Title

The Undesirable Acetylation of Cellulose by the Acetate Ion of 1-ethyl-3-methylimidazolium acetate

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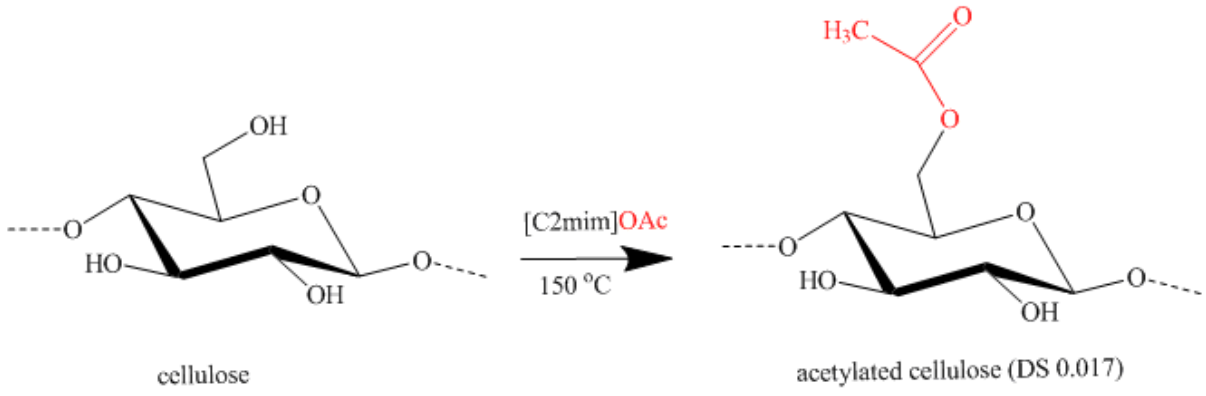
Abstract

The ionic liquid (IL) 1-ethyl-3-methylimidazolium acetate ([C2mim]OAc) is considered to be an inert solvent of cellulose and lignocellulosic biomass. Acetylation (1.7 % mol, or DS 0.017) of cellulose after dissolution in [C2mim]OAc (150 °C for 20 min), is demonstrated by compositional and FTIR analyses, and in [C2min]OAC with ^{13}C enriched acetate by ^{13}C NMR spectroscopy. This acetylation in the absence of acetylating agents has not been reported before and may limit [C2mim]OAC utility in industrial scale biomass processing, even at this low extent. For example, cellulose acetylation may contribute to IL loss in processes where the IL is recovered and reused and inhibit enzyme saccharification of cellulose in lignocellulosic biofuel production processes based on saccharification and fermentation.

Graphical abstract



Introduction

Ionic liquids (ILs) are a class of organic salts that are liquid at temperatures below 100 °C. Recently, the IL 1-ethyl-3-methylimidazolium acetate ([C2mim]OAc) has been in the limelight of biomass processing research. Low melting point, low toxicity, non-volatility and high cellulose- and lignocellulosic biomass-dissolution capacity are among the more attractive characteristics of this IL as a biomass solvent. On the basis of cellulose and lignocellulosic biomass solubility in [C2mim]OAc and the ease of recovery of these solutes, this solvent, among ILs tested and reported to date, is viewed to have the greatest potential utility for industrial scale biomass processing. Reports that the IL can be recovered after biomass dissolution and reused support this view [1, 2]. Furthermore [C2mim]OAc is considered to be an inert solvent in these applications. No studies have addressed the possibility of covalent attachment of the acetate ion of this IL to the cellulosic substrate. In the event of cellulose acetylation, even at very low extents, the industrial relevance of this IL is challenged since IL recycling and inhibition of enzyme saccharification may be significant impediments to its industrial scale use. In this study, the acetylation of cellulose processed by [C2mim]OAc is tested using compositional analysis, FTIR and ^{13}C NMR.

Experimental Part

Chemicals

Ionic liquids (1-butyl-3-methylimidazolium chloride [C4mim]Cl (≥ 95 %) melting point (m.p.) 73 °C, 1-butyl-2,3-dimethylimidazolium chloride [C4mmim]Cl (≥ 97 %) m.p. 96 °C - 99 °C and 1-ethyl-3-methylimidazolium acetate [C2mim]OAc (≥ 90 %) m.p. –20 °C,), Cellulose (Avicel PH-101) and deuterated dimethyl sulphoxide (DMSO-d_6) (99.9 %) were purchased from Sigma-Aldrich (Sydney, NSW). Sodium acetate, enriched with the ^{13}C isotope (1-^{13}C, 99 %), was purchased from Novachem (Melbourne, Australia). Water was Millipore-filtered and deionised (Milli-Q-plus) to a specific resistivity of 18.2 µS at 25 °C. All other solvents and chemicals were analytical grade.

Dissolution and recovery of cellulose

[C2mim]OAc (17 g, in duplicate) in 50 mL glass tubes were placed in an oil bath (clear silicon oil), heated by a hot plate (RET Basic IKA Laboritechnik with an IKA ETS-D5 thermocouple for temperature control) in the open atmosphere and allowed to equilibrate (60 min) at 150 °C. Avicel cellulose (420 mg) was added and the mixture stirred (300 rpm) for 20 min (dissolution appeared complete), then cooled to ca. 80 °C. Water (15 mL) was then added to the mixture in order to precipitate the cellulose. The precipitated cellulose solids were centrifuge-washed (10 x 30 mL, 9000 x g) and freeze dried.

Compositional analysis

Compositional analysis of cellulose before and after reaction with [C2mim]OAc was carried out using the standard NREL procedure for determination of structural carbohydrates in biomass [3]. All IL-treated samples were freeze dried overnight prior to analysis. Each sample (200 mg, in duplicate) was treated with H_2SO_4 (72 % mass) at 30 °C for 1 h. These samples and a sugar recovery standard (SRS, containing known concentrations of glucose) were then exposed to dilute H_2SO_4 (4 %) at 121 °C for 1 h. The hydrolysis products were determined by HPLC (Waters) equipped with a RI detector (Waters 410). Specifically glucose was determined using a Bio-Rad HPX-87H column (at 60 °C, mobile phase: 5 mM H_2SO_4, flow rate: 0.6 mL min^{-1}) and acetic acid was determined (after neutralization of samples with $CaCO_3$) using a Shodex SPO810 column (at 85 °C, mobile phase: deionised water, flow rate: 0.5 mL min^{-1}). The glucose results were corrected for acid decomposition using the % mass recovery from the sugar recovery standard (SRS). The cellulose and acetyl mass content were calculated by conversion of the monosaccharide and acetic acid results with appropriate multiplication factors (0.90 for glucose and 0.699 for acetic acid).

The estimates of standard deviation (absolute) of this analysis (as % dry mass of starting bagasse) are: 1 for glucan and 0.04 for acetyl groups.

ATR-FTIR

A small amount of treated and untreated cellulose, enough to cover the surface of the probe, was placed on the diamond probe of a Thermo Nicolet 870 FTIR (software: OMNIC 7.3). The samples were pressed with an anvil to increase the surface area contacting the probe. Sixty-four scans were acquired for each spectrum and the two

replicate spectra for each sample were overlayed. No differences in the replicate spectra of this study were observed and thus only the first spectrum of each sample was used for analysis.

IL ion concentration determination

The [C2mim]OAc samples were diluted with deionised water and ion concentrations determined by ion chromatography (Metrohm 761 with a conductivity detector). For cation analysis, samples were injected onto a Metrosep C 2 150 (150 mm x 4 mm) column with an aqueous mobile phase (25 % volume acetone, 6 mM tartaric acid and 0.75 mM dipicolinic acid) at 1 mL min^{-1}. For anion analysis samples were injected onto a Metrosep ASupp5 (150 mm x 4 mm) column with an aqueous mobile phase (1 mM $NaHCO_3$ and 3.2 mM Na_2CO_3) at 0.7 mL min^{-1} and suppressed by post-column addition of H_2SO_4 (50 mM). The estimate of relative standard deviation (RSD) of this technique is 0.5 %.

^{13}C enrichment of [C2mim]OAc

Sodium acetate, ^{13}C enriched (99 % at position C1), in aqueous solution was decanted through a cation-exchange resin column (Amberlist IRC-50 (H^+)). The resulting solution (after purification by vacuum distillation) contained ^{13}C acetic acid and traces of sodium acetate (mol 1 % sodium).

[C2mim]OAc in aqueous solution was decanted in an anion-exchange resin column (Amberlist IRA-93 (OH^-)) and the resulting solution was mixed with equimolar amounts of ^{13}C acetic acid to return the IL to the acetate form. The water and any excess

acetic acid was evaporated from the resulting [C2mim]OAc solution (pH 6) using a rotary evaporator and the residual IL dried in a vacuum oven (at 80 °C, ca. 4 mm Hg, > 48 h). A small amount of the resulting ionic liquid (60 mg) was analysed with ion chromatography and found to have the same ionic composition as the starting IL. The final outcome was a mass (4.5 g) of [C2mim]OAc that contained ^{13}C enriched acetate at 8 % mol.

^{13}C NMR sample preparation

The ^{13}C enriched [C2mim]OAc (4.5 g) in a 50 mL round bottom flask was placed in an oil bath in the open atmosphere and allowed to equilibrate (60 min) at 150 °C. Avicel cellulose (130 mg) was then added and the mixture stirred (300 rpm) for 20 min (dissolution appeared complete), then cooled to ca. 80 °C. Water (15 mL) was then added to the mixture in order to precipitate the cellulose. The precipitated cellulose solids were centrifuge-washed (10 x 30 mL, 9000 x g) and freeze dried. A known mass of the resulting solids (83 mg) was added to [C4mim]Cl (510 mg) in the oil bath (120 °C ramping to 160 °C) and stirred (100 rpm) until dissolution appeared complete. The resulting viscous solution was diluted with DMSO-d_6 (ca. 1 g). Chromium acetylacetonate (5 mg mL^{-1}) was added as a relaxation agent and the final solution was transferred to an NMR tube.

^{13}C NMR experiment

NMR spectra were acquired on a 400 MHz (Avance 400) Bruker Biospin (Rheinstetten, Germany) NMR spectrometer, equipped with an inverse (proton coils closest to sample) gradient 5 mm broadband probe tuned to ^{1}H and ^{13}C. The ^{13}C NMR experiments used a 1D sequence with power-gated decoupling and a 30° flip angle (Bruker standard pulse sequence 'zgpg30'). The spectra were acquired with 26 K data points from 0 ppm to 190 ppm using an acquisition time (AQ) of 0.60 sec, an interscan delay (D1) of 2 sec for 38 K scans and a total acquisition time of 28 h.

^{13}C NMR (DMSO-d_6) (atoms as numbered in figure 3): for acetylated cellulose, δ=170.6 (CO acetate or C-7), 102.1 (C-1), 80.0–74.0 (C-2 to C-5), 60.7 (C-6) and for [C4mim]Cl, δ=137.6 (C-3'), 124.0 (C-1'), 123.0 (C-2'), 49.3 (C-5'), 40.4 (DMSO-d_6), 36.0 (C-4'), 32.0 (C-6'), 19.3 (C-7'), 13.6 (C-8').

Results and Discussion

Microcrystalline cellulose (Avicel) was reacted with [C2mim]OAc, recovered and analysed for evidence of acetylation. The presence of acetate impurities from the ionic liquid anion was eliminated by thoroughly washing the reacted cellulose solids and analysing the last washing with ion chromatography. The compositional analysis for cellulose after reacting with [C2mim]OAc showed 0.45 % mass acetyl content and 94 % mass cellulose (*cf* 0.02 % and 92 % for unreacted cellulose). Under the assumption that acetylation takes place only at the C-6 position of cellulose (see figure 3), the degree of substitution (DS) for cellulose reacted with [C2mim]OAc is 0.017.

The apparent acetylation of reacted cellulose is corroborated by ATR-FTIR spectroscopy (see spectra in Figure 1). The absorption at wavenumber 1720 cm^{-1} (C=O stretch), attributed to acetyl groups, is present in the reacted but absent in the unreacted cellulose samples. The other differences between the two spectra in Figure 1 (especially at bands ca. 3250 cm^{-1}, 1420 cm^{-1} and 900 cm^{-1}) are due to the decrystallisation effect the ionic liquid has on cellulose [4, 5].

To confirm acetylation, cellulose was reacted with [C2mim]OAc containing ^{13}C enriched acetate (8 % mol at position C-1) and the recovered cellulose analysed with ^{13}C NMR. As shown in figure 1, the peak at 170 ppm corresponding to the 13C-enriched carbon in acetate is present in the reacted but absent in the unreacted cellulose samples. Although ^{13}C NMR is not well suited to quantitative analysis, integration of the signals for the anomeric sugar carbon (C-1) and the acetate carbon signal (C-7, corrected for overexpression due to ^{13}C enrichment) indicate a degree of substitution of 0.015. At such low levels of acetylation the C-7 signal is observed due to 13C enrichment, but the substituted C-6 signal and the C-8 signals were not observed.

The acetylation demonstated here is unexpected since no acetylating agents were added to these mixtures. In fact, Kohler *et al.*[6] observed acetylation of cellulose (DS 1.86) dissolved in [C2mim]OAc in the presence of the furoylating agent, 2-furoyl chloride. In this case acetates formed rather than the intended furoyl derivatives via a furan-2-carboxylic acetic anhydride intermediate (an acetylating agent). In addition imidazolium ionic liquids have been shown to promote acetylation of carbohydrates with anhydrides and acid chlorides [7]. However the acetylation of cellulose in [C2mim]OAc solutions and in the absence of added acetylating agents is shown here for the first time. Acetic

anhydride can form from acetic acid by dehydration at temperatures ca. 750 °C [8] and, although not confirmed here, it seems likely that a small amount of acetic anhydride may have formed by dehydration of the acetate ion [C2mim]OAc with consequent loss of the C-2 acidic proton of $[C2mim]^+$ and carbene formation.

Conclusion

The undesirable acetylation of cellulose when reacted in [C2mim]OAc, in the absence of added acetylating agents, is shown here for the first time. Given the viewed potential of this ionic liquid for cellulose and biomass processing, this acetylation is noteworthy. Such acetylation may interfere with downstream processing (inhibition of enzymatic sacharification) and recovery of the expensive ionic liquid after use.

Acknowledgements

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Figures

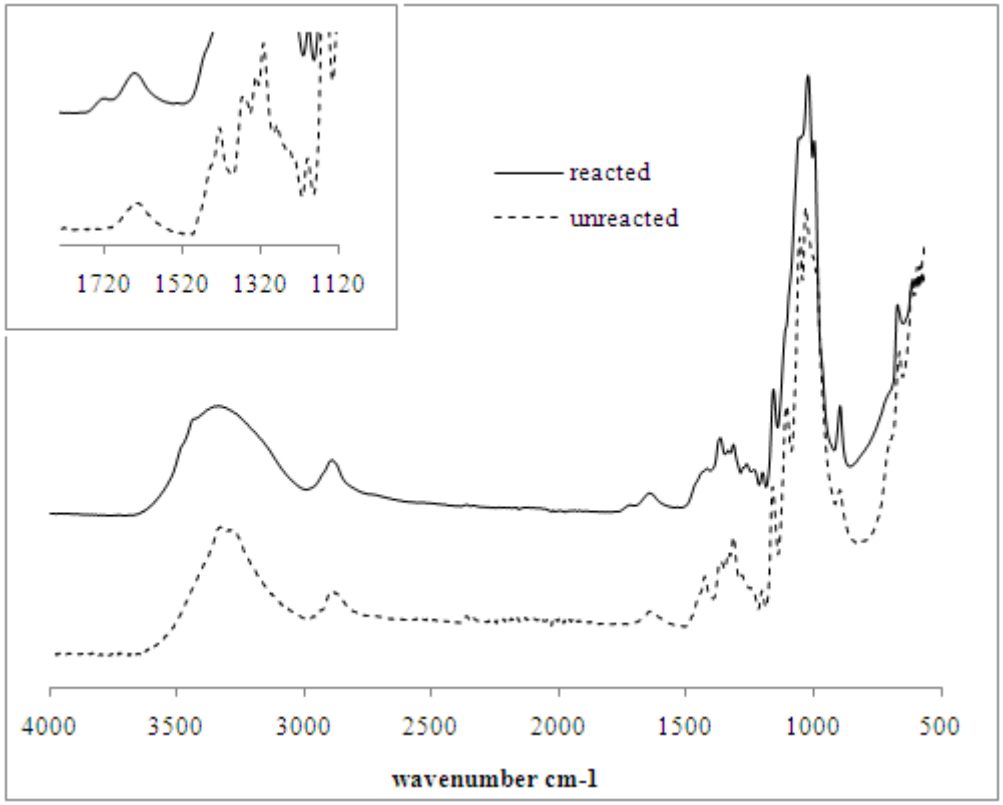

Figure 1: ATR-FTIR of cellulose reacted with [C2mim]OAc and unreacted (normalised and baseline offset)

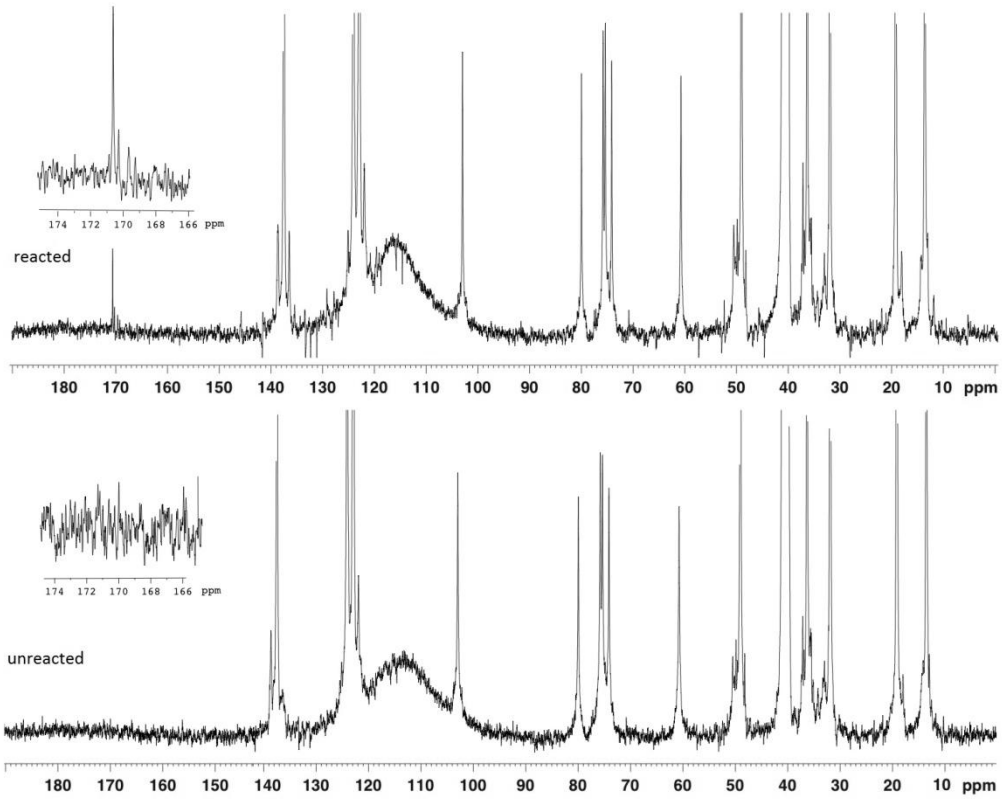

Figure 2: ^{13}C NMR of cellulose reacted with ^{13}C labelled [C2mim]OAc and unreacted (solvents: [C4mim]Cl and DMSO-d_6).

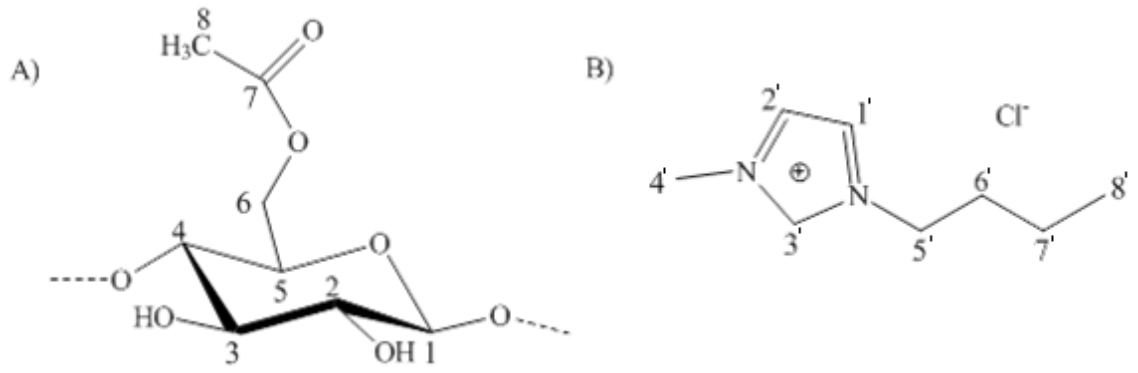

Figure 3: Acetylated cellulose and [C4mim]Cl structures