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(YIP-11) BIOPOLYMER PROCESSING USING IONIC LIQUIDS

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Final Report

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The proposed research will investigate the utilization of ionic liquids (IL's) as solvents and catalysts for the dissolution and degradation of chitin and chitosan. This project will:1) synthesis various IL catalysts to study their effects on depolymerization of chitin and chitosan, 2) synthesis a variety of IL's to invesitgate the effects of dissolution solvents on the rate of reaction and degradation products of the conversion of chitin and chitosan, and 3) investigate the effects of various reaction conditions, such as reaction temperature, and catalyst loading, on the reaction rate and degradation products from the depolymerization of chitin and chitosan.

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Final Report

Contract Number:	FA9550-11-1-0053
Title of Research:	Biopolymer Processing using Ionic Liquids for Feedstock Chemicals
Principal Investigator:	W. Matthew Reichert
Reporting Period:	1 May 2011 to 30 April 2014

I. Abstract

Chitin is the second, cellulose being the first, most abundant biomaterial on the earth. Chitin consists of C-O-C linked *N*-acetylglucosamine groups, which form polymers of various molecular weights and rigidity, based on degree of crosslinking and other components incorporated into the polymer during polymerization. Chitin is not only the main component of the shells of crustaceans, but also exists as a structural polysaccharide of insects, mushrooms, and yeasts. This material represents an untapped source of chemical energy and feedstock chemicals. The proposed research will investigate the utilizing ionic liquids (ILs), which are compounds composed of ions that are liquid at or below 100 °C, as solvents and catalyst for the dissolution and degradation of chitin and chitosan.

One task in this project will investigate the use of a variety of ionic liquids for the dissolution of chitin. The current state of technology has focused on the short-chained imidazolium cations with the chloride and acetate anion. This project will expand this focus to pyrrolidium, piperidinium, ammonium, and phosphonium cations with halide and acetate anions, which literature shows provide the best dissolution results.

Another task in this project is the synthesis of new ionic liquid acid catalysts. The project has focused on incorporating the sulfonic acid functionality in to the ionic liquids with plans to expand into new acidic functional groups. In the past, the synthesis of sulfonic acid functionalized ionic liquids was limited by the sultone starting materials, a new synthetic route has been developed which bypasses this limitation allowing for a more diverse array of acid catalysts. The acid functionalized used for the catalysts was expanded to include the boronic acid and phosphonic acid functionalities. A successful synthetic route to the boronic acid functionalized ionic liquids was achieved. While progress on the phosphonic acid catalysts was made, a successful final product has not been achieved to date.

The final task of this project is the combination of the dissolution of the biomass with the acid catlaysts to depolymerize the biomass into feedstock type chemicals. By using an imidazolium based ionic liquid for the dissolution of chitin and a sulfonic acid functionalized ionic liquid, chitin can be hydrolyzed into its monomer unit, *N*-acetylglucosamine (NAG), as well as other products. The product stream can be controlled by reaction temperature and the structure of the catalyst. Other parameters that might affect the products of the hydrolysis reaction are catalyst loading, water content, and catalyst ions.

II. Technical Section

Technical Objective

Ionic liquids have demonstrated the ability to effectively dissolve biomass,[1,2] including chitin and chitosan, and are excellent media for catalytic processes.[3,4] **The main goal of this project is to demonstrate the ability of ionic liquids, as solvent and catalyst, to convert chitin and chitosan into feedstock chemicals for new biorenewable and biocompatible polymers.**

Technical Approach

The approach to this research effort is to investigate the use of a combination of ionic liquids, one to act as the solvent and the other to act as a catalyst, for the conversion of chitin into feedstock chemicals.

Project Tasks

Task 1: Synthesize and evaluate the dissolution ability of several ionic liquids as solvents for chitin.

Task 2: Develop and synthesize new acid-functionalized ionic liquids for the depolymerization of chitin.

Task 3: Evaluate the depolymerization of chitin and chitosan using in ionic liquids solvent and catalyst in *N*-acetylglucosamine and glucosamine.

The goal of Task 1 will be the synthesis and evaluation of several ionic liquids for the dissolution of chitin and chitosan. The proposed cations to be tested are shown in Figure 1. The anions to be studied will be restricted to acetate and halide anions since these anions have shown the best ability to dissolve chitin.[1,2,5] Two different methods, conventional heating and microwave heating, will evaluate the dissolution of chitin in the ionic liquids. The ionic liquids initially evaluated were the pyrrolidium and piperidinium iodide salts with the acetate salts evaluated next.

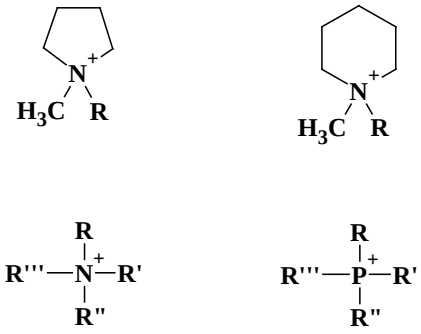
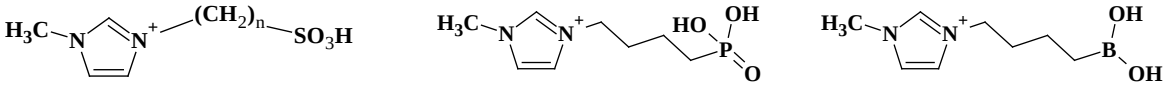


Figure 1. Pyrrolidinium (PYR_{1R}), Piperidinium (PIP_{1R}), Ammonium ($N_{R,R',R'',R'''}$) and Phosphonium ($P_{R,R',R'',R'''}$) cations for evaluation as solvents for chitin and chitosan. R = methyl, ethyl, propyl, butyl, pentyl, hexyl, and heptyl groups.

The goals of Task 2 will be the synthesis of several acid-functionalized ionic liquids. The proposed acid catalysts are shown in Figure 2. Initially the projected focused in the synthesis of in sulfonic acid based ionic liquids, shown in Figure 2a. Now that a new synthesis route to longer alkyl chain tethers is developed, an expansion to the other acid functionalities is under investigation. In order to test the effectiveness of the compounds as catalysts, a simple esterification reaction is used. This study will provide insight into the effectiveness of the acids before evaluation in Task 3.



a b c

Figure 2. Proposed acid functionalized ionic liquids catalysts, a) sulfonic acid functionality, b) phosphoric acid functionality, and c) boronic acid functionality

The goal in Task 3 will be combining results from literature reports and the dissolution study in Task 1 with the catalysts development in Task 2 into an effective deploymerization reaction. The proposed products from the degradation of chitin are shown in Figure 3. The initial depolymerization study will focus on the hydrolysis of chitin in order to produce degradation products. Various reaction conditions will be investigated, such as reaction temperature, catalyst structure, ionic liquid solvent, and catalyst loading. HPLC and LC/MS analysis of the reaction solutions for the monomer and other degradation products will provide insight in the degradation mechanics. The results from the ionic liquid catalyst will be compared to those using mineral acids.

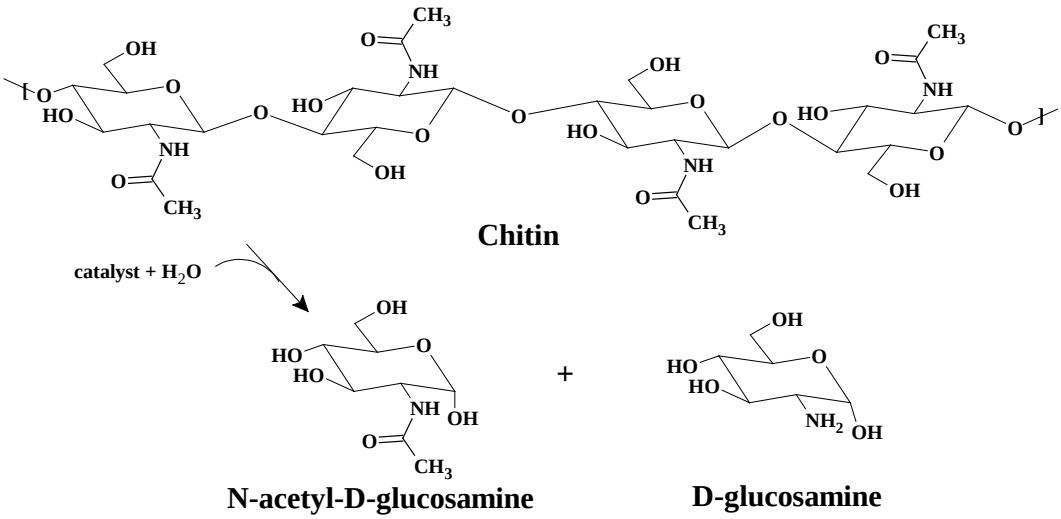


Figure 3. The proposed degradation of chitin into N-acetyl-D-glucosamine (NAG) and D-glucosamine

Progress Statement Summary

This research project is an investigation of ionic liquid technologies for the processing of biomass, specifically chitin. The three tasks in this study are the 1) dissolution of chitin in an ionic liquid, 2) development of an ionic liquid-based acid catalyst, and 3) hydrolysis of chitin utilizing an ionic liquid solvent and an ionic liquid acid catalyst.

A study of the various pyrrolidinium and piperdinium iodide ionic liquids showed little dissolution of the biopolymer even after initial dissolution and reconstitution. The focus of the project shifted to Task 2 and Task 3 using the well-known ionic liquid solvent 1-butyl-3-methylimidazolium chloride, [C_4mim][Cl].

This project focused on the development and utilization of acid functionalized ionic liquids for the depolymerization of chitin into *N*-acetyl-D-glucosamine (NAG) and other dehydration products. Previous reports focused on the synthesis of sulfonic acid functionalized imidazolium and pyrrolidinium cation ionic liquids with the triflate and bis(trifluoromethanesulfonyl)imide anion. This report expands that work to include two other acid functionality, boronic acid. A successful synthetic route for boronic acid functionalized ionic liquids has been developed. This catalyst was then tested using an acid catalyzed transesterification reaction, synthesis of ethyl acetate. The boronic acid ionic liquid proved to be ineffective in catalyzing the reaction. While not useful as a catalyst, literature on boronic acids revealed that they could be used to extract carbohydrates from the reaction mixtures.

In a continuing effort to gain better insight into products formed during the hydrolysis reaction, we utilized a LC/MS instrument and gathered mass spectrum data on the proposed products from the reaction, namely the various oligomers of chitin: NAG, chitobiose, and chitotriose. While the LC method used in this study did not provide separation of the various oligomers, it did allow for their separation from the reaction mixture, [C_4mim][Cl] and the ionic liquid catalysts. The mass spectrum data was collected for the standards: NAG, chitobiose, and chitotriose. Next, the reaction mixtures were tested in order to determine if the products were related to the chitin oligomers. Initial results showed a shift in the mass peaks for the oligomers. Chitobiose, which was original seen at 425 m/z, was offset to 431 m/z while chitotriose was shifted from 628 m/z to 635 m/z. An investigation into the effect of temperature on the depolymerization reaction showed that higher temperatures are more effective for the depolymerization of chitin; the temperatures studied were 80, 100, and 120°C. In addition, the effects of alkyl chain length were investigated. The results of the study showed that the shorter-chained sulfonic acid catalysts depolymerized the chitin faster than longer-chained sulfonic acids. This data coincides with the results from the esterification reaction studies from the last report.

In an effort to understand the product masses and why they are shifted from the standard oligomers, an indepth analysis of the treatment of the reaction mixtures was performed. During this investigation, it became clear that the filters used to remove the insoluble material from the reaction solution were contaminating the LC/MS samples. The contamination was responsible for the shift in the standard peaks and did not allow for the true identification of the reaction products. Since every sample was treated the same way and contaminated in the same manner, the trends seen in the MS data are still believed to be true trends. The unfiltered samples are currently being reanalyzed to determine the true masses for the depolymerization products.

Progress

Task 1 – Synthesis and evaluation of ionic liquids for the dissolution of chitin

With the inability of the pyrrolidium and piperidinium iodide salts to dissolve chitin, we shifted the focus of the task to pretreatment of the biomass in order to facilitate progress on Task 2 and 3.

During the project, we noticed a difficulty in dissolving the chitin in 1-butyl-3-methylimidazolium chloride, namely the time it takes to dissolve using microwave heating. An investigation into this problem was initiated. Literature shows that ionic liquids are used as pretreatments for biomass to be used in other processes[2,6,7] and this should work for our process as well. A pretreatment process was developed the incorporates the 1-ethyl-3-methylimidazolium acetate, [C_2mim][acetate], ionic liquid to dissolve the chitin which is then reconstituted in water to produce an amorphous chitin that is more readily soluble in the [C_4mim][Cl]. Currently this amorphous chitin is used wet, if dried the open chitin infrastructure becomes compact resulting in a material that is hard to dissolve in the ionic liquid. Freeze-drying the pretreated chitin maintains the expanded structure and allows accurate weighing for future percent conversion calculation needed for the degradation study. We are also planning to send a sample to NIST for SEM analysis to investigate the formation of chitin nanofibers, which would be the first evidence of the structures formation using an ionic liquid. We are also preparing a 1% solution of chitin in [C_4mim][Cl] for dynamic light scattering study to understand the structure of the chitin in solution and gain insight into the dissolution process.

We have expanded the sources of chitin used in our experiments as well. By collaborating with local industry, we have obtained raw chitin from crabs. This chitin has been mildly treated to remove the excess meat from the shell and placed in a hammer mill to reduce it to a powder. The powder is then passed through a #200-mesh sieve to obtain a fine powder. Initial dissolution tests show excellent solubility in [C_2mim][acetate] and [C_4mim][acetate]. The next step is to investigate the depolymerization process on this "native" chitin sample.

We have also investigated sources of chitin outside of crustaceans, namely fungi such as mushrooms. The chitin provided from fungi tends to be lower in crystallinity than that of crustacean sources. The lower crystallinity could lead to easier dissolution in the ionic liquid as well as increased reactivity. Mushrooms are an excellent source of chitin and are easily harvested. In collaboration with Dr. Juan Luis Mata, we have harvested the following mushrooms from local environment for testing: *cantharellus lewisii, trametes cubensis, ganoderma lucidum, lactariu corrugis, lentinula raphanica,* and *amanita firma rubescens*. Each species of mushroom is treated with HCl, NaOH, and sodium chlorite to remove the various minerals, protein and glucans, and finally to remove the natural pigments in the fungi. After this treatment, each sample produced a white insoluble material. The materials are awaiting characterization to determine if they are indeed chitin. Preliminary solubility tests have been positive. The material is completely soluble in the ionic liquid [C_4mim][Cl]. Once the insoluble material is characterized, the depolymeization of fungi chitin will be conducted.

Task 2 – Development, synthesis, and characterization of new acid functionalized ionic liquids for the depolymerization of chitin.

Development of new catalysts

We have continued the synthesis of new sulfonic acid functionalized ionic liquids using the thiol-ene technique developed during the first year of this project in collaboration with Dr. Jim Davis's group at the University of South Alabama. With Drs. Richard O'Brien and Arsalan Mirjafari, we have expanded the sulfonic acid cation group to include quaternary ammonium family. This cation should be able to provide four acid sites thus reducing the amount of catalyst needed to perform the hydrolysis reaction. The synthesis of this catalyst is described below.

In addition to this new class of catalysts, the synthesis of two new acid catalysts was explored. The first new family of catalyst investigated was based on the boronic acid functionality. Based on the work of Brown and Gupta, catecholborane was reacted with 1-allyl-3-methylimidazolium [NTf_2] to form 1-(3-catecholborane-propyl)-3-methylimidazolium cation.[8] The catecholborane is then easily hydrolyzed with water to form the boronic acid derivative.

Another new acid catalyst that was developed for this project contained the phosphonic acid functional group. It was believed that the addition of the phosphonic acid group would increase the acidity of the resulting ionic liquid acid catalyst thereby requiring less catalyst for the reaction while maintaining reaction rate. The synthetic strategy for this acid catalyst was based on the work by Jansa and coworkers.[9] Current attempts to synthesis the 4-bromobutylphosphonate intermediate have been unsuccessful due to difficulty in purifying the product.

Synthesis of new catalysts

1-methyl-3-(propaneboronic acid)imidazolium bis(trifyl)imide [mim$C_3B(OH)_2$][NTf_2]

The synthesis of [mim$C_3B(OH)_2$][NTf_2] was performed by following established synthetic routes with new applications. The addition of the boronic acid group proceeds through the reaction of an allyl group with catecholborane. The first step in the synthesis is to attach an allyl group to an imidazole ring. This step is performed by reacting 1-methylimidazole with allyl bromide. The reaction is exothermic and is carried out with the drop-wise addition of allyl bromide into a cold solution of methylimidazole in ethyl acetate.

Once the 1-allyl-3-methylimidazolium bromide has been purified, anion exchange is performed to switch the bromide anion for the [NTf_2] anion. This step is important has the bromide can react with the borane in later step which will result in a deep purple compound. With anion exchange completed, the product is reacted with catecholborane in 20% excess. This step is carried out by the slow addition of a catecholborane/hexane solution to the 1-allyl-3-methylimidazolium [NTf_2]. The solution was allows to stir overnight at room temperature.

After the solvent is removed by vacuum, the resulting borane ester product is hydrolysis by vigorous stirring at room temperature overnight. This step is a deprotection step that breaks the C-O bonds between the catechol and the borane resulting in the formation of the boronic acid functionality.

Preliminary data on the catalyst properties of the boronic acid catalyst indicate that it is not a good candidate for the hydrolysis of chitin. This new catalyst might be an excellent candidate

for the separation of carbohydrates from the reaction solution due to it tendency to complex diols and its ulitization in separating diols in biphasic systems.

Tetra(3-sulfopropyl)ammonium bis(trifyl)imide [$N(C_3SO_3H)_4$][NTf_2]

The synthesis of [$N(C_3SO_3H)_4$][NTf_2] was performed following previous published work on Thiol-ene "click" chemistry.[10,11] The precursor material, tetraallylammonium bromide was synthesized by reacting triallylamine with allyl bromide in a 1:1.4 ratio. The resulting product was a tan solid in good yield. Anion exchange was performed to replace the bromide with the [NTf_2] anion. This step is necessary in order to achieve separation of the product from the reaction solution in later steps. The tetraallylammonium salt is then reacted with thioacetic acid in a 1:8 ratio, which appends a thioacetate group on to the allyl chain. This step is performed under UV radiation with a photo-initiator for 8 hours. After removal of the excess thioacetic acid, the resulting product is a viscous yellow liquid. The thiol is deprotected with acetyl chloride following a procedure established by Prasad.[12] After deprotection, the product is ready for the oxidation step that will convert the thiol into the sulfonic acid. The oxidation of the thiol is conducted using the oxidizer, m-chloroperioxybenzoic acid in a 1:4 ratio. The reaction is cooled to 0 °C and allowed to slowly warm to room temperature and stirred for 48 hours. Preliminary NMR data shows the oxidation of the terminal thiol group. The next step is test the new catalyst in the acid catalyst esterification of ethanol and acetic acid. If the catalyst is successful in producing ethyl acetate, the next step is to test its ability to depolymerize chitin.

Characterization of catalysts

In order to fully implement these catalysts into reactions, the full characterization of their physical properties must be performed. The acquisition of a Karl Fischer titrator and a TGA/MS has allowed for the further characterization of the sulfonic acid catalysts.

Karl Fischer results

The depolymerization reaction of chitin is a hydrolysis reaction, meaning that water is consumed during the reaction. In order to full characterize the reaction solution and the progress of the reaction, the amount of water added to the system needs to be known. The acquisition of a Metrohm 852 Titrando system with volumetric and coulometric Karl Fischer capabilities has allowed the accurate measurement of a wide range of water contents, volumetric for high water contents and coulombic to small water contents. This instrument allowed for the accurate measuring of the water content for the ionic liquids and catalysts used in the experiment. The additional purchase of an oven allowed for the analysis of materials that are not soluble in the Karl Fischer reagent, which is mostly methanol. This addition allowed for the measurement of the water content in the chitin used in the reaction. The oven works by heating a sample placed in a sealed septum vial to a programed temperature. Next, the septum in puncture with a needle and the moisture rich head space gas in transferred via a sealed and heated transfer line of the Karl Fischer titration vessel. The carrier gas is dried air.

The results of the water content measurements showed that the ionic liquid contained % water and the catalyst used in the depolymerization reaction contained less than 0.5 % water. The chitin used in the experiments proved to content % water. These results lead to a modification of the depolymerization reaction. Due to the amount of additional water added from each component on the reaction, no extra water was needed to perform the depolymerization reaction.

Thermal Gravimetric Analysis with Mass Spectrometery (TGA/MS)

Another recent acquisition was software to get a Netzsch TG 209 F1 thermal gravimetric analysis system with a QMS 403C mass spectrometer up and running. This new capability allows for the determination of the temperature limitations for the ionic liquids acid catalyst as well as allow for the monitoring of the decomposition products by mass spectrometry. IN the typical TGA experiment, the samples are heated at 10°/min from room temperature to 600 °C. The sample weights are usually around 10-15 mg and the sample chamber is purged with He to create a non-oxidative environment. This environment provides data on the thermal decomposition of the sample instead of the oxidative decomposition. In Figure 4, the thermal decomposition profile for the catalyst [mimC_5SO_3H][NTf_2] is shown. The curves show two decomposition events with thermal decomposition onset temperatures at 191.5 °C and 352.3 °C. By maintaining the anion and changing the cation to a pyrrolidinium with a propyl tether to the sulfonic acid group, [$PYR_{1,3}SO_3H$][NTf_2], similar mass loss peaks are observed, 190.5 °C and 352.3 °C. The similarities in decomposition temperatures suggest that similar decomposition processes are occurring independent of alkyl chain tether and cation head group.

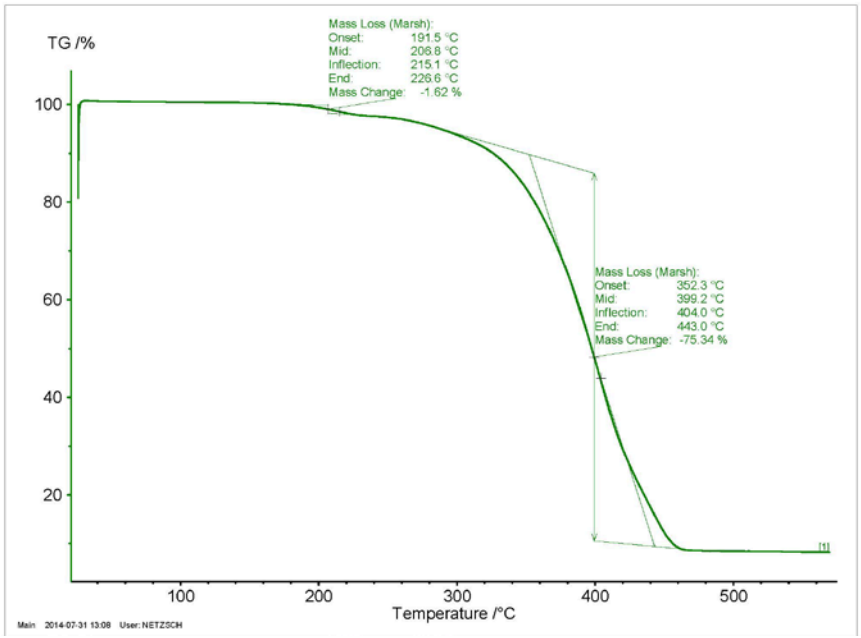


Figure 4. TGA for the acid catalyst [mimC_5SO_3H][NTf_2].

In order to further understand the thermal decomposition pathways, a TGA/MS experiment was performed on the catalyst, [mimC_3SO_3H][OTf]. The TGA of the catalyst [mimC_3SO_3H][OTf], Figure 5, shows three distinct mass loss events, 174.7 °C, 333 °C, and 424 °C. With the TGA and MS working in tandem, we are able to collect MS data during the TGA

experiment by sampling the decomposition gases at various times during the heating profile. This allows for a snapshot of the environment in the TGA sample chamber. For this particular experiment, the MS was set up to scan for masses from 17 m/z to 117 m/z. By incorporating the data from both sets of instruments, a 3-D plot of the mass loss data with the mass spectra in relation to the temperature can be generated, Figure 6. The mass spectrum data shows that the majority of mass loss at the first thermal event is due to water. The highlighted cross section in Figure 6 sows the total ion count for the next thermal event. In this mass loss event, several peaks are observed. As the compound begins to thermal decompose, more water is released. The peaks at 64, 48, and 32 are related to the fragmentation of SO_2, which can be produced from decomposition of the cation or anion. It is also important to point out that the decomposition mechanism does not appear to proceed through the deprotonation of the sulfonic acid group by the triflate anion. If that were the case, there would be a greater mass loss at 162 °C (b.p. of triflic acid) and more masses besides water at lower temperatures.

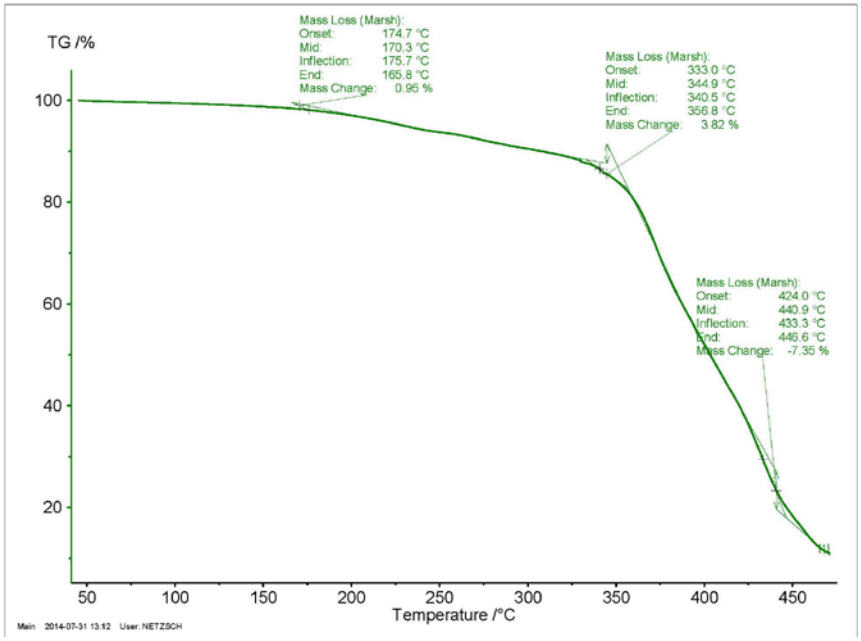


Figure 5. TGA of the acid catalyst, [mimC_3SO_3H][OTf].

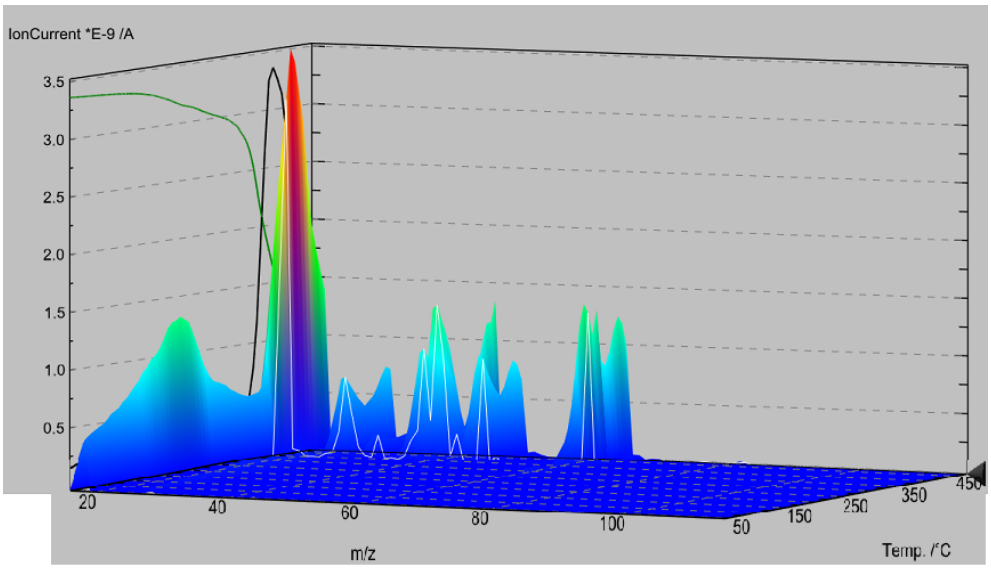


Figure 6. 3D plot of Thermal Degradation of $[C_3SO_3H][OTf]$, highlighted cross-section at 400 °C.

An examination of the mass spectrum at 450 °C, shows a small shift in the abundance of mass fragments. The fragment at 44 m/z increases in intensity while the fragments at 18, 41, and 64 decrease. The peaks reated to the fragmentation of SO2 are still present, they have decreased in intensity. The mass fragments, 44, 41, and 28 are related to the fragmentation of imidazole and the imidazolium cation. This analysis would suggest that the thermal decomposition mechanism is initialed with the degradation of the cation. The lower temperature decomposition at 333 °C is related to the cleaving of the SO_3H group from the imidazolium cation with further decomposition of the ring happening at a higher temperature, 424 °C. Further study of the decomposition process is needed with a focus of larger mass fragments, 100-250 m/z. Also the effects of anion on the decomposition mechanism needs to be explored further.

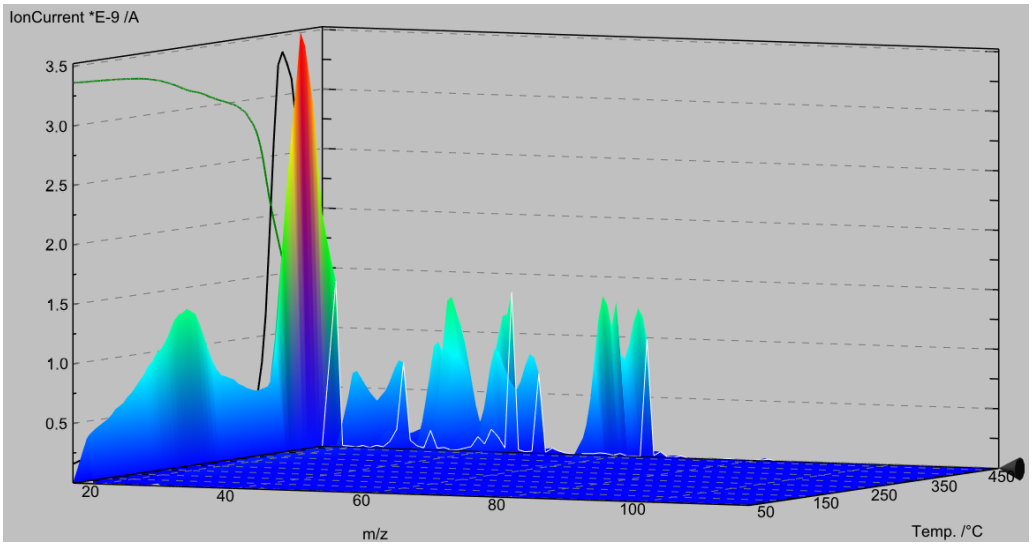


Figure 7. 3D plot of Thermal Degradation of $[C_3SO_3H][OTf]$, highlighted cross-section at 450 °C.

Task 3 – Evaluate the depolymerization of chitin using an ionic liquid solvent and catalyst into *N*-acetylglucosamine

Reaction Experiment

In the depolymerization reactions, 1% (w/w) chitin solutions in [C_4mim][Cl] were prepared aided by microwave radiation. Each chitin solution was heated in a CEM MARS reaction system to 100°C with periodic stirring until complete dissolution was observed.

The depolymerization reactions were performed on 15g of the 1% chitin (w/w) solution in glass jars. The depolymerization reaction was carried out at 120°C, utilizing 1g of each of the catalysts being studied. Since the depolymerization process is a hydrolysis reaction, 0.35 g of water were added to ensure that enough water was present in the reaction vial for complete depolymerization. To monitor the reaction, 0.5 g aliquots of the reaction solution were removed at 10-minute intervals for the first hour starting at 0 seconds. After the first hour, samples were taken every 30 minutes. The reaction was removed from heating after 6 hours of reaction time.

LC/MS sample preparation

To prepare the depolymerization samples for LC/MS analysis, the samples were diluted to 1.5 g total mass with 18MΩ water and shaken. The samples were then diluted by a factor of 1000 and passed through a 0.45μm Whatman GD/X filter. Finally, samples were placed in conical LC autosampler vials.

LC/MS Measurements

The separations were performed on a HPLC system consisting of a pump, autosampler, and column oven. A Phenomenex C18 Luna column (5μm, 100Å, 3.00 x 30 mm) was utilized to separate the components in the reaction mixture. The mobile phase consisted of 0.1 % formic acid in both water and acetonitrile (ACN). The gradient conditions for the mobile phase were 95%:5% water to ACN for the first 2.5 minutes; then a step gradient to 5%:95% water to ACN from 2.5-10 minutes; and finally back to 95%:5% water to ACN for 10-16 minutes to re-equilibrate the column. This gradient profile leads to the separation of the products from the ionic liquid. The samples were separated at 0.5 mL/min flow rate and with an injection volume of 10 μL. The mass spectrometer used for detection was a Thermo-Fisher LTQ-Velos The ionization source for the MS was ESI. The MS parameters were tuned on N-acetylglucosamine in order to maximize sensitivity for the depolymerization product.

To determine the efficiency of ionic liquid acid catalyst on the depolymerization of chitin, we first have to determine the possible products from the reaction. Since the depolymerization of chitin should proceed through the hydrolysis of the ether linkages in the structure, the final product from the depolymerization should be N-acetylglucosamine (NAG). Previous work utilized the Benedict's test and HPLC analysis to follow the production of the reducing sugars, NAG. In that work, one could follow the conversion of chitin into various reducing sugars could be followed but the structure of the products was unknown.

To gain better insight into products formed during the hydrolysis reaction, we utilized a LC/MS instrument and gathered mass spectrum data on the proposed products from the reaction,

namely the various oligomers of chitin: NAG, chitobiose, and chitotriose. Although, the LC method used in this study did not provide separation of the various oligomers, it did allow for their separation from the reaction mixture, [C_4mim][Cl] and the ionic liquid catalysts. The mass specta collected for the standards is shown in Figure 8. This data shows the appearance of the oligomers NAG (222 m/z), chitobiose (425 m/z), and chitotriose (628 m/z) along with the first dehydration products. Recently in the course of tracking down the cause of the shift in the standard peaks for NAG (from 222 m/z to 227 m/z), chitobiose (425 to 431) and chitotriose (628-635), it was discovered that the filters used to separate to insoluble material from the reactions solution had contaminated the samples. The contamination resulted in the peaks at 227, 431, and 635. Since every sample was treated the same way and contaminated in the same manner, the trends seen in the MS data are still believed to be true trends. The unfiltered samples are currently being reanalyzed to determine the true masses for the depolymerization products. Preliminary data from the reanalysis shows that reaction mixture are producing a compound at a mass of 409 m/z. This mass could be related to the chitobiose oligomer through the loss of an oxygen atom during the reaction. We are currently working to resolve this issue and provide better data on the depolymerization reaction of chitin. The following discussion will use our previously recorded data and refer to trends that should still be true.

The graphs in Figure 9 present the extracted ion chromatographs (XIC) for 431 m/z. These graphs are a snapshot of the reaction progressing from t=0 minutes to t=60 minutes. The XIC show an initial decrease the amount of 431m/z at the beginning of the reaction, t=0 to t=20. This decrease maybe related to the catalyst reacting with the shorter oligomers first. At t=30 minutes, there is a sharp increase in the amount of oligomers present as the catalyst starts to react with the long polymer strands of chitin producing the shorter oligomers. Finally, there is another drop in the relative abundance of 431 m/z as the catalyst begins to depolymerize the short chain oligomers further. While the products formed are not the unaltered oligomers themselves (e.g. chitobiose at m/z 425) but related products and contamination (m/z 431), there is clearly a relationship between the masses observed in the reaction solutions and those of the chitin oligomers. As the reaction progresses, the mass peaks grow in and out allowing the observing of the reaction as it progresses. If the masses were entirely associated with the contamination from the filtered, the abundance of the peaks would not change.

A comparison of the changes in catalyst structure on the reaction products is shown in Figure 10. Even with the contamination from the filters, there a large increase in the abundance of mass fragments 227 and 431 at the 30 minutes for the [mimC_6SO_3H][NTf_2] catalyst. This increase signifies an increase in the production of chitobiose and NAG which is quickly consumed in a secondary reaction. By extending the alkyl chain tether from hexyl to octyl, these is a dramatic decrease in the production of the oligomers as well as the reaction taking longer to peak, 30 minutes to 40 minutes. If the alkyl chain tether is decreased in length from hexyl to propyl, the reaction times can by decreased resulting in peak oligomer product in 10 minutes instead of 30 minutes.

Based on these trends and previously reported results, one can control the speed of the reaction and the products with changes to the reaction temperature and the catalyst structure.

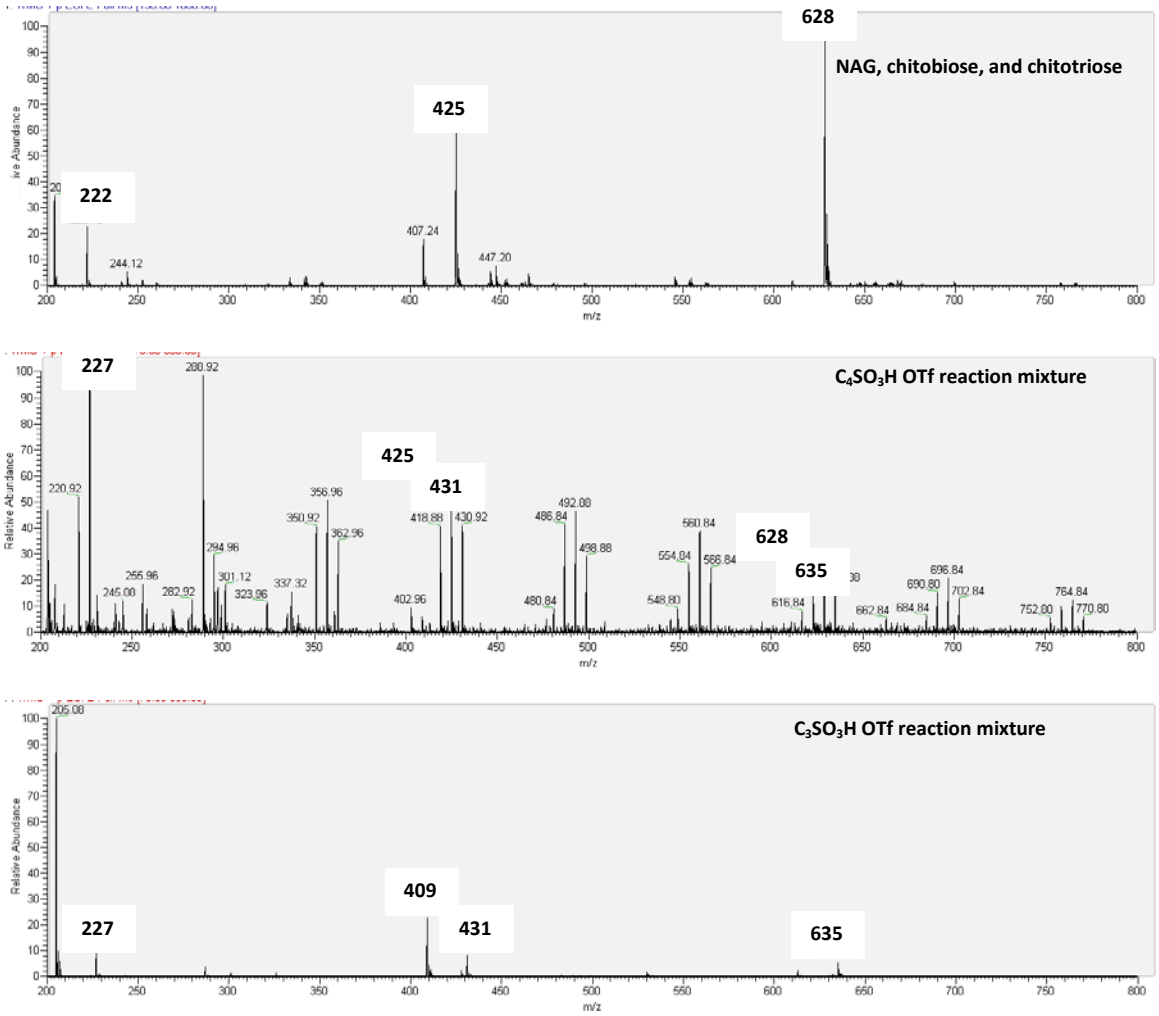


Figure 8. Mass spectra for a mixture of the pure compounds: NAG, chitobiose, and chitotriose in water (top), reaction mixture obtained with [mimC_4SO_3H][OTf] catalyst (middle), and reaction mixture obtained with [mimC_3SO_3H][OTf] (bottom).

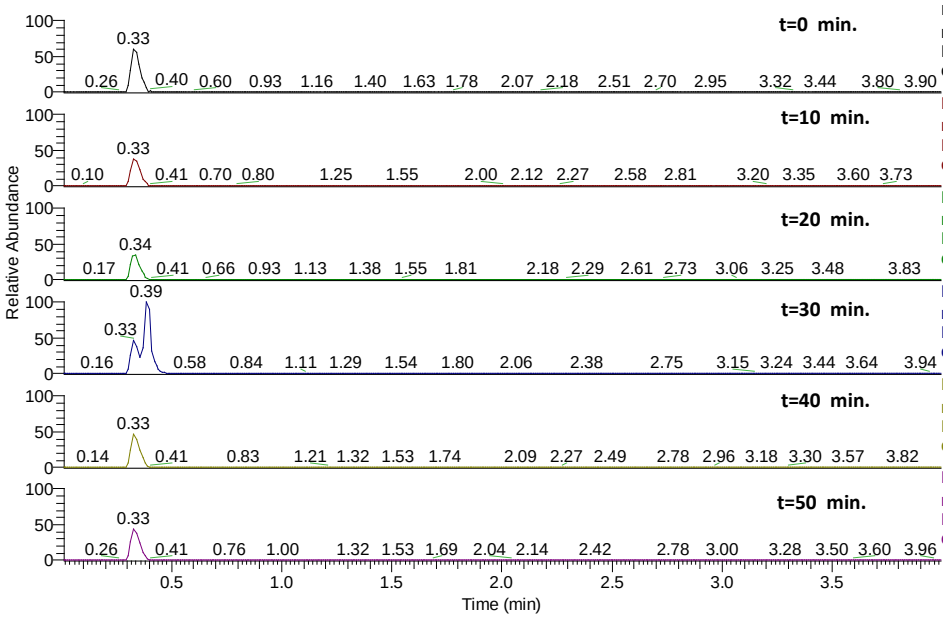


Figure 9. The XIC for 431 m/z in the reaction of chitin with the catalyst $[C_6SO_3H][NTf_2]$ at t=0 minutes through t=50 minutes.

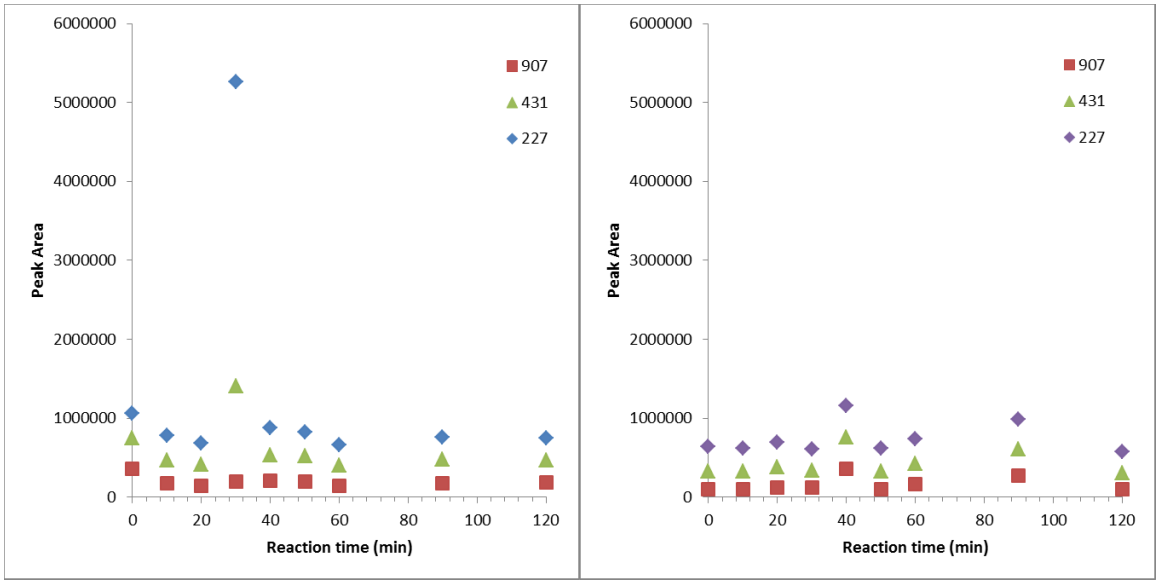


Figure 10. The peak area for the masses, 227, 431, and 907 in the reaction of chitin with the catalysts, $[mimC_6SO_3H][NTf_2]$ and $[mimC_8SO_3H][NTf_2]$ at 120 °C.

Conclusions

Fungi provide an excellent source of chitin that is more amorphous than crustacean chitin, which could lead to increased yields in the depolymerization reactions. We have isolated material from a variety of fungi and are analyzing it for chitin content. The thermal decomposition mechanism for the various catalysts is under investigation. Preliminary data from the TGA/MS shows that the thermal decomposition proceeds through the degradation of the imidazolium cation. It appears that cleavage of the SO_3H group is the first step in the degradation followed by ring decomposition. The TGA data will provide important information for the utilization of the catalyst on a larger scale and possible hazards when the catalysts decompose. Long-term stability tests and oxidative decomposition tests are also needed in order to determine the overall viability of the catalysts. Now that a quaternary ammonium acid catalyst has been synthesized, testing can begin on its catalytic properties. A synthetic route to the product of a phosphonic acid functionalized catalyst is underway.

With the discovery of contamination to the samples, analysis of the reaction solutions was set back. Luckily, the majority of the original non-contaminated reaction solution were kept and refrigerated. Moving forward, the reaction samples need to be reanalyzed for the production of the oligomers and NAG. Based on preliminary results, it appears that the hydrolysis reaction is not only breaking the chitin down into smaller units but also further dehydrating those products. While the previously collected data is contaminated, it is believed that due to the identical treatment of the samples, the trends seen in the reactions are true. Based on these trends, temperature and catalyst structure are important variables in the reaction. The temperature is critical to a successful reaction. This research found that reaction temperatures below 120 °C resulted in no detectable hydrolysis products. The current known detection limit for chitobiose is 1% of total conversion. By changing the catalyst structure, one can also control the reaction rate. The trend above shows that by increasing the alkyl chain tether, the hydrolysis reaction is slowed down and the amount of products decreased. Reanalysis of the reaction solutions should provide strong evidence for the production of the various oligomers and NAG.

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Abstract

This project focused on the development and utilization of acid functionalized ionic liquids for the depolymerization of chitin into N-acetyl-D-glucosamine (NAG) and other dehydration products. Previous reports focused on the synthesis of sulfonic acid functionalized imidazolium and pyrrolidinium cation ionic liquids with the triflate and bis(trifluoromethanesulfonyl)imide anion. This report expands that work to include two other acid functionality, boronic acid. A successful synthetic route for boronic acid functionalized ionic liquids has been developed. This catalyst was then tested using an acid catalyzed transesterification reaction, synthesis of ethyl acetate. The boronic acid ionic liquid proved to be ineffective in catalyzing the reaction. While not useful as a catalyst, literature on boronic acids revealed that they could be used to extract carbohydrates from the reaction mixtures.
In a continuing effort to gain better insight into products formed during the hydrolysis reaction, we utilized a LC/MS instrument and gathered mass spectrum data on the proposed products from the reaction, namely the various oligomers of chitin: NAG,

chitobiose, and chitotriose. While the LC method used in this study did not provide separation of the various oligomers, it did allow for their separation from the reaction mixture, [C4mim][Cl] and the ionic liquid catalysts. The mass spectrum data was collected for the standards, NAG, chitobiose, and chitotriose. Next, the reaction mixtures were tested in order to determine if the products were related to the chitin oligomers. Initial results showed a shift in the mass peaks for the oligomers. Chitobiose, which was original seen at 425 m/z, was offset to 431 m/z while chitotriose was shifted from 628 m/z to 635 m/z. An investigation into the effect of temperature on the depolymerization reaction showed that higher temperatures are more effective for the depolymerization of chitin; the temperatures studied were 80, 100, and 120°C. Also the effects of alkyl chain length were investigated. The results of the study showed that the shorter chained sulfonic acid catalysts depolymerized the chitin faster than longer chained sulfonic acids. This data coincides with the results from the esterification reaction studies from the last report.

In an effort to better understand the product masses and why they are shifted from the standard oligomers, an in depth analysis of the treatment of the reaction mixtures was performed. During this investigation, it became clear that the filters used the remove the insoluble material from the reaction solution was contaminating the LC/MS samples. The contamination was responsible for the shift in the standard peaks and did not allow for the true identification of the reaction products. Since every sample was treated the same way and contaminated in the same manner, the trends seen in the MS data are still believed to be true trends. The unfiltered samples are currently being reanalyzed to determine the true masses for the depolymerization products.

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Reichert, W. M.; Mirjafari, A.; Goode, T.; Williams, N. G.; La, M.; Ho, V.; Yoder, M.; Davis, J. H. Jr. "Synthesis of Long Chain Bronsted Acidic Ionic Liquids," ECS Trans., 2012, 50(11), 623-630.

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Research Objectives

Technical Summary

Funding Summary by Cost Category (by FY, $K)

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Non-Military Government Personnel Costs			
In-house Contractor Costs			
Travel (Be Specific)			
Training (Be Specific)			
Supplies			
Other Expenses (Be Specific)			
Total Resource Requirements			

Report Document

Appendix Documents

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