Ionic liquid mediated biomass deconstruction: from analysis challenges to fermentable sugars

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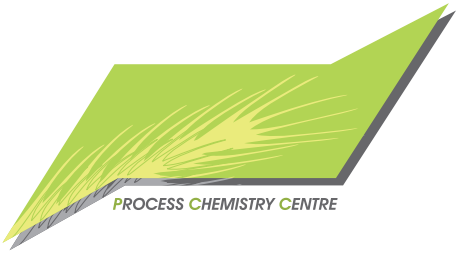

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PREFACE

The present work was carried out at the Laboratory of Industrial Chemistry and Reaction Engineering, Department of Chemical Engineering at Åbo Akademi University and the Analytical Chair, Department of Chemistry at Tallinn University of Technology between 2008 and 2014.

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Turku/Åbo, April 2014

Sari Hyvärinen

ABSTRACT

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Ionic liquid mediated biomass deconstruction: from analysis challenges to fermentable sugars

Doctoral Thesis, Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Department of Chemical Engineering, Åbo Akademi University, 2014

Keywords: Biorefinery, lignocellulosic biomass, ionic liquid-pretreatment, fractionation, deconstruction, hydrolysis, cellulose, hemicelluloses, polysaccharides, monosaccharides, oligosaccharides, 5-(hydroxymethyl)-2-furaldehyde, furfural, gas chromatography, liquid chromatography, capillary electrophoresis

Ionic liquids, ILs, have recently been studied with accelerating interest to be used for a deconstruction/fractionation, dissolution or pretreatment processing method of lignocellulosic biomass. ILs are usually utilized combined with heat. Regarding lignocellulosic recalcitrance toward fractionation and IL utilization, most of the studies concern IL utilization in the biomass fermentation process prior to the enzymatic hydrolysis step. It has been demonstrated that IL-pretreatment gives more efficient hydrolysis of the biomass polysaccharides than enzymatic hydrolysis alone. Both cellulose (especially cellulose) and lignin are very resistant towards fractionation and even dissolution methods. As an example, it can be mentioned that softwood, hardwood and grass-type plant species have different types of lignin structures leading to the fact that softwood lignin (guaiacyl lignin dominates) is the most difficult to solubilize or chemically disrupt.

In addition to the known conventional biomass processing methods, several ILs have also been found to efficiently dissolve either cellulose and/or wood samples – different ILs are suitable for different purposes. An IL treatment of wood usually results in non-fibrous pulp, where lignin is not efficiently separated and wood components are selectively precipitated, as cellulose is not soluble or degradable in ionic liquids under mild conditions. Nevertheless, new ILs capable of rather good fractionation performance have recently emerged. The capability of the IL to dissolve or deconstruct wood or cellulose depends on several factors, (e.g. sample origin, the particle size of the biomass, mechanical treatments as pulverization, initial biomass-to-IL ratio, water content of the biomass, possible impurities of IL, reaction conditions, temperature etc).

The aim of this study was to obtain (fermentable) saccharides and other valuable chemicals from wood by a combined heat and IL-treatment. Thermal treatments alone contribute to the degradation of polysaccharides (e.g. 150 °C alone is said to cause the degradation of polysaccharides), thus temperatures below that should be used, if the research interest lies on the IL effectiveness. On the other hand, the efficiency of the IL-treatment can also be enhanced to combine other treatment methods, (e.g. microwave heating).

The samples of spruce, pine and birch sawdust were treated with either 1-Ethyl-3-methylimidazolium chloride, Emim Cl, or 1-Ethyl-3-methylimidazolium acetate, Emim Ac, (or with ionized water for comparison) at various temperatures (where focus was between 80 and 120 °C). The samples were withdrawn at fixed time intervals (the main interest treatment time area lied between 0 and 100 hours). Double experiments were executed. The selected mono- and disaccharides, as well as their known degradation products, 5-hydroxymethylfurfural, 5-HMF, and furfural were analyzed with capillary electrophoresis, CE, and high-performance liquid chromatography, HPLC. Initially, even GC and GC-MS were utilized.

Galactose, glucose, mannose and xylose were the main monosaccharides that were present in the wood samples exposed to ILs at elevated temperatures; in addition, furfural and 5-HMF were detected; moreover, the quantitative amount of the two latter ones were naturally increasing in line with the heating time or the IL:wood ratio.

REFERAT

Sari Hyvärinen

Joniskvätskemedlat fraktionering av biomassa: från analysutmaningar till fermenterbara sockrar
Doktorsavhandling, Laboratoriet för teknisk kemi och reaktionsteknik, Processkemiska centret, Institutionen för kemiteknik, Åbo Akademi, 2014

Nyckelord: bioraffinaderi, biomassa från lignocellulosa, joniskvätske-förbehandling, fragmentering, hydrolys, cellulosa, hemicellulosor, polysackarider, monosackarider, oligosackarider, 5-hydroximetyl-2-furaldehyd, furfural, gaskromatografi, vätskekromatografi, kapillärelektrofores

Joniska vätskor (förkortas senare i avhandlingen som IL) har nyligen undersökts som ett intressant alternativ för att användas för fraktionering, upplösning eller förbehandling av lignocellulosa. Vanligtvis utnyttjas joniska vätskor i kombination med värme. De flesta studierna gällande joniska vätskor berör lignocellulosamaterialets motståndskraft mot fraktioneringen och utnyttjandet av joniska vätskor i biomassans jäsnings- eller fermenteringsprocess före enzymatiskt hydrolyssteg. Det har visats att jonisk vätskeförbehandling ger en mer effektiv hydrolys av polysackarider i biomassa än vad enbart enzymbehandlingen gör. I synnerhet cellulosa men också lignin är mycket resistenta mot fraktionerings- och även mot upplösningsmetoder. Som ett exempel kan nämnas att barrved, lövved och gräs består alla av olika typ av ligninstrukturer (guajacyl-, guajacyl-syringyl- samt *p*-kumaryl -ligniner) som representerar olika nivåer av motstånd för fraktionering: barrveds guajasyl-dominerande lignin är den svåraste ligninkomponenten att upplösas eller att sönderdelas kemiskt.

Förutom kända konventionella biomassbearbetningsmetoder har också flera joniska vätskor visat sig effektivt kunna lösa upp antingen cellulosa och/eller trä. Beroende på joniska vätskors och biomassasortens egenskaper, lämpar sig joniska vätskor i varierande grad för olika ändamål. Jonisk vätskebehandling av trä resulterar vanligtvis i en icke-upplöst cellulosafraktion - nästan i sin naturliga form men dock närmare sett fibrös massa - medan lignin och hemicellulosor har helt eller delvis nedbrutits. Detta händer även vid konventionell pappersmassatillverkning, t.ex. vid Kraftkokning under starkt alkaliska betingelser. Cellulosa löser sig inte heller i organiska lösningsmedel. Vanligen leder en jonisk vätskebehandling också enbart till fibrös cellulosa, selektiv utfällning av träkomponenter utan någon effektiv separation av lignin. Cellulosa är i regel inte löslig eller nedbrytbar i joniska vätskor under milda kemiska betingelser. Nyligen har dock nya joniska vätskor utvecklats med ganska imponerande fraktioneringsförmåga. Egenskaper hos joniska vätskor, t.ex. förmågan att dekonstruera eller upplösa trä eller cellulosa beror naturligtvis på flera faktorer. Dessa faktorer är biomassaprovets ursprung, partikelstorlek, möjliga mekaniska behandlingar (pulveriseringen med mera), initial biomassa:joniskvätske-förhållande, vattenhalten i biomassan, joniska vätskors möjliga föroreningar, kemiska reaktionsförhållanden, temperatur etc.

Syftet med denna studie var att nå fermenterbara mono- och disackarider samt andra värdefulla kemikalier från ved genom en kombinerad värme-joniskvätskebehandling. Termiska behandlingar bidrar också till nedbrytning av polysackarider (t.ex. 150° C kan ensamt orsaka depolymerisering eller nedbrytning av polysackarider), och därför temperaturer under den skall användas om forskningsintresset ligger på effektiviteten av joniska vätskor. Å andra sidan kan effektiviteten av joniskvätskebehandlingen även förbättras genom att kombinera andra behandlingsmetoder, till exempel uppvärmning i en mikrovågsugn. Det finns dock en stor risk för lokal överhettning för joniska vätskor är salter och därmed sammankopplas ytterst starkt med mikrovågsstrålning.

Prov av gran-, tall-, och björksågspån behandlades antingen med 1-etyl-3-metylimidazoliumklorid, Emim Cl, eller 1-etyl-3-metylimidazoliumacetat, Emim Ac, - eller som referens, även med avjonat vatten för jämförelse - vid olika temperaturer (där fokus var mellan 80 och 120 °C). Proverna togs vid fasta tidsintervall (det huvudsakliga intresset log i tidsintersvallet från 0 till 100 timmar) . Dubbla experiment utfördes. Den valda gruppen av mono- och disackarider, samt deras kända nedbrytningsprodukter - nämligen 5-hydroximetylfurfural, 5-HMF, och furfural - analyserades med hjälp av kapillärelektrofores, CE; högupplösande vätskekromatografi, HPLC och även med gaskromatografi, GC och gaskromatografi-masspektrometri, GC-MS. Galaktos, glukos, mannos och xylos var de dominerande monosackarider som fanns i träproverna efter joniska vätskebehandlingar. Förutom sockrar, fanns det furfural och 5-HMF i proven. Generellt kan man säga att mindre mängder av furfural detekterades än 5-HMF. Den kvantitativa mängden av de två sistnämnda ökar i takt med tiden eller värmepåverkan. Det kan också konstateras att förhållandet jonisk vätska:ved påverkas resultat i hög grad. Många studier har rapporterats gällande optimering av kombination av dessa egenskaper och experimentella betingelser.

LIST OF PUBLICATIONS

The thesis consists of the following publications, which are referred to in the text by their Roman numerals.

I. **S. Hyvärinen**, P. Virtanen, D. Yu. Murzin, J.-P. Mikkola, Towards ionic liquid fractionation of lignocellulosics for fermentable sugars, *Cellulose Chemistry and Technology*, 44, 4–6 (2010) 187–195.
Contribution: performed the experiments, analyzed the samples and wrote the article.

II. R. Pezoa, V. Cortinez, **S. Hyvärinen**, M. Reunanen, J. Hemming, M.E. Lienqueo, O. Salazar, R. Carmona, A. Garcia, D. Yu. Murzin, J.-P. Mikkola, The use of ionic liquids in the pretreatment of forest and agricultural residues for the production of bioethanol, *Cellulose Chemistry and Technology*, 44, 4–6 (2010) 165–172.
Contribution: practical help with GC analysis samples and participating in writing process.

III. **S. Hyvärinen**, P. Damlin, J. Gräsvik, D. Yu. Murzin, J.-P. Mikkola, Ionic liquid fractionation of woody biomass for fermentable monosaccharides, *Cellulose Chemistry and Technology*, 45, 7–8 (2011) 483–486.
Contribution: performing the experiments, analysis and writing the article excluding the experiments concerning pure cellulose, which were performed by P. Damlin.

IV. **S. Hyvärinen**, E. Leino, V. Eta, E. Privalova, E. Salminen, J. Gräsvik, P. Virtanen, P. Mäki-Arvela, J.-P. Mikkola, Ionic liquids as catalytic medium for biomass transformations, in *'Heterogeneous Catalysis in Biomass to Chemicals and Fuels*', Eds. D. Kubička and I. Kubičková, Research Signpost, Kerala, India, ISBN: 978-81-308-0462-0, (2011) 65–102
Contribution: participating in writing the text excluding the parts concerning CO_2 capture methods, which have been written by E. Leino and V. Eta.

V. T. Riittonen, V. Eta, **S. Hyvärinen**, L.J. Jönsson, J.-P. Mikkola, Engineering aspects of bioethanol synthesis in '*Advances in Chemical Engineering - Chemical engineering for renewables conversion*', Ed. D. Yu. Murzin, Burlington: Elsevier, Academic Press, ISBN: 978-0-12-386505-2, Vol. 42 (2013) 1–73.
Contribution: writing pages 5–18.

VI. **S. Hyvärinen**, J.-P. Mikkola, D. Yu. Murzin, M. Vaher, M. Kaljurand, M. Koel, Sugars and sugar derivatives in ionic liquid media obtained from lignocellulosic biomass: Comparison of capillary electrophoresis and chromatographic analysis, *Catalysis Today*, 223 (2014) 18–24.
Contribution: performed the experiments, analyzed the samples and wrote the article.

VII. **S. Hyvärinen**, T. Aid, M. Vaher, M. Kaljurand, M. Koel, J.-P. Mikkola, Sugars and sugar derivatives in ionic liquid mediated lignocellulose, (submitted).
Contribution: performed the experiments, analyzed the samples and wrote the article primarily together with Tiina Aid.

LIST OF OTHER CONTRIBUTIONS RELATED TO THE TOPIC

1. **S. Hyvärinen**, D. Yu. Murzin, J.-P. Mikkola, Bioethanol from lignocellulosics, *Seminar: 'Teknisk kemi - Hållbar utveckling (sommarskola)'*, Umeå and Örnsköldsvik, Sweden, June 10–11, 2008, oral presentation.

2. A. García, **S. Hyvärinen**, R. Carmona, M.E. lienqueo, O. Salazar, Enfoque chromatográfico applicado al pretratamiento biológico de lignocelulosas con miras a la obtención de bioetanol, *II Latin American Congress: Biorefinery, Materials and Energy*, May 4–6, 2009, Concepción, Chile, oral presentation.

3. R. Pezoa, **S. Hyvärinen**, M. Reunanen, J. Hemming, M.E. Lienqueo, D. Yu. Murzin, J.-P. Mikkola, The use of ionic liquids in the pre-treatment of agricultural residues for the production of bioethanol, *2nd Nordic Wood Biorefinery Conference*, September 2–4, 2009, Helsinki, Finland, poster presentation.

4. V. Cortinez, **S. Hyvärinen**, M. Reunanen, J. Hemming, M.E. Lienqueo, D. Yu. Murzin, J.-P. Mikkola, Evaluation of ionic liquid mediated pretreatment for the enzymatic hydrolysis of forest residues for bioethanol production, *2nd Nordic Wood Biorefinery Conference*, September 2–4, 2009, Helsinki, Finland, poster presentation.

5. **S. Hyvärinen**, P. Virtanen, J. Hemming, M. Reunanen, A.-S. Leppänen, A. Pranovich, S. Willför, D. Yu. Murzin, J.-P. Mikkola, Ionic Liquid Fractionation of Lignocellulosics, *2nd Nordic Wood Biorefinery Conference*, September 2–4, 2009, Helsinki, Finland, poster presentation.

6. **S. Hyvärinen**, D. Yu. Murzin, J.-P. Mikkola, Optimal Treatment Process of Lignocelluloses to Bioethanol - (CONICYT), *SusEn Annual Seminar 2009*, October 6–7, 2009, Helsinki, Finland, oral presentation.

7. **S. Hyvärinen**, J.-P. Mikkola, D. Yu. Murzin, CO2 neutral technologies: towards utilization of ionic liquids and micro-organisms in biomass transformation to NextGen bioethanol, *CO2-SUSE –seminar at Åbo Akademi Process Chemistry Centre, Laboratory of Industrial Chemistry and Reaction Engineering*, October 8–9, 2009, Turku/Åbo, Finland, oral presentation.

8. **S. Hyvärinen**, P. Virtanen, A. Pranovich, A.-S. Leppänen, J. Hemming, M. Reunanen, S. Willför, D. Yu. Murzin, J.-P. Mikkola, Towards improved fractionation of lignocellulosics via integrated ionic liquid-thermal pretreatment – The analysis challenge, *XVII Congreso Chileno de Ingeniería Química*, October 25–28, 2009, Viña del Mar, Valparaiso, Chile, poster presentation.

9. **S. Hyvärinen**, D. Yu. Murzin, J.-P. Mikkola, Chemical products and biofuels from biomass, the Finnish experience, *International seminar: Biofuels and their future in the power matrix BIOCOMSA-2*, November 3–5, 2009, Santiago, Chile, oral presentation.

10. **S. Hyvärinen**, D. Yu. Murzin, J.-P. Mikkola, Biofuels and Chemical products from woody biomass, *International workshop/seminar: Taller Grupo Bioetanol-Domeyko-Finlandia*, December 2, 2009, Santiago, Chile, oral presentation.

11. **S. Hyvärinen**, P. Virtanen, R. Pezoa, M.E. Lienqueo, D. Yu. Murzin, J.-P. Mikkola, Ionic liquid fractionation of woody biomass for fermentable monosaccharides, *The 19th International Congress of Chemical and Process Engineering CHISA 2010 and the 7th European Congress of Chemical Engineering ECCE-7*, August 28 - September 1, 2010, Prague, Czech Republic, poster presentation.

12. M.E. Lienqueo, R. Pezoa, V. Cortinez, **S. Hyvärinen**, T. Niklitschek, O. Salazar, R. Carmona, A. Garcia, D. Yu. Murzin, J.-P. Mikkola, Use of ionic liquids for the production of second generation bioethanol, *International Conference and Fair on Education, Research & Employment in the Renewable Energy sector, ICERE 2*, November 11–12, 2010, Santiago, Chile, oral presentation.

13. **S. Hyvärinen**, D. Yu. Murzin, J.-P. Mikkola, K. Peiponen, M.E. Lienqueo Contreras, Ionic liquid mediated pretreatment of lignocellulose, *COST-meeting (CMo903) "Utilisation of Biomass for Sustainable Fuels and Chemicals"*, January 20–21, 2011, Utrecht, The Netherlands, oral presentation.

14. **S. Hyvärinen**, P. Damlin, J. Gräsvik, D. Yu. Murzin, J.-P. Mikkola, Ionic liquid fractionation of woody biomass for fermentable monosaccharides, *The 3rd Nordic Wood Biorefinery Conference*, March 22-24, 2011, Stockholm, Sweden, poster presentation.

15. V. Kontturi, **S. Hyvärinen**, R. Carmona, A. García, D. Yu. Murzin, J.-P. Mikkola and K.-E. Peiponen, Simultaneous detection of the absorption spectrum and refractive index ratio with a spectrophotometer: monitoring contaminants in bioethanol, *Measurement Science and Technology* 22 (2011) 5, 055803/1-055803/6. (Contribution: participating in writing the article).

16. **S. Hyvärinen**, P. Damlin, J. Gräsvik, D. Yu. Murzin, J.-P. Mikkola, Ionic liquid fractionation of woody biomass for fermentable monosaccharides, *Conference on Materials and Technologies for Green Chemistry (SustainChem2011)*, September 5–9, 2011, Tallinn, Estonia, poster presentation.

17. **S. Hyvärinen**, K. Peiponen, M.E. Lienqueo Contreras, D. Yu. Murzin, J.-P. Mikkola, Optimal Treatment Processes of Lignocellulosics to Bio-ethanol, *SusEn Annual Seminar*, September 22, 2011, Helsinki, Finland, oral presentation.

18. **S. Hyvärinen**, P. Damlin, J. Gräsvik, D. Yu. Murzin, J.-P. Mikkola, Ionic liquid fractionation of woody biomass for fermentable monosaccharides, *SusEn Annual Seminar*, September 22, 2011, Helsinki, Finland, poster presentation.

19. **S. Hyvärinen**, J.-P. Mikkola, D. Yu. Murzin, M. Vaher, M. Kaljurand, M. Koel, Capillary Electrophoresis versus HPLC analysis methods used for analyzing sugars and sugar derivatives in ionic liquid media obtained from lignocellulosic biomass, *UBIOCHEM-III: Sustainable production of fuels/energy, materials & chemicals from biomass, the third International Workshop of COST Action CM0903*, November 1–3, 2012, Thessaloniki, Greece, oral presentation.

20. **S. Hyvärinen**, J.-P. Mikkola, D. Yu. Murzin, M. Vaher, M. Kaljurand, M. Koel, Quo Vadis separation science in ionic solutions: CE vs. LC, *Workshop of COST Action CM0903 (UBIOCHEM): Molecules, methods and metrics*, January 24–25, 2013, Palermo, Italy, oral presentation.

21. **S. Hyvärinen**, M. Vaher, D. Yu. Murzin, M.E. Lienque, M. Kaljurand, M. Koel, J.-P. Mikkola, Ionic liquid mediated pretreatment of lignocellulose, *The 5th congress on ionic liquids, COIL-5*, April 21–25, 2013, Algarve, Portugal, poster presentation.

22. **S. Hyvärinen**, J.-P. Mikkola, D. Yu. Murzin, M. Vaher, M. Kaljurand, M. Koel, CE vs. HPLC analysis methods used for analyzing sugars and sugar derivatives in IL media obtained from lignocellulosic biomass, *POKE 1st workshop – kick-off meeting, Sustainable chemistry and process technology in the Northern Baltic Sea region*, June 11–12, 2013, Stockholm, Sweden, oral presentation.

23. **S. Hyvärinen**, M. Vaher, D. Yu. Murzin, M.E. Lienque, M. Kaljurand, M. Koel, J.-P. Mikkola, Ionic Liquid Mediated Pretreatment of Lignocellulose, *Åbo Akademi Process Chemistry Centre Annual Meeting*, August 29, 2013, Åbo/Turku, Finland, poster and flash oral presentation.

24. **S. Hyvärinen**, M. Vaher, D. Yu. Murzin, M.E. Lienque, M. Kaljurand, M. Koel, J.-P. Mikkola, Ionic liquid mediated pretreatment of lignocellulose, *Final meeting of Cost action FP0901 "Biorefinery analytics – Outcomes from FP0901"*, September 17–18, 2013, Åbo/Turku, Finland, poster presentation.

25. **S. Hyvärinen**, J.-P. Mikkola, D. Yu. Murzin, M. Vaher, M. Kaljurand, M. Koel, HPLC analysis methods used for analyzing sugars and sugar derivatives in IL media obtained from lignocellulosic biomass, *POKE symposium (Sustainable chemistry and process technology in the Northern Baltic Sea region)*, December 11–12, 2013, Tallinn, Estonia, oral presentation.

26. **S. Hyvärinen**, M. Vaher, M. Kaljurand, M. Koel, M.E. Lienque, D. Yu. Murzin, J.-P. Mikkola, Capillary electrophoresis in biorefinery analysis, *International Workshop, Algal Biorefinery*, March 17–18, 2014, Santiago, Chile, oral presentation.

27. **S. Hyvärinen**, M. Vaher, M. Kaljurand, M. Koel, M.E. Lienque, D. Yu. Murzin, J.-P. Mikkola, Comparison of chromatographic and capillary electrophoresis analysis methods used for ionic liquid mediated biomass samples, *International Workshop on Ionic Liquids: Melting Temperature, other Properties and Applications*, March 20–21, 2014, La Serena, Chile, oral presentation.

28. **S. Hyvärinen**, M. Vaher, M. Kaljurand, M. Koel, M.E. Lienque, D. Yu. Murzin, J.-P. Mikkola, Deconstruction of lignocellulose to carbohydrates and their derivatives in ionic liquid media, *The 5th NWBC (Nordic Wood Biorefinery Conference),* March 25–27, 2014, Stockholm, Sweden, poster presentation.

CONTENTS

1. INTRODUCTION

1.1 Possibilities for the utilization of lignocellulosic biomass

Besides wood products or pulp making, high amounts of lignocellulosic biomass is currently treated as a waste instead of using it in biorefinery processes: residues from forestry or agriculture, straw and various energy crops could tentatively be utilized by first depolymerizing the polysaccharides (cellulose and hemicelluloses) in the biomass to monosaccharides, which could then be converted further to alcohols, acids, or biopolymers (through chemical and enzymatic catalysis, or conventional fermentation process). Alcohols and acids could further be used in the production of liquid fuels, other chemicals, and bio-based polymers [1, **VI**].

To achieve a high sugar yield from the lignocellulosic biomass (e.g. when producing bioethanol), the biomass is typically pre-treated thermo-chemically before adding enzymes [2]. The existing lignocellulose pre-treatment technology include acid-based methods that may involve the use of catalysts (e.g. sulfuric acid and sulfur dioxide treatments, steam explosion and hydro-thermolysis) and alkaline methods (e.g. ammonia fiber explosion, AFEX) [2]. The biomass pre-treatment method and reaction conditions must be selected depending on the recalcitrance of the feedstock. For example, acid hydrolysis effectively degrades the hemicelluloses and facilitates enzymatic attack on cellulose, being a suitable pre-treatment option for more recalcitrant types of lignocellulosic biomass, such as softwood [2, **VI**].

Apart from the conventional pre-treatment methods discussed above, alternative technologies have also emerged. An example of these is the processing of lignocellulose in ionic liquids, ILs. Efficiency for the dissolution of cellulose, lignin, and even wood has been proven for a range of ionic liquids, (e.g. dialkyl imidazolium derivates [3] and organic super-base derived ILs [4]). Applications of various ionic liquid, IL, mediated treatments, in combination with heat [4—8], have demonstrated a possibility to dissociate wood polysaccharides to monosaccharides rather well. However, despite the efficient solvent properties of some ionic liquids, ILs, wood is not readily soluble in ionic liquids under mild dissolution conditions (e.g. 80 °C, 18 h) [5] — instead, upon IL treatment of wood, non-fibrous pulp is usually obtained, while lignin is not efficiently separated and wood components are selectively precipitated [**VI**]. Nevertheless, there are recent examples demonstrating a possibility of using mild conditions and resulting in fibrillated pulp [**V**, 6, 9].

A possibility to isolate both mono- and polysaccharides obtained from biomass, gives rise to interesting options for generating new products. Some high-value products utilizing biomass-derived mono- or polysaccharides have already been successfully developed and commercialized (e.g. xylitol, furfural and various products based on lignin, cellulose or hemicelluloses) [10—13]. As yet an interesting example, biodegradable films (e.g. derived from xylans and mannans) and other products have been developed, and they are to some extent, expected to be able to compete with plastics in the future [13—15, **VI**].

1.2 Ionic liquids, ILs

Definitions and general characteristics

An ionic liquid, IL, consists of a minimum of two components, a cation and an anion, or it can also consist of multi-ionic substances or compounds with internal charge separation (such as e.g. zwitterions, dual charged ions and deep eutectic solvents), or it can be a mixture of ions and molecular species [9, 16]. Estimations regarding the possible amount of different ion combinations go up to 10^{18}, thus it is evident that their properties also vary, even though there exists a bunch of common generalizations concerning the properties of ILs: they are often said to encompass a low vapor pressure, non-flammability, low toxicity, high thermal stability and are assumed to be liquids at a temperature of 100 °C or below – however, many ILs do not display all of these properties. The toxicity of many ionic liquids remains still to be thoroughly investigated, although many today commonly used ionic liquids have been shown to be toxic - some of them especially to organisms in aquatic ecosystems, which should be kept in mind to hinder a risk for contamination of ground waters while handling water-soluble ILs [9].

In the current work the main ILs that were used were 1-ethyl-3-methylimidazolium chloride (Emim Cl) and 1-ethyl-3-methylimidazolium acetate (Emim Ac), and also for them many manufacturers do not yet indicate thorough toxicity data. However, for example Sigma Aldrich classifies Emim Cl as irritant and gives the hazard statements of the skin irritation, serious eye irritation as well as warning for the possible long lasting harmful effects to aquatic life, while for Emim Ac it is stated that according to company's knowledge the thorough investigation of toxicity does not exist, but the product may be harmful.

Preparing a non-toxic IL sometimes requires multi-step reactions leading to hazardous waste accumulation. Furthermore, as ILs are often prepared from alkyl halide precursors (non-renewable petroleum-based feedstock), their reputation as a 'green chemicals' or 'green solvents' is on trial. However, many ILs with low saturation vapor have been shown to be both technically and environmentally beneficial by minimizing the atmospheric emissions while used instead of traditional organic volatile solvents. Some of them are also biodegradable. Regarding the variety of properties ILs can posses, as mentioned above, it is interesting to mention a couple of opposite examples of them: namely, there are ILs that can be used as hypergolic fuels (3-butyl-1-methyl-pyrrolidinium dicyanamide and 3-butyl-1-methyl-imidazolium dicyanamide etc.), and on the other hand, there are ILs that are flammable and even explode easily at elevated temperatures (3-butyl-1-*H*-imidazolium nitrate and 3-methyl-1-*H*-imidazolium nitrate etc.) [9].

The presence of impurities in ILs, such as water (usually mainly absorption of moisture by the hygroscopic ILs), halides, metals, organic bases or acids or traces of solvents (mainly coming from the synthesis of the ILs) affect both physical and chemical properties of the ILs, (e.g. thermal and chemical stability, melting point, as well as dissolution capabilities) [16]. IL impurities often have a poisoning effect on transition metal catalyzed reactions, and in addition, they may also have a significant role on nanoparticle stability in ILs. In the case of the acid or basic ILs, impurities of

the ILs can dramatically modify their acid-basic properties. The determination of the level of acidity of these ILs has most of time not been determined, which sometimes can lead to misunderstanding in the role of the ILs.

Fractionation of lignocellulosic biomass – the role of ionic liquid, IL

In order to use lignocellulosic biomass as a feedstock for chemical and biofuel production, its deconstruction is the logical first step; solubilization of all lignocellulosic biomass components (lignin, hemicelluloses and cellulose) can be one option, but it is not necessarily needed: for example, dissolution is not needed in dilute acid or organic solvent, Organosolv, treatments or in the traditional wood pulping in paper industry [**V**, 5, 17]. Another option for deconstructing lignocellulosic biomass is disrupting the lignocellulose composite chemically with only partial dissolution of lignin and hemicelluloses and letting the cellulose to remain insoluble but instead just to be fibrillated.

There are several deconstruction methods to choose from, and they all bear their specific pros and cons: the choice depends both on the final biomass utilization target (conventional pulp and paper or valuable platform chemicals and/or biofuel) and the biomass type used (major types being softwood, hardwood and grasses). The deconstruction method that suits for pulp and paper production does not necessarily suit for biorefinery concept – even though there are combined concepts. Softwood, hardwood and grasses all contain seemingly promising species for biorefinery feedstock [17]. The structural differences of the feedstock affect the efficiency of the deconstruction. The affecting properties (e.g. cell wall thickness, pore size and chemical composition) depend on the lignocellulose type. For example, lignin content and composition of it - in other words, whether its main building blocks are guaiacyl (composed principally of coniferyl alcohol units), guaiacyl-syringyl (composed principally of coniferyl and sinapyl alcohol units) or *p*-coumaryl/*p*-hydroxyphenyl subunits (composed principally of *p*-hydroxyphenyl/*p*-coumaryl alcohol units) vary between hardwood, softwood and grasses, which make an important difference in their deconstruction easiness or recalcitrance. Softwood species have mainly guaiacyl lignin, whereas lignin in hardwood species is of type guaiacyl-syringyl lignin, while grasses also contain minor amounts of *p*-coumaryl lignin besides guaiacyl-syringyl lignin. Grasses and hardwood species are generally considered to be easier to deconstruct than softwood species. The recalcitrance of the softwood species' wood against decomposition is caused both by the higher lignin content and by the type of it (higher guaiacyl to syringyl ratio): namely, guaiacyl units form more often C–C cross-links at the C-5 position of the ring - both during lignification and delignification – and the C5 position is substituted in the syringyl unit, which therefore cannot participate in substitution reactions, thus causing the fact that the C–C cross-links cannot be hydrolyzed by base or by acid [17].

Besides the more conventional fractionation methods, such organosolv pulping, chemical pulping (sulfate, sulfite or prehydrolysis sulfate treatment), micro-fluidized pulping, supercritical fluid extraction and acid treatment of pulp, ionic liquid treatments can be applied [**V**]. Regarding the utilization of ILs for lignocellulose biomass fractionation two kinds of approaches have recently emerged [17]. The most widely studied approach emphasizes the solubilization of all the biomass components

(extractives, hemicelluloses, lignin, and cellulose) and the second approach emphasizes the hydrolyzing of the carbohydrate polymers by using acidic or acidified lignocellulose or suitable ionic liquids involving dissolution of hemicelluloses and - more or less partial - lignin leaving the cellulose fraction largely intact.

Concerning cellulose processing, aiming to dissolve cellulose and to obtain cellulose fibers with IL (e.g. targeting to replace *N*-methylmorpholine-*N*-oxide, NMMO, or viscose process), the following properties of ILs should be considered: high decomposition temperature (> 200 °C), low melting point (< 20 °C), possessing stable spinning dopes, non-toxic character, odorless (negligible vapor pressure), low viscosity (making processing easier), and it should not cause deconstruction of cellulose, and in addition to that, the cellulose regeneration should be easy [9, 16]. In order to be able to replace the NMMO or viscose processes, the used IL treatment method should also be more cost effective, and the fiber quality should be at least equal to those achieved by the earlier methods.

When evaluating the effects of the various IL treatment methods or other lignocellulosic biomass fractionation methods, the following aspects can be investigated and compared after treatment: the weight percentage and the composition of solid material, cellulose crystallinity, chemical composition of carbohydrates and lignin in treated solid material or in dissolved material, as well as saccharification yield as hydrolysis kinetics or maximum biomass digestibility [17].

1-Ethyl-3-methylimidazolium acetate, Emim Ac, and some other ionic liquids (sulfonate and sulfate containing ILs) have been shown to be able to partially remove both lignin and hemicellulose fraction [17]. According to critical review by Brandt *et al*., the chloride based ILs did not appear to be as efficient for delignification, and in addition, their use resulted in very low glucose yields after enzymatic saccharification. The presence of chloride anion might enhance the enzyme deactivation even further than plain 'salt effect'. One explanation for obtaining higher concentrations of cellulose in acetate based ILs than in chloride based ones, is said to be the lower viscosity of the acetate-ILs [16]. ILs based on imidazolium cations, (e.g. 1-ethyl-3-methylimidazolium acetate, Emim Ac; 1-allyl-3-methylimidazolium chloride, Amim Cl; and 1-n-butyl-3-methylimidazolium chloride, Bmim Cl), are said to be efficient in dissolving cellulose partially due to their high hydrogen-bond basicity [16, 18], although these ILs are known to deactivate cellulases at fairly low concentrations – the reason why the samples usually are carefully washed away before saccharification step. However, research upon enzymatic development has recently emerged, for example. Shi *et al*. [18] demonstrated a one-pot system for simultaneous IL treatment and enzymatic saccharification for switchgrass with IL-tolerant enzymes.

The hemicellulose and lignin fraction removal or depolymerization – as well as morphology and crystallinity of cellulose, substrate reactivity (and in the case of enzymatic saccharification, also accessibility to enzymes) varies largely even for a single type of biomass, which is seen to result from the lignin (and hemicellulose) solubilization dependence on, or sensitiveness, for the treatment conditions (treatment time, temperature, moisture content of the ionic liquid and/or biomass, biomass loading versus IL ratio) [17, 18]. The longer the treatment time or higher the temperature used, the higher amount of lignin and hemicelluloses appears to be

removed, whereas a higher water content in the system tends to remove less lignin and even to lower the obtained saccharification yields. However, some newly developed IL –systems are capable to remove both hemicelluloses and lignin, even in the presence of large amounts of water [19]. Acidic or less basic environment of non-acetate ILs can sometimes cause hemicelluloses to degrade to furfurals and/or humins, which is not seen for the acetate ionic liquids [17, 18]. Pre-extraction has been suggested for an alternative deconstruction method in order to preserve the hemicelluloses (lignin and cellulose are more resistant towards deconstruction). According to Shi *et al*. regarding the utilization of ILs as pretreatment step before enzymatic saccharification, the severity of the pretreatment conditions can possibly be optimized to reduce the amount of biomass-derived cellulase inhibitors generated during IL pretreatment, thus further increasing sugar yields [18].

Mechanism of dissolution

There is no evident correlation between lignin and wood solubilization: some ILs can dissolve lignin without dissolving wood [16]. A significant increase in wood solubilization can be achieved by using microwaves (albeit with the risk of local overheating). Regarding lignocellulose dissolution reactions, IL anions (especially the chloride anions) are seen responsible for the hydrogen bonding disruption of the lignocellulosic complex matrix. The IL cation can also have an effect on the solubilization by the π-π interactions between the cation and the aromatic compounds of lignin.
Cellulose can be dissolved in IL without its derivatization reactions. The IL anion acts as electron donor center (or H-bond acceptor) interacting with the cellulose OH-group and forming a conceptual electron donor-electron acceptor complex – provided that the anion and cation are located close enough to each other. The cation can be seen as an electron acceptor center via non-bonding or π electron interactions and can additionally prevent the cross-linking of the cellulose.

Precipitation/regeneration of cellulose

By the addition of an anti-solvent, such as water, ethanol or acetone, cellulose dissolved by IL can be precipitated from its solution [16]. The regenerated cellulose is separated by centrifugation or filtration, after which the non-volatile IL can be recovered by the distillation removal of the anti-solvent. Regenerated cellulose could be obtained in different forms such as monoliths, fibers and films. Compared to the native cellulose, the regenerated sample can have the same degree of polymerization, although this much depends on the operating conditions of the treatment. Generally macro- and micro-structure – especially the degree of crystallinity – can be drastically changed and modulated by changing the conditions of regeneration.
Recovery of the ILs is important for future cost-effective and environmentally beneficial processing of cellulosic material, and it needs to be improved through future research.
Concerning the cellulose recovery, there are reports on studies of 2-phase systems using IL/ combined with water, alcohol or supercritical CO_2 – or just simply two immiscible ILs – or even sugars and sugar derivatives as water-IL solution additives. Sucrose is added to a solution of IL in water to separate the IL from the aqueous phase.

Reactivity of lignin in ionic liquids

All deconstruction pretreatment methods of lignocellulosic biomass by utilizing ionic liquids, ILs, cause chemical changes in both lignin and hemicelluloses, while the cellulose usually remains largely chemically unchanged excluding some structural changes [17]. Polymer fragmentation or chemical transformations in the lignin and the hemicellulose fraction can occur. For example, a decrease of the β-O-4 aryl ether bond content in lignin as well as deacetylation of xylan in Emim Ac-treated maple wood has been reported.

Additionally, a study regarding the cleavage of the β-O-4 aryl ether bonds in guaiacylglycerol-β-guaiacyl ether by hydrogen-bond acidic monoalkyl-imidazolium ILs has also been reported; and the reactivity differences depending on the anion were observed: higher yields of the cleavage products had been obtained by using more strongly hydrogen bond-basic anions (Cl^-, Br^- and HSO_4^-) than weakly basic anions. When the ionic liquid contained a coordinating anion, an enol ether product was observed as an intermediate. Less coordinating anions resulted in formation of formaldehyde by the elimination of the γ-hydroxyl methylene group [17].

A profound anion effect on the fragmentation mechanism and the degree of polymerization has been demonstrated - and alkyl sulfate anions had the highest capability to reduce polymer length and fragment the lignin, while the demonstrated order of molecular weight reduction was sulfates > lactate > acetate > chloride > phosphates. It seemed that the functional group of the anion determined the effect, and it had been suggested that the more active anions act as nucleophiles during lignin depolymerization. An increased sulfur content of the lignin after treatment with ionic liquids with sulfur containing anions has also been reported [17].

1.3 Analysis methods for biomass molecules – background

When analyzing sugars or other carbohydrates in wood, paper or pulp samples with gas chromatographic methods, the sample usually needs to be pre-treated: either by means of acid hydrolysis, acid methanolysis or enzymatic hydrolysis, followed by derivatization (silylation) prior to analysis. Both acid methanolysis and acid hydrolysis methods are needed for analysis of the total monosaccharide amounts in polysaccharides (including cellulose, other non-crystalline hemicelluloses, and pectins) because acid hydrolysis also degrades cellulose, unlike acid methanolysis and thus, acid hydrolysis is needed in order to be able to calculate for example the glucose amount released from cellulose. On the other hand, acid methanolysis has been proven to be better method for determination of xylan and uronic acids contents [20—22, **VI**].

When conventional analysis methods cannot be used, for example, due to presence of salts in samples in concentrations exceeding the analytical column tolerances (e.g. ILs or molten salts), the recommendations given above are of minor value; namely the traditional columns designed for carbohydrate analysis in gas chromatography, GC, and high-performance liquid chromatography, HPLC, apparatuses do not tolerate much over 50 ppm of salts. In fact, significant reliability problems have been observed upon use of GC-based methods, particularly in the case of a quantitative determination of monosaccharides in IL-containing samples when highly diluted

sample concentrations were used. The presence of high concentration salt solutions (the ion character of ILs) results in analytical challenges in traditional columns [23, 24]. This issue is particularly important, if the utilized ILs are water-soluble and otherwise soluble in similar solvents as analytes. A typical case is represented by monosaccharides, as the case was in the current work. However, capillary electrophoresis, CE, analysis method appeared as a natural solution and worked well for these types of samples [**VI]**.

Chromatographic analytical methods are generally based on the derivatization of analytes to enable detection by ultraviolet-visible spectroscopy, UV-vis, or even fluorescence spectroscopy. Moreover, neutral sugars have also been analyzed by HPLC using evaporative light-scattering, ELSD; refractive-index, RID; and pulsed-amperometric detectors, PAD. Since in capillary electrophoresis, CE, analysis, one has to overcome the extreme conditions required for the ionization of the carbohydrates and their low sensitivity to absorb UV light [25], alternative CE methods have been developed. One strategy is to add chromophores to the background electrolytes, BGEs, for indirect detection. Many suitable co-ions have been reported earlier and several alternative derivatization techniques have been devised. Nevertheless, ILs have been shown to be suitable BGE additives for the CE analysis of neutral carbohydrates [25]. CE method requires no derivatization, and only dilution is needed for the preparation of the samples. Adding ILs to BGEs therefore serves a dual function. First, the ILs act as chromophores due to their absorbance of UV light, enabling indirect detection. Secondly, ILs interact selectively with the analytes to facilitate their separation [**VI]**.

The basic function of capillary electrophoresis, CE

Figure 1 demonstrates the basic function principles of CE, also depicting the analysis parameters that were used in the current work.

Figure 2 compares CE and HPLC apparatuses and depicts their structural similarities. Unlike in HPLC system there is no high pressure applied with CE apparatus for pumping mobile phase, instead potentials up to 30000 volts can be applied for generating electro-osmotic flow, the driving force for CE. The inner diameter of CE capillary varies typically between 25 and 100 μm, thus allowing the sample volume variation from one nanoliter to 100 nanoliters compared to those of 0.5–1 μl for GC and 1–20 μl for HPLC [26, 27, 28].

The mobile phase “pump” in capillary electrophoresis, CE, is electro-osmotic flow, EOF. EOF, which makes the buffer solution flow from one buffer container to the other, is formed by maintaining the electrical potential across the CE’s capillary tube upon the electrical circuit constituted of the power supply, the electrodes, the capillary, the background electrolyte - also called buffer solution - and the buffer containers/ampoules. The force that drives each of the analytes in CE is a function of the electrical potential across the capillary, ionic charge of each analyte and the “ion mobility” in the BGE. As its simplest form, the function of CE can be explained as follows: the analytes possess different electrophoretic mobilities that depend on the charge and size of analyte. Large analytes with single charge travel more slowly than smaller analytes with single charge, while small ions with double charge travel faster than larger, double-charged ions etc. [26].

In the case of uncoated fused capillary CE column, the EOF "flows" toward the negative electrode: the inner walls of the capillary column have negative charge due to silanol groups (SO_x^-), BGE solution in each ampoule or "buffer container" has an electrode connecting it to power supply and equal amounts of cations and anions, which results to the other electrode to become net negative, the other net positive. While the immobile silanol anions form pairs with mobile buffer cations, a double layer of the BGE cations and anions are built between the capillary wall and the bulk BGE leading to attraction of the remaining BGE cations toward the negative electrode taking bulk buffer solution along. If the opposite direction of the EOF is wished for, one needs a capillary column that is coated with a cationic surfactant. The direction of EOF can also be changed by adding positively charged surfactant to the BGE solution [26].

CE can also use UV absorption detectors, as HPLC. But due to existence of many CE separable analytes with poor UV absorbance, so called indirect detection can sometimes also be utilized: i.e. a UV absorbing species is added to the BGE/buffer, the concentration of which is kept constant giving a "non-stop" UV detector signal, and the signal is obtained as a drop in absorbance when the poorly absorbing analyte arrives at the detector.

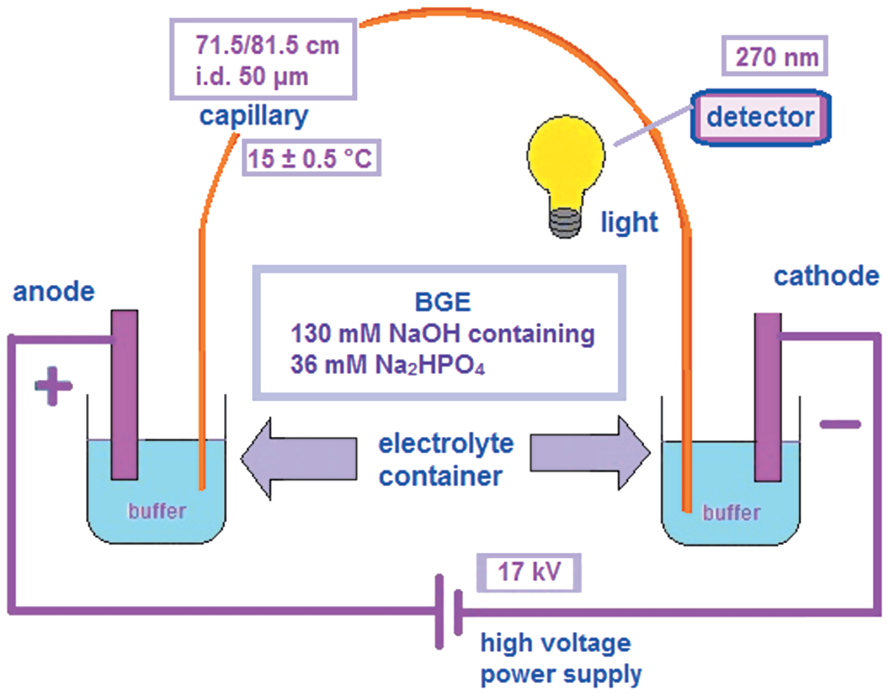


Figure 1. The basic principle of CE with the analysis parameters used in the current work.

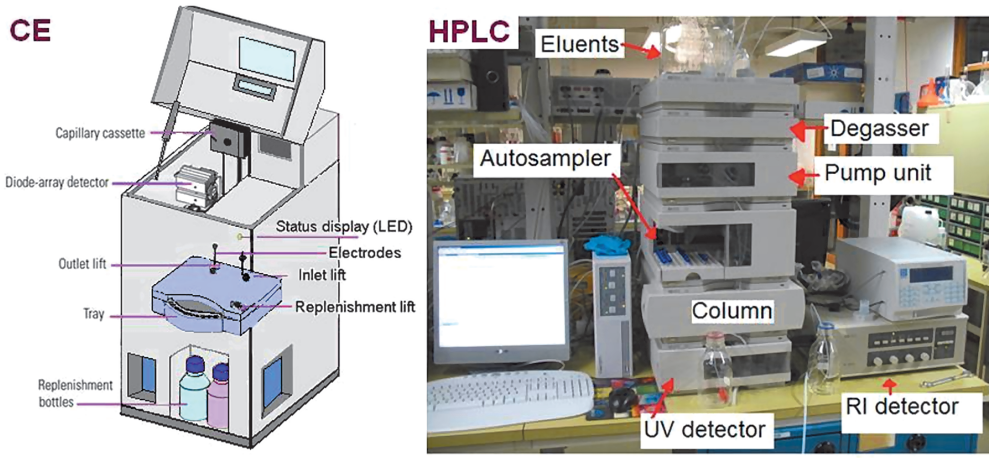


Figure 2. Capillary electrophoresis, CE, and high-performance liquid chromatography, HPLC, analysis apparatuses.

The basic function of gas and liquid chromatography, GC and HPLC

Figure 3 demonstrates one option for HPLC system and provides a separation model for the isocratic system with normal phase column, where more polar compounds adhere more tightly and thus elute later having the longest retention times, RTs, while the non-polar compounds elute first with the fastest RTs (compound A in this example picture). RTs are different for different chemical compounds, and besides the column type, they also depend on the used analysis parameters: column flow rate, temperature, injection volume, and type or types (and e.g. pH) of the used eluents/solvent mixture as well as whether the system is gradient or isocratic [27].

Solvent (eluent) can be chosen according to the polarity opposite the column polarity. Water/acetonitrile or water/methanol solvent mixtures can be chosen as eluent when neutral compounds are analyzed [27]. For lipophilic solutes to be analyzed with normal phase column, eluent should be chosen among organic solvents, such as hydrocarbons, halogenated hydrocarbons or alcohols, whereas eluent for the bio-organic substances to be analyzed with reversed phase, RP, column could be selected among aqueous mixtures with methanol, acetonitrile and additives (buffers, ion-pairs). In addition, for enantiomers (chirality chromatography) aqueous or organic solvents are recommended. For proteins and enzymes (bio-affinity chromatography) aqueous buffers with special additives are advised. Eluent for polymers, proteins and nucleic acids (analyzed by size-exclusion chromatography) should be chosen among aqueous buffers or organic solvents, and eluent for inorganic ions, acids and bases to be analyzed via ion-exchange chromatography is recommended to be selected among aqueous buffers and ionic solutions.

In addition to the isocratic elution system, HPLC separation can be based on a gradient elution. The isocratic elution system has a constant mobile phase composition, while the gradient elution system has a reproducible, stepwise or continuously changing mobile phase composition [27]. With dual pump or mixing-valve etc. combinations for gradient or isocratic systems the HPLC analysis method

possibilities are numerous – not to mention various types of columns, detectors and analytical condition parameters that can be optimized in several ways depending on the sample types and purposes of analysis. High pressure is applied at high-performance liquid chromatography, HPLC, system to pump its mobile phase toward the detector. HPLC detector can be for example a diode array UV detector, DAD; variable wavelength UV detector, VWD; refractive index detector, RID; and various other detector types (e.g. quadrupole MS detector).

There are several HPLC modes and various separation principles (having their own particular types of columns) based on the following types of chromatography: reversed phase, RP, adsorption (most of the bio-organic and bio-active compounds are analyzed with RP columns), ion exchange (e.g. used for bio-organic ions, acids and bases), size-exclusion (e.g. used for proteins, nucleic acids), bio-affinity (for enzymes) and chirality (used for separation of enantiomers). Elution order for normal phase column gives shorter retention times for non-polar and lipophilic solvents, while polar (hydrophilic) solutes elute before non-polar ones in reversed phase elution order columns. The HPLC column should be chosen depending on the purpose of the analysis, the type of the sample and conditions [27].

It is worth noting that the separation ability of the HPLC column is measured by resolution that is influenced by solvent polarity (retention factor), column efficiency (retention factor) and system chemistry (separation factor). Retention is controlled by mobile phase polarity and is convergent; separation is controlled by the chemistry of the column, mobile phase, and compound and by temperature. Efficiency is controlled by particle size, flow rate, and column length. Resolution is proportional to the square root of the number of the plates or column length.

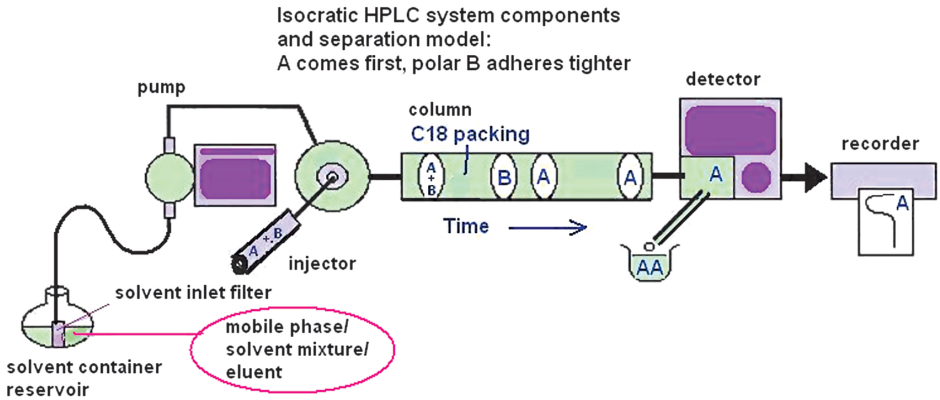


Figure 3. The components for isocratic HPLC system demonstrating separation of example analytes A and B, B being more polar component and thus eluting later (elution order for normal phase HPLC column).

Gas chromatography (see Figure 4) is always carried out in a column. Two kinds of columns are used, (as in liquid chromatography): capillary (open tubular) or packed column. A longer GC column increases the number of theoretical plates, giving higher separation ability [27, 28].

Pressurized gas – called carrier gas - is applied as the "mobile phase" at gas chromatography, GC, apparatus to transfer sample to detector. Typical carrier gases are helium, He; nitrogen, N_2; hydrogen, H_2; carbon dioxide, CO_2; or argon, Ar [28]. A molecular sieve is used to remove water and other impurities. Packed columns contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase. Most packed columns are 1.5–10 m in length and have an internal diameter of 2–4mm. Capillary columns have an internal diameter of a few tenths of a millimeter. The inner walls are coated with thin layer of stationary phase.

The packed column separation principles can be divided to adsorption with normal phase or reversed phase systems. Most of the bio-organic substances are suitable solutes for reversed phased GC adsorption system, while lipophilic solutes, such as oils, fats, lipids and dyes, can be analyzed with normal phase adsorption column. As with HPLC analysis, there are tables available how to choose the right analysis conditions with the right column and separation method [27, 28].

Analyte in GC analysis can be gas or volatile liquids: for example, flavor compounds, essential oils, hydrocarbons, fatty acids, environmental pollutants (e.g. pesticides), and especially modified substances. A compound must have sufficient volatility and thermal stability to be suitable for GC analysis. If all or some of the molecules or other compounds are in the vapor or gas vapor phase at 400-450 °C (or below), without decomposing at the mentioned temperatures, they can usually be analyzed by means of GC [28]. Derivatization (e.g. silylation) can be used to increase compound volatility.

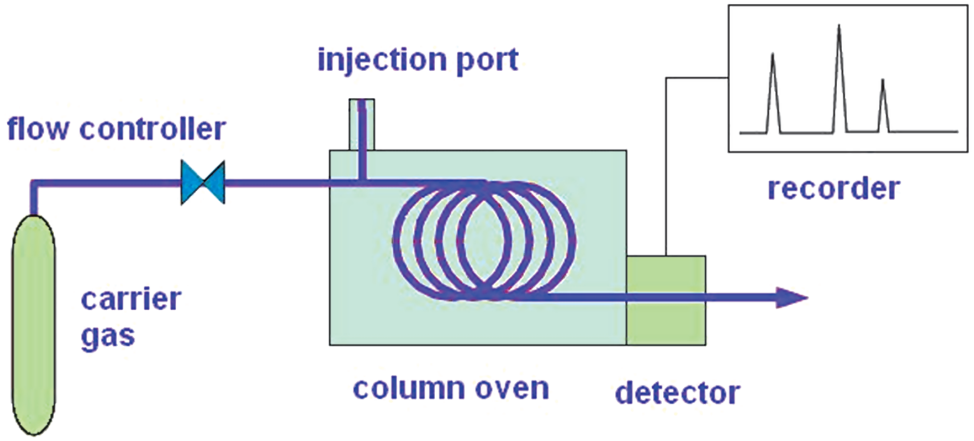


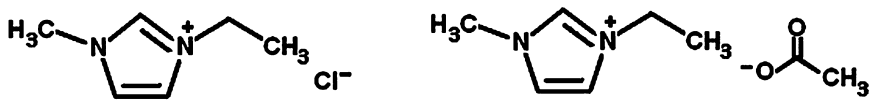
Figure 4. The basic principle of gas chromatography, GC.

2. EXPERIMENTAL

2.1 Sample preparation [VI]

The samples - Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and Silver birch (*Betula pendula*) lignocellulosic biomass sawdust - were grounded and sieved to the particle size of 1—2 mm, and in addition, one industrial Norway spruce wood sample was received with a varying particle size up to ca. 5 mm. The woody biomass was treated with either the ionic liquid, IL, 1-ethyl-3-methylimidazolium chloride, Emim Cl; or 1-ethyl-3-methylimidazolium acetate, Emim Ac; or with deionized water for comparison, at various temperatures: 80, 90 and 100 °C, respectively. In some experiments, even higher temperatures were applied. The wood content in IL (or water) varied between 1.4 and 54.5 wt% (wood in Emim Ac 3.9—11.2 wt% and wood in Emim Cl 1.4—54.5 wt%, respectively). Heat treatment was performed either in an oven in the absence of mixing or with continuous mixing on a heating plate residing on a shaker. Furthermore, an additional method was to apply magnetic stirring while using an oil bath. Some of the samples were oven dried before carrying out the experiments (overnight, at 100 °C). The samples were withdrawn at fixed time intervals (0—880 h). The focus was on the samples exposed to temperature treatment for 2—100 h.

Figure 5 demonstrates the structures of the ILs used in the current work.



1-ethyl-3-methylimidazolium chloride (Emim Cl)

1-ethyl-3-methylimidazolium acetate (Emim Ac)

Figure 5. The structures of the ionic liquids Emim Cl and Emim Ac (ILs, which were also used in the current work).

Since Emim Cl has a melting point of around 80 °C, it was practical to use temperatures above that. During the first experiments, temperatures far above 150 °C were also tried, but those experiments resulted in tar formation and total degradation of all sugars — possibly even leading to the degradation of furfural and 5-hydroxymethylfurfural, 5-HMF, which themselves are the degradation products of pentoses and hexoses, respectively. These elevated temperature experiments were not further pursued, because the focus of the study was to find out whether the utilization of ILs at moderate temperatures in a pre-treatment stage is capable to promote hydrolysis or depolymerization of the investigated lignocellulosic samples to a degree well suited for subsequent fermentations. For the very same reason, any kind of derivatization/silylation or other pre-treatment besides the above explained IL-heat-treatment was not applied to samples prior to HPLC or CE analysis.

Double experiments were always executed. The samples from the earlier experiments were stored in a freezer at temperatures between -18 and -22 °C.

'Simple' sugars (monosaccharides and some disaccharides), 5- HMF and furfural in the liquid part of the sample containing IL were of interest. Due to high IL concentration (over 50 ppm), the utilization of conventional GC and carbohydrate column was not possible; as mentioned earlier, IL-containing lignocellulosic samples led to reliability problems with GC even with very diluted versions of the samples [22, 23]. Instead, the samples were analyzed with a Hewlett Packard 1100 series HPLC, equipped with a RID and a diode array UV detector fitted with an Aminex HPX-87K carbohydrate analysis column and an Agilent 3D CE instrument equipped with a diode array UV/vis detector. For both HPLC and CE analysis, the standard solutions were prepared for all the analytes of interest, as well as for the internal standard, ISTD. Standard sugars were chosen according to the interest of subjects to be analyzed and in order to find an appropriate mixture with maximum separation without overlapping compounds. In addition, the goal was also to obtain results with the highest possible precision.

2.2 The applied ionic liquids, ILs [VI]

The samples were treated either with 1- ethyl-3-methylimidazolium chloride, Emim Cl, or 1-ethyl-3-methylimidazolium acetate, Emim Ac. Emim Cl was purchased from Sigma Aldrich and Merck (assay 98%) and used as such. Emim Ac was prepared by Dr. J. Gräsvik, Umeå University; (purity > 99%) and used as received. Prior to experiments, no water was added. It should be noted that although Emim Cl (as most ILs) is extremely hygroscopic, a glove box was not used for sample handling. The motivation was to study the simplest possible method that would hopefully also be cost-efficient enough to have a potential industrial interest. In addition, it is known that until a certain limit (ca. 1—4% as mentioned in [29]) the presence of water in IL might improve its dissolving/degradation/fibrillation ability, depending on the IL. Thus, the atmospheric moisture was allowed to have an influence — even though excessive moisture-uptake was prevented by weighing and handling Emim Cl as fast as possible.

2.3 CE analysis [VI]

An Agilent 3D capillary electrophoresis, CE, instrument (Agilent Technologies) equipped with a diode array UV/vis detector was used. Uncoated fused silica capillary with the effective length of 71.5 or 81.5 cm and an inner diameter of 50 μm was applied. The temperature of the capillary was 15 + 0.5 °C, the applied voltage was 17 kV and samples were injected under 35 mbar pressure for 10 s. The wavelength of 270 nm was used for detection.

The BGE used in the experiment was 130 mM NaOH containing 36 mM Na_2HPO_4 to provide the required pH. The same BGE mixture has been successfully employed for the analysis of sugar composition in acid hydrolyzed extracts of cellulose fiber samples [30] and for monitoring cellulose degradation in ILs [31].

For quantification purposes stock solutions of 50 mM in Milli-Q water for each sugar and furfurals were prepared. Working standard solutions within an appropriate range of concentration were made by diluting a stock solution with water. Sample preparation for CE analysis has been restricted to diluting supernatant to an

appropriate concentration. All the standards and the samples were analyzed three times for the quantification.

For the identification of the compounds in a sample, a mixture of the following analytes was used (all mentioned according to their CE migration time order): furfural; 5-HMF; sucrose, Suc (used as an internal standard, ISTD); cellobiose, Cel; galactose, Gal; glucose, Glc; mannose, Man; arabinose, Ara; and xylose, Xyl. Fructose has been omitted, because the retention time of it sometimes overlaps with that of arabinose or mannose.

In addition to possible influences from aging, the length and the wall conditions of capillary column — as well as other analysis parameters — do have an effect on how well mannose, fructose and arabinose are separated by CE analysis. At lower concentrations, i.e. 0.1—0.5 mM of mannose, fructose and arabinose could more easily be seen as separate peaks, while at and above the concentration level of 0.5 mM, the peaks started to coalesce more and more.

It is worth noting that CE electropherograms seen in Figures 6 and 7 [**VI**] were obtained when using a different apparatus and capillaries: selectivity and migration times were a little different, although the method was similar. Figure 7 [**VI**] demonstrates the varieties of the electropherograms for fresh and aged standard solutions of mannose, fructose and arabinose at the concentration levels of 2.0 and 0.5 mM: the up-most electropherogram depicts 2.0 mM of galactose, Gal; glucose, Glc; mannose, Man; fructose, Fru; arabinose, Ara; and xylose, Xyl; (the untypical form of the peaks reveal that this concentration is too high for a good, reliable analysis) whereas the two electropherograms below show sample electropherograms of the mentioned sugars at 0.5 mM concentration. The worst overlap for mannose, fructose and arabinose is seen in the third electropherogram, which represents an analysis of an aged sample stored at room temperature overnight.

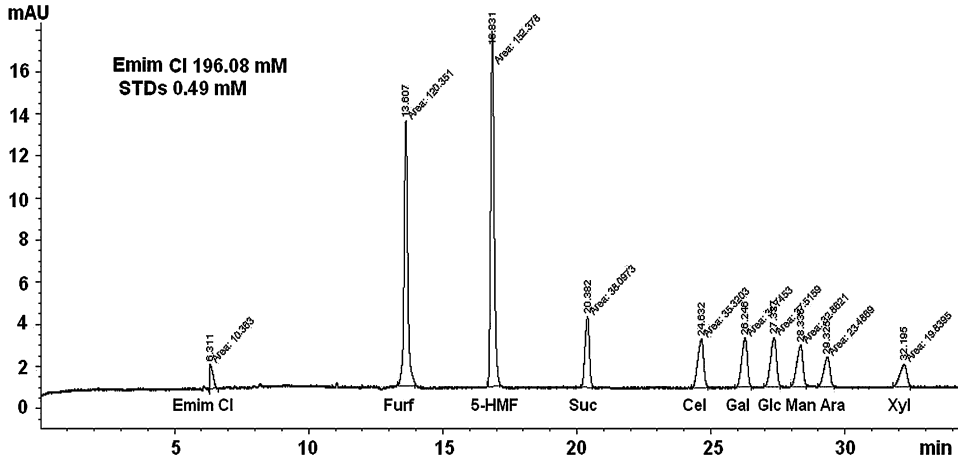


Figure 6. CE electropherogram of calibration analytes (0.5 mM) in Emim Cl (196 mM) [**VI**].

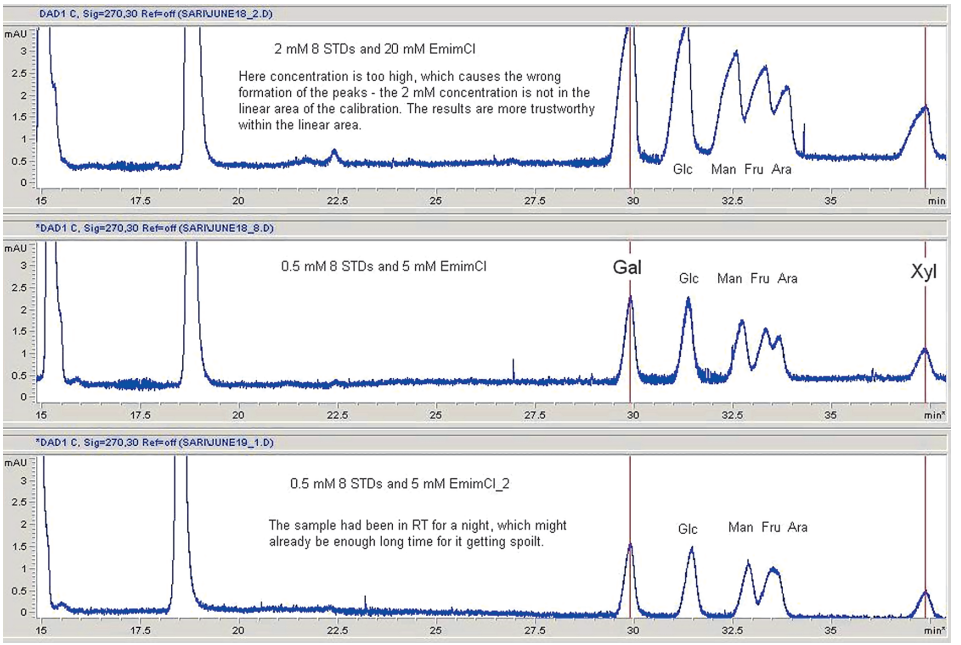


Figure 7. Fresh and aged standard solutions [**VI**].

2.4 HPLC analysis

A Hewlett Packard 1100 series HPLC, equipped with a refractive index detector, RID, and a diode array UV detector, DAD, fitted with an Aminex HPX-87K carbohydrate analysis column were applied simultaneously [**VI**]. However, the present study focused mainly on the signal from the RID. The reason for that was the applied analysis parameters of the detectors, the RID gave clearer and sharper chromatogram peaks, while for DAD the signal-to-noise ratio was often relatively high with the chosen wavelength parameters of 195, 200 and 220 nm, respectively. Consequently, these wavelengths were probably not the optimal ones for DAD. As the mobile phase (eluent), 100% deionized water was used. The eluent was degassed and filtered through a 0.45 µm PVDF membrane filter. All samples were diluted and filtered through a 0.45 µm syringe filter. The column temperature was 80 °C with an injection volume of 10 µL, and the flow rates for mobile phase were 0.300 and 0.400 mL/min. The injection needle was washed by deionized water.

At early stages of the research, other columns and parameters were also applied; one of them was the Supelco LiChrospher RP-8 column with the following column specification: column length 25 cm, particle size 5 µm and pore size 100 µm. Different analysis parameters were tried during HPLC method development: flow rates of 0.1–1 mL/min, temperatures of 25–60 °C, injection volumes of 1–5 µL as well as mixtures of water/acetonitrile (with and without phosphate buffer), water/methanol and water alone and a buffer solution as an alternative mobile phase. In addition, a reversed phase column (RP-C8) was tried (25 °C), applying both a refractive index, RI, detector (35 °C) and a Diode array UV detector, using the eluent flow rate of 0.8 mL/min and the sample injection volume 1.0 µL applying deionized water as the mobile phase (eluent) [**I**, **III**].

For the compound identification, a solution of the following compounds was used as a standard mixture (mentioned in their respective retention time, RT, order in our HPLC method): furfural; 5-HMF; sucrose, Suc (used as an internal standard, ISTD); glucose, Glc; rhamnose, Rha; xylose, Xyl; galactose, Gal; fructose, Fru; mannose, Man; and arabinose, Ara. However, some RT overlapping unfortunately appeared for selected chromatogram peaks [**VI**].

2.5 Gas chromatography, GC [I]

GC analysis was performed directly after sample silylation: the analysis method without acid hydrolysis or acid methanolysis was applied to elucidate the effect of Emim Cl on monosaccharide release, instead of just analyzing the composition of the wood polysaccharide components. Xylitol was used as an internal standard, ISTD.

The monosaccharides were analyzed on a 25 m x 0.2 mm i.d. HP-1 column (film thickness of 0.11 μm), and the chromatograms were handled with the computer software Totalchrom from Perkin-Elmer. The column oven parameters were as follows: 100 °C, 8 min, raised at 2 °C/min to 170 °C and raised at 12 °C/min to 300 °C (7 min); split injector (1:20), 250 °C; flame ionization detector, FID, 300 °C; injection volume, 1 μL. Hydrogen was used as a carrier gas.

3. RESULTS AND DISCUSSION […VII]

GC analysis of the liquid phase of the IL-treated samples could only provide the qualitative results about the nature of monosaccharides and uronic acids present in the samples. Quantitative calculations based on GC analysis were not possible due to the reliability problems caused by ILs, the issue that was demonstrated by the fact that some disappearance of peaks or appearance of extra peaks occurred when comparing samples with and without IL. In addition, analysis had to be performed with much diluted samples, to ensure that IL concentration was not exceeding 50 ppm. GC analysis was performed directly after sample silylation [**VI**].

HPLC could provide some qualitative and even some quantitative information, depending on the concentration of the analytes and IL. However, the best possible separation ability was not achieved in this work, regardless of the different columns and analysis parameters that were investigated. Some of the chromatogram peaks representing furfural and some of the monosaccharides overlapped with each other. In addition, higher concentrations of IL could cause overlapping of the chromatographic peaks representing the ILs applied with the internal standard sugar (sucrose) – or in some cases, even those of other monosaccharides - if the concentration of the IL was high enough in the sample [**VI**]. According to literature, however, there might have been a way to improve the HPLC separation ability via pH manipulation of the eluent towards more acidic side (with the addition of boric acid) [32, **III**].

CE method, in turn, was able to provide both qualitative and quantitative results without problems up to the IL concentration of at least 200 mmol/l (when a higher concentration of 350 mmol/l was present, the peaks on the first electropherograms were fine, but at that point, the capillary had to be washed and regenerated in order to continue analysis — no higher IL concentrations were tried). It is important, however, first to find the right dilution ratio of the samples with deionized water, since only the right dilution ratio in CE analysis gives clearer formed peaks on the electropherogram and much better separation.

Galactose, glucose, mannose, and xylose were the main monosaccharides obtained upon IL-treatment of wood samples, as expected; in addition, 5-HMF and furfural were detected and the quantitative amount of the two latter ones were naturally increasing with respect to the heating time or the ratio of IL to wood. The samples analyzed seemed to include more often 5-HMF (a degradation product of hexoses) than furfural (a degradation product of pentoses), as expected [**VI**], due to the fact that there are more hexoses in hemicellulose building blocks than pentoses. Furfural was more present in long-time birch treatments [**VII**].

Considering the theoretical hexose yields, the obtained 5-HMF yields were low here, less than 10 mg/g fresh wood for both softwoods and fresh hardwoods, respectively (see Figures 8a, 8b and 9 [**VI**]). As expected, some monosaccharides were also obtained, and not all sugars were degraded to 5-HMF or furfural. However, 5-HMF contents obtained in the present study are relatively high considering the fact that they were not calculated against dry solids content – this reporting approach always shows higher concentrations. Furthermore, the obtained 5-HMF content seemed, anyhow, not to be that low when comparing it to the earlier reported results of water soluble non-cellulosic carbohydrate amounts (where the amounts of arabinose, xylose,

galactose, glucose, mannose, rhamnose, glucuronic acid, galacturonic acid, and 4-*O*-methylglucuronic acid were determined[1]) for pre-extracted pine and spruce, which were 4.4-9.5 mg/g dry wood for the tested pine species and 4.7-39.2 mg/g dry wood for spruce species variation depending on the species and whether the tested wood was sapwood or heartwood, in spruce the variation between sapwood and heartwood was not very high sapwood being richer in carbohydrates than heartwood, while on the contrary sapwood in pine contained much less (4.7-6.9 mg/g) water-soluble carbohydrates than pine heartwood (24.2-39.2 mg/g) [33, **VI**].It's worth mentioning though that the earlier in literature reported values were obtained by extraction with distilled water for 3 h at 65±5°C, while in comparison, in the current work Emim Cl treatment of 100 h hours gave 5-HMF ca. 7 mg/g of fresh spruce and pine wood.

The content of 5-HMF varied more in birch than in pine or in spruce. The amounts of 5-HMF for pine wood were higher than for spruce; the lowest values were found for birch, in line with the literature concerning the contents of monosaccharides and uronic acids; and the comparison of their concentrations between the hemicellulose fraction (obtained e.g. by acid methanolysis analysis) and the content in the water-soluble fraction: when furfural is known to be a degradation product of pentoses, C-5 sugars (e.g. arabinose and xylose) and also that of uronic acids, while 5-HMF is a degradation product of hexoses; furthermore, it is natural that pine and spruce gave more 5-HMF than birch if considering that the hemicellulose fraction of birch contains more non-cellulosic xylose, arabinose and uronic acids than spruce or pine (according to the earlier reported values for birch, obtained by acid methanolysis), whereas on the contrary, the contents of water-soluble non-cellulosic xylose, arabinose and uronic acid were reported to be lower in birch than in spruce and pine [**VI**, 33]. In effect, the IL-treatment was demonstrated to be more efficient than plain water extraction, at the very least.

On the other hand, unlike hardwood species that besides guaiacyl units also have richly syringyl units in their lignin structure, softwood species are known to need harsher conditions for delignification - and they are thus more difficult to deconstruct - than hardwoods due to the high content of guaiacyl-lignin and the C-C cross-link formation of guaiacyl units (at C-5 position of the aromatic ring): namely, C-C cross-links cannot be hydrolyzed by acid or base [17]. This might explain why sugars in birch can be obtained faster and can also earlier be turned to their degradation products (furfural and 5-hydroxymethylfurfural).

The obtained furfural and monosaccharide concentrations were low [**VI**]. The results in general suggest that cellulose was not deconstructed or depolymerized to glucose. Surprisingly, furfural and 5-HMF were formed even at relatively short treatment times (e.g. after already 5 h treatment with Emim Cl). In addition, only the lowest amount of ca. 6 mg/g was reached for fresh birch wood by the treatment time of hundred hours. On the contrary, after a similar treatment, the 5-HMF content for the spruce and pine increased from zero to ca. 7 and 8 mg/g (fresh spruce and pine wood), respectively. The first 20 hours seem to be crucial regarding the rate of 5-HMF formation [**VI**]. However, further studies are needed to study the first 20 hours more carefully.

[1] 4-O-methylglucuronic acid is mainly associated with xylans, while galacturonic acid is associated with pectins, and glucuronic acid are often assumed to origin from arabinogalactans.

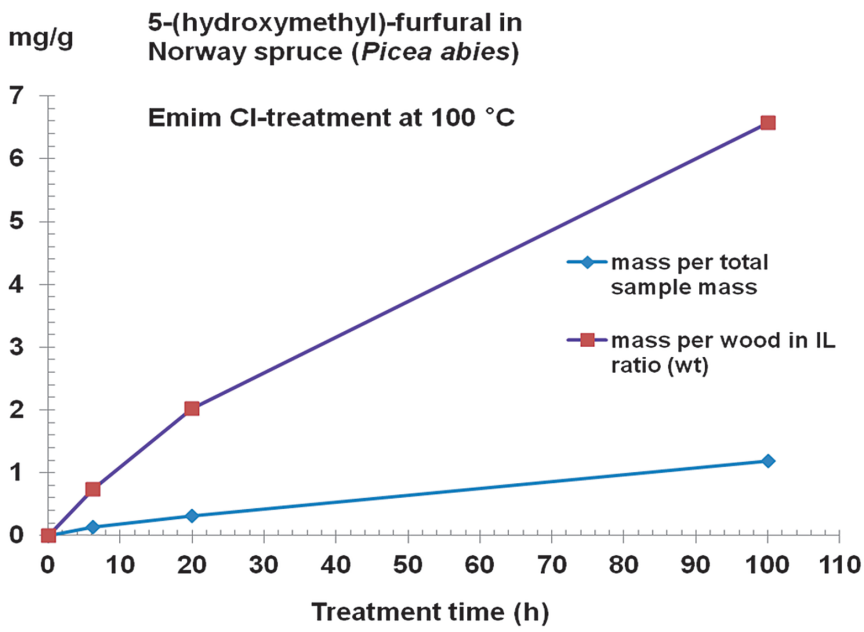


Figure 8a. The amount of 5-HMF obtained upon the IL treatment of Norway spruce [**VI**].

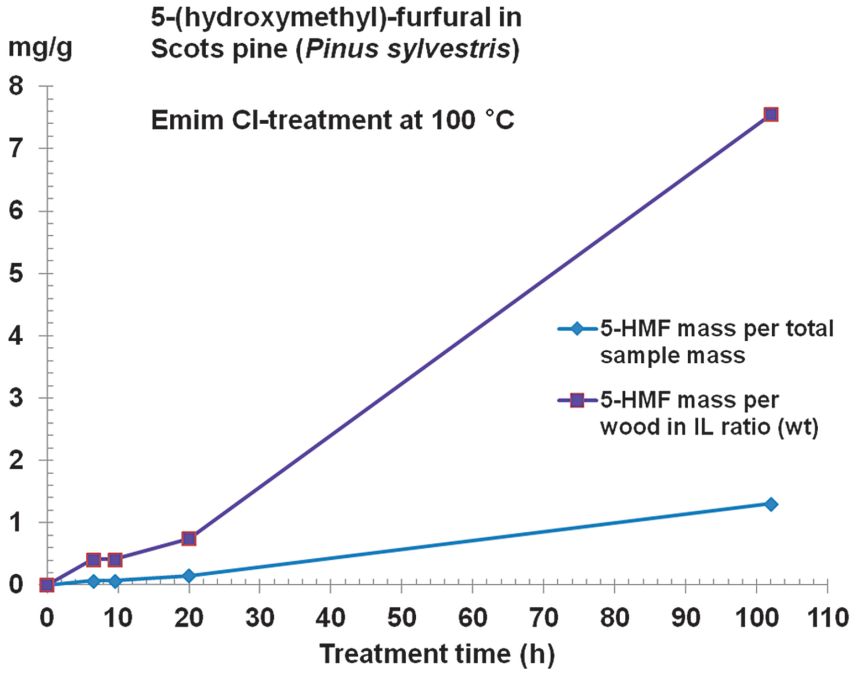


Figure 8b. The amount of 5-HMF obtained upon the IL treatment of Scots pine [**VI**].

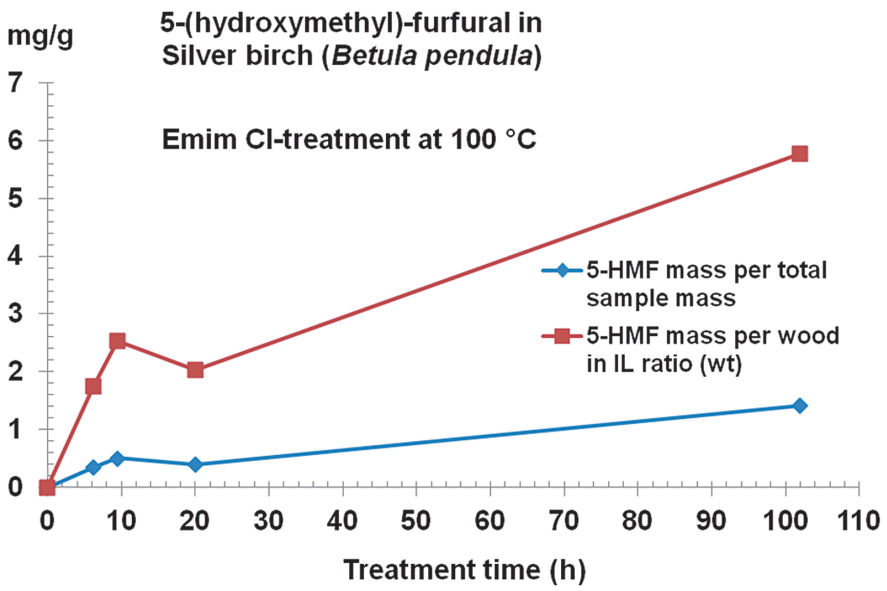


Figure 9. The amount of 5-HMF obtained upon the IL treatment of Silver birch [**VI**].

In this study, when comparing spruce samples treated in Emim Ac to those when Emim Cl was used (treated at 100 °C, >9 h), more analytes were identified in the Emim Cl treated samples than in the Emim Ac treated samples. The latter ones contained practically neither sugars, furfural nor 5-HMF. The reason might be the lower wood-to-IL ratios in the case of Emim Ac. Alternatively, other reasons, such as too harsh experimental conditions causing the sugars in Emim Ac to degrade even beyond 5-HMF and furfural might have occurred, since Emim Ac is known to be an efficient solvent for cellulose. It has even been proposed that Emim Ac could react with lignocellulosic species in the way that enhances the dissolution of cellulose (or wood) [34]. It has also been speculated that the basicity of the anion in the solution influence the reaction via catalyzing the deprotonation step [34, **VI**]. Emim Ac has a higher basicity and affinity to water than Emim Cl, which has been suggested to be the reason for earlier reported significantly faster α,β-dehydration reaction for of tested lignin model compounds that were studied by dissolving in Emim Cl and Emim Ac at 120 °C [17]. Deacetylation of xylan residues in birch and eucalyptus have been reported to occur at 100 °C and 120 °C in 30 h and 3 h, respectively [34, **VI**].

Uronic acids or sugar alcohols were not analyzed; some unknown compounds were, however, seen in the obtained CE electropherograms. The unidentified compounds might presumably be sugar alcohols, some di- and oligosaccharides or other sugar derivatives. It is very plausible that much more happens than just simple hydrolysis/depolymerization/dehydration as in the case of hot water extraction as the utilized ILs are reported to be able to dissolve even wood or cellulose [5]. It should be noted that dissolution of cellulose and/or lignin is often seen as a part of the deconstruction process, although it is not necessarily needed for all deconstruction options (e.g. dilute acid or organic solvent treatments) nor IL treatments [17]. The IL treatments of wood quite often end-up with gaining non-fibrous pulp, while the wood

components are selectively precipitated and lignin is not efficiently removed [5, 6]. Fibrillation is sometimes the reasonable goal for the biomass treatment: in commercial kraft, soda and sulfite pulping, wood chips are also only fibrillated, deconstructed to fibers - sparing cellulose – while nearly all lignin is defragmented and removed [5].

Figure 10 depicts the analysate concentrations after 100-hour-lasting Emim Cl treatment in industrial waste (from Örnsköldsvik, Sweden) spruce sawdust, sieved to the particle size ~5mm, which contains a mixture of heartwood, sapwood as well as residues of knots and bark. Mannose, glucose and galactose were the main monosaccharides obtained after this long treatment. The concentration of the well known hexose degradation product, 5-HMF, was the highest of all. There was no furfural detected for this treatment, and the other measured monosaccharide amounts were mainly only trace amounts. The figure shows even the high standard deviations, which could be expected for this particular sample type constituting of sawdust with varying particle size and especially with varying content including even bark and knots, as well as both sapwood and hardwood.

It is well known that wood component compositions vary between different parts of wood. Particle size of the wood samples has also a great effect on the results. Industrially seen, this type of wood waste is however more relevant research subject than artificially produced completely homogenous samples. Other samples in the current work were of the latter type, and the results were more consistent [**VII**].

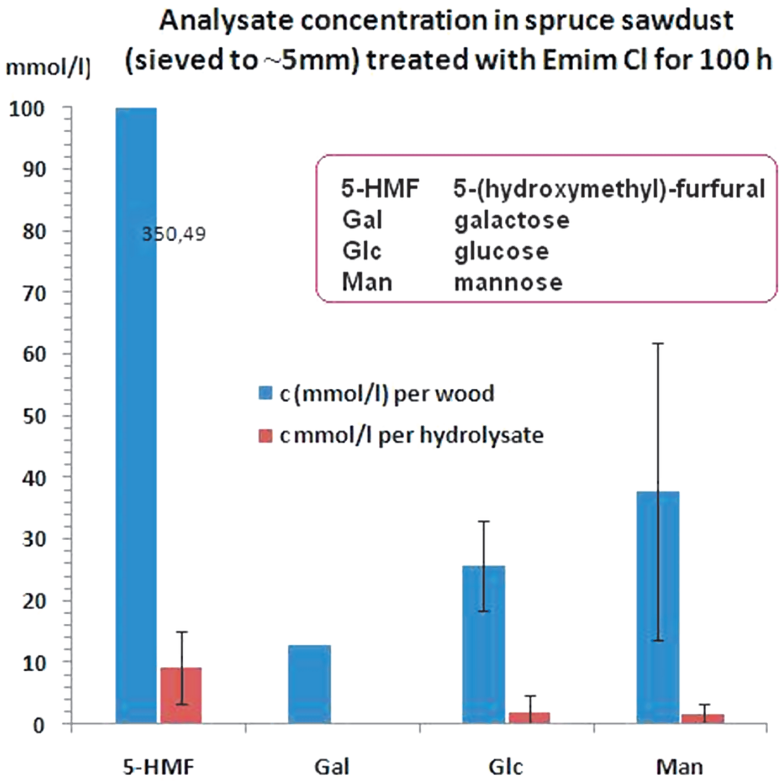


Figure 10. Analysate concentration in industrial spruce sawdust (particle size ~5mm), treated with Emim Cl for 100 h. Seemingly high standard deviations are caused by the variation of this particular spruce sawdust quality (includes sapwood, heartwood and possibly knots and bark residues).

4. CONCLUSIONS

The goal in the current work was to study fractionation of wood by means of an ionic liquid, IL, based pre-treatment, and furthermore, the aim was to obtain monosaccharides, hexoses and pentoses. Softwood sawdust (particle size varying from 2 mm to above 5 mm), Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and Silver birch (*Betula pendula*), were exposed to ionic liquids 1-ethyl-3-methylimidazolium chloride, Emim Cl, and 1-ethyl-3-methylimidazolium acetate, Emim Ac, under thermal treatment (80-150 °C) for various time intervals (0-880 h). The main interest was to study the liquid part of the sample, which contained both the IL and sugars (mono-, di- and oligosaccharides), as well as their degradation products and other carbohydrates depolymerized from polysaccharides – mainly from hemicelluloses.

Some analysis challenges emerged from the fact that both used ILs were completely water-soluble, such as the sugars and due to the lack of higher salt- or IL-concentration tolerance of the traditional carbohydrate analysis columns used in the GC and HPLC apparatuses (the salt tolerance is often less than 50 ppm). Thus, during the project, development of new analysis methods became of essential importance; it was necessary to conduct various trials involving different HPLC analysis parameters for a few different columns and eluents and finally ended up to using the CE method that Dr. Koel *et al*. had been developed earlier. The used CE method is currently relatively novel. During the recent years only relatively few published research reports regard the analysis of samples containing higher concentrations of IL/ILs. Otherwise, the number of research publications concerning IL-based pretreatment methods of lignocellulosic biomass - as well as the knowledge of the treatment process options - has increased exponentially in recent years.

The applied capillary electrophoresis, CE, method clearly demonstrated better separation ability compared to that of HPLC. Moreover, upon CE analysis, the presence of ILs does not result in reliability problems as in the case of traditional GC sugar columns that do not tolerate salts (often less than 50 ppm). Furthermore, the problem of over-lapping chromatogram peaks seen with HPLC could also be avoided using CE.

Galactose, glucose, mannose and xylose were the main monosaccharides obtained after IL-treatment (Emim Cl and Emim Ac) of wood samples. In addition, 5-HMF and furfural were detected, and the quantitative amount of them was increasing with an increase in the heating time or the IL-to-wood ratio. The results suggest that samples contained more often 5-HMF than furfural - furfural was more present in long-time birch treatments, as expected, and this is a reasonable consequence of 5-HMF being the degradation product of hexoses (e.g. glucose, fructose, galactose, mannose, rhamnose) whereas furfural is that of pentoses (e.g. arabinose and xylose). In fact, if all of the polysaccharides - including cellulose - in woody biomass were depolymerized to their monomeric building blocks, the yield of hexoses should be significantly higher than that of pentoses. If cellulose is excluded, xylans in birch would give a significant amount of pentoses. It is also possible that furfural reacts further to its subsequent degradation products, for example possibly to furfuranol (i.e. 2-furanmethanol) via hydration or to furoic acid via oxidation.

Emim Cl-treated (at 100 °C) pine sawdust samples contained more 5-HMF than the similar samples of spruce. The lowest 5-HMF content was found for birch samples. In addition, the content varied more in birch samples than for pine or spruce. This is in line with the literature and expected for reported water-soluble hardwood carbohydrates (monosaccharides and uronic acids). It can be emphasized that at treatment temperatures above 150 °C, similarly longer treatment times (above 100 h) result in tar and/or humins formation and degradation of all sugars. In particular, 0—20 h treatment time seemed to be the most interesting experimental window, provided that mono- or polysaccharides are the main goal. Being sugar degradation products, 5-HMF and furfural are good indicators in revealing when the experimental or treatment conditions are too harsh to obtain ‘simple’ sugars.

Cellobiose was seen in some samples, albeit fucose was in most cases not present. 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. Cellubiose being the only disaccharide that was determined, it can be concluded that di- and oligosaccharides obtained (depolymerized from wood polysaccharides) should also be analyzed in detail in future.

5. NOTATIONS/ABBREVIATIONS

AFEX	ammonia fiber explosion
Amim Cl	1-allyl-3-methylimidazolium chloride
Ar	argon
Ara	arabinose
BGE	background electrolyte
Bmim Cl	1-butyl-3-methylimidazolium chloride
c	concentration, mmol/l
CE	capillary electrophoresis
Cel	cellobiose
CO_2	carbon dioxide
DAD	diode array UV detector
ELSD	evaporative light-scattering detector
Emim Ac	1-ethyl-3-methylimidazolium acetate
Emim Cl	1-ethyl-3-methylimidazolium chloride
FID	flame ionization detector
Fru	fructose
Furf	furfural
Gal	galactose
GalA	galacturonic acid
GC	gas chromatography
Glc	glucose
GlcA	glucuronic acid
h	hour
H_2	hydrogen
He	helium
H-bond	hydrogen bond
5-HMF, HMF	5-hydroxymethylfurfural, i.e. 5-(hydroxymethyl)-2-furaldehyde
HMR	hydroxymatairesinol
HPLC	high-performance liquid chromatography
IL/ILs	ionic liquid/ ionic liquids
ISTD	internal standard
Man	mannose
4-*O*-MeGlcA	4-*O*-methylglucuronic acid
MS	mass spectrometry
N_2	nitrogen
NMMO	*N*-methylmorpholine-*N*-oxide

OH-	hydroxyl
Organosolv	organic solvent
PAD	pulsed-amperometric detector
ppm	parts per million
PVDF	polyvinylidene difluoride
Rha	rhamnose
RI(D)	refractive index (detector)
RP	reverse phase
RT	retention time; (room temperature)
RTIL	room temperature ionic liquid
STD, std	standard
Suc	sucrose
UV	ultraviolet
UV-vis	ultraviolet-visible spectroscopy
VWD	variable wavelength UV detector
Xyl	xylose

6. REFERENCES

[1] P. Maki-Arvela, T. Salmi, B. Holmbom, S. Willfor, D.Yu. Murzin, Chem. Rev. 111 (2011) 5638–5666.

[2] M. Galbe, G. Zacchi, Adv. Biochem. Eng. Biotechnol. 108 (2007) 41–65.

[3] A.W.T. King, J. Asikkala, I. Mutikainen, P. Järvi, I. Kilpeläinen, Angew. Chem. Int. Ed. Engl. 50 (2011) 6301–6305.

[4] I. Anugwom, P. Mäki-Arvela, V. Eta, P. Virtanen, J.-P. Mikkola, Towards optimal treatment procedure upon fractionation of Nordic lignocelluloses using novel alkanol amine-superbase ionic liquid system, a conference abstract, in: The Fourth Nordic Wood Biorefinery Conference (NWBC), Helsinki, October 23–25, 2012.

[5] A. W.T. King, L. Zoia, I. Filpponen, *et al*., J. Agric. Food Chem. 57 (2009) 8236–8243

[6] A. W.T. King, I. Kilpeläinen, S. Heikkinen, P. Järvi, D.S. Argyropoulos, Biomacromolecules 10 (2009) 458–463.

[7] P. Maki-Arvela, E. Salminen, T. Riittonen, P. Virtanen, N. Kumar, J.-P. Mikkola, Int. J. Chem. Eng. 10 (2012) 674761

[8] I. Anugwom, P. Maki-Arvela, P. Virtanen, S. Willfor, R. Sjoholm, J.-P. Mikkola, Carbohydr. Polym. 87 (3) (2012) 2005–2011

[9] J. Gräsvik, D. G. Raut, J.-P. Mikkola 2012] J. Gräsvik, D. G. Raut, J.-P. Mikkola, Challenges and perspectives of ionic liquids vs. traditional solvents for cellulose processing in 'Handbook of ionic liquids' (eds. J. Mun, H. Sim, Nova Science Publishers 2012

[10] M. Käldstrom, N. Kumar, M. Tenho, M.V. Mokeev, Y.E. Moskalenko, D.Yu. Murzin, ACS Catal. 2. (7) (2012) 1381–1393.

[11] J.-P. Mikkola, R. Sjoholm, T. Salmi, P. Maki-Arvela, Catal. Today 48 (1999) 73–78, 1–4

[12] T. Salmi, J. Kuusisto, J. Warna, J.-P. Mikkola, Chim. Ind. 88 (8) (2006) 90–96.

[13] S. Willfor, K. Sundberg, M. Tenkanen, B. Holmbom, Carbohydr. Polym. 72 (2008) 197–210.

[14] K.S. Mikkonen, M.I. Heikkila, S.M. Willfor, M. Tenkanen, Int. J. Polym Sci. (2012) 482810.

[15] K.S. Mikkonen, M. Tenkanen, Trends Food Sci Technol. 28 (2012) 90–102.

[16] H. Olivier-Bourbigou, L. Magna, D. Morvan, Appl. Catal., A 373 (2010) 1–56

[17] A. Brandt, J. Gräsvik, J. P. Halletta, T. Welton, Green Chem. (2013) 15, 550–583

[18] J. Shi, J. M. Gladden, N. Sathitsuksanoh, P. Kambam, L. Sandoval, D. Mitra, S. Zhang, A. George, S. W. Singer, B. A. Simmonsa, S. Singh, Green Chem., 15 (2013) 2579–2589.

[19] V. Eta, I. Anugwom, P. Virtanen, P. Mäki-Arvela, J.-P. Mikkola, Ind. Crop. Prod. 55 (2014) 109–115.

[20] S. Willfor, A. Pranovich, T. Tamminen, *et al*., Ind. Crop. Prod. 29 (2009) 571–580.

[21] S. Willfor, A. Sundberg, J. Hemming, B. Holmbom, Wood Sci. Technol. 39 (2005) 245–258.

[22] S. Willfor, A. Sundberg, A. Pranovich, B. Holmbom, Wood Sci. Technol. 39 (2005) 601–617.

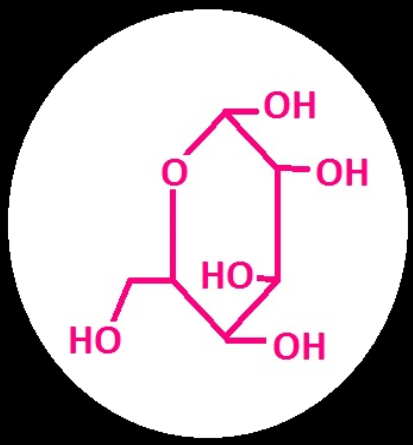
[23] S. Hyvärinen, P. Damlin, J. Grasvik, D.Yu. Murzin, J.-P. Mikkola, Cell. Chem. Technol. 45 (7/8) (2011) 483–486.

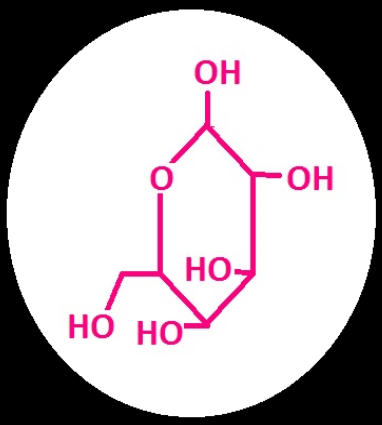
[24] S. Hyvärinen, P. Virtanen, D. Yu. Murzin, J.-P. Mikkola, Cell. Chem. Technol. 44, 4–6 (2010) 187–195.

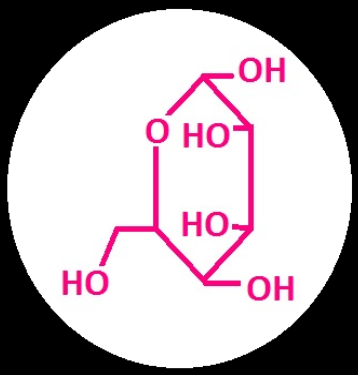
[25] M. Vaher, M. Koel, J. Kazarjan, M. Kaljurand, Electrophoresis 32 (2011) 1068–1073.

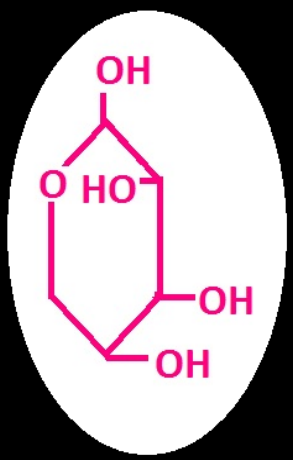
[26] J. Zaia, Capillary electrophoresis-mass spectrometry of carbohydrates in 'Capillary Electrophoresis of Biomolecules, Methods and Protocols' (eds. N. Volpi, F. Maccari), Methods in molecular biology Vol. 984, Humana Press 2013.

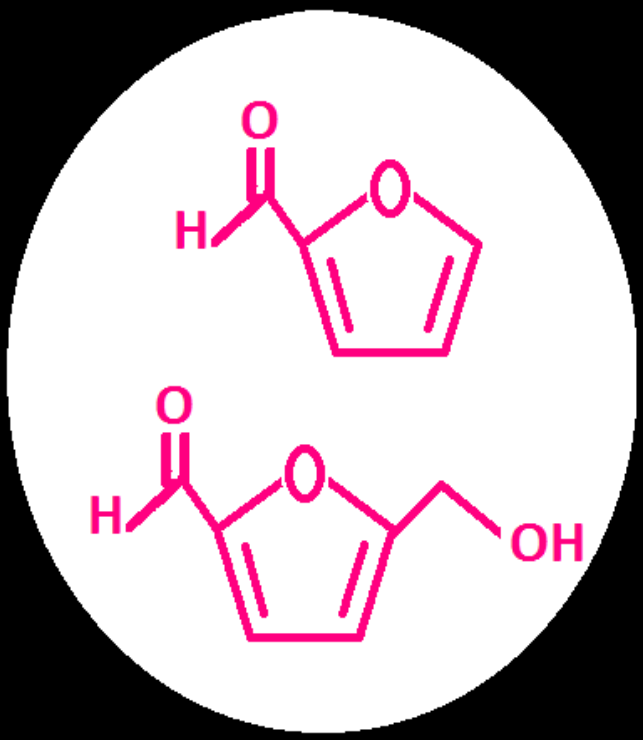
[27] M. C. McMaster, HPLC: A Practical User's Guide, Wiley Interscience, A John Wiley & Sons, Inc. Publication, 2007
[28] J. Franzen, , Theory of GC2 — an unusual approach. J. High Resol. Chromatogr., 2 (1979) 324–328.
[29] C. Sievers, M.B. Valenzuela-Olarte, T. Marzialetti, I. Musin, P.K. Agrawal, C.W. Jones, Ind. Eng. Chem. Res. 48 (2009) 1277–1286.
[30] S. Rovio, H. Simolin, K. Koljonen, H. Siren, J. Chromatogr. A 1185 (2008) 139–144.
[31] M. Vaher, K. Helmja, A. Kasper, M. Kuraˇsin, P. Valjamae, M. Kudrjaˇsova, M. Koel, M. Kaljurand, Catal. Today 196 (2012) 34–41
[32] C. De Muynck, J. Beauprez, W. Soetaert and E. J. Vandamme, J. Chromatogr. A, 1101 (2006) 115.
[33] S. Willfor, A. Sundberg, J. Hemming, B. Holmbom, Wood Sci. Technol. 39 (2005) 245–258.
[34] A. W.T. King, A. Parviainen, P. Karhunen, J. Matikainen, L.K.J. Hauru, H. Sixta, I. Kilpelainen, RSC Adv. 2 (2012) 8020–8026.

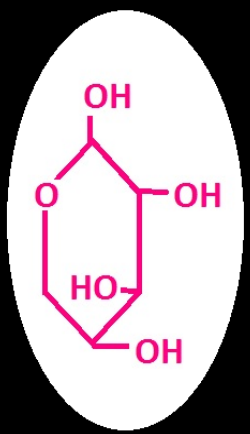












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