Anion Exchange Chromatography
HPLC | High Performance Liquid Chromatography
LG | Levoglucosenone
MALDI | Matrix Assisted Laser Desorption Ionization
MS | Mass Spectroscopy
[NMP][OAc] | n-methylpiperdinium acetate
PAD | Pulsed Amperometric Detector
pK_A | Logarithmic Acid Dissociation Constant, \( \log_{10}(K_A) \)
RID | Refractive Index Detector
r_0 | Initial rate in \( \mu\text{mol cm}^{-3}\text{s}^{-1} \)
rpm | Stir rate in revolutions per minute
T | Reaction Temperature
t | Time
TOF | Time of Flight
Y_max | Maximum Yield
5-HMF | 5-hydroxymethyl furfural
2-FHMK | 2-furylhydroxymethyl ketone
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“You can’t live a perfect day without doing something for someone who will never be able to repay you.” – John Wooden

During the past five years of my graduate education, I’ve been blessed with a network of family, friends, and colleagues whose generosity, encouragement, and support have indebted me in ways I can never fully repay. At 23 years old, I made the decision to pursue a doctoral degree without truly understanding the commitment, dedication, and resolve such an endeavor would require. As I reflect back on my studies here at UC Berkeley, I know the only reasons I survived are the people mentioned (and others carelessly omitted through my own fault) in these few paragraphs.

The smartest decision I made at Berkeley was joining Prof. Alexis T Bell’s research group as a first year graduate student. Alex has guided me in my research, been fair, kind, honest and, most importantly, available at any time to provide feedback, advice, or direction. Whether he offered criticism or encouragement, Alex always pushed me forward. His ability to lead such a large research group, working across so many different areas, is remarkable.

The members of the Bell Group have also played a vital role in my journey through graduate school. During my senior year at Case Western Reserve, my good friend Andrew Behn not only convinced me to attend graduate school but that I belonged at Berkeley. When I joined the group, I underestimated the benefits of working closely with such friendly, intelligent colleagues. The countless chalkboard sessions and non work related discussions I’ve shared with Andrew Behn, Will Vining, Bean Getsoian, Joseph Zakzeski, Fuat Celik, Anton Mlinar, Arne Dinse, David Hanna, Shannon Klaus and Mike Zboray have helped me immensely in my work and been a welcomed distraction from lab scale experimentation.

Within the Bell group, the Energy Biosciences Institute (EBI) has been my home. Chidambaram Mandan and I were the first post doc and graduate student working in the Bell Group EBI team, and together we dealt with the initial lab set up and growing pains associated with starting a new project. So many great ideas have come from debates with both past and present members of our EBI project team including Eric Sacia, Balakrishnan Madhesan, Kris Enslow, and Sasisanker Padmanabhan. I’ve also had the pleasure of working with four undergraduate researchers, Kevin Hwa, Vivien Lee, Siddharth Kola, and Lauren Kim, whose assistance was vital in conducting and completing the experimental work. The EBI has been a wonderful place to work due in large part to the state of the art facilities, seemingly infinite funding support, and tireless dedication of Lab Manager Mara Bryan, Building Manager Zack Philips, Analytical Chemist Stefan Bauer, and Technology Adviser Steve Pietsch.

During my time at Berkeley, I’ve realized that the graduate students in the department are the single most influential group on the experience of higher education. I’ve been very lucky to find friends working across a variety of groups who have all been so welcoming not only to me, but to all the students who enter
the department. Listing all the students names individually would fill up multiple pages, so simply know the parties we enjoyed, lunches we’ve eaten, and softball/basketball games we’ve won and lost have all been wonderful memories of my graduate school experience.

I want to thank my family for all their continued support before, during, and after graduate school. My parents have never wavered in their effort to help me achieve my goals and dreams, and have provided me with a quality education from early childhood, unabashed financial support, and unconditional love. In addition to my parents, I was also fortunate to add a wonderful mother and father-in-law the summer before I attended graduate school that have supported me as one of their own children over the past five years. My extended family: my brother Brian and his wife Sharla, my brothers-in-law Kevin and Mark, and my brother-in-law Jimmy, his wife Kathy, and my beautiful niece Bridget have all helped remind me that with a strong family foundation, anything is possible.

Lastly, I need to thank my beautiful wife Maureen. Five years ago she left her friends, her family, and her home to travel 2,437 miles across the country to start our life together. Graduate school is tough on a marriage, but it has been so rewarding to see our marriage grow and thrive in times of adversity. Her love, tenderness and support were essential to my eventual success. If joining the Bell Group was the smartest decision I made at Berkeley, marrying Maureen was the smartest decision I made in my life.
Chapter 1
Introduction

Lignocellulosic biomass has been identified as a scalable, economically viable, and potentially carbon neutral feedstock for the production of fuels and other petroleum based products.\textsuperscript{[1]} Estimates have shown that 30\% of the United States annual petroleum consumption can be displaced through domestic biomass utilization.\textsuperscript{[2, 3]} Consequently, the US has revised the Renewable Fuels Standard (RFS2) and mandated from 2012 to 2022, fuels from renewable feedstocks will grow from 9\% to 25\% of the total domestic gasoline and diesel consumption.\textsuperscript{[3]} Thus, development of efficient and selective biomass conversion technologies is a key challenge in meeting the growing renewable energy demand.

The transformation of biomass to higher value products is difficult due to the biopolymer macrostructure and crystallinity.\textsuperscript{[4]} Lignocellulose is composed of long microfibrils of crystalline cellulose (20-55 wt\%), a polysaccharide of glucose residues linked through $\beta$-1,4 glycosidic linkages. The microfibrils are aligned with strands of hemicellulose (20-40 wt\%), a slightly branched, analogous biopolymer to cellulose composed of xylose residues. The entire carbohydrate fraction is then encased in lignin (10-30 wt\%), a waxy, highly cross-linked polymer that protects the carbohydrates from chemical and thermal degradation. Much of the recent literature investigating the transformation of biomass to biofuels using homogenous or heterogeneous catalyst has been driven by the need to increase catalyst accessibility by overcoming the recalcitrant nature of the substrate.\textsuperscript{[5]}

Several families of ionic liquids (ILs) have been shown to dissolve cellulose, hemicellulose, lignin, and untreated lignocellulose.\textsuperscript{[6, 7]} ILs are molten salts with melting points below 393 K whose physical properties can be tuned by changing the composition of the anion or cation.\textsuperscript{[6]} Biomass dissolution occurs in ILs because the anions and cations can disrupt the strong internal hydrogen bonding network of the biopolymer by providing electron donor/acceptor pairs, thereby reducing the crystallinity of biomass to facilitate dissolution.\textsuperscript{[8]} In particular, various imidazolium ring based cations, paired with either chloride or acetate anions have shown the highest levels of solubility, with dissolution capacities of up to 20 wt\% for Avicel microcrystalline cellulose, and 8 wt\% for woods and grasses.\textsuperscript{[9]}

After dissolution in ILs, cellulose and hemicellulose can be processed homogeneously to platform molecules as shown in Figure 1.1. In the presence of acidic catalysts such as mineral acids, the carbohydrate fractions can be hydrolyzed to their monomeric sugar residues glucose and xylose. These sugars can either be separated and fermented to alcohols, or subjected to further upgrading. In the presence of the acidic catalysts, glucose and xylose dehydrate to form 5-hydroxymethyl furfural (5-HMF) and furfural respectively. 5-HMF and furfural have been identified as attractive platforms for synthesis of chemicals, fuels, and polymers due to the variety of chemistries that can be applied to the
alcohol and/or aldehyde functionalities attached to the furan ring. Unfortunately, the secondary dehydration to furfurals is very unselective because the sugars and furfurals can also undergo an acid catalyzed cross polymerization condensation reaction to form degradation products called humins.\(^{10}\)

Previous work has focused on either the depolymerization of individual cellulose or hemicellulose model compounds to glucose and xylose \(^{11}\), or the dehydration of glucose to 5-HMF \(^{12-14}\). However, the kinetics of depolymerization and dehydration are largely unreported in the literature and had not been reported in ILs at the time of the initial studies in this dissertation. Additionally, the differences between the simplistic model compounds and the cellulosic and hemicellulosic components in lignocellulosic biomass had not been characterized or considered.

The objective of this dissertation was to understand the kinetics and mechanism of the depolymerization and dehydration of lignocellulosic materials dissolved in ILs. In Chapter 2 kinetic studies were conducted to determine the mechanism, apparent rate law, and activation energy in the hydrolysis of the model compound Avicel microcrystalline cellulose dissolved in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) using H\(_2\)SO\(_4\) as a catalyst. Additional experimentation elaborated on the complex role of water in depolymerization and provided rationale for the increased glucose yield observed when water was added progressively during the initial hydrolysis phase. In Chapter 3, these insights were compared with the kinetics observed for the cellulosic and hemicellulosic components of a perennial grass called miscanthus, dissolved in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]). Using the relationships between the hemicellulosic and cellulosic components of miscanthus and the model compounds, reaction conditions could be altered to optimize the total sugar yield from miscanthus. The effects of pretreatment and extent of delignification on the initial rate of depolymerization using homogenous acid catalysis were also examined and characterized.

After demonstrating the capability to generate sugars in high yield from lignocellulose in ILs, this work focused on further understanding the dehydration of glucose to 5-HMF. Metal chlorides in ILs, specifically chromium chloride (CrCl\(_2\)) dissolved in [Emim][Cl], have been reported as effective catalysts for 5-HMF synthesis from glucose, achieving 70% 5-HMF yield from glucose after 3 h of reaction at 373 K.\(^{13}\) 5-HMF formation from glucose is facilitated through a metal chloride assisted 1,2 intramolecular hydride shift, presumably converting the aldohexose (glucose) to the more reactive ketohexose (fructose) which is readily dehydrated to 5-HMF in [Emim][Cl].\(^{13}\) However, the kinetics of the CrCl\(_2\) catalyzed isomerization of the aldose to the ketose, and the subsequent ketose dehydration to 5-HMF have not been reported. Additionally, the activity of metal chlorides has been shown to vary greatly across different ILs and solvents, indicating that the IL has an unknown benefit for glucose conversion to 5-HMF.\(^{14}\)

In Chapter 4, the activation energy, rate law, and complex relationship between the aldose-to-ketose isomerization and ketose dehydration were determined. Then a kinetic model was developed using Matlab to quantify the kinetic rate constants used to describe the isomerization and dehydration steps.
Interestingly, it was found that the experimental data was more accurately described by a model that involved several intermediates formed before isomerization, suggesting that the reaction mechanism is more complex than a simple two step model. In Chapter 5, in situ Nuclear Magnetic Resonance (NMR) spectroscopy was used to characterize both the solvent effects of [Emim][Cl] on dissolved $^{13}$C labeled glucose and the progression of glucose dehydration catalyzed by metal chlorides. Significant changes in the glucose isomer distribution were observed in [Emim][Cl] compared to D$_2$O. The rate of anomerization, the process by which the structural isomers interconvert, was also found to be significantly faster in the IL. Unfortunately in situ NMR in the presence of CrCl$_2$ produced extremely broad signals, due to the presence of Cr(III) which is both quadrupolar and paramagnetic.$^{[13]}$ Therefore, in situ catalytic studies were conducted in the presence of WCl$_6$, which DFT calculations have shown to be a potentially more active alternative to CrCl$_2$.$^{[15]}$ It was demonstrated that WCl$_6$ also catalyzed a 1,2 intramolecular hydride shift during 5-HMF formation and using the insights gained from the NMR spectra, new intermediates were postulated, a new mechanism was proposed, and additional products and pathways were identified.

![Chemical Structures](image)

**Figure 1.1** Hydrolysis of lignocellulosic biopolymers followed by subsequent dehydration to furfurals

**References**


Chapter 2

A Study of the Acid-Catalyzed Hydrolysis of Cellulose Dissolved in Ionic Liquids and the Factors Influencing the Dehydration of Glucose and the Formation of Humins

Abstract

An investigation was carried out for the hydrolysis of cellulose dissolved in ionic liquids (ILs) 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) and 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) catalyzed by mineral acids. Glucose, cellobiose, and 5-hydroxymethyl furfural (5-HMF) were observed as the primary reaction products. The initial rate of glucose formation was determined to be first order in the concentrations of dissolved glucan and protons, and zero order in the concentration of water. Absence of a dependence on water concentration suggests that cleavage of the β-1,4 glycosidic linkages near chain ends is irreversible. The apparent activation energy for glucose formation is 96 kJ mol\(^{-1}\). The absence of oligosaccharides longer than cellobiose suggests that cleavage of interior glycosidic bonds is reversible, due to the slow diffusional separation of cleaved chains in the highly viscous glucan-IL solution. Progressive addition of water during the course of glucan hydrolysis inhibited the rate of glucose dehydration to 5-HMF and the formation of humins. The inhibition of glucose dehydration is attributed to stronger interaction of protons with water than the 2-OH atom of the pyranose ring of glucose, the critical step in the proposed mechanism for the formation of 5-HMF. The reduction in humin formation associated with water addition is ascribed to the lowered concentration of 5-HMF, since the formation of humin is suggested to proceed through the condensation polymerization of 5-HMF with glucose.

2.1 Introduction

It has been estimated that lignocellulosic biomass could meet ~54% of the annual consumption of oil in the US and that by 2030 biomass-based fuels could supply approximately 20% of the nation’s transportation fuel market.\(^{[1]}\) The challenge, therefore, is to find effective means for converting this important resource into compounds that can be used as building blocks for the production of transportation fuels.

Lignocellulosic biomass is composed of three principal components - lignin, cellulose, and hemicellulose. While the distribution of these components can vary depending on the feedstock source, cellulose and hemicellulose are both biopolymers which account for 70%-90% of the total mass and are the primary sources of products that can be blended into gasoline and diesel.\(^{[2]}\) Cellulose is a crystalline polymer composed of C\(_6\) sugar, whereas hemicellulose is a polymer comprised of C\(_5\) and C\(_6\) sugars. The primary focus of recent investigations has been on the conversion of cellulose to glucose, since this
sugar can be fermented to produce ethanol or butanol for blending with current transportation fuels.\textsuperscript{[1]} It has also been demonstrated that glucose can be converted via non-biological catalytic routes to gasoline or diesel additives by either dehydration of glucose to 5-HMF followed by hydrogenation to lower oxygen content additives such as dimethyl furan or by hydrogenation of glucose to sorbitol followed by dehydration and hydrogenation to hexane.\textsuperscript{[3, 4]}

To obtain glucose, cellulose must be hydrolyzed. Since cellulose is crystalline, it is desirable to first dissolve the cellulose in a suitable solvent in order to make the $\beta$-1,4 glycosidic linkages between glucose residues more readily accessible for hydrolysis. Recent studies have shown that ILs exhibit excellent solubility for cellulose.\textsuperscript{[5]} The high solubility of cellulose in ILs has been attributed to interactions of the anions of the IL with hydroxyl groups within the crystalline polymer structure, thereby disrupting the hydrogen bonding network that stabilizes the crystal.\textsuperscript{[6]}

Several studies of cellulose depolymerization in ILs have been reported. The first of these demonstrated that cellulose dissolved in $[\text{Bmim}]\text{[Cl]}$ could be depolymerized by heating to 373 K for 60 min in the presence of concentrated $\text{H}_2\text{SO}_4$.\textsuperscript{[7]} Overall yields of reducing sugar of 66\% to 81\% were obtained, with glucose yields of 21\% to 39\%. More recent work has shown that the progressive addition of water to cellulose dissolved in $[\text{Emim}]\text{[Cl]}$ can increase the yield of glucose to 89\% and limit the yield of 5-hydroxymethyl furfural to 7\%.\textsuperscript{[8]} In this study $\text{HCl}$ was used as the catalyst and the hydrolysis of cellulose was carried out at 378 K for 4 h. The kinetics of cellulose hydrolysis have been modeled using cellobiose, the dimer of glucose, dissolved in $[\text{Emim}]\text{[Cl]}$.\textsuperscript{[9]} This work showed that the rate of cellobiose hydrolysis is a strong function of water concentration, acid strength and concentration, and temperature. The applicability of these results to cellulose is, however, not fully apparent since there are notable differences between cellulose and cellobiose. For example, it has been reported that the hydrolysis of dissolved cellulose (glucan) could be limited by the accessibility of glycosidic linkages because the glucan strands take on a random coil structure.\textsuperscript{[10]} Likewise, evidence has been presented suggesting that the rate of glucan hydrolysis is dependent on its degree of polymerization because the glycosidic bonds near the ends of a chain of cellulose react twice as fast as those in the interior of the chain.\textsuperscript{[11]}

The present study was undertaken with the aim of developing a deeper understanding of the factors controlling the acid-catalyzed hydrolysis of cellulose dissolved in an IL. Among the questions addressed were the kinetics of hydrolysis, including the temporal evolution of all products, the effects of acid strength and acid concentration, and the effects of water addition on the kinetics of cellulose hydrolysis, glucose dehydration, and formation of humins. Microcrystalline cellulose (Avicel) was used as the substrate and reactions were carried out in either $[\text{Bmim}]\text{[Cl]}$ or $[\text{Emim}]\text{[Cl]}$. 
2.2 Experimental Materials and Methods

2.2.1 Materials

Unless noted otherwise, materials were used as received. Ionic liquids 1-ethyl-3-methylimidazolium chloride, ([Emim][Cl], 98% purity), and 1-butyl-3-methylimidazolium chloride, ([Bmim][Cl], 98% purity) were purchased from Iolitec, Germany. Avicel (microcrystalline cellulose, PH-101, DP < 350), cellobiose (CB), arabinose (Ar), methane sulfonic acid (CH$_3$SO$_3$H, 100% purity), 5-hydroxymethyl furfural (5-HMF, 99% purity), trifluoroacetic acid (CF$_3$COOH, 100% purity), acetic acid (CH$_3$COOH, 100% purity), phosphoric acid (H$_3$PO$_4$, 100% purity), mixed bed resin TMD-8 hydrogen and hydroxide form, and 1-butyl-3-methylimidazolium acetate ([Bmim][CH$_3$COO], 95% purity) were purchased from Sigma Aldrich, USA. Glucose (G, USP Grade) was purchased from Hyclone, USA. Sulfuric acid (H$_2$SO$_4$, 98% purity) was purchased from Acros. Hydrochloric acid (HCl, 37% purity), HPLC grade acetonitrile, and ethyl acetate were purchased from Fisher Scientific, USA.

2.2.2 Cellulose Hydrolysis

The hydrolysis of cellulose was performed using a Symyx Core Module Robot (CMR) equipped with a positive displacement tip. In a typical experiment, [Bmim][Cl] (15mL) was dispensed into a 20 mL vial at 373 K with magnetic tumble stirring at 400 rpm. As the ionic liquid was dispensed, microcrystalline cellulose (750 mg, Avicel) was added to ensure the substrate had good contact with the stir bar. Once the cellulose and ionic liquid had been added, the solution was stirred for 3 h at 373 K in order to assure complete dissolution of the cellulose as determined by the formation of a clear solution. After dissolution the temperature was adjusted to the reaction temperature (usually 363 K) and allowed to equilibrate for 1 h. Mineral acid was diluted with water obtained from a Milli-Q ultrapure water purification system. The diluted acid solution was then added to the IL solution to initiate the reaction. Samples (700 µL) were taken at specified intervals using the Symyx CMR positive displacement tip and added to vials containing 2.1 mL of ultrapure water at room temperature. An aqueous solution of arabinose (700 µL of 10 mg mL$^{-1}$ arabinose in water) was added to this mixture, which served as an internal standard. The sample was then centrifuged, and 500 µL of the supernatant fluid was treated with a mixed bed resin (hydrogen and hydroxide form) to remove the IL. The sample with the solid ion exchange resin was then centrifuged again and the supernatant analyzed by either high performance liquid chromatography using a refractive index detector (HPLC-RID) or high performance anion exchange liquid chromatograph using a pulsed amperometric detector (HPAEC-PAD). The remaining sample that had not been treated with ion exchange resin was extracted with ethyl acetate (5 extractions with 5mL of ethyl acetate) in a pear shaped flask, treated with excess sodium carbonate (NaCO$_3$) to remove water, centrifuged, and analyzed by gas chromatography (GC) with a mass spectrometer (MS) and flame ionization detector (FID).
Reactions in which water was added periodically to the reaction mixture were carried out on the deck of the Symyx CMR. In a typical reaction, [Emim][Cl] (353 µL, 400 ± 20 mg) at 383 K was dispensed manually using a micropipette into a 4 mL screw-top glass vial containing a magnetic stir bar rotating at 250 rpm. Avicel (20 ± 0.5 mg) was added to the reactor and allowed to dissolve at 383 K for 3 h, resulting in a clear solution. The reactor temperature was adjusted to the reaction temperature and the contents of the reactor equilibrated for 1 h. A premade solution of 1.66 M HCl (23.2 µL) was then added to the reactor and the reaction was initiated. After 10, 20, 30, and 60 min, 80 µL, 40 µL, 60 µL, and 100 µL of water (or an equivalent molar amount of acetonitrile) was added to the reactor, which was then resealed. Reactors were removed from the heated CMR deck at specified time intervals and quenched with running room-temperature water for 1 min. The reactor contents were then diluted with ultrapure water (300 µL), and an aqueous solution of arabinose was added (400 µL of 10 mg mL⁻¹ arabinose in water) which served as an internal standard. The sample was then centrifuged, and 500 µL of the supernatant fluid was treated with the mixed bed resin and centrifuged. The supernatant of the sample treated with the solid ion exchange resin was analyzed by HPLC-RID or HPAEC-PAD.

2.2.3 Analytical Techniques

HPLC-RID analysis were performed on a Shimadzu instrument equipped with a Biorad Aminex HPX-87H column maintained at 333 K and eluted with 0.01N H₂SO₄ mobile phase flowing at 0.6 mL min⁻¹. Products were identified by comparison of retention times with those of pure substances. Quantification was determined by dividing the integrated peak areas of hydrolysis products (celllobiose, glucose, 5-HMF), by the integrated peak area of the internal standard (arabinose) and converting the area ratio to a molar concentration using a seven-point calibration curve.

HPAEC-PAD analyses of oligosaccharides were performed on a Dionex ICS-3000 system equipped with a CarboPac PA200 column (3 x 150 mm) maintained at 303 K. Analyses were conducted using a gradient mobile phase flowing at 0.6 mL min⁻¹ in which the concentration of NaOH changed from 30 mM to 100 mM over a 20-min period.

Matrix-assisted laser-desorption ionization time-of-flight mass spectroscopy (MALDI-TOFMS) analyses were conducted on a Shimadzu Biotech AXIMA system operating in reflection mode, using 2,5 dihydroxylbenzoic acid in 70% acetonitrile as the matrix.

Samples extracted into ethyl acetate were injected into a Varian CP-3800 Gas Chromatograph equipped with a FactorFour Capillary Column (UF-5ms 30 m, 0.25 mm, 0.25 µm, P/N CP8944) and analyzed with a Varian triple quadrupole-mass spectrometer and a flame ionization detector. Products identified by mass spectrometry were confirmed by injection of pure substances. For product quantification, the GC peaks detected by a flame ionization detector were integrated and then converted to a molar concentration using a five-point calibration curve.
Product yields were determined on a molar basis using the initial concentration of glucose residues in the starting substrate. Results are reproducible within 5% of the measured value. The initial moles of glucose residues were calculated by dividing the initial substrate concentration by the molecular weight of a glucose residue (162 g mol\(^{-1}\)). The product yield (%) was then determined by multiplying the moles of product by the number of glucose residues contained in the product and then dividing by the initial number of moles of glucose residues. White solids precipitated during dilution of the reaction samples taken prior to the observation of a maximum in glucose yield were assumed to be unreacted Avicel. After the glucose yield reached a maximum, black solids observed in the reactor were assumed to be humins. The yield of humins was estimated on a molar basis by calculating the decrease in glucose yield from the solution and subtracting from it the sum of the measured increase in yield of 5-HMF. Selectivity was calculated by dividing the moles of a product by the sum of the moles of all measured products.

2.3 Results and Discussion

2.3.1 Product Identification and Evolution

Figure 2.1 shows the temporal evolution of products produced during the hydrolysis of cellulose dissolved in [Bmim][Cl] and catalyzed by H\(_2\)SO\(_4\). Glucose and cellobiose are observed as the dominant products during the first 30 min of reaction. For reaction times between 30 min and 100 min, the yield of cellobiose increases and reaches a maximum of 11% after 45 min, whereas the yield of glucose continues to increase, reaching a maximum of 36% at 90 min. During this time interval, the yield of 5-HMF begins to increase, indicating that glucose undergoes dehydration as it accumulates in the reactor. After the maximum in glucose yield was attained, a dark solid material, assumed to be humins, was observed and the yield of 5-HMF continued to increase. With further reaction, the yields of cellobiose and glucose decreased, whereas the yield of humins rose sharply and the yield of 5-HMF increased slightly. In addition to the products shown in Figure 2.1, small amounts of levoglucosenone (LG) and 2-furylhydroxymethyl ketone (2-FHMK) are observed by GC-MS, but the yields of these products never exceeded 3% and 7%, respectively. No evidence for oligosaccharides, other than cellobiose, was observed by MALDI-TOF or HPAEC-PAD. This result differs from an earlier report in which oligosaccharides with 2 to 10 residues were observed during cellulose hydrolysis in purified [Emim][Cl] in the absence of an acid catalyst at 393 K.\(^{[12]}\) It is conceivable that oligosaccharides with more than two units are not formed at the temperatures in this study (363 K), or are formed but very rapidly hydrolyzed to cellobiose and glucose in the presence of the added acid catalyst.

The concurrent appearance of cellobiose and glucose and the absence of longer oligosaccharides during the initial hydrolysis of cellulose suggest that the hydrolysis of the dissolved polymer proceeds from its ends and that the rates of cellobiose and glucose formation are comparable. The continued formation of glucose as the formation of cellobiose reaches a maximum is attributed to
hydrolysis of cellobiose to glucose, a process that occurs about 1.5 times faster
than the hydrolysis of dissolved cellulose as shown in Figure 2.2. It is notable
that for reaction times longer than 100 min, the yield of humins is significantly
higher than the yields of glucose dehydration products (5-HMF, LG, 2-FHMK),
suggesting that the conversion of glucose to humins is faster than the
dehydration of glucose to 5-HMF and 2-FHMK. This conclusion contrasts with
that reported in studies of glucose dehydration carried out in [Bmim][Cl] using
H$_2$SO$_4$ as the catalyst, which showed that after 3 h of reaction at 393 K, 83% of
the products were present as 5-HMF (66%), or 2-FHMK (17%), and only 16% of
the products were humins. The difference between the present results and
those reported earlier is likely due to a higher acid concentration (150 mM H$_2$SO$_4$
compared to 5 mM H$_2$SO$_4$) and lower temperature (363 K compared to 393 K).

2.3.2 Effects of Varying Acid Strength and Concentration

Product Identification and Evolution Experiments similar to those shown in
Figure 1 were carried out with HCl, CH$_3$SO$_3$H, CF$_3$COOH, H$_3$PO$_4$, and
CH$_3$COOH. Table 2.1 compares the pKa in water of each acid, the maximum
glucose yield ($Y_{G\text{Max}}$), the time to reach the maximum glucose yield ($t_{G\text{Max}}$),
the maximum cellobiose yield ($Y_{CB\text{Max}}$), the time to reach the maximum cellobiose
yield ($t_{CB\text{Max}}$), and the maximum 5-HMF yield ($Y_{HMF\text{Max}}$). The data show that acids
with a pKa $\geq 2$ (H$_3$PO$_4$ and CH$_3$COOH) are inactive, whereas acids with pKa $\leq -1.9$
(HCl, H$_2$SO$_4$, and CH$_3$SO$_3$H) show very similar characteristic values for the
maximum yield of product and the time at which the maximum yield is attained.
Only CF$_3$COOH (pKa = 1.0) showed a difference in activity between these two
regimes, with similar maximum yields to those obtained with acids of pKa $\leq -1$,
but longer reaction times to reach maximum yields. These results are consistent
with the findings reported in a recent study of acid-catalyzed hydrolysis of
cellobiose dissolved in [Emim][Cl] carried out at 363 K. In that study it was
observed that the acids with pKa < 0.5 are active for cellobiose hydrolysis, and
that acids with pKa $\leq -1.9$ all behave similarly. The effects of acid strength on the
hydrolysis of both cellulose and cellobiose are best attributable to the extent of
acid dissociation in the IL. Thus, acids with pKa $\leq -1.9$ are expected to be fully
dissociated. Consistent with this interpretation, it was observed that if
[Bmim][CH$_3$COO] was used as the solvent instead of [Bmim][Cl], H$_2$SO$_4$ became
completely inactive because the acetate anions of the IL have a strong affinity for
the protons of the acid. Given these observations further studies were carried out
in chloride containing ILs with strong acids, e.g., H$_2$SO$_4$ and HCl.

The effect of varying H$_2$SO$_4$ concentration on the production of glucose, 5-
HMF, and humins is shown in Figure 2.3, Figure 2.4, and Figure 2.5 respectively
for concentrations ranging from 15 mM H$_2$SO$_4$ (0.05 molar equivalents per
glycosidic linkage) to 300 mM (1.0 molar equivalents per glycosidic linkage).
Increasing the concentration of H$_2$SO$_4$ increased the rate at which all products
were produced. The maximum yield of glucose, shown in Figure 2.3, was
approximately 35% regardless of acid concentration. Similar trends were
observed for cellobiose (not shown), for which the maximum yield was 12%. As
shown in Figures 2.4 and 2.5, as acid concentration increased both the rates and
maximum yields of 5-HMF and humins increased. At the highest acid concentrations, a decrease in 5-HMF yield was observed. The loss of this product is attributed to both humin formation and its degradation to levulinic and formic acid, both of which were observed in small yields by GC-MS.

2.3.3 Activation Energies and Temperature Effects

The hydrolysis of cellulose using H$_2$SO$_4$ in [Bmim][Cl] was studied at temperatures from 343 K to 393 K. The apparent activation energy for the hydrolysis of cellulose to glucose, determined from the Arrhenius plot of the initial rate of glucose formation is 96 kJ mol$^{-1}$ as shown in Figure 2.3. This value agrees well with the apparent activation energy calculated from previously reported results in [Emim][Cl] with CH$_3$SO$_3$H for cellulose and cellobiose hydrolysis, 92 kJ mol$^{-1}$ and 84 kJ mol$^{-1}$, respectively.[9] It is notable that the apparent activation energies for hydrolysis of β-1,4 glycosidic linkages in glucan and cellobiose dissolved in ILs are considerably lower than that reported for cellulose hydrolysis in water catalyzed by sulfuric acid (118-150 kJ mol$^{-1}$).[13, 14]

The glucose selectivity evaluated at the maximum yield of glucose is a weak function of temperature, rising from 60% at 343 K to 64% at 393 K. This insensitivity to temperature suggests that the energy barriers for the hydrolysis of cellulose to glucose and the subsequent dehydration of glucose to 5-HMF are similar. Consistent with this interpretation, it has been reported that the difference between the apparent activation energies for cellulose hydrolysis and glucose dehydration is 7-11 kJ mol$^{-1}$.[9]

2.3.4 The Roles of Water

Two sets of experiments were carried out in order to examine the effects of water addition on the rate of glucan hydrolysis and glucose conversion to 5-HMF and humins. In the first set, it was determined that 8 wt% water (16 molar equivalents per β-1,4 glycosidic linkage) precipitated amorphous cellulose from a 5 wt% glucan solution in [Bmim][Cl]. Therefore, the amount of water added at the start of the experiment was varied between 1 and 10 molar equivalents. The effect of varying initial water content on the total yield of cellobiose and glucose is shown in Figure 2.7. For reaction times shorter than 30 min, the amount of water added had no effect. However the maximum yield of glucose shifted to higher values after longer reaction time. These higher glucose yields resulted in higher 5-HMF yields after 150 min, as shown in Figure 2.8, due to the higher concentration of glucose generated. As discussed below, the amounts of water used in the experiments shown in Figure 2.7 and Figure 2.8 were approximately 20 times lower than the amount required to inhibit glucose dehydration.[8]

In a second set of experiments water was added gradually to a solution of 5 wt% cellulose in [Emim][Cl] containing HCl as the catalyst in accordance with the optimized water addition strategy suggested by Binder and Raines.[8] The initial water content was 5% at 0 min and increased by adding 15 wt%, 5 wt%, 8 wt%, and 10 wt% water at 10, 20, 30, and 60 min, respectively. The effect of water addition on the production of cellobiose, glucose, 5-HMF and humins is shown in Table 2.2. As with the results shown in Figure 2.7, the yield of glucose and
cellobiose after 30 min did not depend on the amount of water added but the yields of 5-HMF and humins after the first hour were reduced with increasing water content (Table 2.2, Experiments 1-5). By following the optimized protocol, glucose yields of up to 74% could be reached in 2 h, with less than a 10% yield of 5-HMF and less than 3% yield of humins (Table 2.2, Experiment 5), which were consistent with the results reported by Binder and Raines.\[8\]

2.3.5 Mechanistic Implications and Rate Law

The mechanism for acid-catalyzed hydrolysis of carbohydrate substrates dissolved in organic solvents can be used to interpret the hydrolysis of glucans dissolved in ionic liquids.\[14, 15\] As illustrated in Figure 2.9, hydrolysis is initiated by the reversible protonation of a β-1,4 glycosidic linkage in a glucan strand. The protonated ether linkage then decomposes to form a molecule of glucose and an oxocarbonium ion.\[14\] The latter species then rapidly reacts with water to form a terminal glucose unit on the glucan strand and a proton. The rate of hydrolysis in IL was determined experimentally to be first order in glucan and acid concentration, but zero order in water concentration as shown in Figure 2.10, suggesting that the protonation of glucan is quasi-equilibrated, but the dissociation of the β-1,4 glycosidic bond is irreversible and, hence, rate limiting.

Only glucose and cellobiose were observed as the initial products of glucan hydrolysis, and no evidence was observed for other oligosaccharides. The absence of longer oligosaccharides can be explained on the basis of the mechanism shown in Figure 2.9. If protonation and cleavage of a β-1,4 glycosidic linkage on the interior of a glucan strand does not result in rapid separation of the chain ends, then the bond can be reestablished. This seems likely since the viscosity of glucan-IL solutions is relatively high. By contrast, the glucose and cellobiose fragments formed upon cleavage of a β-1,4 glycosidic linkage near the end of the glucan strands are able to diffuse away from the newly formed oxocarbonium ion because of their low molecular weight.

The mechanism for the dehydration of sugars to furans has been actively discussed.\[16, 17\] Two pathways have been proposed – one that involves the isomerization of acyclic intermediates and another that proceeds via transformation of ring structures. While several authors have suggested that glucose isomerization to fructose via enolization or hydride shift in the acyclic pathway is required for dehydration,\[17, 18\] we note that fructose was never observed experimentally in this study or our earlier work on the dehydration of glucose.\[4\] Recent computational work has suggested that a precursor to 5-HMF can be formed directly from glucose upon protonation of the C₂ hydroxyl group.\[19\] In a related study concerning the dehydration of xylose to furfural, the same authors concluded that dehydration via the cyclic pathway is more favorable energetically compared to the acyclic pathway.\[20\] For these reasons, we propose that dehydration of glucose in IL proceeds according to the cyclic pathway shown in Figure 2.11.\[19\] After protonation of the C₂-OH hydroxyl, the five-member aromatic ring is formed after the free electrons from O₅ attack C₂ to release water, and the resulting oxocarbonium ion is reduced by aldehyde
formation at C$_1$-OH.$^{[20]}$ Acid-catalyzed protonation and dehydration of the remaining hydroxyl groups on the ring produces 5-HMF.

2.3.6 Understanding Humin Formation

The chemistry of humin formation is very poorly understood. While several authors have suggested that this product is formed by polymerization of furanic compounds,$^{[21]}$ recent experimental work indicates that humin formation occurs by condensation polymerization of sugars and the products of their dehydration.$^{[22]}$ A possible mechanism for this process is shown in Figure 2.12. The proposed mechanism is derived from the cis diol protection of aldehyde functionality in organic synthesis.$^{[23]}$ In the system under investigation, the aldehyde group of 5-HMF can undergo protonation and subsequent reaction with a monosaccharide. The resulting compound can be protonated again to form an oxocarbonium ion that reacts with a second cis hydroxyl group of the monosaccharide to form a cyclic compound. Polymerization then proceeds with the formation of a new oxocarbonium ion either by protonation of the hydroxyl group on 5-HMF or a remaining hydroxyl group on the monosaccharide. This second oxocarbonium ion can then react with the alcohol of a molecule of 5-HMF or sugar to give a new reactive aldehyde group on the opposite side of the molecule to propagate the reaction to higher molecular weight products. While this mechanism is hypothetical it would explain the recent observation that humin formation is first order in the concentrations of sugar and HMF.$^{[22]}$

The influence of water on the progress of glucan hydrolysis, the subsequent dehydration of glucose to 5-HMF, and the formation of humins is complex. As noted in Figure 2.7 and Table 2.2, the concentration of water present during the first 30 min of cellulose hydrolysis has little to no effect on the rate of cellulose hydrolysis, but further addition of water once glucose is formed inhibits the dehydration of glucose and its condensation with 5-HMF to humins.$^{[8]}$ The absence of an effect of water concentration on the initial rate of glucan hydrolysis suggests that the β-1,4 glycosidic linkages in glucan and cellobiose are sufficiently nucleophilic that the presence of water does not affect the interactions of protons from the acid catalyst with these linkages. The results presented in Table 2.2 demonstrate that the principle effect of increasing water concentration during the course of glucan hydrolysis is to limit the extent of glucose dehydration and, thereby, the appearance of 5-HMF and the formation of humins. While it has been suggested that inhibition of glucose dehydration by water could be attributed to Le Chatelier’s principle,$^{[8]}$ this seems unlikely, since the Gibbs free energy change for the dehydration of glucose at 373 K is estimated to be -183 ± 15 kJ mol$^{-1}$. A more plausible interpretation is that the O atom in the hydroxyl group of C$_2$ of the pyranose ring of glucose (2-OH) is less nucleophilic than water and, hence, water effectively diverts protons from protonation of glucose molecules. This view is supported by molecular dynamic simulations which show that water can deprotonate carbohydrates before they begin to dehydrate, and that after deprotonation the acidic proton is more likely to protonate other water molecules than the original carbohydrate.$^{[19]}$ The minimization of humin formation can then be attributed to lower concentrations of
5-HMF resulting from reduction in the dehydration of glucose. The possibility that the reduction in glucose dehydration is caused by a decrease in concentration of protons due to dilution can be ruled out by an experiment in which acetonitrile was added instead of water. The experiment using acetonitrile (Table 2.2, Experiment 6) produced similar results to those without gradual water addition (Table 2.2, Experiment 1), with slightly higher yields of cellobiose, glucose and 5-HMF. While acetonitrile is a polar solvent, its dielectric constant is less than that of water, leading to the conclusion that it is not effective in diverting the protons present in the IL from interacting with glucose and promoting its dehydration.

2.4 Conclusions

The present study has shown that glucan produced by the dissolution of crystalline cellulose in ILs undergoes acid catalyzed hydrolysis to form glucose and cellobiose as the initial products. The initial rate of glucan hydrolysis for Avicel dissolved in [Bmim][Cl] and H$_2$SO$_4$ as the catalyst was determined to be first order in the concentrations of glucan and acid, and zero order in water. The zero order dependence on water indicates that cleavage of glycosidic bonds near the chain end is irreversible. The apparent activation energy for glucan hydrolysis was determined to be 96 kJ mol$^{-1}$. Oligosaccharides longer than cellobiose were not detected under the reaction conditions used, suggesting that the acid-catalyzed cleavage of β-1,4 glycosidic bonds on the interior of glucan strands is likely to be reversible due to slow diffusion of the cleaved ends away from each other. Acids with pKa < 0.5 were active for glucan hydrolysis, and acids with pKa < -2.0 were equally effective independent of anion composition, suggesting that these acids are completely ionized. At higher conversions of glucan, dehydration of glucose to 5-HMF and the formation of humins were observed. The addition of water at the level 1-10 molar equivalents per β-1,4 glycosidic bonds had no effect on the initial rate of glucan hydrolysis, suggesting that the O atoms of the glycosidic linkages are more nucleophilic than the O atoms in water. Addition of larger quantities of water to the reaction mixture, particularly once the formation of substantial amounts of glucose had formed, inhibited the dehydration of glucose to 5-HMF and the formation of humins. The inhibition of glucose dehydration is attributed to preferential protonation of water compared with the C$_2$-OH hydroxyl of glucose. The reduction in humin formation is attributed to the decrease in 5-HMF formation with increasing glucose formation, since humin formation is ascribed to the condensation polymerization of 5-HMF and glucose. This process may be further inhibited by water solvation of protons, making free protons less available for protonation of the O atoms of the carbonyl groups in 5-HMF, the process thought to be responsible for propagation of humin formation.
Figure 2.1 Cellulose hydrolysis product evolution as a function of time. T = 363 K, 15 mL [Bmim][Cl], 0.75 g Avicel, [H$_2$SO$_4$] = 150 mM, [H$_2$O] = 550 mM Circles = glucose, triangles = cellobiose, squares = hydroxymethyl furfural, diamonds = humins. Lines are drawn to guide the eye.
Figure 2.2 Comparison of hydrolysis rates of cellulose and cellobiose; Reaction Conditions: \(T = 363\, K\), 15 mL [Bmim][Cl], 0.75 g Avicel or 0.83 g cellobiose, \([\text{H}_2\text{SO}_4]\) = 150 mM, \([\text{H}_2\text{O}]\) = 550 mM Circles = cellobiose, triangles = cellulose. \(r_0 = m^*t\) with \(m = 0.4137\) for cellobiose, and \(m = 0.2746\) for cellulose.

Table 2.1 Maximum yields and time to reach maximum yields for hydrolysis of cellulose in [Bmim][Cl]. \(T = 363\, K\), 15 mL [Bmim][Cl], 0.75 g Avicel, \([\text{H}_2\text{SO}_4]\) = 150 mM, \([\text{H}_2\text{O}]\) = 550 mM

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<th>(t_{G\text{Max}})</th>
<th>(Y_{C\text{BMax}})</th>
<th>(t_{C\text{BMax}})</th>
<th>(Y_{\text{HMFMax}})</th>
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Figure 2.3 Effect of varying acid strength on glucose production from cellulose. $T = 363$ K, 15 mL [Bmim][Cl], 0.75 g Avicel, varied $[\text{H}_2\text{SO}_4]$ from 15 mM to 300 mM, $[\text{H}_2\text{O}] = 550$ mM. Circles = 15 mM H$_2$SO$_4$, triangles = 30 mM H$_2$SO$_4$, squares = 150 mM H$_2$SO$_4$, diamonds = 300 mM H$_2$SO$_4$. Lines are drawn to guide the eye.
**Figure 2.4** Effect of varying acid strength on 5-HMF production from cellulose. T = 363 K, 15 mL [Bmim][Cl], 0.75 g Avicel, varied [H$_2$SO$_4$] from 15 mM to 300 mM, [H$_2$O] = 550 mM. Circles = 15 mM H$_2$SO$_4$, triangles = 30 mM H$_2$SO$_4$, squares = 150 mM H$_2$SO$_4$, diamonds = 300 mM H$_2$SO$_4$. Lines are drawn to guide the eye.
Figure 2.5 Effect of varying acid strength on Humin production from cellulose. $T = 363$ K, 15 mL [Bmim][Cl], 0.75 g Avicel, varied $[\text{H}_2\text{SO}_4]$ from 15 mM to 300 mM, $[\text{H}_2\text{O}] = 550$ mM. Circles = 15 mM $\text{H}_2\text{SO}_4$, triangles = 30 mM $\text{H}_2\text{SO}_4$, squares = 150 mM $\text{H}_2\text{SO}_4$, diamonds = 300 mM $\text{H}_2\text{SO}_4$. Lines are drawn to guide the eye.
Figure 2.6 Arrhenius plot of rate of cellulose hydrolysis to glucose as a function on inverse temperature. 15 mL [Bmim][Cl], 0.75 g Avicel, [H$_2$SO$_4$] = 150 mM, [H$_2$O] = 550 mM. Linear fit gives $\ln (r_o) = -11,500 T^{-1} + 27$, and $E_A = 95$ kJ mol$^{-1}$. 
Figure 2.7 Effect of varying initial water content on total yield of depolymerization products. T = 363 K, 15 mL [Bmim][Cl], 0.75 g Avicel, [H₂SO₄] = 300 mM, varied [H₂O] from 150 mM to 1500 mM. Circles = 1 molar equivalents of water, triangles = 2 molar equivalents of water, squares = 3.5 molar equivalents of water, diamonds = 10 molar equivalents of water. Lines are drawn to guide the eye.
Figure 2.8 Effect of varying initial water content 5-HMF yield. \( T = 363 \text{ K}, 15 \text{ mL} \) [Bmim][Cl], 0.75 g Avicel, \([\text{H}_2\text{SO}_4] = 300 \text{ mM}, \) varied \([\text{H}_2\text{O}]\) from 150 mM to 1500 mM. Circles = 1 molar equivalents of water, triangles = 2 molar equivalents of water, squares = 3.5 molar equivalents of water, diamonds = 10 molar equivalents of water. Lines are drawn to guide the eye.
Table 2.2. Varying water addition strategy to maximize glucose yield and limit 5-HMF formation. $T = 378$ K, 400 mg [Emim][Cl], 20 mg Avicel, initial $[\text{H}_2\text{SO}_4] = 105$ mM, $[\text{H}_2\text{O}]$ increased from 5 wt% (400 mM) to 43 wt% (5500 mM)

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**Figure 2.9** Proposed reaction mechanism for hydrolysis of cellulose.

**Figure 2.10** Rate law data for cellulose hydrolysis. T = 363 K, 15 mL [Bmim][Cl] Black Circles = varied [H$_2$O] from 150 mM to 1500 mM with 0.75 g Avicel, [H$_2$SO$_4$] = 150 mM, white circles = varied [H$_2$SO$_4$] from 150 mM to 550 mM with 0.75 g Avicel, [H$_2$O] = 550 mM black triangles = varied Avicel loading from 0.15 g to 1.5 g with [H$_2$SO$_4$] = 150 mM, [H$_2$O] = 550 mM. $r_0 = k \times [\text{Acid}]^x [\text{H}_2\text{O}]^y [\text{O-linkage}]^z$ with $x = 1.0038$, $y = 0.0067$, $z = 1.0673$ determined experimentally.
**Figure 2.11** Proposed reaction mechanism for 5-HMF formation.

**Figure 2.12** Proposed reaction mechanism for humin formation.
References


Chapter 3
Effects of Reaction Conditions on the Acid-Catalyzed Hydrolysis of Miscanthus Dissolved in an Ionic Liquid

Abstract
Experiments were conducted to study the effects of reaction conditions on the hydrolysis of miscanthus dissolved in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) catalyzed by H$_2$SO$_4$. It was determined that while there is a small co-inhibition effect associated with the simultaneous hydrolysis of the cellulosic and hemicellulosic portions of miscanthus, the largest rate decreases were observed for the hydrolysis of the hemicellulosic portion. This rate decrease was attributed to the chemical linkage between hemicellulose and lignin in miscanthus, which could be broken with chemical pretreatment. While chemical pretreatment increased the rate of the hydrolysis of the hemicellulosic component, delignification showed no further benefit. The rate of hydrolysis was determined to be first order in concentrations of β-1,4 glycosidic linkage and acid, and zero order in water concentration. The activation energy for the hydrolysis of the glycosidic linkages in the cellulosic and hemicellulosic components were determined to be 95 kJ mol$^{-1}$ and 114 kJ mol$^{-1}$ respectively. Progressive addition of water during the first hour of reaction increased conversion and selectivity to saccharine products, while limiting dehydration of the sugars formed. The conversion of the cellulosic portion of miscanthus could be increased after the first hour of reaction by increasing the reactor temperature. While miscanthus is only partially soluble in [Emim][Cl], it was found that the initial miscanthus loading could be increased to 9 wt% before significant yield decreases attributed to solubility limitations of the cellulosic component were observed. By proper adjustment of reaction conditions, it was possible to achieve yields of sugars approaching 84% from the cellulosic and hemicellulosic components of miscanthus, with minimal dehydration of the sugars to furans.

3.1 Introduction
The sugars comprising the cellulosic and hemicellulosic components of lignocellulosic biomass are attractive starting materials for producing fuels and chemicals; however, gaining access to these components requires thermal or chemical pretreatment of raw biomass. This recalcitrance of biomass is a direct consequence of its structure, which consists of fibrils of crystalline cellulose (25-55 wt%) partially surrounded by strands of amorphous hemicellulose (20-40 wt%) aligned with the cellulose fibrils that are, in turn, encased in lignin (10-30 wt%), a cross-linked, hydrophobic polymer that forms a protective sheath around the carbohydrate fraction.$^{[1]}$

Recent studies have demonstrated that dissolution of lignocellulosic biomass in ionic liquids (ILs) is a potentially attractive means for isolating the
carbohydrate components of biomass. For example, it has been demonstrated that 2-8 wt% of raw or thermally pretreated biomass can be dissolved in imidazolium chloride ILs\[^2\], whereas solubilities of up to 20 wt% can be attained for crystalline cellulose, hemicellulose, and lignin dissolved in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]).\[^3\] A further advantage to using ILs is that the hydrolysis of the dissolved cellulose and hemicellulose can be carried out in this medium using inorganic acids as catalysts.\[^4, 5\] Nevertheless, attainment of high yields of sugars (e.g., glucose, xylose) by acid-catalyzed hydrolysis of cellulose or hemicelluloses is difficult because the sugars can undergo dehydration to produce furanic compounds (e.g., 5-hydroxymethyl furfural, furfural), which can further react with the sugars to form humins.\[^4, 6\] The formation of furans and humins can be minimized, though, by the strategic addition of water as hydrolysis proceeds, resulting, for example, in 70% glucose yield and 79% xylose yield after a two stage HCl-catalyzed hydrolysis of corn stover dissolved in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]).\[^4\]

Miscanthus has been identified as one of several lignocellulosic feedstocks for biofuel production. This perennial grass is particularly attractive because it can be grown with higher annual yields per hectare and with lower water and soil nutrient requirements compared to other biofuel feedstocks, such as corn or poplar.\[^7\] This increased efficiency translates to a biofuel feedstock that does not compete with food crops for nutrient rich soil and can be grown on large areas of land that could be used exclusively for biofuel production.

The aim of this investigation was to establish the effects of reaction conditions on the acid-catalyzed hydrolysis of miscanthus dissolved in the ionic liquid 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]). The influences of temperature, acid concentration, miscanthus concentration, and the strategic addition of water were explored. Complimentary studies of the hydrolysis of cellulose and hemicellulose were carried out in an effort to understand the similarities and differences between the rate of hydrolysis of these pure biopolymers and the corresponding rate of hydrolysis of the cellulosic and hemicellulosic portions of miscanthus. The influence of miscanthus pretreatment in order to cleave the lignin-hemicellulose linkages was also undertaken.

### 3.2 Experimental

#### 3.2.1 Materials

Unless noted otherwise, materials were used as received. Ionic liquids 1-ethyl-3-methylimidazolium chloride, ([Emim][Cl], 98% purity), and 1-ethyl-3-methylimidazolium tosylate ([Emim][Tos], 98% purity), were purchased from Iolitec, Germany. Analytical grade n-methylpiperdinium acetate ([NMP][OAc]) was purchased from BIONIQS, United Kingdom. Miscanthus was supplied by the Energy Biosciences Institute from the University of Illinois Urbana-Champaign. Avicel (microcrystalline cellulose, PH-101, DP < 350), xylose, Xylan from birchwood, 1,6 hexane diol, cellobiose, arabinose, 5-hydroxymethylfurfural (5-HMF, 99% purity), furfural (99% purity), mixed bed resin TMD-8 hydrogen and hydroxide form, and ethylene diamine (EDA, 99% purity) were purchased from
Sigma Aldrich, USA. USP Grade Glucose was purchased from Hyclone, USA. Sulfuric acid (H$_2$SO$_4$, 98% purity) was purchased from Acros, USA.

3.2.2 Miscanthus Pretreatment
All miscanthus was ground using a Retsch Rotor Beater Mill SR300 equipped with an 80 micron filter. For pretreatment of miscanthus in [Emim][Tos], 9 g of IL was heated to 343 K in a 20 mL glass screw top vial to which was added 1 g of ethylene diamine (EDA), and the resulting mixture was stirred at 800 rpm with a magnetic stir bar. Miscanthus (1.25 g) was added to the IL/EDA mixture, which was then stirred for 6-10 h. The solution was allowed to cool, and 10 g of water (Milli-Q ultrapure) was added to precipitate the solids. The sample was centrifuged and the solid sediment was washed with excess water to remove residual IL. All IL was considered to be removed when the wash phase registered a pH = 7 and showed no peak at 220 nm in the UV visible spectra. The washed solid was placed in a vacuum oven at 0.03 bar and 378 K overnight and then stored in a desiccator. Pretreatment of miscanthus in [NMP][OAc] was carried out in an analogous method for 10-12 h at 323 K before precipitation and washing.

The cellulose, hemicellulose, lignin, and ash content of the starting substrates were determined according to the procedure described in the National Renewable Energy Laboratory (NREL) Technical Report NREL/TP-510-42618.[8] The results of these analyses are reported in Table 3.1.

3.2.3 Miscanthus Hydrolysis
Miscanthus (80 µm particle size) was hydrolyzed on the deck of a Symyx Core Module Robot (CMR) equipped with heating and stirring capabilities. In a typical experiment, [Emim][Cl] was heated to 378 K, and the water content of substrates (usually 4-8 wt% water) and [Emim][Cl] (usually 0.5 – 1.5 wt% water) was then measured using a Karl Fischer apparatus (EM Science Equistar C-2000). [Emim][Cl] [390 µL (500 ± 20 mg)] was then dispensed manually using a Rainin micropipette into a 4 mL screw-top glass vial containing a magnetic stir bar. The stir speed was set to 300 rpm, and 22.5 mg of miscanthus was added to the IL. The reactor was sealed and the miscanthus was allowed to dissolve at 378 K for 6 h. After dissolution, the reactor temperature was decreased to 373 K and held at this temperature for 1 h. Reaction was initiated by adding 29 µL of a premade solution of 1.66 M H$_2$SO$_4$ (4.72 mg concentrated H$_2$SO$_4$ and 26 mg H$_2$O). At 10, 20, 30, and 60 min, the reactor was opened and 100 µL, 50 µL, 75 µL and 125 µL of water was added, after which the reactor was resealed. Reactors were removed from the heated CMR deck at specified time intervals and quenched in an ice bath. The reactor was then diluted with 600 µL of ultrapure water containing 10 mg mL$^{-1}$ 1,6 hexane diol to serve as an internal standard. The sample was centrifuged, and 500 µL of the supernatant was treated with a mixed hydrogen and hydroxide form ion exchange resin and centrifuged before the supernatant was analyzed using a high performance liquid chromatography system equipped with a refractive index detector (HPLC-RID).

3.2.4 Analytical techniques
HPLC-RID analysis was conducted using a Shimadzu HPLC equipped with a Biorad Aminex HPX-87H column at 333 K. Samples were eluted with a 0.01N H$_2$SO$_4$ mobile phase at 0.6 mL min$^{-1}$. Products were identified by comparison of retention times with pure substances. Quantification was determined by dividing the integrated peak areas of hydrolysis products (cellobiose, glucose, 5-HMF, xylose, arabinose, furfural), by the integrated peak area of the internal standard (1,6 hexane diol) and converting the area ratio to a molar concentration using a seven-point calibration curve.

Product yields were determined on a molar basis using “dry” weight and were reproducible to within ± 6% of the reported value. The initial substrate weight was multiplied by the dry weight fraction as determined by Karl Fischer analysis to determine the initial “dry” weight. The moles of glucose residues in miscanthus were calculated by multiplying the initial “dry” weight by the cellulose mass fraction determined by the NREL analysis, and dividing by the molecular weight of a glucose residue (162 g mol$^{-1}$). Initial moles of xylose residues were determined using similar methods with the hemicellulose mass fraction and the molecular weight of a xylose residue (132 g mol$^{-1}$). It was assumed that cellobiose, glucose, and 5-hydroxymethyl furfural could only be produced from cellulose, while xylose, arabinose, and furfural could only be produced from hemicellulose. The product yield (%) was then determined by multiplying the moles of a product derived from cellulose or hemicellulose by the number of residues contained in the product and dividing by the initial number of moles of residues. Conversion of cellulose or hemicellulose was calculated as the sum of the respective measured product yields. The yield of humins, degradation products formed via the reaction of sugars with the aldehyde functionality of furfurals, was not quantified and was not accounted for in conversion.

Miscanthus conversion represents the total fraction of hemicellulose and cellulose converted to products. Miscanthus conversion was calculated by multiplying the individual component conversion by their respective weight fraction, and dividing the sum of the terms by the sum of the weight fractions. Saccharide yield represents the yield of products from miscanthus that forms cellobiose, glucose, xylose, or arabinose. Saccharide yield is calculated by multiplying of the total saccharide yield of an individual component by its respective weight fraction, and dividing the sum of the terms by the sum of the weight fractions. Dehydration yield is analogous to saccharide yield, but is defined on the basis of 5-HMF and furfural. Selectivity to saccharide products is defined as the saccharide yield, divided by the miscanthus conversion.

### 3.3 Results and Discussion

#### 3.3.1 Hydrolysis of Model Compounds Compared to Pretreated and Raw Miscanthus

Table 3.2 lists the initial rates of hydrolysis of cellulose (Avicel) and hemicellulose (Xylan) measured under conditions for which the concentrations of β-1,4 glycosidic linkages, acid, and water are identical. For each polymer, the rate of hydrolysis is characterized by an apparent second-order rate coefficient, since rates of cellulose and hemicelluloses hydrolysis are first order with respect...
to the concentration of β-1,4 glycosidic linkages and acid, and zero order in water concentration.\cite{9} Since the ratio of cellulose to hemicellulose differs for the pretreated substrates presented in Table 3.2, examination of the apparent second-order rate coefficient provides a basis for comparing the intrinsic rates of hydrolysis of cellulose and hemicellulose for each of the cases. It is evident that the rate of hemicellulose hydrolysis is approximately 1.4 times faster than the rate of cellulose hydrolysis. It is also apparent that the rate coefficient for hemicellulose hydrolysis is 1.4 times higher than that for cellulose hydrolysis. The rate of hydrolysis of a mixture of Avicel and Xylan from birchwood made up to contain the distribution of these two polymers found in raw miscanthus (see Table 1) was also measured. Table 3.2 shows that while rate coefficients for the hydrolysis of cellulose and hemicelluloses are somewhat smaller than those characterizing the hydrolysis of each substrate separate, the ratio of the rate coefficient for hemicellulose hydrolysis to that for cellulose is 1.7.

The next entry in Table 3.2 compares the initial hydrolysis of raw miscanthus under the same conditions used to measure the rates of cellulose and hemicellulose hydrolysis. As in the case of Avicel and Xylan, the rates of hydrolysis of the cellulosic and hemicellulosic potions of miscanthus are first order in acid concentration and zero order in water concentration as shown in Figure 3.1.\cite{9,10} Since the solubility of miscanthus is limited, we assumed that that the rates of hydrolysis of both biopolymers were first-order in the concentration of β-1,4 glycosidic linkages. It is evident from Table 3.2 that while the rate of hydrolysis of the cellulosic portion of miscanthus is similar to that for the hydrolysis of Avicel contained in a physical mixture of Avicel and Xylan, the rate of hydrolysis of the hemicellulosic portion of miscanthus is significantly lower than that for the hydrolysis of Xylan in the mixture. Likewise, the apparent second-order rate coefficient for the hydrolysis of the cellulosic portion of miscanthus is comparable to that for the Avicel-Xylan mixture, but the apparent rate coefficient for the hydrolysis of the hemicellulosic portion of the miscanthus is significantly lower than that for the hydrolysis of hemicelluloses in the Avicel-Xylan mixture. Additionally, both the rate and rate coefficient of the hemicellulosic portion in miscanthus are lower than the cellulosic portion, a reverse of the trend observed for Xylan and Avicel. A possible cause for the slower hydrolysis of the hemicellulosic portion of miscanthus is its chemical linkage to lignin. To test this hypothesis, miscanthus was pretreated in mixtures of either ethylene diamine and [NMP][OAc] or [Emim][Tos] to cleave the hemicellulose-lignin bonds, and the solid precipitated from these solutions was subjected to hydrolysis under the same conditions as those used for the hydrolysis of raw miscanthus. Table 3.2 shows that both pretreatments raised the apparent rate coefficient for hydrolysis of the hemicellulosic portion of miscanthus to levels comparable to, or greater than, that for hemicellulose present in the Avicel-Xylan mixture. At the same time, pretreatment had little effect on the apparent rate coefficient for the hydrolysis of the cellulosic portion of miscanthus. It is also notable that whether the pretreatment achieved significant or minimal delignification (see Table 3.1) had little effect on the rate coefficients for the hydrolysis of the cellulosic and hemicellulosic portions of pretreated miscanthus.\cite{11}
The apparent activation energies for the hydrolysis of the glycosidic linkages in the cellulosic and hemicellulosic portions of raw miscanthus were determined to be 95 kJ mol\(^{-1}\) and 114 kJ mol\(^{-1}\), respectively as shown in Figure 3.2. While both these values are higher than those for the hydrolysis of Avicel, 84 kJ mol\(^{-1}\), and Xylan, 60 kJ mol\(^{-1}\),\(^{10}\) the activation energy for the hydrolysis of cellulose in miscanthus is only 11 kJ mol\(^{-1}\) higher than that for the hydrolysis of Avicel and within the error bonds of our measurements (± 13 kJ mol\(^{-1}\)). On the other hand, the measured activation energy for hydrolysis of hemicellulose in miscanthus is significantly larger than that for the hydrolysis of Xylan. The higher activation energy for the former case likely reflects the fact that lignin limits the accessibility of protons to the linkages between residues in hemicellulose. Consistent with this reasoning, we note that when the lignin-hemicellulose linkages are broken by pretreatment of miscanthus prior to initiation of hydrolysis, the rate coefficient for the hydrolysis of hemicellulose derived from miscanthus and Xylan become nearly comparable (see Table 3.2).

### 3.3.2 Effects of Water Addition of Miscanthus Hydrolysis

The effect of gradual water addition during the first hour of miscanthus hydrolysis is shown in Figure 3.3. The water content of the reactor solution was 5 wt% at 0 min and then gradually increased upon addition of 15 wt%, 5 wt%, 8 wt%, and 10 wt% water at 10, 20, 30, and 60 min, respectively. This sequence is identical to that used by Binder and Raines, and was found to be optimal for the hydrolysis of both Avicel and corn stover, since it minimized the dehydration of saccharides, without causing precipitation of the substrate.\(^{4}\) As seen in Figure 3.3, the initial yields of saccharides (glucose, cellobiose, xylose, and arabinose) or dehydration products (5-HMF and furfural) were not influenced by water addition. However, after 30 min the addition of water had a dramatic effect on the yields of all products. Gradual addition of water to the reaction mixture drastically increased the yield of saccharides to near 73%, while limiting yield of dehydration products to 4%, with no visual signs of humins after 120 min. The dramatic increase in saccharide yield due to inhibition of dehydration is consistent with trends reported for Avicel and Xylan carried out with gradual addition of water.\(^{4, 9, 10}\)

Comparisons of the hydrolysis the cellulosic and hemicellulosic portions of miscanthus with either Avicel or Xylan with gradual addition of water are shown in Figure 3.4 and Figure 3.5 with detailed product yields shown in Table 3.3. As previously noted in Figure 3.3, experiments with gradual water addition such as those in Figure 3.4 and Figure 3.5, produce primarily saccharide products with very small yields of dehydration products. In Figure 3.4 and Figure 3.5, both Avicel and Xylan react more rapidly than the cellulosic and hemicellulosic portions of miscanthus, consistent with findings reported in Table 3.2. After 60 min, the glucose yield from Avicel was 64%, while the glucose yield from miscanthus was 51%, which accounted for the majority of the 14% difference in conversion between these substrates. This difference in glucose yield increased
gradually from 60 min to 120 min reaching 22% at 120 min (glucose yield from Avicel was 85%, and 63% from miscanthus).

Since Xylan is more reactive than the other substrates, both xylose and furfural accumulated more rapidly in the reactor. After 30 min of reaction, the yields of xylose and furfural from Xylan were 62% and 4%, respectively, as compared to 46% xylose and 3% furfural for the hemicellulosic portion of miscanthus. The increase in furfural and xylose production from Xylan prior to increasing the water concentration to 43 wt% at 60 min resulted in the production of humins. The concentration of humins continued to increase as the xylose yield decreased from 73% at 60 min to 63% at 120 min, while furfural yield only increased from 5% to 7% over the same time period. Conversely, the xylose yield from the hemicellulosic portion of miscanthus stabilized at approximately 73% yield after the gradual water addition was completed at 60 min.

As shown in Table 3.3, the conversion of the hemicellulosic component of miscanthus is faster than the conversion of the cellulosic component under conditions of gradual water addition, in contrast to the relationship in the initial rates of hydrolysis of the miscanthus biopolymers reported in Table 3.2. The linkages between lignin and hemicellulose, as well as the linkages within lignin, could be more amenable to cleavage when water is added to an ionic liquid.\cite{10, 12}

In support of this idea, Ekerdt and coworkers have reported that addition of water to 1-H-3 methylimidazolium chloride increased the rate at which the β-O-4 linkage in a lignin model compound was cleaved.\cite{13} Therefore, we propose that progressive water addition results in increased lignin fractionation and separation from the hemicellulose in miscanthus, which increases proton accessibility to the glycosidic linkages between residues, thereby increasing the rate of hydrolysis.

Table 3.4 lists the distribution of products formed via the hydrolysis of the cellulosic and hemicellulosic portions of miscanthus determined after 150 min of reaction under the conditions reported in Figure 3.4 and Figure 3.5. As previously discussed, while the conversion of the hemicellulosic portion of miscanthus is comparable to Xylan, there is a 22% lower conversion of the cellulosic portion compared to Avicel. Earlier work by Binder and Raines on the hydrolysis of corn stover dissolved in [Emim][Cl] has shown that additional products could be obtained after the unreacted substrate was precipitated with water, washed and dried, redissolved in [Emim][Cl] and then subjected to further hydrolysis.\cite{4} Using a similar strategy, we found that after 90 min of hydrolysis of redissolved miscanthus, overall conversions of 98% and 93% could be achieved for the cellulosic and hemicellulosic portions, respectively, corresponding to an overall miscanthus conversion of 96%, as shown in Table 3.4.

### 3.3.3 Modification of Reactor Conditions to Optimize Saccharide Yield

Attempts to increase conversion by modifying hydrolysis conditions while maintaining the same water addition strategy are summarized in Table 3.5. Selected data from Table 3.3 are shown as entries 1-4 for comparison. Doubling the acid concentration (entry 5-8) increased the conversion of miscanthus from 46% to 64% in 30 min and from 77% to 82% after 120 min, as shown by entries 5 and 8. Moreover, the conversion of the cellulosic portion of miscanthus increased
from 73% to 87% in 120 min. However, doubling the acid concentration caused a decrease in the yield of xylose and an increase in the yield of furfural. As illustrated by entries 4 and 8, the xylose yield decreased from 73% to 52% at the same time that the furfural yield increased from 6% to 14%. The remaining portion of xylose (13%) likely formed humic substances that precipitated with the lignin rich solids upon dilution of the reaction solution for analysis. Since cellulose and hemicellulose react at different rates, it is difficult to adjust the reaction conditions so as to maximize the conversion of both carbohydrate components of biomass. If the reaction is optimized for hemicellulose conversion, the cellulose portion of miscanthus remains incompletely hydrolyzed, and if the reaction conditions are adjusted to optimize cellulose conversion, the hemicellulosic fraction undergoes dehydration, resulting in undesired products.

In an effort to convert the incompletely hydrolyzed cellulosic portion of biomass, the temperature of the reactor was increased at 60 min after the final water addition and maintained as shown in entry 9-13 in Table 3.5. Increasing the temperature of the reactor to 383 K after the first hour slowly increased the conversion of both cellulose and hemicellulose over the next 60 min (Table 3.5, Entry 9-10). However, at 150 min the xylose yield began to decrease due to xylose dehydration to furfural and formation of humins. This process was accelerated if the reaction temperature is adjusted to 393 K at 60 min, enabling the attainment of a maximum miscanthus conversion of 90% with 94% selectivity to saccharides at 90 min (Table 3.5, entry 12). If the reaction was allowed to progress to 120 min, both the glucose and xylose underwent dehydration to furfural and 5-HMF, which then reacted to form humins, thereby decreasing the overall conversion to soluble products (Table 3.5, entry 13). The capability to hydrolyze additional biopolymer after 60 min by increasing temperature is attributed to changes in the water content of the reaction solution. The apparent activation energy for acid catalyzed hydrolysis of dissolved cellobiose calculated from previously published reports is approximately 84 kJ mol\(^{-1}\) in [Emim][Cl] compared to 110 kJ mol\(^{-1}\) in water.\(^5\)\(^,\)\(^14\) In the system under investigation, the water concentration is initially 5 wt%, which equates to approximately 0.5 molecules of water per molecule of [Emim][Cl]. After 60 min of reaction, the water concentration increased to 43 wt%, or about 6 water molecules per molecule of [Emim][Cl]. Therefore, we suggest that increasing the water content of the reaction mixture during the course of hydrolysis may result in an increase in the apparent activation cellulose hydrolysis. Consequently, complete hydrolysis of the cellulose would require progressively higher temperatures as the water concentration is increased. This interpretation might also explain why the conversion of cellulose and hemicellulose plateaus after 60 min.

### 3.3.4 Effects of Pretreatment on Hydrolysis Yield

The two pretreated samples of miscanthus (see Table 3.1) were reacted under the optimal conditions reported Table 3.5 and the results are shown in Table 3.6. Before the temperature was increased at 60 min, both pretreated substrates produced larger yields of cellobiose and yields of products derived
from hemicellulose than was observed with raw miscanthus (Table 3.5: Entry 2, Table 3.6: Entry 2, 7). This is likely a consequence of the pretreatment process, which makes the biomass more amenable to hydrolysis. The increased hemicellulose saccharide yield during the first hour of reaction of pretreated substrates resulted in dehydration of the monosaccharides after the temperature was increased to 393 K at 60 min and led to large amounts of furfural (Table 3.5: Entry 12, Table 3.6: Entry 5, 8). A maximum miscanthus conversion of 93% was obtained using the [NMP][OAc]-pretreated miscanthus, with 94% selectivity to sugars. It should be noted however, that pretreatment resulted in substrate losses as shown in Table 3.7. The miscanthus conversion based on the initial amount of miscanthus that was pretreated (Table 3.6, Entry 9) is actually 65%, because 25% of the cellulose portion and 41% of the hemicellulose portion were lost in the wash phase of the pretreatment process. This observation suggests that pretreatment of miscanthus prior to acid-catalyzed hydrolysis will not result in enhanced yield of saccharides.

Miscanthus, like many lignocellulosic substrates, is only partially soluble in [Emim][Cl] in its raw form.[5,15] To investigate the effect of miscanthus loading on the extent of hydrolysis, experiments were carried out in which the initial concentration of miscanthus in [Emim][Cl] was varied from 4.5 wt% to 18 wt%. Hydrolysis was then carried out at 373 K in the presence of H$_2$SO$_4$ and with the gradual addition of water. The conversions for the cellulosic and hemicellulosic portions of miscanthus are shown in Figure 3.6 and Figure 3.7 with product distributions reported in Table 3.8. The conversion of the cellulosic component decreased from 73% to 66% when the initial weight loading of miscanthus was increased from 4.5 wt% to 9 wt%. When the initial loading was increased further to 18 wt% the cellulosic conversion dropped significantly to 29%. Nevertheless, the selectivity to sugars remained constant at 93% in all cases. As the initial weight percent loading was increased, the decrease in the conversion of the hemicellulosic component was much smaller than the cellulosic component. As shown in Figure 3.7, the hemicellulose conversion decreased from 84% to 73% as the initial weight loading was increased from 4.5 wt% to 18 wt%, while the selectivity to sugars remained constant at 92%. The apparent reaction orders determined from the initial rate data presented in Figure 3.6 and Figure 3.7 follow similar trends. When the 18 wt% weight loading data point was considered, the apparent reaction order for the hemicellulosic portion decreased from 1.02 to 0.88, while the order for the cellulosic portion decreased from 0.75 to 0.33. These findings suggest that the hemicellulosic portion of miscanthus is more soluble and readily hydrolyzed in the ionic liquid compared to the cellulosic portion and the decrease in conversion of the cellulosic portion is due to the onset of solubility limitations.

3.4 Conclusions

The present study has demonstrated that high yields of glucose and xylose with minimal formation of furfurals and humins, can be achieved by the hydrolysis of raw miscanthus dissolved in [Emim][Cl] using H$_2$SO$_4$ as the catalyst. The kinetics of the hydrolysis of the cellulosic and hemicellulosic portions of
miscanthus are first order in acid concentration and zero order in water concentration. The apparent second-order rate coefficients for the hydrolysis of the cellullosic and hemicellulosic portions of miscanthus are about 2.5 times lower than those for Avicel and Xylan, materials used to represent pure cellulose and hemicellulose. These differences are attributed to the inhibiting effects of lignin in raw miscanthus. Consistent with this interpretation, the apparent activation energy for hydrolysis of the cellullosic part of miscanthus is 95 kJ mol\(^{-1}\), whereas that for Avicel is 84 kJ mol\(^{-1}\), and apparent activation energy for the hemicellulosic portion of miscanthus is 114 kJ mol\(^{-1}\), whereas that for Xylan is 60 kJ mol\(^{-1}\).[10] Cleavage of the lignin-hemicellulose linkages by ethylene diamine pretreatment of miscanthus increased the rate of hydrolysis of both the cellullosic and hemicellulosic portions, but delignification showed no additional improvement. The conversion of miscanthus to saccharine products is improved by the gradual addition of water to the reaction mixture, which limits the dehydration of the saccharides to furfurals and the formation of humins. Increasing the concentration of the acid catalyst increases the conversion of the cellullosic portion of miscanthus to glucose but decrease the conversion of the hemicellulosic portion of miscanthus to xylose due to dehydration of this product to furfural and its subsequent condensation with glucose and xylose to form humins. Increasing the reaction temperature from 373 K to 393 K, after 60 min of reaction at the lower temperature, results in significant increases in the yields of saccharides. High yields of saccharides can be achieved with initial miscanthus loadings of up to 9 wt%, but increasing the initial loading to 18 wt% lowered the conversion to soluble products, most significantly for the cellullosic component.
Table 3.1 NREL Composition analysis of initial substrates studied

<table>
<thead>
<tr>
<th>Composition per gram substrate (Dry basis)</th>
<th>Cellulose</th>
<th>Hemicellulose</th>
<th>Lignin</th>
<th>Other (Ash, Pectin, etc.)</th>
</tr>
</thead>
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<tr>
<td>Miscanthus</td>
<td>42%</td>
<td>25%</td>
<td>28%</td>
<td>9%</td>
</tr>
<tr>
<td>Avicel</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Xylan&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>90%</td>
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<td>0%</td>
</tr>
<tr>
<td>NMP Oac&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38%</td>
<td>18%</td>
<td>24%</td>
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<tr>
<td>Emim Tos&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54%</td>
<td>17%</td>
<td>9%</td>
<td>17%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Xylan from Birchwood
<sup>b</sup> 1.27 g of miscanthus was pretreated in 10 g of a solvent mixture of 10 wt% [NMP][OAc] in EDA for 10-12 hrs at 323 K.
<sup>c</sup> 1.21 g of miscanthus was pretreated in 10 g of a solvent mixture of 10 wt% [Emim][Tos] in EDA for 6-10 hrs at 343 K.

Table 3.2 Initial hydrolysis rate and rate constants of initial substrates studied in [Emim][Cl]. T = 373 K, 0.5 mL [Emim][Cl], [H$_2$SO$_4$] = 80 mM, [O-linkage] = 132 mM, [H$_2$O] = 110 mM.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Initial Rate*10$^3$ μmol s$^{-1}$ cm$^{-3}$</th>
<th>Apparent Rate Coefficient k*10$^6$ (cm$^3$ μmol$^{-1}$ s$^{-1}$)</th>
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<tr>
<td></td>
<td>Cellulose</td>
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<tr>
<td>[NMP][OAc]</td>
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<td>10.3</td>
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<tr>
<td>[EMIM][Tos]</td>
<td>13.8</td>
<td>12.7</td>
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</table>
Figure 3.1 Rate law data for miscanthus hydrolysis. \( T = 363 \text{ K}, 600 \text{ mg} \) [Emim][Cl], varied \([\text{H}_2\text{O}]\) from 75 mM to 1500 mM with 15.4 mg miscanthus, \([\text{H}_2\text{SO}_4]\) = 150 mM; varied \([\text{H}_2\text{SO}_4]\) from 15 mM to 300 mM with 15.4 mg miscanthus, \([\text{H}_2\text{O}]\) = 375 mM

Figure 3.2 Arrhenius plot of hydrolysis of hemicellulosic and cellulosic portions of miscanthus as a function of inverse temperature. 600 mg [Emim][Cl], 17.0 mg miscanthus, \([\text{H}_2\text{SO}_4]\) = 150 mM. Rate based on sum of all measured products.
Figure 3.3 Effect of gradual water addition on miscanthus hydrolysis in [Emim][Cl]. T = 373 K, 500 mg [Emim][Cl], 22.5 mg miscanthus, initial $[\text{H}_2\text{SO}_4] = 123$ mM, $[\text{H}_2\text{O}]$ increased from 5 wt% (400 mM) to 43 wt% (5500 mM)
Figure 3.4 Comparison of miscanthus conversion with Avicel using gradual water addition strategy in [Emim][Cl]. T = 373 K, 500 mg [Emim][Cl], 22.5 mg miscanthus or 16.5 mg Avicel, initial [H₂SO₄] = 123 mM, [H₂O] increased from 5 wt% (400 mM) to 43 wt% (5500 mM)
Figure 3.5 Comparison of miscanthus conversion with Xylan from Birchwood using gradual water addition strategy in [Emim][Cl]. T = 373 K, 500 mg [Emim][Cl], 22.5 mg miscanthus or 13.8 mg Avicel, initial [H$_2$SO$_4$] = 123 mM, [H$_2$O] increased from 5 wt% (400 mM) to 43 wt% (5500 mM)
Table 3.3 Individual product yields for hydrolysis of miscanthus, Avicel and Xylan from birchwood. T = 373 K, 500 mg [Emim][Cl], 22.5 mg miscanthus, 16.5 mg Avicel, or 13.8 mg Xylan from Birchwood, initial [H₂SO₄] = 123 mM, [H₂O] increased from 5 wt% (400 mM) to 43 wt% (5500 mM)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (min)</th>
<th>Yield: Cellulose Portion</th>
<th></th>
<th></th>
<th></th>
<th>Yield, Hemicellulose Portion</th>
<th></th>
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<th></th>
<th>Miscanthus Conversion</th>
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<td></td>
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<td>Arabinose</td>
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</table>
Table 3.4 Overall conversion of miscanthus after hydrolyzing precipitate in a second stage. First reaction: \( T = 373 \text{ K} \), 500 mg [Emim][Cl], 22.5 mg miscanthus, initial \([\text{H}_2\text{SO}_4] = 123 \text{ mM}\), \([\text{H}_2\text{O}]\) increased from 5 wt\% (400 mM) to 43 wt\% (5500 mM). Second reaction: \( T = 373 \text{ K} \), 300 mg [Emim][Cl], 6.5 mg residue from first reactor, initial \([\text{H}_2\text{SO}_4] = 123 \text{ mM}\), \([\text{H}_2\text{O}]\) increased from 5 wt\% (400 mM) to 43 wt\% (5500 mM).

<table>
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<tr>
<th>Stage</th>
<th>Time min</th>
<th>Yield; Cellulose Portion</th>
<th>Cellobiose</th>
<th>Glucose</th>
<th>5-HMF</th>
<th>Conversion</th>
<th>Yield, Hemicellulose Portion</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Furfural</th>
<th>Conversion</th>
<th>Miscanthus Conversion</th>
</tr>
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Table 3.5 Optimization of miscanthus hydrolysis by alteration of process conditions. $T = 373 \text{ K}$, 500 mg [Emim][Cl], 22.5 mg miscanthus, initial $[\text{H}_2\text{SO}_4] = 123 \text{ mM}$, $[\text{H}_2\text{O}]$ increased from 5 wt% (400 mM) to 43 wt% (5500 mM), changes in $T$ and $[\text{H}_2\text{SO}_4]$ are noted in key below table

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time min</th>
<th>Yield; Cellulose Portion</th>
<th>Conversion</th>
<th>Yield, Hemicellulose Portion</th>
<th>Conversion</th>
<th>Miscanthus Conversion</th>
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<td>Glucose</td>
<td>5-HMF</td>
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<td>29%</td>
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<td>4</td>
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<tr>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30</td>
<td>12%</td>
<td>53%</td>
<td>4%</td>
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<td>47%</td>
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<tr>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5%</td>
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<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>73%</td>
<td>6%</td>
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<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>76%</td>
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<td>72%</td>
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<td>12&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>79%</td>
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<td>120</td>
<td>2%</td>
<td>59%</td>
<td>6%</td>
<td>67%</td>
<td>75%</td>
</tr>
</tbody>
</table>

a: Initial $[\text{H}_2\text{SO}_4] = 248 \text{ mM}$
b: For first 60 min of reaction, $T$ held at 373 K. At 60 min, $T$ increased from 373 K to 383 K and maintained for the remaining reaction time.
c: For first 60 min of reaction, $T$ held at 373 K. At 60 min, $T$ increased from 373 K to 393 K and maintained for the remaining reaction time.
Table 3.6 Pretreated substrate hydrolysis using optimized process conditions. T = 373 K, 500 mg [Emim][Cl], 22.0 mg [Emim][Tos] pretreated miscanthus or 27.4 mg [NMP][OAc] pretreated miscanthus, initial [H_2SO_4] = 123 mM, [H_2O] increased from 5 wt% (400 mM) to 43 wt% (5500 mM), changes in T and initial substrate are noted in key below table.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (min)</th>
<th>Yield; Cellulose Portion</th>
<th>Conversion</th>
<th>Yield, Hemicellulose Portion</th>
<th>Conversion</th>
<th>Miscanthus Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-HMF</td>
<td>Glucose</td>
<td>Cellobiose</td>
<td>Xylose</td>
<td>Arabinose</td>
</tr>
<tr>
<td>1^a</td>
<td>30</td>
<td>16%</td>
<td>20%</td>
<td>1%</td>
<td>36%</td>
<td>56%</td>
</tr>
<tr>
<td>2^a</td>
<td>60</td>
<td>20%</td>
<td>36%</td>
<td>1%</td>
<td>57%</td>
<td>71%</td>
</tr>
<tr>
<td>3^a</td>
<td>90</td>
<td>22%</td>
<td>45%</td>
<td>1%</td>
<td>67%</td>
<td>82%</td>
</tr>
<tr>
<td>4^a</td>
<td>120</td>
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<td>51%</td>
<td>1%</td>
<td>73%</td>
<td>80%</td>
</tr>
<tr>
<td>5^a,c</td>
<td>90</td>
<td>11%</td>
<td>75%</td>
<td>3%</td>
<td>88%</td>
<td>67%</td>
</tr>
<tr>
<td>6^a,c</td>
<td>120</td>
<td>5%</td>
<td>84%</td>
<td>5%</td>
<td>94%</td>
<td>61%</td>
</tr>
<tr>
<td>7^b</td>
<td>60</td>
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<tr>
<td>9^b,c,d</td>
<td>90</td>
<td>9%</td>
<td>55%</td>
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</tr>
</tbody>
</table>

**Key**:  
- 1: [Emim][Tos] pretreated miscanthus  
- 2: [NMP][OAc] pretreated miscanthus  
- a: For first 60 min of reaction, T held at 373 K. At 60 min, T increased from 373 K to 383 K and maintained for the remaining reaction time.  
- b: Yields based on initial mass of miscanthus subjected to pretreatment  
- c: Yields based on initial mass of miscanthus subjected to pretreatment  
- d: Yields based on initial mass of miscanthus subjected to pretreatment.
Table 3.7 Composition and Recovery of pretreated samples of miscanthus. [Emim][Tos] substrate pretreated in 10 g of a 10 wt% mixture of [Emim][Tos] in ethylene diamine for 6-10 hrs at 343 K; [NMP][OAc] substrate pretreated in 10 g of 10 wt% [NMP][OAc] in ethylene diamine for 10-12 hrs at 323 K

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Miscanthus Mass (g)</th>
<th>Total Mass Recovery</th>
<th>Cellulose Recovery</th>
<th>Hemicellulose Recovery</th>
<th>Lignin Recovery</th>
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<td>Raw Miscanthus</td>
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<td>43%</td>
<td>25%</td>
</tr>
<tr>
<td>[Emim][Tos]</td>
<td>0.87</td>
<td>0.64</td>
<td>54%</td>
<td>91%</td>
<td>49%</td>
</tr>
<tr>
<td>[NMP][OAc]</td>
<td>1.06</td>
<td>0.72</td>
<td>38%</td>
<td>75%</td>
<td>24%</td>
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</tbody>
</table>
Figure 3.6 Conversion of the cellulosic component of miscanthus at various initial miscanthus loadings. \( T = 373 \, \text{K}, \) 500 mg [Emim][Cl], 90 mg (18 wt%), 45 mg (9 wt%) or 22.5 mg (4.5 wt%) miscanthus, initial \([\text{H}_2\text{SO}_4] = 123 \, \text{mM}, \) \([\text{H}_2\text{O}]\) increased from 5 wt% (400 mM) to 43 wt% (5500 mM)
Figure 3.7 Conversion of the hemicellulosic component of miscanthus at various initial miscanthus loadings. T = 373 K, 500 mg [Emim][Cl], 90 mg (18 wt%), 45 mg (9 wt%) or 22.5 mg (4.5 wt%) miscanthus, initial $\left[\text{H}_2\text{SO}_4\right] = 123$ mM, $\left[\text{H}_2\text{O}\right]$ increased from 5 wt% (400 mM) to 43 wt% (5500 mM)
Table 3.8 Individual product yields for hydrolysis of miscanthus at various initial miscanthus loadings. $T = 373 \text{ K, } 500 \text{ mg [Emim][Cl], } 90 \text{ mg (18 wt%), } 45 \text{ mg (9 wt%) or } 22.5 \text{ mg (4.5 wt%) miscanthus, initial } [\text{H}_2\text{SO}_4] = 123 \text{ mM, } [\text{H}_2\text{O}] \text{ increased from 5 wt\% (400 mM) to 43 wt\% (5500 mM)}$

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time min</th>
<th>Cellulose</th>
<th>Glucose</th>
<th>5-HMF</th>
<th>Conversion</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Furfural</th>
<th>Conversion</th>
<th>Miscanthus Conversion</th>
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<td>9%</td>
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<td>29%</td>
<td>63%</td>
<td>5%</td>
<td>5%</td>
<td>73%</td>
<td>45%</td>
</tr>
</tbody>
</table>

a: 22.5 mg miscanthus, 4.5 wt%  
b: 45.0 mg miscanthus, 9 wt%  
c: 95.0 mg miscanthus, 18 wt%
References


Chapter 4

An Investigation of Kinetic Parameters of Chromium Assisted 5-HMF Formation from Glucose in Ionic Liquids

Abstract

A study was undertaken with the goal of quantifying the kinetic parameters used to describe chromium chloride catalyzed dehydration of glucose to 5-HMF in ionic liquids. Chromium chloride was found to catalyze both the forward and reserve aldose-to-ketose isomerization reaction, which transforms the unreactive glucose to fructose. Fructose was readily dehydrated in [Emim][Cl] without an added catalyst, due to the mild acidity of the IL. While CrCl\textsubscript{2} is required to dehydrate glucose to 5-HMF, addition of CrCl\textsubscript{2} to fructose dissolved in ionic liquids lowered 5-HMF selectivity and yield at low initial fructose loadings. Hence, it is important to keep the amount of CrCl\textsubscript{2} in solution low compared to the amount of fructose produced from glucose to optimize 5-HMF formation from glucose dehydration. Isomerization was found to be the rate limiting step, with an activation energy of 94 kJ mol\textsuperscript{-1} compared to the dehydration of fructose with an activation energy of 66 kJ mol\textsuperscript{-1}. Simplification of the model to a series of irreversible steps suggested that the rate of dehydration was 5.5 times faster than the net forward rate of isomerization.

A kinetic model was developed to describe fructose dehydration in an effort to better understand glucose dehydration in ILs. The model predicted apparent rate constants for the forward isomerization, reverse isomerization, and dehydration of \( k_1 = 0.130 \text{ min}^{-1} \), \( k_2 = 0.034 \text{ min}^{-1} \), and \( k_3 = 0.066 \text{ min}^{-1} \). The model, after adjusting for intrinsic rate constants, was consistent with the experimental observation that the rate of dehydration is at least 5.5 times faster than the net forward rate of isomerization. The model developed for fructose was inaccurate at describing glucose dehydration, because at short reaction times, glucose disappears from solution without producing large yields of fructose or 5-HMF. A reconsidered model using a rapid equilibrated intermediate between glucose and fructose improved the fit quality. This leads to further questions regarding unclassified intermediates in the dehydration of glucose to 5-HMF, which require additional in situ experimentation for identification.

4.1 Introduction

The chemical transformation of biorenewable feedstocks to platform molecules remains a key challenge in providing alternative routes to petroleum based synthesis of fuels, chemicals, and plastics.\textsuperscript{(1)} While authors have demonstrated the ability to break energy grasses and corn stover to monosaccharides in ionic liquids (ILs) selectively in high yield, further processing of monosaccharides in ILs is still being developed in the utilization of biorenewable feedstocks.\textsuperscript{(2, 3)} In particular, the dehydration of glucose generated from the cellulosic component of lignocellulosic biomass to form 5-hydroxymethyl
furfural (5-HMF) has been identified as an attractive pathway before further upgrading to commercial products.\[4\]

Metal chlorides in ionic liquids have been reported as highly effective and selective catalytic systems converting glucose to 5-HMF. In 2007, Zhang et al. discovered that CrCl$_2$ in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) could convert glucose to 5-HMF in 70% yield after 3 hr at 373 K.\[5\] Subsequent experimental studies have examined different combinations of metal chlorides and ionic liquids, but CrCl$_2$ in [Emim][Cl] remains one of the most active and selective system for 5-HMF formation from glucose.\[6\]

Previous literature has investigated the role of chromium in the CrCl$_2$ and [Emim][Cl] system. It has been proposed that CrCl$_2$ reacts with the Cl$^-$ anion of the ionic liquid to form CrCl$_3$ as the active species.\[5\] Additionally, CrCl$_3$ has been shown to assist the aldose-to-ketose isomerization via a C$_2$ to C$_1$ intramolecular hydride shift, transforming glucose to fructose which is readily dehydrated to 5-HMF in [Emim][Cl] at elevated temperature.\[7\] This intramolecular hydride shift has been shown to be the rate limiting step in 5-HMF formation from glucose and is independent of the oxidation state of chromium based on density function theory (DFT) calculations.\[8\]

While several authors have elaborated on the mechanistic details of 5-HMF production from glucose with CrCl$_2$ in [Emim][Cl], a detailed experimental kinetic analysis has not been performed. This work aims to further characterize the catalytic system by performing detailed kinetic analysis through experimentation and modeling. First, the activation energies for aldose-to-ketose isomerization and subsequent dehydration to 5-HMF were determined. Then, a kinetic model was developed to estimate rate coefficients from experimental data to further understand the relative rates of isomerization and dehydration. Lastly, the implications of changing the mechanism and the impact on the fit quality of the model were examined.

4.2 Experimental

4.2.1 Materials

Unless noted otherwise, materials were used as received. Ionic liquids 1-ethyl-3-methylimidazolium chloride, ([Emim][Cl], 98% purity) was purchased from Iolitec, Germany. 5-hydroxymethylfurfural (5-HMF, 99% purity), Chromium Chloride (CrCl$_2$, 95% purity), 1,6 hexane diol, mixed bed resin TMD-8 hydrogen and hydroxide form, and D-fructose (99% purity) were purchased from Sigma Aldrich, USA. Glucose (G, USP Grade) was purchased from Hyclone, USA.

4.2.2 Carbohydrate Dehydration

In a typical experiment, 5.0 grams of solid [Emim][Cl] was added to a 20 mL capped vial and heated to 373 K on an Alligator Microplate Tumble Stirrer (V&P Scientific Inc., Model #: VP 710E-2HM-1). After the IL melted, 200 mg of glucose was added to the capped vial and stirred at 400 rpm for 1 hr until fully dissolved. The temperature was then adjusted to the reaction temperature and allowed to equilibrate for 1 hr. Chromium chloride (35 mg, 24 mol% with respect to sugar) was then added to the vial to initiate the reaction. Mole percentages are
always reported with respect to the starting sugar concentration. Samples (500 µL, ~550 mg) were taken using a Rainin micropipette and diluted into 600 µL of ultrapure water containing 10 mg mL⁻¹ 1,6 hexane diol to serve as an internal standard. The sample was centrifuged, and 500 µL of the supernatant was treated with a mixed hydrogen and hydroxide form ion exchange resin and centrifuged before the supernatant was analyzed using a high performance liquid chromatography system equipped with a refractive index detector (HPLC-RID).

4.2.3 Analytical Techniques

HPLC-RID analysis was conducted to measure changes in component concentration using a Shimadzu HPLC equipped with a Biorad Aminex HPX-87H column at 333 K. Samples were eluted at 0.6 mL min⁻¹ with a 0.01N H₂SO₄ mobile phase. Products were identified by comparison of retention times with pure substances. Quantification was determined by dividing the integrated peak areas of products (glucose, fructose, 5-HMF), by the integrated peak area of the internal standard (1,6 hexane diol) and converting the area ratio to a molar concentration using a seven-point calibration curve. Conversion and product yields were calculated by dividing the molar concentration by the initial starting substrate molar concentration.

4.2.4 Modeling Techniques

The concentration data from kinetic experiments was modeled using Matlab. Kinetic equations were assumed to be first order, and the system of equations was solved using the ode23s solver, and allowing the modeling method to vary rate constants. The sum of the coefficients of determination (R²) for all component fit curves was maximized by the model through a minimization of the sum of the individual ratios of the sum of the squares for the residuals (residual sum of squares) to the sum of the squares of the variance (total sum of squares) for each component. Simulations were run for 5 different concentrations (data in Appendix 4.5), and the resulting rate constants were averaged.

4.3 Results and Discussion

4.3.1 Fructose dehydration with and without CrCl₂

The proposed reaction scheme for transforming glucose to 5-HMF with CrCl₂ is shown in Figure 4.1. Authors have speculated that first glucose is isomerized to fructose via a C₂-C₁ hydride shift, which is then readily dehydrated to 5-HMF in [Emim][Cl], an ionic liquid with mild acidity.⁸, ⁷ The resulting rate equations for fructose, 5-HMF, and glucose are shown in equations 1, 2, and 3 with apparent rate constants k₁, k₂, and k₃.

\[
\frac{d[Fructose]}{dt} = k₁[Glu cos e] - (k₂ + k₃)[Fructose] \\
\frac{d[HMF]}{dt} = k₁[Fructose]
\] (1) (2)
Indeed, fructose was readily dehydrated in [Emim][Cl] without addition of an isomerization catalyst as shown in Table 4.1, entry 1-5. As the initial concentration of fructose was increased from 60 mM to 1200 mM, fructose conversion, 5-HMF yield, and 5-HMF selectivity all decreased. There are two possible explanations for this observation. First, as fructose dehydrates to 5-HMF, water is formed as a side product. Water has been shown to inhibit dehydration of carbohydrates in IL’s, because the water molecule is more basic than the hydroxyl molecules of the sugar.\textsuperscript{[3, 9]} Thus, as the concentration of water in the reactor increases, the conversion decreases. The decrease in selectivity is most likely a result of secondary reactions to humins caused by cross condensation between fructose and 5-HMF since both are present in solution at high concentration.\textsuperscript{[3, 10]} This theory is further supported by the observation that the selectivity to 5-HMF from fructose drops suddenly from 80%-90% to 60%-70% when the initial concentration was raised from 60-240 mM to concentrations larger than 600 mM.

After dehydration of fructose without a catalyst was studied, experiments were conducted to understand the effects of adding 35 mg CrCl$_2$ (24 mol%) to solutions of varying initial fructose concentration in [Emim][Cl] and the results are presented in Table 4.1 entries 6-10. It was observed that CrCl$_2$, which has been reported to isomerize glucose to fructose, also catalyzed the reverse ketose-to-aldose transformation. The glucose yield from fructose decreased from 12% to 9% as the initial fructose concentration was increased, suggesting the reverse reaction is first order in initial fructose concentration. The effect of CrCl$_2$ on conversion and 5-HMF yield was much more complicated. The addition of CrCl$_2$ resulted in an increase in fructose conversion, with conversions above 80% for each tested initial condition, which was in stark contrast to the effect of increasing initial fructose loading observed in the absence of CrCl$_2$. This increase in conversion led to higher 5-HMF yields at higher initial fructose loadings, but actually lowered 5-HMF yield at lower fructose concentrations. Likewise, the selectivity to 5-HMF improved at higher initial fructose loadings, but decreased when the initial fructose concentration was low.

To further explore the complicated effect of CrCl$_2$ on 5-HMF formation from fructose, the amount of CrCl$_2$ was varied from 2.0 mg (1.5 mol%) to 56 mg (41 mol%) while the initial fructose concentration kept at 250 mM as shown in Table 4.1, entries 11-15. At all the tested fructose concentrations, conversion remained high in the presence of CrCl$_2$, with conversions ranging between 87%-93%. As the amount of CrCl$_2$ increased for 2.0 mg (1.5 mol%) to 35 mg (24 mol%), glucose production increased from 1% to 11%, but then appeared to plateau with 8% at 56 mg (41 mol%) CrCl$_2$. Increasing the concentration of CrCl$_2$ from 2.0 mg to 56 mg actually lowered the 5-HMF yield and selectivity from 60% to 43%, and 67% to 46% respectively. Thus, CrCl$_2$ has a dual effect on the production of 5-HMF from fructose. At high initial fructose loadings, CrCl$_2$ increases fructose conversion and 5-HMF selectivity thereby improving the yield...
of 5-HMF. However, given a constant concentration of fructose, the dehydration to 5-HMF is actually less efficient with higher catalyst loadings. This could be an indication that CrCl$_2$ must be hydrated to be active, given that CrCl$_2$·6H$_2$O has also been reported as an efficient catalyst for fructose dehydration.[8, 11] Hence, enough fructose must dehydrate in the IL to produce the water necessary to hydrate and activate CrCl$_2$, and if the initial concentration of fructose is too low, or the amount of CrCl$_2$ is too high, 5-HMF production is suppressed. Additionally, characterization of the aldose-ketose isomerization is based solely on comparison of HPLC-RID retention times, which may be inaccurate in describing the key intermediates in the dehydration to 5-HMF in ILs.

4.3.2 Glucose dehydration with CrCl$_2$

The effects of adding CrCl$_2$ to glucose in [Emim][Cl] was studied and the results are reported in Table 4.2. In the absence of CrCl$_2$, glucose is non reactive at the tested conditions (1% conversion, no 5-HMF or fructose observed after 30 min at 373 K). Addition of CrCl$_2$ initiates the reaction, transforming glucose to fructose as a reactive intermediate which is easily dehydrated to 5-HMF in [Emim][Cl] as previously reported.[5, 7] As the initial concentration of glucose was increased from 60 mM to 1200mM in the presence of 35 mg CrCl$_2$ (24 mol%) the amount of fructose observed varied between 10%-14% as shown in Table 4.2, entry 16-20, suggesting the forward aldose-ketose isomerization is also first order in initial substrate concentration. Additionally, the selectivity to 5-HMF increased with increased glucose concentration. As was shown in Table 4.1, the dehydration of fructose to 5-HMF is more selective when the ratio of fructose to CrCl$_2$ is kept high, so at higher glucose concentrations, the resulting fructose concentration is higher, which results in higher selectivity in the fructose to 5-HMF transformation at constant CrCl$_2$ concentration. The conversion of glucose after 30 min of reaction dropped from 86%-59% as the initial glucose concentration was increased from 60 mM to 1200 mM. This effect is further illustrated in Table 4.2 entry 21-25, which showed as the [CrCl$_2$] was decreased from 63 mg (46 mol%) to 2 mg (1.5 mol%) the conversion at 30 min dropped from 80% to 16%. Unlike fructose, glucose conversion is a strong function of CrCl$_2$ catalyst concentration because glucose is unreactive in the absence of CrCl$_2$. Consistent with these observations the yield of fructose increased from 1% to 25% as the concentration of CrCl$_2$ was increased. Hence, there are trade offs associated with increasing CrCl$_2$ concentration for 5-HMF yield from glucose. As previously discussed, increasing CrCl$_2$ concentration decreased the 5-HMF formation from fructose, but as shown in Table 4.2, CrCl$_2$ is required to initiate the reaction by catalyzing the transformation of glucose to fructose. Therefore, it becomes important to balance the CrCl$_2$ concentration such that it is low enough to transform glucose to fructose without hindering the fructose dehydration to 5-HMF.

4.3.3 Comparison of the rates of dehydration and isomerization

A time course showing the evolution of products from glucose in the presence of 9 mg CrCl$_2$ (6 mol%) in [Emim][Cl] at 373 K is represented in Figure
Here the initial concentration of CrCl\(_2\) was lowered in an effort to optimize 5-HMF formation by balancing the competing effects of the catalyst of glucose and fructose chemistries as previously discussed. Glucose rapidly disappears from solution, before large yields of either fructose or 5-HMF are observed. Intermediate products were not observed during this apparent induction period using HPLC-RID. After 5 min, fructose begins to accumulate in the reactor as an intermediate, reaching a maximum yield of 12.6\% after 20 min. Concurrently, 5-HMF is formed as the final product, continues to be produced and reaches a maximum yield of 72\% at 90\% glucose conversion after 3 hr. No traces of levulinic or formic acid, which are degradation products of 5-HMF, were detected at the end of the reaction. These results are in agreement with previous authors who reported 70\% 5-HMF yield at 95\% conversion under equivalent conditions.\[^5\]

Characterizing the relative rates of isomerization and dehydration is critical to understanding the efficiency of metal chloride catalysts on the overall production of 5-HMF from glucose. The activation energies for both fructose dehydration in the absence of CrCl\(_2\) and glucose dehydration assisted with CrCl\(_2\) were determined using an Arrhenius plot shown in Figure 4.3. Glucose isomerization to fructose with CrCl\(_2\) was observed to be the rate limiting step, with an activation energy (E\(_A\)) of 94 ±/− 9 kJ mol\(^{-1}\), compared to 66 ±/− 4 kJ mol\(^{-1}\) for dehydration of fructose to 5-HMF in [Emim][Cl] without a catalyst. The E\(_A\) for isomerization is in good agreement with a DFT study that estimated an energy barrier of 93 kJ mol\(^{-1}\) for Cr(II) complexes in [Emim][Cl] for glucose to fructose isomerization.\[^8\] However, while the E\(_A\) for fructose dehydration without a catalyst in [Emim][Cl] has not previously been reported, a study conducted in [Hmim][Cl] found the E\(_A\) for fructose dehydration to be surprisingly high, 143 kJ mol\(^{-1}\), and similar to the E\(_A\) observed in organic solvents.\[^12\] Therefore, it is possible that [Emim][Cl] plays a more intricate role in these systems that is not currently understood.

The formation of fructose as an intermediate can be used to characterize the ratio of the rate constants of dehydration and isomerization.\[^13\] If the scheme shown in Figure 4.1 was simplified to a series of irreversible steps of Glucose → Fructose → 5-HMF, the maximum concentration of fructose, and time to reach the maximum concentration of fructose, can be described with equations 4 and 5. The apparent rate constants from Figure 4.1 and equations 1, 2, and 3 are related to the apparent rate constants of equations 4 and 5 through \(k'\) and \(k_1\).

\[
[F]_{max} = \frac{[G]_0}{k_1} \frac{k'_3}{k'_1} \frac{k_3}{k'_3 - k'_1} \]  \tag{4}

\[
t_{max} = \frac{1}{k'_3 - k'_1} \ln\left(\frac{k'_3}{k'_1}\right) \]  \tag{5}

Given Figure 4.2, \([F]_{max} = 30 \text{ mmol}, [G]_0 = 240 \text{ mmol}, \text{ and } t_{max} = 20 \text{ min,}\) the simultaneous solution for equations 4 and 5 gives \(k_3 = 0.104 \text{ min}^{-1}\) and \(k'_1 = 30 \text{ mmol}\).
While the apparent rate of dehydration is approximately 5.5 times faster than the apparent rate of isomerization, it is more accurate to determine the intrinsic rates which require the apparent rate constants be normalized by their respective catalyst concentrations, as shown in equations 6, 7, and 8.

\[
k_1 = k_{\text{int},1} \cdot [\text{CrCl}_2] \quad (6)
\]
\[
k_2 = k_{\text{int},2} \cdot [\text{CrCl}_2] \quad (7)
\]
\[
k_3 = k_{\text{int},3} \cdot [H^+] \quad (8)
\]

While the concentration of the isomerization catalyst can be readily calculated (CrCl\(_2\) ~15 mmol), the dehydration catalyst which is assumed to be the C\(_2\) proton of the imidazolium ring of [Emim][Cl], is much more difficult to calculate accurately. As received, 98% pure [Emim][Cl] has a pH between 6.5 - 7.0, and the pKa of the C\(_2\) proton of an imidazolium cation in H\(_2\)O is ~23\([14]\). Therefore, the estimated dissociated proton concentration in the IL would be in picamolar concentrations (75 pmol - 500 pmol). This would suggest that the intrinsic rate constant of dehydration is 6-9 orders of magnitude larger than the net intrinsic rate constant of isomerization. This result further supports the observations that isomerization is the rate limiting step, and is significantly slower than fructose dehydration.

### 4.3.4 Kinetic Modeling Using Comsol

A kinetic model to describe the overall reaction of Figure 4.1 was developed using the data collected and reported in Appendix 4.5.1. First, fructose dehydration in [Emim][Cl] without CrCl\(_2\) was modeled using experimental data, and equations 1, 2, and 3 with \(k_1 = k_2 = 0\) to describe the kinetics. The model predicted an averaged value of \(k_3 = 0.059 +/- 0.021 \text{ min}^{-1}\) compared to an averaged experimental value of \(k_3 = 0.073 +/- 0.030 \text{ min}^{-1}\).

After modeling the 5-HMF formation from fructose without a catalyst, the experimental and model values for \(k_3\) were averaged to obtain \(k_3 = 0.066 \text{ min}^{-1}\), which was substituted into equation 2, and 3. Then experimental data for various initial fructose concentrations dehydrated with 24 mol% CrCl\(_2\) was modeled using Equations 1, 2, and 3, the newly solved \(k_3\) value, and the experimental data in Appendix 4.5.2. The model predicted that the forward isomerization was much more rapid than the reverse, with average values for the isomerization rate constants of \(k_1 = 0.130 +/- 0.022 \text{ min}^{-1}\) and \(k_2 = 0.034 +/- 0.008 \text{ min}^{-1}\,\text{, and } k_1\) consistently between 3.1 to 4.7 times larger than \(k_2\).

Next, the fructose model was applied to experimental data shown in Appendix 4.5.3 where the initial concentration of CrCl\(_2\) was varied with constant initial fructose concentration in an effort to obtain the intrinsic rate coefficients for the isomerization step defined in equations 6 and 7. The intrinsic rate coefficient for dehydration described by equation 8 was left as apparent rate coefficient \(k_3 = 0.066 \text{ min}^{-1}\,\text{, due to the previously discussed picamolar concentration of protons from [Emim][Cl]. As CrCl}_2\) concentration was increased, the model predicted values for apparent rate coefficients \(k_1\), and \(k_2\) increased linearly with a slope
equal to the intrinsic rate coefficient. The results determined $k_{\text{int,1}} = 2.36 \text{ min}^{-1}$, and $k_{\text{int,2}} = 0.426 \text{ min}^{-1}$, which implies that the forward isomerization is 5.5 times faster than the reverse.

In Figure 4.4, the finished model, $k_{\text{int,1}} = 2.36 \text{ min}^{-1}$, $k_{\text{int,2}} = 0.426 \text{ min}^{-1}$, and $k_3 = 0.066 \text{ min}^{-1}$ was applied to the concentration data used to generate Figure 4.2. As seen in Figure 4.4, the fructose model does not accurately describe glucose dehydration. The limitation of the model is the inability to describe the initial disappearance of glucose, without forming fructose or 5-HMF as a product. As shown in Table 4.2, entry 22, after 30 min of reaction, conversion has reached 53%, yet only 22% 5-HMF yield is recorded, and fructose yield is already decreasing from a 12% maximum yield. This causes the model to overestimate 5-HMF and fructose concentration in order to accurately predict the decrease in glucose concentration.

The inability of the model to predict fructose concentrations accurately at short time scales suggested the reaction of glucose to 5-HMF with $\text{CrCl}_2$ is more complex than the reaction proposed in Figure 4.1. Likely, an intermediate is formed from glucose that is in rapid equilibrium with both glucose and fructose, before fructose dehydrates to 5-HMF. A new proposed reaction system, which includes this intermediate, called “$I_1$”, is shown in Figure 4.5. The kinetic rate equations for each component in Figure 4.5 are represented in equations 9, 10, 11, with HMF formation still accurately described with equation 2. New apparent rate coefficients $k_4$, $k_5$, $k_6$, and $k_7$ were used to describe the new equilibrium reactions, and $k_3 = 0.066 \text{ min}^{-1}$ from the previous model.

\[
\frac{d[\text{Fructose}]}{dt} = k_6[I_1] - (k_5 + k_3)[\text{Fructose}] 
\]  
(9)

\[
\frac{d[\text{Glucose}]}{dt} = k_5[I_1] - k_4[\text{Glucose}]
\]  
(10)

\[
\frac{d[I_1]}{dt} = k_5[\text{Fructose}] + k_4[\text{Glucose}] - (k_6 + k_3)[I_1]
\]  
(11)

Equations 2, 9, 10 and 11 were used to model the time course data used to generate Figure 4.2 and the fit is shown in Figure 4.6. The optimized fit generated values of $k_4 = 0.034 \text{ min}^{-1}$, $k_5 = 0.061 \text{ min}^{-1}$, $k_6 = 0.174 \text{ min}^{-1}$, $k_7 = 0.248 \text{ min}^{-1}$. This suggests that the intermediate “$I_1$” is a reactive intermediate, since $k_5 > k_4$ and $k_7 > k_6$. Additionally, since $k_7$ is significantly larger than $k_5$, once “$I_1$” is formed, it is more likely to become fructose than revert back to glucose.

The intermediate “$I_1$”, while seemingly empirical, represents a physical process commonly overlooked in the chemistry of sugar dehydration. Carbohydrates, such as glucose and fructose, actually exist in a variety of isomeric forms in solution. The differences in reactivity of each of these forms is often neglected in both discussion and design of experimentation directed at elucidating catalytic mechanism and activity. However, it was recently observed that fructose dissolved in the ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]), has a drastically different distribution of isomeric forms compared
with water.\textsuperscript{[16]} Therefore, one plausible hypothesis for the physical meaning of \( I_1 \) in Figure 4.5 could be a representation of the anomerization process by which these isomeric forms interconvert in solution. Including multiple intermediates to represent each of the isomers would also lower the predicted observed concentration significantly, making experimental observation of the intermediates with HPLC-RID difficult. The elucidation of these critical interactions and transformations would benefit from further analysis using NMR spectroscopy to characterize the interactions between the substrate, solvent, and catalyst.

4.4 Conclusions

Experimentation was conducted in an effort to elucidate the role of \( \text{CrCl}_2 \) on the dehydration of glucose in [Emim][Cl]. Fructose readily dehydrates in [Emim][Cl] without addition of \( \text{CrCl}_2 \), and the \( E_A \) of fructose dehydration was determined to be 66 kJ mol\(^{-1}\). When \( \text{CrCl}_2 \) was added to fructose dissolved in [Emim][Cl], the ketose to aldose isomerization was observed, confirming that \( \text{CrCl}_2 \) can catalyze both the forward and reverse isomerization. Additionally, addition of \( \text{CrCl}_2 \) increased fructose conversion compared to experimentation with no added catalyst. However, it was also observed that as the ratio of \( \text{CrCl}_2 \) to fructose was increased, 5-HMF yield and selectivity decreased, suggesting that \( \text{CrCl}_2 \) may catalyze secondary pathways that result in soluble humin formation.

Glucose dehydration requires an isomerization catalyst, such as \( \text{CrCl}_2 \), since it isomerizes the unreactive glucose to fructose which facilitates 5-HMF formation. The isomerization step was determined to be rate limiting with an \( E_A = 93 \text{ kJ mol}^{-1} \). As the ratio of \( \text{CrCl}_2 \) to glucose was increased, the amount of fructose in solution increased, suggesting first order kinetics. However, since large concentrations of \( \text{CrCl}_2 \) lower the 5-HMF selectivity from fructose, 5-HMF yield also decreased with increasing \( \text{CrCl}_2 \) concentration. In this manner, the \( \text{CrCl}_2 \) concentration needs to be optimized to maintain high concentrations of fructose relative to the \( \text{CrCl}_2 \) concentration, and controlling the resulting amount of water produced.

Both experimentation and modeling were conducted to understand the forward and reverse rates of isomerization, and the rate of dehydration. Using a simplified irreversible model, the dehydration was determined to be at least 5.5 times faster than the isomerization. Kinetic modeling experiments determined the apparent rate constants for the forward isomerization \( k_1 = 0.130 \text{ min}^{-1} \), reverse isomerization \( k_2 = 0.034 \text{ min}^{-1} \), and \( k_3 = 0.066 \text{ min}^{-1} \). Model results determined the forward isomerization to be between 3.1 and 4.7 times faster than the reverse, a number which was confirmed by determination of the intrinsic rate coefficients \( k_{\text{int},1} = 2.4 \text{ min}^{-1} \) and \( k_{\text{int},2} = 0.43 \text{ min}^{-1} \). The intrinsic rate coefficient for dehydration was not determined because the concentration of free protons in solution was assumed to be between 6-9 orders of magnitude smaller than the concentration of the isomerization catalyst, further suggesting the dehydration step has a much lower energy barrier compared to the isomerization step.
The quality of the model when applied to glucose as a starting substrate instead of fructose decreased significantly. This is because glucose disappears very rapidly from solution, but fructose and 5-HMF are not produced in high yields at short reaction times. Introduction of an intermediate “I₁”, which was assumed to be in equilibrium with both glucose and fructose, greatly improve the quality of the model fit. “I₁” is likely an intermediate which represents the complex process of anomerization, through which carbohydrates interconvert between several distinct isomers in solution. Further experimentation, specifically using NMR spectroscopy, could prove beneficial in understanding the mechanism of metal chloride assisted dehydration of glucose during the initial reaction phase.
Figure 4.1 Proposed mechanism of glucose dehydration to 5-HMF
Table 4.1 Dehydration of various initial concentrations of fructose with and without CrCl$_2$.
$T = 373$ K, 5 gm [Emim][Cl], 20 min time point

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Concentration</th>
<th>CrCl$_2$ (mg)</th>
<th>Conversion</th>
<th>5-HMF Yield</th>
<th>Max Glucose Yield$^a$</th>
<th>5-HMF Selectivity</th>
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<tbody>
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<td>46%</td>
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</tbody>
</table>

$^a$: Reaction time for max glucose yield varies from 5 min – 10 min, since it is a secondary product.
Table 4.2 Dehydration of various initial concentrations of glucose with CrCl\(_2\). \(T = 373\) K, 5 gm [Emim][Cl], 30 min time point

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\(^a\) Reaction time for max fructose yield varies from 10 min – 20 min, since it is a secondary product
Figure 4.2 Glucose dehydration to 5-HMF catalyzed by CrCl$_2$ as a function of time in [Emim][Cl]. T = 373 K, 5.0 g [Emim][Cl], 200 mg Glucose, 8.6 mg CrCl$_2$
Figure 4.3 Arrhenius plot of rates of glucose isomerization and fructose dehydration. 5.0 g [Emim][Cl], 200 mg Glucose or Fructose, 33.0 mg CrCl$_2$ for Glucose experiments (no CrCl$_2$ added in Fructose experiments)
Figure 4.4 Intrinsic model fit for CrCl$_2$ assisted glucose dehydration to 5-HMF in [Emim][Cl]. Experimental: T = 373 K, 5.0 g [Emim][Cl], 8.6 mg CrCl$_2$ (15 mM); Model parameters, $k_1 = 2.4\times[CrCl_2]$, $k_2 = 0.43\times[CrCl_2]$, $k_3 = 0.066$ min$^{-1}$
Figure 4.5 New mechanism for glucose dehydration to 5-HMF with intermediate formation consideration

Figure 4.6 Intermediate model fit for CrCl₂ assisted glucose dehydration to 5-HMF in [Emim][Cl]. Experimental: T = 373 K, 5.0 g [Emim][Cl], 8.6 mg CrCl₂ (15 mM); Model parameters, $k_4 = 0.034 \text{ min}^{-1}$, $k_5 = 0.061 \text{ min}^{-1}$, $k_6 = 0.174 \text{ min}^{-1}$, $k_7 = 0.248 \text{ min}^{-1}$, $k_3 = 0.066 \text{ min}^{-1}$. 
References
## Appendix 4.5
### Appendix 4.5.1 Experimental Data for Fructose Dehydration without CrCl₂

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Chapter 5

An NMR Investigation of Glucose Dehydration Catalyzed by Tungsten Hexachloride in [Emim][Cl]

Abstract
This study was conducted to understand the role of [Emim][Cl] and determine intermediates and products formed during WCl$_6$-catalyzed dehydration of glucose using in-situ $^{13}$C NMR spectroscopy. [Emim][Cl] increases the equilibrium concentrations of the furanose and acyclic isomers of glucose relative to the concentrations of these species present when water is used as the solvent, and increases the rate of anomerization by more than a factor of two. The temporal evolution of intermediates and products formed during WCl$_6$-catalyzed dehydration of glucose was monitored by $^{13}$C NMR of fully $^{13}$C-labeled glucose. The furanose isomers of glucose were found to react more rapidly than the pyranose isomers to form 5-HMF via what is proposed to be an acid-catalyzed mechanism, the acid, HCl, being produced by partial hydrolysis of WCl$_6$. Two intermediate products were observed using either WCl$_6$ or H$_2$SO$_4$ as the catalyst. The $^{13}$C NMR chemical shifts and splitting patterns of these intermediates suggest that they are partially dehydrated enol and keto forms of glucose, rather than fructose. The conversion of these species to 5-HMF was only observed in the presence of WCl$_6$. Deuterium incorporation experiments showed that WCl$_6$ catalyzes a 1,2 intramolecular hydride shift during 5-HMF formation. Two new mechanisms are proposed to explain the pathways by which glucopyranose is converted to 5-HMF. Both begin with the loss of one molecule of water via the acid-catalyzed dehydration of glucopyranose to form a partially dehydrated enol or keto form of glucose, after which WCl$_6$ promotes a hydride shift, followed by further dehydration of the intermediate to form 5-HMF.

5.1 Introduction
The production of fuels and chemicals from renewable resources is a key challenge in reducing global dependence on petroleum. Recent research has focused on the production of 5-hydroxymethyl furfural (5-HMF) from carbohydrates as a potential platform for products traditionally synthesized from nonrenewable feedstocks. While 5-HMF synthesis is readily accomplished via acid catalyzed dehydration of fructose, glucose is a cheaper starting material that can be derived in high yields from lignocellulosic biomass.$^{[1-3]}$ The relative reactivity of glucose and other aldohexoses is, however, much lower than that of ketohexoses, such as fructose.$^{[4]}$ For this reason it has been assumed that catalysts shown to be effective for 5-HMF production from glucose catalyze the aldose-to-ketose isomerization, producing fructose as a reactive intermediate which then dehydrates to 5-HMF.$^{[4-7]}$ An important consequence of this assumption is that isomerization is required for 5-HMF production and must take place before dehydration is initiated.
One of the most active and selective catalytic systems for 5-HMF production from glucose is chromium chloride (CrCl$_2$) dissolved in ILs.$^{[5, 7]}$ Previous research has shown that 70% yield of 5-HMF could be obtained at 90% glucose conversion with CrCl$_2$ dissolved in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) after 3 h of reaction at 373 K.$^{[5]}$ The mechanism of 5-HMF formation was initially proposed to involve a chromium assisted isomerization of glucose to fructose through an enolization pathway, but recent $^1$H NMR experiments suggest that product formation occurs via a chromium catalyzed 1,2 intramolecular hydride shift, from which it is inferred that glucose is transformed from an aldose to a ketose before subsequent dehydration of the ketose to 5-HMF.$^{[4, 5]}$ It should be noted, though, that while it has been claimed that fructose is formed as an intermediate, this conclusion is based solely on of the coincidence of the chromatographic retention times of the formed intermediate and a pure standard of fructose. However, the structure and composition of the intermediate were not confirmed by NMR because NMR spectra acquired in the presence of CrCl$_2$ are difficult to interpret due to strong line broadening from small concentrations of Cr(III) which is both paramagnetic and quadrupolar.$^{[4, 5]}$ Fructose has also been claimed as an intermediate in the dehydration of glucose to 5-HMF catalyzed by GeCl$_4$ dissolved in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]).$^{[6]}$ The basis for this assignment was observation of a peak at 103.6 ppm in the $^{13}$C NMR spectra after glucose was exposed to GeCl$_4$ at 323 K, which disappeared when the temperature was raised to 348 K. The authors suggested that the species giving rise to this peak was a short-lived intermediate, which they attributed to fructose based on suggestions from the literature.

Solvent composition has been noted to affect the CrCl$_2$-catalyzed dehydration of glucose. This conclusion is based on the observation that the activity of CrCl$_2$ diminishes in solvents other than imidazolium based ILs.$^{[7]}$ A possible source of this effect may be the influence of the solvent on the equilibrium distribution of glucose isomers.$^{[4, 8]}$ Glucose can form six distinct isomers in solution as shown in Figure 5.1. In D$_2$O at 298 K glucose is found primarily in the $\beta$-glucopyranose (1: 62.0%) and $\alpha$-glucopyranose (2: 37.6%) forms, with small concentrations of the furanose and acyclic isomers ($\beta$-glucofuranose (3): 0.28%, $\alpha$-glucofuranose (4): 0.11%, acyclic glucose aldehyde (5): 0.004%, and hydrated acyclic glucose (6): 0.006%).$^{[9]}$ Several authors have noted that carbohydrates with higher concentrations of furanose and acyclic isomers tend to form higher 5-HMF yields under acidic conditions.$^{[4, 8]}$ Fructose, for example, also exists primarily in the $\beta$-fructopyranose (68%) form at 303 K in D$_2$O, but has much higher concentrations of furanose and acyclic isomers compared to glucose ($\beta$-fructofuranose: 23%, $\alpha$-fructofuranose: 6.0%, acyclic fructoketose: 0.7%).$^{[8, 10]}$ Consequently, fructose is readily dehydrated to 5-HMF in [Emim][Cl] without an added catalyst.$^{[5, 11]}$ Recent work using $^{13}$C NMR has shown that higher concentrations of the furanose and acyclic isomers are observed for carbohydrates dissolved in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) compared to water.$^{[12]}$ While significantly higher concentrations of $\alpha$-fructofuranose and acyclic fructoketose isomers were observed for fructose, the only isomers observed for glucose were $\beta$-glucopyranose (1) and $\alpha$-
glucopyranose (2), the distribution of which in [Bmim][Cl] (1:2, 65% : 35%) was similar to that observed in D$_2$O (1:2, 61% : 39%) at 353 K. It is likely that the furanose and acyclic glucose isomers were present in the solution tested, but in such low concentrations that these species could not be detected without $^{13}$C labeling and collection of a large number of transients.$^{[8, 9, 13]}$

Given that furanose and acyclic isomers may be more amenable to dehydration, an important secondary process in the catalytic dehydration of glucose to 5-HMF is the replenishment of these isomers as they are consumed. The process converting from one isomeric form to another, called anomerization or mutarotation, is an acid and base catalyzed process. An example using β-glucopyranose is shown in Figure 5.2 and begins with protonation of the ring oxygen.$^{[14]}$ Next, the bond between the anomic carbon and the ring oxygen is broken, and the hydroxyl group of the anomic carbon is reduced to a carbonyl by a base. The bond between the anomic carbon and C$_2$ in glucose (or C$_3$ in fructose) can then rotate, which will determine the α/β orientation upon ring closure. If the ring closes between the C$_5$-C$_1$ position, a glucopyranose isomer is formed, while closure between the C$_4$-C$_1$ position results in a glucofuranose isomer. In water at 293 K, the process of anomerization in Figure 5.2 occurs on the time scale of hours.$^{[15]}$ Hence, under catalytic conditions for glucose dehydration, anomerization could become rate limiting if glucofuranose isomers are more amenable to 5-HMF formation than glucopyranose isomers. The rate of anomerization has been shown to increase significantly in amphoteric solvents, but such measurements have yet to be conducted and reported in IL solvent systems.

The review of the literature presented above indicates that while CrCl$_2$ and other metal chlorides dissolved in ILs can catalyze the dehydration of glucose to 5-HMF, a number of issues remain unanswered. These include the role of the IL, the necessity of forming fructose as an intermediate, and the point along the reaction pathway at which the aldose to ketose conversion occurs. The aim of the present study was to address these issues using $^{13}$C NMR spectroscopy. The tautomeric equilibrium of glucose in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) was determined and the rate of anomerization was measured. Then, in-situ $^{13}$C NMR experiments were conducted in an effort to characterize the species present during the dehydration of glucose to 5-HMF catalyzed by tungsten hexachloride, WCl$_6$, dissolved in [Emim][Cl]. WCl$_6$ was used as the catalyst because tungsten is a spin $\frac{1}{2}$ nucleus, and DFT calculations have suggested it could be a more active catalyst than chromium for glucose dehydration.$^{[16]}$ This work reported here shows definitively that fructose is not the ketose intermediate, and that dehydration is initiated prior to isomerization in the production of 5-HMF from glucose.

5.2 Experimental

5.2.1 Materials

Unless noted otherwise, materials were used as received. The ionic liquid 1-ethyl-3-methylimidazolium chloride, ([Emim][Cl], 98% purity) was purchased from Iolitec, Germany. 5-hydroxymethylfurfural (5-HMF, 99% purity), tungsten
chloride (WCl₆, 99.9% metal basis), 1,6 hexane diol, mixed bed resin TMD-8 hydrogen and hydroxide form, deuterium oxide (D₂O, 100%), formic acid, and D-fructose (99% purity) were purchased from Sigma Aldrich, USA. ¹³C labeled glucose (D-glucose, U-13C₆, 99%), ¹³C labeled fructose (D-fructose, U-13C₆, 99%), deuterium labeled glucose (D-glucose, 2-D, 98%), and dimethyl sulfoxide-d₆ (DMSO-d₆ 99.9%) were purchased from Cambridge Isotopes, USA. Glucose (G, USP Grade) was purchased from Hyclone, USA. Levoglucosenone was purchased from CarboSynth, UK. Levoglucosan was purchased from CarboMer, USA.

5.2.2 NMR Experimentation

NMR analysis was conducted using a Bruker Avance AV-600 MHz NMR spectrometer. All proton decoupled ¹³C spectra were recorded using an inverse-gated pulse program. A typical NMR sample was prepared by adding 30 mg of ¹³C glucose to 478 mg of melted [Emim][Cl] in a 4.0 mL vial and then stirring the contents at 400 rpm for 1 h at 353 K. After dissolution, 100 mg DMSO-d₆ was added to the vial to serve as a lock and internal standard, and the sample was transferred to a NMR sample tube. The capped sample was equilibrated in an oven for 8 h before 10,000 transients were collected. Isomers were identified by comparison of anomeric carbon signals for standards prepared in [Emim][Cl], DMSO-d₆, D₂O, and previously published reports.⁹, ¹⁷ Chemical shifts are reported relative to DMSO-d₆ in solution (40.1 ppm). Quantification was determined by integrating the area of the peak corresponding to the anomeric carbon of each isomer, then normalizing the area by the area of the DMSO-d₆ peak, and summing all the isomer signals to 100%.

Since the acquisition of 10,000 transients requires roughly 10 h, a modified procedure was used to investigate the rate of anomerization and to conduct the in situ study of glucose dehydration. The rate of anomerization was determined by equilibrating a sample for 2 h at 313 K before collecting 300 transients. Then, the temperature was increased to 323 K, and 300 transients were collected every 20 min for the next 2 h. The new equilibrium was considered to have been established when the relative heights of the peaks corresponding to the anomeric carbons of β-glucopyranose and α-glucopyranose remained constant. For in situ catalytic studies a sample was equilibrated for 2 h in an over and 300 transients were collected in the absence of catalyst. 24 mol% of catalyst with respect to glucose (22 mg WCl₆ or 5.6 mg of 98 wt% H₂SO₄) was added to the sample, and 300 transients were collected during the course of the reaction at specified time points. The time labels for the NMR spectra correspond to when the acquisition was finished: “30 min” represents the 300 transients collected from 15-30 min, “60 min” represents the 300 transients collected from 45-60 min, etc.

5.2.3 Carbohydrate Dehydration

The dehydration of glucose was carried out to mimic the experimental conditions for the NMR samples. Heating and stirring were carried out on an Alligator Microplate Tumble Stirrer (V&P Scientific Inc., Model #: VP 710E-2HM-
1). In a typical experiment, 600 mg of glucose was added to 3.0 g of melted [Emim][Cl] in a 20 mL capped vial and dissolved for 1 hr at 373 K with 400 rpm stirring. In a separate 20 mL vial, 320 mg WCl₆ (24 mol% with respect to glucose) was added to an additional 3.0 g of melted [Emim][Cl], and set to stir at 400 rpm, for 1 hr at 373 K. The reaction solution was prepared by mixing 2.5 g of the glucose/[Emim][Cl] solution with 1.0 g of DMSO-d₆ which then equilibrated for 30 min at 353 K before 2.5 g of the WCl₆/[Emim][Cl] solution was added to initiate the reaction.

5.2.4 Analytical Techniques

Sample preparation and analysis were identical to those previously described using a high performance liquid chromatography system equipped with a refractive index detector.[2, 3]

5.3 Results and Discussion

5.3.1 Glucose Equilibrium Isomeric Distribution

Figure 5.3 shows the ¹³C spectrum of completely ¹³C labeled glucose dissolved in [Emim][Cl] containing DMSO-d₆ as an internal standard. All six of the isomers of glucose identified in Figure 5.1 are present in this spectrum. The basis for identification of the glucose isomers was taken from previously published reports for glucose labeled with ¹³C at position C₁ dissolved in D₂O.[9] Quantification of the isomeric distribution for glucose, as well as all other components observed in NMR spectra, was based on the signal for the C₁ carbon in the molecule, because it appears as a doublet, and at higher chemical shift allowing for simpler identification. Solvent peaks associated with the six carbons of the imidazolium ring of [Emim][Cl] were observed as singlets at 15.5, 36.3, 45.6, 122.3, 123.7 and 139.9 ppm, while DMSO-d₆ gave a septuplet peak at 40.1 ppm. The signals corresponding to the C₂-C₆ carbons appear between 60 ppm and 85 ppm range, and are difficult to deconvolute due to peak overlap which arises from spin-spin coupling from the neighboring ¹³C labels and therefore omitted from analysis. However, the signals corresponding to the anomeric carbon of each isomer at the C₁ position in glucose were observed as doublets as shown in the inserts of Figure 5.3. The doublet peak structure is consistent with spin-spin coupling associated with the ¹³C₁-¹³C₂ bond. The three largest signals appear as doublets corresponding to β-glucopyranose, α-glucopyranose, and β-glucofuranose at 97.1, 92.5, and 103.8 ppm respectively as shown in Figure 5.3a. The signal corresponding to α-glucofuranose at 97.5 ppm overlaps the β-glucopyranose peak, which is why only half of the doublet can be observed in Figure 5.3b. A similar signal overlap also occurs between the hydrated acyclic peak at 92.1 ppm and α-glucopyranose at 92.5 ppm as shown in Figure 5.3c. A small signal attributed to the hydrated acyclic glucose isomer at 206.1 ppm was observed in Figure 5.3e, but the low signal made it difficult to isolate the doublet from impurities and background noise.

Table 1 shows that glucose dissolved in [Emim][Cl] is present primarily in the pyranose forms. At 333 K, 61.09% β-glucopyranose and 35.11% α-glucopyranose were observed in [Emim][Cl], compared to 60.02% and 38.95% in
D₂O under equivalent conditions. These results are consistent with a previously published report which observed 65% β-glucopyranose and 35% α-glucopyranose in [Bmim][Cl], compared to 61% and 39% in D₂O at 333 K. While the concentrations of furanose and acyclic isomers are much lower compared to the pyranose forms, the concentrations of these isomers are an order of magnitude higher in [Emim][Cl] compared to D₂O. It is possible that the strong ionic interaction of the IL with the carbohydrate helps stabilize the furanose and acyclic isomers in solution thereby increasing their concentration. Since carbohydrates with larger concentrations of the furanose and acyclic isomers have been proposed to be more amenable to dehydration under acidic conditions, increasing their concentration in the IL may facilitate 5-HMF formation catalyzed by metal chlorides.

5.3.2 Rate of Anomerization in H₂O and [Emim][Cl]

The dynamics of glucose anomerization were determined by equilibrating ¹³C labeled glucose dissolved in D₂O at 313 K for 2 h, after which the temperature was increased to 323 K and the time to establish the new equilibrium determined by monitoring the relative peak heights of the C₁ anomeric carbons of β-glucopyranose and α-glucopyranose. In D₂O, the signal heights stabilized in 40-60 min, suggesting that the time constant for anomerization is about an hour at 333 K. An equivalent perturbation experiment was conducted in [Emim][Cl]. In this case, the peak heights for the C₁ anomeric carbons of β-glucopyranose and α-glucopyranose stabilized after collection of the initial 300 transients during the first 20 min. This suggests that within 20 min, anomerization has occurred and the new equilibrium was established in the IL. It has been reported that the rate of anomerization is faster in amphoteric solvents, which can provide both the acidic and basic functionalities required in the mechanism shown in Figure 5.2. While [Emim][Cl] is not formally classified as amphoteric, it does possess a slightly acidic proton on the C₂ position of the imidazolium ring, as well as a basic chloride anion, which could both catalyze the reactions shown in Figure 5.2.

5.3.3 In Situ NMR Monitoring of Glucose Dehydration Catalyzed by WCl₆

The dehydration of ¹³C labeled glucose in the presence of WCl₆ dissolved in [Emim][Cl] was monitored as a function of time using ¹³C NMR. The anomeric carbon fingerprint region of the ¹³C NMR spectra is shown in Figure 5.4. Before addition of WCl₆, (t = 0 min), the signals for all of the anomeric carbons of the glucopyranose and glucofuranose isomers were observed. Upon addition of WCl₆, several key changes occurred. The signals for α-glucofuranose (shoulder peak at 97.5 ppm) and β-glucofuranose (doublet at 103.8 ppm) disappeared completely after 30 min and did not reappear during 3 h of reaction. The peaks corresponding to the β- and α-glucopyranose isomers at 97.1 and 92.5 ppm decreased in intensity much more slowly than those for the furanose isomers. Figure 5.4 also shows the appearance of two new doublets at 102.8 and 104.2 ppm upon addition of WCl₆. The intensity of these features grew over the first 30 to 60 min of reaction, and then decayed with additional reaction time. The
doublet at 102.8 ppm is assigned to levoglucosan, since the spectrum of the pure compound dissolved in [Emim][Cl] showed a C\textsubscript{1} shift at 102.9 ppm. This product is formed by condensation of the C\textsubscript{1} and C\textsubscript{6} hydroxyl groups of glucopyranose to form levoglucosan, leading ultimately to the formation of levoglucosenone upon further dehydration, as shown in Figure 5.5.\textsuperscript{[2, 19]} A very small peak corresponding to levoglucosenone at 101.1 ppm was also observed in Figure 5.4, further supporting assignment of the peak at 102.8 ppm to levoglucosan.

Additional evidence for reaction products and intermediates are shown in Figures 5.6 and 5.7. For the sake of clarity, the peaks for the ionic liquid (15.5, 35.3, 45.6, 122.3, 123.7 and 136.9 ppm), the region containing the C\textsubscript{2}-C\textsubscript{6} signals for the various isomers of glucose (58-89 ppm), and the anomeric carbon region shown in Figure 5.4, have been removed from Figure 5.6. All six carbon signals corresponding to 5-HMF were observed as indicated by the "◊" markers in Figure 5.6 (C\textsubscript{1} - doublet at 178.7 ppm, C\textsubscript{5} - triplet at 162.8 ppm, C\textsubscript{2} – triplet at 152.0 ppm, C\textsubscript{3} – triplet at 125.5 ppm, C\textsubscript{4} – triplet at 110.2 ppm, and C\textsubscript{6} – doublet at 56.1 ppm). Figure 5.7 presents an expansion of selected regions of the spectrum presented in Figure 5.6. Two singlets attributed to products are observed at 163.1 and 124.5 ppm in addition to the two triplets previously assigned to 5-HMF. These two singlet peaks are attributed to formic acid and CO\textsubscript{2}, respectively. Degradation of 5-HMF in acidic media leads to a 1:1 ratio of levulinic and formic acid.\textsuperscript{[20]} Analysis of pure formic acid in [Emim][Cl] gave a singlet peak at 163.5 ppm, but no evidence was observed for levulinic acid. The peak at 124.5 ppm is tentatively assigned to CO\textsubscript{2}, which is known to exhibit a singlet between 124 and 127 ppm in many solvents.\textsuperscript{[21]} While attempts were made to confirm this assignment, the low solubility of CO\textsubscript{2} in [Emim][Cl] at 353K made it impossible to obtain a clear signal for CO\textsubscript{2}.

In addition to product peaks, new peaks were observed both significantly upfield and downfield of the carbohydrate peaks in Figure 5.3. A doublet at 147.9 ppm, and triplets at 188.6, 135.8 and 38.2 ppm can be seen in Figure 5.6. These peaks, as well as the doublet at 104.2 ppm in Figure 5.4 are all ascribable to reaction intermediates, since the intensities of these features grow during the first 30-60 min of reactions and then pass through a maximum and decrease in intensity. While the peak at 104.2 ppm is very similar in position to the chemical shift for the anomeric carbon of α-fructofuranose (δ = 104.8 ppm), this assignment is not correct. As shown in Figure 5.8 the anomeric carbon for fructose, which is located at the C\textsubscript{2} position, produces a triplet signal due to spin-spin coupling through the C\textsubscript{2}-C\textsubscript{3} and C\textsubscript{2}-C\textsubscript{1} bonds, while the observed intermediate in Figure 5.4 is a doublet. The doublets observed at 104.2 and 147.9 ppm are therefore attributed to C\textsubscript{1} signals of two separate intermediates. The additional triplets at 188.6, 135.8 and 38.2 ppm likely correspond to the C\textsubscript{2}, C\textsubscript{3}, C\textsubscript{4}, or C\textsubscript{5} positions of the two new intermediates. The remaining 7 signals corresponding to the other carbons in the two, six carbon, intermediate molecules are buried in the C\textsubscript{2}-C\textsubscript{6} region (55 – 85 ppm), as a consequence of similarities in chemical environment of these carbons in the intermediates and those of glucose. Unfortunately, it is difficult to isolate and deconvolute the additional peaks corresponding to the intermediates from the multiple peaks.
associated with glucose in the 55 – 85 ppm range. It is notable that the reaction of glucose in [Emim][Cl] catalyzed by H₂SO₄ produced NMR features for intermediate at positions identical to those observed using WCl₆ as the catalyst as shown in Figures 5.9 and 5.10, but the intensities of these features were weaker and did not pass through a maximum. Moreover, the intensities of the observed 5-HMF signals were a factor of two lower when H₂SO₄ rather than WCl₆ was used as the catalyst. These observations suggest that the formation of the intermediates is acid catalyzed but their conversion to 5-HMF is enhanced in the presence of the metal chloride, WCl₆. The source of acid using WCl₆ as the catalyst is likely HCl given the propensity of WCl₆ to hydrolyze with water.¹²² Dissolved in the IL, HCl can be produced in situ after dissociation of WCl₆ and reaction with water to form WCl₆⁻(6-n)(OH)ₙ and nHCl. (WCl₆ ↔ WCl₆⁺(6-n) + nCl⁻; WCl₆⁺(6-n) + nH₂O → nH⁺ + [WCl₆⁻(6-n)(OH)ₙ]; nH⁺ + nCl⁻ ↔ nHCl).

5.3.4 Speculative Structures of Intermediate in Glucose Dehydration

To interpret the peaks observed at 188.6, 147.9, 135.8, 104.2 and 38.2 ppm, we hypothesize that glucose undergoes acid-catalyzed dehydration to form the intermediates shown in Figures 5.11 and 5.12. The possible assignment of the observed NMR shifts with particular carbon atoms is also shown together with the anticipated shift predicted by ChemBioDraw on the basis of a group contribution approach.¹²³ It is unlikely that dehydration involving an OH group attached to C₁, C₅, or C₆ positions will form an intermediate that is a precursor to 5-HMF because these positions correspond to the aldehyde, furan ring, and alcohol oxygen atoms in 5-HMF. The relatively large shift in the C₁ signal of glucose to 147.9 ppm could result from cleavage of the hydroxyl group at the C₂ position, which would produce an enol at C₁. Enols are relatively unstable compared to their keto form, and keto formation at C₁ as shown in Figure 5.11 would give a doublet further up field at 147.9 ppm, as well as the low field triplet signal at 38.2 ppm which would correspond to the CH₂ group at the C₂ position.¹²⁴ The relatively small shift in the C₁ signal of glucose to 104.2 ppm (<+15 ppm for any of the isomers) suggests that the chemical environment of the C₁ carbon of the intermediate is similar to that of the C₁ position of glucose. This minor change could be the result of cleavage of the C₃ or C₄ hydroxyl group which would also form an enol as shown in Figure 5.12. However, the lack of a second triplet at low chemical field suggests that an enol intermediate is stabilized. This might result from aromaticity, or hydrogen bonding with a neighboring functional group (such as the hydroxyl group at C₆) or the solvent.¹²⁵ It would then be expected that the enol would have two upfield triplets corresponding to the alkenol carbon (C₄ at 188.6 ppm) and the second alkene carbon (C₃ at 135.8 ppm).²²⁵ While the ChemBioDraw prediction for the second alkene carbon is slightly lower than the observed shift in Figure 5.6, the 52.8 ppm separation between the two signals is consistent with the predicted 61 ppm shift separation predicted for the two carbons of an alcohol substituted alkene.²²⁴ It is also possible that other isomeric forms could be subjected to partial dehydration to form the observed intermediates, and would need to be considered in further 2-D COSY NMR experimentation.
Plots of the change in the C\textsubscript{1} NMR signal peak area as a function of time are shown in Figure 5.13, together with a plot of the consumption of glucose based on HPLC analysis of the reaction products. As seen in Figure 5.13, while the conversion of furanose isomers (which account for only 3% of the total glucose concentration) is significantly faster than the conversion of pyranose isomers, the curve for the overall conversion closely follows that for the conversion of pyranose isomers and agrees with the measured conversions determined by HPLC-RID. These results further support the assumption that the glucofuranose isomer undergoes dehydration more readily than the glucopyranose isomer.

Figure 5.14 presents the change in the C\textsubscript{1} NMR signal peak area as a function of time for the intermediates and products discussed above. The peak areas for the two intermediates proposed in Figures 5 and 6 and for 5-HMF all grow in intensity much faster than levoglucosan, formic acid, and CO\textsubscript{2}. As the intensities of the peaks associated with intermediates begin to decrease, the 5-HMF signal plateaus, suggesting that these three signals are correlated. As the reaction progresses, larger amounts of formic acid and CO\textsubscript{2} are produced, which may result from the decomposition of 5-HMF to formic acid and levulinic acid, and the subsequent decomposition of formic acid to CO\textsubscript{2}. Interestingly, the intermediates and 5-HMF all appear at the same initial rate. If 5-HMF were produced exclusively from these intermediates in a series of first order elementary steps, then an induction period would have been expected prior to the rise in the concentration of 5-HMF. The rapid initial formation of 5-HMF suggests there is an alternative pathway for 5-HMF formation and, hence, that glucose dehydration may occur via two pathways, one proceeding very rapidly via the glucofuranose isomer and the other proceeding more slowly via the glucopyranose isomer.

To test this hypothesis, we subtracted the expected 5-HMF yield for the glucofuranose (~3%) isomer, and plotted the 5-HMF yield from the glucopyranose isomer as a function of time. As seen in Figure 5.15, an induction period occurs in the production of 5-HMF from the pyranose isomer. As noted in Table 5.2, the initial rates of 5-HMF formation and β-glucofuranose consumption are approximately equivalent whether WCl\textsubscript{6} or H\textsubscript{2}SO\textsubscript{4} is used as the catalyst further supporting the hypothesis that the furanose isomers glucose can react directly to form 5-HMF via an acid-catalyzed pathway.

5.3.5 WCl\textsubscript{6} Mechanistic Details from Deuterium Labeling Experiments

As noted in the Introduction, several authors have concluded that metal chlorides facilitate 5-HMF formation from glucose in ILs by catalyzing a 1,2 intramolecular hydride shift. Experiments were carried out as a part of the present study to assess whether a similar process occurs when glucose dehydration is catalyzed by WCl\textsubscript{6}. \textsuperscript{1}H NMR spectra taken after dehydration of unlabeled glucose catalyzed by WCl\textsubscript{6} dissolved in [Emim][Cl]/D\textsubscript{2}O (molar ratios: D\textsubscript{2}O/Glucose = 1 and D\textsubscript{2}O/[Emim][Cl = 0.051) at 353 K for 3 h, revealed only 2.5% D incorporation at the C\textsubscript{1} position of 5-HMF. Dehydration of glucose with a deuterium label at the C\textsubscript{2} position under equivalent conditions, but with the
replacement of D₂O by H₂O, showed 20% D incorporation at the C₁ position of 5-HMF. These results are similar to those reported for CrCl₂-catalyzed dehydration of glucose leading to the conclusion that a 1,2 hydride shift occurs during the dehydration of glucose to 5-HMF.⁴ While it has been inferred in the study of glucose dehydration catalyzed by CrCl₂ that this phenomenon is an indicator of the isomerization of glucose to fructose, the NMR results obtained in the present study show unequivocally that fructose is not formed when the reaction is catalyzed by WCl₆. Instead, the results presented here suggest that glucose undergoes acid catalyzed dehydration to form reactive intermediates, which then undergo WCl₆ catalyzed intramolecular hydride shift. Hence, dehydration is initiated before the 1,2 intramolecular hydride shift occurs. This distinction, while minor, is very important because catalysts previously thought to be ineffective for dehydration of glucose to 5-HMF, may instead simply lack the acidity required to produce the reactive intermediate. This finding may also shed further light on results which have suggested mixed metal chloride systems, such as CrCl₂ and CuCl₂ in [Emim][Cl], are more effective for 5-HMF formation from cellulose than either used independently.⁵⁶ One metal chloride may assist in the hydrolysis of cellulose to glucose, as well as the dehydration of glucose to partially dehydrated intermediates, while the second is responsible for the intramolecular hydride shift. Reexamination of ineffective metal chlorides in the presence of an added acid source, could potentially lead to an increase in the observed activity for glucose dehydration to 5-HMF.

5.3.6 Mechanism for WCl₆ Catalyzed Dehydration of Glucose to 5-HMF

Figures 5.16, 5.17, and 5.18 describe three possible pathways by which glucose can dehydrate to 5-HMF in the presence of WCl₆. The direct conversion of glucofuranose to 5-HMF is shown in Figure 5.16. Glucofuranose first undergoes partial dehydration at the C₂ position, to form an enol. The enol then rearranges to the keto form, which undergoes ring opening. After ring opening, the C₅ hydroxyl group can rotate and form a furanose ring at the C₂ position. This precursor to 5-HMF then undergoes acid catalyzed dehydration at the C₃ and C₄ positions to form 5-HMF. Figures 5.17 and 5.18 illustrate our proposed pathways for the dehydration of the glucopyranose isomer to 5-HMF. In both cases, the initial substrate is shown as β-glucopyranose deuterium labeled at C₂. First, glucose must undergo dehydration to form the reactive keto or enol intermediates suggested in Figures 5.11 and 5.12, respectively. Conversion of the keto intermediate (Figure 5.11) is shown in Figure 5.17. The reaction proceeds with a concerted ring opening hydride shift step, catalyzed by the metal chloride. In addition to shifting the deuterium label from C₂ to C₁, the pyranose ring breaks, and reforms as a furanose ring with ring closure occurring between the ring oxygen and C₂. After the metal chloride-catalyzed hydride shift, the resulting intermediate, which is now a partially dehydrated ketose, can undergo dehydration to lose the remaining two hydroxyl groups at C₃ and C₄ to form 5-HMF. Figure 5.18 shows the proposed path to 5-HMF starting with the enol intermediate (Figure 5.12). In this case, the ring then opens to form a partially dehydrated acyclic glucoaldehyde intermediate. The intermediate can also...
coordinate with the metal chloride, which shifts the deuterium from C\textsubscript{2} to C\textsubscript{1}, resulting in a partially dehydrated acyclic fructoketose. The furanose ring then forms, and the two hydroxyl groups at C\textsubscript{2} and C\textsubscript{4} are lost in a series of acid-catalyzed dehydration steps. In addition to changing the isomeric distribution of glucose slightly, the IL may also play a significant role in facilitating the ring rearrangement shown in Figures 5.16, 5.17, and 5.18.

5.4 Conclusions

The present study provides further insight into metal chloride catalyzed conversion of glucose dissolved in [Emim][Cl] to 5-HMF. [Emim][Cl] is found to enhance the concentrations of the glucofuranose and acyclic isomers relative those found in water. Glucose anomerization is also faster in [Emim][Cl] compared to water.

In-situ \textsuperscript{13}C NMR was conducted to observe the intermediates and products formed during glucose dehydration catalyzed by WCl\textsubscript{6} dissolved in [Emim][Cl]. The furanose isomers of glucose undergo dehydration to 5-HMF much more rapidly than the pyranose isomers via an acid-catalyzed process, the acid being derived by partial hydrolysis of WCl\textsubscript{6} to form HCl. HCl is also thought to catalyze the partial dehydration of glucose to intermediates, which then undergo a WCl\textsubscript{6} catalyzed 1,2 intramolecular hydride shift before further dehydration to form 5-HMF. Evidence is also found for formic acid and CO\textsubscript{2}, products believed to be formed by the decomposition of 5-HMF. In contrast to previous studies of glucose dehydration catalyzed by CrCl\textsubscript{2} and GeCl\textsubscript{4}, no evidence was found in the present study for fructose as an intermediate.
Figure 5.1 The six structural isomers of glucose. 1: β-glucopyranose, 2: α-glucopyranose, 3: β-glucofuranose, 4: α-glucofuranose, 5: acyclic glucose aldehyde, 6: hydrated acyclic glucose.

Figure 5.2 Process of Anomerization/Mutarotation shown for β-glucopyranose
Figure 5.3 $^{13}$C NMR spectrum showing the presence of the six structural isomers of $^{13}$C glucose dissolved in [Emim][Cl] and DMSO-d$_6$ at 333 K. T = 333 K, 30 mg $^{13}$C glucose, 500 mg [Emim][Cl], 100 mg DMSO-d$_6$, 10,000 transients

a. Anomeric C$_1$ NMR signals for β-glucofuranose δ = 103.8 ppm, β-glucopyranose δ = 97.1 ppm, α-glucopyranose δ = 92.5 ppm;
b. Anomeric C$_1$ NMR signal for α-glucofuranose δ = 97.5 ppm;
c. Anomeric C$_1$ NMR signal for hydrated acyclic glucose δ = 92.1 ppm;
d. Full spectra. [Emim][Cl] δ = 15.5, 36.3, 45.6, 122.3, 123.7, and 136.9 ppm, DMSO-d$_6$ δ = 40.1 ppm, glucose C$_2$-C$_6$ signals between δ = 59 and 85 ppm.
e. Anomeric C$_1$ NMR signal for acyclic glucose δ = 206.1 ppm

Table 5.1 Distribution of glucose isomers dissolved in D$_2$O and [Emim][Cl]. T = 333 K, 30 mg $^{13}$C glucose, 500 mg solvent, 100 mg DMSO-d$_6$, 10,000 transients

<table>
<thead>
<tr>
<th>Isomeric Form</th>
<th>D$_2$O</th>
<th>[Emim][Cl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-pyranose</td>
<td>60.02%</td>
<td>61.09%</td>
</tr>
<tr>
<td>A-pyranose</td>
<td>38.95%</td>
<td>35.11%</td>
</tr>
<tr>
<td>B-furanose</td>
<td>0.62%</td>
<td>2.05%</td>
</tr>
<tr>
<td>A-furanose</td>
<td>0.39%</td>
<td>1.36%</td>
</tr>
<tr>
<td>acyclic</td>
<td>NOB</td>
<td>0.03%</td>
</tr>
<tr>
<td>hydrated acyclic</td>
<td>0.03%</td>
<td>0.35%</td>
</tr>
</tbody>
</table>

NOB – Not observed in spectra
Figure 5.4 Temporal evolution of the $^{13}$C NMR of $^{13}$C glucose dissolved in [Emim][Cl] and DMSO-d$_6$ with WCl$_6$ at 353 K. $T = 353$ K, 42 mg $^{13}$C glucose, 22 mg WCl$_6$, (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$, 300 transients collected at $t = 0$ min before catalyst addition, followed by 300 transients at specified time intervals. β-glucopyranose: $\delta = 97.1$ ppm, α-glucopyranose: $\delta = 92.5$ ppm, β-glucofuranose: $\delta = 103.8$ ppm, Levoglucosan: $\delta = 102.8$ ppm, Intermediate I: $\delta = 104.2$ ppm.

Figure 5.5 Pathway for levoglucosenone formation from β-glucopyranose. 1: β-glucopyranose, 7: levoglucosan, 8: 1,6-anhydro-2,3-dideoxy-β-D-erythro-hexopyranos-2-ulose, 9: levoglucosenone.
Figure 5.6 Temporal evolution of product and intermediates formed during the dehydration of $^{13}$C glucose in [Emim][Cl] and DMSO-d$_6$ with WCl$_6$ at 353 K. $T = 353$ K, 42 mg $^{13}$C glucose, 22 mg WCl$_6$, (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. 300 transients collected at $t = 0$ min before catalyst addition, followed by 300 transients at specified time intervals. HMF peaks (◊ marker) correspond to C$_1$ - doublet at 178.7 ppm, C$_5$ - triplet at 162.8 ppm, C$_2$ – triplet at 152.0 ppm, C$_3$ – triplet at 125.5 ppm, C$_4$ – triplet at 110.2 ppm, and C$_6$ – doublet at 56.1 ppm. Characteristic peaks of intermediates are triplet peaks at 188.6, 135.8 and 38.2 ppm and doublet peak at 147.9 ppm. Two secondary product peaks appear as singlets at 163.1 ppm (formic acid) and 124.5 ppm (CO$_2$).
Figure 5.7 Expansion of a portion of the NMR spectrum shown in Figure 5.6. T = 353 K, 42 mg $^{13}$C glucose, 22 mg WCl$_6$, (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. 300 transients collected at t = 0 min before catalyst addition, followed by 300 transients at specified time intervals. HMF peaks (◊ marker) correspond to C$_5$ - triplet at 162.8 ppm, C$_3$ – triplet at 125.5 ppm. Two secondary product peaks appear as singlets at 163.1 ppm (formic acid) and 124.5 ppm (CO$_2$).
Figure 5.8 $^{13}$C NMR spectrum showing the presence of the five structural isomers of $^{13}$C fructose dissolved in [Emim][Cl] and DMSO-d$_6$. T = 298 K, 30 mg $^{13}$C fructose, 500 mg [Emim][Cl], 100 mg DMSO-d$_6$, 10,000 transients.

a. Full spectra. [Emim][Cl] δ = 15.5, 36.3, 45.6, 122.3, 123.7, and 136.9 ppm, DMSO-d$_6$ δ = 40.1 ppm, fructose C$_2$-C$_6$ signals between δ = 59 and 85 ppm.

b. Anomeric C$_2$ NMR signals for α-fructofuranose δ = 104.8 ppm, β-fructofuranose δ = 102.3 ppm, β-fructopyranose δ = 99.3 ppm;

c. Anomeric C$_1$ NMR signal for acyclic fructose δ = 214.7 ppm

Note: α-fructopyranose appears at 99.2 ppm, but cannot be deconvoluted from the β-fructopyranose peak.
Figure 5.9 Temporal evolution of the $^{13}$C NMR of $^{13}$C glucose dissolved in [Emim][Cl] and DMSO-d$_6$ with H$_2$SO$_4$. T = 353 K, 42 mg $^{13}$C glucose, 4.5 mg 98 wt% H$_2$SO$_4$ (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. 300 transients collected at t = 0 min before catalyst addition, followed by 300 transients at specified time intervals. β-glucopyranose: $\delta = 97.1$ ppm, α-glucopyranose: $\delta = 92.5$ ppm, β-glucofuranose: $\delta = 103.8$ ppm, Levoglucosan: $\delta = 102.9$ ppm, Intermediate 1 $\delta = 104.2$ ppm.
Figure 5.10 Temporal evolution of product and intermediates formed during the dehydration of $^{13}$C glucose in [Emim][Cl] and DMSO-d$_6$ with H$_2$SO$_4$. T = 353 K, 42 mg $^{13}$C glucose, 4.5 mg 98 wt% H$_2$SO$_4$ (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. 300 transients collected at t = 0 min before catalyst addition, followed by 300 transients at specified time intervals. HMF peaks (◊ marker) correspond to C$_1$ - doublet at 178.7 ppm, C$_5$ - triplet at 162.8 ppm, C$_2$ – triplet at 152.0 ppm, C$_3$ – triplet at 125.5 ppm, C$_4$ – triplet at 110.2 ppm, and C$_6$ – doublet at 56.1 ppm. Characteristic peaks of intermediates are Triplet peaks at 188.6, 135.8 and 38.2 ppm and doublet peak at 147.9 ppm. Additional intermediates are observed as triplets at 150.2, 135.9, 119.4, 112.5 ppm and doublets at 148.4, 131.4 ppm.
Figure 5.11 Proposed structure of keto-form intermediate formed by acid-catalyzed dehydration of glucose initiated at the C\textsubscript{2} position (\textsuperscript{13}C NMR shifts predicted by ChemBioDraw are shown in parentheses)

\[ \text{147.9 ppm (169.3 ppm)} \]
\[ \text{38.2 ppm (34.9 ppm)} \]

Figure 5.12 Proposed structure of enol intermediate formed by acid-catalyzed dehydration of glucose initiated at the C\textsubscript{3} position (\textsuperscript{13}C NMR shifts predicted by ChemBioDraw are shown in parentheses)

\[ \text{188.6 ppm (180.1 ppm)} \]
\[ \text{104.2 ppm (107.1 ppm)} \]
\[ \text{135.8 ppm (102.4 ppm)} \]
Figure 5.13 Evolution of NMR glucose peak areas as a function of time. $T = 353$ K, 42 mg $^{13}$C glucose, 22 mg $\text{WC}_6$, (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. Peaks correspond to $\beta$-glucopyranose: $\delta = 97.1$ ppm, $\alpha$-glucopyranose: $\delta = 92.5$ ppm, $\beta$-glucofuranose: $\delta = 103.8$ ppm.
Figure 5.14 Evolution of NMR peak areas as a function of time. $T = 353$ K, 42 mg $^{13}$C glucose, 22 mg WCl$_6$, (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. Peaks correspond to $\beta$-glucopyranose: $\delta$ = 97.1 ppm, $\alpha$-glucopyranose: $\delta$ = 92.5 ppm, $\beta$-glucofuranose: $\delta$ = 103.8 ppm.
Figure 5.15 5-HMF Production from glucopyranose. $T = 353$ K, 42 mg $^{13}$C glucose, 22 mg WCl$_6$, (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. The contribution to the overall yield of 5-HMF from glucofuranose has been subtracted from the data in order to identify the formation of 5-HMF from glucopyranose exclusively.

Table 5.2 Comparison of the initial rates of furanose consumption and 5-HMF formation catalyzed by WCl$_6$ and H$_2$SO$_4$. $T = 353$ K, 42 mg $^{13}$C glucose, 22 mg WCl$_6$ or 5.6 mg 98 wt% H$_2$SO$_4$ (24 mol%), 478 mg [Emim][Cl], 116 mg DMSO-d$_6$. Rates are based on changes in NMR signals at 103.8 ppm for $\beta$-glucofuranose and 178.7 ppm for 5-HMF. Peak areas were converted to concentration based on quantitative analysis done by HPLC-RID.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Rate of $\beta$-glucofuranose Disappearance $\mu$mol cm$^{-3}$ s$^{-1}$</th>
<th>Rate of 5-HMF Appearance $\mu$mol cm$^{-3}$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCl$_6$</td>
<td>0.428</td>
<td>0.429</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>0.407</td>
<td>0.406</td>
</tr>
</tbody>
</table>
**Figure 5.16** Acid-catalyzed dehydration of glucofuranose to 5-HMF

**Figure 5.17** Dehydration of the keto intermediate to form 5-HMF catalyzed by $\text{MCl}_x$. 
Figure 5.18 Dehydration of the enol intermediate to form 5-HMF catalyzed MClx.
References


