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# An optimized capillary electrophoresis method for the simultaneous analysis of biomass degradation products in ionic liquid containing samples☆

Tiina Aid*, Loore Paist, Margus Lopp, Mihkel Kaljurand, Merike Vaher

*Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia*

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ABSTRACT

An indirect capillary electrophoresis method for a quantitative determination of mono-, di- and oligosaccharides was developed to investigate biomass degradation, the isomerization of glucose into fructose and conversion of fructose to 5-hydroxymethylfurfural (5-HMF) in ionic liquids (ILs). Three chromophores, namely 2,6-pyridinedicarboxylic acid (PDC), maleic acid and phthalic acid, were used to perform indirect detection. The electroosmotic flow (EOF) was reversed to reduce analysis time, using 1-tetradecyl-3-methylimidazolium chloride ($C_{14}$MImCl). The simultaneous separation of the underivatized mono-, di- and oligosaccharides was performed using four cellodextrin oligomers (cellotriose, cellotetraose, cellopentaose, cellohexaose), eight carbohydrates (xylose, fructose, glucose, galactose, lactose, cellobiose, raffinose, sucrose), two organic acids (acetic acid, levulinic acid) and 5-HMF. The best performance was obtained using background electrolyte (BGE) composed of 138.2 mM NaOH, 40 mM maleic acid and 5 mM $C_{14}$MImCl, the applied voltage was −21.7 kV. The linear ranges for analyzed compounds were following: organic acids, raffinose and sucrose from 0.20 to 7 mM, cellodextrin oligomers from 0.25 to 5 mM, other analyzed carbohydrates from 0.25 to 7 mM and 5-HMF from 0.05 to 7 mM. The relative standard deviations (RSD) of peak areas varied from 3.47 to 9.62% during a 5-day analysis period and 0.58–5.29% during one day.



## 1. Introduction

As world energy consumption increases rapidly, decreasing the dependence on fossil fuels has become a major concern globally. One of the most potential sources of energy alternative to traditional fossil fuels is an abundant natural polymer—cellulose. Cellulose is one of the main components present in lignocellulosic biomass, in addition to hemicellulose and lignin. Lignocellulose is a generic term used to describe non-edible plant biomass, and it is the most abundant renewable carbon resource, which can be obtained as a waste from the pulp and paper industry, agriculture (e.g. corn stover, sugarcane bagasse, wheat straw, rise husk, etc.), and forestry (different softwood- and hardwood parts) among other sources [1,2]. Cellulose is a semi-crystalline homopolysaccharide that contains glucopyranose residues linked by β-(1,4)-glycosidic bonds. Cellulose chains can form a strong crystalline structure due to the intra- and intermolecular hydrogen bonds between hydroxyl groups. Hemicellulose is a branched amorphous heteropolysaccharide that contains pentose (e.g. xylose and arabinose), hexose (e.g. mannose, glucose and galactose) and sugar acid units. Hemicellulose surrounds the cellulose fibers and is a linkage between cellulose and lignin. Lignin is composed of randomly branched phenylpropenyl units, e.g. coniferyl alcohol, sinapyl alcohol, and coumaryl alcohol. Lignin is surrounding and holding together the cellulose and hemicellulose fibers, resulting in biomass structural rigidity and recalcitrance to chemical and enzymatic hydrolysis. [3,4] To obtain valuable chemicals this rigid material is pre-treated thermochemically and/or physically, followed by its chemical or enzymatic hydrolysis to sugar monomers and further conversion to bioalcohols (ethanol, butanol) [5,6], carboxylic acids (formic acid, acetic acid, levulinic acid) [7], furfural (from pentoses) [8], 5-hydroxymethylfurfural (5-HMF, from hexoses) [9,10] and phenolic compounds (e.g. ferulic acid, syringic acid, vanillic acid, 4-hydroxybenzoic acid, from lignin) [4]. Among common thermochemical and physical pre-treatment methods, a new promising method – pre-treatment with ionic liquids – is gaining popularity.

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* Corresponding author.
*E-mail address:* tiina.aid@ttu.ee (T. Aid).

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ILs are salts with a relatively low melting point (<100 °C) obtained due to the inefficient packing of large irregular organic cations with smaller inorganic or organic anions. ILs have been proposed as greener alternatives to volatile organic solvents because of several advantages, such as negligible vapor pressure, good thermal stability, wide liquid range, good dissolving and extracting ability, excellent microwave-absorbing abilities, designable structures, etc. [11]. Some ILs like imidazolium-based [12–14], pyridinium-based [12,14], and ammonium-based [14] ones combined with chloride, acetate or sulfate anions are capable of dissolving cellulose and/or breaking down glycosidic bonds [15]. When the metal catalyst is added to the cellulose or lignocellulosic biomass to be dissolved in ILs, the most favored platform chemical 5-HMF, a potential feedstock for fuels and chemicals, can be synthesized with remarkably good yield [16–19].

Currently, methods used for carbohydrate analysis include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). The most popular modes of HPLC employed for underivatized carbohydrates analysis are normal phase (HILIC) [20], reversed-phase or anion-exchange modes with refractive index detection or mass spectrometry [21,22], and ultraviolet (UV), or fluorescence detection after precolumn derivatization [23]. In GC, carbohydrates are derivatized by silylation or acetylation before analysis [24]. Unfortunately, GC and HPLC columns used for carbohydrates analysis, tolerate only a limited amount of salts, such as ILs [25,26] and in addition derivatization step is time-consuming. Recently, CE methods to quantify neutral carbohydrates by ionization in strongly alkaline conditions were reported [27,28]. Sugars having a $pK_a$ value in the range from 12 to 13 are weakly acidic, thus a high pH (12–13) solution is needed for deprotonation. The ionized structures are suitable for electrophoretic analysis and identification through indirect UV detection [29,30], direct UV detection [31] and electrochemical [32] or contactless conductivity detection [33].

To choose a rapid and reliable method for monitoring the conversion of cellulose to 5-HMF, several aspects must be taken into account. Firstly, the separation system must tolerate high concentrations of ILs; however, according to literature chromatographic columns for carbohydrates analysis do not endure high concentrations of salts. Secondly, during the biomass conversion process, in addition to mono-, di-, and oligosaccharides some UV inactive by-products such as acetic acid or levulinic acid can be formed. In this point of view the CE method for monitoring the production of 5-HMF from lignocellulosic biomass should include indirect UV detection. The carbohydrates analysis with indirect UV detection typically employs PDC as a chromophore [29]. However, PDC has an absorption maximum at 270 nm, similarly to 5-HMF, resulting thereby in a higher quantification limit. Therefore, the aim of this study was to investigate the applicability of maleic acid (λABS = 210 nm) and phthalic acid (λABS = 230 nm) as chromophores in BGE for a rapid quantification of carbohydrate mono-, di-, and oligomers, acetic acid, levulinic acid, and 5-HMF in ILs solutions, using anodic EOF achieved by adding $C_{14}$MImCl to BGE.

## 2. Materials and methods

### 2.1. Chemicals

D-(+)-xylose, D-(−)-fructose, D-(−)-glucose, D-(+)-galactose, D-(+)-cellobiose, β-lactose, sucrose, D-(+)-raffinose, 5-HMF, furfural, levulinic acid, acetic acid, maleic acid, phthalic acid, PDC, NaOH, hexadimethrine bromide and microcrystalline cellulose were purchased from Sigma-Aldrich and were used as received. Cellotriose (DP3, 97.3%), cellotetraose (DP4, 97.3%), cellopentaose (DP5, 97.5%) and cellohexaose (DP6, 89%) were purchased from Elicityl, France. Ionic liquids, $C_{14}$MImCl (>98%) and 1-ethyl-3-methylimidazolium chloride (EMImCl, >98%), were purchased from IoLiTec, Germany, and were used as received. Stock solutions were prepared taking into account their degree of purity. Chromophores, such as maleic acid, phthalic acid and PDC, were used to prepare stock solutions in concentrations of 100, 35 and 25 mM, respectively.

### 2.2. Electrophoretic conditions

CE separations were performed using an Agilent 3D instrument equipped with a diode array UV/Vis detector. Data acquisition and instrument control were carried out using HP 3D Chemstation software from Agilent Technologies. The optimization procedure was performed employing a fused silica capillary with semi-permanent coating with $C_{14}$MImCl that was added to the background electrolyte, and with an effective length of 61.5 cm (total length of 70 cm) and ID of 22.5 μm (Polymicro Technologies Inc., USA). The sample was injected hydrodynamically under a pressure of 50 mbar for 20 s. Separations were performed at 25 °C at a voltage from −15 to −30 kV. The detection wavelength was 210 nm in the case of maleic acid, 230 nm for phthalic acid and 270 nm for PDC, while 5-HMF was examined at 270 nm in each case. Before each run the capillary was filled with BGE for 7 min and between the runs, the capillary was flushed with 1 M NaOH for 2 min, and ultra-pure water for 3 min. BGE was prepared on the first day and stored at room temperature. All the experimental data were analyzed using Microsoft Office Excel 2007 (Microsoft Corporation) and JMP 12.0 (S.A.S Institute Inc., USA).

The critical micelle concentration for $C_{14}$MImCl in water is 3 mM [34] and therefore the existence of micelles was evaluated. Furfural (potential degradation product of cellulose) was used as micellar marker since it is neutral at used conditions. The separation of furfural was not achieved by using 5 mM of $C_{14}$MImCl containing BGE and at least 10 mM of $C_{14}$MImCl is needed (Fig. S1 in Supporting information) for strongly alkaline BGE (pH 12.7).

A comparison with different surface coating agent – hexadimethrine bromide – was performed. According to obtained results the average efficiency was 20% higher for $C_{14}$MImCl containing BGE and hexadimethrine bromide was further not used in this study.

### 2.3. Cellulose hydrolysis in IL

Cellulose hydrolysis was carried out according to Binder and Raines [35] published paper with slight modifications. 0.2 mg cellulose was dissolved in 2 g EMImCl using conventional heating with thermostat at 105 °C for 4 h, the mixture was stirred periodically. After 4 h, 120 μL 1.7 M HCl was added to the solution, and the mixture was stirred vigorously. After 10 min sample 1 was collected and 400 μL deionized water was added to the solution with vigorous stirring, followed by the addition of deionized water after 20 min thrice: 200, 300 and 500 μL, respectively. Samples 2, 3, 4 were collected before each addition of water and the last sample was collected in the end of the reaction (total reaction time was 6 h).

### 2.4. Validation

Parameters such as linearity, precision, robustness, limit of detection (LoD), and limit of quantification (LoQ) were evaluated. Instrumental LoD and LoQ were experimentally calculated from the analysis of spiked samples giving the signal-to-noise ratio of 3 and 10, respectively. The linearity of calibration curves for each analyte was verified by the coefficient of determination. Precision was evaluated at two levels, repeatability as intra-day precision and reproducibility as inter-day precision. Intra-day precision was