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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

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An investigation was carried out into the hydrolysis of cellulose dissolved in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) and 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) catalyzed by mineral acids. Glucose, cellobiose, and 5-hydroxymethylfurfural (5-HMF) were observed as the primary reaction products. The initial rate of glucose formation was determined to be of first order in the concentrations of dissolved glucan and protons and of zero order in the concentration of water. The 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/ionic liquid 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 humins is suggested to proceed through the condensation polymerization of 5-HMF with glucose.

Introduction

It has been estimated that lignocellulosic biomass could meet about 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 that 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 repeating cellobiose monomers, the dimer of the six-carbon-atom sugar (C_6) D-glucose, while hemicellulose is a polymer comprised of various C_5 and C_6 sugars. The primary focus of recent investigations has been on the conversion of cellulose into glucose, since this sugar can be fermented to produce ethanol or butanol for blending with current transportation fuels.[1] It has also been demonstrated that glucose can be converted by nonbiological catalytic routes to gasoline or diesel additives by either dehydration of glucose to 5-hydroxymethylfurfural (5-HMF) followed by hydrogenation to lower-oxygen-content additives such as dimethylfuran, or by hydrogenation of glucose to sorbitol followed by dehydration and hydrogenation to hexane.[3,4]

To obtain glucose, cellulose must be hydrolyzed. Since cellulose is crystalline, it is desirable to first dissolve cellulose in a suitable solvent to make the β-1,4-glycosidic linkages between glucose residues more readily accessible for hydrolysis. Recent studies have shown that ionic liquids (ILs) exhibit excellent solubility for cellulose.[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.[6]

Several studies of cellulose depolymerization in ILs have been reported. The first of these demonstrated that cellulose dissolved in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) could be depolymerized by heating to 373 K for 60 min in the presence of concentrated H_2SO_4.[7] Overall sugar yields of 66–81% were obtained, with isolated glucose yields of 21–39%. More recent work has shown that the progressive addition of water to cellulose dissolved in 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]) could increase the yield of glucose to 89% and limit the yield of 5-hydroxymethylfurfural (5-HMF) to 7%.[8] In this study, 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 also been modeled using cellobiose dissolved in [Emim][Cl].[9] This work showed that the rate

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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 because 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.[10] Likewise, evidence has been presented that suggests 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.[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 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 [Bmim][Cl] or [Emim][Cl].

Experimental Section

Materials

Unless stated otherwise, materials were used as received. The ILs 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 (100% purity), 5-hydroxymethylfurfural (5-HMF, 99% purity), trifluoroacetic acid (100% purity), acetic acid (100% purity), phosphoric acid (100% purity), mixed-bed resin TMD-8 (hydrogen and hydroxide form), and 1-butyl-3-methylimidazolium acetate ([Bmim][CH_3COO], 95% purity) were purchased from Sigma Aldrich, USA. Glucose (G, USP Grade) was purchased from Hyclone, USA. Sulfuric acid (98% purity) was purchased from Acros. Hydrochloric acid (37% purity), HPLC grade acetonitrile, and ethyl acetate were purchased from Fisher Scientific, USA.

Cellulose hydrolysis

The hydrolysis of cellulose was performed by using a Symyx core module robot (CMR) equipped with a positive displacement tip. In a typical experiment, [Bmim][Cl] (15 mL) was dispensed into a 20 mL vial at 373 K using magnetic tumble stirring at 400 rpm. As the IL was dispensed, microcrystalline cellulose (750 mg, Avicel) was added to ensure the substrate had good contact with the stir bar. Once the cellulose and IL had been added, the solution was stirred for 3 h at 373 K 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. The 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 ultrapure water (2.1 mL) at room temperature. An aqueous solution of arabinose (700 μL of 10 mg mL^{-1} arabinose in water) serving as an internal standard was added to this mixture. The sample was then centrifuged, and the supernatant fluid (500 μL) 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 was analyzed by applying either high-performance liquid chromatography using a refractive index detector (HPLC-RID) or high-performance anion-exchange liquid chromatography using a pulsed amperometric detector (HPAEC-PAD). The remaining sample not treated with ion-exchange resin was extracted with ethyl acetate (5 extractions using 5 mL ethyl acetate) in a pear-shaped flask, treated with excess sodium carbonate ($NaCO_3$) to remove water, centrifuged, and analyzed by using gas chromatography (GC) equipped 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 with 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 was 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, 40, 60, and 100 μL of water, respectively, 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 the supernatant fluid (500 μL) was treated with the mixed-bed resin and centrifuged. The supernatant of the sample treated with the solid ion-exchange resin was analyzed by using HPLC-RID or HPAEC-PAD.

Analytical techniques

HPLC-RID analyses were performed on a Shimadzu instrument equipped with a Biorad Aminex HPX-87H column maintained at 333 K and eluted using a 0.01 N H_2SO_4 mobile phase flowing at 0.6 mL min^{-1}. Products were identified by comparing retention times with those of pure substances. Quantification was determined by dividing the integrated peak areas of hydrolysis products (cellobiose, glucose, or 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 × 150 mm) maintained at 303 K. Analyses were conducted utilizing a gradient mobile phase flowing at 0.6 mL min^{-1}, in which the concentration of NaOH changed from 30 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 ana-

lyzed by using a Varian triple quadrupole mass spectrometer and a flame-ionization detector (GC–MS). The products identified by using 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 into a molar concentration by using a five-point calibration curve.

Product yields were determined on a molar basis by using the initial concentration of glucose residues in the starting substrate. Results were reproducible within 5% of the measured value. The initial number of 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 dividing the total moles of glucose residues contained in the product by the initial number of moles of glucose residues. White solids precipitated during dilution of reaction samples taken prior to a maximum in glucose yield and 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 the sum of the measured increase in the yield of 5-HMF. Selectivity was calculated by dividing the number of moles of a product by the sum of the number of moles of all measured products.

Results and Discussion

The temporal evolution of products produced during the hydrolysis of cellulose, dissolved in [Bmim][Cl] and catalyzed by H_2SO_4, is shown in Figure 1. Glucose and cellobiose are observed as the dominant products during the first 30 min of reaction. For reaction times between 30 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-hydroxymethylfurfural (5-HMF) begins to increase, indicating that glucose undergoes dehydration as it accumulates in the reactor. After the maximum in the glucose yield is reached, a dark solid material, assumed to be humins, is observed, and the yield of 5-HMF continues to increase. With further reaction, the yields of cellobiose and glucose decrease, whereas the yield of humins rises sharply, and the yield of 5-HMF increases slightly. In addition to the products shown in Figure 1, small amounts of levoglucosenone (LG) and 2-furyl hydroxymethyl ketone (2-FHMK) are observed by using gas chromatography equipped with a mass spectrometer (GC–MS), but the yields of these products never exceeded 3 and 7%, respectively. No evidence of oligosaccharides, other than cellobiose, was observed by using matrix-assisted laser-desorption ionization time-of-flight mass spectroscopy (MALDI-TOF) or high-performance anion-exchange liquid chromatography using a pulsed amperometric detector (HPAEC-PAD). This result differs from an earlier report, in which oligosaccharides with 2–10 units were observed during cellulose hydrolysis in purified [Emim][Cl] and in the absence of an acid catalyst at 393 K.[12] It is conceivable that oligosaccharides with more than two units are either not formed at the temperatures applied in this study (363 K) or they are formed, but very rapidly hydrolyzed to cellobiose and glucose in the presence of the added acid catalyst.

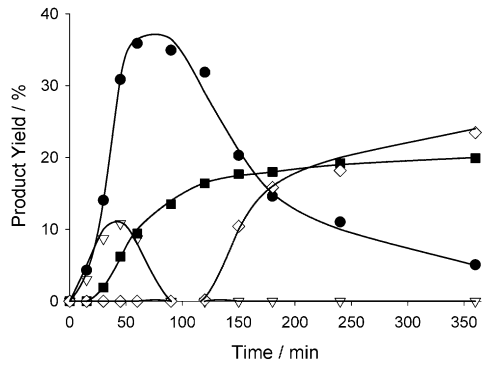


Figure 1. Product evolution as a function of time. Reaction Conditions: 363 K, 15 mM H_2SO_4 (0.5 molar equivalents per glycosidic linkage (MEq)), 0.55 M H_2O (≈3.5 MEq), Avicel (750 mg) dissolved in [Bmim][Cl] (15 mL). ●: glucose, ▽: cellobiose, ■: 5-HMF, and ◇: humins.

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 (see the Supporting Information, Figure S1). At reaction times longer than 100 min, the yield of humins is significantly higher than the yields of the 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 is in contrast to that reported in studies of glucose dehydration carried out in [Bmim][Cl] using H_2SO_4 as the catalyst, which demonstrated that after a reaction at 393 K for 3 h, 83% of the products were present as 5-HMF (66%), or 2-FHMK (17%), and only 16% of the products were humins.[4] The difference between the present results and those reported earlier is probably due to a higher acid concentration (150 mM H_2SO_4 compared to 5 mM H_2SO_4) and lower temperature (363 K compared to 393 K).

Experiments similar to those shown in Figure 1 were performed using HCl, CH_3SO_3H, CF_3COOH, H_3PO_4, and CH_3COOH. Table 1 compares the pK_a values in water of each acid, the maximum glucose (G) yield, the time to reach the maximum glucose yield, the maximum cellobiose (CB) yield, the time to reach the maximum cellobiose yield, and the maximum 5-HMF yield. The data reveal that acids with p$K_a \geq 2$ (H_3PO_4 and CH_3COOH) are inactive, whereas acids with p$K_a \leq -1.9$ (HCl, H_2SO_4, and CH_3SO_3H) show very similar characteristic values for the maximum yields of the product and the time at which the maximum yield is reached. Only CF_3COOH (p$K_a = 1.0$) showed a different activity, which was between these two regimes with maximum yields similar to those obtained using acids with p$K_a \leq -1$, but longer reaction times to reach the maximum of these yields. These results are consistent with the findings reported in a recent study of the acid-catalyzed hydrolysis of cellobiose dissolved in [Emim][Cl] carried out at 363 K.[9] In that study, it was observed that acids with p$K_a < 0.5$ were active for

Table 1. Maximum yields and time to reach maximum yields for the hydrolysis of cellulose in [Bmim][Cl].[a]

Acid	pK_a	Yield [%] G[b]	Time [min] G	Yield [%] CB[c]	Time [min] CB	Yield [%] 5-HMF
HCl	−4.0	33	106	11	54	18
H_2SO_4	−3.0	33	99	11	65	20
CH_3SO_3H	−1.9	32	106	11	54	20
CF_3COOH	1.0	38	208	12	140	17
H_3PO_4	2.1	no activity after 2 h, 363 K, 0.3 M acid				
CH_3COOH	4.5	no activity after 2 h, 363 K, 0.3 M acid				

[a] Reaction conditions: 363 K, 0.55 M H_2O, 15 mM acid, microcrystalline cellulose (750 mg) dissolved in [Bmim][Cl] (15 mL). [b] G = glucose. [c] CB = cellobiose.

cellobiose hydrolysis and that acids with p$K_a \leq -1.9$ all behaved similarly. The effects of acid strength on the hydrolysis of both cellulose and cellobiose are best attributed to the extent of acid dissociation in the IL. Thus, acids with p$K_a \leq -1.9$ are expected to be fully dissociated. Consistent with this interpretation, H_2SO_4 became completely inactive, if [Bmim][CH_3COO] was used as the solvent instead of [Bmim][Cl], because the acetate anions of the IL have a strong affinity for the protons of the acid. Using these observations, further studies were carried out in chloride-containing ILs with H_2SO_4 and HCl.

The effect of varying H_2SO_4 concentration on the production of glucose, 5-HMF, and humins is shown in Figure 2 for concentrations ranging from 15 (0.05 molar equivalents per glycosidic linkage (MEq)) to 300 mM (1.0 MEq). Increasing the concentration of H_2SO_4 increased the rate at which all products were produced. The maximum yield of glucose, shown in Figure 2a, 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 Figure 2b and c, as the 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 was attributed to both humin formation and its degradation to levulinic and formic acids, both of which were observed in low yields by using GC–MS.

The hydrolysis of cellulose using H_2SO_4 in [Bmim][Cl] was studied at temperatures from 343 to 393 K. The apparent activation energy for the hydrolysis of cellulose to glucose, determined from an Arrhenius plot of utilizing the initial rate of glucose formation, was 96 kJ mol^{-1} (see the Supporting Information, Figure S2). This value agrees well with the apparent activation energy calculated from previously reported results in [Emim][Cl] using CH_3SO_3H for the hydrolysis of cellulose and cellobiose, which were 92 and 84 kJ mol^{-1}, respectively.[9] Notably, the apparent activation energies for the 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 was 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 was reported that the difference between the apparent activation energies for cellulose hydrolysis and glucose dehydration is 7–11 kJ mol^{-1}.[9]

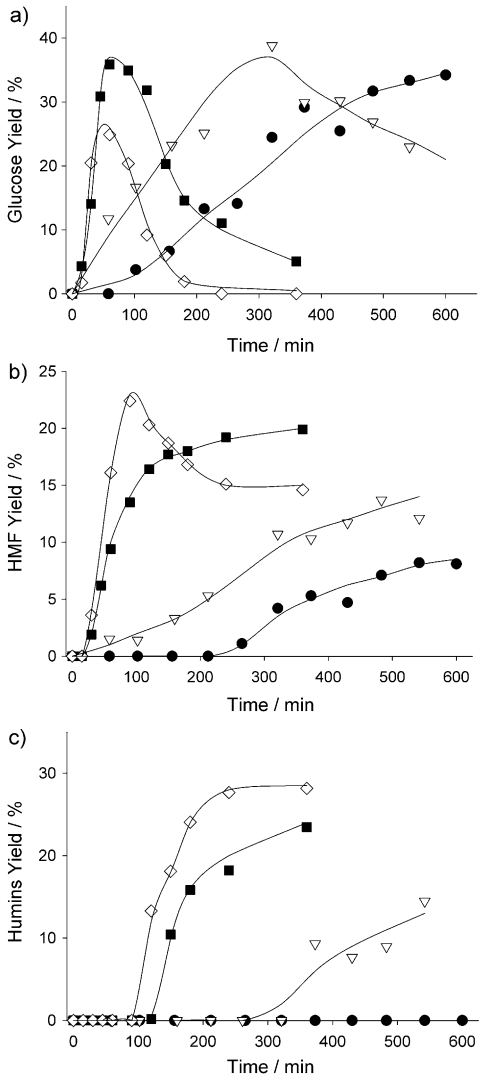


Figure 2. Effect of varying acid strength on a) glucose, b) 5-HMF, and c) humin formation from cellulose. Reaction conditions: 363 K, H_2SO_4 concentration varied from 15 (0.05 molar equivalents per glycosidic residue (MEq)) to 300 mM (1.0 MEq), 0.55 M H_2O (≈3.5 MEq), Avicel (750 mg) dissolved in [Bmim][Cl] (15 mL). ●: 15, ▽: 30, ■: 150, and ◇: 300 mM H_2SO_4.

Two sets of experiments were carried out 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 3. 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

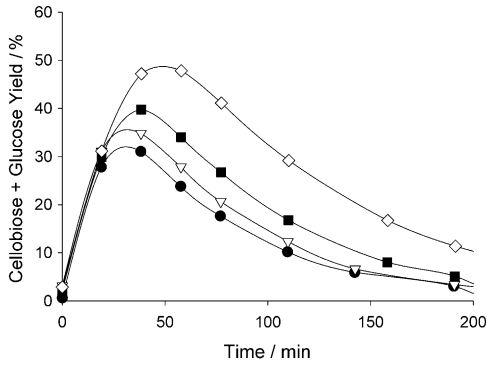


Figure 3. Effect of varying initial water content on the total yield of depolymerization products. Reaction conditions: 363 K, [Bmim][Cl] (15 mL), 0.3 M H_2SO_4 (1.0 molar equivalents per glycosidic linkage (MEq)), water content varied from 0.15 (≈1 MEq) to 1.5 M (≈10 MEq), Avicel (750 mg) dissolved in [Bmim][Cl] (15 mL).

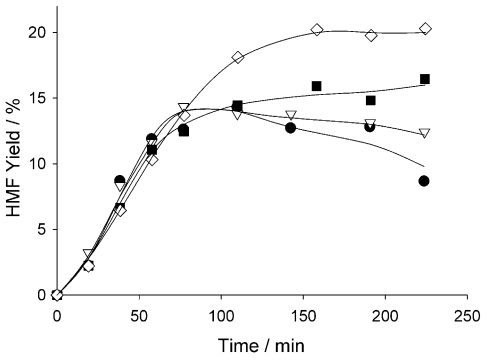


Figure 4. Effect of varying initial water content on the 5-HMF yield. Reaction conditions: 363 K, [Bmim][Cl] (15 mL), 0.3 M H_2SO_4 (1.0 molar equivalents per glycosidic linkage (MEq)), water content varied from 0.15 (≈1 MEq) to 1.5 M (≈10 MEq), Avicel (750 mg) dissolved in [Bmim][Cl] (15 mL). ●: 1, ▽: 2, ■: 3.5, and ◇: 10 MEq.

times. These higher glucose yields resulted in higher 5-HMF yields after 150 min, as shown in Figure 4, due to the higher concentration of glucose generated. As discussed below, the amounts of water used in the experiments shown in Figures 3 and 4 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, which is in accordance with the optimized water-addition strategy suggested by Binder and Raines.[8] The initial water content was 5% at 0 min and was increased by adding 15, 5, 8, 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. As with the results shown in Figure 3, 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, experiments 1–5). By following the optimized protocol, glucose yields of up to 74% could be reached in 2 h, with less than 10% yield of 5-HMF and less than 3% yield of humins (Table 2, experiment 5, entry 20), which was consistent with the results reported by Binder and Raines.[8]

The mechanism for the acid-catalyzed hydrolysis of carbohydrate substrates dissolved in organic solvents can be used to interpret the hydrolysis of glucans dissolved in ILs.[14,15] As illustrated in Scheme 1, 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 the IL was determined experimentally to be of first order in glucan and acid concentration, but zero order in water concentration (see the Supporting Information, Figure S3), suggesting that the protonation of glucan was quasi-equilibrated, whereas the

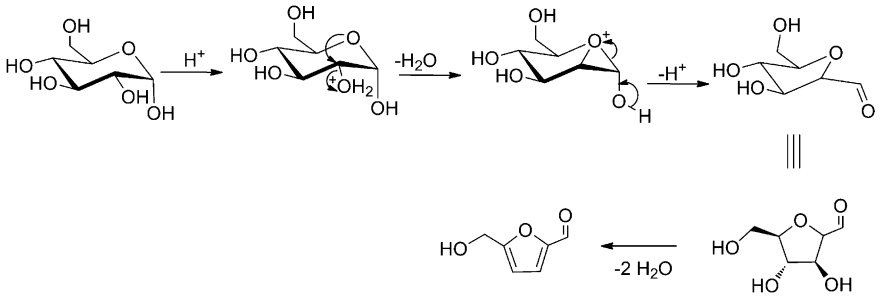
Table 2. Varying water-addition strategy to maximize the glucose yield and limit the 5-HMF formation.[a]

Experiment	Entry	Water added [wt%]					Time	Product yields [%]			
		0	10	20	30	60	[min]	Cellobiose	Glucose	HMF	Humins
1	1	5	–	–	–	–	30	8	51	13	–
	2	5	–	–	–	–	60	2	40	21	3
	3	5	–	–	–	–	90	1	26	24	14
	4	5	–	–	–	–	120	0	17	26	21
2	5	5	15	–	–	–	30	15	51	7	–
	6	5	15	–	–	–	60	5	65	13	–
	7	5	15	–	–	–	90	2	58	19	1
	8	5	15	–	–	–	120	1	46	25	7
3	9	5	15	5	–	–	30	16	48	5	–
	10	5	15	5	–	–	60	7	71	10	–
	11	5	15	5	–	–	90	3	67	14	–
	12	5	15	5	–	–	120	2	59	16	6
4	13	5	15	5	8	–	30	16	43	5	–
	14	5	15	5	8	–	60	2	61	6	–
	15	5	15	5	8	–	90	4	73	9	–
	16	5	15	5	8	–	120	3	74	12	–
5	17	5	15	5	8	10	30	13	54	7	–
	18	5	15	5	8	10	60	8	70	9	–
	19	5	15	5	8	10	90	5	76	10	–
	20	5	15	5	8	10	120	4	74	10	2
6	21	5[b]	15[b]	5[b]	8[b]	10[b]	30	9	46	17	–
	22	5[b]	15[b]	5[b]	8[b]	10[b]	60	4	42	23	–
	23	5[b]	15[b]	5[b]	8[b]	10[b]	90	1	32	26	5
	24	5[b]	15[b]	5[b]	8[b]	10[b]	120	1	27	29	7

[a] Reaction conditions: cellulose (20 mg) in [Emim][Cl] (343 μL, ≈400 mg) at 383 K for 3 h. Reactor temperature reduced to 378 K and allowed to equilibrate for 1 h. 1.66 M HCl (23.2 μL) was added and after 10, 20, 30, and 60 min, 80, 40, 60, and 100 μL of water, respectively, or an equivalent molar amount of acetonitrile, were added to the reactor. [b] Acetonitrile added to IL in place of water.

dissociation of the β-1,4-glycosidic bond was 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 also be explained on the basis of the mechanism shown in Scheme 1. 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 re-established. This seems to be probable because the viscosity of glucan–IL solutions is relatively high. In 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.

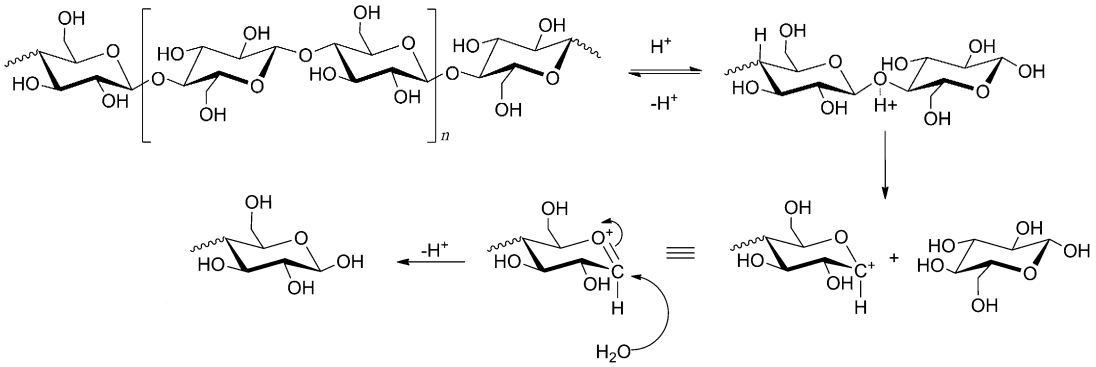


Scheme 2. Proposed reaction mechanism for the formation of 5-HMF.

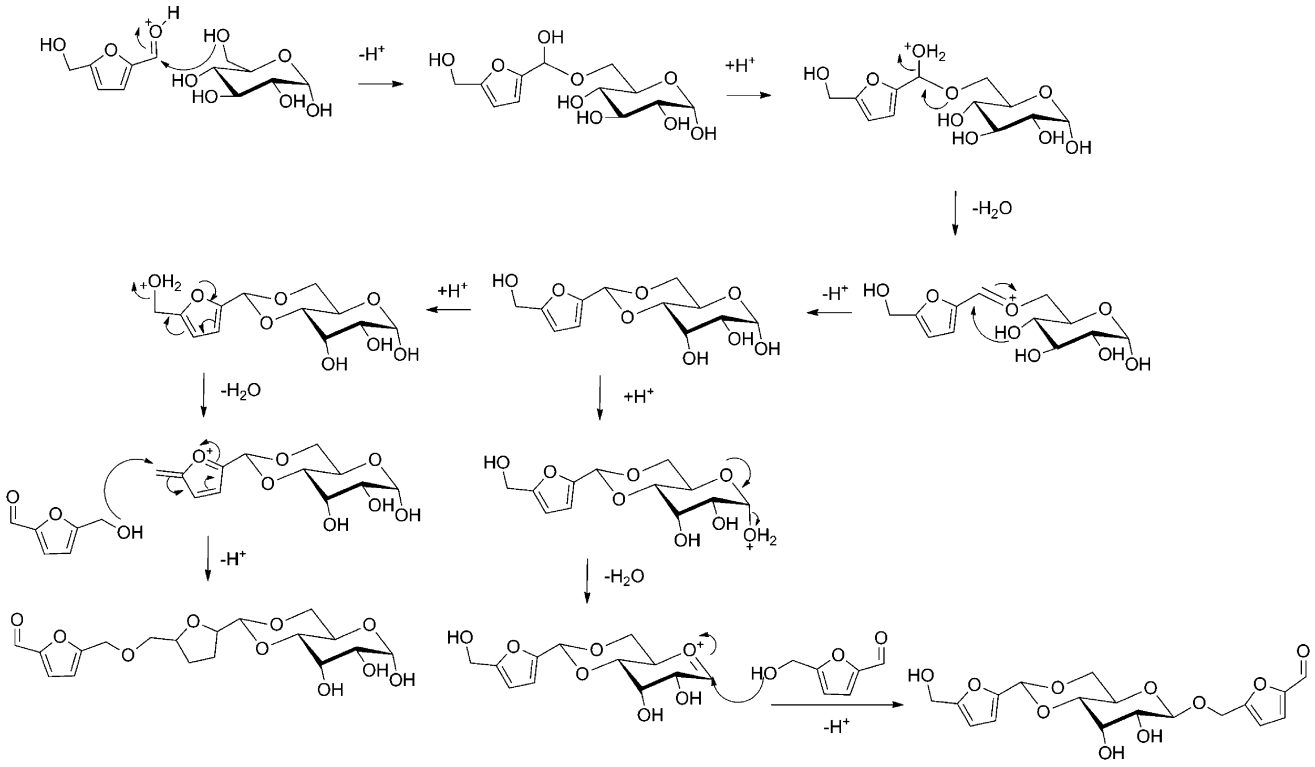
The mechanism for the dehydration of sugars to furans has been actively discussed.[16,17] Two pathways were proposed: one that involves the isomerization of acyclic intermediates and another that proceeds through the transformation of ring structures. While several authors have suggested that glucose isomerization to fructose by 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 nor in our earlier work on the dehydration of glucose.[4] Recent computational work suggested that a precursor to 5-HMF can be formed directly from glucose upon protonation of the 2-OH hydroxyl group.[19] In a related study concerning the dehydration of xylose to furfural, the same authors concluded that dehydration through the cyclic pathway was more energetically favorable than through the acyclic pathway.[20] For these reasons, we propose that dehydration of glucose in IL proceeds according to the cyclic pathway shown in Scheme 2.[19] After protonation of the 2-OH hydroxyl, the five-membered 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 1-OH.[20] Acid-catalyzed protonation and dehydration of the remaining hydroxyl groups on the ring produces 5-HMF.

The chemistry of humin formation is poorly understood. Although several authors suggested that this product is formed by polymerization of furanic compounds,[21] recent experimental work indicated that humin formation occurred through condensation polymerization of sugars with the products of their dehydration.[22] A possible mechanism for this process is shown in Scheme 3. The proposed mechanism is derived from the *cis*-diol protection of an 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. Although this mechanism is hypothetical, it would explain the recent observation that humin formation is of 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 3 and Table 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



Scheme 1. Proposed reaction mechanism for the hydrolysis of cellulose.



Scheme 3. Proposed reaction mechanism for the formation of humins.

formed, inhibits the dehydration of glucose and its condensation with the formed 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 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 $(-183 \pm 15)\,kJ\,mol^{-1}$.[24] A more plausible interpretation is that the oxygen atom in the 2-OH hydroxyl group of the glucose pyranose ring is less nucleophilic than water and, hence, water effectively diverts protons from protonating 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 concentrations of 5-HMF being reduced because of reduction in the dehydration process 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, experiment 6) produced similar results to those without gradual water addition (Table 2, experiment 1), with slightly higher yields of cellobiose, glucose, and 5-HMF. Although acetonitrile is a polar solvent, its dielectric constant is less than that of water, leading to the conclusion that it is not effective in keeping the protons present in the IL from interacting with glucose and promoting its dehydration.

Conclusion

This 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 with H_2SO_4 as the catalyst was determined to be of first order in the concentrations of glucan and acid and of 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 was likely reversible due to slow diffusion of the cleaved ends away from each other. Acids with $pK_a < 0.5$ were active for glucan hydrolysis and acids with $pK_a < -1.9$ were equally effective, independent of the nature of the anion, suggesting that these acids were 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 a level of 1–10 molar

equivalents per β-1,4-glycosidic bonds had no effect on the initial rate of glucan hydrolysis, suggesting that the oxygen atoms of the glycosidic linkages were more nucleophilic than those in water. Addition of larger quantities of water to the reaction mixture, particularly once substantial amounts of glucose had formed, inhibited the dehydration of glucose to 5-HMF and the formation of humins. The inhibition of glucose dehydration was attributed to preferential protonation of water as compared to the 2-OH hydroxyl of glucose. The reduction in humin formation was attributed to the decrease in 5-HMF formation with increasing glucose formation, since humin formation was ascribed to the condensation polymerization of 5-HMF and glucose. This process might be further inhibited by water solvation of protons, making free protons less available for the protonation of the oxygen atoms of the carbonyl groups in 5-HMF, the process thought to be responsible for the propagation of the humin formation.

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Keywords: biomass · cellulose · glucose · ionic liquids · kinetics

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