ABSTRACT: Ionic liquids (ILs) have been widely investigated for the pretreatment and deconstruction of lignocellulosic feedstocks. However, the modes of interaction between IL-anions and cations, and plant cell wall polymers, namely, cellulose, hemicellulose, and lignin, as well as the resulting ultrastructural changes are still unclear. In this study, we investigated the atomic level and suprastructural interactions of microcrystalline cellulose, birch-wood xylan, and organosolv lignin with 1,3-dialkylimidazolium ILs having varying sizes of carboxylate anions. Analysis by $^{13}$C NMR spectroscopy indicated that cellulose and lignin exhibited stronger hydrogen bonding with acetate ions than with formate ions, as evidenced by greater chemical shift changes. Small-angle X-ray scattering analysis showed that while both cellulose and xylan adopted a single-stranded conformation in acetate-ILs, twice as many acetate ions were bound to one anhydroglucose unit than to an anhydroxylose unit. We also determined that a minimum of seven representative carbohydrate units must interact with an anion for that IL to effectively dissolve cellulose or xylan. Lignin is associated as groups of four polymer molecules in formate-ILs and dispersed as single molecules in acetate-ILs, which indicates that it is highly soluble in the latter. In summary, our study demonstrated that 1,3-dialkylimidazolium acetates displayed stronger binding interactions with cellulose and lignin, as compared to formates, and thus have superior potential to fractionate these polymers from lignocellulosic feedstocks.

INTRODUCTION
Lignocellulosic feedstocks, such as agricultural residues, forest biomass, and dedicated bioenergy crops, are abundant and renewable resources composed of three main polymers, i.e., cellulose, hemicellulose, and lignin. Although there have been extensive attempts in the past three decades to use these feedstocks for fuel and value-added products, progress is still hindered by the inherent recalcitrance of lignocellulosic biomass. Physicochemical treatments have been developed to reduce this recalcitrance but were mostly optimized for the isolation and conversion of cellulose. Thus, these biomass conversion routes left behind hemicellulose and lignin, which are potential low-cost feedstocks for the production of bio-based chemicals and materials. Recently, targeted valorization efforts have ensured better utilization of xylan and lignin as raw material in fuels, resins, films, composites, and other applications. However, the effective fractionation of the three most abundant plant cell wall polymers is still a challenge.

One key step in the isolation and utilization of cellulose, hemicellulose, and lignin is designing an efficient solvent system that fractionates these biopolymers without causing significant damage to their native structure and generating undesired products. Solvent systems, such as N-methylmorpholine-N-oxide (NMMO), dimethyl sulfoxide/tetrabutylammonium fluoride, and butanol/acetic acid, have been reported to dissolve one or more of the lignocellulosic components, but they suffer from drawbacks such as low fractionation efficiency, noxious odor, toxicity, and/or volatility. Ionic liquids (ILs), on the other hand, exhibit superior solvation capability, low vapor pressure, and high thermal stability, making them an attractive choice for green processing technologies. These low-melting salts feature an organic cation and an organic or inorganic anion, and their physicochemical properties, such as viscosity and polarity, are customizable by changing the cation/anion combination. ILs containing a chloride anion, i.e., 1-butyl-3-methylimidazolium chloride ([BMMIM][Cl]), have been demonstrated to dissolve up to 25% (w/w) of cellulose. Similarly, imidazolium-based...
ILs containing a methanesulfonate anion could dissolve about 45% (w/w) of milled wood lignin, whereas [BMIM][Cl] could dissolve up to 19% (w/w) of bamboo hemicellulose. Although these ILs have been identified as potential solvents to fractionate lignocellulosic biomass, the underlying mechanism for some IL-ion pairs such as chloride and methanesulfonate to outperform others like bromide, phosphate, and acetate is not well understood. Hence, a comprehensive investigation of the interactions between IL-ion pairs and cellulose, hemicellulose, and lignin is warranted to help select ILs for efficient fractionation of lignocellulosic biomass.

In previous studies, atomic and molecular level interactions between IL-ion pairs and lignocellulosic model systems like microcrystalline cellulose, cellulose, xylan, guaiacol glyceryl ether, and milled wood lignin were probed using experimental (nuclear magnetic resonance (NMR) spectroscopy) and theoretical (density functional theory or DFT) techniques. The moisture content of [EMIM][HCOO], [EMIM][OAc], [AMIM][HCOO], and [AMIM][OAc] was determined to be 10.9 ± 1.4, 8.9 ± 0.7, 6.1 ± 0.4, and 4.6 ± 0.5%, respectively, by heating a representative IL sample to 5 mg in a Pyris 1 thermogravimetric analyzer (TGA, PerkinElmer, Shelton, CT) at 105 °C for approximately 15 min or until constant weight (under air).

Avecil PH-101 microcrystalline cellulose (DP = 350) was purchased from Alfa Aesar (Tewksbury, MA), and birchwood xylan (≥90% xylose) was purchased from Sigma-Aldrich (St. Louis, MO), and used as received. Lignin was produced from hybrid poplar wood chips using an organosolv fractionation process at the Center for Renewable Carbon, University of Tennesse, Knoxville. Briefly, 650 g of wood chips were cooked using a solvent mixture of 50:34:16 (w/w/w) water, ethanol, and methyl isobutyl ketone, containing 0.05 M sulfuric acid as catalyst, at 140 °C for 120 min. The purity of the isolated organosolv lignin, based on acid-soluble lignin and Klason lignin estimation, was 94.6 ± 0.2%, and its molecular weight (Mn) was 37,43 g/mol, with a dispersity of 1.2. The moisture content of Avicel cellulose, birchwood xylan, and organosolv lignin, determined gravimetrically, was 2.9 ± 0.1, 12.5 ± 0.4, and 3.6 ± 0.1%, respectively. Dimethyl sulfoxide-d6 (DMSO-d6, 99.9%) containing tetramethyl silane (0.03%, v/v) was purchased from Cambridge Isotope Laboratories Inc. (Tewksbury, MA).

Preparation of Biopolymer-IL Solutions. The biopolymer-IL solutions were prepared by dissolving 10% (w/w) of the biopolymer, xylan, and lignin in each IL at 80 °C. First, 2 g of ILs were weighed out in a vial and heated at 100 °C for 10 min to remove excess moisture. The temperature was then reduced to 80 °C, and the
biopolymer was added in 20 mg increments until the target mass of 200 mg was reached. The dissolution of the biopolymers was confirmed by placing an aliquot under a bright-field optical microscope (20X magnification); the absence of visible aggregate particles was assumed to indicate complete dissolution. The moisture content at 80 °C of the neat ILs and biopolymer-IL solutions, determined using the TGA (to replicate the NMR testing conditions), are listed in the Supporting Information (Table S1); it was determined that the moisture content for all samples ranged between 0.5 and 3.0% (w/w).

13C NMR Analysis of Biopolymer-IL Solutions. Samples for 13C NMR analysis were prepared by mixing 575 μL of the above-mentioned biopolymer-IL solution and 125 μL of DMSO-d6. A control containing 575 μL of neat IL and 125 μL of DMSO-d6 was also prepared. DMSO-d6 has been shown to aid in the dissociation of IL-cation and anion without altering or participating in interactions with cell wall biopolymers like cellulose. Our preliminary experiments determined that a minimum amount of 125 μL of DMSO-d6 was needed to provide a lock frequency.

NMR spectra were recorded on a Bruker 400 MHz spectrometer (Billerica, MA) at 353 K (80 °C). Both DMSO-d6 and tetramethyl silane were used as internal references. The change in chemical shift for each carbon was calculated by subtracting the chemical shift of neat IL from that of biopolymer-IL solutions. The data were processed (baseline correction and chemical shift assignment) using Mnova software, version 14.2 (Mestrelab Research, A Coruña, Spain). Each experiment was repeated at least three times. A one-way ANOVA was performed to determine any significant differences in the chemical shift changes; the significance level was set at α = 0.05. All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY).

SAXS and WAXS Experiments. Small-angle and wide-angle X-ray scattering (SAXS and WAXS) experiments were conducted at the 12-ID-B beamline of Advanced Photon Source (Argonne National Laboratory, Lemont, IL). All measurements were carried out at room temperature. A 2% (w/w) biopolymer concentration was employed to ensure that individual molecular features were delineated and the differences in IL-anion/cation association with plant polymer components could be discerned. Solutions of 2% (w/w) cellulose, xylan, and lignin were prepared in select ILs, injected into a 2 mm diameter capillary tube, sealed with epoxy, and then used for SAXS analysis. The X-ray wavelength was 0.9322 Å, and a PILATUS (Dectris) detector was used as the area detector. The irradiation time was 10 s (1 s irradiation repeated 10 times). The observed X-ray scattering intensities were normalized to absolute intensity using glassy carbon (a secondary standard sample for intensity calibration). The scattering intensity, I(Q), of cellulose, xylan, and lignin in the different ILs was obtained after subtracting the scaled scattering intensity of the corresponding neat IL. The scaling factor of the neat IL for each sample was determined by comparing the WAXS data of the biopolymer-IL solution and the neat IL, where only the IL background scattering signal was dominant.

SAXS data for cellulose in ILs were analyzed using the model fitting approach implemented in the I200 package in Igor Pro (WaveMetrics, Inc., Portland, OR) software. Cellulose was modeled as cylinders with a Gaussian distribution of radii, which is expressed as follows:

$$I(Q) = I_p(Q)S(Q)$$

(1)

where $I_p$ is the intensity scalar, which is given by the equation below

$$I_p = \frac{\phi}{V_{pol}} (\Delta \rho)^2$$

(2)

where $\phi$ accounts for the cylinder particle concentration with a polydisperse volume and $\Delta \rho$, calculated as $(\rho_{cylinder} - \rho_{solvent})$, is the contrast between cellulose and solvent. $\rho_{cylinder}$ and $\rho_{solvent}$ are the scattering length densities of the cylinder particles (cellulose) and the solvent, respectively. $V_{pol}$, the volume of polydisperse cylinders using the second moment, is given as $V_{pol} = \pi a^2 L (1 + p^2)$, where $r$ and $L$ are the cylinder’s radius and length, respectively. Polydispersity of the cylinder, $p = \sigma / \rho_{cylinder}$, is the ratio of the width of Gaussian distribution to its center. The form factor of a radially polydisperse cylinder, modeled as Gaussian distribution, is expressed as

$$F(q, \alpha) = 2V_{cyl}(qH \cos \alpha)I(qr \sin \alpha)$$

(3)

$$F(q, \alpha) = \frac{1}{\phi} \int_0^{\infty} [1 - \frac{1}{2\sigma^2} (r - r_{cyl})^2] dr$$

(4)

where $F(q, \alpha)$ is the form factor of a cylinder and $G(r)$ is the radial distribution function expressed as

$$I(Q) = (AQ)^{-p} + I_{bkg}$$

(6)

where A is the length scale factor, $I_{bkg}$ is the background intensity of neat IL, and P is the power-law exponent. The lignin SAXS data in IL solution were analyzed using a one- and two-level Unified fit,23,24 which is a combination of Guinier and power-law functions as shown below

$$I(Q) = \sum_{i=1}^{N} \left[ G_i \exp \left( \frac{q^2 R_{I}^2}{3} \right) + B_i \exp \left( \frac{q(R_{I} \sqrt{6})^{-3}}{q} \right) \right] + I_{bkg}$$

(7)

where $N = 1$ for single-level fit and $N = 2$ for two-level fit, $G_i$ is the Guinier prefactor of the $i$th level, $B_i$ is the prefactor of the power-law function of the $i$th level, $P_i$ is the power-law exponent of the $i$th level, $R_{I,i}$ is the radius of gyration of the $i$th level, and $I_{bkg}$ is the background intensity of neat IL. Irrespective of the number of levels used in the fit, the level-1 component on the Unified fit is most important for detailing the lignin particle’s structural characteristics. $R_{I}$ provides a measure of the shape-independent particle size, and exponent $P_1$ is a measure of the polymer conformation in the particle (or bulk chain conformation).

RESULTS AND DISCUSSION

Atomic Interactions of Biopolymers with IL-Cations and Anions. We first investigated the atomic level interactions between IL-ion pairs and representative plant cell wall polymers, i.e., cellulose, xylan, and lignin, using 13C NMR spectroscopy. We selected ILs composed of [EMIM] and [AMIM] cations, and [HCOO] and [OAc] anions, based on previous lignocellulose solubility reports. The 13C NMR spectra of these neat ILs and the corresponding 10% (w/w) solutions of cellulose, xylan, and lignin in the Supporting Information (Figures S1–S16). As seen in Figure 2, carbon atoms in both IL-ion pairs, namely, C2, C4, C5, and C10, exhibited a negative, i.e., upfield chemical shift ($\Delta \delta$) of approximately $-0.22$, $-0.02$, $-0.06$, and $-0.12$ ppm, respectively, in the presence of 10% (w/w) cellulose. This is indicative of an increase in electron density around these atoms and a subsequent increase in shielding. On the other hand, atoms such as C6, C7, and C11 exhibited a consistent positive, i.e., downfield chemical shift ($\Delta \delta$) of approximately 0.09, 0.07,
and 0.24 ppm, respectively (Figure 2), indicating a decrease in electron density around these atoms in the presence of cellulose and a subsequent deshielding. Similar results were observed for all IL-ion pair carbons in the presence of xylan and lignin (Figure 3A,B). The corresponding chemical shift change data are provided in Tables S2-S4.

The marked increase in shielding at C2 in the presence of cellulose is in agreement with the prior studies investigating interactions with [EMIM][OAc],\textsuperscript{15,16} as well as studies using arabinoyl xylan or milled wood lignin as model systems.\textsuperscript{12,18} The shielding effect may be attributed to the polarity-induced charge difference, such as when the C2 atom interacts with the hydroxyl groups of cellulose, xylan, or lignin.\textsuperscript{15-17,25} On average, the increase in shielding was pronounced for the acetate set of ILs when compared to formate-ILs, specifically in the presence of cellulose and lignin (Figures 2 and 3). Between the three biopolymers, the increase in shielding of C2 was the most intense in the presence of cellulose, followed by xylan and then lignin. Other imidazolium ring carbons, namely, C4 and C5 also exhibited an upfield chemical shift and an increase in shielding in the presence of all three biopolymers but at a lower magnitude than C2. The lower magnitude of change is likely due to the lower acidity of C4 and C5 protons and their diminished ability to form hydrogen bonds.

The C6 atom in the methyl group, as well as the C7 and C9 atoms in the ethyl and allyl side chains, exhibited a downfield chemical shift in the presence of all three biopolymers. However, the trend for C8 atom was different in that when possessing an ethyl side chain, C8 exhibited a downfield chemical shift, whereas when substituted with an allyl side chain, it exhibited an upfield chemical shift. The upfield chemical shift of C8 in [AMIM] ILs could be due to hydrogen bonding between their protons and the hydroxyl oxygen of cellulose, xylan, or lignin. This in turn would have led to electron withdrawal from the C9 atom of [AMIM] cations, causing a deshielding effect, as seen in Figures 2 and 3. A previous study had proposed that the C6 and C7 atoms of [AMIM] cations interacted less with the hydroxyl groups of cellulose than the corresponding C8 or C9 atoms because of their proximity to the nitrogen atoms of the imidazolium ring.\textsuperscript{17} Similarly, in our study, the C6 (\(\Delta \delta \sim 0.06\) ppm) and C7 (\(\Delta \delta \sim 0.06\) ppm) atoms of [AMIM] exhibited lower magnitude of chemical shift changes than C8 (\(\Delta \delta \sim -0.10\) ppm) and C9 (\(\Delta \delta \sim 0.14\) ppm) atoms in the presence of cellulose, xylan, and lignin (Figures 2 and 3).

In the case of IL-anions, a downfield chemical shift was observed for the carbonyl C11 atom (Figures 2 and 3) that could be explained based on hydrogen bonding interaction between IL’s oxygen and the hydroxyl proton in cellulose, xylan, and lignin resulting in a significant decrease in electron density. Since the acetate anion is a stronger conjugate base (pK\textsubscript{a} 4.75) than the formate anion (pK\textsubscript{a} 3.75),\textsuperscript{33} the C11 atom of acetate anion could form a stronger hydrogen bond with all three biopolymers. Previous research has expounded on the significance of IL-anions in bringing about the dissolution of cellulose,\textsuperscript{11} but such reports are lacking for xylan and lignin. In this current study, when comparing the absolute changes in chemical shifts of C11, the largest change was observed for

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Changes in chemical shift values (\(\delta_{(cellulose+IL)} - \delta_{(IL)}\)) of all carbon atoms in [EMIM][OAc], [AMIM][OAc], [EMIM][HCOO], and [AMIM][HCOO], each containing 10% (w/w) of cellulose, measured by $^{13}$C NMR spectroscopy at 80 °C.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Changes in chemical shift values (\(\delta_{(xylan/lignin+IL)} - \delta_{(IL)}\)) of all IL carbon atoms in the presence of (A) 10% (w/w) birchwood xylan and (B) 10% (w/w) organosolv lignin, determined using $^{13}$C NMR spectroscopy at 80 °C.
xylan (Δδ ∼ 0.43 ppm), followed by cellulose (Δδ ∼ 0.24 ppm), and then lignin (Δδ ∼ 0.18 ppm).

The C10 in acetate ions exhibited an upfield chemical shift in the presence of all three biopolymers. This increase in shielding is often equated to the redistribution of electronic cloud density. However, in the presence of lignin, the shielding of C10 was far higher (Δδ = −0.25 ppm) than in the presence of xylan (Δδ = −0.15 ppm) or cellulose (Δδ = −0.12 ppm), which suggests that there could be direct interactions between C10 and lignin instead of reorganization of electron density (Figure 3). By virtue of hydrogen bonding with C10 atoms, lignin could depict an increase in interactive strength with acetate-ILs.

The absolute total value of chemical shift changes in the presence of cellulose for the four ILs followed the trend of [EMIM][OAc] > [AMIM][OAc] > [EMIM][HCOO] > [AMIM][HCOO] (Figure 2). Even though our NMR spectroscopic results suggest that both the [AMIM] and [EMIM] cations are involved in hydrogen bonding with cellulose, the basicity of the corresponding anions appears to ultimately determine the strength of these interactions. When comparing all three biopolymers, lignin exhibited the lowest total absolute value of chemical shift changes, and its interactions with the four ILs followed the order of [AMIM][OAc] > [EMIM][OAc] > [EMIM][HCOO] > [AMIM][HCOO]. Xylan strongly interacted with [AMIM]-[OAc] and followed the trend of [AMIM][OAc] > [AMIM][HCOO] ∼ [EMIM][OAc] > [EMIM][HCOO]. Overall, these studies reveal that [AMIM][OAc] exhibits the strongest set of interactions with xylan and lignin, which could be attributed to not only its basic anion but also the electron-negative allyl side chains forming bonus hydrogen bonds.

Suprastructural Conformation of Biopolymers in ILs. While NMR data offered information about the atomic level interactions between ILs and plant polymer components, our SAXS studies investigated their suprastructural conformation in specific ILs. Our selection of ILs for SAXS analysis was based on the magnitude of chemical shift changes observed during the NMR studies.

Cellulose Conformation in ILs. Cellulose displayed the largest and least magnitude of total chemical shift changes with [EMIM][OAc] and [AMIM][HCOO], respectively, during NMR analysis. Therefore, SAXS analysis of cellulose was conducted with these two ILs. As shown in Figure 4, the cellulose SAXS profiles are similar for [EMIM][OAc] and [AMIM][HCOO], except for the overall scattering intensity. The observed monotonic linear increase of scattering intensity with decreasing wave vector, Q−1, implies that the cellulose polymer exhibited a rod-like shape in both ILs. The shoulder feature in the high-Q region (Q > 0.2 Å−1) represents the average cross-sectional radial dimension of the rod-like particle. Consequently, the SAXS data were fitted to a cylindrical shape. The high flux of synchrotron X-rays was necessary to detect such small cross-sectional dimensions of the rod-shaped particles (∼8 Å). The observation of rod-like shape for cellulose is consistent with previous reports.22,23,24

The concentration of cellulose in [EMIM][OAc] at 2% (w/w) is above the polymer overlap regime (∼ 0.8% w/w) but below the level of polymer entanglement (∼3% w/w).22 In this concentration range, cellulose exhibits chain flexibility with a gradual increase in interchain interactions. However, prior to the onset of interchain interactions, cellulose chains are stiff and rod-like23 (Figure 4) for length scales <70 Å (or Q > 0.014 Å−1) due to the rigid β-1,4 bonds connecting individual d-glucose units. And above the entanglement concentration, interchain interactions become dominant, rendering varying degrees of flexibility to cellulose chains.34 For this study, the chosen concentration of 2% (w/w) of cellulose in IL allowed us to produce sufficient scattering signal while maintaining minimal interchain interactions to allow for the kind of analysis described below.

The calculated parameters for cellulose in [EMIM][OAc] and [AMIM][HCOO] are listed in Table 1. The contrast parameter (Δρexp) was fitted after fixing the particle volume fraction (ϕp) of cellulose to that of the value calculated from the weights of cellulose and solvent used to prepare the sample. Table 1 shows that the cross-sectional radii of cellulose cylindrical particles were identical in both ILs, at ∼2.9 Å, and the experimental contrast (Δρexp) was higher than the calculated contrast (Δρcalc). The main distinction in the fit parameters of cellulose in [AMIM][HCOO] and [EMIM]-[OAc] was in the experimentally measured contrast.

Raghuvanshi et al. first observed such a difference between the experimental and calculated contrast between ILs and cellulose and developed a method to quantify the stoichi-

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**Table 1. Summary of Cylindrical Fit Parameters of SAXS Data for a 2% (w/w) Cellulose Solution in Select ILs**

<table>
<thead>
<tr>
<th>fit parameters</th>
<th>[EMIM][OAc]</th>
<th>[AMIM][HCOO]</th>
</tr>
</thead>
<tbody>
<tr>
<td>volume fraction (ϕp)</td>
<td>0.0148</td>
<td>0.0151</td>
</tr>
<tr>
<td>mean cylinder radius (Å)</td>
<td>2.89 ± 0.06</td>
<td>2.85 ± 0.06</td>
</tr>
<tr>
<td>experimental contrast (Δρexp) (x10−6 Å−2)</td>
<td>5.14</td>
<td>4.04</td>
</tr>
<tr>
<td>calculated contrast (Δρcalc) (x10−6 Å−2)</td>
<td>3.42</td>
<td>3.25</td>
</tr>
<tr>
<td>number of anhydroglucose units (AGU) per anion</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>number of anhydroglucose units (AGU) per cation</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

*Calculated from weight percent. bCalculated contrast values. Obtained from eq 8.*
The expression is given as

\[
\text{number of anhydroglucose units per anion (or cation)} = \frac{\left( \sum b_i \right)_{\text{anhydroglucose}}}{1 - \left( \rho_{IL}/\rho_{\text{cellulose}} \right)} \left( \frac{\Delta \rho_{\text{exp}}}{\Delta \rho_{\text{calc}}} - 1 \right)
\]

where \( \left( \sum b_i \right)_{\text{anhydroglucose}} \) and \( \left( \sum b_i \right)_{\text{anion (or cation)}} \) are the scattering lengths of anhydroglucose and the anion (or cation); \( \rho_{IL} \) and \( \rho_{\text{cellulose}} \) are the scattering length densities of the IL and cellulose; and \( \Delta \rho_{\text{exp}} \) and \( \Delta \rho_{\text{calc}} \) are the experimental and calculated contrasts, respectively.

Applying this formula to our system and assuming that only IL ions were involved, we determined that one formate ion binds to 5 AGUs and one acetate ion binds to 3 AGUs (Table 1). Alternatively, the observed increase, in contrast, could be explained by the binding of IL-cations to AGUs. Similar calculations revealed that one [EMIM] cation binds to 6 AGUs and one [AMIM] cation binds to 13 AGUs.

Irrespective of the assumptions, approximately twice as many IL-cations bind to 6 AGUs and one [AMIM] cation binds to 13 AGUs.

Similar calculations revealed that both [EMIM]-[OAc] and [AMIM][HCOO] ions were bound to one cellulose molecule irrespective of the assumptions, approximately twice as many [EMIM][OAc] and [AMIM][OAc] ions were bound to one cellulose molecule.

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where \( \left( \sum b_i \right)_{\text{anhydroglucose}} \) and \( \left( \sum b_i \right)_{\text{anion (or cation)}} \) are the scattering lengths of anhydroglucose and the anion (or cation); \( \rho_{IL} \) and \( \rho_{\text{cellulose}} \) are the scattering length densities of the IL and cellulose; and \( \Delta \rho_{\text{exp}} \) and \( \Delta \rho_{\text{calc}} \) are the experimental and calculated contrasts, respectively.

Applying this formula to our system and assuming that only IL ions were involved, we determined that one formate ion binds to 5 AGUs and one acetate ion binds to 3 AGUs (Table 1). Alternatively, the observed increase, in contrast, could be explained by the binding of IL-cations to AGUs. Similar calculations revealed that one formate ion binds to 5 AGUs and one acetate ion binds to 3 AGUs.

Irrespective of the assumptions, approximately twice as many [EMIM][OAc] and [AMIM][OAc] ions were bound to one cellulose molecule irrespective of the assumptions, approximately twice as many [EMIM][OAc] and [AMIM][OAc] ions were bound to one cellulose molecule.

Similar calculations revealed that both [EMIM][OAc] and [AMIM][OAc] ions were bound to one cellulose molecule irrespective of the assumptions, approximately twice as many [EMIM][OAc] and [AMIM][OAc] ions were bound to one cellulose molecule.

The expression is given as

\[
\text{number of anhydroglucose units per anion (or cation)} = \frac{\left( \sum b_i \right)_{\text{anhydroglucose}}}{1 - \left( \rho_{IL}/\rho_{\text{cellulose}} \right)} \left( \frac{\Delta \rho_{\text{exp}}}{\Delta \rho_{\text{calc}}} - 1 \right)
\]

where \( \left( \sum b_i \right)_{\text{anhydroglucose}} \) and \( \left( \sum b_i \right)_{\text{anion (or cation)}} \) are the scattering lengths of anhydroglucose and the anion (or cation); \( \rho_{IL} \) and \( \rho_{\text{cellulose}} \) are the scattering length densities of the IL and cellulose; and \( \Delta \rho_{\text{exp}} \) and \( \Delta \rho_{\text{calc}} \) are the experimental and calculated contrasts, respectively.
and solvent (\(\Delta \rho = \rho_{\text{particle}} - \rho_{\text{solvant}}\)), as given by eq 2. The volume fraction of xylan chains was fixed to the value calculated from the weights of xylan and solvent in the sample. The volume of a particle (\(V_p\)) was fixed to the calculated diameter of an anhydroxylose unit (AXU). The contrast parameter was the only fit parameter, and the experimentally determined contrast was slightly higher than the calculated contrast values (Table 2). Applying eq 8 with xylan and AXU in place of cellulose and AGU, respectively, the number of [EMIM][OAc] and [AMIM][OAc] (Table 2). Furthermore, the molecular weight of lignin in dilute solutions was determined from SAXS data using the Porod invariant approach. The calculated molecular weights of lignin in [AMIM][OAc] and [AMIM][HCOO] were ~4300 and 17 900 g/mol, respectively, assuming that the molecular density of lignin was 1.35 g/cm³. As mentioned in the Methods section, the \(M_w\) of lignin determined using gel permeation chromatography was ~3743 g/mol. This suggests that lignin dispersed as individual molecules in [AMIM][OAc], whereas approximately four polymer molecules associated together in [AMIM][HCOO]. The fact that the molecular weight of lignin was smaller and closer to that of individual molecules (as determined by GPC) implied that [AMIM][OAc] was a better solvent for lignin than [AMIM][HCOO].

### Table 2. Summary of Fit Parameters of SAXS Data for a 2% (w/w) Solution of Xylan and Lignin in Select ILs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[EMIM][OAc]</th>
<th>[AMIM][OAc]</th>
<th>[AMIM][HCOO]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birchwood Xylan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intensity scalar (cm⁻¹ Å⁻²)</td>
<td>0.0027</td>
<td>0.0028</td>
<td></td>
</tr>
<tr>
<td>(\phi) volume fraction (φ)</td>
<td>0.0146</td>
<td>0.0149</td>
<td></td>
</tr>
<tr>
<td>experimental contrast ((\Delta \rho_{\text{exp}})) (10⁻⁶ Å⁻²)</td>
<td>4.28</td>
<td>4.34</td>
<td></td>
</tr>
<tr>
<td>calculated contrast ((\Delta \rho_{\text{calc}})) (10⁻⁶ Å⁻²)</td>
<td>3.48</td>
<td>3.59</td>
<td></td>
</tr>
<tr>
<td>number of anhydroxylose unit (AXU) per anion</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>number of anhydroxylose unit (AXU) per cation</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Organosolv Lignin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>radius of gyration ((R_g), Å)</td>
<td>18.2 ± 1.2</td>
<td>27.0 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Guinier scalar ((G_1))</td>
<td>0.050</td>
<td>0.071</td>
<td></td>
</tr>
<tr>
<td>Porod scalar ((B_1))</td>
<td>0.00085</td>
<td>0.00024</td>
<td></td>
</tr>
<tr>
<td>power-law exponent ((P_1))</td>
<td>1.7 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

- Calculated from contrast values.
- Obtained from eq 8.

In this study, \(^{13}\)C NMR spectroscopy showed that cellulose, xylan, and lignin polymers interacted differently with select 1,3-dialkylimidazolium ILs. Cellulose exhibited the strongest interactions with [EMIM][OAc], whereas xylan and lignin exhibited significantly higher interactions with [AMIM][OAc]. Significant changes in the \(^{13}\)C chemical shifts of the allyl side chains of [AMIM][HCOO] in the presence of xylan, and C10 of acetate ion in the presence of lignin accounted for these differences. SAXS results suggested that lignin formed particles with either extended or randomly flexible chain conformation depending on the IL. The higher \(^{13}\)C NMR spectroscopic shifts of lignin in [AMIM][OAc] and its self-avoiding conformation determined using SAXS suggested that [AMIM][OAc] is a superior solvent for lignin. In contrast, SAXS analysis of cellulose and xylan showed similar conformations in all tested ILs, albeit significantly different number of IL-anions or cations being.

### CONCLUSIONS

In this study, \(^{13}\)C NMR spectroscopy showed that cellulose, xylan, and lignin polymers interacted differently with select 1,3-dialkylimidazolium ILs. Cellulose exhibited the strongest interactions with [EMIM][OAc], whereas xylan and lignin exhibited significantly higher interactions with [AMIM][OAc]. Significant changes in the \(^{13}\)C chemical shifts of the allyl side chains of [AMIM][HCOO] in the presence of xylan, and C10 of acetate ion in the presence of lignin accounted for these differences. SAXS results suggested that lignin formed particles with either extended or randomly flexible chain conformation depending on the IL. The higher \(^{13}\)C NMR spectroscopic shifts of lignin in [AMIM][OAc] and its self-avoiding conformation determined using SAXS suggested that [AMIM][OAc] is a superior solvent for lignin. In contrast, SAXS analysis of cellulose and xylan showed similar conformations in all tested ILs, albeit significantly different number of IL-anions or cations being.
bound to anhydroglucose (AGU) and anhydroxyleose units (AXU). Twice as many acetates or [EMIM] ions were bound to one AGU as opposed to formate or [AMIM] ions. Moreover, the AGUs exhibited twice as many bonding interactions with acetate anions compared to AXUs. Thus, by combining atomic level interactions and supramolecular conformation of biopolymer/IL systems, 1,3-dialkylimidazolium acetates were shown to exhibit higher set of interactions with all three biopolymers. Finally, parameters developed in this study, namely, the number of IL-ions binding to AGUs/AXUs, could provide critical insights into the design and development of ILs in the future for efficient solubilization of plant polysaccharides.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biomac.3c00047.

Moisture content of the neat and biopolymer-IL solutions; annotated 13C NMR spectra of [AMIM]-[OAc], [AMIM][HCOO], [EMIM][HCOO], and [EMIM][OAc], with and without cellulose, xylan, and lignin; absolute chemical shift changes of cellulose, xylan, and lignin in all tested ILs; and molecular weight, density, and scattering length density of cellulose, xylan, and lignin in all tested ILs (PDF)

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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### REFERENCES


