IONIC LIQUID FRACTIONATION OF WOODY BIOMASS FOR FERMENTABLE MONOSACCHARIDES

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The goal of the present study, devoted to wood fractionation, was to obtain monosaccharides, hexoses and pentoses by means of an ionic liquid (IL) based pre-treatment procedure. Softwood sawdust (maximum particle size of 2 mm) of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) were exposed to ionic liquids – 1-ethyl-3-methylimidazolium acetate (C_2mimAce) and 1-ethyl-3-methylimidazolium chloride (C_2mimCl) – and thermal treatment (80-150 °C), for various time intervals (0-72 h). Furthermore, cellulose of various origins (plants, wood pulps) was dissolved in C_2mimAce and 1-butyl-3-methylimidazolium chloride (C_4mimCl) for the study of the dissolved fractions, stress being laid on monosaccharides and possible by-products, 5-hydroxymethylfurfural and furfural. Knowing the challenges in analysis techniques when ILs and sugars are involved, the present work focuses on the development of suitable analysis methods. To this end, a Hewlett Packard 1100 series HPLC equipped with a refractive index (RI), detector model HP1047 A and a diode array UV detector (DAD) fitted with a carbohydrate column HPX-87K was utilized. Challenges and improvements are discussed.

Keywords: ionic liquid fractionation, cellulose, softwood, fermentable monosaccharides, biomass

INTRODUCTION AND BACKGROUND

The use of ionic liquids (ILs) for an efficient dissolution of cellulose, lignin and wood has been under intensive investigation in recent years and has opened up new possibilities for fractionation, derivatisation and processing of lignocellulosic materials. Several ILs have been found to dissolve both cellulose and wood samples. Moreover, it has been shown that the dissolution capability, as well as the dissolution rates of wood and cellulose in ILs, are influenced by several factors, including the initial biomass-to-IL ratio, biomass particle size and sample origin. However, it has been discovered that a complete dissolution of wood is not necessary for obtaining mono-, di- and oligosaccharides from wood.[1-4]

Nevertheless, the analytics of monosaccharides released from lignocellulosics by means of IL treatment is challenging, since the traditional High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) columns tolerate only very low concentrations of salts, such as ILs. Also, the separation of ILs from sugars is challenging, due to the similar solubility properties (the typical ILs here applicable are *e.g.*, water soluble). The present study focuses especially on the challenging analytics applied for the monosaccharide analysis of IL-pretreated wood and cellulose samples.

The retention times (RTs) for the ILs used in this work and for different monosaccharides – glucose, galactose, mannose, xylose, arabinose, rhamnose and fructose – as well as a disaccharide, sucrose, together with furfural and 5-hydroxymethylfurfural (HMF), were determined. The latter two are often formed as by-products in the fermentation processes of lignocellulosics biomass, as follows: furfural is formed as a degradation product

of pentoses and uronic acids, while HMF is degraded from hexoses, mainly glucose. They are inhibitors for ethanol fermentation and unwanted products while, on the other hand, they are important in many other chemical processes, since compounds, such as HMF, can be used to produce other valuable chemicals (*e.g.* formic and levulinic acid).[5]

EXPERIMENTAL

C_2mimCl was purchased from Merck Chemicals and used as received, while C_4mimCl and C_2mimAce were synthesized at Lomonosov Moscow State University and Umeå University, respectively.

A Hewlett Packard 1100 series HPLC, equipped with a refractive index (RI) detector model HP1047 A and a diode array UV detector (DAD) fitted with a HPX-87K carbohydrate column was utilized for analysis method development. The RI and UV detectors were arranged in series, for enabling their simultaneous use in analysis. An isocratic elution method was evaluated for simultaneous quantification of both ILs and monosaccharides.

100% deionized water was used as a mobile phase (eluent). The eluent was degassed and filtered through a 0.45 μm PVDF membrane syringe filter. The other test parameters were: the flow rate for mobile phase was 0.400 mL/min and the column temperature was of 80 °C, with an injection volume of 10 μL and needle wash by deionized water. From the UV detector, the most interesting signals were detected at 195, 200 and 220 nm (among the tested wavelengths), even if the present study focused mainly on the signal from the RI detector.

The following procedure was applied for dissolving cellulose samples into ILs: 50 mg of cellulose (freeze-dried) and 1 g of IL (opened in glove box) were inserted into a vial and stirred under nitrogen atmosphere at 80 °C (C_4mimCl) and 30 °C (C_2mimAce) for 20 h. After dissolution, 2 mL of absolute EtOH (99.5%) were added to the vial, after which the gel-like product was placed in a glass beaker and an additional portion of alcohol was added. After stirring for 20 min, the mixture was filtered and washed again with fresh EtOH. This washing procedure was repeated 3 times. The collected EtOH was placed in a flask coupled to a rotary evaporator, resulting in the IL left in the flask, which was further analyzed by HPLC. The precipitate was dried in a vacuum oven at 35 °C for about 8 h.

RESULTS AND DISCUSSION

The ILs used in this fractionation study appeared in the HPLC chromatogram before any sugars: the RTs for C_2mimCl and C_4mimCl were of 8.2 min and 8.8 min, respectively. C_2mimAce eluted at 9.7 min, but had a second minor chromatographic response at 23.6 min, when the IL concentration was kept below 1.8 wt%. The higher IL concentrations (over 1.8 wt%) eluted later than the lower IL concentration and the phenomenon was even emphasized in the sugar mixtures: *e.g.* already 1.8 wt% C_2mimAce in water with sugars had an RT several minutes longer than the IL-water solution alone, without any sugars. HMF eluted already after 3.2 min, while the retention time for furfural was around 17.6 min.

Figure 1 illustrates the RI response in HPLC analysis for a sample containing a mixture of different sugars in two different concentrations of IL C_2mimAce (1.8 wt% and 0.1 wt% in deionized water). Also, for the sake of comparison, the RTs for HMF and furfural are shown with signals of various concentrations. HMF and furfural were not included in the sugar mixture, yet their RTs were determined as separate calibration solutions. It can be seen that, if the sample contains both a higher concentration of C_2mimAce ($\geq$ 1.8 wt%) and a lower concentration of sucrose, the chromatogram peak for sucrose would be covered by that of the IL. Figure 2 demonstrates the retention times for the sugars under study. Also, the retention of C_2mimAce, in various concentrations, is shown in the same graph, composed from separately analyzed standard samples. When comparing Figures 1 and 3, it is evident that the RT for C_2mimAce varies, depending on whether it is alone in water solution or included in the sugar mixture. Apparently, when C_2mimAce occurs in sugar mixtures (Figs. 1 and 3), it interacts with the sugars while, when sugars are absent, the RT never exceeds that of sucrose (the first RT for all tested sugars), which unfortunately is the case of higher IL concentrations. It is highly possible that imidazole-type ILs are rather easily "complexing" sugars, thus complicating the analysis procedures. However, the RTs for ILs or HMF do not generally disturb sugar analysis. The RT for HMF is early enough not to disturb sugar analysis, while furfural has a disturbance effect: with the HPLC parameters here used, the RTs for glucose, rhamnose, xylose,

fructose and mannose are very close to each other, and also to RT for furfural. On the other hand, the disadvantage was that, if furfural and HMF co-exist in a sample, a very long post-run (equal to an analysis cycle) is also needed to avoid the occurrence of additional peaks (trace marks of HMF and furfural easily stuck to the system, if pure deionized water is used as an eluent and the washing program is relatively short). When sugar mixtures are analyzed, the freshness of samples and their storing environment/conditions are vital. If the sample stays over night on the HPLC analysis tray and the test run is again run the next day, the results will look different from the original ones (especially if using higher column temperatures, *i.e.* 80 °C, which was the case here, when even the analysis tray nearby easily becomes too warm, if considering the sensitive samples).

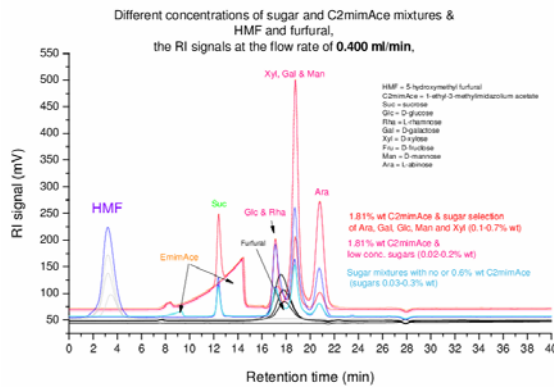

Figure 1: HPLC RI response for a sample of a mixture of different sugars in 2 wt% concentration of IL C_2mimAce in deionized water and for a sample of a mixture of sugars and 0.1 wt% concentration of C_2mimAce. RTs for HMF and furfural are shown (signals of various concentrations). Flow rate – 0.4 ml/min

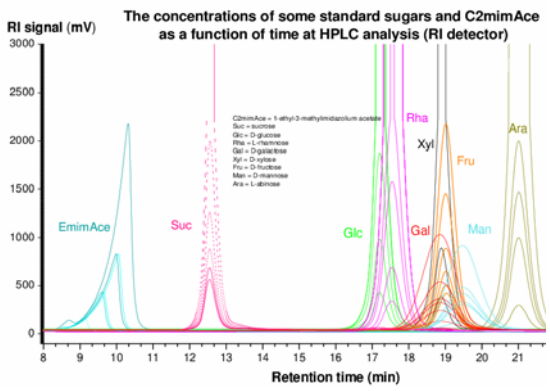

Figure 2: RTs for the tested sugars and C_2mimAce in various concentrations (the graph is made from separately analyzed standard samples). Compared to Fig. 1, it can be seen that RT for C_2mimAce varies depending on whether it is alone in water solution or included in the sugar mixture

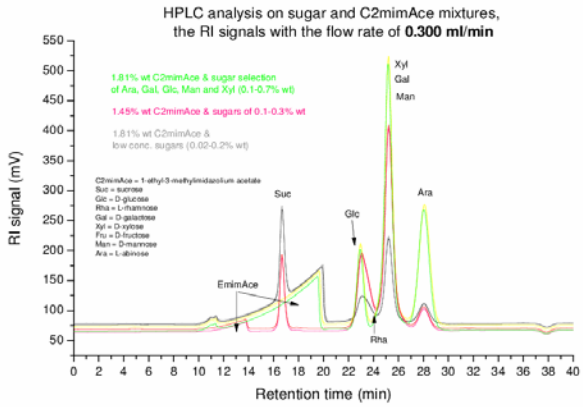

Figure 3: HPLC analysis of sugar and C_2mimAce mixtures, RI signals with flow rate of 0.300 ml/min

A better separation of sugars might be reached by the addition of boric acid to the mobile phase,[6] and/or by reducing the flow rate. For example, reducing the flow rate from 0.400 to 0.300 mL/min alone only gives longer RTs, without better separation. Figure 3 illustrates the RI signals for sugar mixtures, when a lower flow rate is applied.

When liquid fractions (solid material filtered away) of the aged softwood samples had been previously exposed to C_2mimCl and heat over various time intervals, with the addition of deionized water (either before or after the IL and heat treatment), the group of monosaccharides was detected at the RTs determined for arabinose, xylose, fructose,

galactose and glucose. The added distilled water gives rise to more noise (background chromatogram peaks) to the signal. Especially in aged samples with very low sugar concentrations, the quantitative determination of sugar concentrations might not be as reliable as for the fresh samples diluted with deionized water. Instead of distilled water, high quality deionized water is always recommendable for HPLC analysis. However, C_2mimCl was again shown to fractionate the softwood polysaccharides into monosaccharides but, due to the partial overlapping of RTs in the present method, more tests with pH regulation (by the addition of an acid) are required to obtain a clearer separation for all sugars involved.

When looking at the sugar fractions dissolved, upon exposing cellulose of various origins to IL C_4mimCl and heat (80 °C, 20 h), no considerable differences could be observed in the HPLC chromatograms, while the IL used for dissolution had a higher influence on the chromatograms. For the regenerated IL C_4mimCl, the sugars that could be observed in the chromatogram were sucrose, xylose, galactose and arabinose. However, these should originate from the residual hemicellulose – all peaks were small and no glucose that would indicate cellulose depolymerization was observed. Otherwise, C_2mimAce showed the same sugar signals as mentioned above, except the signal for sucrose, which was covered. Although all these sugar signals in cellulose were very low in both cases, interestingly, the signal peaks of the sugars in C_2mimAce were more pronounced than the ones in C_4mimCl. This is understandable, since pure cellulose consists of β-D-glucose units. The reader should be reminded that the difference in the treatment procedure in the case of cellulose appeared in temperature: 80 °C for C_4mimCl and 30 °C for C_2mimAce. The treatment time, 20 h, was quite long. Consequently, the stronger signals and the higher sugar concentrations observed in C_2mimAce can be attributed to the fact that a temperature of 80 °C, together with a relatively long exposure to C_4mimCl, contributes to the degradation of sugars. An alternative reason might simply be the higher efficiency of C_2mimAce in obtaining monosaccharides from hemicelluloses bound into cellulose. In previous studies, the IL exposure was much shorter.

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