Carbohydrate Research 373 (2013) 42–51

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Analysis of mono- and oligosaccharides in ionic liquid containing matrices

Ronny Wahlström *, Stella Rovio, Anna Suurnäkki

VTT Technical Research Centre of Finland, PO Box 1000, FI-02044 VTT, Espoo, Finland

ARTICLE INFO

Article history:
Received 18 June 2012
Received in revised form 2 November 2012
Accepted 12 November 2012
Available online 20 November 2012

Keywords:
Ionic liquid
Saccharide
Derivatization
β-Glucosidase
Endoglucanase
Capillary electrophoresis

ABSTRACT

Ionic liquids (ILs), that is, salts with melting points <100 °C, have recently attracted a lot of attention in biomass processing due to their ability to dissolve lignocellulosics. In this work, we studied how two imidazolium-based, hydrophilic, cellulose dissolving ionic liquids 1,3-dimethylimidazolium dimethylphosphate [DMIM]DMP and 1-ethyl-3-methylimidazolium acetate [EMIM]AcO affect the usually employed analytical methods for mono- and oligosaccharides, typical products from hydrolytic treatments of biomass. HPLC methods were severely hampered by the presence of ILs with loss of separation power and severe baseline problems, making their use for saccharide quantification extremely challenging. Problems in DNS photometric assay and chromatography were also encountered at high ionic liquid concentrations and many capillary electrophoresis (CE) methods did not allow an efficient analysis of saccharides in these matrices. In this paper we describe an optimized CE method with pre-column derivatization for the qualitative and quantitative analysis of mono- and oligosaccharides in sample matrices containing moderate (20–40% (v/v)) concentrations of ILs. The IL content and type in the sample matrix was found to affect both peak shape and quantification parameters. Generally, the presence of high IL concentrations (⩾20% (v/v)) had a dampening effect on the detection of the analytes. IL in lower concentrations of <20% (v/v) was, however, found to improve peak shape and/or separation in some cases. The optimized CE method has good sensitivity in moderate concentrations of the ionic liquids used, with limits of detection of 5 mg/L for cellooligomers up to the size of cellotetraose and 5–20 mg/L for cellopentaose and cellohexaose, depending on the matrix. The method was used for analysing the action of a commercial β-glucosidase in ILs and for analysing saccharides in the IL containing hydrolysates from the hydrolysis of microcrystalline cellulose with *Trichoderma reesei* endoglucanase Cel5A. According to the results, [DMIM]DMP and [EMIM]AcO] showed clear differences in enzyme inactivation.

1. Introduction

Ionic liquids (ILs) have recently received a great deal of interest as a new media for biomass dissolution and chemical modification. ILs are defined as salts with melting points <100 °C.[1] In 2002, Swatloski et al. described the dissolution of cellulose in ionic liquids.[2] The dissolution of wood, in the form of saw dust, has also been demonstrated in ILs.[3] This dissolution ability of ILs can also be exploited in pre-treatment of lignocellulosics prior to enzymatic total hydrolysis and fermentation used in the production of ethanol from renewable feed stocks. Dadi et al.[4] described a pre-treatment process in which the biomass is first dissolved in IL and then precipitated by the addition of an anti-solvent such as water or alcohol. This pre-treatment process was shown to greatly enhance subsequent enzymatic hydrolysis rates. Kamiya et al.[5] reported an alternative process where the cellulosic substrate was first dissolved in ionic liquid and then enzymatically hydrolysed in the same vessel after addition of buffer. After the introduction of these two concepts a number of papers dealing with enzymatic hydrolysis of cellulosics in combination with IL treatments have been published.

Only a few reports have been published on the IL compatibility of analytical methods for saccharides. ILs have, however, been proposed to interact with photometric assays and high performance liquid chromatography (HPLC) methods,[6] which are generally used for analysing the soluble saccharides formed in enzymatic hydrolysis of lignocellulosics. Recently, Hyvärinen et al.[7] discussed difficulties caused by the high salt content in chromatographic saccharide analysis in IL containing sample matrices. On the other hand, ILs have previously been used as auxiliaries in both chromatographic and electrophoretic separation techniques, predominantly as column stationary phases or mobile phase additives (chromatography) or as organic modifiers in background electrolyte solutions (BGEs) and capillary coatings in capillary electrophoresis (CE).[8] Recently, Vaher et al. demonstrated that ILs in low concentrations could act as chromophores for indirect UV detection in the sensitive analysis of small saccharides.[9] According to Vaher et al. the

* Corresponding author. Tel.: +358 40 02 54 073; fax: +358 20 72 27 071.
E-mail address: ronny.wahlstrom@vtt.fi (R. Wahlström).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2012.11.006

presence of low amounts of ILs (10–50 mM) in the BGEs increased the resolution between the saccharide peaks, but increasing the content of IL further led to baseline fluctuation. Another observation was that ILs with long hydrocarbon chains may actually function as surfactants and reverse the electroosmotic flow. High sensitivity saccharide analytical methods in high content IL matrices have not been described previously.

Saccharides are non-ionic compounds in their natural state. To enable the resolution of neutral, non-derivatized saccharides by electric fields as in CE, alkaline borate buffers are used to form charged borate–saccharide complexes.[10–12] The detection (usually measured at 195 nm) is considerably improved by the formation of borate–saccharide complexes.[12] Also indirect detection of saccharides in CE analysis is possible. In this case, a UV absorbing compound (such as sorbic acid[13] or 2,6-pyridinedicarboxylic acid[14]) is added to the BGE and the saccharides are analysed under basic conditions.

The resolving power of CE with pre-column derivatization has previously been demonstrated for mixtures of monosaccharides, including uronic and hexenuronic acids, and small xylo- and cellooligomers, in aqueous solutions.[10,11,15] Maltooligosaccharides with degrees of polymerization (DP) of up to 13 have been separated employing CE techniques.[16] Good separation results for derivatized saccharides have also been obtained employing micellar electrokinetic capillary chromatography (MEKC).[17] CE has been used in the separation of monosaccharide mixtures in matrices containing *N*-methylmorpholine-*N*-oxide (NMMO) used as industrial cellulose solvent.[18] In this study, monosaccharides were analysed in aqueous matrices containing roughly 10% NMMO prior to derivatization and analysis. The presence of NMMO was reported to interfere neither with the derivatization reaction nor with analysis with CE.

Advantages of sample derivatization include a manifold increase in detectability. Usually derivatized saccharides have absorption maxima with wavelengths greater than those of underivatized analytes, which increases also the selectivity of detection. Commonly encountered carbohydrate derivatization reagents are for example, 4-aminobenzoic acid ethyl ester (ABEE),[10,11,15] 4-aminobenzonitrile (ABN),[15] 6-aminoquinoline (6-AQ)[19] and 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS).[16] The derivatization proceeds via reductive amination and needs a free reducing end of the analyte. Reductive amination works well for aldoses, but ketoses such as fructose are not well derivatized.[10,16] Great excesses of derivatization reagent are usually used. In the derivatization method described by Dahlberg et al.,[11] the derivatization reaction is quenched by addition of alkaline borate buffer, which is suggested to form highly water soluble saccharide–borate complexes at the same time as the excess ABEE reagent is precipitated. Alkaline borate buffers are generally employed as BGEs for the separation of ABEE, ABN and similar saccharide derivatives in CE, with some variations in the alkalinity and borate concentration. Occasionally, additives such as surfactants and alcohols are added to the BGEs to improve resolution between adjacent peaks.[15] Both normal[10] and reverse polarity[15] modes have been employed.

Our work on developing IL compatible analytics for saccharide identification and quantification was started to allow us to study the action of hydrolytic enzymes on cellulose in imidazolium-based ILs.[20] The ionic liquids studied were 1,3-dimethylimidazolium dimethylphosphate [DMIM]DMP and 1-ethyl-3-methylimidazolium acetate [EMIM]AcO. The primary aim was to find a method that allows the sensitive quantification of cellooligomers up to the size of cellohexaose in these IL containing matrices. In this paper, we discuss how the presence of [DMIM]DMP and[EMIM]AcO affect the routine methods, such as DNS assay, different chromatography methods and CE in saccharide analysis. We present an optimized method for cellooligomer analysis in significant contents of ILs (20–40% (v/v)) employing CE with pre-column derivatization. The separation power of this method is demonstrated for both mono- and oligosaccharides obtained from wood-derived biomass and results for the quantification of the water soluble cellooligomers glucose, cellobiose, cellotriose, cellotetraose, cellopentaose and cellohexaose in four different matrices are presented. The usefulness of the method is illustrated by two studies, in the first of which the action of a commercial β-glucosidase preparation is studied in [DMIM]DMP and [EMIM]AcO matrices on cellooligomeric substrates, and in the second of which the partial enzymatic hydrolysis of microcrystalline cellulose by an endoglucanase is followed for different time points in IL matrices.

2. Materials and methods

2.1. Chemicals

[DMIM]DMP was prepared as described in the literature.[21] [EMIM]AcO (purity >98%) was purchased from Ionic Liquid Technologies (Heilbronn, Germany) and used without further purifications. The halide content of the [EMIM]AcO determined by ion chromatography was: chloride <100 mg/kg and bromide <50 mg/kg.

Cello-, manno- and xylooligomers in the range of bioses to hexaoses were purchased from Megazyme International (Wicklow, Ireland). Boric acid, sodium hydroxide (NaOH), 1,5-dimethyl-1,5-diaza-undecamethylene polymethobromide (hexadimethrine bromide), xylose, galactose, mannose and arabinose were obtained from Sigma–Aldrich (Steinheim, Germany). Glucose was from VWR International (Leuven, Belgium), galacturonic acid (from citrus origin) was purchased from BDH Chemicals (Poole, UK). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). For the preparation of 3,5-dinitrosalicylic acid (DNS) reagent solution according to Sumner,[22] DNS and potassium sodium tartrate tetrahydrate were acquired from Merck (Darmstadt, Germany). All chemicals were used as received if not otherwise stated.

β-Glucosidase (Novozym 188) was obtained from Novozymes (Bagsvaerd, Denmark) and used as such. β-Glucosidase, xylanase and endoglucanase activities were measured for the crude β-glucosidase preparation and determined to be 5900, 2970 and 740 nkat/mL, respectively. The unit katal (kat) is defined by the International Union for Pure and Applied Chemistry (IUPAC) as the number of catalysed reactions per time unit as mol/s.[23] β-Glucosidase activity was measured according to Bailey and Linko[24] and xylanase activity according to Bailey et al.[25] but at pH 5.0. Endoglucanase activity measurements were carried out according to the HEC assay[26] but using carboxymethylcellulose (CMC) in buffer at pH 5.0.

2.2. Chromatography and DNS assay

Reversed-phase chromatography was carried out based on experimental conditions described by Yasuno et al.[27] Analyses were carried out on a Dionex Ultimate 3000 HPLC system equipped with a Phenomenex C-18 Gemini-NX 3 μm 110A 150 × 2 mm column and a diode array detector. The eluent was a 0.2 M potassium borate buffer at pH 9 with 5% MeOH.

DNS photometric assay was carried out according to the IUPAC standard procedure[26] with the DNS reagent solution prepared as described by Sumner[22] using a Hitachi U-2000 spectrophotometer for absorption measurements at 540 nm. High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was done according to our previously published in-house method.[28]

2.3. Capillary electrophoresis

2.3.1. Derivatization prior to CE analysis

The saccharides were derivatized with 4-aminobenzonitrile (ABN, samples in aqueous solution or containing [DMIM]DMP)