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DOCTORAL THESIS

Catalytic Conversion of Recalcitrant Glycopolymers: Investigation of Saccharification and Isomerisation in Ionic Liquids

Tiina Kontson

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Tiina Kontson

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Tõrksate glükopolümeeride katalüütiline konversioon: sahharifikatsiooni ja isomerisatsiooni uurimine ioonsetes vedelikes

TIINA KONTSON

TAL TECH
KIRJASTUS

Contents

List of publications

The list of author's publications, on the basis of which the thesis has been prepared:

I **T. Aid**, S. Hyvärinen, M. Vaher, M. Koel and J.-P. Mikkola, "Saccharification of lignocellulosic biomasses via ionic liquid pretreatment", *Industrial Crops and Products*, vol. 92, 336−341, 2016.

II **T. Aid**, L. Paist, M. Lopp, M. Kaljurand and M. Vaher, "An optimized capillary electrophoresis method for the simultaneous analysis of biomass degradation products in ionic liquid containing samples", *Journal of Chromatography A*, vol. 1447, 141−147.

III **T. Aid**, M. Koel, M. Lopp and M. Vaher, "Metal-Catalyzed Degradation of Cellulose in Ionic Liquid Media", *Inorganics*, vol. 78 (6), 1−11, 2018.

Author's contribution to the publications

Contribution to the papers in this thesis are:

I The author contributed to designing the wheat straw experiments, performed the experimental work, analysed and interpreted the data, and wrote the manuscript together with Sari Hyvärinen.

II The author contributed to designing the experiments, performed the experimental work (except for the cellulose hydrolysis samples, which were performed by Loore Paist), analysed and interpreted the data, and wrote the manuscript.

III The author contributed to designing the experiments, performed the experimental work, analysed and interpreted the data, and wrote the manuscript.

Other publications by the author (not discussed in this thesis)

- F. Elhi, **T. Aid,** and M. Koel, "Ionic liquids as solvents for making composite materials from cellulose", *Proceedings of the Estonian Academy of Sciences*, vol. 65 (3), 255−266, 2016, DOI: 10.3176/proc.2016.3.09.
- J. Parve, I. Reile, **T. Aid,** M. Kudrjašova, A.-M. Müürisepp, I. Vallikivi, L. Villo, R. Aav, T. Pehk, L. Vares, and O. Parve, "Lipase-catalyzed stereoresolution of long-chain 1,2-alkanediols: a screening of preferable reaction conditions", *Journal of Molecular Catalysis B Enzymatic*, vol. 116, 60−69, 2015, DOI: 10.1016/j.molcatb.2015.03.006.
- **T. Aid,** M. Kaljurand and M. Vaher, "Colorimetric Determination of Total Phenolic Content in Ionic Liquid Extracts by Paper Microzones and Digital Camera", *Analytical Methods*, vol. 7, 3193−3199, 2015, DOI: 10.1039/c5ay00194c.
- A. Paju, T. Kanger, A.-M. Müürisepp, **T. Aid**, T. Pehk and M. Lopp, "Sonogashira cross-coupling of 3-bromo-1,2-diones: an access to 3-alkynyl-1,2-diones", *Tetrahedron*, vol. 70 (35), 5843−5848, 2014, DOI: 10.1016/j.tet.2014.06.037.
- M. Vaher, M. Borissova, A. Seiman, **T. Aid,** H. Kolde, J. Kazarjan, and M. Kaljurand, "Automatic Spot Preparation and Image Processing of Paper Microzone-Based Assays for Analysis of Bioactive Compounds in Plant Extracts", *Food Chemistry*, 143, 465−471, 2014, DOI: 10.1016/j.foodchem.2013.08.007.

Introduction

As world energy demands are growing rapidly, reducing the dependence on fossil fuels has become an important challenge. One of the best potential sources of alternative energy is an abundant natural polymer: cellulose. Cellulose is one of the main components present in lignocellulosic biomass, in addition to lignin and hemicellulose.

Lignocellulose is a generic term used to describe non-edible plant biomass, it is the most abundant renewable carbon resource, and it can be obtained as waste from the pulp and paper industry, agriculture (e.g. corn stover, sugar cane bagasse, wheat straw, rise husk etc.) and forestry (different softwood and hardwood parts), among other sources [1–4]. It has been projected that lignocellulosic biomass has the potential to be a largescale, low-cost, and sustainable feedstock for not only renewable fuels but also for use as a starting material, e.g. 5-hydroxymethylfurfural (5-HMF), for the chemical industry, with the aim of producing fine chemicals and biomaterials. In 2002, Rogers et al. reported that ionic liquids, such as 1-butyl-3-methylimidazolium chloride ([BMIm][Cl]), efficiently dissolve up to 25 wt% of cellulose [5]. This finding provided opportunities for novel processing strategies of biopolymers from homogeneous IL solutions, and a new promising method – pretreatment with ionic liquids – is gaining popularity.

The present dissertation is composed of five main sections. First, there is a literature overview on biorefineries, lignocellulosic biomass, pre-treatment methods to reduce lignocellulose resistance to decomposition, methods to obtain valuable platform chemicals from lignocellulose, and analytical methods for carbohydrates and 5-HMF determination. Chapter 2 explains the main goals of the thesis study and Chapter 3 gives an overview of the chemicals, reagents, sample preparation and instruments used. Chapter 4 introduces the results of Publications I-III:

I Pretreatment efficiency of ILs combined with heat for spruce, birch and pine, as well as winter wheat straw, and the results of IL-treated wheat straw resistance to enzymatic hydrolysis;

II Optimization results of the indirect capillary electrophoresis method for a quantitative determination of mono-, di- and oligosaccharides in an IL medium;

III Isomerisation of glucose to fructose, and the conversion of glucose to 5-HMF in [BMIm][Cl] using metal catalysts ($CrCl_3$, $ZnCl_2$ and $MgCl_2$) as well as tungsten and molybdenum oxide-based polyoxometalates (POMs).

And finally, Chapter 5 gives a general overview of the conclusions that were reached.

Abbreviations

1,4-BDO	1,4-butanediol
[BMIm][Cl]	1-butyl-3-methylimidazolium chloride
[BMIm][Cl]	1-butyl-3-methylimidazolium chloride
[BMIm][OAc]	1-butyl-3-methylimidazolium acetate
[C_{14}MIm][Cl]	1-tetradecyl-3-methylImidazolium chloride
[EMIm][OAc]	1-ethyl-3-methylimidazolium acetate
5-HMF	5-hydroxymethylfurfural
AFEX	Ammonia fiber explosion
Ara	Arabinose
BBD	Box–Behnken design
BGE	Background electrolyte
C_4D	Contactless conductivity detection
CE	Capillary electrophoresis
Cel	Cellobiose
cp	Central points
CTAB	Cetyltrimethylammonium bromide
DES	Deep eutectic solvents
DFF	2,5-diformylfuran
DFT	Density functional theory
DMAc/LiC	*N,N*-dimethylacetamide/lithium chloride
DMF	Dimethylfuran
DMSO	Dimethyl sulfoxide
DMTHF	2,5-dimethyltetrahydrofuran
DP3	Cellotriose
DP4	Cellotetraose
DP5	Cellopentaose
DP6	Cellohexaose
EOF	Electroosmotic flow
FDCA	2,5-furandicarboxylic acid
FID	Flame ionization detector
FL	Fluorescence detection
Fru	Fructose
GC	Gas chromatography
Glc	Glucose
HILIC	Hydrophilic interaction liquid chromatography
HMDS	Hexamethyldisilazane
HPLC	High-performance liquid chromatography

ID	Inner diameter
IL	Ionic liquid
ISTD	Internal standard
k	Number of factors
Lac	Lactose
LHW	Liquid hot water
LoD	Limit of detection
LoQ	Limit of quantification
Man	Mannose
MS	Mass spectormetry
NMMO	N-methyl-morpholine-N-oxide
NPLC	Normal phase liquid chromatography
NTAP	Non-thermal atmospheric plasma
PDC	Pyridinedicarboxylic acid
POM-ILs	Polyoxomethalate-based ionic liquids
POMs	Polyoxometalates
Raf	Raffinose
RID	Refractive index detection
RSM	Response surface methodology
RTIL	Room temperature ionic liquid
SEM	Scanning electron microscopy
STD	Standard deviation
Suc	Sucrose
TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl
TSIL	Task specific ionic liquid
UV/Vis	Ultraviolet/visible
Xyl	Xylose
γ-PGA	Poly-γ-glutamic acid

1 Literature overview

1.1 Circular economy aspects

A circular economy increases the proportion of renewable or recyclable resources and reduces the consumption of raw materials and energy, thereby protecting the environment by cutting emissions and minimizing material loss. Currently, eco-design, sharing, reusing, repairing, refurbishing, and recycling of existing products and materials play a significant role in maintaining the utility of sustainable products, components, and materials [6].

The effective conversion of lignin for different valuable products requires a cost-effective technology.

1.2 Lignocellulose biorefinery

A biorefinery is a refinery that produces energy, biomaterials and other beneficial by-products from biomass. In the literature, Kamm et al. and Van Dyne et al. have defined three different types of biorefineries: the phase I biorefinery (i.e. single feedstock, single process and single product), the phase II biorefinery (i.e. single feedstock, multiple processes and multiple products) and the phase III biorefinery (i.e. multiple feedstocks, multiple processes and multiple products) [7, 8].

An example of a phase I biorefinery is a dry mill ethanol plant, which uses raw materials, such as grains or maize, to produce ethanol. An example of a phase II biorefinery is the current wet milling technology, which uses naturally wet biomass, such as green grass, lucerne, clover or immature grain, to produce various end products, such as starch, high fructose corn syrup, ethanol, corn oil and corn gluten feed. Finally, a phase III biorefinery uses various types of feedstock and processing methods to produce a variety of chemicals, fuels and intermediate or end products for the industrial marketplace from naturally dry raw materials, such as cellulose-containing biomass and wastes [7].

The sustainability of the production of fuels and chemicals from biomass has been widely discussed. For example, there are concerns about the sustainability of the current production of bioethanol, which is produced from starch and sugar crops [9]. The limited supply of such edible crops can lead to competition with food production. Lignocellulosic feedstocks have a crucial advantage over other biomass supplies because they are the non-edible parts of plants and, therefore, they do not compete with food supplies. Additionally, forestry, agricultural and agro-industrial lignocellulosic wastes are accumulated every year in large quantities and disposal of these wastes in soil or landfills causes serious environmental problems. However, they could be utilised to produce several value-added products. From the economic point of view, lignocellulosic biomass can be produced quickly and at lower cost than other agriculturally important biofuel feedstocks such as corn starch, sugar cane and soybeans. It is also significantly cheaper price than crude oil [7, 10].

1.3 The chemical composition of lignocellulosic biomass

Lignocellulosic biomass is typically composed of cellulose, hemicellulose and lignin. Hemicellulose surrounds the cellulose fibres and is a linkage between cellulose and lignin, forming a complex lignocellulosic matrix in native biomass which is highly recalcitrant to depolymerisation [11]. Lignocellulosic biomass can be grouped into energy crops (perennial grasses), forest materials (hard wood, softwood, sawdust, prunings and bark

thinning residues), agricultural residues (cereal straws and bagasse), aquatic plants (water hyacinth) and organic portion of municipal solid wastes [12]. Generally, lignocellulosic biomass consists of 35–50% cellulose, 20–35% hemicellulose, 5–30% lignin and 1-10% other substances, such as proteins, oils and ash [10, 13]. These components can be converted into many platform chemicals, that have great market potential. For example, they can be converted into new bio-based chemicals with various applications (biofuels, medicinal and other high value-added compounds) [14, 15].

The cellulose fragment is composed of a linear chain of glucose units linked by β-1,4- glycosidic bonds (*Figure 1*). These chains tightly aggregate into microfibrils (3 to 5 nm in diameter), which are held together via strong intra- and intermolecular hydrogen bonds and van der Waals forces (pyranose ring stacking). The degree of polymerisation of cellulose varies, depending on its source, between 100 and 10,000. Cellulose usually is present as a crystalline form and a small number of non-organised cellulose chains form amorphous cellulose. In amorphous form, cellulose is more susceptible to enzymatic degradation [16, 17].

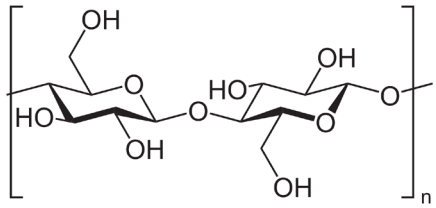

Figure 1. The cellulose fragment, composed of a linear chain of glucose units linked by β-1,4- glycosidic bonds.

Hemicelluloses are complex heteropolysaccharides that can be classified into four structurally distinct classes (*Figure 2*): (a) xylans (β-1,4-xylosyl backbones with arabinose, uronic acid and acetyl side chains), (b) mannans (β-1,4-mannosyl or glucosyl-mannosyl backbones with galactose side chains), (c) β-glucans with mixed linkages (β-1,3-1,4-glucosyl backbones) and (d) xyloglucans (β-1,4-glucosyl backbones with xylose side chains). Unlike cellulose, hemicellulose has a random and amorphous structure and its composition varies depending on cell tissue and plant species and differs in type of glycosidic linkages, side chain compositions and degrees of polymerisation. The most abundant hemicelluloses are glucuronoarabinoxylans and galactoglucomannans [10, 18].

Hemicelluloses differ from each other in composition too; hardwood hemicelluloses contain mostly xylans, whereas softwood hemicelluloses contain mostly glucomannans. Hemicelluloses are embedded in plant cell walls to form a complex network of bonds that provide structural strength by linking cellulose fibres into microfibrils and cross-linking with lignin [10].

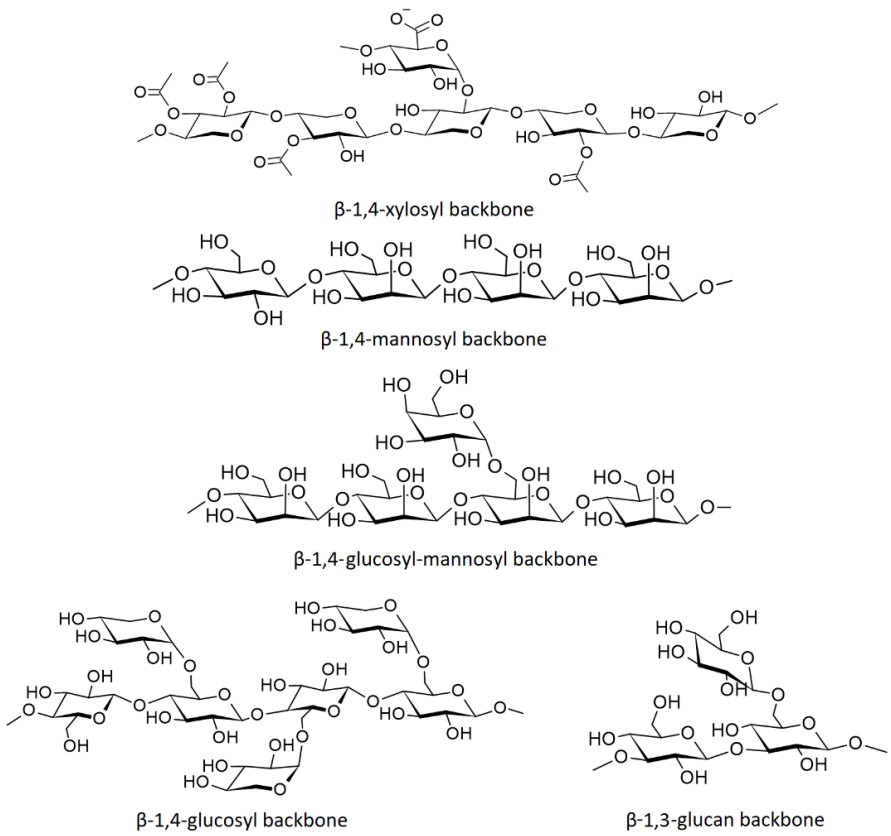

Figure 2. Structure of β-1,4-xylosyl backbone with arabinose, uronic acid and acetyl side chains, β-1,4-mannosyl backbone, β-1,4-glucosyl-mannosyl backbone with galactose side chains, β-1,3-glucosyl backbone and β-1,4-glucosyl backbones with xylose side chains [10, 18, 19].

Lignin is present in the cellular wall to provide a mechanical strength to wood by holding the fibres together and it provides a protective shield against microbial attacks and oxidative stress [9]. Recent studies have focused on the effective utilisation of lignin in many products, such as biosensors, micro- and nanocapsules, biopolymers, platform chemicals, pharmaceutical compounds, electrodes in electrochemistry, re-useable adsorbents and resins. The development of eco-friendly and cost-effective techniques is essential and it can be accomplished through the circular economy approach [20].

Structurally, lignin is an amorphous heteropolymer, non-water soluble and optically inactive; it is composed of randomly branched phenylpropenyl units, e.g. coniferyl alcohol, sinapyl alcohol and coumaryl alcohol (*Figure 3*). The phenylpropenyl units are connected by C–C or C–O–C linkages (more than two-thirds of those units are joined by ether linkages) [21, 22]. The C–C linkages in lignin are the most difficult bonds to break and ether linkages are generally somewhat weaker than C–C linkages. Lignin surrounds and holds together the cellulose and hemicellulose fibres, resulting in biomass structural rigidity and recalcitrance to chemical and enzymatic hydrolysis [21].

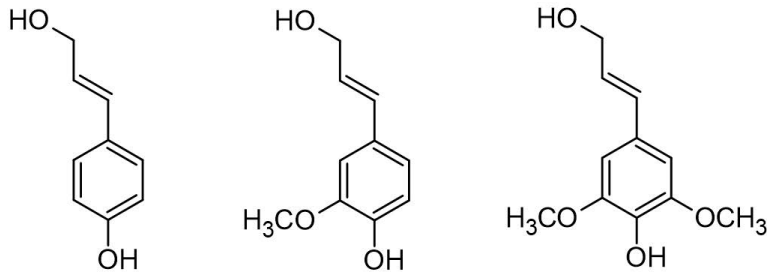

Figure 3. The three phenylpropane units, the building blocks of lignin: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol.

1.4 Pretreatment methods

The general purpose of pretreatment is to break down the complex structures formed by lignin and hemicellulose, disrupt the crystalline structure and reduce the degree of polymerisation of cellulose. Pretreatment has been viewed as one of the most expensive processing steps in the conversion of biomass to fermentable sugars or valuable platform chemicals [4]. Several physical, physicochemical, chemical and biological pretreatment processes have been developed for lignocellulosic biomass decomposition [23].
Biomass hydrolysis and pretreatment solvents can be historically categorised into three generations:

1. First generation: one-step biomass dissolution and hydrolysis with concentrated acids (e.g. sulphuric acid, hydrochloric acid and nitric acid);
2. Second generation: biomass dissolution followed by enzymatic hydrolysis;
3. Third generation: lignocellulose fractionation using cellulose solvents along with other solvents that can dissolve different lignocellulose components (concentrated phosphoric acid (85% (w/w)), ionic liquids (ILs), deep eutectic solvents (DES), NMMO, NaOH/urea and DMAc/LiC) [24].

1.4.1 Physical pretreatment

In physical pretreatment methods, the biomass is mechanically processed to reduce cellulose crystallinity and/or increase the surface area of the material accessible to the reaction via a combination of processes, such as chipping, grinding, or milling [25]. Unfortunately, such processes have high energy demands and that makes them too expensive for large-scale implementation [26]. Physical pretreatment methods also include steam-explosion [27], liquid hot water (LHW) [28], non-thermal atmospheric plasma (NTAP) [29], ultrasound [30] and microwave [31] pre-treatments; in these processes, the lignocellulosic material structure is degraded, and lignin is separated from hemicellulose.

Uncatalysed steam-explosion (also known as autohydrolysis) is the most widely used pretreatment method. It is a cost-effective pretreatment technology that has been advanced to pilot scale demonstration and commercialised application because commercial steam-explosion equipment is available [4]. In this process, the biomass interacts with high-pressure steam at 160 to 260 °C for several minutes and then pressure is quickly reduced, which makes the material undergo an explosive decompression [9].

During pretreatment, the fibre structure is broken and lignocellulose components are partially solubilised. The addition of SO_2, H_2SO_4 or CO_2 in steam explosion has showed good results for breaking the lignin encapsulation, limiting the formation of inhibitory toxic intermediates, and improving hemicellulose removal. The steam explosion method uses about half of the energy of mechanical processing uses, but it is still quite energy intensive [25].

Other methods, such as the ammonia fibre explosion (AFEX) process, are, in principle, like steam explosion, but the yields are relatively lower for lignin-rich biomass. AFEX is also an important pretreatment technology that utilises both chemical (ammonia) and physical (high temperature and pressure) processes to achieve effective results. AFEX promotes cellulose recrystallization, partial hemicellulose depolymerization, reduces the lignin recalcitrance in the treated biomass and increases the surface accessibility for hydrolysis [9].

Thermo-hydrolysis or LHW treatment is another promising method [28, 32, 33]. In LHW pretreatment, pressure is utilized to maintain water in the liquid state at elevated temperatures. Biomass is treated with high temperature in water under high pressure. LHW pretreatment has been reported to have the potential to enhance cellulose digestibility, sugar extraction and pentose recovery, with the advantage of containing little or no inhibitor of sugar fermentation. LHW reduces the need for neutralization of liquid streams and conditioning chemicals since acid is not added [4, 28, 33].

Biomass materials can be comminuted by various grinding, chipping and milling methods. The milling can be further detailed into compression milling, hammer- and ball-milling (wet, dry, vibratory rod/ball milling) [34–36], ball milling/beating, agitation bead milling, pan milling, and other types of milling (fluid energy milling, colloid milling, two roll milling). In addition, attrition [37] and disk refining [38] were also used for pretreatment. Vibratory ball milling was found to be more effective than ordinary ball milling on the improvement of biomass digestibility when used to pretreat spruce and aspen chips. Among all the mechanical comminution techniques, the compression milling is the only process that has been tested in production-scale [4].

1.4.2 Chemical pretreatment

Chemical pretreatments are the most studied pretreatment techniques. They were originally developed for the paper industry for the delignification of cellulosic materials to produce high quality paper products [4]. These pretreatment methods remove lignin and hemicellulose via their reactions with various chemicals, such as dilute acid [39], alkali [40] and organic solvents [41]. In the OrganoSolv process, the internal lignin and hemicellulose bonds are broken down and surface area is increased by pretreatment with ethanol, methanol, ethylene glycol, glycerol, acetic acid or formic acid, with or without catalysts [23, 26]. Acid pretreatment can be conducted either under concentrated acid (e.g. 30–70%) and at low temperatures (e.g. 40 °C), or under diluted acid (e.g. 0.1%) and high temperatures (e.g. 230 °C). Organic and inorganic acids, including H_2SO_4 (the most used), HCl, HNO_3, H_3PO_4, acetic acid and maleic acid, have also been used for acid pretreatment [42].

Alkaline pretreatment is carried out using lower temperatures and pressures than other pretreatment technologies. Alkali pretreatment times are significantly longer: hours or days. Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated. The most used pretreatment agents for alkaline pretreatment are sodium, potassium, calcium and ammonium hydroxides [43].

Another approach is to use biological pretreatment methods (micro-organisms), namely brown, white and soft rot fungi, to produce lignin-degrading enzymes, such as lignin peroxidase, Mn-dependent peroxidise and laccase (monophenol oxidase). This process is advantageous in terms of low energy requirements and involves mild environmental conditions. However, slow kinetics presently limit its application at the industrial level [25].

1.5 Ionic liquids and DES for biomass processing

Ionic liquids were first described in 1914 by Paul Walden and the first reported IL was ethylammonium nitrate [44]. ILs have such internal characteristics as non-flammability, low vapour pressure, high viscosity, high thermal and chemical stability [45] and low conductivity [46]. Owing to their non-volatile and non-flammable properties, they are considered good replacements for the conventional environmentally harmful solvents which are used in catalytic and organic reactions. They also have a wide liquid range, for example [BMIm][Cl] has a melting point of 41 °C and decomposition temperature of 254 °C [47].

Ionic liquids represent chemicals simply defined as organic salts with a melting point below 100 °C. If an IL has a melting point below room temperature, it is often called a room-temperature ionic liquid (RTIL) [48]. The opportunity to pair anions with cations yields an almost endless list of potential ILs. The term “task specific ionic liquid” (TSIL) reveals the nature of this approach. TSILs were introduced as the third generation of ILs, where the required functional group is covalently added to the cationic or anionic part [49].

As early as the 1930s, Graenacher discovered that liquefied quaternary ammonium salts (alone or diluted in suitable solvents) can dissolve cellulose. This was the first successful direct dissolution of cellulose in an organic salt [50]. In the early 2000s, Swatloski et al. reported that [BMIm][Cl] can dissolve cellulose quickly and without derivatisation [51]. Ionic liquid solvents are a promising new approach in the pretreatment of lignocellulosic material because of their ability to dissolve large amounts of cellulose under very mild conditions with almost 100% recovery [52]. Swatloski et al. [51] have studied various ILs, including 1-N-butyl-3-methylimidazolium ($[BMIm]^+$), with different anions and one conclusion of these studies was that the chloride ion, as a small hydrogen bond acceptor, seems to be most appropriate for cellulose dissolution. They also reported that cellulose could be precipitated from an IL solution by the addition of water, or other precipitating solutions, including ethanol and acetone [51].

The cation irregular shapes in ILs result in crystalline structures with significantly lower lattice energies and melting points. The most common cations are imidazolium, tetraalkylammonium, tetraalkylphosphonium, pyridinium and pyrrolidinium. The most common anions are halides, acetate, nitrates, hydrogen sulfate, tetrafluoroborate and hexafluorophospohate [53].

As a result, it is possible to synthesise ionic liquids with specific melting points, densities, viscosities, hydrophilicities and electrical conductivities to be used for certain applications or chemical processes. Imidazolium-based ILs have been the most used ILs for processing wood, lignocellulose and cellulose (*Table 1*) [54].

The impact of the constituting ions on the physicochemical properties of ionic liquids is significant. For instance, [BMIm][Cl] and [EMIm][Cl] are solid at room temperature, while [BMIm][OAc] and [EMIm][OAc] are liquid. [BMIm][BF_4] and [EMIm][BF_4] are liquid at room temperature, while [BnMIm][BF_4] is solid. In terms of viscosity, [BMIm][HSO_4]

has a viscosity of approx. 3100 cP compared to approx. 1500 cP for [EMIm][HSO_4] due to the higher van der Waal forces for longer alkyl chains [54]. In addition, a study of [BMIm] ionic liquids with carboxylates of different chain lengths (formate, acetate, propionate and butyrate) showed that increasing the chain length noticeably increases the viscosity of the ionic liquid [55]. The thermal stability of imidazolium ionic liquids is strongly dependent on the anion. The degradation temperature varies widely, between 200 and 500 °C, and increases in the following order [PF_6] > [BF_4] > [Cl] = [Br] = [I] [56].

Table 1. Summary of the most commonly used imidazolium ILs for wood and cellulose dissolution and fractionation and their physicochemical properties [54].

Ionic Liquid	Melting Temp.(°C)	Density (g/cm3) at 25 °C	Viscosity (cP) at 20–30 °C	Electrical Conduct. (mS/cm) at 25 °C
[BMIm][OAc]	−20	1.1	208	1.4
[BMIm][Cl]	41–70	1.1	Solid	–
[BMIm][Br]	60–81	1.1	Solid	–
[BMIm][I]	−72	1.4–1.5	1110–1183	0.5
[BMIm][HSO_4]	–	1.3	3088	–
[BMIm][BF_4]	−83–74	1.1–1.3	72−233	3.2
[BMIm][PF_6]	11	1.3–1.4	207–450	1.5-4.8
[BMIm][Ace]	30	1.2	800	0.5
[EMIm][OAc]	−45–14	1.0–1.1	91–162	2.5-2.8
[EMIm[Cl]	80–89	1.1–1.2	Solid	–
[EMIm][Br]	65–91	–	Solid	–
[EMIm][I]	79–85	–	Solid	–
[EMIm][HSO_4]	–	1.4	1510	0.5
[EMIm][BF_4]	6–15	1.2–1.4	34–66	13.0-14.1
[EMIm][PF_6]	58–64	1.4	450	5.2
[EMIm][Ace]	34	1.3	556	0.6
[AMIm][Cl]	47	–	–	–
[AMIm][I]	57	–	–	–
[BnMIm][Cl]	75	–	Solid	–
[Bn][BF_4]	78	–	Solid	–
[BnMIm][PF_6]	130–135	–	Solid	–

[BMIm]: 1-butyl-3-methylimidazolium, [EMIm]: 1-ethyl-3-methylimdiazolium, [AMIm] 1-allyl-3-methylimidazolium, [BnMIm]: 1-benzyl-3-methylimidazolium, [OAc]: acetate, [Ace]: acesulfamate, [BF_4]: tetrafluoroborate, [PF_6]: hexafluorophospohate.

The acidity/basicity of imidazolium ionic liquids strongly depends on the acidity/basicity of the anion present. For example, [BMIm][OAc] is considerably more basic than [BMIm][Cl] because the acetate anion is more basic than chloride, while [EMIm][OAc] showed slightly higher basicity than [BMIm][OAc] [57].

Despite the fact that IL pretreatments have been successful for lignocellulose fractionation, the discovery and application of DESs have provided a new approach to lignocellulose fractionation, particularly for lignin extraction. DESs are promising IL

alternatives for biomass fractionation since they have low melting points and low vapour pressure, and they are chemically and thermally stable, non-flammable and highly dissoluble. In addition, DESs are cheaper than ILs, water neutral, low or non-toxic and often biodegradable. DESs, having low viscosity, are desirable considering the economic and technical benefits associated with the downstream processing [58, 59].

1.6 Polyoxometalates for biomass processing

Polyoxometalates are a class of anionic metal-oxygen clusters built by the connection of [MO]x polyhedra of early transition metals in their highest oxidation states [60]. POMs are divided into two categories: isopolyoxometalates (or homopolyoxometalates) and heteropolyoxometalates. The second category is mainly based upon the Keggin structure, which usually is a main group element tetrahedron around which is wrapped an assembly of fused octahedrons [61].

The most studied polyoxo structure-formers are molybdenum (VI) and tungsten (VI), due to the accessibility of empty d-orbitals for metal-oxygen π-bonding and favourable combinations of ionic radius and charge. Polyoxo structures of hexavalent Tc, Re, Ru and Os, pentavalent Cr, Mo, W, Tc and Re, and tetravalent Ti, V, Cr, Mo and W are also well-known and studied [60]. The search for effective catalysts has brought polyoxometalates–anionic metal oxides under consideration because of their unique properties, such as strong Brønsted acidity, good oxidising ability, high water tolerance, low corrosiveness and recoverability. In addition, POMs have exhibited promising performances in the transformation of cellulose into platform chemicals in both homogeneous and heterogeneous systems [62]. A review by Deng et al. [63] highlighted the following good catalytic properties of POMs in the conversion of cellulose to platform chemicals:

1. strong Brønsted acidity;
2. the capability to activate oxidants such as O_2 and H_2O_2 for selective oxidation;
3. high water tolerance;
4. tunable acidity, redox potential and solubility in various media, which allow the rational design of active sites on molecular and atomic scales;
5. high thermal and oxidative stability as compared with common molecular catalysts such as organometallic complexes and enzymes;
6. ease of handling and separation, and the relatively low corrosiveness, possibly owing to the generated corrosion-inhibiting films, which allow them to act as environmentally friendly liquid-phase catalysts, unlike mineral acids.

Another approach is to use TSIL with POMs contained in them. Due to their high negative charge and large metal-oxide framework, POMs can react with a variety of cationic organic groups to form novel functional ILs, polyoxomethalate-based ionic liquids (POM-ILs). IL cations with various structures and properties can provide organic blocks to modify POM catalysts, thanks to their acidity, polarity, solubility, redox properties and surface structures.

Polyoxomethalate-based ionic liquids as solid acid catalysts have been used for the direct conversion of fructose to 5-HMF. The phosphotungstic acid (HPW)-derived IL shows the highest catalytic performance (up to 99% of the yield) in the formation of 5-HMF. In this study, the catalyst afforded a good yield of 5-HMF from inulin (76%) and sucrose (45%) as well [64].

In another study [65], POM-ILs were synthesised and employed for a one-pot dissolution and conversion of powdered switch grass biomass. For comparison purposes,

Avicel Cellulose was also treated under identical conditions. The most promising for biomass conversion was found to be the combination of phosphotungstic acid hydrate and [BMIm][Br]. Avicel Cellulose was then utilised for hydrolysis at 200 °C for 120 min and, as a result, approximately 31 wt% of the biomass and 13 wt% of Avicel Cellulose were converted to water-soluble products, i.e. sugars obtained from the deconstructed cellulose [65].

There are some other situations where heteropolyacids act as efficient catalysts for the conversion of glucose. For example, a 98% conversion of glucose to 5-HMF in ILs and a 99% selectivity of 5-HMF were obtained after a 3 h reaction time at 393 K by using 12-molybdophosphoric acid in a mixture of either [EMIm][Cl] or [BMIm][Cl], with acetonitrile as a co-solvent [66].

1.7 Biomass-derived platform chemicals

Recently there has been a great deal of effort to upgrade raw biomass sugars to fuels other than ethanol. The chemical transformation of biomass into biofuels usually involves catalytic transformation of sugars to fuel components via 5-HMF as an intermediate. *Figure 4* shows possible platform chemicals from C5 and C6 sugars. As shown, 5-HMF can be subsequently upgraded to dimethylfuran (DMF) or diesel-range hydrocarbons. In addition, glucose can be converted to alkanes or to a mixture of light oxygenates and, afterwards, to aromatic gasoline [67, 68]. In addition 5-HMF can be converted to many intermediates, such as 2,5-dimethyltetrahydrofuran (DMTHF), 2,5-diformylfuran (DFF), 2,5-furandicarboxylic acid (FDCA) and levulinic acid. So far, many catalytic systems have been reported to achieve maximum yields of 5-HMF form glucose, and these use various catalysts: mineral and organic acids, salts and zeolites. Conversion is carried out in different solvents: ILs, organic solvents (DMSO and acetone) and bi-phasic solvents [69].

These components can be converted to many platform chemicals that have great market potential, not only as an additive for renewable fuels, but also as a starting material for the chemical industry with the aim of producing fine chemicals, medicine and biomaterials. As shown in *Figure 4*, the main intermediates can be the following compounds: succinic acid, fumaric acid, malic acid, 2,5-furandicarboxylic acid, 3-hydroxypropionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid and glycerol. To that list has recently been added para-xylene, propylene glycol, 1,3-propanediol, lactic acid, isoprene, glycerol, fatty alcohols, 1,3-butadiene, 1,4-butanediol and ethyl lactate [15].

Recently, NatureWorks LLC and BioAmber Inc. started to develop renewable polymers from succinic acid and its derivatives. They formed an alliance (AmberWorks) to investigate the production of completely renewable polyester copolymers of succinic acid and 1,4-butanediol (1,4-BDO). Various polyamides, polyesters and polyester amides can be produced via a condensation reaction of succinic acid or succinic acid diesters with diamines or diols [10].

Another notable intermediate obtained from lignocellulosic biomass is glutamic acid. So far glutamic acid is the most successful intermediate for a commercial polymer: poly-γ-glutamic acid (γ-PGA). γ-PGA is an anionic biopolymer that is formed via gamma-amide linkages [10]. The recent findings of Zhu et al. have shown that the efficient and environmentally friendly production of γ-PGA can be achieved using renewable and low-cost lignocellulosic biomass [70]. γ-PGA is reported to be edible, water-soluble, biocompatible, biodegradable and non-toxic for the environment and

humans. Therefore, γ-PGA and its derivatives have been of interest especially in medicine, food, cosmetics and water treatment industries [71].

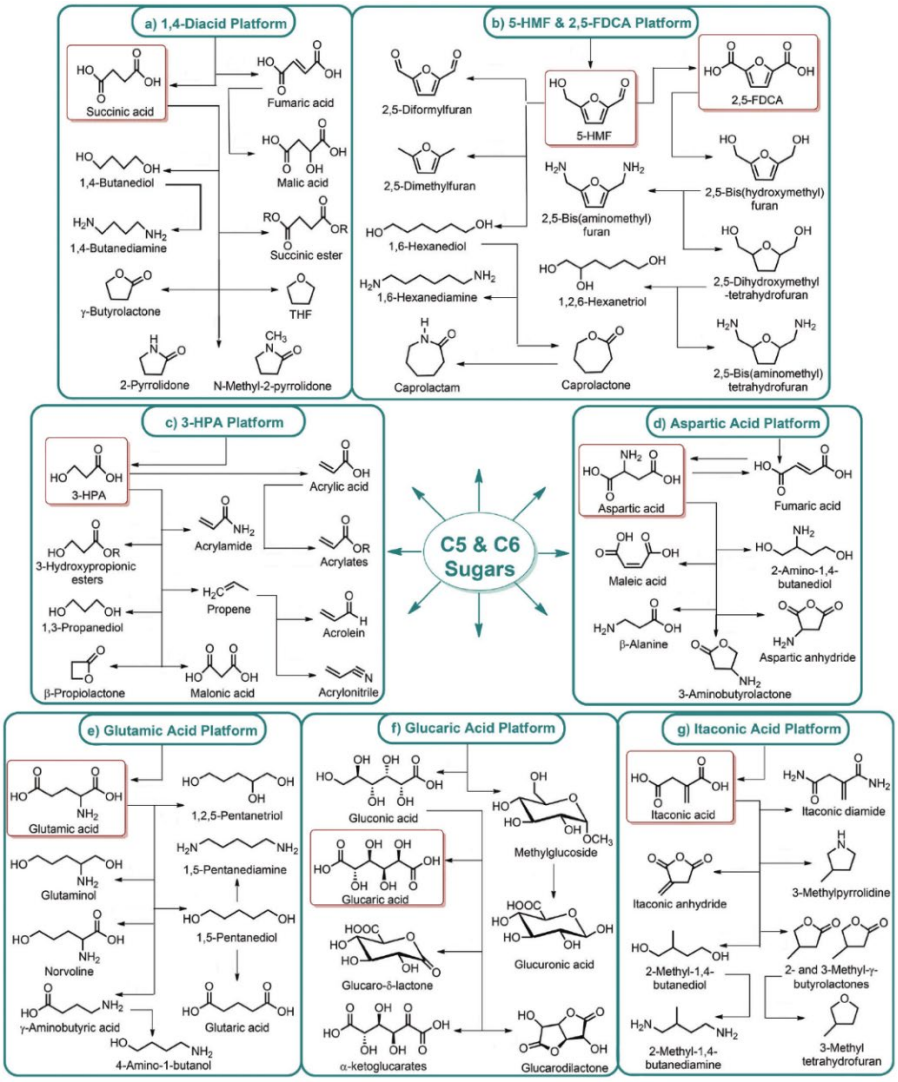

Figure 4. (a) 1,4-diacid, (b) 5-HMF and 2,5-FDCA, (c) 3-HPA, (d) aspartic acid, (e) glutamic acid, (f) glucaric acid and (g) itaconic acid platform chemicals from C5 and C6 sugars [10].

1.8 Determination of carbohydrates

Currently, methods used for carbohydrate analysis include high-performance liquid chromatography (HPLC) [72, 73], gas chromatography (GC) [74] and capillary electrophoresis (CE) (*Table 2*) [75].

Recently, CE methods to quantify neutral carbohydrates by ionisation in strongly alkaline conditions were reported [75, 76]. The poor chromophore properties of carbohydrates present significant challenges to carbohydrate detection. Carbohydrates

can be separated by CE by reaction with borates, after they have been derivatised to make them electrically charged, or in high alkaline (pH above 12) background electrolytes (BGE). Previous CE methods for carbohydrate analysis have used either direct [75] or indirect UV detection [77, 78], contactless conductivity detection (C4D) [79] or complexation with ions, such as borate [80] or copper (II) [81]. Detection with a borate complex, however, suffers from poor sensitivity because detection occurs only at wavelengths close to 190 nm. In addition, copper may induce the formation of supra macromolecular structures with other compounds in biological matrices and leads to poor sensitivity as well [82].

There are a number of chromophores that can be used for indirect detection by CE, including riboflavin and sorbic acid. In one report, Soga and Heiger described a CE method for the simultaneous determination of underivatised amino sugars, sugar alcohols, mono- and disaccharides by indirect UV detection in the presence of 2,6-pyridinedicarboxylic acid (PDC) as the chromophore. A highly alkaline pH (pH 12.1) condition was used to ionise the carbohydrates, and reversed EOF was achieved by adding cetyltrimethylammonium bromide (CTAB) to the BGE. CTAB reversed the EOF from cathodic to anodic by adsorbing to the silanol groups and consequently changing the sign of the zeta potential from negative to positive [83].

Table 2. HPLC, GC and CE experimental conditions for carbohydrate analysis.

Method	Experimental setup	Detection	Other details
HPLC	Normal phase HILIC Reversed-phase Anion-exchange	Refractive index detection (RID) Mass spectrometry (MS) UV/Vis Fluorescence	UV or fluorescence detection requires pre-column derivatization. Max IL conc: 18 000 ppm [84]
GC	Column oven temp: 80 °C	Mass spectrometry (MS) Flame ionisation detector (FID)	Carbohydrates are derivatised by silylation or acetylation before analysis. Max IL conc: 50 ppm [85]
CE	pH 12-13	Indirect UV detection Direct UV detection Electrochemical detection	Max IL conc: 69 000 ppm

The direct UV detection of carbohydrates at 270 nm was initially proposed to be due to an enediolate formation [86]. This mechanism was disproved later by Sarazin et al. because it does not explain the detection of sucrose. They reported that direct UV detection of carbohydrates at 270 nm is made possible instead by a photo-oxidation reaction [87]. In the report, glucose, rhamnose and xylose were shown to have unique UV absorption spectra, leading to the hypothesis of different UV absorbing intermediates for their respective photo-oxidation. This statement was later confirmed in a study by Oliver et al, where they gathered data for glucose, rhamnose and xylose photo-oxidation products by NMR spectroscopy [82]. Capillary electrophoresis with direct UV detection has been shown to be a reliable and robust technique for the analysis of carbohydrates in fruit juices [75], alcoholic beverages, dairy products [88], plant fibre [89], pharmaceuticals [90] and rat brains [91].

Because of the lack of effective chromophores or fluorophores in the structure of carbohydrates, the application of HPLC is greatly limited. The most popular modes of HPLC employed for underivatised carbohydrate analysis are normal phase (NPLC) [92], hydrophilic interaction liquid chromatography (HILIC) [93], reversed-phase or anion-exchange modes with refractive index detection (RID) or mass spectrometry (MS) [73], [94], and UV [95] or fluorescence detection (FL) after pre-column derivatisation [72]. Carbohydrates can be separated with chromatography under the following conditions:

1. anion-exchange column (water containing bases or salts as the eluents),
2. cation-exchange column (water as the eluent),
3. alkyl-bonded silica gel column (water as the eluent),
4. amine-bonded silica gel column (water–acetonitrile as the eluent) [73].

The most used column for carbohydrate separation is an amine-bonded silica gel column [92], but the separation is not always quantitative due to the possible interaction between reducing carbohydrates and an amino group [73]. Besides, specialised carbohydrate columns in combination with RID are often used in the direct HPLC analysis of monosaccharides. However, RID is not as common as a UV or FL detector because the sensitivity is usually not satisfactory.

In GC, the most common derivatives used for carbohydrate determination are methyl ethers, acetates, trifluoroacetates and trimethylsilyl ethers. The good volatility and stability characteristics of the derivatives formed make trimethylsilyl (TMS) ethers the most popular derivatives applied to the GC analysis of carbohydrates and polyalcohols. Silylation generally consists of the use of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) as derivatisating agents and pyridine as a solvent. Currently, there are several derivatisation methods for carbohydrates with one or two steps, which can be satisfactorily carried out. However, the large number of chromatographic peaks per carbohydrate is still mentioned as a major problem and different methods have been developed to reduce this effect [96, 97].

1.8.1 Statistical optimisation of separation conditions

Traditionally, optimisation in analytical chemistry has been performed using the technique called one-variable-at-a-time, meaning that one factor is changed at a time and the other parameters are held constant. However, one-factor optimisation has several disadvantages: it cannot estimate interactions between factors, and it requires more runs for complete optimisation (therefore more reagents and materials are needed). To overcome this problem, the optimisation of analytical procedures has been carried out by using multivariate statistical techniques. Among the most relevant multivariate techniques used in analytical optimisation is response surface methodology (RSM). RSM is an effective statistical and mathematical technique to investigate and/or optimise complex processes using a minimum number of experiments [98].

It is difficult to optimise CE separation conditions, since the wide array of factors, such as temperature, capillary length, buffer composition, applied voltage, ionic strength and injection time, can influence the resolution, separation efficiency and migration time. Box–Behnken design (BBD) is a RSM tool that has been widely used for the optimisation of experimental trials and it can also be used to optimise CE separation conditions [99].

In BBD, the experimental points are located on a hypersphere equidistant from the central point, as exemplified for a three-factor design in *Figure 5*. Its principal characteristics are:

1. it requires an experiment number according to $N=2k(k-1) + cp$, where k is the number of factors and (cp) is the number of the central points;

2. all factor levels have to be adjusted only at three levels (−1, 0, +1), with equally spaced intervals between these levels [98].

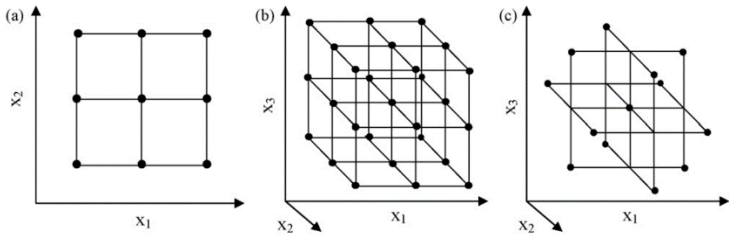

Figure 5. Experimental designs based on the study of all variables on three levels: three-level factorial design for the optimisation of (a) two variables and (b) three variables and (c) BBD for the optimisation of three variables [98].

2 Aims of the study

The aims were to investigate ILs' impact on biomass pretreatment, thereby reducing biomass recalcitrance to enzymatic hydrolysis, to study POMs as catalysts in cellulose degradation, and to develop a precise and fast CE method for carbohydrate determination in an IL medium. The specific aims are the following:

- To study the pretreatment efficiency of ILs combined with heat for lignocellulosic biomass consisting of spruce, birch and pine, as well as winter wheat straw. Also, to investigate IL-treated wheat straw resistance to enzymatic hydrolysis
- The second aim was to develop an indirect capillary electrophoresis method for a quantitative determination of mono-, di- and oligosaccharides to investigate biomass degradation, the isomerisation of glucose into fructose and the conversion of fructose to 5-HMF in ILs.
- The third aim was to research the degradation of commercial cellulose, the isomerisation of glucose to fructose, and the conversion of glucose to 5-HMF in [BMIm][Cl], in the presence of metal catalysts ($CrCl_3$, $ZnCl_2$ and $MgCl_2$), as well as tungsten and molybdenum oxide-based POMs.

3 Experimental

3.1 Reagents and samples

3.1.1 Lignocellulosic biomass samples

The lignocellulosic biomass samples in **Publication I**, Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*), were obtained from Viitasaari, Finland. The samples were ground and sieved to sawdust with a particle size of 1–2 mm. Some Norway spruce was received from Örnsköldsvik, Sweden as sawdust and it contained some larger particles (around 5 mm). The Finnish wood samples were collected at the common sampling height, i.e. 1.5–2 m. The ages of the trees were between 20 and 25 years, based on the year-ring calculation. Sapwood and heartwood were separated and frozen, and the samples were stored in a freezer in separate packages between −18 and −20 °C. The Swedish spruce samples originated from Örnköldsvik's biorefinery cluster (Aditya Birla Domsjö AB) and, being industrial raw material, it also contained some residual bark and knots. The woody biomass sawdust samples (Norway spruce, Scots pine and silver birch) were treated at 100 °C in [EMIm][Cl]; the wood amount in IL was 50 wt%. The wood samples were heated in an oil bath on a magnetic hot plate with continuous mixing. The samples were collected between 9.5–100 h and this experiment was performed in duplicate. Winter wheat straw (Triticum aestivum) was obtained from a local farm in Pärnu-Jaagupi, Estonia. The straw was ground (particle size 0.6–5 mm) and dried at 96 °C to a constant weight before use. The straw was treated with [BMIm][OAc] and [EMIm][Cl] (the amount of straw was 10 wt%) and heating was carried out using a Sanyo Sterilizer MOV-112S thermostat at 100 and 110 °C, without continuous mixing (it was stirred periodically) and samples were collected at fixed time intervals (4, 24, 48 and 72 h) and this experiment was performed in duplicate. Collected samples were washed with deionised water three times and used for enzymatic hydrolysis. Enzymatic hydrolysis for IL-treated wheat straw was performed in a citrate buffer (pH 5) at 50 °C for 24 h, using the enzymes Celluclast 1.5 L and Novozym 188 from Novozymes A/S (Denmark). In the experiment, 0.51 mg of Novozym 188 and 1.02 mg of Celluclast 1.5 L were added to 200 mg substrate (the appropriate amount of enzymes were calculated based on enzyme activity measured with a filter paper assay).

3.1.2 Applied ionic liquids and sample preparations

The biomass samples in **Publication I** were treated with [BMIm][OAc] and [EMIm][Cl]. The [BMIm][OAc] and [EMIm][Cl] were purchased from Sigma Aldrich and Merck (assay 98%) and used as received. Prior to the IL treatments of any samples, no water was added. It is well known that ILs are extremely hygroscopic but, despite that, a glove box was not used when handling the ILs: the idea was to see whether the simplest possible, cost-effective method (which might be attractive to industry) could be used. In addition, the presence of water in many ILs – up to a certain limit (ca. 1–4% has been reported [100]) – is known to improve the dissolving, degradation and fibrillation ability of an IL. The uptake of moisture from the air was not prevented, but the weighing and handling of the ILs (particularly the very hygroscopic [EMIm][Cl]) was carried out as fast as possible.

All of the experiments in **Publication III** were carried out under aerobic conditions using dried ionic liquids; however, the IL was handled and weighed as quickly as possible to prevent further absorption of water. All of the catalysts used were in their hydrated forms. The catalysts $CrCl_3$, $ZnCl_2$, $MgCl_2$, phosphotungstic acid hydrate, phosphomolybdic

acid hydrate and microcrystalline cellulose were obtained from Sigma-Aldrich (Sigma-Aldrich, Steinheim, Germany) and were used as received. [BMIm][Cl] was obtained from IoLiTec (Ionic Liquids Technologies GmbH, Heilbronn, Germany) and was vacuum-dried before use. The IL water content, which was checked by Karl–Fischer titration, was 0.6–0.7%. The catalyst loading was 10 wt% of the substrate in all cases, the substrate loading was 10 wt% from the solvent unless otherwise stated, and the sample typically contained 1 g of IL. The experiments were carried out on a magnetic stirrer plate at 100 or 120 °C in open flasks with constant stirring. First the catalyst was dissolved in IL and after 20 min the cellulose (substrate) was added to the reaction mixture. Samples to compare different catalysts were collected at 24 h after the addition of cellulose. Unusually, a long reaction time (considering the speed of the $CrCl_3$ catalyst) was chosen because preliminary testing showed a slow conversion for molybdenum and tungsten oxide-based POMs.

In 2013, Abia, J. A. and Ozer, R. [65] demonstrated that POM-IL can be used for biomass degradation in 2h at 200 °C to obtain glucose and xylose. In **Publication III**, we tried using POM-IL in a catalytic amount (10 wt%) in [BMIm][Cl] at 100 °C to degrade commercial cellulose to glucose; some glucose was epimerised to mannose and some was converted to 5-HMF.

3.2 Experimental setup

3.2.1 CE with UV/Vis detection

CE separations in **Publications I-III** were performed using an Agilent 3D instrument equipped with a diode array UV/Vis detector.

The electrophoretic conditions in **Publication I** were as follows: uncoated fused silica capillary, 71.5 or 81.5 cm effective length, ID of 50 µm, temperature of the capillary: 15 ± 0.5 °C; applied voltage: 17 kV; hydrodynamic sample injection: 35 mbar for 10 s; detection wavelength: 270 nm. The BGE used in the experiment was 130 mM NaOH, containing 36 mM Na_2HPO_4. For quantification, stock solutions of 50 mM in deionised water for each sugar standard and furfurals were prepared. Working standard solutions within an appropriate range of concentration were prepared by diluting the stock solution with deionised water. The sample preparation for CE analysis was restricted to diluting the supernatant to the appropriate concentration. All of the standards and samples were analysed three times for accurate quantification. For the identification of the compounds in the sample, a mixture of the following analytes was used: furfural, 5-HMF, sucrose (Suc, used as an internal standard, ISTD), D-(+)-cellobiose (Cel), D-(+)-galactose (Gal), D-(+)-glucose (Glc), D-(+)-mannose (Man), arabinose (Ara) and D-(+)-xylose (Xyl) (all mentioned in their CE migration time order). All of the standards used for analysis were purchased from Sigma Aldrich with purity >98%.

The optimisation procedure in **Publication II** was performed employing a fused silica capillary with semi-permanent coating with 1-tetradecyl-3-methylImidazolium chloride ($[C_{14}MIm][Cl]$), which was added to the BGE, 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. The critical micelle concentration for [C_{14}MIm][Cl] in water is 3 mM and therefore the existence of micelles was evaluated. Furfural (a potential degradation product of cellulose) was used as a micellar marker since it is neutral at used conditions. The separation of furfural was not achieved by using 5 mM of [C_{14}MIm][Cl] containing BGE and at least 10 mM of [C_{14}MIm][Cl] was needed for strongly alkaline BGE (pH 12.7). A comparison with a different surface-coating agent – hexadimethrine bromide (HDMB) – was performed. According to the obtained results, the average efficiency was 20% higher for [C_{14}MIm][Cl] containing BGE, and HDMB was not used again in this study.

All of the samples in **Publications II and III** were analysed by CE and the yields were calculated based on calibration curves constructed using authentic standards. The separation was performed using a fused silica capillary with an effective length of 61.5 cm (a total length of 70 cm) and an ID of 22.5 µm (Polymicro Technologies Inc., Wilmington, DE, USA). Capillary walls contained a semi-permanent coating with [C_{14}MIm][Cl] that was added to the BGE. The BGE was composed of 138 mM NaOH, 40 mM maleic acid and 5 mM C_{14}MImCl. The samples were injected hydrodynamically under a pressure of 50 mbar for 20 s and the separations were performed at 25 °C by using a voltage of −21 kV. The detection wavelength was 210 nm in the case of carbohydrates and 270 nm for 5-HMF. Before each run, the capillary was filled with BGE for 7 min and between the runs it was flushed with 1 M NaOH for 2 min and ultrapure water for 3 min. BGE was prepared on the first day and stored at room temperature for one month. The standards used to construct calibration curves and BGE substances, namely D-(+)-glucose, D-(−)-fructose, D-(+)-mannose, D-(+)-cellobiose, sucrose, 5-HMF, maleic acid and NaOH, were obtained from Sigma-Aldrich and were used as received. [C_{14}MIm][Cl] was obtained from IoLiTec and was vacuum-dried before use.

Standards, samples and BGE compounds in **Publication II**, namely 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, HDMB 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.

Scanning electron microscopy (SEM) samples in **Publication I** were prepared as follows: wheat straw and commercial cellulose (from Sigma Aldrich) were treated with [BMIm][OAc], at 100 °C for 1, 6 and 24 h, to investigate the physical changes in structure. The treated material was washed with deionised water, the solvent was replaced with acetone, the material was dried with CO_2 and SEM images were recorded.

3.3 BGE optimisation and validation (Publication II)

Background electrolyte conditions were optimised statistically using a BBD with RS methodology, and the concentrations of compounds were varied as follows: NaOH from 70 to 140 mM, maleic acid from 10 to 40 mM, phthalic acid from 10 to 30 mM, PDC from 10 to 20 mM, and [C14MIm][Cl] from 1 to 5 mM; the voltage was changed from −15 to −30 kV. The carbohydrates xylose, fructose, glucose, galactose, cellobiose, lactose, sucrose and raffinose were used for separation.

The parameters of 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 signal-to-noise ratios of 3 and 10, respectively. The linearity of calibration curves for each analyte were 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 determined by measuring the concentration of the control sample containing 2 mM analytes in five replicates for one day. Inter-day precision was calculated over a 4-day observation period for four replicates.

3.4 Software

Data acquisition and instrument control were carried out using HP 3D Chemstation software from Agilent Technologies. All of the experimental data were analysed using Microsoft Office Excel 2007 (Microsoft Corporation) and the BBD were created with JMP 12.0 (S.A.S Institute Inc., USA).

4 Results and discussion

4.1 IL treatment of lignocellulosic biomass samples (Publication I)

4.1.1 Wood samples

First the samples in **Publication I** were exposed to a [EMIm][Cl] treatment at 100 °C for various time periods. *Figure 6* demonstrates the obtained monosaccharides and their degradation products mmol/L per wood hydrolysate (i.e. the dissolved part of the sample, containing IL and wood components dissolved in the IL) in the sawdust of Norway spruce heartwood and sapwood samples. The concentrations of analytes were 5-HMF, cellobiose, galactose, glucose, mannose, arabinose and xylose. A few unknown compounds could sometimes be seen but could not be identified here. We anticipate that those unknown compounds were either sugar alcohols or uronic acids, galacturonic acid, glucuronic acid or 4-O-methylglucuronic acid [101].

In general, the treatment times and the wood-to-IL ratios were not similar (values were calculated against wood hydrolysate, i.e. the liquid part of the sample where analytes are dissolved in IL) and an interesting trend was observed when comparing heartwood and sapwood. The 5-HMF content seemed to increase more steadily in the treatment times in sapwood samples than with heartwood samples. Galactose and xylose were also more evenly present during the process in heartwood samples than in sapwood samples. The amounts of cellobiose and glucose, together with arabinose, varied in both wood parts. In the case of spruce sapwood, a longer treatment time (100h) resulted in the formation of undetermined compounds that disrupted the CE analysis.

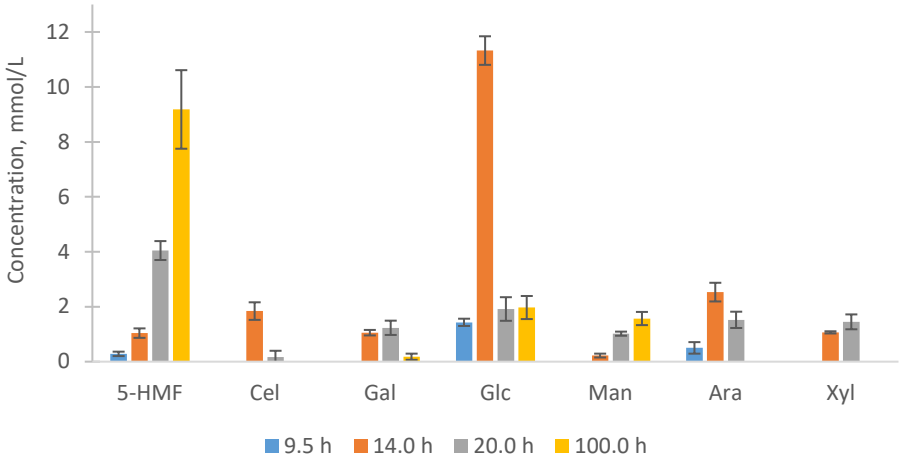

Figure 6. Detected analyte concentrations in [EMIm][Cl]-treated spruce sapwood samples.

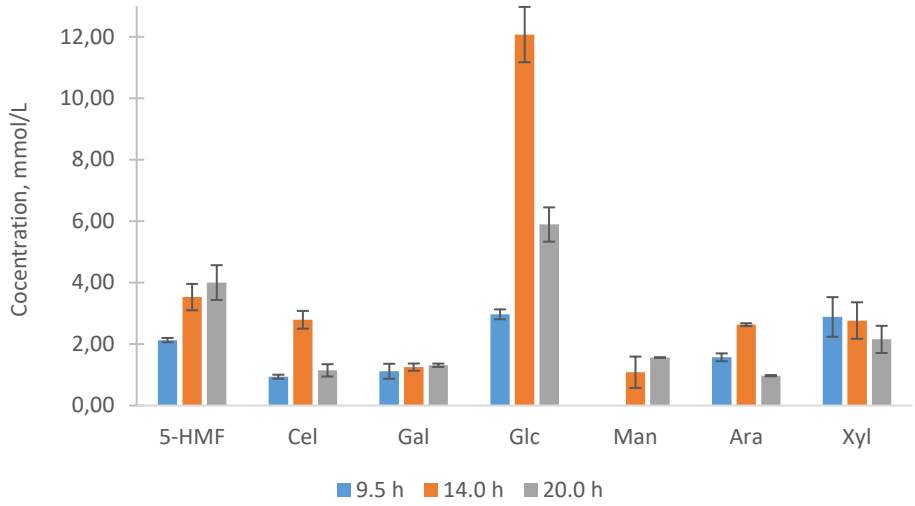

Figure 7. Detected analyte concentrations in [EMIm][Cl]-treated spruce heartwood samples.

Figures 6 and *7* present the analyte concentrations in mmol/L per wood hydrolysate for the fresh wood samples of Norway spruce. These samples were of industrial origin and contained heartwood and sapwood, with some bark and knots. Furthermore, the particle size was different (up to ca. 5 <mm). Heartwood and sapwood have different carbohydrate contents, as do bark and knots. In addition, knots are also known to be rich in antioxidative lignans, flavonoids and stilbenes. Bark contains stilbenes and various polyphenols, e.g. tannins, and is rich in mono- and disaccharides [102]. Considering spruce hemicelluloses, heartwood contains more glucuronoxylan and pectins than sapwood, which should be manifested by an increase in arabinose, xylose, and 4-O-methyl-glucuronic acid amounts among non-cellulosic saccharides. Glucuronoxylans are found in hardwood species in amounts ranging from 15 to 30% of the number of hemicelluloses [103]. Based on this, the main differences can be explained. The content of the known degradation product of hexoses, 5-HMF, increased dramatically with treatment time, as expected. Also, some degradation products of 5-HMF, such as levulinic acid and γ-valerolactone, formed [104].

A comparison of treated Norway spruce and Scots pine sawdust (*Figures 7* and *8*) showed that a wider range of monomeric sugars were detected and the obtained amount of 5-HMF was much less with the 20-hour-treatment, as expected. The shorter treatment time gave higher amounts of glucose, as well as cellobiose, arabinose and xylose. In fact, with longer processing times, these sugars could be transformed to 5-HMF. Sapwood is known to be a richer source of mono- and disaccharides than heartwood and this was confirmed in this study. The IL-treated samples of Scots pine and Norway spruce clearly gave rise to 5-HMF, glucose and galactose. Also, the amount of 5-HMF detected in the spruce samples was much higher since it contains more glucose. Still, treated spruce samples exposed for long treatment times also gave rise to mannose, while pine samples gave rise to cellobiose.

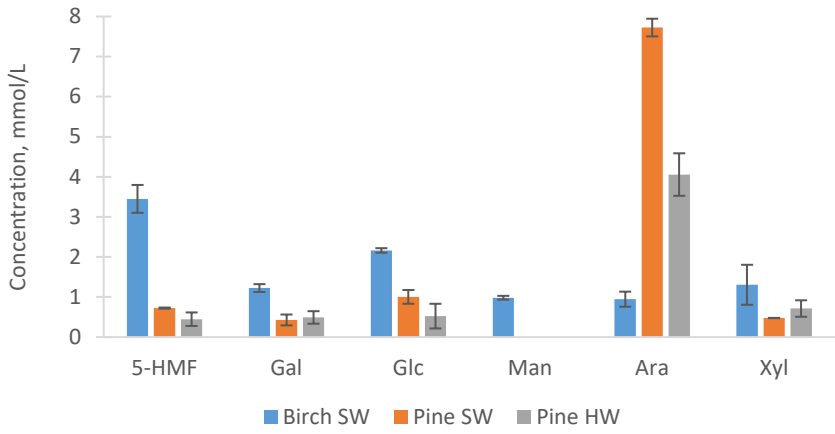

Figure 8. Detected analyte concentrations for [EMIm][Cl] at 100 °C treated birch sapwood (SW, treated for 20h), pine sapwood (treated for 6.5 h) and pine heartwood (HW, treated for 9.5h) samples.

Comparing Norway spruce and Scots pine sapwood sawdust samples (9.5 h treatment time in [EMIm][Cl], at 100 °C (*Figures 7* and *8*), it can be seen that the obtained amounts of 5-HMF, galactose, glucose and xylose were significantly lower with Scots pine than with Norway spruce. However, the amount of arabinose released from Scots pine was clearly higher than from Norway spruce. Softwood is known to contain arabinoglucuronoxylans (5–10% of the hemicellulose yield) at a molar ratio of 10:2:1.3 (Xyl:4-O-MeGlcA:Ara) [103]. Strangely, the spruce samples studied in this work did not release as much xylose and arabinose as the pine samples did. Furthermore, the arabinose content was higher than that of xylose. Arabinogalactans, another type of hemicellulose, are also sources of arabinose, but they are mainly present among hardwood hemicelluloses (especially in the heartwood of the Siberian larch) [103]. The silver birch sawdust samples were treated in a similar way (*Figure 8*). The following mono- and dimeric saccharides were identified: cellobiose (in trace amounts), galactose, glucose, mannose, arabinose and xylose. Furthermore, significant amounts of 5-HMF formed. Some birch samples also contained relatively high amounts of furfural (a known degradation product of pentoses, e.g. that of xylose) after 17 h of [EMIm][Cl] treatment, and some birch samples also produced small amounts of furfural, even after 5 or 6 h of treatment time. Some electropherograms of treated birch also displayed a minor peak immediately on the right side of the furfural peak, this likely being a furfural-related product, for example furfuranol.

The CE method used produced good reducibility and appropriately diluted samples, resulting in low standard deviations (STD <10%). Some samples produced unexpectedly high STDs. Its well known that wood biomass can easily vary about 10% in its composition, depending on, for example, the growing place. Also, the properties and chemical compositions in different parts of stem wood vary a lot. Even though the samples were mostly sapwood and heartwood, the trees processed were quite young. In fact, in young trees, the transition part between the sap- and heartwood is more difficult to identify, and this might be one possible explanation for the STDs being higher than usual.

4.1.2 Wheat straw samples

The wheat straw was pretreated in ILs using conventional heating. After treatment, an IL fraction was removed and analysed with CE. The samples had to be diluted to obtain an appropriate IL concentration for CE analysis (max. 400 mM, 70 ppm) and thus all of the sugars obtained remained under the detection limit. Therefore, the efficiency of the IL treatments was evaluated by determining the sugar contents after the enzymatic hydrolysis step. The main sugars detected were glucose, xylose, arabinose, mannose and cellobiose.

With the use of [EMIm][OAc] IL, higher glucose and xylose concentrations were obtained. In the case of [BMIm][OAc], a plateau was achieved in glucose content after 24 h, while with [EMIm][Cl] no plateau was achieved even after 72 h (see *Figures 9* and *10*). The highest glucose concentrations obtained, 86.17 mmol/L with [BMIm][OAc] and 52.89 mmol/L with [EMIm][Cl], were obtained at 110 °C and 72 h of treatment time. The highest xylose content with the [BMIm][OAc]-treated sample was achieved at 100 °C and 24 h (42.67 mmol/L), while with the [EMIm][Cl] treatment 110 °C and 72 h gave the best result (15.80 mmol/L). The maximum xylose content obtained with pretreatments with acetate-based ILs was 39.40 mmol/L, at 110 °C and 24 h, while prolonged exposure times led to decreasing concentrations. This was probably due to more efficient degradation of hemicelluloses with longer pretreatment times.

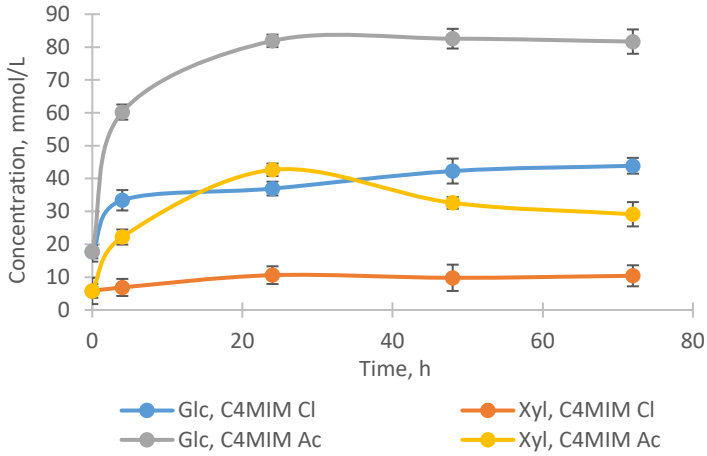

Figure 9. Dynamics of the saccharification process for IL-pretreated wheat straw samples at 100 °C.

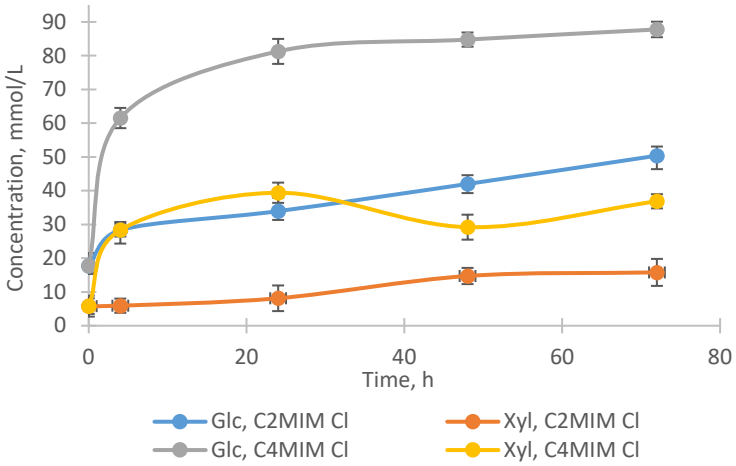

Figure 10. Dynamics of the saccharification process for IL-pretreated wheat straw samples at 110 °C.

4.1.3 IL-pretreated cellulose SEM analysis

SEM images (*Figure 11*) were recorded to visualise the physical changes in the IL-pretreated wheat straw and cellulose. Wheat straw displayed a well-ordered structure after 1 h contact with an IL. Longer treatment times resulted in the wheat straw structure losing some of the fibres, the structure starting to lose its well-organised form and noticeable and homogeneous swelling. Swollen biomass has a larger internal surface area, resulting in better enzyme accessibility and increased binding sites for hydrolysis [105]. The cellulose remained unaffected for 24 h in contrast to the wheat straw. Structural changes in the wheat straw can be explained by its hemicellulose and lignin content, in addition to cellulose.

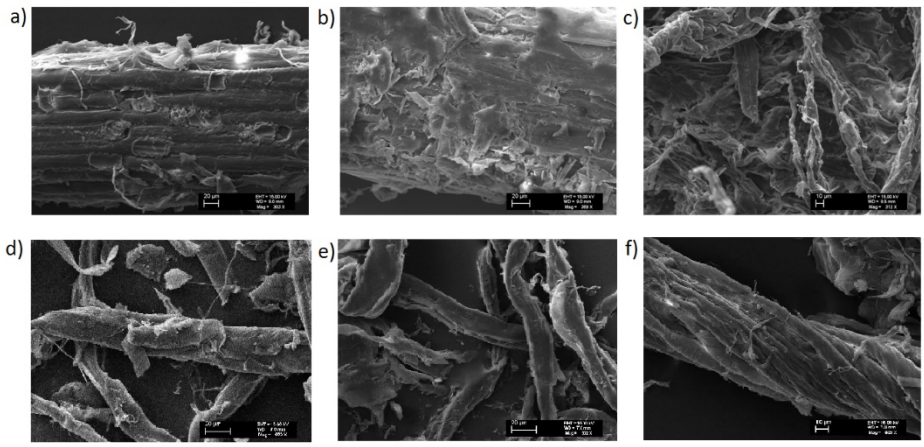

Figure 11. SEM images of [BMIm][OAc]-treated wheat straw samples (at 100 °C) after a) 1, b) 6 and c) 24 h and cellulose samples after d) 1, e) 6 and f) 24h.

4.2 Optimization of the carbohydrate separation method by CE (Publication II)

4.2.1 Capillary choice for noise reduction

Carbohydrates have pKa values between 12 and 13, therefore requiring a high pH of the separation electrolyte. The conductivity of the medium increases with pH, resulting in high electrophoretic current; preliminary measurements with a 50 µm capillary showed that an increase in NaOH concentration caused a low signal-to-noise ratio and unstable noisy baseline (*Figure 12*). The NaOH content was increased with a fixed amount (20 mM in each analysis) and rising electrical current (at 100 mM NaOH concentration about 108 µA), resulting in an unstable and noisy baseline that became too disruptive before the NaOH concentration necessary for complete separation was achieved. After decreasing the capillary ID to 22.5 µm, the baseline became noticeably stable during the analysis; the electrical current was reduced (at 140 mM NaOH concentration of about 27 µA) and the signal-to-noise ratio increased significantly.

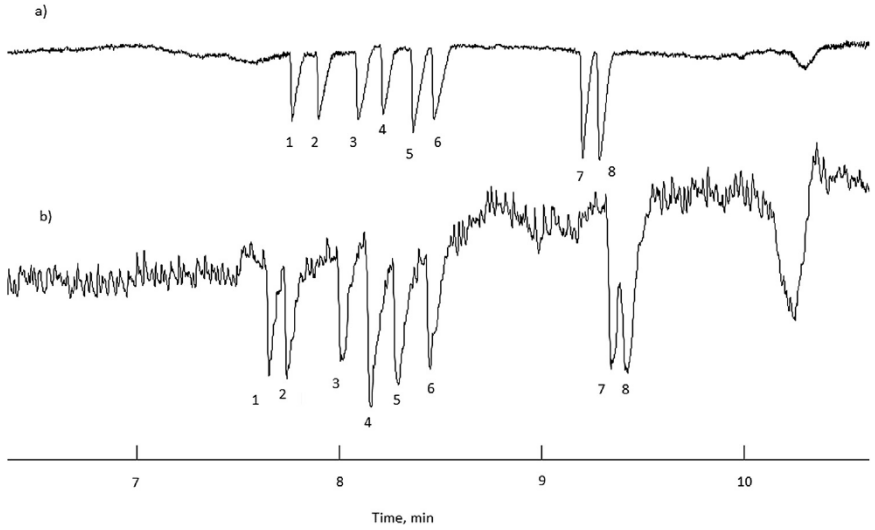

Figure 12. Electropherogram of eight carbohydrates analysed with (a) ID 22.5 µm and (b) ID 50 µm capillary. The CE conditions were the following: a) BGE contained 138.2 mM NaOH 40 mM maleic acid and 5 mM [C_{14}MIm][Cl]; b) BGE contained 100 mM NaOH, 40 mM maleic acid and 5 mM [C_{14}MIm][Cl]; absorbance was measured at 210 nm; capillary length 70/61.5 cm. Identification: 1—Xyl, 2—Fru, 3—Glu, 4—Gal, 5—Cel, 6—Lac, 7—Raf, 8—Suc (analyte concentration: 2 mM of each).

4.2.2 Optimisation of separation conditions

In recent years, chemometric tools have become more and more popular for use in the optimisation of analytical methods due to several advantages, such as the small number of experiments and hence lower consumption of reagents, and less laboratory work. Therefore, in **Publication II**, three BGE conditions were optimised statistically using a BBD with RS methodology. The matrix of the experiments and analysis of the experimental results for optimisation were performed using JMP 12 software. The concentrations of compounds were varied as follows: NaOH from 70 to 140 mM, maleic acid from 10 to 40 mM, phthalic acid from 10 to 30 mM, PDC from 10 to 20 mM, and [C_{14}MIm][Cl] from 1 to 5 mM, while the voltage was changed from -15 to -30 kV. The carbohydrates xylose, fructose, glucose, galactose, cellobiose, lactose, sucrose and raffinose were used for

separation. Optimisation was performed employing a desirability function that was set to maximise resolutions between carbohydrates and minimise the baseline noise. Baseline noise was chosen as a variable since preliminary tests showed that an increase in NaOH and/or surfactant concentration adversely affected this factor.

The predicted conditions for optimal carbohydrate separation for different chromophores are presented in *Table 3*. In all cases, the optimal chromophore content appeared to be the given upper limit, and therefore the solubility of a substance determined the limits of phthalic acid and PDC. The separation of two organic acids, eight carbohydrates and 5-HMF was performed within 12 to 15 min depending on the BGE used and the fastest separation was achieved with maleic acid BGE.

Table 3. Optimised conditions for PDC, maleic acid and phthalic acid BGEs.

Chromophore	NaOH (mM)	Chromophore (mM)	[C_{14}MIm][Cl] (mM)	Voltage (kV)
Maleic acid	138.2	40.0	5.0	−21.7
Phthalic acid	140.0	28.0	4.0	−20.6
PDC	110.7	20.0	1.8	−21.2

The 3D response surface plots for PDC and maleic acid BGEs are presented in *Figure 13* (3D response surface plots for phthalic acid BGE are shown in *Figure 14*). In *Figure 13*, the relationship between concentrations of NaOH, [C_{14}MIm][Cl] and chromophore, and the baseline noise are depicted. In *Figure 13a*, the relationship between the baseline noise and the concentrations of NaOH and [C_{14}MIm][Cl] is shown. The graph shows that the increase in NaOH concentration resulted in a decrease in the signal-to-noise ratio. At the same time, an increase in surfactant concentration resulted in a stabilising effect on the baseline. In *Figure 13b*, the relationship between the concentrations of maleic acid and [C_{14}MIm][Cl] concentration and the baseline noise is shown. The results show that with increasing surfactant concentration the baseline noise increased in the case of low chromophore concentrations. Generally, the disruptive noise caused by high NaOH concentration, which is essential to achieve good resolution of analytes, can be reduced by increasing the concentrations of maleic acid and surfactant in BGE. A similar dependence between components was observed in the case of phthalic acid BGE (*Figure 14*).

Slightly different results were obtained for optimised PDC BGE. At first, the optimal NaOH and surfactant concentrations in PDC BGE were noticeably lower than those in other BGEs. *Figure 13d* shows the higher concentration of surfactant, resulting in higher baseline noise for PDC BGE, in contrast to the case with other BGEs. The relationship between the baseline noise and the concentration of PDC and [C_{14}MIm][Cl] depicted in *Figure 13e* indicates that with maximum chromophore concentration the surfactant mid concentration value is preferable, unlike with other BGEs. As can be seen from *Figure 13f*, the baseline noise increases with increasing NaOH concentration and this can be improved by increasing the PDC concentration, similarly to the case with other BGEs.

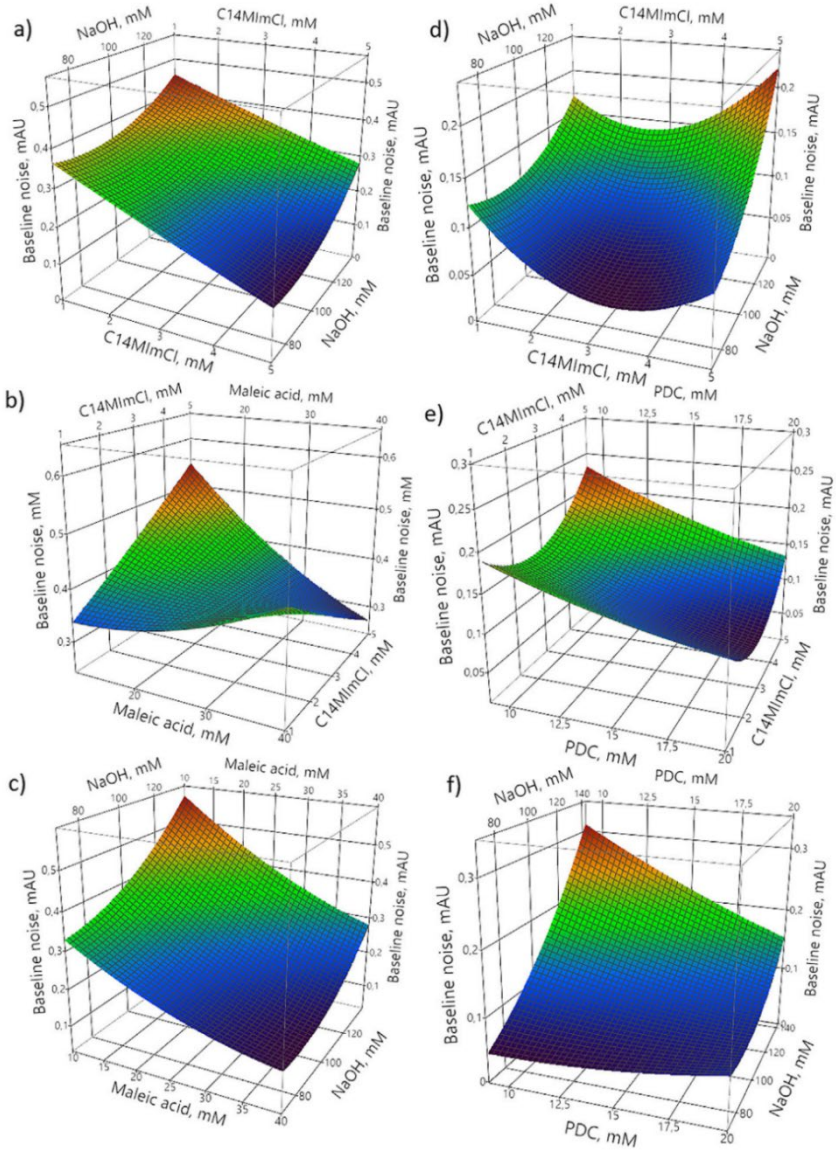

Figure 13. Response surface plots for baseline noise relationships with concentrations of (a) NaOH/[C14MIm][Cl], (b) maleic acid/[C14MIm][Cl], (c) maleic acid/NaOH in maleic acid BGE, (d) NaOH/[C14MIm][Cl], (e) PDC/[C14MIm][Cl] and (f) PDC/NaOH in PDC BGE.

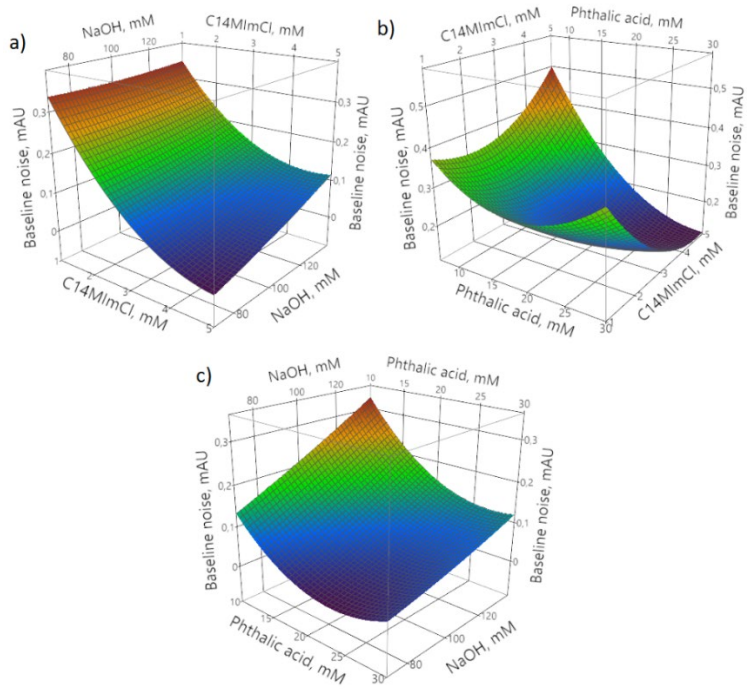

Figure 14. Response surface plots for baseline noise relationships with concentrations of (a) NaOH/[C14MIm][Cl], (b) phthalic acid/[C14MIm][Cl] (c) phthalic acid/NaOH in phthalic acid BGE.

4.2.3 Linearity and precision

Linearity and precision were evaluated using optimised BGEs composed of phthalic and maleic acids. An analysis revealed that cellooligomer standards contained impurities which could be separated only with maleic acid BGE. Hence, maleic acid BGE was used in the next study. The result of regression analysis and the coefficients of determination (R2) for analytes are given in *Table 4*, where the analytes are listed in their migration order. As seen in *Table 4*, the coefficient of determination for all analytes was greater than 0.99, resulting in good linearity. Repeatability was determined for a standard sample that was measured in five replicates on the same day, and reproducibility was determined for four replicates over a four-day observation period. The results show excellent repeatability and reproducibility of the migration times of the analytes, and good repeatability and reproducibility of their peak areas.

4.2.4 Limits of detection and quantification

In the case of sucrose, raffinose, and levulinic and acetic acids, the LoQ was 0.20 mM, while for the other carbohydrates it was 0.25 mM, and the LoD values were between 0.06 and 0.08 mM. Since 5-HMF absorbs well at 270 nm, its LoD and LoQ values were noticeably lower when maleic or phthalic acid BGE was used: – 0.02 and 0.05 mM, respectively. In addition, for 5-HMF PDC absorbs in the same region, giving higher LoD and LoQ values: 0.05 and 0.17 mM, respectively.

Table 4. Linearity and precision for maleic acid BGE.

Analyte	Regression analysis			Precision I (peak areas)		Precision II (migration times)	
	Linear range (mM)	Equation (*y=ax+b*)	R^2	RSD (%) Inter-day	RSD (%) Intra-day	RSD (%) Inter-day	RSD (%) Intra-day
Acetic acid	0.20-7.00	y = 4.5135x – 0.1492	0.9923	2.73	3.47	0.39	1.09
Levulinic acid	0.20-7.00	y = 3.6353x + 0.2880	0.9955	3.20	4.29	0.24	1.21
Xyl	0.25-7.00	y = 2.9891x + 0.2491	0.9976	4.07	9.62	0.37	1.76
Fru	0.25-7.00	y = 3.1838x + 0.3906	0.9964	4.20	7.53	0.05	1.89
Glu	0.25-7.00	y = 3.1948x + 0.4343	0.9961	4.32	7.34	0.11	1.98
Gal	0.25-7.00	y = 3.0800x + 0.7616	0.9954	4.42	6.63	0.10	2.02
Cel	0.25-7.00	y = 4.4243x + 0.6508	0.9969	4.57	8.89	0.25	2.12
Lac	0.25-7.00	y = 4.7923x + 0.3475	0.9962	4.63	9.21	0.18	2.16
DP3	0.25-5.00	y = 4.7025x + 0.3008	0.9928	4.73	9.25	0.34	2.17
DP4	0.25-5.00	y = 4.0007x + 0.1563	0.9902	4.86	8.06	0.06	2.24
DP5	0.25-5.00	y = 4.0037x + 0.2177	0.9936	4.95	7.08	0.47	2.28
DP6	0.25-5.00	y = 4.1686x + 0.0419	0.9942	5.19	6.63	0.23	2.45
Raf	0.20-7.00	y = 5.2987x + 0.0611	0.9963	5.29	6.24	0.17	2.33
Suc	0.20-7.00	y = 6.4173x + 0.0908	0.9974	5.12	8.38	0.22	2.62
5-HMF	0.05-7.00	y = 27.691x + 3.6858	0.9977	0.58	3.49	0.34	2.64

4.2.5 Robustness

Robustness was studied for the IL ([EMIm][Cl]) concentration in the sample. For separation, optimised conditions for maleic acid BGE were used. The analytes acetic acid, levulinic acid, fructose, glucose, cellobiose, cellotriose, cellotetraose, cellohexaose and 5-HMF were used for separation in the presence of [EMIm][Cl]. The concentration of organic acids, 5-HMF, mono-, di- and oligosaccharides was 2 mM each and the IL concentration varied from zero to 400 mM (0, 100, 200, 300 and 400 mM IL). The parameters peak area, peak height and migration times were evaluated, and the results are presented in *Table 5*. *Table 5* shows that the migration times of analytes were not affected by the increase in IL concentration. However, the peak areas and heights varied a lot. All of the observed peak areas decreased in size when the IL concentration was increased to 400 mM. However, the decreased peak areas remained within tolerable limits: between 2.76 and 9.51%. A major problem with the increase in IL concentration was related to the

diminishing peak heights, which led to higher LoD values of analytes in the presence of IL with a high concentration (400 mM). When the IL concentration was ≤ 200 mM, RSDs for peak heights were between 3.41 and 10.53%. Therefore, it is recommended to construct a calibration curve in the presence of an IL with a concentration as in the samples.

Table 5. Influence of IL concentration to analytes peak areas, heights and migration times expressed as RSD values, n=3.

Analyte	Peak area, RSD* (%)	Peak height, RSD* (%)	Migration time, RSD* (%)
Acetic acid	9.51	19.25	0.93
Levulinic acid	7.73	23.08	1.03
Fru	5.18	19.77	1.34
Glu	4.77	21.89	1.36
Cel	2.79	20.25	1.21
DP3	7.86	23.35	1.44
DP4	7.44	21.55	1.17
DP5	6.82	18.72	1.52
DP6	8.39	14.96	2.02
5-HMF	2.76	4.73	2.16

*RSD% are calculated for samples with concentrations of 0, 100, 200, 300 and 400 mM [EMIm][Cl].

4.2.6 Hydrolysis of cellulose in IL

We have demonstrated an efficient separation of cellooligomers obtained from the hydrolysis of cellulose in [EMIm][Cl] using an aqueous HCl solution (*Figure 15*). In addition to carbohydrates, the formation of 5-HMF was also observed. *Table 6* presents the carbohydrate concentrations obtained in the IL solutions. As expected, after the addition of HCl, the cellulose chain started to decompose. At first, cellulose decomposed to longer oligomers, which started to break down to shorter ones, e.g. glucose and cellobiose. This is clearly shown by the results obtained. Sample 2 contained a greater amount of cellopentaose and cellotetraose than samples 4 and 5, and the highest glucose and cellobiose concentrations were obtained in the case of sample 4 and sample 5. Since no additional catalyst was added to the reaction mixture, the highest 5-HMF concentration remained low (>0.54 g/L). Trace amounts of fructose and xylose were detected, as can be seen from the sample electropherogram (concentrations remained under LoQ). The presence of 5-HMF also confirms the formation of fructose since 5-HMF is synthesised via glucose isomerisation to fructose, followed by a fructose dehydration reaction. According to the literature, microcrystalline cellulose may contain a small number of residual hemicelluloses [36], which explains the presence of xylose. The glucose concentration of sample 5 started to decrease after the third addition of water, which was probably related to the presence of an unidentified compound that formed with increasing reaction time. This unknown compound absorbed well at 210 nm but, unfortunately, remained neutral at the pH value of the BGE used, although it was difficult to quantify. As mentioned above, the IL content is essential from the quantification point of view; thus, the IL content for different samples was calculated. In all cases, the IL concentration remained below 255 mM, and had a negligible influence on the results.

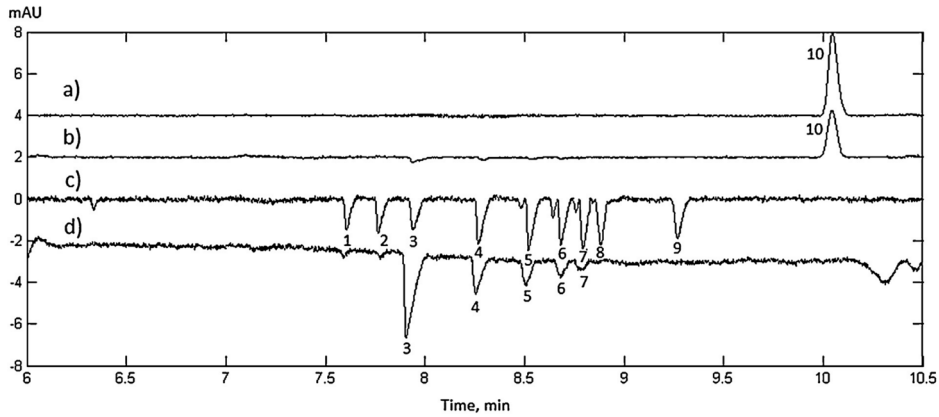

Figure 15. Separation of standards (a, c) and cellulose degradation products (b, d). CE conditions were the following: BGE contained 138.2 mM NaOH, 40 mM maleic acid and 5 mM [C_{14}MIm][Cl]; capillary ID 22.5 µm; absorbance was measured at 210 (a, b) and 270 (c, d) nm; applied voltage was −21.7 kV. Identification: 1–Xyl, 2–Fru, 3–Glu, 4–Cel, 5–DP3, 6–DP4, 7–DP5, 8–DP6, 9–Suc, 10–5-HMF (analyte concentration: 1 mM of each, except 5-HMF, which was 0.5 mM).

Table 6. Hydrolysis of cellulose in [EMIm][Cl], analysed with maleic acid BGE, n = 3.

Analyte	Sample 1 (g/L)	Sample 2 (g/L)	Sample 3 (g/L)	Sample 4 (g/L)	Sample 5 (g/L)
Glucose	n.d.	16.09	19.48	37.46	33.21
Cellobiose	n.d.	7.21	7.81	12.46	9.39
Cellotriose	n.d.	12.38	12.60	14.10	10.42
Cellotetraose	n.d.	12.86	13.85	10.90	7.46
Cellopentaose	n.d.	9.36	9.90	7.47	5.87
5-HMF	n.d.	0.02	0.14	0.54	0.34

n.d. – Not detected.

4.3 Metal-Catalysed Degradation of Cellulose in ILs (Publication III)

Glucose conversion to 5-HMF occurs in two steps: first glucose is isomerised to fructose and then fructose is dehydrated to 5-HMF. The main goal in **Publication III** was to monitor the transformation of cellulose to 5-HMF via glucose and fructose by different widely studied catalysts and compare the results with molybdenum and tungsten oxide-based POMs-ILs. Although the isomerisation and epimerisation of glucose might occur, the formation of fructose predominated. The formation of fructose via isomerisation required some reorganisation of the intermediate, whereas the formation of mannose occurred through rotation around the C2–C3 bond [106]. One of the glucose isomerisation reaction pathways is analogous to that observed in metalloenzymes, such as D-xylose isomerase (XI), which contain two divalent Lewis acid metal centres (Mg^{2+} or Mn^{2+}) confined within a hydrophobic pocket [107], [108]. The reaction pathway includes a ring opening of glucose and coordination with glucose O1 and O2 atoms prior to the isomerisation of the ring-opened glucose chain from position C2 to C1 (via 1,2 intramolecular hydride shift) [109].

Table 7 shows that the conversion of cellulose to 5-HMF in [BMIm][Cl] at 100 °C for 24 h with $ZnCl_2$, $MgCl_2$, $CrCl_3$, molybdenum and tungsten oxide-based POM-ILs as catalysts was completed. In the case of molybdenum and tungsten oxide-based POM-ILs,

the main products were monosaccharides. *Table 7* also shows the total product yields, where all obtained products are summarised.

Table 7. Glucose, mannose and 5-HMF contents in cellulose samples degraded in [BMIm][Cl] using $ZnCl_2$, $MgCl_2$, $CrCl_3$, tungsten (W-POM) and molybdenum (Mo-POM) oxide-based POMs as catalysts.

Catalyst	Glucose, %	Mannose, %	5-HMF, %	Total products yield, %
No catalyst	>0.04	>0.04	>0.04	>0.04
$ZnCl_2$	>0.04	>0.04	4.64	4.64
$MgCl_2$	>0.04	>0.04	3.78	3.78
$CrCl_3$	>0.04	>0.04	55.3	55.3
W-POM	33.7	>0.04	5.82	39.5
Mo-POM	4.96	2.26	2.95	10.2

As expected, the sample with no catalyst did not contain any of the investigated products. The tungsten oxide-based POM showed good activity for cellulose degradation, although the overall yield of products (glucose and 5-HMF) was 29% lower than that obtained with $CrCl_3$ as a catalyst, and the reaction conditions for the POM catalyst were not optimised. The main formed product was glucose instead of 5-HMF. The molybdenum oxide-based POM was the only catalyst that was able to epimerise glucose to mannose, and the latter accounted for 22% of the products obtained. The catalytic activity of the molybdenum oxide-based POM remained remarkably lower and the overall product yield (glucose, mannose and 5-HMF) was 82% less compared to $CrCl_3$. The catalytic ability of $MgCl_2$ and $ZnCl_2$ were the lowest and 5-HMF yields were 6.8% and 8.4%, respectively, of that obtained with $CrCl_3$.

Some metal chlorides were found to be very effective as catalysts for the synthesis of 5-HMF in ILs [110, 111]. The isomerisation of glucose into fructose was favoured by the presence of Lewis acid sites [106]. *Figure 16* shows the mechanism proposed by Zhou et al. for the interaction of metal chlorides with glucose in ILs, such as [BMIm][Cl]. According to this mechanism, the good catalytic performance of $CrCl_3 \cdot 6H_2O$ may be explained by the formation of a more stable metal chloride–glucose complex due to the stronger coordination ability of Cl^- with a chromium centre [110–112].

Ju et al. [113] demonstrated in their 13C NMR study that molybdenum-based POMs are active and selective catalysts for the epimerisation of aldoses. The epimerisation mechanism involves electron transfer from the aldose to the molybdenum oxide octahedra surface units of the POM, followed by an intra-molecular C1→ C2 carbon shift. They also reported that replacing Mo with W in the Kegging structure POM resulted in the loss of epimerisation activity, indicating that the molybdenum octahedral located in the cage-like structure of the POM plays an important role in activating the epimerisation of glucose [113].

Nguyen et al. [114] demonstrated in their 13C NMR and 1H NMR study that glucose epimerisation to mannose using Lewis acids, such as MCl_3 in aqueous phase ($CrCl_3$ was also tested), proceeds via two parallel mechanisms, first a reverse C2 → C1 hydride transfer followed by a C1→ C2 intramolecular carbon shift. They also reported that MCl_3 was also able to epimerise glucose to mannose in low yields and, since fructose formation is predominant, the hydride transfer was a more dominant pathway of glucose conversion [114].

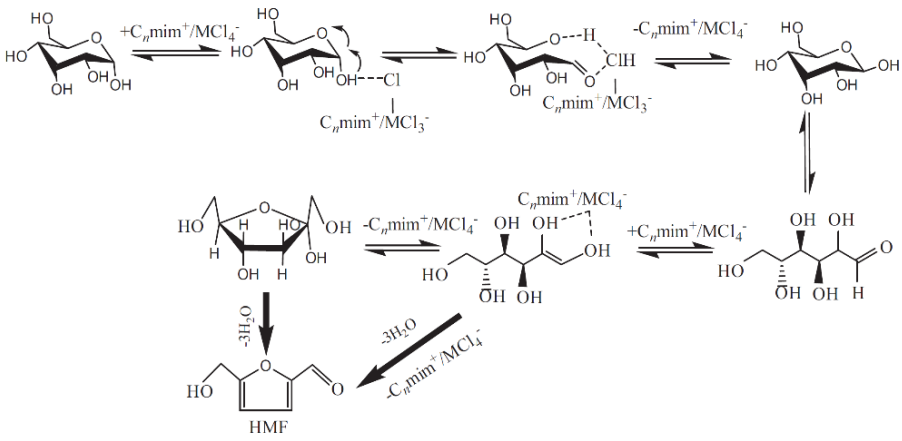

Figure 16. Interaction of metal chlorides with glucose to produce 5-HMF in CnMIM/MCl$_4$ (n= 4, 1-n-butyl-3-methylimidazolium, [BMIm][Cl]; M= Cr, Al and Fe) [30].

4.3.1 Reaction time for POM-IL catalysts

Figure 17 shows the effect of time on the yield of glucose, mannose and 5-HMF from cellulose at 100 and 120 °C for 72 h by using molybdenum and tungsten oxide-based POMs as catalysts. In the case of the molybdenum oxide-based POM, prolonging the reaction time improved the 5-HMF yield at 100 °C, and the plateau in its concentration was not achieved during 72 h. The same trend held true for the yields of glucose and mannose. At 120 °C, glucose and mannose were not detected and the maximum 5-HMF yield remained lower than the yield obtained at 100 °C. The maximum 5-HMF yield, which was achieved at 24 h, was 52% lower, and it started to decrease. In the case of the tungsten oxide-based POM, prolonging the reaction time at 100 °C improved the glucose yield to 24 h and then a plateau was achieved. The 5-HMF yield increased slowly to 72 h. Eminov et al. [115] reported that the highest 5-HMF yield with $CrCl_3 \cdot 6H_2O$ as a catalyst in [BMIm][Cl] was obtained at 120 °C and it was five times higher than the yield obtained at 100 °C. In this work, the temperature was also increased by 20 °C, yet the ability to convert glucose to 5-HMF was not improved. Instead, the opposite effect was observed: glucose was not present in any sample and the maximum 5-HMF yield was achieved at 8 h, remaining 81.4% lower than that obtained at 100 °C. According to the literature, the lower yield could have been caused by humin formation at higher temperatures [116]. Possible humin formation is also supported by the fact that a dark precipitate was formed when the higher temperature was used.

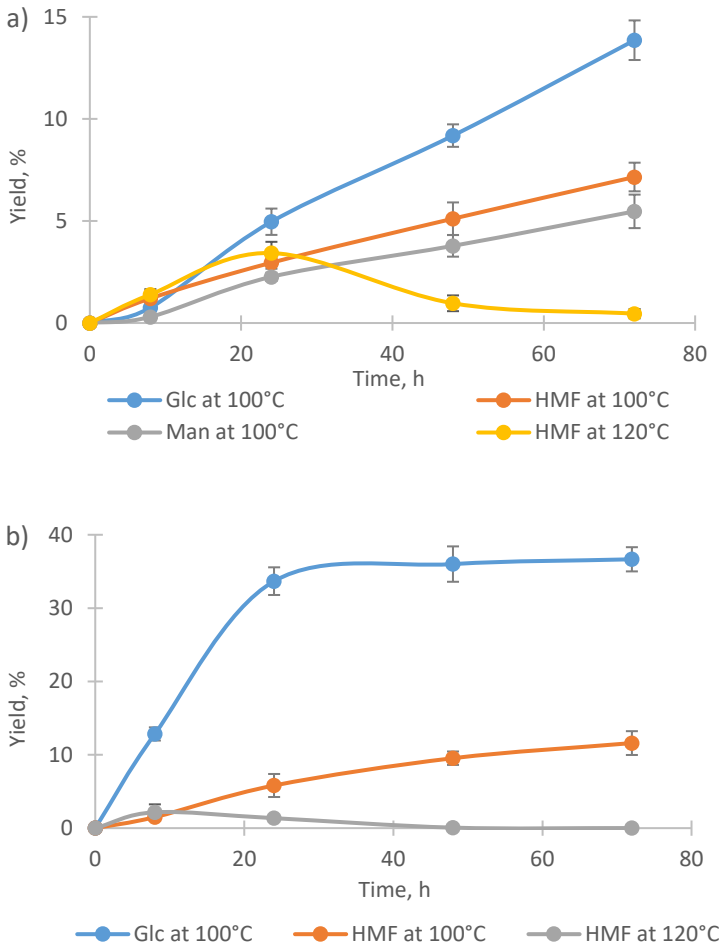

Figure 17. Conversion of cellulose to glucose, mannose and 5-HMF in [BMIm][Cl] at 100 °C and 120 °C by using POMs based on a) molybdenum oxide and b) tungsten oxide.

4.3.2 Cellulose loading and the efficiency of POM formation

Chidambaram and Bell [117] have reported that in [BMIm][Cl] at 120 °C 3% of glucose is converted to humin even without using a catalyst. During the investigated process, humins can also be formed due to the oligomerisation of glucose or fructose with itself, as well as with 5-HMF. However, this inhibits the conversion of cellulose to shorter oligomers and glucose [116, 118]. The formation of glucose and conversion to the other products must be balanced properly to avoid the formation of humins. Thereby, cellulose loadings of 5 and 10 wt% were selected to investigate the effectiveness of 5-HMF formation at 120 °C.

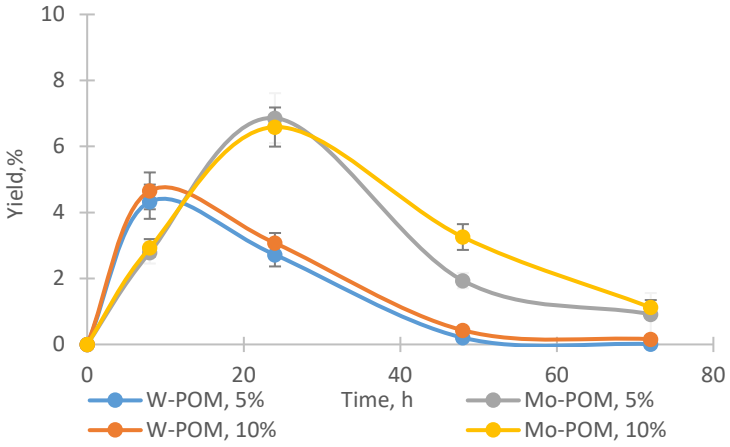

Figure 18. Conversion of cellulose to 5-HMF in [BMIm][Cl] at 120 °C by using tungsten and molybdenum oxide-based POMs at 5 and 10% substrate loading.

Figure *18* shows the results for the conversion of cellulose to 5-HMF in [BMIm][Cl] at 120 °C by using tungsten and molybdenum oxide-based POMs at 5 wt% and 10 wt% substrate loading. The 5-HMF yield followed the same trend despite substrate loading for both of the catalysts used. In the case of the molybdenum oxide-based POM-IL, the maximum 5-HMF yield was achieved at 24 h and formed 52.2% of the maximum yield obtained with a 10 wt% catalyst loading. The maximum 5-HMF yield for the tungsten oxide-based catalyst was achieved during 8 h and formed 46.8% of the yield obtained with a 10% substrate loading. In addition, all samples obtained from 48 and 72 h experiments contained dark precipitates that were most probably humin. Lowering the substrate loading did not improve the conversion to 5-HMF.

Figure 19 shows the cellulose degradation at 100 °C for 72 h to evaluate the efficiency of POM formation in the IL medium. The catalyst (phosphotungstic acid hydrate) was added either 20 min before or 3 h after the substrate. The highest glucose and 5-HMF yields were when the catalyst was added to the sample before the substrate, with the respective yields being 73.7% and 68.4% higher. These results show that the formation of POM was more efficient when the catalyst was added before the substrate.

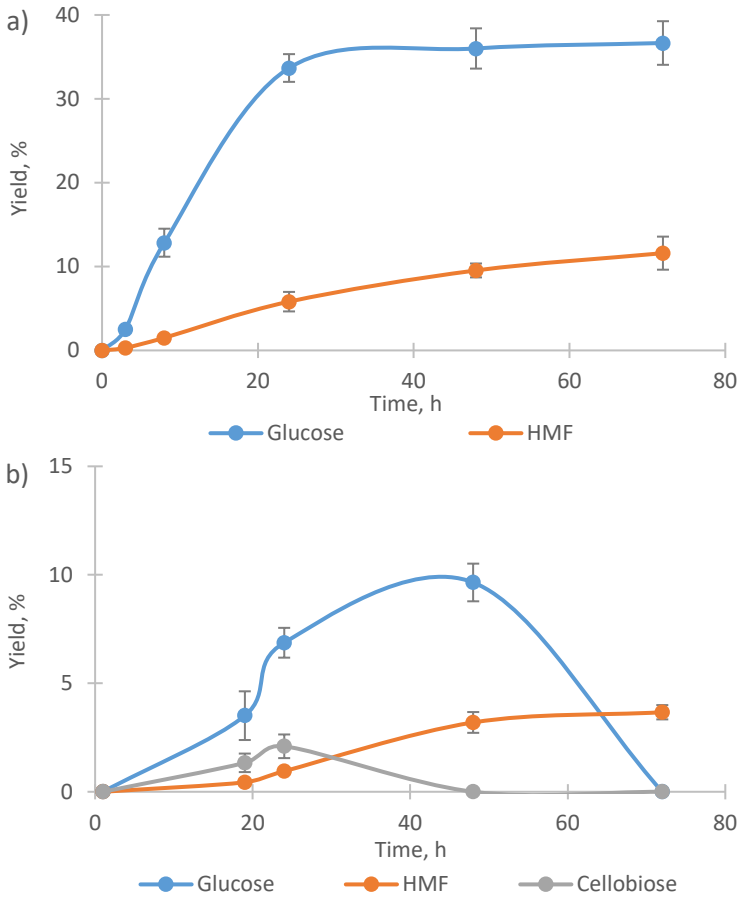

Figure 19. Efficiency of cellulose degradation in [BMIm][Cl] at 100 °C by using phosphotungstic acid hydrate added (a) 20 min before the substrate and (b) 3 h after the substrate.

4.3.3 Effect of the water content of the reaction medium

Li et al. have demonstrated that, according to density functional theory (DFT) calculations, the isomerisation of glucose to fructose over tungsten oxide-based catalysts is possible because of Lewis acid sites (W^{6+}), terminal W-oxo groups that are Lewis basic sites and proton mediators, such as "structural" and physisorbed water on the oxide surface. According to their study, the key aspect of the catalytic mechanism is the proton shift C2 → C1, which is promoted by a synergistic action of the Lewis acid sites and is followed by proton-transfer [121]. No literature data was available regarding either a molybdenum oxide-based catalyst or POM-ILs. The efficiency of 5-HMF conversion and the influence of the reaction medium water content on the process were evaluated at 100 °C for 72 h (*Figure 20*).

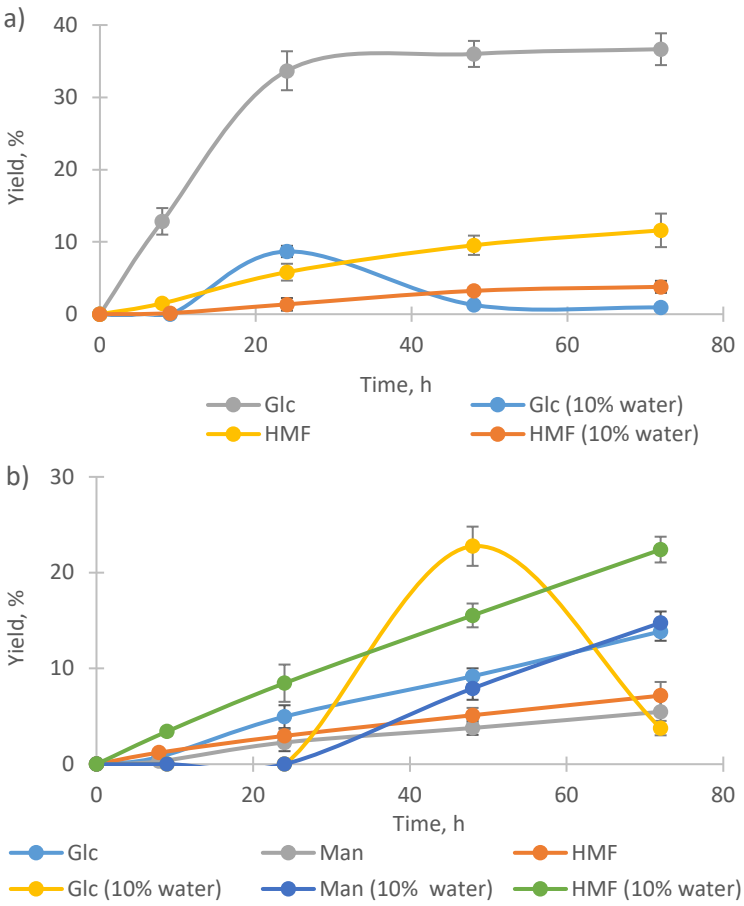

Figure 20. Conversion of cellulose to glucose, mannose and 5-HMF in [BMIm][Cl] at 100 °C in the presence of water (10 wt% of solvent) by using POMs based on (a) tungsten oxide and (b) molybdenum oxide.

In the case of the tungsten oxide-based POM, the highest glucose and 5-HMF yields in the presence of water were 76.3% and 67.4% lower, respectively. The molybdenum oxide-based POM-IL gave the opposite result: the maximum glucose, mannose and 5-HMF yields were obtained in aqueous samples. The highest glucose concentration was achieved with 48 h and the non-water sample content was 59.6% lower. The highest 5-HMF and mannose yields were obtained in the 72 h sample and the yields for the non-water sample was 68.1% and 62.9% lower, respectively. The 5-HMF yield for the molybdenum oxide-based catalyst was 51.8% higher than for the tungsten oxide-based catalyst.

The obtained result confirms that the importance of water in the reaction medium must be further studied:

- Firstly, the water content in reaction medium should be statistically optimised (this can lead to better yields for tungsten oxide-based catalysts).
- Secondly, the reaction temperature should be statistically optimised.
- Thirdly, ILs with different hydrophobicity should be tested.

5 Conclusions

The main conclusions that were made by **Publication I** were following:

- The dominant monosaccharides that were obtained after IL treatments of the wood samples were galactose, glucose, mannose, arabinose and xylose.
- The carbohydrate concentrations depended strongly on the treatment time, temperature and the tree species used.
- Heartwood, sapwood, transition wood, knots, bark etc. all possess different properties and contain chemically different species. Also, hardwood (e.g. silver birch) and softwood species (e.g. Norway spruce and Scots pine) behaved differently, and the obvious reason is their different structures: hardwood species are known to be easier to break down than softwood species, due to the fact that softwood species have guaiacyl lignin as their main lignin type, while the lignin in hardwood species is less recalcitrant, i.e. a combination of guaiacyl and syringyl lignin.
- The hexose's degradation product, 5-HMF, was detected in increasing amounts as a function of the treatment time.
- Treatment temperatures above 150 °C and longer treatment times (above 100 h) resulted in tar and/or humin formation and the degradation of all sugars. In addition, the degradation of sugars was most prominent with the use of [EMIm][OAc].
- Treatment times up to 20 h yielded the best results, considering that the focus was to obtain mono- or polysaccharides.
- Regarding the pretreatment of wheat straw, the following conclusions were reached:Acetate ionic liquids were more efficient in attacking the cellulose, thus helping to achieve higher hydrolysis yields.
- The optimal pretreatment time for wheat straw was 24 h when acetate ILs and convective/conductive heating were applied and, in the case of chloride ILs, no plateau was achieved in 72 h.
- According to SEM images, [BMIm][OAc] treatment resulted in the wheat straw structure becoming disorganised, thereby increasing the ratio of amorphous cellulose.

The conclusions of **Publication II**:

- The simultaneous separation of underivatised mono-, di- and oligosaccharides, acetic acid, levulinic acid and 5-HMF, i.e. products that can be formed upon the combustion of lignocellulosic biomass, was performed employing a BGE composed of 138.2 mM NaOH, 40 mM maleic acid and 5 mM [C_{14}MIm][Cl].
- The BGE conditions were optimised statistically to determine the optimal voltage and concentrations of BGE components. The obtained results confirm that this method, having a wide linear range (therefore smaller number of dilutions), can be used to monitor biomass degradation and 5-HMF formation, offering good precision and fast performance.
- The method demonstrated good robustness for the IL concentration in the sample.

The results of **Publication III** showed that:

- The highest product yield was 55.3% and it was achieved with a $CrCl_3$ catalyst. Tungsten and molybdenum oxide-based POMs were able to decompose cellulose.

The main decomposition products were carbohydrates, such as glucose and mannose.

- The ability of the POMs to convert glucose to 5-HMF was low in the mild conditions used and the overall product yields with the use of the tungsten oxide-based POMs was 28.6% lower than the yield obtained with $CrCl_3$.
- It was expected that increasing the temperature would improve the conversion of 5-HMF but, surprisingly, increasing the temperature by 20 °C did not increase the 5-HMF outcome.
- The 5-HMF yield improved 51.8% when 10 wt% of water was added to the solvent in the case of the molybdenum oxide-based POM.

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Abstract

Catalytic conversion of recalcitrant glycopolymers: investigation of saccharification and isomeristion in ionic liquids

The oil price increase and dependence on dwindling fossil fuel supplies has drawn the world's attention to finding alternative and sustainable energy sources. One of the most promising sources of alternative energy is an abundant natural polymer: cellulose. Cellulose is one of the main components of lignocellulosic biomass. Cellulose is a linear polysaccharide present in the cell membranes of plants, and consists of D-glucose residues connected by β-(1,4)-glucosidic linkages. It has been proposed that lignocellulosic biomass has the potential to be a large-scale, low-cost and sustainable feedstock for important intermediates for chemical and fuel production, e.g. 5-HMF and levulinic acid.

In addition to cellulose, lignocellulosic biomass contains lignin and hemicellulose, which interact with cellulose chains to make them resistant to chemical and enzymatic treatment. Therefore it is necessary to pre-treat the biomass in order to break down the complex interactions between cellulose, hemicellulose and lignin. There are several pre-treatment methods: physical methods (crushing, grinding and irradiation), physico-chemical methods (steam-explosion, treatment with NH_3, CO_2, LHW and organosolvents, acidic and basic hydrolysis, and ozonolysis) and biological methods. The most widely used methods require high temperatures and/or high pressures over long periods of time, increasing the ecological footprint of technology that is meant to promote green thinking. In addition, many inhibitory by-products (e.g. oligosaccharides) for enzymatic degradation form during non-specific reactions. At present, a popular field of research - treatment with ILs - has gained popularity. Ionic liquids have low melting points, and they are characterised by good electrical conductivity, low vapour pressure and high thermal stability. IL pretreatment is usually carried out at an atmospheric pressure of ~100 °C. ILs are easy to synthesise and they can be used both as solvents and catalysts by adding different side chains to the structure.

In **Publication I**, mono- and disaccharide contents were monitored in IL-pretreated wood samples, which contained mainly galactose, glucose, mannose, arabinose and xylose. The contents depended a lot on the pretreatment time and the used temperature, as well as on the wood type. Softwood species (e.g. silver birch) and hardwood species (e.g. Norway spruce and Scots pine) contained different amounts of certain types of monosaccharides and, due to structural differences, carbohydrates in hardwoods decompose more easily to monosaccharides. With a longer reaction time, the hexoses in the sample began to convert to 5-HMF. At temperatures above 150 °C and/or in samples with a long reaction time (100 h), the sugar content decreased significantly and formed products that somewhat disrupted CE analysis.

In addition, the IL pretreatment efficiency was evaluated due to obtained sugar contents on wheat straw samples after enzymatic hydrolysis. The results showed that pretreatment with [BMIm][OAc] was more efficient and the sugar content was 46.9% higher than the maximum content obtained with the pretreatment with [EMIm][Cl]. The optimal pretreatment time with [BMIm][OAc] was 24 h and no plateau was reached during 72 h of [EMIm][Cl] pretreatment. Structural changes in wheat straw were studied via SEM images, and it was concluded from the images that, after pretreatment with

[BMIm][OAc], the structure of wheat straw became more irregular and thus the amount of amorphous cellulose increased.

In the **Publication II**, a CE methodology for the determination of potential lignocellulosic biomass degradation products (mono-, di- and oligosaccharides, acetic acid, levulinic acid and 5-HMF) was developed. The contents of the compounds in the BGE and the voltage for the separation were statistically optimised and the following conditions were obtained: the optimal BGE contained 138.2 mM NaOH, 40 mM maleic acid and 5 mM [C_{14}MIm][Cl], and the separation voltage was −21 kV. The method had a wide linear range, provided fast and accurate results, and was sufficiently robust in the concentration of IL in the sample.

In the **Publication III**, the degradation of commercial cellulose, the isomerisation of glucose to fructose, and the conversion of glucose to 5-HMF in [BMIm][Cl], in the presence of metal catalysts ($CrCl_3$, $ZnCl_2$ and $MgCl_2$), as well as tungsten and molybdenum oxide-based POMs, were evaluated. The highest yield of products was 55.3% and it was obtained when a $CrCl_3$ catalyst was used. The catalytic activity of the used POMs was low and the maximum yield of products was 28.6% lower compared with $CrCl_3$ under the same reaction conditions. In the case of POM catalysts, the maximum yield of 5-HMF was 22.4% and this was achieved with the molybdenum oxide-based POM when 10 wt% of water was added to the solvent. The obtained yields were notably higher under optimised reaction conditions.

Lühikokkuvõte

Tõrksate glükopolümeeride katalüütiline konversioon: sahharifikatsiooni ja isomerisatsiooni uurimine ioonsetes vedelikes

Maailmas, kus pidevalt tõuseb nafta hind ja suureneb sõltuvus vähenevatest fossiilkütuse varudest on suurenenud tähelepanu alternatiivse ja jätkusuutliku energiaallika leidmiseks. Üheks potentsiaalseks taastuvenergia allikaks kütuste, kemikaalide ja materjalide tootmisel on enamlevinud glükopolümeer – tselluloos. Tselluloos, mis on lignotselluloosse biomassi üks põhikomponentidest, on taimede rakukestades esinev lineaarne polüsahhariid, mis koosneb β-(1,4)-glükosiidsete sidemetega ühendatud D-glükoosi jääkidest. Tselluloosi lagunemisel saadakse olulisi vaheprodukte kemikaalide ja biokütuste tootmiseks, sh 5-hüdroksümetüülfurfuraal (5-HMF) ja levuliinhape, kasutades toorainena põllumajanduse-, metsatööstuse-, puidutööstuse-, aianduse- ja muu inimtegevuse ning looduslike protsesside käigus tekkivaid jääke.

Lignotselluloosne biomass sisaldab lisaks tselluloosile ligniini ja hemitselluloosi, mis interakteerudes tselluloosiahelatega muudavad selle keemiliste ja ensümaatiliste mõjutuste suhtes vastupidavaks. Seetõttu on vaja biomassi eelnevalt töödelda. Eeltöötlusmeetodeid on mitmeid: füüsikalised meetodid (purustamine, jahvatamine, kiiritamine), füüsikalis-keemilised meetodid (auruplahvatus-autohüdrolüüs, töötlemine NH_3-ga, CO_2-ga, kuuma veega, organosolventidega, happeline ja aluseline hüdrolüüs, osonolüüs) ja bioloogilised meetodid (seened, aktinomütseedid). Neist enim kasutust leidvad meetodid nõuavad pikema aja jooksul rakendatavat kõrget temperatuuri ja/või rõhku ning suurendavad seeläbi algselt rohelise mõtteviisi propageeriva tehnoloogia ökoloogilist jalajälge oluliselt. Lisaks sellele tekivad mittespetsiifiliste reaktsioonide tõttu palju kõrvalprodukte (nt oligosahhariidid), mis inhibeerivad hüdrolüüsi katalüüsivaid ensüüme. Käesoleval hetkel on nimetatud eeltöötluste hulka lisandunud populaarsust koguv uurimisvaldkond - töötlemine ioonsete vedelikega (IL). Ioonsed vedelikud on madala sulamistäpiga soolad, mida iseloomustavad hea elektrijuhtivus, praktiliselt puuduv aururõhk ja termiline stabiilsus ning IL-eeltöötlus viiakse läbi atmosfäärirõhul ~100 °C juures. Ioonseid vedelikke on kerge sünteesida ning neile on omane erinevate kõrvalahelate lisamisel saavutatav spetsiifilisus, mistõttu saab neid kasutada üheaegselt nii solvendi kui ka katalüsaatorina.

Esimeses publikatsioonis uuriti IL-eeltöötluse järel puidust saadud di- ja monosahhariidide sisaldusi ning proovides esinesid peamiselt galaktoos, glükoos, mannoos, arabinoos ja ksüloos. Sisaldused sõltusid palju eeltöötluse ajast ja kasutatud temperatuurist ning ka kasutatud puidu liigist. Lehtpuuliigid (nt. arukask) ja okaspuukliigid (nt. harilik kuusk ja harilik mänd) sisaldasid erinevates kogustes teatud tüüpi monosahhariide ning struktuuriliste erinevuste tõttu on lehtpuidus sisalduvaid süsivesikuid kergem monosahhariidideks lagundada. Pikema reaktsiooniaja tulemusena hakkasid proovis sisalduvad heksoosid konverteeruma 5-HMF-ks. Temperatuuril üle 150 °C ja/või proovides, mille reaktsiooniaeg oli 100 h, suhkrute sisaldus langes oluliselt ning tekkinud produktide tõttu oli CE analüüs raskendatud.

Lisaks uuriti ka IL-eeltöötlemise efektiivsust nisupõhule ensümaatilise hüdrolüüsi järel saadud suhkrute sisalduse kaudu. Tulemused näitasid, et [BMIm][OAc]-ga tehtud eeltöötlus on efektiivsem ning suhkrute sisaldus oli [EMIm][Cl] eeltöötlusel saadud maksimaalsest tulemusest 46,9% kõrgem. Optimaalne eeltöötluse aeg [BMIm][OAc]-ga

oli 24 h kuid [EMIm][Cl] eeltöötlusel 72 h jooksul platood ei saavutatud. Nisupõhu struktuurilisi muutusi uuriti SEM piltide abil ning piltidelt võis järeldada, et [BMIm][OAc] eeltöötluse järel muutub nisupõhu struktuur korrapäratumaks ning seeläbi suureneb amorfse tselluloosi osakaal.

Teises publikatsioonis töötati välja võimalike lignotselluloosse biomassi laguproduktide (mono-, di- ja oligosahhariidide, äädikhappe, levuliinhappe ning 5-HMF) määramiseks mõeldud CE metoodika. Taustelektrolüüdis sisalduvate ühendite optimaalsed sisaldused ja lahutamisel kasutatav pinge optimeeriti statistiliselt ning saadi järmised tingumused – optimaalne taustelektrolüüt sisaldas 138,2 mM NaOH, 40 mM maleiinhape, ja 5 mM [C_{14}MIm][Cl] ning pinge oli −21 kV. Meetod omab laia lineaarset ala, on piisavalt kiire ja täpne ning robustne proovis sisalduva IL kontsentratsiooni suhtes.

Kolmandas publikatsioonis uuriti tselluloosi lagundamist, glükoosi isomerisatsiooni ja konversiooni 5-HMF-ks [BMIm][Cl]-s, kasutades katalüsaatoritena $CrCl_3$, $ZnCl_2$, $MgCl_2$ ning volfram- ja molübdeenoksiidide põhiseid polüoksometalaate (POM), peamisteks laguproduktideks olid glükoos ja mannoos. Parim produktide saagis oli 55,3% ning see saavutati kasutades CrC_3 katalüsaatorit. Kasutatud POM-de katalüütiline aktiivsus oli madal ning produktide maksimaalne saagis oli $CrCl_3$-ga võrreldes samade reaktsioonitingimuste juures 28,6% madalam. POM katalüsaatoreid kasutades saadud maksimaalne 5-HMF saagis oli 22,4% ning see saavutati molübdeenoksiidi põhise POM kasutamisel kui solvendile oli lisatud 10 wt% vett. Optimeeritud reaktsioonitingimuste juures võivad aga saagised märkimisväärselt paraneda.

Appendix 1

Publication I

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Saccharification of lignocellulosic biomasses via ionic liquid pretreatment

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ABSTRACT

The current work focuses on the pretreatment efficiency of ILs combined with heat for woody biomass consisting of spruce, birch and pine as well as winter wheat straw. The latter was investigated as a comparison and with the aim to enhance its digestibility during enzymatic hydrolysis whereby the influence of IL-treatment to cellulose resistance for hydrolysis was investigated. Considering the wood species, the most common and industrially important wood species in Northern Europe were chosen in the present work and the goal was to obtain fermentable sugars and their degradation product, i.e. 5-hydroxymethylfurfural (5-HMF), which is known valuable platform chemical. Further, the differences in the yields of IL-obtainable carbohydrates between these species were studied. The highest sugar yields were obtained to glucose in the case of spruce and arabinose in the case of pine sapwood, 12.07 and 7.72 mmol/L, respectively. The highest 5-HMF yield was obtained for spruce heartwood (9.18 mmol/L) with longer treatment time, such as 100 h. However, regarding woody biomass, the present work was focused more on the study and analysis of the IL-containing liquid part, wood hydrolysate, after IL-treatment aiming to answer the analysis challenges related to this fraction.

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1. Introduction

Numerous earlier studies of common and industrially important wood species exist in terms of their biological and chemical structure that map the details of wood polysaccharides and other wood constituents (cellulose, lignin, hemicelluloses and even extractives). Also, many authors have proposed possible ways of more efficient wood utilisation (Pickett, 2008; Klemm et al., 2005; Sannigrahi and Ragauskas, 2013; Asakawa et al., 2016; Lienqueo et al., 2016). From industrial point of view, it is important to study samples that are considered as waste by forestry instead of the common academic approach with separated wood parts, together with known original sample properties, like heights, age and condition of the felled tree (fresh and healthy). In light of biorefinery for lignocellulosic biomass, the selection of samples should also include other sources like agricultural and forest residues as well as municipal waste such as organic and paper waste. The use of agricultural residues as cereal straws for biorefinery feedstock contributes in clearly more efficient resource utilization by combining the production of both food and fuels on the same geographical area.

Rogers and co-workers reported that a imidazolium-based ionic liquids (ILs) with chloride anion are able to break down an extensive and well organised hydrogen-bonding network of cellulose and thus promoting dissolution of wood (Fort et al., 2007). The various possibilities to use ILs has awakened a lot of research interest and one of its popular field of applications has become the deconstruction and fractionation of lignocellulosic biomass (Domínguez de María, 2013; Wang et al., 2010; Lan et al., 2011; George et al., 2015). Further use of this upgraded feedstock is more plausible if carried out through the sugar platform that gives rise to industrially important platform chemicals which can be easily converted to biofuels or other bio-based products (FitzPatrick et al., 2010; Silva-Fernandes et al., 2015). The recalcitrance of the lignocellulose toward deconstruction leads to challenges in terms of optimizing and designing any IL-based treatment method. The choice of the suitable deconstruction process depends on the properties and quality of the biomass as well as the targeted end-product. For instance, it is known that lignocellulosic biomass should be exposed to various pretreatment steps to improve the enzymatic hydrolysis

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efficiency in bioethanol production as well as in the case of other fermentation processing (Alvira et al., 2010).

Many studies have been conducted and, therefore, the focus of this study was to investigate the solubility of lignocellulosic biomass in ILs (Mäki-Arvela et al., 2010). On the other hand, complete dissolution is not necessary for most purposes (it is often desirable that cellulose is left unaffected while lignin and/or hemicelluloses are targeted as soluble fractions and removed) and, consequently, chemical disruption effects of ILs were also investigated (Lan et al., 2011).

Typically the process starts by the disruption of the hydrogen bonding and cellulose crystallinity, continued by depolymerization of cellulose (and hemicellulose) to glucose and finally ends with further degradation of glucose to levulinic and formic acid (FA) through 5-hydroxymethylfurfural (5-HMF) as the degradation intermediate. In addition, carbonized structures (humins) are formed by polymerization of 5-HMF (Hassanzadeh et al., 2014). Almost all pretreatment procedures induce, especially if conducted at elevated temperatures, chemical changes in both hemicellulose and lignin. Examples of such changes include polymer fragmentation, chemical transformation or functionalization, while cellulose usually remains chemically unaltered and only some structural modifications of cellulose have been observed (Cheng et al., 2011).

The present work was focused on pretreatment of woody biomass (the most common and industrially important wood species in Northern Europe were chosen) and agricultural residue biomass such as wheat straw in ILs.The aim was to gather more knowledge on the obtained carbohydrate and 5-HMF contents after IL treatment for industrially important lignocellulosic biomasses and the used ILs were well known and widely reported ones. The woody biomass hydrolysate was analysed directly for the monosaccharides content and their degradation products. Also, in case of wheat straw, the IL-treated biomass was followed by enzymatic hydrolysis and then the content on monosaccharides and their degradation products were analysed.

2. Materials and methods

2.1. Lignocellulosic biomass samples

Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and Silver birch (*Betula pendula*) samples were obtained from Viitasaari, Finland. The samples were grounded and sieved to sawdust with a particle size of 1–2 mm. Some Norway spruce was received from Örnsköldsvik, Sweden, as sawdust and it even contained some larger particles (around 5 mm). The Finnish wood samples were collected at the common sampling height, i.e. 1.5–2 m. The ages of the trees were between 20 and 25 years, based on the year ring calculation. Sapwood and heartwood were separated, freeze-frozen and the samples were stored in freezer in separate packages between −18 and −20 °C. The Swedish spruce samples originated from Örnsköldsvik's biorefinery cluster (Aditya Birla Domsjö AB) and being industrial raw material it also contained some residual bark as well as knots.

The woody biomass sawdust samples, such as Norway spruce, Scots pine and Silver birch, were treated at 100 °C in C_2MIM Cl. The amount of wood in IL was 50 wt-%. The wood samples were heated in an oil bath at magnetic hot plate with continuous mixing. The samples were collected between 9.5–100 h and this experiment was performed in duplicate.

Winter wheat (*Triticum aestivum*) straw was obtained from local farm in Pärnu-Jaagupi, Estonia. The straw was grounded (particle size 0.6–5 mm) and dried at 96 °C to constant weight before use. Straw was treated with C_4MIM Ac and C_2MIM Cl (the amount of straw was 10 wt-%) and heating was carried out using Sanyo Sterilizer MOV-112S thermostat at 100 and 110 °C, without continuous mixing (stirred periodically) and samples were collected at fixed time intervals (4, 24, 48 and 72 h) and this experiment was performed in duplicate. Collected samples were washed with deionized water 3 times and used for enzymatic hydrolysis. Enzymatic hydrolysis for IL treated wheat straw was performed in citrate buffer (pH 5) at 50 °C for 24 h, using enzymes Celluclast 1.5 L and Novozym 188 from Novozymes A/S (Denmark). In experiment, 0.51 mg Novozym 188 and 1.02 mg Celluclast 1.5 L was added to 200 mg substrate (the appropriate amount of enzymes were calculated based on enzyme activity measured with filter paper assay).

2.2. Applied ionic liquids

The biomass samples were treated with 1-butyl-3-methylimidazolium acetate (C_4MIM Ac) and 1-ethyl-3-methylimidazolium chloride (C_2MIM Cl). C_4MIM Ac and C_2MIM Cl were purchased from Sigma Aldrich and Merck (assay 98%) and used as received.

Prior to IL-treatments of any samples, no water was added. It is well known that ILs are extremely hygroscopic but despite that a glove box was not used upon handling of the ILs: the focus was to see whether the simplest possible, cost-effective method (that might also interest industry) could be used. In addition, the presence of water in many ILs – until a certain limit (ca. 1–4% has been reported (Hyvärinen et al., 2014; Sievers et al., 2009)) – is known to improve the dissolving, degradation and fibrillation ability of an IL. The uptake of moisture from air was not prevented, even though weighing and handling of the ILs (particularly the very hygroscopic C_2MIM Cl) was carried out as fast as possible.

2.3. Scanning electron microscopy (SEM) samples

Wheat straw and commercial cellulose (from Sigma Aldrich) were treated with C_4MIM Ac, at 100 °C for 1, 6 and 24 h, to investigate the physical changes in structure. The treated material was washed with Milli-Q water, the solvent was replaced to acetone, material was dried with CO_2 and SEM images were recorded.

2.4. CE analysis

An Agilent 3D capillary electrophoresis (CE) instrument (Agilent Technologies) equipped with a diode array UV/Vis detector was used to quantify all obtained samples. Electrophoretic conditions were as follows: uncoated fused silica capillary, 71.5 or 81.5 cm effective length, 50 μm inner diameter, temperature of the capillary: 15 ± 0.5 °C; applied voltage: 17 kV; hydrodynamic sample injection: 35 mbar for 10 s; detection wavelength: 270 nm. The background electrolyte (BGE) used in the experiment was 130 mM NaOH containing 36 mM Na_2HPO_4. Similar BGE mixture was earlier successfully employed in the analysis of sugar compositions of acid hydrolyzed extracts of cellulose fibre samples and for monitoring of cellulose degradation in ILs as explained elsewhere (Hyvärinen et al., 2014; Vaher et al., 2012).

For quantification, stock solutions of 50 mM in Milli-Q water for each sugar standard and furfurals were prepared. Working standard solutions within an appropriate range of concentration were prepared by diluting the stock solution with water. Sample preparation for CE analysis was restricted to diluting of supernatant to the appropriate concentration. All the standards and samples were analyzed three times for accurate quantification. For the identification of the compounds in the sample, a mixture of the following analytes was used: furfural, 5-hydroxymethylfurfural (5-HMF), sucrose (Suc, used as an internal standard, ISTD), cellobiose (Cel), galactose (Gal), glucose (Glc), mannose (Man), arabinose (Ara) and xylose (Xyl) (all mentioned in their CE migration time order). All

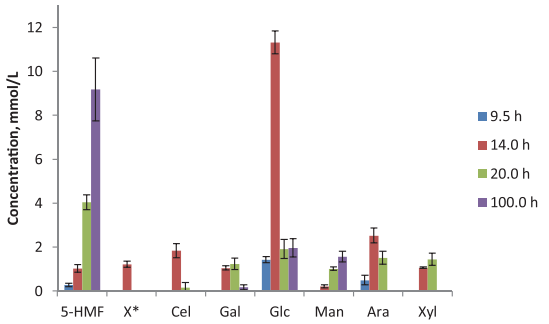

Fig. 1. Detected analytes consentrations in C_2MIM Cl treated spruce sapwood hydrolysates.

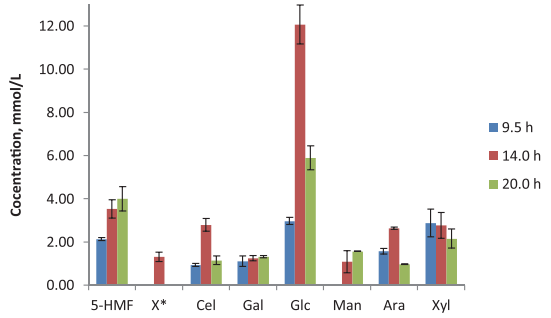

Fig. 2. Detected analytes consentrations in C_2MIM Cl treated spruce heartwood hydrolysates.

the standards used for analysis were purchased from Sigma Aldrich with purity >98%.

3. Results and discussion

At first the samples were exposed to a C_2MIM Cl treatment at 100 °C for various time periods. Fig. 1 demonstrates the obtained monosaccharides and their degradation products mmol/L per wood hydrolysate (i.e. the dissolved part of the sample, containing IL and wood components dissolved in the IL) in sawdust of Norway spruce heartwood and sapwood samples, respectively. The concentrations of analytes: 5-HMF, Cel, Gal, Glc, Man, Ara and Xyl as well as concentration of an unknown compound [here marked with X*, calculated as fucose (Fuc)] are shown. The compound X was expected by migration time as Fuc. Further, a few other unknown compounds could sometimes be seen but could not be identified here. We anticipate that those unknown compounds are either sugar alcohols or uronic acids, galacturonic acid, glucuronic acid or 4-*O*-methylglucuronic acid (Hyvärinen et al., 2014; Riittonen et al., 2013).

In general, the treatment times and the wood-to-IL ratio were not similar (values were calculated against wood hydrolysate, i.e. the liquid part of the sample where analytes are dissolved in IL) and an interesting trend was observed when comparing heartwood and sapwood. For example, 5-HMF content seems to be increasing more steadily among the treatment time in sapwood samples than in case of heartwood samples. Gal and Xyl were also more evenly present during the process in heartwood samples than in sapwood samples. The amounts of Cel and Glc, together with Ara vary in both wood parts. In the case of spruce sapwood, the longer treatment time (100 h) resulted in formation of undetermined compounds that disrupted the CE analysis.

Figs. 1 and 2 represent the analyte concentrations in mmol/L per wood hydrolysate for fresh wood sample of Norway spruce. These samples were of industrial origin and contained heartwood besides sapwood complemented with some bark and knots. Furthermore, the particle size varied from 1 to 5 mm. Heartwood and sapwood have different carbohydrate contents like also bark and knots. In addition, knots are also known to be rich in antioxidative lignans, flavonoids and stilbenes. Bark contains stilbenes and various polyphenols, e.g. tannins and is rich in mono- and disaccharides (Pietarinen et al., 2006; Bertaud and Holmbom, 2004). Considering spruce hemicelluloses, heartwood contains more glucuronoxylan and pectins than sapwood which should be manifested by an increase in Ara, Xyl and 4-*O*-methyl-glucuronic acid amounts among non-cellulosic saccharides. Glucuronoxylans are found in hardwood species in amounts ranging from 15 to 30% of the amount of hemicelluloses (Riittonen et al., 2013; Sjöström, 1993). Based

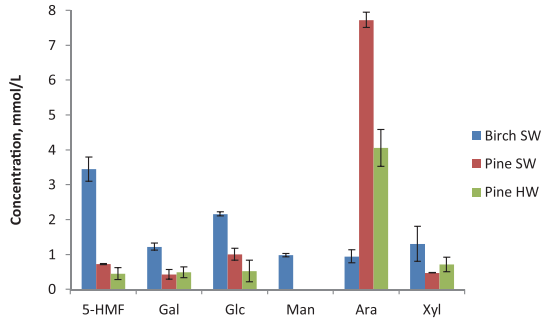

Fig. 3. Detected analytes concentrations for C_2MIM Cl at 100 °C treated birch sapwood (SW, treated for 20 h), pine sapwood (treated for 6.5 h) and pine heartwood (HW, treated for 9.5 h) hydrolysates.

on this the main differences can be explained. The content of the known degradation product of hexoses, 5-HMF, was increasing dramatically with treatment time, as expected. Also some degradation products of 5-HMF, like levulinic acid or γ-valerolactone could form (Centi et al., 2011).

Comparison of treated Norway spruce and Scots pine sawdust (Figs. 2 and 3) shows that a wider range of monomeric sugars could be detected and the obtained amount of 5-HMF was much less upon the 20-h-treatment, as expected. The shorter treatment time gave higher amounts of Glc, as well as Cel, Ara and Xyl. In fact, upon longer processing times, these sugars can be transformed to 5-HMF. Sapwood is known to be richer source for mono- and disaccharides than heartwood as also confirmed in this study. It is evident that the IL-treated samples of Scots pine as well as Norway spruce gave rise to 5-HMF, Glc and Gal. Also, the amount of 5-HMF detected in the spruce samples was much higher since it contains more Glc. Still, treated spruce samples exposed for long treatment times gave rise to also Man whereas, instead, pine samples gave rise to Cel.

When comparing Norway spruce and Scots pine sapwood sawdust samples (9.5 h treatment time in C_2MIM Cl, at 100 °C, Figs. 1 and 3), it can be seen that the obtained amounts of 5-HMF, Gal, Glc and Xyl were significantly lower in case of Scots pine than Norway spruce. However, the amount of Ara released from Scots pine was clearly higher than in case of Norway spruce. Softwood is known to contain arabinoglucuronoxylans (5–10% of the hemicellulose yield) at a molar ratio of 10:2:1.3 (Xyl:4-*O*-MeGlcA:Ara) (Riittonen et al., 2013; Sjöström, 1993). Strangely, the spruce samples studied in this work did not release as much Xyl and Ara as pine samples. Further, the Ara content was higher than that of Xyl. Arabinogalactans, another type of hemicellulose, are also a source

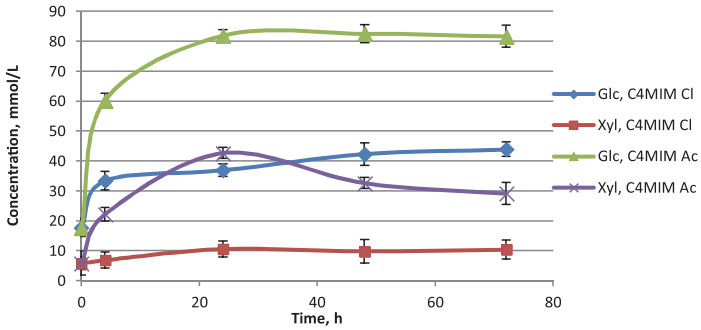

Fig. 4. Dynamics of saccharification process for IL-pretreated wheat straw samples at 100 °C.

for Ara but they are mainly present among hardwood hemicelluloses (especially in heartwood of Siberian larch). Arabinogalactans are built of Gal, Ara and Glc units, in molar ratio at an approximate ratio of 6:3:~0 (Riittonen et al., 2013; Sjöström, 1993). Also, arabinogalactans are highly water-soluble. Upon C_2MIM Cl -treatment at 100 °C for 9.5 h, the Norway spruce sawdust samples released mainly Glc, Ara and 5-HMF. Also, Gal and Xyl could not be determined, unlike in the case of Scots pine (Fig. 3) that underwent a similar treatment.

Further, Silver birch sawdust samples were treated in a similar way (Fig. 3). The following mono- and dimeric saccharides were identified: Cel (in trace amounts), Gal, Glc, Man, Ara and Xyl. Furthermore, significant amounts of 5-HMF was forming. Some birch samples also contained relatively high amounts of furfural (known degradation product of pentoses, e.g. that of Xyl) already after 17 h of C_2MIM Cl treatment and some birch samples also gave rise to small amount of furfural even after 5 or 6 h of treatment time. Some electropherograms of treated birch also displayed a minor peak immediately on the right side of the furfural peak, this likely being a furfural-related product, furfuranol.

The CE method applied gave rise to good reducibility and appropriately diluted samples resulted in low standard deviations (STD < 10%). Herein some samples gave rise to unexpectedly high STDs. Its well known that wood biomass can easily vary in about 10% in its composition, depending on e.g. growing place. Also, the properties and chemical compositions in different parts of stem wood vary a lot. Even though the samples were mostly sapwood and heartwood, the trees processed were quite young. In fact, in young trees, the transition part between the sap- and heartwood is more difficult to identify and this might be one possible explanation for the standard deviations being higher than usual.

C_2MIM Ac is widely recognized for its power in biomass treatments. Nevertheless, the amount of sugars released upon C_2MIM Ac -treatments were really low and lower than what was obtained when C_2MIM Cl was used. The reason might be the long treatment time of 100 h at 100 °C which seemed to be too aggressive for spruce sawdust of 1 mm particle size. Consequently, all the sugars had already degraded and degradation products 5-HMF and furfural dominated as can be seen.

Further, wheat straw was pretreated in ILs using conventional heating. After treatment, IL fraction was removed and analysed with CE. The samples had to be diluted in order to obtain an appropriate IL concentration for CE analysis (max. 400 mM) and, thus all the sugars obtained remained under detection limit. Therefore, the efficiency of the IL treatments was evaluated via determining the sugar contents after enzymatic hydrolysis step. The main sugars detected were Glc, Xyl, Ara, Man and Cel. Upon use of C_2MIM Ac IL, higher Glc and Xyl concentrations were obtained. In case of C_4MIM Ac, a plateau was achieved in Glc content after 24 h while in case of C_2MIM Cl no plateau was achieved even after 72 h (see Figs. 4 and 5). The highest Glc concentrations obtained, 86.17 mmol/L in case of C_4MIM Ac and 52.89 mmol/L in case of C_2MIM Cl, were obtained at 110 °C and 72 h of treatment time. The highest Xyl content in case of C_4MIM Ac treated sample was achieved at 100 °C and 24 h (42.67 mmol/L) whereas in case of C_2MIM Cl treatment 110 °C and 72 h gave the best result (15.80 mmol/L). The maximum Xyl content obtained with pretreatments with acetate based ILs were 39.40 mmol/L, at 110 °C and 24 h, whereby prolonged exposure times led to degreasing concentrations. The plausible reason is

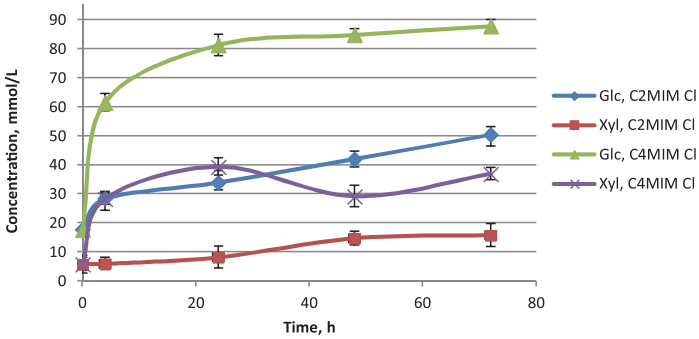

Fig. 5. Dynamics of saccharification process for IL-pretreated wheat straw samples at 110 °C.

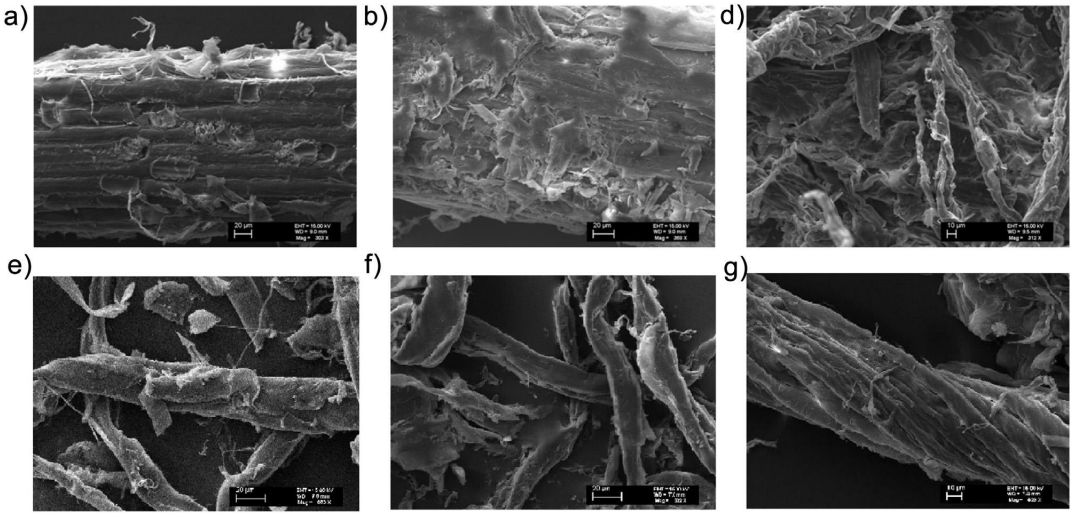

Fig. 6. SEM images of C_4MIM Ac-treated wheat straw samples (at 100 °C) after (a) 1, (b) 6 and (c) 24 h and cellulose samples after (d) 1, (e) 6 and (f) 24 h.

more efficient degradation of hemicelluloses with longer pretreatment times.

3.1. Changes in physical and chemical properties

SEM images (Fig. 6) were recorded to visualize the physical changes in the IL pretreated wheat straw and cellulose. Wheat straw displayed a well ordered structure after 1 h contact with an IL. Longer treatment times resulted in that the wheat strawstructure lost some of the fibres, the structure started to lose its well organized form and there was noticeable and homogeneous swelling. Swollen biomass has bigger internal surface area resulting to better enzyme accessibility and increased binding sites for hydrolysis (Dougherty et al., 2014). Cellulose remained unaffected during 24 h in contrast to wheat straw. Structural changes in wheat straw can be explained due to its hemicellulose and lignin content in addition to cellulose.

4. Conclusions

Galactose, glucose, mannose, arabinose and xylose were the main monosaccharides obtained after IL-treatments of wood samples and the concentrations depended strongly on the treatment time, temperature and the tree species applied. Heartwood, sapwood, transition wood, knots, bark etc. all possess different properties and contain chemically different species. Also, hardwood (e.g. Silver birch) as well as softwood species (e.g. Norway spruce and Scots pine) behaved differently, and obvious reason is their different structure: Hardwood species are known to be easier to deconstruct than softwood species, due to the fact that softwood species have guaiacyl lignin as their main lignin type, while lignin in hardwood species is less recalcitrant, i.e. combination of guaiacyl and syringyl lignin. Also, the hexoses degradation product, 5-HMF, was detected in increasing amounts as a function of the treatment time.

Treatment temperatures above 150 °C as well as longer treatment times (above 100 h) result in tar and/or humins formation and degradation of all sugars. Further, degradation of sugars was most prominent upon use of C_2MIM Ac. Treatment times ranging from 0 to 20 h yielded the best results, considering that the main focus was to obtain mono- or polysaccharides.

Di- and oligosaccharides obtained (depolymerized from wood polysaccharides) should also be analyzed in detail in future, since cellobiose was the only determined disaccharide in the present work. However, cellobiose was seen only occasionally in some samples. The fructose concentration could not always be detected, nor quantitatively determined due to the overlapping tendency of mannose, fructose and arabinose peaks in the electropherograms obtained with the used CE methodology. Rhamnose was determined in the beginning, but the determination of it also suffered from peak overlapping. Thus rhamnose calibration standard was abandoned at an early stage.

The large variability yield of sugars even for a single type of feedstock is probably a sign that the solubilisation of lignin and hemicellulose is very sensitive to treatment conditions such as temperature, time and moisture content of the ionic liquid and/or biomass.

Regarding the pretreatment of wheat straw following conclusions can be made: acetate ionic liquids were more efficient in attacking the cellulose thus helping to achieve higher hydrolysis yields. The optimal pretreatment time for wheat straw was 24 h when acetate ILs and convective/conductive heating were applied and, in case of chloride ILs, no plateau was achieved in 72 h. Regarding SEM images, C_4MIM Ac treatment result in the wheat straw structure to be disorganized and swollen.

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Appendix 2

Publication II

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

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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.

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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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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

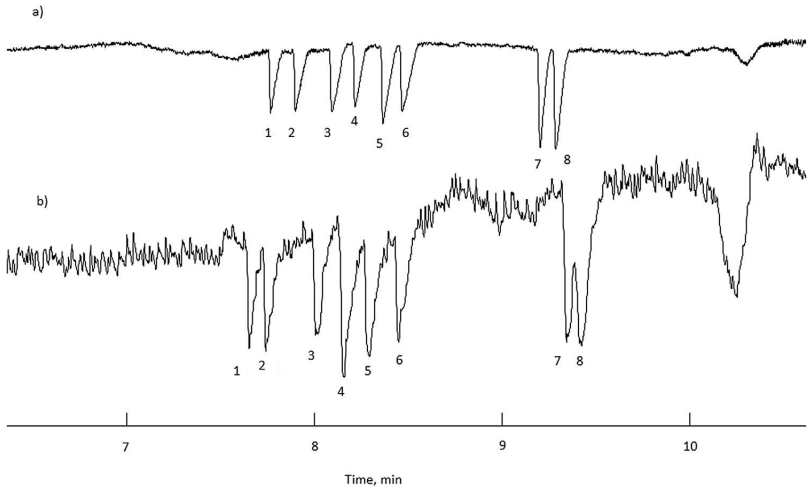

Fig. 1. Electropherogram of 8 carbohydrates analysed with (a) ID 22.5 μm and (b) ID 50 μm capillary. CE conditions were following: a) BGE contained 138.2 mM NaOH, 40 mM maleic acid and 5 mM C_{14}MImCl; b) BGE contained 100 mM NaOH, 40 mM maleic acid and 5 mM C_{14}MImCl; absorbance was measured at 210 nm; capillary length 70/61.5 cm. Identification: 1—xylose, 2—fructose, 3—glucose, 4—galactose, 5—cellobiose, 6—lactose, 7—raffinose, 8—sucrose (analyte concentration: 2 mM of each).

determined by measuring the concentration of the control sample containing 2 mM analytes in five replicates during one day. Inter-day precision was calculated over a 4-day observation period for four replicates.

3. Results and discussions

3.1. Capillary choice for noise reduction

Carbohydrates have pK_a values between 12 and 13, requiring therefore a high pH of the separation electrolyte. The conductivity of the medium increases with pH, resulting in high electrophoretic current; preliminary measurements with a 50 μm capillary showed that an increase in NaOH concentration caused a low signal-to-noise ratio and unstable noisy baseline (Fig. 1). The NaOH content was increased with a fixed amount (20 mM in each analysis) and rising electrical current (at 100 mM NaOH concentration about 108 μA) resulted in unstable and noisy baseline that became too disruptive before the NaOH concentration necessary for complete separation was achieved. After decreasing the capillary ID to 22.5 μm, the baseline became noticeably stable during the analysis, electrical current was reduced (at 140 mM NaOH concentration about 27 μA) and the signal-to-noise ratio increased significantly.

3.2. Optimization of separation conditions

In recent years, chemometric tools have become more and more popular to be used for the optimization of analytical methods, due to their several advantages such as the small number of experiments and hence, lower consumption of reagents, and less laboratory work. Therefore, in this study, the BGE conditions were optimized statistically using a Box-Behnken design with response surface methodology. The matrix of experiments and analysis of experimental results for optimization were performed by JMP 12 software. The concentrations of compounds were varied as follows: NaOH from 70 to 140 mM, maleic acid from 10 to 40 mM, phthalic acid from 10 to 30 mM, PDC from 10 to 20 mM, and C_{14}MImCl from 1 to 5 mM, while the voltage was changed from −15 to −30 kV. Carbohydrates, such as xylose, fructose, glucose, galactose, cellobiose, lactose, sucrose, and raffinose were used for separation. Optimization was performed employing a desirability function that was set to maximize resolutions between carbohydrates and minimize the baseline noise. The baseline noise was chosen to as a variable since preliminary tests showed that increase of NaOH and/or surfactant concentration was adversely affecting this factor.

The predicted conditions for optimal carbohydrates separation for different chromophores are presented in Table 1. In all cases, the optimal chromophore content appeared to be the given upper limit, therefore the solubility of a substance determined the limit for phthalic acid and PDC. The separation of two organic acids, eight carbohydrates and 5-HMF was performed within 12–15 min depending on the BGE used and the fastest separation was achieved with maleic acid BGE (Fig. 2S in Supporting information).

The 3D response surface plots for PDC and maleic acid BGEs are presented in Fig. 2 (3D response surface plots for phthalic acid BGE are shown in Fig. S3 in Supporting information). In the figure, the relationship between the concentrations of NaOH, C_{14}MImCl and chromophore and the baseline noise is depicted. In Fig. 2a, the relationship between the baseline noise, and the concentrations of NaOH and C_{14}MImCl is shown. From that figure it can be seen that, the increase in NaOH concentration resulted in the decrease in the signal-to-noise ratio. At the same time, the increase in surfactant concentration resulted in stabilizing effect for baseline. In Fig. 2b, the relationship between the concentrations of maleic acid and C_{14}MImCl concentration and the baseline noise is shown. From the figure, it can be concluded that with increasing surfactant concentration the baseline noise increases in the case of low chromophore concentrations. In Fig. 2c, the relationship between the concentration of maleic acid and NaOH and the baseline noise is depicted. The surface plot in the figure shows that the signal-to-noise ratio decreases with increasing NaOH concentration. This can be compensated for increasing the maleic acid concentration in BGE. Taken together, the disruptive noise obtained due to high NaOH concentration, which is essential to achieve good resolution of analytes, can be reduced by increasing the concentrations of maleic acid and surfactant in BGE. A similar dependence between components was observed in the case of phthalic acid BGE (Fig. S3 in Supporting information).

Slightly different results were obtained for optimized PDC BGE. At first, the optimal NaOH and surfactant concentrations in PDC

Table 1
Optimized conditions for three BGEs.

Chromophore	NaOH (mM)	Chromophore (mM)	C_{14}MImCl (mM)	Voltage (kV)
Maleic acid	138.2	40.0	5.0	−21.7
Phthalic acid	140.0	28.0	4.0	−20.6
PDC	110.7	20.0	1.8	−21.2

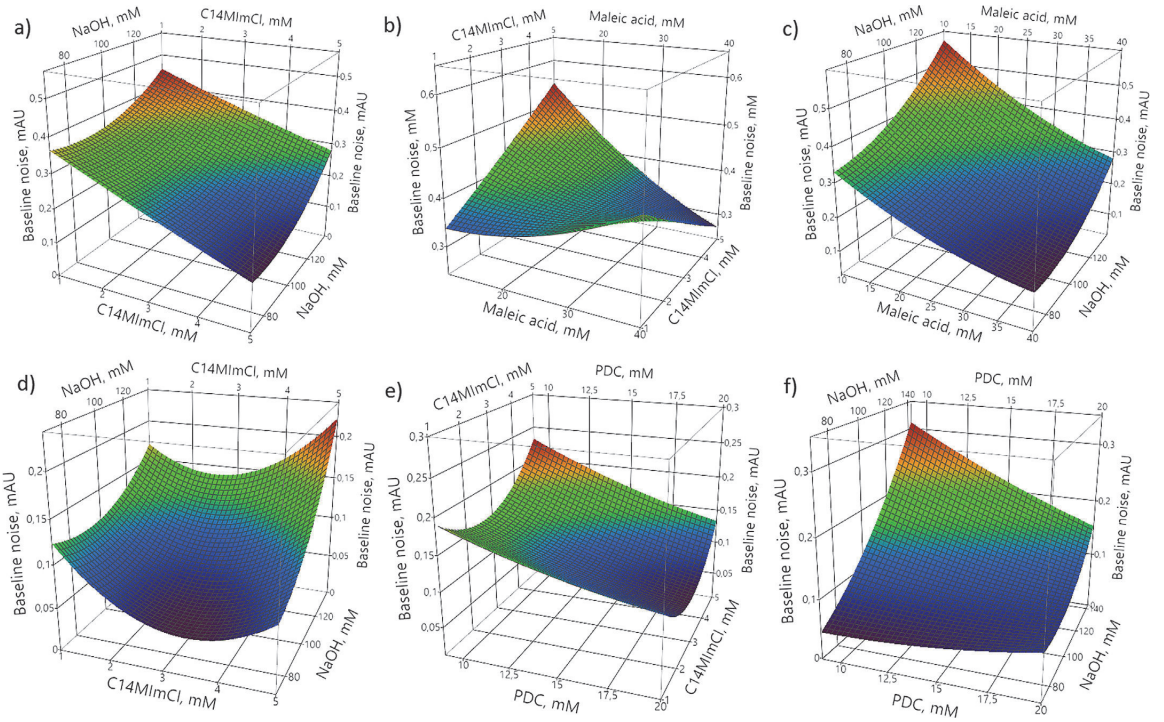

Fig. 2. Response surface plots for baseline noise relationships with concentrations of (a) NaOH/C_{14}MImCl, (b) maleic acid/C_{14}MImCl (c) maleic acid/NaOH in maleic acid BGE, and (d) NaOH/C_{14}MImCl, (e) PDC/C_{14}MImCl and (f) PDC/NaOH in PDC BGE.

BGE were remarkably lower than those in other BGEs. From Fig. 2d it can be seen that the higher concentration of surfactant resulted in higher baseline noise, in contrast to the case with other BGEs. The relationship between the baseline noise and the concentration of PDC and C_{14}MImCl depicted in Fig. 2e indicates that with maximum chromophore concentration the surfactant mid concentration value is preferable unlike other BGEs. As can be seen from Fig. 2f, the baseline noise increases with increasing NaOH concentration and this can be improved by increasing the PDC concentration, similarly to the case with other BGEs.

3.3. Validation of the method

3.3.1. Linearity and precision

Linearity and precision were evaluated using optimized BGEs composed of phthalic and maleic acids. Analysis revealed that cellooligomer standards contained impurity which could be separated only with maleic acid BGE. Hence, maleic acid BGE was preferred in the further study. The result of regression analysis and the coefficients of determination (R^2) for analytes are given in Table 2. In the table, the analytes are listed in their migration order. As seen from the table, the coefficient of determination for all analytes is greater than 0.99 resulting in good linearity, though. Repeatability was determined for a standard sample that was measured in five replicates on the same day and reproducibility was determined for four replicates over a 4-day observation period. The results show excellent repeatability and reproducibility of the migration times of the analytes, and good repeatability and reproducibility of their peak areas.

3.3.2. Limits of detection and quantification

In the case of sucrose, raffinose, and levulinic and acetic acids LoQ was 0.20 mM while for other carbohydrates it was 0.25 mM and LoD values were between 0.06 and 0.08 mM. Since 5-HMF absorbs well at 270 nm, its LoD and LoQ values were remarkably lower when maleic or phthalic acid BGE was used, being −0.02 and 0.05 mM, respectively. In addition, for 5-HMF PDC absorbs in the same region gives higher LoD and LoQ values −0.05 and 0.17 mM, respectively.

3.3.3. Robustness

Robustness was studied for the IL (EMImCl) concentration in the sample. For separation optimized conditions for maleic acid BGE were used. Analytes such as acetic acid, levulinic acid, fructose, glucose, cellobiose, cellotriose, cellotetraose, cellohexaose and 5-HMF were used for the separation in the presence of EMImCl. The concentration of organic acids, 5-HMF, mono-, di- and oligosaccha-

Table 2
Linearity and precision for maleic acid BGE.

Analyte	Regression analysis			Precision I (peak areas)		Precision II (migration times)	
	Linear range (mM)	Equation ($y = ax + b$)	R^2	RSD (%)Inter-day	RSD (%)Intra-day	RSD (%)Inter-day	RSD (%)Intra-day
Acetic acid	0.20–7.00	y = 4.5135x − 0.1492	0.9923	2.73	3.47	0.39	1.09
Levulinic acid	0.20–7.00	y = 3.6353x + 0.2880	0.9955	3.20	4.29	0.24	1.21
D-(+)-xylose	0.25–7.00	y = 2.9891x + 0.2491	0.9976	4.07	9.62	0.37	1.76
D-(−)-fructose	0.25–7.00	y = 3.1838x + 0.3906	0.9964	4.20	7.53	0.05	1.89
D-(−)-glucose	0.25–7.00	y = 3.1948x + 0.4343	0.9961	4.32	7.34	0.11	1.98
D-(+)-galactose	0.25–7.00	y = 3.0800x + 0.7616	0.9954	4.42	6.63	0.10	2.02
D-(+)-cellobiose	0.25–7.00	y = 4.4243x + 0.6508	0.9969	4.57	8.89	0.25	2.12
β-lactose	0.25–7.00	y = 4.7923x + 0.3475	0.9962	4.63	9.21	0.18	2.16
Cellotriose	0.25–5.00	y = 4.7025x + 0.3008	0.9928	4.73	9.25	0.34	2.17
Cellotetraose	0.25–5.00	y = 4.0007x + 0.1563	0.9902	4.86	8.06	0.06	2.24
Cellopentaose	0.25–5.00	y = 4.0037x + 0.2177	0.9936	4.95	7.08	0.47	2.28
Cellohexaose	0.25–5.00	y = 4.1686x + 0.0419	0.9942	5.19	6.63	0.23	2.45
D-(+)-raffinose	0.20–7.00	y = 5.2987x + 0.0611	0.9963	5.29	6.24	0.17	2.33
Sucrose	0.20–7.00	y = 6.4173x + 0.0908	0.9974	5.12	8.38	0.22	2.62
5-HMF	0.05–7.00	y = 27.691x + 3.6858	0.9977	0.58	3.49	0.34	2.64

Table 3
Influence of IL concentration to analytes peak areas, heights and migration times expressed as RSD values, n = 3.

Analyte	Peak area, RSD[a] (%)	Peak height, RSD[a] (%)	Migration time, RSD[a] (%)
Acetic acid	9.51	19.25	0.93
Levulinic acid	7.73	23.08	1.03
Fructose	5.18	19.77	1.34
Glucose	4.77	21.89	1.36
Cellobiose	2.79	20.25	1.21
Cellotriose	7.86	23.35	1.44
Cellotetraose	7.44	21.55	1.17
Cellopentaose	6.82	18.72	1.52
Cellohexaose	8.39	14.96	2.02
5-HMF	2.76	4.73	2.16

[a] RSD% are calculated for samples with concentrations of 0, 100, 200, 300 and 400 mM EMImCl.

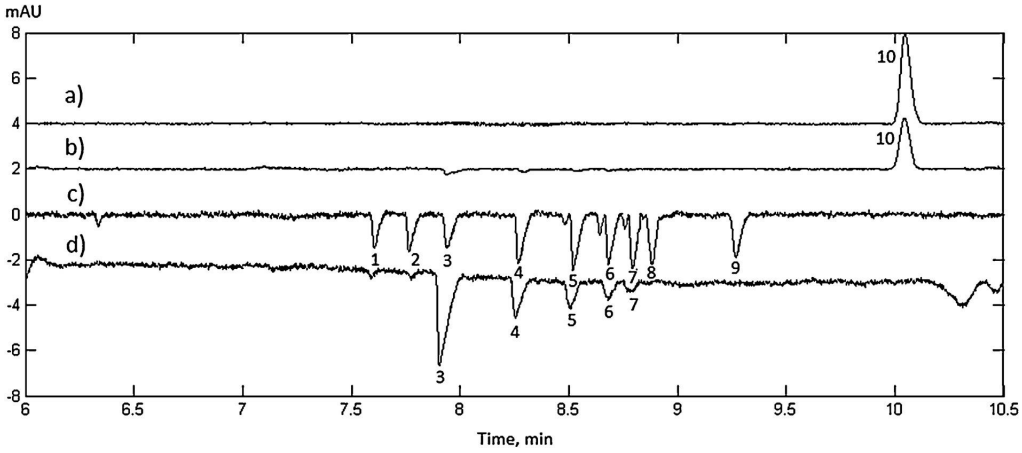

Fig. 3. Separation of standards (a, c) and cellulose degradation products (b, d). CE conditions were following: BGE contained 138.2 mM NaOH, 40 mM maleic acid and 5 mM C_{14}MImCl; capillary ID 22.5 μm; absorbance was measured at 210 (a, b) and 270 (c, d) nm; applied voltage was −21.7 kV. Identification: 1—xylose, 2—fructose, 3—glucose, 4—cellobiose, 5—cellotriose, 6—cellotetraose, 7—cellopentose, 8—cellohexaose, 9—sucrose, 10—5-HMF (analyte concentration: 1 mM of each, except 5-HMF, which was 0.5 mM).

Table 4
Hydrolysis of cellulose in EMImCl, analysed with maleic acid BGE, n = 3.

Analyte	Sample 1 (g/L)	Sample 2 (g/L)	Sample 3 (g/L)	Sample 4 (g/L)	Sample 5 (g/L)
Glucose	n.d.	16.09	19.48	37.46	33.21
Cellobiose	n.d.	7.21	7.81	12.46	9.39
Cellotriose	n.d.	12.38	12.60	14.10	10.42
Cellotetraose	n.d.	12.86	13.85	10.90	7.46
Cellopentaose	n.d.	9.36	9.90	7.47	5.87
5-HMF	n.d.	0.02	0.14	0.54	0.34

n.d.—Not detected.

rides was 2 mM each and the IL concentration varied from zero to 400 mM (0, 100, 200, 300 and 400 mM IL). Parameters like peak area, peak height and migration times were evaluated, the results are presented in Table 3. From Table 3 it can be seen that migration times of analytes were not affected by the increase of IL concentration. However, the peak areas and heights varied a lot. All the observed peak areas decreased in size when the IL concentration was increased to 400 mM. However, the decreased peak areas remained whitin tolerable limits—between 2.76 and 9.51%. A major issue with the increase of IL concentration was related to the diminishing peak heights, which led to higher LoD values of analytes in the presence of IL with a high concentration (400 mM). When the IL concentration was ≤200 mM, RSDs for peak heights were between 3.41 and 10.53%. Therefore, it is recommended to construct a calibration curve in the presence of IL with a concentration similar to that of the samples.

3.3.4. Hydrolysis of cellulose in IL

We have demonstrated an efficient separation of cellooligomers obtained from the hydrolysis of cellulose in EMImCl using aqueous HCl solution (Fig. 3). In addition to carbohydrates, the formation of 5-HMF was also observed. Table 4 presents the carbohydrates concentrations obtained in the ILs solution. As expected, after the addition of HCl, the cellulose chain started to decompose. At first, cellulose decomposed to longer oligomers that started to break down to shorter ones like glucose and cellobiose. This is well proved by the results obtained. Sample 2 contained a greater amount of cellopentaose and cellotetraose compared to samples 4 and 5, and the highest glucose and cellobiose concentrations were obtained in the case of sample 4 and sample 5. Since no additional catalyst was added to the reaction mixture, the highest 5-HMF concentration remained low (>0.54 g/L). Trace amount of fructose and xylose was detected can be seen from the sample electropherogram (concentrations remained under LoQ). The presence of 5-HMF also confirms the formation of fructose since 5-HMF is synthesized via glucose isomerization to fructose followed by fructose dehydration reaction. According to literature, microcrystalline cellulose may contain a small amount of residual hemicelluloses [36] this explains the presence of xylose. The glucose concentration of sample 5 started to decrease after addition of water for the third time, which was probably related to the presence of an unidentified compound that was formed with increasing reaction time. This unknown compound absorbed well at 210 nm but, unfortunately, remained neutral at pH value of BGE used, being though difficult to quantify. As mentioned above, the IL content is essential from the quantification point of view; thus, the IL content for different samples was calculated. In all cases, the IL concentration remained below 255 mM, having a negligible influence on the results.

4. Conclusions

The simultaneous separation of underivatized mono-, di- and oligosaccharides, acetic acid, levulinic acid and 5-HMF, i.e. products that can be formed upon combustion of lignocellulosic biomass as an alternative source for fuels and chemicals, was performed employing a background electrolyte composed of 138.2 mM NaOH, 40 mM maleic acid, and 5 mM C_{14}MImCl. The BGE conditions were optimized statistically, using desirability analysis for determining the optimal voltage and concentrations of BGE components. The obtained results confirm that this method, having a wide linear range (therefore smaller number of dilutions), can be used to monitor biomass degradation and 5-HMF formation, offering good precision and fast performance. Also, the method demonstrates good robustness for IL concentration in the sample.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.04.027.

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Appendix 3

Publication III
T. Aid, M. Koel, M. Lopp and M. Vaher, “Metal-Catalyzed Degradation of Cellulose in Ionic Liquid Media”, *Inorganics*, vol. 78 (6), 1−11, 2018.

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Article

Metal-Catalyzed Degradation of Cellulose in Ionic Liquid Media

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Abstract: Biomass conversion to 5-hydroxymethylfurfural (HMF) has been widely investigated as a sustainable alternative to petroleum-based feedstock, since it can be efficiently converted to fuel, plastic, polyester, and other industrial chemicals. In this report, the degradation of commercial cellulose, the isomerization of glucose to fructose, and the conversion of glucose to HMF in 1-butyl-3-methylimidazolium chloride ([BMIM]Cl]) using metal catalysts ($CrCl_3$, $ZnCl_2$, $MgCl_2$) as well as tungsten and molybdenum oxide-based polyoxometalates (POM) were investigated. Tungsten and molybdenum oxide-based POMs in ionic liquids (IL) were able to degrade cellulose to majority glucose and epimerize glucose to mannose (in the case of the molybdenum oxide-based POM). A certain amount of glucose was also converted to HMF. The tungsten oxide-based POM in IL showed good activity for cellulose degradation but the overall products yield remained 28.6% lower than those obtained using $CrCl_3$ as a catalyst. Lowering the cellulose loading did not significantly influence the results and the addition of water to the reaction medium decreased the product yields remarkably.

Keywords: biorefinery; platform chemicals; cellulose degradation; catalysis

1. Introduction

An efficient utilization of renewable biomass resources, particularly lignocellulosic biomass, is important from the viewpoint of the production of industrial platform chemicals and fuels (Figure 1) [1]. An appropriate technology would efficiently deconstruct the biomass to release cellulose and hemicellulose and hydrolyze cellulosic components to generate oligosaccharides [2].

Of the fundamental building blocks from biorefineries, 5-hydroxymethylfurfural (HMF) is considered a key intermediate for the development of biomass-based products, because a series of compounds such as organic acids, polymer precursors, and biofuels derive from it [3].

Hence the selective catalytic conversion of cellulose to a platform chemical such as glucose, HMF, sorbitol or gluconic acid under mild conditions is the most desirable route in industry [4,5]. However, a high-yield, low-cost, energy-efficient, and direct conversion method for cellulose to HMF is still a challenge to researchers in the field.

Ionic liquids (ILs) are being investigated as effective low-impact solvents for the conversion of cellulose due to being relatively easy to handle, recyclable, and in possession of a negligible vapor pressure. Moreover, ILs have an ability to solubilize low-toxicity metal catalysts for the direct conversion of cellulose to value-added chemicals. However, most published papers focus mainly on the advantages of ionic liquids in the separation of cellulose from lignocellulosic biomass over traditional methods [6–9].

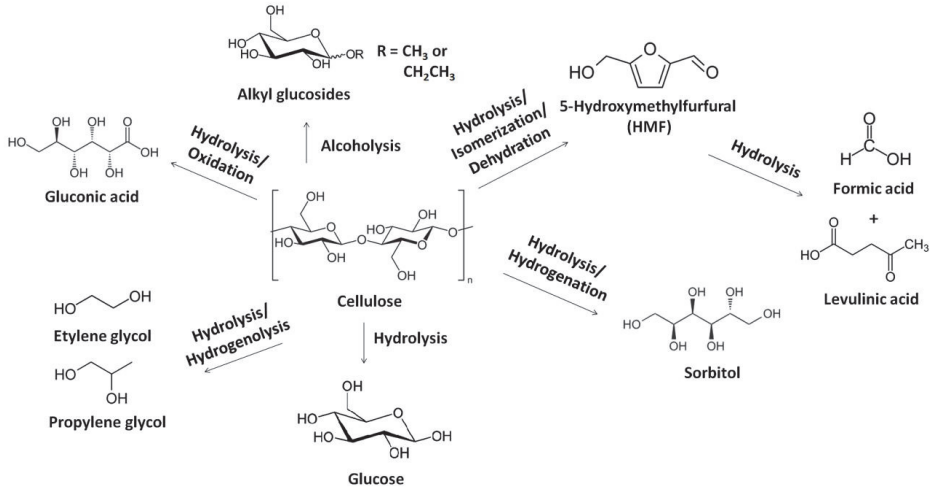

Figure 1. Typical platform chemicals produced from the catalytic transformations of cellulose.

From previous studies it appears that when using cellulose as a feedstock for HMF formation, the key steps are the dissolution and depolymerisation of cellulose to glucose monomers and not the isomerization to fructose and subsequent dehydration of fructose to HMF. Cellulose has an abundant number of intra- and intermolecular hydrogen bonds that make it intrinsically recalcitrant to depolymerize which is why ionic liquids as solvents can provide a solution due to their specific ability to dissolve the compound [10]. ILs appear to be even more advantageous in overcoming these problems as they can act as both solvents and catalysts [7]. The catalytic conversion of biomass-derived carbohydrates to value-added chemicals is a commercially important reaction and requires the use of both Lewis and Brønsted acids. Multifunctional ILs with both types of acidity are promising catalysts as well as solvents for the one-pot conversion of glucose to value-added chemicals.

Until now, the production of HMF with glucose as a feedstock has efficiently used chromium chloride catalyst together with ionic liquids. Zhao et al. [11] were the first to report an unparalleled yield of 69% HMF catalyzed by chromium (II) chloride ($CrCl_2$) in a medium of 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl) at 100 °C for 3 h. Recently, the dehydration of glucose to HMF with a yield of about 67% was achieved in [EMIM]Cl and *N*,*N*-dimethylacetamide (DMA) by using $CrCl_2$ or $CrCl_3$ at 100 °C for 6 h [12]. Dehydration of cellulose to HMF with a yield of about 89% was achieved in [EMIM]Cl by using $CrCl_2$ at 120 °C for 6 h [13]).

The catalytic activity of a $ZnCl_3$-containing IL was found to be highly Lewis acidic, and was the controlling parameter for glucose conversion, resulting in yields of 13.4%, 23.8%, and 67.1% of HMF, levulinic acid (LA) and formic acid (FA), respectively. These multifunctional chlorometallate ILs were found to be recyclable with no loss of metal chloride from their anion [14].

Brønsted acidic ionic liquids (BAILs) showed a very good catalytic effect on the conversion of raw biomass to C5 sugars and furfural. In water medium the remarkably high yield of C5 sugars from bagasse, 88%, was obtained using 1-methyl-3(3-sulfopropyl)-imidazolium hydrogen sulfate ($[C_3SO_3HMIM][HSO_4]$). Similarly, with this BAIL, a high yield (73%) of furfural was obtained in the one-pot method using a water/toluene biphasic solvent system [15].

Calculations revealed the dependence of the catalytic performance of $[C_3SO_3HMIM][HSO_4]$ BAIL on the acidity and nucleophilicity of its constituent ions [16].

1,1,3,3-tetramethylguanidine tetrafluoroborate ($[TMG]BF_4$) as a solvent was confirmed to exhibit excellent catalytic activity in the conversion of C6 carbohydrates to HMF. The HMF yields from the

conversion of fructose, glucose, cellobiose, and microcrystalline cellulose (MCC) were 74%, 27%, 20%, and 18%, respectively [17].

The search for effective catalysts has brought polyoxometalates–anionic metal oxides under consideration because of their unique properties, such as strong Brønsted acidity, good oxidizing ability, high water tolerance, low corrosiveness, and recoverability. In addition, POMs have exhibited a promising performance in the transformation of cellulose into platform chemicals in both homogeneous and heterogeneous systems [18].

A review by Deng et al. [19] highlights the following good catalytic properties of POMs in the conversion of cellulose to platform chemicals:

1. strong Brønsted acidity;
2. the capability to activate oxidants such as O_2 and H_2O_2 for selective oxidation;
3. high water tolerance;
4. tunable acidity, redox potential, and solubility in various media, which allow the rational design of active sites on molecular and atomic scales;
5. high thermal and oxidative stability as compared with common molecular catalysts such as organometallic complexes and enzymes;
6. ease of handling and separation, and the relatively low corrosiveness, possibly owing to the generated corrosion-inhibiting films, which allow them to act as environmentally friendly liquid-phase catalysts, unlike mineral acids.

Another approach is to use task-specific ionic liquids with polyoxometalates (POMs) contained in them. Due to their high negative charge and large metal-oxide framework, POMs can react with a variety of cationic organic groups to form novel functional ionic liquids, POMs-ILs. IL cations with various structures and properties can provide organic blocks to modify POM catalysts, thanks to their acidity, polarity, solubility, redox properties, and surface structures.

Polyoxomethalates-based ionic liquids (POM-IL) as solid acid catalysts have been used for the direct conversion of fructose to HMF. The phosphotungstic acid (HPW)-derived ionic liquid shows the highest catalytic performance (up to 99% of the yield) in the formation of HMF. In this study, the catalyst afforded a good yield of HMF from inulin (76%) and sucrose (45%) as well [20].

In another work [21], POM-IL were synthesized and employed for the one-pot dissolution and conversion of powdered switchgrass biomass. For comparison purposes, Avicel Cellulose was also treated under identical conditions. The most promising for biomass conversion was found to be the combination of phosphotungstic acid hydrate and 1-butyl-3-methylimidazolium bromide. Avicel Cellulose was then utilized for the hydrolysis at 200 °C for 120 min, and as a result, approximately 31 wt % of the biomass and 13 wt % of Avicel Cellulose were converted to water-soluble products; i.e., sugars obtained from the deconstructed cellulose [21].

There are some other examples where heteropolyacids act as efficient catalysts for the conversion of glucose. For example, a 98% conversion of glucose to HMF in ionic liquids and a 99% selectivity of HMF were attained after a 3 h reaction time at 393 K by using 12-molybdophosphoric acid in a mixture of either 1-ethyl-3-methylimidazolium or 1-butyl-3-methylimidazolium chloride with acetonitrile as a co-solvent [22].

Analysis of ILs-containing samples where capillary electrophoresis (CE) is proposed as an effective method for the simultaneous determination of mono-, di- and oligosaccharides, as well as HMF and organic acids (acetic and levulinic acids can be formed during degradation of HMF), is no easy task [23,24]. The objective of this case study was to compare molybdenum and tungsten oxide-based POM-ILs catalytic performance with metal catalysts, such as $CrCl_3$, $ZnCl_2$ and $MgCl_2$ using ILs as solvents.

2. Results and Discussion

The main goal was to monitor the transformation of microcrystalline cellulose to HMF via glucose and fructose by different widely-studied catalysts and compare the results with molybdenum and tungsten oxide-based POMs-ILs. Although the isomerization and epimerization of glucose might occur, the formation of fructose predominated. The formation of fructose via isomerization required some reorganization of the intermediate, whereas the formation of mannose is reported through rotation around the C2–C3 bond [25]. One of the glucose isomerization reaction pathways is analogous to that observed in metalloenzymes, such as D-xylose isomerase (XI), which contain two divalent Lewis acid metal centers (Mg^{2+} or Mn^{2+}) confined within a hydrophobic pocket [26,27]. The reaction pathway includes the ring-opening of glucose and coordination with glucose O1 and O2 atoms prior to the isomerization of the ring-opened glucose chain from position C2 to C1 (via 1,2 intramolecular hydride shift) [28].

Table 1 (Table S1) shows, the conversion of cellulose to HMF in [BMIM]Cl at 100 °C for 24 h with $ZnCl_2$, $MgCl_2$, $CrCl_3$, molybdenum and tungsten oxide-based POM-ILs as catalysts. In the case of molybdenum and tungsten oxides-based POM-ILs the main products were monosaccharides. Table 1 also contains the total product yields where all of the obtained products are summarized.

Table 1. Glucose, mannose and 5-hydroxymethylfurfural (HMF) contents in cellulose samples degraded in [BMIM]Cl using $ZnCl_2$, $MgCl_2$, $CrCl_3$, tungsten (W-POM) and molybdenum (Mo-POM) oxide-based polyoxometalates (POMs) as catalysts. Catalyst loading was 10 wt % from cellulose and cellulose loading was 10 wt %.

Catalyst	Glucose, %	Mannose, %	HMF, %	Total Products Yield, %
No catalyst	>0.04	>0.04	>0.04	>0.04
$ZnCl_2$	>0.04	>0.04	4.64	4.64
$MgCl_2$	>0.04	>0.04	3.78	3.78
$CrCl_3$	>0.04	>0.04	55.3	55.3
W-POM	33.7	>0.04	5.82	39.5
Mo-POM	4.96	2.26	2.95	10.2

As expected, the sample with no catalyst did not contain any of the investigated products. The tungsten oxide-based POM showed good activity for cellulose degradation, however, the overall yield of products (glucose and HMF) remained 29% lower than that obtained with $CrCl_3$ as a catalyst. The main product formed was glucose instead of HMF. The molybdenum oxide-based POM was the only catalyst that was able to epimerize glucose to mannose, while the latter accounted for 22% of the products obtained. The catalytic activity of the molybdenum oxide-based POM remained remarkably lower and the overall product yields (glucose, mannose and HMF) were 82% less compared to $CrCl_3$. The catalytic ability of $MgCl_2$ and $ZnCl_2$ were the lowest and HMF yields were 6.8% and 8.4% of that obtained with $CrCl_3$, respectively.

Some metal chlorides are very effective as catalysts for the synthesis of HMF in ILs [29,30]. The isomerization of glucose into fructose is favored by the presence of Lewis acid sites [25]. Figure 2 shows the mechanism proposed by Zhou et al. for the interaction of metal chlorides with glucose in ILs such as [BMIM]Cl. According to this mechanism, good catalytic performance of $CrCl_3 \cdot 6H_2O$ may be explained by the formation of a more stable metal chloride–glucose complex due to the stronger coordination ability of Cl^- with a chromium center [29–31].

Li et al. [32] demonstrated in their study that according to density functional theory (DFT) calculations, isomerization of glucose to fructose over tungsten oxide-based catalysts is possible because of Lewis acid sites (W6+), terminal W-oxo groups that are Lewis basic sites, and proton mediators, such as "structural" and physisorbed water on the oxide surface. According to their study the key aspect of the catalytic mechanism is the proton shift from C2 → C1 that is promoted by a synergistic action of the Lewis acid sites, which is followed by a proton-transfer [32].

Ju et al. [33] demonstrated in their ^{13}C NMR study that molybdenum-based POMs are active and selective catalysts for the epimerization of aldoses. The epimerization mechanism involves electron transfer from the aldose to the molybdenum oxide octahedra surface units of the POM followed by an intra molecular C1 → C2 carbon shift. They also report that replacing Mo with W in the Kegging structure POM resulted in loss of the epimerization activity, indicating that the molybdenum octahedral located in the cagelike structure of the POM play an important role in activating the epimerization of glucose [33].

Nguyen et al. [34] demonstrated in their ^{13}C NMR and ^{1}H NMR study that glucose epimerization to mannose using Lewis acids, such as MCl_3 in aqueous phase ($CrCl_3$ was also tested), proceeds via two parallel mechanisms: first a reverse C2 → C1 hydride transfer followed by a C1 → C2 intramolecular carbon shift. They also report that MCl_3 are also able to epimerize glucose to mannose in low yields and since fructose formation is predominant, the hydride transfer is the more dominant pathway of glucose conversion [34].

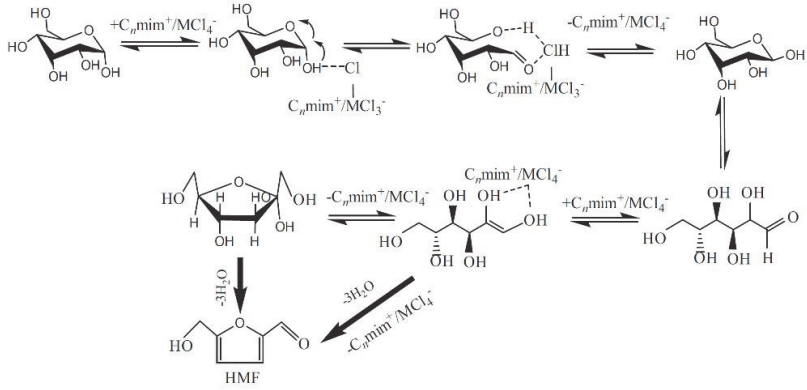

Figure 2. Interaction of metal chlorides with glucose to produce HMF in C_nMIM/MCl_4 (n = 4, 1-n-butyl-3-methylimidazolium, [BMIM]Cl; M = Cr, Al and Fe) [35].

2.1. Reaction Time for POM-IL Catalysts

Figure 3 (Tables S2 and S3) shows the effect of time on the yield of glucose, mannose and HMF from cellulose at 100 and 120 °C for 72 h using tungsten and molybdenum oxide-based POMs as catalysts.

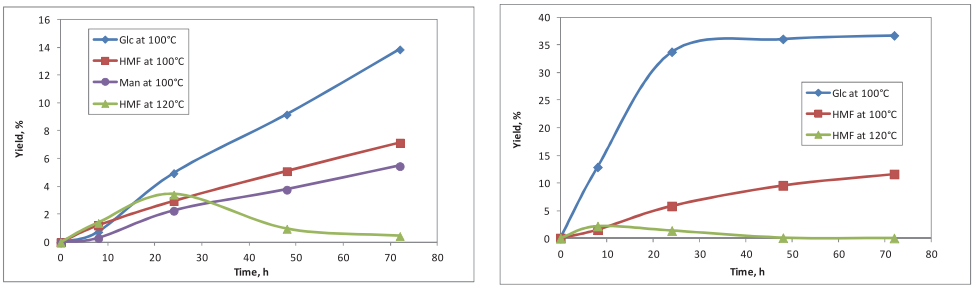

Figure 3. Conversion of cellulose to glucose, mannose and HMF in [BMIM]Cl at 100 and 120 °C by using POMs based on (**a**) molybdenum oxide and (**b**) tungsten oxide. Catalyst loading was 10 wt % from cellulose and cellulose loading was 10 wt % from solvent.

In the case of the molybdenum oxide-based POM, prolonging the reaction time improved the HMF yield at 100 °C and the plateau in its concentration was not achieved by 72 h. The same trend held true for the yields of glucose and mannose. At 120 °C, glucose and mannose were not detected and the maximum HMF yield remained lower than the yield obtained at 100 °C. The maximum HMF yield, which was achieved at 24 h, was 52% lower, and furthermore, it started to decrease with a longer reaction time. In the case of the tungsten oxide-based POM, prolonging the reaction time at 100 °C improved the glucose yield up to 24 h and then a plateau was achieved. The HMF yield increased slowly for 72 h. Eminov et al. [7] reported that the highest HMF yield with $CrCl_3 \cdot 6H_2O$ as a catalyst in [BMIM]Cl was obtained at 120 °C and it was 5 times higher than the yield obtained at 100 °C. In this work, the temperature was also increased by 20 °C, yet the ability to convert glucose to HMF was not improved. Instead, the opposite effect was observed: glucose was not present in any sample and the maximum HMF yield was achieved at 8 h, remaining 81.4% lower than the result obtained at 100 °C. According to the literature the lower yield could be caused by humin formation at higher temperatures [36]. Possible humin formation is also supported by the fact that a dark precipitate was formed when the higher temperature was used.

2.2. Cellulose Loading and the Efficiency of POM Formation

Chidambaram and Bell [22] reported that in [BMIM]Cl at 120 °C 3% of glucose is converted to humin even without using a catalyst. During the investigated process humins can be also formed due to the oligomerization of glucose or fructose with itself as well as with HMF. However, this is inhibiting the conversion of cellulose to shorter oligomers and glucose [36,37]. The formation of glucose and conversion to the other products must be balanced well to avoid the formation of humins. Thereby, cellulose loadings of 5 and 10 wt % were selected to investigate the effectivity of HMF formation at 120 °C.

Figure 4 (Table S4) shows the results for conversion of cellulose to HMF in [BMIM]Cl at 120 °C by using tungsten and molybdenum oxide-based POMs at 5 wt % and 10 wt % substrate loading. The HMF yield followed the same trend in spite of substrate loading for both the catalysts used. In the case of the molybdenum oxide-based POM-IL the maximum HMF yield was achieved at 24 h and was 52.2% of the maximum yield obtained with a 10 wt % catalyst loading. The maximum HMF yield for the tungsten oxide-based catalyst was achieved during 8 h and made 46.8% of the yield obtained with a 10% substrate loading. In addition, all samples obtained from 48 and 72 h experiments contained dark colored precipitate that was most probably humin. Lowering the substrate loading did not improve the conversion to HMF.

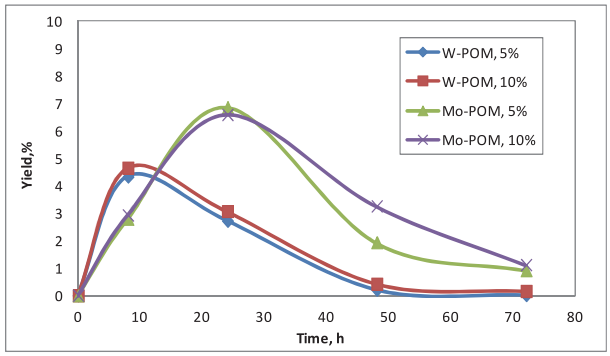

Figure 4. Conversion of cellulose to HMF in [BMIM]Cl at 120 °C by using tungsten and molybdenum oxide-based POMs at 5 and 10% substrate loading. Catalyst loading was 10 wt % from cellulose and cellulose loading was 5 or 10 wt % from solvent.

Figure 5 (Tables S5 and S6) shows the cellulose degradation at 100 °C for 72 h to evaluate the efficiency of POM formation in the IL medium. The catalyst (phosphotungstic acid hydrate) was added either 20 min before or 3 h after the substrate.

The highest glucose and HMF yields were when the catalyst was added to the sample before the substrate, with respective yields at 73.7% and 68.4% higher. These results show that the formation of POM is more efficient when the catalyst was added before the substrate.

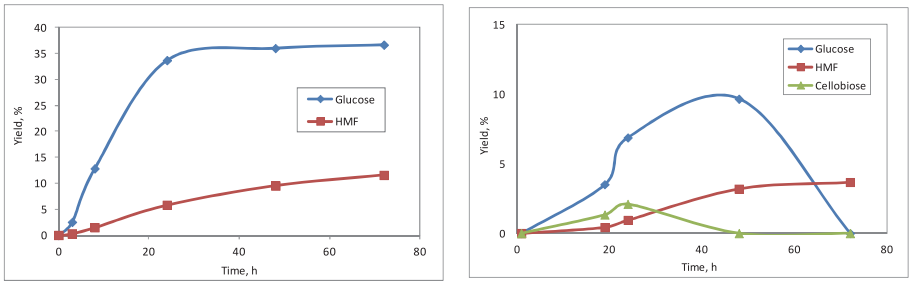

Figure 5. Efficiency of cellulose degradation in [BMIM]Cl at 100 °C by using phosphotungstic acid hydrate added (**a**) 20 min before the substrate, (**b**) 3 h after the substrate. Catalyst loading was 10 wt % from cellulose and cellulose loading was 10 wt % from solvent.

2.3. *Effect of the Water Content of the Reaction Medium on the Conversion of Cellulose to HMF*

Some data is available from the literature on metal catalysts. Zhang et al. [10] reported an increase of HMF yield from glucose in the presence of a higher amount of water in [EMIM]Cl when $CrCl_2$ was used as a catalyst. From the other side, Zhao et al. [12] reported that water has no influence on reaction yields. No data was available for POM-s. Since [BMIM]Cl is a hydroscopic ionic liquid, the influence of water content on the reaction dynamics by using tungsten oxide-based POM as catalysts was studied (Figure 6, Tables S7 and S8). The efficiency of HMF conversion and the influence of the reaction medium water content on the process were evaluated at 100 °C for 72 h.

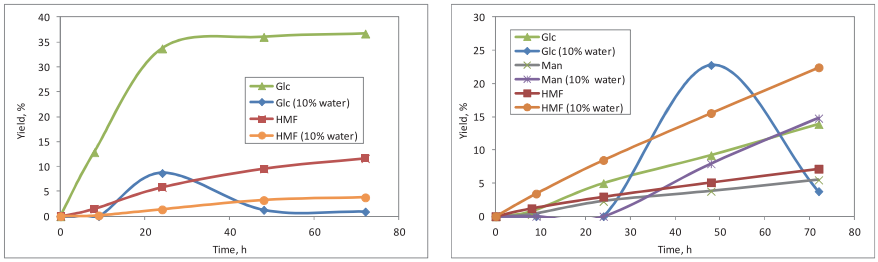

Figure 6. Conversion of cellulose to glucose, mannose and HMF in [BMIM]Cl at 100 °C in the presence of water (10 wt % of solvent) by using POMs based on (**a**) tungsten oxide, (**b**) molybdenum oxide. Catalyst loading was 10 wt % from cellulose and cellulose loading was 10 wt % from solvent.

Tungsten (VI) and molybdenum (VI) are expected to form POMs with imidazolium-based ILs. POMs are a class of anionic metal-oxygen clusters built by the connection of [MO]x polyhedral of the early transition metals in their highest oxidation states [38].

In the case of the tungsten oxide-based POM, the highest glucose and HMF yields in the presence of water were respectively 76.3% and 67.4% lower. The same tendency was observed with the

molybdenum oxide-based POM-IL: the yields of glucose, mannose and HMF were correspondingly 72.7%, 59.2% and 52.8% lower in the presence of water. The experiment showed that increasing the water content resulted in the loss of catalytic activity of both the catalysts used.

3. Materials and Methods

3.1. Catalytic Formation of HMF from Cellulose

All experiments were carried out under aerobic conditions using dried ionic liquids; however, IL was handled and weighed as quickly as possible to prevent further absorption of water. All the catalysts used were in their hydrated form. Catalysts $CrCl_3$, $ZnCl_2$, $MgCl_2$, phosphotungstic acid hydrate, phosphomolybdic acid hydrate and microcrystalline cellulose were obtained from Sigma-Aldrich (Sigma-Aldrich, Steinheim, Germany) and were used as received. [BMIM]Cl was obtained from IoLiTec (Ionic Liquids Technologies GmbH, Heilbronn, Germany) and was vacuum-dried before use. The IL water content, which was checked by Karl–Fischer–Titration, was 0.6–0.7%. Catalyst loading was 10 wt % of the substrate in all cases, the substrate loading was 10 wt % from the solvent in most cases, if not otherwise stated, and the sample typically contained 1 g of IL. The experiments were carried out on a magnetic stirrer plate at 100 or 120 °C in open flasks with constant stirring. All the concentrations of oligo- and monosaccharides and HMF in reaction media were determined by CE and were analyzed at least in triplicate [23]. At first, the catalyst was dissolved in IL and after 20 min the cellulose (substrate) was added to the reaction mixture. The samples to compare different catalysts (Table 1) were collected at 24 h after the addition of cellulose. Unusually, a long reaction time (considering the speed of $CrCl_3$ catalyst) was chosen because preliminary testing showed a slow conversion for molybdenum and tungsten oxide-based POMs.

3.2. Analysis

All the samples were analyzed by capillary electrophoresis and the yields were calculated based on calibration curves constructed using authentic standards. CE separations were performed using an Agilent 3D instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array UV/Vis detector. Data acquisition and instrument control were carried out using Agilent Technologies HP 3D Chemstation software. The separation was performed using a fused silica capillary with an effective length of 61.5 cm (total length 70 cm) and ID of 22.5 μm (Polymicro Technologies Inc., Wilmington, DE, USA). Capillary walls contained a semi-permanent coating with $[C_{14}MIM]Cl$ that was added to the background electrolyte (BGE). The BGE was composed of 138 mM NaOH, 40 mM maleic acid and 5 mM $[C_{14}MIM]Cl$. The samples were injected hydrodynamically under a pressure of 50 mbar for 20 s and the separations were performed at 25 °C by using a voltage of −21 kV. The detection wavelength was 210 nm in the case of carbohydrates and 270 nm for HMF. Before each run the capillary was filled with BGE for 7 min and between the runs it was flushed with 1 M NaOH for 2 min and ultrapure water for 3 min. BGE was prepared on the first day and stored at room temperature for one month. All these conditions were developed in our previous study [23]. Standards used to construct calibration curves and BGE substances, namely D-(+)-glucose, D-(−)-fructose, D-(+)-mannose, D-(+)-cellobiose, sucrose, HMF, maleic acid and NaOH, were obtained from Sigma-Aldrich and were used as received. $[C_{14}MIM]Cl$ was obtained from IoLiTec and was vacuum dried before use.

4. Conclusions

It has been shown that tungsten and molybdenum oxide-based polyoxometalates were able to decompose cellulose. The main decomposition products were carbohydrates such as glucose and mannose. The ability of the polyoxometalates to convert glucose to 5-hydroxymethylfurfural remained low in the mild conditions used and the overall product yields with the use of the tungsten oxide-based polyoxometalates remained 28.6% lower compared to the yield obtained with $CrCl_3$. It was expected that increasing the temperature would improve the conversion of HMF but, surprisingly, increasing

the temperature by 20 °C did not increase the HMF outcome. The increase of the water content of the reaction medium, an influencing factor in fructose dehydration, resulted in the loss of catalytic activity of tungsten and molybdenum oxide-based polyoxometalates.

Supplementary Materials: The following are available online at http://www.mdpi.com/2304-6740/6/3/78/s1, Table S1: Glucose, mannose and HMF contents in cellulose samples degraded in [BMIM]Cl using $ZnCl_2$, $MgCl_2$, $CrCl_3$, tungsten (W-POM) and molybdenum (Mo-POM) oxide-based POMs as catalysts, Table S2: Conversion of cellulose to glucose, mannose and HMF in [BMIM]Cl at 100 and 120 °C by using POMs based on molybdenum oxide, Table S3: Conversion of cellulose to glucose, mannose and HMF in [BMIM]Cl at 100 and 120 °C by using POMs based on tungsten oxide, Table S4: Efficiency of cellulose degradation in [BMIM]Cl at 100°C by using phosphotungstic acid hydrate added 20 min before the substrate, Table S5: Conversion of cellulose to HMF in [BMIM]Cl at 120°C by using tungsten and molybdenum oxides-based POMs at 5 and 10% substrate loading, Table S6: Efficiency of cellulose degradation in [BMIM]Cl at 100°C by using phosphotungstic acid hydrate added 3 h after the substrate, Table S7: Conversion of cellulose to glucose, mannose and HMF in [BMIM]Cl at 100 °C in the presence of water (10 wt % of solvent) by using POMs based on tungsten oxide, Table S8: Conversion of cellulose to glucose, mannose and HMF in [BMIM]Cl at 100 °C in the presence of water (10 wt % of solvent) by using POMs based on (a) tungsten oxide, (b) molybdenum oxide.

Author Contributions: T.A. and M.V. conceived and designed the experiments; T.A. performed the experiments; T.A. and M.V. analyzed the data; M.V. and M.L. contributed reagents/materials/analysis tools. The manuscript was written through contributions of all authors.

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Conflicts of Interest: The authors declare no conflict of interest.

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Täiendusõpe

Kevad 2016	Kursus “Mõõtemääramatuse hindamine keemilises analüüsis”, Tartu Ülikool, Eesti
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